Ethiopia Impact Survey 2017/18

Measuring *control of morbidity* in schistosomiasis and soil transmitted helminthiasis with preventive chemotherapy

Contents

Introduction

This survey protocol describes the background and implementation design for the impact survey that will be conducted in Ethiopia during the 2017/18. Mapping was conducted in December 2013 by the Ministry of Ethiopia which has informed the strategy for the implementation of the preventive chemotherapy (PC) programme for schistosomiasis (SCH) and soil transmitted helminth infections (STH). The aim of this impact survey is to evaluate the effectiveness of the PC in reducing baseline parasitological indicators of infection.

Background to the Impact Survey

Schistosomiasis and soil-transmitted helminthiasis

Schistosomiasis or Bilharzia is a parasitic disease caused by infection with the trematode blood-flukes schistosomes. In sub-Saharan Africa, two major forms of human schistosomiasis occur: intestinal schistosomiasis caused by mainly *Schistosoma mansoni* infection and urogenital schistosomiasis due to *Schistosoma haematobium* infection. Soil-transmitted helminthiasis is caused by infection with a group of intestinal nematode worms, most important of which within much of sub-Saharan Africa are the hookworms (both *Ancylostoma duodenale* and *Necator americanus*), the roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*). Both schistosomiasis and STH are among the neglected tropical diseases (NTDs), which remain serious public health problems, posing unacceptable threats to human health and welfare.

The World Health Assembly resolution 54.19 urges all member states to regularly treat at least 75% of all school aged children who are at risk of morbidity from schistosomiasis and STH. The current control strategy recommended by the World Health Organization is to control the morbidity caused by these parasitic infections through PC with Praziquantel (PZQ) for schistosomiasis and Albendazole or Mebendazole (ALB or MBZ) for STH infection. Schistosome morbidity is mainly caused by the eggs deposited in various parts of the body depending on the species of schistosome, hence the fundamental aim of morbidity control is to reduce prevalence and intensity of infection by drug treatment.

Schistosomiasis and STH in Ethiopia

The national goal is to eliminate Schistosomiasis to a level where it is no longer public health problem by 2020 and to control soil-transmitted helminths where it is no longer a public health problem by 2020.

Two rounds of mapping was completed in the majority of the country prior to starting the national MDA. As a result mapping data was the huge input in determining treatment rounds as well as recruiting sentinel sites. Mapping data from round 1 and round 2 is shown below in Table 1 and 2. Baseline survey in the selected sentinel sites were collected prior to drug distribution.

Table 1. Endemic districts classified by infection category for schistosomiasis.

Endemicity	Pre-school-age	School-age	Adults	Total	Number of Districts / Woredas
Uninfected	5,094,660	14,192,267	25,041,163	45,488,035	382
Low (<10%)	2,129,397	5,931,891	10,466,365	19,012,470	168
Moderate (10-50%)	1,502,031	4,184,229	7,382,750	13,410,990	132
High (>50%)	804,738	2,241,771	3,955,432	7,185,163	61
Unknown	916,262	2,552,443	4,503,590	8,180,908	96
Grand Total	**10,447,087**	**29,102,601**	**51,349,300**	**93,277,566**	**839**

Table 2. Endemic districts classified by infection category for STH.

Endemicity	Pre-school-age	School-age	Adults	Total	Number of Districts / Woredas
Uninfected	392,110	1,092,306	1,927,291	3,500,982	38
Low (<10%)	2,749,546	7,659,449	13,514,508	24,549,515	231
Moderate (10-50%)	2,962,573	8,252,881	14,561,574	26,451,542	221
High (>50%)	3,438,199	9,577,839	16,899,360	30,698,201	254
Unknown	800,402	2,229,691	3,934,117	7,146,444	78
Assumed endemic, prevalence unknown	104,259	290,435	512,451	930,883	17
Grand Total	**10,447,087**	**29,102,601**	**51,349,300**	**93,277,566**	**839**

The first-large scale combined SCH/STH campaign treated 2.9 million school-aged children in 109 districts in April 2015. The bigger round MDA happened in November 2015 treating 4.8 million SAC for SCHs and 12.3 million for STHs. In the 2016/17 MDA a total of 25.4 million SAC and adolescents targeted for treatment of STH, 5.96 million SAC and adolescents and adult was targeted for SCH treatment.

Based on the mapping data the treatment scale up was aggressive and it was to reach 100% of targeted districts in Year 1. Availability of mapping results further informed the frequency of MDA rounds.

Aim

This survey protocol is designed to monitor the impact of PC with PZQ and MEB on the prevalence, intensity and morbidity of SCH and STH infection in SAC during the *Control of Morbidity* phase with the aim of reaching less than 5% heavy intensity of infection across sentinel sites[1].

Ethical approval

Ethical approval for the monitoring and evaluation of the national deworming programme have been granted by the Ethiopian Public Health Institute scientific and Ethical review committee in June 2013. Similar approval was also obtained from Imperial College London.

Objectives

The objectives of the impact survey are:

Survey Objective (SO) 1. **To measure the prevalence of SCH in SAC over time**

SO 2. **To measure the mean intensity of SCH in SAC over time**

SO 3. To measure the percentage of heavily infected SAC with SCH over time

SO 4. **To measure the prevalence of STH in SAC over time**

SO 5. To measure mean intensity of infection of STH over time

SO 6. To measure the percentage of heavily infected children with STH over time

SO 7. To measure macro haematuria in children with *S. haematobium* infection over time

SO 8. To measure micro haematuria in children with *S. haematobium* infection over time

SO 9. To determine demographic and school information

SO 10. To measure Water, Sanitation and Hygiene (WASH) indicators

Study Design

Overview

The observational epidemiological surveys will be used to establish an association between infection status and treatment over time through the national PC programme for SCH and STH. The surveys will use a cross-sectional design whereby a new selection of children from the same age groups will be randomly sampled from the same sentinel schools each year of the survey (minimum 3 years). See

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[1] WHO target for control of morbidity phase (5 to 10 years from inception of treatment) in the progress towards schistosomiasis elimination (WHO, 2013)

Appendix C for a detailed explanation of the statistical approach to the impact surveys, including why cross-sectional sampling was chosen for this survey.

Study Outcomes

The following outcomes will be measured:

- *S. haematobium*: eggs per 10ml of urine using urine filtration method (1 slide per day, repeated over 2 days)
- *S. mansoni*: eggs per 1/24th gram of faeces[2] using the Kato-Katz method (2 slides per day, repeated over 2 days)
- *Ancylostoma duodenale, Necator americanus[3]*: eggs per 1/24th gram of faeces using the Kato-Katz method (2 slides per day, repeated over 2 days)
- *Ascaris lumbricoides*: eggs per 1/24th gram of faeces using the Kato-Katz method (2 slides per day, repeated over 2 days)
- *Trichuris trichiura*: presence of eggs; eggs per 1/24th gram of faeces using the Kato-Katz method (2 slides per day, repeated over 2 days)
- Macrohaematuria: Number of children with visible blood in urine i.e. direct observation of a urine specimen which appears reddish in colour
- Microhaeamturia: Number of children with micro haematuria as detected with a reagent dipstick
- Age, how long lived in the area, and sex
- Water, sanitation and hygiene questions[4]
- School information

The following indicators will be calculated from the measured outcomes (Table 3)

Table 3: Parasitological indicators

Parasitological Indicators			
Parasite group	**Prevalence of infection (%)**	**Intensity of infection (mean number of eggs)**	**Prevalence of intensity of infection (% of SAC within each category)**
S. haematobium (by urine filtration)	Number of infected SAC / total number of SAC examined	Average number of eggs per 10 ml of urine	*Low*: 1 to 49 eggs/10 ml urine *Heavy*: ≥ 50 eggs/10 ml urine
S. mansoni (by Kato-Katz)	As above	Average number of eggs per gram of stool	*Low*: 1 to 99 eggs per gram (epg) *Moderate*: 100 to 399 epg *Heavy*: ≥ 400 epg
Hookworms	As above	Average number of eggs per gram of stool	*Low*: 1 to 1,999 epg *Moderate*: 2,000 to 3,999 epg *Heavy*: ≥ 4,000 epg
A. lumbricoides	As above	Average number of eggs per gram of stool	*Low*: 1 to 4,999 epg *Moderate*: 5,000 to 49,999 epg *Heavy*: ≥ 50,000

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[2] Kato-Katz (KK) is a specific and sensitive tool to diagnose prevalence and intensity of *S. mansoni* infection to use in countries which are in the *control of morbidity* phase.

[3] *A. duodenale* and *N. americanus* (hookworm) need not be monitored where it presents logistical demands if mapping results have shown it to be prevalent at very low frequency.

[4] Waite RC *et. al.* (2017)

T. trichura	As above	Average number of eggs per gram of stool	*Low*: 10 to 999 epg *Moderate*: 1,000 to 9,999 epg *Heavy*: ≥ 10,000 epg

Timing of surveys

The impact survey will be conducted in regions newly initiating large-scale distribution of PZQ and ALB/MEB in 2017/2018 prior to the commencement of treatment activities and on the basis of mapping data. In subsequent years, impact surveys will be conducted just before each future round of PC. Thus if treatment is every two years, surveys will therefore be carried out every other year. This may yield a mixture of annual and biennial data across the sampled schools for all species for the duration of the programme[5].

Study Setting

The population is high and moderate risk SCH communities from the treatment naïve districts[6]. In the mapping exercise in 2013 endemic districts were classified into a risk category (Table 1 and Table 2). WHO guidelines state that high-risk communities (≥50% prevalence) for SCH should receive annual treatment and moderate risk communities (≥10% and <50% prevalence) for SCH, treatment every two years (WHO, 2011). The frequency of treatment will be governed by the highest level of risk from either of the schistosomiasis species.

The STH will also be treated according to the WHO guidelines (WHO, 2011) and will take into consideration other STH control initiatives i.e. child health days and lymphatic filariasis elimination programmes, and will only influence the study settings for selection of sites on a case by case basis.

Number of schools to survey

Sample size calculations indicated that a total of 175 schools are required across both schistosomiasis species to have an 80% chance of detecting a true 40%reduction in S. haematobium prevalence in high and medium prevalence districts, and a true 40% reduction in S. mansoni prevalence in high and medium prevalence districts. Within these 175 schools, 20 schools will monitor *S. haematobium*, and 175 will monitor *S. mansoni.* All 175 schools will be used for monitoring of STH levels[7]. See appendix C for full details of sample size calculations.

Type of schools to survey

The surveys will be conducted in primary schools for a number of reasons, including:

- Higher primary school enrolment in Ethiopia is 87% (world bank,2014) ensures that the majority of children of the desired age group will be included in the sampling frame, minimising selection bias
- Primary schools present a convenient platform for conducting surveys and delivering treatment to the greatest at-risk individuals

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[5] Ideally, we would monitor biennial schools every year. In practice, this would mean treating any individuals who were infected every year (for ethical reasons) and would therefore not represent the national picture. It is therefore proposed that they are only surveyed before each round of PC.
[6] Naïve in terms of never having received national/large-scale treatment at the implementation unit level or with no treatment at then implementation unit for 3 years
[7] Unless there is a case for additional STH impact monitoring for the country

- Infection levels in older primary school children are thought to be an accurate measure of overall infection in the wider community.

Selection of schools to survey

Sentinel schools will be randomly selected from the treatment naïve districts in high and moderate risk SCH communities. The sampling frame will be the list of schools within this population so that all schools have the opportunity to be selected. The sampling will be stratified by risk category for each schistosomiasis species to ensure a balance of schools across the different prevalence categories of schistosomiasis. See Appendix C for full details of stratification and site selection.

What to do if a school cannot be visited

A short list of 'reserve schools' will be provided, such that if a selected school cannot be visited for security or other unpredictable reasons, it can be replaced with another in the same district. Note that selected school should only be replaced with those on the reserve list in extreme circumstances where it is impossible to survey that school, and not for reasons of distance, access difficulty and so on. It is important to document in the field report any school that have been replaced and the reason for this replacement, as this could be a reason for biased impact results.

Selection of children to sample within each school

A total of 120 students will be sampled per school. The SCI protocol is for the ages equating to the highest four grades in primary school to be sampled in equal numbers, as schistosomiasis prevalence generally increases with age in childhood. In Ethiopia, the majority of schools begin primary school in grade 1 at age 7 with children completing grade 6 at the age of 12. Consequently we will sample ages 9, 10, 11 and 12, with 30 children (50% male, 50% female) being sampled from each age group in each school (see Table 4). For details of sample size calculations, please see Appendix C.

Table 4. Age groups of children to be sampled

Year	Age in cross sectional study			
Baseline	9	10	11	12
Follow-up 1	9	10	11	12
Follow-up 2	9	10	11	12
Follow-up 3	9	10	11	12

Data collection and analysis

Paper data collection forms and a double entry system into an excel database for this impact survey will be used. Appendix B contains the school detail form and pupil case record form. Once data has been double entered and cleaned in-country, a copy should be sent to the SCI biostatistician whereupon it will be analysed in conjunction with the in-country stats or technical team, for the specific indicators listed above. If capacity building is required in-country to increase skills in data management and analysis, SCI will tailor a training package based on requests by the MoH. All analyses will be fully shared with collaborators in-country, and the original database will remain with the Ministry of Health.

APPENDIX A: Field team planning manual

Survey team composition

The survey teams composition:

- All regions will be represented in the survey. EPHI and regional focal persons undertake the supervision.
- There will be 35 teams in total. There will be 140 data collectors in total.
- A team will have 4 team members.
- Field days will be 30 days.

Survey team training

Training of teams:

- EPHI will lead the training and SCI contributes to the training.
- Presentations, mobile data collection and laboratory practical sessions will be done in the training. The training will take 3 days.
- The training will take place in October.
- Presentation slides will be prepared following the sentinel site protocol.

The training should will cover the following aspects:
- Rationale and background for conducting the mapping survey
- Essential aspects to maintain unbiased data collection
- Setting up laboratories
- Filling in WASH, school and individual forms
- Health and safety when handling samples
- How to use the microscope
- Preparation of samples: kato-katz, urine filtration and hemastix
- Identification and counting of parasite eggs
- Cleaning up procedures
- Data entry by two clerks (team members) to be checked by the team leader

Timetable of activities

Timeline	Responsibility	Description of activity	Who is involved
2nd week December	EPHI	Training	All data Collectors, SCI Technical Advisor to support (Birhanu)
3rd Week December	EPHI	Data Collection	All Data Collectors for 1 month
3rd Week December	EPHI	Field Supervision	EPHI
3rd Week December	SCI	Survey CTO Supervision	Carolyn
4th Week January	EPHI	Initial Data Cleaning	Birhanu

1st Week Feb	SCI	Data Cleaning and Anaylsis	Bio Stat

Roles and responsibilities

The survey team will be formed by the following team members:

Survey Coordinator - NTD Coordinator, EPHI

The primary duties of the survey coordinator are:

- o Adapt and finalise the survey protocol, including the questionnaire. Obtain TA from SCI as required.
- o Obtain Ethics approval
- o Attend a Technical Working Group Meeting to enable planning.
- o Provide a plan and budget to the FMoH to release the budget in a timely manner
- o If necessary, arrange translation and back translation of questionnaire in local languages
- o Identify the survey team
- o Organise the survey logistics
- o Organise the training of the survey team
- o Oversee the data entry (mobile-based), TA given by SCI.

SCI Program Manager

The primary duties of the SCI program manager are to:

- o Provide Technical Assistance at each stage of: Protocol writing, planning, training, implementation and analysis.
- o Support the use of the data collection platform (Survey CTO)
- o Provide the Technical Support for mobile data collection. To include: set up, training, supervision and download for analysis.

FMoH

The SCH/STH Focal Person should support the process by:

- o Providing the list of districts treated and time of treatment to enable selection.
- o Chair a Technical Working Group Meeting to plan for the survey
- o Processing the plan and releasing the budget in a timely manner
- o Provide feedback on the final report which can be added as notes at the end of the report.

- • Team leader
  - o Contact local schools in the survey area to advise them about the study
  - o Ensure strict adherence to the survey protocol
  - o Locate the schools and fill in the school form
  - o Randomly select the student according to this protocol
  - o Provide the survey teams with necessary materials for daily activities
  - o Review surveys for accuracy and completeness after each school is done.
  - o Review collected data (and eventual upload of data if mobile-based) at the end of each day
  - o Manage daily logistics
  - o Lead a daily debrief with the team
  - o Provide the field report

- Team member
  - Set up the laboratory equipment in each schools
  - Report any issues or concerns to the team leader as they occur
  - Understand the sampling protocol and the necessity of protocol compliance
  - Help in the Team Leader in the process of selection of students
  - Provide the students with container for stool and urine
  - Collect the containers with stool and urine and process them to undertake the quantification of the parasitic load

- SCI Biostatistician
  - Together with the survey coordinator and SCI program manager, adapt and finalise the survey protocol
  - Determine the sampling strategy and number schools and children per school to be surveyed
  - Select the schools to sample
  - Clean the data
  - Analyse the data and produce the report graphs and tables with SCI PM
  - Write the data cleaning notes in the report
- Nurses

APPENDIX B: Data Collection Protocol & Standardised Operating Procedures

2017 YEAR 2 FOLLOW-UP DATA COLLECTION PROTOCOL

Before arriving at the school

The school should be notified of the survey at least one week before the survey is due to start.

- When contacting the state the purpose of the survey and a list of equipment that you need (e.g. tables and chairs) to allow for quick start

Preparations should be made for the survey before arriving at the school:
- Pre-fill pupil identification numbers on the registration forms and slides
- Ensure all equipment is organised
    - Dipsticks cut
    - Cellophane soaking in methyl blue
    - Plastic cut
    - Urine filter holders filled

Arriving at the school

The first thing the team should do when arriving at the school is to seek out the head teacher:

1. Introduce the team and ask for permission to survey
2. The team leader should obtain written permission from the head teacher to survey using the 'head teacher consent form' (appendix B)
3. The team leader should interview the head teacher to complete the 'school information form' and the 'school wash form' (appendix B)
4. The **GPS coordinates** of the school should be entered on **arrival and departure** if data is being collected on paper forms

Parents of children at the school will also be informed of the study through school meetings with the director/head teacher and be requested to provide verbal informed consent for their children to participate within the study. Where requested by parents, an additional meeting with the technical survey staff can be arranged where more detailed information as to why the study is taking place and questions answered by technical staff.

Selecting the students

All students within a school that meet the required ages should be separated into age groups (9 year olds, 10 year olds, 11 year olds and 12 year olds) and assembled in separate lines – one line of boys and one line of girls for each age.

Exclusion criteria: Any child who is unwell (e.g. fever) should not take place in the study and be referred instead to the health workers. Any child whose parents have refused their child's participation in the study should not be included.

15 girls and 15 boys should be selected from each age group

If more than 15 students are present in an age/sex group, they should be selected randomly.

The steps to take for sampling pupils when there are more than 15 in an age/sex group are:
1. Count the total number of students in the line
2. Calculate the sampling fraction (*h*) using the equation below. Non-whole numbers should be rounded up.

$$h = \frac{\textit{Total number of children in line}}{15}$$

3. Select the first child by randomly selecting a number between 1 and *h*. Random number selection can be done in the field by writing numbers on pieces of paper, folding them up, placing them in a container and mixing before drawing one out at random, and then selecting the child that is in this place in line.
4. The second child to sample should be the initial number + *h*.
5. Sampling should then proceed in this manner with every *h*th child being sampled.
6. The selected children should be asked to leave the line to provide samples
7. Sampling should start from be beginning again If the end of the line is reached before 15 pupils are selected. Pupils already selected from the beginning of the line should be excluded from the second selection

Example of selection of children when there are more than 15 children in the age/sex line:
1. There are 25 girls in the line.
2. Therefore *h* = 25/15 = 1.666, which is rounded up to 2
3. The numbers 1 – 2 are written on pieces of paper, folded up and placed in a container and mixed up. The random piece of paper drawn out is 2.
4. The girl second in line is identified and asked to provide samples.
5. The second child to select for sampling is 2 + 2 = 4th in line. The child fourth in line is identified, taken from the line, and asked to provide samples
6. Sampling then continues to children 6 (= 4 + 2), 8, 10, 12, 14, 16, 18, 20, 22, 24 until the end of the line is reached
7. Only 12 children have been selected so far so sampling needs to go back to the beginning of the line excluding children who have already been selected
8. The next child to be sampled is number 1 in line (24 + 2 = back to the first person in line)
9. The next child to be sampled is number 3 in line (1 + 2) now that selected people have been removed, but number 5 in the original line
10. The final child to be sampled in number 5 in the line with selected people removed (3+ 2 = 5) but number 7 in the original line

Random selection to start at girl 2

Sample every second girl to the end of the line

Girl 1	Girl 17
Girl 2	Girl 18
Girl 3	Girl 19
Girl 4	Girl 20
Girl 5	Girl 21
Girl 6	Girl 22
Girl 7	Girl 23
Girl 8	Girl 24
Girl 9	Girl 25
Girl 10	
Girl 11	
Girl 12	
Girl 13	
Girl 14	
Girl 15	
Girl 16	

Sample first girl in line with selected girls removed

Sample every second girl remaining in line until reach 15 girls

Girl 1	Girl 17
Girl 3	Girl 19
Girl 5	Girl 21
Girl 7	Girl 23
Girl 9	Girl 25
Girl 11	
Girl 13	
Girl 15	

A list of the students selected to be in the survey should be given to the school for their records.

What to do if there are not enough students in any age/sex group

If there are less than 15 pupils in any of the desired age/sex groups within the sampled school, sample everybody in that age/sex group. Then 'top-up' the numbers with:

1. Children of the same sex who are in the age groups to be selected but haven't been selected for their age group

2. Children of the different sex who are in the age groups to be selected but haven't been selected for their age group

3. Children of the same sex who are one year younger than the minimum age targeted

4. Children of the different sex who are one year younger than the minimum age targeted

If there are still not enough pupils selected, then sample less than 120 pupils. **Do not sample ages outside the targeted ages and one year younger, and do not go to neighbouring schools to sample more children.** Make sure that the schools information form records the number of children in the school correctly.

> **Example of 'topping up' selection when there are less than 15 children in an age/sex group:**
> If the targeted ages are 10-14 and there are less than 15 girls aged 14. Select:
>
> 1. Girls aged 10-13 who haven't been selected already
> 2. Boys aged 10-14 who haven't been selected already
> 3. Girls aged 9
> 4. Boys aged 9

Collecting the samples

1. Each selected student should be asked for verbal consent to provide urine and stool samples. Urine samples should be collected between 10am and 2pm.
2. Give the selected student empty stool and urine containers and instruct them how to collect sufficient amounts of urine and stool for testing.
3. The team leader registers the student, labels the specimens with an identification number and enters the child's personal details on the individual form (Appendix B).
4. The student submits the stool specimen to the "Kato-Katz" table and proceeds to the "urine" table where the urine sample is submitted.
5. **A separate stool sample should be requested on the second day of the exercise.**
6. *All urine samples should be assessed for macro-haematuria, tested for micro-haematuria and be filtered to be examined for eggs, following the Standard Operating Procedures (SOPs) without deviation. **Urine filtration should be done on all urine samples and not just those positive for micro-haematuria***
7. *All stool samples should be examined for eggs following the Standard Operating Procedures (SOPs) without deviation*
8. *If questions or clarifications are needed please SMS, skype or call the SCI Programme Manager and/or the EPHI Focal Person Gemechu Tadess, Kalkidan Mekete, Birhanu Getachew*

Treatment in schools involved in monitoring process

Schools selected for monitoring surveys **must** be dealt with in exactly the same way as those not included in the survey. This is to ensure the results represent the whole treatment programme, which will not be true if conditions are different for those groups of people involved in the survey.

- **Drug treatments to schools involved in the monitoring survey should be administered <u>at the same time as the national programme</u>**
- Drug treatments to schools involved in the monitoring survey should not be given at the time of the survey
- Drug treatments to schools involved in the monitoring survey should be delivered not more than 2 months after survey

No special care or treatment should be given to those schools/communities involved in monitoring surveys. In particular, the following should be **avoided**:
- Extra drug treatments
- Extra training
- Extra education / Information, Education, and Communication messages

A list of any children testing positive should be kept by the school and the district health officers, such that treatment can take place if there is any unexpected delay to the MDA.

Kato Katz SOP

Diagnosis of: Schistosoma mansoni, Trichuris trichiura, Ascaris lumbricoides, Ancylostoma duodenale and Necator americanus

General Principle: people infected with STH or intestinal schistosomes pass the eggs of the worms with their faeces. By examining a stool specimen under a microscope it is possible to count the number and the type of eggs that are present.

Safety precautions
- The stool should be considered potentially infectious.
- Wear gloves and lab coats whenever handling stool samples.
- Benches, instruments and equipment should be routinely decontaminated with disinfectants after use.
- Materials contaminated with infectious waste should be disinfected before disposal.
- Drinking or eating during laboratory procedures is prohibited.
- Appropriate disinfectant(s) should be used for disposal of contaminated materials, wooden spatulas and specimen containers and for cleaning of workbenches.
- Used specimen containers must be disinfected before washing.

Equipment for Kato Katz

Kato-Katz:
- Stool sample in container (polythene squares tied with grass or plastic pot)
- Microscopic glass slides
- Cellophane sheets (hydrophilic, 30 - 50µm thick)
- Malachite green (or methylene blue)
- Glycerol
- Metal sieve (Endecott Sieve) with 200 - 250µm mesh size
- Slide boxes
- Newspapers
- Wooden or plastic applicators
- Forceps
- Kato-Katz plastic template with a hole of 6mm on a 1.5mm thick template (delivering 41.7mg of faeces)

Microscopic examination:
- Microscope
- Hand tally counter
- Laboratory forms
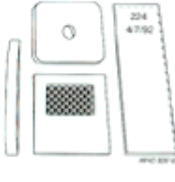
Disinfectants and waste disposal:
- Disinfectant wipes

- Medicated soap
- Methylated spirit
- Waste container (containing disinfectant)

Preparation of Kato Katz Reagents	Images
Step 1: Weigh out 3g of Malachite green powder (or methylene blue).	
Step 2: Dilute it in 100ml of distilled water (this is the **"stock solution"**).	
Step 3: Dilute 60ml of glycerine in 40ml of distilled water*.	
Step 4: Take 1 ml of Malachite green (or methylene blue) **stock solution** and add it to 100ml of the 60% glycerol solution (this is the **"working solution"**).	
Step 5: Cut cellophane into 25mm x 30mm pieces and soak them overnight in the **working solution**.	Fig. 2

*In reference books the ratio is 50% or greater glycerol solution (50ml glycerine and 50ml distilled water). In Uganda they have found this makes too light a solution and thus makes it difficult to read slides after some time has passed.

Kato-Katz Steps	Images
Step 1: Place **two** glass slides alongside each other and label both slides with the sample number and then place a plastic template on top of each.	
Step 2: Place a small amount of the faecal specimen on a newspaper and press through the metal sieve. Using a spatula, scrape the sieved faecal material through the sieve so that only the debris remains on the top.	Fig. 3
Step 3: Scrape up some of the sieved faeces from the underside to fill the hole in the templates, avoiding air bubbles and levelling the faeces off to remove any excess.	

	Images
Step 4: Carefully lift off the templates and place it in a bucket of water mixed with concentrated detergent so that they can be reused.	
Step 5: Place one piece of the cellophane, which has been soaked overnight in the malachite green (or methylene blue) working solution, over the faecal specimen.	
Step 6: Place a clean slide over the top and press it evenly downwards to spread the faeces in a circle (this can be done by inverting the slide onto clean newspaper and pressing firmly). If done well, it should be possible to read newspaper print through the stool smear.	
Step 7: If hookworm is present in the area, the slide should be read within 60 minutes of processing. After that time, the hookworm eggs disappear. The ideal time for observing *S. mansoni* eggs is 24 hours after preparation, however, in bright sunlight the slides clear rapidly and a 24hr delay is not necessary.	

Microscopic Examination for *S. mansoni* and STH	Images
Step 1: After 10 minutes place a little amount of eosin on the slide and place it under microscope using x 10 objective.	
Step 2: Count **ALL** eggs present using a hand tally counter; start in one corner of the sample and systematically scan the whole sample in a 'zig zag' scheme	
Step 3: Record the **number** and the **type of each egg** on a recording form alongside the sample number. If no eggs are seen, record "0".	
Step 4: Remove the faeces and cellophane using a tissue into the waste container and place all slides used when conducting Kato-Katz into the disinfectant. These slides should be cleaned and used again for the survey.	

Note:

The quality control when reading the Kato-Katz slides is important. For example, confirming the agreement % for laboratory technicians to ensure quality (see the agreement % on a specimen collection).

Haemastix SOP

Diagnosis of: Schistosoma haematobium.

All manufactured kits come with instructions on how to use them. It is very important to follow the instructions to ensure the quality of the results.

Equipment for Hemastix test
- Case record form
- Hemastix test strip and Hemastix pot with scale
- Scissors
- Gloves
- Disinfectants and waste disposal

haemastix.MPG

Video demonstration: click on the icon

Steps for Reagent Strips	Images
Step 1: Collect a fresh urine specimen in a clean plastic container. Ensure that the urine is tested in the field within **2 hours** of collection. If there is a delay, refrigerate the specimen if possible.	
Step 3: Remove one strip from its bottle (you can cut the strip in two to save resources) and label the strips with the patient identification.	
Step 4: Completely immerse the reagent areas of the strip into the urine specimen for a few seconds.	

Step 5: When removing the strip, run its edge against the rim of the container to remove any excess urine.	
Step 6: Put the strip horizontally on the table so that the chemicals do not mix together.	
Step 7: Read the strip between 1 and 2 minutes after it has been dipped in the urine specimen.	
Step 8: Match the colour of the strip with the colour chart on the bottle label and record the results on the monitoring form. Record "0" if the result is negative. 1= trace non-haemolysed 2 = trace haemolysed 3 = + 4 = ++ 5 = +++	
Important Note: • DO NOT LAY THE STRIP ON THE COLOUR CHART AS THIS WILL SOIL THE CHART • It is extremely important to read the strip 1-2mins after it has been dipped in the urine sample. Any colour changes that occur after 2 minutes are of no diagnostic value and should be ignored.	

Urine Filtration SOP

Diagnosis of: Schistosoma haematobium

All manufactured kits come with instructions on how to use them. It is very important to follow the instructions to ensure the quality of the results.

Safety precautions
- The urine should be considered potentially infectious.
- Wear gloves and lab coats whenever handling urine samples.

- Benches, instruments and equipment should be routinely decontaminated with disinfectants after use.
- Materials contaminated with infectious waste should be disinfected before disposal.
- Drinking or eating during laboratory procedures is prohibited.
- Appropriate disinfectant(s) should be used for disposal of contaminated specimen containers and for cleaning of workbenches.
- Used specimen containers must be disinfected before washing
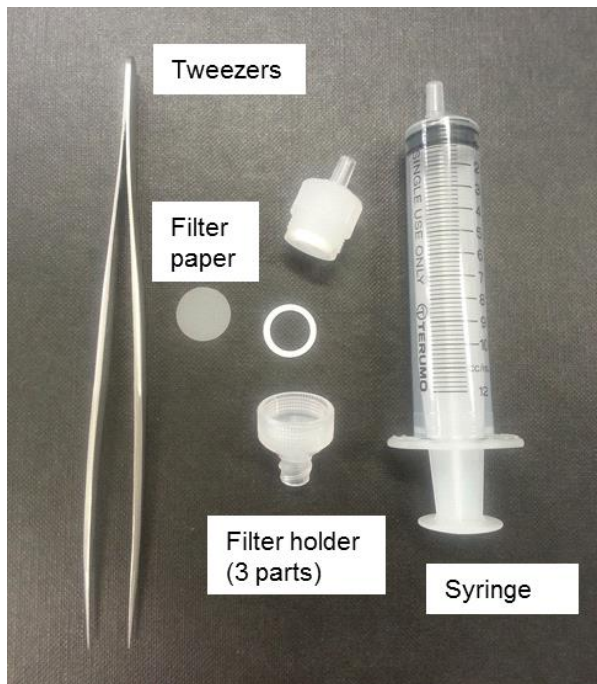
Equipment

General use:
- Gloves
- Laboratory Forms

Urine Filtration:
- Urine pots (250ml)
- Swinnex Filter Holder
- Tweezers/Forceps
- Syringe, plastic, 10ml
- Nucleopore Membrane Filter,
- 13mm diameter and pore size 12µm
- Microscope glass slides
- Lugol's Iodine (5% solution)

Microscopic examination:
- Microscope
- Hand tally counter

Disinfectants and waste disposal:
- Bucket (to discard urine)
- 1% hypochlorite solution (domestic bleach)
- Methylated Spirit
- Medicated soap
- Rubber washing gloves
- Disinfectant wipes
- Waste container (containing disinfectant)

Sample collection:

The number of eggs in the urine varies throughout the day, with the highest between 10am and 2pm. The specimen should be taken between these times and consist of a single urine sample. Since eggs

are more often found at the end of a urine flow, at least 10ml should be collected at the end of urination (the terminal urine). The easiest way to ensure a terminal urine sample is to ask individuals to 'try to fill' a large pot, e.g. 250ml. Note that some children, particularly those who are heavily infected with schistosomiasis, may not be able to provide 10ml of urine. Do not discard these smaller samples, but note the volume (ml) of urine provided. Specimens should be examined as soon as possible after collection as the eggs may hatch and then become invisible, or crystals may form, making a correct diagnosis more difficult.

IMPORTANT NOTE: To increase the volume of urine provided during sample collection, it would be advisable to promote fluid intake and physical exercise prior to micturition (e.g. provide the children with 2 glasses of water, one hour before urine collection, and request the children to participate in 10 minutes of exercise) (Doehring *et al.* 1983).

Steps for Urine Filtration	Images
Step 1: Unscrew the filter holder and insert a nucleopore filter between the two parts of the filter holder. Make sure it is correctly held in place before screwing the unit together again.	
Step 2: Thoroughly shake and mix the urine specimen before drawing a 10ml specimen into the syringe. Then attach the filter unit. **If less than 10ml urine sample is available, withdraw all urine in the sample pot and note the quantity of urine (ml) on the laboratory form next to the ID number**. **Do not discard the urine sample if it is less than 10ml.**	
Step 3: Keeping the syringe and the unit in a vertical position, press the plunger down to push all the urine through the filter and out into a bucket.	

Step 4: Carefully detach the syringe from the filter unit. Draw air into the syringe, reattach the syringe to the filter unit holder and expel the air again. This is important as it removes any excess urine and ensures that the eggs are firmly attached to the filter.	
Step 5: Unscrew the filter holder and use a pair of tweezers to remove the filter and place it inverted, onto the glass microscope slide labeled with a unique identification number. The top side of the filter, where the eggs were captured, should be face-up on the slide. DO NOT DISCARD THE FILTER HOLDER OR SYRINGE.	
Step 6: Add one drop of Lugol's iodine and wait 15 seconds for the stain to penetrate the eggs. This makes the eggs more easily visible.	
Step 7: Immediately examine the whole filter under a microscope at a low power (x40). Schistosome eggs can be seen clearly because they stain orange. Record the *total number of eggs on the filter*.	
Step 8: At the end of the day, wash all reusable equipment (forceps, filter holders, syringes, urine containers, glass slides) in 1% hypocholorite solution (domestic bleach) for use next day, discard used filters and clean the workbench.	
IMPORTANT: Read the slide within an hour of the urine sample being taken otherwise the eggs may be non-viable and become translucent. Do not leave the samples exposed to the sun.	

APPENDIX C: Data Collection Forms

Impact Survey - School Information Form

Date of visit (*DD-MM-YYYY*)	|___|___|-|___|___|___|-|___|___|___|___|	Reporters Initials	|___|___|___|

A. Site Details

1.	Implementation Unit Name	
2.	Implementation Unit Code *(DDD)*	|___|___|___|
3.	Admin level x (sub-district)	
4.	Admin level x (community/s)	

B. GPS (at time of…)

5.	Arrival decimal degrees **east or west**	|___|___|.|___|___|___|___|___|___|___|___|
6.	Arrival decimal degrees **south or north**	|___|___|.|___|___|___|___|___|___|___|___|
7.	Departure decimal degrees **east or west**	|___|___|.|___|___|___|___|___|___|___|___|
8.	Departure decimal degrees **south or north**	|___|___|.|___|___|___|___|___|___|___|___|

C. School details

9.	School Name		
10.	School Code	*(SSS)*	|___|___|___|
11.	Name of Headteacher		
12.	Contact Number of Headteacher		
13.	Have pupils in your school received deworming treatment in the last year?	*0=No* *1=ALB 2=PZQ* *3=PZQ + ALB 4=Don't know*	|___|
14.	**Lowest** Grade taught	*1=One 2=Two 3=Three 4=Four* *5=Five 6=Six 7=Seven 8=Eight*	|___|
15.	**Highest** Grade taught	*1=One 2=Two 3=Three 4=Four* *5=Five 6=Six 7=Seven 8=Eight*	|___|

D. Enrolment numbers

	Boys Enrolled	**Girls** Enrolled
Total	16. |___|___|___|___|	17. |___|___|___|___|
Grade 1	18. |___|___|___|	19. |___|___|___|
Grade 2	20. |___|___|___|	21. |___|___|___|
Grade 3	22. |___|___|___|	23. |___|___|___|
Grade 4	24. |___|___|___|	25. |___|___|___|
Grade 5	26. |___|___|___|	27. |___|___|___|
Grade 6	28. |___|___|___|	29. |___|___|___|
Grade 7	30. |___|___|___|	31. |___|___|___|
Grade 8	32. |___|___|___|	33. |___|___|___|

Mapping and Impact Survey – School WASH Form

Date of survey (*DD-MM-YYYY*)	|___|___|-|___|___|-|___|___|___|___|	Interviewer initials	|___|___|___|
District Name		District Code	|___|___|___|
School Name		School code	|___|___|___|
DDD – implementation unit code, SSS – school code			

A. Observation by Interviewer

	Yes	No	Observations on….
School Water			type/location
An improved water source is located on site			
An improved water source is accessible to all children at school			
School Hygiene			height of station/ease of use
A handwashing station with water and soap is present near the latrines			
A handwashing station with water and soap is present near to kitchen/food preparation area			
A handwashing station with water and soap is accessible to all children at school			
School Sanitation			Physical structure/cleanliness/access for disability students+staff
Latrines are functioning and accessible to all children at school			
Latrine floors (internal and external) are free from excreta			
There is ≥1 latrine per 25 girls			
There is ≥1 latrine per 50 boys			
There is ≥1 latrine for female teacher/staff			
There is ≥1 latrine for male teacher/staff			

B. Answers from Headteacher or Teacher

	Yes	No
School Water		
Do all staff have access to an improved water source at school?		
Do all children have access to an improved water source at school?		
School Hygiene		
Is good hygiene taught at this school?		
School Sanitation		
Did you use a basic sanitation facility last time you defecated at school?		

Form 1: School Information Form notes

The School Form is critical for the survey. It will allow background information required for the survey to be gathered. This form must be filled out upon arrival at each of the schools that are participating in the activities of the SCH and STH programme.

Section A: Site Details

Site Details should be filled out on arrival at the location as outlined on the forms.

> *Date of survey*: To be filled on the day of survey following:
> Day (DD) – Month (MMM) – Year (YYYY)
> Example: (DD-MMM-YYYY): |**2**|**7**|-|**F**|**E**|**B**|-|**2**|**0**|**1**|**1**|
> *Team Leader Initials*: The data collector will record his/her initials in the allocated spot on the form:
> Team Leader Initials |___|___|___|
> Example: John Jones Smith |**J**|**J**|**S**|

1. *Implementation Unit Name*: Record the name of the District here in **BLOCK Capitals** to ensure it is easy to read.
2. *Implementation Unit Code*: Fill in the district code (DDD) in accordance with the assigned codes decided pre-survey this should be a 3 digit number: 001 – XXX.
3. *Admin level x (e.g. sub-district)*: Record the name here.
4. *Admin level x (e.g. community/s)*: Record the name/s here.

Section B: GPS

GPS coordinates must be recorded on site at arrival and departure (stand in the same place for each recording).

Ensure, as per SOP, that the GPS unit is set to decimal degrees format hddd.dddddd

5. *Arrival decimal degrees east or west (as appropriate)*: Record numbers here.
6. *Arrival decimal degrees south or north (as appropriate)*: Record numbers here.
7. *Departure decimal degrees east or west (as appropriate)*: Record numbers here.
8. *Departure decimal degrees south or north (as appropriate)*: Record numbers here.

Section C: School Details

School information will be gathered on site through conversations with the Headteacher who will assist you in the survey activities.

9. *School Name*: Record the name of the school here in **BLOCK Capitals** to ensure it is easy to read
10. *School Code*: Fill in the school code (SSS) in accordance with the assigned codes (see Schools list) this is a 3 digit code: 001– XXX. Schools are numbered (arbitrarily) within each District
11. *Name of Headteacher*: Record the name of the Headteacher here in **BLOCK Capitals** to ensure it is easy to read
12. *Contact Number of Headteacher*: mobile number of Headteacher
13. *Have pupils in the school received deworming treatment in the last year?*: Write the corresponding number in available space.

0=No
1=ALB
2=PZQ
3=PZQ + ALB
4=Don't know

14. *Lowest Class Taught*: Write the corresponding number to the lowest grade taught in the school in the available space.

15. *Highest Class Taught*: Write the corresponding number to the highest grade taught in available space

Section D: Enrolment Numbers

Record the enrolment numbers in the available space. The Headteacher will be able to assist you with this section. The total refers to the total school enrolment.

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|___|___|___|-|___|___|___|	Date of survey (DD-MM-YYYY)	|___|___|-|___|___|-|___|___|___|___|
School Name		Technician initials	|___|___|___|
Sex of children registered on page		Age of children registered on page	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source[1] at school? (Y/N)	Did you use a basic sanitation facility[2] last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
001												
002												
003												
004												
005												
006												
007												
008												
009												
010												
011												
012												
013												
014												
015												

[1] Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
[2] Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|__|__|__|-|__|__|__|	Date of survey (DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|
School Name		Technician initials	|__|__|__|
Sex of children registered on page		Age of children registered on page	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source[1] at school? (Y/N)	Did you use a basic sanitation facility[2] last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
016												
017												
018												
019												
020												
021												
022												
023												
024												
025												
026												
027												
028												
029												
030												

[1] Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
[2] Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|__|__|__|-|__|__|__|	Date of survey (DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|
School Name		**Technician initials**	|__|__|__|
Sex of children registered on page		**Age of children registered on page**	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source[1] at school? (Y/N)	Did you use a basic sanitation facility[2] last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
031												
032												
033												
034												
035												
036												
037												
038												
039												
040												
041												
042												
043												
044												
045												

[1] Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
[2] Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|___|___|___|-|___|___|___|	Date of survey (DD-MM-YYYY)	|___|___|-|___|___|-|___|___|___|___|
School Name		Technician initials	|___|___|___|
Sex of children registered on page		Age of children registered on page	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source¹ at school? (Y/N)	Did you use a basic sanitation facility² last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
046												
047												
048												
049												
050												
051												
052												
053												
054												
055												
056												
057												
058												
059												
060												

¹ Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
² Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|__|__|__|-|__|__|__|	Date of survey (DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|
School Name		Technician initials	|__|__|__|
Sex of children registered on page		Age of children registered on page	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source[1] at school? (Y/N)	Did you use a basic sanitation facility[2] last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
061												
062												
063												
064												
065												
066												
067												
068												
069												
070												
071												
072												
073												
074												
075												

[1] Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
[2] Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|__|__|__|-|__|__|__|	Date of survey (DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|
School Name		Technician initials	|__|__|__|
Sex of children registered on page		Age of children registered on page	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source[1] at school? (Y/N)	Did you use a basic sanitation facility[2] last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
076												
077												
078												
079												
080												
081												
082												
083												
084												
085												
086												
087												
088												
089												
090												

[1] Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
[2] Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|___|___|___|-|___|___|___|	Date of survey (DD-MM-YYYY)	|___|___|-|___|___|-|___|___|___|___|
School Name		Technician initials	|___|___|___|
Sex of children registered on page		Age of children registered on page	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source[1] at school? (Y/N)	Did you use a basic sanitation facility[2] last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
091												
092												
093												
094												
095												
096												
097												
098												
099												
100												
101												
102												
103												
104												
105												

[1] Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
[2] Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Registration Form

School Code (DDD-SSS) (District Code-School Code)	|__|__|__|-|__|__|__|	Date of survey (DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|
School Name		**Technician initials**	|__|__|__|
Sex of children registered on page		**Age of children registered on page**	

ID Number	Name	Age	Sex	Class	How long have you lived here? (years)	Do you have access to an improved water source¹ at school? (Y/N)	Did you use a basic sanitation facility² last time you defecated at school? (Y/N)	Are you taught about good hygiene in school? (Y/N)	Day 1 stool (tick)	Day 1 urine (tick)	Day 2 stool (tick)	Day 2 urine (tick)
106												
107												
108												
109												
110												
111												
112												
113												
114												
115												
116												
117												
118												
119												
120												

¹ Piped water; Public tap or standpipe; Tubewell or borehole; Protected dug well; Protected spring; Rainwater
² Sewer connections, septic system connections, pour-flush latrines, ventilated improved pit latrines and pit latrines with a slab or covered pit

Impact Survey - Individual Parasitological Form

School Code (DDD-SSS) (District Code-School Code)	|__|__|__|-|__|__|__|		Child Code (3 digits 001-120)	
School Name				

Stool Samples: Kato-Katz
record number of eggs found (do not multiply)

Date (DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|		|__|__|-|__|__|-|__|__|__|__|	
Slide	*Day 1 Slide A*	*Day 1 Slide B*	*Day 2 Slide A*	*Day 2 Slide B*
Microscopist initials	|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|
S. mansoni	|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|
Ascaris	|__|__|__|__|__|	|__|__|__|__|__|	|__|__|__|__|__|	|__|__|__|__|__|
Hookworm	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|
Trichuris	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|

Urine Samples: observations, dipsticks and urine filtration

Date	(DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|	(DD-MM-YYYY)	|__|__|-|__|__|-|__|__|__|__|
	Day 1		*Day 2*	
Dipstick result (micro-haematuria)	0 = none 1 = trace non-haemolysed 2 = trace haemolysed 3 = + 4 = ++ 5 = +++	|__|	0 = none 1 = trace non-haemolysed 2 = trace haemolysed 3 = + 4 = ++ 5 = +++	|__|
Visible Haematuria	0 = No 1 = Yes	|__|	0 = No 1 = Yes	|__|
CCA result	0 = negative tr = trace + = 1 ++ = 2 +++ = 3	|__|		
Volume of urine filtered	*(ml)*	|__||__|•|__|	*(ml)*	|__||__|•|__|
Microscopist initials		|__|__|__|		|__|__|__|
S. haematobium Number of eggs	*(ensure zero counts are recorded; if missing leave blank)*	|__|__|__|__|	*(ensure zero counts are recorded; if missing leave blank)*	|__|__|__|__|

Form 3: Individual Form notes

The completion of this form allows each survey participant to be given a unique identification (ID) number comprised of DDD – district code, SSS – school code and NNN – ID number (00-999). This ID allows the individuals' names to be absent from the Individual Form where the diagnostic results are recorded. This form also collects individual level indicators for WASH.

Please complete the information at the <u>top of the page</u>:

> *Date of survey*: To be filled on the day of survey following:
> Day (DD) – Month (MMM) – Year (YYYY)
> Example: (DD-MMM-YYYY): |2|7|-|F|E|B|-|2|0|1|1|
> *Registers Initials*: The data collector will record his/her initials in the allocated spot on the form:
> Registers Initials |___|___|___|
> Example: John Jones Smith |J|J|S|
> *Implementation Unit Name*: Record the name of the District here in **BLOCK Capitals** to ensure it is easy to read.
> *Implementation Unit Code*: Fill in the District code (DDD) in accordance with the assigned codes decided pre-survey this should be a 3 digit number: 001 – XXX.
> *School Name*: Record the name of the School here in **BLOCK Capitals** to ensure it is easy to read.
> *School Code*: Fill in the School code (SSS) in accordance with the assigned codes decided pre-survey this should be a 3 digit number: 001 – XXX.

A. Individual Details
These questions must be filled in when the individual presents their samples on Day 1.
- *Name*: Record the first and last names here in **BLOCK Capitals** to ensure it is easy to read
- *Identification Number (DDD.SSS.NNN)*: Insert the district code (DDD): school code (SSS); and individual number (NNN) which should be a 3 digit number: 001 – 120 which is the sample size.

B. WASH Questions
These questions must be filled in when the individual presents their samples on Day 1.
- *Do you have access to an improved water source at school?*: Tick one box – yes or no
- *Did you use a basic sanitation facility last time you defecated at school?*: Tick one box – yes or no
  Are you taught about good hygiene in school?: Tick one box – yes or no

Form 3: Individual Form

A. Individual
Ask the individual, when they give in their sample, the following questions and record on the form:
1. *Sex*: Record the gender of the individual using the key on the sheet:
    1 = Male; 2 = Female
2. *Grade:* Record the grade of the individual using the key on the sheet:

1 = One; 2 = Two; 3 = Three; 4 = Four; 5 = Five; 6 = Six; 7 = Seven; 8 = Eight

3. *Age*: Record the age of the student in years.

4. *How long have they lived there?* Record the length of time (in years) they have lived in that town.

B: Kato Katz

Samples will be collected on 2 separate days with 2 slides examined from each of the specimen collections.

Date: The date will be recorded on each of the days that the slides are examined. Day 1 and Day

e.g.

Date *(DD-MMM-YYYY)*	**|2|5|-|F|E|B|-|2|0|1|1|**		**|2|6|-|F|E|B|-|2|0|1|1|**	
Slide	*Day 1 Slide A*	*Day 1 Slide B*	*Day 2 Slide A*	*Day 2 Slide B*

Microscopist initials: The microscopist who examines slide A and slide B on each of the days must initial in the provided location.

Microscopist Initials |___|___|___|
Example: John Jones Smith **|J|J|S|**

Slide	*Day 1 Slide A*	*Day 1 Slide B*	*Day 2 Slide A*	*Day 2 Slide B*
Microscopist initials	|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|

Egg count: The egg count for *S. mansoni*, Hookworm, Ascaris and Trichuris needs to be determined and recorded for each slide for each slide.

The number of eggs for each species per slide needs to be recorded.
- Do not multiple this number by 24; ensure that any zeros (0) are recorded and if sample is missing leave blank.
- Where hookworm is not recorded, the record should be scored through or left blank.

S. mansoni*	|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|
Hookworm*	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|
Ascaris*	|__|__|__|__|__|	|__|__|__|__|__|	|__|__|__|__|__|	|__|__|__|__|__|
Trichuris*	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|	|__|__|__|__|

C Urine Filtration and Dipstick Result

Samples will be collected on 2 separate days with 1 slide and dipstick examined from each of the specimen collections.

Date: Record the date that the Urine filtration and dipstick test are carried out *(DD-MMM-YYYY)*

E.g. |2|5|-|F|E|B|-|2|0|1|1|

Dipstick result: Record the dipstick result using the key on the box of the dipsticks and corresponding number on the sheet

        0 = none; 1 = trace non-haemolysed; 2 = trace haemolysed; 3 = +; 4 = ++; 5 = +++

Visible Haematuria: Record the visible haematuria result i.e. can blood in urine be seen using the key on the sheet.

        0 = No; 1 = Yes

Volume of urine: Record the millilitres (ml) of urine collected from the individual. DO NOT THROW OUT URINE IF LESS THAN 10 ml, the volume must still be recorded and sample tested for eggs.

Microscopist initials: The microscopist who examines slide A and slide B on each of the days must initial in the provided location.

        Microscopist Initials |___|___|___|
        Example: John Jones Smith   **|J|J|S|**

Egg Count: Count the number of *S. haematobium* eggs that are found in examination of the filtrated urine

* **ALL urine samples but have urine filtration performed on them**
* Ensure that any zero (0) counts are recorded, if the sample is missing leave blank

Repeat for the Day 2 urine sample.

Form 4: WASH Form

These questions must be filled in prior to the activity by field team on Day 1.
* *Do you have access to an improved water source at school?*: Record ✓ for **YES** or **X** for **NO**

* *Are you taught about good hygiene in school?*: Record ✓ for **YES** or **X** for **NO**

* *Did you use a basic sanitation facility last time you defecated at school?*: Record ✓ for **YES** or **X** for **NO**

Appendix C: Detailed survey methodology & sample size estimation

Deviations from SCI principles in this protocol

SCI/in-country biostatistician to fill in any deviations and reasons for deviations

Sample size details

SCI/in-country biostatistician to fill in details of how sample sizes were determined.

- Estimated initial prevalence in IU's to monitor:
  - *S. haematobium:*
  - *S. mansoni*
- True reduction in prevalence to detect:
  - *S. haematobium:*
  - *S. mansoni:*
- Number of children sampled per school =
- Focalness of schistosomiasis (ICC):
  - Widespread (0.1)
  - Medium (0.25)
  - Very focal (0.4)
- Repeated measures correlation
  - Same children (assuming children only become uninfected, not infected)
  - Same schools, different children (midway between same children and different schools)
  - Different schools (0)
- Alpha: 5%
- Beta: 80%

Statistical approach to impact surveys

> ### Statistical approach to impact survey methodology & sample size estimation
>
> #### Scope
>
> These principles are applicable for those programs that are in the 'control of morbidity' phase of a program, and therefore those with reasonable numbers of implementation units classified as being medium or high prevalence. As a program progresses, it is expected that the prevalence and average intensity of schistosomiasis will decrease. In this instance there are two main options available to a program:
>
> - Remain in the 'control of morbidity' phase but at a smaller scale. This could be achieved by decreasing the size of mapping units (e.g. from district to sub-district level). In this instance, the protocol for impact monitoring would be expected to remain unchanged apart from the implementation units used.
> - Move to the 'elimination of schistosomiasis' phase. This will most likely involve additional monitoring and new protocols being created. Although SCI is involved in some pilot activities that assess methods of moving towards elimination, we do not believe that this is currently a goal of any of our national programs and any additional protocols will be developed as required.
>
> #### Implementation units monitored

SCI impact surveys monitor only in those implementation units that were assessed as being moderate or high prevalence during previous mapping exercises. This is for the following reasons:

- The majority of SCI's activities are in mapping units with medium or high prevalence.

- Achieving a reduction in prevalence and intensity in high and moderate implementation units is imperative to control morbidity.

- The cost of monitoring the number of schools required at low prevalence would be prohibitive and outside the WHO guidelines on the proportion of program activities that should be spent on M&E.

Reasons for cross-sectional sampling

We have elected to use cross-sectional sampling (i.e. different children over time) rather than cohort sampling (i.e. the same children over time) in most SCI surveys for the following reasons:

- We believe that cross-sectional sampling is the most appropriate method for measuring SCI program objectives. Cohort studies are most appropriate when assessing the feasibility of MDA programs and measuring changes in morbidity and nutritional markers, but we now believe that there is sufficient evidence of the individual effectiveness of administering PZQ as part of a national program. Therefore, the question that we are now looking to address is the effectiveness of MDA on the school population rather than on individual children. In this instance, cross-sectional sampling is the most appropriate.

- Non-random drop-out from cohort studies (e.g. those most likely to be infected at follow-up are also those most likely to have dropped out of the study) means that there are statistical issues with analysing cohort data where not all children analysed at baseline have been followed over time. Non-random drop-out can either be for causal reasons such as ill-health or, perhaps more likely, non-causal reasons such as poverty that makes a child both more likely to be infected and to drop out of school. Although there are statistical methods to address drop-out (e.g. mixed models or imputation) we believe that cross-sectional sampling is more robust as it provides a like-for-like comparison of children each year. The assumption of a like-for-like comparison between years requires no external changes between years; however, it should be noted that external changes would likely affect both cohort and cross-sectional sampling equally and therefore there would be no benefit to using cohort sampling above cross-sectional sampling.

- Prevalence of schistosomiasis is believed to increase with age during primary school. However, cohort sampling means that baseline sampling has to take place in the very youngest children to enable them to be followed over multiple years. The consequence of this is that age-related changes and program changes are confounded over time. The use of cross-sectional sampling enables us to sample the oldest children in the school during each survey, avoiding any issues with confounding.

- We found in some countries that drop-out was higher than anticipated in cohort studies. For example, in Malawi approximately 40% of children in a cohort study dropped-out between baseline and the first follow-up. If this pattern were to continue over multiple years then there would not be many children in the final survey.

Sample size calculation

SCI sample sizes calculations find the number of schools required in order to have an 80% chance of detecting a 40% reduction in each schistosomiasis species at the national level for SCH. The parameters used in the calculation are:

- **True reduction in prevalence assumed to have taken place in the population = 40%.** 40% is the minimum target for *S. mansoni* and is less than the ICOSA target for *S. haematobium* of 60%. Therefore this target is somewhat conservative. This is believed to be appropriate as the power calculation itself is relatively low powered at only 80%.

- **Initial prevalence in the population: taken from results of mapping only for those implementation units that were assessed as being moderate or high.** Initial prevalence is a very important parameter in determining the number of schools required and is calculated on a country-by-country basis.

- **Number of children sampled per school = 120.** SCI protocol is to sample 120 children per school. This figure was initially determined to allow for anticipated 'drop-out' from cohort studies. Although sample size calculations for cross-sectional studies have indicated that a lower number of children per school may be acceptable, sampling 120 children per schools allows us to examine differences between ages and gender which is important information for program activities. Additionally, 120 children per school enables easy division of division of children into ages and gender, and it is not believed that sampling less children per school would enable more schools to be assessed due to logistical issues of remaining at each school for two days.

- **Focal nature of schistosomiasis: Intra-class correlation coefficient = 0.25**. Schistosomiasis is often not widespread and occurs in certain areas only, often termed 'hot-spots'. *S. haematobium* is believed to be more focal than *S. mansoni*, and both species are expected to be more focal at low prevalence. How widespread or focal schistosomiasis is can be quantified using the Intra-class Correlation Coefficient (ICC), where a low value indicates widespread disease prevalence and a high value indicates hot-spots. We have used historical data from Zambia and Uganda in order to determine an appropriate ICC of 0.25. SCI plans further research activities to assess ICC across multiple programs and this parameter may change in the future.

- **Repeated measures across schools: variable depending on initial prevalence**. Sampling the same schools over time leads to a lower number of schools being required compared to sampling different schools over time as the children within a school are expected to be correlated – a school with high prevalence at baseline is expected to have relatively high prevalence at follow-up and vice versa. Additionally, cross-sectional sampling of different children in the same schools is expected to require more schools than cohort sampling of the same children over time as the same children are expected to be more correlated than different children in the same school. Parameterising this correlation is somewhat difficult. The minimum correlation would be if we were to sample in different schools and would be exactly 0. The maximum correlation would be if we were to sample the same children in each school when no children go from negative to positive over time and would vary with initial prevalence. We have assumed that the correlation between children in the same school is exactly in the middle between zero and the maximum correlation. Note that we do not use the WHO suggestion of going to some different schools each year as we are not certain that we have sufficient sample sizes for this to be an appropriate technique. However, we hope to test this strategy in future.

- **Alpha = 5%.** This is the level at significance will be assessed and is a standard metric.

- **Beta = 80%.** This is the probability of a significant result being obtained given the true reduction in prevalence aimed for. 80% is a standard metric.

Stratification

Once the number of schools to monitor for each schistosomiasis species has been determined, the next step is to stratify the implementation units for co-endemicity. This is to ensure a balance of schools across the different intensity categories of the other schistosomiasis species. Individual schools can be used to monitor for both *S. haematobium* and *S. mansoni* for schools that meet the criteria for both species. The steps taken when stratifying are as follows:

- Create a list of all *S. haematobium* monitoring units i.e. all implementation units that were categorised as medium or high prevalence. Note the total number of *S. haematobium* monitoring units.

- From the above list, create a summary table showing the number of *S. haematobium* monitoring units in each *S. mansoni* category – high, medium and low prevalence.

- Calculate the proportion of *S. haematobium* monitoring units in each *S. mansoni* category by dividing the number of *S. haematobium* monitoring units in each *S. mansoni* category (step 2) by the total number of *S. haematobium* monitoring units (step 1).

- Calculate the number of schools to monitor for *S. haematobium* in each *S. mansoni* category by multiplying the proportion of *S. haematobium* monitoring units in each *S. mansoni* category (step 3) by the total number of schools to monitor for *S. haematobium*, and rounding to the nearest whole number. Check that the sum of the number of schools to monitor in each category equals the total number of schools to monitor for *S. haematobium*. If not, then increase or decrease the total number of schools to monitor to adjust for any rounding effects.

- Repeat the above steps for *S. mansoni*

- Merge the two tables that contain the infection categories for each species and the number of schools to monitor for each species by the infection categories for each species.

- Create a new column showing the total schools to monitor in each category as the maximum of the number of schools to monitor for each species in that category.

- Find the total number of schools to monitor by summing the total schools to monitor in each category.

Site selection

The final step is the selection of specific schools in which to monitor. The SCI biostatistician will be provided with the sampling frame of a list of all schools and associated implementation unit as per the sampling methodology section above. Sampling is done randomly with no weighting due to school size as sampling proportional to school size may mean that we sample most in urban areas where schistosomiasis prevalence may be less. Additionally, reserve sites are randomly selected from the same implementation unit as the selected site for logistical reasons.

The steps to take for site selection are:

- Remove any schools from the sampling frame that were visited during the mapping exercise. This is because any positive children assessed during mapping should have been treated at the time of assessment.

- Create a subset of the data that includes all of the schools in implementation units that satisfy the first category of the stratification list for *S. haematobium* and *S. mansoni*.

- Randomly select the required number of schools from this infection category.

- Repeat for all infection categories.

- Check that the number of schools selected is equal to the number expected.

- For each implementation unit selected, randomly select two further schools to act as reserve schools for that implementation unit.

Monitoring of STHs

STHs are monitored using the sites selected for schistosomiasis. This is because STH is more widespread than schistosomiasis and therefore the majority of schools selected for schistosomiasis are expected to also have STH infection.

Michelle Clements, Senior Biostatistician, SCI 28/05/15

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