

Document Name: Spot Indole Test

Approved By:

Status: **DRAFT**

**PURPOSE:** The spot indole test is used to determine the ability of an organism to split indole from the tryptophan molecule.

**SAMPLE INFORMATION:**

<b>Type</b>	One, well isolated colony
<b>Source</b>	Blood agar

**REAGENTS and/or MEDIA:**

<b>Type</b>	remel Spot Indole Reagent
<b>Stability and Storage Requirements</b>	<ul style="list-style-type: none"> <li>• Store in original container at 2°C to 30°C until used.</li> <li>• Allow to equilibrate to room temperature before use.</li> <li>• Do not incubate prior to use.</li> <li>• Protect product from light.</li> </ul>

**SUPPLIES:**

- Glass microscope slides
- Filter paper
- Wooden sticks

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FILENAME:

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<b>Document Name: Spot Indole Test</b>	<b>Document Number:</b> MIC52000	
	<b>Version No:</b> 1.0	Page: 2 of 4
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**SPECIAL SAFETY PRECAUTIONS:**

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

**QUALITY CONTROL:**

- Quality control is performed daily:
  - Positive: *Escherichia coli* ATCC 25922
  - Negative: *Proteus mirabilis* ATCC 7002
- A TQC order is automatically generated daily to record the QC results.

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FILENAME:	Print Date:

**PROCEDURE INSTRUCTIONS:**

Step	Action
<b>Performing the spot indole test</b>	
<b>1</b>	Remove the spot indole reagent from the refrigerator and bring to room temperature.
<b>2</b>	Place a piece of filter paper on a glass microscope slide and add 1 or 2 drops of spot indole reagent.
<b>3</b>	Using a wooden stick, pick a colony to be tested and rub the inoculum on the filter paper.
<b>4</b>	Observe for the development of a blue colour within 1 to 3 minutes.

**INTERPRETATION OF RESULTS:**

IF	THEN
Blue colour within 1 to 3 minutes	<b>Spot indole = Positive</b>
Pink colour	<b>Spot indole = Negative</b>



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FILENAME:

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**LIMITATIONS/PRECAUTIONS:**

1. Test only colonies cultured on media without glucose, as glucose inhibits indole production.
2. Organisms from MacConkey agar cannot be tested; indicators in this media may cause a false-positive reaction.
3. Certain strains of *Proteus vulgaris*, *Providencia* spp. and *Aeromonas* spp. will give a false-negative reaction with the spot indole test.
4. Media utilized in this test should be checked with known positive and negative control organisms to ensure adequate tryptophan content necessary for the indole reaction.
5. Because adjacent colonies are likely to take up diffused indole, positive tests are valid only if pure cultures are tested.
6. Do not use Mueller-Hinton agar for test, because tryptophan is destroyed during the acid hydrolysis of casein.

**REFERENCES:**

- remel Spot Indole Reagent package insert, July 17, 2012
- Clinical Microbiology Procedures Handbook, 4<sup>th</sup> edition, ASM Press, 2016

**REVISION HISTORY:**

REVISION	DATE	Description of Change	REQUESTED BY
1.0	8 APR 19	Initial Release	L. Steven

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