

Central TB Division

Ministry of Health and Family Welfare

Date: 18 / 04 / 2024

To

State TB Officers

**Subject: Guidance document on Standard Operating Procedure for Cy-TB skin test for detection of TB infection**

Dear all,

Greetings from the Central TB Division!

In line with our commitment to end TB in India by 2025, the NTEP made a policy decision in 2021 to expand the coverage of Tuberculosis Preventive Treatment (TPT) to include all household contacts (HHC) of pulmonary TB patients and other high-risk groups. Ruling out of active TB and confirming TB infection is essential before initiating TB Preventive Treatment. This would also boost the confidence of both providers and individual in initiating TPT.

One of the key advancements in this regard is the introduction of the Cy-Tb skin test, a novel test recommended for detection of TB infection under the NTEP. Cy-Tb is more specific compared to Tuberculin Skin Test (TST), and not influenced by earlier vaccination. As with the Mantoux technique, it features a single cutoff of more than 5 mm to establish positivity.

The Standard Operating Procedure (SOP) for the Cy-Tb skin test has been developed in accordance with the recommendations of the technical committee and is enclosed herewith. States and districts have already been sensitized to the Cy-Tb skin test procedure during recent training sessions on the Shorter TPT regimen. Additional guidance on cascade training and capacity building for health staff will be provided in due course.

Furthermore, it is emphasized that procurement of Cy-Tb skin test kits is at advanced stage and will soon be made available to all states/ UTs.

I urge all stakeholders to familiarize themselves with the Cy-Tb skin test SOP.

By working together and embracing new technologies and methodologies, we can make significant strides towards achieving our goal of eliminating TB in India.

Warm regards,

  
(Dr. Veena Dhawah)

Additional Commissioner

CVAC & CTD, Ministry of Health & Family Welfare

Copy to,

The State TB Officers - all

Enclosure -Guidance document on Standard Operating Procedure for Cy-TB skin test for detection of TB infection

**National Tuberculosis Elimination Programme**  
**STANDARD OPERATING PROCEDURE OF CY-TB SKIN TEST**  
**FOR DETECTION OF TB INFECTION**

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**Materials needed:**

- Cy-Tb vial: 1ml multi-dose vial (10 tests)
- Syringe: 0.1 ml AD syringe or 1ml single use disposable syringe
- 2X2 gauze pads or cotton balls, alcohol swabs, gloves
- Puncture- resistant sharp disposable container
- Recording form (digital / physical) – Ni-kshay

**Preparation before administration:**

- Cy-Tb vial must be stored in refrigeration 2-8 degree Celsius (DO NOT FREEZE).
  - In the peripheral health facility, the vial should be kept in the refrigerator section of domestic fridge; It should not be stored in freezer section.
- To avoid fluctuations in temperature, do not store in the refrigerator door.
- The vial should preferably be taken out of the refrigerator 5-10 min and kept at room temperature before administering the injection.
- Read the vial label to ensure that the correct solution and strength have been selected.
- Check the vial visually. Vial should be free from particles or aggregates and should be clear and colourless to pale yellow solution.
- After first opening, the vial can be used for up to 28 days provided :  
It has not crossed the expiry date and the VVM has not crossed the discard point. The date and time of opening the vial to be mentioned on the cap of vial.
- Check the expiration date and the date that the vial was opened.
  - The vial should be discarded if it has been open for more than 28 days or the expiration date has passed. (on opening of vial, each
- Select a well-lighted area for administering the test. Check and ensure that necessary materials are handy prior to the test.
- Introduce yourself to the recipient.
- Verify that the correct person receives the test.
- Ask the person if S/he has any allergies.
- Review the person's TB skin test history. Inquire and verify documentation of previous tuberculin skin test results, if any.

- Educate the person and answer questions to address fears, and ease anxieties. Discuss the purpose of the test, testing procedure, and the time frame for returning to have the test read.
  - If the person cannot return 48-72 hours after the test to have the indurations measured and evaluated, do not administer the test. Instead, schedule another time that is more convenient for the person.

**Administration of skin test: (syringes must be filled immediately prior to administration)**

- The administration procedure is identical to intradermal injection for Mantoux technique on forearm.
- Wash your hands with soap and water.
- Wear the gloves.
- On a firm, well-illuminated surface, expose the person's' arm and slightly flex at the elbow. The injection is to be administered on the palm-side-up surface of the forearm (preferably left forearm), about 2 to 4 inches below the elbow. Avoid areas of skin with veins, sores, rashes, scars or excess hair.
- Clean the injection site with an alcohol swab, using circular motion beginning in the centre and working your way outward. Allow the site to dry completely before injection.
- Take the vial and check visually. Vial should be free from particles or aggregates.
- Mix by inverting 2-3 times. Mixing should be performed gently.
- Wipe the top of the vial with a new alcohol swab and allow it to dry thoroughly.
- Fasten the needle tightly on the syringe by holding the cap and twisting it onto the tip of the syringe. Remove the needle cap and make sure that the needle bevel is facing up.
- Hold vial between your thumb and fingers and insert the needle through the stopper. Inject air into the empty space, not the solution, in the vial.
- Invert the vial. With the tip of the needle within the fluid level in the vial, draw out 0.1ml of solution.
- Remove the needle from the vial. Hold the syringe in an upright position and gently tap the syringe to dispel any air bubbles.
- Expel all air from the syringe and excess solution from the needle, leaving exactly 0.1ml of solution in the syringe.
- Stretch the skin taut over the injection site to provide a surface that is easy for the needle to penetrate. This can be accomplished by stretching the skin between the thumb and

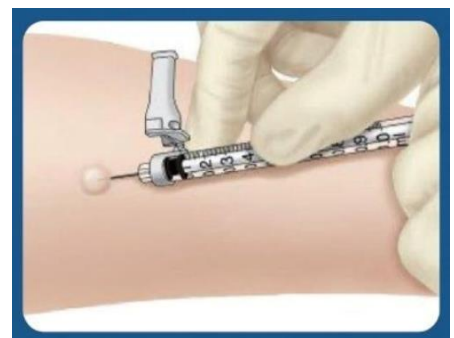
index finger or grasping the patient's forearm and gently pulling the skin from under the arm.

- Hold the syringe between your thumb and index finger with the needle bevel facing up and the syringe parallel to the forearm for intra-dermal injection.

- With the needle against the person's skin, insert the needle slowly at a 5 to 15 degree angle, just below the surface of the skin (you should be able to see the bevel of the needle just below the skin surface).



- Release the stretched skin and hold the syringe in place. Slowly inject the solution, forming a 6-10 mm wheal (pale, raised area with distinct edges, has orange peel appearance and does not disappear immediately).



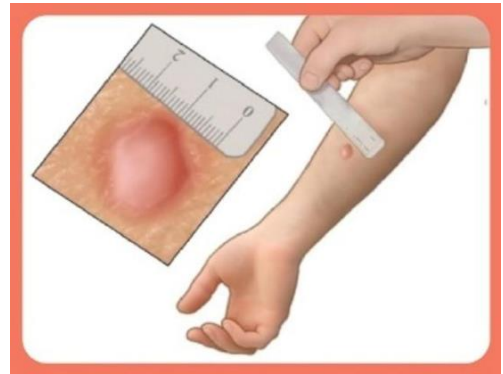
- If no wheal forms or if it is less than 6mm in diameter, repeat the test approximately 2 inches from the original site or on the opposite arm.

- Remove the needle without massaging or pressing the area and immediately discard the used syringe in the sharp's container.
- If minor bleeding occurs, use a 2X2 gauze pad or cotton ball to dab the injection site.
- **Do not cover the site with an adhesive bandage as it could cause irritation.**
- Discard gloves and wash your hands.
- Record the test information in Ni-kshay
- Inform the person that mild itching, swelling, or irritation is normal and usually goes away within 1 week.
- Explain how to care for the injection site: avoid starching the site, keep the site clean and dry and avoid creams, lotions, or adhesive bandages.
- Inform the person that it is important to return within 48 to 72 hours to have the test result read.
- Inform the person to return for the skin test reading.

#### **Reading of skin reaction:**

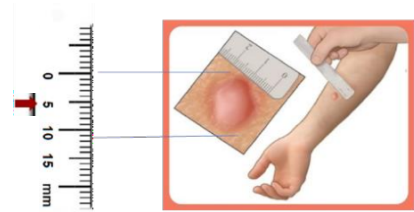
- The test should be read between 48–72 hours (*Not before 48 hours or after 72 hours*).
- Reading should be performed under good illumination, with the forearm slightly flexed at the elbow. Do not measure the redness or bruise.

- Gauge presence of induration (palpable, raised, hardened area or swelling), starting with inspection and then palpation with light, gentle motions.
- Sweep fingertips over the surface of forearm in all four directions to locate margins or edges of induration. Using the fingertip as a guide, mark the widest edges of the induration across the forearm lightly with a fine line or dot (using a ball point pen). If the margins of induration are irregular, mark and measure the longest diameter.
- The diameter of induration is measured across the forearm, from the thumb side of the arm to the little finger side. Using a plastic scale, place the zero-ruler line inside the edge of marked fine line or dot and measure the ruler line inside the right dot or edge of fine line. If the measurement falls between two divisions on the millimetre scale, record the lower division.
- Alternatively, use the ballpoint pen method for reading<sup>1</sup>. A ball point pen line may be drawn on the transverse axis of the forearm, starting 1–2 cm away from the visible skin test reaction and moving slowly towards its centre, exerting moderate pressure against the skin. The point where resistance to pen displacement occurs determines the outer limit of the induration. Mark lightly with a fine line or dot at the widest edges of the induration across the forearm and use a ruler to measure the diameter as above.



### Interpretation of skin reaction:

- Cy-Tb skin test does not measure immunity to TB but the degree of hypersensitivity to the antigens in the Cy-Tb solution. It cannot differentiate between infection and disease.
- **Universal 5mm cut off has been established for Cy-Tb which indicates infection with *Mycobacterium Tuberculosis*.**
- Measure the diameter with the scale, if induration is 5 mm or more, the test is positive.
- Formation of vesicles, bullae, lymphangitis, ulceration and necrosis at the test site should also be noted as they may indicate a high degree of sensitivity and hence the presence of TB infection.



**$\geq 5\text{mm} = \text{Cy-Tb skin test positive}$**

<sup>1</sup> Morán-Mendoza O, Tello-Zavala MC, Rivera-Camarillo M, Ríos-Meza Y. Comparison of different methods and times for reading the tuberculin skin test. Int J Tuberc Lung Dis. 2013;17(10):1273–8

- A negative test may indicate lack of infection with *Mycobacterium tuberculosis* or that the person has acquired infection very recently and not enough time has elapsed for the body to react to the skin test.
- A positive test result indicates infection with *Mycobacterium tuberculosis* regardless of CD4 T-cell count or underlying immune compromised status of person.

**Other consideration on Cy-Tb skin test:**

- **Adverse reaction of test:** Adverse reaction experienced after receiving Cy-TB are generally mild and transient.

<b>Common adverse reaction</b>	Injection site pruritus, pain, hematoma, rash, vesicles, erythema and swelling; mild headache	Reassure the beneficiary. The common adverse reactions are self-limiting. Refer to the nearest health centre if condition worsen.
<b>Uncommon adverse reactions</b>	Injection site induration, pyrexia, haemorrhage, ulcer and discolouration; malaise, influenza like illness and fatigue; Dizziness; Pruritus; Rash; Gastroenteritis; Myalgia; Diarrhoea	Reassure the beneficiary. Refer to the nearest health centre if the condition persists.
<b>Rare adverse reactions</b>	Injection site inflammation and anaesthesia; paraesthesia; head discomfort; urticaria; night sweat; eosinophilia; lymphadenitis; hepatitis; jaundice; nausea; vomiting; arthritis	Reassure the beneficiary. Refer to the nearest health centre if the condition persists.

- Induration and erythema are expected reactions in individuals infected with M. Tuberculosis. Induration more than 50 mm and erythema more than 80 mm may occur in uncommon cases, which normally would decrease after 4 days and disappear within 28 days.
- Active drug safety monitoring (aDSM) review form should be filled and updated in Ni-kshay for major adverse reaction due to test.
- **Boosted reaction:**

- When sensitization to mycobacteria has occurred many years earlier, an initial TST may produce negative or weakly positive response, there being too few sensitized lymphocytes in the circulation to produce a significant local response.
- If the test however is repeated, a larger reading may be obtained due to the immune response being 'recalled' or 'boosted' by the first test.
- Administering such a second test after an initial negative reaction is called two-step testing. Boosting is maximum within one to five weeks of the initial test and may continue for up to two years. The second boosted reading is considered for decision-making or future comparison.
- Thus, in a standard settings, repeat Cy-Tb test can be performed in population with a negative result at interval of at least 6 weeks. This population includes individuals in a screening program such as health care professionals and contacts to active TB cases.
- **Indication of test:**
  - For detection of TB infection for the population age 18 and able.
- **Contraindication of test:**
  - Known hypersensitivity to active substance or any excipients
  - Severe reactions to previous Cy-Tb tests
- **Conversion:**
  - A change in skin test reactivity within two years of a negative test is called conversion, negative to a positive reaction and increased induration  $\geq 5\text{mm}$  compared to the previous reading.
- **Reversion:**
  - Change of the result from positive to negative. This is uncommon among healthy individuals. However, it is noted among the elderly during two-step testing.
- **Repeat test:**
  - A repeat Cy-Tb test can be performed in population with a negative result at interval of at least 6 weeks. This population includes individuals in a screening programme such as health care professions and contacts to active TB.
- **Cy-Tb in persons receiving vaccinations:**
  - Vaccination with live viruses or BCG does not interfere with Cy-Tb skin test result since the test is highly specific.

## Annexure 1: Comparison between Cy-Tb, TST and IGRA

	C-Tb	TST	IGRA
Specificity	High also in BCG vaccinated	Low in BCG vaccinated	High also in BCG vaccinated
Sensitivity	High	High	High
Ease of use	Field friendly, single cut-off allows simple test interpretation	Field friendly, complex test interpretation	Require labs and infrastructure
Cost of test	Low	Low	High
Manufacturing	Robust with high yield, well defined and completely characterized	Complex old product	Complex, multiple components*
<b>Special populations</b>			
Children	More robust	Affected by young age	Affected by young age
PLHIV	More robust with low CD4	Requires info on HIV status for	Affected by HIV and low CD4 count