PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC50500 – Spot Indole Test	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

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

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the spot indole test.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) performing the spot indole test.

SAMPLE INFORMATION:

Type	One, well isolated colony

REAGENTS and/or MEDIA:

Type	remel Spot Indole Reagent
Stability and Storage Requirements	• Store in original container at 2°C to 30°C until used • Allow to equilibrate to room temperature before use • Do not incubate prior to use • Protect product from light

SUPPLIES:
- Glass microscope slides
- Filter paper
- Wooden sticks

SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hang hygiene practices be used.
- 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 of 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. Routine Practices 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

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the spot indole test	
1	Remove the spot indole reagent from the refrigerator and bring to room temperature.
2	Place a piece of filter paper on a glass microscope slide and add 1 or 2 drops of spot indole reagent.
3	Using a wooden stick, pick a colony to be tested and rub the inoculum on the filter paper.
4	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	**Spot indole = Positive**
Pink colour	**Spot indole = Negative**

LIMITATIONS:

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:

1. remel. (2012-07). *Spot Indole Reagent* package insert

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	08 Apr 19	Initial Release	L. Steven
2.0	30 Jun 21	Procedure reviewed and added to NTHSSA policy template	L. Steven