IMMUNOLOGY PRACTICAL– week 10

Detection of specific cell immunity
Skin test
IGRA

Skin tests, IGRA

- Latent tuberculosis is where a patient is infected with *Mycobacterium tuberculosis*, but does not have active tuberculosis disease.
- Patients with latent tuberculosis are not infectious—it is not possible to get TB from someone with latent tuberculosis.

Tests for latent tuberculosis

- two major classes of tests used to identify patients with latent tuberculosis: tuberculin skin tests and IFN-γ (Interferon-gamma) tests.
- The tuberculin skin tests in use include (but are not limited to)
- Mantoux test
- QuantiFERON-TB Gold
- QuantiFERON-TB Gold In-Tube

Skin test – Mantoux test

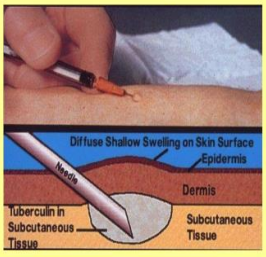
- **Mantoux** – in vivo detection of specific cell immunity after exposition to antigen

- - burden of patient by antigen
- - possible immunodeficiency of patients (anergy, risk of alergic reaction)
- - memory cell after BCG vaccination,
- - exposition to antigen = activation of M
- - interpretation,
- - booster dose of antigen,

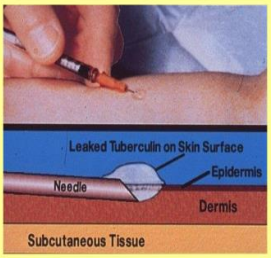
Mantoux test

- The Mantoux test is now standardised by the WHO. 0.1 ml of tuberculin (100 units/ml) is given by intradermal injection into the volar surface of the forearm (subcutaneous injection results in false negatives).

needle to deep



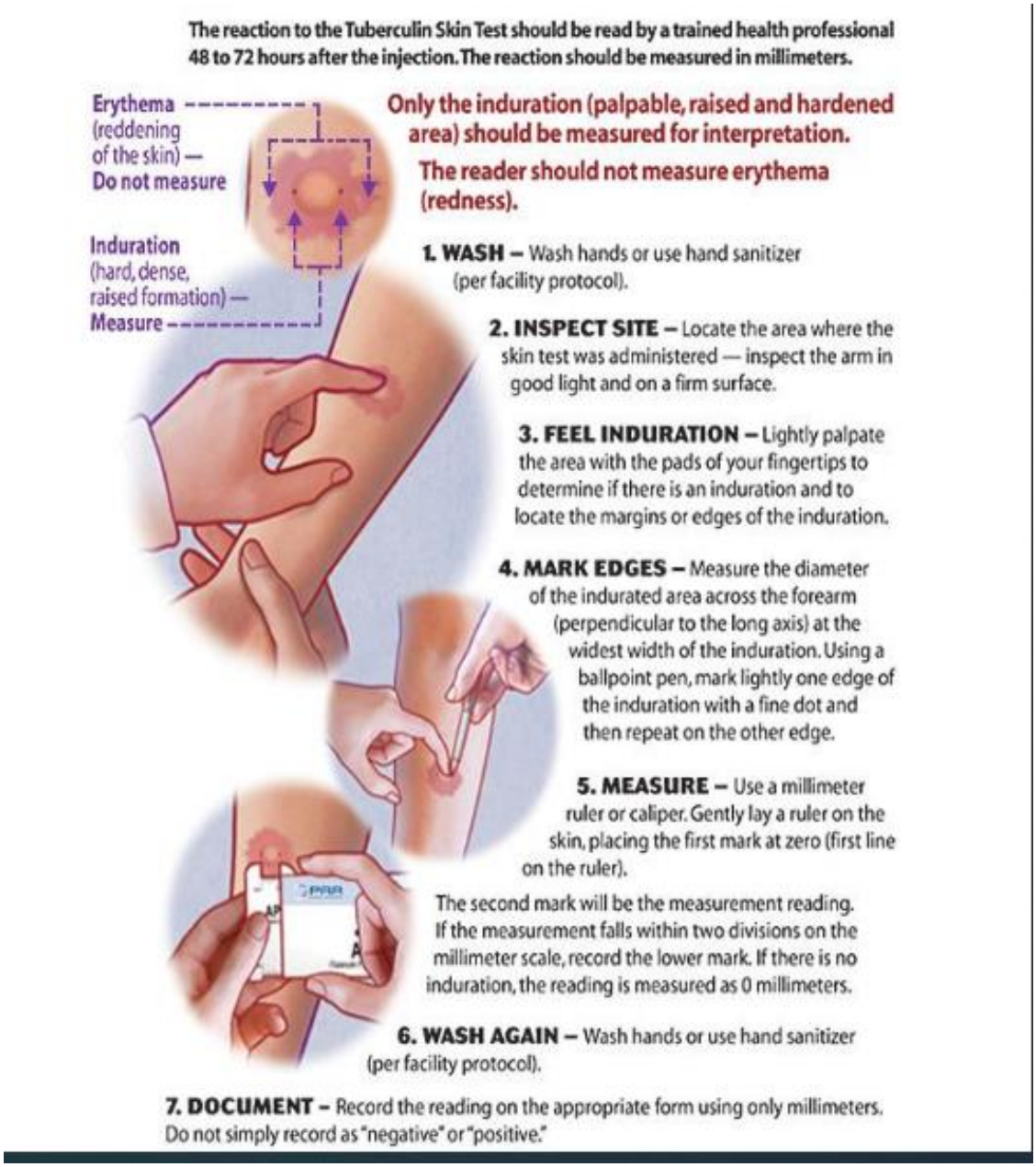
needle to shallow



MANTOUX TUBERCULIN SKIN TEST

The Mantoux Tuberculin Skin Test (TST) is the standard method of determining whether a person is infected with *Mycobacterium tuberculosis.* Tuberculin is purified protein derivative

(PPD), an extract of *Mycobacterium tuberculosis*, *M. bovis*, or *M. avium* that is used in skin testing in animals and humans to identify a tuberculosis infection. PPD is a poorly defined, complex mixture of antigens.Tests based upon PPD are relatively unspecific since many of its proteins are found in different mycobacterial species. The tuberculin skin test is based on the fact that infection with *M. tuberculosis* bacterium produces a delayed-type hypersensitivity skin reaction. The components of the organism are contained in extracts of culture filtrates and are the core elements of the classic tuberculin PPD, that is used for skin testing for tuberculosis. Reaction in the skin to tuberculin PPD begins when T-cells, which have been sensitized by prior infection, are recruited to the skin site where they release lymphokines. These lymphokines induce induration (a hard, raised area with clearly defined margins at and around the injection site) through local vasodilation leading to fluid deposition known as edema, fibrin deposition, and recruitment of other types of inflammatory cells to the area.



Tuberculin Skin Test – Administration

The TST is performed by injecting 0.1 ml of tuberculin-purified protein derivative (PPD) into the inner surface of the forearm. The injection should be made with a tuberculin syringe, with the needle bevel facing upward. The TST is an intradermal injection. When placed correctly, the injection should produce a pale elevation of the skin (a wheal) 6 to 10 mm in diameter.

Tuberculin Skin Test -Reading

The skin test reaction should be read between 48 and 72 hours after administration. A patient who does not return within 72 hours will need to be rescheduled for another skin test. The reaction should be measured in millimeters of the induration (palpable, raised, hardened area or swelling,.The reader should not measure erythema (redness). The diameter of the indurated area should be measured across the forearm (perpendicular to the long axis).

Tuberculin Skin Test -Interpretation

Skin test interpretation depends onthe measurement in millimeters (mm) of the induration andthe person's risk of being infected with TB and/or progression to disease if infected. The following three cut points should be used to determine whether the skin test reaction is positive. A measurement of 0 mm or anything below the defined cut point for each category is considered negative. Tuberculin Skin Test-False-positive ReactionSome persons may react to the TST even though they are not infected with M. tuberculosis. The causes of these false-positive reactions may include, but are not limited to, the following: Infection with nontuberculosis mycobacteria, previous BCG vaccination, incorrect method of TST administration, incorrect interpretation of reaction and incorrect bottle of antigen used.

Interpretation of the Tuberculin Skin Test reading:

Skin test interpretation depends on two factors:

- Measurement of the induration in millimeters
- Person's risk of being infected with TB and of progression to disease if infected

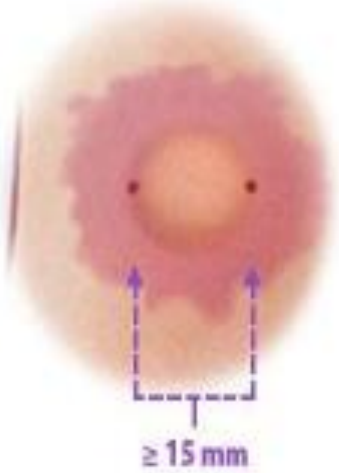
An induration of 5 or more millimeters is considered positive in:

- HIV-infected persons
- Persons who have had a recent contact with another person with TB disease
- Persons with fibrotic changes on chest radiograph consistent with prior TB
- Patients with organ transplants
- Persons who are immunosuppressed for other reasons (e.g., taking the equivalent of ≥15 mg/day of prednisone for 1 month or longer.)

An induration of 10 or more millimeters is considered positive in:

- Recent immigrants (within the last 5 years) from high prevalence countries
- Injection drug users
- Residents and employees of high-risk congregate settings
- Mycobacteriology laboratory personnel
- Persons with clinical conditions that place them at high risk
  Children < 4 years of age, or infants, children, and adolescents exposed to adults at high risk.

An induration of 15 or more millimeters is considered positive in:

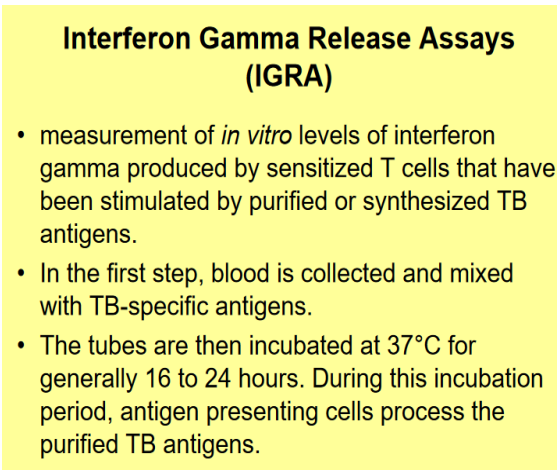


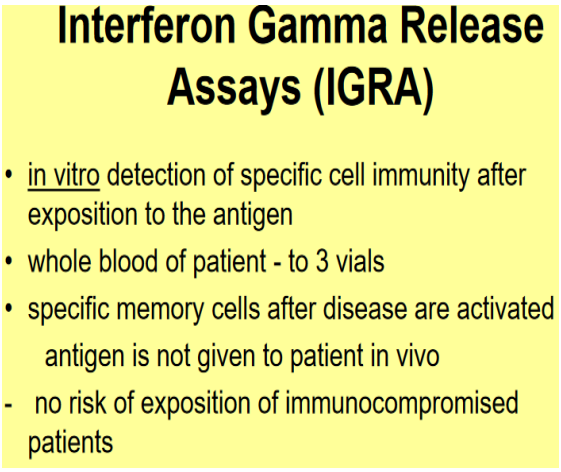
- Persons with no known risk factors for TB.

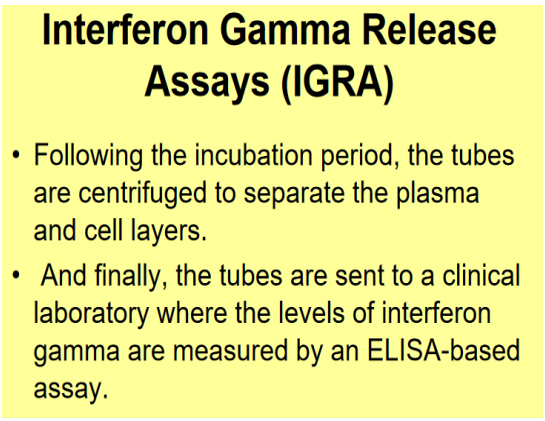
INTERFERON-GAMMA RELEASE ASSAY (IGRA)

Interferon-Gamma Release Assays (IGRAs) are whole-blood tests that can aid in diagnosing *Mycobacterium tuberculosis* infection. They do not help differentiate latent tuberculosis infection (LTBI) from tuberculosis disease. Two IGRAs are commercially available:

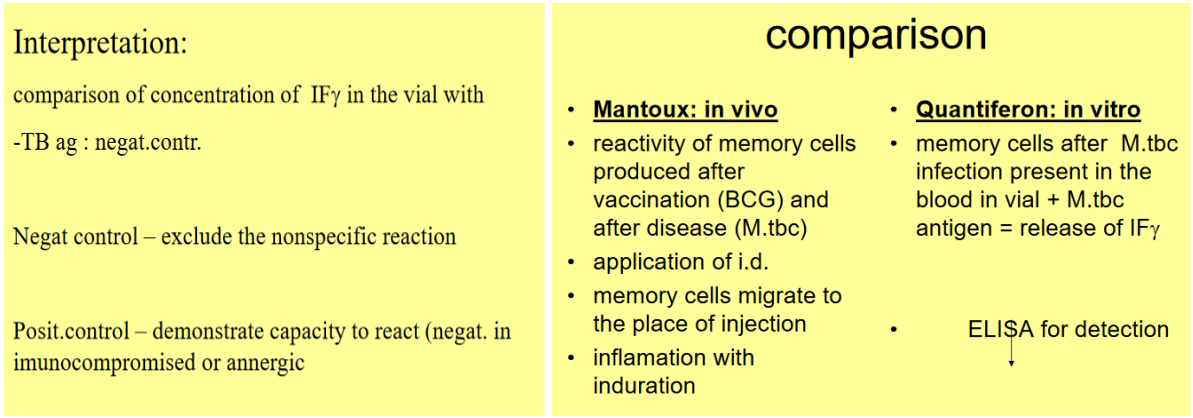
- QuantiFERON®-TB Gold In-Tube test (QFT-GIT)
- T-SPOT®.TBtest (T-Spot)







IGRAs measure a person's immune reactivity to *M. tuberculosis*. White blood cells from most persons that have been infected with *M. tuberculosis* will release interferon-gamma (IFN-γ) when mixed with antigens (substances that can produce an immune response) derived from *M. tuberculosis.* To conduct the tests, fresh blood samples are mixed with antigens and controls.



Positive IGRA:This means that the person has been infected with *M. tuberculosis*. Additional tests are needed to determine if the person has latent TB infection or TB disease. A health care worker will then provide treatment as needed.

Negative IGRA:This means that the person's blood did not react to the test and that latent TB infection or TB disease is not likely.
IGRAs are the preferred method of TB infection testing for the following:
•People who have received bacille Calmette–Guérin (BCG). (BCG is a vaccine for TB disease).
•People who have a difficult time returning for a second appointment to look for a reaction to the TST.
QuantiFERON®-TB Gold In-Tube testThe QuantiFERON®-TB Gold In-Tube (QFT-G) is a blood test for use as an aid in diagnosing *Mycobacterium tuberculosis* infection (both latent tuberculosis infection and active tuberculosis disease).

The QFT-G is an indirect test for M. tuberculosis infection that is based on measurement of a cell-mediatedimmune response. A cocktail of 3 mycobacterial proteins (26 ESAT-6, 27 CFP-10, and TB 7,7) stimulate the patient's T-cells in vitroto release interferon-gamma, which is then measured using ELISA technology. The test detects infections produced by the *M. tuberculosis complex* (including *M. tuberculosis*, *M. bovis*, and *M. africanum* infections). BCG strains and the majority of other non-tuberculosis mycobacteria do not harbor ESAT-6, CFP-10, and TB 7,7 proteins,thus, patients either vaccinated with BCG or infected with most environmental mycobacteria should test negative. Results should always be interpreted in conjunction with other clinical and laboratory findings.

Sources:

Kompaniková, Neuschlová, Sadloňová: Special Bacteriology – Basic Laboratory Tests
Available from: https://www.jfmed.uniba.sk/fileadmin/jlf/Pracoviska/ustav-mikrobiologie-a-imunologie/VLa/Special_Bacteriology_-_basic_laboratory_tests.pdf

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