Collection of human sample material for molecular diagnosis (PCR) of parasites

For all sample materials: do not mix the unpreserved samples in any way with a fixative such as formalin or SAF. Be cautious samples will not be contaminated with any fixative during processing. Use clean tubes and spatula. High quality tubes are important for storage to prevent leakage and/or evaporation. More information about preferable tubes is indicated at the end of this document.

a) Collection of stool sample

Storage if well maintained freezers (≤ -20 °C) and frozen transport are available

1. Handle the unpreserved stool sample preferably within 24 hours after production.
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. Transfer at least 1 gram of stool to a tube (Preferably Greiner 4 ml tubes or otherwise the 2 ml tubes indicated below). Anyhow, do not fill tubes for more than 75%.
4. Only if the stool consistency is very dry, add one small drop of distilled water and mix thoroughly.
5. Close the tubes firmly.
6. Mark the tubes clearly (preferably use pre-printed labels, cryo-babies®).
7. Store at –20 °C (–80 °C is also allowed).
8. Transportation should take place at frozen condition (dry-ice).

Ethanol preservation

1. See point 1 and 2 above.
2. Preferably use Greiner 4 ml tubes. Otherwise the 2 ml tubes indicated below, but these are rather small to handle in combination with ethanol. Anyhow, do not fill tubes for more than 75%. Adjust the indicated volumes accordingly if 2 ml tubes are used. Larger tubes than the Greiner are not practical for long term storage.
3. Add a stool aliquot of approximately 700 µl of volume to 2 ml of ethanol (if available 96% ethanol), resulting in a 1:4 faeces suspension. Volume are rough indications, but never dilute less than 1 part of faeces in 3 parts of ethanol, and never more than 1 part of faeces in 1 part of ethanol. Work as standardized as possible.

4. Make sure the ethanol is well mixed with the stool. This is extremely important.
5. Close the tubes firmly. Preferably seal with parafilm to prevent leakage.
6. These ethanol preserved samples can be stored and transported at any room temperature, however preferably at 4 °C, for a maximum of 3 months before DNA isolation takes place. Samples can be stored –20 °C for an in definitive period. In that case they should also be transported frozen.

b) Collection of urine sample

1. Mix the total amount of collected urine. Use 10 ml of urine for filtration or sedimentation for microscopy detection of *Schistosoma* eggs.
2. Transfer another 10 ml to an appropriate tube. Centrifuge for 5 minutes at 710 x g and discard 9 ml of supernatant. If no centrifuge available, leave the urine for two hours and discard supernatant.
3. Transfer 1 ml sediment to a cryotube. Preferably use Sarstedt 72694. Store frozen (≤ -20 °C).
4. Alternatively (but this has not yet been validated): mix the 0.75 ml of urine sediment with 0.75 ml of ethanol (if available 96% ethanol) and store at room temperature (or if possible at 4 °C). Preferably use Sarstedt 72694.

c) Collection of miscellaneous samples

Preservation of blood

For blood samples (EDTA), store at least 500 µl under frozen conditions without additives. Preferably use Sarstedt 72694 tubes.

Preservation of Baermann and culture sediments

If Baermann and/or culture are used in a specific project for detection of nematodes, it is important that microscopically positive Baermann and culture sediments are preserved in ethanol for molecular confirmation and additional genetic analysis. (For better microscopic identification of the larvae, ethanol can also be used to stop the movement of the larvae, Iodine should not be used). Mix 1 ml of sediment with 1 ml of ethanol (if available 96% ethanol) and store at room temperature (of if possible at 4 °C). Preferably use Sarstedt 72694 tubes.

Preservation of other materials

For example vaginal lavages, sperm and tissue biopsies can be tested for parasite DNA. See also collection of stool sample. Preferably store frozen (≤ -20 °C) and use Sarstedt 72694 tubes. If no freezer available, ask advice from our group beforehand. Depending on the type of material ethanol can be added.

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.
- We experienced that frequent thawing and re-freezing has a strong negative effect on the PCR outcome, in particular for *Strongyloides*. Storage temperatures around +2 to -10 °C should be avoided by any means.
- 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.
- A stool aliquot may also be fixed in SAF fixative for retrospective microscopy. Add an amount of stool of approximately 300 µl to 1 ml of SAF and make sure the SAF is well mixed with the stool. The SAF fixed sample may be stored at room temperature for a long period. Be very careful that no SAF or formalin at all is mixed with a sample predetermined for PCR. Always use clean equipment. Mark all SAF containing samples clearly to avoid confusion with tubes used for PCR analysis.

Additional data for research on stool parasites

We are always interested in the following information:

- gender and age of the patient
- type of intestinal problems
  - diarrhoea (Y/N)
  - duration diarrhoea <1 week / >1 week / > 4 weeks / intermittent
  - severity of diarrhoea <3x per day / 3-7x per day / > 7x per day
- macroscopic examination of stool
  - hard / formed / soft / watery
  - bloody (Y/N)
  - mucoid (Y/N))
- if microscopy done, please indicate procedures used and findings for each samples
  - parasite species detected
  - stages (cysts, trophozoytes, eggs, larvae)
  - intensity of infection (individual egg count per kato-slide or per sample, number of cysts per field or otherwise according to own used procedure)
- recent history of anti-parasitic treatment

In *Schistosoma* endemic regions, including *S. mansoni*, we are always interested in urine samples for circulating antigen (CCA/CAA) determination. Contact us for details about required volumes and storage conditions.

February 2016; Leiden Clinical Parasitology Group
Department of Parasitology; Leiden University Medical Center; The Netherlands

Websites:
https://www.lumc.nl/org/parasitologie/research/
https://www.lumc.nl/org/parasitologie/research/clinical-parasitology/
https://www.lumc.nl/org/parasitologie/research/clinical-parasitology/Diagnostics-Research/
https://www.lumc.nl/org/parasitologie/research/clinical-parasitology/Diagnostics-Research/background/#protocols

Sarstedt tubes – 72694 - 2.0 ml

SARSTEDT

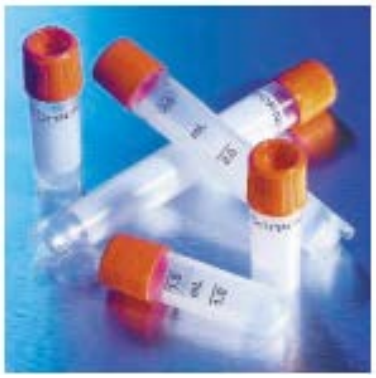
Your partner in medicine and science worldwide...

Description

Screw cap micro tube

Art. No.	72.694
Volume	2 ml
Diameter	10.8 mm
Length	46 mm
Base shape	conical, skirted
Type of closure	screw cap
Material	polypropylene (PP)
Colour	transparent

Corning External Thread Cryogenic Vials - 430659 - 2.0 ml (Round bottom)



Greiner Bio-One B.V. - Scintillation vial, PE, 4ml, 15x57mm, sep screw cap (2000x) – Order No. 146302