Urine Cultures

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

INTRODUCTION

There are several options available to the laboratory that performs urine cultures. The options can be simple or complex depending on the system employed and the degree of specificity desired. This LabFacts includes guidelines for procedures, quality control and proficiency testing as well as information on complexity of testing methods.

First, based on your laboratories' capabilities, determine how specific your laboratory's urine culture results will be. Factors to consider include volume of testing, percentage of positive specimens, level of personnel training, time involved in testing, and costs associated with materials, quality control and proficiency testing.

MODERATE COMPLEXITY

There are two methods that qualify as moderate complexity testing. The first uses media for primary isolation, and all cultures with significant growth are then referred to a reference laboratory for final (definitive) identification and antibiotic susceptibility. Sterile cultures can be reported as "no growth". The second method is a colony count only. The training and time necessary to perform both of these methods are minimal.

HIGH COMPLEXITY

High complexity urine cultures include both initial (presumptive) and definitive identification and/or susceptibilities. There is also an option to perform a colony count and susceptibility without the identification step.

To determine if your personnel meet the standards for moderate or high complexity microbiology testing, consult COLA LabFacts 4 *Personnel*, or call the COLA Information Resource Center at (800) 981-9883.

SPECIMEN COLLECTION AND HANDLING

Regardless of the method, specimen collection and handling are critical steps. Without special preparation of the patient and careful specimencollection and storage, the test results may be compromised.

The most common specimen for microbiologic analysis is the "clean catch" midstream void urine specimen. To guarantee the quality of the specimen, the container for collection must be sterile. Instruct the patient how to collect the specimen and to use care during the collection process because touching the edges or interior of the sterile container to the body could contaminate the specimen.

The patient should thoroughly clean their external genitalia with a mild antiseptic solution, followed by passing the initial stream of urine into the toilet. Collection begins with the midstream portion. The final portion of the urine may be voided directly into the toilet.

Once collection is complete, the container should be tightly closed and transported to the laboratory immediately. Label the container itself, not the lid, with the patients' complete name or some unique identifier. Other urine specimens suitable for culturing include catheterized specimens and suprapubic aspirations. These specimens should be obtained by a qualified practitioner using sterile techniques. It is recommended that urine culture specimens be collected prior to initiation of antibiotic therapy, if possible.

Since urine provides nutrients for the growth of many organisms, it is essential that the specimen be processed within one hour of collection. Refrigeration is necessary if there is a delay in transporting or processing the specimen. Patient specimens may be refrigerated for up to 24 hours at 4^0C without significantly harming bacterial growth. Refrigeration may cause the specimen to become cloudy but this does not adversely affect the culture and is not an indication of bacterial growth.

Before plating the culture, swirl the specimen to be sure it is well mixed. Remember, the results are only as good as the quality of the specimen.

METHODS

I. Combination Media for Colony Count and Presumptive Identification

Prepackaged units, such as Uricult and Bacticult, are more expensive than plated media but serve the same function. These are usually bottled systems containing paddles which hold nutrient and selective media.

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

A. Colony Count (Moderate Complexity)

A colony count is a moderate complexity procedure. The media is inoculated by either dipping the paddle into the urine specimen or pouring urine over the paddle. Follow the manufacturer's instructions for incubation which may range from 18 to 48 hours. To determine the colony count, compare the media to the colony density chart provided by the manufacturer. This comparison gives a range of results e.g. (10,000-25,000) rather than an exact number.

B. Initial (Presumptive) Identification (High Complexity)

The kit systems provide initial (presumptive) identification by comparing growth on a variety of media to a color chart. Characteristics such as color, size, and texture of the colonies and the color of the media are used to make a presumptive identification.

Depending on colony morphology, alone, can be misleading. If there is overlapping growth, it is very difficult to determine the morphology. Also, several organisms produce colonies that are not distinguishable from one another without additional media or biochemical tests. For example, *E. coli*, *Klebsiella*, and *Enterobacter* all cause the nutrient media (CLED) to turn yellow and all three produce black colonies on EMB.

Tightly capping the culture bottle during incubation causes moisture to build up in the vial which may cause the colonies to appear moist, wet, or even mucoid. This can cause confusion, as those characteristics are meant to differentiate between certain organisms. The results you report should reflect the presumptive nature of the answer. Presumptive identification of bacteria is classified as a high complexity procedure.

II. PREPACKAGED MEDIA FOR IDENTIFICATION AND SUSCEPTIBILITY

Bullseye, Biostar and Uro-Plate are examples of products that incorporate identification and susceptibility together. They provide a colony count, presumptive identification and a (direct) susceptibility and are the most expensive option.

A. Colony Count (Moderate Complexity)

Performance of a colony count is a moderate complexity procedure. The colony count is performed on the nutrient media included in the system. The inoculation is the same as that for primary isolation media using a calibrated loop. See Section III A of this LabFacts for more information. The colony count is given as the actual number of colony forming units per milliliter (#CFU/mL).

B. Presumptive Identification (High Complexity)

Performance of presumptive identification is a high complexity procedure. These packages routinely include selective media and one other differential media. The differential media included is usually citrate utilization media. Citrate media allows presumptive identification of many species in the Enterobacteriaceae family. Citrate media has a green color and will change to a blue color if the citrate is used by the bacteria. *E. coli* cannot use citrate so the media stays green while *Klebsiella* and *Enterobacter,* which both use citrate, turn this media bright blue.

C. Direct Susceptibility (High Complexity)

Although unconventional, this high complexity method is popular. This is a non standardized measurement that usually provides results within the first 24 hours. There are several limitations and very extensive quality control requirements for this method.

III. USING MEDIA FOR PRIMARY ISOLATION

Primary isolation media are either nutrient or selective agars. Nutrient media include 5 percent sheep blood agar (SBA) or cysteine lactose electrolyte deficient media (CLED). Eosin methylene blue (EMB) and MacConkey (MAC) are the selective media most frequently used. These can be purchased individually or in combination as a bi-plate. Media for primary isolation are inexpensive and are ideal for performing colony counts using a calibrated loop.

A. How To Perform A Colony Count (Moderate Complexity)

Use a calibrated loop (.01 mL or .001 mL) to inoculate the media. The .001 mL calibrated loop is the most

common and is recommended. Make a single streak down the center of the media. Move the loop across the initial streak in a zigzag pattern to obtain isolated colonies. Turn the plate 90^0, and streak, again in a zigzag pattern, across the first zigzag pattern. Incubate overnight at 35^oC. Count the number of colonies observed on the plate. Multiply by the calibration factor of the loop (100 or 1000 respectively) to get an exact colony count. For example: if the .01 mL loop was used and 20 colonies grew, the colony count would be 20 x 100 = 2000 CFU/mL (colony forming units per milliliter). Any specimens that show no colonies after 24 hours should be re-incubated for an additional 24 hours. This allows for the recognition of organisms that are slow growers or which are partially inhibited by antibiotic therapy.

Reporting an actual number or estimation of organisms present is crucial. A report, of positive (growth) or negative (no growth), is not as useful to the clinician as a colony count. Due to the high incidence of contamination, growth does not necessarily mean there is an infection. On the other hand, an organism that has complex nutritional requirements or one that is slow growing may yield a very low colony count due to the nature of the organism. This may be a true infection and should not be considered negative. A count of 100,000 (10^5) CFU/mL or greater is significant. Growth between 10^4 and 10^5 CFU/mL could signify possible infection. Growth less than 10,000 (10^4) CFU/mL usually suggests contamination.

Determine whether the culture is pure (only one organism) or mixed (multiple organisms). Evidence of three or more organisms usually indicates contamination. If two organisms are present this may represent infection or contamination. The patient's history and the type of specimen are helpful in evaluating this situation.

B. Presumptive Identification (High Complexity)

Growth patterns on primary isolation media allow broad classification of an organism. Nutrient media, such as blood agar, supports the growth of both Gram positive and Gram negative organisms. EMB and MacConkey are selective for Gram negative organisms. These media have additives that inhibit the growth of Gram positive organisms. However, it is not uncommon to see pinpoint or rare colonies on the selective media and significant growth on the nutrient media. This growth pattern represents a Gram positive organism.

Selective media, like EMB and MacConkey, provide more information about the Gram negative organisms. These media also contain lactose. If an organism can utilize lactose, the colonies will turn color due to a pH indicator in the media. *Proteus* and *Pseudomonas,* both non-lactose fermenters, produce clear or colorless colonies on these media. This lack of color change, differentiates the genus *Proteus* and *Pseudomonas* from the lactose fermenting colored colonies of other Enterobacteriaceae species such as *E.coli*, *Klebsiella* sp. and *Enterobacter* sp.

C. Definitive Identification (High Complexity)

Definitive identification is possible when used in combination with additional biochemical tests or commercial identification systems.

GENERAL QUALITY CONTROL

Quality control is necessary to check each step of testing. There are a few quality control procedures that are applicable to both moderate and high complexity testing. These procedures should be performed regardless of the level of testing.

All of the methods require an incubation period. The proper temperature range for incubation is 35^0C to 37^0C. Keep a record of the incubator temperature. To ensure that the temperature is accurate, check your thermometer against a standardized thermometer at least once a year unless your thermometer is a referenced thermometer.

The major component of the test system is the media. The National Committee for Clinical Laboratory Standards (NCCLS) has established criteria to verify that media performs its selective, inhibitory, and nutritive functions:

- Maintain documentation that the commercial media used meets these standards. This information is easily obtained from the manufacturer of the media. In some cases it may be included in the package insert or on the sleeve or box in which the media is shipped. Any media you prepare on site has to be quality controlled for its selectivity, inhibition and growth supporting abilities according to CLIA guidelines.

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

- Visually inspect new lot numbers and shipments of media upon receipt. This includes checking for discoloration, dehydration, contamination, freezing, improperly filled plates, cracked media, and separation of media from the plate. If any deterioration or problems are noticed, do not use the media. Report any problems to the manufacturer. Follow their recommendations for disposal of the media. It is also recommended that you perform a sterility check of the media by incubating one plate overnight. This check ensures proper handling of the media after it left the manufacturer.

- Check each new lot or shipment of reagents, stains, discs, ID systems (including those kits used for presumptive ID), and antisera for positive and negative reactivity upon receipt or concurrent with initial use. To determine the appropriate organism to use as a control consult your package insert or a microbiology text. These organisms do not have to be American Type Culture Collection (ATCC) strains, unless the manufacturer specifically requires them. Any known organism that provides a positive and negative reaction is acceptable. Control organisms may be obtained from local hospitals, reference laboratories, confirmed patient isolates, or commercial sources.

PROFICIENCY TESTING FOR URINE CULTURES

The proficiency testing (PT) requirements, for the specialty of microbiology, are different from other specialties. In microbiology, a laboratory needs to perform a minimum of five samples per test event. These samples should represent all types of microbiology testing the laboratory performs. The proficiency testing samples must be treated in the same manner as patient samples and identified to the same level of identification and susceptibility as patient samples. Those laboratories performing only colony counts, have the option of enrolling in PT for colony counts or performing split sample analysis. For more information see LabFacts 8 *Proficiency Testing*, and LabFacts 9 *Split Sample Analysis*.

All other urine cultures (growth referred, presumptive or definitive ID) and susceptibility testing require enrollment in PT. Most of the PT programs offer a urine culture module. In most cases there are several samples to identify, either presumptively or definitively. One sample is selected by the PT program to have a Gram stain and susceptibility performed.

For example: If patients are only identified as Gram negative rods or Gram positive cocci, report proficiency tests in like fashion. If patients are identified as *Klebsiella pneumonia* and susceptibilities are performed, identify the organism that grew from the PT sample to the same level and determine whether each drug tested is susceptible or resistant. Be sure to follow your PT provider's instructions for the reporting of any drugs not tested in your laboratory. This allows for the best assessment of your system.

BIBLIOGRAPHY

1. Baron, E., Peterson, L.., Finegold, S. *Bailey and Scott's Diagnostic Microbiology.* 9th ed. St. Louis. C.V. Mosby. 1994.

2. Boyd, R., Hoerl, B. *Basic Medical Microbiology.* Boston. Little, Brown, and Company. 1977.

3. Finegold, S., Martin, W., Scott, E. *Bailey and Scott's Diagnostic Microbiology*, 5th ed. St. Louis. C.V. Mosby. 1978

4. National Committee for Clinical Laboratory Standards. *Physician's Office Laboratory Guidelines.* NCCLS Document POL1-T2. 2nd ed. 6/92

5. Sister Graff, L. *A Handbook of Routine Urinalysis.* Philadelphia. J.B. Lippincott. 1983.

6. Unknown, *Urine Under the Microscope.* Nutley, N.J. Hoffman LaRoche. 1973.

ADDITIONAL RESOURCES

1. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* NCCLS Document M2-A4, 4th ed. Approved Standard 1990.

2. National Committee for Clinical Laboratory Standards. *Methods for Dilution, Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.* NCCLS Document M7-A2, 2nd ed. Approved Standard 1990.

3. National Committee for Clinical Laboratory Standards. *Quality Assurance for Commercially Prepared Microbiological Culture Media.* NCCLS Document M22-A, 1990.