FIND

Because diagnosis matters

Zika Diagnostics Product Development Landscape and Needs

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Acknowledgments

Arlene Chua (WHO)

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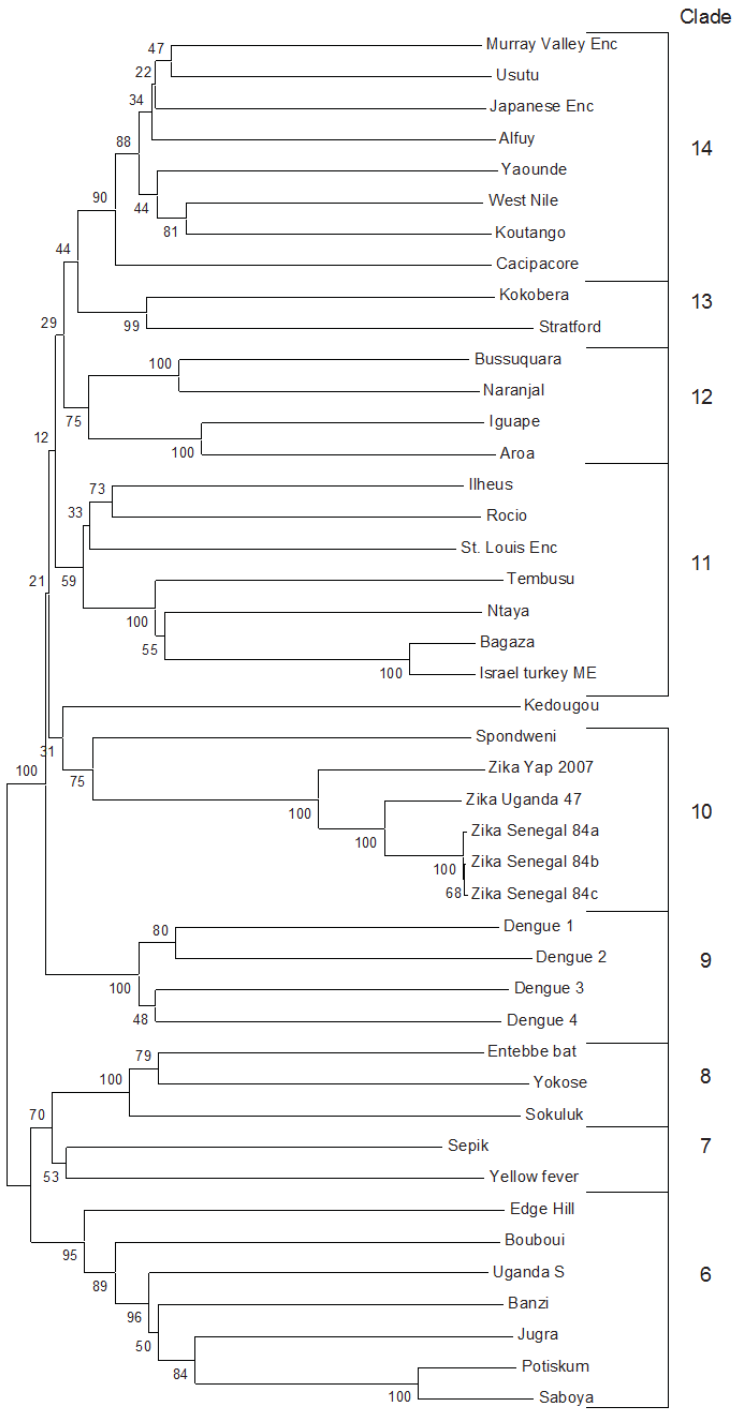

Figure 1. Phylogenetic tree constructed from nucleic acid data from

Source: Lanciotti *et al*, *Emerg Infect Dis* 2008; 8:1232-9

Detection of Zika Virus in Urine

Ann-Claire Gourinat,[1] Olivia O'Connor,[1] Elodie Calvez, Cyrille Goarant, and Myrielle Dupont-Rouzeyrol

We describe the kinetics of Zika virus (ZIKV) detection in serum and urine samples of 6 patients. Urine samples were positive for ZIKV >10 days after onset of disease, which was a notably longer period than for serum samples. This finding supports the conclusion that urine samples are useful for diagnosis of ZIKV infections.

Zika virus (ZIKV) is an emerging mosquito-borne pathogen (family *Flaviviridae*, genus *Flavivirus*) that was isolated in 1947 from a rhesus monkey in the Zika forest in Uganda (*1*). ZIKV is believed to be transmitted to humans by infected *Aedes* spp. mosquitoes (*2,3*). Studies have demonstrated that ZIKV is endemic to Africa and Southeast Asia (*4*). Before 2007, few cases of human infection with ZIKV had been reported. In 2007, an epidemic of ZIKV infection in humans occurred in Yap, Federated States of Micronesia, in the Pacific region. A seroprevalence survey determined that ≤70% of the population had been infected (*5*). During 2007–2013, the few cases of infection with ZIKV reported were in travelers returning from Africa (*6*) or Southeast Asia (*7*).

In humans, ZIKV infection is characterized by mild fever (37.8°C–38.5°C); arthralgia, notably of small joints of hands and feet; myalgia, headache; retroorbital pain; conjunctivitis; and cutaneous maculopapular rash. ZIKV infection is believed to be asymptomatic or mildly symptomatic in most cases (*5*). Thus, Zika can be misdiagnosed during the acute (viremic) phase because of nonspecific influenza-like signs and symptoms. Hemorrhagic signs have not been reported in ZIKV-infected patients (*5*–*7*). However neurologic complications, including Guillain-Barré syndrome, have been observed (*8*).

Biological confirmation of ZIKV infections is based mostly on detection of virus RNA in serum by using reverse transcription PCR (RT-PCR). Although IgM against ZIKV can be detected by ELISA, few laboratories have this ability. Thus, in addition to the nonspecific clinical features of infection with ZIKV, laboratory diagnosis is challenging because of low viremia and cross-reactivity of ZIKV antibodies with other flaviviruses (including dengue), which require confirmation by neutralization assays (*8*) and make rapid serologic confirmation difficult. We investigated the diagnostic utility of urine as a source for detection of ZIKV RNA by real-time RT-PCR.

The Study

In October 2013, a ZIKV outbreak was reported in French Polynesia (*9*). This was the second outbreak of ZIKV infection reported in the Pacific region. In New Caledonia, where ZIKV infection had never been documented, the first cases of ZIKV infection imported from French Polynesia were confirmed by the end of November, and the first autochthonous cases were reported by mid-January 2014. Early in February 2014, the New Caledonia Health Authority declared an outbreak situation. By the end of August 2014, >1,400 cases of ZIKV infection were biologically confirmed, including 34 cases imported from French Polynesia (*10*).

Written informed consent was obtained from all patients in this study. Clinical signs and symptoms of 6 ZIKV-infected patients are shown in the Table. In this study, a cutaneous maculopapular rash of the trunk and extremities was systematically observed and considered a relevant clinical criterion. Complete blood counts showed a discreet perturbation common in many viral infections (mild leukopenia and thrombocytopenia associated with activated lymphocytes).

To detect ZIKV in samples (RNA extracted from 200 μL of serum or urine), we used both sets of primers/probe specific for ZIKV (*11*). A standard curve with serial dilutions of known concentrations of a ZIKV virus stock was used to estimate viral load in samples. All blood samples were also tested for dengue virus and chikungunya virus by real-time RT-PCR and showed negative results. ZIKV was detected in serum of 4 patients (Figure). Urine samples from 2 other patients were also positive for ZIKV, and showed a higher viral load than corresponding serum samples and were positive for ≤7 days (patient 4) and probably >20 days (patient 3) after viremia reached an undetectable level (Figure). Urine samples from 6 healthy patients were also assessed and showed negative results.

Partial sequences of the gene for ZIKV nonstructural protein were obtained (*12*) directly from amplification products from urine or serum samples. Sequences obtained (GenBank accession nos. KJ873160 and KJ873161) had 100% identity with the sequence of a ZIKV strain isolated from a patient who returned from French Polynesia in 2013. As observed previously (*9,13*), sequences also had 98% identity with sequences of ZIKV strains isolated in Cambodia in 2010 and in Yap in 2007.

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Source: Gourinat *et al*, *Emerg Infect Dis* 2015; 21:84-86

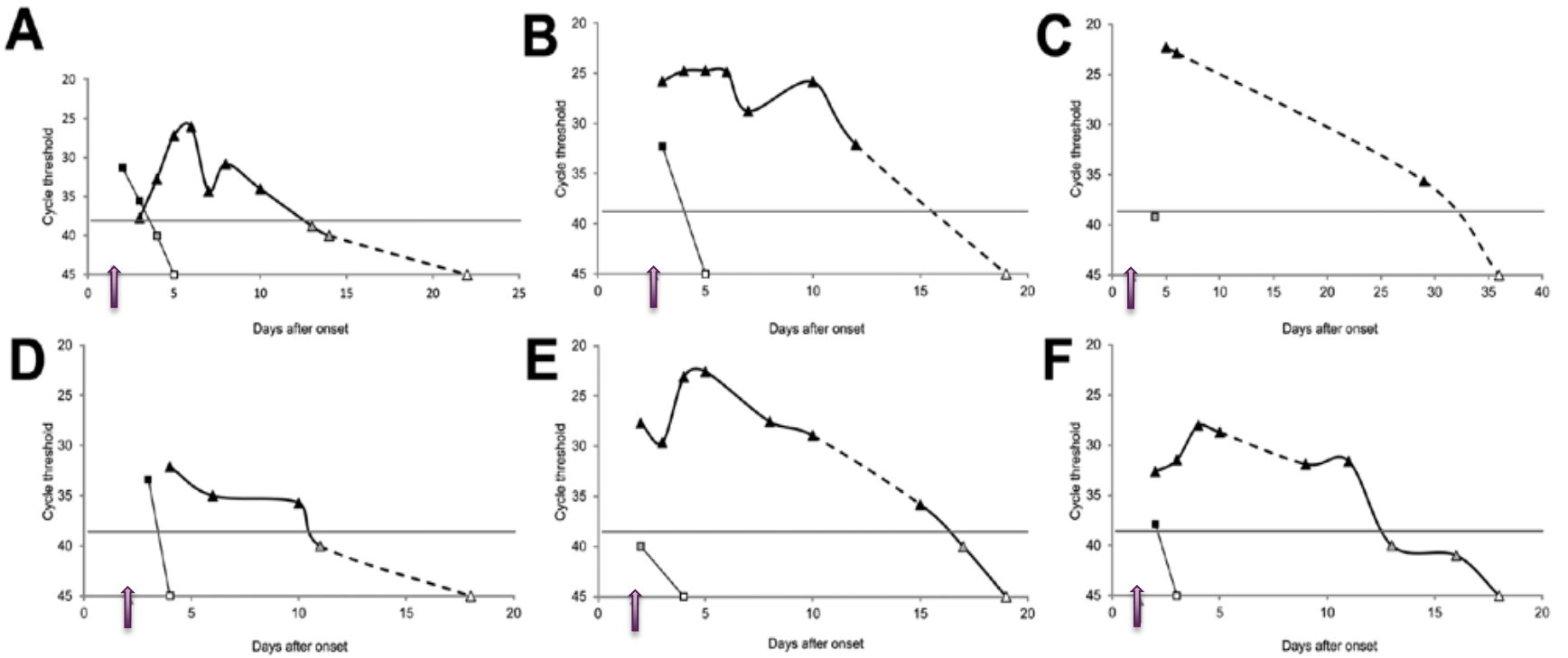

Figure. Detection of Zika virus in blood and urine specimens of 6 patients by using real-time reverse transcription PCR with primers/probe 1086/1162c/1107-Cy5 (*11*) New Caledonia, 2014. A) Patient 1; B) Patient 2; C) Patient 3; D) Patient 4; E) Patient 5; F) Patient 6. Triangles indicate urine samples and squares indicate serum samples. The cutoff cycle threshold (C_t) value is 38.5, as previously reported (*11*) and is indicated by horizontal lines. Black symbols indicate amplifications with C_t <38.5, gray symbols indicate amplifications with C_t ≥38.5, and white symbols indicate negative amplifications. Onset of disease (day 0) was defined retrospectively after questioning patients about initial symptoms. Dashed lines indicate a period >2 days without a sample being obtained. Arrows indicate onset of rash.

Source: Gourinat *et al*, *Emerg Infect Dis* 2015; 21:84-86

Lessons from Ebola

- Many companies have diagnostic technologies in late-stage development or in limited commercial use for nucleic acid, IgG/IgM, and antigen detection, and have a stated willingness to develop a high-priority test

Lessons from Ebola

Explosion in near-patient molecular platforms

PositiveID


STATDiagnostica


Great Basin


Cepheid


BioCartis


IdahoTechnologies


BioFire


Alere


Veredus


Epistem


iCubate


Osmetech


Curetis


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Enigma


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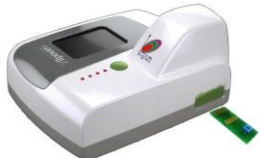
Molbio


Rheonix


QuantumDx


Spartan


Fluidigm


Qiagen


Micronics

Lessons from Ebola

- Many companies have diagnostic technologies in late-stage development or in limited commercial use for nucleic acid, IgG/IgM, and antigen detection, and have a stated willingness to develop a high-priority test

  - More than **70 companies submitted product development plans** for a near-patient Ebola diagnostic test during the course of the epidemic

  - After 2 years, only **6 companies have Ebola test that received Emergency Use Authorization** (EUA) from US FDA or the equivalent designation from WHO Diagnostic Pre-Qualification (plus tests from US CDC and DoD)

  - **No diagnostic products have received regulatory clearance for Ebola through FDA or WHO PQ non-emergency mechanisms**

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Lessons from Ebola

- It is difficult to do diagnostic product development in the middle of an outbreak
  - Clinical and public health needs and TPP specifications have not been established
  - Companies have almost no ability to make realistic risk or reward assessments
  - Regulatory pathways are not established
  - Reference standard assays, reference standard reagents do not exist
  - Clinical specimens become an arena for combat rather than collaboration

Lessons from Ebola

- Outbreaks are not sustainable markets for single-pathogen diagnostic tests
  - **Product development takes time, and outbreaks end** or even worse, lose salience
  - Emergency needs are not identical to long-term clinical, public health, and research needs

Lessons from Ebola

- Data on diagnostic test performance are hard to come by

  - Most companies **lack access to well-characterized samples during the product development phase**

  - For competitive and other reasons, for new products company **data are closely held**

  - Emergency Use Authorizations necessarily allow for **limited product validation studies** in clinical samples (and spiked samples)

Landscape of Zika Diagnostic Tests

Zika only tests

Flavivirus panel test (CHK/DEN/ZIK)

Multi-pathogen fever panel tests (malaria, measles, typhoid, Ebola, Marburg, Lassa...)

- Molecular platforms

- Molecular reagent kits (RT-PCR)

- ELISA
  - IgM/IgG

- Rapid diagnostic tests (RDTs)
  - NS1 Antigen
  - IgG/IgM

Landscape of Zika Diagnostic Tests

Zika only tests

Flavivirus panel test (CHK/DEN/ZIK)

Multi-pathogen fever panel tests (malaria, measles, typhoid, Ebola, Marburg, Lassa...)

Molecular platforms

At least 10 companies with molecular platforms on the market or in late-stage product development (central laboratory or near-patient) have expressed either interest or active product development programs for Zika RNA tests

Landscape of Zika Diagnostic Tests

Zika only tests

Flavivirus panel test (CHK/DEN/ZIK)

Multi-pathogen fever panel tests (malaria, measles, typhoid, Ebola, Marburg, Lassa...)

Molecular reagent kits (RT-PCR)

At least 8 companies have commercially available Zika RT-PCR kits

Multiple additional kits are in development, including multiplex flavivirus kits

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Landscape of Zika Diagnostic Tests

Zika only tests

Flavivirus panel test (CHK/DEN/ZIK)

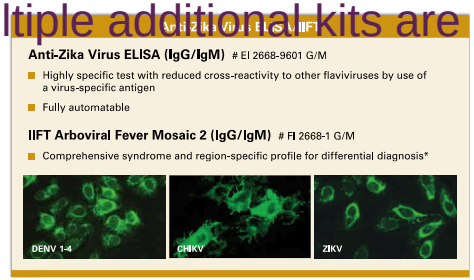
Multi-pathogen fever panel tests (malaria, measles, typhoid, Ebola, Marburg, Lassa...)

ELISA

Two ELISA kits have received regulatory clearance in either the US or Brazil

(US FDA : CDC Zika-MAC ELISA, and Brazil ANVISA: Euroimmune)

Multiple additional kits are in development



Landscape of Zika Diagnostic Tests

Zika only tests

Flavivirus panel test (CHK/DEN/ZIK)

Multi-pathogen fever panel tests (malaria, measles, typhoid, Ebola, Marburg, Lassa...)

Rapid Diagnostic Tests

Two RDTs have received regulatory clearance in either the US or Brazil

(Brazil ANVISA: Biocan and Quibasa)

Multiple additional kits are in development, especially pan-Flavirus tests, and multi-pathogen fever panel tests

Zika: Diagnostic priorities and gaps

Clinical Zika Diagnostics

- Clinically actionable tests? Recovery rate by RT-PCR from people presenting with "classic" Zika symptoms in Brazil and Colombia ~20%.
- Pregnancy risk classification
- Febrile syndromes

Public health/surveillance need

Scientific needs

- **We do not yet understand the virus or the current outbreak, and need qualified diagnostic tests, especially serologic tests, to support:**

Scientific understanding → Clinical implications → Public health implications

Diagnostic priorities and gaps

- Serologic tests (IgG/IgM, including combination tests)
  - Mapping and natural history studies
  - Prevalence studies and immunity in populations
  - Protective effect of immunity?

- Molecular kits
  - Natural history studies
  - Reference standards, for serologies during product development

Diagnostic priorities and gaps

- Product prioritization
- Performance specifications – how good is good enough?
  - Analytic LOD
  - Clinical sensitivity and specificity
  - Cross-reactivity between flaviviruses (especially dengue)
- Guidance on usage
- Accelerated approval

Diagnostic priorities and gaps

Sample Repository

- Highly characterized, curated, accessible samples enriched for Zika
  - Febrile patients
  - Pregnant women (?)

- Critical for companies during **product development**, as most lack access to Zika-positive samples for preliminary testing, and will enter validation studies for regulatory purposes having evaluated tests in <20 samples

- Essential for **validation studies**, to establish analytical and clinical test performance characteristics

Thank you!