ARYLSULFATASE BROTH (3-day and 2-week tests) and ARYLSULFATASE AGAR (3-day test)

INTENDED USE

Remel Arylsulfatase Broths and Arylsulfatase Agar are media recommended for use in qualitative procedures for the biochemical differentiation of rapid and slow-growing *Mycobacterium* species.

SUMMARY AND EXPLANATION

Arylsulfatase is an enzyme that splits free phenolphthalein from the tripotassium salt of phenolphthalein disulfite. The arylsulfatase test is used as an aid in the differentiation of *Mycobacterium* spp. In 1953, Whitehead et al. incorporated the substrate, phenolphthalein sulfate, into a liquid medium to differentiate virulent tubercle bacilli from nonvirulent mycobacteria.[1] In 1958, Wayne et al. reported all mycobacteria, except *M. rhodochrous*, produce arylsulfatase in varying degrees. The differences in enzyme activity were found to be related to differences in growth rate and cell wall permeability of the organism.[2] Wayne et al. formulated a liquid medium and an agar medium to test for arylsulfatase activity.[3] Kubica and Rigdon used the arylsulfatase test as a presumptive differential test to distinguish *M. fortuitum* complex from saprophytic mycobacteria using a 3-day test.[4] These studies have demonstrated, through the use of proper substrate concentration and appropriate choice of incubation time (3 days or 2 weeks) it is possible to subgroup certain mycobacteria on the basis of arylsulfatase activities.

PRINCIPLE

Casein peptone and asparagine supply carbon and nitrogen to support the growth of mycobacteria. Inorganic copper, iron, zinc, and calcium are growth stimulators. Mixtures of sodium and postassium phosphates provide a buffering system. Polysorbate 80 is a nutritional source of essential fatty acids. Bovine plasma albumin protects the tubercle bacilli from the actions of a variety of toxic agents and enhances mycobacterial growth. The arylsulfatase test measures the ability of various *Mycobacterium* spp. to produce an arylsulfatase enzyme which attacks the substrate, tripotassium phenolphthalein disulfate. This reaction releases phenolphthalein resulting in development of a red color in the medium. The 3-day Arylsulfatase Broth and Agar differentiate *M. fortuitum* and *M. chelonae* from other rapid-growing mycobacteria by their ability to produce the arylsulfatase enzyme at a detectable level after 3 days incubation. Other species that produce the enzyme do so at a slower rate. Therefore, the 2-week Arylsulfatase Broth is used to identify the slow-growing *Mycobacterium* species.

REAGENTS (CLASSICAL FORMULAE)*

ARYLSULFATASE BROTH 3-DAY TEST:

Disodium Phosphate (anhydrous)	2.5 g	Magnesium Sulfate	10.0 mg
Asparagine	2.0 g	Calcium Chloride	0.5 mg
Polysorbate 80 10%	2.0 g	Copper Sulfate	0.1 mg
Monopotassium Phosphate	1.0 g	Zinc Sulfate	0.1 mg
Tripotassium Phenolphthalein Disulfate	0.65 g	•ADC Enrichment	100.0 ml
Casein Peptone	0.5 g	Bovine Plasma Albumin (Serum Fraction V) 5%	100.0 ml
Ferric Ammonium Citrate	50.0mg	Demineralized Water	800.0 ml

pH 6.6 ± 0.2 @ 25°C

ARYLSULFATASE BROTH 2-WEEK TEST:

Disodium Phosphate (anhydrous)	2.5 g	Magnesium Sulfate	10.0 mg
Asparagine	2.0 g	Calcium Chloride	0.5 mg
Polysorbate 80 10%	2.0 g	Copper Sulfate	0.1 mg
Tripotassium Phenolphthalein Disulfate	1.95 g	Zinc Sulfate	0.1 mg
Monopotassium Phosphate	1.0 g	•ADC Enrichment	100.0 ml
Casein Peptone	0.5 g	Bovine Plasma Albumin (Serum Fraction V) 5%	100.0 ml
Ferric Ammonium Citrate	50.0mg	Demineralized Water	800.0 ml

pH 6.6 ± 0.2 @ 25°C

•ADC ENRICHMENT:

Bovine Albumin Fraction V	50.0 g	Sodium Chloride	8.5 g
Dextrose	20.0 g	Catalase	0.03 g

ARYLSULFATASE AGAR (BUTT) 3-DAY TEST:

Disodium Phosphate (anhydrous)	2.5 g	Calcium Chloride	0.5 mg
Asparagine	1.0 g	Copper Sulfate	0.1 mg
Monopotassium Phosphate	1.0 g	Zinc Sulfate	0.1 mg
Tripotassium Phenolphthalein Disulfate	0.65 g	Polysorbate 80 10%	25.0 ml
Casein Peptone	0.5 g	Glycerol	10.0 ml
Ferric Ammonium Citrate	50.0mg	Agar	15.0 g
Magnesium Sulfate	10.0mg	Demineralized Water	1000.0 ml

pH 6.6 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

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PROCEDURE

1. Prepare a suspension of an actively growing culture in a liquid medium, such as Dubos Polysorbate 80 Albumin or Middlebrook 7H9 broth, and incubate in ambient air at 35-37°C for 7 days.
2. Inoculate 1 drop of the suspension into the Arylsulfatase Broth or Agar.
3. Incubate the 3-day agar or broth test in ambient air at 35-37°C for 72 hours. Incubate the 2-week broth test in ambient air at 35-37°C for 14 days.
4. After incubation, add 6 drops of Sodium Carbonate (R21267) to each broth or 1 ml to the agar butt.
5. Observe for a pink-red color change. The color usually develops immediately, but in the case of a weak reaction, it may be necessary to wait for 30 minutes in order to be sure of the reaction.[3]

INTERPRETATION

Positive Test - Formation of a pink-red change in the broth or on the surface of the agar is interpreted as follows:

Positive Grade	Color Reaction
+/-	Faint tinge of pink
1+	Pale pink
2+	Pink
3+	Light red
4+	Red
5+	Deep red

Negative Test - No color development in the broth or agar

QUALITY CONTROL

All lot numbers of Arylsulfatase Broth and Arylsulfatase Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Mycobacterium fortuitum ATCC® 6841	Ambient, 3 days or 2 weeks @ 33-37°C	Positive
Mycobacterium tuberculosis ATCC® 25177	Ambient, 3 days or 2 weeks @ 33-37°C	Negative

LIMITATIONS

1. Since many mycobacteria produce arylsulfatase it is important to control the substrate concentration, inoculum size, and time of incubation. If a large inoculum is used with high substrate concentrations and incubated for an extended period of time, even those organisms with low enzyme concentration will produce large amounts of phenolphthalein.[5]
2. The development of any pink color within 30 minutes after the addition of the reagent is regarded as a positive test.
3. An occasional strain of *M. kansasii* may give a positive 3-day reaction. However, it can be differentiated from other mycobacteria by its growth rate and pigment.[5]
4. Check a few tubes of uninoculated substrate medium for free phenolphthalein by adding a few drops of the reagent (R21267). The formation of a pink color indicates the presence of free phenolphthalein. This is caused by premature breakdown of the test medium or the powdered phenolphthalein disulfate salt.[6]

BIBLIOGRAPHY

1. Whitehead, J.E.M., P. Wildy, and H.C. Engback. 1953. J. Pathol. & Bacteriol. 65:451-460.
2. Wayne, L.G., W.J. Juarez, and E.G. Nichols. 1958. J. Bacteriol. 75:367-368.
3. Wayne, L.G. 1961. Am. J. Clin. Pathol. 36:185-187.
4. Kubica, G.P. and L. Rigdon. 1961. Am. Rev. Respir. Dis. 83:737-740.
5. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
6. Kent, P.T. and G.P. Kubica. 1985. Public Health Mycobacteriology - A Guide for Level III Laboratory. U.S. Dept. of H.H.S.and CDC, Atlanta, GA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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