DEVELOPMENT OF DIAGNOSTIC TOOLS FOR EMERGING INFECTIOUS DISEASES

Alice Versiani
DVM, Ph.D.
School of Medicine of Sao Jose do Rio Preto
FAMERP/Brazil
Overview

1. Assay development pathway
   • Definition of intended purpose
   • Controls and samples
   • Validation
   • Types of diagnostic tools (Classic x Novelty)
   • Point-of-care x Laboratory-based tests

2. Brazilian experience
   • Nanotechnology
   • Classic methodologies x new biomolecules
   • New methodologies x classic biomolecules
   • Technologies improvements

3. How to deal with emerging or re-emerging pathogens
   • Scientific networks
• Definition of the intended purpose of the assay:
  • Quantitative x Qualitative assay
  • Biological sample
    • Individual or pooled, matrix composition, host/organism interactions affecting the target analyte…
  • Assay system
    • Physical, chemical, biological, operator-related factors affecting the capacity of the assay to detect a specific analyte in the sample.
  • Teste results interpretation
    • The capacity of a test result, derived from the assay system, to predict accurately the status of the individual or population relative to the purpose for which the assay is applied.

• Selection, collection, preparation, preservation and management of samples are critical variables in design and development of an assay to ensure valid test results.

OIE. 2019. Principles and methods of validation of diagnostic assays for infectious diseases (chapter 1.1.2)
Validation

- Choosing samples:
  - Gold-standard assay (reference method)
  - Controls
  - Open sera bank
  - Disease epidemiology
  - Cross-reaction

<table>
<thead>
<tr>
<th>Reference Samples</th>
<th>Positive</th>
<th>Negative</th>
<th>Inconclusive</th>
<th>Total</th>
<th>PPA</th>
<th>NPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>100%</td>
<td>—</td>
</tr>
<tr>
<td>Presumed Negative</td>
<td>2</td>
<td>59</td>
<td>1</td>
<td>62</td>
<td>—</td>
<td>95.2%</td>
</tr>
</tbody>
</table>

SPECIFICATIONS

- Serological Sensitivity - Secondary infection: 97.9% (92/94)
- Serological Sensitivity - Primary infection: 33.3% (28/84)
- Serological Specificity - Negatives: 100% (108/108)
Assay Development Pathway

OIE. 2019. Principles and methods of validation of diagnostic assays for infectious diseases (chapter 1.1.2)
Types of diagnostic assays

SEROLOGICAL ASSAYS
- ELISA
- Neutralization (PRNT)

MOLECULAR ASSAYS
- PCR
- RT-PCR
- Hybridization

Lateral flow (LFA)
Neutralization (PRNT)
Types of diagnostic assays

**SEROLOGICAL ASSAYS**

- **Biosensors**
  - Luminex (bead array)

**MOLECULAR ASSAYS**

- CRISPR-Cas
- FACS
- LAMP
Types of diagnostic assays

**Point-of-care**
- Can be performed near/at the point of patient care.
- Easy to use;
- Portability;
- Low cost;
- Do not require power or additional reagents;
- Results in minutes.
- Inaccuracy;
- Low sensitivity;
- Single-use device;
- Cross-reactivity.

**Lab-based tests**
- Samples sent to a central laboratory for analysis.
- Accuracy;
- Multiplex assays;
- High diagnostic performance;
- Reproducibility;
- High-end technology.
- Higher costs;
- Needs specialized personal and equipment;
- Requires infrastructure.
Nanomedicine

- **Nanotechnology** → creation, manipulation and exploration of materials on a nanoscale.
  - The physical and chemical properties of matter are, to a large extent, determined by the type of motion of its electrons.

**Gold nanoparticles**
- Strong **optical peak**, which is variable with the particle's morphology;
- Electron-dense and radiopaque;
- Its surface chemistry allows the bonding of organic molecules;
- Low toxicity when introduced into biological systems.
Brazilian experience

- DENV Peptides to avoid ELISA cross-reaction
Brazilian experience

• DENV/ZIKV NS1 Peptides to avoid ELISA cross-reaction


Screening with monoclonal ab’s

Endemic area samples for validation

[A] Tse: 96% TSp: 99%

[B] Tse: 96% TSp: 88%

pepELISA

Brazilian experience

- DENV/ZIKV NS1 Peptides in a multiplex LFA + image processing

Brazilian experience

• LFA signal improvement:
Brazilian experience

• LSPR Nanosensors
  • Localized surface plasmon resonance: when the plasmon frequency is of the same as the incident light, a resonance phenomenon occurs and results in a noticeable optical absorption and generates a sharp electric field on the surface of the metallic nanoparticles. Therefore, any modifications around the nanoparticle, including alterations in their surface, the solvent and particle aggregation, will determine changes in the electronic properties of the nanoparticle’s surface, resulting in alterations in the patterns of the absorption spectrum.
Brazilian experience

- DENV LSPR Nanosensors:

DENV1-4 monoclonal antibody

[A] Normalized relative abs. longitudinal mode

[B] Derivative of the longitudinal mode (nm⁻¹)

Brazilian experience

- DENV LSPR Nanosensors:

**DENV+ HUMAN POOL SERA**

**CONTROLS HUMAN POOL SERA**

**Controls:**
- YFV
- SLEV
- TN

Brazilian experience

• DENV LSPR Nanosensors:

```
DENV+ HUMAN SERA  x  ZIKV+ HUMAN SERA
```

Brazilian experience

- COVID-19 ELISA and LSPR Nanosensor:

IgG ELISA anti-N validation

Brazilian experience

- COVID-19 ELISA and LSPR Nanosensor:


COVID Nanosensor characterization
Brazilian experience

• COVID-19 ELISA and LSPR Nanosensor:

Anti-N polyclonal antibody

Brazilian experience

- COVID-19 ELISA and LSPR Nanosensor:

Brazilian experience

• FACS signal enhancement:

Gold nanoparticles enhance fluorescence signals by flow cytometry at low antibody concentrations*

Daniela S. Reis, Vivian L. de Oliveira, Micael L. Silva, Roberto M. Paniago, Luiz O. Ladeira and Lidia M. Andrade

Sinergic effect of AuNP+Fluorophore
Diagnostic technologies

• How to choose your assay technology?

  • Lab infrastructure x Affected population
    • Cost
    • Time
    • Disease outcome: impact of false-negatives or false-positives results.
    • Co-circulation of similar pathogens that affects diagnosis

• Interdisciplinary research group:
  • Allies technology and biological/medical background

• New diseases
  • Opportunity to well-established academic techniques to gain market place
Emerging or re-emerging pathogens

- How to deal with emerging or re-emerging pathogens?
  - Rapidly develop and deploy diagnostic testing methods;
  - Development of case definitions and testing criteria;
  - Engage public health partners to optimize response capacity and coordination;
  - Establish information sharing processes, procedures, and samples that supports surveillance of new pathogens;
  - Establish genomics and other omics approaches to further enhance infectious disease response capacity.

Establishment of an interdisciplinary research network
Emerging or re-emerging pathogens

- ZikaPLAN brings together 25 leading research and public health organizations in Latin America, North America, Africa, Asia, and Europe, taking a comprehensive approach to tackle the Zika threat.

- CADDE brings together multidisciplinary teams across Brazil and the UK to address critical questions in arbovirus epidemiology and public health in Brazil.

- Brazilian committee that brings together specialists, government representatives, funding agencies, research centers and universities with the aim of integrating initiatives to combat emerging viruses.
COVID-19 INITIATIVES

- Genome sequencing
- 10 granted vaccine projects
- Protocols / Confirmed samples / virus distribution
- 100% national serologic assays
- Cross-validation
The Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics (CREATE-NEO) project will provide a nimble and flexible network of surveillance sites in Central and South America coupled to cutting-edge modeling approaches in order to anticipate and counter emerging arboviruses. Importantly, CREATE-NEO can quickly redirect its mission to address any emerging zoonotic or vector-borne disease.
Acknowledgments

- **LVP/FAMERP group:**
  - Dr. Maurício L. Nogueira
  - Dr. Nathalia Zini
  - Dr. Cássia Estofolete
  - Dr. Livia Sacchetto
  - Students & Hospital staff

- **UTMB-Health group:**
  - Dr. Nikos Vasilakis
  - Dr. Adam Hendy

- **MIT group:**
  - Dr. Lee Gerhke
  - Dra. Irene Bosch

- **NBM research group:**
  - Dr. Luiz Orlando
  - Dr. Lídia Andrade
  - Dr. Estefânia Martins

- **LVBA/UFMG group:**
  - Dr. Flávio da Fonseca
  - Dr. Edel F. Barbosa-Stancioli
  - Dr. Flávia Fonseca
  - MSc. Thais Moraes

- **Financial support:**
  - FAPESP, Capes, CNPq, INCTV, INCT Dengue, NIH.