Spot Indole Reagent

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: PL.391-10

INTENDED USE
For the detection of microorganisms that oxidise the amino acid tryptophan into three major end products: indole, pyruvic acid and ammonia.

SUMMARY AND EXPLANATION
Spot Indole Reagent detects the presence of indole, which indicates tryptophan degradation. Indole production is important in the identification of *Enterobacteriaceae*, a large family of bacteria that includes *Salmonella*, *Shigella* and *Escherichia*. These bacteria are rod-shaped Gram negative facultative anaerobes.

PRINCIPLE OF THE TEST
In certain bacteria, the enzyme tryptophanase breaks down tryptophan into pyruvic acid, ammonia and indole. Indole has the ability to combine with specific aldehydes, which results in the formation of a coloured compound. The active aldehyde in Spot Indole Reagent, 4-dimethylaminocinnamaldehyde, combines with indole to produce a distinct blue colour. In the absence of tryptophanase, indole is not produced, and no colour production will be observed. The presence or absence of colour formation is used for bacterial identification.

MATERIALS PROVIDED
-	PL.391-10	Spot Indole Reagent	10 ml

Per 100ml solution:
- Spot Indole Reagent contains 1g of 4-Dimethylaminocinnamaldehyde.

MATERIALS REQUIRED BUT NOT PROVIDED
- Inoculating loops
- Filter paper
- Cotton-tipped swabs

STABILITY AND STORAGE
Spot Indole Reagent should be stored at 15-25ºC in its original container. Protect from light. Product stored under these conditions will be stable until the expiry date shown on the product label.

PRECAUTIONS
- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Microbial contamination may decrease the accuracy of the test.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Process samples in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.
- Any serious incident that occurs in relation to the device should be reported to the manufacturer and the competent authority of the member state in which the incident occurred.

TEST PROCEDURE
Filter paper method:
1. Dispense 1 to 2 drops of Spot Indole Reagent onto a piece of filter paper (Whatman No. 1 or equivalent).
2. Using an inoculating loop, smear the growth from an actively growing culture onto the reagent-saturated area of the filter paper.
3. Observe the filter paper for the development of a blue colour within 3 minutes.

Swab Method:
1. Dispense 1 to 2 drops of spot indole reagent onto the tip of a cotton swab.
2. Touch the tip of the saturated swab to the top of an actively growing colony on the surface of the agar medium.
3. Observe the cotton tip for the development of a blue colour within 3 minutes.

QUALITY CONTROL PROCEDURE
Internal quality control of the Spot Indole Reagent must be performed regularly on known reference material.

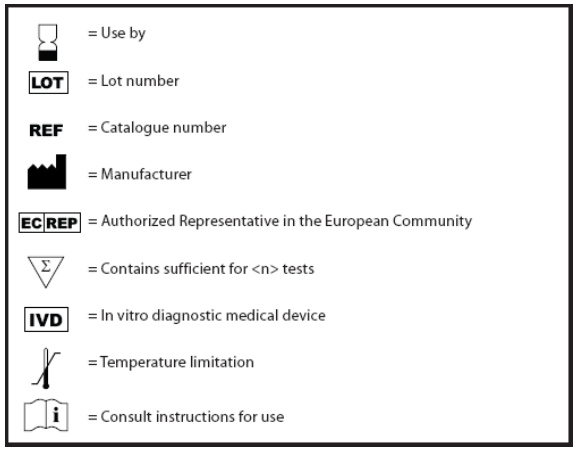
Recommended Quality Control:
Positive - *Escherichia coli* NCTC® 12241/ATCC® 25922* (PLD02)
Negative – *Klebsiella pneumoniae* NCTC® 9633 ATCC® 13883* (PLD04)

INTERPRETATION OF RESULTS
Positive- development of a blue colour within 3 minutes.
Negative- no development of colour within 3 minutes.

LIMITATIONS OF THE PROCEDURE
- Only experienced personnel should carry out the interpretation of stained slides.
- Colonies to be tested must be grown on non-glucose containing media.
- Glucose inhibits Indole production.
- MacConkey (MAC) or eosin-methylene blue agar (EMB) should not be used to culture organisms for the Indole test because they contain indicators which could result in carry over of colour, resulting in false positive colour interpretations.
- Some strains of *Proteus vulgaris*, Providencia and Aeromonas exhibit a false negative reaction with the spot indole test.
- Test colonies must be cultivated on media with adequate tryptophan content which is necessary for the indole reaction.
- Media should be checked with known positive and negative control organisms.
- Only pure cultures of organisms are to be tested. Weak false positive reactions may occur if the inoculum is a mixed culture of indole positive and negative organisms since adjacent colonies are likely to take up diffused indole.

REFERENCES

- Arnold WM, Weaver RH. Quick microtechniques for the identification of cultures. *Journal of Laboratory and Clinical Medicine* 1948; 33:1334-7.
- Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol I Washington, DC: *ASM Press*, 1992.
- Kovacs, N. Eine vereinfachte method zum nachweis der indolbidung durch bakterien. *Z Immunitaetsforsch* 1928; 55: 311-5.



EC REP Advena Ltd. Tower Business Centre, 2nd Floor, Tower Street, Swatar, BKR 4013, Malta.

*NCTC® and NCPF® are trademarks of Public Health England. ATCC® strains are listed for reference only. ATCC® is a registered trademark of the American Type Culture Collection.

HAZARDS IDENTIFICATION
Please refer to Safety Data sheets for full text for all hazard and precautionary statements.

DANGER	PL.391-10	**H302, H314, H331, H332, H335** **P260, P280, P303+P361+P353, P305+P351+P338, P310, P321, P403+P233**

Canada: Toll Free +1 800 268 2341 Tel +1 905 731 0300
20 Mural Street, Unit 4, Richmond Hill, ON, L4B 1K3
www.pro-lab.com

U.S.A: Tel (512) 832-9145
21 Cypress Blvd, Suite 1155, Round Rock, Texas, 78665-1046
www.pro-lab-direct.com

UK: Tel +44 (0)151 353 1613
3 Bassendale Road, Bromborough, Wirral, CH62 3QL
www.pro-lab.co.uk

Revision: 2023 12