India Ink

Principle

Cryptococcus neoformans is an organism capable of producing subacute or chronic mycotic infection involving primarily the brain and meninges and the lungs. The organism is surrounded by a wide, refractile gelatinous capsule. The use of India Ink serves to delineate the large capsule, since the ink particles cannot penetrate the capsular material.

Safety

All specimens, reagents and controls should be handled as though capable of transmitting infectious diseases. Wear appropriate personal protective equipment when running patient samples or performing schedule maintenance. Refer to: Policy and Procedures Safety Manual Infection Control and Procedures 11-085-01.

Materials and Reagents

Microscope
Cover Slip
Glass Slides
India Ink Reagent Droppers
India Ink Capsule Positive (+) Control
India Ink Capsule Negative (-) Control

Specimen

Spinal Fluid (CSF)

Quality Control

The India Ink Positive Control (Scientific Device Laboratory, Cat# 756) is intended to be part of a quality control program to identify the pathogen Cryptococcus neoformans.

The India Ink Negative Control (Scientific Device Laboratory, Cat# 756-1) is to be used to determine the absence of Cryptococcus neoformans.

NOTE: Open control vials are stable until expiration date

Step	Positive and Negative Control
1	Mix one drop of well mixed India Ink reagent with one drop of India Ink Control (Positive and Negative Controls are kept room at temperature).
2	Cover with cover slip and examine with subdued light under the high dry objective. Scan slide under low power with reduce light. Capsules appear as a clear halo. If halo is seen, switch to high power and a positive capsule is one with a yeast-like body in the center of halo. If halo is not observed Cryptococcus is not present on smear.
3	Log the QC results in the India Ink Log.

Procedure

Step	Action
1	Prepare a smear and label the slide with patient's first and last name and date.
2	Mix one drop of spun spinal fluid with one drop of India Ink.
3	Cover with cover slip and examine with subdued light under the high dry objective.
4	Observe for presence or absence of yeast like cells.
5	Log patient result in the India Ink Log.

Note: Cell and parasite identification can be difficult. If you have any doubt of the correct identification, you may consult your co-worker, supervisor, or pathologist for assistance

Result Reporting

Results are manually entered in Cerner under the Microbiology Result Entry (MRE) mode.

The acceptable responses are:

NEYS: No Encapsulated yeast forms seen
EYP1: Encapsulated yeast cells suggestive of
EYP2: Cryptococcus neoformans present

Note: For positive results, both EYP1 and EYP2 should be entered and reported.

For detailed instructions of entering results, please refer to Laboratory Informatics – Cerner Genlab Policies & Procedures Manual, "*Result Entry in the Microbiology Module*" LIS.SCPMG.005 document.

Reference Range

Absence of yeast like cells (Cryptococcus neoformans appears as an oval to spherical, single-budding, thick-walled, yeast-like organism 5 to 15 u in diameter, surrounded by a wide, refractile, gelatinous capsule).

Reference

Bailey and Scott, Diagnostic Microbiology, Third Edition, p. 264.

Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Lab Manager reviewed/ date	Date change Implemented
Minor	Updated format and revised index number.	Julius Salomon, 7/1/17			