SOP for Sample Collection, Screening and Biobank of malaria or uninfected specimens

1- Consumables:

1. Sterile vacuum EDTA tubes (8ml) for blood collection.
2. Medical disposable butterfly needle for blood collection.
3. Disposable gloves, latex-free, unsterile for sample handling.
4. Disposable sterile pipette tips with filter: 10µl, 20µl, 100µl, 200µl and 1000µl.
5. Lab white coat (long sleeve coat is recommended).
6. 70% isopropyl alcohol (2-propanol).
7. Ethyl alcohol (ethanol).
8. Medical masks.
9. Safety glasses.

2- Equipments and Reagents:

1. Cryotubes of 1.8ml for whole blood and serum samples storage.
2. CareStart™ Malaria Pf/Pv Combo Rapid Diagnostic Test kit.
3. CareStart™ Malaria PAN/Pf Combo Rapid Diagnostic Test kit.
4. DNeasy Blood & Tissue Kit (QIAGEN) for genomic DNA extraction.
5. PrimeSTAR® GXL DNA Polymerase (TAKARA) for PCR amplification.
6. Whatman 903 ProteinSaver Cards for serum spots.
7. Whatman FTA Cards for blood spots.
8. Racks and boxes for 1.8ml cryotubes.
9. Pipettes of 100µl, 200µl and 1000µl.
10. Refrigerator (4ºC) and Freezer (-80°C).
11. Appropriate table-centrifuge.

3- Precautions:

1. The research personnel should wear gloves during sample collection.
2. The research personnel should use appropriate barrier precautions, such as lab coat, glove, mask, and safety glass to prevent exposure to skin and mucus membranes when working with known infectious research subjects.

3. All sample containers, equipments and reagents needed should be assembled prior to the procedure.
4. Samples should not undergo freeze-thaw cycles.
5. Freezers need to have a backup generator or other emergency system options.

4- Procedure

4-1 *P. falciparum* (Pf) or *P. vivax* (Pv) infected individual samples from endemic areas

The research personnel should greet the research subject, identify themselves, and then indicate the sample collection procedure to the research subject. The research subject should be correctly identified (through personal and clinical data sheet) prior to sample collection.

1. *Label sterile vacuum blood collection EDTA tubes.
2. Sterilize with isopropyl alcohol 70% the site of skin puncture (arm venipuncture).
3. Collect blood into sterile vacuum blood collection EDTA tubes, mix gently and keep specimens on ice as follow:
   - 5ml of blood from subjects over 6 years old (subjects A)
   - 3ml of blood from subjects less than 7 years old (subjects B)
4. Proceed to malaria rapid diagnostic tests using CareStart™ Malaria Pf/Pv Combo kit according to the manufacturer's instruction. The purpose of this step is to confirm whether specimens are Pf or/and Pv infected.
5. Keep Pf or Pv positive blood specimens at 4ºC (for no longer than 24 hours). We recommend to carry the following steps in the lab.
6. Proceed to parasitological examinations (microscopy) to evaluate the parasitemia and record the density, species and stage of *Plasmodium* parasites.
7. For each blood sample, *label 2 sterile cryotubes with the appropriate sample identification. Take an amount of each sample into the 2 cryotubes as indicated below, and keep on ice before final storage at -80ºC (biobank in Africa):
   - 2ml of blood sample of subject A (aliquot 1ml in each cryotube)
   - 1ml of blood sample of subject B (aliquot 0.5ml in each cryotube)
8. *Label new sterile cryotubes with the appropriate sample identification. Take from the EDTA tubes 1ml (subjects A) or 0.5ml (subjects B) of blood samples into the cryotubes. Keep on ice before storage at -80ºC (for biobank in China).
9. For dried blood samples, *label Whatman FTA cards with the appropriate sample

identification.

10. Drop 50ul of each blood sample (after step 7) onto the card in concentric circular motions within the printed 4 circle areas. Avoid 'puddling' of the liquid sample as it will overload the chemicals on the card.

11. Allow the samples to dry for 1h at room temperature prior to punching.

12. Put dried blood samples in transparent PE zip-lock plastic bags and store at -30ºC.

13. For each specimen, pipette the remaining whole blood from the EDTA tubes into new 1.8ml cryotubes and *label carefully.

14. Centrifuge all the specimens for 5 minutes at 3,000rpm.

Note: If the blood is not centrifuged immediately, the tubes should be refrigerated at 4ºC for no longer than 48 hours.

15. After centrifugation, separate sera from packed cells by pipetting the sera into new sterile cryotubes.

Recommendation: do not pour! Be very careful not to pick up red blood cells. A better serum transfer can be achieved by keeping the pipette above the packed cells layer and leaving a small amount of serum in the tube.

16. Keep packed cells and serum samples on ice before storage at -80ºC.

17. *Label Whatman 903 ProteinSaver cards with the appropriate sample identification. Drop 50ul of each serum sample onto the card in concentric circular motions within the printed 5 circle areas.

18. Allow the samples to dry for 1h at room temperature prior to punching.

19. Put dried serum samples in transparent PE zip-lock plastic bags and store at -30ºC.

20. Freeze the remaining sera immediately at -80°C upright in a cryotube storage box.

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* **Labelling**:

- Time of sample collection
- City or district of malaria endemic area
- Species of *Plasmodium* (Pf of Pv)
- Age of the infected individuals

4-2 Uninfected individual samples from malaria non-endemic areas

The research personnel should greet the research subject, identify themselves, and then indicate the sample collection procedure to the research subject. The research subject should be correctly identified (through personal and clinical data sheet) prior to sample collection.

1. *Label sterile vacuum blood collection EDTA tubes.
2. Sterilize with isopropyl alcohol 70% the site of skin puncture (arm venipuncture).
3. Collect 5ml of blood into sterile vacuum blood collection EDTA tubes, mix gently and keep specimens on ice.
4. Proceed to malaria rapid diagnostic tests using CareStart™ Malaria PAN/Pf Combo kit according to the manufacturer's instruction. The purpose of this step is to screen out *Plasmodium* negative blood specimens.
5. Keep the specimens at 4ºC (for no longer than 24 hours). We recommend to carry the following steps in the lab.
6. Extract genomic DNA using DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's instruction.
7. Perform **PCR amplification (highly sensitive diagnostic test) using DNA extracts.
8. Run 1.2% agarose gel electrophoreses to validate that the specimens are truly *Plasmodium* negative.
9. Identify carefully *Plasmodium* negative blood specimens.
10. For each blood sample, *label 2 sterile cryotubes with the appropriate sample identification. Take 2ml of each sample into the 2 cryotubes (aliquot 1ml in each cryotube) and keep on ice before final storage at -80ºC (biobank in Africa).
11. *Label new sterile cryotubes with the appropriate sample identification. Take from the EDTA tubes 1ml of blood samples into the cryotubes and keep on ice before storage at -80ºC (for biobank in China).
12. For dried blood samples, *label Whatman FTA cards with the appropriate sample identification.
13. Drop 50ul of each blood sample (after step 11) onto the card in concentric circular motions within the printed 4 circle areas. Avoid 'puddling' of the liquid sample as it will overload the chemicals on the card.
14. Allow the samples to dry for 1h at room temperature prior to punching.
15. Put dried blood samples in transparent PE zip-lock plastic bags and store at -30ºC.

16. For each specimen, pipette the remaining blood from the EDTA tubes into new 1.8ml cryotubes and *label carefully.

17. Centrifuge all the specimens for 5 minutes at 3,000rpm.

Note: If the blood is not centrifuged immediately, the tubes should be refrigerated at 4ºC for no longer than 48 hours.

18. After centrifugation, separate sera from packed cells by pipetting the sera into new sterile cryotubes as descrived above.

19. Keep packed cells and serum samples on ice before storage at -80ºC.

20. *Label Whatman 903 ProteinSaver cards with the appropriate sample identification and drop 50ul of each serum sample onto the card as described above.

21. Allow the samples to dry for 1h at room temperature prior to punching.

22. Put dried serum samples in transparent PE zip-lock plastic bags and store at -30ºC.

23. Freeze the remaining sera immediately at -80°C upright in a cryotube storage box.

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* **Labelling**:
  - Time of sample collection
  - City or district of malaria low or non-endemic area
  - Age of the uninfected individual

* * **PCR Amplification**:
  - Using *Plasmodium* genus-specific primers (Zhou Xia et al., *Parasites* 2014; 21-27)

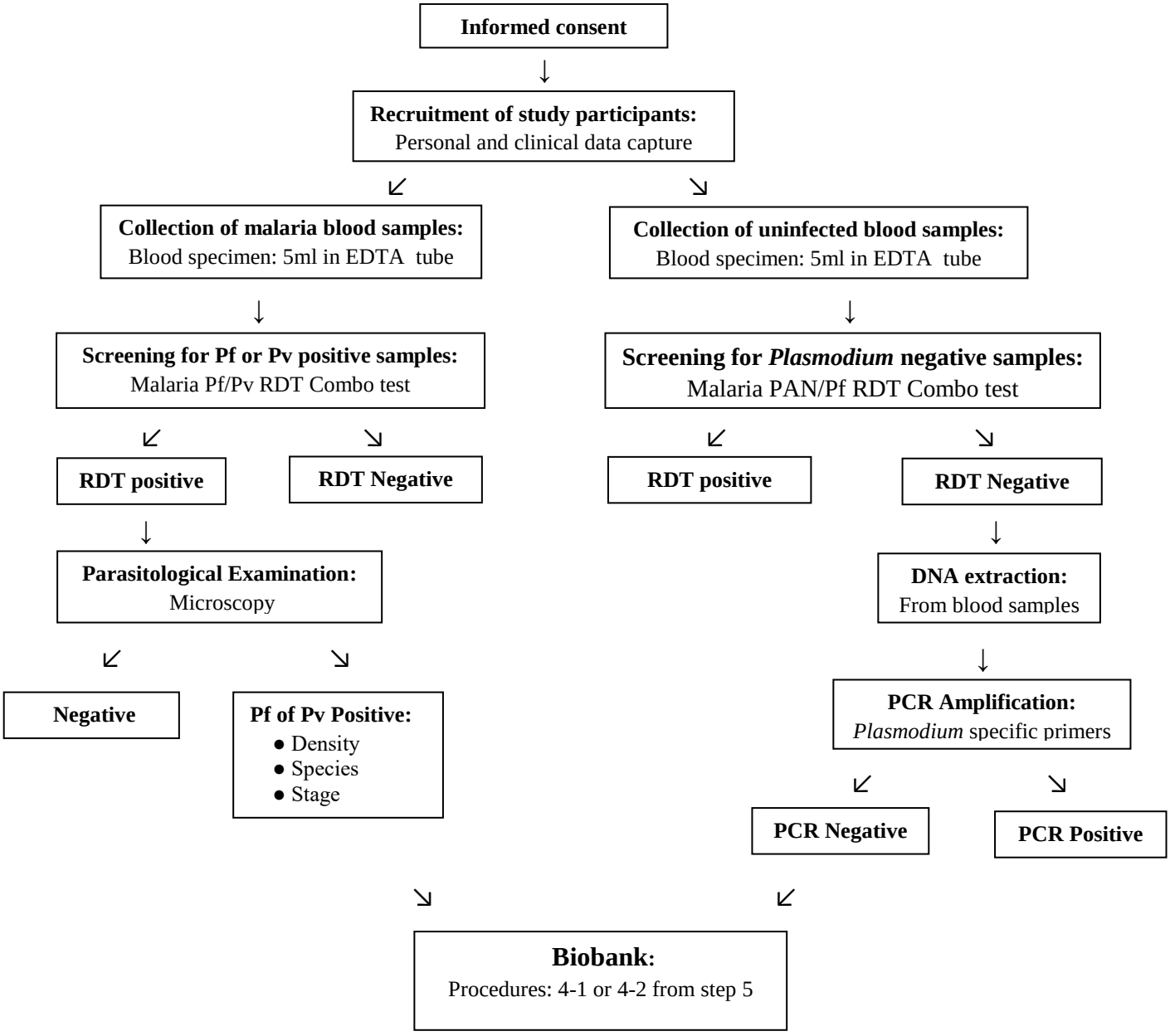
5. Storage of specimens:

1. Before storage, check that all aliquot cryotubes, Whatman FTA and 903 cards are well labelled.

2. Place all aliquots of whole blood samples upright in a specimen box and store at -80ºC.

3. Put all dried samples in transparent PE zip-lock plastic bags at -30ºC.

4. Place all aliquots of packed cells and serum samples upright in a specimen box for cryostorage.



Organization of Specimen Collection from the Field