Tuberculosis continued

This chapter deals with latent tb infection and its managent.

10. What is latent tuberculosis infection?

People can be categorized into three states with respect to tuberculosis- those who are exposed, those who have latent Tb infection and those with active Tb disease.

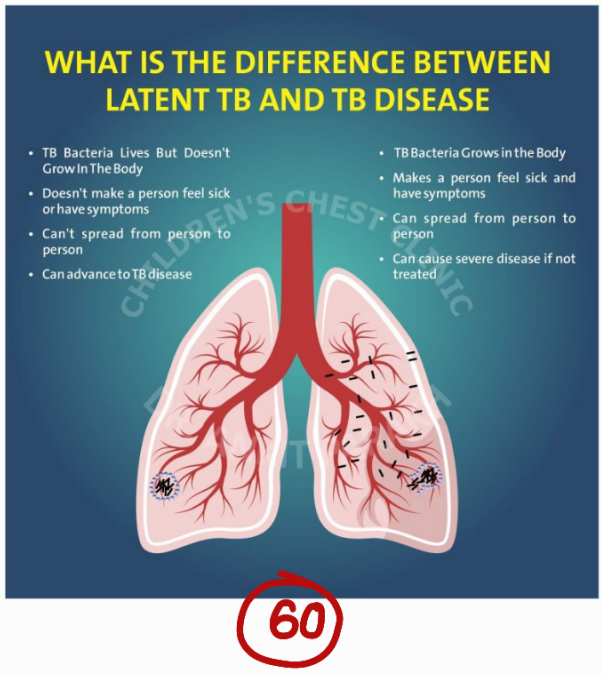
Those who are exposed are not yet infected. Those infected (bacilli have entered the alveoli in lungs and have established infection), may carry Tb bacilli which cannot be demonstrated and without suffering from disease or without manifesting clinical disease; and those with the disease have bacilli which can be demonstrated and they manifest clinical signs and symptoms of disease.

Latent tuberculosis infection (LTBI) is defined by WHO as a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens without evidence of clinically manifested active tuberculosis (TB) disease.

Individuals with LTBI represent a reservoir for active TB cases because 5 to 15% of the infected may suffer from active disease in their life time, the risk being highest

within two years of infection (59). The risk goes up considerably in those with comorbidities like HIV coinfection or diabetes or smoking or malnutrition. The WHO strategy to eliminate TB includes preventing TB disease by managing people already infected with Tb. Undetected active TB and undetected latent TB are the reservoirs of M.tuberculosis. It is estimated that one-quarter of world population is infected with M. tubrrculosis.

If a person has latent TB, the TB bacteria in their body are 'asleep'. The infected are not sick and cannot pass TB on to others. However, the bacteria might 'wake up' in the future, multiply making them ill with active TB. The good news is that latent TB can be treated to prevent this happening.



What are the main differences between active and latent TB? (60)

Latent TB

- TB bacteria are asleep in your body
- you do not feel sick- do not have symptoms
- you cannot spread TB on to others

- the infection can only be detected through a blood test or TB skin test
- can be treated with one or two drugs over three to six months

Active TB

- TB bacteria are awake, are multiplying and making you sick
- you will have symptoms that make you feel unwell
- you can spreadTB to others if it is in your lungs
- it is visible on a chest x-ray if you have TB in the lungs
- can be treated with four or more drugs over at least six months

How does latent TB work?

When a germ enters the body, a battle between the germs and immune cells and their products ensues. Very few people fall ill immediately after they breathe in TB bacteria. If you are in good health, your strong immune system – your body's defence against illness – is likely to remove all the TB bacteria that you breathe in and win the battle. If it is unable to do this, it may at least stun the bacilli to sleep state and prevent you from becoming ill. The bacteria are still in your body,

they are not multiplying and they are not causing damage, and so there are no symptoms.

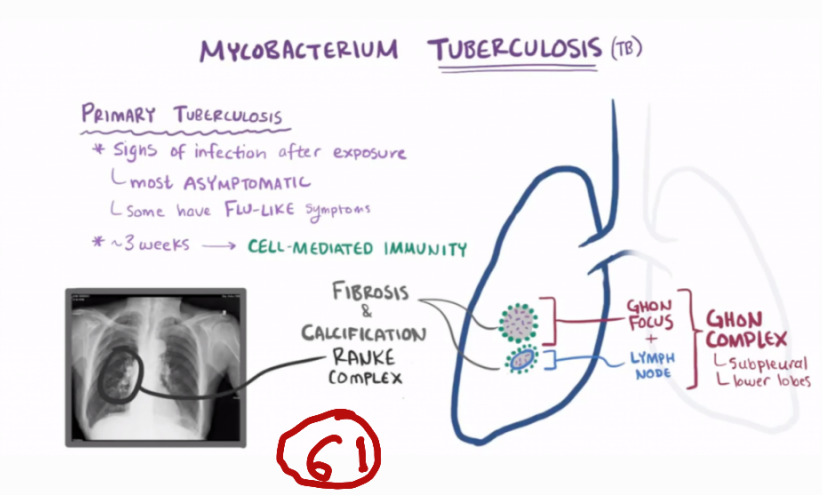
However, latent TB bacteria can ‘wake up’ and become active in the future, making you ill. This can happen many years after you first breathe in TB bacteria. Latent TB bacteria are more likely to wake up if you experience lifestyle stresses or other illnesses that weaken your immune system.

10.1. What are the different stages of M.tb infection?

A. *Exposure*- a person is exposed to the bacilli coming out of the upper respiratory tract (nose/ mouth) of a person suffering from disease. The bacili don't reach the alveoli and they dont multiply.

B. *Infection* - it can be *primary, latent or secondary.*

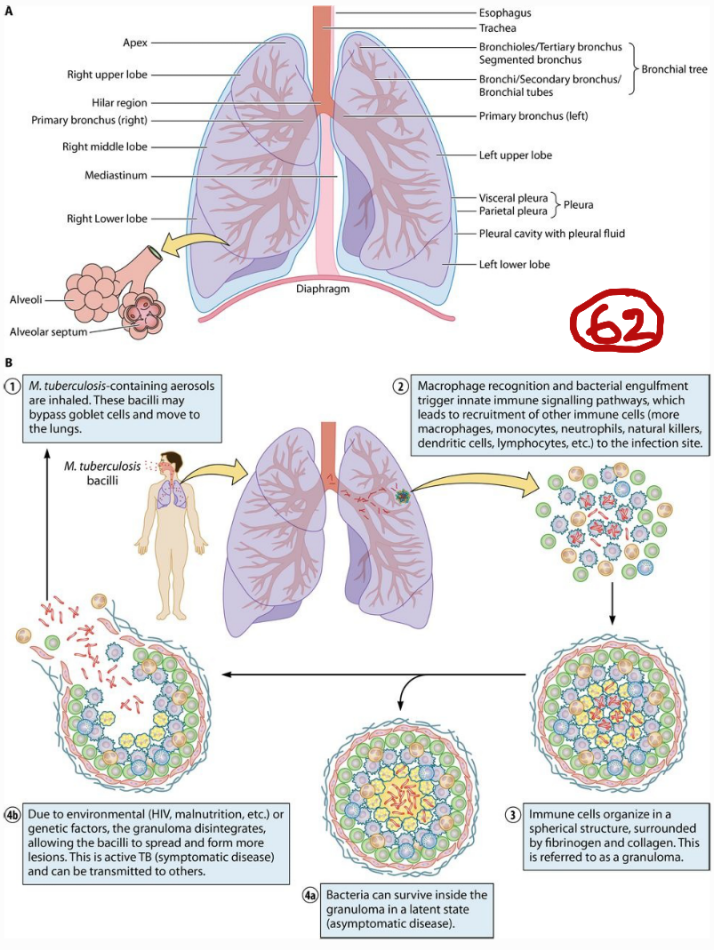
Primary infection- the bacilli reach the alveoli, enter big swallowing macrophages and start multiplying eliciting strong immune response from the body resulting in *granuloma* formation which tries to prevent the spread of bacilli by surrounding the focus with a barrier ring of various immune cells. A few bacilli may escape to regional *lymph node (see my note - part 1)* and infect it



(*primary complex).*

Granuloma is formed in both the lung and lymph node (61). Healing takes place through death and destruction of cells which form a *cheesy* (caseous) centre. Gradually, the focus resolves itself apparently without any residual bacilli or residual sign. The only sign of infection in some people would be white shadow in the lung and the middle of chest (lymph node).

Latent infection- (62)there are three different directions the bacilli may take- before the strong immune response is initiated following infection, a few bacilli go into silent, dormant mode and travel inside macrophages to other parts of the lung especially the upper lobe where oxygen tension is high and stay quiet there waiting for suitable opportunity. The second

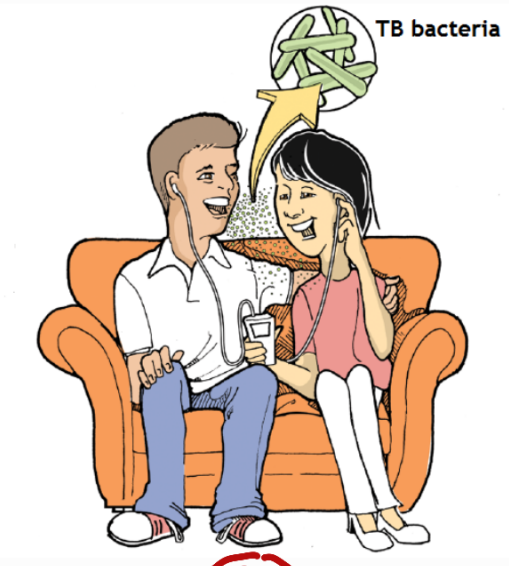


direction is during the initial immune response a few bacilli travel in blood to different organs and stay quiet. The third direction is a few bacilli remain in the primary focus.

In all these situations how is it possible for bacilli to remain inside the macrophage for a long time if the life span of macrophage is a few days to few months? This means the bacilli must occasionally become active, multiply, leave the macrophage, go to new macrophage and stay there quiet. This must happen repeatedly in cycles for the bacilli to be alive. They don't multiply continuously and adapt themselves to the changed environment (which is toxic) and so there is no response from immune system. In all the three cases there will be no evidence of disease or evidence may be in the form of *calcification of focus* (necrotic tissue is either removed totally or calcium is deposited and it becomes firm) which is visible in X-Ray as white spot in lung and lymph node. The bacilli wait for opportune condition to fully awaken themselves and become active again and multiply. About 10% of the individuals with latent infection may develop active disease any time in their life , but most commonly within two years of infection.

Secondary infection- (63)sometimes a person who has

been infected before may be infected again. This is called secondary infection. This appears to be more common in high endemic settings like in Asia and Africa. This means natural infection does not offer lifelong protection. This may be one of the reasons why we have not succeeded in controlling TB epidemic.



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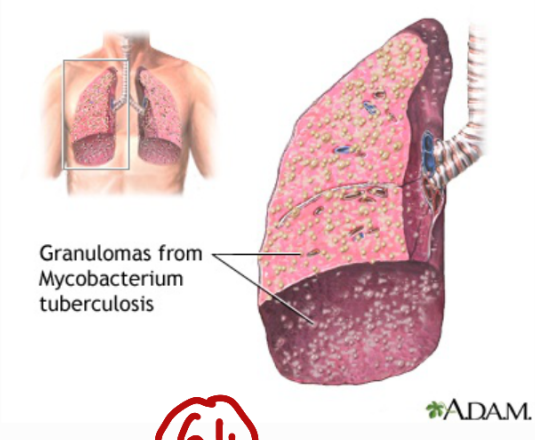
C. Disease- disease is either *progression of primary infection, reactivation of latent infection or progression of secondary infection*.

Progressive primary infection- primary infection in the lung does not go into latent stage but immediately progresses and develops into active lung tuberculosis involving either the lung or the covering of the lung, or it can be Tb disease of any other organ or multiple organs.



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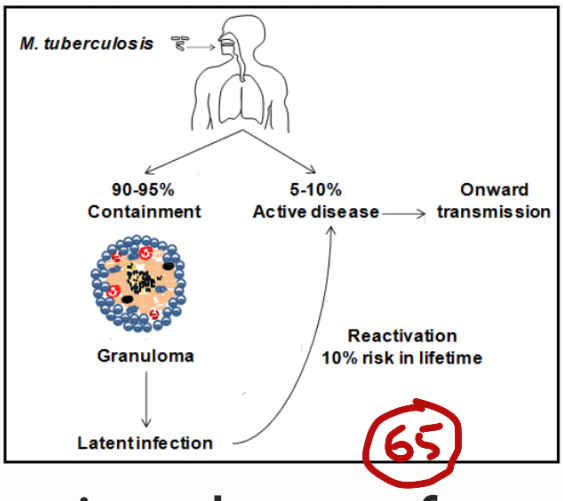
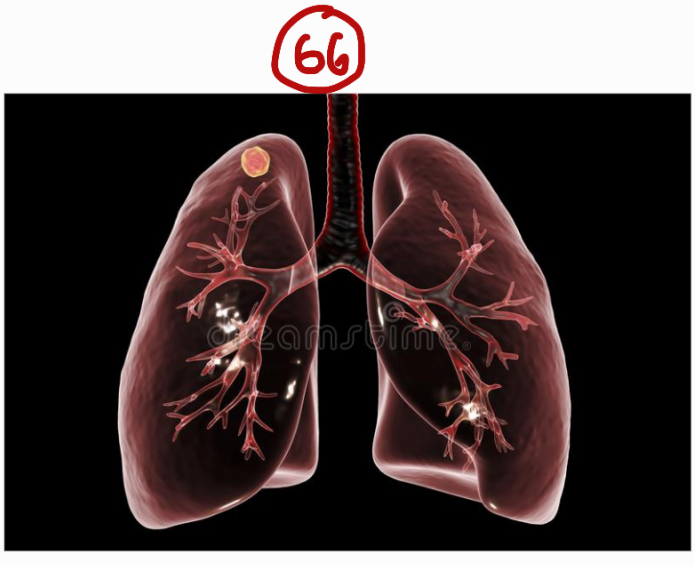
Sometimes, various organs including lungs are studded with small millet like tubercles and the condition is called *miliary tuberculosis* (64)(the tubercles look like millet). Progressive primary is more common in children

just like primary infection.

Reactivation of latent infection-(65) Following latency over a period of time which can range from 2 years to more than 20 years, the dormant bacilli in the initial primary focus (which retain the ability to become active, multiply and to cause active TB if there is a change of immune response) or in the upper lobe or in other organs, start multiplying and produce Tb disease.



Progressive secondary-(66) following secondary infection, the infection may progress giving rise to TB disease. Persons with certain risk factors are more likely to develop reactivated or progressive secondary disease. They include persons with HIV coinfection, children, malnutrition, diabetes, smoking.

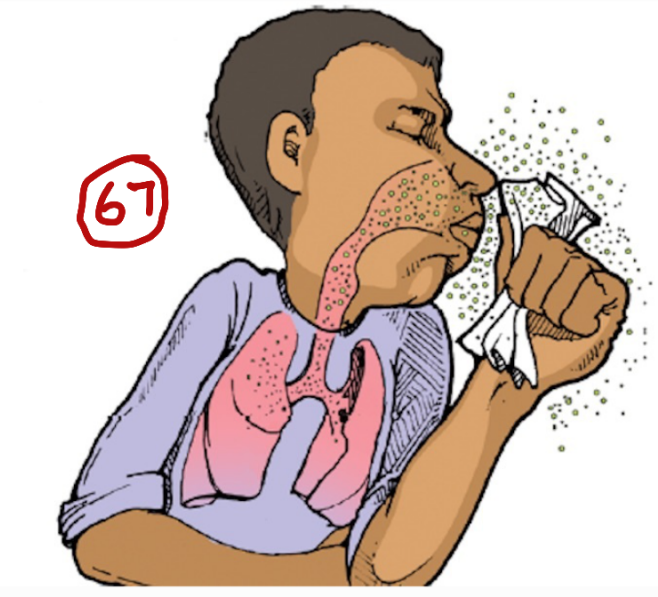


It is clear that the main source of M.tuberculosis is persons with *latent tuberculosis and those with active disease*. Unless both the groups are treated it will be

very difficult to control the epidemic. Let us try to find out more about these two states.

Latent Tuberculosis infection

Active disease patients with sputum smear-positive pulmonary TB are the main source of infection in a community. (patients cough and bring out sputum or phlegm. Since bacilli are expelled during coughing from cavity in the lung which is connected to bronchioles, bronchi and through the respiratory tract to nose and mouth; bacilli can be demonstrated by examining under microscope the stained sputum or phlegm expelled when a person coughs -67). Primary infection with *M. tuberculosis* leads to clinical disease in only ~10% of individuals. In the remaining cases, the resulting immune response stops further growth of *M. tuberculosis*. However, the pathogen is completely removed in only ~10% people, while the immune response in the remaining ~90% individuals only succeeds in containment of infection as some bacilli escape killing by subduing the killing mechanisms of immune cells and remain in nonmultiplying (*dormant or*



latent) state in old lesions. The World Health Organization (WHO) has estimated that one-third of the total world population is latently infected with *M. tuberculosis* and 5%–10% of the infected individuals will develop active TB disease during their life time . However, the risk of developing active disease is 5%–15% every year and lifetime risk is ~50% in HIV coinfected individuals .

In several African and Asian countries, where the transmission of *M. tuberculosis* has been stable or increased in the last few years, the incidence rate is *highest among young adults* with most cases resulting from recent episodes of infection or *reinfection*, rather than reactivation of latent infection. On the contrary, in low TB incidence countries of Western Europe and North America, a higher proportion of cases occur in older patients through *reactivation* or among immigrants from high TB incidence countries. Pulmonary TB or TB in lungs accounts for >85% of active TB cases in high TB incidence countries due to higher rates of active transmission, while extrapulmonary TB or TB in organs other than lungs is also common in low TB incidence countries of the developed world, particularly among HIV-infected individuals and immigrants originating from TB endemic countries .

10.2 .What is the reason for the latency or dormancy?

The exact mechanism whereby M.tuberculosis survives and lies dormant inside macrophages is not clear but there are various hypotheses with some experimental evidence. The irrefutable fact is, unlike other organisms, M.tuberculosis grows well inside *macrophages* which are known to "*grab, kill and eat"* any organism that they discover- M.tb in fact loves to live inside macrophage. However, some bacilli are released from these macrophages when they undergo necrosis (death by damage to cell) or killed by inflammatory response. These bacilli do not multiply. The bacilli become dormant even before an effective immune response has fully been developed due to oxygen poor and acid rich environment outside the macrophage and release of bactericidal enzymes from dead macrophages and neutrophils. These nonreplicating bacilli escape killing and survive because they are not active or multiplying. The actively multiplying bacillary population is eventually killed due to

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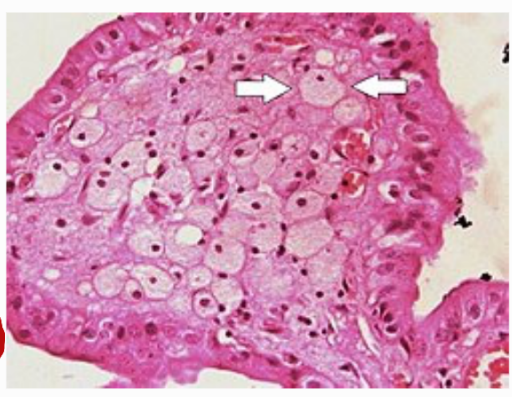
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the development of an effective immune response. Some macrophages develop into *foamy macrophages (68,69)* whose inside is full of vacuoles or vesicles filled with fat. This is due to the fact that being scavenger cells they swallow cellular debris (pieces of killed macrophages and bacilli) which are rich in fat derived from cell membranes of dead cells. The bacilli which are dormant are also swallowed by these foamy macrophages but these bacilli are not killed because they are not multiplying or active. These fatty vesicles provide nourishment to dormant bacilli inside the macrophages. These foamy macrophages filled with fatty vesicles and dormant M.tb bacilli leave granuloma and go to different parts of lungs and may be different organs and seed them. The bacilli begin infection process at this new location again. The upper lobe is preferable location for various reasons (will be explained in later chapters) including high oxygen pressure there and this is where the bacilli are more likely to cause cavitary lesion (which is what the bacilli want because by this they can exit and spread). The bacilli induce a stronger inflammatory response, recruitment of more immune cells, stronger inflammation which leads to tissue destruction, the destroyed cells are turned to liquid in which the bacilli can thrive. This aggravates immune response further, finally resulting in cavity from which bacilli can escape

outside and spread. Formation of cavity is due to aggravated immune response and this is induced by bacilli because this helps in their escape outside!

This process has some parallel in manifestation of Tb disease in HIV infected patients. In HIV infected individuals infected with TB bacilli the immune response is subdued because of low levels of T cell lymphocyte (CD4+ lymphocyte). The presence of bacilli is well tolerated by these patients. Because the immune response is stifled, it does not result in caseous granuloma, cavity formation (cavitary tuberculosis is less common in HIV coinfected individuals). However, the sudden increase in CD4+ T cells in AIDS patients receiving highly active antiretroviral treatment causes an aggressive granulamatous response, caseous necrosis, cavity formation and active TB disease.

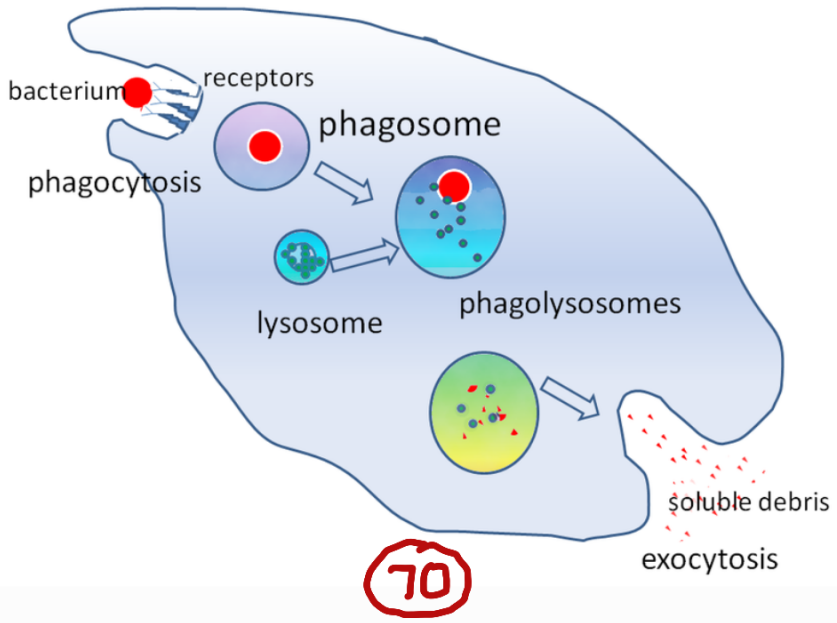
The trick here is when the condition is unfavourable the bacilli go to sleep, since they are not metabolically active they are not affected by immune response. They lie inside the macrophage. When the macrophage dies after its lifespan, the bacilli come out and are swallowed by other macrophages where they continue their dormant life. The cycle is repeated. When the condition becomes favorable because of the change in immune status of infected individual, the sleeping beauty wakes

up, multiplies and causes disease by stimulating aggressive immune respinse.

Therapy for a relatively short time (9 months) with a single drug (isoniazid), active only against actively dividing bacilli , is highly effective for a latent infection that can possibly remain dormant for the entire lifetime of the host. The drug isoniazid which is used in treating LTBI prevents episodes of reinfection by bacilli reactivated from dormancy.

10.3. How do bacilli survive in latency?

Infection with *M. tuberculosis* starts with swallowing of the bacilli by macrophages in alveoli. The bacilli are recognized and swallowed by macrophages which secrete various *cytokines like TNF alpha* and others to recruit more macrophages and other blood cells. Too much cytokine causes damage to neighbouring normal cells. There is a negative feedback mechanism in macrophage which tries to regulate the secretion of cytokines through secretion of certain cytokine suppressing enzymes. M.tuberculosis uses this



mechanism to dampen immune response.

Bacilli *express* several genes (*genes become active)* and produce various proteins and enzymes to neutralize and evade the lethal defenses inside macrophages (70). Their actions are mainly inside the *phagosome/ phagolysosome* because that's where the M.tb killing takes place. Remember, the macrophage puts forth its arms around the bacillus after recognizing it, fuses the arms, forms a vesicle with bacilli inside (*phagosome*) which moves towards the vesicle (*lysosome*)containing toxic enzymes and fuses with it. The enzymes are released into fused *phagolysosome* killing the bacilli. The bacilli are capable of inhibiting these processes. Inside the phagosome - the bacilli

- reduce the acidification which kills,
- the thick cell wall prevents damage from acid,
- prevent fusion of phagosome with lysosome,
- try to suppress the production of lysozymes (enzymes in lysosome) or blunt their action,
- neutralize free oxygen and nitrogen radicals inside the phagolysosome and are highly toxic,
- help themselves move into the more favorable less acidic cytoplasm,
- prevent self death (apoptosis) of phagocyte,
- promote phagocytic necrosis: the released bacilli multiply extracellularly, are swallowed by another

macrophage that also fails to control the growth of *M. tuberculosis,* and likewise are destroyed. The result is granuloma formation at the site of infection; and

- use efflux (expelling) pump to expel toxic substances.

Simply stated, Mtb converts the toxic environment inside into supportive environment and survives; or jumps outside phagosome into cytoplasm and survives; or causes death of macrophage to come out of cell. Or the M.tuberculosis when confronted with adverse environment expresses several genes, induces dormancy , a sleeping state, metabolically inactive, capable of surviving inside hostile acid rich and oxygen depleted necrotic area.

It's main aim in the initial stage is to intensify inflammation so as to form a granuloma with caseous necrosis in the middle so that it can survive in this necrotic material. At a later stage the inflammation is changed to become modulatory, supporting the survival of the organism.

10.4. What exactly is Latent Tuberculosis Infection (LTBI)?

Imagine a situation where bacilli are there in the body but do not produce symptoms, do not multiply, cannot be detected. The only evidence is sensitization of immunity. So, LTBI is a persistent immune response to stimulation by M.tb antigens without evidence of manifestation of disease and with no or very little multiplication of M.tb bacilli. How do we identify this? By looking at the altered immune response which occurs after infection and persists.

It is important to identify infected people because some (10%) of them may develop active disease later in life, suffer the consequences and spread to others. A person infected with TB bacilli can be treated so as to prevent the occurrence of disease and reduce transmission.

10.5. How is latent TB infection diagnosed?

Principle

There is no gold standard test for LTBI. Indeed, because of the presence of very small number of nonmultiplying bacilli, we cannot detect the bacilli or their components by the usual diagnostic tests. The diagnosis of LTBI is rather indirect and relies on evidence of an altered immune response to mycobacterial antigens. You don't detect antigens if there are no bacilli. Presence of

antigen is indicative of presence of bacilli even 8f they are undetectable. There are no perfect methods for the diagnosis of latent tuberculosis infection. The most commonly used tests for LTBI diagnosis are the intradermal tuberculin test (TST) and IGRAs which indirectly measure tuberculosis infection by detecting memory T-cell response, which reveals only sensitization to M. tuberculosis antigens. These tests tell us that the individual has been exposed and infected and does not tell us if the person has active disease or is likely to develop disease. The tests are, therefore, generally considered to be acceptable but imperfect.

Six to eight weeks following infection with M.tuberculosis persons will develop sensitivity to the M.tb proteins because the CD4+T cells are activated by macrophages, multiply, produce clone of cells with memory of M.tb and are primed and ready to deal with M.tb and its antigen (first infection primes immune cells which will be ready to mount immune response when the bacilli or parts of the bacilli enter or introduced into the body). Along with effector T cells which spring into action immediately, memory T cells are produced which lie in wait and become activated, multiply and launch themselves when reexposed to bacilli or their antigens. Anything resembling TB bacilli, even if it is a small part of it, is recognized by the immune system

which launches a reaction to expel the antigen. Since it is not the whole or live bacillus that is used in the test the person will not develop disease. So, an infected person can be identified by doing a tuberculin skin test or IGRA test.

When you inject tuberculin into the skin of a person who is not infected, the macrophage visits the site, sniff at the antigen and says to itself ' I am not familiar with this and this is not dangerous', and rushes off. Nothing happens. If a person is infected, the macrophage which reaches the spot sniffs at the proteins in tuberculin and says to itself, " Ah ha. I recoginse this. An intruder'. It swallows the protein and secretes cytokines which causes the blood vessels in the area to expand and the fluid leaks out into the surrounding tissue along with sensitised immune cells like CD4+ T cells which also secrete cytokines and add to the inflammation. With fluid leak the site becomes swollen, with cytokines the area becomes red and with stimulation of nerve endings the area may become slightly itchy or tender. These are the signs of inflammation. There is usually a delay in this process because of the time required for recruiting CD4+T cells from the lymph nodes. When the offending tuberculin is completely cleared the swelling and other signs of inflammation subside.

In the case of IGRA (Interferon Gamma Release Assay - test which detects the interferon gamma released from T cells when stimulated by TB specific antigens) , a sample of blood is taken, it's T cells are extracted and mixed with TB specific antigens. The T cells, if they are primed by earlier exposure to M.tb, are stimulated by antigens to release cytokine called *gamma interferon.* The amount of interferon produced is measured or the number of interferon producing cells is counted to arrive at diagnosis.

10.5.1. What is tuberculin skin test?

One of the tests used to identify infection with TB bacilli is Tuberculin skin test (TST). The TST was developed more than a 100 years ago (1890) by Robert Koch from killed M.tuberculosis. He did not know the composition of tuberculin but believed that it cured Tuberculosis. He was wrong. It did not cure tuberculosis. Even though it's therapeutic value was disproved later, it's use for diagnosis was recognized. It was called *old tuberculin*.

In 1934, Seibert and Glenn prepared the first batch of a much more purified preparation, which they termed *purified protein derivative* (PPD). *Tuberculin PPD (71)* is a mixture of proteins derived

from filtrate of M.tuberculosis culture killed and purified. It does not have bacilli, live or dead, but only soluble proteins. There are different manufacturers of PPD referred to as international standard (PPD-SI) and other formulations such as PPD RT23 produced by Statens Serum Institut, in Copenhagen, which is the most widely used PPD in the world, and a variability of the potency among PPD may affect the TST result.

In order to standardize the product, Statens Serum Institute, Copenhagen, prepared a batch of PPD from M.tuberculosis. It was extensively tested in animals and a final batch of 670 gm of PPD which was enough to cover 33 million people was prepared. Samples from this batch were supplied to all the countries for their use. India obtained dry powder from SSI and it was reconstituted in BCG lab Guindy, in Chennai, in isotonic buffer solution and supplied in 5ml vials as a ready to use preparation and 0.1 ml of tuberculin corresponds to 1 tuberculin unit (1 TU). Ever since, India has been using this PPD which is called PPD RT23.

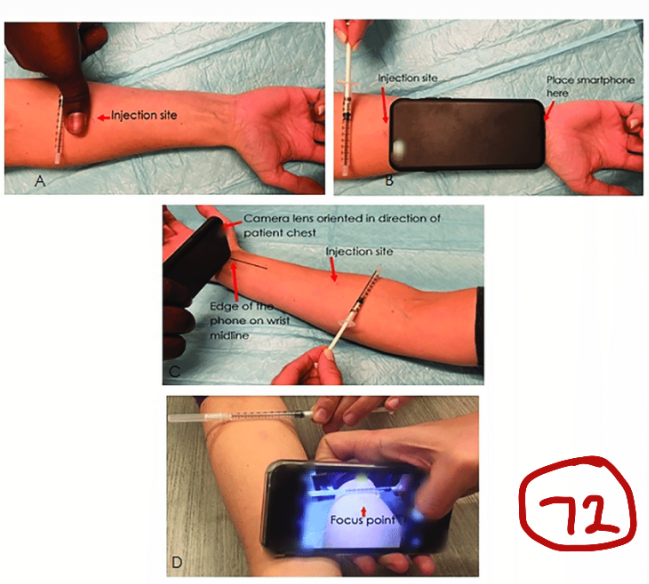
PPD of non-tuberculous (i.e. atypical) mycobacterium are identified by a letter other than S. PPD-A is from *M. avium*; PPD-G from the Gause strain of schotochromogen; PPD-B from the nonphotochromogen Battey bacilli; PPD-F from the rapid grower *M.*

fortuitum and PPD-Y from the yellow photochromogen *M. kanasasii*.

TST is based on hypersensitivity skin reaction to tuberculin PPD. The most prevalent antigenic proteins in PPD are now known to be the bacterial heat shock proteins (or chaperonins). These proteins which constitute 70% of the tuberculin are present in other tuberculous bacteria like BCG and other mycobacteria. This should be kept in mind while interpreting the result of tuberculin skin test. The antigen is prepared in liquid form containing the detergent Tween 80 to decrease adsorption (sticking) of protein to the glass of the vial.

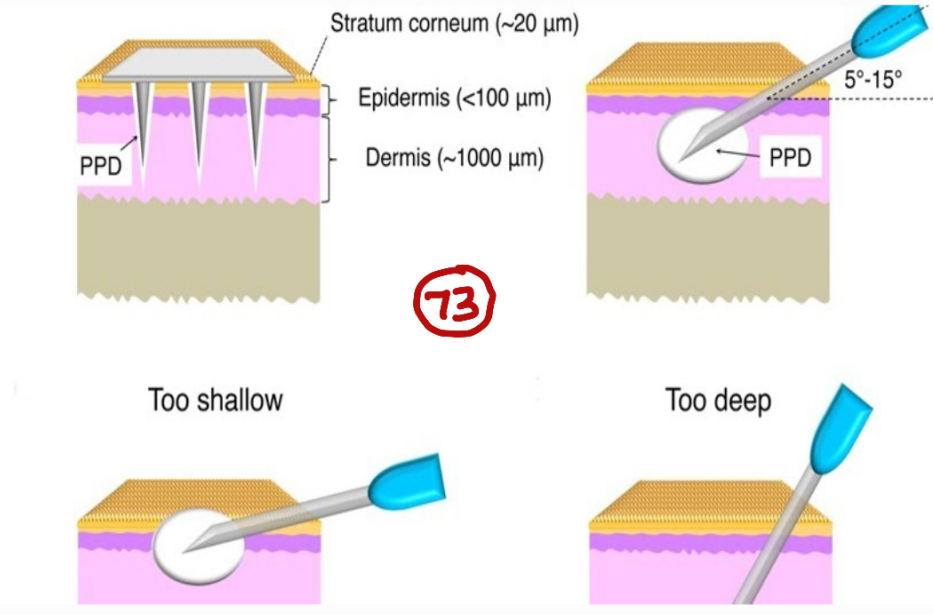
The tuberculin skin test is also called mantoux (pronounced man tou) test and it comprises of two parts: administration of tuberculin and reading of the result.

A. The injection or test



The standard tuberculin test consists of the injection of 0.1 mL of material, which contains 5 *tuberculin units* (TU) of proteins in a solution

obtained from M.tuberculosis (*killed, filtered, purified- that's why it is called purified protein derivative*), into the upper layers of skin, not below the skin (it should not be too shallow or too deep)(72). The site usually chosen is the front surface of the forearm, about 5 to 10 cm below the elbow crease, but any accessible area can be used (the site should be free of scar or blemishes). The forearm is placed palm-up on a firm well-lit surface. A short-beveled 26- or 27-gauge needle should be used with a 1-mL graduated syringe. The needle is inserted slowly into the upper layers of skin (73), bevel up, at an angle of 5–15° (needle bevel should be visible just below skin surface)(74). After injection, a flat bump (in which hair follicles form dimples- looks like the peel of orange) of 8–10 mm diameter should appear(75). If not, the injection is repeated at a site at least 5 cm (2 inches) away from the original site. The information is recorded.



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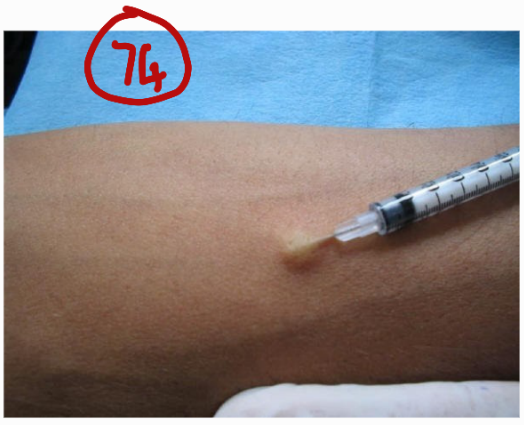
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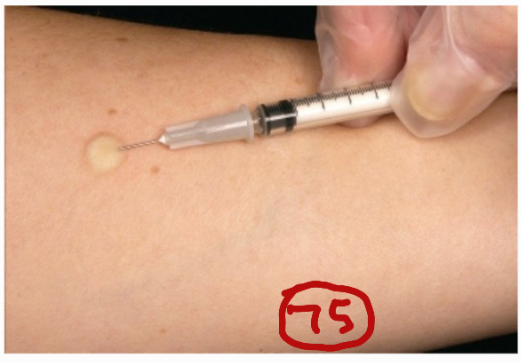
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eg. date and time of test administration, injection site location, lot number of tuberculin.

B. Reading the reaction to the test

The person has to report back to the hospital 48 to 72 hours after the injection for reading. If they do not return within 72 hours, they will probably need to be rescheduled for another TST.

The reaction to the test one expects is formation of redness in the skin with a bump in the middle at the site of injection. The size of the bump or induration is felt first and then measured. The reaction to the test should be read by inspecting and palpating the area where the tuberculin was injected. The size is determined by measuring the diameter of any bump or induration with a ruler. The amount of redness should not be taken into account; only the extent of bump is important. Readings must be recorded accurately in millimeters.

Reading

Inspection

- Inspect injection site under good light, and measure thickening of the skin, not reddening of the skin.

Palpation

- Use your fingertips to find the induration (76). This is a hard, dense, raised formation with definite borders. If there is a firm bump, use a pen to mark the widest edges of the induration on the forearm. The only part that is important for test results is the hard bump. The reddened area or any slight swelling does not count towards the size of the induration. You cannot always see the induration. You must find the induration with your fingertips.

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Mark induration (77)

- Place a mark with a pen on the right and left edges to demarcated the swelling area. Don't include redness in the marked area.

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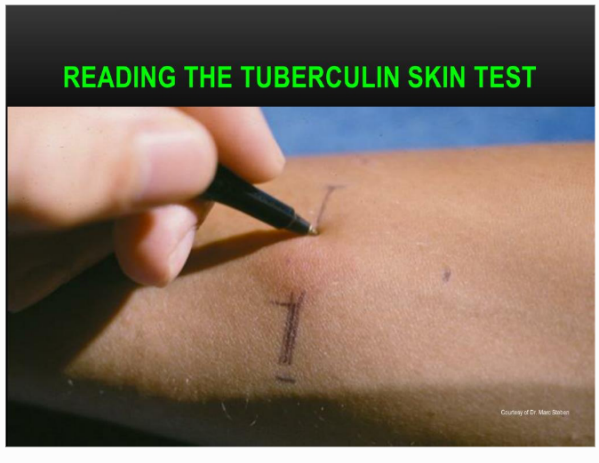
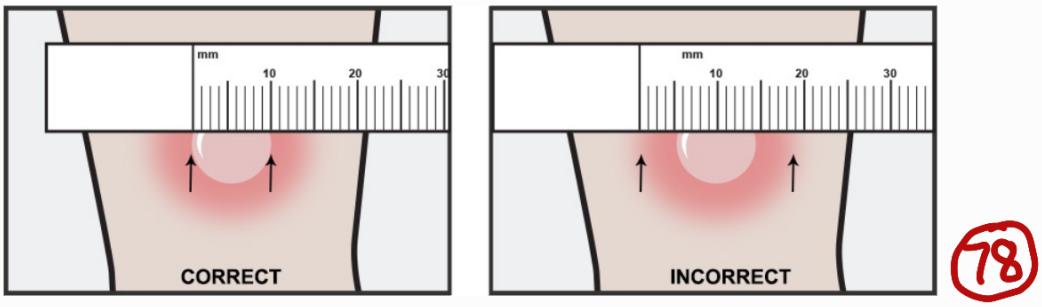


Measure diameter of induration using a clear flexible ruler (78)

- Place “0” of ruler line on the inside left edge of the induration.



78

- Read ruler line on the inside right edge of the induration (use lower measurement if between two gradations on mm scale).

Record diameter of induration

- Do not record as “positive” or “negative”.
- Only record measurement in millimetres.
- If no induration, record as 0 mm.

C. Interpretation

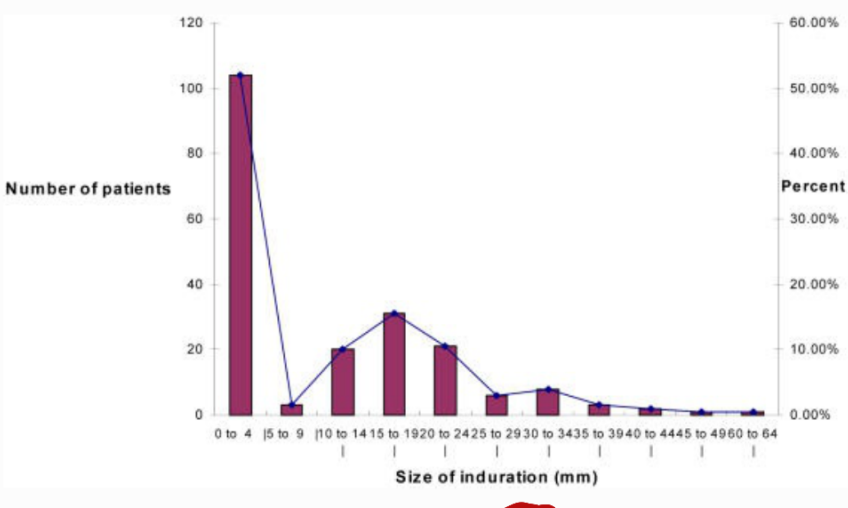
Interpretation of TST depends on two factors:

- size or diameter of the induration;
- person's risk of being infected with TB and of progression to disease if infected.

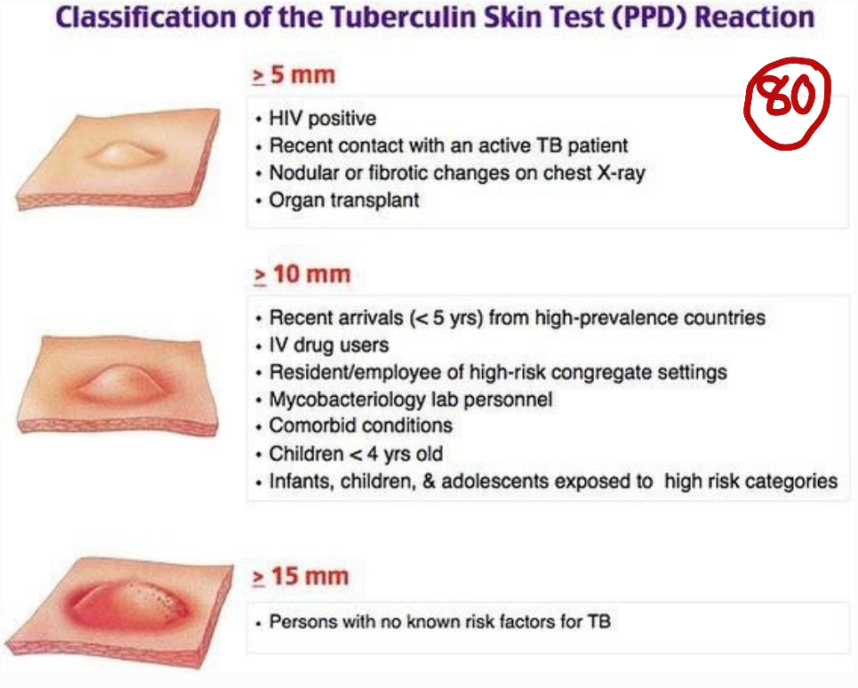
How do we decide on the cut off point of induration by which we can say a person is exposed and infected or not?The interpretation of tuberculin tests requires clinical judgment, as well as an understanding of the test. For this, a sample of children below 5 years is chosen and the swelling to the injection of tuberculin in

these children is measured. The measurements are plotted on a graph.

In a population in which the only M.tuberculosis is the one causing infection the curve describing the distribution of reaction sizes in otherwise healthy infected persons given 5-TU PPD would be bell shaped, having a mode of 17 to 18 mm, with very few reactions less than 10 mm. Thus, defining the minimum reaction size indicative of tuberculous infection would be simple. However, in many parts of the world, a portion of the population is infected with nontuberculous mycobacteria, which induce some degree of sensitization to tuberculin; inoculation with BCG, for many years the world's most commonly used vaccination, has the same enhancing effect on tuberculin reactivity. Although these reactions are on the whole smaller than those caused by *M. tuberculosis,* they blur the distinction between reactions in persons infected with *M. tuberculosis* and those not infected. In such a population where infection with M.tuberculosis and other mycobacteria (including BCG) what you see is a curve with two peaks or modes: an initial larger peak or



mode between 0 and 4 mm, followed by a smaller second peak between 15 to 19 and the trough or antipeak or antimode of 5 mm which is the cut off point that is used(79). Majority of the reaction sizes below 5 mm is due to infection with mycobacteria other than tuberculosis. These mycobacteria called mycobacteria other than tuberculosis (MOTT) are pervasive in the environment in TB endemic regions. The second mode is probably due mostly to infection with M.tuberculosis. Therefore, the antimode of 5 mm is chosen. But then this is linked to the presence or absence of risk factor for infection and progression of infection and the grade of risk. On the basis of a large amount of epidemiologic data and skin testing with antigens prepared from nontuberculous mycobacteria, the best compromise between false-positive and false-negative readings to 5-TU PPD tuberculin tests is 10 mm. Thus, under most circumstances, a reading of 10 mm or more is considered indicative of infection with *M. tuberculosis.* However, in some situations, smaller reactions should be taken to indicate tuberculous



infection. Therefore, risk factors along with size of induration is taken(80).

For example,

- *induration of diameter ≥5 mm is considered positive in:*
  - HIV-positive children;
  - severely malnourished children (with clinical evidence of marasmus or kwashiorkor). And

- *Induration of diameter ≥10 mm is considered positive in:*
  - all other children (whether or not they have received BCG)

A person may have more than one risk factor like less than 4 years and a TB suspect.

D. Pitfalls of PPD

While the TST has been the standard in identifying persons at risk of active TB for the past century, it has several fundamental flaws. It is,therefore, useful but imperfect.

The primary concern with the current test is the high level of *false-positive results (81),* caused by the inability

of the TST to distinguish between *Mtb* infection and either exposure to nontuberculosis mycobacteria or vaccination with Bacille Calmett-Guérin (BCG). Both cases of false-positive responses are generally attributed to an immune response triggered by antigens shared across mycobacteria from either vaccination with BCG or from environmental mycobacteria. Up to 70% of proteins in PPD are shared between M.tb and other mycobacteria including BCG. If you have had BCG or if you are infected with other mycobacteria you may be tuberculin positive without being infected with M.tb. The *BCG vaccine* uses an attenuated, live strain of bovine tuberculosis; it usually is administered to children in most countries for protection against tuberculosis, although protection conferred by the vaccine likely is limited to early childhood (protection against mainly the severe form, not against pulmonary TB and whatever protection it gives it does not last more than ten years). It is

Tuberculin Skin Test Results 81

Causes of false-negatives	Causes of false-negatives *(continued)*
Acquired immunodeficiency syndrome	Systemic viral, bacterial, and fungal infections
Alcoholism	Use of corticosteroids or other immunosuppressant medications
Gastrectomy or intestinal bypass	Zinc deficiency
Hematologic or lymphoreticular disorders	**Causes of false-positives**
Inaccurate reading of induration	Boosting phenomenon†
Live virus vaccines (measles, mumps, and rubella; poliovirus)*	Cross-reaction with nontuberculous mycobacterial antigens
Malnutrition	Error in administering the test
Patient age older than 45 years	Previous bacille Calmette-Guérin vaccination
Renal failure	
Sarcoidosis	

**—When live virus vaccines are administered with the tuberculin skin test, the result is not affected; if these vaccines are given before the tuberculin skin test, results may be false negatives for up to two months.*

†—Institutions may use a two-step approach (test at baseline and again in one to three weeks) to detect boosting reactors and avoid misclassifying them as converters.

Information from references 16 and 17.

assumed that prior BCG vaccination induces tuberculin skin test reactivity, and that a positive TB skin test in a vaccinated person is a sign of the vaccine's effectiveness. However, the cross-reacting response to tuberculin testing following BCG, if one occurs at all, usually is small and it decreases in size rapidly following vaccination. In addition, the degree of actual protection conferred by the vaccination is highly variable, and people who receive BCG are from regions of the world where TB is endemic and a large proportion of the adult population truly is infected with *M. tuberculosis*. For these reasons, a positive skin test response in a high-risk person who has received BCG in the past usually is interpreted as indicating true infection with *M. tuberculosis* .

Sometimes Incorrect administration or incorrect interpretation (redness included) may give false positive result.

False negatives *(81)*are also problematic, particularly in children and immunocompromised individuals . This is attributed to the fact that a positive PPD requires an efficient immune response. Therefore it is likely that PPD fails as an indicator of *Mtb* infection in those populations where good T-cell immunity is lacking.The absence of cell mediated immunity to tuberculin may be

due to the:

- lack of previous sensitization (exposure or infection) or
- to a false-negative result for various reasons or
- to anergy because of immune suppression (HIV, Measles, cancer, malnutrition, immunosuppressive drugs)
- Error in injection, interpretation, recording
- negative for other reasons. Most children with negative result have not been infected with *M. tuberculosis*. A small proportion of otherwise normal children with *M. tuberculosis* infection remain PPD-negative for unknown reasons. From the time of infection to the development of CMI there is a window period of some two to six weeks, when the Mantoux test would be negative. Negative tests can be interpreted to mean that the person has not been infected with the TB bacteria or that the person has been infected recently and not enough time has elapsed for the body to react to the skin test. A repeat test is not advocated before one week as the tuberculin injected for the first test has a booster effect on the subsequent dose. TST may convert to positive ≤eight weeks after *Mycobacterium tuberculosis* infection, an interval that is usually referred to as the “window period”. A negative TST obtained < eight weeks

before does not exclude infection, and a second test is recommended after eight weeks. Also, because it may take longer than 72 h for an elderly individual to develop a reaction, it may be useful to repeat the TB skin test after 96 h and again at one week to adequately screen these individuals.

- Some individuals living in highly endemic areas remain TST negative, suggesting that these individuals are more likely to be naturally resistant to *M. tuberculosis* infection rather than intrinsically deficient in eliciting immune response .

Finally, while the TST can be used detect LTBI, it fails to differentiate between this, active disease or the convalescent patient. Despite these pitfalls, the TST remains the most commonly used tool to detect *Mtb* infection.

E. The booster effect

In some persons who are infected with *M. tuberculosis*, the degree of reaction to tuberculin may wane over time. When given TST years after infection, these persons may have a false-negative reaction. However, the TST may stimulate and prime the immune system, causing a positive or boosted reaction to subsequent tests. Giving

a second TST after an initial negative TST reaction is called *two-step testing.* When sensitization to mycobacteria (infection with M.tb) has occurred many years earlier, an initial intradermal injection of tuberculin may produce a negative or weakly positive response due to there being too few sensitized lymphocytes in circulation to produce a significant local response. If the test is repeated, a larger reading may be obtained due to the immune response being 'recalled' or 'boosted' by the first test. The second boosted reading is the correct one – that is, the result that should be used for decision-making or future comparison. Boosting is maximal if the second test is placed between one and five weeks after the initial test, and it may continue to be observed for up to two years.

F. Mantoux reversion

Reversion is defined as the change to a negative Mantoux result following a previous positive result. Generally this phenomenon is uncommon in healthy individuals, occurring in less than 10% of such people with a previously positive Mantoux.

Reversion is more common

- in older adults (estimated at 8% per year)

- when the initial Mantoux is < 14 mm
- in those where the initial positive reaction was a boosted result (identified by two-step testing).

G. Mantoux conversion

Whereas boosting is a recall of the hypersensitivity response in the absence of new Infection, conversion is the development of new or enhanced hypersensitivity due to infection with tuberculous or non-tuberculous mycobacteria, including BCG vaccination.

Mantoux conversion is defined as a change (within a two-year period) of Mantoux reactivity which meets *either of* the following criteria:

- a change from a negative to a positive reaction
- an increase of ≥ 10 mm.
- Conversion has been associated with an annual incidence of TB disease of 4% in adolescents or 6% in contacts of smear-positive cases.

There is debate about the time required for the immunological changes that produce Mantoux conversion following infection. After inadvertent vaccination with *M. tuberculosis* (the Lubeck disaster), children developed positive reactions in three to seven

weeks. Other studies have shown clinical illness, with a positive tuberculin test, from 19 to 57 days after exposure, with a mean of 37 days.

Therefore, when testing TB contacts for conversion, the second tuberculin test is done eight weeks after the date of last contact with the source case. (In the past, the traditional window period, or interval, of 12 weeks was used.)

H. Situations where Mantoux testing is not recommended

Mantoux testing is not recommended in the following situations:

- Past Mantoux reactions ≥ 15 mm: repeating the test will provide no new diagnostic information and will create discomfort
- Previous TB disease: no useful diagnostic information will be gained and significant discomfort is likely
- Infants under 12 weeks old: a positive reaction is very important, but a negative reaction may indicate that the child is too young to mount a response, and the test will need to be repeated if exposure has occurred.

10.5.2. IGRA (Interferon Gamma Release Assay)

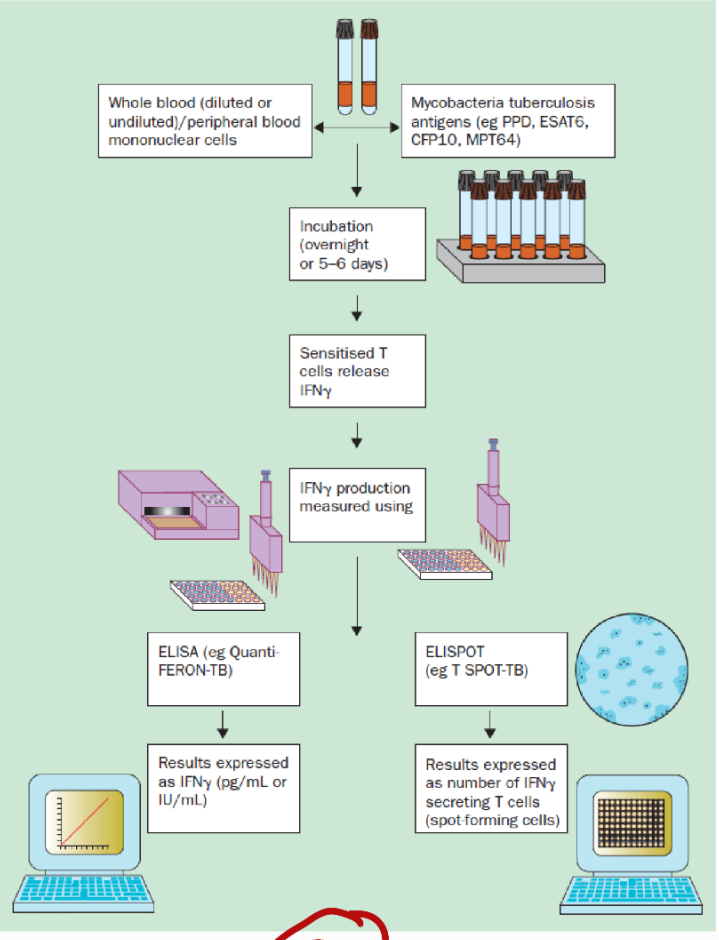
A. What is interferon gamma?

Interferon gamma is a protein or cytokine that is produced by macrophages and lymphocytes. It is produced first by macrophages which secrete several cytokines including interferon gamma and TNF alpha when they are stimulated by pathogen. The mobile macrophage or *dendritic cell* then carries a bit of pathogen and presents it to lymphocytes in the lymph nodes. This results in stimulation of lymphocytes which multiply and form a clone of cells with the memory of pathogen. This clone of T cells in turn produce several cytokines including interferon. The amount of interferon produced depends on the number of T cells. Higher the number the larger the interferon concentration. It recruits more macrophages and monocytes and neutrophils to the site and activates other lymphocytes through the interferon and other cytokines.

B. What are the IGRA tests (82)?

Interferon-Gamma Release Assays (IGRAs) are whole-

blood tests that can aid in diagnosing *Mycobacterium tuberculosis* infection, not disease. They do not help differentiate latent tuberculosis infection (LTBI) from tuberculosis disease. Two IGRAs that have been approved by the U.S. Food and Drug Administration (FDA) are commercially available.



82

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

C. How do they work?

IGRAs measure a person's immune reactivity to *M. tuberculosis*. White blood cells from persons that have been infected with *M. tuberculosis* will release interferon-gamma (IFN-g) when mixed with antigens (substances that can produce an immune response) from *M. tuberculosis (antigens are* ESAT-6, CFP-10 & TB7.7). These antigens are present only in M.tuberculosis, not in BCG and nor in other

Mycobacteria. The tests are therefore not influenced by prior infection with other mycobacteria or prior BCG vaccination.

To conduct the tests, fresh blood samples are drawn from patient and are mixed with antigens (specific to M.tuberculosis) and controls. It requires sophisticated lab equipments and expertise. The results are available after a day.

D. What are the advantages of IGRAs?

- Requires a single patient visit to conduct the test.
- Results can be available within a day.
- Does not boost responses measured by subsequent tests unlike TST.
- Prior BCG (bacille Calmette-Guérin) vaccination does not cause a false-positive IGRA test result.

E. What are the disadvantages and limitations of IGRAs?

- Blood samples must be processed within 8-30 hours after collection while white blood cells are still viable.
- Errors in collecting or transporting blood specimens

or in running and interpreting the assay can decrease the accuracy of IGRAs.

- Lack of predictability- to predict who will progress to TB disease in the future.
- Limited data on the use of IGRAs for:
  - Children younger than 5 years of age;
  - Persons recently exposed to *M. tuberculosis*;
  - Immunocompromised persons; and
  - Serial testing.
- Tests may be expensive.

F. How are the results interpreted?

IGRA interpretations are based on the amount of IFN-g that is released or on the number of cells that release IFN-g.

As with the tuberculin skin tests (TSTs), IGRAs could be used as an aid in diagnosing infection with *M. tuberculosis*. A positive test result suggests that *M. tuberculosis* infection is likely; a negative result suggests that infection is unlikely. An indeterminate result indicates an uncertain likelihood of *M. tuberculosis* infection. A borderline test result (T-Spot only) also indicates an uncertain likelihood of *M. tuberculosis* infection.

A diagnosis of LTBI requires that TB disease be excluded by medical evaluation. This should include checking for signs and symptoms suggestive of TB disease, a chest radiograph, and, when indicated, examination of sputum or other clinical samples for the presence of *M. tuberculosis*. Decisions about a diagnosis of *M. tuberculosis* infection should also include epidemiological and historical information.

G. Recommendations on when to use IGRA tests

- IGRAs can be used in place of (but not in addition to) TST in all situations including contact investigations, testing during pregnancy, and screening of health care workers and others undergoing serial evaluation for *M. tuberculosis* infection.
- Populations in which IGRAs are preferred for testing:
  - Persons who have received BCG (either as a vaccine or for cancer therapy); and
  - Persons from groups that historically have poor rates of return for TST reading.
  - TST is preferred over IGRAs for testing children less than 5 years of age.

- As with TST, IGRAs generally should not be used for

testing persons who have a low risk of infection and a low risk of disease due to *M. tuberculosis*.

H. Pitfalls with IGRA

Similar to what has been described for TST, the performance of the IGRA tests can be affected by several factors, mainly related to an impaired immune response and to technical issues. The clinical accuracy of IGRAs seems to be adversely affected in patients:

- with immune-mediated inflammatory diseases (IMIDs) such as Crohn's disease, where the function of immune cells is suppressed , as well as in patients
- on immunomodulatory drugs such as teriflunomide, which exerts an inhibitory effect on T-cell activation, and outcome in QuantiFERON results changing from positive to negative with marked reduction in IFN-γ. In addition,
- high dose of corticosteroids have been associated a high proportion of indeterminate QTF-GIT results in rheumatoid arthritis patients and inflammatory bowel disease receiving steroids. Patients with these conditions, therefore, should be tested with QTF-GIT prior to steroid treatment
- Interestingly, the IGRA sensitivity is not affected by

diabetes in TB patients;

- The technical variations that may affect IGRA results include those related to blood sampling (time, volume), tube shaking, incubation or processing delay (cell viability in blood may be affected), incubation duration, analytical errors, and manufacturing defects.

I. Can IGRAs be given to persons receiving vaccinations?

As with TST, live virus vaccines might affect IGRA test results. IGRA testing in the context of live virus vaccine administration should be done as follows:

- Either on the same day as vaccination with live-virus vaccine or 4-6 weeks after the administration of the live-virus vaccine
- At least one month after vaccination

J. In What other settings IGRAs are useful?

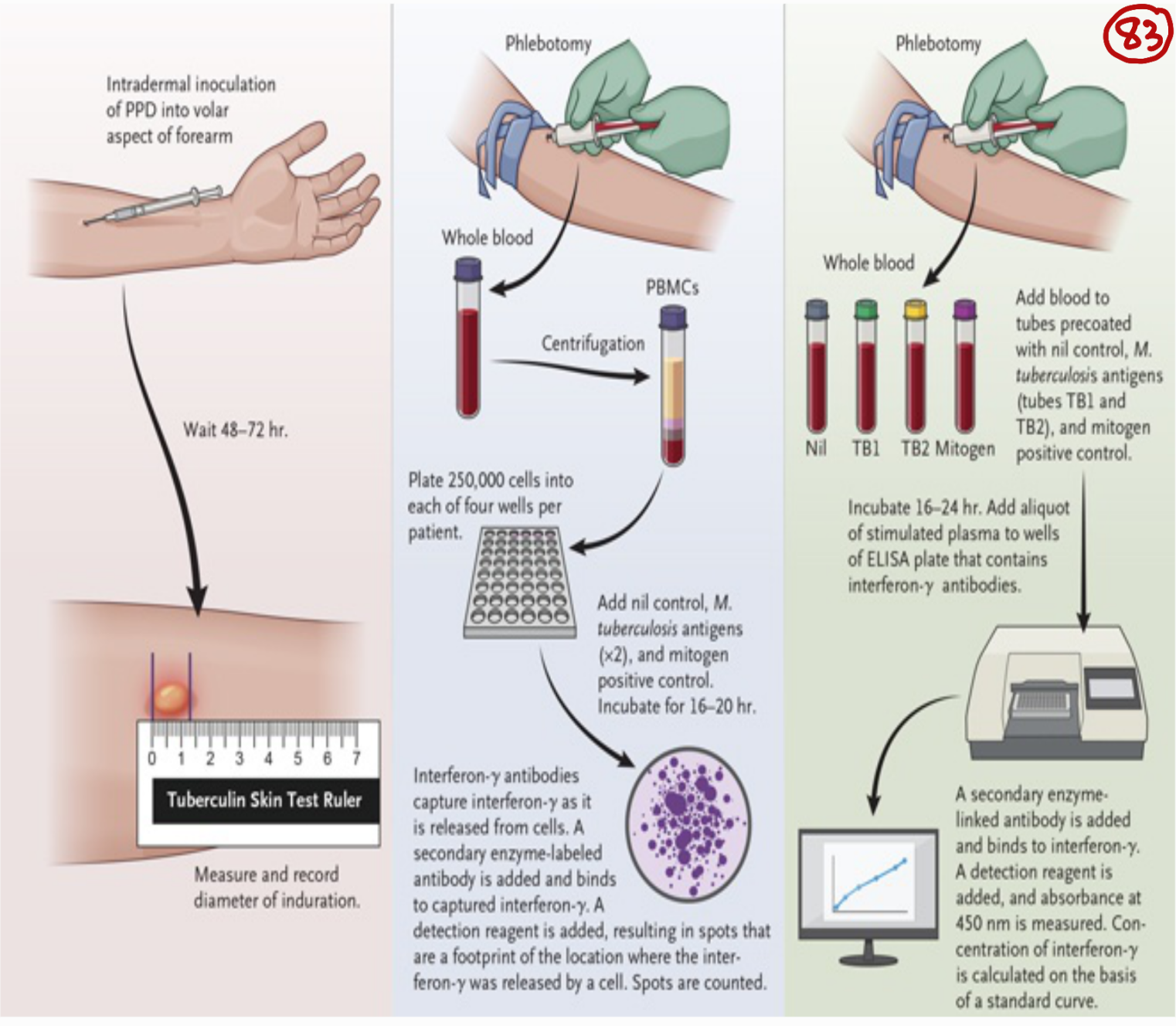
Although IGRAs can not distinguish between LTBI and active TB disease in immunocompetent adults, in high-risk individuals with immunosuppressive conditions and

in children. IGRAs may help in the diagnosis of active disease as additional diagnostic tests, particularly if specimens from the suspected site of infection (such as bronchoalveolar lavage, cerebrospinal fluid) rather than blood is used.

While the results of IGRAs exhibit better correlation with exposure to *M. tuberculosis* in low TB incidence countries; however, their performance is less than adequate in countries with a high TB incidence.

K. Comparison between TST and IGRA (83,84)

- Both TST and IGRA are poor in identifying persons at highest risk of developing active disease. If a person is positive, are they more or less likely to develop the disease is not predicted by these tests. This is the reason why all persons found to be positive for either TST or IGRA are treated. Even strong positive tests do not suggest a higher risk.



- Although both the TST and IGRA are for the diagnosis of LTBI, they, as pointed out earlier, assess different parameters of the immune response that are relevant in immunocompetent individuals.

- Diversity of antigens used in these tests, genetic diversity and differences in immune response

affect the results of these tests. IGRA is more specific in low-risk, BCG-vaccinated individuals and more sensitive in diagnosing *M. tuberculosis* coinfection in HIV-infected patients. Discordant results between TST and IGRA are common in individuals with LTBI. In addition, the correlation of TST and IGRA varies among individuals from settings of high vs. low incidence, perhaps due to the effects of BCG vaccination, exposure to environmental mycobacteria, or the risk of reinfection . This is why TST and IGRA results should be interpreted in the context of tb prevalence and exposure.

- ▢ In addition, false conversions are more commonly seen with IGRAs than with TST in low-risk populations.

- ▢ Generally speaking, both tests exhibit similar limitations. For example, their precision is low in immune-compromised individuals (HIV) being screened for LTBI. This is a crucial constraint, since these individuals are the ones at higher risk of developing TB. Neither TST nor IGRA, QTF-GIT, or QTF are particularly useful in predicting progression to active tuberculosis.

TABLE 1. Interim recommendations for applying and interpreting QuantiFERON®-TB (QFT) (Cellestis Limited, Carnegie, Victoria, Australia)

Reason for testing	Population	Initial screening	Positive results	Evaluation
Tuberculosis (TB) suspect	Persons with symptoms of active TB	Tuberculin skin testing (TST) might be useful; QFT not recommended	Induration ≥5 mm	Chest radiograph, smears, and cultures, regardless of test results
Increased risk for progression to active TB, if infected	Persons with recent contact with TB, changes on chest radiograph consistent with prior TB, organ transplants, or human immunodeficiency virus infection, and those receiving imumnosuppressing drugs equivalent of ≥15 mg/ day of prednisone for ≥1 month*	TST; QFT not recommended	Induration ≥5 mm	Chest radiograph if TST is positive; treat for latent TB infection (LTBI) after active TB disease is ruled out
	Persons with diabetes, silicosis, chronic renal failure, leukemia, lymphoma, carcinoma of the head, neck, or lung, and persons with weight loss of ≥10% of ideal body weight, gastrectomy, or jejunoileal bypass*	TST; QFT not recommended	Induration ≥10 mm	
Increased risk for LTBI	Recent immigrants, injection-drug users, and residents and employees of high-risk congregate settings (e.g., prisons, jails, homeless shelters, and certain health-care facilities)†	TST or QFT	Induration ≥10 mm; percentage tuberculin response ≥15§	Chest radiograph if either test is positive; confirmatory TST is optional if QFT is positive; treat for LTBI after active TB disease is ruled out; LTBI treatment when only QFT is positive should be based on clinical judgment and estimated risk
Other reasons for testing among persons at low risk for LTBI	Military personnel, hospital staff, and health-care workers whose risk of prior exposure to TB patients is low, and U.S.-born students at certain colleges and universities†	TST or QFT	Induration ≥15 mm; percentage tuberculin response ≥30§	Chest radiograph if either test is positive; confirmatory TST if QFT is positive; treatment for LTBI (if QFT and TST are positive and after active TB disease is ruled out) on the basis of assessment of risk for drug toxicity, TB transmission, and patient preference

* QFT has not been adequately evaluated among persons with these conditions; it is not recommended for such populations.
† QFT has not been adequately evaluated among persons aged <17 years, or among pregnant women; it is not recommended for such populations.
§ The following additional conditions are required for QFT to indicate *Mycobacterium tuberculosis* infection: 1) mitogen – nil and tuberculin – nil are both >1.5 IU, and 2) percentage avian difference is ≤10.

Putting all these facts into perspective, it is not difficult to understand why the current TST and IGRA are considered imperfect mainly because of lack of their ability to predict which individuals are more likely to control the infection and who are more likely to progress to active TB.

10.5.3. What is the potential role of antibodies in LTBI diagnosis?

Any infection elicits immune response which is of two types: cell mediated and antibody mediated. Cell mediated response is mainly T lymphocyte centered which coordinate the immune response either directly or through various cytokines they produce. They are best suited to deal with pathogens which prefer to live inside host cells. Antibody mediated response is controlled by B cell which after activation by T cell, converts to plasma cells , multiply and produce a clone of cells producing proteins called antibodies against the pathogen and its antigens. Antibody mediated immunity is best suited to deal with pathogens which are mostly found outside the host cell. The role of antibody response against M.tuberculosis in the protection against TB is marginal, at least compared with that of cell-mediated immunity. This is supported by two kind of observations: the presence of high levels of antibodies in the active form of the disease, suggesting that antibodies do not offer protection and the apparently unaffected risk of TB reactivation of patients receiving antiantibody (rituximab, a human/mouse chimeric anti-CD20 antibody that induces a rapid depletion of normal CD20-expressing B cells) . The detection of significant levels of antibodies to some *M. tuberculosis*-specific proteins has also been noted in latently infected individuals as well as in patients with

active TB disease but not in healthy subjects. However, antibody-based tests have not been used so far for the detection of LTBI.
Although the presence of antibodies in the serum from patients with active TB has led to the development of commercial diagnostic tests, the World Health Organization has not recommended their use as diagnostic tools on the grounds of suboptimal sensitivity and specificity.

10.6. How is LTBI managed?

Eventhough LTBI is not considered a disease in the strict sense of the term, since a significant proportion of those infected will develop active disease in their life time and spread the infection, it is treated with drugs so as to prevent the reactivation of infection into disease.

The aim of the treatment of latent tuberculosis infection is the prevention of progression of infection to active clinical disease.

Isoniazid (INH) administered daily for 6 to 12 months has been the mainstay of treatment, with efficacy ranging from 60 to 90%. The benefit of isoniazid increases progressively when it is administered for up to 9 or 10 months and stabilizes thereafter. As a

consequence, in the absence of controlled, clinical trials comparing isoniazid with placebo, the 9-month isoniazid regimen has been recommended as adequate treatment.

Isoniazid was associated with a reduction in the incidence of tuberculosis among persons with HIV who were receiving antiretroviral therapy, and one study showed the benefit of isoniazid in patients with negative tuberculin skin tests or IGRAs who were also receiving antiretroviral therapy. A recent study from Uganda showed a high rate of conversion from a negative tuberculin skin test to a positive tuberculin skin test (30 cases per 100 person-years) among persons with HIV during the first 6 months of antiretroviral therapy. In geographic areas known for a high rate of transmission of tuberculosis, the protective effect of isoniazid against tuberculosis among people with HIV wanes over time, and continuous protection is maintained through a lifetime duration of treatment for tuberculosis. The World Health Organization recommends that HIV-infected persons in countries with high rates of transmission of tuberculosis receive at least 36 months of isoniazid as a proxy for lifelong treatment.

LTBI Treatment Regimens

MMWR. 2011;60(48):1650-3
JAMA. 2005; 293:2776. (85)

Medication(s)	Recommended Regimen
Isoniazid	**Preferred:** **Isoniazid 300 mg daily x 9 months** Alternative: 300 mg daily x 6 months 900 mg twice weekly x 9 months (DOT) 900 mg twice weekly x 6 months (DOT)
Isoniazid + Rifapentine	**Isoniazid 900 mg weekly x 12 weeks (DOT) +** **Rifapentine once weekly x 12 weeks (DOT)** 10-14 kg 300 mg 14.1-25 kg 450 mg 25.1-32 kg 600 mg 32.1-49.9 kg 750 mg >50 kg 900 mg (maximum dose)
Rifampin	**Rifampin 600 mg daily x 4 months**
Isoniazid + Rifampin	**Isoniazid 300 mg daily x 3 months** **+ Rifampin 600 mg daily x 3 months**

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Other effective regimens are (85):

- Daily rifampin for 3 or 4 months- fewer side effects and better adherence than 9- month isoniazid,

- Daily isoniazid and rifampin for 3 months, and
- Isoniazid (900 mg) and rifapentine (900 mg) once weekly for 12 weeks.
- once-weekly, directly observed isoniazid–rifapentine regimen for 3 months(efficacy similar to that of a 9-month, self-administered regimen of isoniazid alone and was associated with higher

treatment-completion rates (82.1% vs. 69.0%) and less hepatotoxicity (0.4% vs. 2.7%), although permanent discontinuation of the regimen due to side effects was more frequent with the isoniazid–rifapentine regimen (4.9% vs. 3.7%). The 3-month isoniazid–rifapentine regimen may be a cost-effective alternative to the 9-month isoniazid regimen, particularly if the cost of rifapentine decreases and the treatment is self-administered. Currently, the 3-month isoniazid–rifapentine regimen is not recommended for children younger than 2 years of age, persons with HIV infection who are receiving antiretroviral therapy, and women who are pregnant.

A few small studies have explored treatment of latent tuberculosis infection in contacts (both children and adults) of persons with multidrug-resistant tuberculosis on the basis of the results of drug-susceptibility testing of the source patient. However, evidence is lacking on the best treatment approach. Rather, strict observation and monitoring for at least 2 years for the development of active tuberculosis disease are the preferred clinical measures.

Programmatic Approach

For example, in resource -poor countries with a high prevalence and transmission rate of tuberculosis, priority should be given to risk groups with the highest likelihood of active tuberculosis (e.g., persons with HIV and children younger than 5 years of age who are contacts of persons with active tuberculosis). We need not do testing for these. They can be treated as a routine. For others with moderate risk (like diabetes, smoking) and mild risk, one may test and if positive may be treated. For LTBI, two things are important : Exposure to M.tb and infection and therefore asking about history of exposure and *risk factors for exposures* is important; and *risk of progression* once exposed is determined by immune competence and therefore asking about risk factors for progression of infection is also important. All adults and children should have an assessment of risk factors for exposure and progression at least once. Risk of exposure is determined by incidence of tuberculosis. Higher the incidence, higher the risk.