

THE ROLE OF ULTRAFILTRATION MEMBRANES IN THE RECOVERY OF DNA WITH CENTRIFUGAL CONCENTRATORS

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The use of organic extractions followed by diafiltration using centrifugal concentrators for the purification of DNA remains an important tool for forensic laboratories. The purpose of the centrifugal concentrators utilizing ultrafiltration membranes is to both wash away PCR inhibitory substances (such as hematin, humic acids, dyes, detergents, etc.) and also concentrate the nucleic acid in the sample. Therefore, the concentrator has two functions, first to allow low molecular weight inhibitory substances to pass into the filtrate while at the same time retaining the DNA above the membrane in a form that is recoverable. Factors such as membrane type, membrane orientation, and membrane area do not seem to make a large difference in some samples with either high amounts of DNA and/or low amounts of PCR inhibitors. However, for other samples, such as when trace quantities of nucleic acids need to be recovered in the presence of PCR inhibitors, these factors play an important role.

Although polyethersulfone (PES) membranes work well with proteins, membranes made from modified regenerated cellulose (such as Hydrosart®) offer better recoveries of nucleic acids. Membrane area is relevant because nonspecific binding of the sample to the membrane is proportional to membrane area. For the recovery of trace quantities of DNA, less membrane area is better, even with the sacrifice of increased centrifugation time. One surprising finding is that the orientation of the membrane in the centrifugation device may also play a role. We have found that devices that have horizontal oriented membranes offer better recovery and improved removal of inhibitory substances than concentrators with membranes in the near vertical orientation. Moreover, adequate diafiltration of the sample is important to remove substances that are inhibitory to PCR. Simply concentrating the DNA after an organic extraction is not enough, several diavolumes of buffer are necessary to wash the inhibitory substances through the membrane in order to get the high quality STR profiles.