

NWHMC Laboratory (Microbiology) Policy

MICROBIOLOGY SPECIMEN COLLECTION AND HANDLING FOR CLINICIANS

I. APPROPRIATE SPECIMENS:

To ensure collection of appropriate specimens for culture, consideration must be given to the pathogens being looked for. Because 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!**

II. REQUISITIONS/LABELING:

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

III. CONTAINER SELECTION:

A. Culturettes:

Many specimens can be collected using a "**Culturette**" or "**mini-tip Culturette**" swab. 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 anaerobes will survive. **See "Port-A-Cul" or syringe under "D." below if anaerobes are a consideration. 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.

B. Screw cap 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. 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.

D. **Syringes:**

Syringes are excellent (and an alternative to "Port-A-Culs" above), 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.

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. Herpes, respiratory viruses, and Cytomegalovirus (CMV) can all be collected in the viral transport media, and chlamydia only in the chlamydia transport.

F. **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.

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. 10 mL of blood is the maximum for each bottle. Before each venipuncture, meticulous site cleansing must be performed.

IV. **TRANSPORTATION OF SPECIMENS TO THE LABORATORY:**

All specimens, once collected, must be placed in plastic bags to protect personnel who handle them. Sputum 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.

V. **GRAM STAINS:**

The direct gram stain is of value for possible early diagnosis and for culture correlation. Gram stains are not performed on throat and nose specimens. Clinicians have the option of requesting a gram stain with each culture. All wound cultures include a gram stain to help guide culture workup. Ideally, a second swab should be sent if a gram stain is required.

VI. **SENSITIVITIES:**

Sensitivities will be performed when ordered and/or at the discretion of the microbiologists. A battery of antibiotics is tested routinely for gram negative organisms and for staphylococcus species. 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.

VII. 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.

VIII. Specimen Collection Guide:

Test Name	Specimen Collection Instructions
ACTINOMYCES CULTURE	Random specimen using a swab transport system or sputum or Bronchoalveolar Lavage (BAL) in a sterile container. IUD specimens can be submitted in sterile containers.
AFB CULTURE	<p>Sputum: 5-10 mL in a sterile container. Collect early-morning specimen from deep, productive cough on at least 3 consecutive days in the early morning. Best sputum collection is under direct supervision of physician, R.N. or Respiratory Therapist.</p> <p>Urine: Minimum of 40 mL of first morning specimen collected in sterile container on 3 consecutive days.</p> <p>All other types should be submitted in sterile containers, collecting as much material as possible. Swabs are not recommended unless the specimen cannot be collected by other means.</p>
AFB CULTURE - STERILE FLUIDS	Specimens (CSFs and body fluids) are collected by a physician. Collect as much as possible (10-15 mL minimum) in sterile container or syringe.
AFB SMEAR ONLY	See AFB Culture for collection particulars; smears are performed on all sources except urine.
BETA STREP SCREEN (GENITAL)	Collect specimen from cervix or vagina using a swab transport system.
BETA STREP SCREEN (RESPIRATORY)	Swab red or white patches in throat using a swab transport system. Use a tongue depressor to keep tongue out of the way so as not to contaminate the swab with oral flora.
BLOOD CULTURE	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 - see below. 10 mL is optimal for each bottle (5 mL minimum).
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	Collected by a physician in Endoscopy or Surgery. Keep refrigerated until taken to the laboratory.
CEREBRAL SPINAL FLUID (CSF) CULTURE	Collected by a physician. Place fluid in sterile tube(s). Prompt delivery to lab is crucial.
CERVIX CULTURE	Wipe cervix clean of vaginal secretion and mucus. Use speculum and no lubricant. Collect specimen using a 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	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. 1734). 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.
Test Name	Specimen Collection Instructions
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.
CLOSTRIDIUM DIFFICILE TOXIN ASSAY	A random stool specimen, which should be refrigerated until taken to the laboratory. Stool transport media is acceptable and does not require refrigeration.
CLOTEST	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 H.pylori.
CRYPTOSPORIDIUM EXAM	A random stool specimen, which should be refrigerated until taken to the laboratory, or stool specimen in formalin vial, which does not require refrigeration.
CYTOMEGALOVIRUS (CMV) CULTURE	Bronchoalveolar Lavage (BAL) or urine. Refrigerate specimens until taken to the laboratory.
EAR CULTURE	Collect specimen using a swab transport system.
EYE CULTURE	INTERNAL: If specimen is aspirated material, leave in syringe, expelling air. Remove needle and use sterile syringe tip plug. EXTERNAL: The skin around the eye should be cleaned with a mild antiseptic. Collect specimen using swab transport system. Media is available for bedside collection when requested ahead of time (Microbiology Lab 1365)
FAT - QUALITATIVE TEST (Performed on Stool)	Random stool specimen collected in a clean container.
FUNGUS CULTURE	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 petri dish, which the lab can supply, or in a screw cap cup or sterile tube.
G.C. - DNA PROBE	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. 1734). 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.
G.C. CULTURE SCREEN	Wipe cervix clean of vaginal secretion and mucus. Use speculum and no lubricant. Collect specimen using a swab transport system. DO NOT REFRIGERATE SWAB. The viability of this organism decreases after several hours. Transport to the lab ASAP.

GENITAL CULTURE	Collect specimen using a swab transport system. DO NOT REFRIGERATE SWAB. If Neisseria gonorrhoeae is a consideration, the viability of this organism decreases after several hours. Transport to the lab ASAP.
GIARDIA ANTIGEN TEST	Collect random stool specimen in O&P Para-Pak or in "Enteric Pathogen Transport" (culture) vials. Refrigeration not necessary. Fresh stool in clean container is also acceptable. Refrigerate until transport to lab.
GRAM STAIN (ONLY)	Swab transport system directed at the source.
Test Name	Specimen Collection Instructions
HERPES SIMPLEX CULTURE	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.
HERPES SIMPLEX TYPING	Typing for Herpes simplex 1 and 2 confirms the presence of Herpes simplex virus. See Herpes Simplex Virus for specimen collection information.
HERPES ZOSTER CULTURE	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.
INDIA INK MOUNT	Performed on CSF. See Cerebral Spinal Fluid for Collection.
LEGIONELLA CULTURE	Sputum or lower respiratory specimens (lung tissue, pleural fluid, bronchial brushings, bronchial washings, tracheal aspirate) collected by a physician.
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.
NOCARDIA CULTURE	Sputum or lower respiratory specimen (BAL) collected by surgery or by a respiratory therapist.
NOSE CULTURE	Collect specimen from nares using a swab transport system.
OVA AND PARASITES (O&P EXAM)	Collect in 2 vial Para-Pak kit system. One vial is formalin and the other is Zinc-PVA. These solutions preserve the specimen so that parasites, if present, can be detected. A minimum of 3 specimens collected within 10 days is recommended. Fill each 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, without refrigeration.
PINWORM	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.

SKIN CULTURE (SUPERFICIAL)	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	<p>Collect a fresh stool specimen in a sterile screw cap container and submit to the laboratory within 30 minutes. Alternatively, collect a fresh stool and place in a vial of stool culture transport media (red liquid) provided by the laboratory. No refrigeration is needed. 2 stool specimens, collected on separate days are recommended to rule out enteric pathogens.</p> <p>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 C. difficile toxin assay is recommended for these patients.</p>
TEST NAME	SPECIMEN COLLECTION INSTRUCTIONS
THROAT CULTURE	Collect specimen from throat using a 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 exudate or a membrane.
URINE CULTURE	FRESH MIDSTREAM URINE: 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. Specimens collected in the latter manner do 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. Call the Microbiology Lab at 368-1365 for details. Specimen must be collected with a Dacron swab and placed in Viral Transport Media.
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.
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.
VRE SCREEN	Collect specimen of choice using a swab transport system. Random stool specimens are acceptable.
WOUND CULTURE	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	Collect specimen using a swab transport system. Specify SOURCE.
YERSINIA CULTURE	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.
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Reference:

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

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Operational Responsibility/Approval: Director of the Laboratory