

A Novel Method for Nuclei Exclusion in Whole Blood Microbial Sample Preparation

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The field of molecular diagnostics has seen great advances in the last 10 years; however, the difficulty of sampling microbial targets from human blood has been a significant obstacle. Nucleic acid-based methods for detecting low numbers of microbial species in whole blood often require sample concentration to achieve an acceptable limit of detection (LOD). Due to the large contribution of genomic DNA from whole blood, however, concentrating a sample to detect rare targets can be difficult. We have developed a simple yet effective method to selectively remove mammalian genomic DNA from a whole blood sample, while allowing for the purification of microbial targets.

We used a hypotonic lysis buffer with a non-ionic detergent to selectively lyse the outer membrane of mammalian cells, while keeping the nuclear membrane intact. Nuclei were then removed from the lysate by filtration. Total DNA extracted from filtrates was analyzed using qPCR assays specific for nuclear DNA and for bacterial target DNA, fluorescence spectroscopy using Pico Green dye, and gel electrophoresis. To confirm that nuclei remained intact during the cell lysis procedure, lysates were stained prior to filtration with two fluorescent DNA binding dyes, Hoechst 3342 which is membrane permeable, and Propidium iodide which is membrane impermeable.

The dual fluorescent nuclear staining procedure showed that the efficiency of cell lysis in the hypotonic lysis buffer was 97%. The Pico Green assay results showed that 70% (SD = 11.5%) of the total DNA was removed with filtration technique. The qPCR results showed that 98.6% (SD = 0.12%) of the mammalian genomic DNA was removed, while 66% (SD = 12%) of the bacterial DNA was recovered. Agarose gel analysis of the DNA extracts showed no visible genomic DNA band in the filtered samples and clearly visible bands in the non-filtered samples.

We conclude that this simple lysis and filtration procedure is an effective method for excluding mammalian DNA during preparation of whole blood samples. With the mammalian DNA largely removed from the sample, it is much easier to concentrate the remaining DNA and achieve lower LOD in molecular diagnostic assays.

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