ICOSA Malawi

Longitudinal Cohort Study for Monitoring and Evaluation of the Malawi National Schistosomiasis and Soil Transmitted Helminth Control Programme

Integrated Control of Schistosomiasis and Intestinal Helminths (ICOSA)
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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 urinary schistosomiasis due to *Schistosoma haematobium* infection. Soil-transmitted helminthiasis (STH) 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), that 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 Organisation is to control the morbidity caused by these parasitic infections through preventive chemotherapy with Praziquantel (PZQ) for schistosomiasis and Albendazole or Mebendazole (ALB or MEB) for STH infection. Schistosome morbidity is mainly caused by the eggs deposited in various parts of the body depending on the species of schistosomes, hence the fundamental aim of morbidity control is to reduce intensity of infection by drug treatment.

The National Programme for Schistosomiasis and STH Control in Malawi is overseen by the Ministry of Health. In 2009, donations of PZQ from WHO and World Vision, allowed commencement of large-scale preventative chemotherapy, beginning initially in 10 administrative districts, and rapidly scaling up to 18 districts. In 2011, The Schistosomiasis Control Initiative (SCI) sponsored by the UK Department for International Development (DFID) began financial and technical support of the National Control Programmes for Schistosomiasis and STH with the aim of scaling up treatment to National Coverage, under the the ICOSA project (Integrated Control of Schistosomiasis and Intestinal helminths in sub-Saharan Africa). The ICOSA funding currently runs from 2011-2016 and is a partnership between SCI (Imperial College), other implementing agencies and the governments of eight sub-Saharan countries.

Monitoring and evaluation is an integral and crucial part of the National Control Programme. This will allow the implementation process and impact of the programme to be critically and timely assessed. The findings will also help the National Programme management team to take necessary actions to improve the programme implementation and safeguard the success of the control programme. This will include two distinct parts: process monitoring and impact monitoring, the latter being the subject of this protocol.

**Impact monitoring**

Impact monitoring is crucial to assess the impact of the programme on a set of health indicators. It provides a mechanism to measure the control programme’s effectiveness at reducing the burden of disease and its transmission. This enables the programme management team to track progress towards a given control objective, and can provide motivation and guidance to those involved in the programme. It also provides feedback to government and sponsors to encourage their continued support for the programme. Impact monitoring consists of a series of epidemiological surveys. It starts prior to treatment intervention in order to determine the burden and geographic distribution of the diseases before the intervention, and this will provide invaluable baseline measurements which will serve as a reference against which later years can be compared. It continues throughout the life of the control programme. Measurement of prevalence alone is not sufficient as an indicator of impact because of the non-linear relationship between prevalence and intensity of infection and prevalence alone does not reflect morbidity. Therefore, focus should be on both infection (prevalence and intensity of infection) and morbidity. The effectiveness of PZQ against schistosome infection and ALB/MBZ against intestinal worm infections is well established – the aim of impact monitoring is to monitor that this effectiveness is being achieved in the context of the National Programme.

The impact of the control programme on the infection and morbidity can be monitored through longitudinal surveys in combination with cross-sectional surveys. The monitoring surveys will include important baseline data collection and a series of follow-up surveys. A longitudinal survey design requires a baseline data collection prior to the initiation of large-scale distribution of PZQ and ALB/MBZ within schools to be targeted. Follow up surveys will be conducted immediately prior to the treatment for the life of the programme to monitor the impact of the health intervention. A longitudinal survey (where the same
individuals are re-sampled over time) is the strongest survey design to demonstrate that changes in prevalence/intensity are directly attributable to the treatment.

Measurement of impact (M&E) will only be possible in those districts where preventive chemotherapy (PCT) has not yet started. It will also be necessary to have analysed the mapping data in order to sample only from areas that are categorised as high- or moderate-risk for schistosomiasis, and to determine the limits of the distribution of the different schistosome species, which are not currently well understood, especially with respect to *S. mansoni* distribution. Data collection for monitoring and evaluation should follow mapping, which has recently been completed in the 10 districts which are yet to implement mass treatment. The districts are: Balaka, Blantyre, Chiradzulu, Lilongwe, Mwanza, N. and S. Mzimba, Neno, Ntcheu and Ntchisi. A map of these districts is contained within the ‘Study Site’ section of this protocol.

Preliminary results of these mapping surveys, showing the fraction of the district population of school aged population infected with *S. haematobium* and *S. mansoni* (based on a single urine filtration and 2 KK slides from a single stool sample) are shown below. Due to the focal nature of schistosomiasis, individual communities may be more or less affected, with school prevalences varying from 0% to in excess of 80%.

<table>
<thead>
<tr>
<th>Mapping Area</th>
<th><em>S. mansoni</em> prevalence (%) (confidence interval)</th>
<th><em>S. mansoni</em> Risk</th>
<th><em>S. haematobium</em> prevalence (%) (confidence interval)</th>
<th>S.h. risk</th>
<th>Prevalence any 1 STH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balaka</td>
<td>0.59 (0.00-1.49)</td>
<td>Low</td>
<td>14.55 (10.21-18.89)</td>
<td>Moderate</td>
<td>0.39</td>
</tr>
<tr>
<td>Blantyre City</td>
<td>1.37 (0.41-2.32)</td>
<td>Low</td>
<td>10.17 (6.83-13.51)</td>
<td>Moderate</td>
<td>1.42</td>
</tr>
<tr>
<td>Blantyre Rural</td>
<td>7.28 (0.42-14.15)</td>
<td>Moderate</td>
<td>26.32 (18.98-33.67)</td>
<td>Moderate</td>
<td>1.32</td>
</tr>
<tr>
<td>Chiradzulu</td>
<td>6.46 (0.00-15.67)</td>
<td>Moderate</td>
<td>31.76 (21.81-41.71)</td>
<td>Moderate</td>
<td>1.02</td>
</tr>
<tr>
<td>Lilongwe City</td>
<td>8.24 (4.23-12.24)</td>
<td>Moderate</td>
<td>7.64 (4.28-11.0)</td>
<td>Moderate</td>
<td>1.01</td>
</tr>
<tr>
<td>Lilongwe Rural East</td>
<td>13.50 (2.46-24.54)</td>
<td>Moderate</td>
<td>9.56 (4.58-14.55)</td>
<td>Moderate</td>
<td>3.33</td>
</tr>
<tr>
<td>Lilongwe Rural West</td>
<td>2.00 (0.01-3.99)</td>
<td>Low</td>
<td>11.97 (5.85-18.10)</td>
<td>Moderate</td>
<td>3.85</td>
</tr>
<tr>
<td>Mwanza and Neno (initial joint analysis)</td>
<td>1.90 (0.00-4.78)</td>
<td>Low</td>
<td>17.76 (6.52-29.00)</td>
<td>Moderate</td>
<td>0.35</td>
</tr>
<tr>
<td>Mzimba North</td>
<td>0.51 (0.00-1.08)</td>
<td>Low</td>
<td>7.90 (2.44-13.36)</td>
<td>Moderate</td>
<td>3.24</td>
</tr>
<tr>
<td>Mzimba South</td>
<td>0.68 (0.00-1.77)</td>
<td>Low</td>
<td>10.54 (5.15-15.93)</td>
<td>Moderate</td>
<td>2.54</td>
</tr>
<tr>
<td>Mzuzu City</td>
<td>2.83 (0.50-5.17)</td>
<td>Low</td>
<td>2.17 (0.55-3.79)</td>
<td>Low</td>
<td>2.67</td>
</tr>
</tbody>
</table>

**Key Assertion**
Currently there is no comprehensive monitoring and evaluation strategy in place to complement the implementation of large-scale distribution of PZQ for SCH. To fully document and report the impact of the control programme for STH and SCH in Malawi, surveys following a cohort of individuals for the life of the programme should be conducted.
**Objectives**

**General objective**
Monitor the large scale distribution of PZQ and ALB and evaluate the impact on health (morbidity), disease intensity and disease transmission (using parasitological indicators for STH and SCH).

**Specific objectives of ICOSA programme reporting**
SO 1. Monitor coverage of drug distribution

- **Indicators (I):**
  - I 1.1 Number of school age children (enrolled and non enrolled) who have swallowed the drugs in front of the drug distributor / Number of school age children in the implementation zone
    - Goal: at least 75%
  - I 1.2 Number of school age girls (enrolled and non enrolled) who have swallowed the drugs in front of the drug distributor / Number of school age girls in the implementation zone
    - Goal: at least 75%
  - I 1.3 Number of school age boys (enrolled and non enrolled) who have swallowed the drugs in front of the drug distributor / Number of school age boys in the implementation zone
    - Goal: at least 75%
  - I 1.4 Number of non enrolled school age children who have swallowed the drugs in front of the drug distributor / Number of non enrolled school age children in the implementation zone
    - Goal: at least 75%
  - I 1.5 National coverage: number of districts that are implementing treatment algorithm in a year (annual, biannual or twice in a school cycle) / number of districts endemic for Schistosomiasis
    - Goal: 100%

SO 2. Evaluate the intensity of disease (parasitological indicators for STH and SCH): Measure the change on intensity of infection

- **Indicators (I):**
  - I 2.1 Target reductions in mean intensity were established at the outset of the ICOSA programme as shown in the table below:

<table>
<thead>
<tr>
<th>Milestone year</th>
<th>2012</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of countries with disease prevalence and intensity reduced from baseline</td>
<td>3</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Reduction in intensity in Group 1 &amp; 2 countries from baseline (assuming 75% coverage)</td>
<td>S. mansoni</td>
<td>50-65%</td>
<td>65-80%</td>
</tr>
<tr>
<td></td>
<td>S. haematobium</td>
<td>65-80%</td>
<td>75-90%</td>
</tr>
</tbody>
</table>

The following will also provide useful measures of success:

- I 2.2 Percentage of heavily infected children with *S. mansoni*: Number of children with ≥400 eggs per gram in their stool / total number of children tested
- I 2.3 Percentage of moderate infected children with *S. mansoni*: Number of children with between 100 and 399 eggs per gram in their stool / total number of children tested
- I 2.4 Percentage of heavily infected children with *S. haematobium*: Number of children with more than ≥50 eggs per 10ml in their urine / total number of children tested

Measures of STH intensity will also be evaluated.
SO 3. Evaluate the impact of health indicators: Measure the change in SCH infection-related health indicators

- Indicators (I):
  - I 3.1 Anaemia: Average level of haemoglobin measured in school children after implementation of large-scale distribution of PZQ compared to average level of haemoglobin detected in school-aged children prior to initiation of the large-scale distribution of PZQ
  - I 3.2 Percentage of children with anaemia: Number of anaemic children (Hb <115g/litre) / Total number of children investigated for haemoglobin status
  - I 3.3 Percentage of children with severe anaemia: Number of children with Hb <70g/litre / Total number of children investigated for haemoglobin status
  - I 3.4 Haematuria: Levels of haematuria detected in school-aged children after implementation of large-scale distribution of PZQ compared to levels of haematuria detected in school-aged children prior to initiation of the large-scale distribution of PZQ
  - I 3.5 Percentage of stunting: Number of children exhibiting stunting/total number of children measured for height and age.
  - I 3.6 Percentage of wasting: Number of children exhibiting wasting/total number of children measured for height, weight and age.
Study Site (Districts to begin implementation in 2012 are hatched)
Materials and Methods

Study Design

The following outcomes will be measured:

- **S. haematobium**: eggs per 10ml of urine
- **S. mansoni** and soil-transmitted helminths: number of eggs per gram of faeces using the Kato-Katz method (4 slides over 2 days). Hookworm need not be monitored where it presents logistical demands because it is present at very low frequency. KK will only be carried out in districts where **S. mansoni** infection is at moderate or high frequency (Blantyre Rural, Chiradzulu, Lilongwe City and Lilongwe Rural East; awaiting mapping results in Ntcheu and Ntchisi).

- Haematuria: urine dipsticks
- Anaemia: finger prick blood sample and the use of a Hemacue to measure haemoglobin concentration
- Wasting and stunting: height and weight
- Age, how long lived in the area, and sex.

Evaluation will use concurrent longitudinal and cross-sectional studies. The longitudinal study will follow a cohort of randomly sampled primary Standard 1, 2 and 3 (nominally 6, 7 and 8-year-olds) recruited at baseline, carrying out annual measurement. The cross-sectional study will recruit new Standard 1 pupils every year. The aim of the longitudinal study is to monitor prevalence, intensity and morbidity over the course of PCT rounds. The aim of the cross-sectional study is to monitor levels of transmission. If prevalence and intensity decrease over time in the new recruits¹, this could be due to a ‘halo-effect’ of treatment i.e. a reduction in the force of infection.

<table>
<thead>
<tr>
<th>Year</th>
<th>Age* in cross sectional study</th>
<th>Age in longitudinal study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6</td>
<td>6 7 8</td>
</tr>
<tr>
<td>Follow-up 1</td>
<td>6</td>
<td>7 8 9</td>
</tr>
<tr>
<td>Follow-up 2</td>
<td>6</td>
<td>8 9 10</td>
</tr>
<tr>
<td>Follow-up 3</td>
<td>6</td>
<td>9 10 11</td>
</tr>
</tbody>
</table>

*In many countries, the starting age for primary school is fluid so in practice we shall refer to these individuals as in Standard 1.

Sample Size

Depending on the results of the mapping, we could easily see a situation where both **S. mansoni** and **S. haematobium** are present at sufficient levels so as to justify separate evaluations of each. Monitoring is limited to areas with high or medium prevalence of infection. All districts surveyed in the recent mapping fell under this category in the recent mapping and thus will be monitored for **S. haematobium** infection.

To detect a reduction in **S. haematobium** intensity of 65% with 80% power requires an achieved sample of 22 schools, 50 pupils per school. Assuming a 40% follow-up rate within each school over the entire course of the study, this implies a baseline sample of 2,750 pupils i.e. 22 schools, 125 pupils per school. For details of sample size calculations, please see Appendix C. It is proposed that this baseline sample of 125 per school be split roughly evenly between the three lowest Grades: a minimum of 40 Standard 1; 40 Standard 2 and 40 Standard 3 will total 120 pupils, which is adequate. For the cross-sectional study, 40 new Standard 1s will be recruited each year.

¹ There is a possibility that a newly recruited 6-year-old received treatment the previous year through community-wide distribution.
S. mansoni infection was more focal and only present at medium prevalence in Chiradzulu, Blantrye Rural, Lilongwe City and Lilongwe Rural East. Thus schools selected for S. haematobium monitoring in these districts, will also be monitored for S. mansoni infection. The sample was stratified by S. mansoni infection, such that the number of schools selected for S. mansoni monitoring, reflect the frequency of moderate risk areas in the monitoring areas.

STH infection was generally very low. It is difficult to demonstrate impact from low starting values. Thus STH infection will only be monitored where Kato-Katz slides are already being prepared and read for S. mansoni infection.

**Type of schools**
The baseline surveys will be conducted in primary schools for a number of reasons, including:
1. Higher primary school enrolment in Malawi (UNESCO World Data on Education 2010/11) ensures that the majority of children of the desired age group will be included in the sampling frame, minimising selection bias
2. Primary schools present a convenient platform for conducting surveys and delivering treatment to at-risk individuals

**Sampling Methodology**
The population is the annual and biennial (every two years) 2012 PCT target population for treatment naïve districts for S. haematobium which was established from the mapping exercise. High (annual PCT) and moderate-risk (biennial PCT) communities are defined in *Preventive chemotherapy in human helminthiasis* (WHO, 2006). Each district should have been classified into a risk category from the mapping exercise. The sampling frame is the list of schools within this population.

WHO guidelines state that high-risk communities should receive annual treatment and moderate risk communities, treatment every two years. The frequency of treatment will be governed by the highest level of risk from either of the schistosomiasis species.

The method to select primary schools from the sampling frame, is by stratified random sampling. The following is how primary schools should be selected:

- The sampling frame is a list of all primary schools in districts classified as high or medium risk for S. haematobium
- The sampling frame should be as recent and complete as possible (to avoid bias).
- The sampling frame should be stored in electronic (computerised) format, preferably in a Microsoft Access database or alternatively as a Microsoft Excel spreadsheet.
- The sample is stratified by risk of S. mansoni infection.
- The sampling of schools has been conducted by the SCI biostatistician.

**Study Participant Recruitment**
For monitoring and evaluation activities, schools will be contacted as the site of the study. The director of the school will be informed fully about the study and requested to provide informed consent, allowing the study to collect samples from children within the school.

Parents of children at the school will also be informed of the study through school meetings and be requested to provide informed consent for their children to participate within the study. Prior to consent they will be provided with detailed information as to why the study is taking place and questions will be answered by technical staff that are providing the information for the meeting. In addition to this, once children included in the cohort reach age 10, they will also be asked to sign and give informed consent after receiving full information of the study. From those children from which their parents have provided informed consent, random selection will be undertaken by the health workers.

**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.
Frequency of Activities
The baseline survey will be conducted in regions newly initiating large-scale distribution of PZQ in 2012, prior to the commencement of activities and on the basis of mapping data. In subsequent years, monitoring and evaluation surveys will be conducted just before each future round of PCT. Thus if treatment is every two years, surveys will therefore be carried out every other year. This will yield a mixture of annual and biennial data across the sampled schools for both species for the duration of the programme.

Survey Methods
During the survey, cohorts of 125 children aged 6, 7 and 8 years (i.e. from Standards 1, 2 and 3) will be randomly selected, from each of the schools and enrolled into the study. Urinary filtration will be used to determine presence and intensity of infection of urinary schistosomiasis. Furthermore, detection of anaemia and haematuria will also be carried out in children selected to be part of the baseline study. Kato-katz tests will be used to determine STH and intestinal schistosomiasis in the highlighted schools. This group of selected children will be followed for the subsequent years and the same indicators measured annually.

WHO approved protocols (Montresor et al. 2002) will be used for this study and the following individual indicators will be used to measure intensity of disease and morbidity. The child will provide one urine (5-30ml) sample and two stool (2-3g) (one per day) for parasitological examination.

Urine examination:
*S. haematobium* infection intensity will be measured by urinary filtration and optical microscopy. Haematuria will be measured using Haemastix.

Faecal examination (where necessary):
Stool will be examined by standard Kato-Katz procedures (41.7mg template) for prevalence and infection intensity of *S. mansoni*, hookworm, *Trichuris*. Hookworm need not be recorded depending on logistical issues. Two slides will be examined from each stool sample (making 4 samples in total). If slides are read within one hour of preparation, hookworm prevalences may be recorded. If slides are examined after this time, the data entry field for hookworm should be struck-through or left blank.

For Growth measure:
Children height and weight will be taken as an indirect measure of nutritional and health status.

Blood sample:
A fingerprick blood sample will be taken for direct measurement of haemoglobin and detection of anaemia. Hemoglobin level measured. Both cuvette and lancet will be disposed of in a safe manner.

Data collection and analysis
Paper data collection forms will be used and a double entry system into a bespoke database will be adopted using netbooks procured for this programme. Appendices A and B contain a sample school details form and case record form. Once data has been entered, a copy should be sent to the SCI biostatistician whereupon it will be analysed for the specific objectives listed above in order to meet the requirements of the funders of the ICOSA programme. All analyses will be fully shared with collaborators in-country, and the original database will remain with the Ministry of Health.

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2 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 PCT.
BASELINE DATA COLLECTION PROTOCOL

Arriving at the school
The school information form (Appendix A) should be completed by the team leader. The GPS co-ordinates of the school should be entered. Remember also to re-read and re-enter the coordinates at the end of the visit.

Selecting the grades
Students should be selected from Standards 1 (20 boys and 20 girls), 2 (20 boys and 20 girls) and 3 (20 boys and 20 girls). These are minimum numbers; if a Standard contains 45 pupils, for example, it will be more straightforward to sample the entire Standard. If there are less than 120 pupils in Standards 1-3 within the sampled school, it will be necessary to top up the sample from a neighbouring school that occupies similar ecological conditions.

Selecting the students
The students should be separated into Standards and assembled in lines – one line of boys and one line of girls for each grade to be surveyed

- If more than the required number of students is present in a line\(^3\), they should be selected randomly.
- Calculate the sampling interval (SI) for each grade/gender group (i.e., the number of positions in the line after which a child is selected).
- SI = the total number of students in the line divided by the number of students to be surveyed in that grade/gender group, rounded to the nearest whole number.

**Example** There are 105 boys in grade 1 (6 years old), 52 girls in grade 2 (7 years old children) and 22 boys in grade 3 (8 years old children). The SIs are:
- Grade 1 105/20 = 5
- Grade 2 52/20 = 3
- Grade 3 22 is only just above the required number so all boys should be sampled here

- Select an arbitrary "start" number between 1 and the SI, which corresponds to the position of the first student to be selected.
- Subsequent students are selected by adding SI to the position of the previously selected child (in other words, if SI = 5, every 5th child is selected). Continue to the end of the line. This may result in not enough students being selected (e.g. in the grade 2 example above). If this is the case, top up the sample to the required number by taking students from the very end of the line. In other situations, 20 students will be obtained before reaching the end of the line.
- A list of the students selected to be in the cohort should also be given to the school for their records.

Collecting the samples
- Each student is asked for consent to provide urine and stool samples. Urine samples should be collected between 10am and 2pm.
- The student is given empty stool (if appropriate) and urine containers and is instructed on how to collect sufficient amounts of urine and stool for testing.
- The team leader registers the student, labels the specimens with an identification number and enters the student’s personal details on the Case Record Form (Appendix B).
- The student submits the stool specimen to the “Kato-Katz” table and proceeds to the “urine” table where the urine sample is submitted.
- For *S. mansoni*, a separate stool sample should be requested on the second day of the exercise.

\(^3\) The pupils per classroom ratio in Malawian primary schools was 104 in 2007 (UNESCO World Data on Education) so it is likely that sampling will be necessary.
CLASS OPERATING PROCEDURES

Safety precautions
• The stool and urine should be considered potentially infectious.
• Wear gloves whenever handling urine and stool samples plus lab coats when 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 cotton wool, wooden spatulas and specimen containers and for cleaning of workbenches.
• Used specimen containers, filter holders and syringes must be disinfected before washing.

Equipment
• Stool and urine sample collection:
  • Gloves, stool and urine containers.
• Urine filtration:
  • Gloves, filter holders, filters, syringes, flat forceps.
• Testing for micro-haematuria:
  • Gloves, reagent strips (Haemastix).

Kato-Katz:
Template (41.7mg), cellophane, applicator stick, stool sieving mesh, wooden spatula, Whatman blotting paper, glass slides, methylene blue, eosin, glycerol, tissue paper, insecticide.

Microscopic examination
Microscope, hand tally counter, Case Record Forms.
Disinfectants and waste disposal:
  Chloroxylenol (Dettol), chlorhexidene (JIK), medicated soap, methylated spirits.
Waste container (containing disinfectant).

Urine filtration
1. Assemble the filter holder by inserting a filter between the two parts of the filter holder.
2. Mix the urine sample thoroughly and draw up 10ml using a syringe.
3. Pass the urine through the filter and remove the syringe from the filter holder.
4. Discard the used syringe in the waste container. Give the filter holder to the student and instruct him/her to proceed to the microscopy table.
5. Unscrew the lid and remove the filter with flat forceps.
6. Discard the used filter holder in the waste container.
7. Place the filter on a glass slide and quantitatively examine it under the microscope for S haematobium ova.
8. Record the number of eggs on the Case Record Form with the appropriate ID number.
9. Discard the used filter in the waste container.
10. At the end of the day, wash all reusable equipment (forceps, filter holders, syringes, urine containers, glass slides) for use next day, discard used filters and clean the workbench.

Testing for micro-haematuria
1. Take a reagent strip and cut it into two (lengthwise) with a pair of scissors.
2. Dip one half of the reagent strip into the urine sample for a few seconds. (The other half of the reagent strip will be used for the next student’s specimen).
3. Wait for about 2 minutes and compare the colour of the reagent strip to the colours on the label of the reagent strip container.
4. Write down the result on the Case Record Form with the appropriate ID number.
5. Discard the used reagent strip in the waste container.

Preparing Kato-Katz reagents
1. Weigh out 0.5g of Methylene Blue powder.
2. Dilute it in 100ml of distilled water (this is the “stock solution”).
3. Dilute 50ml of glycerine in 50ml of distilled water.
4. Take 1 ml of Methylene blue stock solution and add it to 100ml of the 50% glycerine solution (this is the “working solution”).
5. Cut cellophane into 25mm x 30mm pieces and soak them overnight in the working solution.

Stool processing and Kato-Katz
1. The stool sample is processed immediately while still fresh.
2. Place two glass slides alongside each stool sample.
3. On one end of the slide place a sticker labelled with the ID number from the stool sample container.
4. On the other end of the slide, place a sticker labelled with the date and slide number (1 or 2).
5. Place a template on each of the labelled slides.
6. Take a small portion of stool from the sample container using a wooden spatula.
7. Smear the stool sample on a sieve until sufficient specimen is obtained for processing.
8. Take a small portion of sieved stool with a plastic applicator and transfer it to the slide templates.
9. Remove the templates from the glass slides and discard them in the waste container.
10. Take a piece of prepared cellophane with forceps and place it over each slide’s specimen.
11. Press the slides over blotting paper until there is even spread of the specimen (such that it is transparent) and place the prepared slide into a slide box.
12. Within one hour of preparation, examine the slides quantitatively under the microscope for hookworm eggs. Record the number of eggs on the Case Record Form with the appropriate ID number. If no eggs are seen, record ‘0’. If it is not possible to examine the slides within 1 hour, do not record any value in the hookworm box. (Do not record 0 if the slide was not examined for hookworm eggs). The appropriate box on the Case Record Form can be scored out if desired.
13. Ideally after at least 24 hours, examine the slides quantitatively under the microscope for S mansoni, Ascaris lumbricoides and Trichuris trichiura eggs.

Microscopic examination for S. mansoni and STH
1. Take a Kato-Katz slide, put a little amount of eosin on the slide and place it under microscope using x 10 objective.
2. Read ALL fields of the slide using the vertical ‘zig zag’ scheme.
3. Count all eggs systematically using hand tally counter.
4. Record the counts on the Case Record Form with the appropriate ID number. If no eggs are seen, record ‘0’.

The same Kato-Katz process requires repetition on the following day yielding four counts for each parasite (see Appendix B).

Standard Operating Procedure for using the Hemocue 301
1. Record the individual’s details onto the result sheet
2. Use an alcohol swab/wipe to sterilize one fingertip of the individual
3. Identify a suitable puncture side and ensure that this area is fully cleansed, use a further alcohol swab/wipe if required
4. To activate hold a new and clean lancet onto the sterilised fingertip and depress to force the needle through the skin. An audible click will be heard when this occurs
5. Squeeze the finger to encourage blood flow towards the tip
6. Use a tissue and wipe the first drop of blood off the finger
7. Apply pressure to the finger for more blood flow towards the tip of the finger
8. Apply the microcuvette, for the Hemocue, to the drop of blood. The correct volume of blood (10µl) is drawn into the microcuvette by capillary action
9. Release the finger and provide the individual a cotton ball to apply pressure on the finger
10. Safely discard the lancet into a sharps container
11. After wiping the excess blood from the sides of the microcuvette, place it into the cuvette holder and insert it into the analyser

12. Press the button for the Hemocue reader to conduct the analysis

13. The lab quality result will be displayed automatically onto the screen within approximately 10 seconds

14. Record this reading on the results sheet
15. Dispose of the microcuvette using the appropriate disposal method (clinical waste bin)
16. Provide a plaster for the individual, or apply surgical tape onto the cotton ball to firmly secure it in place

**N.B. No calibration or instrument manipulation is required as the machine is precalibrated and requires minimal maintenance.**

Images from: [http://www.hemocue.com/international/Products/Hemoglobin-1155.html](http://www.hemocue.com/international/Products/Hemoglobin-1155.html) and [http://www.hemocue.com/international/Products/The_Method-1151.html](http://www.hemocue.com/international/Products/The_Method-1151.html)

**Identification of children with severe anaemia**

Any child identified with severe anaemia during the study MUST be referred to local health team for immediate treatment or management as required. The monitoring team should assist as required to ensure this takes place.
Schools/communities selected for monitoring surveys must be dealt with in exactly the same way as those not included in the survey, 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/communities involved in the monitoring survey should be administered at the same time as the national programme
- Drug treatments to schools/communities involved in the monitoring survey should not be given at the time of the survey
- Drug treatments schools/communities involved in the monitoring survey should be delivered not more than 2 months after survey.

Although individuals will be identified as being infected with schistosomes and/or soil-transmitted helminths during the baseline data collection and follow-up surveys, it is essential that these individuals do not receive treatment during data collection to allow extrapolation of the results for this sentinel group to the whole treatment programme. Therefore, it is important to arrange that the data collection in the selected schools and communities be carried out no more than 2 months before the national drug administration takes place. This will ensure that all individuals identified as infected during the survey can receive treatment within 8 weeks of diagnosis.

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 / IEC

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.
## Appendix A

### A. Site Details

<p>| | | | | | | | |</p>
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>1. Date of visit</td>
<td>(DD-MM-YYYY)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2. Team Leader Initials</td>
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<td></td>
</tr>
<tr>
<td>3. District Name</td>
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<tr>
<td>4. Educational District</td>
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</tr>
<tr>
<td>5. Mapping Area Code</td>
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### Arrival

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<tbody>
<tr>
<td>1. east/west</td>
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<td></td>
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</tr>
<tr>
<td>2. north/south</td>
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### Departure

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<tr>
<td>3. east/west</td>
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<tr>
<td>4. north/south</td>
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### School details

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<tr>
<td>1. School Name</td>
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<tr>
<td>2. School Code</td>
<td>(SSS)</td>
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<td></td>
</tr>
<tr>
<td>3. Name of Headteacher</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4. Have pupils in your school received deworming treatment in the last year?</td>
<td>1=Yes 0=No 2=Don't know</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5. <strong>Lowest</strong> Standard taught</td>
<td>1=One 2=Two 3=Three 4=Four 5=Five 6=Six 7=Seven 8=Eight</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>6. <strong>Highest</strong> Standard taught</td>
<td>1=One 2=Two 3=Three 4=Four 5=Five 6=Six 7=Seven 8=Eight</td>
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</tbody>
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### Enrolment numbers

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<td><strong>Boys</strong> Enrolled</td>
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<td></td>
</tr>
<tr>
<td><strong>Girls</strong> Enrolled</td>
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</table>

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<tr>
<td>Whole school</td>
<td>1.</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>2.</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 1</td>
<td>3.</td>
<td></td>
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<tr>
<td></td>
<td>4.</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Standard 2</td>
<td>5.</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>6.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Standard 3</td>
<td>7.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8.</td>
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<td></td>
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</tbody>
</table>
Appendix B
(N.B. for “Grade” read “Standard”. It is acceptable to record “8” in box A.4.

<table>
<thead>
<tr>
<th>A. Individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. First name</td>
</tr>
<tr>
<td>2. Surname</td>
</tr>
<tr>
<td>3. Sex</td>
</tr>
<tr>
<td>4. Grade</td>
</tr>
<tr>
<td>5. Age</td>
</tr>
<tr>
<td>6. How long have you lived here?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Weight</td>
</tr>
<tr>
<td>2. Height</td>
</tr>
<tr>
<td>3. Haemoglobin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Kato Katz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date (DD-MMM-YYYY)</td>
</tr>
<tr>
<td>Slide</td>
</tr>
<tr>
<td>Microscopist initials</td>
</tr>
<tr>
<td>S. mansoni*</td>
</tr>
<tr>
<td>Hookworm*</td>
</tr>
<tr>
<td>Ascaris*</td>
</tr>
<tr>
<td>Trichuris*</td>
</tr>
</tbody>
</table>

*Egg count per slide i.e. do not multiply by 24; ensure zero counts are recorded; if missing leave blank.

<table>
<thead>
<tr>
<th>D. Urine Filtration and Dipstick Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date (DD-MMM-YYYY)</td>
</tr>
<tr>
<td>Egg Count Day 1</td>
</tr>
<tr>
<td>Egg Count Day 2</td>
</tr>
<tr>
<td>Volume of urine</td>
</tr>
<tr>
<td>Dipstick result</td>
</tr>
<tr>
<td>Visible Haematuria</td>
</tr>
</tbody>
</table>
Appendix C

*S. haematobium*
A dataset from Burkina Faso was used for parameter estimation. Baseline *S. haematobium* prevalence was 54% and mean of the log-transformed egg count was 2.20. The value of \( \rho \), the intra-cluster correlation, on log-transformed egg counts was 0.25 for the difference between baseline and first follow-up. The correlation between baseline and follow-up log-transformed egg counts was \( r=0.176 \). By back-transforming effect sizes for the log-transformed egg-counts, comparisons could be made with the ICOSA targets. For the Burkina Faso data, this back-transformation revealed a reduction in intensity of 96%. From the same starting intensity, to detect a reduction in intensity of 65% with 80% power requires an achieved sample of 22 schools, 50 pupils per school. Assuming a 40% follow-up rate within each school over the entire course of the study (as is evidenced from previous SCI work), this implies a baseline sample of 2,750 pupils i.e. **22 schools, 125 pupils per school.**
Standard Operating Procedure: ICOSA Data Collection forms

Form 1: ICOSA - School Form
The Mapping Form is critical for the programme to succeed; it will allow us to gather background information required for the programme. This form must be filled out upon arrival at each of the schools that are participating in the activities of the Schistosomiasis and Soil Transmitted Helminth programme.

Section A: Site Details
Site Details should be filled out on arrival at the location as outlined on the forms.
1. Date of Visit: To be filled on the day of Mapping activities following:
   Day (DD) – Month (MMM) – Year (YYYY)
   Example: (DD-MMM-YYYY): 27-6-2020
2. 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|
3. District Name: Record the name of the District here in BLOCK Capitals to ensure it is easy to read
4. Educational District Name: Record the name of the Educational District here in BLOCK Capitals to ensure it is easy to read. This is our sampling area to be consistent with mapping.
5. Educational District Code: Fill in the district code (DDD) in accordance with the assigned codes (see Schools list) – these are constant with codes used for mapping for consistency, and is a 3 digit number: 001 – 013.

Section B: GPS
The GPS device is likely to arrive with the default setting of degrees and minutes. At first use, you will need to change this to decimal degrees (instructions for the GARMIN eTrex® H):
1. Press PAGE and switch to the menu page
2. Select SETUP and press ENTER
3. Select UNITS and press ENTER
4. Select POSITION FRMT and press ENTER
5. Select the decimal degrees format hddd.ddddd and press ENTER.

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

Section C: School Details
School information will be gathered on site through conversations with the Headteacher who will assist you in the mapping activities.
1. School Name: Record the name of the school here in BLOCK Capitals to ensure it is easy to read
2. School Code: Fill in the school code (SSS) in accordance with the assigned codes (see Schools list) (this is a 3 digit code: 021 – 023. Schools are numbered (arbitrarily) 21 to 23 within each Educational District (can be assigned by field team on arrival in 'Mapping Area'/District, or via Schools list).
3. Name of Headteacher: Record the name of the Headteacher here in BLOCK Capitals to ensure it is easy to read
4. Have pupils in the school received deworming treatment in the last year?: Write the corresponding number in available space.
   $1=Yes$
   $0=No$
   $2=Don’t know$
5. Lowest Standard Taught: Write the corresponding number to the lowest Standard taught in the school in the available space.
6. Highest Standard Taught: Write the corresponding number to the highest grade taught in available space.
## Section D: Enrolment Numbers

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

Example:

<table>
<thead>
<tr>
<th>Boys Enrolled</th>
<th>Girls Enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9.</td>
</tr>
<tr>
<td>Standard 6</td>
<td>11.</td>
</tr>
<tr>
<td>Standard 7</td>
<td>12.</td>
</tr>
<tr>
<td>Standard 8</td>
<td>13.</td>
</tr>
<tr>
<td></td>
<td>14.</td>
</tr>
<tr>
<td></td>
<td>15.</td>
</tr>
<tr>
<td></td>
<td>16.</td>
</tr>
</tbody>
</table>
Form 3: ICOSA – M&E PCE Pupil Form

The M&E form will be filled out throughout the examination of each of the students taking part in the M&E activities for the ICOSA programme. There is one form per student.

Background details:

1. **ID Number**: The ID number must be recorded for every individual partaking in the study in every year; this should be stuck on the form. Identical ID stickers should also be stuck on the child themselves and their sample containers.
   - The ID number must be recorded in the following format: 
     
     \[
     (DDD.SSS.E.G.NN)
     \]
   - The code allocation is as follows:
     - **DDD** – district code
     - **SSS** – school code
     - **E** – year of entry into the study (0 = baseline, 1 = first follow-up etc.)
     - **G** – school Standard at baseline (1 = Standard 1 etc.)
     - **NN** – ID number (00-99)

   - **E.g. 1** At baseline, the first student in Standard 2 in school 1, district 1 will have the ID (i.e. start the ID number, NN, from 1 for each grade):
     \[
     |0|0|1|.|0|0|1|.|0|2|0|1|
     \]
   - **E.g. 2** At all subsequent follow-ups the student above will have *exactly the same ID* since they have been recruited for the longitudinal study
   - **E.g. 3** The first Standard 1 student recruited at second follow-up* as part of the cross-sectional study in the same school will have the ID (i.e. with school Standard at recruitment):
     \[
     |0|0|1|.|0|0|1|.|2|1|0|1|
     \]

   *Note that in medium-risk areas, follow-up may only be necessary every other year. In order to keep the ID codes consistent across the sample new Standard 1 students who are recruited in areas treated every two years at first follow-up (i.e. after two years) should have year of entry = 2.

2. **Date of survey**: To be filled on the day of survey activities following:
   - Day (DD) – Month (MMM) – Year (YYYY)
   - Example: (DD-MM-YYYY): |2|5|F|E|B|2|0|1|1|

At the school: Baseline surveys 2012

1. **ID Number**: The ID number must be recorded for every individual partaking in the study in every year; this should be stuck on the form if stickers are available. Identical ID stickers should also be stuck on the child themselves and their sample containers.
2. **ID numbers should follow the criteria above. Thus for each school at baseline, the ID numbers will be:**
   - DDD.SSS.0.1.01 – DDD.SSS.0.1.40 (Standard 1 children).
   - DDD.SSS.0.2.01 – DDD.SSS.0.2.40 (Standard 2 children) and
   - DDD.SSS.0.3.01 – DDD.SSS.0.3.40 (Standard 3 children).

   The additional 5 children may fall into any of the Standards and ID numbers generated appropriately. If it is not practical to collect data from these 5 children, a sample size of 120 children per school is sufficient.

Section A: Individual

These questions must be filled in when the student presents their samples on day 1.

1. **First Name**: Record the first name here in **BLOCK Capitals** to ensure it is easy to read
2. **Last Name**: Record the last name here in **BLOCK Capitals** to ensure it is easy to read
3. **Sex**: Record the gender of the individual using the key on the sheet:
   - 1 = Male; 2 = Female
4. **Standard**: Record the Standard that the student is in using the following provided key:
   - 1 = one; 2 = two; 3 = three; 4 = four; 5 = five; 6 = six; 7 = seven; 8 = eight
5. **Age**: Record the age of the student in years.
6. How long have they lived there?: Record the length of time (in years) they have lived in that town.

Section B: Observations
This data will be recorded when the student hands in their specimen samples on day 1.

1. Weight: The student’s weight will be taken and recorded in kilograms (kg) with the decimal point recorded.
   - E.g. 45.2
2. Height: The student’s height will be taken and recorded in centimetres (cm) with the decimal point recorded.
   - E.g. 152.7
3. Haemoglobin: The student’s haemoglobin count will be recorded in g/dl with the decimal point recorded.
   - E.g. 09.8

Section C: Kato Katz
Samples will be collected on 2 separate days with 2 slides examined from each of the specimen collections.

1. Date: The date will be recorded on each of the days that the slides are examined. Day 1 and Day 2.
   - E.g.

<table>
<thead>
<tr>
<th>Date (DD-MMM-YYYY)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide</td>
<td>Day 1 Slide A</td>
<td>Day 1 Slide B</td>
<td>Day 2 Slide A</td>
</tr>
</tbody>
</table>

- Microscopist initials: The microscopist who examines slide A and slide B on each of the days must initial in the provided location.
  - Example: John Jones Smith | J | J | S |

<table>
<thead>
<tr>
<th>Slide</th>
<th>Day 1 Slide A</th>
<th>Day 1 Slide B</th>
<th>Day 2 Slide A</th>
<th>Day 2 Slide B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopist initials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Day 1 Slide A</th>
<th>Day 1 Slide B</th>
<th>Day 2 Slide A</th>
<th>Day 2 Slide B</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mansoni*</td>
<td></td>
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<tr>
<td>Hookworm*</td>
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<tr>
<td>Ascaris*</td>
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<td>Trichuris*</td>
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Section D: Urine Filtration and Dipstick Result
1. Date: Record the date that the Urine filtration and dipstick test are carried out
   - E.g. 25-FEB-2011 (DD-MMM-YYYY)
2. Egg Count: Count the number of S. haematobium eggs that are found in examination of the filtrated urine
   - Ensure that any zero (0) counts are recorded, if the sample is missing leave blank
3. Dipstick Results: Record the results from the dipstick test according to the following key that is outlined on the form:
   - 0 = none; 1 = trace haemolysed; 2 = trace non-haemolysed; 3 = +; 4 = ++; 5 = +++
4. Haematuria: Record the presence/absence of visible haematuria in the urine with the provided key:
0 = No; 1 = Yes