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1. Introduction

The Baermann technique is used to separate larvae from faecal material based on the active migration of larvae from an unpreserved stool sample held in a pouch to a freshwater environment. Following a pre-incubation period of 18-24 hrs the pouch with faeces is suspended in lukewarm water. After permitting sufficient time to allow migration, the supernatant is discarded and the sediment is examined microscopically for the presence of *Strongyloides stercoralis* larvae.

2. Scope and application

This Standard Operating Procedure (SOP) provides instructions for preparing and examining fresh stool samples collected from participants of the ALIVE trial for the diagnosis of *Strongyloides stercoralis* infection by a modified Baermann technique. This protocol is applicable to all fresh stool samples collected and received at the laboratory for testing in the context of the ALIVE trial.

3. Responsibilities

Head of Site Laboratory: must ensure that all technicians are well trained and strictly follow this SOP. He/ She must ensure the quality of results.
Laboratory technicians: must know the contents of this SOP and follow it strictly.

4. Safety

Stool samples should be treated as potentially infectious and universal precautions should be always followed, such as:

- ✔ Always wear gloves on the hands during manipulation of biologic materials
- ✔ Take maximum care when pouring off the supernatant in the falcon tube to prevent any splash and contamination
- ✔ Cover slips break easily, so handle them with care to prevent skin cutting
- ✔ Clean the work surface after the work is finished with 1% bleach and 70% alcohol

5. Materials

Equipment

- Microscope
- Scale (digital balance) suitable to weight 1 and 3 grams
- Thermometer to measure the temperature of wet or dry environments
- Incubator in case the room temperature is below 21°C
- Timer

Consumables

- Stool collection cup
- Petri dish
- Spatula
- Gauze: 8 layers – non-sterile, 100% cotton-wool gauze of 5x5 cm
- Wooden applicator stick
- Microscopic slide
- Coverslips
- 50 mL falcon tubes
- Plastic Jar
- Test tube racks
- Laboratory tissue paper
- Labels
- Lukewarm water

NB: lukewarm water can be tap water, as long as it does not contain free living nematodes and is free of chlorine. This should **NOT** be distilled water. Bottled water for consumption is allowed. It is important that the water is lukewarm (around 30°C – 35 °C, maximum 37°C). If needed, use a water bath or incubator to warm the water.

Reagents

- Lugol's Iodine (Iodine-Potassium Iodide) solution (e.g. Sigma-Aldrich; catalog n. 1092611000)
- Activated charcoal (LobaChemie, CAS. 7440-44-0)

Samples

- Fresh (unpreserved) faecal samples
- Process the faecal samples as soon as they arrive at the laboratory. If delay is unavoidable, process them within 24 hours after collection
- Do not refrigerate or freeze the faecal samples before taking the amount needed for the Baermann technique

Other SOPs

- SOP_001_v02 (ST_COLLECT): Stool collection, labelling and transportation to the laboratory
- SOP_002_v02 (ST_FLOW): Stool sample flowchart
- SOP_004_v02 (ST_ALIQ): Reception, aliquoting and storage of stool samples

6. Procedure

Sample preparation

- Using a clean and labelled stool cup, weigh 3 g of stool sample using a suitable scale (digital balance)
- Mix the stool sample with a few drops of water till the consistency becomes smooth (like peanut butter); stool which is too watery cannot be used. The addition of some water to the stool sample enables it to homogenize and make the stool sample smooth. Do not add too much, as this will cause too much leakage through the gauze
- Add 1 g of activated charcoal and mix it until is completely homogenized
- Label a petri dish and place 2 pieces of gauze at the bottom
- Place the faecal material at the centre of the gauze and fully cover the stool sample by folding the gauze up (see pictures A, B and C in Appendix A)
- Close the petri dish and leave it at room temperature (21°C-37°C) for 18 - 24hr. Use an incubator if the room temperature is below 21°C. To confirm the room temperature does not exceed the definite range it is recommended to monitor and record daily the ambient temperature using a calibrated thermometer

Sample filtration

- Open the petri dish
- Make 4 openings at the corners of the gauze and form a pouch containing the faecal material by putting a wooden stick (see picture D in Appendix A)
- Label a 50 mL falcon tube with the sample identification code

- Fill the 50 mL falcon tube with approximately 35 mL of lukewarm water (previously heated if needed)
- Place the pouch with the faecal material in the 50 mL falcon tube making sure the pouch only lightly touches the water but is not fully immersed (see picture E in Appendix A)
- Leave the 50 mL tube to stand for 2 to 3 hours at room temperature (21°C-37°C). Use an incubator if the room temperature is below 21°C[1]
- Decant the supernatant and leave the sediment (approximately 3 mL) to settle for another 30 minutes

Note 1: to confirm the room temperature does not exceed the definite range it is recommended to monitor and record daily the ambient temperature using a calibrated thermometer

Microscopic examination

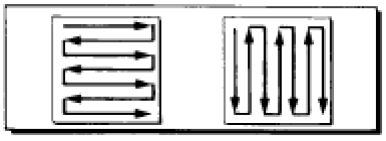
- Check the sample identification code
- Bring a droplet of the sediment using a Pasteur pipette to a microscope slide, do not add a cover slip
- Examine the slide under a microscope at low power (4x10 or 10 x 10)
- As soon as 1 or more larvae are found, add a drop of lugol´s iodine to kill the larvae, add a cover slip and examine further at low (10x10) or high (10x40) power
- If the first slide is negative, examine a new droplet using the same slide and repeat the step until the full sediment has been examined
- Record the total number of *S. stercoralis* larvae seen in the sediment in a semi-quantitative way using the following categories:

0	Negative
1+	1-3 *S. stercoralis* larvae (L1+L3)
2+	4-10 *S. stercoralis* larvae (L1+L3)
3+	10-100 *S. stercoralis* larvae (L1+L3)
4+	> 100 *S. stercoralis* larvae (L1+L3)

- In the recording no differentiation is made between Rhabditiform larvae (L1) and filariform larvae (L3)

Remark:

- Read the slides systematically



Reporting of results

- Use the ***"WHO Bench Aids in the Diagnosis of Intestinal Infections"*** or refer to colour plates of parasites for morphological identification.

Quality control

- Use clean, dry and leak proof containers for sample collection
- As soon as *S. stercoralis* larvae are seen in the sediment, a second microscopist should confirm the determination. The counting of the number of larvae is not confirmed.

7. General remarks

Waste management

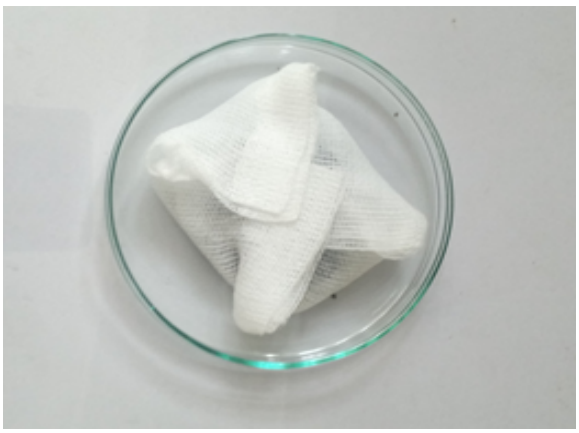
- Testing materials should be disposed of in accordance with local, state and/or federal regulations.

8. References

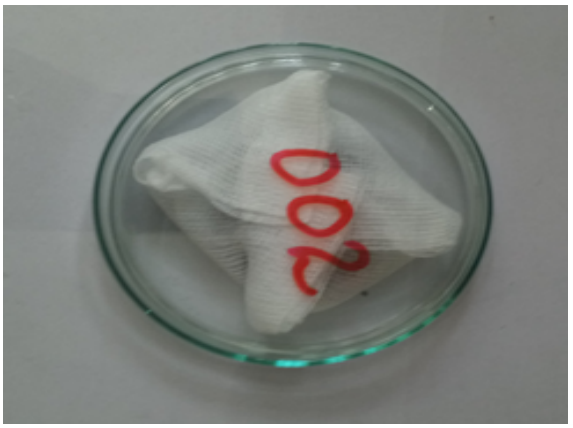
1. Watts, MR., Robertson, G., & Bradbury R. The laboratory diagnosis of *Strongyloides stercoralis*. Microbiol Aust. 2016; (doi:10.1071/ma16003)
2. World Health Organization. Basic laboratory Methods in medical Parasitology, WHO, Geneva, 1991
3. World Health Organization. Bench aids for the diagnosis of intestinal parasites, second edition, WHO, Geneva, 2012
4. Gelaye W, Williams NA, Kepha S, Junior AM, Fleitas PE, Marti-Soler H, et al. (2021). Performance evaluation of Baermann techniques: The quest for developing a microscopy reference standard for the diagnosis of *Strongyloides stercoralis*. PLoS Negl Trop Dis 15(2): e0009076. https://doi.org/10.1371/journal.pntd.0009076

Appendix A: Sample preparation and filtration

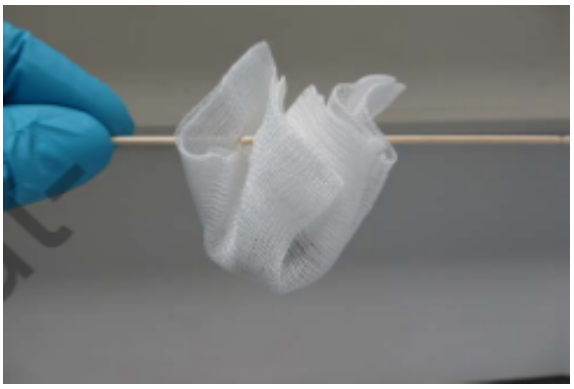
A. Place the stool sample at the centre



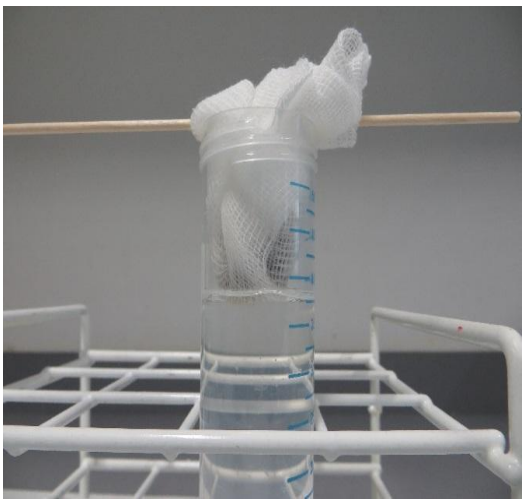
B. Cover the stool sample by folding the gauze up



C. Close the petri dish and incubate



D. Make 4 openings at the edges of the gauze and form a pouch containing the faecal material by putting a wooden stick



E. Place the pouch with the faecal material in the 50 mL falcon tube slightly touching the water