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Cosmetics — Microbiology — Detection of *Candida albicans*

Cosmétiques — Microbiologie — Détection de Candida albicans

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Foreword

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The committee responsible for this document is ISO/TC 217, *Cosmetics*.

This second edition cancels and replaces the first edition (ISO 18416:2007), of which it constitutes a minor revision.

Introduction

Microbiological examinations of cosmetic products are carried out according to an appropriate microbiological risk analysis in order to ensure their quality and safety for consumers.

Microbiological risk analysis depends on several parameters such as the following:

— potential alteration of cosmetic products;

— pathogenicity of microorganisms;

— site of application of the cosmetic product (hair, skin, eyes, mucous membranes);

— type of users (adults, children under 3 years).

For cosmetics and other topical products, the detection of skin pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* may be relevant because they can cause skin or eye infections. The detection of other kinds of microorganism might be of interest since these microorganisms (including indicators of faecal contamination e.g. *Escherichia coli*) suggest hygienic failure during the manufacturing process.

Cosmetics — Microbiology — Detection of *Candida albicans*

1 Scope

This International Standard gives general guidelines for the detection and identification of the specified microorganism *Candida albicans* in cosmetic products. Microorganisms considered as specified in this International Standard might differ from country to country according to national practices or regulations.

In order to ensure product quality and safety for consumers, it is advisable to perform an appropriate microbiological risk analysis to determine the types of cosmetic product to which this International Standard is applicable. Products considered to present a low microbiological (see ISO 29621) risk include those with low water activity, hydro-alcoholic products, extreme pH values, etc.

The method described in this International Standard is based on the detection of *Candida albicans* in a non-selective liquid medium (enrichment broth), followed by isolation on a selective agar medium. Other methods may be appropriate dependent on the level of detection required.

NOTE For the detection of *Candida albicans*, subcultures can be performed on non-selective culture media followed by suitable identification steps (e.g. using identification kits).

Because of the large variety of cosmetic products within this field of application, this method may not be appropriate in every detail for some products (e.g. certain water immiscible products). Other International Standards (ISO 18415) may be appropriate. Other methods (e.g. automated) may be substituted for the tests presented here provided that their equivalence has been demonstrated or the method has been otherwise shown to be suitable.

2 Normative references

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

ISO 21148:2005, *Cosmetics — Microbiology — General instructions for microbiological examination*

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 and definitions

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

3.1
product
portion of an identified cosmetic product received in the laboratory for testing

3.2
sample
portion of the product (at least 1 g or 1 ml) that is used in the test to prepare the initial suspension

3.3
initial suspension
suspension (or solution) of the sample in a defined volume of an appropriate enrichment broth

3.4
sample dilution
dilution of the initial suspension

3.5
specified microorganism
aerobic mesophilic bacterium or yeast that is undesirable in a cosmetic product and is recognized as a skin pathogen species that may be harmful for human health or as an indication of hygienic failure in the manufacturing process

3.6
Candida albicans
yeast that form white to beige, creamy and convex colonies on the surface of a selective medium

Note 1 to entry: The main characteristic for identification is the production of germ tube and/or pseudomycelium and chlamydospore when the test is performed following the method specified in this International Standard.

3.7
enrichment broth
non-selective liquid medium containing suitable neutralizers and/or dispersing agents and demonstrated to be suitable for the product under test

4 Principle

The first step of the procedure is to perform an enrichment by using a non-selective broth medium to increase the number of microorganisms without the risk of inhibition by the selective ingredients that are present in selective/differential growth media.

The second step of the test (isolation) of the test is performed on a selective medium followed by identification tests.

The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganisms.[1] In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and demonstrated (see Clause 11).

5 Diluents and culture media

5.1 General

General instructions are given in ISO 21148. When water is mentioned in this International Standard, use distilled water or purified water as specified in ISO 21148.

The enrichment broth is used to disperse the sample and to increase the initial microbial population. It may contain neutralizers if the specimen to be tested has antimicrobial properties. The efficacy of the neutralization shall be demonstrated (see Clause 11). Information relative to suitable neutralizers is given in Annex B.

The enrichment broth (5.3.3.1), or any of the ones listed in Annex A, is suitable for checking the presence of *Candida albicans* in accordance with this International Standard provided that it has been demonstrated to be suitable in accordance with Clause 11.

Other diluents and culture media may be used if it has been demonstrated that they are suitable for use.

5.2 Diluent for the yeast suspension (tryptone sodium chloride solution)

5.2.1 General

The diluent is used for the preparation of yeast suspension used for the suitability test procedure (see Clause 11).

5.2.2 Composition

— tryptone, pancreatic digest of casein 1,0 g

— sodium chloride 8,5 g

— water 1 000 ml

5.2.3 Preparation

Dissolve the components in water by mixing while heating. Dispense into suitable containers. Sterilize in the autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

5.3 Culture media

5.3.1 General

Culture media may be prepared using the descriptions provided below or from dehydrated culture media in accordance with the manufacturer's instructions. The instructions provided by the supplier of the media should be followed.

NOTE Ready-to-use media can be used when their composition and/or growth yields are comparable to those of the formulae given herein.

5.3.2 Agar medium for suitability test (see Clause 11)

5.3.2.1 Sabouraud dextrose agar (SDA)

5.3.2.1.1 Composition

— dextrose 40,0 g

— peptic digest of animal tissue 5,0 g

— pancreatic digest of casein 5,0 g

— agar 15,0 g

— water 1 000 ml

5.3.2.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by heating. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min. After sterilization the pH shall be equivalent to 5,6 ± 0,2 when measured at room temperature.

5.3.2.2 Other agar media for suitability test

Other agar media for suitability test may be used as appropriate (see Annex A).

5.3.3 Enrichment broth

5.3.3.1 Eugon LT 100 broth

5.3.3.1.1 General

This medium contains ingredients which neutralize inhibitory substances present in the sample: lecithin and polysorbate 80, and dispersing agent octoxynol 9.

5.3.3.1.2 Composition

— pancreatic digest of casein	15,0 g
— papaic digest of soybean meal	5,0 g
— *L*-cystine	0,7 g
— sodium chloride	4,0 g
— sodium sulfite	0,2 g
— glucose	5,5 g
— egg lecithin	1,0 g
— polysorbate 80	5,0 g
— octoxynol 9	1,0 g
— water	1 000 ml

5.3.3.1.3 Preparation

Dissolve the components polysorbate 80, octoxynol 9 and egg lecithin successively in boiling water to complete dissolution. Dissolve the other components by mixing while heating. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

5.3.3.2 Other enrichment broths

Other enrichment broths may be used as appropriate (see Annex A).

5.3.4 Selective agar medium for isolation of *Candida albicans*

5.3.4.1 Sabouraud dextrose chloramphenicol agar

5.3.4.1.1 Composition

— dextrose	40,0 g
— peptic digest of animal tissue	5,0 g

— pancreatic digest of casein 5,0 g

— chloramphenicol 0,050 g

— agar 15,0 g

— water 1 000 ml

5.3.4.1.2 Preparation

Dissolve the components (including the chloramphenicol) or the dehydrated complete medium in the water by mixing while heating. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min. After sterilization the pH shall be equivalent to 5,6 ± 0,2 when measured at room temperature.

5.3.4.2 Other selective agar media

Other selective agar media may be used as appropriate (see Annex A).

5.3.5 Corn meal agar with 1 % polysorbate 80

5.3.5.1 Composition

— infusion from corn meal 50,0 g

— agar 15,0 g

— polysorbate 80 10,0 g

— water 1 000 ml

5.3.5.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by mixing while heating. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min. After sterilization the pH shall be equivalent to 6,0 ± 0,2 when measured at room temperature.

6 Apparatus and glassware

Use the laboratory equipment, apparatus and glassware described in ISO 21148.

7 Strains of microorganisms

For the verification of the test conditions suitability, the following representative strain is used:

Candida albicans ATCC[1)] 10231 or equivalent strain: IP[2)] 48.72 or NCPF[3)] 3179 or NBRC[4)] 1594 or KCTC[5)] 17205, or other equivalent national collection strain.

The culture should be reconstituted according to the procedures provided by the supplier of the reference strain.

1) American Type Culture Collection.

2) Institute Pasteur.

3) National Collection of Pathogenic Fungi.

4) National Biological Resource Center.

5) Korean Collection for Type Culture.

The strain can be kept in the laboratory in accordance with EN 12353.

8 Handling of cosmetic products and laboratory samples

If necessary, store products to be tested at room temperature.

Do not incubate, refrigerate or freeze products and samples before or after analysis.

Sampling of cosmetic products to be analysed should be carried out as described in ISO 21148. Analyse samples as described in ISO 21148 and according to the procedure in Clause 9.

9 Procedure

9.1 General recommendation

Use sterile material, equipment and aseptic techniques to prepare the sample, initial suspension and dilutions. In the case of the preparation of the initial suspension in an appropriate solubilizing agent, the time which elapses between the end of preparation and the moment the inoculum comes into contact with the enrichment broth shall not exceed 45 min, unless specifically mentioned in the established protocols or documents.

9.2 Preparation of the initial suspension in the enrichment broth

9.2.1 General

The enrichment is prepared from a sample (3.2) of at least 1 g or 1 ml of the well-mixed product under test, which is dispersed in at least 9 ml of enrichment broth.

Note *S*, the exact weight or volume of the sample.

The method shall be checked to ensure that the composition (neutralizer eventually added) and the volume of the broth perform satisfactorily (see 11.3).

NOTE In some cases, and when possible, filtration of the cosmetic product through a membrane that is afterwards immersed in the enrichment broth, facilitates the neutralization of the antimicrobial properties of the product (see 11.3.)

9.2.2 Water-miscible products

Transfer the sample, *S*, of product to a suitable container containing an appropriate volume of broth.

9.2.3 Water-immiscible products

Transfer the sample, *S*, of product to a suitable container containing a suitable quantity of solubilizing agent (*e.g. Polysorbate 80*).

Disperse the sample within the solubilizing agent and add an appropriate volume of broth.

9.2.4 Filterable products

Use a membrane filter having a nominal pore size of not greater than 0,45 µm.

Transfer the sample, *S*, on to the membrane in a filtration apparatus (see ISO 21148). Filter immediately and wash the membrane using defined volumes of water and/or diluent.

Transfer and immerse the membrane into a tube or flask of suitable size containing an appropriate volume of broth.

9.3 Incubation of the inoculated enrichment broth

Incubate the initial suspension prepared in broth (see 9.2) at 32,5 °C ± 2,5 °C for at least 20 h (maximum 72 h).

9.4 Detection and identification of *Candida albicans*

9.4.1 Isolation

Using a sterile loop, streak an aliquot of the incubated enrichment broth on to the surface of Sabouraud dextrose chloramphenicol agar in order to obtain isolated colonies.

Invert the Petri dish and then incubate at 32,5 °C ± 2,5 °C for 24 h to 48 h.

Check for characteristic colonies (see Table 1).

Table 1 — Morphological characteristics of *Candida albicans* on selective agar medium

Selective medium	Aspect of the colonies of *Candida albicans*
Sabouraud dextrose chloramphenicol agar	*White to beige, creamy and convex*

9.4.2 Identification of *Candida albicans*

9.4.2.1 General

Candida albicans can appear to be dimorphic and is capable of producing pseudohyphae, some true hyphae, and clusters of round blastoconidia as well as large thick-walled chlamydospores. At low ambient temperature the culture might express this pseudo-mycelial form; however, it can change to the unicellular form at higher temperatures.

Proceed to the following tests for the suspect colonies isolated on the Sabouraud dextrose chloramphenicol agar medium. The presence of *Candida albicans* may be confirmed by other suitable cultural and biochemical tests.

9.4.2.2 Gram's stain

Follow the procedure specified in ISO 21148.

The microscopic observation shall reveal a violet colour, short ovoid or elongated cells, sometimes with budding cells.

9.4.2.3 Germ tube production

9.4.2.3.1 Place 0,5 ml to 1 ml of serum (foetal calf or horse serum) in a small test tube.

9.4.2.3.2 Emulsify a small portion of yeast colony to be tested in the serum.

9.4.2.3.3 Incubate in a water bath, at 37 °C ± 1 °C, for 1,5 h to 2 h, or in an incubator at 37 °C ± 2 °C for 3 h.

9.4.2.3.4 Place a drop of serum on a slide, put on a coverglass and examine microscopically for germ tube production.

Germ tubes appear as cylindrical filaments originating from the blastospore, without any constriction at the point of origin and without obvious swelling along the length of the filament.

The formation of germ tubes characterizes the presence of *Candida albicans*.

If germ tubes were not formed, the colonies shall be examined for production of hyphae, pseudohyphae and chlamydospores in accordance with 9.4.2.4.

9.4.2.4 Culture on corn meal agar with 1 % polysorbate 80

9.4.2.4.1 Remove a small portion of the yeast colony with an inoculating wire and streak-inoculate the surface of the medium across the centre of the plate. Place a sterile coverglass over the inoculum streak.

9.4.2.4.2 Incubate at 32,5 °C ± 2,5 °C for up to 3 d.

9.4.2.4.3 After 24 h, remove the dish lid and examine the growth through the coverglass under the microscope with magnification of 100× to 400×.

Candida albicans produces large, highly refractile, thick-walled chlamydospore which may be seen terminally or on short lateral branches.

10 Expression of the results (detection of *Candida albicans*)

If the identification of the colonies confirms the presence of this species, express the result as:

— "Presence of *Candida albicans* in the sample *S*"

If no growth after enrichment is observed and/or if the identification of the colonies does not confirm the presence of this species, express the result as:

— "Absence of *Candida albicans* in the sample *S*"

11 Neutralization of the antimicrobial properties of the product

11.1 General

The different tests described below demonstrate that the microorganism can grow in analysis conditions.

11.2 Preparation of inoculum

Prior to the test, inoculate the surface of soybean casein digest agar (SCDA) or other suitable (non-selective, non-neutralizing) medium with *Candida albicans*. Incubate the plate at 32,5 °C ± 2,5 °C for 18 h to 24 h.

To harvest the culture use a sterile loop, streak the surface of the culture and re-suspend in the diluent to obtain a calibrated suspension of about 1×10^8 CFU per ml (e.g. using spectrophotometer, ISO 21148:2005, Annex C).

Use this calibrated suspension and its dilutions within 2 h.

11.3 Suitability of the detection method

11.3.1 Procedure

11.3.1.1 In tubes of 9 ml of diluent, prepare a dilution of the calibrated suspension in order to obtain a final count between 100 CFU and 500 CFU per ml. To count the final concentration of viable microorganisms in the diluted calibrated suspension, transfer 1 ml of the suspension into a Petri dish and pour on 15 ml to 20 ml of the melted agar medium kept in a water bath at no more than 48°C. Let solidify and then incubate at 32,5 °C ± 2,5 °C for 20 h to 24 h.

11.3.1.2 Prepare in duplicate, the initial suspension in the conditions chosen for the test (at least 1 g or 1 ml of product under test, defined volume of enrichment broth) in a tube or flask. When using the membrane filtration method filter in duplicate at least 1 ml of product under test and transfer each membrane into a tube or flask containing the enrichment broth in the conditions chosen for the test.

11.3.1.3 Introduce aseptically, 0,1 ml of the diluted calibrated suspension (11.3.1.1) of microorganisms into one tube or flask (suitability test). Mix, then incubate both tubes or flasks (suitability test and non-inoculated control) at 32,5 °C ± 2,5 °C for 20 h to 24 h.

11.3.1.4 Perform an isolation for each tube or flask (suitability test and non-inoculated control). Using a sterile loop, streak an aliquot (same conditions as in the test) of the incubated mixture on to the surface of a Petri dish (diameter 85 mm to 100 mm) containing approximately 15 ml to 20 ml of Sabouraud dextrose chloramphenicol agar medium. Incubate the plates at 32,5 °C ± 2,5 °C for 24 h to 48 h.

11.3.2 Interpretation of suitability test results

Check that the diluted calibrated suspension (11.3.1.1) of bacteria contains between 100 CFU and 500 CFU per ml.

The neutralization is verified and the detection method is satisfactory if a growth characteristic of *Candida albicans* occurs on the suitability test plate and no growth occurs on the control plate.

When growth is detected on the control plate (contaminated products), the neutralization is verified and the detection method is satisfactory if *Candida albicans* is recovered on the suitability test plate.

Failure of growth on the suitability test plates indicates that antimicrobial activity is still present and necessitates a modification of the conditions of the method by an increase in the volume of nutrient broth, the quantity of product remaining the same, or by incorporation of a sufficient quantity of inactivating agent in the enrichment broth, or by an appropriate combination of modifications so as to permit the growth of *Candida albicans*.

If, in spite of the incorporation of suitable inactivating agents and a substantial increase in the volume of broth, it is still not possible to recover viable cultures as described above, indicate that the article is not likely to be contaminated with *Candida albicans*.

12 Test report

The test report shall specify the following information:

a) a reference to this International Standard, i.e. ISO 18416:2015,

b) all information necessary for the complete identification of the product;

c) the method used;

d) the results obtained;

e) all operating details for the preparation of the initial suspension;

f) the description of the method with the neutralizers and media used;

g) the demonstration of the suitability of the method, even if the test has been performed separately;

h) any point not specified in this document, or regarded as optional, together with details of any incidents that may have influenced the results.

Annex A
(informative)

Other media

A.1 Other enrichment broths

A.1.1 Fluid soybean-casein digest medium

A.1.1.1 Composition

—	pancreatic digest of casein	17,0 g
—	papaic digest of soybean meal	3,0 g
—	sodium chloride	5,0 g
—	potassium hydrogen phosphate	2,5 g
—	dextrose	2,5 g
—	water	1 000 ml

A.1.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, heating if necessary. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 7,3 ± 0,2 when measured at room temperature.

Dispense the medium into suitable containers.

A.1.2 Modified letheen broth

A.1.2.1 Composition

—	peptic digest of meat	20,0 g
—	pancreatic digest of casein	5,0 g
—	beef extract	5,0 g
—	yeast extract	2,0 g
—	lecithin	0,7 g
—	polysorbate 80	5,0 g
—	sodium chloride	5,0 g
—	sodium bisulfite	0,1 g
—	water	1 000 ml

A.1.2.2 Preparation

Dissolve successively in boiling water polysorbate 80 and lecithin to complete dissolution. Dissolve the other components by mixing while heating. Mix gently to avoid foam. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 7,2 ± 0,2 when measured at room temperature.

A.1.3 Glucose and peptone added lecithin-polysorbate 80 medium (GPLP 80 broth)

A.1.3.1 Composition

—	glucose	20,0 g
—	yeast extract	2,0 g
—	magnesium sulfate	0,5 g
—	peptone	5,0 g
—	potassium hydrogen phosphate	1,0 g
—	lecithin	1,0 g
—	polysorbate 80	7,0 g
—	water	1 000 ml

A.1.3.2 Preparation

Dissolve the components or the dehydrated complete medium successively in boiling water to complete dissolution. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 5,7 ± 0,2 when measured at room temperature.

A.1.4 D/E neutralizing broth (Dey/Engley neutralizing broth)[5]

A.1.4.1 Composition

—	glucose	10,0 g
—	soybean lecithin	7,0 g
—	sodium thiosulfate pentahydrate	6,0 g
—	polysorbate 80	5,0 g
—	pancreatic digest of casein	5,0 g
—	sodium bisulfite	2,5 g
—	yeast extract	2,5 g
—	sodium thioglycolate	1,0 g
—	bromocresol purple	0,02 g
—	water	1 000 ml

A.1.4.2 Preparation

Dissolve all of these components or dehydrated complete medium, one after another, in boiling water to complete dissolution. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 7,6 ± 0,2 when measured at room temperature.

A.1.5 Soybean-casein-digest-lecithin-polysorbate 80 medium (SCDLP 80 broth)

A.1.5.1 Composition

—	casein peptone	17,0 g
—	soybean peptone	3,0 g
—	sodium chloride	5,0 g
—	potassium hydrogen phosphate	2,5 g
—	glucose	2,5 g
—	lecithin	1,0 g
—	polysorbate 80	7,0 g
—	water	1 000 ml

A.1.5.2 Preparation

Dissolve all of these components or dehydrated complete medium successively in boiling water to complete dissolution. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 7,2 ± 0,2 when measured at room temperature.

A.2 Other agar media for suitability test

A.2.1 Potato dextrose agar medium (PDA)

A.2.1.1 Composition

—	potato extract	4,0 g
—	dextrose	20,0 g
—	agar	15,0 g
—	water	1 000 ml

A.2.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by mixing while heating. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 5,6 ± 0,2 when measured at room temperature.

A.2.2 Soybean-casein digest agar medium (SCDA) or tryptic soy agar (TSA)

A.2.2.1 Composition

—	pancreatic digest of casein	15,0 g
—	papaic digest of soybean meal	5,0 g
—	sodium chloride	5,0 g
—	agar	15,0 g
—	water	1 000 ml

A.2.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by mixing while heating. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min.

After sterilization and cooling of the solution, the pH shall be equivalent to 7,3 ± 0,2 when measured at room temperature.

A.3 Other selective agar medium — Potato dextrose agar medium with antibiotics

A.3.1 Composition

—	potato extract	4,0 g
—	dextrose	20,0 g
—	agar	15,0 g
—	chloramphenicol	0,05 g
—	water	1 000 ml

A.3.2 Preparation

Mix all the components and dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization and cooling of the solution, the pH shall be equivalent to 5,6 ± 0,2 when measured at room temperature.

Alternatively, chloramphenicol may be replaced by use of 0,10 g of benzylpenicillin potassium and 0,10 g of tetracycline per litre of medium, added as a sterile solution just prior to use.

Annex B
(informative)

Neutralizers of antimicrobial activity of preservatives and rinsing liquids

Preservative	Chemical compounds able to neutralize preservative's antimicrobial activity	Examples of suitable neutralizers and of rinse liquids (for membrane filtration methods)
Phenolic compounds: parabens, phenoxyethanol, phenylethanol, etc. anilides	Lecithin, polysorbate 80, ethylene oxide condensate of fatty alcohol, non-ionic surfactants	Polysorbate 80, 30 g/l + lecithin, 3 g/l. Ethylene oxide condensate of fatty alcohol, 7 g/l + lecithin, 20 g/l + polysorbate 80, 4 g/l. D/E neutralizing broth[a] Rinse liquid: distilled water; tryptone, 1 g/l + NaCl, 9 g/l; polysorbate 80, 5 g/l.
Quaternary ammonium compounds, cationic surfactants	Lecithin, saponin, polysorbate 80, Sodium dodecyl sulfate, Ethylene oxide condensate of fatty alcohol	Polysorbate 80, 30 g/l + sodium dodecyl sulfate, 4 g/l + lecithin, 3 g/l. Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l. D/E neutralizing broth[a] Rinse liquid: distilled water; tryptone, 1 g/l + NaCl, 9 g/l; polysorbate 80, 5 g/l.
Aldehydes, formaldehyde-release agents	Glycine, histidine	Lecithin, 3 g/l + polysorbate 80, 30 g/l + L-histidine, 1 g/l. Polysorbate 80, 30 g/l + saponin, 30 g/l + L-histidine, 1 g/l + L-cysteine, 1 g/l. D/E neutralizing broth[a] Rinse liquid: polysorbate 80, 3 g/l + L-histidine, 0,5 g/l.
Oxidizing compounds	Sodium thiosulfate	Sodium thiosulfate, 5 g/l. Rinse liquid: sodium thiosulfate, 3 g/l.
Isothiazolinones, imidazoles	Lecithin, Saponin, amines, sulfates, mercaptans, sodium bisulfite, sodium thioglycollate	Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l. Rinse liquid: tryptone, 1 g/l + NaCl, 9 g/l; polysorbate 80, 5 g/l.
Biguanides	Lecithin, saponin, polysorbate 80	Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l. Rinse liquid: tryptone, 1 g/l + NaCl, 9 g/l; polysorbate 80, 5 g/l.
Metallic salts (Cu, Zn, Hg), organo-mercuric compounds	Sodium bisulphate, L-cysteine sulfhydryl compounds, thioglycollic acid,	Sodium thioglycollate, 0,5 g/l or 5 g/l. L-cysteine, 0,8 g/l or 1,5 g/l. D/E neutralizing broth[a] Rinse liquid: sodium thioglycollate, 0,5 g/l.

NOTE See References[8] and.[11]

[a] D/E neutralizing broth (Dey/Engley neutralizing broth) see Annex A.

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