## ANNEX 2: STANDARD OPERATING PROCEDURE FOR URINE CULTURE

### 1. Purpose
This procedure describes the general procedures for processing a urine culture at a bacteriology laboratory.

### 2. Principle
Most clinicians consider Bacteriuria to be a definitive marker of UTI. Urine is normally a sterile body fluid. However, unless it is collected properly, it can become contaminated with organisms from the perineum, urethra, or vagina. The patient should be given detailed instructions for proper specimen collection.

### 3. Specimen
- Noninvasive urine specimens: Clean-catch or catheterized (indwelling)
- Urine specimens collected by invasive procedures: straight catheter, suprapubic aspiration, cystoscopy, nephrostomy.

The specimen should be stored refrigerated if there is a delay in transport to the laboratory. Do not process the following specimens: 1) specimens delayed more than two hours without refrigeration or preservative; 2) 24-hour urine collection; 3) Foley catheter tips; 4) urine from the bag of a catheterized patient; and 5) duplicate specimens collected on the same day.

### 4. Materials
- Media: Cysteine lactose electrolyte deficiency agar (CLED), Blood agar, MacConkey agar
- Sterile inoculating loop (1µl to deliver 0.001 ml and 10 µl to deliver 0.01 ml)
- Bacterial identification reagents, kits, and susceptibility testing disks.

### 5. Quality Control (QC)
Process the specimen as soon after receipt as possible. If processing is delayed, place the specimen in the refrigerator. Verify that the patient's identifiers on the specimen match those on the accompanying requisition. Ensure that all media and supplies have passed the required QC and are used before expiration.

### 6. Safety Precautions
Standard safety precautions for handling patient specimens must be used when processing these specimens.

### 7. Procedure
**Media Inoculation and Incubation:** Use a sterile loop calibrated to deliver 0.001 ml. Mix urine well. Hold the loop vertically and immerse it just below the surface of the urine. Deliver the loopful onto the plate. Make a straight line down the center of the plate and streak the urine by making a series of passes at 90-degree angles through the inoculum.

- Use a calibrated loop to deliver 0.01 ml for straight catheter, suprapubic, cystoscopy, and nephrostomy specimens.
- Inoculate the sample into BA, MCA and CLED
- Incubate overnight at 35ºC±2ºC.
- Incubate the plates and examine after 16 -24 hours. Isolate potential pathogens and identify using standard tests as per the laboratory protocol. Perform antimicrobial susceptibility

testing (AST) on appropriate organisms. Do not identify resident urogenital flora to the genus or species level.

- If there is no growth at 24 hours, report it as "No growth after 24 hours."
- Re-incubate plates for an additional 24 hours for the following: 1) tiny or scant colonies present that are barely discernible; 2) culture results do not correlate with clinical findings (e.g., the patient has sterile pyuria or symptoms without a positive culture); 3) specimen was collected by an invasive method.

**8. Interpretation**

Determine the colony count of each organism morphotype. With a 0.001 ml loop (1 µl), one colony equals 1,000 CFU/ml.

Interpretation is based on the method of collection and clinical condition:

- Asymptomatic patient; clean-catch or indwelling catheter specimen: report if growth is ≥ 100,000 colonies forming unit (CFU)/ml of the potential pathogen.
- Symptomatic ambulatory patient; clean-catch specimen: report if growth is ≥ 10,000 CFU/ml with one to two species of a potential pathogen. If >two species, urine is considered to be "contaminated," report: "mixed flora."
- Males; clean-catch specimen: report if growth is ≥1,000 CFU/ml of a potential pathogen.
- Specimens obtained by straight catheterization: report growth of ≥100 CFU/ml of any number of species of potential pathogens.
- All patient types report growth of any colony count of potential pathogens for specimens obtained by surgery or bladder aspiration.
- All patient and specimen types: report any isolate of yeast.

Gram-negative bacilli account for most UTIs, specifically *Escherichia coli, Proteus mirabilis, Klebsiella pnuemoniae, and Pseudomonas aeruginosa.* Among the Gram-positive cocci, *Enterococcus* spp*., Staphylococcus saprophyticus,* and GBS (Group B *Streptococcus* or *Streptococcus agalactiae)* are the major etiologic agents. *Staphylococcus aureus* is a rare causative agent of UTI and often represents infection associated with *S. aureus* bacteremia or urinary catheterization.

**For midstream urine and catheter urine**

| **Absolute number of bacteria colonies on the plate (using 1 µl) loop** | **Correspondi ng CFU/ml** | **Interpretation** | **Reporting** |
|---|---|---|---|
| 1 - 9 | $10^3$ | Indicates possible contamination | Report as "No significant bacteria growth" |
| 10 - 99 | $10^4$ | Possible infection or contamination | Report the CFU/ml and proceed with identification and AST |
| ≥ 100 | $10^5$ | Infection **(**significant bacteriuria) | Report the CFU/ml and proceed with identification and AST |

**9. Reporting**

a. Positive Cultures: Report colony count (CFU/ml), full identification, and AST of the pathogen(s). Example: Greater than 100,000 CFU/ml *E. coli.*

b. Mixed cultures, report as: Mixed Flora or contaminated. Example: "Greater than 100,000 CFU/ml mixed growth of Gram-positive and Gram-negative organisms. Culture result is suggestive of contamination. Please resubmit a new specimen."
c. GBS should be reported from women in childbearing years and from known diabetics if ≥ 10,000 CFU/ml.

**10. Procedural Notes**

It is not necessary to inoculate fungal media when yeast cultures are requested. However, it is important to inoculate at least 0.01 ml per plate and hold the cultures for 48-72 hours to recover yeast. Because *E. coli* represents at least 80% of the pathogens in urine cultures, using rapid identification methods (spot indole) to identify this species is acceptable. A mixed culture in an outpatient with uncomplicated UTI likely indicates contamination.

**11. References**


a. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington D.C., USA, 3rd edition, 2010.
b. Cumitech 2C: Laboratory Diagnosis of Urinary Tract Infections. American Society for Microbiology (ASM), Washington D.C., USA. 2009.
c. EJB Urine Culture Flowchart. Basic Microbiology Workshop. ASM