

Simultaneous extraction of mycoplasma, mouse minute virus (MMV), and vesivirus nucleic acids from a single sample

Abstract

Early detection of cell culture contamination is critical to prevent further contamination of facilities and requires fast, reliable assay methods. Here we describe an integrated nucleic acid extraction system that provides quantitative recovery of mycoplasma, MMV, and vesivirus nucleic acids from a single sample. When coupled with real-time PCR, a fast and accurate solution is enabled with a low limit of detection (LOD).

Introduction

Contamination is the most common problem and primary concern of cell culture facilities. Contamination, whether chemical (e.g., media impurities and detergents) or biological (e.g., bacterial, mycoplasmal, fungal, viral, and cross-contamination), poses serious consequences for human safety, product quality, and lost revenue.

Mycoplasmal contamination, first described in 1956 [1], is the most prevalent type of microbial contamination throughout the biopharmaceutical process from research and development to production. It is estimated that 15–35% of established cell cultures are contaminated with mycoplasma [2]. Viral contamination of cell culture and biopharmaceutical products is of equal concern [3]; numerous facilities have been negatively impacted due to contamination with viruses such as vesivirus and mouse minute virus (MMV) [4,5]. Mycoplasmal and viral contaminations are difficult to detect and may reach high titer levels before any detrimental effects are visible in the cell culture. Early detection using rapid, reliable, and accurate assays is critical in order to protect facilities from further contamination.

The Applied Biosystems™ MycoSEQ™ Mycoplasma Detection Kit, ViralSEQ™ MMV Detection System, and ViralSEQ™ Vesivirus Detection Kit are real-time PCR assays designed for early detection of mycoplasma,



Figure 1. The AutoMate Express Nucleic Acid Extraction System.

MMV, and vesivirus. These assays, when coupled with the Applied Biosystems™ PrepSEQ™ Express Nucleic Acid Extraction Kit, provide an integrated system for rapid and sensitive detection of over 90 strains of mycoplasma, 4 known strains of MMV, and all known strains of vesivirus, including vesivirus 2117.

The Applied Biosystems™ AutoMate Express™ Nucleic Acid Extraction System (Figure 1) and the PrepSEQ™ Express Nucleic Acid Extraction Kit offer hands-free automation of the nucleic acid extraction process, providing quantitative and reproducible recovery of nucleic acids from complex samples.

Nucleic acids from mycoplasmas, MMV, and vesivirus may be extracted from a single sample, and real-time PCR performed on the Applied Biosystems™ 7500 Fast Real-Time PCR System providing results in under 5 hours (Figure 2). In this application note, we describe the use of the AutoMate Express Nucleic Acid Extraction System with the PrepSEQ Express Nucleic Acid Extraction Kit to perform simultaneous extraction of nucleic acids from mycoplasma, MMV, and vesivirus spiked into a cell culture matrix or cell culture medium.



Figure 2. Workflow for nucleic acid extraction and analysis.

Materials and methods

Nucleic acids from mycoplasma, MMV, and vesivirus were spiked into cell culture containing 2×10^4 , 2×10^5 , and 2×10^6 cells/mL and extracted simultaneously using the AutoMate Express Nucleic Acid Extraction System (Cat.

No. 4467754) with the PrepSEQ Express Nucleic Acid Extraction Kit (Cat. No. 4466351). Real-time PCR reactions were prepared using the MycoSEQ Mycoplasma Detection Kit (Cat. No. 4460626), ViralSEQ MMV Detection System (Cat. No. 4444415), and ViralSEQ Vesivirus Detection Kit (Cat. No. 4448398C). Real-time PCR was performed on the 7500 Fast Real-Time PCR System operating Applied Biosystems™ AccuSEQ™ Real-Time PCR Software.

The limit of detection (LOD) was determined by spiking a 10-fold dilution series of nucleic acids from mycoplasma, MMV, and vesivirus into cell culture containing 2×10^6 cells/mL, and the nucleic acids were simultaneously extracted using the AutoMate Express system as previously described. Real-time PCR was performed as previously described.

Results

Nucleic acids from mycoplasma, MMV, and vesivirus were spiked into phosphate-buffered saline (PBS) or cell culture containing 2×10^4 , 2×10^5 , and 2×10^6 cells/mL and extracted simultaneously using the AutoMate Express system. Real-time PCR was performed, and the average C_t value of each PCR assay was plotted (Figure 3). The consistent results across all samples and cell densities are indicative of the robustness of the assay.

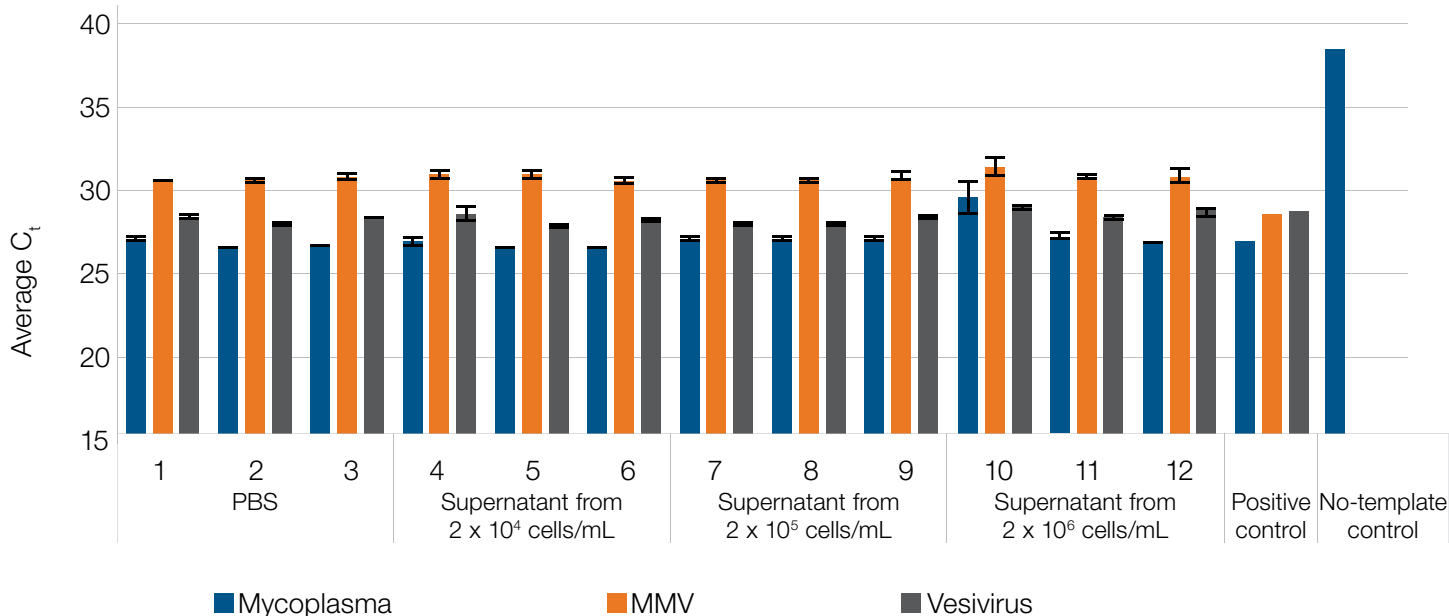


Figure 3. Average C_t values of mycoplasma, MMV, and vesivirus PCR reactions. Nucleic acids were spiked into cell culture containing 2×10^4 , 2×10^5 , and 2×10^6 cells/mL and extracted simultaneously.

A 10-fold dilution series of nucleic acid from mycoplasma, MMV, and vesivirus was also spiked into a cell culture containing 2×10^6 cells/mL and extracted simultaneously using the AutoMate Express system. Real-time PCR was performed as previously described. The average C_t value from each PCR assay was then plotted (Figure 4). The LOD per PCR reaction was determined and is shown in Table 1. As expected, C_t values increase as nucleic acid concentration decreases. The consistent results across replicates within a dilution series are indicative of the repeatability and robustness of the assay.

Table 1. LOD per PCR reaction of mycoplasma, MMV, and vesivirus real-time PCR assays.

Organism	LOD per PCR reaction (after extraction)
Mycoplasma	2 genome copies
MMV	10 genome copies
Vesivirus	20 genome copies

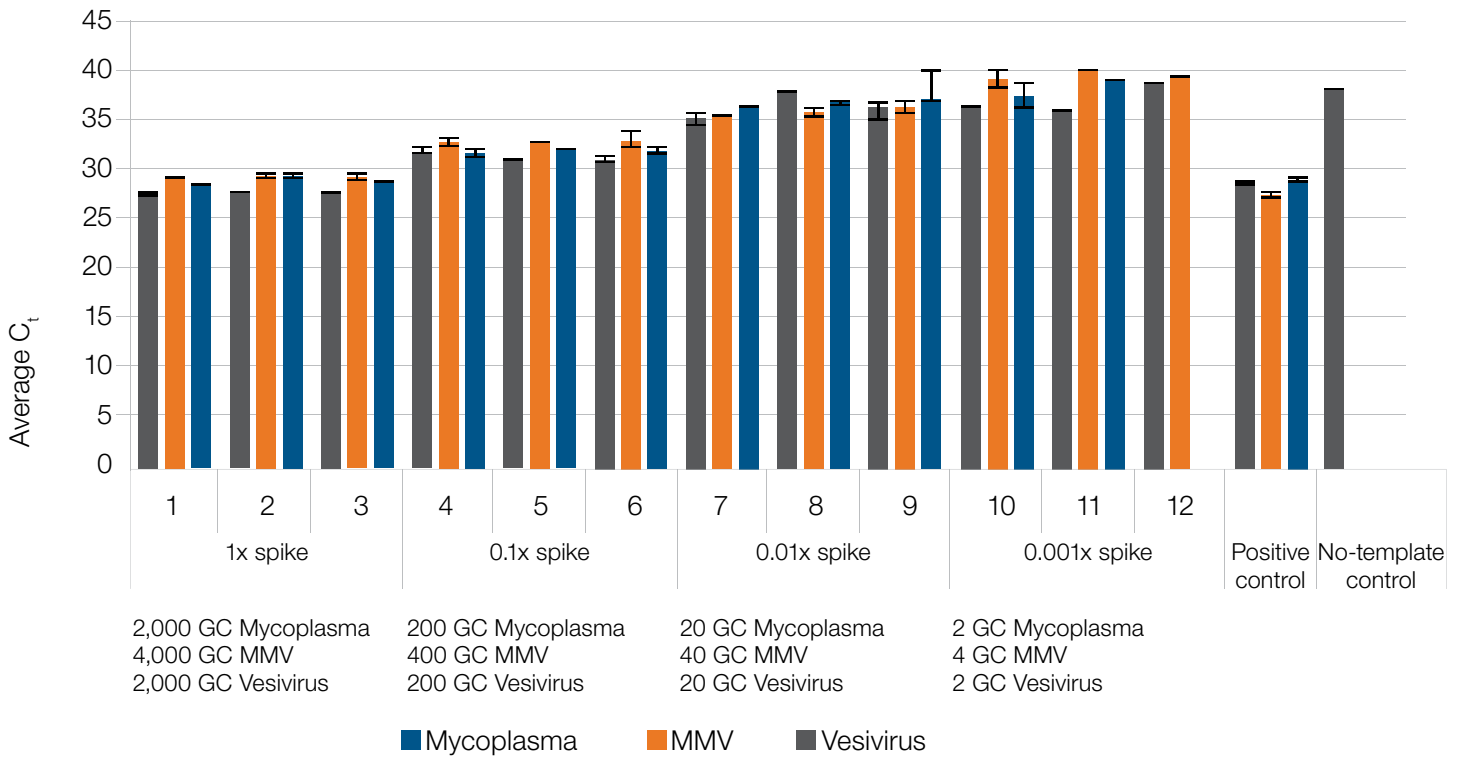


Figure 4. Average C_t values from dilution series of mycoplasma, MMV, and vesivirus nucleic acids. Nucleic acids were spiked into a cell culture containing 2×10^6 cells/mL and extracted simultaneously. The numbers of genome copies (GC) used for each spike are indicated.

Summary

Nucleic acids from mycoplasma, MMV, and vesivirus were successfully extracted from cell culture using the AutoMate Express system and analyzed by real-time PCR. The results indicate that this integrated system enables highly sensitive detection, with an LOD of 2 genome copies per PCR reaction for mycoplasma, 10 genome copies per PCR reaction for MMV, and 20 genome copies per PCR reaction for vesivirus. The results were highly consistent in the presence of varying cell densities and within each dilution, indicating a highly robust and repeatable assay. Implementation of highly sensitive, rapid methods are critical for early detection and remediation of costly cell culture contamination.

References

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