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TECHNICAL SPECIFICATION

colour inside

Electric dishwashers for household use – Methods for measuring the microbiological efficacy of the dishwashing process

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INTERNATIONAL ELECTROTECHNICAL COMMISSION

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ELECTRIC DISHWASHERS FOR HOUSEHOLD USE – METHODS FOR MEASURING THE MICROBIOLOGICAL EFFICACY OF THE DISHWASHING PROCESS

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IEC TS 63331 has been prepared by subcommittee 59A: Electric dishwashers, of IEC technical committee 59: Performance of household electrical appliances. It is a Technical Specification.

The text of this Technical Specification is based on the following documents:

Draft	Report on voting
59A/262/DTS	59A/267/RVDTS

Full information on the voting for its approval can be found in the report on voting indicated in the above table.

The language used for the development of this Technical Specification is English.

This document was drafted in accordance with ISO/IEC Directives, Part 2, and developed in accordance with ISO/IEC Directives, Part 1 and ISO/IEC Directives, IEC Supplement, available at www.iec.ch/members_experts/refdocs. The main document types developed by IEC are described in greater detail at www.iec.ch/publications.

Words in **bold** in the text are defined in Clause 3.

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- reconfirmed,
- withdrawn, or
- revised.

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ELECTRIC DISHWASHERS FOR HOUSEHOLD USE – METHODS FOR MEASURING THE MICROBIOLOGICAL EFFICACY OF THE DISHWASHING PROCESS

1 Scope

This document applies to electric **dishwashers** for household and similar use that are supplied with hot and/or cold water.

This document deals with measurement procedures regarding the reduction of microbial contamination resulting from the use of electric **dishwashers** for household and similar use.

This document specifies methods that enable reproducible measurements. These derived measurement results can only be used for a relative statement. Absolute statements, i.e. health-related claims or conclusions about prevention or treatment of a disease or health improvement, are reserved for explicit regulatory action after a medical assessment.

This document does not apply to appliances intended to be used in medical, veterinary, or pharmaceutical applications.

This document does not address sanitization, disinfection or sterilization measures.

The dishwashing process is a complex **operation** consisting of cleaning dish items and cleaning the **dishwasher** itself. This document only focusses on the dish items to be cleaned.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

IEC 60436:2015, *Electric dishwashers for household use – Methods for measuring the performance*
IEC 60436:2015/AMD1:2020[1]

ISO 607:1980, *Surface active agents and detergents – Methods of sample division*

ISO 15883-1:2006, *Washer-disinfectors – Part 1: General requirements, terms and definitions and tests*

ISO/TS 15883-5:2005[2], *Washer-disinfectors – Part 5: Test soils and methods for demonstrating cleaning efficacy*

ISO 19458:2006, *Water quality – Sampling for microbiological analysis*

NSF/ANSI 184-2019, *Residential Dishwashers*

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[1] A consolidated version of this document exists, comprising IEC 60436:2015 and IEC 60436:2015/AMD1:2020.

[2] This standard has been revised by ISO 15883-5:2021 but the listed edition applies.

EN 10088-1:2014[3], *Stainless steels – Part 1: List of stainless steels*

3 Terms, definitions, symbols and abbreviated terms

3.1 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- IEC Electropedia: available at https://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp

3.1.1
dishwasher
machine that cleans, rinses and dries **tableware** by chemical, mechanical, thermal and electric means

[SOURCE: IEC 60436:2015, 3.1.1, modified – The notes to entry have been omitted.]

3.1.2
microbiological efficacy
antimicrobial action created by the properties of the appliance

3.1.3
test machine
dishwasher under test

[SOURCE: IEC 60436:2015, 3.1.5]

3.1.4
test run
single **cycle** performance assessment of a selected test programme

3.1.5
test series
set of **test runs** which are collectively used to assess the performance

[SOURCE: IEC 60436:2015, 3.1.8]

3.1.6
tableware
dishware, glassware, cutlery and **serving pieces** used according to this standard to test a **dishwasher**

[SOURCE: IEC 60436:2015, 3.1.9]

3.1.7
operation
each event that occurs during the **dishwasher programme** such as cleaning, rinsing or drying

[SOURCE: IEC 60436:2015, 3.1.13]

[3] Withdrawn.

3.1.8
programme
series of **operations** which are pre-defined within the **dishwasher** and which are declared as suitable for specified levels of soil and/or type of load

Note 1 to entry: Usually, an end of programme indicator signals the end of the programme and the user has access to the load.

[SOURCE: IEC 60436:2015, 3.1.14 and IEC 60436:2015/AMD1:2020, 3.1.14]

3.1.9
automatic dispenser
device activated automatically which injects or dispenses **detergent** or **rinse aid**, one or more times into the **dishwasher** at predetermined points in the **dishwasher programme**

[SOURCE: IEC 60436:2015, 3.1.18]

3.1.10
water softener
device which reduces the hardness of water

[SOURCE: IEC 60436:2015, 3.1.20]

3.1.11
rack
support for holding dishware, cutlery, and/or glassware in the **dishwasher**

[SOURCE: IEC 60436:2015, 3.1.22]

3.1.12
place setting
set of **tableware** for the use by one person, not including **serving pieces**

Note 1 to entry: A **place setting** is comprised of different items used for breakfast and lunch (type A); and dessert and dinner (type B). For details, see IEC 60436:2015 and IEC 60436:2015/AMD1:2020.

[SOURCE: IEC 60436:2015, 3.1.10, modified – Note 1 to entry has been modified to add reference to IEC 60436.]

3.1.13
serving pieces
set of items for preparation and serving of food which can include pots, serving bowls, serving cutlery and a platter

[SOURCE: IEC 60436:2015, 3.1.11]

3.1.14
rated dishwasher capacity
whole number of **place settings** together with the **serving pieces** which can be cleaned and dried in one **cycle** when loaded in accordance with the manufacturer's instructions

Note 1 to entry: The **rated dishwasher capacity** is declared by the manufacturer and expressed as a number of **place settings**.

[SOURCE: IEC 60436:2015, 3.1.12]

3.1.15
detergent
cleaning agent for use in **dishwashers** to aid in the removal of food soils by chemical means

Note 1 to entry: A reference **detergent** in powder form is specified for use in IEC 60436:2015, 5.7.

[SOURCE: IEC 60436:2015, 3.1.23, modified – Note 1 to entry has been modified to clarify the reference to IEC 60436.]

3.1.16
rinse aid
chemical agent added to the water in the last rinsing **operation** to improve the drying effect and reduce water marks

Note 1 to entry: A reference **rinse aid** is specified for use in 60436:2015, 5.8.

[SOURCE: IEC 60436:2015, 3.1.24, modified – Note 1 to entry has been modified to clarify the reference to IEC 60436.]

3.1.17
refrigerated
storage of foods at a temperature of (4 ± 3) °C

[SOURCE: IEC 60436:2015, 3.1.32]

3.1.18
freeze
storage of foods at a temperature of (−18 ± 3) °C

[SOURCE: IEC 60436:2015, 3.1.33]

3.1.19
filter
device in the sump of the **dishwasher** to separate soils out of the dishwashing solution

3.1.20
temperature profile
temperature data collected in the **dishwasher** over the duration of the **programme**

3.1.21
demineralized water
water that has been depleted of the salt content by reverse osmosis or ion exchange processes

Note 1 to entry: E.g. by ion exchange or reverse osmosis processes.

3.1.22
bio indicator
standardized test object which has been contaminated with an embedding matrix and test bacteria and is used for checking the microbial efficacy of **dishwashers**

3.1.23
stainless steel strips
standardized test object which is intended to be contaminated with an embedding matrix and test bacteria

3.2 Symbols and abbreviated terms

CFU colony forming unit

N_0 average value of microorganism amount of the three positive controls, before dishwashing (CFU/**bio indicator**)

N average value of microorganism amount per x **bio indicators**, after dishwashing (CFU/**bio indicator**)

RF reduction factor

v^{-1} microorganism amount in a 10 times diluted solution

v^{-x} microorganism amount in a 10^x times diluted solution

s_{N_0} standard deviation of N_0

s_N standard deviation of N

4 List of measurements

This document describes test methods for the measurement of the following parameters:

a) capability to reduce microorganisms in a dishwashing process;

b) microbial inactivation equivalent unit as a simplified method for assessing the reduction of microorganisms under well-defined conditions without performing microbiological tests.

WARNING:

The tests given in this document shall be performed by expert staff trained to handle microorganism-related techniques and in properly equipped laboratories under the supervision of a skilled microbiologist. Some of the test micro-organisms can be facultative pathogens for humans, animals and plants; their handling requires a laboratory of an appropriate biosafety level. National and international safety procedures for working with infectious biomaterials exist to prevent any contamination of laboratory staff, apparatus, the workplace or the environment.

This document does not purport to address all the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national, regional or international regulatory conditions.

5 Test conditions, materials and reagents

5.1 General information

Each dishwashing process and the resulting hygienic condition of the dishes are determined by the interaction of the following main factors:

a) Machine related parameter:
   - **temperature profile** of the **programme**;
   - contact time;
   - mechanics;
   - water quality and volume;
   - treatment agents;
   - rinsing;
   - drying.

b) Procedure related parameter:

- type and quantity of the introduced soil as well as length of time for which and the temperature at which they have been allowed to dry on the dishes;
- shape, surface characteristics, design, degree of soiling and quantity on the dishes;
- shape of the dishes and its loading;

NOTE Due to the interdependency of all the listed parameters, it is crucial to follow the prescribed procedure in the following subclauses. To reach a high repeatability within one laboratory and a high reproducibility between different laboratories, the conditions need to be as similar as possible.

5.2 General purpose and procedure

The purpose of this test is to measure the reduction of the microbial load of **bio indicators** achieved by a selected **dishwasher programme**.

The tests are carried out under conditions described in 5.3. The **test machine** shall be prepared according to 5.4 using a load specified in 5.4.1. The ballast soil to be used during the test is described in 5.4.3. The **bio indicators** shall be prepared according to 6.4. The loading and **operation** of the **dishwasher** is specified in 6.6 and the evaluation is specified in Clause 7.

5.3 Test conditions

5.3.1 General requirements

For all requirements regarding electricity supply, see IEC 60436:2015. Deviations shall be reported in the test report.

5.3.2 Ambient conditions

It is recommended to run the **test machine** in a controlled climate ((23 ± 2) °C ambient temperature and (55 ± 5) % ambient relative humidity) and to record and report the ambient conditions.

For the soiling and preparation of the **stainless steel strips**, similar conditions to the ones mentioned above are recommended.

For the drying of the **bio indicators**, the conditions are described under 6.4.4.

5.3.3 Water conditions

5.3.3.1 Water quality

The inlet water should contain less than 100 CFU/mL at 30 °C. Microorganisms for test purposes as listed in 5.4.8.1 shall not be present in the water. Water sampling shall be done according to ISO 19458 as outlined for the assessment of water quality in the main distributor. The water shall be sampled close to the connecting valve of the **dishwasher**. Aerator and O-rings of the sampling point shall be removed and the water tap disinfected. Water tap shall be rinsed until water temperature is constant before sampling.

5.3.3.2 Water temperature

For the tests cold water as described in IEC 60436:2015, IEC 60436:2015, 5.6.2 is recommended. Deviations shall be reported in the test report.

5.3.3.3 Water quantity

For a valid **test run**, the amount of water in three **test runs** should not exceed a standard deviation of 5 %. In this calculation, intermittently recurring functions according to IEC 60436:2015 are excluded.

5.4 Test equipment and material

5.4.1 Load

The load shall consist of the maximal number of **place settings** as specified by the manufacturer. It is defined in IEC 60436:2015.

The load shall be clean and without imperfection. Dish items shall not be soiled, the ballast soil will be added separately (see 5.4.3).

The composition of test load is described in 6.2.

5.4.2 Test machine

The **dishwasher** manufacturer's instructions regarding installation and use of the **dishwasher** shall be followed.

Manufacturers should provide sufficient information on relevant test conditions for the **test machine**, including installation instructions, **detergent** amounts, **rinse aid** settings, **water softener** settings (if applicable), **filter** type, and loading schemes.

5.4.3 Ballast soil

To simulate the total soil burden of a fully loaded **dishwasher** according to IEC 60436, one portion of frozen ballast soil (100 g for a **dishwasher** ≥ 10 **place settings** and 60 g for < 10 **place settings**) is added to the **dishwasher** load. The ingredients are milk, minced meat, egg, oat flakes, spinach, and margarine according to IEC 60436.

The recipe for a 10 kg ballast soil is specified in Table 1.

Table 1 – Recipe for production of ballast soil

Ingredient		Mass g	
Porridge			2 940
	Oat flakes	141	
	Milk	700	
	Water	2 100	
Margarine			840
Minced spinach			2 870
Minced meat mixture			1 540
	Minced beef	1 155	
	Whole egg (pasteurised)	385	
Egg yolk (pasteurised)			1 960
		Sum	10 150
		~Sum (after evaporation of water during cooking)	~10 000

The preparation steps of the ballast soil are as follows:

a) Mix oat flakes with water and milk. Prepare porridge by bringing the mixture to the boiling point.
b) Add the minced meat mixture and minced spinach and allow to simmer for 10 min.
c) Turn off the heat and add the margarine stirring well.
d) Add the pasteurized egg yolk when the complete mixture is cooled down to a temperature of about 40 °C and stir well.
e) If total mass is less than 10 kg, add water.
f) Fill appropriate portions in containers and **freeze** it at −18 °C.

The frozen ballast soil is placed under a cup/mug/glass **in the front area of the rack**.

5.4.4 Detergent

5.4.4.1 General

The **detergent** to be used is IEC reference **detergent** D according to IEC 60436:2015.

The **detergent** shall be stored in a waterproof container in quantities of no more than 1 kg in a cool and dry atmosphere. It shall be used within six months after production. Before use, the **detergent** shall be homogenized in accordance with ISO 607.

Alternative **detergents** may be used for a specific test aim. Type and name of the **detergent**, recipe (if available) and dosage shall be listed in the test report.

5.4.4.2 Dosage in test run

The quantity of **detergent** for one **test run** shall be 8 g + 1 g per **place setting**. The quantity of **detergent** used during the tests shall be reported.

The **detergent** shall be placed in the **dishwasher** immediately prior to starting the **test run** in the locations specified by the manufacturer. If a **dispenser** is fitted, some or all of the **detergent** dose shall be placed in it according to the manufacturer's instructions. The **dispenser** shall be clean and dry prior to the placement of **detergent**. In the absence of manufacturer's recommendations, the **detergent** shall be placed in the main compartment of the **dispenser**. Alternative dosages may be used for a specific test aim. The dosage shall be listed in the test report.

5.4.5 Rinse aid

The **rinse aid** Formula "III" according to IEC 60436 shall be used.

For **dishwashers** with an adjustable **automatic dispenser**, the setting shall be as recommended by the manufacturer. In the absence of such an indication, the setting shall be used which gives the lowest quantity of **rinse aid**. Any requirement or recommendation to experiment with the setting by the laboratory shall be ignored. For machines without **automatic dispensers**, the **rinse aid** shall be added manually, if so recommended by the manufacturer and in accordance with their instructions.

NOTE Details of a supplier of suitable test materials like **detergent** or **rinse aid** are given in Annex C.

5.4.6 Salt

If the **dishwasher** is equipped with a **water softener** that requires salt, fill the salt reservoir in accordance with the manufacturer's instructions. The salt shall have a purity of > 99,4 % NaCl and insoluble components < 0,05 %.

For **dishwashers** with an adjustable **water softener**, the setting shall be as recommended by the manufacturer for the water hardness used for the test. Where there is no recommendation, use the lowest setting.

5.4.7 General microbiological laboratory equipment

5.4.7.1 General

Test conditions, materials, equipment and instrumentation shall be handled in compliance with good laboratory and microbiology practice.

5.4.7.2 Incubator

The incubator shall be capable of maintaining a constant temperature of 30 ± 1 °C for incubations performed during a test and its control and validation.

5.4.7.3 Autoclave

The autoclave shall be capable of sterilizing equipment and supplies by subjecting them to saturated steam at 121 $^{+3}_{-0}$ °C for at least 15 minutes or at 134 $^{+3}_{-0}$ °C for at least 5 minutes.

5.4.7.4 Test tubes

Test tubes for the extraction of microorganisms from the **bio indicators** shall have dimensions of approximately 160 mm (length) × 16 mm (diameter).

5.4.7.5 Pipettes

Pipettes shall have an adequate nominal volume of 0,1 to 10,0 mL.

5.4.7.6 Electromechanical agitator

A commonly used agitator is the Vortex®[4] mixer.

5.4.7.7 Centrifuge, centrifuge tube

The centrifuge shall be capable of maintaining and be used at a relative centrifugal force of 4 500 g to 5 000 g with 50 mL centrifuge tubes (sterile).

5.4.7.8 Ultrasonic bath

The ultrasonic bath shall have an adequate size for **stainless steel strips/bio indicators** and an operating time of three minutes shall be adjustable.

5.4.7.9 Tilt/roller mixer

The tilt/roller mixer shall be capable of maintaining a controlled speed of 80 r/min. It shall be adequate to extract at least four test tubes simultaneously.

5.4.7.10 PH-meter

The pH-meter should have an inaccuracy of calibration of no more than ± 0,1 pH units (at 20 °C ± 1 °C).

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4 Vortex® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by IEC of this product.

5.4.7.11 Glass beads

Sterile glass beads for extraction shall have a diameter of 3 mm.

5.4.8 Specific microbiological equipment

5.4.8.1 Test strain

For the test purpose, the following non-pathogenic microorganism shall be used:

Micrococcus luteus ATCC 10240 (DSM 1790)

Additional microorganisms may be used, if proven to deliver differentiating results.

NOTE ATCC = American Type Culture Collection; DSM = Deutsche Sammlung von Mikroorganismen (German Collection of Microorganisms).

5.4.8.2 Culture media and solutions

5.4.8.2.1 Culture media

All media and solutions shall be of microbiology grade and sterilized appropriately prior to use. It is recommended to use commercially available and/or water free dry materials for the culture media.

5.4.8.2.2 Tryptic soy agar (TSA)

The composition of tryptic soy agar (TSA) shall be according to Table 2.

Table 2 – Composition of tryptic soy agar

Description	Specification
Casein peptone (pancreatic digest)	15,0 g/L
Soy peptone (papaic digest)	5,0 g/L
Sodium chloride	5,0 g/L
Agar	15,0 g/L
Water (5.4.8.3)	To 1,0 L
Final pH	7,3 ± 0,2

5.4.8.2.3 Tryptic soy broth (TSB)

The composition of tryptic soy broth (TSB) shall be according to Table 3.

Table 3 – Composition of tryptic soy broth

Description	Specification
Tryptone, pancreatic digest of casein	17,0 g/L
Soy peptone, papaic digest of soybean meal	3,0 g/L
Sodium chloride (NaCl)	5,0 g/L
Dipotassium hygrogen phosphate (K_2HPO_4)	2,5 g/L
Glucose	2,5 g/L
Water (5.4.8.3)	To 1,0 L
Final pH	7,3 ± 0,2

5.4.8.3 Water for culture media and solutions

Water may be bi-distilled or **demineralized**. However, the water shall be sterilised prior to use.

5.4.8.4 Diluting agent

As diluting agent for the dilution series physiological sodium chloride solution shall be used. The composition is given in Table 4.

Table 4 – Composition of sodium chloride solution

Description	Specification
Sodium chloride (NaCl)	9,0 g/L
Water (5.4.8.3)	To 1,0 L

5.4.8.5 Neutralisation solution

For the retrieval of test microorganisms after a **test run**, neutralisation solution shall be used in combination with TSB. The composition of a recommended neutralisation solution is given in Table 5.

Table 5 – Composition of neutralisation solution

Description	Specification
Tryptic soy broth	As given in Table 3
"Polysorbate 80" (Tween 80)	30,0 g/L
Lecithin	3,0 g/L
L-Histidine	1,0 g/L
Sodium thiosulphate	5,0 g/L
Water (5.4.8.3)	To 1,0 L

Other neutralisation solutions may be used. The neutralisation solution shall be validated for the tests.

Sterilize the neutralisation solution after preparation. The solution can be kept in the refrigerator for up to 2 months after sterilisation. Alternatively, the freshly prepared solution can be sterilized in extraction tubes directly.

5.4.8.6 Embedding matrix

The used embedding matrix BAMS, a mixture of bovine albumin, mucin and maize starch, is made up as follows:

– 0,6 % bovine albumin (mass fraction),
– 1,0 % mucin (mass fraction),
– 3,0 % maize starch (mass fraction).

For maize starch, use regular starch consisting of 20 % to 25 % amylose and 70 % to 80 % amylopectin from natural cultivation (use as a filler and/or carrier in the pharmaceutical industry). Maize starch shall be used. Refer to CAS 9005-25-8 for the purity and quality standards to be used.

The preparation of BAMS is described in 6.4.2.

5.4.8.7 Stainless steel strips

The stainless steel strip (see Figure 1) is a test specimen which is made of stainless steel with a surface ground to a specified grade, having one drill hole to the left and one to the right of the contamination area for fastening to holders (see Figure 2). It is contaminated with an inoculum of bacteria in an embedding matrix. The prepared **bio indicator** shall not be used for more than 200 **test runs**.

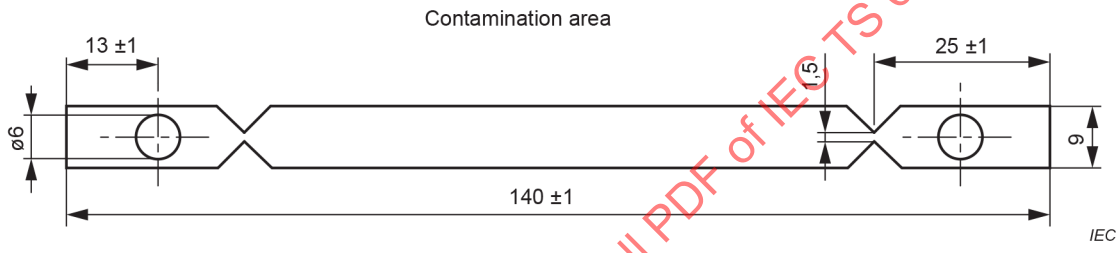
Bio indicators shall not be used if there are scratches or other changes on the surface.

Austenitic steel, material no. 1.4301 as specified in EN 10088-1, thickness: 1 mm.

Surface: 80 grit longitudinally ground.

NOTE Austenitic steel, material no. 4301-304-00-1 as specified in ISO 15510 can be used as substitute, but the longevity could be affected due to subtle differences.

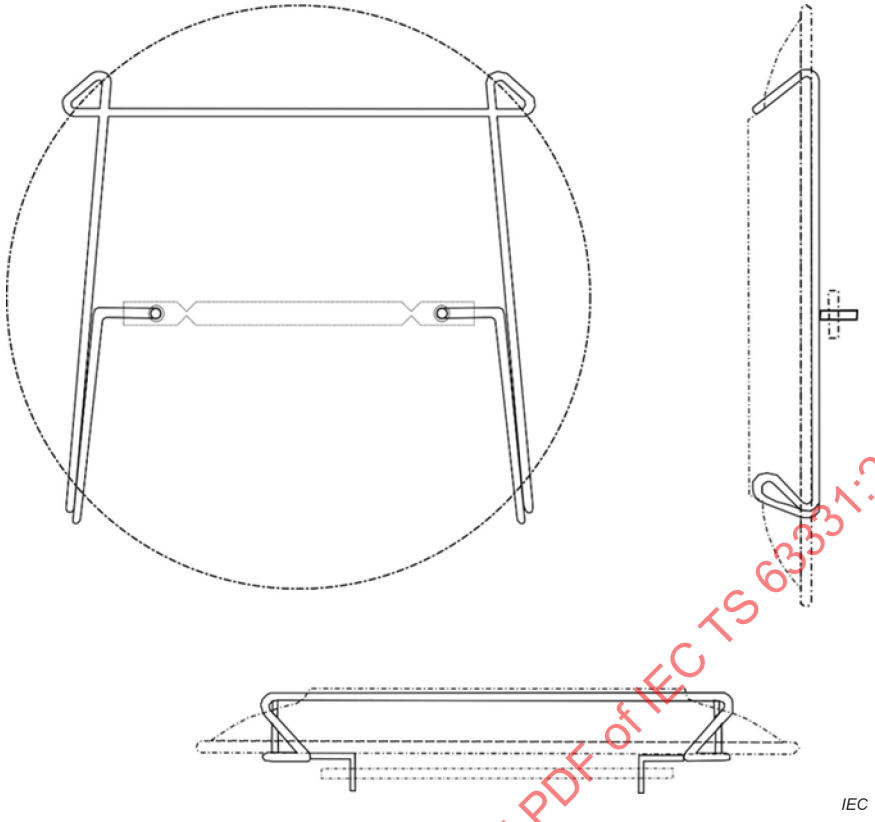
Dimensions in millimetres



NOTE 1 Drawing reproduced from Brands *et al.* 2020 with permission of Britta Brands.

NOTE 2 **Bio indicators** are also described in EN 17735:2022.

Figure 1 – Stainless steel strip / Bio indicator



NOTE Drawing reproduced from Brands *et al.* 2020 with permission of Britta Brands.

Figure 2 – Holders for bio indicators

5.4.9 Measuring equipment for assessing temperature profile

The temperature shall be measured by a temperature sensor placed in the sump of the **dishwasher**. The temperature sensor shall comply with the specifications in Table 6 and shall not influence the **filter** function when installed.

Table 6 – Specifications for data sensor

Description	Specification
Temperature range	0 °C to 100 °C
Accuracy	≤ 0,5 °C over full range
Resolution	≤ 0,1 °C
Response time TC (10 – 90 %) water	≤ 2 min
Response time TC (10 – 90 %) moving air	≤ 5 min
Sampling rate	≤ 10 s
NOTE TC (10 – 90 %) is the time for the sensor to traverse between 10 % and 90 % of its final value. Response time can also be expressed as TC 63 % value. The 63 % figure is the time for the sensor to reach 63 % of its final value. TC (10 − 90 %) and TC 63 % value are approximately of the same order of magnitude for a given sensor.	

5.4.10 Measuring equipment for assessing water consumption

The volume of water inlet shall be measured in litres. Separate metering for hot and cold inlets, where applicable.

Devices using viscosity should be calibrated at the actual nominal temperature ± 5 °C, and the nominal flow rate.

The water consumption of each test **cycle** shall be measured and the mean of a **test series** shall be calculated including standard deviation.

The equipment for measuring the water consumption shall comply with the specifications in Table 7.

Table 7 – Specifications for measuring equipment for water consumption

Description	Specification
Accuracy	≤ ± 2 %
Resolution	≤ 0,1 L

6 Tests

6.1 Test programme

A certain test **programme** is not prescribed, but the selected test **programme** shall be reported.

NOTE Dishwashing **programmes** with main wash temperatures of higher than 60 °C will probably lead to a total microbial reduction of the test strain on the **bio indicators**.

6.2 Load

6.2.1 Composition of the test load

6.2.1.1 General

The composition of the test load follows IEC 60436 except a reduced number of dinner plates, to enable inclusion of the **bio indicators**. The number of dinner plates is calculated in the following way. Use half of the number of declared **place settings** given as **rated dishwasher capacity** or, if the declared number of **place settings** is an uneven number, then use half + 0,5. Each dinner plate is equipped with a holder and a **bio indicator**. The number of dinner plates in use is given in Table 8.

If there is not enough space to position all the plates equipped with holders, additional items may be removed. This has to be noted as a deviation from IEC 60436 is caused by the removal. This topic is currently under review and will be addressed in more detail in a future version of this document.

Table 8 – Quantity of bio indicators according to rated dishwasher capacity

Place settings (rated dishwasher capacity)	Dinner plates to be equipped with bio indicators
15 to 16	8
13 to 14	7
11 to 12	6
9 to 10	5
7 to 8	4
5 to 6	3
4	2
NOTE IEC 60436 does not define the use of dinner plates for **dishwashers** with capacities under 4 **place settings**. This topic is currently under review.	

Figure 3 shows an exemplary loading scheme of the **rack** containing **bio indicators** for a **dishwasher** with a **rated dishwasher capacity** of 13 **place settings**. According to Table 8, seven **bio indicators** need to be fastened to holders on test plates.

IEC

Figure 3 – Exemplary loading scheme of rack containing bio indicators (13 place settings)

6.2.1.2 Conditioning of new load

New load items shall be pre-conditioned by washing them three **times** using **detergent** and **rinse aid**. The type of **detergent** and **rinse aid** specified in 5.4.4 and 5.4.5 is recommended but not required. The **dishwasher** shall dispense **rinse aid** in the final **operation** prior to the next test.

6.2.1.3 Cleaning of used load

Before the **test run**, the load shall be cleaned in a hot **programme** (≥ 60 °C main wash temperature for 15 min) with **detergent**. The type of **detergent** specified in 5.4.4 is recommended but not required. The **dishwasher** shall dispense **rinse aid** in the final **operation** prior to the next test.

NOTE An adequate hygiene status of the test machine can be monitored with appropriate methods, e.g. contact plates.

All **load** items shall be clean and dry prior to the **test run**.

6.3 Test machine

6.3.1 Conditioning of new test machines

Before conducting tests on a new **dishwasher**, it shall be operated at least three **times**, using a **programme** suitable for normally or heavily soiled **tableware**, with reference **detergent** (specified in 5.4.4) and with reference **rinse aid** (specified in 5.4.5), to remove manufacturing residue; a clean load or no load may be used.

6.3.2 Cleaning of test machines

Between two **test runs**, the **test machine** shall be operated at least once, using the **programme** with the highest available temperature, with reference **detergent** (specified in 5.4.4) and with reference **rinse aid** (specified in 5.4.5), to remove residual bacteria and remnants of ballast soil; a clean load or no load may be used.

6.4 Preparation of bio indicators

6.4.1 Preculture and preparation of bacteria suspension

The test strain *M. luteus* ATCC 10240 is cultured on tryptic soy agar. The lyophilized seed culture is handled according to the supplier's instructions: the pellet is rehydrated in (usually 0,5 mL) tryptic soy broth (TSB). After 30 min, 100 µL are inoculated into 10 mL TSB and incubated for 24 h ± 2 h at 30 °C ± 1 °C in a shaking incubator set to a speed 135 r/min ± 15 r/min. From this culture, cryo-cultures for long-term storage are prepared (e.g. by mixing 250 µL with an equal amount of 80 % glycerol). Those cryo-cultures are kept at −80 °C until use.

From the rehydrated culture or cryo-cultures, tryptic soy agar (TSA) is inoculated by streaking an inoculation loop full of material from the (cryo-) culture and incubated for 24 h ± 2 h at 30 °C ± 1 °C. From this first subculture, second and third subcultures are prepared by transferring material to a fresh TSA plate using a sterile inoculation loop, followed by incubation as stated above.

Three plates of the third subculture are used to prepare the bacterial suspension used during the test. Each plate is flooded with 5 mL of sterile 0,9 % sodium chloride solution. The bacteria are detached from the surface of the agar plate by gently moving a sterile Drigalski spatula over the surface. This can be done by either moving the spatula from front to back, in circles or performing a figure-of-eight-movement with the spatula, as long as the bacteria visibly detach from the surface without destruction of the agar. The suspension is transferred to a centrifuge tube using a pipette. The plates are rinsed with an additional 5 mL sterile 0,9 % sodium chloride solution to achieve the highest possible bacterial yield. This solution is added to the centrifuge tube containing the bacterial solution. The bacterial suspension is centrifuged at 4 500 g to 5 000 g for 5 minutes. The supernatant (upper liquid phase) is discarded and the bacterial pellet (the sediment) is used to inoculate the embedding matrix.

6.4.2 Preparation of the embedding matrix

Produce two solutions (A and B) according to ISO/TS 15883-5:2005, H.4.2, H.4.3 and H.4.4 and mix them together in a last step.

For the production of solution A, dissolve 0,3 g mucin in 20 mL sterile H_2O_{dist} on a heating magnetic stirrer and heat up and hold at 55 °C ± 5 °C in a water bath. Add 0,18 g bovine serum albumin and mix until fully dissolved.

Bovine serum albumin is temperature sensitive. It is crucial to keep the temperature of the solution within the set limits. Bovine serum albumin could be a cause for contaminations (depending on the source and pureness); to prevent any contaminations of the matrix, filtration through a sterile **filter** (pore size 0,22 µm) is recommended.

Solution B is produced by heating up 8 mL H_2O_{dist} to the boiling point on the heating magnetic stirrer and adding 0,9 g maize starch, which was before dissolved in 2 mL H_2O_{dist}.The maize starch solution is mixed until it visibly thickened and received a pudding-like consistency.

When solution A and B are cooled down to ambient temperature, they are mixed together resulting in a total of 30 mL BAMS embedding matrix.

NOTE 3 In first test trials, the pudding-like consistency was reached after a simmering time of approximately 6 min at approximately 87 °C.

6.4.3 Cleaning and conditioning of stainless steel strips/bio indicators

Before the first use and after each **test run**, the **stainless steel strips/bio indicators** shall be cleaned in an ultrasonic bath with 5 % cleaning agent for three minutes. After cleaning, the **stainless-steel strips/bio indicators** shall be rinsed twice with **demineralized water** to remove remaining cleaning agent and allow them to dry at ambient conditions. Before usage they shall be autoclaved and cooled down to ambient temperature.

NOTE Malonaldehyde tetrabutylammonium salt, Tetrabutylammonium malondialdehyde enolate, CAS 61869-54-3 or comparable products can be used as a cleaning agent to clean the **bio indicators**.

6.4.4 Contamination of stainless steel strips

The sediment of the test strain from the centrifuge tube is resuspended in 10 mL BAMS embedding matrix and mixed on an electrical agitator. The initial cell count of this inoculum shall be at least 10^8 CFU (minimum) to 10^9 CFU (maximum) *M. luteus* per mL.

To applicate the inoculum, the **stainless steel strips** shall be positioned side by side on a support (e.g. test-tube holder). Place 0,1 mL of the inoculum on the grounded surface (scope 1) of the clean and sterile stainless steel strip and spread evenly using a sterile inoculation loop to cover the complete scope 1 area. Avoid contamination of the back and edge surfaces.

The inoculated **bio indicators** shall be dried under controlled conditions at (22 ± 1) °C and (70 ± 5) % relative humidity for 4 h.

Determination of the initial cell count per **bio indicator**: The initial cell count per **bio indicator** shall be determined according to the procedure described in 6.5.1. The minimum initial count on the **bio indicator** should be 10^7 CFU per **bio indicator**.

The prepared **bio indicators** can be stored **refrigerated** individually in closed test tubes, for up to two months before they are used.

NOTE Storage of **bio indicators** can also be done in a larger storage box, as long as it is ensured that **bio indicators** do not touch each other. However, prevent the storage box from temperature changes (e.g. through removal from the refrigerator) as this could influence the initial cell count and stability of the **bio indicators**.

6.5 Determination of microbial load

6.5.1 Determination of the initial cell count

For each **test run**, three **bio indicators** of the test strain, which have not been washed in the **dishwasher**, shall be examined as reference controls (positive controls). The initial cell count of these **bio indicators** is determined and used as reference to calculate the germ reduction after the dishwashing process.

For the extraction, transfer each **bio indicator** to a test tube containing 5 mL tryptic soy broth with neutralizing solution (see 5.4.8.5) and 2 g glass beads. Homogenize on an electromechanical agitator for 3 s and shake out on a tilt/roller mixer for 10 min with a rolling speed of 80 r/min. Afterwards, remove the **bio indicators** using sterile forceps. The cell count is quantified with the spread-plate or pour-plate method using a decimal dilution series from 10^{-5} to 10^{-7} in double determination. The agar plates shall be incubated at 30 °C ± 1 °C for (48 ± 2) h and colony counting shall be carried out macroscopically.

6.5.2 Determination of the remaining count

After the end of each dishwashing test **programme**, open the door for 10 min or for **dishwashers** with automatic door open system wait 10 min after the end of dishwashing **programme**. The remaining cell count on the **bio indicators** is quantified with the spread-plate or pour-plate method using a decimal dilution series from 10^{0} to 10^{-3} in double determination. The agar plates shall be incubated at 30 °C ± 1 °C for (48 ± 2) h and colony counting shall be carried out macroscopically.

NOTE The end of a dishwashing **programme** is introduced by the end of **programme** indicator (this could be a sound, light or symbol on a display to indicate that the **programme** is complete, and the user has access to the load).

6.6 Loading and operating

6.6.1 Loading

Roll out the **rack** including the dinner plates and remove the dinner plates. Fasten the **bio indicators** to the holders on the dinner plates under aseptic conditions. Place the dinner plates in the **rack** before rolling it back into the **dishwasher**.

Roll out the **rack** including the mugs and remove one of the mugs (in the middle of the upper **rack**, so that the spray arm can reach the ballast soil). Place the frozen ballast soil inside the mug and return it to the **rack**. Roll the **rack** back into the **dishwasher**.

Add the **detergent** to the **detergent** compartment.

6.6.2 Operating

Perform three **test runs** of the **test programme** and clean the **test machine** between the **test runs**. Take out the **filters** prior to the cleaning cycle of the machine for manual pre-cleaning. Make sure that all visible remnants are removed prior to putting the **filter** back into the machine.

Between two successive **test runs** in a **test series**, **test machines** shall be allowed to cool down until they meet the ambient condition requirements of 5.3.2. After the end of each dishwashing **test run**, the door shall be opened, the **rack** including the **bio indicators** shall be rolled out and dried for 10 min. Note that the upper **racks** shall not be rolled out to avoid dripping of residual water drops on the **bio indicators**.

When the process is complete, the **bio indicators** shall be removed from the test plate holders and placed in sterile empty test tubes separately under aseptic conditions. These test tubes shall be sealed and **refrigerated**. The determination of the remaining cell count of *M. luteus* shall be carried out within 4 hours. If the analysis is done in an external laboratory, the test tubes with the **bio indicators** shall be transported under cooled conditions, preferred at a temperature < 4 °C.

7 Evaluation

7.1 General requirements

All individual results shall be recorded.

For each **test run**, three **bio indicators** which have not been washed in the **dishwasher** shall be examined as reference/positive controls. The microbial reduction shall be calculated in relation to the mean value of the reference controls according to 7.2.

7.2 Determination of microbial reduction − Calculation of the reduction factor

The reduction factor RF is calculated according to the following equation:

$$RF = \log_{10} N_0 - \log_{10} N$$

where

RF is the reduction factor;

N_0 is the number of *M. luteus* CFU/reference (positive) control (mean value of three **bio indicators**) before the **test run**;

N is the number of *M. luteus* CFU/**bio indicator** after the dishwashing **test run**.

The standard deviation of N_0, N and the reduction factors shall be calculated.

Predictions of the reduction factor can be calculated using the method in Annex A.

7.3 Results

7.3.1 Expression of results

The results are expressed as dimensionless reduction factors with one position after decimal point. In addition, the results may be indicated as relative reduction in % with two positions after decimal point.

7.3.2 Evaluation of results

This document is not intended to evaluate the achieved microbial reduction or give minimum reduction factors. It only specifies the microbiological reference method for measuring the germ reduction capability of a **dishwasher** in a particular **test programme**.

8 Test report

8.1 Documentation

The individual results of all **test runs** shall be documented. The record sheets in Annex B can be used for this purpose.

The test results shall be compiled in the test report, which shall conclude with an overall assessment of the capability of germ reduction of the **dishwasher**.

8.2 Data in the test report

The following data shall, as a minimum requirement, be documented in the test report:

a) **dishwasher** name and number,
b) positioning of **bio indicators**,
c) chemical and physical data (see record sheet in Annex B),
d) individual results of all **test runs** (see Annex B),
e) **temperature profile** (when calculating MIE unit),
f) signature of the inspector and date.