HYPOXANTHINE AGAR

INTENDED USE
Remel Hypoxanthine Agar is a solid medium recommended for use in qualitative procedures to differentiate aerobic actinomycetes on the basis of hypoxanthine hydrolysis.

SUMMARY AND EXPLANATION
The aerobic actinomycetes are an heterogeneous group of genera and include species of *Nocardia* and *Streptomycces*.[1] As a group, the organisms are gram-positive, catalase-positive, and may be presumptively identified by staining properties, microscopic morphology, and substrate degradation. Mishra et al. developed a taxonomic scheme for these genera on the basis of biochemical characteristics.[2] A simplified scheme for identification of the most commonly isolated actinomycetes was developed by McGinnis et al. and includes the following tests: hypoxanthine, casein, tyrosine, and xanthine.[3]

PRINCIPLE
Beef extract, when combined with peptone, supplies carbohydrates, vitamins, nitrogen compounds, and salts necessary for growth of aerobic actinomycetes. Peptone also provides nutrients in the form of amino acids and peptides. This medium detects the presence of a hydrolytic enzyme that degrades hypoxanthine, resulting in a clearing of the medium surrounding and beneath areas of growth.

REAGENTS (CLASSICAL FORMULA)*

Peptone	5.0 g	Beef Extract	3.0 g
Hypoxanthine	5.0 g	Agar	15.0 g
		Demineralized Water	1000.0 ml

pH 7.0 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Melt the pour tube in a boiling water bath and cool to 45-50°C.
2. Mix to distribute precipitate evenly and dispense into a sterile petri dish. Allow the agar to harden and cool.
3. Heavily inoculate a 10 mm area of the Hypoxanthine Agar surface from a pure culture of the test isolate. Press the inoculum into the agar surface to ensure it remains in place.
4. Seal plate with cellophane tape, Shrink-Seals (REF R522600), or gas permeable strip.
5. Incubate aerobically at 25-30°C for up to 4 weeks.
6. Evaluate the plate weekly for clearing (hydrolysis) around or directly beneath the colony indicating a positive reaction. Plate should be incubated for a full 4 weeks before concluding the reaction is negative.
7. A duplicate set of media may be inoculated and incubated at 35-37°C. Occasionally test results become positive more rapidly at 35-37°C.

INTERPRETATION OF THE TEST
Positive Test - Clearing of hypoxanthine around and/or under growth (hydrolysis)
Negative Test - Growth with no clearing of the medium

QUALITY CONTROL
All lot numbers of Hypoxanthine 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/sample results should not be reported.

CONTROL	INCUBATION	RESULTS
Streptomyces albus ATCC® 17900	Aerobic, up to 10 days, 25-30°C	Positive
Nocardia asteroides ATCC® 19247	Aerobic, up to 10 days, 25-30°C	Negative

BIBLIOGRAPHY

1. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
2. Mishra, S.K., R.E. Gordon, and D.A. Barnett. 1980. J. Clin. Microbiol. 11:728-736.
3. McGinnis, M.R., R.F. D'Amato, and G.A. Land. 1982. Pictorial Handbook of Medically Important Fungi and Aerobic Actinomycetes. Praeger Publishers, New York, NY.

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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