



National Department of Health

Title: India Ink Stain

ID: G_90_T_13_A

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1. Purpose and Scope

This document describes the India ink staining procedure, recommended for microscopic examination of CSF specimen for the presence of encapsulated organisms, in particular *Cryptococcus neoformans*.

2. Principle/Clinical application

India ink staining is a negative staining technique used to determine an organism's cellular morphology. The background is stained whereas the organism remains unstained and the morphology is not distorted in any way. Capsules displace the dye and appear as halos surrounding the organism.

This stain provides a high degree of contrast not available in most other staining procedures. This technique is particularly recommended for the demonstration of the capsule of the yeast, *Cryptococcus neoformans* in CSF specimens as an aid to diagnosis of meningitis.

Responsibilities

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

3. Specimen


- Centrifuged Cerebro Spinal Fluid (CSF) specimen sediment.

4. Safety

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

5. Equipment and Materials

- Remel Bactidrop India Ink stain
- Quality Control organisms: *C. neoformans* ATCC 36556 and *C. albicans* ATCC 10231
- Glass slides and coverslips
- Centrifuge
- Microscope
- Plastic disposable pipettes
- Marking pen
- Gloves

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6. Procedure

6.1 Place a drop of India ink on to a clean glass slide

6.2 Add 1 drop of centrifuged CSF on the slide surface just beside the ink before mixing it into the ink

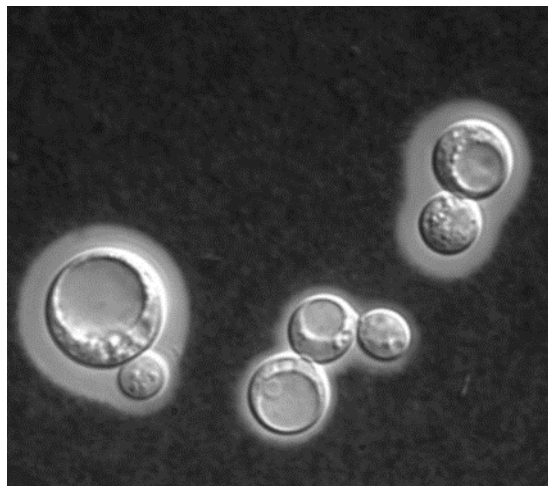
Note: If preparation is too dark, it may be diluted with a small drop of water

6.3 Place a cover slip over the smear avoiding air bubbles, press it down gently so that the film becomes very thin and pale in colour

6.4 Scan under low power, (10x) with reduced lighting

6.5 Switch to high power (40x) to examine for the presence of encapsulated cells

***Cryptococcus neoformans* capsule appears as a halo**




7. Results Recording

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

8. Interpretation

- **Positive** - Organisms possessing a capsule appear highly refractile, surrounded by a clear zone or halo against a dark background. *C. neoformans* appears as a spherical, oval or elongated, single or multiple budding, thick-walled, yeast-like organism 2-15 μm in diameter, usually surrounded by a wide, refractile polysaccharide capsule.
- **Negative**- No clear zone around the organism is observed.

Note: Some Cryptococcus neoformans strains may have insufficient capsule to create a halo with the India ink test.

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- Red and white blood cells may resemble cryptococcal yeasts in an India ink preparation. But the halo has a fuzzy, irregular appearance at the periphery and the cell within the halo has a paler cell wall.

9. Quality Control

- *C. neoformans* ATCC 36556 or an available wild type strain
- *C. albicans* ATCC 10231 or an available wild type strain
- Record the QC results on the Bench Reagent QC Worksheet G_90_WS_1

10. Related Documents

- Bench Reagent QC Worksheet G_90_WS_1_A
- Laboratory Biosafety Info Sheet G_10_Info_3

11. References

- UK Standards for Microbiology Investigations
<https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>
- Bactidrop India Ink stain package insert