CONWAY REGIONAL HEALTH SYSTEM
CLINICAL LABORATORY

Microbiology Specimen Collection Guidelines

PRINCIPLE:

Specimen collection and transportation are critical considerations, as laboratory results are affected by the quality of the specimen and its condition on arrival in the laboratory. Specimens should be obtained to preclude or to minimize the possibility of introducing extraneous (contaminating) microorganisms that are not involved in the infectious process. This is a particular problem with specimens that are already colonized with microorganisms that are not involved in the infectious process. Use of special techniques that bypass areas containing normal flora when this is feasible prevents many problems associated with false-positive results. Likewise, careful skin preperation before procedures such as blood cultures and spinal taps decreases the chance that organisms normally present on the skin will contaminate the specimen. Specimens should be collected during the acute (early) phase of an illness (or within 2 to 3 days for viral infections), and before antibiotics are administered, if possible.

SPECIMEN MANAGEMENT:

Biosafety at the laboratory bench is of primary concern to laboratorians. Health care workers may be unaware of the potential etiologic agent(s) residing in the specimen being transported to the laboratory.

In general, health care workers should comply with the following policies for safety in specimen management:

1. Wear gloves, gowns, and where appropriate, masks and/or goggles when collecting specimens
2. Use leak-proof specimen containers and transport the containers within a sealable, lea-proof plastic bag with a separate compartment for paperwork.
3. Never transport syringes with needles to the laboratory. Instead transfer the contents to a sterile tube or remove the needle with a protective device, recap the syringe, and place it in a sealable, leak-proof plastic bag.
4. Do not transport leaking specimen containers to the laboratory or process them. Notify the physician or the responsible nurse of the leaking container and explain the potential compromised nature of the results if processing is continued; ask for a repeat specimen. If a new specimen is submitted, discard the leaking one. If another specimen cannot be obtained, work with the existing specimen container within a biological safety cabinet.

SPECIMEN: Collection Time and Temp

Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Abscess	Remove surface exudate by wiping with sterile saline or 70% alcohol.					Tissue or fluid is specimen of choice.
Open	Aspirate or pass a swab deep into the lesion and sample the lesions edge	Swab transport system	≤ 2h, RT	≤ 24h, RT	1/day/source	A sample from the base or wall are best.
Closed	Aspirate abscess wall material with syringe. Aseptically transfer all material into anaerobic transport.	Anaerobic transport system ≥ 1ml	≤ 2h, RT	≤ 24h, RT	1/day/source	DO NOT sample surface area (contamination)
Blood Culture	Disinfection of culture bottle apply 70% isopropyl alcohol to rubber stoppers and wait 1 min.	Bacteria: blood cultures vials Adult 10 to 20 ml/set High volume most productive	≤ 2h, RT	≤ 24h RT or per instructions	3 sets in 24 h	Acute Sepsis 2 to 3 sets from seperate sites, all within 10 min. Endocarditis, acute 3 sets from 3 seperate sites. over 1 to 2 h. Endocarditis, subacute 3 sets from 3 seperate sites. Fever of unknown origin. 2 to 3 sets from seperate sites ≥ 1 h apart. If neg. at 24h obtain 2 to 3 more sets.
	Palpate for the vein first					
	Disinfection of venipunture site 1. Cleanse site with 70% alcohol. 2. Swab concentrically, starting at the center, with an iodine preparation. 3. Allow the iodine to dry. *4.* DO NOT *palpate the vein at this point.* 5. Collect blood, 6. After venipuncture, remove iodine from the skin with alcohol.	Infant, 1-5 ml/set. Fungi: 1. Biphasic culture 2. Lysis centrifugation				Some data indicate that an additional aerobic bottle is more productive than an anaerobic bottle.
Bone marrow	Prepare puncture side as for surgical incision.	Inoculate a blood culture bottle	≤24h, RT if culture bottle.	≤24h, RT	1/day	Small volumes of bone marrow may be inoculated directly on culture media.
Burn	Clean and debride the burn wound prior to specimen collection	Place tissue in sterile screw top container. swab exudate.	≤ 2h, RT	≤24hr, RT	1/day/source	A 3-4 punch biopsy is optimum. Process for aerobic culture only. Quantitative cultures may or may not be useful.

SPECIMEN: Collection Time and Temp

Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Catheter: i.v.	1. Cleanse the skin around the catheter site with alcohol. 2. Aseptically remove and clip 5cm of the distal tip of the catheter and place in a sterile cup. 3. Transport directly to the Microbiology lab to prevent drying.	sterile screw-cap container.	≤ 15min, RT	≤ 24h, 4°C	None	Acceptable i.v. catheters for semi-quantitative cultures: central,CVP, Hickman, peripheral, arterial,Broviac,umbilical hyperalimentation, Swan Ganz.
Foley	DO NOT culture since growth represents distal urethral flora					NOT ACCEPTABLE for culture
Cellulitis	1. Cleanse site with 70% alcohol or sterile saline. 2. Aspirate the area of maximum inflammation. (usually the center). With a fine needle and a syringe. 3. Draw small amount of sterile saline into syringe and aspirate into sterile container.	sterile container	≤ 15min, RT	≤ 24h, RT	None	Yield of potential pathogens is 25 to 35%.
CSF	1. Disinfect site with 2% tincture. 2. Insert needle with stylet at L3-L4, L4-L5, L5-S1 interspace 3.Upon reaching the subarachnoid space remove the stylet and collect 1 to 2 ml of fluid into each of 3 leak-proof tubes.	Sterile screw-cap tubes. Minimum amt. Bacteria, ≥1 ml Fungi, ≥2 ml AFB, ≥2 ml Virus, ≥1 ml on ice	Bacteria: NEVER REFRIGERATE ≤ 15min, RT	≤ 24h, RT	None	Obtain blood for culture also. Submit tube #2 to Microbiology. Aspirate of brain abscess or a biopsy may be necessary to detect Anaerobic bacteria or parasites.
Decubitus ulcer	A swab is not the specimen of choice 1. Cleanse surface with sterile saline. 2. If a sample biopsy is not available, swab the base of the lesion. 3. Place swab in appropriate transport system.	Swab transport (aerobic) Tissue: (anaerobic system)	≤ 2h, RT	≤ 24h, RT	1/day/source	A tissue biospy is specimen of choice. A decubitis ulcer swab provides little clinical information. Collection should be discouraged.

SPECIMEN: Specimen type:	**Collection** Guidelines	Device and/or minimal vol.	**Time and Temp** Transport	Storage	Replica limits	Comments
Dental culture: gingival,periodontal, periapical, Vincents stomatitis	1. Carefully cleanse gingival margin and supragingival tooth surface to remove 2. Using a periodontal scaler, carefully remove subgingival lesion material and transfer it to an anaerobic transport system. 3. Prepare for staining smears that have been collected in the same fashion.	Anaerobic transport system	≤ 2h, RT	≤ 24h, RT	1/day	Peridontal lesions should be processed only by labs equipped to provide specialized techniques for the detection enumeration of specific agents.
Ear:						
Inner	1. For intact ear drum, clean ear canal with soap solution and collect fluid via syringe aspiration technique. 2. For ruptured ear drum, collect fluid on swab via an auditory speculum.	Sterile swab or anaerobic system	≤ 2h, RT	≤ 24h, RT	1/day/source	Throat or nasopharyngeal specimens should not be submitted for otitis media.
Outer	1. Use moistened swab to remove any debris or crust from the ear canal. 2. Obtain a sample by firmly rotating swab in the outer ear canal.	Swab transport	≤ 2h, RT	≤ 24h, 4°C	1/day/source	For otitis externa, *vigorous* swabbing is required since surface swabbing may miss streptococcal cellulitis.
Eye						
Conjunctiva	1. Sample both eyes with Seperate swabs (premoistened with sterile saline) by rolling over each conjunctiva. 2. Inoculate medium at time of collection. 3. Smear swabs onto slides for staining.	Direct culture innoculation: BAP CHOC or swab transport	≤ 15min, RT	≤ 24h, RT	None	Swab both eyes even if 1 is not infected. This can serve as a control to compare agents isolated from the infected eye. Gram stain can also be used.
Corneal scrapings	1. Obtain swab specimens As described above. 2. Instill 2 drops of local anesthetic. 3. Using a sterile spatula scrape ulcers or lesions, and inoculate scraping directly onto medium. 4. Apply remaining material to 2 clean glass slides for staining.	Direct culture Inoculation: BHI with 10% sheep bld, CHOC, and IMA	≤ 15min, RT	≤ 24h, RT	None	It is recommended that swab for culture be taken prior to to anesthetic application, whereas corneal scrapings can be obtained afterward.

SPECIMEN: Collection			Time and Temp			
Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Fluid or aspirates	Prepare eye for needle Aspiration of fluid.	Sterile screw-cap container or tube or direct inoculation of small amounts of fluid onto media.	≤ 15min, RT	≤ 24h, RT	1/day	Include fungal media. Anesthetics may be inhibitory to some etilogic agents.
Feces						
Routine culture	Pass directly into a clean dry container. Transport the specimen to microbiology laboratory within 1 h of collection or transfer a visible portion onto a transport swab Or transfer into Cary blair medium	Clean, leak-proof, wide-mouth container or use a swab transport system or Cary Blair medium.	Unpreserved ≤ 1 h, RT Swab transport system ≤ 24h RT.	≤ 24h, 4°C ≤ 48h, RT 4°C	1/day	DO NOT perform routine stool cultures for patients whose length of stay was > 3 days and the admitting diagnosis was not gastroenterititis. Swabs are recommended Only on infants and Patients with diarrhea.
C. difficile	Pass liquid or soft stool directly into clean, dry container. Swab specimen not recommended for toxin testing	Sterile leak-proof wide-mouth Container, ≥ 5 ml	≤ 1h, RT 1-24 h, 4° C > 24h, -20°C	2 days, 4°C for culture 3 days, 4°C or longer at -70°C for toxin test.	½ day	Patients should be passing > 5ml of liquied or soft stools every 24h. Testing of hard or formed stools is not recommended. Freezing at –20 facilitates rapid loss of cytotoxin effect.
E. coli 0157:H7	Pass liquid or bloody stool into a clean dry container.	Sterile, leak proof, wide-mouth container or use swab transport systen ≥ 2ml.	Unpreserved ≤ 1 h, RT. Swab transport system ≤ 24 h RT. or 4° C.	< 24h, 4°C.	1/day	Bloody or liquid stools collected within 6 days of onset among patients with abdominal cramps have the highest yield.
Leukocytes (Not recommended)	Pass feces into a clean dry container. Transport to lab within 1 h. Or transfer to O&P system (10% formaliu or PVA.)	Sterile leak-proof container or 10% formalin and/or PVA > 2ml.	Unpreserved ≤ 1 h, RT. Formalin/PVA: indefinite, RT	≤ 24 h, 4°C Indefinite, RT.	1/day	This procedure should be discouraged. The results are of little clinical value and could be misleading
Rectal swab	1. Carefully insert a swab ≈1 in. beyond the anal sphincter. 2. Gently rotate the swab to sample the anal crypts. 3. Feces should be visible on the swab for detection of pathogens,.	Swab transport	≤ 2h, RT	≤ 24 h, RT	1/day	Reserved for detecting Neiserria gonorrhoeae, Shigella, Campylobacter, and HSV and anal carriage of Group B strep or for patients unable to pass specimen.

SPECIMEN:	Collection		Time and Temp			
Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Fistulas	See abscess.					
Fluids: adominal, amniotic,ascites, bile, joint, paracentesis, pericardial peritoneal, pleural, synovial, thoracentesis	1. Disinfect overlying skin with 2% iodine tincture. 2. Obtain specimen via percutaneous needle aspiration or surgery. 3. Transport immediately to laboratory. 4. Always submit as much fluid as possible NEVER submit a swab dipped in fluid.	Blood culture bottle for bacteria and yeast or sterile screw-cap tube or anaerobic transport system. Bacteria, ≥1 ml	≤15 min, RT	≤ 24 h, RT Pericardial fluid and fluids for fungal cultures ≤ 24 h, 4°C.	None	Amniotic and culdocentsis fluids should be placed in anaerobic system and need not be centrifuged prior to Gram staining . Other fluids are best examined by Gram staining of a cytocentfriuged prep.
Gangrenous tissue	See Abscess					
Gastric: Wash or Lavage	Collect in early morning before patient eats and While they are still in bed. 1. Introduce a nasogastric tube orally or nasally to the stomach. 2. Perform lavage with 25 to 50 ml of chilled, sterile, distilled water. 3. Recover sample and place in a leak-proof, sterile container. 4. Before removing the tube, release suction, clamp it.	Sterile leak-proof container.	≤ 15 min, RT or neutralize within 1 h of collection.	≤ 24h, 4°C	1/day	Tissue biopsy or Aspriates are preferred. Discourage sampling of surface or surface tissue. The specimen must be processed immediately . Mycobacterium die rapidly in gastric washings. Neutralize each 35 to 50 ml of gastric washing with 1.5 ml of 40% anhydrous Na2HPO4.
Genital: female						
Amniotic	1. Aspirate via amniocentesis, cesarean delivery, or intrauterine catheter. 2. Transfer liquid to anaerobic transport system.	Anaerobic transport system, ≥ 1ml	≤ 2 h, RT	≤ 24h, RT	None	Swabbing or aspiration of vaginal membrane is not acceptable because of potential contamination of vaginal flora
Bartholin	1. Disinfect skin with iodine preparation 2. Aspirate fluid from ducts.	Anaerobic transport system, ≥ 1ml	< 2h, RT	< 24h, RT	1/day	

SPECIMEN:	Collection		Time and Temp			
Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Genital (con'd) Cervix	1. Visualize the cervix using a speculum without lubricant. 2. Remove mucus and secretions from the cervix with swab and discard the swab. 3. Firmly yet gently sample the endocervical canal with a newly obtained swab.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	
Cul-de-sac	Submit aspirate or fluid.	Anaerobic transport system. ≥ 1ml	≤ 2h, RT	≤ 24h, RT	1/day	
Endometrial	1. Collect transcervical aspirate via a telescoping catheter. 2. Transfer entire amount to anaerobic transport system.	Anaerobic transport system, ≥ 1ml	≤ 2h, RT	≤ 24h, RT	1/day	
Products of Conception	1. Submit a portion of tissue in a sterile container. 2. If obtained by cesarean delivery, immediately transfer to an anaerobic transport system.	Sterile tube or anaerobic transport system.	≤ 2 h, RT	≤ 24h, RT	1/day	Do not process lochia. This specimen may not provide clinically revelant results.
Urethral	Collect 1 h after patient has urinated. 1. Remove exudate from urethral orifice. 2. Collect discharge material on a swab by massaging the urethra against the pubic symphysis through vagina.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	If no discharge can be obtained wash the extenal urethra with Betadine soap and rinse with water. Insert a urethrogenital swab 2 to 4 cm into the urethra And rotate the swab for 2 sec.
Vaginal	1. Wipe away excessive amount of secretion or discharge. 2. Obtain secretions from the mucosal membrane of the vaginal vault with a sterile swab or pipette. 3. If a smear is also requested use a 2nd swab.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	For inrtrauterine devices, place entire device into a sterile Container and submit at RT. A Gram stain is recommemded for confirmation of bacterial vaginosis. Cultures are often inaccurate and misleading.

SPECIMEN:	Collection		Time and Temp			
Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Genital (con'd) Female and male lesions	1. Clean the lesion with sterile saline and lesion's surface with a sterile scapel blade. 2. Allow transudate to accumulate. 3. While pressing the base of the lesion, firmly sample exudate with a sterile swab.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	
Genital: Male Prostate	1. Cleanse the glans with soap and water. 2. Massage prostate through rectum. 3. Collect fluid on a sterile swab or in a sterile tube.	Swab transport or sterile tube.	≤ 2h, RT	≤ 24h, RT	1/day	Ejaculate may also be cultured.
Urethra	Insert a urethrogenital swab 2 to 4 cm into the urethral lumen, rotate swab, and leave it in place for 2 sec to facilitate absorption.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	
Hair, Dermatophytosis	1. With forceps, collect at least 10 to 12 affected hairs with the base of shaft intact. 2. Place in a clean tube or container.	Clean container, 10 hairs.	≤ 24h, RT		1/day/site	Collect scalp scales, if present, along with scrapings of active borders of lesions. Note any antifungal therapy taken recently.
Nail, Dermatophytosis	1. Wipe nail with 70% alcohol using gauze (not cotton). 2. Clip away a generous portion of the affected area and collect material or debris from under the nail. 3. Place material in a clean container.	Clean container Enough scrapings to cover the head of a thumbtack.	≤ 24h, RT		1/day	
Pilonidal cyst	See abscess					

SPECIMEN: Specimen type:	Collection Guidelines	Device and/or minimal vol.	Time and Temp Transport	Storage	Replica limits	Comments
Respiratory, lower Bronchoalveolar lavage, bronchial brush or wash tracheal aspirate	1. Place aspirate or washing into a sputum trap. 2. Place brush into a sterile container with saline.	Sterile container > 1 ml	≤2h, RT	≤ 24h, 4°C.	1/day	A total of 40 to 80 ml of fluid is needed for quantitative analysis. For quantitative analysis of brushings, place into 0.5 ml of Tryptic Soy Broth.
Sputum, Expectorate	1. Collect specimen under the direct supervision of a Nurse or physician. 2. Have patient rinse or gargle with water to remove superficial flora. 3. Instruct patient to cough deeply to produce a lower respiratory specimen (not postnasal fluid). Collect in a sterile container.	Sterile container > 1 ml. Minimum amounts Bacteria, > 1 ml Fungi, 3-5 ml Mycobacteria 5-10 ml Parasites, 3-5 ml	≤ 2h, RT	≤ 24h, 4°C.	1/day	For pediatric patients a respiratory therapist should collect a speciemn via suction. The best specimen should have ≤ 10 squamous cells/X 100 field.
Sputum, Induced	1. Have patient rinse mouth with water after brushing gums and tongue. 2. With the aid of a nebulizer have patient inhale ≈ 25 ml of 3 to 10% sterile saline. 3. Collect the induced sputum into a sterile container.	Sterile container	≤ 2h, RT	≤ 24h, RT	1/day	
Respiratory, upper Oral	1. Remove oral secretions And debris from the surface of lesion with a swab and then discard. 2. Using a second swab, vigorously sample the lesion, avoiding any areas of normal tissue.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	Discourage sampling of superficial tissue for bacterial evaluation. Tissue biospy specimens or needle aspirates are the specimen of choice.
Nasal	1. Insert a swab, premoistened with sterile saline, ≈ 2 cm into the nares. 2. Rotate the swab against nasal mucosa	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	Anterior nose cultures are reserved for detecting staphylococcal and streptococcal Carriers or for nasal lesions. A nasal speculum may be appropriate.
Nasopharynx	1. Gently insert a calcium alginate swab into the posterior nasopharynx via the nose. 2. Rotate swab slowly for 5 s to absorb secretions. 3. Remove swab and place in transport medium.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	

SPECIMEN:	Collection		Time and Temp			
Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Respiratory: upper (con't) Throat	1. Depress tongue with a tonge depresser. 2. Sample the posterior pharynx, tonsils, and imflamed areas with a sterile swab.	Swab transport	≤ 2h, RT	≤ 24h, RT	1/day	Throat swabs are contraindicated for patients with inflamed epiglottis.
Skin, dermatophytosis	1. Cleanse the affected area with 70% alcohol. 2. Gently scrape the surface of the skin at the active margin of the lesion. *Do Not draw blood.* 3. Place sample in clean container or between 2 clean glass slides.	Clean container enough scrapings to cover the head of a thumbtack.	≤ 24h, RT		1/day/site	If the specimen is submitted between glass slides, tape together and submit them in envelope.
Tissue	1. Submit in a sterile container. 2. For small samples, add severaldrops of sterile saline to keep moist. 3. DO NOT allow tissue to dry out.	Anaerobic transport system or sterile, screw-cap jar. saline may need to be added.	< 15min, RT	≤ 24h, RT	None	Always submit as much tissue as possible.Never submit a swab That has simply been rubbed over the surface. For quantitative study, a sample of 2 by 1 cm, is appropriate.
Urine Female midstream	1. Thoroughly cleanse the urethral area with soap and water. 2. Rinse with wet gauze pads. 3. While holding the labia apart, begin voiding. 4. After several milliliters have passed, collect a midstream portion without stopping the flow of urine.	Sterile, wide-mouth container, ≥ 1ml, or urine transport kit.	Unpreserved: ≤ 2h, RT Preserved: ≤ 24h, RT	≤ 24h, 4°C		1/day
Male, Midstream	1. Cleanse the glans with Soap and water. 2. Rinse with wet gauze pads. 3. While holding the foreskin retracted, begin voiding. 4. After several milliliters have passed, collect a midstream portion without stopping the the flow of urine.	Sterile, wide-mouth container, ≥ 1ml, or urine transport kit	Unpreserved: ≤ 2h, RT Preserved: ≤ 24h, RT	≤ 24h, 4°C		1/day

SPECIMEN:	Collection		Time and Temp			
Specimen type:	Guidelines	Device and/or minimal vol.	Transport	Storage	Replica limits	Comments
Urine (con't)						
Straight catheter	1. Thoroughly cleanse the urethral area with soap and water. 2. Rinse area with wet gauze pads. 3. Aseptically, insert a catheter into the bladder. 4. After allowing ≈ 15 ml to pass, collect urine to be submitted in a sterile container.	Sterile, leak-proof container	Unpreserved: ≤ 2h RT Preserved: ≤ 24h, RT	≤ 24h, 4°C	1/day	
Indwelling Catheter	1. Disinfect the catheter collection port with 70% alcohol. 2. Use a needle and syringe to aseptically collect 5 to 10 ml of urine. 3. Transfer to a sterile tube or container.	Sterile leak-proof container.	Unpreserved: ≤ 2h RT Preserved: ≤ 24h, RT.	≤ 24h, RT	1/day	
Wound	See abscess					

REFERENCE:
1.Journal of Clinical Microbiology 7th edition, Murray, 1999

PREPARED BY: Colleen Janota/Marianne S. Welch 09/13/05

APPROVED BY:_____DATE_____
(Medical Director)

APPROVED BY:_____DATE_____
(Clinical Director)