Antibiotic Media

Product	Description	Standards
Antibiotic Agar No. 1		AOAC, EP, USP
Antibiotic Agar No. 2		AOAC, USP
Antibiotic Agar No. 4		AOAC, USP
Antibiotic Agar No. 5		AOAC, USP
Antibiotic Medium No. 6	can be prepared from Antibiotic Agar No. 2 and 1 g/litre D(+)glucose	
Antibiotic Agar No. 7	corresponds to Antibiotic Agar No. 2 but with pH: 7.0 ± 0.2.	
Antibiotic Agar No. 8	corresponds to Antibiotic Agar No. 2 but with pH: 5.9 ± 0.2.	AOAC, USP
Antibiotic Agar No. 9	can be prepared from CASO Broth, 20 g/litre Agar-Agar	EP, USP
Antibiotic Agar No. 10	can be prepared from CASO Broth, 12 g/litre Agar-Agar and 10 g/litre Tween® 80	EP, USP
Antibiotic Agar No. 11		
Antibiotic Agar No. 12		
Antibiotic Broth (Medium No. 3)		AOAC, EP, USP
SABOURAUD-2 % Dextrose Broth (Medium No. 13)		AOAC, USP

For the microbiological assay of antibiotics in pharmaceutical preparations, body fluids, animal feed preparations, and other materials according to GROVE and RANDALL (1955).

These culture media comply with the recommendations of the United States Pharmacopeia XXVI (2003) and the FDA. Antibiotic agar I also corresponds to medium A of the European Pharmacopeia II.

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Principle

The sample material can be tested by dilution and diffusion methods. The most common method is the agar diffusion test which can be performed in various ways-cylinder, punched-hole or paper-disc tests. It is based on the following principle:

The culture medium is inoculated with the relevant test strain and poured into plates. Defined quantities of the antibiotic under examination and an antibiotic standard are applied as spots (cylinder, punched-hole, paper-discs). On incubation inhibition zones develop around the site of application, there is no microbial growth within these zones and their diameter is a measure of the activity of the antibiotic being tested. The activity of the antibiotic under test is determined by comparing the diameter of its inhibition zone with that of the antibiotic standard.

Typical Composition (g/litre)

Composition of the culture medium (g/l)	Medium No. 1 (MERCK)	Medium No. 2 (MERCK)	Medium No. 3 (MERCK)	Medium No. 4	Medium No. 5 (MERCK)	Medium No. 6	Medium No. 7
Meat extract	1.5	1.5	1.5	1.5	1.5	-	1.5
Yeast extract	3.0	3.0	1.5	3.0	3.0	-	3.0
Peptone from casein	4.0	-	-	-	-	17.0	-
Peptone from meat	6.0	6.0	5.0	6.0	6.0	-	6.0
Peptone from soymeal	-	-	-	-	-	3.0	-
D(+)glucose	1.0	-	1.0	1.0	-	2.5	-
Sodium chloride	-	-	3.5	-	-	5.0	-
di-Potassium hydrogen phosphate	-	-	3.68	-	-	2.5	-
Potassium dihydrogen phosphate	-	-	1.32	-	-	-	-
Agar-agar	15.0	15.0	-	15.0	15.0	-	15.0
Polysorbate 80	-	-	-	-	-	-	-
Manganse sulfate	-	-	-	-	-	0.03	-
Quantity required (g/litre)	30.5	25.5	17.5	26.5	25.5	30.0	25.5
pH at 25 °C	6.6 (± 0.2)	6.6 (± 0.2)	7.0 (± 0.2)	6.6 (± 0.2)	7.9 (± 0.2)	7.0 (± 0.2)	7.0 (± 0.2)

Composition of the culture medium (g/l)	Medium No. 8	Medium No. 9	Medium No. 10	Medium No. 11 (MERCK)	Medium No. 12 (MERCK)	Medium No. 13 (MERCK)
Meat extract	1.5	-	-	1.5	2.5	-
Yeast extract	3.0	-	-	3.0	5.0	-
Peptone from casein	-	17.0	17.0	4.0	-	-
Peptone from meat	6.0	-	-	6.0	10.0	10.0
Peptone from soymeal	-	3.0	3.0	-	-	-
D(+)glucose	-	2.5	2.5	1.0	10.0	20.0
Sodium chloride	-	5.0	5.0	-	10.0	-
di-Potassium hydrogen phosphate	-	2.5	2.5	-	-	-
Potassium dihydrogen phosphate	-	-	-	-	-	-
Agar-agar	15.0	20.0	12.0	15.0	25.0	-
Polysorbate 80	-	-	10.0	-	-	-
Manganse sulfate	-	-	-	-	-	-
Quantity required (g/litre)	25.5	50.0	52.0	30.5	62.5	30.0
pH at 25 °C	5.6 (± 0.2)	7.2 (± 0.2)	7.2 (± 0.2)	8.3 (± 0.2)	6.1 (± 0.2)	5.6 (± 0.2)

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Preparation

Suspend the required quantity of culture medium (see Table), autoclave (15 min at 121 °C), add the test strain of bacteria at 45-50 °C. Pour plates.pH: see table

The ready-to-use plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

1. Cylinder test:

Procedure: Fill Petridishes with 14 ml of the medium to form the base layer, after this has set overlay with 4 ml of the inoculated seed layer. Place steel or glass cylinders on the cooled culture medium under sterile conditions. The ready-to-use test plates can be stored in the refrigerator at +4 °C. Pipette the antibiotic solutions into the cylinders and then incubate at 37 °C for 16-24 hours.

Evaluation: Remove the cylinders, measure the diameters of the inhibition zones (it is best to use a "zone reading instrument") and evaluate them statistically. Draw a standard curve using the values of the standard solutions and read off the activities of the test solutions.

2. Punched-hole test:

Holes are punched out of the inoculated culture medium and the antibiotic solutions are then pipetted into them. All other steps are analogous to those described in the cylinder test.

3. Paper-disc test:

Paper-discs with a diameter of 9 mm are impregnated with the antibiotic solution and placed on the culture medium. The antibiotic can also be applied to the disc after it has been placed on the medium. Plates containing a single layer of medium with a thickness of 2 mm can be used for these tests. Antibiotic agars Nos. 2 or 5 may be employed depending on the pH required. All other steps are analogous to those described in the cylinder test.

4. Serial dilution test:

The antibiotic activity is determined quantitatively by using the known sensitivity of a test strain towards an antibiotic which is expressed numerically as the minimal inhibitory concentration (MIC).

Procedure: Serial dilutions of the antibiotic to be tested are pipetted into the antibiotic broth, this is then inoculated with a defined quantity of the relevant test strain.

Evaluation: The last tube which does not show any turbidity due to microbial growth contains the active antibiotic at a concentration corresponding to the MIC.

5. Turbidimetric test:

This test is more accurate and more sensitive than the serial dilution test.

Procedure: Incubate tubes containing 1 ml aliquots of the antibiotic solution and 9 ml aliquots of the inoculated antibiotic broth for 4 hours at 37 °C in a water bath. The growth of the test bacteria is then stopped by adding 0.5 ml of a dilute formaldehyde solution and the turbidity evaluated photometrically.

Evaluation: The antibiotic concentration is determined by comparing the absorbance of the test solution with that of a previously constructed standard curve.

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Use of antibiotic culture media

Antibiotic		Cylinder test			Turbidimetric test	
			Culture medium		Test strain	Culture medium
	Test strain	Seed culture	Base layer	Seed layer		
Amphomycin	Micrococcus luteus ATCC 14452	Medium No. 1	Medium No. 7	Medium No. 1	-	-
Amphotericin B	Saccharomyces cerevisiae ATCC 9763	Medium No. 13	Medium No. 12	Medium No. 12	-	-
Ampicillin	Micrococcus luteus ATCC 9341	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Bacitracin	Micrococcus luteus ATCC 10240 or Micrococcus luteus ATCC 7468 D	Medium No. 1	Medium No. 2	Medium No. 1	Staph. aureus ATCC 10537	Medium No. 3
Carbomycin	Micrococcus luteus ATCC 9341	Medium No. 3	Medium No. 11	Medium No. 11	-	-
Chloramphenicol	Micrococcus luteus ATCC 9341	Medium No. 3	Medium No. 1	Medium No. 1	-	-
Cephalothin	Staphylococcus aureus ATCC 6538 P	Medium No. 1	Medium No. 2	Medium No. 1	-	-
Colistin	Bordetella bronchiseptica ATCC 4617	Medium No. 9	Medium No. 9	Medium No. 10	-	-
Erythromycin	Micrococcus luteus ATCC 9341	Medium No. 3	Medium No. 11	Medium No. 11	-	-
Gentamicin (Refobacin® Merck)	Bac. subtilis ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	-	-
Kanamycin	Staph. aureus ATCC 6538 P	Medium No. 1	Medium No. 11	Medium No. 11	Staph. aureus ATCC 6538 P	Medium No. 3
Neomycin	Staph. aureus ATCC 6548 P	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Novobiocin	Staph. epidermidis ATCC 12228	Medium No. 1	Medium No. 2	Medium No. 1	-	-
Oleandomycin	Staph. epidermidis ATCC 12228	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Paromomycin	Staph. epidermidis ATCC 12228	Medium No. 1	Medium No. 11	Medium No. 11	Klebsiella pneumoniae ATCC 10031	Medium No. 3
Polymyxin B	Bordetella bronchiseptica ATCC 4617	Medium No. 9	Medium No. 9	Medium No. 10	-	-
Penicillin, oxacillin, methicillin, nafcillin	Staph. aureus ATCC 6538 P	Medium No. 3	Medium No. 2	Medium No. 1	-	-
Streptomycin, dihydrostreptomycin	Bac. subtilis ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	Klebsiella pneumoniae ATCC 10031	Medium No. 3
Tetracycline, oxytetracycline, chlorotetracycline	Bac. cereus ATCC 11778	Medium No. 1	Medium No. 8	Medium No. 8	Staph. aureus ATCC 6538 P	Medium No. 3
Viomycin	Bac. subtilis ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	-	-

Antibiotic Media

Manufacturer	Product
American Type Culture Collection 12301 Parklawn Drive, Rockville Maryland 20852, USA	Test strains
USP Reference Standards 4630 Montgomery Avenue Bethesda, MD 20014, USA	Antibiotic Standards
Schleicher &Schüll GmbH 37586 Dassel, FRG	Paper-discs No. 2628

Literature

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FORSTER, J.W., a. WOODRUF, H.B.: Microbial aspects of penicillin. - **J. Bact., 46**; 187-202 (1943).

GROVE, D.C., a. RANDALL, W.A.: Assay Methods of antibiotics. - **Medical Encyclopedia, N.Y.** (1955).

SCHMIDT, H.W., a. MOYER, A.J.: Penicillin I. Methods of assay. - **J. Bact., 47**; 199-208 (1944).

United States Pharmacopeia XXVI, Chapter "Biological Tests and Assays", 1995.

WALLHÄUSER, K.H., u. SCHMIDT, H.: Sterilisation, Desinfektion, Konservierung, Chemotherapeutica (G. Thieme-Verlag, Stuttgart, 1967).

Ordering Information

Product	Merck Cat. No.	Pack size
Antibiotic Agar No. 1	1.05272.0500	500 g
Antibiotic Agar No. 2	1.05270.0500	500 g
Antibiotic Agar No. 4		
Antibiotic Agar No. 5	1.05271.0500	500 g
Antibiotic Agar No. 6		
Antibiotic Agar No. 7		
Antibiotic Agar No. 8		
Antibiotic Agar No. 9		
Antibiotic Agar No. 10		
Antibiotic Agar No. 11	1.05269.0500	500 g
Antibiotic Agar No. 12	1.10672.0500	500 g
Antibiotic Broth (Medium No. 3)	1.05273.0500	500 g
SABOURAUD-2 % Dextrose Broth (Medium No. 13)	1.08339.0500	500 g
D(+)Glucosemonohydrate	1.08342.1000	1 kg
Agar-agar purified	1.01614.1000	1 kg
Manganese(II) sulfate monohydrate	1.05963.0100	100 g
Tween® 80	8.22187.0500	500 ml
Tryptic Soy Broth	1.05459.0500	500 g

Quality control of Antibiotic Agar No. 1

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 9341	good /very good	Cephalotin, Chloramphenicol and Penicillin/Methicillin
Staphylococcus aureus ATCC 6538-P	good /very good	
Bacillus subtilis ATCC 6633	godd /very good	-
Staphylococcus epidermidis ATCC 12228	good /very good	-
Bacillus cereus ATCC 11778	good /very good	-

Quality control of Antibiotic Agar No. 2

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 10240	fair /good	
Staphylococcus aureus ATCC 6538-P	good /very good	
Staphylococcus epidermidis ATCC 12228	godd /very good	

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Quality control of Antibiotic Agar No. 5

Test strains	Growth	Inhibition zones with
Bacillus subtilis BGA	good / very good	Gentamicin, Streptomycin

Quality control of Antibiotic Agar No. 11

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 9341	good / very good	Ampicillin, Erythromycin
Staphylococcus aureus ATCC 6538-P	good / very good	Kanamycin, Neomycin
Staphylococcus epidermidis ATCC 12228	good / very good	Oleandomycin, Paromomycin

Staphylococcus aureus ATCC 6538_P

Quality control of antibiotic Agar No. 12

Test strains	Growth	Inhibition zones with
Saccharomyces cerevisiae ATCC 9763	good / very good	Amphotericin B (20 µg)

Quality control of antibiotic Broth No. 3

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 9341	good / very good	-
Staphylococcus aureus ATCC 6538-P	good	Kanamycin, Tetracyclin
Klebsiella pneumoniae ATCC 10031	good	Streptomycin