STANDARD OPERATIVE PROCEDURE FOR KATO KATZ TECHNIQUE

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1.0 Distribution list (Controlled copies):

S. No.	Location	Concerned Personnel (sign with date)

2.0 Amendment record:

S. No.	Page no.	Changes made	Amendment date	Authorized by (Sign with date)

3.0 Purpose:

Kato Katz technique is used for qualitative and semi-quantitative diagnosis of soil transmitted helminths(STH) primarily *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, Schistosoma spp. in the stool samples.

4.0 Principle:

People infected with STH or intestinal schistosomes pass the eggs of the worms in their faeces. In the Kato-Katz technique, faeces are pressed through a mesh screen to remove large particles. A portion of the sieved sample is then transferred to the hole of a template 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 and the number is calculated per gram of faeces.

5.0 Materials:

1. Kato-set (template with hole, screen, nylon or plastic, plastic spatula)
2. Wooden applicator
3. Blotting paper or Newspaper
4. Microscope slides
5. Cellophane as coverslip, soaked in a glycerol-malachite green or glycerol-methylene blue solution.
6. Tissue roll
7. Red and yellow biomedical waste bag
8. Stop Watch
9. Microscope

6.0 Procedure:

1. Label a glass slide with the sample number and then place a standard 41.7 mg plastic **template** on top of it.

2. **Homogenize the whole stool sample by stirring it well (not vigorously) for 15-30 sec** with wooden spatula prior to preparing the Kato-Katz thick smear. **Use a new wooden spatula for each sample**.
3. Place a small amount of the fecal sample on a filter paper/ newspaper and press a piece of nylon screen on top. Using a spatula, scrape the sieved fecal material through the screen so that only the debris remains.
4. Scrape up some of the sieved feces to fill the hole in the template, **avoiding air bubbles** and leveling the feces off to remove any excess.
5. Carefully lift off the template and **discard in a Red bio medical waste bag**.
6. Place one piece of the cellophane, which has been soaked overnight in 3% methylene blue/ malachite green glycerol solution, over the fecal sample.
7. Place a clean slide over the top and press it evenly downwards to spread the feces in a circle. Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip. If done well**, it should be possible to read newspaper print through the stool smear.** Place the slide with the cellophane upwards.
8. Keep all prepared Kato Katz **slides on the wet tissue paper** till internal quality control is done.

7.0 Examination and result:

After the preparation of the slide, wait for 15-30 min for the clearing of faecal material around the eggs. Wipe off the slides' bottom surface with dry tissue paper to clean water drops/marks. Place it under a microscope and examine all fields in a **systematic zigzag pattern at low power**. First of all, **screen all slides for Hookworm** at priority, giving **at least 2-3 minutes to each slide** then in second round, read them for other two parasites species using the same time span. Hence, estimated time span should be **at least 7 to 8 minutes to read a Kato Katz slide for all three STH species**. Use stop watch to ensure the given time span. Record the number of eggs of each species on a recording form alongside the sample number. The number of eggs found in the faecal smear should be multiplied by the constant 24 to obtain the number of eggs per gram of faeces.

8.0 Quality Control:

All slides examined under the microscope should not be discarded till internal quality control is done. A total of 10% positive and negative slides will be randomly selected and re-examined by another microscopist at the same time.

9.0 Limitations:

False negative results in case of light intensity of infection or in initial stages of infection (pre- patent) or soon after treatment due to suspension of oviposition.

10.0 Assay time: 35-40 minutes

11.0 Precautions:

Process the specimen using standard safety precautions.
For hookworm detection, the slide should be read **within 30–60 minutes** after the preparation otherwise the hookworm eggs disappear.

Prepare Kato Katz slides in a lot of 10-15 slides depending upon the number of microscopist (5 slides/microscopist).

Keep unprocessed samples in a cold chain (2-8°C) before processing.

Don't discard samples till internal quality control is done. Maintain cold chain for processed samples too.

12.0 References:

1. Bench Aids for the diagnosis of intestinal parasites. World Health Organization (2019).

2. Barbosa CS, Gomes ECS, Marcelino JMR et al.Quality control of the slides by Kato-Katz method for the parasitological diagnosis of schistosomiasis infection by Schistosoma mansoni. J Bras Patol Med Lab, v. 53, n. 2, p. 110-114, April 2017

3. Bosch F, Palmeirim MS, Ali SM et al. Diagnosis of soil-transmitted helminths using the Kato-Katz technique: What is the influence of stirring, storage time and storage temperature on stool sample egg counts? PLoS Negl Trop Dis. 2021 Jan 22;15(1): e0009032.