Laboratory Preparedness and Response Branch  
Biological Response Section  

Specimen Requirements for Real-Time (TaqMan®) RT-PCR Assay for the  
Detection of Mumps Virus RNA in Clinical Samples  

<table>
<thead>
<tr>
<th>Methodology:</th>
<th>Mumps virus Real-time (rti) RT-PCR</th>
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<tbody>
<tr>
<td>Performed:</td>
<td>The real-time (rti) Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) TaqMan® Assay is a CDC-developed test to detect the N gene of the mumps virus. Confirmatory testing may be performed at the CDC.</td>
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<td>Criteria for testing:</td>
<td>Clinical signs and symptoms compatible with mumps.</td>
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<td>Turn-Around-Time:</td>
<td>Specimens meeting the case definition and/or are collected during outbreak investigations by the Disease Investigation Branch (DIB) of the Disease Outbreak Control Division of the Department of Health will be reported within 2-3 business days from DIB approval and receipt of appropriate specimen(s).</td>
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<td>Specimen type required:</td>
<td>The preferred specimen for mumps rti RT-PCR is oral/buccal swab. Specimens must be collected within 9 days of onset of symptoms.</td>
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<td>Specimen storage and transport:</td>
<td>Urine samples collected &gt;4 days after onset of symptoms may also be submitted. Collect a minimum of 10-50 ml. of urine in a sterile cup for testing. Centrifuge urine at 2500 x g for fifteen (15) minutes at 4°C. Re-suspend the sediment in 2 ml of Viral Transport Medium (VTM). Store urine sediment in VTM at 4°C and if possible, ship within 24 hours.</td>
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<td>CSF is an acceptable specimen in the case of suspect mumps meningitis/encephalitis. CSF specimens with DIB approval will be sent to CDC for further testing.</td>
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<td>Use only Dacron® tip swabs with an aluminum or plastic shaft or flocked synthetic swabs. Calcium alginate swabs or cotton swabs with wooden sticks are unacceptable because they may cause PCR inhibition and may contain substances that may inactivate or may be toxic.</td>
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<td>Buccal or oral swabs must be collected in viral VTM, maintained at 2-8 °C and shipped on cold packs within 24</td>
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hours. If there is a delay in shipment (2-3 days from the time of collection), store the sample at 4°C and ship on cold packs or blue ice. Indicate the number of days the specimen was stored at 4°C

Ship urine in VTM (processed as indicated above) on cold packs (i.e. 4°C) within 24 hours. If there is a delay in shipment, store in -70°C freezer and ship on 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 Epidemiology Specialists of the DIB.

Notification of the DIB and the laboratory is requested prior to the submission of specimens.

Criteria for rejection:

- Specimen is received in a container that is leaking.
- Specimen is not collected in a proper container or special handling instruction is not followed.
- Uncentrifuged urine and sediment is not suspended in VTM.
- Urine specimen collected ≤ 4 days after onset of symptoms.
- Viral transport media is expired.
- Use of improper swab or swab not in VTM.
- Specimen is not received at 4°C or packed in blue ice.
- Specimen quantity is insufficient to perform the tests.
- Unlabeled or incomplete specimen labeling and documentation; Submitter will be notified to provide correct information and corrected State Laboratory Division (SLD) Form 81.3.
- Specimen label does not match the requisition.

Stability: All specimens must be refrigerated at 2-8°C immediately after collection. If the specimen cannot be transported to the State Laboratories Division within 48 hours after collection, it should be frozen at -20°C or lower. If a -20°C or lower freezer is not available, keep the sample at 2-8°C. Avoid repeated freeze-thaw cycles. Frozen samples should be shipped on dry ice.
Requisition Form: Each specimen submitted must have a completed SLD Form 81.3. Submitter is responsible for completing SLD Form 81.3 including but not limited to the following information: at least two patient unique identifiers, date of onset of illness, signs and symptoms, travel history, immunization history, test (s) requested, 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 Mumps Virus Nucleic Acid Detected

Result Notification: Laboratory reports are electronically relayed to the submitters via the SLD dashboard. An automatic notification will be relayed when a report is available on the SLD dashboard. Laboratories who do not receive automatic notification will be notified by e-mail on the availability of results on the SLD Dashboard. Submitters who do not have access to electronic reports will be notified via fax or by encrypted e-mail.

Test performed at: Biological Response Section (BRS) Laboratory Preparedness and Response Branch (LPRB) State Laboratories Division Department of Health 2725 Waimano Home Road Pearl City, Hawaii 96782

Contact: Remedios Gose at (808) 453-5993 or (808) 554-9992 or Cheryl Daquip at (808) 453-5984 or (808) 453-6641

Reviewed By:

\[\text{Remedios B. Gose, MSPH, RM (NRCM)}\]
Laboratory Preparedness and Response Branch Chief

Approved By:

\[\text{Edward Desmond, Ph.D.}\]
Administrator, State Laboratories Division