



B133 Comparison of Three Filtration Devices for Recovery of Low Level and Degraded DNA

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After attending this presentation, attendees will understand the use of filter devices available for DNA recovery and which device may produce a better DNA yield for low level and compromised samples and will assist the DNA analyst in making decisions with respect to delicate forensic evidence. This presentation is based on an in-house comparison of the Microcon® 30 MW, Vivacon® 30 MW, and the NucleoSpin® devices for DNA capture using a phenol-chloroform extraction method.

This presentation will impact the forensic science community by demonstrating that an appropriate filter device can impact the outcome of DNA recovered for low level and compromised samples.

There is a strong demand in forensic DNA to analyze smaller and smaller amounts of DNA in casework. Many of these cases may involve “touch” DNA that may have inhibitors present. While phenol extraction is more labor intensive than robotic extraction methods, it permits the analyst to use a filter device to reduce the volume of the DNA extract into the small amplification volume required and increase the chances of template DNA recovery.

The Microcon®, Vivacon®, and NucleoSpin® products all use a membrane-based filtration device for DNA capture. The Microcon® device can hold up to 500µl of volume at a time, the NucleoSpin® can accommodate up to 400µL of volume, and the Vivacon® can hold up to 2mL. All three devices use centrifugal force to filter the volume of the extract and washing buffer through the membrane. The Microcon® and Vivacon® require inversion of the membrane and centrifugal force to release the DNA in the elution buffer. The NucleoSpin® uses a binding buffer which permits washing of the membrane to remove inhibitors and then an elution buffer to release the DNA. The centrifugal time required to spin the buffers and wash is significantly shorter than the Microcon® or Vivacon®.

A series of experiments was set up in a side-by-side comparison of all three devices. An Applied Biosystems® 7500 Real-Time Polymerase Chain Reaction (RT-PCR) instrument with the Applied Biosystems® Quantifiler® Duo Kit was used to estimate the amount of DNA recovered. Only the inhibited and degraded samples were amplified with the Applied Biosystems® Identifiler® Kit and analyzed with an Applied Biosystems® 310 Genetic Analyzer. When comparing known amounts of low level DNA, the Nucleospin® had nearly twice the DNA recovery (91.5pg) compared to the Microcon® (48.6pg), and Vivacon® (50.8pg) at the lowest known concentration of DNA (111.2pg). As the concentrations increased, the Microcon® was comparable and then exceeded the NucleoSpin® at the lowest concentration 111.2pg of known DNA.

Inhibition was studied using different concentrations of soil and denim dye solutions added to the samples after digestion and before extraction since these are commonly encountered inhibitors. The Microcon® and Vivacon® were not able to produce any allele calls whereas the NucleoSpin® produced full profiles at all concentration levels. A degraded blood stain was diluted to create known low level samples to test the recovery ability of the Nucleospin® and Microcon®. The NucleoSpin® generated partial profiles with three different dilutions and the Microcon® produced one partial profile for one dilution and a few allele calls with the remaining dilutions.

In conclusion, the NucleoSpin® was not only more effective at removing inhibitors as anticipated by the literature but was also better at recovering degraded DNA than the Microcon®. The added benefit of the reduced amount of sample handling and shorter centrifuge time makes the NucleoSpin® product a better concentration device for low level and compromised DNA samples.

Low Level DNA, PCR Inhibitors, DNA Filtration Device