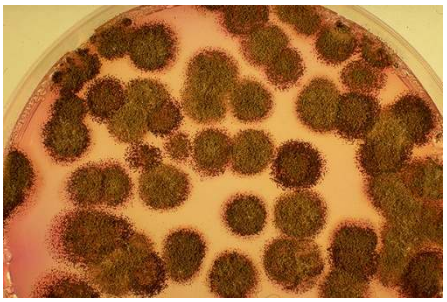
ROSE BENGAL AGAR BASE

Dehydrated culture medium



Rose Bengal Agar with chloramphenicol: colonies of *Aspergillus brasiliensis*

1 - INTENDED USE

For the enumeration of yeasts and moulds in foods, animal feeding stuffs and waters.

2 – COMPOSITIONS

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

Mycological peptone	5.00 g
Dipotassium hydrogen phosphate	1.00 g
Magnesium sulphate	0.50 g
Glucose	10.0 g
Rose bengal	0.05 g
Agar	15.00 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

Rose Bengal Agar is based on the formulation devised by Jarvis[1] and modified by Overcast and Weakley[2], in which chlortetracycline has been replaced by chloramphenicol. It is a selective medium at neutral pH for the enumeration of yeasts and moulds in foods and is recommended for fresh proteinaceous foods whose associated flora is composed mainly by Gram-negative bacilli.[3]
Rose Bengal Agar, supplemented with chlortetracycline, is recommended by APHA[4] for the enumeration of fungi in sewage and polluted waters with spread plate and membrane filter techniques.
The culture medium proposed here is a base to which an antibiotic supplement of the user's choice can be added, to avoid the risks associated with the use of powdered media containing antibiotics.
Mycological peptone provides nitrogen and minerals for microbial growth and colonies pigmentation. Glucose is a source of carbon and energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium. Magnesium sulphate enhances the microbial growth. Rose bengal not only restricts the size and height of mould colonies but assists their enumeration as the colour is taken up by the colonies. Chloramphenicol or chlortetracycline are used as the selective agents to suppress most Gram-positive and Gram-negative bacteria.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 16 g in 500 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of Chloramphenicol Antimicrobic Supplement (REF 4240003) reconstituted with 3 mL of a mixture of sterile distilled water-ethanol (1:1). Mix well and distribute into sterile Petri dishes. The chloramphenicol supplement can also be added to the culture medium before sterilisation. The basic medium can also be supplemented with chlortetracycline hydrochloride 70 mg/L (Dermathopyte Antimicrobic Supplement REF 4240024) after autoclaving.
Avoid exposure of the medium to light.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	bright pink, clear
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Rose Bengal Agar Base	Dehydrated medium	4019912	500 g (15.6 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Foods, animal feeding stuffs and water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Prepare suitable decimal dilutions of the samples.
Add 1 mL to empty Petri dishes using two dishes for each dilution. Pour into each dish approximately 15 mL of melted medium, cooled to 44-47°C. Mix gently, allowing the medium to solidify.
Alternatively, directly inoculate the agar plates using surface spreading technique with 0.1 or 0.2 mL of decimal dilutions.
Alternatively use membrane filter technique: filter an appropriate volume of well-shaken sample or dilution through membrane filters with pore diameter of 0.45 µm and transfer to dishes.
Invert the plates and incubate at 22°C for 5-7 days.

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies
Count colonies on plates that contain an estimated 50-100 colonies. Report as number of yeasts or moulds per gram of food by multiplying the number of colonies by the dilution factor.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, E-mail: export@biolifeitaliana.it;
Web: www.biolifeitaliana.it

11 - 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
Saccharomyces cerevisiae ATCC 9763	25°C/ 72h/A	growth
Aspergillus brasiliensis ATCC 16404	25°C/ 72h/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25°C/ 72h/A	inhibited

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

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Rose Bengal Agar Base supplemented with Chloramphenicol Antimicrobic Supplement is tested for productivity and selectivity by comparing the results with a Reference Batch.
Productivity is tested by semi-quantitative ecometric method with the target strains *S. cerevisiae* ATCC 9763, *C. albicans* ATCC 18804, *P. chrysogenum* ATCC 10106, *A. brasiliensis* ATCC 9642*;* the plates are inoculated by surface spreading technique with decimal dilutions in saline of a colonies' suspension and incubated at 25 °C for 72 hours in air. Target strains exhibit good growth with typical colonies and limited colony spreading.
The 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 following strains: *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633. The growth of the non-target strain is totally inhibited.

13 – LIMITATIONS OF THE METHOD

- 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.[5]
- 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.[5]
- 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.[5]
- For complete identification of isolated microorganisms, it is recommended to perform appropriate tests using pure cultures.

14 - 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.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- 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 Sheet of the product 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

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).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/flasks) and the applied storage conditions (temperature and packaging). According to Baird *et al.* the self-prepared plates with chloramphenicol can be stored in the dark at 2 to 8°C for 7 days.[6] According to APHA the plated medium with chlortetracycline may be held up to 4 weeks at 2 to 8°C.[4]

16 – REFERENCES

1. Jarvis B. Comparison of an improved rose-bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J Appl Bacteriol 1973; 36: 723-727.
2. Overcast WW, Weakley DJ. An aureomycin-rose Bengal agar for the enumeration of yeasts and moulds in cottage cheese. J Milk Food Technol 1969; 32:442.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. APHA Standards Methods for the Microbiological of Water and Wastewater. 23rd, 2017. American Public Health Association, Washington D.C.,
5. 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.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:261-262.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/09

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