Carolinas Laboratory Network

Reference Laboratory

Directory of Services

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Critical Values List

Hematology		
Test	**Lower Limit**	**Higher Limit**
WBC (White Blood Count)	<1000 (This value not called if previous result of <1,000 is documented as called)	> 50,000
Hemoglobin	< 6.0 g/dl < 7.0 g/dl (Newborn - 30 days)	>20.0 g/dl (Greater than 2 weeks old) > 24.0 g/dl (Less than 2 weeks old)
Platelets	< 20,000 < 30,000 (Newborn to 30 days)	>1,000,000
Smear	Previously unreported blasts, intracellular organisms	

Coagulation		
Test	**Lower Limit**	**Higher Limit**
Fibrinogen	< 50 mg/dl	
INR		> 4.0
PTT		> 120 seconds

Urinalysis and Body Fluids	
Test	**Condition**
CSF	Presence of malignant cells, blasts or microorganisms
Ketones	Positive ketones in newborns
Microscopic Exam	Spirochetes resembling Treponema pallidum
Reducing Substance	Newborns - Positive reducing substance when glucose is negative
Sperm	Presence of sperm in female under 13 years of age

Blood Gases (Arterial)		
Test	**Lower Limit**	**Higher Limit**
PCO2	< 20 mmHg	>70 mmHg
pH	< 7.2	>7.6
P02	< 50 mmHg	

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Chemistry		
Test	**Lower Limit**	**Higher Limit**
Alcohol - Ethanol (ETOH)		> 400 mg/dl
Alcohol - Ethylene Glycol		Positive
Alcohol - Volatile Non Ethanol		Positive
Bilirubin		≥ 18.0 mg/dl (Less than 2 weeks old)
Calcium	< 6.0 mg/dl	> 14.0 mg/dl
Calcium, Ionized	< 0.8 mmol/L	> 1.54 mmol/L
CO2	< 10 mmol/L	> 40 mmol/L
Glucose (Blood)	< 40 mg/dl	> 500 mg/dl
Glucose (CSF)	< 40 mg/dl	
K+ (Potassium)	< 2.5 mmol/L	> 7.5 mmol/L (Less than 6 months old) > 6.5 mmol/L (Greater than 6 months old)
Lactic Acid		> 4.0 mmol/L
Magnesium	< 1.0 mg/dl	> 4.7 mg/dl
NA (Sodium)	< 120 mmol/L	> 160 mmol/L
Phosphorous	< 1.5 mg/dl	
Troponin I (CMC Only)		≥ 0.3 ng/ml (Critical not called if previous result of ≥ 0.3 ng/ml is documented as called)
Troponin I (CMC University Only)		≥ 0.07 ng/ml (Critical not called if previous result of ≥ 0.07 ng/ml is documented as called)
Troponin I		≥ 0.5 ng/ml (Critical not called if previous result of ≥ 0.5 ng/ml is documented as called)

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Therapeutic Drugs		
Test	**Lower Limit**	**Higher Limit**
Acetaminophen		> 150 µg/ml
Amikacin		> 45 µg/ml
Caffeine		> 50 µg/ml
Carbamazepine		> 20 µg/ml
Cyclosporine	< 25 ng/ml	> 400 ng/ml
Digoxin		> 3 ng/ml
Dilantin		> 30 µg/ml
Gentamicin		> 12 µg/ml
Lidocaine		> 9 µg/ml
Lithium		> 2 mmol/L
Mysoline		> 24 µg/ml
Phenobarbital		> 60 µg/ml
Salicylate		> 40 µg/ml
Sirolimus	< 2 ng/ml	> 15 ng/ml
Tacrolimus	< 2 ng/ml	> 15 ng/ml
Theophylline		> 25 µg/ml
Tobramycin		> 12 µg/ml
Valproic Acid		> 200 µg/ml
Vancomycin		>30 µg/ml

Blood Bank

- Any cord blood with positive direct coombs
- Incompatible crossmatch with potential for lack of donor blood

TB/Mycology

- All positive acid fast smears from pulmonary secretions
- Dimorphic fungi recovered from any source

Microbiology

- All initial positive blood cultures
- All initial positive results (gram stain or culture) from the following normally sterile body fluids - Vitreous, CSF, Pleural, Pericardial, Peritoneal, Synovial
- Eye cultures positive for *Neisseria gonorrhoeae* , *Pseudomonas* , *Bacillus, Aspergillus, or Fusarium* species
- Isolates of *Clostridium perfringens* or *Clostridium septicum* recovered from wounds or cultures of tissues **(NOTE: Before phoning, discuss with medical director of microbiology,or, in their absence, the on-call pathologist)**
- Isolates of *E.coli* O157:H7 from stool cultures

Critical Values List

Microbiology (continued)
• All possible agents of bioterrorism including the following: *Bacillus anthracis, Francisella tularensis, Brucella spp., Yersenia pestis*
• Isolates of *Neisseria meningitidis from sterile sites*
• Highly unusual or significant organisms or those recovered with low incidence. **(NOTE: Before phoning, discuss with medical director of microbiology, or, in their absence, the on-call pathologist)**

Special Microbiology/Immunology
• All positive acid fast smears from pulmonary secretions
• Positive CSF VDRLs
• All positive RSV cultures (where DFA or EIA was negative)
• Positive RPRs on cord blood
• All positive cryptococcal antigens
• All positive *Pneumocystis* (DFA)
• All positive *Legionella* urinary antigens
• All agents of possible bioterrorism, including *Variola* (Smallpox) virus
• Any positive viral cultures on children under 10 years of age
• Any positive rapid HIV result on maternity patients
• Any positive result for SARS (Severe Acute Respiratory Syndrome) Results provided by Public Health Dept.

Molecular Pathology
• All positive CSF Herpes Simplex Virus (HSV) results by PCR
• All positive CSF Enterovirus results by PCR

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Action	**Signature**	**Date**
Adopted	Edward H. Lipford, MD	8/13/2007
Revised	Edward H. Lipford, MD	8/18/2008
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Review	Edward H. Lipford, MD	2/17/2010
Revise	Edward H. Lipford, MD	12/15/2010
Revise	Edward H. Lipford, MD	5/2/2012
Revise	Edward H. Lipford, MD	5/28/2014
Revise/Review		

Specimen Collection and Handling

I. PRINCIPLE

Laboratory test results are dependent on the quality of the specimen submitted. Patients must be properly prepared so that the best possible specimen can be collected. The specimen must be properly processed, packaged and transported to the laboratory in a timely manner and under environmental conditions that will not compromise the integrity of the specimen. Care, skill, and knowledge when preparing the patient and specimen are essential to the provision of the highest quality standards for testing and services.

II. PROCEDURE

A. Health and Safety Precautions

Occupational Safety and Health Administration (OSHA) has developed guidelines for the handling of clinical specimens. Every specimen should be handled as a potential source of infection. Healthcare personnel are required to comply with recommendations, which enable the safety of both the patient and healthcare personnel. **All specimens should be properly sealed prior to being transported. Leaking containers pose a health hazard. Do not submit needles attached to syringes.**

B. Patient Preparation

Many tests require that the patient be prepared in some specific way to ensure useful results. The best analytical techniques provide results that are only as good as the specimen that has been submitted for analysis.

Fasting requirements

For the majority of tests performed on serum, plasma or whole blood, a fasting specimen is preferred. The fasting specimen provides information that reflects the physiological baseline of the patient. From a practical standpoint, non-fasting specimens are often lipemic, containing high triglycerides from food, which can interfere with many analytical procedures. Patients should fast for the duration of time indicated by their physician.

Blood, serum and plasma specimens

Most blood specimens can be obtained using routine phlebotomy techniques; however, there are some exceptions. The patient's posture, either sitting, standing or lying down, or the time of day relative to the patient's sleep cycle can be important factors in some tests. Refer to the Test Listing and Specimen Requirements Listing for specific patient preparation requirements.

Urine specimens

Many urine tests also require specific preparation of the patient. For routine analysis, the first morning voided (concentrated) specimen is always best. For urine culture specimens, prevention of contamination by normal vaginal, perineal and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen.

C. Specimen Labeling

Each submitted specimen must be labeled with the patient's name and date of collection. When ordering tests in a series (e.g. glucose tolerance):

1. Use one Test Requisition
2. Label each specimen with the patient's name, date and time of collection.
3. Write the number of specimens on the Test Requisition.
4. Submit all specimens within a series together in one specimen bag.

D. Instructions for Packaging Specimens and Test Requisitions

1. Complete the "**Patient Information**" (Patient Name, Date of Birth, Identification number, SS number, Physician name (First and Last), Specimen Collection date and time) and "**Insurance Information**" (Policy holder name, relation, Company name and address, Employer name) sections and check (√) which party will be responsible for payment in the "**Bill To**" section of the requisition form. Enter the ICD9 diagnosis codes that reflect the patient's diagnoses.
2. Collect the specimen(s) in proper transport container. (Refer to the Test Listing and Specimen Requirements worksheet for more information.)
3. The specimen bag has two pouches. Place the specimen(s) in the front ziplock pouch (printed side) and the test requisition form in the back non-ziplock (un-printed side) pouch. This will protect the test requisition form from leakage.
4. FROZEN specimens must be placed in a separate specimen bag along with a separate test requisition form. **Frozen specimens cannot be split for other tests.**

NOTE: PROPER SPECIMEN PACKING HELPS TO EXPEDITE ORDERS.

E. Collection/Processing of Serum, Plasma, Whole Blood and Urine

Serum

The use of serum separator collection tubes is recommended for most analyses. Please refer to the Test Listing and Specimen Requirements worksheet for restrictions.

When using a serum separator tube, follow these instructions:

1. Perform venipuncture as with any other blood collection device.
2. Invert the tube gently no more than five times. Further inversion may cause alterations in sample integrity.
3. Do not remove the stopper at any time. Allow the blood to clot at room temperature for at least 30 minutes, but not longer than 1 hour. Do not centrifuge immediately after drawing blood.
4. Centrifuge at 2200-2500 RPM for at least 15 minutes.

When using serum tubes with no additives, follow these instructions:

1. Perform venipuncture as with any other blood collection device.
2. Allow sample to clot for at least 30 minutes in an upright position, but no longer than 1 hour, before centrifugation.
3. If centrifugation is required, centrifuge within 1 hour of collection at 2200-2500 RPM for at least 15 minutes.
4. If serum requires separation off the red cells, pipette into a clean plastic vial and attach proper labeling. Do not transfer red cells to the vial.

Plasma

Plasma contains fibrinogen and other clotting factors when separated from the red blood cells. Evacuated tubes used to collect plasma specimens contain anticoagulant and frequently, a preservative. The additive in each tube is specified on the label and tube stoppers are color coded according to the additive present. Consult the Test Listing and Specimen Requirements worksheet to determine the correct additive/tube to use.

When using plasma tubes, follow these instructions:

1. Perform venipuncture as with any other blood collection device.
2. Plasma specimens requiring centrifugation, should be centrifuged within 1 hour of collection at 2200-2500 RPM for at least 15 minutes.
3. If plasma requires separation off the red cells, pipette into a clean plastic vial and attach proper labeling. Do not transfer red cells to the vial.

Whole Blood

Collect whole blood according to instructions provided for the individual test. Thoroughly mix the blood with the additives by gently inverting the tube four or five times. Maintain the specimen at ambient temperature before sending to the testing laboratory unless instructed otherwise by the specimen requirements. NEVER FREEZE WHOLE BLOOD unless specifically instructed in the specimen requirements.

24-hour Urine

Because proper collection and preservation of 24-hour urine specimens are essential for accurate test results, patients should be carefully instructed in the correct procedure.

For those analyses requiring the addition of 6N HCl, have the patient collect each voiding in a smaller container and carefully pour the urine into the 24-hour container to avoid any possible acid burns to the patient.

1. Unless the physician indicates otherwise, instruct the patient to maintain the usual amount of liquid intake but to avoid alcoholic beverages.
2. During the collection period, place the 24-hour urine container in a refrigerator or cool place, to prevent growth of microorganisms and possible decomposition of urine constituents.
3. Have the patient empty his/her bladder in the morning into the toilet (not to be included in the 24-hour collection
4. Collect the next voiding and add it as soon as possible to the 24-hour container.

5. Add all subsequent voidings to the container as in (4). The last sample collected should be the first specimen voided the following morning at the same time as the previous morning's first voiding, as in step (3).
6. Mix the contents of the container gently but thoroughly.

F. Specimen Volumes

It is critical that an adequate specimen volume is submitted for analysis. The volume requested is enough for initial analysis as well as any confirmatory tests that must be performed. If an inadequate specimen is submitted, the laboratory may not be able to perform the initial test or required confirmatory procedures. If repeat or confirmatory tests cannot be performed, the report will indicate that specimen quanity submitted was "QNS" (Quantity Not Sufficient) for additional testing.

When serum or plasma is to be submitted for analysis, it is generally good practice to collect a volume that is 2 to 2.5 times the volume of serum or plasma needed for the test. As an example, if 4 ml of serum or plasma is needed for a test, collect 8 to 10 ml of blood.

G. Storing and Transporting Specimens

Specific instructions for storage and transport of specimens for individual tests are detailed in the Test Listing and Specimen Requirements worksheet. Please follow these instructions carefully.

Additional instructions to note:

- **Needles:** Carolinas Laboratory Network is not permitted, by law, to transport needles. They must be removed from syringes prior to submission.

- **Specimen labels:** Each specimen submitted must be properly labeled and must include a completed requisition for testing.

Supplies and Ordering

Supplies for specimen collection and transport are provided without charge for tests referred to our laboratory. Requests for supplies can be made by completing an order request form for either clinical or pathology supplies and faxing to **704-355-3610**. In order to prevent service interruption, please allow 5 to 7 business days for delivery of supplies. STAT requests are accepted and will be delivered with 24 to 48 hours. In rare instances, we may experience difficulties in maintaining inventory due to the manufacturer. In those instances, we will attempt to substitute a similar product.

Questions concerning supplies can be directed to **704-355-9350, option 1.**

Carolinas Laboratory Network
Reference Laboratory Supply Form

Location _____ **Phone** _____ **Date** _____

Qty	Item	Qty	Item
	Bar Code Labels		Urine Containers- Sterile
	Blood Culture Bottles (set of 2 btls)		Urine Culture Transport Tubes
	Blood Collection Needle-21g (box)		Vacutainer Adapters (bag)
	Blood Collection Needle-22g (box)		Viral Culture Transport Tube
	Blood Collection Tubes- Blue 2.7 ml		
	Blood Collection Tubes- Gel SST 6.0ml		**Histology Supplies:**
	Blood Collection Tubes- Gray 3ml		Requisitions, Pathology (pack of 100)
	Blood Collection Tubes - Lavender 3.0 ml		120 ml Specimen Vial (case of 50)
	Blood Collection Tubes - Red 6 ml		60ml Specimen Vial (case of 50)
	CT/GC NAAT Collection Kit-Female		
	CT/GC NAAT Collection Kit- Male		**Cytology Supplies:**
	CT/GC NAAT Collection Kit-Urine		Cytobrushes, snap-offs (100/bag or 500/case)
	Glucose Tolerance Beverage (btl)		Cytolyt Fluid, Cup
	O & P Stool Kits (each)		Cytolyt Fluid, Quart
	Requisitions, Ref Lab IDX (box)		Medscand Combo(brush/ clear handle spatula)
	Requisitions, Ref Lab OBGYN (box)		PapPaks (slides, brushes, folders & spatulas)
	Requisitions, Ref Lab Standard (pack)		Requisitions, Cytology-IDX (100/pack)
	Specimen Bags-Red Top (roll)		Requisitions, Cytology non-IDX (100/pack)
	Specimen Bags-Yellow Top (roll)		Slides, Frosted for FNA (100/pack)
	Specimen Bags-Blue Top (roll)		Spatulas-Blue handle-Puritan (50/bag)
	Specimen Bags-Lg Multi Specimen (roll)		Spray fixative
	Specimen Bags-Red STAT (pack)		Slide transfer boxes- styrofoam
	Stool Containers- Culture		Surepath Brooms (25/bag or 500/case)
	Stool Containers- Plain		Surepath Vials (25/pk or 500/case)
	Swab Culturette		**List Additional Items:**
	Tourniquets, latex free (each)		
	Urine Container-24 hr Soft; Specify additive		
	Urine Container-24 hr Hard; Specify additive		
	Urine Container-Routine		

Please allow 5-7 business days for supplies to arrive in your office.
STAT orders will be filled within 24 -48 hours provided item is in stock.
If you have questions regarding supply orders, call 704-355-9350, option 1.
FAX ORDERS TO 704 355-3610

Blood Culture Collection Guide

Type	Specimen Requirement	Comments
Blood Culture, Routine - Adult	**20.0 mL Blood/Culture**	Never put more than 10.0 mL in one blood culture bottle.
	Set 1(Venipuncture Site #1)	
	8.0-10.0 mL in Plus Aerobic/F (Gray Top)	
	8.0-10.0 mL in Lytic Anaerobic/F (Purple Top)	
	Set 2 (Venipuncture Site # 2)	
	8.0-10.0 mL in Plus Aerobic/F (Gray Top)	
	8.0-10.0 mL in Lytic Anaerobic/F (Purple Top)	
Blood Culture, Routine - Pediatric		
Neonate	**1.0 - 2.0 mL Blood/Culture** - 1.0 mL in Plus Aerobic/F (Gray Top) + 1.0 mL in Lytic Anaerobic/F (Purple Top)	If you collect less than 2.0 mL of blood, put all blood in the aerobic bottle.
1 month - 2 years	**2.0-3.0 mL Blood/Culture** - 1.0 - 1.5 mL in Plus Aerobic/F (Gray Top) + 1.0 - 1.5 mL in Lytic Anaerobic/F (Purple Top)	
2 years - 10 years	**3.0-5.0 mL Blood/Culture** - 1.5 - 2.5 mL in Plus Aerobic/F (Gray Top) + 1.5 - 2.5 mL in Lytic Anaerobic/F (Purple Top)	
10 years - 15 years	**10.0 - 20.0 mL Blood/Culture** - 5.0 - 10.0 mL in Plus Aerobic/F (Gray Top) + 5.0 - 10.0 mL in Lytic Anaerobic/F (Purple Top)	
Blood Culture, Fungus	**1.0 - 5.0 mL** in Myco/F Lytic (White Top/ Red Label)	Do not collect Isolator™ tube
Blood Culture, Bartonella henselae	8.0-10.0 mL in Plus Aerobic/F (Gray Top) 8.0-10.0 mL in Lytic Anaerobic/F (Purple Top)	
Blood Culture, AFB, MAI	**1.0 - 5.0 mL** in Myco/F Lytic (White Top/ Red Label)	
Blood Culture, Histoplasma	Adult - **10.0 mL** in gold/black top Isolator™ Tube Pediatric - **1.5 mL** in gold top Isolator™ tube	
Culture, Stem Cells	**0.5-3.0 mL** in Plus Aerobic/F (Gray Top)	
Culture, Bone Marrow	Bone Marrow iS sent to Special Hematology for inoculation into yellow top SPS Tube	

CAROLINAS LABORATORY NETWORK

Microbiology General Information

I. PURPOSE

This section of the manual is designed to aid physicians, nurses, and other staff in the proper collection and handling of microbiology specimens.

II. POLICY

Any material that is to be submitted to Microbiology will be prepared in a sterile container and labeled in accordance with Carolinas Laboratory Network Policy for Labeling Laboratory Specimens.

III. SPECIAL CONSIDERATIONS

A. Sterility of the specimen must be maintained.

B. Request forms and outside of containers must be clean and free of contamination. Soiled labels and request forms must be discarded and new ones prepared.

C. Specimen containers should not be labeled nor requisitions attached until the specimen is placed in the container.

D. If a swab is used for obtaining the specimen, submit one swab for each type of culture requested (i.e. fungal, anaerobic, routine, acid fast, etc.). **NOTE:** Swabs have been shown to be significantly inferior to tissue specimens for recovery of fungi, mycobacteria, and anaerobes.

Microbiology Collection Guidelines

Specimen Request	Collection/ Transport Instructions	Special Notes
Chlamydia DNA Probe, Eye	Obtain male (blue) Gen-Probe collection kit from laboratory.Use a sterile dacron swab(not in kit) to clean away any discharge present. Do not scrape the conjunctiva while cleaning. Thoroughly swab the lower, then upper conjunctiva 2-3 times with the supplied small tip swab. Insert swab into Gen-Probe transport tube. Snap off shaft at score line. Do not splash. Cap the tube tightly.	The DNA probe method should not be used for detection of GC from the eye, submit specimen for bacterial culture.If both eyes are affected, swab the least affected eye first.
Chlamydia DNA Probe, Genital	Male or Female Collection kit required. **Female:** Remove excess mucus from cervical area using one of the swabs provided in the kit. Discard swab. Insert 2nd swab from kit, 1-1.5 cm into endocervical canal. Rotate swab clockwise for 10 to 30 seconds. Withdraw swab, carefully avoiding any contact with the vaginal mucosa. **Male:** Insert small tip swab from kit, 2-4 cm into urethra. Rotate clockwise 2-3 seconds to ensure contact with all urethral surfaces. Withdraw swab. Fully insert male or female swab into Gen-Probe transport tube. Snap off shaft at score line. Do not splash. Cap tube tightly.	For child abuse or legal situations, obtain specimens for for GC or chlamydia culture, not DNA Probe. Use swabs from kit only. Male patients should not have urinated for at least one (1) hour prior to collection.
Clostridium Difficile Toxin Assay	Collect soft or liquid stool in sterile, wide mouth container.	Rectal swabs and formed stools are not acceptable.
Culture, Anaerobic	Use anaerobic collection device or syringe if collecting fluid sample. See Attachment 1 for a list of unacceptable specimens.	All specimens must be protected from exposure to oxygen, which is toxic to anaerobic organisms. Some sites or specimens are not acceptable for anaerobe culture because anaerobic organisms are part of the usual flora of these sites.
Culture, Blood	See Blood Culture Guidelines	
Culture, Cath Tips	Place tip of catheter in sterile screw top container. Refrigerate at 2-8 ° C if transport is delayed more than 24 hrs	Foley cath tips are not acceptable.
Culture, CSF or Body Fluid (Bacterial)	Submit one (1) mL in sterile screw top vials. Refrigerate at 2-8 ° C if transport is delayed > 24 hrs.	CSF specimens submitted on swabs are unacceptable for CSF culture.
Culture, CSF or Body Fluid (Viral)	Submit one (1) mL CSF or fluid in sterile container. Refrigerate at 2-8 ° C, if transport is delayed > 24 hrs	

Microbiology Collection Guidelines

Specimen Request	Collection/ Transport Instructions	Special Notes
Culture, Exudate from Wound Abcess and Lesions (Bacterial, Viral)	Collect aspirate using needle and syringe. Cleanse site to be cultured thoroughly. If culture site is dry, collect using a culturette swab. Refrigerate at 2-8 ° C if transport is delayed > 24 hrs.	Specimens submitted on culturette swabs are suboptimal for AFB and fungal cultures, but are acceptable. For viral culture, collect an additional swab and place in viral transport media.
Culture, Eye (Bacterial)	Using a small NP swab, touch mucosal surface and place swab in culturette container. Break ampule to moisten swab.	Wash eye with sterile normal saline to remove superficial exudate. Specimens for GC from newborns must be collected prior to the crede procedure.
Culture, Eye (Viral)	Using a small NP swab touch mucosal surface and place swab in viral transport broth. Cap securely.	
Culture, Fungus and KOH Prep (Hair, Nail or Skin)	**Hair** - Remove 10 -12 infected hairs with forceps and place in sterile container or between 2 glass slides. **Skin Scrapings** - Cleanse skin with alcohol sponge. Scrape entire periphery of lesion with scalpel blade or cytology brush. Place scrapings between 2 glass slides or sterile container. **Nail** - Cleanse nail with alcohol sponge. Remove first portion of debris under nail with scalpel and discard. Scrape remaining portion and place between 2 glass slides. Refrigerate at 2-8 ° C if transport is delayed > 24 hrs.	Lesions should be untreated with topical antifungal agents for one week before culturing.
Culture, Genital (Bacterial and Fungal)	Use culturette swab to collect exudate from vaginal or cervical area or from penile discharge. Return swab to holder and crack ampule to preserve specimen. **Do not refrigerate**.	Penile discharge for GC may be spread onto a slide and submitted for gram stain only. Swab for trichomonas should be placed in a sterile tube or urine container with enough sterile saline to keep swab moist (1-2 mL).
Culture, Genital (Viral)	Remove obvious exudate from culture site with sterile swab. Use second swab to scrape mucosal surface well to obtain mucosal cells and then place swab in viral transport media. Refrigerate at 2-8 ° C, if transport is delayed > 2 hrs	When collection a swab, obtain cells from the margin of the lesion. Viruses are found inside human cells.
Culture, *Haemophilus Ducreyi* (Genital)	Use culturette swab to collect exudate from center of soft lesion. Return swab to holder and crack ampule to preserve specimen.	
Culture, *Histoplasma Capsulatum*	One (1) red top tube	
Culture, *Rochalimaea*	One (1) isolator tube	

Microbiology Collection Guidelines

Specimen Request	Collection/ Transport Instructions	Special Notes
Culture, Sputum (Bacterial, AFB, Fungal, Viral)	Submit specimen in sterile, wide mouth container. Refrigerate at 2-8 ° C, if transport is delayed >1 hr	For optimum results, obtain first morning specimen prior to breakfast. A single sputum sample may be used for bacterial, fungal and AFB if there is at least 5 mL of sputum. For viral culture, place a small amount (0.25-0.5 mL) into viral transport broth or send entire specimen. For AFB cultures, sputum culture should be collected on 3 different days.
Culture, Stool (Bacteria)	Use sterile stool container. Requires 10 to 25 gms of specimen. Refrigerate at 2-8 ° C, if transport is delayed > 2 hrs	Specimen must be free of barium, bismuth, and oily suspensions. It cannot be mixed with urine. Diapers are not acceptable.
Culture, Stool (Viral)	Collect a formed stool in sterile container. Swab is also acceptable.	Specimen must be free of barium, bismuth, and oily suspensions. It cannot be mixed with urine. Diapers are not acceptable.
Culture, Throat Screen for Group A Beta Strep	Use culturette swab. Depress tongue with tongue blade and swab tonsillar area, posterior pharynx, and any areas of inflammation.	
Culture, Tissue (Bacterial, Fungal, AFB or Viral)	Remove tissue aseptically and place in sterile container with just enough sterile normal saline to keep tissue moist. Transport to lab within 24 hours	Do not submit entire appendages. Do not use anaerobe collection device. If Bartonella is requested, submit lymph node biopsy or aspirate.
Culture, Upper Respiratory Tract (Bacterial, AFB, Fungal, Viral)	**Nose:** Swab anterior 15 mm of both narces. **Nasopharynx**: Pass a Bradford wire swab back through the nose until it comes in contact with the posterior nasopharyngeal wall, then rotate the swab gently in this position before removing it. **Oropharynx**: See Culture, Throat Screen for Group A Beta Strep. For viral culture, place swab into viral transport broth. Refrigerate at 2-8 ° C, if transport is delayed > 2 hrs	Cultures for the following organisms require additional culture media: C. diphtheriae, B. pertussis, N. meningitidis, N. gonorrhoeae, Fusospirochetal symbiotic disease. Collect an additional swab for each type of culture ordered. When collecting a swab for viral testing, obtain cells from the margin of the lesion. Viruses are found inside human cells.
Culture, Urine (Bacterial)	Use sterile specimen container. Refrigerate at 2-8 ° C, if transport is delayed > 2 hrs or use urine transport tube.	
Culture, Urine (Viral)	Collect 10-20 mL in sterile container	

Microbiology Collection Guidelines

Specimen Request	Collection/ Transport Instructions	Special Notes
DFA , Vesicular Fluid (Herpes Simples, Varicella Zoster)	Obtain 3 acetone cleaned slides from laboratory with slide container. Collect epithelial cells form lesion onto a sterile swab. Transfer cells directly to acetone cleaned slides by rolling swab back and forth over 5 to 10 mm area of slide. Air dry slide completely before placing in slide container.	
DFA, Viral (includes RSV, Influenza, HSV)	Collect 1 to 2 mL of sample into sterile specimen container. Acceptable specimens are lesion basal cell scrapings, tissue, nasal washings, sputum, bronchial washings, tracheal specimens. Culturette swabs also acceptable. Refrigerate at 2-8 ° C, if transport is delayed > 24 hrs.	
Genital Lesion for Donovan Bodies	Cleanse lesion and remove any tissue debris. Place specimen from punch biopsy into sterile container.	
Histoplasma Capsulatum , Antigen Detection Assay, CSF	Submit one (1) mL CSF in sterile screw top vial. Refrigerate at 2-8 ° C, if transport is delayed > 24 hrs	
Histoplasma Capsulatum , Antigen Detection Assay, Serum	Five (5) mL blood in red top tube	
Histoplasma Capsulatum , Antigen Detection Assay, Urine	Ten (10) mL urine in sterile specimen container. Refrigerate at 2-8 ° C, if delayed > 2 hrs	
Ova & Parasite (O & P)	Collect stool in sterile, wide mouth container. If specimen transport to lab is delayed > 30 minutes, place specimen in Parapaks (PVA/formalin fixative transport system)	Stools must be free of barium, bismuth and oils for at least 72 hours. Stools cannot be mixed with urine. Diapers and rectal swabs are not acceptable. A battery of samples is required because some parasites are released in showers; there are intervals in which no organisms can be detected. Routine O& P exam is a screen for Giardia and Cryptosporidium. If other organisms are suspected, order O&P Complete.
Pinworm Exam	Using a 5 inch length of cellophane tape, press a segment of the sticky surface of the tape over the perianal area. Remove tape and attach sticky side down onto a clear glass slide.	Specimen should be collected at night or early morning. Do not use frosted end of the slide. Do not use MAGIC tape, it must be clear tape.
Trichomonas	Collect with sterile culturette swab or sterile swab in sterile specimen container with enough sterile normal saline to keep swab moist (1-2 mL).	

BLOOD CULTURE COLLECTION GUIDE

Type of Blood Culture	Specimen Requirement
Culture, Blood	**Set 1 (Venipuncture Site #1)** 8 – 10 mL in Plus Aerobic/F (gray top) 8 – 10 mL in Lytic Anaerobic/F (purple top) **Set 2 (Venipuncture Site #2)** 8 – 10 mL in Plus Aerobic/F (gray top) 8 – 10 mL in Lytic Anaerobic/F (purple top) If < 5 mL per draw, collect Plus Aerobic/F (gray top) only **NEVER PUT OVER 10 mL IN A BOTTLE**
Culture, Blood-Pediatric	0.5 – 3.0 mL in Plus/Aerobic/F (gray top)
Culture, Blood for Fungus	1.0 – 5.0 mL in Myco/F Lytic (white top, red label) **Note: Do not collect isolator tube**
Culture, Blood for AFB, MAI	1- 5 mL in Myco/F Lytic (white top, red label)
CMV Antigenimia (Replaces CMV Blood Culture)	Two (2) purple top tube – 10 mL
Culture, for *Bartonella henselae*	One (1) Isolator tube
Culture, Stem Cells	0.5 – 3.0 mL in Plus/Aerobic/F (gray top)
Culture, Bone Marrow	Collect I yellow top SPS tube.

CAROLINAS LABORATORY NETWORK

ATTACHMENT 1

SPECIMEN COLLECTION FOR ANAEROBIC CULTURE

Some sites or specimen types are not acceptable for anaerobe culture because anaerobic organisms are part of the usual flora of these sites. A list of unacceptable specimens and corresponding acceptable ones follows:

UNACCEPTABLE	**ACCEPTABLE**
Throat, ear, nasopharynx	None
Sputum, bronchoscopy specimen	Transtracheal aspiration
Tracheostomy site	None
Feces, rectal swab	None (see *Clostridium difficile*)
Voided or cath urine	Suprapubic bladder tap
Urethra	Prostatic or seminal fluid
Superficial wounds, abscess swab	Deep collection, avoiding skin and mucous membranes
Gastrointestinal or abdominal wounds contaminated with feces	None
Vaginal or cervical swabs	Aspirates by culdocentesis

Collection of SurePath Pap Smears V05.1

I. Purpose

The SurePath test pack is a liquid-based thin layer cell preparation process. SurePath slides are intended as a replacement for conventional gynecologic pap smears. SurePath slides are used in the screening and detection of cervical cancer, pre-cancerous lesions, atypical cells and all other cytologic categories as defined by The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses.

II. Materials Needed

- SurePath Preservative Fluid Collection Vial
- Cervex Brush (Rovers Medical Devices BV, Oss – The Netherlands)
- Carolinas Laboratory Network Cytology Gynecologic Requisition
- Specimen Transport Bag
- Pen (for labeling vial and filling out requisition)
- Puritan Spatulas (optional)
- Surgipath Snap-Off C-E Brush (optional)

III. Procedure

A. Complete gynecologic requisition. Every requisition must have the following:
- Full name of patient
- Unique patient identifier (i.e. Soc. Sec #, Hospital #, Chart #)
- Patients date of birth
- First date of last menstrual period
- Clinical history
- Submitting physician's full name
- Date of collection
- Specific specimen site (cervical, vaginal, vulva)
- Attach copy of all insurance information to the requisition
- If HPV testing is desired check either the "HPV if ASCUS Diagnosis" or the "HPV if ASCUS or Negative" box.

B. Label SurePath vial with patient name, collection date, and physician's full name.

C. Insert the Rovers Cervex-Brush into the endocervical canal. Apply gentle pressure until the bristles form against the cervix. Maintain gentle pressure; hold the stem between the thumb and forefinger. Rotate the brush five times in a clockwise direction. Remove the collection device from cervix.

Note: The clockwise direction of rotation is critical for specimen collection.

D. Placing your thumb against the back of the brush pad, simply disconnect the entire brush from the stem into the SurePath preservative vial.

E. Optional specimen collection devices may be used in addition to the Cervex Brush.
1. Surgipath Snap-Off C-E Brush
   a. For adequate specimen collection, insert the C-E brush gently into the endocervix and slowly rotate ½ to 1 full turn. Remove C-E brush.
   b. Snap off the C-E brush tip and deposit the bush into the SurePath vial.
   c. **DO NOT USE** on pregnant patients due to insufficient data.
   d. **DO NOT USE** for endometrial sampling.
2. Puritan Spatulas

a. Sample ectocervix using plastic spatula.
b. Placing your thumb against the back of the spatula, simply disconnect the spatula head and place in SurePath vial.

F. Place the cap on the vial and tighten securely.

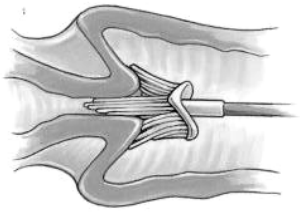
G. Check to be certain vial is labeled

H. Place vial and completed requisition in zip-lock specimen biohazard bag for transport to the laboratory.

Note: All specimen collection devices are designed so that the head/tips of such devices are to be deposited into the SurePath vial and transported to the laboratory. The system is designed so that the laboratory receives 100% of the collected specimen.

SurePath™ test pack

FOUR SIMPLE STEPS



1. Cervical Sample Collection
Insert the Rovers Cervex-Brush® into the endocervical canal. Apply gentle pressure until the bristles form against the cervix. Maintaining gentle pressure, hold the stem between the thumb and forefinger.
NOTE: ROTATE BRUSH FIVE TIMES
Rotate the brush five times in a clockwise direction.

2. Preserve the entire sample
Placing your thumb against the back of the brush pad, simply disconnect the entire brush from the stem into the SurePath™ preservative vial.

3. Cap and label vial
Place the cap on the vial and tighten. Label the vial and lab requisition form with patient name and/or number, physician name and date if desired.

4. Send vial to your lab
Place the vial and requisition into a specimen bag and send to the laboratory.

Directions for Use

SurePath™ Sample Collection Kit

With Pre-scored Detachable Head Devices

for use with SUREPATH™ *liquid-based Pap test*

Cytobrush® Plus GT • Pap Perfect® Plastic Spatula

NON-STERILE

SurePath™ Sample Collection Kit containing Cytobrush® Plus GT cell collector gentle tip and Pap Perfect® plastic spatula are single use devices with detachable heads which remain in the SurePath™ preservative collection vial. Discard the remaining device handle-end after each use.

STORAGE
Nonsterile, to be stored in resealable packaging when not in use.

CAUTION
For use only by medical professionals.

INDICATIONS FOR USE
SurePath™ Sample Collection Kit is used to collect specimens from exo-and-endocervix for use with the SurePath™ liquid-based Pap test. The detachable head devices are to be dropped into the SurePath™ preservative vial to optimize sample collection by enabling 100% of the collected cells to be transferred to the vial for the laboratory to process.

CONTRAINDICATIONS
Do not use Cytobrush® Plus GT on pregnant patients. Do not use Cytobrush® Plus GT for endometrial sampling.

WARNINGS
Insert a Cytobrush® Plus GT device into endocervix until only the bottom-most brush bristles are exposed at the os. To reduce unnecessary bleeding, do not over-rotate device.

ADVERSE REACTIONS
No known adverse reactions.

Manufactured for and Distributed by:
TRIPATH IMAGING®
780 Plantation Drive
Burlington, NC 27215
Toll Free: 1-866-TriPath

Manufactured by:
Medscand®
a CooperSurgical company
95 Corporate Drive, Trumbull, CT 06611
Phone: 203-601-5200 or 800-243-2974
Fax: 800-262-0105

Part #35776 Rev. A 30620:01

Directions for Use

SAMPLING INSTRUCTIONS

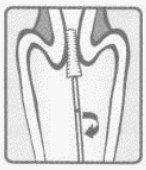
Label a SurePath™ preservative vial with patient, date, lab, and doctor information.

With the patient in lithotomy position, expose cervix using a vaginal speculum moistened with warm water. Visually examine vaginal mucosa and cervix for lesions, ulceration or discharge. Document findings of the examination on patient's record, and communicate the relevant clinical findings to laboratory for optimum cytological interpretation.

1 Select contoured end of Pap Perfect® plastic spatula and rotate 360° around the entire exocervix while maintaining tight contact with exocervical surface. Remove spatula.

2 Visually locate the notched score line on the side of the spatula handle, about 4cm from the contoured collection end. With gloved hand(s) and one single, quick, and firm SNAP, separate the contoured end from the rest of the spatula handle. Do not touch collection end. Drop this contoured collection end into a vial of SurePath™ preservative (supplied by TriPath Imaging, Inc.). Discard remaining device handle end of the spatula after each use. Place cap on vial until step 4; do not tighten cap.

3 Insert Cytobrush® Plus GT device into the endocervix until only the bottom-most bristles are exposed at the os. Slowly rotate 1/4 to 1/2 turn in one direction. To reduce unnecessary bleeding, do not over-rotate brush. Over-rotation may result in poor sample collection. Remove cytobrush device.



4 Visually locate the notched score line on the side of the cytobrush handle, about 4cm from the brush tip. With gloved hand(s) and one single, quick, and firm SNAP, separate the brush head-short handle from the rest of brush handle. Do not touch collection end. Drop brush head-short handle into the same vial of SurePath™ preservative. Discard remaining device handle end of cytobrush.

5 Tighten the SurePath™ vial cap so the torque line on the cap passes the torque line on the vial. Attach to SurePath™ vial an appropriate requisition form and send to lab for PrepStain™ processing.

Collection of Conventional Pap Smears V04.1

IV. Purpose

Sampling of cervical-vaginal areas for cancer detection, pre-cancerous lesions, infectious processes, and benign conditions. The Pap Smear has a proven track record for cancer detection and reduced mortality rate through a systematic screening program. Accurate diagnosis is, however, interdependent upon an adequate sampling technique and quick fixation of the sample. The endocervical brush technique and the spatula technique (for ectocervix) offer an adequate representative sample for evaluation.

V. Material Needed

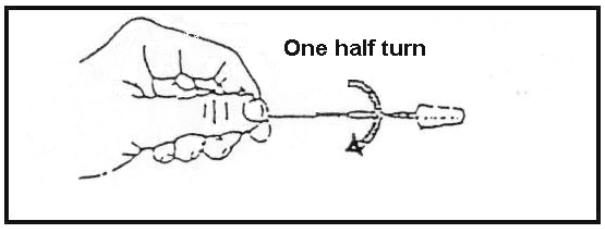
- Gynecologic requisition
- One glass slide (frosted end)
- Lead pencil (pen ink rinses off during processing)
- Endocervical Brush
- Ayre spatula, preferably plastic
- Slide folder for transport
- Fixative Spray

VI. Procedure

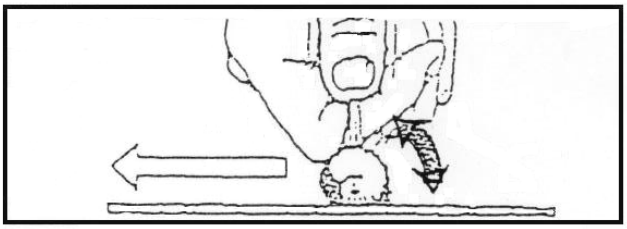
A. Label the frosted end of a glass slide with the patient's full name in pencil.
B. Complete gynecologic requisition. Every requisition must have the following:
   - Full name of patient
   - Unique patient identifier (i.e. Soc. Sec #, Hospital #, Chart #)
   - Patients date of birth (DOB)
   - First date of last menstrual period (LMP)
   - Clinical history
   - Submitting physician's full name
   - Date of collection.
   - Specific specimen site (cervical, vaginal, vulva).
   - Attach copy of all insurance information to the requisition.
C. Single slide pap smear for non-pregnant patients
   1. Sample ectocervix with plastic spatula. Hold spatula. (DO NOT PLACE SPECIMEN ON SLIDE UNTIL ENDOCERVIX SAMPLE IS COMPLETE).
   2. Insert the cytobrush device until only the bottom most fibers are exposed.
   3. Slowly rotate ½ to 1 full turn in one direction. DO NOT OVER-ROTATE. Remove cytobrush.
   4. Hold the brush and place spatula on the corner of the slide and unload specimen and discard spatula.
   5. Immediately roll the brush with moderate pressure on the remaining slide and discard brush.
   6. Immediately fix the glass slide by spraying fixative in a side to side sweeping motion 6-8 inches from slide.
   7. Place slide in specimen folder and close when dry.
   8. Place slide folder and completed requisition in zip-lock specimen biohazard bag for transport to the laboratory.

D. Single slide pap smear for pregnant patients

1. Scrape the cervix lightly using a plastic spatula. Hold spatula. (DO NOT PLACE SPECIMEN ON SLIDE UNTIL ENDOCERVIX SAMPLE IS COMPLETE).
2. Sample the endocervix using a pre-moistened cotton tipped swab by rotating it slightly within the endocervical canal and remove.
3. Hold the swab and place spatula on the corner of the slide and unload specimen and discard spatula.
4. Immediately roll the swab with moderate pressure on the remaining slide and discard swab.
5. Immediately fix the glass slide by spraying fixative in a side to side sweeping motion 6-8 inches from slide.
6. Place slide in specimen folder and close when dry.
7. Place slide folder and completed requisition in zip-lock specimen biohazard bag for transport to the laboratory.



Slowly rotate one half to one full turn



Roll and twist across slide bending bristles slightly

VII. Contraindications for Collecting Conventional Pap Smears

A. Do not use for endometrial sampling
B. Do not use endocervical brush in pregnant patients as insufficient data exists.
C. Do not use an endometrial-sampling device.
D. Patient should not douched within 24 hours.

Note: Clinicians may want to inform patients that thorough sampling of the endocervical canal by the cytobrush may cause spotting for a day or two following the pap test.

Collection of Non-Gynecologic Specimens V05.1

VIII. Purpose

Cytopathology can provide a rapid, simple, and inexpensive means to screen for malignant or pre-malignant diseases, or obtain a variety of other non-neoplastic diagnoses. However, there are many limitations to the interpretation of cytologic specimens. The most common limitations are improperly obtained or inadequately fixed material. The purpose of this procedure is to provide standard cytopreparatory procedures for staff, nurses, and physicians, so a well-preserved specimen is collected.

IX. Materials Needed

- Cytology specimen requisition
- Specimen collection container (see individual procedure below)
- CytoLyt solution (see individual procedure below)

Caution: Do not allow CytoLyt to contact patient – contains methanol. Please observe expiration date and return any expired solution to the Department of Cytology for disposal.

- 95% Alcohol (ETOH) in plastic jars for transport (see individual procedure below)

***** Materials above may be obtained from the cytology department
CMC……(704) 355-4252
CMC Mercy…….(704) 304-5985

X. Procedure

A. Specimen Identification
1. ***Each*** specimen for cytology must be accompanied by a ***separate*** requisition. More than one test may be ordered for a specimen.
2. Each requisition must have the following:
   - Full name of patient.
   - Date of birth
   - Gender
   - Ordering physician's full name
   - Specific specimen type/site (pleural fluid, left nipple discharge)
   - Date and of collection
   - Hospital number/Chart number
   - Pertinent clinical information
3. Attach a copy of insurance information to the requisition.
4. Label the specimen container with patient's full name, specimen type, and ordering physician.
5. Specimens should be transported in a leak proof container inside a plastic biohazard bag. The requisition should be placed in the outside pocket of the bag.
6. Slides must be transported in an appropriate holder to prevent breaking.
7. If slides are submitted, the patient's last name must be written on the frosted end of the slide with a pencil (Ink will not stay on during processing).
8. Only licensed, authorized personnel may submit specimens.

9. If all the above requirements are not met, the specimen will be rejected and returned to the ordering physician. All rejected specimens are recorded in the rejection log located in the cytology department at CMC and CMC-Mercy.

B. Specimen Collection
   1. Mucosal Brushings
      a. Respiratory, Gastrointestinal, and Urinary Brushings. Specimens include but are not limited to Bronchial, Bile Duct, Colonic, Duodenal, Esophageal, Gastric, and Gastroesophageal, Renal, Ureteral Brushings.
      b. Follow specimen identification instructions in Section III, Subsection A of this procedure.
      c. Submit the collection brush by placing the brush directly into a specimen container containing 30ml of CytoLyt solution.
      d. Tightly secure the lid of the specimen container and place into a biohazard transport bag with the matching requisition in the outer pocket.
   2. Mucosal Washings
      a. Respiratory, Gastrointestinal, Pelvic and Urinary Washings. Specimens include but are not limited to Bronchial Alveolar Lavage (BAL) as well as Bronchial, Bladder, Pelvic, Peritoneal, Renal, and Ureteral Washings.
      b. Follow specimen identification instructions in Section III, Subsection A of this procedure.
      c. Collect the specimen using a balanced electrolyte solution.
      d. Add 30ml of CytoLyt Solution to the fresh specimen as soon as possible.
      e. Tightly secure the lid of the specimen container and place into a biohazard transport bag with the matching requisition in the outer pocket.
   3. Sputum
      a. Follow specimen identification instructions in Section III, Subsection A of this procedure.
      b. Instruct the patient to rinse mouth vigorously 3 times with water and then to cough deeply and expectorate into a clean specimen cup.
      c. Add 30ml of CytoLyt Solution to the fresh specimen as soon as possible.
      d. Tightly secure the lid of the specimen container and place into a biohazard transport bag with the matching requisition in the outer pocket.
   4. Serous Effusions
      a. Specimens include Ascitic (Paracentesis), Pericardial, and Pleural Fluid.
      b. Follow specimen identification instructions in Section III, Subsection A of this procedure.
      c. Collect 50-100 ml of fluid into non-glass bottles or syringes (remove needles before sending). **Do not submit serous effusions in glass bottles.**
      d. Tightly secure the lid of the specimen container and place into a biohazard transport bag with the matching requisition in the outer pocket.
      e. Specimen should be transported to the lab fresh and without delay. Refrigerate if transport will be delayed.

      Note: In cases where effusion specimens are not refrigerated, cells may be degenerated and non-diagnostic. A second specimen of rapidly re-accumulating fluid may provide freshly exfoliated diagnostic cells.

5. Urine
    a. Specimens include voided, catheterized, renal, and ureteral urine.
    b. Follow specimen identification instructions in Section III, Subsection A of this procedure.
    c. Collect 50 ml of urine into a specimen cup.
    d. Add 30ml of CytoLyt Solution to the fresh specimen as soon as possible.
    e. Tightly secure the lid of the specimen container and place into a biohazard transport bag with the matching requisition in the outer pocket.
6. Central Nervous System Fluids
    a. Specimens include cerebral spinal fluid (CSF), CNS cyst fluid, and ventric fluid.
    b. Follow specimen identification instructions in Section III, Subsection A of this procedure.
    c. Collect the specimen into a clean specimen container.
    d. Tightly secure the lid of the specimen container and place into a biohazard transport bag with the matching requisition in the outer pocket.
    e. Specimen should be transported to the lab fresh and without delay. Refrigerate if transport will be delayed.
7. Superficial Scrapings
    a. Specimens include Tzank smear, skin lesions, and oral cavity specimens.
    b. Follow specimen identification instructions in Section III, Subsection A of this procedure.
    c. With a pencil, label the frosted end of a slide(s) with patient's name and specimen site.
    d. Rupture an intact vesicle with a sterile blade and scrape the base and edge of the vesicle.
    e. Apply material collected onto the labeled slide(s) as a thin smear.
    f. ***Immediately*** submerge slides in 95% alcohol jar. ***Do not*** allow air-drying of slides.
    g. Tightly secure the lid of the alcohol jar and place into a biohazard transport bag with the matching requisition in the outer pocket.
8. Nipple Discharge
    a. Follow specimen identification instructions in Section III, Subsection A of this procedure.
    b. With a pencil, label the frosted end of a slide with the patient's name and specimen site.
    c. Express fluid on the labeled slide and ***immediately*** submerge slide in 95% alcohol jar. ***Do not*** allow air-drying of slides.
    d. Tightly secure the lid of the alcohol jar and place into a biohazard transport bag with the matching requisition in the outer pocket.
9. Joint Fluid
    a. Follow specimen identification instructions in Section III, Subsection A of this procedure.
    b. Specimen may be sent to the lab in the syringe used for collection provided that the needle is removed and the syringe is recapped and labeled before transport.
    c. Alternatively, the specimen may be transferred to a clean specimen container containing 30 ml of CytoLyt.
    d. Transport the specimen in a biohazard transport bag with the matching requisition in the outer pocket.
10. ARC (Anal-Rectal Cytology)
    a. Rectal Smears should be collected using a SurePath vial and a Dacron swab.
    b. Follow specimen identification instructions in Section III, Subsection A of this procedure.
    c. Moisten the tip of a Dacron swab with tap water.

d. Insert the Dacron swab 5-6cm into the anal canal, past the anal verge, into the rectal vault. This is done without direct visualization of the anal canal.
e. Apply firm lateral pressure to the swab handle as it is rotated and slowly withdrawn from the anal canal, inscribing a cone-shaped arc. Care should be taken to ensure that the transition zone is sampled. A swab or smear of the Peri-Anal skin is an unsatisfactory sample for ARC.

   Note: A cytobrush may be used to collect an ARC, but the cytobrush may be more uncomfortable for the patient. Avoid using cotton swabs on a wooden stick as the handle may break and splinter during collection.

f. Place the swab into a SurePath vial and agitate vigorously several times to release the cellular harvest.
g. If a SurePath vial is not available, the swab can be smeared onto a glass slide and then spray-fixed as per the procedure for conventional cervical Pap smears.
h. Tightly secure the lid of the vial and place into a biohazard transport bag with the matching requisition in the outer pocket.

11. Other

    Please consult with the Department of Cytology at (704) 304-5985 prior to obtaining any specimens not listed above.

C. Specimen Transport
1. CMC
   a. Please deliver cytology specimens directly to the cytology department located in the laboratory on the 4th floor, G wing between the hours of 7:30am and 5pm.
   b. Outside of normal operating hours please deliver cytology specimens to central processing also located in the laboratory on the 4th floor, G wing.
2. CMC–Mercy
   a. Please deliver cytology specimens directly to the cytology department located in the laboratory on the 3rd floor between the hours of 7:00am and 5pm.
   b. Outside of normal operating hours please place specimens into the refrigerator located in histology on the 3rd floor.
3. CMC-Pineville
   a. Please deliver cytology specimens to the CMC-Pineville central processing laboratory area.
   b. A regular courier run transports the specimens to CMC-Mercy for processing.
4. Other facilities
   a. Specimens from other locations are to be sent to the central processing area located at CMC.
   b. Please consult with the courier system for your facility as to their procedures regarding specimen transport to CMC.

QUICK TABLE FOR COLLECTION OF NON-GYNECOLOGIC CYTOLOGY SPECIMENS

Specimen Type	Slides	Fluid
Fine Needle Aspirations	½ slides **immediately** fixed in 95% alcohol ½ slides air-dried	Rinse needle and syringe in 30 ml of CytoLyt.
Bronchial Brushings and Washings Gastrointestinal Brushing and Washing Urinary Brushing and Washing Pelvic/Peritoneal Washing Sputum Urine	None	Specimen container with 30ml of CytoLyt.
Pleural (Thoracentesis) Fluid Ascitic (Paracentesis) Fluid Cerebral Spinal Fluid	None	Send fresh. Do Not Add Fixative! Refrigerate if transport is delayed.
SUPERFICIAL SCRAPINGS: Tzanck test (skin), oral, etc.	**Immediately** submerse slides in 95% alcohol	Do not use CytoLyt
Nipple Discharge	**Immediately** submerse slides in 95% alcohol	Do not use CytoLyt
Rectal Smear	Spray fixed slide if SurePath vial is unavailable	SurePath Vial

Remember:

Materials above may be obtained from the cytology department
CMC……..…(704) 355-4252
CMC-Mercy…….(704) 304-5985
Ratio of 3 parts specimen to 1 part CytoLyt.
Send a completed Non-Gynecologic requisition for each specimen submitted.

Collection of Fine Needle Aspiration Specimens V05.1

XI. Purpose

Fine Needle Aspirations (FNA) can provide a rapid, simple, and inexpensive means to evaluate a mass. There are many limitations to the interpretation of FNA specimens. The most common limitation is improperly obtained or inadequately fixed material. This procedure is to provide standard cytopreparatory procedures for nurses and physicians so that an optimal specimen is collected.

XII. Materials Needed

- Cytology specimen requisition*
- Syringe and needle containing specimen
- Glass slides*
- Plastic slide jar containing 95% alcohol*
- Styrofoam slide carrier*
- Specimen cup containing 30cc of CytoLyt*

*Materials above may be obtained from the cytology department
CMC……. (704) 355-4252
Mercy…… (704) 304-5985

XIII. Procedure

A. Fill out the cytology requisition
   1. Full name of patient
   2. Date of Birth
   3. Gender
   4. Ordering physician's full name
   5. Specific specimen type/site (be specific, include quadrant, right, left, o'clock etc.)
   6. Date of collection
   7. Unique patient identifier (MRN, Hospital Number, SSN, Chart #)
   8. Pertinent clinical data
   9. Number of slides submitted

B. Attach a copy of insurance information to the requisition.

C. Label the frosted end of the glass slides (in pencil) and the specimen cup (marker) with the patient's name and specimen type/site.

D. Collect Optimal Fine Needle Aspiration Specimen

Notes to clinicians:

- When the needle is in the lesion, use a rapid back and forth motion to shear off cells into the needle. A 25-gauge needle should be used in most cases. A 27-gauge needle is useful in vascular organs such as thyroid to decrease obscuring blood.
- When sampling non-cystic (solid) lesions, the aspiration should conclude with no more than a drop of blood in the hub of the needle. A bloody aspirate expressed onto the glass slides obscures diagnostic cells as well as important background clues (i.e., colloid, stroma, etc.).
- For cystic or necrotic lesions, sample the cyst wall or periphery and re-aspirate any remaining mass after cyst drainage.
- Most lesions should be sampled 2 to 4 times with a set of slides prepared from each pass.

- Always submit pairs of smears as one slide immediately submersed in 95% alcohol and one slide air-dried.

- The needle should be rinsed in CytoLyt after each set of slides is prepared to optimize cellular yield. Never reuse a needle rinsed in CytoLyt.
- Thick smears are often Non diagnostic. Prepare smears as indicated in Figures 2 thru 4 using 1-2 drops of aspirated material.
- Contact Dr. McDermott (Medical Director of Cytology) at 355-3471 or page 346-5880 if repeatedly receiving interpretations that are “limited”, “sub optimal”, or “non-diagnostic”.

E. Gently express 1-2 drops of the needle content onto a labeled clean glass slide (Figure 2). The total number of slides depends on number of passes and the amount of material collected.
F. Working quickly to prevent air-drying, prepare smears by placing another labeled clean glass slide face down, on top of the expressed material (Figure 3)
G. Gently Pull slides apart horizontally in opposite directions (Figure 3).
H. Immediately submerse one slide per pair in the plastic jar containing 95% alcohol (Figure 4).
I. Place the other slide into the Styrofoam slide container and allow to air dry (Figure 5).
J. Express and rinse the syringe into the specimen cup containing CytoLyt. Never reuse a needle rinsed in CytoLyt.
K. When the procedure is complete note on the cytology requisition the number of air dried slides, number of alcohol fixed slides, amount of CytoLyt fluid, and the number of passes.
L. Tightly secure the lids of the containers and place all specimen materials into a biohazard bag. Place the requisition in the outer pouch of the specimen bag and transport specimen to laboratory

Tips For Optimal Fine Needle Aspiration

1. Most lesions should be sampled multiple times (2-4x) with a set of slides prepared from each pass. The needle should be rinsed in CytoLyt after each set of slides is prepared to optimize cellular yield, then discarded.

2. When the needle is in the lesion, use a rapid back and forth motion to shear off cells into the needle. A 25-gauge needle should be used in most cases. A 27-gauge needle is useful in vascular organs such as thyroid to decrease obscuring blood.

3. When sampling non-cystic (solid) lesions, the aspiration should conclude with no more than a drop of blood in the hub of the needle. A bloody aspirate expressed onto the glass slides obscures diagnostic cells as well as important background clues (i.e., colloid, stroma, etc.).

4. Always submit paired direct smears as one slide air-dried and one slide immediately alcohol-fixed.

5. For cystic or necrotic lesions, sample the cyst wall or periphery and re-aspirate any remaining mass after cyst drainage.

6. Thick smears are often Non diagnostic. Prepare smears as indicated in Figures 2-6 using 1-2 drops of aspirated material.

7. Contact Dr. McDermott, Medical Director of Cytology, at (704) 355-3471/pager (704) 346-5880 if repeatedly receiving interpretations that are “limited”, “sub optimal”, or “non-diagnostic”.

Figure 2

Express 1-2 drops of specimen onto a glass slide 1/3rd of the way from the frosted end. Repeat for each pass. Quickly work through figures 2-6.

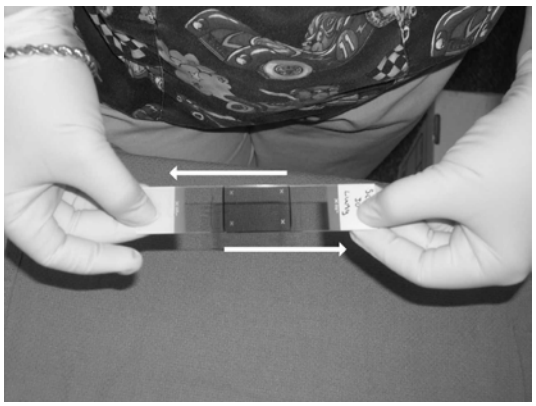


Figure 1

Materials Needed

- Syringe and needle
- Glass slides* (labeled with pencil)
- Plastic slide jar containing 95% alcohol*
- Styrofoam slide carrier*
- Specimen Cup containing 30cc of CytoLyt*

*Materials may be obtained from the cytology department. See Section II of this procedure.

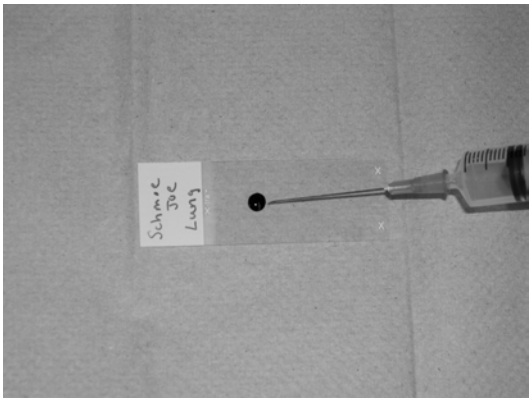


Figure 3

- Place a clean slide face down on top of the expressed material.
- Gently pull slides apart horizontally in opposite directions.
- Immediately submerse one slide per pair in 95% alcohol jar (see figure 4).
- Allow one slide per pair to air dry (see figure 5).

Figure 5

Place one air-dried slide per pair into the Styrofoam slide container.

Figure 4

Immediately submerse one slide per pair in the plastic jar containing 95% alcohol.

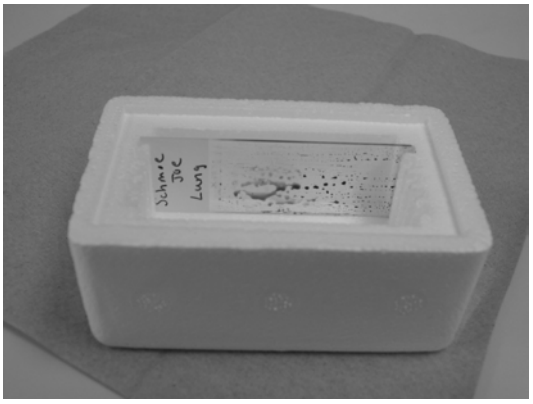


Figure 6

Express the remainder of the specimen into the specimen cup containing CytoLyt. Rinse the needle and syringe by aspirating the CytoLyt into the syringe and expressing it back into the specimen container. Never reuse a needle or syringe rinsed in CytoLyt.

Carolinas Medical Center
Laboratory Procedures Manual
Surgical Pathology/Histology

I. Policy:

A. All material removed from patients during operations in the operating room, hospital floors, emergency room, physician offices and surrounding hospitals will be submitted to the histology laboratory in a properly labeled container accompanied by a properly filled out Surgical Pathology Request Form.

II. Procedures:

A. Each specimen will be placed in a separately labeled containing 10% formalin unless otherwise ordered by the physician (See Procedures for Specific Specimens).

B. Container labels must including the following:

1. Patient's Name: Last, First, Middle
2. History Number
3. Body site or specimen type
4. Collector's initials

C. A Surgical Pathology Request form must accompany and be attached to the specimen. Requisitions must include the following:

1. Patient's Name: Last, First, Middle
2. History Number
3. Patient Location
4. Ordering Physician (Printed)
5. Test to be performed (including any specific instructions)
6. Date and time specimen was collected
7. Specimen type or body site
8. Diagnosis and procedure performed

D. Specimens are delivered to the Histology Laboratory between 5 a.m. until 5:30 p.m. Monday through Friday and 5 a.m. until 12 noon on Saturdays. During other hours, specimens are delivered to Central Processing on the 4th level (phone # 355-5818).

III. Procedure for Specific Specimens:

A. Routine Specimens: Place each specimen in a properly labeled container of 10% formalin and attach a completed Surgical Pathology Request form to the specimen container.

B. Rush Specimens: Place each specimen in a properly labeled container of 10% formalin, attach a properly filled out Surgical Pathology Request form. Mark the **"RUSH"** with a red check. Deliver specimen immediately to the Histology Laboratory.

C. Frozen Section Specimens: Place specimen on saline moistened gauze in a plastic container (never place tissues for frozen section in 10% formalin). Ensure the F/S box on the Surgical Pathology Request form is checked in red. Attach a label and Surgical Pathology Request form to the container. Call 355-3472 (Histology) to alert

Pathologists a frozen section specimen is on the way to the laboratory. Take specimen immediately to the Histology Laboratory. After 5:30 p.m. on weekdays, 12:00 noon Saturdays and all day Sunday. Call 355-5818; Laboratory will notify Pathologist on call.

D. Fresh Specimens: Tissue not preserved in 10% formalin is placed on a saline moistened gauze and placed in a properly labeled plastic impervious container. Attach a filled out Surgical Pathology Request form. Write the word **"Fresh"** on the form in red. Deliver specimen immediately to the Histology Laboratory. The following procedures require a fresh specimen:

   1. Touch Preparation: Note **"Touch Prep"** on Surgical Pathology Request Form.

   2. Muscle and Nerve Biopsies: Place tissue on saline moistened gauze in a properly labeled container and attach Surgical Pathology Request form. Notify Histology (355-3472) that biopsy is being performed. Once obtained, deliver to Histology immediately.

   3. Kidney Biopsy: Submit specimen on saline moistened gauze in a properly labeled container with accompanying Surgical Pathology Request form. Deliver specimen to Histology immediately.

   4. Bone Biopsies for Metabolic Bone Studies: Specimen is delivered fresh in a properly labeled container with filled out Surgical Pathology Request form. Deliver immediately to Histology Laboratory. The Histology Lab will place the specimen in a 70% alcohol solution. If the surgeon wishes, the specimen will be sent to the Orthopedic Hospital. The Histology Lab will handle the request.

E. Liver Biopsy for Quantitative Iron Analyses: Specimen is placed in a properly labeled metal free container either fresh or in 10% formalin. Attach a completed Surgical Pathology Request form.

F. Specimens for Immunofluorescence Studies: Specimen is placed in a properly labeled container filled with transport media which is available upon request from the Histology Laboratory.

G. Testicular Biopsies: Place specimen in a properly labeled container filled with Bouins Fixative (Picric acid and alcohol) which is available from the Histology Laboratory.

H. Large Specimens: (Specimens too large for the largest formalin filled container) Place specimen in a double trash bag with several blue liners or in a basin which is then placed in a double trash bag. Attach a label and a Surgical Pathology Request form to the outside of the bag and deliver immediately to the Histology Laboratory.

CAROLINAS LABORATORY NETWORK

PROCUREMENT OF TISSUE SPECIMENS FOR CHROMOSOMAL ANALYSIS

PURPOSE

To obtain and transport tissue from skin biopsy or pregnancy losses to the Parke Cytogenetics Laboratory.

SPECIMEN

Fetal tissue, extraembryonic membranes, chorionic villi, skins

PROCEDURE

A. **Pregnancy loss:**

1. If material is obtained by way of D&E early in the pregnancy, send the entire specimen (enclosed in sock) so that the technologists can identify fetal tissue, chorionic villi/membranes and avoid maternal decidua.

2. Often the material from D&E's have enough blood and fluids that additional media is not needed, however, if the material is likely to dry out, tissue culture media should be added to it. Media may be obtained from the Parke Cytogenetics Laboratory. Call 355-3848.

3. If chromosome studies were ordered on a stillbirth, a skin biopsy, Achilles tendon and placental tissue from the fetal surface should be obtained in a sterile manner.

4. Wipe the area with alcohol. **DO NOT USE BETADINE** which will render the tissue nonviable. Obtain the specimens by punch biopsy or sterile scalpel blade.

5. The specimen should be placed in media and delivered to the cytogenetics lab.

6. If there is a delay in delivery (e.g. after 5:00 p.m. during the week or on the weekend), place the specimen in a refrigerator. **DO NOT FREEZE**.

B. **Skin biopsy from child or adult:**

1. Disinfect the area from which the biopsy is to be obtained with alcohol. **DO NOT USE BETADINE**.

2. Obtain specimen by punch biopsy or sterile scalpel.

3. Place specimen in tissue culture media.

4. If there is a delay in delivery (e.g. after 5:00 p.m. during the week or on the weekend), place the specimen in a refrigerator. **DO NOT FREEZE**.

C. **Notification and Delivery to the Parke Cytogenetics Laboratory**

1. Specimens can be sent via central processing through the CLN laboratory.

2. To inform cytogenetics that a specimen will be arriving, call the cytogenetics laboratory at **355-3848** during working hours 8-5 Monday through Friday. If after hours during the week M-F, call **355-3159**, and leave a message on voice mail to indicate that we will be receiving a specimen so PCL personnel can follow up on the specimen if it is not received in a timely manner.

3. After 5:00 p.m. on Fridays and anytime on weekends, call 355-3159 and leave a message to indicate that we will be receiving a sample on Monday. If the physician orders a **STAT** processing, page the cytogenetics "on call" technologist at 5011 (dig) and the technologist will pick it up. Once again, place specimens in media, if needed, and place in refrigerator until delivery.

Do Not Put Specimens in Formalin and Do Not Freeze or Place on Ice. Refrigerate Only or cells will be nonviable. Always place in media if there is a possibility of drying out. Saline may be used as a last resort and if delivered the next day.

Specimens should be sent by courier, if possible. Cytogenetics personnel will pick them up if necessary. All tissues should be sent to the Parke Cytogenetics Laboratory with a laboratory lab request form enclosed. Tests may be ordered through Sunquest.