

**Supplementary Table 1.** Sequence of the *Mycoplasma cynos* positive amplification control.

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AGTGTTCCACGTTCAACACGTCCAGTAGCAACTGTTCCACGTCCTGTAATTGTAAA  
TACGTCCTCAACAGCCATTAAGAATGGTTTGTTCATATTCTTTAACAGGTGTTTCAA  
TATATGAATCAACTGCGTCCATTAATTCTAATATTTTTTCTTCGTATTTAGCATCAC  
CTTCAAGTGCTTTTAAAGCTGAACCACGAATAATTGGCGCATTATCTCCATCAAAT  
CCATATTCTGATAAAAGGCTACGAATTTCAACTTCAACTAATTCAATCATTCTTCT  
TCACCTTCTAACATATCAACTTTGTTTAAAGAAAACAACGATACGCGGAACACCAAC  
TTGTTTAGATAAAAGAATGTGTTTCACGTGTTTGAGGCATAGGCCCATCTGTTGCAG  
CAACAA

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**Supplementary Table 2.** Standard curves demonstrating *Mycoplasma cynos* *tuf* assay

performance.

Platform/Day	Slope	$r^2$	Efficiency (%)
7500			
1	$-3.47 \pm 0.06$	0.993	94.3
2	$-3.37 \pm 0.06$	0.994	97.9
3	$-3.44 \pm 0.04$	0.997	95.4
OA			
1	$-2.72 \pm 0.05$	0.986	122
2	$-2.76 \pm 0.07$	0.978	119
3	$-2.76 \pm 0.05$	0.989	120

7500 = ABI 7500 qPCR platform (Thermo Fisher Scientific); OA = QuantStudio OpenArray platform (Thermo Fisher Scientific).

**Supplementary Table 3.** Intra-assay variation of cycle threshold (Ct) values on the ABI 7500 platform.

Day/Copies per $\mu$ l	Mean	SD	%CV (Ct)	%CV ( $2^{-Ct}$ )
1				
$10^2$	31.2	0.08	0.24	5.30
$10^3$	27.7	0.05	0.16	3.14
$10^4$	24.4	0.10	0.39	6.53
2				
$10^2$	30.4	0.05	0.17	3.57
$10^3$	27.0	0.07	0.27	5.21
$10^4$	23.6	0.04	0.19	3.06
3				
$10^2$	31.2	0.08	0.27	5.86
$10^3$	27.9	0.10	0.38	7.25
$10^4$	24.5	0.06	0.24	4.06

CV = coefficient of variation; SD = standard deviation

**Supplementary Table 4.** Intra-assay variation of cycle threshold (Ct) values on the OA platform.

Day/Copies per $\mu$ l	Mean	SD	%CV (Ct)	%CV ( $2^{-Ct}$ )
1				
$10^2$	11.8	0.32	2.72	20.9
$10^3$	9.0	0.27	2.98	16.8
$10^4$	5.8	0.27	4.70	17.3
2				
$10^2$	11.8	0.51	4.35	32.2
$10^3$	8.7	0.32	3.69	20.7
$10^4$	5.7	0.47	8.30	34.2
3				
$10^2$	11.9	0.27	2.24	16.6

10 <sup>3</sup>	8.8	0.28	3.20	17.0
10 <sup>4</sup>	5.9	0.29	4.91	17.2

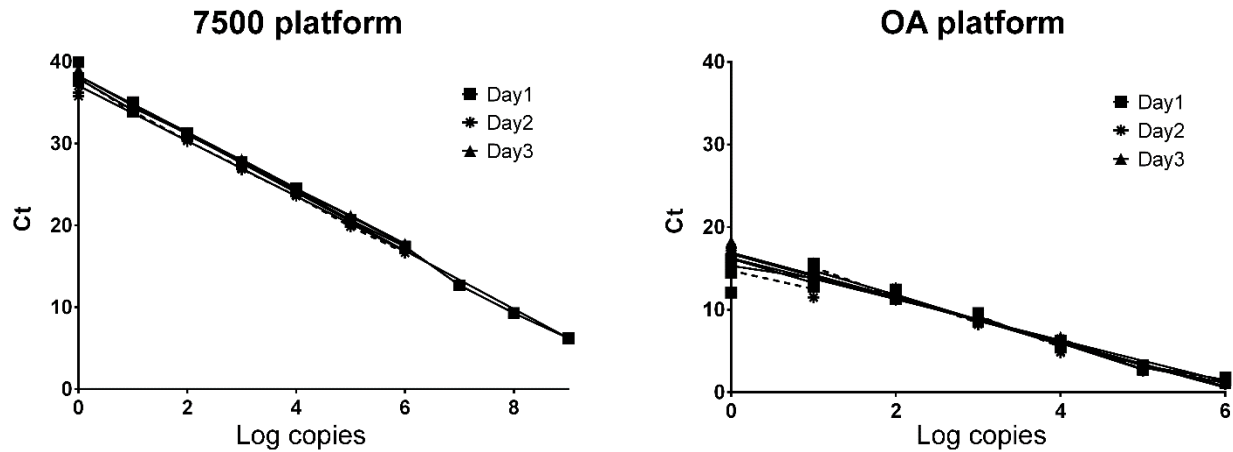
CV = coefficient of variation; OA = QuantStudio OpenArray platform; SD = standard deviation.

**Supplementary Table 5.** Inter-assay variation of cycle threshold (Ct) values.

Platform/Template concentration (per $\mu$ l)	Mean	SD	%CV (Ct)	%CV ( $2^{-Ct}$ )
7500				
10 <sup>2</sup>	30.9	0.49	1.60	36.7
10 <sup>3</sup>	27.5	0.49	1.79	35.9
10 <sup>4</sup>	24.2	0.49	2.03	36.1
OA				
10 <sup>2</sup>	11.9	0.05	0.44	5.3
10 <sup>3</sup>	8.8	0.14	1.55	9.9
10 <sup>4</sup>	5.8	0.12	2.10	10.3

CV = coefficient of variation; OA = QuantStudio OpenArray platform; SD = standard deviation;

7500 = ABI 7500 qPCR platform.



**Supplementary Figure 1.** *Mycoplasma cynos* standard curve dilution series. The *M. cynos* positive amplification control was serially diluted and then amplified with the *tuf* assay on the ABI 7500 and OpenArray (OA) platforms. The log of the *M. cynos* positive amplification control copy number is plotted on the x-axis and the cycle threshold (Ct) is plotted on the y-axis. Dilutions of 0.1 and 0.01 copies per  $\mu\text{L}$  were consistently not detected (not shown).