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St. Mary's Hospital Laboratory Services Manual has been reviewed and approved by:

Paul Holzman, MD.
Laboratory Medical Director

HOW TO USE THIS REFERENCE MANUAL

General information: Information on the laboratory, test requests, billing, and other customer services is found in the beginning of the manual

Specimen collection procedures: General specimen collection procedures follow the general information section. While specific tests may require special collection described in the alphabetical index, the general collection chapter provides basic information based on body site.

Virus and Chlamydia collection: Three specific tables have been included at the end of the specimen collection and handling chapter.

Alphabetical Test Index: A full list of tests and cross references are listed alphabetically. Numbers within the title of the test are ignored in respect to alphabetizing. Bacterial cultures are listed under a culture rather than by organism or body site. Some virus tests have been listed twice, both in the culture area and by the specific virus. Information normally included in an appendix has been placed in the main body of the text for convenience.

Appendix: The appendix contains full copies of forms and a brief CDC summation of PPD 2-step testing and CBC normals by age which were too cumbersome for the alphabetical listing.

GENERAL INFORMATION

Telephone Numbers

St. Mary's Hospital Laboratory personnel are available 24 hours per day, seven days per week to answer inquiries from physicians and clinic personnel.

Specific questions regarding tests may be directed to the department listed below (area code 920):

Laboratory – Clinical 498-4679

Lab Director – Jeff Shadick 884-4773

Lab Supervisor- Renee Pelch 498-4683

LIS Analyst – Peggie Kruswick 498-4680

Bacteriology 498-4675

Blood Bank 498-4672

Laboratory – Anatomic Pathology

Director – Jeff Shadick 884-4773

Pathologist Assistant – Juli Prue 884-3801

Pathologist – Dr. Cutlan 884-4770

Pathologist – Dr. Holzman 884-4770

Pathologist – Dr. Murphy 884-4770

Cytology 498-4664

Histology 884-4770

Pathology Reports 884-4770

Lab Registration 884-4768

If you need to speak to someone at there is no answer at the direct telephone number above, please call the general laboratory for assistance at 498-4679.

General Laboratory Fax Number 498-4751

Pathology/Histology/Cytology Fax Number 884-3566

Referral Testing

Specimens submitted for tests that are not performed in-house will be referred to another accredited reference laboratory. When possible, that laboratory will be listed in the test schedule.

For our customer's convenience, we have listed our primary reference laboratories, their telephone and CLIA numbers.

Quest Diagnostics – Nichols Institute

CLIA #05D0643352

1-800-553-5445

www.questdiagnostic.com

Mayo Laboratories

CLIA #24D0404292

1-800-826-5561

www.mayomedicallaboratories.com

Bellin Hospital Laboratory

CLIA #52D0662050

1-920-433-3650

www.bellin.org

WI State Lab of Hygiene

CLIA #52D0669558

1-800-442-4618

www.slh.wisc.edu

St. Vincent Laboratory

CLIA #52D0689713

1-920-433-8228

www.stvincenthospital.org

Courier Service

A courier service is available through a local courier provider. Specific arrangements for pick-up times and sites can be made through the laboratory (920-498-4679).

Test Request Forms

Each specimen submitted for testing must be listed on a test request form. Forms are available through the laboratory office.

To prevent delays and errors, the forms should be typed or neatly printed. Please indicate the patient's name, date of birth, ordering physician, and test(s) on the test request form. **Medicare/Medicaid patient tests** must be written on a MA & Medicare form. St. Mary's will bill Medicare directly. The following information must be submitted with Medicare patient specimens: Patient's complete name, address, sex, date of birth, patient's Medicare (Medicaid) number, ICD9 code and referring physicians name.

The laboratory will not be able to process specimens that lack ICD9 codes.

Test Additions

Physicians may arrange to have additional testing if sufficient specimen volume remains after the initial tests are completed. To request additional tests, please call the specific department performing the test. Verbal orders must be followed with written orders. These orders should indicate that the specimen is already in the laboratory.

Test Cancellations

Tests can be canceled prior to the initiation of the assay/test without a charge. Cancellations should be called directly to the department performing the test.

Billing

Client invoices will be sent on a monthly basis for procedures performed during the previous month. The bill will include a list of tests performed on all but Medicare/Medicaid patients.

If an invoice is in question, please contact the LIS analyst at 498-4680.

CPT Codes (Current Procedural Terminology) listed with each test description and in the index are provided as a guide to assist you with billing. The CPT codes describe the services ordered and performed, and are subject to change at any time. It is the clinic's responsibility to verify the accuracy of the codes submitted. References for the current CPT Coding include the CPT Coding manual published by the American Medical Association or your local Medicare carrier.

Reporting

Test results are reported immediately upon completion of the tests.

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On special request, results may be called to a physician. STAT tests and critical abnormalities are telephoned or faxed as soon as the results are available.

Special Requests

As a part of our quality assurance program, tests will be repeated when a physician feels that the results are not consistent with the clinical presentation. For most assays, specimens will be retained for at one week.

Special studies and projects can be arranged with the laboratory. Please feel free to contact laboratory personnel if you have any requests.

Laboratory Medicare Compliance Statement

The US federal government has a zero tolerance policy towards fraud and abuse and will use its authority to reduce fraud in Medicare and other federally funded health care programs. St. Mary's Hospital Laboratory had taken steps to help their clients be in compliance. Request forms include areas for symptoms and diagnosis (ICD codes) and documentation check off box for Advanced Beneficiary Notice (ABN).

The Office of Inspector General of the Department of Health and Human Services recognized the physicians must be able to order any test, including screening tests that they believe are appropriate for the treatment of their patients.

A Local Medical Review Policy (LMRP) defines Medicare approved use of a test by indicating what clinical circumstances justify the use of the test. These policies contain ICD diagnosis codes the Medicare Carrier of Fiscal Intermediary will accept for payment. Everything else is considered not reasonable and necessary and claims will be rejected as not medically necessary. If the test does not meet the requirements for medical necessity, the patient, prior to testing must sign an Advanced Beneficiary Notice (ABN). The ABN is to be kept at the facility drawing the test and appropriate box should be checked on the requisition form.

SPECIMEN COLLECTION AND HANDLING

Specimen collection is the foundation upon which all other laboratory procedures are built. Collecting the correct specimen by an appropriate method at the proper time during infection or clinical syndrome significantly enhances isolation frequency and improves the clinical relevance of the laboratory results. This section contains general guidelines for specimen collection. Special instructions for specific tests are provided in the alphabetical test list.

Temperature

Unless otherwise indicated in the alphabetic test list, specimens should be stored at refrigerated temperature. When a test requires that a serum be frozen, the serum should be separated from the red blood cells and frozen as soon as possible.

Needles

Specimens must not be sent to the laboratory with needles.

St. Mary's Laboratory is an approved needle drop off site, provided the needles are contained in an approved hard plastic container with cover.

St. Mary's Laboratory is not an approved medication disposal site.

Acute/Convalescent Testing

Acute phase serum specimens are tested and reported as received unless they are referred to another laboratory.

Convalescent phase serum should be drawn and tested 2-4 weeks following the acute phase.

Specimen Labeling

All specimens submitted to the lab must be labeled in the presence of the patient using 2 identifiers. The label must include the patient's complete name and the date of birth.

The label should also include the type of specimen, date, and time of collection along with the initials of the person collecting the specimen.

The information on the specimen label must match that on the test request form.

Slide Labeling

Please label microscope slide with a pencil. Ink pens, markers, and printed labels come off during fixation.

Patient Demographics: Birth date/Sex

Please indicate the patient age and sex on the test request form. This will help the laboratory assign normal values specific for each patient. **For additional verification of patient identity please include social security number.**

Serum, Plasma or Blood

Draw blood in the color-coded Vacutainer tube indicated in the alphabetical test list. For serum or plasma, draw approximately 2 ½ times of whole blood to yield the specimen requirements. Serum specimens should be allowed to clot at least 30 minutes before separated by centrifugation. Plasma and whole blood collection should completely fill the Vacutainer whenever possible to eliminate dilution from the anticoagulant or preservation and will be immediately mixed by gently inverting the tube 8-10 times. Separate plasma by centrifugation. Transfer the serum or plasma to plastic transport tubes.

It is recommended that a patient fast 8-12 hours before having blood drawn. This decreases the lipemia of the serum which may interfere with test results. Hemolysis may also alter test results and will be noted on a test report.

Safety Concerns:

- Specimens must not be sent with needles.
- No glass transfer tubes.
- No glass Vacutainer tubes.

Vacutainer Tube Contents

Tube top colors may vary with manufacturer.

Blue: Buffered sodium citrate. 2.7 ml must be added to the tube.

Light Blue: Buffered sodium citrate. 1.8 ml must be added to the tube.

Gray: Sodium fluoride and Potassium oxalate

Green: Lithium heparin

Dark Green: Sodium heparin

Lavender: EDTA (K3) liquid

Red: No additive

Red/Gray: SST – serum separator tube

Yellow: Serum separator tube

Mint: Lithium Heparin- plasma separator tube

Order of Draw

Clear Top Tube – No additive, used as a clearing tube before light blue tube

Blue Top Tube – Buffered Sodium Citrate 2.7 mL draw
or

Light Blue Top Tube - Buffered Sodium Citrate 1.8 mL draw

Note: The Buffered Sodium Citrate tubes must be filled to the line.

Under/Over filled tubes will not be accepted.

Red – no additives 6 mL draw

Green – Lithium Heparin 3 mL draw

Lavender - Potassium EDTA (liquid) 3mL draw

Gray – Sodium fluoride and potassium oxalate 2 mL draw

SPECIAL REAGENTS AND SUPPLIES

Swabs

Swab material should be made of sterile cotton, rayon, or Dacron with a wooden shaft. **DO NOT USE CALCIUM ALGINATE SWABS FOR RAPID BACTERIAL, VIRAL, OR CHLAMYDIAL DETECTIONS.** Calcium alginate swabs are toxic for Chlamydia and many enveloped viruses.

Specimens collected with calcium alginate swabs cannot be used for direct fluorescent antibody or membrane EIA testing. Wooden-shafted swabs are not recommended because wooden swabs can contain toxins and formaldehyde, which can inhibit the recovery of viruses and Chlamydia.

In addition, wooden swabs absorb liquid transport media thereby reducing the amount of fluid for culture inoculation.

Transport Medium

Transport media available from St. Mary's Laboratory is Universal Transport Media (called UTM and can be used for ALL viruses). Chlamydia/GC PCR uses the M4RT which is provided in a specialized kit also available from St. Mary's Laboratory.

Blood and body fluids for viral culture should be submitted in sterile tubes without transport media. All other specimens for ALL viruses can be placed in the UTM media as soon as possible after collection.

Pertussis swabs should be collected with a sterile NP swab and placed in a sterile tube for PCR testing.

Chlamydia PCR/GC DNA Probe Swabs

Chlamydia PCR and GC DNA probe tests must be collected using specifically designated collection kits. A small swab is provided in the male collection kit to obtain urethral specimens

A larger swab is provided in the female kit for cervical specimens. To optimize sensitivity, the endocervix should be cleaned first with a cotton swab to remove cervical mucous. Vaginal walls should be avoided if possible.

These collection kits cannot be used for cultures.

The same swab is used for both the Chlamydia PCR and GC DNA tests. Once the swab is placed in the transport tube, it should be broken off, left in the tube, and the tube securely sealed.

SPECIMEN COLLECTION PROCEDURES

Please note: Unless otherwise indicated, viral specimens should be placed in viral transport media and Chlamydia specimens should be placed in Chlamydia transport media. Bacterial and fungal cultures should be placed in an aerobic culturette and the ampule must be broken.

Blood for Virus Isolation

1. Collect 3 ml of whole blood in an EDTA (lavender top) tube.
2. **DO NOT ADD VIRAL TRANSPORT MEDIA.** Blood specimens contain sufficient protein to stabilize most viruses.
3. Send specimens to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 12 hours at 2-8°C. Refrigeration is critical for body fluids because the viruses isolated from blood are often extremely labile.

Blood for Bacterial Isolation

1. **Adults:** Collect 8-10 ml of whole blood for each of 2 BacTec bottle, one PLUS AEROBIC/F (blue capped bottles) and on Lytic/10 ANAEROBIC/F (purple capped bottles)
Pediatrics: Collect 1-3 ml of whole blood for one PEDS PLUS BacTec bottle (pink capped bottle).
2. Remove cap from blood culture bottle.
3. Disinfect rubber stopper with an alcohol pad.
4. Prepare the draw site on the patient by:
 - A. Vigorously scrub site for 15 seconds with Chloraprep pad. Let air dry for 30 seconds.
 - B. Repeat previous step with fresh disinfectant.
 - C. **DO NOT TOUCH** after cleansing.
5. Draw blood using a syringe.
6. Inject blood in each culture bottle by puncturing the rubber stopper with the needle.
Note: Inject the anaerobic bottle first to avoid introducing air from the syringe.
7. **Store bottles at room temperature until transported to St. Mary's laboratory.**
8. Bottles will be forwarded on to St. Vincent Laboratory for testing.

Blood for Fungal Isolation

1. Collect 5 ml of whole blood into a Bactec Myco/F Lytic culture bottle. Culture bottles available from St. Mary's laboratory (498-4679).
2. Remove cap from culture bottle and disinfect rubber stopper with an alcohol pad.
3. Prepare the site on the patient by:
 - A. Vigorously scrub site for 15 seconds with ChloroPrep pad. Let air dry for 30 Seconds.
 - B. Repeat previous step with fresh disinfectant.
 - C. **DO NOT TOUCH** after cleansing.
4. Draw blood by syringe.
5. Inject in culture bottle by puncturing rubber stopper with a new needle or transfer device.
6. Store bottles at room temperature, until transported to St. Mary's Laboratory
7. Bottles will be forwarded on to St. Vincent Laboratory for testing.

Blood for Mycobacterial Isolation

8. Collect 5 ml of whole blood into a Bactec Myco/F Lytic culture bottle. Culture Bottles available from the Microbiology department (498-4679).
9. Remove cap from culture bottle and disinfect rubber stopper with an alcohol pad.
10. Prepare the site on the patient by:
 - D. Vigorously scrub site for 15 seconds with ChloroPrep pad. Let air dry for 30 Seconds.
 - E. Repeat previous step with fresh disinfectant.
 - F. **DO NOT TOUCH** after cleansing.
11. Draw blood by syringe.
12. Inject in culture bottle by puncturing rubber stopper with a new needle or transfer device.
13. Store bottles at room temperature, until transported to St. Mary's Laboratory
14. Bottles will be forwarded on to St. Vincent Laboratory for testing.

Blood (peripheral) for Flow Cytometry

1. Collect 5.0 ml of peripheral blood in EDTA (lavender top), sodium or lithium heparin (green) tubes.

Bone Marrow Aspirates

1. Virus isolation: Submit bone marrow specimens in Heparin (green top) or EDTA (lavender top) Vacutainer tubes to prevent clotting. **Mix well and DO NOT ADD TRANSPORT MEDIA.**
2. Bacterial, fungal or mycobacterial isolation: Submit bone marrow specimen in an appropriate Bactec Blood culture bottle or if bottles are available Submit bone marrow specimens in a sterile tube.
3. Chromosome studies or flow cytometry: Collect 2.0 ml in a heparin syringe and transfer to a green top tube.

Body Fluids for Culture

1. Collect 3-5 ml of CSF, pleural fluids, pericardial fluids, or other body fluids in sterile container.
2. Most body fluids contain enough protein to stabilize viruses, bacteria and fungi. **DO NOT ADD TRANSPORT MEDIA**
3. Send the specimens to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 24 hours at 2-8°C. If longer delays are anticipated, specimens should be frozen at -70°C. Refrigeration is critical for body fluids because the microorganisms isolated from body fluids are often extremely labile.

Body Fluid Counts

1. Collect a minimum of 1.0 ml of CSF, pleural fluid, peritoneal fluid, synovial fluid, or other body fluid in a sterile container.
2. A portion of synovial, pleural, pericardial fluid for counts must be put in an EDTA tube. CSF should be in a syringe or collection container.
3. **Do not refrigerate** fluids for counts. Bring to the laboratory as soon as possible so they may be analyzed fresh.

Coagulation Testing

General Drawing Instructions:

1. Citrated plasma is the only acceptable sample type. All other anticoagulants (heparin, EDTA, oxalate) are not acceptable.
2. Proper blood to anticoagulant ratio is required:
 - A. Vacutainer tubes must be filled to completion to ensure the proper 9:1 blood to anticoagulant ratio is achieved.
 - B. Routine collection requires 2.7 ml blood added to 0.3 ml sodium citrate.
3. To avoid contaminating the sample with tissue thromboplastin or heparin, follow the guidelines below:
 - A. The blood specimen should be collected using universal precautions by venipuncture or from an indwelling catheter. When performing the venipuncture, the coagulation blue top tube should be the second or third tube obtained. If no other tubes are requested, draw a non additive discard tube prior to the blue top tube.
 - B. Collection of blood for coagulation testing through intravenous lines that have been previously flushed with heparin should be avoided, if possible. If the blood must be drawn through an indwelling catheter, possible heparin contamination and specimen dilution should be considered. When obtaining specimens from indwelling lines that may contain heparin, the line should be flushed with 5 ml of saline, and the first 5 ml of blood or 6 times the line volume (dead space volume of the catheter) be drawn off and discarded before the coagulation tube is filled.
4. Mix the sample gently by inverting the tube several times immediately after filling. Do not shake the tube, as this will break down the fibrinogen in the sample.
5. Process the sample as soon as possible, within 60 minutes. Spin down the specimen at 3000 rpm for 10 minutes.
6. Transfer plasma into a plastic tube using a plastic Pasteur pipette.

Eye (Conjunctiva)

1. Moisten a fine dacron, rayon, or cotton swab with sterile physiologic saline.
2. Gently pull the lower eyelid downward and carefully swab the lower conjunctiva to collect both cells and fluids.
3. If both eyes are to be cultured, a separate sterile swab should be used for each eye.
4. Place the swabs in the appropriate (culturette, viral or chlamydial) transport vial and send the vial to the laboratory on wet ice. Specimens may be stored at 2-8°C for up to 48 hours. If longer delays are anticipated, specimens should be frozen at -70°C.

Nasopharyngeal Aspirate

1. Attach a sterile soft polyethylene #8 French feeding tube to a disposable aspiration trap.
2. Using the tube, measure the distance from the patient's nostril to their ear. Mark the distance on the tube using your thumb and forefinger.
3. Gently insert the tube into the nostril until the thumb and forefinger touch the patient's nose.
4. While applying intermittent suction, slowly remove the tube from the nasopharynx.
5. **Viral or chlamydial culture:** Place the end of the tube in a vial containing 2-3 ml of viral or chlamydial transport medium and aspirate the contents into the trap.
Bacterial or fungal culture: Do not add media to the trap.
6. Remove the feeding tube and carefully cap all the orifices on the aspiration trap.
7. Send the aspiration trap to the laboratory on wet ice. Specimens may be stored at 2-8°C for up to 24 hours. If longer delays are anticipated, specimens should be frozen at -70°C.

Nasopharyngeal Smear

1. A dry flexible type 1 aluminum-shafted, cotton-tipped wire swab should be used for this procedure. Separate swabs should be used when specimens are collected from both nostrils. Both swabs should be placed into the same viral transport tube.
2. Using the swab, measure the distance from the patient's nostril to their ear. Mark the distance on the swab using your thumb and forefinger.
3. Gently insert the swab into the nostril until the thumb and forefinger touch the patient's nose.
4. Hold the swab in place for 30-60 seconds then gently rotate the swab three times.
5. Rub the swab onto the wells of an acetone-cleaned slide making sure that the specimen is evenly dispersed over each well. Be sure to roll the swab so that the entire surface of the swab comes into contact with the slide.
6. Allow the slide to air dry.
7. Send the slide to the laboratory in a petri dish or in a slide mailer. Slides may be stored at room temperature or at 2-8°C for up to 48 hours before fixation.

Nasopharyngeal Swab

1. A dry flexible type 1 aluminum-shafted, cotton-tipped wire swab should be used for this procedure. Separate swabs should be used when specimens are collected from both nostrils. Both swabs should be placed into the same transport tube.
2. Using the swab, measure the distance from the patient's nostril to their ear. Mark the distance on the swab using your thumb and forefinger.
3. Gently insert the swab into the nostril until the thumb and forefinger touch the patient's nose.
4. Hold the swab in place for 30-60 seconds then gently rotate the swab three times.
5. Place the swab in transport medium and cut the shaft so that the swab fits into the tube.
6. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 48 hours at 2-8°C. If longer delays are anticipated, specimens should be frozen at -70°C.

Note: Please **do not break the ampule** for Influenza A/B or RSV testing.

Nasopharyngeal Washes

1. Position the patient so that he/she is sitting with his/her head tipped backward.
2. Add up to 5 ml of saline into one nostril and gently close the nasal passage.
3. Ask the patient to expectorate the wash into a collection device or cup.
4. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 72 hours at 2-8°C. If longer delays are anticipated, specimens should be frozen at -70°C.

PAP Test (Thin Prep Pap)

Pre-Collection Steps:

1. Fill out the cytology requisition form.
2. Verify the expiration date on the PreservCyt vial and label the vial with the patient's name and collection date.
3. Obtain the specimen before bimanual examination. Use and unlubricated speculum (saline or warm water may be used) After visualization of the cervix is accomplished, collect the sample. Obtain an adequate sampling of the cervix using a broom-type (e.g. Wallach) collection device or endocervical brush / plastic spatula combination following NCCLS guidelines GP-15 A)

THIN PREP PAP TEST GYNECOLOGIC SPECIMEN COLLECTION

Materials:

This test requires a Cytoc PreserveCyt Solution vial, Endocervical brush/Spatula combination or Broom-like device, cytology requisition card and a marker.

Thin Prep Pap test Broom-like Device Procedure:

1. To obtain an adequate sample form the cervix using a Papette, a broom-like device, insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Rotate the broom, while pushing gently, clockwise direction 5 times.
2. Remove the broom by pulling straight out. **Do not rotate back.** Backwards rotation will cause sample loss.
3. Rinse the broom as quickly as possible in the labeled PreservCyt Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device. It is important to rinse the broom-like device quickly after

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sample collection. Prolonged exposure will cause the cells to adhere to the plastic bristles of the collection device preventing transfer into the solution.

Thin Prep Pap test Endocervical Brush/Spatula Procedure:

1. Obtain an adequate sampling from the cervix using a plastic spatula.
2. Immediately rinse the spatula into the PreservCyt® solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.
3. Obtain and adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate the brush ¼ to ½ turn in one direction.
Do not over-rotate the brush, as this may cause bleeding.
4. Remove the endocervical brush by pulling straight out. Do not rotate back. Backwards rotation will cause sample loss.
5. Immediately rinse the brush in the PreservCyt® solution vial by rotating the device in the solution 10 times while pushing the bristles against the PreservCyt® vial wall. Swirl the brush vigorously to further release material. Discard the brush.
6. Tighten the cap so that the torque line (black line) on the cap passes the torque line (black line) on the vial. Place the vial in the inner pouch of a biohazard bag and place the requisition in the outer pouch to avoid contamination of the paperwork during transport to the laboratory.
7. Label the vial with the patient's name or patient sticker.
8. Fill out the cytology requisition form with the patient name, date of birth, age, LMP, and any other pertinent medical history.
9. Rubber band the requisition around the PreservCyt® vial and transport to the laboratory.

Note: If the specimen is not labeled with the patient's name or patient sticker, the laboratory will return the specimen to the clinic for proper identification. A requisition wrapped around a specimen will NOT constitute a properly labeled specimen.

Rectal Swabs

1. Position the patient so that the anus is readily accessible.
2. Insert a dry cotton, rayon, or dacron swab at least 5 cm into the rectum.
3. Rotate the swab and carefully withdraw it from the rectum. The swab should show signs of fecal material.
4. Place the swab into transport medium and break the shaft of the swab so that it fits into the tube.
5. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 72 hours at 2-8°C. If longer delays are anticipated, specimens should be frozen at -70°C.

Saliva

1. Rub a sterile dry rayon, dacron, or cotton swab over the buccal mucosa opposite the upper molars in the vicinity of the Stensen's ducts and then over the floor of the mouth anterior to the tongue.
2. Place the swab into a vial transport medium and break the shaft of the swab so that it fits into the tube.
3. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 24 hours at 2-8°C. If longer delays are anticipated, specimens should be frozen at -70°C.

Note: Saliva may be collected by aspiration or expectoration into a sterile container. Specimen handling is the same for these specimens as for swab specimens (step 3 above).

Sputum

1. The first morning sputum specimen is desirable.
2. Ask patient to expectorate into a sputum container.
3. After the specimen is collected, cap tube. **Do not use any other transport media.**
4. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 24 hours at 2-8°C.

Stool

1. Collect 10-30 grams of stool (approximately the size of a walnut) in any clean vessel possessing a tight fitting lid. Do not use preservatives for virus isolations.
2. Stool specimens should be refrigerated to retard bacterial growth and sent to the laboratory as soon as possible after collection.

Note: Stools for Chemistry and Hematology tests usually require special collection and handling. Please see the alphabetical test list for more specific collection information.

3. Do not use stool specimen which has fallen into the toilet.
4. Do not urinate into stool collection container.
5. Do not take a laxative before collecting stool specimen.
6. When obtaining specimens from a child in diapers: Stools for Ova and Parasites **should not** be scraped for the diaper. Collect using a plastic barrier as described below.

Note: Highly absorbent diapers will quickly soak up liquid stools and cause viruses to bind irreversibly to the paper fibers. To alleviate this problem, place a piece of plastic wrap in the diaper. Stool collected on the plastic can be placed into clean container.

Stool, Occult Blood

1. Using a wooden applicator, collect small fecal sample.
2. Apply thin smear inside box A of occult blood card.
3. Reuse applicator to obtain second sample from a different part of feces. Apply **thin** smear inside box B.
4. Close cover flap. Properly label and submit to lab. Alternately, stool specimen may be sent to lab in any clean container with tight fitting lid.

Throat Smear

1. A dry cotton, dacron, or rayon swab or a swab moistened with a physiologic saline may be used for this procedure.
2. Vigorously rub the swab across the tonsils and posterior pharynx.
3. Rub the swab on the wells of slide making sure that the specimen is evenly dispersed over each well. Be sure to roll the swab so that the entire surface of the swab comes into contact with the slide.
4. Allow the slide to air dry.
5. Send the slide to the laboratory in a petri dish or in a slide mailer. Slides may be stored at room temperature or at 2-8°C for up to 48 hours before fixation.

Throat Swab

1. A dry cotton, dacron, or rayon swab or a swab moistened with physiologic saline may be used for this procedure.
2. Vigorously rub the swab across the tonsils and posterior pharynx.
3. Place the swab in transport medium and break the shaft of the swab so that it fits in the vial. Break the ampule if present.
4. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 72 hours at 2-8°C. If longer delays are anticipated, specimens should be frozen at -70°C.

Throat Washes

1. Ask the patient to clear all mucous and post-nasal secretions from their throat and mouth.
2. Give patient 2-3 ml of sterile physiological saline and ask them to gargle for 30-60 seconds and expectorate the wash into a clean collection device or cup.
3. Viral and Chlamydial culture: Add the wash to the tube of viral or Chlamydia transport medium.
Bacterial, Fungal, or Mycobacterial culture: Send wash in sealed collection vessel.

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4. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 72 hours at 2-8°C. If longer delays are anticipated, specimens should be frozen at -70°C.

Tissues

1. Collect tissues aseptically, taking care to prevent cross-contamination when specimens are taken from multiple sites.
2. Tissue specimens may be placed into universal transport media or sterile saline.
3. For bacterial, fungal, or mycobacterium culture, submit tissue in sterile container.
4. Send all specimens to the laboratory on wet ice or with a cold pack. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 24 hours at 2-8°C.

Urethral Smears

1. Position the patient so that the urethra is readily accessible.
2. Insert a fine, aluminum-shafted cotton or dacron swab 2-4 cm into the urethra.
3. Carefully rotate the swab three times and remove the swab from the urethra.
4. **Do not** allow the swab to dry.
5. Rub the swab on the well of a slide making sure the specimen is evenly dispersed over the entire well.
6. Allow the slide to air dry.
7. Label the slide and send it to the laboratory in a petri dish or a slide mailer.
8. Slides may be stored at room (20-30°C) or refrigerator (2-8°C) temperature for up to seven days before staining.

Urethral Swabs

1. Position the patient so that the urethra is readily accessible.
2. Insert a fine, aluminum-shafted cotton or dacron swab 2-4 cm into the urethra.
3. Carefully rotate the swab three times and remove the swab from the urethra.
4. Place the swab in the appropriate transport medium and cut the shaft of the swab so that it fits into the tube.
5. Send the specimen to the laboratory on wet ice. **DO NOT FREEZE AT -20°C.** Specimens may be stored for up to 24 hours at 2-8°C.

Note: Urethral discharges are not acceptable specimens.

Note: Patient should not have urinated for at least one hour prior to sampling.

Urine

1. Collect first early morning urine in a sterile container with a tight fitting lid.
2. Refer to individual tests for storage requirements.
3. Urinalysis: Acceptable specimens include clean catch, midstream or catheterized urines. A 10 ml is requested although 1 ml is the minimum requirement. Refrigerate following collection for a maximum of 4 hours.
4. Pregnancy Test: First morning urine is choice. Random urine will be accepted. Minimum volume is 1 ml.

Note: Male urine may be used to test for Chlamydia/GC testing using the PCR methodology. When collecting these specimens, males should not have urinated during the previous 2 hours. There should be 10-50 ml of first catch urine collected into a clean, empty plastic cup without preservatives.

Midstream Clean-Catch Collection of Urine

Specimen Collection Procedure

1. Ask patient to identify him/herself. The name must match the requisition form.
2. Label urine container. Use the permanent marking pen to record date, time, and patient's name on the adhesive label. Affix the label to the urine container, not the cap.
3. Read the patient instructions. Explain instructions to the patient and give him or her a written copy.
4. Guide the patient to the bathroom where the specimen may be collected. Assist any patient who may need assistance, such as the elderly, weak, or obese.

Written instructions for the patient- separate sheets for males and females as follows:

Instructions for Males:

- A. Wash hands with soap and dry them.
- B. Open the urine container and avoid touching the insides.
- C. If uncircumcised, withdraw foreskin.
- D. Using the antiseptic towlette, clean the urethral opening and area around it.
- E. Repeat the cleaning with the second pad.
- F. Begin urinating and pass the first portion into the toilet. Don't stop flow of urine.
- G. Fill the container with the mid-portion.
- H. Pass the remaining urine into the toilet.
- I. Fasten the lid on the urine container.

Instructions for Females:

- A. Wash hands with soap and dry them.
- B. Open the urine container and avoid touching the insides.
- C. Sit on the toilet and spread genital lips with one hand.
- D. Using the towelette, clean the urethral opening and area around it working from front to back.
- E. Repeat the cleaning with the second pad.
- F. Begin urinating and pass first portion into toilet. Do not stop flow of urine.
- G. Fill the urine container with the mid-portion.
- H. Pass the remaining urine into the toilet.
- I. Fasten lid on the urine container.

Urine, 24-hour Collection

Most urine chemistry tests require a 24 hour collection. Record on the test request form any medications that the patient is receiving. The patient should not ingest any alcoholic beverages during the collection period.

1. Instruct patient to empty bladder upon waking. Discard this urine.
2. Collect all urine passed for the next 24 hours, including the first morning urine at the end of the 24 hour period. Collected urine should be stored in the refrigerator during the 24 hour period.
3. The collected urine should be brought to the laboratory that day.
4. In the laboratory, accurately measure total volume and record information on the test request form.
5. Gently and thoroughly mix entire specimen.
6. Pour off 10-20 ml aliquot and refrigerate.

Vesicular Cell Smears

1. Place a small drop of sterile saline on each well of a 2-well microscope slide.
2. Aseptically unroof the lesion and blot the excess fluid with a swab.
3. Scrape the base of the lesion with a swab or a sterile scalpel blade to obtain virus-infected epithelial cells. Gross bleeding should be avoided because red blood cells can interfere with the interpretation of the assay.
4. Transfer the cell scrapings from the scalpel blade to the drops of saline.
5. Spread the exfoliated cells thinly over the wells and allow the slide to air dry at room temperature.
6. Send the slide to the laboratory in a petri dish or in a slide mailer.
7. Slides may be stored for up to 48 hours at 2-8°C.

Vesicle Fluid (swab)

1. Select a vesicle containing clear fluids. The efficiency of virus recovery decreases significantly when pustular fluids are collected.
2. Aseptically unroof the lesion and blot the excess fluid with a swab.
3. Gently rub the base of the lesion with the swab to collect infected epithelial cells.
4. Place the swab in transport media and break off the shaft of the swab so that it will fit into the vial.
5. Send the specimen to the laboratory on wet ice. Do not freeze. Specimens may be stored up to 72 hours in the refrigerator.