Examination of sputum

COLLECTION AND TRANSPORT OF SPUTUM

Sputum for microbiological investigation is collected and transported as follows:

1- Give the patient a clean, dry, wide-necked, *leak-proof* container, and request him or her to cough deeply to produce a **sputum specimen**.
2- Use a phenol-containing disinfectant to wipe the outside of the container after collecting the specimen to prevent the spread of infectious organisms and to **avoid contaminating** the outside of the container.
3- Sputum is best collected in the **morning** soon after the patient wakes and before any mouth-wash is used (the specimen *must* be sputum, not saliva). When pulmonary tuberculosis is suspected, up to three specimens may need to be examined to detect AFB.
4- When it is not possible to obtain sputum from children with suspected pneumonia or bronchopneumonia, pathogens can often be isolated from mucopus **aspirated from the nasopharynx**.
5- **Label** the container with the patient name, age and the date.
6- When pneumonia or bronchopneumonia is suspected, deliver the sputum to the laboratory with as **little delay** as possible because organisms such as ***S. pneumoniae*** **and** ***H. influenzae*** **require culturing as soon as possible**.
7- To ensure the survival of pathogens such as ***S. pneumoniae*** **and** ***H. influenzae***, transfer a purulent part of the sputum to a cotton-wool swab, and insert it in a container of **transport medium (**help the pathogens to survive and avoid the overgrowth of fast-multiplying commensals).
8- Send the sputum specimen and swab with a request form to reach the microbiology laboratory within 6 hours.

LABORATORY EXAMINATION OF SPUTUM

Possible pathogens

BACTERIA

Gram positive	***Gram negative***
Streptococcus pneumoniae	*Haemophilus influenzae*
Staphylococcus aureus	*Klebsiella pneumoniae*
Streptococcus pyogenes	*Pseudomonas aeruginosa*
	Proteus species
	Yersina pestis
	Moraxella catarrhalis

Also *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*.

Commensals

Sputum as it is being collected passes through the pharynx and the mouth. It therefore becomes contaminated with small numbers of commensal organisms from the upper respiratory tract and mouth. These include:

Gram positive	***Gram negative***
Staphylococcus aureus	*Neisseria* species
Staphylococcus epidermidis	*Moraxella catarrhalis*
Enterococci	*Haemophilus influenzae*
Viridans streptococci	Fusobacteria
Streptococcus pneumoniae	Coliforms
Micrococci	
Lactobacilli	
Diphtheroids	
Yeast-like fungi	

Day 1

1- Describe the appearance of the specimen

Describe whether the sputum is:
Purulent: Green-looking, mostly pus
Mucopurulent: Green-looking with pus and mucus
Mucoid: Mostly mucus
Mucosalivary: Mucus with a small amount of saliva When the sputum contains blood, this must also be reported.

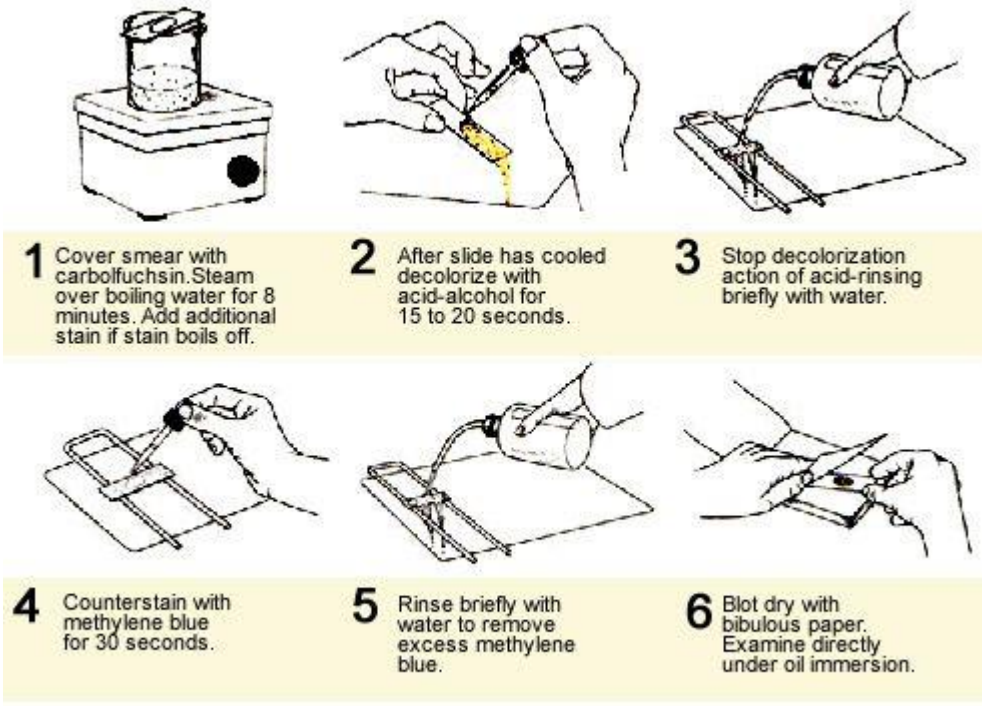
2- Examine the specimen microscopically *Gram smear*

Using a piece of stick, transfer a *purulent* part of the sputum to a glass slide, and make a thin smear. Allow the smear to air-dry in a safe place. Fix, and stain by the Gram technique. Examine the smear for pus cells and *predominant* bacteria. Gram stained smears of sputum must be reported with caution. Cocci, diplococci, streptococci, and rods may be seen in normal sputum because these organisms form part of the normal microbial flora of the upper respiratory tract.

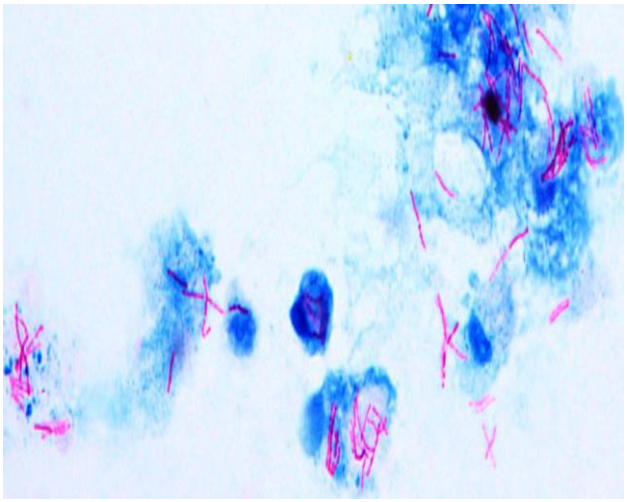
***Note*: When pus cells are present but no bacteria are seen in a Gram stained smear, this may indicate the presence of microorganisms such as *M. tuberculosis, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumophilia* or viruses.**

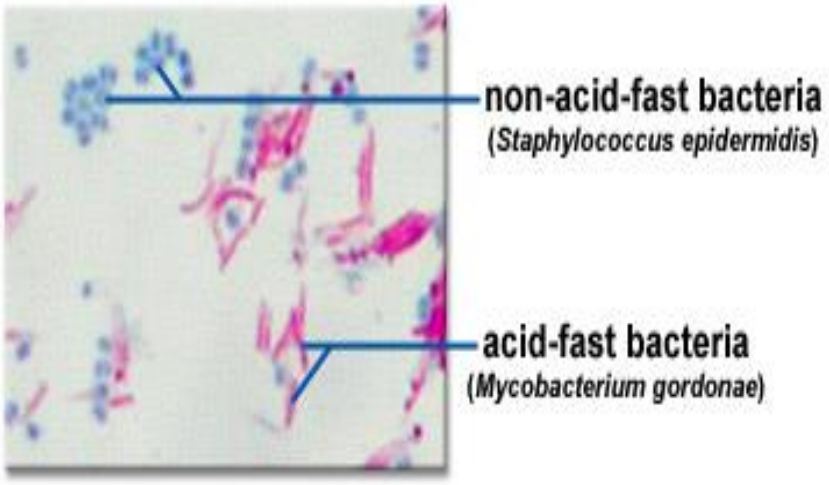
3- Ziehl-Neelsen smear to detect AFB

Studies have shown that the chances of detecting AFB in sputum smears are significantly increased when sputum is first treated with 5% v\v sodium hypochlorite (NaOC1), i.e. bleach, followed by centrifugation or overnight sedimentation. Because NaOC1 kills *M. tuberculosis*, the NaOC1 concentration technique is also safer for laboratory staff. NaOC1 treated sputum cannot be used for culture.



Ziehl-Neelsen acid-fast staining procedure





4- Culture the specimen

Blood agar and chocolate agar

– Wash a purulent part of the sputum in about 5 ml of *sterile* physiological saline.

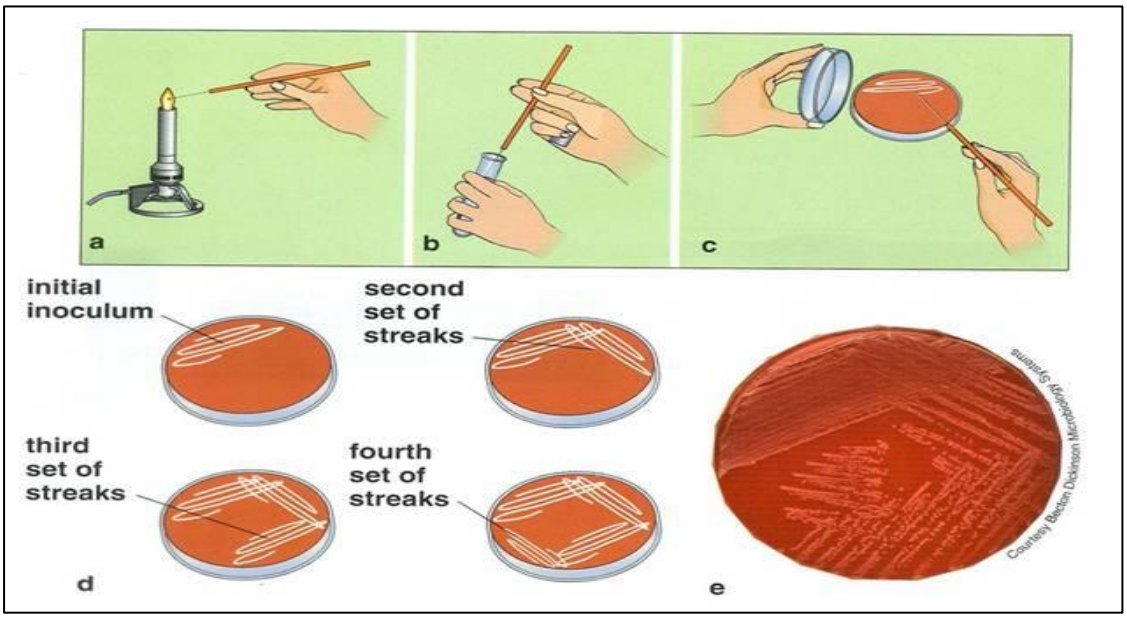
– Inoculate the washed sputum on plates of:

● Blood agar

● Chocolate agar

Inoculation technique to reduce commensal numbers:

Using the technique described in picture below (to inoculate a whole plate of agar), *flame the loop* in between each spread. This will help to obtain a pure growth of the pathogen in the areas of the 3rd and 4th spread. Add an optochin disc to the blood agar plate within the area of 2nd spread. This will help to identify *S. pneumoniae*.



Day 2 and Onwards

1- Examine and report the cultures *Blood agar and chocolate agar cultures*

Look especially for a significant growth of:

_ *Streptococcus pneumoniae* sensitive to optochin .

_ *Haemophilus influenza* .

_ *Staphylococcus aureus*.

Less frequently isolated pathogens

Klebsiella pneumoniae, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*

Streptococcus pyogenes, *Proteus* species, *Candida albicans* .

2- Antimicrobial susceptibility testing

Susceptibility tests should be performed only when the amount of cultural growth of a pathogen is significant. Strains of *S. pneumoniae* should be tested on blood agar for susceptibility to penicillin, tetracycline, and erythromycin. Penicillin susceptibility is best determined using an oxacillin 1 µg disc. A zone size less than 20 mm indicates reduced susceptibility. *H. influenzae* strains should be tested for *beta* lactamase production and susceptibility to ampicillin, tetracycline, and co-trimoxazole.

Summary of Microbiological Examination of Sputum

Day 1

		ADDITIONAL INVESTIGATIONS
1 Describe Specimen	■ ***Report whether specimen:*** – purulent, mucopurulent, mucoid, salivary – contains blood	
2 Examine Microscopically	■ ***Gram smear:*** For pus cells and bacteria ■ ***Zn smear:*** For AFB	■ ***Giemsa smear:*** When pneumonic plague or histoplasmosis is suspected ■ ***KOH preparation:*** When *Aspergillus* infection is suspected ■ ***Toluidine blue-O and Giemsa smears:*** When *Pneumocystis* pneumonia is suspected ■ ***Eosin preparation:*** When an allergic condition requires investigation ■ ***Saline preparation:*** When paragonimiasis is suspected
3 Culture Specimen	■ ***Blood agar*** – Add an optochin disc – Incubate aerobically ■ ***Chocolate agar*** – Incubate in CO_2	■ ***Culture for M. tuberculosis*** (In Reference Laboratory) See text

Day 2 and Onwards

4 Examine and Report Cultures	■ ***Blood and chocolate agar cultures*** Report *significant* growth of: *S. pneumoniae* *H. influenzae* *S. aureus* Less commonly found pathogens: *K. pneumoniae, P. aeruginosa, M. catarrhalis, S. pyogenes, Proteus, C. albicans*	■ Test *H. influenzae* for beta-lactamase production ■ Antimicrobial susceptibility tests as required

Key: Zn = Ziehl-Neelsen, KOH = Potassium hydroxide, CO_2 = Carbon dioxide