Limitations and Future Considerations in Helminth Diagnostics

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Rickettsial diagnostics – moving forward

**STIFA** – From single titers to dynamic serology

**AG / NAAT combined w/serology** – Improved ADM Dg

**STIC** – Robust criteria for diagnostic accuracy

**Baysian Modelling** - IFA results with false positivity?

**PAST** – Antigen detection using protein arrays

**Ag cartography** – Plotting antigenicity on “maps”

**Aims:**

Simple, affordable point-of-care “RDT"

Standardized assay, non-subjective endpoint

Covers antigenic heterogeneity / variation

High accuracy - Sn and Sp in endemic areas (!)

Coverage of complete disease course

Blacksell et al., CID, 2007
Paris DH et al., CMI, 2009
Felgner P et al. PNAS 2009
McGready et al., PNTD, 2010
Paris DH et al, PNTD 2011

Paris DH et al., AJTMH 2013
Lim C. et al., PONE 2015
James S. et al., PNTD, 2016
Weitzel T et al., NEJM 2016
Paris DH et al., COID, 2017
## Context of diagnostics ... schistosomiasis control

<table>
<thead>
<tr>
<th>Programmatic steps</th>
<th>Pre-control</th>
<th>Control</th>
<th>Elimination as public health problem</th>
<th>Interruption of transmission</th>
<th>Post-elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Situation analysis</td>
<td>Preventive chemotherapy (PCT)</td>
<td>PCT and other control measures</td>
<td>PCT, other control measures and surveillance-response</td>
<td>Continued surveillance-response</td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>100% geographical coverage; &gt;75% national coverage; and heavy-intensity infections &lt;5% in sentinel sites</td>
<td>Heavy-intensity infection &lt;1% in all sentinel sites</td>
<td>Reduction of incidence of infection to zero</td>
<td>Incidence of infection remains zero (no autochthonous cases)</td>
<td></td>
</tr>
</tbody>
</table>

2001 World Health Assembly
deworming of school-aged children

praziquantel (safe, effective, inexpensive)

Diagnosis unnecessary, not cost-effective
Little interest in diagnostics R&D

2020 WHO Roadmap to overcome NTDs
-- new era --

“Endgame” - Elimination of schistosomiasis

Importance of diagnostic tools - highly accurate diagnostic assays now required

Utzinger J et al., CMI 2015
Limitations of diagnostics ...

**Questionnaires** (blood in urine)

**Microscopy**
- Urine filtration, fecal smear
- FECT
- Kato-Katz
- FLOTAC
- Mini-FLOTAC

**DNA detection**
- PCR / LAMP

**Antibody detection**
- ELISA
- IHA / IFA

**RDTs**
- POC-CCA

**Antigen detection**
- UCP-LF CAA

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**Table 3. Diagnostic performance of selected signs and symptoms for the diagnosis of *S. mansoni* infection at the community level**

<table>
<thead>
<tr>
<th>Country</th>
<th>S. mansoni prevalence</th>
<th>Questionnaire return rate</th>
<th>No. of children interviewed</th>
<th>No. of children examined</th>
<th>Threshold or high-risk schools</th>
<th>Questions (threshold in %)</th>
<th>Diagnostic performance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Côte d’Ivoire</td>
<td>54.4</td>
<td>12/134 (90)</td>
<td>12.227</td>
<td>50</td>
<td>Blood in stool (22)</td>
<td>88</td>
<td>58</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blood dysentery (14)</td>
<td>88</td>
<td>58</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Schistosomiasis (4)</td>
<td>71</td>
<td>58</td>
<td>73</td>
</tr>
<tr>
<td>Democratic Republic of the Congo</td>
<td>31.2</td>
<td>136/180 (85)</td>
<td>19.362</td>
<td>50</td>
<td>Blood in stool (119)</td>
<td>62</td>
<td>77</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Schistosomiasis (34)</td>
<td>62</td>
<td>69</td>
<td>62</td>
</tr>
<tr>
<td>Kenya</td>
<td>29.4</td>
<td>NA</td>
<td>2312</td>
<td>50</td>
<td>Blood in stool (25)</td>
<td>60</td>
<td>78</td>
<td>43</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>20.9</td>
<td>142/161 (88)</td>
<td>13.756</td>
<td>20</td>
<td>Blood in stool (15)</td>
<td>84</td>
<td>60</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>77</td>
<td>58</td>
<td>96</td>
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<td>Blood dysentery (25)</td>
<td>71</td>
<td>65</td>
<td>76</td>
</tr>
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</table>

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*See footnote a, Table 1.

b The threshold for high-risk schools is the prevalence level at which a school is said to be at high risk. These are the schools that the questionnaire aims to identify.

c See footnote b, Table 1.

d See footnote c, Table 1.

e See footnote d, Table 1.

f Kato–Katz thick smears (2 stool specimens; 1 slide each).

g Kato–Katz thick smears (1 stool specimen; 1 slide).

h NA – not applicable. Questionnaires were not distributed; the work was done by the research team in 46 schools.

i Kato–Katz thick smears (1 stool specimen; 2 slides).

**Bulletin of the World Health Organization 2002, 80 (3)**
Limitations of diagnostics ...

Questionnaires (blood in urine)

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**KK “standard”:**
small amount of stool – good for high egg burden
high transmission setting
quantifiable egg counts - decrease upon therapy
**BUT:** problematic with low egg burden – detection 20-50 EPG

**Mini-FLOTAC:**
Flotation of eggs - no centrifugation – detection 10 EPG
Limitations of diagnostics ...

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Sn and Sp good, with low limit of detection

**BUT:** Poorly standardised for stool testing

“dead” eggs are PCR pos. (!)

Risk of contamination (LAMP)
Limitations of diagnostics ...

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ELISA:
- screening tool – sensitive in unexposed
- BUT: cross-reactivity / cut off titers / species differentiation
- active vs. past infection / endemic areas

IFA:
- confirmation, highly specific - laborious!

Antigens:
- Egg or adult worm based
- lack of ag standardisation / regions / amount

Larva for IFA
- need to establish the cycle
- (dead larva antigens are different!)
Limitations of diagnostics …

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POC-CCA

“appropriate tool for monitoring schisto control programmes”

BUT:
false-positive results
- Urinary tract infection
- Hematuria
- Pregnancy
- Lewis-X trisaccharide
  (common epitope on RBCs and anti-inflammatory cells)
Limitations of diagnostics ...

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Promising ... might be up to 10-fold more sensitive than KK smears
Differential requirements of diagnostic tests

**Diagnosis**
- Acute infection / background titers
- Documentation successful therapy
- Chronic infection

**Microscopy** – egg detection
**PCR** – high sensitivity
**LAMP** – field deployment

**Surveillance**
- Reduction of positivity rates
- Re-emergence of positivity

**Serology** – ideal if “clean”
  i.e. recomb. protein based assay

**Eradication**
- Documentation of true negativity
- New positives

**RDTs (?)** – CCA .... CAA ...
  (ag-capture / semi-quant. / defined cut-off)

**High throughput vs. individual testing**

**Assay with good NPV, low LoD**
Next Generation Protein Microarrays
Schistosomiasis protein array

Proteome microarray consisting of 992 *Schistosoma mansoni* proteins

Selection on the basis of four characteristics:

1. Differentially expressed proteins from the tegument surface of *S. mansoni* (selection by seropositivity, qPCR, WB, ELISA)

2. Bioinformatic prediction by signal peptides and/or transmembrane motifs (cellular localisation)

3. Specific epitope prediction (software, i.e. IEDB)

4. Proteins recognized by *S. mansoni*-infected sera (from endemic areas)

*R.R. de Assis et al., Int Jour Parasitol 2016*
Next-generation proteome array for *Schistosoma mansoni*

Belo Horizonte – Brasil

Previous study:
IgG1 vs. IgE and IgG3, IgG4 responses
-> Surface antigens

N=92 antigens – n=52 shown here

Gaze et al., PLoS Path 2014
R.R. de Assis et al., Int Jour Parasitol 2016
Next-generation proteome array for *Schistosoma mansoni*

**Table 1**

Comparison of antigens recognised by IgG in sera from individuals with chronic and acute *Schistosoma mansoni* between the expanded 992 antigen *S. mansoni* array and the previous 37 antigen *S. mansoni* array (Gaze et al., 2014).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Description</th>
<th>Selection method</th>
<th>Immunoreactivity by infection status</th>
<th>Negative vs chronic</th>
<th>Negative vs acute</th>
<th>Chronic vs acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMP_171190.1</td>
<td>MEG-8 family</td>
<td>Bioinformatic</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMP_014570.2</td>
<td>Saposin,IPR008139 Saposin</td>
<td>Bioinformatic</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>SMP_040790.1</td>
<td>Peptidyl prolyl cis trans isomerase B</td>
<td>Bioinformatic</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMP_048230.1</td>
<td>Solute carrier family 31 (copper transporters)</td>
<td>Surface</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>SMP_002740.2</td>
<td>Hypothetical protein</td>
<td>Expression</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>SMP_030370.1</td>
<td>Calreticulin</td>
<td>Serologic</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>SMP_149590.1</td>
<td>Gamma aminobutyric acid receptor subunit</td>
<td>Bioinformatic</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<tr>
<td>SMP_194970.1</td>
<td>25 kDa integral membrane protein</td>
<td>Surface</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>SMP_194960</td>
<td>Similar to tetraspanin</td>
<td>Expression</td>
<td>Yes</td>
<td>Yes</td>
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</tr>
<tr>
<td>SMP_045200.1</td>
<td>Tegument-allergen-like protein</td>
<td>Surface</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMP_072190.1</td>
<td>Membrane associated protein 29</td>
<td>Surface</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMP_005740.1</td>
<td>Aquaporin 9 (Small solute channel 1)</td>
<td>Bioinformatic</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Proteins also used on the previous described array by Gaze et al. (2014).*
Questions

(i) antibody signatures of **acute** vs. **chronic** infection (**S. mansoni**)  
(ii) antibody signatures of hepatosplenic schistosomiasis vs. chronic infection without **hepatosplenomegaly**  
(iii) antibody signatures of subjects with **immunological resistance** to **S. mansoni** after PZQ vs. those susceptible to reinfection

**Schistosomiasis:** High titres can persist over months to years after treatment
screened with sera to determine antibody signatures indicative of the clinical stages of schistosomiasis and to identify serodiagnostic antigens

Cohort of 120 Schistosomiasis cases treated with Praziquantel (PZQ)
- Heavy (>499 EPG, Eggs/gram feces)
- Medium (100-499 EPG)
- Low (up to 99 EPG) egg burden

Serum specimens at D0 of PZQ treatment and D360 post-treatment

Sera were probed on the *S. mansoni* array

*Breadth and intensity of Ab response directly proportional to egg burden*

*Ab responses declined after clearance of parasite with PZQ at D360*
Antibody responses correlated with parasite burden

USA& BHZ Nonendemic Negative

<99 EPG Low Day0
100-499 EPG Med Day0
>499 EPG Hvy Day0

<99 EPG Low Day360
100-499 EPG Med Day360
>499 EPG Hvy Day360

Log2 FOC

Black: 4-fold SI rise
Red: 32-fold SI rise

n=66/992 proteins

Unpublished data, 2017
Selection process

Top 66 proteins
• Highest average signal intensity at D0
• Lowest signals at D360
• Ideal Ab kinetics 360 days after PZQ

Top 43 differentially reactive antigens
• Significantly more reactive in the heavy egg burden grp compared to other grps / controls
• Heavy burden grp reacted to more antigens (breadth)

Top 18 sero-reactive antigens
• Best combination of high signals intensity and best p-values between grps
• Best correlation between signal intensities, parasite burden and a significant decline after treatment

Unpublished data, 2017
Non-Endemic vs. Low Intensity egg burden

Non-Endemic vs. Medium Intensity egg burden

Non-Endemic vs. High Intensity egg burden

From the top 18 featured proteins:

6 antigens were found to compose the optimal set for low burden
4 antigens for the medium burden
2 antigens for heavy burden

Unpublished data, 2017
Multiplex Serosurveillance Arrays

Endemic diseases / Fever panels
Migration medicine

Sero-Surveillance - Diagnostics
(combined with PCR panels)

Support vaccine design

- Ab isotypes (IgE, IgG1, IgM etc.)
- Ab dynamics
- Cross-reactivities
- Diagnostic vs. vaccine ag
- Protective responses
- Predictive markers
- etc.
Next steps...
Thank you!

“New” Dept. of Medicine (MED)

Prof Phil Felgner, UCI
Dr Huw Davies, UCI