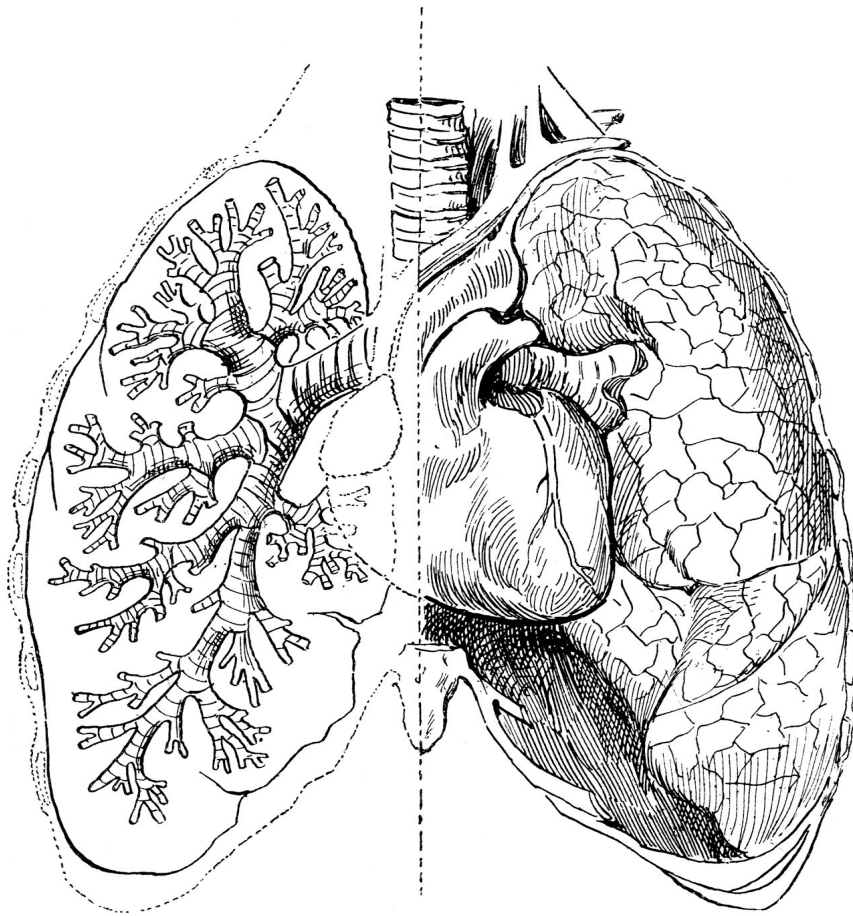


# Microbiology

435's Teamwork  
Respiratory Block

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- 
- Please contact the team leaders for any suggestion, question or correction.
  - Pay attention to the statements highlighted in **bold** and/or **red**.
  - **Footnotes color code:** General | **Females** | **Males**.

[Microbiology.435@gmail.com](mailto:Microbiology.435@gmail.com)

# داء السل Tuberculosis

## - Lecture One -

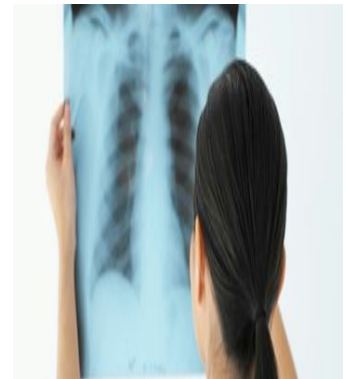
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### Learning Objectives:

- Recognize that tuberculosis as a **chronic** disease mainly affecting the **respiratory system**.
  - Know the **epidemiology** of tuberculosis worldwide and in the kingdom of Saudi Arabia.
  - Understand the **methods of transmission** of tuberculosis and the people at risk.
  - Know the **causative agents** and their characteristic and classification and methods of detection.
  - Understand the **pathogenesis** of tuberculosis.
  - Differentiate between **primary** and **secondary** tuberculosis and the clinical features of each.
  - Understand the method of **tuberculin skin test** and result interpretation.
  - Know the laboratory and radiological **diagnostic methods**.
  - Know the **chemotherapeutic** and other methods of **management** of tuberculosis cases.
  - Describe the methods of **prevention and control** of tuberculosis.
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### Introduction:

Tuberculosis (TB) is one of the oldest and most devastating diseases in human history, which remains a leading cause of deaths worldwide today. It is an ancient<sup>1</sup>, **chronic** disease that affects humans and animals lungs, also, other organs might be affected in one third of cases. If it was properly treated, Tuberculosis can be curable, on the other hand, it is unfortunately fatal if untreated in most cases. TB is typically caused by a *Mycobacterium Tuberculosis*<sup>2</sup> complex, gram positive acid-fast bacilli<sup>3</sup>.



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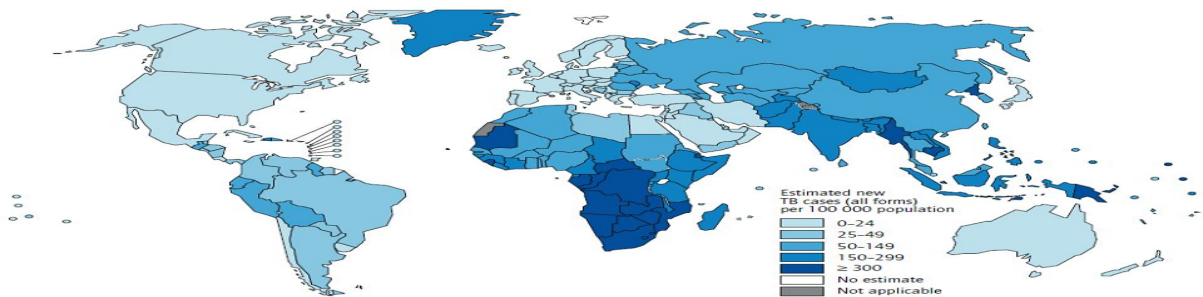
<sup>1</sup> Belonging to the very distant past.

<sup>2</sup> Abbreviation: M. TB.

<sup>3</sup> Explained later on.

## Epidemiology (Read about it more, interesting subject):

- Infection started at the 18th and 19th centuries.
- The attacking rates are still high until today, although morbidity was higher back then.
- TB affects 1/3 of the human race (2 billions) as a latent dormant<sup>4</sup> tuberculosis.
- It is a worldwide disease, but more common in developing countries<sup>5</sup>.
- The poor are the major victims, but all society are at risk.
- The WHO<sup>6</sup> estimated 8.9 million new cases in 2004 and 2-4 million deaths with the following **incidence**:
  - KSA: 32-64 cases/100,000
  - US: 5.2 cases/100,000
  - South East Africa: 290 cases/10,000 due to the coupling with HIV infection<sup>7</sup>.
- **Transmission**:
  - Mainly through inhalation of **airborne<sup>8</sup> droplet**.
  - It can be transmitted by the GIT and skin as well, but rarely<sup>9</sup>.
- **Reservoir<sup>10</sup>**: Patients with open TB. Coughing sneezing makes it more infectious.
  - **Laryngeal TB is the most infectious but pulmonary TB the most common.**<sup>11</sup>
- **Age**: All age groups.
- **People at risk**: Lab technicians, workers in mines, doctors, nurses, **HIV** patients, **diabetics**, end stage renal failure patients, and the people at contact with an index case<sup>12</sup>.



<sup>4</sup> مختفي لا يظهر أية أعراض.

<sup>5</sup> Poor agricultural country that is seeking to become more advanced economically and socially.

<sup>6</sup> World Health Organization.

<sup>7</sup> HIV affects the immune system, which increases the incidence of infectious diseases, especially TB.

<sup>8</sup> Transported by air. (Remember Bloodborne in MSK block?)

<sup>9</sup> بسبب بستره حليب المواشي، الذي يعد بدوره الوسيلة الأكثر شيوعاً لهذا النوع من الانتقال.

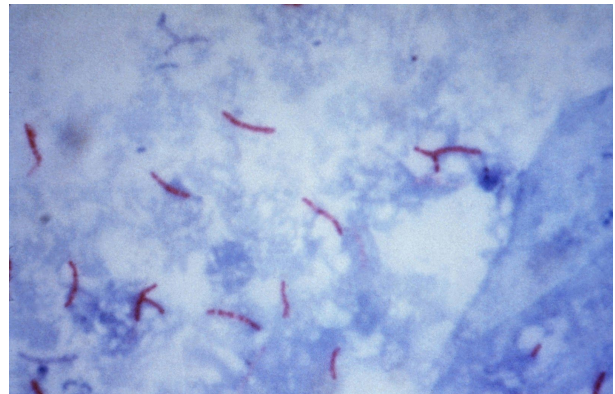
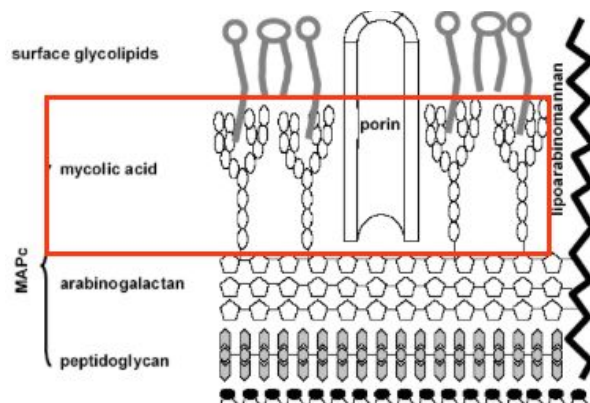
<sup>10</sup> The supply or source of the infection. (Who transmits the disease).

<sup>11</sup> Infection is when the bacteria is destroying but without symptoms. Disease is the end product of infection when the bacteria start causing symptoms.

<sup>12</sup> The first identified case in a group of uninfected people.

## Characteristics of the genus<sup>13</sup> Mycobacteria:

- Gram positive.
- Slim, rod shaped, non-motile, not forming spores.
- **It is called Acid-alcohol fast bacilli; Why?**  
Because it **resists decolorization**<sup>14</sup> with up to **3% HCL** and/or **5% ethanol**.
- **Cannot be stained by Gram stain; Why?**  
**Mycobacteria contain high lipid concentration (Mycolic acid) in its cell wall.**  
**Mycolic acid resist staining. It covers the peptidoglycan so the stain cannot reach it.**
- **Acid Fast Bacilli (AFB):**
  - Stain used is Ziehl-Neelsen stain (ZN stain)<sup>15</sup>.
  - **Strict aerobe**<sup>16</sup>.
  - Multiply intracellularly. (In the alveolar macrophage).
  - Slowly growing (2-8 weeks).
  - Causes delayed hypersensitivity reaction.



- **Mycobacterium Tuberculosis Complex**<sup>17</sup>:
  - 1) *Mycobacterium Tuberculosis* (**Human type**.)
  - 2) *Mycobacterium Bovis* (**Bovine**<sup>18</sup> **type**.)
  - 3) *Mycobacterium Africanum*.
  - 4) *BCG*<sup>19</sup> strains.

All are called **Mycobacterium Tuberculosis Complex** and cause Tuberculosis (TB).

<sup>13</sup> الجنس؛ تصنيف الكائنات الحية (مملكة، فصيلة، جنس، نوع). نناقش هنا خصائص جنس المايكوبكتيريا ككل وليس هذا النوع وحده.

<sup>14</sup> The process of changing to a different, less attractive color.

<sup>15</sup> ZN stain makes the mycobacterium stain bright red and stand out clearly against a blue background.

<sup>16</sup> Need oxygen because they cannot ferment or respire anaerobically.

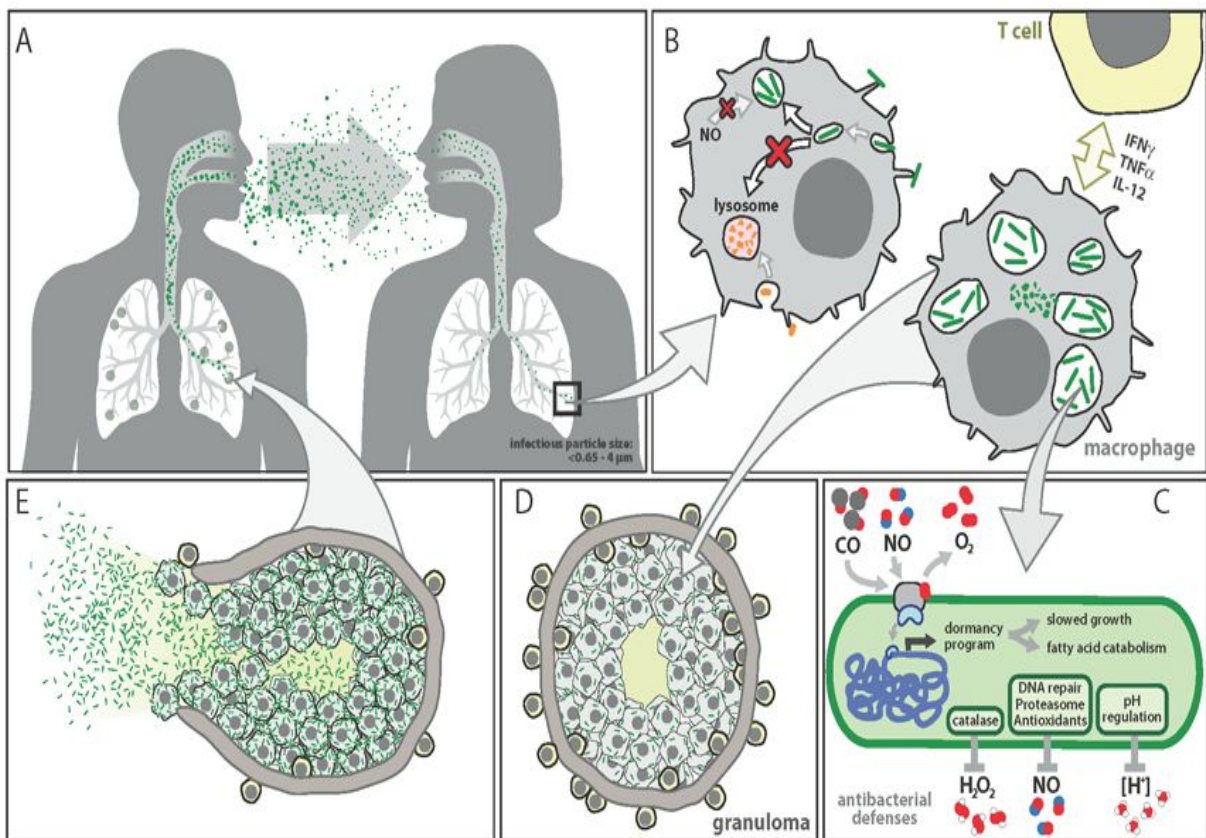
<sup>17</sup> One of them is enough to establish the infection.

<sup>18</sup> الدرن أو السل البقري، يصيب البقر والمواشي.

<sup>19</sup> Bacillus Calmette-Guérin. (Remember the name, we will mention its vaccination later on.)

## Pathogenesis (Better explained in Pathology):

- Mycobacteria, as previously mentioned, is acquired by **airborne droplet**. That droplet reaches the **alveolar macrophages**, and is able to survive their main virulence factor.
- This starts the cell mediated immune response, which controls the multiplication of the organism **but** does not kill it.
- As we studied in foundation's Pathology, this directs the **granuloma formation**.
- The organism now lives in the **dormant state**<sup>20</sup> (latent tuberculosis infection.)
- Patient show evidence of **delayed cell mediated immunity** (type 4), and the disease results due to the **destructive** effect of the immune response.
- Clinically, the disease is divided into:
  - 1) **primary (asymptomatic).**
  - 2) **Secondary (symptomatic).**



## Primary Tuberculosis<sup>21</sup>:

- Occurs in patients **not** previously infected or has no experience with TB.
- **Inhalation of bacilli** → **Phagocytosis**<sup>22</sup> → **Calcification** → **Production of GHON focus**<sup>23</sup>.
- GHON focus or “Primary Complex<sup>24</sup>” occurs at the periphery of the mid zone of the lung.
- The delayed hypersensitivity reaction leads to **causation**. The causation contains many bacilli, enzymes, O<sub>2</sub>, and N<sub>2</sub> intermediates (Nitric Oxide) that leads to the formation of the necrotic centres of granuloma, that ultimately form a cheesy material (**caseous necrosis**).
- Microscopy of the lesion<sup>25</sup> shows **Granuloma**.
- Clinically, primary TB is usually **asymptomatic**, or shows minor illness<sup>26</sup>.
- **Non-pulmonary TB**: May spread from pulmonary infections to other organs:
  - **TB of lymph nodes (Cervical, mesenteric.)**
  - **TB meningitis**<sup>27</sup> (Most serious condition in children.)
  - **TB of bone and joints.**
  - TB of the genitourinary system.
  - TB miliary<sup>28</sup> (Blood and other organs.)
  - TB of soft tissue (cold abscess): lacks inflammation with caseation.

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## Secondary (Reactivated) Tuberculosis:

- Occurs after the **first infection**, later in life.
- The lung is the most common site.
- Requires an immunocompromised patient.
- The lesion is localized in apices<sup>29</sup>.
- **Infectious and symptomatic.**
- Microscopy shows many bacilli, a large area of caseous necrosis in a **cavity** (open TB) with granuloma and caseation.
- Clinically, the patient has **fever, cough, hemoptysis**<sup>30</sup>, **weight loss and general weakness.**
- **Source of secondary TB can be:**
  - **Endogenous:** Reactivation of an old TB in the patient’s body.<sup>31</sup>
  - **Exogenous:** Re-infection in a previously sensitized patient who have had a previous infection with the organism. (After the first infection)

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<sup>21</sup> Initial multiplication is in the alveoli, with spread through lymphatic drainage to the hilar lymph nodes. then to the bloodstream, to the rest of the body.

<sup>22</sup> The ingested bacteria multiplies in the alveolar macrophage.

<sup>23</sup> Grey/white inflammation caused by tuberculosis.

<sup>24</sup> علامة فارقة للتوبركولوسيس لا تظهر إلا في هذا المرض (More)

<sup>25</sup> Which later on heals by fibrosis

Fever and malaise<sup>26</sup> بمعنى أن المريض يكون حامل للميكوبكتيريا لكن لا تظهر عليه أية أعراض درامية للمرض.

<sup>27</sup> In the Cerebrospinal fluid (CSF.) Common in children.

<sup>28</sup> TB of hematogenous (bloodborne) spread. Spread via blood.

<sup>29</sup> Plural form of apex. Single: apex. Plural: apices.

<sup>30</sup> Coughing blood.

<sup>31</sup> AIDS, immune compressive disease or drugs that compromise the immune system.

## Immunity to Tuberculosis (VERY IMPORTANT):

- Cell-mediated immunity is associated<sup>32</sup> with delayed hypersensitivity reaction.
- TB is detected by **tuberculin skin test**.<sup>33</sup>
  - Tuberculin skin test is done to see if you have ever been exposed to tuberculosis. The test is done by putting a small amount of TB protein (antigens) under the top layer of the skin on your inner forearm. If you have ever been exposed to the TB bacteria, your skin will react to the antigens by developing a firm red bump.
  - Tuberculin test takes **2-10** weeks to react to tuberculin and becomes positive.
  - The TB antigens used in the test is called purified protein derivative (**PPD**).
  - A measured amount of **PPD** in a shot is used, that shot is called the Tuberculin Unit (TU) a standard intradermal injection.
  - That activates synthesized lymphocytes to produce cell-mediated immunity which appears as skin induration<sup>34</sup>.
  - The test is **not** specific. It may **not** distinguish between the latent (**inactive**) or **active** TB, except in an individual with a known history of contact with an infected case.
  - Low level activity (**false positive**) may be induced by environmental mycobacteria or a previous vaccination<sup>35</sup>.
- **Procedure of tuberculin test:**
  - Intradermal inoculation of 0.1 ml of PPD, 5 TU<sup>36</sup>.
  - Read after 48-72 hours.
  - We measure the **induration** not the redness.
- **Methods of tuberculin skin test:**
  - 1) **Mantoux test** ([video](#)).
  - 2) Heaf test (screening).



<sup>32</sup> Humans generally have high innate immunity in order to develop the disease.

<sup>33</sup> Also called the “bubble test”.

<sup>34</sup> تيبس، يصبح يابساً.  
<sup>35</sup> في التطعيم ضد السل، نحقن الأنتيجينات الخاصة بالميكوبكتيريا حتى يكون الجسم حصانة ضدها في حال المرض مستقبلاً، بالتالي، عندما نقوم بهذا الاختبار للكشف عن وجود الميكوبكتيريا، فمن الوارد أن يُظهر الشخص السليم ردة فعل كاذبة، إما بسبب أنتيجينات التطعيم كما ذكرنا أو بسبب التعرض للبكتيريا بشكل طبيعي من الجو.

<sup>36</sup> Tuberculin Unit.

( +ve ) Tuberculin Test			( -ve ) Tuberculin Test
>5mm induration <sup>37</sup>	>10mm induration	>15 mm induration	No induration
Recent contact with an active TB <sup>38</sup> .	IV drug user.	Any person.	No previous infection.
HIV.	HIV seronegative patient <sup>39</sup> . Medical condition (diabetes, malignancy)		Pre-hypersensitivity <sup>40</sup> .
High risk for HIV.	Hospital residents and employees specially Mycobacteriology lab personnels <sup>41</sup> . Children < 4 years.	No diabetes or HIV.	Lost TB sensitivity with loss of antigen.

**Note that AIDS patients are susceptible to acquire TB more than others.**



<sup>37</sup> Induration = granuloma.

<sup>38</sup> على اختلاط مباشر بأشخاص مصابين.


<sup>39</sup> The lab result is negative for HIV although the person have it.

<sup>40</sup> It is a delayed response, it needs time to occur.

<sup>41</sup> طاقم العاملين. موظفي مختبر المايكوباكترولوجي.



Laboratory diagnosis of TB<sup>42</sup> (VERY IMPORTANT):

<p><b>Collection of specimens</b></p>	<ul style="list-style-type: none"> <li>● <b>Pulmonary TB:</b> <ul style="list-style-type: none"> <li>- 3 <b>early morning</b><sup>43</sup> <b>sputum samples</b>, induced cough, bronchial lavage, or gastric washing (for infants<sup>44</sup>).</li> </ul> </li> <li>● <b>Renal TB:</b> <ul style="list-style-type: none"> <li>- 3 early morning urine.</li> </ul> </li> <li>● <b>TB meningitis:</b> <ul style="list-style-type: none"> <li>- CSF<sup>45</sup> sample.</li> </ul> </li> <li>● <b>TB of bones and joints:</b> <ul style="list-style-type: none"> <li>- Aspiration.</li> </ul> </li> <li>● <b>TB of Lymph nodes:</b> <ul style="list-style-type: none"> <li>- Pus or tissues NOT swap.</li> </ul> </li> </ul>
<p><b>Microscopy</b></p>	<p>ZN or Auramine<sup>46</sup> stain.</p>
<p><b>Culture</b><sup>47</sup></p>	<p>Culture is the <b>gold standard</b> test for identification and sensitivity.</p> <p><b>Media used:</b> Lowenstein-Jensen media (<b>LJ</b>)</p> <p><b>Media contains</b><sup>48</sup>: Eggs, asparagine, glycerol, pyruvate and malachite green<sup>49</sup>.</p> <p><b>Colonies appear after 2-8 weeks as eugenic, raised, buff, adherent growth.</b></p> <p><b>We differentiate between them by their enhancement</b><sup>50</sup>:</p> <ol style="list-style-type: none"> <li>1) <b>Enhanced by glycerol</b> → <i>M. TB</i>.</li> <li>2) <b>Enhanced by pyruvate</b> → <i>M. Bovis</i>.</li> </ol> <p>Other media <b>plus</b> LJ media may be used:</p> <ul style="list-style-type: none"> <li>- Fluid media (middleBrook).</li> <li>- MGIT<sup>51</sup>.</li> <li>- Automated methods like Bactec MGIT.</li> </ul> 

<sup>42</sup> Two steps process. 1) Take the specimen. 2) Send it to the lab for microscopy and culture.

<sup>43</sup> بمجرد استيقاظ المريض قبل أكله وشربه، لأن تركيز البكتيريا يكون أعلى بعد فترات النوم.

<sup>44</sup> Because they cannot perform any of the previous actions.

<sup>45</sup> Cerebrospinal fluid.

<sup>46</sup> Show acid-fast bacilli using fluorescence microscopy. ([More](#))

<sup>47</sup> Culture takes about 2-6 weeks but you start medication based on your knowledge of the symptoms.

<sup>48</sup> المواد التي ينطلبها المخلوق ليعيش ويتكاثر. يختلف التركيب هنا اختلاف جذري عن طبق الدم، ولكن يسير على المبدأ ذاته.

<sup>49</sup> Inhibits other organisms from growing. (Selective).

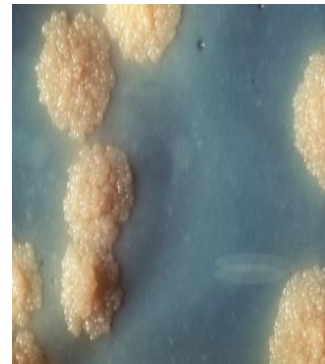
<sup>50</sup> نحضر أنبوبين منفصلين من الميديا، الأول جليسرول والثاني بايروفيت ونحدد النوع بناءً على الأنبوب الذي ينمو به.

<sup>51</sup> Mycobacteria growth indicator test.

## Identification of the organism:

### How can we know for sure that it is *M. TB*?

- 1) Measurement of **Interferon-Gamma (IFN $\gamma$ )** that is secreted from sensitized lymphocytes, challenged by the same mycobacterial proteins in a patient who is previously exposed to the disease. This is a more specific and significant test than the tuberculin skin test.
- 2) If the growth occurred at **37°C** and produced **5-10% CO<sub>2</sub>**
- 3) **PCR**: Molecular test directly from CSF specimens.
- 4) **PropTech<sup>52</sup>**: directly from respiratory samples.
- 5) **Morphology**: Buff, rough and tough.
- 6) **Biochemical tests**: Niacin production and Nitrate test.
- 7) **Sensitivity testing**.
- 8) **Guinea pig inoculation<sup>53</sup>**: Rarely done.



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## Management of a TB case:

- Isolate the patient for 10-14 days if smears were positive.
  - (>1000 organisms/ml of the sputum specimen is considered an infectious case.)
- **Triple regimen<sup>54</sup> of therapy; Why?**
  - To prevent resistant mutants.
  - To cover strains located at different sites of the lung.
  - To prevent relapse.
- Treatment must be guided by antibiotics sensitivity testing.
  - A test that is done to help you choose an antibiotic that will be the most effective against the specific type of bacteria. Some bacteria are resistant to certain antibiotics, which means you must try another antibiotic until the bacteria is finally sensitive to an antibiotic before you prescribe it for the patient.

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<sup>52</sup> Another molecular test that detects the bacterial genes. PCR is a part of it.

<sup>53</sup> حقن الخنازير من فصيلة "غوانا" بالبكتيريا للتأكد ما إذا كانت ردة الفعل المرضية تشير للميكوبكتيريا أم لا. الاختبار غير أخلاقي وقد لا يكاد يستخدم.

<sup>54</sup> A prescribed course of medical treatment.

First Line treatment	Second Line Treatment
<ul style="list-style-type: none"> <li>● Isoniazid (INH.)</li> <li>● Rifampicin (RIF.)</li> <li>● Ethambutol (E.)</li> <li>● Pyrazinamide (P.)</li> <li>● Streptomycin (S.)<sup>55</sup></li> <li>● Directly Observed Therapy<sup>56</sup> (DOT.)</li> </ul>	<ul style="list-style-type: none"> <li>● PASA (Para-Amino Salicylic acid.)</li> <li>● Ethionamide.</li> <li>● Cycloserine.</li> <li>● Kanamycin.</li> <li>● Fluoroquinolones.</li> </ul>
<p>For the first 2 months → INH + RIF + P  For the next 4-6 months → INH + RIF</p>	<p>It is more toxic.  Used if the bacteria was resistant to the first line drugs.</p>

### Why do we use this large amount of drugs? (VERY IMPORTANT)

Because some “**multidrug resistant TB**” is resistant to INH and RIF.

- Also, high bacterial load might lead to resistance.
- It might start to respond to treatment but then develop resistance.
- Addition of pyrazinamide to the INH ± RIF combination on the first 2 months of treatment allows the total duration to be reduced from 8 to 6 months!
- If the patient is resistant to RIF, he will also be resistant to INH, then we should go to second line. However, If the patient is resistant to INH, he don't have to be resistant to RIF.

### Prevention of TB:

- Tuberculin testing of herds<sup>57</sup>.
- Slaughter<sup>58</sup> of infected animals.
- Pasteurization of milk to prevent bovine TB.
- Recognition of new cases.
- Prophylaxis<sup>59</sup> with INH of contacts.
- Follow up cases.
- **Immunization with BCG<sup>60</sup> to all newborns at 1<sup>st</sup> day.**

<sup>55</sup> It is very important because it is non-toxic.

<sup>56</sup> Hospital observation of the patient while taking the medication to prevent mistakes ([Here](#)).

<sup>57</sup> A group of people or animals.

<sup>58</sup> Killing.

<sup>59</sup> Action taken to prevent a disease.

<sup>60</sup> Bacillus Calmette–Guérin vaccine. A vaccine primarily used against tuberculosis.



« قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ »  
سورة الزمر الآية ٩

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## Heartful thanks to Microbiology 435's Team

### Leaders

Sara Alenezy Ali Alzahrani

### Members

Fawzan alOtaibi  
Areb AlOgaiei  
Dalal AlHuzaimi  
Deema AlFaris  
Elham Alzahrani  
Ghaida Aljamili  
Khawla Alammari  
Kowthar almousa  
Lina alshehri  
Maryam Saidan  
Nojood Alhaidri  
Nouf Altwaijri  
Norah Alkhayyal  
Norah Alakeel  
Nurah alqahtani  
Ola alnuhayer  
Samar AlOtaibi  
Shahad Al-Enezi  
Suha Alenazi  
Reema Allhidan