KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date 11 May 2006	SOP-Number MIC.029
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Title: INDIA INK STAIN

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Approvals/Date:

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This SOP has been read and understood by:

Name	Date
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Annual Review	
By	Date

Title: INDIA INK STAIN

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

Title: INDIA INK STAIN

PURPOSE

To demonstrate encapsulated *Cryptococcus neoformans* in CSF specimens, blood cultures or organism suspensions.

PRINCIPLE

A small drop of india ink is added to a drop of CSF, blood culture or culture suspension and examined on a light microscope using reduced light. Yeast cells and capsules of *C. neoformans* are highlighted against a black background.

SCOPE

This Standard Operating Procedure applies to the preparation and examination of India ink stain by technical staff in the microbiology laboratory that have been trained and are competent in performing this procedure.

SAFETY PRECAUTIONS

Dispose of slide in sharps bucket.

MATERIALS

India ink (Store at room temperature)
Glass slide
Coverslip (22x22 mm)
CSF specimen, blood culture or culture suspension

QUALITY CONTROL

Check India Ink Quality:

Frequency: Each time of use or weekly; whichever is less frequent.

Procedure:

1. Mix a drop of India Ink and a drop of distilled water on a slide and coverslip.
2. Examine under 10X objective for clumping of ink particles.

Acceptable Results:

Smooth suspension of ink particles, no clumping.

Corrective Actions for Unacceptable QC Results:

Discard India Ink.

Documentation:

1. Record QC results on INDIA INK QC sheet.
2. Document all corrective action on QC DEVIATION FORM.

3. Review QC results monthly.

CSF SAMPLE PREPARATION

1. Centrifuge CSF at 1500xg/3500 rpm for 10 minutes.
2. Decant supernate into separate sterile tube.

TEST PROCEDURE

1. Place a drop of centrifuged CSF sediment, blood culture or light suspension in saline of organisms from culture on a clean glass slide.
2. Add a small loopful of India Ink, mix and cover drop with coverslip.
3. Examine with reduced light under low and/or high power for budding yeasts with a well defined capsule.
4. Dispose of slide in sharps bucket.

INTERPRETATION

Positive: Round yeast cells 4-20 um in diameter surrounded by clear, well defined capsule.

REPORTING RESULTS

1. Enter results on POSITIVE CULTURE form.
2. Enter results on MICROBBIOLOGY LABORATORY RESULT FORM.
3. Contact Clerical Assistant to pick up lab result form and take to physician in charge of patient.

NOTES

1. White blood cells, particularly lymphocytes, RBCs and bubbles may be mistaken for encapsulated yeast. Nuclear material of lymphocytes has a “grainy” appearance and may be eccentrically located while cryptococci are uniformly smooth. Look carefully for budding forms to differentiate. Cryptococci are perfectly round and the perimeter of the capsule is well defined and demarcated. If numerous organisms are present there should be size variation among the cells.
2. Other *Cryptococcus* spp. and some *Rhodotorula* sp. may also be encapsulated.

Title: INDIA INK STAIN

REFERENCE

LaRocco, ML. 2003. Reagents, Stains and Media: Mycology. In: Manual of Clinical Microbiology. 8th Ed. ASM Press, Washington, DC.

APPENDIX A - India Ink QC sheet

Title: INDIA INK STAIN

APPENDIX A

KCMC Biotechnology Laboratory — Quality Control

Microbiology — **India Ink Stain**

ACCEPTABLE RESULTS: No clumping of ink particles (OK) Year: _____

DATE	BY	INK BRAND	RESULT	DATE	BY	INK BRAND	RESULT

Document all unacceptable results on QC DEVIATION FORM.

Supervisor Review/Date

INDIA INK QC/QC SHEETS