PRODUCT SPECIFICATION SHEET

Acetate Differential Agar (DM421)

Intended Use
Acetate Differential Agar (DM421) is recommended for the differentiation of *Shigella* species from other members of the genus *Escherichia*.

Product Summary and Explanation
Organic acids have been used widely as an aid to the differentiation of *Enterobacteriaceae*, usually in formulae that contained organic nitrogen sources. Most bacteria, however, can use citrate and acetate in the presence of organic nitrogen. The citrate media of Koser[(1)] and Simmons[(2)] were free of organic nitrogen and, therefore, were a true measure of citrate utilization. The differentiation of groups is based on the ability or failure of the test culture to utilize acetate in a medium devoid of trace organic nitrogen. In a further extension of this approach, Trabulsi and Ewing[(3)] developed Acetate Differential Agar Tatum, Ewing and Weaver[(4)] modified the medium by replacing sodium citrate by sodium acetate, which enables the differentiation of *Shigella* species from *Escherichia coli*, particularly anaerogenic, nonmotile biotypes. This medium contains sodium acetate as the sole source of nitrogen. Their basal medium was Simmons Citrate Agar in which sodium acetate was substituted for sodium citrate.

Principles of the Procedure
Acetate differential Agar contains sodium acetate as the sole source of nitrogen. Sodium acetate is utilized as a sole source of carbon by some serobiotypes of *S. flexneri* such as *Shigella flexneri 4a.* Magnesium sulphate is essential ion. Sodium chloride maintains osmotic equilibrium. Monoammonium phosphate and Dipotassium phosphate act as buffering agent. Bromothymol blue is the pH indicator.

Formula / Liter

Ingredients	Gms / Liter
Sodium acetate	2.00
Magnesium sulphate	0.10
Sodium chloride	5.00
Monoammonium phosphate	1.00
Dipotassium phosphate	1.00
Bromothymol blue	0.08
Agar	20.00
Final pH: 6.7 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 29.18 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Distribute in tubes in sufficient amounts to give butt and slant.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Allow the tubes to cool in a slanted position.

Quality Control Specifications

Dehydrated Appearance	Cream to light green homogeneous free flowing powder

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Prepared Medium	Emerald green coloured clear to slightly opalescent gel forms in tubes as slants
Reaction of 2.92% Solution	pH : 6.7 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 2.0% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 25-30°C for upto 1-7 days.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Citrobacter freundii ATCC 8090*	50 -100	good-luxuriant	positive reaction, blue colour
2.	*Enterobacter cloacae ATCC 23355*	50 -100	good-luxuriant	positive reaction, blue colour
3.	*Escherichia coli ATCC 25922*	50 -100	good-luxuriant	positive reaction, blue colour
4.	*Klebsiella pneumoniae ATCC 13883*	50 -100	good-luxuriant	positive reaction, blue colour
5.	*Proteus vulgaris ATCC 13315*	>=10^3	inhibited	
6.	*Salmonella Arizonae ATCC 13314*	50 -100	good-luxuriant	positive reaction, blue colour
7.	*Salmonella Typhi ATCC 19430*	50 -100	poor	negative reaction, green colour
8.	*Shigella sonnei ATCC 25931*	50 -100	none-poor	negative reaction, no change, medium remains green

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate the agar slant surfaces with pure cultures of unknown organisms.
2. Incubate all tubes for up to 7 days at 25-30°C in an aerobic atmosphere.

Results

1. Acetate utilization is indicated by formation of blue colour, which is due to the utilization of sodium acetate and subsequent formation of an alkaline reaction detected by the presence of bromothymol blue indicator.
2. Refer to appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Trabulsi and Ewing demonstrated that *Shigella* and *Proteus* species are unable to utilize acetate and therefore fails to grow.
2. Majority of *Escherichia coli* and closely related organisms grow well within 24-48 hours but some strains grow very slowly and a few strains are unable to utilize acetate as a sole carbon source.
3. Some strains of *Escherichia coli* utilize acetate slowly or not at all and therefore may produce a false negative reaction.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Acetate Differential Agar
Product Code : DM421
Available Pack sizes : 500gm

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References

1. Koser. 1923. J. Bacteriol. *8*:493.
2. Simmons. 1926. J. Infect. Dis. *39*:209.
3. Trabulsi and Ewing. 1962. Public Health Lab. *20*:137.
4. Ewing. 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.

Further Information

For further information please contact your local MICROMASTER Representative.

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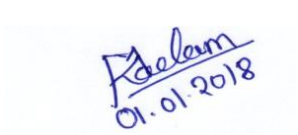		
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