Laboratory Tests for Diagnosis of Dengue: Indications and Pitfalls

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From the clinicians' perspective

In a patient with suspected dengue infection…

– Which test should be ordered?

– What kind of specimens should be sent?

– When will the result be available?

– What does the result mean?

Before making a test request

- Take a good clinical history
  - Clinically compatible
  - Onset of symptoms

NO TEST IS PERFECT

- Clinical history helps
  - Choice of test
  - Interpretation of result

Diagnostic methodologies

1. Direct detection
   - Nucleic acid detection
   - Antigen detection
2. Virus isolation
3. Antibody detection

Antibody detection

- 2 type of tests
  - Anti-dengue IgM antibodies
    - Rapid test
    - ELISA

  - Anti-dengue antibody titre
    - Haemagglutination inhibition assay

Anti-dengue IgM test

- Indication of test
  - Commonly performed test for diagnosis of acute infection
  - Meaningful result on single serum

  What does a single IgM result mean?

Anti-dengue IgM test

- What does a positive anti-dengue IgM result mean?
  - Acute dengue infection
  - Recent dengue infection
  - False positive
    - Cross react with
      - Flavivirus- JE, YF, St. Louis encephalitis, WN virus
      - Non-flavivirus – malaria, leptospirosis, rickettsiosis, etc
      - Connective Tissue Disorder – RA, SLE

Anti-dengue IgM test

- What does a negative anti-dengue IgM result mean?
  - Dengue infection ruled out
  - False negative:
    - Serum taken soon after onset of symptoms
    - Low level of IgM in some secondary cases

Anti-dengue antibody titre

- Haemagglutination-inhibition assay (HAI)
  - Based on the RBC-agglutinating property of dengue virus and anti-dengue antibody's ability to inhibit this agglutination

  - Indication
    - Confirmation of acute dengue infection in cases with diagnostic challenges
    - Differentiation of primary and secondary infection

  - Requires paired samples for interpretation (acute and convalescent samples)

Anti-dengue antibody testing

– Problems:

- Retrospective diagnosis
- Result of single serum not confirmative
- Cross reactivity with other flavivirus infection

Diagnostic methodologies

1. Direct detection
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Nucleic Acid detection

- Reverse transcription- Polymerase Chain Reaction (RT-PCR)
  - Conventional vs Real-time
  - Surrogates the viremic phase
  - Advantages
    - Diagnosis of dengue in the early phase of infection
    - Determination of serotype (molecular epidemiology)
  - Disadvantages
    - High cost
    - Technical expertise

Nucleic Acid detection

- Indication of test
  - Adhoc tests - performed in cases with diagnostic challenges in the early phase of illness
  - Once monthly batch testing for serotyping - Molecular epidemiology

Dengue antigen detection

Dengue NS1 Antigen

- Non-structural protein 1 is a 46- to 50-kilodalton glycoprotein produced by dengue infected mammalian cells

- Function: believed to be involved in viral replication and is recognized as an important immunogen in infection

- 2 forms: expressed in both membrane associated and secreted forms

- The secreted form can be detected in serum

- Correlates well with viremia

Dengue antigen detection

- Methods
  - Rapid tests
  - ELISA tests

- Indication:
  - Diagnosis of acute infection when blood is taken at the early phase of disease (< 1 week)

Virus isolation

- Mosquito cell line: C6/36

- Advantage:
  - Most specific
  - Viability of virus
  - Virus isolate for other studies

- Problems:
  - Technically demanding
  - Long TAT (1-2 weeks)
  - Requires a second test (IFA/PCR) for confirmation of virus growth

- Early disease → direct detection methods
- Late disease → antibody detection methods

Probable	Confirmed	CDC
• Detection of IgM • Single serum sample with HAI titre ≥ 1280	• Virus isolation • Nucleic acid/ Antigen detection • 4 fold rise of HAI titre in paired sera	

To make it simple

- If you have a patient with suspected dengue infection:
  - Take a good history
  - Provide on the request form
    - Onset date
    - Presentation
    - Travel history
  - Order: dengue testing
  - Send: Clotted blood – 3ml
  - Results available : 1-2 days
  - Prepare to receive phone call for any abnormal result
  - Report to CENO when case is suspected

Thank you for your attention