



INMA LABORATORY MANUAL

NUTRITION BIOMARKERS

This manual describes the collection, separation, storage and transport of blood samples for the INMA study. Careful collection of samples is essential to the success of the project, so it is important to follow the protocols exactly every time. Familiarity is assumed with basic laboratory procedures (e.g. how to use a pipette or centrifuge). If any problems are encountered which cannot be resolved locally, please contact the Central Laboratory:

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1. Preparation

To be carried out by INMA Nurse / Midwife for each subject:

Maternal Blood - 12 and 32 weeks gestation (V1 & V2)

- **Laboratory record form** - Complete one at each visit (see Appendix A)
- **Blood tubes** – 2 x 5 ml clotted [red top] and 2 x 5 ml EDTA [purple top].
Label tubes with subject number, visit date and visit type (V1 or V2, where V2 = 12 weeks gestation and V2 = 32 weeks gestation).
- **Sample storage tubes** – Label eight 2 ml sample storage tubes with pre-printed laboratory labels. (These are special labels designed to withstand long-term storage at low temperatures and should not be used for other purposes).

The subject number, visit date and visit type, should be written on each of these tubes using an indelible marker. Place sample storage tubes into a plastic bag.

Cord Blood - Delivery (V3)

- **Laboratory record form** - Complete one form for visit (see Appendix A)
- **Blood tubes** – 1 x 5 ml EDTA [purple top]. Label tube with subject number, visit date and visit type (V3, where V3 = Delivery).
- **Sample storage tubes** – Label two 2 ml sample storage tubes (These are special labels designed to withstand long-term storage at low temperatures and should not be used for other purposes).

The subject number, visit date and visit type, should be written on each of these tubes using an indelible marker. Place sample storage tubes into a plastic bag.

2. Blood collection

See Appendix B for Blood Collection Scheme

Maternal Blood - 12 and 32 weeks gestation (V1 & V2)

Peripheral venous blood samples are to be collected after subjects have been sitting quietly for five minutes. Subjects will be fasting.

Fill 4 bottles in the following order:

- a) 5 ml clotted (red top)
- b) 5ml EDTA (purple top)
- c) 5 ml clotted (red top)
- d) 5ml EDTA (purple top)

Cord Blood - Delivery (V3)

Cord blood is to be collected from the umbilical vein within 10 minutes of placental delivery:

- 5ml EDTA (purple top)

2.1 Sample handling

Maternal Blood - 12 and 32 weeks gestation (V1 & V2)

All samples must be separated within four hours of collection by centrifugation at approximately 3000rpm for 15 minutes.

The exception is the second EDTA tube for which centrifugation is not required.

After collection the samples are handled as described below:

- 10 ml clotted (red top x 2) – store at room temperature in the dark (e.g. in a closed envelope) for at least one hour before centrifugation to allow adequate blood clotting
- 10 ml EDTA (purple top x 2) –
 - Tube 1: store at 4°C (e.g. in fridge or on ice) before centrifugation.
 - Tube 2: store at room temperature (no centrifugation required).

Cord Blood - Delivery (V3)

All samples must be separated within four hours of collection by centrifugation at approximately 3000rpm for 15 minutes.

After collection the samples are handled as described below:

- 5 ml EDTA (purple top x 1) – store at 4°C (e.g. in fridge or on ice) before centrifugation

2.2 Separation of blood

See Appendix D for Sample Processing Scheme

After centrifugation, at approximately 3000rpm for 15 minutes, the samples are handled as follows:

Maternal Blood - 12 and 32 weeks gestation (V1 & V2)

- 10 ml clotted (2 x 5ml red top) – transfer serum into four 2 ml storage tubes (minimum of 0.5 ml to each) using a plastic transfer pipette. Screw **red** caps on storage tubes and write the letter “**S**” (for “serum”) on the side of the storage tube using an indelible marker.
- 5 ml EDTA (purple top): Tube 1
 - Transfer exactly 100 µl of plasma to each of two 2 ml storage tubes using a Gilson graduated pipette (or similar).
Add exactly 900 µl of 5% metaphosphoric acid (see Appendix C) to each tube using the Gilson graduated pipette.
Screw **yellow** caps on storage tubes and mix well by gentle inversion without shaking.
The contents will appear slightly milky.
Write the letter “**V**” (for vitamin) on the side of the storage tube using an indelible marker.

b) Transfer exactly 100 µl of plasma to a 2 ml storage tube using a Gilson graduated pipette (or similar).

Screw a **blue** cap on the storage tube and mix well by gentle inversion without shaking. Write the letter "**AC**" (for antioxidant capacity) on the side of the storage tube using an indelible marker.

c) Transfer the remaining plasma to two 2 ml storage tubes (minimum of 0.5 ml to each) using a plastic transfer pipette. Screw **purple** caps on storage tubes and write the letter "**E**" (for EDTA) on the side of the storage tube using an indelible marker.

▪ 5 ml EDTA (purple top): Tube 2

This tube should not have been centrifuged and should therefore contain whole blood.

At the end of this stage, you should have a total of 10 tubes for each subject:

9 x 2ml storage tubes and one uncentrifuged EDTA blood tube.

- 4 serum tubes (red top)
- 2 vitamin C tubes (yellow top)
- 1 total antioxidant capacity tube (blue top)
- 2 EDTA plasma tubes (purple top)
- 1 uncentrifuged 5 ml EDTA blood tube

If insufficient blood has been collected, follow the protocol as far as possible and note which tubes are missing on the laboratory record sheet.

Cord Blood - Delivery (V3)

- 5 ml EDTA (purple top) – transfer plasma into two 2 ml storage tubes (minimum of 0.5 ml to each) using a plastic transfer pipette. Screw **brown** caps on storage tubes and write the letters "**CE**" (for "cord EDTA") on the side of the storage tube using an indelible marker.

3. Storage of samples

All labelled tubes (9 storage tubes and one 5 ml EDTA blood tube at V1 and V2 **or** 2 storage tubes at V3) from a single subject are to be placed in a single plastic bag and sealed. The plastic bag is then labelled, using an indelible marker, with the subject number, Visit type, date of collection. The bag is transferred to a freezer for storage (-70°C or -20°C if this is not available).

4. Transport to Central Laboratory

Every three months, contact the Nutrition Laboratory in the UMH and the Central Laboratory in Belfast and arrange for the samples to be collected and transported to the reference Laboratories. Along with the samples please send:

1) A list of subject numbers

2) A copy of the laboratory record sheet (appendix A) for each subject.

Please also retain a copy of this locally.

Laboratory record sheet

Subject number:

Date of assessment:

Visit:

(Tick appropriate answer)

- V1 – 12 weeks gestation
- V2 – 32 weeks gestation
- V3 – Delivery – cord blood

Time of blood sample:

Time of centrifugation:

V1 / V2:

All 4 blood bottles filled? Yes No Not applicable
(If no, indicate how much blood obtained)

V3:

Blood bottle filled? Yes No Not applicable
(If no, indicate how much blood obtained)

Freezer:

(Tick appropriate answer –

only store at -20°C when a

-70°C freezer is not available)

 -70°C -20°C

INMA SAMPLE COLLECTION SCHEME

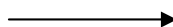
Label all tubes with the subject number, visit date and visit type [V1, V2, V3]
(where, V1 = 12 weeks; V2 = 32 weeks, V3 = Delivery - cord blood)

Maternal Blood - 12 and 32 weeks gestation (V1 & V2)

- Peripheral venous blood
- Fasting subjects
- At rest for 5 minutes
- Collect / Fill tubes in order 1 – 4 (tube colour may vary depending on manufacturer):



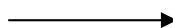
- 1) 5ml Clotted sample
(red top)



Store at room temperature in the dark for at least 1 hour prior to centrifugation to allow clotting



- 2) 5ml EDTA sample
(purple top)



Mix by inversion
Store at 4°C (in fridge or on ice) prior to centrifugation



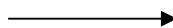
- 3) 5ml Clotted sample
(red top)



Store at room temperature in the dark for at least 1 hour prior to centrifugation to allow clotting



- 4) 5ml EDTA sample
(purple top)

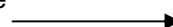


Mix by inversion
Store at room temperature
No centrifugation required

Cord Blood - Delivery (V3)



- 1) 5ml EDTA sample
(purple top)



Mix by inversion
Store at 4°C (in fridge or on ice) prior to centrifugation

Appendix C

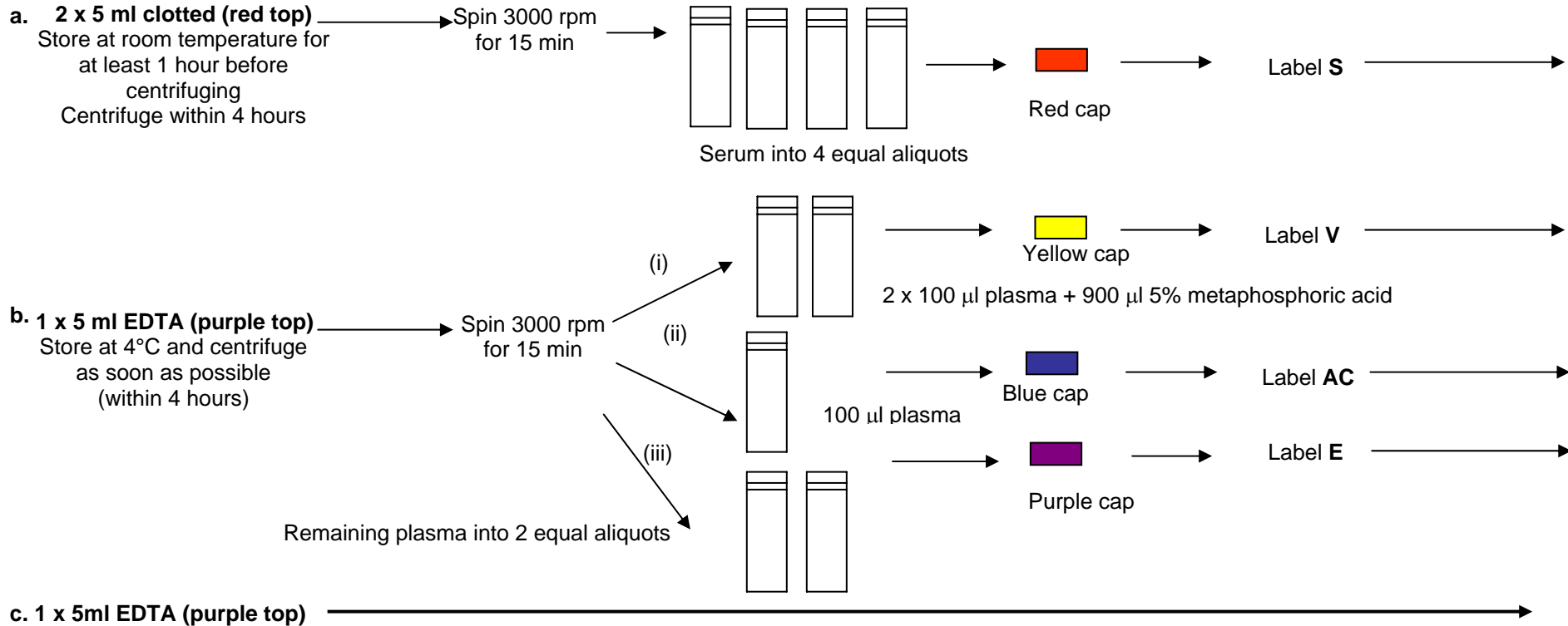
Preparation of metaphosphoric acid solution for stabilization of ascorbate

Metaphosphoric acid crystals can be kept at room temperature. A 5% solution of metaphosphoric acid is made by dissolving 5g of metaphosphoric acid crystals in 100ml of distilled water. The solution should be placed in a dark glass bottle, clearly labelled and kept in a refrigerator at 4°C. The resulting solution is stable for up to two weeks, and a fresh batch **must** be made up at the end of that time.

Appendix D

IMNA - SAMPLE PROCESSING SCHEME

Maternal Blood - 12 and 32 weeks gestation (V1 & V2)



Cord Blood - Delivery (V3)

