CDAlert

Monthly Newsletter of National Institute of Communicable Diseases,
Directorate General of Health Services, Government of India

March-April 2006 Vol.10 : No.3-4

ROLE OF RAPID TECHNIQUES IN DETECTION & SURVEILLANCE OF SOME DISEASES OF PUBLIC HEALTH IMPORTANCE

The traditional method of identifying a pathogen is by culturing it. Many kinds of infectious agents, including mycobacteria, fungi and viruses, either are fastidious and grow slowly or cannot be cultured. In these instances, serologic or histologic methods may identify the infectious agent. Pathologists now have highly sensitive and specific methods of identifying infectious agents but many infections like HIV, Hepatitis B&C, Influenza, and Meningitis are rapidly increasing in areas with limited diagnostic infrastructure. Electricity, equipment and technical expertise to perform sophisticated diagnostic tests is often lacking and on-site clinician support is rare. One strategy that may be used to respond to the challenge of providing healthcare to under privileged population is through integration of newly developed rapid diagnostic tests. These rapid tests can yield results on-site within 10-30 minutes and do not require any elaborate equipment, supplies or electricity. They are easy to read as results are visually interpreted.

APPROPRIATE USES OF RAPID DIAGNOSTIC TESTS

- **Diagnosis:** Rapid tests can be used to identify, confirm or rule out disease in symptomatic patients.
- **Patient Management:** Rapid Tests can be used to accurately prescribe therapeutic drugs and to monitor treatment.
- **Epidemiology:** Community surveillance by rapid tests may be used to monitor the incidence or prevalence of disease for targeting and evaluating health programs. Disease surveillance can also inform the physicians, rapidly, what agents are circulating in the community.
- **Screening:** They can be used in case finding to determine the prevalence of disease in asymptomatic individuals.

The specific performance requirements of a test will vary depending on the intended use.

When to use rapid tests: While considering whether or not to use a rapid diagnostic test in a particular setting, it is important to consider the following:

- Advantages and Disadvantages
- Use algorithms
- Cost
- Barriers and constraints to use.

GENERAL ADVANTAGE OF RAPID DIAGNOSTIC TESTS

- Easy to use, with minimal training required.
- Relatively rapid; same-day results are possible, resulting in fewer patient's lost to follow-up.
- A shelf life as long as 1-2 years at ambient temperatures, with no need for refrigeration.
- Limited or no need for instrumentation.
- No electricity requirement.
- Results can be read visually.
- In some cases, rapid tests are more

accurate than existing reference-level laboratory tests.

GENERAL DISADVANTAGES OF RAPID TESTS

- Cost per test for rapid tests may exceed traditional testing methods .
- They are mainly qualitative, producing only “yes/no” answers.
- They require subjective interpretation, which may result in readers variation.
- In some cases, rapid tests are less sensitive or less accurate.

COST EFFECTIVENESS ANALYSIS

Cost effective analysis is calculation of the costs of a diagnostic test and comparing it to the resulting health outcome. Cost effectiveness of Rapid tests lies in the fact that they are very useful in small blood banks and peripheral health laboratories as test for a single patient can be performed in a short time with minimal infrastructure with no expertise.

USE ALGORITHMS

- These are set of instructions for use in identifying a condition. They consist of questions that can be answered with a “yes” or “No” or indicative arrows to a next greater detail.
- In health care settings they are often used to identify the causative agent of disease and to suggest appropriate theraputic measures.
- Use algorithms consider a number of factors such as:
  - Disease incidence and prevalence.
  - The availability and accuracy of other screening or confirmatory tests.
  - The probable consequences of misdiagnosis.

Some algorithms are excellent when used in clinical practices while others are less accurate and need improvement.

BARRIERS AND CONSTRAINTS TO USE

Before accepting a rapid test technique some barriers and constraints have to be looked into. These can be put into 3 main categories:

Acceptability: Rapid tests need to have sufficient sensitivity and specificity and they need to have adequate predictive values.

Affordability: Decreasing per test costs, carefully designing diagnostic algorithms, and educating end users about the cost savings of more efficient use of therapeutic drugs are important means of maximizing rapid test affordability.

Availability: The availability of Rapid diagnostic tests depends upon its distribution system, quality assurance and its shelf life.

ACCURACY OF DIAGNOSTIC TESTS

Accuracy can be expressed through sensitivity and specificity, positive and negative predictive values, or positive and negative diagnostic likelihood ratios.

Sensitivity: The sensitivity of a test is the probability that it will produce a true positive result when used in an infected population (as compared to a reference or “gold standard”.

Specificity: The specificity of a test is the probability that a test will produce a true negative result when used on a non-infected population (as determined by a reference or “gold standard”

Positive predictive value: The positive predictive values of a test is the probability that a person is infected when a positive test result is observed.

RAPID TEST TECHNOLOGIES

Before considering using a new diagnostic test one should consider how it works. Most of the commercially available rapid tests are based on the following technologies.

- Lateral flow
- Flow through
- Particle Agglutination
- Solid phase (Dipstick Tests)

TESTS BASED ON DIFFERENT TECHNOLOGY PRINCIPALS

Lateral Flow Tests

These tests are also called immunochromatographic strip (ICS) tests. They were introduced in late 1980s. ICS tests are used for the specific qualitative or semi-quantitative detection of many analytes like antigens, antibodies and products of nucleic acid amplification tests. Urine, saliva, serum, plasma or whole blood can be used as specimens. Test sensitivity can be quite good (e.g. hepatitis B surface Ag (HBsAg) ICS tests have claimed a sensitivity of 1.0 ng HBsAg/ml or less). Test specificity can also be very high. The tests use colloidal gold, dye, or latex bead conjugates to generate signal. The assembled strips are stable for months when properly protected from moisture and excessive heat. Test sensitivity ranges from 57-100% and specificity is 85-99%

To perform the test, a sample either alone or with an extraction reagent or running buffer is placed on the sample pad on one end of the strip. The signal reagent is solubilized and binds to the antigen or antibody in the sample and moves through the membrane by capillary action. If specific analyte is present, the signal reagent binds to it, and a second antibody or antigen – immobilized as a line in the nitrocellulose – then captures the complex. If the test is positive, a pink/purple line develops. For example, if antibodies in a serum sample are to be detected, the specific antigen colloidal gold conjugate is immobilized on the nitrocellulose strip in a thin line. If the specific antibodies are present in the serum sample, the antibodies will bind to the colloidal gold complex. The complex then migrates through the nitrocellulose strip by capillary action when it meets the immobilized antigen (test line) forming an antigen-antibody-antigen colloidal gold complex. This forms a pink band indicating the sample is reactive for antibodies. No pink band means sample in negative for antibodies. Validity of the procedure performed is shown by the procedural control line, an additional line of immobilized antibodies (control line) at a distance above the test line on the strip. If the test is performed correctly, this will result in the appearance of pink line upon contact with conjugate. Appearance of 2 lines, therefore, indicates a positive result, while a negative test procedures only one line appears.

Once the specimen is added, the tests can be left unattended until they are read. Results can usually be read in 5 to 15 minutes.

Lateral Flow based test

Flow Through:

Development of flow through tests date from early 1980s. These tests are usually supplied in kits as an individual cassettes with extraction and wash buffers. The test principle involves a flow of fluid containing the analyte through a porous membrane and an adsrobent pad. A second layer or sub-membrane, inhibits “immediate back flow of fluids, which can obscure results.

To detect antibodies or antigens, the corresponding analyte is bound or immobilized as a dot or line on the membrane. This reagent “captures” the analyte as it flows through the membrane.

To perform the test, a sample is applied to the membrane and allowed to wick through by capillary action. Thereafter, sequentially, there is a wash step, addition of the signal reagent and a second wash to clear the membrane. The solutions can be added as rapidly as the previous liquids are absorbed into the cassettes.

Earlier flow through tests used enzyme immunoassay (EIA) principles to generate signal, but more recent tests have successfully used coloured latex particles or colloidal gold.

Advantages:

- A very rapid test procedure with results available in as few as 3 to 5 minutes
- Sensitivity of this test is good for serological assays.

Disadvantages:

- The tests need to be performed individually or in small batches
- These are not “Walk away” tests and require constant attention.

Memberane Flow through test in casette form

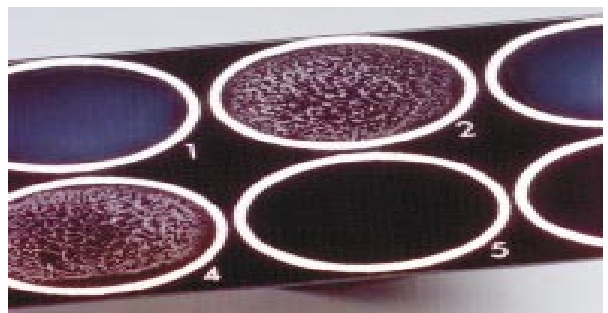
Partice Agglutination

The diagnostic tests based on latex agglutination or passive haemagglutination have been used for many years. They employ white or colored latex particles or gelatin bead, other dyes or colloidal particles, or preserved mammalian/avian blood cells to carry out antigen/antibody assays.

To perform the general version of the test, a specimen is placed on a microscopic slide/card or in a microwell test plate well, and then the carrier particles are added. The mixture is then stirred or agitated and incubated or allowed to settle for a few minutes. The results are visualized by observing agglutination of the carrier particles as they bind to the specific analyte in the sample.

In strong reactions, the interpretation is simple, but in weak or marginal reactions, the interpretations can sometimes be quite difficult, and accurate results are possible only with experience and practice. In the case of micro well plate assays, the pattern of particles that have settled needs to be interpreted to determine the result.

The tests can be qualitative or semi-quantitative, since for the latter, 2 to 10 fold specimen dilutions can be made. The classic agglutination tests often lack sensitivity, and results cannot be stored for reference. Recent agglutination methods using channeled plastic devices and/or automated readers have significantly improved sensitivity with added cost and complicity.



Particle Agglutination Test

Advantages :

- A low test cost.
- Availability of semi quantitative results.
- Relatively short time to obtain results.

Disadvantages

- Need to carefully interpret marginal results.
- Problems with specificity due to interfering substances in many assays.
- A relatively low sensitivity especially for antigen detection tests.

Solid Phase

These assays include the so called “dipstick” or “dipstick comb” tests that date back to the early 1980s.The tests are formatted on solid, non porous supports onto which antigen or antibody is immobilized in order to detect their specific analytes.

To perform the assay, the dipsticks are incubated with patient specimens. This is followed by an initial wash step, addition of signal reagent, and a final wash step. Signal can be generated by binding of latex or colloidal gold particles attached to specific (monoclonal antibodies or recombinant antigens) or non-specific (protein A) reporter molecules. Signal can also be generated by EIA methods.

The sensitivity of serological tests using dipstick is comparable to conventional microwell plate EIAs. However, in case of Ag detection, their sensitivity is, in general, lower than lateral flow or micro well plate EIA methods. Most tests, even those employing EIA principles to generate signal, can be completed in one hour or less.

RAPID TESTS AVAILABLE COMMERCIALLY FOR SOME COMMON INFECTIONS

A new generation of simple and bed-side tests are now available for preliminary rapid diagnosis, at primary treatment location for a number of diseases.. Based on various techniques, these tests can be used to detect both specific antibody and specific antigen.

Rapid Tests for HIV

Increasingly, combinations of rapid tests are being used for the diagnosis of HIV in resource poor settings. Smaller blood transfusion facilities, antenatal/family planning clinics and voluntary counseling and confidential testing (VCCT) programs need simple, rapid tests applicable for individual clients. Because the consequence of a positive HIV test are so severe, the sensitivity and specificity requirements of these tests are close to the “gold standard” – the performance standard of the best laboratory tests. These rapid tests need to be simple to perform, easy to interpret, and false positive results need to be kept to a minimum. They have to be cost-effective also.

Rapid tests for HIV can be less expensive to purchase, require less infrastructure, and do not need trained laboratory staff.

Rapid HIV test formats include:

Agglutination tests: Different types of particles are used to produce clumping or setting patterns of the particles when a specimen is positive for HIV antibodies, An autologus agglutination method detects HIV antibodies with a hybrid antigen-antibody reagent which agglutinates the patient’s red blood cells. A latex particle agglutination detects HIV antibodies by the agglutination of minute latex particles when mixed with the patient’s blood. A newer method uses fluid capillary action to enhance and quicken particle agglutination. Particle adherence

detects HIV antibodies when the settling patterns of small gelatin particles are altered.

Flow through cassettes: or membrane immunoconcentration devices, capture and detect HIV antibodies in a specimen flowing through a porous memberane. A visible dot or line forms on the membrane when HIV antibodies are present.

Solid phase tests: includes the dipstick comb assay. This assay uses a solid plastic matrix to which an HIV antigen is fixed. When HIV antibodies are present, a spot or dot will be visible when processed with a signal reagent.

Immunochromatographic Strip (ICS) tests: utilize a one step method in which the patient's blood specimen is combined with a signal reagent and migrate through a special membrane. A positive reaction is seen as development of line on a membrane. Most tests take less than 15 minutes.

Rapid Test for Syphilis:

Syphilis is one of the important genital ulcer disease (GUD). However, clinical diagnosis is of limited use since chancres may heal or can be atypical, and infected patients may also be asymptomatic. Under the circumstances laboratory diagnosis plays an important role in diagnosis of syphilis.

There are several diagnostic test methods in standard clinical use. Diagnosis can be performed using one or a combination of methods. Rapid syphilis tests have been recently introduced in the immunochromato graphic strip, or lateral flow format. They use finger prick or venous blood, take only 10-15 minutes without needing elaborate laboratory. Health care staff can easily interpret the results. Since all available rapid tests use pallidium recombinant antigens, the results closely reflect those generated by specific confirmatory tests. However, in STD Clinics, a positive rapid test would need to be "confirmed" with a screening test like a RPR.

The RPR (Rapid Plasma Reagin) is a non-treponemal test for serological diagnosis of Syphilis. In this test antibodies are detected in serum samples using particle agglutination technology. It is reliable, economical, reproducible and easy to read with 85 to 90 % sensitivity with primary disease, 100% in secondary, 90-95% in latent and 75% in late or tertiary disease.

However, rapid tests are highly effective in rural outreach programs or in other lower – prevalence settings such as antenatal or family planning clinics. Rapid syphilis tests can be used as a screening, diagnostic or confirmatory test. They can be used in research studies under the correct circumstances

Hepatitis B:

Detection of hepatitis B surface antigen (HBsAg) identifies individuals infected with the Hepatitis-B virus. Rapid tests are intended for qualitative detection of HBsAg in human serum, plasma or whole blood. The majority of rapid tests are based on agglutination or immunochromatgraphic principles.

The sensitivity of the more recently introduced immunochromatographic strips (ICS) test is better than agglutination tests developed earlier e.g. Reverse Passive Haemagglutination Assay (RPHA).

The advantage of ICS method is that it can be completed in 10-20 minutes and performed by nurses or technicians with minimum of training. It is practical for use at the provincial or peripheral health care level, since the test strips are stable for one to two years at ambient temperature. Applications of simple and rapid tests for the detection of HbsAg include case finding and epidemiological surveillance.

Simple and rapid tests for the detection of HBsAg can be used in rural or smaller

clinics, hospitals, or blood banks in resource-poor settings to diagnose chronic hepatitis B infection. Accurate results allow for same-day patient follow up. Intermediate to peripheral level clinics and hospitals and central blood banking facilities can use rapid hepatitis B tests as a back up to standard EIA testing. Secondary applications include case-finding and epidemiological surveillance.

Rapid Tests for Influenza (A+B)

There are several new test kits in the market for the rapid identification of influenza A and B. Rapid diagnosis for influenza is valuable in early diagnosis which enables treatment in time as influenza treatment must be administered within 48 hours of onset of the infection, in order to be effective.

Commercial rapid diagnostic tests are available that can detect influenza viruses within 30 minutes. These rapid tests differ in the types of influenza viruses they can detect and whether they can distinguish between influenza types. Different tests can detect 1) only influenza A viruses: 2) Both influenza A and B viruses but not distinguish between the two types; 3) both influenza A &B and distinguish between them. None of the tests provide any information about influenza A subtypes. All tests are easy to perform and take 15 to 20 minutes time for result except in Zstat Flu test which takes 35 minutes time.

For determination of influenza virus by Rapid tests, appropriate respiratory specimens include throat and nasopharyngeal swabs, aspirates or wash. Nasopharyngeal specimens are more effective than throat swab. Samples should be collected within first 4 days of illness. The specificity and in particular sensitivity of rapid tests are lower than viral cultures. Due to lower sensitivity of the rapid tests, the negative test should be confirmed with viral culture or by other means. In contrast, false positive rapid test results are less likely but can occur during periods of low influenza activity. Therefore when interpreting results of a rapid influenza test the positive and negative predictive values of the test in the context of the level of influenza activity in the community should be considered.

Rapid Tests for Hepatitis C

The Hepatitis C is the major cause of the disease formerly known as non-A, non- B post transfusion Hepatitis. Since the introduction of anti-HCV screening of blood and blood products for donations in most industrialized countries, the incidence of this infection in transfusion recipients has markedly reduced in these countries.

The lab diagnosis of HCV infection is usually made on the basis of the detection of circulating antibodies in the serum. Several simple, easy to use screening tests are available for HCV screening which include agglutination, immunofilteration (flow through), and immunochromatographic lateral flow membrane tests. A positive result is indicated by the appearance of a coloured dot or line, or showing an agglutination pattern. Most of these tests can be performed in 10 minutes with good sensitivity and specificity. These simple rapid tests are most suitable in laboratories that have limited facilities and process a limited number of specimens.

Rapid Tests for Malaria

Development of rapid diagnostic tests for Malaria offers the potential to extend accurate malaria diagnosis to remote areas without microscopy services. Rapid malaria diagnostic tests have been developed in the immunochromatographic strips or lateral flow format. These tests use finger prick or venous blood, take only 10-15 minutes, easily interpretable results. Two types of rapid tests are available, ones that identify the PfHRP-2 antigen of falciparum malaria only and others are based on detection of falciparum malaria and for either vivax malaria or all four malarial species in the same test.

Proper use of quality rapid malaria tests has the potential to prevent and manage severe malaria and malarial epidemics. For travelers and military personnel presenting to medical providers unfamiliar with malaria clinical diagnosis, rapid malaria tests can be equally valuable.

However rapid malaria test performance can be limited by issues such as shelf life and need for strict manufacture's quality control measures.

Commercial rapid test technologies are available for many other viral, bacterial and fungal infections, few of which are listed below:

Infectious agent	Rapid test technology	Analyte/Specimen used
Dengue Virus	Lateral Flow	Specific antibodies in serum
Salmonella Typhi	Agglutination	Qualitative and quantitative estimation of specific antibodies in serum
Leptospira	Agglutination	Specific antibodies in serum
Meningococcal meningitis	Agglutination	Soluble antigens in urine, serum, CSF

The inclusion of these tests in the healthcare program could improve disease surveillance and increase disease detection to allow earlier public health intervention and more effective disease management efforts.

MOLECULAR DIAGNOSTICS

The tools of molecular biology have proven to be readily adaptable for use in the clinical diagnostic laboratory. They are most appropriate for infectious agents that are difficult to detect, identify, grow or test for susceptibility by routine methods or such methods are very time consuming. They can be used for diagnosis, monitoring therapy, epidemiological investigations and infection control.

Nucleic acid based techniques use standard methods for isolating nucleic acids from clinical material and various methods to analyze the DNA and RNA. Since the target DNA and RNA may be present in very small amounts in the sample tested, target and signal amplification techniques can be used before analysis.

Although new molecular techniques are very useful for slow growing or uncultivable organisms, the high cost of these tests is a major concern.

DO's

- Instructions as per the commercial kit insert should strictly be followed for all rapid tests.
- Recommended specimens should be used for detection of Antigens or Antibodies by rapid testing.
- Positive and negative controls should be put up simultaneously as quality control measures for each rapid test.
- Additional follow up testing using available clinical methods is required if the test is negative with persisting clinical symptoms.
- All reactive samples should be confirmed by confirmatory tests.
- Results of rapid tests should always be evaluated in the context of other clinical and epidemiological information available to health care providers.

...about CD*Alert*

CD*Alert* is a monthly newsletter of the National Institute of Communicable Diseases (NICD) , Directorate General of Health Services, to disseminate information on various aspects of communicable diseases to medical fraternity and health administrators. The newsletter may be reproduced, in part or whole, for educational purposes.

Chief Editor: Dr. R. K. Srivastava

Editorial Board: Dr. Shiv Lal, Dr. R. L. Ichhpujani, Dr. Shashi Khare, Dr. A. K. Harit

Guest Editor: Mrs. Inderjeet, Dr. Sunil Gupta & Mr. Ramesh Aggarwal

Publisher: Director, National Institute of Communicable Diseases, 22 Shamnath Marg, Delhi 110 054

Tel: 011-23971272, 23971060 Fax : 011-23922677

E-mail: dirnicd@bol.net.in and dirnicd@del3.vsnl.net.in

Acknowledgement: Financial assistance by WHO/USAID is duly acknowledged.

Printed by Aravali Printers & Publishers Pvt. Ltd., Okhla, Phones: 26388830-32