Childhood Acute Illness & Nutrition Network

KEMRI | Wellcome Trust Clinical Trials

Pancreatic Enzymes and Bile Acids: A Non-Antibiotic approach to Treat Intestinal Dysbiosis in Acutely Ill Severely Malnourished Children

Study Specific Procedure			**SSP No:** LA02 **Version No:** 2.0 **Supersedes:** 1.0 **Effective Date:** 13th April 2022
Title: Blood Processing			
	NAME	**SIGNATURE**	**DATE**
PREPARER	Robert Musyimi		**16th March 2022**
Q.A. AUTHORITY	Aisha Bwika		**12th April 2022**
APPROVING AUTHORITY	Robert Bandsma		**13th April 2022**

1.0 PURPOSE / INTRODUCTION:

The purpose of this SSP is to describe the standard procedures involved in processing and storing of study blood (EDTA for Plasma separation) sample after the sample has been delivered to the laboratory.

2.0 SCOPE / RESPONSIBILITY:

This SSP applies to any study laboratory staff. It is the responsibility of those users to follow the guidelines stipulated herein.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The study laboratory coordinator is responsible for answering questions you may have about the content of this SSP and any other relevant study documentation. Please contact the study laboratory coordinator through your site coordinator. Main CHAIN PB-SAM laboratory coordinator: Caroline Tigoi (email: ctigoi@kemri-wellcome.org) or (rmusyimi@kemri-wellcome.org).

3.0 DEFINITIONS /ABBREVIATIONS:

3.1 PI	Principal Investigator
3.2 EDTA	Ethylene Diamine Tetra Acetic Acid
3.3 CRF	Case Record Form
3.4 SOP	Standard Operating Procedure
3.5 RPM	Revolutions per Minute
3.6 SSP	**Study Specific Procedure**

4.0 MATERIALS

4.1 EDTA purple tops (3 mls)

4.2 Sample Storage vials – **Nunc 1.8 ml cryotubes**

4.3 Pipettes 1ml

4.4 Pipette tips 1ml tips

4.5 -80^{0}C freezer

4.6 Temperature controlled centrifuge machine

4.7 Nalgene cryoboxes system 100 (10 x 10 boxes)

4.8 DNAse/RNAse free filter 300µl tips

5.0 METHODOLOGY:

5.1 General considerations

5.1.1 Samples collected from patients in this study will be for study-specific analyses.

5.1.2 Correct specimen collection bottles and correct request forms must always be used and verified at each collection.

5.1.3 Ensure all samples should be labelled with the Country code, site code, collection time-point code, (see Site Specific Collection Schedule (appendix 7.2), specimen type, patient, and date of collection. For example: 10-A0-P1-XXX-12/10/2014.For sample type, P= plasma from EDTA tube.

5.1.4 Keep samples on ice, with ice packs at all times.

5.1.5 For EDTA Plasma blood, make 4 aliquots of plasma for storage.

5.1.6 If limited amount of sample is collected, P1 and P2 have priority.

5.1.7 There should be a minimum of 300 µl of sample per aliquot before introducing a new aliquot. For example, if there is 500µl of EDTA plasma, put 300 µl in P1 and 200 µl in P2. If sufficient sample divide into two even aliquots.

5.1.8 Store each aliquot in separate 2-inch-high Nalgene system 100 plastic freezer boxes. The idea is that sample aliquots go to specific analytic sites for the specific analyses and are separated at this stage to facilitate an efficient pre-transportation process.

5.1.9 Each freezer box should be labeled on the top and on the side. The label should contain a unique number letter combination (see sample freezer box storage log – Appendix 7.4).

5.1.10 Purposes of the samples are for later investigation on biochemistry, immune and metabolic markers.

5.1.11 Gloves must always be worn when handling specimens. This includes during removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of any spills. Tubes, needles, and pipets must be properly disposed of in biohazard containers, in accordance with institutional requirements.

5.1.12 The time between arrival at the laboratory and freezing at -80^0 C freezer should be maximally 60 minutes. This will be monitored very closely for every site and any deviation on sample transportation and processing time will be communicated. Prolonged delays of sample storage will compromise the integrity of the sample

affecting the quality of lab results and will not be included in data analysis. Temporary storage at -20^{0}C is not allowed.

5.2 Sample rejection criteria

The following sample rejection criteria will be enforced.

5.2.1 Insufficient of < 300 µl - Reject and notify lab manger and clinical team. Fill in sample rejection form

5.2.2 Clotted blood - Reject and notify lab manger and clinical team. Fill in sample rejection form

5.2.3 Two samples with the same specimen number on tube but different numbers in CRF and the vice versa – Reject, discard and notify lab manger and clinical team. Fill in sample rejection form.

5.3 Sample shipment log and registration

5.3.1 At the laboratory where samples are being processed and divided into aliquots, the Sample shipment log MUST be filled out.

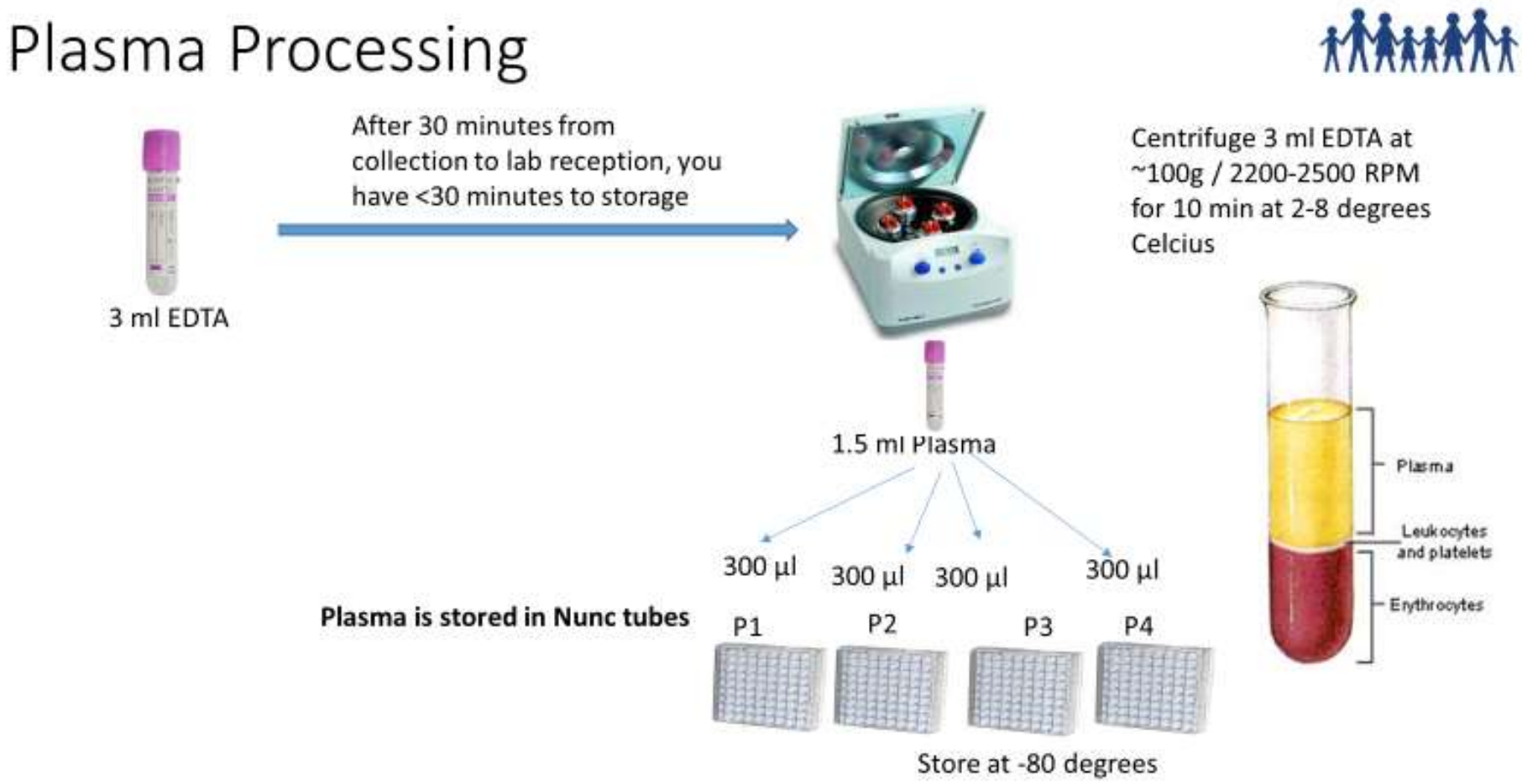
5.3.2 Record time of receiving of sample and freezing of samples on the Sample Shipment Log.

5.3.3 Record in the log if less than the optimal amount of sample is stored (see appendix 7.2) and document the amount of volume stored as specific aliquots.

5.3.4 Hemolysed EDTA sample should be stored, and a comment made in lab CRF

5.3.5 Missing sample - notify lab manager or clinical team

APPENDICES:


Plasma Processing
3 ml EDTA
After 30 minutes from collection to lab reception, you have <30 minutes to storage
Centrifuge 3 ml EDTA at ~100g / 2200-2500 RPM for 10 min at 2-8 degrees Celcius
1.5 ml Plasma
300 µl
300 µl
300 µl
300 µl
P1
P2
P3
P4
Plasma is stored in Nunc tubes
Store at -80 degrees
Plasma
Leukocytes and platelets
Erythrocytes
The Childhood Acute Illness & Nutrition Network

6.0 REFERENCES:

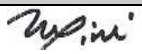
None

7.0 DOCUMENT CHANGE HISTORY

Version Table:

Version 1.0: Title: **Blood Processing**	Dated: **21st October 2021**	SSP No.: **LA02**	No. Pages: **6**
Version 2.0: Title: **Blood Processing**	Dated: **13th April 2022**	SSP No.: **LA02**	No. Pages: **7**
Version Title:	Dated:	SSP No.:	No. Pages:
This document is effective from the date of training/last approval signature and will be reviewed in two years.			

SSP Review and Updating Logs

DATE	NAME OF REVIEWER	SIGNATURE	REASON FOR REVIEW AND CHANGES MADE
17/April /2022	Robert Musyimi		Added appendices on sample processing flow chart

SSP AWARENESS LOG

I, the undersigned below, hereby confirm that I am aware that the accompanying SSP is in existence from the date stated herein and that I shall keep abreast with the current and subsequent SSP versions in fulfillment of Good Clinical Practice (GCP).

Number	Name	Signature	Date (dd/mmm/yyyy)
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