RAPID DIAGNOSIS OF DENGUE OUTBREAKS IN RESOURCE LIMITED FACILITIES

Inam Danish Khan, AK Sahni; Command Hospital, Kolkata, India, titan_afmc@yahoo.com

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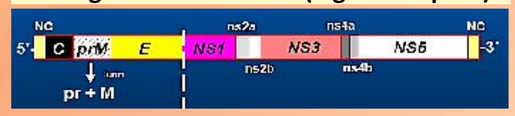
INTRODUCTION

- **Dengue**
  - Emerging public health problem
  - Threatens half of world population
  - Tropical developing world (Americas Africa, Asia-Pacific,, Mediterranean)
  - Regular outbreaks, High morbidity
  - Threat to travelers and military
  - Potential bioweapon
  - No anti-dengue therapy available
  - No licensed vaccines
- **Diagnostic challenges**
  - Endemic regions overwhelmed
  - Cocirculation of endemic viruses
  - JE, West Nile, Yellow F, Chikungunya
  - WHO criteria exacting in time/effort
    - Viral isolation
    - IgM/IgG four fold rising titres
    - Antigen detection by ELISA
    - Immunochemistry
    - Immunofluorescence
    - Nucleic acid amplification
- **Studying the gap in dengue diagnosis**
  - Rapid diagnostic methodology
  - Evaluated with RT-PCR (gold standard)

METHODS

- **100 dengue patients and 100 controls**
  - Random sampling, matched controls
  - Outbreaks of two consecutive years
  - Samples collected on fifth day
  - Immunochromatography, IgM capture ELISA and Reverse transcriptase PCR
  - Kappa (0–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, 0.81–1 perfect agreement)

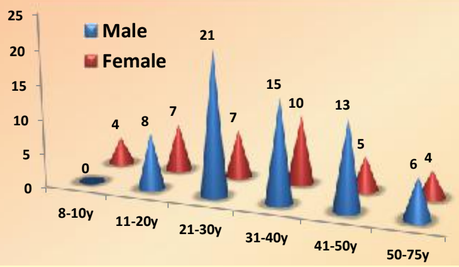
Dengue vector - *Aedes* (Tiger mosquito)



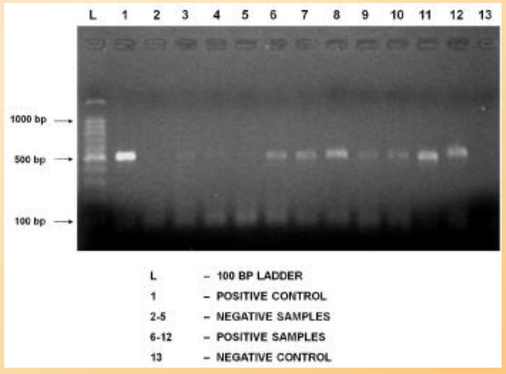
Dengue viral genome

RT-PCR protocol

RESULTS



Age and Sex distribution



RT-PCR for dengue (Amplicon at 511 bp)

Primer	DC-1S	DC-2C
Direction	Sense	Antisense
Nucleotide sequence (5'-3')	TTG-CAC-CAA-CAG-TAC-ATG-TCT-TCA-GGT-TC	TTG-CAC-CAA-CAG-TAC-ATG-TCT-TCA-GGT-TC
Primer length	28 mer	29 mer
Position in genome	D1:132-159,D2:134-161, D3:132-159,D4:136-163	D1:642-614,D2:644-616, D3:642-614,D4:646-618
Product (bp)	511	
Annealing	54°C	

Dengue primer sequences

Test	Positive	95% CI
100 dengue patients		
NS1 antigen (LF-ICT)	58 (58%)	48.33% – 67.67%
IgM by LF-ICT/ELISA	42 (42%)	32.33% – 51.67%
IgG by LF-ICT/ELISA	18 (18%)	10.47% – 25.53%
RT-PCR	65 (65%)	55.65% – 74.35%
IgM & IgG	18 (18%)	10.47% – 25.53%
RT-PCR and NS1	58 (58%)	48.33% – 67.67%
RT-PCR and IgM	7 (7%)	2% -12%
RT-PCR and IgG	3 (3%)	-0.34% – 6.34%
100 dengue controls – No tests were positive		

Results of serological and molecular tests

Antigen/ Antibody	Sensitivity (%)	Specificity (%)	PPV	NPV	Kappa score
NS1 (against RT-PCR)	98.3% (96.5% - 100.1%)	100%	100%	99.3% (98.1% - 100.5%)	0.99 Perfect
IgM (against RT-PCR)	10.8% (6.5% - 15.1%)	74% (68% - 80%)	16.7% (11.5% - 21.8%)	63.29% (56.6% - 69.9%)	-
IgG (against RT-PCR)	4.6% (1.7% - 7.5%)	88.9% (84.5% - 93.2%)	16.67% (11.5% - 21.8%)	65.93% (59.4% - 72.5%)	-

Diagnostic accuracy of serological & molecular tests

DISCUSSION

- **Non-structural antigen – 1 (NS1)**
  - 55 kDa highly conserved glycoprotein
  - Infected cells secrete in early infection
  - High sensitivity and specificity
  - Exhibits limited cross reactivity
  - Day 1 till Day 9 - Low false positive
  - Corroborates with high viral load
  - Low sensitivity - Low viral titres
    - Secondary dengue infection
    - Rising antibodies – Sequestered Ag
- **IgM Antibody**
  - Cross reactivity with flaviviruses
  - Low sensitivity of IgM against RT-PCR
- **RT-PCR (Gold Standard)**
  - Low sensitivity when antibodies appear
  - Exacting in time, expense and effort
  - Not feasible in resource poor facilities
- **Immunochromatography**
  - Rapid, single step, user friendly
  - Stable and reproducible results
  - More sensitivity for primary infections
  - Serotypes cannot be identified
  - Qualitative or semiquantitative results
  - Generating antigen-antibody important
- **Bayesian Latent Class Models**
  - Combination NS1, IgM and IgG testing
  - Sensitivity - 87%, Specificity - 82.8 %
  - PPV - 62%, NPV - 95.2%

CONCLUSION

- Combined NS1, IgM and IgG kits are sensitive, specific, compact and frugal
- Recommended for dengue outbreaks, field conditions & bed side testing