Point-of-care applications for malaria detection

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Faculty Disclosure

Stephanie Yanow

has no relevant financial relationships with commercial interests to disclose.
Learning objectives

1. To identify the criteria required of a diagnostic for malaria in low-resource settings
2. To compare the different settings for a POC malaria diagnostic
3. To discuss a Lab on Chip technology for malaria detection
Malaria

- >300 million cases/year
- >1 million deaths/year

Malaria is treatable
Democratizing Molecular Diagnostics for the Developing World

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Key Words: Molecular diagnostics; Nanotechnology; Developing world; PCR

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DALYs saved with better diagnostics

- STDs
- TB
- Malaria
- Diarrheal Diseases
- HIV
- ALRI

Millions of DALYs Saved
Limited access to diagnostics

<table>
<thead>
<tr>
<th></th>
<th>High access (% of total)</th>
<th>Medium access (% of total)</th>
<th>Low access (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High transmission</td>
<td>10,551,560 (10)</td>
<td>44,044,040 (40)</td>
<td>28,620,160 (26)</td>
</tr>
<tr>
<td>Low transmission</td>
<td>6,632,280 (6)</td>
<td>5,551,290 (5)</td>
<td>14,819,170 (13)</td>
</tr>
</tbody>
</table>

*Population of children aged 0–4 years in sub-Saharan Africa living in areas of malaria transmission according to status quo access to malaria diagnostics and malaria transmission intensity. Children living in areas of no malaria transmission were excluded from this calculation.

Access to a trained provider or microscopy (not POC)
How do we close the gap?

• Strengthen health care systems to support high-tech diagnostics

• Develop better diagnostics for low-resource settings
The ideal diagnostic: ASSURED

- Affordable
- Sensitive (few false-negatives)
- Specific (few false-positives)
- User-friendly (minimal training requirements)
- Rapid (enable immediate treatment) and Robust (no cold chain)
- Equipment-free
- Delivered to those who need it
Diagnosis by microscopy
New diagnostics

RDTs

- Fast
- Easy to use
- Sensitivity: 100 parasites/μL
- Cannot differentiate all species
- Qualitative
- Lack suitable controls (QC)
- Black market RDTs

PCR

- Fast
- Sensitivity: 0.02 parasites/μL
- Can differentiate all species
- Quantitative
- Expensive
- Requires lab infrastructure
- Skilled personnel
Transferring molecular diagnostics to the field
The Accutas System

• Low-cost, portable ‘Lab-on-a-Chip’ device for PCR testing at the point-of-care
• Malaria diagnosis is a priority application
• Our goal: develop a technology that meets the ASSURED criteria

Chip ($1)

Small instrument ($3000)
Our partner

• Aquila Diagnostic Systems Inc. – spin-off company from the University of Alberta

• Have licensed two patents protecting the gel technology for multiple fields of use

• Also developing chips for animal and water testing, and pharmacogenomic markers for drug resistance in breast cancer patients
How do we bring molecular testing to the field?

DNA extraction
Cold chain
Expensive equipment
Field-specific issues

**Specimen processing**
Use special enzymes that work straight from blood, no processing required

**Cold chain/thermostability**
Preserve PCR reagents in a desiccated gel that can be rehydrated directly with blood – “hydrogel PCR”

**Specialized equipment**
Fully automated low-cost instrument with simple user interface

**Skilled technologists**
Sample loading directly onto a plastic chip

**Infrastructure**
Battery powered instrument, disposable self-contained chips with on-board controls
Chips

Liquid PCR reagents with hydrogel

Desiccated hydrogel for storage
Instrument
Our malaria test

• Yes/no screening test

• Amplifies parasite DNA from blood

• Targets DNA that has the same sequence in all species of malaria
Detection of all *Plasmodium* species

![Graphs showing detection of different Plasmodium species](image)
Sensitivity: 2 parasites/μL
Validation with Ugandan samples

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<tr>
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<tbody>
<tr>
<td>Median age [range]</td>
<td>20 years [14 – 42]</td>
</tr>
<tr>
<td>Presentation</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic (n[%])</td>
<td>72 [38.3]</td>
</tr>
<tr>
<td>Clinical malaria (n[%])</td>
<td>116 [61.7]</td>
</tr>
<tr>
<td>Pregnancy status</td>
<td></td>
</tr>
<tr>
<td>In antenatal care (n[%])</td>
<td>184 [97.9]</td>
</tr>
<tr>
<td>At delivery (n[%])</td>
<td>4 [2.1]</td>
</tr>
<tr>
<td>Median parasitemia [range]</td>
<td>440 parasites/μL [1 – 94800]</td>
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### On-chip testing of Ugandan panel

<table>
<thead>
<tr>
<th></th>
<th>Chip PCR</th>
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<tbody>
<tr>
<td></td>
<td>Pos (n)</td>
<td>Neg (n)</td>
<td>Sensitivity* (95% CI)</td>
<td>Specificity* (95% CI)</td>
</tr>
<tr>
<td><strong>Microscopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos (n)</td>
<td>137</td>
<td>5</td>
<td>96.5% (92.0 – 98.5)</td>
<td>63.0% (48.6 – 75.5)</td>
</tr>
<tr>
<td>Neg (n)</td>
<td>17</td>
<td>29</td>
<td></td>
<td></td>
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<tr>
<td><strong>Conventional</strong></td>
<td></td>
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<tr>
<td>real-time PCR</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pos (n)</td>
<td>152</td>
<td>4</td>
<td>97.4% (93.5 – 99.0)</td>
<td>93.8% (79.9 – 98.3)</td>
</tr>
<tr>
<td>Neg (n)</td>
<td>2</td>
<td>30</td>
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*Determined using microscopy or conventional real-time PCR as the gold standard*
Species identification

**P. falciparum**

- Test

**P. vivax**

- Test

**Species identification**

- P. falciparum Mixed infection
- P. vivax Negative
- P. falciparum Mixed infection
- P. vivax Negative

Fluorescence (Arb)

-dF/dT (Arb)

Cycle

Temperature (°C)

P. falciparum

P. vivax

Mixed infection

Negative
Settings for the LOC

- Diagnosis of acute malaria and fever
- Surveillance in elimination settings
- Vaccine trials
Pilot studies in Uganda, 2015

- Diagnosis of malaria in under 5s
- Field trial in Tororo, Uganda (FIND)
- High transmission area
- District hospital settings
- Assess test performance compared with microscopy and RDTs, user experience, and environmental stability
Surveillance

• Sensitivity is key

• Detection of asymptomatic infections with low parasitemias

• Rapid identification of cases to prevent outbreaks and re-emergence

• Detection of non-falciparum species (*P. vivax*)

• Cross-sectional study planned for May, 2015 in the Solomon Islands
Use-scenarios

<table>
<thead>
<tr>
<th>Passive</th>
<th>Case detection</th>
<th>PCD</th>
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<tr>
<td>Case follow-up</td>
<td>Drug resistance testing</td>
<td>Treatment effectiveness testing</td>
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<thead>
<tr>
<th>Active</th>
<th>Reactive</th>
<th>Mobile index case</th>
<th>Network testing</th>
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<tbody>
<tr>
<td></td>
<td>Diagnose and treat</td>
<td>Community index case</td>
<td>Community testing</td>
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<table>
<thead>
<tr>
<th>Proactive</th>
<th>Diagnose and treat</th>
<th>Hotspots</th>
<th>MTAT</th>
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<tr>
<td></td>
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<td>FTAT</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Hotpops</td>
<td>Border (fixed location) testing</td>
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<td></td>
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<td>Time-location testing</td>
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<tr>
<th>Laboratory testing</th>
<th>Confirmation</th>
<th>EQA</th>
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<td></td>
<td>Parasite quantification</td>
<td>Genetic analytics</td>
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Tietje et al., *Trends in Parasitology* 2014
Vaccine trials

• Support vaccine trials by monitoring efficacy on-site
• Sensitivity is critical for volunteer safety
• Specificity is critical to measure vaccine efficacy
• Same day result

• Pilot trials
  • Phase I trial of PlasProtecT (Griffith University)
  • Trials in Africa of PfSPZ (Sanaria)
Advantages of the Accutas

• Simple device for use at the POC in low-resource settings
• Sensitivity is far superior to current diagnostics (>20x)
• Low cost
• Result within 2 hours
• Can support malaria clinical diagnosis, surveillance, and clinical trials for new vaccines or drugs.
• Mobile (active case detection, disaster response)
• Platform technology that can be developed for other applications of clinical importance (eg. Fever chip)
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Dr. Iveth Gonzalez
Dr. Mark Perkins
Dr. Anthony Mbonye
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Self-Assessment Question

Which of the following must be considered in a successful POC diagnostic for malaria?

1. Cost
2. Stability in high temperature
3. Level of skill of the user
4. Sensitivity
5. All of the above