National Department of Health

Title: Spot Indole Test

ID: G_90_T_09_A

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Changes to the last authorized version:

Version	Date issued	Changes

1. Purpose and Scope

This document describes the Spot Indole test procedure used to differentiate Gram negative bacilli.

This document covers the procedure for indole test. The indole test detects tryptophanase production and is an aid in the differentiation of the Enterobacteriaceae and other genera.

2. Principle/Clinical application

The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan.

Tryptophan is hydrolysed by tryptophanase to produce three possible end products – one of which is indole, the others are pyruvate and ammonium ion.
A spot indole test detects rapid indole producing organisms eg E coli.

Organisms to be tested by the spot indole method must be taken from a tryptophan - containing medium (for example blood agar) and never from MacConkey agar as they have pH indicators and pigmentation of lactose-positive colonies which will make interpretation of colour reaction difficult.

3. Responsibilities

- Staff performing Spot Indole require specific training and demonstrated competency.
- Staff performing Spot Indole are responsible for the setup, reading and recording of the coagulase result.
- Staff are required to test and record Spot Indole positive and negative controls daily.

4. Specimen

Test isolated colonies of the organism from Blood agar.

5. Safety

For safety aspects, please review this document G_10_Info_3 Laboratory Biosafety.

6. Equipment and Materials

Depending on the spot indole reagent used for the spot indole test, the resulting colours differ. If using p-methylamino benzaldehyde, the presence of indole is indicated by a red colour and if using pdimethylamino cinnamaledhyde, a blue-green colour is observed.

- Spot indole reagent (1% or 5% p-methylaminobenzaldehyde OR 1% pdimethylaminocinnamaledhyde) or Remel bactidrop Indole -Kovacs'
- Small piece of filter paper placed on a glass slide or petri dish or cotton swabs

- Wooden sticks or nichrome inoculating loop
- Contaminated waste bag
- Quality Control organisms: E. coli ATCC 25922 & Ps aeruginosa ATCC 27853

7. Procedures

Filter Paper Method

7.1 Take one reagent dropper and gently break the glass ampule.

7.2 Moisten filter paper with 1 or 2 drops of reagent.

7.3 Using wooden stick or inoculating loop, take a fresh isolated colony from the SBA plate.

7.4 Smear the colony onto the filter paper.

7.5 Observe a red colour if using **p-methylaminobenzaldehyde** reaction within 1-3 minutes. This is a positive reaction.

Observe a blue/green colour reaction within 1-3 minutes if using **dimethylaminocinnamaledhyde.** This is a positive reaction.

7.6 Test and record results for the QC organisms.

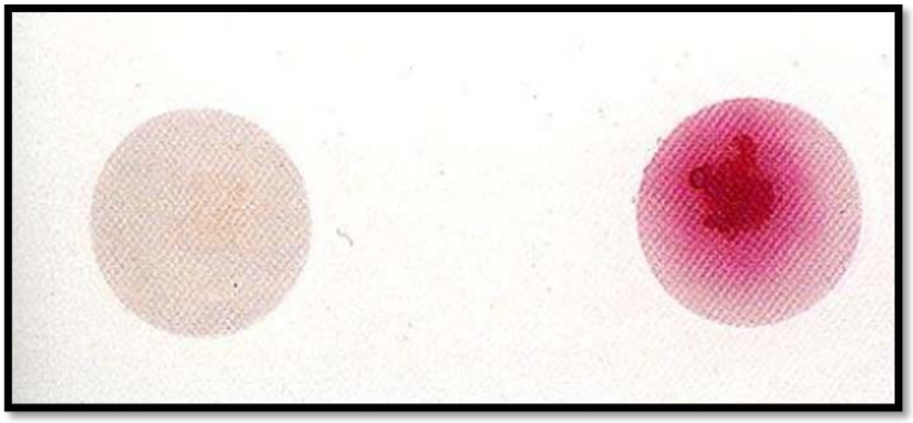
Swab Method

7.7 Moisten a swab stick with 1 or 2 drops of reagent.

7.8 Touch the top of a fresh isolated test colony from an SBA plate.

7.9 Observe for the development of a red or blue/green colour within 1-3 minutes depending on the Indole reagent you are using. This is a positive reaction.

7.10 Test and record results for the QC organisms.



Indole Neg **Indole Pos**

Indole Pos

8. Results Recording

- Record all results onto paper worksheet with registered lab number and patient identification.
- Record results into LIMS.

9. Interpretation

- A positive indole will be demonstrated by the formation of a red or blue green colour depending on the reagent used for testing.

10. Quality Control

- E coli ATCC 25922 – Indole Positive
- Ps aeruginosa ATCC 27853 – Indole Negative
- Record the QC results on the Bench Reagent QC worksheet

11. Related Documents

- Bench Reagent QC Worksheet G_90_WS_1
- Laboratory Biosafety Info Sheet G_10_Info_3

12. References

- DMDP Spot Indole Test SOP- Joanne Letchford 2013
- UK Standards for Microbiology Investigations https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#test-procedures
- Remel BactiDrop Spot Indole reagent dropper Package Insert