Dubos Media
Dubos Broth Base • Dubos Medium Albumin Dubos Oleic Agar Base • Dubos Oleic Albumin Complex • Dubos Broth, Enriched

Intended Use

Dubos Broth Base is used with Dubos Medium Albumin for rapidly cultivating pure cultures of *Mycobacterium tuberculosis*.

Dubos Oleic Agar Base is used with Dubos Oleic Albumin Complex and penicillin for isolating and determining the susceptibility of *M. tuberculosis*.

Dubos Broth, Enriched is a prepared medium used for the cultivation of pure cultures of *M. tuberculosis*.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980s, the number of tuberculosis (TB) cases in the U.S. began increasing. Prior to this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984.[2] Non-tuberculous mycobacterial infections have also increased since the mid 1980s.[3]

Dubos Broth is prepared according to the Dubos, Fenner and Pierce[4] modification of the medium originally described by Dubos and Davis[5] and Dubos and Middlebrook.[6]

Dubos and Middlebrook[6] described Dubos Oleic Medium Albumin as suitable for primary isolation and cultivation of the tubercle bacillus and for studying colony morphology. In comparative studies, Dubos Oleic Albumin Agar Medium was superior to other media studied for primary isolation.[7,8]

There are two types of solid culture media for the primary isolation of mycobacteria, those that have coagulated egg as a base and those that have agar. Lowenstein formulations are examples of media that contain egg; Middlebrook and Dubos formulations contain agar.

Agar based media are not liquefied by contaminating proteolytic organisms but overgrowth may occur. These media are recommended for specimens from nonsterile sites.[9] The medium is clear so colonies of mycobacteria can be viewed through a stereo microscope even if contaminating organisms are present. Colonies can be observed in 10-12 days.

Drugs may be added to Dubos media in exact concentrations because the medium is solidified with agar rather than by inspissation. Also, there is less drug inactivation when egg ingredients are not present.

Mycobacteria grow more rapidly in broth media. Primary culture of all specimens in broth media is recommended.[10] Polysorbate 80 in the medium acts as a surfactant, dispersing the bacilli, which increases growth.

Dubos Broth, Enriched is a modified medium based on the formulation of Dubos et al.[4] This formulation differs from the original in that it has a strong buffering system and an acid pH.[11] The particular value of Dubos Broth, Enriched is that it provides dispersed growth, free of excessive clumps, which can be used to prepare a relatively uniform suspension of mycobacteria for use in bacterial studies. It is also used as a subculture and enrichment medium for the rapid cultivation of *M. tuberculosis* and other mycobacterial species from treated clinical specimens and from direct inoculation of specimens that may yield pure cultures; e.g., cerebrospinal fluid.[12]

Principles of the Procedure

Peptone and asparagine are sources of nitrogen. Disodium phosphate and monopotassium phosphate are sources of phosphates and, along with calcium chloride, help maintain the pH of the medium. Magnesium sulfate, ferric ammonium sulfate, zinc sulfate and copper sulfate are sources of trace metals and sulfates. Polysorbate 80, an oleic acid ester, supplies essential fatty acids for the replication of mycobacteria. Bovine albumin acts as a protective agent by binding free fatty acids that may be toxic to mycobacteria. The albumin is heat-treated to inactivate lipase, which may release fatty acids from the polysorbate 80. Phosphate buffers maintain the pH of the medium. Agar is the solidifying agent.

Formulae

Difco™ Dubos Broth Base

Approximate Formula* Per Liter

Pancreatic Digest of Casein	0.5	g
Asparagine	2.0	g
Polysorbate 80	0.2	g
Monopotassium Phosphate	1.0	g
Disodium Phosphate (anhydrous)	2.5	g
Ferric Ammonium Citrate	50.0	mg
Magnesium Sulfate	10.0	mg
Calcium Chloride	0.5	mg
Zinc Sulfate	0.1	mg
Copper Sulfate	0.1	mg

Difco™ Dubos Medium Albumin

A 5% solution of albumin fraction V from bovine plasma and 7.5% dextrose in normal saline (0.85%).

User Quality Control

Identity Specifications

Difco™ Dubos Broth Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	0.65 g/90 mL solution, soluble in purified water upon boiling. Solution is very light to light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light to light amber, clear, may have a slight precipitate.
Reaction of 0.65 g/90 mL Solution at 25°C:	pH 6.6 ± 0.2

Difco™ Dubos Oleic Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent to opalescent with fine precipitate.
Prepared Appearance:	Light amber, slightly opalescent to opalescent with fine precipitate.
Reaction of 2.0% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Difco™ Dubos Broth Base

Prepare the medium per label directions with added Dubos Medium Albumin (10 mL of albumin to 90 mL base). Inoculate and incubate at 35 ± 2°C with 5-10% CO_2 for up to 3 weeks.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Mycobacterium fortuitum	6841	~10^3	Good
Mycobacterium intracellulare	13950	~10^3	Good
Mycobacterium kansasii	12478	~10^3	Good
Mycobacterium scrofulaceum	19981	~10^3	Good
Mycobacterium tuberculosis H37Ra	25177	~10^3	Good

Difco™ Dubos Oleic Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C with 5-10% CO_2 for up to 3 weeks.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	~10^3	Partial inhibition
Mycobacterium fortuitum	6841	~300	Good
Mycobacterium intracellulare	13950	~300	Good
Mycobacterium kansasii	12478	~300	Good
Mycobacterium scrofulaceum	19981	~300	Good
Mycobacterium tuberculosis H37Ra	25177	~300	Good

Difco™ Dubos Oleic Agar Base

Approximate Formula* Per Liter

Pancreatic Digest of Casein	0.5	g
Asparagine	1.0	g
Monopotassium Phosphate	1.0	g
Disodium Phosphate (anhydrous)	2.5	g
Agar	15.0	g
Ferric Ammonium Citrate	50.0	mg
Magnesium Sulfate	10.0	mg
Calcium Chloride	0.5	mg
Zinc Sulfate	0.1	mg
Copper Sulfate	0.1	mg

Difco™ Dubos Oleic Albumin Complex

A 0.05% solution of alkalinized oleic acid in a 5% solution of albumin fraction V in normal saline (0.85%).

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions[13,14]

1. Biosafety Level 2 practices, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet.
2. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activites in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Directions for Preparation from Dehydrated Product

Difco™ Dubos Broth Base

1. Suspend 1.3 g of the powder in 180 mL of purified water (or 170 mL of purified water and 10 mL glycerol). Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to below 50°C.
4. Aseptically add 20 mL Dubos Medium Albumin and mix thoroughly. Incubate medium for 24 hours to test for microbial load.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Dubos Oleic Agar Base

1. Suspend 4 g of the powder in 180 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to below 50-55°C.
4. Aseptically add 20 mL Dubos Oleic Albumin Complex and 5,000-10,000 units of penicillin (25-50 units per mL of medium). Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The test procedures are recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[13] N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle, but effective digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate text.[3,9,12,13,15]

Specimens that are less likely to be contaminated with other microorganisms (cerebrospinal fluid, pleural fluid, tissue biopsy, etc.) may be inoculated directly into the medium. Consult appropriate texts for recommended procedures.[3,9,12,13,15]

Incubate the tubes at 35 ± 2°C in a CO_2-enriched atmosphere. Keep the tube caps loosened for at least one week to permit circulation of CO_2, but tighten the caps thereafter to prevent dehydration. Loosen briefly once a week to replenish CO_2. Six to eight weeks of incubation may be necessary for evidence of growth of many mycobacteria.

Expected Results

Growth of mycobacterial colonies on the agar medium or in broth media, as indicated by turbidity compared to an uninoculated control.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

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Availability

Difco™ Dubos Broth Base

Cat. No. 238510 Dehydrated – 500 g

Difco™ Dubos Medium Albumin

AOAC

Cat. No. 230910 Tube, 20 mL – Pkg. of 12*

Difco™ Dubos Oleic Agar Base

Cat. No. 237310 Dehydrated – 500 g

Difco™ Dubos Oleic Albumin Complex

Cat. No. 237510 Tube, 20 mL – Pkg. of 12*

BBL™ Dubos Broth, Enriched

Cat. No. 295697 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*