

BD BBL™ Prepared Plated Media for Identification of Aerobic Actinomycetes

Nocardia ID QUAD

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INTENDED USE

The Nocardia ID QUAD plate is a four-sectored plate containing four different media used for differentiation and identification of *Nocardia* species and other aerobic actinomycetes isolated from clinical specimens.

SUMMARY AND EXPLANATION

The most frequently encountered aerobic actinomycetes, members of the order *Actinomycetales*, include the genera *Nocardia*, *Streptomyces*, *Actinomadura*, *Nocardiopsis*, *Rhodococcus* and *Dermatophilus*. The testing algorithm that permits identification of most aerobic actinomycetes consist of direct microscopic techniques and a minimum number of biochemical reactions.¹

Quadrant I contains Casein Agar (skim milk and agar) for determining the ability of isolates to hydrolyze casein.

Quadrant II contains Starch Agar (potato starch and nutrient agar) for testing of isolates for their ability to utilize starch.

Quadrant III contains Tyrosine Agar (tyrosine and nutrient agar) for testing the ability of isolates to decompose tyrosine.

Quadrant IV contains Xanthine Agar (xanthine and nutrient agar) for testing isolates for their ability to decompose xanthine.

PRINCIPLES OF THE PROCEDURE

The four biochemical media contained in the Nocardia ID QUAD plates offer a convenient means of conducting four tests used in the differentiation and identification of *Nocardia* species following observation of staining reactions and microscopic characteristics.^{1,2}

Decomposition of casein in Quadrant I may be detected by observing clear zones in the white, opaque skim milk around the inoculum. Growth without clearing around the inoculum is considered to be a negative test result.

The ability of isolates to break down and utilize starch may be detected in Quadrant II. Starch hydrolysis may be detected by colorless zones surrounding colonies after the plate is flooded with Gram's iodine. Blue or purple zones surrounding colonies indicate a negative test.

The decomposition of tyrosine can be detected in Quadrant III. A clear halo around a colony is a positive test. Growth without the presence of clear halos or growth with the production of melanin-like pigment is a negative test.

The ability of isolates to decompose xanthine may be detected in Quadrant IV. A clear halo around a colony is a positive test. Growth without the presence of clear halos or growth with the production of a melanin-like pigment is a negative test.

REAGENTS

Quadrant I consists of Casein Agar

Approximate Formula* Per Liter Purified Water

Skim Milk, dehydrated75.0 g
Agar20.0 g

Quadrant II consists of Starch Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin5.0 g
Beef Extract3.0 g
Sodium Chloride8.0 g
Potato Starch10.0 g
Agar15.0 g

Quadrant III consists of Tyrosine Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin5.0 g
Beef Extract3.0 g
Sodium Chloride8.0 g
Tyrosine5.0 g
Agar15.0 g

Quadrant IV consists of Xanthine Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin5.0 g
Beef Extract3.0 g
Sodium Chloride8.0 g
Xanthine4.0 g
Agar15.0 g

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC-NIH recommendations, CLSI guidelines or local institution guidelines for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

SPECIMEN COLLECTION AND HANDLING

This medium is not intended to be used directly with specimens or other materials containing mixed microbial flora. Organisms to be tested must first be isolated in pure culture on an appropriate medium. Consult appropriate texts for information.^{1,2}

After use, prepared plates and other contaminated materials should be sterilized by autoclaving.

PROCEDURE

Material Provided: Nocardia ID QUAD

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

Inoculate each sector with a pure culture of the isolate. Use a small sterile spatula to obtain approximately 1 mm of the colony from a pure culture. Using the spatula, cut a small groove through the agar to the bottom of the plate, depositing the inoculum near the bottom of the groove. Alternatively, the tip of a sterile wooden applicator stick can be used to make a well through the agar to the bottom of the plate, depositing the inoculum at the bottom of the well.

Incubate the plates at 30 °C in an inverted position (agar side up) under aerobic conditions and observe every 3 or 4 days for 14 – 21 days.¹

User Quality Control:

1. Examine plates for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that give known, desired reactions. The following test strains are recommended:

MEDIUM	TEST STRAIN	EXPECTED RESULTS
Quadrant I (Casein)	<i>Streptomyces rimosus</i> ATCC™ 10970	Growth with clear zones; positive reaction.
	<i>Nocardia asteroides</i> ATCC 19247	Growth with no clear zones; negative reaction.
Quadrant II (Starch)	<i>Streptomyces rimosus</i> ATCC 10970	Growth with clear zones; positive reaction.
	<i>Nocardia asteroides</i> ATCC 19247	Growth with blue or purple zones; negative reaction.
Quadrant III (Tyrosine)	<i>Streptomyces rimosus</i> ATCC 10970	Growth with clear zones; positive reaction.
	<i>Nocardia asteroides</i> ATCC 19247	Growth with no clear zones; negative reaction.
Quadrant IV (Xanthine)	<i>Streptomyces rimosus</i> ATCC 10970	Growth with clear zones; positive reaction.
	<i>Nocardia asteroides</i> ATCC 19247	Growth with no clear zones; negative reaction.

RESULTS

Examine plates for growth periodically for 14 – 21 days of incubation.

Examine Quadrant I for the presence of a clear halo in the white, opaque medium around the inoculum, which indicates a positive reaction. *S. rimosus* decomposes casein and gives a positive reaction. *N. asteroides* shows a negative reaction or no clearing around the inoculum.

To determine starch utilization, flood Quadrant II with Gram's iodine and observe the plate for colorless zones around the inoculum, which indicates a positive reaction, such as that obtained with *S. rimosus*. *N. asteroides* gives a negative reaction with no clear zones; blue or purple zones surround colonies.

The decomposition of tyrosine in Quadrant III is indicated by clear halos around colonies, which represents a positive reaction obtained with *S. rimosi*. There are no clear halos around colonies in a negative test. *N. asteroides* does not decompose tyrosine.

The ability of isolates to decompose xanthine in Quadrant IV is shown by a clear halo around colonies, such as that obtained with *S. rimosus*. The absence of clear halos or the production of a melanin-like pigment indicates a negative test. *N. asteroides* gives a negative test.

LIMITATIONS OF THE PROCEDURE

For identification, isolates must be in pure culture. Microscopic, biochemical, and other tests may be performed for final identification. Consult appropriate texts for tests and interpretations of results.¹⁻³

AVAILABILITY

Cat. No. Description

298309 BBL™ Nocardia ID QUAD, Pkg. of 10 plates

REFERENCES

1. Land, G.A. 1992. Aerobic actinomycetes, p. 4.0.1-4.1.9. In H.D. Isenberg (ed.), Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.
2. Beaman, B.L., M.A. Saubolle, and R.J. Wallace. 1995. *Nocardia*, *Rhodococcus*, *Streptomyces*, *Oerskovia*, and other aerobic actinomycetes of medical importance, p. 379-399. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual® of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

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