



HC Agar Base

M1388

Intended use:

Recommended for enumeration of moulds in cosmetic products when supplemented with Polysorbate 80.

Composition**

Ingredients	Gms / Litre
Tryptone	2.500
Proteose peptone	2.500
Yeast extract	5.000
Dextrose (Glucose)	20.000
Disodium hydrogen phosphate	3.500
Potassium dihydrogen phosphate	3.400
Ammonium chloride	1.400
Magnesium sulphate	0.060
Sodium carbonate	1.000
Chloramphenicol	0.100
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.46 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Add 20 ml of Polysorbate 80. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Cosmetics do not need to be sterile but they must be adequately preserved. Microbial contamination to cosmetics is a substantial risk to product quality, regulatory compliance and consumer health (1). HC Agar Base, formulated by Mead and O'Neill, is used for enumerating moulds in cosmetic products (5). This medium differs from the traditionally used media for testing cosmetics products by addition of Polysorbate 80 and incubation time of 3 days, rather than 7 days, at 27°C ± 0.5°C to obtain a significant mold count (2).

HC Agar Base contains tryptone and proteose peptone, which serve as sources of carbon, nitrogen, vitamins and minerals. Yeast extract acts as a source of B-complex vitamins that helps to stimulate bacterial growth. Dextrose serves as a source of energy by being the fermentable carbohydrate. Ammonium chloride and magnesium sulphate provide essential ions. Phosphates buffer the medium. Sodium carbonate helps to inactivate the low levels of preservatives if present (e.g. benzoic acid). Chloramphenicol inhibits accompanying bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens*. Polysorbate 80 also neutralizes preservatives and sequesters surfactants that may be present in the sample (5).

Type of specimen

Cosmetic products

Specimen Collection and Handling:

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. The 27±0.5°C incubation temperature is critical for obtaining scrupulously significant mold counts after three days.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Pale yellow to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured with yellow tinge, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.44% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 27.5 ± 0.5°C for 65-72 hours.

Organism

Growth

# <i>Aspergillus brasiliensis</i> ATCC 16404 (00053*)	good
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	none-poor
<i>Serratia marcescens</i> ATCC 8100	none-poor

* Key: Corresponding WDCM numbers. #Formerly known as *Aspergillus niger*

Storage and Shelf Life

Store dehydrated and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Brannan D. K., (Ed.), Cosmetic Microbiology, A Practical Handbook, CRC Press
2. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C. Composition
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Mead C. and O'Neill J., 1986, J. Soc. Cosmet Chem., 37:49-5

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