Version 1.0: March 30, 2010

Standard RT-PCR to Detect the SH Gene of Mumps Virus

Use of trade names or commercial sources is for identification only and does not imply endorsement by CDC.

Primers for Mumps (PCR product will be 675 nucleotides):
Forward SH1, 5' AGT AGT GTC GAT GAT CTC AT 3'
Reverse SH2, 5' GCT CAA GCC TTG ATC ATT GA 3'

This RT-PCR assay can be used to detect mumps RNA in clinical samples or in infected cell culture with the caveat that this assay is less sensitive than real time RT-PCR. The sequence of the PCR product containing the coding region of the SH gene can be used to determine the viral genotype (see Jin et al,. Arch Virol. 2005;150:1903-9).
Primer sequences are from: Jin L, Beard S, Brown DWG. Genetic heterogeneity of mumps virus in the United Kingdom: identification of two new genotypes. J Infect Dis 1999; 180:829 33.
Mumps SH gene. The sequence of the 675 nucleotide amplicon is shown below as cDNA. The primer sequences are in italics and the 318 nt. SH gene is underlined. The SH protein is also shown.

```
    SH1

        AGTAGTGTCGATGATCTCATCAGGTACTAATCTTAGATTGGTGATTCGTCCTGCAATTTT
      1 ---------+---------+---------+---------+---------+---------+ 60
        TCATCACAGCTACTAGAGTAGTCCATGATTAGAATCTAACCACTAAGCAGGACGTTAAAA

        AAAAGATTTAGAAAAAAACTAAAATAAGAATGAATCTCCTAGGGTCGTAACGTCTCGTGA
     61 ---------+---------+---------+---------+---------+---------+ 120
        TTTTCTAAATCTTTTTTTGATTTTATTCTTACTTAGAGGATCCCAGCATTGCAGAGCACT

        CCCTGCCGTCGCACTATGCCGGCAATCCAACCTCCCTTATACCTAACATTTCTAGTGCTA
    121 ---------+---------+---------+---------+---------+---------+ 180
        GGGACGGCAGCGTGATACGGCCGTTAGGTTGGAGGGAATATGGATTGTAAAGATCACGA

a                     M  P  A  I  Q  P  P  L  Y  L  T  F  L  V  L   -

        ATCCTTCTCTATCTCATCATAACCCTGTATGTCTGGACTATATTGACTATTAACTATAAG
    181 ---------+---------+---------+---------+---------+---------+ 240
        TAGGAAGAGATAGAGTAGTATTGGGACATACAGACCTGATATAACTGATAATTGATATTC

a       I  L  L  Y  L  I  I  T  L  Y  V  W  T  I  L  T  I  N  Y  K   -

        ACGGCGGTGCGATATGCAGCACTGTACCAGCGATCCTTCTCTCGCTGGGGTTTTGATCAC
```

```
     241 ---------+---------+---------+---------+---------+---------+ 300
         TGCCGCCACGCTATACGTCGTGACATGGTCGCTAGGAAGAGAGCGACCCCAAAACTAGTG

a        T  A  V  R  Y  A  A  L  Y  Q  R  S  F  S  R  W  G  F  D  H  -

         TCACTCTAGAAAGATCCCCAATTAGGACAAGTCCCGATCCGTCACGCTAGAACAAGCTGC
     301 ---------+---------+---------+---------+---------+---------+ 360
         AGTGAGATCTTTCTAGGGGTTAATCCTGTTCAGGGCTAGGCAGTGCGATCTTGTTCGACG

a        S  L  *

         ATTCAAATGAAGCTGTGCTACCATGAGACATAAAGAAAAAAGCAAGCCAGAACAAACCTA
     361 ---------+---------+---------+---------+---------+---------+ 420
         TAAGTTTACTTCGACACGATGGTACTCTGTATTTCTTTTTTCGTTCGGTCTTGTTTGGAT


         GGATCATAACACAATACAGAATATTAGCTGCTATCACAACTGTGTTCCGGCCACTAAGAA
     421 ---------+---------+---------+---------+---------+---------+ 480
         CCTAGTATTGTGTTATGTCTTATAATCGACGATAGTGTTGACACAAGGCCGGTGATTCTT


         AATGGAGCCCTCGAAACTATTTATAATGTCGGACAATGCCACCTTTGCACCTGGACCTGT
     481 ---------+---------+---------+---------+---------+---------+ 540
         TTACCTCGGGAGCTTTGATAAATATTACAGCCTGTTACGGTGGAAACGTGGACCTGGACA


         TGTTAATGCGGCTGGTAAGAAGACATTCCGAACCTGTTTCCGAATATTGGTCCTATCTGT
     541 ---------+---------+---------+---------+---------+---------+ 600
         ACAATTACGCCGACCATTCTTCTGTAAGGCTTGGACAAAGGCTTATAACCAGGATAGACA


         ACAAGCAGTTATCCTTATATTGGTTATTGTCACTTTAGGTGAGCTTATTAGGATGATCAA
     601 ---------+---------+---------+---------+---------+---------+ 660
         TGTTCGTCAATAGGAATATAACCAATAACAGTGAAATCCACTCGAATAATCCTACTAGTT

         TGATCAAGGCTTGAGC
     661 ---------+------ 676
         ACTAGTTCCGAACTCG (SH2R)
```

Superscript RT-PCR Protocol for Mumps SH

Version 1.0: March 30, 2010

This is a standard RT-PCR reaction. This is a one-tube reaction, so there is a minimum of specimen handling. Some points to consider:

1. Controls.

Positive control should be RNA extracted from cells infected with mumps virus. It is best to use cells infected with a well-characterized strain of mumps, belonging to a genotype not associated with the outbreak. In addition, the use of vaccine virus as a control is not recommended since in an outbreak setting some cases may be referred for testing who have been recently vaccinated. If there is a question of contamination, this will allow sequence studies to determine the possible source of the virus.

Negative controls should include RNA extracted from uninfected cells as well as a control with water substituted for RNA. When doing large numbers of RNA extractions, it is a good idea to include an extraction control. This mock extraction will show that reagents for RNA extraction have not been contaminated by template.

2. **Avoid template contamination**. Use dedicated equipment, rooms and hoods for all pre-PCR procedures. Post amplification analysis and processing should be performed in separate rooms using dedicated equipment. Do not share equipment (including lab coats) between pre-PCR and post-PCR procedures. Use filter tips for all pipetting of pre-PCR procedures and for setting up RT-PCR reactions.

3. Use 5 ul of **RNA** per reaction. Most of the extraction protocols yield 40-50 ul of RNA. We usually extract RNA from one 25 cm^2 flask of infected cells or from 100-200 ul of original viral sample.

MATERIALS:

1. Superscript kit (Invitrogen CAT# 10928-042)
2. RNasin (pancreatic RNase inhibitor)
3. Ice bucket
4. Autoclaved PCR tubes (0.2ul, thin-walled)
5. Forward Primer (20uM working dilution prepared in RNase-free water)
6. Reverse Primer (20uM working dilution prepared in RNase-free water)
7. CDC has prepared aliquots of mumps virus RNA for distribution as a positive control for RT-PCR (contact Paul Rota, prota@cdc.gov).

INSTRUCTIONS:

1. Thaw all kit reagents except enzymes, vortex and place on ice. Keep enzymes (Superscript and RNasin) on ice at all times. Allow RNA samples to thaw on ice and keep on ice while you are setting up the reactions.
2. Label appropriate number of 0.2 ml thin-walled, reaction tubes and place in pre-chilled metal cooling rack. Keep cooling rack on ice for entire protocol.
3. Add appropriate volumes (see worksheet in Appendix 1) of reagents 1 through 5 to a pre-chilled 1.5 ml Eppendorf tube. Vortex and keep tube on ice.
4. Allow time for pre-mix contents to chill, and then add reagents 6 and 7 to pre-mix tube. Vortex and chill briefly on ice.

5. Dispense pre-mix (see worksheet) to each reaction tube.
6. Using a new, clean pipette tip for each transfer, add RNA to each tube and close the cap.
7. Spin the tubes briefly (10,000 rpm for 1 minute) in a chilled microcentrifuge and immediately return the tube to the metal cooling rack.
8. While tubes are spinning start the appropriate file the thermocycler (see parameters in Appendix 2). When the block temperature reaches 55 C, hit the pause button. With the instrument paused, transfer the reaction tubes from the metal cooling rack to the heat block of the thermocycler. Close the cover and press the resume button.
9. Reactions products should be analyzed by agarose gel electrophoresis. A 1% agarose gel is recommended (for example, see Appendix 3).

Version 1.0: March 30, 2010

Appendix 1
SUPERSCRIPT RT-PCR REACTION WORKSHEET:

Date:_____ Operator:_____

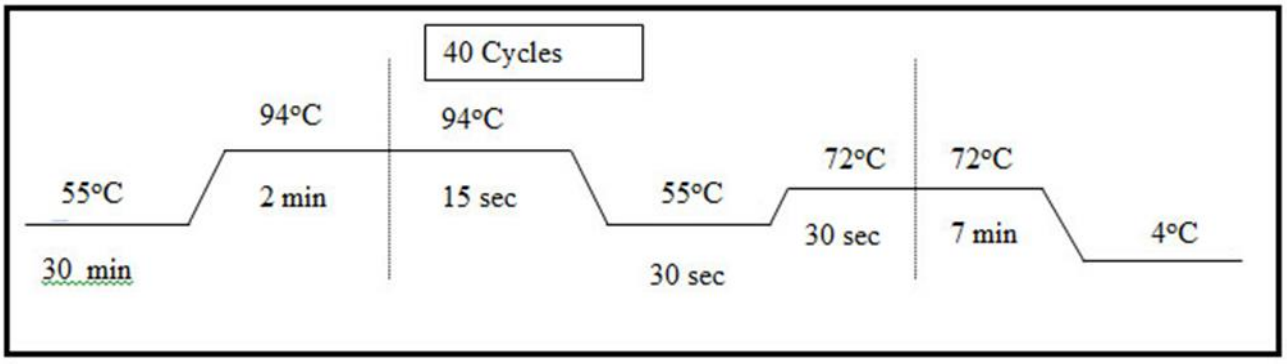
Compxonent	[Final] Conc.	Vol/rxn	# rxns plus 1	Total vol.	Added Y/N
1. 2X Superscript Reaction mix	1X	25 ul			
2. 5mM $MgS0_4$	0.8mM	8 ul			
3. Forward Primer-SH1 (20 uM)	0.2 uM	0.5 ul			
4. Reverse Primer-SH2 (20 uM)	0.2 uM	0.5 ul			
5. ETF water		9.5 ul			
Vortex and place on ice before adding enzymes					
6. Superscript Enzyme		1 ul			
7. RNase Inhibitor (optional)	20 U.	0.5 ul			
Vortex and dispense pre-mix (45 ul) into each chilled reaction tube, then add RNA samples					
8. RNA		5 ul			

Forward Primer:_____ Reverse Primer:_____

Total reaction volume: 50 ul

Version 1.0: March 30, 2010

Appendix 2: Cycling Parameters for Superscript



Appendix 3.
Agarose gel electrophoresis of the reaction products from the standard RT-PCR for the mumps SH gene. Lane 1: molecular weight marker, lane 2: positive sample, lane 3: negative sample, lane 4: negative control, lane 5: positive control