

NWHMC/UW Medicine Laboratory (Microbiology) Policy
MICROBIOLOGY SPECIMEN COLLECTION AND HANDLING FOR CLINICIANS

APPROPRIATE SPECIMENS:

To ensure collection of appropriate specimens for culture, consideration must be given to the pathogens being sought. Since many areas of the body (intestinal, genital, upper respiratory) are colonized with normal flora, care must be taken during collection to bypass these areas. Collection of any specimen after administration of antibiotics is usually futile. If bacterial growth occurs, it may be misleading due to the recovery of resistant, indigenous organisms unrelated to the infection. All specimens should be transported to the laboratory as soon as possible for processing to prevent overgrowth of either pathogenic or non-pathogenic organisms. **In general, a quality specimen yields a quality result and report!**

REQUISITIONS/LABELING:

Requisition forms must be filled out completely with the patient's name, demographic data, doctor name, specimen type and source (anatomical body site) and the date and time of collection. Indicating current antibiotic therapy and specific organisms suspected on the form is helpful. Each specimen must be labeled with two identifiers. Please provide a detailed specimen source to allow for proper interpretation of cultures.

CONTAINER SELECTION:

See [Appendix A](#) for picture chart of containers.

A. Cultures and Swabs:

Many specimens can be collected using a "Culturette" swab. **Culturettes are never refrigerated, and can "hold" organisms at room temperature up to 24 hours.** However, they should be transported to the laboratory as soon as possible, because the nutrients in the specimen can permit growth of commensal organisms thus distorting the proportion of organisms that were originally present. Ideally the culturette should be used for skin, lesion, genital, and upper respiratory sources. Aspirates or tissue are better specimens for deeper sources.

1. **BD Blue top dual swab system:** The dual swab is used for the majority of specimens. The directions for collection are on the wrapper. The swabs, once the specimen is collected, are immersed in a gel that allows for better recovery of organisms, including fastidious anaerobes.
2. **Red Top dual swab culturette:** The culturette is used just for throat specimen collection for Rapid Strep tests, follow-up throat cultures and MRSA screens. The directions for collection are on the wrapper. It is important to make sure that, once the transport media ampule has been crushed, the liquid medium has moistened the cotton ball at the bottom of the tube. This will ensure that the swab, once inserted, will come in contact with the moisture of the transport media and thus preserve the organisms until the swab can be plated on the proper media. Culturettes are used for aerobic organisms, although some aero-tolerant anaerobes will survive.
3. **See "Port-A-Cul" or syringe under "D." below if anaerobes are a consideration.**

B. Sterile containers:

Sterile screw cap containers or containers with secure lids can be used for urine, sputum, and stool specimens. Please make sure lids are screwed on tightly. Specimens should be refrigerated up to 24 hours to prevent overgrowth of normal flora.

Leaky containers are not satisfactory because:

1. Specimens can become contaminated.
2. Hospital and other personnel are exposed to potential disease hazards.

C. Port-A-Cul™:

This is a transport tube used in surgery for collecting swab specimens when anaerobes (and aerobes) are a consideration. The gel-like transport medium can hold organisms up to 24 hours at room temperature. **DO NOT** place tissue or fluids in Port-A-Culs; use a sterile container or syringe instead.

D. Syringes:

Syringes are excellent, and preferred, for collecting fluids or purulent specimens. Expel any air to preserve anaerobes. Before transporting these specimens to the laboratory the needle must be removed and the barrel capped with a rubber plug. Do not refrigerate specimens. Transport the specimen to the laboratory ASAP.

E. Virus and *Chlamydia* transport media:

The transport media for viruses and *Chlamydia* are both pink liquids (1 and ½ mL in a 15 mL screw capped tube) but differ in formulations. *Chlamydia trachomatis*, Herpes, respiratory viruses, and Cytomegalovirus (CMV) can all be collected in the viral transport media, and *Chlamydia pneumoniae* only in the *Chlamydia/Mycoplasma* transport.

F. Stool specimens:

Stool culture and ova and parasite transport media:

Specialized transport medias are recommended over "fresh" specimens for both culture and Ova and Parasite requests. Fresh specimens need to be transported to the laboratory within 30 minutes.

Enteric Transport Media or Cary blair is acceptable for stool cultures and Giardia Antigen.

ECOFIX or a Formalin/PVA combination is acceptable for Ova and Parasite exams, Giardia testing and Cryptosporidium testing.

Sterile containers should be used for *Clostridium difficile* and stool leukocyte testing.

G. Blood cultures:

The Microbiology Laboratory has an automated blood culture system and special bottles (an aerobic and an anaerobic bottle) are collected as a set for each draw. 5-10 mL of blood are recommended for each bottle. Before each venipuncture, meticulous site cleansing must be performed.

H. See [Appendix A](#) below for pictures.

TRANSPORTATION OF SPECIMENS TO THE LABORATORY:

All specimens, once collected, must be placed in plastic bags to protect personnel who handle them. Sputum, fresh stool and urine are the only specimens which can and must be refrigerated until transportation to the laboratory. If left at room temperature, any organisms that are present will double in 15 to 30 minutes, thus altering the concentrations of pathogens and non-pathogens alike.

GRAM STAINS:

The direct gram stain is of value for possible early diagnosis and for culture correlation. Gram stains are not performed on certain specimens such as throat and nose. Clinicians have the option of requesting a gram stain with each culture. Use of the Dual swab system is preferred when gram stains are requested.

SENSITIVITIES:

Sensitivities will be performed when ordered and/or at the discretion of the microbiologists. Generally, when 3 or more pathogens are isolated from any site, sensitivities will not be performed, as such a specimen usually reflects colonization or contamination by the patient's own indigenous flora.

Sensitivities will not be performed on normal skin flora or organisms for which there are no guidelines for reporting

REPORTING:

Preliminary reports are issued the next day on most cultures, or else when there is something of value to report. There often may be more than one preliminary report. Final reports are issued for all cultures, however the time to culture finalization will be different per culture/source.

SPECIMEN COLLECTION GUIDE:

Test Name	Specimen Collection Instructions
ACTINOMYCES CULTURE CXACT	Random specimen using a swab transport system, sputum or Bronchoalveolar Lavage (BAL) in a sterile container. IUD specimens can be submitted in sterile containers. Specimens should be refrigerated and transported immediately to the laboratory.
AFB CULTURE CXAFB	Sputum: 5-10 mL in a sterile container. Collect early-morning specimen from deep, productive cough. Specimens no longer need to be collected 3 consecutive days. Best sputum collection is under direct supervision of physician, R.N. or Respiratory Therapist. Urine: Minimum of 40 mL of first morning specimen collected in sterile container on 3 consecutive days . All other types should be submitted in sterile containers, collecting as much material as possible. Swabs are not recommended. Transport to the laboratory ASAP.
AFB CULTURE - STERILE FLUIDS CXAFB	Specimens (CSF's and body fluids) are collected by a physician. Collect as much as possible (10-15 mL minimum) in sterile container or syringe. Transport ASAP to the laboratory at room temperature.
AFB SMEAR AFGRB	See AFB Culture for collection particulars; smears are always performed on all sources except urine.
BETA STREP SCREEN (GENITAL) CXGRB	Collect specimen from vaginal/rectal site using a BD blue top dual swab transport system.
BETA STREP A SCREEN (RESPIRATORY) CXGRA	Swab red or white patches in throat using a red top dual swab system. Use a tongue depressor to keep tongue out of the way so as not to contaminate the swab with oral flora.
BLOOD CULTURE CXBLD	Blood is drawn by lab when the time is specified by the clinician. The optimum time to draw is just prior to anticipated chills or a temperature spike. Strict protocol exists for venipuncture site cleansing. 10 mL is optimal for each bottle (5 mL minimum).

	Two bottles are drawn per set: one aerobic and one anaerobe. Transport to the laboratory within 2 hours of collection.
BONE MARROW CULTURE	Collect in a sterile yellow top tube containing SPS anti-coagulant. See Blood Culture, AFB Culture, or Fungus Culture for correct Laboratory order number.
BRONCHIAL WASHINGS ROUTINE CULTURE CXRES	Collected by a physician in Endoscopy or Surgery in a sterile container. Keep refrigerated until taken to the laboratory.
CEREBRAL SPINAL FLUID (CSF) CULTURE CXCSF	Collected by a physician. Place fluid in sterile tube(s). Culture is usually performed on the 2nd tube in a series to avoid contamination of normal skin flora. Prompt delivery to lab is crucial. If multiple microbiology tests are required, at least 3 mL of specimen should be sent.
Test Name	Specimen Collection Instructions
CERVIX CULTURE CXGEN (with gram) CXNGN (without gram)	Wipe cervix clean of vaginal secretion and mucus. Use speculum and no lubricant. Collect specimen using a BD blue dual swab transport system. DO NOT REFRIGERATE SWAB. If <i>Neisseria gonorrhoeae</i> (GC) is a consideration, the viability of this organism is very fragile. Transport to the lab ASAP.
CHLAMYDIA – PCR CHPCR	Collection directions for these genital (endocervical or urethral) specimens are printed on the special PROBE-TEC collection kits. There are separate collection kits for females (pink-capped swabs) and males (blue-capped swabs). The kits are obtained from the laboratory (ext. 1344). Urine specimens are also acceptable. Submit at least 10 mL of first part of stream (not mid-stream) in sterile container. Clean catch urines are not ideal.
CHLAMYDIA CULTURE	Collected by a physician. Use Dacron-tipped swabs and special liquid transport media provided by the lab. The site should be vigorously swabbed or scraped, as it is essential that enough epithelial cells, which contain the organism, be collected. This is a send out test and further questions should be directed to the Send Out department at ext. 2042.
<i>CLOSTRIDIUM DIFFICILE</i> TOXIN B PCR CDPCR	A random stool specimen, which should be refrigerated until taken to the laboratory. Stool should be in a sterile container (no preservatives) and refrigerated during transport.
CLOTEST CLO	Specimen is a gastric biopsy collected by a physician and placed in a "Clotest" cupule. The Microbiology Lab interprets the urease test results for the presence of <i>Helicobacter pylori</i> .
<i>CRYPTOSPORIDIUM</i> EXAM CRYPT	A random stool specimen, which should be refrigerated until taken to the laboratory, or stool specimen in ECOFIX, which does not require refrigeration.
<i>CYTOMEGALOVIRUS</i> (CMV) CULTURE	Bronchoalveolar Lavage (BAL) or urine. Refrigerate specimens until taken to the laboratory. This is a send out test and further questions should be directed to the Send Out department at ext 2042.
EAR CULTURE CXEAR	Collect external specimens using a swab transport system. Internal specimens should be collected using a sterile syringe by the physician. Leave the fluid in the syringe for transportation to the laboratory.
EYE CULTURE CXEYE	INTERNAL: If specimen is aspirated material, leave in syringe, expelling air. Remove needle and use sterile syringe tip plug. Transport to the Laboratory ASAP at room temperature. EXTERNAL: The skin around the eye should be cleaned with a mild antiseptic.

	<p>Collect specimen using swab transport system.</p> <p>Media is available for bedside collection when requested ahead of time (Microbiology Lab ext. 1365)</p>
FAT - QUALITATIVE TEST (Performed on Stool)	<p>Random stool specimen collected in a clean container. This is a send out test and further questions should be directed to the Send Out department at ext. 2042.</p>
FUNGUS CULTURE CXFUN	<p>Random sputum or bronchoalveolar lavage collected in a sterile container. Refrigerate until transported to the lab. Hair, skin, and nail scrapings must be placed in a sterile container (please do not place between slides).</p> <p>Fluids or aspirates should be left in the syringe or placed in a sterile container. Culturettes are acceptable for wounds, lesions and other sources.</p>
Test Name	Specimen Collection Instructions
G.C. – PCR GCPCR	<p>Collection directions for these genital (endocervical or urethral) specimens are printed on the special PROBE-TEC collection kit. There are separate collection kits for females (pink) and males (blue). The kits are obtained from the laboratory (ext. 1344). Urine specimens are acceptable. Submit at least 10 mL of first part of stream (not mid-stream) in sterile container. Clean catch urines are not ideal.</p>
G.C. CULTURE SCREEN CXGON	<p>Wipe cervix clean of vaginal secretion and mucus. Use speculum and no lubricant. Collect specimen using a BD blue dual swab transport system. DO NOT REFRIGERATE SWAB. The viability of this organism decreases after several hours. Transport to the lab ASAP.</p> <p>This test should be used in place of PCR for throat, rectal, vaginal and other sources.</p>
GENITAL CULTURE CXGEN (with gram) CXNGN (without gram)	<p>Collect specimen using a BD blue dual swab transport system. DO NOT REFRIGERATE SWAB. If <i>Neisseria gonorrhoeae</i> is a consideration, the viability of this organism decreases after several hours. Transport to the lab ASAP.</p>
GIARDIA ANTIGEN TEST GIARD	<p>Collect random stool specimen in ECOFIX or in "Enteric Pathogen Transport" (culture) vials. Refrigeration not necessary. Fresh stool in clean container is also acceptable. Refrigerate until transport to lab.</p>
GRAM STAIN (ONLY) GRAM	<p>Swab transport system directed at the source.</p>
HERPES SIMPLEX CULTURE CXHSV	<p>Use Dacron-tipped swabs and special liquid Viral Transport Media, both provided by the laboratory. The site should be cleansed with an alcohol wipe (70% alcohol) to remove indigenous flora. The lesion site, which can include vesicles, should then be swabbed. Insert specimen swab in transport media, breaking off end of swab to enable tight closure of cap on transport viral. Refrigerate until transported to lab.</p>
HERPES SIMPLEX TYPING	<p>Typing for <i>Herpes simplex</i> 1 and 2 confirms the presence of <i>Herpes simplex virus</i>. See <i>Herpes Simplex Virus</i> for specimen collection information.</p>
HERPES ZOSTER CULTURE	<p>Use Dacron-tipped swabs and special liquid viral transport media, both provided by the laboratory. The lesion site, which can include vesicles, should be swabbed vigorously. Place swab in transport medium tube. Break off the stick and tighten cap. This is a send out test and further questions should be directed to the Send Out department at ext. 2042.</p>
INDIA INK MOUNT	<p>Test no longer performed. CSF and serum can be sent to a reference lab for Cryptococcal antigen testing. Questions should be referred to the send out department at ext. 2042.</p>

LEGIONELLA CULTURE LEGC	Sputum or lower respiratory specimens (lung tissue, pleural fluid, bronchial brushings, bronchial washings, tracheal aspirate) collected by a physician. This is a send out test and further questions should be directed to the Send Out department at ext. 2042.
MIC/MBC (MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATION)	Isolated organism (usually from a positive blood culture) is tested against one or more antibiotics. Specify antibiotics to be tested.
Test Name	Specimen Collection Instructions
MRSA SCREEN CXMRS (Culture) SAPCR (PCR)	Collect specimen using a Red top dual swab transport system. This culture is used for identifying Methicillin resistant <i>Staphylococcus aureus</i> carriers. If other pathogens are suspected as well, see source specific cultures. Nasal sources will be tested by PCR; all other sources are tested by culture.
NOCARDIA CULTURE CXNOC	Sputum or lower respiratory specimen (BAL) collected by surgery or by a respiratory therapist. Transport refrigerated to the laboratory.
NOSE CULTURE CXNOS CXWND (sinus)	Collect specimen from nares using a swab transport system. Routine nasal cultures are normally performed to look for carriers of specific organisms (i.e. MRSA). Sinus aspirates/swabs should be submitted for infections.
OVA AND PARASITES (O&P EXAM) OVAP	Collect in ECOFIX vial. (Formalin and PVA will also be accepted). These solutions preserve the specimen so that parasites, if present, can be detected. A minimum of 3 specimens collected within 10 days is recommended. However, if the patient is an inpatient, specimens are not recommended after the third day of hospitalization. Fill vial with stool specimen up to the "fill line." No refrigeration of transport vials is necessary. Fresh stool (minimum of 2 mL) may be submitted in clean container. Transport to the lab as soon as possible.
PINWORM PINW	Call laboratory for special collection ("Swube™") paddle. Collect in the morning before the patient arises. Collection directions are provided with the paddle kit.
ROTAVIRUS TEST	Collect a random stool specimen in a clean container. Do not use transport media. Questions should be referred to the Send Outs Department at ext. 2042.
SHIGA-LIKE TOXIN (E. COLI) CXSTO	Collect a fresh stool specimen in a sterile screw cap container and submit to the laboratory within 30 minutes. If delay will be longer, either refrigerate specimens or place it in a vial of stool culture transport media (red liquid) provided by the laboratory. Shiga toxin testing for non-0157 E. colis are performed with each stool culture.
SKIN CULTURE (SUPERFICIAL) CXWND	Before collection, first cleanse the site to remove indigenous flora by using an alcohol wipe (70% alcohol). Collect specimen from site using a swab transport system.
STOOL CULTURE CXSTO	Collect a fresh stool specimen in a sterile screw cap container and submit to the laboratory within 30 minutes. If delay will be longer, either refrigerate specimens or place it in a vial of stool culture transport media (red liquid) provided by the laboratory. 2 stool specimens, collected on separate days are recommended to rule out enteric pathogens. Unacceptable specimens: requests for stool culture on Inpatients after the third day of admission. These patients are normally being treated with broad-spectrum antibiotics

	that could allow for an overgrowth of other flora and also may be the likely cause of diarrhea. A <i>Clostridium difficile</i> toxin assay is recommended for these patients. Also, requests for stool cultures on specimens collected on the same day or more than 2 specimens collected separate days.
Test Name	Specimen Collection Instructions
STOOL WBCS or LEUKOCYTES STLEU	A Fresh stool specimen should be submitted in a clean container (no preservatives).
THROAT CULTURE CXTHR	Collect specimen from throat using a Red top dual swab transport system. Use a tongue depressor to keep the tongue out of the way to better visualize the infected throat. Swab any areas that are red or patchy white or which have exudates or a membrane.
URINE CULTURE CXURN (with gram) CXNUR (without gram)	FRESH MIDSTREAM URINE: Disinfect the urogenital area prior to collection. Collect in a sterile screw cap container and refrigerate until taken to the laboratory. Alternatively, collect the specimen using a Vacutainer Urine C&S Transport Kit, which does not have to be refrigerated.
VIRUS CULTURE	The SOURCE must be provided to ensure the proper media set up for the virus that is being looked for. Specimen must be collected with a Dacron swab and placed in Viral Transport Media. This is a send out test and further questions should be directed to the Send Out department at ext. 2042.
VIRUS CULTURE - RESPIRATORY	Bronchoalveolar lavage (BAL), nasal washings, nasopharyngeal aspirate, or nasopharyngeal swab specimens are required. Transport to the lab immediately, as the specimen must be placed in Viral Transport Media as soon as possible. A DFA is performed along with tissue culture for detection of multiple viral agents. This is a send out test and further questions should be directed to the Send Out department at ext. 2042.
VIRUS CULTURE - VARICELLA ZOSTER VIRUS	Before collection, cleanse site to remove indigenous flora by using an alcohol wipe (70% alcohol). Lesion is swabbed with a Dacron swab and the swab is placed immediately into liquid Viral Transport Media. Break off end of swab and cap tightly. VZV is a labile virus and the specimen should be transported ASAP to the laboratory. This is a send out test and further questions should be directed to the Send Out department at ext. 2042.
VRE SCREEN CXVRE	Collect specimen of choice using a swab transport system or sterile container. Random stool specimens are acceptable. This culture is used to screen for carriers of Vancomycin Resistant <i>Enterococcus</i> . If other pathogens are suspected as well, see specific source culture.
WOUND CULTURE CXWND	Before collection, cleanse site to remove indigenous flora by using an alcohol wipe (70% alcohol). Then swab, or aspirate with a needle and syringe, deep areas rather than the lesion surface. Deep wounds and fluids should be collected by an R.N. or physician. Please note source/anatomic site of specimen for proper culture set up and reporting interpretation.
YEAST SCREEN CXYST	Collect specimen using a swab transport system. Specify SOURCE.





<p>YERSINIA CULTURE CXYER</p>	<p>Collect a fresh stool specimen and submit to the lab in a screw top container within 30 minutes. Alternatively, collect a fresh stool specimen and place it in stool culture transport media, which is provided by the laboratory. Stool transport vial does not require refrigeration.</p>
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REFERENCE:

- Isenberg, Henry D (Ed.). 2004. *Clinical Microbiology Procedures Handbook*, 2nd Edition. Washington, D.C.: ASM Press

APPENDIX A: UW Medicine/NWHMC Microbiology Laboratory Supplies

To request supplies either call the Hospital Stores (inpatients) or Laboratory Support Services (outpatients).

COLLECTION TRANSPORT DEVICE	USES
 <p>BD Dual CultureSwab Plus with gel</p>	<p>For routine bacterial cultures (i.e., wounds, skins, upper respiratory, genital, etc) (Fluids and tissues should be placed in a sterile container)</p> <p>NOT used for MRSA or Rapid Strep Screen</p>
 <p>RED Top dual swabs without gel</p>	<p>For MRSA Screens, Rapid Strep Screens and follow-up throat cultures only.</p> <p>Culturettes with gel interfere with the Rapid Strep testing.</p>
 <p>Port-A-Cul Swab system</p>	<p>For anaerobic cultures (swabs only, tissue and fluids should be placed in sterile container or syringe)</p>
 <p>Stool Preservative vials for Ova & Parasites ECOFIX (green) tube (1 vial)– Currently supplied by laboratory</p>	<p>Ova and parasite exam Cryptosporidium Microsporidia Giardia antigen</p> <p>Not acceptable for <i>Clostridium difficile</i> toxin PCR, Culture and Stool Leukocytes</p>



Stool preservative for Ova and Parasite testing
Pink (formalin) and Gray (pva) tubes (2 vial set) – not currently supplied

Ova and parasite exam
Cryptosporidium
Microsporidia
Giardia antigen

Not acceptable for *Clostridium difficile* toxin
PCR, Culture and Stool Leukocytes



Stool Preservative vial for stool cultures
Orange (enteric transport or Cary Blair)

Stool cultures

Acceptable, but not ideal for Giardia antigen

Not acceptable for *Clostridium difficile* toxin
PCR, Ova & Parasites, Cryptosporidium and Stool
Leukocytes



**Clean Stool Vial – no preservative
(white, no liquid)**

***Clostridium difficile* toxin PCR
Stool Leukocytes**

Can be used for all stool tests
Must refrigerate



Urine Transport tube with preservative

Urine cultures

Not acceptable for CH/GC PCR testing of urine.



**M4, M5 Viral/Chlamydia Transport media – red
top conical tube with pink liquid media**

**Herpes Simplex (HSV)
Varicella Zoster (VZV)
Cytomegalovirus (CMV)
RSV
Viral Culture
Herpes culture
Chlamydia trachomatis culture**

Must refrigerate after collection

Special transports are required for
Mycoplasma/Ureaplasma – contact the send out
department at 206-368-2042



PCR collection Swabs – Pink (female), blue (male)

Chlamydia and GC PCR - for genital specimens
Acceptable Sources:
Cervical/Endocervical and Urethral
For urine specimens, send urine in clean container



Sterile containers - *Must refrigerate*

Urine cultures
Tissue cultures
Nail clippings, skin scrapings, hair for fungus culture
Stool testing for C. difficile and Stool leukocytes (transport vials preferred for other stool tests)
Sputum cultures
Urine for Chlamydia/GC PCR



Sterile Syringe

Aspirates of fluid, abscesses, etc.
Do not expel fluid into a CultureSwab. Remove needle, cap and transport ASAP to laboratory.



BVSCR Affirm collection Kit

Bacterial Vaginosis Screen for Trichomonas, Gardnerella and Candida species (BVSCR)

Only Vaginal specimens accepted



BioMérieux Bac-T Alert Blood Culture bottles
(1 set = 1 aerobic bottle [green] and 1 anaerobic bottle [purple])

For routine blood cultures for bacterial and yeast infections

For routine collection, use both bottles. If patient is hard draw, place blood in green Aerobic bottle only.



For pinworm exams

Pinworm “Swube” Paddle	
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Reviewed: 10/03, 11/05, 12/08, 11/09, 2/10, 04/11 L. Sobotka

Revised: 11/07, 12/08, 11/09, 2/10, 03/11 L. Gilly

Operational Responsibility/Approval:

Laboratory Medical Director
Director of the Laboratory