POTATO DEXTROSE AGAR

Dehydrated and ready-to-use culture medium

1-INTENDED USE

General purpose medium for the isolation, cultivation and enumeration of yeasts and moulds.

2- COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Potato extract	5.0 g
Glucose	20.0 g
Agar	17.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3-PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Potato Dextrose Agar (PDA) is a general-purpose medium for the isolation, cultivation and enumeration of yeasts and moulds. It meets harmonized EP, USP, JP performance specifications[1], where applicable, and corresponds to FDA-BAM medium 127.[2]
PDA is recommended by FDA-BAM[3] for the purification of the colonies enumerated with DG18 agar or DMRC and with the addition of chlortetracycline for the enumeration of yeasts and moulds in cosmetics.[4]
APHA recommends the use of PDA for the detection and enumeration of heat-resistant moulds in foods, because they are not-fastidious in their nutrient requirements and because they will easily form fruiting bodies which enables quick phenotype-based identification.[5]
PDA and PDA with 50 mg/L of chloramphenicol are recommended by ISO 18416[6] and ISO 16212[7] as alternative media to Sabouraud Dextrose Agar with and without chloramphenicol for suitability test and for the detection of *C. albicans* and enumeration of yeasts and moulds in cosmetics.
Potato extract encourages luxuriant fungal growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria. Glucose, at high concentration, is a carbon and energy source.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and pour into sterile Petri dishes. Medium should not be re-melted more than once.
For cosmetics, cool medium to 47-50°C after autoclaving and add 4 mL of filter sterilised 1% chlortetracycline HCl solution (1 g/100 mL) per litre of medium or add 4 mL of the contents of one vial of Dermatophyte Antimicrobic Supplement (REF 4240024) reconstituted with 5 mL of sterile purified water (final concentration in the medium: 40 mg/L). Alternatively add the contents of one vial od Chloramphenicol Antimicrobic Supplement (REF 4240003) to 1 litre of medium before autoclaving (final concentration in the medium: 50 mg/L).

5-PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	very pale yellow, opalescent
Final pH at 20-25 °C	5.6 ± 0.2

6-MATERIALS PROVIDED

Product	Type	REF	Pack
Potato Dextrose Agar	Dehydrated medium	4019352 4019354	500 g (11.9 L) 5 kg (119 L)
Potato Dextrose Agar	Ready-to-use plates	541935D	2 x 10 plates ø 90 mm. Non-vented dishes.

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents. Dermatophyte Antimicrobic Supplement (REF 4240024), Chloramphenicol Antimicrobic Supplement (REF 4240003).

8-SPECIMENS

Foods and cosmetics. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[1-6]

9-TEST PROCEDURE, READING AND INTERPRETATION

Enumeration of yeasts and moulds in cosmetics[4]

1. Use spread plate technique to facilitate recognition of different colony types. Decimally dilute the cosmetic preparation to obtain a complete dilution series from 10^{-1} to 10^{-3}. Mix the dilutions thoroughly and perform all plating in duplicate.
2. Aseptically pipet 0.1 mL of each dilution on pre-poured, solidified Potato Dextrose Agar plates supplemented with 40 mg/L of chlortetracycline and spread inoculum with a sterile, bent glass rod.
3. After the inoculum is absorbed by the medium, incubate the plates at 30 ± 2°C (Do not invert and do not stack more than 3 plates high).
4. Count colonies after 5 days of incubation. If there is no growth after 5 days, re-incubate for another 48 h.

Enumeration of heat-resistant moulds[5]

1. Heat the homogenized sample 30 minutes at 75°C-80°C and cool rapidly to 55°C.
2. Mix thoroughly the cooled suspension with an equal volume of warm (45°C) double strength Potato Dextrose Agar and dispense in 150 mm diameter Petri dishes.
3. Loosely seal the Petri dishes and incubate at 30°C for at least 14 days. Colonies formed by most activated ascospores will be visible in 7-10 days while heat injured or debilitated ascospores require additional time to form colonies.

For other applications, the user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

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10-USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	25°C / 72 h / A	good growth, white yeast-like colonies
A. brasiliensis ATCC 9642	25°C / 72 h / A	good growth, colonies with black hyphae

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11-PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Potato Dextrose Agar (Test Batch:TB) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by semi-quantitative ecometric technique with the following strains *C. albicans* ATCC 18808, *A. brasiliensis* ATCC 9642, *S. cerevisiae* ATCC 9763, *P. chrysogenum* ATCC 10106. After incubation at 25°C for up to 72 hours, the amount of growth on the plates and colonies' characteristics are evaluated and recorded: they shall be comparable in TB and RB.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strain *S. aureus* ATCC 25923. The growth of non-target strain is partially inhibited.

12-LIMITATIONS OF THE METHOD

- Potato Dextrose Agar has poor selective properties; where bacterial overgrowth may be a problem, chloramphenicol (50 mg/L) or chlortetracycline (40 mg/L) are recommended.
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[8]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[8]
- Non-linearity of counts from dilution plating often occurs, i.e., 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[8]
- For complete identification of isolated microorganisms, it is recommended to perform appropriate tests using pure cultures.

13 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

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The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type (plates/tubes/bottles), and the storage method applied (temperature and packaging). According to MacFaddin, the plated medium prepared by the user can be stored at 2-8°C for 6-8 weeks protected from dessication.[9]

15 - REFERENCES

1. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Media M127: Potato Dextrose Agar. Content current as of: 10/17/2017.
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Chapter 18: Yeasts, Molds and Mycotoxins. Content current as of: 11/07/2022.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Chapter 23: Methods for Cosmetics. Content current as of: 12/23/2021.
5. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
6. ISO18416:2015. Cosmetics — Microbiology —Detection of Candida albicans.
7. ISO 16212:2017 Cosmetics — Microbiology — Enumeration of yeast and mould.
8. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/11

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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