

nematodes and cestodes such as schistosoma



SPC | *Life Serves*

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MLS 221-Lab
CLINICAL PARASITOLOGY- Laboratory
Kato-Katz Method for Stool Analysis

San Pedro College
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Learning Objectives



At the end of the laboratory session, students will be able to:

1. Discuss the importance of Kato-Katz technique in diagnosing parasitic infections
2. Explain the processes in the performance of the technique
3. Discuss the advantages and disadvantages of the technique
4. Demonstrate the procedure and the manner of reporting the results



INTRODUCTION



- The Kato-Katz technique facilitates the detection and quantification of helminth eggs that infected subjects pass in their stool.
- Also known as “Cellophane Covered Thick Smear” helminths eggs and schistosoma
- Based on the detection of helminth eggs in small amounts (41.7 mg) of fresh stool.
- A thick smear is prepared on a microscopic slide and helminth eggs are enumerated under a light microscope and recorded separately for each helminth species.

Template may vary. The volume of the stool can vary



INTRODUCTION



- Subsequently, the prevalence and intensity of helminth infections can be determined.
- It is the *diagnostic method recommended for monitoring large-scale treatment programs implemented for the control of soil-transmitted helminth infections* because of its simple format and ease of use in the field.
- Alternative techniques are McMaster and FLOTAC. Commercial diagnostic kits are available for immediate use in the field. (WHO)



INTRODUCTION



- A technique used for qualitative and semi-quantitative diagnosis of intestinal helminthic infestations caused by *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, and especially *Schistosoma* spp.
- WHO recommendation: the Kato Katz technique as the **gold standard** in areas with...
 - Moderate to high transmission rates of soil-transmitted helminths (STH) - i.e. where the proportion of infected individuals is >20 to >50%
 - Intestinal schistosomiasis (>10-50%).
- Where the prevalence of STH is <20%, the specificity of this technique makes it less appropriate, and more sensitive tools should be used.

kato katz technique is good if the area has a moderate to high number of oof cases



KATO-KATZ METHOD

Principle

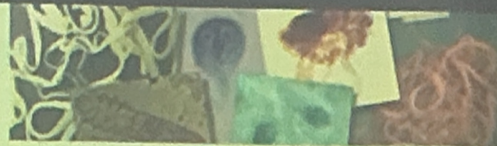


- In the Kato-Katz technique, feces are pressed through a mesh screen to remove large particles.
 - A uniform amount of stool is examined through the use of a template with a uniform-sized hole in the middle.
 - A portion of the sieved sample is then transferred to the hole of a template on a slide.
 - After filling the hole, the template is removed and the remaining sample is covered with a piece of cellophane soaked in glycerol-malachite green.
 - The glycerol *clears* the fecal material from around the eggs while the **malachite green** besides being a dye, is *bactericidal*.
- The eggs are then counted and the number is calculated per gram of feces.



KATO-KATZ METHOD

Quantification of Parasitic Eggs



- Parasite intensity:
 - The number of eggs counted of each STH species per 1 gram of stool.
 - A Kato-Katz smear consists of 41.7 mg of stool. **stool can be 20 mg to 50 mg**
 - To calculate the number of eggs per 1 g of stool (EPG) multiply the egg count from the slide by a factor of 24 ($24 \times 41.7 \text{ mg} \approx 1 \text{ g}$).



KATO-KATZ METHOD

Quantification of Parasitic Eggs



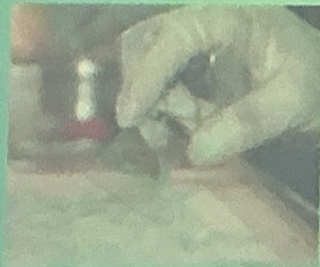
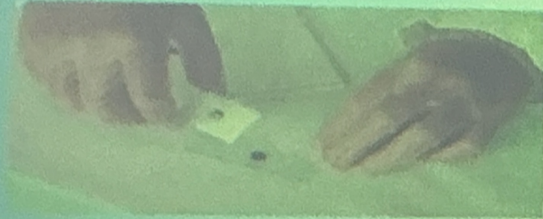
- All eggs seen in the whole preparation are counted.
- Unit used: EPG (Eggs per Gram)
 - It is the estimated number of Eggs Per Gram of stool excreted by the examined infected individual.
- The total egg count is multiplied with a factor depending on the amount of stool used.
 - Multiply the # of counted eggs with **20**, if 50 mg template is used.
 - Multiply the # of counted eggs with **24**, if 41.7 mg template is used.
 - Multiply the # of counted eggs with **50**, if 20 mg template is used.



ADVANTAGES



- Larger quantity of feces can be placed on the slide than the usual direct wet mount or direct fecal smear.
- Satisfactory for detecting medium and large helminth eggs (in feces).
 - *Ascaris* & *Trichuris* eggs are visible at any time
- Several field studies confirm the **simplicity**, **sensitivity**, and **cost effectiveness** of the method.
- The technique can **only be done on fresh-formed stools**.



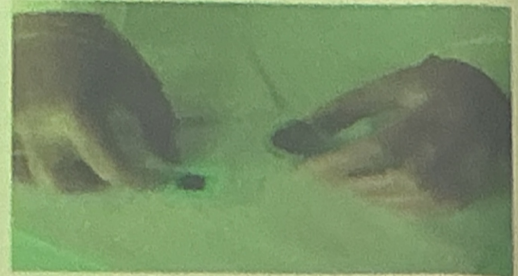
the main determinant for sensitivity is the consistency of the stool. It is used for fresh formed stools, not ideal for watery stools



HPO to LPO only for Ascaris and Trichuris eggs

DISADVANTAGES

- Requires between **1 to 2 hours before the glycerin clears the background of the stool smear** on the slide for accurate visualization of most helminth eggs.
- MAJOR problem: Few hours after the preparation of the slide **hookworm eggs are difficult to recognize** due to over-clearing by glycerin
 - After making the smear with glycerin, microscopic examination should be done within 30-60 minutes.
 - Hookworm eggs are visible 30 minutes after preparation.



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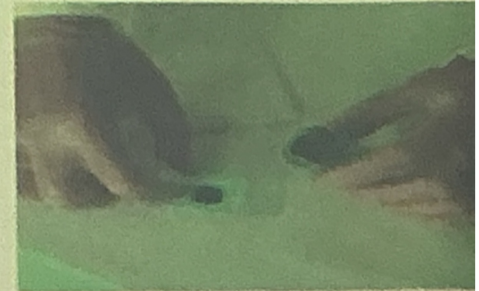
Ascaris and Trichuris compared to hookworms have thick shells



DISADVANTAGES

- Ideal time for observing *Schistosoma* eggs:
 - 24 hours after preparation but with bright sunlight, the slide will clear rapidly and can be examined. (WHO, 1986)
 - For the identification of *Schistosoma* ova, 1% eosin solution can be layered over the cellophane paper. This method can help in the visualization of the miracidium.
- It may NOT be good for small trematode eggs and of little value in detecting protozoan infections.
- This technique is not done on liquid and preserved samples.

WHO - Within 24 hours of after hours but recent studies say it must be within 24 hours



Materials and Reagents

- Wooden applicator sticks.
- Screen (stainless steel, nylon or plastic: 60-105 μm mesh) (Fig. 1).
- Template (stainless steel, plastic, or cardboard) (Fig. 1).
 - A hole of 9 mm on a 1 mm thick template will deliver about 50 mg of feces; a hole of 6 mm on a 1.5 mm thick template, 41.7 mg; and a hole of 6.5 mm on a 0.5 mm thick template, 20 mg.
 - The same size of templates should always be used to ensure repeatability and comparability of prevalence and intensity data.
- Spatula (plastic)
- Microscope slides (75 x 25 mm)

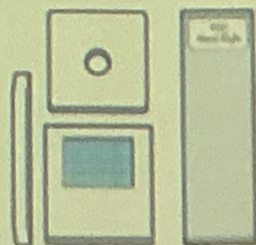
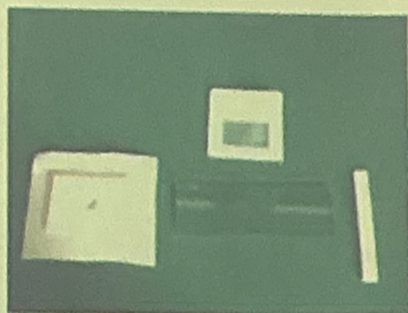


Fig. 1



Materials and Reagents

- Hydrophilic cellophane
 - 40–50 μm thick, strips; 25 x 30 or 25 x 35 mm in size
- Flat-bottom jar with lid
- Forceps
- Toilet paper or absorbent tissue
- Newspaper
- Glycerol-malachite green (1 mL of 3% aqueous malachite green is added to 100 mL of glycerol and 100 ml of distilled water and mixed well).
 - This solution is poured onto the cellophane strips in a jar and left for at least 24 hours prior to use.

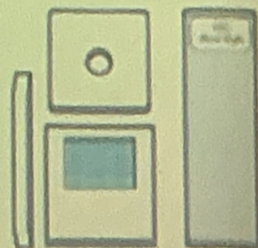
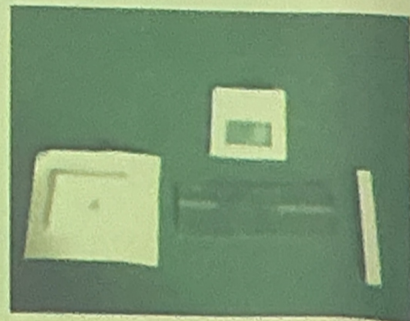


Fig. 1



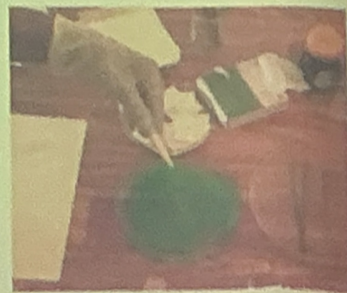
Malachite Green Solution and Cellophane Preparation

A.) To prepare the malachite green solution (3%)

1. Measure 3g of malachite green or methylene blue powder and dissolve it in 100 ml of distilled water.
2. Label with the date of preparation.
3. To store the 3% malachite green or methylene blue solution, avoid exposure to sunlight by covering the beaker with aluminum foil.
 - Renew the solution on a regular basis.

B.) To prepare the glycerol solution (50%)

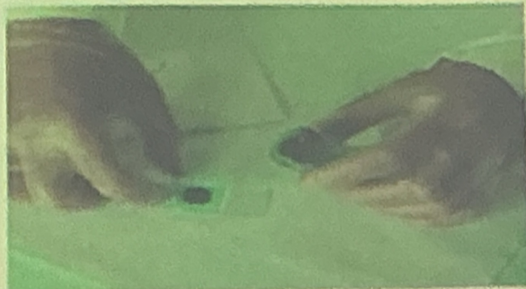
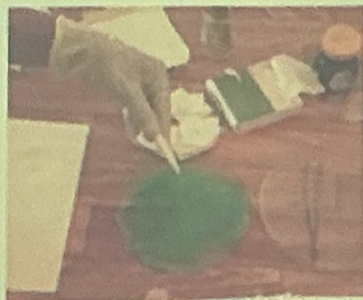
1. Dilute 1 volume of glycerol in the same volume of distilled water.
2. Label with the date of preparation.



Malachite Green Solution and Cellophane Preparation

C.) To prepare the cellophane:

1. 1 ml of the malachite green or methylene blue (3%) solution is mixed with 200 ml of glycerol solution (50%).
2. Shake well to mix the solution.
3. Leave the hydrophilic cellophane in the malachite green solution for at least 24 hours before use.



Kato-Katz Method: Procedure



1. Place a small amount of the fecal sample on a newspaper and press a piece of nylon screen on top.
 - Using a spatula, scrape the sieved fecal material from the screen (Fig. 2).
2. Label a glass slide with the sample number and place a template with hole in the center of a microscope slide.
 - Fill the hole in the template with the ^{Sieved} fecal material, avoiding air bubbles and levelling the feces off to remove any excess material (Fig. 3).

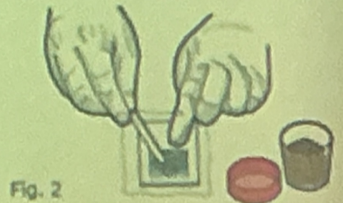


Fig. 2

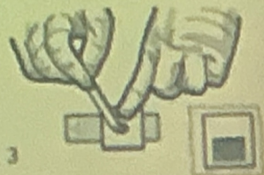


Fig. 3

Kato-Katz Method: Procedure

3. Carefully lift off the template and place it in a bucket of water mixed with concentrated detergent (and disinfectant so that it can be reused.)

4. Place one piece of cellophane, which has been soaked overnight in glycerol solution, over the fecal sample (Fig. 4).

malachite-green glycerol cellophane

5. Invert the microscope slide (Fig. 5) and firmly press the sample against the cellophane strip on another microscope slide or on a smooth hard surface to spread the feces in a circle (Fig. 6).



Fig. 4

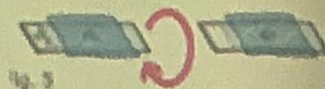


Fig. 5



Fig. 6

Kato-Katz Method: Procedure

6. Carefully pick up the slide again by gently sliding it sideways to avoid separating the cellophane strip or lifting it off.
 - Place the slide on the bench with the cellophane upwards. Water evaporates while glycerol clears the feces. When clarified it should be possible to read newspaper print through the stool smear (Fig. 7).

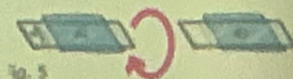


Fig. 5



Fig. 6



Fig. 7



Kato-Katz Method: Procedure

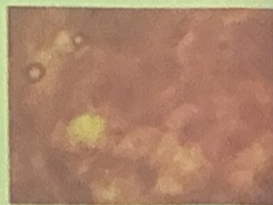


7. For all except hookworm eggs, keep the slide for one or more hours at room temperature to clear the fecal material prior to examination under the microscope.

- To speed up clearing and examination, the slide can be placed in a 40 °C incubator or kept in direct sunlight for several minutes.

8. *A. lumbricoides* and *T. trichiura* eggs will remain visible and recognizable for many months. Hookworm eggs clear rapidly and will no longer be visible after 30–60 minutes.

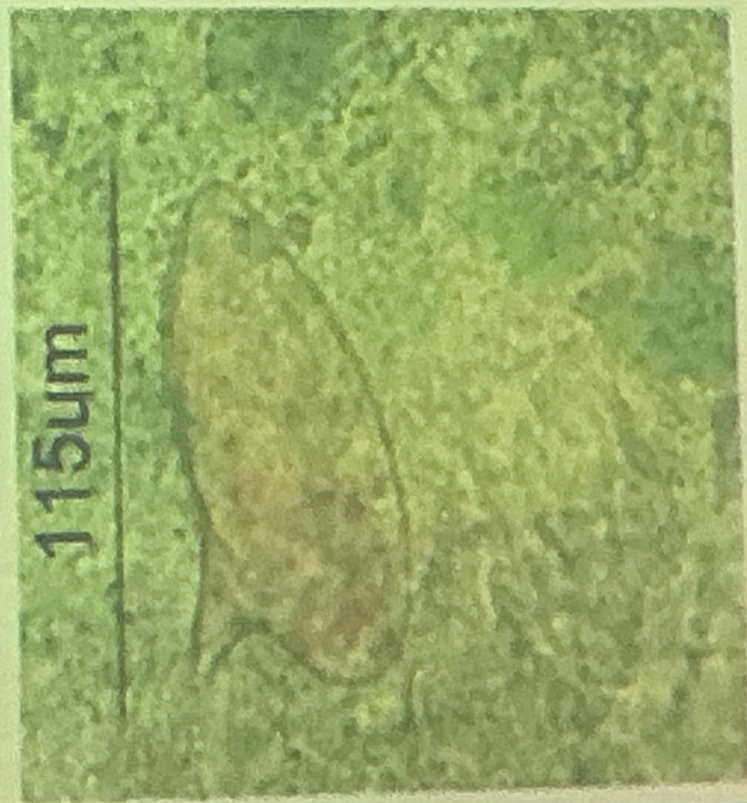
- Schistosome eggs may be recognizable for up to several months but it is preferable to examine the slide preparations within 24 hours. (WHO, 1994)



Ascaris lumbricoides Kato-Katz thick smear in the clearing process. It requires some more minutes to clear completely. A few eggs are hiding in the background.



Ascaris lumbricoides Kato-Katz thick smear in the clearing process. It requires some more minutes to clear completely. A few eggs are hiding in the background

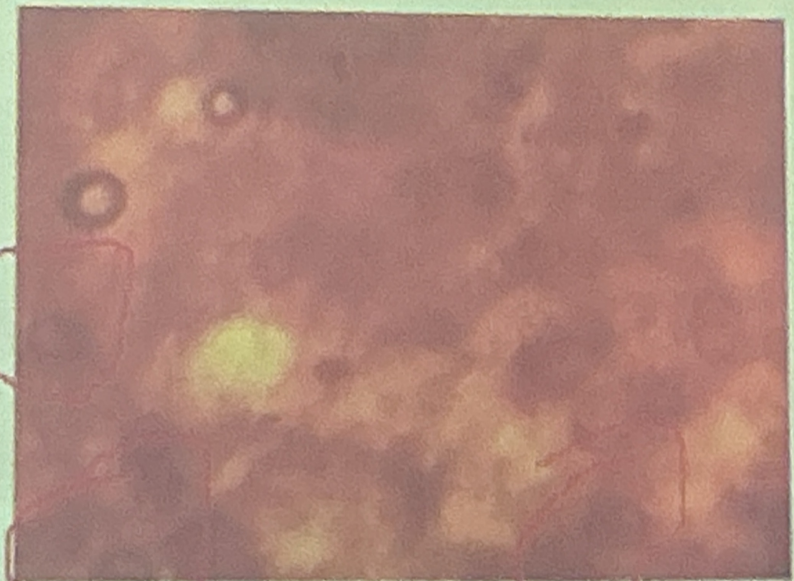


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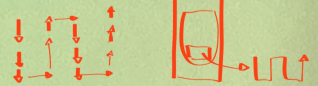
Ascaris lumbricoides Kato-Katz thick smear in the clearing process. It requires some more minutes to clear completely. A few eggs are hiding in the background



Kato-Katz Method: Procedure



Read using
this method



9. The smear should be examined systematically.

10. Then, multiply by the appropriate number to give the number of eggs per gram of feces (by 20 if using a 50 mg template; by 50 for a 20 mg template; and by 24 for a 41.7 mg template)

• Example:

- *Ascaris lumbricoides* ova – 20 x 24
- *Trichuris trichiura* ova – 15 x 24
- Hookworm ova – 10 x 24

• Report:

- *Ascaris lumbricoides* ova – 480 epg
- *Trichuris trichiura* ova – 360 epg
- Hookworm ova – 240 epg



A. lumbricoides (Typical) Trichuris egg as it appears in a Kato-Katz preparation



T. trichiura and *A. lumbricoides* Egg in a Kato-Katz preparation



Table 7.1 WHO Classification of Intensity of Infections with STH and *Schistosoma* spp.



Table 7.1. WHO classification of intensity of infections with soil-transmitted helminths and *Schistosoma* spp.

Organism	Light intensity	Moderate intensity	Heavy intensity
<i>Ascaris lumbricoides</i>	1 – 4,999 epg	5,000 – 49,999 epg	≥ 50,000 epg
<i>Trichuris trichiura</i>	1 – 999 epg	1,000 – 9,999 epg	≥ 10,000 epg
Hookworm	1 – 1,999 epg	2,000 – 3,999 epg	≥ 4,000 epg
<i>Schistosoma japonicum</i> <i>Schistosoma mansoni</i>	1 – 99 epg	100 – 399 epg	≥ 400 epg

KATO-KATZ PHOTOGRAPHS

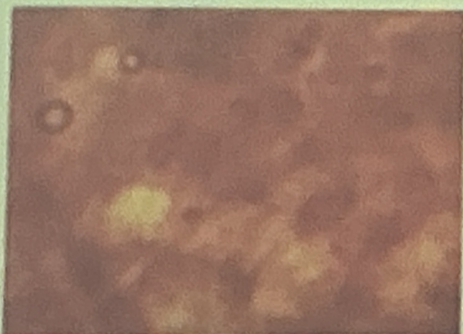


World Health
Organization

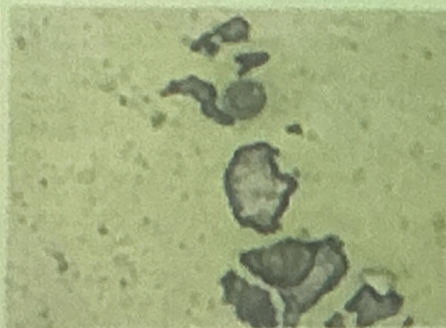
Bench aids for the diagnosis
of intestinal parasites, second edition

Plate

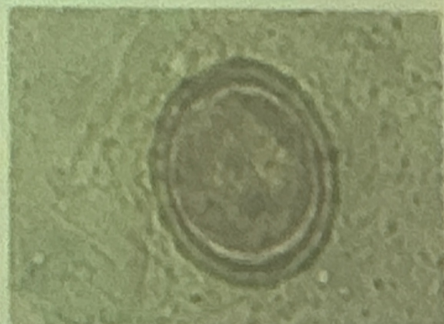
Appearance of some parasite eggs in a Kato-Katz preparation



Ascaris lumbricoides Kato-Katz thick smear in the clearing process. It requires some more minutes to clear completely. A few eggs are hiding in the background



A. lumbricoides Two eggs visible amidst air trapped in the smear. Kato-Katz preparation



A. lumbricoides Typical fertile egg as it appears in a Kato-Katz preparation



Table 7.1 WHO Classification of Intensity of Infections with STH and *Schistosoma* spp.

Table 7
Classes of intensity for schistosome and soil-transmitted helminth infections

By stool examination:

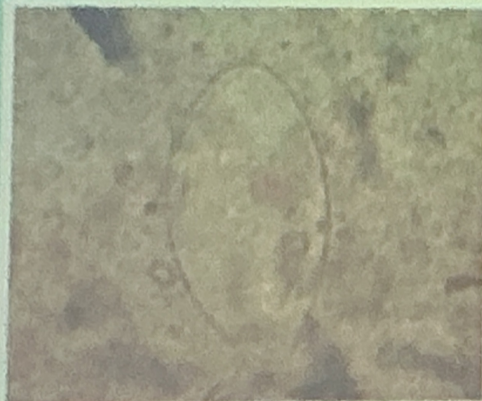
Organism	Light-intensity infections	Moderate-intensity infections	Heavy-intensity infections
<i>A. lumbricoides</i>	1-4999 epg ^a	5000-49 999 epg	≥ 50 000 epg
<i>T. trichiura</i>	1-999 epg	1000-9999 epg	≥ 10 000 epg
Hookworms	1-1999 epg	2000-3999 epg	≥ 4000 epg
<i>S. mansoni</i>	1-99 epg	100-399 epg	≥ 400 epg

By questionnaire or urine filtration:

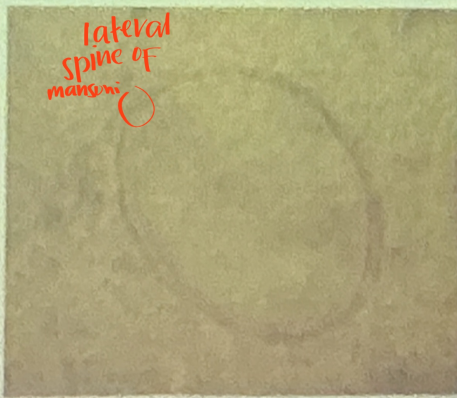
Organism	Light-intensity infections	Heavy-intensity infections
<i>S. haematobium</i>	< 50 eggs/10 ml	Visible haematuria, or ≥ 50 eggs/10 ml

^a epg: eggs per gram (of faeces)

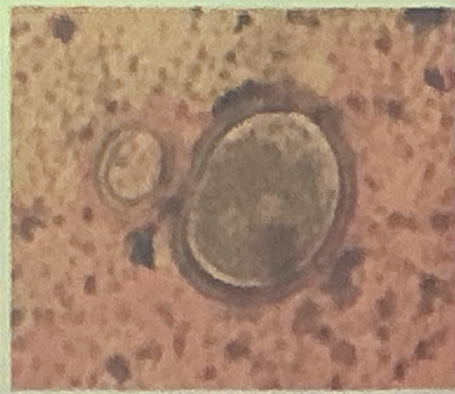
KATO-KATZ PHOTOGRAPHS



Trichostrongylus axei Eggs are easily identified on the basis of their size and shape and the presence of a lateral spine. Kato-Katz preparation



A. suum The spine of the egg is rarely seen and the refringendum quickly becomes apparent. Size and thickness of the shell help identify the species. Kato-Katz preparation

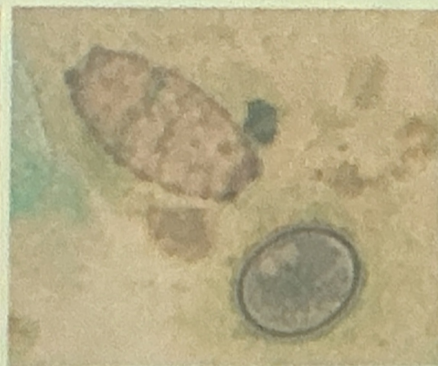


Fasciola spp. and ***A. fuscicollis*** Inside the egg of *Fasciola* the hooks can still be observed. Kato-Katz preparation

KATO-KATZ PHOTOGRAPHS



A. lumbricoides Dehydrated egg in a Kato-Katz preparation



A. lumbricoides Fertile (lower) and infertile egg (upper) in a Kato-Katz preparation



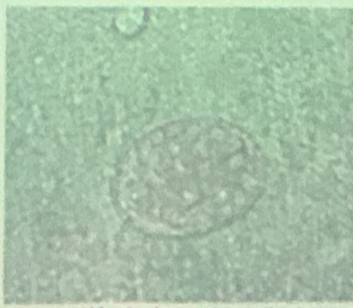
Trichouris trichura Eggs in Kato-Katz preparations may appear larger and more swollen than *A. lumbricoides* observed with other techniques, with degenerated contents. The bipolar prominences are not always sharply defined



KATO-KATZ PHOTOGRAPHS



T. axei and *A. lumbricoides* Egg in a Kato-Katz preparation



Ancylostomidae hookworm Eggs in Kato-Katz preparations are often almost round and become increasingly difficult to see during the clearing process



Ancylostomidae hookworm Egg left too long in Kato-Katz preparation, before examination. Air bubbles trapped and egg almost disappearing



REFERENCES



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Diagnostic Techniques for Intestinal Parasitic Infections (IPI) Applicable to Primary Health Care (PHC) Services. (1986). World Health Organization. Retrieved March 13, 2026, from <https://iris.who.int/server/api/core/bitstreams/40993d21-d527-4d81-aeec-cc5685b137eb/content>



KATO-KATZ METHOD

Templates



Hole diameter	Template Thickness	Amount of stool it will deliver/hold
9 mm	1 mm	50 mg
6 mm	1.5 mm	41.7 mg
6.5 mm	0.5 mm	20 mg



KATO THICK METHOD

Summary



- **Sample:** 50 to 60 mg of stool (two mungo beans)
- **Reagents:**
 - Glycerine
 - Malachite green
 - Cellophane
- Used for mass stool examination
- Detects eggs with thick shells (*Ascaris* and *Trichuris*)
- **Disadvantages:**
 - Not suitable for thin-shelled eggs (Hookworms)
 - Does not detect cysts and trophozoites
 - Limited usefulness with watery or diarrhetic stool
- Best examined **within 10 to 20 minutes**

