Detection and Characterization of Acute Febrile Illness in Western Kenya

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Aims of Study

To collect and characterize specimens from febrile patients who were suspected to have malaria in Kisumu, Kenya.

To pedigree specimens against a panel of nucleic acid and serological markers.

To draw early insights into data that would be generated by a multiplex diagnostic platform.

Pathogen Marker Selection

Figure 1: Impact of markers on sensitivity and specificity in diagnosis for acute fever

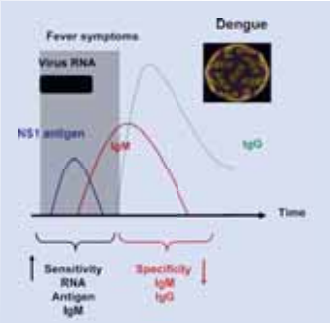

Table 1. Selection of tests on study specimens

	Immunoassay	Real-Time PCR
Malaria	Antigen	DNA/RNA
Typhoid	IgM	DNA/RNA
Rickettsia	IgM/IgG	DNA/RNA
Dengue fever	IgM/IgG	RNA
Measles	IgM	RNA
Influenza	None	RNA

Table 2. Markers detected

Pathogen Group	Antigen*	Gene Target**
Malaria	HRP2 detection	Ribosomal RNA gene
Typhoid	OMP50 (indirect IgM)	Ribosomal RNA gene
Spotted fever	Cell lysate (indirect IgM)	Ribosomal RNA gene
Dengue	Envelope antigen (MAC)	3'-UTR
Measles	Enriched virus (MAC)	Nucleoprotein (N) gene
Influenza	N/A	Matrix gene

*Antigens for antigen detection (malaria), IgM detection (all others)
**Gene targets for PCR detection.

Study Design

Study location: Kisumu, Kenya

Study inclusion criteria:

- Age range: 5 to 10 years
- Febrile status: 38°C or above
- Fever onset within the last 4 days
- No antibiotic use
- Outpatient setting

Total number recruited: 197

Gold standard test:

- Malaria blood smear

Table 3. Average patient profile (n=197)

Average age	7 years
Average temperature	38.8°C
Average days of fever	2.7 days
Average weight	22.2 kg
Percent male/female	54%/46%
Measles vaccination coverage	97%
Malaria positive by blood smear microscopy	67

Results

Figure 2: Test results for malaria diagnosis - comparison of parasitemia as determined by blood smear microscopy to PCR and *Plasmodium falciparum*-specific antigen (HRP2) detection

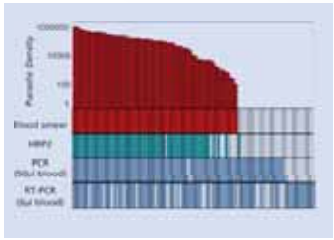

Figure 3: Comparison of IgM, IgG and RNA assay results for dengue virus

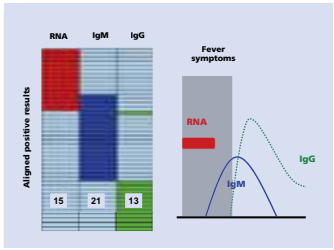

Figure 4: Comparison of IgM, IgG, and DNA assay results for rickettsia

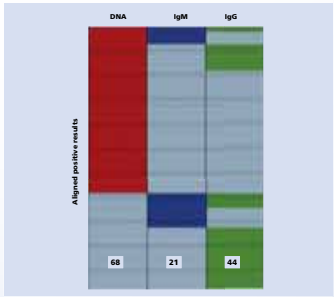

Table 4. Specimens testing positive for multiple pathogens represent a variety of pathogen combinations

Pathogen Combination	Detected by DNA/RNA* assay	Detected by IgM assay
Mal Typ Den Ric	1	
Mal Typ Ric	5	
Mal Den Ric	1	
Typ Den Ric		2
Mal Ric	24	4
Mal Typ	12	
Mal Mea		1
Typ Ric	8	5
Typ Flu	1	
Typ Den		3
Typ Mea		1
Mal Den	7	5
Ric Den	3	
Ric Flu	1	
Mal	41	48
Den	3	11
Ric	24	11
Typ	8	10
Mea	0	0

*DNA was used as template for malaria (Mal), rickettsia (Ric), and S typhi (Typ). RNA was used for dengue (Den), measles (Mea), and influenza (Flu).

Conclusions

Over 80% of specimens tested positive in at least one of the pathogen assays, and many specimens tested positive for two or more pathogens.

A surprising number of specimens were positive or cross-reactive in assays for rickettsia and dengue virus, pathogens generally not diagnosed in the region where the study was performed.

Prospective studies to evaluate multiplex platforms for infectious diseases will be complex, requiring multiple levels of specimen pedigree definition.

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