Malaria or not?
Challenges in diagnosis

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- Funding Somagen Diagnostics
- Funding Phoenix Airmid
- Funding R-Biopharm
- Funding Promega
- Funding Luminex
Malaria is rapidly fatal (even in Canada)

“On June 25, she died from complications of malaria. She was 42.”
Malaria is a multi-organ disease
Microscopy is a visual art

Name this species?
Malaysia - epidemic of mis-diagnosed *P. knowlesi*

- Human infection with *P. knowlesi* was commonly misidentified as the more benign *P. malariae*
- Natural host is the long-tailed macaque
- Widely distributed across Malaysian Borneo and extend to Peninsular Malaysia
- Rapid diagnosis and prompt effective treatment are essential
- Patients who reside in or have traveled to Southeast Asia receive intensive management as appropriate for severe falciparum malaria
Case: Imported *P. knowlesi* to Munich, Germany

73 year old M Myanmar Thailand “rain forest excursions” presented to ED
GCS 7-8, BP 70/50 mmHg, pulse 115/min respiratory frequency 18-20/min, temperature 39.4 °C, SP0₂ 74%

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Creatinine</th>
<th>GFR</th>
<th>Pro-calcitonin</th>
<th>CRP</th>
<th>LDH</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>1.7</td>
<td>40</td>
<td>32.4</td>
<td>300.2</td>
<td>415</td>
<td>1.4</td>
</tr>
<tr>
<td>Day 2</td>
<td>3.1</td>
<td>19</td>
<td>38.7</td>
<td>342.6</td>
<td>586</td>
<td>2.2</td>
</tr>
<tr>
<td>Day 5</td>
<td>5.1 (HD for 5 wks)</td>
<td>11</td>
<td>8.2</td>
<td>149</td>
<td>924</td>
<td>1.5</td>
</tr>
<tr>
<td>Day 30</td>
<td>1.9</td>
<td>32</td>
<td>0.1</td>
<td>5.8</td>
<td>391</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Seilmaier et al, Mal J, 2014
Quality assurance in the laboratory is paramount - Case A
Quality assurance in the laboratory is paramount - Case B
Leapfrog technology
RDTs have played an increasing role in malaria diagnostics
Over 100 manufacturers of RDTs worldwide

hrp2
LDH/aldoase
Limitations of malaria RDTs

- Insensitive for non-\textit{falciparum} malaria especially \textit{P. ovale} (LDH antigen is not stable)
- False positivity in treated patients or gametocytemia
- Spill over bands confounds interpretation of mixed infections
- False positives with rheumatoid factor, co-infections
- Hrp 2 deletions observed in South America and parts of Africa
The problem... how low can you go?

MICROSCOPY & RDT

MOLECULAR

>100,000 parasites per uL (2.5%)

500 parasites per uL (0.0125%)

1 parasites per uL (0.000025%)

0.01 parasites per uL
Clinical and public health impact of Diagnostics

Severe and most symptomatic cases

Asymptomatic cases
Pregnancy
Liver-stage only
Resistant parasites
ERADICATION

>100,000 parasites per uL (2.5%)
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0.01 parasites per uL
What about molecular (NAAT) diagnostics?

• Why do we need it?
  • Asymptomatic or sub-clinical malaria, pregnancy, surveillance, eradication
  • Cheaper gold standard for training/QC purposes
  • Clinical trials for accurate monitoring (MIC studies)

• What formats are feasible?
  • “Lab on a chip”
  • “LAMP”
  • Microfluidics
  • Aptamer technology
  • Nanopore sequencing
Real-time PCR for the detection of malaria:

- RDT: “P. falciparum antigen”
- Microscopy: “Plasmodium species”

11 year old Congolese refugee in Calgary
Nucleic acid-based testing for malaria

• PCR, nested PCR, and real time PCR formats have all been used effectively
• Greater limit of detection than RDT and microscopy especially with multi-copy RNA targets
• High capital and reagent cost, not field-friendly
• Require preventive maintenance of equipment and access to reagents with limited shelf life
• Need to train technologists in molecular diagnostics
LAMP - loop mediated isothermal amplification

• An example of isothermal amplification (others exist such as helicase dependent amplification, strand displacement amplification)
• Relies on a strand-displacing enzyme Bst polymerase
• Relatively resistant to inhibitors in clinical samples permitting crude extraction
• End products are detectable to the naked eye
• RNA can also be used as a target with RT-LAMP and SNP genotyping possible with primer design
Leapfrog technology
LAMP allows molecular amplification detectable by naked eye

- LOD is 1 parasite per uL
- Can detect RNA or repetitive elements to improve LOD

Acta Trop. 2014 Jun;134:52-7
SNP-LAMP POCT to guide chemotherapy

- C580Y
  - +ve samples indicate prolonged therapy (e.g., Co-AKT + MQ) for 7 days.
  - -ve samples indicate regular 3 day ACT or SERCA.

- WT
  - +ve samples indicate regular 3 day ACT or SERCA.
  - -ve samples indicate prolonged therapy (e.g., Co-AKT + MQ) for 7 days.

Unpublished data
Theranostics and Genotyping: critical to drug development

- Theranostics refers to specific diagnostic tests that are required prior to treatment choices.
- Treatment of *P. vivax* and gametocidal drugs are restricted to primaquine and derivatives which require G6PD screening.
- Genotyping of parasites is essential to distinguish recrudescence from re-infection.
- Both theranostics and genotyping methods are not clinically validated and require research and development support.
LAMP training
U Gondar, Ethiopia

Dr. Abebe Bayih
Meslo Sema, MSc student

Malaria Journal 2015 14:44
Active detection of asymptomatic malaria by LAMP in Northwest Ethiopia

• A community based cross-sectional study was conducted in North Gondar, Ethiopia
• A total of 802 study participants were enrolled
• The overall prevalence of asymptomatic malaria using giemsa microscopy was 3.75% compared to LAMP at 5%

Sisay Getie, University of Gondar
Case detection of malaria in pregnancy by LAMP in Northwest Ethiopia

• Study conducted in maternity clinics at rural health centres in NW Ethiopia
• A total of 93 pregnant women suspected of having malaria based on clinical symptoms were enrolled
• The overall prevalence of asymptomatic malaria using giemsa microscopy was 10.8% compared to LAMP at 14%

Banchamlak Tegegne, University of Gondar
# LAMP study in returning travelers (n=140)

Mohon AN et al, DMID, 2015

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%) (95% CI)</th>
<th>Specificity (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LAMP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>100 (93.6-100)</td>
<td>98.6 (91.1-99.9)</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>97.6 (85.9-99.9)</td>
<td>100 (95.3-100)</td>
</tr>
<tr>
<td>non-falciparum</td>
<td>100 (86.3-100)</td>
<td>99.1 (94.3-100)</td>
</tr>
<tr>
<td><strong>RDT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>85.9 (75.2-92.7)</td>
<td>98.6 (91.1-99.9)</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>90.5 (76.5-96.9)</td>
<td>100 (95.3-100)</td>
</tr>
<tr>
<td>non-falciparum</td>
<td><strong>71.0 (51.8-85.1)</strong></td>
<td>99.1 (94.3-100)</td>
</tr>
</tbody>
</table>
Leapfrog technology

New Metagenomic Sequencing Protocol for the Nanopore

MinION (Oxford Nanopore Technologies)

(Greninger, et al., 2015, *Genome Medicine* 7:99)
Undifferentiated fever in sub-Saharan Africa: Precision “omics” diagnostics

Collaboration with Charles Chiu, UCSF
Clinical and public health impact of Diagnostics

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