EMPOWAR Blood sampling collection

Working Practice Document (WPD)

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Revised:	
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1. PURPOSE

The purpose of this WPD is to describe the process for collecting and preparing the blood samples for adult participants in the EMPOWaR study and to ensure that all participating sites are consistent in their methods of collection and storage. This document should be retained in the ISF, section 7

NB: It is import this document is reviewed in conjunction with the current version of the study protocol to ensure all samples collected and all tests required are obtained, as the protocol may be more up to date.

2. DEFINITIONS

Hr – Hour
Inc. - Including
ISF - Investigator Site File
Mins - Minutes
PI - Principle investigator at the site
t - Time
WPD - Working Practice Documents
Trial Research Laboratories – Edinburgh

3. WHY

The specific guidelines for taking blood samples are created to help ensure accuracy and repeatability across the participating sites.

4. WHO

This WPD applies to all staff delegated by the PI for the task of collecting and preparing samples.

5. PROCEDURE

Collected between 10-0 and 16+0 weeks gestation prior to randomisation

1. Prepare the following tubes for sampling at baseline (0hr) and 2 hours (2hr + or- 5 Mins).

Timing	Reagent	Volume	Number	Analysis	Processing
0hr	Fluoride oxalate	2.7mls	1	Glucose	To hospital

					laboratories
0hr	Serum gel	7.5mls	1	Renal function, LFTs, lipid profile, CRP	To hospital laboratories
0hr	Serum gel	7.5mls	1	Cortisol, insulin, NEFA	To trial research laboratories
0hr	EDTA	9mls	1	Adipokines, inflammatory markers Fatty acids in red cell membranes	To trial research laboratories
0hr	Lithium heparin	4.7mls	1	Adipokines, inflammatory markers	To trial research laboratories
2hr	Fluoride oxalate	2.7mls	1	Glucose	To hospital laboratories

TOTAL VOLUME BLOOD (MAX): 34.1mls

2. Label tubes for NHS lab using hospital identification (ID), date and time of collection. For research tubes, write research number on tubes, date of collection and gestation.

3. Check subject has fasted from midnight.

4. Venepuncture

- Identify proposed site of venepuncture and apply a tourniquet to upper arm, 15cms above venepuncture site.
- Find a vein and clean site with antiseptic wipe. Ask patient to clench and open fist three times, using a needle collect blood and fill all tubes required at t=0hr.
- Leaving the needle in place release tourniquet.
- Apply cotton wool to puncture site and withdraw needle, discard into sharps bin.
- Apply pressure to the puncture site until bleeding has stopped. Apply a plaster if required.
- Gently invert each blood tube a few times to mix the blood and reagent. (Shaking too much or violently will lyse the red cells).

Collect the tubes in the following order of priority: fluoride oxalate, serum gel (FOR HOSPITAL LABS), serum gel EDTA, lithium heparin, (FOR TRIAL RESEARCH LABS),

5. Send one fluoride oxalate tube and one serum gel tube to the NHS laboratories and complete the request form asking for glucose, U+E, LFT, lipid profile and CRP.
6. Place remaining tubes on (not in) ice for later processing.
7. Record the t=0hr time and request the subject to drink a 75g oral glucose load within 10Mins
8. At t=2hr (+ or – 5mins) repeat the venepuncture and send the fluoride oxalate tube to NHS hospital lab for analysis of 2hr glucose. Label tube with hospital ID, date and time of collection.
9. Deliver bagged and labelled samples collected for the trial research labs on ice or in a chill bag to the designated local lab for sample processing and storage.

28 weeks gestation

1. Prepare the following tubes for sampling at baseline (0hr) and 2 hours (2hr).

Timing	Reagent	Volume	Number	Analysis	Processing
0hr	Fluoride oxalate	2.7mls	1	Glucose	To hospital laboratories
0hr	Serum gel	7.5mls	1	CRP	To hospital laboratories
0hr	Serum gel	7.5mls	1	Cortisol, insulin, NEFA	To trial research laboratories
0hr	EDTA	9mls	1	Adipokines, inflammatory markers Fatty acids in red cell membranes	To trial research laboratories
0hr	Lithium heparin	4.7mls	1	Adipokines, inflammatory markers	To trial research laboratories
2hr	Fluoride oxalate	2.7mls	1	Glucose	To hospital laboratories

TOTAL VOLUME BLOOD (MAX): 34.1mls

2. Label tubes for NHS lab using hospital ID, date and time of collection. For research tubes, write research number on tubes, date of collection and gestation.

3. Check subject has fasted from midnight

4. Venepuncture

- Identify proposed site of venepuncture and apply a tourniquet to upper arm, 15cms above venepuncture site.
- Find a vein and clean site with antiseptic wipe. Ask patient to clench and open fist three times, using a needle collect blood and fill all tubes required at t=0hr.
- Leaving the needle in place release tourniquet.
- Apply cotton wool to puncture site and withdraw needle, discard into sharps bin.
- Apply pressure to the puncture site until bleeding has stopped. Apply a plaster if required.
- Gently invert each blood tube a few times to mix the blood and reagent. (Shaking too much or violently will lyse the red cells).

Collect the tubes in the following order of priority: fluoride oxalate, serum gel (TO HOSPITAL LAB), serum gel, EDTA and lithium heparin (TO TRIAL RESEARCH LAB).

5. Send one fluoride oxalate tube and one serum gel tube to the NHS laboratories and complete the request form asking for glucose and CRP.

6. Place remaining tubes on (not in) ice for later processing.

7. Record the t=0hr time and request the subject to drink a 75g oral glucose load within 10Mins.

8. At t=2hr repeat (+ or – 5mins) the venepuncture and send the fluoride oxalate tube to hospital lab for analysis of 2hr glucose. Label tube with hospital ID, date and time of collection.

9. Deliver bagged and labelled samples collected for the trial research labs on ice or in a chill bag to the designated local lab for sample processing and storage.

36 weeks gestation

1. Prepare the following tubes for sampling at baseline (0hr) and 2 hours (2hr).

Timing	Reagent	Volume	Number	Analysis	Processing
0hr	Fluoride oxalate	2.7mls	1	Glucose	To hospital laboratories
0hr	Serum gel	7.5mls	1	Renal function, LFTs, lipid profile, CRP	To hospital laboratories

0hr	Serum gel	7.5mls	1	Cortisol, insulin, NEFA	To trial research laboratories
0hr	EDTA	9mls	1	Adipokines, inflammatory markers Fatty acids in red cell membranes	To trial research laboratories
0hr	Lithium heparin	4.7mls	1	Adipokines, inflammatory markers	To trial research laboratories
2hr	Fluoride oxalate	2.7mls	1	Glucose	To hospital laboratories

TOTAL VOLUME BLOOD (MAX): 34.1mls

2. Label tubes for NHS lab using hospital ID, date and time of collection. For research tubes, write research number on tubes, date of collection and gestation.

3. Check subject has fasted from midnight

4. Venepuncture

- Identify proposed site of venepuncture and apply a tourniquet to upper arm, 15cms above venepuncture site.
- Find a vein and clean site with antiseptic wipe. Ask patient to clench and open fist three times, using a needle collect blood and fill all tubes required at t=0hr.
- Leaving the needle in place release tourniquet.
- Apply cotton wool to puncture site and withdraw needle, discard into sharps bin.
- Apply pressure to the puncture site until bleeding has stopped,. Apply a plaster if required.
- Gently invert each blood tube a few times to mix the blood and reagent. (Shaking too much or violently will lyse the red cells).

Collect the tubes in the following order of priority: fluoride oxalate, serum gel (TO HOSPITAL LAB), serum gel, EDTA and lithium heparin (TO TRIAL RESEARCH LABS).

5. Send one fluoride oxalate tube and one serum gel tube to the NHS laboratories and complete the request form asking for glucose and CRP.

6. Place remaining tubes on (not in) ice for later processing.

7. Record the t=0hr time and request the subject to drink a 75g oral glucose load within 10Mins.8..At t=2hr repeat (+ or – 5mins) the venepuncture and send the fluoride oxalate tube to hospital lab for analysis of 2hr glucose. Label tube with hospital ID, date and time of collection.

9. Deliver bagged and labelled samples collected for the trial research labs on ice or in a chill bag to the designated local lab for sample processing and storage.

Procedure FOR COLLECTING CORD BLOODS

1. Ensure that the tubes for cord blood gases required by the NHS Trust/Board and (if the donor is Rhesus Negative) cord blood for Group and Save have been collected.

2. Use the cord clamps to isolate a loop of umbilical cord.

Reagent	Volume	Number	Analysis	Processing
Fluoride oxalate	2.7mls	1	Glucose	To hospital laboratories
Serum gel	2.7mls	1	CRP	To hospital laboratories
EDTA	4.7mls	1	Adipokines, inflammatory markers Fatty acids in red cell membranes	To trial research laboratories
Serum gel	2.7mls	1	Cortisol, C-peptide, NEFA	To trial research laboratories
Lithium heparin	2.7mls	1	Adipokines, inflammatory markers	To trial research laboratories

3. Ideally collect bloods within 15 minutes of delivery.

In order of priority, collect the following tubes from the cord vessels (ideally venous): fluoride oxalate, serum gel (to hospital labs), EDTA, serum gel and lithium heparin (to trial research labs).

4. Ensure tubes appropriately labelled. Store samples on ice and transport to the laboratory.

4. Transport blood samples at 4°C (on ice) collected for the trial research labs to the designated local lab for sample processing and storage.

Sample processing

1. When the bloods are collected they should be processed immediately or at least spun as soon as possible after collection. Samples for Insulin analysis are required to be kept at 4°C.

2. Spin the blood tubes at 2,200rpm for 10 min at +4°C.

3. Remove the tubes from the centrifuge and carefully remove the plasma or serum layer using a pastette pipette and aliquot a minimum of 0.5ml aliquots into pre-labelled 2.0ml screw-top tubes using the maximum of 6 tubes.

5. The white layer in the Plasma EDTA tubes is the buffy coat and is kept for DNA extraction. Pipette carefully this layer into a 2.0ml screw-top tube prelabelled BUFFY. Some red cell or plasma contamination is acceptable. The easiest way to isolate this is to dislodge it from the tube wall using a pipette then slowly suck it out the tube.

6. Aliquot ~0/5ml of remaining red blood cells (RBC) into a separate pre-labelled 2.0 ml screw-top tube.This sample is kept for the analysis of fatty acids in red cell membranes.

In summary the maximum sample set per participant visit should consist of:

6 EDTA plasma samples
6 Lithium Heparin plasma samples
6 Serum samples
1 Buffy sample
1 RBC sample

If only a small amount of blood is collected during a visit less samples tubes may be prepared.

Please ensure all tubes are correctly labelled and freeze them at -20°C or -80°C until subsequent analysis.

NB: Labels should include: the reagent used to collect the sample e.g. EDTA/Lith Hep/serum gel), Buffy (where appropriate), RBC (where appropriate) date of sample collection, subject study ID and gestation

Transfer of frozen samples to the University of Edinburgh

The research team at the University of Edinburgh should be contacted to arrange receipt of the samples before any arrangements are made for transfer (see contact details below).

Samples sent should be transferred with a copy of the EMPOWaR tissue collection log Please review WPD 10 Transport of samples for further details.

Contact details to arrange the collections:

Sonia Whyte

EMPOWaR Trial Manager

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