

Technical Data

Leeds Acinetobacter Agar Base

Intended Use:

Recommended for isolation of *Acinetobacter* species and for selection of MDR (Multi Drug Resistant) *Acinetobacter* with the addition of MDR selective supplement from hospital environment.

Composition**

Ingredients	g / L			
Acicase TM #	15.000			
Soya peptone	5.000			
Sodium chloride	5.000			
Fructose	5.000			
Sucrose	5.000			
Mannitol	5.000			
DL-Phenylalanine	1.000			
Ferric ammonium citrate	0.400			
Phenol red	0.020			
Agar	12.000			
Final pH (at 25°C)	7.0±0.2			

Equivalent to Casein acid hydolysate

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 53.42 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Cool to 45-50°C and add the rehydrated contents of two vials of AC Selective Supplement (FD271) or VCC Selective Supplement (FD335). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Acinetobacter species are ubiquitous bacteria that have been isolated from patients with nosocomial infection, environment, soil, and water. Acinetobacter is mostly found in every type of infections (1). There is an alarming situation as Acinetobacter baumannii is found resistant to commonly used antibiotics including beta-lactams and aminoglycosides (1,2). Immunocompromised patients requiring mechanical respirations are at more risk of infection by Acinetobacter species (3). There are many media developed for the growth of Acinetobacter. Leeds Acinetobacter Medium was developed by Jawad et.al. at the University of Leeds (4).

AcicaseTM and soya peptone provide nitrogenous and carbonaceous compounds, long chain amino acids and vitamins to the organisms. Sucrose, Fructose and Mannitol serve as the carbohydrate source. Sodium chloride maintains the osmotic balance. The phenylalanine serves as the substrate for enzymes which are able to deaminate it to form phenyl pyruvic acid which reacts with ferric ions from ferric ammonium citrate resulting in brown black colonies by species like *Providencia*. The phenol red in the medium serves as a pH indicator. The acidity produced by utilization of carbohydrates results in yellow coloured colonies while the liberation of ammonia ions by the utilization of nitrogenous material in the medium results in pink coloured colonies. Selective supplement helps inhibiting contaminating microflora.

Type of specimen

Clinical samples - Isolated microorganism

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

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Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

3. Further biochemical identification is necessary for confirmation.

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

Light yellow to pink coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plate.

Reaction

Reaction of 5.34% w/v aqueous solution at 25°C. pH : 7.0±0.2

pН

6.80-7.20

Cultural Response

Cultural characteristics observed with added supplement (FD271 or FD335) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Acinetobacter baumannii ATCC BAA-1605	50 -100	luxuriant	>=50 %	pink mucoid colonies with pink color diffused into the medium
Acinetobacter baumannii ATCC BAA-747	>=10 ⁴	inhibited	0 %	
Acinetobacter baumannii ATCC 19606	>=10 ⁴	inhibited	0 %	
Acinetobacter haemolyticus ATCC 19002	>=10 ⁴	inhibited	0 %	
<i>Acinetobacter lwofii</i> ATCC 15309	>=10 ⁴	inhibited	0 %	
<i>Escherichia coli</i> ATCC 25922 (00013*)	>=10 ⁴	inhibited	0 %	
<i>Citrobacter freundii</i> ATCC 8090	>=10 ⁴	inhibited	0 %	
Enterococcus faecalis ATCC 29212 (00087*)	>=10 ⁴	inhibited	0 %	
Burkholderia cepacia ATCC 25416	>=10 ⁴	inhibited	0 %	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Valentine, S.C., et.al. 2008 Phenotypic and molecular characterization of *Acinetobacter baumanni*. Clinical isolates from nosocomial outbreaks in Los Angeles Country, California. J.Clin. Microbiology.; 46:2499-2507.

2.Montefour, K., et.al.2008. *Acinetobacter baumanni* : An Emerging Multidrug Resistant pathogen in critical care Nurse; 28:15-25.

3.Bergogne- Berezin, E., m. L. Joly-Guillou, and J.F. Vieu. 1987. Epidemiology of nosocomial infections due to *Acinetobacter calcoaceticus*, J. Hosp. Infect. 10:105-113. J

4.Jawad A., Hawkey P.M., Description of Leeds Acinetobacter Medium, a New Selective and Differential Medium for Isolation of Clinically Important *Acinetobacter* spp., nad Comparison with Herella and Holton's Agar.

5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 06/2024



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