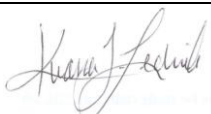


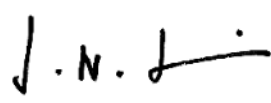


Laboratory Work Practice Document: 6 India Ink

Title of study	High Dose AMBISOME® on a Fluconazole Backbone for Cryptococcal Meningitis Induction Therapy in sub-Saharan Africa: A Phase III Randomized Controlled Non-inferiority Trial		
Acronym	Ambition-cm – AMBIsome Therapy Induction Optimization		
ISRCTN No.:	ISRCTN72509687		
WPD Current version	Version 1.0 12/10/2017		
Author(s)	Kwana Lechiile		12/10/2017
	Timothee Boyer Chamard Clinical Advisor		12/10/2017
Reviewer(s)	David Lawrence Lead clinician		12/10/2017
Approved by	Joseph Jarvis CI		12/10/2017

Revision History:		
Version Number	Effective Date	Reason for Change
1.0		First version

Laboratory Working Practice Document 6: India Ink

Purpose

This document describes the standard operating procedure (SOP) for India Ink.

References

BD India Ink Reagent Droppers insert

Appendices

BD India Ink Reagent Droppers insert

India Ink allows for the visualization of polysaccharide capsules in cryptococcal species. The colloidal carbon particles of the ink are displaced by the capsular material. Because the cryptococcal polysaccharide capsule is resistant to coloration by India Ink, this makes the capsule appear as a clear halo around the microorganisms when viewed under the microscope.

The diagnosis of cryptococcal meningitis is confirmed when the appearance of encapsulated yeast forms in an India Ink smear from CSF. Cryptococci appear as spherical yeast forms that range from 4 μ m to 20 μ m in diameter.

MATERIALS

1. India Ink Reagent dropper
2. Microscope
3. Microscope slide
4. Cover slip

Laboratory Working Practice Document 6: India Ink

PROCEDURE

1. Holding the reagent dropper upright, grasp the middle and gently squeeze to break the ampule inside the dropper
2. Tap the bottom of the dropper against a flat surface a few times, then invert for drop-by-drop dispensing of reagent
3. Centrifuge at 2000xg for 15min prior to preparation of slide and use the **pellet**
4. Place one drop (approximately 20 μ l) of supernatant onto the microscope slide
5. Add one drop of India Ink on the microscope slide next to the drop of CSF. (DO NOT MIX)
6. Gently place the coverslip on top of the two drops
7. Allow for the two drops to spread as a thin film underneath the coverslip
8. Using the microscope, scan the microscope slide under low power
9. Examine under high power for encapsulated yeast forms

RESULTS

A result determined as positive should consist of cryptococcal capsules appearing as clear, refractile halos. The capsules may appear as either broad or narrow. The cryptococcal yeast cells may appear as round, oval, or elongate. The presence or absence of buds may vary.

Fat droplets or lymphocytes should not be interpreted as yeast forms. Fat droplets will not have a well-defined cell wall.

Definitive diagnosis should be accompanied with culture and/or antigenic detection.

Laboratory Working Practice Document 6: India Ink

Training

Each staff member receives or has direct access to applicable Working Practice Documents (WPDs).

Each staff member reviews the applicable WPDs once a year.

All WPD training is documented and tracked in the training log located in the Investigator Site File (ISF)

New staff is trained on applicable WPDs within 30 days of employment and all WPDs within 90 days of employment.

Staff members whose duties fall within this WPD scope are retrained within 14 days of the approval of each WPD revision.

