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Tuberculin Test: A Reappraisal for the Modern Internist

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Abstract

As India is aiming for Tuberculosis elimination, there is an urgent need for strengthening both the diagnostic and therapeutic aspects of the disease. The Tuberculin test is an age-old diagnostic method for tuberculosis infection, both active and latent. But interpretation of the test is still mired in controversy and clinicians often fail to agree on the significance of the test. This article is aimed at resolving some of the controversy surrounding the test and presenting current evidence on its proper use. Interpretation of the test in different clinical settings (like after BCG vaccination and in HIV positive persons) has been discussed. The molecular basis of the test has also been touched upon. A comparison with the IGRA test has been presented. Lastly, rationale for use of the test in the Indian setting has been discussed by the authors.

Key words: Mantoux test, Latent TB, PPD, induration, BCG vaccine.

Introduction

Mycobacterium tuberculosis infection remains one of the main public health problems in developing countries like India. The World Tuberculosis Report, 2022 released by the WHO, shows that India is the country with the highest tuberculosis (TB) burden in the world and the country with the highest proportion of TB deaths[1]. Parallel to the high incidence of TB in the country, another caveat is the rise of multi-drug resistant TB infections[1]. However, in India, a large number of TB patients are still undiagnosed[2]. Out of the projected number of new TB patients in the country, only about 60% are documented each year[2]. So, various operational issues including, but not limited to, the lapses in diagnostic methodology contribute to this large chunk of "missing" TB patients in the country. These patients may act as superspreaders and further propagate the silent epidemic. So, like any other epidemic, control measures must start with proper and timely diagnosis.

Thus, it is imperative that India has a robust method for early diagnosis of TB. The gold standard for diagnosis of TB is microbiological proof of the infection: direct AFB (Acid-Fast Bacilli) stain, mycobacterial culture or genetic tests. Besides this technique, the other major diagnostic process is the tuberculin test[3]. This test has considerable importance from epidemiological point of view but is often under-utilized. As subsequent discussions will show, this age-old test still has a lot of controversies surrounding it and clinicians often have hair-splitting mentality about its utility in clinical care. As India is aiming for a zero-tuberculosis future (Pradhan Mantri TB-Mukt Bharat Abhiyaan), it is imperative that a reappraisal of this time-tested diagnostic method is done and clinicians are amply informed about the nuances of the test.

History

The tuberculin was first described by Dr Robert Koch, way back in 1890[4]. It was he who first identified the tuberculosis bacillus in 1882 and in 1890, he announced the discovery of a substance called "tuberculin", which was a crude extract from the heat-killed bacterial culture. But the subsequent use of "tuberculin" was quite different from what Koch had envisaged in 1890.

Dr Koch thought that tuberculin would be like a vaccine to protect against the TB infection and he advocated serial injections to patients. Although claims about its protective role were soon debunked by his contemporary physicians, and that TB vaccine never really materialized, it was nonetheless found to be useful in diagnosis of the same infection. Soon, the works of Charles Mantoux and Clemens von Pirquet established the groundwork for using this tuberculin extract as a reagent for a skin test to detect TB[3]. This was the tuberculin skin test (TST). The basic tenets of the test have remained unchanged after that. Thus, this test is more than 100-years-old but it still has a lot of unanswered questions.

Types of tuberculin test

As stated, tuberculin test is an intradermal test that elicits a delayed type hypersensitivity reaction. More about the

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molecular basis of the test will be discussed in the next section. But first, a brief discussion on the types of tuberculin test:

1. Mantoux test: The commonest, and probably the only method used now. An intradermal injection of the reagent is given and the subsequent reaction noted (more on this later).
2. Von Pirquet test[5]: Here, the reagent is given by a cutaneous scratch. In the original description of 1909, the scratch was made with a smallpox vaccination lancet.
3. Moro test[6]: This is a skin patch test. In earlier times, a tuberculin ointment was available, which was applied on the skin. This was most suitable for children. At that time, many authors reported that the results of the test had to be read not on the third day, but sixth or seventh day.
4. Calmette test: 10% aqueous solution of tuberculin, applied to the eye, produced marked conjunctivitis (Parker HC, 1908).
5. Trambusti's test[6]: Here, a needle, dipped in 10% tuberculin solution, is thrust into the skin to a depth of 5 mm, twisted 2 - 3 times and withdrawn.
6. Hamburger test[7]: Subcutaneous injection of tuberculin.
7. Craig test[7]: Multiple puncture method, like smallpox vaccination.
8. Stewart test: Single puncture of skin. But unlike Pirquet test, this is done not with a lancet, but with a needle.
9. Tine test: Here, a small button containing 4 - 6 small needles coated with TB antigens is pressed against the skin. Interpretation is similar to Mantoux test.
10. Heaf test: This is also a multiple puncture technique where a spring-loaded gun is used. Here, the diameter of the largest papule is measured. However, this test is no longer applicable because the PPD-S that is available now has been standardised only for the Mantoux test. That solution should not be used for Heaf test. Heaf Test was mainly done with the old tuberculin solution (not available now). This was also called *Sterneedle* Test.

(Except for No. 1, all others are of historical interest only).

The basic premise of all of these tests is the same. A small dose of tuberculin, given to an infected individual, will lead to a visible cutaneous reaction. This reaction will be a marker for the diagnosis of TB infection. But infection does not mean active disease. In many cases, the organism remains latent. So, the test is more important for its negative predictive value. Absence of a reaction (if false negatives can be ruled out: see later) indicates absence of the bacilli in the body. This is important in some cases like before starting biologic therapy in rheumatology.

The reagent used for the test was first prepared by protein extraction from heat-killed cultures of *M. tuberculosis*. This extract was known as old tuberculin (OT)[4]. However, it had a lot of extraneous proteins including beef extracts from the culture. In 1930, Florence Seibert spearheaded the production of a chemically purer form of OT and called it purified protein derivative (PPD). This contained less carbohydrates than the OT and was less prone to non-specific hypersensitivity reactions[4]. Now, for Mantoux test, this PPD is used. In 1941, Seibert prepared a large batch of PPD which was designated as the standard and the USFDA ruled that all subsequent batches of PPD must have a bioassay to demonstrate equal potency to this standard PPD (PPD-S). In 1952, the WHO declared this PPD-S as the standard of quality internationally.

In 1957, the Statens Serum Institute of Copenhagen prepared a large batch of tuberculin for UNICEF. It was called PPD RT 23 (RT: Research Tuberculin)[8]. This is also considered an international standard. 5 units of PPD-S is equivalent to 2 U of PPD-RT 23. At present, both PPD-S and PPD-RT 23 are considered as accepted standards internationally[9]. Any commercially available PPD is mixed with Tween 80, a detergent added to prevent its adsorption on glass. But this does not interfere with test. The testing method along with result interpretation, as described in guidelines, is for PPD mixed with Tween 80 (0.005%).

Now, according to the European Pharmacopoeia, PPD is produced from *M. bovis* and/or *M. tuberculosis*. Production is based on the seed-lot system.

In India, this is manufactured by the BCG Vaccine Laboratory, Guindy, Chennai (Tamil Nadu). But now, other commercial units have also been licensed to produce it.

PPD from other strains of mycobacteria are also prepared (Table I), although their clinical utility is doubtful[3]. They are mainly useful for research purposes like finding the prevalence of environmental mycobacteria.

Table I: Purified protein derivative (PPD) from different mycobacteria species[3].

PPD type	Species of mycobacteria
PPD-S	*M. tuberculosis*
PPD-A	*M. avium*
PPD-G	Gause strain of scotochromogen
PPD-F	*M. fortuitum*
PPD-Y	*M. kansasii*
PPD-B	Non-photochromogen Battey bacilli (*Mycobacterium intracellulare*)

Although culture extracts are still the main source of PPD, there have been attempts to produce recombinant proteins matching *M. tuberculosis* membrane proteins[10]. However, this is still not commercially produced.

Molecular basis of the test

The tuberculin reaction, as utilised in this test, follows the pathway of delayed type hypersensitivity. This is a special type of cell-mediated immunity (CMI). Here, it would be prudent to mention that the same *M. tuberculosis* antigens cause two types of type IV reaction. One is the tuberculin-type reaction, which will be discussed next. And the second one is the granulomatous reaction, which is more severe and found at sites of the disease.

In CMI, the reaction is mainly dependent on T lymphocyte activity, and antibodies or complement have a miniscule role. When the antigen is presented by the antigen presenting cells, the Th0 cells (CD4+) transform into Th1 cells, which drive the CMI response. This transformation takes 12 - 24 hours, hence called "delayed". γ-IFN (secreted by Th1 cell) plays an important role in this pathway.

The tuberculin-type hypersensitivity involves injection of a soluble antigen into the skin with subsequent development of local and systemic (in some cases) reaction. This similar reaction has been documented with *M. tuberculosis*, *M. leprae* and also some other agents like Beryllium. So, the test is not exclusive to TB.

The TST is an example of recall response. Exposure to soluble antigens to which that person had been previously exposed leads to the following cascade of reactions (Fig. 1).

This is where the tuberculin reaction stops and in a few days, the induration also disappears. In this figure, only the local reaction has been described. But very rarely, there may also be some systemic reactions like fever (due to excess TNF-α and IL-1). But if, instead of the soluble antigen, there is live bacilli, then the reaction progresses to form granuloma. This happens because the live bacilli, engulfed by the macrophages, are not killed intracellularly. These macrophages with intracellular live bacilli form the granulomatous reaction by forming giant cells and surrounding necrosis.

Basic technique of tuberculin skin test (TST): Essential points

The proper technique of TST is very important because any deviation from the standard will lead to false results:

1. Only Tuberculin PPD solution for Mantoux test, which has been standardised, should be used for the purpose.
2. The solution is available in four different concentrations:

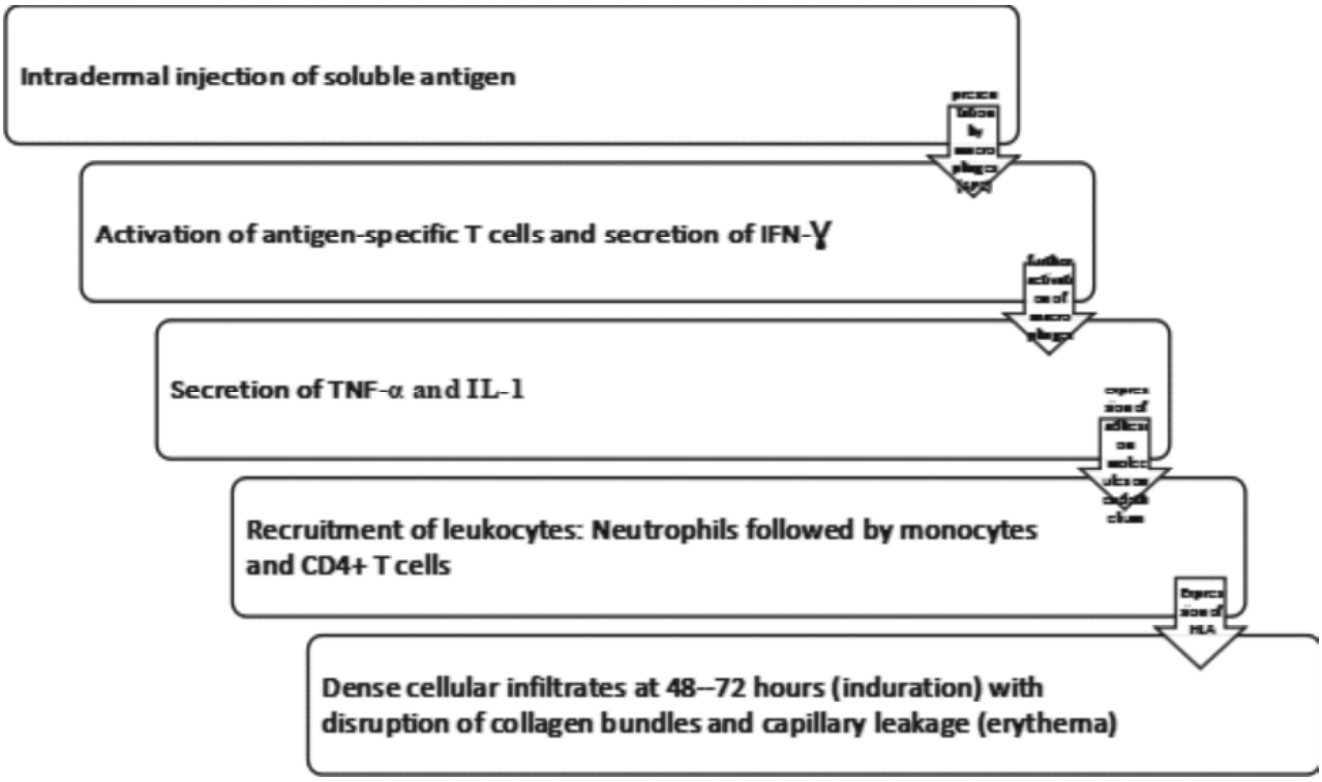

Fig. 1: *The tuberculin reaction physiology (APC: antigen presenting cell).*

1 TU/0.1 mL, 2 TU/0.1 mL, 5 TU/0.1 mL and 10 TU/0.1 mL (TU: Tuberculin unit). 1 TU = 0.02 microgram of PPD-S or PPD-RT23. This means that the volume of the solution injected intradermally will always be 0.1 mL, even if the dose of PPD varies. So, if a patient needs 5 TU of PPD, we will not inject 0.5 ml of 1 TU/0.1 mL solution but we will use the 5 TU/0.1 mL solution only.

3. The tuberculin solution must be stored at 2 - 8° C. It must never be frozen. It must never be exposed to sunlight.
4. The skin test is done on the volar surface of the non-dominant forearm, unless there are problems like local infection or scarring. That particular anatomical area has been chosen for ease of testing only. Thus, if a person has amputated hands, the TST may be done anywhere else in the body.
5. There is no need to sterilize the site before the test, but it should be clean.
6. The injection is given only with the tuberculin syringe supplied for the purpose and not any other syringe.
7. During injection, the needle (26- or 28-gauge) bevel should be facing upwards.
8. Since this is an intradermal injection, if given correctly, there should be no significant bleeding from the site.
9. If the injection technique is correct, a pale wheal of 6 - 10 mm should be raised.
10. If the wheal is not raised properly or is too small on first attempt, the injection of PPD should be repeated 2 inches away from the first site or on the other arm.
11. The patient should be instructed not to apply any chemicals to the area and not to scratch the area. Also, the area should not be covered.
12. Observation is made after 48 - 72 hours. If a patient misses this deadline, the test will be invalidated.
13. The induration at the site of injection is measured perpendicular to the long axis of the arm. Erythema is of no importance in interpretation.
14. The diameter of the induration is measured in millimetres. If there is no induration, it is recorded as "0 mm".
15. The test has not been adequately studied in elderly subjects to make any firm recommendations. But it is generally considered to be similar to young adults. But there is a point of view that in the elderly group, the reading of the Mantoux test may be done at 96 hours instead of 72 (Package insert for Aplisol®; FDA), although this is not a firm recommendation.
16. Although there are 10 TU/0.1 mL strength solutions available, their utility is limited. Some countries like Australia prefer to use the 10 TU dose for TST. But most countries like India use the 5 TU/0.1 mL PPD-S dose.
17. After becoming positive, the induration may remain for up to 1 week.

Adverse reactions of the test

1. Mild pain or pruritus may occur at the injection site for some time. This is usually self-limiting. If excessive, this may be controlled with ice packs.
2. In strongly positive reaction, an ulcer or blister may develop at the site of the test (1 - 2%)[11]. This may later form a scar rarely.
3. Rarely, regional lymphadenitis has been reported[12].
4. Wrong method of injection may lead to formation of a haematoma locally.
5. Immediate allergic reaction (within 12 hours) may develop at the injection site. This is protein allergy, but does not indicate TB infection. Usually, this is self-limiting. Anaphylaxis to the reagent has been reported extremely rarely[12].

Interpretation of TST

When will the test be positive

A Mantoux test will be positive if there is current tuberculosis infection in the body (*M. tuberculosis* or *M. bovis*). However, infection does not always mean active disease. The test cannot differentiate between active and latent infection.

What is a positive test?

A reaction to the tuberculin test, as indicated by induration formation (not erythema), is interpreted according to the Table II below (collected from current CDC guidelines).

A TST becomes positive around two months after TB infection. That is why timing of the test is important to get a meaningful result. As written below, a TST done very early in the course of the disease will be false negative. One should wait at least 8 weeks from the last documented exposure before doing a TST.

Table II: Table showing interpretation of TST induration.

Induration (mm)	Significance
0 - 5	Negative
5 - less than 10	This is considered positive in • HIV positive individuals • Recent contact with an open case of TB • Transplant recipient • Patients on other immunosuppressives like TNF-a antagonists • Children with clinical or radiological suspicion of TB In all other persons, this is considered negative. In BCG-vaccinated children, induration of up to 9 mm may be attributed to the vaccine bacilli for the first 10 years of life.
10 - less than 15	This is considered positive in • Persons coming from TB endemic regions like India • Drug abusers • Microbiological lab workers • Health workers • Persons with chronic conditions like diabetes, silicosis, cancer, etc. • Children younger than 5 years • Children older than 5 years if there is increased exposure.
≥ 15	This is considered positive in any person

Sensitivity of TST in humans is estimated to be around 75%. However, in animals, like cattle, the figure is more than 95%.

False positive

False positive results can occur due to:

- ✓ There is infection with other bacteria of the *M. tuberculosis* complex. This includes *M. africanum* or *M. microtii*.
- ✓ There is previous BCG vaccination.
- ✓ Non-tuberculous mycobacteria infection.
- ✓ Incorrect test reporting and interpretation.

Role of prior BCG vaccination in Mantoux test

Previous BCG vaccination may cause false positive Mantoux test although the reaction is usually weakly positive. BCG is an attenuated strain of *M. bovis* and thus also reacts with the *M. tuberculosis* antigens[13]. This vaccine is included in the universal immunization schedule of high-prevalence countries like India. But the USA and most other Western countries do not make it mandatory, and this vaccine is indicated only for certain groups of people in those places.

Status of prior BCG vaccination must be considered while interpreting tuberculin test result. However, the effect of BCG on tuberculin test wanes after 15 or more years[14]. So, if BCG is given at birth, a tuberculin test of the person in adulthood would probably not be a problem. According to a meta-analysis, the relative risk (RR) of a positive tuberculin skin test (TST) is around 3.5 if the test is performed within 15 years of BCG vaccination; but the RR falls to just 1.4 after 15 years[14]. The TST after BCG is more likely to be positive with PPD-RT23 than with PPD-S[14]. Moreover, a strong reaction with induration > 15 mm even in a BCG vaccinated individual is considered significant[14]. The TST positivity after BCG vaccination usually develops 4 - 8 weeks after the vaccine. In some countries, the TST is used to assess the success of the BCG vaccination. If the TST is negative after BCG vaccine, the vaccine is repeated. But India does not have any such follow-up schedule.

For prior vaccinated individuals, the TB blood test (IGRA) is better as it is not influenced by the BCG[15]. As everyone in India is vaccinated with BCG at birth, the IGRA would be a better test of latent TB in this country (see later)[15].

Mantoux test in the paediatric population

In the paediatric population, the recommended dose of PPD is 1 TU of PPD-RT23[16]. The rest of the procedures are same as in adults. Interpretation is as per Table I.

Repeat test

Usually, unless the test has been conducted non-professionally, there is no indication of a repeat test. If needed, repeat test must be done within 1 week of the first test[15]. This is because, the antigen given during the first test may "boost" the reaction of the repeat test. This may give rise to a false positive result in the repeat test.

This "boosting" effect develops within 2 - 3 weeks and lasts up to 18 months, according to an Indian study[16]. So, the repeat test has to be done either very quickly (within 1 - 2 weeks) or after 18 months. Before interpreting any TST, the history of previous testing must be recorded to avoid false positive reporting.

Two step TST

According to the CDC, Atlanta, there is one situation where "two-step" Mantoux testing is done. This is the baseline test of health workers who will undergo future periodic testing. In short, the protocol for two-step test is as follows:

1. The first TST is done as per protocol. If this comes positive, then the worker is infected already at baseline.
2. Then, no more TST is needed and the worker may be further evaluated for infection.

3. If the first TST is negative, then after 2 - 3 weeks, a second TST is done.
4. If the second is also negative, then there is no baseline TB infection.
5. If this second test comes positive, this may indicate a "boosted" response. This result is documented. If future periodic TST is done, those results will be compared to this "boosted" reaction to look for true Mantoux conversion.

False negative

Some people, even after harbouring the TB bacilli, may show absence of reaction to the TST. Some of the reasons are[3]:

1. Infants.
2. Very recent TB infection (less than 2 months).
3. Severe active TB with high bacterial load.
4. Recent live virus vaccination (within 1 month) (so, if a TST is needed, it should either be given on the same day as the live vaccine or we should wait for 1 month).
5. Incorrect method of the TST.
6. Anergy.
7. Very old TB infection (this negative result may become positive after repeat testing due to "boosting": see previous section).
8. Infection with measles, varicella, etc.
9. In very old age, the reaction may not be fully developed after 72 hours. So, in those cases, the reading should be done after 1 more day to avoid false negative results.
10. Malnutrition.

Reversal of TST

This is defined as conversion of a positive reaction to a negative one during a person's lifetime. This is a very rare phenomenon and is said to occur in old age[17]. This can also occur if the initial induration was borderline positive.

Mantoux conversion

This is defined as changing of a previously negative Mantoux to a positive result[18]. Or, an increase in induration size by ≥ 10 mm compared to a previous test. This usually indicates new infection with the TB bacillus.

When should a Mantoux test be avoided?

Like any other biomedical test, a tuberculin test is also subject to confusion if done indiscriminately. There are some situations where the test becomes superfluous. They include[3]:

- Previous confirmed TB (The test can remain positive for a long time after treatment[19]. The exact duration of immunoreactivity is not known with certainty and thus, Mantoux test after a previously diagnosed episode of TB has doubtful value. The test indicates exposure to the bacillus but does not indicate the outcome: bacterial clearance vs. persistence[20]. This is even true for immunosuppressed individuals. So, the Mantoux test cannot differentiate a previously treated case of TB from an untreated one).
- Infants less than 3 months.
- If the first reaction is > 15 mm.
- Clearly apparent active TB disease.
- Previous severe allergic reaction to the solution.
- **Pregnancy:** Here, TST is not absolutely contra-indicated. But it should be done only if the benefit-risk ratio is favourable.

Mantoux test in pulmonary vs extra-pulmonary TB

Generally speaking, the TST should be positive if there is TB antigen exposure at any anatomical area. But it is found that the Mantoux test is more reliable for pulmonary TB. In many extra-pulmonary cases, the sensitivity of the test drops. For example, using the 10 mm cut-off, the sensitivity of TST for TB meningitis is around 50% in one study[21]. For tubercular pleural effusion, the sensitivity is around 65%[22]. The sensitivity of Mantoux test for extra-pulmonary TB has been reported to be less than 50% in a study involving South Asian subjects[23].

Comparison with IGRA

Another test which is now widely available, is the Interferon Gamma Release Assay (IGRA). The basic principle of this test is exposing peripheral blood lymphocytes to synthetic *M. tuberculosis* antigens and assessing the release of interferon-gamma[24]. The T-lymphocytes of a person, already sensitised to TB antigens, will quickly release large quantities of IFN-γ when exposed to antigens like ESAT-6 and CFP-10. The quantity of this interferon in the sample will tell us about the infection. This test has a lot of advantages over TST, like:

1. Requirement of a single blood sample and no need for the patient to return for reading.

2. Higher sensitivity and specificity.
3. Lack of confounding by previous BCG vaccine.
4. Lack of cross reaction with environmental mycobacteria.
5. Quicker (< 24 hours) reporting.
6. *In-vitro* test.

In various studies, both varieties of the IGRA, i.e., the Quantiferon and TB-spot, have consistently shown better sensitivity compared to TST by more than 10 percentage points[25]. So, this test is likely to supersede the Mantoux test in the future. This may also be used to test for vaccine efficiency in public health surveys[24]. But, in a country like India, where resources are limited, wider uptake of the IGRA is still debatable. So, TST will remain an important tool in clinical and public health setting in this country for the near future.

Conclusion

The Mantoux test is an age-old test with proven merits and demerits. It has its limitations; but in a country like India, the test is still useful from both clinical and public health points of view. A clinician should be aware of the nuances in interpretation of the test. The overall diagnosis of tuberculosis should not be based on the TST alone but this test has important complementary role, along with other microbiological tests, in the overall management of the TB epidemic.

Recommendations

- Tuberculin test is still an essential component of tuberculosis management in India but it should not be used alone.
- There are clear age- and co-morbidity specific guidelines for interpretation of the test. False positives and negatives are possible.
- Proper technique is essential for performing the test. Skill development is necessary.
- Where possible, the IGRA test is better, especially in BCG vaccinated persons.

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