MYCOBACTERIA

Mycobacteria are widespread organisms, typically living in and food sources. Tuberculosis and the leprosy organisms are obligate parasites and are not found as free-living members of the genus. Mycobacteria are aerobic and nonmotile bacteria (except for the species *Mycobacterium marinum*, which has been shown to be motile within macrophages) that are characteristically acid fast. Mycobacteria have an outer membrane. They do not have capsules, and most do not form endospores. The distinguishing characteristic of all *Mycobacterium* species is that the cell wall is thicker than in many other bacteria, which is hydrophobic, waxy, and rich in mycolic acids/mycolates. The cell wall consists of the hydrophobic mycolate layer and a peptidoglycan layer held together by a polysaccharide, arabinogalactan. The cell wall makes a substantial contribution to the hardiness of this genus. The biosynthetic pathways of cell wall components are potential targets for new drugs for tuberculosis.

![Diagram of Mycobacterial cell wall](image)

**Fig. 1** Mycobacterial cell wall: 1-outer lipids, 2-mycolic acid, 3-polysaccharides (arabinogalactan), 4-peptidoglycan, 5-plasma membrane, 6-lipoarabinomannan (LAM), 7-phosphatidylinositol mannoside, 8-cell wall skeleton.

NONTUBERCULOUS MYCOBACTERIA – RUNYON CLASSIFICATION

Runyon classification is a system of identifying mycobacteria on the basis of pigmentation and growth condition of the organisms. The Runyon classification of nontuberculous mycobacteria based on the rate of growth, production of yellow pigment and whether this
pigment was produced in the dark or only after exposure to light. It was introduced by Ernest Runyon in 1959 (Fig. 111). On these bases, the nontuberculous mycobacteria are divided into four groups:

**Photochromogens (Group I)** - produce nonpigmented colonies when grown in the dark and pigmented colonies only after exposure to light and reincubation (M. kansasii, M. marinum, M. simiae).

**Scotochromogens (Group II)** - produce deep yellow to orange colonies when grown in the presence of either the light or the dark (M. scrofulaceum, M. gordonae, M. xenopi, M. szulgai).

**Non-chromogens (Groups III & IV)** - nonpigmented in the light and dark or have only a pale yellow, buff or tan pigment that does not intensify after light exposure (M. tuberculosis, M. avium-intra-cellulare, M. bovis, M. ulcerans, M. fortuitum, M. chelonae).

<table>
<thead>
<tr>
<th>Group</th>
<th>Growth</th>
<th>Pigment</th>
<th>Examples</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>slow</td>
<td>yellow-orange on light (photochromogen)</td>
<td>1. M. kansasii 2. M. marinum</td>
<td>1. similar to TB 2. swimming pool granuloma</td>
</tr>
<tr>
<td>II</td>
<td>slow</td>
<td>yellow-orange in light or dark (scotochromogen)</td>
<td>M. scrofulaceum</td>
<td>cervical adenitis</td>
</tr>
<tr>
<td>III</td>
<td>slow</td>
<td>no pigment</td>
<td>M. avium intracellulare complex (MAC)</td>
<td>similar to TB, esp. in AIDS</td>
</tr>
<tr>
<td>IV</td>
<td>rapid (5 days)</td>
<td>no pigment</td>
<td>M. fortuitum M. chelonae</td>
<td>soft tissue, lung, bone, CNS, eye infections</td>
</tr>
</tbody>
</table>

Fig. 2 Runyon classification.

**MYCOBACTERIUM TUBERCULOSIS (TUBERCULOSIS)**

Tuberculosis (TB) is caused by the infectious agent known as Mycobacterium tuberculosis (MTB). This rod-shaped bacterium, also called Koch's bacillus, was discovered by Dr. Robert Koch in 1882. MTB is a small, slow-growing bacterium that can live only in people. It is not found in other animals, insects, soil, or other nonliving things. MTB is an aerobic bacterium, meaning it needs oxygen to survive. For this reason, during active tuberculous disease, MTB

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1 M. - Mycobacterium
complexes are always found in the upper air sacs of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours, a physiological characteristic that may contribute to its virulence. The bacteria usually attack the lungs, but MTB bacteria can attack any part of the body such as the kidney, spine, and brain. If not treated properly, disease can be fatal. It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease.

**Latent tuberculosis infection (LTBI)**

Latent tuberculosis infection (LTBI) is a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens without evidence of clinically manifested active tuberculosis. The lifetime risk of reactivation for a person with documented LTBI is estimated to be 5–10%, with the majority developing TB disease within the first five years after initial infection.

**Active tuberculosis (TB disease)**

In some people, MTB bacteria overcome the defenses of the immune system and begin to multiply, resulting in the progression from latent tuberculosis infection to TB disease. Some people develop TB disease soon after infection, while others develop TB disease later when their immune system becomes weak.

<table>
<thead>
<tr>
<th>A Person with Latent TB Infection</th>
<th>A Person with TB Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has no symptoms</td>
<td>Has symptoms that may include:</td>
</tr>
<tr>
<td>Does not feel sick</td>
<td>a bad cough that lasts 3 weeks or longer</td>
</tr>
<tr>
<td>Cannot spread TB bacteria to others</td>
<td>pain in the chest</td>
</tr>
<tr>
<td>Usually has a skin test or blood test result indicating TB infection</td>
<td>coughing up blood or sputum</td>
</tr>
<tr>
<td>Has a normal chest x-ray and a negative sputum smear</td>
<td>weakness or fatigue</td>
</tr>
<tr>
<td>Needs treatment for latent TB infection</td>
<td>weight loss</td>
</tr>
<tr>
<td></td>
<td>no appetite</td>
</tr>
<tr>
<td></td>
<td>chills, fever, sweating at night</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Usually feels sick</td>
<td>May spread TB bacteria to others</td>
</tr>
<tr>
<td>Usually has a skin test or blood test result indicating TB infection</td>
<td>Usually has a skin test or blood test result indicating TB infection</td>
</tr>
<tr>
<td>May have an abnormal chest x-ray, or positive sputum smear or culture</td>
<td>Needs treatment to treat TB disease</td>
</tr>
</tbody>
</table>
Extrapulmonary tuberculosis

Extrapulmonary tuberculosis is the infection of any organ in the body other than the lungs by Mycobacterium tuberculosis is called extrapulmonary tuberculosis. It is most commonly a sequel of lung infection by the same organism. The most common sites of extrapulmonary tuberculosis are lymph nodes, pleura, abdomen, bone and joint, spinal cord and the brain and its coverings (Fig. 112).

MYCOBACTERIUM TUBERCULOSIS – SAMPLE COLLECTION

Sputum

The large majority of specimens received for diagnosis are sputum samples. If good specimens are to be obtained, patients must be instructed in how to produce sputum. Specimens should be collected in a separate, ventilated room or preferably outdoors. Keeping both hands on hips, cough forcibly and collect sputum in the mouth; spit the sputum carefully into a wide-mouthed, unbreakable, leakproof container and close the lid tightly. Ideally, a sputum specimen should be 3–5ml in volume, although smaller quantities are acceptable if the quality is satisfactory.
Sputa should be transported to the laboratory as soon as possible. If a delay of a few days cannot be avoided, keep specimens cool (refrigerated but not frozen) Up to a week in cold conditions will not significantly affect the positivity rate of smear microscopy, however, the additional growth of contaminants will result in an increased contamination rate on culture media.

**Laryngeal swab**

Laryngeal swabs may be useful in children and patients who cannot produce sputum or may swallow it. Collect laryngeal swabs in the early morning, before patients eat or drink anything. Use a sterile absorbent cotton swab for collection. Transport each specimen in a container with a few drops of sterile 0.9% saline solution in order to keep the swab wet.

**Other respiratory specimens**

Bronchial secretion (2–5 ml) and BAL (20–40 ml). Pleural effusions (20–50 ml). Transbronchial and other biopsies taken under sterile conditions should be kept wet during transportation by adding few drops of sterile 0.9% saline to the tissue.

**Gastric lavage**

Gastric lavages often contain MOTT and are therefore rarely used for adults, they are indicated for children, however, who produce almost no sputum. Make the collection early in the morning, when the patient has an empty stomach. Neutralize the specimen by adding 100 mg of sodium bicarbonate to the gastric aspirate and transport it immediately to the laboratory.

**Extrapulmonary specimens**

The laboratory may receive a variety of specimens for diagnosis of extrapulmonary TB – body fluids, tissues, urine etc. All liquid specimens should be collected in sterile glass containers without using any preservative. Specimens can be inoculated directly into liquid vials and transported to the laboratory for culture. Specimens must be transported to the laboratory immediately; they should be processed as soon as possible or kept at 2–6 °C. The optimal volumes are at least 3 ml of cerebrospinal fluid and 5–10 ml of blood, collected in citrate blood tubes.

**MYCOBACTERIUM TUBERCULOSIS – ZIEHL-NEELSEN STAIN**

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2 BAL - bronchoalveolar lavage
3 MOTT - mycobacteria other than tuberculosis
M. tuberculosis does not retain any common bacteriological stain due to high lipid content in its wall, and thus is neither Gram-positive nor Gram-negative, hence Ziehl-Neelsen staining, or acid-fast staining, is used. While Mycobacteria do not retain the crystal violet stain, they are classified as acid-fast Gram-positive bacteria due to their lack of an outer cell membrane.

**Ziehl-Neelsen staining procedure**

In the ‘hot’ Ziehl-Neelsen technique, the phenol-carbol fuchsin stain is heated to enable the dye to penetrate the waxy mycobacterial cell wall. The stain binds to the mycolic acid in the mycobacterial cell wall. After staining, an acid decolorizing solution is applied. This removes the red dye from the background cells, tissue fibres, and any organisms in the smear except mycobacteria which retain (hold fast to) the dye and are therefore referred to as acid fast bacilli (AFB). Following decolorization, sputum smear is counterstained with malachite green (or methylene blue) which stains the background material, providing a contrast colour against which the red AFB can be seen. Among the Mycobacterium species, M. tuberculosis and M. ulcerans are strongly acid fast.

![Fig. 4 Ziehl-Neelsen stain - Acid fast bacilli (AFB).](image)

**Reporting of sputum smear**

1. When no ⁴AFB are seen after examining 300 fields, report the smear as ‘No AFB seen’.
2. When very few AFB are seen i.e. when only 1 or 2 AFB are seen after examining 100 fields, request a further specimen to examine (Those AFB might have came from tap water (saprophytic mycobacteria), or it may be scratch of glass slide or by the use of same piece of blotting paper while drying.
3. When any red bacilli are seen, report the smear as ‘AFB positive’ and give an indication of the number of bacteria present as follows:
   1. More than 10 AFB/field at least in 20 fields: report as + + +
   2. 1-10 AFB/field at least in 50 fields: report as + +

⁴AFB - acid fast bacilli
MYCOBACTERIUM TUBERCULOSIS - CULTIVATION

*M. tuberculosis* requires oxygen to grow. *M. tuberculosis* divides every 15-20 hours, which is extremely slow compared to other bacteria, which tend to have division times measured in minutes (*Escherichia coli* can divide roughly every 20 minutes).

*M. tuberculosis* is grown on a selective medium known as Löwenstein-Jensen medium. This method is quite slow, as this organism requires 6-8 weeks to grow, which delays reporting of results. A faster result can now be obtained using Middlebrook medium or BACTEC.

### Löwenstein-Jensen medium

The usual composition applicable to *Mycobacterium tuberculosis* is:

- Malachite green (inhibits most other bacteria)
- Glycerol (enhances the growth of *Mycobacterium tuberculosis*)
- Asparagine
- Potato starch
- Coagulated eggs
- Mineral salt solution (Potassium dihydrogen phosphate, Magnesium sulfate, Sodium citrate)
- Low levels of penicillin and nalidixic acid (to inhibit growth of gram positive and gram negative bacteria)

Löwenstein-Jensen medium doesn't contain any agar, solid consistence is attained by heat coagulation of the egg albumin.

![Mycobacterium tuberculosis (Löwenstein-Jensen medium)](image)

*M. tuberculosis* on Löwenstein-Jensen medium after 6 weeks of cultivation, 37°C form typical nonpigmented, rough, dry colonies. The green color of the medium is due to the presence of malachite green which is one of the selective agents to prevent growth of most other contaminants.

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, Fig. 115). 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 on the measurement in millimeters (mm) of the induration and the person’s risk of being infected with TB and/or progression to disease if infected. The following three cut points (see Fig. 116) 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 reaction**

Some 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.
The reaction to the Tuberculin Skin Test should be read by a trained health professional 48 to 72 hours after the injection. The reaction should be measured in millimeters.

1. **WASH** — Wash hands or use hand sanitizer (per facility protocol).

2. **INSPECT SITE** — Locate the area where the skin test was administered — inspect the arm in good light and on a firm surface.

3. **FEEL INDURATION** — Lightly palpate the area with the pads of your fingertips to determine if there is an induration and to locate the margins or edges of the induration.

4. **MARK EDGES** — Measure the diameter of the indurated area across the forearm (perpendicular to the long axis) at the widest width of the induration. Using a ballpoint pen, mark lightly one edge of the induration with a fine dot and then repeat on the other edge.

5. **MEASURE** — Use a millimeter ruler or caliper. Gently lay a ruler on the skin, placing the first mark at zero (first line on the ruler).

   The second mark will be the measurement reading. If the measurement falls within two divisions on the millimeter scale, record the lower mark. If there is no induration, the reading is measured as 0 millimeters.

6. **WASH AGAIN** — Wash hands or use hand sanitizer (per facility protocol).

7. **DOCUMENT** — Record the reading on the appropriate form using only millimeters. Do not simply record as “negative” or “positive.” Include the date and time the test was read, the name and signature of the person who read the skin test, and the presence or absence of adverse effects.

**Fig. 6** The reaction to Tuberculin Skin Test - results reading.
Fig. 7 Interpretation of the Tuberculin skin test results.

**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®.TB test (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 test**

The 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-mediated immune response. A cocktail of 3 mycobacterial proteins (ESAT-6, CFP-10, and TB 7,7) stimulate the patient's T-cells *in vitro* to 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.

**T-SPOT®.TB test**

The T-SPOT®.TB test is a unique, single-visit blood test, also known as an interferon-gamma release assay (IGRA) for TB infection. The T-SPOT.TB test does not cross react with the bacille Calmette-Guerin (BCG) vaccine and there is no association between T-SPOT.TB test results and immunocompromised status. According to the CDC Guidelines, IGRA's (e.g. the T-SPOT.TB test) may be used in place of a TST in most situations and are preferred for BCG vaccinated individuals. The T-SPOT.TB test enumerates the response of effector T-cells that have been sensitized to *Mycobacterium tuberculosis*. Interferon-gamma is captured and presented as spots from T cells sensitized to TB infection. (Fig. 118).

Fig. 9 T-SPOT®.TB test - principle.

Results of T-SPOT®.TB test are interpreted by subtracting the spot count in the negative (NIL) control from the spot count in Panels A and B.

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5 ESAT-6 - early secreted antigenic target protein 6  
6 CFP-10 - culture filtrate protein-10 kDa  
7 ELISA - enzyme-linked immunosorbent assay
Positive: > 8 spots  
Negative: < 4 spots  
Borderline: 5, 6, or 7 spots

Fig. 10 Results of T-SPOT®.TB.

**PCR-BASED TB DIAGNOSTIC TEST**

The new PCR-based TB diagnostic test called Xpert MTB/RIF is fast, sensitive, and automated. An accurate diagnosis can be obtained in less than 2 hours by adding a reagent to a sputum sample and, 15 minutes later, pipeting it into a cartridge that is inserted into the diagnostic instrument for 1–2 minutes (Fig. 120).

Fig. 11 PCR-based TB diagnostic test - Xpert MTB/RIF.
BCG VACCINE

BCG is a vaccine for tuberculosis (TB) disease. BCG stands for ‘Bacillus Calmette-Guérin’, and is named after the two French scientists who developed the first TB vaccine – Albert Calmette and Camille Guérin. BCG vaccine for percutaneous use contains a live strain of a bacterium closely related to the one that causes TB in humans. The bacterium is an attenuated, live culture preparation of the Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis. It stimulates the immune system but does not cause disease. The vaccine is given intradermally, usually in the left upper arm. This is the recommended site, so that small scar left after vaccination can be easily found in the future as evidence of previous vaccination.

MYCOBACTERIUM LEPRAE - LEPROSY

Leprosy is a chronic infection caused by Mycobacterium leprae. Leprosy is also known as Hansen disease, named after G.A. Hansen, who is credited with the 1873 discovery of M. leprae. The incubation period of M. leprae can range between nine months and twenty years. Leprosy primarily affect superficial tissues, especially the skin and peripheral nerves. Initially, a mycobacterial infection causes a wide array of cellular immune responses. These immunologic events then elicit the second part of the disease, a peripheral neuropathy with potentially long-term consequences.

Individuals who have a vigorous cellular immune response to M. leprae have the tuberculoid form of the disease that usually involves the skin and peripheral nerves. This form of the disease is also referred to as paucibacillary leprosy because of the low number of bacteria in the skin lesions (< 5 skin lesions, with absence of organisms on smear). Results of skin tests with antigen from killed organisms are positive in these individuals.

Individuals with minimal cellular immune response have the lepromatous form of the disease, which is characterized by extensive skin involvement. The organism grows best at 27-30 °C, therefore, skin lesions tend to develop in the cooler areas of the body, with sparing of the groin, axilla, and scalp. This form of the disease is also referred to as multibacillary leprosy because of the large number of bacteria found in the lesions (>6 lesions, with possible visualization of bacilli on smear). Results of skin tests with antigen from killed organisms are nonreactive.

MYCOBACTERIUM LEPRAE - ZIEHL-NEELSEN STAIN

Mycobacterium leprae is a strongly acid-fast rod-shaped organism with parallel sides and rounded ends. In size and shape it closely resembles the tubercle bacillus. It occurs in large numbers in the lesions of lepromatous leprosy, chiefly in masses within the lepra cells, often grouped together like bundles of cigars or arranged in a palisade. Chains are never seen.
Optical microscopy shows *M. leprae* in clumps, rounded masses, or in groups of bacilli side by side, and ranging from 1–8 μm in length and 0.2–0.5 μm in diameter. The bacilli are densely clustered within the cytoplasmic vacuoles of foamy histiocytes. This unique structure called "globi" is demonstrated by the Ziehl-Neelsen stain (Fig. 12).

**Mycobacterium leprae** - Ziehl-Neelsen stain.

**Mycobacterium leprae** – cultivation

The organism has never been successfully grown on an artificial cell culture medium. Instead, it has been grown in mouse foot pads and more recently in nine-banded armadillos because they, like humans, are susceptible to leprosy. This can be used as a diagnostic test for the presence of bacilli in body lesions of suspected leprosy patients. The difficulty in culturing the organism appears to be because it is an obligate intracellular parasite that lacks many necessary genes for independent survival.

**Mycobacterium leprae** - lepromin skin test

Lepromin skin test - although not diagnostic of exposure to or infection with *M. leprae*, this test assesses a patient's ability to mount a granulomatous response against a skin injection of killed *M. leprae*. A sample of inactivated leprosy-causing bacteria is injected just under the skin, usually on the forearm, so that a small lump pushes the skin up. The lump indicates that the antigen has been injected at the correct depth. The injection site is labeled and examined 3 days, and again 28 days, later to see if there is a reaction. Patients with tuberculoid leprosy or borderline lepromatous leprosy typically have a positive response (>5 mm). Patients with lepromatous leprosy typically have no response. People who don't have leprosy will have little or no skin reaction to the antigen.