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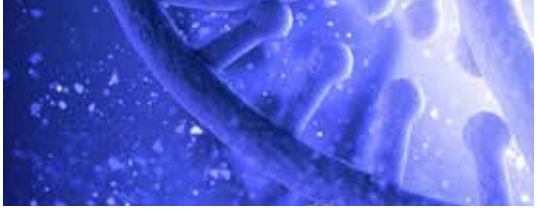

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USE OF QUANTITATIVE MYCOPLASMA DNA CONTROLS IN EVALUATING THE SENSITIVITY OF MOLECULAR-BASED DETECTION SYSTEMS

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Abstract

Novel molecular-based methods offer an effective and efficient means for the detection of mycoplasma contamination in cell cultures. However, for these methods to become widely accepted in research laboratories, they must demonstrate sensitivity, particularly with regard to the limit of detection. Here, we describe the use of quantitative mycoplasma DNA controls in the evaluation of two molecular-based detection systems.

Introduction

Mycoplasma contamination affects roughly 15-35% of continuous cell cultures, resulting in a number of deleterious effects including the induction of chromosomal abnormalities, the disruption of DNA and RNA synthesis, and the inhibition of both cell metabolism and growth rate[1–3]. Historically, the recommended mycoplasma testing protocol for research laboratories included the use of traditional culture-based techniques and DNA staining with fluorochromes[4]. Despite the successful use of these detection methods for many years, their limit of detection remains undefined. Moreover, the entire testing procedure is laborious, costly, and difficult to interpret. However, before an alternative method, such as a molecular-based detection system, can become widely accepted and implemented, this method must exhibit sensitivity with regard to the limit of detection.

To this end, the American Type Culture Collection (ATCC) has designed quantitative mycoplasma DNA controls for use in testing and calibration in ISO 17025 accredited laboratories, inclusivity/exclusivity testing, establishing limits of detection, verification or comparison of test methods, and other molecular applications. Here, we demonstrate the use of these products in evaluating the sensitivity of two different molecular-based detection systems.

Establishing the Sensitivity of Molecular-Based Detection Systems

The limit of detection was established for two molecular-based detection systems comprising a quantitative mycoplasma DNA control (*Acholeplasma laidlawii*, *Mycoplasma orale*, or *Mycoplasma hominis*), a proprietary reaction mixture containing pre-evaluated 16S rRNA-specific primers and probes, and either the Bio-Rad® QX100 Droplet Digital™ PCR (ddPCR) or CFX96 Touch™ Real-Time PCR (QPCR) platform. For each control, predicted input quantities of 1, 10, 100, 1000, 10000, and 100000 were measured using both systems, and the observed sample copy number was plotted (Figure 1). Each assay was performed twice (N=2), and the individual input quantity for each sample was tested in triplicate (n=6). The total mean and standard deviation of the combined samples were calculated at a 95% confidence interval using JMP® Software. (Table 1).

At each of the predicted input copy numbers, the mean observed copy number for the combined samples fell within the 95% confidence interval. In particular, for predicted input copy numbers of 1 and 10, the mean observed copy number was statistically closer in value to the predicted copy number when using the QPCR platform. These results indicate that both molecular-based detection systems are able to sensitively detect and quantify mycoplasma DNA, with the QPCR platform exhibiting more accuracy with lower concentrations of genomic material.

Conclusion

The use of quantitative external DNA controls provides an effective approach for the development and evaluation of novel molecular-based assays for the detection of mycoplasma contamination. Quantitative mycoplasma DNA controls are currently available as certified reference materials from ATCC under the catalog numbers qCRM-15531D, qCRM-17981D, qCRM-19610D, qCRM-19989D, qCRM-23064D, qCRM-23206D, qCRM-23714D, qCRM-23838D, qCRM-25204D, and qCRM-27545D.

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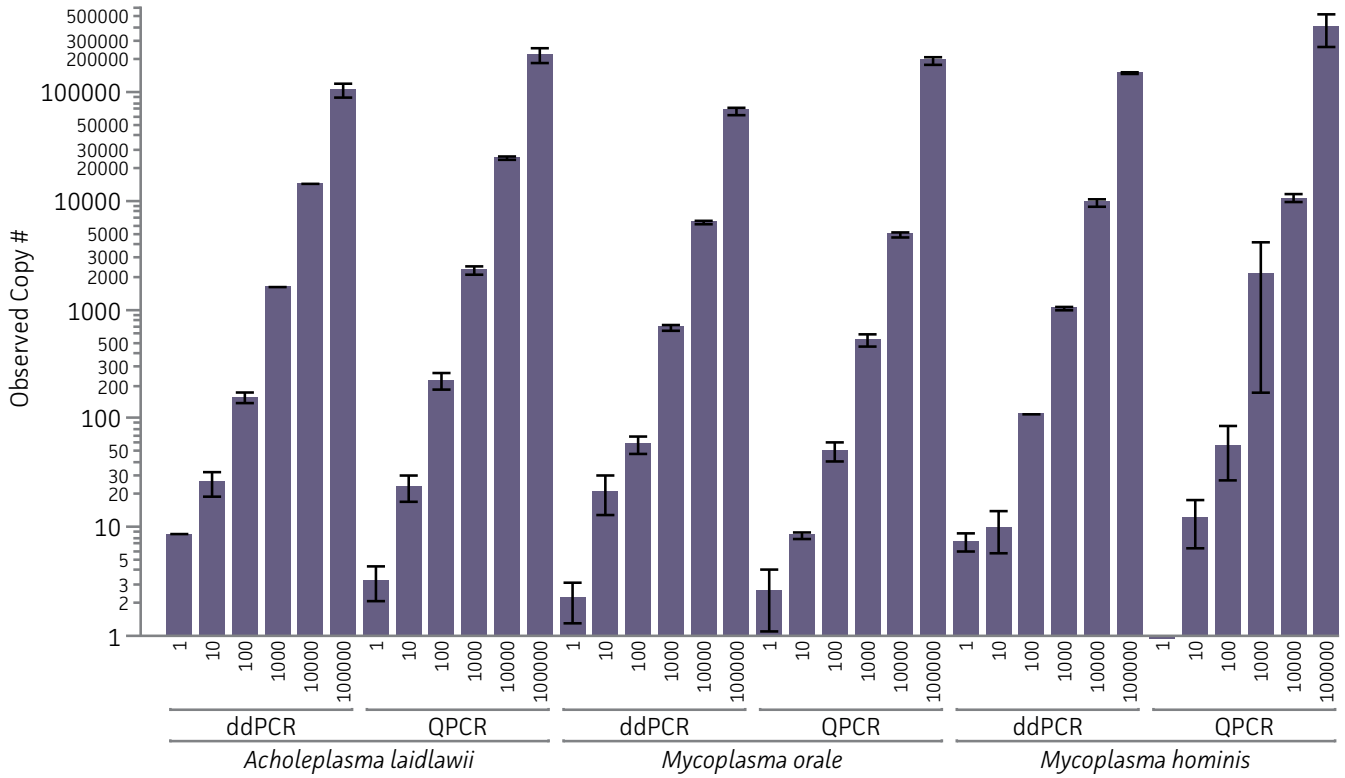

Figure 1. Observed sample copy number relative to predicted input copy number by ddPCR and QPCR for three quantitative mycoplasma DNA controls

Table 1. Observed total mean sample copy number relative to predicted input copy number measured by ddPCR and QPCR (N=2, n=6).

Platform	Predicted Copy #	Mean	Standard Deviation	Confidence Interval (95%)
ddPCR	1	6.21	3.11	3.82 - 8.61
QPCR	1	2.07	1.71	0.766 - 3.39
ddPCR	10	16.61	9.37	12.40 – 26.81
QPCR	10	15.16	8.36	8.74 – 21.59
ddPCR	100	110.78	44.51	76.57 – 144.99
QPCR	100	113.89	30.51	43.54 – 184.20
ddPCR	1000	1145.78	422.40	821.09 – 1470.05
QPCR	1000	1728.94	1367.80	677.56 – 2780.30
ddPCR	10,000	10408.90	3542.83	7685.60 – 13135
QPCR	10,000	13741.70	9094.59	6751 – 20732
ddPCR	100,000	111122	38150	81798 - 140447
QPCR	100,000	280089	119594	188161 – 372017

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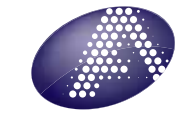

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