KATO-KATZ PREPARATION AND ANALYSIS

1. PURPOSE

Kato-Katz is a diagnostic technique for the detection of helminth eggs in stool using a light microscope. This test is based on the detection of helminth eggs in small amounts (41.7 mg) of fresh stool.

2. INTENDED USERS

Laboratory supervisors, laboratory technicians, laboratory data managers and laboratory assistants.

3. RESPONSIBILITIES

All DeWorm3 laboratory technicians and laboratory supervisors should agree on, understand and follow this SOP during preparation and reading of prepared slides. It is the responsibility of the laboratory supervisor to ensure that all laboratory technicians comply with this SOP.

4. DEFINITIONS

4.1. **Kato Katz:** The Kato-Katz technique facilitates the detection and quantification of helminth eggs that infected subjects pass in their 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. Subsequently, the prevalence and intensity of helminth infections can be determined.

4.2. **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. 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 x 41.7 mg ≈ 1 g).

4.3. **EPG**: EPG is the estimated number of Eggs Per Gram of stool excreted by the examined infected individual.

4.4. **SurveyCTO:** web-based Survey platform for electronic data-collection, based on Open Data Kit.

5. MATERIALS REQUIRED

5.1. Kato Katz template (in the Vestergaard KK-Kit)
5.2. Plastic spatulas (in the Vestergaard KK-Kit)
5.3. Cellophane (in the Vestergaard KK-Kit) or Carramore hydrophilic cellophane
5.4. Nylon screen with a mesh size between 60 to 105 μm (in the Vestergaard KK-Kit), additional nylon mesh can be purchased
5.5. Scrap paper or old newspaper
5.6. Microscope slides
5.7. Binocular Microscope
5.8. Malachite Green or Methylene Blue Solution
    5.8.1. Malachite green or Methylene Blue
    5.8.2. Glycerol
    5.8.3. Distilled water
    5.8.4. Sealable beaker
    5.8.5. Aluminum foil
5.9. Wooden spatulas
5.10. Slide boxes
5.11. Counter
5.12. Stopwatches/timers
5.13. Permanent markers

5.14. Lab coats

5.15. Disposable gloves

5.16. Consumables for preparation and cleaning (buckets, detergent, towels, toilet paper)

5.17. Barcodes/QR code stickers

5.18. Laptop/Desktop with SurveyCTO software and internet connection for label printing

5.19. Brother sticker printer

5.20. Smartphones loaded with lab SurveyCTO forms for logging and recording the results

5.21. 10% bleach solution

5.22. Gauze pads

6. PROCEDURE

6.1. **General point - Safety**
Handle all stool samples as potentially infectious. Wear gloves during the procedure. Be very cautious to not contaminate the environment and workplace. All persons handling and processing stool must have training in GLCP.

6.2. **Kato-Katz Kits**
Each site is provided with 300 Kato-Katz kits from Vestergaard. They consist of the templates, cellophane, spatulas and nylon screen. The templates can be washed and re-used, as well as the spatulas. The cellophane and the Nylon screen are consumables that must be replaced throughout the study.

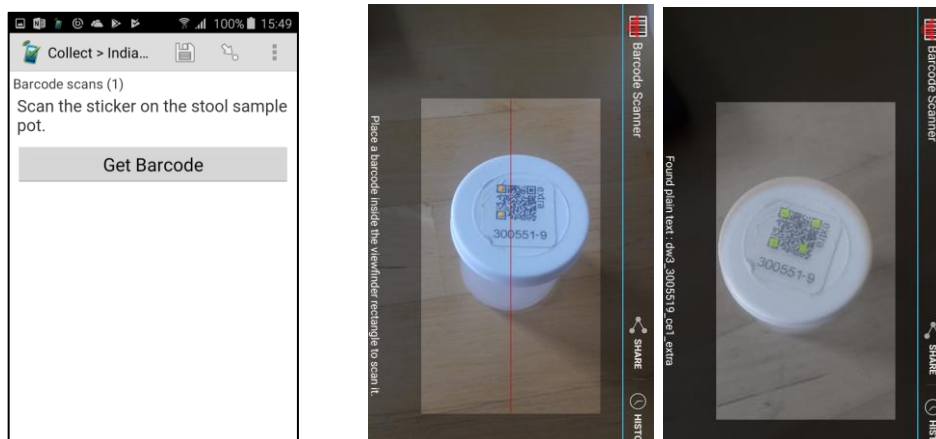6.3. **Malachite green solution and cellophane preparation**

a. To prepare the malachite green solution (3%), measure 3g of malachite green or methylene blue powder and dissolve it in 100 ml of distilled water. Label with the date of preparation.

b. To prepare the glycerol solution (50%) dilute 1 volume of glycerol in the same volume of distilled water. Label with the date of preparation.

c. To prepare the cellophane, 1 ml of the malachite green or methylene blue (3%) solution is mixed with 200 ml of glycerol solution (50%). Shake well to mix the solution. To prepare the cellophane, leave them in the malachite green solution for at least 48 hours before use

d. 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.

6.4. **Receipt and logging of the samples in SurveyCTO to be read by Kato-Katz**

a. When the samples from the field arrive at the laboratory, they must be logged using the "Stool Sample Receipt Form". They can be logged in batches as they are delivered.

b. Using a smartphone Open SurveyCTO and select "Fill Blank Form". Select "Stool Sample Receipt Form" from the list. Swipe left to move to the introduction screen and start the form.
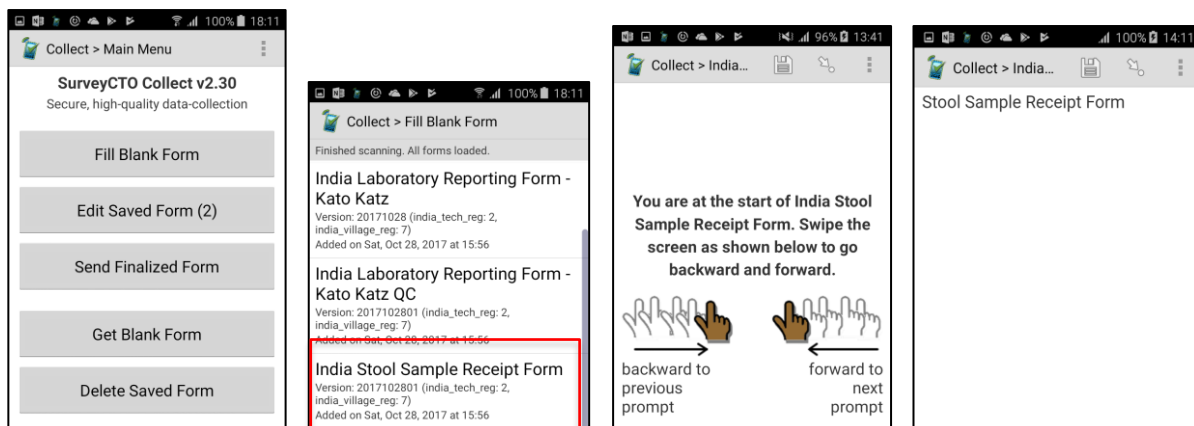
c. Select your name from the list. If your name is not list, select "Technician not listed" at the bottom of the list and you will be prompted to type in your name on the next screen. Please enter your complete name.

d. Enter the number of pots you have received in the batch. The minimum batch size is 1 and the maximum is 25. If you have received more than 25 pots in one go, you will have to fill in more than one form. Make sure you count carefully and enter correctly how many pots you will log in this form as this sets the limits for the rest of the form.

e. Scan the pots on one by one by scanning the barcode sticker on the top of the sample pot. Select "Get Barcode" to scan the sticker. The phone's display will appear as shown below. The box will display the image captured by the phone's camera. Hold the phone level over the sticker so that it is centered in the image on the screen. Make sure you are not covering the camera. The phone will beep (if the sound is on) and the dots will turn from yellow to green when it has successfully read the sticker.


f. It will then display the captured barcode. If this is incorrect, press replace barcode and scan the correct barcode and this will replace the incorrect one. If it is correct, the next screen will ask you to report anything about the pot. This is useful to report anything unusual about the sample's appearance, for example, if the pot is filled with soil, rocks, or an insufficient amount of stool. You can select from the options on the phone screen and you may select multiple options. If there is nothing to report, select [No comment]
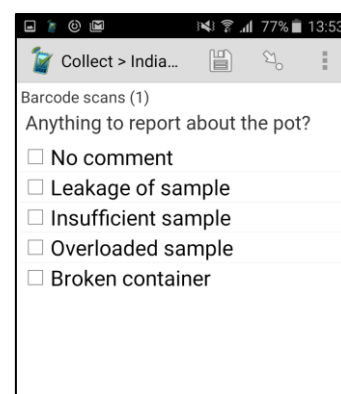
g. If the sample pot barcode sticker is soiled and cannot be scanned, you can manually enter the barcode of the sample. Type in the first 6 digits (household number) of the ID, the 7th check digit, and then the next screen will ask you to enter the last two digits of the ID.
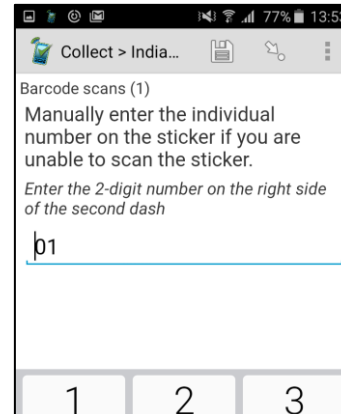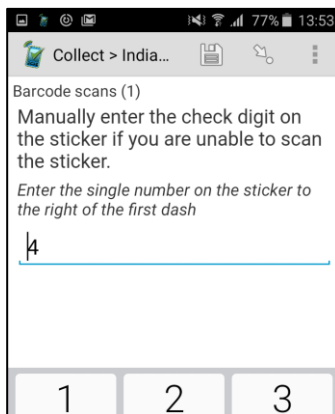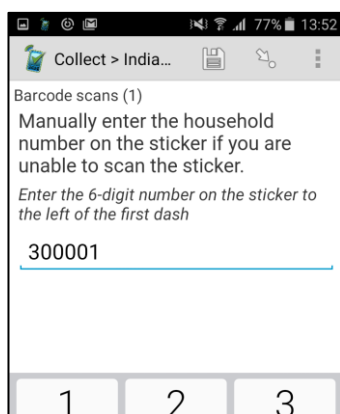

h. The next screens will ask you to select the activity indicated on the sticker. This will automatically be selected as lm1 for longitudinal monitoring survey 1. Then select the sample type. Again, this will already be pre-selected with stool.
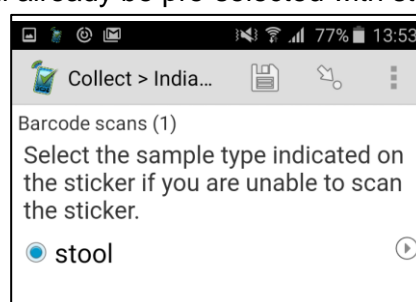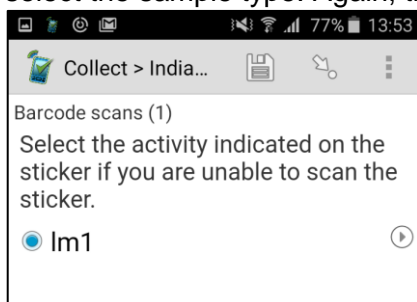
i. The next screen will ask you to confirm the sample ID you have entered. If this is correct, select yes, if it is incorrect select no and go back to the previous screens to re-enter. If the sample ID you have entered has already been scanned/entered in this batch, you will be informed on the screen and you will have to go back and correct the incorrect entry.
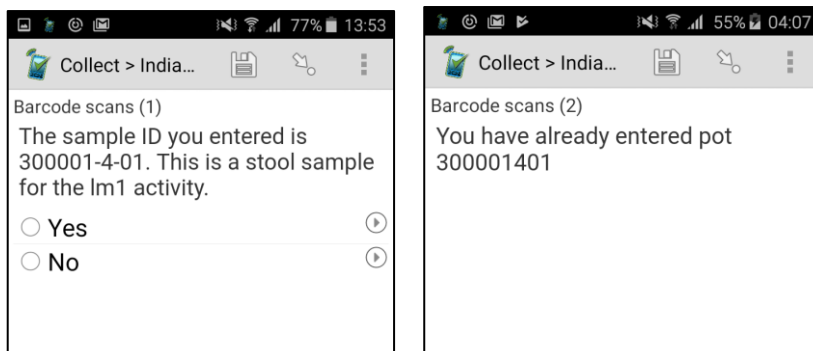
j.   You will repeat this scanning process for all the sample pots in the batch, i.e. if it is a batch of 25, you will scan 25 pots. The small number in brackets after "barcode scans" will tell you which sample number you are currently scanning.

k.   The sticker barcodes on each pot must be scanned to record that you have received the sample. Once you have finished you will be informed and then on the final screen save the form by ensuring the "mark form as finalized" box is ticked. Then press save form and exit.
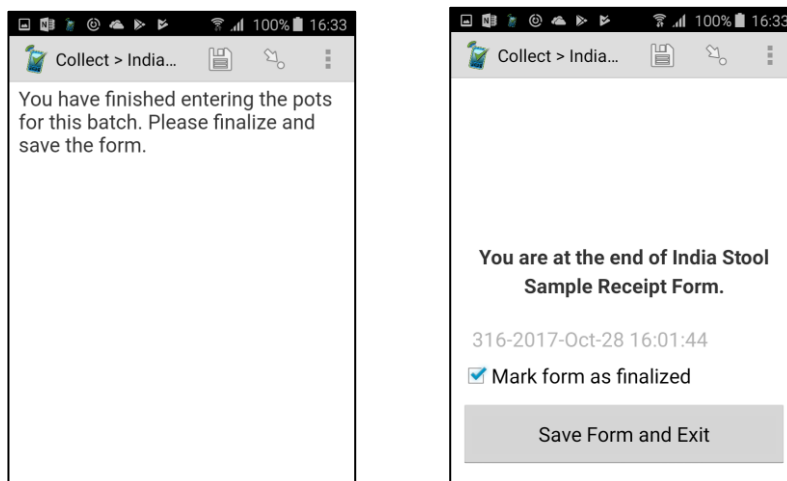
l.   Once all the samples for the batch is scanned, the barcode stickers for the slides can be printed, based on the sample IDs logged.

6.5. **Printing the barcode stickers for the slides with SurveyCTO (Lab Data manager)**

a.   Once the multiple batches of sample pots (or a single batch) have been delivered to the lab and logged using the sample receipt form and the data from these forms have been uploaded to the server, the linked slide barcode stickers can be printed.

b.   One lab data manager at each sub-site laboratory will have access to a computer connected to Wi-Fi with P-touch software installed. (See *SOP 902 Printing household ID barcodes*) and connected to a Brother QL-700 Label Printer. The lab data manager will have login details to the SurveyCTO server.

c.   In the internet search bar enter **deworm3.surveycto.com**. Log in using the details you have been provided separately.

d.   Once logged in you should see four tabs: 1. Design, 2. Collect, 3. Monitor, 4. Export. Click on 4. Export and scroll down to where it says, "your data".
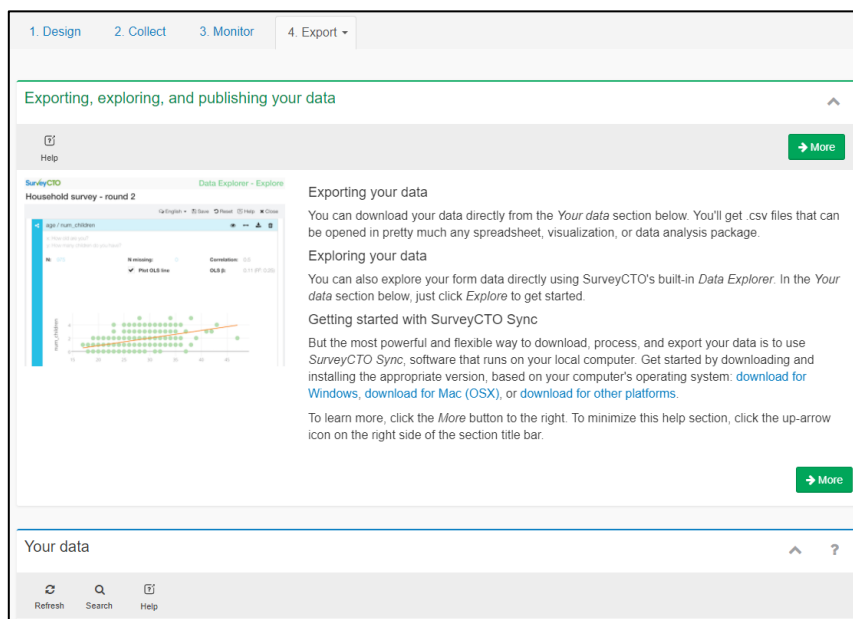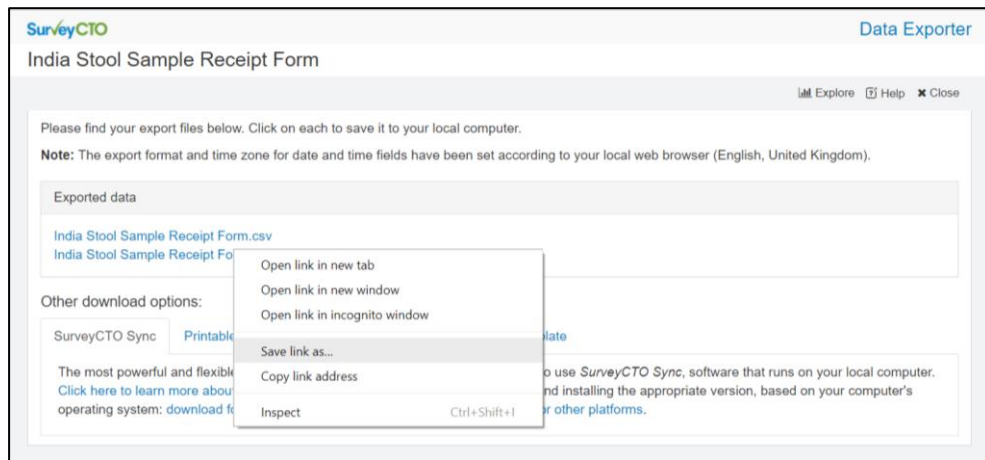
e. Then scroll down to "India Stool Sample Receipt form" and click download on the right-hand side.
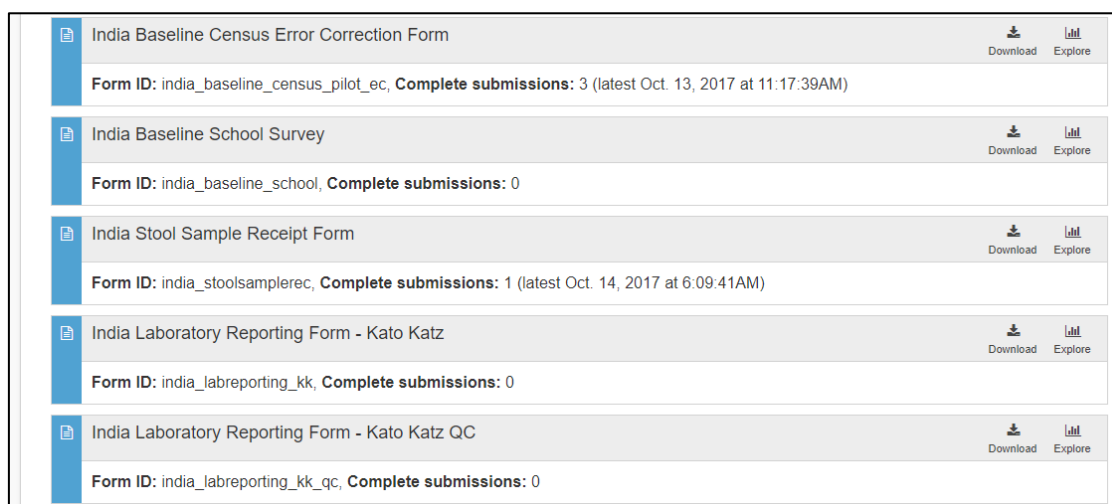

f. Select "Long format" and "All submissions" and click "Download data now"
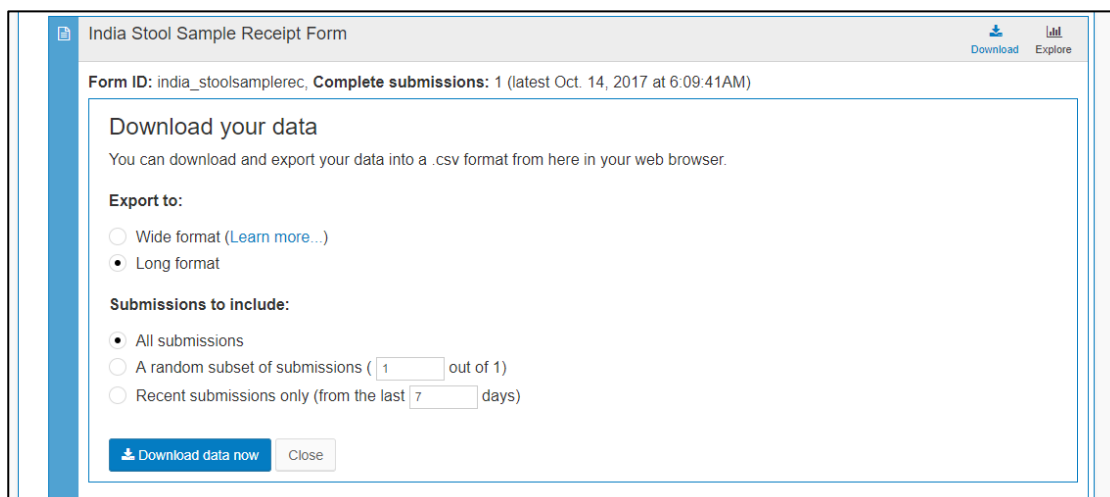
g. Right click on the blue link "India Stool Sample Receipt Form-pot.csv", go to [Save link as].

h. Go to DeWorm3 India – Data Management > Stickers > Kato-Katz. Save the file as "India Stool Sample Receipt Form-pot". **Do not add the date or any other information to the file name.** This will save over and replace the previous day's sticker file. This is OK, as the file is cumulative, so the samples logged on previous days will still be in the database
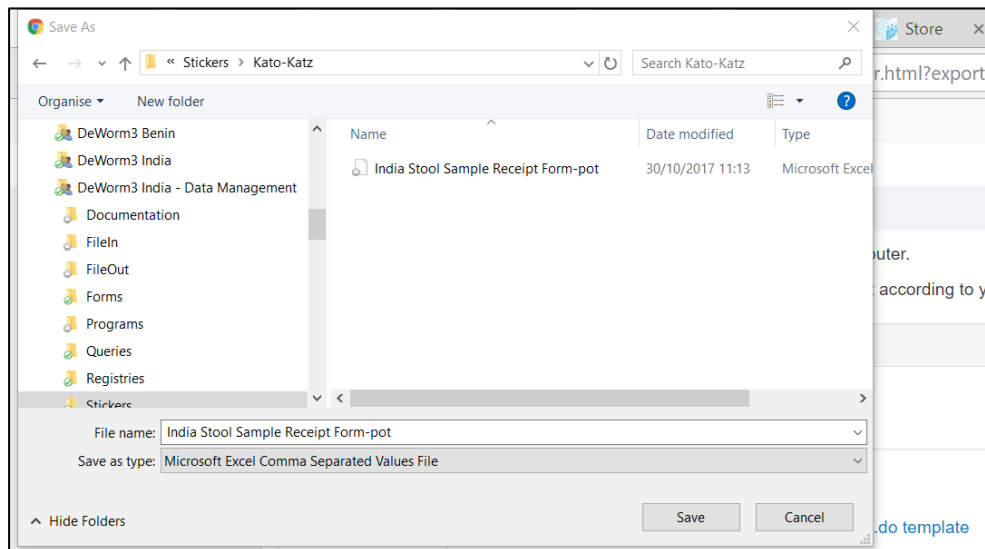
i. Open the folder DeWorm3 India – Data Management > Stickers > Kato-Katz and then double click on "*India kk label template A*". This will open the file in the P-touch software. There will also be a template for B named "*India kk label template B*" you will repeat the same steps with this file after so that you end up with corresponding barcode stickers for slides A and B for each sample.

j. The screen will appear as below with the full database of samples listed.

k. Click on the filter button (highlighted in red) and this will bring up the filter box. Click on the first [Field] drop down menu. If you have logged all the pots that will be coming in to the lab today and you are printing them all at one, you can select "date_pot_received" in the field box.

l. In [Value] write today's date as it appears in the database (e.g. "30/10/17"). Leave the [Condition] as "equals". If you have logged a few batches of samples and you want to print these, but you will also be receiving more samples later and will need to print those, you will need to print the subsequent batches by "time_pot_received" as date would re-print the earlier batches which were printed before. Each batch will have the same value for "time_pot_received". You can print three batches at a time using this variable as there are three filter fields.
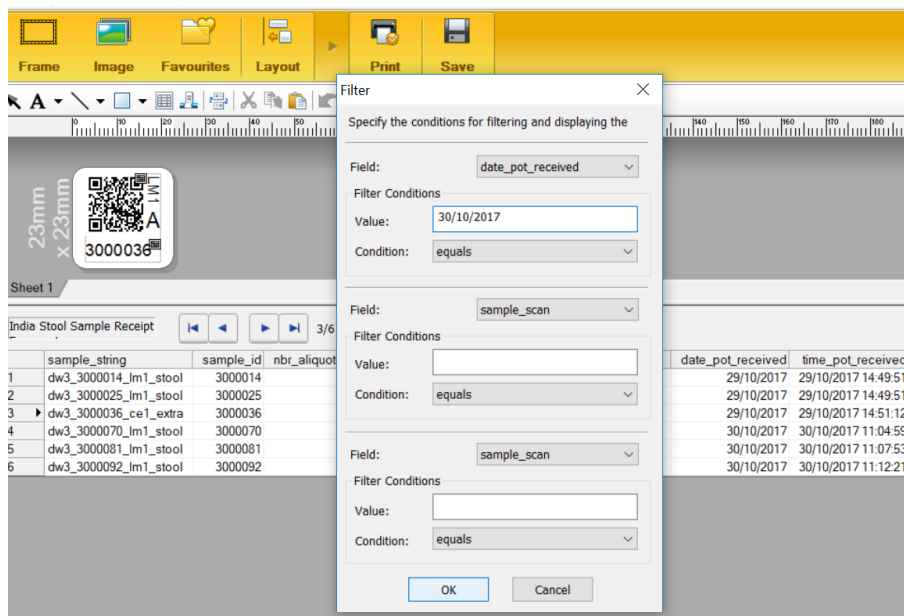
m. Once you have filtered the stickers you need, check the number of rows this is in the database. This is shown at the top or down the left side (highlighted in red).

n. To print the stickers for your activity, go to "File" and select "Print". In the print menu, select "Priority to print quality" and also "Output directly to print driver". Providing you have set the filter to print only the sample IDs that arrived on a set date, or were logged in a set batch, you can select "All records" in [Print Range].
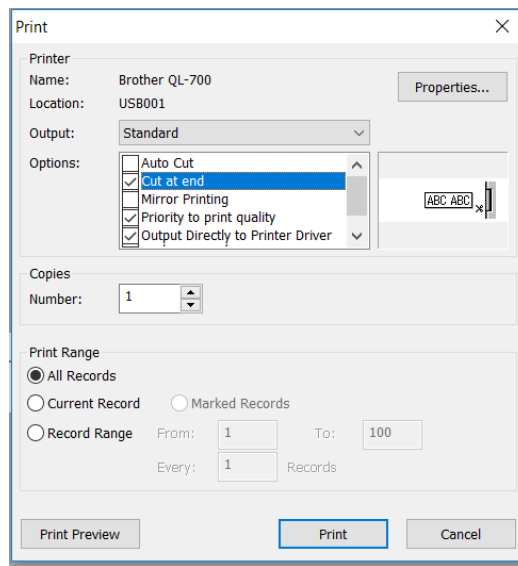
o. To print all the stickers continuously within the range specified, tick the box "cut at end". Alternatively, to print a range of stickers but cut the strip automatically at a set interval (for example, in batches to give to different lab technicians), you can select "Auto Cut" under "Options". Then go to "Properties…" and enter a number under "Cut Every" to specify the interval to be printed. Label here refers to a row in the spreadsheet, not a sample ID.

p. After printing all the A slide stickers, repeat with the B slide template. Two slide barcodes will be printed for each sample that is being collected in the field. One barcode sticker will have "A" and the other will have "B". One will be for each of the two slides prepared.


q. Stick the slide barcodes on the slides and place the labelled slides on top of the sample container until you are ready to prepare the slides.

6.6. **Kato-Katz procedure**

a. Wipe the outside of the stool pot with a clean piece of gauze soaked in 10% bleach to decontaminate the container.

b. Open the stool container and homogenize the stool to ensure eggs are distributed in the sample (if present) with a new clean spatula. If the stool is too hard to process you can add sterile saline solution to homogenize.

c. Place some stool (around 1 gram) on a bit of newspaper/scrap paper. Take the nylon screen and press it on top of the stool to sieve the stool through the nylon screen. Take the spatula and collect the sieved stool.
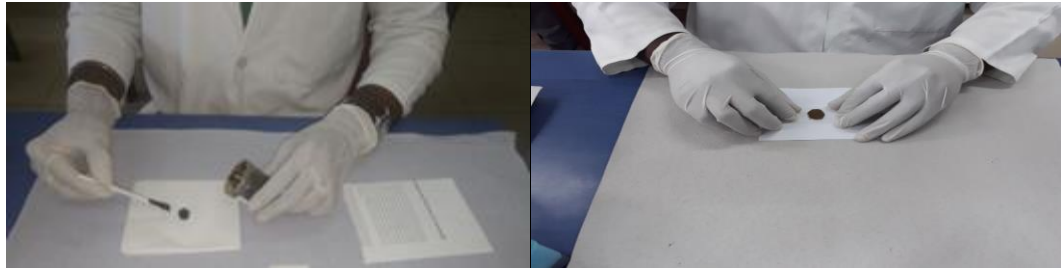
d. Place the Kato-Katz template in the middle of the first slide "A". Completely fill the hole of the template using the stool on the spatula. Make sure there are no air bubbles and remove any excess stool.
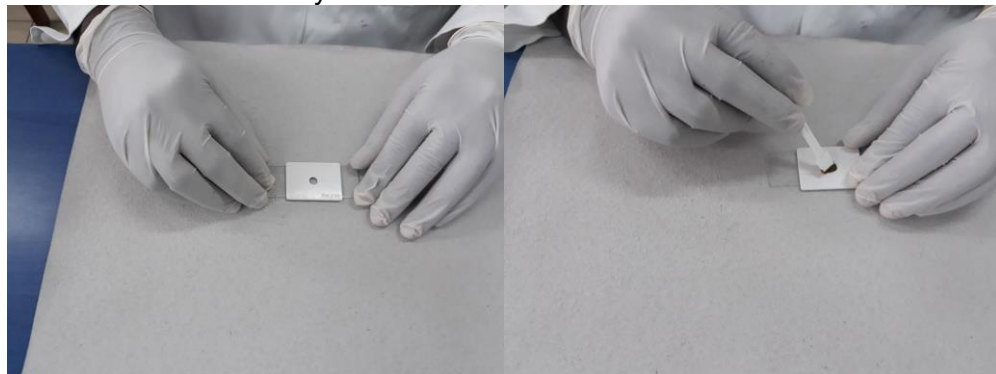

e. Remove the template vertically (avoid any horizontal movement).
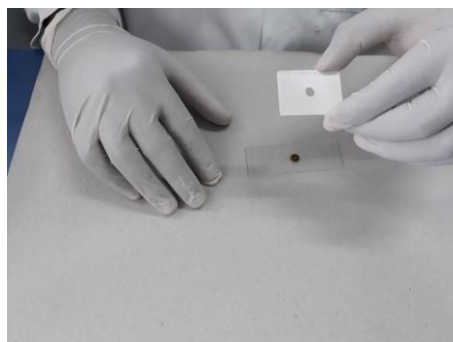
f. Place the Kato-Katz template in the middle of the second slide "B". Completely fill the hole of the template using the stool on the spatula. Make sure there are no air bubbles and remove any excess stool.

g. Add the pre-soaked cellophane on top of the stool cylinders on each slide. Use a second, clean microscope slide or a clean, even surface to press against the cellophane. The faecal material will be spread evenly between the microscope slide and the cellophane strip. Take care to not move, lift or wrinkle the cellophane. Make sure to support the slides to avoid breaking/cracking.
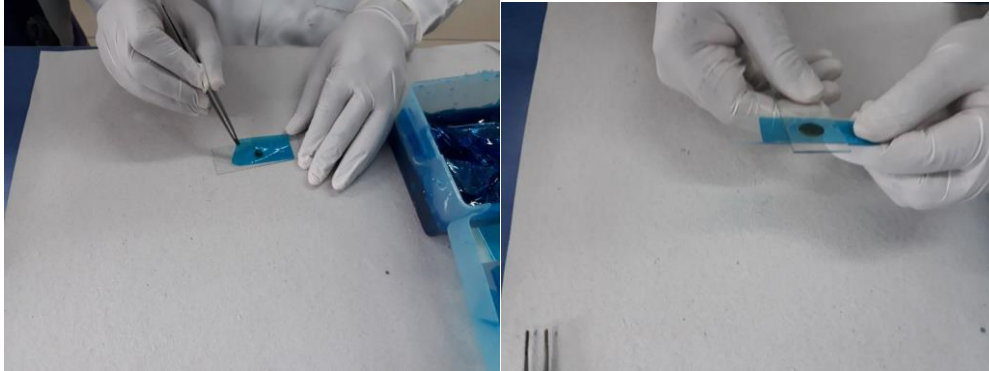
h. Carefully remove the slides by gently sliding them sideways to avoid separating the cellophane strip from the slide. Place the slide on the bench with the cellophane upwards. Water evaporates while glycerol clears the faeces.

i. All the slides must be read **within 30-60 minutes** after they have been prepared.
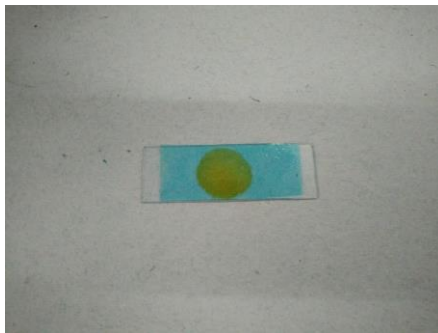
Hookworm eggs clear rapidly and will no longer be visible after 30-60 minutes.

j. Do not prepare more than 10 slides at a time.

k. **TWO SLIDES MUST BE PREPARED FOR EACH STOOL SAMPLE AND READ IN DUPLICATE**

6.7. **Reading procedure**

a. For each stool sample, two slides are prepared for Kato Katz. Examine the whole slide under a light microscope (10-40x magnification). Count the number of helminth eggs and record them for each helminth species separately.

b. The 10% of slides for quality control are randomly selected to be read by a laboratory supervisor, separate to the two slide readers.

6.8. **Survey CTO result entry**

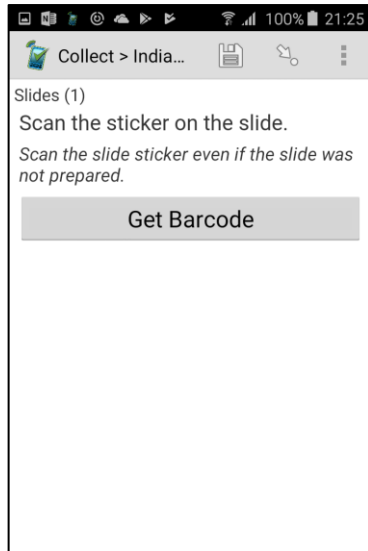a. Each technician reading at a microscope should have a smartphone to record the STH eggs observed in real time.

b. Using a smartphone Open SurveyCTO and select "Fill Blank Form". Select "Laboratory Reporting Form". Swipe left to move to the introduction screen and start the form.
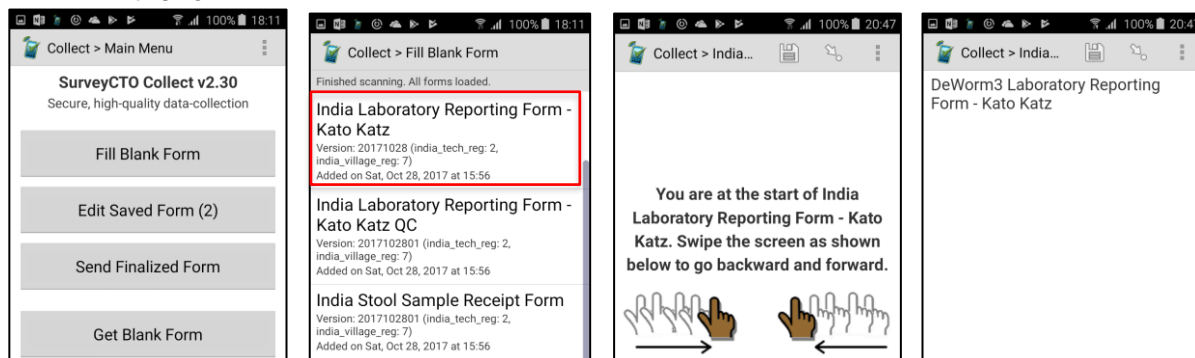
c. Select your name from the list. If your name is not list, select "Technician not listed" at the bottom of the list and you will be prompted to type in your name on the next screen. Please enter your complete name.

d. Enter the number of slides to be read in each batch. For each sample you will read two slides (A and B) so the minimum batch size is 2 and the maximum is 20. Make sure you count carefully and enter correctly how many slides you will log in this form as this sets the limits for the rest of the form.
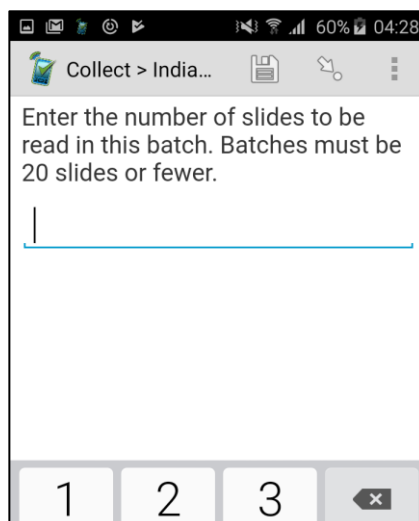
e. Scan the first slide you will read before placing it on the microscope by scanning the barcode sticker on the end of the slide. Select "Get Barcode" to scan the sticker. The phone's display will appear as shown below. The box will display the image captured by the phone's camera. Hold the phone level over the sticker so that it is centered in the image on the screen. Make sure you are not covering the camera. The phone will beep (if the sound is on) and the dots will turn from yellow to green when it has successfully read the sticker.

f. It will then display the captured barcode. If this is incorrect, press replace barcode and scan the correct barcode and this will replace the incorrect one.

g. If the slide barcode sticker cannot be scanned, you can manually enter the barcode of the sample. Type in the first 7 digits (household ID) on sample ID, and then the next screen will ask you to enter the last two digits of the ID. On the third screen, select whether you are reading slide A or B.
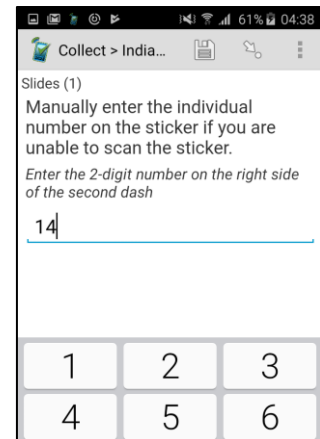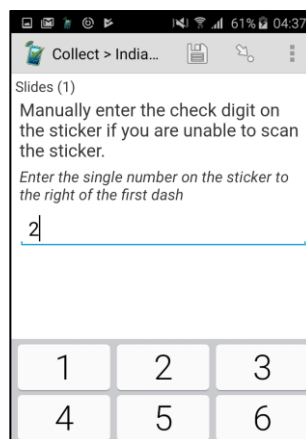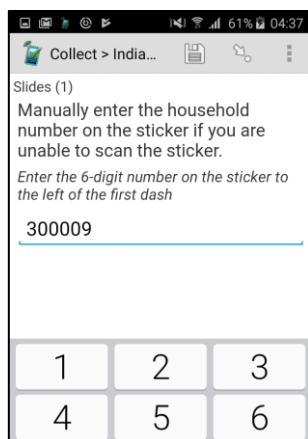
h. The next screens will ask you to select the activity indicated on the sticker. This will automatically be selected as lm1 for longitudinal monitoring survey 1. Then select the sample type. Again, this will already be pre-selected with stool. On the final screen, select whether you are reading slide A or B.
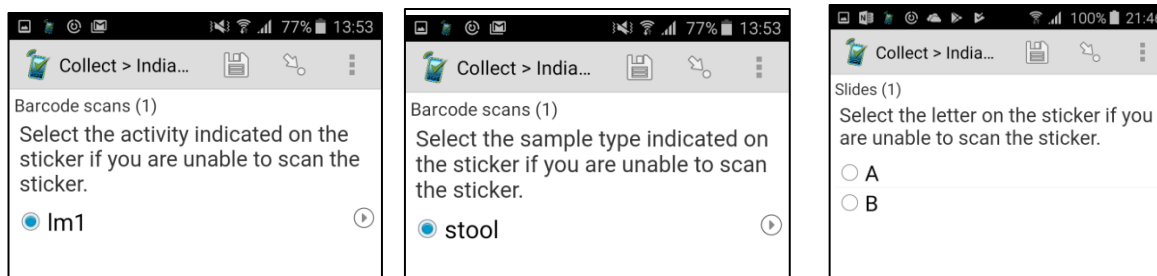

i. The next screen will ask you to confirm the sample ID you have entered. If this is correct, select yes, if it is incorrect select no and go back to the previous screens to re-enter. If the sample ID you have entered has already been scanned/entered in this batch, you will be informed on the screen (as below) and you will have to go back and correct the incorrect entry. This will stop you reading the same slide twice.
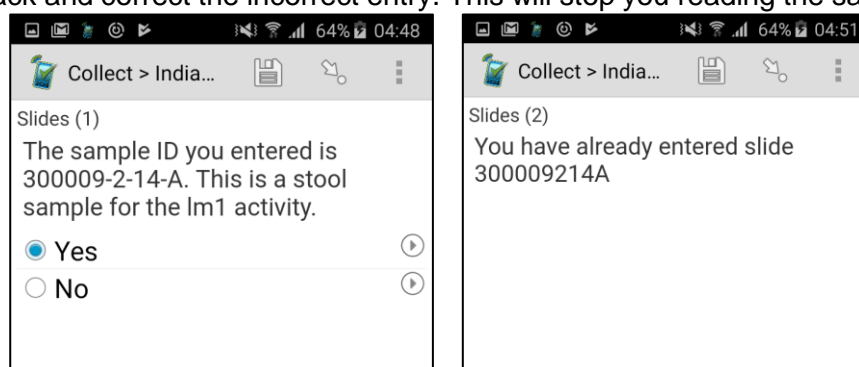
j. Once you have scanned/entered the correct slide ID, you will be asked if you can read the slide. If you select "no" you will be asked to specify why. Select the appropriate reason for the list. If you select "other" you will have to specify the reason why. Once you have recorded the reason why that slide cannot be read, the form will proceed to the next slide. It is very important that the barcode stickers for both slides A and B for each sample are scanned for reading, even if only A or B can be read.
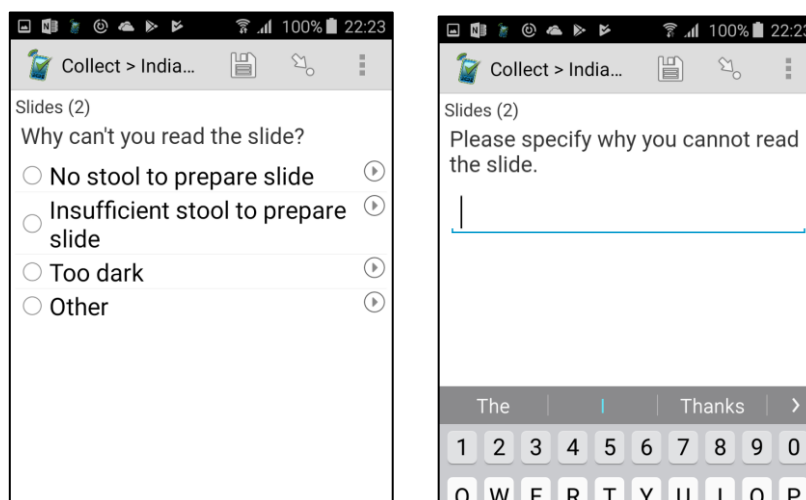
k. If you select "yes" the screen for entering the results will appear. Read the slide according to the instructions in section 6.7. Enter the results for each STH species. You will not be able to proceed to the next screen unless you have entered results for all three. If you did not see any eggs, put "0".
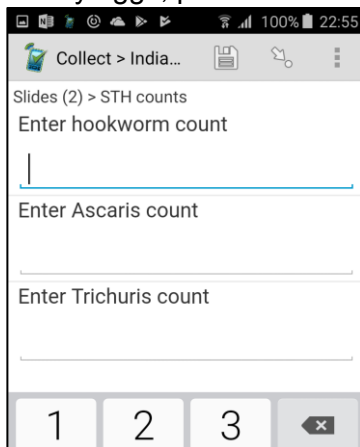
l. The next screen will ask which other species you have observed and are counting. You can select one or more species. Lastly enter any notes or observations about the slide.
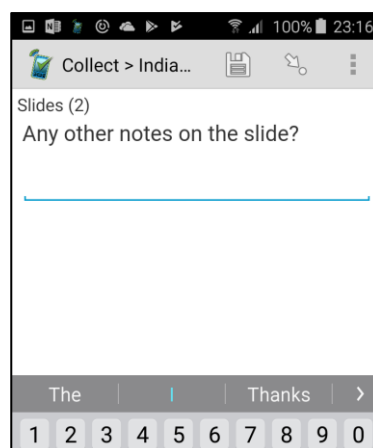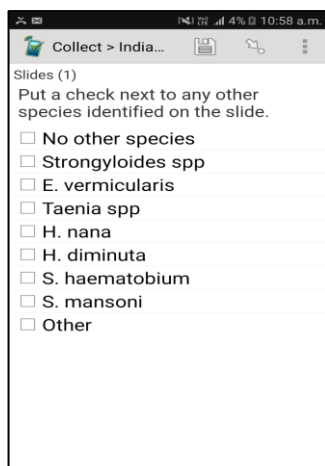
m. After entering this information and swiping left, the form will return to the barcode scanning screen, so you can scan the next slide and continue entering your reading results. Repeat steps f - l for all slides in the batch. If you try to scan the same slide twice, an error message will be displayed. You will not be able to proceed unless you go back.

n. In most batches you enter, approximately 10% (1 in 10 slides read) will be randomly selected for a repeat reading by the quality control (QC) technician. Find these slides in the batch and take them to the quality control technician before swiping forwards.

o. Record whether you gave **all** the selected slides to the quality control technician. If you did not give the selected slides to the quality control technician, record the reason why. If the reason is not listed, select "Other" and write the reason why.

| Did you give your slides to the quality control technician?	Why were you not able to give your slides to the quality control technician?
○ Yes	● QC technician not available
○ No	○ Other

p. You have now finished the survey. On the final screen of the form for each batch, the text in grey displays your technician ID number or your name and the time and date that you started entering each slide in the batch. If you have entered all the required information, then press "Save Form and Exit" to save the form and exit. If you uncheck "Mark form as finalized" you will only be saving the form on your phone, but it will not be finalized or transmitted. Do **not** uncheck the box for this form!
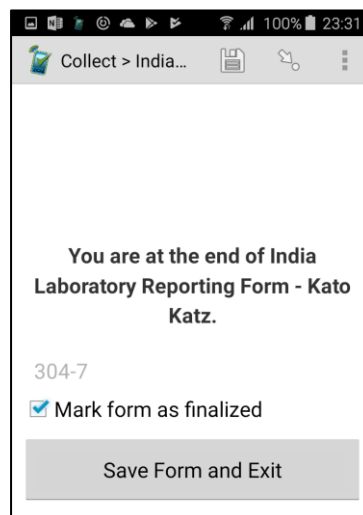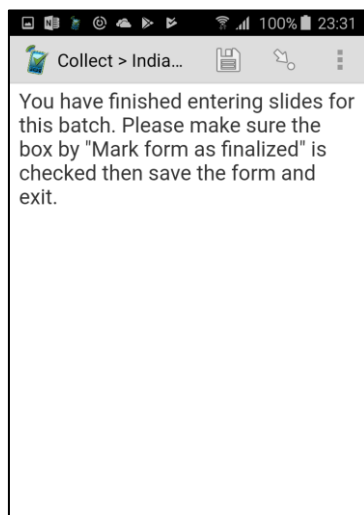

q. Prepare the next batch of slides for reading. Then start a new form for this next batch.

6.9. **Quality Control**

a. Within most batches, 10% of slides will be randomly selected for a second reading by a quality control technician or laboratory supervisor.

b. Once you have finished the first reading of the batch, any selected slides indicated on the screen before finalizing should be brought to the quality control technician.

c. The quality control technician will record the results of each selected slide using the "India Laboratory Reporting Form - Kato Katz QC".

d. The form "India Laboratory Reporting Form - Kato Katz QC" should only be used for recording the result of specific slides randomly selected for reading by the quality control technician. This is not the same as the form "India Laboratory Reporting Form - Kato Katz", which should be used by any technician who is conducting the 'normal' first reading of each batch.

e. The form "India Laboratory Reporting Form - Kato Katz QC" is identical to the form "India Laboratory Reporting Form - Kato Katz" as outlined in Section 6.10 of this SOP. No slides or batches will be selected for further re-reading.

f. There is a difference in how the batch reading works, as the slides will come to the QC technician one at a time. Once you have selected your name, rather than stating how many slides you will read in the batch you will keep selecting "add new slides group" until you have finished all of the slides. Only information for 20 slides can be entered per form. At the end of the reading of each slide after the screen asking if there are any notes on the slide, it will ask [Add a new "slides" group].
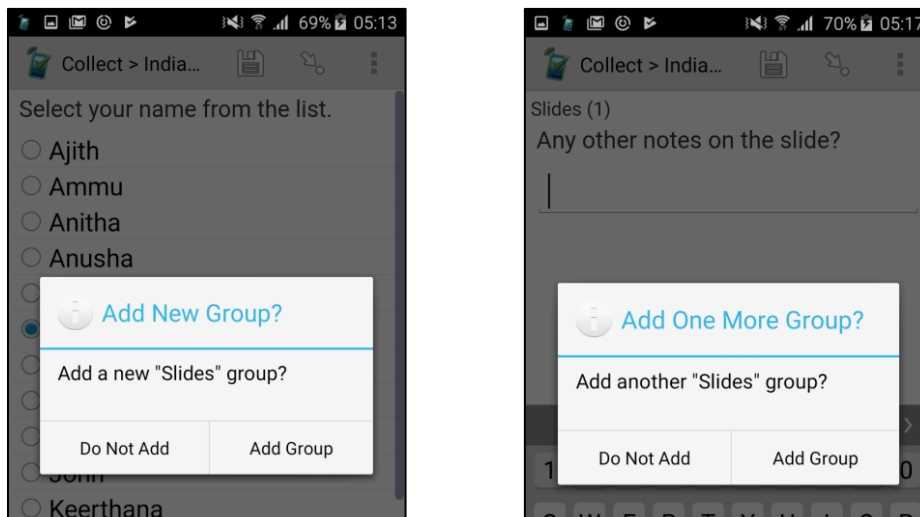
g. If you try to enter 21 slides the form will warn you that you have entered 20 slides, and you will not be able to proceed. The form will instruct you to remove the repeat instance. Do this by holding down on the screen and selecting [remove group].
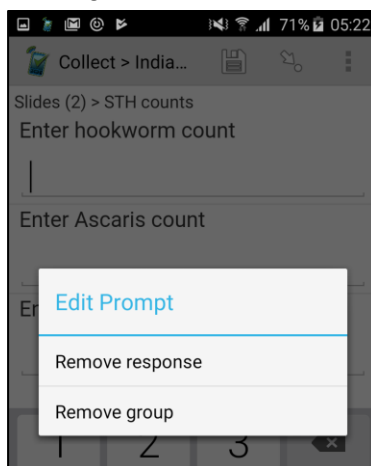
h. The other difference with the "India Laboratory Reporting Form - Kato Katz" are the options for why the slide cannot be read by the QC technician.
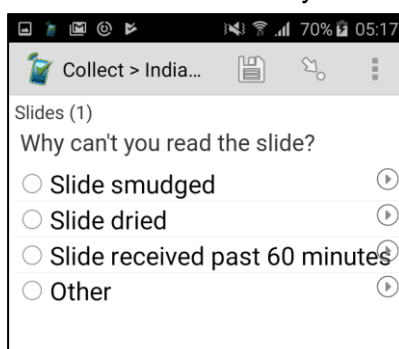
6.10. **Uploading the data**

    a. The data will be saved on the phones and automatically transmitted to the server when they are connected to Wi-Fi. However, the phones must still be checked every evening by the data manager to ensure all the data for the day has been submitted.

6.11. **Additional information**

    a. The lab assistant or technicians can clean the templates and spatulas in water with detergent, rinsed in clean water, and reused. Make sure the detergent is washed away thoroughly as it could break down eggs if it remains on the template.

7. References

- Swiss Tropical Institute, Basel (2005), KATO-Katz technique for helminth eggs.
- WHO, 1994. Bench aids for the diagnosis of intestinal parasites. Geneva: World Health Organization
- Starworms, Website (2017) https://www.starworms.org/src/Frontend/Files/userfiles/files/SOP%2006_%20Duplicate%20Kato-Katz%20smears_v1_0.pdf
- Yap P, Fürst T, Müller I, Kriemler S, Utzinger J, Steinmann P, 2012. Determining soil-transmitted helminth infection and physical fitness of school-aged children. *Journal of Visualized Experiments* (66): e3966