



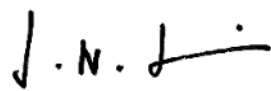


Laboratory Work Practice Document: 5

Pharmacokinetics and Pharmacodynamics 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		
ISRCTN No.:	ISRCTN72509687		
WPD Current version	Version 1.0 02/08/2017		
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Revision History:		
Version Number	Effective Date	Reason for Change
1.0		First version

Laboratory Working Practice Document 5: PK/PD Sample Processing and Storage

Purpose

This document describes the processing and storage of pharmacokinetic blood samples

References

Ambition Trial Protocol

Materials

Lab WPD 1 – Laboratory equipment

Lab WPD 2 – Sample Processing and Storage

Clinical WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study

Section A – PK/PD blood sample procedure

- Blood samples will be taken for PK/PD analysis on study days 1 and 7 in L-AmB arm patients.
 - Please see “WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study” for details.
 - Blood should be drawn directly into a single 6 mL green-top lithium heparin vacutainer tube or similar. The tube must be inverted several times to ensure it is well mixed.
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Section B – PK/PD blood sample processing

1. Separate the plasma from the clotted blood by centrifugation of the sample at 3500rpm for 10 minutes at room temperature.
 2. Centrifugation causes the clotted blood to move to the bottom of the tube, leaving the straw coloured plasma at the top.
 3. For each 6 ml tube the plasma should be carefully removed, taking care not to disturb the red cell interface and divided into 4x2.0 mL cryovials a minimum of 0.2ml per tube.
 4. The remaining blood clot should be discarded.
 5. The tubes containing plasma samples should be fastened securely and clearly labelled using non-water soluble black ink/printed label: Tubes should be labelled with:
 - a. Sample type (PK/PD Plasma)
 - b. Study number
 - c. Patient initials
 - d. Date of sample collection
 - e. Time point (t0, t2, t4, t7, t11, t22)
 - f. Time of sample collection (24 hour notation HH:MM)
 6. Samples should be labelled and transferred to an appropriately labelled PK/PD cryobox. Cryobox should immediately be put in a -80°C freezer until shipment.
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Section C – PK/PD CSF sample procedure

- CSF samples will be taken for PK/PD analysis on study days 1, 7 and 14 in L-AmB arm patients.
- Please see “WPD 19 – Pharmacokinetics and Pharmacodynamics Sub-study” for details.
- CSF should be collected in a sterile collection tube e.g. 5ml

Section D – PK/PD CSF sample processing

1. Spin the CSF samples in collection tubes at 3500rpm for 5 minutes at room temperature
2. Carefully remove the liquid supernatant from the tube, taking care not to disturb the sediment at the bottom of the tube
3. Transfer the supernatant into 2.0mL cryovials with at least 0.5mL per tube
4. Reserve the sediment for subpopulation studies, see SOP 2.5
5. The tubes containing CSF supernatant should be fastened securely and clearly labelled using non-water soluble black ink/printed label. Tubes should be labelled with:
 - a. Sample type (PK CSF)
 - b. Study number
 - c. Patient initials
 - d. Date of sample collection (DD/MM/YY)
 - e. Time of sample in relation to fluconazole dose (“pre” or “post”)
 - f. Time of sample collection (24 hour notation HH:MM)
6. Samples should be labelled and transferred to an appropriately labelled PK/PD cryobox. Cryobox should immediately be put in a -80°C freezer until shipment.

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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.

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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