The dot-enzyme immunoassay (EIA) - TYPHIDOT -is a relatively newer serologic test based upon the presence of specific IgG and IgM antibodies to a specific 50-kD outer membrane protein (OMP) antigen on S. *typhi*.

The test incorporates nitrocellulose strips impregnated with the OMP antigen and separately identifies IgM and IgG antibodies.This test qualitatively detects the presence of IgM class antibodies to Lypopolysaccharide Specific to S. typhi in human serum/ Plasma or whole blood specimens.

Preliminary data have shown sensitivity and specificity of 95% and 86%, respectively.

There are reports that support the contention that the Widal test has poor diagnostic value in children with typhoid fever. Antibiotic therapy has been shown to alter the antibody response to S. typhi infection (titers against O antigens). The Typhidott has been reported as significantly more sensitive than the Widal test. The relative low sensitivity of the blood culture in diagnosing typhoid fever is understandable in the wake of widespread antibiotic use in our country and the difficulties of obtaining large enough blood volumes for cultures from children.

Although bone marrow cultures significantly increase the yield from cultures, they are invasive and difficult to obtain. It must be emphasized that although cultures are associated with a lag period of at least 48 hr for preliminary confirmation of infection, with the recent emergence of drug resistance among S. typhi, they remain an essential investigation.

In many circumstances, especially among partially treated cases presenting to health facilities, combining cultures with a rapid serologic test may reduce the diagnostic difficulty in typhoid fever. Recent data indicate that combining the blood/bone marrow cultures with a Typhidot-Mt will significantly improve the diagnostic yield of these investigations among children who have previously received antibiotics.

We conclude with the thought that neither the Widal nor Typhidott tests are a substitute for cultures in typhoid fever but using these tests in combination increases the negative predictive value.

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THE IMPORTANCE OF CANCER PROTOCOLS IN REPORTING CANCER RESECTION SPECIMENS.

The Cornerstone of oncologic practice and determining optimal patient care is the pathology report for resected cancer specimens. Indeed the culmination of the entire complex process of professional assessment of a cancer resection specimen is the final synopsis or cancer case summary that goes out to the referring clinician embodied in the pathologic report.

In view of the great heterogeneity in the reporting of cancer resection specimens, the cancer committee of the college of American pathologists (CAP) developed a series of cancer protocols for the more common carcinomas, first published in 1998, which are now mandatory for pathologists at all COC (Commission on cancer) approved cancer programs in the United States.

The lack of uniformity and standardization in reporting cancer resections in this country, however leads to serious therapeutic dilemmas among clinicians and oncologists with critical elements required for patient management and prognostication, often missing from the report.

Despite ever increasing work volumes and the inherent complexity of incorporating the large number of formats and protocols essential to cancer reporting, the anatomic pathology wing of Dr. Lal Path Labs has successfully accomplished this through a dedicated customized software incorporating all the CAP – approved checklist for cancer resections. Indeed, we have been routinely using these for more than a year.

The use of standardized reporting enhances our participation in multi-disciplinary superspecialty cancer care, apart from ensuring uniformity of reporting within the department. It also ensures the inclusion of all therapeutically and prognostically relevant data and serves as an important quality parameter.

It is our mission to enhance and augment the role of pathologists as leaders in the practice of oncology in this country and the use of cancer protocols are inextricably and indelibly linked to the achievement of this end.

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FROM THE EDITOR'S DESK

As a health problem, tuberculosis does not enjoy the high profile of HIV/AIDS. Nonetheless Tuberculosis is highly communicable and not far behind HIV/AIDS in fatalities among infected persons. While the Directly Observed Treatment Shortcourse (DOTS), a highly effective low cost technology treatment recommended by the World Health Organization (WHO) has helped to cure millions, incidence of the disease has tripled with high levels of HIV.

Forty years after the first time antimycobacterial treatment became widely available, the world still has to cope with rising levels of TB incidence. There is little doubt that the doomed couple, which TB forms with HIV, has brought this disaster about. On the whole TB cases are rising and although tuberculosis is a curable disease, it is not a cheap disease.

Improving diagnostic testing will go a long way in the combat against tuberculosis. In this issue we give a detailed guideline on the use of Quantiferon TB Gold assay as a newer, more specific diagnostic aid to Latent TB Infection.

Also included in this issue covering new diagnostic aids for infectious diseases are the Dengue NS1 antigen assay and the Typhidot rapid serological test. Early diagnosis aids in the institution of rapid therapy and reduction of morbidity associated with these infections. These rapid tests aid in an end to neglect of what would come across as routine febrile illness at first instance.

Cancer resection specimens are invaluable tools to therapy decisions and to achieve this end, the anatomic pathology department at Dr Lal Path labs has customized reporting protocols for these specimens to incorporate and ensure the inclusion of all therapeutically and prognostically relevant data.

We are proud to be under the expert and able guidance of Dr Arvind Lal, who has been felicitated with the Padma Shree award in recognition of his contribution to the medical field. It will be our endeavour always to maintain the standards he has established.

Your appreciative feedback is very encouraging and we look forward to suggestions to help us improve and provide you the best quality information in the field of diagnostics and their application in clinical diagnosis and treatment. We look forward to your participation and continued support.

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Dr Lal PathLabs
INSIGHT
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PADMA SHREE AWARD FOR DR ARVIND LAL

Dr. Arvind Lal, Chairman & Managing Director of Dr. Lal PathLabs, has been conferred the Padma Shree award by the President of India in recognition of his pioneering efforts in bringing Laboratory services in India at par with the western world.

Under his expert guidance and leadership, Dr. Lal PathLabs, the 60 years old Laboratory service provider has become one of the most reputed laboratories in Asia, having to its credit the highest number of eight accreditations from the National Accreditation Board for Testing and Calibration Laboratories (NABL, Ministry of Science and Technology, Govt. of India). He brought International recognition to the Indian Healthcare Industry in the form of accreditation from the College of American Pathologists (CAP - USA). Dr. Lal PathLabs tests a record number of 9,000 patients every day from the largest test menu in India of over 1,650 tests and panels.

Dr. Lal has revolutionized laboratory medicine by introducing the maximum number of new tests, instruments and ICT systems i.e. Information, Communications-Technology systems in India. His R&D unit is recognized by the Department of Science and Technology, Government of India. This unit has done India proud by developing the latest tests in Molecular Diagnostics like the Real Time PCR for HIV Viral Load Studies, Tuberculosis - India's number one infectious disease and Genotyping for Hepatitis C Virus - the biggest killer of all liver diseases. The aim of the R&D division is to provide highly specialized tests at low cost to the weaker sections of the society.

Dr. Lal is a member of many expert and advisory bodies constituted by the central and the state governments. He is accredited by having the first PPP (Public Private Partnership) in the field of laboratory testing with the Government of Tripura since 2004. Dr. Lal is also an active member of the Confederation of Indian Industries (CII) Healthcare Committee and has played a significant role in promoting the Indian Healthcare sector abroad and in promoting PPP's initiatives with the union government. He is the founder Vice President of ACRO (Association of Clinical Research Organizations) for furthering the cause of Clinical Research in India. Dr. Lal was appointed as the Honorary Physician to the President of India in 2001. He has also been serving as a pathologist to many present and past Prime Ministers of India. His laboratory is the nodal laboratory testing the largest number of government employees from CGHS, ECHS, Ministry of Railways, Delhi Government and many others.

Dr. Lal has been honoured with Lifetime Achievement Award in Medicine in 2003 and the Delhi Ratan Award in 2005 for his extraordinary contribution in the field of Laboratory Medicine. He has also been the recipient of the International Business Council Award and the Indira Gandhi Solidarity Award in 1994. Under his leadership, in July 2008, his laboratory was bestowed, in the healthcare category, with the prestigious CNBC TV 18 and ICICI 'Emerging India Award' presented in London, U.K.

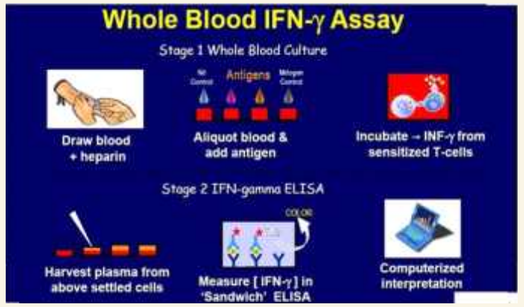
This award is a felicitation to his pioneering efforts and we are honoured to be working under his able guidance.

QUANTI FERON – TB GOLD TEST..... WILL IT REVOLUTIONIZE THE DIAGNOSIS OF TB INFECTION ?

Tuberculosis (T B) Is a highly contagious respiratory disease caused by bacteria of the Mycobacterium tuberculosis complex.

Latent TB infection , which is a non-communicable asymptomatic condition results when a person becomes exposed to Mtb and their body controls, but does not eradicate the infection.

Immunosuppression, can result in re- activation of the Latent tuberculosis and development of tuberculosis disease. Diagnosis of Latent TB infection (LTBI) is an important step in preventing reactivation of tuberculosis, particularly in the risk populations and treatment of Latent TB can markedly reduce the risk of progression to active disease.



The Quantiferon TB Gold assay is an aid in –vitro in the diagnosis of Mtb infection. QFT-G is a type of INF-γ release assay conducted on sensitized white cells after whole blood is incubated with antigen. The FDA approved QFT-G is approved as an aid for diagnosing both active TB disease and LTBI. Previous studies have demonstrated a sensitivity of 80% for the QFT-G in persons with untreated culture-confirmed TB. The predictive value depends on the prevalence of TB in the community.

The test uses three recombinant peptides from Mtb (ESAT-6,CFP-10 and TB7.7) to stimulate T cell interferon gamma production in individuals with Mtb infection. These peptide antigens do not usually stimulate lymphocytes from uninfected, BCG vaccinated persons without disease or risk for latent TB.

Previously, Tuberculin skin test (TST / Mantoux reaction) was the only method for assessing TB infection. Though widely used it has its own limitations like

1. Poor inter-reader reliability - 9 mm (negative) vs. 10mm (positive)?
2. False-positives/specificity
   a. NTM infection
   b. Prior BCG
3. Cost/time of patient visits -
   a. Unread tests
4. Sensitivity?
   a. Reaction wanes over time
   b. Lack of gold standard

These limitations have been addressed in Quantiferon TB Gold assay.

- The stimulation of lymphocytes with Mtb antigens occurs in the phlebotomy tube, rather than in the patient's arm. As a result there is no booster effect from repeated testing as in the case of tuberculin skin test.
- Results of Quantiferon TB Gold are available following a single patient visit without the need for a second visit to evaluate the skin test.
- The in vitro assay is not associated with adverse hypersensitivity reactions.
- The recombinant antigens chosen as stimulants in the new assay are not present in the BCG vaccine. Therefore, the assay is more specific and prior BCG vaccination will not cause false positives in this test, as it does in the tuberculin skin test.
- In addition the interpretation of the lab test is more objective.

This test is more sensitive and specific method for the assessment of tuberculosis infection both latent and in active disease.

Principle - If the patient is infected with M tuberculosis , the white blood cells will release Interferon gamma in response to contact with the TB antigens. The Quantiferon TB gamma results are based on the amount of interferon gamma that is released in response to the antigens.

Each quantiferon TB Gamma result and it's interpretation should be considered in conjunction with other epidemiological, historical, physical, and diagnostic findings.

The advantages of the test are:

1. Requires a single patient visit to draw a blood sample.
2. Result can be available within 24 hours.
3. Does not boost responses measured by subsequent tests , which can happen with tuberculin skin test.
4. Is not subject to reader bias that can occur with tuberculin skin test.
5. Is not affected by prior BCG.

The disadvantages and limitations :

- Blood samples must be incubated with test antigens within 12 hrs after collection while white cells are still viable.

At Dr Lal Path Labs, the sample is directly collected at source in the special tubes coated with test antigens to ensure immediate incubation of the sample.

- There are limited data on the use of Quantiferon –gamma in children younger than 17 yrs of age, among persons recently exposed to M. tuberculosis, patients on immunosuppressive drugs, selected hematological disorders, specific malignancies, diabetes, silicosis, and chronic renal failure.
- Limited data is available on the use of Quantiferon –gamma TB test to determine who is at risk for developing TB disease

Like the TST, QFT-G cannot distinguish infection associated with TB disease from LTBI. For definitive diagnosis of LTBI, TB disease must be ruled out by medical evaluation, which should include ascertaining history of suggestive symptoms and signs, chest x-ray, and examination of sputum or other clinical samples for M. tuberculosis when indicated. As for other diagnostic tests, the prevalence of M. tuberculosis infection in the population being tested affects the predictive value of QFT-G results.

Conclusion

Specificity of Quantiferon TB-Gamma is more than Tuberculin skin test.

Sensitivity of Quantiferon TB- Gamma for TB disease is equal to Tuberculin skin test.

Dr. A.K.Kapoor, Consultant/H.O.D. Front Office, Main Lab

CDC GUIDELINES ON USE OF QUANTIFERON TB GOLD TEST

Use of the QFT-G

- The CDC recommends that the QFT-G be used in all settings in which the TST is currently used, including contact investigations, evaluation of recent immigrants with BCG vaccination, and TB screening of healthcare providers.
- The QFT-G can be used in place of (and not in addition to) the TST.
- A positive QFT-G result should prompt the same medical interventions as a positive TST result.
- Persons with a positive QFT-G result should have active TB excluded before LTBI is diagnosed, at minimum, with a plain chest x-ray.
- No reason exists to follow a positive QFT-G result with a TST.
- Once active TB is excluded, treatment of LTBI should be considered for a positive QFT-G result.

Advantages of the QFT-G vs the TST

- The results can be available within 24 hours without a second visit, whereas the TST requires a second visit for reading at 48 to 72 hours.
- The test responds to antigens more specific for M. tuberculosis and is therefore independent of BCG status unlike the TST.
- The QFT-G is not subject to errors in placement and reading since it is a blood test.
- The QFT-G is also less dependent on previous nontuberculous mycobacterial infection.
- Injection PPD testing can boost subsequent TST responses, while the QFT-G is not affected by boosting from a previous TST.
- The QFT-G can detect both LTBI and active TB.

Disadvantages and uncertainty about the QFT-G

- Errors in collection, transport, and interpretation can reduce the accuracy of the QFT-G.
- A minimum of 5 mL of blood is needed, which may not be acceptable for younger children.
- The specimen has to be analyzed (incubated with test antigens) within 12 hours of collection, which may not be feasible for some settings in which trained laboratory personnel are not locally available and the specimen has to be transported outside the facility.
- The ability of the QFT-G to differentiate LTBI for active TB has not been determined.
- No published data document the performance of the QFT-G in children younger than 17 years.
- Published data for persons recently exposed to TB (eg, contacts) and other populations at high risk for LTBI are not available.
- The performance of the QFT-G has not been evaluated in immunocompromised persons, including those with HIV and those taking immunosuppressive drugs, corticosteroids, and tumor necrosis factor–alpha antagonists.
- The sensitivity of the QFT-G may be less than that of the TST.
- The ability of the QFT-G to predict progression from LTBI to active TB disease has not been determined.

Pearls for Practice

- The QFT-G should be used in all settings in which the TST is currently used, including contact investigations, evaluation of recent immigrants with BCG vaccination, and TB screening of healthcare providers.
- Advantages of the QFT-G vs the TST include specificity for individuals with a history of BCG vaccine and convenience of administration. Data on the sensitivity of the QFT-G for children, immunocompromised individuals, and its ability to predict disease progression are needed.

DENGUE NS1 ANTIGEN

We all remember the mad rush of patients and doctors alike for repeated platelet counts during the dengue season and despite efforts by the authorities to control the mosquito menace dengue fever did grip Delhi and its neighboring areas.

With an aim to establish an accurate diagnosis of acute dengue virus infection early, in order to provide timely information for the management of patients and early public health control of dengue outbreak Dr Lal Path labs added a new rapid test Dengue NS1 antigen to its test menu.

Dengue is the most important arbovirosis in terms morbidity and mortality.

Early laboratory diagnosis of acute dengue virus infection still remains a problem. At present, the three basic methods used by most laboratories for the diagnosis of dengue virus infection are:

1. Viral isolation and identification
2. Detection of viral genomic sequence by a nucleic acid amplification technology assay (rt-pcr)
3. Detection of dengue virus-specific igm antibodies by the igm-capture enzyme-linked immunosorbent assay (MAC-ELISA) and/or the rapid dengue immunochromatographic test (DIT).

Though virus isolation and characterisation are considered as the gold standard of laboratory diagnosis for acute dengue virus infection, it is expensive and it takes at least 6–10 days for the virus to replicate in tissue cell culture or laboratory mosquitoes.

Detection of viral genomic sequence by RT-PCR is also an expensive method and is not widely available in most hospital diagnostic laboratories.

The third method, assay of antidengue specific IgM, depends on the time taken for an infected person's immunological response to produce IgM antibodies against dengue virus antigens.

Thus, both DIT (often considered as the rapid test for diagnosis of dengue infection) and MAC-ELISA do not provide early diagnosis of acute dengue infection, as in most cases, the first detectable IgM only appears on Days 4–5 of the illness. Moreover, a single serological detection of IgM is merely indicative of a recent dengue virus infection, and should not be interpreted as a diagnosis of acute infection without a paired second serum sample.

Recent studies have shown **DENGUE NS1 AG** test gives an:

- Overall higher sensitivity rate than the current three established diagnostic test methods for laboratory diagnosis of acute dengue infection.
- Compared to dengue virus isolation and molecular detection of viral rna, the ns1 antigen-capture elisa gave a higher positive detection within the first four days of illness.
- Also, the ns1 antigen-capture elisa has the added advantage of continuing to give good detection rates up to seven days of the illness.
- It has also been evaluated in some studies that, the NS1 antigen-capture ELISA gave a significantly higher detection rate in acute primary dengue than in acute secondary dengue. Despite the lower detection rate for serum samples from patients with acute secondary dengue, this was still higher than the other dengue diagnostic methods.

Dengue NS1 antigen test is an easy to perform, rapid and specific test is needed to confirm infection during the acute phase of the infection in order to implement appropriate treatment during the early course of the disease. NS1 antigen is a non structural protein recognized as a marker of acute phase of dengue infection, a period for which traditional serological antibodies based methods are of limited value. NS1 antigen has been found circulating in the sample of infected patients from the first day and up to 9 days after onset of fever.

Advantages:

1. The test can be performed in human serum or plasma.
2. It is a disposable test using lateral flow immunochromatographic technique.
3. Rapid test ; result can be read within 15- 30 minutes.
4. It has a high sensitivity of 92.3% and a specificity of 100%.

Take home message

Thus, antigen-capture ELISA should be considered as the test of choice for patients suspected of acute dengue illness, especially those with fever lasting five days or less. For those patients with a history of fever for more than six days and are suspected to have acute dengue infection, the test could also be considered concurrently with an assay of dengue specific IgM.

TYPHIDOT

Another common disease which needs rapid diagnosis and is inherently endemic in Indian settings is Typhoid Fever. In this respect Typhi Dot (Typhi IgM antibody) is an valuable tool in diagnosis of acute stage in typhoid fever.

Typhoid fever is widely recognized as a major public health problem in our country. In the wake of emerging multidrug-resistant strains of bacteria causing typhoid fever, the disorder is known to be associated with significant morbidity and mortality. It is also recognized that a delay in diagnosis and institution of appropriate therapy may significantly increase the risk of adverse outcome and mortality. Although the isolation of Salmonella typhi on blood culture remains the gold standard for diagnosing typhoid fever, the widespread availability and use of antibiotics in the community makes it frequently difficult to isolate the organism on blood cultures and alternative methods such as bone marrow cultures may be required. However, the latter are invasive and difficult to obtain routinely in pediatric patients.

Despite improved methods of bacteriologic isolation, there is a real need for rapid serologic diagnostic tests for typhoid fever.

Accurate diagnosis of typhoid fever at an early stage is not only important for etiological diagnosis but to identify and treat potential carriers and prevent acute typhoid fever outbreaks. Early rising antibodies to lipopolysaccharides O are predominantly IgM in nature. Detection of S. Typhi specific IgM antibodies instead of IgG or both IgG and IgM (as measured by WIDAL TEST) serves as a marker for recent infection.

Dr Lal PathLabs