Specimen Collection Table of Contents

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Basic Specimen Collection Guidelines …… Page 1

Bordetella pertussis Culture and DFA …… Page 2

Bordetella pertussis PCR …… Page 3

Nasopharyngeal Washings …….. Page 5

Nose …… Page 7

Sputum …… Page 8

Stool …… Page 9

Throat ……... Page 11

Urine …… Page 12

Vomitus …… Page 13

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If you have questions about proper specimen collection, please call NPHL Client Services at 402-559-2440 or toll free at 1-866-290-1406.

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SPECIMEN COLLECTION GUIDELINES

The proper collection of a specimen for culture is the most important step in the recovery of pathogenic organisms responsible for infectious disease. A poorly collected specimen may lead to failure in isolating the causative organism(s) and result in the recovery and subsequent treatment of contaminating organisms.

Basic Concepts for Collection

- Collect the specimen from the actual site of infection, avoiding contamination from adjacent tissues or secretions.
- Collect the specimen at optimal times (for example, early morning sputum for AFB culture).
- Collect a sufficient quantity of material.
- Use appropriate collection devices: sterile, leak-proof specimen containers.
- Use appropriate transport media.
- Whenever possible, collect specimens prior to administration of antibiotic or antiviral medication.
- Properly label the specimen and complete the test request form/process. The source of specimen is required.
- Minimize transport time. Maintain an appropriate environment between collection of specimens and delivery to the laboratory. (If special transport media is required please make sure this obtained prior to specimen collection)
- If appropriate, decontaminate the skin surface prior to collecting specimen.

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Bordetella pertussis Culture and DFA

- Specimen of choice is a nasopharyngeal swab or aspirate. Ideally the specimen is collected and plated at bedside and transported to the lab as soon as possible.
- Use a **calcium alginate or Dacron swab** on a flexible wire handle to collect the specimen.
- Seat the patient comfortably. Tilt the head back.
- If available, insert a nasal speculum. Press the swab through the nares until resistance is met due to contact with the nasopharynx.
- Rotate the swab gently and allow the swab to maintain contact with the nasopharynx for 20-30 seconds or until coughing is induced.
- Plate directly onto Charcoal Blood agar or place swab into charcoal transport medium. Label with the patient's name and identification number. Transport to the lab at ambient temperature.
- Repeat the procedure with the second swab if DFA is ordered. Gently roll the swab onto two double-well fluorescent microscope slides. Label both slides with the patient's name, identification number and the date.
- Transport the slides (placed in a microscope slide mailer) at ambient temperature.

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Pertussis PCR

- Nasopharyngeal wash in sterile container or nasopharyngeal swab
- Collect nasopharyngeal swab with Amies, Charcoal, Dacron or cotton swab. (B. Pertussis culture must be submitted on separate Charcoal swab if ordered.) DO NOT USE swabs made with wooden sticks.
- Swabs are available from NPHL. See following collection procedure.
- Transport to lab dry, refrigerated.

Washing:

- Cut off the distal end of the butterfly catheter (needle and butterfly) catheter extension set so that about 2-3 inches of tubing are left attached to the hub.
- Draw up 2-3 mL of saline into a syringe.
- Attach syringe to hub of butterfly catheter. Purge tubing with saline.
- Put on gloves, gown, mask and eye protection.
- Gently remove excess mucous from patient's nose. (If patient is an adult, ask the patient to gently blow nose. For pediatrics, a bulb syringe may be used to remove excess mucous.)
- Position patient in supine position with the head of bed up 30°. The head should be turned to one side and tilted slightly backward.
- Stabilize the patient's head and gently place the catheter into the nare. Placement should be in the nare (nasal wall), not the nasopharynx. Depending on the size of the patient, this should be about 1-2 cm in adults and 0.5 cm to 1.0 cm in children (0.5 cm in neonates). See Figure 1.

Figure 1

- Instill .5 - 2 mls saline (.5 - 1 mls for infants and children, 1 - 2 mls for adults) into the nare and aspirate back mucous, saline and epithelial cells.
- Repeat this process using the same syringe until the sample is cloudy or appears to hold cellular debris. (If the sample is inadequate, the process may be repeated on the opposite nare, using a second sterile syringe and tubing. Usually one nare is sufficient.)
    - NOTE: There may be some blood streaks in the mucous. This is normal and patients/parents should be told this is expected and will stop in a few minutes.
- Transfer contents of tubing and syringe into transport media using the following process: Depress syringe plunger and express fluid from syringe and tubing into transport media. Then withdraw media/fluid back into syringe and tubing. Depress syringe plunger again, expressing fluid from syringe and tubing back into transport media.
    - NOTE: This is necessary to recover any cells adhering to the tubing or syringe.

Nasal Swab:

- Insert a mini-tip culturette swab into the same nares from which the wash was performed approximately 3 cm and gently rub the mucosa.
- Place the swab back into transport media containing aspirated material.
- See Figure 2.
- NOTE: Combining the aspirate and the swab enhances recovery and reduces cost to the patient.
- Deliver entire specimen to the lab immediately.

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Nasopharyngeal Washings/Swab (virus only)

Washing:

- Cut off the distal end of the butterfly catheter (needle and butterfly) catheter extension set so that about 2-3 inches of tubing are left attached to the hub.
- Draw up 2-3 mL of saline into a syringe.
- Attach syringe to hub of butterfly catheter. Purge tubing with saline.
- Put on gloves, gown, mask and eye protection.
- Gently remove excess mucous from patient's nose. (If patient is an adult, ask the patient to gently blow nose. For pediatrics, a bulb syringe may be used to remove excess mucous.)
- Position patient in supine position with the head of bed up 30°. The head should be turned to one side and tilted slightly backward.
- Stabilize the patient's head and gently place the catheter into the nare. Placement should be in the nare (nasal wall), not the nasopharynx. Depending on the size of the patient, this should be about 1-2 cm in adults and 0.5 cm to 1.0 cm in children (0.5 cm in neonates). See Figure 1.

Figure 1

- Instill .5 - 2 mls saline (.5 - 1 mls for infants and children, 1 - 2 mls for adults) into the nare and aspirate back mucous, saline and epithelial cells.

- Repeat this process using the same syringe until the sample is cloudy or appears to hold cellular debris. (If the sample is inadequate, the process may be repeated on the opposite nare, using a second sterile syringe and tubing. Usually one nare is sufficient.)
    - NOTE: There may be some blood streaks in the mucous. This is normal and patients/parents should be told this is expected and will stop in a few minutes.
- Transfer contents of tubing and syringe into transport media using the following process: Depress syringe plunger and express fluid from syringe and tubing into transport media. Then withdraw media/fluid back into syringe and tubing. Depress syringe plunger again, expressing fluid from syringe and tubing back into transport media.
    - NOTE: This is necessary to recover any cells or virus adhering to the tubing or syringe.

Nasal Swab:

- Insert a mini-tip culturette swab into the same nares from which the wash was performed approximately 3 cm and gently rub the mucosa.
- Place the swab back into transport media containing aspirated material.
- See Figure 2.
- NOTE: Combining the aspirate and the swab enhances viral recovery and reduces cost to the patient.
- Deliver entire specimen to the lab immediately.

Figure 2

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Nose

- Gently insert single swab through each nare until it reaches that portion of mucosa that is inflamed or containing exudate. In small children, use a nasopharyngeal swab to facilitate collection.
- Rotate swab quickly in nare and withdraw.
- Swab the opposite nare in same manner. One swab is used for both nares.
- Immediately place swab in culture tube being careful not to touch the swab to the outside of the tube and transport at 2-8 °C.
- Specify if for MRSA or any suspected pathogens on the requisition

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Sputum

- Provide the patient with a sterile specimen container and instruct not to touch inside.
- Instruct the patient to take three to four slow deep breaths and cough forcefully upon exhalation.
- Expectorate directly into the specimen container.
- The specimen should be examined to make sure it contains at least 1ml of thick mucous. **Saliva is not an adequate specimen**. An early morning specimen is preferred.
- Close the lid of the container.
- Send specimen to laboratory at ambient temperature. Refrigerate if a delay of >1 hour is anticipated; freeze for molecular tests.

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Stool

- Collect specimen in a clean bed pan or use plastic wrap placed between the toilet seat and the bowl. Do NOT submit feces contaminated with urine, toilet water, or barium.
- Transfer specimen into a clean, dry container.
- Transport unpreserved stool at ambient temperature within 2 hours of collection for bacterial cultures or at 2-8°C for viral cultures.
- If transport delay > 2 hours is unavoidable, place the specimen in an appropriate preservative or transport media immediately after collection; bacterial stool cultures use enteric transport media (Cary-Blair) at ambient or 2-8ºC; for ova and parasite use Protofix at ambient temperature. Do not fill commercial transport vials above indicator line. Overfilling of transport vial results in improper specimen preservation.
- Stool for antigen testing for C. difficile, toxin, H. pylori antigen, and Rotavirus antigen requires 1 gram (1-10 mL) of unpreserved stool to be transported and stored at Ambient: <2 hours; Refrigerated: 72 hours; Frozen: 1 week.

Notes:

- Only loose or diarrheal stools are recommended for routine bacterial cultures and *C. difficile* toxin testing.
- Bacterial culture orders available include: Culture, Stool (for *Salmonella, Shigella, Campylobacter*); Culture, Hemorrhagic *E. coli* stool (*E. coli* 0157:H7); Culture, Yersinia; Culture, Vibrio.
- Submit 2-3 stools collected at least 24 hours apart for culture.
- Routine stool cultures are not recommended from patients hospitalized for > 3 days, unless the patient is known to be HIV positive or approved by Microbiology Medical Director.
- For parasite testing with **no foreign travel** in the patient's history, order: Ova and Parasite Giardia/Cryptosporidim Antigen Screen
    - Submit one stool for antigen testing for *Giardia* and *Cryptosporidium*. Specimens will be held for 7 days, and a complete parasite exam may be requested on antigen negative stools.
- For complete parasite exam order: Ova and Parasite Complete, Foreign travel,

- Collecting 3 specimens over a 10 day period is optimal. Indicate suspected parasites and patient history if applicable. Specimens analyzed to determine efficacy of treatment should be collected 3-4 weeks after treatment.

- For parasite testing of *Cyclospora*, *Isospora* and *Micorsporidia* by modified acid fast stain order: Ova and Parasite stool for *Cyclospora* and *Ispospora* Stain.
- Test stool for *Clostridium difficile* toxin is recommended for all patients over 6 months of age with clinically significant diarrhea and a history of antibiotic exposure. Consider *C. difficile* testing as an alternative to routine microbiologic studies for inpatients over 6 months of age who have test requests for routine enteric pathogens.

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Throat

- Instruct patient to tilt head backwards, open mouth and say "ah."
- A tongue depressor may be used to depress the tongue and facilitate visualization of pharynx.
- Insert swab without touching lips, teeth, tongue, or cheeks.
- Gently and quickly swab the tonsillar area side to side, making contact with inflamed or purulent sites.
- Carefully withdraw swab without striking oral structures.
- Immediately place swab in culture tube being careful not to touch the swab to the outside of the tube and transport at 2-8 °C.
    - For viral cultures place in viral transport media and transport at 2-8 °C.

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Urine

Midstream or Clean-Catch Urine:

- Assist or allow patient to independently cleanse perineum and collect specimen.
- Clean the urethral opening (and vaginal vestibule in females) with soap and water or wipes included in collection kit. In males, retract the foreskin before micturation.
- Open specimen container and do not touch inside of container.
- After the patient has initiated urine stream, pass urine specimen container into stream and collect midstream portion of urine.
- Replace cap securely on specimen container.
- Transport to laboratory immediately or refrigerate and transport at 2 - 8°C.

First – Void Urine:

- The patient should have not urinated for at least 1 hour prior to specimen collection.
- Collect specimen in a sterile, plastic, preservative- free specimen collection cup.
- The patient should collect 15 – 20mL of voided urine, maximum of 60 ML (the first part of the stream- NOT midstream).
- Label with patient identification, collection time and date.
- Store and transport urine specimen refrigerated.

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Vomitus for Norovirus testing

- Testing will be performed for outbreak investigations only.
- Specimens should be obtained during the acute phase of the illness (within 48-72 hours of onset) while viral shedding is at its highest.
- Collect specimen in a clean bed pan, new gallon Ziploc bag, or a clean Tupperware container. Do NOT submit vomitus contaminated with toilet water, toilet paper, or tissues.
- Transfer specimen into a clean, dry container (multiple Ziploc baggies).
- Transport at 2-8°C.
- Refer to Stool collection sheet for further collection, storage and shipment information.

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