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

No. R. 531 **14 May 1999**

STANDARDS ACT, 1993

COMPULSORY SPECIFICATION FOR FROZEN LOBSTER AND FROZEN LOBSTER PRODUCTS

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 lobster and frozen lobster products 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 rock lobster products published by Government Notice No. R.3964 of 19 December 1969.

ALEXANDER ERWIN
Minister of Trade and Industry

SCHEDULE

COMPULSORY SPECIFICATION FOR FROZEN LOBSTER AND FROZEN LOBSTER PRODUCTS

1 Scope

This specification covers requirements for the handling, preparation, processing, packaging, freezing, storage and quality of frozen lobster tails, frozen whole lobster (cooked or raw) or any other frozen lobster product derived from lobsters of the families Palinuridae and Scyllaridae, and of the family Nephropsidae (genera *Homarus*, *Nephrops* and *Metanephrops*, or any other species of lobster), 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 by-product: A product not intended for human consumption.

2.3 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.

2.4 factory: Any premises in which the product (see 2.17) is prepared or processed or both, and including, to the extent to which the requirements of this specification can be applied, a factory ship on which the product is frozen after preparation and processing.

2.5 freezer: A room or equipment that is specially designed to lower the temperature of a food product through the zone of maximum crystallization (for most products, between –1 °C and –5 °C) and down to an equilibrium temperature of –20 °C or lower, in a period of time that is acceptable for the product.

2.6 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.

2.7 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.

2.8 frozen lobster for catering purposes (catering packs): Packed and frozen lobsters or lobster tails, certain of which might be slightly damaged but all of which are of acceptable quality and in every way fit for human consumption. The product may be presented graded or ungraded.

2.9 frozen lobster tails: Lobster tails that have undergone a freezing process and have been preserved by storage in the frozen state.

2.10 frozen product: A product (see 2.17) that has been preserved by storage in the frozen state.

2.11 frozen whole cooked lobster: A whole cooked lobster that has undergone a freezing process and that has been preserved by storage in the frozen state.

2.12 frozen whole raw lobster: A whole raw lobster that has undergone a freezing process and that has been preserved by storage in the frozen state.

2.13 lobster product: Any commodity made from lobster, with or without other ingredients, and intended for human consumption.

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

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

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

2.17 product: Whole lobster (cooked or raw) or lobster tails or other lobster 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.

2.18 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.

2.19 purge: To hold live lobster in clean, running sea water for at least 72 h in order to clean (empty) the intestine.

2.20 shredded or comminuted lobster: Lobster flesh of which the original muscle structure was broken up during the process of recovery of the flesh from the shell.

2.21 soft-shelled lobster: Lobster in the early post-moult stage, as evidenced by the presence of a soft new shell (exoskeleton) after the shedding of the old, and in which

- the gill-covering region of the carapace (branchiostegite) is still soft (uncalcified) and can be easily torn or damaged or more or less permanently depressed on physical handling, and
- the flesh is high in water content to the extent that it does not possess the characteristic springiness of lobster flesh when in sound condition, and turns mushy or crumbly when cooked.

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

2.23 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, hygienic design and conditions

3.2.1.1 The location of the factory buildings 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 working 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 under side 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 be provided with 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 and 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 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 the 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 change rooms 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

The areas where the raw product and the cooked product are handled shall be physically separated from each other. There shall be no cross-flow of raw and cooked production operations.

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

a) the receipt and storage of raw materials;

b) preparatory operations such as the detailing, removal of the anal canal and washing of lobster;

c) processing operations such as 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 Freezers, chill rooms and freezer storage rooms

3.2.15.1 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 be either 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.

3.2.15.2 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.

3.2.15.3 Freezer storage rooms 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.

3.2.15.4 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.

3.2.16 By-products

Any processing of by-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.

3.2.17 Living quarters

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

3.2.18 Refuse

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

3.2.19 Comfort facilities

3.2.19.1 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.

3.2.19.2 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, separate 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.

3.2.19.3 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 and dressing-rooms.

3.2.20 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 that complies with the requirements of 3.4.2.

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 and 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 a distance sufficient to provide 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 porous material. Wicker baskets shall not be used as containers for lobsters 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 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 product 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, 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-phenylenediamine 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 exceeding 10 °C) immediately after the ice has been manufactured complies with the microbiological 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, 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 directions for use 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 or 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. No food or beverage shall be prepared or consumed by employees in these areas. 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, processing or packaging 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 processing 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. Foot baths shall be drained and cleaned regularly and the disinfectant shall be kept in an 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, breaking pins 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 similarly treated.

3.6.3 Repair

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 10.15.3.2 and 10.15.3.3.

3.6.5 Containers, bins and crates for the handling of raw material 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 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 or appearance of the frozen product in any way shall be present in freezers, chill rooms and 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 area 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 products 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.

3.7 Records

Adequate quality records shall be maintained. Quality records and records of freezer storage temperatures shall be kept for a period of at least two years (see 3.2.15.3).

4 Requirements for the ingredients and the product

4.1 General

4.1.1 Condition of ingredients and the product

4.1.1.1 General

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 Salt

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

4.1.1.3 Seasoning

Seasoning shall be free from foreign material and adulterants.

4.1.1.4 Additives

Only permitted additives shall be used, and then only in the permitted quantities.

4.1.2 Requirements for importing countries

In order to meet the requirements of an importing country, products for export may be packed to requirements that deviate from those laid down in this specification, subject to the following conditions:

a) prior written application for the packaging of the product in accordance with the proposed deviation shall be made to the authority administering this specification, full details of the intended deviation being furnished;

b) the deviation shall not, in the opinion of the authority administering the specification, after consultation with organizations representing the industry, result in the packing and exporting of a product of doubtful quality or of such nature as to affect deleteriously the Republic of South Africa's export image in the market concerned; and

c) the product shall be accurately described on its container or label and the labelling or marking shall not be misleading to the consumer.

4.2 Transportation of whole lobsters and lobster tails to processing and freezing plants

4.2.1 General

Transportation of whole lobster to processing and freezing plants, which shall be done in accordance with the relevant regulations of the Sea Fisheries Act, 1988 (Act 12 of 1988), shall be performed under clean and hygienic conditions, and the product in transit shall be fully protected from contamination by dust and other foreign matter and from the heat of the sun. Lobster shall not be transported under conditions that adversely affect or impair the product. Lobster shall not be transported with other products. Means of transport used for lobster shall not be used for other products likely to impair or contaminate the lobster. The inside surfaces of the means of transport shall be so finished that they do not adversely affect the lobster. The inside surfaces shall be smooth and easily cleaned and disinfected. The lobsters shall be kept moist, cool and alive. Coverings used over unprotected raw material shall not rest on the raw material direct. The method of transportation, and the manner in which lobsters are held during transportation, shall be such that the lobsters are not damaged in any way.

4.2.2 Whole lobsters

Whole lobsters shall be alive upon arrival at the packing plant. For the attainment of this, provision of refrigeration during transportation might be necessary. Neither fresh-water ice nor ice water shall be allowed to come into contact with the lobsters during such transportation.

4.2.3 Lobster tails

Lobster tails shall not be transported in the unfrozen state unless:

a) they have been properly gutted and washed;

b) they are individually well wrapped, graded and packed in their final containers;

c) they are refrigerated to a temperature not lower than –1 °C and not higher than 4 °C and transported to nearby freezing facilities within 5 h after being packaged; and

d) the transportation vehicle is of hygienic construction.

4.3 Packing of lobsters and lobster tails

Only lobsters or lobster tails of the same species shall be packed together in any one container.

4.4 Condition of lobsters

4.4.1 General

A lobster shall at no time be held under adverse conditions of storage, for example in corrugated iron structures that are subjected to direct sunlight. If, after landing, lobsters cannot be removed to processing factories without undue delay, or have to be held at a packing plant, or both, they shall be kept alive either by submersion in clean running sea water or by being held at an ambient temperature in the range 4 °C to 12 °C.

4.4.2 Soundness

Lobsters shall be alive at the time of, or immediately prior to, tailbreaking or, when applicable, immediately prior to cooking, processing and freezing. Lobsters or lobster tails that are only slightly damaged or broken to a minor extent, may be used for catering packs, provided that they are in every other way of acceptable quality and fit for human consumption. Lobsters with hanging tails, uncharacteristic odour or uncharacteristic flesh colour shall be deemed to be unacceptable.

4.4.3 Soft shell (new shell) and berry

A soft-shelled lobster, a lobster in berry or a lobster that has been stripped of berry or that does not comply with statutory requirements shall not be used in the product.

4.5 Frozen raw whole lobster

4.5.1 Before being packed as frozen whole lobster, a live lobster of the *Jasus* species shall be purged. However, should the market require unpurged frozen whole lobster, special dispensation to market lobster of the *Jasus* species in this way shall be requested from the authority administering this specification. In the case of lobsters of other species, the product may consist of unpurged lobsters, subject to the declaration of this fact in the labelling or marking of the product (see 6.1(b)(2) or (3)).

4.5.2 A lobster shall be alive until immediately before being processed, when it shall be killed.

4.5.3 Lobsters shall be neatly and individually wrapped and neatly packed, and then frozen in accordance with 5.3. Alternatively, lobsters may be frozen and then glazed before being wrapped and packed.

4.5.4 Lobsters shall be graded for size and lobsters in any one container shall be acceptably uniform in size. Where lobsters are not graded for size, this fact shall be conspicuously declared on all main panels of immediate containers and master containers.

4.5.5 A lobster may be packed in an ice block, provided that it is completely covered with the ice. There shall be no cracks or other flaws in the ice.

4.6 Frozen cooked whole lobster

4.6.1 The requirements for purging that are applicable to frozen raw whole lobster shall apply (see 4.5.1).

4.6.2 The requirements of 4.5.2 shall apply.

4.6.3 Immediately after being killed, the lobsters shall be cooked, rapidly cooled, scrubbed in cold fresh running water (that complies with all the requirements of 3.4.1) or sea water (that complies with all the requirements of 3.4.2), drained, graded where applicable, neatly and individually wrapped and neatly packed, and then frozen in accordance with 5.3. Alternatively, the product may be frozen and then glazed before being wrapped and packed. When graded, the lobsters in any one container shall be acceptably uniform in size.

4.6.4 After having been thawed, the product shall be such that it is consumable without further cooking.

4.7 Frozen lobster tails

4.7.1 Provided that they comply with the requirements of 4.4.2, lobsters may be held in a chilled condition while awaiting tailbreaking and further processing. The conditions of chilling shall not be such as to affect deleteriously the odour, flavour, colour or appearance of the product.

4.7.2 After the tails have been severed from the bodies, the gut shall be removed immediately in an hygienic manner and the tails very thoroughly washed to remove all traces of free blood. The washing shall be done in running water that complies with the requirements of 3.4.1 or 3.4.2, as relevant. Thereafter, the tails shall be graded where applicable, neatly and individually wrapped, neatly packed, and frozen without delay. Tails shall show no discoloration of whatever nature or origin. When it is necessary to hold the gutted, washed and graded tails under refrigeration awaiting final packing, the chilling of the tails shall begin immediately after gutting and washing, and the final freezing of the product shall start within 5 h of the beginning of the intermediate chilling. The nature and duration of the chilling shall be such as to not affect deleteriously the odour, flavour, colour or appearance of the product.

4.7.3 Whole lobster intended for the packing of tails shall not be subjected to intermediate freezing before further processing, but tails that have been properly gutted, washed, graded and individually wrapped may be frozen as an intermediate step before final packing, mass adjustment and refreezing. Such tails may, with a view to repacking, mass adjustment and final freezing, be warmed (when necessary) in an acceptable way to allow separation of units, provided that the internal temperature of the tails does not rise above –7 °C.

4.7.4 Lobster tails from deep-sea species may, where necessary during preparation for freezing, be treated with an anti-oxidant, such as sulfur dioxide or ascorbic acid, permitted by regulation under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972), in order to prevent melanosis of the flesh, shell and telson (tail fin). The treatment of the tails shall not cause the flavour of the cooked product to be uncharacteristic of the species concerned.

4.7.5 Except in the case of tails intended for packing for catering purposes, tails shall be graded by mass or by length, as appropriate. The count of tails in any container shall be in accordance with the declaration on the container. The tails in any one container shall be acceptably uniform in size and, as far as is practicable within any category, the mass of each tail shall fall within the mass range obtained by dividing the sum of the declared net mass of the appropriate package unit and the minimum overpack (see 4.9) by the corresponding maximum and minimum counts for that category.

4.7.6 Lobster tails in catering packs may be presented graded or ungraded for size, but this shall be declared on the main panel of the label. Provided that the flesh is firm and sound, some minor defects to the shell of such tails may be present. In every other respect, tails in catering packs shall comply with the quality, wrapping, packing and labelling requirements of this specification.

4.8 Shredded and comminuted lobster

Only the flesh from the carapaces of lobsters that were active at the time of tailbreaking shall be used. Unless the lobsters are properly washed immediately and stored in hygienic containers at a temperature not exceeding 4 °C, or in frozen condition while awaiting further processing or transportation in vehicles of hygienic construction to nearby processing plants, the recovery of the flesh from the carapaces shall follow immediately upon the breaking of the tails. The product shall have a characteristic colour, shall show no discoloration of any nature and shall be free from pieces of shell and other foreign matter. Lobsters belonging to different species shall not be used together in the manufacture of the product.

4.9 Overpacking of lobster tails, frozen raw whole lobster and frozen cooked whole lobster

Adequate provision for overpacking shall be made to compensate for loss of mass during frozen storage, transportation and distribution.

4.10 Chemical requirements

When tested in accordance with 9.1 to 9.7, the product shall comply with the relevant requirements of the Foodstuffs, Cosmetics and Disinfectants Act, 1972.

4.11 Microbiological requirements

When tested in accordance with the appropriate methods given in 10.6 to 10.14, the product shall comply with the requirements given in column 2 or column 3 of table 1, as relevant.

Table 1 — Microbiological requirements

1	2	3
Organism	**Contents,** max.	
	Raw products[1)]	**Cooked products**[2)]
Standard plate count	1×10^6/g	1×10^5/g
Enterobacteriaceae	[3)]	100/g
Faecal coliform bacteria	Nil/10 g	Nil/10 g
Staphylococcus aureus	10/g	10/g
Salmonella	Nil/25 g	Nil/25 g
Shigella	Nil/25 g	Nil/25 g
Clostridium perfringens	Nil/25 g	Nil/25 g
Vibrio cholerae	Nil/25 g	Nil/25 g
V. parahaemolyticus	Nil/25 g	Nil/25 g
Listeria monocytogenes	[3)]	Nil/25 g

1) Products that require cooking before being consumed.
2) Products that only require thawing and reheating before being consumed.
3) Not to be tested.

4.12 Antibiotics

Antibiotics shall not be used in the preparation of the product.

5 Packaging, glazing, freezing and storage

5.1 Packaging and wrapping materials and containers

5.1.1 Packaging and wrapping materials

Subject to the relevant requirements of the regulations to 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, inert and of low moisture-vapour permeability, and shall not contain substances deleterious to the product or harmful to health. Wrappers for tails shall bear a true description of the product. Any description that appears on such a wrapper shall not conflict with the requirements of clause 6. Wrappers shall be of such size as to cover the tails adequately, thereby ensuring that, when frozen, the tails do not stick to one another and are protected from freezer burn. 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.

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 containers shall be used. The 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 substances injurious to the product or harmful to health. Outer containers shall be so securely closed as to prevent contamination of the contents by dust or other foreign matter and shall be strong enough to protect the product adequately.

5.2 Glazing

The product may be glazed with 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 products in freezers (other than plate freezers) shall be away from floor and wall surfaces and shall be such that air circulation between packages is not impeded. The freezing capacity of every plant shall be capable of freezing the product to –5 °C or lower within 8 h of its being placed in the freezer. The freezing process shall start with the minimum of delay after preparation and packing. 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 3.2.15.3.)

5.4.2 The product shall be stored and maintained at a temperature of –20 °C or lower. If, at any time during storage, the temperature of the product rises above this temperature, it shall be rapidly lowered to –20 °C. If the temperature rises above –7 °C, the product shall, in addition, be resubmitted for inspection to the authority administering this specification. Products that, before dispatch for sale, are held for periods exceeding those specified in table 2 shall, prior to preparation for dispatch, be resubmitted for inspection to the authority administering this specification.

Table 2 — Maximum storage time for products at specified temperatures without reinspection

1	2	3
Product	**Storage temperature**	
	–20 °C	–25 °C
	Maximum storage time without reinspection months	
Whole lobster packed in gauze	6	8
Whole lobster packed in blocks of ice	12	18
Whole lobster packed in plastics	9	12
Lobster tails wrapped in plastics	12	18

5.5 Condition of frozen product

5.5.1 General

On thawing, the frozen product shall be clean, shall have an attractive, characteristic appearance, shall in every way be sound, intact, and free from defects, and the flesh shall have a springy texture. There shall be no off-odours and other indications of deterioration of the product, or of the use of inferior quality raw materials. The product shall be free from foreign matter, foreign odours, discoloration and "freezer burn" (deep dehydration). There shall be no evidence of an inadequate freezing process or of deterioration. The flavour of the cooked product, whether the product was packed raw or cooked, shall be normal and typical of the species. The texture of the cooked product, whether the product was packed raw or cooked, shall be succulent, firm and springy and otherwise characteristic of the species.

5.5.2 Frozen lobster tails

The shell of lobster tails shall be intact. The telsons (tail fins) shall not be broken or damaged. The colour of the flesh shall be uniform (either pinkish or translucent) and there shall be no loss or change in colour and no discoloration. Gut (anal canal) shall not be present.

5.5.3 Frozen whole lobster (raw, cooked or packed in ice)

Broken legs shall be virtually absent but, when present, shall be packed with the product. Exuded body fluids and spilled feed shall not be perceptible. The shell of lobsters shall be intact. The telson (tail fin) shall not be broken or damaged. The units shall not be distorted or twisted. Frozen whole lobster shall have been adequately purged (see 4.5.1). The units shall be free from discoloration.

6 Marking

6.1 Marking on containers that are not for export (see 6.2)

Except as allowed for in terms of 6.2, the following information shall appear on the outside of each container, in legible and indelible marking in a type face of such size and presentation as prescribed by 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) the appropriate of the following product names, all words being given in bold letters of equal size, except that the word "unpurged" or its equivalent may be in letters of size at least one-third of that in which the product name appears:

1) in the case of frozen rock lobster tails prepared (and packed) from the Cape rock lobster *(Jasus lalandii)*, the inscription "South African rock lobster tails"; in the case of frozen lobster tails prepared (and packed) from South Coast lobster *(Palinurus gilchristi)*, the inscription "South African rock lobster tails, South Coast type"; in the case of frozen lobster tails prepared (and packed) from Natal lobster *(Palinurus delagoae)*, the inscription "Natal rock lobster tails"; in the case of frozen lobster tails prepared (and packed) from slipper lobster (Scyllaridae), the inscription "Slipper lobster tails"; and in the case of frozen lobster tails prepared (and packed) from any other species of lobster, a true and appropriate description that will not mislead the consumer;

2) in the case of frozen raw whole Cape rock lobster *(Jasus lalandii)*, the inscription "Raw whole South African rock lobster"; in the case of frozen raw whole South Coast lobster *(Palinurus gilchristi)*, the inscription "Raw whole South African rock lobster, South Coast type"; in the case of frozen raw whole Natal lobster *(Palinurus delagoae)*, the inscription "Raw whole Natal rock lobster"; in the case of frozen raw whole slipper lobster (Scyllaridae), the inscription "Raw whole slipper lobster"; in the case of frozen raw whole lobster of any other species, a true and appropriate description that will not mislead the consumer; and in the case of frozen raw whole lobster packed unpurged in terms of the allowance made in 4.5.1, the word "unpurged" or its equivalent, given in immediate conjunction with the product name;

3) in the case of frozen cooked whole rock lobster prepared (and packed) from the Cape rock lobster *(Jasus lalandii)*, the inscription "Cooked whole South African rock lobster"; in the case of frozen cooked whole lobster prepared (and packed) from South Coast lobster *(Palinurus gilchristi)*, the inscription "Cooked whole South African rock lobster, South Coast type"; in the case of frozen cooked whole lobster prepared (and packed) from Natal lobster *(Palinurus delagoae)*, the inscription "Cooked whole Natal rock lobster"; in the case of frozen cooked whole lobster prepared (and packed) from slipper lobster (Scyllaridae), the inscription "Cooked whole slipper lobster"; in the case of frozen cooked whole lobster prepared (and packed) from any other species of lobster, a true and appropriate description that will not mislead the consumer; and in the case of frozen cooked whole lobster packed unpurged in terms of the allowance made in 4.5.1, the word "unpurged" or its equivalent, given in immediate conjunction with the product name; and

4) in the case of a frozen rock lobster product other than frozen rock lobster tails, frozen raw whole rock lobster and frozen cooked whole rock lobster, a true and appropriate description of the product, including the name of the product and the presentation of the contents;

c) 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 such code is disclosed to the authority administering this specification (the code may also identify the quota holder on whose behalf the product was packed);

d) 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);

e) in the case of tails other than those in catering packs, the category identification (see 4.7);

f) the country of origin;

g) where applicable, words indicating that the pack is a catering pack;

h) the presence of the anti-oxidant(s), by name, on the immediate containers, and where appropriate, on the master containers;

i) words stating clearly and legibly that the product shall be stored at a temperature of –20 °C or lower;

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

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

– Uncooked (or raw) – Keep frozen

– Partly cooked – Keep frozen. Do not refreeze once thawed

– Cooked – Keep frozen. Do not refreeze once thawed;

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

m) where relevant, directions for use; and

n) any labelling requirement specifically called for by regulation.

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 (k), 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(c) 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 that will not adversely effect the quality of the product.

7.2.2 Delivery for export

The frozen product for export shall be conveyed from the factory to the freezer storage depot and delivered into the transporting vessel's freezer storage facilities 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

The 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 legible from outside the refrigerated compartment.

7.3 Inspection for export

Each consignment of the frozen product intended for export shall be available for inspection at the freezer storage depot from which it is to be shipped. The authority administering this specification shall be notified at least 14 d before the expected date of shipment of the product. Products that do not comply with this specification shall not be kept in those freezer storage rooms from which export is effected, unless clearly identified. The frozen product shall be submitted for re-inspection at the point of shipment if, while the product was stored, whether at the original packing plant or at the point of shipment, or while the product was being transported to the point of shipment, any doubt arose as to the temperature history or the quality of the frozen product.

8 Methods of physical examination

8.1 Organoleptic examination

Examine the product for compliance with the requirements in 5.5 after thawing (see 8.1.1) and after cooking (see 8.1.2).

8.1.1 Procedure for thawing

Thaw the sample unit by enclosing it in a film-type pouch and immersing the pouch in water at room temperature (not exceeding 35 °C). Alternatively, the sample unit may be thawed by exposure to air at ambient temperature of 20 °C ± 5 °C. The complete thawing of the sample is determined by gently squeezing the bag occasionally (so as not to damage the texture of the lobster), until no hard core or ice crystals are left.

8.1.2 Cooking methods

8.1.2.1 Steaming procedure

Wrap the sample unit in aluminium foil and place it on a wire rack suspended over boiling water in a covered container until the internal core temperature of the sample unit is between 65 °C and 70 °C.

8.1.2.2 Boil-in-bag procedure

Place the sample unit into a boilable film-type pouch and seal the pouch. Immerse the pouch in boiling water until the internal core temperature of the sample unit is between 65 °C and 70 °C.

NOTE – The exact times and conditions of cooking or steaming of the sample should be determined by prior experimentation.

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

8.2.1 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.

8.2.2 Remove the packaging material. Wash, dry and determine the mass of the packaging material. Record the difference between the gross mass (see 8.2.1) and the mass of the packaging material as the net mass of the frozen product.

8.3 Determination of the net mass of a glazed product

8.3.1 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.

8.3.2 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 8.3.1) 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.

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

8.4 Determination of count

Examine the product for compliance with 4.7.5.

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 Ascorbic acid content

9.1.1 Reagents

9.1.1.1 Glass-distilled water

9.1.1.2 Metaphosphoric acid (HPO_3)/acetic acid extracting solution

Dissolve, with shaking, 15 g of HPO_3 pellets or freshly pulverized stick HPO_3 in 40 mℓ of glacial acetic acid and 200 mℓ of water and filter rapidly through a fluted paper into a glass bottle of capacity 500 mℓ. Immediately stopper the bottle, using a glass stopper. (HPO_3 slowly hydrolyses to orthophosphoric acid (H_3PO_4), but if stored in a refrigerator, the solution remains satisfactory for 7 d to 10 d.)

9.1.1.3 Ascorbic acid standard solution, 1 mg/mℓ

Accurately weigh out 50 mg of ascorbic acid that has been stored in a desiccator away from direct sunlight, transfer it quantitatively to a 50 mℓ volumetric flask and dilute to volume with water.

NOTE – Prepare this solution afresh immediately before each set of tests.

9.1.1.4 Indophenol standard solution

9.1.1.4.1 Dissolve 50 mg of the sodium salt of 2,6 dichlorophenol (indophenol), that has been stored in a desiccator over soda lime out of direct sunlight, in 50 mℓ of water containing 42 mg of sodium bicarbonate. Shake vigorously and, when the salt is dissolved, transfer quantitatively to a 200 mℓ volumetric flask and dilute to volume with water. Filter through a fluted paper into an amber glass bottle. Immediately stopper the bottle and store in a refrigerator.

NOTE – Decomposition products that make the end-point indistinct occur in some batches of dry indophenol and can also develop with time in the above standard solution. Test the indophenol solution immediately after preparation and at weekly intervals, as follows: add 5,0 mℓ of the extracting solution containing excess ascorbic acid to 15 mℓ of the indophenol standard solution. If the reduced solution is not virtually colourless, discard the old indophenol solution, prepare a fresh indophenol standard solution and retest. If the solid indophenol is at fault, obtain a new supply.

9.1.1.4.2 Transfer three 2,0 mℓ volumes of the ascorbic acid standard solution (see 9.1.1.3) to each of three 50 mℓ Erlenmeyer flasks each containing 5,0 mℓ of the extracting solution (see 9.1.1.2). Titrate rapidly with the indophenol standard solution from a 50 mℓ burette until a light but distinct rose-pink colour persists for at least 5 s. (Each titration usually requires approximately 15 mℓ of indophenol solution, and titres should agree to within 0,1 mℓ.) Similarly titrate three blanks, each consisting of 7,0 mℓ of the extracting solution plus a volume of water approximately equal to the volume of indophenol solution used in the titration of the ascorbic acid solution, and determine the average titre of the blank solutions (usually approximately 0,1 mℓ). Correct the standardization titres by subtracting the average blank titre from each of them and calculate the ascorbic acid equivalent, in milligrams, of 1,0 mℓ of the indophenol standard solution. Standardize the indophenol solution daily against freshly prepared ascorbic acid standard solution.

9.1.2 Preparation of test solution of the product

Shred the product and transfer an appropriate accurately determined mass to a blending machine. Add an appropriate volume of the extracting solution and mix gently until a uniform suspension is obtained. Dilute with the extracting solution to a definite volume V_2, in millilitres, and mix thoroughly.

9.1.3 Procedure

Titrate, with the indophenol standard solution, three aliquots of the test solution, each containing approximately 2 mg of ascorbic acid, and conduct three blank determinations as in 9.1.1.4.2.

NOTE – If the aliquots of test solution are of volume less than 7 mℓ, add, in each case before titration, enough of the extracting solution to raise the final volume to 7 mℓ.

9.1.4 Calculation

Calculate the ascorbic acid content, expressed in milligrams per kilogram of product, using the following formula:

$$(V - V_1) \times \frac{m}{m_1} \times \frac{V_2}{V_3} \times 1000$$

where

V is the average sample titre, in millilitres;

V_1 is the average blank titre, in millilitres;

V_2 is the volume of the test solution (see 9.1.2), in millilitres;

V_3 is the volume of the aliquot of test solution titrated, in millilitres;

m is the mass, of ascorbic acid equivalent to 1,0 mℓ of indophenol standard solution, in milligrams; and

m_1 is the mass of the product in volume V_2 of the test solution, in grams.

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, 1N, 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**: 2,0 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**: 5,0 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**: 3,0 µg Zn/mℓ.

9.2.2.5 Cadmium standard solutions, as follows:

a) **stock standard solution**: 1,5 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 12,5 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 2,0 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 4 mg/kg.

9.2.3.4 Determination of copper

Dilute 10,0 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 5,0 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 50 mg/kg.

9.2.3.5 Determination of zinc

Dilute 1,0 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 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 300 mg/kg.

9.2.3.6 Determination of cadmium

Determine the absorbance of the sample solution, of the reagent blank and of the 1,5 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 standard working solution, the cadmium in the sample is less than 3,0 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 of the nitric acid 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.

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 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 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.

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 aqueous solution of potassium iodide.

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.

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

9.5.1.3 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.

9.5.1.4 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 heating, and diluted to approximately 50 mℓ with water. Remove trace amounts of mercury by bubbling nitrogen through the solution for 10 min.

Dimensions in millimetres

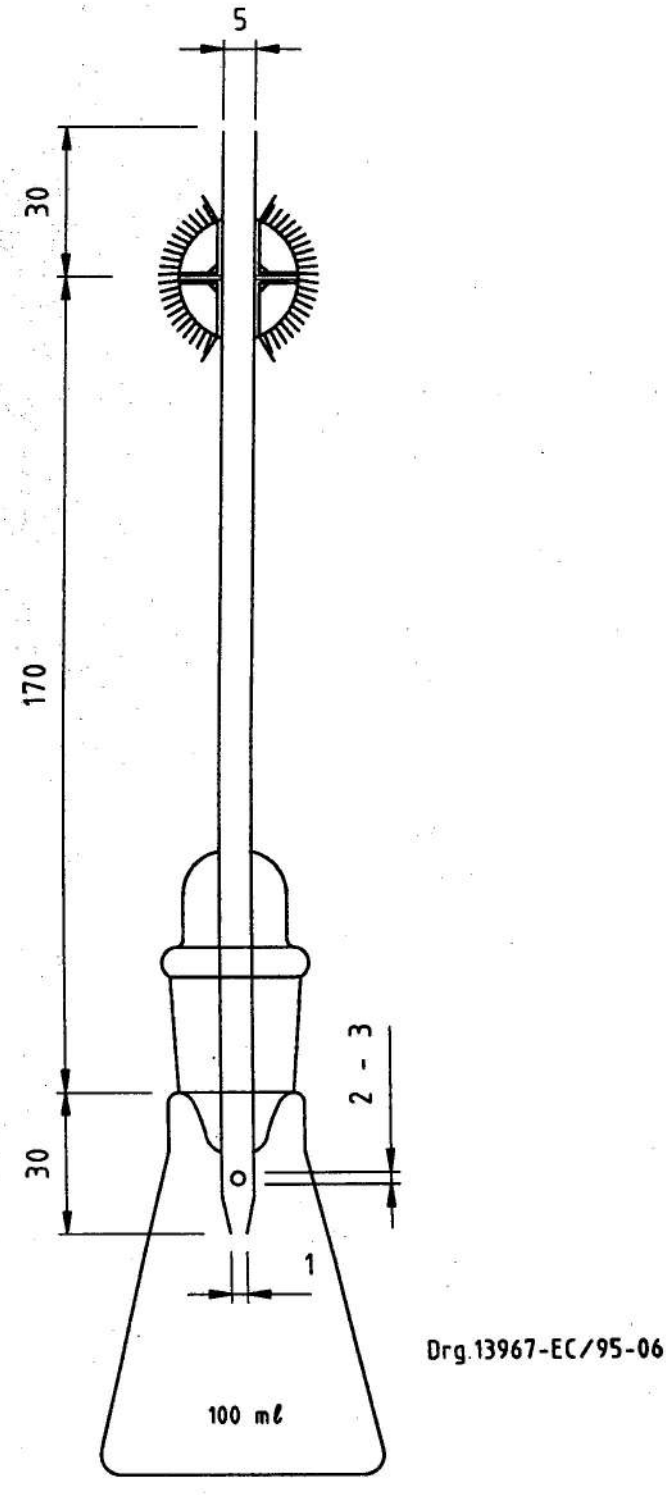
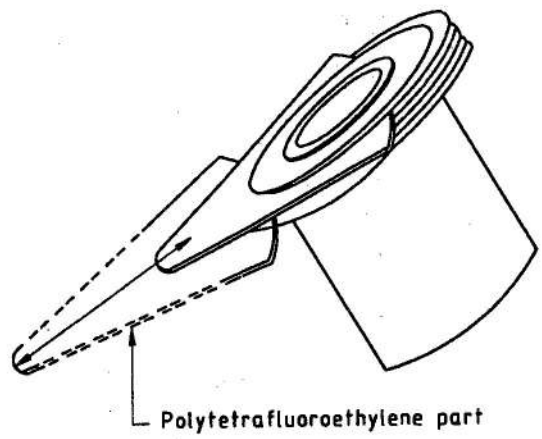


Figure 1 — Apparatus for limit test for arsenic



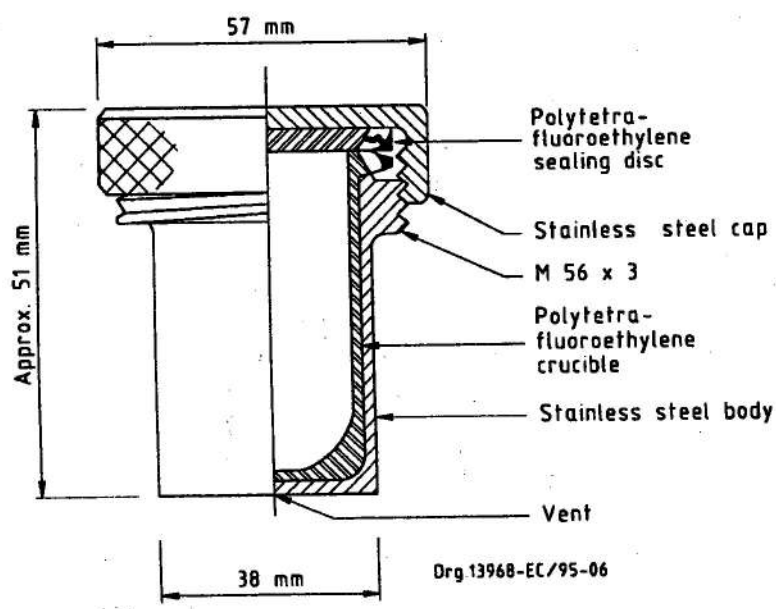


Figure 2 — Digestion vessel

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 9.1.2) (**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 9.5.1.3), 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 2,0 mℓ of the 0,1 µg Hg/mℓ working standard solution to the reaction flask, add 18,0 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 0,2 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 1,0 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.)

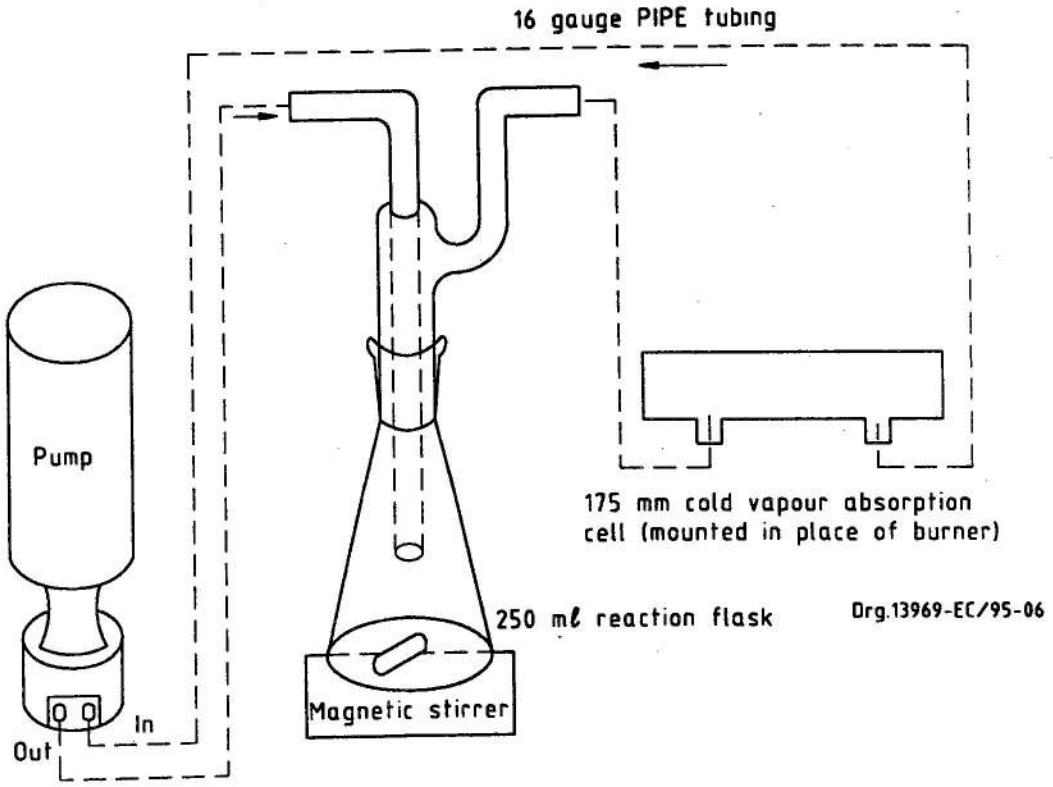


Figure 3 — Apparatus for the determination of mercury content

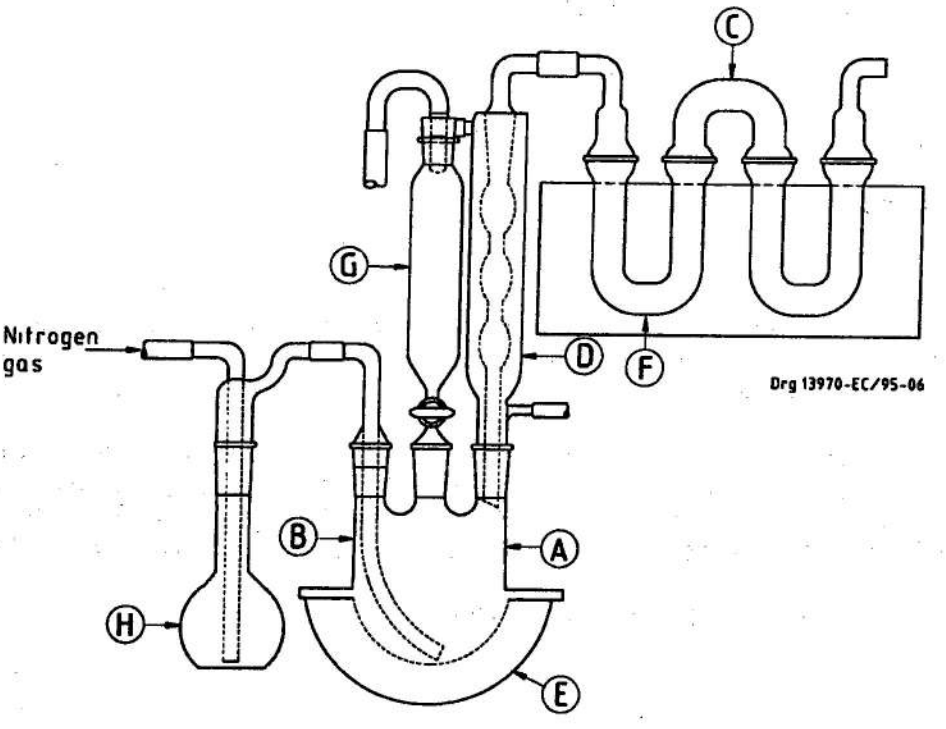


Figure 4 — Apparatus for the determination of sulfur dioxide content

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 Procedure

9.6.3.1 Reagent blank

Prepare a reagent blank.

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

9.6.3.3 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 sulfur dioxide content

9.7.1 Apparatus

9.7.1.1 Distillation apparatus (see figure 4), that consists of a round-bottomed distillation flask (A) of capacity 1 ℓ, that has three parallel necks. A 100 mℓ dropping funnel (G) is fitted into the centre neck and a nitrogen delivery tube (B) passes through one of the side necks to below the level of the liquid in the distillation flask (A). The other side neck is connected to the bottom-end socket of a vertically mounted double-surfaced condenser (D). Connected to the top-end cone of the condenser, there is a set of two U-tubes (F) of 20 mm tubing, connected with a cross-over tube (C).

9.7.1.2 Nitrogen scrubber (see figure 4), that consists of a 250 mℓ gas wash bottle (H) connected by means of silicone tubing to the inlet of the nitrogen delivery tube (B). Both inlet and outlet tubes of the wash bottle are clamped off.

9.7.1.3 Heating mantle, as shown by (E) in figure 4.

9.7.2 Reagents

9.7.2.1 Nitrogen gas.

9.7.2.2 Hydrochloric acid, diluted to one-half of the concentrated strength.

9.7.2.3 Hydrogen peroxide, a 3 % (by volume) solution neutralized to methyl red.

9.7.2.4 Standard sodium hydroxide solution (c(NaOH) = 0,1 mol/ℓ).

9.7.2.5 Pyrogallol/potassium hydroxide (KOH) solution, 65 g of potassium hydroxide dissolved in 85 mℓ of distilled water. Grind 4,5 g of pyrogallol with 5 mℓ of water in a small mortar and transfer to the cooled KOH solution. Repeat the grinding and transfer with two further 5 mℓ volumes of water.

9.7.2.6 Methyl red indicator, 0,25 g of methyl red dissolved in 100 mℓ of ethanol.

9.7.3 Procedure

Transfer the pyrogallol/potassium hydroxide solution into the gas wash bottle (H). Introduce 15 mℓ of the hydrogen peroxide solution to each of the U-tubes (F). Accurately weigh 200 g of the test sample solution, prepared as described in 9.2.3, and transfer this test specimen together with approximately 300 mℓ of water, through the centre neck into the distillation flask (A) and fit the dropping funnel (G). Add 30 mℓ of the hydrochloric acid through the funnel into the distillation flask (A) and close the funnel's stopcock.

Start the nitrogen gas flow at a slow steady stream of bubbles. So heat the distillation flask as to cause refluxing within 20 min to 25 min. Reflux steadily for 1,5 h.

Turn off the water in the condenser (D) and continue heating until the inlet joint of the first U-tube shows condensation and slight warming. Disconnect the condenser and turn off the heat.

When the joint at the top of the condenser cools, remove the cross-over tube (C) and rinse it into the second U-tube. Attach the cross-over tube (C) to the exit joint of the first U-tube and rotate until the open ends touch. Add a drop of the methyl red indicator, and titrate with the standard sodium hydroxide solution while mixing by gentle rocking just until a clear yellow colour occurs. Titrate the second U-tube similarly.

Record the total volume of sodium hydroxide needed for the titration (1 mℓ of a 0,1 mol/ℓ NaOH = 3,203 mg of sulfur dioxide).

9.7.4 Calculation

Calculate the sulfur dioxide content, expressed in milligrams per kilogram of product, using the following formula:

$$\frac{V \times 1000 \times 3,2}{m}$$

where

V is the volume of the standard sodium hydroxide solution used in the titration, in millilitres; and

m is the mass of the test specimen, 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 less than 6×10^{-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 × 160 mm, and

b) 20 mm × 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 × 15 mm,

b) 100 mm × 20 mm, and

c) 150 mm × 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, 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 % and

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 the 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 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 agar)

10.4.4.1 Ingredients

Agar	12 g
Lactose	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 each 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 30 mℓ sterile bottles (see 10.2.2(a)) 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-Vassiliadis 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 using 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. 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 Preparation

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
Water	1 000 mℓ

10.4.33.2 Preparation

Suspend the ingredients in the water. Adjust the pH value 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

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.

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

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

3) Depending on the gel strength of the agar.

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 Preparation

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.

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

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4) Depending on the gel strength of the agar.

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.

10.4.37.2 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
Triptone	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.

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
Triptone	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
Casitone	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 laurel 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 Laurel 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 laurel 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 heating. Add the sodium laurel 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 Laurel 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 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 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 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 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 (see 10.4.50.1) add 0,1 mℓ portions of acriflavine solution (see 10.4.50.2) and ferric ammonium citrate 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 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

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.

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6) Or other peptones of equivalent quality.

7) Depending on the gel strength of the agar.

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 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 g to 18,0 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, 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.

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8) Depending on the gel strength of the agar.

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[8]	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

Disolve 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.

10.4.56 Carbohydrate utilization broth

10.4.56.1 Base

10.4.56.1.1 Ingredients

Proteose peptone	10,0 g
Meat 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.

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8) Depending on the gel strength of the agar.

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 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 calculate the average number of enterobacteriaceae organisms 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 in a water-bath at 44 °C ± 0,25 °C for 16 h to 20 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. 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 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 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 dilution.

Carefully spread the inoculum by means of individual sterile glass spreaders (see 10.3.5) over the surface of each of the eight plates, with as little time delay as possible, trying not to touch the sides of the dish. Allow the plates to dry with their lids on for approximately 15 min at ambient temperature. Invert the plates and incubate them 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 only 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 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 developing 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 that contain 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 the first 18 h to 24 h of the 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 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 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 the lesser of five colonies of each type of suspected *Salmonella* organism on each plate, or all such colonies.

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 the 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 triptone 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 the 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.

Colourless transparent 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	0,0
Voges-Proskauer reaction	30,6
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[9]. 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 at least 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.

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

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

10.13.1 Enrichment

Within 30 min of preparing 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

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 powerful enough to illuminate the plates well and striking the bottom of the plate at an angle of 45°. When examined in this obliquely transmitted light direct 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 (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)[10] 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*)[11]; *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.

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10) Report whether sheep or horse blood was used.

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

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/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 it solidifies. Incubate the plates at 35 °C for 18 h. After incubation, examine the plates and regard the 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 10.14.1.1, 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 whole 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. 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.

10.15.3.1.4 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.

10.15.3.2 Expression of efficacy of cleaning and disinfecting

Allocate one of the symbols S, FS or US to each sample, 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)

10.15.3.3 Calculation

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

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

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 rapidly 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 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 coliforms, 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. 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 coliforms. Non-faecal coliform colonies are grey to cream coloured.

To confirm the presence of faecal coliforms, 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 triptone water (see 10.4.7) and then incubate these subcultures at 44,5 °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 triptone 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 rapidly 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 laurel tryptose broth

Use a sterile pipette to transfer 10 mℓ of the water sample to each of three tubes or bottles that contain double-strength laurel 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 laurel 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 laurel 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. 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 laurel tryptose broth that gives a positive result into a tube or bottle of single-strength brilliant green bile broth (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 laurel tryptose broth that gives a positive result for gas formation, into a bottle of triptone 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 triptone 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.

10.16.2.4 Expression of results

10.16.2.4.1 From the number of tubes or bottles of laurel tryptose broth and confirmatory tests that give positive reactions, calculate, by reference to table 3, 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) yielding 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 give 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 3, 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 3, 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 3.

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 3, the MPN index is 15 and the calculation gives an MPN of $\frac{15}{10} \times 1 \times 100$, i.e. $1,5 \times 10^2$ 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 3, the MPN index is 15 and the calculation gives an MPN of $15 \times 10 \times 100$, i.e. $1,5 \times 10^4$ bacteria per 100 mℓ of water sample.

Example C

Test sample (dilution 10^0) (10 mℓ)	:	2	tubes + ; 1 tube –
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 3, the MPN index is 1,5 and the calculation gives an MPN of $\frac{1,5}{10} \times 1 \times 100$, 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 3, the MPN index is 0,3 and the calculation gives an MPN of $\frac{0,3}{10} \times 1 \times 100$, i.e. 3×10^0 bacteria per 100 mℓ of water sample.

Table 3 — 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 %	≤ 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	1,00
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. 531 **14 Mei 1999**

WET OP STANDAARDE, 1993

VERPLIGTE SPESIFIKASIE VIR BEVRORE KREEF EN BEVRORE KREEFPRODUKTE

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 kreef en bevrore kreefprodukte 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 kreefprodukte, gepubliseer by Goewermentskennisgewing No R.3964 van 19 Desember 1969.

ALEXANDER ERWIN
Minister van Handel en Nywerheid

BYLAE

VERPLIGTE SPESIFIKASIE VIR BEVRORE KREEF EN BEVRORE KREEFPRODUKTE

1 Bestek

Hierdie spesifikasie dek die vereistes vir die hantering, voorbereiding, verwerking, verpakking, bevriesing, bewaring en kwaliteit van bevrore kreefsterte, heel bevrore kreef (gaar of rou) of ander bevrore kreefprodukte van die Palinuridae- en Scyllaridae-familie, en van die Nephropsidae-familie (genera *Homarus, Nephrops* en *Metanephrops*, of enige ander kreefspesie), bedoel vir menslike verbruik. Dit dek ook vereistes vir fabrieke en werknemers wat by die vervaardiging 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 buitehouer (hoofhouer): Die doos, kartonhouer of kis waarin pakkette (toegedraaide of ontoegedraaide) bevrore kreefprodukte vir bewaring en verspreiding verpak word.

2.3 bevrore heel gaar kreef: Heel gaar kreef wat 'n vriesproses ondergaan het en deur bewaring in die bevrore toestand gepreserveer is.

2.4 bevrore heel rou kreef: Heel rou kreef wat 'n vriesproses ondergaan het en deur bewaring in die bevrore toestand gepreserveer is.

2.5 bevrore kreefsterte: Kreefsterte wat 'n vriesproses ondergaan het en deur bewaring in die bevrore toestand gepreserveer is.

2.6 bevrore kreef vir spysenieringsdoeleindes (spysenierspakke): Verpakte en bevrore kreef of kreefsterte waarvan sommige effens beskadig kan wees maar wat almal van aanneemlike kwaliteit en op alle maniere vir menslike verbruik geskik is. Die produk kan gegradeer of ongegradeer aangebied word.

2.7 bevrore produk: 'n Produk (kyk 2.17) wat deur bewaring in die bevrore toestand gepreserveer is.

2.8 fabriek: 'n Perseel waar die produk (kyk 2.17) voorberei of verwerk word, of albei, met inbegrip, in die mate waarin die vereistes van hierdie spesifikasie toegepas kan word, 'n fabriekskip waarop die produk na voorbereiding en verwerking bevries word.

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

2.10 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 bestand is teen blootstelling aan herhaalde skoonmaak, met inbegrip van die gebruik van detergente.

2.11 gesnipperde of fyn kreef: Kreefvleis waarvan die oorspronklike spierstruktuur tydens die proses van herwinning van die vleis uit die dop gebreek is.

2.12 inwendige suiwering: Om lewendige kreef minstens 72 h lank in skoon, lopende seewater te hou om die ingewande skoon (leeg) te maak.

2.13 kreefproduk: 'n Kommoditeit wat van kreef, met of sonder ander bestanddele, gemaak is en vir menslike verbruik bedoel is.

2.14 neweproduk: 'n Produk wat nie vir menslike verbruik bedoel is nie.

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

2.16 preserveer: Om in 'n gebrekvry, eetbare toestand te hou deur die voorkoming van agteruitgang.

2.17 produk: Heel kreef (gaar of rou) of kreefsterte of ander kreefprodukte vir menslike verbruik, in die proses van vervoer, hantering, voorbereiding, verwerking of verpakking vir bevriesing, in die proses van bevriesing, of na bevriesing, soos deur die konteks van die spesifikasie aangedui word.

2.18 produksie: Die hantering, voorbereiding, verwerking of verpakking vir bevriesing, in die proses van bevriesing, of na bevriesing, met inbegrip van die proses van vriesbewaring, soos deur die konteks van die spesifikasie aangedui word.

2.19 sagtedopkreef: Kreef in die vroeë naverdoptyd, wat aangedui word deur die aanwesigheid van 'n sagte, nuwe dop (eksoskelet) na die afwerp van die ou dop en waarin

- die deel van die karapaks (brangiostegiet) wat die kieue bedek nog sag (ongekalsifiseer) is en maklik deur fisiese hantering geskeur of beskadig of min of meer permanent ingedruk kan word, en
- die vleis se waterinhoud só hoog is dat dit nie die kenmerkende elastisiteit van kreefvleis het wanneer dit in 'n ongeskonde toestand is nie en pap of krummelrig word wanneer dit gaargemaak word.

2.20 verkilkamer: 'n Geïsoleerde en verkoelde vertrek wat spesiaal vir die bewaring van voedsel by temperature van laagstens –1 °C en hoogstens 4 °C ontwerp is, met voldoende verkoelvermoë om die verlangde bewaartemperatuur te handhaaf en wat ook voldoende verkoelvermoë kan hê om produkte wat in die verkilkamer geplaas word tot by daardie temperatuur af te koel.

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

2.21 vrieskamer: 'n Vertrek of toerusting wat spesiaal ontwerp is om die temperatuur van 'n voedselproduk deur die sone van maksimum kristallisasie (vir die meeste produkte tussen –1 °C en –5 °C) en laer tot by 'n ewewigstemperatuur van –20 °C of laer te verlaag binne 'n tydperk wat vir die produk aanneemlik is.

2.22 vriesbewaarkamer: 'n Geïsoleerde vrieskamer wat spesiaal ontwerp is vir die bewaring van bevrore voedsel en met voldoende vriesvermoë om 'n produktemperatuur van –20 °C of laer te handhaaf as produkte wat reeds tot by daardie temperatuur gevries is, bewaar word.

OPM – 'n Vriesbewaarkamer is nie vir die bevriesing van produkte ontwerp nie.

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

3 Vereistes vir die fabriek

3.1 Algemeen

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

3.2 Konstruksie en uitleg van toestande in fabriek

3.2.1 Ligging, grootte, higiëniese ontwerp en toestande

3.2.1.1 Die ligging van die fabrieksgeboue moet sodanig wees dat die geboue aanneemlik vry van aanstootlike reuke, rook, stof en ander kontaminasie gehou kan word ten einde aan die toepaslike vereistes ten opsigte van higiëne en sanitasie van die Wet op Gesondheid, 1977, te voldoen. Die fabrieksgeboue moet 'n stewige konstruksie hê, in 'n goeie toestand wees en moet groot genoeg wees om saamdringing van toerusting en werknemers te voorkom 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, asook 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 buite geboue en wat nie werklik 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 geboue moet só ontwerp en gebou wees dat daar voorkom word dat insekte, voëls, knaagdiere en ander ongediertes daar inkom of gehuisves word.

3.2.2 Dakke en plafonne

3.2.2.1 Dakke moet weerbestand wees en moet van nie-absorberende materiaal 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 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 die dak indien daar nie 'n plafon is nie) stofdig wees en beklee wees met 'n geskikte korrosiebestande, ligkleurige en ondeurlatende materiaal wat só gemaak en afgewerk is dat kondensasie, skimmelgroei, afskilfering en die 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 weerbestand wees en ondeurlatend vir water wees. Binneoppervlakke van mure moet met 'n gladde, ligkleurige, wasbare materiaal beklee wees wat waterdig en vry van onnodige uitsteeksels is. Daarbenewens moet die mure in die voorbereidings-, verwerkings- en verpakkingsgebiede tot op 'n hoogte van 2 m bo die vloer met 'n geskikte korrosiebestande, ligkleurige, wasbare en slagbestande materiaal beklee wees, behalwe dat, indien die mure bokant 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 loop. Die lyste moet so klein moontlik wees en vensterbanke moet minstens 1 m bo die vloer wees. In die voorbereidings-, verwerkings- en verpakkingsgebiede en in vrieskamers, verkilkamers en vriesbewaarkamers moet die muur-by-muur- en muur-by-vloeraansluitings met 'n minimum radius van onderskeidelik 25 mm en 40 mm hol afgewerk wees.

3.2.3.2 Deure en deurrame moet beklee wees met of gemaak wees van 'n geskikte korrosiebestande materiaal 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 wyd genoeg wees om kontaminasie van die produk en beskadiging van die deure te voorkom. Alle deure wat van buite af direk in die voorbereidings-, verwerkings- en verpakkingsgebiede oopmaak, moet doeltreffende lugskerms hê of moet sover moontlik self toegaan en dig sluit. Vries-, verkil- en vriesbewaarkamerdeure moet dig sluit.

3.2.4 Vloere

3.2.4.1 Vloere moet gemaak wees van beton of van 'n ander materiaal wat paslik ondeurlatend en korrosiebestand is en maklik skoongemaak kan word, met 'n gelyk oppervlak wat glad maar nie glyerig is nie en wat vry van barste en oop voeë is.

3.2.4.2 Die vloere van die voorbereidings-, verwerkings- en verpakkingsgebiede en van vrieskamers, verkilkamers 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 inkom.

3.2.4.3 Afvoerkanale moet van die oop tipe met, indien nodig, verwyderbare deksels wees en moet ontwerp wees om die maksimum verwagte vloei van vloeistof te hanteer sonder om oor te loop of oorstroming te veroorsaak. Daar mag geen installasie in 'n afvoerkanaal wees wat die vloei van water of skoonmaakwerk belemmer nie. Rioolputsperders moet met maklik verwyderbare siwwe toegerus word. Indien nodig, moet plankmatte van materiaal wat maklik skoongemaak kan word en ondeurlatend is, 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 hulle nie onhigiëniese toestande bevorder nie.

3.2.5.2 Die openinge tussen die treevlakke van trappe in vertrekke waar die produk voorberei, verwerk, verpak en gehanteer word, moet met soliede stygstukke toegemaak wees. Trappe moet soliede relings hê wat hoog genoeg is dat kontaminasie van produkte onder die trappe voorkom 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 die plafonne in voorbereidings-, verwerkings- of verpakkingsgebiede geïnstalleer word nie en mag ook nie op só 'n wyse geïnstalleer word dat toevallige lekkasies die produk kan kontamineer nie. Die afvoer- en rioolpype moet 'n binnediameter van minstens 100 mm hê en moet behoorlik na die buiteatmosfeer ontlug wees.

3.2.7 Verligting

Verligting van minstens 220 lx in algemene werkgebiede en minstens 540 lx by plekke waar die produk noukeurig ondersoek word, moet voorsien word en moet sodanig wees dat dit nie die voorkoms van die kleur van die produk beduidend verander nie. Armature wat hang oor die werkgebiede waar die produk op enige stadium tydens voorbereiding, verwerking of verpakking gehanteer word, moet van die veiligheidstipe wees of andersins só beskerm wees dat kontaminasie van die produk voorkom word indien 'n armatuur of lamp sou breek.

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 lugvloei moet van die meer higiëniese na die minder higiëniese gebiede wees. 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 verwyder kan word en moet van geskikte korrosiebestande materiaal gemaak 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, en op ander gerieflik geleë plekke in die voorbereidings- en verwerkingsgebiede van die fabriek binne maklike bereik van die werknemers, en by die uitgange van toilette:

a) 'n aanneemlike getal handwasbakke met volop warm en koue water of warm lopende water in die temperatuurbestek van 40 °C tot 50 °C en wat aan die vereistes van 3.4.1 voldoen;

b) volop ongeparfumeerde vloeibare seep of aanneemlike detergent in aktiewe toestand;

c) weggooipapierhanddoeke; en

d) krane wat nie met die hande of elmboë beheer word nie, byvoorbeeld krane wat met die knieë of voete beheer word of drukknopkrane met vooraf gestelde volumebeheer.

3.2.9.2 Handontsmettingsbakke, indien voorsien, moet sodanig 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 'n fabriekskip moet minstens een handwasbak in die toiletblok en een in die verwerkings- en verpakkingsgebied lopende warm en koue water hê.

3.2.10 Voetbaddens

Tensy die afwesigheid van voetbaddens in bepaalde omstandighede aanneemlik is of tensy 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 daardie gebiede kan verkry sonder om hul skoeisel te ontsmet nie. Voetbaddens moet só gemaak wees dat hulle voldoende gedreineer en skoongemaak kan word.

3.2.11 Kennisgewings

Kennisgewings moet strategies 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 moet aandui dat spoeg, die gebruik van kougom en enige vorm van tabak en die eet van verversings in daardie gebiede verbied word.

3.2.12 Skeiding van prosesse en fasiliteite

Die gebiede waar die rou produk en die gaar produk gehanteer word, moet fisies van mekaar geskei wees. Daar mag geen kruisvloei van werksaamhede met rou en gaar produkte wees nie.

Afsonderlike vertrekke of duidelik afgebakende gebiede van geskikte grootte moet voorsien word vir:

a) die ontvangs en bewaring van onverwerkte materiaal;

b) voorbereidingswerk soos die stertverwydering, verwydering van die anale kanaal en was van kreef;

c) verwerkingsaktiwiteite soos 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 enige ander bron van kontaminasie wees en moet 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 wat gesluit gehou moet word. Alle gevaarlike materiaal moet opvallend en duidelik geëtiketteer wees en mag nooit met 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 aanwending daarvan moet bewaar word in 'n vertrek waarin geen voedsel, voedselhanteertoerusting, verpakkingsmateriaal of voedselhouers bewaar word nie en mag nooit met voedselhouers, verpakkingsmateriaal, onverwerkte materiaal of die produk in aanraking kom nie. Alle skoonmaak- en ontsmettingsmateriaal moet opvallend en duidelik geëtiketteer wees.

3.2.14 Bewaarfasiliteite vir gereedskap en onderdele

Gereedskap en onderdele wat met die produk in aanraking kom wanneer dit in gebruik is, moet, wanneer dit nie in gebruik is nie, in 'n ontsmettingsoplossing gehou word of op 'n higiëniese wyse bewaar word in 'n droë gebied wat vry van stof en ander bronne van kontaminasie is en plaagdig is. Onderdele vir masjinerie wat die produk kan kontamineer, moet in 'n afsonderlike bewaargebied weg van die verwerkingsgebiede gehou word.

3.2.15 Vrieskamers, verkilkamers en vriesbewaarkamers

3.2.15.1 Verkoelingseenhede, soos kompressors, mag nie geïnstalleer word in 'n gebied waar die produk gehanteer word nie tensy die toerusting 'n integrerende deel van 'n produksie-eenheid is. Indien vrieskamers, verkilkamers en vriesbewaarkamers in verwerkingsgebiede geleë is, moet hul vloere óf 'n integrerende deel van die vloer van die verwerkingsgebied wees óf toereikend aan dié vloer verseël wees. Bewaareenhede moet hoog genoeg bokant die vloer geïnstalleer wees sodat die gebied daaronder maklik en toereikend skoongemaak kan word.

3.2.15.2 Die mure en vloere moet in 'n goeie toestand wees. Die oppervlakke van plafonne, mure en vloere moet van geskikte korrosiebestande materiaal wees, moet waterdig 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.

3.2.15.3 Vriesbewaarkamers moet toegerus wees met outomatiese temperatuurregistreerders 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 tempe-

ratuur moet gehou word en ter insae beskikbaar wees. Temperatuurkaarte moet só ingedeel wees dat elke indeling hoogstens 2 °C in die bewaarbestek verteenwoordig, en moet maklik tot die naaste 1 °C in die bewaarbestek gelees kan word. Produksielotvrieskamers, uitgesonderd plaatbevriesers, moet eksterne meters of ander temperatuuraanwysers hê.

3.2.15.4 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.

3.2.16 Neweprodukte

Die verwerking van neweprodukte 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 bestaan nie.

3.2.17 Woonkwartiere

Woonkwartiere moet heeltemal geskei wees van gebiede waar die produk voorberei, verwerk, verpak of bewaar word.

3.2.18 Vullis

'n Afsonderlike, geskikte vullisfasiliteit moet op die perseel verskaf word en daagliks skoongemaak word.

3.2.19 Fasiliteite

3.2.19.1 '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 die kleedkamers uitgaan. Fasiliteite mag nie direk in 'n voorbereidings-, verwerkings-, verpakkings- of bewaargebied uitgaan nie.

3.2.19.2 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 die kleedkamers geskei is. 'n Voldoende voorraad toiletpapier, lopende warm en koue water, naelborsels, ongeparfumeerde vloeibare seep of 'n aanneemlike detergentoplossing en weggooi-papierhanddoeke moet vir die werknemers beskikbaar wees. Houers moet vir gebruikte handdoeke voorsien word. Vullishouers met 'n higiëniese konstruksie moet voorsien word.

3.2.19.3 Daar moet kennisgewings wees wat vereis dat werknemers hul hande met seep of detergent was nadat hulle die toilet gebruik het. Sluitkaste of beheerde kleremandjies moet verskaf 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 voorbereiding van maaltye gebruik word nie. Eetkamers vir personeel moet van die kleed- en aantrekkamers geskei wees.

3.2.20 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 of in 'n aangewese deel van die voorbereidings-, verwerkings- en verpakkingsgebiede wees waar daar genoeg koue drinkbare water en warm water, indien nodig, of versadigde stoom of skoon seewater is wat aan die vereistes van 3.4.2 voldoen.

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 instandhouding van higiëne en 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 'n geskikte korrosiebestande, nie-absorberende materiaal wees (dws nie van 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 gemaak 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 installasies en toerusting moet só ontwerp wees dat dit die skoonmaak en ontsmetting van die gebiede daaronder vergemaklik. Oop ente en omkrulrande moet bevredigend verseël wees om die aanpak van organiese materiaal en vuiligheid te voorkom. Indien nodig, soos in die geval van toerusting wat nie *in situ* skoongemaak kan word nie, moet dit moontlik wees om die toerusting vir skoonmaak en ontsmetting uitmekaar te haal. 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 weg van die mure en plafonne geïnstalleer word op 'n afstand wat toereikend is om toegang vir skoonmaak en ondersoek te verleen. 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 aanneemlike wyse geïnstalleer wees. Versonke dele moet goed gedreineer wees.

Koper, lood en hul legerings, 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 op 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. Tafelrame moet só ontwerp en gemaak wees dat die ontwikkeling van onhigiëniese toestande en die opbou van bakterieë verhoed word. Die rame moet van gladde, korrosiebestande metaal gemaak 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 van higiëniese konstruksie wees en moet vir skoonmaak verwyder kan word of moet só aan hul rame bevestig wees dat hulle skoongemaak en ontsmet kan word. Indien metaalblaaie by die rande gevou is, moet die voue sodanig gesoldeer, gesweis of met aanneemlike mastiekseëlmiddel verseël wees dat die akkumulasie van organiese materiaal en vuiligheid voorkom word. Alle tafelblaaie moet vinnig en doeltreffend gedreineer kan word en moet vry van barste en splete wees. Alle voeë in tafels moet waterdig gemaak wees.

3.3.3 Snyplanke

Indien snyplanke gebruik word, moet hulle van higiëniese konstruksie wees en van aanneemlike ligkleurige materiaal (uitgesonderd hout of ander absorberende of poreuse materiaal) gemaak wees wat vir gebruik met voedsel geskik is. Snyplanke moet maklik verwyder kan word.

3.3.4 Gereedskap

Messe, skoppe, besems en ander gereedskap mag nie handvatsels van hout of ander poreuse materiaal hê nie. Rottangmandjies mag op geen stadium voor, tydens of na verwerking as houers vir kreef 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 skoonmaak van die 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 produk of hul houers of pakkette 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 ysvervaardigingstoerusting moet deurgaans van 'n higiëniese konstruksie 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 Behoudens 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 kan wees. Daarbenewens moet die water sodanig deur middel van uitvlokking, filtrering, chlorering of 'n ander aanneemlike proses behandel wees om te verseker dat dit aan die volgende vereistes voldoen:

a) **koliforme organismes**: die telling vir koliforme organismes mag hoogstens vyf organismes per 100 mℓ van die water wees (kyk 10.16.1.1 of 10.16.2.3.1); en

b) **fekale koliforme bakterieë**: fekale koliforme bakterieë mag nie in 100 mℓ van die water waarneembaar wees nie (kyk 10.16.1.2 of 10.16.2.3.2).

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 en by 44 °C kan laat fermenteer en indool in triptoonwater kan voortbring.

3.4.1.3 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 middel van die *N,N*-diëtiel-1,4-ℓ-fenileendiamientoets of ander aanneemlike toets met ekwivalente sensitiwiteit bepaal word.

3.4.1.4 Fabrieksinstallasies vir die behandeling van water moet minstens een maal 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 waargeneem kan word nie (kyk 10.16.2.3.2).

3.4.3 Water vir skoonmaak

Water wat vir die skoonmaak van die installasies en toerusting gebruik word, 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. Die vry residuele chloorkonsentrasie moet in alle gevalle deur die *N,N*-diëtiel-1,4-ℓ-fenileendiamientoets of ander aanneemlike toets met ekwivalente sensitiwiteit bepaal word.

3.4.4 Ys

Die suiwerheid van ys moet sodanig wees dat die water wat onmiddellik na vervaardiging daaruit verkry word (deur die ys in aseptiese toestande by 'n temperatuur van hoogstens 10 °C te smelt) aan die mikrobiologiese 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 veroorsaak het by die fabrieks-higiënebeampte aanmeld, wat, indien hy dit nodig ag, toepaslike stappe moet doen om 'n mediese mening oor die werknemer se werkgeskiktheid te verkry. 'n Toepaslike mediese rekord moet van elke werknemer gehou word.

3.5.1.2 Mediese sertifikate 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 'n 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. Sodanige werknemer moet hom onmiddellik by die fabrieksbestuur aanmeld.

3.5.1.4 Indien dit bekend is dat 'n werknemer ly aan 'n siekte wat deur voedsel oorgedra kan word, mag hy nie toegelaat word om in enige deel van die fabriek te werk in 'n hoedanigheid waar die 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 in aanraking met die produk te kom nie tensy die snywond of besering sodanig behandel of verbind is dat die afskeiding van liggaamsvloeistof verhoed word en die wond en sy verband sodanig bedek is dat infeksie of kontaminasie van die produk nie meer moontlik is nie.

3.5.2 Beskermende klere

3.5.2.1 Alle werknemers betrokke by die hantering, voorbereiding en verwerking van die produk tot en met die verpakkingstadium, maar uitgesonderd werknemers wat in vriesbewaar- en verkilkamers werk, moet skoon, ligkleurige, beskermende klere dra sowel as waterdigte voorskote, waterdigte skoenbedekkings of stewels en skoon, wasbare of weggooikopbedekkings wat die hare heeltemal bedek. Wolmusse mag slegs in vriesbewaarkamers gedra word. Oorpakke moet die persoonlike klere van die werknemers heeltemal bedek.

3.5.2.2 Moue mag nie tot onder die elmboë reik nie tensy dit met plastiekoortrekmoue bedek is of wanneer dit in vriesbewaar- en verkilkamers gedra word. Waterdigte beskermende klere moet van plastiek- of rubbermateriaal of 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 van die produk inhou 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 slegs van die perseel verwyder word om in higiëniese toestande gewas te word. Die blyplekke van werknemers word nie as aanneemlik vir dié doel beskou nie.

3.5.2.4 Waterdigte voorskote, oormoue en handskoene moet elke keer as dit verwyder word en so dikwels as wat nodig is, skoongemaak word 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 sowel as toerusting wat by die voorbereiding, verwerking en verpakking van die produk gebruik word, mag slegs uit 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, na elke afwesigheid uit die produksiegebied van die fabriek, met gereelde tussenposes tydens produksie, of te eniger tyd indien dit nodig is, hulle hande met warm water en aanneemlike ongeparfumeerde vloeibare seep of detergent was en in skoon lopende water afspoel. Hulle kan dan hul hande in 'n aanneemlike ontsmettingsoplossing doop en daarna in skoon, lopende water afspoel indien die gebruiksaanwysings op die handdoopmiddel dit vereis. Naellak of naelvernis mag nie op vingernaels gebruik word nie en vingernaels moet kort en skoon gehou word. Juweliersware mag nie gedra word deur werknemers wat onverwerkte materiaal of die onbeskermde produk, of albei, hanteer nie.

3.5.3.2 Werknemers se persoonlike besittings en voedsel mag in geen gebied kom waar die produk en sy bestanddele en verpakkingsmateriaal gehanteer of bewaar word nie. Houers wat by die voorbereiding, verwerking of verpakking van die produk gebruik word, mag vir geen ander doeleindes gebruik word nie.

3.5.3.3 Die gebruik van kougom of tabak in watter vorm ook al is verbode in gebiede waar die produk en die bestanddele en verpakkingsmateriaal daarvan gehanteer en bewaar word. Werknemers mag geen voedsel of drank in hierdie gebiede voorberei of eet 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 werknemers wat die produksie-, verwerkings- of verpakkingsgebiede van die fabriek tydens werkure besoek, moet in dié gebiede aan alle higiënevereistes voldoen en moet skoon beskermende klere dra wat die fabriek moet voorsien.

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 en mag geen toestande bestaan wat vir die produk nadelig is nie. Materiaal wat die produk moontlik kan kontamineer, moet van die verwerkingsgebiede weggehou word. Nie-eetbare materiaal mag nie in dieselfde vertrek as eetbare bestanddele of in die voorbereidings- of verwerkingsgebiede van die fabriek bewaar word nie.

3.6.1.2 Daar mag geen onhigiëniese toestande op die fabrieksperseel wees nie. Rook uit fabriek-skoorstene en uitlaatgasse mag nie 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 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 en 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. Installasies, toerusting en gereedskap moet voor gebruik 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 afgespoel word (kyk 3.4.3) 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 of vreemde materiaal wat van mure, plafonne of oorhoofse strukture op die produk kan val.

3.6.2.2 Vloere en afvoerkanale

Die vloere en die afvoerkanale in die voorbereidings-, verwerkings- en verpakkingsgebiede moet tydens werktydperke 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 die afvoerkanale of op roosters akkumuleer nie. Vloere en afvoerkanale moet so dikwels as wat nodig is en aan die einde van elke dag se werksaamhede deeglik skoongemaak word om 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 voorbereidings-, verwerkings- en verpakkingsgebiede moet, indien nodig, onmiddellik na elke dag se werksaamhede deeglik gewas word en die vertrekke moet so vry moontlik van stof 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 Skoonmaak van waterbehandelingsinstallasies

Fabrieksinstallasies vir die behandeling van water moet een keer per week deeglik volgens 'n aanneemlike metode skoongemaak word.

3.6.2.6 Skoonmaak van die verwerkingstelsel

Die hele verwerkingstelsel moet tydens elke produksie-onderbreking wat langer as 1 h duur, of wanneer dit ook al nodig geag word, skoongemaak word en dit moet aan die einde van elke skof en aan die einde 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, uitbreekpenne en soortgelyke toerusting moet tydens produksie-onderbrekings, na gebruik of te eniger tyd wanneer ontsmetting nodig is, deeglik skoongemaak word en dan met gechloreerde water of deur middel van 'n ander aanneemlike oplossing of 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 sodanig gedreineer word dat water nie opdam en stagneer nie. Sodanige stelsels moet gereeld skoongemaak word om ou materiaal te verwyder en moet voor en na gebruik skoongemaak word. Houtenks moet op soortgelyke wyse behandel word.

3.6.3 Herstelwerk

3.6.3.1 Wanneer instandhouding- of herstelwerk in produksiegebiede uitgevoer is, moet gereedskap en vervangde toerusting onmiddellik uit dié gebiede verwyder word en die betrokke toerusting moet deeglik skoongemaak en ontsmet word.

3.6.3.2 Sweisherstelwerk in gebiede waar die produk gehanteer, voorberei, verwerk of verpak word, mag nie tydens produksie gedoen word nie. Noodherstelwerk mag slegs by onklaarraking en op sodanige wyse gedoen 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 10.15.3.2 en 10.15.3.3 toegeken word.

3.6.5 Houers, bakke en kratte vir die hantering van onverwerkte materiaal en die produk

Houers wat gedeeltelik of heeltemal met onverwerkte materiaal of met die produk gevul is, mag nie op sodanige wyse opgestapel word dat die inhoud van die 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-gebiede 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 word en moet op só 'n wyse 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 bokant die vloer of op pallette en weg van die mure 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 die mure opgestapel word nie. Geen materiaal behalwe die produk of bestanddele van die produk mag in vrieskamers, verkilkamers of vriesbewaarkamers bewaar word nie. Geen toestand en geen voorwerp of stof wat op enige wyse 'n uitwerking op die geur of voorkoms van die bevrore produk kan hê, mag in vrieskamers, verkilkamers en vriesbewaarkamers aanwesig wees nie.

3.6.10 Verwydering van vullis en kreefafval

Daar mag nie toegelaat word dat rommel, afval en oorloop akkumuleer of tot onhigiëniese toestande aanleiding gee nie en daar moet onmiddellik op 'n doeltreffende en higiëniese wyse daarmee weggedoen word. Kreefafval moet op 'n higiëniese wyse uit die verwerkingsgebied verwyder word en houers met kreefafval wat wag om uit die fabriek verwyder te word, moet ver genoeg van verwerkingsgebiede af wees. 'n Afsonderlike vertrek vir vullis of ander aanneemlike vullisfasiliteit moet op die perseel 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. Alle vertrekke waarin onverwerkte materiaal, bestanddele of verpakkingsmateriaal bewaar word, moet daarbenewens knaagdierbestand wees.

3.6.12 Die gebruik van plaagdoders

Plaagdoders mag nie gebruik word in werkgebiede terwyl voorbereiding, verwerking en verpakking aan die gang is nie en voorsorgmaatreëls moet 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 toedraaimateriaal, houers, onverwerkte materiaal of die produk in aanraking kom nie. Die vertrek waarin plaagdoders bewaar word, moet toegesluit 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.

3.7 Rekords

Toereikende kwaliteitsrekords moet gehou word. Kwaliteitsrekords en rekords van vriesbewaartemperature moet vir minstens twee jaar gehou word (kyk 3.2.15.3).

4 Vereistes vir die bestanddele en die produk

4.1 Algemeen

4.1.1 Toestand van bestanddele en die produk

4.1.1.1 Algemeen

Alle bestanddele wat gebruik word, moet val binne die bestek van die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972 (Wet 54 van 1972) en enige regulasie wat daarkragtens uitgevaardig is en moet aan die vereistes daarvan voldoen. Alle bestanddele wat by die voorbereiding van die produk gebruik word, moet skoon, gebrekvry, van goeie kwaliteit en in elke opsig vir menslike verbruik geskik wees. Daarbenewens mag die produk geen stof bevat in hoeveelhede wat gevaar vir menslike gesondheid kan inhou nie.

4.1.1.2 Sout

Sout wat by die voorbereiding van die produk gebruik word, moet eetbaar en vry van verkleuring, onsuiwerhede, bitterheid en ander byreuke en bysmake wees.

4.1.1.3 Geurmiddels

Geurmiddels moet vry van vreemde stowwe en vervalsingsmiddels wees.

4.1.1.4 Bymiddels

Slegs toegelate bymiddels moet gebruik word, en dan slegs in die toegelate hoeveelhede.

4.1.2 Vereistes vir invoerlande

Ten einde aan die vereistes van 'n invoerland te voldoen, kan uitvoerprodukte verpak word volgens vereistes wat afwyk van dié wat in hierdie spesifikasie voorgeskryf word, onderworpe aan die volgende voorwaardes:

a) daar moet vooraf skriftelik by die owerheid wat hierdie spesifikasie administreer, aansoek gedoen word om die produk volgens die voorgestelde afwyking te verpak en volledige besonderhede van die voorgenome afwyking moet verskaf word;

b) die afwyking mag nie, na die mening van die owerheid wat die spesifikasie administreer, na konsultasie met organisasies wat die nywerheid verteenwoordig, tot gevolg hê dat 'n produk van twyfelagtige kwaliteit of van 'n aard wat die Republiek van Suid-Afrika se uitvoerbeeld op die betrokke mark nadelig kan beïnvloed, verpak en uitgevoer word nie; en

c) die produk moet noukeurig op die houer of etiket beskryf word en die etikettering of merke mag nie die verbruiker mislei nie.

4.2 Vervoer van heel kreef en kreefsterte na verwerkings- en bevriesings-installasies

4.2.1 Algemeen

Die vervoer van heel kreef na verwerkings- en bevriesingsinstallasies wat in ooreenstemming met die toepaslike regulasies van die Wet op Seevissery, 1988 (Wet 12 van 1988), gedoen moet word, moet in skoon en higiëniese toestande geskied en die produk moet onderweg ten volle teen kontaminasie deur stof en ander vreemde stowwe en teen die hitte van die son beskerm word. Kreef mag nie vervoer word in toestande wat die produk nadelig beïnvloed of verswak nie. Kreef mag nie saam met ander produkte vervoer word nie. Vervoermiddele wat vir kreef gebruik word, mag nie gebruik word vir ander produkte wat die kreef kan benadeel of kontamineer nie. Die binneoppervlakke van die vervoermiddele moet só afgewerk wees dat dit nie die kreef nadelig beïnvloed nie. Die binneoppervlakke moet glad wees en maklik skoongemaak en ontsmet kan word. Die kreef moet klam, koel en lewendig gehou word. Bedekkings oor onbeskermde onverwerkte materiaal mag nie regstreeks op die onverwerkte materiaal rus nie. Die vervoermetode en die wyse waarop die kreef tydens vervoer gehou word, moet sodanig wees dat die kreef op geen wyse beskadig word nie.

4.2.2 Heel kreef

Heel kreef moet lewendig by die verpakkingsinstallasie aankom. Om dít te bewerkstellig, kan verkoeling tydens vervoer nodig wees. Varswaterys of yswater mag nie tydens sodanige vervoer met die kreef in aanraking kom nie.

4.2.3 Kreefsterte

Kreefsterte mag nie in 'n onbevrore toestand vervoer word nie, tensy:

a) die anale kanaal behoorlik verwyder en die stert gewas is;

b) dit afsonderlik goed toegedraai, gegradeer en in hul finale houers verpak is;

c) dit tot by 'n temperatuur van laagstens –1 °C en hoogstens 4 °C verkoel is en binne 5 h na verpakking na nabygeleë bevriesingsfasiliteite vervoer word; en

d) die voertuig waarin dit vervoer word 'n higiëniese konstruksie het.

4.3 Verpakking van kreef en kreefsterte

Slegs kreef of kreefsterte van dieselfde spesie mag saam in 'n houer verpak word.

4.4 Toestand van die kreef

4.4.1 Algemeen

Kreef mag op geen tydstip in ongunstige toestande gehou word nie, byvoorbeeld in sinkstrukture wat aan direkte sonlig onderwerp word. Indien kreef, nadat dit aan land gebring is, nie sonder oormatige vertraging na verwerkingsfabrieke geneem kan word nie, of by 'n verpakkingsinstallasie gehou moet word, of albei, moet dit lewendig gehou word óf deur onderdompeling in lopende, skoon seewater óf deur hulle by 'n omgewingstemperatuur in die bestek van 4 °C tot 12 °C te hou.

4.4.2 Ongeskondenheid

Kreef moet tydens of onmiddellik voor die uitbreek van die sterte lewendig wees of, indien toepaslik, onmiddellik voordat dit gaargemaak, verwerk en bevries word. Kreef of kreefsterte wat net effens beskadig of in 'n mindere mate gebreek is, kan vir spysenieringspakke gebruik word mits dit op elke ander wyse van 'n aanneemlike kwaliteit is en vir menslike verbruik geskik is. Kreef met hangsterte en met 'n onkenmerkende reuk of vleiskleur moet as onaanneemlik beskou word.

4.4.3 Sagte dop (nuwe dop) en eiers

Sagtedopkreef, eierdraende kreef of kreef waarvan die eiers gestroop is of wat nie aan statutêre vereistes voldoen nie, mag nie in die produk gebruik word nie.

4.5 Bevrore heel rou kreef

4.5.1 Voordat 'n lewendige kreef van die *Jasus*-spesie as bevrore heel kreef verpak word, moet dit inwendig gesuiwer word. Indien die mark egter inwendig ongesuiwerde bevrore heel kreef vereis, moet spesiale vrystelling om kreef van die *Jasus*-spesie op hierdie wyse te bemark, aangevra word van die owerheid wat hierdie spesifikasie administreer. In die geval van kreef van 'n ander spesie kan die produk uit inwendig ongesuiwerde kreef bestaan, onderworpe daaraan dat hierdie feit in die etikettering of merke van die produk verklaar word (kyk 6.1(b)(2) of (3)).

4.5.2 'n Kreef moet tot onmiddellik voor verwerking lewendig wees, wanneer dit doodgemaak moet word.

4.5.3 Kreef moet netjies en afsonderlik toegedraai en netjies verpak word en dan volgens 5.3 bevries word. So nie, kan kreef bevries en verglans word voordat dit toegedraai en verpak word.

4.5.4 Kreef moet volgens grootte gegradeer word en krewe in een houer moet 'n aanneemlik eenvormige grootte hê. Indien kreef nie volgens grootte gegradeer is nie, moet hierdie feit opvallend op alle hoofpanele van onmiddellike houers en hoofhouers vermeld word.

4.5.5 'n Kreef kan in 'n ysblok verpak word mits dit heeltemal deur die ys bedek word. Daar mag geen barste of ander defekte in die ys wees nie.

4.6 Bevrore heel gaar kreef

4.6.1 Die vereistes vir inwendige suiwering wat op bevrore heel rou kreef van toepassing is, geld (kyk 4.5.1).

4.6.2 Die vereistes van 4.5.2 is van toepassing.

4.6.3 Die kreef moet, onmiddellik nadat dit doodgemaak is, gaargemaak, vinnig afgekoel, in koue, lopende vars water (wat aan al die vereistes van 3.4.1 voldoen) of seewater (wat aan al die vereistes van 3.4.2 voldoen) geskrop word en gedreineer word, gegradeer word, indien toepaslik, netjies en afsonderlik toegedraai en netjies verpak word en dan volgens 5.3 bevries word. So nie, kan die produk bevries en dan verglans word voordat dit toegedraai en verpak word. As kreef gegradeer is, moet die krewe in een houer 'n aanneemlik eenvormige grootte hê.

4.6.4 Die produk moet sodanig wees dat dit na ontdooiing sonder verdere gaarmaak geëet kan word.

4.7 Bevrore kreefsterte

4.7.1 Mits kreef aan die vereistes van 4.4.2 voldoen, kan dit voor die uitbreek van die sterte en verdere verwerking in 'n verkilde toestand gehou word. Die verkiltoestande mag nie sodanig wees dat dit die reuk, geur, kleur of voorkoms van die produk benadeel nie.

4.7.2 Nadat die sterte uitgebreek is, moet die anale kanaal onmiddellik op higiëniese wyse verwyder word en die sterte moet baie deeglik gewas word om alle spore van vry bloed te verwyder. Die wasproses moet geskied in lopende water wat aan die vereistes van 3.4.1 of 3.4.2, soos toepaslik, voldoen. Daarna moet die sterte gegradeer word, indien toepaslik, netjies en afsonderlik toegedraai word, netjies verpak word en sonder versuim bevries word. Sterte mag geen verkleuring van watter aard of oorsprong ook al toon nie. Indien dit nodig is om die gewaste en gegradeerde sterte waarvan die anale kanaal verwyder is voor finale verpakking verkoel te hou, moet die verkilling van die sterte onmiddellik na verwydering van die anale kanaal en die was begin, en die finale bevriesing van die produk moet begin binne 5 h nadat tussenverkilling begin het. Die aard en duur van die verkilling moet sodanig wees dat dit nie die reuk, geur, kleur of voorkoms van die produk benadeel nie.

4.7.3 Heel kreef bedoel vir die verpakking van sterte mag nie voor verdere verwerking aan tussenbevriesing onderwerp word nie, maar sterte waarvan die anale kanaal behoorlik verwyder is en wat behoorlik gewas, gegradeer en afsonderlik toegedraai is, kan as 'n tussenstap voor finale verpakking, massaaanpassing en herbevriesing bevries word. Sodanige sterte kan, met die oog op herverpakking, massaaanpassing en finale bevriesing, op aanneemlike wyse verwarm word (indien nodig) sodat die eenhede geskei kan word, mits die interne temperatuur van die sterte nie tot hoër as –7 °C styg nie.

4.7.4 Kreefsterte van diepseespesies kan, indien nodig, tydens voorbereiding vir bevriesing behandel word met 'n anti-oksidant soos swaeldioksied of askorbiensuur wat by regulasie ingevolge die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972 (Wet 54 van 1972) toegelaat word, ten einde melanose van die vleis, dop en telson (stertvin) te voorkom. Die behandeling van die sterte mag nie veroorsaak dat die geur van die gaar produk nie kenmerkend van die betrokke spesie is nie.

4.7.5 Behalwe in die geval van sterte wat vir verpakking vir spysenieringsdoeleindes bedoel is, moet sterte volgens massa of lengte, soos toepaslik, gegradeer word. Die getal sterte in een houer moet volgens die verklaring op die houer wees. Die sterte in een houer moet aanneemlik eenvormig in grootte wees en die massa van elke stert in enige kategorie moet sover moontlik binne die massabestek val wat verkry word deur die som van die verklaarde netto massa van die toepaslike pakketeenheid en die minimum oormaatverpakking (kyk 4.9) deur die ooreenstemmende maksimum en minimum tellings vir daardie kategorie te deel.

4.7.6 Kreefsterte in spysenieringspakke kan gegradeer of ongegradeer vir grootte aangebied word, maar dit moet op die hoofpaneel van die etiket verklaar word. Mits die vleis ferm en ongeskonde is, kan 'n paar klein defekte in die dop van sodanige sterte aanwesig wees. Sterte in spysenieringspakke moet in elke ander opsig aan die kwaliteits-, toedraai-, verpakkings- en etiketteringsvereistes van hierdie spesifikasie voldoen.

4.8 Gesnipperde en fyn kreef

Slegs die vleis van die karapakse van kreef wat tydens die uitbreek van die sterte aktief was, mag gebruik word. Tensy die kreef onmiddellik behoorlik gewas word en by 'n temperatuur van hoogstens 4 °C in higiëniese houers bewaar word, of in 'n bevrore toestand bewaar word voor verdere verwerking of vervoer na nabygeleë verwerkingsinstallasies in voertuie met 'n higiëniese konstruksie, moet die herwinning van die vleis in die karapakse onmiddellik op die uitbreek van die sterte volg. Die produk moet 'n kenmerkende kleur hê, mag geen verkleuring van enige aard toon nie en moet vry van stukke dop en ander vreemde materiaal wees. Kreef wat aan verskillende spesies behoort, mag nie saam by die vervaardiging van die produk gebruik word nie.

4.9 Oormaatverpakking van kreefstert, bevrore heel rou kreef en bevrore heel gaar kreef

Daar moet toereikende voorsiening vir oormaatverpakking gemaak word om vir massaverlies tydens vriesbewaring, vervoer en verspreiding te kompenseer.

4.10 Chemiese vereistes

Volgens 9.1 tot 9.7 getoets, moet die produk aan die toepaslike vereistes van die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972, voldoen.

4.11 Mikrobiologiese vereistes

Volgens die toepaslike metodes in 10.6 tot 10.14 getoets, moet die produk aan die vereistes in kolom 2 of kolom 3 van tabel 1, soos toepaslik, voldoen.

Tabel 1 — Mikrobiologiese vereistes

1	2	3
Organisme	**Inhoud, maks**	
	Onverwerkte produkte[1])	**Gaar produkte[2])**
Standaardplaattelling	1×10^6/g	1×10^5/g
Enterobacteriaceae	[3])	100/g
Fekale koliforme bakterieë	Nul/10 g	Nul/10 g
Staphylococcus aureus	10/g	10/g
Salmonella	Nul/25 g	Nul/25 g
Shigella	Nul/25 g	Nul/25 g
Clostridium perfringens	Nul/25 g	Nul/25 g
Vibrio cholerae	Nul/25 g	Nul/25 g
V. parahaemolyticus	Nul/25 g	Nul/25 g
Listeria monocytogenes	[3])	Nul/25 g

1) Produkte wat gaargemaak moet word voordat dit geëet word.
2) Produkte wat slegs ontdooiing en herverwarming vereis voordat dit geëet word.
3) Moenie getoets word nie.

4.12 Antibiotika

Antibiotika mag nie by die voorbereiding van die produk gebruik word nie.

5 Verpakking, verglansing, bevriesing en bewaring

5.1 Verpakkings- en toedraaimateriaal en houers

5.1.1 Verpakkings- en toedraaimateriaal

Behoudens die toepaslike vereistes van die regulasies van 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 wees met 'n lae waterdampdeurdringbaarheid en dit mag nie stowwe bevat wat skadelik vir die produk of nadelig vir die gesondheid is nie. Omhulsels vir sterte moet 'n ware beskrywing van die produk bevat. Beskrywings wat op sodanige omhulsels verskyn, mag nie met die vereistes van klousule 6 bots nie. Omhulsels moet groot genoeg wees om die sterte toereikend te bedek en daardeur te verseker dat, as die sterte bevrore is, hulle nie aan mekaar vaskleef nie en teen vriesbrand beskerm is. Geen verpakkings- of toedraaimateriaal mag 'n geur na die produk oordra of op enige wyse verkleuring daarvan veroorsaak nie en mag nie self deur aanraking met die produk verkleur word nie.

Verpakkingsmateriaal

a) mag nie sodanig wees dat dit die organoleptiese eienskappe van die produk benadeel nie,

b) mag nie stowwe wat skadelik vir die produk of nadelig vir menslike gesondheid is, kan oordra nie, en

c) moet sterk genoeg wees om die produk toereikend te beskerm.

5.1.2 Buitehouers

Slegs veselbord- of ander aanneemlike houers mag gebruik word. Die houers moet ongebruik (nuut), skoon en heel wees en moet netjies en dig toegemaak wees. Buitehouers van hout mag nie van groenhout gemaak wees nie en mag nie stowwe bevat wat skadelik vir die produk of nadelig vir die gesondheid is nie. Buitehouers moet sodanig dig toegemaak wees dat kontaminasie van die inhoud deur stof of ander vreemde stowwe voorkom word en moet sterk genoeg wees om die produk voldoende te beskerm.

whole South African rock lobster, South Coast type"; in die geval van bevrore gaar heel kreef wat uit Natalse kreef (*Palinurus delagoae*) voorberei (en verpak) is, die opskrif "Cooked whole Natal rock lobster"; in die geval van bevrore gaar heel kreef wat uit sandkreef (*Scyllaridae*) voorberei (en verpak) is, die opskrif "Cooked whole slipper lobster"; in die geval van bevrore gaar heel kreef wat uit enige ander kreefspesie voorberei (en verpak) is, 'n ware en gepaste beskrywing wat nie die verbruiker sal mislei nie; en in die geval van bevrore gaar heel kreef wat ingevolge die toegewing in 4.5.1 inwendig ongesuiwer verpak is, die woord "unpurged" of die ekwivalent daarvan direk by die produknaam; en

4) in die geval van 'n bevrore kreefproduk uitgesonderd bevrore kreefsterte, bevrore rou heel kreef en bevrore gaar heel kreef, 'n ware en gepaste beskrywing van die produk, met inbegrip van die naam van die produk en die aanbieding van die inhoud;

c) die datum van vervaardiging en die identiteit van die fabriek waarin die produk verpak is; die gebruik van 'n kode is toelaatbaar mits die verklaring daarvan bekend gemaak word aan die owerheid wat hierdie spesifikasie administreer (die kode kan ook die kwotahouer vir wie die produk verpak is, identifiseer);

d) in die geval van produkte wat in die Republiek van Suid-Afrika te koop is, die netto massa van die inhoud, indien toepaslik (ooreenkomstig die regulasies wat kragtens die Wet op Handelsmetrologie, 1973, uitgevaardig is);

e) in die geval van sterte, uitgesonderd dié in spysenieringspakke, die kategorie-identifikasie (kyk 4.7);

f) die land van oorsprong;

g) indien toepaslik, woorde wat aandui dat die pak 'n spysenieringspak is;

h) die aanwesigheid van die antioksidant(e), by name, op die onmiddellike houers en, indien toepaslik, op die hoofhouers;

i) woorde wat duidelik en leesbaar meld dat die produk by 'n temperatuur van –20 °C of laer bewaar moet word;

j) indien toepaslik, 'n lys van die bestanddele, in dalende orde van hoeveelheid;

k) 'n verklaring dat die produk gaar of rou is, soos toepaslik, en aanwysings vir bewaring wat op die volgende wyse, soos toepaslik, aangegee word:

- Gaar (of rou) – Hou bevrore
- Halfgaar – Hou bevrore. Moenie herbevries na ontdooiing nie
- Gaar – Hou bevrore. Moenie herbevries na ontdooiing nie;

l) indien die produk met seewater verglans is, moet 'n verklaring te dien effekte opvallend op die hoofpaneel van die etiket direk by die produknaam verskyn;

m) indien toepaslik, gebruiksaanwysings; en

n) enige etiketteringsvereiste wat spesifiek by regulasie vereis word.

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 op elke afsonderlike pakket of op die buite-omhulsel van sodanige pakket verskyn, of op 'n etiket van aanneemlike materiaal wat aan die pakket bevestig is.

6.2.2 Etikette op pakkies moet skoon, netjies en stewig bevestig wees. Hulle mag nie geplaas word oor ander etikette of oor drukwerk wat direk op die pakkette gedruk is nie. Hulle mag slegs deur die fabrikant of sy gemagtigde agent aangebring word.

6.2.3 Etikette of seëlkleefmiddels wat moontlik in die bewaartoestande van die verpakte produkte agteruit sal 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 wat dit bevat en die besonderhede wat deur 6.1(a), (b), (d) en (k) 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 volledige fisiese adres hoef te wees nie, maar dit moet toereikend vir identifikasie-doeleindes 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 word op die buitehouer of op 'n etiket wat stewig aan die buitehouer bevestig is, of op 'n verpakkingstrokie wat in die buitehouer gesit is. 'n Kode kan vir die datum van vervaardiging gebruik word, mits die verklaring van die kode bekend is 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 wees en kan op 'n ander wyse as volgens die vereistes in 6.1 en 6.3 gemerk wees, mits daar geen poging tot wanvoorstelling van die inhoud is nie. Besonderhede soos in 6.1(c) 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 die toepaslike statutêre wette en regulasies.

7.2 Aflewering

7.2.1 Algemeen

Die aflewering van bevrore produkte moet plaasvind in higiëniese toestande wat nie die kwaliteit van die produk nadelig sal beïnvloed nie.

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 afgelewer word in die vriesbewaarfasiliteite van die vaartuig waarin dit vervoer gaan word. Indien die temperatuur van die produk te eniger tyd tydens sodanige vervoer tot hoër as –20 °C styg, moet dit so vinnig moontlik tot by die vereiste temperatuur verlaag word. Die produk moet weer ondersoek 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 die vriesbewaardepot na die kleinhandelsverkooppunt vervoer word. Die temperatuur van die produk tydens plaaslike vervoer moet –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 vir uitvoer moet vir ondersoek beskikbaar wees by die vriesbewaardepot van waar dit 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 aan hierdie spesifikasie voldoen nie, mag nie gehou word in dié vriesbewaarkamers van waar uitvoer geskied nie, tensy dit duidelik geïdentifiseer word. Die bevrore produk moet op die plek van verskeping vir herondersoek aangebied word indien daar, terwyl die produk bewaar is, hetsy by die oorspronklike verpakkingsinstallasie of by die verskepingspunt, of terwyl die produk na die verskepingspunt vervoer is, twyfel oor die temperatuurgeskiedenis of die kwaliteit van die bevrore produk ontstaan het.

8 Metodes van fisiese ondersoek

8.1 Organoleptiese ondersoek

Ondersoek die produk na ontdooiing (kyk 8.1.1) en nadat dit gaargemaak is (kyk 8.1.2) vir voldoening aan die vereistes van 5.5.

8.1.1 Prosedure vir ontdooiing

Ontdooi die monstereenheid deur dit deur 'n filmtipe sakkie te omhul en die sakkie in water by kamertemperatuur (hoogstens 35 °C) te dompel. So nie, kan die monstereenheid ontdooi word deur dit aan lug by 'n omgewingstemperatuur van 20 °C ± 5 °C bloot te stel. Die algehele ontdooiing van die monster word bepaal deur die sak af en toe versigtig te druk (sodat die tekstuur van die kreef nie beskadig word nie) totdat geen harde kern of yskristalle oor is nie.

8.1.2 Gaarmaakmetodes

8.1.2.1 Stoomprosedure

Draai die monstereenheid in aluminiumfoelie toe en plaas dit op 'n draadrak wat in 'n bedekte houer oor kokende water gesuspendeer is totdat die binnekerntemperatuur van die monstereenheid tussen 65 °C en 70 °C is.

8.1.2.2 Kook-in-sakkie-prosedure

Plaas die monstereenheid in 'n kookbare filmtipe sakkie en verseël dit. Dompel die sakkie in kokende water totdat die binnekerntemperatuur van die monstereenheid tussen 65 °C en 70 °C is.

OPM – Die presiese kook- of stoomtye en -toestande van die monster moet vooraf deur eksperimentering bepaal word.

8.2 Bepaling van die netto massa van bevrore produkte, uitgesonderd verglansde produkte

8.2.1 Verwyder ys wat buite aan die pakket kleef onmiddellik nadat dit uit vriesbewaring verwyder is en bepaal die bruto massa van die onoopgemaakte pakket.

8.2.2 Verwyder die verpakkingsmateriaal. Was en droog die verpakkingsmateriaal en bepaal die massa daarvan. Teken die verskil tussen die bruto massa (kyk 8.2.1) en die massa van die verpakkingsmateriaal as die netto massa van die bevrore produk aan.

8.3 Bepaling van die netto massa van 'n verglansde produk

8.3.1 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 inloop. Laat die produk in die water totdat alle oppervlakys gesmelt het. Indien die produk geblokvries is, draai die blok verskeie kere tydens ontglansing om; druk aan die blok en verwyder eenhede uit die water na gelang hulle loskom.

8.3.2 Plaas die inhoud van die houer nadat al die sigbare en voelbare verglansing verwyder is en die eenhede maklik van mekaar loskom, oor (kyk 8.3.1) na 'n geweegde sif met 'n nominale openinggrootte van ongeveer 2 mm. Kantel die sif ongeveer 20° en dreineer 2 min lank.

8.3.3 Teken die massa van die materiaal wat op die sif agterbly as die netto massa van die verglansde produk aan.

8.4 Bepaling van telling

Ondersoek die produk vir voldoening aan 4.7.5.

9 Metodes vir chemiese ontleding

OPM – Gebruik tydens ontleding en tensy 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 gedeioniseerde water.

9.1 Askorbiensuurgehalte

9.1.1 Reagense

9.1.1.1 Glasgedistilleerde water

9.1.1.2 Ekstraheeroplossing van metafosforsuur (HPO_3) en asynsuur

Los 15 g HPO_3-korrels of pas verpoeierde HPO_3-stokkies in 40 mℓ ysasynsuur en 200 mℓ water op deur dit te skud, en filtreer vinnig deur 'n papiertregter in 'n 500-mℓ-glasbottel. Plaas onmiddellik 'n glasprop op die bottel. (HPO_3 hidroliseer stadig tot ortofosforsuur (H_3PO_4), maar indien dit in 'n koelkas bewaar word, bly die oplossing 7 tot 10 d lank bevredigend.)

9.1.1.3 Standaardaskorbiensuuroplossing, 1 mg/mℓ

Weeg noukeurig 50 mg askorbiensuur uit wat in 'n desikkator weg van direkte sonlig af bewaar is, plaas dit kwantitatief na 'n 50-mℓ- volumetriese fles oor en verdun dit tot volume met water.

OPM – Berei 'n vars oplossing onmiddellik voor elke stel toetse voor.

9.1.1.4 Standaardindofenoloplossing

9.1.1.4.1 Los 50 mg van die natriumsout van 2,6-dichloorfenol (indofenol) wat in 'n desikkator oor natronkalk weg van direkte sonlig bewaar is in 50 mℓ water op wat 42 mg natriumbikarbonaat bevat. Skud heftig en plaas dit, wanneer die sout opgelos is, kwantitatief na 'n 200-mℓ- volumetriese fles oor en verdun tot volume met water. Filtreer deur 'n papiertregter in 'n amberglasbottel. Plaas onmiddellik 'n prop op die bottel en bewaar in 'n koelkas.

OPM – Ontbindingsprodukte wat die eindpunt onduidelik maak, kom in sommige lotte droë indofenol voor en kan ook met verloop van tyd in bogenoemde standaardoplossing ontwikkel. Toets die indofenoloplossing soos volg onmiddellik na bereiding en met tussenposes van een week: voeg 5,0 mℓ van die ekstraheeroplossing wat 'n oormaat askorbiensuur bevat by 15 mℓ standaardindofenoloplossing. Indien die gereduseerde oplossing nie feitlik kleurloos is nie, gooi die ou indofenoloplossing weg, berei 'n vars standaardindofenoloplossing en toets weer. Indien die vaste indofenol foutief is, verkry 'n nuwe voorraad.

9.1.1.4.2 Plaas drie 2,0 mℓ volumes standaardaskorbiensuuroplossing (kyk 9.1.1.3) oor na elk van drie 50-mℓ-Erlenmeyerflesse wat elk 5,0 mℓ ekstraheeroplossing bevat (kyk 9.1.1.2). Titreer vinnig met die standaardindofenoloplossing uit 'n 50-mℓ-buret totdat 'n ligte maar duidelike roospienk kleur minstens 5 s lank voorkom. (Elke titrasie vereis gewoonlik ongeveer 15 mℓ indofenoloplossing en titers moet binne 0,1 mℓ ooreenstem.) Titreer op soortgelyke wyse drie blanko oplossings wat elk bestaan uit 7,0 mℓ van die ekstraheeroplossing plus 'n volume water nagenoeg gelyk aan die volume indofenoloplossing wat

by die titrering van die askorbiensuuroplossing gebruik is en bepaal die gemiddelde titer van die blanko oplossings (gewoonlik ongeveer 0,1 mℓ). Korrigeer die standaardiseringstiters deur die gemiddelde blanko titer van elk af te trek en bereken die askorbiensuurekwivalent, in milligram, van 1,0 mℓ van die standaardindofenoloplossing. Standaardiseer die indofenoloplossing daagliks teen vars bereide standaardaskorbiensuuroplossing.

9.1.2 Bereiding van toetsoplossing van die produk

Versnipper die produk en plaas 'n gepaste noukeurig bepaalde massa na 'n vermenger oor. Voeg 'n gepaste volume ekstraheeroplossing by en meng liggies totdat 'n eenvormige suspensie verkry is. Verdun met die ekstraheeroplossing tot 'n bepaalde volume V_2, in milliliter, en meng deeglik.

9.1.3 Prosedure

Titreer, met die standaardindofenoloplossing, drie deelvolumes (alikwotte) van die toetsoplossing wat elk nagenoeg 2 mg askorbiensuur bevat en voer drie blanko bepalings soos in 9.1.1.4.2 uit.

OPM – Indien die volume van die deelvolumes van die toetsoplossing kleiner as 7 mℓ is, voeg in elke geval voor titrering genoeg van die ekstraheeroplossing by om die finale volume op 7 mℓ te bring.

9.1.4 Berekening

Bereken die askorbiensuurgehalte, uitgedruk in milligram per kilogram van die produk, aan die hand van die volgende formule:

$$(V - V_1) \times \frac{m}{m_1} \times \frac{V_2}{V_3} \times 1\,000$$

waar

V die gemiddelde monstertiter is, in milliliter;

V_1 die gemiddelde blanko titer is, in milliliter;

V_2 die volume van die toetsoplossing is (kyk 9.1.2), in milliliter;

V_3 die volume is van die deelvolume van die toetsoplossing wat getitreer is, in milliliter;

m die massa askorbiensuur ekwivalent aan 1,0 mℓ standaardindofenoloplossing is, in milligram; en

m_1 die massa van die produk in volume V_2 van die toetsoplossing is, in gram.

9.2 Bepaling van lood, koper, sink en kadmium (atoomabsorpsiespektrofotometriese metode)

9.2.1 Apparaat

9.2.1.1 Atoomabsorpsiespektrofotometer. (Raadpleeg die fabrikant se verwysingshandleidings vir golflengte, spleetwydte, vlamtoestande, ens.)

9.2.1.2 Kroesie, platinum, inhoudsvermoë 150 mℓ.

9.2.1.3 Waterbad.

9.2.1.4 Temperatuurbeheerde oond.

9.2.2 Reagense

9.2.2.1 Soutsuur, 1N, berei deur 89 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**: 2,0 µg Pb/mℓ.

9.2.2.3 Standaardkoperoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg Cu/mℓ; en

b) **standaardwerkoplossing**: 5,0 µg Cu/mℓ.

9.2.2.4 Standaardsinkoplossings, soos volg:

a) **standaardvoorraadoplossing**: 1 mg Zn/mℓ; en

b) **standaardwerkoplossing**: 3,0 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 12,5 g ± 0,1 g van die monster in die kroesie af (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 by 450 °C. Veras die monster oornag (16 h). Verwyder die kroesie en laat dit afkoel. Voeg 10 mℓ van die 1N-HCl-oplossing by en los die as op deur die kroesie versigtig oor 'n kokende waterbad te verhit. Plaas die inhoud van die kroesie na 'n 25-mℓ- volumetriese fles oor. Verhit die asresidu weer opeenvolgens met twee 5-mℓ-dele van die 1N-HCl-oplossing en voeg dit by die fles. Verkoel, verdun tot volume met die 1N-HCl-oplossing, 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 2,0-µg-Pb/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder as die absorbansie van die standaardwerkoplossing is, is die lood in die monster minder as 4 mg/kg.

9.2.3.4 Bepaling van koper

Verdun 10,0 mℓ van die monsteroplossing met water tot 50,0 mℓ. Bepaal die absorbansie van die monsteroplossing, van die reagensblanko en van die 5,0-µg-Cu/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder as die absorbansie van die standaardwerkoplossing is, is die koper in die monster minder as 50 mg/kg.

9.2.3.5 Bepaling van sink

Verdun 1,0 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-Zn/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder as die absorbansie van die standaardwerkoplossing is, is die sink in die monster minder as 300 mg/kg.

9.2.3.6 Bepaling van kadmium

Bepaal die absorbansie van die monsteroplossing, van die reagensblanko en van die 1,5-µg-Cd/mℓ-standaardwerkoplossing. Indien die absorbansie van die monsteroplossing minus die absorbansie van die reagensblanko minder as die absorbansie van die standaardwerkoplossing is, is die kadmium in die monster minder as 3,0 mg/kg.

9.3 Bepaling van tin (atoomabsorpsiemetode)

9.3.1 Apparaat

Atoomabsorpsiespektrofotometer. (Raadpleeg die fabrikant se verwysingshandleidings vir 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 dit met gedistilleerde water tot 100 mℓ te verdun.

9.3.2.3 Salpetersuur (HNO_3), gekonsentreer. Toets die suiwerheid van 'n lot deur 'n gedeelte met gedistilleerde water tot 1:4 (per volume) te verdun en dit in 'n AA-spektrofotometer te aspireer. Die afwesigheid van 'n Sn-sein dui op die geskiktheid van die salpetersuur vir ontleding.

9.3.3 Bereiding van monster

Weeg 25 g van die monster noukeurig (± 0,01 g) in 'n 250-mℓ-Erlenmeyerfles af. Laat dit in 'n oond by 120 °C droog word.

OPM — Moenie HNO_3 by monsters voeg (kyk hieronder) tensy daar tyd is om hierdie stadium van vertering op dieselfde dag te voltooi nie.

Voeg 30 mℓ van die gekonsentreerde HNO_3 by die fles en verhit versigtig binne 15 min in 'n dampkas om vertering te laat begin. Vermy oormatige skuimvorming. Kook liggies totdat 3 mℓ tot 6 mℓ van die verteersel oorbly of totdat die monster net begin om op die bodem droog te word. Moenie die monster laat verkool nie. Verwyder die fles van die hitte. Gaan sonder versuim soos volg voort en berei gelyktydig twee leë flesse vir reagensblanko's: voeg 25 mℓ gekonsentreerde soutsuur (HCl) by en verhit 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 raam. Plaas die monsteroplossing en die reagensblanko's oor na 25-mℓ- volumetriese flesse. Die monsteroplossing en reagensblanko's kan oornag of langer staan.

Pipetteer 1,0 mℓ van die KCl-oplossing in elke volumetriese fles. Verkoel tot by omgewingstemperatuur 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 ontleding plaasvind.

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-standaardwerkoplossing. 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- waterige kaliumjodiedoplossing.

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 verskaf.

9.4.2.5 Kwik(II)bromied, kwikbromied ($HgBr_2$ = 360,4 analitiese reagensgraad).

9.4.2.6 Kwik(II)bromiedpapier, soos volg berei: plaas in 'n reghoekige bak 'n 5-g/100-mℓ-oplossing kwik(II)bromied in absolute etanol en dompel daarin stukke wit filtreerpapier met 'n gramtal van 80 g/m^2 (Whatman no 1 is geskik) wat elk 200 mm × 15 mm groot en dubbel gevou is. Giet die oortollige vloeistof af en laat die papiere weg van lig droog word deur hulle oor 'n niemetaaldraadjie te hang. Sny die gevoude rande tot 'n wydte van 10 mm weg. Sny die oorblywende strokies in vierkante van 15 mm of skywe met 'n diameter van 15 mm.

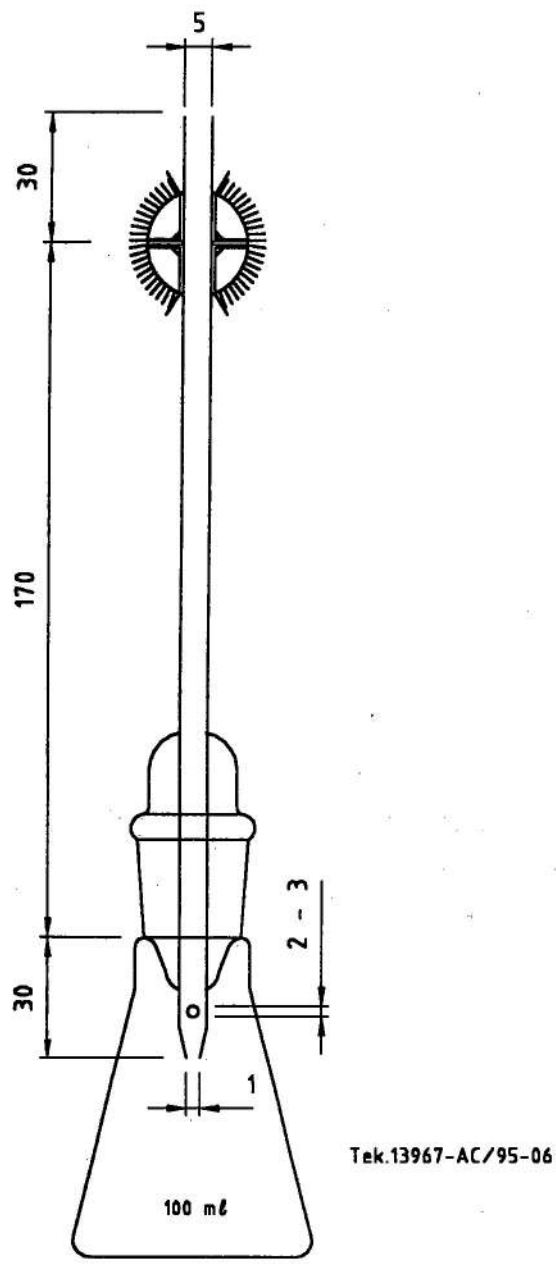
Kwik(II)bromiedpapier moet in 'n houer met 'n glasprop weg van lig gehou word.

9.4.2.7 Gegranuleerde sink

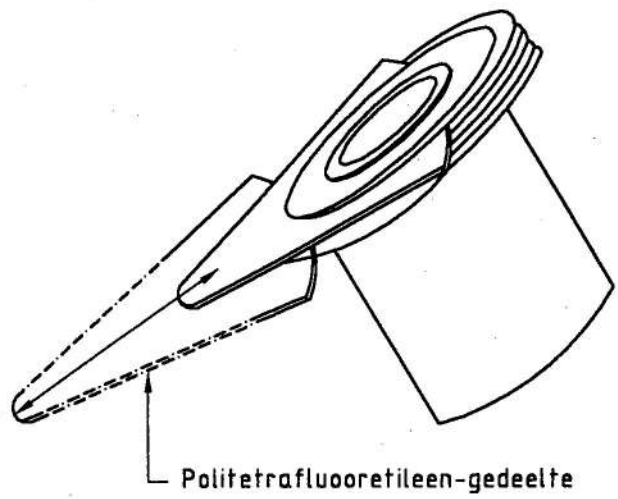
9.4.2.8 Loodasetaatoplossing, 'n 10-g/100-mℓ-oplossing lood(II)asetaat in koolstofdioksiedvry water.

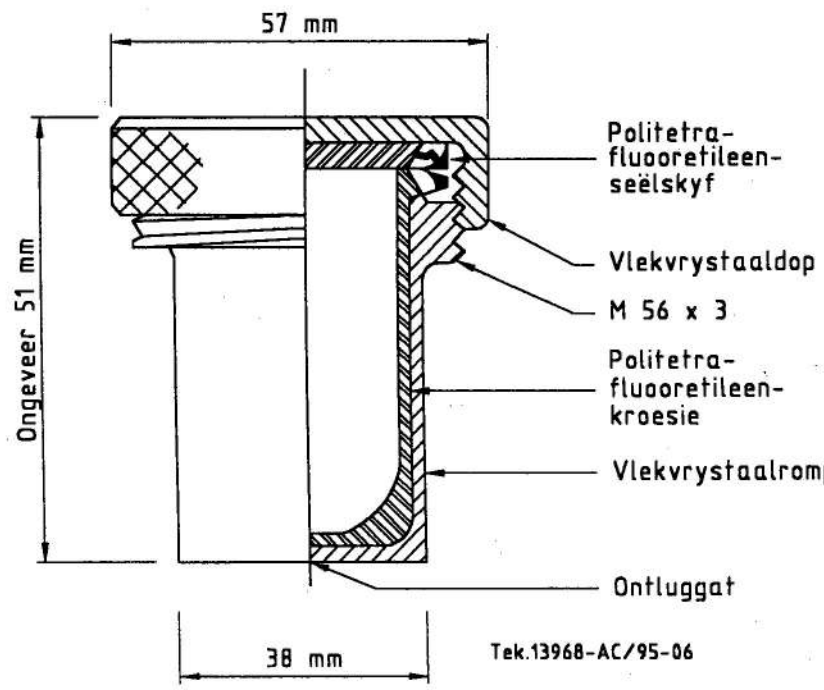
9.4.2.9 Loodasetaatwatte soos volg berei: dompel absorbeerwatte in 'n mengsel van 10 volumes loodasetaatoplossing en 1 volume 2M-asynsuur. Dreineer die oortollige vloeistof deur die watte op verskeie lae filtreerpapier te plaas sonder om die watte te druk. Laat die watte by kamertemperatuur droog word. Loodasetaatwatte moet in 'n lugdigte houer gehou word.

Afmetings in millimeter



Figuur 1— Apparaat vir grenstoets vir arseen





Figuur 2 — Verteerhouer

9.4.3 Prosedure

Neem 5,0 mℓ van die monsteroplossing wat vir die bepaling van tin berei is (kyk 9.3.3). Die apparaat (kyk figuur 1) bestaan uit 'n 100-mℓ- koniese fles, toegemaak 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 tot 'n binnediameter van 1,0 mm getrek en 15 mm van die punt af is 'n syopening met 'n diameter van 2 mm tot 3 mm. Wanneer 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, 'n lengte van 30 mm en 'n soortgelyke plat geslypte oppervlak word in aanraking met en koaksiaal tot die eerste geplaas en word deur twee spiraalvere in posisie gehou. Steek 50 mg tot 60 mg loodasetaatwatte, los gepak, of 'n klein watteproppie en 'n opgerolde stukkie loodasetaatpapier met 'n gekombineerde massa van 50 mg tot 60 mg in die onderste buis. Plaas een van die stukke 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 15 min lank staan en voeg dan 5 g gegranuleerde sink by. Stel die twee dele van die apparaat onmiddellik op en dompel die fles in 'n waterbad by 'n temperatuur wat sodanig is dat 'n egalige ontwikkeling van gas gehandhaaf word. Na minstens 2 h mag geen vlek wat op die kwik(II)bromiedpapier voorkom meer intens wees as dié wat verkry word deur 3 mℓ standaardarseenwerkoplossing (1 µg/mℓ As) op dieselfde wyse met water tot 25 mℓ te verdun nie.

9.5 Bepaling van kwik

9.5.1 Apparaat

9.5.1.1 Atoomabsorpsiespektrofotometer, toegerus met 'n kwikholkatodelamp.

9.5.1.2 Kouedamp-absorpsiesel, in die plek van die brander van die spektrofotometer aangebring (kyk figuur 3).

9.5.1.3 Verteerhouer (kyk figuur 2), wat bestaan uit 'n vlekvrystaalromp wat 'n politetrafluooretileenkroesie steun, en 'n skroefdop met 'n politetrafluooretileen-voering om 'n politetrafluooretileen-seëlopperylak 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.

9.5.1.4 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 soutsuur en 67 mℓ swaelsuur per liter bevat.

9.5.2.5 Verdunde soutsuur, een volume van die soutsuur by nege volumes water.

9.5.2.6 Tindichloriedoplossing, 5 g kristallyne tindichloried ($SnCl_2.2H_2O$) wat deur verhitting in 10 mℓ gekonsentreerde soutsuur opgelos is en met water tot ongeveer 50 mℓ verdun is. 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 1 g ± 0,1 g van die monster noukeurig af (kyk 9.1.2) (**waarskuwing**: moet nie meer as 300 mg droë massa gebruik nie; in die geval van materiaal met 'n hoë vetgehalte, moet hoogstens 200 mg droë massa gebruik word) in die verteerhouer (kyk 9.5.1.3), 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 kantel, 30 min tot 60 min lank of totdat die monsteroplossing helder is in 'n oond wat tot 150 °C voorverhit is. Verwyder die houer en laat dit tot kamertemperatuur afkoel. Skroef die dop los, knip die tuit aan, plaas die inhoud van die houer met behulp van die verdunsuuroplossing (kyk 9.5.2.4) na 'n 100-mℓ- volumetriese fles oor, en verdun tot volume met die suuroplossing.

9.5.4.2 Skakel die kwikholkatodelamp aan en laat die spektrofotometer volledig teen 'n golflengtestelling van 253,7 nm 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 verdunning van die kwikdamp tot die minimum deur buise met die kleinste diameter en kortste lengte moontlik te gebruik. Maak seker dat die afstand tussen die onderste ent 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 die fles onmiddellik, skakel die magnetiese roerder aan, hou 90 s lank aan met roer, skakel die roerder af en skakel die pomp onmiddellik aan. Teken die absorpsielesing aan sodra dit stabiel word. Verwyder die fles en pomp lug deur die stelsel om die kwikdamp te verwyder.

9.5.4.5 Plaas 2,0 mℓ van die 0,1-µg-Hg/mℓ-standaardwerkoplossing na die reaksiefles oor, voeg 18,0 mℓ 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

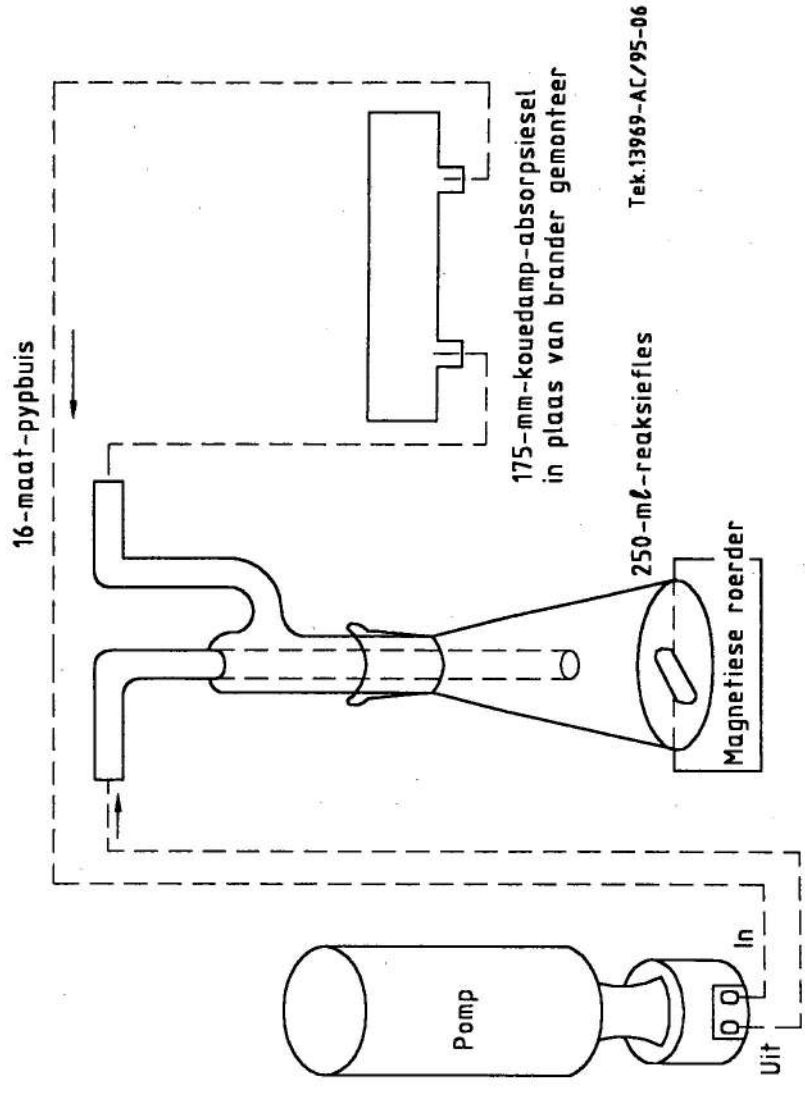
Meet die absorbansie van die 0,2-µg-Hg/mℓ- standaardwerkoplossing, van die reagensblanko en van die monsteroplossing.

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 1,0 mg/kg.

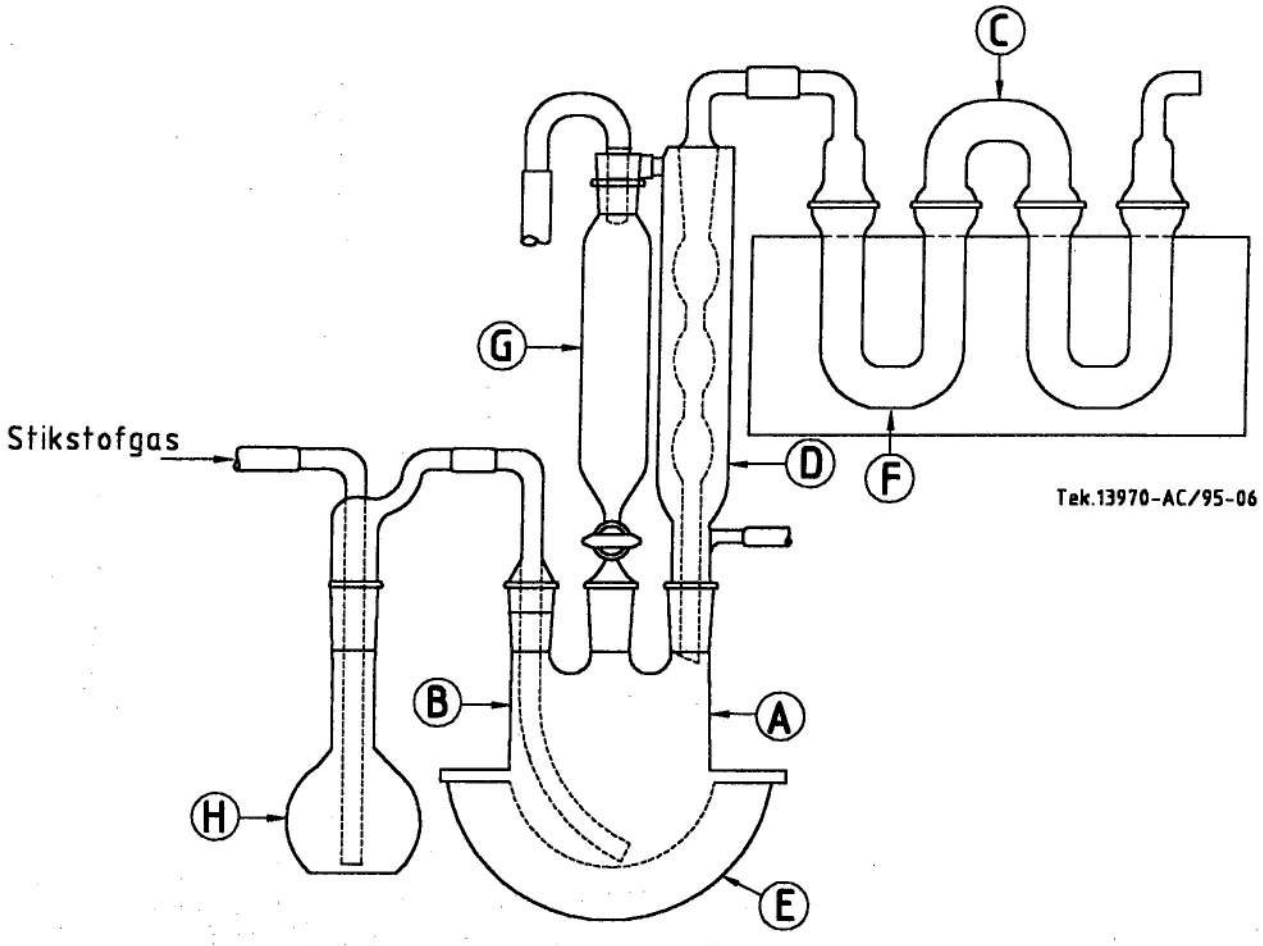
9.6 Bepaling van antimoon

9.6.1 Apparaat

Atoomabsorpsiespektrofotometer. (Raadpleeg die fabrikant se verwysingshandleidings vir golflengte, spleetwydte, vlamtoestande, ens.)



Figuur 3 — Apparaat vir die bepaling van kwikgehalte



Figuur 4 — Apparaat vir die bepaling van swaeldioksiedgehalte

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 Prosedure

9.6.3.1 Reagensblanko

Berei 'n reagensblanko.

9.6.3.2 Volg die apparaatfabrikant se aanwysings vir die hidriedontwikkeling vir antimoon en maak seker dat die antimoon voor ontleding in die SB^{III}-toestand is deur die monster en die standaarde met 'n oormaat kaliumjodied te behandel.

9.6.3.3 Meet die absorbansie van die 0,01-µg-Sb/mℓ- standaardwerkoplossing, van die reagensblanko en van die monsteroplossing (gebruik die monsteroplossing wat by die kwikbepaling 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 van die monster minder as 1 mg/kg.

9.7 Bepaling van swaeldioksiedgehalte

9.7.1 Apparaat

9.7.1.1 Distilleerapparaat (kyk figuur 4) wat bestaan uit 'n distilleerfles met 'n ronde bodem (A),'n inhoudsvermoë van 1 ℓ en met drie parallelle nekke. 'n 100-mℓ-druptregter (G) word in die middelste nek geplaas en 'n stikstofleweringsbuis (B) gaan deur een van die synekke tot onder die vlak van die vloeistof in die distilleerfles (A). Die ander synek word met die onderste sok van 'n vertikaal gemonteerde dubbel-oppervlakkondensator (D) verbind. 'n Stel van twee 20-mm-U-buise (F) word deur 'n verbindbuis (C) met die boonste keël van die kondensator verbind.

9.7.1.2 Stikstofskropper (kyk figuur 4) wat bestaan uit 'n 250-mℓ-gaswasbottel (H) wat deur middel van silikoonbuise met die inlaat van die stikstofleweringsbuis (B) verbind is. Die inlaat- en uitlaatbuise van die wasbottel word toegeklamp.

9.7.1.3 Verhittingsmantel soos deur (E) in figuur 4 aangetoon.

9.7.2 Reagense

9.7.2.1 Stikstofgas

9.7.2.2 Soutsuur, verdun tot die helfte van die gekonsentreerde sterkte.

9.7.2.3 Waterstofperoksied, 'n 3-%(per volume)-oplossing geneutraliseer tot metielrooi.

9.7.2.4 Standaardnatriumhidroksiedoplossing (c(NaOH) = 0,1 mol/ℓ).

9.7.2.5 Pirogallol/kaliumhidroksied(KOH-)oplossing, 65 g kaliumhidroksied opgelos in 85 mℓ gedistilleerde water. Maal 4,5 g pirogallol saam met 5 mℓ water in 'n klein vysel en plaas oor na die afgekoelde KOH-oplossing. Maal weer en plaas oor met nog twee 5-mℓ-volumes water.

9.7.2.6 Metielrooi-indikator, 0,25 g metielrooi opgelos in 100 mℓ etanol.

9.7.3 Prosedure

Plaas die pirogallol/kaliumhidroksiedoplossing in die gaswasbottel (H). Voer 15 mℓ van die waterstofperoksiedoplossing in elk van die U-buise (F) in. Weeg 200 g van die toetsmonsteroplossing, berei soos in 9.2.3 beskryf word, noukeurig af en plaas hierdie toetseksemplaar saam met ongeveer 300 mℓ water deur die middelste nek in die distilleerfles (A) en bring die druptregter (G) aan. Voeg 30 mℓ van die soutsuur deur die tregter by die distilleerfles (A) en maak die tregterafsluitkraan toe.

Begin die stikstofgasvloei teen 'n stadige, bestendige stroom borrels. Verhit die distilleerfles sodanig dat terugvloei binne 20 min tot 25 min begin. Laat dit 1,5 h lank bestendig terugvloei.

Draai die water in die kondensator (D) toe en hou aan verhit totdat die inlaatverbinding van die eerste U-buis kondensasie en effense verhitting toon. Diskonnekteer die kondensator en staak die verhitting.

Sodra die verbinding aan die bopunt van die kondensator afgekoel het, verwyder die verbindbuis (C) en spoel dit in die tweede U-buis in. Sit die verbindbuis (C) aan die uitlaatverbinding van die eerste U-buis vas en roteer dit totdat die oop ente aan mekaar raak. Voeg 'n druppel metielrooi-indikator by en titreer met die standaardnatriumhidroksiedoplossing terwyl dit versigtig gemeng word deur dit liggies te wieg net totdat 'n heldergeel kleur verskyn. Titreer die tweede U-buis op soortgelyke wyse.

Teken die totale volume natriumhidroksied aan wat vir die titrasie nodig is (1 mℓ van 'n 0,1-mol/ℓ-NaOH = 3,203 mg swaeldioksied).

9.7.4 Berekening

Bereken die swaeldioksiedgehalte, uitgedruk in milligram per kilogram van die produk, aan die hand van die volgende formule:

$$\frac{V \times 1\,000 \times 3{,}2}{m}$$

waar

V die volume is van die standaardnatriumhidroksiedoplossing wat in die titrasie gebruik is, in milliliter; en

m die massa van die toetseksemplaar is, in gram.

10 Metodes vir mikrobiologiese ondersoek

10.1 Algemeen

Aseptiese tegnieke moet deurgaans tydens 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 van inhiberende stowwe soos swaar metale en vry alkalië is. 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 '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 die volgende diameter en lengte

a) 16 mm × 160 mm, en

b) 20 mm × 200 mm.

Stop hierdie buise toe met watteproppe of proppe van skuimrubber wat vir outoklavering geskik is. So nie, gebruik skroefdopbuise met soortgelyke 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, wat in eenhede van 0,1 mℓ ingedeel is en in groottes met 'n lewering van 1,0 mℓ, 5,0 mℓ en 10,0 mℓ.

10.2.5 Petribakkies

Petribakkies van glas of benatbare polistireen met 'n diameter en hoogte van

a) 90 mm × 15 mm,

b) 100 mm × 20 mm, en

c) 150 mm × 20 mm.

10.2.6 Volumetriese silinders

Maatsilinders met skaalindelings, met of sonder proppe, en 'n inhoudsvermoë van 5 mℓ, 10 mℓ, 100 mℓ en 1 000 mℓ.

10.2.7 Monsterbottels

Bottels met openings met 'n diameter van 40 mm tot 60 mm, met omruilbare slypglas- of plastiekproppe of gevoerde metaalsluitdoppe en '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, met standaardsluitdoppe van gevoerde metaal of 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 geboor deur die sluitdoppe, 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 so ontwerp is dat hulle 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 'n inhoudsvermoë van 0,9 mℓ tot 1,3 mℓ.

10.3 Toerusting

10.3.1 Outoklaaf

'n Drukhouer wat stoom kan lewer (of wat met 'n sentrale stoombron verbind is), 'n druk van 300 kPa kan deurstaan en binne 10 min na aanvang van die steriliseringsiklus 'n temperatuur van 121 °C ± 2 °C kan bereik.

10.3.2 Inkubators en waterbaddens

Inkubators en waterbaddens met termostaties beheerde verhittings- en verkoelingstoestelle en wat op so 'n wyse van sirkuleermiddele voorsien is dat die temperatuur van die totale ingeslote ruimte tot 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 op so 'n wyse 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 met steriliseerbare houers waarin 'n homogene dispersie van die monster en die voorgeskrewe verdunmiddel gemaak kan word. Die steriliseerbare houers kan van glas, metaal of 'n geskikte plastiekmateriaal wees. Die homogeniseerprosedure mag nie die getal of lewensvatbaarheid van die mikro-organismes in die monster verminder of verlaag 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 bewys is dat hulle volle bakteriese retensie en 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 poriegrootte van hoogstens 0,45 µm.

10.3.6.2 Sterilisasie

Membraanfilters, afsonderlik verpak, moet vooraf deur betroubare handelsfabrikante gesteriliseer word.

Indien membraanfilters gesteriliseer moet word, maak hulle met steriele gedistilleerde water nat (om omkrul te voorkom), 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 in 'n outoklaaf te verhit.

10.3.7 Membraanfilterhouers

10.3.7.1 Tipe

'n Membraanfilterhouer gemaak van niekorroderende, bakteriologies inerte materiaal wat toelaat dat alle vloeistof wat gefiltreer word deur die membraan 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 20 min lank by 121 °C ± 2 °C in 'n outoklaaf, of volgens 'n ander geskikte metode.

10.3.8 Tang

10.3.8.1 Tipe

'n Rondepunttangetjie waarvan die kake gladde binneoppervlakke het.

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 enige 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 dieselfde 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 waarvan die kwaliteit vir mikrobiologiese doeleindes aanneemlik 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 daar anders gespesifiseer word:

a) by temperatuur ..... ± 2 °C

b) by massa ..... ± 1,0 %

c) by volume ..... ± 1,0 % en

d) by pH-waarde ..... ± 0,1 pH-eenheid

10.4.1.4 Ontwaterde kweekmedia

Baie van die kweekmedia wat vereis word, is in ontwaterde vorm verkrygbaar en ter wille van eenvormigheid van resultate word die gebruik van sodanige kweekmedia aanbeveel. Indien sodanige kweekmedia gebruik word, volg die fabrikant se aanwysings vir rekonstituering en sterilisering noukeurig.

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 en, in die geval van kweekmedia, voor sterilisasie aan. 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 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 uit (kyk 10.2.9). Roer die 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 word. Plaas die bottels of, indien toepaslik, die kweekbuise, onmiddellik na sterilisering en terwyl die kweekmedium nog gesmelt is op 'n oppervlak met 'n 1-op-4-helling en laat die kweekmedium styf word.

10.4.1.7 Sterilisasie

Indien sterilisasie in 'n outoklaaf gespesifiseer word en tensy daar anders voorgeskryf word, steriliseer die kweekmedium 15 min lank by 121 °C ± 2 °C in 'n outoklaaf. (Hierdie temperatuur stem ooreen met 'n druk van 103 kPa bo atmosferiese druk by seevlak, dws 207 kPa absoluut.)

10.4.1.8 Kontrolering van bereide kweekmedia

Maak deur geskikte inkubasietoetse seker dat bereide kweekmedia steriel is en die groei van die toepaslike organismes in die gegewe inkubasietoestande kan steun.

10.4.1.9 Bewaring van kweekmedia

Maak seker dat 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 en 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 en suiwer die pH-waarde tot 7,2 aan. Meet 15-mℓ-volumes in 30-mℓ-bottels (kyk 10.2.2(a)) uit en steriliseer in 'n outoklaaf.

10.4.4 Violetrooigalagar (VRG-agar)

10.4.4.1 Bestanddele

Agar	12 g
Laktose	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 die water op deur dit te kook. Verkoel tot by 50 °C 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 1000-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 na bereiding.

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 hierdie 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 gebruik word (kyk 10.2.10) 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 op in die amielalkohol en help die oplossing daarvan aan deur dit in 'n waterbad by 50 °C tot 55 °C te verhit. Verkoel en voeg die suur by. Beskerm teen lig en bewaar by 4 °C. Maak seker dat die reagens liggeel is. (Sekere soorte amielalkohol veroorsaak dat die reagens 'n baie donker kleur het en onbevredigend is.) Bewaar in 100-mℓ-reagensbottels (kyk 10.2.9). Laat 24 h lank staan voor gebruik.

10.4.9 Baird-Parker-agar

10.4.9.1 Bestanddele van basale kweekmedium

Agar	20 g
Glisien	12 g
Triptoon	10 g
Vleisektrak	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. Verkoel 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 by 4 °C vir hoogstens een maand.

Voeg, voordat plate gegiet word, 1 mℓ tellurietoplossing (kyk 10.4.10) asepties by, asook 5 mℓ eiergeel-emulsie (kyk 10.4.11) by elke 90 mℓ basale kweekmedium wat gesmelt en dan tot tussen 45 °C en 50 °C afgekoel is. Meng goed en meet 15-mℓ-volumes asepties uit in steriele petribakkies (kyk 10.2.5(a) of (b)). Laat stol. Gebruik die plate binne 24 h na bereiding. Droog die oppervlak van die kweekmedium minstens 1 h lank by 45 °C voor gebruik 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 in die water op met minimale verhitting. Steriliseer deur filtrering. Bewaar in 'n 100-mℓ-reagensbottel (kyk 10.2.9) by 4 °C vir hoogstens een maand.

10.4.11 Eiergeelemulsie (ongeveer 20 % (per volume))

Was en ontsmet dan die doppe van heel, vars 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 30-mℓ- steriele bottels (kyk 10.2.2(a)) en bewaar hoogstens een maand lank by 4 °C.

10.4.12 Mannitolsout-fenolrooi-agar

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 in steriele petribakkies (kyk 10.2.5(a) of (b)) uit. 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 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

Triptoon	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 in steriele petribakkies (kyk 10.2.5(a) of (b)) uit. 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. **Moenie verhit nie**. Bewaar die oplossing minstens 1 d lank in die donker sodat outosterilisasie kan plaasvind.

10.4.16 Rappaport-Vassiliadis-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 volle inhoud van 'n houer $MgCl_2.6H_2O$ op te los, eerder as om 'n deel van die inhoud te gebruik. Byvoorbeeld, 250 g $MgCl_2.6H_2O$ 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. Meet dit voor gebruik in 10-mℓ-hoeveelhede in proefbuise uit. Steriliseer dit 15 min lank by 115 °C in 'n outoklaaf. 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 behalwe die natriumwaterstofseleniet in ongeveer 800 mℓ water op deur dit te kook en steriliseer dit (in grootmaat) in 'n outoklaaf. Los die natriumwaterstofseleniet in ongeveer 150 mℓ koue water op en steriliseer die oplossing (verkieslik deur filtrering of andersins 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 massa van die bestanddele. Suiwer die pH-waarde tot 7,0 aan en verdun die oplossing tot 1 ℓ met steriele water. Meet 100-mℓ-volumes asepties uit in steriele kweekflesse (kyk 10.2.8). Moenie die kweekmedium verder verhit nie. Die afsaksel wat vorm, sal tot op die bodem van die fles afsak; suspendeer dit weer voordat die kweekmedium gebruik word. Gebruik op die dag van bereiding.

10.4.18 Briljantgroen-fenolrooi-agar (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, behalwe die fenolrooi, die laktose en die sukrose, in ongeveer 800 mℓ water op en steriliseer (in grootmaat) in 'n outoklaaf. Verkoel tot 55 °C. Los die fenolrooi en die suikers in ongeveer 150 mℓ water op en verhit dit 20 min lank by 70 °C in 'n waterbad. Verkoel tot 55 °C 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 tot 1 ℓ met steriele water. Meet 40-mℓ-volumes asepties uit in steriele petribakkies, verkieslik met 'n diameter van 150 mm (kyk 10.2.5(c)). Alhoewel hierdie groter petribakkies verkieslik is, kan kleiner petribakkies (kyk 10.2.5(a) of (b)) gebruik word as die groter bakkies nie beskikbaar is nie, maar berei dan twee maal soveel van hulle as wanneer 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 droog die oppervlak daarvan 30 min lank by 50 °C voordat dit gebruik word. Gebruik die plate op die dag van bereiding.

10.4.19 Sitochroomoksidasetoetsstrokies 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. Verkoel dit tot 50 °C en suiwer die pH-waarde tot 7,4 aan. Meet 10-mℓ-volumes uit in kweekbuise (kyk 10.2.3(a)) en steriliseer 10 min lank in 'n outoklaaf. Laat dit stol in 'n skuins posisie 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, behalwe die ureum, in water op deur dit te kook en verdun die oplossing tot 900 mℓ. Steriliseer hierdie basis (in grootmaat) in 'n outoklaaf en verkoel dit tot 50 °C. 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 tot 1 ℓ met steriele water. Meet 10-mℓ-volumes asepties uit in steriele 30-mℓ-bottels (kyk 10.2.2(a)) en laat dit stol in 'n skuins posisie 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 Bereiding

Los die natriumfosfaat in 15 mℓ water op. Suiwer die pH-waarde tot 7,0 aan met die natriumhidroksiedoplossing. Los die galaktopiranosied in hierdie oplossing op en verdun dit tot 20 mℓ. Bewaar vir hoogstens een maand 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 proefbuisies (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 by omgewingstemperatuur in reagensbottels (kyk 10.2.9).

10.4.26 α-naftoloplossing

Berei 'n waterige oplossing wat 60 g α-naftol per liter bevat met 96 % (per massa) tot 100 % (per massa) etanol as die oplosmiddel. Bewaar hoogstens een maand in reagensbottels (kyk 10.2.9) by omgewingstemperatuur.

10.4.27 Kaliumhidroksiedoplossing

Berei 'n waterige oplossing wat 400 g kaliumhidroksied per liter bevat. Bewaar by omgewingstemperatuur in bottels met alkalibestande plastiekproppe. Moenie glasproppe gebruik nie. Vermy oormatige 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 tipe wat tot nog toe nie teëgekom is nie tot 'n vals negatiewe reaksie kan lei. Groep A tot G moet in alle gevalle toereikend verteenwoordig wees. Volg die antiserumfabrikant se aanwysings vir elke antiserum of antiserummengsel.

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 "l", op te spoor. Volg die antiserumfabrikant se aanwysings vir elke antiserummengsel.

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
Water	1 000 mℓ

10.4.33.2 Bereiding

Suspendeer die bestanddele in die water. Suiwer die pH-waarde tot 7,4 ± 0,2 aan. Verhit terwyl daar dikwels geroer word totdat die kweekmedium kook. **Moenie oorverhit nie**. Plaas onmiddellik oor na 'n waterbad by 50 °C. Gooi 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 teenliggame, van minstens *Shigella*-serotipes 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 ($Na_2S_2O_5$), watervry	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 sodanig aan dat dit na sterilisasie 7,6 sal wees.

Plaas die basis oor na buise, flesse of bottels met 'n inhoudsvermoë van hoogstens 500 mℓ. Steriliseer 10 min lank by 121 °C. Bewaar by 4 °C ± 2 °C in 'n koelkas.

Gooi ongebruikte kweekmedium 2 weke na bereiding weg.

10.4.35.2 D-sikloserienoplossing

10.4.35.2.1 Bestanddele

D-sikloserien (gebruik slegs wit kristalpoeier)	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 (kyk 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.

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1) Die name triptose en sojatoon word tans slegs deur sekere kweekmediafabrikante gebruik. Enige ander pankreaskaseïen- of sojaboonverteersel wat vergelykbare resultate gee, kan gebruik word.

2) Hierdie reagens moet minstens 15 % (per massa) yster bevat.

3) Na gelang van die jelsterkte van die agar.

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
Dinatriumwaterstofortofosfaat (Na_2HPO_4)	2,5 g
Agar[4)]	1 g tot 5 g
Water	1 000 mℓ

10.4.36.2 Bereiding

Los die bestanddele in die water op deur dit te kook. Suiwer die pH-waarde sodanig aan dat dit na sterilisasie 7,3 sal wees. Plaas die kweekmedium in 10-mℓ-hoeveelhede oor na kweekbuise en steriliseer 15 min lank by 121 °C.

Indien die kweekmedium nie op dieselfde dag gebruik word nie, bewaar dit in 'n koelkas by 4 °C ± 2 °C.

Verhit net voor gebruik 15 min lank in kokende water of vloeiende stoom en verkoel dit dan vinnig tot by die inkubasietemperatuur.

Gooi ongebruikte kweekmedium 4 weke na bereiding weg.

10.4.37 Laktose-gelatienkweekmedium

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 fenolrooi, in die water op. Suiwer die pH-waarde sodanig aan dat dit na sterilisasie 7,5 sal wees. Voeg die laktose en fenolrooi by, meet 10-mℓ-hoeveelhede uit in proefbuise en steriliseer 15 min lank by 121 °C.

Indien die kweekmedium nie op dieselfde dag gebruik word nie, bewaar dit by 4 °C ± 2 °C in 'n koelkas.

Verhit dit net voor gebruik 15 min lank in kokende water of vloeiende stoom en verkoel dan vinnig tot by die inkubasietemperatuur.

Gooi ongebruikte kweekmedium 3 weke na bereiding weg.

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4) Na gelang van die jelsterkte van die agar.

5) Die naam triptose word tans slegs deur sekere kweekmediaprodusente gebruik. Enige ander pankreaskaseïenverteersel wat vergelykbare resultate gee, kan gebruik word.

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, 1 g/ℓ, filtergesteriliseer	20 mℓ

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 onder 45 °C afgekoel het. Suiwer weer die pH-waarde tot 8,7 aan en verdun die oplossing tot 1 ℓ met steriele water. Meet 100-mℓ-volumes asepties uit in steriele 250-mℓ-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 d 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. Moenie oorverhit nie. **Moenie in 'n outoklaaf steriliseer nie.** Verkoel tot by 50 °C, suiwer die pH-waarde tot 8,6 aan en verdun die oplossing tot 1 ℓ met steriele water. 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-waterstofsulfiedagar (wat 30 g/ℓ natriumchloried bevat)

10.4.40.1 Bestanddele

Natriumchloried	30 g
Triptoon	15 g
ℓ-lisienwaterstofchloried, 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. Verkoel en suiwer die pH-waarde tot 7,4 aan. Meet 5-mℓ-volumes uit in kweekbuise (kyk 10.2.3(a)). Steriliseer in 'n outoklaaf en sit die proppe styf op die buise om vogverlies te voorkom.

10.4.41 Onaktiveerderoplossing

10.4.41.1 Bestanddele

Polioksiëtileensorbitaanmono-oleaat	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 5-2-ANSA in 100 mℓ 15-%(per volume)-asynsuuroplossing op. Filtreer deur filtreerpapier. Bewaar in 'n bruin bottel met 'n styfpassende prop (verkieslik met 'n boltipe drupper) by 4 °C.

10.4.42.2 Sulfanielsuuroplossing

Los 0,4 g sulfanielsuur in 100 mℓ 15-%(per volume)-asynsuuroplossing op. Filtreer deur filtreerpapier. Bewaar in 'n bruin bottel met 'n styfpassende prop (verkieslik met 'n boltipe drupper) by 4 °C.

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
Kasitoon	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 % (per volume)) by en laat 10 min lank staan. Los die bestanddele heeltemal op deur dit tot kookpunt te verhit. Verkoel tot tussen 45 °C en 50 °C, meet uit in petribakkies 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 by kookpunt te verhit. Verkoel dit tot tussen 45 °C en 50 °C, meet uit in petribakkies 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 deur verhitting in die water op. Voeg die natriumlourielsulfaat by en meng versigtig 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 bevat (kyk 10.2.11). 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 bevat (kyk 10.2.11). 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 die 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 in kweekbuise (kyk 10.2.3(a)) wat elk 'n omgekeerde Durham-buis bevat (kyk 10.2.11). Steriliseer in 'n outoklaaf. So nie, stoom 20 min lank op elk van drie opeenvolgende dae. Toets vir steriliteit deur 24 h lank by 37 °C te inkubeer.

10.4.49 Selektiewe voorverrykingskweekmedium: 1/2 Fraser-boeljon

10.4.49.1 Basis

10.4.49.1.1 Bestanddele

Vleispeptoon (peptiese verteersel van diereweefsel)	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 tot ongeveer 70 °C te verhit. Verdeel die basale kweekmedium in flesse met 'n geskikte inhoudsvermoë om die porsies wat vir die toets nodig is, te verkry. Steriliseer 15 min lank by 121 °C.

10.4.49.2 Akriflavienoplossing

10.4.49.2.1 Bestanddele

Akriflavien	0,125 g
Water	100 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 mℓ

10.4.49.3.2 Bereiding

Los die yster(III)ammoniumsitraat in die water op. Steriliseer deur filtrering.

10.4.49.4 Volledige 1/2 Fraser-boeljon

Voeg onmiddellik voor gebruik 1,0-mℓ-porsies akriflavienoplossing (kyk 10.4.49.2) en ferriammoniumsitraatoplossing (kyk 10.4.49.3) by elke 100 mℓ basale kweekmedium (kyk 10.4.49.1). Meng versigtig.

Suiwer die pH-waarde van die volledige kweekmedium sodanig aan dat dit 7,2 ± 0,2 is.

10.4.50 Selektiewe verrykingskweekmedium: Fraser-boeljon

10.4.50.1 Basis

10.4.50.1.1 Bestanddele

Vleispeptoon (peptiese verteersel van diereweefsel)	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 water op deur dit tot ongeveer 70 °C te verhit. Verdeel 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 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 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 onmiddellik voor gebruik 0,1-mℓ-porsies akriflavienoplossing (kyk 10.4.50.2) en ferriammoniumsitraatoplossing (kyk 10.4.50.3) by elke buis (10-mℓ-volumes) basis (kyk 10.4.50.1). Meng versigtig. Suiwer die pH-waarde van die volledige kweekmedium sodanig 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 (kyk 10.4.51.1). Steriliseer 15 min lank in die outoklaaf wat op 121 °C gestel is. Verkoel tot by 50 °C en voeg die aanvulling asepties by (kyk 10.4.51.2). Suiwer die pH-waarde van die finale kweekmedium sodanig 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

6) Of ander peptone van gelykstaande kwaliteit.

7) Na gelang van die jelsterkte van die agar.

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 dit 15 min lank by 121 °C in 'n outoklaaf. Verkoel tot by 50 °C.

10.4.52.2 Polimiksien-B-sulfaatoplossing

10.4.52.2.1 Bestanddele

Polimiksien-B-sulfaat (100 000 ie) ..... 0,1 g
Water ..... 100 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.

10.4.52.4 Natriumseftasidiempentahidraatoplossing

10.4.52.4.1 Bestanddele

Natriumseftasidiempentahidraat ..... 0,116 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 mℓ
Natriumseftasidiempentahidraatoplossing (kyk 10.4.52.4) ..... 20 mℓ

10.4.52.5.2 Bereiding

Voeg die volgende by die gesmelte basale kweekmedium by 47 °C en meng versigtig tussen elke byvoeging:

– Polimiksien-B-sulfaatoplossing

– Akriflavienwaterstofchloriedoplossing

– Natriumseftasidiempentahidraatoplossing.

Hou die volledige kweekmedium by 47 °C en gooi so vinnig moontlik 15 mℓ in elk van 'n toepaslike getal petribakkies. Laat dit stol.

Droog onmiddellik voor gebruik die oppervlak van die agarplate versigtig, (verkieslik met die deksels af en die agaroppervlak na onder) 30 min lank in 'n oond wat by 50 °C gehou 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 18,0 g
Water	1 000 mℓ

10.4.53.2 Bereiding

Los die komponente of volledig ontwaterde kweekmedium in die water op deur dit te kook. Indien nodig, suiwer die pH-waarde sodanig 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 in 'n skuins posisie stol.

Vir die bereiding van agarplate, steriliseer die vaste kweekmedium in flesse of bottels met 'n geskikte inhoudsvermoë.

Meet die kweekmedium terwyl dit nog vloeibaar is in hoeveelhede van ongeveer 15 mℓ uit in steriele petribakkies 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 komponente of volledige ontwaterde kweekmedium in die water op deur dit te kook. Indien nodig, suiwer die pH-waarde sodanig 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[8)]	12,0 g tot 18,0 g

10.4.55.2 Vloeibare kweekmedia

Water	1 000 mℓ
Gedefibrineerde perde- of skaapbloed	70 mℓ

8) Na gelang van die jelsterkte van die agar.

10.4.55.3 Bereiding

Los die ontwaterde bloedagarbasis no 2 in die water op deur dit te kook. Indien nodig, suiwer die pH-waarde sodanig ná sterilisasie aan dat dit 7,0 by 25 °C is. Meet die kweekmedium uit in buise of flesse met 'n inhoudsvermoë van hoogstens 500 mℓ. Steriliseer die bloedagarbasis 15 min lank by 121 °C in 'n outoklaaf. Verkoel die kweekmedium tot 47 °C. Voeg die gedefibrineerde bloed by en meng goed. Meet die kweekmedium in hoeveelhede van ongeveer 20 mℓ in steriele petribakkies uit en laat dit stol. Vir die bereiding van die agarplate, steriliseer die vaste kweekmedium in flesse of bottels met 'n geskikte inhoudsvermoë. Meet die kweekmedium in hoeveelhede van ongeveer 15 mℓ in steriele petribakkies uit terwyl dit nog vloeibaar is en laat dit stol.

10.4.56 Koolhidraatgebruiksboeljon

10.4.56.1 Basis

10.4.56.1.1 Bestanddele

Proteosepeptoon	10,0 g
Vleisekstrak	1,0 g
Natriumchloried	5,0 g
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. Indien nodig, suiwer die pH-waarde sodanig aan dat dit na sterilisasie 6,8 by 25 °C is. Meet die kweekmedium in buise uit in sodanige hoeveelhede dat 9 mℓ na sterilisasie sal oorbly. 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-ramnoseoplossing en 100 mℓ D-xiloseoplossing)	5,0 g
Water	100 mℓ

10.4.56.2.2 Bereiding

Los elke koolhidraat afsonderlik in 100 mℓ water op. Steriliseer deur filtrering. Indien nodig, suiwer die pH-waarde sodanig aan dat dit na sterilisasie 6,8 by 25 °C is. Meet die kweekmedium in sodanige hoeveelhede in buise uit dat 9 mℓ na sterilisasie sal oorbly. 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 van die basiskweekmedium berei word, voeg ooreenstemmende kleiner volumes van die 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. Indien nodig, suiwer die pH-waarde sodanig aan dat dit na sterilisasie 7,3 ± 0,2 by 25 °C is. Meet die kweekmedium in buise uit in hoeveelhede van ongeveer 5 mℓ. Steriliseer die buise 15 min lank by 121 °C in 'n outoklaaf.

10.4.58 Brein-hartaftrekselboeljon

10.4.58.1 Bestanddele

Brein-hartaftreksel- 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 om die kweekmedium heeltemal op te los. Steriliseer 15 min lank by 121 °C in 'n outoklaaf. Meet uit in steriele 30-mℓ-bottels (kyk 10.2.2(a)).

10.4.59 EDTA-konynplasma

EDTA-konynplasma is gedroogde konynplasma waarby etileendiamientetra-asynsuur (EDTA) as 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 om te rekonstitueer. Meng deur die buisflessie versigtig 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 die minimum beperk word.

10.5.2 Bereiding van die monster

Indien nodig, ontdooi die rou of gaar produk in sy verpakking by 5 °C tot 10 °C totdat al die sigbare ys gesmelt het. Maak seker dat ontdooiing binne 18 h voltooi is. Verwyder 28 g tot 35 g van die produk met 'n steriele mes en tang en plaas dit oor na 'n voorafgeweegde en gesteriliseerde homogeniseerhouer wat geskik is vir gebruik saam met die homogeniseerder (kyk 10.3.4). Voeg genoeg gebufferde isotoniese peptoonwater by (kyk 10.4.2) om 'n 1:10-dispersie van die produk te verkry. Laat die homogeniseerder net lank genoeg volgens die fabrikant se aanwysings werk om 'n homogene dispersie te lewer, dws laat draaitipe homogeniseerders lank genoeg werk sodat die totale getal omwentelinge 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 aldus verkry word vir die toetse wat in 10.6 tot en met 10.14 beskryf word.

10.6 Standaardplaattelling

10.6.1 Gaar produkte

10.6.1.1 Berei 'n oplossing 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 dispersie van die monster by 9 mℓ peptoonwater en voeg 1 mℓ van hierdie mengsel by 'n verdere 9 mℓ peptoonwater. Meng die inhoud van elke bottel deeglik voordat dit gebruik word en gebruik 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 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 verdunning van die 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), gesmelt en tot by 45 °C afgekoel, by die inhoud van elke plaat en meng. Sorg dat niks van die inhoud van die plaat tydens hierdie proses uitstort nie. Die maklikste is om die plaat op 'n tafelblad te plaas en die plaat versigtig te werwel. Laat die agar stol, keer die plate om, etiketteer hulle en inkubeer by 30 °C. Maak seker dat die totale tydperk tussen die bereiding van die verdunnings van die monster en die finale giet van die plate nie 15 min oorskry nie.

Verwyder die plate na 48 h inkubasie uit die inkubator en tel die kolonies wat in die kweekmedium ontwikkel het. Teken hierdie resultate aan en bereken die gemiddelde getal kolonievormende eenhede per gram van die monster.

10.6.2 Rou produkte

Berei 'n 1:1 000-verdunning van die monster soos in 10.6.1.1 beskryf word. Berei 'n verdere 1:10-verdunning deur 1 mℓ van hierdie verdunning met 9 mℓ van die gebufferde isotoniese peptoonwater te meng (kyk 10.4.2), gaan voort soos in 10.6.1.2 beskryf word en gebruik die verdunning wat só verkry is vir plaatvorming, inkubasie en telling.

10.7 Enterobacteriaceae-telling

Pipetteer 2-mℓ-volumes van die monster (kyk 10.5.2) in elk van drie 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 goed 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, etiketteer hulle toepaslik en inkubeer 24 h lank by 37 °C. Ondersoek en tel al die kolonies waarvan die diameter 0,5 mm oorskry en ignoreer al die ander. Beskou al sodanige kolonies as enterobacteriaceae. Teken hierdie resultate aan en bereken die gemiddelde getal enterobacteriaceae-organismes per gram van die monster.

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 in elk van twee bottels van die dubbelsterkte-briljantgroengalkweekmedium (kyk 10.4.6) uit en inkubeer hulle 16 h tot 20 h lank by 37 °C.

10.8.1.2 Indien die kweekmedium gasproduksie toon, soos deur gas in die Durham-buis aangedui word, gebruik 'n platinumdraadlus en verkry subkulture uit elke bottel deur een lus vol na nog 'n bottel met enkelsterkte-briljantgroengalkweekmedium (kyk 10.4.5) en een lus vol na 'n bottel met triptoonwater (kyk 10.4.7) te subkweek, met albei bottels tot 44 °C voorverhit.

10.8.1.3 Inkubeer albei hierdie subkulture 16 h tot 20 h lank in 'n waterbad by 44 °C ± 0,25 °C. 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. Indien 'n rooi kleur ontwikkel en die kultuur in die briljantgroengalkweekmedium tekens van gasproduksie toon, moet dit beskou word dat die kultuur fekale koliforme bakterieë is.

10.8.2 Rou produkte

Meet 100 mℓ van die monster (kyk 10.5.2) asepties in elk van twee bottels dubbelsterkte-briljantgroengalkweekmedium (kyk 10.4.6) uit en inkubeer dit 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 aan- 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 lewer.

10.9 *Staphylococcus aureus*

10.9.1 Plaatinokuleerprosedure

Plaas deur middel van 'n steriele pipet 'n 1-mℓ-volume van die monster (kyk 10.5.2) oor na die oppervlak van drie Baird-Parker-agarplate (kyk 10.4.9) en versprei die enkele volume egalig oor die drie plate. Beskou dié drie plate as een vir die doel van die telprosedure aangesien hulle die 1:10-verdunning van die monster verteenwoordig. Herhaal bostaande prosedure met nog 'n 1-mℓ-volume en nog drie plate. Inokuleer nog twee Baird-Parker-agarplate, elk met 0,1 mℓ van die monster. Elk van dié plate sal die 1:100-verdunning verteenwoordig.

Sprei die inokulum so gou as moontlik versigtig met individuele steriele glasspreiers (kyk 10.3.5) oor die oppervlak van elk van die agt plate en probeer om nie aan die kante van die bakkie te raak nie. Laat die plate met hul deksels op ongeveer 15 min lank by omgewingstemperatuur droog word. Keer die plate om en inkubeer hulle 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 posisie 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 hierdie helder sone voorkom. Inkubeer alle plate weer vir nog 22 h tot 24 h en merk dan die posisie van nuwe tipiese kolonies.

10.9.2.2 Neem vir telling slegs dié plate met tussen 15 en 150 tipiese of atipiese kolonies. 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 1:10-verdunning van die monster (kyk 10.9.1) geïnokuleer is, hou alle plate wat tipiese of atipiese kolonies bevat. Gebruik 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* hang van die volgende bykomende toetse af:

a) Kol-inokuleer elk van die tipiese kolonies wat uit die Baird-Parker-agarplate (kyk 10.9.2.2) gekies is op 'n plaat mannitolsout-fenolrooi-agar (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 presipiteer en veroorsaak dat die kweekmedium troebel word. Helder sones sal rondom positiewe kolonies ontwikkel.

b) Ondersoek die mannitolsout-fenolrooiagarplate vir kolonies wat 'n geel kleur met 'n helder geel sone rondom die kolonie ontwikkel. Dit dui die omskakeling van mannitol in suur aan. Kolonies wat 'n positiewe DNAse-reaksie toon en suur as 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 brein-hartaftrekselboeljon (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 to 6 h lank vir klontvorming. Beskou die toets as positief indien die volume van die klont meer as drie kwart van die oorspronklike volume van die vloeistof beset.

Staphylococcus aureus kan positief geïdentifiseer word indien groei uit 'n gekose kolonie (kyk 10.9.2) 'n positiewe DNAse-reaksie toon, suur as afbreekproduk van mannitol produseer en die gekose kolonies se koagulasereaksie sterk positief is.

10.9.4 Berekening van die *Staphylococcus aureus*-telling

Bereken in die geval van plate wat positief geïdentifiseerde tipiese of atipiese kolonies bevat (kyk 10.9.2), die aantal *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 uit opeenvolgende verdunnings.

OPM – Rond getalle kleiner as 100 tot die naaste veelvoud van vyf af, en getalle bo 100 wat op vyf eindig, tot die naaste veelvoud van 20. Indien die getal groter as 100 is en nie op vyf eindig nie, rond dit tot die naaste veelvoud van 10 af.

Vermenigvuldig die gemiddelde wat só verkry is 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 van die voorverrykte monster (kyk 10.10.1) oor in 'n fles wat 100 mℓ van die R-V-kweekmedium bevat (kyk 10.4.16) en plaas die hele inhoud van die ander fles (kyk 10.10.1) oor in 'n fles met 100 mℓ van die selenietkweekmedium (kyk 10.4.17).

Inkubeer die geïnokuleerde R-V-kweekmedium tot 48 h lank by 43 °C en die geïnokuleerde selenietkweekmedium tot 48 h lank by 37 °C. Gaan na die eerste 18 h tot 24 h van die inkubasie voort met die diagnostiese plaatkweking sonder om die inhoud van die flesse te skud.

10.10.3 Diagnostiese plaatkweking

10.10.3.1 Gebruik 'n platinumdraadlus met 'n binnediameter van 4 mm en verwyder 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 van die briljantgroen-fenolrooi-agar (kyk 10.4.18) en die ander oor die gedroogde oppervlak van 'n VRG-agarplaat (kyk 10.4.4). Meng dan die inhoud van die fles en herhaal die diagnostiese plaatkweking met twee verdere lusse vol op twee verdere plate. Doen die bestryking op só 'n wyse dat verseker word dat goed geïsoleerde kolonies ontwikkel. Bring paslike 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 verrykingskweekmedia migreer. Dit kom dus voor asof monsterneming op die onversteurde oppervlak die moontlikheid van hul 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 selenietkweekmedium- en R-V-kweekmediumflesse vir nog 24 h tot 28 h in hul onderskeie inkubators terug.

10.10.3.4 Herhaal vir elke kultuur aan die einde van hierdie inkubasietydperk die diagnostiese plaatkweking 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 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. Die herkenning van kolonies *Salmonella*-organismes is 'n saak van ondervinding aangesien hul voorkoms verskil op die twee diagnostiese kweekmedia en van spesie tot spesie en van produksielot tot produksielot van die kweekmedium.

10.10.4 Bevestiging van verdagte kolonies

Kies vyf kolonies van elke tipe verdagte *Salmonella*-organisme op elke plaat, 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 dié wyse of die kultuur wat ondersoek word "suiwer" is. Dit is uiters belangrik dat die kultuur wat aan verdere toetse onderwerp gaan word, suiwer moet wees. Indien daar twyfel bestaan, stryk 'n goed geïsoleerde kolonie oor die gedroogde oppervlak van nog 'n plaat VRG-agar. Inkubeer dié plaat 18 h tot 24 h lank by 37 °C en ondersoek soos hierbo. Indien nodig, herhaal dié prosedure totdat die suiwerheid van die kultuur bo redelike twyfel vasgestel is. Onderwerp dié kultuur aan verdere toetse en sorg dat kontaminasie van die kultuur deur ander mikro-organismes vermy 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 ín die toepaslike kweekmedia en toets vir die reaksies wat in 10.10.5.1 tot 10.10.5.7 gegee word.

10.10.5.1 Driesuikerysteragar

Steek die kultuur in die dik ent en stryk dit oor die agarhellingsoppervlak 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, 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 'n 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 verskyn. Moenie die reaksie as negatief beskou voordat 'n inkubasietydperk 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 van die Kovacs-reagens (kyk 10.4.8) by die inhoud van die bottel. Die ontstaan van 'n rooi kleur 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 die twee buise 24 h lank, die een by omgewingstemperatuur en die ander 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 kaliumhidroksiedoplossing (kyk 10.4.27) by elke buis en meng die inhoud na elke byvoeging.

Die ontwikkeling van 'n pienk tot 'n helderrooi kleur binne 15 min dui 'n positiewe reaksie aan.

10.10.5.7 Oksidasereaksie

Wend 'n klein hoeveelheid bakteriemateriaal uit die suiwer kultuur aan op 'n sitochroomoksidasetoetsstrokie (kyk 10.4.19) 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
Indoolproduksie	1,1
Voges-Proskauer-reaksie	0,0
Oksidasereaksie	0,0

Onderwerp alle kulture aan die serologiese bevestigingstoetse, behalwe dié wat op grond van bogenoemde data duidelik nie *Salmonella*-organismes bevat nie.

10.10.7 Serologiese bevestiging

Indien geskikte polivalente anti-*Salmonella* -"O"-, -"H"- en -"Vi"-serums (kyk 10.4.29 tot 10.4.31) beskikbaar is, ondersoek die verdagte kolonies (kyk 10.10.4) vir die aanwesigheid van *Salmonella* -"O"-, -"H"- en -"Vi"-antigene deur glasplaatagglutinasie. Hou egter in gedagte dat daar vir bevestiging nie net op die resultate van serologiese toetse gesteun moet word nie, en dat dit saam met die resultate van biochemiese bevestiging beoordeel moet word.

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 voorheen 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 amper seker dat geen *Salmonella* aanwesig is nie. Die enigste uitsondering is dat 'n kultuur 'n "H"-antigeen kan hê wat nog nie voorheen teëgekom is nie; en

b) indien die resultaat positief is, ag dat die kultuur positief vir *Salmonella* is.

10.10.8.3 Anti-*Salmonella*-"Vi"-serums

Indien die resultate positief is, ag 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 selektiewe verrykingskweekmedium en die XLD-agar (kyk 10.4.33) as die diagnostiese plaatkweekmedium, en inkubeer in albei gevalle by 37 °C.

Kleurlose deursigtige 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	0,0
Voges-Proskauer-reaksie	30,6
Oksidasereaksie	0,0

10.12 *Clostridium perfringens*

10.12.1 Inokulasie en inkubasie (gietplaattegniek)

Plaas deur middel van 'n steriele pipet 1 mℓ van die dispersie van die monster (kyk 10.5.2) oor na elk van twee petribakkies, giet 15 mℓ tot 20 mℓ van die SC-agar (kyk 10.4.35) in elke bakkie en meng goed met die inokulum deur elke bakkie liggies te roteer. Voeg 'n oorlaag van 10 mℓ van dieselfde SC-agar by sodra die kweekmedium gestol het. Laat stol en plaas die plate met die deksel na bo in anaërobiese flesse of ander geskikte houers en inkubeer 20 h lank by 37 °C. Langer inkubasie kan oormatige verswarting langs die onderste rand van die plate tot gevolg hê.

10.12.2 Tel van kolonies

10.12.2.1 Tel na afloop van die gespesifiseerde inkubasietydperk (kyk 10.12.1) die getal kenmerkende kolonies op die plate volgens 10.12.2.2 en teken dit aan. Kolonies *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 dié kolonies volgens 10.12.3.2.

10.12.3.2 Biochemiese bevestiging

10.12.3.2.1 Bevestiging met gebruik van motiliteitsnitraatkweekmedium

Steekinokuleer die gekose kolonies (kyk 10.12.3.1) in motiliteitsnitraatkweekmedium (kyk 10.4.36). 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. Toets vir die aanwesigheid van nitriet deur 0,2 mℓ tot 0,5 mℓ van die nitrietopspoorreagens (kyk 10.4.42) by elke buis motiliteitsnitraatkweekmedium[9)] te voeg. Die ontstaan van 'n rooi kleur bevestig die reduksie van nitraat tot nitriet.

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9) Om gesondheidsredes kan dit wenslik wees om hierdie toets onder 'n dampkap uit te voer.

Indien daar nie binne 15 min 'n rooi kleur ontstaan het nie, voeg 'n klein hoeveelheid sinkstof by (kyk 10.4.60) en laat 10 min lank staan. Indien 'n rooi kleur na die byvoeging van sinkstof ontstaan, het geen nitraatreduksie plaasgevind nie.

10.12.3.2.2 Bevestiging met behulp van laktose-gelatienkweekmedium

Inokuleer die gekose kolonies (kyk 10.12.3.1) in laktose-gelatienkweekmedium (kyk 10.4.37). Inkubeer 24 h lank in anaërobiese toestande by 37 °C.

Ondersoek die buise laktose-gelatienkweekmedium vir die aanwesigheid van gas en 'n geel kleur (weens suur), wat die fermentasie van laktose aandui. Verkil die buise minstens 1 h lank by 5 °C en gaan na vir gelatienvervloeiing. Indien die kweekmedium gestol het, inkubeer weer vir 'n bykomende 24 h en gaan weer na vir gelatienvervloeiing.

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 binne 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 monster berei is (kyk 10.5.2) 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 dié 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 die kulture te skud en stryk elke lus vol oor 'n diagnostiese *Vibrio*-agarplaat (kyk 10.4.39) op só 'n wyse dat die ontwikkeling van goed geïsoleerde kolonies verseker word. Keer die plate om en inkubeer hulle 18 h tot 24 h lank by 37 °C.

Ondersoek die geïnkubeerde plate vir die aanwesigheid van *Vibrio spp* aan die hand van die volgende eienskappe:

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-waterstofsulfiedagar (kyk 10.4.40) en na ureumagarhellings (kyk 10.4.21) en inkubeer dié 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 groei oor na 'n sitochroomoksidasetoetsstrokie (kyk 10.4.19) en bepaal of die kolonies sitochroomoksidasepositief is (kyk 10.10.5.7).

10.13.3.2 Ondersoek ook die lisien-indool-motiliteit-waterstofsulfiedagarkulture en, indien daar vermoed word dat enige organisme *Vibrio cholerae* of *Vibrio parahaemolyticus* is, stuur 'n monster na 'n aanneemlike toetslaboratorium vir verdere identifikasie.

10.14 Opsporing van *Listeria monocytogenes*

WAARSKUWING – Die opsporing van *Listeria monocytogenes* mag slegs in behoorlik toegeruste laboratoriums onder die beheer van 'n bedrewe mikrobioloog onderneem word en groot sorg moet by die wegdoen van alle geïnkubeerde materiaal gedra word.

10.14.1 Prosedure

10.14.1.1 Bereiding van die monster

Indien nodig, ontdooi die rou of gaar produk in sy verpakking by 5 °C tot 10 °C totdat al die sigbare ys gesmelt het. Maak seker dat ontdooiing binne 18 h voltooi is. Verwyder 25 g tot 35 g van die produk met 'n steriele mes en tang en plaas dit oor na 'n voorafgeweegde en gesteriliseerde homogeniseerhouer 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 net lank genoeg volgens die fabrikant se aanwysings werk om 'n homogene dispersie te lewer, 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 aldus verkry word 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 na 'n buis wat 10 mℓ selektiewe verrykingskweekmedium bevat (kyk 10.4.50). 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) sodanig 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 Keer die bakke om wat in 10.14.1.4.1 en 10.14.1.4.2 verkry is. Plaas hulle in 'n inkubator wat op 37 °C gestel is. PALCAM-agarplate word óf mikro-aërobies in 'n fles met 5 % tot 12 % (per volume) CO_2, 5 % tot 15 % (per volume) O_2 en 75 % (per volume) N_2 geïnkubeer, óf dit word aërobies geïnkubeer.

10.14.1.4.4 Ondersoek die bakke ná inkubasie van 24 h tot 48 h lank soos volg vir die aanwesigheid van tipiese kolonies *Listeria* spp:

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 omring deur swart halo's. Na 48 h is die kolonies swart met 'n diameter van 2 mm tot 3 mm, het die swart halo's en is dit in die middel ingeval.

b) *PALCAM-agar*: laat PALCAM-agarplate 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, is soms swart in die middel, maar het altyd swart halo's.

10.14.1.4.5 Indien groei min is of indien geen kolonies na 24 h inkubasie aanwesig is nie, inkubeer die bakke nog 18 h tot 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 as tipies of verdag beskou word. 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 gedroogde plate triptoonsojagis-ekstrakagar (TSGEA) (kyk 10.4.53) op só 'n wyse dat goed geïsoleerde kolonies sal ontwikkel. Plaas die plate 24 h lank of totdat 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 algehele rand. Indien nodig, ondersoek 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 gemengde kulture sigbaar is, kies 'n tipiese *Listeria*-spp-kolonie en subkweek dit na 'n verdere plaat TSGEA. 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 3-%(per volume)-waterstofperoksiedoplossing op 'n skyfie. *Listeria* spp is katalasepositief, wat deur die onmiddellike vorming van gasborrels gedemonstreer word.

10.14.1.7 Kleureienskappe en morfologie

10.14.1.7.1 Gramkleuring

Voer die Gramkleuring op 'n tipiese kolonie TSGEA (kyk 10.14.1.5.1.2) uit. *Listeria* spp is Gram-positiewe stafies.

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 met TSGEB (kyk 10.4.54). 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 bogenoemde kultuur tussen skyfies en ondersoek die kultuur met 'n mikroskoop. Die *Listeria* spp kom voor as slanke, kort stafies met 'n duidelike tuimelmotiliteit.

Kulture wat by temperature hoër as 25 °C gekweek is, sal dalk nie dié beweging vertoon nie. Vergelyk hulle altyd met 'n bekende kultuur. Cocci, groot stawe of stawe met vinnige swemmotiliteit is nie *Listeria* spp nie. Gebruik as alternatiewe toets vir motiliteit '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 steekplek. *Listeria* spp is motiel en het 'n tipiese sambreelagtige groeipatroon. Indien 'n negatiewe resultaat verkry word, inkubeer vir nog 5 d en neem die steekplek weer 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)[10] om die hemolitiese reaksie te bepaal. Droog die agaroppervlak goed voor gebruik. Trek 'n rooster op die bodem van die plaat en merk 20 tot 25 spasies per plaat. Neem 'n tipiese

10) Meld of skaap- of perdebloed gebruik is.

kolonie uit die TSGEA-plaat (kyk 10.14.1.5.1.2) en steek met 'n lus een spasie vir elke kultuur. Steek gelyktydig positiewe en negatiewe kontrolekulture (*L. monocytogenes, L. ivanovii, L. seeligeri* en *L. innocua*).

Ondersoek ná inkubasie van 24 h tot 48 h by 37 °C die toetsstamme en -kontroles. *L. monocytogenes* toon smal, helder, ligte sones (ß-*haemolysis*)[11]. *L. innocua* behoort geen helder sone rondom die steekplek te toon nie. *L. seeligeri* toon 'n swak sone van hemolise. *L. ivanovii* toon gewoonlik breë, duidelik gedelinieerde sones van ß-*haemolysis*. Lig die plate teen 'n helder lig om toetskulture met kontroles te vergelyk.

10.14.1.7.4 Koolhidraatgebruik

Inokuleer die koolhidraatgebruiksboeljonne (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 deur 'n geel kleur aangedui 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 moliteit 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-*haemolysis* in bloedagarkulture. Van die drie hemolitiese *Listeria* spp, gebruik slegs *L. monocytogenes* nie xilose nie en is positief vir ramnosegebruik.

10.15 Toets vir 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 deur een ent van 'n houtstaaf met 'n lengte ongeveer 140 mm en 'n diameter van ongeveer 2 mm met 'n ronde pluisie absorbeerwatte met 'n massa van 30 mg tot 50 mg te bedek. 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/houers en steriliseer hulle 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 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 dit 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 soos in 10.14.1.1 beskryf word en toets weer.

10.15.2 Monsternemingsprosedure

10.15.2.1 Algemeen

Neem indien moontlik monsters uit minstens 15 verskillende oppervlakke, elk met 'n oppervlakte van nagenoeg 10 cm^2. Teken in vierkante sentimeter die oppervlakte aan van elke oppervlak waaruit 'n monster geneem is.

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11) Dit kan makliker gesien word deur 'n kolonie wat op die oppervlak van die agar rondom die inokulummerk groei, te verwyder.

10.15.2.2 Monsterneming met deppers

Maak 'n depperhouer asepties oop en, indien 'n plastieksakkie gebruik is, maak dit aan die kant weg van die watteplusie oop. Maak seker dat die vingers van die monsternemer nooit tydens die hantering van 'n 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 terselfdertyd die depper sodanig dat die hele oppervlak van die pluisie deeglik in aanraking kom met die oppervlak waarvan 'n monster geneem word. Plaas die depper terug in sy houer en, indien 'n plastieksakkie gebruik is, verseël dit weer. Merk die houer sodanig dat dit met die monsternemingspunt geïdentifiseer kan word.

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 af in 'n 30-mℓ-bottel met gebufferde isotoniese peptoonwater (kyk 10.4.2) deur die bottelnek as hefboom te gebruik. Skud die bottel deeglik. Dié 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. Voeg een 15-mℓ-volume van die plaattellingagar (kyk 10.4.3), gesmelt en tot 45 °C afgekoel, by elke plaat, en meng die inhoud van die plaat deur dit liggies te werwel. Laat stol, keer die plate om en inkubeer 72 h lank by 25 °C.

10.15.3.1.3 Vertolking

Tel die getal bakteriekolonies wat op die twee plate ontwikkel het aan die einde van die inkubasietydperk en teken dit aan. Neem vyf maal dié getal as die getal lewensvatbare bakterieë op die toetsoppervlak waarop die monster geneem is en korrigeer vir 'n oppervlakte van 10 cm^2, indien nodig.

10.15.3.1.4 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 in 10.8.

10.15.3.2 Uitdrukking van skoonmaak- en ontsmettingsdoeltreffendheid

Ken een van die simbole B, RB of OB soos volg aan elke monster toe volgens die getal lewensvatbare bakterieë of die aanwesigheid van fekale koliforme bakterieë:

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)

10.15.3.3 Berekening

Bereken die persentasie skoonmaak- en ontsmettingsdoeltreffendheid aan die hand van die volgende formule:

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

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 'n steriele tangetjie (kyk 10.3.8) 'n steriele membraanfilter (kyk 10.3.6) oor die poreuse plaat, met die roosterkant na bo. Sit die houer weer aanmekaar.

Meng die watermonster deeglik deur die monsterhouer ongeveer 10 keer (met 'n vinnige beweging van die pols) om te keer en regop te bring. Plaas 100 mℓ van die monster asepties oor 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 plaas die membraanfilter met die roosterkant na bo asepties met 'n steriele tangetjie (kyk 10.3.8) oor 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 hulle 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 van hulle (dws kolonies met 'n pienk tot donkerrooi kleur met 'n metaalagtige goudgroen skynsel) in buise of bottels met laktosepeptoonwater (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. 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 verdunningsfaktor 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 in 'n waterdigte houer in 'n waterbad by 44 °C ± 0,25 °C. 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 van hulle (dws kolonies wat blou middelpunte met 'n deurskynende omtrek het) 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 gasvorming toon, soos deur gas in die Durham-buis aangedui word, gebruik 'n platinumdraadlus om uit elke bottel een lus vol in 'n bottel triptoonwater (kyk 10.4.7) te subkweek en inkubeer dié subkulture dan 16 h tot 20 h lank in 'n waterbad by 44,5 °C ± 0,25 °C. 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 per 100 mℓ van die watermonster 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 om te keer en regop te bring (deur 'n vinnige beweging van die pols). Die tyd wat verloop tussen die meng en die 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 vars pipet om 1 mℓ van die primêre oplossing in 'n ander bottel met 9 mℓ steriele peptoonwater (kyk 10.4.47) oor te plaas en meng deeglik. Herhaal hierdie prosedure 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 Inokulasie 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 vars steriele pipet vir elke verdunning. Inkubeer die geïnokuleerde buise of bottels 48 h lank by 37 °C ± 0,5 °C.

Ondersoek die kulture na inkubasie en beskou dié wat troebelheid weens bakteriële groei en gasvorming toon as positiewe reaksies. Gasvorming word aangedui deur 'n hoeveelheid gas wat minstens voldoende is om die holte aan die bopunt van die Durham-buis te vul. Tel en teken vir elke verdunning die getal buise of bottels aan wat 'n positiewe reaksie toon.

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 lourieltriptoseboeljon wat 'n positiewe resultaat gee, in 'n buis of bottel met enkelsterkte-briljantgroengalboeljon (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 wat 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 en bevestig die aanwesigheid van fekale koliforme bakterieë.

10.16.2.4 Uitdrukking van resultate

10.16.2.4.1 Bereken met behulp van tabel 3 en aan die hand van die getal buise of bottels lourieltriptoseboeljon en bevestigende toetse wat positiewe reaksies gee, die waarskynlikste getal koliforme en fekale koliforme bakterieë.

10.16.2.4.2 Kies van verdunnings

Kies vir elke monster wat ondersoek word drie opeenvolgende verdunnings volgens een van die volgende reëls, soos toepaslik:

a) kies die hoogste verdunning (dws met die laagste konsentrasie of die kleinste hoeveelheid van die monster) wat positiewe resultate gee saam met die 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 dié verdunning en die een wat hoër en een wat laer is (kyk 10.16.2.4.4, voorbeeld D), behalwe as 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 uit tabel 3 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 die eerste drie kolomme van tabel 3 te raadpleeg, die toepaslike volgorde van die nommers, bv 3, 2, 1, en lees in die vierde kolom die MPN-indeks, wat in hierdie geval 15 is.

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 met 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 mag van 10 is. Vertrouensgrense word in tabel 3 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 aldus 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 vermenigvuldig met 100, die gespesifiseerde verwysingsvolume, om die getal bakterieë per 100 mℓ van die 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	buise + ; 3 buise –
(verdunning 10^{-3})(1 mℓ)	:	0	buise + ; 3 buise –

Volgens tabel 3 is die MPN-indeks 15 en die berekening gee 'n MPN van $\frac{15}{10} \times 1 \times 100$, dws $1,5 \times 10^2$ 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	buise + ; 3 buise –

Volgens tabel 3 is die MPN-indeks 15 en die berekening gee 'n MPN van $15 \times 10 \times 100$, dws $1,5 \times 10^4$ 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	buise + ; 3 buise –
(verdunning 10^{-2})(1 mℓ)	:	0	buise + ; 3 buise –
(verdunning 10^{-3})(1 mℓ)	:	0	buise + ; 3 buise –

Volgens tabel 3 is die MPN-indeks 1,5 en die berekening gee 'n MPN van $\frac{1,5}{10} \times 1 \times 100$, dws $1,5 \times 10^1$ 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	buise + ; 3 buise –
(verdunning 10^{-2})(1 mℓ)	:	0	buise + ; 3 buise –
(verdunning 10^{-3})(1 mℓ)	:	0	buise + ; 3 buise –

Volgens tabel 3 is die MPN-indeks 0,3 en die berekening gee 'n MPN van $\frac{0,3}{10} \times 1 \times 100$, dws 3×10^0 bakterieë per 100 mℓ van die watermonster.

Tabel 3 — 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 %	≤ 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	1,00
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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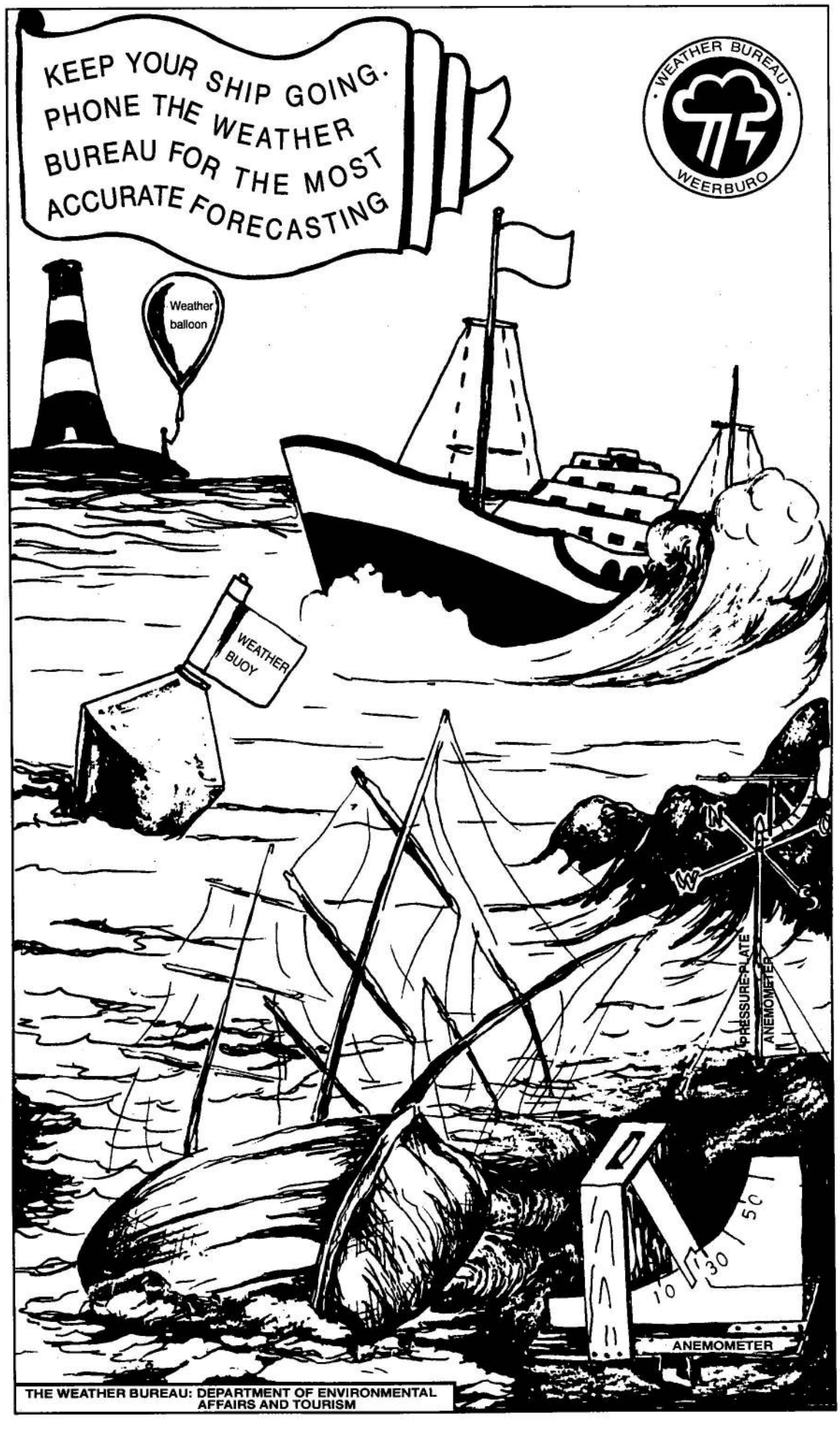
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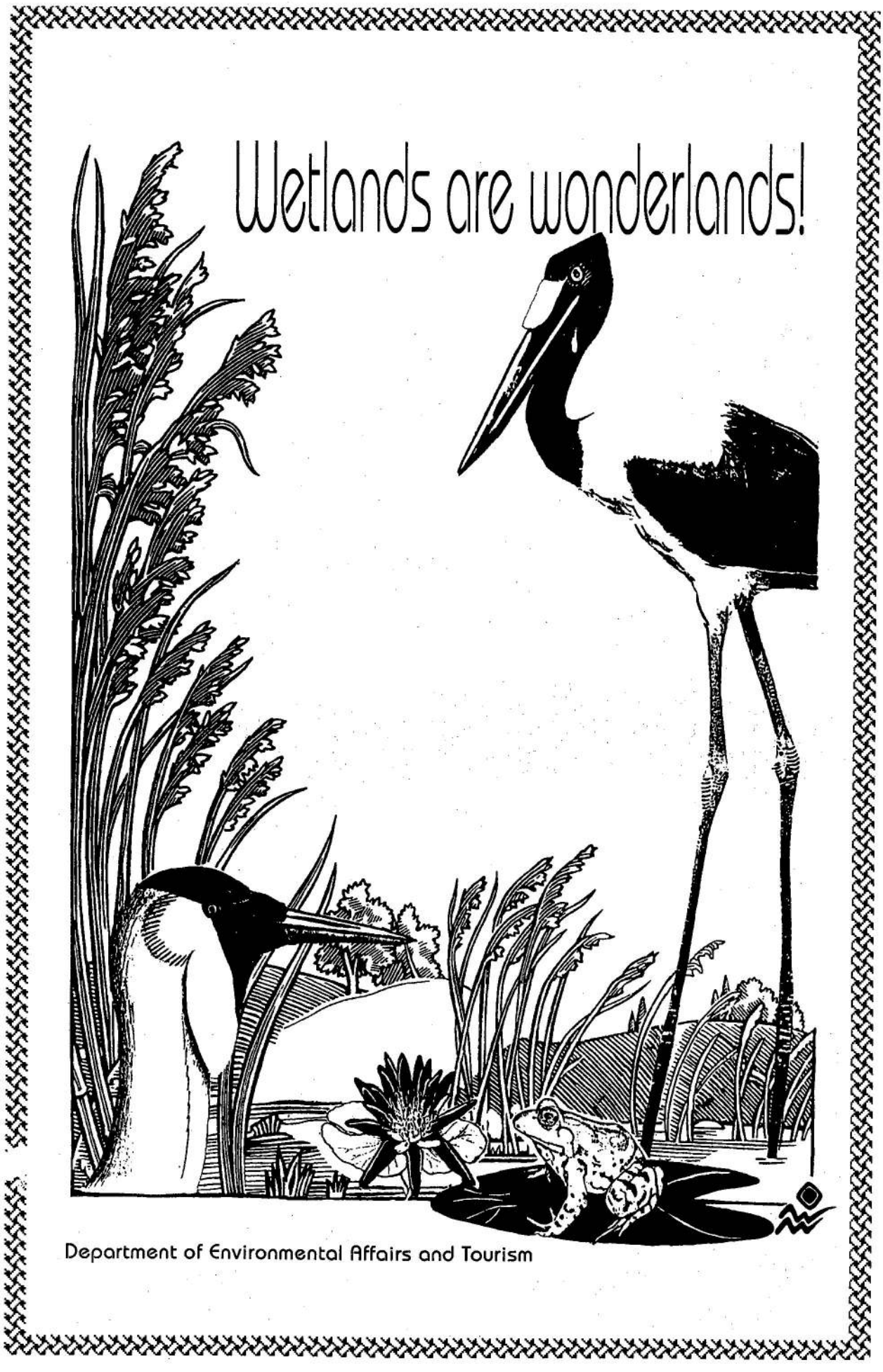
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