

User Quality Control:

1. Examine the reagent for signs of deterioration (See "Product Deterioration").
2. Positive (*Escherichia coli* ATCC® 25922) and negative (*Klebsiella pneumoniae* ATCC 33495) controls should be run simultaneously with the organism to be tested.

LIMITATIONS OF THE PROCEDURE

Allow at least 30 sec for color to develop before considering the indole reagent test negative. Pigmented organisms may yield ambiguous results.

REFERENCES

1. Balows, A., W.J. Hausler, Jr., K.L. Herrmann, H.D. Isenberg, and H.J. Shadomy (ed.). 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
2. Shigei, J. 1992. Test methods used in the identification of commonly isolated aerobic gram-negative bacteria. Part 1, Indole test, p. 1.19.13-1.19.16. In H.D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
3. Arnold, W.M., and R.H. Weaver. 1948. Quick microtechniques for the identification of cultures. J. Clin. Med. 33:1334-1337.

TECHNICAL SERVICES: In the United States, telephone Technical Services, toll free 800-638-8663.

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MADE
IN
U.S.A.

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BBL® Indole
Reagent Droppers

Cat. No. 4361185
Packaged 50 droppers/carton

INTENDED USE

BBL® Indole Reagent Droppers (modified Kovacs' reagent) are used in determining the ability of bacteria to produce indole by the deamination of tryptophan.

SUMMARY AND EXPLANATION

The production of indole has been widely used as an aid in differentiating certain genera and species within the same genus.

BBL Indole Reagent may be used for tube or extraction procedures or with a variety of biochemical kits requiring Kovacs' or Ehrlich's reagents.^{1,2}

Each dropper is good for one day's use after the ampule has been broken.

PRINCIPLES OF PROCEDURE

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.

REAGENTS

BBL Indole Reagent Droppers contain 0.5 ml of 5% *p*-dimethylaminobenzaldehyde dissolved in a solution of 25% hydrochloric acid and 75% isobutyl alcohol.

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Precautions: For In Vitro Diagnostic Use.

Follow proper laboratory procedures in handling and disposing of infectious material. Indole reagent is very acidic. Avoid contact with the skin. Rinse thoroughly with water if spilled.

Storage Instructions: Store at room temperature 15 to 30°C (59 to 86°F). Protect from light.

Product Deterioration: This 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.

PROCEDURE

Material Provided: BBL Indole Reagent Droppers, Cat. No. 4361185.

Materials Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Directions for Use and Results: To Use BBL Indole Reagent Droppers, hold upright and **POINT AWAY FROM YOURSELF**. Grasp the middle with thumb and forefinger and squeeze gently to break glass ampule inside the dropper. **Caution: Break ampule close to its center one time only. Do not manipulate dropper any further as the plastic may puncture and injury may occur.** Tap the bottom on the tabletop a few times. When ready for use, invert for convenient drop-by-drop dispensing of reagent for the required test procedure.

A. Rapid Tube Method (Arnold and Weaver)³

1. Heavily inoculate a pure culture of organisms to be tested into 1 ml of prewarmed broth of 0.03% tryptophan, 0.1% peptone, and 0.5% dipotassium phosphate (K_2HPO_4) in purified water. Broth should be adjusted to pH 7.4 to 7.8.
2. Incubate tubes in 35 to 37°C water bath for 2 h.
3. Add contents of one reagent dropper.
4. The appearance of a red ring at the surface of the broth within 30 sec indicates a positive reaction for indole production. A yellow color is negative.

B. Standard Tube Method

1. Inoculate a pure culture of the organism to be tested into a culture tube containing 4 ml of **Trypticase[®] Soy Broth** (Cat. No. 4397482).
2. Incubate for 18 to 24 h at 35 to 37°C.
3. Add the contents of one reagent dropper. Shake gently.
4. The appearance of a red ring at the surface of the broth within 30 sec indicates a positive reaction for indole production. A yellow color is negative. *Note:* For a more sensitive reaction, 1 to 2 ml of the culture should be aseptically removed from the culture tube and incubated for an additional 24 h. Retest. A negative reaction at 48 h indicates indole has not been produced.

C. Extraction with Xylene

1. Inoculate a pure culture of the organism to be tested into 4 ml of appropriate test medium. For *Enterobacteriaceae* use **BBL Tryptophan (Trypticase) 1% Solution** (Cat. No. 4321717). For others use **BBL Indole Nitrite Medium** (Cat. No. 4321655).
2. Incubate for 18 to 24 h at 35 to 37°C.
3. Add 1 ml of xylene. Mix well.
4. When layers have begun to separate, add the contents of one **BBL Indole Reagent Dropper**.
5. The appearance of a red ring at the surface of the xylene within 30 sec indicates a positive reaction for indole production. A yellow color is negative. *Note:* For a more sensitive reaction, 1 to 2 ml of the culture should be aseptically removed from the culture tube and incubated for an additional 24 h. Retest. A negative reaction at 48 h indicates indole has not been produced.

D. Other Methods: BBL Indole Reagent Droppers may be used in modifications of the indole test which employ Kovacs' or Ehrlich's indole reagent. For spot tests use **BBL DMACA Indole Reagent Droppers** (Cat. No. 4361187).