SPUTUM TEST

Sputum is bronchial secretions hyperlinks utilized to clean the respiratory passageways from foreign substances and microbes outstanding defensive and the immunological response. It is an aqueous liquid composed of **(95%)** water and the remaining material are plasma, mucus, normal flora, defensive Immune cells and sometimes it contains some solids. During expectoration (Coughing), sputum gets contaminated with normal bacterial flora and cells from pharynx and mouth.

Examination of sputum is mainly carried out for:

- **Identification of causative agent or organism** associated with a particular suspected infection of the lower respiratory tract, e.g.
  – Suspected tuberculosis– Suspected fungal infection
  –Chronic disease like bronchiectasis & Pneumonia
- **Cytological examination** for the investigation of malignant cells and viral infections**.**

The following instructions should be considered when collecting sputum sample.

1. A mouthwash is done at first to remove any bacterial or fungal contamination.
2. Preferred collection of sputum in the early morning because the pulmonary secretions may be accumulate overnight.
3. **Induction of Sputum**: If the patient is not able to expectorate (Cough) the sputum spontaneously (especially Children), inhaling aerosol of 15% sodium chloride (NaCl) and 20% propylene glycol (C3H8O2) for 20 minutes can induce expectoration. Sputum can also be induced by inhaling distilled water in association with chest physiotherapy or by inhaling nebulized hypertonic saline.
4. Sputum sample is collected in a sterile, clean, dry and wide-mouthed plastic container with a securely fitting screw cap. Mix the sample with sterilize wooden sticks in preparation for examination.
5. Sample consisting of only the saliva (watery appearance, clear, and foamy) is not acceptable for laboratory investigations; in such case, another sample should be collected.

Sputum test include the following examinations:

A- Physical Examination:

Physical appearance of sputum is often indicative and symptomatic of the underlying pathologic process which include:

1) **Consistency and Appearances:** Natural sputum is aqueous composition, colorless and clear. The existence of any glitter بريق which means the presence of cellular substances and in pathological cases sputum became Mucoid in texture, Purulent, or a mixture of both (mucopurulent), and Bloody.

2) **Color:**

Normal sputum is colorless.

Abnormal colors are:

a) Bloody: Hemoptysis (Spitting blood) blood in sputum means 1 of 3 (Ts):

❶ Pulmonary tuberculosis (**T**.B). ❷ **T**umor (bronchogenic carcinoma).

❸**T**rauma causing (Hemorrhage).

***b)* Rusty:** It means the decomposition of hemoglobin and presence of Pneumococcal lobar pneumonia *(Klebsiella pneumoniae).*

c) Yellow or Purulent: Lung abscess (Pus Cells).

d) Green: *Pseudomonas* infection

e) Pink, frothy (air bubbles): Pulmonary edema استسقاء رئوي

3) **Odor:**

Normal sputum is **odorless**.

Pathology:

Putrid odor: Associated with **Gangrene**.

Fecal odor: Associated with **liver abscess.**

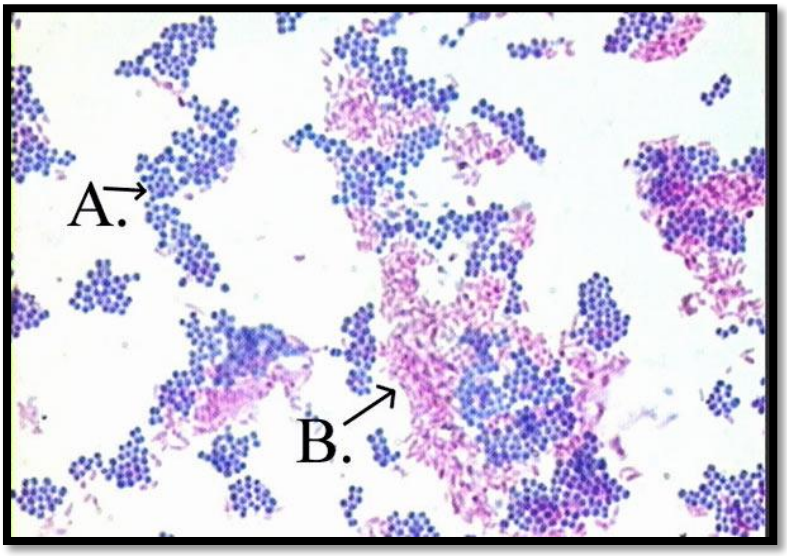
B- Microscopic examination:

i- Unstained film: Sputum is taken directly by mixing with normal saline only in order to observe; pus cells, Red blood cell and fungi.

ii- Stained film: Sputum is stained in different stains procedures according to the provisional diagnosis, such as **Albert stain** in case of whooping cough, or Gram's stain in ordinary bacteria, and **Ziehl-Neelsen's** stain (Acid fast stain) in case of pulmonary tuberculosis (Mycobacterium tuberculosis) which is illustrated as follows.

Ziehl-Neelsen technique: This technique is very simple, rapid and inexpensive.

1- Prepare bacterial smear on clean and grease free slide, using sterile technique.
2- Allow smear to dry in air and then fix by heat.
3- Cover the smear with carbol fuchsin stain.
4- Heat the stain by alcohol flame until vapor begins to rise (i.e. about 60 C). Do not overheat. Allow the heated stain to remain on the slide for 5 minutes.
5- Wash off the stain with clean distilled water.
6- Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolorized, i.e. pale pink.
7- Wash well with clean distilled water.
8- Cover the smear with malachite green stain for 1–2 minutes, using the longer time when the smear is thin.
9- Wash off the stain with clean water.
10- Wipe the back of the slide clean, and place it in a draining rack to air-dry.
11- Examine the smear microscopically, using the 100 X oil immersion objective.



(B)-Acid fast: Bright red to intensive purple red. **(A)**-Non-acid fast: Blue color (A).

With Ziehl-Neelsen staining, mycobacteria appear as bright red straight or slightly curved orb roads (0.2-0.5 µ in width and 2-4 µ in length) against a green or blue background (see Figure). Mycobacteria, both acid- and alcohol-fast are termed as acid-fast bacilli (AFB). Minimum 100 fields are examined before reporting the smear as negative. If acid-fast bacilli are seen, their number should be reported.
A **negative sputum smear** does not rule out the diagnosis of tuberculosis since smear may be of poor quality or organisms may be small in number, or sputum sample may not have been collected properly.

Concentration technique of Sputum:

In concentration technique, a solution of concentrated sodium hypochlorite (NaOCl) is added to the sputum sample, which causes the ① liquefaction of mucus and ② killing of mycobacteria.

The sample is centrifuged for sedimentation, then from the sediment of sputum a thin smear is prepared for staining with Ziehl-Neelsen staining and examined.

C-Sputum culture:

Culture media used are:
① Blood agar. ② MacConkey agar. ③ Chocolate agar.

After inoculating media dishes ❶ (blood agar & chocolate agar) are incubated anaerobically with (5-10 %) **CO2** gas using a Candle jar. ❷ But the MacConkey agar is incubated aerobically. Then all Plates are incubated in **37º C** incubator for 24 hours inoculated agar plates are examined for bacterial growth developing.

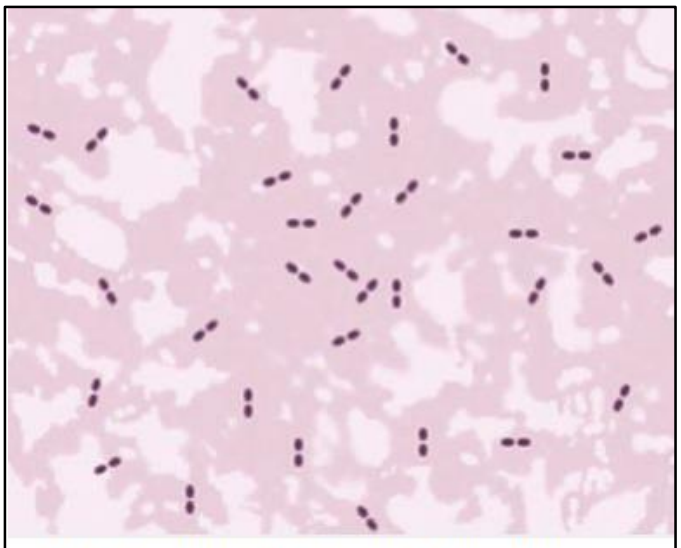
Types of the most common bacteria that cause bronchitis:

1- *Streptococcus pneumoniae;* gram-positive diplococci.
2- *Streptococcus pyogenes*
3- *Haemophilus influenzae; Gram-negative coccobacilli.*
4- *Klebsiella pneumoniae*
5- *Pseudomonas aeruginosa*

After all selecting a suitable culture media is required for some types of bacteria especially for *Mycobacterium tuberculosis* which is cultured on Lowenstein -Jensen medium.

Sputum is cultured in a slant small bottles, incubated at (35 – 37ºC) for (2 – 3 days). Positive culture may not appear and should be left in incubator for 6 weeks and checked every 3 days for growth, because Mycobacteria has a long generation time (18-20 hours) thus it needs a long time period to grow.

Note: Sensitivity test is carried out only if the amount of bacterial growth is significant.



Gram stained smear of sputum showing gram-positive diplococci (Streptococcus pneumoniae)