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The Role Of Ultrafiltration Membranes In The Recovery Of DNA With Centrifugal Concentrators

Michelle Beckwith[1], Andrew Backer[1], Andrew Robertson[1], Whitney Phillips[1], Malena Jimenez[2], Brittany Baldwin[2], and Michael Vagell[3]
[1]Paternity Testing Corporation, Columbia, MO,; [2]Missouri State Highway Patrol, Crime Laboratory Division, Jefferson City, MO;
[3]Sartorius Stedim North America, Inc., Bohemia, NY
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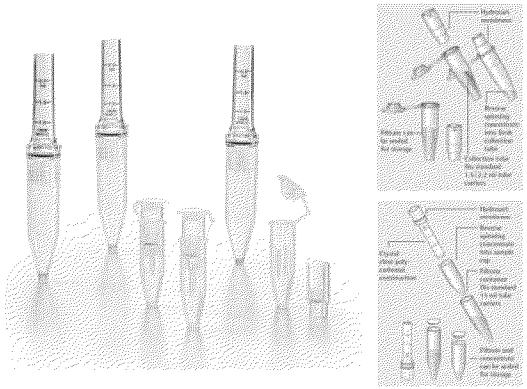
Abstract

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.

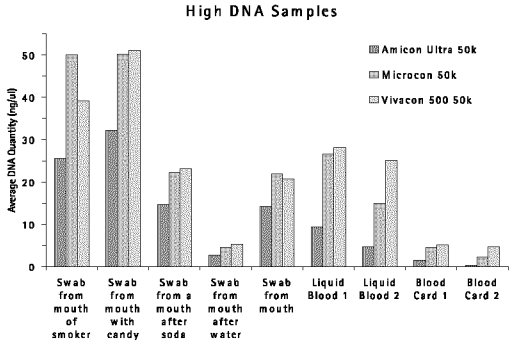
Although polyethersulfone (PES) membranes work well with proteins, membranes made from modified regenerated cellulose (such as Hydrosart®) offer better recoveries of nucleic acids. Membrane area is relevant because nonspecific binding of the sample to the membrane is proportional to membrane area. For 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 short tandem repeat (STR) profiles.

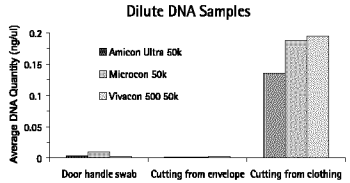
Vivacon® 500 and 2 Concentrators



Experiment 1: Recovery of DNA

Samples were extracted by Paternity Testing Corporation (PTC) according to the organic extraction method validated at that laboratory. 100 µl of the extract was split between the Amicon® Ultra 0.5 50k, Microcon® 50k, and the Vivacon® 500 50k. These samples were quantified in duplicate using Quantifiler™ Human at the Missouri State Highway Patrol (MSHP) Crime Laboratory, Jefferson City location. They were not amplified.





Conclusion

Recovery of concentrated DNA from the Vivacon® 500 is comparable to the Microcon®, but better than the Amicon® Ultra. Possible reasons for the difference in DNA recovery could be due greater membrane area in the Amicon® Ultra 0.5 or its membrane orientation (near vertical).

Experiment 2: DNA Profiles

Vaginal swabs were collected in duplicate approximately 8 and 36 hours post-coital. Approximately ½ of each swab was extracted using the MSHP differential extraction procedure, generating eight total samples (four sperm and four nonsperm). Nonsperm samples were extracted with an extraction buffer containing sodium dodecyl sulfate (SDS) and sexual assault samples were extracted using an extraction buffer containing Sarkosyl. 100 µl of the extracts were split between the Amicon® Ultra 50k, the Microcon® 50k, and the Vivacon® 500 50k. These samples were quantified in duplicate using Quantifiler™ Human and amplified using the average quantification value at the Promega PowerPlex™16 genetic loci. They were injected for five seconds on an Applied Biosystems™ 3130 Genetic Analyzer.

Sample Name	Device	Sample Type	Average Quantity (ng/µl)	Amp. Amount (µl)	Profile Obtained
#1	Amicon Ultra 50k	Nonsperm	117.8	dil - 1:118	full
	Microcon 50k		196.17	dil - 1:196	full
	Vivacon 500 50k		121.51	dil - 1:121	full
#2	Amicon Ultra 50k		166.41	dil - 1: 166	full
	Microcon 50k		300.07	dil - 1:300	full
	Vivacon 500 50k		310.67	dil - 1:310	full
#1	Amicon Ultra 50k		46.83	dil - 1:46	full
	Microcon 50k		60.21	dil - 1:60	full
	Vivacon 500 50k		55.65	dil - 1:56	full
#2	Amicon Ultra 50k		58.61	dil - 1:58	full
	Microcon 50k		89.21	dil - 1:89	full
	Vivacon 500 50k		81.15	dil - 1:81	full
#1	Amicon Ultra 50k	Sperm	0.4	2.5	partial (8/16)
	Microcon 50k		0.95	1	full
	Vivacon 500 50k		1	1	full
#2	Amicon Ultra 50k		0.61	2	partial (14/16)
	Microcon 50k		1.34	1	full
	Vivacon 500 50k		1.53	1	full
#1	Amicon Ultra 50k		0.01	19.2	none
	Microcon 50k		0.03	19.2	full
	Vivacon 500 50k		0.03	19.2	partial (14/16)
#2	Amicon Ultra 50k		0.02	19.2	none
	Microcon 50k		0.04	19.2	full
	Vivacon 500 50k		0.04	19.2	full

Conclusion

The Vivacon® 500 50k performed comparably to the Microcon® 50k in regards to sample yields and the profiles developed from both the sperm and non-sperm fractions of semen containing samples. The samples concentrated using Amicon® Ultra 50k filtration devices generally had lower yields than those concentrated with the other filters. Additionally, the sperm fraction from several samples concentrated using Amicon® Ultra 50k filtration devices failed to produce a full profile while their Microcon® 50 and Vivacon® 500 50kDa counterparts did. This was similar to the amplification inhibition observed in casework samples.

Experiment 3: Consistent Recovery

A 0.04 ng/µl solution of 9947A human control DNA was used in this experiment. Each type of extraction reagent with concentrations mimicking casework samples were processed through a phenol-chloroform extraction step and then concentrated. 400 µl of the Straight Extraction and Sperm (S) Extraction Reagents and 450 µl of the Nonsperm (NS) Extraction Reagents were placed into the Vivacon® 500 50 kDa ultrafiltration device. These samples were quantified in duplicate using Quantifiler™ Human and amplified using the average quantity at the Promega PowerPlex™ 16 genetic loci. They were injected for five or ten seconds on an Applied Biosystems™ 3130 Genetic Analyzer.

Sample Name	Average Quant (ng/µl)	IPC CT Value	Amp. Amount (µl)	Total input (ng)	Profile Obtained
1 Straight Ext Reagents	0.0566	27.85	18	1.02	full
2 Straight Ext Reagents	0.0353	27.89	19.2	0.68	full
3 Straight Ext Reagents	0.0267	27.96	19.2	0.51	full
1 NS Ext Reagents	0.0316	27.98	19.2	0.61	none
2 NS Ext Reagents	0.0322	27.9	19.2	0.62	none
3 NS Ext Reagents	0.0302	27.99	19.2	0.58	partial (8/16)
1 S Ext Reagents	0.0258	27.94	19.2	0.49	full
2 S Ext Reagents	0.0536	27.81	19.2	1.03	full
3 S Ext Reagents	0.0535	27.78	19.2	1.03	none

Conclusion

Quantitation values reasonably mimicked the known amount of DNA input (0.04ng/µl). All internal positive control (IPC) CT values were acceptable and therefore, no inhibition was apparent.

Full profiles were developed from the Straight Extraction Reagent samples. However, the Nonsperm and Sperm Reagent samples demonstrated the inability to yield full profiles on more than half of the samples tested. This may indicate that there is inhibition present in the differential extraction reagents (presumably detergents).

It should be noted that samples concentrated using the Vivacon® 500 50k filters in other experiments did not show signs of amplification inhibition. However, all nonsperm fractions in the other experiments were diluted prior to amplification. Dilution may remove enough of the inhibitor to allow for sufficient profile development. Based on this study, there is potential for PCR inhibition if the maximum allowable extract is amplified for differentially extracted samples. Diluting samples wherever possible is recommended.

Across the sample set, the average quantity obtained was 0.0384 ng/µl with a standard deviation of 0.0138 ng/µl. All samples obtained yields within two standard deviations of the mean. This data suggests the Vivacon® 500 50k filters yield precise and accurate results.

For further information: mike.vagell@sartorius-stedim.com