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GOVERNMENT NOTICE
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DEPARTMENT OF TRADE AND INDUSTRY
DEPARTEMENT VAN HANDEL EN NYWERHEID

No. R. 530 **14 May 1999**

STANDARDS ACT, 1993

COMPULSORY SPECIFICATION FOR FROZEN FISH, FROZEN MARINE MOLLUSCS AND FROZEN PRODUCTS DERIVED THEREFROM

I, Alexander Erwin, Minister of Trade and Industry, hereby under Section 22(1)(a)(I) of the Standards Act, 1993 (Act No. 29 of 1993), and on the recommendation of the Council of the South African Bureau of Standards, declare the specification for frozen fish, frozen marine molluscs and frozen products derived therefrom, as set out in the Schedule, to be compulsory with effect from the date 2 months after the date of publication of this notice, with the simultaneous withdrawal of the compulsory specification for frozen fish, frozen marine molluscs and frozen fish and frozen marine mollusc products, published by Government Notice No. R.35 of 12 January 1973.

ALEXANDER ERWIN
Minister of Trade and Industry

SCHEDULE

COMPULSORY SPECIFICATION FOR FROZEN FISH, FROZEN MARINE MOLLUSCS AND FROZEN PRODUCTS DERIVED THEREFROM

1 Scope

This specification covers the requirements for the handling, preparation, processing, packaging, freezing, storage and quality of frozen fish, frozen marine molluscs, frozen fish products and frozen marine mollusc products intended for human consumption. It also covers requirements for factories and employees involved in the production.

2 Definitions

For the purposes of this specification, the following definitions apply:

2.1 acceptable: Acceptable to the authority administering this specification.

2.2 brine pre-freezing: A process by which fish are rapidly frozen by total immersion in refrigerated brine at a temperature of –7 °C or lower.

NOTE – Brine pre-freezing does not complete the process of freezing defined below as the "freezing process" (see 2.11).

2.3 by-product: A product not intended for human consumption.

2.4 chill room: An insulated and refrigerated room that is specially designed for the storage of foods at temperatures not lower than –1 °C and not higher than 4 °C, that has sufficient refrigeration capacity to maintain the desired storage temperature and that could also have sufficient refrigeration capacity that products placed in the chill room are cooled to that temperature.

NOTE – Where the product is to be stored with ice in a chill room, the above definition is not applicable.

2.5 factory: Any premises, vehicle or vessel on or in which fish (see 2.6) or fish products (see 2.8) are handled or treated to prepare them for freezing for commercial purposes. This definition excludes fish shops, hotels, boarding houses, restaurants or other eating houses.

2.6 fish: Any edible cold-blooded aquatic (marine or freshwater) vertebrate that has gills throughout its life, and that has any limbs modified into fins, and all marine molluscs and cephalopods (see 2.13).

2.7 fish bone defect: A fish bone of length equal to or exceeding 10 mm, or of diameter equal to or exceeding 1 mm. A bone of length not exceeding 5 mm is not considered a defect if its diameter does not exceed 2 mm. The foot of a bone (where it has been attached to the vertebra) can be disregarded if its width does not exceed 2 mm, or if it can easily be stripped off with a fingernail.

2.8 fish product: Any fish product, whether in a processed form or not, wholly or partly derived from fish (see 2.6), and intended for human consumption.

2.9 freezer: A room or equipment that is specially designed to lower the temperature of a food product through the zone of maximum crystallization (see 2.11) and down to an equilibrium temperature of –20 °C or lower in a period of time that is acceptable for the product.

2.10 freezer storage room: An insulated freezer room that is specially designed for the storage of frozen foods and that has sufficient freezing capacity to maintain a product temperature of –20 °C or lower when products that have already been frozen to that temperature are being stored.

NOTE – A freezer storage room is not designed to freeze products.

2.11 freezing process: The continuous process whereby the temperature of the product is lowered through the zone of maximum crystallization (for most products, between –1 °C and –5 °C), at a rate of at least 6 mm of product thickness per hour, and that is only completed when the temperature of the entire product, after thermal stabilization, has reached –20 °C or lower.

NOTE – The process whereby a product that has undergone brine pre-freezing is so further frozen that the temperature of the entire product, after thermal stabilization, is –20 °C or lower, is considered a continuous process in terms of this specification, provided that the temperature of the product is not raised above –7 °C during this process.

2.12 frozen fish product: A fish product (see 2.8), smoked or unsmoked, cooked or uncooked, that has undergone a freezing process and has been preserved by storage in the frozen state.

2.13 marine mollusc: Any marine invertebrate of the phylum Mollusca, that has a soft unsegmented body and often a shell, secreted by a fold of skin (the mantle). The group includes bivalves (such as clams and mussels) and cephalopods (such as squid).

2.14 minced fish: Minced particles of skeletal muscle of fish that have been separated from, and that are acceptably free from, fish bones and skin.

2.15 outer container (master container): The box, carton or case into which packages of frozen fish products (with or without wrappers) are packed for storage and distribution.

2.16 package (immediate container): The immediate carton, plastics pouch or other container in which the product is packed for storage and distribution.

2.17 preserve: To maintain in sound edible condition by the prevention of deterioration.

2.18 process: The course of operations during production (see 2.20) of the product.

2.19 product: Fish, marine molluscs, fish products or marine mollusc products for human consumption, in the course of transportation, handling, preparation, processing or packaging for freezing, in the course of being frozen, or after having been frozen, as indicated by the context of the specification.

2.20 production: The handling, preparation, processing or packaging for freezing, in the course of being frozen, or after having been frozen, including the process of frozen storage, as indicated by the context of the specification.

2.21 quick-freezing process: The continuous process whereby the temperature of the product is lowered through the zone of maximum crystallization (for most products, between –1 °C and –5 °C), within 4 h at a rate of at least 25 mm of product thickness per hour, and that is only completed when the temperature of the entire product, after thermal stabilization, has reached –20 °C or lower.

2.22 quick-frozen product: A fish product (see 2.8), smoked or unsmoked, cooked or uncooked, that has been frozen by the quick-freezing process.

2.23 skewer: A pin for holding cuts of fish (with or without vegetables or fruit or any combination thereof) and that may be made of wood, metal or a plastics material specially suitable to be used in contact with food.

2.24 smoke; wood smoke: Smoke that is derived from wood or from woody plants that are acceptably free from gum and resin, paint, timber preservative and other added substances, in a state of combustion or friction and that can be used to smoke fish in kilns designed for the purpose, or that can be condensed in, or absorbed into, a suitable food grade liquid or powder for the preparation of a smoke dip.

2.25 suitable: Acceptable and complying with the requirements for the intended purpose.

2.26 suitable corrosion-resistant material: Impermeable material that has smooth surfaces (free from pits, crevices and scale), that is non-toxic and that is unaffected by sea water, ice, fish slime and any other corrosive substance with which it is likely to come into contact and that is capable of withstanding exposure to repeated cleaning, including the use of detergents.

3 Requirements for the factory

3.1 General

All the statutory requirements contained in the Occupational Health and Safety Act, 1993 (Act 85 of 1993), in the Health Act, 1977 (Act 63 of 1977), in the Perishable Products Export Control Act, 1983 (Act 9 of 1983), and in any other relevant Acts shall be complied with.

3.2 Factory construction, layout and conditions

3.2.1 Location, size and hygienic design and conditions

3.2.1.1 The location of the factory shall be such that the buildings can be kept acceptably free from objectionable odours, smoke, dust and other contamination, in order to comply with the relevant requirements for hygiene and sanitation of the Health Act, 1977. The factory buildings shall be of sound construction, in good repair, and large enough to prevent crowding of equipment and employees and to permit adequate cleaning and the maintenance of product quality and hygiene.

3.2.1.2 The factory premises shall be well drained and adequately fenced to keep out larger animals, such as cats and dogs, and also unauthorized persons and vehicles. Outdoor work areas and roads and pathways on the premises shall have a permanent surface of concrete, brick, bitumen or other durable material. Areas outside buildings and not in actual use shall either be covered by lawn or have a surface that is not liable to produce dust and that does not contain toxic substances.

3.2.1.3 The factory and equipment shall be so designed as to permit the processing of raw materials without undue delay. The buildings shall be so designed and constructed as to prevent the entry and harbouring of insects, birds, rodents and other vermin.

3.2.2 Roofs and ceilings

3.2.2.1 Roofs shall be weatherproof and made of non-absorbent material. Roofs and, where applicable, ceilings shall fit tightly to the walls and shall be at least 2,4 m above the floor. In the preparation, processing and packaging areas, the roof and, where applicable, the ceiling shall be at least 300 mm above any equipment and high enough to allow the free movement of mobile equipment and moving parts of other equipment.

3.2.2.2 In the preparation, processing and packaging areas and in storage areas for ingredients and

packaging materials for the product, the ceiling (or, where no ceiling is provided, the roof) shall be dustproof and faced with a suitable corrosion-resistant, light-coloured and impermeable material that is so constructed and finished as to minimize condensation, mould development, flaking and the lodgement of dirt, and that is capable of being cleaned without damage. The underside shall have a smooth surface. Areas where sauce is prepared or where the cooked product is handled, or where ingredients and packaging materials are stored, shall have a ceiling.

3.2.3 Walls and doors

3.2.3.1 Outer walls shall be weatherproof and impermeable to water. Interior wall surfaces shall be faced with a smooth, light-coloured, washable material that is impermeable to water and free of unnecessary projections. In addition, the walls in the preparation, processing and packaging areas shall be faced with a suitable corrosion-resistant, light-coloured, washable and impact-resistant material to a height of 2 m above the floor, except that when soiling of the walls could occur above this height, the facing shall be continued to a higher level. All ledges on the inside of walls and all windowsills shall be sloped towards the floor at an angle of at least 45°. The ledges shall be kept to a minimum size and windowsills shall be at least 1 m above floor level. In the preparation, processing and packaging areas and in freezers, chill rooms and freezer storage rooms, the wall-to-wall and wall-to-floor junctions shall be coved, the minimum radius of the coving being 25 mm and 40 mm respectively.

3.2.3.2 Doors and door frames shall be sheathed with, or made from, a suitable corrosion-resistant material and shall have seamless, light-coloured, impermeable and washable surfaces. If wood is used, it shall be sheathed to render it impermeable to water. Doors through which the product is moved between the preparation, the processing and the packaging areas shall be wide enough to prevent contamination of the product and damage to the doors. All doors that open direct from the outside atmosphere into the preparation, processing and packaging areas shall be provided with effective air screens or shall, as far as is practicable, be self-closing and tight-fitting. Freezer, chill room and freezer storage room doors shall be tight-fitting.

3.2.4 Floors

3.2.4.1 Floors shall be constructed of concrete or other material that is suitably impermeable, corrosion-resistant and easy to clean, and that has an even surface that is smooth but not slippery, and that is free from cracks and open joints.

3.2.4.2 Floors of the preparation, processing and packaging areas and of freezers, chill rooms and freezer storage rooms shall be suitably sloped and shall be drained to external gullies, sumps and sewers. Outlets shall have, immediately outside the factory walls, a trap that prevents the entry of rodents.

3.2.4.3 Drainage channels shall be of the open type with removable covers, where necessary, and shall be designed to cope with the maximum expected flow of liquid without overflowing or causing flooding. There shall be no installations in a drainage channel that could obstruct the flow of water or the cleaning activities. Gully traps shall be fitted with easily removable strainers. Where necessary, duckboards of easily cleaned, impermeable material shall be provided. Wooden duckboards shall not be used in wet areas. Floors and drains shall be maintained in good condition and repair.

3.2.5 Lift cages and staircases

3.2.5.1 The inside surfaces of lift cages shall be suitably corrosion resistant, and lift shafts shall be properly drained and accessible for cleaning. Mesh doors may be used, provided that they are not such as to be conducive to unhygienic conditions.

3.2.5.2 Staircases in rooms where the product is prepared, processed, packaged or handled shall have the spaces between treads closed in with solid risers. Staircases shall have solid balustrades of such a height as to prevent contamination of products underneath the stairs.

3.2.6 Cables and pipes

3.2.6.1 Cables and pipes shall, where applicable, be:

a) fixed above ceilings; or

b) chased into walls; or

c) fixed away from walls or ceilings and above the floor, and spaced in such a way that the ceilings, walls, floor, cables and pipes can be easily cleaned and maintained in a hygienic condition; or

d) carried under the floor.

3.2.6.2 Drainage and sewer pipes shall not be installed above ceilings in preparation, processing or packaging areas, nor shall they be installed in such a way that accidental leakages could contaminate the product. The drainage and sewer pipes shall have an inside diameter of at least 100 mm and shall be properly vented to the outside atmosphere.

3.2.7 Illumination

An illumination of at least 220 lx in general work areas and of at least 540 lx at points where close examination of the product is carried out, shall be provided and shall be such that it does not significantly alter the appearance of the colour of the product. Luminaires suspended over the work areas where the product is handled at any stage during preparation, processing or packaging shall be of the safety type or otherwise so protected as to prevent contamination of the product in the event of breakage of a luminaire or lamp.

3.2.8 Ventilation

The ventilation shall be such that it keeps the air fresh, and removes excess water vapour, and that it prevents the build-up of excessive heat, the formation of condensate and the growth of mould on overhead structures. The air shall be free from noxious fumes, vapours, dust and contaminating aerosols. The air flow shall be from the more hygienic to the less hygienic areas. Natural ventilation shall be augmented, where necessary, by mechanical means.

Windows that open for ventilation purposes shall be insect-screened. The screens shall be easily removable for cleaning and shall be made from suitable corrosion-resistant material.

3.2.9 Hand-washing facilities

3.2.9.1 The following shall be provided at those entrances to the preparation and processing areas of the factory that are used by the employees, and at other conveniently situated places in the preparation and processing areas of the factory within easy reach of the employees, and at the toilet exits:

a) an acceptable number of wash-hand basins, with an abundant supply of hot and cold or warm running water in the temperature range 40 °C to 50 °C and that complies with the requirements of 3.4.1;

b) an ample supply of unscented liquid soap or acceptable detergent in active condition;

c) disposable paper towels; and

d) taps operated by means other than the hands or elbows, for example knee-operated or foot-operated taps, or push-button taps with pre-set volume control.

3.2.9.2 Disinfectant hand dips, where provided, shall be of such a design that they can be adequately cleaned. Access to hand-washing facilities shall at all times be unobstructed. The wash-hand basins shall be of a suitable corrosion-resistant material, shall have a smooth finish and shall drain into drainage channels direct.

3.2.9.3 In the case of a factory ship, at least one wash-hand basin in the toilet block and one in the processing and packaging area shall be supplied with hot and cold running water.

3.2.10 Footbaths

Unless their absence in particular circumstances is acceptable, or unless alternative acceptable cleaning and disinfecting facilities are provided, footbaths that contain a suitable disinfectant solution shall be provided at each entrance to the preparation, processing and packaging areas that is used by employees, and shall be so located that employees cannot obtain access to those areas without disinfecting their footwear. Footbaths shall be so constructed that they can be adequately drained and cleaned.

3.2.11 Notices

Notices shall be strategically displayed in the preparation, processing, packaging and storage areas, in the changerooms and in the toilet facilities. The notices shall require that hands be washed with soap or detergent and shall indicate that spitting, the use of chewing gum and of tobacco in any form, and the taking of refreshments are prohibited in those areas.

3.2.12 Separation of processes and facilities

Separate rooms or well-defined areas of suitable size shall be provided for:

a) the receipt and storage of raw materials;

b) preparatory operations such as the heading, gutting and washing of fish;

c) processing operations such as filleting, steaking and freezing;

d) packaging; and

e) the storage of the product.

3.2.13 Stores

3.2.13.1 General

The production area of the factory shall not be used for storage purposes.

3.2.13.2 Edible ingredients

Storage facilities for edible ingredients used in the preparation of the frozen product shall be dry, free from dust and any other source of contamination, and shall be verminproof.

3.2.13.3 Packing and packaging materials

Clean, dustproof, verminproof and dry storerooms shall be provided for the storage of packaging materials.

3.2.13.4 Storage facilities for poisonous and other harmful materials

3.2.13.4.1 Storage facilities for pesticides or other poisonous and harmful materials

Pesticides or other poisonous and harmful materials and the equipment for their application shall be stored in a room in which no foodstuff, food-handling equipment, packaging material or food containers are stored and which shall be kept locked. All dangerous materials shall be prominently and distinctly labelled and shall at no time come into contact with food containers, packaging materials, raw materials or the product.

3.2.13.4.2 Storage facilities for cleaning and disinfecting materials

Cleaning and disinfecting materials and the equipment for their application shall be stored in a room in which no foodstuff, food-handling equipment, packaging material or food containers are stored and shall

at no time come into contact with food containers, packaging materials, raw materials or the product. All cleaning and disinfecting materials shall be prominently and distinctly labelled.

3.2.14 Storage facilities for utensils and spare parts

Utensils and spare parts that, when in use, come into contact with the product, shall, when not in use, be kept in a disinfectant solution or stored in a hygienic manner in a dry area that is free from dust and any other source of contamination, and that is verminproof. Spare parts for machinery that are capable of contaminating the product shall be kept in a separate storage area away from the processing areas.

3.2.15 Smoke room

Doors used during the firing of smoke rooms shall not open direct into processing areas, unless the smoke generator is so designed as to obviate pollution of these areas. Separate facilities shall be provided for the storage of smoke-generating materials.

3.2.16 Freezers, chill rooms and freezer storage rooms

3.2.16.1 Refrigeration units, such as compressors, shall not be installed in an area where the product is handled, with the exception of equipment that is an integral part of a production unit. Where freezers, chill rooms and freezer storage rooms are located in processing areas, their floors shall either be an integral part of the floor of the processing area or adequately sealed to that floor. Any storage units shall be installed high enough above the floor to permit easy and adequate cleaning of the area under them.

3.2.16.2 The walls and floors shall be in good condition. The surfaces of ceilings, walls and floors shall be of suitable corrosion-resistant material, shall be impermeable to water and shall be smooth, and free from cracks, crevices and flaking of surface material. The floors shall be drainable, and the floors of chill rooms shall be sloped to effect complete draining.

3.2.16.3 Freezer storage rooms in factories other than factory ships shall be equipped with automatic temperature recorders that have enough suitably placed sensing elements to monitor the overall air temperature. The temperature in freezer storage rooms shall be automatically and continuously monitored and a record of the temperature shall be kept and shall be available for inspection. Temperature charts shall be so graduated that each division represents not more than 2 °C within the storage range, and shall be easily readable, to the nearest 1 °C, within the storage range. Batch freezers, other than plate freezers, shall be fitted with external gauges or other temperature indicators.

3.2.16.4 Freezer storage rooms on fishing vessels should have temperature recorders but shall have at least external gauges or temperature indicators and the indicated temperatures shall be recorded every 4 h.

3.2.16.5 The entrances to freezers, chill rooms and freezer storage rooms shall be protected from the inflow of warm air by the provision of an ante-room or a mechanical air curtain or strip curtains or self-closing shutters.

3.2.17 By-products

Any processing of by-products and non-fish products that are not intended for human consumption shall be conducted in buildings that are physically separated from the factory in such a way that there is no possibility of contamination of the product.

3.2.18 Living quarters

Living quarters shall be completely separated from areas where the product is prepared, processed, packaged or stored.

3.2.19 Refuse

A separate, suitable refuse facility shall be provided on the premises and shall be cleaned daily.

3.2.20 Comfort facilities

3.2.20.1 An acceptable number of suitable change rooms, shower baths, wash-hand basins whose taps operate as described in 3.2.9, toilets (separate for each sex) and, where appropriate, urinals, shall be provided within practical distance from the factory processing areas. Shower baths shall connect direct to the change rooms. Comfort facilities shall not open direct into a preparation, processing, packaging or storage area.

3.2.20.2 Toilets shall be completely separate from change rooms, the only permissible access being through close-fitting, self-closing doors. Toilet blocks shall have their own hand-washing facilities, separated from those provided in change rooms. An ample supply of toilet paper, hot and cold running water, nailbrushes, unscented liquid soap or an acceptable detergent solution, and disposable paper towels shall be available to employees. Receptacles shall be provided for used towels. Refuse bins of hygienic construction shall be provided.

3.2.20.3 Notices shall be posted requiring employees to wash their hands with soap or detergent after they have used the toilet. Lockers or controlled clothes baskets shall be provided, and the layout and equipment shall be such as to permit proper cleaning and maintenance. The comfort facilities shall be kept clean and tidy. The comfort facilities shall be adequately ventilated. Change rooms and dressing-rooms shall not be used as living quarters or for the preparation of meals. Staff dining-rooms shall be separate from the change rooms or dressing-rooms.

3.2.21 Facilities for cleaning and disinfecting portable equipment

Facilities with proper drainage shall be provided for the cleaning and disinfecting of portable equipment. Such facilities shall be located in a separate room or in a designated area in the preparation, processing and packaging areas where there is an ample supply of cold potable water, and hot water where required, or saturated steam, or clean sea water, at adequate pressure, that complies with the requirements of 3.4.2.

3.2.22 Specific requirements for fishing vessels

3.2.22.1 General considerations

Fishing vessels shall be designed for the rapid and efficient handling of fish, and for easy cleaning and disinfecting. All surfaces with which the fish might come into contact shall be impermeable and, where practicable, shall be of suitable corrosion-resistant material. The surfaces shall be easily cleanable and shall have no projections or other features that could cause damage to the product.

Deck pounds, pen stanchions and dividing boards shall be constructed of suitable corrosion-resistant material and shall be easily removable. Their number and height shall be such as to hold the estimated amount of fish and to prevent movement and crushing of the fish as a result of excess mass of fish or the vessel's motion. Where practicable, wood shall be sheathed with a suitable corrosion resistant material such as fibreglass, or shall be so treated as to be impermeable to water. Metalwork, other than stainless steel or galvanized steel or aluminium, that does not come into contact with the product shall be coated with corrosion-resistant and non-toxic paint or other protective coating.

A suitable drainage system shall be provided. Areas where the product is prepared, processed, packaged or stored shall be well isolated from grease, oil, fuel, heat, fumes, food for crew, storage areas for material other than the product, and from the engine room and other sources of contamination.

3.2.22.2 Storage facilities for fish

If fish are unloaded on land within 8 h of the catch, the vessel shall have facilities that will at least protect the fish from the direct rays of the sun and keep the fish cool (at a temperature of 20 °C or lower) and moist.

If fish are to be kept at sea for more than 8 h but not more than 10 d, facilities for chilling or freezing (or both) shall also be provided. Vessels on which fish are to be kept for more than 10 d shall have facilities for freezing the fish.

All vessels that are equipped for chilling or freezing (or both) shall have a suitable drainage system that is able to remove the meltwater into a sump as fast as it accumulates.

3.2.22.3 Sea water and brine storage tanks

In vessels that use refrigerated sea water or refrigerated brine systems for chilling or stowing the catch, all tanks, heat exchangers, pumps and associated piping shall be made of, or coated with, suitable corrosion-resistant material. A space of at least 60 mm shall be provided between cooling coils and the tank sides, to allow for the scrubbing and flushing of fish debris from the wells. The tanks or wells shall be so designed that the fish can first be washed and pre-cooled with refrigerated sea water, and then frozen by brine pre-freezing. Pipes other than cooling coils shall be completely sunk in and covered.

3.2.22.4 Sea water intake and waste disposal

Deck hoses shall be supplied with clean sea water, at an acceptable pressure, by a pump used only for clean sea water. Subject to good naval architectural practice, the point of intake of sea water for cooling and cleaning the product shall be situated at the deepest practicable point on one side of the vessel, and the sewage and waste water disposal and engine cooling discharge shall be disposed of at the shallowest point practicable, on the opposite side of the vessel.

The water supply pipes and waste disposal lines that service the vessel's toilets, wash-handbasins and kitchen sinks shall be capable of carrying peak loads, shall be watertight and shall not pass through spaces where the catch is prepared, processed, packaged or stored. Piping for the supply of clean sea water shall have no cross-connections with the engine or condenser cooling system and shall be so constructed as to prevent any possibility of back-syphoning from the kitchen sinks or the toilets.

3.2.22.5 Water supplies

An acceptable supply of cold potable water or clean sea water at an acceptable pressure shall be available at an acceptable number of points throughout the fishing vessel. On vessels engaged in processing other than cleaning, a supply of hot water at a temperature of at least 60 °C shall be available for use. Where practicable, an acceptable water-treatment system (such as exposure to ultraviolet light) shall be provided for the treatment of sea water that is used in the processing of the product.

3.3 Equipment for production

3.3.1 General

3.3.1.1 Processing areas shall be so designed, equipped and staffed as to allow free movement of workers to facilitate cleaning and the maintenance of both hygiene and product quality.

3.3.1.2 All plant, equipment and utensils that come into contact with the product shall be smooth-surfaced, light-coloured and of a suitable corrosion-resistant, non-absorbent material (i.e. not wood or other absorbent or porous material), which may have an acceptable plastics-coated surface suitable for use with food but should preferably be made of stainless steel. They shall be of hygienic design, with no open joints or crevices and shall be so constructed as to facilitate their cleaning and disinfecting. Plant or equipment shall be so designed as to facilitate the cleaning and disinfecting of the areas under them. Open ends and curled edges shall be satisfactorily sealed to prevent the accumulation of organic material and dirt. Where necessary, as in the case of equipment that cannot be cleaned *in situ*, it shall

be possible to dismantle the equipment for cleaning and disinfecting. Surfaces with which the product comes into contact shall not be painted.

3.3.1.3 All parts of stationary equipment or equipment that is not readily movable shall be installed away from the walls and ceilings at distances sufficient to allow access for cleaning and inspection. All permanently mounted equipment shall be either installed high enough above the floor to allow access for cleaning and inspection, or shall be completely sealed to the floor.

3.3.1.4 Equipment shall preferably not be sunk into the floor but if this is unavoidable, the installation of the equipment shall be such as to be acceptable. Sunken areas shall be well drained.

Copper, lead and their alloys (other than solder) and other metals or materials detrimental to health or to the product, shall not be used in the construction of equipment that comes into contact with the raw materials or with the unprotected product at any stage of its processing. The use of solder in equipment shall be minimized.

3.3.2 Tables

Wooden tables shall not be used in processing areas. Table frames shall be of a design and construction that will not allow the development of unhygienic conditions and bacterial build-up. The frames shall be made of smooth corrosion-resistant metal or shall have been so coated as to protect them from corrosion. Table tops shall be of seamless stainless metal or other seamless, corrosion-resistant, smooth, impermeable material that possesses similar surface characteristics. They shall be of hygienic construction and shall be either removable for cleaning, or so secured to their frames as to allow cleaning and disinfecting. Where metal tops are folded at the edges, the folds shall be so soldered, welded or sealed with an acceptable mastic sealant as to prevent the accumulation of organic matter and dirt. All table tops shall allow rapid and effective drainage, and shall be free from cracks and crevices. All joints in tables shall have been made watertight.

3.3.3 Cutting boards

If cutting boards are used, they shall be of hygienic construction and shall be made of acceptable light-coloured material (other than wood or other absorbent or porous material), suitable for use with food. Cutting boards shall be easily removable.

3.3.4 Utensils

Knives, shovels, brooms and other utensils shall not have handles of wood or of other absorbent or porous material. Wicker baskets shall not be used as containers for fish at any stage before, during or after processing.

3.3.5 Disinfecting and cleaning facilities

Disinfecting facilities for gloves and knives shall be available at convenient and acceptable points. Cleaning and disinfecting materials, hot and cold running water or saturated steam, hose pipes, spray nozzles, brushes, scrapers and other equipment needed for the cleaning of the fishing vessel, plant, equipment and utensils shall be available. These materials and equipment shall not be stored in a room where food-handling equipment is stored and shall at no time come into contact with raw materials, the products, or their containers or packages.

3.3.6 Ice-making equipment

All surfaces of ice-making equipment that come into contact with the ice shall be of suitable corrosion-resistant material. The ice-making equipment shall be of hygienic construction throughout. Whenever ice is transferred, stored or transported, it shall be effectively protected from contamination.

3.4 Water

3.4.1 Potable water

3.4.1.1 Subject to the provisions of 3.4.2, every factory shall have an adequate supply of clean potable water that is free from suspended matter and substances that could be deleterious to the product or harmful to health. In addition, the water shall have been so treated, by flocculation, filtration, chlorination or other acceptable process, as to ensure compliance with the following requirements:

a) **coliform organisms**: the count of coliform organisms shall not exceed five organisms per 100 mℓ of the water (see 10.16.1.1 or 10.16.2.3.1); and

b) **faecal coliform bacteria**: faecal coliform bacteria shall not be detectable in 100 mℓ of the water (see 10.16.1.2 or 10.16.2.3.2).

3.4.1.2 For the purposes of the water examination, the coliform group shall include all Gram-negative, non-spore-forming rods capable of fermenting lactose with the production of acid and gas at 37 °C in less than 48 h. Faecal coliform bacteria shall be regarded as Gram-negative, non-spore-forming rods capable of fermenting lactose with the production of acid and gas at both 37 °C and 44 °C in less than 48 h, and of producing indole in tryptone water.

3.4.1.3 Chlorinated water that could have any deleterious effect on the product shall be dechlorinated immediately before use. In all cases, the free residual chlorine concentration shall be determined by the *N,N*-diethyl-1,4-ℓ-phenylene diamine test or other acceptable test that has equivalent sensitivity.

3.4.1.4 Factory installations for the treatment of water shall be thoroughly cleaned at least once a week by an acceptable method.

3.4.2 Sea water

Clean, uncontaminated, freshly pumped sea water may be used for any purpose in the factory, provided that the count of coliform organisms does not exceed 50 organisms per 100 mℓ of the water (see 10.16.2.3.1) and no faecal coliform bacteria are detectable in 100 mℓ of the water (see 10.16.2.3.2).

3.4.3 Water for cleaning

Water used for the cleaning of the plant and equipment shall comply with the requirements of 3.4.1 or 3.4.2, as relevant. Chlorinated water that could have any deleterious effect on the product shall be dechlorinated immediately before use. In all cases, the free residual chlorine concentration shall be determined by the *N,N*-diethyl-1,4-ℓ-phenylene diamine test or other acceptable test that has equivalent sensitivity.

3.4.4 Ice

The purity of ice shall be such that the water derived from it (by melting the ice under aseptic conditions at a temperature not higher than 10 °C) immediately after the ice has been manufactured, complies with the requirements of 3.4.1 or 3.4.2, as relevant.

3.5 Requirements for employees engaged in the handling, preparation, processing, packaging and storage of the product

3.5.1 Health

3.5.1.1 Before being engaged, employees shall pass an appropriate medical examination to ensure that they are free from communicable diseases, and they shall thereafter pass an annual medical examination. In the case of any absence of more than one day owing to illness, the employee shall,

before resuming duty, report the nature of the illness which necessitated the absence to the factory hygiene officer who shall, should he deem it necessary, take the appropriate steps to obtain a medical opinion on the employee's fitness for work. An appropriate medical record of each employee shall be kept.

3.5.1.2 Any medical certificate submitted by an employee of a factory shall be available for inspection by the authority administering this specification.

3.5.1.3 No employee who is a carrier of, or is suffering from, any communicable disease, especially a carrier of *Salmonella* or *Shigella*, or one who shows symptoms of, or is suffering from, gastro-enteritis or an enterobacterial infection or a disorder or condition that causes discharge of fluid from any part of the skin or body, shall be allowed to come into contact with the product. Any such employee shall immediately report to the factory management.

3.5.1.4 No employee who is known to be affected with a disease that is capable of being transmitted through food shall be permitted to work in any part of the factory in a capacity in which there is a likelihood that the employee will contaminate the product with pathogenic organisms.

3.5.1.5 No employee who is suffering from any cut or injury shall be allowed to come into contact with the product unless the cut or injury has been so treated or dressed that the discharge of body fluid has been prevented, and the wound and its dressing have been so covered as to ensure that infection or contamination of the product is no longer possible.

3.5.2 Protective clothing

3.5.2.1 All employees engaged in the handling, preparation and processing of the product up to and including the packaging stage, but excluding employees operating within freezer storage rooms and chill rooms, shall wear clean, light-coloured, protective clothing, waterproof aprons, waterproof slipovers or boots, and clean, washable or disposable headgear that completely covers their hair. Woollen caps may be worn in freezer storage rooms only. Overalls shall completely cover the personal clothing of the employees.

3.5.2.2 Sleeves shall not extend below the elbows, except when covered by plastics sleevelets or when worn in freezer storage rooms and chill rooms. Waterproof protective clothing shall be of a plastics or rubber material or a similar acceptable material. All protective clothing shall be of hygienic design, shall have no external pockets, shall be in good repair and shall not constitute a source of contamination to the product.

3.5.2.3 Protective clothing, other than waterproof aprons, sleevelets and gloves, shall not be stored in work areas; when not in use, it shall be kept in change rooms and shall not be removed from the premises except for laundering under hygienic conditions. The homes of employees shall not be regarded as acceptable for this purpose.

3.5.2.4 Waterproof aprons, sleevelets and gloves shall be cleaned at each time of removal and as frequently as necessary, and shall be hung on hooks or pegs at exits from production areas during intervals between work and during visits to the toilet. Gloves shall be thoroughly cleaned and then disinfected by the use of chlorinated water or other acceptable solution or procedure. Waterproof aprons, sleevelets and gloves, and also all equipment used in the preparation, processing and packaging of the product, shall not be removed from the work areas except for repairs and for cleaning under hygienic conditions.

3.5.3 Personal hygiene

3.5.3.1 Before starting work, and after each absence from the factory production area, at regular intervals during production, or at any time when necessary, employees shall wash their hands with warm water and acceptable unscented liquid soap or detergent and rinse them in clean, running water. They may then dip their hands in an acceptable disinfectant solution, after which they shall rinse their hands in clean running water, if so required by the usage directions of the hand dip. Neither varnish nor lacquer shall be used on fingernails, and fingernails shall be kept short and clean. Jewellery shall not be worn by employees who handle raw materials or the unprotected product or both.

3.5.3.2 Neither employees' personal effects nor their food shall be present in any area where the product and its ingredients and packaging materials are handled and stored. Containers used in the preparation, processing or packaging of the product shall not be used for any other purpose.

3.5.3.3 The use of chewing gum and of tobacco in any form shall not be permitted within the areas where the product and its ingredients and packaging materials are handled or stored. Spitting shall not be allowed anywhere within the factory premises. Notices to these effects shall be posted strategically (see 3.2.11).

3.5.4 Visitors

Any person, including employees who visit or enter the production areas of the factory during the hours of operation, shall, when in those areas, comply with all hygiene requirements and shall wear clean protective clothing that shall be provided by the factory.

3.6 Hygienic operating requirements

3.6.1 General

3.6.1.1 In relation to the handling, transportation, processing, packaging, freezing and storage of the product, no operation(s) shall be performed and no conditions shall exist that are detrimental to the product. Materials liable to contaminate the product shall be kept away from the processing areas. Non-edible materials shall not be stored in the same room as edible ingredients or in the preparation or packaging areas of the factory.

3.6.1.2 There shall be no unhygienic conditions on the factory premises. Smoke from factory chimneys and exhaust fumes shall not be allowed to enter the factory building(s) in a quantity or manner that is offensive, injurious or dangerous to health, or that causes contamination of the product at any stage during the processing of the product.

3.6.2 Cleaning and disinfecting

3.6.2.1 Physical facilities

3.6.2.1.1 The building, premises, plant, equipment, utensils and all other physical facilities of the factory shall be kept clean and in good repair and shall be maintained in an orderly hygienic condition. The cleaning and disinfecting of the preparation, processing and packaging areas of factories and of all auxiliary equipment and utensils shall be organized on a regular basis and shall be carried out by trained employees. Before use, plant, equipment and utensils shall be thoroughly cleaned with a detergent or other cleaning agent and disinfected. A detergent-disinfectant may be used. Immediately before the start of any operations, equipment shall be thoroughly rinsed with water (see 3.4.3) to remove any dust and any disinfectant (if used).

3.6.2.1.2 The processing and packaging areas, storage rooms, chill rooms, freezer storage rooms and freezers shall be kept free from mould, dust, dirt, flaking paint and other loose or extraneous material that could fall onto the product from walls, ceilings or overhead structures.

3.6.2.2 Floors and drainage channels

During periods of operation, the floors and the drainage channels in the preparation, processing and packaging areas shall be kept clean by regular sweeping, scrubbing and flushing with water. Refuse shall not be permitted to accumulate in drainage channels or on grids. Thorough cleaning of floors and drainage channels shall take place as often as is necessary and at the end of each day's operations, in order to maintain hygienic conditions. Footbaths shall be drained and cleaned regularly and the disinfectant shall be kept in active condition.

3.6.2.3 Walls of preparation, processing and packaging areas

The walls of preparation, processing and packaging areas shall, where necessary, be thoroughly washed immediately after each day's operations and the rooms shall be kept as free from dust as possible.

3.6.2.4 Cleaning and disinfecting materials

Cleaning and disinfecting materials, hot and cold running water that complies with the requirements of 3.4.3, saturated steam, hosepipes, brushes and other materials and equipment necessary for the cleaning of the factory, equipment and utensils shall be available. Cleaning materials, such as scouring wool, that could contaminate the product shall not be used.

3.6.2.5 Cleaning of water treatment installations

Factory installations for the treatment of water shall be thoroughly cleaned once a week by an acceptable method.

3.6.2.6 Cleaning of the processing system

The entire processing system shall be cleaned during each break in production that lasts for more than 1 h or whenever it is deemed to be necessary, and shall be effectively cleaned, at the end of each shift and at the end of each day's operations. It shall be clean at the time of further use.

3.6.2.7 Cleaning of utensils

Knives and similar items of equipment shall, during breaks in production, after use, or at any time when disinfection is necessary, be thoroughly cleaned and then disinfected by the use of chlorinated water or other acceptable solution or procedure. When the factory is in operation, equipment and utensils shall not be removed from the work area except for repair, cleaning or replacement.

3.6.2.8 Cleaning of the discharge system

Any discharge system at the jetty and any conveyance system to the factory shall be so drained that stagnant water does not collect. Such systems shall be regularly cleaned of stale material and cleaned before and after use. Holding tanks shall be similary treated.

3.6.3 Repairs

3.6.3.1 Whenever maintenance or repairs have been carried out in production areas, tools and replaced equipment shall be immediately removed from these areas and the affected equipment shall be thoroughly cleaned and disinfected.

3.6.3.2 Welding repairs in the areas where the product is handled, prepared, processed or packaged shall be performed when the plant is not in production or as emergency work during breakdown only, and in such a way that the product is not exposed to welding fumes, splatter or slag particles.

3.6.4 Efficacy of cleaning

The efficacy of the cleaning and disinfecting process specified in 3.6.2 shall be such that, in samples taken in accordance with 10.15.2, the percentage efficacy of cleaning and disinfecting in the sample, determined in accordance with 10.15.3, is acceptable when scored by the system set out in 10.15.3.3 and 10.15.3.4.

3.6.5 Containers, bins and crates for the handling of raw materials and the product

When filled or partly filled with raw material or with the product, containers shall not be stacked in a way that allows contact of the contents of a container with the bottom of the container stacked above it. Containers that hold edible materials shall not be stacked direct on the floor or against the wall, and whenever they are moved, they shall be effectively protected from contamination. Containers that hold edible materials shall be stored at least 250 mm above floor level. Where pallets are used instead of racks, shelves or stands, there shall be a clearance of at least 100 mm above floor level. Containers shall be of hygienic design and shall either be light-coloured or have a bright metallic finish. Non-edible materials shall not be stored in the same room as edible ingredients or in the preparation or processing areas of the factory.

3.6.6 Wrapping materials

Wrapping materials used during the packaging of the product shall be kept in corrosion-resistant containers of hygienic construction, and shall be so dispensed that the wrappers require only minimum handling.

3.6.7 Packaging materials

Materials for the packaging of the product shall be stored on racks/shelves at a distance of at least 250 mm from the floor or on pallets, and away from the walls.

3.6.8 Spare parts

Spare parts for machinery, and other items that are capable of contaminating the product, shall be stored away from the preparation, processing, packaging and product storage areas.

3.6.9 Freezers, chill rooms, freezer storage rooms and their equipment and instruments

Freezers, chill rooms, freezer storage rooms and their equipment and instruments shall operate efficiently and shall be kept clean and in a hygienic condition. The temperature in freezer storage rooms shall be automatically and continuously monitored and a record of the temperature shall be kept and shall be available for inspection. Products shall not be stacked direct on the floor or against the walls. No material other than the product or ingredients of the product shall be stored in freezers, chill rooms or freezer storage rooms. No condition and no object or matter that could affect the flavour, odour or appearance of the frozen product in any way shall be present in freezers, chill rooms or freezer storage rooms.

3.6.10 Removal of refuse and offal

Litter, waste and overflow shall not be allowed to accumulate or to give rise to unhygienic conditions, and shall be disposed of promptly in an efficient and hygienic way. Offal shall be removed from the processing area in a hygienic manner, and containers for offal awaiting removal from the factory shall be well separated from the processing areas. A separate refuse room or other acceptable refuse facility shall be provided on the premises, and shall be cleaned at least once a day.

3.6.11 Vermin control

All buildings in which raw materials, ingredients and the product are stored, or in which the product is handled, prepared, processed or packaged, shall be kept free from insects, birds, rodents and other

vermin. All rooms in which raw materials, ingredients or packaging materials are stored, shall, in addition, be rodentproof.

3.6.12 The use of pesticides

Pesticides shall not be used in work areas while preparation, processing and packaging are in progress, and precautions shall be taken to ensure that equipment and work surfaces are kept free from pesticide residues. Pesticides and cleaning chemicals shall at no time be allowed to come into contact with wrapping material, containers, raw materials or the product. The room in which pesticides are stored shall be kept locked and the materials contained in it shall be handled only by employees trained in their use.

3.6.13 Animals

Animals, including birds, shall not be allowed in any part of the factory.

3.6.14 Operations aboard fishing vessels

Shipboard handling, chilling, processing and freezing of the product shall be conducted under conditions of sanitation and hygiene equal to those of the equivalent procedures and practices of shore establishments. Before any product comes on board, and between hauls, the deck, deck pounds, pen stanchions and dividing boards, and all other deck equipment that will come into contact with the product, shall be hosed down with clean sea water. The deck shall be scrubbed to remove all visible dirt and debris at least once a day. During fishing trips, the hold bilge sump of the vessel shall be drained regularly. All gear shall be thoroughly cleaned when fishing has ceased. The gut of ripped fish shall be removed immediately, and the fish shall be washed in clean water that is free from entrails and liver particles. When practicable, the deck shall be cleared before the next haul is landed on deck. The deck, hold and processing areas of fishing vessels shall be thoroughly cleaned and disinfected immediately after discharge of the cargo.

3.7 Records

On fishing vessels with freezing facilities, or with both freezing and packaging facilities, and in factories on land, adequate quality records shall be maintained. Quality records and records of freezer storage temperatures on fishing vessels and in factories on land shall be kept for a period of at least two years (see 3.2.16.3 and 3.2.16.4).

3.8 The handling, preparation, processing, packaging, transportation and storage of the product

3.8.1 General

From the time the catch comes on board and during preparation, processing, packaging, transportation and storage, the product shall be protected from heat, the direct rays of the sun, frost, the drying effect of wind, and contamination by birds, dust, oil, fuel and noxious fumes. The product shall be processed as soon as possible after having been caught. Except where intended to be frozen in the round, fish shall be gutted as soon as possible and shall be thoroughly washed in clean water to remove all blood, slime and pieces of gut.

3.8.2 Fishing vessels not equipped to chill or freeze

The catch shall be transported to land, where the chilling or freezing process shall be started within 8 h of catching.

In cases where the fish are immediately processed or packed on board such a vessel, the requirements of 3.8.1 shall apply. The temperature of fish on board shall not exceed 20 °C.

3.8.3 Chilling vessels

On board fishing vessels that are equipped with chilling facilities, fish intended for processing shall, where practicable, be chilled or iced as soon as possible after they have been landed on deck. Fish that have not been chilled within 8 h of having been landed on deck shall not be processed. Fish that are to be kept at sea for between 8 h and 10 d shall, as a minimum requirement, be stowed in ice. Flaked ice, cubed ice or finely crushed cobbled ice, of diameter less than 5 cm, shall be used, and staging or shelves shall be so installed as to limit the depth of bulk storage to a maximum of 1 m. Individual layers of fish shall be separated from one another and from the floor, ship-sides and bulkheads by acceptable layers of ice. Species of fish that could have a detrimental effect on one another shall be stored in separate holds or containers. Vessels that are equipped to chill but not to freeze shall not be used to keep fish at sea for more than 10 d. Fish shall not be kept iced for longer than good manufacturing practice permits. In the case of tuna, vessels that are not equipped to freeze shall keep their catch on ice or in chilled or refrigerated sea water and shall transport the catch to factories on land within 5 d.

3.8.4 Tuna

Tuna may be kept on ice for a period not exceeding 5 d (see 3.8.3). Brine pre-freezing of tuna at –7 °C is adequate for a period not exceeding 14 d. For longer periods of storage, the fish shall be frozen and kept frozen at a temperature of –20 °C or lower. If brine pre-freezing is employed, the total chloride (determined as NaCl) content of the tuna, determined in accordance with 9.8, shall not exceed 2 % (by mass).

3.8.5 Transportation by road and rail

Transportation of the product by road shall be carried out in covered and insulated or refrigerated vehicles constructed and equipped to protect the product adequately. Under dusty conditions or conditions that might affect the fish adversely, the transportation by road shall be in a vehicle with a dustproof storage hold. If the duration of road transport is longer than 1 h:

a) unfrozen fish shall be transported in an insulated and refrigerated truck that can keep the fish at the temperature of melting ice, or in an insulated truck where the fish is on ice; and

b) a frozen product shall be conveyed in an insulated and refrigerated storage hold in a truck, in accordance with the requirements in 7.2.2 and 7.2.3.

Transportation of the product by rail shall be carried out in insulated and refrigerated vehicles. The frozen final product awaiting transportation or loading shall not be kept unrefrigerated for longer than 1 h. Adequate precautions shall be taken to prevent the product from becoming physically damaged, for example as a result of pressure or movement during transportation. The product shall not be transported with other products that can contaminate or impair the product. The means of transport used for the product shall not at any time be used for transporting other products likely to impair or contaminate the product. The inside surfaces of the means of transport shall be so finished that they do not adversely affect the product, shall be smooth and shall be easy to clean and disinfect. If ice is used to chill the product, adequate drainage shall be provided in order to ensure that the water from the melted ice does not stay in contact with the product.

3.8.6 Freezing of the product before processing

If the product is to be frozen before processing, the freezing shall start:

a) soon after the catch is landed on deck; or

b) in the case of white fish and flat fish and similar species with a low fat content, within 8 h of being caught, on condition that the fish temperature is never allowed to rise above 20 °C; or

c) where the temperature of the fish in (b) above has been brought down to the temperature of melting ice, within 10 d; or

d) in the case of highrisk fatty fish (including *Clupeidae* and *Scombridae*), within 72 h of landing on board, provided that the fish temperature is brought down to 3 °C or lower within 6 h after being caught and to 0 °C within 16 h of landing on board.

3.8.7 Thawing of the frozen product for processing

When being thawed for processing, the frozen product shall not be exposed to ambient temperatures higher than 20 °C. Thawing shall be as rapid as possible and shall be completed in less than 20 h. Unless the processing is started before or immediately after thawing is complete, the chilling of the thawed fish to a temperature as close to 0 °C as possible shall be started immediately. Freezing after processing shall, unless the processing requires a longer period, be started within 8 h of the completion of the thawing process.

3.9 Special requirements relating to marine molluscs

Marine molluscs shall be protected from heat and contamination, shall be kept moist (and alive, where possible) while on board the vessel and shall be acceptably protected during transportation. Where marine molluscs, other than abalone, cannot be kept alive, they shall either be frozen, or shall be kept in ice for a period not exceeding 2 d before being processed or frozen.

NOTE – Octopus and squid may be preserved in ice for 4 to 5 days, provided that the melted ice water is properly drained.

Processing of abalone shall start while the abalone is still alive. The flesh shall be cleaned under hygienic conditions that ensure freedom from contamination. The gut and the mouth shall be removed. The fringes and the foot need not be removed, but the epithelium between the fringes and the foot shall be brushed off. The cleaning operation shall be followed by thorough washing in running water that complies with the requirements of 3.4.1 or 3.4.2. The flesh shall be free from unsightly fork marks or other damage.

4 Requirements for the ingredients and the product

4.1 Condition of ingredients and the product

4.1.1 General

4.1.1.1 All ingredients used shall fall within the scope of, and shall comply with the requirements of, the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972), and any regulations promulgated thereunder. All ingredients used in the preparation of the product shall be clean, sound, of good quality and in every way fit for human consumption. In addition, the product shall not contain any substance in amounts that might present a hazard to human health.

4.1.1.2 Wood smoke that is used for the smoking of the product shall be obtained by the use of wood that is acceptably free from gum and resin, paint, timber preservative and other added substances.

4.1.1.3 All units and pieces of fish presented in one of the forms described in 4.2.1 to 4.2.16 (inclusive) shall comply with the relevant description. Scraps of fish shall not be included. If frozen fish or frozen fish pieces are glazed with chilled water before packing, the temperature of the water used for glazing shall be 5 °C or lower.

4.1.1.4 Fish products described in 4.2.17 to 4.2.23 (inclusive) shall be free from viscera, heads and gills, and acceptably free from scales, fins and tails.

4.1.2 Fish

4.1.2.1 Frozen fish

Frozen product shall be prepared and inspected in accordance with 8.1.

4.1.2.2 General characteristics

The fish used in the preparation of the product and not previously frozen shall have the following general characteristics:

a) a characteristic fresh appearance, colour and odour, without any perceptible rancidity or sourness;

b) where applicable, prominent, bright, clear and moist eyes;

c) where slime is present and natural to the species, transparent or creamy white slime;

d) where applicable, bright red gills; gill odours shall be characteristically fresh for the species;

e) where applicable, bright abdominal blood;

f) firm and elastic flesh adhering to the bone; and

g) when the fish is cooked, an odour and flavour that is characteristically fresh for the species and a firm but tender and succulent texture.

4.1.2.3 Tuna

In the case of tuna, the following more specific and additional characteristics shall apply:

a) **skin mucus**: preferably transparent but may also be milky; the colour iridescent to bright;

b) **eyes**: brilliant colour, convex to slightly convex in shape, but not flat; the pupils black to duller black and transparent clear to slightly bloody;

c) **gills**: bright-red, pale-red or brown-red in colour; characteristic neutral-to-sweet odour;

d) **gill odour**: fresh, strong seaweady, metallic, like freshly cut grass, very slightly musty, slightly peppery or oily. Definitely not acetic, butyric, fruity, faecal, sulfurous, sour, beery, malty, lactic or predominantly musty;

e) **abdominal wall**: intact to slack, but not soft or damaged;

f) **peritoneum**: preferably, but not necessarily, intact, but not torn or dissolved;

g) **colour of flesh**: light pink to dark red, depending on species;

h) **colour of flesh along backbone**: same as that of the surrounding flesh;

i) **physical damage**: slight deformity and mutilation acceptable, but the fish shall not be split; and

j) **cooked fish**: a flavour characteristic of the species, but not insipid, bitter or peppery. There shall be no excessive protein curd (coagulated substances of denatured protein) on the surface of the flesh and no separation of the muscle layers (honeycombing). The colour shall be off-white or pinkish to light brown, depending on the species. There shall be no orange or green discoloration of the meat.

4.1.3 Salt

Salt used in the preparation of the product shall be edible, free from bitterness and other off-odours and off-flavours, discoloration and impurities.

4.1.4 Seasoning

Seasoning ingredients shall be free from foreign matter and adulterants.

4.1.5 Additives

Only permitted additives (see 4.1.1.1) shall be used, and then only in the permitted quantities.

4.2 Presentation of fish and fish products

Fish or fish products shall be presented whole or in other acceptable forms, such as those given in 4.2.1 to 4.2.23 (inclusive).

4.2.1 Whole (round) fish

A fish as taken from the sea and not yet processed, or a fish that has been bled only.

4.2.2 Gutted fish

A round fish that has been eviscerated.

4.2.3 Gilled and gutted fish

A gutted fish (see 4.2.2) from which the gills have been removed.

4.2.4 Headed and gutted fish

A gutted fish (see 4.2.2) from which the head has been removed.

4.2.5 Dressed fish

A headed and gutted fish from which the scales have been removed.

4.2.6 Cutlets

Crosswise cuts, of thickness not exceeding 40 mm, from fish from which all viscera and blood have been removed and in which large fish bones do not protrude conspicuously.

4.2.7 Fillet (wings on)

The fleshy side of a whole fish cut lengthwise as close to the backbone as possible and including the pectoral fin, belly flap, pin bones, neck bones and skin. It shall be an intact, unragged section of fish and shall be acceptably free from scales.

4.2.8 Fillet (single fillet)

The fleshy side of a whole fish as described in 4.2.7, but with the pectoral fin and shoulder girdle removed.

4.2.9 Skinned fillet

A fillet as described in 4.2.8, but with the skin removed.

4.2.10 Skinned and boned fillet (skinless, deboned fillet)

A fillet as described in 4.2.9, but with the fish bones removed.

4.2.11 Double fillet (butterfly fillet)

The two fillets from a single fish, as described in 4.2.8, 4.2.9 or 4.2.10, but joined together along either the back or the belly.

4.2.12 Fish blocks

Blocks of fish prepared in any acceptable form and interleaved when necessary. Subject to appropriate labelling (see 6.1(b)), the blocks may also consist of minced fish. Fish blocks shall, as far as practicable, be free from bulging, voids, surface discoloration, dehydration and "freezer burn" (deep dehydration).

4.2.13 Trawled or trawl-marked whole fish

Whole fish of good quality, showing trawl marks such as depleted skin, some bruising and some surface cuts and that are suitable only for further processing, and that, if packed, shall be marked accordingly.

4.2.14 Smoked fish

Any of the fish or fish cuts described in 4.2.1 to 4.2.13 and that have been smoked (see 4.1.1.2).

4.2.14.1 Hot-smoked fish

Fish smoked at a sufficiently high temperature and for such a period of time as to ensure the heat coagulation of the protein throughout.

4.2.14.2 Cold-smoked fish

Fish smoked at a temperature at which the product does not show any signs of heat coagulation of protein.

4.2.15 Smoke-flavoured fish

Any of the fish or fish cuts described in 4.2.1 to 4.2.13 and that have been dipped in a smoke-flavoured liquid or dry material.

4.2.16 Fish fingers (sticks) and fish portions

Fish fingers (sticks) (see 4.2.16.1) and fish portions (see 4.2.16.2) prepared from blocks of frozen skinned and boned fish flesh, or formed from unfrozen fish flesh, with or without additional ingredients, into portions that are acceptably uniform in size and shape.

4.2.16.1 Fish finger (stick)

A product, including one with a bread coating or a batter coating, of unit mass not less than 20 g and not more than 50 g, and the length of which does not exceed three times its greatest width.

4.2.16.2 Fish portion

A product, with or without a bread coating or a batter coating, of any shape and size; if the mass of the product is less than 50 g, the qualifying word "portion" shall be acceptably inserted on the main panel of

the package. Fish portions prepared from unskinned or incompletely boned fish shall be described as "skin-on" portions or "not boneless" portions respectively, or both, or shall be given another description with the same meaning.

4.2.17 Fish kebabs

Cuts of fish, with or without vegetables or fruit or any combination thereof, threaded on skewers.

4.2.18 Fish cakes, fish balls and fish patties

Cakes, balls and patties that have been prepared from edible fish flesh, with or without other ingredients, and that are acceptably uniform in shape, size and texture.

4.2.19 Fish sausages

Sausages that have been prepared, with or without other ingredients, from edible minced fish flesh or boned fish fillet and that may have been smoked. Fish sausages may have been formed in natural or artificial casings. Inedible casings shall have been completely stripped off before packing, where appropriate. Fish sausages shall be uniform in size, shape and texture, and shall be free from discoloration and from ragged ends. There shall be no off-cuts or burst, distorted or cut-marked units. Cross-cut units shall have been cleanly cut at right angles to the longitudinal axes.

4.2.20 Fish in sauce

Solid fish or pieces of fish packed in sauce, together with permitted seasoning (see 4.1.4), spices or other appropriate ingredients.

4.2.21 Fish with vegetables or cereals or both, with or without sauce

Solid fish or pieces of fish packed with vegetables or cereals or both, with or without sauce, together with permitted seasoning, spices or other appropriate ingredients.

4.2.22 Curried fish

Solid fish or pieces of fish, with or without batter, packed in curry sauce, together with permitted seasoning and spices.

4.2.23 Pickled fish

Solid fish or pieces of fish, with or without batter, packed with onions and curry sauce, together with permitted seasoning and spices.

4.3 Physical requirements

4.3.1 Net mass

The net mass of a frozen product, determined in accordance with 8.3 or 8.4, as relevant, shall comply with the relevant requirements of the Trade Metrology Act, 1973 (Act 77 of 1973).

4.3.2 Packing

The product shall be acceptably packed. (See also 5.1.)

4.3.3 Uniformity of size

In packs where more than one unit is packed in a package, the units shall, on visual inspection, be acceptably uniform in size except that one filler piece may be used. If packs contain units of varying sizes, this shall be declared on the label.

4.3.4 Colour and appearance

The product shall be attractive in appearance and characteristic in colour. In any one package, the units shall be acceptably uniform in colour. In flesh packs, raggedness, blood clots, blood columns, staining and discoloration shall not be present to the extent that they detract from the appearance of the pack. Roes shall be intact unless labelled as broken roes and shall have the characteristic colour of fresh roes.

4.3.5 Texture

In flesh packs, the flesh shall not be bruised and shall have the firmness of texture that is characteristic of the species.

4.3.6 Odour and flavour

The state of the raw material, the production process and the packaging shall ensure that, after the frozen product has been thawed to a temperature above 10 °C, and the units have been separated where necessary, the odour is fresh and characteristic of the product, and that off-odours and other indications of deterioration or of the use of inferior raw materials are absent. The odour and flavour of the cooked product shall also be fresh and characteristic.

4.3.7 Freedom from defects

4.3.7.1 The product shall be acceptably free from bruised or otherwise damaged material, from detached scales and from sand, grit, dirt, pieces of shell, other extraneous matter and visible parasites. The product shall be acceptably free from slime, from "freezer burn" (deep dehydration) that cannot easily be removed by scraping, and from discoloration and blemishes.

4.3.7.2 A product described as boneless shall contain not more than one fish bone defect (see 2.7) per kilogram of the fish component of the product, and shall be free from cartilage or fish bone that, after being cooked, is capable of piercing or hurting the palate.

4.3.7.3 A product described as boned or deboned shall not contain more than five fish bone defects (see 2.7) per kilogram of the fish component of the product.

4.3.7.4 A product described as skinless shall have no residual skin tissue or surface damage that materially detracts from its appearance. A product described as being "skin-on" shall be substantially free from skin damage or surface damage that materially detracts from its appearance.

4.3.8 Other physical requirements

When tested in accordance with the methods referred to in column 4 of table 1, the product shall comply with the appropriate requirements given in column 3.

4.4 Chemical requirements

When tested in accordance with 9.2 to 9.8, the product shall comply with the relevant requirements of this specification and of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972).

4.5 Microbiological requirements

When tested in accordance with the methods given in 10.6 to 10.14, the product shall comply with the requirements given in columns 2, 3 and 4 of table 2.

5 Packaging, glazing, freezing and storage

5.1 Packaging materials and outer containers

5.1.1 Packaging and wrapping materials

Subject to the relevant requirements of the regulations promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972), packaging and wrapping materials for the unprotected product shall be unused (new), clean, non-toxic and inert and of low moisture-vapour permeability, and shall not contain substances deleterious to the product or harmful to health. No packaging or wrapping material shall impart a flavour to, or in any way cause discoloration of, the product, or be itself discoloured by contact with the product. Unless the fish product is glazed (see 5.2), it shall be packed in a material of acceptably low permeability to moisture and oxygen.

Table 1 — Physical requirements

1	2	3	4
Product	**Property**	**Minimum requirement** % (by mass)	**Test method subclause**
Battered or crumbed fish products (raw)	Fish content	60[1)]	8.2.1
Battered or crumbed fish products (partly cooked, or fried)	Fish content	50[1)]	8.2.1
Fish cakes	Fish content	37,5	9.7
Fish sausages, fish balls and fish patties	Fish content	50	9.7
Fish in sauce	Washed mass	50[1)]	8.2.2
Fish with vegetables or fruit or cereals or any combination thereof	Washed mass (i.e. all solid components) Washed mass of fish component	60[1)] 35[1)]	8.2.2 8.2.3
Curried fish	Washed mass (i.e. all solid components) Fish content	50[1)] 40[1)2)]	8.2.2 8.2.1
Pickled fish	Washed mass (i.e. all solid components) Mass of fish component including batter Fish content Onion	50[1)] 45[1)] 35[1)2)] 5[1)]	8.2.2 8.2.3 8.2.1 8.2.5
Fish pies and similar products	Fish content of filling Mass of filling (raw) Mass of filling (cooked)	50 45 25	9.7 8.2.4 8.2.4
Fishpaste and chopped fish with vegetables, with or without cereals	Fish content	50	9.7
Breaded or battered marine molluscs (including calamari rings)	Mollusc content	40[1)]	8.2.1

1) Expressed as a percentage of the declared net mass of the product.
2) Only tested in the event of concern about the fish content of the coated product.

Table 2 — Microbiological requirements

1	2	3	4
Organism	**Contents, max.**		
	Raw products[1] (with or without added ingredients)	**Cooked products[2] or hot smoked products (or both)**	**Cold smoked products[3]**
Standard plate count	1×10^6/g	1×10^5/g	1×10^5/g
Enterobacteriaceae	[4]	100/g	100/g
Faecal coliform bacteria	Nil/10 g	Nil/ 10 g	Nil/10 g
Staphylococcus aureus	10/g	10/g	10/g
Salmonella	Nil/25 g	Nil/25 g	Nil/25 g
Shigella	Nil/25 g	Nil/25 g	Nil/25 g
Clostridium perfringens	Nil/25 g	Nil/25 g	Nil/ 25 g
Vibrio cholerae	Nil/25 g	Nil/25 g	Nil/ 25 g
Vibrio parahaemolyticus	Nil/25 g	Nil/25 g	Nil/25 g
Listeria monocytogenes	[4]	Nil/25 g	Nil/25 g

1) Products that require cooking before being consumed.
2) Products that only require thawing and reheating before being consumed.
3) Intended for consumption without cooking.
4) Not to be tested.

Packaging materials shall

a) not be such as to impair the organoleptic characteristics of the product,

b) not be capable of transmitting substances injurious to the product or harmful to human health, and

c) be strong enough to protect the product adequately.

5.1.2 Outer containers

Only fibreboard or other acceptable outer containers shall be used. Outer containers shall be unused (new), clean and intact, and shall be neatly and securely closed. Wooden outer containers shall not be made of green wood and shall not contain any substance that is injurious to the product or harmful to health. Outer containers shall be so closed as to prevent contamination of the contents by dust or foreign matter and shall be strong enough to protect the product adequately.

5.2 Glazing

The product may be glazed using chilled water or other acceptable glazing agent as a substitute for wrapping, provided that the glaze is maintained in an acceptable condition up to and including the final point of sale. When the product is glazed, the coating of ice shall cover the product completely to ensure that dehydration and oxidation are minimized. Water used for glazing shall comply with the requirements for potable water (see 3.4.1) or sea water (see 3.4.2), and its temperature shall be 5 °C or lower.

5.3 Freezing

The stacking of the product in freezers (other than plate or brine freezers) shall be away from floor and

wall surfaces and shall be such that air circulation between packages is not impeded. The product shall be subjected to either the freezing or the quick-freezing process as soon as possible after processing. When the product is packed into containers before freezing, it shall not be exposed to excessively high temperatures and the delay between packaging and freezing shall not exceed 3 h. The temperature of a product, other than a smoked or a precooked product or a product processed within 8 h of having been landed on deck, shall not exceed 20 °C during processing. Freezing capacity shall not be overtaxed. The freezing and the frozen storage of the product shall be carried out in a way that will obviate freezer burn.

5.4 Frozen storage

5.4.1 Records of the temperature of freezer storage rooms shall be retained for at least two years from the date of recording, and shall be available for inspection by the authority administering this specification. (See also 3.2.16.3.)

5.4.2 The product shall be stored and maintained at a temperature of –20 °C or lower. The practical storage life of flat fish, lean fish, fatty fish and tuna at various temperatures is given in table 3, and any product stored in excess of the appropriate period shall be liable to re-inspection at the discretion of the authority administering this specification.

Table 3 — Practical storage life

1	2	3	4
Product	**Storage temperature**		
	–20 °C	–25 °C	–30 °C
	Maximum storage life months		
Flat fish, e.g. sole	15	21	25
Lean fish, e.g. hake, kingklip, skate, squid, fish sticks and fish portions	12	18	21
Fatty fish – glazed	6	9	12
– vacuum-packed	10	12	15
Tuna	6	9	12

5.4.3 If at any time during storage, the temperature of the product rises above the appropriate storage temperature, it shall be rapidly lowered to that temperature. If the temperature of the product rises above –7 °C, the product shall, in addition, be submitted to the relevant authority for re-inspection.

6 Marking

6.1 Marking on packages that are not for export (see 6.4)

Except as allowed for in terms of 6.4, the following information shall appear on each package, in legible and indelible marking and in accordance with 6.2, in a typeface of such size and presentation as prescribed by the regulations promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972), and the Trade Metrology Act, 1973 (Act 77 of 1973):

a) the name and full physical address of the manufacturer, producer, proprietor or controlling company, or, in the case of containers packed for any other person or organization, the name and full physical address of that person or organization;

b) a true description of the product, including the name of the product and the presentation of the contents. In addition, if so desired by the person or organization referred to in (a) above, in the case of a quick-frozen product (see 2.22), the words "Quick frozen". The name or designation that is used

in the labelling of the product shall not be misleading, and the variety of fish, except in the case of fish sticks, fish portions, fish cakes, fish balls, fish patties and fish sausages, shall be stated. The method of preparation and the presentation of the contents shall be in accordance with the description on the label or package. Where applicable, the description shall be in accordance with the relevant description given in 4.2;

c) where applicable, a list of the ingredients, in descending order of content;

d) a statement that the product is cooked or uncooked, as applicable, and instructions for storage, given in the following manner:

- Uncooked (or raw) – Keep frozen
- Partly cooked – Keep frozen. Do not refreeze once thawed
- Cooked – Keep frozen. Do not refreeze once thawed;

e) in the case of products for sale in the Republic of South Africa, the net mass of the contents, where applicable (in accordance with the regulations promulgated under the Trade Metrology Act, 1973); where the product has been glazed, the net mass declaration of the product shall be exclusive of the glaze;

f) the country of origin;

g) if the product has been glazed with seawater, a statement to this effect prominently displayed on the main panel of the label, in immediate conjunction with the name of the product;

h) where relevant, directions for use;

i) any labelling requirement specifically called for by regulation; and

j) the date of manufacture and the identity of the factory in which the product was packed. The use of a code is permissible, provided that the key to the code is disclosed to the authority administering this specification.

The trade name of a product shall not be misleading to the consumer.

6.2 Labels

6.2.1 The information required by 6.1 shall be printed on each individual package or on the overwrap covering such a package, or on a label of acceptable material attached to the package.

6.2.2 Labels on packages shall be clean and neat and securely attached. They shall not be superimposed on other labels or on matter printed direct on the packages. They shall not be applied by any person other than the manufacturer or his authorized agent.

6.2.3 Labels or sealing adhesives that are liable to deteriorate under the conditions of storage of the packaged products shall not be used.

6.3 Marking on outer containers that are not for export (see 6.4)

6.3.1 Outer containers shall be clean, neat and unbroken, and on every such container (carton, box, etc.) shall be printed or stencilled the quantity and size or net mass of the packages it contains and the information required by 6.1(a), (b), (d) and (e), except that the physical address required by 6.1(a) need not be the full physical address but shall be sufficient for identification purposes. The method of preparation need not be given on the outer container.

6.3.2 The date of manufacture, the identity of the factory and the batch number (if applicable) shall be

stamped or otherwise indelibly marked on the outer container or on a label securely attached to the outer container, or on a packing slip inserted into the outer container. A code may be used for the date of manufacture, provided that the key to the code is disclosed to the authority administering this specification.

6.4 Marking on outer containers and packages for export

Outer containers and packages for export shall be marked in accordance with the requirements of the importing country and may be marked differently from the requirements of 6.1 and 6.3, provided that there is no attempt to misrepresent the contents. Details as required by 6.1(j) shall be printed on each outer container and package.

7 Delivery and inspection

7.1 General

The requirements of 7.2 and 7.3 shall be subject to the requirements of the applicable statutory Acts and regulations.

7.2 Delivery

7.2.1 General

The delivery of frozen products shall take place under hygienic conditions.

7.2.2 Delivery for export

Frozen product for export shall be conveyed from the factory to the freezer storage depot and delivered into the transporting vessel's freezer storage holds at a temperature of –20 °C or lower. If, at any time during this transportation, the temperature of the product rises above –20 °C, it shall be lowered to the required temperature as rapidly as possible. The product shall be re-inspected if the temperature has risen above –7 °C.

7.2.3 Delivery for local sale

Frozen product for local distribution shall be conveyed in refrigerated or insulated trucks from the factory or the freezer storage depot to the point of retail sale. The temperature of the product during local transportation shall, except at the outer surfaces of a stack, be –20 °C or lower. Refrigerated trucks shall be fitted with at least one thermometer that is so installed as to be readable from outside the refrigerated compartment.

7.3 Inspection for export

Each consighment of frozen product shall be available for inspection at the freezer storage depot from which it is to be shipped for export. The authority administering this specification shall be notified at least 14 d before the expected date of shipment of the product. Products not accepted for export shall, if stored in freezer storage rooms together with products approved for export, be segregated and clearly identified. The frozen product shall be submitted for re-inspection at the point of shipment if, while the product was being held for shipment, doubt arose as to its temperature history.

8 Methods of physical examination

8.1 Physical examination of frozen fish for its general characteristics

8.1.1 Smaller fish may be thawed whole. In the case of a large fish, cut a sample wedge of suitable size from the back of the fish just behind the head and extending down to the backbone or, alternatively, cut a cross-section piece at least 50 mm thick through the fish, just behind the head or in front of the tail. Where possible, ensure that the mass of each sample piece is at least 1 kg. Thaw the sample and examine it for odour and colour (see 4.1.2.2(a)). Cartilaginous fish such as sharks and rays should be thawed in air to facilitate the detection of any ammoniac odours.

8.1.2 In the case of tuna, thaw the sample and remove the blood by rinsing the sample in tap water. Then examine each sample for colour in general (see 4.1.2.3(g)), and for colour along the backbone (see 4.1.2.3(h)). Steam the sample until a core temperature of at least 70 °C to 75 °C has been reached and, in terms of the requirements of 4.1.2.3(j), examine for:

a) flavour;

b) protein curd on the surface of the flesh;

c) separation of muscle layers; and

d) colour.

8.1.3 Visually examine the fish for the remainder of its general characteristics in terms of the applicable requirements given in 4.1.2.

8.2 Determination of the fish content, filling content and washed mass

8.2.1 Determination of the fish content of bread-coated or batter-coated products

8.2.1.1 Record the declared net mass (m_0) printed on the package. Place the contents of the package in a water-bath maintained at 47 °C to 49 °C and allow the units to remain in the water until the breading or batter (as applicable) becomes soft and can easily be removed from the still-frozen fish flesh by means of a round-tipped spatula or table knife.

NOTE – Several preliminary trials may be necessary to determine the optimum immersion time required for "de-breading" or "de-battering" the units in a package. **For these trials only**, a saturated solution of copper (II) sulfate may be used in place of the water. The optimum immersion time is the minimum time of immersion in the copper sulfate solution required before the breading or batter (as applicable) can easily be scraped off, leaving only a slight trace of blue colour on the surface of the "de-breaded" or "de-battered" fish units.

8.2.1.2 Remove the units from the water-bath and blot them lightly with paper towelling. Scrape and remove the breading or batter from the fish flesh by means of the spatula, removing the coating from narrow sides and ends first and then from wide flat surfaces. If the coating of a unit is difficult to remove, immerse the unit for up to a further 5 s and remove the residual coating. Ensure that the total immersion time does not exceed 15 s. Determine the mass (m_1) of all the "de-breaded" or "de-battered" units.

8.2.1.3 Calculate the fish content, expressed as a mass percentage of the product, using the following formula:

$$\frac{m_1}{m_0} \times 100$$

where

m_0 is the declared net mass of the package, in grams; and

m_1 is the total mass of all the "de-breaded" or "de-battered" units, in grams.

8.2.2 Washed mass of a product that contains sauce

8.2.2.1 Record the declared net mass (m_0) printed on the package. Place the contents of the package on a tared sieve of nominal aperture size approximately 2 mm, and rinse the product with water, pre-warmed, if necessary, to a temperature not higher than 38 °C, until free of sauce. Drain for 2 min and then determine and record the mass of the material remaining on the sieve as the washed mass (m_1) of the fish component.

8.2.2.2 Calculate the percentage washed mass of the product by using the formula given in 8.2.1.3.

8.2.3 Washed mass of fish component of a product that contains vegetables or fruit or cereals (or any combination of them)

8.2.3.1 Record the declared net mass (m_0) printed on the package. Place the contents of the package on a tared sieve of aperture size approximately 2 mm and rinse with water, pre-warmed, if necessary, to a temperature not higher than 38 °C. Remove the vegetables or fruit or cereals, as applicable. Drain for 2 min and then determine and record the mass of the material remaining on the sieve as the washed mass (m_1) of the fish component.

8.2.3.2 Calculate the percentage washed mass of the product by using the formula given in 8.2.1.3.

8.2.4 Mass of pie filling

Thaw five fish pies and then determine their total mass (m_0). Remove the crust and determine the mass of the filling (m_1). Calculate the mass of the filling, expressed as a mass percentage of the product, using the following formula:

$$\frac{m_1}{m_0} \times 100$$

where

m_0 is the mass of five pies, in grams; and

m_1 is the mass of the filling of five pies, in grams.

8.2.5 Mass of onion of pickled fish

8.2.5.1 Record the declared net mass (m_0) printed on the package. Place the contents of the package on a tared sieve of aperture size approximately 2 mm and rinse with water, pre-warmed, if necessary, to a temperature not higher than 38 °C, until free of sauce. Remove the fish. Drain for 2 min and then determine and record the mass of the material remaining on the sieve as the mass (m_1) of the onion.

8.2.5.2 Calculate the percentage mass of the onion in the product by using the formula given in 8.2.1.3.

8.3 Determination of the net mass of frozen products other than glazed products

8.3.1 Immediately after removal of the package from frozen storage, remove any ice adhering to the outside of the package and determine the gross mass of the unopened package.

8.3.2 Remove the packaging material. Wash, dry and determine the mass of the packaging material. Record the difference between the gross mass (see 8.3.1) and the mass of the packaging material as the net mass of the frozen product.

8.4 Determination of the net mass of a glazed product

8.4.1 Immediately after removal of the package from frozen storage, place the contents of the package in a container into which fresh potable water (see 3.4.1) at ambient temperature is introduced from the bottom at a flow rate of approximately 5 ℓ/min. Leave the product in the water until all surface ice has melted. If the product is block-frozen, turn the block over several times during deglazing; probe the block and remove units from the water as they become loose.

8.4.2 After all the glaze that can be seen or felt has been removed and the units separate easily, transfer the contents of the container (see 8.4.1) to a tared sieve of nominal aperture size approximately 2 mm. Incline the sieve at an angle of approximately 20° and drain for 2 min.

8.4.3 Record the mass of the material remaining on the sieve as the net mass of the glazed product.

9 Methods of chemical analysis

NOTE – During the analysis and unless otherwise specified, use only reagents of recognized analytical grade or (when such a grade is unobtainable) of the purest grade available, and use only distilled or deionized water.

9.1 Preparation of product sample

Thaw the product in its packaging and then remove the packaging. Where applicable, for example in the case of fish pies, remove the crust/casing after thawing. Pass the remaining contents of the sample twice through a meat grinder and mix the minced sample thoroughly. Transfer an appropriate quantity of the minced sample to a glass container that has a lid or screw cap that can be tightly closed. Store in a refrigerator until the tests are carried out.

9.2 Determination of lead, copper, zinc and cadmium (atomic absorption spectrophotometric method)

9.2.1 Apparatus

9.2.1.1 Atomic absorption spectrophotometer. (Refer to the manufacturer's reference manuals for wavelength, slit width, flame conditions, etc.)

9.2.1.2 Crucible, platinum, of capacity 150 mℓ.

9.2.1.3 Water-bath.

9.2.1.4 Temperature controlled furnace.

9.2.2 Reagents

9.2.2.1 Hydrochloric acid, 1 N, prepared by diluting 89 mℓ of HCl to 1 ℓ with distilled water.

9.2.2.2 Lead standard solutions, as follows:

a) **stock standard solution**: 1 mg Pb/mℓ; and

b) **working standard solution**: 1,0 µg Pb/mℓ.

9.2.2.3 Copper standard solutions, as follows:

a) **stock standard solution**: 1 mg Cu/mℓ; and

b) **working standard solution**: 3,0 µg Cu/mℓ.

9.2.2.4 Zinc standard solutions, as follows:

a) **stock standard solution**: 1 mg Zn/mℓ; and

b) **working standard solution**: 2,0 µg Zn/mℓ.

9.2.2.5 Cadmium standard solutions, as follows:

a) **stock standard solution**: 1 mg Cd/mℓ; and

b) **working standard solution**: 1,0 µg Cd/mℓ.

9.2.3 Methods

9.2.3.1 Preparation of sample solution

Weigh 25 g ± 0,1 g of sample into the crucible (see 9.2.1.2), and dry for 2 h at 135 °C to 150 °C. Transfer the crucible to a cold, temperature-controlled furnace and slowly raise the temperature to 450 °C. Ash the sample overnight (16 h). Remove the crucible and allow it to cool. Add 10 mℓ of the 1N HCl and dissolve the ash by heating the crucible cautiously on a boiling water-bath. Transfer the contents of the crucible to a 25 mℓ volumetric flask. Heat the ash residue again successively with two 5 mℓ portions of the 1N HCl and add it to flask. Cool, dilute to volume with the 1N HCl, and mix.

9.2.3.2 Reagent blank

Prepare a reagent blank.

9.2.3.3 Determination of lead

Determine the absorbance of the sample solution, of the reagent blank and of the 1,0 µg Pb/mℓ working standard solution. If the absorbance of the sample solution minus the absorbance of the reagent blank is less than the absorbance of the working standard solution, the lead in the sample is less than 1 mg/kg.

9.2.3.4 Determination of copper

Dilute 5,0 mℓ of the sample solution to 50,0 mℓ with water. Determine the absorbance of the sample solution, of the reagent blank and of the 3,0 µg Cu/mℓ working standard solution. If the absorbance of the sample solution minus the absorbance of the reagent blank is less than the absorbance of the working standard solution, the copper in the sample is less than 30 mg/kg.

9.2.3.5 Determination of zinc

Dilute 5,0 mℓ of the sample solution to 100,0 mℓ with water. Determine the absorbance of the sample solution, of the reagent blank and of the 2,0 µg Zn/mℓ working standard solution. If the absorbance of the sample solution minus the absorbance of the reagent blank is less than the absorbance of the working standard solution, the zinc in the sample is less than 40 mg/kg.

9.2.3.6 Determination of cadmium

Determine the absorbance of the sample solution, of the reagent blank and of the 1,0 µg Cd/mℓ working standard solution. If the absorbance of the sample solution minus the absorbance of the reagent blank is less than the absorbance of the working standard solution, the cadmium in the sample is less than 1 mg/kg.

9.3 Determination of tin (atomic absorption method)

9.3.1 Apparatus

Atomic absorption spectrophotometer. (Refer to the manufacturer's reference manuals for wavelength, slit width, flame conditions, etc.)

9.3.2 Reagents

9.3.2.1 Tin standard solutions, as follows:

a) **stock standard solution**: 1 mg Sn/mℓ; and

b) **working standard solution**: 40,0 µg Sn/mℓ.

9.3.2.2 Potassium chloride solution, 10 mg K/mℓ, prepared by dissolving 1,91 g of KCl and diluting to 100 mℓ with distilled water.

9.3.2.3 Nitric acid (HNO_3), concentrated. Test the purity of a lot by diluting a portion to 1:4 (by volume) with distilled water and aspirating into an AA spectrophotometer. The absence of an Sn signal indicates suitability for analysis.

9.3.3 Preparation of sample

Accurately (± 0,01 g) weigh 25 g of the sample into a 250 mℓ Erlenmeyer flask. Dry in an oven at 120 °C.

NOTE – Do not add HNO_3 to samples (see below) unless there is time to complete this stage of digestion on the same day.

Add 30 mℓ of the concentrated HNO_3 to the flask and, within 15 min, heat gently in a hood to initiate digestion, avoiding excess frothing. Gently boil until 3 mℓ to 6 mℓ of digest remains or until the sample just begins to dry on the bottom. Do not allow the sample to char. Remove the flask from the heat. Without delay, continue as follows, simultaneously preparing two empty flasks for reagent blanks: add 25 mℓ of concentrated hydrochloric acid (HCl), and heat gently for about 15 min until sample bumping from the evolution of chlorine (Cl_2) stops. Increase the heat, and boil until a volume of 10 mℓ to 15 mℓ remains. Use a similar flask that contains 15 mℓ of water, to estimate the remaining volume. Transfer the sample solution and the reagent blanks to 25 mℓ volumetric flasks. The sample solution and reagent blanks may stand overnight or longer.

Pipette 1,0 mℓ of the KCl solution into each volumetric flask. Cool to ambient temperature and dilute to volume with water. Mix well and filter the sample solution only through dry, medium porosity paper into a dry polypropylene or polyethylene screw-cap bottle. Transfer the blanks to similar bottles. Cap the bottles until analysis. Solutions are stable for several months.

9.3.4 Reagent blank

Prepare a reagent blank.

9.3.5 Procedure

Determine the absorbance of the sample solution, of the reagent blank and of the 40,0 µg Sn/mℓ working solution. If the absorbance of the sample solution minus the absorbance of the reagent blank is less than the absorbance of the working standard solution, the tin in the sample is less than 40 mg/kg.

9.4 Determination of arsenic (Gutzeit method)

9.4.1 Apparatus

See figure 1 and 9.4.3.

9.4.2 Reagents

9.4.2.1 Arsenic standard solutions, as follows:

a) **stock standard solution**: 1 mg As/mℓ; and

b) **working standard solution**: 1,0 µg As/mℓ.

9.4.2.2 Hydrochloric acid, concentrated.

9.4.2.3 Potassium iodide solution, a 16,6 g/100 mℓ solution.

9.4.2.4 Tin (II) chloride solution

Dissolve 33 g of tin (II) chloride (free from arsenic) in 10 mℓ of hydrochloric acid and sufficient water to produce 100 mℓ.

Dimensions in millimetres

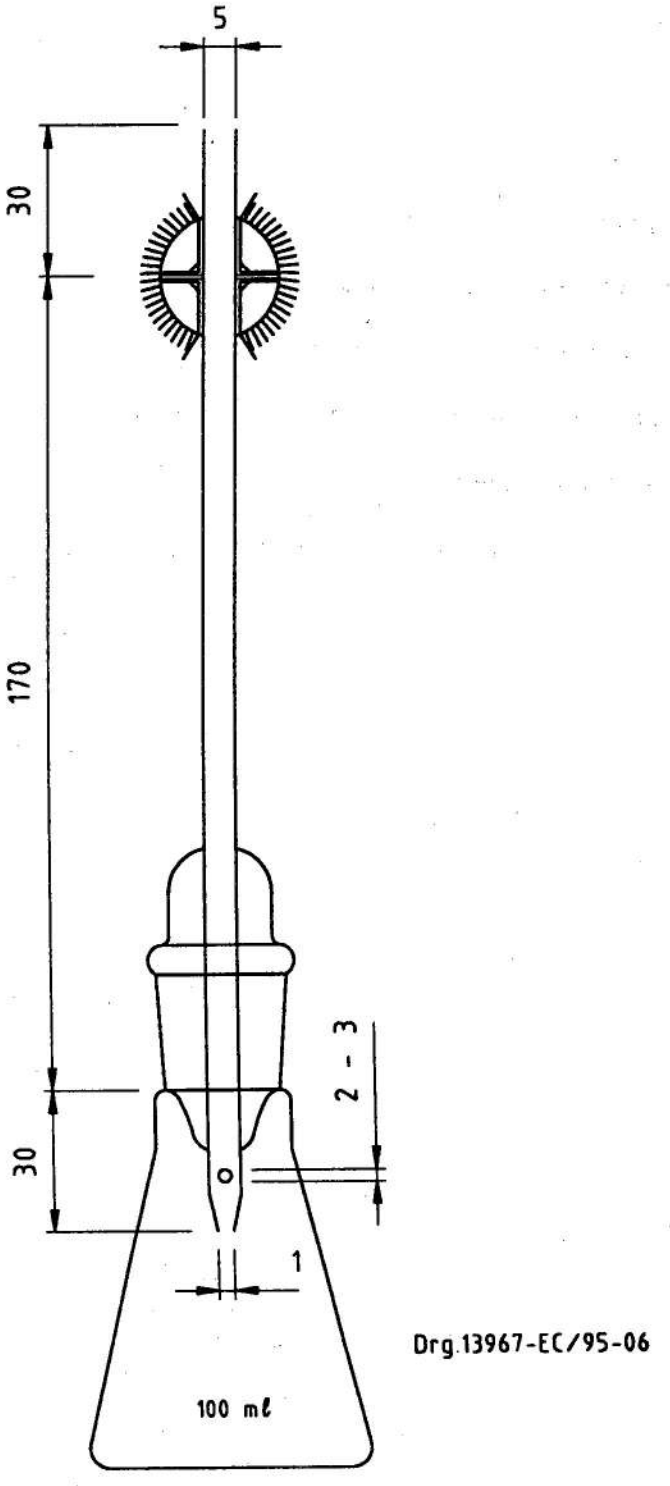

Figure 1 — Apparatus for limit test for arsenic

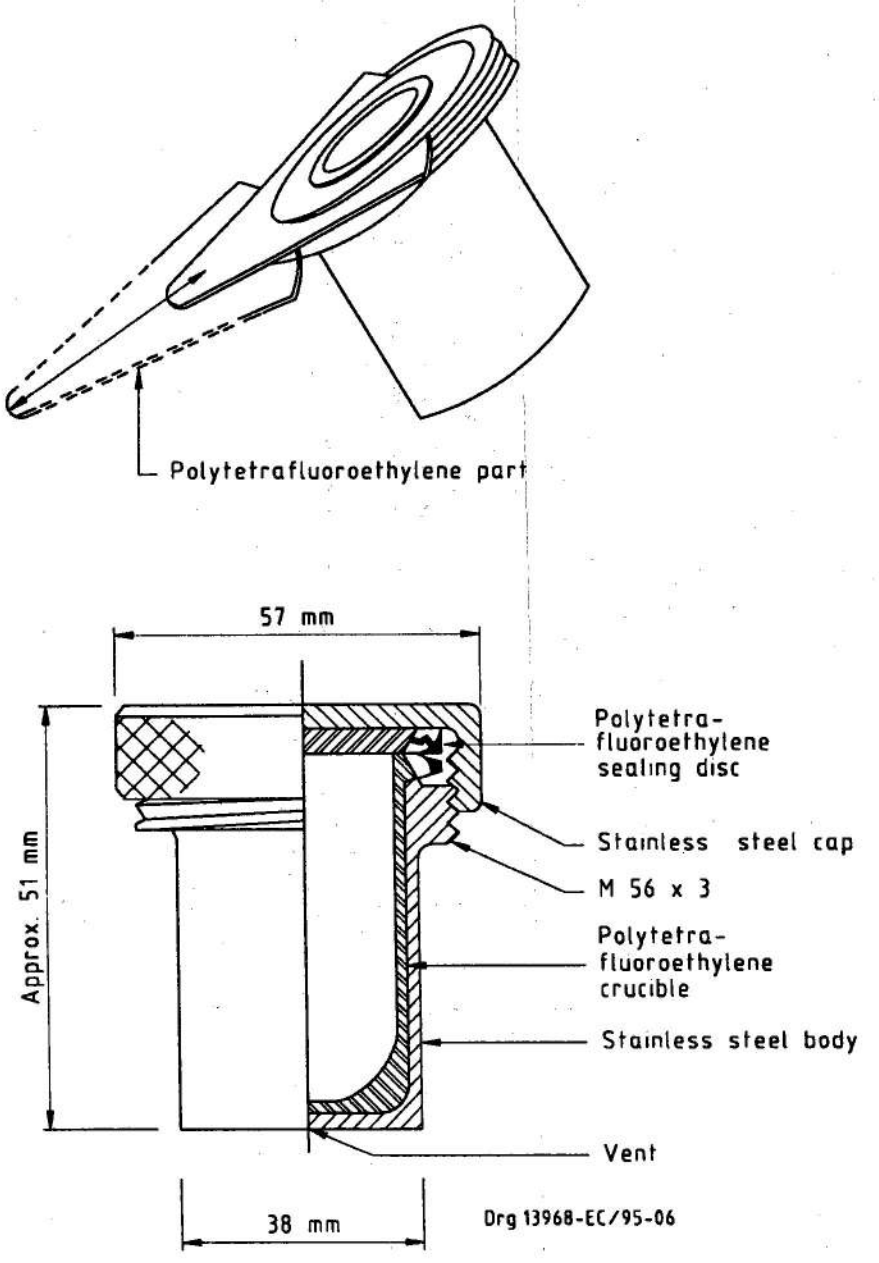

Figure 2 — Digestion vessel

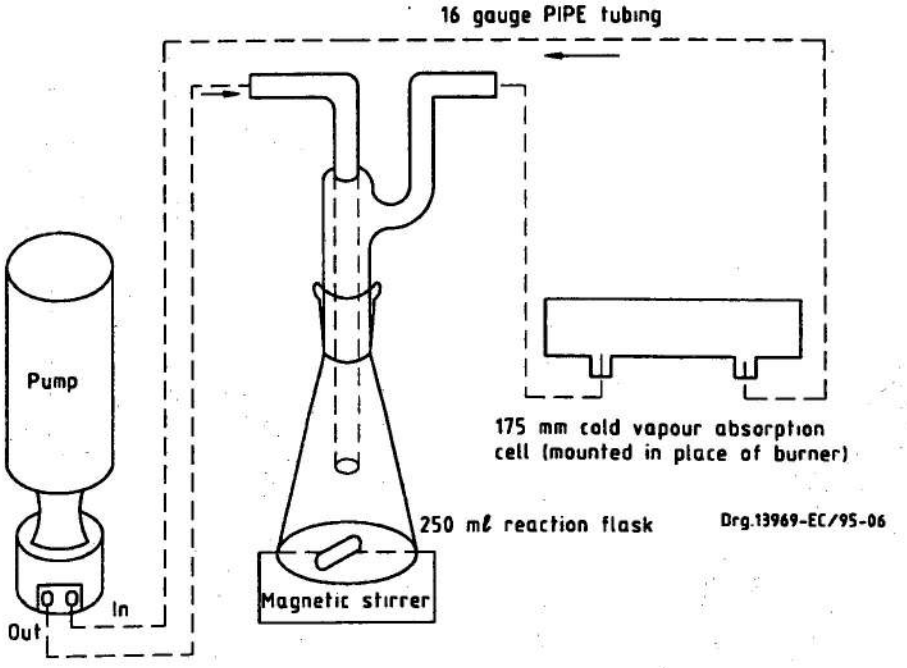

Figure 3 — Apparatus for the determination of mercury content

9.4.2.5 Mercury (II) bromide, mercuric bromide ($HgBr_2$ = 360,4 Analytic reagent grade).

9.4.2.6 Mercury (II) bromide paper, prepared as follows: in a rectangular dish, place a 5 g/100 mℓ solution of mercury (II) bromide in absolute ethanol and immerse in it pieces of white filter paper of grammage 80 g/m^2 (Whatman No. 1 is suitable), each measuring 200 cm x 15 mm or 200 mm x 15 mm and folded in two. Decant the excess liquid and allow the papers to dry, protected from light, by suspending them over a non-metallic thread. Cut away the folded edge to a width of 10 mm. Cut the remaining strips into 15 mm squares or discs of diameter 15 mm.

Mercury (II) bromide paper should be kept in a glass-stoppered container and protected from light.

9.4.2.7 Zinc, granulated.

9.4.2.8 Lead acetate solution, a 10 g/100 mℓ solution of lead (II) acetate in carbon dioxide-free water.

9.4.2.9 Lead acetate cotton, prepared as follows: immerse absorbent cotton in a mixture of 10 volumes of lead acetate and 1 volume of 2M acetic acid. Drain off the excess liquid by placing the cotton on several layers of filter paper, without squeezing the cotton. Allow the cotton to dry at room temperature. Lead acetate cotton should be kept in an airtight container.

9.4.3 Procedure

Take 5,0 mℓ of the sample solution prepared for the determination of tin (see 9.3.3).

The apparatus (see figure 1) consists of a 100 mℓ conical flask closed with a ground-glass stopper through which passes a glass tube of length approximately 200 mm and of internal diameter 5 mm. The lower part of the tube is drawn to an internal diameter of 1,0 mm and at a distance of 15 mm from its tip is a lateral orifice of diameter 2 mm to 3 mm. When the tube is in position in the stopper, the lateral orifice should be 2 mm to 3 mm below the lower surface of the stopper. The upper end of the tube has a perfectly flat, ground surface at right angles to the axis of the tube. A second glass tube of the same internal diameter and of length 30 mm, with a similar flat ground surface, is placed in contact and co-axially with the first, and is held in position by two spiral springs. Into the lower tube, insert 50 mg to 60 mg of lead acetate cotton, loosely packed, or a small plug of cotton and a rolled piece of lead acetate paper of combined mass 50 mg to 60 mg. Between the flat surfaces of the tubes, place one of the pieces of mercury (II) bromide paper (see 9.4.2.6). In the conical flask, dilute 5,0 mℓ of the sample solution to 25 mℓ with water. Add 15 mℓ of concentrated hydrochloric acid, 0,1 mℓ of tin (II) chloride solution, and 5 mℓ of potassium iodide solution, allow to stand for 15 min and then add 5 g of granulated zinc. Immediately assemble the two parts of the apparatus and immerse the flask in a water-bath at a temperature such that a uniform evolution of gas is maintained. After not less than 2 h, any stain produced on the mercury (II) bromide paper shall be not more intense than that obtained by treating 1 mℓ of arsenic working standard solution (1 µg/mℓ As) diluted to 25 mℓ with water in the same way.

9.5 Determination of mercury

9.5.1 Apparatus

9.5.1.1 Atomic absorption spectrophotometer, fitted with a mercury hollow cathode lamp.

9.5.1.2 Digestion vessel (see figure 2), that consists of a stainless steel body that supports a polytetrafluoroethylene crucible, and a screw-on cap that has a polytetrafluoroethylene liner to provide a polytetrafluoroethylene sealing surface or a similar digestion vessel.

A polytetrafluoroethylene spout is snapped on the outside rim of the vessel to permit the quantitative transfer of the contents without contact with metal parts.

9.5.1.3 Cold vapour absorption cell, fitted in place of the burner of the spectrophotometer (see figure 3).

9.5.1.4 Diaphragm pump.

9.5.2 Reagents

9.5.2.1 Hydrochloric acid, concentrated.

9.5.2.2 Nitric acid, concentrated.

9.5.2.3 Sulfuric acid, concentrated.

9.5.2.4 Diluting acid solution, an aqueous solution that contains 58 mℓ of the nitric acid and 67 mℓ of the sulfuric acid per litre.

9.5.2.5 Dilute hydrochloric acid, one volume of the hydrochloric acid added to nine volumes of water.

9.5.2.6 Stannous chloride solution, 5 g of crystalline stannous chloride ($SnCl_2.2H_2O$) dissolved in 10 mℓ of the concentrated hydrochloric acid by warming, and diluted to approximately 50 mℓ with water. Remove trace amounts of mercury by bubbling nitrogen through the solution for 10 min.

9.5.2.7 Mercury standard solutions, as follows:

a) **stock standard solution**, 1 mg Hg/mℓ; and

b) **working standard solution**, 0,1 µg Hg/mℓ: dilute 1,0 mℓ of the stock standard solution (see (a) above)to 100 mℓ with the dilute hydrochloric acid (HCl). Then dilute 1,0 mℓ of this solution to 100 mℓ with the dilute HCl. Prepare this solution daily.

9.5.3 Reagent blank

Prepare a reagent blank.

9.5.4 Procedure

9.5.4.1 Accurately weigh out 1 g ± 0,1 g of the sample (see 9.2) (**caution**: do not use more than 300 mg dry mass; for materials with a high fat content, do not use more than 200 mg dry mass) into the digestion vessel (see 9.5.1.2), add 5,0 mℓ of the concentrated nitric acid HNO_3, and close the vessel by tightening the screw cap. Place the vessel, without tilting, into an oven preheated to 150 °C for 30 min to 60 min or until the sample solution is clear. Remove the vessel and allow it to cool to room temperature. Unscrew the cap, snap on the spout, transfer the contents of the vessel with the aid of the diluting acid solution (see 9.5.2.4) to a 100 mℓ volumetric flask, and dilute to volume with acid solution.

9.5.4.2 Switch on the mercury hollow cathode lamp, and allow the spectrophotometer to equilibrate fully at a wavelength setting of 253,7 nm. Pipette 20 mℓ of the 100 mℓ sample solution (see 9.5.4.1) into the reaction flask. Place a magnetic follower in the flask and connect the absorption cell, the reaction flask and the diaphragm pump in series and in a closed system by means of polytetrafluoroethylene (PTFE) tubing (see figure 3), minimizing the dilution of the mercury vapour by using tubing of the smallest diameter and of the shortest length practicable. Ensure that the distance between the lower end of the inlet tube and the surface of the sample solution in the reaction flask is at least 10 mm.

9.5.4.3 Switch on the magnetic stirrer and the pump. Adjust the absorption reading on the spectrophotometer to zero. Switch off the stirrer and the pump.

9.5.4.4 Disconnect the flask, add 1 mℓ of the stannous chloride solution to the sample solution, immediately reconnect the flask, switch on the magnetic stirrer, continue stirring for 90 s, switch off the stirrer and then immediately switch on the pump. Record the absorption reading as soon as it becomes stable. Remove the flask and pump air through the system to remove the mercury vapour.

9.5.4.5 Transfer 1,0 mℓ of the 0,1 µg Hg/mℓ working standard solution to a reaction flask, add 19,0 mℓ of distilled water and repeat the procedure given in 9.5.4.2, 9.5.4.3 and 9.5.4.4.

9.5.5 Calculation

Measure the absorbance of the 0,1 µg Hg/mℓ working standard solution, of the reagent blank and of the sample solution.

If the absorbance of the sample solution minus the absorbance of the reagent blank is less than the absorbance of the working standard solution, the mercury content of the sample is less than 0,5 mg/kg.

9.6 Determination of antimony

9.6.1 Apparatus

Atomic absorption spectrophotometer. (Refer to the manufacturer's reference manuals for wavelength, slit width, flame conditions, etc.)

9.6.2 Reagents

9.6.2.1 Potassium iodide.

9.6.2.2 Antimony standard solutions, as follows:

a) **stock standard solution**: 1 mg Sb/mℓ; and

b) **working standard solution**: 0,01 µg Sb/mℓ.

9.6.3 Reagent blank

Prepare a reagent blank.

9.6.4 Procedure

9.6.4.1 Follow the apparatus manufacturer's instructions for the hydride generation for antimony, ensuring that the antimony is in the Sb^{111} state before analysis, by treating the sample and standards with an excess of potassium iodide.

9.6.4.2 Measure the absorbance of the 0,01 µg Sb/mℓ working standard solution, of the reagent blank and of the sample solution (using the sample solution obtained in the mercury determination). If the absorbance of the sample solution minus the absorbance of the reagent blank is less than the absorbance of the working standard solution, the antimony content of the sample is less than 1 mg/kg.

9.7 Determination of protein nitrogen content and calculation of fish content

9.7.1 Reagents

During the analysis, use only reagents of recognized analytical grade that are free from nitrogen, and use only distilled water or water of equivalent purity.

9.7.1.1 Hydrogen peroxide solution, 30 % (by volume).

9.7.1.2 Mercuric oxide (HgO).

9.7.1.3 Potassium sulfate or anhydrous sodium sulfate.

9.7.1.4 Sulfuric acid, concentrated (d at 25 °C/25 °C is 1,84).

9.7.1.5 Zinc granules.

9.7.1.6 Boric acid solution, 40 g/ℓ.

9.7.1.7 Sodium hydroxide-sodium thiosulfate solution, 45 %: 450 g of sodium hydroxide and 80 g of sodium thiosulfate ($Na_2S_2O_3.5H_2O$) dissolved in water and diluted to 1 ℓ.

9.7.1.8 Standardized sulfuric acid solution ($c(H_2SO_4)$ = 0,1 mol/ℓ).

9.7.1.9 Screened methyl red indicator, 0,125 g of methyl red and 0,083 g of methylene blue dissolved in 100 mℓ of ethanol (96 % (by volume)), and filtered.

9.7.2 Procedure

9.7.2.1 Accurately weigh out approximately 2 g of the prepared sample (see 9.1) and transfer this test specimen to a 500 mℓ Kjeldahl digestion flask. Add a few glass beads, 7 g of the potassium sulfate (or anhydrous sodium sulfate), 0,3 g of the mercuric oxide and 15 mℓ of the concentrated sulfuric acid. Slowly and carefully add 5 mℓ of the hydrogen peroxide solution and leave until the reaction has subsided.

9.7.2.2 Heat gently until frothing ceases and then heat strongly until the solution clears. Continue the digestion for at least another 30 min (approximately 1 h is required for complete digestion).

9.7.2.3 Cool and dilute the contents of the flask with approximately 250 mℓ of water. Cool to ambient temperature and run 75 mℓ of the sodium hydroxide-sodium thiosulfate solution down the side of the flask so that it forms a separate layer at the bottom and does not immediately mix with the acid solution. Add a few granules of zinc.

9.7.2.4 Connect the flask to a Kjeldahl distillation unit, mix the contents of the flask by gentle swirling, and then distil the ammonia (i.e. approximately 150 mℓ of distillate) into an Erlenmeyer flask that contains 50 mℓ of the boric acid solution and three or four drops of the screened methyl red indicator.

9.7.2.5 Titrate the ammonia in the Erlenmeyer flask with the standardized sulfuric acid solution.

9.7.2.6 Carry out a blank determination under identical conditions, but omit the test specimen.

9.7.3 Calculation

9.7.3.1 The protein nitrogen content, expressed as a percentage by mass of the product, is given by the following formula:

$$\frac{(V - V_1) \times c \times 2 \times 1,4}{m}$$

where

V is the sample titre of sulfuric acid, in millilitres;

V_1 is the blank titre of sulfuric acid, in millilitres;

c is the concentration of the standardized sulfuric acid, in moles per litre; and

m is the mass of the test specimen, in grams.

9.7.3.2 Calculate the fish content, expressed as a percentage by mass of the product, by multiplying the protein nitrogen content by 37,5.

NOTE – Where the product contains proteinaceous matter other than fish, apply appropriate corrections when assessing the fish content.

9.8 Determination of chloride content (as sodium chloride)

9.8.1 Reagents

During the analysis, use only reagents of recognized analytical grade and use only distilled water or water of equivalent purity.

9.8.1.1 Nitrobenzene.

9.8.1.2 Nitric acid, diluted to one-half of the concentrated strength.

9.8.1.3 Sodium carbonate solution, saturated.

9.8.1.4 Standard potassium thiocyanate solution (c(KCNS) = 0,1 mol/ℓ).

9.8.1.5 Standard silver nitrate solution ($c(AgNO_3)$ = 0,1 mol/ℓ), accurately standardized.

9.8.1.6 Ferric alum indicator, a cold saturated solution of ferric ammonium sulfate ($NH_4Fe(SO_4)_2.12H_2O$) to which a few drops of the dilute nitric acid (see 9.8.1.2) have been added.

9.8.2 Procedure

9.8.2.1 Take a sample of fish as described in 9.1 and prepare the sample in accordance with 9.1.

9.8.2.2 Accurately weigh a suitable quantity of the prepared sample into an evaporating basin or crucible, moisten this test specimen with the sodium carbonate solution, and dry on a water-bath. Char the dried specimen and ash it at a temperature not higher than 500 °C.

9.8.2.3 Extract the residue with the dilute nitric acid and filter into a 100 mℓ volumetric flask. Repeat the extraction and filtration once, wash the filter thoroughly with the dilute nitric acid, dilute the solution in the flask to volume with the dilute nitric acid, and mix.

9.8.2.4 To a suitable aliquot in a 250 mℓ Erlenmeyer flask, add 25 mℓ of the standard silver nitrate solution, 5 mℓ of the nitrobenzene and 1 mℓ of the ferric alum indicator, and shake well. Titrate the excess silver nitrate with the standard potassium thiocyanate solution until a permanent reddish colour persists for 15 s. Carry out a blank determination under identical conditions, but omit the test specimen. The difference between titrations of the blank determination and of the test is the volume (V) of silver nitrate used in the determination.

9.8.3 Calculation

Determine the chloride content (as sodium chloride), expressed as a percentage by mass of the product, using the following formula:

$$\frac{V \times c \times 5{,}845}{m}$$

where

V is the volume of standard silver nitrate solution used in the determination, in millilitres;

c is the concentration of the standard silver nitrate solution, in moles per litre; and

m is the mass of the original test specimen represented by the aliquot used in the titration, in grams.

10 Methods of microbiological examination

10.1 General

Use aseptic techniques throughout the examination.

10.2 Laboratory glassware

10.2.1 General

Ensure that all glassware used is resistant to repeated heat sterilization and that the glass is free from inhibitory substances such as heavy metals and free alkalis. Borosilicate glass with an expansion coefficient of less than $6 \times 10^{-6}\ k^{-1}$ is recommended.

10.2.2 Bottles (universal)

Bottles that have standard plastics or metal screw caps, and that have a nominal capacity of

a) 30 mℓ,

b) 100 mℓ,

c) 250 mℓ,

d) 500 mℓ, and

e) 1 000 mℓ.

10.2.3 Culture tubes

Rimless cylindrical tubes that have hemispherical ends and a nominal wall thickness of 1,5 mm, and of diameter and length

a) 16 mm x 160 mm, and

b) 20 mm x 200 mm.

Plug these tubes with cotton wool plugs or with plugs of a foam rubber suitable for autoclaving. Alternatively, use screw-capped tubes of similar dimensions.

10.2.4 Graduated pipettes

Total delivery pipettes for bacteriological purposes only, that have an outflow opening of diameter 2 mm

to 3 mm, are graduated in units of 0,1 mℓ, and are of sizes to deliver 1,0 mℓ, 5,0 mℓ and 10,0 mℓ.

10.2.5 Petri dishes

Petri dishes made of glass or of wettable polystyrene, and of diameter and height

a) 90 mm x 15 mm,

b) 100 mm x 20 mm, and

c) 150 mm x 20 mm.

10.2.6 Volumetric cylinders

Graduated measuring cylinders, with or without stoppers, and of capacities 5 mℓ, 10 mℓ, 100 mℓ and 1 000 mℓ.

10.2.7 Sample bottles

Bottles that have mouths of diameter 40 mm to 60 mm, with interchangeable ground-glass or plastics stoppers or lined metal closures, and of capacity 250 mℓ to 300 mℓ, of diameter 70 mm to 80 mm and of height 120 mm to 150 mm.

10.2.8 Culture flasks

Flasks or bottles of capacity 200 mℓ, that have standard lined metal or plastics closures similar to those described in 10.2.2 and 10.2.7 but with holes of diameter 12 mm to 15 mm, drilled through the closures, that can be plugged with cotton wool or other bacteria-trapping filters.

10.2.9 Reagent bottles

Bottles of capacity 50 mℓ and 100 mℓ and that have polypropylene or other plastics stoppers of such design that they can be used to deliver drops of the reagent.

10.2.10 Small test tubes

Rimless cylindrical tubes that have hemispherical ends, a nominal wall thickness of 0,5 mm, a diameter of 6 mm to 7 mm, a length of 100 mm and a capacity of 2,5 mℓ to 3 mℓ. These tubes can also be used as long Durham tubes.

10.2.11 Durham tubes

Tubes as described in 10.2.10 but of length 35 mm to 45 mm and of capacity 0,9 mℓ to 1,3 mℓ.

10.3 Equipment

10.3.1 Autoclave

A pressure vessel that is capable of producing steam (or that is connected to a central steam source) and is capable of withstanding a pressure of 300 kPa and of attaining a temperature of 121 °C ± 2 °C within 10 min of the beginning of the sterilization cycle.

10.3.2 Incubators and water-baths

Incubators and water-baths that have thermostatically controlled heating and cooling devices, and that are so fitted with means of circulation that the temperature of the total enclosed space is maintained to within 2 °C of the thermostat setting.

10.3.3 Hot air oven (for sterilization by means of dry heat)

A thermostatically controlled oven, heated by electricity or gas and so fitted with means of circulation that the temperature of the total enclosed space is maintained at 170 °C ± 5 °C, the heat supply being such that the working temperature is regained within 10 min of the momentary opening and closing of the oven door.

10.3.4 Homogenizer

A mechanical mixing apparatus of either a rotating or a pulsating type, and that has sterilizable containers in which a homogeneous dispersion of the sample and the prescribed diluent can be produced. The sterilizable containers may be of glass, metal or a suitable plastics material. The homogenizing procedure shall not reduce the number or viability of the micro-organisms in the sample.

10.3.5 Glass spreaders

Glass spreaders ("hockey sticks") made from glass rods of diameter 3,5 mm and of length 200 mm, by bending each rod at right angles approximately 30 mm from one end. Smooth the cut ends by heating in a flame.

10.3.6 Membrane filters

10.3.6.1 Types

Membrane filters (preferably marked with a grid) that have been proved to provide full bacterial retention and satisfactory speed of filtration, to be stable in use, and to be free from chemicals that retard the growth and development of bacteria. Use membrane filters that have a maximum pore size not exceeding 0,45 µm.

10.3.6.2 Sterilization

Individually packed membrane filters should have been presterilized by reliable commercial manufacturers.

If membrane filters are to be sterilized, moisten them with sterile distilled water (to prevent curling), interleave with filter paper, and pack them tightly into Petri dishes that are kept closed. Sterilize by immersing in boiling water for 1 h or by autoclaving at 115 °C for 15 min.

10.3.7 Membrane filter holders

10.3.7.1 Type

A membrane filter holder constructed of non-corrosive, bacteriologically inert material that permits all fluid being filtered to pass through the membrane.

10.3.7.2 Sterilization

Assemble the filter holder loosely, ensuring that the porous plate is seated flush with the top surface of its receptacle. Wrap the assembled filter holder in brown paper or other suitable material and sterilize by autoclaving at 121 °C ± 2 °C for 20 min or by any other suitable method.

10.3.8 Forceps

10.3.8.1 Type

Round-tipped forceps that have smooth inner surfaces to their jaws.

10.3.8.2 Sterilization

Sterilize by dipping in methylated spirits or technical methanol and then igniting the adherent liquid. Alternatively, use any other suitable method.

10.4 Media and reagents

10.4.1 General

10.4.1.1 Water

Use only glass-distilled water, or demineralized water of equivalent purity, that is clear, colourless and free from visible suspended matter, and of which the pH value, measured at 25 °C, is in the range 5,0 to 7,5.

10.4.1.2 Quality of ingredients

In the preparation of the media and reagents, use only ingredients of quality acceptable for microbiological purposes. Use anhydrous salts unless otherwise specified.

10.4.1.3 Accuracy

Except where otherwise specified, allow the following tolerances:

a) on temperatures ..... ± 2 °C

b) on masses ..... ± 1,0 %

c) on volumes ..... ± 1,0 %

d) on pH value ..... ± 0,1 pH unit.

10.4.1.4 Dehydrated media

Many of the media required are obtainable in dehydrated form and, for uniformity of results, the use of such media is recommended. If such media are used, follow the manufacturer's instructions strictly regarding reconstitution and sterilization.

10.4.1.5 Adjustment of pH value

Where the final pH value of a medium or reagent is specified, so adjust the pH value that it is correct at 25 °C. If necessary, adjust the pH value during preparation and, in the case of media, before sterilization. Unless otherwise specified, use a solution of hydrochloric acid (c(HCl) = 1 mol/ℓ) or of sodium hydroxide (c(NaOH) = 1 mol/ℓ), as appropriate, to adjust the pH values.

10.4.1.6 Dispensing

Where specified quantities of media are to be dispensed into bottles, use 30 mℓ universal bottles (see 10.2.2(a)) or 16 mm diameter culture tubes (see 10.2.3(a)). Where bulk sterilizing is required, use any suitable glass container of the required quality (see 10.2.1). Dispense reagents into reagent bottles (see 10.2.9). Stir media constantly while dispensing. Whenever the preparation of slopes for surface cultivation is required, dispense the medium in 10 mℓ volumes and sterilize as specified. Immediately after

sterilization, and while the medium is still melted, place the bottles or, when relevant, the culture tubes, on a 1-in-4 sloped surface and allow the medium to solidify.

10.4.1.7 Sterilization

When sterilization by autoclaving is specified, and unless otherwise directed, autoclave the medium at 121 °C ± 2 °C for 15 min. (This temperature corresponds to a pressure of 103 kPa above atmospheric pressure at sea level, i.e. 207 kPa absolute.)

10.4.1.8 Control of prepared media

Ensure, by suitable incubation tests, that prepared media are sterile and are capable of supporting the growth of the relevant organisms under the stated conditions of incubation.

10.4.1.9 Storage of media

Ensure that prepared media are carefully protected from exposure to heat and sunlight and have not evaporated or changed in concentration or in pH value, and that, unless otherwise specified, they are used within three months of preparation.

10.4.2 Buffered isotonic peptone water (diluent)

10.4.2.1 Ingredients

Peptone	10 g
Sodium chloride	5 g
Sodium phosphate dibasic dodecahydrate	3,5 g
Potassium phosphate monobasic	1,5 g
Water	1 000 mℓ

10.4.2.2 Preparation

Dissolve the ingredients in water and, adjust the pH value to 7,0. Dispense as follows:

a) 9 mℓ volumes into 30 mℓ bottles (see 10.2.2(a));

b) 99 mℓ volumes into 250 mℓ bottles (see 10.2.2(c)); and

c) larger volumes into bulk containers.

Sterilize by autoclaving.

10.4.3 Plate-count agar

10.4.3.1 Ingredients

Agar	15 g
Tryptone	5 g
Yeast extract	2,5 g
Glucose	1 g
Water	1 000 mℓ

10.4.3.2 Preparation

Dissolve the ingredients in the water by boiling. Cool to 50 °C and, adjust the pH value to 7,2. Dispense 15 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)), and sterilize by autoclaving.

10.4.4 Violet red bile agar (VRB glucose agar)

10.4.4.1 Ingredients

Agar	12 g
Glucose	10 g
Peptone	7 g
Sodium chloride	5 g
Yeast extract	3 g
Bile salts	1,5 g
Neutral red	0,03 g
Crystal violet	0,002 g
Water	1 000 mℓ

10.4.4.2 Preparation

Dissolve the ingredients in the water by boiling. Cool to 50 °C and adjust the pH value to 7,4. Aseptically dispense as follows:

a) 15 mℓ volumes into sterile Petri dishes (see 10.2.5(a) or (b)); and

b) 500 mℓ into a 1 000 mℓ sterile bottle (see 10.2.2(e)).

Place the bottles in a water-bath maintained at 45 °C, and allow the plates to solidify. Use the medium within 4 h of preparation.

10.4.5 Brilliant green bile medium (single strength)

10.4.5.1 Ingredients

Desiccated ox bile	20 g
Lactose	10 g
Peptone	10 g
Brilliant green	0,013 g
Water	1 000 mℓ

10.4.5.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,4. Dispense 10 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)), each containing an inverted Durham tube (see 10.2.11), and sterilize by autoclaving.

10.4.6 Brilliant green bile medium (double strength)

Prepare this medium as described in 10.4.5, but use double the quantities of ingredients. Dispense 100 mℓ volumes into 250 mℓ bottles (see 10.2.2(c)), each containing an inverted small test tube that is used as a long Durham tube (see 10.2.10), and sterilize by autoclaving.

10.4.7 Tryptone water

10.4.7.1 Ingredients

Tryptone ..... 10 g
Sodium chloride ..... 5 g
Water ..... 1 000 mℓ

10.4.7.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,5. Dispense 9 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)), and sterilize by autoclaving.

10.4.8 Kovacs reagent

10.4.8.1 Ingredients

p-methylaminobenzaldehyde dibasic ..... 5 g
Amyl alcohol (pyridine-free) ..... 75 mℓ
Hydrochloric acid, concentrated ..... 25 mℓ

10.4.8.2 Preparation

Dissolve the *p*-methylaminobenzaldehyde in the amyl alcohol, aiding solution by warming in a water-bath at 50 °C to 55 °C. Cool and add the acid. Protect from light and store at 4 °C. Ensure that the reagent is light yellow. (Certain brands of amyl alcohol cause the reagent to be a very dark colour and to be unsatisfactory.) Store in 100 mℓ reagent bottles (see 10.2.9). Allow to stand for 24 h before use.

10.4.9 Baird-Parker agar

10.4.9.1 Basal medium ingredients

Agar ..... 20 g
Glycine ..... 12 g
Tryptone ..... 10 g
Meat extract ..... 5 g
Lithium chloride ..... 5 g
Yeast extract ..... 1 g
Water ..... 1 000 mℓ

10.4.9.2 Preparation

Dissolve the ingredients in the water by boiling. Cool to 50 °C and adjust the pH value to 7,2. Dispense 90 mℓ volumes into 250 mℓ bottles (see 10.2.2(c)) and sterilize by autoclaving. Store at 4 °C for not longer than one month.

Before pouring plates, aseptically add 1 mℓ of tellurite solution (see 10.4.10) and 5 mℓ of egg yolk emulsion (see 10.4.11) to every 90 mℓ of the basal medium, that has been melted and then cooled to between 45 °C and 50 °C. Mix well and aseptically dispense 15 mℓ volumes into sterile Petri dishes (see 10.2.5(a) or (b)). Allow to solidify. Use the plates within 24 h of preparation. Dry the surface of the medium at 45 °C for at least 1 h before use and, just before use, spread 0,5 mℓ of sodium pyruvate solution (see 10.4.13) over the surface.

10.4.10 Tellurite solution

10.4.10.1 Ingredients

Potassium tellurite ..... 1 g
Water ..... 100 mℓ

10.4.10.2 Preparation

Dissolve the potassium tellurite in the water with minimal heating. Sterilize by filtration. Store in a 100 mℓ reagent bottle (see 10.2.9) at 4 °C for not longer than one month.

10.4.11 Egg yolk emulsion (approximately 20 % (by volume))

Wash and then disinfect the shells of unbroken fresh hen's eggs. Break the shells, aseptically separate the yolks from the whites and collect the yolks in a sterile beaker. Add water in the ratio of four volumes of water to one volume of egg yolk, mix thoroughly and heat in a water-bath at 45 °C for 2 h. Remove the precipitate by centrifuging, or by allowing the mixture to stand overnight in a refrigerator and decanting the supernatant fluid. Sterilize the supernatant fluid by filtration. Dispense 5 mℓ volumes into 250 mℓ sterile bottles (see 10.2.2(c)) and store at 4 °C for not longer than one month.

10.4.12 Mannitol salt phenol red agar

10.4.12.1 Ingredients

Sodium chloride ..... 75 g
Agar ..... 15 g
Mannitol ..... 10 g
Peptone from meat ..... 10 g
Meat extract ..... 1 g
Phenol red ..... 0,025 g
Water ..... 1 000 mℓ

10.4.12.2 Preparation

Dissolve the ingredients in the water by boiling and adjust the pH value to 7,4. Sterilize by autoclaving and aseptically dispense 15 mℓ volumes into sterile Petri dishes (see 10.2.5(a) or (b)). Allow to solidify. Use the plates on the day of preparation.

10.4.13 Sodium pyruvate solution

Prepare an aqueous solution that contains 200 g of sodium pyruvate per litre and sterilize by filtration. Preferably use only a freshly prepared solution. Alternatively, store the solution at 4 °C for not more than 3 d.

10.4.14 DNAse test agar

10.4.14.1 Ingredients

Tryptose ..... 20 g
Agar ..... 12 g
Sodium chloride ..... 5 g
Deoxyribonucleic acid ..... 2 g
Water ..... 1 000 mℓ

10.4.14.2 Preparation

Dissolve the ingredients in the water by boiling, and adjust the pH value to 7,4. Sterilize by autoclaving and aseptically dispense 15 mℓ volumes into sterile Petri dishes (see 10.2.5(a) or (b)). Allow to solidify. Use the plates on the day of preparation.

10.4.15 Brilliant green solution

10.4.15.1 Ingredients

Brilliant green ..... 0,5 g
Sterile water ..... 100 mℓ

10.4.15.2 Preparation

Dissolve the brilliant green in the water in a sterile flask. **Do not heat.** Store the solution in the dark for at least 1 d to effect autosterilization.

10.4.16 Rappaport-Vassiladis magnesium-chloride malachite-green medium (R-V medium)

10.4.16.1 Solution A

10.4.16.1.1 Ingredients

Tryptone ..... 5,0 g
Sodium chloride ..... 8,0 g
Potassium dihydrogen phosphate (KH_2PO_4) ..... 1,6 g
Water ..... 1 000 mℓ

10.4.16.1.2 Preparation

Dissolve the ingredients in the water by heating to about 70 °C. Prepare solution A on the day of preparation of the complete R-V medium (see 10.4.16.4).

10.4.16.2 Solution B

10.4.16.2.1 Ingredients

Magnesium chloride hexahydrate ($MgCl_2.6H_2O$) ..... 400,0 g
Water ..... 1 000 mℓ

10.4.16.2.2 Preparation

Dissolve the magnesium in the water. As this salt is very hygroscopic, it is advisable to dissolve the entire contents of a container of $MgCl_2.6H_2O$ rather than use part of the contents. For instance, 250 g of $MgCl_2.6H_2O$ added to 625 mℓ of water gives a solution of total volume 795 mℓ and a concentration of about 31,5 g per cent of $MgCl_2.6H_2O$. The solution may be kept in a brown glass bottle at room temperature.

10.4.16.3 Solution C

10.4.16.3.1 Ingredients

Malachite green oxalate ..... 0,4 g
Water ..... 100 mℓ

10.4.16.3.2 Preparation

Dissolve the malachite green oxalate in the water.

The solution may be kept in a brown glass bottle at room temperature.

10.4.16.4 Complete R-V medium

10.4.16.4.1 Ingredients

Solution A (see 10.4.16.1) ..... 1 000 mℓ
Solution B (see 10.4.16.2) ..... 100 mℓ
Solution C (see 10.4.16.3) ..... 10 mℓ

10.4.16.4.2 Preparation

To 1 000 mℓ of solution A, add 100 mℓ of solution B and 10 mℓ of solution C. So adjust the pH value, if necessary, that, after sterilization, it is 5,2. Distribute before use into test tubes in 10 mℓ quantities. Autoclave at 115 °C for 15 min. Store the prepared medium in a refrigerator.

10.4.17 Selenite medium (Stokes and Osborne)

10.4.17.1 Ingredients

Mannitol ..... 5 g
Peptone ..... 5 g
Yeast extract ..... 5 g
Sodium hydrogen selenite ..... 4 g
Potassium phosphate dibasic ..... 2,62 g
Potassium phosphate monobasic ..... 1,36 g
Sodium taurocholate ..... 1 g
Brilliant green solution (see 10.4.15) ..... 1 mℓ

10.4.17.2 Preparation

Dissolve the solid ingredients, except the sodium hydrogen selenite, in approximately 800 mℓ of water by boiling, and sterilize in bulk by autoclaving. Dissolve the sodium hydrogen selenite in approximately 150 mℓ of cold water and sterilize the solution (preferably by filtration, or otherwise by heating in steam at 100 °C for 10 min). Aseptically add this solution and the 1 mℓ of brilliant green solution to the sterilized and cooled bulk of the ingredients. Adjust the pH value to 7,0 and dilute the solution to 1 ℓ with sterile water. Aseptically dispense 100 mℓ volumes into sterile culture flasks (see 10.2.8). Do not heat the medium further. The sediment that forms will settle at the bottom of the flask; resuspend it before the medium is used. Use on the day of preparation.

10.4.18 Brilliant green phenol red agar (Edel and Kampelmacher)

10.4.18.1 Ingredients

Agar ..... 12 g
Peptone ..... 10 g
Lactose ..... 10 g
Sucrose ..... 10 g
Meat extract ..... 4 g
Sodium chloride ..... 3 g
Sodium phosphate dibasic ..... 0,8 g

Sodium phosphate monobasic ..... 0,6 g
Phenol red ..... 0,09 g
Brilliant green solution (see 10.4.15) ..... 1 mℓ

10.4.18.2 Preparation

Dissolve the solid ingredients, except the phenol red, the lactose and the sucrose, in approximately 800 mℓ of water and sterilize in bulk by autoclaving. Cool to 55 °C. Dissolve the phenol red and the sugars in approximately 150 mℓ of water and heat in a water-bath at 70 °C for 20 min. Cool to 55 °C and add this solution, together with the 1 mℓ of brilliant green solution, to the bulk of the ingredients and mix. Adjust the pH value to 7,0 and dilute the solution to 1 ℓ with sterile water. Aseptically dispense 40 mℓ volumes into sterile Petri dishes of diameter preferably 150 mm (see 10.2.5(c)). Although these larger Petri dishes are preferable, if they are not available use smaller Petri dishes (see 10.2.5(a) or (b)) but prepare twice as many of them as when larger Petri dishes are used and use a volume of medium that will give the same depth of medium as in the large Petri dishes. Allow the medium to solidify and dry the surface of the medium at 50 °C for 30 min before use. Use the plates on the day of preparation.

10.4.19 Cytochrome oxidase test strips or cytochrome oxidase reagent

Commercially available.

10.4.20 Triple sugar iron agar

10.4.20.1 Ingredients

Peptone ..... 20 g
Agar ..... 12 g
Lactose ..... 10 g
Sucrose ..... 10 g
Sodium chloride ..... 5 g
Meat extract ..... 3 g
Yeast extract ..... 3 g
Glucose ..... 1 g
Ferric citrate ..... 0,3 g
Sodium thiosulfate pentahydrate ..... 0,3 g
Phenol red ..... 0,024 g
Water ..... 1 000 mℓ

10.4.20.2 Preparation

Dissolve the ingredients in the water by boiling. Cool to 50 °C and adjust the pH value to 7,4. Dispense 10 mℓ volumes into culture tubes (see 10.2.3(a)) and sterilize by autoclaving for 10 min. Allow to solidify in a sloping position that will give a butt of depth approximately 25 mm and a sloped surface of length at least 30 mm long. The medium should have an orange-red colour.

10.4.21 Urea agar (Christensen)

10.4.21.1 Ingredients

Urea (50 mℓ of a 400 g/ℓ solution) ..... 20 g
Agar ..... 15 g
Sodium chloride ..... 5 g
Potassium phosphate dibasic ..... 2 g
Glucose ..... 1 g
Peptone ..... 1 g
Phenol red ..... 0,012 g

10.4.21.2 Preparation

Dissolve the ingredients, except the urea, in water by boiling, and dilute the solution to 900 mℓ. Sterilize this base in bulk by autoclaving and cool to 50 °C. Add 50 mℓ of a filter-sterilized solution that contains 400 g of urea per litre, and mix well. Adjust the pH value to 6,8 and dilute the solution to 1 ℓ with sterile water. Aseptically dispense 10 mℓ volumes into 30 mℓ sterile bottles (see 10.2.2(a)), and allow to solidify in a sloping position that will give a butt of depth approximately 25 mm and a sloped surface of length at least 30 mm.

10.4.22 Lysine decarboxylation medium (Taylor)

10.4.22.1 Ingredients

ℓ-lysine hydrochloride monobasic ..... 5 g
Yeast extract ..... 3 g
Glucose ..... 1 g
Bromocresol purple ..... 0,015 g
Water ..... 1 000 mℓ

10.4.22.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 6,8. Dispense 10 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)) and sterilize by autoclaving.

10.4.23 ß-galactosidase reagent

10.4.23.1 Ingredients

Sodium phosphate monobasic ..... 0,69 g
Ortho-nitrophenyl ß-*d*-galactopyranoside ..... 0,08 g
Sodium hydroxide solution, 0,4 g/ℓ ..... 3 mℓ approx.

10.4.23.2 Procedure

Dissolve the sodium phosphate in 15 mℓ of water. Adjust the pH value to 7,0 with the sodium hydroxide solution. Dissolve the galactopyranoside in this solution, and dilute to 20 mℓ. Store at 4 °C for not longer than one month.

10.4.24 Voges-Proskauer medium

10.4.24.1 Ingredients

Peptone ..... 7 g
Glucose ..... 5 g
Potassium phosphate dibasic ..... 5 g
Water ..... 1 000 mℓ

10.4.24.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 6,9. Dispense 0,2 mℓ volumes into small test tubes (see 10.2.10) and sterilize by autoclaving.

10.4.25 Creatine solution

Prepare an aqueous solution that contains 5 g of creatine hydrate monobasic per litre. Store in reagent bottles (see 10.2.9) at ambient temperature for not longer than one month.

10.4.26 α-naphthol solution

Using 96 % (by mass) to 100 % (by mass) of ethanol as the solvent, prepare an aqueous solution that contains 60 g of α-naphthol per litre. Store in reagent bottles (see 10.2.9) at ambient temperature for not longer than one month.

10.4.27 Potassium hydroxide solution

Prepare an aqueous solution that contains 400 g of potassium hydroxide per litre. Store at ambient temperature in bottles fitted with alkali-resistant plastics stoppers. Do not use glass stoppers. Avoid undue exposure to the atmosphere.

10.4.28 Saline solution

10.4.28.1 Ingredients

Sodium chloride ..... 8,5 g
Water ..... 1 000 mℓ

10.4.28.2 Preparation

Dissolve the sodium chloride in the water. Dispense 9 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)) and sterilize by autoclaving.

10.4.29 Polyvalent anti-*Salmonella* "O" serum

Use commercial anti-sera against the somatic antigens of a sufficiently large number of *Salmonella* serotypes, to make it unlikely that an as-yet-unencountered type would lead to a false negative reaction. In all cases, the groups A to G should be adequately represented. For each anti-serum or mixture of anti-sera, follow the instructions of the anti-serum manufacturer.

10.4.30 Polyvalent anti-*Salmonella* "H" serum

Use commercial anti-sera against the flagellar antigens of a sufficiently large number of *Salmonella* serotypes to detect both specific and non-specific factors, excluding factor "I". For each mixture of anti-sera, follow the instructions of the anti-serum manufacturer.

10.4.31 Polyvalent anti-*Salmonella* "Vi " serum

Use commercial anti-sera. Follow the manufacturer's instructions strictly.

10.4.32 Gram-negative medium

10.4.32.1 Ingredients

Polypeptone ..... 20 g
Sodium chloride ..... 5 g
Sodium citrate ..... 5 g
Potassium phosphate dibasic ..... 4 g
Mannitol ..... 2 g
Potassium phosphate monobasic ..... 1,5 g
Glucose ..... 1 g
Sodium desoxycholate ..... 0,5 g
Water ..... 1 000 mℓ

10.4.32.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,0. Dispense 100 mℓ volumes into culture flasks (see 10.2.8) of capacity at least 200 mℓ and sterilize by autoclaving at 115 °C for 20 min.

10.4.33 XLD medium

10.4.33.1 Ingredients

Yeast extract	3,0 g
ℓ-lysine HCl	5,0 g
Xylose	3,75 g
Lactose	7,5 g
Sucrose	7,5 g
Sodium desoxycholate	1,0 g
Sodium chloride	5,0 g
Sodium thiosulfate	6,8 g
Ferric ammonium citrate	0,8 g
Phenol red	0,08 g
Agar	12,5 g

10.4.33.2 Preparation

Suspend the ingredients in the water. Adjust the pH to 7,4 ± 0,2. Heat with frequent agitation until the medium boils. **Do not overheat.** Transfer immediately to a water-bath at 50 °C. Pour into plates as soon as the medium has cooled.

It is important to avoid preparing large volumes which will cause prolonged heating.

10.4.34 Polyvalent anti-*Shigella* "O" serum

Use commercial polyvalent anti-sera against the somatic antigens, including antibodies, of at least *Shigella* serotypes 1 to 15.

10.4.35 Egg-yolk-free tryptose-sulfite-cycloserine agar (SC agar)

10.4.35.1 Base

10.4.35.1.1 Ingredients

Tryptose[1)]	15,0 g
Soytone[1)]	5,0 g
Yeast extract	5,0 g
Disodium disulfite ($Na_2S_2O_5$), anhydrous	5,0 g
Ammonium iron (III) citrate[2)]	1,0 g
Agar[3)]	12 g to 18 g
Water	1 000 mℓ

1) The names tryptose and soytone are used at present only by certain producers of media. Any other pancreatic casein or soybean digest that gives comparable results may be used.

2) This reagent should contain at least 15 % (by mass) or iron.

3) Depending on the gel strength of the agar.

10.4.35.1.2 Preparation

Dissolve the ingredients in the water by boiling. So adjust the pH value that it will be 7,6 after sterilization. Transfer the base to tubes or flasks or bottles of capacity not more than 500 mℓ. Sterilize for 10 min at 121 °C. Store in a refrigerator at 4 °C ± 2 °C.

Discard unused medium 2 weeks after preparation.

10.4.35.2 D-Cycloserine solution

10.4.35.2.1 Ingredients

D-Cycloserine (use white crystalline powder only) ..... 4,0 g
Water ..... 100 mℓ

10.4.35.2.2 Preparation

Dissolve the D-Cycloserine in the water and sterilize the solution by filtration.

10.4.35.3 Complete SC agar medium

Before plating (see 10.12.1), add 1 mℓ of the sterilized D-Cycloserine solution (see 10.4.35.2) to each 100 mℓ of sterile melted base (see 10.4.35.1) at 50 °C.

10.4.36 Motility-nitrate medium

10.4.36.1 Ingredients

Peptone ..... 5,0 g
Meat extract ..... 3,0 g
Galactose ..... 5,0 g
Glycerol ..... 5,0 g
Potassium nitrate (KNO_3) ..... 1,0 g
Disodium hydrogenorthophosphate (Na_2HPO_4) ..... 2,5 g
Agar[4] ..... 1 g to 5 g
Water ..... 1 000 mℓ

10.4.36.2 Procedure

Dissolve the ingredients in the water by boiling. So adjust the pH value that it will be 7,3 after sterilization. Transfer the medium to culture tubes in 10 mℓ quantities and sterilize at 121 °C for 15 min. If not used the same day, store the medium in a refrigerator at 4 °C ± 2 °C.

Just prior to use, heat in boiling water or flowing steam for 15 min and then cool rapidly to the incubation temperature.

Discard unused medium 4 weeks after preparation.

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4) Depending on the gel strength of the agar.

10.4.37 Lactose-gelatine medium

10.4.37.1 Ingredients

Tryptose[5]	15,0 g
Yeast extract	10,0 g
Lactose	10,0 g
Gelatine	120,0 g
Phenol red	0,05 g
Water	1 000 mℓ

10.4.37.2 Preparation

Dissolve the ingredients, except the lactose and phenol red, in the water. So adjust the pH value that it will be 7,5 after sterilization. Add the lactose and phenol red, dispense 10 mℓ quantities into test tubes and sterilize at 121 °C for 15 min.

If not used the same day, store the medium in a refrigerator at 4 °C ± 2 °C.

Just prior to use, heat in boiling water or flowing steam for 15 min and then cool rapidly to the incubation temperature.

Discard unused medium 3 weeks after preparation.

10.4.38 *Vibrio* enrichment medium (double strength)

10.4.38.1 Ingredients

Sodium chloride	40 g
Tryptone	20 g
Sodium taurocholate	10 g
Sodium carbonate	2 g
Gelatine	2 g
Potassium tellurite solution, 1 g/ℓ, filter-sterilized	20 mℓ

10.4.38.2 Preparation

Dissolve all the ingredients, except the potassium tellurite solution, in approximately 900 mℓ of water by boiling. Adjust the pH value to 8,7 and sterilize the resulting basal medium by autoclaving. After cooling to below 45 °C, aseptically add the potassium tellurite solution. Re-adjust the pH value to 8,7 and dilute the solution to 1 ℓ with sterile water. Aseptically dispense 100 mℓ volumes into 250 mℓ sterile bottles (see 10.2.2(c)). Use the medium within 2 h of adding the potassium tellurite solution.

The basal medium, i.e. the medium without the potassium tellurite solution, is stable at 4 °C for up to 3 d only.

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5) The name tryptose is used at present only by certain producers of media. Any other pancreatic casein digest that gives comparable results may be used.

10.4.39 *Vibrio* diagnostic agar

10.4.39.1 Ingredients

Sucrose	20 g
Agar	15 g
Sodium chloride	10 g
Sodium citrate	10 g
Sodium thiosulfate pentahydrate	10 g
Special peptone	10 g
Desiccated ox bile	5 g
Yeast extract	5 g
Sodium taurocholate	3 g
Ferric citrate	1 g
Bromothymol blue	0,04 g
Thymol blue	0,04 g
Water	1 000 mℓ

10.4.39.2 Preparation

Dissolve the ingredients in water by boiling. Do not overheat. **Do not autoclave.** Cool to 50 °C, adjust the pH value to 8,6 and dilute the solution to 1 ℓ with sterile water. Aseptically dispense 15 mℓ volumes into sterile Petri dishes (see 10.2.5(a) or (b)). Allow to solidify. Use the plates within 3 h of preparation.

10.4.40 Lysine-indole-motility-hydrogen-sulfide agar (containing 30 g/ℓ of sodium chloride)

10.4.40.1 Ingredients

Sodium chloride	30 g
Tryptone	15 g
ℓ-lysine hydrochloride monobasic	5 g
Meat peptone	5 g
Meat extract	3 g
Yeast extract	3 g
Agar	2 g
Glucose	1 g
Ferric ammonium citrate	0,5 g
Sodium thiosulfate pentahydrate	0,3 g
Bromocresol purple	0,016 g
Water	1 000 mℓ

10.4.40.2 Preparation

Dissolve the ingredients in the water by boiling. Cool, and adjust the pH value to 7,4. Dispense 5 mℓ volumes into culture tubes (see 10.2.3(a)). Sterilize by autoclaving and stopper the tubes tightly to prevent loss of moisture.

10.4.41 Inactivator solution

10.4.41.1 Ingredients

Polyoxyethylene sorbitan mono-oleate	2 g
Sodium taurocholate	1 g
Gelatine	1 g
Sodium thiosulfate pentahydrate	0,3 g
Potassium phosphate monobasic	0,1 g
Sodium citrate	0,1 g
Water	1 000 mℓ

10.4.41.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,2. Dispense 9 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)). Sterilize by autoclaving.

10.4.42 Nitrite-detection reagent

10.4.42.1 5-amino-2-napthalenesulfonic acid (5-2 ANSA) solution

Dissolve 0,1 g of 5-2 ANSA in 100 mℓ of 15 % (by volume) acetic acid solution. Filter through filter paper. Store in a well-stoppered brown bottle (preferably with a bulb-type dropper) at 4 °C.

10.4.42.2 Sulfanilic acid solution

Dissolve 0,4 g of sulfanilic acid in 100 mℓ of 15 % (by volume) acetic acid solution. Filter through filter paper. Store in a well-stoppered brown bottle (preferably with a bulb-type dropper) at 4 °C.

10.4.42.3 Preparation of complete reagent

Mix equal volumes of the two solutions (see 10.4.42.1 and 10.4.42.2) just before use.

Discard unused reagent immediately.

10.4.43 m-Endo agar LES

10.4.43.1 Ingredients

Agar	15,0 g
Lactose	9,4 g
Tryptose	7,5 g
Tryptone	3,7 g
Sodium chloride	3,7 g
Thiopeptone	3,7 g
Dipotassium phosphate	3,3 g
Sodium sulfite	1,6 g
Yeast extract	1,2 g
Monopotassium phosphate	1,0 g
Basic fuchsin	0,8 g
Sodium desoxycholate	0,1 g
Sodium lauryl sulfite	0,05 g
Water	1 000 mℓ

10.4.43.2 Preparation

Suspend the ingredients in the water. Add 20 mℓ of ethanol (95 % (by volume)) and allow to stand for 10 min. Dissolve the ingredients completely by heating to boiling. Cool to between 45 °C and 50 °C and dispense into Petri dishes, ensuring that the depth of the medium in each plate is at least 3 mm.

10.4.44 mFC agar

10.4.44.1 Ingredients

Agar	13 g
Lactose	12,5 g
Tryptose	10 g
Proteose peptone No. 3	5 g
Sodium chloride	5 g
Yeast extract	3 g
Bile salts No. 3	1,5 g
Aniline blue (water blue)	0,1 g
Water	1 000 mℓ

10.4.44.2 Preparation

Suspend the ingredients in the water and dissolve completely by heating to boiling. Cool to between 45 °C and 50 °C and dispense into Petri dishes, ensuring that the depth of the agar in each plate is at least 3 mm.

10.4.45 Lauryl tryptose broth (single strength)

10.4.45.1 Ingredients

Tryptose	20 g
Lactose	5 g
Sodium chloride	5 g
Dipotassium phosphate	2,75 g
Monopotassium phosphate	2,75 g
Sodium lauryl sulfate, specially pure	0,1 g
Water	1 000 mℓ

10.4.45.2 Preparation

Dissolve the tryptose, lactose, sodium chloride and phosphates in the water by warming. Add the sodium lauryl sulfate and mix gently to avoid froth formation. Adjust the pH value to 6,8 and dispense 10 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)), each containing an inverted Durham tube (see 10.2.11). Sterilize by autoclaving.

10.4.46 Lauryl tryptose broth (double strength)

Prepare this medium as described in 10.4.45, but use double the quantities of ingredients. Dispense 10 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)), each containing an inverted Durham tube (see 10.2.11). Sterilize by autoclaving.

10.4.47 Peptone water

10.4.47.1 Ingredients

Peptone ..... 10 g
Sodium chloride ..... 5 g
Water ..... 1 000 mℓ

10.4.47.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,5. Dispense 9 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)), and sterilize by autoclaving.

10.4.48 Lactose peptone water

10.4.48.1 Ingredients

Peptone ..... 10 g
Lactose ..... 10 g
Sodium chloride ..... 5 g
Phenol red solution (0,4 g of phenol red per
100 mℓ of water) ..... 2,5 mℓ
Water ..... 100 mℓ

10.4.48.2 Preparation

Dissolve the ingredients in the water, adjust the pH value to 7,5 and add the phenol red solution. Dispense 10 mℓ volumes into 30 mℓ bottles (see 10.2.2(a)) or into culture tubes (see 10.2.3(a)), each containing an inverted Durham tube (see 10.2.11). Sterilize by autoclaving. Alternatively, steam for 20 min on each of three successive days. Test for sterility by incubation at 37 °C for 24 h.

10.4.49 Selective pre-enrichment medium: ½ Fraser broth

10.4.49.1 Base

10.4.49.1.1 Ingredients

Meat peptone (peptic digest of animal tissue) ..... 5,0 g
Tryptone peptic digest of casein ..... 5,0 g
Meat extract ..... 5,0 g
Yeast extract ..... 5,0 g
Sodium chloride ..... 20,0 g
Di-sodium hydrogen phosphate ($2H_2O$) ..... 12,0 g
Potassium di-hydrogen phosphate ..... 1,35 g
Aesculin ..... 1,0 g
Lithium chloride ..... 3,0 g
Sodium salt of nalidixic acid ..... 0,01 g
Water ..... 1 000 mℓ

10.4.49.1.2 Preparation

Dissolve the dehydrated base components or the complete dehydrated base in the water by heating to about 70 °C. Distribute the basal medium in flasks of suitable capacity to obtain the portions necessary for the test. Sterilize at 121 °C for 15 min.

10.4.49.2 Acriflavine solution

10.4.49.2.1 Ingredients

Acriflavine ..... 0,125 g
Water ..... 100,0 mℓ

10.4.49.2.2 Preparation

Dissolve the acriflavine in the water. Sterilize by filtration.

10.4.49.3 Ferric ammonium citrate solution

10.4.49.3.1 Ingredients

Iron (III) ammonium citrate ..... 5,0 g
Water ..... 100,0 mℓ

10.4.49.3.2 Preparation

Dissolve the iron (III) ammonium citrate in the water. Sterilize by filtration.

10.4.49.4 Complete ½ Fraser broth

Immediately before use, add 1,0 mℓ portions of acriflavine solution (see 10.4.49.2) and ferric ammonium citrate solution (see 10.4.49.3) to each 100 mℓ of basal medium (see 10.4.49.1). Mix gently. So adjust the pH value of the complete medium that it is 7,2 ± 0,2.

10.4.50 Selective enrichment medium: Fraser broth

10.4.50.1 Base

10.4.50.1.1 Ingredients

Meat peptone (peptic digest of animal tissue) ..... 5,0 g
Tryptone peptic digest of casein ..... 5,0 g
Meat extract ..... 5,0 g
Yeast extract ..... 5,0 g
Sodium chloride ..... 20,0 g
Di-sodium hydrogen phosphate ($2H_2O$) ..... 12,0 g
Potassium di-hydrogen phosphate ..... 1,35 g
Aesculin ..... 1,0 g
Lithium chloride ..... 3,0 g
Sodium salt of nalidixic acid ..... 0,02 g
Water ..... 1 000 mℓ

10.4.50.1.2 Preparation

Dissolve the dehydrated base components or the complete dehydrated base in the water by heating to about 70 °C. Distribute the basal medium in 10 mℓ volumes in 30 mℓ bottles (see 10.2.2(a)). Sterilize for 15 min at 121 °C.

10.4.50.2 Acriflavine solution

10.4.50.2.1 Ingredients

Acriflavine ..... 0,25 g
Water ..... 100,0 mℓ

10.4.50.2.2 Preparation

Dissolve the acriflavine in the water. Sterilize by filtration.

10.4.50.3 Ferric ammonium citrate solution

10.4.50.3.1 Ingredients

Iron (III) ammonium citrate ..... 5,0 g
Water ..... 100,0 mℓ

10.4.50.3.2 Preparation

Dissolve the iron (III) ammonium citrate in the water. Sterilize by filtration.

10.4.50.4 Complete Fraser broth

Immediately before use, to each tube (10 mℓ volumes) of base (10.4.50.1) add 0,1 mℓ portions of acriflavine solution (see 10.4.50.2) and ferric ammonium solution (see 10.4.50.3). Mix gently. So adjust the pH value of the complete medium that it is 7,2 ± 0,2.

10.4.51 First selective plating-out medium: Oxford agar

10.4.51.1 Agar base

10.4.51.1.1 Ingredients

Columbia agar base ..... 39,0 g
Aesculin ..... 1,0 g
Iron (III) ammonium citrate ..... 0,5 g
Lithium chloride ..... 15,0 g
Water ..... 1 000 mℓ

10.4.51.1.2 Preparation

Dissolve the solid ingredients in the water by boiling. Sterilize at 121 °C for 15 min.

10.4.51.2 Supplement for 500 mℓ medium

10.4.51.2.1 Ingredients

Cycloheximide ..... 200,0 mg
Colistin sulfate ..... 10,0 mg
Acriflavine ..... 2,5 mg
Cefotetan ..... 1,0 mg
Fosfomycin ..... 5,0 mg
Ethanol ..... 2,5 mℓ
Water ..... 2,5 mℓ

10.4.51.2.2 Preparation

Dissolve the solid ingredients in the ethanol/water mixture and sterilize by filtration.

10.4.51.3 Preparation of complete Oxford agar medium

Take 500 mℓ of the agar base (see 10.4.51.1). Sterilize in the autoclave set at 121 °C for 15 min. Cool to 50 °C and aseptically add the supplement (see 10.4.51.2). So adjust the pH value of the final medium that it is 7,0 at 25 °C.

10.4.52 Second selective plating-out medium: PALCAM agar

10.4.52.1 Agar base

10.4.52.1.1 Ingredients

Pancreatic peptone of casein[6]	23,0 g
Starch	1,0 g
Sodium chloride	5,0 g
Agar[7]	9,0 g to 18 g
Yeast extract	3,0 g
D-glucose	0,5 g
D-mannitol	10,0 g
Aesculin	0,8 g
Iron (III) ammonium citrate	0,5 g
Phenol red	0,08 g
Lithium chloride	15,0 g
Water	1 000 mℓ

10.4.52.1.2 Preparation

Dissolve the dehydrated base components or the complete dehydrated base in 960 mℓ of the water by boiling. Adjust the pH value to 7,2 ± 0,1. Sterilize by autoclaving at 121 °C for 15 min. Cool to 50 °C.

10.4.52.2 Polymyxin B sulfate solution

10.4.52.2.1 Ingredients

Polymyxin B sulfate (100 000 iu)	0,1 g
Water	100,0 mℓ

10.4.52.2.2 Preparation

Dissolve the Polymyxin B sulfate in the water. Sterilize by filtration.

10.4.52.3 Acriflavine hydrochloride solution

10.4.52.3.1 Ingredients

Acriflavine	0,05 g
Ethanol	50,0 mℓ
Water	50,0 mℓ

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6) Or other peptones of equivalent quality.

7) Depending on the gel strength of the agar.

10.4.52.3.2 Preparation

Mix the ethanol with the water and dissolve the acriflavine in the ethanol/water mixture. Sterilize by filtration.

10.4.52.4 Sodium ceftazidime pentahydrate solution

10.4.52.4.1 Ingredients

Sodium ceftazidime pentahydrate ..... 0,116 g
Water ..... 100 mℓ

10.4.52.4.2 Preparation

Dissolve the sodium ceftazidime pentahydrate in the water. Sterilize by filtration.

10.4.52.5 Complete PALCAM medium

10.4.52.5.1 Ingredients

Basal medium (see 10.4.52.1) ..... 960,0 mℓ
Polymyxin B sulfate solution (see 10.4.52.2) ..... 10 mℓ
Acriflavine hydrochloride solution (see 10.4.52.3) ..... 10,0 mℓ
Sodium ceftazidime pentahydrate solution
(see 10.4.52.4) ..... 20 mℓ

10.4.52.5.2 Preparation

To the molten basal medium at 47 °C, make the following additions, mixing gently between each addition:

– Polymyxin B sulfate solution

– Acriflavine hydrochloride solution.

– Sodium ceftazidime pentahydrate solution.

Maintain the complete medium at 47 °C, and as quickly as possible, pour 15 mℓ into each of an appropriate number of Petri dishes. Allow to solidify.

Immediately before use, dry the surface of the agar plates carefully (preferably with the lids off and the agar surface downwards) in an oven controlled at 50 °C for 30 min, or until the surface of the agar is dry.

10.4.53 Solid culture medium: Tryptone soya yeast extract agar (TSYEA)

10.4.53.1 Ingredients

Tryptone soya broth ..... 30,0 g
Yeast extract ..... 6,0 g
Agar[8)] ..... 12,0 to 18 g
Water ..... 1 000 mℓ

10.4.53.2 Preparation

Dissolve components or complete dehydrated medium in the water by boiling. So adjust the pH value,

8) Depending on the gel strength of the agar.

if necessary, that, after sterilization it is 7,3 at 25 °C. Dispense quantities of about 6 mℓ of the solid culture medium into tubes. Autoclave the tubes at 121 °C for 15 min. Allow to set in a sloping position.

For the preparation of agar plates, sterilize the solid medium in flasks or bottles of suitable capacity. Dispense the medium, while still liquid, in quantities of about 15 mℓ into sterile Petri dishes and allow to solidify.

10.4.54 Liquid culture medium: Tryptone soya yeast extract broth (TSYEB)

10.4.54.1 Ingredients

Tryptone soya broth ..... 30,0 g
Yeast extract ..... 6,0 g
Water ..... 1 000 mℓ

10.4.54.2 Preparation

Dissolve components or complete dehydrated medium in the water by boiling. So adjust the pH value, if necessary, that, after sterilization, it is 7,3 at 25 °C. Dispense quantities of about 6 mℓ of the medium into tubes. Autoclave the tubes at 121 °C for 15 min.

10.4.55 Blood agar

10.4.55.1 Blood agar base No. 2

Meat peptone ..... 15,0 g
Liver digest ..... 2,5 g
Yeast extract ..... 5,0 g
Sodium chloride ..... 5,0 g
Agar[9] ..... 12,0 g to 18 g

10.4.55.2 Liquid media

Water ..... 1 000 mℓ
Horse or sheep defibrinated blood ..... 70 mℓ

10.4.55.3 Preparation

Dissolve the dehydrated blood agar base No. 2 in the water by boiling. So adjust the pH value, if necessary, that, after sterilization, it is 7,0 at 25 °C. Dispense the medium into tubes or flasks of capacity not more than 500 mℓ. Autoclave the blood agar base at 121 °C for 15 min. Cool the medium to 47 °C. Add the defibrinated blood and mix well.

Dispense the medium in quantities of about 20 mℓ into sterile Petri dishes and allow to solidify. For the preparation of agar plates, sterilize the solid medium in flasks or bottles of suitable capacity. Dispense the medium, while still liquid, in quantities of about 15 mℓ into sterile Petri dishes and allow to solidify.

9) Depending on the gel strength of the agar.

10.4.56 Carbohydrate utilization broth

10.4.56.1 Base

10.4.56.1.1 Ingredients

Proteose peptone ..... 10,0 g
Beef extract ..... 1,0 g
Sodium chloride ..... 5,0 g
Bromocresol purple ..... 0,02 g
Water ..... 1 000 mℓ

10.4.56.1.2 Preparation

Dissolve the components in the water by boiling. So adjust the pH value, if necessary, that, after sterilization, it is 6,8 at 25 °C. Dispense the medium into tubes in such quantities that, after sterilization, 9 mℓ will remain. Autoclave the tubes at 121 °C for 15 min.

10.4.56.2 Carbohydrate solutions

10.4.56.2.1 Ingredients

Carbohydrate (100 mℓ of L-rhamnose solution and
100 mℓ of D-xylose solution) ..... 5,0 g
Water ..... 100 mℓ

10.4.56.2.2 Preparation

Dissolve separately each carbohydrate in 100 mℓ of water. Sterilize by filtration. So adjust the pH value, if necessary, that, after sterilization, it is 6,8 at 25 °C. Dispense the medium into tubes in such quantities that, after sterilization, 9 mℓ will remain. Autoclave the tubes at 121 °C for 15 min.

10.4.56.3 Complete carbohydrate utilization broth

For each carbohydrate, aseptically add 1 mℓ of carbohydrate solution (see 10.4.56.2) to 9 mℓ of the base medium (see 10.4.56.1). If smaller volumes of base medium are prepared, add correspondingly smaller volumes of the carbohydrate solution.

10.4.57 Motility medium

10.4.57.1 Ingredients

Casein peptone ..... 20,0 g
Meat peptone ..... 6,1 g
Agar ..... 3,5 g
Water ..... 1 000 mℓ

10.4.57.2 Preparation

Dissolve the components in the water by boiling. So adjust the pH value, if necessary, that, after sterilization, it is 7,3 ± 0,2 at 25 °C. Dispense the medium into tubes in quantities of about 5 mℓ. Autoclave the tubes at 121 °C for 15 min.

10.4.58 Brain heart infusion broth

10.4.58.1 Ingredients

Brain heart infusion solids	17,5 g
Tryptose	10,0 g
Dextrose	2,0 g
Sodium chloride	5,0 g
Disodium phosphate	2,5 g
Water	1 000 mℓ

10.4.58.2 Preparation

Suspend the ingredients in the water. Boil to dissolve the medium completely. Autoclave at 121 °C for 15 min. Dispense into sterile 30 mℓ bottles (see 10.2.2(a)).

10.4.59 EDTA rabbit plasma

EDTA rabbit plasma is desiccated rabbit plasma to which ethylenediaminetetra-acetic acid (EDTA) has been added as the anticoagulant. It is commercially available in freeze-dried form in vials. To reconstitute add 3 mℓ of sterile water to the contents of the vial. Mix by gently rotating the vial end over end.

10.4.60 Zinc dust.

Commercially available.

10.5 Preparation of the sample

10.5.1 Storage of the product

Store the product, of mass at least 200 g, for the minimum practicable period under such conditions that changes in composition are prevented or minimized.

10.5.2 Preparation of the sample

When necessary, thaw the raw or cooked product in its packaging at 5 °C to 10 °C until all the visible ice has melted. Ensure that thawing is completed within 18 h. Using a sterile cutter and forceps, remove 28 g to 35 g of the product and transfer it to a previously tared and sterilized homogenizing container suitable for use with the homogenizer (see 10.3.4). Add enough of the buffered isotonic peptone water (see 10.4.2) to obtain a 1:10 dispersion of the product. Operate the homogenizer in accordance with the manufacturer's instructions for just long enough to produce a homogeneous dispersion, i.e. operate rotating homogenizers for such a time that the total number of revolutions of the macerator blades is 15 000 to 20 000, but in no case for longer than 2,5 min. Use the 1:10 dispersion of the product so obtained for the tests described in 10.6 to 10.14 (inclusive).

10.6 Standard plate count

10.6.1 Cooked products

10.6.1.1 Prepare a dilution of one volume of the sample in 1 000 volumes of diluent by mixing 1 mℓ of the sample (see 10.5.2) with 99 mℓ of the buffered isotonic peptone water (see 10.4.2) in a bottle (see 10.2.2). Alternatively, add 1 mℓ of the dispersion of the sample to 9 mℓ of peptone water and add 1 mℓ of this mixture to a further 9 mℓ of peptone water. Mix the contents of each bottle thoroughly before using them, using the appropriate of the following methods of mixing:

a) use a suitable mechanical mixer, preferably of the vibratory type;

b) where the dilution is contained in a screw-capped bottle, mix by inverting and righting the bottle by hand 10 times; or

c) where the dilution is contained in a capped or cotton-wool-plugged container, reciprocally roll the container in an upright position at least 20 times between the palms of the hand.

10.6.1.2 From the dilution of the sample so obtained, pipette a 1 mℓ volume into each of two sterile Petri dishes (see 10.2.5(a) or (b)). To the contents of each plate, add one 15 mℓ volume of the plate-count agar (see 10.4.3), melted and cooled to 45 °C, and mix. Avoid spilling any of the contents of the plate during this process. This is best achieved by placing the plate on a table top and gently swirling the plate. Allow the agar to solidify, invert the plates, label them and incubate at 30 °C. Ensure that the total period between the preparation of the dilutions of the sample and the final plating does not exceed 15 min. After 48 h of incubation, remove the plates from the incubator and count the colonies that have developed in the medium. Record these results and calculate the average number of colony-forming units per gram of the sample.

10.6.2 Raw products

Prepare a 1:1 000 dilution of the sample as described in 10.6.1.1. Prepare a further 1:10 dilution by mixing 1 mℓ of this dilution with 9 mℓ of the buffered isotonic peptone water (see 10.4.2), and then proceed as described in 10.6.1.2, using the dilution so obtained for plating, incubating and counting.

10.7 Enterobacteriaceae count

Pipette 2 mℓ volumes of the dispersion of the sample (see 10.5.2) into each of two sterile Petri dishes (see 10.2.5(a)). To the contents of each plate, add at least 15 mℓ of the VRB glucose agar (see 10.4.4), and mix. Mix well with the inoculum by gently rotating each dish. When the medium has solidified, add an overlayer of the same VRB glucose agar. Avoid spilling any of the contents of the plate during this process. Allow the agar to solidify, invert the plates, label them appropriately, and incubate at 37 °C for 24 h. Examine and count all colonies of diameter exceeding 0,5 mm, ignoring all others. Regard all such colonies as those of enterobacteriaceae. Record these results and multiply the number of enterobacteriaceae colonies by 2,5 to obtain the enterobacteriaceae count per gram of sample.

10.8 Faecal coliform bacteria

10.8.1 Cooked products

10.8.1.1 Aseptically measure 100 mℓ of the sample (see 10.5.2) into each of two bottles of the double-strength brilliant green bile medium (see 10.4.6) and incubate these at 37 °C for 16 h to 20 h.

10.8.1.2 If the medium shows the production of gas, as indicated by gas in the Durham tube, use a platinum wire loop to subculture from each bottle one loopful into a further bottle of single-strength brilliant green bile medium (see 10.4.5) and one loopful into a bottle of tryptone water (see 10.4.7), both preheated to 44 °C.

10.8.1.3 Incubate both these subcultures at 44 °C ± 0,25 °C for 16 h to 20 h in a water-bath. Add 0,1 mℓ to 0,5 mℓ of Kovacs reagent (see 10.4.8) to the culture in the tryptone water. Mix by gently shaking the bottle. If a red colour develops and the culture in the brilliant green bile medium shows the production of gas, consider the culture to be faecal coliform bacteria.

10.8.2 Raw products

Aseptically measure 100 mℓ of the dispersion sample (see 10.5.2) into each of two bottles of double-strength brilliant green bile medium (see 10.4.6) and incubate these at 37 °C for 16 h to 20 h. Examine and confirm suspect cultures as described in 10.8.1.2 and 10.8.1.3.

NOTE – This method determines the presence or absence of viable faecal coliform bacteria in 10 g of product. This implies that as little as one such organism per 10 g will give a positive result.

10.9 *Staphylococcus aureus*

10.9.1 Plating procedure

Transfer, by means of a sterile pipette, a 1 mℓ volume of the dispersion sample (see 10.5.2) to the surface of three Baird-Parker agar plates (see 10.4.9), evenly distributing the single volume over the three plates. Regard these three plates as one during the counting procedure, since they represent the 1:10 dilution of the sample. Repeat the above procedure with a further 1 mℓ volume and three further plates. Inoculate each of a further two Baird-Parker agar plates with 0,1 mℓ of the sample. Each of these plates will represent the 1:100 dilutions. Carefully spread the inoculum by means of individual sterile glass spreaders (see 10.3.5) over the surface of each of the plates with as little time delay as possible, trying not to touch the sides of the dish. Incubate the plates at 43 °C for 24 h to 48 h.

10.9.2 Selection procedure

10.9.2.1 After 24 h to 26 h of incubation, mark on the bottom of the plates the positions of any typical colonies present. Typical colonies are black, shining and convex (of diameter 1 mm to 1,5 mm) and surrounded by a clear zone that could be partially opaque. An opalescent ring, immediately in contact with the colonies, could appear in this clear zone. Re-incubate all plates at 43 °C for a further 22 h to 24 h and then mark the position of any new typical colonies.

10.9.2.2 Take for enumeration those plates that contain between 15 and 150 typical or atypical colonies. Select for confirmation (see 10.9.3) five typical or five atypical colonies, as the case may be, from each plate. If there are fewer than 15 typical or atypical colonies present on the plates inoculated with the 1:10 dilution of the sample (see 10.9.1), retain all plates that contain any typical or atypical colonies. Select all such colonies for confirmation (see 10.9.3).

10.9.3 Confirmation tests

The selection procedure (see 10.9.2) is dependent on the use of elevated temperatures (43 °C) for incubation, and will facilitate the confirmation of the identity of *Staphylococcus aureus*.

The confirmation of the presence of *Staphylococcus aureus* is dependent on the following additional tests:

a) Spot-inoculate each of the typical or atypical colonies selected from the Baird-Parker agar plates (see 10.9.2.2) onto a plate of mannitol salt phenol red agar (see 10.4.12) and a plate of DNAse test agar (see 10.4.14). Use a heavy inoculum. Incubate the plates at 37 °C for 48 h.

   After incubation, flood the surface of the DNAse test agar plate with dilute hydrochloric acid of concentration c(HCl) = approximately 1 mol/ℓ. The DNA will precipitate and cause the medium to become turbid. Clear zones will develop around positive colonies.

b) Inspect the mannitol salt phenol red agar plates for colonies that develop a yellow colour with a clear yellow zone around the colony. This would indicate conversion of mannitol to acid. Those colonies that show a positive DNAse reaction and that produce acid as a breakdown product of mannitol, are then subjected to the coagulase test.

c) Use a sterile loop to remove an inoculum from the surface of each selected colony and transfer it to a bottle of brain heart infusion broth (see 10.4.58). Incubate at 37 °C for 24 h. Aseptically add 0,1 mℓ of each culture to 0,3 mℓ of EDTA rabbit plasma (see 10.4.59) in small sterile tubes and incubate at 37 °C. Examine for clotting for 4 h to 6 h. Consider the test to be positive if the volume of the clot occupies more than three-quarters of the original volume of the liquid.

Staphylococcus aureus can be positively identified if growth from a selected colony (see 10.9.2) shows

a positive DNAse reaction, produces acid as a breakdown product of mannitol and the selected colonies are strongly positive for the coagulase reaction.

10.9.4 Calculation of the *Staphylococcus aureus* count

In the case of plates containing positively identified typical or atypical colonies (see 10.9.2), calculate the number of *Staphylococcus aureus* for each dilution from the percentage of *Staphylococcus aureus* identified from the selected colonies during the confirmation tests (see 10.9.3). Calculate the average number of *Staphylococcus aureus* from the duplicate plates or from consecutive dilutions.

NOTE – Round those numbers of less than 100 to the nearest multiple of five, and round those numbers that exceed 100 and end in five, to the nearest multiple of 20. If the number exceeds 100 and does not end in five, round it to the nearest multiple of 10.

Multiply the average so obtained by the reciprocal of the inoculum volume and then by the reciprocal of the corresponding dilution of the test sample, to obtain the number of *Staphylococcus aureus* per gram of sample.

10.10 *Salmonella*

10.10.1 Pre-enrichment

Transfer 25 mℓ volumes of the sample (see 10.5.2) into each of two sterile 250 mℓ culture flasks (see 10.2.8). Incubate the flasks at 37 °C for 16 h to 20 h.

10.10.2 Selective enrichment

Transfer the entire contents of one of the flasks of the pre-enriched sample (see 10.10.1) into a flask that contains 100 mℓ of the R-V medium (see 10.4.16), and transfer the entire contents of the other flask (see 10.10.1) into a flask that contains 100 mℓ of the selenite medium (see 10.4.17).

Incubate the inoculated R-V medium at 43 °C for up to 48 h and the inoculated selenite medium at 37 °C for up to 48 h. After 18 h to 24 h of incubation and without shaking the contents of the flasks, proceed with the diagnostic plating.

10.10.3 Diagnostic plating

10.10.3.1 Using a platinum wire loop of internal diameter 4 mm, remove two loopsful of the culture in the R-V medium from the surface of the medium and streak one over the surface of a plate of the brilliant green phenol red agar (see 10.4.18) and the other over the dried surface of a VRB glucose agar plate (see 10.4.4). Then mix the contents of the flask and repeat the diagnostic plating with two further loopsful on two further plates. Perform the streaking in a way that will ensure the development of well-isolated colonies. Suitably label the diagnostic plates to identify which of the two methods of sampling was used. Invert the plates and incubate them at 37 °C for 18 h to 24 h.

NOTE – It is claimed that motile *Salmonella* organisms migrate to the surface of the enrichment media. Sampling the undisturbed surface would therefore appear to increase the probability of their detection.

10.10.3.2 Streak similar plates in the same way with the culture in the selenite medium.

10.10.3.3 Return the cultures in both the selenite medium and the R-V medium flasks to their respective incubators for a further 24 h to 28 h.

10.10.3.4 At the end of this incubation period, repeat, for each culture, the diagnostic plating onto a further series of plates and incubate these plates at 37 °C for 18 h to 24 h.

10.10.3.5 After incubation, examine the plates for presumptive colonies of *Salmonella* organisms. If growth on the plates is scant or if no suspect colonies are present, incubate the plates for a further 20 h to 24 h and re-examine them. Subject any suspect colony to further examination. The recognition of colonies of *Salmonella* organisms is a matter of experience, because their appearance differs on the two diagnostic media, and from species to species, and from batch to batch of medium.

10.10.4 Confirmation of suspect colonies

Select five colonies of each type of suspected *Salmonella* organism on each plate, or all such colonies, whichever are less.

Streak each of the selected colonies onto the dried surface of a VRB agar plate (see 10.4.4) in a way that will ensure the development of well-isolated colonies. Incubate the plates at 37 °C for 18 h to 24 h. Examine the colonies developing on the plates for uniformity of characteristics, and in this way establish whether the culture under examination is "pure". It is of paramount importance that culture that is to be subjected to further test work be pure. If in doubt, streak a well-isolated colony onto the dried surface of a further plate of VRB agar. Incubate this plate at 37 °C for 18 h to 24 h and examine as above. If necessary, repeat this procedure until the purity of the culture is established beyond reasonable doubt. Subject this culture to further tests, taking care to avoid contamination of the culture with other micro-organisms. Use only lactose-negative (colourless) colonies for further tests.

10.10.5 Biochemical confirmation

Using a platinum needle, subculture the pure culture (see 10.10.4) onto or into relevant media and test for the reactions given in 10.10.5.1 to 10.10.5.7.

10.10.5.1 Triple sugar iron agar

Stab the culture into the butt and streak it onto the agar slope surface of the triple sugar iron agar (see 10.4.20). Incubate at 37 °C for 24 h to 48 h and examine. Classify the results as follows:

Butt:

Yellow colour	Glucose converted (acid)
Red colour or no change	Glucose not converted
Black colour	Hydrogen sulfide produced
Gas bubbles or cracks	Gas produced from glucose

Slope:

Yellow colour	Aerobic conversion of lactose or sucrose or both (acid)
Red colour or no change	Neither lactose nor sucrose converted.

10.10.5.2 Urease production

Streak the pure culture (see 10.10.4) onto the agar slope surface of urea agar (see 10.4.21). Incubate at 37 °C for 24 h to 48 h and examine. The splitting of urea produces ammonia, which changes the colour of the medium to pink, and later to cherry red.

10.10.5.3 Lysine decarboxylation

Inoculate a loopful of the pure culture (see 10.10.4) just below the surface of the lysine decarboxylation medium (see 10.4.22), incubate at 37 °C for 18 h to 24 h and examine. The decarboxylation of lysine produces cadaverine, which changes the colour of the medium to purple. A yellow colour or an unchanged medium indicates the absence of lysine decarboxylation.

10.10.5.4 ß-galactosidase production

Suspend a small quantity of the bacterial material from the pure culture (see 10.10.4) in 0,25 mℓ of the saline solution (see 10.4.28) in a small sterile test tube. Add a drop of toluene to this suspension, and heat the tube for 5 min in a water-bath maintained at 37 °C. Add 0,25 mℓ of the ß-galactosidase reagent (see 10.4.23) to the suspension and mix. Incubate the tube at 37 °C for at least 24 h and examine at intervals. A yellow colour, indicating a positive reaction, might occur within 20 min. Do not regard the reaction as negative until incubation for 24 h is completed.

10.10.5.5 Indole production

Inoculate a bottle of tryptone water (see 10.4.7) with the pure culture. Incubate at 37 °C for 24 h. After incubation, add 0,5 mℓ of the Kovacs reagent (see 10.4.8) to the contents of the bottle. The formation of a red colour indicates a positive reaction.

10.10.5.6 Voges-Proskauer reaction

Inoculate each of two tubes of Voges-Proskauer medium (see 10.4.24) with the pure culture. Incubate one tube at ambient temperature and the other at 37 °C for 24 h. After incubation, add two drops of the creatine solution (see 10.4.25), three drops of the α-naphthol solution (see 10.4.26) and then two drops of the potassium hydroxide solution (see 10.4.27) to each tube, mixing the contents after each addition. The development of a pink to bright red colour within 15 min indicates a positive reaction.

10.10.5.7 Oxidase reaction

Apply a small quantity of bacterial material from the pure culture to a cytochrome oxidase test strip (see 10.4.19) and rub it well into the reaction area. Allow to stand for approximately 30 s. A blue colour indicates a positive reaction.

10.10.6 Interpretation of results of biochemical confirmation tests

Reaction	Percentage of *Salmonella* types showing a positive reaction
Acid from glucose	100,0
Gas from glucose	91,9
Acid from lactose	0,8
Acid from sucrose	0,5
Hydrogen sulfide production	91,6
Urease production	0,0
Lysine decarboxylation	94,5
ß-galactosidase production	1,5
Indole production	1,1
Voges-Proskauer reaction	0,0
Oxidase reaction	0,0

Subject all cultures to the serological confirmation tests, except those that, on the basis of the above data, clearly do not contain *Salmonella* organisms.

10.10.7 Serological confirmation

Where suitable polyvalent anti-*Salmonella* "O" and "H" sera and "Vi" sera (see 10.4.29 to 10.4.31) are available, examine the suspect colonies (see 10.10.4) for the presence of *Salmonella* "O" and "H" antigens and for "Vi" antigens by slide agglutination. Bear in mind, however, that the results of serological tests should not be solely relied upon for confirmation and should be assessed together with the results obtained by biochemical confirmation.

10.10.8 Interpretation of results of serological confirmation tests

10.10.8.1 Polyvalent anti-*Salmonella* "O"serum

Interpret the results as follows:

a) where the result is negative, it is almost certain that no *Salmonella* is present. The only exception is that a culture might have an as-yet-unencountered "O" antigen; and

b) where the result is positive, it is only an indication that the culture could be from the genus *Salmonella*.

10.10.8.2 Polyvalent anti-*Salmonella* "H" serum

Interpret the results as follows:

a) where the result is negative, it is almost certain that no *Salmonella* is present. The only exception is that a culture might have an as-yet-unencountered "H" antigen; and

b) where the result is positive, consider the culture to be positive for *Salmonella*.

10.10.8.3 Anti-*Salmonella* "Vi" sera

Where results are positive, consider the culture to be positive for *Salmonella*.

10.11 *Shigella*

10.11.1 Detection

Proceed as described in 10.10 for *Salmonella*, but use the selenite medium (see 10.4.17) as the selective enrichment medium and the XLD agar (see 10.4.33) as the diagnostic plating medium and, in both cases, incubate at 37 °C.

Red colonies on XLD agar are suspect *Shigella* organisms.

Salmonella spp. and *Salmonella typhi* can also be detected with the use of XLD agar and this method supplements the method described in 10.10.

10.11.2 Confirmation

Subject each suspect colony to the biochemical tests described in 10.10.5 and to a serological test as described in 10.10.7 and 10.10.8, but use polyvalent anti-*Shigella* "O" serum (see 10.4.34).

10.11.3 Interpretation of results

Reaction	Percentage of *Shigella* types showing a positive reaction
Acid from glucose	100,0
Gas from glucose	2,1
Acid from lactose	0,2
Acid from sucrose	0,6
Hydrogen sulfide production	0,0
Urease production	0,0
Lysine decarboxylation	0,0
ß-galactosidase production	38,3
Indole production	30,6
Voges-Proskauer reaction	0,0
Oxidase reaction	0,0

10.12 *Clostridium perfringens*

10.12.1 Inoculation and incubation (poured plate technique)

By means of a sterile pipette, transfer 1 mℓ of the dispersion of the sample (see 10.5.2), to each of two Petri dishes, pour 15 mℓ to 20 mℓ of the SC agar (see 10.4.35) into each dish and mix well with the inoculum by gently rotating each dish. When the medium has solidified, add an overlayer of 10 mℓ of the same SC agar. Allow to solidify and place the plates, with the lid uppermost, in anaerobic jars or other suitable containers and incubate at 37 °C for 20 h. Longer incubation can result in excess blackening along the bottom rim of the plates.

10.12.2 Counting of colonies

10.12.2.1 After the specified period of incubation (see 10.12.1), count and record the number of characteristic colonies on the plates in accordance with 10.12.2.2. Colonies of *C. perfringens* are black.

10.12.2.2 Count the characteristic colonies on each plate and record the arithmetic mean of the counts from the two plates.

10.12.3 Confirmation

10.12.3.1 Selection of colonies for confirmation

Select a total of 10 characteristic colonies from the plates counted in accordance with 10.12.2.2. If fewer than 10 colonies are available on the plates counted, select all the characteristic colonies present. Confirm these colonies in accordance with 10.12.3.2.

10.12.3.2 Biochemical confirmation

10.12.3.2.1 Confirmation using motility-nitrate medium

Stab-inoculate the selected colonies (see 10.12.3.1) into motility-nitrate medium (see 10.4.36). Incubate under anaerobic conditions at 37 °C for 24 h.

Examine the tubes of motility-nitrate medium for the type of growth along the stab line. Motility is evident from diffuse growth out into the medium away from the stab line. Test for the presence of nitrite by adding 0,2 mℓ to 0,5 mℓ of the nitrite-detection reagent (see 10.4.42) to each tube of motility-nitrate medium.[10] The formation of a red colour confirms the reduction of nitrate to nitrite.

If no red colour is formed within 15 min, add a small amount of zinc dust (see 10.4.60) and allow to stand for 10 min. If a red colour is formed after the addition of zinc dust, no reduction of nitrate has taken place.

10.12.3.2.2 Confirmation using lactose-gelatine medium

Inoculate the selected colonies (see 10.12.3.1) into lactose-gelatine medium (see 10.4.37). Incubate under anaerobic conditions at 37 °C for 24 h.

Examine the tubes of lactose-gelatine medium for the presence of gas and of a yellow colour (owing to acid), indicating fermentation of lactose. Chill the tubes at 5 °C for 1 h and check for gelatine liquefaction. If the medium has solidified, reincubate for an additional 24 h and again check for gelatine liquefaction.

10.12.3.3 Interpretation

Bacteria that produce black colonies on SC medium are non-motile, reduce nitrate to nitrite, produce acid and gas from lactose, and liquify gelatine in 48 h are considered to be *C. perfringens*.

Cultures that show a faint reaction for nitrite (i.e. a pink colour) should be eliminated, since *C. perfringens* consistently gives an intense and immediate reaction.

10.13 Pathogenic *Vibrio* (*Vibrio cholerae* and *Vibrio parahaemolyticus*)

10.13.1 Enrichment

Within 30 min of preparing the dispersion of the sample (see 10.5.2), prepare two cultures, each consisting of 100 mℓ of the sample mixed with 100 mℓ of the double-strength *Vibrio* enrichment medium (see 10.4.38). Incubate these cultures for 18 h to 24 h, one at 37 °C and the other at 42 °C.

10.13.2 Diagnostic plating

Without shaking the cultures, remove a loopful from the surface of each culture, and streak each loopful onto a *Vibrio* diagnostic agar plate (see 10.4.39) in a way that will ensure the development of well-isolated colonies. Invert the plates and incubate them at 37 °C for 18 h to 24 h.

Examine, in terms of the following characteristics, the incubated plates for the presence of *Vibrio* spp.:

Description of colonies	Colony diameter mm	Presumptive identification
Flat, yellow and round	2 to 3	*Vibrio cholerae*
Smooth and green (sucrose negative)	3 to 5	*V. parahaemolyticus*
Flat, yellow and round	4 to 6	*V. alginolyticus*
Round and blue	0,5 to 1	*Pseudomonas, Aeromonas*
Transparent	0,1 to 0,5	*Proteus* or other enterobacteria

10) For health reasons, it may be desirable to carry out this test under a fume hood.

Transfer suspect colonies to the lysine-indole-motility-hydrogen-sulfide agar (see 10.4.40) and to urea agar slopes (see 10.4.21), and incubate these cultures at 37 °C for 18 h to 24 h.

10.13.3 Confirmation

10.13.3.1 Examine the urea agar slopes and, if no urease has been produced, transfer some of the growth to a cytochrome oxidase test strip (see 10.4.19) and determine whether the colonies are cytochrome oxidase positive (see 10.10.5.7).

10.13.3.2 Also examine the lysine-indole-motility-hydrogen-sulfide agar cultures and, if any organism is suspected to be *Vibrio cholerae* or *Vibrio parahaemolyticus*, send a sample to an acceptable testing laboratory for further identification.

10.14 Detection of *Listeria monocytogenes*

Warning: Detection of *Listeria monocytogenes* shall only be undertaken in properly equipped laboratories under the control of a skilled microbiologist, and great care shall be taken in disposal of all incubated materials.

10.14.1 Procedure

10.14.1.1 Preparation of the sample

When necessary, thaw the raw or cooked product in its packaging at 5 °C to 10 °C until all the visible ice has melted. Ensure that thawing is completed within 18 h. Using a sterile cutter and forceps, remove 25 g to 35 g of the product and transfer it to a previously tared and sterilized homogenizing container suitable for use with the homogenizer (see 10.3.4). Add enough of the selective pre-enrichment medium (see 10.4.49) to obtain a 1:10 dispersion of the product. Operate the homogenizer in accordance with the manufacturer's instructions for just long enough to produce a homogeneous dispersion, i.e. operate rotating homogenizers for such a time that the total number of revolutions of the macerator blades is 15 000 to 20 000, but in no case for longer than 2,5 min. Use the 1:10 dispersion of the product so obtained for the tests for the detection of *Listeria monocytogenes*.

10.14.1.2 Primary enrichment

Incubate the initial suspension (see 10.14.1.1) at 30 °C for 24 h.

10.14.1.3 Secondary enrichment

After the primary enrichment, transfer 0,1 mℓ of the culture obtained in 10.14.1.2 to a tube containing 10 mℓ of selective enrichment medium (see 10.4.50). Incubate the inoculated medium at 37 °C for 48 h.

10.14.1.4 Plating out and identification

10.14.1.4.1 From the primary enrichment culture (see 10.14.1.2), take, by means of a loop, a streak from the culture and so inoculate the surface of the first selective plating-out medium (Oxford agar) (see 10.4.51) that well-isolated colonies will be obtained.

Proceed in the same way with the second selective plating-out medium (PALCAM agar) (see 10.4.52).

10.14.1.4.2 From the secondary enrichment medium (see 10.14.1.3), repeat the procedure described in 10.14.1.4.1 with the two selective plating-out media.

10.14.1.4.3 Invert the dishes obtained in 10.14.1.4.1 and 10.14.1.4.2. Place them in an incubator set at 37 °C. PALCAM agar plates are incubated either micro-aerobically in a jar that contains 5 % to 12 % (by volume) of CO_2, 5 % to 15 % (by volume) of O_2 and 75 % (by volume) of N_2, or aerobically.

10.14.1.4.4 After incubation for 24 h to 48 h, examine the dishes for the presence of typical colonies of *Listeria* spp. as follows:

a) **Oxford agar**: typical colonies of *Listeria* spp. grown on Oxford agar for 24 h are small (of diameter 1 mm) dark-brown colonies surrounded by black halos. After 48 h, the colonies are black, of diameter 2 mm to 3 mm, and have black halos and sunken centres.

b) **PALCAM agar**: after incubation, allow PALCAM agar plates to regain their pink to purple colour by exposure to air for 1 h. *Listeria* spp. grow as small green colonies, of diameter 1,5 mm to 2 mm, sometimes with black centres, but always with black halos.

10.14.1.4.5 If growth is slight or if no colonies are present after 24 h incubation, continue to incubate the dishes at 37 °C as described in 10.14.1.4.3, for a further 18 h to 24 h and re-examine for the presence of *Listeria* spp.

10.14.1.5 Confirmation

10.14.1.5.1 Selection of colonies for confirmation

10.14.1.5.1.1 For confirmation, take from each plate of each selective medium (see 10.14.1.4.3 and 10.14.1.4.4), five colonies considered to be typical or suspect. If on one plate there are fewer than five typical or suspect colonies, take for confirmation all the typical or suspect colonies.

10.14.1.5.1.2 Streak the selected colonies onto the surface of pre-dried plates of tryptone soya yeast extract agar (TSYEA) (see 10.4.53) in a way that will allow well-isolated colonies to develop. Place the plates in an incubator at 37 °C for 24 h or until growth is satisfactory.

10.14.1.5.1.3 Typical colonies are of diameter 1 mm to 2 mm, colourless, convex and opaque with an entire edge. If necessary, examine the plates, using a source of beamed white light that is powerful enough to illuminate the plates well and strikes the bottom of the plate at an angle of 45°. When examined in this obliquely transmitted light directly from above the plate, colonies of *Listeria* spp. exhibit a bluish colour and a granular surface. If mixed cultures are apparent, pick a typical *Listeria* spp. colony and subculture to a further plate of TSYEA. Carry out the following tests, using colonies of a pure culture on the TSYEA.

10.14.1.6 Catalase reaction

Take a typical colony and suspend it in a drop of 3 % (by volume) of hydrogen peroxide solution on a slide. *Listeria* spp. are catalase positive, which is demonstrated by the immediate formation of gas bubbles.

10.14.1.7 Staining properties and morphology

10.14.1.7.1 Gram staining

Perform the Gram stain on a typical colony on TSYEA (see 10.14.1.5.1.2). *Listeria* spp. are Gram-positive rods.

10.14.1.7.2 Motility test (if necessary)

Choose a typical colony on TSYEA (see 10.14.1.5.1.2) and suspend it in a tube containing TSYEB broth (see 10.4.54). Incubate at 20 °C to 25 °C for 8 h to 24 h until a cloudy medium is observed.

Use a loop to deposit a drop of the above culture between slides and examine the culture, using a microscope. The *Listeria* spp. appear as slim, short rods with pronounced tumbling motility. Cultures grown at above 25 °C might not exhibit this motion. Always compare them to a known culture. Cocci, large rods, or rods with rapid, swimming motility are not *Listeria* spp. As an alternative test for motility, use an inoculating needle to stab the motility broth (see 10.4.57) with a culture taken from a typical colony on TSYEA (see 10.14.1.5.1.2) and incubate it at 25 °C for 48 h. Examine for growth around the stab. *Listeria*

spp. are motile, giving a typical umbrella-like growth pattern. If a negative result is obtained, incubate for an additional 5 d and observe the stab again.

10.14.1.7.3 Haemolysis test

If the morphological and physiological characteristics and catalase reaction are indicative of *Listeria* spp. inoculate the blood agar plates (see 10.4.55)[11] to determine the haemolytic reaction. Dry the agar surface well before use. Draw a grid on the plate bottom, marking 20 to 25 spaces per plate. Take a typical colony from the TSYEA (see 10.14.1.5.1.2) plate and, using a loop, stab one space for each culture. Simultaneously stab positive and negative control cultures (*L. monocytogenes, L. ivanovii, L. seeligeri* and *L. innocua*).

After incubation at 37 °C for 24 h to 48 h, examine the test strains and controls. *L. monocytogenes* shows narrow, clear light zones (β-haemolysis)[12]. *L. innocua* should show no clear zone around the stab. *L. seeligeri* shows a weak zone of haemolysis. *L. ivanovii* usually shows wide, clearly delineated zones of β-haemolysis. Hold plates up to a bright light to compare test cultures with controls.

10.14.1.7.4 Carbohydrate utilization

Inoculate the carbohydrate utilization broths (see 10.4.56) with one loopful or 0,1 mℓ of the TSYEB culture (see 10.14.1.7.2). Incubate at 37 °C for up to 7 d. Positive reactions (acid formation) are indicated by a yellow colour and occur mostly within 24 h to 48 h.

10.14.1.8 Interpretation of morphological and physiological properties and of biochemical reaction

All *Listeria* spp. are small, Gram-positive rods (only with 24-h-old cultures) that demonstrate motility in wet mount and in the motility medium. They are catalase positive. *L. monocytogenes* utilizes rhamnose but not xylose. *L. monocytogenes, L. ivanovii* and *L. seeligeri* (weak reaction) produce β-haemolysis in blood agar cultures. Of the three haemolytic *Listeria* spp., only *L. monocytogenes* fails to utilize xylose and is positive for rhamnose utilization.

10.15 Test for efficacy of cleaning and disinfecting of plant, equipment and utensils

10.15.1 Sampling equipment

10.15.1.1 Preparation of swabs

Prepare each swab by capping one end of a wooden rod, of length approximately 140 mm and of diameter approximately 2 mm, with a rounded bud of absorbent cotton wool of mass 30 mg to 50 mg. Dip the bud of each swab into a beaker containing inactivator solution (see 10.4.41) and then place the swab in an individual plastics bag or other suitable container that is capable of withstanding the subsequent sterilizing without damage. Seal the bags or containers and sterilize by autoclaving or by other acceptable means. Store the swabs in a cool dark place until used.

10.15.1.2 Absence of substances that inhibit growth of micro-organisms

Melt and cool to 45 °C the contents of two bottles of plate-count agar (see 10.4.3). Inoculate one bottle with *Escherichia coli* and the other bottle with *Bacillus subtilis*, so that a heavy growth will be ensured. Mix the contents in each bottle thoroughly and pour into each of two sterile Petri dishes (see 10.2.5(a) or (b)). Aseptically remove the buds of two swabs and immerse a bud in the agar in each of the plates before the agar solidifies. Incubate the plates at 35 °C for 18 h. After incubation, examine the plates and regard the

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11) Report whether sheep or horse blood was used.

12) This is more readily seen by removing any colony grown on the surface of the agar around the inoculum mark.

swabs as unsuitable if there is any sign of inhibition of growth of organisms around and under either of the swabs. In such a case, prepare a fresh batch of swabs as described in 10.15.1.1, and retest.

10.15.2 Sampling procedure

10.15.2.1 General

Where possible, take samples from at least 15 different surfaces, each of area approximately 10 cm^2. Record, in square centimetres, the area of each surface sampled.

10.15.2.2 Sampling with swabs

Aseptically open a swab container and, if a plastics bag was used, open it on the side away from the cotton wool bud. Ensure that throughout the handling of a swab, the fingers of the sampler do not touch the bud or the adjacent part of the stem. Vigorously rub the bud of the swab over the area to be sampled. While doing this, so rotate the swab as to bring the entire surface of the bud into intimate contact with the surface being sampled. Replace the swab in its container and, if a plastics bag was used, reseal it. So mark the container as to identify it with the point sampled.

10.15.3 Test procedure

10.15.3.1 Testing of swabs

10.15.3.1.1 Rinse suspension

Remove the swab from the container and break off the bud into a 30 mℓ bottle of buffered isotonic peptone water (see 10.4.2) by using the neck of the bottle for leverage. Shake the bottle well. This rinse suspension is a 1:10 dilution of the sample.

10.15.3.1.2 Inoculation and incubation

Aseptically pipette a 1 mℓ volume of the rinse suspension into each of two Petri dishes (see 10.2.5(a) or (b). To each plate, add one 15 mℓ volume of the plate-count agar (see 10.4.3), melted and cooled to 45 °C, and mix the contents of the plate by gentle swirling. Allow to solidify, invert the plates and incubate at 25 °C for 72 h.

10.15.3.1.3 Interpretation

At the end of the incubation period, count and record the total number of bacterial colonies developed on both plates. Take five times this number as the number of viable bacteria on the test area sampled and, if necessary, correct to an area of 10 cm^2.

10.15.3.2 Test for the presence of faecal coliform bacteria

Incubate the remainder of the rinse suspension (see 10.15.3.1.1) at 37 °C for 6 h to 8 h. Then proceed as described in 10.8.

10.15.3.3 Expression of efficacy of cleaning and disinfecting

Allocate to each sample one of the symbols S, FS or US, in accordance with the viable bacteria count or the presence of faecal coliform bacteria, as follows:

Count	Symbol
0 to 15/10 cm^2: faecal coliform bacteria absent	S (Satisfactory)
16 to 75/10 cm^2: faecal coliform bacteria absent	FS (Fairly satisfactory)
Over 75/10 cm^2: or presence of faecal coliform bacteria, or both	US (Unsatisfactory)

10.15.3.4 Calculation

Calculate the percentage efficacy of cleaning and disinfecting, using the following formula:

$$\frac{(2X + Y) \times 100}{2T}$$

where

X is the number of S results;

Y is the number of FS results; and

T is the total number of samples.

10.16 Microbiological examination of water

10.16.1 Membrane filter method

10.16.1.1 Examination for total coliform bacteria

Immediately before use, attach a sterile membrane filter holder (see 10.3.7) to a filter flask. Dismantle the holder and, using sterile forceps (see 10.3.8), place a sterile membrane filter (see 10.3.6) over the porous plate, grid-side uppermost. Re-assemble the holder.

Thoroughly mix the water sample by inverting and righting the sample container approximately 10 times (by rapid movement of the wrist). Aseptically transfer 100 mℓ of the sample to the assembled membrane filter holder (see 10.3.7) and filter by applying suction to the filter flask.

After the 100 mℓ water has been filtered, rinse the funnel three times with 20 mℓ to 30 mℓ volumes of sterile water. Carefully dismantle the holder and, using sterile forceps (see 10.3.8), aseptically transfer the membrane filter, grid-side uppermost, to a plate of m-Endo agar LES (see 10.4.43). Ensure that the surface of the m-Endo agar LES (see 10.4.43) in the Petri dish is free from excess moisture. Ensure that no air bubble is trapped between the membrane filter and the surface of the agar and that good wetting contact is maintained.

Invert and incubate the m-Endo agar LES plates at 35 °C ± 0,5 °C for 18 h to 24 h in a dark container. Examine the plates and count the number of coliform colonies, i.e. colonies that have a pink to dark-red colour, with a golden-green metallic sheen. If more than one filter was used to filter the 100 mℓ water sample, count all the coliform suspect colonies on all the filters. The sheen area could vary in size from a small pinhead to complete coverage of the colony surface. Colonies that lack sheen are considered to be non-coliform.

If it is suspected that the sample could contain more than 30 coliform bacteria per 100 mℓ, suitable dilutions may be prepared and filtered. To confirm the presence of coliform bacteria, subculture each colony or a representative number of them (i.e. colonies that have a pink to dark-red colour, with a golden-green metallic sheen) into tubes or bottles of lactose peptone water (see 10.4.48) and incubate at 37 °C ± 0,5 °C for 48 h. Examine for gas formation. Gas formation is indicated by an amount of gas at least sufficient to fill the concavity of the top of the Durham tube (see 10.2.11). The formation of gas confirms

the presence of coliform bacteria.

Calculate the number of coliform bacteria per 100 mℓ of water sample as follows:

$$\frac{N \times D}{V} \times 100$$

where

N is the total number of colonies counted;

D is the dilution factor; and

V is the volume of sample filtered, in millilitres.

Carry out the test in duplicate and preferably in triplicate.

NOTE – It is recommended that the most probable number method (MPN) (see 10.16.2.3.1) be used for determining the number of coliform bacteria in sea water.

10.16.1.2 Examination for faecal coliform bacteria

Proceed as in 10.16.1.1, but use a plate of mFC agar (see 10.4.44), and incubate the mFC agar at 44 °C ± 0,25 °C for 18 h to 24 h in a watertight container in a water-bath. Examine the plates and count the number of colonies that exhibit a blue centre with a translucent periphery. These colonies are suspect faecal coliform bacteria. Non-faecal coliform colonies are grey to cream coloured. To confirm the presence of faecal coliform bacteria, subculture each colony or a representative number of them (i.e. colonies that exhibit a blue centre with a translucent periphery) into tubes or bottles of lactose peptone water (see 10.4.48) and incubate at 44 °C ± 0,25 °C for 24 h. Examine for gas formation. Gas formation is indicated by an amount of gas at least sufficient to fill the concavity of the top of the Durham tube. If the medium shows the formation of gas, as indicated by gas in the Durham tube, use a platinum wire loop to subculture, from each bottle, one loopful into a bottle of tryptone water (see 10.4.7) and incubate these subcultures at 44,5 °C ± 0,25 °C for 16 h to 20 h in a watertight container in a water-bath. Add 0,1 mℓ to 0,5 mℓ of Kovacs reagent (see 10.4.8) to the culture in the tryptone water. Mix by gently shaking the bottle. The development of a red colour denotes the presence of indole and confirms the presence of faecal coliform bacteria.

Use the same equation as in 10.16.1.1 to calculate the number of faecal coliform bacteria per 100 mℓ of water sample.

NOTE – It is recommended that the most probable number (MPN) method (see 10.16.2.3.2) be used for determining the number of faecal coliform bacteria in sea water.

10.16.2 Most probable number (MPN) method

10.16.2.1 Preparation of the test portion and tenfold dilution series

Thoroughly mix the water sample by inverting and righting the sample container approximately 10 times (by rapid movement of the wrist). The interval between mixing and removal of the test portion should not exceed 3 min. Remove 1 mℓ of the sample with a sterile pipette and add to 9 mℓ of sterile peptone water (see 10.4.47). Thoroughly mix this primary dilution by rapidly inverting and righting the container approximately 10 times. Use a fresh pipette to transfer 1 mℓ of the primary dilution into another bottle containing 9 mℓ of sterile peptone water (see 10.4.47), and mix thoroughly. Repeat these operations to obtain a tenfold dilution series. Prepare a sufficient number of dilutions to ensure that all bottles containing the final dilution will yield a negative result.

10.16.2.2 Inoculation of lauryl tryptose broth

Use a sterile pipette to transfer 10 mℓ of the water sample to each of three tubes or bottles that contains double-strength lauryl tryptose broth (see 10.4.46). Use a sterile pipette to transfer 1 mℓ of the water to each of three tubes or bottles that contain single-strength lauryl tryptose broth (see 10.4.45). Transfer 1 mℓ of each of the subsequent dilutions (see 10.16.2.1) into each of three tubes or bottles that contain single-strength lauryl tryptose broth (see 10.4.45). Use a fresh sterile pipette for each dilution. Incubate the inoculated tubes or bottles at 37 °C ± 0,5 °C for 48 h.

Examine the cultures after incubation and regard as positive reactions those that show turbidity due to bacterial growth and gas formation. Gas formation is indicated by an amount of gas at least sufficient to fill the concavity of the top of the Durham tube (see 10.2.11). For each dilution, count and record the number of tubes or bottles that show a positive reaction.

10.16.2.3 Confirmatory tests

10.16.2.3.1 Coliform bacteria

To confirm the presence of coliform bacteria, subculture, from each tube or bottle of lauryl tryptose broth that gives a positive result, into a tube or bottle or single-strength brilliant green bile medium (see 10.4.5) and incubate at 37 °C ± 0,5 °C. Examine for gas formation within 48 h. The formation of gas confirms the presence of coliform bacteria.

10.16.2.3.2 Faecal coliform bacteria

Subculture from each tube or bottle of lauryl tryptose broth that gives a positive result for gas formation, into a bottle of tryptone water (see 10.4.7). Incubate in a water-bath, maintained at 44,5 °C ± 0,25 °C, for 24 h. Add 0,1 mℓ to 0,5 mℓ of Kovacs reagent (see 10.4.8) to the culture in the tryptone water. Mix by gently shaking the bottle. The development of a red colour denotes the presence of indole.

10.16.2.4 Expression of results

10.16.2.4.1 From the number of tubes or bottles of lauryl tryptose broth and confirmatory tests that gives positive reactions, calculate, by reference to table 4, the most probable number of coliform and faecal coliform bacteria.

10.16.2.4.2 Selection of dilutions

For each sample examined, select three consecutive dilutions in accordance with one of the following rules, as appropriate:

a) select the highest dilution (i.e. that having the lowest concentration or the smallest amount of sample) that gives positive results together with the two preceding sets of dilutions (see 10.16.2.4.4, examples A and B);

b) if fewer than three sets of dilutions give positive results, start with the set that contains the lowest dilution (i.e. that having the highest concentration or the largest amount of sample) (see 10.16.2.4.4, example C); or

c) if only one set of dilutions gives a positive result, use this dilution and the one higher and lower (see 10.16.2.4.4, example D), except when the set of dilutions that gives a positive result is found at the level of the first dilution prepared. In this case, it is necessary to select the first three dilutions for the calculation of the MPN.

10.16.2.4.3 Determination of the MPN index

From table 4, determine the MPN index by the number of positive tubes or bottles in each of the three consecutive dilutions (selected in accordance with 10.16.2.4.2(a) to (c)).

Example: should the number of positive tubes or bottles for the three consecutive dilutions read 3, 2, 1 (see 10.16.2.4.4, example B), then, by consulting table 4, find, in the first three columns, the appropriate sequence of numbers, i.e. 3, 2, 1 and in the fourth column, read the MPN index, which, in this case, is 15.

10.16.2.4.4 Calculation of the most probable number

Calculate the number of coliform and faecal coliform bacteria per 100 mℓ of the water sample by multiplying the MPN index (see 10.16.2.4.3) by the reciprocal of the lowest dilution selected (i.e. that having the highest sample concentration), multiplied by 100. When the lowest dilution selected corresponds to the tube or bottle inoculated with 10 mℓ, first divide the MPN index by 10. The result can be expressed as a number between 1,0 and 9,9 multiplied by 10^x, where x is the appropriate power of 10. Confidence limits are given in table 4.

Example: with reference to example A in the text, the MPN index of 15 (see 10.16.2.4.3) is divided by 10, i.e. the volume of the inoculum in the lowest dilution selected. The quotient thus obtained, i.e. 1,5, is multiplied by 1,0, i.e. the reciprocal of the lowest dilution (10^0) selected. The product 1,5 is then multiplied by 100, the specified reference volume, to obtain the number of bacteria per 100 mℓ of water sample.

Example A

Test sample (dilution 10^0) (10 mℓ) :	3	tubes +; 0 tubes –
Test sample (dilution 10^0) (1 mℓ) :	2	tubes + ; 1 tube –
Test sample Dilution 10^{-1} (1 mℓ) :	1	tube + ; 2 tubes –

Dilution 10^{-2} (1 mℓ) : 0 tube + ; 3 tubes –
Dilution 10^{-3} (1 mℓ) : 0 tube + ; 3 tubes –

From table 4, the MPN index is 15 and the calculation gives an MPN of $\frac{15}{10}$ x 1 x 100, i.e.1,5 x 10^2 bacteria per 100 mℓ of water sample.

Example B

Test sample (dilution 10^0) (10 mℓ) :	3	tubes + ; 0 tubes –
Test sample (dilution 10^0) (1 mℓ) :	3	tubes + ; 0 tubes –
Test sample		
(dilution 10^{-1}) (1 mℓ) :	3	tubes + ; 0 tubes –
(dilution 10^{-2}) (1 mℓ) :	2	tubes + ; 1 tube –
(dilution 10^{-3}) (1 mℓ) :	1	tube + ; 2 tubes –
(dilution 10^{-4}) (1 mℓ) :	0	tube + ; 3 tubes –

From table 4, the MPN index is 15 and the calculation gives an MPN of 15 x 10 x 100, i.e. 1,5 x 10^4 bacteria per 100 mℓ of water sample.

Example C

Test sample (dilution 10^0) (10 mℓ) :	2	tubes + ; 1 tubes –
Test sample (dilution 10^0) (1 mℓ) :	1	tube + ; 2 tubes –
Test sample (dilution 10^{-1}) (1 mℓ) :	0	tube + ; 3 tubes –

(dilution 10^{-2}) (1 mℓ) : 0 tube + ; 3 tubes –
(dilution 10^{-3}) (1 mℓ) : 0 tube + ; 3 tubes –

From table 4, the MPN index is 1,5 and the calculation gives an MPN of $\frac{1,5}{10}$ x 1 x 100, i.e. $1,5 \times 10^1$ bacteria per 100 mℓ of water sample.

Example D

Test sample (dilution 10^0) (10 mℓ) :	0	tubes + ; 3 tubes –
Test sample (dilution 10^0) (1 mℓ) :	1	tube + ; 2 tubes –
Test sample (dilution 10^{-1}) (1 mℓ) :	0	tube + ; 3 tubes –

Dilution 10^{-2} (1 mℓ) : 0 tube + ; 3 tubes –
Dilution 10^{-3} (1 mℓ) : 0 tube + ; 3 tubes –

From table 4, the MPN index is 0,3 and the calculation gives an MPN of $\frac{0,3}{10}$ x 1 x 100, i.e. 3×10^0 bacteria per 100 mℓ of water sample.

Table 4 — MPN index and confidence limits

1	2	3	4	5	6
Number of positive tubes for the three dilutions selected			MPN index	Confidence limits	
First	Second	Third		> 95 %	
0	0	0	< 0,30	0,00	0,94
0	0	1	0,30	0,01	0,95
0	1	0	0,30	0,01	1,0
0	1	1	0,61	0,12	1,7
0	2	0	0,62	0,12	1,7
0	3	0	0,94	0,35	3,5
1	0	0	0,36	0,02	1,7
1	0	1	0,72	0,12	1,7
1	0	2	1,1	0,4	3,5
1	1	0	0,74	0,13	2,0
1	1	1	1,1	0,4	3,5
1	2	0	1,1	0,4	3,5
1	2	1	1,5	0,5	3,8
1	3	0	1,6	0,5	3,8
2	0	0	0,92	0,15	3,5
2	0	1	1,4	0,4	3,5
2	0	2	2,0	0,5	3,8
2	1	0	1,5	0,4	3,8
2	1	1	2,0	0,5	3,8
2	1	2	2,7	0,9	9,4
2	2	0	2,1	0,5	4,0
2	2	1	2,8	0,9	9,4
2	2	2	3,5	0,9	9,4
2	3	0	2,9	0,9	9,4
2	3	1	3,6	0,9	9,4
3	0	0	2,3	0,5	9,4
3	0	1	3,8	0,9	10,4
3	0	2	6,4	1,6	18,1
3	1	0	4,3	0,9	18,1
3	1	1	7,5	1,7	19,9
3	1	2	12	3	36
3	1	3	16	3	38
3	2	0	9,3	1,8	36
3	2	1	15	3	38
3	2	2	21	3	40
3	2	3	29	9	99
3	3	0	24	4	99
3	3	1	46	9	198
3	3	2	110	20	400
3	3	3	> 110		

No. R. 530 **14 Mei 1999**

WET OP STANDAARDE, 1993

VERPLIGTE SPESIFIKASIE VIR BEVRORE VIS, BEVRORE SEESKULPDIERE EN BEVRORE PRODUKTE WAT DAARUIT VERKRY WORD

Ek, Alexander Erwin, Minister van Handel en Nywerheid, verklaar hierby kragtens artikel 22(1)(a)(i) van die Wet op Standaarde, 1993 (Wet No 29 van 1993), en op aanbeveling van die Raad van die Suid-Afrikaanse Buro vir Standaarde, die spesifikasie vir bevrore vis, bevrore seeskulpdiere en bevrore produkte wat daaruit verkry word, ooreenkomstig die besonderhede in die Bylae uiteengesit, tot verpligte spesifikasie met ingang van die datum 2 maande na die datum van publikasie van hierdie kennisgewing, met die gelyktydige terugtrekking van die bestaande verpligte spesifikasie vir bevrore vis, bevrore seeskulpdiere en produkte van bevrore vis en bevrore seeskulpdiere, gepubliseer by Goewermentskennisgewing No R.35 van 12 Januarie 1973.

ALEXANDER ERWIN
Minister van Handel en Nywerheid

BYLAE

VERPLIGTE SPESIFIKASIE VIR BEVRORE VIS, BEVRORE SEESKULPDIERE EN BEVRORE PRODUKTE WAT DAARUIT VERKRY WORD

1 Bestek

Hierdie spesifikasie dek die vereistes vir die hantering, voorbereiding, verwerking, verpakking, bevriesing, bewaring en kwaliteit van bevrore vis, bevrore seeskulpdiere, bevrore visprodukte en bevrore seeskulpdierprodukte wat vir menslike verbruik bedoel is. Dit dek ook vereistes vir fabrieke en werknemers wat by die produksie betrokke is.

2 Woordbepaling

Die volgende woordbepalings geld vir die doel van hierdie spesifikasie:

2.1 aanneemlik: Aanneemlik vir die owerheid wat hierdie spesifikasie administreer.

2.2 voorbevriesing in pekel: 'n Proses waarvolgens vis vinnig bevries word deur middel van totale onderdompeling in verkoelde pekel by 'n temperatuur van –7 °C of laer.

OPM – Voorbevriesing in pekel voltooi nie die bevriesingsproses wat hieronder as die "bevriesingsproses" (2.11) omskryf word nie.

2.3 neweprodukt: 'n Produk wat nie vir menslike verbruik bedoel is nie.

2.4 verkilkamer: 'n Geïsoleerde en verkoelde kamer wat spesiaal ontwerp is vir die bewaring van voedsel by temperature van laagstens –1 °C en hoogstens 4 °C, wat voldoende verkoelvermoë het om die gewenste bewaartemperatuur te handhaaf en wat ook voldoende verkoelvermoë kan hê om die produkte wat daarin geplaas word tot die genoemde temperatuur te verkoel.

OPM – Indien die produk met ys in 'n verkilkamer bewaar moet word, is die woordbepaling hierbo nie van toepassing nie.

2.5 fabriek: 'n Perseel, voertuig of vaartuig waarop of waarin vis (kyk 2.6) of visprodukte (kyk 2.8) gehanteer of behandel word om dit vir bevriesing vir handelsdoeleindes voor te berei. Hierdie woordbepaling sluit viswinkels, hotelle, losieshuise, restourante of ander eetplekke uit.

2.6 vis: Enige eetbare, koudbloedige, gewerwelde waterdier (see- of varswaterdier) wat vir sy hele lewensduur kiewe het en waarvan ledemate tot vinne ontwikkel het, asook alle seeskulpdiere en kefalopodes (kyk 2.13).

2.7 visgraatdefek: 'n Visgraat wat 10 mm of langer is, of 'n diameter van 1 mm of meer het. 'n Graat wat hoogstens 5 mm lank is, word nie as 'n defek beskou indien sy diameter hoogstens 2 mm is nie. Die voet van 'n graat (waar dit aan die rugwerwel vas is) kan verontagsaam word indien sy breedte nie 2 mm oorskry nie, of as dit maklik met 'n vingernael afgestroop kan word.

2.8 visproduk: Enige visproduk, in 'n verwerkte vorm al dan nie, wat heeltemal of gedeeltelik uit vis verkry is (kyk 2.6) en vir menslike verbruik bedoel is.

2.9 vrieskamer: 'n Kamer of toerusting wat spesiaal ontwerp is om die temperatuur van 'n voedselproduk deur die sone van maksimum kristallisasie (kyk 2.11) en laer tot by 'n ewewigstemperatuur van –20 °C of laer te verlaag binne die tydperk wat ten opsigte van die produk aanneemlik is.

2.10 vriesbewaarkamer: 'n Geïsoleerde vrieskamer wat spesiaal ontwerp is vir die bewaring van bevrore voedsel en wat voldoende vriesvermoë het vir die handhawing van 'n produktemperatuur van –20 °C of laer, as produkte wat reeds tot dié temperatuur bevries is, daarin bewaar word.

OPM – 'n Vriesbewaarkamer is nie ontwerp om produkte te bevries nie.

2.11 bevriesingsproses: Die deurlopende proses waarvolgens die temperatuur van die produk teen 'n tempo van minstens 6 mm van die produk se dikte per uur deur die sone van maksimum kristallisasie (vir die meeste produkte tussen –1 °C en –5 °C) verlaag word en wat slegs voltooi is wanneer die temperatuur van die hele produk na termiese stabilisering –20 °C of laer bereik het.

OPM – Die proses waarvolgens 'n produk wat deur voorbevriesing in pekel bevries is, verder bevries word totdat die temperatuur van die hele produk na termiese stabilisering –20 °C of laer is, word vir die doel van hierdie spesifikasie as 'n deurlopende proses beskou, mits die temperatuur van die produk tydens hierdie proses nie hoër as –7 °C styg nie.

2.12 bevrore visproduk: 'n Visproduk (kyk 2.8), gerook of ongerook, gaar of nie gaar nie, wat bevries is en deur vriesbewaring in die bevrore toestand gepreserveer is.

2.13 seeskulpdier: Enige ongewerwelde seedier van die filum molluske, met 'n sagte ongesegmenteerde liggaam en wat dikwels 'n skulp het wat deur 'n vou van die vel (die mantel) versteek is. Die groep sluit tweekleppige diere (byvoorbeeld gapermossels en mossels) en kefalopodes (byvoorbeeld inkvis) in.

2.14 fyngemaakte vis: Fyngemaakte stukkies visskeletspier wat van die visgrate en vel losgemaak en aanneemlik vry van visgrate en vel is.

2.15 buitehouer (hoofhouer): Die doos, kartonhouer of kis waarin pakkette (toegedraaide of ontoegedraaide) bevrore visprodukte vir bewaring en verspreiding verpak word.

2.16 pakket (onmiddellike houer): Die onmiddellike kartonhouer, plastieksakkie of ander houer waarin die produk vir bewaring en verspreiding verpak word.

2.17 preserveer: In 'n gebrekvry, eetbare toestand hou deur die voorkoming van agteruitgang.

2.18 proses: Die reeks bewerkings tydens produksie (kyk 2.20) van die produk.

2.19 produk: Vis, seeskulpdiere, vis- of seeskulpdierprodukte vir menslike verbruik, tydens vervoer, hantering, voorbereiding, verwerking of verpakking vir bevriesing, in die proses van bevriesing of nadat dit reeds bevries is, soos uit die verband van die spesifikasie blyk.

2.20 produksie: Die hantering, voorbereiding, verwerking of verpakking vir bevriesing, in die proses van bevriesing of nadat dit reeds bevries is, met inbegrip van die proses van vriesbewaring, soos uit die verband van die spesifikasie blyk.

2.21 snelbevriesingsproses: Die deurlopende proses waarvolgens die temperatuur van die produk teen 'n tempo van minstens 25 mm van die produk se dikte per uur binne 4 h deur die sone van maksimum kristallisasie (vir die meeste produkte tussen –1 °C en –5 °C) verlaag word en wat slegs voltooi is wanneer die temperatuur van die hele produk na termiese stabilisering –20 °C of laer bereik het.

2.22 snelbevrore produk: 'n Visproduk (kyk 2.8), gerook of ongerook, gaar of nie gaar nie, wat volgens die snelbevriesingsproses bevries is.

2.23 pen: 'n Pen waarop vissnitte (met of sonder groente of vrugte of enige kombinasie daarvan) bymekaargehou kan word en wat van hout of metaal kan wees of van plastiekmateriaal wat geskik is om in aanraking met voedsel gebruik te word.

2.24 rook; houtrook: Rook wat verkry word van hout of houtagtige plante wat aanneemlik vry van gom, hars, verf, houtverduursamingsmiddel en ander bygevoegde stowwe is, wat in 'n staat van verbranding of wrywing is en wat gebruik kan word om vis te berook in oonde wat vir dié doel ontwerp is, of wat gekondenseer of geabsorbeer kan word in 'n geskikte voedselgraad vloeistof of poeier vir die voorbereiding van 'n rookdoopmiddel.

2.25 geskik: Aanneemlik en wat aan die vereistes vir die beoogde doel voldoen.

2.26 geskikte korrosiebestande materiaal: Ondeurlatende materiaal met gladde oppervlakke (vry van gaatjies, splete en skaal), wat nietoksies is en nie aangetas word deur seewater, ys, visslym en enige ander korroderende stof waarmee dit waarskynlik in aanraking sal kom nie en wat teen blootstelling aan herhaalde skoonmaak, met inbegrip van die gebruik van detergente, bestand is.

3 Vereistes vir die fabriek

3.1 Algemeen

Daar moet voldoen word aan al die statutêre vereistes van die Wet op Beroepsgesondheid en Veiligheid, 1993 (Wet 85 van 1993), die Wet op Gesondheid, 1977 (Wet 63 van 1977), die Wet op Reëling van die Uitvoer van Bederfbare Produkte, 1983 (Wet 9 van 1983), of enige ander toepaslike wet.

3.2 Konstruksie en uitleg van en toestande in fabriek

3.2.1 Ligging, grootte en higiëniese ontwerp en toestande

3.2.1.1 Die ligging van die fabriek moet sodanig wees dat die geboue aanneemlik vry van aanstootlike reuke, rook, stof en ander kontaminasie gehou kan word sodat dit aan die toepaslike vereistes vir higiëne en sanitasie van die Wet op Gesondheid, 1977, voldoen. Die fabrieksgeboue moet 'n stewige konstruksie hê, in 'n goeie toestand wees en groot genoeg wees om te verhoed dat toerusting en werknemers saamgedring word en om toereikende skoonmaak en die handhawing van produkkwaliteit en higiëne moontlik te maak.

3.2.1.2 Die fabrieksperseel moet goed gedreineer en toereikend omhein wees om groter diere soos katte en honde, sowel as ongemagtigde persone en voertuie, uit te hou. Buitenshuise werkgebiede, paaie en voetpaaie op die perseel moet 'n permanente oppervlak van beton, baksteen, bitumen of ander duursame materiaal hê. Gebiede wat rondom geboue geleë is en nie gebruik word nie, moet met grasperke bedek wees of 'n oppervlak hê wat waarskynlik nie stof sal veroorsaak nie en nie toksiese stowwe bevat nie.

3.2.1.3 Die fabriek en toerusting moet só ontwerp wees dat onverwerkte materiaal sonder oormatige vertraging verwerk kan word. Die ontwerp en konstruksie van die geboue moet só wees dat daar voorkom word dat insekte, voëls, knaagdiere en ander ongediertes die gebou binnekom of skuiling daarin vind.

3.2.2 Dakke en plafonne

3.2.2.1 Dakke moet weerbestand wees en moet van 'n nie-absorberende materiaal gemaak wees. Dakke en, indien toepaslik, plafonne moet styf teen die mure pas en moet minstens 2,4 m bo die vloer wees. In die voorbereidings-, verwerkings- en verpakkingsgebiede moet die dak en, indien toepaslik, die

plafon minstens 300 mm bo enige toerusting wees en hoog genoeg wees om die vrye beweging van mobiele toerusting en bewegende dele van ander toerusting toe te laat.

3.2.2.2 In die voorbereidings-, verwerkings- en verpakkingsgebiede en in bewaargebiede vir bestanddele en verpakkingsmateriaal vir die produk moet die plafon (of, indien daar nie 'n plafon is nie, die dak), stofdig wees en beklee wees met geskikte korrosiebestande, ligkleurige en ondeurlatende materiaal waarvan die konstruksie en afwerking sodanig is dat kondensasie, skimmelvorming, afskilfering en vaskleef van vuiligheid tot 'n minimum beperk word en sonder beskadiging skoongemaak kan word. Die onderkant moet 'n gladde oppervlak hê. Gebiede waar sous berei word of waar die gaar produk gehanteer word, of waar bestanddele en verpakkingsmateriaal bewaar word, moet 'n plafon hê.

3.2.3 Mure en deure

3.2.3.1 Buitemure moet weerdig en waterdig wees. Binneoppervlakke van mure moet met gladde, ligkleurige, wasbare, waterdigte materiaal beklee wees en moet vry wees van onnodige uitsteeksels. Hierbenewens moet die mure in die voorbereidings-, verwerkings- en verpakkingsgebiede tot op 'n hoogte van 2 m bo die vloer met geskikte korrosiebestande, ligkleurige, wasbare en slagbestande materiaal beklee wees, behalwe dat waar mure bo hierdie hoogte vuilgesmeer kan word, die bekleding tot op 'n hoër hoogte moet strek. Alle lyste aan die binnekant van mure en alle vensterbanke moet met 'n hoek van minstens 45° na die vloer afloop. Die lyste moet so klein moontlik gehou word en vensterbanke moet minstens 1 m bo die vloervlak wees. In die voorbereidings-, verwerkings- en verpakkingsgebiede en in vries-, verkil- en vriesbewaarkamers moet die aansluitings tussen mure en die aansluitings tussen vloere en mure met 'n minimum radius van onderskeidelik 25 mm en 40 mm gerond wees.

3.2.3.2 Deure en deurrame moet met geskikte korrosiebestande materiaal beklee wees of daarvan gemaak wees en moet naatlose, ligkleurige, ondeurlatende en wasbare oppervlakke hê. Indien hout gebruik word, moet dit beklee wees om dit waterdig te maak. Deure waardeur die produk tussen die voorbereidings-, verwerkings- en verpakkingsgebiede beweeg, moet breed genoeg wees om kontaminasie van die produk en beskadiging van die deure te voorkom. Alle deure wat van buite direk in die voorbereidings-, verwerkings- en verpakkingsgebiede oopgaan, moet doeltreffende lugskerms hê en moet sover dit prakties moontlik is vanself toegaan en dig sluit. Die deure van vries-, verkil- en vriesbewaarkamers moet dig sluit.

3.2.4 Vloere

3.2.4.1 Vloere moet gemaak wees van beton of van ander paslik ondeurlatende materiaal wat korrosiebestand is, maklik skoongemaak kan word en 'n gelyk oppervlak het wat glad maar nie glyerig is nie en vry van barste en oop voeë is.

3.2.4.2 Vloere van die voorbereidings-, verwerkings- en verpakkingsgebiede en van vries-, verkil- en vriesbewaarkamers moet 'n geskikte helling hê en moet na buiterioolputte, opvangputte en -riole afvoer. Uitlate moet net buite die fabrieksmure 'n sperder hê wat voorkom dat knaagdiere binnedring.

3.2.4.3 Afvoerkanale moet van die oop tipe met, indien nodig, verwyderbare deksels wees en moet ontwerp wees om die maksimum verwagte vloeistofvloei te hanteer sonder om oor te loop of oorstroming te veroorsaak. Daar mag geen installasie wat die vloei van water en skoonmaakwerk belemmer in 'n afvoerkanaal wees nie. Rioolputsperders moet toegerus wees met siwwe wat maklik verwyder kan word. Indien nodig, moet plankmatte van ondeurlatende materiaal wat maklik skoongemaak kan word, voorsien word. Houtplankmatte mag nie in nat gebiede gebruik word nie. Vloere en afvoerders moet in 'n goeie toestand gehou word.

3.2.5 Hysbakke en trappe

3.2.5.1 Die binneoppervlakke van hysbakke moet paslik korrosiebestand wees en hysbakskagte moet behoorlik gedreineer en vir skoonmaakdoeleindes toeganklik wees. Maasdeure kan gebruik word, mits dit nie onhigiëniese toestande bevorder nie.

3.2.5.2 By trappe in vertrekke waar die produk voorberei, verwerk, verpak of gehanteer word, moet die openinge tussen treevlakke met soliede stygstukke toegemaak wees. Trappe moet soliede relings hê

wat hoog genoeg is om te voorkom dat produkte onder die trap gekontamineer word.

3.2.6 Kabels en pype

3.2.6.1 Kabels en pype moet, indien toepaslik,

a) bokant plafonne bevestig wees, of

b) in mure ingelaat wees, of

c) weg van mure en plafonne en bokant die vloer bevestig wees en só gespasieer wees dat die plafonne, mure, vloer, kabels en pype maklik skoongemaak en in 'n higiëniese toestand gehou kan word; of

d) onder die vloer deurloop.

3.2.6.2 Afvoer- en rioolpype mag nie bokant plafonne in voorbereidings-, verwerkings- of verpakkingsgebiede geïnstalleer word nie en ook nie op só 'n wyse dat toevallige lekkasie die produk kan kontamineer nie. Die afvoer- en rioolpype moet 'n binnediameter van minstens 100 mm hê en moet behoorlik na buite ontlug wees.

3.2.7 Verligting

In die algemene werkgebiede moet verligting van minstens 220 lx voorsien word en op plekke waar die produk noukeurig ondersoek word, moet die verligting minstens 540 lx wees. Die verligting moet sodanig wees dat dit nie die voorkoms van die kleur van die produk beduidend verander nie. Armature wat hang bokant werkgebiede waar die produk tydens enige stadium van voorbereiding, verwerking of verpakking gehanteer word, moet van die veiligheidstipe wees of andersins só beskerm wees dat kontaminasie van die produk, indien 'n armatuur of lamp sou breek, voorkom word.

3.2.8 Ventilasie

Die ventilasie moet sodanig wees dat dit die lug vars hou en oormatige waterdamp verwyder en dat dit die opbou van oormatige hitte, die vorming van kondensaat en skimmelgroei op oorhoofse strukture voorkom. Die lug moet vry van skadelike walms, dampe, stof en kontaminerende aërosol wees. Die lug moet van meer higiëniese na minder higiëniese gebiede vloei. Natuurlike ventilasie moet, indien nodig, deur meganiese middele aangevul word.

Vensters wat vir ventilasiedoeleindes oopgemaak word, moet insekskerms hê. Die skerms moet maklik vir skoonmaakdoeleindes afgehaal kan word en moet van geskikte korrosiebestande materiaal wees.

3.2.9 Handwasfasiliteite

3.2.9.1 Die volgende moet voorsien word by die ingange na die voorbereidings- en verwerkingsgebiede van die fabriek wat deur die werknemers gebruik word, asook op ander gerieflik geleë plekke in die voorbereidings- en verwerkingsgebiede van die fabriek waar die werknemers dit maklik kan bereik, en by die uitgange van toilette:

a) 'n aanneemlike getal handwasbakke met volop warm en koue lopende water of warm water in die temperatuurbestek 40 °C tot 50 °C en wat aan die vereistes van 3.4.1 voldoen;

b) volop ongeparfumeerde vloeibare seep of aanneemlike detergent in 'n aktiewe toestand;

c) weggooipapierhanddoeke; en

d) krane wat nie met die hand of elmboog beheer word nie, bv knie- of voetbeheerde krane of drukknopkrane met vooraf gestelde volumebeheer.

3.2.9.2 Handontsmettingsbakke, indien voorsien, moet só ontwerp wees dat hulle goed skoongemaak

kan word. Toegang tot handwasfasiliteite moet te alle tye onbelemmer wees. Die handwasbakke moet van geskikte korrosiebestande materiaal wees, 'n gladde afwerking hê en regstreeks in afvoerkanale dreineer.

3.2.9.3 In die geval van fabriekskepe moet minstens een handwasbak in die toiletblok en een in die verwerkings- en verpakkingsgebied lopende warm en koue water voorsien.

3.2.10 Voetbaddens

Tensy die afwesigheid van voetbaddens in bepaalde omstandighede aanneemlik is of alternatiewe aanneemlike skoonmaak- en ontsmettingsfasiliteite voorsien word, moet voetbaddens met 'n geskikte ontsmettingsoplossing voorsien word by elke ingang na die voorbereidings-, verwerkings- en verpakkingsgebiede wat deur werknemers gebruik word en dit moet só geplaas wees dat werknemers nie toegang tot dié gebiede kan verkry sonder om hulle skoeisel te ontsmet nie. Voetbaddens moet só gemaak wees dat hulle voldoende gedreineer en skoongemaak kan word.

3.2.11 Kennisgewings

Kennisgewings moet op strategiese plekke in die voorbereidings-, verwerkings-, verpakkings- en bewaargebiede, in die kleedkamers en in die toiletfasiliteite vertoon word. Die kennisgewings moet vereis dat hande met seep of detergent gewas word en dit moet aandui dat spoeg, die gebruik van kougom en enige vorm van tabak en die eet of drink van verversings in hierdie gebiede verbode is.

3.2.12 Skeiding van prosesse en fasiliteite

Afsonderlike vertrekke of duidelik afgebakende gebiede van geskikte grootte moet voorsien word vir:

a) die ontvangs en bewaring van onverwerkte materiaal;

b) voorbereidingswerk soos verwydering van die kop, uithaal van ingewande en was van vis;

c) verwerkingsaktiwiteite soos filering, skyfsny en bevriesing;

d) verpakking; en

e) die bewaring van die produk.

3.2.13 Bewaarplekke

3.2.13.1 Algemeen

Die produksiegebied van die fabriek mag nie vir bewaardoeleindes gebruik word nie.

3.2.13.2 Eetbare bestanddele

Bewaarfasiliteite vir eetbare bestanddele wat by die voorbereiding van die bevrore produk gebruik word, moet droog, vry van stof en ander bronne van kontaminasie, en plaagdig wees.

3.2.13.3 Pakket- en verpakkingsmateriaal

Skoon, stofvry, plaagdigte en droë pakkamers moet vir die bewaring van verpakkingsmateriaal voorsien word.

3.2.13.4 Bewaarfasiliteite vir giftige en ander skadelike materiaal

3.2.13.4.1 Bewaarfasiliteite vir plaagdoders of ander giftige en skadelike materiaal

Plaagdoders of ander giftige en skadelike materiaal en die toerusting vir die toediening daarvan moet bewaar word in 'n vertrek waarin geen voedsel, voedselhanteertoerusting, verpakkingsmateriaal of

voedselhouers bewaar word nie en dit moet gesluit gehou word. Alle gevaarlike materiaal moet opvallende en duidelike etikette hê en mag nooit met die voedselhouers, verpakkingsmateriaal, onverwerkte materiaal of die produk in aanraking kom nie.

3.2.13.4.2 Bewaarfasiliteite vir skoonmaak- en ontsmettingsmateriaal

Skoonmaak- en ontsmettingsmateriaal en die toerusting vir die toediening daarvan moet bewaar word in 'n vertrek waarin geen voedsel, voedselhanteertoerusting, verpakkingsmateriaal of voedselhouers bewaar word nie en dit mag nooit met voedselhouers, verpakkingsmateriaal, onverwerkte materiaal of die produk in aanraking kom nie. Alle skoonmaak- en ontsmettingsmateriaal moet opvallende en duidelike etikette hê.

3.2.14 Bewaarfasiliteite vir gereedskap en onderdele

Gereedskap en onderdele wat tydens gebruik met die produk in aanraking kom, moet, wanneer dit nie gebruik word nie, in 'n ontsmettingsoplossing gehou word of op 'n higiëniese wyse bewaar word in 'n droë gebied wat vry van stof en enige ander bron van kontaminasie is en wat plaagdig is. Onderdele van masjinerie wat die produk moontlik kan kontamineer, moet in 'n afsonderlike bewaargebied weg van die verwerkingsgebiede bewaar word.

3.2.15 Rookkamer

Deure wat gedurende die stook van rookkamers gebruik word, mag nie direk in verwerkingsgebiede oopgaan nie tensy die rookgenereerder só ontwerp is dat dit besoedeling van hierdie gebiede uitskakel. Afsonderlike fasiliteite moet vir die bewaring van rookgenererende materiaal voorsien word.

3.2.16 Vrieskamers, verkilkamers en vriesbewaarkamers

3.2.16.1 Verkoelingseenhede, soos kompressors, mag nie geïnstalleer word in 'n gebied waar die produk gehanteer word nie, tensy die toerusting 'n integrerende deel van die produksie-eenheid is. Indien vrieskamers, verkilkamers en vriesbewaarkamers in verwerkingsgebiede geleë is, moet hul vloere 'n integrerende deel van die vloer van die verwerkingsgebied wees of op toereikende wyse aan die vloer verseël wees. Bewaareenhede moet hoog genoeg bokant die vloer geïnstalleer wees sodat die gebied daaronder maklik en deeglik skoongemaak kan word.

3.2.16.2 Die mure en vloere moet in 'n goeie toestand wees. Die oppervlakke van plafonne, mure en vloere moet van geskikte korrosiebestande, waterdigte materiaal wees en moet glad en vry van barste, splete en afskilfering van oppervlakmateriaal wees. Die vloere moet gedreineer kan word en die vloere van verkilkamers moet skuins wees sodat dit heeltemal gedreineer kan word.

3.2.16.3 Vriesbewaarkamers in fabrieke, uitgesonderd fabriekskepe, moet outomatiese temperatuur-registreerders hê met genoeg sensorelemente op plekke wat geskik is om die totale lugtemperatuur te moniteer. Die temperatuur in vriesbewaarkamers moet outomaties en deurlopend gemoniteer word en 'n rekord van die temperatuur moet gehou word en ter insae beskikbaar wees. Temperatuurkaarte moet só ingedeel wees dat elke indeling hoogstens 2 °C in die bewaarbestek verteenwoordig en dit moet maklik tot die naaste 1 °C in die bewaarbestek gelees kan word. Produksielotvrieskamers, uitgesonderd plaatbevriesers, moet eksterne meters of ander temperatuuraanwysers hê.

3.2.16.4 Vriesbewaarkamers op visvaartuie behoort temperatuurregistreerders hê, maar dit moet minstens eksterne meters of temperatuuraanwysers hê en die aangeduide temperature moet elke 4 h aangeteken word.

3.2.16.5 Die ingange na vries-, verkil- en vriesbewaarkamers moet teen die invloei van warm lug beskerm word deur die voorsiening van 'n voorvertrek of 'n meganiese luggordyn of strookgordyne of luike wat self toegaan.

3.2.17 Neweprodukte

Die verwerking van neweprodukte en nievisprodukte wat nie vir menslike verbruik bedoel is nie, moet gedoen word in geboue wat fisies só van die fabriek geskei is dat daar geen moontlikheid van kontaminasie van die produk is nie.

3.2.18 Woonkwartiere

Woonkwartiere moet heeltemal geskei wees van gebiede waar die produk voorberei, verwerk, verpak of bewaar word.

3.2.19 Vullis

'n Afsonderlike, geskikte vullisfasiliteit moet op die perseel voorsien word en moet daagliks skoongemaak word.

3.2.20 Fasiliteite

3.2.20.1 'n Aanneemlike getal geskikte kleedkamers, storthokkies, handwasbakke waarvan die krane werk soos in 3.2.9 beskryf word, toilette (afsonderlik vir elke geslag) en, indien toepaslik, urinale, moet binne 'n praktiese afstand van die fabriek se verwerkingsgebiede voorsien word. Storthokkies moet direk in kleedkamers uitgaan. Fasiliteite mag nie regstreeks in 'n voorbereidings-, verwerkings-, verpakkings- of bewaargebied uitgaan nie.

3.2.20.2 Toilette moet heeltemal afsonderlik van kleedkamers wees en die enigste toelaatbare toegang moet wees deur deure wat dig sluit en self toegaan. Toiletblokke moet hul eie handwasfasiliteite hê wat van dié in kleedkamers geskei is. Genoeg toiletpapier, lopende warm en koue water, naelborsels, ongeparfumeerde vloeibare seep of 'n aanneemlike detergentoplossing en weggooipapierhanddoeke moet vir werknemers beskikbaar wees. Houers vir gebruikte handdoeke moet voorsien word. Vullishouers met 'n higiëniese konstruksie moet verskaf word.

3.2.20.3 Daar moet kennisgewings wees wat vereis dat werknemers hulle hande na gebruik van die toilet met seep of detergent was. Sluitkaste of beheerde kleremandjies moet voorsien word en die uitleg en toerusting moet sodanig wees dat dit behoorlik skoongemaak en in stand gehou kan word. Die fasiliteite moet skoon en netjies gehou word. Die fasiliteite moet toereikend geventileer wees. Kleedkamers en aantrekkamers mag nie as woonkwartiere of vir die bereiding van maaltye gebruik word nie. Eetkamers vir personeel moet afsonderlik van kleedkamers wees.

3.2.21 Fasiliteite vir die skoonmaak en ontsmetting van draagbare toerusting

Fasiliteite met behoorlike dreinering moet vir die skoonmaak en ontsmetting van draagbare toerusting voorsien word. Sodanige fasiliteite moet in 'n afsonderlike vertrek wees of in 'n aangewese deel van die voorbereidings-, verwerkings- en verpakkingsgebiede waar daar genoeg koue drinkbare water en warm water, indien nodig, of versadigde stoom of skoon seewater is wat onder toereikende druk is en aan die vereistes van 3.4.2 voldoen.

3.2.22 Spesifieke vereistes vir visvaartuie

3.2.22.1 Algemene oorwegings

Visvaartuie moet vir vinnige en doeltreffende hantering van vis en vir maklike skoonmaak en ontsmetting ontwerp wees. Alle oppervlakke waarmee die vis moontlik in aanraking kan kom, moet ondeurlatend wees en moet, indien dit prakties uitvoerbaar is, van geskikte korrosiebestande materiaal wees. Die oppervlakke moet maklik skoongemaak kan word en mag geen uitsteeksels of ander eienskappe hê wat die produk kan beskadig nie.

Dektenks, penstaanders en skeiplanke moet van geskikte korrosiebestande materiaal gemaak wees en moet maklik verwyder kan word. Die getal en hoogte daarvan moet sodanig wees dat dit die geraamde hoeveelheid vis kan hou en dat daar voorkom word dat die vis beweeg en plat gedruk word as gevolg van

oormatige massa vis of die skip se beweging. Indien dit prakties uitvoerbaar is, moet hout met 'n geskikte korrosiebestande materiaal soos veselglas beklee wees, of moet dit só behandel wees dat dit waterdig is. Metaalwerk, uitgesonderd vlekvrystaal of gegalvaniseerde staal of aluminium wat nie met die produk in aanraking kom nie, moet met korrosiebestande, nietoksiese verf of 'n ander beskermende deklaag geverf wees.

'n Geskikte afvoerstelsel moet voorsien wees. Gebiede waar die produk voorberei, verwerk, verpak of bewaar word, moet deeglik van ghries, olie, brandstof, hitte, dampe, voedsel vir die bemanning, bewaargebiede vir ander materiaal as die produk, en van die enjinkamer en ander bronne van kontaminasie geïsoleer wees.

3.2.22.2 Bewaarfasiliteite vir vis

Indien vis binne 8 h nadat dit gevang is aan land gebring word, moet die vaartuig fasiliteite hê om die vis minstens teen direkte sonstrale te beskerm en om die vis koel (by 'n temperatuur van 20 °C of laer) en klam te hou.

Indien vis langer as 8 h maar hoogstens 10 d ter see gehou word, moet fasiliteite vir verkilling of bevriesing (of albei) ook voorsien word. Vaartuie waarop vis langer as 10 d gehou word, moet bevriesingsfasiliteite vir die vis hê.

Alle vaartuie wat vir verkilling of bevriesing (of albei) toegerus is, moet 'n geskikte afvoerstelsel hê wat die gesmelte water na 'n opvangput kan afvoer so vinnig as wat dit akkumuleer.

3.2.22.3 Seewater- en pekelbewaartenks

Op vaartuie waarin van 'n stelsel van verkoelde seewater of verkoelde pekel gebruik gemaak word om die vangs te verkil of te bewaar, moet alle tenks, hitteruilers, pompe en bybehorende pype van geskikte korrosiebestande materiaal gemaak wees of daarmee bedek wees. 'n Ruimte van minstens 60 mm moet tussen koelkronkels en tenkkante gelaat word om die skrop en uitspoel van visoorblyfsels uit die putte moontlik te maak. Die tenks of putte moet só ontwerp wees dat die vis eers met verkoelde seewater gewas en voorverkoel kan word en dan deur middel van pekelbevriesing voorbevries kan word. Pype, uitgesonderd koelkronkels, moet heeltemal versink en bedek wees.

3.2.22.4 Inneem van seewater en wegdoen van afval

Dekslange moet van skoon seewater onder aanneemlike druk voorsien word deur 'n pomp wat slegs vir skoon seewater gebruik word. Onderworpe aan goeie skeepsargitektuur, moet die inlaatpunt vir seewater vir die verkoeling en skoonmaak van die produk so diep as moontlik aan die een kant van die vaartuig geleë wees en die wegdoen van rioolvuil en afvalwater en enjinverkoelingsuitlaat moet so vlak as moontlik aan die teenoorgestelde kant van die vaartuig geskied.

Die watertoevoerpype en afvalafvoerpype vir die vaartuig se toilette, handwasbakke en kombuis-opwasbakke moet spitsbelastings kan dra, moet waterdig wees en mag nie deur ruimtes gaan waar die vangs voorberei, verwerk, verpak of bewaar word nie. Pype vir die voorsiening van skoon seewater mag geen kruisverbindings met die enjin- of kondensatorverkoelstelsel hê nie en die konstruksie daarvan moet sodanig wees dat enige moontlikheid van terugheweling van die kombuisopwasbakke of die toilette voorkom word.

3.2.22.5 Watervoorsiening

Genoeg koue drinkbare water of skoon seewater onder aanneemlike druk moet op 'n aanneemlike getal plekke oral op die visvaartuig beskikbaar wees. Op vaartuie waar ander verwerking as skoonmaak gedoen word, moet warm water by 'n temperatuur van minstens 60 °C vir gebruik beskikbaar wees. Indien dit prakties uitvoerbaar is, moet 'n aanneemlike waterbehandelingstelsel (bv blootstelling aan ultraviolet lig) voorsien word vir die behandeling van seewater wat by die verwerking van die produk gebruik word.

3.3 Produksietoerusting

3.3.1 Algemeen

3.3.1.1 Verwerkingsgebiede moet só ontwerp, toegerus en beman wees dat werkers vryelik kan beweeg om skoonmaak en die handhawing van higiëne sowel as produkkwaliteit te vergemaklik.

3.3.1.2 Alle installasies, toerusting en gereedskap wat met die produk in aanraking kom, moet 'n gladde oppervlak hê, lig van kleur wees en van geskikte korrosiebestande, nie-absorberende materiaal wees (dws nie hout of ander absorberende of poreuse materiaal nie), wat 'n aanneemlike plastiekbedekte oppervlak geskik vir gebruik met voedsel kan hê, maar dit moet verkieslik van vlekvrystaal wees. Dit moet 'n higiëniese ontwerp sonder oop lasse of splete hê en moet só gemaak wees dat dit die skoonmaak en ontsmetting daarvan vergemaklik. Die installasie of toerusting moet só ontwerp wees dat dit die skoonmaak en ontsmetting van die gebiede daaronder vergemaklik. Oop ente en omkrulrande moet bevredigend verseël wees ten einde die aanpak van organiese materiaal en vuiligheid te verhoed. Indien nodig, soos in die geval van toerusting wat nie in situ skoongemaak kan word nie, moet die toerusting vir skoonmaak en ontsmetting uitmekaar gehaal kan word. Oppervlakke waarmee die produk in aanraking kom, mag nie geverf wees nie.

3.3.1.3 Alle dele van vaste toerusting of toerusting wat nie maklik geskuif kan word nie moet só ver van die mure en plafonne af geïnstalleer wees dat daar toegang vir skoonmaak en ondersoek is. Alle permanent gemonteerde toerusting moet óf hoog genoeg bo die vloer geïnstalleer wees om toegang vir skoonmaak en ondersoek te verleen óf moet heeltemal teen die vloer verseël wees.

3.3.1.4 Toerusting moet verkieslik nie in die vloer versink wees nie maar indien dit onvermydelik is, moet die toerusting op 'n aanneemlike wyse geïnstalleer wees. Versonke dele moet goed gedreineer wees.

Koper, lood en legerings daarvan (uitgesonderd soldeersel) en ander metale of materiaal wat nadelig vir die gesondheid of vir die produk is, mag nie gebruik word in die konstruksie van toerusting wat in enige stadium van verwerking met die onverwerkte materiaal of met die onbeskermde produk in aanraking kom nie. Die gebruik van soldeersel in toerusting moet tot 'n minimum beperk word.

3.3.2 Tafels

Houttafels mag nie in verwerkingsgebiede gebruik word nie. Die ontwerp en konstruksie van tafelrame moet sodanig wees dat die ontwikkeling van onhigiëniese toestande en die opbou van bakterieë verhoed word. Die rame moet van gladde korrosiebestande metaal wees of moet sodanig bedek wees dat hulle teen korrosie beskerm is. Tafelblaaie moet van naatlose vlekvrymetaal of ander naatlose, korrosiebestande, gladde, ondeurlatende materiaal met soortgelyke oppervlakeienskappe wees. Hulle moet 'n higiëniese konstruksie hê en moet vir skoonmaakdoeleindes verwyder kan word of moet só aan hul rame bevestig wees dat skoonmaak en ontsmetting moontlik is. Indien metaalblaaie by die rande gevou is, moet die voue sodanig gesoldeer, gesweis of met 'n aanneemlike mastiekseëlmiddel verseël wees dat die aanpak van organiese stof en vuiligheid voorkom word. Alle tafelblaaie moet vinnig en doeltreffend kan dreineer en moet vry van barste en splete wees. Alle lasse in tafels moet waterdig gemaak wees.

3.3.3 Snyplanke

Indien snyplanke gebruik word, moet hulle 'n higiëniese konstruksie hê en van 'n aanneemlike ligkleurige materiaal (uitgesonderd hout of ander absorberende of poreuse materiaal) gemaak wees en vir gebruik met voedsel geskik wees. Snyplanke moet maklik verwyder kan word.

3.3.4 Gereedskap

Messe, skoppe, besems en ander gereedskap mag nie handvatsels van hout of ander absorberende of poreuse materiaal hê nie. Rottangmandjies mag op geen stadium voor, tydens of na verwerking as houers vir vis gebruik word nie.

3.3.5 Ontsmettings- en skoonmaakfasiliteite

Ontsmettingsfasiliteite vir handskoene en messe moet op gerieflike en aanneemlike plekke beskikbaar wees. Skoonmaak- en ontsmettingsmateriaal, lopende warm en koue water of versadigde stoom, waterslange, spuitkoppe, borsels, skrapers en ander toerusting wat vir die skoonmaak van die visvaartuig, installasie, toerusting en gereedskap nodig is, moet beskikbaar wees. Hierdie materiaal en toerusting mag nie bewaar word in 'n vertrek waar voedselhanteertoerusting bewaar word nie, en mag nooit met onverwerkte materiaal, die produkte of die houers of pakkette daarvan in aanraking kom nie.

3.3.6 Ysvervaardigingstoerusting

Alle oppervlakke van ysvervaardigingstoerusting wat met die ys in aanraking kom, moet van geskikte korrosiebestande materiaal wees. Die konstruksie van die ysvervaardigingstoerusting moet deurgaans higiënies wees. Wanneer ys ook al oorgeplaas, bewaar of vervoer word, moet dit doeltreffend teen kontaminasie beskerm wees.

3.4 Water

3.4.1 Drinkbare water

3.4.1.1 Onderworpe aan die bepalings van 3.4.2, moet elke fabriek 'n toereikende voorraad skoon drinkbare water hê wat vry is van stowwe in suspensie en bestanddele wat skadelik vir die produk of nadelig vir die gesondheid is. Daarbenewens moet die water deur middel van uitvlokking, filtrering, chlorering of 'n ander aanneemlike proses sodanig behandel wees dat voldoening aan die volgende vereistes verseker word:

a) **koliforme organismes**: die telling vir koliforme organismes mag nie vyf organismes per 100 mℓ van die water (kyk 10.16.1.1 of 10.16.2.3.1) oorskry nie; en

b) **fekale koliforme bakterieë**: daar mag geen fekale koliforme bakterieë in 100 mℓ van die water (kyk 10.16.1.2 of 10.16.2.3.2) waarneembaar wees nie.

3.4.1.2 Vir die doeleindes van die waterondersoek sluit die koliforme groep alle Gram-negatiewe, niespoorvormende stawe in wat laktose, met die voortbring van suur en gas, in minder as 48 h by 37 °C kan laat fermenteer. Fekale koliforme bakterieë moet beskou word as Gram-negatiewe, niespoorvormende stawe wat laktose, met die voortbring van suur en gas, in minder as 48 h by 37 °C sowel as 44 °C kan laat fermenteer en indool in triptoonwater kan voortbring.

3.4.1.3 Gechloreerde water wat die produk nadelig kan beïnvloed, moet onmiddellik voor gebruik ontchloor word. In alle gevalle moet die vry residuele chloorkonsentrasie dmv die *N,N*-diëtiel-1,4-ℓ-fenileendiamientoets of 'n ander aanneemlike toets met 'n ekwivalente sensitiwiteit bepaal word.

3.4.1.4 Fabrieksinstallasies vir die behandeling van water moet minstens een keer per week deeglik volgens 'n aanneemlike metode skoongemaak word.

3.4.2 Seewater

Skoon, ongekontamineerde, vars gepompte seewater kan vir enige doel in die fabriek gebruik word, mits die telling vir koliforme organismes nie 50 organismes per 100 mℓ van die water oorskry nie (kyk 10.16.2.3.1) en geen fekale koliforme bakterieë in 100 mℓ van die water (kyk 10.16.2.3.2) waarneembaar is nie.

3.4.3 Water vir skoonmaak

Water wat gebruik word vir die skoonmaak van die installasie en toerusting moet aan die vereistes van 3.4.1 of 3.4.2, soos toepaslik, voldoen. Gechloreerde water wat 'n nadelige uitwerking op die produk kan hê, moet onmiddellik voor gebruik ontchloor word. In alle gevalle moet die vry residuele

chloorkonsentrasie deur die *N,N*-diëtiel-1,4-ℓ-fenileendiamientoets of 'n ander aanneemlike toets met 'n ekwivalente sensitiwiteit bepaal word.

3.4.4 Ys

Die suiwerheid van ys moet sodanig wees dat die water wat daaruit verkry word direk nadat die ys vervaardig is (deur die ys in aseptiese toestande by 'n temperatuur van hoogstens 10 °C te smelt) aan die vereistes van 3.4.1 of 3.4.2, soos toepaslik, voldoen.

3.5 Vereistes vir werknemers wat by die hantering, voorbereiding, verwerking, verpakking en bewaring van die produk betrokke is

3.5.1 Gesondheid

3.5.1.1 Voordat werknemers in diens geneem word, moet hulle 'n toepaslike mediese ondersoek slaag om te verseker dat hulle nie aan aansteeklike siektes ly nie en hulle moet daarna jaarliks 'n mediese ondersoek slaag. In geval van afwesigheid van meer as een dag weens siekte moet die werknemer, voordat hy/sy weer begin werk, die aard van die siekte wat die afwesigheid genoodsaak het by die fabriekshigiënebeampte aanmeld, wat, indien hy dit nodig ag, die toepaslike stappe moet doen om mediese advies oor die werknemer se geskiktheid vir werk in te win. 'n Toepaslike mediese rekord moet van elke werknemer gehou word.

3.5.1.2 'n Mediese sertifikaat wat deur 'n fabriekswerker ingedien word, moet ter insae beskikbaar wees vir die owerheid wat hierdie spesifikasie administreer.

3.5.1.3 Geen werknemer wat 'n draer is van of ly aan 'n aansteeklike siekte, veral 'n draer van *Salmonella* of *Shigella*, of een wat simptome toon van of ly aan gastro-enteritis of enterobakteriese infeksie of 'n siekte of toestand wat 'n afskeiding van vloeistof uit enige deel van die vel of liggaam veroorsaak, mag toegelaat word om met die produk in aanraking te kom nie. Enige sodanige werknemer moet hom onmiddellik by die fabrieksbestuur aanmeld.

3.5.1.4 Indien dit bekend is dat 'n werknemer aan 'n siekte ly wat deur voedsel oorgedra kan word, mag hy nie toegelaat word om in enige deel van die fabriek te werk in 'n hoedanigheid waar daar 'n moontlikheid bestaan dat hy die produk met patogene organismes kan kontamineer nie.

3.5.1.5 Geen werknemer met 'n snywond of besering mag toegelaat word om met die produk in aanraking te kom nie tensy die snywond of besering só behandel of verbind is dat die afskeiding van liggaamsvloeistof verhoed word en die wond en die verband só bedek is dat daar verseker word dat besmetting of kontaminasie van die produk nie meer moontlik is nie.

3.5.2 Beskermende klere

3.5.2.1 Alle werknemers wat by die hantering, voorbereiding en verwerking van die produk tot en met die verpakkingstadium betrokke is, maar uitgesonderd werknemers wat in vriesbewaarkamers en verkilkamers werk, moet skoon, ligkleurige, beskermende klere dra sowel as waterdigte voorskote, waterdigte skoenbedekkings of stewels en skoon, wasbare of weggooitipe kopbedekkings wat die hare heeltemal bedek. Wolmusse mag slegs in vriesbewaarkamers gedra word. Oorpakke moet die werknemers se persoonlike klere heeltemal bedek.

3.5.2.2 Moue mag nie tot onder die elmboë reik nie tensy dit met plastiekoormoue bedek is of in vriesbewaarkamers en verkilkamers gedra word. Waterdigte beskermende klere moet van plastiek- of rubbermateriaal of 'n soortgelyke aanneemlike materiaal wees. Alle beskermende klere moet 'n higiëniese ontwerp hê, mag geen buitesakke hê nie, moet heel gehou word en mag nie 'n bron van kontaminasie vir die produk wees nie.

3.5.2.3 Beskermende klere, uitgesonderd waterdigte voorskote, oormoue en handskoene, mag nie in werkgebiede gebêre word nie; wanneer dit nie gebruik word nie, moet dit in kleedkamers gehou word en mag nie van die perseel af verwyder word nie, behalwe om in higiëniese toestande gewas te word.

Die wonings van werknemers word nie as aanneemlik vir hierdie doel beskou nie.

3.5.2.4 Waterdigte voorskote, oormoue en handskoene moet skoongemaak word elke keer as dit afgehaal word en so dikwels as wat nodig is, en moet tydens werkpouses en besoeke aan die toilet aan hake of penne by die uitgange van produksiegebiede opgehang word. Handskoene moet deeglik skoongemaak word en dan deur middel van gechloreerde water of 'n ander aanneemlike oplossing of prosedure ontsmet word. Waterdigte voorskote, oormoue en handskoene asook alle toerusting wat by die voorbereiding, verwerking en verpakking van die produk gebruik word, mag slegs uit die werkgebiede verwyder word vir herstelwerk en om in higiëniese toestande skoongemaak te word.

3.5.3 Persoonlike higiëne

3.5.3.1 Werknemers moet voordat hulle begin werk en na elke afwesigheid uit die produksiegebied van die fabriek, met gereelde tussenposes tydens produksie of te eniger tyd wanneer dit nodig is, hulle hande met warm water en 'n aanneemlike ongeparfumeerde vloeibare seep of detergent was en dit daarna in skoon lopende water afspoel. Daarna kan hulle hul hande in 'n aanneemlike ontsmettingsoplossing doop, waarna hulle hul hande in skoon lopende water moet afspoel indien dit deur die gebruiksaanwysings van die handdoopmiddel vereis word. Naellak of naelvernis mag nie op vingernaels gebruik word nie en vingernaels moet kort en skoon gehou word. Werknemers wat onverwerkte materiaal of die onbeskermde produk, of albei, hanteer, mag nie juweliersware dra nie.

3.5.3.2 Die persoonlike besittings en voedsel van werknemers mag in geen gebied kom waar die produk en die bestanddele daarvan en verpakkingsmateriaal gehanteer en bewaar word nie. Houers wat gebruik word by die voorbereiding, verwerking of verpakking van die produk mag vir geen ander doel gebruik word nie.

3.5.3.3 Die gebruik van kougom en tabak in enige vorm mag nie toegelaat word in die gebiede waar die produk en die bestanddele daarvan en verpakkingsmateriaal gehanteer of bewaar word nie. Daar mag nêrens op die fabrieksperseel gespoeg word nie. Kennisgewings te dien effekte moet op strategiese plekke aangebring wees (kyk 3.2.11).

3.5.4 Besoekers

Enige persoon, met inbegrip van die werknemers wat die produksiegebiede van die fabriek tydens werkure besoek of binnegaan, moet, terwyl hy/sy in dié gebiede is, aan al die higiënevereistes voldoen en skoon, beskermende klere dra wat deur die fabriek verskaf moet word.

3.6 Vereistes vir higiëniese werk

3.6.1 Algemeen

3.6.1.1 Met betrekking tot die hantering, vervoer, verwerking, verpakking, bevriesing en bewaring van die produk, mag geen werk gedoen word of toestande bestaan wat nadelig vir die produk is nie. Materiaal wat die produk moontlik kan kontamineer, moet van verwerkingsgebiede weggehou word. Nie-eetbare materiaal mag nie in dieselfde vertrek as eetbare bestanddele of in die voorbereidings- of verpakkingsgebiede van die fabriek bewaar word nie.

3.6.1.2 Daar mag geen onhigiëniese toestande op die fabrieksperseel wees nie. Daar mag nie toegelaat word dat rook van fabriekskoorstene en uitlaatgasse die fabrieksgebou(e) binnedring in hoeveelhede of op 'n wyse wat aanstootlik, nadelig of gevaarlik vir die gesondheid is of in enige stadium van die verwerking van die produk kontaminasie van die produk kan veroorsaak nie.

3.6.2 Skoonmaak en ontsmetting

3.6.2.1 Fisiese fasiliteite

3.6.2.1.1 Die gebou, perseel, installasies, toerusting, gereedskap en alle ander fisiese fasiliteite van die fabriek moet skoon en in 'n goeie toestand gehou word en moet netjies en higiënies gehou word. Die voorbereidings-, verwerkings- en verpakkingsgebiede van fabrieke en alle hulptoerusting en gereedskap moet gereeld deur opgeleide werknemers skoongemaak en ontsmet word. Voordat die installasie, toerusting en gereedskap gebruik word, moet dit deeglik met 'n detergent of ander skoonmaakmiddel skoongemaak en ontsmet word. 'n Detergentontsmettingsmiddel kan gebruik word. Onmiddellik voordat daar met werksaamhede begin word, moet toerusting deeglik met water (kyk 3.4.3) afgespoel word om stof en ontsmettingsmiddel (indien dit gebruik is) te verwyder.

3.6.2.1.2 Die verwerkings- en verpakkingsgebiede, bewaarkamers, verkilkamers, vriesbewaarkamers en vrieskamers moet vry gehou word van skimmel, stof, vuiligheid, verf wat afskilfer en ander los vreemde materiaal wat van mure, plafonne en oorhoofse strukture op die produk kan val.

3.6.2.2 Vloere en afvoerkanale

Tydens werktydperke moet die vloere en afvoerkanale in die voorbereidings-, verwerkings- en verpakkingsgebiede skoon gehou word deur dit gereeld uit te vee, te skrop en met water af te spoel. Daar mag nie toegelaat word dat vullis in afvoerkanale of op roosters ophoop nie. Vloere en afvoerkanale moet so dikwels as wat nodig is en na afloop van elke dag se werksaamhede deeglik skoongemaak word ten einde higiëniese toestande te handhaaf. Voetbaddens moet gereeld gedreineer en skoongemaak word en die ontsmettingsmiddel moet in 'n aktiewe toestand gehou word.

3.6.2.3 Mure van voorbereidings-, verwerkings- en verpakkingsgebiede

Die mure van die voorbereidings-, verwerkings- en verpakkingsgebiede moet, indien nodig, onmiddellik na elke dag se werksaamhede deeglik gewas word en die vertrekke moet so stofvry moontlik gehou word.

3.6.2.4 Skoonmaak- en ontsmettingsmateriaal

Skoonmaak- en ontsmettingsmateriaal, lopende warm en koue water wat aan die vereistes van 3.4.3 voldoen, versadigde stoom, waterslange, borsels en ander materiaal en toerusting wat vir die skoonmaak van die fabriek, toerusting en gereedskap nodig is, moet beskikbaar wees. Skoonmaakmateriaal soos skuurwol, wat die produk kan kontamineer, mag nie gebruik word nie.

3.6.2.5 Die skoonmaak van waterbehandelingsinstallasies

Fabrieksinstallasies vir die behandeling van water moet een maal per week volgens 'n aanneemlike metode deeglik skoongemaak word.

3.6.2.6 Skoonmaak van die verwerkingstelsel

Die hele verwerkingstelsel moet tydens elke produksieonderbreking wat langer as 1 h duur, skoongemaak word of wanneer dit ook al nodig geag word, en dit moet aan die einde van elke skof en na afloop van elke dag se werksaamhede doeltreffend skoongemaak word. Dit moet skoon wees as dit weer gebruik word.

3.6.2.7 Skoonmaak van gereedskap

Messe en soortgelyke toerustingsitems moet tydens produksieonderbrekings, na gebruik en te eniger tyd wanneer ontsmetting ook al nodig is, deeglik skoongemaak word en dan met gechloreerde water of 'n ander aanneemlike oplossing of volgens 'n ander prosedure ontsmet word. Wanneer die fabriek in werking is, mag toerusting en gereedskap nie uit die werkgebied verwyder word nie, behalwe vir herstelwerk, skoonmaak of vervanging.

3.6.2.8 Skoonmaak van die aflaaistelsel

Aflaaistelsels by die aanlêplek en vervoerstelsels na die fabriek moet só gedreineer word dat water nie opdam en stagneer nie. Sodanige stelsels moet gereeld skoongemaak word om ou materiaal daaruit te verwyder, en moet ook voor en na gebruik skoongemaak word. Houtenks moet op dieselfde wyse behandel word.

3.6.3 Herstelwerk

3.6.3.1 Elke keer as instandhoudings- of herstelwerk in produksiegebiede uitgevoer word, moet die gereedskap en die toerusting wat vervang word onmiddellik uit hierdie gebiede verwyder word en die betrokke toerusting moet deeglik skoongemaak en ontsmet word.

3.6.3.2 In die gebiede waar die produk gehanteer, voorberei, verwerk of verpak word, mag sweis-herstelwerk slegs uitgevoer word as die installasie nie in produksie is nie of as noodherstelwerk by onklaarraking en dit moet op só 'n wyse uitgevoer word dat die produk nie aan sweisdampe, spatsels of slakdeeltjies blootgestel word nie.

3.6.4 Skoonmaakdoeltreffendheid

Die doeltreffendheid van die skoonmaak- en ontsmettingsproses wat in 3.6.2 gespesifiseer word, moet sodanig wees dat, by monsters wat volgens 10.15.2 geneem is, die persentasie skoonmaak- en ontsmettingsdoeltreffendheid in die monster, volgens 10.15.3 bepaal, aanneemlik is as punte volgens die stelsel in 10.15.3.3 en 10.15.3.4 toegeken word.

3.6.5 Houers, bakke en kratte vir die hantering van onverwerkte materiaal en die produk

Houers wat heeltemal of gedeeltelik met onverwerkte materiaal of met die produk gevul is, mag nie só op mekaar gestapel word dat die inhoud van 'n houer met die bodem van die houer wat daarop gestapel is in aanraking kan kom nie. Houers met eetbare materiaal mag nie regstreeks op die vloer of teen die muur opgestapel word nie en wanneer hulle verskuif word, moet hulle doeltreffend teen kontaminasie beskerm word. Houers met eetbare materiaal moet minstens 250 mm bokant vloervlak bewaar word. In gevalle waar palette in plaas van rakke of standers gebruik word, moet daar 'n vry ruimte van minstens 100 mm bokant vloervlak wees. Houers moet 'n higiëniese ontwerp hê en moet lig van kleur wees of 'n blink metaalafwerking hê. Nie-eetbare materiaal mag nie in dieselfde vertrek as eetbare bestanddele of in die voorbereidings- of verwerkingsgebiede van die fabriek bewaar word nie.

3.6.6 Toedraaimateriaal

Toedraaimateriaal wat by die verpakking van die produk gebruik word, moet in korrosiebestande houers met 'n higiëniese konstruksie gehou en moet só uitgemeet word dat die toedraaimateriaal die minimum hantering vereis.

3.6.7 Verpakkingsmateriaal

Materiaal vir die verpakking van die produk moet op rakke minstens 250 mm van die vloer af of op palette en weg van die mure af bewaar word.

3.6.8 Onderdele

Onderdele vir masjinerie en ander items wat die produk kan kontamineer, moet weg van die voorbereidings-, verwerkings-, verpakkings- en produkbewaargebiede bewaar word.

3.6.9 Vrieskamers, verkilkamers, vriesbewaarkamers en hul toerusting en instrumente

Vrieskamers, verkilkamers, vriesbewaarkamers en hul toerusting en instrumente moet doeltreffend werk en skoon en in 'n higiëniese toestand gehou word. Die temperatuur in vriesbewaarkamers moet outomaties en deurlopend gemoniteer word en 'n rekord van die temperatuur moet gehou word en ter

insae beskikbaar wees. Produkte mag nie regstreeks op die vloer of teen mure opgestapel word nie. Geen materiaal behalwe die produk of produkbestanddele mag in vrieskamers, verkilkamers of vriesbewaarkamers bewaar word nie. Geen toestand en geen voorwerp of stof wat die geur, reuk of voorkoms van die bevrore produk op enige wyse kan beïnvloed, mag in vrieskamers, verkilkamers of vriesbewaarkamers aanwesig wees nie.

3.6.10 Verwydering van vullis en visafval

Daar mag nie toegelaat word dat rommel, afval en oorloop ophoop of onhigiëniese toestande veroorsaak nie en dit moet vinnig op 'n doeltreffende en higiëniese wyse weggedoen word. Visafval moet op 'n higiëniese wyse uit die verwerkingsgebied verwyder word en houers met visafval wat wag om uit die fabriek verwyder te word, moet ver van die verwerkingsgebiede af wees. 'n Afsonderlike vulliskamer of ander aanneemlike vullisfasiliteit moet op die terrein voorsien word en moet minstens een maal per dag skoongemaak word.

3.6.11 Plaagbeheer

Alle geboue waarin onverwerkte materiaal, bestanddele en die produk bewaar word of waarin die produk gehanteer, voorberei, verwerk of verpak word, moet vry van insekte, voëls, knaagdiere en ander ongediertes gehou word. Hierbenewens moet alle vertrekke waarin onverwerkte materiaal, bestanddele of verpakkingsmateriaal bewaar word, knaagdierdig wees.

3.6.12 Die gebruik van plaagdoders

Plaagdoders mag nie in werkgebiede gebruik word terwyl voorbereiding, verwerking en verpakking aan die gang is nie en daar moet voorsorgmaatreëls getref word om te verseker dat toerusting en werkoppervlakke vry van plaagdoderresidu's gehou word. Daar mag op geen tydstip toegelaat word dat plaagdoders en skoonmaakchemikalieë met verpakkingsmateriaal, houers, onverwerkte materiaal of die produk in aanraking kom nie. Die vertrek waarin plaagdoders bewaar word, moet gesluit gehou word en die materiaal daarin mag slegs gehanteer word deur werknemers wat in die gebruik daarvan opgelei is.

3.6.13 Diere

Diere, met inbegrip van voëls, mag in geen deel van die fabriek toegelaat word nie.

3.6.14 Werksaamhede aan boord van visvaartuie

Die hantering, verkilling, verwerking en bevriesing van die produk aan boord van die visvaartuig moet in dieselfde sanitêre en higiëniese toestande geskied as wat vir die prosedures en praktyke in fabrieke aan land geld. Voordat 'n produk aan boord kom en tussen vangste moet die dek, dektenks, penstaanders en skeiplanke en alle ander dektoerusting wat met die produk in aanraking sal kom met skoon seewater afgespuit word. Die dek moet minstens een maal per dag geskrop word om alle sigbare vuiligheid en afval te verwyder. Tydens visvaarte moet die opvangput in die ruim van die vaartuig gereeld gedreineer word. Alle gereedskap moet na afloop van die visvangs deeglik skoongemaak word. Die ingewande van oopgesnyde vis moet dadelik verwyder word en die vis moet gewas word in skoon water wat geen ingewande en lewerdeeltjies bevat nie. Indien dit prakties uitvoerbaar is, moet die dek leeggemaak word voordat die volgende vangs daarop gestort word. Die dek, ruim en verwerkingsgebiede van visvaartuie moet deeglik skoongemaak en ontsmet word onmiddellik nadat die vrag afgelaai is.

3.7 Rekords

Op visvaartuie met bevriesingsfasiliteite of met bevriesings- sowel as verpakkingsfasiliteite, en in fabrieke aan land moet toereikende kwaliteitsrekords gehou word. Kwaliteitsrekords en rekords van vries-bewaringstemperature op visvaartuie en in fabrieke aan land moet vir minstens twee jaar gehou word (kyk 3.2.16.3 en 3.2.16.4).

3.8 Die hantering, voorbereiding, verwerking, verpakking, vervoer en bewaring van die produk

3.8.1 Algemeen

Vanaf die oomblik dat die vangs aan boord kom en tydens voorbereiding, verwerking, verpakking, vervoer en bewaring moet die produk teen hitte, regstreekse sonstrale, ryp, die uitdrogende uitwerking van wind en kontaminasie deur voëls, stof, olie, brandstof en skadelike dampe beskerm word. Die produk moet verwerk word so gou moontlik nadat dit gevang is. Die ingewande van vis moet so gou moontlik verwyder word en die vis moet deeglik in skoon water gewas word om alle bloed, slym en stukkies ingewande te verwyder, behalwe in die geval van vis wat bedoel is om as ronde vis bevries te word.

3.8.2 Visvaartuie wat nie toegerus is om te verkil of te bevries nie

Die vangs moet aan land gebring word waar die verkil- of bevriesingsproses moet begin binne 8 h nadat die vis gevang is.

In gevalle waar die vis onmiddellik aan boord van sodanige vaartuig verwerk of verpak word, geld die vereistes van 3.8.1. Die temperatuur van vis aan boord mag hoogstens 20 °C wees.

3.8.3 Visvaartuie met verkilfasiliteite

Aan boord van visvaartuie met verkilfasiliteite, moet vis wat vir verwerking bedoel is, indien prakties uitvoerbaar, verkil of op ys geplaas word so gou moontlik nadat dit aan boord gebring is. Vis wat nie verkil is binne 8 h nadat dit aan boord gebring is nie, mag nie verwerk word nie. Vis wat tussen 8 h tot 10 d op see gehou word, moet, as 'n minimum vereiste, in ys bewaar word. Ysvlokkies, ysblokkies of fyngedrukte ysblokkies, met 'n diameter van minder as 5 cm, moet gebruik word en stellasies of rakke moet só geïnstalleer word dat die diepte van grootmaatbewaring tot 'n maksimum van 1 m beperk word. Afsonderlike lae vis moet van mekaar en van die vloer, die skeepswande en skotte geskei word deur aanneemlike lae ys. Visspesies wat 'n nadelige uitwerking op mekaar kan hê, moet in afsonderlike ruime of houers bewaar word. Vaartuie wat toegerus is om te verkil maar nie om te bevries nie, mag nie gebruik word om vis langer as 10 d op see te hou nie. Vis mag nie vir langer as wat goeie vervaardigingspraktyk toelaat op ys gehou word nie. In die geval van tuna moet die vangs van vaartuie wat nie toegerus is om te bevries nie op ys of in verkilde of verkoelde seewater gehou word en die vangs moet binne 5 d na fabrieke aan land vervoer word.

3.8.4 Tuna

Tuna mag vir 'n tydperk van hoogstens 5 d (kyk 3.8.3) op ys bewaar word. Voorbevriesing van tuna in pekel by –7 °C is toereikend vir 'n tydperk van hoogstens 14 d. In die geval van langer bewaartydperke moet die vis bevries word en by 'n temperatuur van –20 °C of laer in 'n bevrore toestand gehou word. Indien voorbevriesing in pekel gebruik word, mag die totale chloriedgehalte (bepaal as NaCl) van die tuna, volgens 9.8 bepaal, hoogstens 2 % (volgens massa) wees.

3.8.5 Pad- en spoorvervoer

Padvervoer van die produk moet geskied in toe en geïsoleerde of verkoelde voertuie wat gebou en toegerus is om die produk toereikend te beskerm. In stowwerige toestande of toestande wat die vis nadelig kan beïnvloed, moet padvervoer in 'n voertuig met 'n stofvrye bewaarruim geskied. Indien padvervoer langer as 1 h duur, moet:

a) onbevrore vis vervoer word in 'n geïsoleerde en verkoelde vragwa wat die vis by die temperatuur van smeltende ys kan hou, of in 'n geïsoleerde vragwa waarin die vis op ys is; en

b) 'n bevrore produk in 'n geïsoleerde en verkoelde bewaarruim in 'n vragwa vervoer word, volgens die vereistes in 7.2.2 en 7.2.3.

Spoorvervoer van die produk moet in geïsoleerde en verkoelde voertuie geskied. Die bevrore finale

produk wat nog vervoer of gelaai moet word, mag nie langer as 1 h onverkoel gelaat word nie. Daar moet toereikende voorsorgmaatreëls getref word om fisiese beskadiging van die produk, bv as gevolg van druk of beweging tydens vervoer, te voorkom. Die produk mag nie vervoer word saam met ander produkte wat die produk kan kontamineer of laat agteruitgaan nie. Die vervoermiddele wat vir die produk gebruik word, mag op geen tydstip vir die vervoer van ander produkte gebruik word wat moontlik die produk kan laat agteruitgaan of kontamineer nie. Die binne-oppervlakke van die vervoermiddel moet só afgewerk wees dat dit nie die produk nadelig beïnvloed nie; dit moet glad wees en maklik om skoon te maak en te ontsmet. Indien ys gebruik word om die produk te verkil, moet toereikende dreinering voorsien word ten einde te verseker dat die water van die gesmelte ys nie in aanraking met die produk bly nie.

3.8.6 Bevriesing van die produk voor verwerking

Indien die produk voor verwerking bevries moet word, moet die bevriesing begin:

a) gou nadat die vangs aan boord gebring is; of

b) binne 8 h nadat die vis gevang is in die geval van wit vis of plat vis en soortgelyke visspesies met 'n lae vetgehalte, op voorwaarde dat die temperatuur van die vis nooit tot hoër as 20 °C mag styg nie; of

c) binne 10 d indien die temperatuur van die vis in (b) hierbo tot die temperatuur van smeltende ys verlaag is; of

d) binne 72 h nadat dit aan boord gebring is in die geval van hoërisiko- vetterige vis (met inbegrip van *Clupeidae* en *Scombridae*), mits die temperatuur van die vis binne 6 h nadat dit gevang is tot 3 °C of laer verlaag word en tot 0 °C verlaag word binne 16 h nadat dit aan boord gebring is.

3.8.7 Ontdooiing van die bevrore produk vir verwerking

Terwyl die bevrore produk vir verwerking ontdooi word, mag dit nie aan omgewingstemperature van hoër as 20 °C blootgestel word nie. Die ontdooiing moet so vinnig moontlik wees en moet in minder as 20 h afgehandel wees. Tensy daar voor of onmiddellik nadat die ontdooiing afgehandel is met die verwerking begin word, moet daar onmiddellik begin word om die ontdooide vis tot so na as moontlik aan 0 °C te verkil. Bevriesing na verwerking moet begin binne 8 h nadat die ontdooiingsproses afgehandel is, tensy die verwerking 'n langer tydsduur vereis.

3.9 Spesiale vereistes met betrekking tot seeskulpdiere

Seeskulpdiere moet teen hitte en kontaminasie beskerm word, moet klam (en, indien moontlik, lewendig) gehou word terwyl hulle aan boord van die vaartuig is en moet gedurende vervoer op 'n aanneemlike wyse beskerm word. Indien seeskulpdiere, uitgesonderd perlemoen, nie lewendig gehou kan word nie, moet hulle bevries word of voor verwerking of bevriesing vir hoogstens 2 d in ys gehou word.

OPM – Seekat en inkvis kan 4 tot 5 d lank in ys gepreserveer word, mits die gesmelte yswater behoorlik gedreineer word.

Verwerking van perlemoen moet begin terwyl dit nog lewendig is. Die vleis moet skoongemaak word in higiëniese toestande wat verseker dat kontaminasie verhoed word. Die ingewande en mond moet verwyder word. Die baardrande en die poot hoef nie verwyder te word nie maar die epitelium tussen die baardrande en poot moet afgeborsel word. Nadat dit skoongemaak is, moet dit deeglik gewas word in lopende water wat aan die vereistes van 3.4.1 of 3.4.2 voldoen. Die vleis moet vry van lelike vurkmerke of ander beskadiging wees.

4 Vereistes vir die bestanddele en die produk

4.1 Toestand van bestanddele en die produk

4.1.1 Algemeen

4.1.1.1 Alle bestanddele wat gebruik word, moet binne die bestek val van en moet voldoen aan die vereistes van die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972 (Wet 54 van 1972), en enige regulasie wat daarkragtens uitgevaardig word. Alle bestanddele wat by die voorbereiding van die produk gebruik word, moet skoon, gebrekvry, van goeie kwaliteit en in elke opsig geskik vir menslike verbruik wees. Hierbenewens mag die produk geen stof bevat in hoeveelhede wat gevaarlik vir die mens se gesondheid kan wees nie.

4.1.1.2 Houtrook wat gebruik word om die produk te rook, moet verkry word van hout wat aanneemlik vry van gom en hars, verf, houtverduursamingsmiddels en ander bygevoegde stowwe is.

4.1.1.3 Alle eenhede en stukke vis wat aangebied word in een van die vorms wat in 4.2.1 tot en met 4.2.16 beskryf word, moet aan die toepaslike beskrywing voldoen. Afvalstukkies vis mag nie ingesluit word nie. As bevrore vis of bevrore stukkies vis voor verpakking met verkilde water verglans word, moet die temperatuur van die water wat vir die verglansing gebruik word 5 °C of laer wees.

4.1.1.4 Visprodukte wat in 4.2.17 tot en met 4.2.23 beskryf word, mag geen ingewande, koppe en kiewe bevat nie en moet aanneemlik vry wees van skubbe, vinne en sterte.

4.1.2 Vis

4.1.2.1 Bevrore vis

Bevrore produkte moet volgens 8.1 voorberei en ondersoek word.

4.1.2.2 Algemene eienskappe

Die vis wat by die voorbereiding van die produk gebruik word en nie voorheen bevries is nie, moet die volgende algemene eienskappe hê:

a) 'n kenmerkende vars voorkoms, kleur en reuk, sonder enige waarneembare galsterigheid of suurheid;

b) indien toepaslik, prominente, blink, helder en vogtige oë;

c) waar slym aanwesig is en 'n natuurlike eienskap van die spesie is, deurskynende of roomwit slym;

d) indien toepaslik, helder rooi kiewe; die reuk van die kiewe moet kenmerkend vars vir die spesie wees;

e) indien toepaslik, helderkleurige buikbloed;

f) vleis wat ferm en elasties is en aan die grate vaskleef; en

g) as die vis gaargemaak is, moet die reuk en geur kenmerkend vars vir die spesie wees en die tekstuur daarvan moet ferm maar sag en sappig wees.

4.1.2.3 Tuna

In die geval van tuna geld die volgende meer spesifieke en bykomstige kenmerke:

a) **velslym**: verkieslik deursigtig, maar kan ook melkerig wees; die kleur iriserend tot helder;

b) **oë**: glansende kleur, konvekse tot effe konvekse vorm, maar nie plat nie; die pupille swart tot

dowwer swart en deursigtig helder tot effe bloederig;

c) **kiewe**: helderrooi, bleekrooi en bruin-rooi van kleur; kenmerkende neutrale tot soet reuk;

d) **reuk van kiewe**: vars, sterk seewieragtig, metaalagtig, soos vars gesnyde gras, baie effe muwwerig, effe peperagtig of olierig. Beslis nie soos asyn, botter, vrugte, ontlasting, swael, suur, bier, mout, melk of oorwegend muf nie;

e) **buikwand**: ongeskonde tot slap, maar nie sag of beskadig nie;

f) **buikvlies**: verkieslik maar nie noodwendig ongeskonde nie, maar nie geskeur of opgelos nie;

g) **vleiskleur**: ligpienk tot donkerrooi, na gelang van die spesie;

h) **vleiskleur met ruggraat langs**: dieselfde as dié van die omringende vleis;

i) **fisiese beskadiging**: effense vervorming en skending is aanneemlik, maar die vis mag nie oopgebars wees nie; en

j) **gaar vis**: die geur moet kenmerkend van die spesie wees maar nie laf, bitter of peperagtig nie. Daar mag geen oormatige proteïenstolsel (gestolde stowwe van gedenatureerde proteïen) op die oppervlak van die vleis wees nie en die spierlae mag nie van mekaar wegtrek nie (ruitvorming). Die kleur moet naaswit of pienkerig tot ligbruin wees, na gelang van die spesie. Daar mag geen oranje of groen verkleuring van die vleis wees nie.

4.1.3 Sout

Sout wat by die voorbereiding van die produk gebruik word, moet eetbaar en vry van bitterheid en ander byreuke en bygeure, verkleuring of onsuiwerhede wees.

4.1.4 Geurmiddels

Geurmiddels moet vry wees van vreemde stowwe en vervalsingsmiddels.

4.1.5 Bymiddels

Slegs toegelate bymiddels (kyk 4.1.1.1) moet gebruik word, en dan slegs in die goedgekeurde hoeveelhede.

4.2 Aanbieding van vis en visprodukte

Vis of visprodukte moet heel aangebied word of in ander aanneemlike vorms, soos dié in 4.2.1 tot en met 4.2.23.

4.2.1 Heel (ronde) vis

'n Vis soos dit uit die see gehaal is en nog nie verwerk is nie of 'n vis wat slegs gebloei is.

4.2.2 Vis waarvan die ingewande verwyder is

'n Ronde vis waarvan die ingewande verwyder is.

4.2.3 Vis waarvan die kiewe en die ingewande verwyder is

'n Vis waarvan die ingewande (kyk 4.2.2) en die kiewe verwyder is.

4.2.4 Vis waarvan die kop en die ingewande verwyder is

'n Vis waarvan die ingewande (kyk 4.2.2) en die kop verwyder is.

4.2.5 Gedresseerde vis

'n Vis waarvan die kop en die ingewande verwyder is en wat ontskub is.

4.2.6 Kotelette

Dwarssnitte, hoogstens 40 mm dik, van vis waarvan alle ingewande en bloed verwyder is en wat geen groot grate bevat wat opvallend uitsteek nie.

4.2.7 Filet, lengtesnit

Die vleiskant van 'n heel vis in die lengte en so na as moontlik aan die ruggraat afgesny. Dit sluit die borsvin, pensgedeelte, losgraatjies, nekgrate en vel in. Dit moet 'n ongeskonde, skoon gesnyde stuk vis wees wat aanneemlik sonder skubbe is.

4.2.8 Filet (enkelfilet)

Die vleiskant van 'n heel vis soos in 4.2.7 beskryf, maar met die borsvin en skouergordel verwyder.

4.2.9 Ontvelde filet

'n Filet soos in 4.2.8 beskryf is, waarvan die vel verwyder is.

4.2.10 Ontvelde en ontgrate filet (filet sonder vel en sonder grate)

'n Filet soos in 4.2.9 beskryf is, waarvan die visgrate verwyder is.

4.2.11 Dubbele filet (vlinderfilet)

Die twee filette van een vis, soos in 4.2.8, 4.2.9 of 4.2.10 beskryf is, maar wat by die rug of maag aan mekaar vas is.

4.2.12 Visblokke

Blokke vis wat in enige aanneemlike vorm voorberei is met tussenblaaie, indien nodig. Onderworpe aan gepaste etikettering (kyk 6.1(b)), mag die blokke ook uit gemaalde vis bestaan. Indien prakties uityoerbaar, moet visblokke vry wees van uitdying, holtes, oppervlakverkleuring, ontwatering en "vriesbrand" (diep ontwatering).

4.2.13 Getreilde vis of heel vis met treilmerke

Heel vis van goeie kwaliteit wat treilmerke, soos 'n beskadigde vel, geringe kneusing en geringe oppervlaksnymerke toon en wat slegs vir verdere verwerking geskik is en dienooreenkomstig gemerk moet word indien dit verpak word.

4.2.14 Gerookte vis

Enigeen van die vis of vissnitte wat in 4.2.1 tot 4.2.13 beskryf word en gerook is (kyk 4.1.1.2).

4.2.14.1 Warmgerookte vis

Vis wat by 'n toereikend hoë temperatuur gerook is vir sodanige tydsduur dat hittestolling van die proteïen dwarsdeur verseker is.

4.2.14.2 Koudgerookte vis

Vis wat gerook is by 'n temperatuur waarby die produk geen teken van hittestolling van die proteïen toon nie.

4.2.15 Rookgegeurde vis

Enigeen van die vis of vissnitte wat in 4.2.1 tot 4.2.13 beskryf word en in rookgegeurde vloeistof of droë materiaal gedoop is.

4.2.16 Visvingers en visporsies

Visvingers (kyk 4.2.16.1) en visporsies (kyk 4.2.16.2) wat voorberei is uit blokke bevrore ontvelde en ontgrate visvleis, of gevorm is uit onbevrore visvleis, met of sonder bykomende bestanddele, in porsies wat aanneemlik eenvormig in grootte en fatsoen is.

4.2.16.1 Visvinger

'n Produk, met inbegrip van 'n bedekking van broodkrummels of beslag, met 'n eenheidsmassa van minstens 20 g en hoogstens 50 g en waarvan die lengte hoogstens drie maal sy grootste breedte is.

4.2.16.2 Visporsie

'n Produk, met of sonder 'n bedekking van broodkrummels of beslag, met enige fatsoen en grootte; indien die massa van die produk minder as 50 g is, moet die kwalifiserende woord "porsie" op aanneemlike wyse op die hoofpaneel van die pakket ingevoeg word. Visporsies wat uit vis met vel of gedeeltelik ontgrate vis voorberei word, moet onderskeidelik as porsies "met vel" of "gedeeltelik ontgraat", of albei, beskryf word, of 'n ander beskrywing met dieselfde betekenis moet daaraan gegee word.

4.2.17 Vissosaties

Vissnitte met of sonder groente of vrugte of enige kombinasie daarvan, ingeryg op penne.

4.2.18 Viskoekies en visfrikadelle

Viskoekies en -frikadelle wat 'n aanneemlik eenvormige fatsoen, grootte en tekstuur het en wat uit eetbare visvleis met of sonder ander bestanddele voorberei is.

4.2.19 Viswors

Wors wat met of sonder ander bestanddele voorberei is van eetbare gemaalde visvleis of ontgrate visfilet en wat gerook kan wees. Viswors kan in natuurlike of kunsderm gestop wees. Indien toepaslik, moet oneetbare kunsderm voor verpakking heeltemal verwyder word. Viswors moet eenvormig van grootte, fatsoen en tekstuur wees en moet vry wees van verkleuring en van toiingrige ente. Daar mag nie afvalstukkies of oopgebarste, vervormde eenhede en eenhede met snymerke wees nie. Dwarsgesnyde eenhede moet haaks op hul langsasse skoon gesny wees.

4.2.20 Vis in sous

Soliede vis of visstukke wat in sous verpak is met toelaatbare geurmiddels (kyk 4.1.4), speserye of ander geskikte bestanddele.

4.2.21 Vis met groente of graan of albei, met of sonder sous

Soliede vis of visstukke wat met groente of graan of albei verpak is, met of sonder sous, met toelaatbare geurmiddels, speserye of ander geskikte bestanddele.

4.2.22 Kerrievis

Soliede vis of visstukke, met of sonder beslag, verpak in kerriesous met toelaatbare geurmiddels en speserye.

4.2.23 Ingelegde vis

Soliede vis of visstukke, met of sonder beslag, verpak met uie en kerriesous met toelaatbare geurmiddels en speserye.

4.3 Fisiese vereistes

4.3.1 Netto massa

Die netto massa van 'n bevrore produk, bepaal volgens 8.3 of 8.4, soos toepaslik, moet aan die toepaslike vereistes van die Wet op Handelsmetrologie, 1973 (Wet 77 van 1973), voldoen.

4.3.2 Verpakking

Die produk moet aanneemlik verpak wees. (Kyk ook 5.1.)

4.3.3 Eenvormige grootte

In pakke waarin meer as een eenheid in 'n pakket verpak is, moet die eenhede by visuele ondersoek aanneemlik eenvormig van grootte wees, behalwe dat een vulstuk gebruik mag word. Indien pakke eenhede van verskillende groottes bevat, moet dit op die etiket gemeld word.

4.3.4 Kleur en voorkoms

Die produk moet aantreklik lyk en moet 'n kenmerkende kleur hê. Al die eenhede in 'n pakket moet 'n aanneemlik eenvormige kleur hê. In die geval van vleispakke moet die vleis sodanig vry van toiingrigheid, bloedklonte, bloedkolomme, vlekke en verkleuring wees dat dit nie aan die voorkoms van die pak afbreuk doen nie. Viskuit moet ongeskonde wees, tensy die etiket aandui dat dit gebreekte viskuit is en dit moet die kenmerkende kleur van vars viskuit hê.

4.3.5 Tekstuur

In vleispakke mag die vleis nie gekneus wees nie en dit moet die fermheid van tekstuur hê wat kenmerkend van die spesie is.

4.3.6 Reuk en smaak

Die toestand van die onverwerkte materiaal, die vervaardigingsproses en die verpakking moet verseker dat, nadat die bevrore produk tot 'n temperatuur hoër as 10 °C ontdooi is en die eenhede, indien nodig, van mekaar geskei is, die reuk vars en kenmerkend van die produk is en daar geen byreuke en ander aanduidings van agteruitgang of van die gebruik van onverwerkte materiaal van minderwaardige kwaliteit aanwesig is nie. Die reuk en smaak van die gaar produk moet ook vars en kenmerkend wees.

4.3.7 Vryheid van defekte

4.3.7.1 Die produk moet aanneemlik vry wees van gekneusde of andersins beskadigde materiaal, los skubbe, sand, grint, vuiligheid, stukkies skulp, ander vreemde stowwe en sigbare parasiete. Die produk moet aanneemlik vry wees van slym en "vriesbrand" (diep ontwatering) wat nie maklik afgekrap kan word nie, en van verkleuring en vlekke.

4.3.7.2 'n Produk wat as graatloos beskryf word, mag hoogstens een visgraatdefek (kyk 2.7) per kilogram van die viskomponent van die produk hê en moet vry wees van kraakbeen of visgrate wat, nadat dit gaar is, in die verhemelte kan steek of dit kan seermaak.

4.3.7.3 'n Produk wat as ontgraat beskryf word, mag hoogstens vyf visgraatdefekte (kyk 2.7) per kilogram van die viskomponent van die produk bevat.

4.3.7.4 'n Produk wat as ontvel beskryf word, mag geen oorblywende velweefsel of oppervlakbeskadiging toon wat die voorkoms daarvan wesenlik benadeel nie. 'n Produk wat as "met vel" beskryf word, moet wesenlik vry wees van vel- of oppervlakbeskadiging wat die voorkoms daarvan wesenlik benadeel.

4.3.8 Ander fisiese vereistes

By die toets van die produk volgens die metodes waarna in kolom 4 van tabel 1 verwys word, moet dit aan die toepaslike vereistes in kolom 3 voldoen.

4.4 Chemiese vereistes

Volgens 9.2 tot 9.8 getoets, moet die produk aan die toepaslike vereistes van hierdie spesifikasie en aan dié van die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972 (Wet 54 van 1972), voldoen.

4.5 Mikrobiologiese vereistes

By die toets van die produk volgens die metodes in 10.6 tot 10.14, moet dit aan die vereistes in kolom 2, 3 en 4 van tabel 2 voldoen.

5 Verpakking, verglansing, bevriesing en bewaring

5.1 Verpakkingsmateriaal en buitehouers

5.1.1 Verpakkings- en toedraaimateriaal

Behoudens die toepaslike vereistes van die regulasies uitgevaardig kragtens die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972 (Wet 54 van 1972), moet verpakkings- en toedraaimateriaal vir die onbeskermde produk ongebruik (nuut), skoon, nietoksies en inert en van lae vogdampdeurlatendheid wees en dit mag geen stof bevat wat nadelig vir die produk of nadelig vir die gesondheid is nie. Geen verpakkings- of toedraaimateriaal mag 'n bysmaak aan die produk verleen of dit enigsins verkleur nie en mag nie self as gevolg van aanraking met die produk verkleur nie. Tensy die visproduk verglans is (kyk 5.2), moet dit verpak word in materiaal met 'n aanneemlik lae vog- en suurstofdeurlatendheid.

Verpakkingsmateriaal moet

a) nie sodanig wees dat dit die organoleptiese eienskappe van die produk benadeel nie,

b) nie stowwe wat skadelik vir die produk of nadelig vir menslike gesondheid is, kan oordra nie, en

c) sterk genoeg wees om die produk toereikend te beskerm.

Tabel 1 — Fisiese vereistes

1	2	3	4
Produk	**Eienskap**	**Minimum vereiste % (volgens massa)**	**Toetsmetode-subidousule**
Visprodukte, gekrummel of met beslag (rou)	Visgehalte	60[1)]	8.2.1
Visprodukte, gekrummel of met beslag (gedeeltelik gaar, of gebraai)	Visgehalte	50[1)]	8.2.1
Viskoekies	Visgehalte	37,5	9.7
Viswors en visfrikadelle	Visgehalte	50	9.7
Vis in sous	Gewaste massa	50[1)]	8.2.2
Vis met groente of vrugte of graan of enige kombinasie daarvan	Gewaste massa (dws alle soliede komponente) Gewaste massa van viskomponent	60[1)] 35[1)]	8.2.2 8.2.3
Kerrievis	Gewaste massa (dws alle soliede komponente) Visgehalte	50[1)] 40[1)2)]	8.2.2 8.2.1
Ingelegde vis	Gewaste massa (dws alle soliede komponente) Massa van viskomponent met beslag Visgehalte Uie	50[1)] 45[1)] 35[1)2)] 5[1)]	8.2.2 8.2.3 8.2.1 8.2.5
Vispasteie en soortgelyke produkte	Visgehalte van vulsel Massa van vulsel (rou) Massa van vulsel (gaar)	50 45 25	9.7 8.2.4 8.2.4
Vissmeer en gekapte vis met groente, met of sonder graan	Visgehalte	50	9.7
Seeskulpdiere met broodkrummels of beslag (met inbegrip van calamariringe)	Skulpdiergehalte	40[1)]	8.2.1

1) Uitgedruk as 'n persentasie van die verklaarde netto massa van die produk.
2) Slegs getoets in die geval van besorgdheid oor die visgehalte van die bedekte produk.

5.1.2 Buitehouers

Slegs veselbord- of ander aanneemlike buitehouers moet gebruik word. Buitehouers moet ongebruik (nuut), skoon en heel wees en moet netjies en stewig toegemaak wees. Buitehouers van hout mag nie van groen hout gemaak wees nie en mag geen stof bevat wat skadelik vir die produk of nadelig vir die gesondheid is nie. Buitehouers moet só toegemaak wees dat kontaminasie van die inhoud deur stof of vreemde stowwe voorkom word en dit moet sterk genoeg wees om die produk toereikend te beskerm.

5.2 Verglansing

Die produk kan verglans word met verkilde water of 'n ander aaneemlike verglansingsmiddel in plaas daarvan om dit toe te draai, mits die verglansing tot by en met die finale verkooppunt in 'n aanneemlike toestand bly. Indien die produk verglans word, moet die yslaag die produk heeltemal bedek om te verseker dat ontwatering en oksidasie tot die minimum beperk word. Water wat vir verglansing gebruik word, moet aan die vereistes vir drinkbare water (kyk 3.4.1) of seewater (kyk 3.4.2) voldoen, en die temperatuur daarvan moet 5 °C of laer wees.

Tabel 2 — Mikrobiologiese vereistes

1	2	3	4
Organisme	Inhoud, maks		
	Rou produkte[1] (met of sonder bygevoegde bestanddele)	Gaar produkte[2] of warmgerookte produkte (of albei)	Koudgerookte produkte[3]
Standaardplaattelling	1×10^6/g	1×10^5/g	1×10^5/g
Enterobacteriaceae	[4]	100/g	100/g
Fekale koliforme bakterieë	Nul/10 g	Nul/ 10 g	Nul/10 g
Staphylococcus aureus	10/g	10/g	10/g
Salmonella	Nul/25 g	Nul/25 g	Nul/25 g
Shigella	Nul/25 g	Nul/25 g	Nul/25 g
Clostridium perfringens	Nul/25 g	Nul/25 g	Nul/25 g
Vibrio cholerae	Nul/25 g	Nul/25 g	Nul/25 g
Vibrio parahaemolyticus	Nul/25 g	Nul/25 g	Nul/25 g
Listeria monocytogenes	[4]	Nul/25 g	Nul/25 g

1) Produkte wat voor gebruik gaargemaak moet word.
2) Produkte wat voor gebruik slegs ontdooi en herverhit moet word.
3) Bedoel vir gebruik sonder gaarmaak.
4) Moet nie getoets word nie.

5.3 Bevriesing

In vrieskamers (uitgesonderd plaat- of pekelbevriesers) moet die produk weg van die vloer- en muuroppervlakke gestapel word op sodanige wyse dat lugsirkulasie tussen pakkette nie belemmer word nie. Die produk moet so gou moontlik na verwerking bevries of snelbevries word. Wanneer die produk voor bevriesing in houers verpak word, mag dit nie aan uitermate hoë temperature blootgestel word nie en daar mag hoogstens 3 h tussen die verpakking en bevriesing verloop. Die temperatuur van 'n produk, uitgesonderd 'n gerookte of vooraf gaargemaakte produk of 'n produk wat verwerk is binne 8 h nadat dit aan boord gebring is, mag gedurende die verwerkingsproses nie 20 °C oorskry nie. Vriesvermoë mag nie oorbelas word nie. Die bevriesing en vriesbewaring van die produk moet só uitgevoer word dat dit vriesbrand sal uitskakel.

5.4 Vriesbewaring

5.4.1 Rekords van die temperatuur van vriesbewaarkamers moet minstens twee jaar lank vanaf die aantekendatum gehou word en moet ter insae beskikbaar wees vir die owerheid wat hierdie spesifikasie administreer. (Kyk ook 3.2.16.3.)

5.4.2 Die produk moet by 'n temperatuur van –20 °C of laer bewaar en gehou word. Die praktiese bewaarduur van plat vis, maer vis, vetterige vis en tuna by verskillende temperature word in tabel 3 aangegee en enige produk wat langer as die toepaslike tydperk bewaar word, is onderhewig aan herondersoek na goeddunke van die owerheid wat hierdie spesifikasie administreer.

Tabel 3 — Praktiese bewaarduur

1	2	3	4
Produk	Bewaartemperatuur		
	–20 °C	–25 °C	–30 °C
	Maksimum bewaarduur maande		
Plat vis, bv tongvis	15	21	25
Maer vis, bv stokvis, koningklip, rog, inkvis, visvingers en visporsies	12	18	21
Vetterige vis – verglans	6	9	12
– vakuumverpak . .	10	12	15
Tuna	6	9	12

5.4.3 Indien die temperatuur van die produk te eniger tyd tydens bewaring tot hoër as die toepaslike bewaartemperatuur styg, moet dit vinnig tot dié temperatuur verlaag word. As die temperatuur van die produk tot hoër as –7 °C styg, moet die produk hierbenewens weer vir ondersoek aan die betrokke owerheid voorgelê word.

6 Merke

6.1 Merke op pakkette wat nie vir uitvoer bedoel is nie (kyk 6.4)

Behalwe soos ingevolge 6.4 toegelaat word, moet die volgende besonderhede leesbaar en onuitwisbaar en in ooreenstemming met 6.2 op elke pakket aangebring wees in druk wat só groot is en só uiteengesit is soos voorgeskryf in die regulasies uitgevaardig kragtens die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972 (Wet 54 van 1972), en die Wet op Handelsmetrologie, 1973 (Wet 77 van 1973):

a) die naam en volledige fisiese adres van die fabrikant, produsent, eienaar of beherende maatskappy of, in die geval van houers wat vir 'n ander persoon of organisasie verpak is, die naam en volledige fisiese adres van dié persoon of organisasie;

b) 'n juiste beskrywing van die produk, met inbegrip van die naam van die produk en die vorm waarin die inhoud aangebied word. Hierbenewens, indien die persoon of organisasie wat in (a) hierbo genoem word dit verlang, in die geval van 'n snelbevrore produk (kyk 2.22) die woord "Snelbevrore". Die naam of aanwysing wat by die etikettering van die produk gebruik word, mag nie misleidend wees nie en die soort vis, behalwe in die geval van visvingers, visporsies, viskoekies, visfrikadelle en viswors, moet aangedui word. Die voorbereidingsmetode en die vorm waarin die inhoud aangebied word, moet in ooreenstemming met die beskrywing op die etiket of pakket wees. Indien toepaslik, moet die beskrywing in ooreenstemming met die toepaslike beskrywing in 4.2 wees;

c) indien toepaslik, 'n lys van die bestanddele in dalende volgorde van hoeveelheid;

d) 'n verklaring dat die produk gaar of rou is, soos toepaslik, en bewaaraanwysings, soos volg aangegee:

Rou – Hou bevrore
Halfgaar – Hou bevrore. Moenie herbevries na ontdooiing nie
Gaar – Hou bevrore. Moenie herbevries na ontdooiing nie:

e) in die geval van produkte vir verkoop in die Republiek van Suid-Afrika, die netto massa van die inhoud, indien toepaslik (ingevolge die regulasies uitgevaardig kragtens die Wet op Handelsmetrologie, 1973); as die produk verglans is, moet die verklaring van die netto massa van die produk nie die glanslaag insluit nie;

f) die land van oorsprong;

g) indien die produk met seewater verglans is, 'n verklaring te dien effekte, wat opvallend op die hoofpaneel van die etiket direk saam met die naam van die produk vertoon word;

h) indien toepaslik, gebruiksaanwysings;

i) enige etiketvereiste wat spesifiek by regulasie vereis word; en

j) die datum van vervaardiging en identiteit van die fabriek waarin die produk verpak is. Die gebruik van 'n kode is toelaatbaar mits die verklaring van die kode verstrek word aan die owerheid wat hierdie spesifikasie administreer.

Die handelsnaam van 'n produk mag nie vir die verbruiker misleidend wees nie.

6.2 Etikette

6.2.1 Die besonderhede wat in 6.1 vereis word, moet gedruk wees op elke individuele pakket of op die buiteomhulsel wat so 'n pakket bedek of op 'n etiket van aanneemlike materiaal wat aan die pakket bevestig is.

6.2.2 Etikette op pakkette moet skoon, netjies en stewig bevestig wees. Hulle mag nie geplaas word oor ander etikette of oor drukwerk wat regstreeks op die pakkette gedruk is nie. Hulle mag slegs deur die fabrikant of sy gemagtigde agent aangebring word.

6.2.3 Etikette en seëlkleefmiddels wat moontlik in die bewaartoestande van die verpakte produkte kan agteruit gaan, mag nie gebruik word nie.

6.3 Merke op buitehouers wat nie vir uitvoer bedoel is nie (kyk 6.4)

6.3.1 Buitehouers moet skoon, netjies en heel wees en die hoeveelheid en grootte of netto massa van die pakkette daarin asook die besonderhede wat in 6.1(a), (b), (d) en (e) vereis word, moet op elke sodanige houer (karton, doos, ens) gedruk of gestensil word, behalwe dat die fisiese adres wat in 6.1(a) vereis word nie die volle fisiese adres hoef te wees nie, maar dit moet voldoende vir identifikasiedoeleindes wees. Die voorbereidingsmetode hoef nie op die buitehouer aangegee te word nie.

6.3.2 Die datum van vervaardiging, die identiteit van die fabriek en die produksielotnommer (indien toepaslik) moet gestempel of andersins onuitwisbaar gemerk wees op die buitehouer of op 'n etiket wat stewig aan die buitehouer bevestig is of op 'n verpakkingstrokie wat in die buitehouer geplaas is. 'n Kode kan vir die datum van vervaardiging gebruik word, mits die verklaring van die kode verstrek word aan die owerheid wat hierdie spesifikasie administreer.

6.4 Merke op buitehouers en pakkette wat vir uitvoer bedoel is

Buitehouers en pakkette vir uitvoer moet volgens die vereistes van die invoerland gemerk word en kan op 'n ander wyse as volgens die vereistes in 6.1 en 6.3 gemerk wees, mits daar nie gepoog word om 'n wanvoorstelling van die inhoud te gee nie. Besonderhede soos in 6.1(j) vereis word, moet op elke buitehouer en pakket gedruk word.

7 Aflewering en ondersoek

7.1 Algemeen

Die vereistes in 7.2 en 7.3 is onderworpe aan die vereistes van toepaslike statutêre wette en regulasies.

7.2 Aflewering

7.2.1 Algemeen

Die aflewering van bevrore produkte moet in higiëniese toestande geskied.

7.2.2 Aflewering vir uitvoer

Die bevrore produk vir uitvoer moet by 'n temperatuur van –20 °C of laer van die fabriek na die vriesbewaardepot vervoer word en in die vaartuig waarin dit vervoer gaan word se vriesbewaarruim afgelewer word. Indien die temperatuur van die produk te eniger tyd gedurende sodanige vervoer tot hoër as –20 °C styg, moet dit so vinnig moontlik tot die vereiste temperatuur verlaag word. Die produk moet herondersoek word indien die temperatuur tot hoër as –7 °C gestyg het.

7.2.3 Aflewering vir plaaslike verkoop

Die bevrore produk vir plaaslike verspreiding moet in verkoelde of geïsoleerde vragwaens van die fabriek of vriesbewaardepot na die kleinhandelverkooppunt vervoer word. Tydens plaaslike vervoer moet die temperatuur van die produk –20 °C of laer wees, behalwe by die buiteoppervlakke van 'n stapel. Verkoelde vragwaens moet minstens een termometer hê wat só geïnstalleer is dat dit buite die verkoelde kompartement gelees kan word.

7.3 Ondersoek vir uitvoer

Elke besending van die bevrore produk moet vir ondersoek beskikbaar wees by die vriesbewaardepot van waar dit vir uitvoer verskeep sal word. Die owerheid wat hierdie spesifikasie administreer, moet minstens 14 d voor die verwagte verskepingsdatum van die produk in kennis gestel word. Produkte wat nie vir uitvoer aangeneem word nie, moet eenkant gehou en duidelik geïdentifiseer word as dit saam met produkte wat vir uitvoer goedgekeur is in vriesbewaarkamers bewaar word. Die bevrore produk moet op die plek van verskeping vir herondersoek voorgelê word indien daar twyfel oor die temperatuurgeskiedenis daarvan ontstaan het terwyl dit op verskeping gewag het.

8 Metodes vir fisiese ondersoek

8.1 Fisiese ondersoek van bevrore vis vir die algemene eienskappe daarvan

8.1.1 Kleinerige vis kan heel ontdooi word. Sny in die geval van 'n groot vis 'n monsterwig van 'n geskikte grootte uit die rug van die vis net agter die kop tot by die ruggraat of, so nie, 'n dwarssnitstuk van minstens 50 mm dik deur die vis net agter die kop of net voor die stert. Verseker, indien moontlik, dat die massa van elke monster minstens 1 kg is. Laat die monster ontdooi en ondersoek dit vir reuk en kleur (kyk 4.1.2.2(a)). Kraakbeenagtige vis soos haaie en rog moet in lug ontdooi word om die opsporing van 'n ammoniakreuk te vergemaklik.

8.1.2 In die geval van tuna moet die monster ontdooi word en die bloed met kraanwater afgespoel word. Ondersoek dan elke monster vir kleur in die algemeen (kyk 4.1.2.3(g)), en vir kleur met die ruggraat langs (kyk 4.1.2.3(h)). Stoom die monster tot 'n kerntemperatuur van minstens 70 °C tot 75 °C bereik is en ondersoek die volgende volgens die vereistes van 4.1.2.3(j):

a) geur;

b) proteïenstolsel op die oppervlak van die vleis;

c) lostrek van spierlae; en

d) kleur.

8.1.3 Ondersoek die vis visueel vir die oorblywende algemene eienskappe daarvan volgens die toepaslike vereistes in 4.1.2.

8.2 Bepaling van die visgehalte, vulselgehalte en gewaste massa

8.2.1 Bepaling van die visgehalte van produkte bedek met broodkrummels of beslag

8.2.1.1 Teken die verklaarde netto massa (m_0) aan wat op die pakket gedruk is. Plaas die inhoud van die pakket in 'n waterbad waarvan die temperatuur by 47 °C tot 49 °C gehou word en laat die eenhede in die water bly totdat die broodkrummels of beslag (soos toepaslik) sag word en maklik met 'n rondepuntspatel of 'n tafelmes van die steeds bevrore visvleis verwyder kan word.

OPM – Dit kan nodig wees om verskeie voorlopige toetse uit te voer om die optimum onderdompeltyd te bepaal wat nodig is om die broodkrummels en beslag van die eenhede in 'n pakket te verwyder. **Slegs vir hierdie toetse** kan 'n versadigde oplossing van koper(II)sulfaat in plaas van water gebruik word. Die optimum onderdompeltyd is die kortste onderdompeling in die kopersulfaatoplossing wat nodig is om die broodkrummels of die beslag (soos toepaslik) maklik te kan afkrap en slegs 'n geringe spoor van blou te laat op die oppervlak van die viseenhede waarvan die broodkrummels of beslag verwyder is.

8.2.1.2 Haal die eenhede uit die waterbad en druk liggies met 'n papierhanddoek droog. Skraap die broodkrummels of beslag met die spatel van die visvleis af, eers van die smal kante en ente en dan van die breë plat oppervlakke en verwyder dit. Indien dit moeilik is om die bedekking van 'n eenheid af te haal, onderdompel die eenheid vir tot nog 5 s en verwyder die res van die bedekking. Maak seker dat die totale onder-dompeltyd hoogstens 15 s is. Bepaal die massa (m_1) van al die eenhede waarvan die broodkrummels of beslag verwyder is.

8.2.1.3 Bereken die visgehalte, uitgedruk as 'n persentasie van die massa van die produk, aan die hand van die volgende formule:

$$\frac{m_1}{m_0} \times 100$$

waar

m_0 die verklaarde netto massa van die pakket is, in gram; en

m_1 die totale massa is van al die eenhede waarvan die broodkrummels of beslag verwyder is, in gram.

8.2.2 Gewaste massa van 'n produk wat sous bevat

8.2.2.1 Teken die verklaarde netto massa (m_0) aan wat op die pakket gedruk is. Plaas die inhoud van die pakket in 'n geweegde sif met 'n nominale openinggrootte van ongeveer 2 mm en spoel die produk af met water wat, indien nodig, voorverwarm is tot 'n temperatuur van hoogstens 38 °C, totdat die produk vry van sous is. Dreineer 2 min lank en bepaal dan die massa van die materiaal wat in die sif agterbly as die gewaste massa (m_1) van die viskomponent, en teken dit aan.

8.2.2.2 Bereken die persentasie gewaste massa van die produk aan die hand van die formule in 8.2.1.3.

8.2.3 Gewaste massa van die viskomponent van 'n produk wat groente of vrugte of graan (of enige kombinasie daarvan) bevat

8.2.3.1 Teken die verklaarde netto massa (m_0) aan wat op die pakket gedruk is. Plaas die inhoud van die pakket in 'n geweegde sif met 'n openinggrootte van ongeveer 2 mm en spoel af met water wat, indien nodig, tot 'n temperatuur van hoogstens 38 °C voorverwarm is. Verwyder die groente of vrugte of graan, soos toepaslik. Dreineer 2 min lank en bepaal dan die massa van die materiaal wat in die sif agterbly as die gewaste massa (m_1) van die viskomponent, en teken dit aan.

8.2.3.2 Bereken die persentasie gewaste massa van die produk aan die hand van die formule in 8.2.1.3.

8.2.4 Massa van pasteivulsel

Laat vyf vispasteie ontdooi en bepaal dan hulle totale massa (m_0). Verwyder die kors en bepaal die massa van die vulsel (m_1). Bereken die massa van die vulsel, uitgedruk as 'n persentasie van die massa van die produk, aan die hand van die volgende formule:

$$\frac{m_1}{m_0} \times 100$$

waar

m_0 die massa van vyf pasteie is, in gram; en

m_1 die massa van die vulsel van vyf pasteie is, in gram.

8.2.5 Massa van uie in ingelegde vis

8.2.5.1 Teken die verklaarde netto massa (m_0) aan wat op die pakket gedruk is. Plaas die inhoud van die pakket in 'n geweegde sif met 'n openinggrootte van ongeveer 2 mm en spoel die produk af met water wat, indien nodig, voorverwarm is tot 'n temperatuur van hoogstens 38 °C, totdat dit vry van sous is. Haal die vis uit. Laat dit 2 minute lank dreineer en bepaal dan die massa van die materiaal wat in die sif agterbly as die massa (m_1) van die uie, en teken dit aan.

8.2.5.2 Bereken die persentasie massa van die uie in die produk aan die hand van die formule in 8.2.1.3.

8.3 Bepaling van die netto massa van ander bevrore produkte as verglansde produkte

8.3.1 Verwyder, onmiddellik nadat die pakket uit vriesbewaring gehaal is, alle ys wat aan die buitekant van die pakket kleef en bepaal die bruto massa van die onoopgemaakte pakket.

8.3.2 Verwyder die verpakkingsmateriaal. Was en droog die verpakkingsmateriaal en bepaal die massa daarvan. Teken die verskil tussen die bruto massa (kyk 8.3.1) en die massa van die verpakkingsmateriaal as die netto massa van die bevrore produk aan.

8.4 Bepaling van die netto massa van 'n verglansde produk

8.4.1 Plaas die inhoud van die pakket onmiddellik nadat dit uit vriesbewaring gehaal is in 'n houer waarin vars drinkbare water (kyk 3.4.1) by omgewingstemperatuur van onder af teen 'n vloeitempo van ongeveer 5 ℓ/min ingelaat word. Laat die produk in die water totdat al die oppervlakys gesmelt het. Indien die produk geblokvries is, draai die blok 'n paar maal tydens ontglansing om; druk die blok en verwyder eenhede uit die water na gelang hulle losraak.

8.4.2 As al die verglansing wat gesien of gevoel kan word, verwyder is en die eenhede maklik van mekaar geskei kan word, plaas die inhoud van die houer (kyk 8.4.1) oor na 'n geweegde sif met 'n

nominale openinggrootte van ongeveer 2 mm. Hou die sif skuins met 'n hoek van ongeveer 20° en dreineer 2 min lank.

8.4.3 Teken die massa van die materiaal wat in die sif agterbly as die netto massa van die verglansde produk aan.

9 Metodes vir chemiese ontleding

OPM – Gebruik tydens ontleding en tensy daar anders gespesifiseer word, slegs reagense van erkende analitiese graad of (indien sodanige graad onverkrygbaar is) van die suiwerste graad wat beskikbaar is en gebruik slegs gedistilleerde of gedeïoniseerde water.

9.1 Bereiding van produkmonster

Laat die produk in sy verpakking ontdooi en verwyder dan die verpakking. Indien toepaslik, bv in die geval van vispasteie, verwyder die kors/deklaag na ontdooiing. Maal die oorblywende inhoud van die monster twee maal in 'n vleismeul en meng die gemaalde monster deeglik. Plaas 'n geskikte hoeveelheid van die gemaalde monster oor na 'n glashouer met 'n deksel of skroefdop wat styf toegemaak kan word. Bewaar in 'n koelkas totdat die toetse uitgevoer word.

9.2 Bepaling van lood, koper, sink en kadmium (atoomabsorpsiespektrofotometriese metode)

9.2.1 Apparaat

9.2.1.1 'n Atoomabsorpsiespektrofotometer (Raadpleeg die fabrikant se verwysingshandleidings mbt golflengte, spleetwydte, vlamtoestande, ens).

9.2.1.2 Kroesie, platinum, met 'n inhoudsvermoë van 150 mℓ.

9.2.1.3 Waterbad.

9.2.1.4 Temperatuurbeheerde oond.

9.2.2 Reagense

9.2.2.1 Soutsuur, 1 N, berei deur 98 mℓ HCl met gedistilleerde water tot 1 ℓ te verdun.

9.2.2.2 Standaardloodoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg Pb/mℓ; en

b) **standaardwerkoplossing:** 1,0 µg Pb/mℓ.

9.2.2.3 Standaardkoperoplossings, soos volg:

a) **standaardvoorraadoplossing:** 1 mg Cu/mℓ; en

b) **standaardwerkoplossing:** 3,0 µg Cu/mℓ.

9.2.2.4 Standaardsinkoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg Zn/mℓ; en

b) **standaardwerkoplossing**: 2,0 µg Zn/mℓ.

9.2.2.5 Standaardkadmiumoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg Cd/mℓ; en

b) **standaardwerkoplossing**: 1,0 µg Cd/mℓ.

9.2.3 Metodes

9.2.3.1 Bereiding van monsteroplossing

Weeg 25 g ± 0,1 g van die monster af in die kroesie (kyk 9.2.1.2) en laat dit 2 h lank by 135 °C tot 150 °C droog word. Plaas die kroesie in 'n koue, temperatuurbeheerde oond en verhoog die temperatuur stadig tot 450 °C. Laat die monster oornag (16 h) veras. Haal die kroesie uit en laat dit afkoel. Voeg 10 mℓ van die 1N HCl by en laat die as oplos deur die kroesie versigtig oor 'n kokende waterbad te verhit. Plaas die inhoud van die kroesie in 'n 25-mℓ- volumetriese fles. Verhit die asresidu weer agtereenvolgens met twee 5-mℓ-porsies van die 1N HCl en voeg dit by die fles. Laat afkoel, verdun volgens volume met die 1N HCl en meng.

9.2.3.2 Reagensblanko

Berei 'n reagensblanko.

9.2.3.3 Bepaling van lood

Bepaal die absorbansie van die monsteroplossing, van die reagensblanko en van die 1,0-µg-Pb/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder is as die absorbansie van die standaardwerkoplossing, is die lood in die monster minder as 1 mg/kg.

9.2.3.4 Bepaling van koper

Verdun 5,0 mℓ van die monsteroplossing met water tot 50,0 mℓ. Bepaal die absorbansie van die monsteroplossing, van die reagensblanko en van die 3,0-µg-Cu/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder is as die absorbansie van die standaardwerkoplossing, is die koper in die monster minder as 30 mg/kg.

9.2.3.5 Bepaling van sink

Verdun 5,0 mℓ van die monsteroplossing met water tot 100,0 mℓ. Bepaal die absorbansie van die monsteroplossing, van die reagensblanko en van die 2,0-µg-Zn/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder is as die absorbansie van die standaardwerkoplossing, is die sink in die monster minder as 40 mg/kg.

9.2.3.6 Bepaling van kadmium

Bepaal die absorbansie van die monsteroplossing, van die reagensblanko en van die 1,0-µg-Cd/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder is as die absorbansie van die standaardwerkoplossing, is die kadmium in die monster minder as 1 mg/kg.

9.3 Bepaling van tin (atoomabsorpsiemetode)

9.3.1 Apparaat

Atoomabsorpsiespektrofotometer. (Raadpleeg die fabrikant se verwysingshandboeke mbt golflengte, spleetwydte, vlamtoestande, ens).

9.3.2 Reagense

9.3.2.1 Standaardtinoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg Sn/mℓ; en

b) **standaardwerkoplossing**: 40,0 µg Sn/mℓ.

9.3.2.2 Kaliumchloriedoplossing, 10 mg K/mℓ, berei deur 1,91 g KCl op te los en met gedistilleerde water tot 100 mℓ te verdun.

9.3.2.3 Salpetersuur (HNO_3), gekonsentreer. Toets die suiwerheid van 'n lot deur 'n porsie met gedistilleerde water tot 1:4 (volgens volume) te verdun en aspireer dit in 'n AA-spektrofotometer. Die afwesigheid van 'n Sn-sein dui op geskiktheid vir ontleding.

9.3.3 Bereiding van monster

Weeg 25 g (± 0,01 g) van die monster noukeurig in 'n 250-mℓ-Erlenmeyerfles af. Laat dit by 120 °C in 'n oond droog word.

OPM – Moet nie HNO_3 by monsters (kyk onder) voeg nie tensy daar tyd is om hierdie stadium van vertering op dieselfde dag te voltooi.

Voeg 30 mℓ van die gekonsentreerde HNO_3 by die fles en verhit dit binne 15 min versigtig in 'n dampkas om vertering te laat begin en vermy oormatige skuimvorming. Laat dit liggies kook totdat 3 mℓ tot 6 mℓ van die verteersel oorbly of totdat die monster net begin droog word op die bodem. Moet nie die monster laat verkool nie. Verwyder die fles van die hitte. Gaan dadelik soos volg te werk en berei tegelykertyd twee leë flesse vir reagensblanko's: voeg 25 mℓ gekonsentreerde soutsuur (HCl) by, verhit ongeveer 15 min lank liggies totdat die onegalige gekook van die monster as gevolg van die ontwikkeling van chloor (Cl_2) ophou. Verhoog die hitte en kook totdat 'n volume van 10 mℓ tot 15 mℓ oorbly. Gebruik 'n soortgelyke fles met 15 mℓ water om die oorblywende volume te skat. Plaas die monsteroplossing en die reagensblanko's na 25-mℓ- volumetriese flesse oor. Die monsteroplossing en die reagensblanko's kan oornag of langer staan.

Pipetteer 1,0 mℓ van die KCl-oplossing in elke volumetriese fles. Laat dit tot omgewingstemperatuur afkoel en verdun met water tot volume. Meng goed en filtreer slegs die monsteroplossing deur droë papier met 'n medium porositeit in 'n droë polipropileen- of poliëtileenskroefdopbottel. Plaas die blanko's na soortgelyke bottels oor. Maak die bottels toe totdat dit ontleed word. Oplossings is etlike maande lank stabiel.

9.3.4 Reagensblanko

Berei 'n reagensblanko.

9.3.5 Prosedure

Bepaal die absorbansie van die monsteroplossing, van die reagensblanko en van die 40,0-µg-Sn/mℓ-werkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder as die absorbansie van die standaardwerkoplossing is, is die tin in die monster minder as 40 mg/kg.

9.4 Bepaling van arseen (Gutzeit-metode)

9.4.1 Apparaat

Kyk figuur 1 en 9.4.3.

9.4.2 Reagense

9.4.2.1 Standaardarseenoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg As/mℓ; en

b) **standaardwerkoplossing**: 1,0 µg As/mℓ.

9.4.2.2 Soutsuur, gekonsentreer.

9.4.2.3 Kaliumjodiedoplossing, 'n 16,6-g/100-mℓ-oplossing.

9.4.2.4 Tin(II)chloriedoplossing

Los 33 g tin(II)chloried (arseenvry) in 10 mℓ soutsuur en genoeg water op om 100 mℓ te lewer.

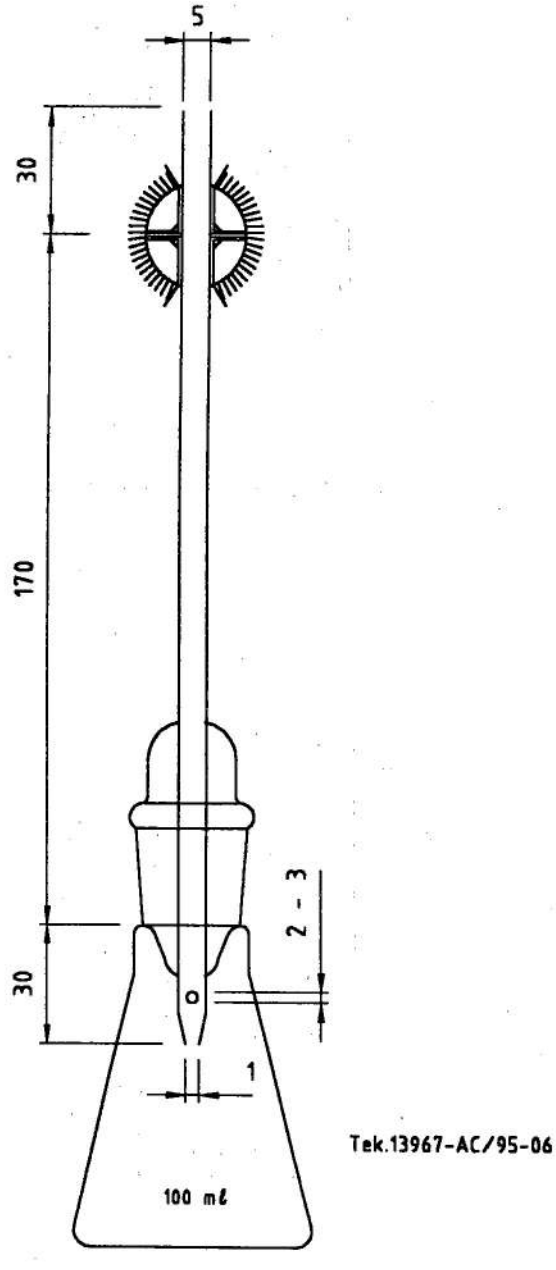
9.4.2.5 Kwik(II)bromied, kwikbromied ($HgBr_2$ = 360,4 analitiese reagensgraad).

9.4.2.6 Kwik(II)bromiedpapier, wat soos volg berei word: plaas in 'n reghoekige bak 'n 5-g/100-mℓ-oplossing kwik(II)bromied in absolute etanol en dompel daarin stukkies wit filtreerpapier met 'n gramtal van 80 g/m^2 (Whatman no 1 is geskik), elk met afmetings 200 cm x 15 mm of 200 mm x 15 mm en dubbel gevou. Giet die oortollige vloeistof af en laat die papiere weg van lig droog word deur dit oor 'n niemetaaldraad te hang. Sny die gevoude rand af tot 'n breedte van 10 mm. Sny die oorblywende stroke in 15-mm-vierkante of skywe met 'n diameter van 15 mm.

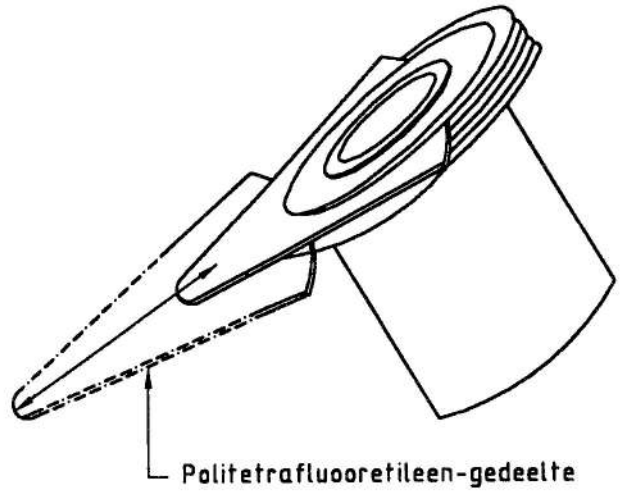
Kwik(II)bromiedpapier moet in 'n houer met 'n glasprop gehou word en teen lig beskerm word.

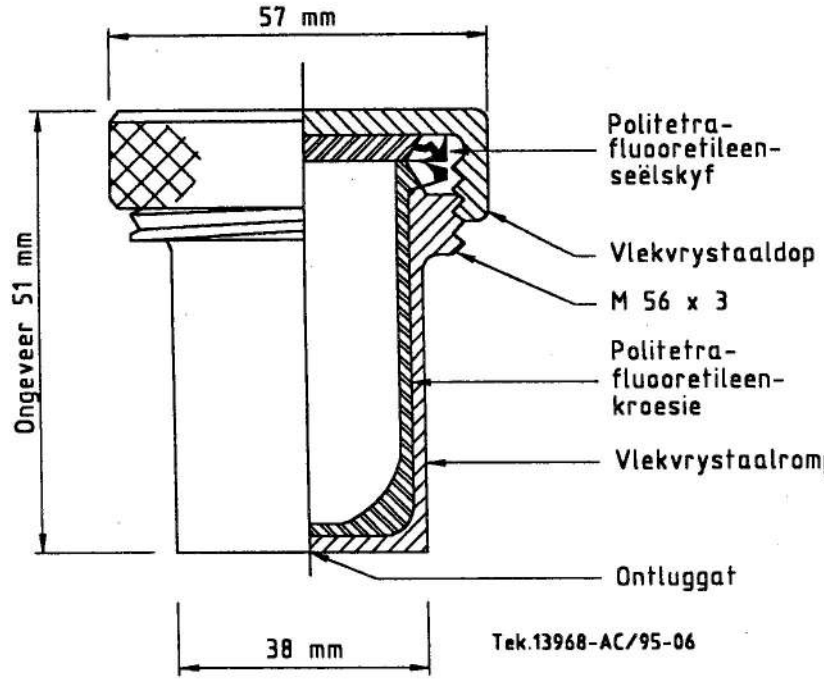
9.4.2.7 Sink, gegranuleer.

9.4.2.8 Loodasetaatoplossing, 'n 10-g/100-mℓ-oplossing van lood(II)asetaat in water wat vry van koolstofdioksied is.

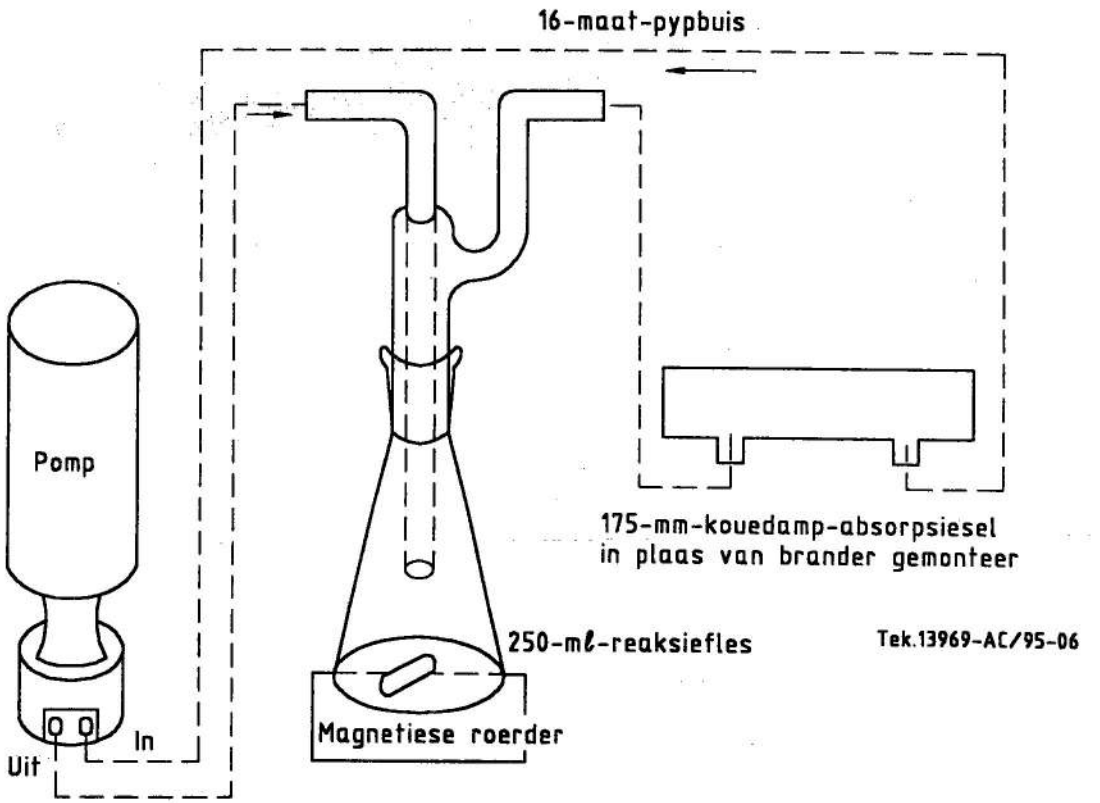


Figuur 1 — Apparaat vir grenstoets vir arseen





Figuur 2 — Verteerhouer



Figuur 3 — Apparaat vir die bepaling van kwikgehalte

9.4.2.9 Loodasetaatwatte, wat soos volg berei word: dompel absorbeerwatte in 'n mengsel van 10 volumes loodasetaat en 1 volume 2M-asynsuur. Laat die oortollige vloeistof wegdreineer deur die watte op etlike lae filtreerpapier te plaas, sonder om die watte te druk. Laat die watte by kamertemperatuur droog word. Loodasetaatwatte moet in 'n lugdigte houer gehou word.

9.4.3 Prosedure

Neem 5,0 mℓ van die monsteroplossing wat vir die bepaling van tin (kyk 9.3.3) berei is.

Die apparaat (kyk figuur 1) bestaan uit 'n 100-mℓ- koniese fles met 'n slypglasprop waardeur 'n glasbuis met 'n lengte van ongeveer 200 mm en 'n binnediameter van 5 mm gaan. Die onderste deel van die buis is getrek tot 'n binnediameter van 1,0 mm en 15 mm van die punt daarvan is 'n syopening met 'n diameter van 2 mm tot 3 mm. As die buis in die prop in posisie is, moet die syopening 2 mm tot 3 mm onder die onderste oppervlak van die prop wees. Die boonste ent van die buis het 'n volkome plat, geslypte oppervlak wat reghoekig op die as van die buis is. 'n Tweede glasbuis met dieselfde binnediameter en 'n lengte van 30 mm, met 'n soortgelyke plat geslypte oppervlak, word in aanraking met en koaksiaal tot die eerste buis geplaas en deur twee spiraalvere in posisie gehou. Plaas 50 mg tot 60 mg loodasetaatwatte, los gepak, of 'n klein watteproppie en 'n opgerolde stukkie loodasetaatpapier met 'n gesamentlike massa van 50 mg tot 60 mg in die onderste buis. Plaas een van die stukkies kwik-(II)bromiedpapier (kyk 9.4.2.6) tussen die plat oppervlakke van die buise. Verdun 5,0 mℓ van die monsteroplossing in die koniese fles met water tot 25 mℓ. Voeg 15 mℓ gekonsentreerde soutsuur, 0,1 mℓ tin(II)-chloriedoplossing en 5 mℓ kaliumjodiedoplossing by, laat dit 15 min lank staan en voeg dan 5 g gegranuleerde sink by. Stel dadelik die twee dele van die apparaat op en dompel die fles in 'n waterbad by 'n temperatuur wat sodanig is dat 'n egalige ontwikkeling van gas volgehou word. Na minstens 2 h mag geen vlek wat op die kwik(II)-bromiedpapier voortgebring word intenser wees as dié wat verkry is as 1 mℓ van die standaard-arseenwerkoplossing (1 µg/mℓ As) op dieselfde wyse met water tot 25 mℓ verdun word nie.

9.5 Bepaling van kwik

9.5.1 Apparaat

9.5.1.1 Atoomabsorpsiespektrofotometer, voorsien van 'n kwikholkatodelamp.

9.5.1.2 Verteerhouer (kyk figuur 2), wat bestaan uit 'n vlekvrystaalromp wat 'n politetrafluooretileen-kroesie steun en 'n skroefdop met 'n politetrafluooretileen-voering om 'n politetrafluooretileen-seëloppervlak te verskaf, of 'n soortgelyke verteerhouer.

'n Politetrafluooretileen-tuit word aan die buitenste rand van die houer vasgeknip om dit moontlik te maak om die inhoud kwantitatief oor te plaas sonder dat dit met metaaldele in aanraking kom.

9.5.1.3 Kouedamp-absorpsiesel, wat in die plek van die brander van die spektrofotometer (kyk figuur 3) aangebring is.

9.5.1.4 Diafragmapomp.

9.5.2 Reagense

9.5.2.1 Soutsuur, gekonsentreer.

9.5.2.2 Salpetersuur, gekonsentreer.

9.5.2.3 Swaelsuur, gekonsentreer.

9.5.2.4 Verdunsuuroplossing, 'n waterige oplossing wat 58 mℓ salpetersuur en 67 mℓ swaelsuur per liter bevat.

9.5.2.5 Verdunde soutsuur, een volume van die soutsuur by nege volumes water gevoeg.

9.5.2.6 Tindichloriedoplossing, 5 g kristallyne tindichloried ($SnCl_2.2H_2O$), in 10 mℓ van die gekonsentreerde soutsuur opgelos deur dit te verwarm en met water tot ongeveer 50 mℓ te verdun. Verwyder spoorhoeveelhede kwik deur stikstof 10 min lank deur die oplossing te laat borrel.

9.5.2.7 Standaardkwikoplossings, soos volg:

a) **standaardvoorraadoplossing**, 1 mg Hg/mℓ; en

b) **standaardwerkoplossing**, 0,1 µg Hg/mℓ: verdun 1,0 mℓ van die standaardvoorraadoplossing (kyk (a) hierbo) met die verdunde soutsuur (HCl) tot 100 mℓ. Verdun dan 1,0 mℓ van hierdie oplossing met die verdunde HCl tot 100 mℓ. Berei hierdie oplossing daagliks.

9.5.3 Reagensblanko

Berei 'n reagensblanko.

9.5.4 Prosedure

9.5.4.1 Weeg noukeurig 1 g ± 0,1 g van die monster af (kyk 9.2) (**waarskuwing**: moet nie meer as 300 mg droë massa gebruik nie; gebruik hoogstens 200 mg droë massa in die geval van materiaal met 'n hoë vetgehalte) in die verteerhouer (kyk 9.5.1.2), voeg 5,0 mℓ van die gekonsentreerde salpetersuur HNO_3 by en maak die houer toe deur die skroefdop vas te draai. Plaas die houer, sonder om dit te laat kantel, 30 min tot 60 min lank of totdat die monsteroplossing helder is in 'n oond wat tot 150 °C voorverhit is. Haal die houer uit en laat dit tot kamertemperatuur afkoel. Skroef die dop af, knip die tuit aan, plaas die inhoud van die houer met behulp van die verdunsuuroplossing (kyk 9.5.2.4) oor na 'n 100-mℓ-volumetriese fles en verdun tot volume met die suuroplossing.

9.5.4.2 Skakel die kwikholkatodelamp aan en laat die spektrofotometer toe om ten volle teen 'n golflengtestelling van 253,7 nm te ekwilibreer. Pipetteer 20 mℓ van die 100-mℓ-monsteroplossing (kyk 9.5.4.1) in die reaksiefles. Plaas 'n magnetiese volger in die fles en verbind die absorpsiesel, die reaksiefles en die diafragmapomp in serie en in 'n geslote stelsel deur middel van 'n politetrafluooretileen(PTFE)-buis (kyk figuur 3), en beperk die verdunning van die kwikdamp tot 'n minimum deur buise met die kleinste diameter en kortste lengte moontlik te gebruik. Maak seker dat die afstand tussen die onderpunt van die inlaatbuis en die oppervlak van die monsteroplossing in die reaksiefles minstens 10 mm is.

9.5.4.3 Skakel die magnetiese roerder en die pomp aan. Stel die absorpsielesing op die spektrofotometer op zero. Skakel die roerder en die pomp af.

9.5.4.4 Diskonnekteer die fles, voeg 1 mℓ van die tindichloriedoplossing by die monsteroplossing, herverbind onmiddellik die fles, skakel die magnetiese roerder aan, laat dit 90 s lank roer, skakel die roerder af en skakel dan onmiddellik die pomp aan. Teken die absorpsielesing aan sodra dit stabiliseer. Verwyder die fles en pomp lug deur die stelsel om die kwikdamp te verwyder.

9.5.4.5 Plaas 1,0 mℓ van die 0,1-µg-Hg/mℓ-standaardwerkoplossing na 'n reaksiefles oor, voeg 19,0 mℓ van die gedistilleerde water by en herhaal die prosedure in 9.5.4.2, 9.5.4.3 en 9.5.4.4.

9.5.5 Berekening

Meet die absorbansie van die 0,1-µg-Hg/mℓ-standaardwerkoplossing, van die reagensblanko en van die monsteroplossing.

Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder as die absorbansie van die standaardwerkoplossing is, is die kwikgehalte van die monster minder as 0,5 mg/kg.

9.6 Bepaling van antimoon

9.6.1 Apparaat

Atoomabsorpsiespektrofotometer. (Raadpleeg die fabrikant se verwysingshandleidings mbt golflengte, spleetwydte, vlamtoestande, ens).

9.6.2 Reagense

9.6.2.1 Kaliumjodied.

9.6.2.2 Standaardantimoonoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg Sb/mℓ; en

b) **standaardwerkoplossing**: 0,01 µg Sb/mℓ.

9.6.3 Reagensblanko

Berei 'n reagensblanko.

9.6.4 Prosedure

9.6.4.1 Volg die apparaatfabrikant se aanwysings vir die hidriedgenerering vir antimoon en maak seker dat die antimoon voor ontleding in die Sb^{111}-toestand is deur die monster en standaardoplossings met 'n oormaat kaliumjodied te behandel.

9.6.4.2 Meet die absorbansie van die 0,01-µg-Sb/mℓ-standaardwerkoplossing, van die reagensblanko en van die monsteroplossing (deur middel van die monsteroplossing wat by die bepaling van die kwik verkry is). Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder as die absorbansie van die standaardwerkoplossing is, is die antimoongehalte in die monster minder as 1 mg/kg.

9.7 Bepaling van proteïenstikstofgehalte en berekening van visgehalte

9.7.1 Reagense

Gebruik tydens ontleding slegs reagense van 'n erkende analitiese graad wat vry van stikstof is en gebruik slegs gedistilleerde water of water van ekwivalente suiwerheid.

9.7.1.1 Waterstofperoksiedoplossing, 30 % (volgens volume).

9.7.1.2 Kwikoksied (HgO).

9.7.1.3 Kaliumsulfaat of anhidriese natriumsulfaat.

9.7.1.4 Swaelsuur, gekonsentreer, (d by 25 °C/25 °C is 1,84).

9.7.1.5 Sinkkorrels.

9.7.1.6 Boorsuuroplossing, 40 g/ℓ.

9.7.1.7 Natriumhidroksied-natriumtiosulfaatoplossing, 45 %: 450 g natriumhidroksied en 80 g natriumtiosulfaat ($Na_2S_2O_35H_2O$) opgelos in water en verdun tot 1 ℓ.

9.7.1.8 Gestandaardiseerde swaelsuuroplossing, ($c(H_2SO_4)$ = 0,1 mol/ℓ).

9.7.1.9 Gemaskeerde metielrooi-indikator, 0,125 g metielrooi en 0,083 g metileenblou, opgelos in 100 mℓ etanol (96 % (volgens volume)), en gefiltreer.

9.7.2 Prosedure

9.7.2.1 Weeg noukeurig ongeveer 2 g van die bereide monster (kyk 9.1) af en plaas hierdie toetseksemplaar in 'n 500-mℓ-Kjeldahl-verteerfles. Voeg 'n paar glaskrale, 7 g van die kaliumsulfaat (of anhidriese natriumsulfaat), 0,3 g van die kwikoksied en 15 mℓ van die gekonsentreerde swaelsuur by. Voeg 5 mℓ van die waterstofperoksiedoplossing stadig en versigtig by en laat staan totdat die reaksie bedaar het.

9.7.2.2 Verhit versigtig totdat dit ophou skuim en verhit dan sterk totdat die oplossing helder word. Gaan minstens nog 30 min lank met die vertering voort (ongeveer 1 h is nodig vir algehele vertering).

9.7.2.3 Laat die inhoud van die fles afkoel en verdun dit met ongeveer 250 mℓ water. Laat tot kamertemperatuur afkoel en laat 75 mℓ van die natriumhidroksied-natriumtiosulfaatoplossing teen die kant van die fles inloop sodat dit 'n afsonderlike laag op die bodem vorm en nie onmiddellik met die suuroplossing meng nie. Voeg 'n paar sinkkorrels by.

9.7.2.4 Verbind die fles met 'n Kjeldahldistilleereenheid, meng die inhoud van die fles deur dit liggies te werwel en distilleer dan die ammoniak (dws ongeveer 150 mℓ distillaat) in 'n Erlenmeyerfles wat 50 mℓ van die boorsuuroplossing en drie of vier druppels van die gemaskeerde metielrooi-indikator bevat.

9.7.2.5 Titreer die ammoniak in die Erlenmeyerfles met die gestandaardiseerde swaelsuuroplossing.

9.7.2.6 Voer 'n blanko bepaling in identiese toestande uit, maar laat die toetseksemplaar weg.

9.7.3 Berekening

9.7.3.1 Die proteïenstikstofgehalte, uitgedruk as 'n persentasie volgens massa van die produk, word aan die hand van die volgende formule aangegee:

$$\frac{(V - V_1) \times c \times 2 \times 1,4}{m}$$

waar

V die monstertiter swaelsuur is, in milliliter;

V_1 die blanko titer swaelsuur is, in milliliter;

c die konsentrasie gestandaardiseerde swaelsuur is, in mol per liter, en

m die massa van die toetseksemplaar is, in gram.

9.7.3.2 Bereken die visgehalte, uitgedruk as 'n persentasie volgens massa van die produk, deur die proteïenstikstofgehalte met 37,5 te vermenigvuldig.

OPM – As die produk ander proteïenbevattende materiaal buiten vis bevat, moet toepaslike korreksies by die beoordeling van die visgehalte aangebring word.

9.8 Bepaling van chloriedgehalte (uitgedruk as natriumchloried)

9.8.1 Reagense

Gebruik tydens die ontleding slegs reagense van erkende analitiese graad en slegs gedistilleerde water of water van ekwivalente suiwerheid.

9.8.1.1 Nitrobenseen.

9.8.1.2 Salpetersuur, verdun tot die helfte van die gekonsentreerde sterkte.

9.8.1.3 Natriumkarbonaatoplossing, versadig.

9.8.1.4 Standaardkaliumtiosianaatoplossing (c(KCNS) = 0,1 mol/ℓ).

9.8.1.5 Standaardsilwernitraatoplossing ($c(AgNO_3)$ = 0,1 mol/ℓ), noukeurig gestandaardiseer.

9.8.1.6 Ferrialuin-indikator, 'n koudversadigde ferriammoniumsulfaatoplossing ($NH_4Fe(SO_4)_2.12H_2O$) waarby 'n paar druppels verdunde salpetersuur (kyk 9.8.1.2) gevoeg is.

9.8.2 Prosedure

9.8.2.1 Neem 'n monster van die vis soos in 9.1 beskryf word en berei die monster volgens 9.1.

9.8.2.2 Weeg 'n geskikte hoeveelheid van die bereide monster noukeurig in 'n indampbakkie of kroes af, bevogtig hierdie toetseksemplaar met die natriumkarbonaatoplossing en laat dit oor 'n waterbad droog word. Verkool die gedroogde eksemplaar en veras dit by 'n temperatuur van hoogstens 500 °C.

9.8.2.3 Ekstraheer die residu met die verdunde salpetersuur en filtreer dit in 'n 100-mℓ- volumetriese fles. Herhaal die ekstraksie en filtrasie een maal, was die filter deeglik met die verdunde salpetersuur, verdun die oplossing in die fles tot volume met die verdunde salpetersuur en meng.

9.8.2.4 Voeg 25 mℓ van die standaardsilwernitraatoplossing, 5 mℓ nitrobenseen en 1 mℓ ferrialuin-indikator by 'n gepaste alikwot in 'n 250-mℓ-Erlenmeyerfles en skud goed. Titreer die oormaat silwernitraat met die standaardkaliumtiosianaat-oplossing totdat 'n permanente rooierige kleur 15 s lank voorkom. Voer 'n blanko bepaling in identiese toestande uit, maar laat die toetseksemplaar weg. Die verskil tussen die titrasies van die blanko bepaling en van die toets is die volume (V) silwernitraat wat by die bepaling gebruik is.

9.8.3 Berekening

Bereken die chloriedgehalte (as natriumchloried), uitgedruk as 'n persentasie volgens massa van die produk, aan die hand van die volgende formule:

$$\frac{V \times c \times 5{,}845}{m}$$

waar

V die volume standaardsilwernitraatoplossing is wat by die bepaling gebruik is, in milliliter;

c die konsentrasie standaardsilwernitraatoplossing is, in mol per liter; en

m die massa van die oorspronklike toetseksemplaar is, verteenwoordig deur die alikwot wat by die titrasie gebruik is, in gram.

10 Metodes vir mikrobiologiese ondersoek

10.1 Algemeen

Aseptiese tegnieke moet deurgaans in die ondersoek gevolg word.

10.2 Laboratoriumglasware

10.2.1 Algemeen

Maak seker dat alle glasware wat gebruik word teen herhaalde hittesterilisasie bestand is en dat die glas vry is van inhiberende stowwe soos swaar metale en vry alkalieë. Boorsilikaatglas met 'n uitsettingskoëffisiënt van minder as $6 \times 10^{-6}k^{-1}$ word aanbeveel.

10.2.2 Bottels (universeel)

Bottels met standaardskroefdoppe van plastiek of metaal en met 'n nominale inhoudsvermoë van

a) 30 mℓ,

b) 100 mℓ,

c) 250 mℓ,

d) 500 mℓ, en

e) 1 000 mℓ.

10.2.3 Kweekbuise

Liplose silindriese buise met halfronde ente en 'n nominale wanddikte van 1,5 mm en met 'n diameter en lengte van

a) 16 mm x 160 mm, en

b) 20 mm x 200 mm.

Stop hierdie buise toe met watteproppe of met proppe van skuimrubber wat vir gebruik in 'n outoklaaf geskik is. So nie, gebruik skroefdopbuise met dieselfde afmetings.

10.2.4 Pipette met skaalindelings

Totalelewering-pipette, slegs vir bakteriologiese doeleindes, waarvan die uitvloei-opening 'n diameter van 2 mm tot 3 mm het en wat in eenhede van 0,1 mℓ ingedeel is, in groottes wat 1,0 mℓ, 5,0 mℓ en 10,0 mℓ kan lewer.

10.2.5 Petribakkies

Petribakkies van glas of benatbare polistireen, met 'n diameter en hoogte van

a) 90 mm x 15 mm,

b) 100 mm x 20 mm, en

c) 150 mm x 20 mm.

10.2.6 Volumetriese silinders

Maatsilinders met skaalindelings, met of sonder proppe, en met 'n inhoudsvermoë van 5 mℓ, 10 mℓ, 100 mℓ en 1 000 mℓ.

10.2.7 Monsterbottels

Bottels waarvan die bek 'n diameter van 40 mm tot 60 mm het, met omruilbare slypglas- of plastiekproppe of gevoerde metaalsluitdoppe en met 'n inhoudsvermoë van 250 mℓ tot 300 mℓ, 'n diameter van 70 mm tot 80 mm en 'n hoogte van 120 mm tot 150 mm.

10.2.8 Kweekflesse

Flesse of bottels met 'n inhoudsvermoë van 200 mℓ en standaardsluitdoppe van gevoerde metaal of van plastiek soortgelyk aan dié wat in 10.2.2 en 10.2.7 beskryf word, maar met gate met 'n diameter van 12 mm tot 15 mm deur die sluitdoppe geboor wat met watte of ander bakterievangfilters toegestop kan word.

10.2.9 Reagensbottels

Bottels met 'n inhoudsvermoë van 50 mℓ en 100 mℓ en met polipropileen- of ander plastiekproppe wat só ontwerp is dat dit gebruik kan word om druppels van die reagens te lewer.

10.2.10 Klein proefbuise

Liplose silindriese buise met halfronde ente, 'n nominale wanddikte van 0,5 mm, 'n diameter van 6 mm tot 7 mm, 'n lengte van 100 mm en 'n inhoudsvermoë van 2,5 mℓ tot 3 mℓ. Hierdie buise kan ook as lang Durham-buise gebruik word.

10.2.11 Durham-buise

Buise soos in 10.2.10 beskryf word, maar met 'n lengte van 35 mm tot 45 mm en met 'n inhoudsvermoë van 0,9 mℓ tot 1,3 mℓ.

10.3 Toerusting

10.3.1 Outoklaaf

'n Drukhouer wat stoom kan voortbring (of met 'n sentrale stoombron verbind is), 'n druk van 300 kPa kan weerstaan en 'n temperatuur van 121 °C ± 2 °C kan bereik binne 10 min nadat daar met die steriliseersiklus begin is.

10.3.2 Inkubators en waterbaddens

Inkubators en waterbaddens wat termostaties beheerde verhittings- en verkoelingstoestelle het en wat só met sirkuleermiddele toegerus is dat die temperatuur van die totale ingeslote ruimte binne 2 °C van die termostaatstelling gehou word.

10.3.3 Warmlugoond (vir sterilisasie deur middel van droë hitte)

'n Termostaties beheerde oond wat deur elektrisiteit of gas verhit word en só met sirkuleermiddele toegerus is dat die temperatuur van die totale ingeslote ruimte by 170 °C ± 5 °C gehou word en waarvan die hittetoevoer sodanig is dat die werktemperatuur weer bereik word binne 10 min nadat die oonddeur kortstondig oop- en toegemaak is.

10.3.4 Homogeniseerder

'n Meganiese mengapparaat van die draai- of pulseertipe wat steriliseerbare houers het waarin 'n homogene dispersie van die monster en die voorgeskrewe verdunmiddel gemaak kan word. Die steriliseerbare houers kan van glas, metaal of geskikte plastiekmateriaal wees. Die homogeniseer-prosedure mag nie die getal mikro-organismes in die monster of die lewensvatbaarheid daarvan verminder nie.

10.3.5 Glasspreiers

Glasspreiers ("hokkiestokke") wat van glasstawe met 'n diameter van 3,5 mm en 'n lengte van 200 mm gemaak is deur elke staaf ongeveer 30 mm van een ent af haaks te buig. Maak die gesnyde ente glad deur dit in 'n vlam te verhit.

10.3.6 Membraanfilters

10.3.6.1 Tipes

Membraanfilters (verkieslik met 'n roosterpatroon) ten opsigte waarvan daar bewys is dat dit volkome bakteriële retensie en 'n bevredigende filtreerspoed bied, stabiel tydens gebruik is en vry is van chemikalieë wat die groei en ontwikkeling van bakterieë vertraag. Gebruik membraanfilters met 'n maksimum porie-grootte van hoogstens 0,45 µm.

10.3.6.2 Sterilisasie

Membraanfilters wat afsonderlik verpak is, moet vooraf deur betroubare handelsfabrikante gesteriliseer word.

Indien membraanfilters gesteriliseer moet word, maak hulle met steriele gedistilleerde water nat (om te voorkom dat hulle opkrul), plaas hulle tussen lae filtreerpapier en pak hulle styf in petribakkies wat toe gehou word. Steriliseer deur hulle 1 h lank in kokende water te dompel of 15 min lank by 115 °C te outoklaveer.

10.3.7 Membraanfilterhouers

10.3.7.1 Tipe

'n Membraanfilterhouer wat van niekorroderende, bakteriologies inerte materiaal gemaak is en wat al die vloeistof wat gefiltreer word deur die membraan laat gaan.

10.3.7.2 Sterilisasie

Sit die filterhouer losweg aanmekaar en maak seker dat die poreuse plaat gelyk met die boonste vlak van sy houer is. Draai die aanmekaargesitte filterhouer in bruinpapier of ander geskikte materiaal toe en steriliseer dit deur dit 20 min lank by 121 °C ± 2 °C in 'n outoklaaf te plaas, of volgens 'n ander geskikte metode.

10.3.8 Tang

10.3.8.1 Tipe

Rondepunttangetjies waarvan die binnekant van die kake glad is.

10.3.8.2 Sterilisasie

Steriliseer deur dit in brandspiritus of tegniese metanol te doop en dan die vloeistof wat daaraan vaskleef aan die brand te steek. So nie, volg 'n ander geskikte metode.

10.4 Kweekmedia en reagense

10.4.1 Algemeen

10.4.1.1 Water

Gebruik slegs glasgedistilleerde water of gedemineraliseerde water van ekwivalente suiwerheid wat helder, kleurloos en vry van sigbare stowwe in suspensie is en waarvan die pH-waarde, by 25 °C gemeet, in die bestek van 5,0 tot 7,5 is.

10.4.1.2 Kwaliteit van bestanddele

Gebruik slegs bestanddele van 'n kwaliteit wat aanneemlik vir mikrobiologiese doeleindes is by die bereiding van kweekmedia en reagense. Gebruik anhidriese soute tensy daar anders gespesifiseer word.

10.4.1.3 Noukeurigheid

Laat die volgende toleransies toe, tensy anders gespesifiseer word:

a) by temperatuur ..... ± 2 °C

b) by massas ..... ± 1,0 %

c) by volume ..... ± 1,0 %

d) by pH-waarde ..... ± 0,1 pH-eenheid

10.4.1.4 Ontwaterde kweekmedia

Baie van die kweekmedia wat vereis word, is in 'n ontwaterde vorm verkrygbaar en die gebruik van sodanige kweekmedia word aanbeveel ter wille van eenvormige resultate. Indien sodanige kweekmedia gebruik word, moet die fabrikant se aanwysings vir rekonstituering en sterilisering streng gevolg word.

10.4.1.5 Aansuiwering van pH-waarde

Indien die finale pH-waarde van 'n kweekmedium of reagens gespesifiseer word, suiwer die pH-waarde só aan dat dit by 25 °C korrek is. Indien nodig, suiwer die pH-waarde tydens bereiding aan en, in die geval van kweekmedia, voor sterilisasie. Tensy daar anders gespesifiseer word, gebruik 'n oplossing van soutsuur (c(HCl) = 1 mol/ℓ) of natriumhidroksied (c(NaOH) = 1 mol/ℓ), soos toepaslik, om die pH-waardes aan te suiwer.

10.4.1.6 Uitmeting

Indien gespesifiseerde hoeveelhede van die kweekmedia in bottels uitgemeet moet word, gebruik 30-mℓ-universele bottels (kyk 10.2.2 (a)) of kweekbuise met 'n diameter van 16 mm (kyk 10.2.3 (a)). Indien grootmaatsterilisasie vereis word, gebruik enige geskikte glashouer van die vereiste kwaliteit (kyk 10.2.1). Meet reagense in reagensbottels (kyk 10.2.9) uit. Roer kweekmedia aanhoudend terwyl dit uitgemeet word. As die bereiding van hellings vir oppervlakkweking vereis word, meet die kweekmedium in 10-mℓ-volumes uit en steriliseer soos gespesifiseer. Plaas die bottels of, indien toepaslik, die kweekbuise onmiddellik na sterilisasie en terwyl die kweekmedium nog gesmelt is op 'n oppervlak met 'n helling van 1 op 4 en laat die kweekmedium stol.

10.4.1.7 Sterilisasie

Indien sterilisasie in 'n outoklaaf gespesifiseer word en tensy daar anders aangewys word, steriliseer die kweekmedium 15 min lank in 'n outoklaaf by 121 °C ± 2 °C. (Hierdie temperatuur stem ooreen met 'n druk van 103 kPa bo atmosferiese druk by seespieël, dws 207 kPa absoluut.)

10.4.1.8 Kontrole van bereide kweekmedia

Maak dmv gepaste inkubasietoetse seker dat bereide kweekmedia steriel is en die groei van toepaslike organismes in die gegewe inkubasietoestande kan steun.

10.4.1.9 Bewaring van kweekmedia

Maak seker dat die bereide kweekmedia sorgvuldig teen blootstelling aan hitte en sonlig beskerm word, dat dit nie verdamp het nie, dat die konsentrasie of pH-waarde daarvan nie verander het nie en dat, tensy daar anders gespesifiseer word, dit binne drie maande na bereiding gebruik word.

10.4.2 Gebufferde isotoniese peptoonwater (verdunmiddel)

10.4.2.1 Bestanddele

Peptoon	10 g
Natriumchloried	5 g
Natriumfosfaatdodekahidraat, dibasies	3,5 g
Kaliumfosfaat, monobasies	1,5 g
Water	1 000 mℓ

10.4.2.2 Bereiding

Los die bestanddele in water op, suiwer die pH-waarde tot 7,0 aan. Meet soos volg uit:

a) 9-mℓ-volumes in 30-mℓ-bottels (kyk 10.2.2(a));

b) 99-mℓ-volumes in 250-mℓ-bottels (kyk 10.2.2(c)); en

c) groter volumes in grootmaathouers.

Steriliseer in 'n outoklaaf.

10.4.3 Plaattellingagar

10.4.3.1 Bestanddele

Agar	15 g
Triptoon	5 g
Gisekstrak	2,5 g
Glukose	1 g
Water	1 000 mℓ

10.4.3.2 Bereiding

Los die bestanddele in die water op deur dit te kook. Laat dit tot 50 °C afkoel en suiwer die pH-waarde tot 7,2 aan. Meet 15-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) en steriliseer in 'n outoklaaf.

10.4.4 Violetrooigalagar (VRG-agar)

10.4.4.1 Bestanddele

Agar	12 g
Glukose	10 g
Peptoon	7 g
Natriumchloried	5 g
Gisekstrak	3 g
Galsoute	1,5 g
Neutraalrooi	0,03 g
Kristalviolet	0,002 g
Water	1 000 mℓ

10.4.4.2 Bereiding

Los die bestanddele in water op deur dit te kook. Laat dit tot 50 °C afkoel en suiwer die pH-waarde tot 7,4 aan. Meet asepties soos volg uit:

a) 15-mℓ-volumes in steriele petribakkies (kyk 10.2.5(a) of (b)); en

b) 500 mℓ in 'n 1 000-mℓ- steriele bottel (kyk 10.2.2(e)).

Plaas die bottels in 'n waterbad wat by 45 °C gehou word en laat die plate stol. Gebruik die kweekmedium binne 4 h nadat dit berei is.

10.4.5 Briljantgroengalkweekmedium (enkelsterkte)

10.4.5.1 Bestanddele

Gedroogde beesgal	20 g
Laktose	10 g
Peptoon	10 g
Briljantgroen	0,013 g
Water	1 000 mℓ

10.4.5.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,4 aan. Meet 10-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) wat elk 'n omgekeerde Durham-buis (kyk 10.2.11) bevat en steriliseer in 'n outoklaaf.

10.4.6 Briljantgroengalkweekmedium (dubbelsterkte)

Berei die kweekmedium soos in 10.4.5 beskryf word, maar gebruik dubbelhoeveelhede van die bestanddele. Meet 100-mℓ-volumes uit in 250-mℓ-bottels (kyk 10.2.2(c)) wat elk 'n omgekeerde klein proefbuisie bevat wat as 'n lang Durham-buis (kyk 10.2.10) gebruik word en steriliseer in 'n outoklaaf.

10.4.7 Triptoonwater

10.4.7.1 Bestanddele

Triptoon	10 g
Natriumchloried	5 g
Water	1 000 mℓ

10.4.7.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,5 aan. Meet 9-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) en steriliseer in 'n outoklaaf.

10.4.8 Kovacs-reagens

10.4.8.1 Bestanddele

p-metielaminobensaldehied, dibasies	5 g
Amielalkohol (piridienvry)	75 mℓ
Soutsuur, gekonsentreer	25 mℓ

10.4.8.2 Bereiding

Los die *p*-metielaminobensaldehied in die amielalkohol op en help die oplos daarvan aan deur dit in 'n waterbad by 50 °C tot 55 °C te verhit. Laat dit afkoel en voeg die suur by. Beskerm teen lig en bewaar by 4 °C. Maak seker dat die reagens liggeel van kleur is. (Sommige soorte amielalkohol veroorsaak dat die reagens 'n baie donker kleur het en onbevredigend is.) Bewaar in 100-mℓ-reagensbottels (kyk 10.2.9). Laat dit voor gebruik 24 h lank staan.

10.4.9 Baird-Parker-agar

10.4.9.1 Bestanddele van basale kweekmedium

Agar	20 g
Glisien	12 g
Triptoon	10 g
Vleisekstrak	5 g
Litiumchloried	5 g
Gisekstrak	1 g
Water	1 000 mℓ

10.4.9.2 Bereiding

Los die bestanddele in die water op deur dit te kook. Laat dit afkoel tot 50 °C en suiwer die pH-waarde tot 7,2 aan. Meet 90-mℓ-volumes uit in 250-mℓ-bottels (kyk 10.2.2(c)) en steriliseer in 'n outoklaaf. Bewaar hoogstens een maand lank by 4 °C.

Voeg, voordat plate gegiet word, 1 mℓ tellurietoplossing (kyk 10.4.10) en 5 mℓ eiergeelemulsie (kyk 10.4.11) asepties by elke 90 mℓ van die basale kweekmedium, wat vooraf gesmelt en daarna tot tussen 45 °C tot 50 °C afgekoel is. Meng goed en meet 15-mℓ-volumes asepties in steriele petribakkies (kyk 10.2.5(a) of (b)) uit. Laat stol. Gebruik die plate binne 24 h nadat dit berei is. Droog die oppervlak van die kweekmedium minstens 1 h lank by 45 °C voordat dit gebruik word en sprei net voor gebruik 0,5 mℓ natriumpiruvaatoplossing (kyk 10.4.13) oor die oppervlak.

10.4.10 Tellurietoplossing

10.4.10.1 Bestanddele

Kaliumtelluriet	1 g
Water	100 mℓ

10.4.10.2 Bereiding

Los die kaliumtelluriet met die minimum verhitting in die water op. Steriliseer deur filtrering. Bewaar hoogstens een maand lank by 4 °C in 'n 100-mℓ-reagensbottel (kyk 10.2.9).

10.4.11 Eiergeelemulsie (ongeveer 20 % (volgens volume))

Was en ontsmet die doppe van vars heel hoendereiers. Breek die doppe, skei die geel asepties van die wit en vang die geel in 'n steriele beker op. Voeg water by in die verhouding van vier volumes water tot een volume eiergeel, meng deeglik en verhit 2 h lank in 'n waterbad by 45 °C. Verwyder die presipitaat deur sentrifugering of deur die mengsel oornag in 'n koelkas te laat staan en gooi die bodrywende vloeistof af. Steriliseer die bodrywende vloeistof deur filtrering. Meet 5-mℓ-volumes uit in 250-mℓ- steriele bottels (kyk 10.2.2(c)) en bewaar hoogstens een maand lank by 4 °C.

10.4.12 Mannitolsout-fenolrooiagar

10.4.12.1 Bestanddele

Natriumchloried	75 g
Agar	15 g
Mannitol	10 g
Vleispeptoon	10 g
Vleisekstrak	1 g
Fenolrooi	0,025 g
Water	1 000 mℓ

10.4.12.2 Bereiding

Los die bestanddele in die water op deur dit te kook en suiwer die pH-waarde tot 7,4 aan. Steriliseer in 'n outoklaaf en meet 15-mℓ-volumes asepties uit in steriele petribakkies (kyk 10.2.5(a) of (b)). Laat stol. Gebruik die plate op die dag van bereiding.

10.4.13 Natriumpiruvaatoplossing

Berei 'n waterige oplossing wat 200 g natriumpiruvaat per liter bevat en steriliseer dit deur filtrering. Gebruik verkieslik slegs 'n vars bereide oplossing. So nie, bewaar die oplossing hoogstens 3 d lank by 4 °C.

10.4.14 DNAse-toetsagar

10.4.14.1 Bestanddele

Triptose	20 g
Agar	12 g
Natriumchloried	5 g
Deoksiribonukleïensuur	2 g
Water	1 000 mℓ

10.4.14.2 Bereiding

Los die bestanddele in die water op deur dit te kook en suiwer die pH-waarde tot 7,4 aan. Steriliseer in 'n outoklaaf en meet 15-mℓ-volumes asepties uit in steriele petribakkies (kyk 10.2.5(a) of (b)). Laat stol. Gebruik die plate op die dag van bereiding.

10.4.15 Briljantgroenoplossing

10.4.15.1 Bestanddele

Briljantgroen	0,5 g
Steriele water	100 mℓ

10.4.15.2 Bereiding

Los die briljantgroen in 'n steriele fles in die water op. **Moet nie verhit nie.** Bewaar die oplossing minstens een dag lank in die donker sodat outosterilisasie kan plaasvind.

10.4.16 Rappaport-Vassiladis-magnesiumchloriedmalagietgroen kweekmedium (R-V-kweekmedium)

10.4.16.1 Oplossing A

10.4.16.1.1 Bestanddele

Triptoon	5,0 g
Natriumchloried	8,0 g
Kaliumdiwaterstoffosfaat (KH_2PO_4)	1,6 g
Water	1 000 mℓ

10.4.16.1.2 Bereiding

Los die bestanddele in die water op deur dit tot ongeveer 70 °C te verhit. Berei oplossing A op die dag waarop die volledige R-V-kweekmedium (kyk 10.4.16.4) berei word.

10.4.16.2 Oplossing B

10.4.16.2.1 Bestanddele

Magnesiumchloriedheksahidraat ($MgCl_2.6H_2O$) ..... 400,0 g
Water ..... 1 000 mℓ

10.4.16.2.2 Bereiding

Los die magnesium in die water op. Aangesien hierdie sout baie higroskopies is, is dit raadsaam om die hele inhoud van 'n houer $MgCl_2.6H_2O$ op te los eerder as om 'n deel van die inhoud te gebruik. Byvoorbeeld, 250 g van die $MgCl_2.6H_2O$ gevoeg by 625 mℓ water gee 'n oplossing met 'n totale volume van 795 mℓ en 'n konsentrasie van ongeveer 31,5 g persent $MgCl_2.6H_2O$. Die oplossing kan in 'n bruin glasbottel by kamertemperatuur gehou word.

10.4.16.3 Oplossing C

10.4.16.3.1 Bestanddele

Malagietgroenoksalaat ..... 0,4 g
Water ..... 100 mℓ

10.4.16.3.2 Bereiding

Los die malagietgroenoksalaat in die water op.

Die oplossing kan in 'n bruin glasbottel by kamertemperatuur gehou word.

10.4.16.4 Volledige R-V-kweekmedium

10.4.16.4.1 Bestanddele

Oplossing A (kyk 10.4.16.1) ..... 1 000 mℓ
Oplossing B (kyk 10.4.16.2) ..... 100 mℓ
Oplossing C (kyk 10.4.16.3) ..... 10 mℓ

10.4.16.4.2 Bereiding

Voeg 100 mℓ van oplossing B en 10 mℓ van oplossing C by 1 000 mℓ van oplossing A. Suiwer die pH-waarde, indien nodig, só aan dat dit na sterilisasie 5,2 is. Verdeel dit voor gebruik in 10-mℓ-hoeveelhede in proefbuise. Steriliseer dit 15 min lank in 'n outoklaaf by 115 °C. Bewaar die bereide kweekmedium in 'n koelkas.

10.4.17 Selenietkweekmedium (Stokes en Osborne)

10.4.17.1 Bestanddele

Mannitol ..... 5 g
Peptoon ..... 5 g
Gisekstrak ..... 5 g
Natriumwaterstofseleniet ..... 4 g
Kaliumfosfaat, dibasies ..... 2,62 g
Kaliumfosfaat, monobasies ..... 1,36 g
Natriumtourocholaat ..... 1 g
Briljantgroenoplossing (kyk 10.4.15) ..... 1 mℓ

10.4.17.2 Bereiding

Los die vaste bestanddele, uitgesonderd die natriumwaterstofseleniet, in ongeveer 800 mℓ water op deur dit te kook en steriliseer dit by die groot maat in 'n outoklaaf. Los die natriumwaterstofseleniet in ongeveer 150 mℓ koue water op en steriliseer die oplossing (verkieslik deur filtrering of, so nie, deur dit 10 min lank in stoom by 100 °C te verhit). Voeg hierdie oplossing en die 1-mℓ-briljantgroenoplossing asepties by die gesteriliseerde en afgekoelde hoofmassa van die bestanddele. Suiwer die pH-waarde tot 7,0 aan en verdun die oplossing met steriele water tot 1 ℓ. Meet 100-mℓ-volumes asepties uit in steriele kweekflesse (kyk 10.2.8). Moet nie die kweekmedium verder verhit nie. Die afsaksel wat vorm, sal tot onder in die fles afsak; suspendeer dit weer voordat die kweekmedium gebruik word. Gebruik op die dag van bereiding.

10.4.18 Briljantgroen-fenolrooiagar (Edel en Kampelmacher)

10.4.18.1 Bestanddele

Agar	12 g
Peptoon	10 g
Laktose	10 g
Sukrose	10 g
Vleisekstrak	4 g
Natriumchloried	3 g
Natriumfosfaat, dibasies	0,8 g
Natriumfosfaat, monobasies	0,6 g
Fenolrooi	0,09 g
Briljantgroenoplossing (kyk 10.4.15)	1 mℓ

10.4.18.2 Bereiding

Los die vaste bestanddele, uitgesonderd die fenolrooi, die laktose en die sukrose, in ongeveer 800 mℓ water op en steriliseer dit by die groot maat in 'n outoklaaf. Laat dit tot 55 °C afkoel. Los die fenolrooi en die suikers in ongeveer 150 mℓ water op en verhit 20 min lank in 'n waterbad by 70 °C. Laat dit tot 55 °C afkoel en voeg hierdie oplossing, saam met die 1 mℓ briljantgroenoplossing, by die hoofmassa van die bestanddele en meng. Suiwer die pH-waarde tot 7,0 aan en verdun die oplossing met steriele water tot 1 ℓ. Meet 40-mℓ-volumes asepties uit in steriele petribakkies, verkieslik met 'n diameter van 150 mm (kyk 10.2.5(c)). Hoewel hierdie groter petribakkies verkieslik is, kan kleiner petribakkies (kyk 10.2.5(a) of (b)) ook gebruik word as die groter bakkies nie beskikbaar is nie, maar berei dan twee maal soveel bakkies voor as wanneer die groter petribakkies gebruik word en gebruik 'n volume kweekmedium wat dieselfde diepte kweekmedium as in die groot petribakkies sal gee. Laat die kweekmedium stol en laat die oppervlak daarvan voor gebruik 30 min lank by 50 °C droog word. Gebruik die plate op die dag waarop dit berei is.

10.4.19 Sitochroomoksidasetoetsstroke of sitochroomoksidasereagens

In die handel verkrygbaar.

10.4.20 Driesuikerysteragar

10.4.20.1 Bestanddele

Peptoon	20 g
Agar	12 g
Laktose	10 g
Sukrose	10 g
Natriumchloried	5 g
Vleisekstrak	3 g
Gisekstrak	3 g
Glukose	1 g
Ferrisitraat	0,3 g
Natriumtiosulfaatpentahidraat	0,3 g
Fenolrooi	0,024 g
Water	1 000 mℓ

10.4.20.2 Bereiding

Los die bestanddele in die water op deur dit te kook. Laat dit tot 50 °C afkoel en suiwer die pH-waarde tot 7,4 aan. Meet 10-mℓ-volumes uit in kweekbuise (kyk 10.2.3(a)) en steriliseer dit 10 min lank in 'n outoklaaf. Laat dit in 'n skuins posisie stol, wat 'n dik ent met 'n diepte van ongeveer 25 mm en 'n skuins oppervlak met 'n lengte van minstens 30 mm sal gee. Die kweekmedium moet 'n oranjerooi kleur hê.

10.4.21 Ureumagar (Christensen)

10.4.21.1 Bestanddele

Ureum (50 mℓ van 'n 400-g/ℓ-oplossing)	20 g
Agar	15 g
Natriumchloried	5 g
Kaliumfosfaat, dibasies	2 g
Glukose	1 g
Peptoon	1 g
Fenolrooi	0,012 g

10.4.21.2 Bereiding

Los die bestanddele, uitgesonderd die ureum, in water op deur dit te kook en verdun die oplossing tot 900 mℓ. Steriliseer hierdie basis by die groot maat in 'n outoklaaf en laat dit tot 50 °C afkoel. Voeg 50 mℓ van 'n filtergesteriliseerde oplossing wat 400 g ureum per liter bevat by en meng goed. Suiwer die pH-waarde tot 6,8 aan en verdun die oplossing met steriele water tot 1 ℓ. Meet 10-mℓ-volumes asepties uit in 30-mℓ- steriele bottels (kyk 10.2.2(a)) en laat dit in 'n skuins posisie stol, wat 'n dik ent met 'n diepte van ongeveer 25 mm en 'n skuins oppervlak met 'n lengte van minstens 30 mm sal gee.

10.4.22 Lisiendekarboksileringskweekmedium (Taylor)

10.4.22.1 Bestanddele

ℓ-lisienhidrochloried, monobasies	5 g
Gisekstrak	3 g
Glukose	1 g
Broomkresolpers	0,015 g
Water	1 000 mℓ

10.4.22.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 6,8 aan. Meet 10-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) en steriliseer in 'n outoklaaf.

10.4.23 ß-galaktosidasereagens

10.4.23.1 Bestanddele

Natriumfosfaat, monobasies	0,69 g
Ortonitrofeniel-ß-*d*-galaktopiranosied	0,08 g
Natriumhidroksiedoplossing, 0,4 g/ℓ	ongeveer 3 mℓ

10.4.23.2 Prosedure

Los die natriumfosfaat in 15 mℓ water op. Suiwer die pH-waarde tot 7,0 aan met die natriumhidroksied-oplossing. Los die galaktopiranosied in hierdie oplossing op en verdun tot 20 mℓ. Bewaar hoogstens een maand lank by 4 °C.

10.4.24 Voges-Proskauer-kweekmedium

10.4.24.1 Bestanddele

Peptoon ..... 7 g
Glukose ..... 5 g
Kaliumfosfaat, dibasies ..... 5 g
Water ..... 1 000 mℓ

10.4.24.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 6,9 aan. Meet 0,2-mℓ-volumes uit in klein proefbuise (kyk 10.2.10) en steriliseer in 'n outoklaaf.

10.4.25 Kreatienoplossing

Berei 'n waterige oplossing wat 5 g kreatienhidraat (monobasies) per liter bevat. Bewaar hoogstens een maand lank by omgewingstemperatuur in reagensbottels (kyk 10.2.9).

10.4.26 α-naftoloplossing

Gebruik 96 % (volgens massa) tot 100 % (volgens massa) etanol as oplosmiddel en berei 'n waterige oplossing wat 60 g α-naftol per liter bevat. Bewaar hoogstens een maand lank by omgewingstemperatuur in reagensbottels (kyk 10.2.9).

10.4.27 Kaliumhidroksiedoplossing

Berei 'n waterige oplossing wat 400 g kaliumhidroksied per liter bevat. Bewaar by omgewingstemperatuur in bottels met alkalibestande plastiekproppe. Moet nie glasproppe gebruik nie. Vermy onnodige blootstelling aan die atmosfeer.

10.4.28 Soutoplossing

10.4.28.1 Bestanddele

Natriumchloried ..... 8,5 g
Water ..... 1 000 mℓ

10.4.28.2 Bereiding

Los die natriumchloried in die water op. Meet 9-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) en steriliseer in 'n outoklaaf.

10.4.29 Polivalente anti-*Salmonella*-"O"-serum

Gebruik kommersiële antiserums teen die somatiese antigene van 'n groot genoeg getal *Salmonella*-serotipes om dit onwaarskynlik te maak dat 'n vals negatiewe reaksie verkry sal word agv 'n tot nog toe onbekende tipe. In alle gevalle moet die groepe A tot G toereikend verteenwoordig wees. Volg in die geval van elke antiserum of antiserummengsel die aanwysings van die antiserumfabrikant.

10.4.30 Polivalente anti-*Salmonella*-"H"-serum

Gebruik kommersiële antiserums teen die flagellêre antigene van 'n groot genoeg getal *Salmonella*-serotipes om spesifieke sowel as niespesifieke faktore, uitgesonderd faktor "I", op te spoor. Volg in die geval van elke antiserummengsel die aanwysings van die antiserumfabrikant.

10.4.31 Polivalente anti-*Salmonella*-"Vi"-serum

Gebruik kommersiële antiserums. Volg die fabrikant se aanwysings noukeurig.

10.4.32 Gram-negatiewe kweekmedium

10.4.32.1 Bestanddele

Polipeptoon	20 g
Natriumchloried	5 g
Natriumsitraat	5 g
Kaliumfosfaat, dibasies	4 g
Mannitol	2 g
Kaliumfosfaat, monobasies	1,5 g
Glukose	1 g
Natriumdesoksicholaat	0,5 g
Water	1 000 mℓ

10.4.32.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,0 aan. Meet 100-mℓ-volumes uit in kweekflesse (kyk 10.2.8) met 'n inhoudsvermoë van minstens 200 mℓ en steriliseer dit 20 min lank by 115 °C in 'n outoklaaf.

10.4.33 XLD-kweekmedium

10.4.33.1 Bestanddele

Gisekstrak	3,0 g
ℓ-lisien HCl	5,0 g
Xilose	3,75 g
Laktose	7,5 g
Sukrose	7,5 g
Natriumdesoksicholaat	1,0 g
Natriumchloried	5,0 g
Natriumtiosulfaat	6,8 g
Ferriammoniumsitraat	0,8 g
Fenolrooi	0,08 g
Agar	12,5 g

10.4.33.2 Bereiding

Suspendeer die bestanddele in die water. Suiwer die pH-waarde tot 7,4 ± 0,2 aan. Verhit en roer dikwels totdat die kweekmedium kook. **Moet dit nie oorverhit nie.** Plaas dit onmiddellik na 'n waterbad by 50 °C oor. Giet in plate sodra die kweekmedium afgekoel het.

Dit is belangrik om nie groot volumes wat langdurige verhitting sal meebring, te berei nie.

10.4.34 Polivalente anti-*Shigella*-"O"-serum

Gebruik kommersiële polivalente antiserums teen die somatiese antigene, met inbegrip van teen-liggaampies, van minstens *Shigella*-serotipe 1 tot 15.

10.4.35 Eiergeelvry triptosesulfietsikloserienagar (SC-agar)

10.4.35.1 Basis

10.4.35.1.1 Bestanddele

Triptose[1] ..... 15,0 g
Sojatoon[1] ..... 5,0 g
Gisekstrak ..... 5,0 g
Dinatriumdisulfiet ($Na_2S_2O_5$), anhidries ..... 5,0 g
Ammoniumyster(III)sitraat[2] ..... 1,0 g
Agar[3] ..... 12 g tot 18 g
Water ..... 1 000 mℓ

10.4.35.1.2 Bereiding

Los die bestanddele in die water op deur dit te kook. Suiwer die pH-waarde só aan dat dit na sterilisasie 7,6 sal wees. Plaas die basis na buise of flesse of bottels met 'n inhoudsvermoë van hoogstens 500 mℓ oor. Steriliseer dit 10 min lank by 121 °C. Bewaar in 'n koelkas by 4 °C ± 2 °C.

Gooi die ongebruikte kweekmedium 2 weke na bereiding weg.

10.4.35.2 D-sikloserienoplossing

10.4.35.2.1 Bestanddele

D-sikloserien (gebruik slegs wit kristallyne poeier) ..... 4,0 g
Water ..... 100 mℓ

10.4.35.2.2 Bereiding

Los die D-sikloserien in die water op en steriliseer die oplossing deur filtrering.

10.4.35.3 Volledige SC-agarkweekmedium

Voeg voor plaatkweking (10.12.1) 1 mℓ van die gesteriliseerde D-sikloserienoplossing (kyk 10.4.35.2) by elke 100 mℓ steriele gesmelte basis (kyk 10.4.35.1) by 50 °C.

10.4.36 Motiliteitsnitraatkweekmedium

10.4.36.1 Bestanddele

Peptoon ..... 5,0 g
Vleisekstrak ..... 3,0 g
Galaktose ..... 5,0 g
Gliserol ..... 5,0 g
Kaliumnitraat (KNO_3) ..... 1,0 g

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1) Die name triptose en sojatoon word tans slegs deur sekere fabrikante van kweekmediums gebruik. Enige ander pankreaskaseïen- of sojaboonverteersel wat vergelykbare resultate gee, kan gebruik word.

2) Hierdie reagens moet minstens 15 % (volgens massa) yster bevat.

3) Na gelang van die jelsterkte van die agar.

Dinatriumwaterstofortofosfaat (Na_2HPO_4) ..... 2,5 g
Agar[4] ..... 1 g tot 5 g
Water ..... 1 000 mℓ

10.4.36.2 Prosedure

Los die bestanddele in die water op deur dit te kook. Suiwer die pH-waarde só aan dat dit na sterilisasie 7,3 sal wees. Plaas die kweekmedium in 10-mℓ-hoeveelhede na kweekbuise oor en steriliseer dit 15 min lank by 121 °C. Bewaar die kweekmedium in 'n koelkas by 4 °C ± 2 °C indien dit nie op dieselfde dag gebruik word nie.

Verhit die kweekmedium net voor gebruik 15 min lank in kookwater of vloeiende stoom en koel dit dan vinnig tot die inkubasietemperatuur af.

Gooi die ongebruikte kweekmedium 4 weke na bereiding weg.

10.4.37 Laktosegelatienkweekmedium

10.4.37.1 Bestanddele

Triptose[5] ..... 15,0 g
Gisekstrak ..... 10,0 g
Laktose ..... 10,0 g
Gelatien ..... 120,0 g
Fenolrooi ..... 0,05 g
Water ..... 1 000 mℓ

10.4.37.2 Bereiding

Los die bestanddele, behalwe die laktose en die fenolrooi, in die water op. Suiwer die pH-waarde só aan dat dit na sterilisasie 7,5 sal wees. Voeg die laktose en fenolrooi by, meet 10-mℓ-hoeveelhede in proefbuise uit en steriliseer dit 15 min lank by 121 °C.

Bewaar die kweekmedium in 'n koelkas by 4 °C ± 2 °C indien dit nie op dieselfde dag gebruik word nie.

Verhit die kweekmedium net voor gebruik 15 min lank in kookwater of vloeiende stoom en koel dit dan vinnig tot inkubasietemperatuur af.

Gooi die ongebruikte kweekmedium 3 weke na bereiding weg.

10.4.38 *Vibrio*-verrykingskweekmedium (dubbelsterkte)

10.4.38.1 Bestanddele

Natriumchloried ..... 40 g
Triptoon ..... 20 g
Natriumtourocholaat ..... 10 g
Natriumkarbonaat ..... 2 g
Gelatien ..... 2 g
Kaliumtellurietoplossing, 1g/ℓ, filtergesteriliseer ..... 20 mℓ

4) Na gelang van die jelsterkte van die agar.

5) Die naam triptose word tans slegs deur sekere fabrikante van kweekmediums gebruik. Enige ander pankreaskaseïenverteersel wat vergelykbare resultate lewer, kan gebruik word.

10.4.38.2 Bereiding

Los al die bestanddele, behalwe die kaliumtellurietoplossing, in ongeveer 900 mℓ water op deur dit te kook. Suiwer die pH-waarde tot 8,7 aan en steriliseer die resulterende basale kweekmedium in 'n outoklaaf. Voeg die kaliumtellurietoplossing asepties by nadat die kweekmedium tot laer as 45 °C afgekoel het. Suiwer die pH-waarde weer tot 8,7 aan en verdun die oplossing met steriele water tot 1 ℓ. Meet 100-mℓ-volumes asepties uit in 250-mℓ- steriele bottels (kyk 10.2.2(c)). Gebruik die kweekmedium binne 2 h nadat die kaliumtellurietoplossing bygevoeg is.

Die basale kweekmedium, dws die kweekmedium sonder die kaliumtellurietoplossing, is hoogstens 3 dae lank by 4 °C stabiel.

10.4.39 Diagnostiese *Vibrio*-agar

10.4.39.1 Bestanddele

Sukrose	20 g
Agar	15 g
Natriumchloried	10 g
Natriumsitraat	10 g
Natriumtiosulfaatpentahidraat	10 g
Spesiale peptoon	10 g
Gedroogde beesgal	5 g
Gisekstrak	5 g
Natriumtourocholaat	3 g
Ferrisitraat	1 g
Broomtimolblou	0,04 g
Timolblou	0,04 g
Water	1 000 mℓ

10.4.39.2 Bereiding

Los die bestanddele in water op deur dit te kook. Moet dit nie oorverhit nie. **Moet dit nie in 'n outoklaaf steriliseer nie.** Laat dit tot 50 °C afkoel, suiwer die pH-waarde tot 8,6 aan en verdun die oplossing met steriele water tot 1 ℓ. Meet 15-mℓ-volumes asepties uit in steriele petribakkies (kyk 10.2.5(a) of (b)). Laat dit stol. Gebruik die plate binne 3 h na bereiding.

10.4.40 Lisien-indool-motiliteit-waterstof-sulfiedagar (wat 30 g/ℓ natriumchloried bevat)

10.4.40.1 Bestanddele

Natriumchloried	30 g
Triptoon	15 g
ℓ-lisienhidrochloried, monobasies	5 g
Vleispeptoon	5 g
Vleisekstrak	3 g
Gisekstrak	3 g
Agar	2 g
Glukose	1 g
Ferriammoniumsitraat	0,5 g
Natriumtiosulfaatpentahidraat	0,3 g
Broomkresolpers	0,016 g
Water	1 000 mℓ

10.4.40.2 Bereiding

Los die bestanddele in die water op deur dit te kook. Laat dit afkoel en suiwer die pH-waarde tot 7,4 aan. Meet 5-mℓ-volumes uit in kweekbuise (kyk 10.2.3(a)). Steriliseer dit in 'n outoklaaf en prop die buise styf toe om vogverlies te voorkom.

10.4.41 Onaktiveerderoplossing

10.4.41.1 Bestanddele

Polioksiëtileensorbitaanmonoöleaat	2 g
Natriumtourocholaat	1 g
Gelatien	1 g
Natriumtiosulfaatpentahidraat	0,3 g
Kaliumfosfaat, monobasies	0,1 g
Natriumsitraat	0,1 g
Water	1 000 mℓ

10.4.41.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,2 aan. Meet 9-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)). Steriliseer in 'n outoklaaf.

10.4.42 Nitrietopspoorreagens

10.4.42.1 5-amino-2-naftaleensulfoonsuur (5-2-ANSA)-oplossing

Los 0,1 g van die 5-2-ANSA in 100 mℓ van 15-%(per volume)-asynsuuroplossing op. Filtreer deur filtreerpapier. Bewaar by 4 °C in 'n bruin bottel wat styf toegeprop is (verkieslik met 'n boltipe drupper).

10.4.42.2 Sulfanielsuuroplossing

Los 0,4 g sulfanielsuur in 100 mℓ van 15-%(per volume)-asynsuuroplossing op. Filtreer deur filtreerpapier. Bewaar by 4 °C in 'n bruin bottel wat styf toegeprop is (verkieslik met 'n boltipe drupper).

10.4.42.3 Bereiding van volledige reagens

Meng gelyke volumes van die twee oplossings (kyk 10.4.42.1 en 10.4.42.2) net voor gebruik.

Gooi ongebruikte reagens onmiddellik weg.

10.4.43 m-Endo-agar LES

10.4.43.1 Bestanddele

Agar	15,0 g
Laktose	9,4 g
Triptose	7,5 g
Triptoon	3,7 g
Natriumchloried	3,7 g
Tiopeptoon	3,7 g
Dikaliumfosfaat	3,3 g
Natriumsulfiet	1,6 g
Gisekstrak	1,2 g
Monokaliumfosfaat	1,0 g
Basiese fuchsien	0,8 g
Natriumdesoksicholaat	0,1 g
Natriumlourielsulfiet	0,05 g
Water	1 000 mℓ

10.4.43.2 Bereiding

Suspendeer die bestanddele in die water. Voeg 20 mℓ etanol (95 % (volgens volume)) by en laat 10 min

lank staan. Los die bestanddele heeltemal op deur dit tot kookpunt te verhit. Laat afkoel tot tussen 45 °C en 50 °C, meet dit in petribakkies uit en maak seker dat die diepte van die kweekmedium in elke plaat minstens 3 mm is.

10.4.44 mFC-agar

10.4.44.1 Bestanddele

Agar	13 g
Laktose	12,5 g
Triptose	10 g
Proteosepeptoon no 3	5 g
Natriumchloried	5 g
Gisekstrak	3 g
Galsout no 3	1,5 g
Anilienblou (waterblou)	0,1 g
Water	1 000 mℓ

10.4.44.2 Bereiding

Suspendeer die bestanddele in die water en los heeltemal op deur dit tot kookpunt te verhit. Laat dit tot tussen 45 °C en 50 °C afkoel, meet dit in petribakkies uit en maak seker dat die diepte van die agar in elke plaat minstens 3 mm is.

10.4.45 Lourieltriptoseboeljon (enkelsterkte)

10.4.45.1 Bestanddele

Triptose	20 g
Laktose	5 g
Natriumchloried	5 g
Dikaliumfosfaat	2,75 g
Monokaliumfosfaat	2,75 g
Natriumlourielsulfaat, spesiaal suiwer	0,1 g
Water	1 000 mℓ

10.4.45.2 Bereiding

Los die triptose, laktose, natriumchloried en fosfate in die water op deur dit te verhit. Voeg die natriumlourielsulfaat by en meng liggies om skuimvorming te voorkom. Suiwer die pH-waarde tot 6,8 aan en meet 10-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) wat elk 'n omgekeerde Durham-buis (kyk 10.2.11) bevat. Steriliseer in 'n outoklaaf.

10.4.46 Lourieltriptoseboeljon (dubbelsterkte)

Berei hierdie kweekmedium soos in 10.4.45 beskryf word, maar gebruik dubbel die hoeveelheid bestanddele. Meet 10-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) wat elk 'n omgekeerde Durham-buis (kyk 10.2.11) bevat. Steriliseer in 'n outoklaaf.

10.4.47 Peptoonwater

10.4.47.1 Bestanddele

Peptoon	10 g
Natriumchloried	5 g
Water	1 000 mℓ

10.4.47.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,5 aan. Meet 9-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) en steriliseer in 'n outoklaaf.

10.4.48 Laktosepeptoonwater

10.4.48.1 Bestanddele

Peptoon	10 g
Laktose	10 g
Natriumchloried	5 g
Fenolrooi-oplossing (0,4 g fenolrooi per 100 mℓ water)	2,5 mℓ
Water	100 mℓ

10.4.48.2 Bereiding

Los die bestanddele in water op, suiwer die pH-waarde tot 7,5 aan en voeg die fenolrooi-oplossing by. Meet 10-mℓ-volumes uit in 30-mℓ-bottels (kyk 10.2.2(a)) of kweekbuise (kyk 10.2.3(a)) wat elk 'n omgekeerde Durham-buis (kyk 10.2.11) bevat. Steriliseer in 'n outoklaaf. So nie, stoom dit 20 min lank op drie opeenvolgende dae. Toets vir steriliteit deur dit 24 h lank by 37 °C te inkubeer.

10.4.49 Selektiewe voorverrykingskweekmedium: ½ Fraser-boeljon

10.4.49.1 Basis

10.4.49.1.1 Bestanddele

Vleispeptoon (peptiese verteersel van dierweefsel)	5,0 g
Triptoon- peptiese verteersel van kaseïen	5,0 g
Vleisekstrak	5,0 g
Gisekstrak	5,0 g
Natriumchloried	20,0 g
Dinatriumwaterstoffosfaat ($2H_2O$)	12,0 g
Kaliumdiwaterstoffosfaat	1,35 g
Eskulien	1,0 g
Litiumchloried	3,0 g
Natriumsout van nalidiksiensuur	0,01 g
Water	1 000 mℓ

10.4.49.1.2 Bereiding

Los die ontwaterde basiskomponente of die volledige ontwaterde basis in die water op deur dit tot ongeveer 70 °C te verhit. Versprei die basale kweekmedium in flesse met 'n geskikte inhoudsvermoë om die porsies te verkry wat vir die toets nodig is. Steriliseer 15 min lank by 121 °C.

10.4.49.2 Akriflavienoplossing

10.4.49.2.1 Bestanddele

Akriflavien	0,125 g
Water	100,0 mℓ

10.4.49.2.2 Bereiding

Los die akriflavien in die water op. Steriliseer deur filtrering.

10.4.49.3 Ferriammoniumsitraatoplossing

10.4.49.3.1 Bestanddele

Yster(III)ammoniumsitraat ..... 5,0 g
Water ..... 100,0 mℓ

10.4.49.3.2 Bereiding

Los die yster(III)ammoniumsitraat in die water op. Steriliseer deur filtrering.

10.4.49.4 Volledige ½ Fraser-boeljon

Voeg 1,0-mℓ-porsies akriflavienoplossing (kyk 10.4.49.2) en ferriammoniumsitraatoplossing (kyk 10.4.49.3) net voor gebruik by elke 100 mℓ basale kweekmedium (kyk 10.4.49.1). Meng liggies. Suiwer die pH-waarde van die volledige kweekmedium só aan dat dit 7,2 ± 0,2 is.

10.4.50 Selektiewe verrykingskweekmedium: Fraserboeljon

10.4.50.1 Basis

10.4.50.1.1 Bestanddele

Vleispeptoon (peptiese verteersel van dierweefsel) ..... 5,0 g
Triptoon- peptiese verteersel van kaseïen ..... 5,0 g
Vleisekstrak ..... 5,0 g
Gisekstrak ..... 5,0 g
Natriumchloried ..... 20,0 g
Dinatriumwaterstoffosfaat ($2H_2O$) ..... 12,0 g
Kaliumdiwaterstoffosfaat ..... 1,35 g
Eskulien ..... 1,0 g
Litiumchloried ..... 3,0 g
Natriumsout van nalidiksiensuur ..... 0,02 g
Water ..... 1 000 mℓ

10.4.50.1.2 Bereiding

Los die ontwaterde basiskomponente of die volledige ontwaterde basis in die water op deur dit tot ongeveer 70 °C te verhit. Versprei die basale kweekmedium in 10-mℓ-volumes in 30-mℓ-bottels (kyk 10.2.2(a)). Steriliseer 15 min lank by 121 °C.

10.4.50.2 Akriflavienoplossing

10.4.50.2.1 Bestanddele

Akriflavien ..... 0,25 g
Water ..... 100,0 mℓ

10.4.50.2.2 Bereiding

Los die akriflavien in die water op. Steriliseer deur filtrering.

10.4.50.3 Ferriammoniumsitraatoplossing

10.4.50.3.1 Bestanddele

Yster(III)ammoniumsitraat ..... 5,0 g
Water ..... 100,0 mℓ

10.4.50.3.2 Bereiding

Los die yster(III)ammoniumsitraat in die water op. Steriliseer deur filtrering.

10.4.50.4 Volledige Fraser-boeljon

Voeg 0,1-mℓ-porsies akriflavienoplossing (kyk 10.4.50.2) en ferriammoniumoplossing (kyk 10.4.50.3) net voor gebruik by elke buis (10-mℓ-volumes) van die basis (10.4.50.1). Meng liggies. Suiwer die pH-waarde van die volledige kweekmedium só aan dat dit 7,2 ± 0,2 is.

10.4.51 Eerste selektiewe plaatkweekmedium: Oxford-agar

10.4.51.1 Agarbasis

10.4.51.1.1 Bestanddele

Columbia-agarbasis	39,0 g
Eskulien	1,0 g
Yster(III)ammoniumsitraat	0,5 g
Litiumchloried	15,0 g
Water	1 000 mℓ

10.4.51.1.2 Bereiding

Los die vaste bestanddele in die water op deur dit te kook. Steriliseer 15 min lank by 121 °C.

10.4.51.2 Aanvulling vir 500 mℓ kweekmedium

10.4.51.2.1 Bestanddele

Sikloheksimied	200,0 mg
Kolistiensulfaat	10,0 mg
Akriflavien	2,5 mg
Sefotetaan	1,0 mg
Fosfomisien	5,0 mg
Etanol	2,5 mℓ
Water	2,5 mℓ

10.4.51.2.2 Bereiding

Los die vaste bestanddele in die etanol-water-mengsel op en steriliseer deur filtrering.

10.4.51.3 Bereiding van volledige Oxford-agarkweekmedium

Neem 500 mℓ van die agarbasis (10.4.51.1). Steriliseer 15 min lank in 'n outoklaaf wat op 121 °C gestel is. Laat dit tot 50 °C afkoel en voeg die aanvulling (kyk 10.4.51.2) asepties by. Suiwer die pH-waarde van die finale kweekmedium só aan dat dit 7,0 by 25 °C is.

10.4.52 Tweede selektiewe plaatkweekmedium: PALCAM-agar

10.4.52.1 Agarbasis

10.4.52.1.1 Bestanddele

Pankreaspeptoon van kaseïen[6]	23,0 g
Stysel	1,0 g
Natriumchloried	5,0 g
Agar[7]	9,0 g tot 18 g
Gisekstrak	3,0 g
D-glukose	0,5 g
D-mannitol	10,0 g
Eskulien	0,8 g
Yster(III)ammoniumsitraat	0,5 g
Fenolrooi	0,08 g
Litiumchloried	15,0 g
Water	1 000 mℓ

10.4.52.1.2 Bereiding

Los die ontwaterde basiskomponente of die volledige ontwaterde basis in 960 mℓ van die water op deur dit te kook. Suiwer die pH-waarde tot 7,2 ± 0,1 aan. Steriliseer 15 min lank by 121 ° C in 'n outoklaaf. Laat dit tot 50 °C afkoel.

10.4.52.2 Polimiksien-B-sulfaatoplossing

10.4.52.2.1 Bestanddele

Polimiksien-B-sulfaat (100 000 ie)	0,1 g
Water	100,0 mℓ

10.4.52.2.2 Bereiding

Los die polimiksien-B-sulfaat in die water op. Steriliseer deur filtrering.

10.4.52.3 Akriflavienwaterstofchloriedoplossing

10.4.52.3.1 Bestanddele

Akriflavien	0,05 g
Etanol	50,0 mℓ
Water	50,0 mℓ

10.4.52.3.2 Bereiding

Meng die etanol met die water en los die akriflavien in die etanol-water-mengsel op. Steriliseer deur filtrering.

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6) Of ander peptoon van ekwivalente kwaliteit.

7) Na gelang van die jelsterkte van die agar.

10.4.52.4 Natriumseftasidiempentahidraatoplossing

10.4.52.4.1 Bestanddele

Natriumseftasidiempentahidraat ..... 1,116 g
Water ..... 100 mℓ

10.4.52.4.2 Bereiding

Los die natriumseftasidiempentahidraat in die water op. Steriliseer deur filtrering.

10.4.52.5 Volledige PALCAM-kweekmedium

10.4.52.5.1 Bestanddele

Basale kweekmedium (kyk 10.4.52.1) ..... 960,0 mℓ
Polimiksien-B-sulfaatoplossing (kyk 10.4.52.2) ..... 10 mℓ
Akriflavienwaterstofchloriedoplossing (kyk 10.4.52.3) ... 10,0 mℓ
Natriumseftasidiempentahidraatoplossing
(kyk 10.4.52.4) ..... 20 mℓ

10.4.52.5.2 Bereiding

Voeg die volgende byvoegings by die gesmelte basale kweekmedium by 47 °C en meng liggies tussen elke byvoeging:

- Polimiksien-B-sulfaatoplossing.
- Akriflavienwaterstofchloriedoplossing.
- Natriumseftasidiempentahidraatoplossing.

Hou die volledige kweekmedium by 47 °C en giet so gou moontlik 15 mℓ in elk van 'n toepaslike getal petribakkies. Laat dit stol.

Droog onmiddellik voor gebruik die oppervlak van die agarplate sorgvuldig (verkieslik met die deksels af en die agaroppervlak na onder) in 'n oond wat 30 min lank by 50 °C beheer word, of totdat die oppervlak van die agar droog is.

10.4.53 Vaste kweekmedium: Triptoonsojagisekstrakagar (TSGEA)

10.4.53.1 Bestanddele

Triptoonsojaboeljon ..... 30,0 g
Gisekstrak ..... 6,0 g
Agar[8)] ..... 12,0 g tot 18 g
Water ..... 1 000 mℓ

10.4.53.2 Bereiding

Los komponente of volledige ontwaterde kweekmedium in die water op deur dit te kook. Suiwer die pH-waarde, indien nodig, só aan dat dit na sterilisasie 7,3 by 25 °C is. Meet hoeveelhede van ongeveer 6 mℓ van die vaste kweekmedium in buise uit. Steriliseer die buise 15 min lank by 121 °C in 'n outoklaaf. Laat dit in 'n skuins posisie stol.

8) Na gelang van die jelsterkte van die agar.

Steriliseer die vaste kweekmedium in flesse of bottels met 'n geskikte inhoudsvermoë vir die bereiding van agarplate. Meet die kweekmedium, terwyl dit nog in vloeistofvorm is, in hoeveelhede van ongeveer 15 mℓ in steriele petribakkies uit en laat dit stol.

10.4.54 Vloeibare kweekmedium: Triptoonsojagisekstrakboeljon (TSGEB)

10.4.54.1 Bestanddele

Triptoonsojaboeljon	30,0 g
Gisekstrak	6,0 g
Water	1 000 mℓ

10.4.54.2 Bereiding

Los die komponente of volledige ontwaterde kweekmedium in die water op deur dit te kook. Suiwer die pH-waarde, indien nodig, só aan dat dit na sterilisasie 7,3 by 25 °C is. Meet hoeveelhede van ongeveer 6 mℓ van die kweekmedium in buise uit. Steriliseer die buise 15 min lank by 121 °C in 'n outoklaaf.

10.4.55 Bloedagar

10.4.55.1 Bloedagarbasis no 2

Vleispeptoon	15,0 g
Lewerverteersel	2,5 g
Gisekstrak	5,0 g
Natriumchloried	5,0 g
Agar[9)]	12,0 g tot 18 g

10.4.55.2 Vloeibare kweekmedia

Water	1 000 mℓ
Gedefibrineerde perde- of skaapbloed	70 mℓ

10.4.55.3 Bereiding

Los die ontwaterde bloedagarbasis no 2 in die water op deur dit te kook. Suiwer die pH-waarde, indien nodig, só aan dat dit na sterilisasie 7,0 by 25 °C is. Meet die kweekmedium in buise of flesse met 'n inhoudsvermoë van hoogstens 500 mℓ uit. Steriliseer die bloedagarbasis 15 min lank in 'n outoklaaf by 121 °C. Laat die kweekmedium tot 47 °C afkoel. Voeg die gedefibrineerde bloed by en meng deeglik.

Meet die kweekmedium in hoeveelhede van ongeveer 20 mℓ uit in steriele petribakkies en laat dit stol. Steriliseer die vaste kweekmedium in flesse of bottels met 'n geskikte inhoudsvermoë vir die bereiding van die agarplate. Meet die kweekmedium, terwyl dit nog vloeibaar is, in hoeveelhede van ongeveer 15 mℓ in steriele petribakkies uit en laat dit stol.

10.4.56 Koolhidraatgebruiksboeljon

10.4.56.1 Basis

10.4.56.1.1 Bestanddele

Proteosepeptoon	10,0 g
Beesvleisekstrak	1,0 g
Natriumchloried	5,0 g

9) Na gelang van die jelsterkte van die agar.

Broomkresolpers ..... 0,02 g
Water ..... 1 000 mℓ

10.4.56.1.2 Bereiding

Los die komponente in die water op deur dit te kook. Suiwer die pH-waarde, indien nodig, só aan dat dit na sterilisasie 6,8 by 25 °C is. Meet die kweekmedium in sodanige hoeveelhede in buise uit dat dit na sterilisasie 9 mℓ sal bly. Steriliseer die buise 15 min lank by 121 °C in 'n outoklaaf.

10.4.56.2 Koolhidraatoplossings

10.4.56.2.1 Bestanddele

Koolhidraat (100 mℓ L-ramnose-oplossing en
100 mℓ D-xilose-oplossing) ..... 5,0 g
Water ..... 100 mℓ

10.4.56.2.2 Bereiding

Los elke koolhidraat afsonderlik in 100 mℓ water op. Steriliseer deur filtrering. Suiwer, indien nodig, die pH-waarde só aan dat dit na sterilisasie 6,8 by 25 °C is. Meet die kweekmedium in sodanige hoeveelhede in buise uit dat dit na sterilisasie 9 mℓ sal bly. Steriliseer die buise 15 min lank by 121 °C in 'n outoklaaf.

10.4.56.3 Volledige koolhidraatgebruiksboeljon

Voeg vir elke koolhidraat 1 mℓ koolhidraatoplossing (kyk 10.4.56.2) asepties by 9 mℓ van die basiskweekmedium (kyk 10.4.56.1). Indien kleiner volumes basiskweekmedium berei word, voeg dienooreenkomstig kleiner volumes koolhidraatoplossing by.

10.4.57 Motiliteitskweekmedium

10.4.57.1 Bestanddele

Kaseïenpeptoon ..... 20,0 g
Vleispeptoon ..... 6,1 g
Agar ..... 3,5 g
Water ..... 1 000 mℓ

10.4.57.2 Bereiding

Los die komponente in die water op deur dit te kook. Suiwer die pH-waarde, indien nodig, só aan dat dit na sterilisasie 7,3 ± 0,2 by 25 °C is. Meet die kweekmedium in hoeveelhede van ongeveer 5 mℓ in buise uit. Steriliseer die buise 15 min lank by 121 °C in 'n outoklaaf.

10.4.58 Breinhartaftrekselboeljon

10.4.58.1 Bestanddele

Breinhartaftreksel- vaste stowwe ..... 17,5 g
Triptose ..... 10,0 g
Dekstrose ..... 2,0 g
Natriumchloried ..... 5,0 g
Dinatriumfosfaat ..... 2,5 g
Water ..... 1 000 mℓ

10.4.58.2 Bereiding

Suspendeer die bestanddele in die water. Kook dit om die kweekmedium heeltemal op te los. Steriliseer

dit 15 min lank by 121 °C in 'n outoklaaf. Meet dit in steriele 30-mℓ-bottels uit (kyk 10.2.2(a)).

10.4.59 EDTA-konynplasma

EDTA-konynplasma is gedroogde konynplasma waarby etileendiamientetra-asynsuur (EDTA) as die antistolmiddel gevoeg is. Dit is in die handel in gevriesdroogde vorm in buisflessies beskikbaar. Voeg 3 mℓ steriele water by die inhoud van die buisflessie vir rekonstituering. Meng deur die buisflessie liggies ent oor ent te draai.

10.4.60 Sinkstof.

In die handel verkrygbaar.

10.5 Bereiding van die monster

10.5.1 Bewaring van die produk

Bewaar die produk, met 'n massa van minstens 200 g, vir die minimum praktiese tydperk in sodanige toestande dat veranderings in samestelling voorkom word of tot 'n minimum beperk word.

10.5.2 Bereiding van die monster

Indien nodig, laat die rou of gaar produk in sy verpakking by 5 °C tot 10 °C ontdooi totdat al die sigbare ys gesmelt het. Maak seker dat die ontdooiing binne 18 h voltooi is. Gebruik 'n steriele mes en tang, verwyder 28 g tot 35 g van die produk en plaas dit oor na 'n vooraf geweegde en gesteriliseerde homogeniseerhouer wat geskik is vir gebruik saam met die homogeniseerder (kyk 10.3.4). Voeg genoeg van die gebufferde isotoniese peptoonwater (kyk 10.4.2) by om 'n 1:10-dispersie van die produk te verkry. Laat die homogeniseerder volgens die fabrikant se aanwysings net lank genoeg werk om 'n homogene dispersie te verkry, dws laat draaitipe homogeniseerders lank genoeg werk sodat die totale getal omwentelings van die snylemme 15 000 tot 20 000 is, maar in geen geval langer as 2,5 min nie. Gebruik die 1:10-dispersie van die produk wat só verkry is vir die toetse in 10.6 tot en met 10.14.

10.6 Standaardplaattelling

10.6.1 Gaar produkte

10.6.1.1 Berei 'n verdunning van een volume van die monster op 1 000 volumes verdunmiddel deur 1 mℓ van die monster (kyk 10.5.2) met 99 mℓ van die gebufferde isotoniese peptoonwater (kyk 10.4.2) in 'n bottel (kyk 10.2.2) te meng. So nie, voeg 1 mℓ van die dispersiemonster by 9 mℓ peptoonwater en voeg 1 mℓ van hierdie mengsel by nog 9 mℓ peptoonwater. Meng die inhoud van elke bottel deeglik voordat dit gebruik word volgens die toepaslike van die volgende mengmetodes:

(a) gebruik 'n geskikte meganiese menger, verkieslik van die vibreertipe;

(b) indien die verdunning in 'n skroefdopbottel is, meng deur die bottel 10 maal met die hand om te keer en weer regop te bring; of

(c) indien die verdunning in 'n houer met 'n dop of watteprop is, rol die houer minstens 20 maal in 'n regop posisie heen en weer tussen die handpalms.

10.6.1.2 Pipetteer 'n 1-mℓ-volume van die verdunde monster wat aldus verkry is in elk van twee steriele petribakkies (kyk 10.2.5(a) of (b)). Voeg een 15-mℓ-volume van die plaattellingagar (kyk 10.4.3), wat gesmelt is en tot 45 °C afgekoel het, by die inhoud van elke plaat en meng. Sorg dat niks van die inhoud van die plaat tydens die proses uitstort nie. Die maklikste manier is om die plaat op 'n tafelblad te plaas en die plaat versigtig te werwel. Laat die agar stol, keer die plate om, bring etikette daarop aan en inkubeer by 30 °C. Maak seker dat die totale tyd wat verstryk vandat die verdunnings van die monster berei is totdat die finale plate gegiet word hoogstens 15 min is. Haal die plate na 48 h inkubasie uit die inkubator en tel die kolonies wat in die kweekmedium ontwikkel het. Teken die resultate aan en bereken die gemiddelde getal kolonievormende eenhede per gram van die monster.

10.6.2 Onverwerkte produkte

Berei 'n 1:1 000-verdunning van die monster soos in 10.6.1.1 beskryf word. Berei nog 'n 1:10-verdunning deur 1 mℓ van hierdie verdunning met 9 mℓ van die gebufferde isotoniese peptoonwater (kyk 10.4.2) te meng en gaan dan te werk soos in 10.6.1.2 beskryf word deur die verdunning wat só verkry is vir plaatkweking, inkubering en plaattelling te gebruik.

10.7 Enterobacteriaceae-telling

Pipetteer 2-mℓ-volumes van die dispersiemonster (kyk 10.5.2) in elk van twee steriele petribakkies (kyk 10.2.5(a)). Voeg minstens 15 mℓ van die VRG-glukoseagar (kyk 10.4.4) by die inhoud van elke plaat en meng. Meng deeglik met die inokulum deur elke bakkie liggies te roteer. Voeg 'n oorlaag van dieselfde VRG-glukoseagar by wanneer die kweekmedium gestol het. Sorg dat niks van die inhoud van die plaat tydens hierdie proses uitstort nie. Laat die agar stol, keer die plate om, bring gepaste etikette daarop aan en inkubeer 24 h lank by 37 °C. Ondersoek en tel alle kolonies met 'n diameter wat 0,5 mm oorskry en verontagsaam al die ander. Beskou al sodanige kolonies as dié van enterobacteriaceae. Teken hierdie resultate aan en vermenigvuldig die getal enterobacteriaceae-kolonies met 2,5 om die enterobacteriaceae-telling per gram van die monster te verkry.

10.8 Fekale koliforme bakterieë

10.8.1 Gaar produkte

10.8.1.1 Meet 100 mℓ van die monster (kyk 10.5.2) asepties uit in elk van twee bottels met dubbelsterkte-briljantgroengalkweekmedium (kyk 10.4.6) en inkubeer die bottels 16 h tot 20 h lank by 37 °C.

10.8.1.2 Indien die kweekmedium gasproduksie toon, soos aangetoon deur gas in die Durham-buis, gebruik 'n platinumdraadlus en verkry subkulture van elke bottel deur een lus vol na nog 'n bottel met enkelsterkte-briljantgroengalkweekmedium (kyk 10.4.5) te subkweek en een lus vol na 'n bottel met triptoonwater (kyk 10.4.7), met albei bottels tot 44 °C voorverhit.

10.8.1.3 Inkubeer albei hierdie subkulture 16 h tot 20 h lank by 44 °C ± 0,25 °C in 'n waterbad. Voeg 0,1 mℓ tot 0,5 mℓ van die Kovacs-reagens (kyk 10.4.8) by die kultuur in die triptoonwater. Meng deur die bottel liggies te skud. Indien 'n rooi kleur ontwikkel en die kultuur in die briljantgroengalkweekmedium tekens van gasontwikkeling toon, moet die kultuur as fekale koliforme bakterieë beskou word.

10.8.2 Onverwerkte produkte

Meet asepties 100 mℓ van die dispersiemonster (kyk 10.5.2) uit in elk van twee bottels met die dubbelsterkte-briljantgroengalkweekmedium (kyk 10.4.6) en inkubeer die bottels 16 h tot 20 h lank by 37 °C. Ondersoek en bevestig verdagte kulture soos in 10.8.1.2 en 10.8.1.3 beskryf word.

OPM – Hierdie metode bepaal die aanwesigheid of afwesigheid van lewensvatbare fekale koliforme bakterieë in 10 g van die produk. Dit impliseer dat so min as een sodanige organisme per 10 g 'n positiewe resultaat sal gee.

10.9 *Staphylococcus aureus*

10.9.1 Plaatinokuleerprosedure

Plaas met behulp van 'n steriele pipet 'n 1-mℓ-volume van die dispersiemonster (kyk 10.5.2) oor na die oppervlakke van drie Baird-Parker-agarplate (kyk 10.4.9) en versprei die enkele volume eweredig oor die drie plate. Beskou hierdie drie plate as een vir die doel van die plaattellingsprosedure, aangesien hulle 'n 1:10-verdunning van die monster verteenwoordig. Herhaal die prosedure hierbo met nog 'n 1-mℓ-

volume en nog drie plate. Inokuleer elk van nog twee Baird-Parker-agarplate met 0,1 mℓ van die monster. Elk van hierdie plate sal die 1:100-verdunning verteenwoordig. Sprei die inokulum sorgvuldig met behulp van individuele steriele glasspreiers (kyk 10.3.5) so gou as moontlik oor die oppervlak van elk van die plate en probeer om nie aan die kante van die bakkie te raak nie. Inkubeer die plate 24 h tot 48 h lank by 43 °C.

10.9.2 Kiesprosedure

10.9.2.1 Merk na inkubasie van 24 h tot 26 h die posisies van tipiese kolonies wat aanwesig is op die onderkant van die plate. Tipiese kolonies is swart, blink en konveks (met 'n diameter van 1 mm tot 1,5 mm) en is omring deur 'n helder sone wat gedeeltelik ondeursigtig kan wees. 'n Opaliserende kring wat direk in kontak met die kolonies is, kan in die helder sone voorkom. Herinkubeer al die plate 'n verdere 22 h tot 24 h lank by 43 °C en merk dan die posisies van nuwe tipiese kolonies.

10.9.2.2 Neem vir telling díe plate wat tussen 15 en 150 tipiese of atipiese kolonies bevat. Kies vir bevestiging (kyk 10.9.3) vyf tipiese of vyf atipiese kolonies, na gelang van die geval, uit elke plaat. Indien daar minder as 15 tipiese of atipiese kolonies aanwesig is op die plate wat met die verdunning van 1:10 van die monster (kyk 10.9.1) geïnokuleer is, hou al die plate wat tipiese of atipiese kolonies bevat. Kies al sodanige kolonies vir bevestiging (kyk 10.9.3).

10.9.3 Bevestigingstoetse

Die kiesprosedure (kyk 10.9.2) is afhanklik van die gebruik van verhoogde temperature (43 °C) vir inkubasie en sal die bevestiging van die identiteit van *Staphylococcus aureus* vergemaklik.

Die bevestiging van die aanwesigheid van *Staphylococcus aureus* is afhanklik van die volgende bykomende toetse:

a) Doen 'n kol-inokulasie van elk van die tipiese of atipiese kolonies wat uit die Baird-Parker-agarplate (kyk 10.9.2.2) gekies is op 'n plaat mannitolsout-fenolrooiagar (kyk 10.4.12) en 'n plaat DNAse-toetsagar (kyk 10.4.14). Gebruik 'n swaar inokulum. Inkubeer die plate 48 h lank by 37 °C.

   Oorstroom die oppervlak van die DNAse-toetsagarplaat na inkubasie met verdunde soutsuur met 'n konsentrasie van c(HCl) = ongeveer 1 mol/ℓ. Die DNA sal neerslaan en veroorsaak dat die kweekmedium troebel word. Helder sones sal rondom positiewe kolonies ontwikkel.

b) Ondersoek die mannitolsoutfenolrooi-agarplate vir kolonies wat 'n geel kleur met 'n helder geel sone rondom die kolonie ontwikkel. Dit is 'n aanduiding dat mannitol in suur omgesit is. Dié kolonies wat 'n positiewe DNAse-reaksie toon en wat suur as 'n afbreekproduk van mannitol produseer, word dan aan die koagulasetoets onderwerp.

c) Gebruik 'n steriele lus om 'n inokulum van die oppervlak van elke gekose kolonie te verwyder en plaas dit oor na 'n bottel breinhartaftrekselboeljon (kyk 10.4.58). Inkubeer 24 h lank by 37 °C. Voeg 0,1 mℓ van elke kultuur asepties by 0,3 mℓ EDTA-konynplasma (kyk 10.4.59) in klein steriele buise en inkubeer by 37 °C. Ondersoek 4 h tot 6 h lank vir klontvorming. Beskou die toets as positief as die volume van die klont meer as drie kwart van die oorspronklike volume van die vloeistof in beslag neem.

Staphylococcus aureus kan positief geïdentifiseer word as groei van 'n gekose kolonie (kyk 10.9.2) 'n positiewe DN-ase-reaksie toon en suur as afbreekproduk van mannitol produseer en die gekose kolonies sterk positief vir die koagulasereaksie is.

10.9.4 Berekening van *Staphylococcus aureus*-telling

Bereken in die geval van plate wat positief geïdentifiseerde tipiese of atipiese kolonies (kyk 10.9.2) bevat, die getal *Staphylococcus aureus* vir elke verdunning aan die hand van die persentasie *Staphylococcus aureus* wat tydens die bevestigingstoetse (kyk 10.9.3) uit die gekose kolonies geïdentifiseer is. Bereken die gemiddelde getal *Staphylococcus aureus* aan die hand van die duplikaatplate of opeenvolgende verdunnings.

OPM – Rond getalle kleiner as 100 tot die naaste veelvoud van vyf af en getalle bo 100 wat op 5 eindig, tot die naaste veelvoud van 20. As 'n getal groter as 100 is en nie op 'n 5 eindig nie, rond dit tot die naaste veelvoud van 10 af.

Vermenigvuldig die gemiddelde wat só verkry word met die resiprook van die inokulumvolume en dan met die resiprook van die ooreenstemmende verdunning van die toetsmonster om die getal *Staphylococcus aureus* per gram van die monster te verkry.

10.10 *Salmonella*

10.10.1 Voorverryking

Plaas 25-mℓ-volumes van die monster (kyk 10.5.2) oor in elk van twee steriele 250-mℓ-kweekflesse (kyk 10.2.8). Inkubeer die flesse 16 h tot 20 h lank by 37 °C.

10.10.2 Selektiewe verryking

Plaas die hele inhoud van een van die flesse met die voorverrykte monster (kyk 10.10.1) oor in 'n fles wat 100 mℓ van die R-V-kweekmedium (kyk 10.4.16) bevat en plaas die hele inhoud van die ander fles (kyk 10.10.1) oor in 'n fles wat 100 mℓ van die selenietkweekmedium (kyk 10.4.17) bevat.

Inkubeer die geïnokuleerde R-V-kweekmedium tot 48 h lank by 43 °C en die geïnokuleerde seleniet-kweekmedium tot 48 h lank by 37 °C. Gaan na 18 h tot 24 h inkubasie en sonder om die inhoud van die flesse te skud met die diagnostiese plaatkweking voort.

10.10.3 Diagnostiese plaatkweking

10.10.3.1 Verwyder met behulp van 'n platinum-draadlus met 'n binnediameter van 4 mm twee lusse vol van die kultuur in die R-V-kweekmedium van die oppervlak van die kweekmedium en stryk een oor die oppervlak van 'n plaat met briljantgroen-fenolrooiagar (kyk 10.4.18) en die ander oor die drooggemaakte oppervlak van 'n plaat met VRG-glukoseagar (kyk 10.4.4). Meng dan die inhoud van die fles en herhaal die diagnostiese plaatkweking met nog twee lusse vol op nog twee plate. Doen die bestryking op só 'n wyse dat die ontwikkeling van goed geïsoleerde kolonies verseker word. Bring gepaste etikette op die diagnostiese plate aan om te identifiseer watter een van die twee monsternemingsmetodes gebruik is. Keer die plate om en inkubeer hulle 18 h tot 24 h lank by 37 °C.

OPM – Daar word beweer dat motiele Salmonella-organismes na die oppervlak van die verrykingskweekmedium migreer. Dit kom dus voor asof monsterneming uit die onversteurde oppervlak die moontlikheid van opsporing vergroot.

10.10.3.2 Bestryk soortgelyke plate op dieselfde wyse met die kultuur in die selenietkweekmedium.

10.10.3.3 Plaas die kulture in die seleniet- sowel as die R-V-kweekmediumflesse vir 'n verdere 24 h tot 28 h in hulle onderskeie inkubators terug.

10.10.3.4 Herhaal na afloop van die inkubasietydperk die diagnostiese plaatkweking met elke kultuur op 'n verdere reeks plate en inkubeer hierdie plate 18 h tot 24 h lank by 37 °C.

10.10.3.5 Ondersoek die plate na inkubasie vir vermeende kolonies *Salmonella*-organismes. Indien daar min groei op die plate is of indien daar geen verdagte kolonies aanwesig is nie, inkubeer die plate nog 20 h tot 24 h lank en ondersoek hulle weer. Onderwerp enige verdagte kolonie aan verdere ondersoek. Ondervinding is nodig om kolonies *Salmonella*-organismes te herken omdat hul voorkoms verskil op die twee diagnostiese kweekmedia en van spesie tot spesie, sowel as van produksielot tot produksielot van die kweekmedia.

10.10.4 Bevestiging van verdagte kolonies

Kies op elke plaat vyf kolonies van elke tipe verdagte *Salmonella*-organisme, of al sodanige kolonies, wat ook al die minste is.

Stryk elk van die gekose kolonies oor die gedroogde oppervlak van 'n VRG-agarplaat (kyk 10.4.4) op só

'n wyse dat die ontwikkeling van goed geïsoleerde kolonies verseker word. Inkubeer die plate 18 h tot 24 h lank by 37 °C. Ondersoek die kolonies wat op die plate ontwikkel vir eenvormigheid van eienskappe en bepaal op hierdie wyse of die kultuur wat ondersoek word "suiwer" is. Dit is uiters belangrik dat die kultuur wat aan verdere toetswerk onderwerp moet word, suiwer moet wees. Indien twyfel bestaan, stryk 'n goed afgesonderde kolonie oor die gedroogde oppervlak van nog 'n plaat VRG-agar. Inkubeer hierdie plaat 18 h tot 24 h lank by 37 °C en ondersoek soos hierbo. Indien nodig, herhaal hierdie prosedure totdat die suiwerheid van die kultuur bo redelike twyfel vasgestel is. Onderwerp hierdie kultuur aan verdere toetse en dra sorg dat kontaminasie van die kultuur met ander mikro-organismes verhoed word. Gebruik slegs laktose-negatiewe (kleurlose) kolonies vir verdere toetse.

10.10.5 Biochemiese bevestiging

Subkweek die suiwer kultuur (kyk 10.10.4) met 'n platinumnaald óp of in die toepaslike kweekmedia en toets vir die reaksies in 10.10.5.1 tot 10.10.5.7.

10.10.5.1 Driesuikerysteragar

Maak 'n steekkultuur in die dik ent en stryk dit oor die agarhellingoppervlak van die driesuikerysteragar (kyk 10.4.20). Inkubeer 24 h tot 48 h lank by 37 °C en ondersoek. Klassifiseer die resultate soos volg:

Dik ent:

Geel kleur	Glukose omgesit (suur)
Rooi kleur of geen verandering	Glukose nie omgesit nie
Swart kleur	Waterstofsulfied geproduseer
Gasborrels of barste	Gas uit glukose geproduseer

Helling:

Geel kleur	Aërobiese omsetting van laktose of sukrose, of albei (suur)
Rooi kleur of geen verandering	Geen laktose en geen sukrose omgesit nie.

10.10.5.2 Produksie van urease

Stryk die suiwer kultuur (kyk 10.10.4) oor die skuins ureumagaroppervlak (kyk 10.4.21). Inkubeer 24 h tot 48 h lank by 37 °C en ondersoek. Die splitsing van ureum produseer ammoniak, wat die kleur van die kweekmedium na pienk en later na kersierooi verander.

10.10.5.3 Lisiendekarboksilering

Inokuleer 'n lus vol van die suiwer kultuur (kyk 10.10.4) net onder die oppervlak van die lisiendekarboksileringskweekmedium (kyk 10.4.22), inkubeer 18 h tot 24 h lank by 37 °C en ondersoek. Die dekarboksilering van lisien produseer kadawerien, wat die kleur van die kweekmedium na pers verander. 'n Geel kleur of onveranderde kweekmedium dui die afwesigheid van lisiendekarboksilering aan.

10.10.5.4 Produksie van ß-galaktosidase

Suspendeer 'n klein hoeveelheid van die bakteriemateriaal van die suiwer kultuur (kyk 10.10.4) in 0,25 mℓ van die soutoplossing (kyk 10.4.28) in 'n klein, steriele proefbuis. Voeg 'n druppel tolueen by hierdie suspensie en verhit die buis 5 min lank in 'n waterbad wat by 37 °C gehou word. Voeg 0,25 mℓ van die ß-galaktosidasereagens (kyk 10.4.23) by die suspensie en meng. Inkubeer die buis minstens 24 h lank by 37 °C en ondersoek met tussenposes. 'n Geel kleur, wat 'n positiewe reaksie aandui, kan binne 20 min voorkom. Moet nie die reaksie as negatief beskou voordat die inkubeertydperk van 24 h verstryk het nie.

10.10.5.5 Indoolproduksie

Inokuleer 'n bottel triptoonwater (kyk 10.4.7) met die suiwer kultuur. Inkubeer 24 h lank by 37 °C. Voeg

na inkubasie 0,5 mℓ Kovacs-reagens (kyk 10.4.8) by die inhoud van die bottel. 'n Rooi verkleuring dui 'n positiewe reaksie aan.

10.10.5.6 Voges-Proskauer-reaksie

Inokuleer elk van twee buise van die Voges-Proskauer-kweekmedium (kyk 10.4.24) met die suiwer kultuur. Inkubeer een buis 24 h lank by omgewingstemperatuur en die ander een by 37 °C. Voeg na inkubasie twee druppels van die kreatienoplossing (kyk 10.4.25), drie druppels van die α-naftoloplossing (kyk 10.4.26) en dan twee druppels van die kaliumhidroksiedoplossing (kyk 10.4.27) by elke buis en meng die inhoud na elke byvoeging. Indien 'n pienk tot helderrooi kleur binne 15 minute ontwikkel, dui dit 'n positiewe reaksie aan.

10.10.5.7 Oksidasereaksie

Wend 'n klein hoeveelheid bakteriemateriaal uit die suiwer kultuur op 'n sitochroomoksidasetoetsstrook (kyk 10.4.19) aan en vryf dit goed in die reaksiegebied in. Laat dit ongeveer 30 s lank staan. 'n Blou kleur dui 'n positiewe reaksie aan.

10.10.6 Vertolking van resultate van biochemiese bevestigingstoetse

Reaksie	Persentasie *Salmonella*-tipes wat 'n positiewe reaksie toon
Suur uit glukose	100,0
Gas uit glukose	91,9
Suur uit laktose	0,8
Suur uit sukrose	0,5
Produksie van waterstofsulfied	91,6
Produksie van urease	0,0
Lisiendekarboksilering	94,5
Produksie van ß-galaktosidase	1,5
Produksie van indool	1,1
Voges-Proskauer-reaksie	0,0
Oksidasereaksie	0,0

Onderwerp alle kulture, behalwe dié wat op grond van bogenoemde data klaarblyklik nie *Salmonella*-organismes bevat nie, aan serologiese bevestigingstoetse.

10.10.7 Serologiese bevestiging

Indien geskikte polivalente anti-*Salmonella*-"O"- en -"H"-serums en -"Vi"-serums (kyk 10.4.29 tot 10.4.31) beskikbaar is, ondersoek die verdagte kolonies (kyk 10.10.4) deur middel van glasplaatagglutinasie vir die aanwesigheid van *Salmonella*-"O"- en -"H"-antigene en vir -"Vi"-antigene. Hou egter in gedagte dat daar nie uitsluitlik op die resultate van die serologiese toetse staatgemaak moet word vir bevestiging nie en dat die resultate beoordeel moet word in samehang met die resultate wat deur middel van biochemiese bevestiging verkry is.

10.10.8 Vertolking van resultate van serologiese bevestigingstoetse

10.10.8.1 Polivalente anti-*Salmonella*-"O"-serum

Vertolk die resultate soos volg:

a) indien die resultaat negatief is, is dit feitlik seker dat geen *Salmonella* aanwesig is nie. Die enigste uitsondering is dat 'n kultuur 'n "O"-antigeen kan hê wat nog nie vantevore teëgekom is nie; en

b) indien die resultaat positief is, is dit slegs 'n aanduiding dat die kultuur van die *Salmonella*-genus kan wees.

10.10.8.2 Polivalente anti-*Salmonella*-"H"-serum

Vertolk die resultate soos volg:

a) indien die resultaat negatief is, is dit feitlik seker dat geen *Salmonella* aanwesig is nie. Die enigste uitsondering is dat 'n kultuur 'n "H"-antigeen kan hê wat nog nie tevore teëgekom is nie; en

b) indien die resultaat positief is, beskou dit dat die kultuur positief vir *Salmonella* is.

10.10.8.3 Anti-*Salmonella* "Vi"-serums

Indien die resultaat positief is, beskou dit dat die kultuur positief vir *Salmonella* is.

10.11 *Shigella*

10.11.1 Opsporing

Volg die prosedure wat in 10.10 vir *Salmonella* beskryf word, maar gebruik die selenietkweekmedium (kyk 10.4.17) as die selektieweverrykingskweekmedium en die XLD-agar (kyk 10.4.33) as die kweekmedium vir diagnostiese plaatkweking en inkubeer in albei gevalle by 37 °C.

Rooi kolonies op XLD-agar is verdagte *Shigella*-organismes.

Salmonella spp en *Salmonella typhi* kan ook met behulp van XLD-agar opgespoor word en hierdie metode vul die metode in 10.10 aan.

10.11.2 Bevestiging

Onderwerp elke verdagte kolonie aan die biochemiese toetse in 10.10.5 en aan 'n serologiese toets soos in 10.10.7 en 10.10.8 beskryf, maar gebruik polivalente anti-*Shigella*-"O"-serum (kyk 10.4.34).

10.11.3 Vertolking van resultate

Reaksie	Persentasie *Shigella*-tipes wat 'n positiewe reaksie toon
Suur uit glukose	100,0
Gas uit glukose	2,1
Suur uit laktose	0,2
Suur uit sukrose	0,6
Produksie van waterstofsulfied	0,0
Produksie van urease	0,0
Lisiendekarboksilering	0,0
Produksie van ß-galaktosidase	38,3
Indoolproduksie	30,6
Voges-Proskauer-reaksie	0,0
Oksidasereaksie	0,0

10.12 *Clostridium perfringens*

10.12.1 Inokulasie en inkubasie (gietplaattegniek)

Plaas met behulp van 'n steriele pipet 1 mℓ van die dispersie van die monster (kyk 10.5.2) na elk van twee petribakkies oor, giet 15 mℓ tot 20 mℓ van die SC-agar (kyk 10.4.35) in elke bakkie en meng deeglik met die inokulum deur elke bakkie liggies te roteer. Voeg 'n oorlaag van 10 mℓ van dieselfde SC-agar by wanneer die kweekmedium gestol het. Laat dit stol en plaas die plate, met die deksel bo, in anaërobiese flesse of ander geskikte houers en inkubeer 20 h lank by 37 °C. 'n Langer inkubasietydperk kan daartoe lei dat dit langs die onderste rand van die plate oormatig swart word.

10.12.2 Tel van kolonies

10.12.2.1 Tel na die gespesifiseerde inkubasietydperk (kyk 10.12.1) die getal kenmerkende kolonies op die plate volgens 10.12.2.2 en teken dit aan. Kolonies van *C. perfringens* is swart.

10.12.2.2 Tel die kenmerkende kolonies op elke plaat en teken die rekenkundige gemiddelde van die tellings van die twee plate aan.

10.12.3 Bevestiging

10.12.3.1 Kies van kolonies vir bevestiging

Kies 'n totaal van 10 kenmerkende kolonies uit die plate wat volgens 10.12.2.2 getel is. Indien minder as 10 kolonies beskikbaar is op die plate wat getel is, neem al die kenmerkende kolonies wat aanwesig is. Bevestig hierdie kolonies volgens 10.12.3.2.

10.12.3.2 Biochemiese bevestiging

10.12.3.2.1 Bevestiging met behulp van motiliteitsnitraatkweekmedium

Steekinokuleer die geselekteerde kolonies (kyk 10.12.3.1) in die motiliteitsnitraatkweekmedium (kyk 10.4.36) in. Inkubeer 24 h lank in anaërobiese toestande by 37 °C.

Ondersoek die buise motiliteitsnitraatkweekmedium vir die tipe groei langs die steeklyn. Motiliteit blyk uit verspreide groei in die kweekmedium in en weg van die steeklyn af. Toets vir die aanwesigheid van nitriet deur 0,2 mℓ tot 0,5 mℓ van die nitrietopspoorreagens (kyk 10.4.42) by elke buis van die motiliteits-nitraatkweekmedium[10)] te voeg. Die ontstaan van 'n rooi kleur bevestig die reduksie van nitraat tot nitriet.

Indien daar nie binne 15 min 'n rooi kleur ontstaan het nie, voeg 'n klein hoeveelheid sinkstof (kyk 10.4.60) by en laat dit 10 min lank staan. Indien 'n rooi kleur na die byvoeging van sinkstof ontstaan, het geen reduksie van nitraat plaasgevind nie.

10.12.3.2.2 Bevestiging met behulp van laktosegelatienkweekmedium

Inokuleer die gekose kolonies (kyk 10.12.3.1) in laktosegelatienkweekmedium (kyk 10.4.37). Inkubeer 24 h lank in anaërobiese toestande by 37 °C.

Ondersoek die buise met laktosegelatienkweekmedium vir die aanwesigheid van gas en 'n geel kleur (weens die suur), wat fermentasie van die laktose aandui. Verkil die buise 1 h lank by 5 °C en gaan na vir gelatienvervloeiing. Indien die kweekmedium gestol het, inkubeer weer 24 h lank en gaan weer na vir gelatienvervloeiing.

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10) Om gesondheidsredes kan dit wenslik wees om hierdie toets onder 'n dampkap uit te voer.

10.12.3.3 Vertolking

Bakterieë wat swart kolonies op SC-kweekmedium produseer, niemotiel is, nitraat tot nitriet reduseer, suur en gas uit laktose produseer en gelatien in 'n tydperk van 48 h laat vervloei, word as *C. perfringens* beskou.

Kulture wat 'n flou reaksie vir nitriet toon (dws 'n pienk kleur), moet uitgeskakel word, aangesien *C. perfringens* konstant 'n intense en onmiddellike reaksie gee.

10.13 Patogene *Vibrio (Vibrio Cholerae* en *Vibrio parahaemolyticus)*

10.13.1 Verryking

Berei, binne 30 min nadat die dispersie van die monster (kyk 10.5.2) berei is, twee kulture wat elk bestaan uit 100 mℓ van die monster gemeng met 100 mℓ van die dubbelsterkte-*Vibrio*-verrykingskweekmedium (kyk 10.4.38). Inkubeer hierdie kulture 18 h tot 24 h lank, die een by 37 °C en die ander by 42 °C.

10.13.2 Diagnostiese plaatkweking

Verwyder 'n lus vol van die kultuur van die oppervlak van elke kultuur sonder om dit te skud en stryk elke lus vol oor 'n diagnostiese *Vibrio*-agarplaat (kyk 10.4.39) op só 'n wyse dat dit die ontwikkeling van goed geïsoleerde kolonies sal verseker. Keer die plate om en inkubeer hulle 18 h tot 24 h lank by 37 °C.

Ondersoek die geïnkubeerde plate aan die hand van die volgende eienskappe vir die aanwesigheid van *Vibrio spp*:

Beskrywing van kolonies	Diameter van kolonie mm	Vermoedelike identifikasie
Plat, geel en rond	2 tot 3	*Vibrio cholerae*
Glad en groen (sukrose-negatief)	3 tot 5	*V. parahaemolyticus*
Plat, geel en rond	4 tot 6	*V. alginolyticus*
Rond en blou	0,5 tot 1	*Pseudomonas, Aeromonas*
Deursigtig	0,1 tot 0,5	*Proteus* of ander enterobakterieë

Plaas verdagte kolonies oor na die lisien-indool-motiliteit-waterstof-sulfiedagar (kyk 10.4.40) en na die ureumagarhellings (kyk 10.4.21) en inkubeer hierdie kulture 18 h tot 24 h lank by 37 °C.

10.13.3 Bevestiging

10.13.3.1 Ondersoek die ureumagarhellings en, indien geen urease geproduseer is nie, plaas van die groeisel oor na 'n sitochroomoksidasetoetsstrook (kyk 10.4.19) en bepaal of die kolonies sitochroom-oksidase-positief is (kyk 10.10.5.7).

10.13.3.2 Ondersoek ook die lisien-indool-motiliteit-waterstof-sulfiedagarkulture en indien enige organisme 'n vermeende *Vibrio cholerae* of *Vibrio parahaemolyticus* is, stuur 'n monster vir verdere identifisering na 'n aanneemlike toetslaboratorium.

10.14 Opsporing van *Listeria monocytogenes*

Waarskuwing: Die opsporing van *Listeria monocytogenes* moet slegs in behoorlik toegeruste laboratoriums onder beheer van 'n bekwame mikrobioloog onderneem word en daar moet met groot sorg te werk gegaan word met die wegdoen van alle geïnkubeerde materiaal.

10.14.1 Prosedure

10.14.1.1 Bereiding van die monster

Ontdooi, indien nodig, die onverwerkte of gaar produk in sy verpakking by 5 °C tot 10 °C totdat alle sigbare ys gesmelt het. Maak seker dat die produk binne 18 h klaar ontdooi. Verwyder met 'n steriele skêr en tang 25 g tot 35 g van die produk en plaas dit oor na 'n vooraf geweegde en gesteriliseerde homogeniseer-houer wat geskik is vir gebruik saam met die homogeniseerder (kyk 10.3.4). Voeg genoeg van die selektiewe voorverrykingskweekmedium by (kyk 10.4.49) om 'n 1:10-dispersie van die produk te verkry. Laat die homogeniseerder volgens die fabrikant se aanwysings net lank genoeg werk om 'n homogene dispersie te verkry, dws laat draaitipe homogeniseerders lank genoeg werk sodat die totale getal omwentelings van die snylemme 15 000 tot 20 000 is, maar in geen geval langer as 2,5 min nie. Gebruik die 1:10-dispersie van die produk wat só verkry is vir die toetse vir die opsporing van *Listeria monocytogenes*.

10.14.1.2 Primêre verryking

Inkubeer die aanvanklike suspensie (kyk 10.14.1.1) 24 h lank by 30 °C.

10.14.1.3 Sekondêre verryking

Plaas, na die primêre verryking, 0,1 mℓ van die kultuur wat in 10.14.1.2 verkry is oor in 'n buis wat 10 mℓ van die selektiewe verrykingskweekmedium (kyk 10.4.50) bevat. Inkubeer die geïnokuleerde kweekmedium 48 h lank by 37 °C.

10.14.1.4 Plaatkweekmedium en identifikasie

10.14.1.4.1 Neem uit die primêre verrykingskultuur (kyk 10.14.1.2) met 'n lus 'n strook uit die kultuur en inokuleer die oppervlak van die eerste selektiewe plaatkweekmedium (Oxford-agar) (kyk 10.4.51) só dat goed geïsoleerde kolonies verkry sal word.

Gaan op dieselfde wyse met die tweede selektiewe plaatkweekmedium (PALCAM-agar) (kyk 10.4.52) te werk.

10.14.1.4.2 Herhaal met die sekondêre verrykingskweekmedium (kyk 10.14.1.3) die prosedure in 10.14.1.4.1 met die twee selektiewe plaatkweekmedia.

10.14.1.4.3 Draai die bakkies wat in 10.14.1.4.1 en 10.14.1.4.2 verkry is, om. Plaas hulle in 'n inkubator wat op 37 °C gestel is. PALCAM-agarplaatkweekmedia word óf mikro-aërobies geïnkubeer in 'n fles wat 5 % tot 12 % (per volume) CO_2, 5 % tot 15 % (per volume) O_2 en 75 % (per volume) N_2 bevat, óf dit word aërobies geïnkubeer.

10.14.1.4.4 Ondersoek na inkubasie vir 24 h tot 48 h die bakkies vir die aanwesigheid van tipiese kolonies *Listeria* spp. soos volg:

a) **Oxford-agar**: tipiese kolonies *Listeria* spp. wat 24 h lank op Oxford-agar gekweek is, is klein (met 'n diameter van 1 mm) donkerbruin kolonies wat omring is deur swart halo's. Na 48 h is die kolonies swart, met 'n diameter van 2 mm tot 3 mm, het swart halo's en is in die middel ingeval.

b) **PALCAM-agar**: laat PALCAM-agarplaatkweekmedia na inkubasie hul pienk tot pers kleur herwin deur hulle 1 h lank aan lug bloot te stel. *Listeria* spp. groei as klein groen kolonies met 'n diameter van 1,5 mm tot 2 mm, soms met swart middelpunte, maar altyd met swart halo's.

10.14.1.4.5 Indien die groei gering is of as daar na 24 h inkubasie geen kolonies aanwesig is nie, inkubeer die bakke nog 18 h to 24 h lank by 37 °C soos in 10.14.1.4.3 beskryf word en ondersoek weer vir die aanwesigheid van *Listeria* spp.

10.14.1.5 Bevestiging

10.14.1.5.1 Kies van kolonies vir bevestiging

10.14.1.5.1.1 Neem vir bevestiging uit elke plaat van elke selektiewe kweekmedium (kyk 10.14.1.4.3 en 10.14.1.4.4) vyf kolonies wat tipies of verdag is. Indien daar op een plaat minder as vyf tipiese of verdagte kolonies is, neem al die tipiese of verdagte kolonies vir bevestiging.

10.14.1.5.1.2 Stryk die gekose kolonies oor die oppervlak van vooraf drooggemaakte plate met triptoon-sojagisekstrakagar (TSGEA) (kyk 10.4.53) op so 'n wyse dat goed geïsoleerde kolonies sal ontwikkel. Plaas die plate 24 h lank of totdat die groei bevredigend is in 'n inkubator by 37 °C.

10.14.1.5.1.3 Tipiese kolonies het 'n diameter van 1 mm tot 2 mm, is kleurloos, konveks en ondeursigtig met 'n volle rand. Ondersoek, indien nodig, die plate met 'n gestraalde wit lig wat sterk genoeg is om die plate goed te verlig en wat die bodem van die plaat met 'n hoek van 45° tref. Indien daar van reg bokant die plaat in hierdie skuins invallende lig ondersoek ingestel word, vertoon kolonies *Listeria* spp. 'n blouerige kleur en 'n korrelrige oppervlak. Indien daar duidelik gemengde kulture is, kies 'n tipiese *Listeria*-spp.-kolonie en maak nog 'n plaat met TSGEA-subkulture. Voer die volgende toetse met kolonies van 'n suiwer kultuur op die TSGEA uit.

10.14.1.6 Katalasereaksie

Neem 'n tipiese kolonie en suspendeer dit in 'n druppel van 3-%(per volume)-waterstof-peroksiedoplossing op 'n skyfie. *Listeria* spp. is katalasepositief, wat bewys word deur die onmiddellike vorming van gasborrels.

10.14.1.7 Kleureienskappe en morfologie

10.14.1.7.1 Gram-kleuring

Voer die Gram-kleuring op 'n tipiese kolonie op TSGEA (Kyk 10.14.1.5.1.2) uit. *Listeria* spp. is Gram-positiewe stawe.

10.14.1.7.2 Motiliteitstoets (indien nodig)

Kies 'n tipiese kolonie op TSGEA (kyk 10.14.1.5.1.2) en suspendeer dit in 'n buis wat TSGEB (kyk 10.4.54) bevat. Inkubeer 8 h tot 24 h lank by 20 °C tot 25 °C totdat 'n wolkerige kweekmedium waargeneem word.

Gebruik 'n lus en plaas 'n druppel van die kultuur hierbo tussen skyfies en ondersoek die kultuur met 'n mikroskoop. Die *Listeria* spp. lyk soos slanke, kort stawe met duidelike tuimelmotiliteit. Kulture wat by temperature hoër as 25 °C gekweek is, sal moontlik nie hierdie beweging vertoon nie. Vergelyk hulle altyd met 'n bekende kultuur. Cocci, groot stawe, of stawe met 'n vinnige swemmotiliteit is nie *Listeria* spp. nie. As 'n alternatiewe toets vir motiliteit, gebruik 'n inokuleernaald en steek 'n kultuur uit 'n tipiese kolonie op TSGEA (kyk 10.14.1.5.1.2) in die motiliteitsboeljon (kyk 10.4.57) en inkubeer dit 48 h lank by 25 °C. Ondersoek vir groei rondom die steekmerk. *Listeria* spp. is motiel en het 'n tipiese sambreelagtige groeipatroon. Indien 'n negatiewe resultaat verkry word, inkubeer nog 5 d lank en neem weer die steekmerk waar.

10.14.1.7.3 Hemolisetoets

Indien die morfologiese en fisiologiese eienskappe en katalasereaksie *Listeria* spp. aandui, inokuleer die bloedagarplate (kyk 10.4.55)[11)] om die hemolitiese reaksie te bepaal. Maak die agaroppervlak voor gebruik deeglik droog. Trek 'n rooster op die bodem van die plaat en merk 20 tot 25 spasies per plaat.

11) Meld of skaap- of perdebloed gebruik is.

Neem 'n tipiese kolonie uit die TSGEA-plaat (kyk 10.14.1.5.1.2) en steek een opening vir elke kultuur met behulp van 'n lus. Steek terselfdertyd positiewe en negatiewe kontrolekulture (*L. monocytogenes, L. ivanovii, L. seeligeri* en *L. innocua*).

Ondersoek die toetsstamme en -kontroles na inkubasie van 24 h tot 48 h lank by 37 °C. *L. monocytogenes* toon smal, helder ligte sones (ß-hemolise)[12]. *L. innocua* moet geen helder sone rondom die steekmerk toon nie. *L. seeligeri* toon 'n swak sone van hemolise. *L. ivanovii* toon gewoonlik breë, duidelik gedelinieerde sones van ß-hemolise. Hou die plate teen 'n helder lig om die toetskulture met die kontroles te vergelyk.

10.14.1.7.4 Koolhidraatgebruik

Inokuleer die koolhidraatgebruiksboeljons (kyk 10.4.56) met een lus vol of 0,1 mℓ van die TSGEB-kultuur (kyk 10.14.1.7.2). Inkubeer tot 7 d lank by 37 °C. Positiewe reaksies (suurvorming) word aangedui deur 'n geel kleur en kom meestal binne 24 h tot 48 h voor.

10.14.1.8 Vertolking van morfologiese en fisiologiese eienskappe en van biochemiese reaksie

Alle *Listeria* spp. is klein, Gram-positiewe stawe (slegs in die geval van 24 h oue kulture) wat motiliteit in nat montasie en in die motiliteitskweekmedium toon. Hulle is katalasepositief. *L. monocytogenes* gebruik ramnose maar nie xilose nie. *L. monocytogenes, L. ivanovii* en *L. seeligeri* (swak reaksie) produseer ß-hemolise in bloedagarkulture. Van die drie hemolitiese *Listeria* spp. gebruik slegs *L. monocytogenes* nie xilose nie en is positief vir gebruik van ramnose.

10.15 Toets vir die doeltreffendheid van die skoonmaak en ontsmetting van installasies, toerusting en gereedskap

10.15.1 Monsternemingstoerusting

10.15.1.1 Bereiding van deppers

Berei elke depper voor deur 'n ronde pluisie absorbeerwatte met 'n massa van 30 mg tot 50 mg op die een ent van 'n houtstaaf met 'n lengte van ongeveer 140 mm en 'n diameter van ongeveer 2 mm aan te bring. Doop die pluisie van elke depper in 'n beker met onaktiveerderoplossing (kyk 10.4.41) en plaas dan die depper in 'n afsonderlike plastieksakkie of ander geskikte houer wat die daaropvolgende sterilisering sonder beskadiging sal deurstaan. Verseël die sakkies of houers en steriliseer in 'n outoklaaf of op 'n ander aanneemlike manier. Bewaar die deppers in 'n koel, donker plek totdat dit gebruik word.

10.15.1.2 Afwesigheid van stowwe wat die groei van mikro-organismes inhibeer

Smelt die inhoud van twee bottels plaattellingagar (kyk 10.4.3) en verkoel dit tot 45 °C. Inokuleer een bottel met *Escherichia coli* en die ander bottel met *Bacillus subtilis* só dat 'n digte groei verseker is. Meng die inhoud in elke bottel deeglik en giet dit in elk van twee steriele petribakkies (kyk 10.2.5(a) of (b)). Verwyder die pluisies van twee deppers asepties en dompel 'n pluisie in die agar in elk van die plate voordat die agar stol. Inkubeer die plate 18 h lank by 35 °C. Ondersoek die plate na inkubasie en beskou die deppers as nie geskik nie indien daar enige teken is dat die groei van organismes om en onder enigeen van die deppers geïnhibeer is. Berei in sodanige geval 'n vars lot deppers voor soos in 10.15.1.1 beskryf word en toets weer.

10.15.2 Monsternemingsprosedure

10.15.2.1 Algemeen

Indien moontlik, neem monsters van minstens 15 verskillende oppervlakke, elk met 'n oppervlakte van

12) Dit kan makliker gesien word deur 'n kolonie wat op die oppervlak van die agar rondom die inokuleermerk groei, te verwyder.

ongeveer 10 cm^2. Teken die oppervlakte aan, in vierkante sentimeter, van elke oppervlak waarop 'n monster geneem is.

10.15.2.2 Monsterneming met deppers

Maak 'n depperhouer asepties oop en, indien 'n plastieksakkie gebruik is, maak dit oop aan die kant weg van die watteplusie. Maak seker dat die vingers van die monsternemer nooit tydens die hantering van die depper aan die pluisie of aan die aanliggende deel van die steel raak nie. Vryf die pluisie van die depper deeglik oor die oppervlakte waarvan 'n monster geneem moet word. Roteer die depper terselfdertyd só dat die hele oppervlak van die pluisie deeglik in aanraking kom met die oppervlak waarvan 'n monster geneem word. Plaas die depper terug in die houer en, indien 'n plastieksakkie gebruik is, verseël dit weer. Merk die houer só dat dit die plek waar die monster geneem is, identifiseer.

10.15.3 Toetsprosedure

10.15.3.1 Toets van deppers

10.15.3.1.1 Spoelsuspensie

Haal die depper uit die houer en breek die pluisie in 'n 30-mℓ-bottel met gebufferde isotoniese peptoonwater (kyk 10.4.2) af deur die bottelnek as hefboom te gebruik. Skud die bottel deeglik. Hierdie spoelsuspensie is 'n 1:10-verdunning van die monster.

10.15.3.1.2 Inokulasie en inkubasie

Pipetteer 'n 1-mℓ-volume van die spoelsuspensie asepties in elk van twee petribakkies (kyk 10.2.5(a) of (b)). Voeg 'n 15-mℓ-volume van die plaattellingagar (kyk 10.4.3) wat gesmelt en tot 45 °C afgekoel is by elke plaat en meng die inhoud van die plaat deur dit liggies te werwel. Laat dit stol, keer die plate om en inkubeer 72 h lank by 25 °C.

10.15.3.1.3 Vertolking

Tel die totale getal bakteriekolonies wat aan die einde van die inkubasie-tydperk op die twee plate ontwikkel het en teken dit aan. Neem vyf maal hierdie getal as dié getal lewensvatbare bakterieë op die toetsoppervlak waarop die monster geneem is en korrigeer vir 'n oppervlakte van 10 cm^2, indien nodig.

10.15.3.2 Toets vir die aanwesigheid van fekale koliforme bakterieë

Inkubeer die oorblywende spoelsuspensie (kyk 10.15.3.1.1) 6 h tot 8 h lank by 37 °C. Volg dan die prosedure wat in 10.8 beskryf is.

10.15.3.3 Uitdrukking van skoonmaak- en ontsmettingsdoeltreffendheid

Ken een van die simbole B, RB of OB, volgens die telling van lewensvatbare bakterieë of die aanwesigheid van fekale koliforme bakterieë soos volg aan elke monster toe:

Telling	Simbool
0 tot 15/10 cm^2: fekale koliforme bakterieë afwesig	B (Bevredigend)
16 tot 75/10 cm^2: fekale koliforme bakterieë afwesig	RB (Redelik bevredigend)
Bo 75/10 cm^2: of fekale koliforme bakterieë aanwesig, of albei	OB (onbevredigend)

10.15.3.4 Berekening

Bereken die persentasie skoonmaak- en ontsmettingsdoeltreffendheid aan die hand van die volgende formule:

$$\frac{(2X + Y) \times 100}{2T}$$

waar

X die getal B-resultate is;

Y die getal RB-resultate is; en

T die totale getal monsters is.

10.16 Mikrobiologiese ondersoek van water

10.16.1 Membraanfiltermetode

10.16.1.1 Ondersoek vir totale koliforme bakterieë

Sit 'n steriele membraanfilterhouer (kyk 10.3.7) onmiddellik voor gebruik op 'n filtreerfles vas. Haal die houer uitmekaar en plaas met behulp van 'n steriele tangetjie (kyk 10.3.8) 'n steriele membraanfilter (kyk 10.3.6), met die roosterkant bo, oor die poreuse plaat. Sit die houer weer aanmekaar.

Meng die watermonster deeglik deur die monsterhouer ongeveer 10 keer (deur 'n vinnige beweging van die polsgewrig) om te keer en regop te bring. Plaas 100 mℓ van die monster asepties in die saamgestelde membraanfilterhouer (kyk 10.3.7) en filtreer deur suiging op die filtreerfles uit te oefen.

Spoel die tregter drie maal met 20-mℓ- tot 30-mℓ-volumes steriele water uit nadat die 100 mℓ water gefiltreer is. Haal die houer versigtig uitmekaar en gebruik 'n steriele tangetjie (kyk 10.3.8) om die membraanfilter, met die roosterkant bo, asepties oor te plaas na 'n plaat met m-Endo-agar LES (kyk 10.4.43). Maak seker dat die oppervlak van die m-Endo-agar LES (kyk 10.4.43) in die petribakkie vry van oortollige vog is. Maak seker dat geen lugborrel tussen die membraanfilter en die oppervlak van die agar vasgevang word nie en dat goeie benattingskontak gehandhaaf word.

Keer die m-Endo-agar-LES-plate om en inkubeer 18 h tot 24 h lank by 35 °C ± 0,5 °C in 'n donker houer. Ondersoek die plate en tel die getal koliforme kolonies, dws kolonies met 'n pienk tot donkerrooi kleur met 'n metaalagtige goudgroen skynsel. Indien meer as een filter gebruik is om die 100-mℓ-watermonster te filtreer, tel al die verdagte koliforme kolonies op al die filters. Die skynselgebied kan wissel van die grootte van 'n klein speldekop tot die hele oppervlak van die kolonie. Kolonies sonder 'n skynsel word as niekoliform beskou.

Indien daar vermoed word dat die monster meer as 30 koliforme bakterieë per 100 mℓ bevat, kan geskikte verdunnings berei en gefiltreer word. Ten einde die aanwesigheid van koliforme bakterieë te bevestig, subkweek elke kolonie of 'n verteenwoordigende getal daarvan (dws kolonies met 'n pienk tot donkerrooi kleur met 'n metaalagtige goudgroen skynsel) in buise of bottels met laktose peptoonwater (kyk 10.4.48) en inkubeer 48 h lank by 37 °C ± 0,5 °C. Ondersoek vir gasvorming. Gasvorming word aangedui deur 'n hoeveelheid gas wat genoeg is om minstens die holte aan die bopunt van die Durham-buis te vul (kyk 10.2.11). Gasvorming bevestig die aanwesigheid van koliforme bakterieë.

Bereken die getal koliforme bakterieë per 100 mℓ van die watermonster soos volg:

$$\frac{N \times D}{V} \times 100$$

waar

N die totale getal getelde kolonies is;

D die verdunfaktor is; en

V die volume van die gefiltreerde monster is, in milliliter.

Voer die toets in duplikaat en verkieslik in triplikaat uit.

OPM – Daar word aanbeveel dat die waarskynlikstegetal(MPN)-metode (kyk 10.16.2.3.1) gebruik word om die getal koliforme bakterieë in seewater te bepaal.

10.16.1.2 Ondersoek vir fekale koliforme bakterieë

Gaan soos in 10.16.1.1 te werk, maar gebruik 'n mFC-agarplaat (kyk 10.4.44) en inkubeer die mFC-agar 18 h tot 24 h lank by 44 °C ± 0,25 °C in 'n waterdigte houer in 'n waterbad. Ondersoek die plate en tel die getal kolonies wat 'n blou middelpunt met 'n deurskynende omtrek toon. Hierdie kolonies is verdagte fekale koliforme bakterieë. Niefekale koliforme kolonies is grys- tot roomkleurig. Ten einde die aanwesigheid van fekale koliforme bakterieë te bevestig, subkweek elke kolonie of 'n verteenwoordigende getal daarvan (dws kolonies met 'n blou middelpunt met 'n deurskynende omtrek) in buise of bottels met laktosepeptoonwater (kyk 10.4.48) en inkubeer 24 h lank by 44 °C ± 0,25 °C. Ondersoek vir gasvorming. Gasvorming word aangedui deur 'n hoeveelheid gas wat genoeg is om minstens die holte aan die bopunt van die Durham-buis te vul. Indien die kweekmedium die vorming van gas toon, soos aangedui deur gas in die Durham-buis, gebruik 'n platinumdraadlus om uit elke bottel een lus vol in 'n bottel triptoonwater (kyk 10.4.7) te subkweek en inkubeer hierdie subkulture 16 h tot 20 h lank by 44,5 °C ± 0,25 °C in 'n waterdigte houer in 'n waterbad. Voeg 0,1 mℓ tot 0,5 mℓ Kovacs-reagens (kyk 10.4.8) by die kultuur in die triptoonwater. Meng deur die bottel liggies te skud. Die ontwikkeling van 'n rooi kleur dui die aanwesigheid van indool aan en bevestig die aanwesigheid van fekale koliforme bakterieë.

Gebruik dieselfde vergelyking as in 10.16.1.1 om die getal fekale koliforme bakterieë wat in 100 mℓ van die watermonster aanwesig is, te bereken.

OPM – Daar word aanbeveel dat die waarskynlikstegetal(MPN)-metode (kyk 10.16.2.3.2) gebruik word om die getal fekale koliforme bakterieë in seewater te bepaal.

10.16.2 Waarskynlikstegetal(MPN)-metode

10.16.2.1 Bereiding van die toetsgedeelte en reeks tienvoudige verdunnings

Meng die watermonster deeglik deur die monsterhouer ongeveer 10 keer met 'n vinnige beweging van die polsgewrig om te keer en regop te bring. Die tyd wat verloop tussen die meng en uithaal van die toetsgedeelte mag hoogstens 3 min wees. Verwyder 1 mℓ van die monster met 'n steriele pipet en voeg dit by 9 mℓ steriele peptoonwater (kyk 10.4.47). Meng hierdie primêre verdunning deeglik deur die houer ongeveer 10 keer vinnig om te keer en regop te bring. Gebruik 'n skoon pipet om 1 mℓ van die primêre verdunning na 'n ander bottel met 9 mℓ steriele peptoonwater (kyk 10.4.47) oor te plaas en meng deeglik. Herhaal hierdie prosedures om 'n reeks tienvoudige verdunnings te verkry. Berei 'n voldoende getal verdunnings om te verseker dat al die bottels wat die finale verdunning bevat, 'n negatiewe resultaat sal gee.

10.16.2.2 Inokulering van lourieltriptoseboeljon

Gebruik 'n steriele pipet om 10 mℓ van die watermonster oor te plaas na elk van drie buise of bottels wat dubbelsterkte-lourieltriptoseboeljon (kyk 10.4.46) bevat. Gebruik 'n steriele pipet om 1 mℓ van die water oor te plaas na elk van drie buise of bottels wat enkelsterkte-lourieltriptoseboeljon (kyk 10.4.45) bevat. Plaas 1 mℓ van elk van die daaropvolgende verdunnings (kyk 10.16.2.1) oor in elk van drie buise of bottels wat enkelsterkte-lourieltriptoseboeljon (kyk 10.4.45) bevat. Gebruik 'n skoon steriele pipet vir elke verdunning. Inkubeer die geïnokuleerde buise of bottels 48 h lank by 37 °C ± 0,5 °C.

Ondersoek die kulture ná inkubasie en beskou dié wat troebelheid as gevolg van bakteriële groei en gasvorming toon, as positiewe reaksies. Gasvorming word aangedui deur 'n hoeveelheid gas wat genoeg is om minstens die holte aan die bopunt van die Durham-buis (kyk 10.2.11) te vul. Tel vir elke verdunning die getal buise of bottels wat 'n positiewe reaksie toon en teken dit aan.

10.16.2.3 Bevestigende toetse

10.16.2.3.1 Koliforme bakterieë

Ten einde die aanwesigheid van koliforme bakterieë te bevestig, subkweek uit elke buis of bottel louriel-triptoseboeljon wat 'n positiewe resultaat gee, in 'n buis of bottel met enkelsterkte-briljantgroengalkweekmedium (kyk 10.4.5) en inkubeer by 37 °C ± 0,5 °C. Ondersoek binne 48 h vir gasvorming. Gasvorming bevestig die aanwesigheid van koliforme bakterieë.

10.16.2.3.2 Fekale koliforme bakterieë

Subkweek uit elke buis of bottel lourieltriptoseboeljon wat 'n positiewe resultaat vir gasvorming gee, in 'n bottel triptoonwater (kyk 10.4.7). Inkubeer 24 h lank in 'n waterbad waarvan die temperatuur by 44,5 °C ± 0,25 °C gehou word. Voeg 0,1 mℓ tot 0,5 mℓ Kovacs-reagens (kyk 10.4.8) by die kultuur in die triptoonwater. Meng deur die bottel liggies te skud. Die ontwikkeling van 'n rooi kleur dui die aanwesigheid van indool aan.

10.16.2.4 Uitdrukking van resultate

10.16.2.4.1 Bereken, deur tabel 4 te raadpleeg, aan die hand van die getal buise of bottels met louriel-triptoseboeljon en bevestigende toetse wat positiewe reaksies gee, die waarskynlikste getal koliforme en fekale koliforme bakterieë.

10.16.2.4.2 Kies van verdunnings

Kies drie opeenvolgende verdunnings ooreenkomstig een van die volgende reëls vir elke monster wat ondersoek word, soos toepaslik:

a) kies die hoogste verdunning (dws dié met die laagste konsentrasie of kleinste hoeveelheid van die monster) wat positiewe resultate gee saam met twee voorafgaande stelle verdunnings (kyk 10.16.2.4.4, voorbeeld A en B);

b) indien minder as drie stelle verdunnings positiewe resultate gee, begin met die stel met die laagste verdunning (dws dié met die hoogste konsentrasie of die grootste hoeveelheid van die monster) (kyk 10.16.2.4.4, voorbeeld C); of

c) indien slegs een stel verdunnings 'n positiewe resultaat gee, gebruik hierdie verdunning en die een wat hoër en laer is (kyk 10.16.2.4.4, voorbeeld D), behalwe wanneer die stel verdunnings wat 'n positiewe resultaat gee, gevind word op die vlak van die eerste verdunning wat berei is. In sodanige geval is dit nodig om die eerste drie verdunnings vir die berekening van die MPN te kies.

10.16.2.4.3 Bepaling van die MPN-indeks

Bepaal volgens tabel 4 die MPN-indeks aan die hand van die getal positiewe buise of bottels in elk van die drie opeenvolgende verdunnings (volgens 10.16.2.4.2(a) tot (c) gekies).

Voorbeeld: Indien die getal positiewe buise of bottels vir die drie opeenvolgende verdunnings 3, 2, 1 is (kyk 10.16.2.4.4, voorbeeld B), kry dan, deur tabel 4 te raadpleeg, in die eerste drie kolomme die toepaslike volgorde van die nommers, dws 3, 2, 1, en lees die MPN-indeks, wat in hierdie geval 15 is, in die vierde kolom af.

10.16.2.4.4 Berekening van die waarskynlikste getal

Bereken die getal koliforme en fekale koliforme bakterieë per 100 mℓ van die watermonster deur die

MPN-indeks (kyk 10.16.2.4.3) te vermenigvuldig met die resiprook van die laagste verdunning wat gekies is (dws dié met die hoogste konsentrasie van die monster), vermenigvuldig met 100. Indien die laagste verdunning wat gekies is, ooreenkom met die buis of bottel wat met 10 mℓ geïnokuleer is, moet die MPN-indeks eers deur 10 gedeel word. Die resultaat kan uitgedruk word as 'n getal tussen 1,0 en 9,9 vermenigvuldig met 10^x, waar x die toepaslike sterkte van 10 is. Vertrouensgrense word in tabel 4 aangegee.

Voorbeeld: met verwysing na voorbeeld A in die teks word die MPN-indeks van 15 (kyk 10.16.2.4.3) deur 10 gedeel, dws die volume van die inokulum in die laagste verdunning wat gekies is. Die kwosiënt wat só verkry word, dws 1,5, word met 1,0 vermenigvuldig, dws die resiprook van die laagste verdunning (10^0) wat gekies is. Die produk 1,5 word dan met 100, die gespesifiseerde verwysingsvolume, vermenigvuldig om die getal bakterieë per 100 mℓ watermonster te kry.

Voorbeeld A

Toetsmonster (verdunning 10^0) (10 mℓ) :	3	buise +; 0 buise –
Toetsmonster (verdunning 10^0) (1 mℓ) :	2	buise + ; 1 buis –
Toetsmonster Verdunning 10^{-1} (1 mℓ) :	1	buis + ; 2 buise –

Verdunning 10^{-2} (1 mℓ) : 0 buis + ; 3 buise –
Verdunning 10^{-3} (1 mℓ) : 0 buis + ; 3 buise –

Volgens tabel 4 is die MPN-indeks 15 en die berekening gee 'n MPN van $\frac{15}{10}$ x 1 x 100, dws 1,5 x 10^2 bakterieë per 100 mℓ van die watermonster.

Voorbeeld B

Toetsmonster (verdunning 10^0) (10 mℓ) :	3	buise + ; 0 buise –
Toetsmonster (verdunning 10^0) (1 mℓ) :	3	buise + ; 0 buise –
Toetsmonster (verdunning 10^{-1}) (1 mℓ) :	3	buise + ; 0 buise –
(verdunning 10^{-2}) (1 mℓ) :	2	buise + ; 1 buis –
(verdunning 10^{-3}) (1 mℓ) :	1	buis + ; 2 buise –
(verdunning 10^{-4}) (1 mℓ) :	0	buis + ; 3 buise –

Volgens tabel 4 is die MPN-indeks 15 en die berekening gee 'n MPN van 15 x 10 x 100, dws 1,5 x 10^4 bakterieë per 100 mℓ van die watermonster.

Voorbeeld C

Toetsmonster (verdunning 10^0) (10 mℓ) :	2	buise + ; 1 buis –
Toetsmonster (verdunning 10^0) (1 mℓ) :	1	buis + ; 2 buise –
Toetsmonster (verdunning 10^{-1}) (1 mℓ) :	0	buis + ; 3 buise –

(verdunning 10^{-2}) (1 mℓ) : 0 buis + ; 3 buise –
(verdunning 10^{-3}) (1 mℓ) : 0 buis + ; 3 buise –

Volgens tabel 4 is die MPN-indeks 1,5 en die berekening gee 'n MPN van $\frac{1,5}{10}$ x 1 x 100, dws 1,5 x 10^1 bakterieë per 100 mℓ van die watermonster.

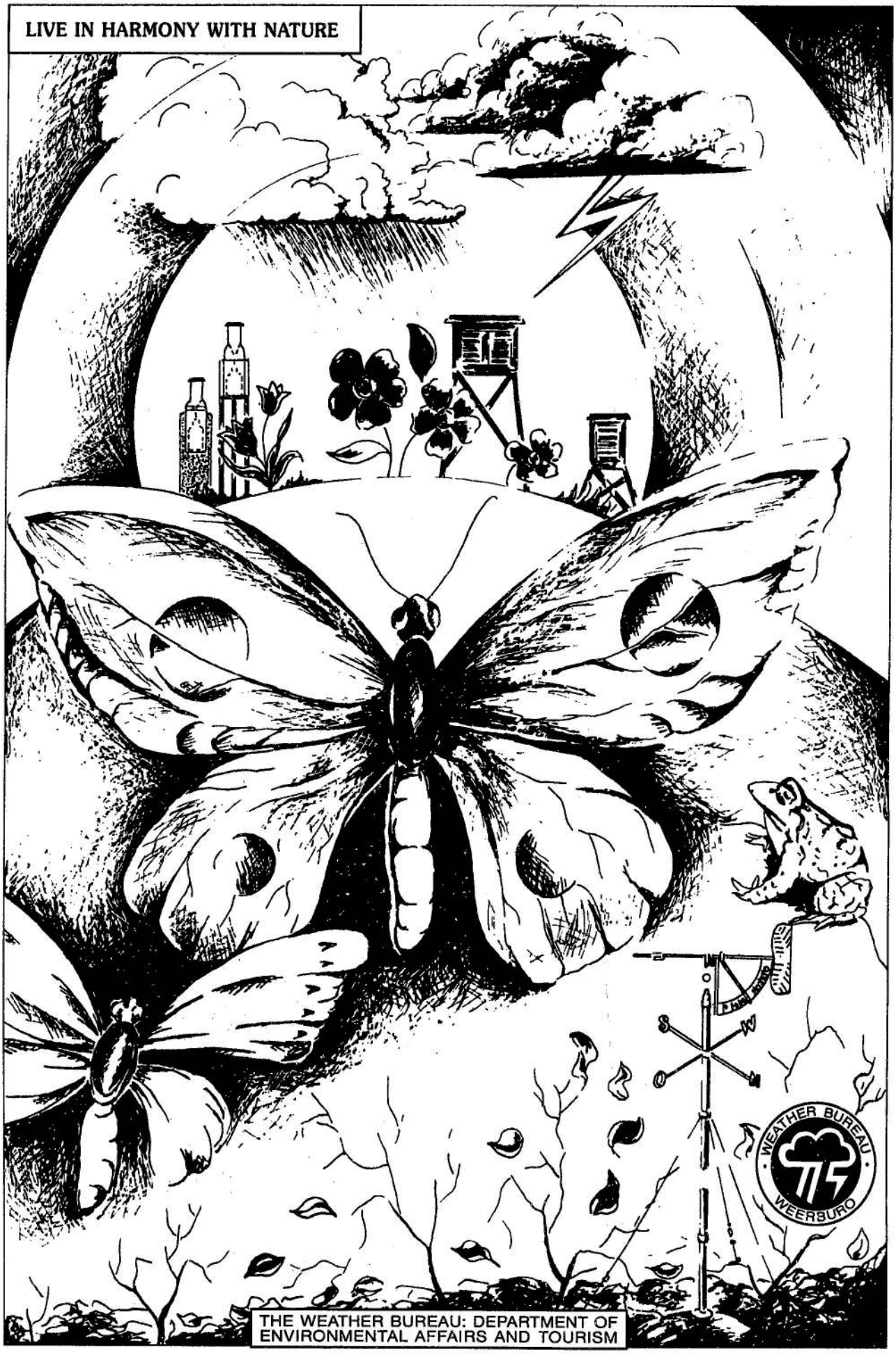
Voorbeeld D

Toetsmonster (verdunning 10^0) (10 mℓ) :	0	buise + ; 3 buise –
Toetsmonster (verdunning 10^0) (1 mℓ) :	1	buis + ; 2 buise –
Toetsmonster (verdunning 10^{-1}) (1 mℓ) :	0	buis + ; 3 buise –

Verdunning 10^{-2} (1 mℓ) : 0 buis + ; 3 buise –
Verdunning 10^{-3} (1 mℓ) : 0 buis + ; 3 buise –
Volgens tabel 4 is die MPN-indeks 0,3 en die berekening gee 'n MPN van $\frac{0,3}{10}$ x 1 x 100, dws 3 x 10^0 bakterieë per 100 mℓ van die watermonster.

Tabel 4 — MPN-indeks en vertrouensgrense

1	2	3	4	5	6
Getal positiewe buise vir die drie verdunnings wat gekies is			MPN-indeks	Vertrouensgrense	
Eerste	Tweede	Derde		> 95 %	
0	0	0	< 0,30	0,00	0,94
0	0	1	0,30	0,01	0,95
0	1	0	0,30	0,01	1,0
0	1	1	0,61	0,12	1,7
0	2	0	0,62	0,12	1,7
0	3	0	0,94	0,35	3,5
1	0	0	0,36	0,02	1,7
1	0	1	0,72	0,12	1,7
1	0	2	1,1	0,4	3,5
1	1	0	0,74	0,13	2,0
1	1	1	1,1	0,4	3,5
1	2	0	1,1	0,4	3,5
1	2	1	1,5	0,5	3,8
1	3	0	1,6	0,5	3,8
2	0	0	0,92	0,15	3,5
2	0	1	1,4	0,4	3,5
2	0	2	2,0	0,5	3,8
2	1	0	1,5	0,4	3,8
2	1	1	2,0	0,5	3,8
2	1	2	2,7	0,9	9,4
2	2	0	2,1	0,5	4,0
2	2	1	2,8	0,9	9,4
2	2	2	3,5	0,9	9,4
2	3	0	2,9	0,9	9,4
2	3	1	3,6	0,9	9,4
3	0	0	2,3	0,5	9,4
3	0	1	3,8	0,9	10,4
3	0	2	6,4	1,6	18,1
3	1	0	4,3	0,9	18,1
3	1	1	7,5	1,7	19,9
3	1	2	12	3	36
3	1	3	16	3	38
3	2	0	9,3	1,8	36
3	2	1	15	3	38
3	2	2	21	3	40
3	2	3	29	9	99
3	3	0	24	4	99
3	3	1	46	9	198
3	3	2	110	20	400
3	3	3	> 110		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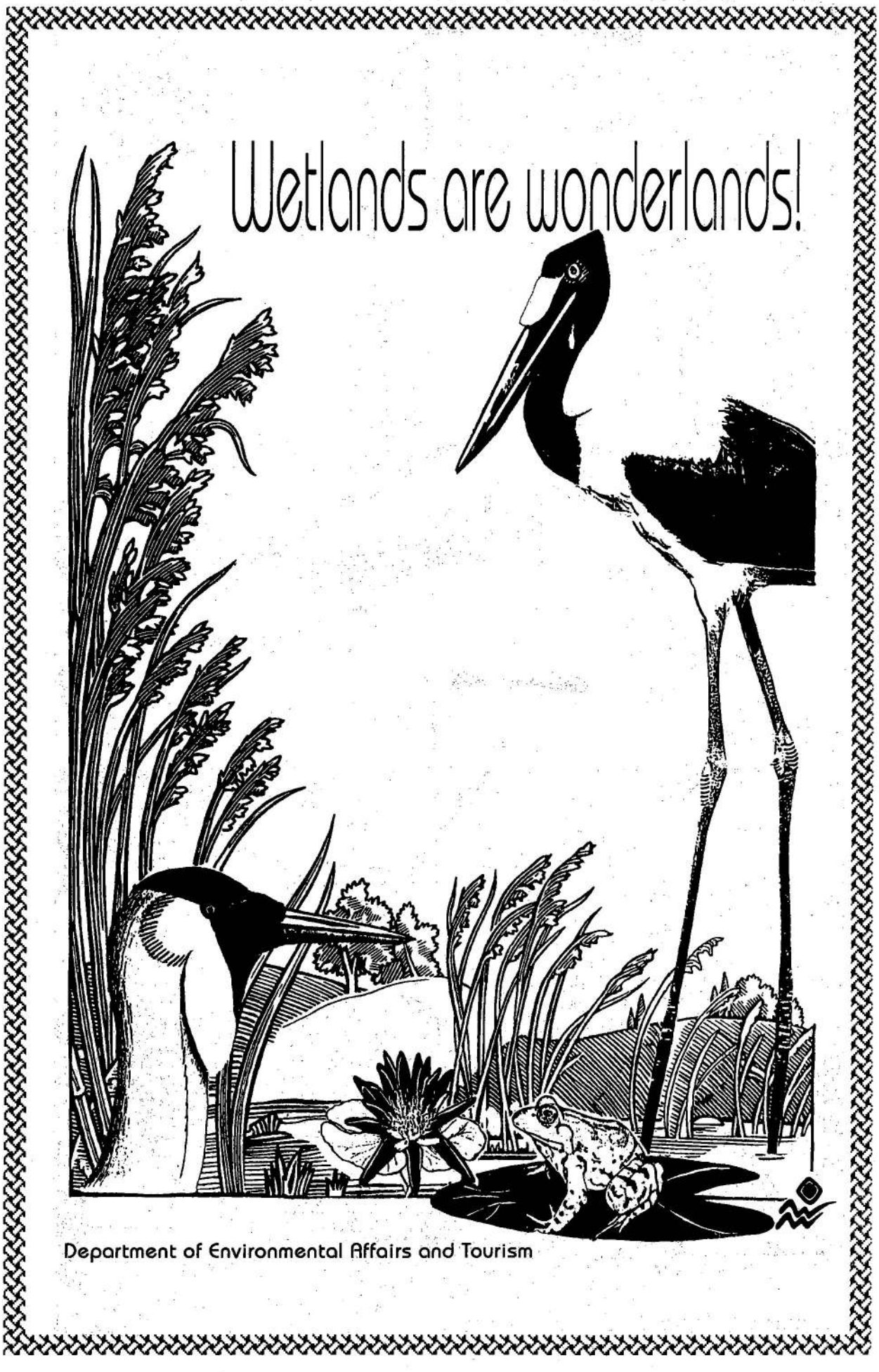
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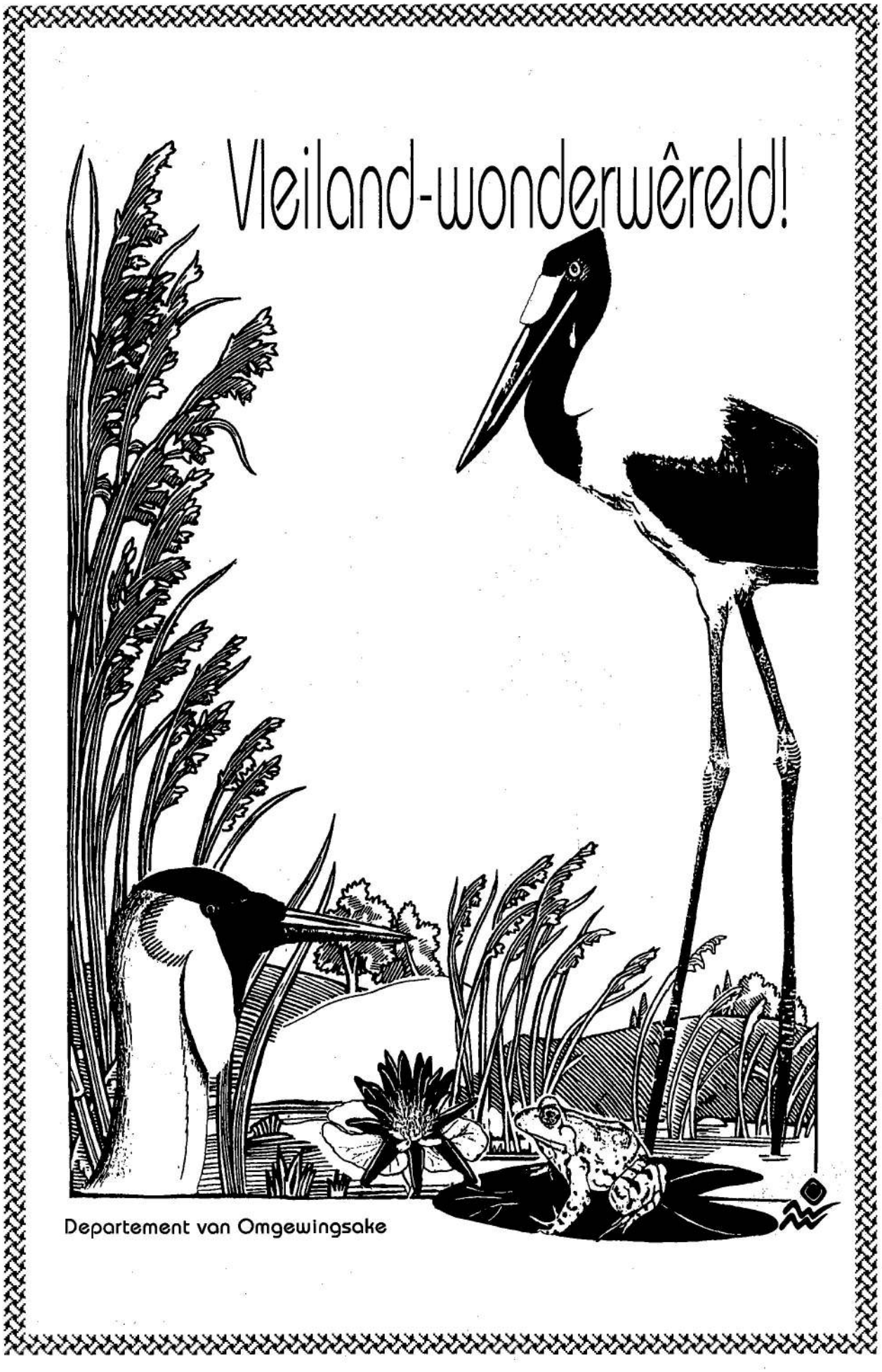
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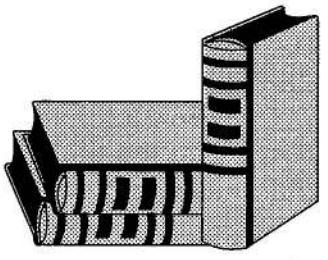

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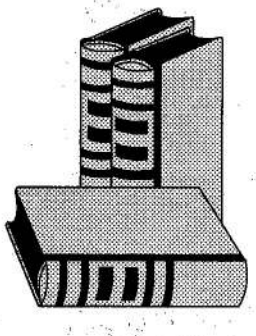

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