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DEPARTMENT OF TRADE AND INDUSTRY
DEPARTEMENT VAN HANDEL EN NYWERHEID

No. R. 529 **14 May 1999**

STANDARDS ACT, 1993

COMPULSORY SPECIFICATION FOR DISINFECTANTS AND DETERGENT-DISINFECTANTS

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 disinfectants and detergent-disinfectants as set out in the Schedule, to be compulsory with effect from the date 2 months after the date of publication of this notice.

ALEXANDER ERWIN
Minister of Trade and Industry

SCHEDULE

COMPULSORY SPECIFICATION FOR DISINFECTANTS AND DETERGENT-DISINFECTANTS

1 Scope

This specification covers requirements for disinfectants and detergent-disinfectants intended for use on inanimate surfaces.

NOTES

1 All disinfectants and detergent-disinfectants in the Republic of South Africa are covered by this compulsory specification. In addition to the requirements in this specification, the requirements promulgated by the Registrar, Agriculture Pests Act, 1983 (Act 36 of 1983), and those in respect of substances subject to the Medicines and Related Substances Control Act, 1965 (Act 101 of 1965), also apply. Biocides for use in water treatment are excluded.

2 As a result of specific applications or assertions made on the label or in the directions for use, it may be necessary also to register the disinfectants or detergent-disinfectants under Act 36 or Act 101, depending on the purpose for application.

3 Using the compulsory specification, it is not possible to determine the bactericidal activity of the undiluted product. Some dilution is always produced by the addition of inoculum, hard water or sterile skimmed milk.

4 Should virucidal efficacy be claimed with reference to this specification, reference may not be made to a specific virus, but a general reference thereto may be made on the label.

5 If a product complies with the requirements of this compulsory specification, it can be considered to be bactericidal or virucidal, but it should not necessarily be inferred that the product is a suitable disinfectant for a defined purpose.

2 Definitions

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

2.1 acceptable: Acceptable to the authority administering this specification.

2.2 address: An address in the Republic of South Africa, which includes the street or road number (if a number has been allocated), the name of the street or road and the name of the suburb, village or town, and in the case of a farm, the name of the farm and of the magisterial district in which it is situated. In the case of imported disinfectants or detergent-disinfectants, "address" means the address of the manufacturer or supplier or importer.

2.3 bacteriophage; phage: A virus that infects bacteria.

NOTE – In this compulsory specification, phage is used as a model to assess general virucidal efficacy.

2.4 batch: That quantity of sealed containers of disinfectant or detergent-disinfectant that have been filled from one homogeneous blend or, in the case of continuous production processes, that have been filled from one day's production.

2.5 clean conditions: Conditions where surfaces are physically clean before the application of the disinfectant or detergent-disinfectant.

2.6 creaming: A phenomenon associated with a concentration of the dispersed phase in one portion

of an emulsion, at the expense of the dispersed phase in the remaining portion of the emulsion.

2.7 critical dilution: The highest dilution with water of a coal-tar type disinfectant or of the standard phenol, which, when tested in accordance with 5.9, permits growth of *Salmonella typhi* after exposure to it of 2,5 min and 5,0 min, but not after exposure of 7,5 min.

2.8 detergent-disinfectant: A product that cleans and disinfects simultaneously.

2.9 dirty conditions: Conditions where organic material is present on surfaces to such an extent that a higher than normal concentration of the disinfectant or detergent-disinfectant is needed to cancel the inactivating effect of the organic material and still ensure disinfection.

2.10 disinfectant: A chemical agent that kills most vegetative forms of pathogenic and other micro-organisms (but not necessarily all bacterial and fungal spores, mycobacteria, rickettsiae or viruses) on inanimate surfaces.

2.11 factory: Any premises in which the disinfectant or detergent-disinfectant is manufactured, produced or repacked or handled in such a way that the quality of the disinfectant or detergent-disinfectant could be affected by the activities.

2.12 inanimate surface: Any surface other than live human or live animal tissue.

2.13 plaque: A clear zone of lysis on a lawn of bacteria, that results from the lytic infection of one bacterium by one bacteriophage, and subsequent infection of surrounding bacteria by the offspring of that phage.

2.14 plaque count: The total number of plaques counted on a lawn of bacteria that develop on a plate of bacterial growth medium incubated at 37 °C.

3 General requirements for the factory and for employees

3.1 General

All the statutory requirements of the Occupational Health and Safety Act, 1993 (Act 85 of 1993), and the Health Act, 1977 (Act 63 of 1977), shall be complied with.

3.2 Factory construction, layout and conditions

3.2.1 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. A system of control shall be maintained to keep the factory free from birds, rodents, insects and other vermin, and to ensure that the disinfectant or detergent-disinfectant is not contaminated and that the quality of the disinfectant or detergent-disinfectant is not compromised in any way.

3.2.2 Roof and ceilings

The roof shall be weatherproof and the ceiling (or underside of the roof if there is no ceiling) shall be smooth and reasonably dustproof.

3.2.3 Walls, floors and doors

Outer walls shall be weatherproof and impermeable to water. Doors and door frames shall be made from corrosion-resistant material or protected to prevent corrosion. Floors shall be constructed of concrete or other durable, impervious and non-slip material that is resistant to wear and corrosion and easy to clean. Provision shall be made for adequate drainage.

3.2.4 Illumination

General illumination shall be such as to permit efficient operations during the manufacture and production of the disinfectant or detergent-disinfectant.

3.2.5 Ventilation

Provision shall be made for an adequate supply of fresh air and the prevention of a build-up of toxic gases. Natural ventilation shall be augmented, if necessary, by mechanical means.

3.2.6 Storage facilities for packaging materials

Containers, closures, cartons and labels for the packing and packaging of the disinfectant of detergent-disinfectant shall be stored in clean, reasonably dustproof, dry storage facilities.

3.2.7 Storage facilities for finished disinfectants or detergent-disinfectants

Finished disinfectants or detergent-disinfectants awaiting dispatch shall be stacked, but not direct upon the floor, in well-ventilated storage facilities. A separate quarantine area shall be provided for the storage of rejected materials.

3.2.8 Refuse

A separate refuse room or other equally adequate refuse facility shall be provided on the premises. Litter and waste shall be disposed of promptly and efficiently in a way that will not compromise the environment and will comply with the requirements of the local authorities.

3.2.9 Housekeeping

The factory and its equipment shall be cleaned and maintained in such a way that the quality of the disinfectant or detergent-disinfectant can be maintained and the safety of personnel ensured.

3.2.10 Water

3.2.10.1 Potable water

Every factory shall have an adequate supply of clean potable water that is free from suspended matter and substances that are deleterious to the disinfectant or detergent-disinfectant or injurious to health.

3.2.10.2 Water for cleaning

Water used for the cleaning of the factory and equipment shall comply with the requirements of 3.2.10.1.

3.2.11 Comfort facilities

Adequate dressing rooms, wash-hand basins, shower baths with hot and cold running water, and sanitary facilities shall be provided.

3.2.12 Testing facilities

The manufacturer shall have access to, and shall use, acceptable testing facilities to ensure that the disinfectant or detergent-disinfectant complies with the requirements of section 4.

3.2.13 Records

Suitable records of the results of the tests carried out shall be available to the administering authority for at least three years.

4 General requirements for the disinfectant or detergent-disinfectant

4.1 Type

The various types of disinfectant or detergent-disinfectant are shown in table 1. These are provided as examples and do not limit the development of new formulations or types.

4.2 Disinfecting efficacy

When the disinfectant or detergent-disinfectant is tested in accordance with the relevant test procedures given in 5.6 to 5.13 and 6.4 (see table 1), at the prescribed concentration, the claims made in regard to the efficacy of the disinfectant or detergent-disinfectant on the package, in the package leaflet or on the label attached to the package (see 7.2(f)) or in product support information, shall be regarded as the disinfecting efficacy requirement for the disinfectant or detergent-disinfectant.

NOTES

1 Should new disinfectants or detergent-disinfectants be developed and formulated, and these methods not be suitable for use on the new disinfectants or detergent-disinfectants, additional test methods might have to be developed and validated before implementation and use. In most cases, however, new disinfectants or detergent-disinfectants can be tested using existing procedures with or without slight modifications.

2 Where more than one active ingredient is used, the substance with the highest concentration determines the type of test.

4.3 Corrosiveness

Where applicable, when the disinfectant or detergent-disinfectant is tested in accordance with 6.1 at the prescribed concentration, it shall not cause more than slight dulling of the surface of the aluminium and the test strip shall show no evidence of pitting, etching or discoloration. If claims are made for non-corrosiveness of a specific material, the manufacturer shall substantiate his claim.

4.4 Water-insoluble-matter content

Where applicable, when the disinfectant or detergent-disinfectant is tested in accordance with 6.2 at the prescribed concentration, the water-insoluble-matter content shall not exceed the appropriate of the following:

a) liquid disinfectants or detergent-disinfectants: 5,0 g/ℓ; or

b) solid disinfectants or detergent-disinfectants: 2,5 % (by mass).

4.5 Rinsing properties of detergent-disinfectants

When the detergent-disinfectant is tested in accordance with 6.3 at the prescribed concentration, it shall be free-rinsing.

NOTE – Although the test for rinsing properties in the case of quaternary ammonium compounds can show that the detergent-disinfectant is free-rinsing, non-rinsable residues might still be retained on the treated surface.

4.6 Cleaning efficacy of detergent-disinfectants

When the detergent-disinfectant is tested in accordance with 6.4 at the prescribed concentration, except in the case of a chlorine type, its mean cleaning efficacy shall be at least 80 % of the mean cleaning efficacy of the standard detergent-disinfectant. In the case of a chlorine type detergent-disinfectant, the mean cleaning efficacy shall be at least 60 % of that of the standard detergent-disinfectant.

Table 1 — Disinfecting efficacy test methods

1	2	3	4	5	6	7	8
Test subsection	**Product**	**Type 1**	**Type 2**	**Initial test organism suspension** per mℓ	**Final organism contact load** per mℓ	**Efficacy limit**	**Test exposure time**
5.6	Disinfectants and detergent-disinfectants based on chlorhexidine gluconate	Liquid	Solid	10^7 - 10^8	10^6	99,9 % or log reduction > 3	1 min
5.7	Disinfectants and detergent-disinfectants based on glutaral-dehyde and other aldehydes for general use	A homogeneous stabilized liquid	A homogeneous liquid, with an activator that could be either a liquid or a solid, supplied separately, which, after mixing, will constitute the disinfectant	10^5	10^4	99,9 %	Pse[1], Esc[2], Sta[3] 5 min Asp[4] 15 min
5.7	Disinfectants based on glutaraldehyde for use on medical instruments	A homogeneous stabilized liquid	A homogeneous liquid, with an activator that could be either a liquid or a solid, supplied separately, which, after mixing, will constitute the disinfectant	10^7	10^6	99,99 %	Pse[1] 5 min Asp[4] 15 min Spores[5] 4 h
5.8	Disinfectants and detergent-disinfectants based on phenolics (Kelsey Sykes)	A homogeneous liquid	A solid	10^8 - 10^{10}	Not applicable	> 2/5 tubes no growth	8 min 18 min
5.9	Coal-tar type disinfectant liquids (black and white) (RW-coefficient)	Black disinfectant liquid	White disinfectant liquid	Not applicable	Not applicable	Not applicable	2,5 min 5 min 7,5 min
5.10	Disinfectants and detergent-disinfectants based on iodophors	Without added acid	With an acceptable acid added	10^5	10^3	99,9 %	5 min
5.10	Disinfectants and detergent-disinfectants based on organic or inorganic halogen com-pounds (other than iodine compounds)	Foaming	Non-foaming	10^5	10^3	99,9 %	30 s

Table 1 *(concluded)*

1	2	3	4	5	6	7	8
Test subsection	**Product**	**Type 1**	**Type 2**	**Initial test organism suspension per mℓ**	**Final organism contact load per mℓ**	**Efficacy limit**	**Test exposure time**
5.10	Disinfectants and detergent-disinfectants based on non-oxidizing surfactants, such as quaternary ammonium compounds, acid-anionics and amphoterics	A homogeneous liquid	A solid	10^5	10^3	99,9 %	5 min
5.10	Disinfectants and detergent-disinfectants based on stabilized inorganic chlorine compounds	Foaming	Non-foaming	10^5	10^3	99,9 %	Recommended minimum exposure period on label
5.11	Peroxygen disinfectants and detergent-disinfectants which may be based on blends such as peroxygen compounds, surfactants, organic acids and buffer systems	A liquid	A solid	10^8	10^6	99,9 %	Pse[1] 5 min Asp[4] 15 min Spores[5] 4 h and 6 h
5.12	Active ingredients not listed elsewhere	As relevant	As relevant	10^8 Bacteria 10^7 Asp[4]	10^7 Bacteria 10^6 Asp[4]	Bacteria log 5 Spores log 1	5 min
5.13	Disinfectants and detergent-disinfectants for which virucidal claims are made	As relevant	As relevant	10^7	10^6	99,9 %	20 min
6.4	Detergent – claims	As relevant	As relevant	–	None	> 80 % of std. but > 60 % of std. for a chlorine type	Not applicable

1) *Pseudomonas aeruginosa.*
2) *Escherichia coli.*
3) *Staphylococcus aureus.*
4) *Aspergillus niger.*
5) *Bacillus subtilis* var. *globigii.*

4.7 Storage stability

4.7.1 Detergent-disinfectants based on iodophors

When the detergent-disinfectant is tested in accordance with 6.5.1:

a) no visible separation shall occur during either the 24 h cooling period or the 24 h warming period; and

b) the highest of the four values of the available iodine content shall not differ from the lowest one by more than 10 % of the lowest value.

4.7.2 Coal-tar type disinfectants

4.7.2.1 Stability before dilution

When coal-tar type disinfectants are tested in accordance with 6.5.2.1, the disinfectant liquid shall not, during either of the two tests, show more than traces of separation of oil at either the top or the bottom. A creamed liquid (see 2.6) that can be rendered uniform by inverting the cylinder and restoring it to an upright position three times, shall not be deemed to have undergone separation.

NOTE – The quick test given in 6.5.2.2 may not be regarded as a substitute for the normal test given in 6.5.2.1, but it may be used to give an indication of the stability of a white disinfectant liquid. In this test, stability is indicated if the diameter of a deposit does not exceed 10 mm.

4.7.2.2 Stability after dilution

When coal-tar type disinfectants are tested in accordance with 6.5.2.3, the disinfectant liquid shall be miscible with the hard water (see 5.4.18.4) at both the highest and the lowest dilutions recommended on the container and the emulsions shall show no sign of separation and not more than traces of separation of oil at either the top or the bottom.

4.7.3 Disinfectants and detergent-disinfectants based on glutaraldehyde

4.7.3.1 When tested in accordance with 6.5.3.1, a type 1 disinfectant or detergent-disinfectant and the liquid component of a type 2 disinfectant or detergent-disinfectant shall remain homogeneous and free-flowing and shall comply with 4.2.

4.7.3.2 When tested in accordance with 6.5.3.2, the solid component of a type 2 disinfectant or detergent-disinfectant shall remain free-flowing, and the type 2 disinfectant or detergent-disinfectant that it activates shall still comply with 4.2.

4.7.3.3 After a type 1 disinfectant or detergent-disinfectant has been diluted or after the two components of a type 2 disinfectant or detergent-disinfectant have been mixed to the prescribed concentration and the disinfectant has been stored in closed dark containers at 25 °C for the effective life as stated on the label (see 7.2(j)), the resulting dilution shall still comply with 4.2.

4.7.4 Disinfectants or detergent-disinfectants based on chlorhexidine gluconate

4.7.4.1 When stored in accordance with 6.5.4.1, the disinfectant or detergent-disinfectant shall remain homogeneous. After standing for a further 24 h at a temperature of 20 °C ± 5 °C, the disinfectant or detergent-disinfectant shall show no sign of precipitation or separation.

4.7.4.2 When stored in accordance with 6.5.4.2, the disinfectant or detergent-disinfectant shall show no sign of precipitation or separation and shall still comply with all the requirements of 4.2.

4.7.5 Disinfectants or detergent-disinfectants based on quaternary ammonium compounds, stabilized inorganic chlorine compounds and organic or inorganic halogen compounds (other than iodine compounds)

4.7.5.1 When a liquid disinfectant or detergent-disinfectant is tested in accordance with 6.5.5.1, it shall remain homogeneous and free-flowing.

4.7.5.2 When a solid disinfectant or detergent-disinfectant is tested in accordance with 6.5.5.2, it shall not cake into hard lumps.

4.8 pH value

Where applicable, when tested in accordance with 6.6 at the prescribed concentration, the pH value shall be as specified on the container (see 7.2(g)).

4.9 Freedom from visible impurities of solid disinfectants or solid detergent-disinfectants based on quaternary ammonium compounds, stabilized inorganic chlorine compounds and organic or inorganic halogen compounds (other than iodine compounds)

When a solid disinfectant or solid detergent-disinfectant is tested in accordance with 6.7, the number of visible specks of impurities shall not exceed five.

4.10 Added colouring matter for disinfectants or detergent-disinfectants based on iodophors

When tested in accordance with 6.8, a disinfectant or detergent-disinfectant shall not contain any added colouring matter.

5 Methods of microbiological testing

5.1 General

The tests shall be undertaken by persons experienced in microbiological techniques, using aseptic techniques.

NOTES

1 In order to ensure accuracy of these tests, it is recommended that each test be repeated.

2 Before these tests are carried out, the efficacy of the inactivating system should be checked to ensure that it adequately inactivates the disinfectants or detergent-disinfectants to be tested.

3 For the purpose of checking the resistance of the test organisms and the other test conditions, it is advisable to include a reference standard. It is essential that it be a disinfectant or detergent-disinfectant based on the relevant active ingredient, but, because a universal standard is difficult to select, each laboratory should make its own choice of material.

5.2 Laboratory ware

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

5.2.1 Universal container culture bottles

Bottles made of glass, fitted with standard screwed metal caps with rubber liners and of nominal capacity:

a) 30 mℓ; and

b) 110 mℓ.

Do not use plastics containers or glass containers fitted with plastics tops.

5.2.2 Culture tubes

Rimless cylindrical tubes that have hemispherical ends and a nominal wall thickness of 1,5 mm and that are of the following sizes:

a) diameter and length 16 mm x 160 mm; and

b) diameter and length 20 mm x 200 mm.

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

5.2.3 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.

5.2.4 Volumetric cylinders

Graduated measuring cylinders with or without stoppers and of capacities 5 mℓ, 10 mℓ, 100 mℓ, 500 mℓ and 1 000 mℓ.

5.2.5 Culture flasks

Culture flasks of capacities 250 mℓ, 500 mℓ and 1 ℓ.

5.2.6 Erlenmeyer flasks

Erlenmeyer flasks of capacities 250 mℓ, 2 ℓ and 3 ℓ.

5.2.7 Petri dishes

Petri dishes of diameter and height 100 mm x 20 mm and made of glass or of wettable polystyrene.

5.2.8 Reagent bottles

Bottles of capacities 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.

5.2.9 Inoculating loop

A length of platinum or platinum-iridium alloy wire of diameter 0,376 mm, mounted in a holder that consists of a thin metal rod or tube. The end of the wire is formed into a loop of diameter 4 mm at a distance of 38 mm from the holder. The loop is at such an angle to the axis of the wire that it can be kept in the horizontal plane while being lifted vertically off the surface of the liquid.

5.2.10 Droplet pipette

A droplet pipette that delivers 0,2 mℓ in about five droplets.

5.2.11 Micropipetter

A micropipetter, high precision, adjusted to dispense 20 µℓ accurately, and sterile tips suitable for use with this micropipetter.

5.3 Equipment

5.3.1 Autoclave

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

5.3.2 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.

5.3.3 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.

5.3.4 Stop-watch

A stop-watch accurate to within 1 s per hour.

5.4 Media and reagents

5.4.1 General

5.4.1.1 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.

5.4.1.2 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.

5.4.1.3 Accuracy

Except where otherwise specified, allow the following tolerances:

a) on temperatures ..... ± 2 °C

b) on masses ..... ± 1,0 %

c) on volumes ..... ± 1,0 %

d) on pH value ..... ± 0,1

5.4.1.4 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 these are used, follow the manufacturer's instructions strictly with regard to reconstitution and sterilization.

5.4.1.5 Filtration of media

Whenever it is necessary to filter a medium in the course of its preparation, proceed as follows:

a) filter a medium that does not contain a solidifying agent, i.e. a liquid medium or broth, through a medium-speed filter paper; or

b) if the medium contains a solidifying agent (for example, agar) filter it through a 10 mm to 15 mm thick layer of pre-wetted absorbent cotton wool. To prevent solidification of the medium during filtration, use a steam-jacketed funnel. Alternatively, carry out the filtration in a steam chamber.

5.4.1.6 Adjustment of the pH value of media

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

5.4.1.7 Dispensing

Where specified quantities of media are to be dispensed into bottles, use 30 mℓ universal bottles (see 5.2.1(a)). Where bulk sterilizing is required, use any suitable glass container of the required quality or suitably stoppered culture tubes (see 5.2.2(a)). Dispense reagents into reagent bottles (see 5.2.8). 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, place the bottles or, when relevant, the culture tubes, on a 1-in-4 sloped surface and allow the agar to solidify.

5.4.1.8 Sterilization

When sterilization by autoclaving is specified and unless otherwise directed, autoclave the medium at 121 °C for 15 min.

5.4.1.9 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.

5.4.1.10 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.

5.4.2 Bottom layer agar

5.4.2.1 Ingredients

Tryptone	13,0 g
Agar	11,0 g
Sodium chloride	8,0 g
Glucose	1,0 g
Water	1 000 mℓ

5.4.2.2 Preparation

Dissolve the ingredients in the water by warming. Cool to 45 °C to 50 °C and dispense into Petri dishes of nominal diameter 90 mm, ensuring that the depth of the agar in each plate is at least 3 mm.

5.4.3 Bovine albumin solution

5.4.3.1 Ingredients

Albumin (bovine)	15,0 g
Water	1 000 mℓ

5.4.3.2 Preparation

Dissolve the albumin in the water. Sterilize by passing through a filter with maximum effective pore size of 0,45 µm. Dispense 10 mℓ volumes into bottles (see 5.2.1(a)).

5.4.4 Cetrimide inactivator

5.4.4.1 Ingredients

Polyoxyethylene sorbitan mono-oleate	8,0 g
Sodium taurocholate	8,0 g
Sodium thiosulfate	1,5 g
Potassium phosphate, monobasic	0,5 g
Sodium citrate	0,5 g
Water	1 000 mℓ

5.4.4.2 Preparation

Dissolve the ingredients in the water by heating. Dispense 20 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving.

5.4.5 Diluent used in the "5,5,5" test

5.4.5.1 Ingredients

Albumin (bovine)	0,3 g
Sodium chloride	9,0 g
Water	1 000 mℓ

5.4.5.2 Preparation

Dissolve the ingredients in the water. Sterilize by passing through a filter with maximum effective pore size of 0,45 µm. Dispense 10 mℓ volumes into bottles (see 5.2.1(a)).

5.4.6 Hard water used in preparing the yeast suspension

5.4.6.1 Ingredients

Calcium chloride ($CaCl_2$)	1,0 g
Magnesium chloride ($MgCl_2.6H_2O$)	8,5 g
Water	1 000 mℓ

5.4.6.2 Preparation

Dissolve the ingredients in the water. Dispense 100 mℓ volumes into bottles (see 5.2.1(b)) and sterilize by autoclaving.

5.4.7 Inactivator media

5.4.7.1 Inactivator medium No. 1

5.4.7.1.1 Ingredients

Monopotassium phosphate (KH_2PO_4)	0,5 g
Sodium citrate ($Na_3C_6H_5O_7.3H_2O$)	0,5 g
Sodium taurocholate	8,0 g
Sodium thiosulfate ($Na_2S_2O_3.5H_2O$)	1,5 g
Polyoxyethylene sorbitan mono-oleate	8,0 g
Water	1 000 mℓ

5.4.7.1.2 Preparation

Dissolve the ingredients in the water by heating. Dispense 20 mℓ volumes into bottles (see 5.2.1(a)). Sterilize by autoclaving.

NOTE – This inactivator has been found suitable for disinfectants and detergent-disinfectants based on iodophors, organic halogen compounds (other than iodine compounds) and most quaternary ammonium compounds.

5.4.7.2 Inactivator medium No. 2

5.4.7.2.1 Ingredients

Polyoxyethylene sorbitan mono-oleate	30,0 g
Beef extract	20,0 g
Peptone	20,0 g
Sodium chloride	10,0 g
Water	1 000 mℓ

5.4.7.2.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7.1. Dispense 9 mℓ and 10 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving.

NOTE – This inactivator has been found suitable for disinfectants and detergent-disinfectants based on glutaraldehyde.

5.4.7.3 Inactivator medium for the "5,5,5" test

5.4.7.3.1 Ingredients

Lecithin (made from soya, purified)	3,0 g
ℓ-histidine	1,0 g
Phosphate buffer 0,25N (see 5.4.12)	10,0 mℓ
Sodium thiosulfate ($Na_2S_2O_3.5H_2O$)	5,0 g
Polyoxyethylene sorbitan mono-oleate	30,0 mℓ
Water	1 000 mℓ

5.4.7.3.2 Preparation

Dissolve the ingredients in the water by heating. Dispense 20 mℓ volumes into bottles (see 5.2.1(a)).

Sterilize by autoclaving.

5.4.7.4 Neutralizer broth medium

Use TSB (see 5.4.22) but to each 100 mℓ of broth, add 3 g of soy lecithin (azolectin) and 20 g of polyoxyethylene sorbitan mono-oleate. When testing an antiseptic that contains cetrimide, also add 20 mℓ of cetrimide inactivator (see 5.4.4). Adjust the pH value to between 7,0 and 7,4. Dispense 9 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving for 20 min.

NOTES

1 In order to dissolve the azolectin and polyoxyethylene sorbitan mono-oleate in the TSB, use half the volume of TSB in a large container and add the azolectin and polyoxyethylene sorbitan mono-oleate while stirring. Boil for 30 min to 60 min with constant stirring until all the azolectin granules are dissolved. Allow to cool before diluting to the final volume with TSB. Adjust the pH value and dispense as required. Do not fill the bottles to more than half their volume before sterilizing, to prevent boiling over of the contents, which will alter the azolectin/polyoxyethylene sorbitan mono-oleate ratio.

2 Sometimes the azolectin/polyoxyethylene sorbitan mono-oleate emulsion settles to the bottom of the container. If this happens, storage of approximately one week at room temperature usually allows the solids to redissolve. If the neutralizer is required sooner, heating in a water-bath at 100 °C followed by occasional swirling during cooling will redissolve the solids.

3 This inactivator has been found suitable for antiseptics based on chlorhexidine gluconate and for some disinfectants and detergent-disinfectants based on quaternary ammonium compounds and glutaraldehyde.

5.4.8 Malt extract agar

5.4.8.1 Ingredients

Malt extract	30,0 g
Agar	15,0 g
Soya peptone	5,0 g
Water	1 000 mℓ

5.4.8.2 Preparation

Dissolve the ingredients in the water. Dispense 10 mℓ and 15 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving. Allow only the 10 mℓ volumes to solidify in a sloped position.

5.4.9 Nutrient agar

5.4.9.1 Ingredients

Agar	15,0 g
Peptone	5,0 g
Sodium chloride	5,0 g
Yeast extract	2,0 g
Beef extract	1,0 g
Water	1 000 mℓ

5.4.9.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,1. Dispense 10 mℓ and 15 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving. Allow only the 10 mℓ volumes to solidify in a sloped position.

5.4.10 Nutrient broth No. 2 (double strength)

5.4.10.1 Ingredients

Peptone ..... 10,0 g
Sodium chloride ..... 5,0 g
Refined meat extract ..... 10,0 g[1)]
Water ..... 1 000 mℓ

5.4.10.2 Preparation

Dissolve the ingredients in water, adjust the pH value to 7,1 and dilute the solution to 1 ℓ. Dispense 5 mℓ volumes into culture tubes (see 5.2.2(a)) and sterilize by autoclaving.

5.4.11 Nutrient medium

5.4.11.1 Ingredients

Peptone ..... 5,0 g
Sodium chloride ..... 5,0 g
Yeast extract ..... 2,0 g
Beef extract ..... 1,0 g
Water ..... 1 000 mℓ

5.4.11.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,1. Dispense 10 mℓ and 50 mℓ volumes into bottles (see 5.2.1(a) and 5.2.1(b), respectively) and sterilize by autoclaving.

5.4.12 Phosphate buffer 0,25N

5.4.12.1 Ingredients

Monopotassium phosphate (KH_2PO_4) ..... 34,0 g
Water ..... 500 mℓ

5.4.12.2 Preparation

Dissolve the monopotassium phosphate in the water. Adjust the pH value to 7,2 with 1N NaOH. Dispense into 30 mℓ bottles (see 5.2.1(a)) and sterilize by autoclaving.

5.4.13 Physiological saline solution

5.4.13.1 Ingredients

Sodium chloride ..... 9,0 g
Water ..... 1 000 mℓ

5.4.13.2 Preparation

Dissolve the sodium chloride in the water. Dispense into 250 mℓ culture flasks (see 5.2.5) and sterilize by autoclaving.

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1) Meat extract made from specially selected raw materials of a light colour adjusted to neutrality and dried to a fine powder.

5.4.14 Reculture medium

5.4.14.1 Ingredients

Beef extract ..... 10 g
Peptone ..... 10 g
Sodium chloride ..... 5 g
Polyoxyethylene sorbitan mono-oleate ..... 30 g
Water ..... 1 000 mℓ

5.4.14.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7,5. Dispense 10 mℓ volumes into culture tubes (see 5.2.2(a)) and sterilize by autoclaving.

5.4.15 Sodium thiosulfate (20 g/ℓ)

5.4.15.1 Ingredients

Sodium thiosulfate ..... 20 g
Water ..... 1 000 mℓ

5.4.15.2 Preparation

Dissolve the sodium thiosulfate in the water, dispense 20 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving.

NOTE – This inactivator has been found suitable for disinfectants and detergent-disinfectants based on stabilized inorganic chlorine compounds and stabilized chlorine compounds.

5.4.16 Sodium thiosulfate (10 g/ℓ)

5.4.16.1 Ingredients

Sodium thiosulfate ..... 10 g
Water ..... 1 000 mℓ

5.4.16.2 Preparation

Dissolve the sodium thiosulfate in the water, dispense 20 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving.

5.4.17 Sporulation medium SM 1

5.4.17.1 Ingredients

Agar ..... 12,0 g
Manganese sulfate ($MnSO_4.4H_2O$) ..... 0,03 g
Dipotassium phosphate ..... 4,0 g
Nutrient broth ..... 3,125 g
Water ..... 1 000 mℓ

5.4.17.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 6,6. Dispense 20 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving. Allow the medium to solidify in a sloped position.

5.4.18 Standard hard water

5.4.18.1 Standard hard water No. 1

5.4.18.1.1 Ingredients

Calcium chloride ..... 2,1 g
Water ..... 7 500 mℓ

5.4.18.1.2 Preparation

Dissolve the calcium chloride in the water. Dispense 97 mℓ volumes into 110 mℓ bottles (see 5.2.1(b)) and sterilize by autoclaving.

NOTE – This hard water has been found suitable for the microbiological testing of disinfectants and detergent-disinfectants based on glutaraldehyde, organic halogen compounds (other than iodine compounds), quaternary ammonium compounds, stabilized inorganic chlorine compounds and stabilized chlorine compounds.

5.4.18.2 Standard hard water used for the microbiological testing of disinfectants and detergent-disinfectants based on iodophors

5.4.18.2.1 Ingredients

Magnesium chloride ..... 18,5 g
Calcium chloride ..... 7,9 g
Sodium bicarbonate ..... 22,4 g
Water ..... 2 500 mℓ

5.4.18.2.2 Preparation

Dissolve the magnesium chloride and calcium chloride in water, adjust the pH value to between 7,6 and 8,0 and dilute to 1 ℓ with water. Sterilize by autoclaving (solution A). Dissolve the sodium bicarbonate in water, adjust the pH value to between 7,6 and 8,0 and dilute to 1 ℓ with water. Sterilize by filtration (solution B). Add 1 mℓ of each of solutions A and B to 95 mℓ of sterile water in an Erlenmeyer flask (see 5.2.6).

5.4.18.3 Standard hard water for use in the Kelsey-Sykes test

5.4.18.3.1 Ingredients

Calcium chloride ($CaCl_2$) ..... 0,304 g
Magnesium chloride ($MgCl_2.6H_2O$) ..... 0,139 g
Water ..... 1 000 mℓ

5.4.18.3.2 Preparation

Dissolve the ingredients in the water. Dispense 100 mℓ volumes into 110 mℓ bottles (see 5.2.1(b)) and sterilize by autoclaving.

5.4.18.4 Standard hard water used in the Rideal-Walker test

5.4.18.4.1 Ingredients

Calcium chloride ..... 0,15 g
Magnesium sulfate ..... 0,15 g
Water ..... 1 000 mℓ

5.4.18.4.2 Preparation

Dissolve the ingredients in the water. Dispense 100 mℓ volumes into 110 mℓ bottles (see 5.2.1(b)) and sterilize by autoclaving.

5.4.19 Standard phenol solution (50 g/ℓ)

Using pure phenol that has a crystallizing point not lower than 40,5 °C, prepare a 50 g/ℓ stock solution in water and use this for making the control dilutions (see 5.10.2).

NOTE – It is important that pure phenol be used, because cresol has approximately three times the bactericidal efficacy of phenol, and error resulting from its presence (as indicated by reduction of the crystallizing point to below 40,5 °C) might be considerable.

5.4.20 Sterile skimmed milk

5.4.20.1 Blend 50,0 g of skimmed milk powder with 400 mℓ of water in a high-speed blender for 5 min to 7 min. Dilute to 500 mℓ with water.

5.4.20.2 Filter through a coarse filter paper that has been previously wetted, or centrifuge for 10 min at a resultant centrifugal force of 6 kN/kg. If there is a visible residue on the filter paper or in the centrifuge tube, repeat the procedure with a different batch of skimmed milk.

5.4.20.3 Dispense 15 mℓ to 25 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving for 10 min. Store in a refrigerator maintained at 4 °C.

5.4.20.4 Test for freedom from growth-inhibiting factors

5.4.20.4.1 Using sterile water, prepare a suspension of *Staphylococcus aureus* (see 5.5.2.1). So standardize the suspension, by using a spectrophotometer in conjunction with a standard curve, a haemocytometer, Petroff-Häusser counting chamber or any other suitable means, that it contains approximately 100 million organisms (10^8) per millilitre. Use the suspension within three hours of preparation.

5.4.20.4.2 Mix 0,12 mℓ of this suspension with 15 mℓ of melted nutrient agar (see 5.4.9) that has been cooled to 45 °C and pour the mixture into a flat-bottomed Petri dish of diameter 100 mm (see 5.2.7), placed on a level surface. After the agar has set, place the Petri dish for at least 15 min in a refrigerator maintained at 2 °C to 5 °C. Put three sterile, polished, stainless steel cylinders (penicillin cups) of outer diameter 8 mm ± 0,5 mm, inner diameter 6 mm ± 0,5 mm and length 10 mm ± 1 mm on the agar and fill each with the milk to be tested.

5.4.20.4.3 Leave the Petri dish in the refrigerator for 2 h and then incubate the Petri dish at 37 °C for 16 h to 20 h. Remove the cylinders from the Petri dish and examine the set agar plate for zones of growth inhibition.

5.4.20.4.4 Repeat the procedure given in 5.4.20.4.1 to 5.4.20.4.3 (inclusive), using:

a) *Escherichia coli* as the test organism, with:

   1) a suspension containing 650 million ($6,5 \times 10^8$) organisms per millilitre; and
   2) an inoculum of 0,15 mℓ in 15 mℓ of melted nutrient agar; and

b) *Pseudomonas aeruginosa* as the test organism, with:

   1) a suspension containing 500 million ($5,0 \times 10^8$) organisms per millilitre; and
   2) an inoculum of 0,15 mℓ in 15 mℓ of melted nutrient agar.

5.4.20.4.5 If no zones of growth inhibition are visible on any of the three sets of plates, the milk may be used for the test.

5.4.21 Top layer agar

5.4.21.1 Ingredients

Tryptone	10,0 g
Sodium chloride	8,0 g
Agar	6,0 g
Glucose	3,0 g
Water	1 000 mℓ

5.4.21.2 Preparation

Dissolve the ingredients in the water by warming. Dispense 90 mℓ volumes into 110 mℓ bottles (see 5.2.1(b)) and sterilize by autoclaving.

5.4.22 Tryptone soy broth (TSB)

5.4.22.1 Ingredients

Tryptone	17,0 g
Sodium chloride	5,0 g
Soy peptone	3,0 g
Potassium hydrogen dibasic-phosphate	2,5 g
Dextrose	2,5 g
Water	1 000 mℓ

5.4.22.2 Preparation

Dissolve the ingredients in the water, heating if necessary. Adjust the pH value to 7,3, dispense 10 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving.

5.4.23 Wright and Mundy medium (synthetic broth AOAC)

5.4.23.1 Part A

5.4.23.1.1 Ingredients

ℓ-cystine	0,05 g
d ℓ-methionine	0,37 g
ℓ-arginine hydrochloride	0,4 g
d ℓ-histidine hydrochloride	0,3 g
ℓ-lysine hydrochloride	0,85 g
ℓ-tyrosine	0,21 g
d ℓ-threonine	0,5 g
d ℓ-valine	1,0 g
ℓ-leucine	0,8 g
d ℓ-isoleucine	0,44 g
Glycine	0,06 g
d ℓ-serine	0,61 g
d ℓ-alanine	0,43 g
ℓ-glutamic acid hydrochloride	1,3 g

ℓ-aspartic acid	0,45 g
d ℓ-phenylalanine	0,26 g
d ℓ-tryptophan	0,05 g
ℓ-proline	0,05 g
Water	500 mℓ

5.4.23.1.2 Preparation

Dissolve the ingredients in the water and add 18 mℓ of a sodium hydroxide (c(NaOH) = 1 mol/ℓ) solution.

5.4.23.2 Part B

5.4.23.2.1 Ingredients

Sodium chloride	3,0 g
Potassium chloride	0,2 g
Magnesium sulfate	0,05 g
Potassium phosphate	1,5 g
Disodium phosphate	4,0 g
Thiamine hydrochloride	0,01 g
Nicotinamide	0,01 g
Water	500 mℓ

5.4.23.2.2 Preparation

Dissolve the ingredients in the water. Mix Parts A and B. If necessary, adjust the pH value to 7,1. Dispense half of the medium in 10 mℓ ± 0,2 mℓ volumes and the other half in 6 mℓ ± 0,2 mℓ volumes into bottles (see 5.2.1(a)) and sterilize by autoclaving.

Before use, add to each tube in the two sets of bottles 0,1 mℓ and 0,06 mℓ, respectively, of a sterile 100 g/ℓ solution of glucose.

5.4.24 Yeast suspension

5.4.24.1 Preparation of 20 % (by mass) of moist yeast suspension

NOTES

1 Whenever possible, the 20 % (by mass) of moist yeast suspension should be sterilized on the day the yeast is received. If this is not feasible, the unopened yeast package should be stored at a temperature not higher than 5 °C for not longer than 48 h before use.

2 Information regarding sources of yeast can be obtained from the South African Bureau of Standards, Private Bag X191, PRETORIA, 0001.

Crumble approximately 500 g of baker's yeast by hand into a previously tared 1 ℓ beaker and determine the mass of the moist yeast. Cream the yeast by adding a small volume of hard water (see 5.4.6) while stirring the mixture. Carefully transfer the creamed portion to an Erlenmeyer flask of capacity 2 ℓ (see 5.2.6) and add a further small volume of hard water to any lumpy residue remaining in the beaker. Continue this process until all the yeast has been transferred from the beaker to the flask and the concentration of the yeast suspension in the flask has been reduced to approximately 40 % (by mass) of moist yeast. Shake the contents of the flask vigorously and remove large particles by passing the suspension through a sieve of aperture size 140 µm, supported in a funnel in an Erlenmeyer flask of capacity 3 ℓ (see 5.2.6). Add enough hard water to reduce the concentration of the yeast to approximately 20 % (by mass) of moist yeast. Shake thoroughly and, while agitating, dispense 100 mℓ volumes into bottles (see 5.2.1(b)). Sterilize by autoclaving and store at 4 °C until required for use.

5.4.24.2 Determination of moisture content

Pipette 25 mℓ of the sterilized yeast suspension (see 5.4.24.1) into a dry tared dish and dry to constant mass in a hot air oven maintained at 100 °C. Use this mass to determine the additional volume of hard water that must be added to each bottle of sterilized yeast suspension to make a suspension that contains exactly 5 % (by mass) of dry yeast.

5.4.24.3 Adjustment of pH value

Using a sodium hydroxide (c(NaOH) = 1 mol/ℓ) solution, adjust the pH value of 100 mℓ of the 20 % (by mass) of moist yeast suspension (see 5.4.24.1) to 7,0, and note the volume of the sodium hydroxide solution required.

5.4.24.4 Preparation of 5 % (by mass) of dry yeast suspension

Immediately before use add to 100 mℓ of 20 % (by mass) of moist yeast suspension (see 5.4.24.1), the volume of hard water (see 5.4.6) necessary to make a suspension that contains exactly 5 % (by mass) of dry yeast and enough sodium hydroxide (c(NaOH) = 1 mol/ℓ) solution (see 5.4.24.3) to adjust the pH value to 7,0. Store the yeast suspension at 4 °C for not longer than 7 d before use.

5.5 Test organisms[2)]

Use the following test organisms:

a)	*Staphylococcus aureus*	:	SABS TCC Sta 53 and SABS TCC Sta 59;
b)	*Escherichia coli*	:	SABS TCC Esc 25;
c)	*Escherichia coli* K-12 Hfr, NCTC12486	:	SABS TCC Esc 36;
d)	*Escherichia coli* 36 with bacteriophage MS2	:	SABS TCC Phg-C1 in cases where virucidal claims are made;
e)	*Escherichia coli* ATCC 13706	:	SABS TCC Esc 37;
f)	*Escherichia coli* 37 with bacteriophage ΦX174	:	SABS TCC Phg-C2 in cases where virucidal claims are made;
g)	*Pseudomonas aeruginosa*	:	SABS TCC Pse 2 and SABS TCC Pse 16;
h)	*Salmonella typhi*	:	SABS TCC Sal 10 (NCTC 786, Lister strain)
I)	*Aspergillus niger*	:	SABS TCC 355 in cases where fungicidal claims are made; and
j)	*Bacillus subtilis* var. *globigii*	:	SABS TCC Bac 35 in cases where sporicidal claims are made.

Organisms that have survived the action of a disinfectant or detergent-disinfectant shall under no circumstances be used in a test.

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2) Obtainable from the South African Bureau of Standards, Private Bag X191, PRETORIA, 0001.

NOTES

1 Additional organisms may be used if so desired.

2 The extreme importance of using the standard strain is emphasized.

5.5.1 Maintenance of test organisms

5.5.1.1 *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa*

5.5.1.1.1 From a newly opened freeze-dried culture or recently received agar culture, subculture the test organisms into bottles of 10 mℓ nutrient medium (see 5.4.11).

(Use tryptone soy broth (see 5.4.22) in the case of disinfectants based on chlorhexidine gluconate.)

5.5.1.1.2 Incubate the bottles at 37 °C for 24 h. Make subcultures from the cultures in the bottles onto slopes of nutrient agar (see 5.4.9). Incubate the slopes at 37 °C for 24 h.

5.5.1.1.3 From each of these slope cultures, prepare four subcultures (stock cultures) of each test organism on 10 mℓ nutrient agar slopes (see 5.4.9). Incubate the stock cultures at 37 °C for 24 h and then store them in a refrigerator maintained at 4 °C.

NOTE – Take not more than six serial subcultures from each stock culture before resorting to a new freeze-dried culture.

5.5.1.2 *Aspergillus niger*

Inoculate slopes of malt extract agar (see 5.4.8) with *Aspergillus niger* and incubate at 25 °C for 7 d.

5.5.1.3 *Bacillus subtilis* var. *globigii*

5.5.1.3.1 Inoculate slopes of sporulation medium SM 1 (see 5.4.17) with *B. subtilis* var. *globigii* and incubate at 30 °C for 7 d and then at ambient temperature until 80 % to 100 % spores are present (i.e. approximately 5 d).

5.5.1.3.2 Determine the degree of sporulation microscopically. When 80 % to 100 % spores are present, harvest the spores by adding sterile water and gently scraping the agar surface. Alternatively, use a sterile glass rod or sterile beads to rub the spores off.

5.5.1.3.3 Centrifuge the suspension for 20 min to 30 min. Decant the supernatant liquid. Wash the spores 3 times to 4 times with sterile water until the supernatant liquid is completely clear. Re-suspend the spores in sterile water and heat the suspension for 10 min at 80 °C. Cool the suspension rapidly and store at 4 °C for 7 d.

5.5.1.3.4 Finally, wash the spore suspension once more in sterile distilled water and so standardize the suspension, by using a spectrophotometer in conjunction with a standard curve, a haemocytometer, Petroff-Häusser counting chamber or any other suitable means, that it contains at least 10 million (10^7) spores per millilitre.

5.5.1.3.5 Dispense into sterile bottles (see 5.2.1(a)) and store the spore suspension at 4 °C until needed.

5.5.2 Preparation of cultures for test suspensions

5.5.2.1 *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*

5.5.2.1.1 For each of the test organisms, inoculate a nutrient agar slope (see 5.4.9) from a stock culture kept at 4 °C (see 5.5.1.1.3) and incubate at 37 °C for 24 h.

5.5.2.1.2 For the test, use a 24 h culture that has been subcultured for two successive days. After six subcultures, restart the process, using a fresh stock culture (see 5.5.1.1.3).

NOTE – The physiological condition of the test organisms is important and might influence inter-laboratory and intra-laboratory variations in test results.

5.5.2.1.3 Using 10 mℓ of sterile water, wash the bacterial growth resulting from 24 h incubation from the slope (see 5.5.2.1.1), scraping the agar surface if necessary. Carefully decant the suspended growth into a sterile Erlenmeyer flask (see 5.2.6) and shake vigorously to suspend all growth in the water. So standardize the suspension, by using a spectrophotometer in conjunction with a standard curve, a haemocytometer, Petroff-Häusser counting chamber or any other suitable means, that it contains the required number of organisms per millilitre as stipulated in the individual microbiological efficacy tests. Use the suspension within three hours of preparation.

5.5.2.2 *Salmonella typhi*

5.5.2.2.1 Transfer a small portion of growth from a stock culture (see 5.5.1.1.3) to 5 mℓ of Nutrient broth No. 2 (double strength) (see 5.4.10) and incubate at 37 °C for 24 h.

5.5.2.2.2 Continue subculturing into fresh tubes of Nutrient broth No. 2 (double strength) at daily intervals, always transferring one standard loopful of the culture. After two weeks of subculturing as described, begin the process again with a fresh stock culture.

5.5.2.2.3 A subculture made on any day between the third day and the fifteenth day (inclusive) may be used for the test, subject to the following provisions:

a) omission of one daily subculturing operation requires no special reorganization of procedure but, if subculturing cannot take place on two successive days, three successive daily subculturing operations shall be carried out after the break before organisms suitable for the test are obtained; and

b) over week-ends, the following procedure may be adopted: on the Friday make a subculture at about 10:00 and incubate at 37 °C until 16:00. Transfer the subculture to an incubator maintained at 22 °C, and leave it there for the week-end. At 08:00 on the following Monday, transfer it back, for at least 2 h, to the incubator maintained at 37 °C, and then prepare the usual subculture for use on the Tuesday.

NOTE – Discard cultures that show signs of clumping or pellicle formation.

5.5.2.3 *Aspergillus niger*

Using 10 mℓ of a sterile 0,5 g/ℓ solution of polyoxyethylene sorbitan mono-oleate, wash the growth resulting from 7 d incubation from the slope (see 5.5.1.2), scraping the agar surface if necessary. Carefully decant the suspended growth into a sterile bottle (see 5.2.1(a)) and shake vigorously to suspend all growth in the water. So standardize the suspension, by using a haemocytometer, Petroff-Häusser counting chamber or any other suitable means, that it contains the required number of organisms per millilitre as stipulated in the individual microbiological efficacy tests. Use the suspension within three hours of preparation.

5.5.2.4 *Bacillus subtilis* var. *globigii*

Prepare the suspension in accordance with 5.5.1.3.

5.5.2.5 Bacteriophage

NOTE – The preparation refers to bacteriophage MS2 strain (see 5.5(d)). The preparation of bacteriophage ΦX174 strain is identical, except that a different bacterial culture, i.e. SABS TCC Esc 37, is used.

5.5.2.5.1 Preparation of the bacteriophage stock

Use either the plate method or the broth method.

5.5.2.5.1.1 Plate method

5.5.2.5.1.1.1 Inoculate a dilution of bacteriophage MS strain that produced a semi-confluent lysis on an agar plate with *Escherichia coli* SABS TCC Esc 36 into tubes containing top layer agar (see 5.4.21). Pour the suspension onto bottom layer agar (see 5.4.2), and incubate at 37 °C for 18 h.

5.5.2.5.1.1.2 Dispense 2,5 mℓ of nutrient medium (see 5.4.11) aseptically onto each plate and remove the nutrient medium with top layer agar by scraping it from the bottom layer agar, using a sterile glass rod.

5.5.2.5.1.1.3 Homogenize the suspension by vigorous shaking and then centrifuge the suspension at 7 000 r/min for 10 min.

5.5.2.5.1.1.4 Add 10 % (by volume) of chloroform to the bacteriophages in the supernatant and store this stock solution at 4 °C.

5.5.2.5.1.2 Broth method

5.5.2.5.1.2.1 Add the bacteriophage MS strain to a nutrient medium (see 5.4.11) culture of *Escherichia coli* SABS TCC Esc 36 which is near the end of the log phase to ensure that virtually every bacterial cell is infected simultaneously.

5.5.2.5.1.2.2 Centrifuge the suspension at 7 000 r/min for 10 min after lysis has occurred (visible as a marked drop in turbidity).

5.5.2.5.1.2.3 Add 10 % (by volume) of chloroform to the bacteriophages in the supernatant and store this stock solution at 4 °C.

5.5.2.5.2 Titration of the bacteriophage suspension

5.5.2.5.2.1 Inoculate a nutrient agar slope from a stock culture of *Escherichia coli* SABS TCC Esc 36 (see 5.5.1.1.3) and incubate at 37 °C for 24 h.

5.5.2.5.2.2 Using 2 mℓ of nutrient medium (see 5.4.11), wash the bacterial growth from the 24 h culture from the slope, scraping the agar surface if necessary. Carefully decant the suspended growth into a sterile screw-top glass bottle and vortex to suspend all the growth in the medium.

5.5.2.5.2.3 Add 0,5 mℓ of the solution obtained in 5.5.2.5.2.2 to 50 mℓ of nutrient medium (see 5.4.11) and incubate for 2 h at 37 °C.

5.5.2.5.2.4 Verify that the absorbance measured at 620 nm ± 20 nm through cells of optical path 1 cm is between 0,05 and 0,10 (inclusive), representing a bacterial concentration of approximately 10^8 organisms per millilitre.

5.5.2.5.2.5 Prepare a tenfold dilution series of the bacteriophage suspension, using, for each step, 9 mℓ volumes of sterile distilled water and, to 0,1 mℓ of each of the dilutions obtained, add 0,9 mℓ of the 2 h *E. coli* culture (see 5.5.2.5.2.4).

5.5.2.5.2.6 Place the suspensions in a water-bath or incubator at 37 °C for 15 min ± 1 min and then add 5,0 mℓ of top layer agar, which had been melted and kept in a water-bath at 44 °C to prevent solidification of the agar.

5.5.2.5.2.7 Vortex and immediately spread the mixture over the surface of the bottom layer agar in appropriately labelled Petri dishes, and swirl gently.

5.5.2.5.2.8 Allow the agar to solidify, invert and incubate the Petri dishes at 37 °C for 24 h.

5.5.2.5.2.9 After incubation, examine the plates for plaque formation. Count and record the plaques on each plate that contains between 10 and 100 plaques.

5.5.2.5.2.10 A bacteriophage titre of at least 10^7 per millilitre is recommended.

5.6 Disinfecting efficacy of disinfectants and detergent-disinfectants based on chlorhexidine gluconate

5.6.1 Inoculate a bottle of tryptone soy broth (see 5.4.22) from a daily subculture of each of the *Staphylococcus aureus* (SABS TCC Sta 59) and *Pseudomonas aeruginosa* (SABS TCC Pse 16) (see 5.5.2.1.2) test organisms.

5.6.2 Incubate the inoculated tryptone soy broth at 37 °C for 24 h and proceed as in 5.5.2.1.3, so standardizing the suspension that it contains at least 10 million (10^7) but not more than 100 million (10^8) organisms per millilitre.

5.6.3 Test procedure

5.6.3.1 Using sterile distilled water, prepare a relevant dilution of the disinfectant or detergent-disinfectant (see 7.2(f)).

5.6.3.2 Dispense 9 mℓ volumes of this sample dilution aseptically into sterile test tubes and place for 10 min in a water-bath maintained at 37 °C. Repeat this procedure but use 9 mℓ of sterile distilled water as a control.

5.6.3.3 Melt the contents of a sufficient number of bottles (containing 15 mℓ volumes) of nutrient agar (see 5.4.9), cool to 45 °C and maintain them at this temperature.

5.6.3.4 Using a clean, sterile pipette, dispense 1 mℓ of the *Staphylococcus aureus* suspension (see 5.6.2) into one of the tubes of each disinfectant or detergent-disinfectant to be tested and to the control. Mix well, using a vortex type mixer and maintain the suspension in the water-bath for the duration of the test.

5.6.3.5 Remove a 1 mℓ volume from each tube after 1 min and add each volume to separate 9 mℓ volumes of neutralizer broth medium (see 5.4.7.4) and stir well.

5.6.3.6 Prepare a tenfold dilution series of the sample, using for each step 9 mℓ volumes of sterile distilled water as diluent. Four to five serial dilutions are recommended for the control.

5.6.3.7 Using a clean, sterile pipette, dispense 1 mℓ of each dilution (see 5.6.3.5 and 5.6.3.6) onto two appropriately labelled, sterile plates (Petri dishes). Add 15 mℓ of nutrient agar (see 5.6.3.3) to each of the plates and swirl gently to ensure an even distribution of colonies after incubation. Avoid spilling any of the contents of the plates during this process.

5.6.3.8 Allow the agar to solidify, invert the plates and incubate at 37 °C for 48 h.

5.6.3.9 After incubation, examine the plates for growth. Count and record the colonies on each plate (of the test solution and of the control) that contains between 30 and 300 colonies. If the least diluted sample (with the highest concentration) yields less than 30 colonies, count all the colonies. Ensure that the colonies that have been counted are derived from survivors of the test organisms and not from contamination.

5.6.3.10 Take the dilution factor (*DF*) for the test sample and for the control as the inverse of the dilution, for example if the sample or the control has been diluted to 1/1 000, take *DF* as 1 000.

5.6.3.11 The percentage kill is given by the following formula:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

where

A is the number of organisms counted after contact with the samples (see 5.6.3.9);

B is the number of organisms counted in the control (see 5.6.3.9);

DF_s is the dilution factor for the sample as calculated in 5.6.3.10; and

DF_c is the dilution factor for the control as calculated in 5.6.3.10.

5.6.3.12 Repeat the procedure described in 5.6.3.1 to 5.6.3.11, using *Pseudomonas aeruginosa* as the test organism.

5.6.4 Interpretation of results

Deem the disinfectant or detergent-disinfectant to comply with the requirements of 4.2 if, subject to the following conditions:

a) at a concentration of 2 % of chlorhexidine gluconate (in cases where the supplied disinfectant or detergent-disinfectant contains 5 % of chlorhexidine gluconate or more); or

b) at the prescribed concentration, i.e. in an undiluted form (see 7.2(f)) (in cases where the supplied disinfectant or detergent-disinfectant contains less than 5 % of chlorhexidine gluconate),

the disinfectant or detergent-disinfectant kills at least 99,9 % of each organism tested.

5.7 Disinfecting efficacy of disinfectants and detergent-disinfectants based on glutaraldehyde

5.7.1 Survivor count method

The following distinctions are made:

a) disinfectants or detergent-disinfectants intended for general use; and

b) disinfectants intended for use on medical instruments.

5.7.1.1 Preparation of test organism suspensions

5.7.1.1.1 Disinfectants and detergent-disinfectants based on glutaraldehyde, intended for general use

Prepare the test organism suspensions as described in 5.5.2.1 and 5.5.2.3 and ensure that each millilitre of growth medium contains:

a) at least 100 000 (10^5), but not more than 1 million (10^6) *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* organisms; and

b) 100 000 (10^5) *Aspergillus niger* organisms,

respectively.

5.7.1.1.2 Disinfectants based on glutaraldehyde, intended for use on medical instruments

Prepare the test organism suspensions as described in 5.5.2.1, 5.5.2.3 and 5.5.2.4, and ensure that each millilitre of growth medium contains:

a) at least 10 million (10^7), but not more than 100 million (10^8) *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* organisms;

b) 10 million (10^7) *Aspergillus niger* organisms; and

c) at least 10 million (10^7) *Bacillus subtilis* var. *globigii* spores,

respectively.

5.7.1.2 Preparation of control and test solutions

5.7.1.2.1 Control solutions

5.7.1.2.1.1 Disinfectants and detergent-disinfectants based on glutaraldehyde, intended for general use

Dispense 8,9 mℓ of standard hard water No. 1 (see 5.4.18.1) into each of three sterile glass bottles and to each add 0,1 mℓ of sterile skimmed milk (see 5.4.20).

5.7.1.2.1.2 Disinfectants based on glutaraldehyde, intended for use on medical instruments

Dispense 9 mℓ of sterile distilled water into each of three sterile glass bottles.

5.7.1.2.2 Test solution

5.7.1.2.2.1 Disinfectants and detergent-disinfectants based on glutaraldehyde, intended for general use

Prepare the prescribed concentration of the test sample as stated on the label (see 7.2(f)), using standard hard water No. 1 (see 5.4.18.1). Dispense 8,9 mℓ of the test solution into each of three sterile glass bottles and to each add 0,1 mℓ of sterile skimmed milk (see 5.4.20). Use the test solution on the day of preparation.

5.7.1.2.2.2 Disinfectants based on glutaraldehyde, intended for use on medical instruments

Prepare the relevant concentration of the test sample as stated on the label (see 7.2(f)), using sterile distilled water. Dispense 9 mℓ of the test solution into each of three sterile glass bottles. Use the test solution on the day of preparation.

5.7.1.3 Temperature adjustment

Label the bottles that contain the control, the test solutions and the test organism suspensions and place them for at least 30 min in a water-bath maintained at 22 °C ± 1 °C.

5.7.1.4 Test procedure for disinfectants and detergent-disinfectants based on glutaraldehyde, intended for general use

NOTE – For the sake of brevity, the procedure for testing the disinfectant solution against one organism is given. For each organism, a control test is carried out, using the relevant solution (see 5.7.1.2.1.1 and 5.7.1.2.1.2) instead of the disinfectant solution. Each control runs concurrently with its associated test (with a time lapse of about 30 s between associated actions). Use a stop-watch for this purpose.

5.7.1.4.1 Melt the contents of a sufficient number of bottles (containing 15 mℓ volumes) of nutrient agar (see 5.4.9), cool to 45 °C and maintain them at this temperature.

5.7.1.4.2 Using a clean, sterile pipette, dispense 1 mℓ of the *Pseudomonas aeruginosa* test suspension (see 5.7.1.1.1(a)) into the test solution and to the control (without removing the bottles from the water-bath). Remove the bottles from the water-bath, vortex them to mix the contents thoroughly and immediately return them to the water-bath.

5.7.1.4.3 At the end of a 5 min exposure period (actual contact time), transfer 1 mℓ of the test solution containing the organism to 9 mℓ of inactivator medium No. 2 (see 5.4.7.2) and mix well into a uniform suspension (first dilution). Within 30 s, repeat this procedure with the control.

5.7.1.4.4 Using a clean, sterile pipette, transfer 1 mℓ of the inactivator medium containing the organism (see 5.7.1.4.3) to a Petri dish and then 1 mℓ to the first of a series of bottles, each containing 9 mℓ of sterile distilled water. Mix well into a uniform suspension (second dilution).

5.7.1.4.5 Repeat the procedure in 5.7.1.4.4, but transfer the suspension obtained after the second dilution to the second of the series of bottles. Dilute further until an end dilution of 1:1 000 of the test sample is obtained.

5.7.1.4.6 Consecutively prepare, as described above, a dilution series using the control.

5.7.1.4.7 From each dilution of the test sample, transfer 1 mℓ to each of two appropriately labelled, sterile plates (Petri dishes). Do the same for each dilution of the control. Add 15 mℓ of nutrient agar (see 5.7.1.4.1) to each of the plates and swirl gently to ensure an even distribution of colonies after incubation. Avoid spilling any of the contents of the plates during this process.

5.7.1.4.8 Allow the agar to solidify, invert the plates and incubate them at 37 °C for 48 h.

5.7.1.4.9 After incubation, examine the plates for growth. Count and record the colonies on each plate (of the test solution and of the control) that contains between 30 and 300 colonies. If the least diluted sample (with the highest concentration) yields less than 30 colonies, count all the colonies. Ensure that the colonies that have been counted are derived from survivors of the test organisms and not from contamination.

5.7.1.4.10 Take the dilution factor (*DF*) for the test sample and for the control as the inverse of the dilution, for example if the sample or the control has been diluted to 1/1 000, take *DF* as 1 000.

5.7.1.4.11 The percentage kill is given by the following formula:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

where

A is the number of organisms counted after contact with the sample (see 5.7.1.4.9);

B is the number of organisms counted in the control (see 5.7.1.4.9);

DF_s is the dilution factor for the sample as calculated in 5.7.1.4.10; and

DF_c is the dilution factor for the control as calculated in 5.7.1.4.10.

NOTE – The same formula is applied to determine the percentage kill of *Escherichia coli* after 5 min contact time (see 5.7.1.4.12), *Staphylococcus aureus* after 5 min contact time (see 5.7.1.4.13) and *Aspergillus niger* after 15 min contact time (see 5.7.1.4.14).

5.7.1.4.12 Repeat the procedure described in 5.7.1.4.1 to 5.7.1.4.11 (inclusive) with the *Escherichia coli* suspension (see 5.5.2.1).

5.7.1.4.13 Repeat the procedure described in 5.7.1.4.1 to 5.7.1.4.11 (inclusive) with the *Staphylococcus aureus* suspension (see 5.5.2.1).

5.7.1.4.14 Repeat the procedure described in 5.7.1.4.1 to 5.7.1.4.11 (inclusive) with the *Aspergillus niger* test organism suspension (see 5.5.2.3), but

a) use an exposure period (actual contact time) of 15 min,

b) add malt extract agar (see 5.4.8) to the suspensions on the plates, and

c) incubate for 7 d at 25 °C.

5.7.1.5 Test procedure for disinfectants based on glutaraldehyde, intended for use on medical instruments

5.7.1.5.1 *Pseudomonas aeruginosa* and *Aspergillus niger*

Carry out the procedure in 5.7.1.4.1 to 5.7.1.4.11 (inclusive), using the *Pseudomonas aeruginosa* test suspension (see 5.7.1.1.1(a)) and the *Aspergillus niger* test suspension (see 5.5.2.3).

5.7.1.5.2 *Bacillus subtilis* var. *globigii*

Carry out the procedure in 5.7.1.4.1 to 5.7.1.4.11 (inclusive), using the *Bacillus subtilis* var. *globigii* test suspension (see 5.5.2.4), but:

a) use an exposure period (actual contact time) of 4 h; and

b) incubate at 37 °C for 48 h.

5.7.1.6 Interpretation of results

Deem the samples to comply with the requirements of 4.2 if, for each organism tested, a result of at least a 99,99 % kill was obtained.

5.7.2 Sporicidal activity (Kelsey-Sykes test modified)

5.7.2.1 Medium

Use the inactivator medium No. 2 described in 5.4.7.2.

5.7.2.2 Test organism suspension

Use the spore suspension of *B. subtilis* var. *globigii* (see 5.5.2.4).

5.7.2.3 Preparation of test solution

Prepare the prescribed concentration of the test solution as stated on the label (see 7.2(f)), using sterile distilled water. Use the test solution on the day of preparation.

5.7.2.4 Test procedure

5.7.2.4.1 Dispense 3 mℓ of the test solution into a sterile glass bottle.

5.7.2.4.2 Label the bottles containing the test solution and the test organism suspensions and place them for at least 30 min in a water-bath maintained at 22 °C ± 1 °C.

5.7.2.4.3 Then, without removing the bottle containing the test solution from the water-bath and using a clean, sterile pipette, add 1 mℓ of the test organism suspension to the test sample. Simultaneously start a stop-watch. Remove the bottle from the water-bath, mix the contents well and immediately return it to the water-bath.

5.7.2.4.4 After exactly 4 h, transfer 0,02 mℓ of the suspension obtained in 5.7.2.4.3 to each of five tubes containing 10 mℓ of inactivator medium No. 2 (see 5.4.7.2).

5.7.2.4.5 Incubate the inoculated tubes of inactivator medium at 30 °C for 48 h.

5.7.2.4.6 After incubation, examine the tubes of inactivator medium for growth and record the results.

5.7.2.5 Interpretation of results

Deem the sample to comply with the requirements of 4.2 if no growth of the test organism is detectable in any of the five tubes of inactivator medium.

5.7.3 Kelsey-Sykes test

5.7.3.1 Procedure

Follow the procedure in 5.8, but use only *Pseudomonas aeruginosa* as the test organism.

5.7.3.2 Interpretation of results

Deem the sample to comply with the requirements of 4.2 if the initial concentration of disinfectant (concentration B) (see 5.8.2.6(a)) shows no growth of the test organism in at least two of the five tubes of reculture medium in sets inoculated at:

a) the eighth minute after the addition of the initial inoculum; and

b) the eighteenth minute after the addition of the initial inoculum.

An example of a series of test results and their interpretation is given in table 2.

5.8 Kelsey-Sykes test for detergent-disinfectants based on phenolics

5.8.1 Minimum inhibitory concentration test

5.8.1.1 Preparation of the test suspensions

Prepare a 1:10 dilution in Wright and Mundy medium (see 5.4.23) of a freshly grown subculture of each of the test organisms (see 5.5.2.1).

NOTE – Before diluting a *Pseudomonas aeruginosa* culture, filter it through a coarse filter paper.

5.8.1.2 Preparation of the test sample dilutions

5.8.1.2.1 To 5 mℓ of test sample in a glass bottle of capacity 30 mℓ (see 5.2.1(a)), add 5 mℓ of Wright and Mundy medium (see 5.4.23). Mix well and transfer 5 mℓ of this dilution of test sample to a further 5 mℓ of Wright and Mundy medium. Repeat the procedure until 10 doubling dilutions of the test sample (from 1:2 to 1:1 024) have been prepared. Discard 5 mℓ of the last dilution (so that each bottle will contain 5 mℓ of a dilution of the test sample).

5.8.1.2.2 Repeat 5.8.1.2.1 until three sets of 10 doubling dilutions of the test sample have been prepared.

5.8.1.3 Test procedure

5.8.1.3.1 To each of the 10 dilutions of the test sample (see 5.8.1.2.2), add 0,02 mℓ of the *Staphylococcus aureus* test suspension (see 5.8.1.1). Incubate the inoculated bottles at 30 °C for 72 h.

Examine the bottles for growth. The minimum inhibitory concentration is the highest dilution (minimum concentration) not showing growth.

5.8.1.3.2 Repeat the procedure given in 5.8.1.3.1 but use, successively, the *Escherichia coli* and *Pseudomonas aeruginosa* (see 5.8.1.1) test suspensions.

5.8.1.4 Interpretation of results

Determine which of the three test organisms is most resistant to the test sample, i.e. the organism for which the minimum inhibitory concentration is the highest. Use this organism for the remainder of the test (see 5.8.2).

5.8.2 Remainder of test

5.8.2.1 Selection of the test organism for the determination

Using the minimum inhibitory concentration test (see 5.8.1), determine which of the test organisms is the most resistant, and use it as the test organism for the determination.

5.8.2.2 Preparation of culture for test organism suspensions

On the day before the test is due to be carried out, inoculate a tube containing 10 mℓ of Wright and Mundy medium (see 5.4.23) from a daily subculture of the appropriate test organism (see 5.5.2.1) and incubate the inoculated medium at 37 °C for 24 h.

5.8.2.3 Preparation of test organism suspension for the test under "clean" conditions

5.8.2.3.1 After incubation (see 5.8.2.2), centrifuge the culture of the test organism for 15 min at a resultant centrifugal force of 6 kN/kg. Using a sterile Pasteur pipette, remove and discard the supernatant liquid and resuspend the organism in 10 mℓ of hard water prepared for use in this test (see 5.4.18.3).

5.8.2.3.2 Transfer this suspension to a sterile glass bottle of capacity 30 mℓ (see 5.2.1(a)).

5.8.2.3.3 Add a few sterile glass beads and shake for 1 min.

5.8.2.4 Preparation of test organism suspension for the test under "dirty" conditions

Obtain a suspension that contains 5 % (by mass) of dry yeast by adding 6 mℓ of the culture of the test organism (see 5.8.2.3) to 4 mℓ of 5 % (by mass) of the dry yeast suspension (see 5.4.24.4) contained in a sterile glass bottle of capacity 30 mℓ (see 5.2.1(a)). Add a few sterile glass beads to the mixture and vortex for 1 min.

5.8.2.5 Estimation of the number of viable organisms in the test organism suspension

So standardize the suspension by using a spectrophotometer in conjunction with a standard curve, a haemocytometer, Petroff-Häusser counting chamber or any other suitable means, that it contains at least 100 million (10^8) but not more than 10^{10} organisms per millilitre. Use the suspension within three hours of preparation.

5.8.2.6 Preparation of test solutions

Using the hard water prepared for this test (see 5.4.18.3) and glass bottles of capacity 30 mℓ (see 5.2.1(a)), prepare three different concentrations A, B and C of the test sample that are such that:

a) concentration B is that which is expected or claimed to pass the test;

b) concentration A is half of concentration B; and

c) concentration C is one-and-a-half-times concentration B.

For example, if a test sample is expected to pass the test at a concentration of 1 %, concentration A is a 0,5 % concentration, B is a 1 % concentration and C is a 1,5 % concentration.

5.8.2.7 Test procedure under "clean" conditions

5.8.2.7.1 Dispense 3 mℓ of each concentration of the test sample (see 5.8.2.6) into glass bottles of capacity 30 mℓ (see 5.2.1(a)), and label these bottles A, B and C, as relevant.

5.8.2.7.2 Place these bottles and the bottle containing the test organism suspension (see 5.8.2.3) for at least 30 min in a water-bath maintained at 22 °C ± 1 °C. To maintain the reproducibility of the test, adhere strictly to the temperature stated.

5.8.2.7.3 Then, without removing the bottle containing test concentration A from the water-bath, add 1 mℓ of the test suspension (see 5.8.2.3) and at the same time start a stop-watch (zero time). Remove the bottle from the water bath, mix well and immediately return it to the water-bath.

5.8.2.7.4 One minute after zero time, add, in the same way, 1 mℓ of the test suspension to the bottle containing test concentration B.

5.8.2.7.5 Five minutes after zero time, add, in the same way, 1 mℓ of the test suspension to the bottle containing test concentration C.

5.8.2.7.6 Eight minutes after zero time, transfer 0,02 mℓ from the bottle containing test concentration A to each of five tubes of reculture medium (see 5.4.14) each of which has been labelled A1.

5.8.2.7.7 Ten minutes after zero time, add, in the same way as described in 5.8.2.7.3 above, a further 1 mℓ of test suspension (see 5.8.2.3) to the bottle containing test concentration A.

5.8.2.7.8 Eighteen minutes after zero time, transfer 0,02 mℓ from the bottle containing test concentration A to each of five tubes of reculture medium each of which has been labelled A2.

Table 2 — Kelsey-Sykes test timetable

1	2	3	4	5	6
Time min	**Test sample suspension A**	**Time** min	**Test sample suspension B**	**Time** min	**Test sample suspension C**
0	1 mℓ test suspension to A	1	1 mℓ test suspension to B	5	1 mℓ test suspension to C
8	0,02 mℓ transferred from A to reculture medium A1	9	0,02 mℓ transferred from B to reculture medium B1	–	–
10	1 mℓ test suspension to A	11	1 mℓ test suspension to B	13	0,02 mℓ transferred from C to reculture medium C1
–	–	–	–	15	1 mℓ test suspension to C
18	0,02 mℓ transferred from A to reculture medium A2	19	0,02 mℓ transferred from B to reculture medium B2	23	0,02 mℓ transferred from C to reculture medium C2

5.8.2.7.9 Concurrently with 5.8.2.7.1 to 5.8.2.7.8, treat the test concentrations B and C in the same way, but base the time intervals on the times at which the first additions of test suspensions were made, and label the sets of tubes of reculture medium B1 and B2, and C1 and C2 (respectively). Thus, in the case of test concentration B, addition and transference will be made one minute later than the times given for test concentration A (i.e. at 9 min, 11 min and 19 min after zero time) and, in the case of test concentration C, additions and transference will be made at 13 min, 15 min and 23 min after zero time.

NOTE – In order to obviate errors in transference, a copy of the test timetable (see table 2) should be used during each test. Each step of the test should be ticked off on the timetable as it is carried out.

5.8.2.7.10 Incubate all the inoculated tubes of reculture medium at 30 °C for 48 h.

5.8.2.7.11 After incubation, examine the tubes of reculture medium for growth and record the results as shown in the example given in table 3.

Table 3 — Test sample tested under "clean" conditions
Test organism: *Pseudomonas aeruginosa*

1	2	3	4	5	6
Test No.	**Test sample concentration % (by volume)**	**Test concentration organisms** mℓ	**Reculture medium** 1	**Reculture medium** 2	**Result**
1	0,8	$6,9 \times 10^8$	+ + + + +	+ + + + +	Fail
	1,6	$6,9 \times 10^8$	− − + + +	− + + + +	Fail
	2,4	$6,9 \times 10^8$	− − − − −	− − − − −	Pass
Test solution at 1,6 % (by volume) concentration has failed. Test repeated at higher concentrations.					
1	0,9	$8,4 \times 10^8$	− + + + +	+ + − + −	Fail
	1,8	$8,4 \times 10^8$	− − − − +	− − + + +	Pass
	2,7	$8,4 \times 10^8$	− − − − −	− − − − −	Pass
2	0,9	$2,1 \times 10^9$	− + + + +	+ + + + +	Fail
	1,8	$2,1 \times 10^9$	− − − − +	− − + + +	Pass
	2,7	$2,1 \times 10^9$	− − − − −	− − − − −	Pass
3	0,9	$5,7 \times 10^8$	− − + + +	+ + + + +	Fail
	1,8	$5,7 \times 10^8$	− − − − −	− − − + +	Pass
	2,7	5.7×10^8	− − − − −	− − − − −	Pass

5.8.2.7.12 If test concentration B passes the test (see 5.8.2.8), repeat steps 5.8.2.7.1 to 5.8.2.7.11 on two subsequent days.

5.8.2.8 Test procedure under "dirty" conditions

Carry out the procedure described in 5.8.2.7 but use the test organism suspension described in 5.8.2.4.

5.8.2.9 Interpretation of results

Deem the initial concentration of disinfectant or detergent-disinfectant (concentration B) (see 5.8.2.6(a)) to have passed the test if there is no growth of the test organism in at least two of the five tubes of reculture medium in sets inoculated at:

a) the eighth minute after the addition of the initial inoculation; and

b) the eighteenth minute after the addition of the initial inoculation.

An example of a series of test results and their interpretation is given in table 3.

5.9 Disinfecting efficacy of coal-tar type disinfectant liquids (black and white) — Rideal-Walker coefficient test

5.9.1 Preparation of test organism suspension

Mix thoroughly a culture of *Salmonella typhi* (see 5.5.2.2) in nutrient broth No. 2 (double strength) (see 5.4.10) that has been incubated for 24 h at 37 °C and, before use, place the tube for 30 min ± 5 min in the water-bath (see 5.3.2) maintained at 22 °C.

5.9.2 Preparation of control and test solution

5.9.2.1 Standard phenol control solution

From the 50 g/ℓ of standard phenol solution (see 5.4.19), make five phenol control dilutions that contain 1 g of pure phenol in each of 95 mℓ, 100 mℓ, 105 mℓ, 110 mℓ and 115 mℓ of solution.

NOTE – These dilutions may be stored in the dark for not more than one week before use.

5.9.2.2 Stock solution of the test sample (1:100)

5.9.2.2.1 Immediately before withdrawing any portion for testing, thoroughly mix a composite sample of the disinfectant to be tested, ensuring that air is not beaten into or shaken into the sample.

5.9.2.2.2 Withdraw the test portion from the middle of the sample by means of a 5 mℓ capacity pipette (see 5.2.3). Fill the pipette to just above the mark, wipe it clean on the outside with sterile cotton wool and then adjust the contents to the mark. Allow the contents of the pipette to discharge below the surface of about 480 mℓ of water at a temperature of 18 °C, contained in a measuring cylinder.

5.9.2.2.3 Rinse the pipette out three times (or more in the case of viscous liquids) by drawing up and returning some of the dilution.

5.9.2.2.4 Make the solution up to 500 mℓ with water, stopper the cylinder and thoroughly mix the contents by inverting, with a corkscrew motion, 50 times.

5.9.2.3 Test solutions

From the stock solution (see 5.9.2.2), prepare five suitable test solutions (see tables 4 and 6) based on the nominal RW coefficient stated on the label (see 7.2(m)). Place 5 mℓ of each of the five chosen solutions in sterile culture tubes (see 5.2.2(a)). Mark and place these tubes in sequence in a rack in the water-bath (see 5.3.2), with the strongest disinfectant solution on the left.

5.9.2.4 Phenol solutions

Prepare five culture tubes (see 5.2.2(a)), each containing 5 mℓ of a different phenol control solution (see 5.9.2.1), and mark and arrange them in the same way in the water-bath as the solutions of the disinfectant (see 5.9.2.3).

5.9.2.5 Culture medium

5.9.2.5.1 Mix thoroughly a culture of *Salmonella typhi* (see 5.5.2.2) in Nutrient broth No. 2 (double strength (see 5.4.10) that has been incubated at 37 °C for 24 h and, before use, place the tube for 30 min ± 5 min in a water-bath maintained at 22 °C.

5.9.2.5.2 Place two sets of 15 tubes (marked sequentially "1" to "30"), each containing 5 mℓ of Nutrient broth No. 2 (double strength) (see 5.4.10), in the water-bath.

5.9.3 Test procedure

5.9.3.1 Starting exactly at zero time (use the stop-watch (see 5.3.4) for timing all operations), add 0,2 mℓ of the test organism suspension (see 5.9.1) from the droplet pipette (see 5.2.10) to the leftmost test tube containing the disinfectant solution (see 5.9.2.3). Ensure that all of the culture added is pipetted straight into the disinfectant solution and not onto the wall of the test tube. Shake the tube; 30 s after this, inoculate the next tube to the right with 0,2 mℓ of culture in a similar manner. Inoculate each successive tube, at intervals of 30 s, until the fifth tube has been inoculated.

5.9.3.2 Thirty seconds after this last addition, i.e. 2,5 min after zero time, withdraw a representative loopful (see 5.2.9) of the well-shaken contents of the tube on the extreme left and add this to the tube marked "1" and containing 5 mℓ of the nutrient broth No. 2 (double strength) (see 5.4.10). Immediately after inoculation, shake the tube containing the inoculated broth. Thirty seconds after this loopful has been withdrawn, transfer, in the same way, a loopful of the contents of the second test tube to the tube of broth marked "2". Repeat this procedure at intervals of 30 s, working from left to right, until all five test tubes have been so treated.

NOTE – In each withdrawal, ensure that the loop is removed vertically from the surface of the liquid, with its plane horizontal. Sterilize the loop by flaming before each withdrawal and ensure that the loop is cold before being used again.

5.9.3.3 Starting again in each case with the left hand tube, perform two further cycles of withdrawals until three sets of cultures (15 tubes) have been made, i.e. at 2,5 min, 5 min and 7,5 min intervals, respectively, after exposure.

5.9.3.4 Repeat the procedure given in 5.9.3.1 to 5.9.3.3 with the five tubes containing the phenol control dilutions (see 5.9.2.3) using the Nutrient broth No. 2 (double strength) tubes numbered "16" to "30".

5.9.3.5 Incubate all the tubes prepared in accordance with 5.9.3.1 to 5.9.3.4 at 37 °C for not less than 48 h.

5.9.4 Recording of results

Record in which tubes growth of *Salmonella typhi* occurs and ensure that the growth did not originate from a contaminant. It is convenient to use a "+" sign to refer to a tube that shows growth, and a "–" sign to refer to a tube that shows no growth.

The test is satisfactory when at least one of the phenol control dilutions shows a critical dilution response, i.e. a "+ + –" growth pattern; if not, discard the test results and repeat the test.

NOTE – Where the critical dilution figure of phenol is 115, it is advisable to repeat the test using five phenol control dilutions containing 1 g of pure phenol in each of 100 mℓ, 105 mℓ, 110 mℓ, 115 mℓ and 120 mℓ of solution.

5.9.5 Rideal-Walker coefficient

5.9.5.1 Calculation

Obtain the Rideal-Walker coefficient of the disinfectant by dividing the critical dilution figure of the disinfectant by the critical dilution figure of phenol in the range 95 to 115. When none of the dilutions of the sample shows the critical dilution response, it is permissible to obtain a result by interpolation but not by extrapolation.

5.9.5.2 Example

A typical set of results is shown in table 4.

Table 4 — Typical set of results in calculating the Rideal-Walker coefficient

1	2	3	4	5	6	7	8
Dilution of sample	Exposure time min			Dilution of phenol (5 %)	Exposure time min		
	2,5	5	7,5		2,5	5	7,5
1:1 000	–	–	–	1:95	–	–	–
1:1 100	+	–	–	1:100	+	–	–
1:1 200	+	+	–	1:105	+	+	–
1:1 300	+	+	–	1:110	+	+	+
1:1 400	+	+	+	1:115	+	+	+
Rideal-Walker coefficient = $\frac{1\ 300}{105}$ = 12,4							

Table 5 — RW coefficients and total dilution volumes

1	2	3	4	5	6	7
Critical dilution of sample	Total volume of diluted sample mℓ	RW coefficient for corresponding critical dilution of the phenol				
		95	100	105	110	115
1:2 500	125	26,3	25,0	23,8	22,7	21,7
1:2 400	120	25,3	24,0	22,9	21,8	20,9
1:2 300	115	24,2	23,0	21,9	20,9	20,0
1:2 200	110	23,2	22,0	21,0	20,0	19,1
1:2 100	105	22,1	21,0	20,0	19,1	18,3
1:2 000	100	21,1	20,0	19,0	18,2	17,4
1:1 900	95	20,0	19,0	18,1	17,3	16,5
1:1 800	90	18,9	18,0	17,1	16,4	15,7
1:1 700	85	17,9	17,0	16,2	15,5	14,8
1:1 600	80	16,8	16,0	15,2	14,5	13,9
1:1 500	75	15,8	15,0	14,3	13,6	13,0
1:1 400	70	14,7	14,0	13,3	12,7	12,2
1:1 300	65	13,7	13,0	12,4	11,8	11,3
1:1 200	60	12,6	12,0	11,4	10,9	10,4
1:1 100	55	11,6	11,0	10,5	10,0	9,6
1:1 000	50	10,5	10,0	9,5	9,1	8,7
1: 900	45	9,5	9,0	8,6	8,2	7,8
1: 800	40	8,4	8,0	7,6	7,3	7,0
1: 700	35	7,4	7,0	6,7	6,4	6,1
1: 600	30	6,3	6,0	5,7	5,5	5,2
1: 500	25	5,3	5,0	4,8	4,6	4,3
1: 400	20	4,2	4,0	3,8	3,6	3,5

Table 6 — RW coefficients and total dilution volumes

1	2	3	4	5	6	7
Critical dilution of sample	**Total volume of diluted sample mℓ**	**RW coefficient for corresponding critical dilution of the phenol**				
		95	**100**	**105**	**110**	**115**
1:350	70	3,7	3,5	3,3	3,2	3,0
1:300	60	3,2	3,0	2,9	2,7	2,6
1:250	50	2,6	2,5	2,4	2,3	2,2
1:200	40	2,1	2,0	1,9	1,8	1,7
1:150	30	1,6	1,5	1,4	1,4	1,3
1:100	20	1,1	1,0	–	–	–

5.9.6 Rideal-Walker coefficient for dilutions of disinfectant of 1:100 to 1:2 500

5.9.6.1 Dilutions of disinfectant

The stock solution of disinfectant (see 5.9.2.2) contains 5 mℓ of disinfectant fluid in 500 mℓ of solution (dilution of 1:100).

5.9.6.1.1 To obtain a dilution between 1:2 500 and 1:400 (see column 1 of table 5), dilute 5 mℓ of the stock dilution by the addition of sterile water to make the appropriate total volume shown in column 2.

5.9.6.1.2 To obtain a dilution between 1:100 and 1:350 (see column 1 of table 6), dilute 20 mℓ of the stock dilution by the addition of sterile water to make the appropriate total volume shown in column 2.

5.9.6.2 Rideal-Walker coefficients

The Rideal-Walker coefficient for a sample dilution occurring in column 1 of table 5 or table 6 (as relevant), relative to one of the phenol dilutions given in the heading to columns 3 to 7 (inclusive), is given in the appropriate column.

NOTE – These tables are intended to facilitate the calculation of the results and should not be regarded as imposing any limits on the disinfectant dilutions to be used. They may be extended as desired.

5.10 Disinfecting efficacy — "Use dilution" test

5.10.1 General

The following disinfectants or detergent-disinfectants, with the necessary adjustments, as indicated, are tested using this procedure:

a) disinfectants or detergent-disinfectants based on iodophors, using the hard water described in 5.4.18.2 and inactivator medium No. 1 as described in 5.4.7.1;

b) disinfectants or detergent-disinfectants based on organic halogen compounds (other than iodine compounds), using the hard water described in 5.4.18.1 and the inactivator medium No. 1 as described in 5.4.7.1;

c) disinfectants or detergent-disinfectants based on quaternary ammonium compounds, using the hard water described in 5.4.18.1 and inactivator medium No. 1 as described in 5.4.7.1;

NOTE – Owing to the tendency for quaternary ammonium compounds to be adsorbed onto the surface of glassware, care must be taken to ensure that no residues are present on the glassware at the beginning of the test.

d) disinfectants or detergent-disinfectants based on stabilized inorganic chlorine compounds, using the hard water described in 5.4.18.1 and the sodium thiosulfate inactivator as described in 5.4.15; and

e) disinfectants and detergent-disinfectants based on stabilized chlorine compounds, using the hard water described in 5.4.18.1 and the sodium thiosulphate inactivator described in 5.4.15.

5.10.2 Preparation of controls and test solutions

5.10.2.1 Milk and hard water mixture

Using a clean, sterile pipette, dispense 1 mℓ of sterile skimmed milk (see 5.4.20) aseptically into each of two bottles containing 97 mℓ of hard water (see 5.4.18.1). When solid detergent-disinfectants are tested, prepare only one such mixture.

5.10.2.2 Control solution

Add 1 mℓ of sterile water to the contents of one bottle of skimmed milk and hard water mixture (see 5.10.2.1).

5.10.2.3 Test solution

5.10.2.3.1 Liquid disinfectant or detergent-disinfectant

Prepare a solution of the test sample in sterile water of such concentration that a 1:100 dilution of this solution will correspond with the dilution given on the label (see 7.2(f)) and add 1 mℓ of this solution to the other milk and hard water mixture (see 5.10.2.1). Alternatively, if the labelled dilution does not exceed 1 in 100, add the required quantity of sample straight to such a volume of the mixture as to produce a final volume of 99 mℓ.

5.10.2.3.2 Solid disinfectant or detergent-disinfectant

Weigh straight into a dry, sterile 110 mℓ bottle a quantity of the sample that will produce the dilution given on the label (see 7.2(f)) after adding:

a) 1 mℓ of sterile water;

b) 1 mℓ of sterile skimmed milk (see 5.4.20); and

c) sufficient sterile hard water (see 5.4.18.1) to bring the total volume to 99 mℓ.

Each pipette used for the measurement of a solution that contains a quaternary ammonium compound, shall, before use, be pre-rinsed at least twice with that solution.

5.10.2.4 Temperature adjustment

Immediately after the dilution has been prepared, mix well but gently (to minimize foaming). Before testing, take the bottles containing the control, the test solutions and the test organism suspensions and place them for at least 30 min in a water-bath maintained at 22 °C ± 1 °C. To maintain reproducibility of the test results, adhere strictly to the temperature as stated.

5.10.3 Preparation of test organism suspensions

Prepare *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* test organism suspensions as described in 5.5.2.1 and ensure that each millilitre of growth medium contains at least 100 000 (10^5) but not more than 1 million (10^6) organisms. Use the suspensions within three hours of preparation.

5.10.4 Test procedure

5.10.4.1 Melt the contents of a sufficient number of bottles (containing 15 mℓ volumes) of nutrient agar (see 5.4.9), cool to 45 °C and maintain them at this temperature. Dispense 1 mℓ ± 0,5 mℓ volumes of the appropriate inactivator solution (see 5.4.7) aseptically into each of two appropriately marked, sterile Petri dishes.

5.10.4.2 Using a clean, sterile pipette, dispense 1 mℓ of the *Staphylococcus aureus* test suspension (see 5.10.3) into the test solution (see 5.10.2.3.1 in the case of a liquid disinfectant or liquid detergent-disinfectant and 5.10.2.3.2 in the case of a solid disinfectant or solid detergent-disinfectant). Shake the bottle to mix the test suspension and the test solution thoroughly. Start a stop-watch. About 20 s before the end of the recommended exposure period (see 7.2(f)), shake the test solution gently, draw up 1 mℓ of the test solution aseptically into a 1 mℓ pipette, and, at the exact end of the exposure time, expel this solution into the inactivator solution in one of the Petri dishes. Immediately mix the solutions thoroughly.

5.10.4.3 Repeat the procedure given in 5.10.4.2 with the control solution.

5.10.4.4 Add 15 mℓ of melted agar (see 5.10.4.1) to each of the Petri dishes and swirl the plates gently on a table top to ensure an even distribution of colonies after incubation. Avoid spilling any of the contents of the plates during this process. Allow the agar to solidify, and incubate the plates at 37 °C for 48 h.

5.10.4.5 After incubation, examine the plates for growth. Count and record the colonies on each plate (of the test solution and of the control) that contains between 30 and 300 colonies. If the least diluted sample (with the highest concentration) yields less than 30 colonies, count all the colonies. Ensure that the colonies that have been counted are derived from survivors of the test organisms and not from contamination.

5.10.4.6 Take the dilution factor (*DF*) for the sample and the control as the inverse of the dilution, for example if the sample has been diluted to 1/1 000, take *DF* as 1 000.

5.10.4.7 The percentage kill is given by the following formula:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

where

A is the number of organisms counted after contact with the sample (see 5.10.4.5);

B is the number of organisms counted in the control (see 5.10.4.5);

DF_s is the dilution factor for the sample as calculated in 5.10.4.6; and

DF_c is the dilution factor for the control as calculated in 5.10.4.6.

NOTE – The same formula is applied to determine the percentage kill of *Escherichia coli* and *Pseudomonas aeruginosa*.

5.10.4.8 Repeat the procedure described in 5.10.4.1 to 5.10.4.7 (inclusive), using, successively, the *Escherichia coli* and the *Pseudomonas aeruginosa* test suspensions.

5.10.4.9 Repeat the entire test described in 5.10.4.1 to 5.10.4.8 (inclusive) on two subsequent days.

5.10.5 Interpretation of results

Deem the sample to comply with the requirements of 4.2 if, for each organism tested at the recommended concentration, exposure (contact) time (see 7.2(f)) and inactivator used (see 5.4.7), a result of at least 99,9 % kill was obtained.

5.11 Disinfecting efficacy of disinfectants and detergent-disinfectants based on a blend of peroxygen compounds, a surfactant, organic acids and an inorganic buffer system

5.11.1 Preparation of control and test solution

5.11.1.1 Test solution

5.11.1.1.1 Using sterile distilled water, prepare a concentration of double the relevant dilution of the disinfectant or detergent-disinfectant (see 7.2(f)).

5.11.1.1.2 Dispense 5 mℓ volumes of this sample dilution and 4 mℓ volumes of sterile distilled water aseptically into sterile test tubes and maintain at room temperature.

5.11.1.2 Control solution

Use 9 mℓ of sterile distilled water as a control.

5.11.2 Test procedure

5.11.2.1 Melt the contents of a sufficient number of bottles (containing 15 mℓ volumes) of nutrient agar (see 5.4.9), cool to 45 °C and maintain them at this temperature.

5.11.2.2 Using a clean, sterile pipette, add 1 mℓ of the *Bacillus subtilis* var. *globigii* spore suspension prepared as in 5.5.2.4 aseptically to the tubes of each disinfectant or detergent-disinfectant to be tested and to the control. Mix well, using a vortex type mixer, and maintain at room temperature for the duration of the test.

5.11.2.3 Remove 1 mℓ volumes from each tube after 1 h and add each volume to a separate 9 mℓ volume of sterile distilled water as diluent. (For the control, four to five serial dilutions are recommended.)

5.11.2.4 Plate out each dilution by pipetting 1 mℓ of the dilution onto an appropriately labelled, sterile Petri dish. Add 15 mℓ of nutrient agar (see 5.11.2.1) to each of the plates and swirl gently to ensure an even distribution of colonies after incubation. Avoid spilling any of the contents of the plates during this process. Allow the agar to solidify.

5.11.2.5 Repeat the procedure described in 5.11.2.1 to 5.11.2.4 after 2 h, 3 h and 4 h contact times.

5.11.2.6 Invert the plates and incubate at 37 °C for 48 h.

5.11.2.7 After incubation, examine the plates for growth. Count and record the colonies on each plate (of the test solution and of the control) that contains between 30 and 300 colonies. If the least diluted sample (with the highest concentration) yields less than 30 colonies, count all the colonies. Ensure that the colonies that have been counted are derived from survivors of the test organisms and not from contamination.

5.11.2.8 Take the dilution factor (*DF*) for the sample and the control as the inverse of the dilution, for example if the sample has been diluted to 1/1 000, take *DF* as 1 000.

5.11.2.9 The percentage kill is given by the following formula:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

where

A is the number of organisms counted after contact with the sample (see 5.11.2.7);

B is the number of organisms counted in the control (see 5.11.2.7);

DF_s is the dilution factor for the sample as calculated in 5.11.2.8; and

DF_c is the dilution factor for the control as calculated in 5.11.2.8.

5.11.3 Interpretation of results

Deem the disinfectant or detergent-disinfectant to comply with the requirements of 4.2 if, after 4 h reaction time, a result of at least a 99,9 % kill was obtained.

5.12 "5,5,5" method for testing disinfecting efficacy of detergents and detergent-disinfectants

5.12.1 Preparation of the test organism suspensions

Prepare the test organism suspensions as described in 5.5.2.1, 5.5.2.3 and 5.5.2.4, respectively, and ensure that each millilitre of growth medium contains:

a) 400 million (4×10^8) *Escherichia coli, Staphylococcus aureus* and *Pseudomonas auruginosa* organisms;

b) 10 million (10^7) *Aspergillus niger* organisms; and

c) 800 000 (8×10^5) *Bacillus subtilis* var. *globigii* spores,

respectively.

Use the suspensions within three hours of preparation.

5.12.2 Preparation of control and test solutions

5.12.2.1 Control solution

Dispense 9,0 mℓ of sterile water into sterile glass bottles and to each, add 0,5 mℓ of bovine albumin solution (see 5.4.3).

5.12.2.2 Test solution

Prepare the prescribed concentration of the test sample as stated on the label (see 7.2(f)), using sterile water. Dispense 9,0 mℓ of the test solution into sterile glass bottles and to each, add 0,5 mℓ of bovine albumin solution. Use the test solution on the day of preparation.

5.12.3 Temperature adjustment

Label the bottles containing the control, the test solutions and the test organism suspensions and place them for at least 30 min in a water-bath maintained at 22 °C.

5.12.4 Test procedure

NOTE – For the sake of brevity, the procedure for testing the disinfectant solution against one organism is given. For each organism, a control test is carried out, using sterile distilled water instead of the disinfectant solution. Each control runs concurrently with its associated test (with a time lapse of about 30 s between associated actions). Use a stop-watch for this purpose.

5.12.4.1 Melt the contents of a sufficient number of bottles (containing 15 mℓ volumes) of nutrient agar (see 5.4.9), cool to 45 °C and maintain them at this temperature.

5.12.4.2 Start the test after the temperature of the control, test solutions and the test organism suspensions have adjusted to 22 °C. Using a clean, sterile pipette, add 0,5 mℓ of the *Pseudomonas aeruginosa* test suspension (see 5.12.1(a)) to the test solution and the control (without removing the bottles from the water-bath). Remove the bottles from the water-bath, shake them to mix the contents thoroughly and immediately return them to the water-bath.

5.12.4.3 At the end of a 5 min exposure period (actual contact time), transfer 1 mℓ of the test solution containing the organism to 9 mℓ of inactivator medium for the "5,5,5" test (see 5.4.7.3) and mix well into a uniform suspension (first dilution). Within 30 s, repeat this procedure with the control.

5.12.4.4 Using a clean, sterile pipette, transfer 1 mℓ of the inactivator medium containing the organism (see 5.12.4.3) to the first of a series of bottles, each containing 9 mℓ of sterile diluent (see 5.4.5). Mix well into a uniform suspension (second dilution).

5.12.4.5 Repeat the procedure in 5.12.4.4, but transfer the suspension obtained after the second dilution to the second of the series of bottles. Dilute further until an end dilution of 1:1 000 of the test sample is obtained.

5.12.4.6 Consecutively prepare, as described above, a dilution series using the control.

5.12.4.7 From each dilution of the test sample, transfer 1 mℓ to each of two appropriately labelled, sterile plates (Petri dishes). Do the same for each end dilution of the control. Add 15 mℓ of nutrient agar (see 5.12.4.1) to each of the plates and swirl gently to ensure an even distribution of colonies after incubation. Avoid spilling any of the contents of the plates during this process. Allow the agar to solidify.

5.12.4.8 Invert the plates and incubate them at 37 °C for 48 h.

5.12.4.9 After incubation, examine the plates for growth. Count and record the colonies on each plate (of the test solution and of the control) that contains between 30 and 300 colonies. If the least diluted sample (with the highest concentration) yields less than 30 colonies, count all the colonies. Ensure that the colonies that have been counted are derived from survivors of the test organisms and not from contamination.

5.12.4.10 Take the dilution factor (*DF*) for the test sample and for the control as the inverse of the dilution, for example if the sample or the control has been diluted to 1/1 000, take *DF* as 1 000.

5.12.4.11 Calculate the log reduction factor, using the following formula:

$$\log (B \times DF_c) - (A \times DF_s)$$

where

A is the number of organisms counted after contact with the sample (see 5.12.4.9);

B is the number of organisms counted in the control (see 5.12.4.9);

DF_s is the dilution factor for the sample as calculated in 5.12.4.10; and

DF_c is the dilution factor for the control as calculated in 5.12.4.10.

NOTE — The same formula is applied to determine the log reduction factor of *Escherichia coli* and *Staphylococcus aureus* after 5 min contact time (see 5.12.4.12), *Aspergillus niger* after 15 min contact time (see 5.12.4.13) and *Bacillus subtilis* var. *globigii* after 1 h contact time (see 5.12.4.14).

5.12.4.12 Repeat the procedure described in 5.12.4.1 to 5.12.4.11 (inclusive) with the *Escherichia coli* and *Staphylococcus aureus* suspensions (see 5.12.1(a)).

5.12.4.13 Repeat the procedure described in 5.12.4.1 to 5.12.4.11 (inclusive) with the *Aspergillus niger* test organism suspension (see 5.12.1(b)), but:

a) use an exposure period (actual contact time) of 15 min;

b) add malt extract agar (see 5.4.8) to the suspensions on the plates; and

c) incubate for 7 d at 25 °C.

5.12.4.14 Repeat the procedure described in 5.12.4.1 to 5.12.4.11 (inclusive) with the *Bacillus subtilis* var. *globigii* spore suspension (see 5.12.1(c)), but use an exposure period (actual contact time) of 1 h.

5.12.5 Interpretation of results

Deem the sample to comply with the requirements of 4.2 if, for each vegetative organism tested, a log reduction value of at least 5, and for the *Bacillus subtilis* var. *globigii* spore, a log reduction value of at least 1 was obtained.

5.13 Determination of the virucidal activity, using bacteriophages as indicator organisms

5.13.1 Inactivator

Choose the most suitable inactivator for each product as determined in the tests for bactericidal efficacy.

5.13.2 Hard water

Choose the hard water for each product as determined in the tests for bactericidal efficacy.

5.13.3 Preparation of the test bacterial suspensions

Prepare *Escherichia coli* SABS TCC 36 and *Escherichia coli* SABS TCC 37 cultures as in 5.5.2.5.2.1 to 5.5.2.5.2.4 (inclusive).

5.13.4 Preparation of test bacteriophage suspensions

The MS2 and ΦX174 bacteriophages (see 5.5.2.5) are brought, immediately before use, to a titre between 10^7 and 10^8 plaque-forming units per millilitre by dilution in the nutrient medium (see 5.4.11).

5.13.5 Preparation of control and test solution

5.13.5.1 Control solution

Dispense 9,9 mℓ of hard water (see 5.13.2) or sterile distilled water, depending on the type of disinfectant or detergent-disinfectant (see tests for bactericidal efficacy), into a sterile glass bottle.

5.13.5.2 Test solution

Using hard water or sterile distilled water (see tests for bactericidal efficacy), prepare the prescribed concentration of the test sample as stated on the label (see 7.2(f)). Dispense 9,0 mℓ of the test solution

into a sterile glass bottle. Use the test solution on the day of preparation.

5.13.6 Temperature adjustment

Label the bottles containing the control, the test solutions and the test organism suspensions and place them for at least 30 min in a water-bath maintained at 22 °C.

5.13.7 Test procedure

NOTE – For the sake of brevity, the procedure for testing the disinfectant solution against bacteriophage strain MS2 is given. Each control runs concurrently with its associated test (with a time lapse of about 30 s between associated actions). Use a stop-watch for this purpose.

5.13.7.1 Melt the top layer agar (see 5.4.21) and aseptically dispense 5 mℓ volumes into sterile culture tubes (see 5.2.2(a)), which should be kept in a water-bath maintained at 44 °C, to prevent solidification of the agar.

5.13.7.2 Using a clean, sterile pipette, dispense 1 mℓ of the MS2 bacteriophage test suspension (see 5.13.4) into the test solution and to the control (without removing the bottles from the water-bath). Remove the bottles from the water-bath, vortex their contents and immediately return them to the water-bath.

5.13.7.3 At the end of a 15 min exposure period (actual contact time), transfer 1 mℓ of the test solution containing the bacteriophage (see 5.13.7.2) to 9 mℓ of inactivator (see 5.13.1) and vortex into a uniform suspension (first dilution). Within 30 s, repeat this procedure with the control.

5.13.7.4 Using a clean, sterile pipette, transfer 1 mℓ of the inactivator medium containing the bacteriophage (see 5.13.7.3) to the first of a series of bottles, each containing 9 mℓ of sterile physiological saline (see 5.4.13). Vortex into a uniform suspension (second dilution).

5.13.7.5 Repeat the procedure in 5.13.7.4, but transfer the suspension obtained after the second dilution to the second of the series of bottles. Dilute further until an end dilution of 1:100 of the test sample is obtained.

5.13.7.6 Consecutively prepare, as described above, a dilution series using the control until an end dilution of 1:100 000 is obtained.

5.13.7.7 From each dilution of the test sample, transfer 0,1 mℓ to each of 0,9 mℓ of the 2 h *E. coli* SABS TCC Esc 36 culture (see 5.13.3). Do the same for the end dilutions of the control.

5.13.7.8 Place the suspensions in an incubator at 37 °C for 15 min ± 1 min and then add 5,0 mℓ of the top layer agar (see 5.13.7.1).

5.13.7.9 Vortex and immediately spread the mixture over the surface of the bottom layer agar (see 5.4.2) in labelled Petri dishes and swirl gently to ensure an even distribution of organisms after incubation.

5.13.7.10 Allow the agar to solidify, invert and incubate the Petri dishes at 37 °C for 24 h.

5.13.7.11 After incubation, examine the plates for plaque formation. Count and record the plaques on each plate (of the test solution and of the control) that contains between 30 and 100 plaques. If the least diluted sample (with the highest concentration) yields less than 30 plaques, count all the plaques.

5.13.7.12 Take the dilution factor (*DF*) as the inverse of the dilution, for example if the solution has been diluted to 1/1 000, take *DF* as 1 000.

5.13.7.13 The percentage kill is given by the following formula:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

where

A is the number of plaques counted after contact with the sample (see 5.13.7.11);

B is the number of organisms counted in the control (see 5.13.7.11);

DF_s is the dilution factor for the sample as calculated in 5.13.7.12; and

DF_c is the dilution factor for the control as calculated in 5.13.7.12.

NOTE – The same formula is applied to determine the percentage kill of ϕX174 bacteriophage strain.

5.13.7.14 Repeat the procedure described in 5.13.7.1 to 5.13.7.13 (inclusive) with the ϕX174 bacteriophage strain.

5.13.7.15 Repeat the procedure described in 5.13.7.1 to 5.13.7.14 (inclusive) on two different days.

5.13.8 Interpretation of results

Deem the sample to comply with the requirements of 4.2 if, for each bacteriophage tested, a result of at least a 99,99 % kill was obtained.

6 Methods of physical and chemical examination

6.1 Corrosiveness

6.1.1 Test strip

One brightly finished uncoated aluminium strip that complies with BS 1470:1987, *Specification for wrought aluminium and aluminium alloys for general engineering purposes: plate, sheet and strip,* of size 75 mm x 19 mm x 1 mm, degreased by washing in acetone and drying at ambient temperature.

6.1.2 Procedure

6.1.2.1 Using a sharp instrument, divide the strip into equal halves by scoring a line across the strip through its midpoint.

6.1.2.2 Using a 100 mℓ measuring cylinder, completely immerse the strip in the test sample. Stopper the cylinder and maintain at 23 °C for 24 h. After 24 h, remove enough of the test sample to bring the level in the measuring cylinder to the midpoint line of the test strip. Restopper the cylinder and keep it at 23 °C for a further 24 h.

6.1.2.3 Remove the test strip, rinse it thoroughly first with water, then with acetone and allow to air dry. Examine the strip for compliance with 4.3.

6.2 Water-insoluble-matter content

6.2.1 Procedure

6.2.1.1 Pipette 5,0 mℓ of a liquid disinfectant or liquid detergent-disinfectant (or place 2 g of a solid disinfectant or solid detergent-disinfectant) into a beaker and add 250 mℓ of hard water (see 5.4.18.1).

6.2.1.2 Heat in a steam bath with frequent stirring until the sample is completely dispersed.

6.2.1.3 Filter the solution immediately, under suction, through a tared glass fibre filter and ensure that the insoluble matter is quantitatively transferred to the filter.

6.2.1.4 Wash the beaker and the residue five times with 20 mℓ volumes of hot hard water.

6.2.1.5 Allow the solution to drain completely and dry the residue at 105 °C until constant mass is attained. Cool in a desiccator and weigh.

6.2.2 Calculation

6.2.2.1 Solid disinfectants or solid detergent-disinfectants

Calculate the water-insoluble matter content S as a percentage by mass, using the formula:

$$S = \frac{m_2}{m_1} \times 100$$

where

m_1 is the mass of the test sample taken, in grams; and

m_2 is the mass of the residue after it has been dried, in grams.

6.2.2.2 Liquid disinfectants or liquid detergent-disinfectants

Calculate the content of water-insoluble-matter in the test solution, expressed in grams per litre, using the formula:

$$\frac{m}{V}$$

where

m is the mass of the residue after it has been dried, in grams; and

V is the volume of the test solution, in litres.

Check for compliance with 4.4.

6.3 Rinsing properties for detergent-disinfectants

6.3.1 Using a syringe, pipette 0,4 mℓ or weigh 0,4 g of the undiluted test sample into a thoroughly cleaned 500 mℓ conical flask and add 200 mℓ of standard hard water (see 5.4.18). Stopper the flask and shake it vigorously for 1 min. In the case of detergent-disinfectants based on organic halogen compounds (other than iodine compounds), phenolics and stabilized chlorine compounds, use 2,0 mℓ of each test sample in the case of a liquid detergent-disinfectant and 2 g of each test sample in the case of a solid detergent-disinfectant. In the case of detergent-disinfectants based on iodophors and quaternary ammonium compounds, test the smallest dilution recommended on the label (see 7.2(f)).

6.3.2 Pour out the solution and rinse the flask by adding 200 mℓ of the standard hard water, shaking vigorously for 1 min and pouring off the water. Invert the flask and allow to dry.

6.3.3 Carry out a blank by repeating the above procedure but omitting the test sample.

Compare the two flasks.

6.3.4 Consider the sample to comply with the requirements of 4.5 if the streaks and marks on the flask used for the test do not exceed those on the flask used for the blank.

6.4 Cleaning efficacy of detergent-disinfectants

6.4.1 Apparatus

6.4.1.1 Six test panels of anodized aluminium, machined from aluminium that complies with SABS 989:1994, *Aluminium and aluminium alloy casting ingots for remelting* (alloy designation Al-99,5: alloy code SA2). The panels are of the dimensions given in figure 1 and have indentations as shown in the figure.

6.4.1.2 Six suitable clamps, so fitted to the stirrers (see 6.4.1.3) as to allow the panel to be gripped and rotated about a vertical axis.

6.4.1.3 Six mechanical stirrers, capable, when supporting a test panel, of operating smoothly and continuously at 60 r/min to 62 r/min.

6.4.1.4 Water-bath, that is thermostatically controlled, capable of maintaining a temperature of 30 °C and fitted with a cover that has a row of six holes to accommodate the test beakers (see 6.4.1.5).

6.4.1.5 Six test beakers, each of capacity 800 mℓ, of internal diameter approximately 90 mm and of height approximately 135 mm.

6.4.1.6 Six flat-bottom glass crystallizing basins, each of capacity 175 mℓ, of diameter approximately 80 mm and of height approximately 40 mm to hold the six test panels.

6.4.1.7 Six tall-form beakers, each of capacity 150 mℓ.

6.4.1.8 Spatula, fitted with a flexible straight-edge steel blade.

6.4.1.9 Watch glasses.

6.4.2 Materials

6.4.2.1 Standard liquid detergent-disinfectant.[3)]

6.4.2.2 Margarine.[3)]

6.4.2.3 Standard hard water.

6.4.2.4 Ingredients

Calcium chloride ($CaCl_2.2H_2O$) ..... 0,880 g
Magnesium sulfate ($MgSO_4.7H_2O$) ..... 0,987 g
Water ..... 5 000 mℓ

6.4.2.5 Preparation

Dissolve the calcium chloride and magnesium sulfate in the water.

3) Obtainable from the South African Bureau of Standards, Private Bag X191, PRETORIA, 0001.

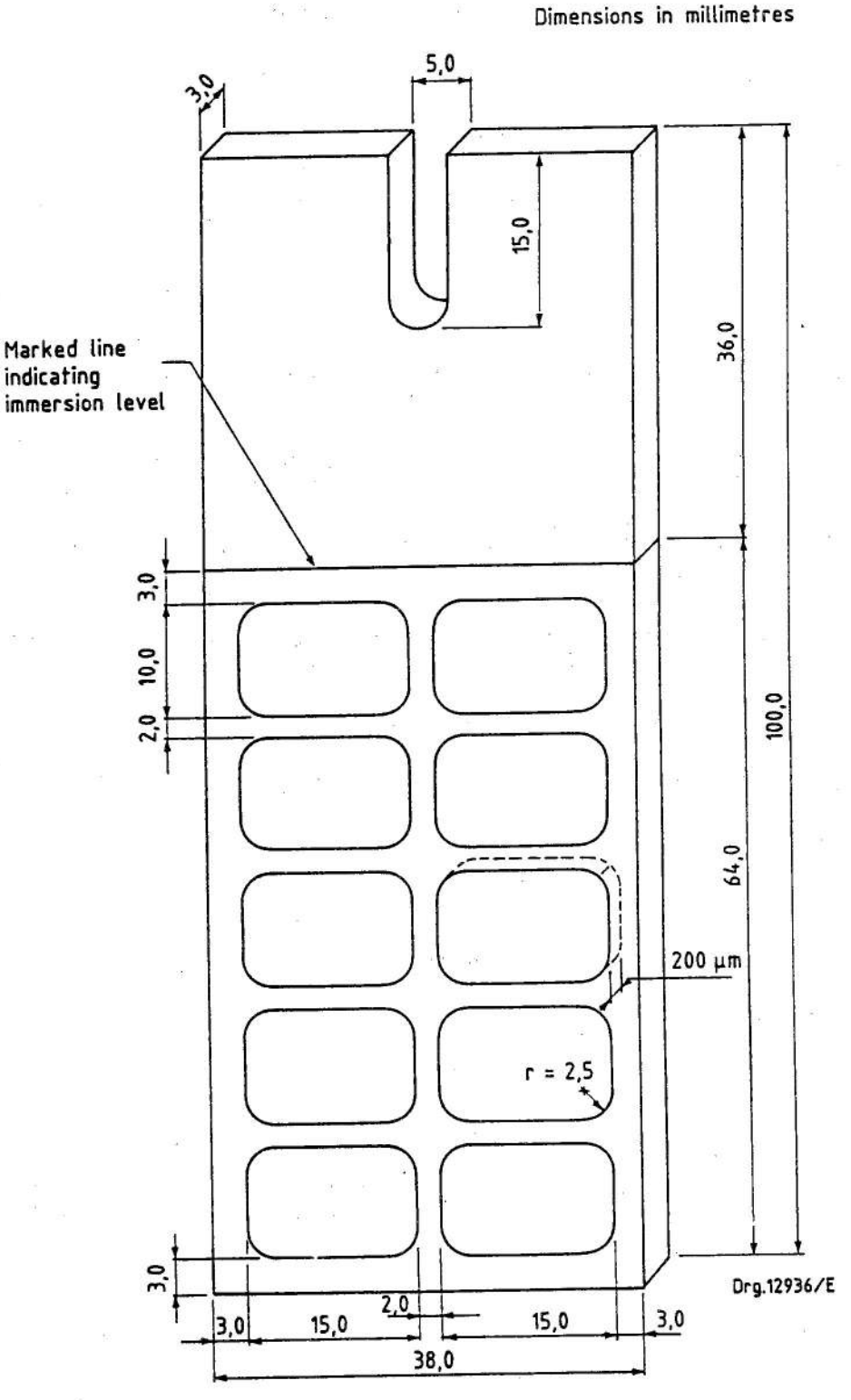

Figure 1 — Aluminium test panel

6.4.3 Procedure

6.4.3.1 Accurately weigh 15,0 g of the standard detergent-disinfectant into each of three beakers (see 6.4.1.5) and the appropriate mass of test sample into each of the other three beakers in order to obtain the required dilution (in the 750 mℓ of hard water mentioned in 6.4.3.2), as stipulated by the manufacturer. In the case of detergent-disinfectants based on iodophors, test a dilution that contains 75 mg/ℓ of free iodine. In all other cases, test the greatest dilution (smallest concentration) recommended on the label for cleaning and disinfection.

6.4.3.2 Add sufficient volumes of hard water to each beaker to bring the volume in each up to 750 mℓ. So place the beakers in the water-bath (see 6.4.1.4), maintained at 30 °C, that the three beakers containing the standard detergent-disinfectant are in the first, third and fifth holes and the three beakers containing the test samples are in the remaining holes. Cover the beakers with watch glasses (see 6.4.1.9).

6.4.3.3 Thoroughly clean the test panels (see 6.4.1.1) with a suitable detergent (for example, 1,0 % detergent that complies with SABS 892:1993, *General purpose detergent (beads, granules and powders)*, dissolved in hot water) and allow to air dry.

6.4.3.4 Place each panel in its glass crystallizing basin, clearly identify the panel-and-basin combination and place the basins and panels in an oven at 105 °C ± 3 °C for 1 h.

Remove the basins and panels from the oven and, after allowing them to cool in a desiccator, determine the mass of each basin together with its panel.

6.4.3.5 Homogenize the margarine by mixing it with the spatula.

6.4.3.6 Use the spatula to pack each panel with approximately 1,3 g of the margarine, ensuring that the mass of each panel does not differ by more than 0,2 g from any of the others and that the exposed surfaces of the margarine are smooth and level. When the panels are inspected, no pin holes, air bubbles or other inclusions shall be visible.

6.4.3.7 When each panel has been packed with margarine, place the panel in its glass crystallizing basin and determine the combined mass.

NOTE – Ensure that, throughout the procedure, the portion of the panel containing the margarine is not touched.

6.4.3.8 So fit each panel into a clamp of the stirrer (see 6.4.1.2) that the jaws of the clamp are positioned at the end of the panel above the soiled areas. So adjust each panel that it is vertical.

6.4.3.9 Lower a panel assembly carefully into the centre of each of the six beakers containing 750 mℓ of detergent-disinfectant solution (see 6.4.3.1), maintained at 30 °C, until the solution level just reaches the marked line above the soiled area (see figure 1).

6.4.3.10 Switch on the stirrers and, after stirring for 180 min at 60 r/min to 62 r/min, remove each panel (in turn) from the detergent solution and place it temporarily in a vertical position in an empty 150 mℓ tall-form beaker (see 6.4.1.7).

6.4.3.11 Remove any traces of margarine on the flat surfaces of the panels by using absorbent paper (for example, toilet paper), taking care that the margarine in the indentations of the panels is not touched. Remove any traces of margarine on the flat surfaces between the indentations of the panels, by scraping the flat surfaces with a straight-edge spatula (see 6.4.1.8).

6.4.3.12 Place each panel in its glass crystallizing basin, clearly identify the panel-and-basin combination and place the basins and panels into an oven at 105 °C ± 3 °C for 3 h. After allowing the glass basins and panels to cool in a desiccator, determine the mass of each glass basin together with its panel.

6.4.4 Calculation

6.4.4.1 Calculate the cleaning efficacy C (as a percentage by mass) of each test detergent and of each portion of standard detergent, as follows:

$$C = \frac{m_2 - m_3}{m_2 - m_1} \times 100$$

where

m_1 is the mass of the panel and the glass basin, in grams;

m_2 is the initial mass of the panel, margarine and glass basin, in grams; and

m_3 is the final mass of the panel, margarine and glass basin, in grams.

6.4.4.2 Calculate the mean cleaning efficacy of the test samples and of the standard detergent-disinfectant samples, subject to the following conditions:

a) use all three results (calculated as in 6.4.4.1) to calculate a mean if no two results differ by more than three percentage units difference; or

b) use any two results (calculated as in 6.4.4.1) that do not differ by more than three percentage units difference to calculate the mean; or

c) if the individual results (calculated as in 6.4.4.1) above do not conform to (a) or (b) above, discard all the results and repeat the test.

Check for compliance with 4.6.

6.5 Storage stability

6.5.1 Detergent-disinfectants based on iodophors

6.5.1.1 Reagents

6.5.1.1.1 0,02N sodium thiosulfate ($Na_2S_2O_3$) solution, accurately standardized.

6.5.1.1.2 10 g/ℓ starch solution.

6.5.1.2 Procedure

6.5.1.2.1 Transfer 200 mℓ of the sample to a 250 mℓ stoppered measuring cylinder. Cool to 10 °C ± 1 °C, maintain at this temperature for 24 h and then examine the test sample visually for signs of separation.

6.5.1.2.2 If visible separation has not occurred, allow the test sample to return to ambient temperature and then take from it two test specimens, one from near the surface and one from near the bottom of the contents of the cylinder.

6.5.1.2.3 Determine the available iodine content of each of these test specimens as follows:

Pipette accurately 1 mℓ of the test specimen into a 250 mℓ Erlenmeyer flask (see 5.2.6), add 100 mℓ of distilled or demineralized water and titrate with the 0,02N sodium thiosulfate (see 6.5.1.1.1) solution until a straw-coloured titration mixture has been reached. Add a small amount of the starch indicator (see 6.5.1.1.2) and take the end point at a starch indicator colour change from blue to colourless.

NOTE – As some iodophors release iodine very slowly as the end point is neared, a reasonable time should be allowed before the titration is regarded as completed.

6.5.1.2.4 Repeat the test with another 200 mℓ test sample but in the stage described in 6.5.1.2.1 above, warm and maintain the test sample at a temperature of 40 °C ± 1 °C for 24 h instead of cooling it.

6.5.1.3 Calculations

Calculate, as follows, the available iodine contents *I* (as a percentage) of each of the four test specimens:

$$I = A \times N \times 12{,}69$$

where

A is the volume of the $Na_2S_2O_3$ solution used in the titration, in millilitres; and

N is the normality of the $Na_2S_2O_3$ solution.

6.5.1.4 Assessment of results

Examine the results for compliance with the relevant requirements of 4.7.1.

6.5.2 Coal-tar type disinfectant liquids

6.5.2.1 Stability before dilution (normal test)

6.5.2.1.1 Pour 100 mℓ of the composite test sample into a 100 mℓ stoppered measuring cylinder. Cool to 5 °C ± 1 °C and maintain at this temperature for 7 d, ensuring that, during this period, the liquid is not exposed to direct sunlight or to the possibility of localized overheating. With the minimum disturbance of the liquid, place the cylinder in front of a strong beam of light and examine carefully for separation of the ingredients and the presence of any sediment. If, in the case of a white disinfectant liquid, a creamed liquid is present, invert the measuring cylinder and restore it to an upright position three times and then examine the liquid for uniformity.

6.5.2.1.2 Repeat the procedure described in 6.5.2.1 with another 100 mℓ of the disinfectant liquid, but keep the cylinder for 7 d in a water-bath maintained at 45 °C ± 1 °C.

6.5.2.1.3 In the case of a black disinfectant liquid, repeat the procedure described in 6.5.2.1.1 with a further 100 mℓ of the disinfectant liquid, but cool the liquid to 0 °C ± 1 °C and maintain it at this temperature for 1 h.

6.5.2.1.4 Examine for compliance with 4.7.2.1.

6.5.2.2 Stability before dilution (quick test) (white disinfectant liquids only)

NOTE – This test may not be regarded as a substitute for the normal test of 6.5.2.1.

Pour 10 mℓ of the liquid into 90 mℓ of water at 20 °C to 22 °C contained in a clean boiling tube of nominal diameter 38 mm and that has a hemispherical end. Seal the tube with a suitable stopper and invert it, using a corkscrew motion, six times (i.e. through three complete end-over-end cycles). Allow to stand for 6 h at 20 °C to 22 °C away from direct light and then measure the depth of any deposit in the bottom of the tube.

6.5.2.3 Stability after dilution

6.5.2.3.1 Adjust the temperature of the test sample and hard water (see 5.4.18.4) to 25 °C ± 1 °C and then prepare at least 100 mℓ of the highest dilution recommended on the container, by pouring the appropriate volume of sample into the appropriate volume of hard water contained in a stoppered measuring cylinder. Form an emulsion by inverting and righting the stoppered cylinder (using a corkscrew motion) through 30 complete end-over-end cycles. Transfer to a 100 mℓ stoppered measuring

cylinder, allow to stand undisturbed for 6 h at 25 °C ± 1 °C, then examine the emulsion against a strong beam of light for signs of breaking up of the emulsion and the presence of more than just traces of oil at the top or at the bottom.

6.5.2.3.2 Repeat the procedure given in 6.5.2.3.1 but prepare the lowest dilution recommended on the container.

6.5.2.3.3 Examine for compliance with 4.7.2.2.

6.5.3 Disinfectants and detergent-disinfectants based on glutaraldehyde

6.5.3.1 Store type 1 (see table 1) disinfectants or detergent-disinfectants and the liquid component of type 2 (see table 1) disinfectants or detergent-disinfectants in their original unopened bottles at 5 °C ± 1 °C for 24 h. Test the contents of 50 % of the bottles in the sample for compliance with 4.7.3.1.

6.5.3.2 Store the solid components of type 2 detergent-disinfectants in their original unopened bottles under ambient conditions for 6 months. Test the contents of 50 % of the bottles in the sample for compliance with 4.2 and 4.7.3.2.

6.5.4 Disinfectants and detergent-disinfectants based on chlorhexidine gluconate

6.5.4.1 Separately store samples of the disinfectant or detergent-disinfectant in their original unopened bottles at 5 °C ± 1 °C and at 43 °C ± 1 °C for 48 h. Test the contents of the bottles for compliance with 4.7.4.1.

6.5.4.2 Store the disinfectants or detergent-disinfectants at a temperature of 20 °C ± 5 °C for a period of 12 months from the date of manufacture and test the contents of the bottles for compliance with 4.2 and 4.7.4.2.

6.5.5 Disinfectants or detergent-disinfectants based on quaternary ammonium compounds, stabilized inorganic chlorine compounds and organic or inorganic halogen compounds (other than iodine compounds)

6.5.5.1 Liquid disinfectants or liquid detergent-disinfectants

Store each disinfectant or detergent-disinfectant in its original unopened bottle at 5 °C ± 1 °C for 12 h. Check for compliance with 4.7.5.1.

6.5.5.2 Solid disinfectants or solid detergent-disinfectants

Store the disinfectant or detergent-disinfectant in its original unopened bottle at ambient temperature under normal conditions for 6 months. Check for compliance with 4.7.5.2.

6.6 Determination of pH value

6.6.1 Dilution of disinfectants and detergent-disinfectants

6.6.1.1 Stabilized inorganic chlorine compounds

Dissolve 1 g of a solid test sample or 1 mℓ of a liquid test sample in 100 mℓ of carbon-dioxide-free distilled water.

6.6.1.2 Iodophors

Dissolve 1 mℓ of the test sample in 100 mℓ of carbon-dioxide-free distilled water.

6.6.1.3 Phenolics

Dissolve 1 g of a solid test sample or 1 mℓ of a liquid test sample in 100 mℓ of carbon-dioxide-free distilled water.

6.6.1.4 Organic halogen compounds (other than iodine compounds)

Dissolve 1 g of a solid test sample or 1 mℓ of a liquid test sample in 100 mℓ of carbon-dioxide-free distilled water.

6.6.1.5 Quaternary ammonium compounds

Prepare a 1/100 dilution of the test sample in carbon-dioxide-free distilled water.

6.6.1.6 Chlorhexidine gluconate

Dilute the test sample in carbon-dioxide-free distilled water to the prescribed concentration (see 7.2(f)).

6.6.2 Procedure

Use a suitable, acceptable pH meter that has been appropriately calibrated to determine the pH value of the sample.

6.6.3 Expression of results

Express the results as "pH at 25 °C".

6.6.4 Interpretation of results

Check for compliance with 4.8.

6.7 Freedom from visible impurities of solid disinfectants or solid detergent-disinfectants based on quaternary ammonium compounds, stabilized inorganic chlorine compounds and organic or inorganic halogen compounds (other than iodine compounds)

Spread approximately 50 g of each test sample over the bottom of a 150 mm diameter Petri dish. Check for compliance with 4.9 by viewing the sample from approximately 600 mm.

6.8 Added colouring matter for disinfectants or detergent-disinfectants based on iodophors

6.8.1 Reagent

Sodium thiosulphate, 50 g/ℓ aqueous solution.

6.8.2 Procedure

Using a pipette, transfer 5 mℓ of the test sample to a clean test tube. Using a separate pipette, add 5 mℓ of the sodium thiosulphate solution and mix well.

6.8.3 Assessment of results

If the colour of the mixture in the tube is no darker than a pale straw colour 30 s after mixing, deem the detergent-disinfectant to comply with 4.10.

7 Packaging and marking

7.1 Packaging

Methods of packaging that are suitable for the disinfectant or detergent-disinfectant as described in SABS 0229:1990, *Packaging of dangerous goods for road and rail transportation in South Africa*, and the relevant regulations under the Trade Metrology Act, 1973 (Act 77 of 1973), shall be complied with. The bottles (including the closures) in which the disinfectant or detergent-disinfectant is packed shall not interact chemically or physically with the disinfectant or detergent-disinfectant and shall be strong enough to protect the disinfectant or detergent-disinfectant adequately during normal handling, storage and transportation. The closure shall not be made of cork or of any material containing cork.

7.2 Marking

The following information shall appear on each container, or on a label securely attached to each container, in prominent, legible and indelible marking in type of such size and presentation as is 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 registration number and full name and address of the manufacturer, producer, proprietor, or controlling company or, in the case of bottles packed for any other person or organization, the full name and address of that person or organization;

b) words indicating the type of disinfectant or detergent-disinfectant and its active disinfecting ingredient (see table 1) and whether it is suitable for use for cleaning-in-place operations;

c) the nominal volume or mass (as appropriate) of the contents, in plain type and in a colour that affords a distinct contrast to the colour of the container or label;

d) the batch identification; or

e) the production date of the batch (or both);

f) general instructions for use for the various purposes for which the disinfectant or detergent-disinfectant is suitable, including the recommended dilutions that allow the product to comply with the requirements of 4.2 (disinfecting efficacy), for the exposure period and test organism stated in the test method for each purpose;

g) where applicable, the pH value at the recommended dilution (see (f) above);

h) the first-aid treatment and the name of any antidote that may be used;

i) where applicable, warnings that:

   1) the disinfectant or detergent-disinfectant should be stored in closed bottles in a dry place at a temperature not exceeding 30 °C, protected from intense light and apart from flammable materials, food products and packaging material;

   2) contact of the undiluted disinfectant or detergent-disinfectant and its fumes with skin and eyes should be avoided;

   3) rubber gloves and where necessary, goggles, should be worn when the disinfectant or detergent-disinfectant is being handled;

   4) unless recommended by the manufacturer, the disinfectant or detergent-disinfectant should not be mixed with other incompatible substances (such as soap and other anionic detergents) where relevant;

5) some disinfectants, such as iodophors, could stain certain porous materials (such as plastics) and corrode certain metals (such as copper, iron, silver alloys and aluminium); and

6) the efficacy of the disinfectant could be compromised if surfaces are soiled;

j) the storage period or shelf life of the diluted disinfectant or detergent-disinfectant, when diluted to the lowest recommended concentration (highest use dilution);

k) the expiry date of the disinfectant or detergent-disinfectant;

l) if, in the case of disinfectants based on glutaraldehyde, the disinfectant or detergent-disinfectant is supplied in the form of a homogeneous liquid and an activator, a statement that the liquid and the activator shall be mixed before use as a disinfectant;

m) the strength designation and, in the case of coal-tar type disinfectants, the nominal Rideal-Walker coefficient (see 5.9.5);

n) in the case of bottles for black coal-tar type disinfectants, the following statement: "Black coal-tar type disinfectant liquid is not recommended for use with exceptionally hard water";

o) a substantiation of specific virucidal claims;

p) the product name (which shall not be misleading to the consumer); and

q) where applicable, a statement that the disinfectant or detergent-disinfectant has to be shaken before use.

No. R. 529

14 Mei 1999

WET OP STANDAARDE, 1993

VERPLIGTE SPESIFIKASIE VIR ONTSMETTINGSMIDDELS EN DETERGENTONTSMETTINGSMIDDELS

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 ontsmettingsmidddels en detergentontsmettingsmiddels 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.

ALEXANDER ERWIN
Minister van Handel en Nywerheid

BYLAE

VERPLIGTE SPESIFIKASIE VIR ONTSMETTINGSMIDDELS EN DETERGENTONTSMETTINGSMIDDELS

1 Bestek

Hierdie spesifikasie dek vereistes vir ontsmettingsmiddels en detergentontsmettingsmiddels wat bedoel is vir gebruik op nielewende oppervlakke.

OPM

1 Alle ontsmettingsmiddels en detergentontsmettingsmiddels in die Republiek van Suid-Afrika word deur hierdie verpligte spesifikasie gedek. Benewens die vereistes in hierdie spesifikasie is die vereistes afgekondig deur die Registrateur, Wet op Landbouplae, 1983 (Wet 36 van 1983), sowel as dié met betrekking tot stowwe onderworpe aan die Wet op Beheer van Medisyne en Verwante Stowwe, 1965 (Wet 101 van 1965), ook van toepassing. Biododers vir gebruik by waterbehandeling word uitgesluit.

2 As gevolg van spesifieke gebruike of verklarings wat op die etiket of in die gebruiksaanwysings voorkom, kan dit nodig wees om ook die ontsmettingsmiddels of detergentontsmettingsmiddels ingevolge Wet 36 of Wet 101, na gelang van die doel van die gebruik, te registreer.

3 Dit is nie moontlik om die bakteriedodende aktiwiteit van die onverdunde produk aan die hand van die verpligte spesifikasie te bepaal nie. Die byvoeging van inokulum, harde water of steriele afgeroomde melk gee altyd 'n mate van verdunning.

4 Indien daar met verwysing na hierdie spesifikasie op virusdodende doeltreffendheid aanspraak gemaak word, mag daar nie na 'n spesifieke virus verwys word nie, maar 'n algemene verwysing daarna kan op die etiket aangebring word.

5 Indien 'n produk aan die vereistes van hierdie verpligte spesifikasie voldoen, kan daar geoordeel word dat dit bakteriedodend of virusdodend is, maar daar moet nie noodwendig afgelei word dat die produk 'n geskikte ontsmettingsmiddel vir 'n bepaalde doel is nie.

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 adres: 'n Adres in die Republiek van Suid-Afrika wat die straat- of padadres (indien 'n nommer toegeken is), die naam van die straat of pad en die naam van die voorstad, dorp of stad en, in die geval van 'n plaas, die naam van die plaas en van die landdrosdistrik waarin dit geleë is, insluit. In die geval van ingevoerde ontsmettingsmiddels of detergentontsmettingsmiddels beteken "adres" die adres van die fabrikant of leweransier of invoerder.

2.3 bakteriofaag (faag): 'n Virus wat bakterieë besmet.

OPM – In hierdie verpligte spesifikasie word faag gebruik as 'n model om algemene virusdodende doeltreffendheid te beoordeel.

2.4 produksielot: Die hoeveelheid verseëlde houers ontsmettingsmiddel of detergentontsmettingsmiddel wat met een homogene mengsel gevul is of, in die geval van deurlopende produksieprosesse, uit een dag se produksie gevul is.

2.5 skoon toestande: Toestande waar oppervlakke fisies skoon is voordat die ontsmettingsmiddel of detergentontsmettingsmiddel aangewend word.

2.6 skuim: 'n Verskynsel wat verband hou met 'n konsentrasie van die gedispergeerde fase in een deel van 'n emulsie ten koste van die gedispergeerde fase in die oorblywende deel van die emulsie.

2.7 kritiese verdunning: Die hoogste verdunning met water van 'n koolteertipe ontsmettingsmiddel of van die standaardfenol wat, volgens 5.9 getoets, toelaat dat *Salmonella typhi* groei nadat dit 2,5 min en 5,0 min lank daaraan blootgestel is maar nie nadat dit 7,5 min lank daaraan blootgestel is nie.

2.8 detergentontsmettingsmiddel: 'n Produk wat tegelyk skoonmaak en ontsmet.

2.9 vuil toestande: Toestande waarin daar in só 'n mate organiese materiaal op oppervlakke aanwesig is dat 'n hoër konsentrasie ontsmettingsmiddel of detergentontsmettingsmiddel as normaalweg nodig is om die inaktiveringsuitwerking van die organiese materiaal te kanselleer en steeds ontsmetting te verseker.

2.10 ontsmettingsmiddel: 'n Chemiese middel wat die meeste vegetatiewe vorms van patogeniese en ander mikroörganismes (maar nie noodwendig alle bakterie- en fungusspore, mikobakterieë, rickettsiae of virusse nie) op nielewende oppervlakke doodmaak.

2.11 fabriek: 'n Perseel waar die ontsmettingsmiddel of detergentontsmettingsmiddel op só 'n wyse vervaardig, geproduseer of herpak of gehanteer word dat die aktiwiteite 'n uitwerking op die kwaliteit van die ontsmettingsmiddel of detergentontsmettingsmiddel kan hê.

2.12 nielewende oppervlak: 'n Ander oppervlak as lewende mense- of lewende diereweefsel.

2.13 plaket: 'n Duidelike sone lise op 'n laag bakterieë wat die resultaat is van die lisebesmetting van een bakterie deur een bakteriofaag, asook daaropvolgende besmetting van omliggende bakterieë deur die nageslag van dié faag.

2.14 plakettelling: Die totale getal plakette wat getel word op 'n laag bakterieë wat ontwikkel op 'n plaat bakteriekweekmedium wat by 37 °C geïnkubeer is.

3 Algemene vereistes vir die fabriek en vir werknemers

3.1 Algemeen

Daar moet aan al die statutêre vereistes van die Wet op Beroepsgesondheid en Veiligheid, 1993 (Wet 85 van 1993), en die Wet op Gesondheid, 1977 (Wet 63 van 1977) voldoen word.

3.2 Konstruksie, uitleg en toestande van fabriek

3.2.1 Die fabrieksgeboue moet van 'n goeie konstruksie en in 'n goeie toestand wees en dit moet groot genoeg wees om opeenhoping van toerusting en werknemers te verhoed en om toereikende skoonmaak en die handhawing van produkkwaliteit moontlik te maak. 'n Beheerstelsel moet in stand gehou word om die fabriek vry van voëls, knaagdiere, insekte en ander ongediertes te hou en om te verseker dat die ontsmettingsmiddel of detergentontsmettingsmiddel nie gekontamineer word nie en dat die kwaliteit van die ontsmettingsmiddel of detergentontsmettingsmiddel op geen wyse in die gedrang kom nie.

3.2.2 Dak en plafon

Die dak moet weerbestand wees en die plafon (of onderkant van die dak as daar geen plafon is nie) moet glad en redelik stofdig wees.

3.2.3 Mure, vloere en deure

Buitemure moet weerbestand en waterdig wees. Deure en deurkosyne moet van korrosiebestande materiaal wees of só beskerm wees dat korrosie verhoed word. Vloere moet van beton of ander duursame, ondeurlatende en glyvaste materiaal wees wat teen slytasie en korrosie bestand is en maklik skoongemaak kan word. Daar moet voorsiening vir toereikende dreinering gemaak word.

3.2.4 Verligting

Die algemene verligting moet sodanig wees dat daar tydens die vervaardiging en produksie van die ontsmettingsmiddel of detergentontsmettingsmiddel doeltreffend gewerk kan word.

3.2.5 Ventilasie

Daar moet voorsiening gemaak word vir 'n toereikende toevoer van vars lug en die voorkoming van die opbou van toksiese gasse. Natuurlike ventilasie moet, indien nodig, deur meganiese middele aangevul word.

3.2.6 Bergfasiliteite vir verpakkingsmateriaal

Houers, proppe, kartonne en etikette vir die pak en verpakking van die ontsmettingsmiddels of detergent-ontsmettingsmiddels moet in skoon, redelik stofdigte, droë bergfasiliteite geberg word.

3.2.7 Bergfasiliteite vir klaar ontsmettingsmiddels of detergentontsmettingsmiddels

Klaar ontsmettingsmiddels of detergentontsmettingsmiddels wat op versending wag, moet in goed geventileerde bergfasiliteite gestapel word maar nie regstreeks op die vloer nie. 'n Afsonderlike kwarantyngebied moet vir die berging van afgekeurde materiaal voorsien word.

3.2.8 Afval

'n Afsonderlike kamer vir afval of ander ewe geskikte fasiliteit vir afval moet op die perseel voorsien word. Afvalmateriaal moet stiptelik en doeltreffend weggedoen word op 'n wyse wat nie die omgewing in die gedrang bring nie en dit moet aan die vereistes van die plaaslike owerhede voldoen.

3.2.9 Huishouding

Die fabriek en sy toerusting moet op só 'n wyse skoongemaak en in stand gehou word dat die kwaliteit van die ontsmettingsmiddel of detergentontsmettingsmiddel gehandhaaf kan word en die veiligheid van die personeel verseker word.

3.2.10 Water

3.2.10.1 Drinkbare water

Elke fabriek moet 'n voldoende toevoer van skoon, drinkbare water hê wat vry is van gesuspendeerde stowwe en stowwe wat nadelig vir die ontsmettingsmiddel of detergentontsmettingsmiddel of skadelik vir die gesondheid is.

3.2.10.2 Skoonmaakwater

Water wat vir die skoonmaak van die fabriek en toerusting gebruik word, moet aan die vereistes van 3.2.10.1 voldoen.

3.2.11 Geriefsfasiliteite

Voldoende kleedkamers, handewasbakke, stortbaddens met warm en koue lopende water, en sanitêre fasiliteite moet voorsien word.

3.2.12 Toetsfasiliteite

Die fabrikant moet toegang hê tot aanneemlike toetsfasiliteite en moet dit ook gebruik om te verseker dat die ontsmettingsmiddel of detergentontsmettingsmiddel aan die vereistes van afdeling 4 voldoen.

3.2.13 Rekords

Geskikte rekords van die resultate van die toetse wat uitgevoer is, moet minstens drie jaar lank vir die administratiewe owerheid beskikbaar wees.

4 Algemene vereistes vir die ontsmettingsmiddel of detergentontsmettingsmiddel

4.1 Tipe

Die verskillende tipes ontsmettingsmiddels of detergentontsmettingsmiddels word in tabel 1 aangegee. Dit word as voorbeelde verskaf en beperk nie die ontwikkeling van nuwe formulerings of tipes nie.

4.2 Ontsmettingsdoeltreffendheid

By die toets van die ontsmettingsmiddel of detergentontsmettingsmiddel volgens die toepaslike toetsprosedures in 5.6 tot 5.13 en 6.4 (kyk tabel 1) teen die voorgeskrewe konsentrasie, moet die aanspraak wat op die doeltreffendheid van die ontsmettingsmiddel of detergentontsmettingsmiddel op die verpakking, in die verpakkingsblaadjie of op die etiket op die verpakking (kyk 7.2(f)) of in die steuninligting van die produk gemaak word, as die ontsmettingsdoeltreffendheidsvereiste vir die ontsmettingsmiddel of detergentontsmettingsmiddel beskou word.

OPM

1 Indien nuwe ontsmettingsmiddels of detergentontsmettingsmiddels ontwikkel en geformuleer word en hierdie metodes nie geskik is vir gebruik met die nuwe ontsmettingsmiddels of detergentontsmettingsmiddels nie, sal bykomende toetsmetodes moontlik voor die implementering en gebruik daarvan ontwikkel en gevalideer moet word. In die meeste gevalle kan nuwe ontsmettingsmiddels of detergentontsmettingsmiddels egter aan die hand van bestaande prosedures met of sonder geringe modifikasies getoets word.

2 In gevalle waar meer as een aktiewe bestanddeel gebruik word, word die tipe toets deur die stof met die hoogste konsentrasie bepaal.

4.3 Korroderendheid

By die toets van die ontsmettingsmiddel of detergentontsmettingsmiddel volgens 6.1 teen die voorgeskrewe konsentrasie, mag dit, indien toepaslik, nie meer as 'n geringe dofwording op die oppervlak van die aluminium veroorsaak nie en die toetsstrook mag geen tekens van invreting, etsing of verkleuring toon nie. Indien daar aanspraak gemaak word op niekorroderendheid van 'n spesifieke materiaal, moet die fabrikant die aanspraak bewys.

4.4 Gehalte aan wateronoplosbare stof

By die toets van die ontsmettingsmiddel of detergentontsmettingsmiddel volgens 6.2 teen die voorgeskrewe konsentrasie, mag die gehalte aan wateronoplosbare stof, indien toepaslik, hoogstens die toepaslike van die volgende wees:

a) vloeibare ontsmettingsmiddels of detergentontsmettingsmiddels: 5,0 g/ℓ; of

b) vaste ontsmettingsmiddels of detergentontsmettingsmiddels: 2,5 % (volgens massa).

Tabel 1 — Toetsmetodes vir ontsmettingsdoeltreffendheid

1	2	3	4	5	6	7	8
Toets-onder-afdeling	**Produk**	**Tipe 1**	**Tipe 2**	**Aanvanklike toets-organisme-suspensie** per mℓ	**Finale organisme-kontaklas** per mℓ	**Doeltreffend-heidsgrens**	**Toets-blootstel-tyd**
5.6	Ontsmettingsmiddels en detergentontsmettingsmiddels met chloorheksidienglukonaat as basis	Vloeistof	Vaste stof	10^7 - 10^8	10^6	99,9 % of log-reduksie > 3	1 min
5.7	Ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied en ander aldehiede as basis vir algemene gebruik	'n Homogene gestabiliseerde vloeistof	'n Homogene vloeistof met 'n aktiveerder wat 'n vloeistof of 'n vaste stof kan wees wat afsonderlik voorsien word en na vermenging die ontsmettingsmiddel sal uitmaak	10^5	10^4	99,9 %	Pse[1], Esc[2], Sta[3] 5 min Asp[4] 15 min
5.7	Ontsmettingsmiddels met glutaaraldehied as basis vir gebruik op mediese instrumente	'n Homogene gestabiliseerde vloeistof	'n Homogene vloeistof met 'n aktiveerder wat 'n vloeistof of 'n vaste stof kan wees wat afsonderlik voorsien word en na vermenging die ontsmettingsmiddel sal uitmaak	10^7	10^6	99,99 %	Pse[1] 5 min Asp[4] 15 min Spore[5] 4 h
5.8	Ontsmettingsmiddels en detergentontsmettingsmiddels met fenol (Kelsey Sykes) as basis	'n Homogene vloeistof	'n Vaste stof	10^8 - 10^{10}	Nie van toepassing nie	> 2/5 buise geen groei	8 min 18 min
5.9	Koolteertipe ontsmettings-vloeistowwe (swart en wit) (RW-koëffisiënt)	Swart ont-smettingsvloeistof	Wit ontsmettingsvloeistof	Nie van toepassing nie	Nie van toepassing nie	Nie van toepassing nie	2,5 min 5 min 7,5 min
5.10	Ontsmettingsmiddels en detergentontsmettingsmiddels met jodofore as basis	Sonder bygevoegde suur	Met 'n aanneemlike suur bygevoeg	10^5	10^3	99,9 %	5 min
5.10	Ontsmettingsmiddels en detergentontsmettingsmiddels met organiese of anorganiese halogeenverbindings (uitgesonderd jodiumverbindings) as basis	Skuimvormend	Nieskuimvormend	10^5	10^3	99,9 %	30 s

Tabel 1 *(einde)*

1	2	3	4	5	6	7	8
Toets-onder-afdeling	**Produk**	**Tipe 1**	**Tipe 2**	**Aanvanklike toets-organisme-suspensie** per mℓ	**Finale organisme-kontaklas** per mℓ	**Doeltreffend-heidsgrens**	**Toets-blootstel-tyd**
5.10	Ontsmettingsmiddels en detergentontsmettingsmiddels met nieoksiderende surfaktante, byvoorbeeld kwaternêre ammoniumverbindings, suuranione en amfotere as basis	'n Homogene vloeistof	'n Vaste stof	10^5	10^3	99,9 %	5 min
5.10	Ontsmettingsmiddels en detergentontsmettingsmiddels met gestabiliseerde anorganiese chloorverbindings as basis	Skuimvormend	Nieskuim-vormend	10^5	10^3	99,9 %	Aanbevole minimum blootsteltydperk op etiket
5.11	Peroksigeenontsmettingsmiddels en -detergentontsmettings-middels wat mengsels soos peroksigeenverbindings, surfaktante, organiese sure en bufferstelsels as basis kan hê	'n Vloeistof	'n Vaste stof	10^8	10^6	99,9 %	Pse[1] 5 min Asp[4] 15 min Spore[5] 4 h en 6 h
5.12	Aktiewe bestanddele wat nie elders aangegee word nie	Soos toepaslik	Soos toepaslik	10^8 Bakterieë 10^7 Asp[4]	10^7 Bakterieë 10^6 Asp[4]	Bakterieëlog 5 Spoorlog 1	5 min
5.13	Ontsmettingsmiddels en detergentontsmettingsmiddels wat na bewering virusdodend is	Soos toepaslik	Soos toepaslik	10^7	10^6	99,9 %	20 min
6.4	Detergent – bewerings	Soos toepaslik	Soos toepaslik	–	Geen	> 80 % van standaard maar > 60 % van standaard vir 'n chloortipe	Nie van toepassing nie

1) *Pseudomonas aeruginosa.*
2) *Escherichia coli.*
3) *Staphylococcus aureus.*
4) *Aspergillus niger.*
5) *Bacillus subtilis* var *globigii.*

4.5 Afspoeleienskappe van detergentontsmettingsmiddels

By die toets van die detergentontsmettingsmiddel volgens 6.3 teen die voorgeskrewe konsentrasie, moet dit maklik afspoelbaar wees.

OPM – Hoewel die toets vir afspoeleienskappe in die geval van kwaternêre ammoniumverbindings kan toon dat die detergentontsmettingsmiddel maklik afspoelbaar is, kan nieafspoelbare residu's moontlik op die behandelde oppervlak agterbly.

4.6 Skoonmaakdoeltreffendheid van detergentontsmettingsmiddels

By die toets van die detergentontsmettingsmiddel volgens 6.4 teen die voorgeskrewe konsentrasie, behalwe in die geval van 'n chloortipe, moet sy gemiddelde skoonmaakdoeltreffendheid minstens 80 % van die gemiddelde skoonmaakdoeltreffendheid van die standaarddetergentontsmettingsmiddel wees. In die geval van 'n chloortipe detergentontsmettingsmiddel moet die gemiddelde skoonmaakdoeltreffendheid minstens 60 % van dié van die standaarddetergentontsmettingsmiddel wees.

4.7 Bewaarstabiliteit

4.7.1 Detergentontsmettingsmiddels met jodofore as basis

By die toets van die detergentontsmettingsmiddel volgens 6.5.1:

a) mag geen sigbare skeiding tydens die 24 h afkoeltydperk of die 24 h verhittingstydperk plaasvind nie; en

b) mag die hoogste van die vier waardes van die beskikbare gehalte aan jodium nie van die laagste een met meer as 10 % van die laagste waarde verskil nie.

4.7.2 Koolteertipe ontsmettingsmiddels

4.7.2.1 Stabiliteit voor verdunning

By die toets van koolteertipe ontsmettingsmiddels volgens 6.5.2.1, mag die ontsmettingsvloeistof tydens geeneen van die twee toetse meer as net tekens van skeiding van olie aan die bo- of onderkant toon nie. Daar word nie geag dat 'n skuimvloeistof (kyk 2.6) wat weer eenvormig word as die silinder drie maal omgekeer en weer regop gedraai word, skeiding ondergaan het nie.

OPM – Die vinnige toets in 6.5.2.2 kan nie as plaasvervanger vir die normale toets in 6.5.2.1 beskou word nie, maar dit kan dien as aanduiding van die stabiliteit van 'n wit ontsmettingsvloeistof. In hierdie toets word stabiliteit aangedui as die diameter van 'n neerslag hoogstens 10 mm is.

4.7.2.2 Stabiliteit na verdunning

By die toets van koolteertipe ontsmettingsmiddels volgens 6.5.2.3, moet die ontsmettingsvloeistof in die hoogste sowel as die laagste verdunnings wat op die houer aanbeveel word, met harde water (kyk 5.4.18.4) mengbaar wees, en die emulsies mag geen teken van skeiding toon nie en net tekens van skeiding van olie aan die bo- of onderkant.

4.7.3 Ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis

4.7.3.1 Volgens 6.5.3.1 getoets, moet 'n tipe 1-ontsmettingsmiddel of -detergentontsmettingsmiddel en die vloeibare komponent van 'n tipe 2-ontsmettingsmiddel of -detergentontsmettingsmiddel homogeen en vryvloeiend bly en moet dit aan 4.2 voldoen.

4.7.3.2 Volgens 6.5.3.2 getoets, moet die vaste komponent van 'n tipe 2-ontsmettingsmiddel of -detergentontsmettingsmiddel vryvloeiend bly en die tipe 2-ontsmettingsmiddel of -detergentontsmettingsmiddel wat daardeur geaktiveer word, moet steeds aan 4.2 voldoen.

4.7.3.3 Nadat 'n tipe 1-ontsmettingsmiddel of -detergentontsmettingsmiddel verdun is of nadat die twee komponente van 'n tipe 2-ontsmettingsmiddel of -detergentontsmettingsmiddel tot die voorgeskrewe konsentrasie gemeng is en die ontsmettingsmiddel in toe donker houers by 25 °C bewaar is vir die effektiewe gebruiksduur wat op die etiket gemeld word (kyk 7.2(j)), moet die resulterende verdunning steeds aan 4.2 voldoen.

4.7.4 Ontsmettingsmiddels of detergentontsmettingsmiddels met chloorheksidienglukonaat as basis

4.7.4.1 Volgens 6.5.4.1 bewaar, moet die ontsmettingsmiddel of detergentontsmettingsmiddel homogeen bly. Nadat die ontsmettingsmiddel of detergentontsmettingsmiddel nog 24 h lank by 'n temperatuur van 20 °C ± 5 °C gestaan het, mag dit geen teken van neerslag of skeiding toon nie.

4.7.4.2 Volgens 6.5.4.2 bewaar, mag die ontsmettingsmiddel of detergentontsmettingsmiddel geen teken van neerslag of skeiding toon nie en dit moet steeds aan al die vereistes van 4.2 voldoen.

4.7.5 Ontsmettingsmiddels en detergentontsmettingsmiddels met kwaternêre ammoniumverbindings, gestabiliseerde anorganiese chloorverbindings en organiese of anorganiese halogeenverbindings (uitgesonderd jodiumverbindings) as basis

4.7.5.1 By die toets van 'n vloeibare ontsmettingsmiddel of detergentontsmettingsmiddel volgens 6.5.5.1, moet dit homogeen en vryvloeiend bly.

4.7.5.2 By die toets van 'n vaste ontsmettingsmiddel of detergentontsmettingsmiddel volgens 6.5.5.2, mag dit nie harde klonte vorm nie.

4.8 pH-waarde

Volgens 6.6 teen die voorgeskrewe konsentrasie getoets, moet die pH-waarde, indien toepaslik, wees soos op die houer gespesifiseer word (kyk 7.2(g)).

4.9 Vryheid van sigbare onsuiwerhede van vaste ontsmettingsmiddels of vaste detergentontsmettingsmiddels gebaseer op kwaternêre ammoniumverbindings, gestabiliseerde anorganiese chloorverbindings en organiese of anorganiese halogeenverbindings (uitgesonderd jodiumverbindings)

By die toets van 'n vaste ontsmettingsmiddel of vaste detergentontsmettingsmiddel volgens 6.7, mag die getal sigbare spikkels onsuiwerheid hoogstens vyf wees.

4.10 Bygevoegde kleurstof vir ontsmettingsmiddels of detergentontsmettingsmiddels met jodofore as basis

Volgens 6.8 getoets, mag 'n ontsmettingsmiddel of detergentontsmettingsmiddel geen bygevoegde kleurstof bevat nie.

5 Metodes vir mikrobiologiese toetse

5.1 Algemeen

Die toetse moet onderneem word deur persone wat ervaring het van mikrobiologiese tegnieke, en aseptiese tegnieke moet gebruik word.

OPM

1 Ten einde die noukeurigheid van hierdie toetse te verseker, word daar aanbeveel dat elke toets herhaal word.

2 Voordat die toetse uitgevoer word, moet die doeltreffendheid van die inaktiveringstelsel nagegaan word om te verseker dat dit die ontsmettingsmiddels of detergentontsmettingsmiddels wat getoets moet word op toereikende wyse inaktiveer.

3 Ten einde die weerstand van die toetsorganismes sowel as die ander toetstoestande na te gaan, is dit raadsaam om 'n verwysingstandaard in te sluit. Dit is noodsaaklik dat dit 'n ontsmettingsmiddel of detergentontsmettingsmiddel met die betrokke aktiewe bestanddeel as basis moet wees, maar omdat dit moeilik is om 'n universele standaard te kies, moet elke laboratorium sy eie materiaal kies.

5.2 Laboratoriumware

Maak seker dat alle glasware bestand is teen herhaalde hittesterilisasie en dat die glas vry is van inhiberende stowwe soos swaar metale en vry alkali's. Boorsilikaatglas met 'n uitsettingskoëffisiënt van minder as $6 \times 10^{-6} k^{-1}$ word aanbeveel.

5.2.1 Universelehouer-kweekbottels

Bottels van glas met standaardmetaalskroefdeksels met rubbervoerings en met 'n nominale inhoudsvermoë van:

a) 30 mℓ; en

b) 110 mℓ.

Moet nie plastiekhouers of glashouers met plastiekdeksels gebruik nie.

5.2.2 Kweekbuise

Randlose silindriese buise met halfronde ente en 'n nominale wanddikte van 1,5 mm van die volgende groottes:

a) diameter en lengte 16 mm x 160 mm; en

b) diameter en lengte 20 mm x 200 mm.

Stop watteproppe in hierdie buise of proppe van skuimrubber wat geskik is vir outoklavering of gebruik skroefdekselbuise met dieselfde afmetings.

5.2.3 Meetpipette

Totalelewering-pipette slegs vir bakteriologiese doeleindes wat 'n uitvloei-opening met 'n diameter van 2 mm tot 3 mm het, in eenhede van 0,1 mℓ afgemeet is en só groot is dat dit 1,0 mℓ, 5,0 mℓ en 10,0 mℓ kan lewer.

5.2.4 Volumetriese silinders

Maatsilinders met skaalindelings met of sonder proppe en met 'n inhoudsvermoë van 5 mℓ, 10 mℓ, 100 mℓ, 500 mℓ en 1 000 mℓ.

5.2.5 Kweekflesse

Kweekflesse met 'n inhoudsvermoë van 250 mℓ, 500 mℓ en 1 ℓ.

5.2.6 Erlenmeyerflesse

Erlenmeyerflesse met 'n inhoudsvermoë van 250 mℓ, 2 ℓ en 3 ℓ.

5.2.7 Petribakkies

Petribakkies met 'n diameter en 'n hoogte van 100 mm x 20 mm wat van glas of benatbare polistireen gemaak is.

5.2.8 Reagensbottels

Bottels met 'n inhoudsvermoë van 50 mℓ en 100 mℓ met polipropileen- of ander plastiekproppe en van sodanige ontwerp dat hulle gebruik kan word om druppels reagens te lewer.

5.2.9 Inokuleerlus

'n Stuk platinum- of platinum-iridiumlegeringdraad met 'n diameter van 0,376 mm, gemonteer in 'n houer wat uit 'n dun metaalstaaf of -buis bestaan. Die ent van die draad is gevorm in 'n lus met 'n diameter van 4 mm op 'n afstand van 38 mm van die houer af. Die lus is met só 'n hoek met betrekking tot die as van die draad dat dit in die horisontale vlak gehou kan word terwyl dit vertikaal van die oppervlak van die vloeistof opgelig word.

5.2.10 Druppipet

'n Druppipet wat 0,2 mℓ in ongeveer vyf druppels lewer.

5.2.11 Mikropipetter

'n Mikropipetter van hoë presisie wat ingestel is om 20 µℓ noukeurig uit te meet, en steriele punte wat geskik is vir gebruik met hierdie mikropipetter.

5.3 Toerusting

5.3.1 Outoklaaf

'n Drukhouer wat stoom kan voortbring of met 'n sentrale stoombron verbind is en 'n druk van 300 kPa kan weerstaan. Die outoklaaf kan binne 10 min na die begin van die steriliseersiklus 'n temperatuur van 121 °C bereik.

5.3.2 Inkubators en waterbaddens

Inkubators en waterbaddens met termostaties beheerde verhit- en afkoeltoestelle en wat sirkuleermiddele het wat sodanig is dat die temperatuur van die totale ingeslote ruimte binne 2 °C van die termostaatstelling gehou word.

5.3.3 Warmlugoond (vir sterilisering met droë hitte)

'n Termostaties beheerde oond wat deur elektrisiteit of gas verhit word en sirkuleermiddele het wat sodanig is dat die totale ingeslote ruimte by 170 °C ± 5 °C gehou word, terwyl die hittetoevoer sodanig is dat die werktemperatuur herwin word binne 10 min nadat die oonddeur vinnig oop- en toegemaak is.

5.3.4 Stophorlosie

'n Stophorlosie wat tot binne 1 s per uur noukeurig is.

5.4 Media en reagense

5.4.1 Algemeen

5.4.1.1 Water

Gebruik slegs glasgedistilleerde water of gedemineraliseerde water van ekwivalente suiwerheid, wat helder, kleurloos en vry van sigbare gesuspendeerde stowwe is en waarvan die pH-waarde, gemeet by 25 °C, in die bestek van 5,0 tot 7,5 is.

5.4.1.2 Kwaliteit van bestanddele

Gebruik by die bereiding van die media en reagense slegs bestanddele van 'n kwaliteit wat vir mikrobiologiese doeleindes aanneemlik is. Gebruik anhidriese soute tensy daar anders gespesifiseer word.

5.4.1.3 Noukeurigheid

Laat die volgende toleransies toe, behalwe as daar anders gespesifiseer word:

a) op temperature ..... ± 2 °C

b) op massas ..... ± 1,0 %

c) op volumes ..... ± 1,0 %

d) op pH-waarde ..... ± 0,1

5.4.1.4 Ontwaterde media

Baie van die media wat vereis word, is in ontwaterde vorm beskikbaar en ter wille van eenvormige resulate word die gebruik van sodanige media aanbeveel. Indien hulle gebruik word, moet die fabrikant se aanwysings met betrekking tot die hersamestelling en sterilisering streng gevolg word.

5.4.1.5 Filtrering van media

Gaan soos volg te werk wanneer dit ook al nodig is om 'n medium tydens die bereiding daarvan te filtreer:

a) filtreer 'n medium wat nie 'n stolmiddel bevat nie, dws 'n vloeibare medium of boeljon, deur 'n medium-spoedfiltreerpapier; of

b) indien die medium 'n stolmiddel (byvoorbeeld agar) bevat, filtreer dit deur 'n laag vooraf natgemaakte absorbeerwatte wat 10 mm tot 15 mm dik is. Gebruik 'n stoommanteltregter om stolling van die medium tydens filtrering te voorkom. So nie, voer die filtrering in 'n stoomkamer uit.

5.4.1.6 Aansuiwering van die pH-waarde van media

In gevalle waar die finale pH-waarde van 'n medium of reagens gespesifiseer word, suiwer die pH-waarde, indien nodig, tydens die bereiding en, in die geval van media, voor sterilisering só aan dat die vereiste pH-waarde, gemeet by 25 °C, na bereiding verkry word. Tensy daar anders gespesifiseer word, gebruik 'n soutsuuroplossing (c(HCl) = 1 mol/ℓ) of natriumhidroksied, (c(NaOH) = 1 mol/ℓ, soos toepaslik, om die pH-waardes aan te suiwer.

5.4.1.7 Uitmeting

Gebruik 30 mℓ universele bottels (kyk 5.2.1(a)) in gevalle waar gespesifiseerde hoeveelhede media in bottels uitgemeet moet word. Indien grootmaatsterilisering vereis word, gebruik 'n geskikte glashouer van die vereiste kwaliteit of kweekbuise met geskikte proppe (kyk 5.2.2(a)). Meet reagense in reagensbottels (kyk 5.2.8) uit. Roer die media deurentyd tydens uitmeting. Indien die bereiding van hellings vir oppervlak-kweking vereis word, meet die medium in 10 mℓ volumes uit en steriliseer dit soos gespesifiseer word. Plaas die bottels of, indien toepaslik, die kweekbuise, onmiddellik na sterilisering op 'n oppervlak met 'n helling van 1 op 4 en laat die agar stol.

5.4.1.8 Sterilisering

Indien sterilisering in 'n outoklaaf gespesifiseer word en tensy daar anders voorgeskryf word, plaas die medium 15 min lank by 121 °C in 'n outoklaaf.

5.4.1.9 Beheer oor bereide media

Verseker deur middel van geskikte inkubasietoetse dat bereide media steriel is en dat dit die groei van die betrokke organismes in die gemelde inkubasietoestande kan steun.

5.4.1.10 Bewaring van media

Maak seker dat bereide media 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.

5.4.2 Onderlaagagar

5.4.2.1 Bestanddele

Triptoon	13,0 g
Agar	11,0 g
Natriumchloried	8,0 g
Glukose	1,0 g
Water	1 000 mℓ

5.4.2.2 Bereiding

Los die bestanddele deur verhitting in die water op. Laat dit tot 45 °C tot 50 °C afkoel en meet dit uit in petribakkies met 'n nominale diameter van 90 mm en maak seker dat die diepte van die agar in elke plaat minstens 3 mm is.

5.4.3 Beesalbumienoplossing

5.4.3.1 Bestanddele

Albumien (bees)	15,0 g
Water	1 000 mℓ

5.4.3.2 Bereiding

Los die albumien in die water op. Steriliseer dit deur dit deur 'n filter met 'n maksimum effektiewe poriegrootte van 0,45 µm te laat gaan. Meet 10 mℓ volumes in bottels uit (kyk 5.2.1(a)).

5.4.4 Setrimiedinaktiveerder

5.4.4.1 Bestanddele

Polioksiëtileensorbitaanmonoöleaat	8,0 g
Natriumtourocholaat	8,0 g
Natriumtiosulfaat	1,5 g
Kaliumfosfaat, monobasies	0,5 g
Natriumsitraat	0,5 g
Water	1 000 mℓ

5.4.4.2 Bereiding

Los die bestanddele deur verhitting in die water op. Meet 20 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf.

5.4.5 Verdunmiddel wat in die "5,5,5"-toets gebruik word

5.4.5.1 Bestanddele

Albumien (bees)	0,3 g
Natriumchloried	9,0 g
Water	1 000 mℓ

5.4.5.2 Bereiding

Los die bestanddele in die water op. Steriliseer dit deur dit deur 'n filter met maksimum effektiewe poriegrootte van 0,45 µm te laat gaan. Meet 10 mℓ volumes in bottels uit (kyk 5.2.1(a)).

5.4.6 Harde water wat by die bereiding van die gissuspensie gebruik word

5.4.6.1 Bestanddele

Kalsiumchloried ($CaCl_2$)	1,0 g
Magnesiumchloried ($MgCl_2.6H_2O$)	8,5 g
Water	1 000 mℓ

5.4.6.2 Bereiding

Los die bestanddele in die water op. Meet 100 mℓ volumes in bottels uit (kyk 5.2.1(b)) en steriliseer dit in 'n outoklaaf.

5.4.7 Inaktiveermedia

5.4.7.1 Inaktiveermedium no 1

5.4.7.1.1 Bestanddele

Monokaliumfosfaat (KH_2PO_4)	0,5 g
Natriumsitraat ($Na_3C_6H_5O_7.3H_2O$)	0,5 g
Natriumtourocholaat	8,0 g
Natriumtiosulfaat ($Na_2S_2O_3.5H_2O$)	1,5 g
Polioksiëtileensorbitaanmonoöleaat	8,0 g
Water	1 000 mℓ

5.4.7.1.2 Bereiding

Los die bestanddele deur verhitting in die water op. Meet 20 mℓ volumes in bottels uit (kyk 5.2.1(a)). Steriliseer dit in 'n outoklaaf.

OPM – Daar is gevind dat hierdie inaktiveerder geskik is vir ontsmettingsmiddels en detergentontsmettingsmiddels wat gebaseer is op jodofore, organiese halogeenverbindings (uitgesonderd jodiumverbindings) en die meeste kwaternêre ammoniumverbindings.

5.4.7.2 Inaktiveermedium no 2

5.4.7.2.1 Bestanddele

Polioksiëtileensorbitaanmonoöleaat	30,0 g
Beesvleisekstrak	20,0 g
Peptoon	20,0 g
Natriumchloried	10,0 g
Water	1 000 mℓ

5.4.7.2.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,1 aan. Meet 9 mℓ en 10 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf.

OPM – Daar is gevind dat hierdie inaktiveerder geskik is vir ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis.

5.4.7.3 Inaktiveermedium vir die "5,5,5"-toets

5.4.7.3.1 Bestanddele

Lesitien (gemaak van soja, gesuiwer)	3,0 g
ℓ-histidien	1,0 g
Fosfaatbuffer 0,25N (kyk 5.4.12)	10,0 mℓ
Natriumtiosulfaat ($Na_2S_2O_3.5H_2O$)	5,0 g
Polioksiëtileensorbitaanmonoöleaat	30,0 mℓ
Water	1 000 mℓ

5.4.7.3.2 Bereiding

Los die bestanddele deur verhitting in die water op. Meet 20 mℓ volumes in bottels uit (kyk 5.2.1(a)). Steriliseer dit in 'n outoklaaf.

5.4.7.4 Neutraliseerboeljonmedium

Gebruik TSB (kyk 5.4.22) maar voeg 3 g sojalesitien (asolektien) en 20 g polioksiëtileensorbitaanmonoöleaat by elke 100 mℓ boeljon. By die toets van 'n antiseptikum wat setrimied bevat, voeg ook 20 mℓ setrimiedinaktiveerder by (kyk 5.4.4). Suiwer die pH-waarde tot tussen 7,0 en 7,4 aan. Meet 9 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit 20 min lank in 'n outoklaaf.

OPM

1 Om die asolektien en polioksiëtileensorbitaanmonoöleaat in die TSB op te los, gebruik die helfte van die volume TSB in 'n groot houer en voeg die asolektien en polioksiëtileensorbitaanmonoöleaat by terwyl daar geroer word. Kook 30 min tot 60 min lank en roer aanhoudend totdat al die asolektienkorrels opgelos is. Laat dit afkoel voordat dit tot die finale volume met TSB verdun word. Suiwer die pH-waarde aan en meet dit uit soos vereis. Moet nie die bottels voor sterilisering tot meer as die helfte van hul volume vul nie om te voorkom dat die inhoud oorkook, wat die asolektien/polioksiëtileensorbitaanmonoöleaat-verhouding sal verander.

2 Soms sak die asolektien/polioksiëtileensorbitaanmonoöleaat-emulsie tot op die bodem van die houer af. Indien dit gebeur, sal die vaste stowwe gewoonlik weer oplos as dit ongeveer een week lank by kamertemperatuur bewaar word. Indien die neutraliseerder vroeër benodig word, kan die vaste stowwe weer opgelos word deur dit by 100 °C in 'n waterbad te verhit en daarna tydens afkoeling af en toe te werwel.

3 Daar is gevind dat hierdie inaktiveerder geskik is vir antiseptikums met chloorheksidienglukonaat as basis, asook vir sommige ontsmettingsmiddels en detergentontsmettingsmiddels met kwaternêre ammoniumverbindings en glutaaraldehied as basis.

5.4.8 Moutekstrakagar

5.4.8.1 Bestanddele

Moutekstrak ..... 30,0 g
Agar ..... 15,0 g
Sojapeptoon ..... 5,0 g
Water ..... 1 000 mℓ

5.4.8.2 Bereiding

Los die bestanddele in die water op. Meet 10 mℓ en 15 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf. Laat slegs die 10 mℓ volumes in 'n skuins posisie stol.

5.4.9 Voedingsagar

5.4.9.1 Bestanddele

Agar ..... 15,0 g
Peptoon ..... 5,0 g
Natriumchloried ..... 5,0 g
Gisekstrak ..... 2,0 g
Beesvleisekstrak ..... 1,0 g
Water ..... 1 000 mℓ

5.4.9.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,1 aan. Meet 10 mℓ en 15 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf. Laat net die 10 mℓ volumes in 'n skuins posisie stol.

5.4.10 Voedingsboeljon no 2 (dubbelsterkte)

5.4.10.1 Bestanddele

Peptoon ..... 10,0 g
Natriumchloried ..... 5,0 g
Verfynde beesvleisekstrak ..... 10,0g[1)]
Water ..... 1 000 mℓ

5.4.10.2 Bereiding

Los die bestanddele in die water op, suiwer die pH-waarde tot 7,1 aan en verdun die oplossing tot 1 ℓ. Meet 5 mℓ volumes in kweekbuise uit (kyk 5.2.2(a)) en steriliseer dit in 'n outoklaaf.

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1) Beesvleisekstrak gemaak van spesiaal uitgesoekte grondstowwe met 'n ligte kleur wat tot 'n neutrale kleur verander is en tot 'n fyn poeier gedroog is.

5.4.11 Voedingsmedium

5.4.11.1 Bestanddele

Peptoon ..... 5,0 g
Natriumchloried ..... 5,0 g
Gisekstrak ..... 2,0 g
Beesvleisekstrak ..... 1,0 g
Water ..... 1 000 mℓ

5.4.11.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,1 aan. Meet 10 mℓ en 50 mℓ volumes in bottels uit (kyk onderskeidelik 5.2.1(a) en 5.2.1(b)) en steriliseer dit in 'n outoklaaf.

5.4.12 Fosfaatbuffer 0,25N

5.4.12.1 Bestanddele

Monokaliumfosfaat (KH_2PO_4) ..... 34,0 g
Water ..... 500 mℓ

5.4.12.2 Bereiding

Los die monokaliumfosfaat in die water op. Suiwer die pH met 1N NaOH tot 7,2 aan. Meet dit in 30 mℓ bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf.

5.4.13 Fisiologiese soutoplossing

5.4.13.1 Bestanddele

Natriumchloried ..... 9,0 g
Water ..... 1 000 mℓ

5.4.13.2 Bereiding

Los die natriumchloried in die water op. Meet dit in 250 mℓ kweekflesse uit (kyk 5.2.5) en steriliseer dit in 'n outoklaaf.

5.4.14 Herkweekmedium

5.4.14.1 Bestanddele

Beesvleisekstrak ..... 10 g
Peptoon ..... 10 g
Natriumchloried ..... 5 g
Polioksiëtileensorbitaanmonoöleaat ..... 30 g
Water ..... 1 000 mℓ

5.4.14.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 7,5 aan. Meet 10 mℓ volumes in kweekbuise uit (kyk 5.2.2(a)) en steriliseer dit in 'n outoklaaf.

5.4.15 Natriumtiosulfaat (20 g/ℓ)

5.4.15.1 Bestanddele

Natriumtiosulfaat ..... 20 g
Water ..... 1 000 mℓ

5.4.15.2 Bereiding

Los die natriumtiosulfaat in die water op, meet 20 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf.

OPM – Daar is gevind dat hierdie inaktiveerder geskik is vir ontsmettingsmiddels en detergentontsmettingsmiddels met gestabiliseerde anorganiese chloorverbindings en gestabiliseerde chloorverbindings as basis.

5.4.16 Natriumtiosulfaat (10 g/ℓ)

5.4.16.1 Bestanddele

Natriumtiosulfaat ..... 10 g
Water ..... 1 000 mℓ

5.4.16.2 Bereiding

Los die natriumtiosulfaat in die water op, meet 20 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf.

5.4.17 Spoorvormingsmedium SM 1

5.4.17.1 Bestanddele

Agar ..... 12,0 g
Mangaansulfaat ($MnSO_4.4H_2O$) ..... 0,03 g
Dikaliumfosfaat ..... 4,0 g
Voedingsboeljon ..... 3,125 g
Water ..... 1 000 mℓ

5.4.17.2 Bereiding

Los die bestanddele in die water op en suiwer die pH-waarde tot 6,6 aan. Meet 20 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf. Laat die medium in 'n skuins posisie stol.

5.4.18 Standaard- harde water

5.4.18.1 Standaard- harde water no 1

5.4.18.1.1 Bestanddele

Kalsiumchloried ..... 2,1 g
Water ..... 7 500 mℓ

5.4.18.1.2 Bereiding

Los die kalsiumchloried in die water op. Meet 97 mℓ volumes in 110 mℓ bottels uit (kyk 5.2.1(b)) en steriliseer dit in 'n outoklaaf.

OPM – Daar is gevind dat hierdie harde water geskik is vir mikrobiologiese toetse op ontsmettingsmiddels en detergent-ontsmettingsmiddels wat gebaseer is op glutaaraldehied, organiese halogeenverbindings (uitgesonderd jodiumverbindings), kwaternêre ammoniumverbindings, gestabiliseerde anorganiese chloorverbindings en gestabiliseerde chloorverbindings.

5.4.18.2 Standaard- harde water wat gebruik word vir mikrobiologiese toetse op ontsmettingsmiddels en detergentontsmettingsmiddels met jodofore as basis

5.4.18.2.1 Bestanddele

Magnesiumchloried ..... 18,5 g
Kalsiumchloried ..... 7,9 g
Natriumbikarbonaat ..... 22,4 g
Water ..... 2 500 mℓ

5.4.18.2.2 Bereiding

Los die magnesiumchloried en kalsiumchloried in water op, suiwer die pH-waarde tot tussen 7,6 en 8,0 aan en verdun tot 1 ℓ met water. Steriliseer dit in 'n outoklaaf (oplossing A). Los die natriumbikarbonaat in water op, suiwer die pH-waarde tot tussen 7,6 en 8,0 aan en verdun tot 1 ℓ met water. Steriliseer dit deur filtrering (oplossing B). Voeg 1 mℓ van oplossing A en van oplossing B by 95 mℓ steriele water in 'n Erlenmeyerfles (kyk 5.2.6).

5.4.18.3 Standaard- harde water vir gebruik in die Kelsey-Sykes-toets

5.4.18.3.1 Bestanddele

Kalsiumchloried ($CaCl_2$) ..... 0,304 g
Magnesiumchloried ($MgCl_2.6H_2O$) ..... 0,139 g
Water ..... 1 000 mℓ

5.4.18.3.2 Bereiding

Los die bestanddele in die water op. Meet volumes van 100 mℓ in 110 mℓ bottels uit (kyk 5.2.1(b)) en steriliseer dit in 'n outoklaaf.

5.4.18.4 Standaard- harde water wat in die Rideal-Walker-toets gebruik word

5.4.18.4.1 Bestanddele

Kalsiumchloried ..... 0,15 g
Magnesiumsulfaat ..... 0,15 g
Water ..... 1 000 mℓ

5.4.18.4.2 Bereiding

Los die bestanddele in die water op. Meet volumes van 100 mℓ in 110 mℓ bottels uit (kyk 5.2.1(b)) en steriliseer dit in 'n outoklaaf.

5.4.19 Standaardfenoloplossing (50 g/ℓ)

Berei met gebruik van suiwer fenol met 'n kristalliseerpunt wat nie laer as 40,5 °C is nie 'n 50 g/ℓ voorraadoplossing in water en gebruik hierdie oplossing om die kontroleverdunnings te berei (kyk 5.10.2).

OPM – Dit is belangrik dat suiwer fenol gebruik word omdat kresol ongeveer drie maal die bakteriedodende doeltreffendheid van fenol het en 'n fout as gevolg die aanwesigheid daarvan (soos aangedui deur verlaging van die kristalliseerpunt tot laer as 40,5 °C) kan aansienlik wees.

5.4.20 Steriele afgeroomde melk

5.4.20.1 Meng 50,0 g afgeroomde melkpoeier 5 min tot 7 min lank met 400 mℓ water in 'n hoëspoedmenger. Verdun dit tot 500 mℓ met water.

5.4.20.2 Filtreer deur growwe filtreerpapier wat vooraf natgemaak is, of sentrifugeer dit 10 min lank teen 'n resulterende sentrifugale krag van 6 kN/kg. Indien daar 'n sigbare residu op die filtreerpapier of in die sentrifugeerbuis is, herhaal die prosedure met 'n ander produksielot afgeroomde melk.

5.4.20.3 Meet volumes van 15 mℓ tot 25 mℓ in bottels uit (kyk 5.2.1(a)) en steriliseer dit 10 min lank in 'n outoklaaf. Bewaar in 'n koelkas wat by 4 °C gehou word.

5.4.20.4 Toets vir vryheid van groei-inhiberende faktore

5.4.20.4.1 Gebruik steriele water en berei 'n suspensie van *Staphylococcus aureus* (kyk 5.5.2.1). Standaardiseer die suspensie só met behulp van 'n spektrofotometer saam met 'n standaardkenkromme, 'n hemositometer, Petroff-Häusser-telkamer of 'n ander geskikte middel, dat dit ongeveer 100 miljoen organismes (10^8) per milliliter bevat. Gebruik die suspensie binne drie uur nadat dit berei is.

5.4.20.4.2 Meng 0,12 mℓ van hierdie suspensie met 15 mℓ gesmelte voedingsagar (kyk 5.4.9) wat tot 45 °C afgekoel is en giet die mengsel in 'n platboompetribakkie met 'n diameter van 100 mm (kyk 5.2.7) wat op 'n gelyk oppervlak geplaas is. Plaas die petribakkie nadat die agar gestol het minstens 15 min lank in 'n koelkas wat by 2 °C tot 5 °C gehou word. Plaas drie steriele gepoleerde vlekvrystaalsilinders (penisilliensilinders) met 'n buitediameter van 8 mm ± 0,5 mm, 'n binnediameter van 6 mm ± 0,5 mm en 'n lengte van 10 mm ± 1 mm op die agar en vul elkeen met die melk wat getoets moet word.

5.4.20.4.3 Laat die petribakkie 2 h lank in die koelkas staan en inkubeer dan die petribakkie 16 h tot 20 h lank by 37 °C. Haal die silinders uit die petribakkie en ondersoek die gestolde agarplaat vir groei-inhibisiesones.

5.4.20.4.4 Herhaal die prosedure in 5.4.20.4.1 tot en met 5.4.20.4.3, en gebruik:

a) *Escherichia coli* as die toetsorganisme, met:

1) 'n suspensie wat 650 miljoen ($6,5 \times 10^8$) organismes per milliliter bevat; en
2) 'n inokulum van 0,15 mℓ in 15 mℓ gesmelte voedingsagar; en

b) *Pseudomonas aeruginosa* as die toetsorganisme, met:

1) 'n suspensie wat 500 miljoen ($5,0 \times 10^8$) organismes per milliliter bevat; en
2) 'n inokulum van 0,15 mℓ in 15 mℓ gesmelte voedingsagar.

5.4.20.4.5 Indien geen groei-inhibisiesones op enigeen van die drie stelle plate sigbaar is nie, kan die melk vir die toets gebruik word.

5.4.21 Bolaagagar

5.4.21.1 Bestanddele

Triptoon	10,0 g
Natriumchloried	8,0 g
Agar	6,0 g
Glukose	3,0 g
Water	1 000 mℓ

5.4.21.2 Bereiding

Los die bestanddele deur verhitting in die water op. Meet 90 mℓ volumes in 110 mℓ bottels uit (kyk 5.2.1(b)) en steriliseer dit in 'n outoklaaf.

5.4.22 Triptoonsojaboeljon (TSB)

5.4.22.1 Bestanddele

Triptoon	17,0 g
Natriumchloried	5,0 g
Sojapeptoon	3,0 g
Dibasiese kaliumwaterstoffosfaat	2,5 g
Dekstrose	2,5 g
Water	1 000 mℓ

5.4.22.2 Bereiding

Los die bestanddele in die water op, met verhitting indien dit nodig is. Suiwer die pH-waarde tot 7,3 aan, meet 10 mℓ volumes in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf.

5.4.23 Wright-en-Mundy-medium (sintetiese boeljon AOAC)

5.4.23.1 Deel A

5.4.23.1.1 Bestanddele

ℓ-sistien	0,05 g
d ℓ-metionien	0,37 g
ℓ-arginienhidrochloried	0,4 g
d ℓ-histidienhidrochloried	0,3 g
ℓ-lisienhidrochloried	0,85 g
ℓ-tirosien	0,21 g
d ℓ-treonien	0,5 g
d ℓ-valien	1,0 g
ℓ-leusien	0,8 g
d ℓ-isoleusien	0,44 g
Glisien	0,06 g
d ℓ-serien	0,61 g
d ℓ-alanien	0,43 g
ℓ-glutamiensuurhidrochloried	1,3 g
ℓ-aspartiensuur	0,45 g
d ℓ-fenielalanien	0,26 g
d ℓ-triptofaan	0,05 g
ℓ-prolien	0,05 g
Water	500 mℓ

5.4.23.1.2 Bereiding

Los die bestanddele in die water op en voeg 18 mℓ van 'n natriumhidroksied (c(NaOH) = 1 mol/ℓ) oplossing by.

5.4.23.2 Deel B

5.4.23.2.1 Bestanddele

Natriumchloried	3,0 g
Kaliumchloried	0,2 g
Magnesiumsulfaat	0,05 g
Kaliumfosfaat	1,5 g
Dinatriumfosfaat	4,0 g
Tiamienhidrochloried	0,01 g
Nikotienamied	0,01 g
Water	500 mℓ

5.4.23.2.2 Bereiding

Los die bestanddele in die water op. Meng deel A en deel B. Suiwer die pH-waarde tot 7,1 aan, indien nodig. Meet die helfte van die medium in volumes van 10 mℓ ± 0,2 mℓ en die ander helfte in volumes van 6 mℓ ± 0,2 mℓ in bottels uit (kyk 5.2.1(a)) en steriliseer dit in 'n outoklaaf.

Voeg voor gebruik by elke buis in die twee stelle bottels onderskeidelik 0,1 mℓ en 0,06 mℓ van 'n steriele 100 g/ℓ glukoseoplossing by.

5.4.24 Gissuspensie

5.4.24.1 Bereiding van 20 % (volgens massa) klam gissuspensie

OPM

1 Wanneer dit ook al moontlik is, moet die 20 % (volgens massa) klam gissuspensie gesteriliseer word op die dag waarop die gis ontvang word. Indien dit nie moontlik is nie, moet die onoopgemaakte gispakkie voor gebruik hoogstens 48 h lank by 'n temperatuur van hoogstens 5 °C bewaar word.

2 Inligting oor gisverskaffingsbronne kan van die Suid-Afrikaanse Buro vir Standaarde, Privaatsak X191, PRETORIA, 0001 verkry word.

Krummel ongeveer 500 g bakkersgis met die hand in 'n vooraf geweegde 1 ℓ beker en bepaal die massa van die klam gis. Verroom die gis deur 'n klein volume harde water (kyk 5.4.6) by te voeg terwyl die mengsel geroer word. Plaas die verroomde gedeelte sorgvuldig oor na 'n Erlenmeyerfles met 'n inhoudsvermoë van 2 ℓ (kyk 5.2.6) en voeg nog 'n klein volume harde water by enige klonterige residu wat in die beker oorbly. Herhaal hierdie proses totdat al die gis van die beker na die fles oorgeplaas is en die konsentrasie van die gissuspensie in die fles tot ongeveer 40 % (volgens massa) van die klam gis verlaag is. Skud die inhoud van die fles goed en verwyder groot stukke deur die suspensie te sif deur 'n sif met 'n openinggrootte van 140 µm, wat gesteun word in 'n tregter in 'n Erlenmeyerfles met 'n inhoudsvermoë van 3 ℓ (kyk 5.2.6). Voeg genoeg harde water by om die konsentrasie van die gis tot ongeveer 20 % (volgens massa) klam gis te verlaag. Skud deeglik en, terwyl daar geskud word, meet 100 mℓ volumes in bottels uit (kyk 5.2.1(b)). Steriliseer in 'n outoklaaf en bewaar dit by 4 °C totdat dit vir gebruik benodig word.

5.4.24.2 Bepaling van voggehalte

Pipetteer 25 mℓ van die gesteriliseerde gissuspensie (kyk 5.4.24.1) in 'n droë geweegde bakkie en laat dit tot 'n konstante massa droog word in 'n warmlugoond wat by 100 °C gehou word. Gebruik hierdie massa om die bykomende volume harde water te bepaal wat by elke bottel gesteriliseerde gissuspensie gevoeg moet word om 'n suspensie te verkry wat presies 5 % (volgens massa) droë gis bevat.

5.4.24.3 Aansuiwering van pH-waarde

Gebruik 'n oplossing van natriumhidroksied (c(NaOH) =1 mol/ℓ) en suiwer die pH-waarde van 100 mℓ van die 20 % (volgens massa) klam gissuspensie (kyk 5.4.24.1) tot 7,0 aan en teken die volume aan van die natriumhidroksiedoplossing wat vereis word.

5.4.24.4 Bereiding van 5 % (volgens massa) droë gissuspensie

Voeg net voor gebruik by die 100 mℓ 20 % (volgens massa) klam gissuspensie (kyk 5.4.24.1), die volume harde water (kyk 5.4.6) wat nodig is om 'n suspensie te verkry wat presies 5 % (volgens massa) droë gis en genoeg natriumhidroksied (c(NaOH) = 1 mol/ℓ) oplossing (kyk 5.4.24.3) bevat om die pH-waarde tot 7,0 aan te suiwer. Bewaar die gissuspensie voor gebruik hoogstens 7 d lank by 4 °C.

5.5 Toetsorganismes[2)]

Gebruik die volgende toetsorganismes:

a) *Staphylococcus aureus*	:	SABS TCC Sta 53 en SABS TCC Sta 59;
b) *Escherichia coli*	:	SABS TCC Esc 25;
c) *Escherichia coli* K-12 Hfr, NCTC12486	:	SABS TCC Esc 36;
d) *Escherichia coli* 36 met bakteriofaag MS2	:	SABS TCC Phg-C1 in gevalle waar daar op virusdodende eienskappe aanspraak gemaak word;
e) *Escherichia coli* ATCC 13706	:	SABS TCC Esc 37;
f) *Escherichia coli* 37 met bakteriofaag ϕX174	:	SABS TCC Phg-C2 in gevalle waar daar op virusdodende eienskappe aanspraak gemaak word;
g) *Pseudomonas aeruginosa*	:	SABS TCC Pse 2 en SABS TCC Pse 16;
h) *Salmonella typhi*	:	SABS TCC Sal 10 (NCTC 786, Lister-stam)
i) *Aspergillus niger*	:	SABS TCC 355 in gevalle waar daar op swamdodende eienskappe aanspraak gemaak word; en
j) *Bacillus subtilis* var *globigii*	:	SABS TCC Bac 35 in gevalle waar daar op spoordodende eienskappe aanspraak gemaak word.

Organismes wat die inwerking van 'n ontsmettingsmiddel of 'n detergentontsmettingsmiddel oorleef het, mag in geen omstandighede in 'n toets gebruik word nie.

OPM

1 Bykomende organismes kan gebruik word, indien dit verlang word.

2 Daar word beklemtoon dat dit van die uiterste belang is om van die standaardstam gebruik te maak.

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2) Verkrygbaar by die Suid-Afrikaanse Buro vir Standaarde, Privaatsak X191, PRETORIA, 0001.

5.5.1 Instandhouding van toetsorganismes

5.5.1.1 *Escherichia coli, Salmonella typhi, Staphylococcus aureus* en *Pseudomonas aeruginosa*

5.5.1.1.1 Gebruik 'n pas oopgemaakte gevriesdroogde kultuur of agarkultuur wat onlangs ontvang is en subkweek die toetsorganismes in bottels met 10 mℓ voedingsmedium (kyk 5.4.11).

(Gebruik triptoonsojaboeljon (kyk 5.4.22) in die geval van ontsmettingsmiddels met chloorheksidien-glukonaat as basis.)

5.5.1.1.2 Inkubeer die bottels 24 h lank by 37 °C. Kweek subkulture van die kulture in die bottels op voedingsagarhellings (kyk 5.4.9). Inkubeer die hellings 24 h lank by 37 °C.

5.5.1.1.3 Berei uit elk van hierdie hellingkulture vier subkulture (voorraadkulture) van elke toetsorganisme op 10 mℓ voedingsagarhellings (kyk 5.4.9). Inkubeer die voorraadkulture 24 h lank by 37 °C en bewaar hulle dan in 'n koelkas wat by 4 °C gehou word.

OPM – Neem hoogstens ses reekssubkulture uit elke voorraadkultuur voordat 'n nuwe gevriesdroogde kultuur gebruik word.

5.5.1.2 *Aspergillus niger*

Inokuleer moutekstrakagarhellings (kyk 5.4.8) met *Aspergillus niger* en inkubeer 7 d lank by 25 °C.

5.5.1.3 *Bacillus subtilis* var *globigii*

5.5.1.3.1 Inokuleer hellings van spoorvormingsmedium SM 1 (kyk 5.4.17) met *B. subtilis* var *globigii* en inkubeer 7 d lank by 30 °C en dan by omgewingstemperatuur totdat daar 80 % tot 100 % spore is (dws ongeveer 5 d).

5.5.1.3.2 Bepaal die graad van spoorvorming mikroskopies. Wanneer daar 80 % tot 100 % spore is, oes die spore deur steriele water by te voeg en die agaroppervlak saggies te skraap. So nie, gebruik 'n steriele glasstaaf of steriele krale om die spore af te vryf.

5.5.1.3.3 Sentrifugeer die suspensie 20 min tot 30 min lank. Giet die bovloeistof af. Was die spore drie maal tot vier maal met steriele water totdat die bovloeistof heeltemal helder is. Hersuspendeer die spore in steriele water en verhit die suspensie 10 min lank by 80 °C. Laat die suspensie vinnig afkoel en bewaar dit 7 d lank by 4 °C.

5.5.1.3.4 Was die spoorsuspensie nog een keer in steriele gedistilleerde water en standaardiseer die suspensie só met behulp van 'n spektrofotometer saam met 'n standaardkenkromme, 'n hemositometer, Petroff-Häusser-telkamer of 'n ander geskikte middel dat dit minstens 10 miljoen (10^7) spore per milliliter bevat.

5.5.1.3.5 Meet in steriele bottels uit (kyk 5.2.1(a)) en bewaar die spoorsuspensie by 4 °C totdat dit benodig word.

5.5.2 Bereiding van kulture vir toetssuspensies

5.5.2.1 *Escherichia coli, Staphylococcus aureus* en *Pseudomonas aeruginosa*

5.5.2.1.1 Inokuleer vir elk van die toetsorganismes 'n voedingsagarhelling (kyk 5.4.9) uit 'n voorraadkultuur wat by 4 °C gehou word (kyk 5.5.1.1.3) en inkubeer dit 24 h lank by 37 °C.

5.5.2.1.2 Gebruik vir die toets 'n 24 h kultuur wat vir twee opeenvolgende dae gesubkweek is. Begin die proses na ses subkwekings weer met 'n nuwe voorraadkultuur (kyk 5.5.1.1.3).

OPM – Die fisiologiese toestand van die toetsorganismes is belangrik en kan moontlik interlaboratorium- en intralaboratoriumwisselings in toetsresultate beïnvloed.

5.5.2.1.3 Gebruik 10 mℓ steriele water en was die bakterieë wat na 24 h inkubasie gegroei het van die helling (kyk 5.5.2.1.1) af, en skraap die agaroppervlak indien dit nodig is. Giet die gesuspendeerde groei sorgvuldig in 'n steriele Erlenmeyerfles (kyk 5.2.6) en skud goed om al die groei in die water te suspendeer. Standaardiseer die suspensie só met behulp van 'n spektrofotometer saam met 'n standaardkenkromme, 'n hemositometer, Petroff-Häuser-telkamer of 'n ander geskikte middel dat dit die vereiste getal organismes per milliliter bevat wat in die individuele mikrobiologiese doeltreffendheidstoetse voorgeskryf word. Gebruik die suspensie binne drie uur na bereiding.

5.5.2.2 *Salmonella typhi*

5.5.2.2.1 Plaas 'n klein porsie van die groei van 'n voorraadkultuur (kyk 5.5.1.1.3) oor na 5 mℓ voedingsboeljon no 2 (dubbelsterkte) (kyk 5.4.10) en inkubeer 24 h lank by 37 °C.

5.5.2.2.2 Hou aan om voedingsboeljon no 2 (dubbelsterkte) met daaglikse tussenposes in vars buise te subkweek, en plaas altyd een standaardlus vol van die kultuur oor. Begin die proses weer met 'n vars voorraadkultuur na twee weke van subkweking soos dit beskryf is.

5.5.2.2.3 'n Subkultuur wat op enige dag tussen die derde dag tot en met die vyftiende dag gemaak is, kan vir die toets gebruik word, onderworpe aan die volgende voorwaardes:

a) as een daaglikse subkweking oorgeslaan word, vereis dit geen spesiale herorganisasie van die prosedure nie, maar indien subkweking op twee opeenvolgende dae nie kan plaasvind nie, moet drie opeenvolgende daaglikse subkwekings na die onderbreking uitgevoer word voordat organismes wat vir die toets geskik is, verkry word; en

b) oor naweke kan die volgende prosedure gevolg word: subkweek ongeveer om 10:00 op die Vrydag en inkubeer tot 16:00 by 37 °C.

Plaas die subkultuur oor na 'n inkubator wat by 22 °C gehou word en laat dit daar vir die naweek. Plaas dit om 08:00 op die volgende Maandag minstens 2 h lank terug in die inkubator wat by 37 °C gehou word en berei dan die subkultuur vir gebruik op die Dinsdag.

OPM – Gooi die kulture weg wat tekens van klontvorming of vliesvorming toon.

5.5.2.3 *Aspergillus niger*

Gebruik 10 mℓ van 'n steriele 0,5 g/ℓ oplossing van polioksiëtileensorbitaanmonoöleaat, was die groei wat na 7 d inkubasie ontstaan het van die helling (kyk 5.5.1.2) af, en skraap die agaroppervlak indien dit nodig is. Giet die gesuspendeerde groei sorgvuldig in 'n steriele bottel (kyk 5.2.1(a)) en skud goed om al die groei in die water te suspendeer. Standaardiseer die suspensie só met behulp van 'n hemositometer, Petroff-Häusser-telkamer of 'n ander geskikte middel dat dit die vereiste getal organismes per milliliter bevat wat in die individuele mikrobiologiese doeltreffendheidstoetse voorgeskryf word. Gebruik die suspensie binne drie uur na bereiding.

5.5.2.4 *Bacillus subtilis* var *globigii*

Berei die suspensie volgens 5.5.1.3.

5.5.2.5 Bakteriofaag

OPM – Die bereiding verwys na die bakteriofaag-MS2-stam (kyk 5.5(d)). Die bereiding van die bakteriofaag-ϕX174-stam is dieselfde, behalwe dat 'n ander bakteriekultuur, nl SABS TCC Esc 37, gebruik word.

5.5.2.5.1 Bereiding van bakteriofaagvoorraad

Volg die plaatmetode of die boeljonmetode.

5.5.2.5.1.1 Plaatmetode

5.5.2.5.1.1.1 Inokuleer 'n verdunning van die bakteriofaag-MS-stam wat 'n halfsaamvloeiende lise geproduseer het op 'n agarplaat met *Escherichia coli* SABS TCC Esc 36 in buise met bolaagagar (kyk 5.4.21). Giet die suspensie op onderlaagagar (kyk 5.4.2) en inkubeer dit 18 h lank by 37 °C.

5.5.2.5.1.1.2 Meet 2,5 mℓ voedingsmedium (kyk 5.4.11) asepties op elke plaat uit en verwyder die voedingsmedium met bolaagagar deur dit met behulp van 'n steriele glasstaaf van die onderlaagagar af te skraap.

5.5.2.5.1.1.3 Homogeniseer die suspensie deur dit goed te skud en sentrifugeer die suspensie dan 10 min lank teen 7 000 r/min.

5.5.2.5.1.1.4 Voeg 10 % (volgens volume) chloroform by die bakteriofae in die bovloeistof en bewaar hierdie voorraadoplossing by 4 °C.

5.5.2.5.1.2 Boeljonmetode

5.5.2.5.1.2.1 Voeg die bakteriofaag-MS-stam by 'n voedingsmediumkultuur (kyk 5.4.11) van *Escherichia coli* SABS TCC Esc 36 wat naby die einde van die logfase is om seker te maak dat feitlik elke bakteriesel tegelyk besmet word.

5.5.2.5.1.2.2 Sentrifugeer die suspensie 10 min lank teen 7 000 r/min nadat lise voorgekom het (sigbaar as 'n opmerklike afname in troebelheid).

5.5.2.5.1.2.3 Voeg 10 % (volgens volume) chloroform by die bakteriofae in die bovloeistof en bewaar hierdie voorraadoplossing by 4 °C.

5.5.2.5.2 Titrering van die bakteriofaagsuspensie

5.5.2.5.2.1 Inokuleer 'n voedingsagarhelling uit 'n voorraadkultuur van *Escherichia coli* SABS TCC Esc 36 (kyk 5.5.1.1.3) en inkubeer dit 24 h lank by 37 °C.

5.5.2.5.2.2 Gebruik 2 mℓ van die voedingsmedium (kyk 5.4.11), was die bakteriegroei van die 24 h kultuur van die helling af, en skraap die agaroppervlak indien dit nodig is. Giet die gesuspendeerde groei sorgvuldig in 'n steriele skroefdekselglasbottel en werwel om al die groei in die medium te suspendeer.

5.5.2.5.2.3 Voeg 0,5 mℓ van die oplossing wat in 5.5.2.5.2.2 verkry is by 50 mℓ van die voedingsmedium (kyk 5.4.11) en inkubeer dit 2 h lank by 37 °C.

5.5.2.5.2.4 Verifieer dat die absorbansie wat by 620 nm ± 20 nm gemeet word deur selle met 'n optiese pad van 1 cm tussen 0,05 tot en met 0,10 is, wat 'n bakteriekonsentrasie van ongeveer 10^8 organismes per milliliter verteenwoordig.

5.5.2.5.2.5 Berei 'n tienvoudigeverdunning-reeks van die bakteriofaagsuspensie en gebruik vir elke stap 9 mℓ volumes gesteriliseerde gedistilleerde water en voeg 0,9 mℓ van die 2 h *E. coli* kultuur (kyk 5.5.2.5.2.4) by 0,1 mℓ van elk van die verdunnings wat verkry is.

5.5.2.5.2.6 Plaas die suspensies 15 min ± 1 min lank by 37 °C in 'n waterbad of inkubator en voeg dan 5,0 mℓ bolaagagar by, wat gesmelt is en in 'n waterbad by 44 °C gehou is om stolling van die agar te voorkom.

5.5.2.5.2.7 Werwel die mengsel en smeer dit dan onmiddellik oor die oppervlak van die onderlaagagar in petribakkies met toepaslike etikette en werwel dit saggies.

5.5.2.5.2.8 Laat die agar stol, keer die plate om en inkubeer die petribakkies 24 h lank by 37 °C.

5.5.2.5.2.9 Ondersoek die plate na inkubasie vir plaketvorming. Tel die plakette op elke plaat wat tussen 10 en 100 plakette bevat en teken dit aan.

5.5.2.5.2.10 'n Bakteriofaagtiter van minstens 10^7 per milliliter word aanbeveel.

5.6 Ontsmettingsdoeltreffendheid van ontsmettingsmiddels en detergentontsmettingsmiddels met chloorheksidienglukonaat as basis

5.6.1 Inokuleer 'n bottel triptoonsojaboeljon (kyk 5.4.22) uit 'n daaglikse subkultuur van elk van die toetsorganismes *Staphylococcus aureus* (SABS TCC Sta 59) en *Pseudomonas aeruginosa* (SABS TCC Pse 16) (kyk 5.5.2.1.2).

5.6.2 Inkubeer die geïnokuleerde triptoonsojaboeljon 24 h lank by 37 °C en gaan voort soos in 5.5.2.1.3 en standaardiseer die suspensie só dat dit minstens 10 miljoen (10^7) maar hoogstens 100 miljoen (10^8) organismes per milliliter bevat.

5.6.3 Toetsprosedure

5.6.3.1 Gebruik steriele gedistilleerde water en berei 'n toepaslike verdunning van die ontsmettingsmiddel of detergentontsmettingsmiddel (kyk 7.2(f)).

5.6.3.2 Meet 9 mℓ volumes van hierdie monsterverdunning asepties in steriele toetsbuise uit en plaas dit 10 min lank in 'n waterbad wat by 37 °C gehou word. Herhaal hierdie prosedure maar gebruik 9 mℓ steriele gedistilleerde water as kontrole.

5.6.3.3 Smelt die inhoud van 'n voldoende getal bottels (wat 15 mℓ volumes bevat) voedingsagar (kyk 5.4.9), koel dit af tot 45 °C en hou hulle by hierdie temperatuur.

5.6.3.4 Gebruik 'n skoon, steriele pipet, meet 1 mℓ van die *Staphylococcus aureus*-suspensie (kyk 5.6.2) uit in een van die buise van elke ontsmettingsmiddel of detergentontsmettingsmiddel wat getoets moet word, sowel as in die kontrole. Meng goed met behulp van 'n werweltipe menger en hou die suspensie vir die duur van die toets in die waterbad.

5.6.3.5 Haal na 1 min 'n 1 mℓ volume uit elke buis en voeg elke volume by afsonderlike 9 mℓ volumes van die neutraliseerboeljonmedium (kyk 5.4.7.4) en roer goed.

5.6.3.6 Berei 'n tienvoudigeverdunning-reeks van die monster en gebruik vir elke stap 9 mℓ volumes van die steriele gedistilleerde water as verdunmiddel. Vier tot vyf reeksverdunnings word vir die kontrole aanbeveel.

5.6.3.7 Gebruik 'n skoon, steriele pipet en meet 1 mℓ van elke verdunning (kyk 5.6.3.5 en 5.6.3.6) uit op twee toepaslik geëtiketteerde, steriele plate (petribakkies). Voeg 15 mℓ van die voedingsagar (kyk 5.6.3.3) by elk van die plate en werwel saggies om egalige verspreiding van kolonies na afloop van inkubasie te verseker. Verhoed dat die inhoud van die plate tydens hierdie proses stort.

5.6.3.8 Laat die agar stol, keer die plate om en inkubeer hulle 48 h lank by 37 °C.

5.6.3.9 Ondersoek die plate na afloop van inkubasie vir groei. Tel die kolonies op elke plaat (van die toetsoplossing sowel as van die kontrole) wat tussen 30 en 300 kolonies bevat en teken dit aan. Indien die laagste verdunning (hoogste konsentrasie) minder as 30 kolonies oplewer, tel al die kolonies. Maak seker dat die kolonies wat getel is afkomstig is van oorlewendes van die toetsorganismes en nie van kontaminasie nie.

5.6.3.10 Beskou die verdunningsfaktor (*VF*) vir die toetsmonster en vir die kontrole as die omgekeerde van die verdunning; indien die monster of die kontrole byvoorbeeld tot 1/1 000 verdun is, beskou *VF* as 1 000.

5.6.3.11 Die doodpersentasie word deur die volgende formule aangegee:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

waar

A die getal organismes is wat na kontak met die monsters getel is (kyk 5.6.3.9);

B die getal organismes is wat in die kontrole getel is (kyk 5.6.3.9);

DF_s die verdunningsfaktor vir die monster is soos in 5.6.3.10 bereken; en

DF_c die verdunningsfaktor vir die kontrole is soos in 5.6.3.10 bereken.

5.6.3.12 Herhaal die prosedure in 5.6.3.1 tot 5.6.3.11 en gebruik *Pseudomonas aeruginosa* as die toetsorganisme.

5.6.4 Vertolking van resultate

Ag dat die ontsmettingsmiddel of detergentontsmettingsmiddel aan die vereistes van 4.2 voldoen, indien, onderworpe aan die volgende voorwaardes:

a) teen 'n konsentrasie van 2 % chloorheksidienglukonaat (in gevalle waar die ontsmettingsmiddel of detergentontsmettingsmiddel wat gelewer is 5 % of meer chloorheksidienglukonaat bevat); of

b) teen die voorgeskrewe konsentrasie, dws in 'n onverdunde vorm (kyk 7.2(f)) (in gevalle waar die ontsmettingsmiddel of detergentontsmettingsmiddel wat gelewer is minder as 5 % chloorheksidienglukonaat bevat),

die ontsmettingsmiddel of detergentontsmettingsmiddel minstens 99,9 % van elke organisme wat getoets is, doodmaak.

5.7 Ontsmettingsdoeltreffendheid van ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis

5.7.1 Oorlewendetelling-metode

Die volgende onderskeid word getref:

a) ontsmettingsmiddels of detergentontsmettingsmiddels vir algemene gebruik; en

b) ontsmettingsmiddels vir gebruik op mediese instrumente.

5.7.1.1 Bereiding van toetsorganismesuspensies

5.7.1.1.1 Ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis vir algemene gebruik

Berei die toetsorganismesuspensies soos in 5.5.2.1 en 5.5.2.3 beskryf word en maak seker dat elke milliliter groeimedium onderskeidelik die volgende bevat:

a) minstens 100 000 (10^5), maar hoogstens 1 miljoen (10^6) *Escherichia coli-*, *Staphylococcus aureus-* en *Pseudomonas aeruginosa*-organismes; en

b) 100 000 (10^5) *Aspergillus niger*-organismes.

5.7.1.1.2 Ontsmettingsmiddels met glutaaraldehied as basis vir gebruik op mediese instrumente

Berei die toetsorganismesuspensies soos in 5.5.2.1, 5.5.2.3 en 5.5.2.4 beskryf word en maak seker dat elke milliliter groeimedium onderskeidelik die volgende bevat:

a) minstens 10 miljoen (10^7) maar hoogstens 100 miljoen (10^8) *Escherichia coli-*, *Staphylococcus aureus-* en *Pseudomonas aeruginosa*-organismes;

b) 10 miljoen (10^7) *Aspergillus niger*-organismes; en

c) minstens 10 miljoen (10^7) *Bacillus subtilis* var *globigii*-spore.

5.7.1.2 Bereiding van kontrole- en toetsoplossings

5.7.1.2.1 Kontroleoplossings

5.7.1.2.1.1 Ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis vir algemene gebruik

Meet 8,9 mℓ standaard- harde water no 1 (kyk 5.4.18.1) in elk van drie steriele glasbottels uit en voeg 0,1 mℓ steriele afgeroomde melk by elkeen (kyk 5.4.20).

5.7.1.2.1.2 Ontsmettingsmiddels met glutaaraldehied as basis vir gebruik op mediese instrumente

Meet 9 mℓ steriele gedistilleerde water in elk van drie steriele glasbottels uit.

5.7.1.2.2 Toetsoplossing

5.7.1.2.2.1 Ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis vir algemene gebruik

Berei die voorgeskrewe konsentrasie van die toetsmonster soos op die etiket gemeld word (kyk 7.2(f)) en gebruik standaard- harde water no 1 (kyk 5.4.18.1). Meet 8,9 mℓ van die toetsoplossing in elk van drie steriele glasbottels uit en voeg 0,1 mℓ steriele afgeroomde melk (kyk 5.4.20) by elkeen. Gebruik die toetsoplossing op die dag waarop dit berei is.

5.7.1.2.2.2 Ontsmettingsmiddels met glutaaraldehied as basis vir gebruik op mediese instrumente

Berei die toepaslike konsentrasie van die toetsmonster soos op die etiket gemeld word (kyk 7.2(f)) en gebruik steriele gedistilleerde water. Meet 9 mℓ van die toetsoplossing in elk van drie steriele glasbottels uit. Gebruik die toetsoplossing op die dag waarop dit berei is.

5.7.1.3 Temperatuurstelling

Etiketteer die bottels wat die kontrole, die toetsoplossings en die toetsorganismesuspensies bevat en plaas hulle minstens 30 min lank in 'n waterbad wat by 22 °C ± 1 °C gehou word.

5.7.1.4 Toetsprosedure vir ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis vir algemene gebruik

OPM – Kortheidshalwe word die prosedure vir die toets van die ontsmettingsmiddeloplossing vir een organisme aangegee. Vir elke organisme word 'n kontroletoets met gebruik van die toepaslike oplossing (kyk 5.7.1.2.1.1 en 5.7.1.2.1.2) in plaas van die ontsmettingsmiddeloplossing uitgevoer. Elke kontrole val saam met die gepaardgaande toets (en ongeveer 30 s verloop tussen gepaardgaande stappe). Gebruik 'n stophorlosie vir hierdie doel.

5.7.1.4.1 Smelt die inhoud van 'n voldoende getal bottels (wat 15 mℓ volumes bevat) voedingsagar (kyk 5.4.9), laat tot 45 °C afkoel en hou hulle by hierdie temperatuur.

5.7.1.4.2 Gebruik 'n skoon, steriele pipet en meet 1 mℓ van die *Pseudomonas aeruginosa*-toetssuspensie (kyk 5.7.1.1.1(a)) in die toetsoplossing en in die kontrole uit (sonder om die bottels uit die waterbad te haal). Haal die bottels uit die waterbad, werwel hulle om die inhoud deeglik te meng en plaas hulle onmiddellik terug in die waterbad.

5.7.1.4.3 Plaas na verloop van die blootsteltydperk van 5 min (werklike kontaktyd) 1 mℓ van die toetsoplossing wat die organisme bevat oor na 9 mℓ inaktiveermedium no 2 (kyk 5.4.7.2) en meng goed totdat die suspensie eenvormig is (eerste verdunning). Herhaal hierdie prosedure binne 30 s met die kontrole.

5.7.1.4.4 Gebruik 'n skoon, steriele pipet en plaas 1 mℓ van die inaktiveermedium wat die organisme bevat (kyk 5.7.1.4.3) oor na 'n petribakkie en plaas dan 1 mℓ oor na die eerste van 'n reeks bottels wat elk 9 mℓ steriele gedistilleerde water bevat. Meng goed totdat die suspensie eenvormig is (tweede verdunning).

5.7.1.4.5 Herhaal die prosedure in 5.7.1.4.4, maar plaas die suspensie wat na die tweede verdunning verkry is oor na die tweede van die reeks bottels. Verdun verder totdat 'n finale verdunning van 1:1 000 van die toetsmonster verkry word.

5.7.1.4.6 Berei agtereenvolgens, soos hierbo beskryf word, 'n verdunningsreeks met die kontrole.

5.7.1.4.7 Plaas 1 mℓ van elke verdunning van die toetsmonster oor na elk van twee toepaslik geëtiketteerde steriele plate (petribakkies). Doen dieselfde ten opsigte van elke verdunning van die kontrole. Voeg 15 mℓ voedingsagar (kyk 5.7.1.4.1) by elk van die plate en werwel hulle saggies om egalige verspreiding van kolonies na inkubasie te verseker. Verhoed dat die inhoud van die plate tydens hierdie proses stort.

5.7.1.4.8 Laat die agar stol, keer die plate om en inkubeer hulle 48 h lank by 37 °C.

5.7.1.4.9 Ondersoek die plate na inkubasie vir groei. Tel die kolonies op elke plaat (van die toetsoplossing en van die kontrole) wat tussen 30 en 300 kolonies bevat en teken dit aan. Indien die laagste verdunning (hoogste konsentrasie) minder as 30 kolonies oplewer, tel al die kolonies. Maak seker dat die kolonies wat getel is afkomstig is van oorlewendes van die toetsorganismes en nie van kontaminasie nie.

5.7.1.4.10 Beskou die verdunningsfaktor (*VF*) in die geval van die toetsmonster en in die geval van die kontrole as die omgekeerde van die verdunning; indien die monster of die kontrole byvoorbeeld tot 1/1 000 verdun is, beskou *VF* as 1 000.

5.7.1.4.11 Die doodpersentasie word deur die volgende formule aangegee:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

waar

A die getal organismes is wat na kontak met die monster getel is (kyk 5.7.1.4.9);

B die getal organismes is wat in die kontrole getel is (kyk 5.7.1.4.9);

DF_s die verdunningsfaktor vir die monster is soos in 5.7.1.4.10 bereken; en

DF_c die verdunningsfaktor vir die kontrole is soos in 5.7.1.4.10 bereken.

OPM – Dieselfde formule word gebruik om die doodpersentasie van *Escherichia coli* na 5 min kontaktyd (kyk 5.7.1.4.12), *Staphylococcus aureus* na 5 min kontaktyd (kyk 5.7.1.4.13) en *Aspergillus niger* na 15 min kontaktyd (kyk 5.7.1.4.14) te bepaal.

5.7.1.4.12 Herhaal die prosedure in 5.7.1.4.1 tot en met 5.7.1.4.11 met die *Escherichia coli*-suspensie (kyk 5.5.2.1).

5.7.1.4.13 Herhaal die prosedure in 5.7.1.4.1 tot en met 5.7.1.4.11 met die *Staphylococcus aureus*-suspensie (kyk 5.5.2.1).

5.7.1.4.14 Herhaal die prosedure in 5.7.1.4.1 tot en met 5.7.1.4.11 met die *Aspergillus niger*-toets-organismesuspensie (kyk 5.5.2.3), maar

a) gebruik 'n blootsteltyd (werklike kontaktyd) van 15 min,

b) voeg moutekstrakagar (kyk 5.4.8) by die suspensies op die plate, en

c) inkubeer 7 d lank by 25 °C.

5.7.1.5 Toetsprosedure vir ontsmettingsmiddels met glutaaraldehied as basis vir gebruik op mediese instrumente

5.7.1.5.1 *Pseudomonas aeruginosa* en *Aspergillus niger*

Voer die prosedure in 5.7.1.4.1 tot en met 5.7.1.4.11 uit en gebruik die *Pseudomonas aeruginosa*-toetssuspensie (kyk 5.7.1.1.1(a)) en die *Aspergillus niger*-toetssuspensie (kyk 5.5.2.3).

5.7.1.5.2 *Bacillus subtilis* var *globigii*

Voer die prosedure in 5.7.1.4.1 tot en met 5.7.1.4.11 uit en gebruik die *Bacillus subtilis* var *globigii*-toets-suspensie (kyk 5.5.2.4), maar:

a) gebruik 'n blootsteltydperk (werklike kontaktyd) van 4 h; en

b) inkubeer 48 h lank by 37 °C.

5.7.1.6 Vertolking van resultate

Ag dat die monsters aan die vereistes van 4.2 voldoen indien 'n doodresultaat van minstens 99,99 % verkry is vir elke organisme wat getoets word.

5.7.2 Spoordodende aktiwiteit (Kelsey-Sykes-toets, gemodifiseer)

5.7.2.1 Medium

Gebruik die inaktiveermedium no 2 in 5.4.7.2.

5.7.2.2 Toetsorganismesuspensie

Gebruik die spoorsuspensie van *B. subtilis* var *globigii* (kyk 5.5.2.4).

5.7.2.3 Bereiding van toetsoplossing

Berei die voorgeskrewe konsentrasie van die toetsoplossing soos op die etiket gemeld word (kyk 7.2(f)) en gebruik steriele gedistilleerde water. Gebruik die toetsoplossing op die dag waarop dit berei is.

5.7.2.4 Toetsprosedure

5.7.2.4.1 Meet 3 mℓ van die toetsoplossing in 'n steriele glasbottel uit.

5.7.2.4.2 Etiketteer die bottels wat die toetsoplossing en die toetsorganismesuspensies bevat en plaas hulle minstens 30 min lank in 'n waterbad wat by 22 °C ± 1 °C gehou word.

5.7.2.4.3 Voeg dan, sonder om die bottel wat die toetsoplossing bevat uit die waterbad te haal, met behulp van 'n skoon, steriele pipet, 1 mℓ van die toetsorganismesuspensie by die toetsmonster. Sit die stophorlosie terselfdertyd aan. Haal die bottel uit die waterbad, meng die inhoud goed en plaas dit onmiddellik terug in die waterbad.

5.7.2.4.4 Plaas 0,02 mℓ van die suspensie wat in 5.7.2.4.3 verkry is na presies 4 h oor na elk van vyf buise wat 10 mℓ inaktiveermedium no 2 (kyk 5.4.7.2) bevat.

5.7.2.4.5 Inkubeer die geïnokuleerde buise inaktiveermedium 48 h lank by 30 °C.

5.7.2.4.6 Ondersoek na afloop van inkubasie die buise inaktiveermedium vir groei en teken die resultate aan.

5.7.2.5 Vertolking van resultate

Ag dat die monster aan die vereistes van 4.2 voldoen indien geen groei van die toetsorganisme in enigeen van die vyf buise inaktiveermedium bespeur kan word nie.

5.7.3 Kelsey-Sykes-toets

5.7.3.1 Prosedure

Volg die prosedure in 5.8, maar gebruik slegs *Pseudomonas aeruginosa* as die toetsorganisme.

5.7.3.2 Vertolking van resultate

Ag dat die monster aan die vereistes van 4.2 voldoen indien die aanvanklike konsentrasie van die ontsmettingsmiddel (konsentrasie B) (kyk 5.8.2.6(a)) geen groei van die toetsorganisme toon nie in minstens twee van die vyf buise herkweekmedium in stelle wat geïnokuleer is op:

a) die agtste minuut na die byvoeging van die aanvanklike inokulum; en

b) die agtiende minuut na die byvoeging van die aanvanklike inokulum.

'n Voorbeeld van 'n reeks toetsresultate en die interpretasie daarvan word in tabel 2 aangegee.

5.8 Kelsey-Sykes-toets vir detergentontsmettingsmiddels met fenol as basis

5.8.1 Toets vir minimum inhiberende konsentrasie

5.8.1.1 Bereiding van die toetssuspensies

Berei 'n 1:10-verdunning van 'n nuutgekweekte subkultuur van elkeen van die toetsorganismes (kyk 5.5.2.1) in Wright-en-Mundy-medium (kyk 5.4.23).

OPM – Filtreer 'n *Pseudomonas aeruginosa*-kultuur voor verdunning deur growwe filtreerpapier.

5.8.1.2 Bereiding van die toetsmonsterverdunnings

5.8.1.2.1 Voeg 5 mℓ Wright-en-Mundy-medium (kyk 5.4.23) by 5 mℓ van die toetsmonster in 'n glasbottel met 'n inhoudsvermoë van 30 mℓ (kyk 5.2.1(a)). Meng goed en plaas 5 mℓ van hierdie verdunning van die toetsmonster oor na nog 5 mℓ Wright-en-Mundy-medium. Herhaal die prosedure totdat 10 verdubbelverdunnings van die toetsmonster (van 1:2 tot 1:1 024) berei is. Verontagsaam 5 mℓ van die laaste verdunning (sodat elke bottel 5 mℓ van 'n verdunning van die toetsmonster bevat).

5.8.1.2.2 Herhaal 5.8.1.2.1 totdat drie stelle van 10 verdubbelverdunnings van die toetsmonster berei is.

5.8.1.3 Toetsprosedure

5.8.1.3.1 Voeg 0,02 mℓ van die *Staphylococcus aureus*-toetssuspensie (kyk 5.8.1.1) by elk van die 10 verdunnings van die toetsmonster (kyk 5.8.1.2.2). Inkubeer die geïnokuleerde bottels 72 h lank by 30 °C.

Ondersoek die bottels vir groei. Die minimum inhiberende konsentrasie is die hoogste verdunning (minimum konsentrasie) wat nie groei toon nie.

5.8.1.3.2 Herhaal die prosedure in 5.8.1.3.1, maar gebruik agtereenvolgens die *Escherichia coli*- en *Pseudomonas aeruginosa*-toetssuspensie (kyk 5.8.1.1).

5.8.1.4 Vertolking van resultate

Bepaal watter van die drie toetsorganismes die meeste weerstand teen die toetsmonster bied, dws die organisme waarvoor die minimum inhiberende konsentrasie die hoogste is. Gebruik hierdie organisme vir die oorblywende deel van die toets (kyk 5.8.2).

5.8.2 Oorblywende deel van toets

5.8.2.1 Keuse van die toetsorganisme vir die bepaling

Bepaal deur middel van die toets vir minimum inhiberende konsentrasie (kyk 5.8.1) watter van die toetsorganismes die meeste weerstand bied en gebruik dit as die toetsorganisme vir die bepaling.

5.8.2.2 Bereiding van kultuur vir toetsorganismesuspensies

Inokuleer 'n buis wat 10 mℓ van die Wright-en Mundy-medium (kyk 5.4.23) bevat uit 'n daaglikse subkultuur van die toepaslike toetsorganisme (kyk 5.5.2.1) op die dag voor die toets uitgevoer moet word en inkubeer die geïnokuleerde medium 24 h lank by 37 °C.

5.8.2.3 Bereiding van toetsorganismesuspensie vir die toets in "skoon" toestande

5.8.2.3.1 Sentrifugeer die kultuur van die toetsorganisme 15 min lank teen 'n resulterende sentrifugale krag van 6 kN/kg na afloop van die inkubasie (kyk 5.8.2.2). Gebruik 'n steriele Pasteurpipet, verwyder die bodrywende vloeistof en doen dit weg en hersuspendeer die organisme in 10 mℓ harde water wat vir gebruik in hierdie toets (kyk 5.4.18.3) berei is.

5.8.2.3.2 Plaas hierdie suspensie oor na 'n steriele glasbottel met 'n inhoudsvermoë van 30 mℓ (kyk 5.2.1(a)).

5.8.2.3.3 Voeg 'n paar steriele glaskrale by en skud 1 min lank.

5.8.2.4 Bereiding van toetsorganismesuspensie vir die toets in "vuil" toestande

Verkry 'n suspensie wat 5 % (volgens massa) droë gis bevat deur 6 mℓ van die kultuur van die toetsorganisme (kyk 5.8.2.3) by 4 mℓ van 5 % (volgens massa) van die droë gissuspensie (kyk 5.4.24.4) in 'n steriele glasbottel met 'n inhoudsvermoë van 30 mℓ (kyk 5.2.1(a)) te voeg. Voeg 'n paar steriele glaskrale by die mengsel en werwel 1 min lank.

5.8.2.5 Beraming van die getal lewensvatbare organismes in die toetsorganismesuspensie

Standaardiseer die suspensie só met behulp van 'n spektrofotometer saam met 'n standaardkenkromme, 'n hemositometer, Petroff-Häusser-telkamer of 'n ander geskikte middel dat dit minstens 100 miljoen (10^8) maar hoogstens 10^{10} organismes per milliliter bevat. Gebruik die suspensie binne drie uur nadat dit berei is.

5.8.2.6 Bereiding van toetsoplossings

Gebruik die harde water wat vir hierdie toets berei is (kyk 5.4.18.3) en glasbottels met 'n inhoudsvermoë van 30 mℓ (kyk 5.2.1(a)) en berei drie verskillende konsentrasies A, B en C van die toetsmonster wat sodanig is dat:

a) konsentrasie B die een is wat na verwagting of na bewering die toets sal slaag;

b) konsentrasie A die helfte van konsentrasie B is; en

c) konsentrasie C een en 'n half maal konsentrasie B is.

Indien daar byvoorbeeld verwag word dat 'n toetsmonster met 'n 1 % konsentrasie die toets sal slaag, is konsentrasie A 'n 0,5 % konsentrasie, B 'n 1 % konsentrasie en C 'n 1,5 % konsentrasie.

5.8.2.7 Toetsprosedure in "skoon" toestande

5.8.2.7.1 Meet 3 mℓ van elke konsentrasie van die toetsmonster (kyk 5.8.2.6) uit in glasbottels met 'n inhoudsvermoë van 30 mℓ (kyk 5.2.1(a)) en etiketteer hierdie bottels A, B en C, soos toepaslik.

5.8.2.7.2 Plaas hierdie bottels en die bottel wat die toetsorganismesuspensie bevat (kyk 5.8.2.3) minstens 30 min lank in 'n waterbad wat by 22 °C ± 1 °C gehou word. Hou streng by die gemelde temperatuur om die reproduseerbaarheid van die toets te handhaaf.

5.8.2.7.3 Voeg dan, sonder om die bottel wat toetskonsentrasie A bevat uit die waterbad te haal, 1 mℓ van die toetssuspensie (kyk 5.8.2.3) by en sit terselfdertyd 'n stophorlosie (zero-tyd) aan die gang. Haal die bottel uit die waterbad, meng goed en plaas dit onmiddellik terug in die waterbad.

5.8.2.7.4 Voeg op dieselfde wyse een minuut na zero-tyd 1 mℓ van die toetssuspensie by die bottel wat toetskonsentrasie B bevat.

5.8.2.7.5 Voeg op dieselfde wyse vyf minute na zero-tyd 1 mℓ van die toetssuspensie by die bottel wat toetskonsentrasie C bevat.

5.8.2.7.6 Plaas agt minute na zero-tyd 0,02 mℓ uit die bottel wat toetskonsentrasie A bevat oor na elk van vyf buise herkweekmedium (kyk 5.4.14) wat A1 geëtiketteer is.

5.8.2.7.7 Voeg tien minute na zero-tyd op dieselfde wyse as in 5.8.2.7.3 hierbo nog 1 mℓ van die toetssuspensie (kyk 5.8.2.3) by die bottel wat toetskonsentrasie A bevat.

5.8.2.7.8 Plaas agtien minute na zero-tyd 0,02 mℓ uit die bottel wat toetskonsentrasie A bevat oor na elk van vyf buise herkweekmedium wat A2 geëtiketteer is.

Tabel 2 — Kelsey-Sykes-toetstydtafel

1	2	3	4	5	6
Tyd min	**Toetsmonster-suspensie A**	**Tyd** min	**Toetsmonster-suspensie B**	**Tyd** min	**Toetsmonster-suspensie C**
0	1 mℓ toetssuspensie volgens A	1	1 mℓ toetssuspensie volgens B	5	1 mℓ toetssuspensie volgens C
8	0,02 mℓ oorgeplaas van A na herkweek-medium A1	9	0,02 mℓ oorgeplaas van B na herkweek-medium B1	–	–
10	1 mℓ toetssuspensie volgens A	11	1 mℓ toetssuspensie volgens B	13	0,02 mℓ oorgeplaas van C na herkweek-medium C1
–	–	–	–	15	1 mℓ toetssuspensie volgens C
18	0,02 mℓ oorgeplaas van A na herkweek-medium A2	19	0,02 mℓ oorgeplaas van B na herkweek-medium B2	23	0,02 mℓ oorgeplaas van C na herkweek-medium C2

5.8.2.7.9 Behandel op dieselfde tyd as wat 5.8.2.7.1 tot 5.8.2.7.8 uitgevoer word die toetskonsentrasies B en C op dieselfde wyse maar baseer die tussenposes op die tye waarop die eerste byvoegings van die toetssuspensies gedoen is en etiketteer die stelle buise herkweekmedium (onderskeidelik) B1 en B2, en C1 en C2. In die geval van toetskonsentrasie B sal byvoeging en oorplasing dus een minuut later gedoen word as die tye wat vir toetskonsentrasie A aangegee word (dws op 9 min, 11 min en 19 min na zero-tyd) en, in die geval van toetskonsentrasie C, sal byvoegings en oorplasing op 13 min, 15 min en 23 min na zero-tyd gedoen word.

OPM – Ten einde foute by oorplasing te voorkom, moet 'n kopie van die toetstydtafel (kyk tabel 2) tydens elke toets gebruik word. Elke stap van die toets moet op die tydtafel afgemerk word terwyl dit uitgevoer word.

5.8.2.7.10 Inkubeer al die geïnokuleerde buise herkweekmedium 48 h lank by 30 °C.

5.8.2.7.11 Ondersoek die buise herkweekmedium na inkubasie vir groei en teken die resultate aan soos in die voorbeeld in tabel 3 aangetoon word.

Tabel 3 — Toetsmonster getoets in "skoon" toestande
Toetsorganisme: *Pseudomonas aeruginosa*

1	2	3	4	5	6
Toets No	**Toetsmonster-konsentrasie % (volgens volume)**	**Toets-konsentrasie organismes** mℓ	**Herkweekmedium** 1	2	**Resultaat**
1	0,8 1,6 2,4	$6,9 \times 10^8$ $6,9 \times 10^8$ $6,9 \times 10^8$	+ + + + + - - + + + - - - - -	+ + + + + - + + + + - - - - -	Faal Faal Slaag
Toetsoplossing met 'n 1,6 % konsentrasie (volgens volume) het gefaal. Toets herhaal met hoër konsentrasies.					
1	0,9 1,8 2,7	$8,4 \times 10^8$ $8,4 \times 10^8$ $8,4 \times 10^8$	- + + + + - - - - + - - - - -	+ + - + - - - + + + - - - - -	Faal Slaag Slaag
2	0,9 1,8 2,7	$2,1 \times 10^9$ $2,1 \times 10^9$ $2,1 \times 10^9$	- + + + + - - - - + - - - - -	+ + + + + - - + + + - - - - -	Faal Slaag Slaag
3	0,9 1,8 2,7	$5,7 \times 10^8$ $5,7 \times 10^8$ 5.7×10^8	- - + + + - - - - - - - - - -	+ + + + + - - - + + - - - - -	Faal Slaag Slaag

5.8.2.7.12 As toetskonsentrasie B die toets slaag (kyk 5.8.2.8), herhaal stap 5.8.2.7.1 tot 5.8.2.7.11 op twee agtereenvolgende dae.

5.8.2.8 Toetsprosedure in "vuil" toestande

Voer die prosedure in 5.8.2.7 uit maar gebruik die toetsorganismesuspensie wat in 5.8.2.4 beskryf word.

5.8.2.9 Vertolking van resultate

Ag dat aanvanklike konsentrasie ontsmettingsmiddel of detergentontsmettingsmiddel (konsentrasie B) (kyk 5.8.2.6(a)) die toets geslaag het as daar geen groei van die toetsorganisme is nie in minstens twee van die vyf buise herkweekmedium in stelle wat geïnokuleer is op:

a) die agtste minuut na die byvoeging van die aanvanklike inokulasie; en

b) die agtiende minuut na die byvoeging van die aanvanklike inokulasie.

'n Voorbeeld van 'n reeks toetsresultate en die interpretasie daarvan word in tabel 3 aangegee.

5.9 Ontsmettingsdoeltreffendheid van koolteertipe ontsmettingsvloeistowwe (swart en wit) — Rideal-Walker-koëffisiënt-toets

5.9.1 Bereiding van toetsorganismesuspensie

Meng deeglik 'n kultuur van *Salmonella typhi* (kyk 5.5.2.2) in voedingsboeljon no 2 (dubbelsterkte) (kyk 5.4.10) wat 24 h lank by 37 °C geïnkubeer is en plaas die buis voor gebruik 30 min ± 5 min lank in die waterbad (kyk 5.3.2) wat by 22 °C gehou word.

5.9.2 Bereiding van kontrole- en toetsoplossing

5.9.2.1 Standaardfenolkontroleoplossing

Berei van die 50 g/ℓ standaardfenoloplossing (kyk 5.4.19) vyf fenolkontroleverdunnings wat 1 g suiwer fenol in elke 95 mℓ, 100 mℓ, 105 mℓ, 110 mℓ en 115 mℓ van die oplossing bevat.

OPM – Hierdie verdunnings kan hoogstens een week lank voor gebruik in die donker bewaar word.

5.9.2.2 Voorraadoplossing van die toetsmonster (1:100)

5.9.2.2.1 Meng 'n saamgestelde monster van die ontsmettingsmiddel wat getoets moet word deeglik net voordat 'n deel vir die toets uitgetrek word en maak seker dat lug nie in die monster opgeneem word nie.

5.9.2.2.2 Trek die toetsdeel uit die middel van die monster met behulp van 'n pipet (kyk 5.2.3) met 'n inhoudsvermoë van 5 mℓ. Maak die pipet tot net bokant die merk vol, vee dit aan die buitekant skoon met steriele watte en verminder die inhoud dan tot by die merk. Laat die inhoud van die pipet uitloop onder die oppervlak van ongeveer 480 mℓ water by 'n temperatuur van 18 °C in 'n maatsilinder.

5.9.2.2.3 Spoel die pipet drie maal uit (of meer in die geval van viskeuse vloeistowwe) deur 'n bietjie van die verdunning daarin op te trek en weer terug te laat loop.

5.9.2.2.4 Vul die oplossing met water tot 500 mℓ aan, prop die silinder toe en meng die inhoud deeglik deur dit 50 maal met 'n kurktrekkerbeweging om te keer.

5.9.2.3 Toetsoplossings

Berei van die voorraadoplossing (kyk 5.9.2.2) vyf geskikte toetsoplossings (kyk tabel 4 en 6) wat gebaseer is op die nominale RW-koëffisiënt wat op die etiket gemeld word (kyk 7.2(m)). Plaas 5 mℓ van elk van die vyf gekose oplossings in steriele kweekbuise (kyk 5.2.2(a)). Merk hierdie buise en plaas hulle in volgorde in 'n rak in die waterbad (kyk 5.3.2), met die sterkste ontsmettingsmiddeloplossing aan die linkerkant.

5.9.2.4 Fenoloplossings

Berei vyf kweekbuise (kyk 5.2.2(a)) wat elk 5 mℓ van 'n ander fenolkontroleoplossing (kyk 5.9.2.1) bevat en merk en rangskik hulle op dieselfde wyse in die waterbad as die oplossings van die ontsmettingsmiddel (kyk 5.9.2.3).

5.9.2.5 Kweekmedium

5.9.2.5.1 Meng 'n kultuur van *Salmonella typhi* (kyk 5.5.2.2) deeglik in voedingsboeljon no 2 (dubbelsterkte) (kyk 5.4.10) wat 24 h lank by 37 °C geïnkubeer is en plaas die buis voor gebruik 30 min ± 5 min lank in 'n waterbad wat by 22 °C gehou word.

5.9.2.5.2 Plaas twee stelle van 15 buise (wat opeenvolgend van "1" tot "30" gemerk is) wat elk 5 mℓ voedingsboeljon no 2 (dubbelsterkte) (kyk 5.4.10) bevat, in die waterbad.

5.9.3 Toetsprosedure

5.9.3.1 Begin presies op zero-tyd (gebruik die stophorlosie (kyk 5.3.4) om die tyd van alle werksaamhede te meet) en voeg 0,2 mℓ van die toetsorganismesuspensie (kyk 5.9.1) met die druppipet (kyk 5.2.10) by die heel linkerkantste toetsbuis wat die ontsmettingsmiddeloplossing bevat (kyk 5.9.2.3). Maak seker dat al die kultuur wat bygevoeg word direk in die ontsmettingsmiddeloplossing gepipetteer word en nie teen die wand van die toetsbuis nie. Skud die buis; inokuleer 30 s hierna op soortgelyke wyse die volgende buis aan die regterkant met 0,2 mℓ van die kultuur. Inokuleer elke daaropvolgende buis met tussenposes van 30 s totdat die vyfde buis geïnokuleer is.

5.9.3.2 Trek 30 s na hierdie laaste byvoeging, dws 2,5 min na zero-tyd, 'n verteenwoordigende lusvol (kyk 5.2.9) van die inhoud van die buis heel links nadat dit goed geskud is en voeg dit by die buis wat "1" genommer is en 5 mℓ voedingsboeljon no 2 (dubbelsterkte) (kyk 5.4.10) bevat. Skud die buis met die geïnokuleerde boeljon onmiddellik na afloop van die inokulasie. Plaas dertig sekondes nadat hierdie lusvol uitgetrek is op dieselfde wyse 'n lusvol van die inhoud van die tweede toetsbuis oor na die buis met boeljon wat "2" genommer is. Herhaal hierdie prosedure met tussenposes van 30 s van links na regs totdat al vyf toetsbuise so behandel is.

OPM – Maak elke keer tydens die uittrekproses seker dat die lus vertikaal uit die oppervlak van die vloeistof gehaal word met die vlak daarvan horisontaal. Steriliseer die lus voor elke uittrekproses deur dit in 'n vlam te hou en maak seker dat die lus koud is voordat dit weer gebruik word.

5.9.3.3 Begin weer in elke geval met die linkerkantste buis en voer nog twee siklusse onttrekkings uit totdat drie stelle kulture (15 buise) gekweek is, dws met tussenposes van onderskeidelik 2,5 min, 5 min en 7,5 min na blootstelling.

5.9.3.4 Herhaal die prosedure in 5.9.3.1 tot 5.9.3.3 met die vyf buise wat die fenolkontroleoplossings bevat (kyk 5.9.2.3) en gebruik die buise met voedingsboeljon no 2 (dubbelsterkte) wat van "16" tot "30" genommer is.

5.9.3.5 Inkubeer al die buise wat ooreenkomstig 5.9.3.1 tot 5.9.3.4 berei is minstens 48 h lank by 37 °C.

5.9.4 Aantekening van resultate

Teken aan in watter buise groei van *Salmonella typhi* voorkom en maak seker dat die groei nie uit 'n kontaminant ontstaan het nie. Dit is gerieflik om met 'n "+"-teken te verwys na 'n buis wat groei toon en met 'n "–"-teken te verwys na 'n buis wat geen groei toon nie.

Die toets is bevredigend indien minstens een van die fenolkontroleverdunnings 'n kritieseverdunningsresponsie, dws 'n "+ + –"-groeipatroon toon; indien dit nie die geval is nie, verontagsaan die toetsresultate en herhaal die toets.

OPM – In gevalle waar die kritieseverdunningsyfer van fenol 115 is, is dit raadsaam om die toets te herhaal met vyf fenolkontroleverdunnings wat 1 g suiwer fenol in elke oplossing van 100 mℓ, 105 mℓ, 110 mℓ, 115 mℓ en 120 mℓ bevat.

5.9.5 Rideal-Walker-koëffisiënt

5.9.5.1 Berekening

Bereken die Rideal-Walker-koëffisiënt van die ontsmettingsmiddel deur die kritieseverdunningsyfer van die ontsmettingsmiddel te deel deur die kritieseverdunningsyfer van fenol in die bestek van 95 tot 115. Indien geeneen van die verdunnings van die monster die kritieseverdunningresponsie toon nie, is interpolasie toelaatbaar om 'n resultaat te verkry, maar nie ekstrapolasie nie.

5.9.5.2 Voorbeeld

'n Tipiese stel resultate word in tabel 4 aangegee.

Tabel 4 — Tipiese stel resultate by die berekening van die Rideal-Walker-koëffisiënt

1	2	3	4	5	6	7	8
Verdunning van monster	Blootsteltyd min			Verdunning van fenol (5 %)	Blootsteltyd min		
	2,5	5	7,5		2,5	5	7,5
1:1 000	-	-	-	1:95	-	-	-
1:1 100	+	-	-	1:100	+	-	-
1:1 200	+	+	-	1:105	+	+	-
1:1 300	+	+	-	1:110	+	+	+
1:1 400	+	+	+	1:115	+	+	+
Rideal-Walker-koëffisiënt = $\frac{1\ 300}{105}$ = 12,4							

Tabel 5 — RW-koëffisiënte en totale volume van verdunnings

1	2	3	4	5	6	7
Kritiese verdunning van monster	Totale volume van verdunde monster mℓ	RW-koëffisiënt vir ooreenstemmende kritiese verdunning van die fenol				
		95	100	105	110	115
1:2 500	125	26,3	25,0	23,8	22,7	21,7
1:2 400	120	25,3	24,0	22,9	21,8	20,9
1:2 300	115	24,2	23,0	21,9	20,9	20,0
1:2 200	110	23,2	22,0	21,0	20,0	19,1
1:2 100	105	22,1	21,0	20,0	19,1	18,3
1:2 000	100	21,1	20,0	19,0	18,2	17,4
1:1 900	95	20,0	19,0	18,1	17,3	16,5
1:1 800	90	18,9	18,0	17,1	16,4	15,7
1:1 700	85	17,9	17,0	16,2	15,5	14,8
1:1 600	80	16,8	16,0	15,2	14,5	13,9
1:1 500	75	15,8	15,0	14,3	13,6	13,0
1:1 400	70	14,7	14,0	13,3	12,7	12,2
1:1 300	65	13,7	13,0	12,4	11,8	11,3
1:1 200	60	12,6	12,0	11,4	10,9	10,4
1:1 100	55	11,6	11,0	10,5	10,0	9,6
1:1 000	50	10,5	10,0	9,5	9,1	8,7
1: 900	45	9,5	9,0	8,6	8,2	7,8
1: 800	40	8,4	8,0	7,6	7,3	7,0
1: 700	35	7,4	7,0	6,7	6,4	6,1
1: 600	30	6,3	6,0	5,7	5,5	5,2
1: 500	25	5,3	5,0	4,8	4,6	4,3
1: 400	20	4,2	4,0	3,8	3,6	3,5

Tabel 6 — RW-koëffisiënte en totale volume van verdunnings

1	2	3	4	5	6	7
Kritiese verdunning van monster	**Totale volume van verdunde monster** mℓ	**RW-koëffisiënt vir ooreenstemmende kritiese verdunning van die fenol**				
		95	**100**	**105**	**110**	**115**
1:350 1:300 1:250	70 60 50	3,7 3,2 2,6	3,5 3,0 2,5	3,3 2,9 2,4	3,2 2,7 2,3	3,0 2,6 2,2
1:200 1:150 1:100	40 30 20	2,1 1,6 1,1	2,0 1,5 1,0	1,9 1,4 –	1,8 1,4 –	1,7 1,3 –

5.9.6 Rideal-Walker-koëffisiënt vir verdunnings van ontsmettingsmiddel van 1:100 tot 1:2 500

5.9.6.1 Verdunnings van ontsmettingsmiddel

Die voorraadoplossing van die ontsmettingsmiddel (kyk 5.9.2.2) bevat 5 mℓ ontsmettingsvloeistof in 500 mℓ van die oplossing (verdunning van 1:100).

5.9.6.1.1 Ten einde 'n verdunning van tussen 1:2 500 en 1:400 (kyk kolom 1 van tabel 5) te verkry, verdun 5 mℓ van die voorraadverdunning deur steriele water by te voeg om dit op die toepaslike totale volume in kolom 2 te bring.

5.9.6.1.2 Ten einde 'n verdunning van tussen 1:100 en 1:350 te verkry (kyk kolom 1 van tabel 6), verdun 20 mℓ van die voorraadverdunning deur steriele water by te voeg om dit op die toepaslike totale volume in kolom 2 te bring.

5.9.6.2 Rideal-Walker-koëffisiënte

Die Rideal-Walker-koëffisiënt vir 'n monsterverdunning wat in kolom 1 van tabel 5 of tabel 6 (soos toepaslik) voorkom met betrekking tot een van die fenolverdunnings boaan kolom 3 tot en met kolom 7, word in die toepaslike kolom aangegee.

OPM – Hierdie tabelle is bedoel om die berekening van die resultate te vergemaklik en hulle plaas geen beperking op die ontsmettingsverdunnings wat gebruik moet word nie. Hulle kan na wens uitgebrei word.

5.10 Ontsmettingsdoeltreffendheid — Gebruiksverdunningtoets

5.10.1 Algemeen

Die volgende ontsmettingsmiddels of detergentontsmettingsmiddels, met die nodige aanpassings, soos aangedui, word met behulp van hierdie prosedure getoets:

a) ontsmettingsmiddels of detergentontsmettingsmiddels met jodofore as basis, met gebruik van die harde water wat in 5.4.18.2 beskryf word en inaktiveermedium no 1 wat in 5.4.7.1 beskryf word;

b) ontsmettingsmiddels of detergentontsmettingsmiddels met organiese halogeenverbindings (uitgesonderd jodiumverbindings) as basis, met gebruik van die harde water wat in 5.4.18.1 beskryf word en inaktiveermedium no 1 wat in 5.4.7.1 beskryf word;

c) ontsmettingsmiddels of detergentontsmettingsmiddels met kwaternêre ammoniumverbindings as basis, met gebruik van die harde water wat in 5.4.18.1 beskryf word en inaktiveermedium no 1 wat in 5.4.7.1 beskryf word;

OPM – Weens die geneigdheid van kwaternêre ammoniumverbindings om op die oppervlak van glasware geadsorbeer te word, moet daar sorg gedra word dat daar aan die begin van die toets geen residu's op die glasware is nie.

d) ontsmettingsmiddels of detergentontsmettingsmiddels met gestabiliseerde anorganiese chloorverbindings as basis, met gebruik van die harde water wat in 5.4.18.1 beskryf word en die natriumtiosulfaatinaktiveerder wat in 5.4.15 beskryf word; en

e) ontsmettingsmiddels of detergentontsmettingsmiddels met gestabiliseerde chloorverbindings as basis, met gebruik van die harde water wat in 5.4.18.1 beskryf word en die natriumtiosulfaatinaktiveerder wat in 5.4.15 beskryf word.

5.10.2 Bereiding van kontrole- en toetsoplossings

5.10.2.1 Mengsel van melk en harde water

Gebruik 'n skoon, steriele pipet en meet 1 mℓ van die steriele afgeroomde melk (kyk 5.4.20) asepties uit in elkeen van twee bottels wat 97 mℓ harde water (kyk 5.4.18.1) bevat. By die toets van vaste detergentontsmettingsmiddels, berei slegs een sodanige mengsel.

5.10.2.2 Kontroleoplossing

Voeg 1 mℓ steriele water by die inhoud van die mengsel afgeroomde melk en harde water van een bottel (kyk 5.10.2.1).

5.10.2.3 Toetsoplossing

5.10.2.3.1 Vloeibare ontsmettingsmiddel of detergentontsmettingsmiddel

Berei 'n oplossing van die toetsmonster in steriele water waarvan die konsentrasie sodanig is dat 'n 1:100-verdunning van hierdie oplossing sal ooreenstem met die verdunning aangegee op die etiket (kyk 7.2(f)) en voeg 1 mℓ van hierdie oplossing by die ander mengsel van melk en harde water (kyk 5.10.2.1). So nie, indien die geëtiketteerde verdunning nie 1 op 100 oorskry nie, voeg die vereiste hoeveelheid van die monster direk by sodanige volume van die mengsel om 'n finale volume van 99 mℓ te verkry.

5.10.2.3.2 Vaste ontsmettingsmiddel of detergentontsmettingsmiddel

Weeg direk in 'n droë, steriele 110 mℓ bottel 'n hoeveelheid van die monster af wat die verdunning op die etiket (kyk 7.2(f)) sal lewer nadat die volgende bygevoeg is:

a) 1 mℓ steriele water;

b) 1 mℓ steriele afgeroomde melk (kyk 5.4.20); en

c) genoeg steriele harde water (kyk 5.4.18.1) om die totale volume op 99 mℓ te bring.

Elke pipet wat gebruik word vir die meet van 'n oplossing wat 'n kwaternêre ammoniumverbinding bevat, moet voor gebruik minstens twee maal met dié oplossing uitgespoel word.

5.10.2.4 Temperatuurstelling

Meng die verdunning goed maar saggies (om skuimvorming tot 'n minimum te beperk) onmiddellik nadat dit berei is. Plaas die bottels met die kontrole, die toetsoplossings en die toetsorganismesuspensies voor die toets minstens 30 min lank in 'n waterbad wat by 22 °C ± 1 °C gehou word. Om reproduseerbaarheid van die toetsresultate te behou, moet daar streng by die gemelde temperatuur gehou word.

5.10.3 Bereiding van toetsorganismesuspensies

Berei *Escherichia coli-*, *Staphylococcus aureus-* en *Pseudomonas aeruginosa*-toetsorganismesuspensies soos in 5.5.2.1 beskryf en maak seker dat elke milliliter groeimedium minstens 100 000 (10^5) maar hoogstens 1 miljoen (10^6) organismes bevat. Gebruik die suspensies binne drie uur nadat dit berei is.

5.10.4 Toetsprosedure

5.10.4.1 Smelt die inhoud van 'n voldoende getal bottels (wat 15 mℓ volumes bevat) voedingsagar (kyk 5.4.9), koel dit af tot 45 °C en hou hulle by hierdie temperatuur. Meet 1 mℓ ± 0,5 mℓ volumes van die toepaslike inaktiveeroplossing (kyk 5.4.7) asepties in elk van twee toepaslik gemerkte steriele petribakkies uit.

5.10.4.2 Gebruik 'n skoon, steriele pipet en meet 1 mℓ van die *Staphylococcus aureus*-toetssuspensie (kyk 5.10.3) in die toetsoplossing uit (kyk 5.10.2.3.1 in die geval van 'n vloeibare ontsmettingsmiddel of vloeibare detergentontsmettingsmiddel en 5.10.2.3.2 in die geval van 'n vaste ontsmettingsmiddel of vaste detergentontsmettingsmiddel). Skud die bottel om die toetssuspensie en die toetsoplossing deeglik te meng. Sit die stophorlosie aan die gang. Skud die toetsoplossing saggies ongeveer 20 s voor die einde van die aanbevole blootsteltydperk (kyk 7.2(f)), trek 1 mℓ van die toetsoplossing asepties in 'n 1 mℓ pipet op en los hierdie oplossing presies aan die einde van die blootsteltyd in die inaktiveeroplossing in een van die petribakkies. Meng die oplossings onmiddellik daarna deeglik.

5.10.4.3 Herhaal die prosedure in 5.10.4.2 met die kontroleoplossing.

5.10.4.4 Voeg 15 mℓ gesmelte agar (kyk 5.10.4.1) by elk van die petribakkies en werwel die plate saggies op 'n tafelblad om 'n egalige verspreiding van kolonies na inkubasie te verseker. Verhoed dat die inhoud van die plate tydens hierdie proses stort. Laat die agar stol en inkubeer die plate 48 h lank by 37 °C.

5.10.4.5 Ondersoek die plate na inkubasie vir groei. Tel die kolonies op elke plaat (van die toetsoplossing en van die kontrole) wat tussen 30 en 300 kolonies bevat en teken dit aan. Indien die laagste verdunning (hoogste konsentrasie) minder as 30 kolonies oplewer, tel al die kolonies. Maak seker dat die kolonies wat getel is, afkomstig is van oorlewendes van die toetsorganismes en nie van kontaminasie nie.

5.10.4.6 Beskou die verdunningsfaktor (*VF*) in die geval van die monster en in die geval van die kontrole as die omgekeerde van die verdunning; indien die monster byvoorbeeld tot 1/1 000 verdun is, beskou *VF* as 1 000.

5.10.4.7 Die doodpersentasie word deur die volgende formule gegee:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

waar

A die getal organismes is wat na kontak met die monster getel is (kyk 5.10.4.5);

B die getal organismes is wat in die kontrole getel is (kyk 5.10.4.5);

DF_s die verdunningsfaktor vir die monster is soos in 5.10.4.6 bereken; en

DF_c die verdunningsfaktor vir die kontrole is soos in 5.10.4.6 bereken.

OPM – Dieselfde formule word gebruik om die doodpersentasie van *Escherichia coli* en *Pseudomonas aeruginosa* te bepaal.

5.10.4.8 Herhaal die prosedure in 5.10.4.1 tot en met 5.10.4.7 en gebruik agtereenvolgens die *Escherichia coli*- en die *Pseudomonas aeruginosa*-toetssuspensies.

5.10.4.9 Herhaal die hele toets beskryf in 5.10.4.1 tot en met 5.10.4.8 op twee opeenvolgende dae.

5.10.5 Vertolking van resultate

Ag dat die monster aan die vereistes van 4.2 voldoen indien 'n doodresultaat van minstens 99,9 % verkry is vir elke organisme wat teen die aanbevole konsentrasie vir die aanbevole blootsteltyd (kontaktyd) (kyk 7.2(f)) en met die aanbevole inaktiveerder getoets is (kyk 5.4.7).

5.11 Ontsmettingsdoeltreffendheid van ontsmettingsmiddels en detergentontsmettingsmiddels met 'n mengsel van peroksigeenverbindings, 'n surfaktant, organiese sure en 'n anorganiese bufferstelsel as basis

5.11.1 Bereiding van kontrole- en toetsoplossing

5.11.1.1 Toetsoplossing

5.11.1.1.1 Gebruik steriele gedistilleerde water en berei 'n konsentrasie van dubbel die toepaslike verdunning van die ontsmettingsmiddel of detergentontsmettingsmiddel (kyk 7.2(f)).

5.11.1.1.2 Meet 5 mℓ volumes van hierdie monsterverdunning en 4 mℓ volumes steriele gedistilleerde water asepties in steriele toetsbuise uit en hou dit by kamertemperatuur.

5.11.1.2 Kontroleoplossing

Gebruik 9 mℓ steriele gedistilleerde water as kontrole.

5.11.2 Toetsprosedure

5.11.2.1 Smelt die inhoud van 'n voldoende aantal bottels (wat 15 mℓ volumes bevat) voedingsagar (kyk 5.4.9), koel hulle af tot 45 °C en hou hulle by hierdie temperatuur.

5.11.2.2 Gebruik 'n skoon, steriele pipet, voeg 1 mℓ van die *Bacillus subtilis* var *globigii*-spoorsuspensie wat soos in 5.5.2.4 berei is, asepties by die buise van elke ontsmettingsmiddel of detergentontsmettingsmiddel wat getoets moet word, sowel as by die kontrole. Meng goed met behulp van 'n werveltipe menger en hou dit vir die duur van die toets by kamertemperatuur.

5.11.2.3 Haal 1 mℓ volumes na 1 h uit elke buis en voeg elke volume by 'n afsonderlike 9 mℓ volume steriele gedistilleerde water as verdunmiddel. (In die geval van die kontrole word vier tot vyf reeksverdunnings aanbeveel.)

5.11.2.4 Plaas elke verdunning op 'n plaat uit deur 1 mℓ van die verdunning op 'n toepaslik geëtiketteerde steriele petribakkie te pipetteer. Voeg 15 mℓ van die voedingsagar (kyk 5.11.2.1) by elk van die plate en werwel saggies om 'n egalige verspreiding van kolonies na inkubasie te verseker. Verhoed dat die inhoud van die plate tydens hierdie proses stort. Laat die agar stol.

5.11.2.5 Herhaal die prosedure in 5.11.2.1 tot 5.11.2.4 na 'n kontaktyd van 2 h, 3 h en 4 h.

5.11.2.6 Keer die plate om en inkubeer hulle 48 h lank by 37 °C.

5.11.2.7 Ondersoek die plate na inkubasie vir groei. Tel die kolonies op elke plaat (van die toetsoplossing en van die kontrole) wat tussen 30 en 300 kolonies bevat en teken dit aan. Indien die laagste verdunning (hoogste konsentrasie) minder as 30 kolonies oplewer, tel al die kolonies. Maak seker dat die kolonies wat getel is, afkomstig is van oorlewendes van die toetsorganismes en nie van kontaminasie nie.

5.11.2.8 Beskou die verdunningsfaktor (*VF*) in die geval van die monster en in die geval van die kontrole as die omgekeerde van die verdunning; indien die monster byvoorbeeld tot 1/1 000 verdun is, beskou *VF* as 1 000.

5.11.2.9 Die doodpersentasie word deur die volgende formule aangegee:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

waar

A die getal organismes is wat na kontak met die monster getel is (kyk 5.11.2.7);

B die getal organismes is wat in die kontrole getel is (kyk 5.11.2.7);

DF_s die verdunningsfaktor vir die monster is soos in 5.11.2.8 bereken; en

DF_c die verdunningsfaktor vir die kontrole is soos in 5.11.2.8 bereken.

5.11.3 Vertolking van resultate

Ag dat die ontsmettingsmiddel of detergentontsmettingsmiddel aan die vereistes van 4.2 voldoen indien 'n doodpersentasie van minstens 99,9 % na 'n reaksietyd van 4 h verkry is.

5.12 "5,5,5"-metode vir die toets van ontsmettingsdoeltreffendheid van detergente en detergentontsmettingsmiddels

5.12.1 Bereiding van die toetsorganismesuspensies

Berei die toetsorganismesuspensies soos in onderskeidelik 5.5.2.1, 5.5.2.3 en 5.5.2.4 beskryf word en maak seker dat elke milliliter groeimedium onderskeidelik die volgende bevat:

a) 400 miljoen (4×10^8) *Escherichia coli-*, *Staphylococcus aureus-* en *Pseudomonas aeruginosa*-organismes;

b) 10 miljoen (10^7) *Aspergillus niger*-organismes; en

c) 800 000 (8×10^5) *Bacillus subtilis* var *globigii*-spore.

Gebruik die suspensies binne drie uur nadat dit berei is.

5.12.2 Bereiding van kontrole- en toetsoplossing

5.12.2.1 Kontroleoplossing

Meet 9,0 mℓ steriele water in steriele glasbottels uit en voeg by elkeen 0,5 mℓ van die beesalbumien-oplossing (kyk 5.4.3).

5.12.2.2 Toetsoplossing

Berei die voorgeskrewe konsentrasie van die toetsmonster soos op die etiket (kyk 7.2(f)) gemeld en gebruik steriele water. Meet 9,0 mℓ van die toetsoplossing in steriele glasbottels uit en voeg by elkeen 0,5 mℓ beesalbumienoplossing. Gebruik die toetsoplossing op die dag waarop dit berei is.

5.12.3 Temperatuurstelling

Etiketteer die bottels wat die kontrole, die toetsoplossings en die toetsorganismesuspensies bevat en plaas hulle minstens 30 min lank in 'n waterbad wat by 22 °C gehou word.

5.12.4 Toetsprosedure

OPM – Kortheidshalwe word die prosedure vir die toets van die ontsmettingsmiddeloplossing vir een organisme aangegee. Vir elke organisme word 'n kontroletoets uitgevoer met gebruik van steriele gedistilleerde water in plaas van die ontsmettingsmiddeloplossing. Elke kontrole val saam met die gepaardgaande toets (met 'n tydsverloop van ongeveer 30 s tussen gepaardgaande stappe). Gebruik 'n stophorlosie vir hierdie doel.

5.12.4.1 Smelt die inhoud van 'n voldoende aantal bottels (wat 15 mℓ volumes bevat) voedingsagar (kyk 5.4.9), koel hulle tot 45 °C af en hou hulle by hierdie temperatuur.

5.12.4.2 Begin die toets nadat die temperatuur van die kontrole, toetsoplossings en die toetsorganismesuspensies 22 °C bereik het. Gebruik 'n skoon, steriele pipet, voeg 0,5 mℓ van die *Pseudomonas aeruginosa*-toetssuspensie (kyk 5.12.1(a)) by die toetsoplossing en by die kontrole (sonder om die bottels uit die waterbad te haal). Haal die bottels uit die waterbad, skud hulle om die inhoud deeglik te meng en plaas hulle onmiddellik terug in die waterbad.

5.12.4.3 Plaas na 'n blootsteltydperk van 5 min (werklike kontaktyd) 1 mℓ van die toetsoplossing wat die organisme bevat oor na 9 mℓ inaktiveermedium vir die "5,5,5"-toets (kyk 5.4.7.3) en meng goed tot 'n eenvormige suspensie (eerste verdunning). Herhaal hierdie prosedure binne 30 s met die kontrole.

5.12.4.4 Gebruik 'n skoon, steriele pipet en plaas 1 mℓ van die inaktiveermedium wat die organisme bevat (kyk 5.12.4.3) oor na die eerste van 'n reeks bottels wat elk 9 mℓ steriele verdunmiddel (kyk 5.4.5) bevat. Meng goed tot 'n eenvormige suspensie (tweede verdunning).

5.12.4.5 Herhaal die prosedure in 5.12.4.4, maar plaas die suspensie wat na die tweede verdunning verkry is oor na die tweede van die reeks bottels. Verdun verder totdat 'n finale verdunning van 1:1 000 van die toetsmonster verkry word.

5.12.4.6 Berei agtereenvolgens, soos hierbo beskryf, 'n verdunningsreeks met gebruik van die kontrole.

5.12.4.7 Plaas 1 mℓ van elke verdunning van die toetsmonster oor na elk van twee toepaslik geëtiketteerde, steriele plate (petribakkies). Doen dieselfde met elke finale verdunning van die kontrole. Voeg 15 mℓ voedingsagar (kyk 5.12.4.1) by elk van die plate en werwel saggies om 'n egalige verspreiding van kolonies na inkubasie te verseker. Verhoed dat die inhoud van die plate tydens hierdie proses stort. Laat die agar stol.

5.12.4.8 Keer die plate om en inkubeer hulle 48 h lank by 37 °C.

5.12.4.9 Ondersoek die plate na afloop van inkubasie vir groei. Tel die kolonies op elke plaat (van die toetsoplossing en van die kontrole) wat tussen 30 en 300 kolonies bevat en teken dit aan. Indien die laagste verdunning (hoogste konsentrasie) minder as 30 kolonies oplewer, tel al die kolonies. Maak seker dat die kolonies wat getel is, afkomstig is van oorlewendes van die toetsorganismes en nie van kontaminasie nie.

5.12.4.10 Beskou die verdunningsfaktor (*VF*) in die geval van die toetsmonster en in die geval van die kontrole as die omgekeerde van die verdunning; indien die monster of die kontrole byvoorbeeld tot 1/1 000 verdun is, beskou *VF* as 1 000.

5.12.4.11 Bereken die logreduksiefaktor aan die hand van die volgende formule:

$$\log (B \times DF_c) - (A \times DF_s)$$

waar

A die getal organismes is wat na kontak met die monster getel is (kyk 5.12.4.9);

B die getal organismes is wat in die kontrole getel is (kyk 5.12.4.9);

DF_s die verdunningsfaktor vir die monster is soos in 5.12.4.10 bereken; en

DF_c die verdunningsfaktor vir die kontrole is soos in 5.12.4.10 bereken.

OPM – Dieselfde formule word gebruik vir die bepaling van die logreduksiefaktor van *Escherichia coli* en *Staphylococcus aureus* na 5 min kontaktyd (kyk 5.12.4.12), van *Aspergillus niger* na 15 min kontaktyd (kyk 5.12.4.13) en van *Bacillus subtilis* var *globigii* na 1 h kontaktyd (kyk 5.12.4.14).

5.12.4.12 Herhaal die prosedure in 5.12.4.1 tot en met 5.12.4.11 met die *Escherichia coli*- en *Staphylococcus aureus*-suspensie (kyk 5.12.1(a)).

5.12.4.13 Herhaal die prosedure in 5.12.4.1 tot en met 5.12.4.11 met die *Aspergillus niger*-toets-organismesuspensie (kyk 5.12.1(b)), maar:

a) gebruik 'n blootsteltydperk (werklike kontaktyd) van 15 min;

b) voeg moutekstrakagar (kyk 5.4.8) by die suspensies op die plate; en

c) inkubeer dit 7 d lank by 25 °C.

5.12.4.14 Herhaal die prosedure in 5.12.4.1 tot en met 5.12.4.11 met die *Bacillus subtilis* var *globigii*-spoorsuspensie (kyk 5.12.1(c)), maar gebruik 'n blootsteltyd (werklike kontaktyd) van 1 h.

5.12.5 Vertolking van resultate

Ag dat die monster aan die vereistes van 4.2 voldoen indien 'n logreduksiewaarde van minstens 5 vir elke vegetatiewe organisme wat getoets is en 'n logreduksiewaarde van minstens 1 vir die *Bacillus subtilis* var *globigii*-spoor verkry is.

5.13 Bepaling van die virusdodende aktiwiteit met behulp van bakteriofae as indikatororganismes

5.13.1 Inaktiveerder

Kies die geskikste inaktiveerder vir elke produk soos in die toetse vir bakteriedodende doeltreffendheid bepaal is.

5.13.2 Harde water

Kies die harde water vir elke produk soos in die toetse vir bakteriedodende doeltreffendheid bepaal is.

5.13.3 Bereiding van die toetsbakteriesuspensies

Berei *Escherichia coli* SABS TCC 36- en *Escherichia coli* SABS TCC 37-kulture soos in 5.5.2.5.2.1 tot en met 5.5.2.5.2.4.

5.13.4 Bereiding van toetsbakteriofaagsuspensies

Die MS2- en ϕX174-bakteriofae (kyk 5.5.2.5) word onmiddellik voor gebruik deur verdunning tot 'n titer van tussen 10^7 en 10^8 plaketvormende eenhede per milliliter in die voedingsmedium (kyk 5.4.11) gebring.

5.13.5 Bereiding van kontrole- en toetsoplossing

5.13.5.1 Kontroleoplossing

Meet 9,9 mℓ harde water (kyk 5.13.2) of steriele gedistilleerde water, na gelang van die tipe ontsmettingsmiddel of detergentontsmettingsmiddel (kyk toetse vir bakteriedodende doeltreffendheid), in 'n steriele glasbottel uit.

5.13.5.2 Toetsoplossing

Gebruik harde water of steriele gedistilleerde water (kyk toetse vir bakteriedodende doeltreffendheid) en berei die voorgeskrewe konsentrasie van die toetsmonster soos op die etiket gemeld (kyk 7.2(f)). Meet 9,0 mℓ van die toetsoplossing in 'n steriele glasbottel uit. Gebruik die toetsoplossing op die dag waarop dit berei is.

5.13.6 Temperatuurstelling

Etiketteer die bottels wat die kontrole, die toetsoplossings en die toetsorganismesuspensies bevat en plaas hulle minstens 30 min lank in 'n waterbad wat by 22 °C gehou word.

5.13.7 Toetsprosedure

OPM – Kortheidshalwe word die prosedure vir die toets van die ontsmettingsmiddeloplossing vir bakteriofaagstam MS2 aangegee. Elke kontrole val saam met die gepaardgaande toets (met 'n tydsverloop van ongeveer 30 s tussen gepaardgaande stappe). Gebruik 'n stophorlosie vir hierdie doel.

5.13.7.1 Smelt die bolaagagar (kyk 5.4.21) en meet asepties 5 mℓ volumes uit in steriele kweekbuise (kyk 5.2.2(a)) wat gehou moet word in 'n waterbad wat by 44 °C gehou moet word om stolling van die agar te verhoed.

5.13.7.2 Gebruik 'n skoon, steriele pipet en meet 1 mℓ van die MS2-bakteriofaagtoetssuspensie (kyk 5.13.4) uit in die toetsoplossing en in die kontrole (sonder om die bottels uit die waterbad te haal). Haal die bottels uit die waterbad, werwel die inhoud daarvan en plaas hulle onmiddellik in die waterbad terug.

5.13.7.3 Plaas na afloop van die blootsteltydperk van 15 min (werklike kontaktyd) 1 mℓ van die toetsoplossing wat die bakteriofaag bevat (kyk 5.13.7.2) oor na 9 mℓ inaktiveerder (kyk 5.13.1) en werwel totdat die suspensie eenvormig is (eerste verdunning). Herhaal binne 30 s hierdie prosedure met die kontrole.

5.13.7.4 Gebruik 'n skoon, steriele pipet en plaas 1 mℓ van die inaktiveermedium wat die bakteriofaag bevat (kyk 5.13.7.3) oor na die eerste van 'n reeks bottels wat elkeen 9 mℓ steriele fisiologiese soutoplossing (kyk 5.4.13) bevat. Werwel totdat die suspensie eenvormig is (tweede verdunning).

5.13.7.5 Herhaal die prosedure in 5.13.7.4, maar plaas die suspensie wat na die tweede verdunning verkry is oor na die tweede van die reeks bottels. Verdun verder totdat 'n finale verdunning van 1:100 van die toetsmonster verkry word.

5.13.7.6 Berei agtereenvolgens, soos hierbo beskryf, 'n verdunningsreeks met gebruik van die kontrole totdat 'n finale verdunning van 1:100 000 verkry word.

5.13.7.7 Plaas 0,1 mℓ van elke verdunning van die toetsmonster oor na elk van 0,9 mℓ van die 2 h *E. coli* SABS TCC Esc 36 kultuur (kyk 5.13.3). Doen dieselfde ten opsigte van die finale verdunnings van die kontrole.

5.13.7.8 Plaas die suspensies 15 min ± 1 min lank by 37 °C in 'n inkubator en voeg dan 5,0 mℓ van die bolaagagar by (kyk 5.13.7.1).

5.13.7.9 Werwel die mengsel en smeer dit onmiddellik oor die oppervlak van die onderlaagagar (kyk 5.4.2) in geëtiketteerde petribakkies en werwel saggies om 'n egalige verspreiding van organismes na inkubasie te verkry.

5.13.7.10 Laat die agar stol, keer die plate om en inkubeer die petribakkies 24 h lank by 37 °C.

5.13.7.11 Ondersoek die plate na inkubasie vir plaketvorming. Tel die plakette op elke plaat (van die toetsoplossing en van die kontrole) wat tussen 30 en 100 plakette bevat en teken dit aan. Indien die laagste verdunning (hoogste konsentrasie) minder as 30 plakette oplewer, tel al die plakette.

5.13.7.12 Beskou die verdunningsfaktor (*VF*) as die omgekeerde van die verdunning; indien die oplossing byvoorbeeld tot 1/1 000 verdun is, beskou *VF* as 1 000.

5.13.7.13 Die doodpersentasie word deur die volgende formule aangegee:

$$\frac{(B \times DF_c) - (A \times DF_s)}{B \times DF_c} \times 100$$

waar

A die getal plakette is wat na kontak met die monster getel is (kyk 5.13.7.11);

B die getal organismes is wat in die kontrole getel is (kyk 5.13.7.11);

DF_s die verdunningsfaktor vir die monster is soos in 5.13.7.12 bereken; en

DF_c die verdunningsfaktor vir die kontrole is soos in 5.13.7.12 bereken.

OPM – Dieselfde formule word gebruik om die doodpersentasie van ϕX174-bakteriofaagstam te bepaal.

5.13.7.14 Herhaal die prosedure in 5.13.7.1 tot en met 5.13.7.13 met die ϕX174-bakteriofaagstam.

5.13.7.15 Herhaal die prosedure in 5.13.7.1 tot en met 5.13.7.14 op twee verskillende dae.

5.13.8 Vertolking van resultate

Ag dat die monster aan die vereistes van 4.2 voldoen indien 'n doodresultaat van minstens 99,99 % verkry is vir elke bakteriofaag wat getoets word.

6 Metodes vir fisiese en chemiese ondersoek

6.1 Korroderendheid

6.1.1 Toetsstrook

Een blinkafgewerkte onbestrykte aluminiumstrook wat voldoen aan BS 1470:1987, *Specification for wrought aluminium and aluminium alloys for general engineering purposes: plate, sheet and strip*, met 'n grootte van 75 mm x 19 mm x 1 mm, wat ontvet is deur dit in asetoon te was en by omgewingstemperatuur te laat droog word.

6.1.2 Prosedure

6.1.2.1 Gebruik 'n skerp instrument en verdeel die strook in twee deur 'n keep deur die middelpunt daarvan te trek.

6.1.2.2 Gebruik 'n 100 mℓ maatsilinder en dompel die strook heeltemal in die toetsmonster. Prop die silinder toe en hou dit 24 h lank by 23 °C. Verwyder na 24 h soveel van die toetsmonster dat die vlak in die maatsilinder gelyk met die middellyn van die toetsstrook lê. Prop die silinder weer toe en hou dit nog 24 h lank by 23 °C.

6.1.2.3 Haal die toetsstrook uit, spoel dit deeglik eers met water en daarna met asetoon af en laat dit lugdroog word. Ondersoek die strook vir voldoening aan 4.3.

6.2 Gehalte aan wateronoplosbare stof

6.2.1 Prosedure

6.2.1.1 Pipetteer 5,0 mℓ van 'n vloeibare ontsmettingsmiddel of vloeibare detergentontsmettingsmiddel (of plaas 2 g vaste ontsmettingsmiddel of vaste detergentontsmettingsmiddel) in 'n beker en voeg 250 mℓ harde water (kyk 5.4.18.1) by.

6.2.1.2 Verhit in 'n stoombad en roer dikwels totdat die monster heeltemal gedispergeer het.

6.2.1.3 Filtreer die oplossing onmiddellik onder suiging deur 'n geweegde glasveselfilter en maak seker dat die onoplosbare stof kwantitatief na die filter oorgeplaas word.

6.2.1.4 Was die beker en die residu vyf maal met 20 mℓ volumes warm harde water.

6.2.1.5 Laat die oplossing heeltemal afloop en droog die residu by 105 °C totdat konstante massa bereik word. Laat in 'n desikkator afkoel en weeg.

6.2.2 Berekening

6.2.2.1 Vaste ontsmettingsmiddels of vaste detergentontsmettingsmiddels

Bereken die gehalte aan wateronoplosbare stof S as 'n persentasie volgens massa aan die hand van die volgende formule:

$$S = \frac{m_2}{m_1} \times 100$$

waar

m_1 die massa is van die toetsmonster wat geneem is, in gram; en

m_2 die massa van die residu is nadat dit gedroog is, in gram.

6.2.2.2 Vloeibare ontsmettingsmiddels of vloeibare detergentontsmettingsmiddels

Bereken die gehalte aan wateronoplosbare stof in die toetsoplossing, uitgedruk in gram per liter, aan die hand van die volgende formule:

$$\frac{m}{V}$$

waar

m die massa van die residu is nadat dit gedroog is, in gram; en

V die volume van die toetsoplossing is, in liter.

Gaan na vir voldoening aan 4.4.

6.3 Afspoeleienskappe van detergentontsmettingsmiddels

6.3.1 Pipetteer met behulp van 'n spuit 0,4 mℓ of weeg 0,4 g van die onverdunde toetsmonster af in 'n deeglik skoongemaakte koniese fles van 500 mℓ en voeg 200 mℓ standaard- harde water (kyk 5.4.18) by. Prop die fles toe en skud dit 1 min lank goed. In die geval van detergentontsmettingsmiddels met organiese halogeenverbindings (uitgesonderd jodiumverbindings), fenol en gestabiliseerde chloorverbindings as basis, gebruik 2,0 mℓ van elke toetsmonster in die geval van 'n vloeibare detergentontsmettingsmiddel en 2 g van elke toetsmonster in die geval van 'n vaste detergentontsmettingsmiddel. In die geval van detergentontsmettingsmiddels met jodofore en kwaternêre ammoniumverbindings as basis, toets die kleinste verdunning wat op die etiket (kyk 7.2(f)) aanbeveel word.

6.3.2 Gooi die oplossing uit en spoel die fles uit deur 200 mℓ van die standaard- harde water daarin te gooi, dit 1 min lank goed te skud en die water af te giet. Keer die fles om en laat dit droog word.

6.3.3 Voer 'n blanko toets uit deur die bostaande prosedure te herhaal maar die toetsmonster weg te laat.

Vergelyk die twee flesse.

6.3.4 Ag dat die monster aan die vereistes van 4.5 voldoen indien die strepe en merke op die fles wat vir die toets gebruik is nie meer is as dié op die fles wat vir die blanko toets gebruik is nie.

6.4 Skoonmaakdoeltreffendheid van detergentontsmettingsmiddels

6.4.1 Apparaat

6.4.1.1 Ses toetspanele van geanodiseerde aluminium, gemasjineer uit aluminium wat aan SABS 989:1994, *Gietblokke van aluminium- en aluminiumlegerings vir oorsmelting* (legeringaanwysing Al-99,5: legeringkode SA2) voldoen. Die panele, met afmetings soos in figuur 1, het holtes soos in die figuur aangetoon word.

6.4.1.2 Ses geskikte klampe, wat só aan die roerders (kyk 6.4.1.3) aangebring is dat die paneel vasgehou en om 'n vertikale as gedraai kan word.

6.4.1.3 Ses meganiese roerders wat, indien hulle 'n toetspaneel ondersteun, gladweg en deurlopend teen 60 r/min tot 62 r/min kan werk.

6.4.1.4 Waterbad, wat termostaties beheer word, 'n temperatuur van 30 °C kan handhaaf en toegerus is met 'n dekstuk met ses gate in 'n ry waarin die toetsbekers (kyk 6.4.1.5) geplaas word.

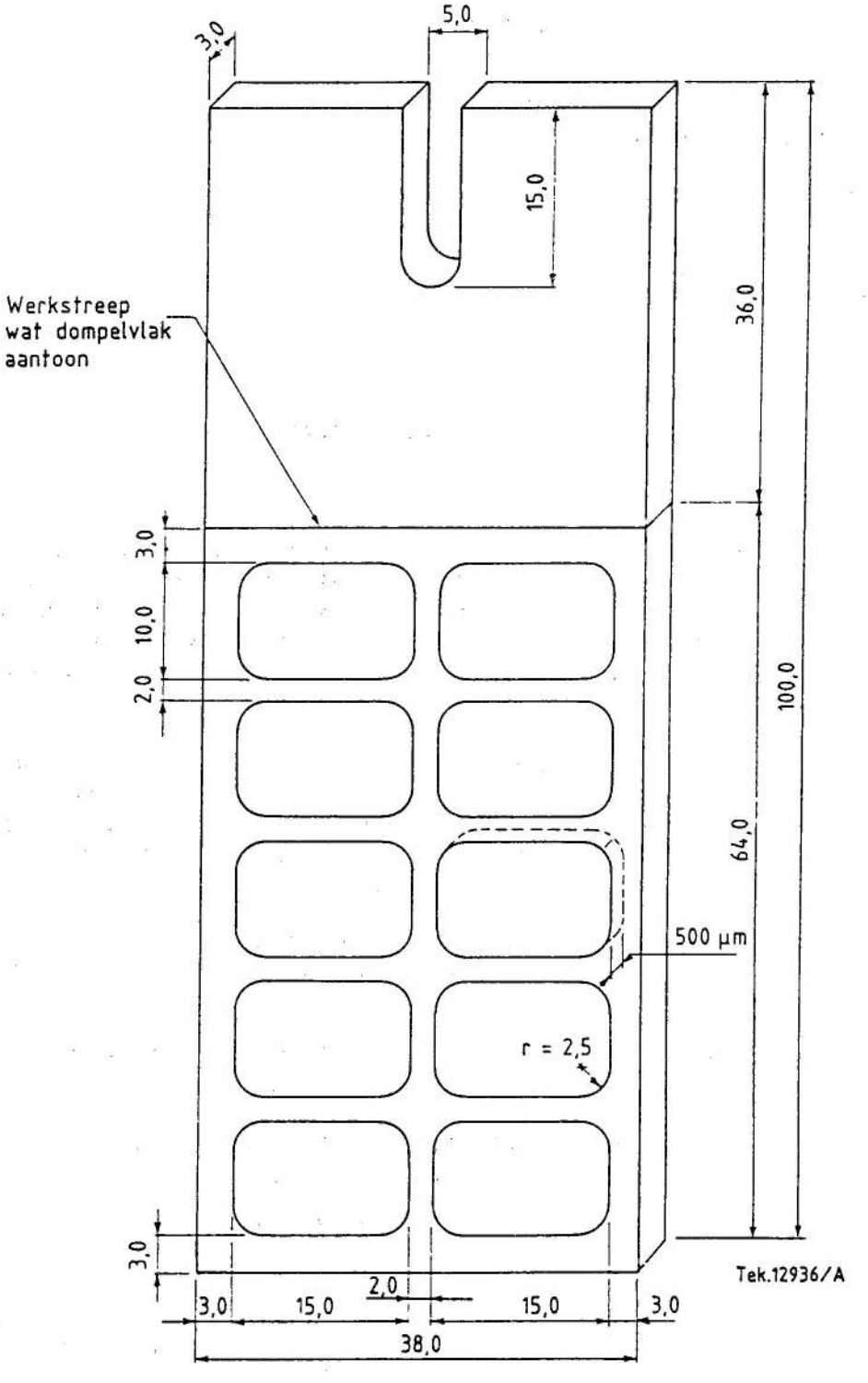
6.4.1.5 Ses toetsbekers, elk met 'n inhoudsvermoë van 800 mℓ, 'n binnediameter van ongeveer 90 mm en 'n hoogte van ongeveer 135 mm.

6.4.1.6 Ses platboom-kristalliseerbakkies van glas, elk met 'n inhoudsvermoë van 175 mℓ, 'n diameter van ongeveer 80 mm en 'n hoogte van ongeveer 40 mm, om die ses toetspanele te hou.

6.4.1.7 Ses hoëvormbekers, elk met 'n inhoudsvermoë van 150 mℓ.

6.4.1.8 Spatel, met 'n buigsame staallem met 'n reguit kant.

Afmetings in millimeter



Figuur 1 — Aluminiumtoetspaneel

6.4.1.9 Horlosieglase.

6.4.2 Materiaal

6.4.2.1 Vloeibare standaarddetergentontsmettingsmiddel.[3)]

6.4.2.2 Margarien.[3)]

6.4.2.3 Standaard- harde water.

6.4.2.4 Bestanddele

Kalsiumchloried ($CaCl_2.2H_2O$) ..... 0,880 g
Magnesiumsulfaat ($MgSO_4.7H_2O$) ..... 0,987 g
Water ..... 5 000 mℓ

6.4.2.5 Bereiding

Los die kalsiumchloried en die magnesiumsulfaat in die water op.

6.4.3 Prosedure

6.4.3.1 Weeg 15,0 g van die standaarddetergentontsmettingsmiddel noukeurig af in elk van drie bekers (kyk 6.4.1.5) en die toepaslike massa van die toetsmonster in elk van die ander drie bekers ten einde die vereiste verdunning (in die 750 mℓ harde water wat in 6.4.3.2 genoem word) te verkry, soos die fabrikant bepaal. In die geval van die detergentontsmettingsmiddels met jodofore as basis, toets 'n verdunning wat 75 mg/ℓ vry jodium bevat. Toets in alle ander gevalle die grootste verdunning (kleinste konsentrasie) wat op die etiket vir skoonmaak en ontsmetting aanbeveel word.

6.4.3.2 Voeg voldoende volumes harde water by die inhoud van elke beker om die volume in elk tot 750 mℓ aan te vul. Plaas die bekers só in die waterbad (kyk 6.4.1.4) wat by 30 °C gehou word, dat die drie bekers wat die standaarddetergentontsmettingsmiddel bevat in die eerste, die derde en die vyfde gat is en die drie bekers wat die toetsmonsters bevat in die oorblywende gate is. Bedek die bekers met horlosieglase (kyk 6.4.1.9).

6.4.3.3 Maak die toetspanele (kyk 6.4.1.1) deeglik skoon met 'n geskikte detergent (byvoorbeeld 1,0 % detergent wat aan SABS 892:1993, *Detergent vir algemene doeleindes (krale, korrels en poeier)* voldoen en in warm water opgelos is) en laat dit lugdroog word.

6.4.3.4 Plaas elke paneel in sy glaskristalliseerbakkie, identifiseer die paneel-en-bakkie-kombinasie duidelik en plaas die bakkies en panele 1 h lank in 'n oond by 105 °C ± 3 °C.

Haal die bakkies en panele uit die oond en bepaal die massa van elke bakkie saam met sy paneel nadat hulle in 'n desikkator afgekoel het.

6.4.3.5 Homogeniseer die margarien deur dit met die spatel te meng.

6.4.3.6 Gebruik die spatel om ongeveer 1,3 g van die margarien op elke paneel te pak en maak seker dat die massa van elke paneel hoogstens 0,2 g van dié van enigeen van die ander verskil en dat die blootgestelde oppervlakke van die margarien glad en gelyk is. As die panele ondersoek word, mag geen speldpuntgaatjies, lugblasies of ander insluitsels sigbaar wees nie.

6.4.3.7 Sodra die margarien op elke paneel gepak is, plaas die paneel in sy glaskristalliseerbakkie en bepaal die gesamentlike massa.

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3) Verkrygbaar by die Suid-Afrikaanse Buro vir Standaarde, Privaatsak X191, PRETORIA, 0001.

OPM – Maak seker dat die gedeelte van die paneel wat die margarien bevat gedurende die prosedure nie aangeraak word nie.

6.4.3.8 Bring elke paneel só in 'n klamp van die roerder (kyk 6.4.1.2) aan dat die kake van die klamp by die ent van die paneel, bo die vuil dele, geplaas is. Stel elke paneel só op dat dit vertikaal is.

6.4.3.9 Laat sak 'n paneelsamestel sorgvuldig in die middel van elk van die ses bekers met 750 mℓ detergentontsmettingsmiddeloplossing (kyk 6.4.3.1) wat by 30 °C gehou word totdat die vlak van die oplossing net gelyk met die merkstreep bokant die vuil deel is (kyk figuur 1).

6.4.3.10 Skakel die roerders aan en haal, nadat daar 180 min lank teen 60 r/min tot 62 r/min geroer is, elke paneel (om die beurt) uit die detergentoplossing en plaas dit tydelik in 'n vertikale posisie in 'n leë 150 mℓ hoëvormbeker (kyk 6.4.1.7).

6.4.3.11 Verwyder alle tekens van margarien van die plat oppervlakke van die panele met absorberende papier (byvoorbeeld toiletpapier) en sorg dat die margarien in die holtes van die panele nie aangeraak word nie. Verwyder alle tekens van margarien op die plat oppervlakke tussen die holtes van die panele deur die plat oppervlakke met 'n reguitkantspatel (kyk 6.4.1.8) te skraap.

6.4.3.12 Plaas elke paneel in sy glaskristalliseerbakkie, identifiseer die paneel-en-bakkie-kombinasie duidelik en plaas die bakkies en panele 3 h lank in 'n oond by 105 °C ± 3 °C. Bepaal die massa van elke glasbakkie saam met sy paneel nadat die glasbakkies en panele in 'n desikkator afgekoel het.

6.4.4 Berekening

6.4.4.1 Bereken die skoonmaakdoeltreffendheid C (as 'n persentasie volgens massa) van elke toetsdetergent en van elke deel standaarddetergent soos volg:

$$C = \frac{m_2 - m_3}{m_2 - m_1} \times 100$$

waar

m_1 die massa van die paneel en die glasbakkie is, in gram;

m_2 die aanvanklike massa van die paneel, die margarien en die glasbakkie is, in gram; en

m_3 die finale massa van die paneel, die margarien en die glasbakkie is, in gram.

6.4.4.2 Bereken die gemiddelde skoonmaakdoeltreffendheid van die toetsmonsters en van die monsters standaarddetergentontsmettingsmiddel, behoudens die volgende voorwaardes:

a) gebruik al drie resultate (bereken soos in 6.4.4.1) om 'n gemiddelde te bereken indien geen twee resultate met meer as drie persentasie-eenhede verskil nie; of

b) gebruik enige twee resultate (bereken soos in 6.4.4.1) wat met hoogstens drie persentasie-eenhede verskil om die gemiddelde te bereken; of

c) indien die afsonderlike resultate (bereken soos in 6.4.4.1) hierbo nie met (a) of (b) hierbo ooreenstem nie, verontagsaam al die resultate en herhaal die toets.

Gaan na vir voldoening aan 4.6.

6.5 Bewaarstabiliteit

6.5.1 Detergentontsmettingsmiddels met jodofore as basis

6.5.1.1 Reagense

6.5.1.1.1 0,02N natriumtiosulfaat($Na_2S_2O_3$) oplossing, noukeurig gestandaardiseer.

6.5.1.1.2 10 g/ℓ styseloplossing.

6.5.1.2 Prosedure

6.5.1.2.1 Plaas 200 mℓ van die monster oor na 'n 250 mℓ maatsilinder met 'n prop. Laat dit afkoel tot 10 °C ± 1 °C, hou dit 24 h lank by hierdie temperatuur en ondersoek dan die toetsmonster visueel vir tekens van skeiding.

6.5.1.2.2 Indien geen sigbare skeiding plaasgevind het nie, laat die toetsmonster na omgewingstemperatuur terugkeer en neem dan twee toetseksemplare daaruit, een van naby die oppervlak en een van naby die bodem van die inhoud van die silinder.

6.5.1.2.3 Bepaal die gehalte aan beskikbare jodium van elk van hierdie toetseksemplare soos volg: Pipetteer noukeurig 1 mℓ van die toetseksemplaar in 'n 250 mℓ Erlenmeyerfles (kyk 5.2.6), voeg 100 mℓ gedistilleerde of gedemineraliseerde water daarby en titreer dit met die 0,02N natriumtiosulfaatoplossing (kyk 6.5.1.1.1) totdat 'n strooikleurige titreermengsel verkry word. Voeg 'n klein hoeveelheid van die styseloplossing (kyk 6.5.1.1.2) by en neem die eindpunt as 'n styselindikatorkleurverandering van blou tot kleurloos.

OPM – Aangesien sommige jodofore jodium baie stadig vrylaat namate die eindpunt genader word, moet 'n redelike tyd toegelaat word voordat die titrasie as voltooi beskou word.

6.5.1.2.4 Herhaal die toets met nog 'n 200 mℓ toetsmonster, maar in die stadium wat in 6.5.1.2.1 hierbo beskryf word, moet die toetsmonster verhit en 24 h lank by 'n temperatuur van 40 °C ± 1 °C gehou word in plaas daarvan om dit te laat afkoel.

6.5.1.3 Berekenings

Bereken die gehalte aan beskikbare jodium *I* (as 'n persentasie) van elk van die vier toetseksemplare soos volg:

$$I = A \times N \times 12{,}69$$

waar

A die volume is van die $Na_2S_2O_3$-oplossing wat in die titrasie gebruik is, in milliliter; en

N die normaliteit van die $Na_2S_2O_3$-oplossing is.

6.5.1.4 Beoordeling van resultate

Ondersoek die resultate vir voldoening aan die toepaslike vereistes van 4.7.1.

6.5.2 Koolteertipe ontsmettingsvloeistowwe

6.5.2.1 Stabiliteit voor verdunning (normale toets)

6.5.2.1.1 Giet 100 mℓ van die saamgestelde toetsmonster in 'n 100 mℓ maatsilinder met 'n prop. Verkoel dit tot 5 °C ± 1 °C en hou dit 7 d lank by hierdie temperatuur en maak seker dat die vloeistof gedurende hierdie tydperk nie aan direkte sonlig blootgestel word nie en dat plaaslike oorverhitting nie kan plaasvind

nie. Plaas die silinder met die mins moontlike versteuring van die vloeistof in 'n sterk straal lig en ondersoek die monster noukeurig vir skeiding van die bestanddele en vir die aanwesigheid van 'n afsaksel. Indien 'n skuimvloeistof in die geval van 'n wit ontsmettingsvloeistof voorkom, keer die maatsilinder drie maal om en draai dit weer regop en ondersoek dan die vloeistof vir eenvormigheid.

6.5.2.1.2 Herhaal die prosedure in 6.5.2.1 met nog 100 mℓ van die ontsmettingsvloeistof, maar hou die silinder 7 d lank in 'n waterbad wat by 45 °C ± 1 °C gehou word.

6.5.2.1.3 Herhaal in die geval van 'n swart ontsmettingsvloeistof die prosedure in 6.5.2.1.1 met nog 100 mℓ van die ontsmettingsvloeistof, maar verkoel die vloeistof tot 0 °C ± 1 °C en hou dit 1 h lank by hierdie temperatuur.

6.5.2.1.4 Ondersoek vir voldoening aan 4.7.2.1.

6.5.2.2 Stabiliteit voor verdunning (vinnige toets) (slegs wit ontsmettingsvloeistowwe)

OPM – Hierdie toets kan nie as 'n plaasvervanger vir die normale toets in 6.5.2.1 beskou word nie.

Giet 10 mℓ van die vloeistof by 90 mℓ water by 20 °C tot 22 °C in 'n skoon kookbuis met 'n nominale diameter van 38 mm en 'n halfronde ent. Verseël die buis met 'n geskikte prop en keer dit ses maal met 'n kurktrekkerbeweging om (dws keer dit deur drie volledige sirkelbewegings om). Laat dit 6 h lank by 20 °C tot 22 °C weg van direkte lig af staan en meet dan die diepte van enige neerslag in die bodem van die buis.

6.5.2.3 Stabiliteit na verdunning

6.5.2.3.1 Bring die temperatuur van die toetsmonster en die harde water (kyk 5.4.18.4) tot 25 °C ± 1 °C en berei dan minstens 100 mℓ van die hoogste verdunning wat op die houer aanbeveel word deur die toepaslike volume van die monster by die toepaslike volume harde water in 'n maatsilinder met 'n prop te giet. Vorm 'n emulsie deur die toegepropte silinder (met 'n kurktrekkerbeweging) deur 30 volledige sirkelbewegings om te keer. Plaas oor na 'n 100 mℓ maatsilinder met 'n prop, laat dit 6 h lank onversteurd by 25 °C ± 1 °C staan en ondersoek dan die emulsie in 'n sterk ligstraal vir tekens van opbreek van die emulsie en vir die aanwesigheid van meer as net tekens van olie aan die bo- of onderkant.

6.5.2.3.2 Herhaal die prosedure in 6.5.2.3.1 maar berei die laagste verdunning wat op die houer aanbeveel word.

6.5.2.3.3 Ondersoek vir voldoening aan 4.7.2.2.

6.5.3 Ontsmettingsmiddels en detergentontsmettingsmiddels met glutaaraldehied as basis

6.5.3.1 Bewaar tipe 1-ontsmettingsmiddels of -detergentontsmettingsmiddels (kyk tabel 1) en die vloeibare komponent van tipe 2-ontsmettingsmiddels of -detergentontsmettingsmiddels (kyk tabel 1) 24 h lank in hul oorspronklike onoopgemaakte bottels by 5 °C ± 1 °C. Toets die inhoud van 50 % van die bottels in die monster vir voldoening aan 4.7.3.1.

6.5.3.2 Bewaar die vaste komponente van tipe 2-detergentontsmettingsmiddels 6 maande lank in hul oorspronklike onoopgemaakte bottels in omgewingstoestande. Toets die inhoud van 50 % van die bottels in die monster vir voldoening aan 4.2 en 4.7.3.2.

6.5.4 Ontsmettingsmiddels en detergentontsmettingsmiddels met chloorheksidienglukonaat as basis

6.5.4.1 Bewaar monsters van die ontsmettingsmiddel of detergentontsmettingsmiddel 48 h lank afsonderlik in hul oorspronklike onoopgemaakte bottels by 5 °C ± 1 °C en by 43 °C ± 1 °C. Toets die inhoud van die bottels vir voldoening aan 4.7.4.1.

6.5.4.2 Bewaar die ontsmettingsmiddels of detergentontsmettingsmiddels vir 'n tydperk van 12 maande vanaf die datum van vervaardiging by 'n temperatuur van 20 °C ± 5 °C en toets die inhoud van die bottels vir voldoening aan 4.2 en 4.7.4.2.

6.5.5 Ontsmettingsmiddels of detergentontsmettingsmiddels met kwaternêre ammoniumverbindings, gestabiliseerde anorganiese chloorverbindings en organiese of anorganiese halogeenverbindings (uitgesonderd jodiumverbindings) as basis

6.5.5.1 Vloeibare ontsmettingsmiddels of vloeibare detergentontsmettingsmiddels

Bewaar elke ontsmettingsmiddel of detergentontsmettingsmiddel 12 h lank in sy oorspronklike onoopgemaakte bottel by 5 °C ± 1 °C. Gaan na vir voldoening aan 4.7.5.1.

6.5.5.2 Vaste ontsmettingsmiddels of vaste detergentontsmettingsmiddels

Bewaar die ontsmettingsmiddel of detergentontsmettingsmiddel 6 maande lank in sy oorspronklike onoopgemaakte bottel in normale toestande by omgewingstemperatuur. Gaan na vir voldoening aan 4.7.5.2.

6.6 Bepaling van pH-waarde

6.6.1 Verdunning van ontsmettingsmiddels en detergentontsmettingsmiddels

6.6.1.1 Gestabiliseerde anorganiese chloorverbindings

Los 1 g van 'n vaste toetsmonster of 1 mℓ van 'n vloeibare toetsmonster in 100 mℓ koolstofdioksiedvry gedistilleerde water op.

6.6.1.2 Jodofore

Los 1 mℓ van die toetsmonster in 100 mℓ koolstofdioksiedvry gedistilleerde water op.

6.6.1.3 Fenole

Los 1 g van 'n vaste toetsmonster of 1 mℓ van 'n vloeibare toetsmonster in 100 mℓ koolstofdioksiedvry gedistilleerde water op.

6.6.1.4 Organiese halogeenverbindings (uitgesonderd jodiumverbindings)

Los 1 g van 'n vaste toetsmonster of 1 mℓ van 'n vloeibare toetsmonster in 100 mℓ koolstofdioksiedvry gedistilleerde water op.

6.6.1.5 Kwaternêre ammoniumverbindings

Berei 'n 1/100-verdunning van die toetsmonster in koolstofdioksiedvry gedistilleerde water.

6.6.1.6 Chloorheksidienglukonaat

Verdun die toetsmonster in koolstofdioksiedvry gedistilleerde water tot die voorgeskrewe konsentrasie (kyk 7.2(f)).

6.6.2 Prosedure

Gebruik 'n geskikte, aanneemlike pH-meter wat op toepaslike wyse gekalibreer is om die pH-waarde van die monster te bepaal.

6.6.3 Uitdrukking van resultate

Druk die resultate as "pH by 25 °C" uit.

6.6.4 Vertolking van resultate

Gaan na vir voldoening aan 4.8.

6.7 Vryheid van sigbare onsuiwerhede van vaste ontsmettingsmiddels of vaste detergentontsmettingsmiddels gebaseer op kwaternêre ammoniumverbindings, gestabiliseerde anorganiese chloorverbindings en organiese of anorganiese halogeenverbindings (uigesonderd jodiumverbindings)

Smeer ongeveer 50 g van elke toetsmonster oor die boom van 'n petribakkie met 'n diameter van 150 mm. Gaan na vir voldoening aan 4.9 deur vanaf ongeveer 600 mm na die monster te kyk.

6.8 Bygevoegde kleurstof vir ontsmettingsmiddels of detergentontsmettingsmiddels met jodofore as basis

6.8.1 Reagens

Natriumtiosulfaat, 50 g/ℓ wateroplossing.

6.8.2 Prosedure

Gebruik 'n pipet en plaas 5 mℓ van die toetsmonster oor na 'n skoon toetsbuis. Gebruik 'n afsonderlike pipet en voeg 5 mℓ van die natriumtiosulfaatoplossing by en meng goed.

6.8.3 Beoordeling van resultate

Indien die kleur van die mengsel in die buis nadat dit 30 s lank gemeng is nie donkerder as 'n dowwe strooikleur is nie, oordeel dat die detergentontsmettingsmiddel aan 4.10 voldoen.

7 Verpakking en merke

7.1 Verpakking

Daar moet voldoen word aan die verpakkingsmetodes wat geskik is vir die ontsmettingsmiddel en detergentontsmettingsmiddel soos beskryf in SABS 0229:1990, *Verpakking van gevaarlike goedere vir pad- en spoorvervoer in Suid-Afrika*, en die toepaslike regulasies ingevolge die Wet op Handelsmetrologie, 1973 (Wet 77 van 1973). Die bottels (met inbegrip van die proppe) waarin die ontsmettingsmiddel of detergentontsmettingsmiddel verpak is, mag nie chemies of fisies op die ontsmettingsmiddel of detergentontsmettingsmiddel inwerk nie en moet sterk genoeg wees om die ontsmettingsmiddel of detergentontsmettingsmiddel toereikend tydens normale hantering, berging en vervoer te beskerm. Die proppe mag nie van kurk of enige materiaal wat kurk bevat, wees nie.

7.2 Merke

Die volgende besonderhede moet in opvallende, leesbare en onuitwisbare merke aangebring wees op elke houer of op 'n etiket wat stewig aan elke houer bevestig is in 'n lettertipe van sodanige grootte en aanbieding as wat voorgeskryf word deur regulasies uitgevaardig kragtens die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, 1972 (Wet 54 van 1972), en die Wet op Handelsmetrologie, 1973 (Wet 77 van 1973):

a) die registrasienommer en volle naam en adres van die fabrikant, produsent, eienaar of beheermaatskappy of, in die geval van bottels wat vir enige ander persoon of organisasie verpak word, die volle naam en adres van dié persoon of organisasie;

b) woorde wat die tipe ontsmettingsmiddel of detergentontsmettingsmiddel en die aktiewe ontsmettingsbestanddeel (kyk tabel 1) daarvan aandui, en of dit geskik is om vir lokale skoonmaakwerk te gebruik;

c) die nominale volume of massa (soos toepaslik) van die inhoud, in gewone letters en in 'n kleur wat duidelik van die kleur van die houer of etiket verskil;

d) die produksielotidentifikasie; of

e) die produksiedatum van die produksielot (of albei);

f) algemene gebruiksaanwysings vir die verskillende doeleindes waarvoor die ontsmettingsmiddel of detergentontsmettingsmiddel geskik is, met inbegrip van die aanbevole verdunnings wat dit moontlik maak dat die produk vir die blootsteltydperk en toetsorganisme, vir elke doel wat in die toetsmetode gemeld word, aan die vereistes van 4.2 (ontsmettingsdoeltreffendheid) kan voldoen;

g) indien toepaslik, die pH-waarde by die aanbevole verdunning (kyk (f) hierbo);

h) die noodhulpbehandeling en die naam van enige teenmiddel wat gebruik kan word;

i) indien toepaslik, waarskuwings dat:

1) die ontsmettingsmiddel of detergentontsmettingsmiddel in toe bottels in 'n droë plek by 'n temperatuur van hoogstens 30 °C bewaar moet word, beskerm teen skerp lig en weg van vlambare materiaal, voedselprodukte en verpakkingsmateriaal;

2) aanraking van die onverdunde ontsmettingsmiddel of detergentontsmettingsmiddel en die dampe daarvan met die vel en oë vermy moet word;

3) rubberhandskoene en, indien nodig, skermbrille gedra moet word wanneer die ontsmettingsmiddel of detergentontsmettingsmiddel hanteer word;

4) tensy die fabrikant dit aanbeveel, die ontsmettingsmiddel of detergentontsmettingsmiddel, indien toepaslik, nie met ander niesaambruikbare stowwe (byvoorbeeld seep en ander anioniese detergente) gemeng moet word nie;

5) sommige ontsmettingsmiddels, soos jodofore, sekere poreuse materiaal (soos plastiek) kan vlek en sekere metale (soos koper, yster, silwerlegerings en aluminium) kan korrodeer; en

6) die doeltreffendheid van die ontsmettingsmiddel in die gedrang kan kom indien oppervlakke vuil is;

j) die bergtydperk of rakleeftyd van die verdunde ontsmettingsmiddel of detergentontsmettingsmiddel, indien dit tot die laagste aanbevole konsentrasie (verdunning met die hoogste gebruik) verdun is;

k) die vervaldatum van die ontsmettingsmiddel of detergentontsmettingsmiddel;

l) indien, in die geval van ontsmettingsmiddels met glutaaraldehied as basis, die ontsmettingsmiddel of detergentontsmettingsmiddel in die vorm van 'n homogene vloeistof en 'n aktiveerder voorsien word, 'n verklaring dat die vloeistof en die aktiveerder gemeng moet word voordat dit as 'n ontsmettingsmiddel gebruik word;

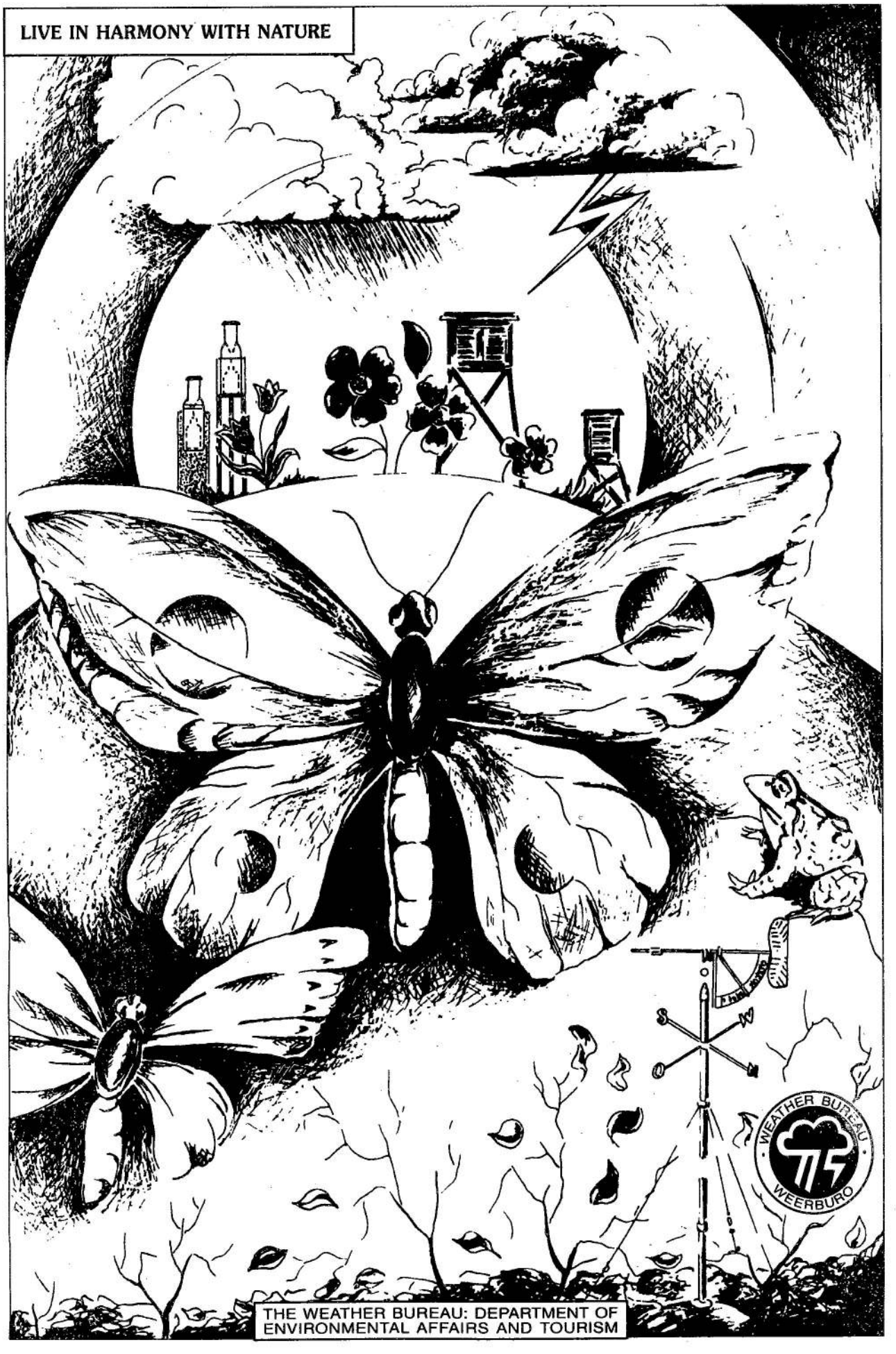
m) die sterkteaanwysing en, in die geval van koolteertipe ontsmettingsmiddels, die nominale Rideal-Walker-koëffisiënt (kyk 5.9.5);

n) in die geval van bottels vir swart koolteertipe ontsmettingsmiddels, die volgende verklaring: "Swart koolteertipe ontsmettingsvloeistof word nie vir gebruik met besonder harde water aanbeveel nie";

o) stawing van spesifieke virusdodende aansprake;

p) die naam van die produk (wat nie vir die verbruiker misleidend mag wees nie); en

q) indien toepaslik, 'n verklaring dat die ontsmettingsmiddel of detergentontsmettingsmiddel voor gebruik geskud moet word.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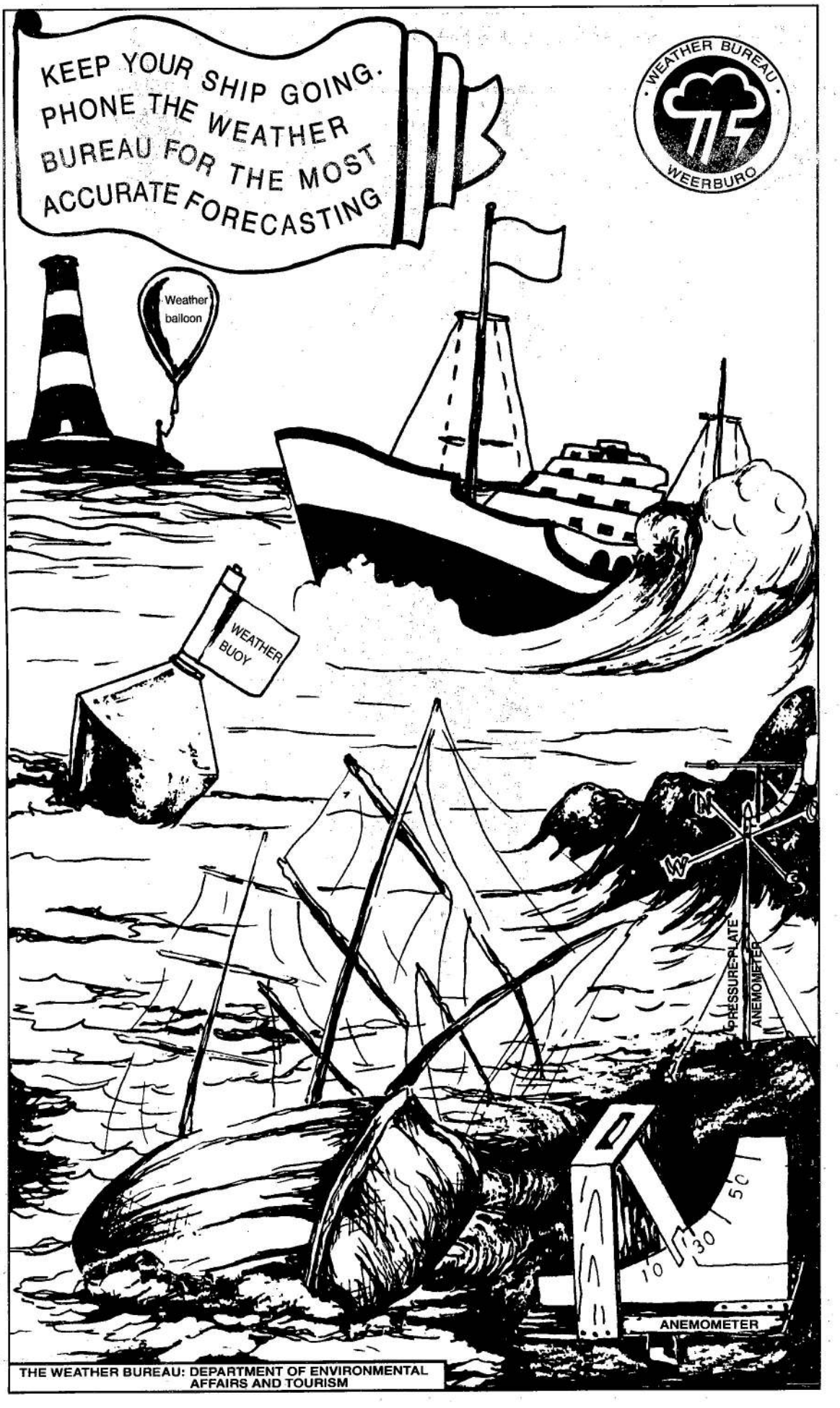
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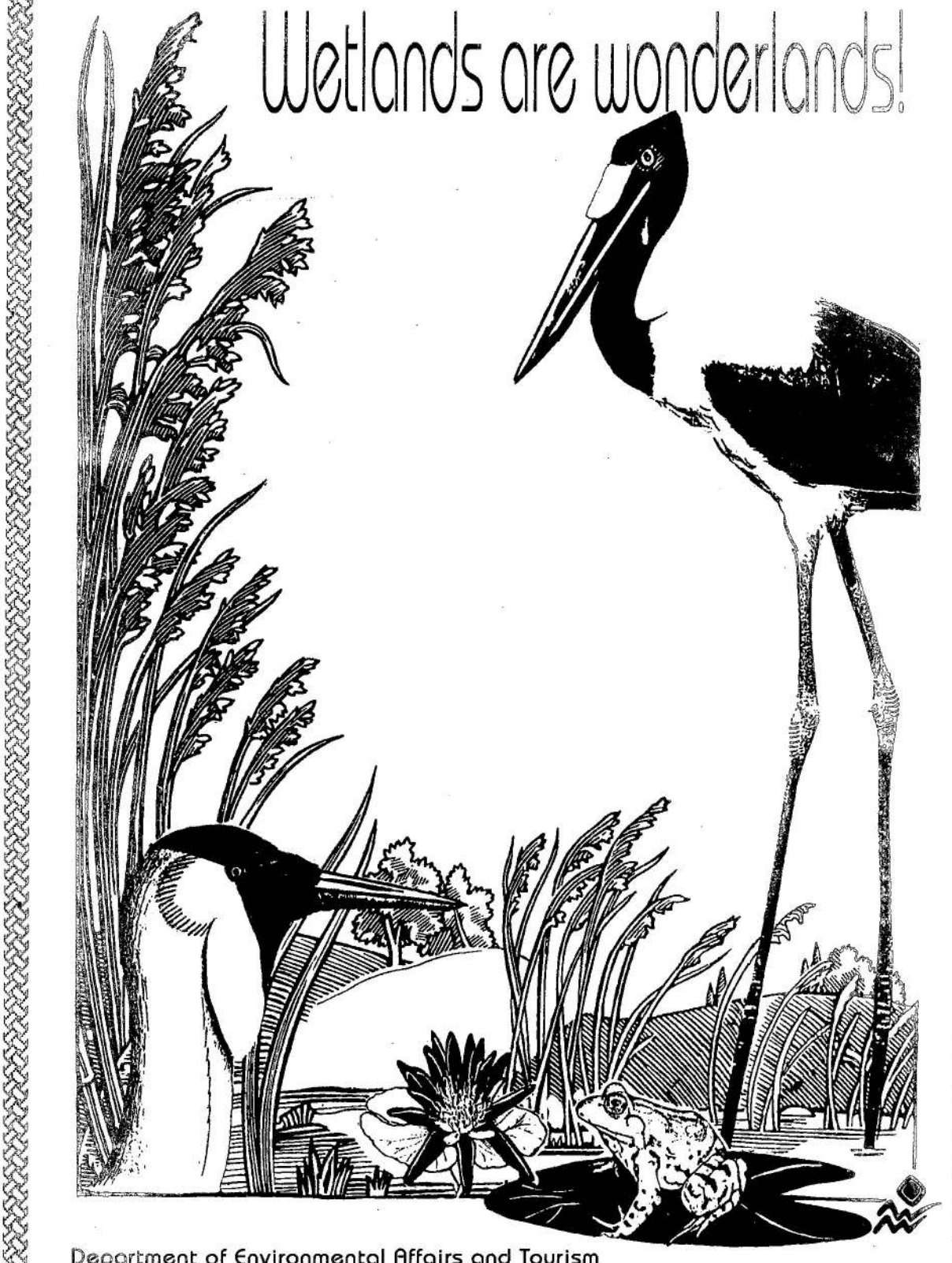
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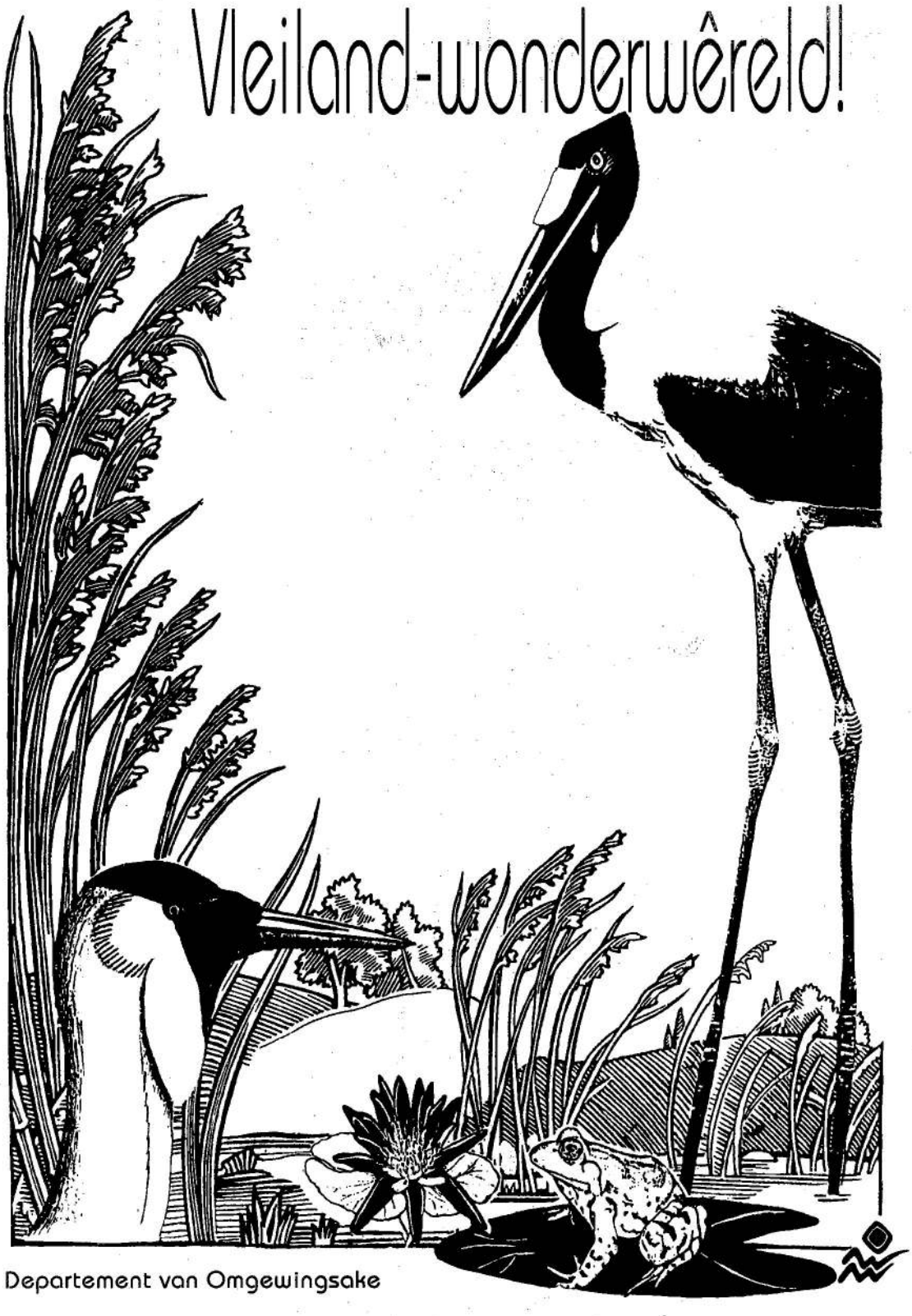
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