World Health Organization

Diagnostic methods for the control of strongyloidiasis

Virtual meeting, 29 September 2020

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This publication contains the report of the meeting on thge diagnostic methods for the control of strongyloidiasis and does not necessarily represent the decisions or policies of WHO.

Contents

1 Background

A meeting on diagnostic methods for the control of strongyloidiasis was organized by the World Health Organization (WHO) Department of Control of Neglected Tropical Diseases (Geneva, Switzerland) in collaboration with the WHO Collaborating Center on strongyloidiasis and other neglected tropical diseases (Negrar, Italy) and the Federation University (Melbourne, Australia). Children Without Worms (Decatur (GA), USA) provided logistic support. The agenda is attached as *Annex 1* and the list of participants as *Annex 2*.

Dr Zeno Bisoffi and Dr Antonio Montresor welcomed the participants and reported that there were no conflicts of interest to be declared by participants.

In his opening presentation, Dr Montresor shared the good progress in preventive chemotherapy coverage for *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms. The main reason why no specific activities for *Strongyloides stercoralis* had been implemented so far was because of the poor availability of ivermectin outside the context of elimination programmes for lymphatic filariasis and onchocerciasis. He expects this to change soon, as two generic formulations of ivermectin are in the prequalification process with WHO. Moreover, strongyloidiasis is now included in the WHO road map for neglected tropical diseases for 2021–2030 as an additional soil-transmitted helminth parasite targeted for control.

WHO hopes to enable rapid expansion of strongyloidiasis control programmes using the existing infrastructure for other neglected tropical disease (NTD) control or elimination programmes, as was done to add schistosomiasis.

The preliminary steps for implementing a strongyloidiasis control programme were shared, namely:

- gain knowledge of the epidemiology of *S. stercoralis*;
- conduct a field evaluation of the proposed intervention. Pilot interventions should evaluate the impact and feasibility of the proposed strategy (a pilot study is planned in Ethiopia); and
- find a standard diagnostic tool to enable assessment of the public health burden of the disease and exchange of information among different research and control groups; for many countries there is no epidemiological information at all, so we need recommendations for assessment of baseline prevalence.

2 Objective of the meeting

The meeting addressed the last key area, that is, determining the best method or combination of diagnostic methods for a control programme for *S. stercoralis* infections in humans.

Dr Montresor's presentation highlighted that while there is currently no "gold standard" for the diagnosis of *S. stercoralis*, there is a felt urgency to optimize diagnostic regimens that are currently available, and in the context of population-based testing (as opposed to individual focused diagnostics in clinical settings). In other words, the diagnostic test(s) should have good accuracy, but we should remember that in public health we do not aim at individual diagnosis: rather, we need a tool that should help to estimate the prevalence in a population (see *Annex 3: presentation 1*).

3 Presentations

After the meeting was opened and its objective was clarified the invited experts presented their perspectives and analysis of each category of currently available diagnostic tests.

3.1 Molecular diagnostics

Dr Richard Bradbury presented an overview of molecular diagnostics, the highlights of which are summarized below (see also *Annex 3: presentation 2*).

1. We should consider not only the predictive values of the tests but also their ease of use for field deployment.
2. Many molecular tests have not been clinically validated. For instance, two loop-mediated isothermal amplification (LAMP) tests and a real-time polymerase chain reaction (PCR) by Pilotte have been implemented, but all were compared only to Verweij's PCR. None has been widely validated in population-based settings, which is a limitation.
    (a) LAMP (Watts) offers good analytical sensitivity but is not yet fully clinically validated; LAMP (Fernández-Soto) is promising but not yet widely validated, though an early version has been used in field trials in Angola.
3. The evaluation of test sensitivity depends on the method used for comparison (only the Verweij real-time PCR has been tested against high sensitivity and specificity parasitological methods such as Baermann and agar plate culture). Several studies have evaluated the diagnostic sensitivity and specificity of the Verweij PCR using either the agar plate culture or the Baermann sedimentation as the reference standard. These resulted in a different range of sensitivity results between 17.4% and 97.2%, the majority reporting a sensitivity of between 84.7% and 97.2%.
4. A factor in the wide variation in the reported sensitivity of Verweij's real-time PCR across different studies may be the use of non-standardized DNA extraction and PCR reagents and standard operating procedures.
5. It is crucial to standardize the DNA extraction method to avoid variable and unreliable results. A specific extraction should be recommended along with the PCR test. **Quality control schemes become critical** for programme-wide implementation, so results are comparable across laboratories. *Caution:* Using a single DNA extraction method for all laboratories will be challenging as each laboratory will likely have a preferred extraction method already employed in their laboratory.

6. To avoid false–negative results due to DNA inhibitors, an internal DNA extraction/inhibition control is recommended. This would require adding a DNA extraction/inhibition control LAMP, so two LAMPs, or a multiplex PCR approach with both the *S. stercoralis* target and an extraction/inhibition control target. The group of Fernández-Soto tested LAMP in school-aged children in Angola (unpublished data), which demonstrated good accuracy. LAMP has advantages over PCR for use at district laboratory level. A hand-held LAMP device has also been developed.
7. LAMP can be used in a district level laboratory with only a water bath or heat block, while real-time PCR is only available at the reference laboratory level as it requires a real-time PCR cycler, and specialized capacity and competencies are not available in every country.
8. Cost is a factor to be considered for molecular diagnostics. In a work by Sultana, the Qiagen PowerSoil kit was found to be the optimal extraction method; unfortunately, it is expensive.

3.2 Serological diagnostics

Professor Siddhartha Mahanty presented an overview of the available serological tests and their advantages and disadvantages for public health diagnostic use in a strongyloidiasis control programme. The key points are summarized below (see also *Annex 3: presentation 3*).

1. Serology offers high sensitivity, but specificity is less assured. Additionally, there are concerns about cross-reactivity. Professor Mahanthy showed the accuracy of various assays (see presentation 3).
2. There is a question about whether there has been any independent assessment of quality assurance of serological diagnostics. This assessment is needed before deployment of currently used diagnostic assays.
3. Serological methods should be considered in the context of the different phases of the immunological response, with immunoglobulins developing at different stages of an infection (unlike in most other parasitic infections, post-treatment reversion of seropositivity has been demonstrated for strongyloidiasis, so serology can be used for response monitoring in target populations following treatment).
4. Serology has several advantages for deployment in the field: easy to establish and maintain in small laboratories; high sensitivity and specificity that is acceptable, albeit not optimal; high throughput testing is possible; point-of-care testing is possible; it can measure the effect of treatment.
5. There are two types of target antigens in use at present for serological diagnosis: (i) extracts of the larval stages of *S. stercoralis* or related parasites (referred to as "crude antigens") or (ii) *S. stercoralis* genetically engineered proteins (referred to as "recombinant antigens"). The assays can be performed on samples of serum and blood spots (the latter is particularly attractive for use in control programmes).

Recently, lateral flow tests have been implemented, and they would be ideal for control programmes but still need to be validated.

6. Issues are with diagnostic confidence with serological assays: (i) diagnostic accuracy is hampered by the lack of a gold standard for diagnosis; and (ii) accuracy depends on the antigen used; there is trade-off between higher sensitivity and acceptable specificity among available assays.

3.3 Coprological methods

Dr Alejandro Krolewiecki presented the various coprological diagnostic methods available, their comparison with the current one used for other soil-transmitted helminth parasites (Kato–Katz) and the advantages and disadvantages. These are summarized below *(Annex 3: presentation 4)*.

1. Diagnostic tools utilized for other control programmes are not totally accurate (for example, Kato–Katz has only 64% sensitivity for *A.s lumbricoides*) but nevertheless allow a proper estimation of baseline prevalence and proper monitoring of the control programme.
2. For *S. stercoralis*, Kato–Katz is not suitable, and neither is FLOTAC, direct smear or McMaster (they all have very low sensitivity). Could Baermann be done initially for a basic assessment in view of integration with other programmes for control of soil-transmitted helminthiases?
3. Koga agar plate and Baermann have better sensitivity, but they do not permit quantification and need fresh stool. However, quantification of larval load is not needed for strongyloidiasis control in public health; it is more for clinical use for individuals. It can be used for baseline monitoring and evaluation purposes.
4. Harmonization of protocols is required for Baermann and agar plate culture: there are different protocols under the same name, resulting in incomparable results raising quality control issues.
5. The agar plate method takes a long time, and the Baermann method requires a large space in the laboratory. However, a modified Baermann method showed at least equal sensitivity than a traditional one and is less demanding in terms of laboratory space.
6. Key aspects to be considered: accuracy, reproducibility, cost, time and laboratory space. Sensitivity is not the key issue for a control programme. Stools are ideal for integration with other soil-transmitted helminthiases but inconvenient for other NTDs.

3.4 Panel discussion

Dr Montresor asked the panellists for their opinion about the best diagnostic tool to be used. Their responses are summarized below.

R. Bradbury considers the NIE enzyme-linked immunosorbent assay (ELISA) to be the most efficient method for population screening for the following reasons:

- a finger-prick is easily obtained;
- the test it is relatively simple to perform;
- recombinant antigen can be easily produced at large scale, enabling production of the needed quantity of kits for a control programme of global scale;
- prices can be probably negotiated for large quantities;
- equipment for conducting ELISA tests is available in all endemic countries, even if not always at very peripheral level; and
- additional faecal tests may be added to this in specific areas according to the programme or epidemiological situation.

Z. Bisoffi favours serology plus a faecal test because:

- serology is sensitive and good for assessment, but once a low prevalence is reached the lower specificity of serology would find a high proportion of false−positives;
- there is still not enough evidence for identifying the best serological method. NIE has advantages (supply for instance) but there are no studies supporting its accuracy as high;
- the NIE luciferase immunoprecipitation system is better in terms of accuracy, but the technology is not affordable for in-field evaluation;
- modified Baermann could play a role;
- collection of faeces for PCR could be implemented, and they could both be tested in a reference laboratory.

For **S. Mahanty**:

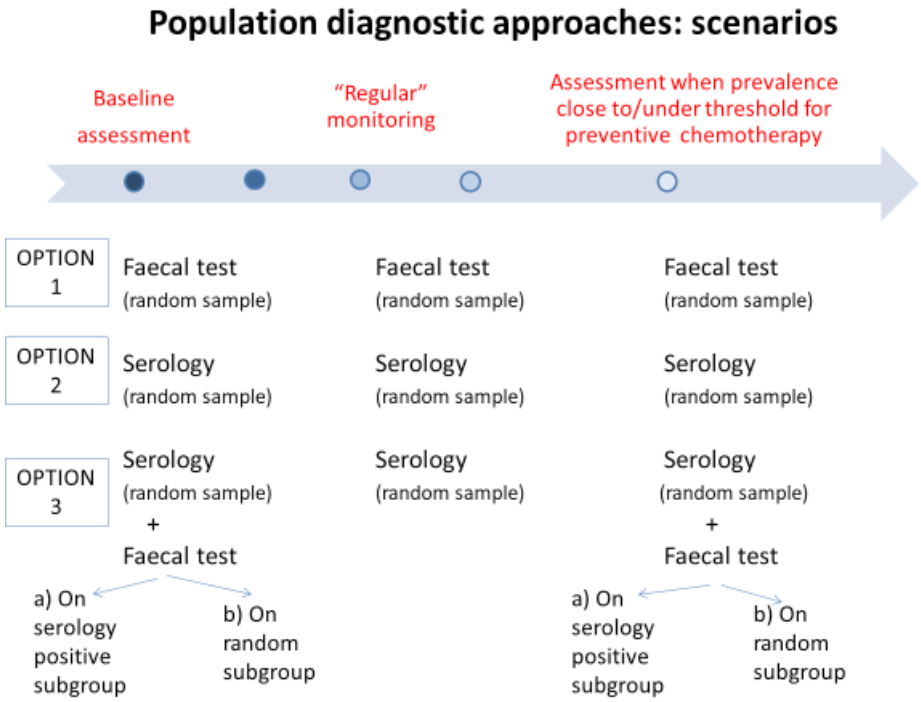
- serology alone can likely accomplish the goals of estimating prevalence and monitoring intervention. However, because of issues with specificity, once low prevalence is achieved, it should be combined with a direct parasitological diagnostic method (such as molecular diagnosis).

According to **A. Krolewiecki**:

- integration of *S. stercoralis* testing may be more efficient and feasible with NTDs other than soil-transmitted helminthiases as serology offers that option (the collection of serum on filter paper is already done for other NTDs);
- NIE ELISA seems the best candidate but others can be considered;
- simplicity and availability to upscale the tests should be kept in mind.

Dora Buonfrate presented three possible diagnostic scenarios for the participants to choose which would be most applicable and accurately predictive for a population-based control programme (Fig. 1).

Fig. 1. Suggested scenarios for *S. stercoralis* diagnostic approaches



3.5 General discussion

The following points emerged from the general discussion.

1. *Serology may be the best initial approach, with additional tests added in later phases of the control programme*. However, no single serological test was identified clearly as outstanding in accuracy.
2. *Additional faecal testing can be added* to a smaller sample of serological tests for increased accuracy. A combination of methods would be important for the comparison of prevalence at baseline and later on. Programmes need more sensitive tests when prevalence gets low. However, a pragmatic approach is essential, and we do not need a perfect test initially. Also, screening more people may be more important that having two tests (higher accuracy) on a smaller sample.
   A flexible approach, with a minimum standard that is cognizant of cost and feasibility should be recommended. It should have flexibility and comparability to include additional technologies later according to the programme phases and diagnostic advances.
3. *Samples collected for other NTD programs could be tested for S. stercoralis also.*
   (a) Integration with other NTD programmes is important.

(b) Can consider collecting samples for multi-NTD diagnostics and, possibly,

(c) Existing bio-banks could be utilized to assess *S. stercoralis*. This will help the investment case.

4. Quality control for DNA extraction and PCR and external quality assurance services are essential areas for investment to support any diagnostic strategy. A preliminary step to compare different methods and compare results across laboratories will be highly advisable.
5. While selecting the recommended diagnostic approach, the threshold for action (for interventions by countries) should be considered. Diagnostic approaches should be linked to action thresholds.
6. Another approach to consider is CASCADE (available from the World Gastroenterological Organizaton): based on availability of resources and programme phases, different combination of tests can be applied.
7. An independent assessment of the quality of different serological tests should be conducted.
8. There are opportunities to assess impact indirectly where ivermectin was used for other purposes.

Dr Montresor closed the meeting by thanking all the participants from across the many time zones. He will share the draft report for participants' input when ready and then proceed with agreed next steps.

After the virtual meeting, the organizing committee considered the different issues discussed and issued the following suggestions that were shared with all the participants for discussion and approval.

4 Summary of considerations and decisions

Lack of a standard diagnostic approach for strongyloidiasis is presently a major impediment for the direct comparison of research results from different groups and for the conduct and evaluation of pilot activities.

The participants considered it essential to provide guidance to researchers and managers of NTD control programme potentially interested in including strongyloidiasis in the portfolio of the NTDs targeted based on the available diagnostics. If more efficient diagnostic methods will be developed in the future, these will be evaluated and incorporated in the suggestions.

The following points should therefore be considered as interim guidance with the aim of facilitating the conduct of operational research activities and comparison of the results obtained and as the starting of pilot control activities targeting strongyloidiasis with the available tools.

1. Serological assessment is the best available option, even though no perfect serological test is available yet. In addition, the serological approach would enable easy assessment of the prevalence of *S. stercoralis* in areas where sera banks have been established for other NTDs.
2. Currently, the best choice among the different serological tests is considered to be NIE ELISA because the kit is available commercially and can be produced in large quantities for field use. Also, because it is based on recombinant antigen, it can be procured at an affordable cost.
3. Whenever possible, the serological assessment of *S. stercoralis* prevalence should be accompanied by a Baermann or agar-plate method (according to the preference of the laboratory). However, if two methods are coupled (i.e. serology plus faecal examination), the results should be reported separately for each method (to facilitate comparability with other interventions where for example only serology is used).

It is suggested to conduct pilot projects applying preventive chemotherapy interventions when the serological prevalence of *S. stercoralis* exceeds 15% with the present sensitivity of the NID ELISA test. This would correspond to a 10% actual prevalence in the population and a test with 91% sensitivity and 94% specificity (Bisoffi et al., 2014).

5 Next steps

1. The WHO Department of Control of Neglected Tropical Diseases, in collaboration with the WHO Collaborating Centre in Negrar (Italy), will conduct an evaluation of the NIE ELISA and other serological tests in two ways by:
   (a) assessing the sensitivity and specificity of the commercial kit on sera bank specimens available in the Center (protocol to be developed by the Collaborating Centre); and
   (b) organizing a multi-centre longitudinal evaluation study on migrants and in endemic countries (protocol to be developed by the Collaborating Centre and shared with potentially interested research groups).
2. The University of Salta (Argentina) and the Federation University (Australia) will develop standard operating procedures for Baermann, modified Baermann and Harada-Mori methods. The procedures will be developed from the existing standard operating procedures in the *WHO bench aids for the diagnosis of intestinal parasites*.
3. The WHO Department of Control of Neglected Tropical Diseases will contact the group commercializing the NIE ELISA and discuss the production capacities and preferential prices.
4. The WHO Department of Control of Neglected Tropical Diseases will continue to follow up the prequalification process of generic ivermectin and discuss possible preferential prices for large-scale use in control programmes and for use in research settings. Endemic countries and all the participants of this meeting will be kept informed about the results.
5. The WHO Department of Control of Neglected Tropical Diseases will promote pilot projects for the control of strongyloidiasis to validate the suggested diagnostic approach and supportive evidence for suggested action thresholds.
6. The WHO Department of Control of Neglected Tropical Diseases will promote the collection of epidemiological information on strongyloidiasis whenever epidemiological collection of other NTDs is conducted. For this purpose, WHO will issue guidance on the specimen to be collected, the laboratory analysis to be conducted to identify strongyloidiasis and the interpretation of the results.
7. The WHO Department of Control of Neglected Tropical Diseases will promote integration of control of strongyloidiasis with other preventive chemotherapy programmes targeting other soil-transmitted helminthiases or other NTDs.

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Presentation 2 (R. Bradbury)

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Presentation 3 (S. Mahanty)

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Presentation 4 (A. Krolewiecki)

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Annex 1
Agenda

Time	Topic	Speaker
13:25	Login to meeting	Chair: Z. Bisoffi
13:30–13:45	Welcome (including declaration of conflict of interest); opening remarks on diagnostic need from a public health perspective	A. Montresor
13:45–14:00	Current molecular diagnostics (advantages and cons)	R. Bradbury
14:00–14:15	Current serological diagnostics (pro and cons)	S. Mahanty
14:15–14:30	Current coprological diagnostics (pro and cons)	A. Krolewiecki
14:30–14:45	**Break**	
14:45–15:30	Panel discussion: Which is the best diagnostic method (or combination of diagnostic methods) to respond to the needs of a control programme?	A. Montresor D. Buonfrate R. Bradbury S. Mahanty A. Krolewiecki
15:15–16:15	Discussion (continued) contribution from participants: Which is the best diagnostic method (or combination of diagnostic methods) to respond to the needs of a control programme?	Chair
16:15–16:30	Summary and next steps	Chair
16:30–16:45	Close of meeting	Zeno Bisoffi Antonio Montresor

Annex 2
List of participants

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Annex 3
Presentations

Presentation 1 (Antonio Montresor)

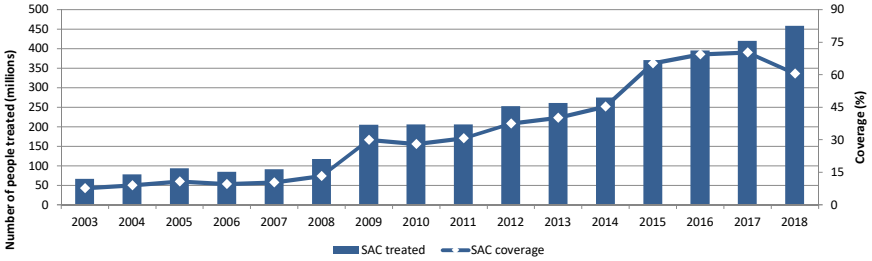
Standardizing diagnostic approaches for the control of strongyloidiasis

Antonio Montresor
Department of Control of Neglected Tropical Diseases
World Health Organization, Geneva

World Health Organization

Control of STH

In the last 10 years:
- Good progress in control of *A. lumbricoides*, *T. trichiura* and hookworms



- No specific control activities for strongyloidiasis…

The limitation factor for the establishment of a strongyloidiasis control programmes was the poor availability of ivermectin.

At the moment 2 generic ivermectin for human use are in prequalification and the drug is expected to be available at accessible cost soon.

World Health Organization

Window of opportunity

WHO/NTD included the control of strongyloidiasis among the targets of the Road Map for 2030 because the expected availability of ivermectin open a window of opportunity for the control of the infection:

Several PC programmes are in place reaching million of individuals with other drugs, this would provide a no cost infrastructure to distribute ivermectin.

The establishment and scaling up of a strongyloidiasis control programme could be much more rapid than for other NTD

World Health Organization

Additional tools needed to establish PC control programme

- Knowledge of the global epidemiology
  to estimate needs and discuss with potential donors
- Field evaluation of the proposed intervention
  to convince potential donors and MoH in the endemic countries in investing on it
- Standard diagnostic methods
  to decide if the control intervention is needed and to monitor the impact

World Health Organization

Important characteristics of a diagnostic method for use in control programme

The ideal diagnostic tool should:

- have good sensitivity and specificity
- have stable performances
- be relatively simple to perform
- be available in sufficient quantities
- have affordable cost

World Health Organization

Diagnostic needs in public health are different than in clinical practice

Sensitivity/specificity:

- In clinical practice we are interested that a positive individual is constantly correctly identified by the diagnostic tool.
- In public health we need that a population at risk is correctly classified by a survey conducted with the diagnostic tool.

In public health, even if a diagnostic tool is not completely sensitive, we can take this into account and estimate the "real" prevalence

EXAMPLE (method with 70% sensitivity)
if the prevalence measured by this diagnostic in a population is 10%
we can estimate the "real" prevalence at 14.5% :

we do not need to know which individual is positive.

World Health Organization

A the moment no standard method for diagnosis of *S. stercoralis* is identified.

There are multiple coprological, serological and molecular diagnostic methods described, mainly developed for clinical use.

Objective of this meeting

To determine which of the existing diagnostic methods (or combination of methods) will be the best for use in a strongyloidiasis control programme based on PC.

World Health Organization

Urgency

Strongyloidiasis is causing enormous suffering in endemic countries.

Waiting years for the perfect diagnostic tool would exclude a generation of infected individuals from the benefits of the control intervention.

We feel that, despite not being perfect, the available diagnostics are sufficiently performant to guide decisions in the establishment and monitoring of a control programme.

If new and more performant tools will became available, these will be evaluated and incorporated in the control programme.

World Health Organization

Additional slide on cost

Low cost is always an advantage.

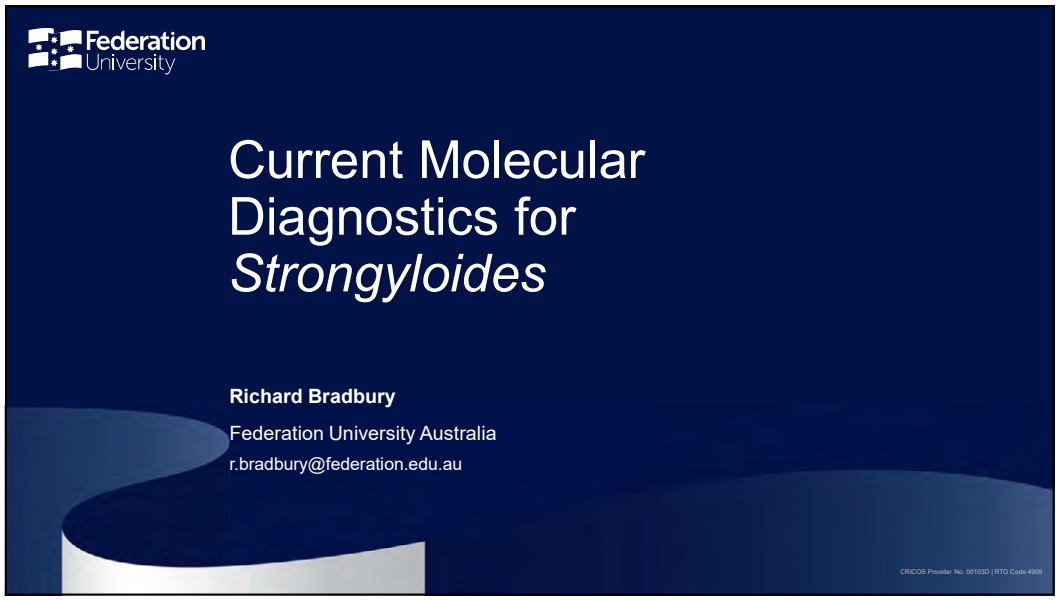
However, we should consider that we are conducting the survey in a fraction of the population benefiting for the intervention.

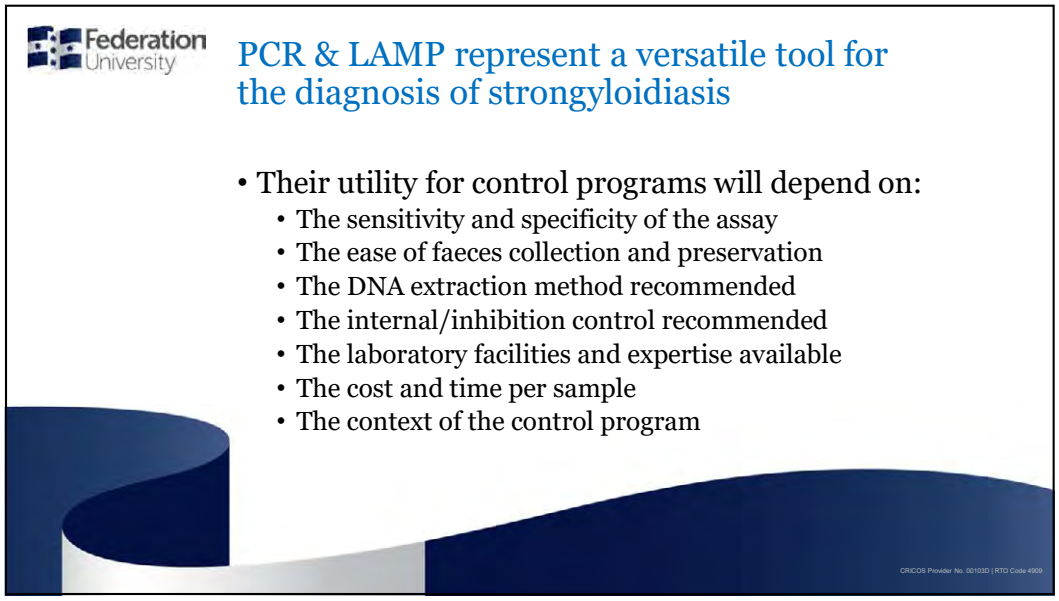
Investing 10$ for each diagnostic test in a survey (for example covering 250 individuals) may be acceptable if this provide information on a much larger population (for example 50 000 individuals).

In this example the diagnostic cost for each individual benefiting from the intervention will be of 5 cent

World Health Organization

Presentation 2 (Richard Bradbury)



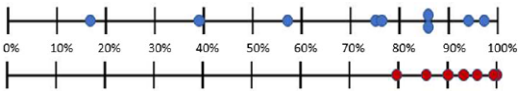


Commonly Recognised Molecular Diagnostic Tests

- The only clinically validated Molecular Test, with the most rigorous analytical specificity testing is the real-time PCR described by Verweij, et al. in 2009.
- A real-time PCR published by Pilotte, et al. in 2016 has been scientifically validated, but not yet clinically validated
- Two LAMP assays have been described, both have been scientifically validated, but not yet clinically validated

Real-time PCR by Verweij, et al. 2009:

- Target: *18s rRNA*
- Analytical sensitivity (LoD) on a single larva: 10^{-2} (4/5)
- Sensitivity*:
- Specificity*:



- Cost estimate**: USD$7-10

Has been used extensively in surveillance studies globally

* Validated against Baermann and/or agar plate culture

**Including DNA extraction

Real-time PCR by Pilotte, et al. 2016 :

- Target: Repetitive DNA elements
- Analytical sensitivity (LoD) on a single larva : 10^{-3*}
- Sensitivity: nd**
- Specificity: nd**
- Cost: USD$7-10

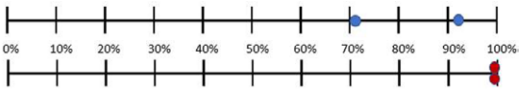
Has been used in surveillance studies on samples from the Mississippi, Kenya and Bangladesh

* Calculated – based on estimated concentration of 1 pg DNA per larvae

** Good percentage agreement to Verweij 2009 real-time PCR in 78 samples but insufficient positive DNA samples (n=1) were available for accurate calculation

LAMP by Watts, et al. 2014:

- Target: *28s rRNA*
- Analytical sensitivity (LoD) on a single larva: 10^{-1} (4/5)
- % +ve Agreement*:
- % -ve Agreement*:



- Cost estimate**: USD$6-9

% Agreement to Verweij, et al. 2009 real-time PCR on stored samples from samples from Australia and Bangladesh

* Compared to Verweij 2009 real-time PCR

**Including DNA extraction

LAMP by Fernandez-Soto, et al. 2016:

- Target: *18s rRNA*
- Analytical sensitivity (LoD) : 10 larvae*
- % +ve Agreement**: 100%§
- % -ve Agreement**: not performed
- Cost estimate¶: USD$6-9

No validation on large number of samples from endemic region

* Calculated – based on estimated concentration of 1 pg DNA per larvae
**Compared to Verweij 2009 real-time PCR
§ One LAMP positive in a real-time PCR negative sample
¶ Including DNA extraction

DNA extraction protocols affect results!

Comparison of five DNA extraction protocols for *Strongyloides* real-time PCR

Extraction Method	**No. *S. ratti* third-stage larvae spiked in stool (no. positive/no. tested)**		
	10	5	1
	Ct (n+/n)	Ct (n+/n)	Ct (n+/n)
PowerSoil kit (Qiagen)	25 (4/4)	28 (4/4)	29 (4/4)
Ultra Clean Fecal DNA kit (MoBio)	27 (4/4)	27 (4/4)	30 (3/4)
QIAamp Tissue kit (Qiagen; modified with polyvinylpolypyrrolidone)	28 (4/4)	30 (4/4)	30 (4/4)
Repeated bead beating plus Ultra Clean Fecal DNA kit (MoBio)	34 (2/4)	36 (1/4)	36 (2/4)
semi-automated DNA extraction method using repeated bead beating combined with NucliSENS easyMAG	31 (4/4)	32 (4/4)	32 (3/4)

Taken from: Sultana, et al. 2013. Real-Time Polymerase Chain Reaction for Detection of *Strongyloides stercoralis* in Stool. *Am J Trop Med Hyg* 88: 1048-1051.

Pros and Cons – Real-time PCR

Pros:

- Good sensitivity & high specificity
- Can be performed on preserved stool (not formalin) ?urine
- Reproducible & comparable across sites
- No specialist parasitology skills required

Cons:

- Cost (~$7-10 per test with extraction)
- DNA extraction labour intensive
- Requires technical skills in molecular biology
- Requires expensive specialist equipment (reference lab level)
- Requires faecal sample
- Pilotte method needs clinical validation

Pros and Cons - LAMP

Pros:

- High analytical specificity
- Can be performed on preserved stool (not formalin) ?urine
- Reproducible & comparable across sites
- No specialist parasitology skills required
- Does not requires expensive specialist equipment (district lab level)

Cons:

- Needs clinical validation
- Current validations are vs Verweij 2009 PCR
- Cost (~$6-9 per test with extraction)
- DNA extraction labour intensive
- Requires technical skills in molecular biology
- Requires faecal sample

Further important considerations

- Internal extraction/inhibition controls are important
  - ~5% faecal samples inhibitory, local diet dependent
- This means multiplex PCR or 2 LAMPs per sample to ensure against false negatives
- Require specialised kits and reagents, including resupply and cold chain
- Require technical expertise to perform
- Susceptible to laboratory contamination (false positives) unless strict protocols are followed

Summary

- The Verweij 2009 real-time PCR is the only comprehensively validated molecular assay.
  - A wide range of sensitivity values have been reported
- The Pilotte real-time PCR and the Watts LAMP have only been compared to the Verweij PCR. The Fernandez-Soto LAMP has had very limited comparison to other methods thus far.
  - All three of these assays require validation before they could be considered
- Molecular methods are relatively expensive, need fresh or preserved stool, and require molecular laboratory expertise
- Any recommended PCR method must also include a standard kit and protocol for DNA extraction

Federation University

Questions?

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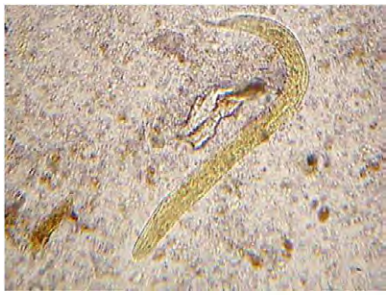


Presentation 3 (Siddhartha Mahanty)

WHO Meeting on the Diagnostic Methods for the Control of Strongyloidiasis

Serological Diagnosis of *Strongyloides* infections

Siddhartha Mahanty

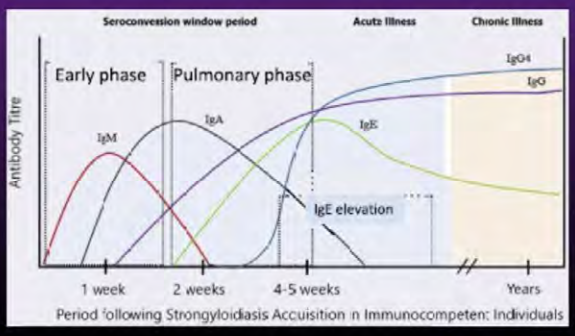
Associate Professor, Department of Medicine

The Peter Doherty Institute for Infection and Immunity

Royal Melbourne Hospital
University of Melbourne
Melbourne, Australia

Doherty institute

The Human Antibody Response to *Strongyloides*



- IgM and IgA detectable in acute infection
- IgE and IgG rise together and IgE declines while IgG persists during chronic illness
- IgG declines ~12 months post-treatment

Arifin 2019

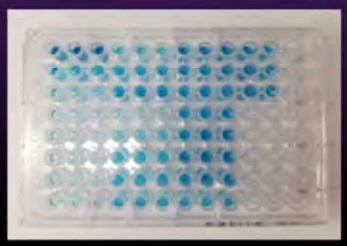
Why should we use serological assays for diagnosis?

In individuals with Strongyloides infections, antibody detection tests --

- Have high diagnostic sensitivity and specificity
- Potential for point-of-care translation
- Can identify infection at all stages (early and late)
- Can demonstrate effect of treatment

Serological diagnosis: Methodology

- Antibody ELISA
  - Larval extracts ("crude" Ags) – IgG, IgG4, IgE
    IVD/DRG (Ss L3), Bordier™ (Sr L3)
  - Recombinant Ags
    - NIE
    - SsIR
- Formats
  - Serum
  - Blood spots
  - Lateral flow ("strip test")
    - IgG, IgE



Key publications

- Effectiveness of Screening and Treatment Approaches for Schistosomiasis and Strongyloidiasis in Newly-Arrived Migrants from Endemic Countries in the EU/EEA: A Systematic Review. Agbata E, et al. Int J Environ Res Public Health. 16(1): 11-51, 2018.
- Accuracy of five serologic tests for the follow up of Strongyloides stercoralis infection. Buonfrate D, et al; PLoS Negl Trop Dis. 2015 Feb 10;9(2):e0003491.
- Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. Bisoffi Z et al. PLoS Negl Trop Dis. 8(1):e2640, 2014
- Strongyloidiasis screening in migrants living in Spain: systematic review and meta-analysis. Salvador F, et al. Trop Med Int Health. 25(3):281-290, 2020
- Use of dried blood spots to define antibody response to the *Strongyloides stercoralis* recombinant antigen NIE. Mounsey K, et al. Acta Trop. 138:78-82, 2014

Comparison of 5 serologic assays ç

- N=399
- 3 study groups (confirmed POS, exposed, HIV POS)
- 5 assays
  - IFAT (in-house, filariform larvae)
  - IVD ELISA (Ss somatic Ag)
  - Bordier ELISA (Sr somatic Ag)
  - NIE ELISA (rNIE)
  - NIE LIPS (Ruc-NIE fusion protein)

Table 2. Test accuracy on samples from subjects with certain diagnosis (denominator for sensitivity: 114 subjects with Ss larvae in stool; denominator for specificity: 115 subjects with no Ss larvae in stool and no exposure).

TEST	Sensitivity (IC 95%)	Specificity (IC 95%)
NIE ELISA	75.4 (67.5–83.3)	94.8 (90.7–98.9)
NIE LIPS	85.1 (78.6–91.6)	100.0 (100.0–100.0)
IFAT	93.9 (89.5–98.3)	92.2 (87.3–97.1)
IVD ELISA	91.2 (86.0–96.4)	99.1 (97.4–100.0)
BORDIER ELISA	89.5 (83.8–95.1)	98.3 (95.9–100.0)

doi:10.1371/journal.pntd.0002640.t002

Bisoffi PLoS NTD 2015

Comparison of diagnostic serology for *Strongyloides*

TABLE 2. Accuracy of serological methods for *Strongyloides stercoralis* diagnosis according to selected studies published after 2005

Reference (year)	Test(s) assessed	Sensitivity (%)	Specificity (%)	Study characteristics
[36] (2014)	Bordier ELISA[a]	90.8	94.1	Study using a composite reference standard of faecal and serological tests (denominator for positives: samples with positive stool or ≥3/5 positive serological tests) to cope with the lack of a reference standard. Limits: retrospective study design
	IVD-ELISA[b]	92.3	97.4	
	NIE-ELISA[c]	70.8	91.1	
	IFAT[d]	94.6	87.4	
	NIE-LIPS[e]	83.8	99.5	
[37] (2014)	SciMedx ELISA[a]	85.5	82.6	Study mainly aimed at assessing percentage agreement of the three tests. Reference standard for positives: samples with ≥2/3 positive serological tests—faecal results not considered
	InBios-Strongy ELISA[f]	83.6	91.3	
	NIE-LIPS[e]	89.1	89.1	
[41] (2007)	AMC-ELISA[g]	93.3	95.0	Study using a panel of serum specimens from a population composed of patients with proven strongyloidiasis, healthy controls, and patients with various parasitic and other diseases. Faecal results used as a unique reference standard
	Dipstick[f]	91.1	97.7	
	Bordier ELISA[a]	83.3	97.2	
	IVD-ELISA[b]	88.9	97.2	
[42] (2010)	IVD-ELISA[b]	91.2	93.3	As above
[44] (2010)	Crude Ag-ELISA[a]	97.0	100	Specificity predetermined at 100% for all methods by the use of cut-off values obtained from ROC curves for 90 samples from patients with positive stools and ten healthy controls from a non-endemic area
	NIE-ELISA[c]	84.0	100	
	NIE-LIPS[e]	97.8	100	
[43] (2008)	NIE-ELISA[c]	97	95	Prospective design. Specificity assessed in a small group of healthy controls, but also confirmed (for LIPS) in samples from patients with filarial and other helminth infections
	NIE-LIPS[e]	97	100	
[47] (2007)	IFAT[d]	74.1	98.4	Accuracy (high titre) determined with latent class analysis to cope with the lack of a reference standard. Coprological methods used for the model not optimal for *S. stercoralis*
[49] (2006)	IFAT[d]	97.4	97.9	Reference standard for sensitivity: samples from patients with *S. stercoralis* larvae in stools. Reference standard for specificity: samples from controls with no risk of infection and negative stools. Cross-reactivity assessed separately
[55] (2011)	IFAT[d]	73	NA	Study on HIV-positive subjects, with average CD4 count of 373/μL

Ag, antigen; HIV, human immunodeficiency virus; IFAT, immunofluorescence antibody test; LIPS, luciferase immunoprecipitation system; NA, not available; ROC, receiver operating characteristic.
[a]Crude Ag (*S. ratti*), commercially available.
[b]Crude Ag (*S. stercoralis*), commercially available.
[c]Recombinant Ag, in-house.
[d]Crude Ag (*S. stercoralis*), in-house.
[e]Commercially available for study purpose only.
[f]Recombinant Ag, commercially available.

Buonfrate Clin Micro Infect, 2015

Diagnostic performance of serological tests

Assay	Sensitivity (%) TP/TP+FN	Specificity (%) TN/FP+TN
Bordier	91 (86-96)	93 (90-96)
IVD (DRG)	92 (87-97)	97 (96-99)
NIE Elisa	95 (75-97)	93 (90-96)
NIE-Luminex	53	98

References:

Agbata, 2018 PMID 30577567
Bisoffi, 2014 PMID 2442732
Buonfrate, 2015 25668740
Rascoe. 2015 25860665

Which diagnostic assay is best for a control program?
Sensitivity vs. specificity

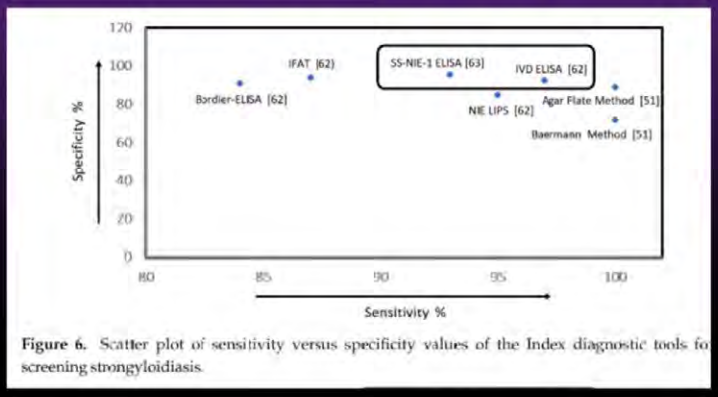
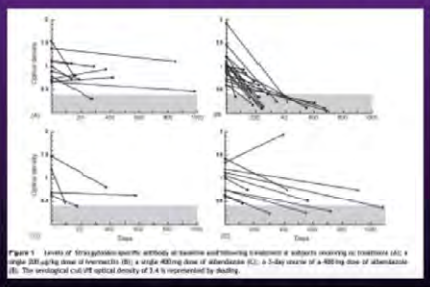


Figure 6. Scatter plot of sensitivity versus specificity values of the Index diagnostic tools fo screening strongyloidiasis.

Agbata et al., 2018

Effect of treatment on seropositivity



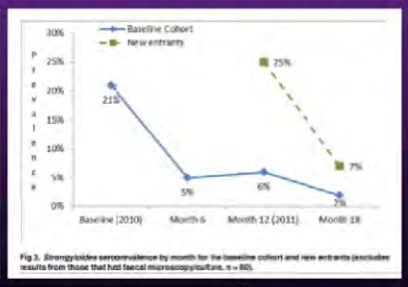


Table 2. Number of samples which demonstrated cure at follow up for each test.

Test	Positives at baseline	Response	%	Negativization	%
FAT	91	79	86.8	36	39.6
WE-LIPS	82	65	79.3	35	42.7
WE-ELISA	69	56	81.2	50	72.5
VCI	88	79	89.8	48	54.5
Bordier	86	71	82.6	47	54.7

Page 2006, Buonfrate, 2015, Kearns 2017,

The Trouble with Serodiagnosis for Control Programs

• Issues with diagnostic 'confidence' Sensitivity 85-95% Specificity 29-99%	Highly dependent on Ag used
• Absence of gold standard	Difficulty in calculating diagnostic efficiency

Pros and cons of serological diagnostic assays

Pros

- ✔ High sensitivity
- ✔ Can be performed on dried blood spots
- ✔ Commercial kits
- ✔ Easy harmonization between labs

Cons

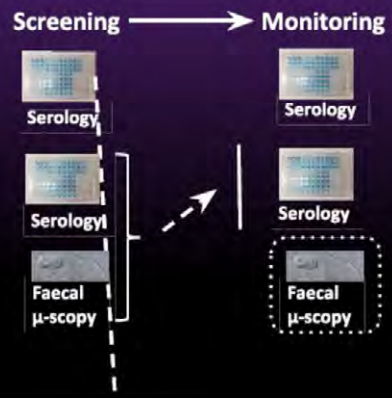
- ✔ Possible cross-reactions
- ✔ Accuracy depends on type of assay
- ✔ Needs equipment (reader) not present in all labs

Potential applications of serology in elimination programs

- What does serology uniquely offer? Standardised, sensitive tests for prevalence screening
- What is it better for than direct diagnostic techniques? High throughput screening for prevalence
- How can serology be applied to control programs?

  To determine prevalence and monitor effectiveness of interventions

Potential application in control programs



Summary

Serological diagnosis has a useful role in monitoring infection in control programs

- More sensitive than direct diagnostic tests
- High throughput/less labour-intensive

Limitations of serological diagnostic testing

- Supply chain for reagents
- High sensitivity/suboptimal specificity of crude Ag-based assays
- Sensitivity of recombinant Ag-based ELISAs

Questions for discussion

- Why should serology be considered in a control program?
- What circumstances recommend use of serology (vs direct diagnostics)?
- What are the best strategies for combining serology with other diagnostic assays?

Thank you for your attention!

Questions?

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Presentation 4 (Alejandro Krolewiecki)

Summary

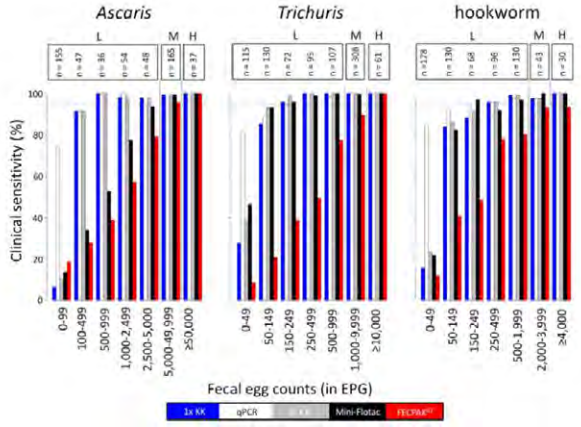
- Stool methods should be fresh for detection of *S. stercoralis*.
- Sensitivity is improved by multiple sampling.
- Intermittent larval expulsion lowers sensitivity of any stool method.
- Methods aiming for eggs are useless.
- Different protocols are used under the same name.

What is the sensitivity required for implementation?

Number of comparisons	*Ascaris lumbricoides* 63		*Trichuris trichiura* 67		Hookworm 94	
Method	Sensitivity (%)	95%BCI	Sensitivity (%)	95%BCI	Sensitivity (%)	95%BCI
1-slide Kato-Katz	63.8	59.1–68.6	82.2	80.1–84.5	59.5	56.9–62.2
2-slide Kato-Katz	64.6	59.7–69.8	84.8	82.5–87.1	63.0	59.8–66.4
2-sample Kato-Katz	69.2	63.2–74.6	89.7	86.3–92.6	74.2	70.6–78.1
3-sample Kato-Katz	70.4	64.9–75.6	90.5	87.6–93.1	74.3	70.8–78.2
Direct microscopy	52.1	46.6–57.7	62.8	56.9–68.9	42.8	38.3–48.4
Formol-ether concentration (FEC)	56.9	51.1–63.5	81.2	73.0–89.2	53.0	48.6–57.5
FLOTAC	79.7	72.8–86.0	91.0	88.8–93.5	92.4	87.6–96.2
Mini-FLOTAC	75.5	54.0–95.9	76.2	33.9–99.4	79.2	72.7–85.9
McMaster	61.1	56.3–65.9	81.8	79.6–84.2	58.9	55.7–62.2
Specificity	99.6		97.5		98.0	

Nikolay *et al*. IJP, 2014

Sensitivity on different infection intensities for STH



Cools *et al*. PNTD, 2019

What is available on coproparasitologic methods for *S. stercoralis*?

- ~~Direct smear.~~
- ~~Kato Katz.~~
- ~~McMaster`s~~
- ~~MiniFLOATAC~~
- Formol-Ether Concentration.
- Spontaneous sedimentation.
- Harada Mori.
- **Koga`s Agar plate.**
- **Baermann.**
- **Bearmann with charcoal preincubation.**

Requirements

- Methods looking for larvae rather than eggs.
- Fresh stools.
- No SAF or formalin.
- No need for quantification.

Harmonization of protocols

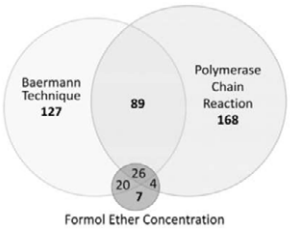
Everybody in every lab following the same protocol with the same materials

The Baermann example:

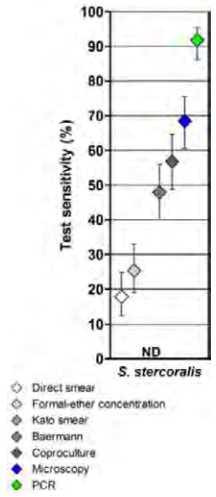
High variability of estimations and little correlation

	Baermann test		Total
	Positive	Negative	
Koga agar plate test positive	19	0	19
Koga agar plate test negative	2	159	161
Total	21[a]	159	180

[a]The sensitivity of the Koga agar plate method was 90.5% and that of the Baermann method was 100% if the combined results from both tests are considered as diagnostic 'gold' standard.



Parasites*	Lutz		Formalin-ethyl acetate		Baermann		Agar plate culture		Harada-Mori culture		Total no. parasite infections
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	(n=101)
Helminths											
S. stercoralis	14	42.4	16	48.5	16	48.5	23	69.7	8	24.0	33



Amor, 2020. PNTD; Meurs, 2017.PNTD; Steinmann, 2007. PNTD; Marchi-Blatt, 2003 BJID

Baermann: principle

- The technique is used based on the active migration or movement of larvae downward from fresh stool samples to water of warmer temperature.
- Feces are suspended in water
- After permitting sufficient time to allow migration, the warm water is drained off, centrifuged, and examined microscopically for the presence of the larvae

STOP

STOP Consortium SOPs. https://stoptheworm.org/

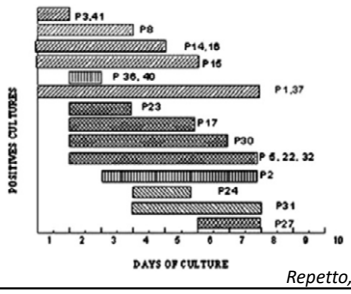
A modified Baermann

- Simplified procedure.
- Less lab space.
- Shorter time.
- Improved sensitivity with charcoal preincubation.

Meurs et al, 2017. PNTD

Agar Plate

- Petri dish
- 3grams of fresh stools.
- Incubation for 2 to 9 days.
- Daily review of plates.
- Requires microscopic identification



Repetto, 2010. AJTMH

Key aspects to be considered

- Features
  - ✓ *accuracy, reproducibility, cost, time, lab space, complexity.*
- Integration capacity.
  - ✓ *stools are ideal for integration with STH, but inconvenient for any other NTDs.*
- Epidemiologic question.
  - ✓ *baseline, assess progress, approaching control.*

Thanks

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		Gold standard (Composite result)			
		Positive (%)	Negative (%)	Total (%)	Sensitivity (95% CI)
	Total	131 (100)	233 (100)	364 (100)	
Traditional	Positive	35 (26.7)	0 (0)	35 (9.6)	26.7 (19.9– 34.9)
	Negative	96 (73.3)	233 (100)	329 (90.4)	
Modified	Positive	29 (22.1)	0 (0)	29 (8)	22.1 (15.9 - 30.0)
	Negative	102 (77.9)	233 (100)	335(92)	
Modified+charcoal preincubation	Positive	114(87)	0 (0)	114 (31.7)	87 (80.2 – 91.7)
	Negative	17(13)	233 (100)	250 (68.7)	


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