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Laboratory Work Practice Document: 3 (Kampala and Mbarara) Sample Processing and Storage

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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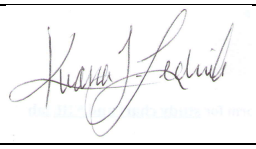
Author(s)	Kwana Lechiile Lab Scientist		10/09/2019
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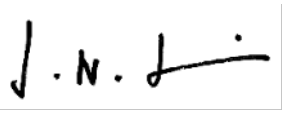
Approved by	Joseph Jarvis CI		10/09/2019

Revision History:		
Version Number	Effective Date	Reason for Change
1.0	14/06/2018	First version
1.1	05/12/2018	Mbarara added to the sites, Harare removed
1.2	10/09/2019	Lilongwe and Cape Town removed from document

Laboratory Working Practice Document 3: Sample Processing and Storage

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Purpose

This document describes the day to day laboratory duties in Kampala and Mbarara

References

Ambition Phase III Trial Protocol

Materials

Lab WPD 1 – Laboratory equipment
Lab WPD 2 – Quantitative Cryptococcal cultures
Clinical WPD 15- Timing of evaluation and tests

Appendices

Lab Processing Chart: Kampala and Mbarara

Section A – Lab maintenance, quality control

Monitoring of freezer and fridge temperatures, reagents/agar expiration dates, working order of autoclaves, incubators and other laboratory equipment, should all be performed in line with on-site laboratory quality control and health and safety procedures

Weekly stock check of lab consumables for trial. Inform trial team on-site and trial manager when replacements required. See Laboratory WPD 1 – Laboratory equipment

Laboratory Working Practice Document 3: Sample Processing and Storage

Section B – Samples required

All results should be recorded with patient study number, date, and study day.

All results should be communicated to the study team.

– Study bloods

The following must be performed according to the trial protocol

- CD4 at baseline if not done within 3 months
- FBC and ALT on days 1, 7, 14, 28
- Creatinine, electrolytes on days 1, 3, 5, 7, 10, 12, 14, 28

– CSF

- India Ink examination AND/OR Cryptococcal antigen (IMMY CrAg) on D1
- CSF cell count and differential, protein, glucose and routine culture on D1 for all patients and additional LPs, as indicated
- Quantitative fungal culture will be conducted on D1, 7, and 14

Table 1: **Timings of study bloods and CSF analyses**

TABLE 6.1. Event Schedule	Screening	Week 1							Week 2							Wk 4
Study Day	≤D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	
Clinical labs																
HIV testing (if status unknown)*		X														
Pregnancy Test (Urine/Serum) (2)		X														
Full Blood Count		X						X							X	X
CD4 count & Viral load (if needed)*		X														
ALT		X						X							X	X
Urea, creatinine and electrolytes		X		X		X		X			X		X		X	X
CSF																
Opening pressure		X						X							X	
Cell count and differential*		X														
Protein, glucose*		X														
Routine culture *		X														
India ink examination* (3)		X														
Cryptococcal antigen* (3)		X														
Quantitative fungal culture		X						X							X	
Immune parameters (4)		X						X							X	

Laboratory Working Practice Document 3: Sample Processing and Storage

Section C - Procedures for sample processing and saving - Please see Appendix: Lab Processing Chart

Day 1 (or 0)

Required samples:

- *2 yellow (or red) top (1 for biochemistry, 1 for serum storage)*
- *≥3 purple top (1 for FBC, 1 for whole blood storage, 1 for plasma and buffy coat storage and other samples may be for CD4, viral load and resistance testing)*
- *CSF sample in 3 x white top tube (5 mls each)*

DAY 1 CSF SAMPLES

NB CSF must be processed within **4 HOURS** of receipt

1. Perform protein and glucose on CSF
2. Perform India Ink, CrAg (if required), cell count (on day 0/1 sample)
3. Plate out day 1 unspun CSF dilutions 0-4 (neat, one in 10, 100, 1,000 and 10,000) for quantitative culture (see WPD 2: Quantitative Cryptococcal Cultures)

   Store isolate once grown in 2 Microbank vials.
4. Vortex CSF and save: divide into 3 x 2ml Cryovials - min 200 µL each. Label and place into freezer box.

TB culture only if clinically indicated (will be performed in TB lab, and is not mandated by trial protocol)

DAY 1 BLOOD SAMPLES

1. Take blood for CD4, viral load and resistance testing (if indicated), whole blood, plasma and buffy coat (all purple tops), and serum (yellow or red top). Total required = ≥ 3 purple tops (4 ml each) and 2 yellow or red top (4 ml).
2. Perform FBC, ALT, creatinine and electrolytes on Day 1
3. WHOLE BLOOD: take 2x cryovials and pipette 900 uL of WHOLE BLOOD (unspun sample) into each (should be mixed well in purple top tubes prior to transfer). Label and place into whole blood storage box.
4. PLASMA AND BUFFY COAT:

   Spin blood from purple top tube at 800xg for 15 minutes. Avoiding the buffy coat and red cells at the bottom, remove as much of the plasma as possible using a sterile Pasteur pipette and divide into 2 cryovials.

   Buffy coat is the small region between the plasma and red blood cells. After removing the plasma, use a sterile Pasteur pipette to carefully aspirate the buffy coat in a circular movement trying not to disturb the red blood cell layer then store in a single cryovial
5. SERUM: Spin yellow (or red) top sample at 800xg for 15 minutes. In the yellow top, gel now separates cells from serum. Use a sterile Pasteur pipette to remove as much of the serum possible and divide into 2 cryovials.

Day 3

Required samples:

- *1 purple top (for plasma and buffy coat storage)*
- *1 yellow/red top tube (4ml)*

DAY 3 BLOOD SAMPLES

1. Perform urea, creatinine and electrolytes on Day 3
2. PLASMA AND BUFFY COAT

Day 5

Required samples:

- *1 yellow/red top tube (4ml)*

DAY 5 BLOOD SAMPLES

1. Perform urea, creatinine and electrolytes on Day 5

Day 7

Required samples:

- *2 purple top (1 for FBC, and 1 for plasma and buffy coat storage)*
- *1 yellow/red top tube (4ml)*
- CSF sample in 2 x white top tubes (5 mls each)

DAY 7 CSF SAMPLES

1. Perform quantitative cultures on Day 7 LP.
2. Save remaining CSF, divide into 3 Cryovials.
3. Save Cryptococcus isolate in 2 Microbank vial if growth from Day 7 LP

DAY 7 BLOOD SAMPLES

1. Perform FBC, ALT, urea, creatinine and electrolytes on Day 7
2. PLASMA AND BUFFY COAT

Day 10

Required samples:

- *1 yellow/red top tube (4ml)*

DAY 10 BLOOD SAMPLES

2. Perform urea, creatinine and electrolytes on Day 10

Day 12

Required samples:

- *1 yellow/red top tube (4ml)*

DAY 12 BLOOD SAMPLES

3. Perform urea, creatinine and electrolytes on Day 12

Day 14

Required samples:

- *2 purple top (1 for FBC, and 1 for plasma and buffy coat storage)*
- *1 yellow/red top tube (4ml)*
- *CSF sample in 2 x white top tube (5 mls each)*

DAY 14 CSF SAMPLES

1. Perform quantitative cultures on Day 14 LP. Save remaining CSF, divide into 3 Cryovials.
2. Save Cryptococcus isolate in 2 Microbank vial if growth from Day 14 LP

DAY 14 BLOOD SAMPLES

1. Perform FBC, ALT, urea, creatinine and electrolytes on Day 14
2. PLASMA AND BUFFY COAT

Day 28

Required samples:

- *1 purple top (for FBC)*
- *1 yellow/red top tube (4ml)*

DAY 28 BLOOD SAMPLES

1. Perform FBC, ALT, urea, creatinine and electrolytes on Day 28

Laboratory Working Practice Document 3: Sample Processing and Storage

PROCESSING AND LABELLING

SPINNING: For all samples use 800xg for 15 mins, unless stated otherwise on LPC.

LABELLING: Each sample must be labelled with study number (e.g. AMB 1-1-001), nature of sample (e.g. CSF/ plasma etc), study day (e.g. d3), date and time (time specimen taken, not time specimen received in lab).

The study day is derived from **the first day the patient receives study drugs**. That is day 1. That is not necessarily the day of hospital admission.

STORAGE: Cryovials containing samples should be stored in appropriately labelled cryoboxes. Cryoboxes should be stored in -80°C freezer

CRYPTOCOCCAL ISOLATE STORAGE: Cryptococcal isolates are stored in Microbank beads in an appropriately labelled cryobox and stored in -80°C freezer.

PROCEDURE FOR STORING ON MICROBANK BEADS:

1. Using a permanent marker, label with the study number, date and day of study (e.g. AMB 1-1-001, 12.10.15, d7)
2. Under aseptic conditions open the screw cap cryovial.
3. Inoculate the cryopreservative fluid with young colonial growth (18-24 hours) picked from a pure culture to approximately a 3-4 McFarland standard.
4. Close vial tightly and invert 4-5 times to emulsify organism. DO NOT VORTEX!
5. At this point the microorganisms will be bound to the porous beads. The excess cryopreservative fluid should be well aspirated leaving the inoculated beads as free of liquid as possible. Close the vial finger tight.
6. Record on the storage log.
7. Store the inoculated cryovial in -80°C freezer.

Section D - Summary of samples to be saved

Day 1:
Serum (stored in 2 Cryovials)
Plasma (divided into 2 Cryovials)
Buffy coat (1 Cryovial)
Whole blood (divided into 2 cryovials)
CSF (divided into 3 Cryovials)
Crypto isolate (in 2 Microbank vials)

Day 3:
Plasma (divided into 2 Cryovials)
Buffy coat (1 Cryovial)

Day 7:
Plasma (divided into 2 Cryovials)
Buffy coat (1 Cryovial)
CSF (divided into 3 Cryovials)
Crypto isolate (in 2 Microbank vials – if growth from day 7 LP)

Day 14:
Plasma (divided into 2 Cryovials)
Buffy coat (1 Cryovial)
CSF (divided in 3 Cryovials)
Crypto isolate (in 2 Microbank vial (if growth from Day 14 LP)

APPENDIX

Lab Processing Chart: Kampala and Mbarara

AMBITION-cm

High Dose AMBISOME on a Fluconazole Backbone for Cryptococcal Meningitis Induction Therapy in sub-Saharan Africa: A Phase 3 Randomised Controlled Non-inferiority Trial

(As per Protocol version 2.1: 07/11/2017)

Protocol Version: 2.1 07/11/2017 LPC Version: 1.1: 05/12/2018 Revised/Updated:

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Laboratory Working Practice Document 3: Sample Processing and Storage

Table 1: Schedule of Events (as per Study Protocol)

TABLE 6.1. Event Schedule	Screening	Week 1							Week 2							Wk 4	Wk 6	Wk 8	Wk 10	Wk 16
Study Day	≤D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14					
Consent forms																				
PIS and signed consent	X	X																		
Follow up																				
Screening and randomisation	X	X																		
Clinical review		X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Outpatient follow-up																X	X	X	X	
Week 16 telephone																				X
Clinical labs																				
HIV testing (if status unknown)*		X																		
Pregnancy Test (Urine/Serum) (2)		X																		
Full Blood Count		X						X							X	X				
CD4 count & Viral load (if needed)*		X																		
ALT		X						X							X	X				
Urea, creatinine and electrolytes		X		X		X		X			X		X		X	X				
Clinical evaluation																				
Chest X-ray (1)		X																		
CSF																				
Opening pressure		X						X							X					
Cell count and differential*		X																		
Protein, glucose*		X																		
Routine culture *		X																		
India ink examination* (3)		X																		
Cryptococcal antigen* (3)		X																		
Quantitative fungal culture		X						X							X					
Immune parameters (4)		X						X							X					

*Part of routine care. 1. If clinically indicated. 2. For women of childbearing age. 3. India ink or CrAg required for inclusion. 4. As part of sub-studies at limited sites.

Table 2: Specimen Log

Evaluation	Specimen	Tests	Special Notes
Haematology	EDTA	FBC	All patients, when applicable
CD4	EDTA	CD4 count	Should be performed if it has not been done within 3 months or if there is reason to suspect it is required.
Viral Load	EDTA	Viral load	Should be performed if it has not been done within 3 months or if there is reason to suspect it is required.
Resistance	EDTA	Resistance testing	If patient is on ART, has a detectable viral load, and reports good adherence or their ART history gives reason to suspect resistance, then resistance testing will be performed if possible at the site.
Whole Blood	EDTA	For further analysis and sub-studies	Storage at -80°C until shipment
Plasma	EDTA	For further analysis and sub-studies	Storage at -80°C until shipment
Chemistry	Yellow/Red Top Tube	ALT, Creatinine, Electrolytes	All patients, when applicable
Serum	Yellow/Red Top Tube	For further analysis and sub-studies	Storage at -80°C until shipment
Chemistry – CSF	CSF tube	Protein, Glucose	All patients, when applicable
Microbiology – CSF	CSF tube	India Ink, CrAg LFA, Gram stain, Cell count and differential, Routine culture (for D1 CSF and if clinically indicated thereafter) Quantitative Cryptococcal culture (for all CSF)	Store isolate and any unused sample at -80°C until shipment

Classification of samples:

Routine Tube: Part of routine clinical care, tests performed by usual laboratory and not required to be in an ISO accredited lab. Not curated by Ambition.

Monitoring Tube: As part of protocol driven monitoring, stored if samples remain and curated by Ambition.

Study Tube: For specified sub-studies or storage for future research. Curated by Ambition.

Laboratory Working Practice Document 3: Sample Processing and Storage

Table 3: Study lay-out

Visit	Specimen	Tests Conducted	Storage	Special Notes
A number of samples on Day 1 are routine tests which may be handled by other laboratories *Store any unused samples, if possible* *Aim for a minimum of 5ml CSF from each LP*				
Day 1	CSF (5ml) x 3	Routine Tube 1: perform protein and glucose, cell count and differential, CrAg LFA, India Ink, Gram stain, routine culture	No storage for study required.	May need to separate into two: biochemistry and microbiology
		Study Tube 1: perform quantitative cryptococcal culture in duplicates	Vortex and divide remaining CSF into 3x2ml cryovials (min. 200µl each) and store at -80°C	Store isolate once grown in 2 Microbank vials and keep at -80°C
		Study Tube 2: Sample for storage	General CSF storage: Unspun sample	Store at -80°C
	EDTA (4ml)	Optional Routine Tube 1, 2 and 3: CD4 and/or VL/Resistance Test (one tube for each test)		
		Monitoring Tube 1: FBC		
		Study Tube 1: Whole blood	Invert 8-10 times and pipette into 2x2ml cryovials (min. 900µl each) and store at -80°C	
		Study Tube 2: Spin tube at 800^{x}g for 15min and remove plasma	Divide plasma into 2x2ml cryovials. Remove buffy coat and put into 1 x 2ml cryovial	Store at -80°C
	Yellow/Red Top Tube (4ml) x 2	Monitoring Tube 1: ALT, urea, creatinine, and electrolytes		
		Study Tube 1: Spin tube at 800^{x}g for 15min and remove serum	Divide serum into 2x2ml cryovials and store at -80°C	
Day 3	Yellow/Red Top Tube (4ml)	Monitoring Tube 1: Urea, creatinine and electrolytes		

	EDTA (4ml)	Study Tube 1: Spin tube at 800^{x}g for 15min and remove plasma	Divide plasma into 2x2ml cryovials. Remove buffy coat and put into 1 x 2ml cryovial	Store at -80°C
Day 5	Yellow/Red Top Tube (4ml)	Monitoring Tube 1: Urea, creatinine and electrolytes		
Day 7	CSF (5ml) x 2	Study Tube 1: Perform quantitative cryptococcal culture in duplicates	Vortex and divide remaining CSF into 3x2ml cryovials (min. 200µl each) and store at -80°C	Store isolate once grown in 2 Microbank vials and keep at -80°C
		Study Tube 2: Sample for storage	General CSF storage: Unspun sample	Store at -80°C
	EDTA (4ml) x 2	Monitoring Tube 1: FBC		
		Study Tube 1: Spin tube at 3500rpm/ 800^{x}g for 15min and remove plasma	Divide plasma into 2x2ml cryovials. Remove buffy coat and put into 1 x 2ml cryovial	Store at -80°C
	Yellow/Red Top Tube (4ml)	Monitoring Tube 1: ALT, urea, creatinine, and electrolytes		
Day 10	Yellow/Red Top Tube (4ml)	Urea, creatinine and electrolytes		
Day 12	Yellow/Red Top Tube (4ml)	Monitoring Tube 1: Urea, creatinine and electrolytes		
Day 14	CSF (5ml) x 2	Study Tube 1: Perform quantitative cryptococcal culture in duplicates	Vortex and divide remaining CSF into 3x2ml cryovials (min. 200µl each) and store at -80°C	Store isolate once grown in 2 Microbank vials and keep at -80°C
		Study Tube 2: Sample for storage	General CSF storage: Unspun sample	Store at -80°C
	EDTA (4ml) x 2	Monitoring Tube 1: FBC		
		Study Tube 1: Spin tube at 800^{x}g for 15min and remove plasma	Divide plasma into 2x2ml cryovials. Remove buffy coat and put into 1 x 2ml cryovial	Store at -80°C

	Yellow/Red Top Tube (4ml)	Monitoring Tube 1: ALT, urea, creatinine, and electrolytes		
Day 28	EDTA (4ml)	Monitoring Tube 1: FBC		
	Yellow/Red Top Tube (4ml)	Monitoring Tube 1: ALT, urea, creatinine, and electrolytes		

Table 4: Shipments

Specimen	Destination	Shipment Date	Special Notes
CSF			
Crypto Isolates			
Serum			
Whole Blood			
Plasma			
Buffy Coat			

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.

Laboratory Working Practice Document 3: Sample Processing and Storage

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

Date	Name (Please print)	Signature