PYRAZINAMIDASE AGAR

INTENDED USE
Remel Pyrazinamidase Agar is a solid medium recommended for use in qualitative procedures for differentiation of *Mycobacterium* spp. on the basis of pyrazinamidase activity.

SUMMARY AND EXPLANATION
The clinical use of pyrazinamide (PZA) for treatment of tuberculosis was hindered by the difficulty in performing reliable susceptibility tests. The drug has maximal activity in vitro at pH 5.5, however, many strains of *Mycobacterium tuberculosis* grow poorly or not at all in an acidic medium. Investigators have reported, PZA-susceptible *M. tuberculosis* strains possess the enzyme pyrazinamidase (PZase) which deaminates PZA to pyrazinoic acid and ammonia; PZA-resistant strains lack this enzyme.[1-4] In 1953, Allen et al. described a technique for estimating pyrazinoic acid concentrations of solutions, based on development of a colored complex with ferrous ion.[5] In 1974, Wayne described a method for detecting PZase activity in mycobacteria using an agar medium and adding freshly prepared ferrous ammonium sulfate solution.[6]

PRINCIPLE
Casein peptone and asparagine supply essential nutrients for the growth of mycobacteria. Inorganic salts supply ions required for mycobacterial metabolism. Polysorbate 80 is an oleic ester which supplies fatty acids for the replication of mycobacteria. Pyrazinamide is the substrate which can be deaminated by *M. tuberculosis, Mycobacterium avium* complex, and *Mycobacterium marinum*, whereas *Mycobacterium bovis* and *Mycobacterium kansasii* do not possess the enzyme pyrazinamidase.

REAGENTS (CLASSICAL FORMULA)*

Disodium Phosphate	2.5 g	Ferric Ammonium Citrate	50.0 mg
L-Asparagine	2.0 g	Magnesium Sulfate	10.0 mg
Pyruvic Acid, Sodium Salt	2.0 g	Calcium Chloride	0.5 mg
Monopotassium Phosphate	1.0 g	Zinc Sulfate	0.1 mg
Casein peptone	0.5 g	Copper Sulfate	0.1 mg
Polysorbate 80	0.2 g	Agar	15.0 g
Pyrazinamide	0.1 g	Demineralized Water	1000.0 ml

pH 6.6 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate the surface of 2 tubes of Pyrazinamidase Agar using a heavy inoculum of the test isolate from an actively growing culture. Use enough inoculum so that it is readily visible. Do not stab the medium.
2. Incubate the tubes along with 2 uninoculated control tubes aerobically at 33-37°C.
3. After 4 days incubation, remove one inoculated tube and one control tube from the incubator and add 1.0 ml of freshly prepared 1% ferrous ammonium sulfate to the tubes.
4. Allow the tubes to equilibrate at room temperature for 30 minutes, then refrigerate at 2-8°C to prevent growth of contaminants.
5. After 4 hours, examine for a pink color development by holding the tube against a white background using incandescent room light.
6. The control must be negative for the test to be valid.
7. After 7 days incubation at 33-37°C, remove the second inoculated tube and control tube from the incubator and proceed as described above.
8. If the 4-day test is positive, it is not necessary to hold the second set of tubes for the 7-day test.

INTERPRETATION OF THE TEST
Positive Test - Pink band at the surface of the agar
Negative Test - No color development

QUALITY CONTROL
All lot numbers of Pyrazinamidase 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 intracellulare ATCC® 13950	Aerobic, 4 & 7 days, @ 33-37°C	Positive
Mycobacterium bovis ATCC® 19210	Aerobic, 4 & 7 days, @ 33-37°C	Negative

LIMITATIONS
1. A large inoculum size must be used or false-negative readings may result.[4,8]
2. Do not incubate in a CO_2 atmosphere, as it will counteract the growth-inhibiting effects of PZA.[7,8]

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BIBLIOGRAPHY

1. Konno, K., F.M. Feldman, and W. McDermott. 1967. Am. Rev. Respir. Dis. 95:461-469.
2. Brander, E. 1972. Tubercle. 53:128-131.
3. Tatar, J. 1974. Gruzlica. 42:773-777.
4. McClatchy, J.K., A.Y. Tsang, and M.S. Cernich. 1981. Antimicrob. Agents Chemother. 20:556-557.
5. Allen, W.S., S.M. Aronovic, L.M. Brancone, and J.N. Williams. 1953. Anal. Chem. 25:895.
6. Wayne, G.L. 1974. Am. Rev. Respir. Dis. 109:147-151.
7. Butler, W.R. and J.O. Kilburn. 1982. J. Clin. Microbiol. 16:1106-1109.
8. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press, Washington, D.C.

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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