Clinical Specimen Collection Manual for Trainers

Version Date: July, 2019

The Clinical Specimen Collection Manual provided by The Ohio State University is intended as general guidance and education to clinicians, laboratory personnel, and others involved in the collection of clinical specimens. Patient choice and clinical judgment must remain central to the selection of individual clinical specimen collection, diagnostic tests, and therapy. The clinical management and selection of culture specimens for an individual patient always rests upon the clinical decision making of their providers.

Authors, technical advisors and editors from The Ohio State University include:
1. Joan-Miquel Balada-Llasat, PhD;
2. Ashley Bersani, MPH, CPH; Kurt B. Stevenson, MD, MPH;
3. Shu-Hua Wang, MD, MPH, TB; Jennifer Kue, PhD;
4. Getnet Yimer, MD, PhD;
5. Wondwossen A. Gebreyes, DVM, PhD, DACVPM.
Layout by Marc Hardman.
EPHI Contributors in the order of their contribution
1.Rajiha Abubeker, PhD candidate
2. Gebrie Alebachew,MSc
3.Surefel Fantaw, MSc
4.Amete Mihirete, PhD candidate
5.Eyasu Tigabu , PhD
6.Estifanos Tsige, MSc
7.Ebba Abte, PhD

Technical advisors from the United States Centers for Disease Control and Prevention include:
1. Carmen Hazim, MPH;
2.Matthew Westercamp, PhD;
3. Michael Omondi, MS; Denise Kirley, RN;
4. Daniel Vanderende, MD;
5. Benjamin J. Park, MD4.

This document was supported by Cooperative Agreement Number GH001752, funded by the United States Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the United States Centers for Disease Control and Prevention nor the United States Department of Health and Human Services.

Clinical Specimen Collection Manual for Trainers

Table of Contents

List of Abbreviations and Acronyms

AFB	acid-fastbacilli
AMR	antimicrobialresistance
AREA	autonomy, readiness, experience, action
ASAP	as soon aspossible
ASP	antimicrobial stewardshipprogram
BAL	bronchial –alveolarlavage
BSI	bloodstreaminfections
CDC	CentersforDiseaseControlandPrevention
CLABSI	catheter-associatedbloodstreaminfection
CFU	colony-formingunit
CM	centimeter
CO_2	carbondioxide
CVC	central venouscatheter
CVAD	central venous accessdevice
DOB	date ofbirth
EG	forexample
EIA	enzymeimmunoassay
ELISA	enzyme-linked immunofluorescentimmunoassay
GI	gastrointestinal
HR	hour
ID	identification
IE	thatis
MIN	minute
ML	milliliter
MRSA	methicillin-resistant *staphylococcusaureus*
MSSA	methicillin-sensitive *staphylococcusaureus*
NAAT	nucleic acid amplificationtest
NAOH	sodium hydroxide, also known as lye
PED(S)	pediatric(s)
PMN	polymorphonuclearleukocytes
PPE	personal protectiveequipment
SOP	standard operatingprocedure
SPS	sodium polyanetholesulfonate,glass
STAT	urgent or rush,immediately
TB	tuberculosis
TBC	to beconfirmed
TBD	to bedetermined
ToT	training oftrainer(s)
WBC	white bloodcell
WHO	World Health Organization

Definition of Terms

Aerobe	amicroorganismthatgrowsinthepresenceofairorrequiresoxygenfor growth
Anaerobe	anorganismthatgrowswithoutair,orrequiresoxygen-freeconditionstolive
Antecubital	bend of theelbow
CentralVenousCatheter	atubethatispassedthroughaveintoendupinthethoracic(chest)portion of the vena cava (the large vein returning blood to the heart) or in the right atrium of the heart; it may be inserted for the short term or long term (synonym: central line)
Differential	of, showing,ordependingonadifference;differingorvaryingaccordingto circumstances or relevantfactors
Inoculate (aninfectiveagent) immunity	introduce (cells or organisms) into a culture medium;introduce intoanorganism;treat(apersonoranimal)withavaccinetoproduce against adisease
Pathogenesis	the manner of development of adisease
PersonalProtectiveEquipment	necessary items worn to minimize exposure to hazards that causeserious workplace injuries and illness; may include items such as gloves, safety glasses and shoes, earplugs or muffs, hard hats, respirators, or coveralls, vests and full body suits
Peripheralvein	aveininthearms,hands,legsandfeetthatleaddeoxygenatedbloodfrom the capillaries in the extremities back to the heart
Standardprecautions	asetofinfectioncontrolpracticesusedtopreventtransmissionofdiseases that can be acquired by contact with blood, body fluids, non-intact skin (including rashes), and mucous membranes
Venipuncture	the puncture of a vein as part of a medical procedure, typically towithdraw a blood sample or for an intravenous injection (synonym: peripheral blood draw)

Overview of Clinical Specimen Collection Training Package

Training Package Components:

- ***Trainers Manual:***Designed for use with the Training of Trainers course on Clinical Specimen Collection.Containsinstructions, recommended course agenda, core modules, and assessment toolsfor bothMaster Trainers andparticipants (Facility-level trainers).The first sections of this manual will provide some guidance on the theories of adult learning and how to best teach learners at different levels. The appendices contain copies of the entire lecture slide set provided to prepare for on-the–job training.
- ***ReferenceManual:***Designed for use in on-the-job training (group-based or self-study) of front-line healthcareworkers responsible for the collection of clinical specimens. Containsallofthereference and instructional material for the Facility-level trainers and participants.
- ***Assessment Tools:***Assessment Tools and Competency Checklists are provided for trainers and participants to assess their understanding of the training sessions and materials. Eachassessment maybe administered following the completion of each module. Competency Checklists may also be administered to monitor improvement asa competency measure. Directions on how to administer the assessments are provided in each module.
- ***Core Module PowerPoint Slides:***Presentation slides are available to be used as adjunct and supplement to the training sessions.
  - Module 1: Specimen collection, transportand processing
  - Module 2: Bloodcollection
  - Module 3: Wound (skin and soft tissue)culture collection
  - Module 4: Urine culturecollection
  - Module 5: Respiratory culturecollection
  - Module 6: Stool culturecollection
- ***Supplemental Module PowerPoint Slides***: Supplemental content for training Master Trainersand Facility-LevelTrainers on Adult Learning Methods
  - Theory of AdultLearning
  - GeneratinganEnvironmentforEffective Learning

Options for Implementation:
The Clinical Specimen Collection Training Package may be used in a variety of ways depending on the audience, time and resources available for training.

Training of Trainer (Group-Based) Course:
A basic two-day course to be used in training Master Trainers and Facility-level Trainers responsible for cascading trainings on clinical specimen collection to healthcare workers at the pre-service, in-service, or national-levels.This group-based course combines lectures with demonstrations of proper methods including general instructions for specimen collection, transport, and processing, and specific instructions on blood, wound, urine, respiratory, and stool cultures. The course also provides the opportunity for group learning with role-playing and hands-on practice of the methods. Intended to use with the Trainer's Manual, Assessment Tools, Core Module PowerPoint Presentations, and Supplemental Module PowerPoint Presentations provided in Training Package. Additional information including recommended course agenda can be found in the section: *Information for Master Trainers.*

On-the-Job Training Course (Group Based or Individual Review):
An in-service training for front-line healthcareworkers responsible for the collection of clinical specimens. The course uses the Reference Manual,which contains basic instructions for specimen collection, transport, and processing and specific instructions on blood, wound, urine, respiratory, and stool cultures. May be used with Core Module PowerPoint Slides and Assessment Tools if time and resources permit.The participants can readily return to the materials for review or foruse during specimen collection procedures.

This training course may be administered in a group-based setting or may be used in self-study and individual review. Using a modular approach, the course allows modules to be taught individually (stand-alone) or combined (full or partial course) based on participants'needs (type of specimen collection training needed) and time availability (e.g., 1 day, 4 hours, or 1 hour).Training sessions for healthcare workers may be as short as 30-45 minutes.The trainer may adapt the training schedule and outline according to the specific needs and time constraints of the audience.

Purpose:
The **purpose** of this specimen collection training is to improve proper specimen collection practices among front line staff at healthcare facilities across Ethiopia. The **short-term outcome** of this training is to create master trainers who will then develop a cadre of facility-level trainers. **Long-term outcomes** are to reduce specimen contamination and rejection, and build a sustained program of training facility trainers and front line healthcare facility staff.

Information for Master Trainers

Welcome to this Training of Trainers course on clinical specimen collection. You have been selected to teach this class because of your knowledge and leadership in promoting excellence in clinical patient care. You also have a responsibility, as part of your clinical duties, to collect samples for microbiologic cultures

from patients. This is of primary importance because culture results provide critical information to guide the optimal treatment of infections with antibiotics.

One of the major reasons to address the proper collection of clinical samples for culture is to guide the best choice of antibiotics in an effort to prevent the development of Antibiotic Resistance.

Resistance to antibiotics has been increasing significantly in the hospital and clinic setting. One of the biggest contributors to thisresistance development and transmission is exposure of antibioticdrugs.

When culture information from a patient with an infection is not known, broad-spectrum antibiotics are utilized. When cultures are obtained, and yield positive results, antibiotics can be narrowed and the amount of antibiotic exposure reduced. Thus, your job in obtaining timely and proper specimen collection is very important. By performing this part of your job well you are contributingsignificantly to the care and well-being of the patient. The supplementary modules summarized in the last section are provided as additional reference materials to provide background and foundational knowledge regarding the need for accurate and appropriate specimen collection in the context of the increasing frequency and burden of antimicrobial resistance.

Goals of Train the TrainerCourse

- To outline the need for proper specimen collectiontooptimizethetreatmentofpatients with infectiousdiseases.
- To provide the participant with training on the standard and basic methods to obtain blood, wound, urine, respiratory, and stool samples for cultures.
- To provide basic information on skills neededto protect healthcare workers and patients from transmission of infectious agents (e.g. proper hand hygiene, proper specimen handling and transport,etc.)

Course Description:

The Training of Trainer coursewill be group-based and you will be the leader of these group-based learning sessions. The group-based classes are designed to combine lectures with demonstrations of proper methods including general instructions for specimen collection, transport, and processing and specific instructions on blood, wound, urine, respiratory and stool cultures. The class will also provide the opportunity for group learning with role playing and hands-on practice of the methods. Modules 1-6 are designed for all participants to be use dfor administering on -the-job training and may be reviewed individually or with co-workers at the time of the specimen collection. It is important to emphasize that each module my be taugh individually as a stand alone training if needed.

.

Table 1: Core Training Modules Overview.

Module	Description	Session topics covered
1	Specimen Collection, Transport and Processing	• General Concepts and Overview of SpecimenCollection • Good Clinical Specimens • Keeping Specimens Free of Contamination • Collection Specimens at Right (Optimal) Time • Collecting Specimens in the Right Container • Collecting the Right Amount • Ensuring Specimens are Correctly Labeled • Safety andTransport • Rejection Criteria andDisposal
2	Blood Culture Collection	• Blood StreamInfections • Blood Cultures andMethods • Blood CultureCollection from Peripheral Vein • Blood Culture Collection from Central Venuos Catheter
3	Wound (Skin and Soft Tissue) Culture Collection	• WoundCultures • Collection Methods • Collection Procedures
4	Urine Culture Collection	• UrineCultures • Contamination Prevention • Types of Collection ▪ Female ▪ Male ▪ Indwellingcatheters ▪ PediatricPatients
5	Respiratory Culture Collection	• RespiratoryCultures • Type of Collection ▪ Nares ▪ Nasopharyngeal ▪ Throat ▪ Lower Respiratory
6	Stool Culture Collection	• Overview of Stool and Gastrointestinal TractCultures • Stool CollectionKit • Stool Cultures andCollection

Exercises and Demonstration:

Information and instructions for demonstration and teach-back exrecisesare provided at the end of each module. Generally, people will work in pairs, following the protocol for each step. For example: one person is the specimen collector, one person in patient; then reverse roles. Allow 15-30 minutes for the exercises and demonstrations Additional details are provided in the respective section for each module.

Course Preparation:

A list of materials, equipment, and supplies for facilitating the training is provided. A number of preparations must be considered prior to the start of the course:

- Prepare and review the class register
- Print the Registration Form (see Appendix B)
- Name tags may be provided to each participant during the registration or at the training entrance
- Prepare enough copies of the Trainer's Manual for each participant (Prepare Reference Manuals if conducting On-the-Job Trainings)

- Set up classroom for the number of participants scheduled to attend
- Set up a table at the front of the room with all specimen collection supplies and materials displayed
- Set up each participant work space with a Trainer's Manual, writing pad, pen, and name card (for folding and writing participants' names)
- If planning to conduct group-learning activities and demonstrations, ensure there is adequate work space for groups of 2 – 4 participants for activities and role playing
- If using the Training Package PowerPoint Slides, ensure computer and projector work properly (Optional)

A recommended course agenda is provided for use or adaptation based on the participants need and time. At the start of the course, some initial activities are required prior to the delivery of the core training content. The following should be conducted:

- Allow time for introductions for both the trainers and participants
- Review the course agenda and course goals
- Hand out the registration form and give instructions for completion
- Distribute, review, and discuss the materials used in the course, and allow time for questions
- Discuss general class rules including punctuality, participation, questions, respect of others' opinions, and use of electronic devices (i.e. cellular phones)

Recommended Course Agenda for Training of Trainers

Day 1

Topic	Description	Estimated Time
Opening Remarks	• Welcome • Introductions, Sign-InSheet • Participants'expectations	10 minutes
Overview of the Training	• Goals andobjectives • Approach to training; *Theory of Learning* and *Generatingan Environment for EffectiveLearning* • Review ofmaterials	15 minutes
Module 1: Specimen Collection, Transport and Processing	• Presentation, Discussion, Activities, Check-InQuestions • Review • Assessment • Teach-back, Demonstrations, Activities	60-90 minutes
	LUNCH BREAK	60 minutes
Module 2: Blood Culture Collection	• Presentation, Discussion, Activities, Check-InQuestions • Review • Assessment • Teach-back, Demonstrations, Activities	60-90 minutes
	BREAK	10 minutes
Module 3: Wound (Skin and Soft Tissue) Culture Collection	• Presentation, Discussion, Activities, Check-InQuestions • Review • Assessment • Teach-back, Demonstrations, Activities	45-60 minutes
Day 1 Summary	• General questions, comments	10-15 minutes

Day 2

Topic	Description	Estimated Time
Warm-up and Review of Day 1	• Module 1: Specimen Collection, Transport andProcessing • Module 2: Blood CultureCollection • Module 3: Wound (Skin and Soft Tissue) CultureCollection	15 minutes
Module 4: Urine Culture Collection	• Presentation, Discussion, Activities, Check-InQuestions • Review • Assessment • Teach-back, Demonstrations, Activities	45-60 minutes
	BREAK	10 minutes
Module 5: Respiratory Culture Collection	• Presentation, Discussion, Activities, Check-InQuestions • Review • Assessment • Teach-back, Demonstrations, Activities	30-45 minutes
Module 6: Stool Culture Collection	• Presentation, Discussion, Activities, Check-InQuestions • Review • Assessment • Teach-back, Demonstrations, Activities	30-45 minutes
	LUNCH BREAK	60 minutes
Review of Days 1-2	• Module 1: Specimen Collection, Transport andProcessing • Module 2: Blood CultureCollection • Module 3: Wound (Skin and Soft Tissue) CultureCollection • Module 4: Urine CultureCollection • Module 5: Respiratory CultureCollection • Module 6: Stool CultureCollection	30 minutes
Training Discussion	• Discussion on how to use training package materials to train others • Monitoring and EvaluationApproach • IndicatorsDiscussion	30 minutes
Closing	• Acknowledgments • NextSteps • Post-training questionnaire	50 minutes

Checklist of Materials, Equipment and Supplies forFacilitating the Training

To conduct the 2-day Training of Trainer (ToT) Course, trainers will need the materials, equipment, and supplies listed in the table below. Availability of these may depend on local resources. Some of these materials are optional for the On-the-Job Training Course and may be provided based on included activities, resources, and time.

Table 2: Checklist for Materials, Equipment and Supplies.

Materials	Quantity	Person Responsible	Status
Group-Based Course			
Trainer's or Reference Manuals (Print out in advance if possible)	One copy per participant and per trainer		
Class Roster with names of participants			
Name tags	One per participant and per trainer		
Folders, writing pads and pens	One per participant and per trainer		
Flip chart and easels with markers	One of each		
Flash drive containing Trainer's or Reference Manual and Slide Set (optional)	One per participant and per trainer (optional)	n/a for front line staff	
Demonstration supplies			
Tourniquets	1 for every 4 participants		
Gloves: Sizes small, medium and large	One box of each size		
Skin antisepsis materials (alcohol or chlorhexidine)	Samples - Enough for participant groups for demonstrations		
Alcohol-based hand rub for hand washing demonstra-tions	Samples - Enough for participant groups for demonstrations		
Needles, syringes tubing for blood collection **Blood culture bottles**	Samples- Enough for participant groups for demonstrations		
Wound swabs	Samples- Enough for participant groups for demonstrations		
Urinary catheter set up	Samples- Enough for participant groups for demonstrations		
Collection containers for urine, sputum, and stool	Samples- Enough for participant groups for demonstrations		
Clean Plastic Bag and Spoon	1 for every 4 participants		
Audiovisual Equipment Needed for Facilitating the Training (Optional)			
Overhead projector and /or laptop and LCD projector and extra bulbs	One of each		
Remote control for projector and batteries	One of each		
Laser pointer	One of each		
Screen or white surface for projecting slides	One of each		
Extension cords	As needed		
Portable microphone	Optional depending on group size		

Supplemental Module: Adult Learning Methods

Module Preparation

This material on adult learning methods is provided to give each Master and Facility-level Trainer an overview of the theories and methods to optimize the learning of adultsin each participant class. The materials in these modules can be applied to large groups or to one-on- one training sessions when providing training oftrainers. The learning theory slide sets provided with this manual can assist with training of trainer training sessions for Master Trainers and Facility-level Trainers but are typically not used with in-service trainings of front-line healthcare staff.

Materials Needed:

- SeeChecklist for Materials, Equipment and Supplies
- *PowerPoint Slides: Theory of AdultLearning*
- *PowerPoint Slides: Generating anEnvironment for EffectiveLearning*

Time:approximately 1 hour

Instructions for Trainer:
Ideal class size is 5-25. Classes of 10 or more participants should be sub-divided into groups of 4 or 5 for discussions and activities. Allow time at beginning for introductions.Plan for group discussions at the end of each characteristic of learning to discuss how the principles discussed can be implemented in the regular classroom training of participants.

Theory of Adult Learning

Malcolm Knowles developed the Theory of Andragogy to outline the theoretical and practical methods for teaching based on the six characteristics of adult learners. These characteristics are:

- The Need to Know
- The Learners' Self Concept
- The Role of the Learners' Experience

  Readiness to Learn
- Orientation to Learning

  Motivation

The Need to Know
This concept centers on each participant recognizing why appropriate culturing and specimen collection is important. The SupplementaryMaterialssectioncontainsinformation on stewardship outlining importance of AMR, principles of optimizing antibiotic use, and the need for surveillance. These can be reviewed by each Master Trainer and Facility Trainer and can be presented during Day 1 or 2 if time allows.

Be certain that you understand the reasons for the clinical specimen collection and AMR surveillanceso that you can convey it to your trainees. The slides in the Supplementary Materials section can be used and adapted to your audienceneeds.

The Learners' Self Concept

This concept recognizes that most adults believe they are responsible for their lives and need to have the freedom to choose which skills to improve. Thus, you need to treat your trainees as being very capable and self-directed. This can be accomplished as you create an environment for self- directed learning.

The Role of the Learners' Experience

Each participant comes to class with varied levels of training and experience. The trainees inyour sessions will have differences in background, learning style, needs, interests, and goals. They will learn best with a variety of learning experiences: discussions, simulations, problem-solving activities, and case methods.

Readiness to Learn

Readiness to learn is influenced by how the participant views the materials taught as being applicable to their daily work situation. Thematerials in these training modules are important because it can be applied in the day-to-day activities and duties of each of the participants in the sessions. The trainees will be much more engaged in the training as they are taught the practical techniques of clinical specimencollection.

Orientation to Learning

Most adults are task- centered and problem-solving. That is why demonstrations and group role-playing as each specimen collection method is learned is highly critical. That is the best way the learners will retain the methods being taught. Also remind the participants that the pictures in the REFERENCE

MANUAL serve as a great reminder of each step in the process that they are learning. In fact, they can follow each step outlined in REFERENCE MANUAL as they role play each specimen collection.

Motivation

The motivation of each participant in the classes will be variable. Some participants will right away see the importance of the training and others will not. You need to assess the class in the beginning of each session to determine the approach required for the motivation level of that class. The greatest motivation for learning is internal with a desire for increased job satisfaction and self-esteem. It is
important that they are motivated by understanding the critical importance of AMR and the principles of antimicrobial and diagnostic stewardship and recognizing that early and appropriate culture specimen collection will greatly enhance patient care and outcomes. Hopefully, this understanding will engage them and motivate them to want to learn.

Another tool to understanding the theory of adult learning is the mnemonic AREA (Autonomy, Readiness, Experience, and Action). This tool emphasizes the principles already outlined in this section. Autonomous and active participation by each trainee will promote better learning and retention. The readiness to learn of each trainee is dependent upon them seeing that learning proper clinical specimen collection is a practical

and important part of their daily activities that will greatly improve the lives of their patients. Utilizing theexperience of the trainees will promote their participation in the learning. They want to contribute to the improvement in patient care. Learning the new skills contained in the training modules will encourageperformance.

Generating an Environment for Effective Learning

Generating the correct environment for learning puts

- The Law ofAction
- The Law ofDiscovery
- The Law ofBuilding
- The Law ofRelevance
- The Law ofRepetition
- The Law ofSharing
- The Law ofFun

All of us learn better by doing than by listening and watching.

- Incorporate small group discussions, hands on learning with simulation, and other modes ofparticipation.
- For each specimen collection method provide ademonstrationreferringtothepicturesinthe Reference Manual and then divide the class into small groups of 4-5 individuals toallow for individual practice and role-playing. The specimens cannot actually be collected but the method can best be demonstrated this way.
- Allow for many questions to be asked before, during, and after learning about eachspecimen collectionmethod.
- Spend time reviewing past training oneach specimen collection method and beginwith probingquestions.
- Have the class members review what theyhave learned setting the foundation for the next skill set to beacquired.
- Continue to emphasize the importance and relevance of obtaining appropriate cultures to successfullyovercomeantimicrobialresistance and to greatly improve patientcare.
- Do not hesitate to continually repeat andreview. Frequently review because repetition improves recall and enhanceslearning.

- Group participation and sharing of ideas and experiences also enhances thelearning environment.
- Encourage open discussions, small group activities, question and answer sessions. We learn best when we try and when we takerisks. We are more likely to take risks when we are comfortable andrelaxed.
- Makesurethatalltraineesfeelcomfortableand relaxed to enhance the learning environment. Make certain that they are havingfun.

Here are some additional actions that can betaken in each learning session to enhance andencourage learning:

- Review previous learning. Briefly summarize what was learned in lastmodules
- Exploratory exercises. Explore what the trainees already know about the new material to betaught
- Theoretical discussion. Briefly present didactic training on the new module to providefoundation and context to thematerial
- Application Exercise. Demonstrate the clinical culture method to bemastered
- Hands on learning, simulation. In small groups, practice the methods to belearned
- Always review thepresentation

Module 1: Specimen Collection, Transport and Processing

Module Preparation

Materials Needed:

- See Checklist for Materials, Equipment and Supplies
- PowerPoint Slides: Module 1
- Alcohol-based hand rub
- Access to sink,soap, and towels if available

Time: approximately 60-90 minutes

Instructions for Trainer: Ideal class size is 5-25. Classes of 10 or more participants should be sub-divided into groups of 4 or 5 for discussions and activities. Plan for group discussions and check-in questions throughout the module. Allow time at beginning for introductions.

Discussion and Check-In Questions:
Check-In and Discussion Questions are provided below. Answers can be found at the end of the module on pages 35-36.

- Why are sample transport delays problematic?
- What is the most important step in sample management?
- How long should the antiseptic remain in contact with the skin surface?
- Should specimens be collected BEFORE or AFTER administration of antibiotics?
- Name two rejection criteria.
- When multiple tubes or containers of the same specimen are collected, how many labels are required?
- Name two outcomes of improper collection.

Topics to Cover:

- Objectives
- Introduction
- Key Points to Highlight
- Processing
- Journey of a Specimen
- Timing and Containers
- Volume, Labeling and Transportation
- Insitution Policies
- Rejection Criteria andDisposal
- Summary
- Check-In Questions

Key Points to Highlight

This module covers extensive content on Specimen Collection, Transport, and Processing. During the delivery and presentation of the training content, it is important that trainers convey the key points listed in the following table along with their page numbers for reference. Focus on the step-by-step protocol.

Key Points to Convey	Page Number in Module
Culture results are dependent on the quality of the specimen submitted, therefore it is essential that appropriates specimens be collected.	22
Good Clinical Specimens are: • Free of contamination • Collected at the righttime • Collected in the right container • The rightamount • Transported to the lab quickly • Correctly labeled and with the right forms	26
Each patient needs to be properly identified to ensure the specimen is being collected from the correct patient	24
There are three main ways specimens are contaminated: 1. From you, the person collecting the specimen 2. From the patient, usually from the skin around where the specimen was collected 3. From the collection container, usually because the container was broken or not stored properly	26
The best way to prevent microorganisms from you contaminating a specimen is to wash your hands with either soap and water (preferred) or using an alcohol based hand rub.	26
Collect the specimen from the actual site of infection, avoiding contamination from adjacent tissues or secretions. If appropriate, decontaminate the skin surface.	29
Collect specimens before antibiotics are given. Collect specimens during acute phases of illness (e.g., when a patient is febrile)	29
If the patient is expected to collect the sample themselves (e.g. urine self-collection, the clinician should provide clear instructions on the procedures for collection with emphasis on steps to minimize contamination.	25
A sufficient quantity of the specimen must be obtained to perform all the requested tests.	30
Always follow SOPs and when in doubt contact the laboratory.	31
At the time of collection, properly label the specimen and complete the test request form per the facility SOP.	31
Minimize transport time. If transport time increases, microorganisms can die and others can overgrow	31

Objectives

- Introduce the importance of proper specimen collection and transport, and describe common pitfalls
- Stress the importance of timely communication between the microbiology laboratory and those collecting specimens
- Discuss rules or principles to follow in order to collect microbiology specimens which will accurately reflect the development of the disease (pathogenesis) of the microbiological agent

Introduction to Specimen Collection, Transport and Processing

Diagnosis and effective treatment of a sick patient consistofestablishingalinkbetweenthelaboratory findings, syndromes and patient's clinical condition. Detection of the pathogen in the specimen is the first step to make the connection.

Laboratory resultsinfluencetherapeuticdecisionsandcanhave significant impact on patient care and outcomes. It is important to provide accurate laboratory results inordertoassuregoodtreatment.Properspecimen collection is essential for optimal use of laboratory services. The quality of the specimen collection processes affect the accuracy of results. As a result, proper collection of a specimen for culture is the most important step in the recovery of pathogenic organisms. If the specimens have been poorly collected, it leads to failure in isolation and/ or recovery of contaminating organisms. Thus, high quality specimens must be collected in order to provide the most clinically relevant results for the patients.Theresultsfromthelaboratoryarelimited by the quality of thespecimen.

To ensure that specimens are collected in an appropriate way, specimens should be obtained in a manner to minimize contamination. Contamination can be attributed to the introduction of indigenous microbiota which is the human body's normal flora that does not typically cause infections.

The microbiota is constituted by microorganisms such as bacteria, protozoa, and fungi that are found on or in specific areas of the body. The skin and mucous membranes of the oral cavity, intestines, upper respiratory tract, and vagina have specific and permanent flora. They are harmless, even beneficial, in their usual sites, and they inhibit the growth of pathogens, but they can cause infection if they are introduced into sterile sites. Some microbes are able to produce illness in humans. They are never part of the normal flora but may cause subclinical infection,*(e.g. M.tuberculosis)*.

Others are part of the normal microbiota, they can acquire extra virulence factors and they can produce illness (pathogenic), e.g. *E. coli*. Sometimes, microorganisms part of the normal microbiota can cause disease if they gain access to deep tissuesby trauma, surgery, lines, e.g. *S. epidermis*. In immunocompromised patients, many free-living bacteria and microbiota can cause disease, especially if introduced into deep tissues, e.g. *Acinetobacter spp*.

Because of the presence of microbiota, when performing bacterial cultures, we need to assess if bacteria is producing a specific disease. Collection

of specimen following aseptic techniques is done to avoid contamination of specimens with normal flora, and limit erroneous culture results.

An organism is clinically present if they are isolated in abundance, the only microorganism growing (pure culture), if isolated multiple times from the same site (on more than one occasion), or from normallysterile sites; if there is evidence of local inflammation or immune response to the microorganism, or if the finding fits with the clinical picture.

The clinician should determine the differential diagnosis based on patient presentation, select and collect the appropriate specimens, order desired testing, interpret the results and act on them.

Journey of a Specimen

The journey of the specimen:

1. Collection of thespecimen
2. Transportation to the central processing area orlaboratory
3. Registration of the specimens
4. Transfer to testing locationsdependingonthetestsordered

There are three phases in any specimen testing:

- Pre-analytic phase,
- Analytic phase
- Post-analytic phase.

The pre-analytic testing phase occurs first and

involves all processes that happen before specimen testing: test order confirmation, patient identification / specimen collection, storage /transportation to lab and receiving/verification and issuing lab unique accession number.

This phase may include specimen handling issues that occur even prior to the time the specimen is received in the laboratory. Important errors can occur during the pre-analytic phase with specimen collection, handling and identification. Therefore, the pre-analytical phase must have rigorous control measures to avoid unwittingly allowing problems or errors to travel further "downstream."

The second is the analytic phase. This phase includes what is usually considered the "actual" laboratory testing or the diagnostic procedures, processes, and products that ultimately provide results.

The post-analytic phase is the final phase of the laboratory process. This phase culminates in the production of a final value, result, a diagnostic report.

The interpretation of the microbiology results directly depends on the quality of the specimens receivedfor analysis. Because all microbes grow, multiply, and die very quickly, if these occur during specimen collection, transport, storage, or processing, the results of analysis will be compromised and the interpretation of the results will be misleading.

Proper management of samples is critical to the accuracy and reliability of testing and the results that will be generated. Remember that the journey starts with a good specimen. If garbage is sent to the lab, garbage results will be generated.

Identifying and Preparing the Patient

Prior to specimen collection, each patient needs to be properly identified to ensure the specimen is being collected from the correct patient. In general, the confirmation of patient identification should take place during the initial screening and admission (registration, appointment scheduling, phone screening, pre-operation), for diagnostic tests and procedures/ transfer between care areas, giving or receiving results.

Before proceeding with the collection, ask the patient to state identifying information. In order to avoid mistakes, you need to use at least two (2) identifiers. Patient identification includes any two of the following:

- patientname
- medical recordnumber
- date ofbirth
- government-issuedphotographidentification (usually used inoutpatients)

If the patient's identification is not confirmed appropriately the following outcomes could happen:

- Apatient'slabresultsthatwereputinthewrong patient's chart resulted in that patient getting incorrectmanagement.
- A patient got someone else'streatment.
- Two different patients had tests that were not intended for them, since there was a mixedup.
- A patient got the wrong test. For instance, a patient got a lumbar puncture that was not needed because the test results that weretaken were for anotherpatient.

Some tests require that the patient be fasting prior to specimen collection. There may also be special timing issues for tests such as blood cultures. Sample collection and preservation will vary, depending on the test and the type of sample to be collected. The institution must carefully define a sample collection process for all tests it performs.

If the patient is expected to collect the sample themselves (e.g. urine self-collection, the clinician should provide clear instructions on the procedures for collection with emphasis on steps to minimize contamination. The institution should have a set of standard operating procedures or protocols to ensure that appropriate collection kits with instructions

for collection, safety precautions and labeling are available for their patients. It is suggested that instructions for the patients be in the languages for the community the institution is serving, or

presented as simple, easy-to-understand graphics.

Table 3: Specimen Types -This manual will discuss six (6) common specimen types:

Specimen	Description	Notes/Considerations
Blood	Considered a sterile body fluid – Should appearaconsistentredcolorbutcanseparate allowed to sit aftercollection	Usually drawn from a vein (venipuncture)
Stool	Stool can range from watery and loose to firm and well formed	Commonly collected after patient defecates into a clean/dry receptacle
Urine	Color can range from pale yellow to dark brown and be clear or cloudy. Urine can appear pink/red if blood is present	Ensure clean catch specimens are free from contamination.
Sputum	Sputum is generally thick, cloudy and yellow/green/white in color and isproduced by the lower respiratorytract	Saliva is generally thin and clear, is produced in the mouth, and is not the same as sputum
Purulent drainage / Aspirate	Drainage can be serous, sanguineous, purulent. Thick consistency, may appear “milky” in appearance; green, yellow, brown or white color.	Ideal specimens include actual drainage usually collected by syringe. A sterile swab can also be used.
Tissue	Necrotic, purulent, granulomas.	Must be kept moist

Ideal Clinical Specimens

Culture results are dependent on the quality of the specimen submitted, therefore it is essential that good quality specimens are collected.Good clinical specimens are defined as being:

- Free of contamination: Microorganismsshould not be accidentally introduced into aspecimen duringcollection
- Collected at the righttime
- Collected in the right container: Everyspecimen has a correct container and only that container should be used. If you do not know which container to use or cannot find the correct container –ASK
- The rightamount
- Transported to the lab quickly: How quickly a specimen must get to the lab will depend on the type of specimen and the test ordered. Ingeneral, the faster you can get a specimen to the lab the better.
- Correctly labeled and with the right forms:While each laboratory request form is different it is important each form be completed clearly and correctly

While this manual will provide you with general guidance for good specimen collection, it is also important to follow the Standard Operating Procedures (SOPs) used where you work. When available, SOPs will provide details specific to specimen collection at your facility.

Keeping Specimens Free of Contamination

Acontaminantisamicroorganismthatwasputintoa specimen when, or after, the specimen wascollected. When contaminants are put into aspecimen, that specimen has been contaminated. Because microorganisms are almost everywhere, special care must be taken to avoid contaminating the specimens you collected.

Contaminants are not what is making the patient sick and make understanding culture results difficult.

There are three main ways specimens are contaminated:

1. From you, the person collecting thespecimen
2. From the patient, usually from the skinaround where the specimen wascollected
3. From the collection container, usually because the container was broken or not storedproperly

***Preventing contamination from you*:**

The best way to prevent microorganisms from you contaminating a specimen is to wash your handswith either soap and water (preferred) or using an alcohol based hand rub.In addition, Personal Protective Equipment plays a role. Protective personnel equipment should always be worn, such as gloves, gown and in some instances masks.

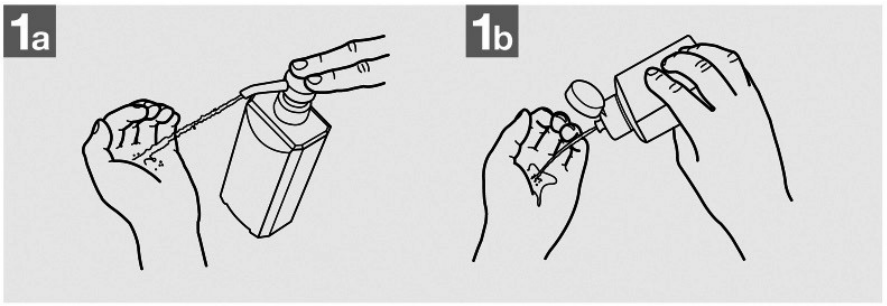
Figure 1: Hand washing using soap and water.

Duration of entire procedure: 40-60 seconds

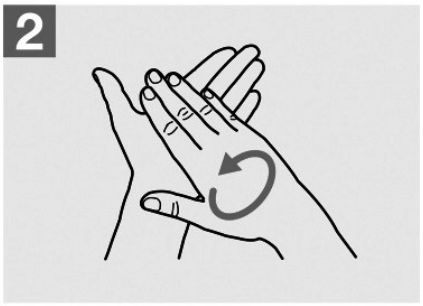
Figure 2: Hand washing using alcohol based hand rub.

Duration of entire procedure: 20-30 seconds

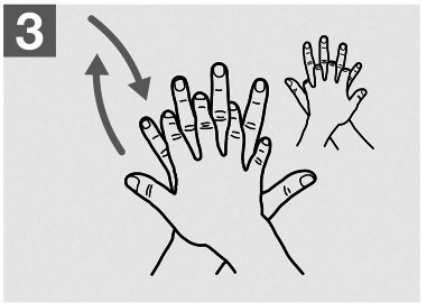
When decontaminating hands with an alcohol- based hand rub, use an amount sufficient to cover all surfaces of hands. If hands are visibly soiled, use soap and water to wash hands.



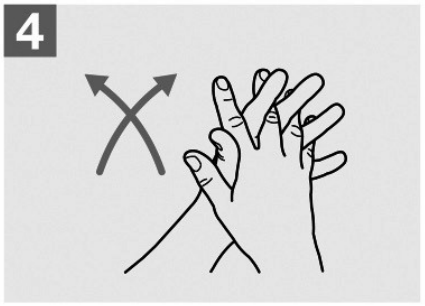
Apply a palmful of the product in a cupped hand, covering all surfaces;



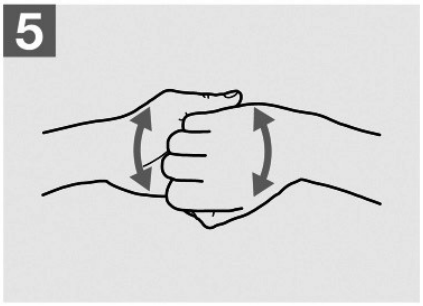
Rub hands palm to palm;



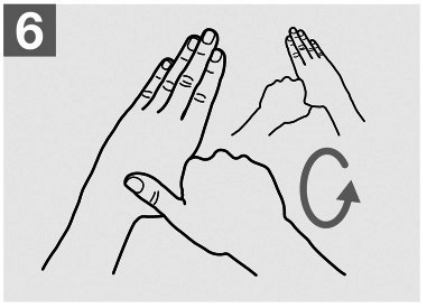
Right palm over left dorsum with interlaced fingers and vice versa;



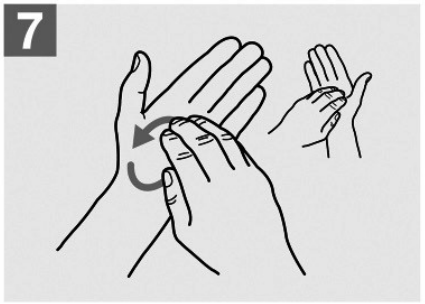
Palm to palm with fingers interlaced;



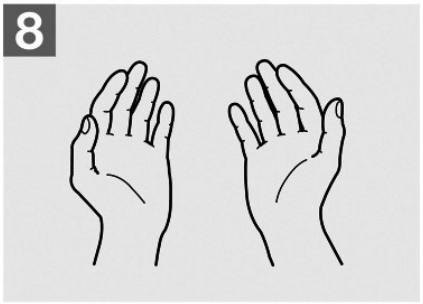
Backs of fingers to opposing palms with fingers interlocked;



Rotational rubbing of left thumb clasped in right palm and vice versa;



Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;



Once dry, your hands are safe.

Preventing contamination from the patient:

If appropriate, decontaminate the skin surface. Keep in mind that microorganisms live on the skin. If specimens are collected through the skin (e.g., blood) or may contact the skin (e.g., urine) the skin is cleaned to prevent microorganisms on the skin from contaminating a specimen. Table 4provides agents commonly used agents used to clean and prepare skin for specimen collection.

Collect the specimen from the actual site of infection, avoiding contamination from adjacent tissues or secretions.

Table 4: Agents commonly used agents used to clean and prepare skin for specimen collection.

Agent	Contact time	Notes/Considerations
70-95% alcohol	Allow to dry. 30 sec.-2 min	The immediate antimicrobial activity of alcohol is stronger and kills more quickly than chlorhexidine gluconate or povidone iodine, but has no residual effect
2% chlorhexidine	Allow to dry. 30 sec.-2 min	Chlorhexidine binds to the top layer of the skin, which results in persistent activity and does not becomeinactivated in the presence of organic material
1-2% tincture of iodine	Allow to dry. 30 sec.-2 min	Iodine is inactivated by organic material so should only be applied to clean skin

Preventing contamination from the collection container:

The specimen collection container itself may become contaminated if that container has been opened before the specimen is collected or the container is broken. Do not use a specimen collection container, which:

- Has been previously opened, or was since it was last cleaned/sterilized (often a broken sealshows a container has beenopened)
- Is cracked or does not close properly — Even a small crack in a container canallow contamination.
- Showsevidenceofhavinggottenwetorother signs of poorstorage.

Specimen collection containers are cheap when compared to a patient's wellbeing. Never put a patient at risk by collecting their specimen in a possibly contaminated container.

Collecting Specimens in the Right Container

The container used for specimen collection is often important. The shape of a specimen container can help prevent contamination and may limit specimen volume (amount). Certain specimen containers include chemicals that while needed for certain tests but will ruin the specimen for others. Additionally, certain microbiology specimens will require specific transport media to help protect microorganisms so they can be successfully cultured (grown) in the lab.

Every specimen must be collected in the appropriate, clean, leak proof-sterile containers. Check expiration date before inoculating collection device. If a delay in processing is expected contact the laboratory and receive instructions on what to do with the specimen. In these cases, a transport medium mustbeusedthatcontainbufferstomaintainconstant pH, semisolidtominimizespills&oxidation,non-nutritive to prevent overgrowth of rapid growers,charcoalto neutralize toxic substances in the specimen, ex. Stuart's, Cary- Blair, Amie

Timing: Collecting Specimens at the Right

(Optimal) Time

The time at which a specimen is collected can affect how well the laboratory to able to detect an organism in a specimen. A few general rules:

- Collect specimens before antibiotics aregiven. If specimen is collected after the treatment has started, this will affect the recovery ofthe microorganism). Organisms that wouldotherwise be isolated may not necessarily grow after exposure to an antibiotic agent, even though that antibiotic is not optimal for treatment, i.e. selection of resistant organism that are in fewer quantities.
- Collect specimens during acute phases of illness (e.g., when a patient is febrile) as etiologicagents are more likely to bedetected.
- Collect urine and sputum specimens early inthe morning (right after waking) ideally as the first urination or productive cough of theday

While good to follow these general rules when possible, facility guidelines as well as physician orders should be followed. In some instances, it may not be ideal to wait for the next day since it may delay diagnosis.

Table 5: Optional collection times for commonly collected specimens

Specimen	Optimal Time of Collection	Notes/Considerations
Urine for culture	First morning specimen	Collect from start of urination and patient should not have urinated in at least the last hour
Blood for culture	Prior to taking antibiotics	
Sputum for culture	Early morning specimen preferred.	
Purulent drainage or aspirate for culture	None	

Additional Considerations:

- If there is suspicion of bacterial endocarditis andinitialculturesarenegativeat48hoursthen collect 2-3 additional cultures fromdifferentsites. If bacteremia or fungemia with persistently negative blood cultures is suspected, consider alternative blood culture methods designed to enhance recovery of mycobacteria, fungi, and other rare and fastidious microorganisms.
- To rule out tuberculosis, acid fast bacteria (AFB) culture is used. Three consecutive sputum specimens collected 8-24 hours apart areneeded, with at least one being an early AMspecimen.
- For Gonorrhea/Chlamydia diagnosis from urine, first voided urine of day is the optimal specimen and the first stream of urine optimal. Less sensitive: patient should not have urinated
- for at least 1 hour. Do not use nucleic acid amplification methods as “proof of cure”. Not midstreamurine.

Volume: Collecting the Right Amount

A sufficient quantity of the specimen must be obtained to perform all the requested tests. The amount will depend on the laboratory requirements. For some specimens (e.g., urine and purulent drainage) lack of specimen is generally not a problem. For specimens that are more difficult (e.g., sputum) and/or painful to collect (e.g., blood and cerebral spinal fluid), specimens that are too small are often an issue.

Small specimens are especially problematic. For blood where the sensitivity of culture (ability to find the organism) is directly related to the amount (volume) of blood submitted. For adults, the idealvolume of blood specimen for culture is usually 10 mL per bottle. If swabs are used to collect the
specimen, a separate swab needs to be used for each body site or wound to be cultured and enough material must be submitted for Gram stain if required.

Always follow SOPs and when in doubt contact the laboratory to determine the amount of specimen that should be collected.

For orders with more than one test, ensure that the proper transport is utilized and that enough specimen is sent to be able to full fill all the orders. If there is not enough specimen volume, contact the laboratory to set the preference of tests to be performed.Forexample,anaerobicculturerequests need to be submitted in anaerobic transport media. If more than one blood tube is ordered, the orderof collection is asfollows:

Microbiology → coagulation test → chemistry → immunology → serology → blood bank

Labeling: Ensuring Specimens are Correctly Labeled and with the Right Forms

Labeling of the sample should occur at the time of

collection. Before sending any specimen to the lab make certain that EVERY individual container has a label and that all request forms and paperwork are completed. Specimens received in the lab without labels or with missing/incomplete SHOULD be rejected.

As the person collecting a specimen it is your responsibility to apply specimen labels and complete all request form correctly per your facility SOPs.

Properly label the specimen and complete the test request form. These steps are very important in order to perform the correct test on the right specimen and patient. If the specimen requisition form is initiated by the ordering physician, it is then that the patient is identified and collection is done per clinician's order.

Transporting Specimens to the Laboratory

Frequently, specimens are collected outside the laboratory, and must be transported for testing. Generally, specimen transport involves only theshort distance from the patient care area (unit) to the facility laboratory with transport done immediately after specimen collection. If a specimen cannot be taken to the laboratory immediately, the specimen must be stored correctly to protect the organisms that lab will later try to identify. In all cases, transport must be managed carefully in order to maintain integrity of the sample, giving attention to temperature, preservation needs, special transport containers, and timelimitations.

Minimize transport time. If transport time increases, microorganisms can die and others can overgrow

There are several common methods used to protect specimens that are not taken immediately to a laboratory for testing:

- **Refrigerated**: Specimen in placed in arefrigerator (about +4°C). No food should be stored in a refrigerator used to storespecimens.
- **Frozen**: Specimen is kept frozen (below-20°C)
- **Placed in transport media**: Use appropriate transport media (anaerobe transport vials, universal transport media). Different transport media will be used depending on thespecimen and organisms that will be tested for. The lab should provide you with the correct transport media if it should be used. Be aware that some specimen collection containers have transport mediaincluded.

Ideally, all specimens should be promptly transported to the lab within 2 to 24 hours. However, certain specimens should be transported immediately (sterile body fluids- CSF). Most samples are transportedtothelabatroomtemperature,however urine needs to be refrigerated if transport time exceeds 2 hours and in some instances preservatives are used. Depending on the specimens, there are maximum transport times if no transport media is used or if it is used. Most specimens are okay ifnot

transport media is used for up to one hour, if longer, transport media should be used (Table 6 and 7).

Some samples may require special handling, such as immediate refrigeration, protection from light, or prompt delivery to the laboratory. Anyimportant safety precautions should be explained. It isalso important to ensure the safety of those handling the material before, during, and after transport.

All samples should be handled as if they were infectious and standard precautions need to be followed. Standard precautions refer to the practice of avoiding contact with patients' bodily fluids, by means of the wearing of nonporous articles such as medical gloves and lab coats.

Table6: TransportTimebyPresenceorAbsenceofTransportMedia

Specimen	Maximum Transport Time not in Transport Media	Maximum Transport Time in Transport Media
All Specimens	Process within one hour	Interpretation of one positive culture problematic, especially if isolate is coagulase negative Staphylococcus. Consider alternative blood culture methods designed to enhance recovery of mycobacteria, fungi, and other rare and fastidious microorganisms
Stool Culture	2 hours	Cary-Blair 48 hours
GC Cultures	Immediately place swab in Amies with charcoal or other GC approved transport medium	Not more than 24 hours in Amies with charcoal. Store/ transport at ambient temperature
Respiratory Viral Cultures	Nasopharyngeal secretions or aspirates, BAL; 24 hours at 4°C	Not more than 48 hours if specimen transferred to Viral Transport Media
Clostridium difficile Toxin Assay	2 hours at ambient temperature 72 hours at 4°C; 1 week, frozen	Cary-Blair one week (check with manufacturer)
Urine for CMV	24 hours. Store at 4°C	Not recommended

Table 7: SuggestedTransport Media and Utility

Medium	Utility	Comments
Stuart's Medium	Most aerobic and some facultative anaerobes	Good general purpose media. Dual swabs most convenient
Amies Medium	Most anaerobic and facultative anaerobes	Good general purpose media. Yield for facultative anaerobes may be higher than from Stuart's
Amies with Charcoal	GC	Best media for GC
Cary-Blair	General purpose medium for transport of stool pathogens (Salmonella, Shigella, Vibro, Campylobacter, Yersinia, (C. difficile toxin A/B – some assays)	All stool specimens that cannot be setup within 1 hour should be placed in Cary-Blair media Cary-Blair media especially useful for Campylobacter
Anaerobic Transport Media	Many Types	Recommend media with oxygen indicator. General transport media are not good for strict anaerobes. Do not refrigerate
Ova and Parasite media (PVA, SAF, 10% formalin, Alcohol based – Ecofix)	Protozoa quickly lost in unpreserved stool	Media that do not contain mercury or formalin are more environmentally friendly
Viral Transport Media	Many types	Most contain antibiotics which renders then unusable for bacterial culture

Institution Policies

Theinstitutionneedstoprovidethefollowing information before the collection of the specimen:

1. Laboratory operationhours,
2. Contact personinformation
3. List oftestsperformedroutinely(laboratoryguide) including the time to results or days of testing (batched test and STAT (immediate)testing)
4. Sample collection information for healthcare personnel at the collection site, with appropriate containers and collection suppliesandlabeling system.
5. Sample acceptance andrejection criteria.
6. Laboratory request form.(See Appendix A for sample requisition form). Each specimen must be accompanied bya requisition form. Correct, legible, complete clinical information is required on all requisitions. Missing or illegible patient and physician information is a significant contributor to delayed reporting or possible rejection. A completed requisition should include the following demographic and test order information:

- Patient's name and identificationnumber (patient's uniqueidentifier)
- Patient's address, location (hospitalward, clinic,etc.)
- Patient's age, gender, phone number
- Description and source ofspecimen
- Date and time of specimencollection
- Name of collector and who to contactwith results.

Table 8: Specimen Processing Guide for Less Frequently Ordered Bacteriology Culture

Specimen	Collection	Transport Container	Transport Time	Comments
Conjunctiva/ Eye	Cleanse skin around the eye with a mild antiseptic and swab infected area	Swab in Transport Media	Within 2 hours of collection	Submit a swab from the uninfected eye for comparison
Ear (Otitis Externa)	Remove debris; collect specimen by rotating the swab in the ear canal	Swab in Transport Media	Refrigerate if >2 hour delay in transport	Inner ear specimens are collected by a physician
Genital Females	Cervix and vagina-use a non-lubricated speculum and swab affected site	Swab in Transport Media	For diagnosis of N. gonorrhoeae transport immediately Vaginal swabs- refrigerate if > 2-hour delay in transport	For diagnosis of N. gonorrhoeae submit swab in charcoal Transport Media. Do not refrigerate
Genital Males & females	Swab urethra, collect discharge	Swab in charcoal Transport Media	For diagnosis of N. gonorrhoeae transport immediately	For diagnosis of N. gonorrhoeae submit swab in charcoal Transport Media. Do not refrigerate

Table 9: Examples of proper collection/transport of specimens

Specimen	Source of Contamination	Storage and Transport	Solution/Monitor	Education
Respiratory Culture	Improper mouth care prior to collection of specimen. Lack of deep cough to obtain lower respiratory material.	Ambient for 1 hour. Refrigerated 24 hours. Some organisms, such as Haemophilus influenzae are susceptible to drying or low temperature.	Monitor % rejected sputum. % with oral contamination (epithelial cells; multiple Strep species, usually in clumps on gram stain and culture results). Sputum culture Vs. blood culture results?	All sputum samples are contaminated to varying degrees with oropharyngeal flora. Rinse mouth with sterile saline/water immediately before expectoration reduces number of contaminating bacteria. Timely feedback to individuals who collected specimen. Sputum samples of <2 mL should not be processed unless obviously purulent.
Wound Culture	Improper cleaning of wound site prior to collection.	In transport container. Ambient for no longer than 24 hours. Maximize transport time.	Number of squamous epithelial cells Vs. PMNs seen on Gram stain. Presence of squamous epithelial cells associated with a superficial specimen. The representative specimen is taken from the advancing margin of the wound.	Superficial: cleanse with 70% alcohol; aspirate or swab fluid Deep: cleanse with 70% alcohol, use syringe, surgical procedure. Tissue: aspirate or 5-10 mm piece of tissue.
Mycobacteria Culture	Sputum: Impropermouth care prior to collection of specimen	Ambient for 8 hours. Refrigerated 24 hours.	Contamination rate; track % NAOH required for decontamination. Culture redigests.	Timely feedback to individuals who collected specimen.

Specimen Rejection

The laboratory establishes rejection criteria. It is the responsibility of the laboratory to enforce its policies on sample rejection so that patient care is not compromised.

These are the conditions upon which a sample that the clinician collected may be rejected:

- Unlabeledsample
- Broken or leakingtube/container
- Insufficient patientinformation
- Sample label and patient name on thetest request form do notmatch
- Hemolyzed sample (depending on thetest)
- Sample collected in a wrong tube,wrong preservative

The following are examples of samples that should be rejected:

- inadequate volume for the quantity of preservative. The specimen added to thetube
- if it contains either media or preservative, it should be proportional. Containers usually have label indicating how much specimen needs tobe collected.
- Insufficient quantity for the test requested. Itwill affect the performance of the test if a minimum volume isrequired.
- Prolonged transport time, or other poorhandling during transport. Because bacteria candie
- or overgrow, if the transport time is above the acceptable limit, specimen should notbe processed.
- Duplicate specimens from same body site received on the same day (unless physician has given specific orders). Usually anadded expenditure.
- Obviously contaminatedspecimen
- Dryswabs

Disposal

Waste generated during collection should be handled in a safe manner. To ensure proper disposal of patient samples, the hospital should develop a policy for sample disposal. Local, as well as country regulations for disposal of medical waste need to be followed.

Also, the hospital should establish and follow procedures to disinfect samples prior to disposal.

Summary

Culture results are dependent on the quality of the specimen submitted, therefore it is essential that appropriates specimens be collected. This includes:

- Collection from the correct anatomicsite
- Use the proper technique and therequired supplies
- Using the appropriate container that willenable the pathogens to survive e.g. transportmedia.
- The specimen must be packaged appropriately and transported to the laboratory in a timelyand safemanner.
- Relevant clinical information such as diagnosis and current, or planned, antimicrobialtreatment will help to guide the laboratory staff in the processing and reporting of appropriate results. When inappropriate or poor quality specimens aresubmitted,theresultsobtainedmaynothelp in correct patientmanagement.
- Specimensshouldbecollectedbytrainedstaff using appropriate personalprotective
- Equipment such as gown, mask and gloveswhen required, this minimizes the chance of specimen contamination.
- Provide a laboratory handbook withcollection information to allusers.
- Have a system for tracking samples.
- Establish and implement a policy forsample storage and sample disposal.
- Maintain sample integrity.
- Assure that all transport regulationsand

requirements aremet.

- Always follow standardprecautions.

Check-In Questions

1. **Why are sample transport delaysproblematic?**

   Answer:Delaysintransportmaycompromiseth
   e
   qualityofthesampleandresultininaccuratetes
   t results

2. **What is the most important step insample management?**

   Answer: Proper collection of a specimen for culture

3. **How long should the antiseptic remain incontact with the skinsurface?**

   Answer: 30 seconds - 2 minutes.

4. **Should specimens be collected BEFORE orAFTER administration ofantibiotics?**

   Answer: Before administration of antibiotics

5. Name two rejectioncriteria.

Answer: Any of the following:

- Unlabeledsample
- Broken or leakingtube/container
- Insufficient patientinformation
- Sample label and patient name on thetest request form do notmatch
- Hemolyzed sample (ruptured red bloodcells)
- Sample collected in a wrong tube,wrong preservative
- Inadequate volume for the requiredpreservative
- Insufficient quantity for the testrequested
- Prolonged transporttime
- Duplicate specimens from same body site receivedonthesameday(unlessphysicianhas given specificorders)
- Obviously contaminatedspecimen
- Dryswabs

6. When multiple tubes or containers of thesame specimen are collected, how many labels are required?

Answer: A label is needed for EVERY individual tube, container sent to the lab

7. Name two out comes of improper collection.

Answer: Any of the following

- Delays in reporting testresults
- Unnecessaryre-draws/re-tests
- Decreased customersatisfaction
- Increasedcosts
- Incorrect diagnosis /treatment
- Injury
- Death

Module 2: Blood Culture Collection

Module Preparation

Materials Needed:

- See Checklist for Materials, Equipment and Supplies
- PowerPoint Slides: Module 2
- Alcohol-based hand rub
- Gloves
- Needles
- Syringes
- Blood culture bottles
- Skin antisepsis materials
- Blood Culture Collection Checklist
- Module 2 Assessment Form

Time: approximately 60-90 minutes

Instructions for Trainer:

Ideal class size is 5-25. Classes of 10 or more participants should be sub-divided into groups of 4 or 5 for discussions and activities. Plan for group discussions and check-in questions throughout the module. Allow time at beginning for introductions and pre-assessment, with time at end for demonstration / teach-back exercise and post-assessment. Conduct Pre and post-assessments using the assessment tool provided at end of module on pages 52-53.

Discussion and Check-In Questions:

Check-In and Discussion Questions are provided below. Answers can be found at the end of the module on page 51.

- What is the single most important factor in blood culture collection?
- What is the correct number of blood cultures and how many hours apart should the cultures be collected for acute patients?
- What equipment and supplies are needed before blood collection takes place?
- True/False: All blood culture samples should be refrigerated.

Demonstration and Teach-Back Exercise:

Information and instructions for the module activity are provided at the end of the module on pages50-51.

Topics to Cover:

- Key Points to Highlight
- Objectives
- Introduction
- Equipment and Supplies
- Blood Draw from PeriperhalVien
- Blood Draw from Central Venuous Catheter
- Additional Information
- Summary
- Check-In Questions
- Demonstration and Teach Back Exercises
- Assessments/Checklists

Key Points to Highlight:

This module covers extensive content on Blood Culture Collection. During the delivery and presentation of the training content, it is important that trainers convey the key points for participants to remember, listed in the following table along with their page numbers for reference.

Key Points	Page Number in Module
Blood bottles may be made in-house or be pre- manufactured (commercially prepared). If manual bottles are used, ensure that the stopper is not loose;if it is, do not use the bottle. Use a different one that the sterility is not compromised.	39
Samples are usually collected in sets – except for some pediatric patients. A set is defined as: • One (1) anaerobic and one (1) aerobic bottle (2 bottles total) • OR two (2) aerobic bottles Always collect the anaerobic bottle first	40
To rule out contamination, at least one blood culture should be drawn through the peripheral vein (venipuncture). Peripheral vein samples should be collected first.	41
Blood cultures should be collected before antibiotics are administered.	41
The venipuncture site must be properly disinfected and allowed to dry to avoid contamination of the blood culture with skin flora.	44
Do not palpate the vein after disinfecting the site to avoid contamination. If the site is touched, repeat the disinfection.	44
Major sources of contamination are: • Improper cleaning of skin or catheter prior to drawing specimen (major issue) • Transfer collected blood from tube to blood bottle culture. • Collection from catheter	41

Objectives

- Specify most common blood collection procedures, focusing on equipment and supplies used.
- Collection guidelines to follow to rule outsepsis, catheter sepsis, acute/subacuteendocarditis.

Introduction

Bloodstream infections (BSI) are commonreasons for hospitalization, and they are usually causedby a complication from a device, implant or graft.A blood infection typically originates from some other specific site within the body, spreading from that site when a person has a severe infection and/or the immune system cannot confine it to its source. BSI is associated with high mortality and health care cost. BSI are difficult to treat; traditional diagnostic methods can take days.

Blood cultures are procedures that identify a disease-causing organism in the blood. Infections of the bloodstream are most commonly caused by bacteria (bacteremia) but can also be caused by yeastsor other fungi (fungemia) or by a virus (viremia). Although blood can be used to test for viruses, this module focuses on the use of blood cultures to detect and identify bacteria and fungi in the blood. A blood culture is being done to determine which specific organism is causing the problem and how besttocombatit.Results direct the health practitioner to appropriate antimicrobial therapy for the specific microorganism present in the blood.Thetestisrelativelysimpleforthe patient and involves a simple blooddraw.

Collection Containers

Two Methods for Blood Cultures exist, and there are two types of manual bottles used. It is important to ensure that the stopper is not loose, if it is, do not use the bottle.

- Blood BottleCultures
- IsolatorTube

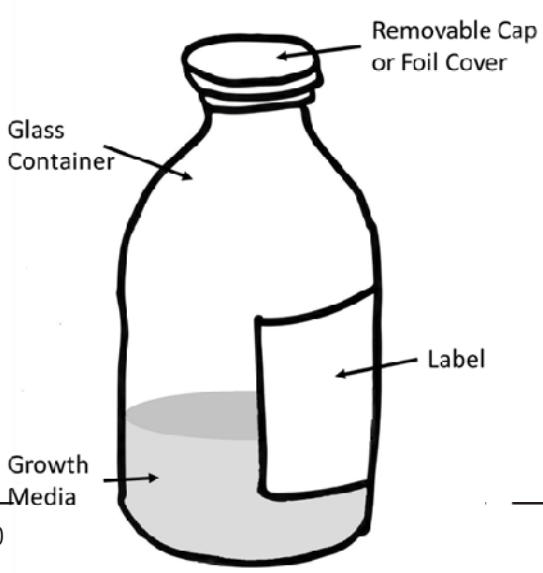
Blood bottle cultures use enrichment bottles that are checked manually for growth (Figure 3) or bottles that are pressurized and allow direct collection of blood into them.

They have media to enrich for growth and can be anaerobic or aerobic. Thebottles contain a fluorometric matrix at the bottom that changes if the CO2 increases (CO2 is produced when bacteria grow).

Blood bottles may be made in-house or be pre-manufactured (commercially prepared). Problems may arise if manual bottles are used. It is important to ensure they are prepared properly and ensure that the stopper is not loose.

When using manual culture methods bottles are incubated at 37 ºC for 5-7 days. When using automatized systems, there is a continuousmonitoring, every 10 minutes that will alert if the system detects a change in the matrix. Ensure that the blood culture bottles are at ambient temperature and should be incubated within 12 hours of collection.

Figure 3. Example of manual "in house" bottle



As an alternative method for blood cultures, an isolator tube can be used. The tube contains saponins, once the blood is added, the blood cells will lyse. The tube then is centrifuged and the pellet is plated on media and incubated for growth. This tubeisusuallyusedtoisolatemycobacteriaandfungi and plates are usually incubated 4-6 weeks. Volume collected using isolator tubes differs for pediatric patients from adultpatients.

Number of Samples to Collect

For blood cultures, multiple blood samples are usually collected for testing and from different veins to increase the likelihood of detecting the bacteriaor fungi that may be present in small numbers and/or may enter the blood intermittently. This is also done to help ensure that any bacteria or fungi detected are the ones causing the infection and are not contaminants.

Samples are usually collected in sets – except for some pediatric patients. A set is defined as:

- One(1)anaerobicandone(1)aerobicbottle(2 bottlestotal)
- OR two (2) aerobicbottles

Depending on the patient weight, the minimum number of blood culture sets that should be drawn per patient are two (4 bottles).

Collection can be done by:

- Venipuncture (collection from the peripheral vien)
- Via the central venous catheter (central line).

Collection by venipuncture is preferred. If one blood culture set is collected via the central venous catheter, the second set must be collected from the peripheral vein. Peripheral vein samples should be collected first.

The number of positive sets correlates with true sepsis (except for coagulase negative Staphylococcus that may represent contamination during collection).

Volume to Collect

The volume collected is the single most important factor to ensure bacteria recovery. Volume collected using blood bottles differs for pediatric patients from adult patients.

- For adults:8-10 ml per bottle (20–30 mL of blood per set)
- For children: an age- and weight-appropriate volume of blood should be cultured (see Table 10).

If less than 10 mL of blood is collected, inoculate only the aerobic bottle.

Sensitivity of a blood culture is directly related to the volume of blood submitted. Two blood culture sets (10 mL in both aerobic and anaerobic bottles) before administration of antibiotics is 98% sensitive (J. Clan. Microbiol. 1998 36: 657- 661).

Timing of Blood Cultures

The timing of blood culture orders should be dictated by patient acuity.Blood cultures should be collected before antibiotics are administered.If patient is already on antibiotics, do not draw blood from a lumen that has had antibiotics administered through it during the previoushour.

In urgent situations, 2 or more blood culture sets can be obtained sequentially over a short time interval, after which empiric therapy can be initiated.

In less urgent situations, obtaining blood culture sets may be spaced over several hours or more.

Also,an important determinant is the number of blood culture sets performed during a given septic episode. Generally, in adults with a suspicion of BSI 2 blood culture sets should be obtained in the evaluation of each septic episode.

Timing and number of cultures will depend on the cause of BSI, for example:

- Acute patients: 2 cultures (set) collected from separate venipuncture sites within a10-minute period.
- Endocarditis: 3 cultures over a 1 to 2-hourperiod (3 more cultures collected 24 hours later if the first 3 werenegative)
- Fever of unknown origin: 2 to 3 culturescollected at least 1 hour apart (2 to 3 collected 24 to 36 hours later if the first werenegative)

Table 10. Weight-appropriate volume of blood that should be collected for blood culture in pediatric patients.

Weight of Patient (kg)	Recommended Volume of Blood for Culture (mL)		Total Volume for Culture (mL)
	Culture set Bottle #1	Culture set Bottle #2	
≤1	2*	. . .	2
1.1–2	2*	2*	4
2.1–12.7	4*	2*	6
12.8–36.3	10	10	20
>36.3	20–30	20–30	40–60
* because the low volumeinoculate into a single aerobic blood culture bottle.			

Preventing Contamination

When collecting blood for a bacterial, mycobacterial or fungal culture, be aware that the major sources of contamination are:

- Improper cleaning of skin or catheter priorto drawing specimen (majorissue)
- Transfer the collected blood to the blood bottle culture.
- Collection froma central venous catheter

Catheterdrawnbloodsamplesaremorelikelyto becolonized. To rule out contamination:

- At least one blood culture set should be drawn through theperipheral vein
- Peripheral vein samples should be collectedfirst

In order to avoid contamination during venipuncture collection itis very important to:

- disinfect the venipuncture site with 70% alcohol
- allow alcoholto dry and follow by applying povidone iodine or chlorhexidine.
- Inoculate the blood into the blood culture bottle immediately and transport to the lab without refrigeration.

If Coagulase Negative Staphylococcus are found in more than one bottle, then this may be considered pathogenic rather than contamination.

It is important to have an ongoing education program, to monitor contamination rates.

Equipment and Supplies

- Blood culture bottles (withlabels)
- Gauze and adhesive bandages (e.g.BAND-AIDS)
- Material for skin disinfection: Iodine and70% alcohol, orChlorhexidine
- 20 mL syringes (1 syringe for each set ofblood cultures; lower volume for pediatric patient may be used)
- Sterileneedles(gaugesizedependsoninfantvs. adultpatient)
- Gloves
- Tourniquet

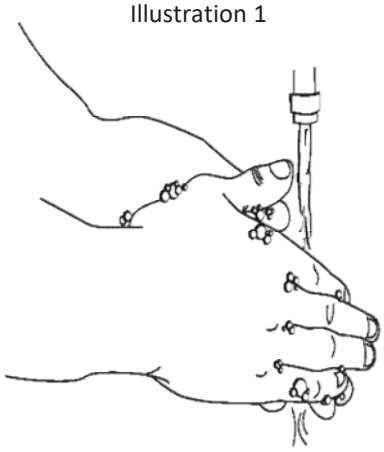
Blood Draw from Peripheral Vein

From AdultPatients

1. Fill the requisition by ordering physician prior to obtaining blood for culturing (physician orders,patientidentified,samplecollectedand requisition form filled out indicating timeof collection, site of collection, type of blood culture bottle filled (aerobic or anaerobic)
2. Prepare equipment andsupplies.

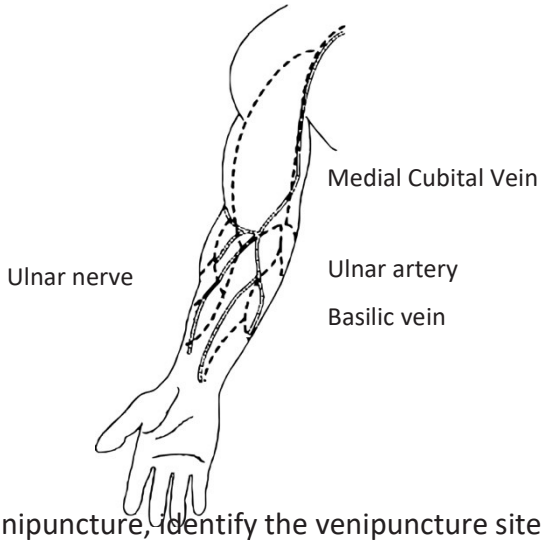
3. A set of blood cultures comprise of one aerobic and one anaerobic bottle. Determine the typeof culturebottlestoutilize(aerobicandanaerobic). If necessary, discuss timing of cultures, sites, need for any special instructions etc., with the physician if blood collected by other health professional.
4. Explaintheproceduretothepatientand/ortheir significant other ifpresent.
5. Verifythepatient'sidentificationbyusingtwo patient identifiers perpolicy.
6. Follow Standard Precautions for all patients. Use appropriatepersonalprotectiveequipment(PPE) such as gloves, masks, and/or faceshields.
7. Labelallbottleswithpatientinformationand date/time of bloodcollection.
8. Wash hands thoroughly with soap and water, and dry hands with a clean papertowel.

Illustration 1



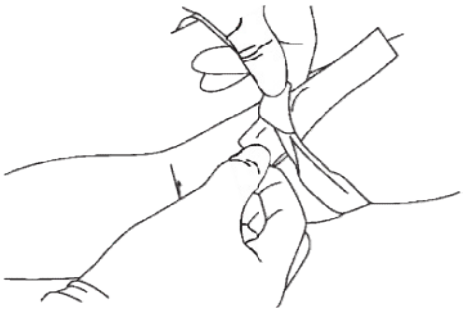
9. Put ongloves.
10. Use alcohol to disinfect tops of blood culture bottles.Donotapplyiodinetobottletops.Allow to drycompletely.
11. Forambulatorypatients,positionpatientina chair with aback.
12. Select the site, preferably at the antecubital area (i.e. the bend of the elbow). Warming the arm with a hot pack, or hanging the hand down may make it easier to see the veins. Palpate the area to locate the anatomic landmarks. DO NOT touch the site once alcohol or another antiseptic has been applied.

Illustration 2



13. For venipuncture, identify the venipuncture site by tying a tourniquet around patient's arm to locate a vein. About 4–5 finger widths above the selected venipuncture site. Then untie the tourniquet.

Illustration 3

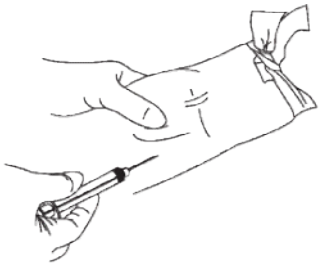


14. Ask the patient to form a fist so that the veins are more prominent.

Illustration 4

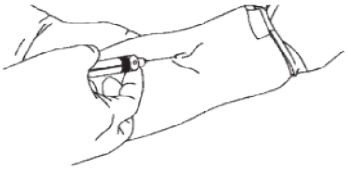
15. The venipuncture site must be properly disinfectedtoavoidcontaminationoftheblood culture with skinflora.
16. Prepare the site by usingeither:
    - 70-95%alcohol
    - Chlorhexidine
    - 70% alcohol followed by 2% tincture ofIodine
17. Rub the site vigorously in a concentric manner, start at the center of the site and moveoutward. If using chlorhexidine perform back and forth motion.
18. Wait at least 30 seconds for the area todry.
    - NOTE:Donotpalpatetheveinafterdisinfecting the site to avoid contamination. If the site is touched, repeat thedisinfection.
19. Anchor the vein by holding the patient's armand placing a thumb BELOW the venipuncturesite

Illustration 5



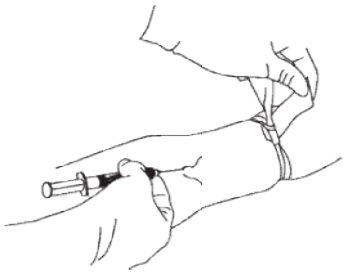
20. Enter the vein swiftly at a 30-degree angle

Illustration 6



21. Draw the required amount of blood usingneedle andsyringe.
22. Oncesufficientbloodhasbeencollected,release the tourniquet BEFORE withdrawing theneedle.

Illustration 7



23. Withdraw the needle gently and applygentle pressure to the site with clean gauze. Cover gauze with cleanbandage.

Illustration 8

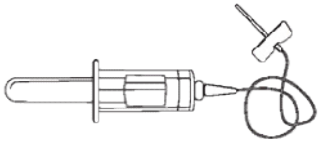
24. Inoculate the culture bottles by piercing the stopper on the culture bottle with the needle directly above the tube using slow,steady pressure. **Anaerobic always first.**Roll/shake bottles afterwards.Rubber stoppers need to be cleaned with alcohol wipe prior to inserting needle.
    - NOTE: If manual bottles are used, ensure that the stopper is not loose, if it is, do not usethe bottle. Use a different one that the sterility is notcompromised.

25. Discard the used needle and syringe intoa puncture-resistant sharpscontainer.
26. Discard the supplies used into the rightdisposal bin.
27. Remove gloves, wash hands thoroughly with soap and water, or 2% alcohol-based gycerol. Dry hands with a clean paper towel as needed.
28. Fill out the requisition forms, check foraccuracy
29. Transport the blood cultures to theLaboratory promptly, if delayed; they can be at room temperature for not more than 12hours.
30. Do notrefrigerate

From Pediatric Patients

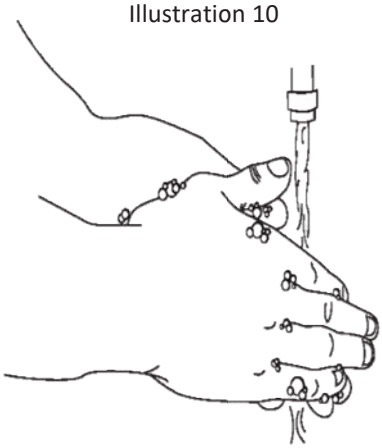
1. Fill the requisition by ordering physician prior to obtaining blood for culturing (physician orders,patientidentified,samplecollectedand requisition form filled out indicating timeof collection, site of collection, type of blood culture bottle filled (aerobic or anaerobic)
2. Prepare equipment andsupplies.

Illustration 9



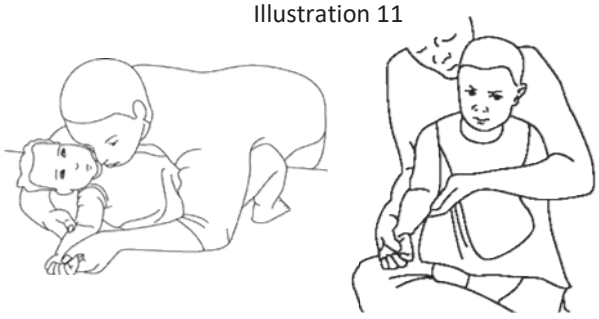
3. Use a winged steel needle, usually 23 or 25 gauge, with an extension tube (butterfly). Keep the tube and needle separate until the needleis in thevein
4. Explain the procedure to the patientand/or parents.
5. Verifythepatient'sidentificationbyusingtwo patient identifiers perpolicy.
6. Follow Standard Precautions for all patients. Use appropriate protective equipment suchas gloves, masks, and/or faceshields.
7. A set of blood cultures comprise of one aerobic and one anaerobic bottle. Determine the typeof culturebottlestoutilize(aerobicandanaerobic). If necessary, discuss timing of cultures, sites, need for any special instructions etc., with the physician if blood collected by other health professional.
8. Labelallbottleswithpatientinformationand date/time of bloodcollection.
9. Wash hands thoroughly with soap and water, and dry hands with a clean papertowel.

Illustration 10



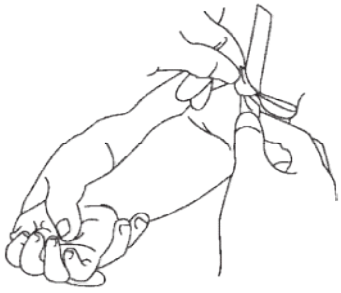
10. Put ongloves.
11. Use alcohol to disinfect tops of blood culture bottles.Donotapplyiodinetobottletops.Allow to drycompletely.
12. Immobilize the baby orchild

Illustration 11



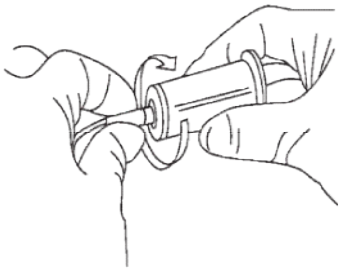
13. Put tourniquet on the patient about twofinger widths above the venipuncturesite

Illustration 12



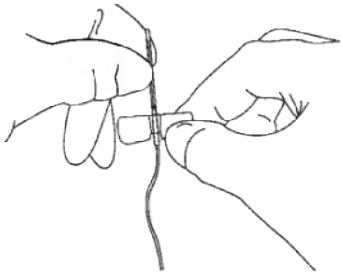
14. Attach the end of the winged infusion set to the end of the vacuum tube and insert thecollection tube into the holder until the tube reaches the needle.

Illustration 13



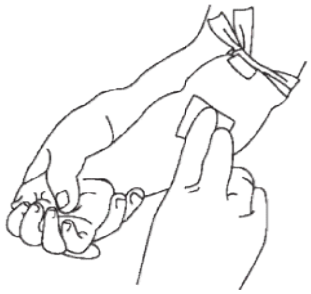
15. Remove the plastic sleeve from the end ofthe butterfly

Illustration 14



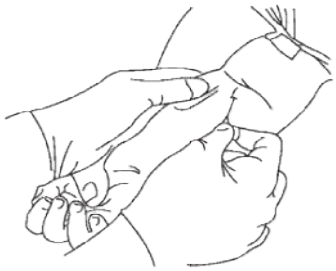
16. The venipuncture site must be properly disinfectedtoavoidcontaminationoftheblood culture with skinflora.
17. Prepare the site by using either: Chlorhexidine or 70% alcohol followed by 2% tincture oflodine
18. Rub the site vigorously in a concentric manner, start at the center of the site and move outward. If using chlorhexidine perform back and forth motion.

Illustration 15



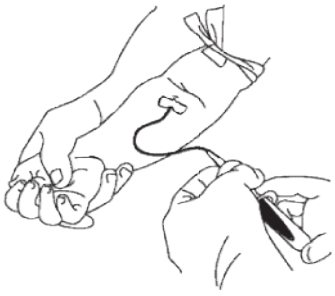
19. Wait at least 30 seconds for the area todry.
    - NOTE:Donotpalpatetheveinafterdisinfecting the site to avoid contamination. If the site is touched, repeat thedisinfection.
20. Use a thumb to draw the skin tight, abouttwo finger widths below the venipuncturesite

Illustration 16



21. Push the vacuum tube completely ontothe needle.

Illustration 17



22. Blood should begin to flow into the tube. Fill the tube until it is full or until the vacuum is exhausted; if filling multiple tubes, carefully remove the full tube and replace with another tube, taking care not to move the needle inthe vein.
23. After the required amount of blood hasbeen collected, release thetourniquet.
24. Place gauze over the venipuncture site and slowly withdraw the needle. Ask parent to continue applying mildpressure
25. Remove butterfly from vacuumholder
26. Inoculate the culture bottles.**Anaerobic always first.**Roll/shake bottles afterwards.Rubber stoppers need to be cleaned with alcohol wipe prior to inserting needle. Piercethe stopper on the culture bottle with the needle directly above the tube using slow, steady pressure.
    - NOTE: If manual bottles are used, ensure that the stopper is not loose, if it is, do not usethe bottle. Use a different one that the sterility is notcompromised.
27. Discard the used needle and syringe intoa puncture-resistant sharpscontainer.
28. Discard the supplies used into the rightdisposal bin.
29. Remove gloves, wash hands thoroughly with soap and water, and dry with a clean papertowel
30. Fill out the requisition forms, check foraccuracy
31. Transport the blood cultures to theLaboratory promptly, if delayed; they can be at room temperature for not more than 12hours.
32. Do notrefrigerate

Blood Draw from Central Venous Catheter

1. Fill the requisition by ordering physician prior to obtaining blood for culturing (physician orders,patientidentified,samplecollectedand requisition form filled out indicating timeof collection, site of collection, type of blood culture bottle filled (aerobic or anaerobic)
2. Prepare equipment andsupplies.
3. Explain the procedure to the patientand/or their significant other ifpresent.
4. Verifythepatient'sidentificationbyusingtwo patient identifiers perpolicy.
5. Follow Standard Precautions for all patients. Use appropriate protective equipment suchas gloves, masks, and/or faceshields.
6. A set of blood cultures comprise of one aerobic and one anaerobic bottle. Determine the type of culture bottles to utilize (aerobic and anaerobic). If necessary, discuss timing of cultures, sites, need for any specialinstructions etc., with the physician if blood collected by other healthprofessional.
7. Catheter site must be properly disinfected to avoid contamination of the blood culturewith skinflora.
8. Labelallbottleswithpatientinformationand date/time of bloodcollection,
9. Wash hands thoroughly with soap and water, and dry hands with a clean papertowel.
10. Put ongloves.
11. Use alcohol to disinfect tops of blood culture bottles.Donotapplyiodinetobottletops.Allow to drycompletely.
12. Fromcentralvenouscatheter(CVC)alwaysclean the cap with alcohol for 15-30 seconds and allow cap to dry completely before accessingit.

13. Inoculate the culture bottles. **Anaerobic always first.** Roll/shake bottles afterwards.Rubber stoppers need to be cleaned with alcohol wipe prior to inserting needle. Piercethe stopper on the culture bottle with the needle directly above the tube using slow, steady pressure.
    - NOTE: If manual bottles are used, ensure that the stopper is not loose, if it is, do not usethe bottle. Use a different one that the sterility is notcompromised.
14. Discard the used needle and syringe intoa puncture-resistant sharpscontainer.
15. Discard the supplies used into the correctdisposal bin.
16. Remove gloves, wash hands thoroughly with soap and water, and dry with a clean papertowel
17. Fill out the requisition forms, check foraccuracy
18. Transport the blood cultures to theLaboratory promptly, if delayed; they can be at room temperature for not more than 12hours.
19. Do notrefrigerate

Four different methods can be used to draw blood from Central Venuous Catheter (CVC) (see Table 11)

Table 11. Different methods of blood collection from CVC (procedure if prone to contamination)

Discard	Remove a specified amount of blood from CVC via a syringe. 10mL is ideal volume to be flashed. Use a new syringe for the sample. Flush the CVAD with sodium chloride 0.9%	Removes potential contaminants. No blood is returned that might introduce pathogens	Potential blood loss with frequent blood samples
Push - Pull	Flush the CVC with sodium chloride 0.9%. Without removing the syringe, aspirate 6ml of blood, then push it back into the CVC. Repeat this process x 3. Remove the empty syringe andattach a new syringe to obtain bloodsample. Flush CVC with sodium chloride0.9%	Requires mixing the blood back and forth in asyringe several times to eliminate contaminants. Limits blood loss	May be difficult to obtain enough blood for three to four push-pull sequences particularly with malfunctioning catheters. Risk of hemolysis with the agitation of blood
Re-infusion	Aspirate 6mls of blood into a syringe and attach a sterile cap. Obtain blood sample via a syringe. Re-infuse the discard from the first syringe	Involves returning the discard specimen after obtaining the samples. Minimizes blood loss	Potential to re-infuse clots. Potential for contamination of the blood being reinfused. Potential for error including the possibility of confusing the discard syringe with the blood sample
Dead space	Withdraw until blood enters the syringe. Discard. Repeat and take appropriate sample amount	Reduces nosocomial blood loss. Reduction in the potential for infection	Potential for contamination of the blood; as no discard blood taken. Higher risk of erroneous samples

Additional Information

*For ruling out catheter sepsis (*Catheter associated blood stream infection (CLABSI)

Draw two blood culture sets, one set is obtained from the suspected catheter and a second set must be from a peripheral site. Always note time of collection.

Blood cultures should not be drawn through an intravenouscatheteratthetimeofcatheterinsertion. Drawing blood for cultures through an indwelling intravascular catheter should be avoided whenever possible, since ports are frequently colonized with skinflora.

For acute endocarditis

Specialty collection procedure (invasive) skilled or trained personnel can draw.

Acute endocarditis most often occurs when an aggressive species of skin bacteria, especially *Staphylococcus aureus*, enters the bloodstream and attacks a normal, undamaged heart valve. Once *S. aureus* begins to multiply inside the heart, they may send small clumps of bacteria called septic emboli into the bloodstream to spread the infection to other organs, especially to the kidneys, lungs and brain. Some patients have a higher risk to develop acute endocarditis (no previous heart valve damage) some risk factors include: artificial (prosthetic) heart valves or heart valves repaired with artificial material, history of endocarditis, certain types of congenital heart defects, abnormalities of the heart valves resulting from heart transplantation, intravenous drug users.

In order to rule out acute endocarditis:

1. Draw 2-3 culture sets from separate siteswithin 30 minutes of each other and before beginning antimicrobialtherapy.
2. Begin therapy after cultures are obtained establishing a specific microbialdiagnosis.

For subacute endocarditis

Subacute endocarditis tends to involve heart valves that already are damaged in some way, and it usually is less likely to cause septic emboli than acute endocarditis.

These are the most frequent organisms causing subacute endocarditis:

- ¥ Viridans group*: S. mutans, S. sanguis*; witha history of dental work withoutprophylaxis.
- ¥ Group D strep: *S. bovis, Enterococcus*;with history of gastrointestinal or genital-urinary cancer.
- ¥ *S. epidermidis, Candida spp, Aspergillus spp, Pseudomonas* spp., Viridans group: IV drugusers

To rule out subacute endocarditis

1. Draw 2-3 blood culture sets on day 1, spaced30-60 minutes apart. This may help to document a continuousbacteremia.
2. If all are negative, additional sets can be drawn on days 2 and 3 (no more than 4 sets in a 24-hour period).

Immediate antibiotics are less important than establishing a specific microbial diagnosis

Summary

The role of the health worker in collecting, labeling, and ensuring the timely and proper delivery of specimens to the laboratory is very important in the hospital setting.

With this, the health worker should be knowledgeable enough about the hospital's policy and procedures for specimen collection. However, they should not only possess the right knowledge, but as well as the skill and understanding in performing necessary procedures in accordance with the organization's protocols, policies, and guidelines.

References

Clinical Microbiology Procedures Handbook.2nd Edition. . HD Isenberg ed. ASM. Cumitechs. ASM Press. Wash.DC.

Manual of Clinical Microbiology,10 Edition. ASM Press. Wash. DC. 2011.Miller MJ.

A Guide To Specimen Management in Clinical Microbiology. ASM Press. Wash. DC. 1999.

AMR Surveillance in Ethiopia Protocols (2017- 2019)

Bailey & Scott's Diagnostic Microbiology. Mosby, Inc. St Louis, Missouri, USA, 13th edition, 2013

IDSA guidelines. CID 2013:57 (15 August). Baron et al

Ohio State University Wexner Medical Center Specimen Collection Guidelines

Johns Hopkins Medical Microbiology Specimen Collection Guidelines

Check-In Questions

1. What is the single most important factor inblood culturecollection?

a. Answer: Blood volume is the singlemost importantfactor

b. Optimal blood to broth ratio is 1:5 to1:10

c. Obtain 20 mL of blood per culture. (Oneculture set consists of one bottle of aerobic culture medium and one bottle of anaerobic culture medium or two aerobicbottles)

d. Inoculate 10 mL into eachbottle

e. If only 10 mL or less is obtained, inject theblood into the aerobic bottle only. Attempt a repeat venipuncture. Note volume of bloodobtained

2. What is the correct number of blood culturesand how many hours apart should the cultures be collected for acutepatients?

a. 2-3 cultures at least 1 hourapart

b. Answer:2-3culturesfromseparatevenipuncture sites within a 10-minuteperiod

c. 3 cultures over a 1 to 2-hourperiod

d. None of theabove

3. What equipment and supplies are neededbefore blood collection takesplace?

a. Answer: all of the following, Blood culture bottles, Gauze and adhesive bandages(e.g. BAND-AIDS),

b. Material for skin disinfection: Iodine and70% alcohol, orChlorhexidine

c. 20 mL syringes (1 syringe for each set ofblood cultures)

d. Sterileneedles

e. Gloves

f. Tourniquet

4. True/False: All blood culture samples should be refrigerated?

- Answer: False, blood culture samples should be kept at ambient (room temperature), but mustbe incubated in automated system within 12hours.

Demonstration and Teach-Back Exercises

Objective
Participants will learn that:

- Collection of clinical specimens is critical for making the appropriate diagnosis of infection.
- Correct collection of clinical specimens leads to appropriate selection of antimicrobials.
- Correct collection of clinical specimens prevents contamination of specimens that leads to incorrect prescribing of antibiotics.

Time: approximately 30 minutes

Materials:
Refer to the materials sections in each module (e.g. materials and supplies listed, such as gloves). Materials need to be available based on the number of participants.

Instructions for the Trainer
Provide each participant with a paper copy of the graphic illustrated instructions (translated into Amharic) for each type of specimen collection. The participants are instructed to keep ready access to their copy of their graphic illustration instruction sheets for quick review when they are called upon to actually collect a clinical specimen. They can take the copy with them to use as guidance to follow when collecting specimens to be certain that all steps are conducted properly.

The training for each collection specimen will be conducted in three phases.
Phase One is the didactic phase where the instructor outlines the steps of each specimen collection as illustrated graphically in each of the modules.

- Each participant will have a paper copy of the illustrated instructions (translated into Amharic) for each type of specimen collection.
- Participants are instructed to follow along as each step is explained.
- Give participants an opportunity to ask questions regarding each individual step.

Phase Two is role playing by two participants with all other participants observing and critiquing.

- The instructor will select two participants to provide this role play; one will be the patient and the other the specimen collector.
- Using the materials provided, they will demonstrate each step on the graphic illustration instruction sheet short of actually collecting the specimen. For steps that they cannot actually perform (blood collection, urine collection, stool collection, etc.) they can explain what they would do as the specimen collector.
- The other participants will follow each step on their graphic illustration instruction sheet and critique how the role players performed. For example, did they forget a step?

Blood Culture Collection Assessment Form

1. Blood cultures are intendedto:

a) Help determine the source ofinfections

b) Identify the microbial etiology ofthe bloodstreaminfection

c) Guide antimicrobialtherapy

d) All of theabove

2. At least two sets of blood cultures shouldbe drawn: True or False?

3. The timing of blood cultures is moreimportant than the volume of blood: True orFalse?

4. What is the number of blood cultures and how many hours apart should the cultures becollected for acutepatients?

a) 2-3 cultures at least 1 hourapart

b) 2-3 cultures from separate venipuncturesites within a 10-minuteperiod

c) 3 cultures over a 1 to 2-hourperiod

d) None of theabove

5. What is the blood collection volume foradults per bottle?

a) 1-2 ml/bottle based on weight ofperson

b) 5-10 ml/bottle per septicepisode

c) 8-10 ml/bottle and 2-3 cultures perseptic episode

d) 10-20 ml/bottle

6. At a minimum, how many identifiers should be used to verifya patient?

a) 1

b) 2

c) 5

d) 0

7. The optimal blood-to-broth ratiois:

a) 1:1 to 1:10

b) 1:10 to 1:20

c) 1:5 to 1:10

d) None of the above

8. A properly collected sample, that is free of contaminants, is key to providing accurateand reliable blood culture results: True orFalse?

9. Which item is a potential sourceof contamination?

a) Improper cleaning ofskin

b) Transfer of collected blood from tube toblood bottleculture

c) Improperly disinfected venipuncturesite

d) All of theabove

10. Blood cultures should not be drawnthrough intravenouscatheteratthetimeofcatheter insertion: True orFalse?

Instructions for Use

Trainers can use the assessment questions to assess participants' knowledge gained from the Blood Culture Collection Module. Assessment should be conducted at the end of each module. Each question is worth 2 points. A score of 90% or above is passing. If participant does not pass, a thorough review of the module is required. Trainers should conduct a second assessment as needed.

Answer Key: Blood Culture Collection AssessmentForm

1. Blood cultures are intendedto:
    a. Help determine the source ofinfections
    b. Identify the microbial etiology ofthe bloodstreaminfection
    c. Guide antimicrobialtherapy
    d. All of theabove

    Answer = D

2. At least two sets of blood cultures shouldbe drawn: True or False?

    Answer = True

3. The timing of blood cultures is moreimportant than the volume of blood: True orFalse?

    Answer = False

4. What is the number of blood cultures and how many hours apart should the cultures becollected for acutepatients?
    a. 2-3 cultures at least 1 hourapart
    b. 2-3culturesfromseparatevenipuncturesites within a 10-minuteperiod
    c. 3 cultures over a 1 to 2-hourperiod
    d. None of theabove

    Answer = B

5. What is the blood collection volume foradults?
    a. 1-2 ml/bottle based on weight ofperson
    b. 5-10 ml/bottle per septicepisode
    c. 8-10 ml/bottle and 2-3 cultures perseptic episode
    d. 10-20 ml/sterilesyringe

    Answer = C

6. How many identifiers should be used to verifya patient?
    a. 1
    b. 2
    c. 5
    d. 0

    Answer = B

7. The optimal blood-to-broth ratiois:
    a. 1:1 to 1:10
    b. 1:10 to 1:20
    c. 1:5 to 1:10
    d. None of the above

    Answer = C

8. A properly collected sample, that is free of contaminants, is key to providing accurateand reliable blood culture results: True orFalse?

    Answer = True

9. Which item is a potential sourceof contamination?
    a. Improper cleaning ofskin
    b. Transfer of collected blood from tube to blood bottle culture
    c. Improperly disinfected venipuncturesite
    d. All of theabove

    Answer =D

10. Blood cultures should not be drawnthrough intravenouscatheteratthetimeofcatheter insertion: True orFalse?

    Answer = True

Blood Culture Collection Competency Checklist

Name:
Date:

Site Location:

Evaluator/Instructor's Name:

General Guidelines for Specimen Collection	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Gather all necessary equipment and supplies.	☐	☐	☐
2. Label bottles with participant's information per identification policy and date/time of blood collection before starting the procedure.	☐	☐	☐
3. Wash hands and put on gloves.	☐	☐	☐
4. Prepare patient by telling them what will be done and provide written instructions.	☐	☐	☐

Comments:

Blood Culture Collection Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Prepare equipment to obtain sample.	☐	☐	☐
a. Use alcohol to disinfect rubber stopper on blood culture bottles.			

Comments:

2. Indentify the venipuncture site by applying tourniquet around patient's arm.	☐	☐	☐

Comments:

3. Prepare venipuncture site by using either cholorhexidine or 70% alcohol follwed by 2% tincture of iodine.	☐	☐	☐
a. Wait 1 minute for the area to dry			

Comments:

4. Draw the required amount of blood using needle and syringe.	☐	☐	☐

Comments:

Blood Culture Collection Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
5. Withdraw needle gently and apply gentle pressure to the site with clean gauze. Cover gauze with clean bandage. *Comments:*	☐	☐	☐
6. Inoculate the culture bottles. Pierce the stopper on the culture bottle with the needle directly above the tube using slow, steady pressure. *Comments:*	☐	☐	☐
7. Drawing blood from a central line: a. Wipe bottle septums with alcohol swabs and allow todry. b. Place a new injection cap on the port prior toaccessing. c. Clean the new cap with alcohol for 15 seconds andallow cap todry. d. Attacha20mLsyringeandaspirate16-20mLofbloodfor a single set of bloodcultures. e. Flush each port that was drawn from with 20 mL ofsterile saline, using the push-pause flushing technique to help maintainpatency. f. Use blood transfer device, place 8-10 mL of bloodinto each bottle, inoculating anaerobic bottlefirst. *Comments:*	☐	☐	☐
8. Check culture bottles for accurate labeling. *Comments:*	☐	☐	☐
9. Properly fill out requisition form. *Comments:*	☐	☐	☐
10. Discard used supplies appropriately. *Comments:*	☐	☐	☐
11. Remove gloves and wash hands. *Comments:*	☐	☐	☐

Blood CultureCollectionProcedure	**Able to Perform**	**Able to Performwith Assistance**	**Unable to Perform**
12. Transport specimens tolaboratory promptly. a. Place specimen in a plastic bag with biohazardsign. b. Completedlabrequisitionformplacedontheoutsideof the transportbag.	☐	☐	☐

Comments:

Recommendation:☐Pass ☐ Needs morepractice

Comments:

Instructions for Use: Blood Culture Collection Procedures Competency Checklist

It is the Laboratory Director or Site Coordinator's responsibility to monitor compliance and ensure that competency evaluations are performed on a routine basis. Laboratory personnel must demonstrate competency in accordance with the following schedule:

- Initial competency evaluation upon hiringor learning a newprocedure.
- Six months following the initialcompetency evaluation.
- Annuallythereafter.

Each procedure must be directly observed by an instructor, trainer, nurse, site coordinator, or laboratory director (depending on the site) and evaluatedaccordingtotheperformancecriteria listed on each Competency Checklist. The evaluator must be someone who is trained in the procedure and has demonstrated proficiency.

Evaluation Instructions:

1. Record name of the individual to beevaluated, date, site location, and evaluator'sname.
2. The evaluator, instructor, or site coordinator will directly observe the individual performing each clinicalprocedure.
3. The evaluator will rate procedure criteria evaluated as either: *able to perform, able to performwithassistance,orunabletoperform.*
4. The evaluator will note additional feedbackunder "*Comments*".
5. The evaluator will determine if the individual received an overall score of *Pass* or *Needsmore practice*,andanycorrectiveactionorretraining required.
6. Thesitecoordinatororlaboratorydirectorwill maintain the Competency Checklistform.

All samples are to be collected and tested exactly as routine clinical materials. Evaluation of competency samples, as well as proficiency testing samples, must be made by the individual without consultation with other staff. **A minimum of two successful competency assessments are required.**

Module 3: Wound (Skin and Soft Tissue) Culture Collection

Module Preparation

Materials Needed:

- See Checklist for Materials, Equipment and Supplies
- PowerPoint Slides: Module 3
- Alcohol-based hand rub
- Gloves
- Wound swabs
- Specimen collection containers
- Skin antisepsis materials
- Wound Culture Collection Checklist
- Module 3 Assessment Form

Time: approximately 45-60 minutes

Instructions for Trainer:

Ideal class size is 5-25. Classes of 10 or more participants should be sub-divided into groups of 4 or 5 for discussions and activities. Plan for group discussions and check-in questions throughout the module. Allow time at beginning for introductions and pre-assessment, with time at end for demonstration / teach-back exercises and post-assessment. Conduct Pre and post-assessments using the assessment tool provided at end of module on page 70.

Discussion and Check-In Questions:
Check-In and Discussion Questions are provided below. Answers can be found at the end of the module on page 66.

- True/False: Type and or origin of wound should be noted on the label.
- What are the two preferred methods of wound culture collection?
- True/False: If the wound surface is dry, the swab can be pre- moist- ened in the transport media before swabbing the wound.
- When collecting a specimen from an abscess, how much material (aspirate) is needed?

Demonstration and Teach-Back Exercises
Information and instructions for the module activity are provided at the end of the module on page 66.

Topics to Cover:

- Objectives
- Introduction
- Equipment andSupplies
- Pre-Collection, Collection and Procedures
- Summary
- Check-In Questions
- Demonstration and Teach-Back Exercises
- Assessments/Checklists

Objectives

- Specify most common specimencollection procedures: equipment andsupplies.
- Use of correct practices forcollection.
- Focus on different collection techniques based on wound types (tissue, abscess, bullae,vesicles, closed wounds, cellulitis, openwounds).

Introduction

Cultures of wounds provide information on the type of microorganism causing the infection and susceptibility to guide treatment. A wound can be open or closed and can contain pus or purulentmaterial.

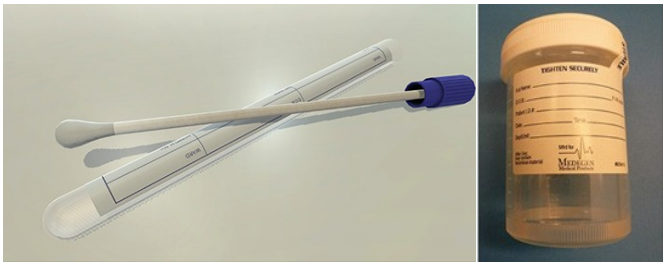
Wound specimens may involve the following:

- Abscess: pus under pressure
- Bullae: a bubble-like cavity filled with air or fluid
- Tissue
- Vesicles: a fluid-filled or air-filled cavity or sac

Specimens can be collected using the following methods:

- Swab –rayon/dacron swabs are preferred over cotton; swabs should be kept moist
- Aspiration (using a needle)
- Biopsy to collect portion of tissue

Figure 4. Types of specimen containers used for collection and transporting wound sample



The ordering physician will decide upon the type of specimen needed for culture. Needle aspiration and tissue biopsy are the preferred methods, however they are invasive and may require skilled personnel.

Wound swabs are acceptable as they are practical, non–invasive and cost effective. However, keep in mind that swabs dry easily and can only obtain a limited amount of material. Wound swabs should not be allowed to dry out.

When collecting wound swabs, only viable wound tissue must be swabbed rather than necrotic tissue or pus, since these may contain normal microbiota. Swabbing necrotic tissue or pus may produce false results which can lead to inappropriate antibiotic treatment. The area must be cleansed with sterile normal saline or sterile water prior to collection to avoid contamination with skin microbiota.

Swabs are not accepted for mycobacterial cultures, perirectal abscesses, and oral abscesses.
Gram stains cannot be provided from a single swab. If a Gram stain is needed, collect two swabs.

Labeling wound specimens is very important. Do not use the label “wound” alone. Be specific about body site and type of wound (forexample “human bite wound, knuckle”). Do not ask the laboratory to report everything that grows. See sample Requisition Form (from Ethiopia) in Appendix A.

Some considerations to improve the patient’s outcome: knowing the type of wound (source and type) can help anticipate the most common infecting bacteria.

- Skin surface wounds: *Staphylococcusaureus*
- Bites: respiratory flora, *Pasteurellamultocida*
- Burns: *Pseudomonas spp*, othernonfermenters, *S. aureus*
- Abdominal wounds: GI flora(Enterobacteriaceae, *Enterococcus spp*, etc)

Equipment and Supplies

	Wound Swab	Tissue Biopsy	Aspirate of Abscess and Closed Wounds
Clean or sterile gloves	✓ 3 sets needed for cleansing the wound, taking the swab,and applying the new dressing.	✓	✓
Sterile Gauze and supplies required for cleansing and redressing thewound	✓	✓	✓
Sterile normal saline or sterile water (60-120ml)	✓		
Sterile swab with transport media for collection of the specimen	✓		
Sterile non- bacteriostaticsaline		✓	
Surgical soap		✓	
70-95% alcohol and 1-2% tincture of iodine		✓	✓
Sterile syringe and needle		✓	
Surgical materials as needed for biopsy as designated by clinician		✓	

Before Collecting the Specimen

1. Prepare equipment andsupplies. Ensure labels used.
2. Explaintheproceduretothepatientand/ortheir significant other ifpresent
3. Complete requisition form with type of specimen, time of collection, site of collection, you name (name of person collecting specimen), patient's location, etc.

   NOTE: When filling "type of specimen", do not use the label "wound" alone. Be specific about body site and type of wound (for example "human bite wound, knuckle").
4. Verifythepatient'sidentificationbyusingtwo patient identifiers perpolicy
5. Remember to always label the specimen with the required information:
   - Collection Date and Time
   - Patient Identification (Name, DOB, etc.)
6. Wash hands thoroughly with soap and water, dry hands on a paper towel.
7. Follow Standard Precautions for all patients. Use appropriate protective equipment such asgloves, masks, and/or faceshields

Procedure Using Wound Swab

The following procedure should be used when collecting with a swab. If a Gram stain is needed, collect two swabs.If there are 2 or more wounds in the same location, use a separate swab for each wound.

1. Label tubes with patient information and date/time of collection.
2. Wash hands thoroughly with soap and water, dry hands on a papertowel.
3. Put on (sterile)gloves.
4. Remove the cover dressing of the woundusing forceps or sterilegauze.
5. Remove the gloves and perform hand hygiene then put on another pair of (clean)gloves.
6. Cleanse the wound with at least 60 – 120mL sterile normal saline or sterilewater.
7. Rotate the tip of the swab over 1 - 2 cmarea ofviabletissuefor5secondsusingsufficient pressure to extract fluid from the wound tissue.Avoid touching the wound edge or peri-wound skin with the swab.

    - NOTE: If the wound surface is dry, the swab can be pre- moistened in thetransport media before swabbing the wound. If there are two or more wounds in the same location, use a separate swab for eachwound.
    - NOTE: Do not swab necrotic tissue or pus

8. Place the swab into the tube with transport medium and rotate to close. Ensure the swab tip is in contact with the liquid transport medium at the base of the tube.
9. Remove gloves. Wash hands.

(adding additional illustration(s); will need to reorder accordingly)

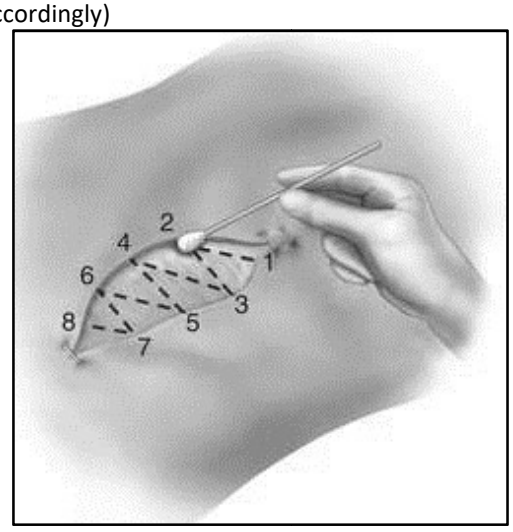


Illustration 18: Procedure using wound swab

Collection of Tissue

Collectionisaninvasiveprocedureandrequires surgery. This may require skilled personnel, or the procedure may need to be conducted by a trained professional.

1. Collect at least 1 -2 cm area of viable tissueaseptically.
2. Cleanse the superficial area thoroughl with seterile saline, changing sponges with each application. Remove all superficial exudates. Remove overlying debris with scalpel and swabs or sponges.
3. Includematerialfrom both the center and the edge of thelesion.
4. Place the specimen in a sterile containeron sterile gauze moistened with sterile non- bacteriostaticsaline.
5. Transport in less than an hour at ambient temperature, in a manner to ensure recoveryof anaerobicorganisms.

NOTE: Do not submit tissue informalin.DonotjamthetissueintoaCulturetteusin gthe swab; this is not an acceptable transportdevice.

Collection of Abscess Material

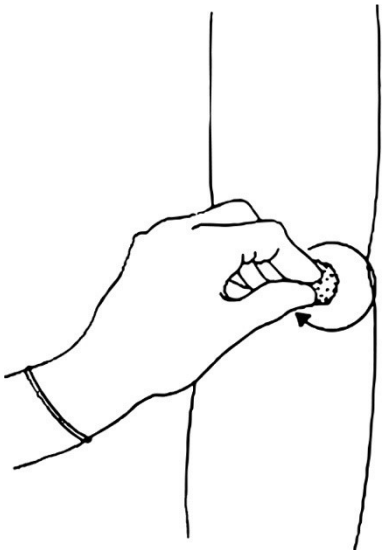
Needle aspiration is an invasive procedure and may require skilled personnel. The procedure may need to be conducted by a trained professional.

1. Decontaminate the surface with 70-95%alcohol and 1-2% tincture ofiodine
2. For an undrained abscess, use a sterile needle and syringe to collect purulent material aseptically.
    - NOTE: Open miliary abscesses should be opened with a sterile scalpel and then the expressed material should be collected witha sterile needle andsyringe.
3. Transfer 5-10 ml of the aspirated material to an anaerobic transport vial. Transport immediately.
    - NOTE: Anaerobic transport media is notrecommended for AFB culture. If requesting AFB culture, transfer at least 1 ml of the aspirated material into a sterilecontainer.

Collection of Bullae, Vesicles, Closed Wounds

1. Cleanse the skin. Prepare the site by usingONE of the following:
    - 70-95%alcohol
    - Chlorhexidine
    - 70% alcohol followed by 2% tincture oflodine
2. Rub the site in a concentric manner, start atthe center of the site and move outward.If using chlorhexidine perform back and forthmotion.

Illustration 18: rubbining in concentric manner



3. Wait at least 30 seconds for the area todry
4. Aspirate the fluid/purulent material usinga sterile needle andsyringe
5. Ifanaspirateisobtained,placeinanappropriate transportmedia.
6. If no material is obtained, open the vesicle or bullous lesion and use a swab to collect cells from the base of the lesion. Place inappropriate transportmedia.
7. For vesicles, select a fresh vesicle, wipe gently with alcohol, dry thoroughly with sterilegauze.
8. Using small needle (26 g x 1/2 inch)aspirate vesicularfluid.

9. Transfer the fluid to the transport medium by filling the remainder of the syringe with the medium, then flush the solution intothe transport tube or by swabbing the vesicle and breaking off the swab into tube of transport media.
10. USE STERILE TECHNIQUE AT ALLTIMES
11. Usinga sterile needle andsyringe
12. Ifanaspirateisobtained,placeinanappropriate transportmedia.
13. If no material is obtained, open the vesicle or bullous lesion and use a swab to collect cells from the base of the lesion. Place inappropriate transportmedia.
14. For vesicles, select a fresh vesicle, wipe gently with alcohol, dry thoroughly with sterilegauze.
15. Using small needle (26 g x 1/2 inch)aspirate vesicularfluid.
16. Transfer the fluid to the transport medium by filling the remainder of the syringe with the medium, then flush the solution intothe transport tube or by swabbing the vesicle and breaking off the swab into tube of transport media.
17. USE STERILE TECHNIQUE AT ALLTIMES

Procedure for Cellulitis

Cellulitis should be treated empirically. Swabs and leading-edge aspirates fail to yield etiologic agents in the majority of cases. If an unusual organism issuspected, a leading-edge (advancing margin) punch biopsy is the recommended specimen of choice.

Place tissue biopsy in sterile container with small volume of saline without preservatives.

Procedure for Sinus Tract

A sinus tract is a narrow opening or passageway underneath the skin that can extend in any direction through soft tissue and reults in dead space with potential for abscess formation.

Preferredspecimens:aspirationofmaterial obtained by needle, catheterization or curettings from the lining of the sinustract.Swabs of the sinus tracts are acceptable only if the above cannot be obtained, however swabs maynotaccuratelyreflecttheunderlying diseaseprocess.

1. Do not submit cultures of superficial lesions for anaerobic culture. Biopsy of advancing marginof wound is the preferred specimen for anaerobes, mycobacteria andfungi.
2. Clean the sinus tract opening of the wound surface mechanically, without using agermicidal agent, to remove as much of the superficial flora as possible. Surgical soap or 70% ethyl or isopropylalcohol.
3. Attempt to collect the base or edges ofthe wound to avoid collecting “normal flora” organisms.

Summary

The role of the health worker in collecting, labeling, and ensuring the timely and proper delivery of specimens to the laboratory is very important in the hospital setting.

With this, the health worker should be knowledgeable enough about the hospital's policy and procedures for specimen collection. However, they should not only possess the right knowledge, but as well as the skill and understanding in performing necessary procedures in accordance with the organization's protocols, policies, and guidelines.

References

Clinical Microbiology Procedures Handbook.2nd Edition. . HD Isenberg ed. ASM. Cumitechs. ASM Press. Wash.DC.

Manual of Clinical Microbiology,10 Edition. ASM Press. Wash. DC. 2011.Miller MJ.

A Guide To Specimen Management in Clinical Microbiology. ASM Press. Wash. DC. 1999.

AMR Surveillance in Ethiopia Protocols (2017-2019)

Bailey & Scott's Diagnostic Microbiology. Mosby, Inc. St Louis, Missouri, USA, 13th edition, 2013

IDSA guidelines. CID 2013:57 (15 August). Baron et al

Ohio State University Wexner Medical Center Specimen Collection Guidelines

Johns Hopkins Medical Microbiology Specimen Collection Guidelines

Check-In Questions

1. **True/False:** Type and or origin of wound should be noted on the label. Answer: True. Do not use the label "wound" alone. Be specific about body siteandtypeofwound(forexample"humanbite wound,knuckle").

   2. What are the preferred two methods ofwound culturecollection?
   Answer: Needle aspiration and tissue biopsy are preferred methods but swab is acceptable as it is practical, non–invasive and cost effective

   3. True/False: If the wound surface is dry, the swab can be pre- moistened in the transport media before swabbing the wound. Answer:True

   4. When collecting a specimen from an abscess, how much material (aspirate) is needed? Answer: Transfer 5-10 ml of the aspirated material to an anaerobic transport vial.Transport immediately. Anaerobic transport media is not recommended for Acid- Fast Bacilli (AFB) culture. If requestingAFB culture, transfer at least 1 ml of the aspirated material into a sterilecontainer

Demonstration and Teach-Back Exercises

Objective:
Participants will learn that:
Collection of clinical specimens is critical for making the appropriate diagnosis of infection. Correct collection of clinical specimens leads to appropriate selection of antimicrobials. Correct collection of clinical specimens prevents contamination of specimens that leads to incorrect prescribing of antibiotics.

Time: approximately 30 minutes

Materials:
Refer to the materials sections in each module (e.g. materials and supplies listed, such as gloves). Materials need to be available based on the number of participants.
Provide each participant with a paper copy of the graphic illustrated instructions (translated into Amharic) for each type of specimen collection. The participants are instructed to keep ready access to their copy of their graphic illustration instruction sheets for quick review when they are called upon to actually collect a clinical specimen. They can take the copy with them to use as guidance to follow when collecting specimens to be certain that all steps are conducted properly.

Instructions for the Trainer:
The training for each collection specimen will be conducted in three phases.
Phase One is the didactic phase where the instructor outlines the steps of each specimen collection as illustrated graphically in each of the modules.
Each participant will have a paper copy of the illustrated instructions (translated into Amharic) for each type of specimen collection.
Participants are instructed to follow along as each step is explained.
Give participants an opportunity to ask questions regarding each individual step.
Phase Two is role playing by two participants with all other participants observing and critiquing.
The instructor will select two participants to provide this role play; one will be the patient and the other the specimen collector.
Using the materials provided, they will demonstrate each step on the graphic illustration instruction sheet short of actually collecting the specimen. For steps that they cannot actually perform (blood collection, urine collection, stool collection, etc.) they can explain what they would do as the specimen collector.
The other participants will follow each step on their graphic illustration instruction sheet and critique how the role players performed. For example, did they forget a step?

Wound Culture Collection Assessment Form

1. **Knowing the source and type of wound canhelp the microbiologist anticipate the most common infecting bacteria. True orFalse?**

2. **The preferred method of wound culturecollection is:**
   a. Swab
   b. Tissue biopsy andswab
   c. Needle aspiration and tissuebiopsy
   d. All of theabove

3. **Viable microorganism causing the infection are usually in the wound tissue and must beswabbed rather than necrotic tissue or pus. True orFalse?**

4. **Which statement isfalse?**
    a. Swabbing is the most effective methodfor collecting mycobacterial cultures and oral abscesses.
    b. Swabbing is non-invasive and costeffective.
    c. If a Gram stain is needed, collect twoswabs.
    d. Swabbingnecrotictissueorpusmayproduce falseresults.

5. **The proper way to store and transportcollected tissue for cultureis:**
    a. Onformalin
    b. In a sterile plasticcontainer
    c. Place tissue into a culturette using aswab
    d. Place the specimen in a sterilecontainer on sterile gauze moistened with sterile nonbacteriostaticsaline

6. **When collecting culture from an open wound, attempt to culture the base or edges of the wound to avoid collecting normal floraorganisms. True orFalse?**

7. **If swab is collected, which is the proper wayto place the swab in the transportmedium?**
    a. Wrap the swab in tissue then place intransport medium
    b. Place the swab into the transport medium ensuring that the swab tip is in contact with the liquid transport medium at the base of thetube
    c. Place the tissue into a Culturette using theswab
    d. None of theabove

8. **When collecting tissue aseptically, include material from both the center and the edge ofthe lesion.True or False?**

9. **When collecting specimen from an abscess,which statement istrue.**
    a. Collect purulent material aseptically from an undrained abscess using a sterile needle and syringe.
    b. Transfer 5-10ml of the aspirated material to an aerobic transport vial.
    c. None of theabove

    d. A+B

Instructions for Use

Trainers may use the assessment questions to assess participants' knowledge gained from the Wound Culture Collection Module. Assessment should be conducted at the end of each module. Each question is worth 2 points. A score of 90% or above is passing. If participant does not pass, a thorough review of the module is required. Trainers should conduct a second assessment as needed.

Answer Key: Wound Culture Collection Assessment Form

1. Knowing the source and type of wound canhelp the microbiologist anticipate the most common infecting bacteria. True orFalse?

Answer = True

2. The preferred method of wound culturecollection are:

a. Swab

b. Tissue biopsy andswab

c. Needle aspiration and tissuebiopsy

d. All of theabove

Answer = C

3. Viable microorganism causing the infection are usually in the wound tissue and must beswabbed rather than necrotic tissue or pus. True orFalse?

Answer = True

4. Which statement isfalse?

a. Swabbingisthemosteffectivemethodfor collecting mycobacterial cultures and oral abscesses.

b. Swabbing is non-invasive and costeffective.

c. If a Gram stain is needed, collect twoswabs.

d. Swabbingnecrotictissueorpusmayproduce falseresults.

Answer = A

5. The proper way to store and transportcollected tissue for cultureis:

a. Onformalin

b. In a sterile plasticcontainer

c. Place tissue into a culturette using aswab

d. Place the specimen in a sterilecontainer on sterile gauze moistened with sterile nonbacteriostaticsaline

Answer =D

6. When collecting culture from an open wound, attempt to culture the base or edges of the wound to avoid collecting normal floraorganisms. True orFalse?

Answer = True

7. If swab is collected, which is the proper wayto place the swab in the transportmedium?

a. Wrap the swab in tissue then place intransport medium

b. Place the swab into the transport medium ensuring that the swab tip is in contact withthe liquid transport medium at the base of thetube

c. Place the tissue into a Culturette using theswab

d. None of theabove

Answer =B

8. When collecting tissue aseptically, include material from both the center and the edge ofthe lesion. True orFalse?

Answer = True

9. When collecting specimen from an abscess,which statement istrue.

a. Collect purulent material aseptically froman undrained abscess using a sterile needleand syringe.

b. Transfer 5-10ml of the aspirated material toan anaerobic transportvial.

c. None of theabove

d. A+B

Answer =D

Wound Culture Collection Procedures Competency Checklist

Name:
Date:

Site Location:

Evaluator/Instructor's Name:

General Guidelines for Specimen Collection	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Gather all necessary equipment and supplies.	☐	☐	☐
2. Label culturette tube with participant's information per identification policy and date/time of specimen collection before starting the procedure.	☐	☐	☐
3. Wash hands and put on gloves.	☐	☐	☐
4. Prepare participant by telling them what will be done.	☐	☐	☐

Comments:

Swab Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Prepare equipment (i.e., gloves, saline, sterile swab) to obtain sample.	☐	☐	☐

Comments:

2. Wash hands thoroughly with soap and water, dry hands, and put on clean gloves.	☐	☐	☐

Comments:

3. Remove the soiled dressing. Dispose gloves and soiled dressing in appropriate container (e.g., biohazard bag).	☐	☐	☐

Comments:

4. Wash hands thoroughly with soap and water, dry hands, and put on clean gloves.	☐	☐	☐

Comments:

5. Cleans the wound with at least 60-120 mL sterile normal saline or sterile water.	☐	☐	☐

Comments:

Swab Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
6. Rotate the tip of the swab over 1-2 cm area of viable tissue for 5 seconds to extract fluid from the wound tissue. *Comments:*	☐	☐	☐
7. Insert the swab into the culturette tube, making sure it does not make contact with opening of tube upon insertion. a. Check the swab tip is in contact with the liquidtransport medium at the base of thetube. b. Twist the cap to secure thetube *Comments:*	☐	☐	☐
8. Apply a clean dressing to the wound as ordered. *Comments:*	☐	☐	☐
9. Discard used supplies appropriately. *Comments:*	☐	☐	☐
10. Remove gloves and wash hands. *Comments:*	☐	☐	☐
11. Transport specimen to laboratory promptly. a. Place specimen in a plastic bag with biohazardsign. *b.* Completedlabrequisitionformplacedontheoutsideof the transportbag. *Comments:*	☐	☐	☐

Recommendation:☐Pass ☐ Needs morepractice
Comments:

Instructions for Use: Wound Culture Collection Procedures Competency Checklist

It is the Laboratory Director or Site Coordinator's responsibility to monitor compliance and ensure that competency evaluations are performed on a routine basis. Laboratory personnel must demonstrate competency in accordance with the following schedule:

- Initial competency evaluation upon hiringor learning a newprocedure.
- Six months following the initialcompetency evaluation.
- Annuallythereafter.

Each procedure must be directly observed by an instructor, trainer, nurse, site coordinator, or laboratory director (depending on the site) and evaluatedaccordingtotheperformancecriteria listed on each Competency Checklist. The evaluator must be someone who is trained in the procedure and has demonstrated proficiency.

Evaluation Instructions:

1. Record name of the individual to beevaluated, date, site location, and evaluator'sname.
2. The evaluator, instructor, or site coordinator will directly observe the individual performing each clinicalprocedure.
3. The evaluator will rate procedure criteria evaluated as either: able to perform, able to performwithassistance,orunabletoperform.
4. The evaluator will note additional feedbackunder "Comments".
5. The evaluator will determine if the individual receivedanoverallscoreofPassorNeedsmore practice, and any corrective action or retraining required.
6. Thesitecoordinatororlaboratorydirectorwill maintain the Competency Checklistform.

All samples are to be collected and tested exactly as routine clinical materials. Evaluation of competency samples, as well as proficiency testing samples, must be made by the individual without consultation with other staff. **A minimum of two successful competency assessments are required.**

Module 4: Urine Culture Collection

Module Preparation

Materials Needed:

- See Checklist for Materials, Equipment and Supplies
- PowerPoint Slides: Module 4
- Alcohol-based hand rub
- Gloves
- Urine collection containers
- Urinary catheter set
- Skin antisepsis materials
- Urine Culture Collection Checklist
- Module 4 Assessment Form

Time: approximately 45-60 minutes

Instructions for Trainer:

Ideal class size is 5-25. Classes of 10 or more participants should be sub-divided into groups of 4 or 5 for discussions and activities. Plan for group discussions and check-in questions throughout the module. Allow time at beginning for introductions and pre-assessment, with time at end for demonstration / teach-back exercises and post-assessment. Conduct Pre and post-assessments using the assessment tool provided at end of module on pages80.

Discussion and Check-In Questions:
Check-In and Discussion Questions are provided below. Answers can be found at the end of the module on page79.

- True/False: Urine cultures are one of the few clinical specimens which is cultured quantitatively.
- Identify the main points of storing and transporting urine specimens.
- Which specimens are unacceptable for culture?
- In groups of 3-4 people review the procedure and differences of collecting urine specimens from male, female and pediatric patients.

Demonstration and Teach-Back Exercises
Information and instructions for the module activity are provided at the end of the module on page87.

Topics to Cover:

- Objectives
- Introduction
- Equipment andSupplies
- Pre-Collection/Collection/Procedures
  - Female
  - Male
  - Indwellingcatheters
  - PediatricPatients
- Summary
- Check-In Questions
- Demonstration and Teach-Back Exercises
- Assessments/Checklists

Objectives

- Specify most common specimencollection procedures: equipment andsupplies.
- Use of correct practices forcollection.
- Describe which techniques to use depending on midstream urine collection (male versus female), indwelling catheter and pediatric urinecollection.

Introduction

The urine culture is a test that detects and identifies bacteria and yeast in the urine, which may be causing a urinary tract infection.

Best Practices

This module describes the best practices for collection. Urine needs to be collected in a sterile wide-mouth container and contamination from normal flora needstobeavoided.Urinecanbefrom:

- Mid-stream, “clean catch”
- Catheter

Ensure that there is sufficient volume to perform the tests. If insufficient volume, instruct the patient to collect a new sample. Transport the specimen to the laboratory immediately, or refrigerate the specimen if transport is delayed more than 2 hours. Do not freeze the specimen. Refrigerated specimens must be cultured within 24 hours of collection.

Contaminating bacteria will replicate if specimen is not quickly transferred to a preservative tube or stored (4°C). If a urine preservative tube is used, transfer urine to the tube within 10 minutes of collection (good for 48 hrs. at ambient temp.),

otherwise refrigerate. When collecting urine always instruct for proper cleansing and collection of “clean catch” urine samples and transport or refrigerate immediately or place in preservative container.

24hoururines (continuous collection) andthose from catheter bags are not acceptable forculture. The following specimens are unacceptable and they should be rejected:

- 24-hour urinecollections
- collections from urinary catheterbags
- Foley cathetertips
- collections from bedpan orurinal

Cleansing and Preventing Contamination

The specimen must be collected with minimum contamination.Contaminating organisms can grow and cause misleading culture results. Urine is normally sterile but can be easily contaminated during collection with organisms from the area between the anus and the scrotum or vulva (perineum) during collection. All nonsurgical samples become contaminated with urogenital microbiota during collection.

If patient is self collecting, healthcare provider should instruct patient on proper collection and how to minimize contamination to specimen and container. Patients must be instructed to properly cleanse the peri-urethral genital skin area prior to collectionof the mid-stream portion of the urine stream in order to get an “clean catch” and obtain accurate urine culture result. These illustrations can also be provided to the patient as a guide for collection.

One specimen is adequate to diagnose infections in patients who have symptoms of infection: frequency, urgency and pain on urination; this may be accompanied by fever.

The laboratory processes the specimens in a quantitative manner, to enable significant growth to be detected. Individuals with urinary tract infections typically have $>10^4$cfu/ml. As a result, urine cultures are one of the few clinical specimens which are cultured quantitatively.

Up to 60 to 80% of urine specimens received for culture are negative or contain only contaminants (Diphtheroids, Coagulase Negative Staphylococcus, Micrococcus). The laboratory can use rapid methods to check for infection:

- Microscopy (Gram stain, only if requested). Not optimalsinceitrequiresconcentration,laboratory expertise and timeconsuming

- Nitrates,leukocyteesterase.Greatprescreening tools, however can miss someinfections

Equipment and Supplies

	Mid-Stream Collection	Catheter Collection
Sterile urine collection containers, 50mL (cups for collection and transport).	✓	✓
Label for specimen container	✓	✓
Sterile wipe/cleansing towelette	✓	
Visual aid/instructions for patient self-collection	✓	
Sterile syringe 30 mL		✓
Sterile needle 23- or 25-gauge		✓
Alcohol swabs		✓
Gloves		✓

Before Collecting the Specimen

1. Prepare equipment andsupplies. Container should be labeled
2. Explaintheproceduretothepatientand/ortheir significant other ifpresent.
3. Complete requisition form with type of specimen, time of collection, site of collection, you name (name of person collecting specimen), patient's location, etc.
4. Verifythepatient'sidentificationbyusingtwo patient identifiers perpolicy.
5. Remember to always label the specimen with the required information:
   - Collection Date and Time
   - Patient Identification (Name, DOB, etc.)
   - Collection Method. The requisition must be filled out and the exact source of the specimen indicated e.g. "midstream urine".
   - Test required.
6. Wash hands thoroughly with soap and water and dry the hands with a paper towel.
7. Follow Standard Precautions for all patients. Use appropriate protective equipment such asgloves, masks, and/or faceshields.

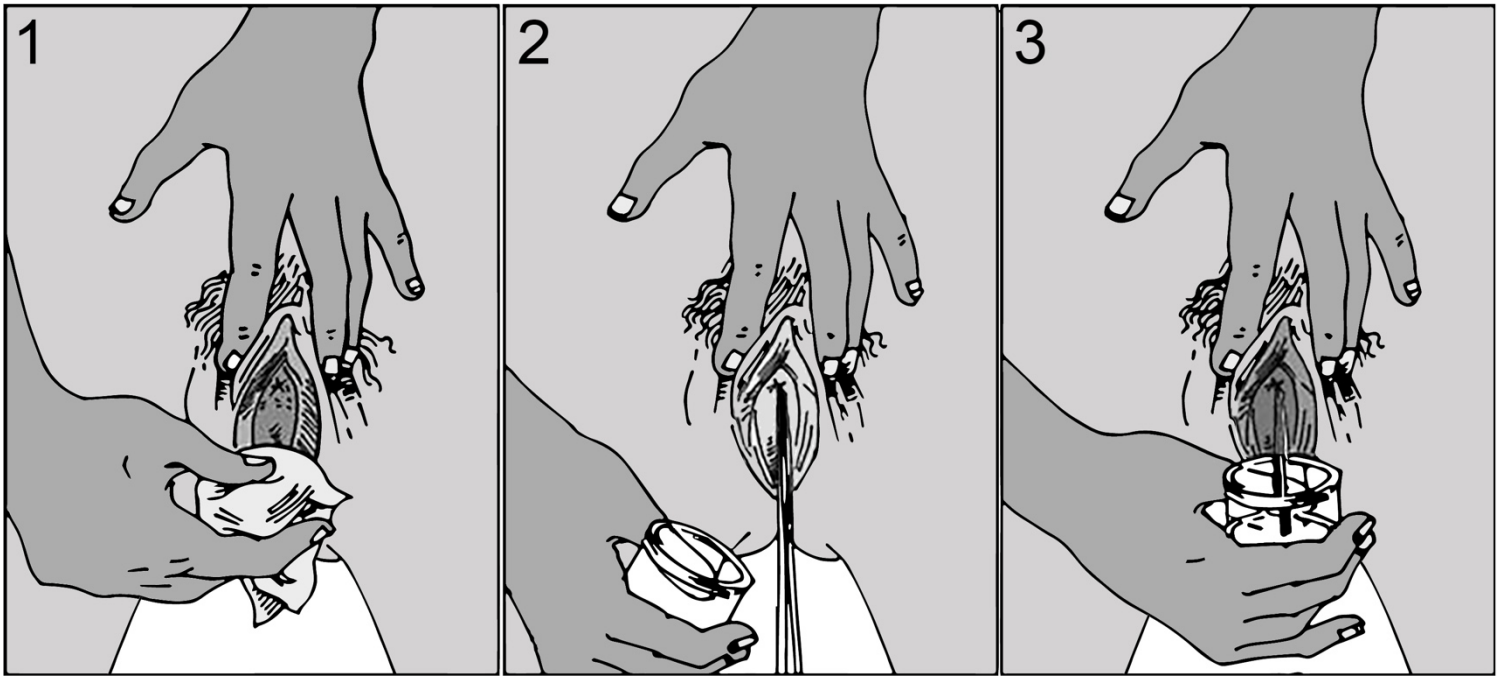
Midstream Urine Collection

From Females

The following protocol should be followed:

1. Wash hands thoroughly with soap and waterand dry the hands with a papertowel.
2. With one hand spread the labia and holdthem apart(1) and use sterile wipe/cleansing towelette to clean the meatus from front toback.
3. Start voiding urine, after the first portion of the urineispassed(2);collectaportionoftheurine by voiding into the sterile container(3).
4. Avoid contact between the container and the legs, vulva, or clothing. Do not touch the insideof the container orlid.
5. Stop collection when container is abouthalf-full.
6. Screw cap oncontainer.
7. Wash hands thoroughly with soap and water, and dry with a clean papertowel.
8. Give sample topersonnel.

Figure 5. Midstream urine collection from female

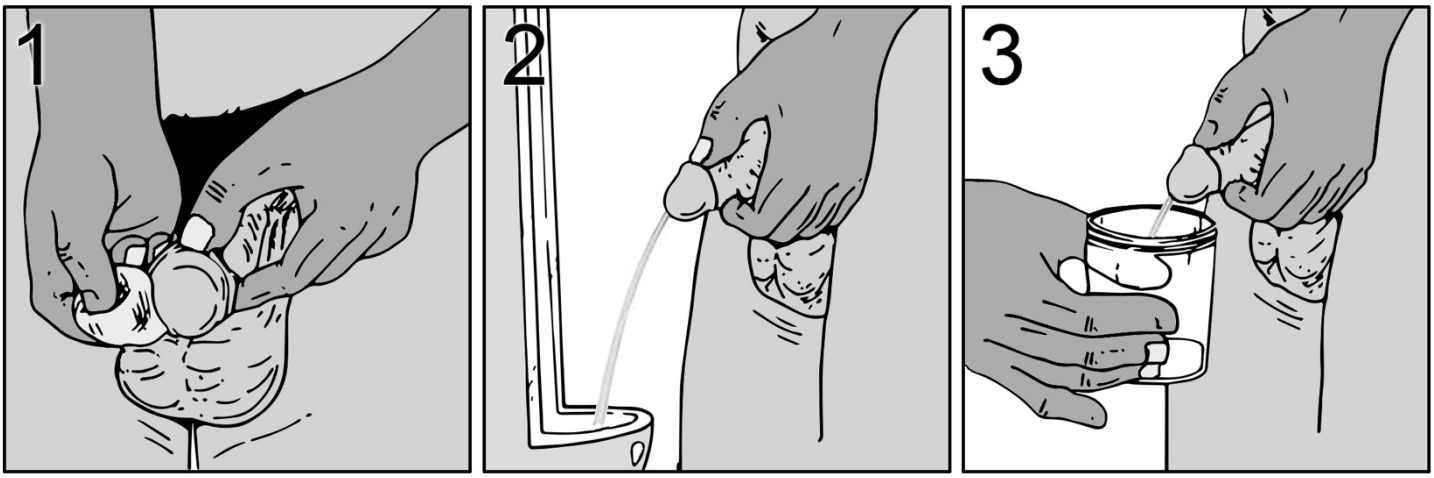


From Males

The following protocol should be followed:

1. Wash hands thoroughly with soap and waterand then dry hands with a clean papertowel.
2. If the patient is uncircumcised, instruct himto retracttheforeskin,holdingitbackduringthe entireprocedure.
3. If possible, instruct the patient to clean the area around the penis opening (glans penis) by starting at the tip of the penis andcleaning downward (using a towelette) and also cleaning directly across the meatus with a different towelette (1).
4. Passtheinitialportionofurineintothetoilet bowl(2).
5. Pass some of the remaining urine into thesterile, screw-cap plastic cup provided. Do not touch the inside of the container or lid(3).
6. Stop collection when container is abouthalf-full.
7. Carefully screw cap oncontainer.
8. Wash hands thoroughly with soap and water, and dry with a clean papertowel.
9. Give sample topersonnel.

Figure 6. Midstream urine collection from males



When the urine is collected to rule out Chlamydia trachomatis or Neisseria gonorrhea by a nucleic acid amplification test (NAAT), the collection of urine is different from midstream collection. The patient must not have urinated during the previous two hours and the first 10 to 30 ml of the urine stream should be collected in a clean, empty plastic cup without preservatives. Because depending on the test used, there are special tubes, transfer 2 ml of urine in test-specific transport media.

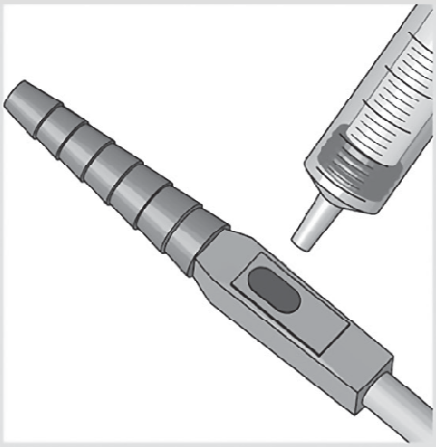
Collection of Urine from Indwelling Catheter

It should only be collected by trained personnel, such as nurses.

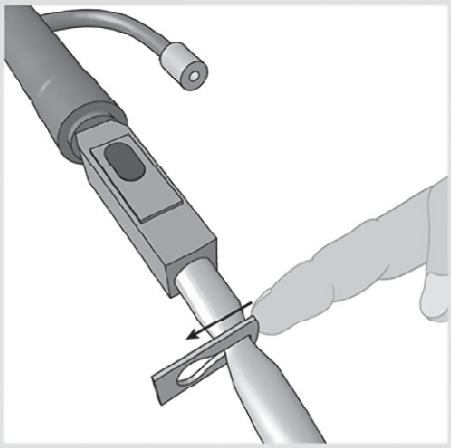
The following steps should be followed:

1. Wash hands thoroughly with soap and waterand dry the hands with a papertowel.
2. Identify the collection port on thecatheter.
3. Put on new pair of cleangloves.
4. Clamp the catheter for 15 minutes-this willresult in urine collecting in the cathetertube.
5. Disinfect the port using 70% alcohol. Allow alcohol to dry before obtaining thespecimen.
6. Collect approximately 10 ml of urine usinga syringe and needle; use aseptictechnique.
7. Transfer the specimen into the sterile container.
8. Dispose of any needles into a sharpsbox.
9. Disinfect needle entrance site with alcoholswab.
10. Unclasp catheter.
11. Label the specimen with the requiredinformation.
12. Remove gloves and wash hands thoroughly with soap andwater.

Figure 7. Collecting a catheter specimen of urine.

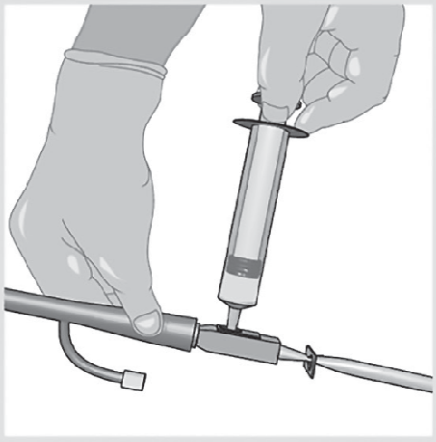


1a. A sampling port can be found on the tubing of the catheter drainage bag – urine should only be obtained from this point.



1b. Clamp the catheter below the port so that urine can collect above it in the tubing. Some catheter bags have an integral clamp.

1c. Swab the sampling port with an alcohol-impregnated swab following local policy to reduce the risk of cross infection and contamination of the specimen.



1d. Insert the syringe tip into the sampling port and withdraw the urine following manufacturer's instructions.

1e. Place sample in the specimen pot, avoiding contact with the syringe. Secure top to prevent leakage and contamination, then label, place in a specimen bag and seal.

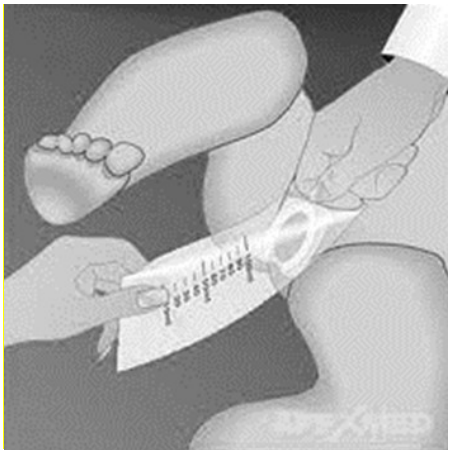
1f. If the sample is taken from a catheter valve, the valve must be cleaned with an alcohol-impregnated swab first to reduce the risk of cross infection.

Pediatric Patients

When a clean catch cannot be collected from a pediatric patient:

1. Clean theperineum.
2. Tape a small sterile plastic bag to the perineum, so that the specimen can be collected at thenext urination.
3. Transfer the specimen to a sterilecontainer.
4. Label the specimen with therequired information.

Figure 8: Urine collection from pediatric patient



Straight urethral catheterization for pediatric patients:

Note: straight urethral catheterization of pediatric patients is associated with a small risk of introducing bacteria from the perineal area to the bladder.

1. Catheterize the bladder using aseptic technique when the patient's bladder is full.
2. Discard the initial 15-30 mL of urine.
3. Collect a sample from the mid- or later flow ofurine into a sterile container.
4. Label the specimen with the requiredinformation.

Summary

The role of the health worker in collecting, labeling, and ensuring the timely and proper delivery of specimens to the laboratory is very important in the hospital setting.

With this, the health worker should be knowledgeable enough about the hospital's policy and procedures for specimen collection. However, they should not only possess the right knowledge, but as well as the skill and understanding in performing necessary procedures in accordance with the organization's protocols, policies, and guidelines.

References

Clinical Microbiology Procedures Handbook.2nd Edition. . HD Isenberg ed. ASM. Cumitechs. ASM Press. Wash.DC.

Manual of Clinical Microbiology,10 Edition. ASM Press. Wash. DC. 2011.Miller MJ.

A Guide To Specimen Management in Clinical Microbiology. ASM Press. Wash. DC. 1999.

AMR Surveillance in Ethiopia Protocols (2017- 2019)

Bailey & Scott's Diagnostic Microbiology. Mosby, Inc. St Louis, Missouri, USA, 13th edition, 2013

IDSA guidelines. CID 2013:57 (15 August). Baron et al

Ohio State University Wexner Medical Center Specimen Collection Guidelines

Johns Hopkins Medical Microbiology Specimen Collection Guidelines

Check-In Questions

1. **True/False: Urine cultures are one of the few clinical specimens which is cultured quantitatively.**

   Answer: True. The laboratory processes the specimens in a quantitative manner, to enable significant growth to be detected. Individuals with urinary tract infections typically have $>10^4$cfu/ ml. As a result, urine cultures are one of the few clinical specimens which is cultured quantitatively.

2. Identify the main points of storingand transporting urinespecimens.

Answer: Transfer urine to a Urine Preservativetube within 10 minutes of collection (good for 48 hrs. at ambient temp. Less optimal: store/transporturines at 4° C for up to 24hrs.

3. Specimens that are unacceptable forculture include:

- **Foley cathetertips**
- **Collections frombedpan**
- **Collections from urinary catheterbags**

Answer: All of the three listed answers are unacceptable for culture.

4. In groups of 3-4 people review the procedureand differences of collecting urine specimens from male, female and pediatricpatients.

Answer: Midstream urine collection - females collecting specimens to be certain that all steps are conducted properly.

Demonstration and Teach-Back Exercises

Objective:
Participants will learn that:

- Collection of clinical specimens is critical for making the appropriate diagnosis of infection.
- Correct collection of clinical specimens leads to appropriate selection of antimicrobials.
- Correct collection of clinical specimens prevents contamination of specimens that leads to incorrect prescribing of antibiotics.

Time: approximately 30 minutes

Materials:
Refer to the materials sections in each module (e.g. materials and supplies listed, such as gloves). Materials need to be available based on the number of participants.
Provide each participant with a paper copy of the graphic illustrated instructions (translated into Amharic) for each type of specimen collection. The participants are instructed to keep ready access to their copy of their graphic illustration instruction sheets for quick review when they are called upon to actually collect a clinical specimen. They can take the copy with them to use as guidance to follow when

Instructions for the Trainer
The training for each collection specimen will be conducted in three phases.
Phase One is the didactic phase where the instructor outlines the steps of each specimen collection as illustrated graphically in each of the modules.

- Each participant will have a paper copy of the illustrated instructions (translated into Amharic) for each type of specimen collection.
- Participants are instructed to follow along as each step is explained.
- Give participants an opportunity to ask questions regarding each individual step.

Phase Two is role playing by two participants with all other participants observing and critiquing.

- The instructor will select two participants to provide this role play; one will be the patient and the other the specimen collector.
- Using the materials provided, they will demonstrate each step on the graphic illustration instruction sheet short of actually collecting the specimen. For steps that they cannot actually perform (blood collection, urine collection, stool collection, etc.) they can explain what they would do as the specimen collector.

- The other participants will follow each step on their graphic illustration instruction sheet and critique how the role players performed. For example, did they forget a step?

Urine Culture Collection Assessment Form

1. Which of the following statements aretrue?

a. Allurinecollectionand/ortransportcontainers should be clean and free ofcontaminants.

b. Specimen containers should not bereused.

c. Always instruct proper cleansing andcollection of clean catch urinesamples.

d. All of theabove.

e. None of theabove.

2. Urine specimens do not have to be refrigerated or placed in preservation tube if not culturing immediately. True orFalse?

3. Urine specimen can be collectedfrom:

a. Mid-stream

b. Cleancatch

c. Catheter

d. All of theabove

4. Specimens that are unacceptable forculture include:

a. Foley cathetertips

b. Collections frombedpan

c. Collections from urinary catheterbags

d. All of theabove

e. None of theabove

5. Urine specimen that has been frozen can be used for culture. True or False?

6. Refrigerated urine specimen must be cultured within 24 hours of collection. True orFalse?

7. Proper labeling techniqueincludes:

a. Patient identification (name, date ofbirth, patient IDnumber)

b. Collection date andtime

c. Collection method and testrequired

d. All of theabove

e. None of theabove

8. Collecting urine specimen from a bedpanis appropriate to use. True orFalse?

9. One specimen is adequate to diagnose infections in patients who have symptoms of infection such as frequency, urgency and pain on urination. True orFalse?

10. Approximately 60% to 80% of urine specimens received for culture are negative or contain only contaminants. True or False?

Instructions for Use

Trainers can use the assessment questions to assess participants' knowledge gained from the Urine Culture Collection Module. Each question is worth 2 points. A score of 90% or above is passing.Assessment should be conducted at the end of each module. Each question is worth 2 points. A score of 90% or above is passing. If participant does not pass, a thorough review of the module is required. Trainers should conduct a second assessment as needed.

Answer Key: Urine Culture Collection AssessmentForm

1. Which of the following statements aretrue?

a. Allurinecollectionand/ortransportcontainers should be clean and free ofcontaminants.

b. Specimen containers should not bereused.

c. Always instruct proper cleansing andcollection of clean catch urinesamples.

d. All of theabove.

d. None of theabove.

Answer = D

2. Urine specimen do not have to be refrigerated or placed in preservation tube if not culturing immediately. True orFalse?

Answer = False

3. Urine specimen can be collectedfrom:

a. Mid-stream

b. Cleancatch

c. Catheter

d. All of the above

Answer =D

4. Specimens that are unacceptable forculture include:

a. Foley cathetertips

b. Collections frombedpan

c. Collections from urinary catheterbags

d. All of theabove

e. None of theabove

Answer = D

5. Urine specimen that has been frozen is can be used for culture. True orFalse?

Answer = False

6. Refrigerated urine specimen must be cultured within 24 hours of collection. True orFalse?

Answer = True

7. Proper labeling techniqueincludes:

a. Patient identification (name, date ofbirth, patient IDnumber)

b. Collection date andtime

c. Collection method and testrequired

d. All of theabove

e. None of theabove

Answer = D

8. Collecting urine specimen from a urinalis appropriate to use. True orFalse?

Answer = False

9. One specimen is adequate to diagnose infections in patients who have symptoms of infection such as frequency, urgency and pain on urination. True orFalse?

Answer = True

10. Approximately 60% to 80% of urine specimens receivedforculturearenegativeorcontainonly contaminants. True or False?

Answer = True

Urine Culture Collection Competency Checklist

Name:
Date:

Site Location:

Evaluator/Instructor's Name:

General Guidelines for Specimen Collection	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Gather all necessary equipment and supplies.	☐	☐	☐
2. Label specimen cup with participant's information per identification policy and date/time of specimen collection before starting the procedure.	☐	☐	☐
3. Wash hands and put on gloves.	☐	☐	☐
4. Prepare patient by telling them what will be done by providing verbal instructions and give written procedure instructions.	☐	☐	☐

Comments:

Catheter Collection Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Prepare equipment (i.e., gloves, alcohol wipes, sterile syringe, needle) to obtain sample.	☐	☐	☐

Comments:

2. Wash hands thoroughly with soap and water, dry hands, and put on clean gloves.	☐	☐	☐

Comments:

3. Identify the collection port on the catheter.	☐	☐	☐

Comments:

4. Clamp the catheter for 15 minutes to collect urine in the catheter tube.	☐	☐	☐

Comments:

5. Disinfect the port using 70% alcohol.	☐	☐	☐

Comments:

Catheter Collection Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
6. Collect 10 mL of urine through the samling port by using sterile syringe and needle. *Comments:*	☐	☐	☐
7. Transfer specimen into sterile specimen cup. *Comments:*	☐	☐	☐
8. Dispose needles into a sharps box. *Comments:*	☐	☐	☐
9. Label the specimen with required patient information. *Comments:*	☐	☐	☐
 Comments:	☐	☐	☐
11. Remove gloves and wash hands thoroughly with soap and water. *Comments:*	☐	☐	☐
12. Give sample to lab personnel promptly. *Comments:*	☐	☐	☐

Recommendation:☐Pass ☐ Needs morepractice
Comments:

Instructions for Use: Urine Culture Collection Procedures Competency Checklist

It is the Laboratory Director or Site Coordinator's responsibility to monitor compliance and ensure that competency evaluations are performed on a routine basis. Laboratory personnel must demonstrate competency in accordance with the following schedule:

- Initial competency evaluation upon hiringor learning a newprocedure.
- Six months following the initialcompetency evaluation.
- Annuallythereafter.

Each procedure must be directly observed by an instructor, trainer, nurse, site coordinator, or laboratory director (depending on the site) and evaluatedaccordingtotheperformancecriteria listed on each Competency Checklist. The evaluator must be someone who is trained in the procedure and has demonstrated proficiency.

Evaluation Instructions:

1. Record name of the individual to beevaluated, date, site location, and evaluator'sname.
2. The evaluator, instructor, or site coordinator will directly observe the individual performing each clinicalprocedure.
3. The evaluator will rate procedure criteria evaluated as either: able to perform, able to performwithassistance,orunabletoperform.
4. The evaluator will note additional feedbackunder "Comments".
5. The evaluator will determine if the individual receivedanoverallscoreofPassorNeedsmore practice, and any corrective action or retraining required.
6. Thesitecoordinatororlaboratorydirectorwill maintain the Competency Checklistform.

All samples are to be collected and tested exactly as routine clinical materials. Evaluation of competency samples, as well as proficiency testing samples, must be made by the individual without consultation with other staff. A minimum of two successful competency assessments is required.

Module 5: Respiratory Culture

Module Preparation

Materials Needed:

- See Checklist for Materials, Equipment and Supplies
- PowerPoint Slides: Module 5
- Alcohol-based hand rub
- Personal Protective Equipment (e.g. gloves)
- Specimen collection containers
- Swabs
- Respiratory Culture Collection Checklists
- Module 5 Assessment Form

Time: approximately 45-60 minutes

Instructions for Trainer:

Ideal class size is 5-25. Classes of 10 or more participants should be sub-divided into groups of 4 or 5 for discussions and activities. Plan for group discussions and check-in questions throughout the module. Allow time at beginning for introductions and pre-assessment, with time at end for demonstration / teach-back exercises and post-assessment. Conduct Pre and post-assessments using the assessment tool provided at end of module on page 93.

Discussion and Check-In Questions:
Check-In and Discussion Questions are provided below. Answers can be found at the end of the module on page92.

- Before collecting a sputum sample, what step must be taken first?
- What are special considerations to keep in mind if pneumonia is suspected in the patient?
- Identify the different types of respiratory tract specimens.
- Which organisms require to be introduced into (inoculated) onto culture media or special transport medium?

Demonstration and Teach-Back Exercises
Information and instructions for the module activity are provided at the end of the module on page 87.

Topics to Cover:

- Objectives
- Introduction
- Equipment/Supplies
- Pre-Collection/Collection
    - Nares/nasopharyngeal
    - Throat
    - Sputum
    - Bronchoalveolar lavage
- Summary
- Check-In Questions
- Demonstration/Teach Back Exercises
- Assessments/Checklist

Objectives

- Specify most common specimencollection procedures: equipment andsupplies
- Collection guidelines to rule out respiratory infectionswhenusingupperorlowerrespiratory specimens
- Safety and special collection to ruleout tuberculosis

Introduction

Respiratory cultures are performed to rule out respiratory infections.

Depending on the infection, upper or lower respiratory specimens should be collected. Upper respiratory tract specimens can be collected from:

- Nose hairs (Nares for detection of MRSA carriers)
- Nasopharyngeal swabs and washings (diagnosis of viral disease)
- Throat swabs (commonly used to diagnose bacterial infections in the throat).

Lower respiratory tract specimens include:

- Sputum
- Bronchial brush or wash
- Bronchial-alveolar lavage

For the different respiratory specimens, collection and transport containers used vary. See Table 12

These are somekeypoints for the laboratory diagnosis of respiratory infection:

- First morning sputum is always best forculture.
- Blood cultures that accompany sputumspecimens may occasionally be helpful, particularly in high risk community acquired pneumoniapatients.
- The laboratory should be contacted for specific instructions prior to collection of specimens for fastidiouspathogenssuchasBordetellapertussis, bacteria can only grow if special nutrients are present in the culturemedium.
- In the immunocompromised host, a broad diagnosticapproachbasedoninvasivelyobtained specimens issuggested.

When deciding what specimen to collect, these are some hints to follow:

- Blood, not sputum, may be specimen of choicefor diagnosing bacterialpneumonia.
- Patients must rinse mouth with water, and coughdeepforcollectionoflowerrespiratory specimens.
- Bronchial-alveolar lavages are better than bronchial washings for diagnosing pneumonia. Performed by physicians, consists of injecting liquid to the lungs to wash the area andcollect.

Table 12: Types of respiratory specimens, collection and transport containers

Specimen	Collection	Transport Container	Comments
Nares/Nasopharyngeal	Swab of nostril/pharynx	Swab in Transport Media	Avoid mucus
Throat	Swab of pharynx and tonsils	Swab in Transport Media	Avoid touching mouth or gums with the swab
Sputum	Deep breath and cough to achieve a deep sputum	Sterile container	Rinse mouth
Bronchoalveolar Lavage	Performed by a health professional	Sterile container	Performed by a health professional

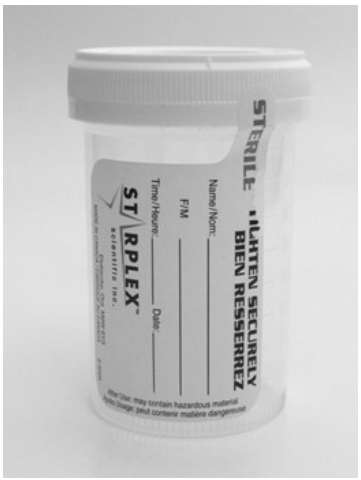
Equipment and Supplies

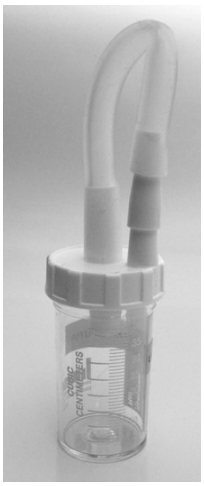
- Alcohol hand rub
- Personal protective equipment (e.g. gloves)
- Swabs
- Culture containers

Before Collecting the Specimen

1. Prepare equipment andsupplies.Container should be labeled
2. Explaintheproceduretothepatientand/ortheir significant other ifpresent.
3. Complete requisition form with type of specimen, time of collection, site of collection, you name (name of person collecting specimen), patient's location, etc.
4. Verifythepatient'sidentificationbyusingtwo patient identifiers perpolicy.
5. Remember to always label the specimen with the required information:
    - Collection Date and Time
    - Patient Identification (Name, DOB, etc.)
    - Collection Method. The requisition must be filled out and the exact source of the specimen indicated e.g. "Bronchial Wash".
    - Test required
6. Wash hands thoroughly with soap and water and dry the hands with a paper towel.
7. Follow Standard Precautions for all patients. Use appropriate protective equipment such asgloves, masks, and/or faceshields

Figure 8: Types of specimen containers used for collection and transport of respiratory culture specimen.



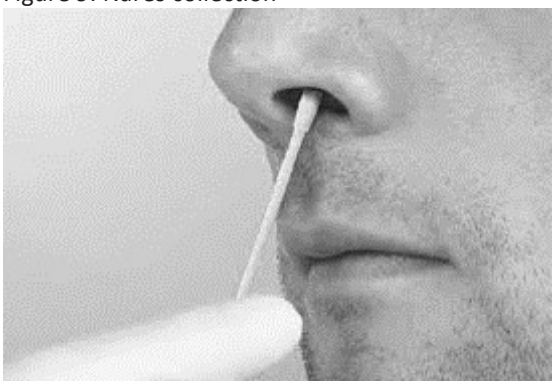


Nares Specimen Collection

Nares swabs are only acceptable for MSSA/MRSA surveillance, not routineculture.

1. Wash hands thoroughly with soap and water and dry the hands with a paper towel. Wear gloves.
2. Graspstheswabcapwithfingers.Becarefulto avoid contacting the swab or stick with your fingers.
3. Withdraw the swab; sweep around the interior surface of the anterior nares. (Do both sideswith oneswab.)
4. Carefullyplaceswabincollectioncontainerand snap off shaft of swab. Make sure the cap is securelyfastened.
5. Label the tube with the patient's name,specimen or specimen bar-code (nares culture) anddate.
6. Send to microbiology lab with a requisitionslip.
7. Remove Gloves and Wash hands thoroughly.

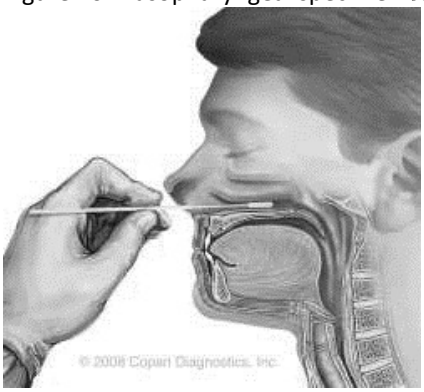
Figure 9: Nares collection



Nasopharyngeal Specimen Collection

1. Wash hands thoroughly with soap and water and dry the hands with a paper towel. Wear gloves.
2. Peel open the pouch containing the collection swab and remove the swab.
3. Holding the swab near the patient's head, visualize the distance fromthepatient'snostriltothefrontoftheear.
4. Use the thumb and forefinger of a gloved handto grip the swab shaft at a point equivalent to half the distance measured in Step 3. This distance approximates the mid-inferior turbinatesampling site.
5. Tilt the head of the patient backwards slightly. Have the patient close their eyes as this helps minimize discomfort.
6. Gently insert the swab throughoneofthenostrilsandhorizontallyinto the nasal passage up to the measured distance on the swab shaft or until resistance is met.
7. Rotate the swab 2 or 3 times and then hold the swab in place for 5-10 seconds to absorb the samplematerial.Remove the swab and insert into the Transport Medium Tube. Break the plastic shaft swab at the break point line. Replace cap and screw on tightly.
8. Apply label.
9. Place in biohazardtransport bag and send tolab.
10. Remove Gloves. Wash hands thoroughly

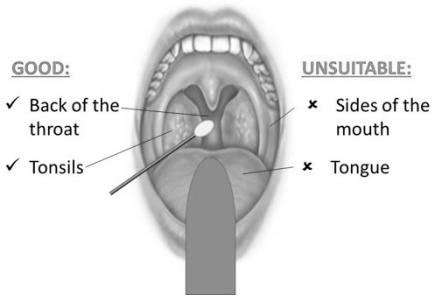
Figure 10: Nasopharyngeal specimen collection



Throat Specimen Collection

1. Wash hands thoroughly with soap and water and dry the hands with a paper towel. Wear gloves.
2. Useacotton,dacron(forviralculture),calcium alginate swab, or eSwab forcollection.
3. Forthroat,useatonguebladeandagoodlight source to ensure goodvisualization.
4. Reach behind the uvula and swab. Swab theback of the throat and tonsils, not the sides of the mouth ortongue.
    - both tonsillarfauces
    - the posteriorpharynx
    - anyulceration,exudate,lesion,orarea of inflammation
5. Place the swab into the appropriatetransport media and transport at ambienttemperature.
6. Remove Gloves. Wash hands thoroughly

S. pyogenes is a common agent of bacterial pharyngitis,followedbyGroupCor*GStreptococcus, Arcanobacteriumhaemolyticum, Neisseria gonorrhoeae, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Corynebacterium diphtheriae* and *Bordetella pertussis*. While *S.pyogenes*, Group C or *G Streptococcus*, *Arcanobacteriumhaemolyticum*and *Corynebacterium diphtheriae* can be collected with swabs, to increase recovery of *N. gonorrhea* or *B. pertussis*, specimen should be directlyinoculated in culture media. For *Chlamydophilapneumoniae* and *Mycoplasma pneumoniae* place specimen in



transportmedia.

Figure 11. Collection of throat specimen from patient.

Lower Respiratory Tract Specimen Collection

Sputum, tracheal aspirate, bronchoalveolar wash, bronchoalveolar lavage are collected to assessinfection. Expectoratedsputumisacceptableforbacterial, mycobacterial, and fungal cultures. Not acceptable for viralcultures.

Lower respiratory specimens should be collected in sterile, leak proof, disposable containers.Do not use waxed containers, they may result in false positive smears. Avoid contamination with tap water or other fluid which may contain environmental bacteria.

Refrigerate the collected specimen if transport time is >1h. Never use swabs for the collection of lower respiratory specimens.

Sputum Collection

1. Instruct the patientto:
    - Rinse mouth with tap water to remove food particles
    - Breathe deeply (in and out 3 times) and cough several times to achieve a deep specimen
    - Expectorate into dry, sterile container
2. Transport immediately at ambient temperature. Refrigerate if a delay of more than one hour is anticipated.

Tuberculosis patients should expect or ate sputum in the early morning, into a sterile container with lid sealed tightly.Patients with clinical and chest x-ray findings compatible with Tuberculosis should collect 3 first morning sputum specimens (on 3 separate days) for acid-fast bacilli (AFB) culture.

Figure 12. How to collect a sputum sample.

Bronchial Brush/Wash/Lavage

Collection of bronchial brush/wash/lavage is a medical procedure in which a bronchoscope is passed through the mouth or nose into the lungs and fluid is squirted into a small part of the lung and then collected for examination. This technique is performed by experienced individuals. Usually only performed in immunosuppressed patients. Samples shouldbecollectedinasterilecontainerandkeepat ambienttemperature.

Note: bronchial wash/lavage collection is an invasive procedure that may require skilled personnel or respiratory technologist to collect the sample.

Summary

The role of the health worker in collecting, labeling, and ensuring the timely and proper delivery of specimens to the laboratory is very important in the hospital setting.

With this, the health worker should be knowledgeable enough about the hospital's policy and procedures for specimen collection. However, they should not only possess the right knowledge, but as well as the skill and understanding in performing necessary procedures in accordance with the organization's protocols, policies, and guidelines.

References

Clinical Microbiology Procedures Handbook. 2nd Edition. HD Isenberg ed. ASM. Cumitechs. ASM Press. Wash. DC.

Manual of Clinical Microbiology,10 Edition. ASM Press. Wash. DC. 2011.Miller MJ.

A Guide To Specimen Management in Clinical Microbiology. ASM Press. Wash. DC. 1999.

AMR Surveillance in Ethiopia Protocols (2017- 2019)

Bailey & Scott's Diagnostic Microbiology. Mosby, Inc. St Louis, Missouri, USA, 13th edition, 2013

IDSA guidelines. CID 2013:57 (15 August). Baron et al

Ohio State University Wexner Medical Center Specimen Collection Guidelines

Johns Hopkins Medical Microbiology Specimen Collection Guidelines

Check-In Questions

1. Before collecting a sputum sample, whatstep must be takenfirst?

Answer: Patient must rinse mouth first.

2. What are special considerations to keep in mindif pneumonia is suspected in thepatient?

Answer: Blood cultures that accompany sputum specimens may occasionally be helpful, particularly in high risk community acquired pneumonia patients. Blood, not sputum, may be specimen of choice for diagnosing bacterial pneumonia

3. Identifythedifferenttypesofrespiratorytract specimens.

Answer: Nose detection of Methicillin-resistant Staphylococcus aureus

Nasopharyngeal swabs - diagnosis of Bordetella pertussis,

Nasopharyngeal swabs and washings diagnosis of viral disease,

Throat swab

4. Which organisms require to be introduced into (inoculated) onto culture media orspecial transportmedium?

Answer: N. gonorrhea or B. pertussis, specimen should be directly inoculated in culture media. For Chlamydophila pneumoniae and Mycoplasma pneumoniae place specimen in transport media

Demonstration and Teach-Back Exercises

Objective:
Participants will learn that:

- Collection of clinical specimens is critical for making the appropriate diagnosis of infection.
- Correct collection of clinical specimens leads to appropriate selection of antimicrobials.
- Correct collection of clinical specimens prevents contamination of specimens that leads to incorrect prescribing of antibiotics.

Time: approximately 30 minutes

Materials:
Refer to the materials sections in each module (e.g. materials and supplies listed, such as gloves). Materials need to be available based on the number of participants.
Provide each participant with a paper copy of the graphic illustrated instructions (translated into Amharic) for each type of specimen collection. The participants are instructed to keep ready access to their copy of their graphic illustration instruction sheets for quick review when they are called upon to actually collect a clinical specimen. They can take the copy with them to use as guidance to follow when collecting specimens to be certain that all steps are conducted properly.

Instructions for the Trainer
The training for each collection specimen will be conducted in three phases.
Phase One is the didactic phase where the instructor outlines the steps of each specimen collection as illustrated graphically in each of the modules.

- Each participant will have a paper copy of the illustrated instructions (translated into Amharic) for each type of specimen collection.
- Participants are instructed to follow along as each step is explained.
- Give participants an opportunity to ask questions regarding each individual step.

Phase Two is role playing by two participants with all other participants observing and critiquing.

- The instructor will select two participants to provide this role play; one will be the patient and the other the specimen collector.
- Using the materials provided, they will demonstrate each step on the graphic illustration instruction sheet short of actually collecting the specimen. For steps that they cannot actually perform (blood collection, urine collection, stool collection, etc.) they can explain what they would do as the specimen collector.
- The other participants will follow each step on their graphic illustration instruction sheet and critique how the role players performed. For example, did they forget a step?

Respiratory Culture Collection Assessment Form

1. A bacterial sputum culture is used to detect and diagnose bacterial lower respiratorytract infections such as bacterial pneumonia or bronchitis. True orFalse?

2. Lower respiratory tract specimen doesnot include:

a. Sputum

b. Throat

c. Trachealaspirate

d. Bronchoalveolarwash

3. Too many epithelial cells indicate upper respiratory specimen contamination. Trueor False?

4. Which of the following statement isfalse?

a. Patient should rinse mouth out with water prior tosputum collection.

b. Collect specimen in a sterile, leakproof, disposablecontainer

c. Bacterial sputum culture does not have tobe refrigerated if collected within 24hours

d. Specimen should be placed into theappropriate transport media and transport at ambient temperature

5. A fresh sputum sample usually collected first thing in the morning is best for culture. True or False?

6. The laboratory should be contacted for specific instructions prior to collection of specimens for pathogens such as Bordetella pertussis. True or False?

7. Upper respiratory tract specimen iscollected from:

a. Nasopharyngeal swabs andwashings

b. Bronchoalveolarlavage

c. Bronchial wash

d. Sputum

8. Which is considered a good throatspecimen collection:

a. Swabs from the sides of the mouth andtongue

b. Swabs from the back of the throat andtonsils

c. Swabs from the tongue andthroat

d. All of theabove

9. Wax specimen containers may result infalse positive smearsfor acid-fast bacilli (AFB).True or False?

Instructions for Use

Trainers can use the assessment questions to assess participants' knowledge gained from the Respiratory Culture Collection Module. Assessment should be conducted at the end of each module. Each question is worth 2 points. A score of 90% or above is passing. If participant does not pass, a thorough review of the module is required. Trainers should conduct a second assessment as needed.

Answer Key: Respiratory Culture Collection Assessment Form

1. A bacterial sputum culture is used to detect and diagnose bacterial lower respiratorytract infections such as bacterial pneumonia or bronchitis. True orFalse?

Answer = True

2. Lower respiratory tract specimen doesnot include:

a. Sputum

b. Throat

c. Trachealaspirate

d. Bronchoalveolarwash

Answer = B

3. Too many epithelial cells indicate upper respiratory specimen contamination. Trueor False?

Answer = True

4. Which of the following statement isfalse?

a. Patient should rinse mouth out with water prior tocollection.

b. Collect specimen in a sterile, leakproof, disposablecontainer

c. Bacterial sputum culture does not have tobe refrigerated if collected within 24 hours

d. Specimen should be placed into theappropriate transport media and transport at ambient temperature

Answer = C

5. A fresh sputum sample usually collected first thing in the morning is best for culture. Trueor False?

Answer = True

6. The laboratory should be contacted for specific instructions prior to collection of specimensfor pathogens such as Bordetella pertussis. True or False?

Answer = True

7. Upper respiratory tract specimen iscollected from:

a. Nasopharyngeal swabs andwashings

b. Bronchoalveolarlavage

c. Throat

d. Sputum

Answer = A

8. Which is considered a good throatspecimen collection:

a. Swabs from the sides of the mouth andtongue

b. Swabs from the back of the throat andtonsils

c. Swabs from the tongue andthroat

d. All of theabove

Answer = B

9. Wax specimen containers may result infalse positive smears. True or False?

Answer = True

Respiratory Culture Competency Checklist:Nasopharyngeal Procedure

Name:
Date:

Site Location:

Evaluator/Instructor's Name:

General Guidelines for Specimen Collection	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Gather all necessary equipment and supplies.	☐	☐	☐
2. Label transport medium tube with participant's information per identification policy and date/time of specimen collection before starting the procedure.	☐	☐	☐
3. Wash hands and put on gloves.	☐	☐	☐
4. Prepare participant by telling them what will be done and give procedure instructions.	☐	☐	☐

Comments:

Nasopharyngeal Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Prepare equipment (i.e., gloves, alcohol wipes, swabs) to obtain sample.	☐	☐	☐

Comments:

2. Wash hands thoroughly with soap and water, dry hands, and put on clean gloves.	☐	☐	☐

Comments:

3. Open pouch containing the collection swab and remove swab.	☐	☐	☐

Comments:

4. Seat the patient comfortably.	☐	☐	☐
a. Patient's head should be tilted backwards slightly for proper specimencollection.			
b. Have the patient close theireyes.			

Comments:

Nasopharyngeal Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
5. Use the thumb and forefinger of a gloved hand to grip the swab shaft. a. Gently insert the swab through one of the nostrils and horizontallyintothenasalpassageuptothemeasured distance on the swab shaft or until resistance ismet b. Rotate the culture swab gently and allow the swabto remain for at least 5-10seconds. c. Remove the culture swab gently. *Comments:*	☐	☐	☐
6. Using the same culture swab, repeat the procedure in the other nostril. *Comments:*	☐	☐	☐
7. Place the swab into the viral transport medium tube. a. Break the plastic shaft swab at the break point line. *Comments:*	☐	☐	☐
8. Replace cap and screw on tightly. *Comments:*	☐	☐	☐
9. Label viral transport medium tube with participant's information per identification policy and date/time of specimen collection. *Comments:*	☐	☐	☐
10. Transport specimen to laboratory promptly. a. Place specimen in a plastic bag with biohazardsign. b. Completedlabrequisitionformplacedontheoutsideof the transportbag. *Comments:*	☐	☐	☐

Recommendation:☐Pass ☐ Needs more practice
Comments:

Instructions for Use: Naso pharyngeal Procedure Competency Checklist

It is the Laboratory Director or Site Coordinator's responsibility to monitor compliance and ensure that competency evaluations are performed on a routine basis. Laboratory personnel must demonstrate competency in accordance with the following schedule:

- Initial competency evaluation upon hiringor learning a newprocedure.
- Six months following the initialcompetency evaluation.
- Annuallythereafter.

Each procedure must be directly observed by an instructor, trainer, nurse, site coordinator, or laboratory director (depending on the site) and evaluatedaccordingtotheperformancecriteria listed on each Competency Checklist. The evaluator must be someone who is trained in the procedure and has demonstrated proficiency.

Evaluation Instructions:

1. Record name of the individual to beevaluated, date, site location, and evaluator'sname.
2. The evaluator, instructor, or site coordinator will directly observe the individual performing each clinicalprocedure.
3. The evaluator will rate procedure criteria evaluated as either: able to perform, able to performwithassistance,orunabletoperform.
4. The evaluator will note additional feedbackunder "Comments".
5. The evaluator will determine if the individual receivedanoverallscoreofPassorNeedsmore practice, and any corrective action or retraining required.
6. Thesitecoordinatororlaboratorydirectorwill maintain the Competency Checklistform.

All samples are to be collected and tested exactly as routine clinical materials. Evaluation of competency samples, as well as proficiency testing samples, must be made by the individual without consultation with other staff. A minimum of two successful competency assessments is required.

Respiratory Culture Competency Checklist: Throat Procedure

Name:
Date:

Site Location:

Evaluator/Instructor's Name:

General Guidelines for Specimen Collection	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Gather all necessary equipment and supplies.	☐	☐	☐
2. Label transport medium tube with participant's information per identification policy and date/time of specimen collection before starting the procedure.	☐	☐	☐
3. Wash hands and put on gloves.	☐	☐	☐
4. Prepare participant by telling them what will be done and give procedure instructions.	☐	☐	☐

Comments:

Throat Procedure	Able to Perform	Able to Perform with Assistance	Unable to Perform
1. Prepare equipment (i.e., gloves, alcohol wipes, swabs) to obtain sample.	☐	☐	☐

Comments:

2. Wash hands thoroughly with soap and water, dry hands, and put on clean gloves.	☐	☐	☐

Comments:

3. Use cotton, Dacron, calcium alginate or eSwab for collection.	☐	☐	☐

Comments:

4. Use a tongue blade and a good light source to ensure visualization.	☐	☐	☐

Comments:

Throat Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
5. Reach behind the uvula and swab: a. Both tonsillarfauces b. The posteriorpharynx c. Any ulceration, exudate, lesion, or area ofinflammation *Comments:*	☐	☐	☐
6. Place the swab into the appropriate transport system. *Comments:*	☐	☐	☐
7. Remove gloves and wash hands thoroughly with soap and water. *Comments:*	☐	☐	☐
8. Transport specimen to laboratory promptly. a. Place specimen in a plastic bag with biohazardsign. b. Completedlabrequisitionformplacedontheoutsideof the transportbag. *Comments:*	☐	☐	☐

Recommendation:☐Pass ☐ Needs morepractice
Comments:

Instructions for Use: Throat Procedure Competency Checklist

It is the Laboratory Director or Site Coordinator's responsibility to monitor compliance and ensure that competency evaluations are performed on a routine basis. Laboratory personnel must demonstrate competency in accordance with the following schedule:

- Initial competency evaluation upon hiringor learning a newprocedure.
- Six months following the initialcompetency evaluation.
- Annuallythereafter.

Each procedure must be directly observed by an instructor, trainer, nurse, site coordinator, or laboratory director (depending on the site) and evaluatedaccordingtotheperformancecriteria listed on each Competency Checklist. The evaluator must be someone who is trained in the procedure and has demonstrated proficiency.

Evaluation Instructions:

1. Record name of the individual to beevaluated, date, site location, and evaluator'sname.
2. The evaluator, instructor, or site coordinator will directly observe the individual performing each clinicalprocedure.
3. The evaluator will rate procedure criteria evaluated as either: able to perform, able to performwithassistance,orunabletoperform.
4. The evaluator will note additional feedbackunder "Comments".
5. The evaluator will determine if the individual receivedanoverallscoreofPassorNeedsmore practice, and any corrective action or retraining required.
6. Thesitecoordinatororlaboratorydirectorwill maintain the Competency Checklistform.

All samples are to be collected and tested exactly as routine clinical materials. Evaluation of competency samples, as well as proficiency testing samples, must be made by the individual without consultation with other staff. A minimum of two successful competency assessments is required.

Respiratory Culture Competency Checklist: Sputum Collection

Name:

Date:

Site Location:

Evaluator/Instructor's Name:

General Guidelines for Specimen Collection	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Gather all necessary equipment and supplies.	☐	☐	☐
2. Label transport medium tube with participant's information per identification policy and date/time of specimen collection before starting the procedure.	☐	☐	☐
3. Prepare participant by telling them what will be done and give procedure instructions.	☐	☐	☐

Comments:

Sputum Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Verbally instruct patient on how to collect sputum culture.	☐	☐	☐

Comments:

2. Ask patient to rinse mouth with water before attempting collection.	☐	☐	☐

Comments:

3. Instruct patient to breathe deeply and cough several times to produce lower respiratory tract specimen (not post nasal fluid).	☐	☐	☐

Comments:

4. Instruct patient to cough or spit into a dry, sterile container.	☐	☐	☐

Comments:

Sputum Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
5. Instruct patient to transport the specimen to the lab immediately or to refrigerate the specimen if there is any delay in transporting the specimen. a. Place specimen in a plastic bag with biohazardsign. b. Completedlabrequisitionformplacedontheoutsideof the transportbag.	☐	☐	☐

Comments:

Recommendation:☐Pass ☐ Needs more practice

Comments:

Instructions for Use: Sputum Collection Competency Checklist

It is the Laboratory Director or Site Coordinator's responsibility to monitor compliance and ensure that competency evaluations are performed on a routine basis. Laboratory personnel must demonstrate competency in accordance with the following schedule:

- Initial competency evaluation upon hiringor learning a newprocedure.
- Six months following the initialcompetency evaluation.
- Annuallythereafter.

Each procedure must be directly observed by an instructor, trainer, nurse, site coordinator, or laboratory director (depending on the site) and evaluatedaccordingtotheperformancecriteria listed on each Competency Checklist. The evaluator must be someone who is trained in the procedure and has demonstrated proficiency.

Evaluation Instructions:

1. Record name of the individual to be evaluated, date, site location, and evaluator'sname.
2. The evaluator, instructor, or site coordinator will directly observe the individual performing each clinical procedure.
3. The evaluator will rate procedure criteria evaluated as either: able to perform, able to perform with assistance,or unable to perform.
4. The evaluator will note additional feed back under "Comments".
5. The evaluator will determine if the individual received an overall scoreof Passor Needs more practice, and any corrective action or retraining required.
6. The site coordinatoror laboratory director will maintain the Competency Check list form.

All samples are to be collected and tested exactly as routine clinical materials. Evaluation of competency samples, as well as proficiency testing samples, must be made by the individual without consultation with other staff. A minimum of two successful competency assessments is required.

Module 6: Stool Culture Collection

Module Preparation

Materials Needed:

- See Checklist for Materials, Equipment and Supplies
- PowerPoint Slides: Module 6
- Alcohol-based hand rub
- Personal Protective Equipment (e.g. gloves)
- Specimen collection containers
- Plastic bag
- Spoon
- Stool Collection Checklist
- Module 6 Assessment Form

Time: approximately 45-60 minutes

Instructions for Trainer:

Ideal class size is 5-25. Classes of 10 or more participants should be sub-divided into groups of 4 or 5 for discussions and activities. Plan for group discussions and check-in questions throughout the module. Allow time at beginning for introductions and pre-assessment, with time at end for demonstration / teach-back exercises and post-assessment. Conduct Pre and post-assessments using the assessment tool provided at end of module on page 111.

Discussion and Check-In Questions:
Check-In and Discussion Questions are provided below. Answers can be found at the end of the module on page110.

- Identify the threshold for transport time in which a stool culture must be refrigerated.
- If a stool culture specimen is contaminated by urine or toilet water, what is the proper protocol to follow?
- How many stool samples may be collected in a 24-hour period?
- Name at least 2 of the 4 acceptable patient identifiers for the container label.

Demonstration and Teach-Back Exercises
Information and instructions for the module activity are provided at the end of the module on page 87.

Topics to Cover:

- Introductions
- Equipment and Supplies
- Stool Collection Procedures
- Summary
- Check-In Questions
- Demonstration and Teach-Back Exercises
- Assessments/Checklists

Objectives

- Specify most common specimencollection procedures: equipment andsupplies.
- Self-collection, practices to collect without contaminationandsamplingofstoolsamples.

Introduction

Stool specimens are collected for culture when patients are exhibiting ongoing symptoms of gastrointestinal infections.Gastrointestinal infections include a wide variety of disease presentations as well as infectiousagents from esophagitis, gastritis, gastroenteritis to proclitic. For many of these infections, particularly non-inflammatory diarrhea and acute gastroenteritis of short duration, no laboratory testing is recommended. However, for the laboratory approach to establishing a gastroenteritis diagnosis, stool culture can be used.

Freshly passed stool should be collected on a sterile clean container. Stool samples should be refrigerated if there are more than two-hour delay in transport.

Stool samples collected on patients hospitalized longer than 3 days prior to collection are not acceptable for routine enteric culture. Only loose or diarrheal stools are recommended for routine bacterial and C. difficile cultures.

Various types of stool collection kits include:

- Commercially prepared kits (different colored vials)
- Kits made in-house for stool collection

Commercially prepared kits consist of different vials depending on the test to be ordered. The vials can have fixative (i.e- formalin, used for ova-parasite exam), Cary- Blair (bacterial stool culture) and without fixatives (multiple uses, including culture, molecular, antigen detection, fecal fat).

Kits made in- house for stool collection should include:

- Sterile container
- Lid
- Label
- Spoon

Collection vessels may include:

- A bed pan
- Clean, unused plastic bag
- Unused plastic wrap placed over a toilet seat.

Do not collect more than one stool specimen in 24 hours unless the specimen was improperly collected (e.g. contaminated with urine or toilet water).

Patients that are self-collecting should be given clear instructions and a visual aid (handout).

See visual aids later in this module for details.

Rectal swabs in transport media are acceptable, but less preferred. Keep in mind that the volume of specimen that can be collected using rectal swabs is limited.

Based on the prevalence of gastrointestinal(GI) pathogens, the laboratory will assess the media to inoculate the stool. Selection of routine and specialized media will depend on patient history, population, geographic area and hospital size.

Some of the organisms that commonly cause gastro intestinal disease are:

- Campylobacter jenuni/coli,
- Salmonella spp,
- Shigella spp,
- E. coli [Shiga toxin screen (EIA or PCR)],
- Yersinia spp,
- Pleisomonas/Aeromonas spp,
- Vibrio spp.

Equipment and Supplies

- Sterile container with lid
- Bed pan/plastic wrap/plastic bag (to collect sample)
- Personal protective equipment (e.g. gloves)
- Label
- Spoon

Before Collecting the Specimen

1. Prepare equipment and supplies.Ensure labels available.
2. Explaintheproceduretothepatientand/ortheir significant other ifpresent.
3. Complete requisition form with type of specimen, time of collection, site of collection, you name (name of person collecting specimen), patient's location, etc.
4. Verify the patient'side notification by using two patient identifiers per policy.
5. Remember to always label the specimen with the required information:
    - Collection Date and Time
    - Patient Identification (Name, DOB, etc.)
    - Collection Method
    - Test required
6. Wash hands thoroughly with soap and water and dry the hands with a paper towel.

7. Follow Standard Precautions for all patients. Use appropriate protective equipment such asgloves, masks, and/or faceshields.

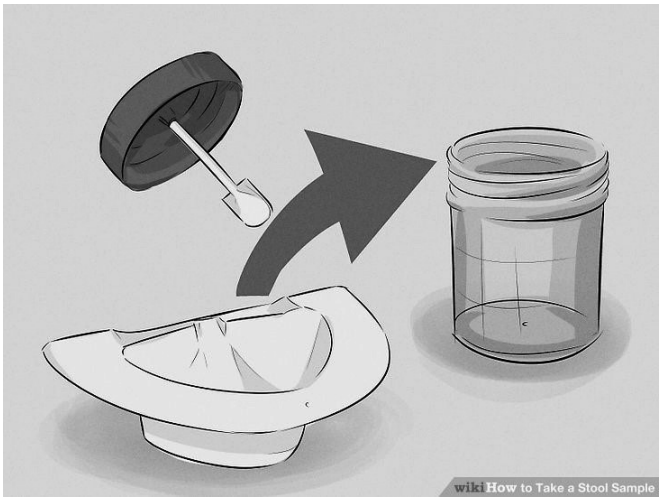
Stool Culture Collection

Remember:If the patientis self-collecting, the healthcare provider should instruct patient on proper collection and how to minimize contamination to specimen and container

1. Wash hands thoroughly with soap and water and dry the hands with a paper towel.
2. Collect specimen in one of the following:
   - A clean bed pan
   - Unused plastic wrap (if available) placed between the toilet seat and thebowl.
   - A clean, un used plastic bag

NOTE: Do not submit feces contaminated with urine or toilet water.

Figure 12. Stool culture collection preparation



3. Transfer the stool to the vial / sterile clean container using the spoon if available; otherwise use another tool thatis clean, fill until the specimen reaches the fillline. Remove the spoon from lid and discard.
   - NOTE: Always place the specimen in an appropriate stool preservative or transport media, immediately after collection. If no transport media is included, add the stool in thesterile container.
4. Replace cap on vial and shake for a minute.
5. Wash hands thoroughly with soap and water and dry the hands with a paper towel.
6. Transport at ambient temperature within two hours ofcollection. If more than 2 hours then place vial in refrigerator until ready to transport .

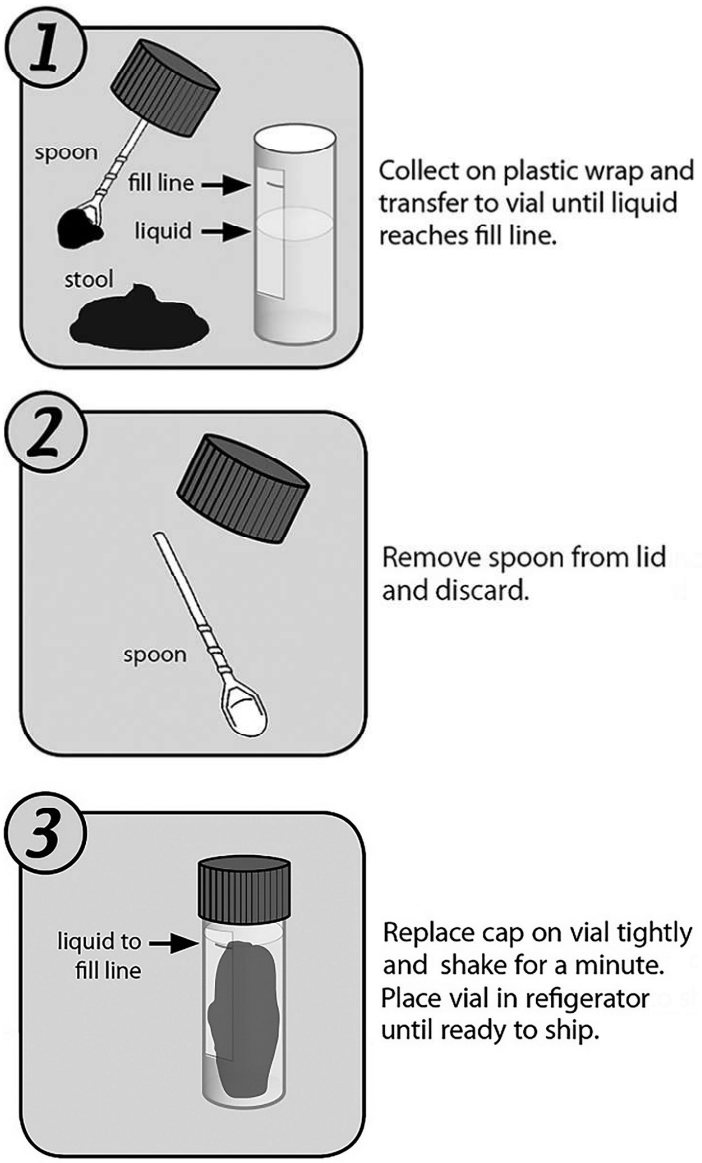


Figure 13. Stool culture collection

If a stool specimen is not available, thefollowing are suitable alternatives for culture (less preferable):

- A swab of rectalmucus
- A rectal swab inserted one inch into the anal canal (not acceptable for Rotavirus/Adenovirus EIA or C. difficiletesting).

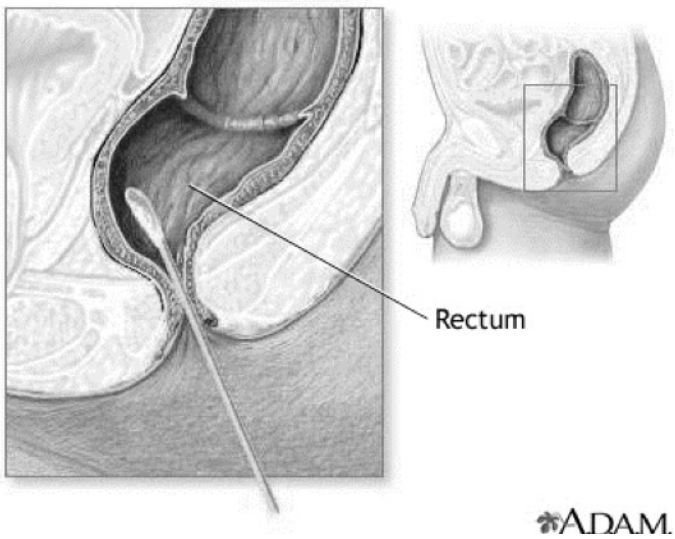
These should be transported to the laboratory at ambient temperature within two hours ofcollectionmust be submitted in fixative. It is not acceptable to send more than one specimen collected on any given day.

Summary

The role of the health worker in collecting, labeling, and ensuring the timely and proper delivery of specimens to the laboratory is very important in the hospital setting.

With this, the health worker should be knowledgeable enough about the hospital's policy and procedures for specimen collection. However, they should not only possess the right knowledge, but as well as the skill and understanding in performing necessary procedures in accordance with the organization's protocols, policies, and guidelines.

Figure 14: Rectal swab procedure



References

Clinical Microbiology Procedures Handbook.2nd Edition. . HD Isenberg ed. ASM. Cumitechs. ASM Press. Wash.DC.

Manual of Clinical Microbiology,10 Edition. ASM Press. Wash. DC. 2011.Miller MJ.

A Guide To Specimen Management in Clinical Microbiology. ASM Press. Wash. DC. 1999.

AMR Surveillance in Ethiopia Protocols (2017-2019)

Bailey & Scott's Diagnostic Microbiology. Mosby, Inc. St Louis, Missouri, USA, 13th edition, 2013

IDSA guidelines. CID 2013:57 (15 August). Baron et al

Ohio State University Wexner Medical Center Specimen Collection Guidelines

Johns Hopkins Medical Microbiology Specimen Collection Guidelines

WikiHow.com/how-to-take-a-stool-sample

Check-In Questions

2. Identify the threshold for transport time inwhich a stool culture must be refrigerated.

Answer: refrigerate specimen if greater than 2-hour delay in transport

3. If a stool culture specimen is contaminated by urine or toilet water, what is the properprotocol tofollow?

Answer: Reject the specimen (discard it appropriately, review proper specimen disposal methods for the typical setting) and collect another clean sample for processing

4. How many stool samples may be collected ina 24-hour period?

Answer: 1 sample in a 24-hour period

5. Name at least 2 of the 4 acceptablepatient identifiers for the containerlabel.

Answer:

- Patient name
- Date ofbirth
- Medical recordnumber
- Government-issued photographidentification

Demonstrations and Teach-Back Exercises

Objective:
Participants will learn that:

- Collection of clinical specimens is critical for making the appropriate diagnosis of infection.
- Correct collection of clinical specimens leads to appropriate selection of antimicrobials.
- Correct collection of clinical specimens prevents contamination of specimens that leads to incorrect prescribing of antibiotics.

Time: approximately 30 minutes

Materials:
Refer to the materials sections in each module (e.g. materials and supplies listed, such as gloves). Materials need to be available based on the number of participants.
Provide each participant with a paper copy of the graphic illustrated instructions (translated into Amharic) for each type of specimen collection. The participants are instructed to keep ready access to their copy of their graphic illustration instruction sheets for quick review when they are called upon to actually collect a clinical specimen. They can take the copy with them to use as guidance to follow when collecting specimens to be certain that all steps are conducted properly.

Instructions for the Trainer
The training for each collection specimen will be conducted in three phases.
Phase One is the didactic phase where the instructor outlines the steps of each specimen collection as illustrated graphically in each of the modules.

- Each participant will have a paper copy of the illustrated instructions (translated into Amharic) for each type of specimen collection.
- Participants are instructed to follow along as each step is explained.
- Give participants an opportunity to ask questions regarding each individual step.

Phase Two is role playing by two participants with all other participants observing and critiquing.

- The instructor will select two participants to provide this role play; one will be the patient and the other the specimen collector.
- Using the materials provided, they will demonstrate each step on the graphic illustration instruction sheet short of actually collecting the specimen. For steps that they cannot actually perform (blood collection, urine collection, stool collection, etc.) they can explain what they would do as the specimen collector.
- The other participants will follow each step on their graphic illustration instruction sheet and critique how the role players performed. For example, did they forget a step?

Stool Culture Collection Assessment Form

1. How many stool samples can be collected withina 24-hour period?

a. 3samples

b. 2samples

c. 1sample

d. As many asneeded

2. Stool that has passed into the toilet or mixedwith urine cannot be used for culture. True orFalse?

3. Fungal cultures of stool are clinically useful.True orFalse?

4. If a stool specimen is not available,suitable alternatives for cultureinclude:

a. A swab of rectalmucus

b. Urinespecimen

c. A swab of thecervix

d. None of theabove

5. Stoolcollectionforova&parasitetestingmustbe:

a. Collected in a sterilecontainer

b. Stool must be placed in the vial within 2 hoursof collection

c. Can remain at room temperature orrefrigerated if not culturingimmediately

d. All of theabove

e. None of theabove

6. Unpreserved stool in a sterile container is required for Norovirus testing. True orFalse?

7. Only hard stools are recommended forroutine bacterial and C. difficile cultures andPCR.True or False?

8. For bacterial culture, transfer stool collected on plastic wrap and transfer using the spoon tovial until liquid reached fill line. True orFalse?

9. Stool cultures can be routinely performedon:

a. outpatients

b. allinpatients

c. inpatients admitted for <3days

d. a andb

e. a andc

10. Lab assesses the patient history, population,geographic area & hospital size todetermine the selective media to inoculate routinely & the specialized media to inoculate onlyupon request. True or False?

Instructions for Use

Trainers can use the assessment questions to assess participants' knowledge gained from the Stool Culture Collection Module. Assessment should be conducted at the end of each module. Each question is worth 2 points. A score of 90% or above is passing. If participant does not pass, a thorough review of the module is required. Trainers should conduct a second assessment as needed.

AnswerKey:StoolCultureCollectionAssessmentForm

1. **How many stool samples can be collected withina 24-hour period?**
   a. 3samples
   b. 2samples
   c. 1 sample
   d. As many asneeded
   Answer = C

2. **Stool that has passed into the toilet or mixedwith urine cannot be used for culture. True orFalse?**
   Answer = True

3. **Fungal cultures of stool are clinically useful.True orFalse?**
   Answer = False

4. **If a stool specimen is not available,suitable alternatives for cultureinclude:**
   a. A swab of rectalmucus
   b. Urinespecimen
   c. A swab of thecervix
   d. None of theabove
   Answer = A

5. **Stool cultures for Ova & Parasite testing mustbe:**
   a. Collected in a sterilecontainer
   b. Stool must be placed in the vial within 2 hoursof collection
   c. Can remain at room temperature orrefrigerated if not culturingimmediately
   d. All of theabove
   e. None of theabove
   Answer = D

6. **Unpreserved stool in a sterile container is required for Norovirus testing. True orFalse?**
   Answer = True

7. **Only hard stools are recommended forroutine bacterial and C. difficile cultures andPCR. True or False?**
   Answer = False

8. **Forbacterialculture,transferstoolcollectedon plastic wrap and transfer using the spoon tovial until liquid reached fill line. True orFalse?**
   Answer = True

9. **Stool cultures can be performedon:**
   a. outpatients
   b. allinpatients
   c. inpatients admitted for <3days
   d. a andb
   e. a and c
   Answer = E

10. **Lab assesses the patient history, population, geographic area & hospital size todetermine the selective media to inoculate routinely & the specialized media to inoculate onlyupon request. True or False?**
    Answer = True

Stool Culture Collection Competency Checklist

Name:

Date:

Site Location:

Evaluator/Instructor's Name:

General Guidelines for Specimen Collection	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Gather all necessary equipment and supplies.	☐	☐	☐
2. Label transport medium tube with participant's information per identification policy and date/time of specimen collection before starting the procedure.	☐	☐	☐
3. Prepare participant by telling them what will be done and give written procedure instructions.	☐	☐	☐
Comments:			

Stool Collection Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
1. Prepare equipment (i.e., specimen cup) to obtain sample.	☐	☐	☐
Comments:			
2. Verbally instruct patient on how to collect stool specimen at home.	☐	☐	☐
Comments:			
3. Instruct patient to to wash hands before and after collecting the specimen so as not to spread infection.	☐	☐	☐
Comments:			
4. Instruct patient to to wash hands before and after collecting the specimen so as not to spread infection.	☐	☐	☐
a. Instruct patient to collect either solid or liquidstool.			
b. Instructpatienttocollectdiarrheainabagandputinto the specimencup.			
Comments:			

Stool Collection Procedure	Able to Perform	Able to Performwith Assistance	Unable to Perform
5. Instruct patient to place the lid on the specimen cup and label it with his/her name and date the stool was collected. a. Instructpatienttodeliverstoolspecimenthesamedaythe stool is collected whenpossible. b. Instruct patient to refrigerate stool specimen if specimen cannot be transported to the lab within 2 hours of collection. c. Instructpatienttowashhandsthoroughlywithsoapand water after stool specimencollection. *Comments:*	☐	☐	☐
6. In the lab: Wash hands thoroughly with soap and water, dry hands, and put on clean gloves. *Comments:*	☐	☐	☐
7. Transfer specimen into a Cary-Blair transport medium container for routine bacterial stool culture or the appropriate container for other tests. *Comments:*	☐	☐	☐
8. Instruct patient to transport the specimen to the lab immediately or to refrigerate the specimen if there is any delayintransportingthespecimen.(Maycombinewith#5) Also suggest adding section for stool collection for HCW following CDC guidelines as listed in https://www.cdc.gov/urdo/downloads/ speccollectionguidelines.pdf page#7. *a. Place specimen in a plastic bag with biohazardsign.* *b. Completed lab requisition form placed on the outsideof the transport bag.* *Comments:*	☐	☐	☐

Recommendation:☐Pass ☐ Needs morepractice
Comments:

Instructions for Use: Stool Culture Collection Procedures Competency Checklist

It is the Laboratory Director or Site Coordinator's responsibility to monitor compliance and ensure that competency evaluations are performed on a routine basis. Laboratory personnel must demonstrate competency in accordance with the following schedule:

- Initial competency evaluation upon hiringor learning a newprocedure.
- Six months following the initialcompetency evaluation.
- Annuallythereafter.

Each procedure must be directly observed by an instructor, trainer, nurse, site coordinator, or laboratory director (depending on the site) and evaluatedaccordingtotheperformancecriteria listed on each Competency Checklist. The evaluator must be someone who is trained in the procedure and has demonstrated proficiency.

Evaluation Instructions:

1. Record name of the individual to beevaluated, date, site location, and evaluator'sname.
2. The evaluator, instructor, or site coordinator will directly observe the individual performing each clinicalprocedure.
3. The evaluator will rate procedure criteria evaluated as either: able to perform, able to performwithassistance,orunabletoperform.
4. The evaluator will note additional feedbackunder "Comments".
5. The evaluator will determine if the individual receivedanoverallscoreofPassorNeedsmore practice, and any corrective action or retraining required.
6. Thesitecoordinatororlaboratorydirectorwill maintain the Competency Checklistform.

All samples are to be collected and tested exactly as routine clinical materials. Evaluation of competency samples, as well as proficiency testing samples, must be made by the individual without consultation with other staff. A minimum of two successful competency assessments is required.

Supplementary Materials

This additional section provides an overview ofantimicrobialresistance(AMR),principlesof Antimicrobial Stewardship, and applicationof the World Health Organization (WHO) Diagnostic Stewardship methods. The content of these slides willnotbereviewedindetailherewiththeexception of some important key points. (CDC to advise if the accompanying slides should be furtherdeveloped.)

The Master Trainers, however, are encouraged to review these slides in depth and consider which portions may be used in their training of participants in the regular specimen collection training sessions.

In addition to the content of the AMR slides, the Master Trainers may also consider reviewing the CDC Antibiotic Resistance Threats PDF or provide this to selected participants. This provides a great overview of targeted resistance patterns. The materials in these supplementary slides can be considered for presentation in their entirety to the Master Trainers to provide the needed background and foundational understanding for why proper specimen collection is critical.

https://www.cdc.gov/drugresistance/threat-report-2013/index.html

Slides and materials on developing antimicrobial stewardshipprogramsarenotreviewedherebut again are provided as backgroundinformation. Effective antimicrobial stewardship programs rely on proper specimen collection and the use of culture results to guide therapeutic decisions.

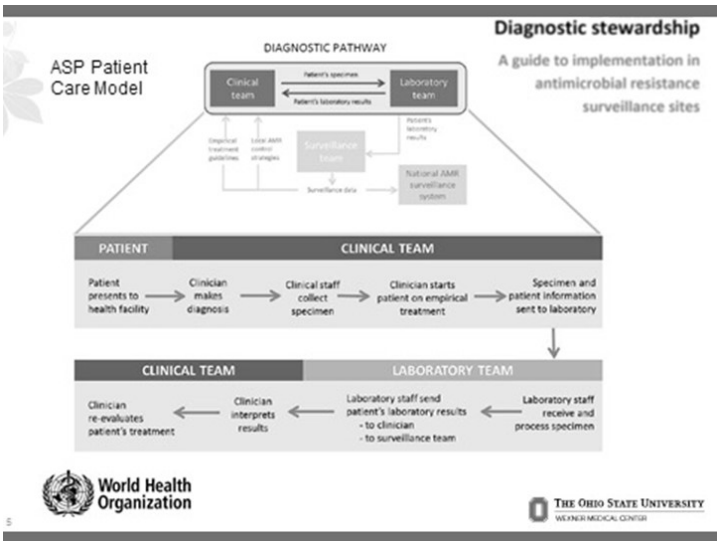
There are multiple key points from supplementary slides that should be continually emphasized during the training.

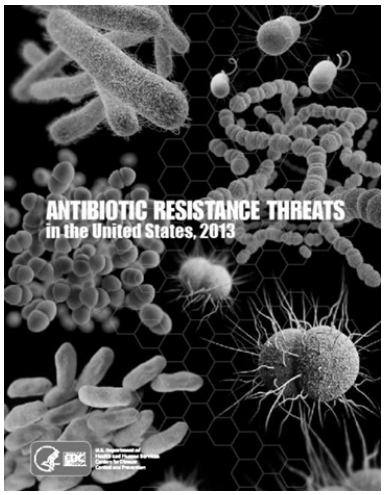
Key point 1 is that antimicrobial resistance has increased to dramatic levels and there are now some organisms that may be susceptible to only one or no antibiotics making treatment very difficult and most often unsuccessful.

Key point 2 is that overuse or indiscriminate use of antibiotics (“antibiotic pressure”) leads to the selection and development of these highly resistant pathogens.

Other key principles to be emphasized during the training are found in slides on WHO Diagnostic Stewardship and will be reviewed in more detail here. Strong consideration should be made to at least utilizing this group of slides as an introductory part of the training.

The typical sequence of events in the evaluation of a patient with an infection and collection of appropriate specimens is found in the WHO Diagnostic Stewardship Model.





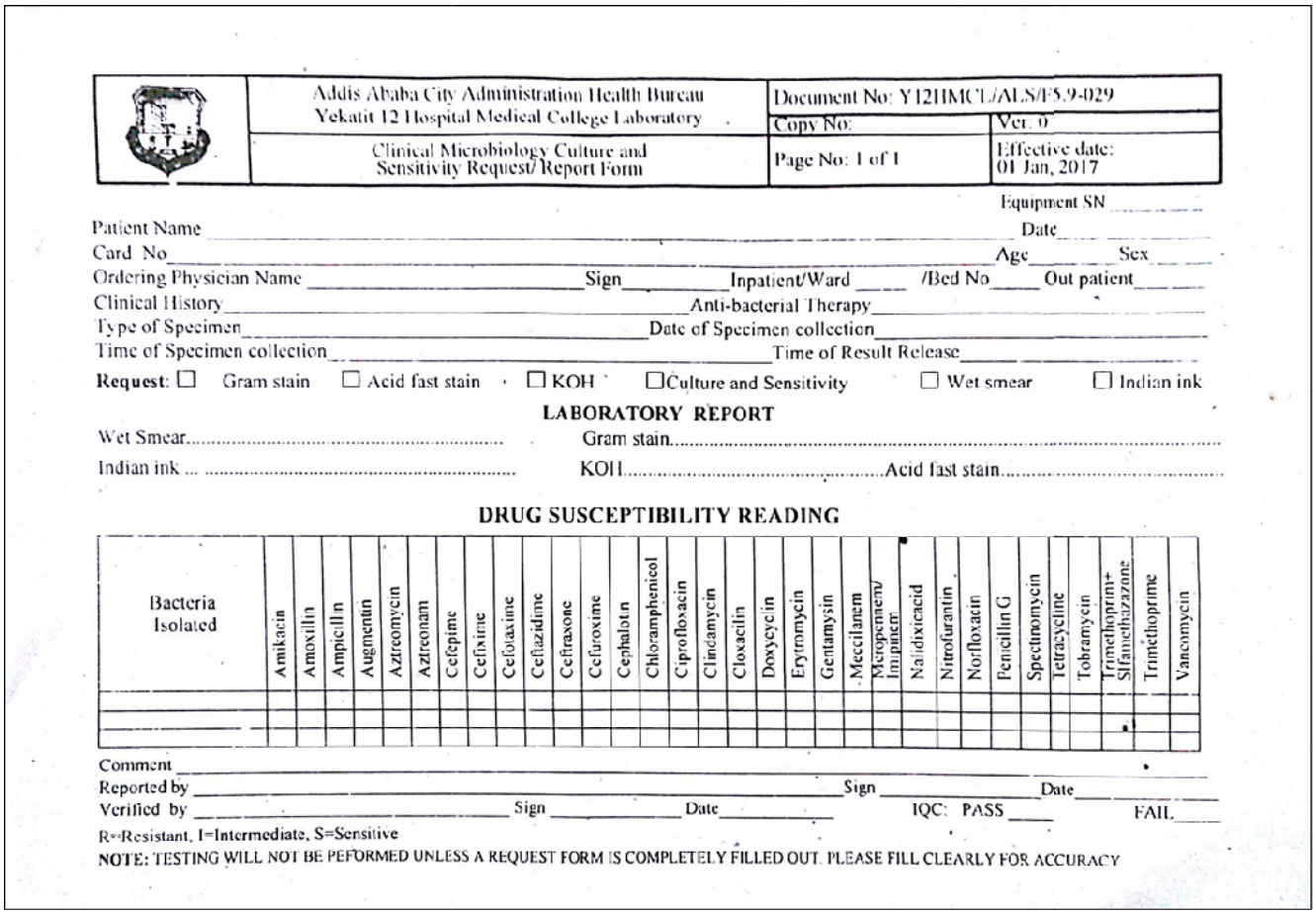
The steps are summarized here and should be emphasized repeatedly during the training sessions as each culture method is taught and reviewed. Educate on the basic principles of antimicrobial stewardship—"culture-driven prescribing":

- Assess patient by clinicians as the patientpresents to the healthcaresystem
- Preliminary diagnosis ismade
- Obtain appropriate cultures based onthe preliminarydiagnosis
- Start empiric antibiotics, typically broadspectrum to cover all the potentialorganisms
- Modify and narrow antibiotics based onculture results
- Only treat for the minimal durationrequired

Thus, **key point 3** is that the main principle of appropriate antibiotic management and stewardship is "culture-driven therapy". Antibiotics are started broadly to cover all of the potential organisms but are narrowed quickly based on the culture results. Failure to obtain appropriate specimens results in no cultures. No cultures mean continued use of broad spectrum drugs that increase antibiotic pressure and increase selection and dissemination of resistance.

Key Point 4 is that appropriate choice and duration of antibiotics can only be determined when the organism causing the infection is known. The etiologic organism can only be determined when appropriate cultures are obtained before antibiotics are started. Be certain to repeatedly emphasize with each training on each type of specimen collection that the cultures should be obtained before antibiotics are everstarted.

Appendix A: Sample Requisition Form, Ethiopia



Addis Ababa City Administration Health Bureau Yekatit 12 Hospital Medical College Laboratory	Document No: Y12HMCL/ALS/F5.9-029	
	Copy No:	Ver. 0
Clinical Microbiology Culture and Sensitivity Request/ Report Form	Page No: 1 of 1	Effective date: 01 Jan, 2017

Equipment SN _____

Patient Name _____ Date_____

Card No_____ Age_____ Sex_____

Ordering Physician Name _____ Sign_____ Inpatient/Ward _____ /Bed No_____ Out patient_____

Clinical History_____ Anti-bacterial Therapy_____

Type of Specimen_____ Date of Specimen collection_____

Time of Specimen collection_____ Time of Result Release_____

Request: ☐ Gram stain ☐ Acid fast stain ☐ KOH ☐ Culture and Sensitivity ☐ Wet smear ☐ Indian ink

LABORATORY REPORT

Wet Smear..... Gram stain.....

Indian ink ..... KOH..... Acid fast stain.....

DRUG SUSCEPTIBILITY READING

Bacteria Isolated	Amikacin	Amoxillin	Ampicillin	Augmentin	Aztreomycin	Aztreonam	Cefepime	Cefixime	Cefotaxime	Ceftazidime	Ceftraxone	Cefuroxime	Cephalotin	Chloramphenicol	Ciprofloxacin	Clindamycin	Cloxacilin	Doxycycin	Erytromycin	Gentamysin	Meccilanem	Meropenem/ Imipinem	Nalidixicacid	Nitrofurantin	Norfloxacin	Penicillin G	Spectinomycin	Tetracycline	Tobramycin	Trimethoprim+ Sfamethazazone	Trimethoprime	Vancomycin

Comment _____

Reported by _____ Sign _____ Date_____

Verified by _____ Sign _____ Date_____ IQC: PASS ____ FAIL____

R=Resistant, I=Intermediate, S=Sensitive

NOTE: TESTING WILL NOT BE PEFORMED UNLESS A REQUEST FORM IS COMPLETELY FILLED OUT. PLEASE FILL CLEARLY FOR ACCURACY

Appendix B: Sample Training Registration Form and Sign-In Sheet

(next page)

ACTIVITY TITLE: Clinical Specimen Collection Training

INSTRUCTOR: _____

DATE(S): _____

VENUE: _____

REGISTERED PARTICIPANTS & SIGN-IN SHEET

No.	Name	Institute Affiliation & Role	Email	Initials Day 1	Initials Day 2	Follow -Up	Follow -Up	Follow -Up
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								
32								
33								
34								
35								

Notes:

Lead Contact/Instructor: _____

Facilitators: _____

Sponsors: _____

Appendix C: PowerPoint Slides and Step-by-Step Collection Instruction Guides for Reference

Please see separate, individual slide decks and collection guidelines for Modules 1-6as available.

List of other contributors

EPHI clinical bacteriology and mycology case team staff

1. Semira Ibrahim, MSc
2. DegefuBeyene, MSc
3. ZelekeAyenew, MSc
4 DejeneShiferaw, MSc
5 MeseretAssefa, MSc
6 Elias siyum, MSc
7 DawitAssefa, BSc
8 . . YonasMekonen, BSc
9 YohannisYitagesu, BSc
10 Tesfa Addis, BSc
11. Mulushewa, BSc
12. . Yibralem,Diploma
13. Ebsa Fekede, Diploma
14. Nega Assamen, PhD candidate
15 Abebe Assefa, PhD candidate
16 AberaAbdeta, MSc candidate
17. EtsehiwotAdamu, MSc candidate

Master trainers

1.Dr. Daniel Yilma
2.Sr. Sara kahassy
3.Sr. HiwotFisshea
4.Dr. Kinfe Lemma
5.Aynalem Mohammed
6.Dr. AyneshetAdane
7.Dr. Zekariasgessew
8.Aregwi Haileslasse
9. Zemeny Workenh
10.Dr. WondossenAmogne
11.Ato TesfayeSeboka
12.Hailay Teklu