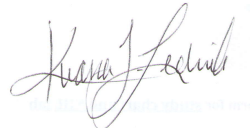


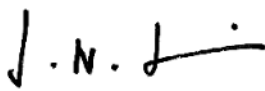


# Laboratory Working Practice Document 3: Sample Processing and Storage

Ambition Trial Coordinating Centre  
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## Laboratory Work Practice Document: 3 (Blantyre) Sample Processing and Storage

|                            |  |   |            |
|----------------------------|--|---|------------|
| <b>Title of study</b>      | High Dose AMBISOME® on a Fluconazole Backbone for Cryptococcal Meningitis Induction Therapy in sub-Saharan Africa: A Phase III Randomized Controlled Non-inferiority Trial |   |            |
| <b>Acronym</b>             | Ambition-cm – AMBIsome Therapy Induction OptimizatiON  |   |            |
| <b>ISRCTN No.:</b>         | ISRCTN72509687   |   |            |
| <b>WPD Current version</b> | Version 1.3 10/09/2019   |   |            |
| <b>Author(s)</b>           | Kwana Lechiile<br>Lab Scientist  |   | 10/09/2019 |
|                            | Timothée Boyer Chammard<br>Clinical Advisor  |   | 10/09/2019 |
| <b>Reviewer(s)</b>         | David Lawrence<br>Lead Clinician   |  | 10/09/2019 |
| <b>Approved by</b>         | Joseph Jarvis<br>CI  |  | 10/09/2019 |

| Revision History: |                |   |
|-------------------|----------------|---|
| Version Number    | Effective Date | Reason for Change   |
| 1.0               | 05/07/2018     | First version   |
| 1.1               | 22/08/2018     | Addition of semi-quantitative CrAg at D1 and India ink at D14 |
| 1.2               | 01/11/2018     | Addition of STOP-CM Immunology                                |
| 1.3               | 10/09/2019     | Addition of PAXgene Specimen Collection and Storage           |
|                   |                |   |

# Laboratory Working Practice Document 3: Sample Processing and Storage

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## **Purpose**

This document describes the day to day laboratory duties

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## **References**

Ambition Phase III Trial Protocol

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## **Materials**

Lab WPD 1 – Laboratory equipment

Lab WPD 2 – Quantitative Cryptococcal cultures

Lab WPD 5 – PK Sampling

Lab WPD 7 – Quantitative Cryptococcal PCR sub-study

Lab WPD 8 – Cryptococcal Semi-Quantitative Antigen

Lab WPD 9 - PAXgene Specimen Collection and Storage

Clinical WPD 15- Timing of evaluation and tests

Clinical WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study

Clinical WPD 20 – Quantitative PCR Sub-study

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## **Appendices**

Lab Processing Chart: Blantyre

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## **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

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## **Section B – Samples required**

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**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
- Biosynex CryptoPS CrAg on whole blood on D1  
(please see Lab WPD 8 – Cryptococcal Semi-Quantitative Antigen)
- PK samples on days 1 and 7  
(please see Appendix, Lab WPD 5 – PK sampling & Clinical WPD 19 – PK/PD Sub-study)
- qPCR samples (5ml of blood – EDTA tubes): D1 pre-dose, 24 hours post-dose, D3, D7 and D14  
(please see Appendix, Lab WPD7 & Clinical WPD 20 - Quantitative PCR Sub-study)
- Zymosan assay on whole blood (min 1 ml) D1, D7, D14
- Luminex sample (min 750ml of stored plasma) D1, D7, D14
- PAXgene tube on D1 (2.5mL of blood)  
(please see Appendix and Lab WPD 9- PAXgene Specimen Collection and Storage)

### **– CSF**

- India Ink examination and Cryptococcal antigen (IMMY CrAg) on D1
- Biosynex CryptoPS CrAg on CSF on D1  
(please see Lab WPD 8 – Cryptococcal Semi-Quantitative Antigen)
- 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
- CSF for PK/PD and immune parameters on D1, 7, and 14  
(please see Appendix, Lab WPD 5 – PK sampling & WPD 19 – PK/PD Sub-study)
- CSF for qPCR (1ml): D1 (pre-dose), D7 and D14  
(please see Appendix, Lab WPD7 & Clinical WPD 20 - Quantitative PCR Sub-study)

## Laboratory Working Practice Document 3: Sample Processing and Storage

- CSF for luminex (min 750ml stored supernatant) D1, D7, D14
- PAXgene tube on D1 (2.5mL of CSF)  
(please see Appendix and Lab WPD 9- PAXgene Specimen Collection and Storage)

Table 1: Timings of study bloods and CSF analyses

| 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 |      |
| <b>Clinical labs</b>                |           |        |    |    |    |    |    |    |        |    |     |     |     |     |     |      |
| HIV testing (if status unknown)*    | X         |        |    |    |    |    |    |    |        |    |     |     |     |     |     |      |
| Pregnancy Test (Urine/Serum) (1)    | 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   | X    |
| Blood for drug levels (2)           | X         |        |    |    |    |    |    |    | X      |    |     |     |     |     |     |      |
| <b>CSF</b>                          |           |        |    |    |    |    |    |    |        |    |     |     |     |     |     |      |
| 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   |      |
| CSF Drug levels (2)                 | X         |        |    |    |    |    |    | X  |        |    |     |     |     |     | X   |      |
| Immune parameters (2)               | X         |        |    |    |    |    |    | X  |        |    |     |     |     |     | X   |      |

\*Part of routine care. 1. For women of childbearing age. 2. Sub-studies at limited sites 3. India ink or CrAg required for inclusion.

## Laboratory Working Practice Document 3: Sample Processing and Storage

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### ***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)
- $\geq 3$  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 ml each)
- PK samples

*(Please see Lab WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study)*

- qPCR samples

*(Please see WPD 20 – Quantitative PCR sub-study)*

- STOP-CM Immunology samples
- 2 PAXgene tubes (1 for blood, 1 for CSF)

*(Please see WPD 9 - PAXgene Specimen Collection and Storage)*

#### **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)  
Store isolate once grown in 3 Microbank vials (1 for STOP-CM Sub Study).
4. Vortex CSF and save: divide into 3 x 2ml Cryovials - min 200  $\mu$ L each. Label and place into freezer box.
5. PK SAMPLES: Please see WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study
6. qPCR SAMPLES: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub-study
7. Biosynex CryptoPS CrAg: Please see Lab WPD 8 – Cryptococcal semi-quantitative antigen
8. STOP-CM: Store CSF supernatant for Luminex
9. PAXgene tube: Please see WPD 9 - PAXgene Specimen Collection and Storage

*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/or resistance testing (if indicated), whole blood, plasma and buffy coat (all purple tops) and serum (yellow or red top). Total required = 2 yellow or red top (4 ml) and  $\geq 3$  purple tops (4 ml each)

## Laboratory Working Practice Document 3: Sample Processing and Storage

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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.
6. PK SAMPLES: Please see WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study
7. qPCR SAMPLES: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub-study
8. Biosynex CryptoPS CrAg: Please see Lab WPD 8 – Cryptococcal semi-quantitative antigen
9. STOP-CM: Additional whole blood sample (lithium heparin) for zymosan assay
10. STOP-CM: Additional stored aliquot of plasma (lithium heparin) for luminex
11. PAXgene tube: Please see WPD 9 - PAXgene Specimen Collection and Storage

### **24 hours post-dose**

#### ***Required samples:***

- *qPCR blood: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub Study*

### **Day 3**

#### ***Required samples:***

- *1 purple top (for plasma and buffy coat)*
  - *1 yellow/red top tube (4ml)*
  - *qPCR samples*
- (Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR sub-study)*

### **DAY 3 BLOOD SAMPLES**

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

## Laboratory Working Practice Document 3: Sample Processing and Storage

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3. qPCR SAMPLES: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub-study

### 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, 1 for plasma and buffy coat storage)
- 1 yellow/red top tube (4ml)
- CSF sample in 2 x white top tubes (5 ml each)
  
- PK samples  
(Please see Lab WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study)
- qPCR samples (Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR sub-study)
- STOP-CM Immunology samples

#### **DAY 7 CSF SAMPLES**

1. Perform quantitative cultures on Day 7 LP.
2. Save remaining CSF, divide into 3 x 2ml Cryovials.
3. Save Cryptococcus isolate in 2 Microbank vials if growth from Day 7 LP (1 for STOP-CM Sub Study)
4. PK SAMPLES: Please see WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study
5. qPCR SAMPLES: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub-study
6. STOP-CM: Store CSF supernatant for luminex

#### **DAY 7 BLOOD SAMPLES**

1. Perform FBC, ALT, urea, creatinine and electrolytes on Day 7
2. PLASMA AND BUFFY COAT
3. PK SAMPLES: Please see WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study
4. qPCR SAMPLES: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub-study

## Laboratory Working Practice Document 3: Sample Processing and Storage

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5. STOP-CM: Additional whole blood sample (lithium heparin) for zymosan assay
6. STOP-CM: Additional stored aliquot of plasma (lithium heparin) for luminex

### Day 10

#### **Required samples:**

- 1 yellow/red top tube (4ml)

#### **DAY 10 BLOOD SAMPLES**

1. Perform urea, creatinine and electrolytes on Day 10

### Day 12

#### **Required samples:**

- 1 yellow/red top tube (4ml)

#### **DAY 12 BLOOD SAMPLES**

1. Perform urea, creatinine and electrolytes on Day 12

### Day 14

#### **Required samples:**

- 2 purple top (1 for FBC, 1 for plasma and buffy coat storage)
- 1 yellow/red top tube (4ml)
- CSF sample in 2 x white top tube (5 ml each)
- PK samples  
(Please see Lab WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study)
- qPCR samples (including India ink)  
(Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR sub-study)
- STOP-CM: Immunology samples

#### **DAY 14 CSF SAMPLES**

1. Perform quantitative cultures on Day 14 LP. Save remaining CSF, divide into 2 Cryovials.
2. Save Cryptococcus isolate in 2 Microbank vial if growth from Day 14 LP (1 for STOP-CM Sub Study)
3. PK SAMPLES: Please see WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study
4. qPCR & India ink SAMPLES: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub-study for qPCR sample processing and storage and for India ink realization – also see Lab WPD6 – India ink
5. STOP-CM: Store CSF supernatant for luminex



# Laboratory Working Practice Document 3: Sample Processing and Storage

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## DAY 14 BLOOD SAMPLES

1. Perform FBC, ALT, urea, creatinine and electrolytes on Day 14
2. PLASMA AND BUFFY COAT
3. PK SAMPLES: Please see WPD 5 – PK sampling & WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study
4. qPCR SAMPLES: Please see Lab WPD7 & Clinical WPD 20 – Quantitative PCR Sub-study
5. STOP-CM: Additional whole blood sample (lithium heparin) for zymosan assay
6. STOP-CM: Additional stored aliquot of plasma (lithium heparin) for luminex

## 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

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## **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 on 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.

# Laboratory Working Practice Document 3: Sample Processing and Storage

## **Section D - Summary of samples to be saved**

- Day 1:** Serum (stored in 2 Cryovials)  
Plasma (divided into 2 Cryovials)  
Buffy coat (stored in 1 Cryovial)  
Whole blood (divided into 2 cryovials)  
CSF (divided into 3 Cryovials)  
Crypto isolate (in 3 Microbank vials)  
qPCR whole blood (stored in 1 ceramic beaded tube)  
qPCR plasma (stored in 1 eppendorf tube)  
qPCR CSF supernatant (stored in 1 cryovial)  
qPCR CSF pellet (stored in 1 cryovial)  
PK plasma and CSF  
STOP-CM: Immunology whole blood, plasma and CSF  
Blood PAXgene tube  
CSF PAXgene tube
- 24HR:** qPCR whole blood (stored in 1 ceramic beaded tube)  
qPCR plasma (stored in 1 eppendorf tube)
- Day 3:** Plasma (divided into 2 Cryovials)  
Buffy coat (stored in Cryovial)  
qPCR whole blood (stored in 1 ceramic beaded tube)  
qPCR plasma (stored in 1 eppendorf tube)
- Day 7:** Plasma (divided into 2 Cryovials)  
Buffy coat (stored in 1 Cryovial)  
CSF (divided into 3 Cryovials)  
Crypto isolate (in 2 Microbank vials – if growth from day 7 LP)  
qPCR whole blood (stored in 1 ceramic beaded tube)  
qPCR plasma (stored in 1 eppendorf tube)  
qPCR CSF supernatant (stored in 1 cryovial)  
qPCR CSF pellet (stored in 1 cryovial)  
PK plasma and CSF  
STOP-CM: Immunology whole blood, plasma and CSF
- Day 14:** Plasma (divided into 2 Cryovials)  
Buffy coat (stored in 1 cryovial)  
CSF (divided in 3 Cryovials)  
Crypto isolate (in 2 Microbank vial (if growth from Day 14 LP)  
qPCR whole blood (stored in 1 ceramic beaded tube)  
qPCR plasma (stored in 1 eppendorf tube)

## Laboratory Working Practice Document 3: Sample Processing and Storage

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qPCR CSF supernatant (stored in 1 cryovial)  
qPCR CSF pellet (stored in 1 cryovial)  
PK plasma and CSF  
STOP-CM: Immunology whole blood, plasma and CSF

**On D1, D3, D7 and D14 if you are participating in the PK/PD, qPCR or semi-quantitative CrAg sub-studies please refer to the corresponding WPDs.**

## APPENDIX

### Lab Processing Chart: Blantyre

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

Protocol Version: 2.1 07/11/2017

LPC Version: 1.1: 01/11/2018

Revised/Updated:

Revised/Updated: Version 1.2 10/09/2019 : addition of PAXgene specimen collection and storage at D1

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## Laboratory Working Practice Document 3: Sample Processing and Storage

Table 1: Specimen Log – Blood

| Evaluation           | Specimen                         | Tests   | Special Notes   |
|----------------------|----------------------------------|---|---|
| Haematology          | EDTA                             | FBC   | All patients, when applicable   |
| Haematology          | 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.  |
| Virology             | EDTA                             | HIV Viral load  | Should be performed if it has not been done within 3 months or if there is reason to suspect it is required.  |
| Virology             | EDTA                             | HIV 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. |
| Chemistry            | Yellow/Red Top Tube              | ALT, Creatinine, Electrolytes                                       | All patients, when applicable   |
| Whole Blood          | EDTA                             | For semi-quantitative CrAg and for further analysis and sub-studies | Storage at -80°C until shipment   |
| Whole Blood          | Lithium heparin tube             | For zymosan assay (STOP-CM: Immunology)                             | Processed at MLW in real time   |
| Plasma               | EDTA                             | For further analysis and sub-studies                                | Storage at -80°C until shipment   |
| Plasma               | Lithium heparin tube (green top) | For luminex (STOP-CM: Immunology)                                   | Storage at -80°C until analysis at MLW  |
| Plasma for PK        | Lithium Heparin tube (green top) | PK analysis in both study arms                                      | Storage at -80°C until shipment   |
| Serum                | Yellow/Red Top Tube              | For further analysis and sub-studies                                | Storage at -80°C until shipment   |
| Whole blood for qPCR | EDTA                             | For qPCR sub-study in blood   | Storage at -80°C until shipment   |
| Blood PAXgene        | PAXgene tube                     | For PAXgene tube sub-study  | Storage immediately at 18-25°C, then at -20°C or at -80°C   |


  
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Table 2: Specimen Log – CSF

|                    |              |  |   |
|--------------------|--------------|--|---|
| CSF Chemistry      | CSF tube     | Protein, Glucose   | All patients, when applicable                               |
| CSF Microbiology   | CSF tube     | India Ink, CrAg LFA, Gram stain, Cell count and differentiation, Routine culture (for D1 CSF and if clinically indicated thereafter) and semi-quantitative CrAg<br>Quantitative cryptococcal culture (for all CSF) | Store isolate and any unused sample at -80°C until shipment |
| CSF for PK         | CSF tube     | PK analysis in both study arms   | Storage at -80°C until shipment                             |
| CSF for qPCR       | CSF tube     | For qPCR sub-study in CSF  | Storage at -80°C until shipment                             |
| CSF for immunology | CSF tube     | For luminex (STOP-CM: Immunology)  | Storage at -80°C until analysis at MLW                      |
| CSF PAXgene        | PAXgene tube | For PAXgene tube sub-study   | Storage immediately at 18-25°C, then at -20°C or at -80°C   |

**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  |
|---|---------------|--|---|--|
| <b>A number of samples on Day 1 are routine tests which may be handled by other laboratories</b><br><b>*Store any unused samples*      *Aim for a <u>minimum</u> of 5ml CSF from each LP*</b> |               |  |   |  |
| 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, semi-quantitative CrAg (Biosynex CryptoPS) | 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<br><br>STOP CM: Store isolate in one additional Microbank vial |
|   |               | Study Tube 2: Sample for storage   | General CSF storage:<br>Unspun sample<br><br>qPCR sub-study: centrifuge CSF at 2000g for 10 min and store supernatant and pellet in different cryovials at -80°C<br><br>PK sub-study: Centrifuge and divide CSF into 2 x 2mL cryovials (min. 500µl each)<br><br>STOP-CM study:<br>1. 2 x 1ml unspun sample for RNA sequencing<br>2. 1 x 750ml supernatant for luminex | Store at -80°C<br><br><br><br><br><br><br><br><br><br>Store at -80°C<br><br><br>Store -80°C                                    |



## Laboratory Working Practice Document 3: Sample Processing and Storage

|  |                  |  |  |  |
|--|------------------|--|--|--|
|  |                  |  | 3. Use sediment to subculture and store isolates for phenotypic and genotypic analyses   |  |
|  | CSF PAXgene tube | Immediately after adding CSF, gently invert the PAXgene tube 8-10 times.<br><br>Samples do not require any processing. | Immediately after collecting specimens, store the PAXgene tube at 18-25°C for a minimum of 2 hours and a maximum of 72 hours.<br><br>The PAXgene tubes can then be stored at -20°C or below. If you would prefer to store at -80°C then it will be necessary to first store at -20°C for at least 24 hours before transferring to the deep freeze. | When storing in the freezer the preference is to store on a wire rack: do not freeze tubes upright in a Styrofoam tray as this may cause the tubes to crack. If you cannot store on a wire rack then keep the tubes in a freezer bag and store them in a safe, secure position in the freezer. |
|  | EDTA (4ml)       | Optional Routine Tube 1 and 2: CD4 and/or VL/Resistance Test (one tube for each test)                                  |  |  |
|  |                  | Monitoring Tube 1: FBC   | After FBC, spin at 800g for 15min and store remaining plasma as below  | If being performed in research laboratory.   |
|  |                  | Study Tube 1: Whole blood<br>Perform semi-quantitative CrAg (Biosynex CryptoPS)  | Invert 8-10 times and pipette into 2x2ml cryovials (min. 900µl each) and store at -80°C  |  |
|  |                  | Study Tube 2: Spin tube at 800g for 15min and remove plasma  | Divide plasma into 2x2ml cryovials. Remove buffy coat and put into 1 x 2ml cryovial  | Store at -80°C   |
|  |                  | Study Tube 3: qPCR sub-study   | Remove 1.2ml and store in 2ml cryovial containing ceramic beads; centrifuge remaining 3.8ml at 2000g for 10min and store plasma in 1.5ml Eppendorf tube  | Store at -80°C   |

## Laboratory Working Practice Document 3: Sample Processing and Storage

|                 |                               |  |  |  |
|-----------------|-------------------------------|--|--|--|
|                 | Yellow/Red Top Tube (4ml) x 2 | Monitoring Tube 1: ALT, urea, creatinine, and electrolytes   | Spin and store remaining serum as below.   | If being performed in research laboratory.   |
|                 |                               | Study Tube 1: Spin tube at 800g for 15min and remove serum   | Divide serum into 2x2ml cryovials and store at -80°C   |  |
|                 | Lithium Heparin (4ml) x 5     | Study Tubes: Spin tubes at 3500rpm/800g for 15min and remove plasma  | For the first sample: divide plasma into 3x2ml cryovials (1* minimum 750mcl for luminex, 2* minimum 500mcl for PK). For each subsequent blood sample, divide plasma into 2x2ml cryovials (minimum 500mcl, for PK).   | PK tubes are collected at 5 different time points: within 1 h of t2, t4, t7, t12, t23<br>Store at -80°C  |
|                 | Lithium Heparin               | 1ml whole blood for zymosan assay  | Assay done at MLW in real time, immunology lab   | No storage   |
|                 | Blood PAXgene tube            | Immediately after adding the blood, gently invert the PAXgene tube 8-10 times.<br><br>Samples do not require any processing. | Immediately after collecting specimens, store the PAXgene tube at 18-25°C for a minimum of 2 hours and a maximum of 72 hours.<br><br>The PAXgene tubes can then be stored at -20°C or below. If you would prefer to store at -80°C then it will be necessary to first store at -20°C for at least 24 hours before transferring to the deep freeze. | When storing in the freezer the preference is to store on a wire rack: do not freeze tubes upright in a Styrofoam tray as this may cause the tubes to crack. If you cannot store on a wire rack then keep the tubes in a freezer bag and store them in a safe, secure position in the freezer. |
| 24hrs post-dose | EDTA (4ml) x 1                | Study Tube 1: qPCR sub-study   | Remove 1.2ml and store in 2ml cryovial containing ceramic beads; centrifuge remaining 3.8ml at 2000g for 10min and store plasma in 1.5ml Eppendorf tube  | Store at -80°C   |
| Day 3           | Yellow/Red Top Tube (4ml)     | Monitoring Tube 1: Urea, creatinine and electrolytes   | Spin and store remaining serum.  | If being performed in research laboratory.   |

## Laboratory Working Practice Document 3: Sample Processing and Storage

|       |                           |   |  |  |
|-------|---------------------------|---|--|--|
|       | EDTA (4ml) x 2            | Study Tube 1: Spin tube at 800 <sup>x</sup> 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   |
|       |                           | Study Tube 2: qPCR sub-study  | Remove 1.2ml and store in 2ml cryovial containing ceramic beads; centrifuge remaining 3.8ml at 2000g for 10min and store plasma in 1.5ml Eppendorf tube  |  |
| Day 5 | Yellow/Red Top Tube (4ml) | Monitoring Tube 1: Urea, creatinine and electrolytes                      | Spin and store remaining serum.  | If being performed in research laboratory.   |
| 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<br><br>STOP CM: Store isolate in one additional Microbank vial |
|       |                           | Study Tube 2: Sample for storage  | General CSF storage:<br>Unspun sample<br><br>qPCR sub-study: centrifuge CSF at 2000g for 10min and store supernatant and pellet in different cryovials at -80°C<br><br>PK sub-study: Centrifuge and divide CSF into 2 x 2mL cryovials (min. 500µl each)<br><br>STOP-CM study:<br>1 x 750ml supernatant for luminex | Store at -80°C   |


## Laboratory Working Practice Document 3:

### Sample Processing and Storage

|        |                           |   |  |  |
|--------|---------------------------|---|--|--|
|        |                           |   | Use sediment to subculture and store isolates for phenotypic and genotypic analyses  |  |
|        | EDTA (4ml) x 3            | Monitoring Tube 1: FBC  | After FBC, spin at 800g for 15min and store remaining plasma as below  | If being performed in research laboratory.   |
|        |                           | Tube 2: Spin tube at 3500rpm/ 800g for 15min and remove plasma        | Divide plasma into 2x2ml cryovials. Remove buffy coat and put into 1 x 2ml cryovial  | Store at -80°C   |
|        |                           | Tube 2: qPCR sub-study  | Remove 1.2ml and store in 2ml cryovial containing ceramic beads; centrifuge remaining 3.8ml at 2000g for 10min and store plasma in 1.5ml Eppendorf tube  |  |
|        | Yellow/Red Top Tube (4ml) | Monitoring Tube 1: ALT, urea, creatinine, and electrolytes            | Spin and store remaining serum.  | If being performed in research laboratory.   |
|        | Lithium Heparin (4ml) x 6 | Study Tubes: Spin tubes at 3500rpm/800g for 15min and remove plasma   | For the first sample: divide plasma into 3x2ml cryovials (1* minimum 750mcl for luminex, 2* minimum 500mcl for PK). For each subsequent blood sample, divide plasma into 2x2ml cryovials (minimum 500mcl, for PK). | PK tubes are collected at 6 different time points; t0 and then within 1h of: t2, t4, t7, t12, t22<br>Store at -80°C            |
|        | Lithium Heparin           | 1ml whole blood for zymosan assay                                     | Assay done at MLW in real time, immunology lab   | No storage   |
| Day 10 | Yellow/Red Top Tube (4ml) | Urea, creatinine and electrolytes                                     | Spin and store remaining serum.  | If being performed in research laboratory.   |
| Day 12 | Yellow/Red Top Tube (4ml) | Monitoring Tube 1: Urea, creatinine and electrolytes                  | Spin and store remaining serum.  | If being performed in research laboratory.   |
| 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<br><br>STOP CM: Store isolate in one additional Microbank vial |
|        |                           | Study Tube 2: Sample for storage                                      | General CSF storage:<br>Unspun sample  | Store at -80°C   |

## Laboratory Working Practice Document 3: Sample Processing and Storage

|        |                           |   |  |  |
|--------|---------------------------|---|--|--|
|        |                           |   | <p>qPCR sub-study: centrifuge CSF at 2000g for 10min. Store supernatant in cryovials at -80°C<br/>Use 20µl of the pellet to perform India ink<br/>Store the remaining pellet in a different cryovial at -80°C</p> <p>PK sub-study: Centrifuge and divide CSF into 2 x 2mL cryovials (min. 500µl each)</p> <p>STOP-CM study:<br/>1 x 750ml supernatant for luminex</p> <p>Use sediment to subculture and store isolates for phenotypic and genotypic analyses</p> |  |
|        | EDTA (4ml) x 3            | Monitoring Tube 1: FBC  | After FBC, spin at 800 <sup>x</sup> g for 15min and store remaining plasma as below  | If being performed in research laboratory. |
|        |                           | Study Tube 1: Spin tube at 800 <sup>x</sup> 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                             |
|        |                           | Study Tube 2: qPCR sub-study  | Remove 1.2ml and store in 2ml cryovial containing ceramic beads; centrifuge remaining 3.8ml at 2000g for 10min and store plasma in 1.5ml Eppendorf tube  |  |
|        | Yellow/Red Top Tube (4ml) | Monitoring Tube 1: ALT, urea, creatinine, and electrolytes                | Spin and store remaining serum.  | If being performed in research laboratory. |
|        | Lithium Heparin           | 1ml whole blood for zymosan assay   | Assay done at MLW in real time, immunology lab   | No storage                                 |
| Day 28 | EDTA (4ml)                | Monitoring Tube 1: FBC  | After FBC, spin at 800 <sup>x</sup> g for 15min and store remaining plasma at -80°C  | If being performed in research laboratory. |
|        | Yellow/Red Top Tube (4ml) | Monitoring Tube 1: ALT, urea, creatinine, and electrolytes                | Spin and store remaining serum at -80°C.   | If being performed in research laboratory. |


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AMBIsome Therapy Induction OptimizatiON

## Laboratory Working Practice Document 3: Sample Processing and Storage

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Table 4: Shipments

| Specimen                    | Destination | Shipment Date | Special Notes |
|-----------------------------|-------------|---------------|---------------|
| CSF                         |             |               |               |
| CSF – PK/PD Study           |             |               |               |
| Crypto Isolates             |             |               |               |
| STOP CM Isolates            |             |               |               |
| qPCR CSF                    |             |               |               |
| qPCR bloods                 |             |               |               |
| PK Plasma                   |             |               |               |
| Serum                       |             |               |               |
| Whole Blood                 |             |               |               |
| CSF and Blood PAXgene tubes |             |               |               |

## Laboratory Working Practice Document 3: Sample Processing and Storage

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### 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 |
|------|---------------------|-----------|
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