Developing a point-of-care multiplexed diagnostic system for low-resource settings in developing countries

Challenges in target marker selection and evaluation

Project Objectives

- To develop a POC multiplexed diagnostic platform for the detection of pathogens in clinical specimens in low-resource settings in developing countries.

The DxBox platform is being developed by Micronics as part of a public-private consortium lead by Paul Yager, Department of Bioengineering, University of Washington.

Infectious disease and marker selection

- Fever symptoms
- Dengue
- Typhoid IgM DNA
- Rickettsia IgM DNA
- Measles IgM RNA
- Influenza None RNA

Study Design

- Age range: 5-10 years old
- Fever status: 38 °C or higher
- No recent history of antibiotics
- Outpatient setting

Total number recruited: 197 Children

Average Patient Profile (n=197)

<table>
<thead>
<tr>
<th>Average age</th>
<th>7.01 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg temperature</td>
<td>38.8 °C</td>
</tr>
<tr>
<td>Avg days-of-fever</td>
<td>2.69 days</td>
</tr>
<tr>
<td>Avg Weight</td>
<td>22.2 kg</td>
</tr>
<tr>
<td>Percent Male/Female</td>
<td>54% / 46%</td>
</tr>
<tr>
<td>Measles Vaccination Coverage</td>
<td>97%</td>
</tr>
<tr>
<td>Malaria positive by blood smear microscopy</td>
<td>67</td>
</tr>
</tbody>
</table>

Summary of IgM and antigen (for malaria) detection/immunoassay results

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number Positive</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>63</td>
<td>31.5%</td>
</tr>
<tr>
<td>Dengue</td>
<td>20</td>
<td>10.0%</td>
</tr>
<tr>
<td>Typhoid</td>
<td>30</td>
<td>15.0%</td>
</tr>
<tr>
<td>Measles</td>
<td>3</td>
<td>3.5%</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>22</td>
<td>11.0%</td>
</tr>
<tr>
<td>Influenza</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Summary of PCR results

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number Positive</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>89</td>
<td>45.2</td>
</tr>
<tr>
<td>Dengue</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Typhoid</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Measles</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Influenza (in nasal swabs)</td>
<td>20</td>
<td>10.1</td>
</tr>
</tbody>
</table>

Number of specimens positive for one or more pathogens by immunoassays

- Specimens Positive for 1 Pathogen: 83
- Specimens Positive for 2 Pathogens: 17
- Specimens Positive for 3 Pathogens: 1
- Specimens Positive for 4 Pathogens: 1

Results

Diagnosis of malaria by blood smear microscopy, PCR and HRP2 detection. The data is sorted by Parasitemia. Matched PCR and HRP2 positives are shown.

Conclusions

PCR showed superior sensitivity for identification of malaria infections at low parasitemia densities.

- Immunossays identified other pathogens on the panel PCR.
- Immunossay results should not be interpreted as confirmatory of each other but rather as complementary.
- Immunossay results may need to be confirmed to increase result specificity.
- The impact of combining PCR with antigen detection or immune response detection on the sensitivity or specificity needs to be determined on an individual disease basis.

Prospective studies to evaluate multiplex platforms for infectious diseases will be complex, requiring multiple levels of specimen pedigree definition.
Developing a POC multiplexed diagnostic system for low resource settings in developing countries: Challenges in target marker selection and evaluation

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Emerging technologies such as lab-on-a-chip and micro-array-based platforms allow the simultaneous detection of multiple disease markers from a single specimen. This offers the promise of greater capability to correctly diagnose and treat clinical syndromes such as infectious disease-related fever in the developing world. Selection of the appropriate markers for the intended syndrome and evaluation of their performance in the target populations is particularly challenging in low resource settings since laboratory-based etiology data of common diseases may not exist.

For infectious diseases the most common markers for infection are pathogen-specific RNA/DNA, antigen or IgM and IgG antibody response. In this study, a panel of protein and nucleic acid markers for infectious diseases that were perceived to cause acute febrile illness (AFI) in children presenting to outpatient clinics in Kisumu, western Kenya were selected. In this malaria holoendemic region, children with AFI would normally be treated presumptively for malaria. Our data illustrate the likely challenges to be encountered when evaluating a panel of infectious disease markers for a multiplex diagnostic platform in low resource settings. We also discuss the potential complexities of interpreting the results from such a platform.