



ASSESSING THE EFFICACY OF ANTHELMINTHIC DRUGS AGAINST SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHIASES



**World Health
Organization**



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WHO Library Cataloguing-in-Publication Data

Assessing the efficacy of anthelmintic drugs against schistosomiasis and soil-transmitted helminthiases.

1.Schistosomiasis – drug therapy. 2.Helminthiasis – drug therapy. 3.Anthelmintics – therapeutic use. 4.Soil – parasitology. 5.Neglected diseases. I.World Health Organization.

ISBN 978 92 4 156455 7

(NLM classification: QV 253)

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Printed in France.

WHO/HTM/NTD/PCT/2013.4



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ACKNOWLEDGEMENTS

The World Health Organization (WHO) expresses special thanks to all those who contributed to the preparation of this manual. The writing committee comprised: Antonio Montresor, WHO, Geneva, Switzerland; Bruno Levecke, Ghent University, Merelbeke, Belgium; Marco Albonico, Ivo de Carneri Foundation, Milan, Italy; Piero Olliaro, WHO, Geneva, Switzerland; and Jozef Vercruysse, Ghent University, Merelbeke, Belgium.

Thanks are also extended to the following people for their contributions, suggestions and support: David Addis, Children Without Worms, Atlanta, Georgia, USA; Lester Chitsulo, WHO, Geneva, Switzerland; Jean Tenena Coulibaly, Swiss Tropical and Public Health Institute, Basel, Switzerland; Nilanthi De Silva, University of Kelaniya, Kelaniya, Sri Lanka; Dirk Engels, WHO, Geneva, Switzerland; Albis Gabrielli, WHO, Geneva, Switzerland; Jiagang Guo, WHO, Geneva, Switzerland; Theresa Gyorkos, McGill University, Montreal, Canada; Stefanie Knopp, Swiss Tropical and Public Health Institute, Basel, Switzerland; Simone Magnino, WHO, Geneva, Switzerland; Pamela Mbabazi, WHO, Geneva, Switzerland; Maria Rebollo, Liverpool School of Tropical Medicine, Liverpool, United Kingdom; Valerio Reggi, WHO, Geneva, Switzerland; Mussa Sacko, Institut National de Recherche en Santé Publique, Bamako, Mali; Russell Stothard, Liverpool University, Liverpool, United Kingdom; Juerg Utzinger, Swiss Tropical and Public Health Institute, Basel, Switzerland; and Aya Yajima, WHO, Geneva, Switzerland.

1. BACKGROUND AND OBJECTIVES

Background

Schistosomiasis caused by *Schistosoma haematobium*, *S. mansoni*, *S. japonicum* and *S. mekongi* and soil-transmitted helminthiasis caused by *Ascaris lumbricoides*, *Necator americanus*/*Ancylostoma duodenale* (the hookworms) and *Trichuris trichiura* are among the most prevalent neglected tropical diseases (Berthony et al., 2006; Hall et al., 2008; Brooker, 2010). The main strategy for controlling the morbidity caused by these diseases is preventive chemotherapy with periodic administration of single-dose anthelmintics: praziquantel at 40 mg/kg for schistosomes, and albendazole at 400 mg or mebendazole at 500 mg for soil-transmitted helminthiasis (WHO, 2006; Gabrielli et al., 2011).

Significant progress has been made in the control of both schistosomiasis and soil-transmitted helminthiasis over the past few decades, and WHO has drawn up a roadmap to guide implementation of the policies and strategies set out in *Accelerating work to overcome the global impact of neglected tropical diseases* (WHO, 2012a), and more than 70 governments, NGOs and pharmaceutical companies committed themselves to support implementation of this roadmap in the *London Declaration on Neglected Tropical Diseases* on 31 January 2012.¹

A considerable increase in the number of individuals treated with preventive chemotherapy is expected in the next few years (WHO, 2012b, 2012c); this may result in the development of anthelmintic resistance in the parasites targeted. The limited number of studies in the public domain that have reported reduced efficacy of anthelmintic drugs were confounded by methodological flaws (De Clercq et al., 1997; Reynoldson et al., 1997; Sacko et al., 1999; Flohr et al., 2007; Humpries et al., 2011; Soukhathammavong et al., 2012) and do not yet provide conclusive evidence of anthelmintic resistance among helminths that infect humans. The most important confounding factors in studies of anthelmintic drug efficacy are listed in *Annex 1*.

Guidelines on monitoring anthelmintic drug efficacy were issued by WHO (WHO, 1999). Since the publication of those guidelines, studies have provided new insight into: indicators of drug efficacy (Montresor, 2011), thresholds for reduced efficacy (Vercruysse et al., 2011; Levecke et al., 2012a), sample size (Levecke et al. 2011a, 2012b), length of follow-up (Scherrer et al., 2009) and statistical analysis of data on drug efficacy (Dobson et al., 2009; Vercruysse et al., 2011; Levecke et al., 2011b), indicating that revision of the current guidelines is warranted.

¹ NTD Partner Website. *Uniting to combat neglected tropical diseases. Ending the neglect and reaching 2020 goals.*
<http://www.unitingtocombatntds.org/>

Objectives

The objective of the present document is to provide national control programmes with up-to-date guidelines on monitoring the efficacy of anthelmintic drugs administered in preventive chemotherapy programmes against schistosomiasis and soil-transmitted helminthiasis. Guidance is provided on when and how to assess the efficacy of anthelmintics, including detailed recommendations on indicators of efficacy, sample size, follow-up period, laboratory methods, statistical analysis and final interpretation of data collected, and also on how to respond when drug efficacy is reduced. In addition, examples are provided of an information letter for schools, an informed consent form, standard operating procedures for all recommended laboratory methods and a form for data collection.

The method described here is for evaluating the efficacy of a single anthelmintic drug against a group of parasites. It is not recommended for drug combinations (e.g. albendazole + praziquantel or albendazole + ivermectin).



2. WHEN TO ASSESS THE EFFICACY OF ANTHELMINTHIC DRUGS

We suggest that the efficacy of anthelmintic drugs used in preventive chemotherapy be assessed in one of two scenarios:

1. An assessment should be undertaken each time the programme manager suspects reduced treatment performance, despite satisfactory preventive chemotherapy coverage and compliance. For example, in case of:

- unexpected persistence of parasite-attributable morbidity (e.g. haematuria, malnutrition or anaemia) in the target population after several rounds of treatment;
- unexpected persistence of schistosome and/or soil-transmitted helminth infections of high intensity in the target population; or
- an insufficient drop in prevalence and intensity of infections in the target population.

2. Independently of whether drug failure is suspected, the efficacy of an anthelmintic drug should be evaluated when it has been administered in a preventive chemotherapy programme for 4 years or more.

3. ASSESSMENT OF THE EFFICACY OF ANTHELMINTIC DRUGS

Assessment of anthelmintic efficacy consists of five consecutive steps, presented schematically in *Figure 1*. Details of the steps are discussed below.

3.1 School selection and communication with school personnel and families

As the aim of the survey is not to represent the epidemiological situation of schistosomiasis or soil-transmitted helminthiasis in an area, it is not necessary to sample schools randomly. One or more schools should be selected in an area where reduced drug efficacy is reported or suspected or where helminth infections are expected to be most prevalent. Other criteria in selecting schools are the distance from the laboratory and the cooperation of school personnel.

A letter of introduction (an example is given in *Annex 3*) should be sent well in advance to the selected schools (i) to explain the purpose and details of the survey, (ii) to inform the principals of the proposed date for collecting specimens and (iii) to instruct teachers to obtain consent from the parents of the children involved in the study (see *Annex 4* for an example of an informed consent form).

3.2 Sample size and selection of children

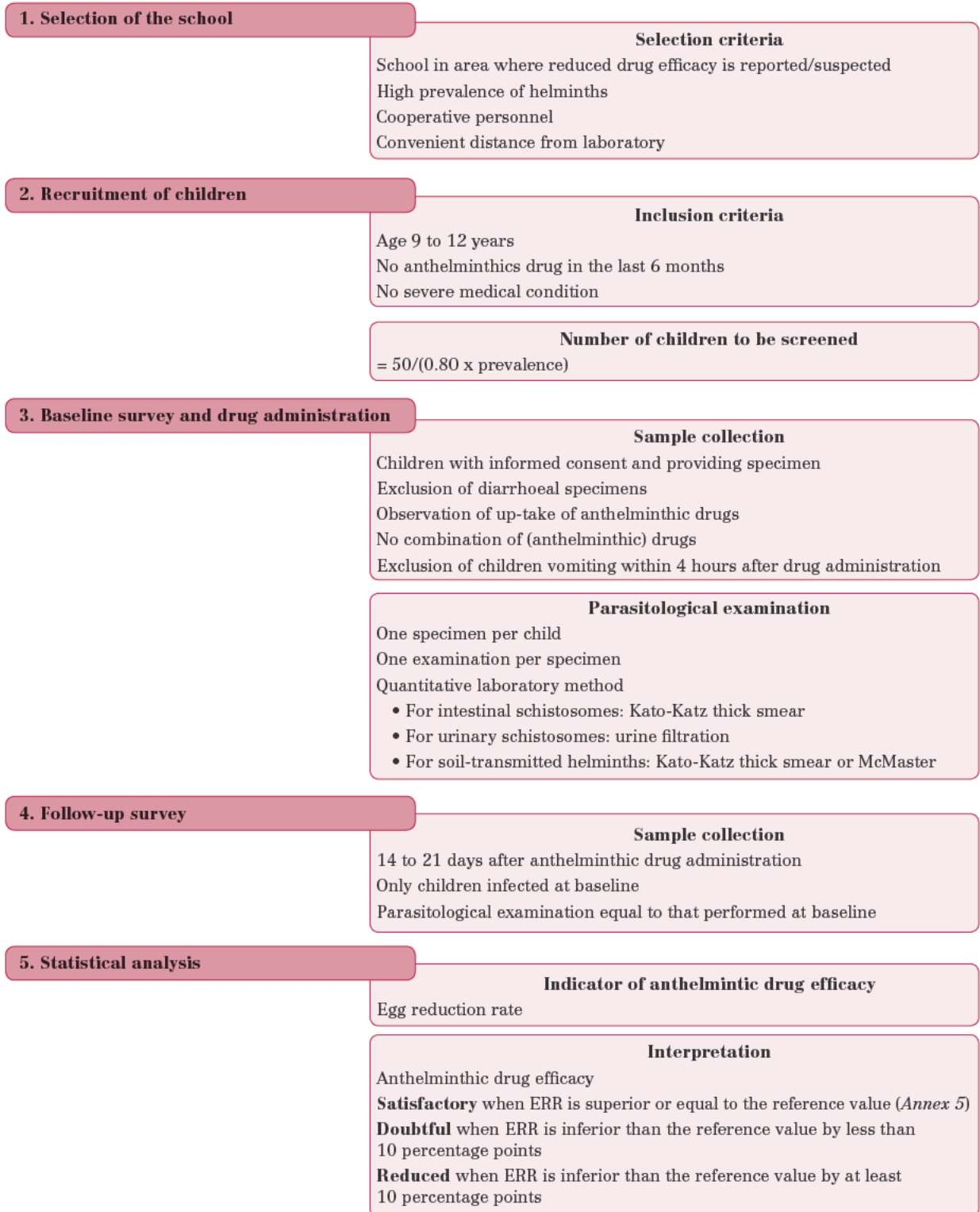
To increase the possibility of finding infected children, children aged 9–12 years should be selected and a baseline survey should be conducted at least 6 months after the last round of anthelmintic drug administration.

A sample of 50 children positive for each of the parasites targeted (i.e. 50 children positive for *A. lumbricoides*, 50 children positive for *T. trichiura* and 50 children positive for hookworm) by the investigation is sufficient to evaluate the efficacy of the investigated drug (Levecke et al., 2011a, 2012b). In programmes running for several years in which the majority of children are free from infection, a large number will have to be screened to obtain at least 50 positive cases for each parasite.

The numbers to be screened according to the prevalence in the area is shown in *Table 1*, with a compliance rate conservatively estimated at 80%.

If one parasites is present at a low prevalence (e.g. less than 10%), it is not considered to be of public health importance, and assessment of anthelmintic drug efficacy against this parasite is probably unnecessary.

FIGURE 1. Visual presentation of the 5 consecutive steps to assess anthelmintic efficacy



A team composed of a leader and three or four experienced laboratory technicians can usually collect and analyse more than 65 specimens a day, so that the baseline and follow-up surveys can be completed according to the timing shown in *Figure 2*. A local (district) education officer could introduce the health team to the school personnel, and teachers could assist in data recording and in managing the flow of children. If experienced personnel are not available at country level, assistance can be requested from the Department of Control of Neglected Tropical Diseases at WHO (see *Annex 5* for direct contact).

TABLE 1. Numbers of children to be screened to obtain 50 positive specimens, according to prevalence*

Prevalence (%)	No. of children to be screened
80	78
70	90
60	104
50	125
40	156
30	208
20	314
10	625

* The number of children to be screened is estimated from:

$$\text{No. of children to be screened} = \frac{\text{No. of infected children}}{\text{Compliance rate} \times \text{Prevalence}}$$

The compliance rate is the percentage of the children identified as positive at baseline who provide a stool specimen at follow-up and it is conservatively estimated at 80% in this table.

3.3 Baseline survey

Each day, 80 schoolchildren are given a plastic stool container and asked to provide a faecal or urine specimen. The aim is to receive at least 65 specimens a day. The specimen should be approximately 10 g of faeces and/or 50 ml of urine.

The containers for faecal specimens should be distributed to the children either on the day of collection or the previous day. The number of specimens returned is usually higher if the containers are distributed the previous day, but the first option simplifies the logistics.

The containers for urine specimens should be distributed to the children on the day of collection. Specimens should be collected between 10:00 and 14:00 as urinary excretion of the eggs follows a daily rhythm with a peak around noon. Physical exercise and fluid intake have been shown to increase egg output significantly, and it is therefore useful to ask the children to do some short physical exercise before collecting the urine samples (Doehring et al., 1983).

Each child who returns a faecal or urine specimen should be identified on a form (see example in *Annex 6*) that clearly states the name and family name and bears the serial number that is on the container in order to identify the specimen. Liquid or diarrhoeic stool samples should be discarded because the condition interferes with standard evaluation of faecal egg counts.

3.4 Drug administration

It is recommended that each child be given a light snack (e.g. a slice of bread or a biscuit) before the drug under investigation is administered. It is essential that the drugs administered are within the expiry date and properly stored. WHO can provide limited quantities of drugs for evaluations of efficacy (see address in *Annex 5*). The recommended doses of the anthelmintics commonly used in preventive chemotherapy programmes are listed in *Annex 2*. The tablet should be ingested under direct observation by the distributor, and the child should be maintained under observation for approximately 4 hours. The children can remain at school and continue their usual activities, but they should rapidly report any side-effect to a member of the investigation team.

A child who vomits after drug administration should be excluded from the analysis because the precise amount of anthelmintic drug he or she consumed will be unknown.

At the end of the survey, after collection of follow-up data, it is suggested that the anthelmintic drug is administered to all the children in the school who did not provide a specimen. Children should also be given treatment for the parasites identified during microscopic evaluation against which the drug under investigation is not effective. For example, if during evaluation of a drug against soil-transmitted helminthiasis a high prevalence of *S. mansoni* is detected, praziquantel should be administered to the entire school after the return of the second specimen.

3.5 Parasitological examination

3.5.1 Laboratory methods

The indicator of choice for drug efficacy is the egg reduction rate (ERR). Specimens should therefore be examined by quantitative parasitological methods to determine the number of parasite eggs per gram of faeces or per 10 ml of urine. A single sample from the specimen provided by each child is sufficient to calculate the number of eggs. Multiple sampling does not improve the mean egg count estimate (Levecke et al., unpublished data, Olliaro et al., unpublished data).

One of the following quantitative methods is currently recommended:

- *Kato–Katz thick smear method* (WHO, 1991): The main advantage of this technique is its extensive use in medical parasitology, the confidence acquired by laboratory technicians, the minimal need for supplies and equipment to perform it, and the capacity of the method to identify soil-transmitted helminths and intestinal schistosomes. Disadvantages of this method are its sensitivity to variation in the specific weight of different faecal samples and difficulty to process hard or loose faecal samples. Furthermore, in tropical climates, hookworm eggs disappear (over-clarify) 30–60 min after preparation. Reading of slides should be carefully planned to respect this interval.
- *McMaster method*: This is the standard reference method for evaluating drug efficacy in veterinary parasitology and has recently been evaluated for human helminths (Levecke et al., 2011c). It is not, however, suitable for diagnosis of schistosome eggs. The main advantage of this method is that it is more rapid and slides are cleaner, easier to read and robust, so that they can be reused several times.
- *Urine filtration* (WHO, 1991): This is the only method that allows the recovery and enumeration of *S. haematobium* eggs in urine.

Other methods are being evaluated (e.g. mini FLOTAC) as possible alternatives to the Kato–Katz and McMaster egg-counting method.

Details of the methods are presented in *Annex 7*. Addresses for procuring laboratory materials are given in *Annex 5*.

3.5.2 Quality control

In order to ensure the accuracy of the egg counts conducted by laboratory technicians, quality control should be performed on a number of slides randomly selected. Quality control can be organized in many ways. One simple method is re-reading 10% of the slides of each laboratory technician by an expert microscopist. If the expert identifies a difference in the egg count per gram of more than 10% and more than four eggs¹, he or she should re-read the slide with the microscopist and discuss the reasons for the discrepancy.

3.5.3 Repository of samples

It is recommended that a pooled sample be prepared by mixing a standard quantity of each positive faecal specimen in a single container and adding a standard quantity of fixative (for details of the preparation of a pooled sample, see *Annex 7*). This sample can be stored at room temperature and will be useful for future reference. It should be clearly labelled, recorded in an inventory and stored.

3.6 Follow-up survey

An interval of 14–21 days between the treatment and the collection of follow-up data increases data standardization and avoids the risk that eggs identified in a specimen are from parasites that infected the individual after drug administration. The scheme presented in *Figure 2* allows:

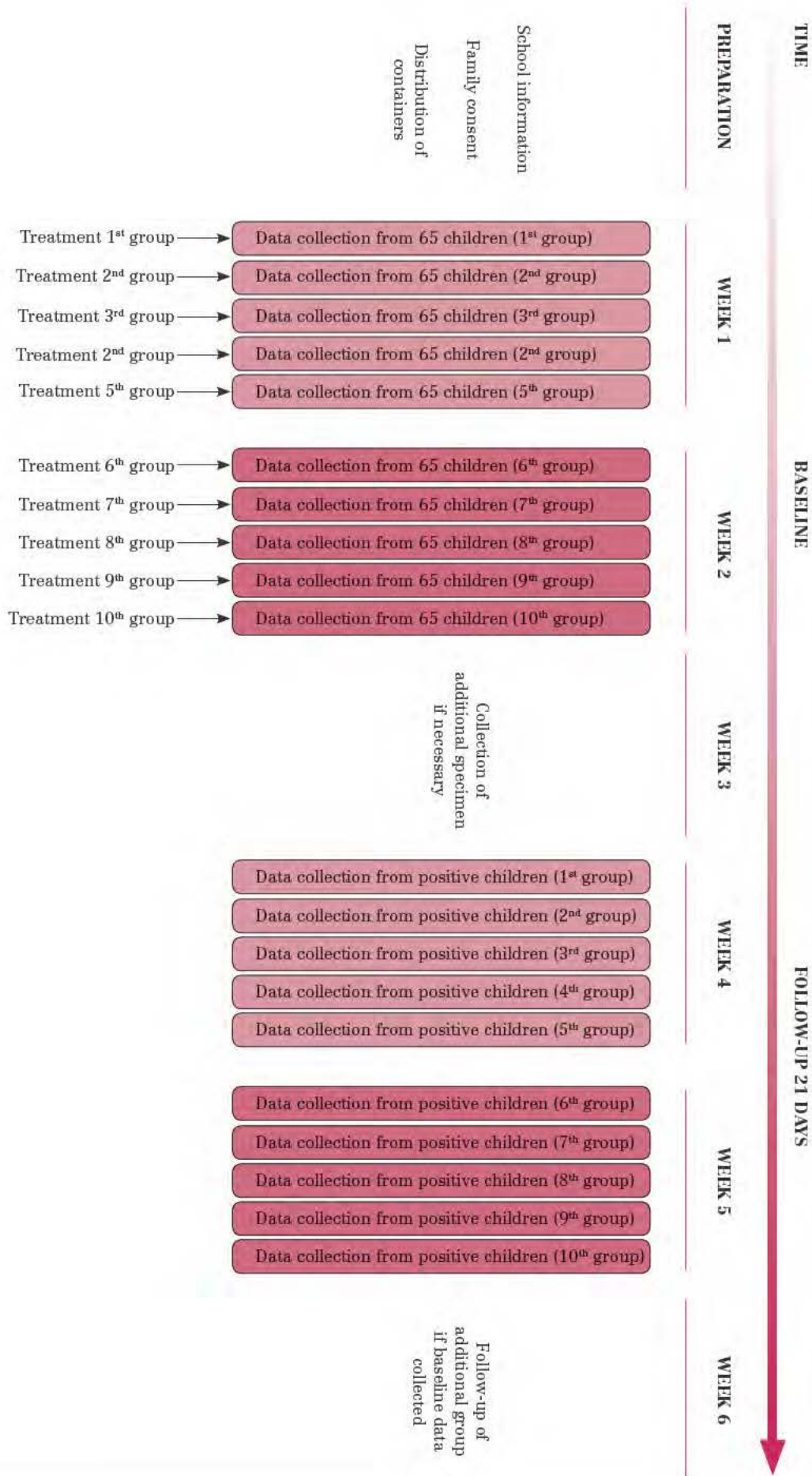
- a regular working schedule for 5 days each week, with collection of faecal specimens and treatment in the morning and laboratory examination in the afternoon;
- the collection and examination of additional specimens if the number of children infected with the different species present in the first 5–10 days would result in fewer than 50 positive results per species investigated; and
- maintaining a 21-day interval between treatment and follow-up.

Only children that had a positive specimen at baseline will be requested to provide a second specimen after 14–21 days. Schools will be followed-up in the same order as in the baseline survey. Children who do not attend school on the follow-up day or do not bring a specimen can be followed-up 1 or 2 days later.

The laboratory method used in the baseline survey should be used in the follow-up survey. It is recommended that a pooled sample be prepared of the positive samples at follow-up (for details of the preparation of a pooled sample, see *Annex 7*).

¹ A difference of a few eggs among readers is acceptable, but in case of low egg count this could represent a significant percentage (e.g. a difference of 1 egg in the case of 2 eggs per slide corresponds to a 50% difference). To avoid misinterpretation on this point, we suggest the difference between the readings should exceed 10% **and** more than 4 eggs to entail re-reading.

FIGURE 2. Schematic presentation of the timing for the baseline survey, treatment and follow-up survey. The suggested timing allows the collection of 65 specimens per day (650 in two weeks, that is the maximum number needed according to *Table 1*).



4. STATISTICAL ANALYSIS

4.1 Calculation of egg reduction rate

Analysis of the egg reduction rate (ERR) will be calculated on children found positive at the baseline survey and who returned a stool sample at the follow up survey.

The ERR is the appropriate parasitological indicator for evaluating the efficacy of anthelmintic drugs (Montresor, 2011; Vercruysse et al., 2011). The following formula should be used to calculate the ERR for each helminth of interest:

$$\text{ERR (\%)} = 100 \times \left(1 - \frac{\text{arithmetic mean egg counts at follow-up}}{\text{arithmetic mean egg counts at baseline}} \right)$$

4.2 Evaluation of the results

The efficacy of the anthelmintic drug under examination can be classified into different levels by comparing the observed ERR with the reference value for each parasite species (*Annex 2*).

Anthelmintic drug efficacy is:

- satisfactory if the ERR is superior or equal to the reference value;
- doubtful if the ERR is inferior to the reference value by less than 10 percentage points; and
- reduced if the ERR is inferior to the reference value by at least 10 percentage points.

For example, for albendazole (400 mg) against *A. lumbricoides*, in case of:

- ERR = 98%: the drug efficacy is considered satisfactory;
- ERR = 90%: the drug efficacy is considered doubtful;
- ERR = 80% the drug efficacy is considered reduced.



5. INSTITUTIONS TO BE CONTACTED IN CASE OF REDUCED DRUG EFFICACY

If reduced efficacy is observed, it is mandatory to contact WHO and the collaborating centre (see *Annex 5*) to discuss further action, including identification of possible confounding factors, data analysis and interpretation, inventory of specimens and anthelmintic drug investigated.

The response to observed reduced drug efficacy depends on i) the drug found to be poorly effective, ii) the extent of poor response, iii) the parasite identified to respond poorly, iv) the epidemiology in the country of the parasite in question. The response also depends on progress in finding new alternative approaches (based on drugs and administration models).

The local drug authority should also be informed about doubtful or reduced drug efficacy and of any further investigation or corrective measure established in collaboration with WHO.

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ANNEXES

ANNEX 1. POSSIBLE CONFOUNDING FACTORS IN THE ASSESSMENT OF ANTHELMINTHIC DRUG EFFICACY

The evaluation of anthelmintic drug efficacy may be confounded by various factors, which can be roughly classified into confounders inherent to (i) the statistical analysis of the data collected, (ii) the drug regimens, (iii) the host and (iv) the parasite. The following is adapted from the review by Vercruysse et al. (2011a). This is not an exhaustive listing, and many confounders remain to be identified.

Confounder inherent to statistical analysis of collected data

Selection of an inappropriate indicator can confound an evaluation of drug efficacy. As it is more difficult to eliminate all the parasites in high-intensity infections, the cure rate is less satisfactory than when the same drug is used against low-intensity infections (Montresor, 2011). *To reduce the influence of this confounding factor it is therefore recommended that the egg reduction rate (ERR) be used rather than the cure rate for evaluating the efficacy of an anthelmintic drug (Montresor, 2011; Vercruysse et al., 2011b).*

Formula to calculate egg reduction rate. Different formulae could be applied to calculate ERR. These formulae are based on different statistical units (individual vs. group) and on different ways to calculate the mean egg number (arithmetic mean or geometric mean). *To increase comparability and avoid the distortion caused by geometric mean, it is recommended to assess drug efficacy using the group-based ERR and arithmetic mean: group based formula provides more accurate and precise ERR results compared to individual ERR (Levecke et al., 2011), and is robust (Vercruysse et al., 2011b); arithmetic mean of egg count provides more accurate ERR results, whereas ERR determined from geometric mean egg counts may under- or overestimate drug efficacy (Dobson et al., 2009).*

Confounders inherent to the drug regimen

The variable quality of the anthelmintic agent is an important confounding factor. *It is important to ensure the quality of the anthelmintic drug used in the control programme. To eliminate this confounding factor, programmes should only use drugs manufactured in conformity with Good Manufacturing Practices (GMP) certified by a regulatory authority member of the Pharmaceutical Inspection Co-operation Scheme (PIC/S).¹ If this is not possible, appropriate quality testing should be conducted by the appropriate drug regulatory authority of the recipient country. Testing should be done preferably before medicines are*

¹ The list of PIC/S members is available at <http://www.picscheme.org/>

shipped to the destination country and on samples collected by staff independent from the concerned manufacturers and/or procurement agents. WHO can provide advice on sampling and testing.

Reduction in drug quality can occur with inappropriate storing or handling. *To reduce the influence of this confounding factor, it is important to use new drugs of good quality when evaluating possible loss of drug efficacy.*

Suboptimal drug regimens are the rule in widescale treatment for public health. For logistical reasons, anthelmintics are administered as a single dose and may never achieve 100% efficacy (Geary et al., 2010). *To reduce the influence of this confounding factor, the efficacy of an anthelmintic drug should be evaluated against the standard reference of drug efficacy (Annex 2).*

Confounders inherent to the host

Factors that affect intestinal transit. The anthelmintic activity of drugs relies on the extended presence of effective concentrations at the location of the parasite (Lacey, 1990). Some clinical conditions (e.g. gastrointestinal diseases, malnutrition and immunodeficiency) and some drugs (e.g. anti-inflammatory drugs or antibiotics) that alter the intestinal transit will alter the exposure of parasites to anthelmintics and thus affect drug efficacy (Sanchez et al., 2006). *To reduce the influence of this confounding factor, children with diarrhoea or other relevant medical conditions or who are taking other drugs should be excluded from the statistical analysis.*

Factors that affect drug absorption and bioavailability. The bioavailability of praziquantel and albendazole is increased by concomitant administration of lipids and glucides (Castro et al., 2010). *To reduce the influence of this confounding factor, it is suggested that children be given a snack before drug administration.*

Episodes of vomiting after drug administration interfere with drug intake. *To reduce the influence of this confounding factor, children who vomit shortly after drug administration (within 4 hours) should be excluded from the evaluation of drug efficacy.*

Confounders inherent to the parasite

Density-dependent fecundity. It was reported that a reduction in adult canine hookworm (*Ancylostoma caninum*) counts after preventive chemotherapy did not always result in a proportional reduction in egg counts because of increased fecundity in the small residual worm population that survived the anthelmintic treatment (Kotze and Kopp, 2008). *There is little evidence that this phenomenon has a major impact on the assessment of drug efficacy against human helminths by the ERR (Vercruysse et al., 2011b).*

Day-to-day variation in egg excretion. Daily egg excretion varies considerably. This may thwart evaluations of drug efficacy, particularly when efficacy is assessed from individual ERRs. As a consequence, egg counts after drug administration might be higher than those at baseline, hence underestimating drug efficacy. *To reduce the influence of this confounding factor, it is suggested to calculate drug efficacy by means of group-based rather than individual-based ERR (Vercruysse et al., 2011b).*

Slow release of remnant schistosome eggs in tissues may result during the first few days after treatment, even when the parasite has been destroyed by the action of the drug. *To reduce the influence of this confounding factor, an appropriate follow-up (not less than 14 days) should be used.*

Continued excretion of non-viable eggs for *S. haematobium*, has been reported three weeks after treatment (Tchuem-Tchuenté et al. 2004) and there is a need for distinguishing the viability of eggs that may continue to be excreted after worm death. *A possible suggestion could be as follows: Samples collected after treatment, when positive (at least one egg detected), should be also analysed for egg viability using in sequence two different criteria: i) adding a drop of water on the same filter and checking for miracidium movements or flame cell activity; ii) performing a miracidial hatching test.*

The poor efficacy of praziquantel against immature schistosomes (Botros et al., 2005) means that these parasites can mature and produce eggs a few weeks after treatment. *To reduce the influence of this confounding factor, an appropriate follow-up (not more than 21 days) should be used.*

References

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ANNEX 2. RECOMMENDED DOSE AND REFERENCE EFFICACY (EGG REDUCTION RATE) FOR SELECTED ANTHELMINTHIC DRUGS

Albendazole (chewable tablet, 400 mg)

General information: Albendazole is a benzimidazole derivative that interferes with microtubular assembly and blocks glucose uptake by intestinal nematodes. It is poorly absorbed by the gastrointestinal tract; the absorbed fraction is rapidly metabolized and eliminated in the bile.

Dosage: The drug is used as a single administration of a standard dose (400 mg). A half dose (200 mg) is suggested for children aged 1–2 years (WHO, 2002).

Use: In view of its simple administration and excellent safety record, the drug is frequently used in preventive chemotherapy for the control of soil-transmitted helminthiasis and lymphatic filariasis. Efficacy against soil-transmitted helminthiasis: the egg reduction rate (ERR) shown in *Table A2.1* was measured in a series of investigations conducted in seven countries with the McMaster egg counting method and other methods described in this manual (Vercruysse et al., 2011). The performance of the Kato–Katz test has been evaluated to be similar to the ones obtained with the McMaster egg counting method (Albonico et al., 2012).

TABLE A2.1 Reference drug efficacy of albendazole (chewable tablet, 400 mg)

	Reference efficacy (egg reduction rate, %)*
<i>A. lumbricoides</i>	≥ 95
Hookworms	≥ 90
<i>T. trichiura</i>	≥ 50**

* Estimated from a study involving 1834 individuals in Brazil, Cambodia, Cameroon, Ethiopia, India, the United Republic of Tanzania and Viet Nam (Vercruysse et al., 2011).

** The ERR in cases of infection with *T. trichiura* is significantly lower than that for the other soil-transmitted helminthiasis; however, when the drug is used at regular intervals, as in school health programmes, it is sufficient to eliminate high-intensity infections and progressively reduce prevalence. Mebendazole performs better than albendazole in case of infections of higher intensity.

References

- Albonico M et al. (2012). Comparison of Kato–Katz thick smear and McMaster egg counting method for monitoring drug efficacy against soil-transmitted helminths in school children of Pemba Island, Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 106:199–201.
- Vercruysse J et al. (2011). Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. *PLoS Neglected Tropical Diseases*, 5:e948.
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Mebendazole (500 mg)

General information: Mebendazole is a benzimidazole derivative that blocks glucose uptake by many intestinal and tissue nematodes. It is excreted in the faeces largely unchanged. The small amounts absorbed are rapidly metabolized in the liver into inactive metabolites.

Dosage: The drug is given as a single administration of the same dose (500 mg) for people over 1 year of age.

Use: In view of its simple administration and lack of side-effects, the drug is frequently used in preventive chemotherapy programmes (e.g. school health programmes) (WHO, 2002).

Efficacy against soil-transmitted helminthiasis: The ERR intervals presented in Table A2.2 result from a series of investigations conducted in seven countries with the McMaster egg counting method and standard operating procedures (Vercruysse et al., in preparation).

The performance of the Kato-Katz method has been evaluated to be similar to that obtained with the McMaster egg counting method (Abonico et al., 2012).

TABLE A2.2 Reference drug efficacy of mebendazole (500-mg tablet)

Reference efficacy (egg reduction rate, %)*	
<i>A. lumbricoides</i>	≥ 95
Hookworms	≥ 70
<i>T. trichiura</i>	≥ 50**

* The ERR was estimated from a study involving individuals in Brazil, Cambodia, Cameroon, Ethiopia, the United Republic of Tanzania and Viet Nam (Vercruysse et al., in preparation).

** Mebendazole performs better than albendazole in case of infections of higher intensity.

References

Abonico M et al. (2012). Comparison of Kato-Katz thick smear and McMaster egg counting method for monitoring drug efficacy against soil-transmitted helminths in school children of Pemba Island, Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 106:199–201.

Vercruysse J et al. (in preparation).

WHO (2002). *Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Report of a WHO expert committee*. Geneva, World Health Organization.

Praziquantel (600 mg)

General information: Praziquantel is structurally unrelated to the other anthelmintics and is highly active against a wide range of trematodes, including all species of schistosomes. It is well absorbed after oral intake. Immediately after exposure, the schistosomes contract, lose their anchorage on blood vessels and gradually disintegrate.

Dosage: The drug is given as a single administration at a dosage of 40 mg/kg to children over 4 years of age (WHO, 2001). In community-based interventions, where scales are not available, the appropriate dose of praziquantel is determined using a 'dose pole' (Montresor et al., 2001). However, for the assessment of praziquantel efficacy it is suggested to provide tablets to children based on their weight. *Table A2.3* shows the number of tablets of praziquantel to be provided to school-aged children according to their weight to assure a minimal dose of 40 mg/kg.

TABLE A2.3 Praziquantel dosage by weight in school-age children.

Body weight range (kg)	Number of tablets (praziquantel 600 mg)	Dose range (mg/kg)
15–22.4	1½	60–40
22.5–29.9	2	53–40
30–37.4	2½	50–40
37.5–44.9	3	48–40
45–59.9	4	53–40

Use: In view of its simple administration and minor and self-limiting side-effects, the drug is frequently used in preventive chemotherapy programmes (e.g. school health programmes) (WHO, 2002).

Efficacy against schistosomes: Evidence for an efficacy threshold from published studies is currently weak. Cochrane systematic reviews identified relatively few randomized controlled trials of ERR, and all were based on geometric mean egg counts for *S. haematobium* (Danso-Appiah et al., 2007) and *S. mansoni* (Danso-Appiah et al., 2013). Tentative ERR values are presented in *Table A2.4*.

TABLE A2.4 Reference drug efficacy of praziquantel (600-mg tablet)

Reference efficacy (egg reduction rate, %)	
<i>S. haematobium</i> ¹	≥ 90
<i>S. mansoni</i> ²	≥ 90
<i>S. japonicum</i> ³	≥ 90
<i>S. mekongi</i> ⁴	≥ 90

1. Cochrane systematic review (Danso-Appiah et al., 2007) and an analysis of data for individual patients in five studies with 1813 evaluable patients (Olliaro et al., manuscript in preparation).
2. Cochrane systematic review (Danso-Appiah et al., 2013) and an analysis of data for individual patients in 11 studies with 1226 evaluable patients (Olliaro et al., manuscript in preparation).
3. One study with 200 patients (Olliaro et al., 2011), also included in Olliaro et al., manuscript in preparation; no systematic review currently available.
4. One study with 93 patients (Lovis et al., 2012); no systematic review currently available.

Various diagnostic approaches (in terms of number of stool samples, mean used and interval between baseline and follow-up) were used to measure the ERR reported here.

Additional data are presently collected in a standardized way; more precise ERR values for praziquantel will be provided as soon these data have been analysed.

References

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ANNEX 3. EXAMPLE OF INFORMATION LETTER FOR SCHOOLS

Evaluation of the drug efficacy of the school deworming programme

Schoolmaster of _____ Primary School, address, district

Information for school personnel

In the context of an evaluation of the efficacy of the medicines used to control intestinal (or urinary) parasites, an evaluation will be conducted in your school. A team from the Ministry of Health will visit the school on:

- _____ dd/mm/yy
- _____ dd/mm/yy
- _____ dd/mm/yy

and will invite a group of children in the school to provide a specimen (of faeces and/or urine).

The children recruited will receive a dose of medicine. The medicine is recommended by WHO for use in school control programmes and is considered safe.

Some children may experience some minor and temporary side-effects as the worms are destroyed by the drug.

Side-effects include mild abdominal pain, nausea, vomiting, diarrhoea and fatigue, and do not normally require medical treatment.

An additional specimen (of faeces and/or urine) will be collected again after few weeks, i.e. on:

- _____ dd/mm/yy
- _____ dd/mm/yy
- _____ dd/mm/yy

The participation of the children in this evaluation is voluntary, and teachers are invited to inform and obtain consent from the parents (see attached example).

Children participating in the evaluation will receive medicine for any parasite identified by the faecal examination. The individual results of any investigation will remain confidential.

You are invited to contact the organizer of the evaluation if the proposed dates are not suitable or in case you need any additional information.

Thank you in advance for your collaboration,

The evaluation organizer

Dr _____

Department _____

Ministry of Health

Contacts Email: _____@_____; Office phone: _____, Mobile phone: _____

ANNEX 4. EXAMPLE OF INFORMED CONSENT FORM

To be copied by children in their exercise book in the local language.

Between _____ (dd/mm/yy) and _____ (dd/mm/yy), the children of the school will be requested to provide a stool (or urine) sample to medical personnel from the Ministry of Health and will receive medicine against intestinal (or urinary) worms.

The medicine is recommended by WHO for use in school control programmes and is considered safe.

Some children may experience some minor and transient side-effects as the worms are destroyed by the drug.

Side-effects include mild abdominal pain, nausea, vomiting, diarrhoea and fatigue, and do not normally require medical treatment.

The results of this investigation are important to determine whether the medicine is still active against the worms.

You are kindly request to approve the participation of your child.

Signature of parents: _____

ANNEX 5. USEFUL ADDRESSES

Whom to contact in case of reduced drug efficacy:

World Health Organization

Department of Control of Neglected Tropical Diseases
20 Av. Appia
1211 Geneva
Switzerland
e-mail: wormcontrol@who.int

Direct contact: Dr A. Montresor: montresora@who.int

WHO Collaborating Centre for the monitoring of anthelmintic drug efficacy for soil-transmitted helminthiasis

Ghent University, Department of Virology, Parasitology & Immunology
Salisburylaan 133
B-9820 Merelbeke
Belgium

Direct contact, Professor J.Vercruysse: jozef.vercruysse@ugent.be

Where to procure laboratory material:

Kato-Katz kits

- Vestergaard Frandsen Group
<http://www.vestergaard-frandsen.com/>
E-mail: sales@vestergaard-frandsen.dk
- Neolab – hydrophilic cellophane for Kato-Katz
<http://www.neolab.de>

McMaster slides

- Focal point
<http://www.mcmaster.co.za/>
- Chalex Corporation
<http://www.vetslides.com/>

Urine filtration equipment

- Millipore for filter holders
<http://www.millipore.com/catalogue/module/C160>
- Sefar for filters
<http://www.sefar.com>
E-mail: hans-peter.brunner@sefar.ch
- Sterlitech schistosome test kit
<http://www.sterlitech.com/membrane-disc-filters/polycarbonate-membranes/schistosome-test-kit.html>
E-mail: hans-peter.brunner@sefar.ch

ANNEX 6. FORM FOR COLLECTING PERSONAL DATA AND INFORMATION ON PARASITES

STANDARD OPERATING PROCEDURE FOR THE EVALUATION OF DRUG EFFICACY

INDIVIDUAL DATA COLLECTION FORM

Date [dd/mm/yy]___/___/___

DRUG TESTED

Albendazole ☐

Mebendazole ☐

Praziquantel ☐

I PERSONAL DATA

ID Number _____

School _____

Child's name _____

Age _____ (years)

Sex M ☐ F ☐

(in case of praziquantel evaluation weight _____)

II EXCLUSION

Does the child have diarrhoea Yes ☐ No ☐

Did the child take other drug(s) in the past 6 months Yes ☐ No ☐ I do not know ☐

III PARASITOLOGICAL DATA: BASELINE ☐ FOLLOW-UP ☐

(a) Stool examination	Lab technician		Quality control (on 10% of slides) To be completed by the senior lab technician	
	eggs/slide	eggs/g	eggs/slide	eggs/g
<i>Ascaris lumbricoides</i>				
<i>Trichuris trichiura</i>				
Hookworms				
<i>Schistosoma mansoni/japonicum</i>				
Other parasites identified:				
(b) Urine filtration	Lab technician		Quality control (on 10% of slides) To be completed by the senior lab technician	
	eggs/10 ml urine		eggs/10 ml urine	
<i>Schistosoma haematobium</i>				

IV DRUG ADMINISTRATION

(in case of praziquantel evaluation: number of tablets administered _____)

The child swallowed the drug under observation Yes ☐ No ☐

Episodes of vomiting occurred after drug administration Yes ☐ No ☐

ANNEX 7. STANDARD OPERATING PROCEDURES FOR LABORATORY METHODS

Kato–Katz thick smear method

Materials and reagents

- applicator sticks;
- screen, stainless-steel, nylon or plastic: 60–105 mesh size;
- template, stainless-steel, plastic or cardboard. Templates of different sizes have been produced in different countries. A hole of 9 mm on a 1-mm thick template will deliver 50 mg of faeces; a hole of 6 mm on a 1.5-mm thick template, 41.7 mg; and a hole of 6.5 mm on a 0.5-mm thick template, 20 mg. The templates should be standardized, and the same size template should always be used to ensure repeatability and comparability of prevalence and intensity data;
- spatula, plastic;
- microscope slides (75 x 25 mm);
- hydrophilic cellophane, 40–50 g, strips 25 x 30 or 25 x 35 mm;
- flat-bottom jar with lid;
- forceps;
- toilet paper or absorbent tissue;
- newspaper;
- glycerol–malachite green or glycerol–methylene blue solution (1 ml of 3% aqueous malachite green or 3% methylene blue added to 100 ml of glycerol and 100 ml of distilled water and mixed well). This solution is poured onto the cellophane strips in a jar and left for at least 24 h before use.

Procedure

- place a small mound of faecal material on newspaper or scrap paper and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top;
- scrape the flat-sided spatula across the upper surface of the screen to collect the sieved faeces;
- place the template with hole on the centre of a microscope slide and add faeces from the spatula so that the hole is completely filled. Pass the side of the spatula over the template to remove excess faeces from the edge of the hole;
- remove the template carefully so that the cylinder of faeces is left on the slide;
- cover the faecal material with the pre-soaked cellophane strip. The strip must be very wet if the faeces are dry and less so if the faeces are soft. If excess glycerol solution is present on the upper surface of cellophane, wipe with toilet paper;
- invert the microscope slide and firmly press the faecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface. The faecal material will be spread evenly between the microscope slide and the cellophane strip. It should be possible to read newspaper print through the smear after clarification;
- carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporates while glycerol clears the faeces;
- read the slide after 30–60 min at ambient temperature;

- the smear should be examined in a systematic manner and the number of eggs of each species recorded;
- the multiplication factors used to obtain the number of eggs/g from the number of eggs/slide are: 20 if using a 50-mg template, 50 if using a 20-mg template and 24 if using a 41.7-mg template.

Reference

WHO (1991). *Basic laboratory methods in medical parasitology*. Geneva, World Health Organization.
http://www.who.int/wormcontrol/documents/benchmarks/training_manual/n/#.

McMaster egg counting method

Materials and reagents

- 60-ml containers;
- digital scales (precise to 0.01 g);
- stirring device (fork, spatula, tongue depressor, spoon);
- measuring cylinder;
- Pasteur pipettes and rubber teats;
- strainer;
- saturated NaCl solution to be prepared at least 1 day before use and kept at room temperature (specific density = 1.2 can be verified with a densitometer);
- McMaster slides;
- compound microscope;
- 5 l distilled water;
- 3 kg NaCl.

Procedure

Flotation solution (to be prepared 24 h before processing samples):

- heat 5 l water to 50 °C;
- gently add NaCl while stirring the suspension;
- stop adding NaCl when a sediment appears;
- keep the solution at room temperature.

McMaster egg counting method

- place a 60-ml container on the electric scale;
- tare the scale (the display should show 0.00 g);
- homogenize the stool with a wooden spatula;
- weigh exactly 2 g of stool on the scale;
- add 30 ml of saturated NaCl;
- homogenize and pour the faecal suspension three times through a tea strainer to withhold large debris. During the last sieving step, the filtrate must be squeezed dry;
- rinse the McMaster slide and tap it on a hard surface;
- homogenize the suspension filtrate by pouring it 10 times from one beaker to another, and fill one chamber of a regular McMaster slide using a Pasteur pipette. Repeat for the other side. Minimize the time between taking the suspension up in the pipette and transferring it into one of the chambers of the McMaster slide;
- allow the McMaster slide to stand for 2 min, place under a light microscope and examine with 100x magnification. Count all the eggs under the two separate grids (representing a volume of 2 x 0.15 ml). If the slides are read before 2 min, the eggs will not have reached the surface of the slide;
- calculate the number of eggs per gram of faeces by multiplying the total number of eggs under the two grids by 50. This is done for each parasite species.

References

Levecke B et al. (2011). A comparison of the sensitivity and fecal egg counts of the McMaster egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. *PLoS Neglected Tropical Diseases*, 5:e1201.

For further details, see: <http://www.youtube.com/watch?v=UZ8tzswA3tc>.

Urine filtration

Materials and reagents

- coverslip;
- filter holder (diameter, 13 or 16 mm);
- forceps;
- plastic syringe, 10 ml;
- filter;
- polycarbonate or nylon filter (pore size, 20 µm) or paper filter (Whatman No. 51 or No. 1).

Procedure

- place a polycarbonate (or nylon or paper) filter in the holder and close it. Agitate the urine sample by shaking it gently or by filling and emptying the syringe twice;
- draw 10 ml of urine into the syringe and attach the filter holder to the syringe;
- expel the urine from the syringe into the filter holder over a bucket or sink;
- carefully remove the filter holder from the syringe, draw air into the syringe, re-attach the filter and expel the air. This is important as it helps to remove excess urine and also makes sure the eggs, if present, are attached to the filter;
- remove the filter holder from the syringe, open it, seize the filter with the forceps and place it (top side up) on a microscope slide. Add one drop of Lugol's iodine and wait for 15 s for the stain to penetrate the eggs;
- examine the whole filter under the microscope immediately at low power (x 40). Record the number of eggs.

If it is necessary to preserve the sample, the filter hydrophilic cellophane can be soaked in glycerol–malachite green or glycerol–methylene blue solution used in the Kato–Katz method.

References

WHO (1991). *Basic laboratory methods in medical parasitology*. Geneva, World Health Organization.
http://www.who.int/wormcontrol/documents/benchmarks/training_manual/en/#.

Repository samples

Materials and reagents

- 60-ml containers;
- electric scale (precise to 0.01 g);
- stirring device (fork, spatula, tongue depressor);
- measuring cylinder;
- 70% ethanol;
- Falcon tubes with screw top (50 ml);
- parafilm.

Procedure

- place a 60-ml container on the electric scale.

For each positive specimen:

- tare (zero) the scale (display should show 0.00 g);
- homogenize the stool sample with a wooden spatula;
- weigh 1 g of stool on the scale;
- repeat these steps for each positive sample examined that day. For example, if 10 stool samples are found to be positive, these steps will be repeated 10 times for a total volume of 10 g;
- thoroughly mix the pooled sample with a stirring device;
- place a pre-labelled Falcon tube on the electric scale;
- weigh 2.5 g of the pooled sample;
- add 70% ethanol up to a volume of 25 ml;
- homogenize the suspension by thoroughly shaking the Falcon tube;
- seal the Falcon tube with parafilm to prevent evaporation of ethanol;
- store the samples at room temperature.

