Version 3.0 08/20/21

CDC Mumps rRT-PCR Assay vs 2 for the Detection of Mumps Virus RNA in Clinical Samples

Changes compared to version 2: Version 2 listed incorrect cycling conditions. Version 3 lists the correct condition. The change is highlighted in green.

This rRT-PCR assay is designed to detect both mumps vaccine and wild type strains. This assay targets the mumps N-gene. Version 1 of the assay (*Rota et al., Clin. Vaccine Immunol. 20(3)* ***2013*** *p391*) occasionally produced false-negative results with specimens in which the mumps forward primer and probe binding sites had nucleotide substitutions. The new version of the assay improves detection in these specimens through introduction of degenerate positions in the forward primer and probe. In addition, the enzyme mix was changed to the QuantiTect kit to allow use of the same enzyme that is used for the CDC measles and MeVA rRT-PCR assays. The reagents, instruments, and other information listed below have been validated by CDC for use in their CLIA-approved assays, but each laboratory should conduct their own validation and establish their own SOPs.

Disclaimer
Use of trade names and commercial sources in this protocol does not imply endorsement by the Centers for Disease Control and Prevention (CDC).

Background
The mumps virus genome is a single-stranded RNA that contains seven genes encoding the nucleoprotein (N), phosphoprotein (P), membrane (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and large (L) proteins. The SH gene is the most variable region of the mumps genome and is used to genotype mumps strains (see standard RT-PCR protocol for mumps for methods used to amplify the SH gene for viral detection and genotyping). Transcripts from the N gene are the most abundant transcript in cells infected with mumps virus. Therefore, the N gene provides a good target for detection of viral RNA from mumps in clinical samples and infected cells.

The human RNase P gene primer and probe set serves as a positive control to monitor sample quality, RNA extraction, and presence of inhibitors of the PCR reaction. This control cannot be used as a control for reverse transcription since it will detect both RNA and DNA.

Test Specificity
The mumps virus primer/probe set included in this protocol has been shown to detect mumps strains in genotypes C, H, N, K, G, and A. There was no cross-reaction with viral cultures from human specimens positive for parainfluenza virus type 4A, measles virus, or rubella virus.

Controls
CDC can provide synthetic mumps virus N-gene RNA that can be used as a positive control.

Protocol Use Limitations
These protocols were validated at CDC using ABI Taqman® one-step probe rRT-PCR chemistry on ABI 7500 and ABI 7500 fast dx thermocyclers. However, this assay has been adapted for use on other platforms.

The sequence of the N gene varies between strains of wild-type viruses. This variability may result in mismatches between the primer or probe sequences and the target sequence. Therefore, this assay may not be able to detect RNA from some wild-type strains.

Version 3.0 08/20/21

Positive rRT-PCR results should be interpreted in conjunction with signs, symptoms, and recent MMR vaccine history due to the potential detection of the vaccine strain in clinical specimens.

Kit

QuantiTect Probe RT-PCR Kit (Qiagen #204443 (100 reactions) #204445 (1000 reactions))

MuV Primers and Probe (*targets the Mumps N-gene)*

MuVN687Fv2	5' GTA TGA CAG CDT ACG ACC AAC CT 3'
MuVN668R	5' –GCG ACC TTG CTG CTG GTA TT-3'
MuVN622Pv2	5' FAM-CYG GRT CTG CTG ATC GGC GAT-BHQ 3'

RNaseP Primers and Probe

RNP_F	5' -AGA TTT GGA CCT GCG AGC G- 3'
RNP_R	5' -GAG CGG CTG TCT CCA CAA GT- 3'
RNP_P	5' -FAM-TTC TGA CCT GAA GGC TCT GCG CG-BHQ1- 3'

Prepare Primers/Probe mix

Rehydrate Primers & Probes in TE buffer to 100µM concentration

Mumps Probe/Primer mix

91.5µL H_2O
3µL Forward Primer
3µL Reverse Primer
2.5µL Probe

RNP Probe/Primer mix

93µL H_2O
3µL Forward Primer
3µL Reverse Primer
1µL Probe

The final concentrations of the primers are probes are:

MuV_F	0.3µM	**RNP_F**	0.3µM
MuV_R	0.3µM	**RNP_R**	0.3µM
MuV_P	0.25µM	**RNP_P**	0.1µM

Master Mix (for Qiagen QuantiTect kit)

Component	Vol (µl)/Well
RNase free water (qs to 25 µl)	5.6
2x Qiagen RT-PCR MM	10
Primer/Probe Mix	2
RNase Inhibitor	0.2
QuantiTect RT Mix	0.2

RNA	2
TOTAL	**20**

Assay Set-up

All specimens are tested for both mumps and RNaseP RNA. Specimens are run in duplicate for mumps and a single well for RNaseP. Appropriate controls must be used.

CDC uses the following:

- RNaseP positive control - Human Reference Total RNA (Stratagene #750500)
  1 ng per well
- Mumps positive control – synthetic RNA transcript of the N gene of mumps (CDC)
  Run as a 10^5 and 10^3 copies/per µL High-Low control
- NTCs for both RNaseP and mumps
- RNA Negative Extraction Control run for both RNaseP and mumps
- RNA Positive Extraction control for RNaseP

Instrumentation and Cycling

ABI 7500 & ABI 7500 Fast DX (in standard mode)

Reverse transcription	50°C for 20 min
PCR initial activation	95°C for 15 min
40 cycles	
Denaturation	95°C for 5 sec
Anneal/extend	60°C for 1 min

Interpretation

MuV	RNaseP	Interpretation
Ct <40	Ct <40	Positive
Ct <40	Undetermined	Positive
Undetermined	Ct<40	Negative
Undetermined	Undetermined	Indeterminate
Discordant (neg/pos) duplicates	Ct<40	Indeterminate

rRT-PCR should be repeated for specimens with discordant duplicates (duplicates more than 1.5 Ct apart or that are negative/positive) and specimens that are indeterminate (mumps negative and RNaseP negative). If the result is again discordant, the rRT-PCR should be repeated using re-extracted RNA when possible.