Detailed standard operating procedure for the collection, processing and storage of blood specimens

NOTES

- This SOP applies to plasma collection using EDTA or lithium-heparin coagulation tubes, and serum with (SST) or without separator, collected via venepuncture.
- This SOP does not cover blood withdrawal techniques/procedures (qualified personnel should follow standard withdrawal procedures).
- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines**.**
- For a summary version of this protocol with side-by-side standard vs. minimal protocol step comparisons, please see "WERF EPHect SOP Fluid Collection Table".
- As this protocol applies to different processing and storage methods (e.g. the use of RNA stabilising fluid), keep a copy of the exact step-by-step protocol used in your lab.

Processing and storage materials

1. Biospecimen form (page 24);
2. Log sheet to record sample-related data;
3. Blood collection tubes with EDTA and heparin for plasma, SST or no SST for serum
4. Crushed ice if a delay is anticipated
5. Appropriate racks to hold tubes in upright position
6. Centrifuge
7. Volume adjustable pipette
8. Transfer pipettes
9. Labels suitable for long-term freezer storage, and IDs printed using 2D barcoding
10. Aliquot vials with screw top gasket closure
11. Freezers: -80C or liquid nitrogen (LN_2)

1. Blood collection

1.1. Blood collection should be performed by a licensed phlebotomist, nurse, anaesthesiologist, or medical doctor.

1.2. Collection should be performed in an adequate setting, e.g. in the phlebotomy room, or on the ward. Blood collection in the operating theatre should be avoided, if possible.

1.3. Time of sample collection in the clinic:

1.3.1. ***Standard collection:*** Collect blood samples before induction of anaesthesia (before pre-med is given).

1.3.2. ***Required minimum:*** Record whether blood is collected (1) prior to pre-medication; (2) after pre-medication but before anaesthesia; or (3) after anaesthesia.

1.4. Fasting status at sample collection:

1.4.1. ***Standard collection:*** Collect only samples after fasting for at least 10 hours. Record on the log sheet the time since the study participant ate or drank anything except plain water (Fasted since: __:__pm/am).

1.4.2. ***Required minimum:*** Record on the log sheet the time since the study participant ate or drank anything except plain water (Fasted since: __:__pm/am).

1.5. Preparation of sample collection tubes:

1.5.1. ***Standard collection:*** Label each blood collection tube with a 2D barcode in addition to a human readable unique identifier, participant ID, date of collection and type of sample. Record on the log sheet the date and time of sample collection (Date: __/__/__ and __:__am/pm).

1.5.2. ***Required minimum:*** Label each blood collection tube with a unique identifier, participant ID, date of collection, and type of sample. Record on the log sheet the date and time of sample collection (Date: __/__/__ and __:__am/pm).

1.6. Order of sample type collection:

1.6.1. ***Standard collection:*** Prioritise: 1) EDTA plasma; 2) SST serum; 3) other tube types, in a pre-determined order of priority. Keep a record of the order in your adapted SOP.

1.6.2. ***Required minimum:*** Collect collection tubes always in the same order of priority and keep a record of the order in your adapted SOP.

1.7. It is important that tubes with anti-coagulants (e.g., EDTA, heparin), which need to be inverted after blood draw, are gently inverted 8-10 times (no vigorous shaking). Place in the collection rack in an upright position.

1.8. Temperature and waiting conditions of samples until processed in the lab:

1.8.1. ***Standard collection:*** Place samples on wet ice/in refrigerator immediately if there will be more than 1 hour before processing (maximum time to processing: 4 hours). If the time to processing is less than 1 hour, samples can be kept at room temperature.

1.8.2. ***Required minimum:*** Place samples on wet ice/in refrigerator if there will be more than 2 hour before processing (maximum time to processing: 4 hours). If the time to processing is less than 2 hours, samples can be kept at room temperature.

2. Sample processing in the laboratory

2.1. Allow any SST tubes (red top) to clot for 30-60 minutes in an upright position at room temperature in the first 30 minutes from collection of the sample. SST tubes should be placed on wet ice after 1 hour if there will be more than 2 hours until processing.

2.2. Time until samples processed in the lab:

2.2.1. ***Standard collection:*** Record on the log sheet the time sample processing started in the laboratory. Blood samples should be centrifuged within 1 hour of blood collection.

2.2.2. ***Required minimum:*** Record on the log sheet the time sample processing started in the laboratory. Blood samples should be centrifuged within 4 hours of blood collection.

2.3. Centrifugation of samples:

2.3.1. ***Standard collection:*** Centrifuge samples for 10 minutes at 2500g at 4°C. Keep a record of the standard time and g in your centre's SOP.

2.3.2. ***Required minimum:*** Centrifuge samples for 10 minutes at 2500g at room temperature. Keep a record of the standard time and g in your centre's SOP.

2.4. After centrifugation of samples:

2.4.1. ***Standard collection:*** Place the spun tubes on a rack in upright position and on wet ice during aliquotting.

2.4.2. ***Required minimum:*** Place the spun tubes on a rack in upright position at room temperature during aliquotting.

2.5. Aliquotting of samples:

2.5.1. ***Standard collection:*** Have a set number of aliquot tubes and sizes for each sample type that will be collected. Pre-label aliquot vials (see section 3). Put empty aliquot vials into a rack that sits on wet ice before aliquotting. Aliquot into small volumes between 100-500uL to minimize later freeze-thaw cycles.

2.5.2. ***Required minimum:*** Have a set number of aliquot tubes and sizes for each sample type that will be collected. Pre-label aliquot vials (see section 3).

2.6. Aspirate plasma/serum using an appropriate transfer pipette (ideally a volume-adjustable pipette) being careful not to disturb the cell layer below, holding the tube at a 45° angle.

2.7. Transfer plasma/serum to appropriately sized aliquot vial with screw top gasket closure (do not use tubes with a push top as they are not airtight) and fill as close to full as possible to minimise exposure to air.

2.8. Repeat steps 2.6 and 2.7 until all the plasma and serum has been transferred. Record volume of sample in each aliquot of plasma or serum.

2.9. If cells are accidentally mixed with the plasma/serum, the aliquot vial can be re-centrifuged as before and the plasma/serum can be transferred to a new aliquot vial.

2.10. White Blood Cell (WBC) aliquotting: Using a transfer pipette take the buffy coat layer from the collection tube. Aspirate slowly and carefully using a circular motion to remove all the visible buffy coat and transfer to an appropriate sized aliquot vial with screw top gasket closure. Record the volume of sample in each aliquot of WBC.

2.11. Red Blood Cell (RBC) aliquotting: Using a transfer pipette, gently mix the remaining erythrocytes and aspirate in another appropriate sized aliquot vial with screw top gasket closure and fill as close to full as possible to minimise surface area. Record the volume of sample in each aliquot of RBC.

3. Labelling aliquots and storage

3.1. Use special labels and ink that do not disintegrate when stored in very low temperature freezers (i.e. do not use laser printers or most ink-printers as they disintegrate when frozen).

3.2. Preparation of sample aliquot tubes:

3.2.1. ***Standard collection:*** Label the aliquot tubes with the participant ID number followed by a unique aliquot ID number. For example: ENDO-123456-U654321-P-01: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (P for plasma), aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points. Furthermore, include the above information in human readable format and in a 2D barcode on the label.

3.2.2. ***Required minimum:*** Label the aliquot tubes with the participant ID followed by a unique aliquot ID number. For example: ENDO-123456-U654321-P-01: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (P for plasma), aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points.

3.3. Time until sample aliquots are put into freezers for storage:

3.3.1. ***Standard collection:*** Samples should be processed and stored into freezers within a maximum of 1 hour and time should be recorded on the log sheet. Also record the type, number and volume of aliquots prepared.

3.3.2. ***Required minimum:*** Samples should be processed and stored into freezers within a maximum of 4 hours, and time of storage should be recorded on the log sheet. Also record the type, number and volume of aliquots prepared.

3.4. Sample storage in freezers:

3.4.1. ***Standard collection:*** Store serum, plasma and WBC/RBC aliquots in liquid nitrogen (LN_2) freezers, which have less temperature fluctuations than -80°C freezers.

3.4.2. ***Required minimum:*** Store serum, plasma and WBC/RBC aliquots in -80°C or lower freezers.

3.5. Record on the log sheet any variations or deviations from the SOP, problems, or issues (e.g., hemolysis, vial cracked during processing).

3.6. Record the location of each sample in the freezer including freezer number, rack, box, and position in the box along with all other sample attributes in a database. If possible avoid using a spreadsheet format, but preferably use a relational database.

4. Freezer check

4.1.1. ***Standard collection:*** Split aliquots from the same sample type and individual between freezers in case of a freezer breaking down. Check freezers bi-weekly and keep a written-log of checks. Have alarm systems setup on all freezers in addition to human bi-weekly checks.

4.1.2. ***Required minimum:*** Manually check freezers bi-weekly and keep a written-log of checks.

5. Data recording check list

5.1. Record protocol, specifying which steps are adhered to (standard or minimum).

5.2. Record the time since the study participant ate or drank anything except plain water (fasted since: __:__pm/am).

5.3. For each sample, record:

5.3.1. Date and time of blood collection (Date: __/__/__ and __:__am/pm).

5.3.2. Start time of sample processing in the laboratory (__:__am/pm).

5.3.3. Type, number and volume of aliquots prepared.
5.3.4. Date and time aliquots stored into freezers (Date: __/__/__ and __:__am/pm).

5.3.5. Any variations or deviations from the SOP, problems, or issues.

5.4. In the long-term, record:

5.4.1. Any freeze-thaw that occurs with a sample for any reason.

5.4.2. Any change of location of a sample, including sending a sample out to an assay lab for processing.

5.4.3. Any new samples created from the original aliquots (i.e., a sub-aliquot) in the same manner as described above.

5.5. Keep a bi-weekly log of freezer checks.

Detailed standard operating procedure for the collection, processing and storage of urine specimens

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines**.**
- For a summary version of this protocol with side-by-side standard vs. minimal protocol step comparisons, please see "WERF EPHect SOP Fluid Collection Table".
- As this protocol applies to different processing and storage methods, keep a copy of the exact step-by-step protocol used in your lab.

Processing and storage materials

1. Biospecimen form (page 24);
2. Log sheet to record sample-related data;
3. Sterile urine collection container with a wide mouth and a leak-proof cap
4. Crushed ice if a delay is anticipated
5. Transfer pipette
6. Dipstick for urine analysis
7. Volume adjustable pipette
8. Centrifuge
9. Labels suitable for long-term freezer storage, and IDs printed using 2D barcoding
10. Aliquot vials with screw top gasket closure
11. Freezers: -80C or liquid nitrogen (LN_2)

1. Urine collection

1.1. Sample collection method:

1.1.1. ***Standard collection:*** Obtain a clean catch, mid-stream, first morning void*, urine sample from the patient. Instruct them to collect the urine sample first thing in the morning when they get out of bed. Provide the patient with a sterile specimen container with a wide mouth and a leak-proof cap and written instructions on how to collect clean catch midstream urine sample.

1.1.2. ***Required minimum:*** Obtain a clean catch urine sample from the patient in the clinic or at the patient's home by providing the patient with a sterile specimen container with a wide mouth and a leak-proof cap and written instructions on how to collect clean catch midstream urine sample.

*Patient instruction for first morning clean catch urine sample[1]:

1. Collect the first urine that occurs as the first void after waking up.
2. Wash your hands with soap and warm water.
3. Sit on the toilet with your legs spread apart. Use two fingers to spread open your labia.
4. Use an antiseptic wipe to clean the inner folds of the labia. Wipe from the front to the back.
5. Use a second antiseptic wipe to clean over the opening where urine comes out (urethra), just above the opening of the vagina.
6. Keeping your labia spread open, urinate a small amount into the toilet bowl, then stop the flow of urine.
7. Hold the urine cup a few inches from the urethra and urinate until the cup is about half full.
8. You may finish urinating into the toilet bowl.

[1] http://www.nlm.nih.gov/medlineplus/ency/article/007487.htm

9. Screw the cap on to the collection cup securely and place in the refrigerator until taking the sample to the clinic.

1.2. If the sample is collected in the clinic, the research nurse should record the time of sample collection. If the sample is a first morning void brought into clinic with the patient, the patient should record the time and type of sample collection and report to the research nurse.

1.3. If the sample is collected in clinic, it should be put on wet ice immediately. If the patient collects the sample at home, it should be maintained in a refrigerator and delivered in an ice pack to the clinic (at 4°C).

1.4. Record on the log sheet the time since the study participant ate or drank anything except plain water (Fasted since: __:__pm/am). Record what time the urine sample was collected, whether this was a first morning void or spot urine, and whether the study participant has urinated during the night time if the collected sample is a first morning void.

1.5. Labelling of the collection cup:

1.5.1. ***Standard collection:*** Label the collection cup before giving to the patient for collection, with a 2D barcode in addition to human-readable the unique identifier of the patient and sample identifier.

1.5.2. ***Required minimum:*** Once the collection cup is collected from the patient, label the sample with the unique identifier of the patient and sample identifier.

2. Sample processing in the laboratory, labelling aliquots and storage

2.1. Time until samples processed in the lab:

2.1.1. ***Standard collection:*** Keep the sample refrigerated until processed and complete processing within 2 hours. Record on the log sheet the time sample processing started in the laboratory.

2.1.2. ***Required minimum:*** Keep the sample refrigerated until processed and complete processing within a maximum of 48 hours. Record on the log sheet the time sample processing started in the laboratory.

2.2. Discard the sample if it contains blood, and record.

2.3. Mix the sample by either swirling the cup or pipetting the urine up and down a couple of times.

2.4. Perform dipstick urine analysis and record result including specific gravity. If specific gravity is lower than 1.001 or greater than 1.032, retest for accuracy. Record on the log sheet, the results, including specific gravity and that retest is performed.

2.5. Aspirate required amount of unprocessed urine and label the aliquot vials with screw top gasket closures.

2.6. Preparation of sample aliquot tubes:

2.6.1. ***Standard collection:*** Label the aliquot vials with the participant ID number followed by a unique aliquot ID number. For example: ENDO-123456-U654321-U-01: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (U for urine) and aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points. Further, include the above information in human readable format and in a 2D barcode on the label.

2.6.2. ***Required minimum:*** Label the aliquot vials with the participant ID followed by the sample aliquot number. Also include date of sample creation on the label. For example: ENDO-123456-U-01: Center identifier (ENDO), participant ID (123456), type of sample (U for urine), aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points.

2.7. Sample storage in freezers:

2.7.1. ***Standard collection:*** Store the unprocessed urine aliquots in liquid nitrogen (LN_2) freezers, which have less temperature fluctuations.

2.7.2. ***Required minimum:*** Store the unprocessed urine aliquots at -80°C or lower.

2.8. Fill a sterile tube with the remaining urine in the sample collection container and centrifuge at 1000-3000g at 4°C for 5 minutes.

2.9. Place the sample on wet ice and aspirate the supernatant into required number of aliquots. Label the aliquots as in 2.6. and store the processed urine aliquots as in 2.7.

2.10. Duration until sample aliquots are put into freezers for storage:

2.10.1. ***Standard collection:*** Samples should be processed and stored into freezers within a maximum of 2 hours and time should be recorded on the log sheet. Also record the type, number and volume of aliquots prepared.

2.10.2. ***Required minimum:*** Samples should be processed and stored into freezers within a maximum of 48 hours and time should be recorded on the log sheet. Also record the type, number and volume of aliquots prepared.

2.11. Record on the log sheet any variations or deviations from the SOP, problems, or issues.

2.12. Record the location of each sample in the freezer including freezer number, rack, box, and position in the box along with all other sample attributes in a database. If possible, avoid using a spreadsheet format, but preferably use a relational database.

3. Freezer check

3.1.2. ***Standard collection:*** Split aliquots from the same sample type and individual between freezers in case of a freezer breaking down. Check freezers bi-weekly and keep a written-log of checks. Have alarm systems setup on all freezers in addition to human bi-weekly checks.

3.1.1. ***Required minimum:*** Manually check freezers bi-weekly and keep a written-log of checks.

4. Data recording check list

4.1. Record protocol, specifying which steps are adhered to (standard or minimum).

4.2. Record the time since the study participant ate or drank anything except plain water (Fasted since: __:__pm/am).

4.3. For each sample, record:

4.3.1. Date and time of urine collection (Date: __/__/__ and __:__am/pm).

4.3.2. Type of urine collection (e.g. Clean catch spot urine or clean catch first morning void urine).

4.3.3. Start time of sample processing in the laboratory (__:__am/pm).

4.3.4. Results of dipstick urinalysis including specific gravity and that retest is performed.
4.3.5. Type, number and volume of aliquots prepared.

4.3.6. Date and time aliquots stored into freezers (Date: __/__/__ and __:__am/pm).

4.3.7. Any variations or deviations from the SOP, problems, or issues.

4.4. In the long-term, record:

4.4.1. Any freeze-thaw that occurs with a sample for any reason.

4.4.2. Any change of location of a sample, including sending a sample out to an assay lab for processing.

4.4.3. Any new samples created from the original aliquots (i.e., a sub-aliquot) in the same manner as described above.

4.5. Keep a bi-weekly log of freezer checks.

Detailed standard operating procedure for the collection, processing and storage of saliva specimens

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines**.**
- For a summary version of this protocol with side-by-side standard vs. minimal protocol step comparisons, please see Supplemental Table 3.
- As this protocol applies to different processing and storage methods, keep a copy of the exact step-by-step protocol used in your lab.

Processing and storage materials

1. Biospecimen form (Supplemental Appendix VII);
2. Log sheet to record sample-related data;
3. Sterile saliva collection container or is collecting sample for DNA the manufacturer's provided collection container
4. Crushed ice if a delay is anticipated.
5. Transfer pipette
6. Volume adjustable pipette
7. Centrifuge
8. RNA stabilizer fluid *(optional if planning RNA studies)*
9. Labels suitable for long-term freezer storage, and IDs printed using 2D barcoding
10. Aliquot vials with screw top gasket closure
11. Freezers: -80C or liquid nitrogen (LN_2)

1. Saliva collection

1.1. Record whether the patient has fasted and for how long.

1.2. Record recent (last 24 hours) exposures to toothpaste, gum, cigarettes, alcohol, meal including fish, spicy food, medication.

1.3. Fasting status at sample collection:

1.3.1. ***Standard collection:*** Collect sample after a fast of at least 6 hours.

1.3.2. ***Required minimum:*** Collect sample at least 1 hour after brushing teeth, at least 1 hour after eating a meal, at least 12 hours after last alcohol consumption, and at least 20 minutes after consuming acidic food (e.g., citrus fruits) or high sugar food.

1.4. Rinse mouth before collection of the sample.

1.5. Sample collection method:

1.5.1. ***Standard collection:*** Ask patient to drool into a sterile specimen container without the action of spitting. Saliva production can be enhanced by showing the patient mouth watering images such as lemons (no salivary stimulants). If collecting sample for DNA (e.g., Oragene®) follow the manufacturer's protocol for collection.

1.5.2. ***Required minimum:*** Ask patient spit or drool into a sterile specimen container. Saliva production can be enhanced by showing the patient mouth watering images such as lemons (no salivary stimulants). Record the collection technique.

1.6. Amount of sample collection:

1.6.1. ***Standard collection:*** Obtain 2ml of saliva apart from foam/bubbles.

1.6.2. ***Required minimum:*** Obtain at least 1ml of saliva apart from foam/bubbles. If many bubbles form during collection, the participant can cap the container and gently tap the collection container on a hard surface.

1.7. Labelling of sample collection tubes:

1.7.1. ***Standard collection:*** Label the sample with a 2D barcode in addition to human-readable, showing the unique identifier of the patient and sample identifier. Record on the log sheet the date and time of sample collection (Date: __/__/__ and __:__am/pm).

1.7.2. ***Required minimum:*** Label the sample with the unique identifier of the patient and sample identifier. Record on the log sheet the date and time of sample collection (Date: __/__/__ and __:__am/pm).

2. Sample processing in the laboratory, labelling aliquots and storage

2.1. Place samples on wet ice or in refrigerator if there will be more than 1 hour before processing. If the time to processing is less than 1 hour, samples can be kept at room temperature.

2.2. Record on the log sheet the time of the sample processing started in the laboratory.

2.3. Pipette an appropriate amount of unprocessed saliva into a screw top vial with gasket closure and put on wet ice.

2.4. Preparation of sample aliquot tubes:

2.4.1. ***Standard collection:*** Label the aliquot vials with the participant ID number followed by a unique aliquot ID number. For example: ENDO-123456-U654321-S-01: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (S for saliva) and aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points. Further, include the above information in human readable format and in a 2D barcode on the label.

2.4.2. ***Required minimum:*** Label the aliquot vials with the participant ID followed by the sample aliquot number. For example: ENDO-123456-S-01: Center identifier (ENDO), participant ID (123456), type of sample (S for saliva), aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points.

2.5. Sample storage in freezers:

2.5.1. ***Standard collection:*** Store the unprocessed saliva aliquots in liquid nitrogen (LN_2) freezers, which have less temperature fluctuations.

2.5.2. ***Required minimum:*** Store the unprocessed saliva aliquots in a freezer of -80°C or lower.

2.6. Centrifuge the remaining saliva sample in the collection tube at 1000g for two minutes at 4°C.

2.7. Aliquot appropriate amount of supernatant into an appropriate sized screw top vial with gasket closure. Label the aliquots as in 2.4. and store the processed saliva aliquots as in 2.5.

2.8. If interested in RNA extraction: Aliquot from the remaining supernatant in the collection tube after centrifugation to a different aliquot vial, which contains an RNA stabilizer [commercially available products: Allprotect Tissue Reagent® (Qiagen); DNA / RNA Shield™ (Zymoresearch); ProtectRNA™ (Sigma-Aldrich); RiboLock™ (Thermoscientific); RNAlater® (Qiagen); Ambion® RNAsecure™ Reagent (Life-technologies); SUPERase•In™ (Life-technologies); PAXgene Tissue Containers (Qiagen)]. Label the aliquots as in 2.4. and store the processed RNA saliva aliquots as in 2.5.

2.9. Duration until sample aliquots are put into freezers for storage:

2.9.1. ***Standard collection:*** Samples should be processed and stored into freezers within maximum of 4 hours and time should be recorded on the log sheet. Also record the type, number and volume of aliquots prepared.

2.9.2. ***Required minimum:*** Record on the log sheet, the time of the sample processing completion/ time put into the freezer and type, number and volume of aliquots prepared.

2.10. Record any variations or deviations from the SOP, problems, or issues.

2.11. Record the location of each sample in the freezer including freezer number, rack, box, and position in the box along with all other sample attributes in a database. If possible, avoid using a spreadsheet format, but preferably use a relational database.

3. Freezer check

3.1.2. ***Standard collection:*** Split aliquots from the same sample type and individual between freezers in case of a freezer breaking down. Check freezers bi-weekly and keep a written-log of checks. Have alarm systems setup on all freezers in addition to human bi-weekly checks.

3.1.1. ***Required minimum:*** Manually check freezers bi-weekly and keep a written-log of checks.

4. Data recording check list

4.1. Record protocol, specifying which steps are adhered to (standard or minimum).

4.2. Record the time since the study participant ate or drank anything except plain water (Fasted since: __:__pm/am). Is it at least 6 hours? Yes/No.

4.3. Record whether it has been less than, (1) 1 hour after brushing teeth, (2) 1 hour after eating a meal, (3) 12 hours after last alcohol consumption, (4) 20 minutes after consuming acidic foods (e.g., citrus fruits) or high sugar foods.

4.4. For each sample, record:

4.4.1. Date and time of saliva collection (Date: __/__/__ and __:__am/pm).

4.4.2. Start time of sample processing in the laboratory (__:__am/pm).

4.4.3. Type, number and volume of aliquots prepared.

4.4.4. Date and time aliquots stored into freezers (Date: __/__/__ and __:__am/pm).

4.4.5. Any variations or deviations from the SOP, problems, or issues.

4.5. In the long-term, record:

4.5.1. Any freeze-thaw that occurs with a sample for any reason.

4.5.2. Any change of location of a sample, including sending a sample out to an assay lab for processing.

4.5.3. Any new samples created from the original aliquots (i.e., a sub-aliquot) in the same manner as described above.

4.6. Keep a bi-weekly log of freezer checks.

Detailed standard operating procedure for the collection, processing and storage of endometrial fluid specimens

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines**.**
- For a summary version of this protocol with side-by-side standard vs. minimal protocol step comparisons, please see "WERF EPHect SOP Fluid Collection Table".
- As this protocol applies to different processing and storage methods, keep a copy of the exact step-by-step protocol used in your lab.

Processing and storage materials

1. Biospecimen form (page 24);
2. Log sheet to record sample-related data;
3. Embryo-transfer catheter and a 20ml syringe
4. Normal saline solution
5. Liquid nitrogen or dry ice
6. Crushed ice if a delay is anticipated
7. Transfer pipette
8. Volume adjustable pipette
9. Centrifuge
10. Labels suitable for long-term freezer storage, and IDs printed using 2D barcoding
11. Aliquot vials with screw top gasket closure
12. Freezers: -80C or liquid nitrogen (LN_2)

1. Endometrial fluid collection

1.1. Time of sample collection in the clinic:

1.1.1. ***Standard collection:*** The sample is collected before any pre-medication/anesthesia administration.

1.1.2. ***Required minimum:*** Record whether sample is collected 1) prior to pre-medication, 2) after pre-medication but before anesthesia, or 3) after anesthesia.

1.2. Sample collection is performed by either (1) Aspiration method: Embryo-transfer catheter connected to a 20ml syringe to apply vacuum. (2) Lavage method: Slowly infuse and withdraw 4ml of saline solution into the uterine cavity. This uterine lavage fluid (ULF) can be processed as endometrial fluid, but the supernatant from ULF should be regarded with caution (see main text for discussion).

1.3. Record the time, date, volume and method of sample collection.

1.4. If the sample is collected using the embryo-transfer catheter, cut the tip of the catheter, insert into an aliquot tube and snap freeze immediately in liquid nitrogen.

1.5. If the lavage method is used Sample treatment until transported to the lab:

1.5.1. ***Standard collection:*** Transfer the sample into a screw top vial. If the amount of sample is small (not enough for centrifugation), put the sample immediately into liquid nitrogen or dry ice. If

the amount of sample is enough for centrifugation put the sample on wet ice and transfer to the laboratory within 30 minutes.

1.5.2. ***Required minimum:*** Transfer the sample into a screw top vial. If the amount of sample is small (not enough for centrifugation), put the sample immediately into liquid nitrogen or dry ice. If the amount of sample is enough for centrifugation put the sample on wet ice and transfer to the laboratory as soon as possible.

2. Sample processing in the laboratory

2.1. Record on the log sheet the start time of sample processing in the laboratory.

2.2. Record colour, clarity and volume of the sample.

2.3. Centrifugation of samples:

2.3.1. ***Standard collection:*** Centrifuge samples for 5 minutes at 900g at 4°C. Keep a record of the standard time and g in your adapted SOP.

2.3.2. ***Required minimum:*** Centrifuge samples for 5 minutes at 900g at room temperature. Keep a record of the standard time and g in your adapted SOP.

2.4. Transfer the supernatant to appropriate sized aliquot vial with screw top gasket closure (not 'push fit top' as they are not airtight) and fill as close to full as possible to minimise surface area. If ULF method is used for collection, be cautious in downstream analyses of the supernatant (See main text for discussion).

2.5. Transfer the pellet to appropriate sized aliquot vial with screw top gasket closure (not 'push fit top' as they are not airtight).

3. Labelling aliquots and storage

3.1. Use special labels and ink that do not disintegrate when stored in very low temperature freezers (i.e. Do not use laser printers or most ink-printers as they disintegrate when frozen).

3.2. Preparation of sample aliquot tubes:

3.2.1. ***Standard collection:*** Label the aliquot vials with the participant ID number followed by a unique aliquot ID number. For example: ENDO-123456-U654321-EF-01: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (EF for endometrial fluid) and aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points. Further, include the above information in human readable format and in a 2D barcode on the label.

3.2.2. ***Required minimum:*** Label the aliquot vials with the participant ID followed by the sample aliquot number. For example: ENDO-123456-EF-01: Center identifier (ENDO), participant ID (123456), type of sample (EF for endometrial fluid), aliquot number (01). Also, include date of

sample creation on the label to be able to distinguish samples from the same participant collected at different time points.

3.3. Record the time of the sample processing completion/ time put into the freezer. Also record the type, number and volume of aliquots prepared.

3.4. If storing unprocessed small amount of sample in freezers:

3.4.1. ***Standard collection:*** Transfer the small, unprocessed sample from dry ice or liquid nitrogen to liquid nitrogen (LN_2) freezers for long-term storage.

3.4.2. ***Required minimum:*** Transfer the small unprocessed sample from dry ice or liquid nitrogen to -80°C or lower freezers for long term storage.

3.5. If storing processed sample separated into supernatant and pellet in freezers:

3.5.1. ***Standard collection:*** Store fluid aliquots in liquid nitrogen (LN_2) freezers, which have less temperature fluctuations.
3.5.2. ***Required minimum:*** Store fluid aliquots at -80°C or lower freezers for long term storage.

3.6. Record any variations or deviations from the SOP, problems, or issues.

3.7. Record the location of each sample in the freezer including freezer number, rack, box, and position in the box along with all other sample attributes in a database. If possible avoid using a spreadsheet format, but preferably use a relational database.

4. Freezer check

4.1.1. ***Standard collection:*** Split aliquots from the same sample type and individual between freezers in case of a freezer breaking down. Check freezers bi-weekly and keep a written-log of checks. Have alarm systems setup on all freezers in addition to human bi-weekly checks.

4.1.2. ***Required minimum:*** Manually check freezers bi-weekly and keep a written-log of checks.

5. Data recording check list

5.1. Record protocol, specifying which steps are adhered to (standard or minimum).

5.2. For each sample, record:

5.2.1. Date and time of fluid collection (Date: __/__/__ and __:__am/pm).

5.2.2. Start time of sample processing in the laboratory (__:__am/pm).

5.2.3. Type, number and volume of aliquots prepared.

5.2.4. Date and time aliquots stored into freezers (Date: __/__/__ and __:__am/pm).

5.2.5. Any variations or deviations from the SOP, problems, or issues.

5.3. In the long-term, record:

5.3.1. Any freeze-thaw that occurs with a sample for any reason.

5.3.2. Any change of location of a sample, including sending a sample out to an assay lab for processing.

5.3.3. Any new samples created from the original aliquots (i.e., a sub-aliquot) in the same manner as described above.

5.4. Keep a bi-weekly log of freezer checks.

Detailed standard operating procedure for the collection, processing and storage of peritoneal fluid specimens

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines**.**
- For a summary version of this protocol with side-by-side standard vs. minimal protocol step comparisons, please see "WERF EPHect SOP Fluid Collection Table".
- As this protocol applies to different processing and storage methods, keep a copy of the exact step-by-step protocol used in your lab.

Processing and storage materials

1. Biospecimen form (page 24);
2. Log sheet to record sample-related data;
3. 20ml suction device or laparoscopic needle and a 20ml syringe
4. Normal saline solution
5. Crushed ice if a delay is anticipated
6. Transfer pipette
7. Volume adjustable pipette
8. Centrifuge
9. Labels suitable for long-term freezer storage, and IDs printed using 2D barcoding
10. Aliquot vials with screw top gasket closure
11. Freezers: -80C or liquid nitrogen (LN_2)

1. Peritoneal fluid collection

1.1. The sample is collected after premedication/anesthesia administration.

1.2. Sample collection is performed by either (1) 2 20ml suction devices (2) laparoscopic needle and manual aspiration using a syringe. Always use the same device and record the device used for collection in the SOP.

1.3. If no peritoneal fluid or a very small amount of peritoneal fluid is found, the pelvis can be washed with 20ml sterile normal saline solution using laparoscopic needle and manual aspiration using a syringe under direct visual control. This peritoneal lavage fluid (PLF) can be processed as peritoneal fluid, but the supernatant from PLF should be regarded with caution (see main text for discussion).

1.4. Record the time, date, volume and method of sample collection.

1.5. Sample treatment until transported to the lab:

1.5.1. ***Standard collection:*** Transfer the sample into a screw top vial and put the sample on wet ice and transfer to the lab within 30 minutes.

1.5.2. ***Required minimum:*** Transfer the sample into a screw top vial and put the sample on wet ice and transfer to the lab as soon as possible.

2. Sample processing in the laboratory

2.1. Record on the log sheet the start time of sample processing in the laboratory.

2.2. Record colour, clarity and volume of the sample.

2.3. Centrifugation of samples:

2.3.1. ***Standard collection:*** Centrifuge samples for 5 minutes at 900g at 4°C. Keep a record of the standard time and g in your adapted SOP.

2.3.2. ***Required minimum:*** Centrifuge samples for 5 minutes at 900g at room temperature. Keep a record of the standard time and g in your adapted SOP.

2.4. Transfer the supernatant to appropriate sized aliquot vial with screw top gasket closure (not 'push fit top' as they are not airtight) and fill as close to full as possible to minimise surface area. If PLF method is used for collection, be cautious in downstream analyses of the supernatant (See main text for discussion).

2.5. Transfer the pellet to an appropriate sized aliquot vial with screw top gasket closure (not 'push fit top' as they are not airtight).

3. Labelling aliquots and storage

3.1. Use special labels and ink that do not disintegrate when stored in very low temperature freezers (i.e. do not use laser printers or most ink-printers as they disintegrate when frozen).

3.2. Preparation of sample aliquot tubes:

3.2.1. ***Standard collection:*** Label the aliquot vials with the participant ID number followed by a unique aliquot ID number. For example: ENDO-123456-U654321-PF: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (PF for peritoneal fluid) and aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points. Further, include the above information in human readable format and in a 2D barcode on the label.

3.2.2. ***Required minimum:*** Label the aliquot vials with the participant ID followed by the sample aliquot number. For example: ENDO-123456-PF-01: Center identifier (ENDO), participant ID (123456), type of sample (PF for peritoneal fluid) and aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points.

3.3. Record the time of the sample processing completion/ time put into the freezer. Also record the type, number and volume of aliquots prepared.

3.4. Sample storage in freezers:

3.4.1. ***Standard collection:*** Store fluid aliquots in liquid nitrogen (LN_2) freezers, which have less temperature fluctuations, for long term storage.

3.4.2. ***Required minimum:*** Store fluid aliquots at -80°C or lower freezers for long term storage.

3.5. Record any variations or deviations from the SOP, problems, or issues.

3.6. Record the location of each sample in the freezer including freezer number, rack, box, and position in the box along with all other sample attributes in a database. If possible avoid using a spreadsheet format, but preferably use a relational database.

4. Freezer check

4.1.1. ***Standard collection:*** Split aliquots from the same sample type and individual between freezers in case of a freezer breaking down. Check freezers bi-weekly and keep a written-log of checks. Have alarm systems setup on all freezers in addition to human bi-weekly checks.

4.1.2. ***Required minimum:*** Manually check freezers bi-weekly and keep a written-log of checks.

5. Data recording Check list

5.1. Record protocol, specifying which steps are adhered to (standard or minimum).

5.2. For each sample, record:

5.2.1. Date and time of fluid collection (Date: __/__/__ and __:__am/pm).

5.2.2. Start time of sample processing in the laboratory (__:__am/pm).

5.2.3. Type, number and volume of aliquots prepared.

5.2.4. Date and time aliquots stored into freezers (Date: __/__/__ and __:__am/pm).

5.2.5. Any variations or deviations from the SOP, problems, or issues.

5.3. In the long-term, record:

5.3.1. Any freeze-thaw that occurs with a sample for any reason.

5.3.2. Any change of location of a sample, including sending a sample out to an assay lab for processing.

5.3.3. Any new samples created from the original aliquots (i.e., a sub-aliquot) in the same manner as described above.

5.4. Keep a bi-weekly log of freezer checks.

Detailed standard operating procedure for the collection, processing and storage of menstrual effluent (blood) specimens

NOTES

- This SOP does not cover safety procedures for the collection and processing of these samples and personnel must follow institutional biosafety guidelines**.**
- For a summary version of this protocol with side-by-side standard vs. minimal protocol step comparisons, please see "WERF EPHect SOP Fluid Collection Table".
- As this protocol applies to different processing and storage methods, keep a copy of the exact step-by-step protocol used in your lab.

Processing and storage materials

1. Biospecimen form (page 24)
2. Log sheet to record sample-related data
3. A diaphragm or a mixing cannula
4. Sterile closed container for transfer of the sample to the lab
5. Crushed ice if a delay is anticipated
6. Transfer pipette
7. Volume adjustable pipette
8. Centrifuge
9. EDTA/heparin tubes if plasma is going to be collected from the sample
10. Labels suitable for long-term freezer storage, and IDs printed using 2D barcoding
11. Aliquot vials with screw top gasket closure
12. Freezers: -80C or liquid nitrogen (LN_2)

1. Menstrual effluent (blood) collection

1.1. Collect menstrual effluent sample with a diaphragm or mixing cannula.

1.2. Record on the log sheet the day of the menstrual cycle of the sample collection (Day __) and the date and time of menstrual effluent collection (Date: __/__/__ and __:__am/pm).

1.3. Labelling of sample collection container:

1.3.1. ***Standard collection:*** Once the collection container is collected from the patient label the sample with a 2D barcode in addition to human-readable the unique identifier of the patient and sample identifier.

1.3.2. ***Required minimum:*** Once the collection container is collected from the patient, label the sample with the unique identifier of the patient and sample identifier.

1.4. Sample treatment until transported to the lab:

1.4.1. ***Standard collection:*** The sample is put in a closed container and transferred and processed in the laboratory within 1 hour on wet ice.

1.4.2. ***Required minimum:*** The sample is put in a closed container and transferred and processed in the laboratory within 1 hour at room temperature.

2. Sample processing in the laboratory, labelling aliquots and storage

2.1. Record start time of sample processing in the laboratory. Sample should be processed within a maximum of 1 hour.

2.2. In the laboratory, if an unprocessed sample is required for the study, transfer sample into appropriate sized aliquot vial with screw top gasket closure.

2.3. Preparation of sample aliquot tubes:

2.3.1. ***Standard collection:*** Label the aliquot vials with the participant ID number followed by a unique aliquot ID number. For example: ENDO-123456-U654321-ME: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (ME for menstrual effluent) and aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points. Further, include the above information in human readable format and in a 2D barcode on the label.

2.3.2. ***Required minimum:*** Label the aliquot vials with the participant ID number followed by a unique aliquot ID number. For example: ENDO-123456-U654321-ME: Center identifier (ENDO), participant ID (123456), unique aliquot vial ID (U654321), sample type (ME for menstrual effluent) and aliquot number (01). Also, include date of sample creation on the label to be able to distinguish samples from the same participant collected at different time points.

2.4. Sample storage in freezers:

2.4.1. ***Standard collection:*** Store the unprocessed menstrual effluent aliquots in liquid nitrogen (LN_2) freezers, which have less temperature fluctuations.

2.4.2. ***Required minimum:*** Store the unprocessed menstrual effluent aliquots at -80°C or lower freezers.

2.5. Samples should be stored into freezers within maximum of 1 hour and time should be recorded on the log sheet. Also record the type, number and volume of aliquots prepared.

2.6. If a plasma sample is required for the study, the remaining sample from the collection container is transferred into EDTA/heparin tubes and placed on wet ice. Record which tubes is used.

2.7. Centrifuge sample for 10 minutes at 2500 x g at 4°C.

2.8. Aspirate plasma using an appropriate transfer pipette (or ideally a volume-adjustable pipette) into appropriate sized aliquot vials. Label the aliquots as in 2.3, store the processed plasma aliquots as in 2.4 and keep the record of time and type of aliquots prepared as in 2.5.

2.9. Record any variations or deviations from the SOP, problems, or issues.

2.10. Record the location of each sample in the freezer including freezer number, rack, box, and position in the box along with all other sample attributes in a database. If possible avoid using a spreadsheet format, but preferably use a relational database.

3. Freezer check

3.1.2. ***Standard collection:*** Split aliquots from the same sample type and individual between freezers in case of a freezer breaking down. Check freezers bi-weekly and keep a written-log of checks. Have alarm systems setup on all freezers in addition to human bi-weekly checks.

3.1.1. ***Required minimum:*** Manually check freezers bi-weekly and keep a written-log of checks.

4. Data recording Check list

4.1. Record protocol, specifying which steps are adhered to (standard or minimum).

4.2. Record date and time of menstrual effluent collection (Date: __/__/__ and __:__am/pm).

4.3. Record cycle day on the menstrual effluent collection (Day __).

4.4. For each sample, record:

4.4.1. Date and time of fluid collection (Date: __/__/__ and __:__am/pm).

4.4.2. Start time of sample processing in the laboratory (__:__am/pm).

4.4.3. Type, number and volume of aliquots prepared.

4.4.4. Date and time aliquots stored into freezers (Date: __/__/__ and __:__am/pm).

4.4.5. Any variations or deviations from the SOP, problems, or issues.

4.5. In the long-term, record:

4.5.1. Any freeze-thaw that occurs with a sample for any reason.

4.5.2. Any change of location of a sample, including sending a sample out to an assay lab for processing.

4.5.3. Any new samples created from the original aliquots (i.e., a sub-aliquot) in the same manner as described above.

4.6. Keep a bi-weekly log of freezer checks.

EPHect BIOSPECIMEN COLLECTION FORM (to be completed by research nurse)

Date and time sample collected: (DD/MM/YYYY) ___ / ___ / _____ Time: ____:____ AM/PM

What was the first day of your last menstrual period? (DD/MM/YYYY) ___ / ___ / _____

Are your periods regular? (Predictable within one week)
 Yes No

Specify range of days :____ [regular range: 21-35days]

If you have not had a menstrual period in the past 90 days, please tell us why:
☐ Taking hormones continuously (*e.g. the Pill, injections, Mirena, HRT*)
☐ Pregnant
☐ Breastfeeding
☐ Unsure
☐ Other (*Please describe*) _____

Are you currently having a menstrual period/vaginal bleeding *(including spotting for which you only need a panty liner)*?
☐ No
☐ Yes, menstrual period
☐ Yes, irregular bleeding/spotting

Do you currently have a coil [IUD] in place?
☐ No
☐ Yes → If yes, what kind of IUD? ☐ Progesterone containing IUD (Mirena)
☐ Other coil/intrauterine device

When was the last time you had something to eat?
____ : ____ am/pm ☐ Today ☐ Yesterday

When was the last time you had something to drink (other than plain water) and what did you drink?
____ : ____ am/pm ☐ Today ☐ Yesterday
_____

Clinical Measurements:

Height: _____ in cm, or _____ in inches

Weight: _____ in kg, or _____ in pounds

Hip circumference: _____ in cm, or _____ in inches

Waist circumference: _____ in cm, or _____ in inches
See WHO guidelines on how to take measurements (also included on page 30):
www.who.int/nutrition/publications/obesity/WHO_report_waistcircumference_and_waisthip_ratio/en/

If saliva samples are being collected:

Please indicate whether or not you have used the following in the last 24 hours and what time you used each item.

Toothpaste	☐ No ☐ Yes →	___ : ___ AM/PM	☐ Today ☐ Yesterday
Gum	☐ No ☐ Yes →	___ : ___ AM/PM	☐ Today ☐ Yesterday
Cigarettes	☐ No ☐ Yes →	___ : ___ AM/PM	☐ Today ☐ Yesterday
Alcohol	☐ No ☐ Yes →	___ : ___ AM/PM	☐ Today ☐ Yesterday

In the past 24 hours have you eaten:

Spicy food? ☐ No ☐ Yes
Fish? ☐ No ☐ Yes

If urine samples are being collected:

When did you last urinate (prior to providing the sample)?

____ : ____ am/pm ☐ Today ☐ Yesterday

What time was the urine sample produced?

____ : ____ am/pm

Is this urine sample your first morning void?

☐ No
☐ Yes → If yes, did you get up during the night to urinate? ☐ No ☐ Yes

In collecting this sample, did you follow a clean catch protocol?

☐ No
☐ Yes

If undergoing an operation:

Was any pre-med taken before blood, urine, saliva, endometrial fluid and eutopic endometrium/ myometrium collection? (NB. EPHect recommends sample taking prior to pre-med administration)

 No
 Yes

If yes, tick which samples were taken after pre-med administration:

 Blood Urine Saliva Endometrial fluid
 Eutopic endometrium/ myometrium

Time pre-med was administered: ____am/pm

Please specify the type of pre-med was administered _____

Was anaesthetic administered before blood, endometrial fluid and eutopic endometrium collection?

- ☐ No
- ☐ Yes

If yes, tick which samples were taken after anaesthesia administration:

☐ Blood ☐ Endometrial fluid ☐ Eutopic endometrium

If yes, time anaesthetic was administered: ____am/pm

Please specify the type of pre-med was administered: _____

Method(s) of excision:

Ectopic endometrium

- ☐ Electrosurgery
- ☐ Harmonic scalpel
- ☐ Laser [CO_2, NdYag and others]
- ☐ Cold scissors/scapels

Eutopic endometrium

- ☐ Endometrial sampling device
- ☐ Curettage with cervical dilation
- ☐ Brushing

Myometrium

- ☐ Laser [CO_2, NdYag and others]
- ☐ Electrosurgery
- ☐ Cold scissors/scapels
- ☐ TruCut biopsy

Peritoneum

- ☐ laser [CO_2, NdYag and others]
- ☐ Electrosurgery
- ☐ Ultrasound energy
- ☐ Harmonic scalpel
- ☐ Cold scissors/scapels
- ☐ Brushing

Method(s) of collection:

Peritoneal fluid

- ☐ No lavage. Amount collected ___ml
- ☐ Lavage method with 10ml sterile saline solution. Amount of peritoneal lavage fluid (PLF) ___ml

Endometrial fluid

- ☐ No lavage. Amount collected ___ml
- ☐ Lavage method with 4ml sterile saline solution. Amount of uterine lavage fluid (ULF) ___ml

Use of prescription drugs, non-prescription drugs, vitamins or supplements in the past 30 days.

Type of drug	**Have you ever taken this drug every day for over a month?**	**At what age did you first take this drug every day for over a month?**	**In total, how many years you have taken this drug? Please estimate, and enter "0 total years" if less than 1 year.**	**Are you currently taking this drug every day?**	**Please write down the specific name of the drug you have used most recently if known:**
PRESCRIPTION DRUGS	✓ *if yes*	*Age 1st*	*Years taken:*	✓ *if yes*	*Name of drug:*
a. Hormonal medications	☐	__ __	__ __	☐	
Birth control pill	☐	__ __	__ __	☐	
Progestin injection/shot	☐	__ __	__ __	☐	
Transdermal patch/dot	☐	__ __	__ __	☐	
Vaginal ring	☐	__ __	__ __	☐	
Progesterone containing coil/IUD	☐	__ __	__ __	☐	
Hormonal implant	☐	__ __	__ __	☐	
Oral progestins to regulate cycle	☐	__ __	__ __	☐	
GnRH agonist infection/shot	☐	__ __	__ __	☐	
Norethindrone acetate	☐	__ __	__ __	☐	
Danazol	☐	__ __	__ __	☐	
Hormone replacement therapy (HRT)	☐	__ __	__ __	☐	
Other: ……	☐	__ __	__ __	☐	
b. Pain medications	☐	__ __	__ __	☐	
Paracetamol/acetaminophen	☐	__ __	__ __	☐	
Aspirin	☐	__ __	__ __	☐	
Ibuprofen	☐	__ __	__ __	☐	
COX-2 inhibitors (e.g. celebrex, vioxx)	☐	__ __	__ __	☐	
Other anti-inflammatory analgesics (e.g. naproxen, mefanamic acid, aleve, naprosyn, relafen, keoprofen, anaprox)	☐	__ __	__ __	☐	
Narcotic analgesics (e.g. hydrocodone+ paracetamol, codeine, morphine)	☐	__ __	__ __	☐	
Muscle relaxants (e.g. diazepam/ temazepam, buscopan)	☐	__ __	__ __	☐	
Other: ……	☐	__ __	__ __	☐	
c. Diuretic (water pill)	☐	__ __	__ __	☐	
d. Diabetic tablets	☐	__ __	__ __	☐	
e. Insulin	☐	__ __	__ __	☐	
f. Thyroid drugs	☐	__ __	__ __	☐	
g. Drugs for epilepsy	☐	__ __	__ __	☐	
h. Sleeping tablets / tranquilisers	☐	__ __	__ __	☐	
i. Anti-depressants	☐	__ __	__ __	☐	
j. Other drugs to treat mental illness	☐	__ __	__ __	☐	
k. Drugs for osteoporosis ("brittle bones")	☐	__ __	__ __	☐	

l. Drugs for rheumatoid arthritis	☐	__ __	__ __	☐	
m. Antibiotics for a month or more	☐	__ __	__ __	☐	
n. Antacids	☐	__ __	__ __	☐	
o. Drugs for stomach ulcer / gastritis	☐	__ __	__ __	☐	
p. Drugs for high cholesterol	☐	__ __	__ __	☐	
q. Drugs for allergies (antihistamines)	☐	__ __	__ __	☐	
r. Steroids (oral, inhaled, or nasal)	☐	__ __	__ __	☐	
s. Chemotherapy for cancer	☐	__ __	__ __	☐	
t. Tamoxifen for cancer	☐	__ __	__ __	☐	
u. Blood pressure drugs	☐	__ __	__ __	☐	
v. Drugs for angina (chest pain)	☐	__ __	__ __	☐	
w. Other drugs for a heart condition	☐	__ __	__ __	☐	
x. Inhaler for asthma	☐	__ __	__ __	☐	
y. Warfarin / heparin to thin blood	☐	__ __	__ __	☐	
z. Migraine tablets/injections	☐	__ __	__ __	☐	
Other 1: …….	☐	__ __	__ __	☐	
Other 2: ……..	☐	__ __	__ __	☐	
Other 3: ……..	☐	__ __	__ __	☐	
Other 4: ……..	☐	__ __	__ __	☐	
Other 5: ……..	☐	__ __	__ __	☐	

Type of drug	**Have you taken this drug in the past 30 days?**	**Have you taken this drug in the past 48 hours (2 days)?**	**In the past 30 days, on how many days have you taken this drug?**	**Please write down the specific name of the drug if known:**
NON-PRESCRIPTION DRUGS	✓ *if yes*	✓ *if yes*	*Number of days:*	*Name of drug:*
a. Aspirin	☐	☐	__ __ days	
b. Paracetamol	☐	☐	__ __ days	
c. Ibuprofen	☐	☐	__ __ days	
d. Other anti-inflammatory analgesics (e.g. naproxen)	☐	☐	__ __ days	
e. Herbal pain medication: ……..	☐	☐	__ __ days	
f. Other pain medication: ……..				
g. Migraine tablets	☐	☐	__ __ days	
h. Antihistamine for allergies	☐	☐	__ __ days	
i. Cold medicine / lemsip	☐	☐	__ __ days	
j. Decongestant	☐	☐	__ __ days	
k. Cough syrup	☐	☐	__ __ days	
l. Antacids	☐	☐	__ __ days	
m. Sleeping tablets	☐	☐	__ __ days	
n. Eye drops	☐	☐	__ __ days	
o. Vaginal thrush treatments (cream or tablets)	☐	☐	__ __ days	
p. Cystitis treatments / cymalon	☐	☐	__ __ days	
q. Mouth ulcer treatments	☐	☐	__ __ days	
r. Nicotine replacement treatments	☐	☐	__ __ days	
Other 1:.....	☐	☐	__ __ days	
Other 2:.....	☐	☐	__ __ days	
Other 3:.....	☐	☐	__ __ days	
Other 4:.....	☐	☐	__ __ days	
Other 5:.....	☐	☐	__ __ days	
VITAMINS & SUPPLEMENTS				
#1:	☐	☐	__ __ days	
#2:	☐	☐	__ __ days	
#3:	☐	☐	__ __ days	
#4:	☐	☐	__ __ days	
#5:	☐	☐	__ __ days	
#6:	☐	☐	__ __ days	
#7:	☐	☐	__ __ days	
#8:	☐	☐	__ __ days	
#9:	☐	☐	__ __ days	
#10:	☐	☐	__ __ days	

Measurement of waist and hip circumference

I. Waist circumference should be measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest, using a stretch-resistant tape that provides a constant 100 g tension;

II. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor.

For both measurements, the subject should stand with feet close together, arms at the side and body weight evenly distributed, and should wear little clothing. The subject should be relaxed, and the measurements should be taken at the end of a normal expiration. Each measurement should be repeated twice; if the measurements are within 1 cm of one another, the average should be calculated. If the difference between the two measurements exceeds 1 cm, the two measurements should be repeated.