TECHNICAL SPECIFICATION

ISO/TS 15883-5

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Washer-disinfectors —

Part 5: Test soils and methods for demonstrating cleaning efficacy

Laveurs désinfecteurs —

Partie 5: Terrains d'essai et méthodes pour démontrer l'efficacité de nettoyage

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 15883-5 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

ISO 15883 consists of the following parts, under the general title *Washer-disinfectors*:

— *Part 1: General requirements, terms and definitions and tests*

— *Part 2: Requirements and tests for washer-disinfectors employing thermal disinfection for surgical instruments, anaesthetic equipment, bowls, dishes, receivers, utensils, glassware, etc.*

— *Part 3: Requirements and tests for washer-disinfectors employing thermal disinfection for human waste containers*

— *Part 4: Requirements and tests for washer-disinfectors employing chemical disinfection for thermolabile endoscopes*

— *Part 5: Test soils and methods for demonstrating cleaning efficacy* [Technical Specification]

Introduction

Verification of cleaning efficacy is a key aspect of establishing satisfactory performance of a washer-disinfector. The current state of knowledge has not permitted development of a single internationally acceptable test method. As an interim measure, the Technical Committees responsible for the ISO 15883 series of standards on washer-disinfectors (ISO/TC 198 and CEN/TC 102) have decided that the cleaning efficacy of washer-disinfectors claiming compliance with the ISO 15883 series of standards be demonstrated by referring to the test soils and methods that are currently used in a number of different countries. For the convenience of the user of the ISO 15883 series of standards, these test soils and methods are described in this Technical Specification. It should be noted that it remains the intention of the Technical Committees to develop a single test method.

Washer-disinfectors —

Part 5:
Test soils and methods for demonstrating cleaning efficacy

1 Scope

This Technical Specification includes the test soils and methods that can be used to demonstrate the cleaning efficacy of washer-disinfectors (WD) according to the ISO 15883 series of standards.

The inclusion of the test soils and methods in this Technical Specification does not indicate that they are of equivalent sensitivity in their determination of cleaning efficacy.

Acceptance criteria are included, based on visual inspection and/or a microbiological end-point as stated for each method. Where chemical detection of residual soiling is required/sought, methods can be complemented by the specific determination of a residual component of the applied test soil.

NOTE 1 The test soils and methods included in this Technical Specification are sourced from national standards and published documents submitted by member bodies of the Technical Committee preparing this Technical Specification. They have been edited only to provide a uniform format within this Technical Specification.

NOTE 2 An example of this is the use of the peroxidase test (see Annex J) to detect residual blood (haemoglobin) from the test soil applied to surgical instruments or flexible endoscopes (e.g. using the method described in Annex G). See also ISO 15883-1:2005, Annex D.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3166-1, *Codes for the representation of names of countries and their subdivisions — Part 1: Country codes*

ISO 15883-1:2005, *Washer-disinfectors — Part 1: General requirements, terms and definitions and tests*

3 Applicability

3.1 Where any of the test methods specified below deviate from the test method for cleaning efficacy specified in ISO 15883-1, the method given in ISO 15883-1 shall be used (see ISO 15883-1:2005, 6.10). Cleaning efficacy, for example, shall be determined after exposure to only the cleaning part of the operating cycle.

3.2 Table 1 includes a summary of the test soils which are included in this Technical Specification. The test soils are listed for the specific type of WD loads for which they were specified; the same test soils may be used also for other types of loads: for example, soils specified for surgical instrument may be used for other metal components.

Table 1 — Summary of test soils including their allocation to the type of load

Load type	Country code[a]	Reference in Bibliography	Constituents of soil	Annex in this Technical Specification
Surgical instruments (including rigid endoscopes)	AT	[34]	Heparinzed sheep blood coagulated with protamine	Annex A
	DE	[32], [33]	Sheep blood, *E. faecium*[b] Egg yolk, *E. faecium*[b] Semolina, butter, sugar, milk powder, *E. faecium*[b]	Annex G
	DE	[41], [42], [43]	Tetramethylbenzidine, hydrogen peroxide solution, bovine haemoglobin	Annex J
	NL	[39]	Bovine serum albumin fraction 5, porcine gastric mucin type 3, bovine fibrinogen fraction 1, bovine thrombin	Annex K
	SE	[24]	Citrated cattle blood coagulated with calcium chloride	Annex M
	UK	[28], [30]	Defibrinated horse/sheep blood, egg yolk, dehydrated hog mucin	Annex N
	US	[31] [47]	Protein/organic soil (user preference), *B. atrophaeus* endospores Albumin, haemoglobin, fibrinogen, thrombin	Annex S
Bowls, dishes, receivers	SE	[24]	Citrated cattle blood coagulated with calcium chloride	Annex M
	UK	[28], [30]	Defibrinated horse/sheep blood, egg yolk, dehydrated hog mucin	Annex N
Anaesthesia equipment / accessories	AT	[36]	Nigrosin, wheat flour, hens egg	Annex B
	DE	[32], [33]	Sheep blood, *E. faecium*[b]	Annex G
	SE	[24]	Citrated cattle blood coagulated with calcium chloride	Annex M
	UK	[28], [30]	Glycerol, dehydrated hog mucin, horse serum, unbleached plain flour, aqueous safranine solution, water	Annex O
Infant feeding bottles	DE	[32], [33]	Sheep blood, *E. faecium*[b] egg yolk, *E. faecium*[b] semolina, butter, sugar, milk powder, *E. faecium*[b]	Annex G
Baby bottles and suction bottles	SE	[24]	Citrated cattle blood coagulated with calcium chloride	Annex M

Table 1 (*continued*)

Load type	Country code[a]	Reference in Bibliography	Constituents of soil	Annex in this Technical Specification
Bedpans	AT	[36]	Nigrosin, wheat flour, hens egg, instant potato flakes,	Annex C
	DE	[22], [23], [38]	Bovine albumin, mucin, maize starch *E. faecium*[b]	Annex H
	SE	[24]	Citrated cattle blood coagulated with calcium chloride	Annex M
	UK	[27], [30]	Unbleached plain flour, water soluble adhesive wallpaper paste, hens egg, black Indian ink, water	Annex P
Urine bottles	AT	[36]	Nigrosin, wheat flour, hens egg,	Annex D
	SE	[24]	Citrated cattle blood coagulated with calcium chloride	Annex M
	UK	[27], [30]	Defibrinated horse/sheep blood, water soluble adhesive wallpaper paste, hens egg, black Indian ink, water	Annex Q
Flexible endoscopes	AT	[34], [44]	Nigrosin, wheat flour, hens egg, *E. faecium*[b]	Annex E
	DE	[34], [35]	Blood, *E. faecium*[b]	Annex I
	DE	[41], [42], [43]	Tetramethylbenzidine, hydrogen peroxide solution, bovine haemoglobin	Annex J
	FR	[37]	Biofilm formed by *Pseudomonas aeruginosa*	Annex F
	NL	[40]	Bovine serum albumin, procine mucin, bovine thrombin, bovine fibrinogen	Annex L
	UK	[30]	Glycerol, dehydrated hog mucin, horse serum, unbleached plain flour, aqueous safranine solution, water	Annex R
	US	[31] [47]	Protein/organic soil (user preference), *B. atrophaeus* endospores Bacteria, protein, carbohydrate, endotoxin, haemoglobin	Annex S
Stainless steel items (including bedpans, urine bottles)	NL	[39]	Bovine albumin fraction 5, porcine gastric mucin type 3, bovine fibrinogen fraction 1, bovine thrombin	Annex K
Wash bowls	SE	[24]	Calcium stearate generated *in situ* from soap and calcium chloride solution	Annex M
Reusable medical instruments including flexible endoscopes	US	[31]	Protein/organic soil (user preference), *B. atrophaeus* endospores	Annex S

[a] Country code as specified in ISO 3166-1.

[b] The test soils and methods may also be used for microbial testing of disinfection efficacy of WDs according to the ISO 15883 series when requested by the user.

Annex A
(normative)

Test soil and method for surgical instruments (Austria)

A.1 Reference

The test methods using a heparinized blood test soil for testing and evaluating the cleaning efficacy of automated WDs for surgical instruments as an optional type test and operational test are based on Reference [34] and were adapted or complemented for the presentation in this Technical Specification.

A.2 Materials

— **Blood from a laboratory sheep**.

— **Heparin**[1)].

— **Protamine sulphate** or **hydrochloride**[1)].

Optional:

— **Cleaning-indicators for ordinary surgical instruments**[1)].

— **Cleaning-indicators for instruments for minimally invasive surgery**[1)].

A.3 Apparatus

— **Normal laboratory equipment**.

— **Paintbrush**, 25 mm in width and 4 mm of thickness.

— **Syringes,** of 20 ml capacity.

A.4 Preparation of test soil

A.4.1 Heparinized sheep blood

Add 0,1 ml heparin per 100 ml of sheep blood immediately after the blood is drawn (heparinized sheep blood).

A.4.2 Completion of the test soil

Directly before use bring the blood to room temperature.

Pour the heparinized blood into a clean and dry bowl, add 0,15 ml of protamine sulphate to each 10 ml of blood and mix well. The blood should coagulate within approximately 10 min to 20 min.

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1) Guidance on suitable commercially available products may be obtained from Austrian Standards Institute, Heinestr. 38, 1020 Vienna, Austria.

A.5 Storage

Store the blood and the protamine sulphate (or hydrochloride) in a refrigerator at 4 °C to 8 °C and according to the manufacturer's instructions respectively.

A.6 Test pieces

A.6.1 Ordinary surgical instruments

Surgical instruments with joints (scissors with joints and clamps with box locks at a ratio of 1:1) in sufficient numbers to provide a full load of the WD under test when using 20 test pieces per tray.

A.6.2 Instruments for minimally invasive surgery

As a surrogate for rigid endoscopes, dummies made of stainless steel tubing should be used with a wall thickness of approximately 1 mm and either:

— a length of 150 mm, inner diameter of 8 mm; or

— a length of 300 mm, inner diameter of 4 mm and 6 mm.

A.7 Inoculation of test pieces

A.7.1 Ordinary surgical instruments

Allow the blood to equilibrate to room temperature before use. Clean and dry the test instruments thoroughly. Apply the test soil to joints and corrugate surfaces of the instruments at ambient temperature using a paintbrush. Take care that the blood is used within approximately 10 min (in any case before complete coagulation). The total amount of the test soil should be about 0,05 % of the amount of water for the cleaning phase in the tank of the WD (e.g. 20 l water; 10 ml blood).

Place 20 pieces of the soiled instruments horizontally and at random on each of the trays.

All instruments shall be prepared and arranged on the tray within 30 min.

Leave the instruments on the tray to dry at ambient temperature and humidity for approximately 30 min. Then take each of the instruments and check them for excessive test soil (e.g. coagulated test soil spots $\geqslant$ 5 mm in diameter on the surface of the instruments) which shall be removed by means of an absorbent pad. Then place the instruments upside down on another tray and leave them to dry for at least 30 min but not more than 60 min.

A.7.2 Instruments for minimally invasive surgery

Allow the blood to equilibrate to room temperature before use. Fill the lumens with the test soil in a way that the inner surfaces are completely wetted. Take care that the blood is used within approximately 10 min (in any case before complete coagulation). Make sure that the lumens are open after this procedure (e.g. by blowing through the lumens with compressed air). Then, apply a thin layer of blood to the outer surfaces of the dummies using a paintbrush.

Connect the soiled dummies to the appropriate nozzles and luer-locks (at least three per connection type) and place them on or in the load carrier according to the manufacturer's instructions.

All instruments shall be prepared and arranged on the load carrier within 30 min.

Leave the instruments on the load carrier to dry for at least 60 min but not more than 90 min.

A.8 Test method

A.8.1 Ordinary surgical instruments

Load the WD with the test instruments on their tray and start the WD with a full load. Run the cleaning cycle of the “surgical instrument” programme in accordance with the manufacturer’s instructions.

Immediately after the cleaning cycle, interrupt the programme and unload the WD.

For each type of load, at least three cycles shall be run in the WD.

If there are not enough test instruments available to provide a full load, run as many cycles as necessary to check every position possible in the WD and fill the blank positions with clean items on their trays according to the manufacturer’s instructions.

In addition, suitable industrially produced cleaning-indicators may be used which should be placed on the trays and evaluated after completion of the cleaning cycle in accordance with the manufacturer’s instructions.

A.8.2 Instruments for minimally invasive surgery

Load the WD with test instruments and start the WD with a full load. Run the cleaning cycle of the adequate programme in accordance with the manufacturer’s instructions.

Immediately after the cleaning cycle, interrupt the programme and unload the WD.

For each type of load, at least three cycles, shall be run in the WD.

Blank nozzles shall be connected to clean items according to the manufacturer’s instructions.

In addition, suitable industrially produced cleaning-indicators may be used. At least one of them should be connected to each type of connection nozzle and evaluated after completion of the cleaning cycle in accordance with the manufacturer’s instructions.

A.9 Results

A.9.1 Ordinary surgical instruments

A.9.1.1 Detection of residual soil

After cleaning in the WD, examine the instruments visually. Examine every single instrument by opening and closing box locks and joints. Record the number of clean (no remains of blood visible to the naked eye at normal light with any optical corrections required for normal visual acuity) and not clean instruments. Calculate the ratio of the test pieces with residual soil to the originally soiled instruments. Express the result in percent.

Items other than the inoculated test pieces shall not be considered.

In cases of doubt, protein detection tests (e.g. biuret reaction) should be carried out to confirm whether the visible residue is due to the test soil.

If applicable, examine the cleaning-indicators and check the results for compliance with the manufacturer's instructions.

A.9.1.2 Acceptance criteria

The cleaning efficacy of the WD shall be regarded as satisfactory if

— at least 95 % of all test pieces show no visible residue of the test soil,

— the amount of protein on the instruments is below the detection level or within the limits of the acceptance criteria given by the manufacturer of the test as applicable (see also ISO 15883-1:2005, Annex C),

— the results of the cleaning-indicators are within limits of the acceptance criteria of the manufacturer, if applicable.

A.9.2 Instruments for minimally invasive surgery

A.9.2.1 Detection of residual soil

Examine the outer surfaces of the instruments for minimally invasive surgery visually (see above). Record the number of clean (no remains of blood visible to the naked eye at normal light with any optical corrections required for normal visual acuity) and not clean instruments.

In addition, examine the inner surfaces by swabbing the tubes and examining the swabs for visible contamination. If no contamination is visible, check the swab for protein with protein detection tests (e.g. biuret reaction). Evaluate the tests in accordance with the manufacturer's instructions.

Items other than the inoculated test pieces shall not be considered.

A.9.2.2 Acceptance criteria

The cleaning efficacy of the WD should be regarded as satisfactory if

— none of the test pieces show visible residue of the test soil on the outer surfaces,

— the amount of protein in the lumen instruments is below the detection level or within the limits of the acceptance criteria given by the manufacturer of the test as applicable (see also ISO 15883-1:2005, Annex C), and

— the results of the cleaning-indicators are within limits of the acceptance criteria of the manufacturer, if applicable.

A.10 Safety considerations

A.10.1 Personal protective equipment

When preparing the test soil, inoculating the test pieces, loading the inoculated test pieces into the WD or examining the processed devices for residual protein, the operator should wear a protective gown (or apron) and gloves.

A.10.2 Disposal

All chemicals and test soils can be disposed of as non-hazardous, non-clinical waste.

A.10.3 Environmental spillage

Environmental surfaces that have been contaminated with test soil shall be wiped clean using a cloth moistened with a solution of an appropriate detergent/disinfectant in accordance with local policies and procedures.

Annex B
(normative)

Test soil and method for anaesthesia equipment (Austria)

B.1 Reference

The MNE[2)] test soil for testing and evaluating the cleaning efficacy of automated WDs for anaesthesia equipment is described in Reference [36].

B.2 Materials

— **Nigrosine** (1 % aqueous suspension).

— **Wheat flour**.

— **Hens' eggs**.

B.3 Apparatus

— **Normal laboratory apparatus.**

— **Paintbrush**, 25 mm in width.

— **Syringes** (20 ml or more).

B.4 Preparation of test soil

B.4.1 Nigrosine suspension

Add 6 g nigrosine powder to 600 ml lukewarm tap water, heat the mixture to approximately 80 °C and dissolve while stirring continuously.

B.4.2 Wheat flour suspension

Add 115 g of wheat flour to 800 ml cold tap water, heat while stirring continuously; bring to the boil and boil for 3 min.

B.4.3 MN mixture

Mix 600 ml nigrosine suspension (B.4.1) with 800 ml of wheat flour suspension (B.4.2).

This mixture may be prepared in larger amounts.

2) MNE from German: Mehl, Nigrosin, Ei.

B.4.4 Completion of the test soil

Immediately before use, warm up 700 g of the MN mixture (B.4.3) to approximately 35 °C. Add the white and yolk of three middle sized raw eggs and mix thoroughly (MNE mixture). Allow the test soil to equilibrate to room temperature before use. Adjust to room temperature again, if necessary.

B.5 Storage

The test soil base (B.4.3) can be stored in a refrigerator and may be kept for up to 3 days.

B.6 Test pieces

— **Anaesthesia equipment**, of the type to be used in routine practice in sufficient numbers to provide a full load of the WD under test (preferably transparent/translucent tubing).

B.7 Inoculation of test pieces

If the soil has been stored, allow it to equilibrate to room temperature before use. Clean and dry the test pieces thoroughly. Apply the soil to the inner surface of the larger test pieces (breathing tubes, etc.) by pouring the soil into the items or using a syringe; place the test pieces on a horizontal surface and roll them to distribute the soil over the inner surface. Hold the test pieces vertically to allow excess soil to drain off the surface. Then, apply an even layer of the test soil to the outer surface using the paintbrush. Smaller test pieces, such as endotracheal tubes and connectors, should be treated similarly.

The complete anaesthesia equipment shall be prepared and arranged on the load carrier within 30 min.

Leave the soiled equipment on the load carrier to dry at ambient temperature and humidity for at least 60 min but not more than 90 min.

B.8 Test method

Load the WD with the test pieces and start the WD with a full load. Run the cleaning cycle of the relevant programme in accordance with the manufacturer's instructions.

Immediately after the cleaning cycle interrupt the programme and unload the WD.

For each type of load at least two cycles shall be run in the WD.

B.9 Results

B.9.1 Detection of residual soil

Examine the outer and inner surfaces of the test pieces visually. Report the number of clean (no remains of test soil visible to the naked eye at normal light with any optical corrections required for normal visual acuity) and not clean test pieces.

In addition, examine the inner surfaces as far as possible by swabbing the tubes and examining the swabs for visible contamination. If no contamination is visible, check the swab for protein with protein detection tests (e.g. biuret reaction). Evaluate the tests in accordance with the manufacturer's instructions.

Items other than the inoculated test pieces shall not be considered.

B.9.2 Acceptance criteria

The cleaning efficacy of the WD should be regarded as satisfactory if

— none of the test pieces show visible residue of the test soil on the outer and inner surfaces,

— the amount of protein in the lumen instruments is below the detection level or within the limits of the acceptance criteria given by the manufacturer of the test as applicable (see also ISO 15883-1:2005, Annex C).

B.10 Safety considerations

B.10.1 Personal protective equipment

When preparing the test soil, inoculating the test pieces, loading the inoculated test pieces into the WD and examining the processed devices for residual protein, the operator should wear a protective gown (or apron) and gloves.

B.10.2 Disposal

All chemicals, and test soils can be disposed of as non-hazardous, non-clinical waste.

B.10.3 Environmental spillage

Environmental surfaces that have been contaminated with test soil shall be wiped clean using a cloth moistened with a solution of an appropriate detergent/disinfectant in accordance with local policies and procedures.

Annex C
(normative)

Test soil and method for bedpans (Austria)

C.1 Reference

The KMNE[3)] test soil for the evaluation of bedpan cleaning by automated WDs is described in Reference [36].

The method described may also be used for chamber walls, load carriers and containers for instruments. (See, however, 2.1.)

C.2 Materials

— **Nigrosine** (1 % aqueous suspension).

— **Wheat flour** (plain).

— **Hens' eggs**.

— **Instant potato flakes**.

C.3 Apparatus

— **Normal laboratory apparatus**.

— **Paintbrush**, 40 mm in width.

— **Egg beater**, with six to seven coils made of 1 mm diameter steel wire forming a head of about 70 mm in diameter.

C.4 Preparation of test soil

C.4.1 Nigrosine suspension

Add 6 g nigrosine powder to 600 ml lukewarm tap water, heat to approximately 80 °C and dissolve while stirring continuously.

C.4.2 Wheat flour suspension

Add 115 g wheat flour (grain) to 800 ml cold tap water, heat while stirring continuously; bring to a boil and continue boiling for 3 min.

3) KMNE from German: Kartoffelflocken, Mehl, Nigrosin, Ei.

C.4.3 MN mixture

Mix 600 ml nigrosine suspension (C.4.1) with 800 ml of wheat flour suspension (C.4.2).

This mixture may be prepared in larger amounts.

C.4.4 Completion of the test soil

Immediately before use, warm up 700 g of the MN mixture (C.4.3) to approximately 35 °C. Add the white and yolk of three middle sized raw eggs and mix thoroughly. Allow the test soil to equilibrate to room temperature before use. Adjust to room temperature again, if necessary.

Add approximately 100 g of instant potato flakes in small amounts while stirring continuously until the required consistency is reached (KMNE test soil).

To assess consistency, dip the egg beater approximately 70 mm deep into the mixture, rotate it slowly and lift it carefully out of the mixture. When the mixture is of the right consistency, it will flow slowly downwards between the coils and, after 5 s to 10 s, the lump of test soil remaining in the head of the beater should measure 40 mm to 50 mm in diameter.

C.5 Storage

The test soil base (C.4.3) can be stored in a refrigerator and may be kept for up to 3 days.

The KMNE test soil should be used as soon as it is prepared.

C.6 Test pieces

— **Bedpans**, of the type to be used in routine practice and in sufficient numbers to provide a full load of the WD under test.

C.7 Inoculation of test pieces

Clean and dry the test piece thoroughly and warm it up to approximately 35 °C. Load the test piece with 200 g to 300 g of test soil and distribute it evenly to give a layer of approximately 20 mm thickness. This is to simulate the main stool portion in the appropriate position.

Using the paintbrush apply the test soil to all the inner surfaces of the test piece to result in a layer of approximately 2 mm thickness.

In addition, to simulate a heavily soiled bedpan, apply the test soil to the outer surface that would be in contact with the patient's skin to result in a layer of approximately 2 mm thickness and apply the test soil to the remaining outer surfaces, including the handles, to result in a layer of approximately 1 mm thickness.

Prepare sufficient test pieces to provide a full load in the WD being tested. Allow the soil to rest at ambient temperature and humidity for not less than 5 min and not more than 10 min.

C.8 Test method

Place the soiled test pieces in the WD and run the WD with a full load using the "bedpan" programme in accordance with the manufacturer's instructions.

C.9 Results

C.9.1 Detection of residual soil

After cleaning in the WD, examine the bedpans visually.

C.9.2 Acceptance criteria

For the cleaning process to be regarded as satisfactory, there shall be no visible residue of the test soil.

C.10 Safety considerations

C.10.1 Personal protective equipment

When preparing the test soil, inoculating the test pieces, or loading the inoculated test pieces into the WD, the operator should wear a protective gown (or apron) and gloves.

C.10.2 Disposal

All chemicals and test soils can be disposed of as non-hazardous, non-clinical waste.

C.10.3 Environmental spillage

Environmental surfaces that have been contaminated with test soil shall be wiped clean using a cloth moistened with a solution of an appropriate detergent/disinfectant in accordance with local policies and procedures.

Annex D
(normative)

Test soil and method for urine bottles (Austria)

D.1 Reference

The MNE[4)] test soil for the evaluation of cleaning of urine bottles by automated WDs is described in Reference [36].

D.2 Materials

— **Nigrosine** (1 % aqueous suspension).

— **Wheat flour** (plain).

— **Hens' eggs**.

D.3 Apparatus

— **Normal laboratory apparatus**.

D.4 Preparation of test soil

D.4.1 Nigrosine suspension

Add 6 g nigrosine powder to 600 ml lukewarm tap water, heat the mixture to approximately 80 °C and dissolve while stirring continuously.

D.4.2 Wheat flour suspension

Add 115 g wheat flour to 800 ml cold tap water heat while stirring continuously; bring to a boil and continue boiling for 3 min.

D.4.3 MN mixture

Mix 600 ml nigrosine suspension (D.4.1) with 800 ml of wheat flour suspension (D.4.2).

This mixture may be prepared in larger amounts.

D.5 Storage

The test soil base (D.4.3) can be stored in a refrigerator and may be kept for up to 3 days.

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4) MNE from German: Mehl, Nigrosin, Ei.

D.6 Completion of the test soil

Immediately before use, warm up 700 g of the MN mixture (D.4.3) to approximately 35 °C. Add the white and yolk of three middle sized raw eggs and mix thoroughly. Adjust the temperature to approximately 35 °C if necessary (MNE test soil).

D.7 Test pieces

— **Urine bottles**, of the type to be used in routine practice in sufficient numbers to provide a full load of the WD under test.

D.8 Inoculation of test pieces

If the soil has been stored, allow it to equilibrate to room temperature before use. Pour 15 ml to 20 ml of the test soil into each urine bottle at ambient conditions. Shake and rotate the bottles to ensure that the test soil is distributed evenly on all the inner surfaces of the bottles. This should include the neck region of the bottle where, during use, the surface is likely to be in contact with the skin.

Allow the soil to rest at ambient temperature and humidity for not less than 5 min and not more than 10 min.

D.9 Test method

Place the soiled urine bottles in the WD and run the WD with a full load using the “urine bottle” programme in accordance with the manufacturer’s instructions.

D.10 Results

D.10.1 Detection of residual soil

After cleaning in the WD, examine the urine bottles visually.

D.10.2 Acceptance criteria

For the cleaning process to be regarded as satisfactory there shall be no visible residue of the test soil.

D.11 Safety considerations

D.11.1 Personal protective equipment

When preparing the test soil, inoculating the test pieces, or loading the inoculated test pieces into the WD, the operator should wear a protective gown (or apron) and gloves.

D.11.2 Disposal

All chemicals, and test soils can be disposed of as non-hazardous, non-clinical waste.

D.11.3 Environmental spillage

Environmental surfaces that have been contaminated with test soil shall be wiped clean using a cloth moistened with a solution of an appropriate detergent/disinfectant in accordance with local policies and procedures.

Annex E
(normative)

Test soil and method for flexible endoscopes (Austria)

E.1 References

The MNE[5)] test soil and method for testing and evaluating the cleaning efficacy of automated WDs for flexible endoscopes are based on References [34] and [44] and were adapted or complemented for this presentation.

E.2 Materials

— **Nigrosine** (1 % aqueous suspension).

— **Wheat flour** (plain).

— **Hens eggs**.

— **Casein peptone – soya meal peptone agar** (CSA).

— **Casein peptone – soya meal peptone agar** (CSB).

— **Selective agar** (e.g. kanamycin esculin azide agar).

— **Sterile 0,9 % physiological saline solution**.

— **Suitable test organism** (e.g. *E. Faecium* ATCC 6057, DSM 2146).

E.3 Apparatus

— **Normal laboratory apparatus**.

— **Laboratory blender**.

— **Paintbrush**.

— **Sterile syringes** (10 ml and 20 ml).

— **Sterilized tube adapters**, for soiling the endoscope channels.

— **Connectors**, to link the test pieces to the WD ports.

E.4 Preparation of test soil

E.4.1 Nigrosine suspension

Add 6 g nigrosine powder to 600 ml lukewarm tap water, heat the mixture to approximately 80 °C and dissolve while stirring continuously.

5) MNE form German: Mehl, Nigrosin, Ei.

E.4.2 Wheat flour suspension

Add 115 g of wheat flour to 800 ml cold tap water, heat the mixture while stirring continuously; bring to a boil and continue boiling for 3 min.

E.4.3 MN mixture

Mix 600 ml nigrosine suspension (E.4.1) with 800 ml of wheat flour suspension (E.4.2).

This mixture may be prepared in larger amounts.

Homogenize this test soil in a laboratory blender to give a micro-dispersed test soil.

Clumps in the test soil can block the endoscope channels.

E.4.4 Bacterial suspension and subcultures

Prepare a subculture by passing the test organism (e.g. *E. faecium*) twice over CSB (or appropriate selective broth) at (36 ± 1) °C for 24 h. Using a Drigalski spatula, plate 0,1 ml of this subculture onto CSA (or appropriate selective agar) and incubate at (36 ± 1) °C for 48 h (or as appropriate for the relevant test organism). Typically, two Petri dishes to three Petri dishes need to be washed off with sterile 0,9 % physiological saline solution to produce 10 ml to 20 ml test soil. Then, centrifuge the bacterial suspension for 10 min at approximately 3 000 r/min and wash the resulting sediment by resuspending it in 0,9 % physiological saline solution.

NOTE Bacterial subcultures are prepared from stock cultures. A maximum of three subcultures are allowed. For details regarding the maintenance of microbiological stock cultures, see EN 12353.

E.4.5 Completion of the test soil

Immediately before use, warm up 700 g of the MN mixture (E.4.3) to approximately 35 °C. Add the suspension of microorganisms and mix well before the eggs are added. Add the white and yolk of three middle sized raw eggs and mix thoroughly. Adjust the temperature to approximately 35 °C if necessary.

E.5 Storage

The test soil base (E.4.3) can be stored in a refrigerator and may be kept for up to 3 days.

E.6 Test pieces

E.6.1 Tubes for use as test pieces

— **Polytetrafluoroethylene** (PTFE) **tubes**, having a length of 2 m and inner diameters of 1,0 mm and 2,0 mm respectively.

E.6.2 Endoscopes for use as test pieces

— **Colonoscope**.

— **Gastroscope**.

— **Bronchoscope**.

— **Duodenoscope**.

The additional use of endoscopes as test objects is mandatory for type tests and optional for operational and routine testing.

NOTE The kinds of endoscopes to be used in type testing are limited to the ones which are intended by the WD manufacturer to be processed in the WD.

The test with endoscopes as test pieces shall only be performed if the test with PFTE tubes has yielded satisfactory results.

Endoscopes which are used in daily clinical routine shall not be used as test pieces.

E.7 Inoculation of test pieces

E.7.1 Tubes for use as test pieces

Apply the test soil by flushing through the tubing, then purge the tube with 20 ml of air using a syringe.

Store the test piece horizontally for 1 h at room temperature.

NOTE The test pieces serve as surrogates for endoscope channels. The number of test pieces required varies according to the capacity of the WD.

E.7.2 Endoscopes for use as test pieces

Inject 10 ml of the test soil through the biopsy channel and the air/water channel by inserting a syringe at the supply socket. With a second syringe purge 20 ml of air through the channels in the same direction to ensure they are not blocked. Apply the test soil to the outer surfaces using a paintbrush.

Store the contaminated endoscopes horizontally for 1 h at room temperature.

E.8 Test method

E.8.1 General

Load the soiled test pieces (tubes or endoscopes) into the WD according to operating instructions. Start the programme after fixing the test pieces in the WD using adequate connectors. Following completion of the processing stage under evaluation or at the end of the programme, but prior to the drying stage, remove the test pieces from the WD.

Immediately flush each tube (or channel of the endoscope) with 10 ml CSB containing suitable neutralizers to enable the quantitative determination of recoverable test organisms; flush the test pieces from the same direction as previously applied in the WD. Dilute the rinsing solution adequately and plate 0,1 ml of each dilution stage onto selective agar plates appropriate for the organisms under test.

Determine the total bacterial count in the bacterial suspension (E.4.5), test soils (E.7), rinse fluids and the final rinse water (E.8.2) by means of surface culture. (Plate onto selective agar plates appropriate for the organisms under test and incubate at (36 ± 1) °C for 48 h, or as appropriate for the relevant test organism.)

The lumens of control test pieces not exposed to the process shall also be flushed with 10 ml CSB. According to the high bacterial counts of the untreated controls, cultures of higher dilution steps are necessary.

If only the cleaning efficacy is to be evaluated, remove the test pieces from the WD after the cleaning stage and determine the achieved reduction factor of the test organism count.

If the cleaner has no disinfecting properties, the cleaning efficacy can be evaluated without the use of neutralizers.

E.8.2 Final rinse water

Draw at least 200 ml of the final rinse water out of the tank of the WD. One-hundred (100) ml of the final rinse water are membrane filtered. Place the filters on selective agar plates appropriate for the organisms under test. Incubate at (36 ± 1) °C for 48 h (or as appropriate for the relevant test organism).

Performance qualification tests should include confirmation of absence of *Legionella pneumophila*, *Pseudomonas aeruginosa* and other relevant microorganisms (as applicable).

E.9 Results

E.9.1 Detection of residual soil

After cleaning in the WD, examine the tubes and outer surfaces of the endoscopes as well as the rinse fluids visually.

E.9.2 Determination of reduction factor

Calculate the reduction factor (F_{red}) by comparing the bacterial counts in the rinse fluids with controls (i.e. test pieces not exposed to the procedure):

$$F_{red} = \log_{10} C_{cfu1} - \log_{10} C_{cfu2} \quad \text{(E.1)}$$

where

F_{red} is the reduction factor;

C_{cfu1} is the number of colony forming units on test pieces not exposed to process (control);

C_{cfu2} is the number of colony forming units on test piece exposed to process.

E.9.3 Acceptance criteria

The cleaning efficacy of the WD shall be regarded as satisfactory if the following requirements are met.

— At the end of the cleaning stage the test pieces and the rinse fluids shall be visually clean.

— The F_{red} of the test organism count shall be $\geqslant 4$.

— The test organism shall not be detectable in 100 ml of the final rinse water (after completion of the cycle but before the drying stage).

E.10 Safety considerations

E.10.1 Personal protective equipment

When preparing the test soil, inoculating the test pieces, loading the inoculated test pieces into the WD or examining the processed devices for residual protein, the operator should wear a protective gown (or apron), gloves, eye protection and a face mask.

E.10.2 Disposal

All chemicals and test soils can be disposed of as non-hazardous, non-clinical waste.

E.10.3 Environmental spillage

Environmental surfaces that have been contaminated with test soil shall be wiped clean using a cloth moistened with a solution of an appropriate detergent/disinfectant in accordance with local policies and procedures.

Annex F
(normative)

Test soil and method for flexible endoscopes (France)

F.1 Reference

The French test method[37] uses tubing contaminated with biofilm to evaluate the cleaning cycle in an endoscope WD. The method is also used for the evaluation of the endoscope WD self-disinfection cycle. A similar British method published in HTM 2030 [30] has some differences, for example regarding the methods used to contaminate test pieces and to recover bacteria. Where they exist, such differences are described in specific notes.

F.2 Materials

F.2.1 Chemicals and growth media.

— **Liquid growth medium**, consisting of phosphate buffer (containing 1,2 g/l sodium phosphate, dibasic and 0,5 g/l potassium phosphate, monobasic) containing 0,25 g/l casamino acids, 0,1 g/l yeast extract, 0,2 g/l $MgSO_4 \cdot 2\ H_20$, 0,000 5 g/l $FeSO_4 \cdot 7\ H_20$, 0,025 g/l lactose.

— **Ringer's solution**, 1/4 strength, supplemented with 0,05 % polysorbate 80.

— **Nutrient agar**, trypticase soy agar.

NOTE For the test method described in HTM 2030:

— nutrient agar supplemented with 1 g/l sodium desoxycholate and 0,025 g/l of 2,4,4'-trichlor-2'-hydroxydiphenylether;

— liquid growth medium: consisting of phosphate buffer (containing 1,2 g/l sodium phosphate, dibasic and 0,5 g/l potassium phosphate, monobasic) containing 0,25 g/l sodium glutamate and 0,1 g/l citric acid.

F.2.2 Microorganisms.

— *Pseudomonas aeruginosa* (CIP A22).

NOTE For the test method described in HTM 2030, use *Pseudomonas aeruginosa* ATCC 25619.

F.3 Apparatus

— **Peristaltic pump**.

— **Incubator,** capable of being maintained at (30 ± 2) °C.

— **Conical flask**, of 1 l capacity, fitted with rubber bung, air vent and two glass tubes.

— **Connecting tubing**.

— **Tubing made of polytetrafluoroethylene** (PTFE) having a length of 1,5 m to 2,0 m and an inner diameter of 6 mm.

— **Normal laboratory glassware**.

NOTE An example of the test equipment for biofilm formation is given in Figure F.1.

F.4 Preparation of test soil

Inoculate a Petri dish containing supplemented nutrient agar with *Pseudomonas aeruginosa* and incubate at (30 ± 2) °C for 36 h to 48 h.

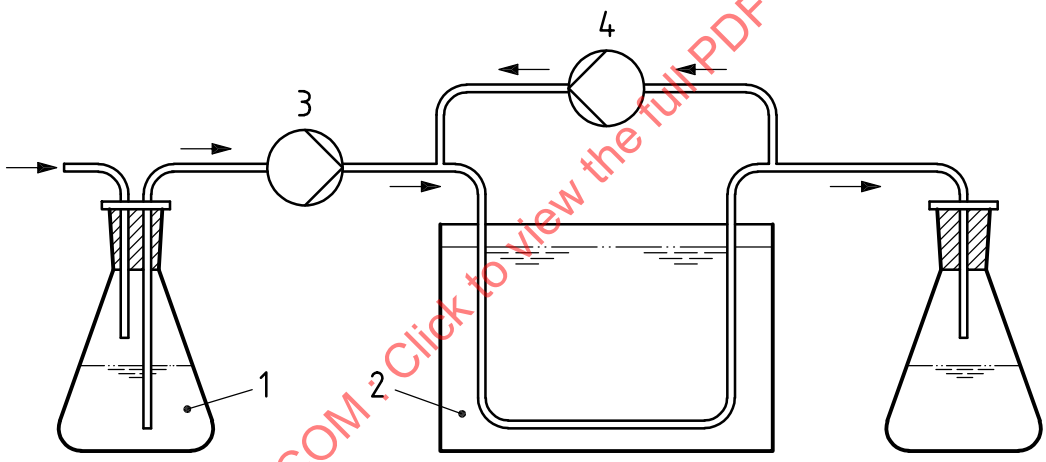
Fit the flask with a bung through which an air vent fitted with a 0,22 µm filter passes, and two glass tubes one of which reaches to the bottom of the flask and one of which terminates above the level of liquid in the flask.

Connect the glass tubes via a peristaltic pump (P1 or 3 in Figure F.1) and short lengths of flexible tubing to the PTFE tubing. Pump the liquid growth medium through the tubing system at 2 ml/min to 3 ml/min throughout the incubation period. Keep the content of the loop in agitation using another peristaltic pump running at above 100 ml/min (P2 or 4 in Figure F.1).

Inoculate the loop with 5 ml to 10 ml *Pseudomonas aeruginosa* bacterial suspension containing about 10^8 bacteria per millilitre.

Maintain the system in an incubator at (30 ± 2) °C for 72 h to 96 h.

NOTE For the test method described in HTM 2030 the liquid growth medium is inoculated with mucoid colonies of *Pseudomonas aeruginosa* from the agar plate, incubated at (30 ± 2) °C for 18 h to 24 h and the culture is pumped round the tubing system at 50 ml/min to 75 ml/min throughout the incubation period. The system is maintained in an incubator at (30 ± 2) °C for 72 h to 96 h.



Key

1 liquid growth
2 water bath 30 °C
3 peristaltic pump P1
4 peristaltic pump P2

Figure F.1 — Test equipment for biofilm formation

F.5 Test pieces

The test system consists of two tubes of 300 mm lengths cut from the tubing with biofilm grown on the inner surface during the procedure described in F.4. Connect the two tubes of 300 mm lengths via isolating valves and Y-piece connectors in place of a section of pipework of the WD.

F.6 Inoculation of test pieces

See F.4 and F.5.

F.7 Test method

Subject a 300 mm section of the tubing (T_1) prepared with biofilm to the recovery procedure described below.

Remove a section of the piping in the endoscope channel irrigation system of the WD and replace it with the test system consisting of two 300 mm lengths of the biofilm test piece tubing connected via isolating valves and Y-piece connectors. With the valves open, set the WD to operate the test cycle ("self-disinfect" cycle for evaluation of the self-disinfection cycle or "standard cycle" for the evaluation of cleaning efficacy).

At the end of any wash stage and any intermediary rinse stage but immediately before the start of the chemical disinfection stage, close the valves isolating one of the test pieces (T_2). On completion of the disinfection stage and any subsequent rinse stage, remove both test pieces (T_2 and T_3) and carry out the recovery procedure described below.

Replace the test pieces with two more sections of the tubing with biofilm and carry out a further cycle. Isolate one of the test pieces (T_4) at the end of the disinfection stage and before any rinsing process. On completion of the cycle, remove both test pieces (T_4 and T_5) and carry out the following recovery procedure.

Cut the 300 mm length of tube into six portions each of approximately 50 mm in length. Transfer three of these pieces into individual universal containers containing nutrient broth and incubate at (30 ± 2) °C for 72 h.

Cut the remaining three sections in half longitudinally and transfer each pair to 10 ml of 1/4-strength Ringer's solution containing 0,05 % polysorbate in a thin-walled universal container with 5 g sand. Vortex each test tube for 1 min. Allow the sand to settle briefly before performing serial dilutions.

NOTE For the test method described in HTM 2030, the remaining three sections are cut in half longitudinally and each pair is transferred to 10 ml of 1/4-strength Ringer's solution containing 0,05 % polysorbate in a thin-walled universal container. The container is ultrasonicated for 10 min at 30 MHz to 50 MHz.

Prepare tenfold serial dilutions of the eluate obtained and use these for the enumeration of the surviving organisms by the spread plate technique. Carry out all determinations in duplicate.

For the evaluation of cleaning efficacy, a determination of the residual amounts of proteins and polysaccharides remaining on the test piece shall be performed. In order to do that, repeat the test as described above. Cut each 300 mm length of test pieces collected (T'_1, T'_2, T'_3, T'_4, T'_5) into six portions each of approximately 50 mm in length. Transfer each piece into individual universal containers containing 10 ml of sterile distilled water. Scrape thoroughly each piece with sterile scalpel. Vortex each test tube for 1 min. Determine the residual amount of proteins and polysaccharides in the eluate using the Lowry[45] and Dubois[46] methods or other methods found equivalent and scientifically proven.

F.8 Results

F.8.1 General

The data obtained provide the following information:

— T_1 is the recoverable population on the original test piece;

— T_2 is the recoverable population after the washing stage and intermediary rinse stage(s);

— T_3 is the recoverable population after wash, disinfect and rinse stages;

— T_4 is the recoverable population after wash and disinfect stages;

— T_5 is the recoverable population after wash, disinfect and rinse stages;

— T_3 and T_5 should have the same population within the limits of experimental error; the difference between them is a measure of the reproducibility of the system;

— $T_1 - T_2$ is the loss of microorganisms during the washing stage;

— $T_4 - T_3$ and $T_4 - T_5$ are the losses during the post-disinfection rinse;

— $T_2 - T_4$ is the loss due to the disinfection process;

— T'_1 is the concentration of protein and polysaccharide per surface unit on the original test piece;

— T'_2 is the concentration of protein and polysaccharide per surface unit on the test piece after washing;

— T'_3 is the concentration of protein and polysaccharide per surface unit on the test piece after wash, disinfect and rinse stages;

— T'_4 is the concentration of protein and polysaccharide per surface unit on the test piece after wash and disinfect stages;

— T'_5 is the concentration of protein and polysaccharide per surface unit on the test piece after wash, disinfect and rinse stages;

— T'_3 and T'_5 should have the same value within the limits of experimental error; the difference between them is a measure of the reproducibility of the system;

— $T'_1 - T'_2$ is the loss of protein and polysaccharide during the wash stage;

— $T'_4 - T'_3$ and $T'_4 - T'_5$ are the losses of protein and polysaccharide during the post disinfection rinse;

— $T'_2 - T'_4$ is loss of protein and polysaccharide due to the disinfection process.

F.8.2 Acceptance criteria

On testing the full self-disinfect cycle there shall be no recovery of organisms from T_3, T_4 and T_5. When this is the case, the test shall be repeated with the exposure time reduced to half the normal value to determine the limit of capability for the self-disinfect cycle.

NOTE Recovery of organisms at half the exposure time indicates a low or marginal capability for the self-disinfect cycle.

On testing the cleaning efficacy of the wash stage there shall be as follows:

— no recovery of organisms from T_3 and T_5;

— no residual proteins and polysaccharides from T_3 and T_5 (taking into account the sensitivity of the test method used);

— the reduction of the amount of residual proteins and polysaccharides after the wash stage ($T_1 - T_2$) shall be at least 90 %.

F.9 Safety considerations

Normal microbiological laboratory safety procedures should be used. All material used should be autoclaved before disposal as non-hazardous waste.

Annex G
(normative)

Test soil and method for surgical instruments, glassware and anaesthesia equipment (Germany)

G.1 References

The methods described hereafter are based on References [32] and [33] and have been adapted or complemented for this presentation. Test pieces (screws and tubes) contaminated with blood, semolina pudding or egg yolk test soils and *E. faecium* ATCC 6057 (DSM 2146), a test organism with proven heat resistance, are loaded into the WD to be tested. Following the processing stage under evaluation or completion of the process (as applicable) the test pieces are removed from the WD, inspected for visual cleanliness and then tested microbiologically.

G.2 Materials

G.2.1 Screws for use as test pieces.

— **Stainless steel screws**, see ISO 1207 M6 × 20.

G.2.2 Tubes for use as test pieces.

— **Soft rubber tubes**, with 6 mm inner diameter, 2 mm wall thickness, of red colour, pyrogen-free, rolled.[6)]

G.2.3 Blood for use as test soil.

— **Defibrinated sheep blood**.[6)]

— *E. faecium* (ATCC 6057).

G.2.4 Semolina pudding for use as test soil.

— **Skimmed milk powder**.

— **Butter**.

— **Sugar**.

— **Semolina** (durum wheat meal).

— **Tap water**.

— *E. faecium* (ATCC 6057).

6) Guidance on suitable commercially available products may be obtained from Deutsches Institut für Normung e. V. (DIN), Burggrafenstr. 6, 10787 Berlin, Germany.

G.2.5 Egg yolk for use as test soil.

— **Eggs**.

— *E. faecium* (ATCC 6057).

G.2.6 Casein peptone — soya bean meal peptone broth (CSL)[7].

— Casein peptone	17,0 g/l
— Soya bean meal peptone agar	3,0 g/l
— Sodium chloride	5,0 g/l
— Dipotassium hydrogenphosphate	2,3 g/l
— Dextrose	2,5 g/l
— Distilled water	1 000 ml
— pH	7,3 ± 0,1

G.2.7 Casein peptone — soya bean meal peptone agar (CSA)[7].

— Casein peptone	15,0 g/l
— Soya bean meal peptone agar	5,0 g/l
— Sodium chloride	5,0 g/l
— Agar	12,0 g/l to 15,0 g/l (depending on manufacturer)
— Distilled water	1 000 ml
— pH	7,3 ± 0,1

G.2.8 Physiological saline solution (0,9 % NaCl).

G.2.9 Calcium chloride ($CaCl_2$).

G.2.10 Potassium carbonate (K_2CO_3).

G.2.11 Silica gel.

G.3 Apparatus

Normal microbiological laboratory apparatus, including the following.

— **Steam sterilizer** (e.g. in accordance with EN 285).

— **Water bath**, capable of being controlled at (36 ± 1) °C.

— **Incubator**, capable of being controlled at (36 ± 1) °C.

— **pH-meter**, having an accuracy of calibration of 0,1 pH units at 25 °C.

7) Guidance on suitable commercially available products may be obtained from Deutsches Institut für Normung e. V. (DIN), Burggrafenstr. 6, 10787 Berlin, Germany.

— **Stopwatch**.

— **Vortex mixer**.

— **Test tubes** and **flasks**, of suitable capacity.

— **Graduated pipettes**, of 10 ml and 1 ml capacities.

— **Petri dishes**, of diameter 90 mm to 100 mm.

— **Glass beads**, of diameter 3 mm to 4 mm.

— **Ultrasonic bath**.

— **Centrifuge**.

— **WD**.

G.4 Preparation of test soils

G.4.1 Bacterial suspension and subcultures

Prepare a subculture by passaging *E. faecium* twice over CSL at (36 ± 1) °C for 24 h. Using a Drigalski spatula, plate 0,1 ml of this subculture onto CSA and incubate at (36 ± 1) °C for 48 h. A minimum of 12 Petri dishes are usually required to obtain 100 ml test soil. Remove the colony-forming units (cfu) from the agar surface of the Petri dish using 0,9 % NaCl solution. Centrifuge the bacterial suspension for 10 min at approximately 3 000 r/min and wash the resulting sediment through resuspension in 0,9 % NaCl solution.

Prepare bacterial subcultures from stock cultures. The number of subcultures shall not exceed three. For details regarding the maintenance of microbiological stock cultures, see EN 12353.

G.4.2 Blood test soil

Suspend the test microorganism sediment obtained (G.4.1) in blood. Approximately 50 ml of blood are required to contaminate 80 to 100 test pieces.

G.4.3 Semolina pudding test soil

Stir 10 g of skimmed milk powder with 100 ml tap water. Allow the powder to dissolve, then add 5 g of sugar and 4 g of butter. Heat the solution to the boiling point in a waterbath. Then, stir 4 g of semolina into the boiling solution. Heat the mash for 20 min in the boiling waterbath, stir from time to time and then steam sterilize at 121 °C for 15 min. Approximately 100 ml of semolina pudding are required to prepare 80 test pieces to 100 test pieces. Mix the test microorganism sediment (see G.4.1) with the semolina pudding mash thus prepared.

Semolina pudding prepared aseptically (e.g. under laminar flow conditions) as well as test pieces contaminated therewith can be stored under recontamination proof conditions in a refrigerator at 7 °C to 10 °C without occurrence of any significant change in the bacterial count present on the test piece.

G.4.4 Egg yolk test soil

Maintain the eggs for 30 min at a temperature of 20 °C in a waterbath. Heat the eggs for 4 min with steam at 100 °C, then cool the eggs in water with a temperature of 20 °C for 5 min. Open the eggs under aseptic conditions. Ensure that the albumen is firm and the egg yolk still fluid. Separate the egg yolk from the albumen. Approximately 15 eggs are required to prepare 100 ml of test soil. Around 50 ml of egg yolk are required to contaminate 80 to 100 test pieces. Mix the test microorganism sediment (G.4.1) with the egg yolk thus prepared.

G.5 Preparation of test pieces

G.5.1 Screws for use as test pieces

Before use, thoroughly clean and degrease the screws in a WD or ultrasonic bath or by means of a commercial cleaner designed for laboratory purposes. For ultrasonic cleaning use only neutral cleaners. Rinse the screws thoroughly with distilled water following both ultrasonic and manual cleaning. After drying steam sterilize the screws at 121 °C and then store them under dry conditions over silica gel. The screws are reusable several times.

G.5.2 Tubes for use as test pieces

Cut the tubes into 70 mm long segments. Pre-clean the tube segments before use either by means of a WD at 93 °C or by boiling for 10 min using a commercial cleaner designed for laboratory purposes. Rinse the pre-cleaned tube segments thoroughly with distilled water. After drying, steam sterilize the test segments at 121 °C and then store them under dry conditions over silica gel. Dispose of the tube segments immediately after a single use.

G.6 Contamination of test pieces

G.6.1 Screws

Immerse the screws in the respective test soil for at least 1 min in such a manner that the screws become fully wetted (use approximately 0,8 g semolina pudding or egg yolk or approximately 0,1 ml blood per screw). Then, place the screws head down on filter paper in order to eliminate excessive fluid and allow the screws to dry in a standing position in a glass container for 24 h. Condition the screws for 24 h at 45 % relative air humidity (e.g. over saturated K_2CO_3 solution).

The screws can also be exposed in an incubator at (36 ± 1) °C for 4 h.

For special testing the test soil also can directly be spread on the surfaces to be tested.

G.6.2 Tubes

Immerse the tube segments in the respective test soil for at least 1 min in such a manner that the tube segments become fully wetted. Then, place the tube segments into a glass container and allow them to dry in a horizontal position for 24 h. Condition the tube segments for 24 h at 45 % relative air humidity (e.g. over saturated K_2CO_3 solution).

NOTE 1 The tube segments can also be exposed in an incubator at (36 ± 1) °C for 4 h.

NOTE 2 For special testing, the test soil can also directly be spread on the surfaces to be tested.

G.7 Tests for various fields of application

G.7.1 Instruments and laboratory glass

Load the items to be tested into the WD according to operating instructions. Twenty screws per test soil contaminated with blood, egg yolk and semolina pudding test soils are used as test pieces. Distribute the test pieces throughout the WD, preferably in the corners of the sieve baskets. After completion of the programme, remove the test pieces aseptically from the WD and inspect them for cleanliness. Record areas where no cleanliness has been achieved. Then transfer the test pieces to test tubes containing 10 ml CSL and incubate the test tubes for 7 days at (36 ± 1) °C. If turbidity develops, identify the test organism through subcultivation and/or microscopy.

If only the cleaning efficacy is to be evaluated, remove the test pieces from the WD after completion of the cleaning stage and assess the achieved cleanliness visually; in addition the achieved reduction factor of the test organism count can be determined.

If only the disinfecting efficacy is to be evaluated, place the test pieces in locations where they are not directly hit by the spray jet. It is recommended that the test pieces be placed into small flat baskets that are then positioned in the front corners of the upper insert basket. This ensures protection of the test pieces from the spray jet by the items loaded into the WD.

G.7.2 Anaesthesia equipment

Load the items to be tested into the WD according to operating instructions. Use tube segments and screws contaminated with blood test soil as test pieces. Put the tube segments on the jets designed for the flushing of tubes. Place the screws horizontally into utensil baskets and distribute them evenly throughout the WD. After completion of the programme, remove the test pieces aseptically from the WD and inspect them for cleanliness. Record areas where no cleanliness has been achieved. Then transfer the test pieces to test tubes containing 10 ml CSL. Incubate the test tubes for 7 days at (36 ± 1) °C. If turbidity develops, identify the test organism through subcultivation and/or microscopy.

If only the cleaning efficacy is to be evaluated, remove the test pieces from the WD after completion of the cleaning stage and assess the achieved cleanliness visually; in addition the achieved reduction factor of the test organism count can be determined.

If only the disinfecting efficacy is to be evaluated, place the test pieces in locations where they are not directly hit by the spray jet. It is recommended to place the test pieces into small flat baskets which are then positioned in the front corners of the upper insert basket. This ensures protection of the test pieces from the spray jet by the items loaded into the WD.

G.7.3 Baby bottles

Load the items to be tested into the WD according to operating instructions. Use screws contaminated with the blood, egg yolk and semolina pudding test soils as test pieces. Distribute the test pieces throughout the WD. After completion of the programme, remove the test pieces aseptically from the WD and inspect them for cleanliness. Record areas where no cleanliness has been achieved. Then transfer the test pieces to test tubes containing 10 ml CSL. Incubate the test tubes for 7 days at (36 ± 1) °C. If turbidity develops, identify the test organism through subcultivation and/or microscopy.

If only the cleaning efficacy is to be evaluated, remove the test pieces from the WD after completion of the cleaning stage and assess the achieved cleanliness visually; in addition the achieved reduction factor of the test organism count can be determined.

If only the disinfecting efficacy is to be evaluated, place the test pieces in locations where they are not directly hit by the spray jet. It is recommended that the test pieces be placed into small flat baskets that are then positioned in the front corners of the upper insert basket. This ensures protection of the test pieces from the spray jet by the items loaded into the WD.

G.8 Control and comparison tests

G.8.1 Determination of bacterial count

Determine the bacterial count on the test pieces. Transfer the test pieces to test tubes containing 10 ml of 0,9 % NaCl solution. Vortex the test tubes with glass beads for 10 min at a speed of 300 Hz·to 400 Hz. Determine the total bacterial count by means of surface culture [incubate on CSA at (36 ± 1) °C for 24 h]. The bacterial count shall be $> 1 \times 10^8$ colony-forming units (cfu) per screw and $> 1 \times 10^7$ cfu per tube segment.

G.8.2 Determination of the heat resistance of *E. faecium*

Check the heat resistance of *E. faecium* at least every six months and record the results. Heat 20 test tubes containing about 10 ml CSL to 70 °C in a waterbath. Once the CSL tubes have reached a temperature of 70 °C load each test tube with 20 test piece sections. Allow the test tubes to remain in the waterbath for 10 min, then take the test tubes out and either place them onto ice or immediately cool them under cold running water. Incubate the test tubes at (36 ± 1) °C for 24 h. Heat resistance shall be considered adequate if 90 % of the test tubes still yield viable *E. faecium* cells.

G.9 Acceptance criteria

The procedure shall be deemed efficient if the following requirements are met.

— At the end of the procedure the test pieces shall be visually clean.

— The test organism shall be detectable on less than 5 % of the total number of test pieces.

G.10 Safety considerations

G.10.1 Disposal

All chemicals, blood as well as items to be eliminated may be disposed of as non-hazardous and non-clinical waste. All waste contaminated with *E. faecium* (a microorganism considered non-pathogenic) should undergo thermal disinfection in accordance with regional practices and procedures.

G.10.2 Environment

Environmental surfaces contaminated with test soil should undergo wipe-disinfection using a suitable surface disinfectant in accordance with regional practices and procedures.

Annex H
(normative)

Test soil and method for bedpans (Germany)

H.1 Reference

The methods described hereafter are based on References [22], [23] and [38] and have been adapted or complemented for this presentation. Test pieces contaminated with *E. faecium*, a test organism with proven heat resistance, are loaded into the WD to be tested. Following the processing stage under evaluation or completion of the process (as applicable), the test pieces are removed from the WD, inspected for visual cleanliness and then tested microbiologically.

H.2 Materials

H.2.1 RAMS test soil.

— **RAMS test soil,** consisting of

  — bovine albumin;

  — mucin;

  — maize starch;

  — *E. faecium* (ATCC 6057, DSM 2146).

H.2.2 Test pieces.

— **Stainless steel plates**, X5CrNi1810 (see EN 10088-1) with two attachment ends each, contamination field approximately 100 mm × 10 mm.

H.2.3 Casein peptone — soya bean meal peptone broth (CSL)[8].

— Casein peptone	17,0 g/l
— Soya bean meal peptone agar	3,0 g/l
— Sodium chloride	5,0 g/l
— Dipotassium hydrogenphosphate	2,3 g/l
— Dextrose	2,5 g/l
— Distilled water	1 000 ml
— pH	7,3 ± 0,1

8) Guidance on suitable commercially available products may be obtained from Deutsches Institut für Normung e. V. (DIN), Burggrafenstr. 6, 10787 Berlin, Germany.

H.2.4 Casein peptone — soya bean meal peptone agar (CSA)[9].

— Casein peptone	15,0 g/l
— Soya bean meal peptone agar	5,0 g/l
— Sodium chloride	5,0 g/l
— Agar	12,0 g/l to 15,0 g/l (depending on manufacturer)
— Distilled water	1 000 ml
— pH	7,3 ± 0,1

H.2.5 Kanamycin esculin azide agar[9].

— Casein peptone	20,0 g/l
— Yeast extract	5,0 g/l
— Sodium chloride	5,0 g/l
— Sodium citrate	1,0 g/l
— Esculin	1,0 g/l
— Ammonium iron(III)-citrate	0,5 g/l
— Sodium azide	0,15 g/l
— Kanamycin sulphate	0,02 g/l
— Agar	10,0 g/l to 15,0 g/l (depending on manufacturer)
— Distilled water	1 000 ml
— pH	7,1 ± 0,2

H.2.6 Kanamycin esculin azide broth[9].

— Casein peptone	20,0 g/l
— Yeast extract	5,0 g/l
— Sodium chloride	5,0 g/l
— Sodium citrate	1,0 g/l
— Esculin	1,0 g/l
— Ammonium iron(III)-citrate	0,5 g/l
— Sodium azide	0,15 g/l
— Kanamycin sulphate	0,02 g/l
— Distilled water	1 000 ml
— pH	7,1 ± 0,2

9) Guidance on suitable commercially available products may be obtained from Deutsches Institut für Normung e. V. (DIN), Burggrafenstr. 6, 10787 Berlin, Germany.

H.2.7 Physiological saline solution.

— **Physiological saline solution** (0,9 %NaCl).

H.3 Apparatus

Normal microbiological laboratory apparatus, including the following:

— **Steam sterilizer** (e.g. in accordance with EN 285).

— **Waterbath**, capable of being controlled at (36 ± 1) °C.

— **Incubator**, capable of being controlled at (36 ± 1) °C.

— **pH-meter**, having an accuracy of calibration of 0,1 pH units at 25 °C.

— **Stopwatch**.

— **Vortex mixer**.

— **Test tubes** and **flasks,** of suitable capacity.

— **Graduated pipettes,** of 10 ml and 1 ml capacities.

— **Petri dishes,** of diameter 90 mm to 100 mm.

— **Glass beads,** of diameter 3 mm to 4 mm.

— **Ultrasonic bath**.

— **Centrifuge**.

— **WD**.

H.4 Preparation of test soil

H.4.1 Bacterial suspension and subcultures

Prepare a subculture by passaging *E. faecium* ATCC 6057 twice over CSL at (36 ± 1) °C for 24 h. Using a Drigalski spatula, plate 0,1 ml of this subculture onto CSA and incubate for 48 h at (36 ± 1) °C. The bacterial growth is eluted with sterile 0,9 % physiological saline solution to produce 10 ml to 20 ml of test soil. Then, centrifuge the bacterial suspension for 10 min at approximately 3 000 r/min and wash the resulting sediment through resuspension in 0,9 % physiological saline solution.

Prepare bacterial subcultures from stock cultures. The number of subcultures shall not exceed three. For details regarding the maintenance of microbiological stock cultures, see EN 12353.

H.4.2 Test soil solution A

Dissolve 3,0 g mucin in 200 ml distilled water. Keep this mucin solution in a water bath with a temperature of 50 °C to 60 °C and stir. Add 1,8 g of bovine albumin to the mucin solution. Mix the mucin solution.

NOTE Alternatively, a magnetic stirrer with a temperature of 50 °C to 60 °C can be used.

It is recommended to use glass beads to improve the solubility of mucin.

If the test soil is to be stored for several days, steam sterilize the mucin solution at 121 °C. The bovine albumin solution is prepared separately and sterilized by filtration (pore size 0,5 µm).

H.4.3 Test soil solution B

Heat 80 ml of water to boiling point (e.g. in a waterbath). Suspend 9 g of maize starch in 20 ml cold distilled water and add to the hot water. Stir the starch solution until a noticeable thickening develops.

If the test soil is to be stored for several days, steam sterilize the starch solution at 121 °C.

H.4.4 RAMS test soil

Allow solution A (H.4.2) and solution B (H.4.3) to cool to room temperature, then mix the two solutions.

If the test soil is to be used within one day following its preparation, it need not be sterilized. Test soils prepared from sterilized solutions may be stored for no more than 4 weeks at temperatures above 7 °C.

H.5 Preparation of test pieces

Before use, thoroughly clean and degrease the test pieces in a WD or ultrasonic bath or by means of a commercial cleaner designed for laboratory purposes. For ultrasonic cleaning use only neutral cleaners. Rinse the test pieces thoroughly with distilled water following both ultrasonic and manual cleaning. After drying the test pieces, steam sterilize them at 121 °C and then store them under dry conditions over silica gel. The test pieces are reusable several times.

NOTE If rust appears it can be removed by means of a cleaner containing phosphoric acid which is designed for surgical stainless steel instruments.

H.6 Contamination of test pieces

In order to contaminate 50 to 100 test pieces, thoroughly mix the bacterial suspension described in H.4.1 with 20 ml RAMS (H.4.4). To contaminate the test pieces, pipette 0,1 ml RAMS onto the middle field of the polished side of the test pieces and evenly spread it without contaminating the attachment holes and the lateral surfaces. Allow the test pieces to dry for 5 h to 12 h at 18 °C to 24 °C and 50 % to 70 % relative humidity horizontally in a dust-proof place, preferably on metal sheets. Subsequently the test soil shall be dry.

Swabs are unsuitable for spreading the test soil since they may alter the total amount of test soil.

The test pieces can be stored at 7 °C to 10 °C for no more than 2 months.

For special testing the test soil can also be applied directly onto the surfaces to be tested.

H.7 Test procedure

Fix the test pieces onto the load and the WD. Load the WD with the items to be tested according to operating instructions and start the cycle. Record the positions of the test pieces. After completion of the cycle remove the test pieces aseptically from the WD and inspect them for cleanliness. Record areas where no cleanliness has been achieved. Transfer the test pieces to test tubes containing 10 ml CSL or kanamycin esculin azide broth, shake for 10 min at a speed of 300 Hz/s to 400 Hz/s and incubate for 48 h at (36 ± 1) °C on a growth medium (e.g. kanamycin esculin azide agar) to obtain a surface culture for determining the bacterial count.

If only the cleaning efficacy is to be evaluated, remove the test pieces from the WD after completion of the cleaning stage and assess the achieved cleanliness visually; in addition the achieved reduction factor of the test organism count can be determined.

In order to increase the sensitivity of the method test tubes containing the test pieces in CSL or kanamycin esculin azide broth may be incubated for up to 7 days at (36 ± 1) °C. If turbidity develops, identify the test organism through subcultivation.

H.8 Control and comparison tests

H.8.1 Determination of bacterial count

Determine the bacterial count on the test pieces. Transfer the test pieces to test tubes containing 10 ml of CSL or kanamycin esculin azide broth. Vortex the test tubes for no less than 20 min at a speed of 300 Hz/s to 400 Hz/s. Determine the total bacterial count by means of surface culture [incubate on kanamycin esculin azide agar at (36 ± 1) °C for 48 h]. The bacterial count shall not be $> 1 \times 10^7$ colony-forming units (cfu) per test piece.

H.8.2 Calculation of reduction factor

The reduction factor (F_{red}) is calculated as follows:

$$F_{red} = \log_{10} \cdot C_{cfu1} - \log_{10} \cdot C_{cfu2} \quad \text{(H.1)}$$

where

F_{red} is the reduction factor;

C_{cfu1} is the number of colony forming units on test pieces not exposed to process (control);

C_{cfu2} is the number of colony forming units on test piece exposed to process.

H.8.3 Determination of the heat resistance of *E. faecium*

Check the heat resistance of *E. faecium* at least every six months and record the results. Heat 20 test tubes containing about 10 ml CSL to 70 °C in a waterbath. Once the CSL tubes have reached a temperature of 70 °C load each test tube with 20 test piece sections. Allow the test tubes to remain in the waterbath for 10 min, then take the test tubes out and either place them onto ice or immediately cool them under cold running water. Incubate the test tubes at (36 ± 1) °C for 24 h. Heat resistance shall be considered adequate if 90 % of the test tubes still yield viable *E. faecium* cells.

H.9 Acceptance criteria

The procedure shall be considered efficient if the following requirements are met:

— Following the procedure, test pieces shall be visually clean.

— No more than one test piece per test batch shall yield a $F_{red} \leqslant 5$. However, a $F_{red} \geqslant 4$ is required for all test pieces.

H.10 Safety considerations

H.10.1 Disposal

All chemicals, blood as well as items to be eliminated may be disposed of as non-hazardous and non-clinical waste. All waste contaminated with *E. faecium* (a microorganism considered non-pathogenic) shall undergo thermal disinfection in accordance with regional practices and procedures.

H.10.2 Environment

Environmental surfaces contaminated with test soil shall undergo wipe-disinfection using a suitable surface disinfectant in accordance with regional practices and procedures.

Annex I
(normative)

Test soil and method for flexible endoscopes (Germany)

I.1 Reference

The methods described hereafter are based on References [34] and [35] and have been adapted or complemented for this presentation. Test pieces contaminated with *E. faecium*, a test organism with proven heat resistance, are loaded into the WD to be tested. Following the processing stage under evaluation or completion of the process (as applicable), the test pieces are removed from the WD, inspected for visual cleanliness and then tested microbiologically.

I.2 Materials

I.2.1 Test soil.

— **Blood,** freshly drawn from a laboratory sheep.[10)]

— **Protamine hydrochloride**.[10)]

— **Heparin**.[10)]

I.2.2 Polytetrafluoroethylene tubes.

— **Polytetrafluoroethylene tubes** (PTFE), having a length of 2 m and inner diameters of 1,0 mm and 2,0 mm.

I.2.3 Endoscopes.

— **Colonoscope**.

— **Gastroscope**.

— **Bronchoscope with forward-oblique telescope**.

— **Duodenoscope with forward-lateral telescope**.

I.2.4 Connectors.

— **Connectors**, to link the test pieces to the WD ports.

I.2.5 Test organisms.

— *E. faecium* (ATCC 6057, DSM 2146).

In case a microorganism other than *E. faecium* proves to be more resistant to the process temperature (see I.10) used in the quantitative suspension test, all tests shall be performed using this specific microorganism in place of *E. faecium*.

10) Guidance on suitable commercially available products may be obtained from Deutsches Institut für Normung e. V. (DIN), Burggrafenstr. 6, 10787 Berlin, Germany.

I.2.6 Physiological saline solution.

— **Physiological saline solution** (0,9 % NaCl).

I.2.7 Casein peptone — soya bean meal peptone broth (CSL)[11].

— Casein peptone	17,0 g/l
— Soya bean meal peptone agar	3,0 g/l
— Sodium chloride	5,0 g/l
— Dipotassium hydrogenphosphate	2,3 g/l
— Dextrose	2,5 g/l
— Distilled water	1 000 ml
— pH	7,3 ± 0,1

I.2.8 Casein peptone — soya bean meal peptone agar (CSA)[11].

— Casein peptone	15,0 g/l
— Soya bean meal peptone agar	5,0 g/l
— Sodium chloride	5,0 g/l
— Agar	12,0 g/l to 15,0 g/l (depending on manufacturer)
— Distilled water	1 000 ml
— pH	7,3 ± 0,1

I.2.9 Kanamycin esculin azide agar[11].

— Casein peptone	20,0 g/l
— Yeast extract	5,0 g/l
— Sodium chloride	5,0 g/l
— Sodium citrate	1,0 g/l
— Esculin	1,0 g/l
— Ammonium iron(III)-citrate	0,5 g/l
— Sodium azide	0,15 g/l
— Kanamycin sulphate	0,02 g/l
— Agar-agar	10,0 g/l to 15,0 g/l (depending on manufacturer)
— Distilled water	1 000 ml
— pH	7,1 ± 0,2

11) Guidance on suitable commercially available products may be obtained from Deutsches Institut für Normung e. V. (DIN), Burggrafenstr. 6, 10787 Berlin, Germany.

I.3 Apparatus

Normal microbiological laboratory apparatus, including the following:

— **Steam sterilizer** (e.g. in accordance with EN 285).

— **Waterbath,** capable of being controlled at (70 ± 1) °C.

— **Incubator,** capable of being controlled at (36 ± 1) °C.

— **pH-meter,** having an accuracy of calibration of 0,1 pH units at 25 °C.

— **Stopwatch**.

— **Vortex mixer**.

— **Test tubes** and **flasks,** of suitable capacity.

— **Graduated pipettes**, of 10 ml and 1 ml capacities.

— **Petri dishes,** of diameter 90 mm to 100 mm.

— **Glass beads,** of diameter 3 mm to 4 mm.

I.4 Preparation of test soil

I.4.1 Bacterial suspension and subcultures

Prepare a subculture by passaging *E. faecium* ATCC 6057 twice over CSL at (36 ± 1) °C for 24 h. Using a Drigalski spatula, plate 0,1 ml of this subculture onto CSA and incubate for 72 h at (36 ± 1) °C. A minimum of 50 Petri dishes are usually required to obtain 120 ml of test soil. Remove the colony forming units from the agar surface of the Petri dish using sterile physiological saline solution. Then homogenize the bacterial suspension using glass beads. Filter it through glass wool (if necessary) and centrifuge it for 10 min at approximately 3 000 r/min. Wash the resulting sediment by resuspension in 0,9 % physiological saline solution. The final bacterial count shall yield at least 1×10^{11} cfu/ml.

Prepare bacterial subcultures from stock cultures. The number of subcultures shall not exceed three. For details regarding the maintenance of microbiological stock cultures, see EN 12353.

I.4.2 Test pieces with 2,0 mm inner diameter

Draw 100 ml sheep blood onto 0,1 ml heparin. Immediately prior to testing, mix 9,5 ml of heparinized blood with 0,35 ml of bacterial suspension and 0,15 ml of protamine hydrochloride. The latter shall be added immediately prior to contaminating the test pieces. Determine the total bacterial count by means of surface culture.

I.4.3 Test pieces with 1,0 mm inner diameter

Draw 100 ml sheep blood onto 0,1 ml heparin. Immediately prior to testing, mix 2,0 ml of heparinized blood with 0,35 ml of bacterial suspension and 7,65 ml of CSL. Determine the total bacterial count by means of surface culture.

I.5 Contamination of test pieces

I.5.1 Test pieces with 2,0 mm inner diameter

Inject 10 ml of test soil (I.4.2) through the test piece, then purge the test piece with 20 ml of air using a syringe. Take care that the air is pushed through the test piece in such a way that the formation of blood clots of 100 mm to 200 mm length is prevented. Stretch the test piece and store it horizontally for 1 h at room temperature.

NOTE 1 The test pieces serve as surrogates for endoscope channels. The number of test pieces required varies according to the capacity of the WD.

NOTE 2 For special testing the test soil can also directly be spread on the surfaces to be tested.

I.5.2 Test pieces with 1,0 mm inner diameter

Inject 10 ml of test soil (I.4.3) through the test piece, then purge the test piece with 20 ml of air using a syringe. Take care that the air is pushed through the test piece in such a way that the formation of blood clots of 100 mm to 200 mm length is prevented. Stretch the test piece and store it horizontally for 1 h at room temperature.

NOTE 1 The test pieces serve as surrogates for endoscope channels. The number of test pieces required varies according to the capacity of the WD.

NOTE 2 For special testing the test soil can also directly be spread on the surfaces to be tested.

I.5.3 Endoscopes for use as test pieces

Inject 10 ml of test soil through the endoscope working channel, then purge the test piece with 20 ml of air using a syringe. Turn over the test piece once and store it horizontally for 1 h at room temperature. Then perform a patency check by means of guide wires (diameter = 50 % of working channel diameter).

I.6 Procedure

I.6.1 General

The temperature and the disinfectant concentration required for the following processing methods are established by quantitative suspension tests (I.10).

I.6.2 PTFE tubes for use as test pieces

Load the test pieces into the WD according to operating instructions. Use PTFE pieces contaminated with the respective test soil as test pieces. Start the programme after fixing the test pieces in the WD. Following completion of the processing stage under evaluation or after the end of the programme, but prior to the drying stage, remove the test pieces from the WD, assess them qualitatively for visual cleanliness and immediately flush with 50 ml CSL containing suitable neutralizers to enable the quantitative determination of recoverable test organisms. The test pieces shall be flushed from the same direction as previously applied in the WD. Also flush the control test pieces not exposed to the process using 50 ml CSL.

If only the cleaning efficacy is to be evaluated, remove the test pieces from the WD after completion of the cleaning stage including rinsing and assess the achieved cleanliness visually. Determine the achieved reduction factor of the test organism count.

If only the disinfecting efficacy is to be evaluated, expose the test pieces to the process excluding the cleaning stage. Following completion of the processing stage under evaluation, but prior to the drying stage, remove the test pieces from the WD, assess them qualitatively for visual cleanliness, and determine the achieved reduction factor of the test organism count.

If the cleaner has no disinfecting properties, the cleaning efficacy can be evaluated without the use of neutralizers.

I.6.3 Endoscopes for use as test pieces

Tests with endoscopes used as test pieces shall only be performed if the tests with PTFE tubes have yielded a satisfactory efficacy.

Load the endoscopes (test pieces) inoculated with the corresponding test soil into the WD according to operating instructions. Start the programme after fixing the test pieces in the WD. Following completion of the processing stage under evaluation or at the end of the programme, but prior to the drying stage, remove the test pieces from the WD and immediately flush with 50 ml CSL containing suitable neutralizers to enable the quantitative determination of recoverable test organisms. The test pieces shall be flushed from the same direction as previously applied in the WD. Then flush each of the other endoscope channels with 10 ml CSL containing suitable neutralizers from the same direction as previously applied in the WD. Also flush the working channels of control test pieces not exposed to the process using 50 ml CSL.

If only the cleaning efficacy is to be evaluated, remove the test pieces from the WD after completion of the cleaning stage including rinsing and assess the achieved cleanliness visually. Determine the achieved reduction factor of the test organism count.

If only the disinfecting efficacy is to be evaluated, expose the test pieces to the process excluding the cleaning stage. Following completion of the processing stage under evaluation, but prior to the drying stage, remove the test pieces from the WD and determine the achieved reduction factor of the test organism count. Verify on a case-by-case basis whether this is a workable procedure in view of the disinfectant used.

If the cleaner has no disinfecting properties, the cleaning efficacy can be evaluated without the use of neutralizers.

I.6.4 Final rinse water

Test 200 ml of the aseptically taken final rinse water for the presence of the respective test organism.

Performance qualification tests should include confirmation of absence of *Mycobacteria*, *Legionella pneumophila*, *Pseudomonas aeruginosa* and other relevant microorganisms (as applicable).

I.7 Control and comparison tests

I.7.1 Total bacterial counts

Determine the total bacterial count in the bacterial suspension (I.4.1), test soils (I.4.2 and I.4.3), rinse fluids (I.6.2 and I.6.3) and the final rinse water (I.6.4) by means of surface culture [plate onto kanamycin esculin azide agar and incubate at (36 ± 1) °C for 48 h].

Handle all liquids used as enrichment cultures with aseptic laboratory procedures.

I.7.2 Enrichment cultures

I.7.2.1 Rinse fluids

Incubate residual rinse fluids in enrichment cultures at (36 ± 1) °C for 72 h. If turbidity develops, identify the test organism by subcultivation.

Rather than testing the whole residual rinse fluid volume, it is preferable to test smaller aliquots or different dilutions. Incubate 1 ml rinse fluid in 9 ml CSL at (36 ± 1) °C for 72 h.

I.7.2.2 Final rinse water

50 ml of final rinse are filtered through a sterilization grade filter. Examine the filters by means of enrichment culture.

I.7.3 PTFE tubes for use as test pieces

Once flushed, fill the test pieces with liquefied kanamycin esculin azide agar cooled to 60 °C and incubate at (36 ± 1) °C for 24 h. *E. faecium* colonies still remaining in the test piece are identifiable and countable through their black colour.

I.7.4 Determination of the heat resistance of *E. faecium*

Check the heat resistance of *E. faecium* at least every six months and record the result. Cut 10 mm segments off the contaminated tubes. Heat 20 test tubes containing about 10 ml CSL to 70 °C in a waterbath. Once the CSL tubes have reached a temperature of 70 °C load each test tube with 20 test piece sections. Allow the test tubes to remain in the waterbath for 10 min, then take the test tubes out and either place them onto ice or immediately cool them under cold running water. Incubate the test tubes at (36 ± 1) °C for 24 h. Heat resistance shall be considered adequate if 90 % of the test pieces still yield viable *E. faecium* cells.

I.8 Results

I.8.1 Calculation of the reduction factor

Calculate the reduction factor (F_{red}) by comparison with control test pieces which have not been exposed to the procedure. At the end of the respective drying time, flush test pieces serving as controls without exposing them to the process. Calculate the F_{red} as follows:

$$F_{red} = \log_{10} \cdot C_{cfu1} - \log_{10} \cdot C_{cfu2} \quad \text{(I.1)}$$

where

F_{red} is the reduction factor;

C_{cfu1} is the number of colony forming units on test pieces not exposed to process (control);

C_{cfu2} is the number of colony forming units on test piece exposed to process.

NOTE Usually about 0,1 % of the test organisms to 1,0 % of the test organisms introduced into the test piece are recoverable in control test pieces.

I.8.2 Acceptance criteria

The procedure shall be deemed effective if the following requirements are met.

— At the end of the cleaning stage, the test pieces shall be visually clean.

— The F_{red} of the test organism count achieved by cleaning alone shall be $\geqslant 4$.

— At the end of the process, the test organism shall not be detectable in rinse fluids.

— At the end of the process, the test organism shall not be detectable in the final rinse water.

— At the end of the process, the test pieces shall only contain a few isolated bacterial colonies (< 10 cfu) recognizable through their black colour on kanamycin esculin azide agar.

— The test system shall be designed to enable a F_{red} of the test organism count of $\geqslant 9$.

NOTE 1 Numerous publications show that endoscopes may contain bacterial counts higher than 10^9 cells after patient use. Hence cleaning and disinfecting procedures are needed that lead to a microbial depletion of this magnitude. To date it is still technically impossible to set the microbial load of the test soil at a value that allows the determination of $F_{red} > 9$ in a labour-saving way. However, the results of the cleaning and disinfecting stages and of the whole process enable an assessment.

NOTE 2 Usually the routine testing of individual processing stages is unreasonably labour-intensive.

I.9 Safety considerations

I.9.1 Disposal

All chemicals, blood as well as items to be eliminated may be disposed of as non-hazardous and non-clinical waste. All waste contaminated with *E. faecium* (a microorganism considered non-pathogenic) shall undergo thermal disinfection in accordance with regional practices and procedures.

I.9.2 Environment

Environmental surfaces contaminated with test soil shall undergo wipe-disinfection using a suitable surface disinfectant in accordance with regional practices and procedures.

I.10 *In vitro* pre-tests

I.10.1 Determining a suitable neutralizer

I.10.1.1 General

A suitable neutralizer should be determined in accordance with the current DGHM guidelines[51] or corresponding standards (see EN 13624, EN 13727, EN 14348, EN 14476, prEN 14561, prEN 14562, prEN 14563, prEN 14885). A series of disinfectant or cleaner concentrations are used. The test series is performed with all test organisms listed under I.10.1.2. Concentration mixtures are chosen in such a manner that the limits of efficacy become recognisable. This concentration is used for all subsequent testing procedures.

I.10.1.2 Test organisms

— *E. faecium* (ATCC 6057, DSM 2146).

— *Candida albicans* (ATCC 10231, DSM 1386).

— *Staphylococcus aureus* (ATCC 6538, DSM 799).

— *Pseudomonas aeruginosa* (ATCC 15442, DSM 939).

I.10.2 Quantitative suspension test

I.10.2.1 Efficacy against bacteria and yeast-like fungi

The methodology used follows the DGHM Guidelines[51] except that the tests are performed at the process temperatures specified for the WD under evaluation or corresponding standards (see EN 13624, EN 13727,

prEN 14885). Carry out the suspension tests under clean conditions, i.e. with the addition of 0,3 % bovine albumin. Add the test organisms only once the process temperature has been reached. As a minimum, use the use concentration as well as one concentration above and one below the use concentration. At process temperatures above 60 °C, test only *E. faecium*. Choose exposure times of 5 min, 10 min and 15 min.

I.10.2.2 Test organisms

— *E. faecium* (ATCC 6057, DSM 2146).

— *Mycobacterium terrae* (ATCC 15755, DSM 43227).

— *Candida albicans* (ATCC 10231, DSM 1386).

— *Staphylococcus aureus* (ATCC 6538, DSM 799).

— *Pseudomonas aeruginosa* (ATCC 15442, DSM 939).

I.10.2.3 Antiviral efficacy

The tests should be carried out in accordance with the Guideline of the German Federal Health Office (Bundesgesundheitsamt[52] and of the German Association against Virus Diseases, Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten[53] [54]) pertaining to the determination of the antiviral efficacy of chemical disinfectants by means of quantitative suspension tests without challenge at the process temperature. The virus suspension test can currently be omitted at temperatures above 60 °C.

I.10.2.4 Acceptance criteria

In quantitative suspension tests a reduction factor of the test organism count of $F_{red} \geqslant 5$ for bacteria and yeast-like fungi and of $F_{red} \geqslant 4$ for viruses shall be achievable when applying the use concentration throughout the corresponding exposure time at the process temperature selected. However, it should be noted that a process may not claim to be “generally virucidal” on the basis of the results of suspension tests for antiviral efficacy conducted at process temperature.

I.11 Safety considerations

I.11.1 Disposal

All chemicals, blood as well as items to be eliminated may be disposed of as non-hazardous and non-clinical waste. All waste contaminated with *E. faecium* and other test microorganisms shall undergo thermal disinfection in accordance with regional practices and procedures.

I.11.2 Environment

Environmental surfaces contaminated with test soil shall undergo wipe-disinfection using a suitable surface disinfectant in accordance with regional practices and procedures.

Annex J
(normative)

Test soil and method for surgical instruments and flexible endoscopes, Peroxidase test (Germany)

J.1 Reference

See References [41], [42] and [43].

Traces of residual blood can be detected due to the (pseudo)peroxidase activity of haemoglobin.

A colour change of guajac resin or benzidine gives positive test result for blood diluted 1:1 000 000 [41]. Other methods as well as the Kastle-Meyer-test are described in Reference [42].

The peroxidase activity of haemoglobin in blood catalyses the oxidation of a chromogen in the presence of a peroxide (hydrogen peroxide) to form a coloured reaction product which can easily be detected visually.

Tetramethyl-benzidine (TMB) can be used to replace poisonous benzidine as chromogen. This reaction can indicate blood residues in liquids or on surfaces by a colour change to blue. This peroxidase reaction in blood will still show a positive result even after the influence of heat, alkalines or aldehydes. Oxidizing process chemicals will interfere with this detection method.

J.2 Materials

— **TMB-test,** consisting of:

  — 0,1 % tetramethyl benzidine (TMB) in 5 % acetic acid;

  — 3 % hydrogen peroxide solution.

— **Bovine haemoglobin.**

NOTE An example of a ready-to-use TMB solution is available from suppliers of biochemicals. Other compounds can also be used for the peroxidase reaction (see References [41] and [42]).

J.3 Apparatus

— **Test vials**.

— **Cotton swabs** (peroxidase free).

— **Pipettes,** of 1 ml capacity.

— **Syringes**.

J.4 Test pieces

— **Transparent polytetrafluoroethylene (PTFE) tubes**.

NOTE Naturally contaminated devices and/or simulated devices previously contaminated with blood can also be used.

J.5 Sampling

J.5.1 Direct method

Fill transparent polytetrafluoroethylene (PTFE) tubes with the activated TMB solution (see J.6) in order to detect blood residues inside the lumen. The activated TMB solution can also be brought into direct contact with surfaces by means of a pipette or swab (see J.5.2) in order to detect residues directly on the actual spot.

J.5.2 Swab method

A swab can be used for sampling non-transparent lumens or surfaces. Take care that the swab does not react with the test solution by performing a blind control. If surfaces are dry, the swab is moistened with a drop of water or with 1 % SDS solution.

J.5.3 Rinsing method

Another method for sampling may be rinsing of a lumen using a small amount of 1 % SDS solution (see ISO 15883-1:2005, C.2) and detection of haemoglobin in the eluate by the use of microhaematuria test sticks or similar which are based on the same reaction. See Reference [43].

J.6 Test procedure

Activate 1 ml TMB solution with 4 drops of 3 % hydrogen peroxide solution which is then used to detect blood residues. Blood residues are indicated within seconds by an intense blue colour. Contaminated surfaces (e.g. instruments or test objects like PTFE tubes) will change the colour of the test solution to blue. Tests showed that even 0,1 µg of dried and heat denatured blood will give a colour change easily visible with the activated TMB solution.

Fill the tubes with the test solution by means of a syringe (J.5.1) or apply the test solution directly to the surfaces of instruments using a swab (see J.5.2).

It is recommended to use appropriate concentrations of bovine haemoglobin dissolved in water as colour reference samples. 1 µl of a solution of 100 mg bovine haemoglobin dissolved in 100 ml water will contain 1 µg haemoglobin which will give an intense blue colour. 1 µg of haemoglobin can be applied on a surface or in a tube as a positive control in order to test sampling methods.

NOTE The Peroxidase test can be used to detect blood residues or residues of haemoglobin-containing test soils. However blood residues having been in contact with oxidizing chemicals (for example peracetic acid or hydrogen peroxide) may not be detected because of the destruction of peroxidase activity.

J.7 Acceptance criteria

The process shall be considered acceptable if after following the test procedure in J.6, there is no blue colour observed in the eluate.

If the colour of the activated TBM solution in contact with the surface of the instrument to be tested or with the cotton after swabbing turns to clear blue, the result shall not be accepted.

Using the microhaematuria test sticks detection method for the eluate, a result of more than 10 molecules of haemoglobin per micromillilitre (µl) on reference scale indicate residual soiling.

J.8 Safety considerations

J.8.1 Safe handling of chemicals

The information provided by the manufacturer of chemicals, for example in the Material Safety Data Sheet, shall be applied and the appropriate personal protective equipment worn.

J.8.2 Disposal

All chemicals can be disposed of as non-hazardous waste.

The tested instruments or objects can be reprocessed in a WD. Instruments and objects which had direct contact with the TMB solution or SDS solution should be rinsed with water first.

Annex K
(normative)

Test soil and method for stainless steel items including surgical instruments (Netherlands)

K.1 Reference

See Reference [39].

K.2 Materials

K.2.1 Test soil.

Test soil consisting of:

— **Bovine serum albumin**, fraction 5.

— **Porcine gastric mucin**, type 3.

— **Bovine fibrinogen**, fraction 1.

— **Bovine thrombin**.

— **Phosphate buffer**, 0,05 mol/l (pH = 7,4. 6,85 g/l disodium hydrogen phosphate and 1,6 g/l sodium dihydrogen).

— **Amino black solution** 0,1 %.

K.2.2 Residual protein detection.

— **Sodium dodecyl sulphate** (SDS), 1 % aqueous solution.

— **Ninhydrin reagent**.

— **Swabs**.

K.3 Apparatus

— **Normal laboratory apparatus**.

— **Centrifuge**.

— **Incubator**, capable of being maintained at 104 °C.

K.4 Preparation of test soil

Prepare two protein solutions in 0,05 mol/l phosphate buffer:

— solution A to give a concentration of 1 % albumin, 1 % mucin and 0,2 % fibrinogen;

— solution B to give a concentration of 0,01 % thrombin.

For use with lumen instruments, both solutions should first be centrifuged for 10 min at 4 600 g to remove undissolved particles.

K.5 Storage

The prepared solution may be stored refrigerated for a week.

Shake well before use.

K.6 Test pieces

Devices that are representative of the loads to be processed shall be used.

K.7 Inoculation of test pieces

Both protein solutions shall be applied in equal amounts.

Apply solution A on the test pieces and allow them to dry for 2 h at 104 °C. Subsequently apply solution B on the test pieces and allow them to dry for 2 h at 104 °C. Then apply amino black solution on the test pieces and leave for 30 min. Rinse the test pieces with water and allow them to dry for 2 h at 104 °C.

Whenever practicable, inoculate the devices by immersion in the test soil. Ensure that all surfaces of the devices are covered with the test soil. Special attention shall be paid to box joints and corrugated surfaces.

When the test soil is used on the chamber walls, it shall be allowed to dry at ambient temperature.

Inoculate instruments with lumens by sucking the test soil through the internal parts of the device during a period of at least 30 min. Colouring with amino black may be omitted.

K.8 Test method

Process the test pieces in the WD in accordance with the manufacturer's instructions.

K.9 Results

K.9.1 Detection of residual soil

After exposure to the cleaning process, check the test pieces for visual contamination.

Check accessible surfaces of the test pieces for residual protein by swabbing and visualising protein residues using ninhydrin reagent. The method used should be validated and it should be demonstrated that it is capable of detecting at least 8 µg of bovine serum albumin on a glass surface. (See ISO 15883-1:2005, C.1).

Check the inner surface of lumen instruments by eluting the lumen with a 1 % solution of SDS at ambient temperature for a period of not less than 15 min. The volume of eluate shall be as low as practicable for the dimensions of the device under test. Measure the protein content of the eluate using a validated technique capable of detecting at least 10 µg protein/ml (calibrated using bovine serum albumin. (See ISO 15883-1:2005, C.2).

NOTE The limits given (8 µg/ml and 10 µg/ml) refer to the sensitivity of the test method and not to the concentration of protein per unit surface area of the cleaned device.

K.9.2 Acceptance criteria

The process shall be deemed to be acceptable if:

— no visual contamination is present;

— the swab test does not give more than 3 % positives (to allow for false positives);

— the amount of protein in the eluate from lumen instruments is less than the detection level.

K.10 Safety considerations

K.10.1 Personal protective equipment

When preparing the test soil, inoculating the test pieces, loading the inoculated test pieces into the WD or examining the processed devices for residual protein, the operator should wear a protective gown, surgical gloves, eye protection and a face mask.

Use heat protective gloves for the removal of the inoculated test pieces from the incubator.

K.10.2 Disposal

All chemicals, and test soils can be disposed of as non-hazardous, non-clinical waste.

K.10.3 Environmental spillage

Environmental surfaces that have been soiled with test soil shall be wiped clean using a cloth moistened with a solution of an appropriate detergent/disinfectant in accordance with local policies and procedures.

Annex L
(normative)

Test soil and method for surrogate devices for endoscope channels (Netherlands)

L.1 Reference

See Reference [40].

L.1.1 Test soil

Test soil consisting of:

— **Bovine serum albumin**.

— **Porcine mucin**.

— **Bovine thrombin**.

— **Bovine fibrinogen**.

— **Phosphate buffer** (pH = 7,4, 1,32 g/l sodium dihydrogen phosphate, 5,4 g/l disodium hydrogen phosphate).

— **Amido black stain**, ρ = 0,1 % in 25 % aqueous isopropanol and 10 % aqueous acetic acid.

L.1.2 Residual protein detection

— **Ninhydrin reagent**.

— **Swabs**.

L.2 Apparatus

— **Normal laboratory apparatus**.

— **Incubator**, capable of being maintained at 104 °C.

L.3 Preparation of test soil

Mix four separately prepared protein solutions to give the test soil in phosphate buffer. Dissolve thrombin and fibrinogen by heating the solution up to 40 °C. The final solution consists of mass concentration of 1 % bovine serum albumin, 1 % porcine mucin, 0,2 % bovine fibrinogen and 0,2 % bovine thrombin.

L.4 Storage

The shelf life is 1 week when stored at room temperature.

Shake the solution well before use.

L.5 Test pieces

The surrogate device consists of authentic trumpet valve cylinders in combination with three tubes of polytetrafluoroethylene (PTFE), simulating the water channel (inner diameter of 2 mm, length 1 500 mm in both directions), the air channel (inner diameter of 2 mm, length 1 500 mm in both directions) and the biopsy/suction channel (inner diameter 4 mm, length 1 500 mm in both directions, 100 mm tube between the biopsy port and the suction valve). Add a separate tube to simulate the elevator channel (inner diameter 1 mm, with a stainless steel wire with an outer diameter of 0,7 mm, length 2 000 mm).

L.6 Inoculation of test pieces

Connect the three channels of the surrogate device to a peristaltic pump and circulate the test soil through the channels for 2 min. After the circulation, reverse the flow and press the test soil out of the tubes. Drain the tubes of the surrogate device and subsequently dry them in an incubator for 2,5 h at 104 °C positioned horizontally on absorption paper.

After this period, check the tubes for remaining moisture. If moisture is still present, prolong the drying period. Repeat the soiling procedure once.

Colour the protein soil inside the tubing with amido black stain, by sucking it into the tubes with a peristaltic pump and leave it to rest in the tubes for 2 min. After this period, reverse the flow and press the amido black stain solution out of the tubes. Dry the tubes for 2,5 h in an incubator at 104 °C and finally flush with compressed air.

The inoculated surrogate device may be used for a maximum period of 4 weeks.

L.7 Verification of test soil

To assess the degree of fixation of the test soil, soil three separate tubes and dry them according the soiling procedure. Evaluate the quality of the test soil using three rinsing fluids: distilled water, 1 % SDS and 2 mol/l sodium hydroxide. The test soil fulfils the requirements if:

— rinsing with distilled water for 3 min shows no removal of test soil;

— rinsing with SDS removes part of the test soil (25 % to 75 %) by visual check;

— rinsing with NaOH removes all of the test soil.

Red to brown residues of the test soil may remain in the tube even after rinsing with NaOH. This may indicate that the drying temperature may be too high or that the drying period may be too long. One should experiment with the drying conditions to find the optimum drying conditions. Alternatively drying may be performed under vacuum or by blow through.

L.8 Test method

Process the inoculated surrogate device in the WD in accordance with the manufacturer's instructions.

L.9 Results

L.9.1 Detection of residual soil

Inspect the tubing for the presence of any visible contamination.

Disassemble the tubing and swab the area of the PTFE tube. Examine the swab for visible contamination. If no contamination is visible, check the swab for protein with ninhydrin (see ISO 15883-1:2005, C.1).

L.9.2 Acceptance criteria

The process shall be acceptable if:

— no contamination is visible on the inside of the PTFE tubes; and

— the swabs are clean and give no reaction with ninhydrin.

L.10 Safety considerations

L.10.1 Personal protective equipment

When preparing the test soil, inoculating the test pieces, loading the inoculated test pieces into the WD or examining the processed devices for residual protein the operator should wear a protective gown, surgical gloves, eye protection and a face mask.

Heat protective gloves for removing the surrogate devices from the incubator.

L.10.2 Disposal

All chemicals, and test soils can be disposed of as non-hazardous, non-clinical waste.

L.10.3 Environmental spillage

Environmental surfaces that have been soiled with test soil shall be wiped clean using a cloth moistened with a solution of an appropriate detergent/disinfectant in accordance with local policies and procedures.

Annex M
(normative)

Test soils and methods for surgical instruments, wash bowls, bedpans, urine bottles, anaesthesia equipment, baby bottles and suction bottles (Sweden)

M.1 Reference

SIS – TR 3:2002, see Reference [24].

M.2 Materials

M.2.1 Test soils.

M.2.1.1 Citrated blood.

Blood from live, healthy cows, with an addition of 3,8 g sodium citrate per 1 000 ml blood.

M.2.1.2 Diluted citrated blood.

Citrated blood (M.2.1.1) diluted with an equal amount of saline solution (9 g NaCl/l).

M.2.1.3 Deionized water.

M.2.1.4 Soap solution, a solution of 1 part per mass soap and 99 parts per mass deionized water.

The water should be heated to approximately 40 °C for the soap to be easily dissolved.

The soap should be of the “superfatted” type.[12)]

M.2.1.5 Calcium chloride solution, 250 mmol/l $CaCl_2$ in distilled water.

M.2.2 Detergent, to be chosen by the tester from the manufacturer’s list of recommended products for the WD under test.

M.3 Apparatus

— **Low, rectangular flat-based bowl**, approximately 150 mm × 200 mm bottom surface.

— **Flat brush**, width approximately 35 mm.

— **Graduated measuring cylinder**, of 25 ml capacity, and measuring error ± 0,5 ml.

12) Guidance on suitable commercially available products may be obtained from SIS, Swedish Standards Institute, St. Paulsgatan 6, 118 80 Stockholm, Sweden.

M.4 Preparation of test soil

Use the citrated blood (M.2.1.1 or M.2.1.2) between the fifth and the fourteenth day after drawing. Prepare the soap solution (M.2.1.4) at ambient temperature.

M.5 Storage

Citrated blood shall be stored refrigerated at +2 °C to +6 °C.

M.6 Test pieces

M.6.1 General.

For a complete test at least five trays with surgical instruments and/or at least six of each of the other test objects should be tested on each five separate runs for each type of load.

M.6.2 Surgical instruments.

One instrument tray with:

— 20 Mayo scissors, curved, 14 cm, stainless steel, polished clean, not lubricated;

— 20 forceps, Crile, curved, 14 cm, stainless steel, polished clean, not lubricated.

Interiors of box-locks should be polished so that sharp scratches, burrs, edges, etc., are smoothed.

M.6.3 Wash bowls, having a diameter of 320 mm.

M.6.4 Bedpans, size A, with lid.

M.6.5 Urine bottles, propene or similar, with lid.

M.6.6 Anaesthesia equipment.

Anaesthesia set containing:

— 2 wrinkled rubber hoses, length 1 050 mm, diameter 22 mm.

— 1 wrinkled rubber hose, length 1 050 mm, diameter 26 mm.

— 1 mask, simple, rubber, size 3.

— 1 bellow, rubber, 2 l.

— 1 surplus valve.

— 1 Y-piece.

M.6.7 Baby bottles.

— **Baby bottles, polycarbonate** or similar, 300 ml, complete with teat of natural rubber and ring and cover of polyamide or similar.

— **Baby bottles, glass**, 200 ml, with teat of natural rubber.

M.6.8 Suction bottles.

— **Suction bottles 1 l**, sulphonate or similar, diameter 90 mm, height 170 mm.

— **Suction bottles 2 l**, sulphonate or similar, diameter 135 mm, height 235 mm.

— **Suction bottles 5 l**, sulphonate or similar, diameter 165 mm, height 335 mm.

M.7 Inoculation of test pieces

M.7.1 General

Perform all inoculations with test soils containing citrated blood (M.2.1.1 or M.2.1.2) at 20 °C to 22 °C, and under such conditions (air humidity and air circulation) that the blood will not at all, or only where it has formed a thin film, have dried within 2,5 h.

Perform the inoculation with the soap solution (M.2.1.4) at ambient temperature.

M.7.2 Surgical instruments

Pour citrated blood (M.2.1.1) at room temperature into a bowl. Add $CaCl_2$ solution (M.2.1.5) to a calcium ion content in the final solution of 2,5 mmol/l. The required quantity of $CaCl_2$ solution should be defined in preliminary tests where the blood is titrated by $CaCl_2$ solution and the calcium ion content is determined by ion selective electrometrics. Mix carefully.

Immerse the instruments (M.6.2) completely in the mixture. Cover all surfaces, including joints, by the mixture by opening and closing the instruments under the surface. Then take up the instruments and put them horizontally and randomly on the instrument tray (M.6.2). All instruments should be so prepared and arranged within 15 min. Leave the instruments on the tray to dry for 2,5 h.

M.7.3 Wash bowls

Pour 1 l deionized water at approximately 40 °C into the wash bowl (M.6.3). Add 150 ml soap solution (M.2.1.4) at the same temperature to the water. The temperature of the final soap solution should be (40 ± 3) °C. Whip to obtain a foam so that the wash bowl is filled to the brim.

Add 30 ml $CaCl_2$ solution (M.2.1.5) in portions of approximately 10 ml by stirring. After each portion is added, splash the mixture about in the bowl and around the brim. After the last addition of $CaCl_2$ the mixture forms a thick flocculent deposit.

Let the wash bowl with its content stand for 10 min. Then slowly pour the mixture out of the wash bowl while turning it round so that the thick flocculent deposits on the brim are flushed off.

Let the wash bowl air dry for 30 min in a vertical position.

When the wash bowl is dry, it shall have a remaining grey film including fine deposits.

M.7.4 Bedpans

Mix a suitable volume of citrated blood (M.2.1.1) with a $CaCl_2$ solution (M.2.1.5) as in M.7.2. Stir the mixture thoroughly. Pour it into bedpans (M.6.4) and spread it with a brush on the interiors and the upper part of the brim. The remaining quantity of the test soil on the surface of the bedpan should be 12 ml to 14 ml, any surplus is poured out of the bedpan. All test objects in a test run should be so treated within 15 min. All test bedpans in the run should be air-dried for approximately 2,5 h in a horizontal position.

M.7.5 Urine bottles

Mix a suitable volume of diluted citrated blood (M.2.1.2) with $CaCl_2$ solution (M.2.1.5) as in M.7.2. Stir thoroughly. Pour the mixture into a urine bottle (M.6.5). Put the lid on to the bottle and turn the bottle around so that its inside is totally covered with the blood mixture. Pour the remaining blood mixture into the next urine bottle to be prepared, and handle it in the same way. All urine bottles tested simultaneously shall be prepared within 15 min. Then let the bottles air dry for approximately 2,5 h without their lids and positioned vertically with the openings downwards.

M.7.6 Anaesthesia equipment

Mix a suitable volume of citrated blood (M.2.1.1) with $CaCl_2$ solution (M.2.1.5) as in M.7.2. Smear the inside and the outside of the mask (M.6.6) with the blood mixture with the help of a brush. Do not inoculate the other parts of the set.

Inoculate all masks tested simultaneously within 15 min. Let them air-dry for approximately 2,5 h.

M.7.7 Baby bottles and suction bottles

Prepare in the same manner as for urine bottles (M.7.5).

M.8 Test method

M.8.1 General

Perform the test in an environment at ambient temperature.

Select data for services to the WD to be tested (water pressure, water temperature, voltage, etc.) at the lower limits specified by the manufacturer in order to create the most unfavourable conditions acceptable.

Start all tests with the test objects soiled with citrated blood (M.2.1.1 or M.2.1.2) within 15 min after drying is completed.

When tested, fill the WD with items for which the programme under test is intended by the manufacturer to the maximum load specified by the manufacturer.

M.8.2 Surgical instruments

Load the WD with the test instruments on the tray. If the WD can take additional instrument trays, fill it with clean surgical instruments on the trays according to the manufacturer's instructions. If the WD can take more than four but less than nine instrument trays simultaneously, two of these should be with test instruments according to M.7.2. If it can take more than eight trays simultaneously, three of these should be with test instruments according to M.7.2.

Run as many tests as required to test all possible positions of the test tray(s) (or at least five runs).

M.8.3 Wash bowls

Load the wash bowls prepared according to M.7.3 into the WD according to the manufacturer’s instructions. If the WD can take additional goods, fill it with clean items according to the manufacturer's instructions.

Run the test sufficient times that a minimum of 30 test items have been tested (at least five runs).

M.8.4 Bedpans

Perform the test as for wash bowls (M.8.3). If the WD also takes bedpan lids, use those for filling up the WD if necessary.

M.8.5 Urine bottles, anaesthesia equipment, baby bottles and suction bottles

Perform the test as for wash bowls (M.8.3).

M.9 Results

M.9.1 Detection of residual soil

Upon completion of the test, examine the cleanliness of all test items visually. The number of clean (no remains of blood or other soil visible to the naked eye at normal light with any optical correction required for normal visual acuity) and not clean instruments is counted and documented. Do not consider items other than the inoculated test items. Do not consider lids to inoculated bedpans or urine bottles.

On surgical instruments, pay special attention to box-locks and joints.

M.9.2 Acceptance criteria

For the cleaning efficiency of the WD to be deemed acceptable, at least 95 % of all test objects for each test object type under test should be clean.

M.10 Safety considerations

M.10.1 Protective attire

Use a protective gown, surgical gloves, eye protection and a face mask when preparing the test soil, inoculating the test pieces and loading them into the WD.

M.10.2 Disposal of chemicals, blood and used disposables

All chemicals, blood and disposable items used can be disposed of in the normal way, unless national requirements stipulate special methods of disposal.

M.10.3 Cleaning of environment

Environmental surfaces that have been visibly soiled with blood should be wiped clean with a cloth (cloths) moistened with an appropriate detergent-disinfectant in accordance with local, regional or national rules or guidelines for the use of detergents and disinfectants.