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AMBITION-*cm*
AMBIsome Therapy Induction OptimizatioN

Laboratory Work Practice Document: 8
Cryptococcal Semi-Quantitative Antigen – CryptoPS

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		
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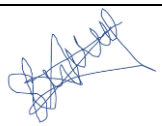
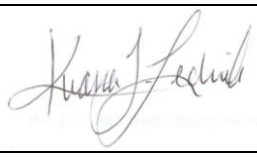
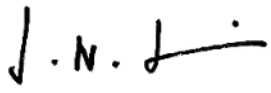
	Aude Sturny-Leclere Lab Engineer, Institut Pasteur		22/11/2017
Reviewer(s)	David Lawrence Lead clinician		22/11/2017
	Kwana Lechiile Lab Scientist		22/11/2017
Approved by	Joseph Jarvis CI		22/11/2017

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

Laboratory Working Practice Document 8: Cryptococcal Semi-Quantitative Antigen – CryptoPS

Purpose

This document describes the standard operating procedure (SOP) for the Cryptococcal semi-quantitative antigen "BioSynex® CryptoPS" assay.

References

BioSynex® CryptoPS package insert

Appendices

BioSynex® CryptoPS package insert

General

1. The aim of this sub-study is to evaluate a newly developed point of care, lateral flow semi-quantitative cryptococcal antigen test, CryptoPS, in patients with cryptococcal meningitis.
2. This test will be performed in real time to determine antigen titer at baseline, in blood and CSF, and compare results to the currently established point of care test from IMMY.
3. This sub-study will recruit all patients from each arm, from two study sites, Blantyre (Malawi) and Gaborone (Botswana).
4. There will be 160 patients recruited from each treatment arm to this sub-study.

MATERIALS

EQUIPMENT PROVIDED

- Test cassettes, individually packaged in a pouch with a desiccant

- Capillary tubes (20 µl)

- Dropper bottle of diluent

- Bottle of diluent for the titration protocol

- Positive control bottle

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STORAGE

Store in the original packaging at room or refrigerated temperature (2-25°C).
The test must remain in its sealed pouch until it is used.

PROCEDURE

1. Biosynex CryptoPS test will be performed along with IMMY CrAg LFA on blood on D1 (pre-dose).
2. Biosynex CryptoPS test will be performed along with IMMY CrAg LFA on CSF on D1 (diagnostic LP).

SEMI-QUANTITATIVE PROCEDURE (Figure 1)

1. Open the pouch and take out the cassette; then put it on a flat, horizontal surface.
   Write down the ID, number or REF sample on the cassette.
2. Put 20 µl of the sample (serum, plasma, whole blood or CSF) in the sample well (S) of the cassette.
3. Open the dropper bottle containing the diluent. Pour 3 drops of diluent into the sample well by applying slight pressure on the sides of the bottle. Avoid adding air bubbles to the cassette sample well and tipping the liquid into the result reading window.
4. Start the timer. As the test progresses a reddish colour migration front appears and migrates along the membrane.
5. Read the result after **10 minutes** migration. Very positive results will be seen more quickly. Do not interpret any test band appearing 15 minutes after the sample is deposited in the cassette.
6. Record the results on the electronic data capture system.
7. After reading, dispose of the test components and the sample in accordance with the established procedure for disposal of biological waste.

Figure 1: Procedure

EQUIPMENT to run the CryptoPS test:

* Personal equipment (Lab coat, gloves)
* Trash can
* Crypto P/S cassette (BioSynex provided)
* Dropper bottle of diluent (BioSynex provided)
* Capillary tubes (20µl) or pipette/tips
* Timer

PROCEDURE

1/ Write down your sample's number/reference on the cassette

2/ Put 20µl of sample

+

3/ Add 3 drops of diluent

4/ 10mins of incubation

5/ Read the visual results and write down all the datas

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INTERPRETATION OF THE RESULTS

POSITIVE:
- The test line **T1** appears at 25 ng/ml of capsular antigen.
- The test line **T2** appears at 2.5 µg/ml of capsular antigen.

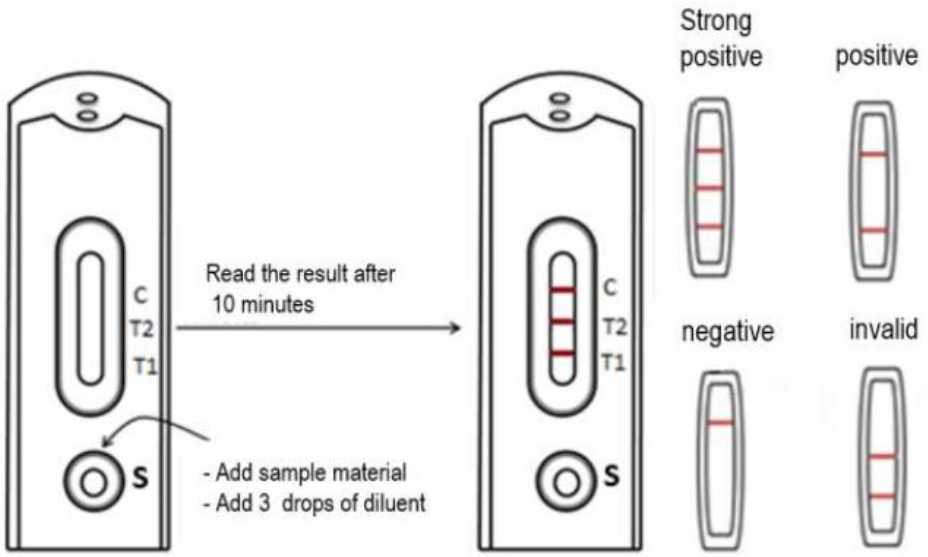
If there is positivity in T2 the T1 line is also present.

- **Positive (T1)**: presence of 2 distinct coloured lines. A line appears in the control line C and a coloured line (even of low intensity) appears in the test line T1.

- **Strong positive (T1&T2)**: presence of 3 distinct coloured lines. A line appears in the control line C and two-coloured lines (even of low intensity) appear in the test lines T1 and T2.

NEGATIVE: A coloured line appears in the control line (C). No line appears in the test lines T1 and T2.

INVALID: No visible coloured line in the control line C, even if bands appear in T1 and T2.
- **The results of a test without a control line must be discarded.**
- **Review the procedure and repeat the test with a new cassette.**
- **If there is a line in T2 and in C, without a line in T1, do not interpret, and repeat the test with a new cassette.**



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.

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Staff signatures: (signing below indicates that you have read this SOP and understand the material contained in it)

Date	Name (Please print)	Signature