

# Overview

Welcome to Memorial Health System. Our laboratory is a full-service laboratory offering comprehensive clinical and anatomic pathology testing to the medical community of the Mid-Ohio Valley. We strive to maintain excellence in laboratory services and to provide you with the best professional assistance in the field of Laboratory Medicine.

Our laboratory employs more than 60 highly-trained professionals, medical laboratory technologists and technicians, and support staff. We offer state-of-the-art instrumentation, techniques, and data processing advancements that enable us to provide our clients with quality results.

This Laboratory User's Guide represents the efforts of managers, supervisors, pathologists, technologists, and clerical staff to develop a practical and comprehensive guide to Memorial Health System's Laboratory services. We trust that our services will exceed your expectations and truly enhance the care you provide your patients. **EVERY TEST IS OUR MOST IMPORTANT!**

# Personnel

Marietta Memorial Hospital, Selby General Hospital and Belpre Medical Campus is part of the total health care program of Marietta Memorial Hospital. The laboratory uses the most modern technology to provide a variety of tests and service for inpatients as well as outpatients. Outpatient services from MMH lab are dedicated to providing the community with cost –effective laboratory testing, continuously improving and expanding available services and total customer satisfaction. Marietta Memorial Hospital, Selby General Hospital and Belpre Medical Campus is a technologically advanced laboratory staffed by experienced medical technologists providing services **24 hours a day, 365 days a year.**

## **Quality Control**

Marietta Memorial Hospital participates in various quality control programs such as the College of American Pathologists Proficiency Testing Program and the Ohio Department of Health Testing Program.

## **Accreditation and Licensing**

- Department of Health and Human Services (DHHS)
  - College of American Pathologist (CAP)

## **Professional Staff**

- Associate Pathologist            F.R. Macatol, MD, FACP, FCAP (374-1499)
- Chief Pathologist                Matthew Macatol, MD, (374-1490)
- Laboratory Director            Heidi Willil, MT, ASCP (374-1439)
- Blood Bank                        Shelly Baylor, MT, ASCP (374-1432)
- Chemistry/Toxicology          Clarenda Fragale, MT, ASCP (374-1676)
- Hematology                        Christie Flanigan, MT, ASCP (374-1523)
- Histology/Cytology              Ken Schoolcraft, MT, ASCP (374-1696)
- Microbiology/Immunology      Suzanne Williams, MT, ASCP (374-1401)
- Specimen Processing            Heather Butler, MLT, ASCP (568-5640)

# Scope of Service

## **Billing Services**

Our commitment is to maintain customer satisfaction. We look forward to helping you with your billing questions.

The billing office is open from 8:00 a.m. to 5:00 p.m. Monday through Friday. Please contact us through our Client Services at (740) 374-1431. For billing questions, please contact the Cashiers Office at 374-1476 or Kim Ward at 374-1403.

## **Courier Services**

Courier services are available for transporting specimens to throughout MHS from your location. This service provides delivery of specimens (including frozen) under controlled conditions. Special courier services will be established if appropriate arrangements can be made. Pick-up frequency is determined by referral volume.

## **Laboratory Tours**

We are proud of our laboratory, our technical capabilities and the people who work together to provide the highest quality laboratory services to the medical community. We welcome the opportunity to show our lab to current and prospective clients and their staff members. Please call the laboratory to arrange a visit with us.

## **Referral Testing**

Our laboratory is a full service lab. We perform a wide variety of tests at our own facilities; however, a few highly complex procedures are referred to reliable reference labs, primarily Quest Diagnostics.

## **Repeat Testing**

Repeat determinations will be performed at no charge if, in the opinion of the physician, a distinct variance exists between the clinical picture and the laboratory result. For this reason, we routinely store most of the serum specimens for 3 days. Other specimens are kept for shorter time periods according to their stability. Please contact the laboratory for details regarding repeat testing.

## **Supply Request**

Marietta Memorial Laboratory provides all forms and supplies necessary for the collection and transport of our specimens for testing. Please completely fill out one of our Supply Order Forms and return it to the lab by courier or by fax:

Fax: 740-374-1766

We attempt to process and deliver your orders as quickly as possible.

# Test Requisition Information

Marietta Memorial provides requisition forms for your convenience; one to be used for clinical testing and the other for cytology or surgical pathology testing. The forms are preprinted with the client's name, address, and client number to facilitate test ordering and billing.

## Billing Information

Marietta Memorial Laboratory routinely bills most major medical insurance carriers, as well as smaller local carriers. In addition, we participate in many managed healthcare delivery systems.

Please check the appropriate boxes and submit all necessary billing information on the test requisition form.

### Private Patient

- Name of Patient
- Patient's Social Security Number
- Date of Birth
- Sex
- Name of responsible party, if other than patient
- Current address
- Telephone number with area code
- Written diagnosis and/or ICD-9 code

### Private Insurance

- Name of Patient
- Patient's Social Security Number
- Date of Birth
- Sex
- Current address
- Telephone number with area code
- Name and address of insurance company
- Written diagnosis and/or ICD-9 code
- Employer's name and address
- Copy of both sides of insurance card

### Medicare

- Name of Patient
- Patient's Social Security Number
- Date of Birth
- Sex
- Current address
- Telephone number with area code
- Medicare number
- Written diagnosis and/or ICD-9 code
- Copy of both sides of insurance card

# Test Requisition Information

## Filling Out The Laboratory Requisition Forms

- If there is more than one physician in practice, please circle or check box by the name of the referring physician to avoid delays in receiving results
- Clearly print the patient's full name, address, sex, date of birth, and social security number in the appropriate spaces.
- Check the appropriate billing category and complete the required information.
- Write the ICD-10 code(s) and any other pertinent clinical information, including medications, in the appropriate space.
- Specify tests to be performed by checking the appropriate boxes or writing miscellaneous tests in the space provided. Try to avoid using abbreviations for miscellaneous tests to avoid delays in processing.

# Specimen Collection and Preparation

## Handling and Processing of Blood Specimens

There are multiple factors associated with the handling and processing of laboratory specimens that can introduce test result inaccuracy both before the specimen has been obtained and after it has been collected. These pre-analytical factors can produce pre-analytical changes that result in erroneous lab test results. Examples include:

- Failure to draw a patient at correct time (fasting, post prandial, pre or post medication)
- Failure to centrifuge specimens in a timely manner
- Hemolysis secondary to venipuncture technique or specimen mishandling
- Analyte concentration changes due to evaporation
- Incorrect storage temperature
- Using improper Vacutainer tube with inappropriate additive
- Incorrect transport
- Improper amount of blood in tube
- Clotted or partial clotted specimens for tests requiring whole blood

## Labeling Specimens

Label the specimen(s) appropriately with the following information at bedside:

- Two patient identifiers (Patient's name and date of birth or ssn#)
- Date of collection
- Time of collection
- Collector's initials

## Specimen Packaging

### Specimens

OSHA requires that all shipments containing clinical specimens be marked with a "Biohazard" label. Bags and labels for shipments sent to Marietta Memorial will be provided.

### Ambient Temperature (room temp)

Our standard specimen bags are designed to transport serum and urine specimens that do not require special temperatures.

### Exposure to Light

It is important to avoid exposing blood specimens for photosensitive analytes to artificial or sunlight for any length of time. Examples are Vitamin A, B6, and porphyrins. These specimens are to be protected with an aluminum wrap or equivalent.

### Refrigerated Specimens

Place specimens in the refrigerator for storage before being picked up by the courier. When packing for transport, place specimen tube or urine tube into zip-lock portion of bag with the requisition being put into the outer pouch. Place coolant in box along with any specimens in a way so that there is no direct contact of the specimens with the coolant.

# **Specimen Collection and Preparation**

## **Frozen Specimens**

Place specimen in the freezer for storage before pick up by the courier. Each pour off tube must be labeled urine, plasma, serum, etc. When packing for transport, place specimen tube into zip-lock portion of bag with the requisition being put into the outer pouch. Place coolant in box along with any specimens in a way so that there is direct contact of the specimen with the coolant.

## **Specimen Transport**

Biohazard zip-lock bags are available and must be used for the transport of all laboratory specimens.

Each bag should contain the following:

1. One patient **ONLY** per bag.
2. Requisition legibly filled out with all patient demographics, billing information, ICD-10 codes and tests ordered.
3. Labeled Specimens

## **Rejection of Specimens**

As part of our quality assurance program and as a part of requirements of various certifying agencies, we have developed the following list of specimen rejection criteria. These criteria were developed with the intention of ensuring accurate, meaningful patient results.

### **Unsatisfactory Information**

- All specimens must be properly identified by full name. All specimens for blood group and type testing must be labeled with the patient's name, date of birth or social security number, date/time and initials of phlebotomist.
- All specimens must be accompanied by a requisition that includes name, birthdate, sex, date/time of collection, and name of ordering physician.
- The source of the specimen should be noted when appropriate.
- A specimen not labeled properly may in many circumstances be discarded.

### **Inadequate Specimen Due to Collection and Transportation Problems**

- Contamination of the specimen
- Insufficient specimen for test requested, such as quantities less than those stated in the manual.
- Collection in improper container (incorrect anticoagulant, unsterile containers for cultures, improper preservatives, etc.)
- Failure to follow special instructions (draw on ice, protect from light, separate immediately)
- Prolonged delay in transportation.
- Marked hemolysis of serum/plasma (due to trauma to cells/difficulty drawing)
- Clotted specimen in plasma tubes.
- Hemolysis secondary to venipuncture technique or specimen mishandling
- Clotted or partial clotted specimens for tests requiring whole blood

# Specimen Collection and Preparation

## Inadequate Specimen Due To Patient Preparation

- Non-fasting patient for testing that requires fasting state.
- Incorrect preparation of patient for test.
- Specimen drawn at incorrect time (drug levels for peak and trough, glucose tolerance)

If a compromised specimen is accepted, a note will be made on the final report as to the nature of the problem and caution should be used when interpreting the results. If a specimen is rejected, the client will be contacted for recollection.

All specimens are examined upon receipt by the lab to ensure suitability for analysis. If the specimen volume is insufficient or if the specimen has been improperly handled, the reliability of the results could be compromised and the specimen will not be processed. The client will be contacted for recollection.

## Blood Collection – Performance of Venipuncture

Most lab tests are performed on anticoagulated whole blood, plasma, or serum.

- Plasma: Draw a sufficient amount of blood with the indicated anticoagulant to yield the necessary plasma volume. Gently mix the blood collection tube by inverting 8-10 times immediately after collection. If required, separate the plasma from cells by centrifuging within 30 minutes.
- Serum: Draw a sufficient amount of blood to yield the necessary serum volume. Gently mix the blood 5 times if SST tube is used. Allow blood to clot at room temperature for approximately 20 minutes. Separate serum from clot by centrifugation within 60 minutes.
- Whole Blood: Draw a sufficient amount of blood with the indicated anticoagulant. Gently mix the blood collection tube by inverting 8-10 times immediately after collection.

Blood samples used for laboratory testing are typically obtained by venipuncture. The proper procedures for routine venipuncture are outlined below. All these procedures should be conducted observing OSHA's "Universal Precautions." When collecting, processing, or handling specimens, they should be considered a biohazard source with the potential of transmitting infectious diseases.

## Venipuncture Procedure

Properly identify the patient by checking **two** identifiers (the armband, ask the patient to state his or her full name, birthday, etc.) Prepare the tubes and other equipment needed:

- Gloves
- Tourniquet
- Alcohol prep pads
- Dry cotton balls or gauze
- Appropriate evacuated tubes for testing ordered
- Holder or syringe and needle
- Adhesive pressure strip or Band-Aids
- Biohazard waste container

# Specimen Collection and Preparation

Review the request form or physician order to determine that you have the appropriate evacuated tubes. Check for diet restrictions. If the test requires that the patient be fasting, make sure that these requirements have been followed.

Position the patient so that the arm is supported by the stationary object, such as a drawing chair, drawing table, or bed. Never draw blood from a standing patient. Do not draw blood from a compromised limb (due to mastectomy, stroke, surgery, etc.) **Do not draw above an intravenous infusion!!**

Always wear gloves and work quickly so that the tourniquet does not remain on the patient's arm longer than one minute. Apply the tourniquet approximately 2-4 inches above the elbow, snug but not tight. Ask the patient to make and hold a fist.

Palpate (feel) for a vein. The most commonly used veins are the median cubital, cephalic, and basilic veins. A vein should have an elastic feel and "gives" under pressure.

Clean the chosen puncture site using the alcohol pad, starting at the center of the site, moving in a circular motion. Allow the skin to dry. Place the index finger on the vein above the puncture site, the thumb on the vein below the puncture site, and pull the skin tight to prevent the vein from "rolling."

With the needle bevel facing upward, line up the needle with the vein at an upward angle of approximately 15-30°. Puncture the vein in a rapid smooth motion, without penetrating through the vein. Push the evacuated tube forward until the back of the needle punctures the rubber stopper.

Reassure the patient. Explain that there will be slight pain associated with the procedure. Never tell the patient no matter what age that "this will not hurt."

## Order of Draw

When drawing for multiple specimen types, establish the correct order of draw to avoid contamination with additives. Draw the tubes in the following order:

1. Blood cultures
2. Red top
3. Light Blue (citrate)
4. Marble (SST)
5. Green (heparin)
6. Lavender (EDTA)
7. Pink (EDTA for Blood Bank)
8. Gray (K-Oxalate)

Fill the light blue tube until the vacuum is exhausted. Partially filled citrate tubes are unacceptable. Never pour the contents of one tube into another.

Remove the tourniquet and ask the patient to relax his/her hand. Do not keep the tourniquet on the arm for more than 1 minute. After the tube has completed filling, remove and insert other tubes as needed into the tube holder. Immediately and gently invert all additive tubes after filling.

Remove the needle and place a clean gauze on the puncture site and apply a slight pressure. Activate safe needle device immediately post draw. Dispose of the needle into an appropriate sharps container. Request that the patient hold the gauze with pressure. After labeling the tubes,

# Specimen Collection and Preparation

inspect the puncture site. After bleeding has stopped, apply an adhesive strip over the gauze. Instruct the patient to leave the bandage in place for at least 15 minutes. Dispose of all contaminated items appropriately.

At the completion of the venipuncture, be sure that the bleeding has stopped. If blood flow has not stopped, apply pressure with a fresh gauze until it does. This is critical with patients receiving anticoagulants.

## Skin Puncture Procedure

Avoid a finger that is cold, swollen, or inflamed.

1. A fresh pair of gloves must be worn.
2. With your left thumb and index finger, grasp either the patient's long or ring finger about 3 inches from the tip of the finger. Moving your left hand toward the tip of the patient's finger, apply a massaging motion to the fleshy portion of the finger.
3. Repeat this massaging process five or six times.
4. Cleanse the ball or pad of the finger with an alcohol swab. Do not use iodine to cleanse the skin.
5. Dry the ball or pad of the finger with dry gauze, to avoid hemolysis due to residual alcohol.
6. Pick up a sterile lance and remove the lancet from package.
7. With your right hand, grasp the lancet.
8. Depress the button on top of the lancet, making a deep cut on the side of the ball of the finger. The cut should be across the fingerprints.
9. If the blood flows freely, wipe away the first drop with a clean gauze. If it doesn't flow freely, hold the finger downward and apply gentle pressure just above the puncture site. If the blood does not flow easily after gentle massage, make another puncture at a different site.
10. Fill EDTA microtainer quickly, then stopper and mix thoroughly. Do not scrape the blood specimen from the finger as it may cause hemolysis. Mix well by inverting 8-10 times. Fill other microtainers as ordered.
11. Each filled microtainer tube should have the patient's name written on it. Then place microtainer tube in an empty plain red top tube for transport. Label the red top tube with the proper patient identification and your initials, date and time of draw.

## Glucose Tolerance Testing

Unless the physician tell the patient otherwise, for 3 days prior to testing the patient should eat three balanced meals each day; include bread, starches, or sweets. Beginning after dinner on the night before the test, the patient should not eat or drink anything except water until coming to the laboratory. Patients who smoke should abstain from smoking from the time they go to bed the night before until completion of the entire testing procedure.

## Specimen Preparation

### Pre-Centrifugation Phase

Strict adherence to all phases of collection and processing is essential for accurate test results.

Plasma specimens are obtained using a Vacutainer tube containing an anticoagulant. These specimens can be centrifuged within minutes after collection. Any vacuum tube containing an

# Specimen Collection and Preparation

anticoagulant should be inverted gently 8-10 times after blood collection to ensure the intended action of the additive.

Serum specimens are obtained from tubes when the blood has been allowed to clot. Prior to centrifugation, the specimen must be thoroughly clotted.

## Clotting Instructions

Clotting instructions with minimum clotting time recommendations:

- Non-additive tubes (red/plastic) – 30 minutes
- SST tubes – 30 minutes

Recommended times are based upon intact clotting process. Patients with abnormal clotting due to disease, or those receiving anticoagulant therapy require more time for complete clot formation. Separation of serum or plasma from cells should take place within 2 hours of collection to prevent erroneous test results.

When specimen requirements call for a chilled specimen, the specimen is to be immediately placed in a small plastic bag, tied and then placed in another bag of crushed ice or a mixture of ice and water. Examples requiring chilled specimen include ammonia, and lactic acid.

## Centrifugation

Blood specimens should be adequately clotted prior to centrifugation. They should be centrifuged with the stoppers in place for 10 minutes at 3,200 rpm.

All specimens collected in tubes with gel barriers should be properly centrifuged prior to transport.

When gel tubes are drawn, after centrifugation the serum is separated from the cells by the gel barrier. It is recommended that the serum be physically separated from contact with cells as soon as possible. After proper centrifugation, serum can be left in contact with the gel barrier of SST tubes for up to 5 days with proper storage.

## Safety

With normal operation, the centrifuge does not present any safety hazards. It is important to follow the listed safety precautions while operating the centrifuge:

- Never open the lid while the rotor is moving
- Balance centrifuge before operating
- If a tube spills or breaks, clean instrument by using approved cleansing procedure

## Specimen Storage

Any specimen that must be stored for more than 1 hour prior to pickup should be refrigerated, unless otherwise indicated under specimen requirements. Do not refrigerate unspun specimens.

# Specimen Collection and Preparation

Any specimen that requires freezing should be frozen as soon as possible after collection. Always freeze specimens in a plastic vial, never glass. Confirm that the specimens are properly spun, properly labeled, and accompanied by a requisition. Place the corresponding specimen(s) and requisitions into a specimen transport bag.

## Specimen Transport

Transportation should occur at correct temperature so that the specimen integrity is always maintained. Some tests require that the specimen be shielded from light. These specimens, such as those being assayed for Vitamin a, B6, and porphyrins, should be protected from light by wrapping the specimen with foil or using amber transfer tubes.

**Table 1: Marietta Memorial Hospital Lab Specimen Tube Guide**

Tube Top Color	Optimum Volume	Minimum Volume	Additive	Special Instructions	Comments
<b>Adults Blood Cultures</b>					
BD Bactec Purple and Blue top	10 mL per bottle	3 mL per bottle		NOTE: Recovery of bacteria is directly related to the volume of blood collected. Collect as much as possible to the 10 mL/btl as possible.  Special prep for puncture site: 1. Cleanse with sterile alcohol swab, outward with a circular motion. 2. Cleanse with chlorhexidine prep and allow site to dry. The tops of bottle MUST be cleansed with alcohol before dispensing the blood.	
<b>Pediatric Blood Cultures</b>					
BD Bactec Pink Top	3 mL per bottle	0.5 mL per bottle		Same as above	
<b>Other Specimen Containers:</b>					
Red top	9 mL	2 mL	None	DO NOT invert	Used for test requiring serum
Blue top (Na. Citrate) Light blue top	2.7 mL  1.8 mL	2.7 mL  1.8 mL	Sodium Citrate	Must fill blue to capacity, gently invert 8-10 times and transport to lab ASAP	Used for coagulation tests
Gold Top	6 mL	1 mL	Gel barrier	Invert 5 times	Used for test requiring serum
Mint Green Top	6 mL	1 mL	Gel barrier	Invert 8-10 times	Used for test requiring plasma
Green top	4 mL	2 mL	Sodium Heparin	Invert 8-10 times	If used for ammonia level must be

## Specimen Collection and Preparation

					collected on ice and transported to lab ASAP
<b>Tube Top Color</b>	<b>Optimum Volume</b>	<b>Minimum Volume</b>	<b>Additive</b>	<b>Special Instructions</b>	<b>Comments</b>
Lavender top	4 mL 3 mL	2 mL 1.5 mL	K2 EDTA	Gently invert 8-10 times	Must be filled at least 50% for hematology testing 100% for BNP tests
Pink top	5.5 mL	2.5 mL	EDTA	Gently invert 8/10 times	Used for Blood bank
Gray top	2.5 mL	1.0 mL	Potassium oxalate/sodium fluoride	Gently invert 8-10 times	Used for chemistry testing

Vigorous mixing of tubes may cause hemolysis. Insufficient mixing or delayed mixing in tubes with anticoagulants may result in clotting, platelet clumping and incorrect test results.

**Table 2: Commonly Used Medical Acronyms**

Acronym	Proper Name	Acronym	Proper Name
CBC	Complete Blood Count	LYTES	Electrolytes
CR	Creatinine	MG	Magnesium
CR CL	Creatinine Clearance	MN	Manganese
C/S	Culture and Sensitivity	PB	Lead
DIG	Digoxin	PO4	Phosphorous
DIL	Dilantin	PROG	Progesterone
E2	Estradiol	PROL	Prolactin
E3	Estriol	PSA	Prostate Specific Antigen
ESR	Erythrocyte Sedimentation Rate	PLT	Platelet Count
ESTRO	Estrogen	PT	Protime
FERR	Ferritin	PTT / APTT	Activated Partial Thromboplastin Time
FBS	Fasting Blood Sugar	RETIC	Reticulocyte
H&H	Hemoglobin and Hematocrit	SENS	Sensitivities
HCT	Hematocrit	TEG	Tegretol
HG	Mercury	THY	Thyroid
HGB	Hemoglobin	TP	Total Protein
K	Potassium	TSH	Thyroid Stimulating Hormone
LFT	Liver Function Tests	UA	Urinalysis
LI	Lithium	WBC	White Blood Cell Count

## Specimen Collection and Preparation

**Table 3: Quick Reference Guide For Frequently Ordered Lab Tests**

Laboratory Test	Container Type	Special Instructions
CBC H&H Sed Rate	4 mL Purple (minimum 2 mL) or 3 mL Purple (minimum 1.5 mL)	Immediately invert 8-10 times
PT PTT Fibrinogen D-Dimer Antithrombin	Blue (citrate) 2.7 mL or Light Blue (citrate) 1.8 mL	Gently invert 8-10 times after filling Must be filled to capacity
Electrolytes BMP (Chem 7) ADP (Chem 12) Sodium Potassium BUN Creatinine CPK CKMB Magnesium Glucose Amylase Lipase Digoxin Gentamycin Thyroid Panel PSA	Mint Green Lithium Heparin Tube 6mL	Gently invert 8-10 times
Ammonia	Green 4 mL	Gently invert 3-5 times and immediately put tube in bag and then in a bag of ice and transport to lab ASAP.
Blood Cultures	<b>Volume is critical</b> <i>Standard:</i> blue and purple top with 8-10 mL in each bottle <i>Pediatric:</i> pink top with 0.5-5 mL with a minimum of 0.5 ml	Preparation: - Cleanse site with sterile alcohol - Cleanse with 2% ChloroPrep - Cleanse top of bottle with sterile alcohol before dispensing blood.

# Specimen Collection and Preparation

## Urine Collection

### Clean Catch Urine:

- Remove lid of container and take care to handle outside of container only.
- Wash hands with soap and water, rinse and dry.
- For *males*, retract foreskin and cleanse the glans penis with towellete provided in clean catch kit. For *females*, spread the labia and wash the area from front to back with towellet provided in clean catch kit.
- Pass the first portion of urine into toilet and without stopping, catch the remaining urine into sterile container.
- Place the lid securely on the container and give the specimen to the technician or nurse for proper handling.

### 12 or 24 Hour Urine Collection

For testing that requires 12 or 24 hours urine collection, we provide the appropriate urine jugs. For such testing, patients should be instructed to time the collection accurately during the collection period and to collect all urine voided during the time period as described in the procedure below. If additives are needed, make sure the patient is notified of potentially hazardous preservatives that have been added to the urine jug.

1. Upon rising in the morning, urinate into the toilet and empty bladder completely. Do not collect this sample.
2. Write down (on the jug) the time, from this time forward, collect all urine voided for either 12 or 24 hours in container. Direct contact with preservatives in the collection jug may be hazardous. Patients should void into a clean container and pour the urine into the collection jug.
3. Refrigerate the collected urine between voiding.
4. At exactly the same time the next morning, void completely again and add this sample to the jug. Write down the time on the container.
5. Keep the 12 or 24 hour urine specimen refrigerated and bring to the laboratory as soon as possible.

## Microbiology Specimen Collection

### General Guidelines:

- All specimens must be collected in sterile containers.
- Collect specimen before administering antimicrobial agents when possible.
- Collect from actual infection site with as little contamination from indigenous microbial flora as possible to ensure that the sample yields reliable results.
- Collect an adequate amount of sample.
- Non-specific terms such as “wound”, “eye”, and “genital” to describe a specimen are not as useful to the lab as the names of specific anatomic location and diagnosis. Source must be on the specimen and requisition.
- The optimal times for collecting specimens must be based on both the type of infectious disease process and the ability of the lab to process the sample. The Microbiology Lab is fully staffed from 6:30 a.m. to 11:30 p.m. Monday through Friday and 6:30 a.m. to 3:00

# Specimen Collection and Preparation

p.m. on Saturday and Sunday. They are able to receive and process the specimen in a more appropriate manner.

- The first morning sputum and urine samples are optimal for recovery of acid-fast bacteria, fungi, and other pathogens because they are more concentrated and more likely to contain larger numbers of the pathogen.
- If a specimen is to be collected through intact skin, cleanse the skin with alcohol followed by 1-2% tincture iodine to prepare the site, allow a contact time of two minutes to maximize the antiseptic effect.
- 24-hour specimens are usually unacceptable except for the recovery of parasites.
- Dried, delayed, inadequate amount, externally contaminated samples, duplicate samples, leaking samples, or material sent in the wrong preservative or transport medium will be rejected at the discretion of the laboratory personnel.
- Properly label the specimen and complete the requisition.

## General Transport Guidelines

- When possible, deliver all specimens to the laboratory promptly, preferable with 1-2 hours of collection. Prompt processing minimizes loss in viability of pathogens and ensures an accurate appraisal of the different flora present.
- If a delay in transport is anticipated, a transport medium must be used.
- Wound specimens for anaerobic workup must be submitted in an anaerobic transport medium in addition to the aerobic medium. The culturettes with the gel base covers both aerobic and anaerobic conditions.
- Most specimens can be refrigerated with these exceptions:
  - Blood cultures
  - Purple tops
  - Genital specimens for *N. gonorrhoeae*
  - CSF and other body fluids except urine
  - Stool in preservative or transport medium
  - Eye and inner ear specimens
  - Specimens inoculated onto primary culture media at bedside or at a doctor's office

These specimens must remain at ambient temperature.

- Never transport syringes with needles to the lab. Transfer the contents to a sterile tube or container or cap off the syringe after safely removing the needle.

## Specific Procedure for Microbiology Specimen Collection

- Indicate suspected organisms on request for if unusual
- Try to collect the maximum volume required for each type of bottle to avoid false negative results.
- Use only the type of blood culture bottle provided by MMH laboratory. Since the BACTEC continuous monitoring system is used, bottles from other systems cannot be analyzed by our automated system.
- If you have the minimum amount of blood collected from an adult, always divide blood between BOTH bottles.
- Most cases of bacteremia are detected by using two or three sets of separately collected cultures. More than three sets yield little additional information. Conversely, a single blood culture may miss bacteremia and make it difficult to interpret the clinical significance of certain isolate organisms.

# Specimen Collection and Preparation

- In order to collect a good and representative blood culture, one must use the appropriate skin disinfection method and collect a sufficient volume of blood.

## Collection Procedure:

1. Select a different site for each culture drawn, preferable opposite sides of the body.
2. Apply the tourniquet and palpate a vein, release tourniquet.
3. Cleanse the skin with sterile alcohol. Starting in center, working outward in a circular motion.
4. Scrub the site again with iodine swab or chloro prep kit. Allow the site to dry completely.
5. Swab the rubber stopper with alcohol and allow to dry. **DO NOT TOUCH THE STERILE STOPPER AFTER IT HAS BEEN WIPED. DO NOT USE IODINE ON THE STOPPERS.**
6. Carefully reapply the tourniquet. Do not retouch the vein with your fingers.
7. Perform venipuncture, withdrawing 8-10 mLs of blood for adults and 1-5 mLs for pediatric patients.
8. Do not overfill bottles.
9. If performing a syringe draw, use a transfer device to dispense blood into blood culture bottles.
10. Each bottle has a unique identification barcode, **DO NOT** cover barcodes with the collection or ID label.
11. Check puncture site for bleeding.
12. Bandage the site, and instruct patient to leave bandage on for 15-30 minutes.
13. Transport the bottle to the laboratory at ambient temperature. **DO NOT** refrigerate or incubate bottles before transporting.

## Gastrointestinal Tract Specimens

- For a routine culture, our lab screens for Salmonella, Shigella, Campy, and E. coli 0157. Isolation of Vibrio, Yersinia, and Aeromonas require special media. Please specify on the requisition or notify the lab before specimen submission.
- Stool specimens are **NOT** recommended for screening high risk or exposed patients for Vancomycin Resistant Enterococci (VRE). Proper specimen is a rectal swab for VRE. Please specify on the requisition if stool is submitted for VRE only.
- Anaerobic studies are not performed on fecal specimens.
- Do not culture any patient that has been hospitalized for >3 days for Salmonella, Shigella, Campy, or Yersinia. Consider C-diff toxin assay.
- When stools are ordered to be collected X3, do not collect all specimens on the same day. For stool cultures, collect one specimen a day for three consecutive days. For parasites, collecting three specimens within a 7-10 day period is adequate. Multiple specimens within a 24 hour period will be rejected.
- For specimens that can be delivered within one hour to the Microbiology laboratory, routine bacterial culture for enteric pathogens and O&P examination may be performed on the same stool sample. If a stool specimen cannot be transported to the lab within one hour, use transport media with preservatives. Refer to the table below for transport media used for various stool tests.

# Specimen Collection and Preparation

- For O & P Exam: Specimens received from patients after the 4<sup>th</sup> hospital day will be rejected for O & P without prior consultation

**Table 6: Stool Specimens Transport/Suitability Guidelines**

<i>Test</i>	<i>Fresh Specimen</i>	<i>Carey Blair</i>	<i>Formalin</i>
Culture	B	Yes	No
Giardia Antigen	C	No	Yes
Cryptosporidium Antigen	C	No	Yes
Cyclospora	C	No	Yes
Microsporidium	C	No	Yes
Rotavirus Antigen	D	No	No
O&P	B	No	Yes
Clos. Difficile Toxin	D	No	No
H. Pylori Antigen	D	No	No

**B: Deliver within 1 hour at ambient temp. DO NOT REFRIGERATE.**

**C: Transport media recommended. If unable to obtain; fresh specimens can be refrigerated overnight and delivered within 24 hours at 2-8°C.**

**D: Deliver at 2-8°C within 2 hours of collection.**

## Collection Procedure

Have patient obtain stool specimen by one of the following methods:

1. Pass stool directly into a sterile, wide mouth, leak proof container with a tight fitting screw cap lid.
2. Pass stool into a clean, dry bedpan, and transfer into a sterile container with a screw cap lid.
3. Place clean plastic wrap between seat and the bowl to collect stool, then transfer to a sterile container with a screw cap lid.
4. For young pediatric patients, diapers can be lined with plastic wrap to collect stool, then transfer to a sterile container with a screw cap lid.

\*Containers must be labeled with patient information and time of collection

## Procedure Notes

DO NOT use toilet paper to collect stool. It may be impregnated with barium salts, which are inhibitory for some pathogens.

DO NOT contaminate the stool with toilet water or urine, which may interfere with the analysis.

## Transport

Transport fresh stools within 1 hour to the laboratory at ambient temperature. Use transport media if a delay is anticipated. Deliver stool in transport media at ambient temperature within 72 hours for O&P, and within 24 hours for all other tests.

## Rectal/Anal Swabs

- These specimens are submitted primarily for the detection of N. Gonorrhoeae, HSV, and anal carriage of Beta Streptococcus group A.
- Rectal swabs are acceptable only for culture of diarrheal pathogens from infants or from patients who are acutely ill. These swabs must show feces otherwise they will be rejected.
- Anal swabs are unacceptable for culture of bacterial diarrheal agents.
- Swabs are unacceptable for C. Diff toxin, O&P, and other methods for detecting parasites.

# Specimen Collection and Preparation

## Collection

**Rectal Swab:** pass the tip of the sterile swab 1 inch beyond the anal sphincter. Carefully rotate the swab, remove it, and place in transport medium. The swab should show feces.

**Anal Swab:** For *N. gonorrhoeae* cultures, swab the anal crypts inside the anal ring. Avoid fecal contamination as much as possible. Place swab in transport medium as soon as possible, or place directly onto selective medium.

Label all specimens with patient information and time of collection.

## Transport

Deliver all swabs in transport medium. Dry swabs will be rejected. For *N. gonorrhoeae*, deliver to the lab as soon as possible, preferably within 1 hour at ambient temperature. Do not refrigerate. For all other tests, deliver at ambient temperature within 24 hours.

## Genital Tract Specimens

General Considerations:

Genital specimens are submitted primarily for the detection of sexually transmitted diseases. The most common agents are *N. gonorrhoeae*, *C. trachomatis*, HSV, *Trichomonas*, and *C. albicans*. Special attention must be paid to specimen collection and selection due to the fact that most genital specimens are taken from sites harboring large numbers of normal flora. Anaerobic studies are only performed on appropriate specimen sites. If an anaerobic infection is suspected, transport specimen in an anaerobic transport tube.

Nucleic Acid detection is needed for GC and Chlamydia detection. Collection kits are available. Refer to the kit package for collection instructions.

**Table 7: Genital Specimens for Aerobic and Anaerobic Cultures**

Source	Not cultured for anaerobes	Cultured for anaerobes
Female	Cervical Swab Endocervix Perineum Urethra Vagina Vaginal/anorectum Vulva	Amniocentesis Bartholin's cyst Cervical aspirate Culdocentesis Endometrium Fallopian tube IUD Pelvic abscess Placenta from C-section Products of conception Ovary Uterus
Male	Penis Prostatic fluid Seminal fluid Urethra	

## Specimen Collection and Preparation

- Endocervical specimens are recommended for chlamydia detection by nucleic acid methodology.
- Vaginal specimens are inferior to cervical specimens due to the increased presence of normal flora that interferes with culture interpretations.
- It is recommended that Chlamydia tests be ordered with each GC request, since the two infections often occur together.
- For the detection of Group B Strep. in women, new public health guidelines suggest obtaining one or two swabs of the vaginal introitus and the anorectum. Cervical specimens are not acceptable due to a much lower recovery rate.
- Diagnosis of gonorrhea in males can often be confirmed by gram stain of the urethral exudates. For females, confirmation by gram stain cannot be done because some nonpathogenic species in the vagina may resemble the diplococcal morphology of gonorrhea.
- For GC culture requests, use Dacron swabs. Cotton fibers contain fatty acids, which are inhibitory to *N. gonorrhoeae*. DO NOT use swabs with wooden sticks. Wood resin can be toxic for chlamydia, ureaplasma, micoplasma and viruses.

### Collection procedure for females

- Amniotic Fluid: Aspirate fluid by catheter, at cesarean section, or amniocentesis. Label and transport tube in anaerobic container.
- Bartholin Gland: Decontaminate the skin with iodine. Aspirate the material from the duct. Pus from gland abscesses can sometimes be collected from the ducts with digital palpation.
- Cervix/Endocervix: DO NOT use lubricant during procedure. Warm water can be substituted. Moisten the speculum with warm water and insert speculum. Remove excess mucus from cervix and surrounding mucosa using a cotton ball. Insert a Dacron swab into the distal portion of the cervix. Rotate the swab and allow to remain 30 seconds in endocervical canal to ensure adequate sampling. Replace swab and place in transport medium. Label with patient information and deliver promptly to lab.

**Table 8: Collection Considerations for Genital Tract**

Suspected Agent	Recommended Specimens
<i>N. gonorrhoeae</i>	Cervical, urethral, anal, vaginal, urine for male/female
Group B Strept.	Vaginal/anorectum
Other bacteria	Prostatic fluid, cervical, vaginal
<i>H. ducreyi</i>	Ulcers of genitalia, perianal area, inguinal nodes
Anaerobes	Epididymis aspirate, amniotic fluid, abscess fluid
Fungus	Anal, vaginal, cervical
Herpes Simplex virus	Genital or perianal lesions ; Serum
<i>C. trachomatis</i>	Urethral, vulval, cervical; Urine for male and females
<i>M. hominis</i>	Cervical, urethral, prostatic fluid, endometrial tissue
<i>U. urealyticum</i>	Cervical, urethral, epididymis, prostatic fluid

# Specimen Collection and Preparation

- Cul-De-Sac:
  - Submit aspirate or fluid
- Endometrium:
  - Place the patient in the lithotomy position
  - Insert speculum and visualize the cervix
  - Place a narrow lumen catheter within the cervix.
  - Insert the tip of a culture swab through the catheter and collect the endometrial specimen. This method prevents the touching of the cervical mucosa and reduces the chance for contamination.
  - Place the culture swab into anaerobic transport medium and send to the lab at ambient temperature.
  - Endometrium should never be collected through the cervix with an unprotected swab. This technique will contaminate the swab with cervical and vaginal flora, the same organisms that cause endometritis.
- Fallopian Tubes and Ovaries:
  - Obtain aspirates or materials during surgery.
  - Put the specimen in an anaerobic transport media or a sterile screw cap container.
  - Label with patient information and deliver to lab promptly.
- Intrauterine Device:
  - Remove surgically to prevent cervical or vaginal contamination.
  - Place the entire device, including exudates, into a sterile container.
  - Label with patient information and deliver to lab promptly.
- Products of Conception:
  - Submit portion of tissue in a screw cap sterile container
  - Specimens obtained from a C-section must be submitted in an anaerobic transport system.
- Urethra:
  - Collect the specimen one or more hours after patient has urinated.
  - Stimulate discharge by gently massaging the urethra against the pubic symphysis through the vagina.
  - Collect the discharge with a sterile swab and insert into the appropriate transport medium.
  - If discharge cannot be obtained, wash the external urethra with betadine soap and rinse with water.
  - Insert a urethrogenital swab 2-4 cm into the endourethra, gently rotate swab, and leave in place for 1-2 seconds. Withdraw swab and submit in appropriate transport medium.
  - Label with patient information and deliver to the lab immediately.
  - Neisseria/Gonhorea/Chlamydia 1<sup>st</sup> 2-50 mL obtained without cleaning
- Vulva:
  - Clean the surface of the lesion with .85% NaCl. If there is a crust on the lesion, remove it.
  - Scrape the lesion until serous fluid emerges.
  - Wipe away fluid and debris with sterile gauze.
  - Press the base of lesion until clear fluid is expressed.
  - Aspirate vesicular fluid with a 26 or 27 gauge needle OR unroof the vesicle, and collect fluid with a sterile swab (for HSV detection) OR scrape the base of an open vesicle with a sterile scalpel blade, and then rub the base with a sterile swab (for HSV and H. ducreyi detection.)

# Specimen Collection and Preparation

## Collection Procedures for Males

- Anal/Rectal Swab
  - Refer to gastrointestinal specimens, rectal/anal swabs.
- Penile Lesion:
  - Clean the surface of lesion with .85% NaCl. If there is a crust on the lesion, remove it.
  - Scrape the lesion until serous fluid emerges.
  - Wipe away fluid and debris with sterile gauze.
  - Press the base of lesion until clear fluid is expressed.
  - Aspirate vesicular fluid with a 20 or 27 gauge needle.
  - Remove needle, recap the syringe with a sterile cap and transport the specimen to the lab immediately in the syringe.
- Prostatic Fluid:
  - This site is used primarily for the diagnosis of prostatitis. Gram negative enteric rods are the most frequently encountered pathogens for both acute and chronic form of this disease.
  - Perform a digital massage through the rectum.
  - Collect the specimen in a sterile tube.
- Urethra:
  - This is the most commonly cultured male genital site.
  - Do not allow the patient to urinate for at least one hour before specimen collection.
  - Remove the external skin flora of the urethral meatus as in preparation for a urine specimen.
  - Express exudates from the urethra, and collect the exudates on a Dacron swab, place the swab into the transport medium.
  - If exudate is unavailable, insert a urethro-genital swab about 2 cm into the urethra, gently rotate it, remove and place in transport medium.
  - For gram stain, collect additional exudates on a second swab, and use this swab to prepare a slide for staining. Roll the swab over 2-3 cm of the slide's surface, and label the slide with patient information.

## Transport of genital specimens

- *N. gonorrhoeae* is nutritionally fastidious and environmentally fragile, and cannot tolerate cold temperature or lack of CO<sub>2</sub>. *N. gonorrhoeae* and GC swabs in medium should be delivered to the lab within 24 hours.
- For routine genital specimens use transport medium. Deliver swabs in transport media within 24 hours to the lab. Genital specimens with anaerobes surgically obtained can be handled as a wound/misc. specimen.
- DO NOT refrigerate.
- DRY SWABS will be REJECTED.
- Transport inoculated culture within 15 minutes to the lab at ambient temperature.
- Gen-Probe tubes are transported at room temperature.

# Specimen Collection and Preparation

**Table9: Recommended Transport Media For Genital Specimens**

Test	Specimen Type	Transport Medium	Transport Temperature	Storage Time
Culture, GC	Swab	Gel Swab	RT	<24 hr
Culture, Genital	Swab	Gel Swab	RT	<24 hr
Culture, Genital	Fluid/Tissue	Sterile container or Anaerobic transport	RT	<24 hr
Culture, Beta Strep Group B	Swab	Swab transport or Todd-Hewitt Medium/LIM Broth/NEL Broth/Carrot Broth	2-8 °	< 48 hr
GC/Chlamydia by NAAT	Swab	Urine unpreserved or Cepheid collection kit	2-8°	7 days

## Respiratory Specimens

### General Considerations

- Careful specimen collection is important because respiratory specimens can be easily contaminated with oropharyngeal flora, thus making the culture results clinically irrelevant.
- Microbiology Laboratory reserves the right to reject unsatisfactory samples based on gram stain results.
- Routine bacterial cultures do not include the screening for the following organisms that require special media: *C. diphtheriae*, *A. haemolyticum*, *B. pertussis*, *L. pneumophila*, and *M. pneumoniae*.
- First morning specimens are recommended for AFB and fungal cultures.
- Routine bacterial, fungal, and AFB cultures may all be performed on the same sputum provided that: the specimen is fresh (<1 hour old), it is adequate (5-10 ml), and it is good quality (not saliva).

**Table 10: Respiratory Specimen Collection/Transport Guidelines**

Test Name	Specimen	Transport Media	Temperature	Storage Time
Culture, throat	Throat swab	Swab transport	RT	<24 hrs
Culture, herpes	Throat swab	Viral Transport	2-8° C	< 24 hrs
Culture, Respiratory	Sputum	Sterile Container	RT/2-8° C	<2 hrs/<24 hrs
Culture, AFB	Sputum	Sterile Container	RT/2-8° C	<2 hrs/<24 hrs
Culture, Fungus	Sputum	Sterile Container	RT/2-8° C	<2 hrs/<24 hrs
Flu A&B Antigen Screen/ RSV Antigen	Nasal Washing or Aspirate	Sterile Container	2-8° C	<24 hrs
Flu A&B by NAAT RSV	Nasal Washing or Cepheid Swab	Sterile Container	Room Temperature	<24 hrs

# Specimen Collection and Preparation

## Specimen Collection for Respiratory Specimens

### Sputum:

- Have patient rinse and gargle with clean water.
- Have patient cough up specimen from deep in chest, expectorate the specimen into a sterile, specimen with lid.
- Observe the specimen to see that it is not just saliva or bubbly spit, if so, collect another specimen.
- For bacterial culture collect 1-2 mL, for AFB please submit 5-10 mL.

### Throat/Pharyngeal:

- Have the patient say “ahhh.”
- Depress tongue gently with depressor.
- Reach behind the uvula and swab tonsillar area, pharynx, or any ulceration, lesion or area of inflammation.
- Avoid touching the cheek, tongue, teeth, or lips.
- Insert the swab into the transport medium.
- Label swab with patient information.

### Nasopharyngeal Swab:

- Used primarily for detection of *B. pertussis*, or viruses.
- Remove excess secretions from the anterior nares. Use a separate aluminum shaft NP swab for each nostril.
- Gently pass the swab through one nostril and into nasopharynx. When the swab is inserted far enough, patient will show discomfort with tears, and urge to sneeze.
- Rotate swab on the nasopharyngeal membrane and allow to sit for 10-15 seconds.
- Remove the swab carefully.
- For culture, insert the swab in the appropriate transport medium.

### Nasal Washings:

- Used for the detection of RSV and Flu A/B antigen.
- For 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 in nose, directed posteriorly and toward the opening of the external ear.
- Apply suction, using a rotating movement, slowly withdraw catheter.
- For washings, suction 3-5 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 a sterile, screw cap container or viral transport medium.
- Label with patient information and transport 2-8°C.
- There is a syringe/catheter collection kit available for ease of use.

### Nasal Specimens:

- Nasal swabs are submitted primarily for the detection of Staphylococcal carriers.

## Specimen Collection and Preparation

- Insert a sterile swab into the nose until resistance is met at the level of the turbinates (about 1" into the nose).
- Rotate swab against nasal mucosa.
- Remove swab and insert into medium.
- Repeat the steps for other nostril if indicated.
- Label with patient information and transport to lab.

### Mouth:

- Used primarily for detection of yeast and HSV.
- Rinse mouth with sterile saline.
- Wipe the lesion with dry sterile gauze and swab or scrape area of exudation or ulceration.
- Place specimen on a sterile swab and place in transport medium.
- Label with patient information and transport to lab.

### Transporting Respiratory Specimens

#### Sputum:

- Transport fresh sputum and tracheal specimens to lab within 1-2 hours at ambient temperature.
- Refrigerate if a delay of >2 hours is anticipated
- Swabs are unacceptable specimens.

#### Bronchoscopy, Lung, Sinus, and Tracheal Aspirates:

- Deliver to lab within 1-2 hours at ambient temperature.
- DO NOT refrigerate.

#### Swabs of Nose, Throat, Nasopharynx, Mouth:

- Use regular swab transport system for routine bacterial and fungal cultures. Use viral culturette for viral cultures.
- Transport to lab within 24 hours at ambient temperature.

#### Nasopharyngeal Washings/Aspirates:

- Transport in sterile container or in a multipurpose or viral transport medium for viral studies.
- Deliver to lab at 2-8° C.

## Urine Specimens

### General Considerations:

- Never collect urine from a bedpan, urinal, or catheter bags at bedside.
- Routine cultures with colony counts are done on fresh random urine specimens collected into a sterile container or a B/D urine C&S transport tube with preservative.
- Clean urethral opening prior to specimen collection to ensure that the specimen obtained is not contaminated with colonizing microorganisms in this area.
- Foley catheter specimens should be submitted only after a new catheter had been put in place.

# Specimen Collection and Preparation

- For AFB cultures, submit the entire first morning voided specimen. Obtain special container and instructions from the lab. For patients with a foley, change bag and collect 2 hours of specimen.
- In symptomatic patients, one specimen is usually adequate for diagnosis and another is taken 48 to 72 hours after institution of therapy. In asymptomatic patients, two or three specimens may be necessary. In cases of suspected renal tuberculosis, three consecutive first morning specimens should be submitted.
- A 24-hour urine is unacceptable for culture, as is more than one specimen in a 24-hour period.
- Suprapubic aspirate is the only acceptable specimen for anaerobic studies.

## Collection of Urine:

- Clean Catch Urine
  - If the patient is collecting their own urine, supply them with clear verbal or written instructions to wash hands, use the cleaning material supplies, wipe the vaginal area carefully from front to back and between folds of skin for females and retract the foreskin and clean the glans (head of penis) for males. Use each cleaning towelette only once and throw away.
  - Hold cup with fingers on outside, pass a small amount of urine into toilet and then pass midstream portion of urine into cup.
  - Place lid on cup and tighten. Label with patient information and submit to lab.
- Straight Catheter Urine
  - These urine specimens are useful when clean-catch urines cannot be obtained or when results from clean-catch specimens are equivocal and a diagnosis is critical.
  - Prior to catheterization, the patient should force fluids until bladder is full.
  - Clean urethral opening with soap. Rinse with water.
  - Using a sterile technique, pass a catheter into bladder.
  - Collect the initial 15-30 mL of urine, and discard.
  - Collect a sample from the mid to later flow of urine in a sterile container.
  - Label with patient information and specify method of collection on container.
- Indwelling (Foley) Catheter Urine
  - Routine processing of urine from patients with chronic indwelling catheters may be of no value except epidemiologically. Large numbers of potential pathogens are common in these patients.
  - Collect urine from sampling port with a needle, or needless sampling port is available.
  - Clean port with alcohol wipe and puncture the port with a syringe and withdraw at least 10 mL of urine.
  - Transfer into a sterile container and tighten lid.
  - Label with patient information and specify method of collection on container.

## Transport of Urine Specimens

- Transport all fresh, unpreserved urine specimens to the lab within 1 hour at ambient temperature.
- If a delay of > 1 hour is anticipated, refrigerate the urine and deliver to lab within 24 hours after collection time.

# Specimen Collection and Preparation

- Specimens collected in a B/D urine C&S tube is NOT suitable for UA and microscopic studies.

## Wounds And Other Miscellaneous Specimens

### General Considerations:

- Distinguish between surface wounds and deep or surgical wounds.
- If a specimen is to be collected through the intact skin, clean first with 70% alcohol followed by iodine solution to prepare site.
- For anaerobic studies, the specimen of choice is an aspirate, not swab. An anaerobic transport medium must be used to ensure viability.
- For encrusted lesions, culture is not recommended unless an exudate is present.

### Collection Procedure for Wounds and Miscellaneous Specimens

- **Abscess, open**
  - Remove as much of the superficial flora as possible by decontaminating the skin.
  - Remove exudates, and firmly sample the advancing margin and base of the lesion with a swab.
  - Submit the swab in the appropriate transport medium or submit aspirate in a sterile container.
  - Label with required patient information and deliver to lab.
  - Open abscesses and catheter tips are unacceptable for anaerobic studies.
- **Abscess, closed**
  - This is a better specimen than a ruptured abscess.
  - Decontaminate the skin overlying the abscess.
  - Aspirate the deepest portion of abscess contents with a 3-5 mL syringe.
  - Submit aspirate in anaerobic transport tube, sterile tube, or sterile cup.
  - If collected during surgery, submit a portion of the abscess wall for culture.
  - If an aspirate is unobtainable, submit two swabs and preserve one anaerobically.
  - Label with required patient information and submit to lab.
- **Bite Wound**
  - DO NOT culture fresh, uninfected bite wounds, since infectious agents will not likely be recovered from these sites.
- **Bone**
  - Obtain a small piece at surgery.
  - Submit in sterile container.
  - DO NOT use Formalin.
- **Burns**

*NOTE: The surface of burn wounds will become colonized by the patient's microbial flora or by environmental organisms. When the organism load is large, infection of underlying tissue may occur and bacteremia may occur. Cultures of the burn surface alone are misleading and therefore, biopsies of deeper tissue are often indicated.*

  - Disinfect with 70% alcohol and then with iodine solution. Allow the iodine to dry.
  - Physician will collect a punch biopsy sample.
  - Label with required patient information and deliver to lab.
  - Also monitor patient condition with blood cultures.
- **Catheter, IV**

# Specimen Collection and Preparation

- Clean skin around catheter site with alcohol and then with iodine.
- Remove the clip 5-cm distal tip of catheter directly into sterile tube or cup.
- Label with required patient information and deliver to lab.
- Transport promptly to lab to prevent drying.
  
- **Cellulitis**
  - Clean site by wiping first with sterile alcohol then iodine.
  - Aspirate area of maximum inflammation with fine needle and syringe.
  - Draw a small amount of sterile saline into syringe.
  - Remove needle (with protective device), recap, and submit to lab.
  
- **Ear, Inner**

*Note: Tympanocentesis is reserved for complicated, recurrent, or chronic persistent otitis media.*

  - For intact eardrum clean ear canal with antiseptic solution.
  - The patient may be given a general anesthetic since the incision causes great pain.
  - The physician surgically incises the eardrum and collects as much fluid as possible into a syringe.
  - Submit aspirate in anaerobic transport, sterile tube or sterile cup.
  
- **Ear, Outer**
  - Clean the ear canal with a disinfectant and rinse with sterile saline to remove debris or crust.
  - Obtain sample by firmly rotating swab in outer canal. If there are lesions, swab over them.
  - Insert swab into a transport medium and deliver to lab.
  
- **Hair for dermatophytes**
  - With forceps, collect 10-12 affected hair with bases of shafts intact.
  - Submit in dry, sterile container.
  
- **Nail for dermatophytes**
  - Wipe nail with 70% alcohol with gauze not cotton.
  - Clip away generous portion of affected area, and collect material or debris from under nail.
  - Place in dry, sterile container.
  
- **Skin punch biopsy**
  - Disinfect the skin surface with 70% alcohol and then with iodine.
  - Collect 3-4 mm sample with punch.
  - Submit in a sterile container.
  - DO NOT use formalin to preserve sample.
  
- **Skin for dermatophytes**
  - Clean surface with 70% alcohol.
  - Scrape surface of skin at active margin of lesion. Do not draw blood.
  - Place in dry, sterile container.
  
- **Tissue**

*Note: Do not place tissue specimens for culturing in formalin. Processing of accidentally formalinized specimens will not be attempted.*

  - Submit in sterile container.
  - If sample is small, add a few drops of sterile saline to keep the specimen moist or submit in an anaerobic transport medium.

# Specimen Collection and Preparation

- Any anaerobes in an intact piece of large tissue will be protected from exposure to air until the specimen is minced and ground up during processing before culturing.
- **Wounds (see abscesses)**

## Transport of Wounds and Miscellaneous Specimens

- **Swabs**
  - Transport in appropriate transport media at ambient temperature within 24 hours. Dry swabs will be rejected.
  - If anaerobes are suspected, a gel swab must be used
- **Aspirates and other fluids**
  - Submit in anaerobic transport medium, sterile tube or cup.
  - Transport syringes in a leak-proof, sealable bag.
  - Transport at ambient temperature within 2 hours of collection if the specimen is not in transport medium.
  - Transport within 24 hours at ambient temperature if the specimen is in transport media.
- **Tissue and Biopsies**
  - Submit in sterile container at ambient temperature immediately.
  - Submit in anaerobic transport medium at ambient temperature within 24 hours.
- **Hair, Nail, and Skin for Dermatophytes**
  - Transport within 24 hours at ambient temperature.

## Specimen Collection and Preparation

**Table 11: Specimen Containers, Transport, and Storage**

Specimen Type	Container	Transport	Storage	Remarks
Abscess (open)	Swab Transport	< 2 hrs, RT	< 24 hrs, RT	
Abscess (closed)	Anaerobic Transport or Syringe	< 2 hrs, RT	< 24 hrs, RT	
Blood Cultures	BD BACTEC blood culture bottles	< 2 hrs, RT	< 24 hrs, RT	Do Not refrigerate
Acid-fast Bacteria - Sputum - Urine	Sterile Container Sterile Container	< 2 hrs, RT < 2 hrs, RT	<24 h 2-8°C <24 h 2-8°C	For Sputum & Urine submit specimens 3 consecutive days.
Body Fluid (sterile) (other than blood, CSF, and Urine)	Sterile Container or Anaerobic Transport Tube	< 15 min, RT < 2 hrs, RT	< 24 hrs, RT < 24 hrs, RT	Do Not Refrigerate
Bronchoscopy	Sterile Container	< 2 hrs, RT	<24 h 2-8°C	
Catheter, I.V.	Sterile Container	< 2 hrs, RT	<24 h 2-8°C	
CSF	Sterile Container	< 15 min, RT	< 24 hrs, RT	Do Not Refrigerate
Cervix	Swab Transport or Charcoal Swab	< 2 hrs, RT	< 24 hrs, RT	Do Not Refrigerate
Chlamydia Culture	Multipurpose Transport medium / CVM	<2 hrs, 2-8°C	<48 h 2-8°C	
C. Difficile Toxin	Sterile Container	< 1 hr, RT	<24 h 2-8°C	
Ear, Inner	Sterile Container or Anaerobic Transport Tube	< 2 hrs, RT < 2 hrs, RT	< 24 hrs, RT < 24 hrs, RT	
Ear, Outer	Swab Transp System	< 2 hrs, RT	< 24 hrs, RT	
Eye	Swab Transport or Direct Inoculation	< 2 hrs, RT < 15 min, RT	< 24 hrs, RT < 4 hrs, RT	
Fungus Culture	Sterile Container or Swab Transport System	< 24 hrs, RT < 24 hrs, RT		
GC Culture (genital)	Swab Transport	< 2 hrs, RT	< 24 hrs, RT	Do Not Refrigerate

## Specimen Collection and Preparation

Giardia EIA	Sterile Container or Formalin	< 1 hrs, RT < 2 hrs, RT	<24 h 2-8°C <24 hrs, RT	
Hair	Sterile Container	< 24 hrs, RT		
Nail	Sterile Container	< 24 hrs, RT		
Nasopharynx	N/P Swab Transport or Fluid in Sterile Container	<2 hrs, 2-8°C <2 hrs, 2-8°C	<24 h 2-8°C <24 h 2-8°C	
Nose	Swab Transport System	< 2 hrs, RT	< 24 hrs, RT	
Ova and Parasites	Sterile Container or Formalin / PVA vials	< 1 hr, RT < 24 hrs, RT	< 1 hr, RT < 1 week, RT	
Rectal Swab	Swab Transport System	< 1 hr, RT	< 24 hrs, RT	
Skin	Sterile Container or Swab Transport System	< 2 hrs, RT < 2 hrs, RT	< 24 hrs, RT < 24 hrs, RT	
Sputum	Sterile Container	< 2 hrs, RT	<24 hrs 2-8°C	
Stool	Sterile Container, C&S medium or Cary Blair Medium	< 1 hrs, RT < 2 hrs, RT	< 24 hrs, RT <24 hrs 2-8°C	
Throat	Swab Transport System	< 2 hrs, RT	< 24 hrs, RT	
Tissue	Sterile Container or Anaerobic Transport System	< 15 min, RT < 2 hrs, RT	< 24 hrs, RT < 24 hrs, RT	
Transtracheal Asp.	Sterile Container	< 2 hrs, RT	<24 hrs 2-8°C	
Urethra	Charcoal Swab or Swab Transport System	< 2 hrs, RT	< 24 hrs, RT	
Urine	Sterile Container or special C&S tube	< 1 hrs, RT < 2 hrs, RT	<24 hrs 2-8°C < 24 hrs, RT	
Vaginal	Swab Transport or Charcoal Swab	< 2 hrs, RT < 2 hrs, RT	< 24 hrs, RT < 24 hrs,	

## Specimen Collection and Preparation

			RT	
Virus	Viral Culture System	< 2 hr, 2-8°C	<48 hrs, 2-8°C	

# Specimen Collection and Preparation

## Cytopathology/Gynecologic Collection

### Introduction

The cervical pap smear is a proven screening technique for the detection of premalignant and malignant lesions of the uterine cervix. The success of the pap depends on careful quality of the screening program by experienced cytotechnologists and pathologist. We believe that communication between the referring physician and the Cytology lab enhances the success of our program.

The Cytology Department recognizes that concern for the welfare of the community and patient is a principle element of our profession. It is our goal to deliver top quality technical services with focus on accuracy, thoroughness and timelines. We now offer pap tests using ThinPrep the conventional pap smear. We employ a Revised Bethesda System Terminology for Cytology Reporting and recommend that the provisional guidelines used by the National Cancer Institute be used in follow up of abnormal cervical cytopathologic findings. The cervical pap smear is a SCREENING technique and is not recommended as the sole means for diagnosing or excluding malignant or premalignant lesions.

### Specimen Collection For Conventional Pap Smear

A large part of the success of gynecologic cytology depends upon the quality of the smear. Even the most astute cytotechnologist or pathologist cannot interpret a smear that is not adequately collected and prepared. The following is intended as guidelines for obtaining an optimal smear:

- The patient should be instructed not to use a vaginal douche or any vaginal medication or lubricant for at least 24 hours before smear is to be collected. The patient should also be instructed to refrain from sexual intercourse during this period of time.
- The slide should be properly identified before the procedure begins. The patient's full name must be written with pencil of the frosted end of the slide. This is a federal regulatory requirement and the lab cannot accept any specimen that is not identified properly.
- A completed request form supplied by the lab should accompany the specimen. Complete as indicated on the form.
- The patient is prepared for the procedure according to the physician's protocol. No lubricant should be used for introduction of the speculum. The sampling should be done before the pelvic examination.
- In general, the ectocervix is sampled with a spatula after removing excess mucus. The posterior vaginal fornix may also be sampled as clinically indicated.
- The endocervical sample is usually obtained with a commercially available endocervical brush. Use a single 90-180° turn on the endocervical brush once it is properly positioned in the endocervical canal; then remove and smear. Do not pull the brush back and forth.

The smears should be spread on a single slide. Two separate slides can be used, but the cost/benefit ratio favors a prepared single slide. The sampling for the single slide technique is performed in the usual manner. First, if clinically indicated, a scraping is obtained from the posterior vaginal fornix with a tongue depressor or spatula. The material is kept on the

# Specimen Collection and Preparation

instrument. Then a scraping with a second spatula is made from the ectocervix, with special care to sample the transformation zone. Again, the material is kept on the instrument. Lastly, the endocervical sample is obtained with endocervical brush.

All two or three samples must then be quickly transferred to the labeled slide. The endocervical brush is rolled onto the slide, from one edge to the other near the end farthest from the label; the ectocervical samples are spread in the middle, and the posterior vaginal vault material is placed at the end nearest the label of the patient's name on the frosted end. This must be done swiftly to leave no time for drying.

Faulty sampling may result in smears with scant cellularity, or smears that are too thick to be adequately interpreted. Gentle spreading of the cell sample is important. If too much force is applied, cells will become elongated and nuclear streaks will appear on the smear. If lubricant is used to introduce the vaginal speculum before sampling, the smear will contain an opaque amorphous material that obscures the cellular detail. If the sample does not include material from the transformation zone, no elements from that area will be detected on the smear (i.e. columnar endocervical cells or metaplastic cells.).

As soon as the three samples have been deposited, the slide is immediately sprayed with fixative provided in an environmentally safe pump bottle or the packet of fixative is poured over the specimen. Incorrect or delayed fixation results in poor cellular staining. Totally air dried smears must be reported out as unsatisfactory if the drying artifact completely obscures cellular details.

After the slide has been properly fixed, allow the fixative to dry before closing the plastic or cardboard slide holder. The slide will adhere to the container if not allowed to dry thoroughly.

When the slide has dried, place it in the slide holder and close and secure the closure with the crack and peel label from the patient's requisition form. Please write the patient's name on the label to assure proper patient identification. Place slide holder in biohazard bag and place requisition form in the side pouch of the bag. Forward to the lab for processing.

## Specimen Collection for ThinPrep® Pap Test™

The ThinPrep® Pap Test™ enhances the quality of the pap smear. There is an increase of abnormality detections and a reduction in number of unsatisfactory slides.

### Patient Education

- The patient should be instructed not to use a vaginal douche or any vaginal medication or lubricant for at least 24 hours prior to the specimen being taken. The patient should also be instructed to refrain from sexual intercourse during this time period.

### Requisition

- A completed request form supplied by MMH should accompany the specimen. Complete as indicated on the form.

### Sampling

- The patient is prepared for the procedure according to physician's protocol. No lubricant should be used for the introduction of the speculum. The specimen should be done before any pelvic exam.
- For a sample obtained using endocervical brush/plastic spatula:

# Specimen Collection and Preparation

- Obtain an adequate sampling from the ectocervix using a plastic spatula.
  - Rinse the spatula into the PreservCyt® Solution vial by swirling the spatula vigorously in the vial 10 times. Discard spatula.
  - Obtain an adequate sampling from the endocervix using an endocervical brush. Insert the brush into the cervix until only the bottommost fibers are exposed. Slowly rotate ¼ to ½ turn in one direction. DO NOT over rotate.
  - Rinse the brush in the PreservCyt® Solution by rotating the device 10 times while pushing against the vial wall. Swirl the brush vigorously to further release material. Discard the brush.
  - Tighten the cap so that the torque line on the cap passes the torque line on the vial.
  - Record the patient's name and identification number on vial.
  - Record the patient information and medical history on the cytology req. form.
  - Place the vial and requisition in the specimen bag and forward to the lab.
- **For a sample obtained using a broom-like device**
    - Obtain an adequate sampling from the cervix using 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. Push gently, and rotate the broom in a clockwise direction 5 times.
    - Rinse the broom into the 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.
    - Tighten cap so that the torque line on the cap passes the torque line on vial.
    - Record the patient's name and identification number on vial.
    - Record the patient information and medical history on the cytology req. form.
    - Place the vial and requisition in the specimen bag and forward to the lab.

## Reporting System

The Marietta Memorial Laboratory Cytopathology report consists of the following:

1. Adequacy of Specimen
2. General Diagnostic Category
3. Additional Finding or Comments

### Adequacy of Specimen

- Satisfactory for evaluation (presence or absence of endocervical cells)
- Unsatisfactory for evaluation (specify reason)

### General Diagnostic Category

- Negative for Intraepithelial Lesion (specify within normal limits or benign changes)
- Epithelial Cell Abnormalities (specify cell abnormality)
- Other (endometrial cells in a post menopausal patient inconsistent with history)
- Unsatisfactory for evaluation

### Benign Changes

Infection:

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida sp.
- Predominance of coccobacilli consistent with shift in vaginal flora
- Other

## **Specimen Collection and Preparation**

Reactive Cellular changes associated with:

- Inflammation
- Endometrial cells present (in woman over 40 yrs.)
- Other

Other Findings:

- atrophy
- marked estrogen effect
- moderate estrogen effect
- slight estrogen effect
- progesterone effect

### **Epithelial Cell Abnormalities**

Squamous Cell

- Atypical squamous cells of undetermined significance (ASCUS) (ACS-H)
- Low grade intraepithelial lesion (HPV, Condyloma, Mild dysplasia/ CIN I)
- High grade intraepithelial lesion (Moderate and Severe dysplasia, carcinoma-in-situ, CIN II and CIN III, CIS)
- Squamous cell carcinoma

Glandular Cell

- Atypical glandular cells of undetermined significance (specify type AGUS)
- Adenocarcinoma (specify type) NOS
- Endocervical Adenocarcinoma in-situ

Other Malignant Neoplasm (specify)

# Specimen Collection and Preparation

Unsatisfactory specimens include insufficient epithelial cells present on the slide for evaluation, slide preparation too thick for evaluation, cellularity obscured by inflammation or blood, and poor fixation resulting in extensive cellular degeneration.

## Cytopathology Services: Non-Gyn

MMH lab provides a full spectrum of cytology services on non-gynecological specimens. For requirements and collection questions, please call the Cytology Department at 5090.

### Sputum for Cytology

To obtain cellular material from a productive cough to look for malignant cells.

Label a sterile container with patient's name, doctor, date and time of collection. Instruct the patient that the first a.m. specimen is best. Explain that expectorate from a deep cough is needed for testing. Cover specimen tightly with lid and bring to the lab with appropriate order.

### Urine for Cytology

To obtain cellular material from a urine to look for malignant cells.

Explain to the patient that the first a.m. voided urine is needed. Collect 100 cc of urine (minimum volume 30mL) in a sterile urine cup labeled with patient's name, date and time of collection, and doctor. Bring specimen immediately to lab with appropriate order. If there is a delay between collection and delivery to the lab, please instruct patient to refrigerate specimen.

### Fine Needle Aspiration for Cytology

To obtain cellular material from palpable lesions (breast, thyroid, etc.) for cytological evaluation.

- Make sure to completely fill out requisition form and label slides on frosted end.
- Palpate mass, localize and clean area using alcohol prep pads. With plunger depressed, place needle in mass or area to be aspirated. Then create pressure by pulling plunger back and make several "sawing" motions with needle. Making multidirectional passes to increase the cellularity and the likelihood to sample all the areas of the mass.
- Before withdrawing needle, replace plunger and remove.
- Place approximately one drop of aspirate material on a labeled slide. Take a second slide and pull in opposite directions to make two smears. Spray fix both slides or place in vial containing at least 95% ethanol and repeat process with additional material or place remainder in small labeled container containing 10% formalin for a cell block.
- Allow slides to dry completely and send in carrier with completed cytology form to the lab for evaluation.

# Specimen Collection and Preparation

## Histopathology Services

### Introduction

A full spectrum of surgical pathology and consultative services are available. All pathologists affiliated with MMH are board certified by the American Board of Pathology. All requests for examination and diagnosis are viewed as a request for consultation by another physician and, as such, you are welcome to contact our pathologists concerning their findings.

### Submission Requirements

- A completed request form supplied by MMH lab must accompany the specimens. Please complete this form as indicated on the requisition.
- Submit each specimen separately in a plastic container filled with 10% neutral buffered formalin.
- The container must be labeled with the patient's name. For large specimens, use large containers with enough formalin to achieve a ratio of 5 parts fixative to 1 part tissue.
- All specimen requests should be stated in the "comments" area of the request form.
- Small specimens are to be placed in sealable plastic bag. The folded request form is placed in the pocket separate from the specimen container to avoid possible contamination from leaks.
- Forward the specimen to the lab where the specimen will be processed overnight for slide preparation and diagnosis the next day.
- For procedures and requirements on non-routine specimens, contact a pathologist at the laboratory for instruction.

# Specimen Collection and Preparation

## Critical (Panic) Values

**Note: ALL panic values will be called to physician regardless if they have been called before!!**

DEPT	TEST	CRITICAL LOW	CRITICAL HIGH	UNITS
<b>BLOOD BANK</b>	Cord Blood DAT	All positive cord blood DATs		
	Crossmatch	Any delay in providing blood components		
<b>CHEMISTRY</b>	Glucose	<40	>400	mg/dL
	Potassium	<2.5	>6.5	MEQ/L
	Calcium	<7.0	>13.0	mg/dL
	Sodium	<120	>160	MEQ/L
	Digoxin		>3.0	NG/dL
	U. Protein (OB patients only)		> Or = 300	mg/dL
	Troponin I		>0.3	ng/mL
	Lactic Acid		≥ 2.0	mmol/L
<b>COAGULATION</b>	INR		>5.0	
	APTT		>170	seconds
<b>HEMATOLOGY</b>	White blood cell	<2.0	>40.0	X10 <sup>3</sup> /uL
	Hemoglobin	<7.0		g/dL
	Hematocrit	<15.0		%
	Platelet	<50		X10 <sup>3</sup> /uL
<b>Microbiology</b>	CSF Culture		All positives	
	CSF Bacterial Antigen Testing		All positives	
	All Cultures		All positives	
	Stool Salmonella/ Shigella Campylobacter Antigen		All positives	
	Stool C. Diff		All positives	
	Acid-Fast Bacilli		All positives	
	Culture		Any culture considered to be of immediate medical significance	