INDOLE SPOT REAGENT
(1% CINNAMALDEHYDE)

- For in vitro use only - Catalogue No. RI40

Our Indole Spot Reagent is used to detect the presence of indole, which is one of the degradation products of the bacterial metabolism of tryptophan.

Tryptophan is an amino acid that can be oxidized by some bacteria to form three major end products: indole, pyruvic acid, and ammonia. Detection of indole indicates tryptophan degradation and can be accomplished by the addition of certain aldehydes to form colored end products. Indole Reagent contains the active ingredient, p-dimethylaminocinnamaldehyde (PACA), which reacts with indole to form a blue-green compound. Other available reagents for the detection of indole include Ehrlich's reagent and Kovac's Reagent (Dalynn RK75) both of which contain the aldehyde p-dimethylamino-benzaldehyde. Comparatively, Indole Reagent (PACA) is the most sensitive reagent and can detect as little as 3.0µg of indole per mL.

Indole Reagent is the reagent of choice for performing the spot indole test. The spot indole test is a rapid procedure designed by Vracko and Sherris. It can be used to presumptively characterize *Escherichia coli*, and to differentiate swarming *Proteus* species. It has also been found useful for examining anaerobic organisms. More specifically it can be used:

<u>To aid in differentiation between genera:</u>

1. Separate *Escherichia coli* (+) from members of *Klebsiella* (V-), *Enterobacter* (V-), *Hafnia* (-), *Serratia* (V-), and *Pantoea* (-)

<u>To aid in differentiation between species:</u>

1. *Paenibacillus alvei* (+) from other *Bacillus* spp. (-)
2. *E. coli* (+), *E. fergusonii* (+), *E. hermanii* (+), from *E. vulneris* (-), *E. blattae* (-)
3. *Proteus vulgaris* (+), *P. inconstans* (+), *P. rettgeri* (+) from other *Proteus* spp. (-)
4. *Klebsiella oxytoca* (+), *K. ornithinolytica* (+) from other *Klebsiella* spp. (usually -)

<u>Other uses:</u>

1. Along with other tests (urease and ornithine) subdivides *Haemophilus influenzae* and *Haemophilus parainfluenzae* into biotypes
2. Along with sialidase, α and β-glucosidase, α-fructosidase, differentiates between black-pigmented anaerobes: *Porphyromonas asaccharolyticus* (+), *P. endodontalis* (+), *P. gingivalis* (+), and *Prevotella intermedia* (+) from *Prevetolla corporis* (-), *P. denticola* (-), *P. loesheii* (-), *P. melaninogenica* (-), and *Porphyromonas levii* (-)

Formulation per 100 mL

p-Dimethylaminocinnamaldehyde ..... 1.0 g
Hydrochloric Acid (10%)..... 100.0 mL

Recommended Procedure (Spot Test)

1. Place a piece of filter paper (Whatman no.1) into a sterile petri dish.
2. Saturate the filter paper with Indole Reagent.
3. From an overnight culture grown on a tryptophan-containing agar plate (i.e. TSA with or without blood) select well-isolated colonies for testing.
4. Using a sterile loop or wooden applicator, rub a portion of the colony onto the filter paper. If a large filter paper is used, many tests may be performed on a single filter paper.
5. Observe and document any color changes that occur within 30 seconds.

Interpretation of Results

Positive: Blue to blue-green color (within 30s)

Negative: Colorless or light pink

- *Do not use media that contain an indicator such as MacConkey or EMB since the dye may obscure results*
- *Do not use Mueller-Hinton agar since acid hydrolysis of casein causes destruction of tryptophan*
- *Do not use glucose-containing media since the presence of glucose and the acid produced from fermentation will inhibit indole production*
- *For a mixed culture, ensure that the colonies being tested are at least 5 mm from other surrounding colonies since indole is a diffusible product*
- *Some organisms form indole but break it down as rapidly as it is produced and therefore false-negative reactions may occur. This mainly occurs with some Clostridium species*
- *Weakly indole-positive organisms such as Cardiobacterium hominis and Suttonella indologenes cannot be tested using the rapid spot test. These organisms must be tested using the xylene extraction procedure*
- *The tube method with xylene extraction is the most sensitive indole test*
- *Kovac's Reagent can also be used for the spot test but is less sensitive*

Quality Control

Organism	**Expected Results**	
Escherichia coli ATCC 25922	+ve	Blue-green color
Pseudomonas aeruginosa ATCC 27853	-ve	No color change

Storage and Shelf Life

Our Indole Reagent should be stored at 4°C to 8°C and protected from light. Under these conditions, it has a shelf life of 26 weeks from the date of manufacture.

References

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4. Welch DF, Ahlin PA, Matsen JM. Differentiation of *Haemophilus* spp. in respiratory isolate cultures by an indole spot test. J Clin Micro 1982; 15:216-219.
5. Sutter VL, Citron DM, Edelstein MAC et al. Wadsworth anaerobic bacteriology manual. 4th ed. Belmont: Star, 1985.
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8. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St Louis: Mosby, 1998.
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