

Supplementary Material

Tables and Graphs

	1	2	3	4	5	6	7	8	9	10
KY969482_California_2017(G)		98.84	98.84	95.95	97.69	95.95	95.38	95.95	95.95	86.13
KY680537_Canada_2017(G)	98.84		100.00	97.11	98.84	97.11	96.53	97.11	97.11	87.28
MF965227_Massachusetts_2017(G)	98.84	100.00		97.11	98.84	97.11	96.53	97.11	97.11	87.28
KF738113_India_2016(G)	95.95	97.11	97.11		97.11	97.69	94.80	95.38	95.38	86.71
KY604739_India_2017(G)	97.69	98.84	98.84	97.11		97.11	96.53	97.11	97.11	86.13
AF280799_UK_2001(G)	95.95	97.11	97.11	97.69	97.11		94.80	95.38	95.38	87.28
KM597072_Gabon_2015(G)	95.38	96.53	96.53	94.80	96.53	94.80		94.80	94.80	85.55
MG765427_MuVi_Dunedin.NZL_32.17(G)	95.95	97.11	97.11	95.38	97.11	95.38	94.80		100.00	85.55
MG765426_MuVi_NewPlymouth.NZL_30.17(G)	95.95	97.11	97.11	95.38	97.11	95.38	94.80	100.00		85.55
FJ211584 strain RIT4385 NZ vaccine strain (A)	86.13	87.28	87.28	86.71	86.13	87.28	85.55	85.55	85.55	

Table S1. Pairwise comparison of hemagglutinin-neuraminidase (HN) protein sequences of selected Mumps viruses. Genotype is indicated in parenthesis.

Sample number	CDC* method (Ct)	modified method (Ct)	Difference (Ct)
3886	30	26	4
3887	26	23	3
3888	39	34	5
4176	29	25	4
4178	34	31	3
4199	35	31	4
4200	34	31	3
4247	39	33	6
4248	31	27	4
4249	28	25	3
4250	32	28	4
4251	32	28	4
4316	38	34	4
4318	34	30	4
4369	34	30	4
4390	>40	39	>1
4467	34	31	3
4523	>40	39	>1
4525	29	24	5
4526	32	29	3
4585	28	27	1
4702	33	29	4
4770	33	29	4
4771	>40	34	>6

4837	33	29	4
5551	34	31	3
5752	35	31	4
5921	>40	38	>2
5947	29	26	3
5949	33	30	3
6057	32	29	3
6058	28	25	3
6059	30	26	4
6105	39	37	2
6107	34	28	6
6132	39	34	5
6133	31	28	3
6167	33	28	5
6169	28	23	5
6236	40	34	6
6237	37	33	4
6238	27	22	5
6240	34	29	5
6396	37	33	4
6397	34	32	2
6398	34	31	3
6494	27	24	3
6564	28	24	2
6648	Undetermined	41	
Mumps (A) positive control (MMR)	25	25	0
Mumps (A) positive control (MMR)	26	27	1
NTC	Undetermined	Undetermined	
NTC	Undetermined	Undetermined	

Table S2. Real-time RT-PCR comparison for genotype G positive samples including duplicate tests for positive control (genotype A MMR) and negative control (water).

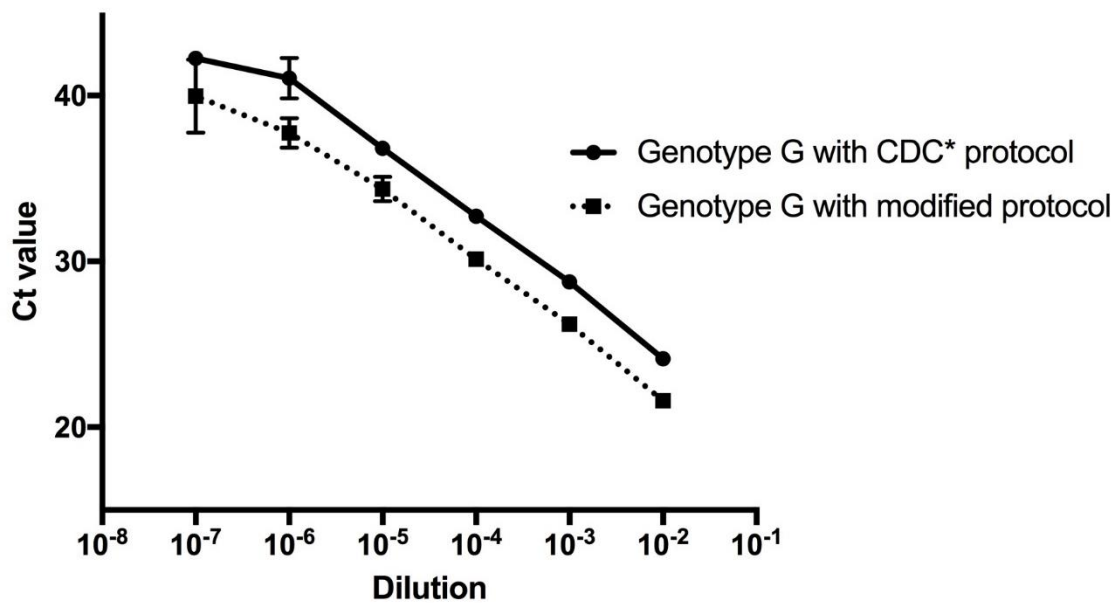
Dilution	Genotype G with CDC* protocol						Mean	STD	No. of repli.	No. of pos.	% pos.	Probit value
1.00E-02	23.91	23.8	24.09	24.16	24.51	24.32	24.13	0.26	6	6	100	NA
1.00E-03	28.93	28.59	28.63	28.65	29.02	28.71	28.76	0.18	6	6	100	NA
1.00E-04	32.86	32.88	32.51	32.66	32.83	32.62	32.73	0.15	6	6	100	NA
1.00E-05	36.82	36.75	36.55	37.2	36.8	36.81	36.82	0.21	6	6	100	NA
1.00E-06	40.92	38.94	40.49	41.91	42.23	41.79	41.05	1.22	6	6	100	NA
1.00E-07	n.d.	42.24	n.d.	n.d.	n.d.	n.d.	42.24	NA	6	1	17	4.033

1.00E-08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NA	NA	6	0	0	NA
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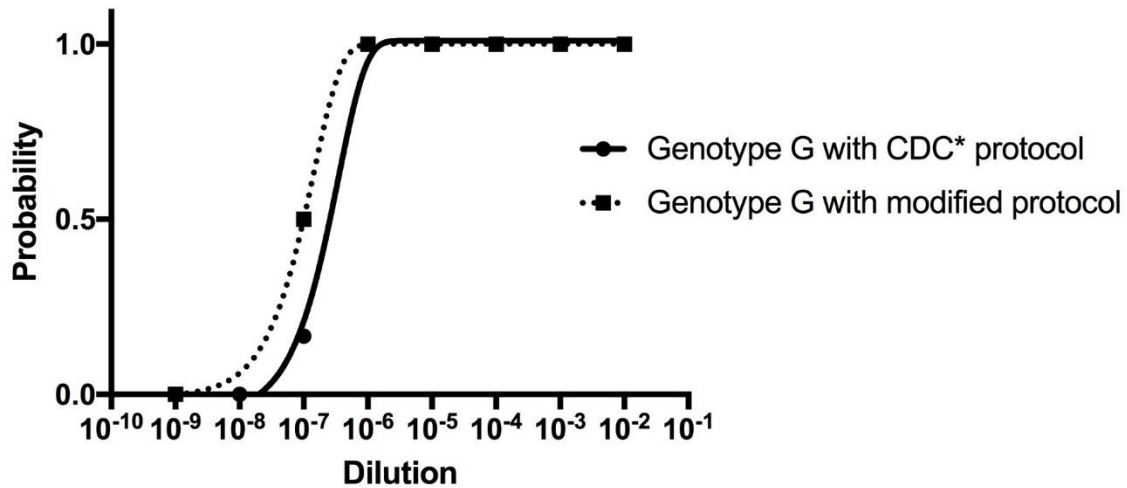
Table S3. Sensitivity testing of mumps virus genotype G with the CDC* protocol (n.d. = not detected, NA = not assessed).

Dilution	Genotype G with modified protocol						Mean	STD	No. of repli.	No. of pos.	% pos.	Probit value
1.00E-02	21.97	21.9	20.84	21.58	21.54	21.7	21.59	0.40	6	6	100	NA
1.00E-03	26.54	26.22	25.73	26.2	26.3	26.28	26.21	0.27	6	6	100	NA
1.00E-04	30.29	30.55	30	30.06	29.84	30.07	30.14	0.25	6	6	100	NA
1.00E-05	34	34.4	35.8	33.8	34.3	33.86	34.36	0.74	6	6	100	NA
1.00E-06	37.83	38.13	37.41	38.96	37.85	36.26	37.74	0.89	6	6	100	NA
1.00E-07	n.d.	38.6	42.51	38.78	n.d.	n.d.	39.96	2.21	6	3	50	5.0
1.00E-08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NA	NA	6	0	0	NA

Table S4. Sensitivity testing of mumps virus genotype G with the modified assay (n.d. = not detected, NA = not assessed).



Graph S1: Comparison of analytical sensitivity on 10-fold dilutions of genotype G RNA (GraphPad Prism v7). Depicted are mean values and standard deviations.



Graph S2: Probit regression analysis on 10-fold dilutions of Genotype G RNA (GraphPad Prism v7). The LoD (95% of samples test positive) for the CDC* protocol is at a dilution factor of 4.452×10^{-7} , and for the modified assay at a dilution factor of 2.666×10^{-7} .

Virus	Sample number	original Ct value or delta cycle (DC)	Ct value with modified Mumps real-time PCR
Measles	0570	29	n.d.
	6382	23	n.d.
VZV	0352	24	n.d.
	1467	25	n.d.
Parvovirus	0502	14	n.d.
	8251	12	n.d.
Enterovirus	7705	28	n.d.
	1262	27	n.d.
Adenovirus	8354	24	n.d.
	846335	26	n.d.
Influenza A, H1N1	2979	19	n.d.
Influenza B	05314	15	n.d.
RSV	5541	32	n.d.
	5548	22	n.d.
Parainfluenza 1	5638	27	n.d.
Parainfluenza 2	5579	18	n.d.
HSV	6001	DC 6.35	n.d.
	8666	DC 7.35	n.d.
MMR positive control			25.17
negative control			n.d.

Table S5. Assessment of analytical specificity of the modified mumps virus real-time PCR (DC = delta cycle, n.d. = not detected).

	Genotype	Ct value 1	Ct value 2	Ct value 3
QCMD MM17S-07	H	32.34	32.54	32.76
QCMD MM17S-08	H	28.94	29.03	28.95
QCMD MM17S-09	H	36.69	36.83	36.19
QCMD MM17S-10	H	25.92	25.88	25.95

Table S6. Assessment of the accuracy of the modified mumps virus real-time PCR using proficiency QCMD mumps virus samples.