Active tuberculosis develops only in some people after infection. Almost all tuberculous infections are asymptomatic and remain latent. Latent tuberculosis infection (LTBI) itself progresses to active disease in approximately 5-10% of infected persons. LTBI represents a vast reservoir of potential cases of tuberculosis around the world. This reservoir of LTBI is therefore a major barrier to the ultimate control and elimination of tuberculosis. The rate of progression of LTBI to active disease is much more in immunocomprised patients. Diagnosis and treatment of LTBI are therefore very critical. LTBI until very recently has been diagnosed exclusively by tuberculin skin testing (TST). The TST is fraught with problems including relatively poor sensitivity and specificity. There is a need for tests that give more rapid diagnosis, allowing for earlier treatment of cases, decreased transmission of infection and decreased expenditure of resources. This is especially for the developing countries where tuberculosis and HIV are rampant. There is also a need for increased sensitivity of testing so that cases do not go unrecognized, and for increased specificity and negative predictive value to decrease the cost of having a high suspicion for this disease. Newer tests for LTBI offer the promise of greatly improved diagnostic accuracy\(^1,2\).

The new generation tests for the diagnosis of LTBI are:
1. QuantiFERON-TB (QFT)
2. QuantiFERON-TB Gold (QFN Gold)
3. T SPOT-TB test
4. Nucleic Acid Amplification (NAA) assays
5. Adenosine deaminase (ADA) levels
6. Newer culture technologies
7. Rapid diagnosis of Multi-drug resistant tuberculosis (MDR TB)
8. Genotyping Studies which are mainly used epidemiological studies.

**QuantiFERON-TB Test\(^3\)**

This test is an in vitro diagnostic aid that measures a component of cell-mediated immune reactivity to *M. tuberculosis*. The basis of this test is the detection in serum of the release of IFN-gamma on stimulation of sensitized T cells by antigens of *M. tuberculosis*.

Whole blood is incubated overnight with purified protein derivative (PPD) from *M. tuberculosis* and control antigens. The IFN-\(\gamma\) released from sensitized lymphocytes is subsequently quantified by ELISA. The antigens included in the test kits are PPD from *M. tuberculosis* (tuberculin) and PPD from *Mycobacterium avium* (avian sensitin). The kits also include phytohemagglutinin (a mitogen used as a positive assay control), and saline (negative control or nil).

**Interpretation**

QFT results are based on the proportion of IFN-\(\gamma\) released in response to tuberculin as compared with mitogen. Interpretation of QFT results is stratified by estimated risk for infection with *M. tuberculosis*, in a manner similar to that used for interpreting TST with different cut-off values. A positive result suggests that *M. tuberculosis* infection is likely; a negative result suggests that infection is unlikely; and indeterminate result suggests QFT results cannot be interpreted as a result of low mitogen response or high background response. Each QFT result and its interpretation should be considered in...
conjunction with other epidemiological, historical, physical, and diagnostic findings.

PPD antigens are shared across mycobacterial species, including *M. bovis* BCG. A positive response, therefore, lacks specificity for *M. tuberculosis* infection and may reflect infection with NTM or vaccination with BCG. However, early studies were encouraging, and demonstrated decreased false positive results with QFT relative to the TST in BCG-vaccinated individuals and those exposed to NTM. Thus the sensitivity and specificity of QFT was better than TST.

**When should the test be used?**

QFT can be used in all circumstances in which the tuberculin skin test (TST) is currently used, including contact investigations, evaluation of recent immigrants who have had BCG vaccination, and TB screening of health care workers and others who are at an increased risk for LTBI.

Before the QFT is conducted, arrangements should be made with a qualified laboratory and courier service, if needed, to ensure prompt and proper processing of blood. Blood collected for QFT should be transported to the concerned laboratory within 12 hours.

**Advantages**

- Requires a single patient visit to draw a blood sample
- Results can be available within 24 hours
- Does not boost responses measured by subsequent tests, which can happen with tuberculin skin tests (TST)
- Is not subject to reader bias that can occur with TST
- Is not affected by prior BCG (Bacille Calmette-Guérin) vaccination

**Disadvantages and Limitations**

- Blood samples must be processed within 12 hours after collection while white blood cells are still viable.
- There are limited data on the use of QFT in children younger than 17 years of age, among persons recently exposed to *M. tuberculosis*, and in immunocompromised persons (e.g., impaired immune function caused by HIV infection, current treatment with immunosuppressive drugs, selected hematological disorders, specific malignancies, diabetes, silicosis, and chronic renal failure).
- Errors in collecting or transporting blood specimens or in running and interpreting the assay can decrease the accuracy of QFT.
- Limited data on the use of QFT to determine who is at risk for developing TB disease.

**QuantiFERON-TB Gold (QFN Gold)**

This test is a more specific form of QFT. Instead of PPD, the test uses antigens that are highly specific to *M. tuberculosis*. These antigens include the early secretory antigenic target 6 (ESAT-6) and culture filtrate protein (CFP-10). These low molecular weight proteins, encoded within the region of difference 1 (RD1) of the *M. tuberculosis* genome, are more specific to *M. tuberculosis* than PPD, and they are not shared with the BCG sub strains and most NTM species (with the exception of *M. kansasii*, *M. marinum* and *M. szulgai*). Tests based on these TB specific antigens are called RD1 based IFN-γ assays. With the introduction of RD1 based assays, PPD based IFN-γ assays are already being phased out.

**T SPOT-TB Test**

This is a new test that detects both active and latent tuberculosis infection. It uses a new technique called ELISPOT. It is an ex vivo T-cell-based assay for the detection of cell-mediated immunity, for use in detecting *M. tuberculosis*. The technique detects and enumerates peripheral blood gamma-interferon secreting T cells in response to TB antigens. These t cells are counted as spots on a test plate. The T-SPOT.TB is more accurate at detecting latent TB infection than the tuberculin test.

**Advantages**

1. It can also be used in immunocompromised patients and produces accurate results for HIV-positive people,
2. It is a highly sensitive test (sensitivity being about 85-95%).
   a. It correlates significantly better with the degree of exposure.
   b. In healthy BCG-vaccinated individuals, this test was not confounded by BCG. Previous BCG vaccination might trigger false-positive results from the tuberculin test because it does not distinguish whether a person has TB infection or has produced antibodies in response to the BCG vaccination, according to the study.
3. The specificity of this test is also much more than TST.
   a. In patients infected with non-tuberculous mycobacteria, no significant positive association has been found.
Nucleic Acid Amplification (NAA) Assays

The NAA assays amplify *M. tuberculosis*-specific nucleic acid sequences using a nucleic acid probe. NAA assays enable direct detection of *M. tuberculosis* in clinical specimens. Such assays complement the conventional laboratory approach to the diagnosis of active disease.

AFB smears are rapid, but lack sensitivity and specificity. Culture is both sensitive and specific, but results are obtained after 2-8 weeks. NAA assays, however, allow rapid, sensitive and specific diagnosis of *M. tuberculosis*. The sensitivity of NAA assays is at least 80% in currently used tests and the specificity is 98-99%. The sensitivity of these assays in AFB smear-negative samples is lower than for smear-positive samples.

The NAA assays that are currently commercially used are the AMPLICOR *M. tuberculosis* Test, the Amplified Mycobacterium Tuberculosis Direct (MTD) Test and its reformulated form – AMTDII or E-MTD – for enhanced MTD.

1. AMPLICOR *M. tuberculosis* Test: uses DNA polymerase chain reaction (PCR) to amplify nucleic acid targets.
2. The MTD assay is an isothermal strategy for detection of *M. tuberculosis* rRNA. The E-MTD has an improved sensitivity, especially in smear-negative specimens. The positive predictive value of E-MTD is low (59%) in persons with low risk for tuberculosis, but high (almost 100%) in persons with intermediate and high risk. The negative predictive value is high (>90%). These results compared favorably with the AFB smears. Therefore E-MTD is particularly helpful for confirming disease in intermediate- and high-risk persons and for excluding cases in low-risk patients.

CDC Recommendations (2000)

AFB smear and NAA should be performed on the first sputum sample collected.

- If smear and NAA are both positive, diagnosis of pulmonary tuberculosis is certain.
- If smear is positive and NAA is negative, the sputum should be tested for inhibitors by spiking the sputum sample with an aliquot of lysed mycobacterium. Tuberculosis and repeating the assay. If inhibitors are not detected, the process is repeated on additional specimens. If the sputum remains positive, inhibitors are not detected, and NAA is negative, it can be assumed that the patient has infection with NTM.
- If a sputum smear is negative, E-MTD is positive, additional samples should be tested. If further samples are E-MTD positive, it is assumed that the patient has pulmonary tuberculosis.
- If both sputum smear and E-MTD are negative, an additional specimen should be tested by E-MTD. If negative, it can be assumed that the patient does not have infectious pulmonary tuberculosis.

CDC concludes that clinical judgment is final and that definitive diagnosis rests on response to therapy and culture results. In persons with intermediate or high risk for pulmonary tuberculosis, if sputum smear is negative, a presumptive diagnosis of tuberculosis is made if NAA is positive. NAA should not be performed on sputum samples from cases who have negative sputum smear and a low risk for tuberculosis.

Limitations

1. It is expensive and hence cannot be used in the developing world where tuberculosis is rampant.
2. NAA does not give drug sensitivity information.
3. It detects nucleic acids from both dead and living organisms. Hence may be false positive for active disease in patients who have a recent history of infection and have been adequately treated.

NAA assay that use *M. tuberculosis* mRNA are under study. *M. tuberculosis* mRNA has a half-life of only minutes. Hence its assays are positive only while viable mycobacteria are present, and therefore are useful as sensitive indicators of adequate treatment and for rapid detection of drug susceptibility.

NAA assays have a role in the diagnosis of extrapulmonary tuberculosis. The overall sensitivity in non-respiratory specimens ranges from 67-100%. The sensitivity is more in cerebrospinal fluid than pleural fluid.

Adenosine Deaminase (ADA) levels

ADA levels have shown great promise in the diagnosis of extrapulmonary tuberculosis especially pleural effusion and pericardial effusion. Its value for diagnosing tuberculous meningitis is limited.

Cultures

There are three types of conventional culture media: solid, which include egg-based media (Lowenstein-Jensen), agar-based and liquid-based media. Newer culture technologies are in development. One such medium is TK Medium. TK Medium uses multi-color dye indicators that identify *M. tuberculosis* rapidly. It can also be used for drug-susceptibility testing, and can differentiate a contaminated specimen.
**Multidrug-resistance Tuberculosis**

The detection of rifampicin resistance may be used as a surrogate for uncovering multi resistance, because most rifampicin-resistant isolates are also isoniazid-resistant. Rifampicin resistance signals the need for treatment with secondary drugs. Rapid detection of rifampicin resistance can be detected by identifying genotypic abnormalities by linear probe assays or molecular beacons; or phenotypic abnormalities by phage amplification.

**Genotyping**

TB genotyping results, when combined with epidemiologic data, help to:

- Distinguish TB patients who are involved in the same chain of recent transmission.
- Identify TB patients whose disease is the result of reactivation of a TB infection that was acquired in the past.
- Monitor our progress toward eliminating TB transmission.

TB genotyping identifies genetic links between *Mycobacterium tuberculosis* isolates from different TB patients. If two TB patients have isolates with non-matching genotypes, it indicates that the two patients are not involved in the same chain of recent transmission (recent transmission is defined as TB transmission that has occurred within the previous 2 years). When two patients have isolates with matching genotypes, in some of these situations, the two patients will be involved in the same chain of recent transmission, but in other situations these patients will not be involved in the same chain of recent transmission. The key to determining if TB patients with matching genotypes are involved in the same chain of recent transmission is to investigate whether the patients share epidemiologic links that can explain where and how they might have transmitted TB among themselves.

By helping to identify TB patients who are involved in recent transmission, TB genotyping will have the following impact in prevention and control of tuberculosis:

- Outbreaks will be detected earlier and controlled more rapidly.
- Incorrect TB diagnoses based on false-positive culture results will be identified more easily.
- Unsuspected relationships between cases and new and unusual transmission settings will be discovered.
- Transmission that occurs between patients who reside in different jurisdictions will be detected more readily.
- TB programs will be able to evaluate completeness of routine contact investigations and progress toward TB elimination by monitoring surrogate measures of recent TB transmission.

**Conclusion**

Current evidence suggests that both the TST and IFN-γ assays have advantages and limitations. Both tests appear to be useful. No single test will suit all conditions. The development of IFN-γ assays has increased the diagnostic tools available for LTBI. It is important to consider both tests as part of an expanding armamentarium of TB diagnostics. The test to be selected will depend on the population, the goal of testing, and the resources available e.g. the RD1 based IFN-γ assay might be a more appropriate test for serial monitoring of health care workers in many developed countries as a key component of nosocomial TB control. It will eliminate the need for repeat visits and two steps TST testing, avoid boosting, and minimize interpretational difficulties. IFN-γ assays will be helpful in low endemic populations where cross reactivity due to BCG and NTM pose problems in TST interpretation because they have higher specificity. It is also likely to reduce false positives and enhance targeted LTBI treatment, particularly in low incidence settings. Cross reactivity due to BCG appears to be an important issue in some populations where BCG vaccination is not given at birth, but later on in life. IFN-γ assays may be helpful in screening populations such as HIV-infected individuals, homeless individuals, and injection drug users in whom the low positive rates for TST is a major concern.

In high-burden and resource-limited settings such as India, where even access to simple technology such as sputum microscopy may be poor, the TST might continue to serve a useful purpose. In the Indian context, a 15 year follow up of 280,000 subjects from the south Indian BCG vaccine trial has showed that TST response is significantly associated with development of active TB. This study and many other Indian studies suggest that the TST remains a useful test that is also cost-effective. Also, data from several studies in India suggest that BCG vaccination may not pose a major problem in the interpretation of TST results. BCG might adversely affect TST results in other populations, depending on BCG strain used, timing of vaccination, frequency, and time elapsed since vaccination. It is therefore important to consider these issues carefully before selecting the appropriate test.

The utility of IFN-γ assays in high burden countries such as India needs further evaluation. Long term studies are needed to determine the association between positive IFN-γ results and the subsequent risk of active TB. If these
studies consistently demonstrate a strong association between positive IFN-γ results and risk of active TB, and if the effect is stronger than that of TST, it can be inferred that IFN-γ assays have a better ability to identify those at higher risk for progressing to active disease. Till then, it is probably a good strategy to keep both TST and IFN-γ assays on the LTBI diagnostic menu, and select the appropriate test based on the population, the purpose of testing, and the resources available.

REFERENCES

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