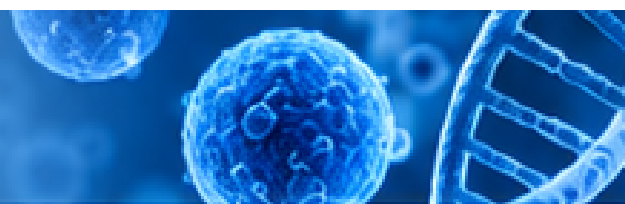




Mycoplasma nucleic acid (DNA) detection

Performance Evaluation





1. Detection Limit

1: Sample preparation

- 1) Take 6 sensitivity samples, dissolve and mix them with 6mL of diluent to prepare a 100 CFU/mL sample;
- 2) Take 9 mL of diluent and 1 mL of 100 CFU/mL sample to prepare a 10 CFU/mL sample;
- 3) Take 5 mL of diluent and 5 mL of 10 CFU/mL sample to prepare a 5 CFU/mL sample;
- 4) Take 5 mL of diluent and 5 mL of 5 CFU/mL sample to prepare a 2.5 CFU/mL sample;
- 5) Take 5 mL of diluent and 5 mL of 2.5 CFU/mL sample to prepare a 1.25 CFU/mL sample.

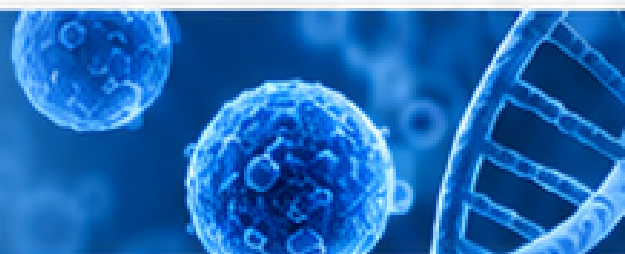
1.1: Sample addition and detection

The samples (100 CFU/mL, 10 CFU/mL, 5 CFU/mL, 2.5 CFU/mL, 1.25 CFU/mL) were tested according to the standard procedure of the reagent kit. Each sample was tested 20 times, with a negative control set up for each test.

1.2: Results

The sensitivity evaluation results are as follows:

serial number	Detect CT values at different concentrations (CFU/ mL)									
	100		10		5		2.5		1.25	
1	27.6	26.62	30.74	29.33	31.78	31.48	32.96	33.9	35.83	36.93
2	25.82	25.72	30.65	30.45	31.58	30.51	34.04	34.69	35.67	36.69
3	27.54	25.94	30.26	30.71	31.06	30.95	32.93	34.75	35.17	35.55
4	26.76	26.28	29.95	29.31	31.77	31.93	32.4	34.43	36.83	36.76
5	26.88	27.65	29.85	30.17	30.25	30.15	32.94	33.99	35.44	36.77
6	27.96	27.5	30.18	30.68	30.22	30.86	32.26	32.75	34.85	35.13
7	25.65	25.51	30.56	29.32	30.15	31.54	34.78	33.29	34.6	35.69
8	27.16	26.98	30.14	29.45	30.63	31.48	34.71	33.08	∞	34.58
9	26.71	26.79	30.16	29.8	31.59	31.92	32.35	32.3	36.76	36.61
10	25.28	27.82	30.26	30.65	30.09	31.06	32.05	32.08	34.42	35.33
negative detection	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
rate	100%		100%		100%		100%		95%	



Based on the above results, we can see that samples with a concentration of 2.5 CFU/mL can be stably detected as positive. We also analyzed experimental data from other experiments and further supported our conclusion. After summarizing a large amount of statistical data, we conservatively set the detection sensitivity of the reagent kit at 2.5 CFU/mL.

2: Repetitiveness

2.1 Sample addition and detection

The samples (enterprise reference products B065-R1 and B065-R2) were tested according to the standard procedure of the reagent kit. Each sample was tested 10 times, with a negative control set up for each test.

2.2 Result

The results of the repeatability evaluation are as follows:

serial number	R1		R2	
	FAM	ROX	FAM	ROX
1	23.93	23.48	28.96	28.45
2	22.85	22.36	27.89	26.46
3	22.58	22.55	29.56	29.38
4	22.06	21.72	29.51	28.45
5	24.06	24.43	27.95	26.13
6	23.39	23.36	28.72	28.43
7	23.01	23.79	29.55	28.95
8	22.44	21.74	28.03	26.51
9	24.60	24.61	28.94	28.04
10	22.22	22.69	28.65	28.44
negative	∞	∞	∞	∞
CV	3.70%	4.45%	2.27%	4.07%

Based on the test results of the aforementioned samples, it can be observed that the repeatability is within 5%. We also analyzed experimental data from other experiments and further substantiated our conclusions. After summarizing a substantial amount of statistical data, we conservatively set the intra-batch precision of the reagent kit at 5%.

3.0 Specificity evaluation data

3.1 Sample addition and detection

The detection of samples (*Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*) was conducted according to the standard procedure of the reagent kit. Each sample was tested four times, with negative and positive controls (B065-R2) set up for each test.

3.2 Result

The results of the specific cross-evaluation are as follows:

serial number	Escherichia coli		Staphylococcus aureus		Candida albicans	
	FAM	ROX	FAM	ROX	FAM	ROX
1	∞	∞	∞	∞	∞	∞
2	∞	∞	∞	∞	∞	∞
3	∞	∞	∞	∞	∞	∞
4	∞	∞	∞	∞	∞	∞
negative	∞	∞	∞	∞	∞	∞
positive	28.96	28.45	28.96	28.45	28.96	28.45