

Microbiology and Virology

I. Hours of Operation

Microbiology: 7:00 AM – 11:30 PM; Monday - Friday
7:00 AM – 4:00 PM; Saturday
7:00 AM – 3:00 PM; Sunday
Virology: 7:00 AM – 3:30 PM; Monday - Friday
7:00 AM – 11:00 AM; Saturday

II. Contact Section

Microbiology 449-5850
Virology 449-5238

III. Scope of Testing

Microbiology detects and identifies microorganisms causing infectious diseases. Specialized testing is available for bacteria, fungi, viruses, mycobacteria, parasites and anaerobic organisms. *In vitro* testing for antimicrobial susceptibility is available for selected organisms.

IV. Anaerobic Cultures:

Collect tissue, body fluid (remove bubbles), deep abscess aspirate (remove bubbles). Swabs specimens are of minimal value but, if necessary, use anaerobic transport tube. Transport to laboratory as soon as possible.

V. Bacterial Cultures:

Abscess: Collect by aseptic aspiration (preferred), or use a Culturette II.

Body Fluids: Collect in a syringe or sterile sealed container. Seal tightly to avoid leakage. Swab specimens are of minimal value.

Body Fluid (Anaerobic): Collect aseptically, use a syringe, express all air bubbles, and cap. Swab specimens are of minimal value.

Blood: Specimen Collection: The volume of blood cultured is critically important for the recovery of potential bacterial pathogens. 20 mL should be collected to ensure maximum detection of positive cultures. The following chart should be used to determine the volume of blood to be placed in the appropriate blood culture bottle:

Blood Collected	BacT/ALERT FA	BacT/ALERT SN
20 mL	10 mL	10 mL
8-19 mL	Split	Split
1-7 mL ²	All	None
<1 mL	Unacceptable	Unacceptable

1. Optimal amount, which is strongly recommended for routine blood cultures. Other volumes listed are for difficult draws only.
2. Place the entire specimen (1-7 mL) in a BacT/ALERT SN bottle if anaerobic bacteremia is suspected. Notify laboratory if anaerobic bacteremia is suspected.

Notify the laboratory if Brucella or any other unusual or rare pathogen is suspected.

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Blood cultures should be collected according to the following guidelines:

Severe, life-threatening infection: two or three separate cultures by separate venipunctures (each arm) collected before initiation of antimicrobial therapy.

NOTE: Two or three blood cultures/day have maximal sensitivity (greater than 99%) for the detection of bacteremia. Therefore, ordering only one blood culture or greater than three blood cultures per day is not recommended.

Patient on antimicrobial therapy: collect prior to next dose of antibiotics whenever possible.

Prior to venipuncture, cleanse skin with chlorhexidine concentrically, starting from the proposed puncture site outward away from the site for at least 30 seconds. Allow to air dry for a minimum of 30 seconds. Perform venipuncture and inject blood into the bottles in a timely manner so that blood does not clot.

Interpretation:

Negative cultures: preliminary "no growth to date" reports are issued after 24 hrs. Subsequent reports are not issued until final report of "no growth" at 5 days.

Positive cultures: reports are issued, as information becomes available.

Bronchoscopy: Collect in a sterile container. Seal tightly to avoid leakage. Avoid contamination with oropharyngeal material.

Catheter Tip (CVP/Swan Ganz): Prep the catheter insertion site and catheter noting the location where the line passes through the skin. Remove the entire catheter and keep it from touching the skin. Using a sterile scissors cut off a 5-6 cm segment of the distal tip of the catheter and send in a dry, sterile container to Microbiology. Using a sterile scissors cut off 5-6 cm of the intracutaneous segment of the catheter and send in a dry, sterile container to Microbiology. Bacterial colonies are quantitated and colony counts greater than 15 may suggest catheter related sepsis.

Cerebrospinal Fluid: Collect 1 mL (minimum) in sterile screw-capped tubes (tube #2 preferred). Seal tightly to avoid leakage. **Do not refrigerate.** Transport to laboratory immediately.

Cervix or Cervical Os: Swab with a Culturette II using a speculum while collecting (minimize vaginal contamination).

Ear Tympanocentesis: Collect in a sterile screw-capped tube. Seal tightly to avoid leakage. Cultures of the external ear surface are of minimum value.

Endometrial: Collect tissue (see below) if possible or use a Culturette II.

Eye: Collect purulent material and/or swab conjunctiva. Use a Culturette II.

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Nose (Anterior Nares): Insert Culturette II 1-2 cm into nares. Not useful for diagnosis of sinusitis. Used for determination of colonization, e.g. with MRSA (see special requests).

Pus (Anaerobic): Use deep aseptic aspiration using a syringe and express all air bubbles, or use anaerobic transport tube. Swab specimens are of minimal value.

Rectal (Stool Specimen Preferred): Collect with 2 swabs in a Culturette II. *Salmonella*, *Shigella*, *Campylobacter*, *E. coli O157:H7* are routinely sought. *Yersinia enterocolitica* is routinely sought in children 6 years old and under. Notify Microbiology if *Vibrio*, *Yersinia*, *Aeromonas*, or *Plesiomonas* are suspected.

Sputum: Collect deep cough specimen free from saliva or oropharyngeal material. Use a sterile screw cap container. Seal tightly to avoid leakage. Suitability for culture and extent of workup is dependent on quality of specimen.

Stool: Collect in clean, dry container. *Salmonella*, *Shigella*, *Campylobacter*, *E. coli O157:H7* are routinely sought. *Yersinia* is routinely sought in children 6 years old and under. Notify Microbiology if *Vibrio*, *Yersinia*, *Aeromonas*, or *Plesiomonas* is suspected.

Throat: Thoroughly swab posterior pharynx with a Culturette I or II. Processed for β -hemolytic strep only.

Tissue: Collect aseptically and place a reasonably sized (1 cm³) piece of tissue in a sterile container and seal. Keep exposure of the specimen to a minimum. Swab specimens are of minimal value.

Urine: Collect clean catch midstream urine in a sterile screw-capped container. Seal tightly to avoid leakage. Refrigerate until sent to Laboratory (minimum volume = 1 mL). Bacterial colonies are quantitated. Pure culture isolates are identified and susceptibilities are performed at counts greater than 10,000 colonies/mL, if appropriate.

Vagina: Swab vagina using a Culturette II. Workup limited to potential pathogens; notify Microbiology if gonorrhea is suspected.

Wound: Swab affected area with a Culturette II or a biopsy placed in a sterile container.

VI. Special Requests:

Specify in "Comments" section of requisition. These procedures are included as part of the routine culture when indicated, and should be collected as described.

Actinomycetes: Collect a deep aseptic aspirate of pus; seal it in a syringe or anaerobic transport tube.

Bordetella Culture (Whooping Cough): Paired nasopharyngeal and throat specimens are recommended, use Calgiswab or dacron swab for the nasopharyngeal specimens. Inoculate

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Regan-Lowe agar plates at bedside. Regan-Lowe agar plates are available in Microbiology. PCR is test of choice.

Bordetella PCR: Preferred method for the diagnosis of pertussis. Use small Dacron wire swab. Swab specimen from deep nasopharynx. Place swab in M4, Amies, or Stuart's transport media or use green capped culturette. Calgiswabs are unacceptable. Detects both *B. pertussis* and *B. parapertussis* and differentiates between them. (see section XI).

Brucella: Collect blood, bone marrow, or deep abscess material. Culture is performed as a **Blood Culture** or a **Routine Culture**, as appropriate, but must be specially handled.

Gonorrhea (Thayer-Martin) Culture: Specimen Collection: genital, urethral, rectal, or throat swabs. Use a Jembec plate (warmed to room temperature), swab the plate, add the CO₂ generating tablet, and seal the plate in the special zip lock bag provided.

Legionella Culture: Collect pleural fluid, lung tissue, bronchoscopy material, or sputum. See also LEGIONELLA, URINE ANTIGEN.

MRSA Colonization (Methicillin-Resistant *Staphylococcus aureus*): Collect anterior nares, nasopharyngeal, or other specimens. Reported only as the presence or absence of Methicillin-resistant *Staphylococcus aureus*.

STGB Colonization (Streptococcus Group B): Collect vaginal and/or rectal specimen using Culturette I or II. Reported only as the presence or absence of STGB. CDC guidelines (MMWR 2002:51; RR-11) specify that all pregnant females be screened by culture for vaginal and/or rectal colonization with *Streptococcus* Group B (STGB). Specimens for screening culture should be collected at 35-37 weeks gestation. Appropriate specimens are vaginal and rectal swabs. Both should be collected using a single swab by inserting into vagina then inserting into rectum.

Vibrio: Collect stool, blood, or wound material.

VRE Colonization (Vancomycin Resistant Enterococcus): Collect a rectal swab. Reported as the presence or absence of VRE.

VII. Mycology-Fungus

Notify Microbiology if *Histoplasma* or *Coccidioides* are suspected.

Abscess: Aspirate material into a sterile syringe, cap, and transport to the Laboratory as soon as possible. If aspiration is impossible, collect with a Culturette II.

Blood: Collect using DuPont isolator system (lysis centrifugation) - obtain from microbiology. Obtain one specimen per day for three days. A routine blood culture should be ordered concurrently to rule out bacterial pathogens.

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Bone Marrow: Aseptically collect bone marrow in a sterile syringe and deliver to the Laboratory immediately before the specimen clots. If this is impossible, transfer specimen to a sterile heparinized container.

Bronchial: Collect in a sterile screw-capped container. Seal tightly to avoid leakage. Deliver promptly to the Laboratory.

CSF: Collect in a sterile tube. At least 0.5 mL is necessary for adequate mycological study. Immediately deliver to the laboratory.

Fluids: Collect in a sterile container. Seal tightly to avoid leakage.

Hair: Collect several suspicious or fluorescent hairs with a sterile forceps and place in a sterile container.

Nails: Clean nails with 70% ethanol. Scrape from the proximal to the distal end of the nail. Discard the first four or five scrapings. Put subsequent scrapings and debris from under the nail plate into a sterile container.

Skin Scrapings: Clean lesion with 70% ethanol or sterile distilled water. Collect scrapings and any moist exudate in a sterile container.

Sputum: Collect 10-15 mL of first morning sputum in a wide-mouth sterile plastic cup with a screw cap. Seal tightly to avoid leakage. Patient should be instructed to brush teeth and rinse mouth prior to collection. Clearly label nebulized specimens as "induced".

Tissue: Put in a sterile container **without** preservatives and deliver to the Laboratory immediately to avoid dehydration.

Urine: Collect 25-50 mL of urine in a sterile container. Seal tightly to avoid leakage. Indicate if specimen is a suprapubic, catheterized, or clean catch urine.

Notes: Turnaround time: negative result requires 6 weeks. Definitive identification performed when applicable. Susceptibility testing only after consultation between the Microbiology Laboratory and the clinician.

VIII. Mycobacteria (TB Culture, AFB Culture)

Blood: Collect using Isolator tube (obtained from laboratory). Patient type restricted to severely immunocompromised or HIV infection. One specimen per day for three days is recommended.

Body Fluids (CSF, pleural, peritoneal, synovial, bone marrow, etc.): Collect aseptically using aspiration techniques or surgical procedures. Large amounts of specimen are preferable and should be submitted in sterile containers. Seal tightly to avoid leakage. *NOTE: Bone marrow biopsy specimens are preferable to bone marrow aspiration for the isolation of mycobacteria.*

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Bronchial Aspirates, Washings, Brushings, Transtracheal Aspirates, Bronchoalveolar Lavage: Collect in a sterile screw-capped plastic container. Seal tightly to avoid leakage. Deliver to the Laboratory immediately. Nucleic acid amplification for *M. tuberculosis* complex also available (see section XI).

Feces: SPECIAL REQUEST: Stool specimens for the culture of acid-fast organisms will be accepted from patients suspected of, or having HIV infection. Otherwise, stool is an unacceptable specimen. Submit in a sterile screw-capped container, in a sealed plastic bag. Label appropriately for this type of patient.

Gastric Lavage: Collect to obtain sputum from patients unable to cooperate with sputum induction (e.g., infants and children). Collect early in the morning before the patient eats or gets out of bed. Collect in a sterile screw-capped plastic container. Seal tightly to avoid leakage. Deliver to the Laboratory immediately. NOTE: When sputum induction is followed in 30 minutes by gastric lavage, the combined results yield more positive cultures than either method alone.

Sputum: Submit three early morning specimens with minimal amounts of oral or nasal secretions. A maximum of 3 specimens collected on separate days will be accepted. Collect in a sterile screw-capped plastic container. Seal tightly to avoid leakage. NOTE: *Patient should be instructed to rinse mouth with water prior to sputum collection.* Nucleic acid amplification for *M. tuberculosis* complex also available (see section XI).

Aerosol Induced Sputum: Clearly label specimen as "induced" since this type of specimen resembles saliva.

Swab Specimens: Material collected on swabs is not optimal for the recovery of acid-fast organisms. Swabs will be accepted if there is no other means of collection. Dry swabs will not be accepted.

Tissue and Biopsy: Collect aseptically and place in sterile container without fixatives or preservatives. Deliver immediately to the Laboratory for prompt processing. If delivery must be delayed, place specimen in sterile water or saline (enough to cover) to prevent dehydration and refrigerate.

Urine: Wash the external genitalia prior to collection. Collect an early morning midstream urine of approximately 100 mL in a sterile screw-capped container. Seal tightly to avoid leakage. A maximum of 3 specimens obtained on separate days will be accepted. Catheter and suprapubic urines should be clearly labeled as such.

Note: Turnaround time - 8 weeks (negative result). Definitive identification and susceptibility testing performed when applicable.

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IX. Parasitology

Blood (for malarial and other parasite): Collect 3 mL in EDTA tube. Fingerstick is also acceptable. Collect between fever spikes for malaria, at fever spike for *Leishmania/Trypanosoma*, and at noon and midnight for microfilariae.

Stool: Collect 5 mL in clean container. Add entire 5 mL of stool to Alpha-Tec ProtoFix™ CLR vial (white cap). One specimen/day for 3 days is recommended.

X. Virology

Specimens for virus culture are processed daily (Monday-Saturday).

Virus Culture Instructions:

- A. Consult virus isolation guides (charts on following pages) to determine the type(s) of specimen(s) to be collected.
- B. Determine the appropriate specimen transport system by consulting the chart (on following pages).
- C. Label the specimen transport system (patient name, specimen source, and date/time collected).
- D. Check Section IV. Specimen Collection for Virus Isolates for specific instructions.
- E. Keep the specimen on wet ice or refrigerated, until transported to the Microbiology or Virology Laboratory. **Do not freeze specimens.** (*Exception is peripheral blood, which is kept at room temperature.*)
- F. For virus cultures, isolation is optimal when specimens are collected early in the course of illness.

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Virus Isolation Guides

VIRAL RESPIRATORY DISEASE

DISEASE	SPECIMENS	VIRUS ASSOCIATED
1. Influenza	Nasal wash or swab Throat wash or swab	Influenza A, B Influenza A, B
2. Acute respiratory disease Syndromes: rhinitis, pharyngitis, laryngitis, laryngotracheobronchitis (croup), tracheobronchitis, bronchiolitis, viral pneumonia	Nasal wash or swab Throat wash or swab	Influenza A, B Adenoviruses Respiratory syncytial virus, Parainfluenza viruses, Coxsackie viruses A & B ³ , Echoviruses ³ , Herpes simplex virus ³ , Coronaviruses ^{1,3} , Rhinoviruses, Epstein-Barr Virus ^{1,2}
3. Misc. respiratory involvement: Herpangina virus; Stomatitis	Throat wash or swab, stool (Enteroviruses)	Coxsackie Viruses Echoviruses, Herpes simplex virus

NOTES

¹ *Viral isolation not performed*

² *Serodiagnosis available*

³ *Less common*

VIRAL DERMATOLOGIC DISEASE

DISEASE	SPECIMENS	VIRUS ASSOCIATED
1. Chickenpox (varicella)	Vesicle fluid, lesion scrapings ¹	Varicella zoster virus
2. Shingles (zoster)	Vesicle fluid, lesion scrapings ¹	Varicella zoster virus
3. Oral herpes simplex	Vesicle fluid, throat or mouth swab	Herpes simplex virus
4. Genital herpes simplex	Vesicle fluid, vaginal, urethral, cervical or rectal swab	Herpes simplex virus
5. Rubella (German Measles) ²	N/A	Rubella virus ²
6. Measles	Blood, urine, throat swab or wash, nasopharyngeal swab, conjunctival swab	Measles virus
7. Vaccinia	Blood (pre-eruptive), vesicle/pustule dome, vesicle fluid, crusts, throat wash or swab	Vaccinia virus
8. Miscellaneous	Throat washings or swab, stool, vesicle fluid	Echoviruses, Coxsackie viruses, Herpes simplex virus

NOTES:

¹ *Direct immunofluorescence for VZV on lesion preferred test for diagnosis of Varicella zoster infections.*

² *Viral isolation not performed; diagnosis can be established by serodiagnosis. Neonatal anti-rubella virus IgM levels can be determined on cord blood for rapid diagnosis.*

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VIRAL CENTRAL NERVOUS SYSTEM DISEASE

DISEASE	SPECIMENS	VIRUS ASSOCIATED
1. Encephalitis (meningoencephalitis)	CSF, acute phase blood (arboviruses), urine (Mumps virus), brain biopsy (herpes simplex viruses), throat wash or swab	Mumps, Herpes simplex ³ , Arboviruses ¹ , Measles, Rubella ² , Rabies, Vaccinia (post-vaccination), Epstein-Barr ² , Cytomegalovirus
2. Aseptic meningitis	CSF, stool or rectal swab, throat wash or swab, urine (Mumps virus)	Coxsackie ³ , Echoviruses ³ Less common: Mumps, Herpes simplex ³ , Varicella zoster, Polio, Lymphocytic choriomeningitis, Epstein-Barr ² , Arboviruses ¹
3. Paralysis	CSF, stool/rectal swab, throat wash/swab	Polioviruses, Coxsackie viruses, Echoviruses

NOTES:

¹ Attempts to isolate arboviruses generally have not been successful. Serodiagnosis is available for arboviruses common to this geographic area. Consult the Virology Laboratory (449-5238)

² Serodiagnosis is available; consult the Serology Laboratory (449-4956)

³ PCR is the method of choice (see section XI).

PERINATAL VIRAL DISEASE

DISEASE	SPECIMENS	VIRUS ASSOCIATED
1. Rubella (German Measles) ¹	N/A	Rubella virus ¹
2. Cytomegalic inclusion disease, congenital or perinatal CMV infection	Blood, urine, throat swab, maternal cervical or vaginal swab	Cytomegalovirus ³
3. Herpes neonatorum ²	Vesicle fluid, CSF, throat, urine, conjunctival swab, blood, maternal cervical swab	Herpes simplex virus ³

NOTES:

¹ Viral isolation is not performed; diagnosis can be established by serodiagnosis. Neonatal anti-rubella IgM levels can be determined in cord blood for rapid diagnosis.

² For rapid diagnosis, direct immunofluorescence for HSV can be performed on lesion scrapings except for eyes & genitalia.

³ PCR also available (see section XI, part C).

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VIRAL OCULAR DISEASE

DISEASE	SPECIMENS	VIRUS ASSOCIATED
1.Primary or recurrent Herpes simplex	Conjunctival swab, corneal scrapings	Herpes simplex virus
2.Zoster ophthalmitis	Conjunctival swab, corneal scrapings	Varicella zoster virus
3.Pharyngeal conjunctival fever, keratoconjunctivitis	Conjunctival swab, corneal scrapings, throat wash or swab, stool	Adenoviruses, Echoviruses

VIRAL GASTROINTESTINAL DISEASE

DISEASE	SPECIMENS	VIRUS ASSOCIATED
Acute gastroenteritis	Stool, rectal swab	Rotavirus ¹ , Coxsackie viruses, Echoviruses, Norwalk-like agent ² , Enteric adenoviruses ² , Caliciviruses ²

NOTES:

¹ Rotaviruses do not grow in conventional culture systems; see ROTAVIRUS, ANTIGEN in the alphabetic list in this manual.

² No culture system is available for these agents.

SYSTEMIC AND MISCELLANEOUS VIRAL DISEASE

DISEASE	SPECIMENS	VIRUS ASSOCIATED
1.Mumps ¹	Saliva, throat swab (Stenson duct), urine, CSF	Mumps virus
2.Hepatitis ²	N/A	Hepatitis A, B, C, and D viruses
3.Myocarditis	Throat wash or swab, stool, blood	Coxsackie viruses ³ , Echoviruses ³
4.Pericarditis	Throat wash or swab, stool pericardial fluid, blood	Coxsackie viruses ³ , Echoviruses ³
5.Pleurodynia	Throat wash or swab, stool, blood	Coxsackie viruses ³ , Echoviruses ³

NOTES:

¹ See above for list of specimens when CNS is involved

² No culture systems are available for hepatitis viruses. Serological assays for all viruses are available through the Serology Laboratory (449-4956). Refer to specific viral serologic test for specimen collection instructions.

³ PCR also available (see section XI, part D).

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Specimen Collection Systems ^{1,2}

SYSTEM	CONTAINER	SPECIMEN
Sterile specimen Container	Sterile plastic cup with screw cap	Stool, urine, large biopsy tissue
Virus Transport Media	Sterile screw cap tube containing transport medium for virus and chlamydia	All swab specimens, biopsy tissue, nasal, nasopharyngeal, & throat washes, vesicle fluid
Sterile CSF Tube	Sterile screw cap tube	CSF
Chlamydia DNA PCR and/or <i>Neisseria gonorrhoeae</i> DNA PCR kit	Swabs with transport tube in a kit form; Sterile urine container	Endocervical and urethral swabs <20 mL first-voided urine

NOTES:

1 Contact Supply department for specimen containers.

2 All specimen containers should be clearly identified (patient name, address/ward, specimen type, date obtained, and physician).

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Specimen Collection For Virus Isolation

A. Throat Swab

1. Vigorously swab pharynx, tonsils, and facial pillars with sterile dacron swabs - two swabs per site. *NOTE: For mumps virus, also swab area around the Stensen duct orifices.*
2. Place specimen into transport medium: rapidly rotate swab in the transport medium several times and break off the swab depositing the swab tips in the medium
3. Cap tightly, label, and place inside a transport bag.
4. Place specimen in a water/crushed ice (wet ice) bath or at 4°C.
5. Deliver to the Laboratory on wet ice.

B. Nasal Swab

1. Pass wire dacron nasopharyngeal swab into nostril parallel with the palate and gently rotate.
2. Place swab into transport medium. Can cut wire shaft with scissors before capping tightly.
3. Proceed as in A3-A5 (above)

C. Nasal Wash (Adult)

1. Instill 5 mL of sterile saline into each nostril while patient sits with head tipped back and closes the airway by beginning to say "car".
2. Tip head forward collecting fluid in a screw cap plastic cup. Add the contents of a tube of Virus transport medium to the cup. Label as "Virus Transport Media Added".
3. Proceed as A3-A5 above.

D. Nasopharyngeal Wash (Child)

1. Wipe away excess rhinorrhea from outside the nose.
2. Gently rub the mucosal surface of the nares with a dacron thin-wire swab. Place swab in virus transport media and break or cut off excess wire shaft.
3. Using 3-4 inches of tubing (broken off from a butterfly infusion set) and syringe, insert between 1-2 mL saline into nose, draw out into syringe and expel the washings into a tube of Virus transport medium. *Note: The washings should contain congestive material.*
4. Proceed as in A3-A5 above.

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E. Throat Wash

1. Patient should gargle with 2-3 ml sterile saline and deposit wash in sterile screw cap plastic cup. Add the contents of a tube of Virus transport medium to the cup. Label as such.
2. *Note: The washings should contain congestive material.*
3. Proceed as in A3-A5 above.

F. Conjunctival Swab

1. Swab lower conjunctival sac of the eye five times with a sterile premoistened sterile dacron swab.
2. Proceed as in A2-A5 above.

G. Corneal Scrapings

1. Remove suspect corneal epithelial cells with a sterile dacron swab and place in a tube of Virus transport medium.
2. Proceed as in A3-A5 above.

H. Urine (Excluding Chlamydia/Gonorrhea PCR Specimens - *See below*)

1. Collect clean voided midstream sample (10-20 mL) in a sterile container. Bagged or catheterized urine is acceptable for infants/young children.
2. Proceed as in A3-A5 above.

I. Rectal Swab

1. Insert a sterile dacron swab into the rectum and gently rotate.
2. Proceed as in A3-A5 above.

J. Cerebrospinal Fluid (CSF)

1. Collect CSF aseptically and place in a sterile screw-cap tube.
2. Proceed as in A3-A5 above.

K. Biopsy Specimens

1. Collect fresh specimen under sterile conditions. Separate instruments should be used for each specimen.
2. If the specimen is small, place it in a tube of Virus transport medium.

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3. If the specimen is large, place it in a sterile container and add the contents of a tube of Virus transport medium to the container with the specimen.
4. Proceed as in A3-A5 above.

L. Cervical Swab

1. Using a speculum to visualize the cervix, uterus - swirl a sterile dacron swab firmly on the external os of the cervical canal.
2. Proceed as in A2-A5 above.

M. Vaginal Swab

1. Insert a sterile dacron swab in the vagina until resistance is encountered, and swirl in place.
2. Proceed as in A2-A5 above.

N. Vesicle Fluid

1. Collect vesicle fluid on a dacron swab after un-roofing the vesicle with a sterile needle. If desired, use a separate swab and rub vigorously at the base of the lesion.
2. Proceed as in A2-A5 above.
3. Alternatively, using a 26 g or 27 g needle fitted to a tuberculin syringe, aspirate fluid from the vesicle after rinsing the vesicle surface with sterile saline.
4. Place the fluid in a tube of Virus transport medium. Rinse the syringe several times with transport medium to remove all vesicle fluid inside the syringe.

O. Cell Scrapings for VZV or HSV Direct Immunofluorescence

1. Eye and genital specimens are not acceptable.
2. Clean the outer surface of the vesicle carefully with an alcohol wipe. Allow to dry.
3. Un-roof the vesicle with a sterile needle. Vesicular fluid should be collected for a back up virus culture, as described above.
4. Collect cells at the base of the lesion by rubbing vigorously with a sterile swab, or by scraping gently with a scalpel.
5. Rub the swab or scalpel scrapings evenly over each of 3 clearly separated areas on a clean microscope slide. The three spots should each be approximately 1 cm in diameter. Three well slides are available in Virology.

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6. DO NOT FIX THE SLIDE WITH CYTOLOGY SPRAY FIXATIVE
7. Allow the slide to dry. Place the slide in a cardboard holder and transport the slide immediately to the Microbiology Laboratory.

P. Stool

1. Place 4-8 g of fecal material in a screw-cap plastic cup.
2. Proceed as in A3-A5 above.

Q. Peripheral Blood Leukocytes

1. Collect 5-10 mL of blood in a green top Vacutainer tube (sodium heparin).
2. Label and place inside a transport bag.
3. Deliver to the laboratory at room temperature within 24 hours of collection.

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Specimen Collection for Chlamydia Cultures

A. ACCEPTABLE SPECIMENS

DISEASE	SPECIMEN
Urethritis	urethral swab
Cervicitis	endocervical swab
Conjunctivitis	conjunctival swab
Trachoma	conjunctival swab
Infant pneumonitis	nasopharyngeal (infants), throat and nasopharyngeal (young children)
Lymphogranuloma venereum ¹	bubo aspirate
Fitz-Hugh-Curtis syndrome (perihepatitis)	peritoneal fluid, endocervical swab
Pelvic inflammatory disease (PID)	peritoneal fluid, tissue, endocervical swab
Psittacosis ¹	sputum, lung tissue
Pneumonitis, upper respiratory tract infections of adults and older children ¹	throat or nasopharyngeal swab, endotracheal aspirate or wash, bronchoalveolar lavage fluid, lung tissue
Sexual abuse (prepubescent female)	vaginal, rectal, throat, urethral swab
Sexual abuse (male)	Rectal, throat, urethral swab

¹ Serum for acute and convalescent titers should be collected for diagnosis of lymphogranuloma venereum, psittacosis, and *Chlamydia pneumoniae* infections.

B. UNACCEPTABLE SPECIMENS

- Saliva
- Semen
- Urine
- Blood
- Vaginal specimens (except for prepubescent females or women with previous history of a hysterectomy)

C. SPECIMEN COLLECTION

1. Collect swab specimens using a dacron-tipped plastic shafted swab. Do not use wooden shaft swabs or calgiswabs, as they are toxic to the *Chlamydia*.
2. After collection, place the swab in a thawed tube of transport media, cut the plastic shaft leaving the swab in the tube, and recap the tube.

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3. Other specimens, such as tissue and fluids can be added directly to a tube of transport media.
4. Place the specimen in a water/crushed ice (wet ice) bath or at 4°C.
5. Transport to the laboratory on wet ice.

XI. Molecular Infectious Disease Testing

A. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* PCR

1. Use only the Microtest M4-RT media and swab kit. Calcium alginate, dacron-tipped swabs are not acceptable. The instructions below apply to both *Chlamydia trachomatis* and *Neisseria gonorrhoeae* detection, with the exception of urine specimens.
2. **Urethral Specimens (Males)**
 - a. The patient should not have urinated for at least 1 hour prior to sampling.
 - b. Insert the small-tipped swab into the urethra and rotate 3-5 seconds.
 - c. Immediately withdraw swab. Insert swab into Microtest M4-RT transport tube and break at score line.
 - d. Replace cap and securely tighten until cap clicks into place.
 - e. Refrigerate and transport as soon as possible to the laboratory. Refrigerated specimens can be tested up to seven days after collection.
3. **Endocervical Specimens (Females)**
 - a. Remove excess mucus from the exocervix with a large swab. **DISCARD**. Do **NOT** place the large swab into the transport tube.
 - b. Insert the small-tipped swab into the endocervix and rotate for 15-30 seconds. Avoid touching the vaginal walls when inserting and removing the swab.
 - c. Insert swab into the Microtest M4-RT transport tube and break at score line.
 - d. Replace cap and securely tighten until cap clicks into place.
 - e. Refrigerate and transport as soon as possible to the laboratory. Refrigerated specimens can be tested up to seven days after collection.
4. **Urine Specimens (Males and Females):**

Note: *Neisseria gonorrhoeae* PCR testing on female urine specimens is not FDA approved. However this test was independently validated in our lab. Its sensitivity is not optimal and endocervical specimens are preferred.

 - a. The patient should not have urinated for at least 1 hour prior to sampling.

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- b. Collect 15-20 mL of **first** voided urine in a sterile urine specimen cup (there is no need to cleanse the urethral meatus prior to collection). Do **NOT** put the urine in the transport tube.
- c. *Note: This specimen cannot be used for urine culture or urinalysis.*
- d. Refrigerate urine specimen within 1 hour.
- e. Transport refrigerated to the laboratory as soon as possible. Refrigerated specimens can be tested up to seven days after collection.

Note: PCR testing is not acceptable when a legally valid (as opposed to diagnostically valid) result is required, such as in cases of suspected sexual assault or abuse. Culture is the only legally acceptable test for such situations.

B. *Bordetella pertussis* & *parapertussis* PCR

- 1. Nasopharyngeal swab specimen or aspirate.
- 2. Use Dacron Wire swab or green capped culturette.
- 3. Insert swab into posterior nasopharynx and hold for 15-30 seconds. Remove.
- 4. Place swab or aspirate into M4, Stuart's or Amies transport media.
- 5. Transport refrigerated to the laboratory as soon as possible.
- 6. *B. pertussis* & *B. parapertussis* are detected simultaneously and are differentiated.

C. Herpes simplex virus type 1 and 2 PCR

- 1. Blood – collect whole blood 7.0 mL RED-CLOT or 5 mL EDTA
- 2. CSF – 1 mL
- 3. Lesion – collect using dacron swab. Place in viral transport medium
- 4. Respiratory – 1 mL BAL
- 5. Other – tissue, body fluid
- 6. Transport refrigerated to the laboratory as soon as possible
- 7. HSV 1 and HSV 2 are detected simultaneously and are differentiated

D. Enterovirus PCR

- 1. Blood – collect whole blood 7.0 mL RED-CLOT or 5 mL EDTA
- 2. CSF – 1 mL
- 3. Transport refrigerated to the laboratory as soon as possible.

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4. Detects coxsackie viruses, echoviruses, and other enteroviruses.
- E. *Mycobacterium tuberculosis* Complex Amplification**
1. Respiratory specimens – sputum, BAL, etc.
 2. Complex includes *M. tuberculosis*, *M. africanum*, *M. bovis*. Test detects all three but does not differentiate among them.