

1 **Online Resource**

2 **Endpoint Genotyping of A2063G and A2064G Mutations in *Mycoplasma pneumoniae***

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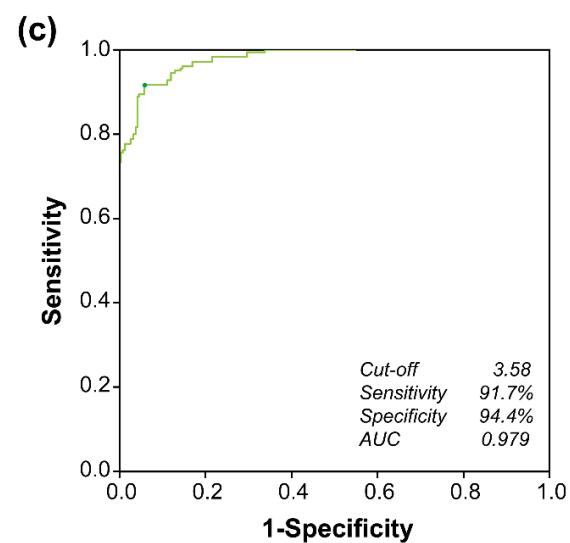
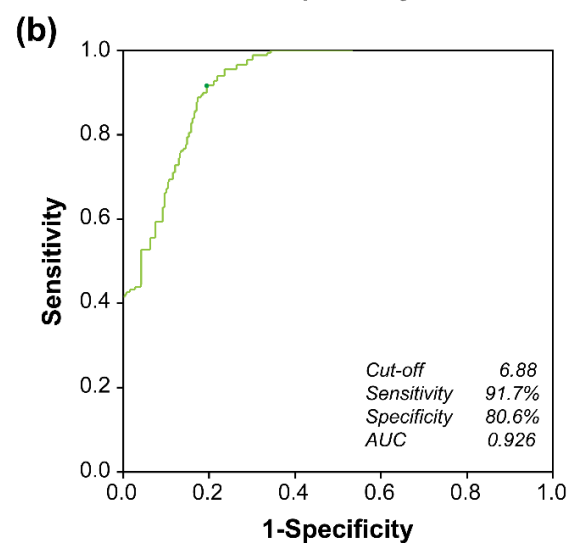
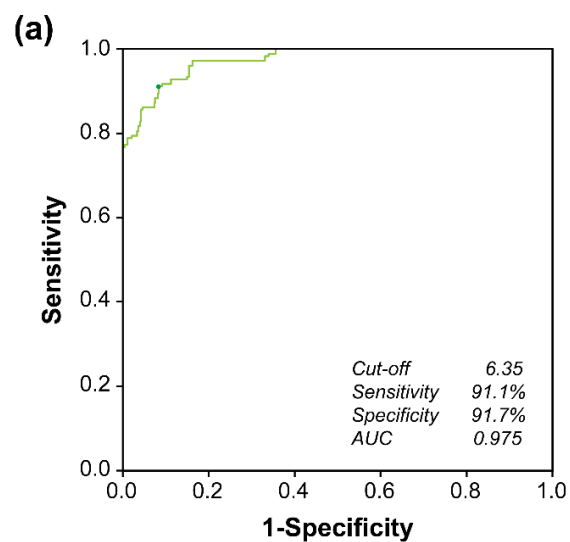
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14 **Fig. S1** Receiver operating characteristic (ROC) analysis and corresponding cutoff values for the (a) ATTO, (b) FAM,

15 and (c) HEX channels

16 AUC, area under the curve

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18 **Table S1** *In silico* analysis of primer and probe alignment to *Mycoplasma pneumonia*.

Target	Oligonucleotide	No. of target strains detected	No. of non-target strains detected
<i>M. pneumoniae</i>	Forward primer	401/401	0
	Reverse primer	784/784	0
	WT probe	91/91	0

19 WT, wild type

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21 **Table S2** *In silico* analysis of primer and probe alignment to 21 organisms.

Organisms	NCBI taxid	Alignment			Expected cross-reactivity
		F (bp/bp)	R (bp/bp)	WT P (bp/bp)	
MERS-CoV	1335626	10/21	13/29	9/20	None
Human coronavirus 229E	11137	10/21	13/29	9/20	None
Human coronavirus OC43	31631	9/21	11/29	9/20	None
Human coronavirus NL63	277944	8/21	11/29	9/20	None
Human coronavirus HKU1	290028	9/21	12/29	8/20	None
RSV type A	1439707	10/21	12/29	0/20	None
Human parainfluenza virus 1	12730	10/21	10/29	10/20	None
Human parainfluenza virus 2	2560525	9/21	11/29	9/20	None
Human parainfluenza virus 3	11216	10/21	11/29	9/20	None
Human parainfluenza virus 4	2560526	11/21	12/29	10/20	None
Enterovirus D68	42789	13/21	10/29	9/20	None
Rhinovirus B14	12131	11/21	12/29	10/20	None
Human adenovirus 1	10533	13/21	9/29	9/20	None
Human metapneumovirus	162145	11/21	13/29	10/20	None
<i>Streptococcus pneumoniae</i>	1313	13/21	19/29	13/20	None
<i>Haemophilus influenzae</i>	727	12/21	18/29	12/20	None
<i>Chlamydophila pneumoniae</i>	83558	10/21	14/29	11/20	None
<i>Legionella pneumophila</i>	446	14/21	15/29	12/20	None
<i>Bordetella pertussis</i>	520	15/21	0/29	15/20	None

<i>Bordetella parapertussis</i>	519	14/21	12/29	12/20	None
Human bocavirus	329641	9/21	12/29	10/20	None

22 NCBI, National Center for Biotechnology Information; WT, wild type

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24 **Table S3** PCR results of 44 clinical specimens using the developed MGB probe-based PCR assay and PowerChek™

25 MPDR real-time PCR kit.

Sample #	MGB probe-based PCR				PowerChek™ Mp-DR real-time PCR kit			
	Channel-specific end-point fluorescence intensity (a.u.)			Interpretation	Channel-specific Ct value			Interpretation
	ATTO	FAM	HEX		ROX	FAM	VIC	
1	0.3	0.7	0.9	Negative	ND ^a	ND	ND	Negative
2	0.3	0.7	1	Negative	ND	ND	ND	Negative
3	0.3	0.7	1.1	Negative	ND	ND	ND	Negative
4	0.3	0.7	1	Negative	ND	ND	ND	Negative
5	1.1	3.5	2.9	Negative	ND	ND	ND	Negative
6	0.3	0.6	0.9	Negative	ND	ND	ND	Negative
7	0.3	0.9	1.3	Negative	ND	ND	ND	Negative
8	0.3	0.7	1	Negative	ND	ND	ND	Negative
9	0.2	0.5	0.8	Negative	ND	ND	ND	Negative
10	0.2	0.7	1	Negative	ND	ND	ND	Negative
11	0.2	0.5	0.8	Negative	ND	ND	ND	Negative
12	0.5	1.5	1.2	Negative	ND	ND	ND	Negative
13	0.3	0.7	0.9	Negative	ND	ND	ND	Negative
14	0.3	0.6	0.9	Negative	ND	ND	ND	Negative
15	0.3	0.6	0.8	Negative	ND	ND	ND	Negative
16	0.3	0.6	0.9	Negative	ND	ND	ND	Negative
17	0.3	0.7	1	Negative	ND	ND	ND	Negative
18	0.3	0.9	1.3	Negative	ND	ND	ND	Negative
19	0.3	0.8	1.2	Negative	ND	ND	ND	Negative
20	0.3	0.7	1.1	Negative	ND	ND	ND	Negative
21	0.3	0.6	1.3	Negative	ND	ND	ND	Negative
22	0.4	0.8	1	Negative	ND	ND	ND	Negative
23	44.7	12.3	3	WT	21.6	ND	ND	WT
24	32.4	8.3	2.9	WT	33.4	ND	ND	WT
25	48	12.9	3	WT	18.6	ND	ND	WT
26	6.3	26.3	2.1	A2063G	33.6	33.4	ND	A2063G
27	7.2	29	2.5	A2063G	23.1	23.4	ND	A2063G
28	7.3	27.1	2.5	A2063G	29.7	30	ND	A2063G
29	6.7	20.8	2.2	A2063G	27.9	28.1	ND	A2063G

30	7.1	25	2.6	A2063G	25.4	25.7	ND	A2063G
31	6.3	22.9	2.1	A2063G	28.5	28.8	ND	A2063G
32	9	19.5	2.9	A2063G	26.5	27	ND	A2063G
33	11.1	21.2	3	A2063G	25.8	25.4	ND	A2063G
34	9.2	18.7	2.6	A2063G	28.8	28.5	ND	A2063G
35	2.3	8.5	1.3	A2063G	34.6	34.5	ND	A2063G
36	7.8	29.7	2.8	A2063G	24.8	24.9	ND	A2063G
37	3.2	10.2	2	A2063G	33.7	34	ND	A2063G
38	9.8	16.2	2.4	A2063G	20.9	20.4	ND	A2063G
39	2.4	12.2	1.6	A2063G	36.2	37.1	ND	A2063G
40	10.5	23.3	2.7	A2063G	23.4	23.7	ND	A2063G
41	6.7	23.2	2.5	A2063G	28.9	29.3	ND	A2063G
42	5.6	20.9	2.5	A2063G	30.9	31.1	ND	A2063G
43	8.2	29.5	2.9	A2063G	23.2	23.2	ND	A2063G
44	6.7	23.1	3	A2063G	29.9	30	ND	A2063G

26 ^aND, not detected; MGB, minor groove binder; PCR, polymerase chain reaction; a.u., arbitrary units; Ct, cycle
27 threshold; MPDR, *Mycoplasma pneumoniae* drug resistance; TM, trademark.

28