
remel

INDOLE (EHRlich'S)

INTENDED USE

Remel Indole (Ehrlich's) is a reagent recommended for use in qualitative procedures to detect indole production by microorganisms grown in indole broth, especially nonfermenters and anaerobes.

SUMMARY AND EXPLANATION

The use of p-Dimethylaminobenzaldehyde in the indole test was first reported by Vracko and Sherris.¹ Ehrlich's reagent is recommended for nonfermenting, gram-negative bacilli and for anaerobes.^{2,4}

PRINCIPLE

Tryptophanases are intracellular enzymes that mediate the production of indole by hydrolytic activity against the amino acid, tryptophan. Indole combines with dimethyl-aminobenzaldehyde to form a red compound. The reaction is a condensation process formed by an acid splitting of the protein. When using Ehrlich's reagent, small amounts of indole produced from the breakdown of tryptophan by tryptophanase must first be extracted with xylene or a xylene substitute before they are detectable.⁵ Optimal results depend on the medium in which the indole test is performed. Use of an inadequate peptone broth or a glucose-containing medium may cause aberrant results.⁶

REAGENTS (CLASSICAL FORMULA)*

p-Dimethylaminobenzaldehyde (CAS 100-10-7) 8.68 g
Hydrochloric Acid (Conc.) (CAS 7647-01-0) 174.0 ml
Ethyl Alcohol 95% (CAS 64-17-5) 826.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

DANGER! POISON, may be fatal or cause blindness if swallowed. Cannot be made nonpoisonous. Vapor harmful. **CORROSIVE**, may cause burns or irritation to skin, eyes and respiratory tract. Avoid breathing vapor and eye/skin contact. **FLAMMABLE**, keep away from heat, sparks and flame.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Read and follow directions carefully. Refer to Material Safety Data Sheet for additional information.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-30°C until used. Allow product to equilibrate to room temperature before use and protect from light.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.²

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Xylene or xylene substitute, (7) Pipette, (8) Glass test tubes.

PROCEDURE

1. Aliquot 2 ml of indole broth, incubated at 35-37°C for 24-72 hours, to a separate tube.
2. Add 1.0 ml of xylene or xylene substitute and shake the tube.
3. Allow tube to stand so that solvent rises to the surface.
4. Dispense 5 drops (0.5 ml) of Indole (Ehrlich's) reagent down the side of the tube. Do not shake.
5. Observe for pink-red color development in the form of a ring interfaced between broth and solvent within 15 minutes.

INTERPRETATION

Positive Test - Pink-red ring between broth and solvent within 15 minutes
Negative Test - No color development in the form of a ring within 15 minutes

QUALITY CONTROL

All lot numbers of Indole (Ehrlich's) reagent have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Bacteroides
thetaiotaomicron
ATCC® 29741

Clostridium sordelii
ATCC® 9714

Elizabethkingia
meningoseptica
ATCC® 13253

Prevotella
melaninogenica
ATCC® 25845

Pseudomonas
aeruginosa
ATCC® 27853

Veillonella parvula
ATCC® 10790

INCUBATION

Anaerobic, 48h
@ 35-37°C

Anaerobic, 48h
@ 35-37°C

Ambient, 18-24h
@ 35-37°C

Anaerobic, 48h
@ 35-37°C

Ambient, 18-24h
@ 35-37°C

Anaerobic, 48h
@ 35-37°C

RESULTS

Positive

Positive

Positive

Negative

Negative

Negative

LIMITATIONS

1. Qualify peptone broths (other than tryptophan broth) with a known indole-positive organism before using for the indole test.
2. Peptone broth containing glucose must not be used for indole detection.⁶
3. Some organisms (e.g., *Clostridium* spp.) form indole and break it down as rapidly as it is produced. A false-negative result may occur.
4. Indole (Ehrlich's) Reagent is not specific for indole. By utilizing a solvent, such as xylene, to extract indole, the red color obtained in the solvent layer only occurs with indole and skatole.⁶
5. The optimal pH for typtophanase activity is one that is slightly alkaline (pH 7.4 to 7.8). A decrease in pH (toward acidity) may result in a false-negative or a weak false-positive reaction.⁶
6. Cultures tested for indole production must be incubated aerobically. Indole production decreases under reduced oxygen tension.⁶

BIBLIOGRAPHY




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4. Holdeman, L.V., E.P. Cato, and W.E.C. Moore. 1977. Anaerobe Laboratory Manual. 4th ed. Virginia Polytechnic Institute, Blacksburg, VA.

5. Gubash, S.M. and E.E. Bennett. 1989. J. Clin. Microbiol. 27:2136-2137.
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PACKAGING

REF R21213, Indole (Ehrlich's).....25 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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