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AMBITION-*cm*
AMBIsome Therapy Induction OptimizatioN

# Laboratory Work Practice Document: 7
# Quantitative Cryptococcal PCR sub-study
# Sample Processing and Storage

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|---|---|---|---|
| **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 | | |
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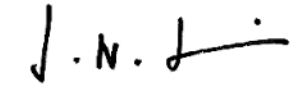
| **Reviewer(s)** | David Lawrence<br>Lead Clinician |  | 22/08/2018 |
| **Approved by** | Joseph Jarvis<br>CI |  | 22/08/2018 |

| Revision History: | | |
|---|---|---|
| Version Number | Effective Date | Reason for Change |
| 1.0 | 21/09/2017 | First version |
| 1.1 | 14/06/2018 | Blood tubes storage details and CSF volume |
| 1.2 | 22/08/2018 | Addition of India ink at Day 14 |
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# Laboratory Working Practice Document 7: qPCR Sample Processing and Storage

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**Purpose**

This document describes the processing and storage of qPCR sub-study blood and CSF samples. These samples will be analysed at the Institut Pasteur in Paris, France.

**References**

Ambition Trial Protocol

**Materials**

- Lab WPD 1 – Laboratory equipment
- Lab WPD 2 – Sample Processing and Storage
- Lab WPD 6 – India ink
- Clinical WPD 20 – Quantitative Cryptococcal PCR Sub-study

***Section A1 – qPCR blood sample procedure***

- Blood samples will be taken for qPCR analysis on study days 1, 2, 3, 7 and 14.
- Timings of bloods are as follow: D1 – pre-dose, 24 hours post-dose, D3, D7, D14.
- Blood should be drawn directly into a single 5 mL purple-top EDTA vacutainer tube or similar. The tube must be inverted several times to ensure it is well mixed.

***Section A2 – qPCR blood sample processing***

1. Take 1.2 ml of blood and put it in a 2ml tube with 0.1 mm silica beads (Ref: MP Biomedicals® Lysing Matrix B Tubes, Ref: 116911500) and freeze the tube at -80°C [fungemia related-DNA or RNA]
2. Centrifuge the initial tube containing 3.8 ml of blood at 2000g, 10min
3. Collect 1.5 ml of plasma in an Eppendorf tube and freeze it at -80°C [circulating RNA+DNA + CrAg screening]

If the sample received is less than 5ml, priority is to store minimum 1ml of whole blood in silica beads tube.

***Section B1– qPCR CSF sample procedure***

- CSF samples will be taken for qPCR analysis on study days 1, 7 and 14.
- Timings of CSF are as follow: D1 – pre-dose, D7, D14.
- CSF should be collected in a sterile collection tube e.g. 5ml
- A minimum volume of 1ml of CSF is needed at Day 1 and Day 7; and a minimum volume of 1.5ml is needed on Day 14.

# Laboratory Working Practice Document 7: qPCR Sample Processing and Storage

### *Section B2 – D1 and D7 qPCR CSF sample processing*

1. Note the volume of CSF collected
2. Centrifuge the CSF (2000g)
3. Carefully remove the liquid supernatant from the tube, taking care not to disturb the sediment at the bottom of the tube
4. Freeze supernatant at -80°C [free RNA+DNA and CrAg screening]
5. Freeze the pellet at -80°C [CSF fungal load DNA or RNA+DNA]

### *Section B2 – D14 qPCR CSF sample processing in BLANTYRE*

1. Note the volume of CSF collected
2. Centrifuge the CSF (2000g)
3. Carefully remove the liquid supernatant from the tube, taking care not to disturb the sediment at the bottom of the tube
4. Freeze supernatant at -80°C [free RNA+DNA and CrAg screening]
5. Use 20µl of the pellet to perform India ink – according to Lab WPD 6
6. Freeze the remaining pellet at -80°C [CSF fungal load DNA or RNA+DNA]

### *Section B2 – D14 qPCR CSF sample processing in GABORONE*

1. Centrifuge a tube of 500 µl of CSF (2000g) for India ink
2. Note the remaining volume of CSF collected and also centrifuge the CSF (2000g)
3. Carefully remove the liquid supernatant from each tube, taking care not to disturb the sediment at the bottom of the tube
4. Freeze the pellet of the 500µl tube of CSF at -80°C for India ink
5. Freeze supernatant of the remaining volume at -80°C [free RNA+DNA and CrAg screening]
6. Freeze the pellet of the remaining volume at -80°C [CSF fungal load DNA or RNA+DNA]

# Laboratory Working Practice Document 7: qPCR Sample Processing and Storage

**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 7: qPCR 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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