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QUALITY CONTROL METHOD - 7

Oxidase Test

PURPOSE

The oxidase test is a qualitative test for the identification of nonfermenters and miscellaneous Gram-negative bacteria. The test screens colonies suspected of being one of the Winterobacteriaceae (all Gram-negative). The cytochrome oxidase test uses dyes that substitute for oxygen as artificial electron acceptors. In the reduced state, the dye is colorless; however, in the presence of cytochrome oxidase and atmospheric oxygen, p-phenylendiamine is oxidized, forming indophenol blue. This procedure should be performed by a trained laboratory technician.

EQUIPMENT

1. Becton-Dickinson Reagent Droppers
2. Filter paper
3. Plastic or platinum loop

PROCEDURE

1. If using this test is a part of the Salmonella and Shigella procedure (Alken QC-3), treat all oxidase negative colonies as suspect *Salmonella* or *Shigella* organisms. All items in this test contaminated with suspect colonies should be autoclaved before disposal.

2. Add a few drops of the reagent to a filter paper or equivalent.

3. Using a plastic or platinum loop, tooth pick or wooden applicator stick or equivalent, pick a well-isolated colony approximately 24 hours old and smear the colony into the reagent zone of the paper. Stainless steel or nichrome inoculating loops or wires should not be used for this because surface oxidation products formed when flame sterilizing may result in false-positive reactions.

4. Do not use refrigerated cultures without allowing them to reach room temperature.

5. The reagent appears to be more responsive if it is not allowed to dry out on the paper. Therefore, if several colonies are to be tested, fresh reagent may need to be added to the filter paper periodically.

INTERPRETATION

1. Most bacterial colonies having cytochrome oxidase activity develop a deep rose to purple color at the inoculation site within 10 seconds.

2. However, Becton-Dickinson recommends allowing 10-30 seconds for a positive reaction to occur. Any organism producing a blue color in 10-60 seconds probably is NOT *Salmonella* or *Shigella*.

3. Any further delayed positive reaction should be considered negative.

4. Small colonies that are actually oxidase (+) positive, may sometimes give a negative reaction. Therefore, it is sometimes necessary to use several well-isolated colonies to obtain sufficient biomass to obtain a positive reaction.

ALTERNATIVE PROCEDURE WITH HOME PREPARED REAGENT

INGREDIENTS:

N,N,N',N'-Tetramethyl-p-phenylenediamine. 2HCL 0.4 g
Distilled water 40 ml
10 µl yellow sterile individually wrapped loop (1 per test)

EQUIPMENT & SUPPLIES:

sterile swabs (medical style on sticks)
weighing pan (avoid metal pans)
3 to 10 cc syringes
aluminum foil
beaker full of disinfectant to soak used swabs

PROCEDURE:

1. Mix ingredients together in weighing pan and mix thoroughly.

2. Draw reagent solution into syringes and cover syringe with aluminum foil to seal out light. Be sure to expel all air from syringe. Use freshly prepared reagent. Can be stored in a covered syringes under refrigeration for one week.

3. Dip sterile swab into culture grown in Tryptic Soy Broth (QC-22).

4. Add reagent to the dipped swab. Add more reagent as necessary to keep paper from drying out.

5. If a dark purple color develops in 10 to 30 seconds, the test is positive.

6. Alternative procedure: Apply freshly prepared reagent directly to young culture (24 hours) on either agar plate or slant. Oxidase-positive colonies develop a pink color and progressively turn dark purple. If cultures are to be preserved, complete the transfer from plates to which reagent has been added within 3 minutes, since the reagent is toxic to the organisms.

REFERENCES:

1. FDA Bacteriological Analytical Manual - 8th Edition/1995