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PROTOCOLS

MICROBIOLOGICAL ANALYSIS IN THE COSMETIC INDUSTRY

ALL PROCEDURES ACCORDING TO ISO REGULATIONS

Inspired by knowledge

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Microbiological risk in the cosmetic industry

Nowadays, when the margin of health risk of marketed products is continuously narrowing, **microbiological testing** of **cosmetic products** seems **crucial** to guarantee consumer safety and product quality. Such measure is of vital importance in order to maintain a good image by the marketing or manufacturing company in the market.

To date, there is no official method for the microbiological control of cosmetics, but international organizations such as the **ISO Standards** (International Standard Organization) aim to harmonize and establish reproducible control parameters to ensure the reliability of the obtained results.

There is a series of ISO Standards whose function is to establish pollution risk analysis as well as acceptance or rejection criteria of the manufactured lots, standardizing the control in cosmetic products. The criteria of acceptance/ rejection of lots according to **ISO 17516: 2016** follows:

Microorganisms	**Children under the age of 3, products used in the eye are, mucous membranes**	**Other products**
Aerobic mesophilic bacteria (yeast and molds are also detectable)	≤ 1 x 10^2 UFC per g or ml[a]	≤ 1 x 10^3 UFC per g or ml[b]
Escherichia coli	Absence per 1 g or ml	Absence per 1 g or ml
Pseudomonas aeruginosa	Absence per 1 g or ml	Absence per 1 g or ml
Staphylococcus aureus	Absence per 1 g or ml	Absence per 1 g or ml
Candida albicans	Absence per 1 g or ml	Absence per 1 g or ml

Due to the variability inherent in the plate counting method, according to Chapter 61 of the USP or chapter 2.6.12 of the EP. Interpretation of results, the results are considered outside the limit if a> 200 CFU / g or ml b> 2000 CFU / g or ml

NOTE: When bacterial colonies are detected on Saboraud Dextrose agar, Saboraud Dextrose Agar can be used with antibiotics

From **Condalab** we put at your disposal all our **culture media according to ISO formulations** in order to facilitate the microbiological test analysis in cosmetics.

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Recount of *Total Mesophilic Aerobics*

Procedure as defined by ISO 21149:2017

Introduction

The analysis of this bacterial group includes all microorganisms capable of developing in the presence of oxygen and at a temperature between 20 °C and 45 °C. The count of mesophilic aerobic microorganisms, under these conditions, would estimate the total microflora of the product but would not specify nor identify the type of microorganism.

A low count of these microorganisms does not guarantee, or indeed indicate, the absence of pathogens or their toxins, in the same way as a high count would not indicate the presence of pathogenic flora. However, a high count is not desirable as this could mean:

- Excessive contamination of the raw material.
- Incorrect handling during the manufacturing process.
- The possibility that pathogens are present (since they are mesophilic microorganisms)
- An immediate impairment of the product.

Method

ENRICHMENT AND BLOCKING

1 ml/g of sample + 9 ml of Eugon LT 100 Broth (**CAT. 2110**)
Incubation: 32.5 °C ± 2.5 °C – 20 h/72 h

Other enrichening broths are Dey-Engley Neutralizing Broth (CAT. 2003), and Letheen Broth Modified (CAT. 1244)

If your product does not need neutralizers, you can dilute it directly in Buffered Peptone Water (CAT. 1402)

PRESUMPTIVE ISOLATION

Trypticasein Soy Agar (TSA) (**CAT. 1068**)
Incubation: 32.5 °C ± 2.5 °C – 24 h/48 h

Another isolating medium may be used such as Eugon LT 100 Agar (CAT. 2152)

READING THE RESULTS

A CFU plate count is performed and the decision to accept/reject is taken following the criteria established in ISO 17516:2004

Bibliography

COLIPA. *Guidelines on Microbial Quality Management*, 1997 Published by the European Cosmetic, Toiletry and Perfumery Association (COLIPA).

UNE-EN-ISO 21149:2017. *Cosmetics. Microbiology. Enumeration and detection of aerobic mesophilic bacteria.*

E P. *Microbiological Examination of non-sterile products*, 4th edition, published by the European Pharmacopeaia, 2002.

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Enumeration of *Yeasts and Molds*

Procedure as defined by ISO 16212:2017

Introduction

Yeasts and mold are classified as a kingdom Fungi, which is separate from the other eukaryotic life kingdoms of plants and animals. They come in multiple forms, including mushrooms, molds and microscopic organisms (like yeasts). Commonly the term mold is given to certain multicellular filamentous fungi, equipped with a microscopic mycelium and whose growth on foodstuffs is easily recognized by its velvety or cotton-like appearance.

Fungi are strict aerobe, eukaryotic, characteristically mycelial and heterotrophic microorganisms, obtaining nourishment by absorption. They develop in a pH range from 2 to 9, at a temperature between 10 to 35 °C and can grow in relatively low water activity conditions (a_w).

Method

ENRICHMENT AND BLOCKING

1 ml/g of sample + 9 ml of Eugon LT 100 Broth (**CAT. 2110**)
Incubation: 32.5 °C ± 2.5 °C – 20 h/72 h

Other enrichening broths are Dey-Engley Neutralizing Broth (CAT. 2003), and Letheen Broth Modified (CAT. 1244)

If your product does not need neutralizers, it can be diluted directly in Buffered Peptone Water (CAT. 1402)

PRESUMPTIVE ISOLATION

Sabouraud Dextrose Agar + Chloramphenicol (**CAT. 1134**)
Incubation: 25 °C ± 2.5 °C – 3/5 days

READING THE RESULTS

A CFU plate count is performed and the decision to accept/reject is taken following the criteria established in ISO 17516:2014

Bibliography

COLIPA. *Guidelines on Microbial Quality Management*, 1997 Published by the European Cosmetic, Toiletry and Perfumery Association (COLIPA).

UNE-EN-ISO 16212:2017. *Cosmetics. Microbiology. Enumeration of yeast and mould.*

EN 13624:2003, *Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants for instruments used in the medical area. Test method and requirements (phase 2, step 1).*

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Identification and isolation of *Escherichia Coli*

Procedure as defined by ISO 21150:2015

Introduction

This is a microorganism belonging to the Enterobacteriaceae family, a Gram-negative bacillus, with mobility and no spores. Furthermore, it is lactose positive (i.e. it ferments the carbohydrate in question) and oxidase negative.

It is a ubiquitous bacterium in the intestines of human beings and warm-blooded animals. Due to its high presence in the intestinal tract and in feces, it is a microorganism indicative of poor hygiene practices followed during the manufacturing process of cosmetic products.

Method

ENRICHMENT AND BLOCKING

1 ml/g of sample + 9 ml of Eugon LT 100 Broth (**CAT. 2110**)
Incubation: 32.5 °C ± 2.5 °C – 20 h/72 h

Other enrichment broths are Dey-Engley Neutralizing Broth (CAT. 2003), Letheen Broth Modified (CAT. 1244), and Lactose Broth (CAT. 1206)

Should you require further information on how to neutralize the different preservatives, please contact us

PRESUMPTIVE ISOLATION

MacConkey Agar (**CAT. 1052**)
Incubation: 32.5 °C ± 2 °C – 24 h/48 h

READING THE RESULTS

The colonies show up brick red in color, bile precipitates

BIOCHEMICAL TESTS

Selecting well-separated copies, carry out:
Gram stain (bacilli gram-negative) (**CAT. 4600**)

CONFIRMATION

Selective isolation in Levine Agar (EMB) (**CAT.1050**)
Incubation: 32.5 °C ± 2 °C – 24 h/48 h

READING THE RESULTS

E. coli colonies appear with a metallic sheen under reflected light and bluish-black under transmitted light

Bibliography

UNE-EN-ISO 21150:2015. *Cosmetic – Microbiology – Detection of Escherichia coli.*

Geis, PA (ed.). *Cosmetic Microbiology. A practical approach.* Taylor and Francis. 2nd edition. US (2006).

Entidad Nacional de Acreditación (ENAC). *Análisis microbiológicos. Documento aclaratorio.* NT-32 Rev. 2. (2010).

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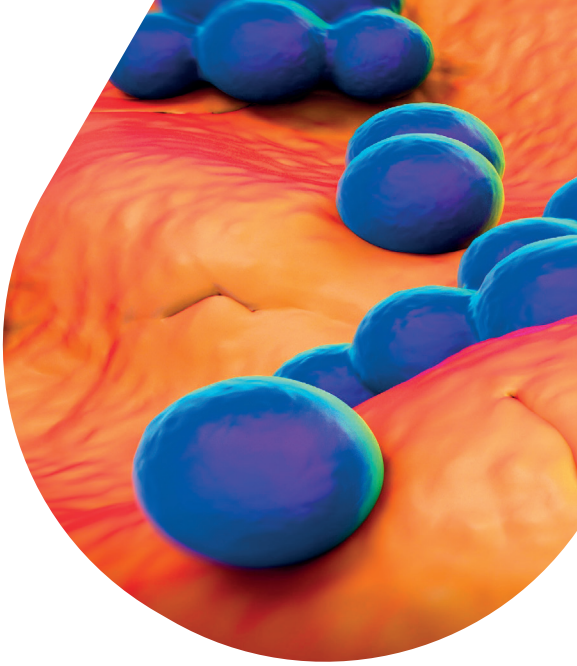
Identification and isolation of *Staphylococcus Aureus*



Procedure as defined by ISO 22718:2015

Introduction

They are bacteria belonging to the Staphylococcaceae family. They are facultative anaerobe cocci grouped in clusters, gram-positive, immobile with no spores. Furthermore, they produce virulence factors such as coagulase and catalase which are used as confirmatory proofs after isolation.

It is a bacterium found worldwide that feeds on the human epithelium. It is also an opportunistic pathogen, meaning that its identification in cosmetic products is crucial to ensure the health and well-being of consumers.

Method

ENRICHMENT AND BLOCKING

1 ml/g of sample + 9 ml of Eugon LT 100 Broth (**CAT. 2110**)
Incubation: 32.5 °C ± 2 °C – 20 h/72 h

Other enrichment broths can also be used, such as:
Dey-Engley Neutralizing Broth (CAT. 2003)
Letheen Broth Modified (CAT. 1244)

Should you require further information on how to neutralize the different preservatives, please contact us

PRESUMPTIVE ISOLATION

Baird Parker Agar (**CAT. 1100** + **CAT. 5129**)
Incubation: 32.5 °C ± 2 °C – 24 h/48 h

There are other culture media contemplated in Standards such as: Mannitol Salt Agar (MSA) (Chapman Medium) (CAT. 1062), Vogel-Johnson Agar (CAT. 1079)

READING THE RESULTS

Colonies show up in a brilliant black color, surrounded by a clear zone (2 – 5 mm)

BIOCHEMICAL TESTS

Selecting well-separated colonies, carry out:

- Gram stain (Gram-positive cocci grouped in clusters) (**CAT. 4600**)
- Catalase Assay (+)
- Coagulase Test (+)

Bibliography

Harris LG, Foster SJ, Richards RG. *An introduction to Staphylococcus aureus, and techniques for identifying and quantifyings S.aureus adhesins in relations to adhesion to biomaterials: Review.* Eur Cells Mater. 2002 jul-dec; 4(2): 39-60.

Bustos-Martínez JA, Hamdan-Partida A, Gutiérrez-Cárdenas M. *Staphylococcus aureus: la reemergencia de un patógeno en la comunidad.* Rev Biomed. 2006 oct-dic; 17(4): 287-305.

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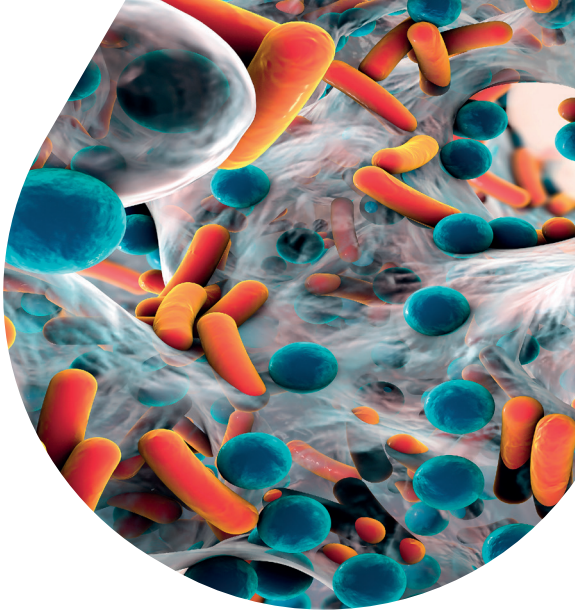

Identification and isolation of *Pseudomonas Aeruginosa*

Procedure as defined by ISO 22717:2015

Introduction

They are bacteria belonging to the Pseudomonadaceae family. They are straight or curved bacilli, strict aerobes, Gram-negative, motile with no spores. They are, furthermore, catalase positive, using this characteristic for their identification.

This group of bacteria are capable of producing different pigments such as pyocyanin and fluoresceine. In fact, several culture media were developed to promote the production of these pigments and for their identification.

Method

ENRICHMENT AND BLOCKING

1 ml/gr of sample + 9 ml of Eugon LT 100 Broth (**CAT. 2110**)
Incubation: 32.5 °C ± 2.5 °C – 20 h/72 h

Other enrichment broths can also be used, such as:
Dey-Engley Neutralizing Broth (CAT. 2003)
Letheen Broth Modified (CAT. 1244)

Should you require further information on how to neutralize the different preservatives, please contact us

PRESUMPTIVE ISOLATION

Cetrimide Agar (**CAT. 1102**)
Incubation: 32.5 °C ± 2.5 °C – 24 h/48 h

READING THE RESULTS

Colonies show up with greenish-yellow pigments (procyonine) with florescence under UV light

BIOCHEMICAL TESTS

selecting well-separated colonies, carry out:

- Gram stain (Gram-negative bacilli) (**CAT. 4600**)
- Oxidase test (+)

CONFIRMATION

Isolation of the suspicious colonies grown in Cetrimide Agar in Pseudomonas Agar P (**CAT. 1531**)
Incubation: 32.5 °C ± 2.5 °C – 24 h/48 h/72 h

READING THE RESULTS

Check on growth on 24, 48 and 72 h. *P. aeruginosa* forms colonies surrounded by a greenish-blue area (pyocyanin) or red/brown (pyorubin)

Bibliography

SINGER S. *The Use of Preservative Neutralizers in Diluents and Plating Media*. Cosmetics and Toiletries. 1987, December, 102 p 55.

E P. *Microbiological Examination of non-sterile products*, 4th edition, published by the European Pharmacopeaia, 2002.

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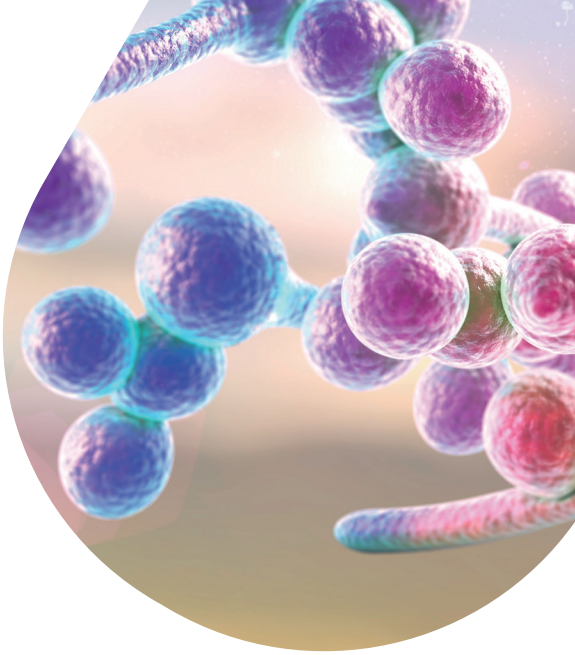

Identification and isolation of *Candida Albicans*

Procedure as defined by ISO 18416:2015

Introduction

These are yeasts belonging to the Saccharomycetaceae family and the *Candida* genus. These fungi develop in a unicellular form and are saprophytes of human beings. It is extremely common to find them in oral cavities, gastrointestinal tract and vaginal area. Although they perform a very important function in in the fermentation of certain sugars for the organism, there are occasions when this microorganism acts as an opportunistic pathogen.

On account of this, cosmetic products must be analyzed to identify the presence or not of this microorganism to avoid the unwanted consequences of an infection of *C. albicans* to the consumer.

Method

ENRICHMENT AND BLOCKING

1 ml/g of sample + 9 ml of Eugon LT 100 Broth (**CAT. 2110**)
Incubation: 32.5 °C ± 2.5 °C – 20 h/72 h

Other enrichment broths can also be used, such as:
Dey-Engley Neutralizing Broth (CAT. 2003)
Letheen Broth Modified (CAT. 1244)

Should you require further information on how to neutralize the different preservatives, please contact us

PRESUMPTIVE ISOLATION

Agar Dextrosa Saboraud with Cloranfenicol (**CAT. 1134**)
Incubation: 32,5 °C ± 2,5 °C – 24 h/48 h

There are other culture media contemplated in Standards such as: Potato Dextrose Agar (CAT. 1022)

There are also other routine ones not contemplated in Standards such as Biggy Agar (CAT. 1006)

READING THE RESULTS

The colonies are white to beige, creamy and convex

IDENTIFICATION

Selecting well-separated colonies, carry out:

- Gram stain (**CAT. 4600**): short or elongated ovoid cells, violet in color with possible germinating cells
- Germination test (+)

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KELLY, J.P., and FUNIGIELLO, F. *Candida albicans: A study of media designed to promote chlamydospore production*, J. Lab. & Clin. Med., 53, 1959, pp 807 – 809.

GORDON, M.A and LITTLE G.N., *Effective dehydrated media with surfactants for identification of Candida albicans*. J. of Int. Soc. for Human and Animal Mycol. 2, 1963, pp 171 – 175.

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