# URINARY TRACT CULTURES

Assuming that there is no kidney infection (nephritis), urine comes out of the kidney sterile. The sterile urine moves down the ureters into the urinary bladder for storage. The bladder has a couple of excellent defense mechanisms to keep microbial growth down---a mucin protein layer and IgA from the immune system. There are other defense mechanisms of the urinary tract---the free flow of urine, antimicrobial chemicals in urine (urea, ammonia), antimicrobial chemicals in semen. Most of the organisms that might produce a bladder infection (cystitis) or urethritis come from the anal area. As the urine moves into the distal urethra, it picks up urethral flora. The bacterial normal flora can include *Staphylococcus, Streptococcus, Corynebacterium, Lactobacillus*, and members of the family of Enterobacteriaceae, while the fungus *Candida* (yeast) may also be present.

If urine is taken from a healthy person as a catheterized sample or a midstream clean catch, the urine should be essentially free of organisms. The presence of more than 100,000 cells/ml of urine indicates a urinary tract infection (UTI). Less than 10,000 indicates no infection, although a count between 10,000-1000,000/ml does not absolutely rule out an infection. In a sample of voided urine (no precautions about how the sample is taken), upwards of 1000 cells may be contaminants from the urethra. Another key diagnostic test for real infection is the number of white blood cells seen in the microscopic field of vision using a high power (40X) lens. Less than 5 WBC/high power field is normal.

In this lab, you will compare a clean catch midstream urine specimen to a normally voided urine specimen. Selectively differential media (CNA and EMB agar) will be used to separate out gram + (*Staph, Strep*) from gram – (Enterobacteriaceae), as well as lactose fermenters (E. coli) from nonfermenters (*Pseudomonas, Proteus,* etc.).

**Instructions for Clean Catch Midstream Urine Collections**

**You have been given towelettes and a urine specimen cup to obtain a sample of your urine.**

1. Unscrew the cap of the urine specimen cup. Place the cap on the counter with the "straw" facing upward. **To avoid contamination, do not touch the inside of the cup, cap or straw.**
2. Cleanse yourself with towelettes as follows:
   **Males** - Wipe the head (end) of your penis in a single motion with the first towelette. Repeat this with the second towelette. If you are not circumcised, hold the foreskin back before cleansing and continue to hold it back when you are collecting the urine sample.
   **Females** - Separate the labia, which are the folds of skin on either side of the area from which you urinate. Wipe the inner folds of skin from front to back in a single motion with the first towelette. Then wipe down through center of labial folds with the second towelette. Make sure to keep the labia separated while you are collecting the urine sample.
3. Urinate a small amount into toilet.
4. Place the collection cup under the stream of urine and continue to urinate into the cup. Once the collection cup is full, finish urinating into toilet.
5. Replace the cap on the cup, and tighten the cap securely.

**MATERIALS NEEDED: per table**

Urine sample

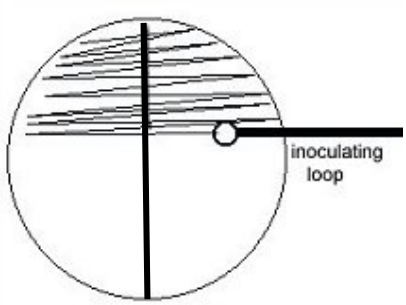
0.01 (1/100) ml sample calibrated sterile inoculating loops

1 CNA plate

1 EMB plate

Antimicrobial towellettes

Containers for urine




**PROCEDURES:**

1. Some tables will be designated as midstream clean catch specimens and other tables designated as normal void specimens.
2. Dip the 0.01 ml loop into the urine same (just into the urine, without submererging the plastic sample.
3. Place a straight line down the center of the agar plate, then streak in a dense zig-zag pattern back and forth across the plate to the bottom.
4. Repeat this previous step for the other agar plate, using a new inoculums.
5. Incubate plates at 37 degrees C.

2nd session:

1. Check your plates for growth. Calculate the number of gram + and gram – bacteria in the urine sample by multiplying the colony count X the dilution of the calibrated loop (1/100).

   **If there arehave 50 colonies on the EMB, 50 X 100 dilution = 5000 cells/ml of urine**

2. It may be that your count is very sparse and the colony count will be easily determined. However, if the colonies extend more than ¾ of the way down the plate, you can guesstimate it at over 100,000 total.
3. Gram stain the predominant colony type on each medium.
4. If there is a predominant coccus on the CAN plate, run a catalase test. Remember that *Staph* is cat+ while *Strep* and *Enterococcus* are cat-.

**INTERPRETATION:**

Refer back to the exercise on isolation of bacteria from the sponge and ENT, where the CNA and EMB media are described. There is a description of demolytic types in the ENT exercise. EMB is a selective medium, allowing the growth of gram – bacteria (the dyes eosin and methylene blue inhibit gram +). In addition, lactose sugar is in this medium, along with the 2 dyes which act as pH indicators. If the bacterium uses lactose an acid is produced, which then changes the pH in the medium, causing the colonies to become dark-centered or have a purple-green sheen (the methylene blue due precipitates out). Non-fermenters of lactose will not make acid, nor change their colony color, so the colonies are transparent against the medium color.

**Common microbes:**

- Gram – bacilli = probable Enterobacteriaceae family
- Gram + cocci = probable *Staph* or *Strep*
- Gram + rods = probable *Lactobacillus*
- Budding yeast = *Candida*

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**QUESTIONS:**

1. What will E. coli colonies appear like on EMB?
2. What is the inhibitory agent in CNA?
3. What is the critical number of cells/ml of urine, over which it is designated to be a UTI?

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