NITRATE BROTH

I. PRINCIPLE

Organisms that reduce nitrate have the ability to extract oxygen from nitrates to form nitrites and other reduction products. The presence of nitrites in the medium is detected by the formation of a red diazonium dye, p-sulfobenzeneazo-α-naphthylamine, following the addition of naphthylamine and sulfanilic acid. If no color develops after adding the reagents, this indicates that nitrates have not been reduced (a true negative reaction) or that they have been reduced beyond the oxidation level of nitrite to products such as ammonia, nitrogen gas (denitrification), nitric oxide (NO), or nitrous oxide (N_2O) and hydroxylamine. Since the test reagents detect only nitrites, the latter process would lead to a false-negative result. Therefore, it is necessary to add a small amount of zinc dust to all negative reactions. Because zinc ions reduce nitrates to nitrites, the development of a red color after adding zinc dust indicates the presence of residual nitrates and confirms a true negative reaction.

II. MATERIALS AND REAGENTS

A. Nitrate Broth (store at 2-8°C until stated expiration date)
B. Sterile inoculating loop
C. Test tube
D. Nitrate Reagent A (0.8% sulfanilic acid), store at 2-8°C until stated expiration date
E. Nitrate Reagent B (0.6% N,N dimethyl-L-naphthylamine), store at 2-8°C until stated expiration date
F. Zinc dust, store at room temperature
G. Applicator sticks

III. SAFETY

A. Do not splash N, N dimethyl-L-naphthylamine or sulfanilic acid or allow zinc dust to come into contact with skin or eyes. They may burn the skin or eyes. Rinse affected area with water upon exposure. Avoid breathing vapors.
B. Do not store zinc dust in the refrigerator. Condensation may result in an explosive compound.

IV. QUALITY CONTROL

A. Quality control is performed with each new lot or shipment.
B. The organisms to be used and their expected reactions are as follows:
    1. *E coli* ATCC 25922 = positive (red within 30 sec of adding reagents A and B)
    2. *A. baumannii* ATCC 19606 = negative (color development only after addition of zinc dust)

3. *Ps. aeruginosa* ATCC 27853 = (no color development within 10 min after the addition of zinc)

C. If controls do not display expected results, quality control must be repeated. Notify the supervisor.

V. PROCEDURE

A. Using a sterile inoculating loop, inoculate the Nitrate Broth medium with 2-3 colonies of the organism to be tested.
B. With the cap loosened, incubate the tube at 35°C in a non-CO_2 incubator for 24-48 h.
C. Examine the tube for growth. When the broth is visibly turbid, use a sterile pipette to transfer 3 ml into a sterile tube.
D. Add 5 drops of Nitrate Reagent A to the broth.
E. Add 5 drops of Nitrate Reagent B to the broth.
F. Observe for the production of a pink to red color within 30 sec.
G. If no color change occurs within 30 sec, use an applicator stick to add a small amount of zinc dust.
H. Observe for the production of a pink to red color within 10 min.

VI. INTERPRETATION

A. Before the addition of zinc dust:
   1. Positive = pink to red within 30 sec. Nitrate was reduced to nitrite.
   2. Negative = no color change. Add zinc before recording the final result.

B. After the addition of zinc dust:
   1. Positive = no color change within 10 min. Nitrate was reduced to nitrogen gas.
   2. Negative = pink to red color within 10 min. Nitrate was not reduced to either nitrite or nitrogen gas.

VII. REFERENCES

A. Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria. U.S. Department of Health and Human Services. Center for Disease Control. 1980. p. 18.
B. BBL Quality Control and Product Information Manual for Tubed Media. Becton Dickinson Microbiology Systems. Cockeysville, MD, 1993.
C. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn. 1992. Diagnostic Microbiology. 4th ed. J.B. Lippincott Company, Philadelphia.

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