SOP Title : Aliquots of human stool for molecular diagnosis (PCR) of parasites.

Study title: Repeated doses of Praziquantel in Schistosomiasis Treatment (RePST): An open label, randomized controlled trial of single vs. multiple treatments of praziquantel in intestinal African schistosomiasis in Cote d'Ivoire.

1. Scope and application

Stool aliquot collection for further molecular diagnosis (PCR) of parasites.

2. Responsibilities

- Laboratory technician or person assigned.
- To take a stool aliquot from the stool container before or after Kato-Katz is done (every two weeks, from Week 0 to Week 8).

3. Material

3.1. Safety

- Handle all samples as potentially infectious. Wear gloves and lab coats during the procedure.
- Practice safety precautions for handling and disposal of infectious materials.

3.2. Materials and samples

3.2.1. Materials required

- Cryotubes 2 mL
- Ethanol 96%
- Micropipette to transfer 1 mL of ethanol
- Wooden spatula to transfer stool
- Gloves
- Labels

3.2.2. Samples

- Stool
- Samples should be aliquoted the day of collection, if possible.

4.Procedures

1. Handle the unpreserved stool sample preferably within 24 hours after collection. If not possible, store the samples at 4^0 C.

Always, mix very well the stool before taking the aliquot using a spatula.

2. Preferably sieve the stool (similar to the Kato-slide preparation or use the stool already sieved during the Kato procedure) to remove large debris; if this is too laborious avoid transfer of large particles, stones, seeds etc.

3. Use the 2 mL cryotubes provided (see Figures below). Do not fill the tubes more than 75%.

4. Add 1 mL of ethanol to the tube (see Figure 1). Tubes with ethanol can be prepared before the aliquot collection in order to save time. Always close the tube firmly to avoid ethanol leakage.

5. Add a stool aliquot (see Figure 2) resulting in 1:3 faeces suspension. Volume are rough indications (see Figures 3 and 4), but never dilute less than 1 part of faeces in 4 parts of ethanol, and never more than 1 part of faeces in 2 parts of ethanol. Work as standardized as possible.

Note 1: if diarrhoea, then add 500 µL of the stool and 500 µL of ethanol with the help of a pipette or micropipette.

Note 2: if very hard stool, first transfer an amount to another container and add a little amount of ethanol. Mix it very well to softener the sample. From there, take the aliquot to a 2 mL tube as point 5 explains.

6. Make sure the ethanol is well mixed with the stool. This is extremely important. No big parts of stool should be visible, and when mixing tube up and down should look brown and liquid ish.

7. Close the tubes firmly.

Before storage, always check that the tube has a label with the ID and the week number and that is correlated to the original container with the stool sample.

8. Storage of samples should ideally be at 4 °C. If a fridge is not available, storage at –20 °C is also possible. Keep the tubes standing inside the small white cardboard boxes.

9. If samples are stored –20 °C (which they can be for an in definitive period) then they should also be transported frozen during the whole chain (observe difficulties when doing field work).

10. If they are kept at –20 °C during the study but cannot be stored at –20 °C when transporting to Abidjan, then they should not be frozen again when arriving to Abidjan but kept at 4 °C ideally. If there are no fridges available, the samples can be stored at room temperature, last case.

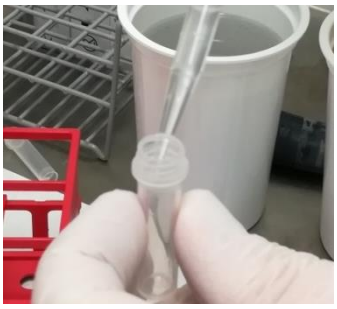

Figure 1 Add 1 mL ethanol

Figure 2. Add a stool aliquot into the tube

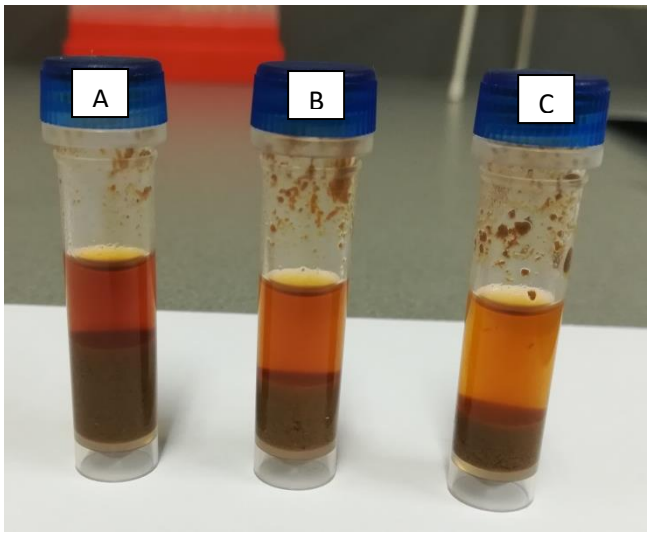

Figure 3. Volume of stool and ethanol ratio.

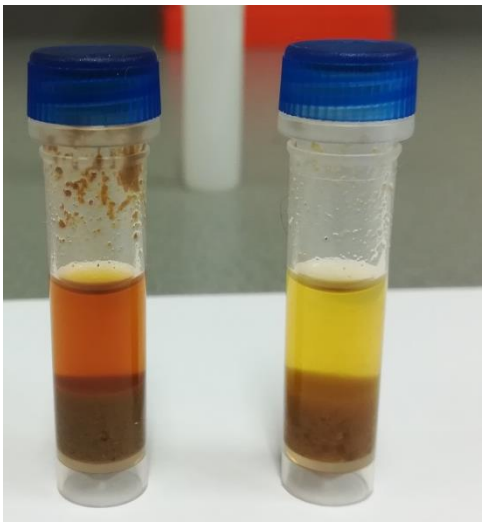

Figure 4. Perfect stool volume.

Tube A has the maximum stool volume allowed.

Tube B has the perfect stool volume (1:3).

Tube C has the minimum stool volume allowed.

General remarks on sample collection

- By any mean use tubes with an external thread screw cap and tubes made of soft-plastic.

The quality of the cap is crucial, in particular for ethanol-preservation.

- Preservation in ethanol means an additional washing procedure before actual DNA isolation.

This is laborious and more expensive. On the other hand PCR results seem to be somewhat more reliable.

- Storage at room temperature in ethanol under tropical conditions still means that temperatures > 40 °C should always be avoided (also during transportation!). Storage at 4 °C is always preferable.

4.1.Waste management

Dispose remaining potentially contaminated material without contaminating the local environment.

4.2. Precautions

1. For all sample materials: do not mix the unpreserved samples in any way with a fixative such as formalin or SAF.
2. Use clean tubes and spatula.
3. High quality tubes are important for storage to prevent leakage and/or evaporation.
4. All human stool products should be handled as potentially infectious material.

2. Waste disposal. Testing materials should be disposed of in accordance with local, state and/or federal regulations.

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