Point of Care Tests for Diagnosis of Dengue Infection

Bangkok, Thailand
November - December, 2007

Milton R. Tam, Ph.D.
Diagnostics Consultant
Pediatric Dengue Vaccine Initiative
Definition - Point of Care Test

“An analytical test undertaken by a member of the health care team or by a non-medical individual in a setting distinct from a normal hospital laboratory.”  R. Cramb – Royal Coll. Pathologists

- Simple and rapid tests
- Small hand-held analyzers
- Larger desktop or portable analyzers
Diagnostic testing trends - the haves vs. the have-nots

- **Resource-rich**
  - Developed countries
  - Remote testing
  - In central hospital or commercial labs
  - Centralized, automated equipment
  - High-volume, complex test methods
  - Near-patient (POC) testing with simple or sophisticated devices

- **Resource-limited**
  - Developing countries
  - Near-patient (POC), rapid testing
  - In health clinics with no or limited lab
  - Decentralized, manual, few supplies and simple equipment
  - Low-volume, simple tests
  - Replaces syndromic diagnosis

Adapted from H. Lee, 2006
# POC Test Applications

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urgent treatment</td>
<td>Life-threatening illness</td>
</tr>
<tr>
<td>Starting treatment</td>
<td>At-risk patients</td>
</tr>
<tr>
<td>Containment</td>
<td>Undefined outbreaks</td>
</tr>
<tr>
<td>Failure to return</td>
<td>Patients lost to follow-up</td>
</tr>
</tbody>
</table>
Why POC testing? “The diagnostic paradox”

- **Example 1:** RT-PCR (~1 – 2 weeks)
  - Obtain sample → ship to lab → refrigerate → batch + run test → get result → send result back to physician → notify patient → patient returns to clinic → treat. Test sensitivity 90% x Patient return 70% = 63% treated

- **Example 2:** Rapid POC test (~1 hour)
  - Obtain sample → test on-site → get result in <1 hr → treat. Test sensitivity 70% x Patient return 100% = 70% treated

Adapted from Gift et al 1999.
“ASSURED” – Characteristics of ideal Dx tests for the developing world

- **A**ffordable by those at risk of infection
- **S**ensitive (few false negatives)
- **S**pecific (few false positives)
- **U**ser-friendly (simple to perform)
- **R**apid treatment/ **R**obust
- **E**quipment-free (no large instruments)
- **D**elivered, available to those who need it

D. Mabey et al., 2004
Ideal POC tests are:

- Rapid, replace waiting for lab results
- Validated in clinical trials
- Demonstrated to improve clinical outcome
  - Accurate, differential diagnosis
  - Rapid, appropriate treatment immediate
- Cost-effective in treating patients
- Used by wide range of trained personnel
- Part of a total quality system
Test needs for Dengue vaccine and control programs

- Accurate, simple, rapid tests at POC
- Early detection of primary infection
- Distinguish primary vs. secondary infection
- Determine subtypes 1 – 4
- Test for protective antibodies
- Prediction of long-term “immune memory” to immunization
“Typical” Dengue infection course

Primary infection

Secondary infection

Antibody levels

NS1

virus

IgM

IgG

Testing period

From: Paul Young-WHO/TDR/PDVI Workshop on Dengue Diagnostics Oct 2004
Opportunities to detect primary Dengue infection

- **Early detection** - viral antigen or RNA
- **Serology** – IgM antibody $>5$ days or 4-fold rise in titer
- **Culture** – Virus isolation
Our current Dengue diagnostic “toolbox”

- Virus isolation
- RT- PCR or NASBA
- Plaque reduction neutralization
- Hemagglutination inhibition
- IgM, IgG, NS1 ELISA
- Simple/rapid IgM, IgG tests
  - particle agglutination
  - solid-phase “dipsticks”
  - lateral flow tests

Technically complex - reference laboratory-based

Relatively simple, clinical laboratory-based and/or potentially point-of-care
All current methods have deficiencies for use at POC

<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th>PRN/HI</th>
<th>NAAT</th>
<th>ELISA</th>
<th>Rapid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td><strong>S</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-?</td>
</tr>
<tr>
<td><strong>S’</strong></td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+/-?</td>
</tr>
<tr>
<td><strong>U</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>R’</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>E’</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>D’</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
</tr>
</tbody>
</table>

(Affordable, Sensitive, Specific, User-friendly, Rapid/robust, Equipment-free, Delivered)
Some Rapid Point-of-Care Tests

Early results were promising!
Solid-phase “dipsticks”

ELISA on a stick
Multi-step
Multi-analyte

PanBio
(Orgenics also)
Pentax particle agglutination test

- Simple methodology
- Colored hydroxyapatite beads
- Microwell Agglutination test
- Test takes ~60 minutes

Pentax, Tokyo, Japan
Lateral flow (LF) test
(immunochromatographic strips)

- Simple, “Walk-away” test
- IgM and/or IgG
- Results in 5 – 10 minutes
- Initial results were promising
PanBio IgM/IgG rapid LF test

Primary and secondary infections may be recognized by interpretation of results.

From PanBio’s web site
But sensitivity and/or specificity can be suboptimal

- For example, sensitivity limits of HBsAg tests:
  - ELISA: 0.1 – 0.2 ng/ml
  - Rapid LF test: 1 – 2 ng/ml

- Antigens or antibodies may not be dengue-specific and/or nonspecific or low-affinity antibody can lower specificity
Comparative evaluation of commercial rapid IgM LF tests

- Blacksell et al., 2006, Lab evaluation
- Blacksell et al., 2007, Prospective evaluation in Laos
- PDVI-WHO/TDR Dengue serum panel
Rapid IgM LF tests fall short of manufacturers’ claims

<table>
<thead>
<tr>
<th>Company</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>22.9</td>
<td>98.8</td>
</tr>
<tr>
<td>Diazyme</td>
<td>17.8</td>
<td>98.2</td>
</tr>
<tr>
<td>Globalemed</td>
<td>62.9</td>
<td>69.1</td>
</tr>
<tr>
<td>Minerva</td>
<td>8.6</td>
<td>100</td>
</tr>
<tr>
<td>PanBio</td>
<td>65.3</td>
<td>97.6</td>
</tr>
<tr>
<td>Standard</td>
<td>21.8</td>
<td>98.8</td>
</tr>
<tr>
<td>Teco</td>
<td>9.5</td>
<td>97.0</td>
</tr>
<tr>
<td>Tulip</td>
<td>2.9</td>
<td>96.3</td>
</tr>
</tbody>
</table>

S. Blacksell et al, 2006. Laboratory evaluation of 491 positive, 491 negative sera
Rapid IgM LF tests fall short of manufacturers’ claims

<table>
<thead>
<tr>
<th>Company</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>13.0</td>
<td>98.8</td>
</tr>
<tr>
<td>Diazyme</td>
<td>5.8</td>
<td>98.8</td>
</tr>
<tr>
<td>Globalemed</td>
<td>33.3</td>
<td>74.4</td>
</tr>
<tr>
<td>Minerva</td>
<td>8.6</td>
<td>93.9</td>
</tr>
<tr>
<td>PanBio</td>
<td>21.7</td>
<td>96.3</td>
</tr>
<tr>
<td>Standard</td>
<td>10.2</td>
<td>96.3</td>
</tr>
<tr>
<td>Teco</td>
<td>17.4</td>
<td>97.0</td>
</tr>
<tr>
<td>Tulip</td>
<td>6.4</td>
<td>99.4</td>
</tr>
</tbody>
</table>

S. Blacksell et al, 2007. 151 positive, 151 negative specimens
From prospective study in Laos
WHO/TDR – PVDI Dengue IgM ELISA and rapid test evaluation

Averaged results from 7 test sites
Rapid Dengue IgM LF tests

- Many commercial tests available
- All tested fell short of manufacturers’ claims
- All need higher sensitivity
- Sensitivity lower during “window” period of infection
- Specificity could be better?
- Not useful for secondary infections
Why do Dengue IgM LF tests fall short of claims?

- No IVD regulation in majority of developing countries
- No manufacturing and QC standards
- Stability of many tests uncertain
- Clinical evaluation, data processing standards lacking
- Few validations of manufacturers’ claims
- Reference serum panels lacking
NS1 Antigen Detection – Earlier Diagnosis of Primary Infection?
“Typical” dengue infection

Primary infection

Secondary infection

Antibody levels

Testing period

NS1

IgG

IgM

Days

Fever onset

Fever onset

From: Paul Young-WHO/TDR/PDVI Workshop on Dengue Diagnostics Oct 2004
Dengue NS1 Tests

- Detection of NS1 antigen in “window” period
- Can be positive when IgM or NAAT are negative
- Commercial ELISA include:
  - Platelia (BioRad), pan-E Dengue Early ELISA (PanBio)
- Rapid NS1 LF test – in development
  - Publications?
  - Sensitivity, specificity?
- Serotype-specific anti-NS1 antibody test?
Improvements to Current POCT

- **Sample preparation** – whole blood, dried blood spots (?), oral fluids (?), immune complexes (?)
- **Sensitivity** – higher affinity antibodies, new Mabs, aptamers, mimitopes, multi-antigenic recombinant antigens
- **Specificity** – neutralize cross-reactive Abs, use more specific antibodies, antigens
- **Materials** – Higher quality, consistency
Improvements to Current POCT

- **Manufacturing** – better protocols, QA/QC, packaging, stability, consistency
- **Better signal reagents** – fluorescent beads, paramagnetic beads, LF-EIA
- **Hand-held readers** – small, battery-powered devices, allow quantitative readout
- **Detection of PCR products by LF**
- **Combination tests** – IgM + NS1
POC tests in development
Improving Lateral Flow Tests

- Higher sensitivity and specificity
- Individual tests for serotypes 1-4?
- Tests for other flaviviruses?
- Tests for neutralizing or protective antibody?
- Semi-quantitative assays
Improving Lateral Flow Tests
Diagnostics for the Real World

### Concentration of Chlamydial LPS (picograms)

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>420</th>
<th>125</th>
<th>40</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal signal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signal amplified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Improved antigen concentration device and “enhanced” lateral flow Test for Chlamydia – Helen Lee, Cambridge University
Biosensor tests?

Many versions currently In development
What biosensors can do:

- Integrate specimen processing, assay, read-out steps
- Bring NAAT and immunoassays to the POC
- Reduce sample and reagent volumes, and costs
- Reduce risk of contamination
- Contain waste on disposable card
- Reduce assay time
PATH’s “Lab on a Chip” Platform

BMGF project with UW, PATH, Micronics on Fevers Panel including dengue. From B. Weigl, PATH
UC Berkeley Magnetic Bead Sensor

Magnetic Detection

- The magnetic bead acts as highly specific bio/electro interface
- Hall sensor detects only immobilized beads
- The other beads are collected by the magnet
**Homogeneous Assays**

A paradigm shift for POCT?

From Hazell, 2006

Kinetic assay. Colored product develops from colorless enzyme substrate.
Other examples with Reflected light off colloidal Gold particles
Conclusion - No optimal POC test for dengue primary infection exists right now

- **Shorter term**
  - Improve IgM rapid test sensitivity, specificity
  - Develop, validate NS1 rapid tests
  - Combine improved IgM + NS1 tests?
Conclusion - No optimal POC test for dengue primary infection exists right now

- **Longer term**
  - Develop POC and lab-based tests for neutralizing antibody, vaccine vs. wild type immunity, subtyping, secondary infection, and DSS/DHF
  - Monitor, promote development of biosensors and new technologies such as homogeneous assays
What we need to do

- Continue to work with government labs, NGOs, and academia
- Engage and support industry:
  - Develop/sustain collaborations
  - Provide reagents, specimen panels
  - Assist product development
What we need to do

- Engage and support industry (continued):
  - IP assistance: patents and licensing
  - Continue to provide comparative lab and field evaluations
  - Promote end-user acceptance
  - Identify and/or create markets
Questions / Discussion