



Antibiotic Assay Medium No. 36

M1666

Intended Use:

A general purpose medium used with or without blood or other enrichment, for isolating a wide variety of fastidious microorganisms.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired aseptically add 5% v/v defibrinated blood in previously cooled medium at 45 - 50°C. Mix well before pouring.

Principle And Interpretation

The composition of this medium is in accordance to CFR (1). This medium is recommended for sterility testing (2) Antibiotic Assay Medium No. 36 is widely employed as seed agar for agar diffusion assay for antibiotic bleomycin. The test organism *Mycobacterium smegmatis* is also maintained in this medium. This medium is employed for cultivation and isolation of fastidious or nonfastidious microorganisms. This medium is also used as maintenance medium of *Pseudomonas aeruginosa* for plate assay of ticarcillin. The medium like the conventional medium is used for a multitude of purposes including maintenance of stock cultures, plate counting, isolation of microorganisms from a variety of specimen types and a base for media containing blood (3,4).

Tryptone and Soya peptone gives the essential nutrients for maintenance and growth of the test organisms used. Osmotic balance is maintained by sodium chloride. Agar provides excellent medium for antibiotic diffusion and gives well defined zones of inhibition. Freshly prepared plates should be used for antibiotic assays.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling:

Test organisms are inoculated in sterile seed agar pre-cooled to 40-45°C and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important step for the good results.

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. Freshly prepared medium plates must be used or it may result in erroneous results.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of 5-7% w/v sterile defibrinated blood :
Cherry red coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.0% w/v aqueous solution at 25°C (after sterilization). pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added 5-7% w/v sterile defibrinated blood after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Growth w/ blood	Recovery w/ blood
Growth at 30-35°C for <= 3 days					
<i>Bacillus subtilis</i> ATCC 6633	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Staphylococcus aureus</i> ATCC25923	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Staphylococcus aureus</i> ATCC 6538	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Escherichia coli</i> ATCC 8739	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Escherichia coli</i> NCTC 9002	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Pseudomonas aeruginosa</i> ATCC 9027	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Salmonella</i> Abony NCTC 6017	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Micrococcus luteus</i> ATCC 9341	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Salmonella</i> Typhimurium ATCC 14028	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Streptococcus pneumoniae</i> ATCC 6305	50-100	luxuriant	>=70%	luxuriant	>=70%
Growth at 20-25°C for <= 5 days					
* <i>Aspergillus brasiliensis</i> ATCC 16404	50-100	luxuriant		luxuriant	
<i>Candida albicans</i> ATCC 2091	50-100	luxuriant	>=70%	luxuriant	>=70%
<i>Candida albicans</i> ATCC 10231	50-100	luxuriant	>=70%	luxuriant	>=70%

Reference

1. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242-259, (April 1).
2. Wright and Welch, 1959-60, Antibiotic Ann., 61.
3. MacFaddin 1985, Media for isolation-cultivation-identification-maintenance medical bacteria Vol, I, Williams, & Wilkins, Baltimore, MD
4. Forbes BA, Sahm DF, Weissfeld AS, 2002, Bailey and Scott's Diagnostic Microbiology, 11th ed.

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