Simplifying Progress

Ethylene Oxide Treatment as a Method for Introducing PCR Grade Vivacon® Centrifugal Concentrators

Rik McRae
Sartorius Stedim Lab Ltd, Stonehouse, Gloucestershire GL10 3UT, UK

Background

There have been a number of recent forensic investigations in both Europe and the United States where the results of DNA profiling based on STR analysis suggested apparent links between unconnected cases from different geographical areas. Subsequent investigation revealed that contaminant human DNA had been inadvertently introduced during manufacture of plastic ware consumables and reaction products used in the DNA analysis. (1)

Presently, most laboratory materials and reagents associated with the isolation of DNA from evidential items for subsequent DNA profiling are handled in a stringent 'clean' room environment. However, 'clean' does not necessarily mean that an item is free from contamination with extraneous human DNA.

Previous studies have shown that ethylene oxide (EtO) treatment appears to significantly reduce the amplification of residual extraneous DNA on items for forensic analysis, and that EtO treatment does not affect any downstream forensic DNA analysis. (2)

Materials and Methods

Vivacon® centrifugal UF concentrators
Vivacon® concentrators are disposable ultrafiltration devices optimally suited for DNA concentration. For best performance with DNA samples, they are equipped with the patented regenerated cellulose membrane 'Hydrosart'. Vivacon® 2 mL concentrators with 30 K and 100 K MWCO membrane were chosen for this study.

Preparing Vivacon® devices with 'spiked' human DNA
Briefly, 3 devices were spiked with 5 ng high molecular weight human genomic DNA. DNA sample was applied directly onto the UF membrane in a laminar flow hood. These 'spiked' devices were placed into gas sterilising pouches with Tyvek panels to allow the sterilising EtO gas to penetrate. Pouches were heat sealed. 2 duplicate sets of 'negative control' devices with no DNA were packed in similar heat sealed sterilising pouches.

Modified EtO treatment
Both 'spiked' and clean Vivacon® 2 centrifugal concentrators were sent for a modified EtO sterilisation regime at Isotron, UK. The total exposure time to EtO gas was up to 6 hours under modified conditions. [2].

Real-Time Quantitative PCR (qPCR) Analysis
PCR amplification and detection was carried out on a 5-channel, 96-well block Stratagene MX3005P real-time instrument. Human DNA quantification was performed in 20 uL assays using Brilliant low ROX reaction mastermix and human genomic DNA specific Taqman hydrolysis probe chemistry across 55 cycles of amplification. The limit of detection was found to be 12–16 pg of DNA and offered sensitivity as low as a single cell.

Evaluation of DNA removal from 'spiked' devices
For DNA quantification, devices were loaded with 2 mL of molecular biology grade water and centrifuged according to the manufacturers recommendations. After backspin, concentrate was transferred to PCR clean 1.5 mL tubes. 5 µL of the concentrate was used in a 20 µL qPCR reaction over 55 cycles to detect any residual human DNA present in the devices.

Performance of EtO vs. non-EtO Vivacon® devices
For DNA quantification, devices were loaded with 1.0 ng of human genomic DNA in 2 mL of molecular biology grade water and centrifuged according to the manufacturers recommendations. After backspin, concentrate was transferred to PCR clean 1.5 mL tubes. The volume of concentrate was determined gravimetrically and 5 µL of the concentrate was used in a 20 µL qPCR reaction for DNA quantification.

Results

Table 1. EtO treatment has no significant effect on speed of concentration.

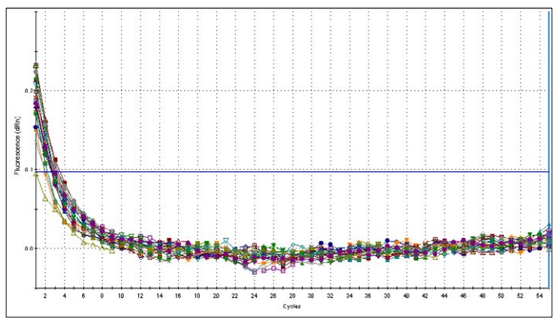
Device MWCO	EtO treated?	Qty of DNA Loaded	Initial volume	Initial volume Spin speed / time	Final volume
30 K	No	1.0 ng	2 mL	2,500 xg 60 min	45 µL
30 K	Yes	1.0 ng	2 mL	2,500 xg 60 min	45 µL
100 K	No	1.0 ng	2 mL	2,500 xg 30 min	59 µL
100 K	Yes	1.0 ng	2 mL	2,500 xg 30 min	58 µL

Table 2. EtO treatment has no significant effect on recovery of high molecular weight DNA.

Device MWCO	EtO treated?	Qty of DNA Loaded	Initial volume	Initial volume Spin speed / time	DNA recovery
30 K	No	1.0 ng	2 mL	2,500 xg 60 min	31 %
30 K	Yes	1.0 ng	2 mL	2,500 xg 60 min	38 %
100 K	No	1.0 ng	2 mL	2,500 xg 30 min	23 %
100 K	Yes	1.0 ng	2 mL	2,500 xg 30 min	18 %

Figure 1. (a) DNA-free results of EtO treated Vivacons® – No human genomic DNA amplification was observed after 55 qPCR cycles using Taqman probe chemistry. (b) Pure DNA is obtained after ultrafiltration – Reproducibly low variation is observed in real-time PCR cycle threshold (Ct) values of internal control. Baseline = no internal control.

a)



b)

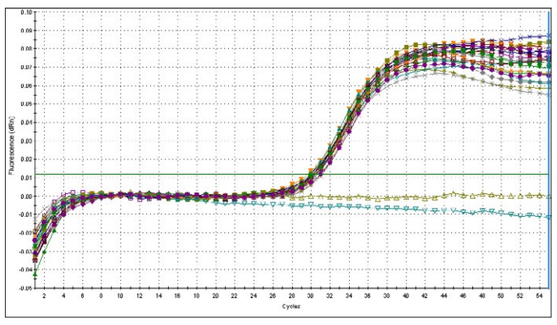


Table 3. EtO eliminates DNA contamination in devices

Amount DNA spiked in device	EtO treated?	Contaminating DNA detected
Clean – no DNA	Yes	No
Spiked (5.0 ng)	No	Yes (Ct = 42.82)
Spiked (5.0 ng)	Yes	No

Conclusion

This study shows that an EtO treatment regime is capable of reducing DNA contamination, by preventing amplification of contaminant extraneous DNA, such that database loadable contaminant DNA profiles should not be possible after gas sterilisation.

Our results show that EtO does not significantly affect the key speed or recovery performance metrics of Vivacon® centrifugal concentrators.

This study also suggests that Vivacon® centrifugal concentrators are ideally suited to concentration of DNA samples for stringent PCR amplification and analysis applications such as forensic DNA testing.

Vivacon® 500
- 0.5 mL volume
- Hydrosart® RC membranes
- Fits 1.5 | 2.2 mL rotors
- Backspin concentrate recovery
- 2–100 K MWCO options
- Pre PCR sample concentration
- Post PCR primer removal
- Post sequencing dye removal

References

1. T. Howitt, Ensuring the integrity of results: a continuing challenge in forensic DNA analysis (2003). Document link: http://www.promega.com/geneticidproc/ussymp14proc/oralpresentation/ Howitt.pdf.
2. K. Shaw et. al., Comparison of the effects of sterilisation techniques on subsequent DNA profiling. (2008) Int. J. Legal Med. 122 29–33.

Acknowledgements

The author would like to thank Dr Mike Brownleader from Generon Ltd, Maidenhead, United Kingdom for discussions and also Dr Naveed Anwar for providing scientific consultation and forensic expertise to both Sartorius Stedim Biotech and Generon Ltd.