Lab-5:

Specimen Collection & Processing - Part 2:

Collection of Clinical Materials:

Urine Culture:

- Urine cultures are performed primarily when Urinary Tract Infection (UTI) is suspected.

- A midstream specimen (voided midstream urine), voided after washing the external orifice of glans, is used for urine cultures.

- In special situations such as neonates or elderly, suprapubic aspiration or catheterization may be required to obtain a specimen.

- In cases of STD, the first pass of urine should be used.

A 10 ml urine must be collected in sterile screw capped tube (preferable a morning sample) or another sterile container. Because urine is a good culture medium, it is essential that the cultures be done within 1 hour after collection or stored in a refrigerator at 4°C for no more than 18 hours.

Gross examination of urine by direct examination is includes color, odor, cloudiness and pH are important in diagnosis.

For staining and culturing; the urine sample should be centrifuged at 3000 rpm for 5 minute. The sediment of urine is used for cell counting, gram-staining, and culturing of bacteria.

- The presence of 10 WBC/ml indicates infection (**pyelonephritis, cystitis**).
- A urine sample that has abundant squamous epithelial cells suggests that it is contaminated and the results of the culture are not reliable.
- For detection of parasites and fungi in urine, the specimen should be centrifuged, and wet-preparation, microscopic examination and culturing should be done from sediment.

For colonies counting; because contamination of samples can occur as urine passes through the urethra, a numeric threshold of colony-forming units (CFU/ml) has been established to confirm the infection.

Quantitative Urine Culture

Quantitative urine culture is a standard, inexpensive diagnostic test that is widely undertaken to provide lab evidence of UTI. For quantitative urine cultures, the urine is **Not centrifuged**, but it performed by two ways:

(1) A calibrated loop inserted into urine cup (holds 0.01 mL of urine) can be used to streak the surface of culture media.

- **Example:** if the 0.01 mL loop was used and 20 colonies grew after urine culture, the colony count would be 20 x 100 = 2000 CFU/mL.
- In general, if 0.01ml was used and 10-100 colonies were developed, this suggest contamination while if **>100** colonies, indicate infection.

(2) Serial 10-fold dilutions can be made and the samples from the dilutions streaked on culture media.

The number of CFU/ml can be calculated by the following formula:

$$\text{CFU/ml} = \frac{\text{Colony count}}{\text{Size of sample used}} \times \text{Urine dilution used}$$

The results of urine culture can be interpreted by **Three categories** for determination of normal and abnormal status of UTI:

Category-1: If number of bacteria per ml of urine **$>10^5$ organisms /ml** is indicate **significant infection (bacteriurea)** with UTI.

Category-2: If **10^4 - 10^5 organism/ml** present is considered to be **suspected infection**.

Category-3: if **10^3 organism/ml** is suggest **normal state**. If repeated cultures reveal different organisms, indicate contamination.

It is commonly accepted that a bacterial count of at least 100,000/mL must be found to conclude that **significant bacteriuria** is present in asymptomatic persons. There is evidence that as few as 100/mL are significant in symptomatic patients.

Note: Only dilution of agar plates with acceptable count (30-300 cfu/ml) is used for calculation of the concentration of the original bacterial culture of urine. Counts more than or less than the acceptable count (30-300 cfu/ml) should be neglected.

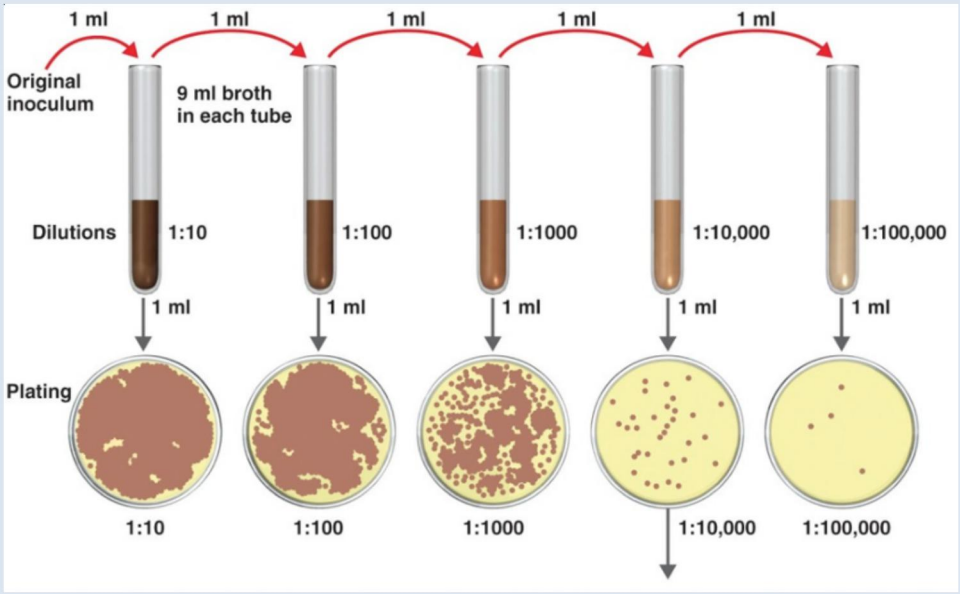
Intellectual Questions on Urine Culture:

Q1. What is the purpose of collecting a midstream specimen for urine culture?

Q2. What is quantitative urine culture?

Q3. Use the data below to calculate of the concentration of original bacterial culture of urine sample:

A. No. of bacteria on each agar plate :
B. Dilution of agar plate with acceptable count :
C. calculate of the concentration of the original bacterial culture of urine:
D. Can you suggest the patient state as significant bacteriurea, suspected infection, or normal state?



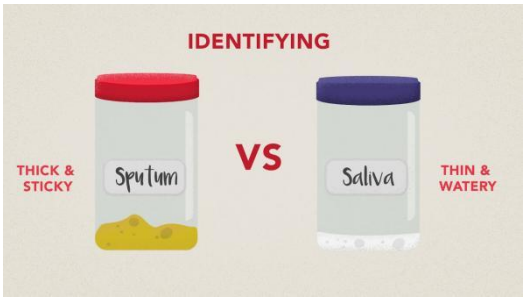
Sputum Culture:

In Upper Respiratory Tract (URT), the **swab cultures** are used primarily to detect URT infections especially common cold, sore throat, pharyngitis, tonsillitis, sinusitis, epiglottitis and oral thrush.

The **sputum cultures** are performed primarily to detect Lower Respiratory Tract (LRT) infections when pneumonia or tuberculosis is suspected.

How to collect a sample of sputum?

1. Rinse mouth with water (Do not use antiseptic mouthwash).
2. Take a very deep breath and hold the air for 5 seconds and cough hard until some sputum comes up into mouth. Spit out the sputum into the sample cup (or into sterile Petri dish) with minimize contamination by saliva.
3. At least 10 ml of sputum must be collected from patient in morning if possible, and transported immediately to laboratory. If the patient cannot cough, induction of sputum, transtracheal aspirate, bronchial lavage, or lung biopsy may be necessary.
4. **Homogenization of sputum:** mix equal volume of sputum with sputolysin agent such as dithiothreitol (DTT) in bottle, then the sputum can be liquefied by vortexing with the aid of glass beads.
5. **Decontamination:** mix equal volume of 4% NaOH and incubate at 37°C for 15-30 minutes, then centrifuge it at 3000 rpm for 15 min. the supernatant is discard.
6. **Neutralization:** the sediment is neutralized with phosphate buffer solution (PBS).
7. **Concentration:** centrifuge it again. Discard the supernatant. The sediment is taken for staining and culturing.



It is important that the specimen for culture should be sputum (mucoid), not saliva (watery).

- The sputum of patient with bacterial infection may purulent containing blood, and may be putrid in odor. A reliable specimen has more than 25 leukocytes and fewer than 10 epithelial cells per 100× field.
- Gram stain and acid-fast stained smears, and culture make from homogenized sputum.

Intellectual Question on Sputum Culture:

Q1. Why sputum must be collected from patient in the morning?