Rapid Diagnostic Tests in Childhood Infections

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Presumptive treatment of infections often results in irrational antimicrobial use resulting in detrimental spread of drug resistance and untoward side effects. A rapid diagnostic test (RDT) is a test that delivers a result earlier than conventional testing methods employed in the past to identify the offending microorganism. RDTs help in early definitive therapy, reduction in hospital stay and cost, and in degree of morbidity and mortality associated with the infection. To select a proper RDT, one should consider how specific and sensitive the test is. Most RDTs gives a qualitative result not quantitative; hence disease severity, monitoring of the disease, prognostication and therapeutic efficacy cannot be assessed. A RDT should be easy to perform, should not require sophisticated machines, and kits should be stable in extremes of temperature. RDTs may be of immense help in remote places where conventional diagnostic facilities are unavailable or lack quality. RDTs hold promise of reasonable diagnostic accuracy if done in a optimal clinical background. They should never be ordered as a shotgun approach to exclude all possible infections but should be used judiciously with appropriate interpretation.

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Traditionally, patients with infectious diseases are treated with antimicrobials empirically, and definitive therapy is started only after the offending organism grows in cultures and sensitivity test results are available, which takes considerable time. The clinical distinction between viral and bacterial infection may not be possible always, which frequently results in overuse of antibiotics. Almost half of the patients in outpatient clinics with upper respiratory infection receive antibiotics [1,2]. Overuse of antibiotics causes development of resistance, reducing the already dwindling number of effective antibiotics, drug toxicity and increases the chances of resilient and opportunistic infections like *C difficile*. Therefore, it is vital to diagnose the causative organism and its sensitivity/resistance pattern in order to ensure positive patient management, adequate antimicrobial stewardship and limit further outbreak.

Conventional tests for diagnosis of infectious diseases and for determining drug-susceptibility of microorganisms were based upon microscopy and culture methods. These tests are more trustworthy but are time consuming, cumbersome and also require logistics and skills, which may not be available in peripheral health care setting. Average time required to identify the microorganism and its drug-susceptibility to antibiotics is about 40 hours [3]. Rapid diagnostic tests (RDTs) are quick, easy to perform, and results are available earlier than conventional methods, with a usual turn-around time of few hours. For rapid immunochromatic card test it takes 15 to 20 minutes whereas ELISA test requires 5 to 6 hours time. Due to the ease and feasibility of RDTs, they may be available at the point-of-care setting *i.e.*, in the office or in the emergency room. This has immense value in deciding whether the patient will need inpatient care or may be treated as an outpatient [4]. The qualities of an ideal RDT are outlined in ***Box* 1**.

COMMON METHODS USED IN RDT

Pathogenic organisms in rapid tests are detected by identifying the non-visual biological signal they generate. These signals include structural components of bacteria, viruses, protozoa and fungi, specific antigen and antibodies, metabolic end products, DNA and RNA base sequence, enzymes, toxins or surface polysaccharides [5].

BOX 1 QUALITIES OF AN IDEAL RAPID DIAGNOSTIC TEST

- Rapid turnaround time from sample collection to result.
- Can be performed in usual samples such as blood, urine, stool and cerebrospinal fluid.
- High sensitivity, specificity and predictive values.
- Diagnosis should be pathogen-specific with information about drug resistance.
- Should be available at different settings like hospitals, outpatient clinics, and in remote places.
- Results should be reproducible.
- Test should be cost-effective.
- It should utilize minimal logistics, and require minimal skill to perform.
- It should withstand extremes of temperature.
- Results are obtained in a single visit.

Detectors are used to pick up the right signal from numerous background signals in the sample into meaningful information to clinch the diagnosis.

Antigen Detection Methods

Agglutination test: Patient's specimen containing the bacterial antigen with antibodies directed against it will result in visible precipitation. If quantity of antibody is significantly higher or lower than antigen there will be no precipitation – known as prozone and postzone effect, respectively. As these tests have low sensitivity, they have been mostly abandoned.

Latex agglutination assay: Latex coated antibodies are used to detect the antigen.

Enzyme immunoassay (EIA) or Enzyme linked immunoassay (ELISA): These are highly sensitive rapid tests to detect viral antigens, toxins and organisms. Antibodies are bonded to enzymes, and if antigen-antibody reaction occurs, the enzyme catalyzes the reaction to produce a visible colored end product.

Detection of Antibodies by Serological Methods

EIA and other serological methods for detection of antibodies can be used but have their own limitations. Presence of antibodies may indicate either a recent or past infection. A 4-fold rise in antibody titers following infection or raised IgM antibodies can indicate early acute phase response. However, this response may be inhibited by elevated IgG, which competes with IgM for binding at the same antigen site, thereby resulting in false negative test.

Nucleic Acid Tests

These are used for the following purposes : (*i*) direct detection and quantification of pathogens in patients' specimen; (*ii*) identification of microorganism grown in culture; (*iii*) characterization of microorganism beyond basic identification *eg*, detecting genes encoding for resistance; and (*iv*) identification of species of organisms and strain typing useful to locate the source of an outbreak of infection.

Nucleic acid hybridization method: This method involves coupling of a probe that contains known nucleic acid sequences against target nucleic acid regions present in patient's specimen [6]. A negative hybridization test indicates either the absence of a target organism or presence below the limit of detection by hybridization.

Nucleic acid amplification methods: At times, the target in the specimen might not have sufficient nucleic acid to be detected by the above technique. Hence amplification of the target to greater number of copies can be performed with Nucleic acid amplification test (NAAT), which uses Polymerase chain reaction (PCR). Quantitative PCR (qPCR) is a significant advancement where one is able to detect the number of targets in the original clinical specimen. This helps to establish the disease burden, assess effectiveness of therapy, prognosticate and monitor disease progression. In multiplex PCR, more than one primer or probe is used to detect different target in one reaction; *eg*, multiplex PCR-containing probes for herpes simplex and enteroviruses to be used to detect the responsible organism in viral meningitis.

Gas Liquid Chromatography

Once the pathogen is grown, it can be identified quickly by its metabolic end products, such as short-chain fatty acids, with use of gas liquid chromatography. It can also be used to identify the long-chain fatty acids present in the cell wall and membranes of different organisms.

***Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry* (MALDI – TOFMS)**

In this technique, the organism grown is mixed with a chemical matrix, and laser is applied, which cause desorption of proteins and ionization. These ions are separated on the basis of time they travel in a charged vacuum tube, known a flight tube, to a detector. The patterns they produce are compared to known patterns of different organisms stored in the database.

RAPID DIAGNOSTIC TESTS IN COMMON DISEASES

Malaria

Immunochromatographic tests are available to detect malaria antigen by monoclonal antibodies targeted to it. They are simple to perform with results in few minutes, without any sophisticated devices. The antigens targeted in current test kits are:

Histidine rich protein 2 (HRP2): This is specific to *P. falciparum* produced by asexual stage and young gametocytes [7]. Antigen positivity may persist for weeks even after successful treatment.

Parasite lactate dehydrogenase (pLDH): It is present both in asexual and sexual (gametocyte) stage. Different isomers of this antigen are present. One is found in all the four species of malaria known as pan-specific. Other antigens are either *P. falciparum* or *P. vivax* specific. Unlike HRP2, these disappear following successful treatment but as gametocytes also produce pLDH, it may remain positive after clearance of the asexual stage [8].

Plasmodium aldolase: This is an enzyme of the glycolytic pathway produced by all four species of malaria parasite; the kits based on this test are hence panspecific [9].

P. falciparum and *P. vivax* malaria occur nearly in equal numbers as a single species infection in India. Their treatment differs; hence, differentiation between these two species with an appropriate RDT is essential. Accuracy of detection should also be noted. World Health Organization (WHO) has suggested the detection score against *P. falciparum* and *P. vivax* to be at least 75% at parasite level of 200/μL. False positive rate should be less than 10% and invalid result should be less than 5% [10]. These tests can even detect parasites sequestered in deep vascular compartment where microscopy would fail. As these antigens might persist even after successful treatment, these are not useful to monitor treatment or to quantify the parasite. Thus, they are not useful to prognosticate or judge therapeutic efficacy of antimalarial drugs. They are useful where microscopic diagnosis is not available or is not of acceptable standard. RDT can also serve as screening diagnosis where microscopy can be reserved for resolution of doubtful cases or confirmation of a negative RDT in spite of high clinical suspicion of malaria. Serological methods that detect antibodies against malaria are also available but should never be used for treatment decisions [11].

Typhoid Fever

Widal test: This test, used extensively to diagnose typhoid, is an outdated particle agglutination test. It detects antibodies in the patient's serum against the O an H antigen of *Salmonella typhi* and H antigens of paratyphi A and B [12]. Conventionally, a positive Widal test implies rising titer in paired blood sample 10 to 14 days apart, which is too long period for diagnosis. This test has suboptimal sensitivity and specificity [13]. Poor sensitivity is due to negative results early in infection, prior antibiotic therapy and poor immune response in certain individuals. Poor specificity is due to presence of baseline antibodies in endemic areas, cross reactivity with other gram negative enterobactericae and non typhoidal salmonella, and anamnestic reaction with unrelated infections like malaria.

Typhidot test: It measures both IgM and IgG antibodies against 50kDa outer membrane protein (OMP) in ELISA format. If IgM is positive, the test is considered positive whereas if IgM is negative and IgG is positive, it is considered indeterminate. In TyphidotM**,** only IgM antibodies are measured against the above mentioned antigen after completely removing the IgG molecules. Overall typhidot tests have a sensitivity and specificity close to 80%. However, these tests are not accurate enough to replace the gold standard blood culture for typhoid [15].

Tuberculosis

The problem of bacteriological diagnosis of tuberculosis (TB) in children is multiple. Children have difficulty in expectorating sputum, and they usually suffer from paucibacillary disease in childhood making the bacteriological diagnosis difficult. The expert MTB / RIF test is a major advance in the diagnosis of TB and detection of resistance to rifampicin. It is a cartridge-based fully automated NAAT for TB case detection. It purifies, concentrates, amplifies and identifies the targeted nucleic acid sequences in the organisms genome in a short time, providing results within 2 hours. It is a real time PCR molecular testing as it fully integrates and automatizes three processes – sample preparation, amplification and detection. WHO policy recommendation states that it should be used as an initial diagnostic test in all children suspected of having TB, acknowledging resource implication. It should be done in children suspected of having multidrug resistant TB (MDR) or HIV-associated TB [16]. The test has high sensitivity (88%) in detecting TB with negative predictive value more than 98% in both high and low prevalence TB settings. The false positive results are linked to detection of dead bacilli, which would not be picked up by culture [17]. The test can be performed in respiratory specimens like sputum, induced sputum and gastric aspirate. For extra pulmonary TB, CSF, lymph nodes and other tissues may be used. Performance of this test is relatively poor in pleural fluid. This test is not suitable for monitoring TB patients as they detect both live and dead bacilli. The test is not recommended for stool, urine or blood samples.

Dengue

Dengue produces symptoms and signs that resemble other viral infections and are non-specific. Patients may progress to severe dengue very early and supportive measures taken early can be life-saving; hence an early laboratory diagnosis is helpful. Virus isolation and nucleic acid detection is time consuming and needs sophisticated instruments, which are mostly not available. NS1 antigen is non structural protein that can be detected as early as first day of onset of symptoms to about 7 days, both in primary and secondary dengue. In secondary dengue, as patients have pre-existing IgG, they form immune complexes with the viral antigen. Two types of rapid tests are available for diagnosis of dengue infections [18-20]:

Card test: This is a rapid agglutination test where results are available in 20-30 minutes. It is a solid phase immunochromatographic test for qualitative detection of NS1 antigen and IgM and IgG antibody against dengue. In secondary dengue, as immune complex are formed with NS1 antigen, it may fail to detect the antigen. This test has poor performance as compared to ELISA test.

KEY MESSAGES

- Rapid diagnostic tests ensure definitive diagnosis of infections, thereby preventing unnecessary presumptive treatment.
- These usually provide qualitative results, and are not useful in monitoring treatment.
- These tests require minimum skill making them useful in places without sophisticated laboratory infrastructure.
- These tests must always be ordered on the basis of clinical setting, and not as a battery.

ELISA test: It takes longer time, about 5 to 6 hours, to detect NS1 antigen and IgG and IgM antibodies against dengue. This test can detect antigens that have formed immune complex with IgG antibodies, and thus has much higher sensitivity as compared to card test. There are high IgM and low IgG in primary dengue infection unlike in secondary infection. A 4-fold rise in IgG between acute and convalescent sera is diagnostic but has the drawback of need of two paired samples that results in delay in confirmation. Serum IgM may be undetectable in secondary dengue making the interpretation of the test difficult. The best time to test for antibodies is five days or more after the onset of symptoms.

TORCH Infections

TORCH is an acronym which groups together congenital or perinatally transmitted non-bacterial infections in neonates that share certain clinical and laboratory features. As detection of 'other infections' – which stand for the letter 'O' in TORCH – is increasing with time, doubts have been raised whether indiscriminate testing for a big group needs to be done. Immunochromato-graphic tests are available that detect IgG and IgM antibodies against the organisms [21]. However, these are not useful for CMV and HSV. If IgG is positive in the baby, it can be due to infection or could be transplacental antibody from the mother without true infection. On the other hand, if IgM is positive, it indicates recent infection. A negative test does not rule out infection as IgM may not be positive for weeks in infections like Herpes. It is always prudent to look for specific signs of individual infection and direct the investigation accordingly.

Viral Hepatitis

ELISA tests to detect IgG and IgM antibodies to hepatitis A virus may be done of which IgM indicates current active disease [22]. For Hepatitis B detection, multiple antigen and antibodies are required to establish the disease type. ELISA testing to detect antibodies against Hepatitis C denotes chronic disease but cannot diagnose acute disease. For Hepatitis E, both IgG and IgM antibodies can be detected in acute disease by ELISA test. In areas where hepatitis E is not endemic, nucleic acid testing must be done to confirm.

Diarrhea

Immunochromatographic tests are used to detect group A rotavirus antigens and adenovirus antigens from stools samples. False negative results are possible and test does not exclude coinfection with other pathogens. For *Clostridium difficile* associated diarrhea, detection of cytotoxin by ELISA test is done, but it does not establish the diagnosis unequivocally.

Meningitis and Meningoencephalitis

Latex agglutination test in the CSF is used for rapid detection of capsular polysaccharides of common meningeal pathogens – pneumococcus, *H. influenzae* type b and meningococcus. One of the advantages is that prior administration of antibiotics does not influence the test. However, both false positive and negative results can occur [23]. These tests may be recommended for patient with negative gram stain and CSF culture [24]. For Japanese encephalitis, detection of IgM antibodies in the CSF by ELISA is diagnostic.

Others

Rapid test are available to detect antibodies to HIV 1/2 and 'O' subtypes and HIV1 p24 antigen in serum or plasma. However, they should never be used alone to diagnose or exclude HIV infection. Detection of scrub typhus by IgM ELISA test is available but should be interpreted in the context of clinical presentation and other laboratory findings. Rapid tests are also done to detect antibodies to recombinant antigen RK39 for Kala azar. Tests are also available to detect Mycoplasma IgM, amebic liver abscess, Hydatid disease and Chikungunya. Rapid tests can diagnose respiratory syncytial virus (RSV) from nasopharyngeal samples to prevent unnecessary antibiotics in infants with respiratory distress. Similarly Group A streptococcus (GAS) can be diagnosed from pharyngeal specimens; though RDTs cannot distinguish between true infection and colonization.

Multiplex PCR, which combines several probes, can identify 25 or more common bacterial and fungal pathogens responsible for blood stream infections. In addition, it can point out presence of resistance genes like *mec A, van A/B* and carbapenem resistance [4]. This not only reduces the use of multiple antibiotics but also promotes targeted therapy.

CONCLUSION

Numerous rapid tests are now available, which can rationalize antibiotic therapy and halt the relentless march of drug resistance. With more and more RDTs being available, it is the duty of treating physician to select the right RDT based on the clinical presentation, prevalence of the organism in the community or hospital, and the cost and time to result. Shot gun approach to do the entire battery of tests at one go will only defeat the purpose of the tests.

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