

Specimen Collection, Handling, and Special Instructions

Specimen Collection and Handling Guidelines:

Specimen Collection General Guidelines:

The accuracy of any laboratory test result is dependent upon the integrity of the specimen on which it is performed. This section gives guidelines to follow for collecting and transporting blood and body fluids. Specific test requirements are found in the Alphabetical Test Listing in this catalog. Please feel free to call your *Avera LabNet* service center with any questions.

FASTING SPECIMENS:

An overnight (12-16 hour) fast is required for most fasting specimens. If individual tests require specific fasting requirements, the requirements will be outlined in the Collection Notes Section of the individual test listing.

SERUM OR PLASMA:

Draw blood into tube appropriate for the test(s) required. The amount of blood should be 2.5 times the volume of serum/plasma required for testing. Allow the tube to fill properly. Tubes with anticoagulant need to be inverted 5-6 times to avoid clot formation. Do not shake. Centrifuge the specimen and separate the serum/plasma as soon as possible (< 1 hour from time of collection) into a plastic transport tube, being careful not to transfer any cells. Carefully tighten cap. On the transport tube clearly identify the specimen as serum or plasma (and anticoagulant when used). Rare testing may require different timing from collection to sample separation. Special requirements will be clearly identified in the individual test listings under Collection Notes.

Platelet-Poor Plasma: Refer to Coagulation Special Instructions Section

Platelet-Rich Plasma: Refer to "Stypven - Collection Note Section" in alphabetical test listing

WHOLE BLOOD:

Draw blood using correct anticoagulant tube appropriate for the test(s) required. Allow the tube to fill completely and invert 5-6 times to facilitate mixing with anticoagulant and avoid clot formation. Submit original collection tube, completely labeled, for testing.

CSF:

Transfer CSF to leakproof plastic vial for transport. Conventional CSF screw cap collection tubes generally leak and are not recommended. If multiple specimens are being submitted for different types of testing, be sure to label specimen transport tubes with the original specimen container number (tube #1, tube #2, etc.).

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THERAPEUTIC DRUG MONITORING:

Specimen collection and storage on a gel barrier can decrease the concentration of certain drugs in the specimen. Depending on the specimen volume and the storage time, the decreases may be clinically significant. **We do not recommend the use of a serum separator tubes or gels for collection of therapeutic drug testing.**

Peak, Trough and Random Levels: Reference ranges for certain therapeutic drugs are based on the time the specimens are drawn according to time guidelines around the time of the drug dose/infusion (Peak and Trough Levels). If a drug level is offered as a "Peak or Trough" level, it is recommended to follow the specimen timed drawing guidelines listed under the Collection Notes for the individual test. If the ordering physician does not specify which level to draw, contact the physician to confirm which level is clinically indicated. Random levels are recommended to only be used in cases of suspected toxicity or if the drug level required does not list specific peak and trough drawing guidelines.

VENIPUNCTURE COLLECTION GUIDELINES:

- Follow all "exposure control" guidelines required by your facility [i.e. gloves, lab coat, proper sharps disposal, etc.]
- Assemble all supplies necessary for the venipuncture
- Properly identify the patient
- Apply tourniquet around arm, 3-4 inches above the preselected venipuncture site
- Cleanse site with alcohol or approved site preparation solution (certain procedures will require that a non-alcohol germicide be used in cleansing the site)
- Allow cleansed area to air dry to prevent burning sensation or hemolysis of specimen
- Grasp patient's arm firmly, placing thumb 1-2 inches below the chosen site to draw the skin tight (this technique will also assist in anchoring the vein for access)
- Perform venipuncture with needle bevel side up
- Collect specimen(s) utilizing a vacuum tube system or syringe method
- When using vacuum tube system for collecting the specimens, assure that all tubes containing anticoagulant are filled to required volumes (exhaustion of vacuum) and are gently inverted 5-6 times immediately after collection. DO NOT SHAKE specimens
- Release tourniquet as soon as possible after venous access is successful
- Remove needle from site, apply direct pressure with cotton ball or gauze
- Assure that site has stopped bleeding, apply bandaid as applicable
- Label all specimens according to protocol prior to leaving the drawing area

Drawing Order For Vacuum Tube Collection System:

- Blood culture tubes, sterile tubes
- Tubes for coagulation studies
- Tubes without additives
- Other additives in the following order: Light Blue, Gold, Light Green, Lavender, Gray

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ORDER OF DRAW FOR MULTIPLE TUBE COLLECTIONS:

| <u>CLOSURE COLOR</u> | <u>COLLECTION TUBE</u> | <u>MIX BY INVERTING</u> |
|----------------------|--|-------------------------|
| Blood Cultures | N/A | N/A |
| Light Blue | Citrate Tube | 3-4 times |
| Red | SST Gel Separator Tube | 5 times |
| Light Green | PST Gel Separator Tube with Li Heparin | 8-10 times |
| Lavender | EDTA (plastic) | 8-10 times |
| Gray | Sodium fluoride | 8-10 times |

CLSI ORDER OF DRAW GUIDELINES:

The following order-of-draw is recommended when drawing multiple specimens for clinical laboratory testing during a single venipuncture. Its purpose is to avoid possible test result error due to cross contamination from tube additives. This procedure should be followed for both glass and plastic venous blood collection tubes.

1. Blood culture tube
 2. Coagulation tube (e.g. blue closure)
 3. Serum tube with or without clot activator, with or without gel (e.g. red closure)
 4. Heparin tube with or without gel plasma separator (e.g. green closure)
 5. EDTA (e.g. lavender closure)
 6. Glycolytic inhibitor (e.g. gray closure)
- The order of draw has been revised to reflect the increased use of plastic blood collection tubes. Plastic serum tubes containing a clot activator may cause interference in coagulation testing. Glass nonadditive serum tubes may be drawn before the coagulation tube.
 - When using a winged blood collection set for venipuncture and a coagulation tube is the first tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection tubing dead space and to assure maintenance of the proper anticoagulant/blood ratio and need not be completely filled. The discard tube should be a nonadditive or a coagulation tube.

Reference: CLSI DOCUMENT H3-A5, Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard-5th edition, Vol. 23, No. 32

VACUUM TUBE SYSTEM REMINDERS:

1. Tubes with powdered anticoagulants should be tapped near the stopper to dislodge any anticoagulant that may be between the stopper and the tube wall.
2. All tubes with liquid anticoagulants should be filled to the exhaustion of the vacuum to ensure proper ratio of anticoagulant to blood.

Specimen Labeling Guidelines:

Label all specimens (collection tubes and specimen transport tubes) with:

- Patient's full name
- Collection date/time
- Phlebotomist name or initials.
- When available, place pre-printed # from the request form on all tubes. **DO NOT USE THE NUMBERED LABEL IN LIEU OF PATIENTS FULL NAME.**

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Blood Bank Specimen Labeling Requirements:

Mandatory blood bank specimen labeling requirements are required at each Avera LabNet Service Center. Obtain your specific Service Center's guidelines prior to submitting specimens for Blood Bank testing.

Specimen Storage and Transport Guidelines:

Individual test listing will specify correct specimen storage and transport temperatures required.

Specimens should be refrigerated until courier pick up or mailing unless otherwise specified in the test listing. Specimens requiring refrigeration during transport should be sent with chilled "cool packs".

Certain tests will list temperature requirement as "Frozen (< -20C); Refrigerate LIMITED TIME ONLY - See Notes". This type of storage requirement will allow for storage and transport of specimen at refrigerated temperature ONLY if transport/storage of specimen will not exceed a certain time frame. If there is any possibility that time frame from specimen collection to time prepared specimen is received by the Service Center for testing will exceed time limits, please freeze specimen. Contact your Service Center with any questions relating to frozen specimen integrity.

Specimens which require freezing should be frozen ASAP in a PLASTIC TRANSPORT TUBE [unless Collection Note Section includes different instructions], allowing room for expansion during freezing. Transport with frozen "cool packs" in an insulated container may be adequate provided the specimen will be delivered to the Service Center within 4 hours. Otherwise, dry ice should be used in transport.

Send a separate sample for each test requiring a frozen specimen. This will prevent compromising the specimen if thawing and refreezing the sample is required due to testing being performed on different days or at different locations.

All specimens must be placed in a sealed leakproof biohazard transport bag prior to transporting with courier.

Unacceptable Specimen Guidelines

General Criteria for Unacceptable Specimens:

Specimens may be rejected for many reasons including the following:

- Hemolysis
- Lipemia
- Insufficient quantity
- Improper preservative
- Unlabeled specimen
- Mislabeled specimen
- Improper specimen collection

[Unacceptable Specimens - Continued Next Page]

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Individual test listings will outline certain biological criteria that will determine a sample to not be suitable for testing [listed under Collection Notes Section]. You will be notified of specimen rejection ASAP. No specimen will be discarded due to rejection until the ordering physician or client has been notified. If recollection of the specimen is impossible or would compromise patient care, it may be possible in some cases to provide a result WITH THE FULL UNDERSTANDING OF THE PHYSICIAN that, the validity of the results may be questionable.

Unlabeled/Mislabeled Specimens:

Avera LabNet's Service Centers will make every attempt to correctly identify specimens. However, when we find an improperly labeled specimen, we will telephone to notify you. If you are able to identify/verify that the specimen is the correct specimen and was mislabeled, we will accept it after a verification form is completed [test may be reported with disclaimer statement]. If you cannot positively identify a specimen, we will ask that you recollect. If we receive two unlabeled specimens of the same type (both serum), both specimens will be rejected and you will be asked to recollect.

24 - Hour Urine Collection Instructions:

Collection General Guidelines:

1. 24-hour collection containers and transfer tubes are available from Avera LabNet Service Centers. Refer to the Alphabetical Test Listing for preservatives, storage, transport and special handling instructions.
2. Instruct the patient to empty their bladder and discard this urine. Note the time. This is the start time. Collect and save all urine for the next 24 hours. At the same time the following day empty the bladder and collect this urine. This is the end time.
3. Refrigerate urine during collection unless otherwise indicated under Collection Note Section.
4. Record the 24-hour urine total volume on the Test Requisition Form. The total volume is required in the calculation of results. [If unable to measure the total volume accurately, the entire specimen may be submitted to the Service Center for testing.]
5. After the collection is complete, mix the specimen and prepare specimen as outlined in Collection Note Section. All specimens must be prepared for transport in leakproof screw cap containers and closed tightly to avoid leakage.

Provide each patient with collection instructions. For convenience, you may copy "Patient 24-Hour Urine Collection Instructions" on next page.

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Patient 24-Hour Urine Collection Instructions:

IMPORTANT:

Please Read All Instructions Before Starting Collection Of Urine

The accurately timed urine collection, which you are about to make, is an important part of your examination. Decisions important to your health may depend on it.

The test that has been ordered on you is valid **ONLY** if the collection includes **ALL** urine that you pass in a 24-hour period. If for any reason some of the urine passed during the collection time is **NOT** put into the container for collection, the test will **NOT** be accurate and a new collection should be scheduled. Please contact your laboratory immediately if you will need to begin a new collection and will require a new collection container.

You will be supplied with one container. This container is capped. It may or may not contain some liquid. If liquid is present, do not discard. This liquid is a preservative that is necessary for the accuracy of the specific test your doctor has ordered. Do not allow the liquid to come in contact with your skin. If such accidentally happens, wash the involved area immediately with a large amount of water. If irritation occurs, contact your physician.

Be sure to keep the collection container closed and in a cool place, preferably refrigerated.

INSTRUCTIONS:

1. Start the 24-hour collection period at 7:00 am or when you get up in the morning. Empty your bladder at this time and discard this urine [do not put this specimen into the collection container].
2. Collect all urine that you pass for the next 24-hours, until 7:00 am or the specific time you began the collection.
3. At exactly 7:00 am, or the specific time you began the urine collection, again empty your bladder and place this collected specimen in the container. This is the last specimen that should be added to the container.
4. Should you have a bowel movement during the 24-hour period, try to pass your urine prior to the bowel movement to avoid loss of the urine that may be passed at this time. Do not allow any of the feces passed to contaminate the urine being collected. If feces does contaminate the urine specimen, the collection must be restarted.
5. As soon as collection is complete, return the specimen to the laboratory that testing has been ordered through.

Laboratory Name: _____ Location: _____

Patient Name: _____ Test Ordered: _____

To Be Filled In By Patient:

Date & Time Collection Began: _____

Date & Time Collection Ended: _____

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INSTRUCCIONES PARA PACIENTES EXTERNOS PARA LA COLECCIÓN CRONOMETRADA DE ORINA POR _____ HORAS

(Patient Instructions for Outpatient Timed _____ Hour Urine Collection, Spanish)

1. El laboratorio proveerá una botella desechable, limpia, plástica, y de abertura ancha, que tiene una tapa y es suficiente grande para contener 2-3 litros para toda la muestra de orina. El bote de orina tiene que ser etiquetado antes de que salga del laboratorio con el nombre del paciente y la prueba que se llevará a cabo.
 2. Un conservante podía haber sido añadido al contenedor de la colección para mantener la integridad de la muestra durante la recogida y el periodo de pruebas. Si un conservante fuera añadido, una etiqueta de aviso será fijado al bote de la colección. Es necesario que se tome precaución para evitar el contacto con los conservantes de orina. Muchos conservantes de orina son soluciones ácidas que pueden causar irritaciones de la piel. Se puede pedir a usted que refrigere la muestra (mantenerla sobre hielo durante la colección) si no se usa conservantes al comienzo de la colección. Los requisitos de la conservación dependen de cual prueba se haga.
 3. Todos los pacientes recibirán un aparato aparte para cada muestra de orina para que no orine directamente en el bote de colección de las 24 horas. Es especialmente importante que los pacientes no orinen directamente en los recipientes que contienen conservantes de orina. En vez de eso, se usa estos aparatos apartes para cada muestra de orina, y luego se vacian ellos en el contenedor de las 24 horas de colección.
 - Pacientes varones recibirán un recipiente pequeño de rutina.
 - Pacientes mujeres SIEMPRE recibirán un aparato "gorra" que se queda en el asiento de retrete para coleccionar la orina.
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4. Empezar la colección de 24 horas por vaciar la vejiga a una hora predeterminada. Deshaga de esta muestra inicial, y apunte a que hora se lo hace. Esta es la hora de empezar la colección.

LA HORA DE EMPEZAR ES _____.

5. Después de la hora de empezar, TODA LA ORINA tiene que ser coleccionada y vaciada en el recipiente proveído.

La colección termina exactamente _____ horas después de la muestra inicial desechada.

LA HORA DE TERMINAR ES _____.

Al tiempo de terminar, completamente vacie la vejiga y agregue esa muestra a la colección. Con ella, la colección está completa.

Si en cualquier momento la colección de orina se contamina con deposición, favor de llamar al Laboratorio de Avera McKennan al 322-7120 para instrucciones.

Si la cantidad de orina es grande, por favor llame al Laboratorio al 322-7120 para obtener otro bote. Es muy importante que toda la orina vaciada durante el tiempo de colección sea guardada y puesta en el recipiente de colección.

Favor de devolver la colección cronometrada de orina al Laboratorio lo más pronto posible después de que se termine la colección.

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Coagulation Special Instructions:

Testing to evaluate the hemostatic mechanism are extremely sensitive to methods of sample collection and processing. Test results are a direct reflection of sample integrity. Specimens should be processed, and sent according to acceptable protocol.

PLATELET-POOR PLASMA PREPARATION – DOUBLE CENTRIFUGATION METHOD:

1. General Specimen Drawing Instructions:
 - a. Vacuum tubes must be filled to completion to ensure the proper 9:1 ratio of blood to anticoagulant is achieved.
 - b. Routine collection requires 4.5 mL blood added to 0.5 mL 3.2% sodium citrate. (3.2% Sodium Citrate Tube – Light Blue Top)
 - c. When drawing specimen avoid contaminating sample with tissue thromboplastin or heparin as they may alter testing results:
 - Venipuncture must be clean, with no trauma
 - Hemolyzed specimens are not acceptable
 - First 5 mL of blood drawn should not be used for coagulation testing [either discard or use for other testing]
 - If drawn through an indwelling catheter, the first 20 mL of blood must be discarded or used for other testing as appropriate
 - d. Draw appropriate number of tubes to provide required volume of plasma for testing [required specimen volumes listed in “Alphabetical Test Listing”].
 - e. Invert collection tubes gently 5-6 times to mix blood with anticoagulant. DO NOT SHAKE tubes. Process specimens immediately.
2. Centrifuge for 10-15 minutes at 3000 RPM.
3. With a plastic transfer pipet carefully remove plasma, place in a plastic tube and centrifuge again [10-15 minutes at 3000 RPM]. **Platelet-poor plasma must have a final platelet count of <10,000. Please validate centrifugation and time guidelines to your facility’s equipment by completing a platelet count on a plasma specimen prepared with these guidelines. If platelet count is >10,000, adjust times, centrifuge rpm, or complete a third centrifugation step prior to submitting specimen for testing.**
4. With a plastic transfer pipet, transfer plasma to plastic transport tube being careful to avoid aspirating the buffy coat.
5. Each individual coagulation test ordered should be prepared and submitted as an individual specimen. DO NOT submit multiple test specimens in one tube. Coagulation Consultation Study testing also requires submission of “normal control specimens” – Refer to the following section on Coagulation Consultation Special Collection Instructions.
6. Label specimen. Include anticoagulant.
7. Freeze immediately at < - 40° C.
8. Specimen must remain frozen and be received within 24 hours. Specimens not received in the frozen state will be rejected for testing.
9. Ship on dry ice.

[Coagulation Special Instructions - Continued Next Page]

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AVERA LABNET SIOUX FALLS SERVICE CENTER:

COAGULATION CONSULTATION STUDY - SPECIAL COLLECTION INSTRUCTIONS:

1. A "Coagulation Consultation Request Form" must be completely filled out and submitted with the specimen(s) and test requisition at the time of the test order.
2. A normal control sample must accompany the patient specimen and be handled exactly as outlined for patient samples. Testing is performed on the control sample and if the results are not considered to be normal, it implies that possibly some process involved in collection, processing, shipping, and/or handling may have been improperly performed and therefore the patient results may not be valid.
3. **Patient and normal control must avoid warfarin (Coumadin) therapy for 2 weeks and heparin therapy for 2 days prior to collection of specimens for testing.**
4. Follow all "Platelet-Poor Plasma" collection and preparation instructions previously outlined.
5. 2-4 5mL 3.2% Sodium Citrate tubes should be drawn on both the patient and the normal control depending the required number of individual plasma specimens for testing (5-8 1 mL aliquots frozen platelet-poor plasma specimens). Number of required 1.0 mL aliquots is outlined in the "Alphabetical Test Listing" for the specific type of Coagulation Consult ordered.

Fetal Fibronectin Collection, Precaution and Warning Guidelines:

Proper specimen collection and handling is essential in providing high quality Fetal Fibronectin testing results. All individuals responsible for the collection of these specimens are requested to follow all precautions, warnings and instructions listed in the package insert of the specialized specimen collection kit.

GENERAL SPECIMEN COLLECTION PRECAUTIONS AND WARNINGS:

1. Specimens for Fetal Fibronectin testing should be collected prior to collection of culture specimens.
2. Specimens should be obtained prior to digital cervical examination or vaginal probe ultrasound examination as manipulation of the cervix may cause the release of Fetal Fibronectin.
3. Specimens should not be collected if the patient has had sexual intercourse within 24 hours prior to the sampling time because semen and/or sperm present in the sample may increase the possibility of the test giving a false positive result.
4. Specimens will not be tested if the specimen transport tubes have leaked in transit.
5. Care must be taken not to contaminate the swab or cervicovaginal secretions with lubricants, soaps, or disinfectants [i.e. K-Y Jelly lubricant, Betadine disinfectant, Monistat cream, hexachlorophene]. These substances may interfere with absorption of the specimen by the Dacron collection swab or with the antibody-antigen reaction of the test analysis.
6. Fetal Fibronectin tests are not intended for use in the management of patients with moderate or gross vaginal bleeding. The presence of vaginal bleeding judged by the caregiver to be moderate or gross in amount may contribute to difficulty in interpreting the analytical result. [Continued Next Page]

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7. Rupture of membranes should be ruled out prior to specimen collection since Fetal Fibronectin is found in both amniotic fluid and the fetal membranes.
8. Specimens should not be obtained from patients with suspected or known placental abruption, placental previa, or patients with cancers of the reproductive tract.
9. There is insufficient information characterizing the association of Fetal Fibronectin expression to delivery in asymptomatic women with HIV/AIDS.
10. Collected specimens should always be stored and transported in temperatures < 25 C. Refrigerated (2-8° C) temperatures are preferred.
11. Use only one Specimen Collection Device per patient sample and DO NOT use collection kits past their expiration date.

FETAL FIBRONECTIN GENERAL COLLECTION INSTRUCTIONS:

1. Always use special collection kits specific for Fetal Fibronectin testing and follow Specimen Collection Kit specific instructions.
2. Collection from Symptomatic Women: During sterile speculum exam, prior to any examination or manipulation of the cervix or vaginal tract, lightly rotate the collection kit swab across the posterior fornix of the vagina for approximately 10 seconds to absorb cervicovaginal secretions. Subsequent attempts to saturate the swab may invalidate the test.
Collection from Asymptomatic Women: During sterile speculum exam, prior to any examination or manipulation of the cervix or vaginal tract, lightly rotate the collection kit swab across either the posterior fornix of the vagina or the ectocervical region of the external cervical os for approximately 10 seconds to absorb cervicovaginal secretions. Subsequent attempts to saturate the swab may invalidate the test.
3. Remove swab and immerse Dacron tip in buffer. Break the shaft [at the score] even with the top of the transport tube.
4. Align the shaft with the hole inside the tube cap and push down tightly over the shaft, sealing the tube. WARNING – the shaft must be aligned to avoid leakage of the specimen.
5. Specimens must be stored at refrigerated temperatures and have testing completed within 3 days of collection. Do not collect specimens after routine courier pickups on Fridays or prior to extended holiday weekends.

FETAL FIBRONECTIN INDETERMINATE TEST RESULTS - CAUSES AND ACTIONS:

On rare occasions, the testing of a fetal fibronectin specimen will result in an "indeterminate or invalid" reading on the instrument used for testing. In this instance, the method is unable to determine if the specimen is positive or negative for fetal fibronectin.

Possible reasons for "invalid/indeterminate" results include the following:

1. Atypically high concentration of fibronectin due to a presence of amniotic fluid. [The assay is intended to be used on women with intact fetal membranes.]
2. Interfering substances [may include soaps and lubricants] are present in the specimen.
3. Sample matrix abnormality.

Summary of actions taken when "invalid/indeterminate" results occur:

- Result will be reported as indeterminate and will be called to you by our Service Center.
- Specimen will be referred out for further testing that will result in a positive or negative determination. This result will be reported and called to you as soon as available.
- If the quantity of the remaining specimen is not sufficient for the referred testing, you will be contacted and requested to collect another specimen. If requested to collect another specimen, you must wait 24 hours after the original collection [or since the most recent digital exam] to recollect or the test results will be inaccurate.

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Pre-Glucose Tolerance Test Carbohydrate Diet:

For certain types of Glucose Tolerance Tests, a carbohydrate enriched diet is required for 3 days prior to Glucose Tolerance Test collection. At a minimum, a 150 gram carbohydrate diet is required. The amount of carbohydrates consumed during this time frame may always be more than the minimum required amount.

Remind the patient that no food or liquid, except water, should be consumed after 10:00 pm, prior to the morning of the test.

Example 300 Gram Carbohydrate Diet:

- Breakfast:** Fruit (1 banana, 1 orange, 1/2 grapefruit or 1/2 c. juice)
Cereal (1/2 cup)
Bread (1 slice)
Milk (1/2 cup)
Sugar (2 teaspoonfuls)
- Lunch:** Meat, cheese or egg (amount desired)
Bread (2 slices) -or- Spaghetti, macaroni, rice (1 cup cooked) -or-
Noodles (1 cup cooked)
Dessert (fruit, cake, pie or cookies)
Milk (1 cup)
** Meat, tomatoes, vegetables, etc. may be added as desired
- Dinner:** Potato (1 medium)
Vegetable (at least 1/2 cup)
Bread (1 slice)
Meat (as desired)
Milk (1 cup)
Dessert (tapioca or rice pudding, fruit)
- Snacks:** Are permitted in any quantity or type

Microbiology Collection Guidelines & Special Instructions:

Microbiological procedures used are those recommended by the American Society for Microbiology, American Society of Clinical Pathologists, College of American Pathologists, and National Committee for Clinical Laboratory Standards.

For the collection and evaluation of microbiology specimens, it is necessary to understand that any given body site develops its own "usual/normal" flora. When collecting cultures from sites, special care must be taken to bypass contaminating flora. Examples of such sites are skin, upper respiratory tract, intestinal tract, female genital area, and open draining wounds. Submit aspirated material whenever possible.

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Culture Collection Basics:

- Collect the specimen at optimal times and prior to antimicrobial therapy, if possible.
- Collect sufficient quantity.
- Use proper collection containers and transport media. Always assure that transport containers are leakproof.
- If required, prepare site to assure uncontaminated collection. Submit aspirated material whenever possible.
- Indicate source and collection time information on the requisition.
- Minimize transport time.

Reflex Testing General Guidelines:

Certain microbiology procedures require reflex testing to provide appropriate information to the ordering physician allowing for result interpretation and therapy intervention decisions.

Reflex Testing Guidelines are identified under each test in the "Alphabetical Test Listing". Reflex testing required will be billed appropriately.

Examples of reflex testing on potential pathogenic cultures include, but may not be limited to:

- Bacterial Identification(s)
- Susceptibility Testing(s)
- Monoclonal Typing(s)

It is the responsibility of the ordering physician to specifically order "reflex testing is not to be completed" if at the time of the test request reflex testing is not clinically indicated.

If reflex testing is not required due to culture results or if the physician has specifically requested, testing will not be completed or billed.

Microbiology Routine Culture Collection Guidelines:

Acid-Fast Bacilli Culture and/or Smear:

Acceptable Specimen Guidelines:

- **Pulmonary specimens:**
 1. Spontaneously produced sputum - specimen of choice; collect approximately 5-10 mL of a first morning expectorated specimen; collect in sterile container
 - To raise sputum, patient must be instructed to take a deep breath, hold it momentarily, and then cough deeply and vigorously
 2. Induced sputum - should be collected by appropriate personnel following facility procedures; collect in sterile container
 3. Bronchoscopy, bronchial washings, lavages, brushings, etc. - should be collected by appropriate personnel following facility procedures; collect approximately 5-10 mL if possible; collect in sterile container
 4. Transtracheal aspiration or laryngeal swabbing- should be collected by appropriate personnel following facility procedures; collect in sterile container

Note: A series of three specimens collected on three separate days is recommended.

[Acid-Fast Acceptable Specimens - Continued Next Page]

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- **Gastric specimen:** Gastric lavage may be used to collect specimens from patients who have swallowed their sputum during the night; specimen should be collected before patient arises in the morning; in sterile collection container collect 20-25 mL of gastric contents; series of three specimens collected on three separate days is recommended
- **Urine specimen:** Specimen should be collected as outlined in Urine Culture; early morning specimen is preferred; collect 50 mL of urine and submit in sterile collection container; series of three specimens collected on three separate days is recommended
- **Cerebrospinal fluid specimen:** Specimen volume for testing is critical to assure isolation of the AFB; 10 mL of CSF is recommended; collect in sterile container
- **Fecal specimen:** Feces are not routinely cultured for AFB unless being ordered on an HIV positive patient suspected of having Mycobacterium avium infection; collect approximately 50 grams of stool specimen in a clean, leakproof container
- **Blood specimen:** Call Service Center Microbiology Department for specific collection instructions and appropriate collection tubes
- **Tissue, Pus, Exudate specimen:**
 1. Pus or Exudate: may collect utilizing culture swab system; specimen may also be collected on small pieces of sterile bandage material place in sterile leakproof container
 2. Tissue biopsy: send in sterile leakproof container with a small amount of sterile saline added to prevent drying
- **Other body fluids specimens:** Specimens such as pleural, pericardial, and joint fluid may be tested; collect in sterile container such as a syringe; cap syringe appropriately - do not submit with needle attached

General AFB Collection Reminders:

1. Collect in a sterile, leakproof container. [Exception - fecal specimen, see previous section]
2. A series of three specimens collected on three separate days is recommended for urine, gastric, and sputum specimens.
3. Transport specimen within 24 hours.
4. If specimen will not be transported immediately, refrigerate specimen.
5. Ship whole blood at ambient temperature.

Anaerobe Culture:

- Acceptable Anaerobe Culture Sources/Sites:
 1. Any closed abscess not of bowel origin [Aspiration by needle and syringe; surgically obtained tissue]
 2. Urine [Suprapubic needle aspiration of the bladder]
 3. Pulmonary [Percutaneous transtracheal aspiration of lower respiratory secretions – protected bronchial brush catheter is of questionable utility due to possible anaerobic contamination]
 4. Female Genital Tract [Peritoneal fluid by culdocentesis; Endometrium via protected catheter]
 5. Soft tissue, bone and joint [Percutaneous needle aspiration –after prior surface decontamination, preferably an uninvolved surface; surgically obtained tissue]
 6. Sinus tract and deep wound [Aspiration by needle or plastic intravenous type catheter threaded into infected site – after prior surface decontamination]
 7. Blood and other normally sterile body fluids other than urine [Anaerobic bottle for blood; syringe or anaerobic transport tube for other fluids]

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- **Unacceptable Anaerobe Culture Sources/Sites: [Will not be tested]**
 1. Abscesses of bowel origin including appendiceal and perirectal abscesses
 2. Feces, rectal swabs and colostomy discharge [when clinically indicated, these types of specimens may be used for the diagnosis of botulism and for intestinal disease caused by *Clostridium difficile* and *Clostridium perfringens*]
 3. Gastric specimens
 4. Superficial skin lesions, skin ulcers and pilonidal sinus
 5. Abdominal wounds contaminated with feces [eg. open fistulas] and exudative wounds not properly collected [must exclude skin contamination]
 6. Surgical drain sites
 7. Voided or catheterized urine and Foley catheter tips
 8. Vaginal or cervical specimens including lochia
 9. Prostatic or seminal fluid
 10. Throat and nasopharyngeal swabs and oral secretions
 11. Sputum and bronchoscopic specimens
 12. Gingival swabs

Anaerobe Collection Guidelines:

- **Needle or Catheter Aspiration Collection:**

1. Aspirated material is preferred.
2. Aspirate material with a long needle or intravenous-type catheter into a sterile syringe. Remove needle or catheter, tightly cap syringe, and submit for testing. DO NOT transport syringe with needle attached.
3. Soft tissue infections may be cultured by injection of 1-2 mL sterile saline into the infected site and then aspirate saline/tissue fluid with syringe system.
4. Portion of aspirated material may also be transferred to anaerobic swab collection system and handled as outlined under "Swab Collection".
5. Aspiration Method Reference:

| <u>Source</u> | <u>Method</u> |
|---|--|
| Lower Respiratory Secretions | Percutaneous transtracheal aspiration |
| Closed Abscess or Body Fluid | Aspiration by needle and syringe. Swab method may also be used if material is scanty in amount |
| Urine | Suprapubic aspiration |
| Sinus tract, uterine cavity, deep wound, etc. | Decontaminate site with Betadine; Syringe aspiration using plastic intravenous type catheter threaded into infected site |
| Endometrial Technique | Through a speculum, a catheter is introduced into the cervical os and a swab extended through the catheter into the endometrial cavity |
| Pelvic Inflammatory Disease | Swab vagina with betadine and aspirate through posterior vaginal wall (culdocentesis) |

Specimen Collection, Handling, and Special Instructions

- **Swab Collection:**

Note: Submission of fluids for anaerobic culture via aspirate in a syringe with air expelled is far superior to swabs, which readily allow oxygen exposure and decrease anaerobic culture yield.

1. Only use approved anaerobe culturette swabs. Swabs can be obtained by contacting your Service Center. Swabs should only be used to collect scanty material. Aspiration specimens are preferred when amount of specimen allows for aspiration.
2. Collect as much specimen as possible so that culturette tip is completely saturated. Place swab immediately into culturette container. Follow all culturette collection instructions on package.

Specimen General Reminders:

- Label specimen and transport as soon as possible
- When transport delay is unavoidable, specimen should be held at room temp.
- Specimen collected for anaerobic culture are also suitable for aerobic, AFB, and fungal cultures. Make sure that quantity of specimen collected is adequate for all testing requested

Blood Culture:

- Follow all "exposure control" guidelines required by your facility [i.e. gloves, lab coat, proper sharps disposal, etc.]
- Prepare venipuncture site using appropriate technique. Example technique:
 1. Palpate vein and select venipuncture site. Clean site with isopropanol scrub solution for 60 seconds or 70% Isopropyl Alcohol. Allow to air dry.
 2. Apply 2% tincture of iodine to venipuncture site starting at the center of the collection site and moving outward in concentric circles to the periphery. Allow to dry.
 3. If you must palpate after skin prep is completed, the finger of your glove must be disinfected or sterile glove must be worn.
- Draw blood culture specimens using sterile syringe or butterfly needles and sterile technique.
- Collect a minimum of 10-20 ml blood from adults (1-3 ml for pediatric). For Adult specimen, inject specimen equally into two bottles. Swab bottle cap/septum with alcohol prior to inoculation of bottle.
- Use blood culture bottles provided by your Avera LabNet Service Center.
- Label bottles with specific patient information and collection site information. Transport to Service Center as soon as possible at ambient temperature. DO NOT REFRIGERATE.

Eye Culture:

- Acceptable specimen sources: conjunctiva, cornea, eyelid margin, aqueous or vitreous. Specify "right" or "left" eye.
- Use a sterile swab from a culturette collection swab. After collection, insert the swab into the culturette holder, break the ampule at the bottom of the holder.
- Ship at ambient temperature within 24 hours of collection.

Ear Culture:

- Acceptable specimen sources: fluid obtained by tympanocentesis, scrapings from external ear.
- Transport aspirated material or material collected by needle and syringe in a sterile tube. Send scrapings in a sterile screw cap tube.
- If eardrum has ruptured and fluid is draining, cleanse the external ear canal and use a small swab to collect material. After collection insert the swab into the culturette holder and break the ampule at the bottom of the holder to release the culture holding media.
- Ship at ambient temperature within 24 hours.

Specimen Collection, Handling, and Special Instructions

Fungus Culture:

- Acceptable specimens: respiratory tract fluids (sputum, bronchial washing, transtracheal aspirates, bronchoalveolar lavage, bronchial brushings), urine, CSF, exudates, abscess contents, secretions, vaginal material, skin, nails, hair, tissue and whole blood. Swab specimens are acceptable on vaginal specimens only.
- Collect specimen in a sterile, leakproof container.
- Tissue specimens should be placed in a small amount of sterile saline to prevent dehydration.
- Contact Service Center Microbiology Department for special blood collection instructions and/or appropriate supplies.
- All specimens should be sent within 24 hours of collection.
- Ship respiratory fluids, urine, secretion, exudates and aspirates on cold pack.
- Ship tissue, skin, nails, hair, CSF and whole blood at ambient temperature.

Genital Culture:

- Collect vaginal, cervical or urethral specimen using the swab from a culturette collection swab.
- After collection, insert the swab into culturette holder and break the ampule at the bottom of the holder to release the culture holding media.
- Identify collection site on test requisition.
- Ship at ambient temperature within 24 hours.

Miscellaneous Culture (Wound, Body Fluid, Aspirates, etc.):

- Acceptable specimen sources: deep wounds, abscesses, aspirates, CSF and other body fluids or sites.
- Collect specimen using the swab from a culturette collection swab, by aspiration (depending on specimen source) or by placing collected fluid specimen in a sterile leakproof container.
- Swab Specimen: After collection, insert the swab into culturette holder and break the ampule at the bottom of the holder to release the culture holding media.
- Ship specimen at ambient temperature as soon as possible, preferably within 24 hours.

Sputum Culture:

- Patient should gargle and rinse mouth with water prior to collection.
- Collect 5-10 ml early morning sputum in a sterile, leakproof container. Specimen collected using deep cough technique is preferred.
- Specimen submitted for testing should not appear to be only "clear saliva". Specimen must contain "thick, purulent like" material.
- Transport specimen as soon as possible.

Stool Culture:

- Specimen of choice: fresh stool collected as soon after onset of symptoms as possible (3-5 days).
- Collect 1-2 grams fresh stool in a clean, dry container. Do not let urine or water from the toilet touch the specimen. Place specimen in Modified Cary Blair transport media.
- Ship at ambient temperature as soon as possible, preferably within 24 hours.

Throat/Nose Culture:

- Rub posterior of the tonsils, soft palate and back wall of the lower pharynx or swab nares with a sterile culturette collection swab.
- After collection, insert the swab into a culturette holder and break the ampule at the bottom of the holder to release the culture holding media.
- Ship specimen at ambient temperature as soon as possible, preferably within 24 hours.

Specimen Collection, Handling, and Special Instructions

Urine Culture:

- Preferred specimens include: first morning specimen collected as a clean-catch midstream specimen or catheterized specimen. All non-catheterized specimens must be collected as clean-catch midstream specimens.
- Patient instruction on correct procedure to follow for obtaining a midstream collection is very important. Example collection instructions on next page may be copied and used for patient education as needed.
- Transport as soon as possible. Refrigerate immediately following collection and send the same day. Or transfer an aliquot to a urine culture transport kit and transport within 24-48 hours.

Specimen Collection, Handling, and Special Instructions

Male Patient Midstream Urine Collection Instructions:

IMPORTANT:

Please Read All Instructions Before Starting Collection Of Urine

1. Hold foreskin back, if present. Wash the end of the penis with a soap towelette or gauze pad provided and then rinse the end of the penis with two more towelettes or gauze pads moistened with warm water. DO NOT throw towelettes or gauze pads in the toilet. Discard in wastebasket.
2. Continue to hold the foreskin back, if present, and begin to urinate into the toilet. After the first few teaspoons of urine has passed, place the urine collection cup under the urine stream and collect the rest of the urine in the container or until the cup is close to full. Do not overflow the cup. The amount collected is not critical, even one-fourth cup is an adequate sample for the test.
3. Do not touch the inside of the sterile cup before or after the specimen is collected. Always hold the cup on the outside surface.
4. When you have finished, replace the lid on the cup and tighten securely. Wash any spilled urine from the outside of the cup.
5. Make sure that your full name is correctly written on the cup.
6. Notify the facility personnel that the specimen has been collected.

GUÍA PARA COLECCIONAR MUESTRA DE ORINA LIMPIA:

(Guidelines for Collecting Clean Catch Urine Specimens (Males, Spanish))

Lea todo el procedimiento antes de empezar.

Note: La colección apropiada de su muestra nos ayudará a proveerle a su doctor resultados más precisos.

CABALLEROS:

1. Obtenga lo siguiente del estante por encima del lavabo:
 - a. toallita de jabón (soap towelette)
 - b. 2 gasas 4x4 toallitas remojadas en agua tibia
 - c. 1 gasa 4x4 toallita seca
2. Jale para atrás el prepucio y detengalo con una mano si es necesario. Use la toallita de jabón para lavar la punta de su pene. Luego tirela en la basura, no en la taza del baño.
3. Continúe deteniendo la piel hacia arriba y con cuidado limpie la punta del pene con una gasa remojada en agua tibia, y luego con la otra también. Hechelas en la basura y con la almohadita seca, seque la punta de su pene.
4. Continúe deteniendo hacia atrás su piel y empiece a orinar en la taza del baño. Después de orinar un chorrillo, coloque el envase en el chorro y colecciona el resto de la orina sin dejar que este vacee por favor. Después que termine, asegure bien la tapadera del contenedor y devuélvalo al empleado

Specimen Collection, Handling, and Special Instructions

Female Patient Midstream Urine Collection Instructions:

IMPORTANT:

Please Read All Instructions Before Starting Collection Of Urine

1. With two fingers of one hand, hold the outer folds of the vagina apart. With the other hand gently wash the vaginal area from front to back with a soap towelette or gauze pad provided and then rinse the area front to back with two more towelettes or gauze pads moistened with warm water. DO NOT throw towelettes or gauze pads in the toilet. Discard in wastebasket.
2. Continue to hold the outer folds of the vagina apart and begin to urinate into the toilet. Lean forward slightly so that the urine flows directly into the toilet without running along the skin surface. After the first few teaspoons of urine has passed, place the urine collection cup under the urine stream and collect the rest of the urine in the container or until the cup is close to full. Do not overflow the cup. The amount collected is not critical, even one-fourth cup is an adequate sample for the test.
3. Do not touch the inside of the sterile cup before or after the specimen is collected. Always hold the cup on the outside surface.
4. When you have finished, replace the lid on the cup and tighten securely. Wash any spilled urine from the outside of the cup.
5. Make sure that your full name is correctly written on the cup.
6. Notify the facility personnel that the specimen has been collected.

GUÍA PARA COLECCIONAR MUESTRA DE ORINA LIMPIA:

(Guidelines for Collecting Clean Catch Urine Specimens (Females, Spanish))

Lea todo el procedimiento antes de empezar.

Note: La colección apropiada de su muestra nos ayudará a proveerle a su doctor resultados más precisos.

DAMAS:

1. Obtenga lo siguiente del estante por encima del lavabo:
 - a. toallita de jabón (soap towelette)
 - b. dos gasas 4x4 toallitas remojadas en agua tibia
 - c. 1 gasa 4x4 toalla seca
2. Baje su ropa interior o calzón abajo de sus rodillas de esa manera no interferirá con la colección. Con dos dedos de su mano, detenga la parte de afuera de su vagina. Con la otra mano con cuidado lave su vagina de enfrente hacia atrás usando la toallita de jabón (mire figura). Tire la toallita en la basura, no en la taza del baño.
3. Todavía agarrando la parte de afuera de su vagina, retirándola del orificio por donde orina, enjuage el área de enfrente hacia atrás usando la primera gasa remojada 4x4 con agua tibia y prosiga hacer lo mismo con la segunda. Por último, séquese el área de enfrente hacia atrás con la gasa seca. Luego tire todas las toallas o gasas en la basura.
4. Continúe agarrando su parte de afuera de la vagina y empiece a orinar en la taza del baño. Hagase un poquito hacia en frente para que la orina caiga directo hacia abajo sin mojar su piel. Después de orinar un chorrillo, coloque el envase en el chorro y colecte el resto de la orina. No permita que rebose.
5. Después que termine, asegure la tapadera del contenedor bien cerrada y devuélvalo al empleado.

Specimen Collection, Handling, and Special Instructions

Microbiology Miscellaneous Special Instructions:

Pin Worm Cellophane Tape Collection General Instructions:

- Specimens are best obtained a few hours after the patient has retired, or the first thing in the morning before a bowel movement or bath. If the patient is a child still in diapers, the specimen should be collected between 10:00 p.m. and midnight [child should have not just defecated prior to collection].
- To assure recovery of parasitic elements that may be passed intermittently and in fluctuating numbers, it is recommended to collect three specimens on different days [multiple specimens should not be collected on the same day]. The number of specimens to be tested must be defined by the ordering physician.
- Supply the patient with the appropriate amount of wooden tongue depressors, glass slides, and clear cellophane tape [or pinworm collection kits] to collect the required number of specimens.
- General instructions for collection should include:
 1. Attach cellophane tape to the wooden tongue depressor with the sticky side out. Approximately 2-3 inches of sticky surface should be sufficient.
 2. Hold the tongue depressor in one hand. With the other hand, separate the patient's buttocks.
 3. Firmly press the tape collection area to all the skin directly around the anus.
 4. Remind the person collecting the specimen that the eggs are not visible to the naked eye.
 5. Remove the tape from the tongue depressor avoiding contact with the sticky tape area as much as possible.
 6. Spread the tape, sticky side down, on the microscope slide provided. Smooth the tape down with a cotton ball or tissue.
 7. Remind the person collecting the specimen, to wash their hands, including under the nails, after collecting the specimen.
 8. If multiple specimens are being collected, specimens may kept until the last specimen is collected and then all returned to the laboratory at the same time.
 9. Instruct the person collecting the specimen to label each slide collected with the patient's full name and date of collection.

Ova & Parasite General Instructions:

- Patient instruction on correct procedure to follow for obtaining specimen utilizing the appropriate ova & parasite collection system is very important. Package inserts or collection instructions on next page may be copied and used for patient instruction as needed.
- The patient should be cautioned against the use of antacids, barium, bismuth, antidiarrheal medication, or oily laxatives prior to collection of the specimen.
- The patient should be reminded that the specimens collected should not come into contact with urine or toilet water.
- To assure recovery of parasitic elements that may be passed intermittently and in fluctuating numbers, it is recommended to collect three specimens on different days [multiple specimens should not be collected on the same day]. The number of specimens to be tested must be defined by the ordering physician.

Specimen Collection, Handling, and Special Instructions

Patient Ova & Parasite Collection Instructions:

IMPORTANT:

Please Read All Instructions Before Specimen Collection Is Completed

You have been given a collection kit, which will help you conveniently collect a stool specimen for testing that your physician has ordered. All directions must be closely followed to assure the best possible specimen for testing. The kit you have been given may include multiple containers. Please be sure that you have put some of your specimen in all the tubes. If you are instructed to collect multiple specimens on different days, you will be given the appropriate number of collection kits for the days required.

CAUTION:

- Solutions in the collection containers are poisonous. DO NOT DRINK. Keep them out of the reach of children.
 - Antidote If Swallowed: Dilute by drinking 2-4 glasses of water. Immediately contact an emergency facility, poison information center or a physician to receive medical attention. Save the collection container; label information will be helpful for determining appropriate medical treatment.
- If any liquid from the collection containers gets on your skin or in your eyes, flush with plenty of water. If irritation develops, consult your physician.

Collection Instructions:

1. The stool should be passed into a clean, DRY container. Use a bed pan or place a large plastic bag into a waste basket to catch the specimen. A clean margarine tub, clean wide-mouthed jar or clean milk carton with the top cut off can also be used.
2. Do not urinate in the container. The stool specimen must not come into contact with urine or toilet water. Do not pass the specimen directly into the collection kit containers.
3. After the stool is collected, open the kit container. Using the collection spoon built into the lid of the container [or wooden sticks, if supplied] place small scoops of the stool from areas which appear bloody, slimy or watery into the container. If the stool is formed [hard], please try to sample small amounts from each end and the middle. Continue to add specimen level reaches the "fill to here" line or indicator. DO NOT contaminate the outside of the collection container with the stool sample.
4. Mix the stool sample with the liquid in the container with the spoon or wooden sticks. Twist the cap tightly closed and shake the container vigorously until the content is well mixed.
5. Repeat steps 3 and 4 until all kit containers have been filled with stool specimen. After all containers have been filled, the remaining stool specimen may be discarded.
6. Double check all caps to be sure they are tightly closed.
7. Fill in all information required on each container. Be sure to check the box on the container which describes the consistency of the specimen you collected. [Formed = distinct shape and hard; Soft = distinct shape but soft; Loose = no distinct shape, thick sludge-like; Watery = very loose, liquid-like]
8. Wash hands thoroughly after collection is complete.
9. Store collected specimens at room temperature and return them to the laboratory as soon as all required specimens have been collected.

Specimen Collection, Handling, and Special Instructions

Virology Collection Guidelines & Special Instructions:

Viral specimens should be collected early in the illness. Certain types of viral agents associated with different types of clinical syndromes are more easily isolated out of different types of specimens. The "Practical Medical Virology: Guide to Specimen Collection" chart included in this section is provided to help guide you and your ordering physician to proper specimen collections. This guide will also outline appropriate tests your physician may order [culture vs. direct staining techniques/DFA] to identify viral agents.

With the exception of sterile body fluids, specimens should be inoculated into a viral transport medium [or utilization of Viral Culturette system]. You Avera LabNet Service Center will provide either Viral Transport Media or Viral Culturette system depending on specific reference lab requirements. In situations where no viral transport medium is available, the laboratory will accept specimens on moist swabs or in clean containers if they are kept cool and are transported to the service center within 6 hours of collection. Because many viruses are labile, it is always best to collect the specimen just prior to transport to the Service Center.

NOTE: Culture site is required on request form for processing. Indicate the specific viruses or clinical syndromes testing is to be completed for. Transport specimens within 24 hours of collection.

Suitable Collection Sites for Viral Culture:

1. Oral-pharyngeal swabs (deep throat, not nasopharyngeal swabs)
2. Throat washings
3. Nasopharyngeal aspirates
4. Stool or rectal swabs
5. Spinal or other body fluids
6. Urine
7. Vesicular fluid
8. Eye exudate
9. Biopsy or autopsy tissue
10. Buffy coat from blood or anticoagulated whole blood
11. Sputum
12. Skin or mucous membrane lesions

Unacceptable Specimens for Viral Culture:

The following specimens will be rejected:

1. Swabs that have dried
2. Specimens not in viral transport medium or collected with viral culturette system (moist swab specimen exceeds 6 hour time limit)
3. Specimens determined to be of no clinical value after consultation and concurrence with the attending physician

Specimen Collection, Handling, and Special Instructions

Site Specific General Guidelines:

Oral-pharyngeal Specimens:

- Deep throat swabs are collected by vigorously rubbing the tonsils and posterior nasal passages with a sterile swab [may use viral culturette if available from your Service Center].
- The swab is then placed in the Viral Transport Medium for 30 minutes or follow appropriate viral culturette system instructions.
- After the 30 minutes, the swab is wrung out several times by pressing the swab against the side of the vial. After completion of this step, **the swab must be removed and discarded.**
- Throat washing may be collected and placed in the Viral Transport Medium.
- Nasopharynx specimens may be obtained by inserting a flexible N-P wire collection swab into the posterior nasopharynx or by aspiration of the secretions with a one-ounce bulb. - **Note: Nasal aspirates yield better results due to the increase of cell volume collected.**

Nasopharyngeal aspirates:

- Collect nasopharyngeal aspirates with a suction catheter into a sterile container.
- A nasopharyngeal wash may be collected by placing 5 ml of sterile saline delivered into one nostril and aspirated to collect the wash. Repeat with the other nostril.
- General Collection Procedure:

Aspirate:

- Attach mucus trap to suction pump and catheter, leaving wrapper on suction catheter. Turn on suction and adjust to suggested pressure.
- Without applying suction, insert catheter into the nose, directed posteriorly and toward the opening of the external ear. Note: Depth of insertion necessary to reach posterior pharynx is equivalent to distance between anterior nares and external opening of the ear.
- Apply suction. Using a rotating movement, slowly withdraw the catheter.
- Label and transport to laboratory at refrigerated temperature [2-8 C].

Washing:

- Suction 3.0-5.0 ml of sterile saline into a new sterile bulb.
- Insert bulb into one nostril until nostril is occluded.
- Instill saline into one nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
- Empty bulb into suitable, dry, sterile specimen container.
- Label and transport to the laboratory at refrigerated temperature [2-8 C].
- Inoculate collected specimen into viral transport medium if a viral culture is requested.
- If only a DFA, Influenza A Antigen or RSV is requested then send the specimen in a sterile container.
- If both culture and DFA are requested, split specimen and handle as outlined.

Stool or Rectal Specimens:

- Collect 2-5 grams of stool in a clean container.
- Place a small pea-size portion of freshly passed stool into the viral transport medium and break up with an applicator stick.
- A rectal swab is acceptable only if unable to collect a stool for viral culture. To collect, insert a moistened swab 2-3 cm into the anal orifice and rotate. Place swab in viral transport medium for 30 minutes. After 30 minutes, wring out several times by pressing the swab against the side of the vial. **Remove swab and discard.**
- If sending a specimen for Electron Microscopy, do not place in viral transport medium. Place specimen in sterile leakproof container. DO NOT freeze.

Specimen Collection, Handling, and Special Instructions

Urine:

- A voided urine should be collected in a sterile container.
- Immediately transfer 2 mLs of the urine into viral transport medium.
- Send both the inoculated viral transport medium and the remaining urine in the sterile container for testing.

Spinal Fluid or Other Body Fluids:

- Body fluids should be collected in appropriate sterile tubes.
- As soon as possible send specimen to the Service Center.
- If the fluid is thought to be contaminated with bacteria, 0.2 to 0.3 ml of viral transport medium may be added to the specimen.
- If spinal fluid is to be submitted, collect at least 1 mL of CSF; 2-3 mL is preferred.

Vesicular Fluid and Lesions:

- Collect the specimen within 3 days of the eruption.
- Carefully wash the surface of vesicle with 70% ethanol.
- Aspirate the vesicle fluid with a tuberculin syringe.
- Place the aspirated fluid in viral transport medium or utilize viral culturette system.
- A vesicle may be opened up with a sterile blade and then the lesion rubbed with a swab. Place the swab in viral transport medium for 30 minutes. After 30 minutes, wring out swab by pressing the swab against the side of the vial. **Remove swab and discard.**
- Swabs from vesicles may be placed in viral transport medium already containing vesicle fluid.

Eye Exudates:

- Eye exudates from the palpebral conjunctivae are collected on a sterile swab or utilize viral culturette system if available from your Service Center.
- Place the swab in viral transport medium for 30 minutes. After 30 minutes, wring out swab by pressing the swab against the side of the vial. **Remove swab and discard.**

Sputum:

- Collect a deep cough sputum specimen in a sterile container.
- Place a sterile swab in the sputum specimen and coat the swab with the specimen.
- Place the swab in viral transport medium for 30 minutes. After 30 minutes, wring out swab by pressing the swab against the side of the vial. **Remove swab and discard.**

Skin or Mucous Lesions:

- Vesicles or pox should be ruptured and the base of the underlying ulcer scraped with a sterile swab to obtain both cells and vesicle fluid or may utilize viral culturette system if available from your Service Center.
- Place the swab in viral transport medium for 30 minutes. After 30 minutes, wring out swab by pressing the swab against the side of the vial. **Remove swab and discard.**

Biopsy of Autopsy Tissue:

- If possible, collect tissue specimen aseptically.
- Place specimen in viral transport medium. **Do not place tissue in formalin.**

Blood - Buffy Coat:

- 10 ml of blood is collected in EDTA (K₃) vacutainer or sodium heparin tube may be used.
- This specimen must reach the lab within 24 hour from collection and the laboratory should be called prior to transport of specimen.
- Lesser amounts of blood may be suitable in case of small infants.

Specimen Collection, Handling, and Special Instructions

Practical Medical Virology: A Guide to Specimen Collection & Testing

Reference: USD-SM Clinical Research Virology Laboratory March 25, 1998

| <u>Clinical Syndrome</u> | <u>Viral Agent</u> | <u>Specimen</u> | <u>Test</u> | |
|----------------------------------|---------------------|------------------------|------------------|---------------|
| Respiratory | | | | |
| Common Cold | Rhinovirus (common) | NS, TS | Culture | |
| | Coronavirus | NS, TS | Culture | |
| | RSV | NPA, NW | DFA | |
| | Parainfluenza | NPA, NW, TS | DFA, Culture | |
| | Influenza A, B | NPA, NW, TS | DFA, Culture | |
| | Enterovirus (rare) | TS, Stool | Culture | |
| | Adenovirus (rare) | NPA, NW, TS | DFA, Culture | |
| | Pharyngitis | Adenovirus (common) | NPA, NW, TS | DFA, Culture |
| | | EBV | | |
| | | Enteroviruses | TS, Stool | Culture |
| HSV | | TS | Culture | |
| RSV | | NPA, NW | DFA | |
| Measles | | TS | Culture | |
| Influenza | | NPA, NW, TS | DFA, Culture | |
| Parainfluenzas | | NPA, NW, TS | DFA, Culture | |
| Laryngitis | | Influenza | NPA, NW, TS | DFA, Culture |
| | | Parainfluenzas | NPA, NW, TS | DFA, Culture |
| | Rhinovirus | NS, TS | Culture | |
| | Adenovirus | NPA, NW, TS | DFA, Culture | |
| | Parainfluenzas | NPA, NW, TS | DFA, Culture | |
| Croup | RSV | NPA, NW | DFA | |
| | Influenza | NPA, NW, TS | DFA, Culture | |
| | Adenovirus | NPA, NW, TS | DFA, Culture | |
| | RSV (common) | NPA, NW | DFA | |
| Bronchitis | Parainfluenzas | NPA, NW, TS | DFA, Culture | |
| | Influenza | NPA, NW, TS | DFA, Culture | |
| | Adenovirus (rare) | NPA, NW, TS | DFA, Culture | |
| | Measles (rare) | TS | Culture | |
| | RSV | NPA, NW | DFA | |
| Bronchiolitis | Parainfluenza | NPA, NW, TS | DFA, Culture | |
| | Influenza | NPA, NW, TS | DFA, Culture | |
| | Adenovirus | NPA, NW, TS | DFA, Culture | |
| | Adenovirus | NPA, NW, TS | DFA, Culture | |
| Pertussislike | Influenza A & B | NPA, NW, TS | DFA, Culture | |
| | RSV | NPA, NW | DFA | |
| Influenza | Influenza | NPA, NW, TS, BAL | DFA, Culture | |
| | Adenovirus | NPA, NW, TS, BAL | DFA, Culture | |
| Pneumonia | Parainfluenzas | NPA, NW, TS, BAL | DFA, Culture | |
| | CMV | BAL, Lung tissue | Rapid Culture | |
| Healthy Adult | VZV | BAL, Lung tissue | Culture | |
| | Adenovirus | BAL, Lung tissue | DFA, Culture | |
| | Influenza | BAL, Lung tissue | DFA, Culture | |
| | HSV | BAL, Lung tissue | Culture | |
| | RSV | BAL, Lung tissue | DFA | |
| | Parainfluenzas | BAL, Lung tissue | DFA, Culture | |
| | Immunosuppressed | CMV | BAL, Lung tissue | Rapid Culture |
| | | VZV | BAL, Lung tissue | Culture |
| | | Adenovirus | BAL, Lung tissue | DFA, Culture |
| | | Influenza | BAL, Lung tissue | DFA, Culture |
| HSV | | BAL, Lung tissue | Culture | |
| RSV | | BAL, Lung tissue | DFA | |
| Parainfluenzas | | BAL, Lung tissue | DFA, Culture | |
| Cutaneous/Mucous Membrane | | | | |
| Vesicular | | HSV | Lesion swab | Culture, DFA |
| | | VZV | Lesion swab | DFA, Culture |
| | Enterovirus | TS, Stool, Lesion swab | Culture | |
| Exanthematous | Measles | | | |
| | Rubella | | | |
| | Adenovirus | TS, Stool | Culture | |
| | Enterovirus | TS, Stool | Culture | |
| | CMV | TS, Urine | Rapid Culture | |

Specimen Collection, Handling, and Special Instructions

Ocular

| | | | |
|----------------|--------------------------|--|---|
| Conjunctivitis | Adenovirus HSV VZV | Conjunctival swab Conjunctival swab Conjunctival swab | Culture Culture, DFA Culture, DFA |
| Keratitis | Adenovirus HSV VZV | Corneal scraping Corneal scrap, lesion Corneal scrap, lesion | Culture Culture, DFA Culture, DFA |

Infectious Mono.

| | | | |
|------------------------|--|------------------|---------------|
| EBV | | | |
| CMV (heterophile neg.) | | TS, Urine, Blood | Rapid Culture |
| HIV | | | |

CNS

| | | | |
|---|--|--|--|
| Encephalitis | HSV VZV Togavirus (EEE, WEE, etc.) Adenovirus Mumps Influenza Enterovirus HIV | Brain biopsy CSF CSF, TS, Stool CSF, Urine TS, CSF TS, CSF, Stool | Culture Culture Culture Culture Culture Culture |
| Meningitis | Enterovirus HSV Mumps | CSF, TS, Stool CSF, lesion swab CSF, Urine | Culture Culture Culture |
| Progressive multifocal Leukoencephalopathy | Papovavirus (JC) | | |

Cardiovascular

| | | | |
|--------------------------|---------------------------------|---|-------------------------------|
| Myocarditis/Pericarditis | Enterovirus CMV Influenza | TS, Stool, Endocardial bx. TS, Urine, Endocardial bx. TS, Endocardial bx. | Culture Culture Culture |
|--------------------------|---------------------------------|---|-------------------------------|

Digestive Tract

| | | | |
|-----------------|--|---|---|
| Gastroenteritis | Rotavirus Adenovirus Norwalk Enterovirus Astrovirus Calcivirus CMV | Stool Stool Stool Stool Stool Stool Stool | EM EM, Culture EM EM, Culture EM EM Rapid Culture |
| Colitis | CMV | Stool | Rapid Culture |
| Proctitis | HSV | Lesion, rectal swab | Culture |
| Hepatitis | Hepatitis A Hepatitis B EBV CMV Adenovirus HSV | Liver bx. biopsy Liver bx. biopsy Liver bx. biopsy | Rapid Culture Culture Culture |

Abbreviation Key:

| | | | |
|----------------|------------------------------|----------------------|-----------------------------|
| BAL | bronchoalveolar lavage | Lg | lung tissue |
| CMV | cytomegalovirus | NPA | nasopharyngeal aspirate |
| CSF | cerebrospinal fluid | NS | nasal swab |
| Culture | viral culture | NW | nasal wash |
| DFA | immunofluorescent assays | Rapid Culture | Rapid CMV Culture |
| EBV | Epstein-Barr virus | RSV | respiratory syncytial virus |
| EEE | eastern equine encephalitis | TS | throat swab |
| EM | electron microscopy | VZV | varicella-zoster virus |
| HIV | human immunodeficiency virus | WEE | western equine encephalitis |
| HSV | herpes simplex virus | | |