

Product Insert

INDOLE REAGENT

Products

AS-701 Indole Reagent 1 bottle / pkg

Intended Use

Indole reagent is intended for use in the Spot-Indole Test for detection of indole production by anaerobic bacteria.

Summary

Indole Reagent is used for the determination of the organism's ability to produce indole from deamination of tryptophan by the enzyme tryptophanase. Indole Reagent is intended to be used in the Spot-Indole Test, and was shown to be 10 to 100 times more sensitive than Kovac's or Ehrlich's reagents in detecting indole and indole derivatives. Indole combines with p-Dimethylaminocinnamaldehyde (DMACA) to produce a blue/blue-green compound.

Formulation

p-Dimethylaminocinnamaldehyde	10.00	G
Hydrochloric Acid	100.00	mL
DI Water	890.00	mL

Final volume: 30.0 mL ± 3.0 mL

Precautions

For *IN VITRO DIAGNOSTIC USE* only. Approved biohazard precautions and aseptic techniques should be observed when using this product. This product is for use only by properly-trained and qualified personnel. Sterilize all biohazard waste prior to disposal.

Storage and Shelf Life

Storage: Upon receipt, store at room temperature in original packaging until use. Do not use the product if there are signs of deterioration to the package. The expiration date applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the label.

Shelf Life: 1 year from date of manufacture.

Procedure

Spot-Indole Test: Inoculate an agar medium that contains sufficient tryptophan, such as Egg Yolk Agar (AS-511) or Brucella Blood Agar (AS-111). Place a sterile filter paper disk on an area of heavy growth for 5 minutes. Remove to an empty petri dish and a drop of Indole Reagent to the disk. Development of a blue/blue-green color on the filter disk within 30 seconds is positive for indole production. Dark-pigmented organism must be examined carefully since the pigmentation may mask the color development.

An alternative method is place a piece of filter paper in a clean petri dish moistened with Indole Reagent. Do not oversaturate the paper with liquid. Remove several colonies from the agar with a loop/wooden applicator and rub into the filter paper. It is preferred to use a heavy inoculum when testing anaerobes, since the reaction can sometimes be weak.

Materials Required, But Not Provided

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, disinfectant, other culture media, and serological / biochemical reagents.

Interpretation of Results

Development of a blue/blue-green color on the filter disk or around the inoculum within 30 seconds is positive for indole production. Dark-pigmented organism must be examined carefully since the pigmentation may mask the color development. No change in color or pink color development within 30 seconds is negative for indole production. Any change in color after 30 seconds should be disregarded.

Limitations

Growth medium that contains tryptophan is mandatory when testing for indole production. The enzyme that degrades tryptophan is diffusible in agar and it is necessary to have only one culture of an organism per plate or false-positives may be observed. Do not use a plate that has a Nitrate Disk on it; a positive nitrate response may interfere with the spot-indole test by giving false-negative results. Indole Reagent will not provide complete information for the identification of bacterial isolates. Additional test procedures and media are required for complete identification. Consult reference materials for additional information.

Quality Control

The following organisms are routinely used for quality control testing at Anaerobe Systems.

Organism Tested	ATCC #	Expected Reaction
Bacteroides fragilis	25285	Negative
Clostridium perfringens	13124	Negative
Fusobacterium necrophorum	25286	Positive
Propionibacterium acnes (indole + strains)	6919	Positive

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If the reactivity capacity of this media is to be tested for performance, it is recommended that the following ATCC organisms be evaluated, growing on Brucella Blood Agar plates, for reactivity.

Organism	ATCC#	Expected Reaction
B. fragilis	25285	Negative
F. necrophorum	25286	Positive

Physical Appearance: Indole Reagent should appear as a transparent amber liquid within an opaque plastic bottle.

References

1. Sutter, V. L. and W. T. Cater. 1972. Evaluation of media and reagents in indole-spot test in anaerobic bacteriology. *Am. J. Clin. Path.* 58: 335 – 338.
2. Lombard, G. L. and V. R. Dowell, Jr. 1983. Comparison of three reagents for detecting indole production by anaerobic bacteria in microtest systems. *J. Clin Microbiol.* 18: 609 – 613.
3. Jousimies-Somer, H. R., Summanen, P., Citron, D. M., Baron, E. J., Wexler, H. M. and S. M. Finegold. 2002. *Wadsworth – KTL Anaerobic Bacteriology Manual*. Star Publishing Co., Belmont, CA 94002.
4. Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1977. *Anaerobe Laboratory Manual*. Virginia Polytechnic Institute and State University. Blacksburg, VA 24061
5. Isenberg, H. D. 1992. *Clinical Microbiology Procedures Handbook*. American Society for Microbiology Publishing, Washington, D.C. 20005.
6. Murray, R. P., et al. 1999. *Manual of Clinical Microbiology*. American Society for Microbiology Publishing, Washington, D.C. 20005.

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