

CASEIN AGAR

INTENDED USE

Remel Casein Agar is a solid medium recommended for use in qualitative procedures to differentiate aerobic actinomycetes on the basis of casein hydrolysis.

SUMMARY AND EXPLANATION

The aerobic actinomycetes are a heterogeneous group of genera and include species of *Nocardia* and *Streptomyces*.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: casein, hypoxanthine, tyrosine, and xanthine.³

PRINCIPLE

Casein Agar consists of agar and skim milk, a source of casein. Some aerobic actinomycetes produce a hydrolytic enzyme that degrades casein, resulting in a clearing of the medium surrounding and beneath areas of growth.

REAGENTS (CLASSICAL FORMULAE)*

Casein A:

Skim Milk..... 100.0 g
Demineralized Water..... 1000.0 ml

Casein B:

Agar.....20.0 g
Demineralized Water..... 1000.0 ml

*Adjusted as required to meet performance standards.

PROCEDURE

1. Melt the tube of Casein B in a boiling water bath and cool to 45-50°C.
2. Add cooled Casein B to the Casein A tube and mix well. Dispense into a sterile petri dish and allow the agar to harden and cool.
3. Heavily inoculate a 10 mm area of the Casein 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 14 days.
6. Evaluate the plate at 3-4 day intervals for clearing (hydrolysis) around or directly beneath the colony indicating a positive reaction. Incubate for a full 14 days before being 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 casein around and/or under growth (hydrolysis)
Negative Test - Growth with no clearing of the medium

QUALITY CONTROL

All lot numbers of Casein 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

Nocardia brasiliensis ATCC® 19297
Nocardia asteroides ATCC® 19247

INCUBATION

Aerobic, 2 weeks @ 25-30°C
Aerobic, 2 weeks @ 25-30°C

RESULTS

Positive
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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IFU 8210, Revised September 20, 2007

Printed in U.S.A.

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