Proc.# **2013.010**

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| Procedure | **Rapid Spot Indole** |

**Principle**: A positive reaction indicates the presence of the enzyme tryptophanase that reacts with tryptophan to produce indole. The indole produced reacts in the acid medium with p-dimethylaminobenzaldehyde of the indole test reagent to form a quinoidal red-violet compound.

**Specimen**:

1. ***Patient Preparation***: NA
2. ***Type of Specimen***: Pure culture or colony of bacteria for spot indole testing
3. ***Specimen Handling***: Culture is to be handled with routine laboratory safety practices for infectious agents.

**Equipment and Materials**:

1. ***Equipment***: NA
2. ***Materials***: Indole Reagent Droppers (Becton Dickinson, Cockeysville, MD) with 5% p-dimethylaminobenzaldehyde dissolved in a solution of 25% hydrochloric acid and 75% isobutyl alcohol. Also needed are applicator sticks and filter paper.
3. ***Materials*** ***Preparation***: None
4. ***Performance Parameters***: The reagent is hermetically sealed in an ampule, which affords protection of the solution from chemical instability until the expiration date. Change in color of the reagent from light yellow to brown indicates improper storage which may cause weaker reactions.
5. ***Storage Requirements***: Store at room temperature (15 to 30°C). Protect from light.

**Calibration**: Not applicable

**Quality Control**: On each day a test is performed, run a positive and negative control. Record in the quality control log book under “Miscellaneous Tests.” Use *E. coli* ATCC 25922 for the positive control and *Ps. aeruginosa* ATCC 27853 for the negative control.

**Procedure**:

1. Using a wooden applicator stick or a plastic dispo pipette, run a heavy portion of the colony to be tested onto the Whatman filter paper.
2. Crush the ampule of the Spot Indole Reagent Dropper holding the dropper upright and pointing away. Break the ampule close to its center one time only. Do not manipulate dropper any further as the plastic may puncture and injury may occur.
3. Add 2-3 drops of the reagent onto the filter paper.
4. Interpret results as follows:
   1. Positive – Development of a blue color within 30 seconds
   2. Negative – A white to yellow color.

**Calculations**: Not applicable

**Reporting Results**: Used to aid in identification of various bacteria

**Medical Alert Values**: Not applicable

**Notes**:

1. ***Reference Ranges***: NA
2. ***Abnormal Results***: NA
3. ***Reporting Format***: Results of test are written on the microbiology worksheet.

**Limitations**:

1. In general, the indole test is useful in differentiating between species within the genera of *Bacteroides*, *Fusobacterium, Clostridium*, and *Peptococcus*. See anaerobe identification references for specifics.
2. The spot indole test may also be used to differentiate swarming *Proteus* sp. and to presumptively identify *E. coli* along with colony morphology.

**References**:

Finegold, S. et el, *Diagnostic Microbiology*, 7th Ed., CV Mosby Co., St. Louis, 1986

*Manual of Clinical Microbiology*, 5th Ed., Washington, DC, 1985

Package inserts from BBL Spot Indole Reagent Droppers, Becton Dickinson, Cockeysville, MD

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