PRO-LAB DIAGNOSTICS

SPOT INDOLE REAGENT

(for *in vitro* diagnostic use)

PRODUCT CODE PL.391-10

Σ 250

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INTENDED USE

Pro-Lab's Spot Indole Reagent is to be used in the qualitative method to determine the ability of an organism to split indole from the tryptophan molecule.

SUMMARY AND EXPLANATION

Spot Indole Reagent was used by Vracko and Sherris in 1963 for the presumptive differentiation of *Proteus* species and *Escherichia coli*[1]. The work of Lowrence, Reich and Traub in 1969, indicated that p-diemethyl-aminocinnamaldeyde is the most sensitive indole reagent, capable of detecting 3 mcg of indole per millilitre of medium[2].

PRINCIPLE OF THE PROCEDURE

The amino acid tryptophan can be oxidized by certain bacteria using intracellular enzymes collectively called 'tryptophanase', resulting in the production of indole. The indole is detected by the p-dimethylaminocinnamaldehyde, which involves a chemical combination producing a distinct blue colour. The presence or absence of indole formation is used for bacterial identification.

MATERIALS SUPPLIED

Pro-Lab Spot Indole Reagent PL.391-10 is supplied as 10 ml of liquid reagent in an amber dropper bottle. The reagent is ready for use.

FORMULA

p-Dimethylaminocinnamaldehyde	10 g
Hydrochloric Acid	100 ml
Deionized Water	900 ml

PRECAUTIONS

1. Pro-Lab Spot Indole Reagent PL.391-10 is intended for *in vitro* diagnostic use only.
2. Do not use the reagent after the expiry date shown on the product label.
3. The reagent should not be used if the colour has changed.
4. Safety precautions should be taken in handling, processing and discarding all clinical specimens as a pathogenic organism may be present.
5. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid information.

STORAGE

The Pro-Lab Spot Indole Reagent should be stored at 2-30°C in its original container. Do not freeze or overheat. Protect from light. Keep the screw cap tightly closed. Product stored under these conditions will be stable until the expiry date shown on the product label.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

Clinical specimens should be inoculated onto appropriate isolation media to obtain well-defined isolated colonies for testing. For specific procedures regarding specimen collection and preparation of primary cultures refer to a standard microbiology textbook.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Inoculating loops
2. Filter paper (Whatman No. 1 or equivalent)
3. Cotton-tipped swabs
4. Incubator
5. Supplemental media
6. Quality control organisms

PROCEDURE

Allow the reagent to come to room temperature prior to use.

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 a colony, from an actively growing culture, 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

For laboratory quality control, the following reference strains are recommended:

ORGANISM	EXPECTED RESULT
Bacteroides ovatus ATCC #8483	positive
Escherichia coli ATCC #25922	positive
Prevotella melaninogenica ATCC #25845	negative
Proteus mirabilis ATCC #12453	negative

Each lot of Spot Indole Reagent is subject to quality control at Pro-Lab using a test panel which includes the above organisms.

INTERPRETATION OF RESULTS

Positive reaction:	The development of a blue colour within 3 minutes.
Negative reaction:	The development of a pink colour within 3 minutes.

LIMITATIONS OF THE PROCEDURE

1. Colonies to be tested must be grown on non-glucose containing media, since glucose inhibits indole production.
2. MacConkey (MAC) or eosin-methylene blue agar (EMB) cannot be used to culture organisms for the indole test, since they contain indicators which could result in carryover of colour, resulting in false positive colour interpretations.
3. Some strains of *Proteus vulgaris*, *Providencia* and *Aeromonas* exhibit a false negative reaction with the Spot Indole test[3].
4. 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.
5. Only pure cultures of organisms are to be tested. Weakly 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[4].

REFERENCES

1. Vracko, R. and J.C. Sherris. (1963). Am. J. Clin. Path. 39:429-432.
2. Lowrance, B.L., P. Reich and W.H. Traub. (1969). Appl. Microbiol. 17:923-924.
3. Balzevic, D.J. and G.M. Ederer. (1975). Principles of Biochemical Tests in Diagnostic Microbiology. John Wiley & Sons, New York, NY.
4. Sutter, V.L. and W.T. Carter. (1972). Am. J. Clin. Path. 58:335-338.

	= Use by
LOT	= Lot number
REF	= Catalogue number
	= Manufacturer
IVD	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use
Σ	= Contains sufficient for <n> tests

EU	C Corrosive Hazardous ingredient: HCl (hydrochloric acid) R34 - Causes burns. S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection. S45 - In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
US	WARNING: May be fatal if inhaled. Causes severe respiratory tract burns. Causes eye and skin burns. May be harmful if swallowed. Contains material that may cause target organ damage, based on animal data.

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