ANTIBIOTIC MEDIA

ANTIBIOTIC SEED AGAR A1
POLYMIXIN BASE AGAR A9
POLYMIXIN SEED AGAR A10
NEOMYCIN ASSAY AGAR A11

Dehydrated culture media

1 - INTENDED USE

Culture media for microbiological agar diffusion assay of antibiotics.

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

TABLE 1

Medium	Antibiotic Seed Agar A1	Neomycin Assay Agar A11	Polymyxin Base Agar A9	Polymyxin Seed Agar A10
REF	4010752	4017752	4019202	4019252
Peptone	6.0	6.0		
Tryptone	4.0	4.0	17.0	17.0
Yeast extract	3.0	3.0		
Beef extract	1.5	1.5		
Soy peptone			3.0	3.0
Glucose	1.0	1.0	2.5	2.5
Sodium chloride			5.0	5.0
Dipotassium hydrogen phosphate			2.5	2.5
Agar	15.0	15.0	20.0	12.0
Quantity required (g/L)	30.5	30.5	50.0	42.0
Tween 80 (added to the base)				10

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Antibiotic media are prepared with formulations in compliance with the FDA publication 21 CFR.[1] The antibiotic media are identified numerically with names assigned by Grove and Randall.[2] Antibiotic media are intended for microbiological assay of antibiotics and for the quantitative determination of antibiotics in pharmaceutical preparations, foods, animal feed preparations, and other materials. The most common method for microbiological assay of antibiotics is the agar diffusion test performed by cylinder, perforated hole or paper disc tests. The culture medium is inoculated with the test strain and poured into dishes. Defined quantities of antibiotic under test and an antibiotic standard are applied as spots onto the plates. During incubation, inhibition zones of microbial growth develop around the application site, and their diameter is a measure of the activity of the antibiotic tested, compared with the inhibition zones given by standard solutions of the same antibiotic.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend the required quantity of culture medium (see Table 1) in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121° C for 15 minutes. Cool to 47-50°C and add the microbial test strain. Mix well and distribute in sterile Petri dishes. Polymyxin Seed Agar A10 requires the addition of 10 g/L of Tween 80 before autoclaving.

5 - PHYSICAL CHARACTERISTICS

TABLE 2

Medium	Antibiotic Seed Agar A1	Neomycin Assay Agar A11	Polymyxin Base Agar A9	Polymyxin Seed Agar A10
REF	4010752	4017752	4019202	4019252
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder	beige, fine, homogeneous, free-flowing powder	beige, fine, homogeneous, free-flowing powder	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	yellow, limpid	yellow, limpid	yellow, limpid	yellow, limpid
Final pH at 20-25 °C	6.5 ± 0.1	7.8 ± 0.2	7.3 ± 0.2	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Antibiotic Seed Agar A1	Dehydrated medium	4010752	500 g (16.4 L)
Neomycin Assay Agar A11	Dehydrated medium	4017752	500 g (16.4 L)
Polymyxin Base Agar A9	Dehydrated medium	4019202	500 g (10 L)
Polymyxin Seed Agar A10	Dehydrated medium	4019252	500 g (11.9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, test strains, steel or porcelain assay cylinders or paper discs, ancillary culture media and reagents.

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8 – SPECIMENS

Pharmaceutical preparations, foods, animal feed preparations and other materials.

9 - TEST PROCEDURE

Preparation of the microbial suspension[1]

Maintain organisms on agar slants containing 10 mL of the appropriate medium (generally Antibiotic Seed Agar A1). Incubate the slants at 32°C–35°C for 24 hours. Using 3 mL of sterile saline solution, wash the growth from the agar slant onto a large agar surface, such as a Roux bottle, containing 250 mL of the appropriate medium. Spread the suspension of organisms over the entire surface of the Roux bottle with the aid of sterile glass beads. Incubate the Roux bottle at 32°C–35°C. Wash the resulting growth from the agar surface with 50 mL of sterile saline solution. Determine the dilution factor that will give a 25% light transmission at a wavelength of 580 millimicrons using a suitable photoelectric colorimeter and a 13 mm diameter test tube as an absorption cell. It may be necessary to adjust the suspension. Determine the amount of suspension to be added to each 100 mL of agar by the use of test plates. Store the test organism suspension under refrigeration.

This general working scheme is valid for all test strains, with these exceptions:

1) For *Bacillus subtilis* centrifuge the growth from the Roux bottle, decant the supernatant, take up the microorganisms in 50-70 mL of saline and heat the resulting suspension at 70°C for 30 minutes.

2) For *Bacillus cereus* var. *mycoides*, use the general method with the modifications described for *Bacillus subtilis*, but heat the microbial suspension before centrifugation and wash the spore suspension three times with 25-30 mL of sterile distilled water. After the last wash, resuspend the spores in 50-70 mL of sterile distilled water.

3) For *Microsporum gypseum* incubate an Erlenmeyer flask containing 200 mL of Sabouraud Broth with 20% glucose added, inoculated with the microorganism, for 6-8 weeks at 25°C. Check the degree of sporification and when it exceeds 80%, collect the spores from the mycelial layer with a spatula: the spores are on the surface of the material floating in the broth. Suspend the collected spores in 50 mL of sterile saline and store in a refrigerator.

4) Keep *Enterococcus faecalis* in 10 mL of Antibiotic Broth A3. To carry out the assay, prepare a fresh subculture by transferring an aliquot of the stock cultures into 100 mL of Antibiotic Broth A3 and incubate 16-18 hours at 37°C.

5) For *Saccharomyces cerevisiae* incubate the slant of Antibiotic Seed Agar A1 at 30°C for 24 hours and the Roux bottle at 30°C for 48 hours. Microbial suspensions prepared as described can be used without further dilution to inoculate the turbidimetric and diffusion dosing medium.

Microbiological agar diffusion assay

The activity of antibiotics is estimated by comparing the inhibition of growth of a sensitive microorganisms produced by 5 doses of reference substance and 1 dose of antibiotic to be examined.[3]

Prepare the inoculated suitable medium with a a known quantity of a suspension of test microorganism sensitive to the antibiotic to be examined.

Mix the medium and the inoculum and pour into Petry dishes a quantity to form a layer 2-5 mm thick. Alternatively, the medium may consist of 2 layers, only the upper layer being inoculated. For each Petri dish, 21 mL of base layer and 4 mL of the seed layer may be generally suitable.

Prepare the solutions of the reference substance and of the antibiotic to be examined having known concentrations.

Pipette the antibiotic solutions into the cylinders or into the punched holes or on paper-discs with a diameter of 9 mm placed on the culture medium.

Incubate the inoculated plates for 16 to 18 hours at the appropriate incubation temperature for each antibiotic.

10 - READING AND INTERPRETATION

After incubation, measure the diameters of the zones of inhibition using an appropriate measuring device such as a millimetre rule, callipers, or an optical projector. Draw a standard curve using the values of the standard solutions and read off the activities of the test solutions. Refer to appropriate procedures for results reading and interpretation.[1,3]

11 - USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing 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 inoculated by poured plate method, useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Antibiotic Seed Agar A1		
Staphylococcus aureus ATCC 6538P	35-37°C/18-24 H/A	good growth
Neomycin Assay Agar A11		
Micrococcus luteus ATCC 9341	35-37°C/24-48 H/A	good growth
Staphylococcus epidermidis ATCC 12228	35-37°C/24-48 H/A	good growth
Polymyxin Base Agar A9, Polymyxin Seed Agar A10		
Bordetella bronchiseptica ATCC 4617	35-37°C/40-48 H/A	good growth

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

12 - PRECAUTIONS AND WARNINGS

- These products are for microbiological control and for professional use only; they are 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.
- These culture media contain 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 the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture media 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture media 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 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.

13 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, they 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 products 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/tubes/flasks) and the applied storage conditions (temperature and packaging).

14- REFERENCES

1. Food and Drug Administration. 21 CFR, Part 436 – Tests and Methods of Assay of Antibiotic and Antibiotic Containing Drugs. Subpart D—Microbiological Assay Methods. April 1, 1996
2. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopaedia, Inc. New York, N.Y.
3. USP 31 <81> Antibiotic, Microbial Assay

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 1	Updated layout and content	2022/06

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

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