

Clinical Testing

Bacteriology

General Submission Guidelines for Bacteriology Specimens

1. Collect the specimen before administering antimicrobial agents when possible.
2. Collect the specimen with as little contamination from indigenous microbiota as possible to ensure that the sample will be representative of the infected site.
3. Use sterile equipment and aseptic technique to collect specimens and to prevent introduction of microorganisms during invasive procedures.
4. Collect an adequate specimen (refer to the *Manual of Services* for amount necessary to perform each test).
5. Assure the specimen container is clearly and correctly labeled with the patient name, date and time of collection, source and/or specific site so that the proper culture media will be selected during processing in the laboratory.
6. Complete all information on the test requisition. The unique patient identifier on the test requisition must match the unique identifier on the specimen.
7. Transport all specimens to the laboratory promptly to ensure the survival and isolation of fastidious organisms, to prevent overgrowth by more hardy bacteria, to shorten the duration of specimen contact with some local anesthetics used in collection procedures that may have antibacterial activities, and to provide more accurate diagnosis.

Specimen Collection

The Illinois Department of Public Health, Bacteriology Section, accepts isolates of aerobic bacteria for identification and susceptibility testing. However, the laboratory does not accept clinical specimens from hospitals such as tissues or fluids. The laboratory will accept a swab for *Corynebacterium*. Please call for additional instructions.

Note: Do not submit specimens that may have anaerobic or both anaerobic and aerobic bacteria.

Female Genital Tract

Genital tract specimens are submitted primarily for the detection of sexually transmitted pathogens. Bacteriology accepts specimens for the isolation and identification of *Neisseria gonorrhoeae*. Illinois Department of Public Health does not perform anaerobic bacteriology.

Cervix

1. Do not use lubricant during procedure.
2. Wipe the cervix clean of vaginal secretion and mucus.
3. Rotate a sterile swab, and obtain an exudate from the endocervical glands.
4. If no exudate is seen, insert a sterile swab into the endocervical canal, and rotate the swab.

Vagina

1. Use a speculum without lubricant.
2. Collect secretion from the mucosa high in the vaginal canal with sterile pipette or swab.

Male

Urethra

Used primarily to detect *N. gonorrhoeae*

1. Collect specimens at least two hours after the patient has urinated.
2. Insert a thin urethrogenital swab 2 cm to 4 cm into the endourethra, gently rotate it, leave it in place for one to two seconds and withdraw it.

Ocular Specimens

1. Obtain viral and chlamydia samples before topical anesthetics are instilled.
2. Obtain samples for chlamydia with calcium alginate swabs and viral cultures with Dacron® swabs or cotton swabs with non-wood shafts.
3. Send prepared smears and inoculated media to the laboratory immediately.

Conjunctival Scraping

1. Instill one or two drops of topical anesthetic.
2. Scrape the lower tarsal conjunctiva with a sterilized kimura spatula.
3. Inoculate the appropriate media directly.
4. Prepare smears by applying the scraping in a circular manner to a clean glass slide or by compressing material between two glass slides and pulling the slides apart.
5. Alternatively, use a calcium alginate swab or cotton-tipped applicator to swab the inferior tarsal conjunctiva (inside the surface of an eyelid) and the fornix of the eye. However, organisms are more readily detected in scraping than from a swab.

Respiratory Specimens

1. Twenty-four hour sputum collections are not recommended for culture.

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2. If *Corynebacterium diphtheriae*, *Achromobacterium haemolyticum*, *Neisseria gonorrhoeae* or *Legionella* are suspected, the physician should contact the clinical bacteriology laboratory prior to specimen collection because special techniques and/or media are required for the isolation of these agents.

Upper Respiratory Tract Infections

Throat (Pharyngeal Specimens)

Submitted primarily for the detection of group A streptococci (also can be used to detect *Neisseria gonorrhoeae*, *Haemophilus influenza*, and *Achromobacterium haemolyticum*)

1. Do not obtain throat samples if epiglottis is inflamed, as sampling may cause serious respiratory obstruction.
2. Depress tongue gently with tongue depressor.
3. Extend a sterile swab between the tonsillar pillars and behind the uvula. (Avoid touching the cheeks, tongue, uvula or lips).
4. Sweep the swab back and forth across the posterior pharynx, tonsillar areas and any inflamed or ulcerated area to obtain a sample.

Nasal Swabs

Submitted primarily for the detection of staphylococcus carriers

1. Insert a sterile swab into the nose until resistance is met at the level of the turbinates (approximately 1 inch into the nose).
2. Rotate the swab against the nasal mucosa.
3. Repeat the process on the other side.

Nasopharyngeal Suction

Submitted for the detection of carriers of *Staphylococcus pyogenes*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, and *Pertussis* (performed by PCR)

1. Suction material from the nasopharynx, and collect it in a sterile container.

Nasopharyngeal Swabs

Submitted primarily for the detection of carriers of *Neisseria meningitidis*

1. Carefully insert a flexible-wire calcium alginate-tipped swab through the nose into the posterior nasopharynx, and rotate the swab.
2. Keep the swab near the septum and floor of the nose.

Urine Culture Specimens

General considerations:

1. Never collect urine from a bedpan or urinal.
2. Thoroughly clean the urethral opening (and the vaginal vestibule in females) prior to collection procedures to ensure that the specimen obtained is not contaminated with colonizing microorganisms.
3. Soap rather than disinfectant is recommended for cleaning the urethral area. If disinfectants are introduced into the urine during collection, it may be inhibitory to the growth of microorganisms.
4. Transport specimens to the laboratory within two hours of collection. If it cannot be transported within two hours of collection, the urine specimens should be refrigerated. (Bacterial counts remain stable for at least 24 hours at 4 C.) Do not freeze.
5. The Illinois Department of Public Health uses a boric acid preservative that maintains the organism for a limited period of time.
6. Any urine collection procedure involving catheterization must be done with scrupulous aseptic technique to avoid introducing microorganisms.
7. Send the first morning voided urine. Three consecutive first morning urine specimens are recommended for mycobacterial culture.
8. Do not submit 24-hour urine collections for culture.

Urine Collection Techniques

Clean Catch Urine Specimens (Female)

1. Thoroughly wash hands with soap and water, rinse, and dry before collecting the specimen. If the patient is collecting the specimen, provide her with detailed instructions, including diagrams or a pictorial display.
2. Cleanse the urethral opening and vaginal vestibule area with soapy water or clean gauze pads soaked with liquid soap.
3. Rinse the area well with water or wet gauze wipes.
4. Hold labia apart during voiding.
5. Allow a few milliliters of urine to pass (Do not stop the flow of urine).

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6. Collect the midstream portion of urine in a sterile container.

Clean Catch Urine Specimens (Male)

1. Thoroughly wash hands with soap and water, rinse, and dry before collecting the specimen. If the patient is collecting the specimen, provide him with detailed instructions, including diagrams or a pictorial display.
2. Cleanse the penis, retract the foreskin (if not circumcised) and wash with soapy water.
3. Rinse the area well with sterile water.
4. Keeping the foreskin retracted (to minimize contamination with skin flora), allow a few milliliters of urine to pass. (Do not stop the flow of urine).
5. Collect the midstream portion of urine in a sterile container.

Straight Catheter Urine (In/Out Catheter Urine Specimens)

In/out catheter urine specimens are useful when clean catch urine cannot be obtained or when results from clean catch urine specimens are equivocal and a diagnosis is critical.

1. Prior to catheterization, the patient should force fluids until the bladder is full (forcing fluids may reduce organism number).
2. Clean the patient's urethral opening (and in females, the vaginal vestibule) with soap, and carefully rinse the area with water.
3. Using sterile technique, pass a catheter into the bladder.
4. Collect the initial 15 mL to 30 mL of urine, and discard it from the mouth of the catheter.
5. Collect a sample from the middle or later flow of urine in a sterile container.

Indwelling Catheter Urine

Indwelling catheters are placed in patients who are unable to pass urine.

1. Clean the catheter collection port with a 70 percent alcohol wipe.
2. Using sterile technique puncture the collection port with a needleless syringe; do not collect urine from a collection bag.
3. Aspirate the urine, and place it in a sterile container.

Suprapubic Bladder Aspiration (SPA) of the Urinary Bladder

SPA is useful in determining urinary infection in adults in whom infection is suspected and for whom results from routine procedure have been equivocal and diagnosis is critical. SPA is also useful in pediatric patients when clean catch urine specimens are difficult to obtain.

1. Before SPA, the patient should force fluids until the bladder is full.
2. Shave and disinfect the suprapubic skin overlying the urinary bladder.
3. The physician will make a small lance wound through the epidermis, just above the symphysis pubis.
4. Aspirate urine from the bladder by using a needle aspiration technique.

Transport Medium

Genital

1. The specimen is collected with a swab then plated directly to a modified Thayer Martin plate. Streak the plate for isolation followed by incubation in a 5 percent carbon dioxide incubator. The following day, ship the specimen to the Illinois Department of Public Health laboratory.
2. The specimen should be kept moist and plated within 12 hours.
3. Dry specimens are rejected.
4. The preferable method of transport for *Neisseria gonorrhoeae* is a transport medium such as modified Thayer Martin plates. The submitter may submit an Amies Charcoal swab **with prior approval**.

Eye and Ear

1. The specimen is collected with a swab transport system.
2. The ampule of the kit must be broken to keep the specimen moist.
3. Dry specimens are not acceptable.

Throat (Strep A) and Nasal

1. The specimen is collected with a swab transport system.
2. Dry specimens are rejected.

Urine

Submit in a urine collection tube (BD, Sage).

Nasopharyngeal

1. The specimen is collected with a nasopharyngeal swab.
2. Put swab into swab transport system and then send to the laboratory.

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Wound/Abscess

1. The specimen is collected with a swab transport system.
2. Do not send if an infection by anaerobic bacteria is suspected.

Ship all specimens as Category B.

Ship to:

Illinois Department of Public Health

Bacteriology Laboratory

2121 W. Taylor St.

Chicago, IL 60612-4224

Phone: 312-793-4760

Fax: 312-793-7216

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Table 1. Bacteriology

Bacterial Agent	Performed at	TAT (Days)
<i>Alcaligenes species</i>	Ch	6
<i>Actinomyces species</i>	Ch	12
<i>Actinomycetes</i>	Ch	20
<i>Acinetobacter species</i>	Ch	12
<i>Aerococcus species</i>	Ch	12
<i>Aeromonas species</i>	Ch	12
<i>Bacillus anthracis</i> ¹	Ca, Ch, Sp	5
<i>Bacillus species</i>	Ch	12
<i>Bordetella species</i>	Ch	20
<i>Brucella species</i> ¹	Ch	5
<i>Burkholderia mallei</i> ¹	Ca, Ch, Sp	5
<i>Burkholderia pseudomallei</i> ¹	Ca, Ch, Sp	5
<i>Burkholderia species</i>	Ch	20
<i>Chryseobacterium species</i>	Ch	20
<i>Corynebacterium diphtheriae</i> ¹	Ch	8
<i>Corynebacterium species</i>	Ch	20
<i>Enterococcus species</i> /Vancomycin Resistant <i>Enterococcus</i> (VRE)	Ch	7
<i>Erysipelothrix</i>	Ch	5
<i>Francisella tularensis</i> ^{1,2}	Ca, Ch, Sp	7
<i>Garnerella vaginalis</i>	Ch	5
<i>Haemophilis influenzae</i>	Ch	2
<i>Haemophilis species</i>	Ch	5
<i>Legionella species</i> ²	Ch	N/A
<i>Listeria monocytogenes</i> ²	Ch	5
<i>Listeria species</i>	Ch	5
<i>Micrococcus species</i>	Ch	3
<i>Moraxella species</i>	Ch	20
<i>Neisseria gonorrhoeae</i>	Ch	5
<i>Neisseria meningitidis</i>	Ch	3
<i>Neisseria species</i>	Ch	3
<i>Nocardia species</i>	Ch	20
<i>Pasteurella species</i>	Ch	5
Pneumococcus	Ch	5
<i>Pseudomonas species</i>	Ch	3
<i>Staphylococcus aureus</i> /Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA) ³	Ch, Sp	5
<i>Staphylococcus species</i>	Ch	3
<i>Streptococcus species</i>	Ch	6
<i>Streptomyces species</i>	Ch	20
Gram positive non-spore formers	Ch	7
Gram positive spore formers	Ch	7
Non-enteric gram negative bacilli	Ch	7
Other Gram negative bacteria	Ch	8
Unclassified bacteria	Ch	8

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¹Refer to Appendix J. Reportable Diseases

²Illinois Department of Public Health will ship to CDC; refer to “Services Available at U.S. Centers for Disease Control and Prevention.”

³Vancomycin Intermediate *Staphylococcus aureus* (VISA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) are sent to CDC for confirmation; refer to “Services Available at U.S. Centers for Disease Control and Prevention.”

Shipping Instructions

1. By ground transport
 - a. Wrap specimen(s) individually in absorbent material.
 - b. Place wrapped specimen(s) into a biohazard labeled bag and seal securely.
 - c. Place the test requisition(s) in the biohazard bag outside pouch so that it does not come in contact with the specimen sealed inside the bag.
 - d. Place the sealed biohazard bag and test requisition(s) inside the shipping container.
 - e. The shipping container must be rigid such as a cooler and labeled with the UN 3373 Biological Substance Category B marking.
 - f. Close securely.
2. Commercial carrier by ground/air transport
 - a. Wrap specimen(s) individually in absorbent material.
 - b. Place the wrapped specimen(s) inside a biohazard labeled 95 kPa bag and seal following the instructions on the bag.
 - c. Place the test requisition(s) in the 95 kPa bag outside pouch so that it does not come in contact with the specimen sealed in the bag.
 - d. Place the sealed 95 kPa bag and completed test requisitions(s) inside the outer shipping container and close securely.
 - e. Label the outer shipping container with the appropriate Illinois Department of Public Health laboratory address.
 - f. Complete the return address section to include the name of the person shipping the package, business name and address and a business phone number.
 - g. The shipping container must include the UN3373 Biological Substance Category B marking.