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TECHNICAL SPECIFICATION

Washing machines for household use – Method for measuring the microbiological performance

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INTERNATIONAL
ELECTROTECHNICAL
COMMISSION

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WASHING MACHINES FOR HOUSEHOLD USE – METHOD FOR MEASURING THE MICROBIOLOGICAL PERFORMANCE

FOREWORD

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IEC TS 63429 has been prepared by subcommittee 59D: Performance of household and similar electrical laundry appliances, of IEC Technical Committee 59: Performance of household and similar electrical appliances. It is a Technical Specification.

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

Draft	Report on voting
59D/498/DTS	59D/503A/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.

In this document, the following print type is used:

– terms defined in Clause 3: **bold type**.

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.

The committee has decided that the contents of this document will remain unchanged until the stability date indicated on the IEC website under webstore.iec.ch in the data related to the specific document. At this date, the document will be

- reconfirmed,
- withdrawn,
- replaced by a revised edition, or
- amended.

INTRODUCTION

This Technical Specification describes a method to measure the microbial contamination reduction performance of household **washing machines**. Microbial reduction due to any potential or claimed antimicrobial action of detergents other than the base powder of the required detergent, as well as of bleach systems or any other additives, is not addressed.

This first edition has been developed to provide a globally applicable and agreed method to measure the reduction of the microbial contamination of household **washing machines** and their **programmes**, to be measured with textile pieces contaminated with test microorganisms under standardized conditions.

The reduction of the microbial contamination is just one of the performance parameters measurable for a **washing machine**. Therefore, it is not used as the only parameter to describe the performance of a washing **programme,** but it is measured along with the other performance parameters considered for that **programme**.

NOTE The use of the reference **washing machine** as defined in IEC 60456 as a reference process for the reduction of microbial contamination is under consideration.

The test microorganisms requested for this Technical Specification require BSL-1 and 2 laboratories. BSL-1 laboratories are restricted to the use of *Staphylococcus arlettae* and *Saccharomyces cerevisiae*, whereas BSL-2 laboratories shall use *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans*, or all five test microorganisms.

Classification of test microorganisms can change and can differ on national levels. It is the responsibility of the user to ensure compliance with applicable national classification.

Different type strains of the same microbial species described in this Technical Specification can be used if the achievement of comparable results is proven.

Additional test microorganisms can be used besides the test strains described in this Technical Specification, but their result cannot be used to claim compliance with the Technical Specification and shall not be reported in the same test report.

The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collections (ATCC). This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by IEC of the product named.

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.

A warning about the use of this document is included in Clause 4.

WASHING MACHINES FOR HOUSEHOLD USE – METHOD FOR MEASURING THE MICROBIOLOGICAL PERFORMANCE

1 Scope

This Technical Specification provides a test method for measuring the microbial contamination reduction performance of household **washing machines** with textile pieces contaminated with test microorganisms under standardized conditions. The microbial numbers on the contaminated textile pieces are measured before and after the washing **programme** and the reduction is calculated. Furthermore, a potential **cross contamination** from contaminated to uncontaminated textile pieces within the washing **programme** is measured.

This document does not address the microbial contamination reduction due to any potential or claimed antimicrobial action of detergents as well as of bleach systems or any additives.

This document applies to **washing machines** for household use, within the meaning that the scope of TC 59 indicates for household use, including the washing related functions of **washer-dryers.**

This document does not apply to professional **washing machines** nor to commercial laundry **operations** associated with food service, hospital linens or other non-residential applications.

This document deals with measurement procedures regarding the reduction of microbial contamination resulting from the use of electrical appliances 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.

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 60456:2010, *Clothes washing machines for household use – Methods for measuring the performance*
IEC 60456:2010/AMD1:2022

ISO 2267, *Surface active agents – Evaluation of certain effects of laundering – Methods of preparation and use of unsoiled cotton control cloth*

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

EN 12353, *Chemical disinfectants and antiseptics. Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

3 Terms, definitions and symbols

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

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

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

3.1 Terms and definitions

3.1.1
washing machine
appliance for cleaning and rinsing of textiles using water which may also have a means of extracting excess water from the textiles

[SOURCE: IEC 60456:2010, 3.1.1]

3.1.2
test run
performance assessment of one **programme** execution in the test **washing machine**

3.1.3
test series
set of **test runs** which are collectively used to assess performance

[SOURCE: IEC 60456:2010, 3.1.12]

3.1.4
operation
each performance of a function that occurs during the **washing machine programme** such as pre-wash, washing, rinsing, draining, or spinning

[SOURCE: IEC 60456:2010, 3.1.13]

3.1.5
programme
series of **operations** which are pre-defined within the **washing machine,** and which are declared by the manufacturer as suitable for washing certain textile types for hygiene purposes

[SOURCE: IEC 60456:2010, 3.1.14, modified – "for hygiene purposes" has been added to the end of the definition.]

3.1.6
cycle
complete washing process, as defined by the **programme** selected, consisting of a series of **operations** (wash, rinse, spin, etc.) and including any **operations** that occur after the completion of the **programme**

[SOURCE: IEC 60456:2010, 3.1.6, modified – The note has been omitted.]

3.1.7
base load
textile load used for testing without **biomonitor cloths** and **biomonitors**

3.1.8
cross contamination
transfer of microorganisms from contaminated fabrics to uncontaminated fabrics during one **test run**

3.1.9
biomonitor
microorganism carrier inoculated with microorganisms to be used to monitor the reduction of microbial contamination

3.1.10
biomonitor cloth
cotton cloth with six individual pockets that fixes **biomonitors** and temperature loggers during a **test run** in a **washing machine**

3.1.11
temperature profile
time-temperature data representing the water temperature in the **washing machine** during the **test run**

3.1.12
washer-dryer
washing machine which includes both a spin extraction function and also a means for drying the textiles, usually by heating and tumbling

[SOURCE: IEC 60456:2010, 3.1.4, modified – The note has been omitted.]

3.2 Symbols

cfu	colony forming unit
N_0	average value of microorganism amount of the three positive controls, before exposition to the test **programme** (cfu/**biomonitor**)
N	average value of microorganism amount per 5 **biomonitors**, after exposition to the test **programme** (cfu/**biomonitor**)
$\log(N_0/N)$	reduction factor per microorganism type
v^{-1}	microorganism amount in a 10 times diluted solution
v^{-2}	microorganism amount in a 100 times diluted solution

4 Requirements

This document specifies a test method for measuring the reduction of microbial contamination in clothes **washing machines** and of possible **cross contamination** from contaminated **biomonitors** to sterile cotton swatches.

This document does not specify safety requirements and does not deal with performance of **washing machines** measured under IEC 60456 nor with effects on fabrics.

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 microorganisms 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.

5 Test conditions, materials, equipment and instrumentation

5.1 Test conditions

Ambient conditions: electricity supply, water supply, and ambient temperature shall be in accordance with IEC 60456:2010. The **base load** shall be the cotton **base load** as specified in IEC 60456:2010.

Hard water with a total water hardness of (2,5 ± 0,2) mmol/l or soft water with a total water hardness of (0,5 ± 0,2) mmol/l as defined in IEC 60456 shall be used for testing.

The water supplied to the **washing machine** shall contain less than 100 cfu/ml total microbial counts at an incubation temperature of 30 °C. Microorganisms for test purposes as listed in 5.2.1 should not be present in the supplied water. The water supplied to the **washing machine** shall not contain more than 0,3 mg/l of chlorine.

When checking for the presence of microorganisms in the supplied water, the following shall be considered: *Staphylococcus aureus, Staphylococcus arlettae, Candida albicans, Saccharomyces cerevisiae* are normally not present in the water system and shall not be looked for. *Pseudomonas aeruginosa* can be present in small numbers (< 10 *Pseudomonas aeruginosa* counts per 100 ml), and at these levels does not significantly influence measurements in accordance with this document.

The water supplied to the **washing machine** shall contain less than 10 cfu/100 ml *Pseudomonas aeruginosa*. If higher numbers of *Pseudomonas aeruginosa* are detected, measures to decontaminate the water system should be taken and tests should be repeated.

Water sampling shall be done in accordance with ISO 19458:2006, 4.4.1.3 as outlined for the assessment of water quality in the main distributor. The water shall be sampled from the water tap in the water system which is closest to the connecting valve of the **washing machine**. The aerator and O-rings of the sampling point shall be removed, and the water tap disinfected. The water tap shall be rinsed until water temperature is constant before sampling. Sampling volume shall be 150,0 ml.

The microbiological quality of the water supplied to the **washing machine** is determined in accordance with 6.5.3.

5.2 Materials and reagents

5.2.1 Microorganisms for test purposes

For test purposes, the following strains shall be used as test microorganisms:

- *Pseudomonas aeruginosa* ATCC 15442
- *Staphylococcus aureus* ATCC 6538
- *Candida albicans* ATCC 10231

NOTE 1 This combination of microorganisms can require a Biosafety Level 2 laboratory.

Alternative test microorganisms that can be used are:

- *Staphylococcus arlettae* ATCC 43957
- *Saccharomyces cerevisiae* ATCC 9763

NOTE 2 This combination of microorganisms can require a Biosafety Level 1 laboratory.

NOTE 3 The use of additional risk group 1 test microorganisms (e.g., Escherichia coli) is under consideration.

The three test microorganisms *P. aeruginosa*, *S. aureus* and *C. albicans* or the two test strains *S. arlettae* and *S. cerevisiae* shall be used in a **test series**. The use of all five test microorganisms in one **test series** is permitted.

NOTE 4 This combination of microorganisms can require a Biosafety Level 2 laboratory.

NOTE 5 For indications about possible suppliers of ready-to-use **biomonitors**, see Annex B.

5.2.2 Culture media and solutions

5.2.2.1 Culture media

5.2.2.1.1 General

All media and solutions shall be of microbiological grade and sterilized appropriately prior to use. It is recommended to use commercially available or water-free dry materials for the culture media. The specifications in 5.2.2.1.2 to 5.2.2.1.6 refer to water-free products.

5.2.2.1.2 Tryptone soy agar (TSA)

The composition of tryptone soy agar (TSA) shall be in accordance with Table 1.

Table 1 – Composition of TSA

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
Final pH	7,3 ± 0,2

5.2.2.1.3 Sabouraud dextrose agar with chloramphenicol

The composition of Sabouraud dextrose agar with chloramphenicol shall be in accordance with Table 2.

Table 2 – Composition of Sabouraud dextrose agar with chloramphenicol

Description	Specification
Pancreatic digest of casein	5,0 g/l
Peptic digest of animal tissue	5,0 g/l
Dextrose monohydrate	40,0 g/l
Chloramphenicol	0,05 g/l
Agar	15,0 g/l
Final pH	5,6 ± 0,2

5.2.2.1.4 Tryptic soy broth (TSB)

The composition of tryptic soy broth (TSB) shall be in accordance with Table 3.

Table 3 – Composition of Tryptic Soy Broth (TSB)

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 hydrogen phosphate (K_2HPO_4)	2,5 g/l
Glucose	2,5 g/l
Final pH	7,3 ± 0,2

5.2.2.1.5 Baird-Parker agar

The composition of Baird-Parker agar shall be in accordance with Table 4.

Table 4 – Composition of Baird-Parker agar

Description	Specification
Tryptone	10,0 g/l
Beef extract	5,0 g/l
Yeast extract	1,0 g/l
Glycine	12,0 g/l
Sodium pyruvate	10,0 g/l
Lithium chloride	5,0 g/l
Agar	20,0 g/l
Final pH	6,8 ± 0,2

Autoclave the agar at 121 °C for 15 min. After cooling down to 50 °C, add aseptically 50,0 ml/l of egg yolk tellurite emulsion.

5.2.2.1.6 Cetrimide agar

The composition of cetrimide agar shall be in accordance with Table 5.

Table 5 – Composition of cetrimide agar

Description	Specification
Pancreatic digest of gelatine	20,0 g/l
Potassium sulphate	10,0 g/l
Magnesium chloride	1,4 g/l
Cetyltrimethylammonium chloride	0,3 g/l
Agar	13,6 g/l
Final pH	7,2 ± 0,2

Dissolve the components of the cetrimide agar in 1,0 L water, as specified in 5.2.2.2. Add 10,0 ml of glycerol and boil to dissolve completely. Autoclave the agar at 121 °C for 15 min.

5.2.2.2 Water for culture media and solutions

Bi-distilled or demineralised water may be used. However, water for culture media and solutions shall be sterilized prior to use.

Sterilization is not required if the water will be used for preparation of culture media and is subsequently sterilized.

5.2.2.3 Diluting agent

The composition of the diluting agent shall be in accordance with Table 6.

Table 6 – Composition of diluting agent

Description	Specification
Tryptone, pancreatic digest of casein	1,0 g/l
Sodium chloride (NaCl)	8,5 g/l
Polysorbate 80	1,0 g/l
Final pH	7,0 ± 0,2

5.2.2.4 Neutralisation solution

For the retrieval of test microorganisms after a **test run**, a neutralisation solution shall be used in combination with TSB. The composition of one recommended neutralisation solution is given in Table 7.

Table 7 – Composition of neutralisation solution

Description	Specification
Polysorbate 80	30,0 g/l
Lecithin	3,0 g/l
L-Histidine	1,0 g/l
Natrium thiosulfate pentahydrate	5,0 g/l
Water (5.2.2.2)	To 1,0L

5.2.3 Detergent

5.2.3.1 General

The detergent to be used in the main test shall be the base powder of the standard powder detergent IEC-P. The detergent to be used to clean the **washing machine** and decontaminate load and **biomonitor cloths** shall be the standard powder detergent IEC-P with all three components. The standard powder detergent IEC-P and its base powder are defined in IEC 60456:2010/AMD1:2022 Annex B.

5.2.3.2 Detergent dosage in main test

The detergent dosage shall be 8 g +4 g base powder / kg load for horizontal axis machines and 8 g +4 g base powder / kg load for vertical axis machines in the main wash.

NOTE 1 The composition of the base powder of reference detergent A* in IEC 60456:2010 Edition, Annex B is identical to that of the base powder of the standard powder detergent (IEC-P) in IEC 60456:2010/AMD1:2022, Annex B.

If a detergent dispenser is present, the detergent dose specified shall be placed in the dispenser. If there is no detergent dispenser, all detergent is added into the **washing machine** at the base of the drum.

NOTE 2 Only a small amount of detergent is used in this Technical Specification to allow the measurement of the impact of the **washing machine** on microbial contamination reduction. The detergent base powder is used to avoid any microbiocidal effect caused by the bleach of the complete detergent formulation. Due to the low amount of detergent base powder, an addition of soil is not necessary for the measurement of microbial contamination reduction.

5.3 Equipment

5.3.1 General

Test conditions, materials, equipment, and instrumentation shall be in accordance with IEC 60456:2010, unless otherwise specified, and handled in compliance with good microbiology laboratory practice.

5.3.2 Incubator

The incubator shall be capable of maintaining a constant temperature of (36 ± 1) °C for bacterial strains and (30 ± 1) °C for the yeast strain, and (25 ± 1) °C for the drying of the contaminated **biomonitors**.

5.3.3 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 min or at (134 +3/−0) °C for at least 5 min.

5.3.4 Cotton carrier

The cotton carriers are swatches made of standard cotton fabric in accordance with ISO 2267. The dimension shall be 1,0 cm × 1,0 cm.

Cotton fabric shall be boiled three times in distilled water, dried at room temperature, and ironed. Swatches of 1,0 cm × 1,0 cm are cut, autoclaved, dried at room temperature, and kept sterile prior to use.

5.3.5 Biomonitor cloth

Biomonitor cloth is made of cotton fabric in accordance with ISO 2267. The dimensions shall be 210 mm × 70 mm, with 6 pockets with 35 mm × 35 mm per pocket for the **biomonitors** and the temperature logger. The cloth is folded in the middle with pockets on the inner sides and fixed either with hook-and-loop fasteners or press buttons. The **biomonitor cloth** is illustrated in Figure 1.

Dimensions in millimetres

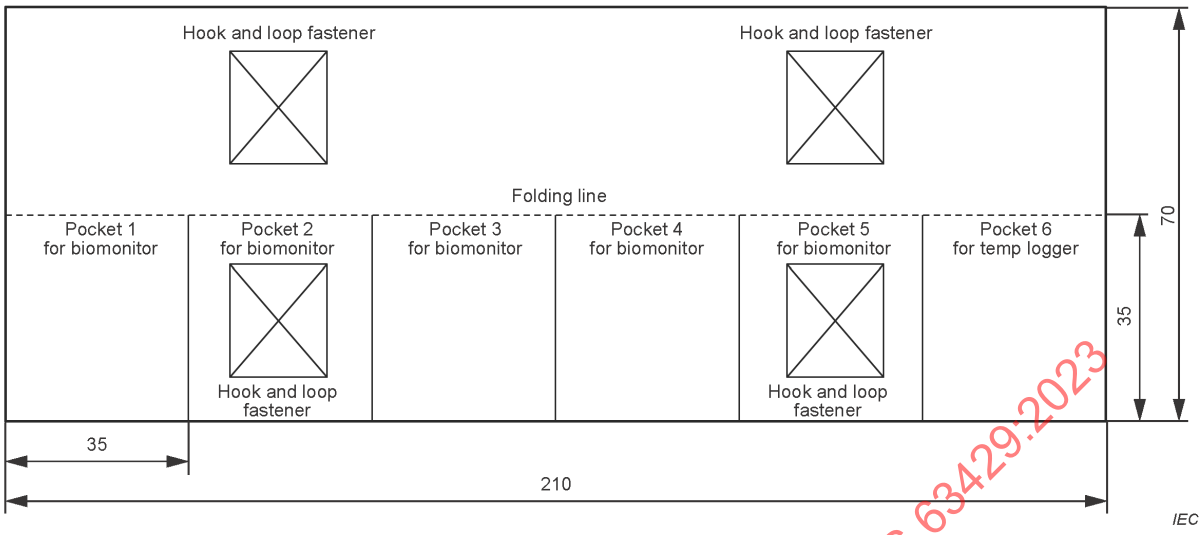

Figure 1 – Biomonitor cloth

The **biomonitor cloth** shall not be used for more than 100 washing **cycles**, including the preparation and disinfection **cycles**.

Before and after a **test run**, the **biomonitor cloths** shall be washed with a wash **programme** that ensures a water temperature ≥ 80 °C in the main wash for at least 30 min without detergent followed by at least 4 rinse **operations**. Measuring equipment as specified in 5.3.9 may be used to ensure temperature and holding time. The **biomonitor cloths** are then dried and sterilized in the autoclave. The dry and sterilized **biomonitor cloths** shall be stored under appropriate conditions to avoid re-contamination.

If it is not possible to perform a wash **programme** that ensures a water temperature ≥ 80 °C in the main wash, a cotton 60 °C wash **programme** in the reference **washing machine** with standard powder detergent (IEC-P; dosage according to IEC 60456:2010/AMD1:2022) followed by a cotton 60 °C wash **programme** without any detergent shall be performed, to appropriately prepare and decontaminate the **biomonitor cloths**.

NOTE Treatment of **biomonitor cloths** and **base load** (5.3.8) before and after a **test run** can be done in the same wash **programme**.

5.3.6 Pipettes

Pipettes shall have a nominal volume of 0,1 ml to 10,0 ml.

5.3.7 Electromechanical agitator

An electromechanical agitator commonly used in laboratories may be used for the purposes of this document.

5.3.8 Base load

The **base load** shall not be used for more than 100 washing **cycles** including the preparation and disinfection **cycles**. Compliance with IEC 60456:2010, Annex H is not required.

Before and after a **test run**, the **base load** shall be washed with a wash **programme** that ensures a water temperature ≥ 80 °C in the main wash for at least 30 min without detergent followed by at least 4 rinse **operations**. Measuring equipment as specified in 5.3.9 may be used to ensure temperature and holding time. The **base load** is dried and sterilized in the autoclave.

The dried and sterilized **base load** shall be stored under appropriate conditions to avoid re-contamination.

If it is not possible to perform a wash **programme** that ensures a water temperature ≥ 80 °C in the main wash, a cotton 60 °C wash **programme** in the reference **washing machine** with the standard powder detergent (IEC-P; dosage according to IEC 60456:2010/AMD1:2022) followed by a cotton 60 °C wash **programme** without any detergent shall be performed, to appropriately prepare and decontaminate the **base load**.

NOTE Treatment of **biomonitor cloths** (5.3.5) and **base load** before and after a **test run** can be done in the same wash **programme**.

5.3.9 Measuring equipment for assessing temperature profile

The **temperature profile** shall be measured by temperature loggers placed in the **biomonitor cloths** during a **test run**. The temperature loggers shall comply with the specifications in Table 8.

Table 8 – Temperature logger specification

Temperature range	At least (0 to 85) °C
Accuracy	≤ 0,5 K at 0 °C to 60 °C; ≤ 2 K above 60 °C
Resolution	≤ 0,2 K
Response time TC(10%-90%) water	≤ 2 min
Sampling rate	≤ 10 s
Max mass	30 g
Dimensions	max 35 mm in each dimension
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.	

5.3.10 Measuring equipment for water consumption

Equipment for measuring water volume shall comply with the specifications of IEC 60456:2010.

6 Tests

6.1 Test method principles

Biomonitors are used to determine the microbial reduction in the domestic washing processes and the possible **cross contamination** of microorganisms.

Test conditions, materials, equipment, and instrumentation shall be in accordance with IEC 60456:2010, unless otherwise specified.

6.2 Preparation of washing machine

Prior to each **test run**, the **washing machine** shall be conditioned to remove all microorganisms from **washing machine** parts and standing water inside the test appliance.

Different cleaning methods for **washing machines** with and without a heating system are provided in Annex A.

6.3 Preparation of test microorganisms and biomonitors

6.3.1 Cultures

6.3.1.1 General

The test microorganisms and their stock cultures shall be prepared and stored according to EN 12353.

6.3.1.2 Working cultures of *P. aeruginosa, S. aureus* and *S. arlettae*

Streaking onto at least two plates containing TSA shall be used to prepare working cultures of *P. aeruginosa, S. aureus*, and *S. arlettae* subcultures from the stock culture.

After 24 h of incubation at (36 ± 1) °C, a second subculture shall be prepared from the first subculture in the same way and incubated. The second subculture is used to prepare the **biomonitors**.

6.3.1.3 Bacterial test suspension (*P. aeruginosa, S. aureus* and *S. arlettae*)

The following procedure shall be followed.

a) Take a suitable amount of tryptic soy broth (see 5.2.2.1.4) and place in a vial or a flask.

NOTE 20 ml test suspension is sufficient for approximately 50 to 60 **biomonitors.**

Take the working culture (see 6.3.1.2) and transfer a loop of the cells into the tryptic soy broth by rubbing the loop against the wet wall of the flask to dislodge the cells before immersing in the tryptic soy broth. Shake the flask for 3 min using an electromechanical agitator (see 5.3.7).

b) Adjust the number of cells in the suspension from $1,5 \times 10^9$ cfu/ml to $5,0 \times 10^9$ cfu/ml using the tryptic soy broth (see 5.2.2.1.4), estimating the number of cfu by any suitable means. Maintain this test suspension at 4 °C and use within 2 h.

The use of a spectrophotometer is recommended for adjusting the number of cells, at a wavelength of about 620 nm and with a cuvette of 10 mm path length. In this case, each laboratory shall produce calibration data for each test organism, knowing that suitable values of optical density are generally found at 2,000 ± 0,100. To achieve reproducible results for the number of cells in the test suspension, it is recommended to dilute the test suspension, for example tenfold.

NOTE A colorimeter is a suitable alternative.

c) For counting, prepare 10^{-6}, 10^{-7} and 10^{-8} dilutions of the test suspension using the diluting agent (see 5.2.2.3). Mix with an electromechanical agitator (see 5.3.7). Take a sample of each dilution and inoculate using the pour-plate or the spread-plate technique.

- When using the pour-plate technique, transfer each 1 ml sample into separate Petri dishes and add 15 to 20 ml melted TSA (see 5.2.2.1.2), cooled to (45 ± 1) °C.
- When using the spread-plate technique, spread each 0,1 ml sample on a surface-dried plate containing TSA (see 5.2.2.1.2)

Incubate the plates at (36 ± 1) °C for 24 h to 48 h. Appropriate colony counts of each dilution shall be used for microorganism quantification (15 cfu to 300 cfu per plate).

6.3.1.4 Working cultures of *C. albicans* and *S. cerevisiae*

Streaking onto at least two plates containing Sabouraud dextrose agar (see 5.2.2.1.3) shall be used to prepare a working subculture of *C. albicans* and *S. cerevisiae* from the stock.

After incubation of 42 h to 48 h at (30 ± 1) °C, a second subculture shall be prepared from the first subculture in the same way and incubated. The second subculture is used to prepare the **biomonitors**.

6.3.1.5 Yeast test suspensions (*C. albicans* and *S. cerevisiae*)

The following procedure shall be followed.

a) Take a suitable amount of tryptic soy broth (see 5.2.2.1.4) and place in a vial or flask.

NOTE 1 20 ml test suspension is sufficient for approximately 50 to 60 **biomonitors.**

Take the working culture (see 6.3.1.4) and transfer a loop of the cells into the tryptic soy broth (see 5.2.2.1.4) by rubbing the loop against the wet wall of the flask to dislodge the cells before immersing in the tryptic soy broth. Shake the flask for 3 min using an electromechanical agitator (see 5.3.7).

b) Adjust the number of cells in the suspension to $1,5 \times 10^8$ cfu/ml to $5,0 \times 10^8$ cfu/ml using the tryptic soy broth (see 5.2.2.1.4), estimating the number of cfu by any suitable means. Maintain this test suspension at 4 °C and use within 2 h.

The use of a spectrophotometer is recommended for adjusting the number of cells, at a wavelength of about 620 nm and with a cuvette of 10 mm path length. In this case, each laboratory shall produce calibration data for each test organism, knowing that suitable values of optical density are generally found at 3,500 ± 0,100. To achieve reproducible results for the number of cells in the test suspension, it is recommended to dilute the test suspension, for example tenfold.

NOTE 2 A colorimeter is a suitable alternative.

c) For counting, prepare 10^{-5}, 10^{-6} and 10^{-7} dilutions of the test suspension using the diluting agent (see 5.2.2.3). Mix with an electromechanical agitator (see 5.3.7). Take a sample of each dilution and inoculate using pour-plate or spread-plate technique.

- When using the pour-plate technique, transfer each 1 ml sample into separate Petri dishes and add 15 to 20 ml melted Sabouraud dextrose agar with Chloramphenicol (see 5.2.2.1.3), cooled to (45 ± 1) °C.
- When using the spread-plate technique, spread each 0,1 ml sample on a surface-dried plate containing Sabouraud dextrose agar with Chloramphenicol (see 5.2.2.1.3).

Incubate the plates at (30 ± 1) °C for 48 h. Appropriate colony counts of each dilution shall be used for microorganism quantification (15 cfu to 300 cfu per plate).

6.3.2 Biomonitors

6.3.2.1 Cotton carriers

Cotton carriers are described in 5.3.4.

6.3.2.2 Inoculation of the carriers

Eight cotton carriers shall be used per microorganism strain and **test run**.

The cotton carriers are dipped in the test microorganism suspension and repeatedly turned by sterilized tweezers to wet them completely and remove all air bubbles.

The cotton carriers shall rest for (30 ± 2) min in the suspension. Afterwards, cotton carriers are taken out of the suspension with tweezers. To remove excess suspension, cotton carriers are pressed three times against the wall of the tube and are then transferred in open Petri dishes for drying for a minimum of 3 h in an **incubator** at a temperature of (25 ± 1) °C until **biomonitors** are dry. Record drying temperature and time.

The required microbial load of the **biomonitors** is defined in 7.3 and shall be verified directly after the drying process as described in 6.4.2.

NOTE 1 In order to guarantee satisfactory drying after 3 h, it is necessary to ensure a low humidity (< 50% RH) in the incubator at the beginning of the drying procedure. Incubators filled with other materials such as agar plates can have a high humidity delaying the drying of **biomonitors**.

Five **biomonitors** per microorganism strain shall be used per **test run**. In addition, 3 **biomonitors** for each test microorganism shall be prepared to be used for validation (see 6.5.1).

Store the **biomonitors** at −20 °C in air-tight vessels and use within one week after drying.

As alternative, ready-to-use **biomonitors** can be used. In this case, the **biomonitors** shall be stored in accordance with the manufacturer's recommendations.

NOTE 2 For indications about possible suppliers of ready-to-use **biomonitors**, see Annex B.

6.3.2.3 Biomonitor placement in biomonitor cloths

For each test strain, one **biomonitor cloth** with six pockets shall be used. Five **biomonitors** per test strain are transferred by sterilized tweezers into separate pockets. A temperature logger (see 5.3.9) may be added to the **biomonitor cloth** into the sixth pocket. **Biomonitor cloths** shall be marked with an appropriate method, to allow the identification of test strains after the test.

6.3.2.4 Sterile carrier placement in biomonitor cloth

For the assessment of **cross contamination**, place five sterile cotton carriers (see 5.3.4) with sterilized tweezers into the individual pockets of one **biomonitor cloth**. A temperature logger (see 5.3.9) shall be added into the sixth pocket. The **biomonitor cloth** shall be marked with an appropriate method, to allow the identification of negative control after the test.

6.4 Main test

6.4.1 General

The **programme** and size of the **base load** used for the **test series** shall be selected according to manufacturer’s specification.

If the **base load** mass is other than the maximum rated capacity of the **washing machine**, the mass of the **base load** used, and its percentage of the maximum capacity shall be clearly stated in the report.

Split the dry **base load** into two similar parts. Fill one part into the **washing machine** drum, place the **biomonitor cloths** (see 5.3.5) with the **biomonitors** of the different test microorganisms and the temperature loggers (see 5.3.9), as well as the **biomonitor cloth** with the sterile cotton carriers and the temperature logger (see 5.3.9), in the middle and finish loading with the second part of the **base load**.

Run the tested **programme** and analyse the **biomonitor** in accordance with 6.4.2.

At least three valid **test runs** shall be performed for the tested **programme**. Requirements for a valid **test run** and **test series** are outlined in 7.3.

6.4.2 Evidence of test microorganisms

6.4.2.1 General

To determine the amount of microorganisms on the **biomonitors**, each **biomonitor** is placed into 10 ml of neutralisation solution (see 5.2.2.4) and mixed vigorously with an electromechanical agitator (see 5.3.7) for 3 min before plating. This test-tube with the shake-off solution is marked as 10^0 test-tube.

6.4.2.2 Determination of microorganisms before washing (unwashed reference biomonitors, N_0)

A serial dilution in diluting agent (see 5.2.2.3) is performed up to dilution v^{-7} (10^{-7} test-tube) for *P. aeruginosa, S. aureus*, and *S. arlettae*, and up to v^{-6} (10^{-6} test-tube) for *C. albicans* and *S. cerevisiae*. Mix with an electromechanical agitator (see 5.3.7). Take a sample of the dilutions v^{-3} to v^{-7} for *P. aeruginosa, S. aureus*, and *S. arlettae*, and of the dilutions v^{-2} to v^{-6} for *C. albicans* and *S. cerevisiae*. Inoculate the samples using pour-plate or spread-plate techniques.

- When using the pour-plate technique, transfer each 1 ml sample into separate Petri dishes and add 15 to 20 ml melted Tryptic soy agar (see 5.2.2.1.2) or appropriate selective media (see 5.2.2.1.5 and 5.2.2.1.6) – for **biomonitors** containing *P. aeruginosa, S. aureus* and *S. arlettae* – or Sabouraud dextrose agar with Chloramphenicol (see 5.2.2.1.3) – for **biomonitors** containing *C. albicans* or *S. cerevisiae*- cooled to (45 ± 1) °C.
- When using the spread-plate technique, spread each 0,1 ml sample on a surface-dried plate containing Tryptic soy agar (see 5.2.2.1.2) or appropriate selective media (see 5.2.2.1.5 and 5.2.2.1.6) for **biomonitors** containing *P. aeruginosa*, *S. aureus* and *S. arlettae* – or Sabouraud dextrose agar with Chloramphenicol (see 5.2.2.1.3) – for **biomonitors** containing *C. albicans* or *S. cerevisiae*. In addition, also spread 1,0 ml sample on an appropriate agar plate from the 10^0 test-tube to achieve a detection limit of < 10 cfu/**biomonitor**.

NOTE Experienced laboratory personnel can identify and distinguish the test microorganisms by the visual occurrence of their colonies on Tryptic soy agar respectively Sabouraud dextrose agar. If necessary for identification, selective culture media (5.2.2.1.5 and 5.2.2.1.6) can be used to determine the microbial counts on the washed **biomonitors** and on the former-sterile **biomonitors** to measure **cross contamination**.

Incubate the plates with *P. aeruginosa, S. aureus,* and *S. arlettae* at (36 ± 1) °C for 24 h to 48 h. The plates with *C. albicans* and *S. cerevisiae* shall be incubated at (30 ± 1) °C for 48 h.

Appropriate colony counts of each dilution shall be used for microorganism quantification (15 cfu to 300 cfu per plate).

If necessary for identification, some colonies can be sub-cultivated on appropriate selective culture media (see 5.2.2.1.5 and 5.2.2.1.6).

6.4.2.3 Determination of microorganisms after washing (washed biomonitors, N)

After a serial dilution (see 6.4.2.2), take a sample of the dilutions v^0 to v^{-6} for the bacterial strains, and of the dilutions v^0 to v^{-5} for the yeast strains. Follow the procedure for inoculation of the samples, incubation of the agar plates and counting of the colonies as outlined in 6.4.2.2.

6.4.2.4 Determination of cross-contamination during the washing cycle (uncontaminated biomonitors)

After serial dilution (see 6.4.2.2) up to dilution v^{-2} (10^{-2} test-tube), take a sample of the dilutions v^0 to v^{-2}. Inoculate the samples using the pour-plate or the spread-plate technique. Follow the procedure for inoculation of the samples, incubation of the agar plates and counting of the colonies as outlined in 6.4.2.2.

6.5 Validation

6.5.1 Enumeration of microorganisms before washing N_0 (reference biomonitor)

For each **test run**, the microorganism content of three **biomonitors** per test microorganism strain shall be determined before exposure to the tested **programme**. The determination of microorganism counts on the carriers is performed as described in 6.4.2. The weighted average mean value ($\log_{10}$) of the three **biomonitors** is the basis for the calculation of reduction factors R (see 7.1) for the tested **programme**.

6.5.2 Negative control (cross contamination)

For each **test run**, the microorganism content of five former sterile cotton carriers (see 6.3.2.4) shall be assessed as described in 6.4.2 to detect **cross contamination** of a **test run**.

6.5.3 Determination of water quality

To ensure appropriate microbiological water quality, supplied water is sampled prior to a **test series**, as described in 5.1.

Transfer 1,0 ml and 0,1 ml water sample into two separate Petri dishes and add 15 ml to 20 ml of melted Tryptic soy agar (see 5.2.2.1.2) cooled to (45 ± 1) °C to determine the total colony count. When using the spread-plate technique, spread a 1,0 ml and a 0,1 ml water sample on two separate surface-dried plates containing Tryptic soy agar (see 5.2.2.1.2). Incubate the plates at (30 ± 1) °C for 24 to 48 h.

Filter a 100,0 ml water sample through a sterile membrane filter made of cellulose ester with a medium pore size of 0,45 µm. Membrane filters are laid on Cetrimide agar (see 5.2.2.1.6) for the detection of *P. aeruginosa*. Incubate the plates at (36 ± 1) °C for 24 h to 48 h.

Appropriate colony counts shall be used for microorganism quantification (15 cfu to 300 cfu per plate).

Pseudomonas aeruginosa threshold values for the water supplied to the **washing machine** are given in 5.1.

6.5.4 Determination of water quantity in the main wash

The amount of water in the main wash for each **test run** of the tested **programme** can be determined as described in IEC 60456.

7 Evaluation

7.1 Log reduction

The reduction factor for each **test run** and test microorganism is calculated as $R = \log(N_0 / N)$

where

N_0 is the average value of the microbial counts of 3 **biomonitors** before washing (see 6.4.2.2);

N is the average value of the microbial counts of the 5 **biomonitors**, after exposure to the tested **programme** (see 6.4.2.3).

The standard deviation shall be calculated for at least 3 **test runs** for N_0, N and the reduction factors R.

7.2 Cross contamination

Any growth of microorganisms after the incubation period of the 5 control swatches (see 6.4.2.4) shall be reported in cfu/sterile cotton carrier.

If no growth could be detected, this shall also be stated as < 10 cfu/sterile cotton carrier.

7.3 Validity of test run and test series

When the conditions below are satisfied, each **test run,** or **test series** is deemed valid. If any of these conditions are not met, any invalid **test run** shall be repeated. Invalid **test runs** shall be documented in the test report.

- Water quality of the water supplied to the test **washing machine** shall meet the conditions outlined in 5.1, assessed once within a **test series**.
- The initial microbial counts of the **biomonitors** after drying shall exceed 10^6 cfu per carrier for *P. aeruginosa*, *S. aureus*, and *S. arlettae*, and exceed 10^5 cfu per carrier for *C. albicans* and *S. cerevisiae* (refers to 6.3.2.2).
- Standard deviation of the initial counts of the **biomonitors** $\log(N_0)$ and the microbial counts after exposition to the tested **programme** $\log(N)$ shall not exceed 0,5 within one **test run**.
- Standard deviation of the initial counts of the **biomonitors** $\log(N_0)$ and the microbial counts after exposition to the tested **programme** $\log(N)$ shall not exceed 1 within a **test series**.

8 Test report

The test report shall contain the following information:

- Reference to this Technical Specification
- Date/period of testing
- Type of **washing machine** according to IEC 60456
  - Supplier's name or trademark
  - Suppliers model identifier
- **Programme** tested
- **Base load mass** in kg, and in percentage of the maximum capacity
- Ambient conditions: voltage and frequency of electricity supply and ambient temperature
- Water quality of supplied water: Aerobic mesophilic counts (30 °C), *Pseudomonas aeruginosa* counts in 100 ml water
- Test microorganisms used
- Drying temperature and drying time of **biomonitors**
- Number of **test runs** (valid and invalid)
- Microorganism number per microorganism type and **biomonitor**, before test
- Microorganism number per microorganism type and **biomonitor**, after test
- Average microbial numbers on carriers before washing expressed as $\log(N_0)$, per **test run** and **test series** for the individual test microorganisms
- Standard deviations of average microbial numbers on carriers before washing expressed as $\log(N_0)$, per **test run** and **test series** for the individual test microorganisms
- Average microbial numbers on carriers after exposure to the tested **programme**, expressed as $\log(N)$, per **test run** and **test series** for the individual test microorganisms.
- Standard deviations of average microbial numbers on carriers after exposure to the tested **programme**, expresses as $\log(N)$, per **test run** and **test series** for the individual test microorganisms.
- Reduction factor R per microorganism type (including standard deviation)
- Microorganism transfer to sterile carriers (**cross contamination**)
- **Temperature profile** during **test runs**
- Water hardness
- Production data of **biomonitors** (for commercially available **biomonitors**: Lot-number, production date and quality control data)
- Special comments

The test report may contain additional information e.g., determined in accordance with IEC 60456. Examples are:

- Energy consumption
- Water consumption (total, main wash, rinsing)
- **Programme** time
- Water inlet temperature
- Maximum washing temperature
- Residual moisture content