BORDETELLA PERTUSSIS /B. PARAPERTUSSIS POLYMERASE CHAIN REACTION (PCR) DETECTION

I. GENERAL CONSIDERATIONS:

The Molecular Microbiology Laboratory offers testing of nasopharyngeal secretions by the PCR technique for laboratory diagnosis of B. pertussis and B. parapertussis infection. The procedures below should be followed to insure proper specimen processing.

II. SPECIMEN COLLECTION:

- (1) Check M4 collection kit to ensure that transport tube with medium is not beyond expiration date; M4 Collection transport medium is <u>preferred</u> to be stored refrigerated, but it is acceptable at room temperature. Once the sample has been collected in the media, the sample <u>must</u> be refrigerated. Collection kits may be obtained from Laboratory Services.
- (2) Collect ONE posterior nasopharyngeal specimen using a mini-tip plastic-shaft Nylon flocked swabs (included in the kit). Only experienced medical personnel should perform NP collections; such collections may induce paroxysmal coughing.

Place the swab into screw-capped tube containing M4 transport medium and break the plastic shaft at the scoring leaving the swab tip in the tube. Make sure that no part of the swab remains above the top of the tube. Tighten the cap securely.





M4 Collection Kit Guidelines

When your child needs a hospital, everything matters."

GENERAL COLLECTION INFORMATION

1. LABEL AND TRANSPORT INSTRUCTIONS

- Assure proper labeling of specimen. Standard labeling requirements include:
 - 1. Patient's full legal name complete first and last names correctly spelled
 - 2. Test Requisition Barcode, Medical Record Number or Date of Birth
 - 3. Date & time of collections (documented on sample or requisition)
 - 4. Name of collector (documented on sample or requisition)
 - 5. Source of specimen
- These collection guidelines serve only as general instructions. Only trained personnel should perform these procedures.
- M4 collection transport medium is used to transport all types of swab specimens for viral, Chlamydial and Mycoplasma cultures, PCR testing, and Respiratory Infection Array (FARVPP).
- Prior to sample collection, check the collection kit to ensure that the transport medium has not passed the expiration date. Also, check the color of the media to make sure it is pink. If it is yellow or purple, do not use.
- M4 collection transport medium is preferred to be stored refrigerated, but is acceptable at room temperature.
- Once the sample has been collected in the media, the sample **MUST** be refrigerated and transported on ice or cold-pack.

2. COLLECTION KIT CONTENTS

- Sterile polyester- tipped swabs (enclosed in paper envelope) and sterile flocked swabs (enclosed in paper envelope)
- M4 transport media (pink vial)
- Specimen labels

3. GENERAL SWAB COLLECTION INSTRUCTIONS

- Apply appropriate but significant pressure to the body site to be cultured while rotating the swab.
- This ensures that adequate cells (viruses and chlamydial species are intracellular) are obtained.
- Agitate swab briskly in vial containing M4 transport media.
- Cut the swab shaft leaving tip in vial and secure the lid tightly.

RESPIRATORY SITE COLLECTION INSTRUCTIONS

1. <u>THROAT SWAB COLLECTION FOR RESPIRATORY VIRAL CULTURE OR MYCOPLASMA PNEUMONIAE DETECTION</u> <u>BY PCR</u>

- Use sterile polyester-tipped swab provided in M4 Collection Kit.
- Swab posterior oropharynx and tonsillar tissues firmly in a manner similar as for streptococcal throat culture.
- If specific lesions are seen (suggesting HSV, enterovirus, etc), use a second swab to samples the lesions.
- Agitate swab briskly in vial containing M4 transport media.
- Cut the swab shaft leaving tip in vial and secure the lid tightly.
- Note: Viral throat culture is also good specimen type for recovery of adenovirus but not RSV or influenza.

2. POSTERIOR NASOPHARYNX SWAB COLLECTION FOR MYCOPLASMA PNEUMONIAE AND BORDETELLA PERTUSSIS/ BORDETELLA PARAPERTUSSIS BY PCR, CHLAMYDIA TRACHOMATIS CULTURE, RESPIRATORY VIRAL CULTURE, RESPIRATORY INFECTION ARRAY (FARVPP) FOR INFLUENZA A & B VIRUSES, RSV, HUMAN METAPNEUMOVIRUS, RHINOVIRUS/ENTEROVIRUS, CORONAVIRUS, PARAINFLUENZAVIRUS 1-4, ADENOVIRUS, CHLAMYDIOPHILA PNEUMONIAE, BORDETELLA PERTUSSIS AND MYCOPLASMA PNEUMONIA.

- Use sterile flocked swab provided in M4 Collection Kit.
- Have patient lie down on his/ her back or sit in a chair.
- Immobilize patient's head and gently insert sterile flocked swab into one naris while lifting the nostrils.
- Continue inserting the swab with a rotating back and forth motion until it reaches the posterior nasopharynx where resistance is met. This will require the swab to bend along the curvature of the nasopharyngeal space. The distance inserted to reach the posterior nasopharynx should be at least a few inches, depending on the age of the patient.
- Leave the swab in place for 5-10 seconds. This will likely induce tearing and coughing.
- Withdraw the swab with a rotating motion to loosen and collect cellular material while in contact with the mucosal surfaces of the mid-inferior portion of the inferior turbinate.
- · Agitate swab briskly in vial containing M4 transport media.
- Cut the swab shaft leaving tip in vial and secure the lid tightly.



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Note: If ordering multiple respiratory tests, one NP sample for Respiratory Infection Array (FARVPP) should be submitted as a separate sterile flocked swab in M4 media. Additional molecular respiratory testing (PCR) can be combined into a second NP sample and submitted as a sterile flocked swab in M4 media.

NON-RESPIRATORY BODY SITE COLLECTION INSTRUCTIONS

1. ANAL SWAB COLLECTION FOR VIRAL CULTURE

- Wet sterile **polyester-tipped swab** with sterile saline.
- Firmly swab rectal mucosa while rotating swab.
- Agitate swab briskly in vial containing M4 transport media.
- Cut the swab shaft leaving tip in vial and secure the lid tightly.

2. CERVICAL, VAGINAL, OR URETHRAL SWAB COLLECTION FOR VIRAL OR C. TRACHOMATIS CULTURE

- Firmly swab desired area with sterile polyester-tipped swab.
- Agitate swab briskly in vial containing M4 transport media.
- Cut the swab shaft leaving tip in vial and secure the lid tightly.

3. CONJUNCTIVAL SWAB COLLECTION FOR VIRAL OR C. TRACHOMATIS CULTURE OR HSV/VZV BY PCR

- Firmly swab conjunctiva with sterile polyester-tipped swab.
- Left and right eye may be collected with separate swabs and pooled into one M4 vial.
- Agitate swab briskly in vial containing M4 transport media.
- Cut the swab shaft leaving tip in vial and secure the lid tightly.

4. LESION/ VESICLE SWAB COLLECTION FOR VIRAL CULTURE OR HSV/VZV BY PCR

- Newly developed, unbroken vesicles provide best yield.
- Break vesicle and firmly swab fluid and vesicle base (to obtain cells) with sterile polyester-tipped swab.
- If vesicle is already broken, select vesicle with moist base, scrape outer layer and firmly swab base.
- More than one vesicle/lesion may be sampled and the specimens pooled in one vial.
- Agitate swab briskly in vial containing M4 transport media.
- Cut the swab shaft leaving tip in vial and secure the lid tightly.

5. TISSUE FOR VIRAL CULTURE

• Autopsy or biopsy tissue obtained for viral culture should be placed into M4 vials.

LABEL AND TRANSPORT INSTRUCTIONS

- Assure proper labeling of specimen. Standard labeling requirements include::
 - 1. Patient's full legal name complete first and last names correctly spelled
 - 2. Test Requisition Barcode, Medical Record Number or Date of Birth
 - 3. Date & time of collections (documented on sample or requisition)
 - 4. Name of collector (documented on sample or requisition)
 - 5. Source of specimen
- Transport the vial with swab in a biohazard zip-lock transport bag to the laboratory as soon as possible.
- After sample collection, specimens MUST be refrigerated and transported on ice or cold-pack.

Contacting Laboratory Services

Virology Laboratory personnel can be consulted for technical and collection procedure information by calling the phone number above.