IFU

HARDY DIAGNOSTICS
A Culture of Service®

Instructions for Use

Leeds Acinetobacter Medium

Cat. no. G261	Leeds Acinetobacter Medium, 15x100mm Plate, 18ml	10 plates/bag

Intended Use

Leeds Acinetobacter Medium is a selective and differential medium intended for use as a plating technique to obtain a pure culture of *Acinetobacter*. The test is performed with a mixed population of microorganisms from a laboratory sample. Leeds Acinetobacter Medium is not intended for use in the identification of colonization with *Acinetobacter* to aid in the prevention and control of such bacteria in healthcare settings. Leeds Acinetobacter Medium is not intended to diagnose *Acinetobacter* infections, guide or monitor treatment for infections, or provide susceptibility results to antibiotics for which any strains of *Acinetobacter* are resistant. Sub-culturing is necessary for microorganism identification, susceptibility testing, and epidemiological typing.

Summary and Principles of the Procedure

The selective components in Leeds Acinetobacter Medium are traditionally cefsulodin and cephradine, which inhibit the growth of non-*Acinetobacter* gram-negative bacteria, and vancomycin, which inhibits the growth of gram-positive bacteria.[1] The presence of fructose and sucrose and neutral red, a pH indicator, allows the differentiation of bacteria based on the products released when the bacteria use fructose and sucrose as a nutrient source. The colonies of those that release acidic products are yellow, while the colonies of those that release alkaline products are red.

Formula and Ingredients

Ingredients per liter of deionized water:*

Leeds Acinetobacter Medium:	
Casein Acid Hydrolysate	15.0gm
Soy Peptone	5.0gm
Sodium Chloride	5.0gm
Fructose	5.0gm
Sucrose	5.0gm
Mannitol	5.0gm
Phenylalanine	1.0gm
Ferric Ammonium Citrate	0.4gm
Phenol Red	0.02gm
Selective Agents	0.035gm
Agar	12.0gm

* Adjusted and/or supplemented as required to meet growth and/or inhibitory properties.

Final pH 7.0 +/- 0.2 at 22.5 degrees C +/- 2.5 degrees C.

Warnings or Precautions

For *in vitro* diagnostic use.

This product is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory samples should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.[3]

Sterilize all biohazard waste before disposal.

Storage and Shelf Life

Storage: Upon receipt store at 2-8 degrees C away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date applies to the product in its intact packaging when stored as directed.

Sample Collection and Handling

Consult the listed references for information on the collection and handling of laboratory samples: *Cumitech 3B*,[4] *Bailey & Scott's Diagnostic Microbiology*,[5] *Clinical Microbiology Procedures Handbook*,[6] and *Manual of Clinical Microbiology*.[7]

Test Procedure

Method of Use: Prior to inoculation, the plates should be brought to room temperature. Inoculate the medium with the laboratory sample and streak for isolation. Incubate aerobically at 35 degrees C for 24-48 hours. Do not incubate in an atmosphere supplemented with CO_2. Examine plates for colonies showing typical morphology and color after 24 to 48 hours. Do not re-incubate if negative at 48 hours.

Materials Provided

Leeds Acinetobacter Medium.

Materials Required But Not Provided

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

Quality Control

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method	Incubation			Results
		Time	Temperature	Atmosphere	
Leeds Acinetobacter Medium:					
Acinetobacter baumannii ATCC® BAA-747*	A	24hr	35°C	Aerobic	Growth; pink mucoid colonies with pink to mauve color diffused into the medium
Citrobacter freundii CV5	B	24hr	35°C	Aerobic	Partial inhibition; small yellow colonies with yellow color diffused into the medium
Burkholderia cepacia ATCC® 25416	B	24hr	35°C	Aerobic	Partial inhibition; small pink colonies with pink to mauve color diffused into the medium
Escherichia coli ATCC® 25922*	B	24hr	35°C	Aerobic	Inhibited
Enterococcus faecalis ATCC® 29212	B	24hr	35°C	Aerobic	Inhibited
Candida albicans ATCC® 10231	B	24hr	35°C	Aerobic	Inhibited

* Recommended QC strains for User Quality Control according to CLSI document M22, when applicable.[8]

Method A

To test the Nutritive Capacity of plated or tubed media.

1. Resuspend a lyophilized pellet of the desired organism in an appropriate broth (see manufacturer's product insert).

2. Transfer several drops to an appropriate plate and streak for isolation. Incubate the plate for the appropriate time (24 to 72 hours) in the correct temperature and atmosphere.

3. Alternatively, a stock culture can be taken from a frozen culture, or from lyophilized strains available in "KWIK-STIK™" or "LYFO DISK®" configurations.

4. Suspend three to five isolated colonies in a small volume of Tryptic Soy Broth (TSB; Cat. no. R30) and incubate for 4 to 5 hours. Adjust the turbidity to match that of a 0.5 McFarland standard (Cat. no. ML05). This basic suspension should contain approximately 10^7 to 10^8 CFU/ml. Alternately, a direct suspension can be made if the culture is 18 to 24 hours old (or depending on isolate).

5. For testing the nutritive capacity of a nutrient medium, dilute the cell suspension to 1:100 in TSB or normal saline.

6. Inoculate the test plate or tube with a 10 µl calibrated loop of the diluted suspension. This will provide approximately 10^3 to 10^4 CFU per plate or tube. Plates are streaked in four quadrants for isolation. If this does not provide isolated colonies for the media being tested, use a tenfold lighter inoculum.

Method B

To test the inhibitory capacity of plated and tubed media.

1. Use the same cell suspension (equivalent to a 0.5 McFarland standard) described in "Method A" and dilute to 1:10 in Tryptic Soy Broth (TSB).

2. Inoculate the inhibitory medium, as described in "Method A," with a 10 µl calibrated loop onto the plate or tube. This should result in 10^4 to 10^5 CFU per plate or tube. A tenfold lighter inoculum may be required to avoid overwhelming some selective media. A non-inhibitory plate is also inoculated at the same time, to serve as a positive control.

User Quality Control

Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction, and at least one organism to demonstrate inhibition or a negative reaction (where applicable).

Interpretation of Results

On Leeds Medium:

Acinetobacter spp. will produce light pink mucoid colonies with a pink to mauve color diffused into the medium. The colonies are circular, convex, smooth, and opaque with entire margins of 1 to 2 mm in diameter after 24 hours at 35 degrees C.

Stenotrophomonas maltophilia will produce light pink colonies with a pink to mauve diffused into the medium. The colonies are opaque and flat with rugose surfaces and crenated margins of 1 to 2 mm in diameter after 24 hours at 35 degrees C.

Burkholderia cepacia will produce light pink colonies with a pink to mauve color diffused into the medium. 90% of *B. cepacia* strains are oxidase positive.

On Leeds Medium (referring to MDR Acinetobacter Medium, these organisms are inhibited on MDR Acinetobacter Medium):

Citrobacter spp. will produce yellow colonies with a yellow color diffused into the medium.

Providencia alcalifaciens will produce brown colonies with a brown-black color diffused into the medium.

Serratia marcescens will produce pink colonies with yellow margins.

Limitations of the Procedure

Leeds Acinetobacter Medium is a selective and differential medium limited to use as a plating technique to obtain a pure culture of *Acinetobacter*. Sub-culturing is required for identification as *Acinetobacter* or antibiotic-resistant, *e.g.*, by biochemical profiling or antibiotic susceptibility testing. If antibiotic susceptibility testing is necessary, one of the Clinical and Laboratory Standards Institute (CLSI) reference methods, *e.g.*, as described in the CLSI Document M2[9] or M7,[10] or M100,[11] should be used; alternatively, a commercial antibiotic susceptibility test cleared for use by the Food and Drug Administration (FDA) can be substituted.

Burkholderia cepacia and/or *Stenotrophomonas maltophilia* may grow on this medium producing pink colonies. Differentiation can by made by colonial morphology and/or an oxidase test.

White colonies present at 24 hours may turn pink after 48 hours of incubation.

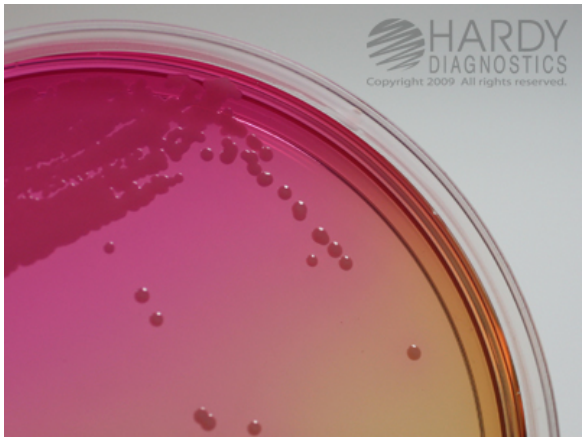
Color-blind individuals may encounter difficulty in distinguishing the color differences on Acinetobacter Medium.

Minimize exposure of Acinetobacter Medium to light before and during incubation, as light can be destructive.

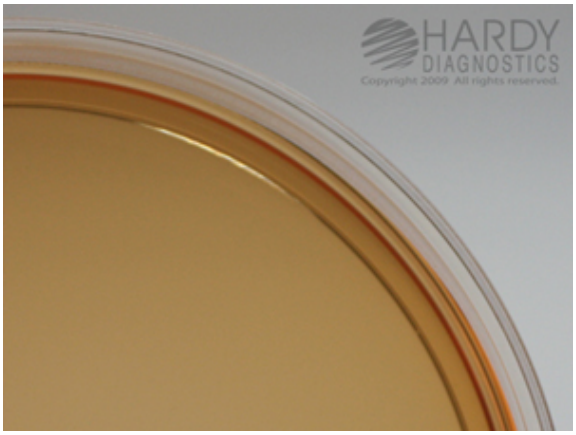
Acinetobacter spp. will produce light pink colonies and pink to mauve coloration of the medium, *Stenotrophomonas maltophilia* will likewise produce light pink colonies and pink to mauve coloration of the medium, and *Burkholderia cepacia* will similarly produce light pink colonies and pink to mauve coloration of the medium. Thus the microorganism species cannot be identified directly from source material.

Physical Appearance

Acinetobacter Media should appear clear to slightly opalescent, and light peach in color.



Acinetobacter baumannii (ATCC® BAA-747) colonies growing on Leeds Acinetobacter Medium (Cat. no. G261). Incubated aerobically for 24 hours at 35 deg. C.



Uninoculated plate of Leeds Acinetobacter Medium (Cat. no. 261).

ATCC is a registered trademark of the American Type Culture Collection.

References

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11. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Testing; Twenty-Second Informational Supplement*. CLSI document M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

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