

G163 Comparative Analysis of Size Specific DNA Concentrators Following Organic DNA Extraction

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After attending this presentation, attendees will understand some principles of DNA analysis, the differences between Sartorius Stedim Vivacon and Millipore Amicon concentrators, and how the addition of PCR inhibitory substances influences the recovery of DNA following PCIA organic extraction. Attendees will be introduced to principles behind organic DNA extraction methodologies, including the practical benefits of selecting function-specific DNA concentrators. The current study will demonstrate the efficiency of DNA recovery using four commercially available centrifugal DNA concentrators following PCIA organic DNA extraction of forensic type samples.

This presentation will impact the forensic science community by presenting data suggesting the practical benefit of size-specific DNA concentrators for the optimal recovery of DNA from forensic casework type samples. The results of this study demonstrate a significant difference in concentrator brands' abilities to recover nucleic acids, which may cause forensic laboratories to reconsider which concentrator units are currently being utilized.

The use of organic extractions followed by centrifugal concentrators for the purification of DNA is an important tool for forensic laboratories. Centrifugal concentrators both remove PCR inhibitory substances and concentrate the nucleic acid in a sample.^{1,2} They are designed to speed up the evaporation of solvents from DNA samples so that purified DNA can be collected and further tested. The objective of the current study was to determine which centrifugal concentrator pore size would recover the most DNA by analyzing samples of whole blood dilutions in deionized water ranging in concentration from 0.2 - 40%. Internal Positive controls for all samples were compared in order to assess the efficiency with which each concentrator removed inhibitory substances.

The purpose of this study was to determine which DNA concentrator (filter and pore size) was better suited for organic extractions based on percent recovery and quality of profile generated. The two DNA concentrator brands under evaluation included Sartorius Stedim Vivacon 500 (size YM-50 and YM-100 Nominal Molecular Weight Limit in Daltons) (Goettingen, Germany) and Millipore Amicon^å Ultra (size 50 and 100 NMWL) (Darmstad, Germany). The filter membranes of Amicons have a vertical design allowing for a greater membrane surface area, promising to provide fast sample processing and high sample recovery.³ The Vivacon filters utilize a horizontal filter design which is equipped with the patented regenerated cellulose Hydrosart^å membrane.⁴

In brief, compared to Vivacon filters, Amicon filters demonstrated reduced DNA recovery, on average recovering 52% less nucleic acids. Furthermore, the data indicated decreased amplification efficiency indicating a failure to remove all inhibitory substances. The use of Vivacon 100 NMWL concentrators following PCIA organic extraction has the practical benefit of optimum recovery of DNA and total removal of PCR inhibitory substances, which is particularly advantageous when dealing with forensic samples.

References:

- ^{1.} Beckwith M, Backer A, Robertson A, Phillips W, Jimenez M, Baldwin B. The Role of Ultrafiltration Membranes In The Recovery of DNA With Centrifugal Concentrators. Sartorius Stedim Lab, Itd., San Antonio, Texas. Poster
- ² Beckwith, M. Side by Side Comparison and Validation of the Vivacon, Microcon and Amicon Ultra 0.5 Centrifuge Filtration Devices. Mid America Forensic DNA Conference, Toronto, Canada. (2010).
- ^{3.} Amicon Ultra-0.5 Centrifugal Filter Devices: for volumes up to 500mL. *Millipore Corporation*. (2009): 1-25.
- ^{4.} Technical data and operating instructions: Vivacon 500. Sartorius Stedim. (2010): 1-7.

Forensic Science, DNA Analysis, Concentrator