Development of Rapid Assays for Common Infectious Diseases

HC Mody*, Shailesh Y*. Parab* and PK Desai*

INTRODUCTION

Last two decades have witnessed a sea change in various diagnostic techniques and tests available for various infectious diseases. While nucleic acid based diagnostic tests have added new dimension to the sensitivity and specificity of hitherto available diagnostic tests for diseases, these tests have not gained widespread usage because of their cost, complexity and infrastructure requirements. On the other hand Rapid Immunodiagnostic tests have revolutionized the laboratory practice to an extent beyond imagination. These tests have become commonplace, easily available and affordable and usable in any ordinary, even mofussil laboratories without any specialized equipments or training.

Basic Scientific advances in three areas have been primarily responsible for these developments:
1. Monoclonal antibodies
2. Recombinant antigens
3. Colour development systems based on colloidal gold, selenium etc.,

Judicious and ingenious combination of above three ingredients have been used in present day Rapid Diagnostics Tests (RDTs) to achieve unprecedented sensitivity, specificity and speed of various tests available in the market.

WHAT IS A RAPID ASSAY?

Rapid Tests are Immuno diagnostics tests used for wide range of diseases. They provide quick results, without requiring sophisticated facilities, equipments or specialized training. They are also cost effective and easy to interpret.

As per World Health Organization (WHO) the rapid test or assay should have following characteristics:

- High quality
- Ease in use.
- Quick, i.e. interpretation of results within 10 mins to 2 hours.
- Require a little or no instrumentation or specialized gadgets.
- Feasibility of room temperature storage of such tests, mainly in countries with resource poor settings.
- Results should be obtained on the same day thereby getting advantage of timely medical intervention.
Rapid assays or tests are very well suitable and compatible with the laboratories in resource poor settings where numerous specialized gadgets and equipments, as would be required for other test formats like Enzyme Linked Immuno-Sorbant Assay (ELISA), Polymerase Chain Reaction (PCR), Microarrays etc. Following are the features of these tests which have justified use of rapid tests or assays in clinical Laboratory:

- **Robustness**
  - Majority of these tests are stable even at room temperature throughout its shelf life.
  - Retain their quality even at higher ambient temperatures for short exposures.
  - Packing of 1 test/pack.
  - Good resistance during transportation.
  - Some offers dried reagents which offers greater stability.

- **Affordability**
  - Cost of the test is very reasonable.
  - Proves to be less expensive when small number of samples is needed to be tested.
  - Less wastage and hence easy to dispose off.
  - Less cost with respect of sample collection.

Rapid Tests have various advantages from clinicians point of view:

- **Simplicity**
  - No need for any kind of specialized instruments.
  - Highly competent person not needed to perform the test.
  - Mostly results are read visually.
  - Easy to dispose off after the completion of the test.

- **Rapid tests are available in various formats which are as follows:**
  - Agglutination assays
  - Solid Phase Immobilization Assays (SPIA)
  - Immuno-chromatography Assays
    - Immuno-filtration Assays
    - Lateral Flow Assays

Table 1 gives the comparison of various formats available for rapid tests.

In common infectious diseases like Malaria, Filariasis, Acquired Immuno-Deficiency Syndrome (AIDS), Tuberculosis etc., Rapid tests have
outperformed other techniques with respect to its simplicity, robustness and affordability. Rapid tests for malaria have simplified the diagnosis by replacing age-old, cumbersome microscopy techniques. Some of the rapid tests can also differentiate between the Plasmodium species. Mostly antigen detection is preferred. The comparative chart of different antigen detection tests for malaria is shown in Table 2.

proved their usefulness due to their simplicity as well as dependability. The brief summary of rapid tests for different infectious diseases is given below:

**MALARIA DIAGNOSIS BY RAPID TEST**

There are different diagnostics tests available for Malaria detection which mainly include: Microscopy, Qualitative Buffy Coat (QBC), PCR and Rapid tests. Although Rapid tests do not have more sensitivity than that of other tests, it has

<table>
<thead>
<tr>
<th>Principle</th>
<th>Agglutination Assay</th>
<th>SPIA</th>
<th>Immunofiltration Assay</th>
<th>Lateral flow Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugates</td>
<td>No Need</td>
<td>Colloidal gold or coloured particle conjugates</td>
<td>Colloidal gold or coloured particle conjugates</td>
<td>Colloidal gold or coloured particle conjugates</td>
</tr>
<tr>
<td>Results</td>
<td>Read Visually</td>
<td>Read Visually</td>
<td>Read Visually</td>
<td>Read Visually / or by reflectometry</td>
</tr>
<tr>
<td>Methodology</td>
<td>Mostly one step</td>
<td>Multi-step</td>
<td>Multi-step</td>
<td>Mostly One step</td>
</tr>
<tr>
<td>Cost</td>
<td>Inexpensive</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate to Inexpensive</td>
</tr>
<tr>
<td>Temperature stability</td>
<td>Needs ambient temperature for stability</td>
<td>Need Room temperature (upto 30°C storage) for stability</td>
<td>Need Room temperature (upto 30°C storage) for stability</td>
<td>Stable at wide range of temperature</td>
</tr>
<tr>
<td>Quantitative/qualitative</td>
<td>Quantitative/ Semi-quantitative</td>
<td>Quantitative</td>
<td>Quantitative</td>
<td>Quantitative / semi-quantitative</td>
</tr>
<tr>
<td>Multi-analytes testing</td>
<td>Not possible</td>
<td>Possible to some extent</td>
<td>Possible</td>
<td>Possible</td>
</tr>
</tbody>
</table>

Table 1. Comparison of different test formats of Rapid tests
be obviated, as it offers the advantage that one can use day time samples, as well, for diagnosis. Here also the Rapid tests are available in two different formats: 1. Antigen detection and 2. Antibody

Table 2. Comparative chart of different antigens detected in Rapid diagnostic tests for Malaria

<table>
<thead>
<tr>
<th>Target Antigen</th>
<th>Pf HRP-2 Test</th>
<th>Pf HRP-2 &amp; PMA Test</th>
<th>pLDH Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Format</td>
<td>2 Lines</td>
<td>3 Lines</td>
<td>3 Lines</td>
</tr>
<tr>
<td>Capability</td>
<td>Only detects <em>P. falciparum</em></td>
<td><em>P. falciparum &amp; other Plasmodium species</em></td>
<td>All the four species</td>
</tr>
<tr>
<td>Differentiation between the species</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Indication of recentness of infection</td>
<td>Not possible</td>
<td>Not possible</td>
<td>If positive indicates viable parasitemia</td>
</tr>
</tbody>
</table>

**Pf**: *Plasmodium falciparum*; **HRP**: Histidine Rich Protein; **PMA**: PAN Malaria Antigen; **pLDH**: *Plasmodium* Lactate Dehydrogenase

**DIAGNOSIS OF FILARIASIS BY RAPID TEST**

Rapid tests for Filariasis have offered not only simplicity but also feasibility of sample collection where conventional practice of the night sample collection can be obviated, as it offers the advantage that one can use day time samples, as well, for diagnosis. Here also the Rapid tests are available in two different formats: 1. Antigen detection and 2. Antibody
RAPID TESTS FOR TUBERCULOSIS

Diagnostic tests for Tuberculosis are still based on old proven techniques and have serious shortcomings. Even today culture technique is considered gold standard for diagnosis of Tuberculosis. Antibody detection tests incorporate antigens like Lipoarabinomann (LAM), Antigen-60 (A-60) and 38KDa antigen in different formats. Rapid tests for Tuberculosis still pose a challenge to the researchers and test developers. Although the serology of antigen or antibody test is simple, cheap and rapid, still there are problem relating to sensitivity and specificity. There is still a need for a perfect antigen which can detect accurately the notoriously variable antibody response. Presently not a single rapid test is available which can boast of enough sensitivity and specificity.

RAPID TESTS FOR HIV

Major tests available for HIV are indirect tests available in various formats, in which rapid test is one. Rapid tests for HIV are very well established, and most of these tests offer performance equivalent to that of ELISA with respect to sensitivity, specificity and seroconversion index. The antigens mainly employed are envelope glycoproteins, against which the antibodies are detected.
themselves. Although there are many challenges ahead of us, yet from research and developmental that is occurring worldwide, one can be assured that RDTs will have bright future in the arena of Diagnostics.

REFERENCES


