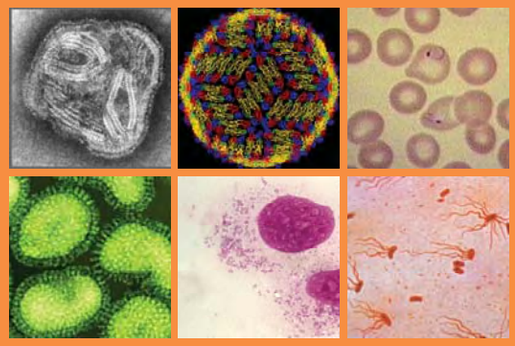
Developing a point-of-care multiplexed diagnostic system for low-resource settings in developing countries

Challenges in target marker selection and evaluation



PATH
A catalyst for global health

Gerlach[1], JL, Anyona[2], SB, Koros[2], J, Waitumbi[2], JN, Polhemus[2], ME, Watts[1], K, Singhal[1], M, Crudder[1], C, Steele[1], M, and Domingo[1], GJ

[1] PATH, Seattle, WA, USA

[2] WRAIR/KEMRI, Kisumu, Kenya

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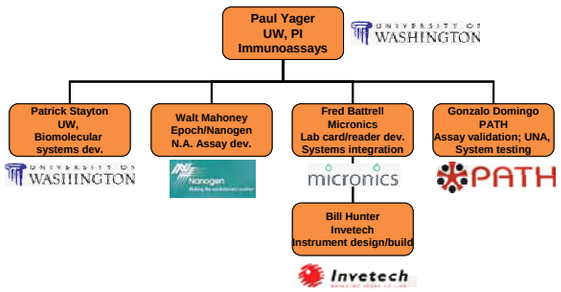
Project Objectives

- 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.



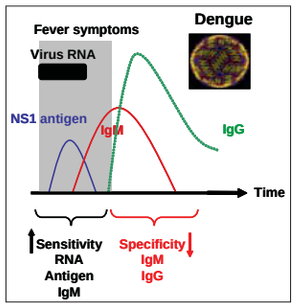
Purpose of prospective clinical study

What are the infectious disease causes of acute fever in a pediatric population in a malaria-holoendemic area?

To simulate the potential output of a diagnostic platform with capabilities to detect multiple pathogen-specific markers for multiple diseases.

Disease-specific markers for multiplex diagnostic platform		
	Immunoassay	Real-Time PCR
Malaria	Antigen	DNA
Typhoid	IgM	DNA
Rickettsia	IgM	DNA
Dengue Fever	IgM	RNA
Measles	IgM	RNA
Influenza	None	RNA

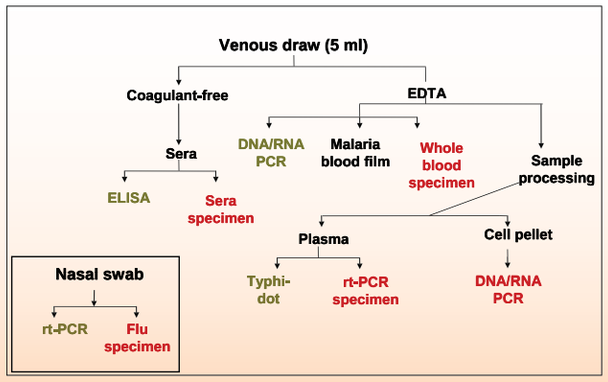
Infectious disease and marker selection



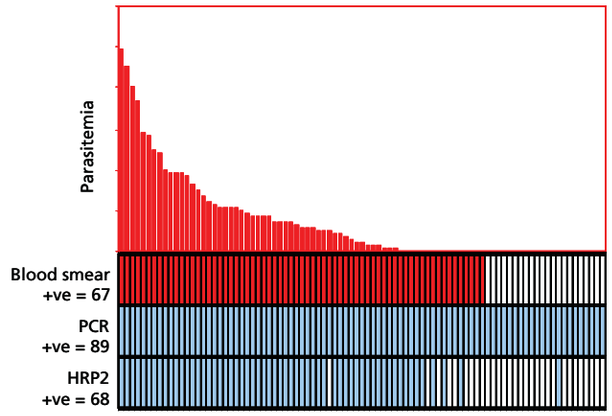
Study Design

Study Inclusion criteria:
Age range: 5-10 years old
Febrile status: 38 °C or higher
No recent history of antibiotics
Outpatient setting
Total number recruited: 197 Children

Average Patient Profile (n=197)	
Avg age	7.01 years
Avg temperature	38.8 °C
Avg days-of-fever	2.69 days
Avg Weight	22.2 kg
Percent Male/Female	54% / 46%
Measles Vaccination Coverage	97%
Malaria positive by blood smear microscopy	67



Results



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

Summary of IgM and antigen (for malaria) detection immunoassay results		
n = 197	Number Positive	Percent Positive
Malaria	63	31.5%
Dengue	20	10.0%
Typhoid	30	15.0%
Measles	3	1.5%
Rickettsia	22	11.0%
Influenza	N/A	N/A

Summary of PCR results		
n = 197	Number Positive	Percent Positive
Malaria	89	45.2
Dengue	0	0
Typhi	0	0
Measles	0	0
Rickettsia	0	0
Influenza (in Nasal swabs)	20	10.1

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

Conclusions

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

Immunoassays identified other pathogens on the panel PCR.

Immunoassay results should not be interpreted as confirmatory of each other but rather as complementary.

Immunoassay 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.

This project is funded by the Bill & Melinda Gates Foundation under its Grand Challenges in Global Health Program

Developing a POC multiplexed diagnostic system for low resource settings in developing countries: Challenges in target marker selection and evaluation

Gerlach[1], JL, Anyona[2] SB, Koros[2], J, Waitumbi[2], JN, Polhemus[2], ME, Watts[1], K, Singhal[1], M, Crudder[1], C, Steele[1], M, and Domingo[1]*, GJ.

1. PATH, Seattle, Washington, USA
2. WRAIR/KEMRI, Kisumu ,Kenya

* gdomingo@path.org

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.