APPLICATION OF REAL-TIME PCR FOR ROUTINE MYCOPLASMA SURVEILLANCE IN CELL LINES USED IN VIROLOGY LABORATORIES

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Abstract

Mycoplasma contamination compromises cell culture reliability, physiology, and reproducibility, making rapid and sensitive detection essential for quality research. This study assessed Mycoplasma contamination in routinely maintained cell lines using real-time PCR and evaluated Oxford Nanopore sequencing for genomic characterization of a Mycoplasma-positive patient sample. DNA from in-use BHK-21 and C6/36 cell lines was extracted using a silica membrane-based method and screened for Mycoplasma by real-time PCR targeting the 16S–23S rRNA spacer region. Both tested cell lines produced negative results, suggesting the absence of detectable Mycoplasma nucleic acids. During the experimental period, fungal contamination compatible with Candida spp. was identified in old and newly maintained C6/36 cultures by manual microscopic identification using Gram staining in Clinical Bacteriology and Molecular Biology. The fungal contamination was not investigated or confirmed by Nanopore sequencing. To avoid possible cross-contamination, the newly acquired BHK-21 cell line was kept untouched and was not subjected to Mycoplasma detection; therefore, its Mycoplasma status could not be assessed. Oxford Nanopore sequencing was performed exclusively on a Mycoplasma-positive patient sample and did not involve the BHK-21 or C6/36 cell lines. Prior to sequencing, conventional PCR followed by agarose gel electrophoresis showed no visible amplification band. However, subsequent real-time PCR produced a characteristic amplification curve, confirming the presence of Mycoplasma DNA. Following real-time PCR confirmation, the sample was subjected to whole-genome sequencing using the Oxford Nanopore MinION platform and analyzed using the Oxford Nanopore bacterial genome workflow. Although sequencing generated insufficient read depth for complete genome assembly and species-level characterization, the findings demonstrate the sensitivity and reliability of real-time PCR for Mycoplasma detection and emphasize the importance of molecular confirmation before downstream genomic analysis.

Keywords: Mycoplasma contamination, Cell culture, Oxford Nanopore Technology, Whole genome sequencing, Virology laboratory