


STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: Spot Indole	Revision Date: 20-April-2018	Issue Date: 20-April-2016
Document Number: MIC52000	Status: Approved	
Distribution: Microbiology Test Manual	Page: 1 of 4	
Approved by: S. Asmussen, Manager of Diagnostic Services	Signed by: 	

INTRODUCTION:

The spot indole test reagent is used to detect the rapid production of indole by bacteria. The test must be performed from colonies growing on a tryptophan containing medium, such as sheep blood agar as indole is one of the degradation products of the bacteria's metabolism of the amino acid tryptophan.

PURPOSE:

To ensure indole test is being performed correctly for:

- Presumptive identification of *Escherichia coli*
- Differentiation of swarming proteus
- Differentiation between *Klebsiella pneumoniae* and *Klebsiella oxytoca*

SAMPLE INFORMATION:

Type	Well-isolated colonies that are 18-24hrs old.
Source	Sheep Blood Agar (tryptophan containing medium)

REAGENTS and/or MEDIA:

Type	Indole Spot Test Reagent
Stability	Do not use beyond expiry date
Storage Requirements	2-8°C in the dark

SUPPLIES:

- Filter Paper (Whatman no.3)
- Sterile Loop or wooden stick

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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 where 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.

Test reagent is corrosive; apply the use of proper personal protective equipment as outlined in indole test reagent MSDS sheet.

QUALITY CONTROL:

Quality Control is set up each day the test is performed using the following control organisms:

Positive:	<i>Escherichia coli</i>	ATCC # 25922
Negative:	<i>Klebsiella pneumonia</i>	ATCC # 13883

A QC order is generated in the TQC system: Resulting Worklist→MICS→SI

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PROCEDURE INSTRUCTIONS:

Step	Action
Performing a Spot Indole	
1	Wet filter paper with indole test reagent
2	Using sterile loop or end of stick, pick well-isolated colony from Sheep Blood Agar plate.
3	Smear colony onto wet filter paper
4	Observe for color change within 2 minutes

INTERPRETATION OF RESULTS:

- **Positive:** Bluish/green colour change
- **Negative:** No colour change



NOTES AND PRECAUTIONS:

1. Colonies should not be tested from media containing dyes such as MacConkey, as dye carry-over may suggest positive results.
2. False-positive reactions may occur if testing colonies from a mixed culture due to the diffusion of indole into the medium from adjacent indole-positive colonies.
3. Peptone media containing glucose or other carbohydrates must not be used for the detection of indole.
4. Mueller-Hinton Blood Agar does not contain tryptophan and cannot be used for spot indole testing.

REFERENCES:

- Murray, P., Baron, E. J., Jorgensen, J., Landry, M. L., & Pfaller, M. (2007). *Manual of Clinical Microbiology* (9 ed., Vol. 1).

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- PMLmicrobiologicals. (2008, January). Indole Spot Test Reagent-Material Safety Data Sheet. *MSDS#: 130* .
- PMLmicrobiologicals. (2001, January). Indole Test Reagents Biochemical Identification. *Technical Data Sheet #845 Rev.2* .

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	03-NOV-2010	Initial Release	M-L Dufresne
2.0	31-Dec-13	LIS steps/pictures	A.Darrach
2.1	30-April-14	Review- No Change	C. Russell
3.0	31-Mar-16	Update of "Special Safety Precautions" to reflect risk assessment recommendations.	C. Russell