

NHRC DEPARTMENT OF RESPIRATORY DISEASES RESEARCH Specimen Collection

1. PURPOSE

This SOP standardizes specimen collection which is essential to our ability to isolate and identify pathogens. Diagnostic and investigational information developed at the Naval Health Research Center (NHRC) Department of Respiratory Diseases Research (DRDR) relies on the integrity of the specimens received.

2. SCOPE

This procedure applies primarily to the NHRC DRDR personnel who collect, store and ship specimens for identification, as well as personnel who support these efforts.

3. GENERAL CONSIDERATIONS

Some of the collection procedures described in Section 6. may not be applicable for every study or need. At times, NHRC DRDR may be involved with new diagnostics which may have unique specimen collection equipment and / or supplies. During such times, additional training and instruction will be given to ensure continuity of quality.

3.1. PPE

Specimen collectors wear appropriate personal protective equipment (PPE) determined by site regulations, Study Coordinator and site Principle Investigator (PI). Collectors should be aware of each site's "Exposure Control Plan."

List of PPE includes:

- Laboratory coat or gown
- Gloves (non-latex); changed and hands washed between subjects
- Laboratory goggles or glasses (optional)
- Face shield or respirator (optional)

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3.1.1. ASEPTIC TECHNIQUES

Aseptic techniques, defined as “a set of specific practices and procedures performed under carefully controlled conditions with the goal of minimizing contamination by pathogens” should be followed during all specimen collections. (Refer to SOP # G-0.322 Aseptic Techniques.)

- Clean bench or table to be used in specimen collection with 10% bleach solution or 70% ethanol prior to each subject and after collection of all specimens. Sterile field or absorbent pads can be used if applicable.
- Transport media and collection tubes using no media must be opened with one hand. Avoid touching rim or inside tube.
- When using swabs for specimen collection, do not let the swab touch any exterior surface or the outside of tube.
- For throat swabs, a tongue depressor can be employed to secure the tongue to ensure the swab touches only areas designated for sampling.
- For blood collection, be sure to use only self-sheathing needles.

3.1.2. UNIVERSAL PRECAUTIONS

Always practice universal precautions when handling subjects or subject specimens. Always wear PPE. (See 3.2.) Use 10% bleach or 70% ethanol to decontaminate surfaces where collection is taking place. (Refer to SOP # S-0.34 BSL2 Response Plan.)

Note: Both 70% ethanol and 10% bleach (sodium hypochlorite) are effective disinfectants. When disinfecting with bleach, wait 10 minutes before proceeding. 70% ethanol is fast acting and is very effective against influenza, HSV I and II and adenovirus.

4. DEFINITIONS/ABBREVIATIONS

BAP:	Blood Agar Plates
C:	(degrees) Celsius
cm:	centimeter

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CRF:	Case Report Forms
CSF:	Cerebrospinal Fluid
EDTA:	Ethylenediaminetetraacetic Acid Buffer
g:	standard gravity
HEPES:	N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid buffer
HIPAA:	Health Insurance Portability and Accountability Act
HSV I and II:	Herpes Simplex Virus (Type I and Type II)
IRB:	Internal Review Board
ITCF:	Infectious Tissue Culture Fluid
L3 and L4:	Spinal lumbar location
ml:	milliliter
Multi-Microbe Media (M4):	(5 ml) Multi-Microbe Media (M4) manufactured by Micro Test, Inc, REMEL, Lenexa, KS, is used in specimen collection for virus isolation to prevent drying, maintain viral viability between collection and inoculation, and to retard bacterial and fungal growth.
NHRC DRDR:	Naval Health Research Center Department of Respiratory Diseases Research
PCR:	Polymerase Chain Reaction
PI:	Principle Investigator
PPE:	Personal Protective Equipment
QA:	Quality Assurance
QC:	Quality Control

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REMEL:	A global provider of a wide range of high quality Microbiology products used by clinical, industrial, research, and academic laboratories.
RUO:	Research Use Only
SP-4 Broth:	<i>Mycoplasma</i> Transport Media: SP-4 is a glucose media manufactured by REMEL and is used in the specimen collection of <i>Mycoplasma pneumoniae</i> .
SOP:	Standard Operating Procedure
SSN:	Social Security Number
SST:	Serum Separator Tube
TE Buffer:	Tris and EDTA Buffer
TSB:	Trypticase Soy Broth with 15% glycerol
VTM:	Viral Transport Media: VTM components are Hank's Balanced Salts, Bovine Serum Albumin, Gelatin, sucrose, L-glutamic acid, Phenol Red (indicator), HEPES buffer, Vancomycin, Amphotericin B and Colistin.
UTM:	Universal Transport Media: UTM consists of Hank's Balanced Salts, Bovine Serum Albumin, L-Cysteine, Gelatin, Sucrose, L-Glutamic Acid, HEPES Buffer, Vancomycin, Amphotericin B, Colistin and Phenol Red. It has a pH of 7.3 +/- 0.2@25°C.

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5. RESPONSIBILITIES

5.1. THE SPECIMEN COLLECTOR

The specimen collector must have the required level of training and expertise to collect specimens as reflected on a signed training certificate for each type of collection procedure, as well as training and understanding of aseptic technique, media QC and equipment QC, if applicable. Once training is complete, a training certificate will be signed by the physician or designee. (Refer to Culture Collection Certification, Form # QA-4.470 and Serum Collection Certification, Form # QA-4.480.) The collector is responsible for sending the completed site personnel training records to the NHRC Safety and Logistics office.

Additionally, the specimen collector is responsible for:

- the collection and labeling of specimens.
- coordinating the shipping of specimens to NHRC DRDR.
- completing and signing Laboratory Request Form # QA-4.40 and Laboratory Specimen Log, Form # 4.50. (Refer to SOP # QA-0.315, Preparation of Laboratory Request Forms.)
- completing and signing forms specific to individual investigational diagnostic studies.
- performing inventory for collection supplies.
- training and understanding emergency procedures for adverse reactions, (see SOP # QA-0.345, Medical Device Adverse Event Reporting), and other subject care and information, including HIPAA.

5.2. THE SHIPPER

The shipper is responsible for ensuring that the proper procedures are followed when packing, labeling and shipping specimens for diagnostic testing. (Refer to SOP # QA-0.309 Biological Substance Category B Specimens: How to Package, Label, and Ship.)

Additionally, the shipper ensures that the NHRC DRDR Shipping and Receiving personnel have been notified of a tracking number when the shipment is sent. (Refer to SOP # G-0.325 Specimen Tracking.)

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5.3. THE STUDY COORDINATOR OR SITE PRINCIPLE INVESTIGATOR (PI)

The Study Coordinator or the site Principle Investigator is responsible for:

- providing this SOP or appropriate guidelines to the study or outbreak sites.
- ensuring that all collectors/shippers at each site are properly trained.
- providing feedback to the sites on the quality of specimens received by NHRC DRDR.

5.4. NHRC DRDR SHIPPING AND RECEIVING PERSONNEL

The NHRC DRDR Shipping and Receiving personnel will provide the necessary collection materials, when appropriate.

5.5. SAFETY AND LOGISTICS AT NHRC DRDR

The Safety and Logistics is responsible for reviewing and updating the NHRC DRDR Specimen Collection SOP annually, or as change in regulation/policy occurs.

Safety and Logistics will ensure that sites have and use PPE as appropriate to each study.

Additionally, the Safety and Logistics will keep copies of the Specimen Collection Training Certificates and Serum Collection Training Certificates.

When shipments arrive, Safety and Logistics evaluate incoming specimens, and will provide feedback directly to the site or Study Coordinator that will include in what condition the shipment appears.

6. PROCEDURE

6.1. SPECIMEN COLLECTION SUPPLIES

Collection supplies will be provided to each collection site when necessary. (Please contact NHRC DRDR Shipping and Receiving personnel on the first Monday of every month to order additional kit supplies. Please order enough supplies for at least a one month period.)

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Specimen collection supplies consist of the following:

- Personal protective equipment, including gloves (non-latex)
- 10% bleach solution, or 70% ethanol, or sterile field or absorbent pads, if applicable
- Transport media and swabs/cultures (if applicable)
- Collection containers (if applicable)
- Shipping materials
- Alcohol prep pads/gauze
- Needles, Vacutainer™ hubs, butterfly sets, and tourniquets, if applicable
- Flashlights and tongue depressors
- Investigational diagnostic kits and/or supplies, as appropriate
- Parafilm® (if applicable)
- Sharps containers
- Coban

6.2. QUALITY CONTROL OF SUPPLY COMPONENTS

Companies developing point-of-care investigational diagnostics may impose their own quality control methods on parts and supply components.

6.2.1. TRANSPORT MEDIA TUBES

Upon receipt of a new shipment box of media, a Transport Media Quality Control Site Worksheet - Worksheet #1, Form # QA-5.13, should be initiated and each tube should be inspected. If more than 15% of tubes in a box fail QC, then the entire box must be discarded.

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6.2.1.1. pH QUALITY CONTROL DETERMINATION

pH QC determination is made by performing the following visual color test:

MEDIA	COLOR	RESULT
VTM	Salmon pink (neutral)	Pass
	Blue violet (alkaline)	Fail
	Yellow (acidic)	Fail
UTM	Light orange-red	Pass
	Other color	Fail

6.2.1.2. CLARITY QUALITY CONTROL DETERMINATION

Clarity QC determination is made by performing the following visual test:

CLARITY	RESULT
Clear	Pass
Cloudy	Fail

6.2.1.3. QC STATUS

If specimen passes on pH and clarity, the overall QC status should be assigned as "pass". Otherwise, it should be assigned "fail".

6.2.1.4. EXPIRATION DATE

Media must be used prior to the expiration date or be discarded.

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6.2.1.5. DOCUMENTING QC RESULTS OF MEDIA AND/OR TUBES

Information is recorded on the correct row of Form # QA-5.13, Transport Media Quality Control Site Worksheet - Worksheet #1. Record the following:

- Media type (VTM, UTM, SP4, TE, Agar, TSB, Other)
- Date received
- Lot #
- Manufacturer
- Quantity received
- Quantity of tubes which fail pH QC
- Quantity of tubes which fail clarity and color QC
- Total tubes which fail QC (discarded)
- Date discarded
- Date new box was inspected for collection use
- Date and initials of site technician

After each new box of media is received and a quality check is performed, a copy of Form # QA-5.13 should be sent back with a specimen shipment to NHRC. The original form is kept in a binder labeled according to study procedures.

One unused tube of media from each box received by the site must be sent back to NHRC DRDR with the next shipment for quality control assessment. This tube should be listed on the NHRC DRDR Specimen Log, Form # QA-4.50 and Laboratory Requisition Form # QA-4.40 as a specimen using the Lot # as the ID for original specimen, (i.e. 123456) and the expiration date as a second identifier. If an ID number is not assigned at the site, the study coordinator will assign one.

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6.2.1.5.1. REORDERING MEDIA

Contact NHRC DRDR for additional media.

6.2.2. UNIVERSAL TRANSPORT MEDIA (UTM) SYSTEM

All lot numbers of UTM media are tested for microbial contamination, toxicity to host cells and the ability to maintain viability of desired agents.

6.2.3. TE BUFFER

Prior to specimen collection, 2 ml cryogenic vials of TE buffer should always be stored at room temperature or refrigerated. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.2.4. TSB WITH 15% GLYCEROL

Prior to specimen collection, 2 ml cryogenic vials or 15 ml plastic vials should always be refrigerated. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.2.5. CULTURETTES

Prior to specimen collection, culturettes⁵ should always be stored at room temperature or refrigerated. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.2.6. SST TUBES

Prior to specimen collection, SST tubes should always be stored at room temperature. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

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6.2.7. AGAR PLATES

Prior to collection, BAP, Chocolate, Jembec, Regan-Lowe agar plates should always be refrigerated. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.2.8. SLANTS

Prior to collection, BAP, chocolate, Stuart and Amies and Regan-Lowe slants should always be refrigerated. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.2.9. SWABS

Swabs must be made of Dacron™ (polyester) material or other sterile material approved for use with human subjects. Wooden or metal swabs should not be used. Check the integrity (i.e., no tears, seal intact) of the swab pouch to ensure sterility. Some swabs have perforated sticks which must be broken off after placing in the tube. Developers of investigational equipment may provide unique swabs to be used with point-of-care diagnostics. These may be approved for use by the study PI and may require IRB approval, as well. Swabs should be stored at room temperature.

6.2.10. STERILE 50 ML TUBES

Prior to specimen collection, ensure that tubes are stored in sealed bag. Do not use tubes if there is glass damage, i.e., cracks, etc.

6.3. QUALITY CONTROL OF SPECIMENS AND ASSAYS

Provided that only freshly collected and properly stored specimens are obtained by approved aseptic collection procedures, none of these media or collection components will interfere with virus/bacteria isolation, serological antibody identification, or PCR testing.

Blank samples should be included with test sets for QC/comparison for assay quality.

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6.4. SUBJECT / STUDY PARTICIPANT DOCUMENTATION

All personal identifiers and clinical information should be recorded on appropriate study documents (e.g., study questionnaire or Case Report Form (CRF) and specimen logs as applicable) prior to specimen collection. In situations in which a study case report form is not required, such as samples collected during special studies, (i.e., clinical trials, outbreaks), an NHRC DRDR requisition form at a minimum is required.

An NHRC DRDR requisition form must be completed for every set of specimens sent to NHRC DRDR. (See Forms # QA-4.40 and 4.50 and refer to SOP # QA-0.315 Preparation of Laboratory Request Forms.) Original subject or study participant personal information will be tracked by assigning an identifier number and a secondary identifier number to each specimen. When assigning identifier number to subject specimens, HIPAA regulations are observed. (See to NHRC IRB HIPAA training <https://net.nhrc.navy.mil/Corporate/training/training.html>.)

Fill out the NHRC DRDR Laboratory Requisition Form # QA-4.40 including the requesting clinician or designee signature (or research assistant as appropriate).

All NHRC DRDR study CRFs, if applicable, must accompany specimens sent to NHRC.

6.5. SPECIMEN COLLECTION TYPES

6.5.1. THROAT SWAB

6.5.1.1. SELECTION

Choose correct transport media and swab based on possible pathogen or study determination.

Transport Media	Tube Type/color	Pathogen
VTM or UTM with Dacron™ swab or similar approved type	3 ml tube media/salmon pink	Microbiology culture and PCR
SP-4 with Dacron™ swab, or similar approved type	1.8 ml tube/silver top	Mycoplasma Culture and PCR
TE Buffer with Dacron™ swab, or similar approved type	1.8 ml tube/pink top	PCR for Virus and bacteria

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Culturette		Bacteria Culture

6.5.1.2. SUPPLIES AND EQUIPMENT

Tongue depressor

Flashlight

Transport media (VTM/UTM kits with swabs)

Dacron™, rayon, nylon or other sterile approved for human subject use swab appropriate to media

Gloves (non-latex)

Refrigerator

Ultra-low freezer

Ice (crushed)

6.5.1.3. METHOD

Request subject/patient to open his/her mouth. If oropharynx is not easily visualized (determined by whether most of the uvula is visualized), employ one or more of the following techniques:

- Instruct subject/patient to say “Ahhh” (making a low pitched open sound, elevating the soft palate and lowering the posterior tongue).
- Instruct subject/patient to visualize his/her own mouth in front of a mirror (if one readily available) and open mouth so that the back is visible.
- Using aseptic technique, hold down the tongue with a sterile tongue depressor to prevent it from contaminating the swab with saliva.

Rub the sterile swab 3 times across each tonsillar fossa (e.g., with a down-up-down motion) while twirling the swab in your hand, followed by making a complete circular motion with the swab tip against the posterior pharynx. Make sure to swab any areas that are inflamed or ulcerated. (See Attachment 7.1.)

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Do not touch cheeks, teeth, gums, or saliva with swab as you withdraw from the mouth.

Place swab in transport media tube.

If all 3 areas (both tonsillar fossae and the oropharynx) cannot be swabbed due to inability to visualize the area or due to subject/patient gagging or non-cooperation, but at least one area could be swabbed, document this on the Laboratory Specimen Log (Form # QA-4.50). Likewise, document if the swab touched cheeks, teeth, gums, saliva, or anything else prior to placing it in the transport media. In both cases the specimen will still be accepted for testing.

6.5.1.3.1. SPECIMENS IN TRANSPORT MEDIA

For specimens requiring VTM or UTM, keep swab inside the tube and break off the shaft of the swab to fit into transport media. Specimen must have swab inside tube for acceptance at NHRC DRDR. (Follow sponsor-provided diagnostic kit instructions for collection during clinical trial phase and/or store according to study protocol or outbreak investigation procedures.)

6.5.1.3.2. CULTURETTE SPECIMENS

For culturette specimens, return swab to transport shaft and break moisture packet at the base. For investigative diagnostic kits which don't involve media, the collection method based on the study protocol will be used. Follow manufacturer's recommendations for temperature and storage.

6.5.1.3.3. SP-4, TE BUFFER AND TSB SPECIMENS

For SP-4 and TE buffer specimens, place swab inside transport media and agitate swab inside the media, express the media out of the swab, discard swab into a biohazard sharps container. (Note: to express the media out, firmly roll swab in the inner transport media container of the media several times).

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TSB, SP-4, and TE specimens should be kept cold immediately after collection (e.g. on crushed ice) until they can be placed in the storage freezer ultra-low or according to study-specific procedures. These specimens should only remain on ice for a maximum of 60 minutes.

Store specimen in an ultra-low freezer at for at least 24 hours until ready to ship to the NHRC DRDR. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.5.2. NASOPHARYNGEAL SWAB

6.5.2.1. SELECTION

Sterile Dacron™ or other polyester, or swabs of similar materials with flexible shafts and transport media or container appropriate for the study and pathogen must be used. Two swabs should be used if specimens are to be collected from both nostrils. Both swabs should be placed into the same transport tube.

6.5.2.2. SUPPLIES AND EQUIPMENT

Nasal speculum

Nasopharyngeal swab (Rayon, Dacron™ or other polyester or similar material) or appropriate transport media or container

Gloves (non-latex)

Refrigerator

Ultra-low freezer

Ice (crushed)

6.5.2.3. METHOD

Insert nasal speculum, if applicable.

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Using the swab as a guide, measure the distance from the subject's nostril to their ear. Mark the distance on the swab using your thumb and forefinger.

Gently insert the swab into the nostril until the thumb and forefinger touch the subject's nose (it will help to gently rotate the swab as it is inserted).

Hold the swab in place for 2-3 seconds then gently rotate the swab as you slowly remove it.

Place the swab in transport media tube or container and cut the shaft so that the swab fits into the tube.

6.5.2.3.1. UTM, VTM, SP-4 AND TE SPECIMENS

UTM, VTM, SP-4, and TE specimens should be kept cold immediately after collection (e.g. on crushed ice) until they can be placed in the ultra-low storage freezer or according to study-specific procedures. These specimens should only remain on ice for a maximum of 60 minutes.

If specimens are required to be frozen, store specimen in an ultra-low freezer for at least 24 hours to ensure the specimen is completely frozen prior to shipping it to NHRC DRDR. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.5.3. NASAL SWAB

6.5.3.1. SELECTION

Sterile Dacron™ polyester or other polyester; rayon, (or materials approved for use with human subjects), with flexible plastic shafts and transport media or containers appropriate for the study and pathogen must be used. Two swabs should be used if specimens are to be collected from both nostrils. Both swabs should be placed into the same transport tube or container.

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6.5.3.2. SUPPLIES AND EQUIPMENT

Nasal speculum (if applicable)

Nasal swab (Dacron™ polyester, or other polyester, rayon or materials for use with human subjects) or appropriate transport media containers

Refrigerator

Ultra-low freezer

Ice (crushed)

Dry ice (refer to appropriate shipping SOP for further instructions.)

6.5.3.3. METHOD

If only one nostril is to be sampled, choose the nostril with the most secretions or the nostril dictated by the study (consult study requirements). Carefully insert a sterile swab into nostril 1-2 cm (until the fibrous portion is no longer visible and touching mucosal surface). Without advancing or retracting swab, twirl it between fingers as you sweep across the septum and floor of nasal cavity 3 times. If both nostrils are to be sampled, consult study coordinator or protocol for guidance regarding number of swabs and media tubes to use.

Place the swab in transport media tube or container and break or cut the shaft so that the swab fits into the tube.

6.5.3.3.1. VTM, UTM, SP-4, TE, TSB SPECIMENS

VTM, UTM, SP-4, TE, TSB specimens should be kept cold immediately after collection (e.g. on crushed ice) until they can be placed in the ultra-low storage freezer or according to study-specific procedures. These specimens should only remain on ice for a maximum of 60 minutes.

If specimens are required to be frozen, store specimen in an ultra-low freezer for at least 24 hours to ensure the specimen is completely frozen prior to shipping it to NHRC DRDR.

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6.5.4. THROAT AND NASAL COMBINATION SPECIMEN

A throat and nasal specimen are collected separately according to sections 6.5.1. and 6.5.3. The swabs are placed into the same transport media vial and processed according to study-specific procedures.

6.5.5. NASAL WASH PROCEDURE

6.5.5.1. MATERIALS/SUPPLIES

PPE (see section 3.1.)

Facial tissue

Sterile nonbacteriostatic saline (eg. bullets)

Sealable biohazard bags

Closable sterile specimen container (eg. sterile urine cup)

Shipping container with cold pack (off-site clinics or outside clients)

Syringe method:

- 3-5 ml sterile disposable syringe
- Sterile soft catheter, eg. # 8 soft French feeding tube. Base syringe size and tubing length on age and size of patient.
- Bulb method: 1-2 oz tapered tip sterile rubber bulb

6.5.5.2. PROCEDURE PREPARATION

All patients should be informed about the nature of the procedure and the risks associated with it: nasal discomfort, mild bleeding, and brief drowning sensation. Execute procedure quickly, as patient will be unable to breathe effectively until fluid is removed from nasal cavity.

6.5.5.3. NON-ASPIRATION METHOD (ADULT AND OLDER CHILD)

Position patient comfortably in sitting position, neck slightly hyper-extended

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- While wearing appropriate PPE, have the patient blow their nose.
- Draw 3 - 5 ml of sterile saline (depending on patient size) into a sterile syringe, then attach sterile soft catheter. Catheter is optional, but provides more effective distribution of saline to the posterior nasal cavity.
- Tuck a piece of absorbent material into shirt to act as a bib. Position patient comfortably in sitting position, neck slightly hyper-extended, and instruct patient to close back of throat by making a “K” sound so that he/she is unable to inhale through nose. Instruct patient not to swallow during the procedure.
- Inject the whole volume of saline quickly (but not forcefully) into one nostril while holding the other closed. If possible, have the patient retain the saline for a few seconds.
- Place specimen container directly under the nose with slight pressure on the upper lip.
- Tilt the head forward and allow the fluid to flow into the specimen container.
- Repeat procedure on other nostril, collecting fluid into the same container. You may use the same syringe and catheter.
- Offer the patient tissues.

Label specimen container with Patient ID, while in the presence of the patient.

6.5.5.4. ASPIRATION METHOD

6.5.5.4.1. ADULT AND OLDER CHILD

Don PPE. Wipe excess mucus from patient’s nose with facial tissue.

Fill syringe with 3-5 ml saline. Amount depends on size and age of patient. Attach tubing to syringe tip.

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Tilt patient's head back slightly beyond "sniff position." Instruct older patients to make a "K" sound so that he/she is unable to inhale through nose, and not to swallow during the procedure.

Instill saline into one nostril while holding second nostril closed.

Aspirate fluid gently while withdrawing and rotating tube to dislodge cells and secretions. NOTE: Recovery must occur rapidly or instilled fluid will rapidly drain down the throat.

Inject specimen into the container. Repeat with other nostril and inject specimen into same container. You may use the same syringe and catheter.

Offer the patient tissues.

6.5.5.4.2. INFANT AND YOUNGER CHILD

Sit child on parent's lap facing forward, with the child's back against the parent's chest. The parent should wrap one arm around the child in a manner that will restrain the child's body and arms.

While wearing appropriate PPE, wipe patient's nose with tissue.

Fill bulb syringe with 1-3 ml of sterile saline (depending on patient size).

Tilt head back and instill entire volume of saline into one nostril.

Immediately release the pressure on the bulb to aspirate the specimen back into the bulb.

Transfer the specimen into specimen container.

Repeat procedure on other nostril, transferring second specimen into the same specimen container.

Offer tissues as appropriate.

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6.5.5.5. PROCESSING OF SPECIMEN

Label specimen container with Patient ID, collector's initials and date while in the presence of the patient. Place container in sealed biohazard bag. Discard syringe and tubing into biohazard waste. Place specimen on ice or in refrigerator within 20 minutes of collection.

6.5.5.6. STORAGE

Consult study protocol for the appropriate storage and shipping methods. In general, storage at 2-8°C is appropriate if testing will commence within 72 hours of collection. If testing will be delayed beyond 72 hours, store in ultra-low freezer. Storage at -20°C is not appropriate.

6.5.6. SPUTUM (FOR RESEARCH ONLY)

6.5.6.1. SELECTION

Lower respiratory tract secretions from infected subjects are confirmed by noting the presence of large number of leukocytes in the absence of epithelial cells. Epithelial cells in the specimen signal gross contamination. The first early morning specimen is preferred.

6.5.6.2. SUPPLIES AND EQUIPMENT

Sterile screw-cap sputum collection cup or sterile 50 ml tubes

Gloves (non-latex)

Refrigerator

Parafilm[®]

6.5.6.3. METHOD

Have subject rinse out mouth with water.

Have the subject create a deep cough.

Collect sputum from cough directly into the sterile container.

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Secure cap and seal with Parafilm®.

Temporarily store in refrigerator until shipment to NHRC DRDR. (For longer storage, store in an ultra-low freezer). Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.5.7. RECTAL SWAB (RUO)

6.5.7.1. SELECTION

Swabs are submitted for culture of enteric pathogens or to assess gastrointestinal shedding of non-enteric pathogens.

6.5.7.2. SUPPLIES AND EQUIPMENT

Transport media

Dacron™ swab

Gloves (non-latex)

Ultra-low freezer

Ice (crushed)

6.5.7.3. METHOD

Use sterile Dacron™ swab.

Gently insert the swab 4-6 cm into rectum, rotate the swab against the mucosa one complete circle, remove and place in transport media tube.

6.5.7.3.1. UTM, VTM, SP-4 AND TE SPECIMENS

UTM, VTM, SP-4, and TE specimens should be kept cold immediately after collection (i.e. crushed ice) until they can be placed in the ultra-low storage freezer or according to study-specific procedures. These specimens should only remain on ice for maximum of 60 minutes.

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If specimens are required to be frozen, store specimen in an ultra-low freezer for at least 24 hours to ensure the specimen is completely frozen prior to shipping it to NHRC DRDR.

6.5.8. TISSUE SAMPLES

6.5.8.1. SELECTION

Autopsy cases – Refer to SOP # QA-0.337 Fatal Case Procedures (RUO).

6.5.8.2. SUPPLIES AND EQUIPMENT

Sterile instruments, sterile collection kits

Ultra-low freezer

NHRC DRDR autopsy collection and shipping kit

6.5.8.3. METHODS

Using safety precautions, place small sterile fresh sample of the below six specimens into water-tight primary container sterile 50 ml tube provided or sterile zip-lock bag (with plastic jar container). NHRC DRDR requests at least a specimen from each of the represented areas below, if possible. The kit will allow for six specimens.

- Representative pulmonary parenchyma from right lung
- Representative pulmonary parenchyma from left lung
- Tissue from organ system most affected, at sites demonstrable micro or macro pathology
- Trachea
- Sample of respiratory secretions preferably from lower segmented bronchial
- Other abnormal fluids with or without clear evidence of infection, (abscesses, pulmonary effusions, ascites, etc.)

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Place small tissue samples (10-18 grams) of each specimen above into sterile 50 ml tubes /or sterile plastic bag (with plastic jar container) and freeze in an ultra-low freezer for at least 24 hours to ensure the specimen is completely frozen prior to shipping it to the NHRC DRDR. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.5.9. ISOLATES

6.5.9.1. SELECTION

Growth and Transport media selection is based on the following chart:

Transport/Growth Media	Type of container	Pathogen
TSB w/15% glycerol	Tube	<i>Streptococcus pyogenes</i> <i>Streptococcus pneumoniae</i> <i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i> <i>Bordetella pertussis</i>
Blood Agar Plate or Slant	Plate or Tube	<i>Streptococcus pyogenes</i> <i>Streptococcus pneumoniae</i> <i>Staphylococcus aureus</i>
Chocolate Agar Plate or Slant	Plate or Tube	<i>Haemophilus influenzae</i>
JEMBEC Plate, Stuart Slant, Amies Slant	Plate or Tube	<i>Neisseria meningitidis</i>
Regan-Lowe Slant or plate	Tube	<i>Bordetella pertussis</i>
VTM/UTM Media or frozen (-80°C) ITCF Infectious Tissue Culture Fluid	Tube	For all virus isolates (ITCF)

6.5.9.2. SUPPLIES

Appropriate transport media

Sterile loop, pick, or device

Incubator

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Ultra-low freezer

Parafilm®

6.5.9.3. METHODS

Consult site microbiology laboratory SOPs for detailed instructions on how to isolate cultures.

Re-sub isolate colonies using sterile instrument into fresh media plate or slant.

Incubate plate or slant for 24 hours.

Observe for growth and if present ship to NHRC DRDR.

6.5.9.3.1. PLATES AND SLANTS

Plates and Slants should be sealed with Parafilm® and shipped to NHRC DRDR at room temperature. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.5.9.3.1.1. ALIQUOTS

TSB w/15% glycerol should be aliquotted (if not already done by NHRC DRDR) into 3 plastic cryogenic vials frozen in an ultra-low freezer for at least 24 hours to ensure the specimen is completely frozen prior to shipping.

If no growth is present re-incubate for another 24 hours.

If no growth still exists repeat above steps.

6.5.10. SERUM

6.5.10.1. SELECTION

Serum Separator tubes are used with a Vacutainer™ system to draw blood. All supplies are used within their expiration date and stored per manufacturer's instructions.

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6.5.10.2. SUPPLIES AND EQUIPMENT

Gloves (non-latex)

Tourniquet

Vacutainer hub

Vacutainer needle

Serum Separator Tube (SST or marble top)

Alcohol swabs (70% isopropyl alcohol)

Band-aid® or coban

Centrifuge

Three plastic cryogenic vials

Ultra-low

Freezer

6.5.10.3. METHOD

Before any specimens are drawn, verification of the subject must be performed by asking the subject's name and SSN if applicable.

Collect samples prior to antibiotic therapy. (Note: Pneumoniae Surveillance study – only enroll subjects that were given antibiotics 24 hours or less before collection. If they did receive antibiotics, be sure to note the name and time antibiotics were given on the CRF. Only write in antibiotic names if the antibiotics were BEFORE specimen collection. Do NOT write in names of antibiotics that were prescribed to be taken AFTER specimen collection)

Prepare tube and Vacutainer™ needle assembly.

Apply tourniquet several inches above antecubital vein and find a suitable vein.

Cleanse area with 70% isopropyl alcohol in a circular motion (starting towards the center and moving to the outside).

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Insert needle into the vein at 15 degree angle with bevel side up.

Hold Vacutainer™ needle secure in the vein and carefully ease the SST tube forward into the hub.

When tube is full, release the tourniquet prior to removing tube from the hub.

Withdraw needle and place cotton or Band-Aid® on the site and apply pressure.

Dispose of needle into a biohazard sharps container.

Secure cotton with tape on subject's arm.

Label SST tube.

Allow tubes to clot for 5-10 minutes (at room temp) then place in centrifuge at 1500 g for 10 minutes.

Aliquot serum from SST tube using a sterile pipette into 3 labeled cryogenic vials.

Store specimen in an ultra-low freezer for at least 24 hours to ensure the specimen is completely frozen prior to shipping it to the NHRC Department of Respiratory Diseases Research. Please refer to the Laboratory Temperature Ranges Form # E-5.24 for appropriate storage temperatures.

6.5.10.3.1. ADDITIONAL BLOOD STUDY NOTES AND CAUTIONS

Convalescent serum is drawn 2-4 weeks after acute serum.

Keep specimens stored in an ultra-low freezer or dry ice until shipment to the NHRC DRDR.

Avoid freeze-thaw cycles.

Specimens should be tightly capped and if applicable sealed with Parafilm®.

Pack, label and ship in compliance with SOP # QA-0.309 Biological Substance Category B Specimens: How to Package, Label, and Ship.

NHRC DEPARTMENT OF RESPIRATORY DISEASES RESEARCH Specimen Collection

Upon receipt, specimens must meet acceptance criteria established by the NHRC DRDR. Refer to SOP # QA-0.310 Specimen Receipt and Evaluation for criteria. A QA Challenge Form # QA-1.53 will be completed for specimens that do not meet acceptance criteria.

If it is determined that specimen collection is being performed incorrectly, the study coordinator is to be notified immediately and the deficient site will be notified via email and/or telephone. Any necessary follow-up will be completed by the study coordinator.

All blood draws are performed with a licensed physician or phlebotomist easily located for questions or emergencies.

All those drawing blood are to make every effort to draw a minimal amount of blood. No more than the required amount for the study is to be drawn.

Semi-annual refresher training is provided for all phlebotomists, which includes information on how to care for patients who experience adverse reactions from phlebotomy, including fainting, seizures and injuries.

6.6. LABELING

Identify the subject before collecting the specimen.

All specimens should be properly labeled after collection. The NHRC DRDR can only accept specimens that are properly labeled.

Use a label that is legible and resistant to ultra-low temperature and moisture on specimen tubes or containers.

Specimens should be labeled with the following:

- Primary identifier (unique)
- Secondary identifier
- Other identifiers as applicable to the study
- Date of collection
- Serum samples must also be labeled with the initials of the collector.

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Any mistakes made on labels should be corrected by drawing a single line through the error, initial and date next to it, and writing the corrected information.

6.7. SAFETY

Besides PPE, it is advised to take every safety precaution.

6.7.1. SHARPS

Any contaminated sharp object, if applicable, should be placed in a red biohazard sharps container. Follow collection site guidelines for biohazardous and chemical hazardous waste collection and disposal.

Contaminated non-sharp items should be placed in a red biohazardous bag.

6.7.2. SPECIMEN SPILLS

Cover spill with 10% bleach for 10 minutes.

Cover area with absorbent paper (i.e. paper towels.)

Wearing PPE, clean up the spill and absorbent paper and place in the biohazardous waste red bag.

If sharps are involved in the spill, clean up using a sharps dust pan and broom.

6.8. MONITORING OF EQUIPMENT PERFORMANCE AT THE SITES

6.8.1. REFRIGERATORS AND FREEZERS

Daily temperature recording of refrigerators and ultra-low freezers used to store transport media and specimens are monitored each day by the persons responsible for collection of samples at the sites (Refer Temperature Reading NHRC DRDR SOP # E-0.153).

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The freezer and refrigerator temperatures must be taken each day and recorded on the temperature log developed by the site, or a template can be provided by NHRC DRDR, or downloaded from a suitable logger system.

Once a month the temperature log must be replaced with a new temperature log and the study PI or designee must review the log for accuracy and consistency.

Once everything is correct, the log should be faxed to the NHRC DRDR QA Section, as well as filed on-site in a site binder labeled "Temperature Logs" with dividers for freezers, refrigerators and any other equipment.

When freezer or refrigerator temperature is out-of-range, contact the Study Coordinator and NHRC DRDR QA Specialist at 619.553.8950 or other appropriate number.

6.9. SHIPPING

Refer SOP # QA-0.309 Biological Substance Category B Specimens: How to Package, Label, and Ship.

7. ATTACHMENTS

7.1. THROAT SWAB COLLECTION DIAGRAM

8. REFERENCES

SOP # G-0.322 Aseptic Techniques

SOP # S-0.34 BSL2 Response Plan (See section on Universal Precautions.)

NHRC IRB HIPAA training:

<https://net.nhrc.navy.mil/Corporate/training/training.html>

SOP # QA-0.315 Preparation of Laboratory Request Forms

SOP # QA-0.345 Medical Device Adverse Event Reporting

SOP # QA-0.309 Biological Substance Category B Specimens: How to Package, Label and Ship.

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SOP # G-0.325 Specimen Tracking

SOP # QA-0.310 Specimen Receipt and Evaluation

SOP # QA-0.337 Fatal Case Procedures (RUO)

SOP # E-0.153 Temperature Reading

Form # QA-4.470 Culture Collection Certification

Form # QA-4.480 Serum Collection Certification

Form # QA-4.40 Laboratory Requisition Form

Form # QA-4.50 Laboratory Specimen Log Form

Form # QA-1.53 QA Challenge

Form # QA-5.13 Transport Media Quality Control Site Worksheet –
Worksheet #1

Form # E-5.24 Laboratory Temperature Ranges

A Guide to Specimen Management in Clinical Microbiology. Miller, JM. 2nd
edition. 1999.

Clinical Microbiology Procedures Handbook. Isenberg. H. Volume 3, 2nd
edition. ASM Press 2004. Section 10.4.2.

9. REVISION HISTORY

Version	Brief general description and justification of changes	Effective Date
A	New SOP to combine all collection SOPs	5/17/2006
B	Added section 6.3 for specimen container test interference comments. Updated SOP format to new template.	8/02/07
C	Added new Header: Department changed names Added new Purpose and Scope: Sections needed additional focus. Revised entire main body, including updating NRDL to be NHRC Department of Respiratory Disease Research.	11/08/07

Approved: 11.24.2010

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	<p>Additional definitions: Updated orientation and flow. Updated to reflect Department name change.</p> <p>Updated number system for re-ordering VTM/SP-4 Tubes.</p> <p>QC of Specimens and Assays (6.3): Additional information was needed to ensure document accuracy.</p> <p>Removed diagram from main body and placed as Attachment 7.1. per new SOP format.</p> <p>Detached forms; made them separate documents per new SOP format.</p> <p>Added new References.</p> <p>Changed order of References per new SOP format.</p>	
D	<p>Header: Department name change. Added McDonough. Removed Little; added Ellorin. Removed Faix, added Blair.</p> <p>The following changes have been made to accommodate protocol changes and investigational trials.</p> <p>1. Purpose: Added "and investigational" after diagnostic to accommodate testing of investigational devices.</p> <p>3. General Considerations; added: "At times, NHRC may be involved with new diagnostics which may have unique specimen collection equipment and / or supplies. During such times, additional training and instruction will be given to ensure continuity of quality.</p> <p>3.2. Bulleted section; added: . . ."using no media" (referring to collection tubes.)</p> <p>4. Definitions, added: UTM Added bullet: "completing and signing forms specific to individual investigational diagnostic studies"</p> <p>Universal: Deleted form # 0.960. Added Form # QA-5.13. Renamed QA-0.309. (See Reference section.) Added SOP # QA-0.337 Fatal Case Procedures (RUO)</p> <p>References: Added NHRC Intranet address.</p>	11.29.2009
E	<p>4.: Added BAP, CRF, IRB, QA, PI, and RUO. Deleted KS. Combined L3 and L4. New section title and 'definitions/abbreviations'.</p> <p>5.1.: Change QA section to Safety and Logistics section for sending complete site personnel training records.</p> <p>6.1.: Added following materials: coban and sharps containers.</p> <p>6.5.1.1.: Added color of VTM/UTM media on chart</p> <p>6.5.1.3.3.: Added procedure on how to 'express the media out'.</p> <p>6.5.5.: Added new section – nasal wash procedure. Moved other sub-sections down one number.</p> <p>6.5.6.3.: Switched the first 2 procedures to correct method.</p> <p>6.5.10.3.: Added information on subjects that have taken antibiotics before blood draw per study coordinator for 'Pneumoniae Surveillance' study.</p> <p>6.5.10.3.1.: Added semi-annual refresher training for phlebotomists.</p> <p>Added form # E-5.24 throughout SOP including section 8 – reference section. Deleted actual temperature ranges on SOP and reference form # E-5.24. Change 'freezer' to ultra-low freezer. Changes per Safety and Logistics.</p> <p>Deleted Bordet-Gengou agar media throughout SOP and replaced with Regan-Lowe per Microbiology supervisor.</p> <p>Corrected spelling, grammatical, punctuation and format errors</p>	11.30.2010

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	throughout SOP.	
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7.1. THROAT SWAB COLLECTION DIAGRAM

