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Written by	Augusto Messa Jr.
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Reviewed by	Stella Kepha
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Read, reviewed and approved by	Name and Date
BDU (Wendemagen Enbiale)	Wendemagegn Enbiale, 8th December, 2021
FM- CISM (Inácio Mandomando)	Inacio Mandomando, December 20, 2021
KEMRI (Charles Mwandawiro)	Charles Mwandawiro, 7th December 2021
LUMC (Lisette van Lieshout)	Lisette van Lieshout, 7th December 2021

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1. Introduction

The Kato-Katz technique is a copro-microscopic method for the diagnosis of soil transmitted helminths (STH) currently considered the gold-standard for the diagnosis of these parasites. It facilitates the detection and quantification of helminth eggs that infected subjects pass in their stools. In this method, stools are sieved through a mesh screen to remove large particles and transferred to a standard hole of a template (41.7 mg) on a slide. After filling the hole, the template is removed, and the remaining sample is covered with a piece of cellophane soaked in glycerol. The glycerol clears the faecal material around the eggs. The eggs are then counted under a light microscope and the infection rate will be calculated as eggs per gram of stool (EPG).

The Kato–Katz technique is the diagnostic method recommended by the World Health Organization for monitoring large-scale treatment programmes implemented for the control of STH infections because of its simple format and ease of use in the field, as well as the possibility of determining the EPG which allows the estimation of Cure Rates (CR) or Egg Reduction Rates (ERR) after treatment. To increase the sensitivity of the method, 2 slides of each sample are analysed.

The following nematode species can be detected by Kato-Katz:

- *Ascaris lumbricoides*;
- *Trichuris trichiura*;
- Hookworms, without differentiating species (i.e. *Necator americanus*; *Ancylostoma duodenalis* and *A. ceylanicum*);
- *Schistosoma mansoni*.

2. Scope and application

This Standard Operating Procedure (SOP) describes the procedures for Soil Transmitted Helminths eggs screening and quantitation by the Kato-Katz technique from stool samples for participants in the ALIVE Trial. This protocol is applicable to all fresh stool samples collected in the context of the ALIVE trial and received in the Parasitology Laboratory for testing.

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;
- ✓ Clean the work surface after the work is finished with 1% bleach and 70% alcohol.

5. Materials

Equipment

- Light microscope with a maintenance certificate

Consumables

- Standard Kato-Katz plastic template with a punched hole for 41.7 mg of stool; make clear notes if the used templates have a deviant size
- Spatulas (plastic or wooden)
- Mesh (metallic or nylon) with pore size 60-105 µm
- Hydrophilic cellophane paper, 40-50 mm, 25 x 30 mm (or 25 x 35 mm)
- Microscope slides
- Counters
- Stopwatches or timers
- Tweezers
- Laboratory tissue paper
- Aluminium foil or a piece of plastic sheet or a Petri dish
- Scrap paper or old newspapers
- Labels
- Gloves
- Toothbrushes to clean the template
- Toilet paper
- Buckets and basins
- Towels
- Phones and their chargers
- Pencils and marker pens
- Bin liners
- Slide boxes

Reagents

- Malachite green/methylene blue powder
- Glycerol
- Standard saline solution (0.9% NaCl)
- Distilled water

Preparation of solutions

Kato Katz stock solution:

- Add 3 g of malachite green/methylene blue powder to 100 mL of distilled water in a sterile glass bottle;
- Mix well and store at room temperature away from direct light
- Label as “3% malachite green (or methylene blue) stock solution” and add the date of preparation.

Kato-Katz working solution:

- Add 3 mL of the Kato-Katz stock solution to a mixture of 100 mL glycerol + 100 mL of distilled water.

- Mix well and store at room temperature away from direct light
- Label as “Glycerol-malachite green (or methylene blue) working solution” and add the date of preparation.

Remarks:

1. The solutions (stock and working) should be stored at room temperature in a place protected from direct light. Renew the working solution regularly (on a weekly basis) and make sure there is enough solution at least 24 hours before receiving any stool samples
2. Pre-soak the cellophane paper pieces in the Kato-Katz’s solution at least 24 hours prior to usage.

Samples

- Only fresh faecal samples should be used, not older than 24 hours.
- Ideally all the samples should be analysed on the day of stool passage and collection, but if circumstances require, they must be analyzed within 24 hours after receiving them in the laboratory.
- Faecal specimens for Kato-Katz can be temporarily stored at 2-8°C before processing, but the priorities defined in SOP_002 should be followed strictly.
- For each participant stool sample two Kato-Katz slides will be prepared.
- If the stool sample is too hard, add a few drops of standard saline solution (0.9% NaCl) and mix to soften the sample (till the consistency becomes smooth like peanut butter), then continue with the procedures described on the sample preparation section.
- If the stool sample is liquid, skip steps 4 to 6 from the sample preparation section and use a Pasteur pipette to transfer the volume of stool to fill the Kato-Katz template and proceed from step 7.

Other SOPs

- SOP_001 (ST_COLLECT): Stool collection, labelling and transportation to the laboratory
- SOP_002 (ST_FLOW): Sample flowchart
- SOP_004 (ST_ALIQ): Reception, aliquoting and storage of stool samples

6. Procedure**Sample preparation**

1. Label the 2 microscope slides, using preprinted labels with the unique sample identifier and indicate slide A and slide B
2. Place the standard Kato-Katz template on top of the labelled microscope slide
3. Using a spatula, stir the stool sample thoroughly until it is fully homogenized: the colour, consistency and content of the whole stool sample must look and/or feel the same (as described in SOP_004 (ST_ALIQ): Reception, aliquoting and storage of stool samples)

Sample sieving

1. Place 2 g of stool samples on aluminium foil or a piece of plastic sheet or a Petri dish
2. Place the mesh (metallic or plastic) on the stool sample, press strongly on the stool so that it is

sieved through the mesh

3. Use the spatula to scrape the sieved faecal material
4. Fill the template hole with the sieved faecal material and make sure no air bubbles are formed.
5. Remove the plastic template by lifting it vertically (avoid horizontal movements)
6. Use the tweezers to cover the cylinder of stool sample on the slide with a cellophane membrane pre-soaked with the Kato's solution
7. Turn over the slide and press it on a smooth surface covered with 2-3 layers of tissue or filter paper in order to spread the sample between the cellophane cover and the microscope slide as much as possible (be careful to avoid leakages and breaking the microscope slide)
8. Turn over the slide again, taking care to not leave the cellophane paper on the tissue and make sure the sample is well extended (to verify, try to read a sheet through the slide)
9. Allow the slide to clear for 30-60 minutes, protected from direct sunlight (in a drawer for example)
10. Proceed with the microscopic examination

Remarks: If hookworm is present in the community under investigation, it is essential to read the slides shortly after a clearing time of 30 minutes, with a maximum clearing time of 60 minutes. In the event that circumstances make it impossible to read the samples on the same day (for e.g. too many samples received late in the day), the slides should be examined for hookworm within 30 min and a second reading should be performed to identify *Trichuris* and *Ascaris* ideally within 24-48 hours.

Microscopic examination

1. Check the sample identification.
2. Observe the preparation under the microscope starting with the 10X objective.
3. Observe all the slide fields systematically and carefully (see Figure 1).
4. Count all the eggs observed for each helminth species and record the result by slide. Record egg counts separately for slide A and slide B. The two slides should be read independently by two different technicians.
5. Repeat the procedure for all the samples to be examined.

Quality control

Each day, 10% of the slides should be randomly selected to be read by an independent reader (e.g. laboratory supervisor or expert technician assigned for QC), who is not any of the two slide readers, for quality control purposes.

Discrepant/ inconsistent results will be considered if there is a difference:

- In presence/absence of a specific helminth species; or
- Differences in egg counts exceed:
 - 10 eggs for Kato-Katz slides with ≤ 100 eggs.
 - Exceed 20% for Kato-Katz slides with more than 100 eggs.

If a discrepancy is detected between the first readings and the QC reading, the third technician should read the slide and his/her reading is considered the valid one. All discrepancies should be discussed daily with all the technicians involved, to improve the quality of the results.

Reporting results

Each trial site must set-up an internal mechanism to timely report the screening Kato-Katz results to the investigators or operational team (study coordinator or delegated team member) in charge of the field activities in order to schedule the randomization/baseline visit of eligible participants.

Likewise, if a sample gets damaged during processing and Kato-Katz results cannot be obtained, the operational team must be immediately informed in order to ensure a new sample is collected within

the protocol allowed time window. Same is applicable for the follow up samples collected during the final study visit on day 21.

7. General remarks

Waste management

Dispose of potentially contaminated material without contaminating the local environment.

Precautions

Ensure that local SOPs on screening and sample delivery are available to staff.

8. References

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3. Speich B, Ali SM, Ame SM, Albonico M, Utzinger J, Keiser J. Quality control in the diagnosis of *Trichuris trichiura* and *Ascaris lumbricoides* using the Kato-Katz technique: Experience from three randomised controlled trials. *Parasites and Vectors*. 2015;8: 1–8. doi: 10.1186/s13071-015-0702-z
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Appendix A: Bench aid for sample preparation

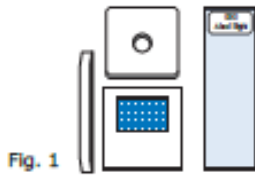


Fig 1 - 7. Adapted from WHO (2019) - Bench aids for the diagnosis of intestinal parasites.