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# INDOLE BROTH

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## INTENDED USE

Remel Indole Broth is a liquid medium recommended for use in qualitative procedures to determine indole production by members of the *Enterobacteriaceae*.

## SUMMARY AND EXPLANATION

Indole Broth contains casein peptone, which is rich in the amino acid, tryptophan. Microorganisms that possess the enzyme tryptophanase have the ability to hydrolyze and deaminate tryptophan with the production of indole, pyruvic acid, and ammonia.<sup>1</sup> Indole, which has split from the tryptophan molecule, can be detected by addition of a reagent, p-dimethylaminobenzaldehyde, forming a distinct red color.<sup>2</sup> Indole production is an important characteristic in the identification of many species of bacteria, in particular, enteric gram-negative rods. Indole Broth is recommended by the American Public Health Association (APHA) and in the *Identification of Enterobacteriaceae* for use in the differentiation of enteric gram-negative rods.<sup>3,4</sup>

## PRINCIPLE

Casein peptone supplies essential growth factors, trace elements, and is the source of tryptophan in this medium. Sodium chloride maintains osmotic equilibrium. Indole is released when certain gram-negative rods hydrolyze tryptophan. Dimethylaminobenzaldehyde reagent is added which combines with the indole and produces a distinct red color.

## REAGENTS (CLASSICAL FORMULA)\*

Casein Peptone.....	20.0 g	Sodium Chloride.....	5.0 g
		Demineralized Water .....	1000.0 ml

pH 7.0 ± 0.2 @ 25°C

\*Adjusted as required to meet performance standards.

## PROCEDURE

1. Use a pure, 18-24 hour culture of the test isolate. Lightly inoculate Indole Broth from the center of a well-isolated colony.
2. Incubate aerobically at 33 - 37°C for 24 - 48 hours.
3. Using aseptic technique, aliquot 2 ml of broth to a separate tube.
4. Dispense 5 drops (0.5 ml) of Indole (Kovacs') reagent (REF R21227) down the side of the tube and shake gently.
5. Observe for a pink to red color development in the form of a ring interfaced between the broth and the reagent, within 3-5 minutes.
6. If the test is negative for indole production at 24 hours, reincubate the original tube for an additional 24 hours and repeat the test.

## INTERPRETATION OF THE TEST

Positive Test - Pink to red colored ring at the interface of the broth and the reagent  
Negative Test - No color development

## QUALITY CONTROL

All lot numbers of Indole Broth 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

*Escherichia coli* ATCC® 25922  
*Salmonella enterica* serovar Typhimurium ATCC® 14028

### INCUBATION

Aerobic, 24 - 48 h @ 33 - 37°C  
Aerobic, 24 - 48 h @ 33 - 37°C

### RESULTS

Positive  
Negative

## LIMITATIONS

1. Do not use peptone media containing glucose for indole detection.<sup>1</sup>
2. Peptone broths (other than tryptophan broth) should be qualified for use by testing with a known positive indole-producing organism.<sup>1</sup>
3. The optimal pH for tryptophanase activity is one that is slightly alkaline. An acidic pH may result in a false negative or a weakly false positive reaction.<sup>1</sup>

## BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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