

**Specimen Submission Requirements for *Bordetella pertussis*,  
*Bordetella parapertussis*, *Bordetella holmesii*, and *Bordetella  
bronchiseptica* Detection and Identification by Real-time  
Polymerase Chain Reaction (PCR)**

- Methodology:** Real time PCR
- Performed:** Real time PCR is used to detect *B. pertussis*, *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica* using the repetitive insertion sequences IS481, IS1001, and BPP-Tox in nucleic acid extracted from a clinical material.
- Turn-Around-Time:** Testing of samples will be batched and results(s) will be reported within 48 hours from completion of the test. All specimens will be forwarded to the Bacteriology Section for possible culture and isolation of the agent.
- Acceptable Specimens:** Nasopharyngeal (NP) aspirates or washes and NP swabs. Bronchoalveolar lavage is acceptable in cases where pneumonia is suspected. Submit at least 0.5 ml in a sterile container. Swabs in Regan-Lowe media are also acceptable.
- Specimen Collection:** Use only Dacron tip swabs with an aluminum or plastic shaft. Calcium alginate swabs or cotton swabs with wooden sticks are unacceptable because they may cause PCR inhibition.
- For NP swabs-** Insert swab into the nostril until the posterior nares is reached. Leave swab in place for 10 seconds.  
Place swabs immediately in a dry sterile vial or in Regan-Lowe transport media. Break the shaft and tighten the cap of the vial. Label each specimen with a unique identifier, type of specimen and date of collection.
- Keep specimens at 4°C. If transport is to be delayed for longer than 72 hours, dry swabs should be frozen at -20°C and transported in the laboratory in dry ice.
- For NP wash/aspirate-** Have the patient sit with the head tilted slightly backward. Instill 1-1.5 ml of non-bacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2-3 ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate NP secretions. Repeat this procedure with each nostril.

Collect NP/OP wash or aspirate in sterile vials. Label each specimen with a unique identifier, type of specimen and date of collection.

Specimen storage,  
packing and transport:

Ship specimens with cold packs to keep the sample at 4°C. If the specimen has been frozen at -20°C, ship specimens in dry ice.

Follow packing and shipping instructions of the U.S. Department of Transportation (U.S. DOT), and the International Air Transport Association (IATA) for Biological Substance, Category B.

Specimen submission:

Submitters: Clinical Laboratories and the Disease Investigation Branch (DIB)

Submitters must notify Dayna Ornellas of the Medical Microbiology Branch (MMB) at 453-6711, or Norman O'Connor, Bacteriology, Medical Microbiology Branch (MMB) at 453-6706 (for culture and isolation) prior to the submission of specimens.

**Note:** It is the responsibility of the submitter to track the arrival of the specimens along with Form 81.3 at the State Laboratories Division to ensure that these specimens are received at the respective testing laboratories.

Criteria for rejection:

**Specimen without prior approval from the State Epidemiologist or DIB Epidemiologist Investigator**

- Specimen is received in a container that is leaking. Specimen **will not be processed** if the safety of the laboratory worker is compromised;
- Specimen is not collected in a proper container or special handling instruction is not followed;
- Transport media is expired;
- Specimens collected with swabs with cotton tips or calcium alginate, and swabs with wooden shafts;
- Specimen is not received at 4°C or packed in blue ice;
- Specimen quantity is insufficient to perform the tests;
- Unlabeled specimens;
- Incomplete specimen labeling and documentation;
- Specimen label does not match the requisition.

**Stability:** All specimens must be refrigerated at 2-8°C immediately after collection. If liquid specimens cannot be transported to the State Laboratories Division within 72 hours after collection, they should be kept frozen at -20°C (**for PCR detection**). Specimens in Regan-Lowe media must be maintained at 2-8°C. Do not freeze.

**Requisition Form:** Each specimen submitted must have a completed Form 81.3. (<http://health.hawaii.gov/statelab/files/2013/05/sld-sld81-3.pdf>). Submitter is responsible for completing Form 81.3 (including but not limited to the following information: patient identifier, date of onset of illness, signs and symptoms, travel history, immunization history, name and address of submitter).

Requisition forms shall be placed in a separate bag and shall not be packed with the specimen(s).

**Normal Value:** No *B. pertussis*, *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica* nucleic acid detected.

**Result Notification:** Laboratory results are reported to the submitters by electronic reporting system or via FAX. Laboratory reports for the Disease Investigation Branch (DIB) of the DOH Disease Outbreak Control Division (DOCD) will be posted to the DOCD SharePoint.

**Test performed at:** State Laboratories Division  
Medical Microbiology Branch  
2725 Waimano Home Road  
Pearl City, Hawaii 96782

**Contact:** For PCR Testing:  
Dayna Ornellas (808) 453-6711  
Derek Harauchi at (808) 453-6705  
Lance Chinna at (808) 453-6706  
**OR**  
For Culture and Isolation:  
Norman O'Connor  
808-453-6706

Approved by:

Edward P. Desmond

Edward Desmond, Ph.D.  
Administrator, State Laboratories Division

5/6/2019

Date