Filter Apparatus Verification, 0.45 µm PES Filter Material Including Comparison of Double Swab Method and M-Vac with Saliva Stained Cotton

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A verification test was conducted to evaluate the use of a filter apparatus with a 0.45 µm PES filter as a viable means for concentrating M-Vac samples. The verification also included a comparison of the double swab and M-Vac sampling methods. Black cotton swatches were stained with diluted saliva and dried. The swatches were sampled using both methods. The M-Vac samples were concentrated using a Nalgene filter apparatus. The swatch preparation, sampling and sample processing was done by third party forensics personnel. The 0.45 µm PES filter was found to collect the DNA material from the liquid M-Vac sample and is a viable means for concentrating M-Vac samples. The M-Vac collected 39 times more DNA material than the double swab method.

Objective

Verify the use of a 0.45 µm polyethersulfone (PES) filter and disposable sterile filtering apparatus as a sample concentration means for M-Vac samples via the sampling of mock DNA evidence and the use of traditional DNA processing methods.

Procedures

Sample Preparation – 4 swatches of clean black cotton were prepared for the verification. Two swatches were left unstained. They were placed and sealed in an evidence bag as reference swatches. Two swatches were stained with 2mL of a 10:1 dilution of saliva and dried. They were placed and sealed in an evidence bag. The swatches were prepared by Sorenson Forensics.

Sampling – The sampling was conducted at the West Jordan Police Department's forensic lab by West Jordan and Draper Utah CSI. They processed the mock evidence utilizing standard evidence handling procedures. Two swatches, one reference and one stained, were sampled with the double swab method. The swabs were boxed, labeled, sealed and set aside to dry. The other two swatches, one reference and one stained, were sampled utilizing the M-Vac System. In addition, the swatch that had been stained and sampled with the double swab method was sampled again with the M-Vac to see how much more DNA material would be collected after swabbing. Following each M-Vac sample, the sample bottle was removed from the separation unit, capped and labeled. A new M-Vac was used for each sample.

The M-Vac samples were concentrated utilizing a filter apparatus. There are a number of filter apparatus options available. Nalgene Rapid Flow Sterile Disposable Filter Units with a 0.45 µm PES filter were used in this test. The filter diameter is 50 mm.

The filter apparatus is compatible with the M-Vac Systems support equipment (SEC). It was connected to the SEC vacuum port by disconnecting the vacuum tube from the M-Vac and connecting it to the vacuum fitting on the filter apparatus. The vacuum was turned ON and the protective cover was removed from the top of the filter. The sample bottle was opened, swirled and

poured into the filter funnel. The protective cover was replaced. The vacuum pump remained ON until all the solution had been pulled through the filter. The bottle of filtrate was removed, capped, sealed, labeled and refrigerated. The filter was labeled, sealed and set aside to dry. The process was repeated for each M-Vac sample.

The identity of the samples, swab, filter and filtrate, were blinded from the analysts with a numerical key. The analysts did not know which samples they were processing, reference or stained.

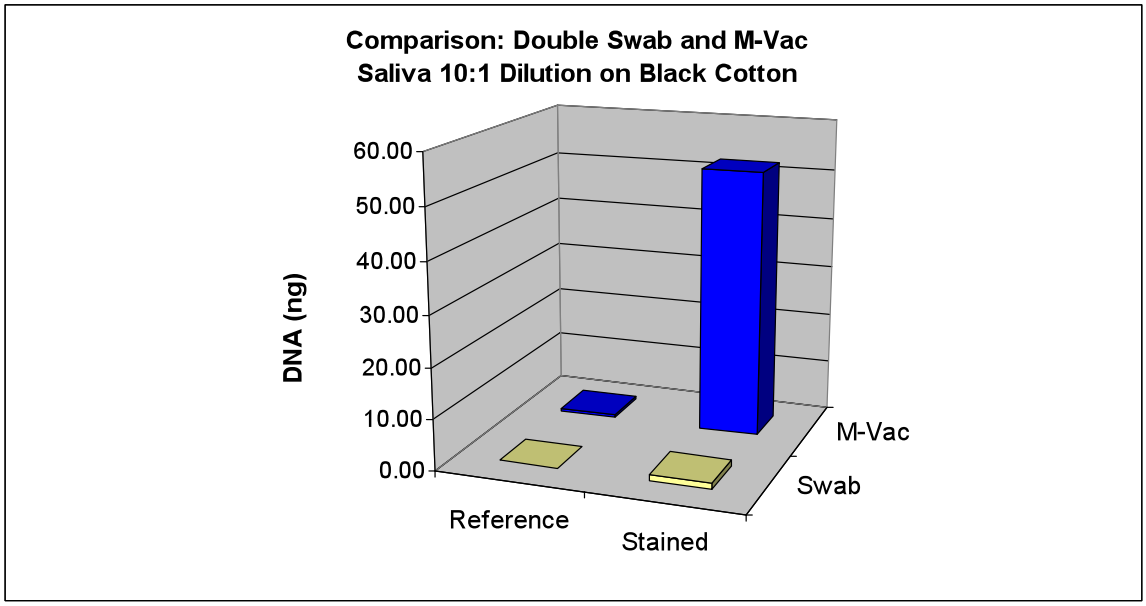
Analysis – The swabs, filters and filtrate were processed by Sorenson Forensics. The filters were removed from the filter apparatus with a scalpel and cut into strips. The bottles of filtrate were concentrated/pelleted utilizing the normal M-Vac solution processing procedure. The swabs, filters & pellets were processed using a Chelex extraction and quantitated with a Plexor HY System.

Results

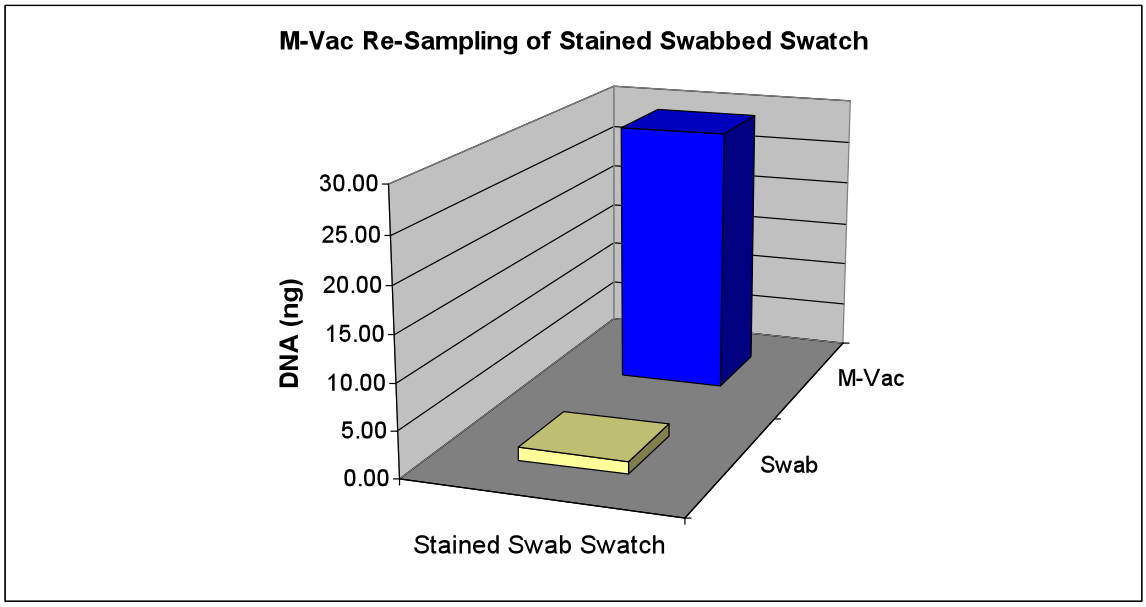
Graph 1 shows a comparison of the double swab and M-Vac method. Table 1 contains all of the data from the verification including the M-Vac sampling of the stained swab sample and the filtrate data. All of the filtrates were free of any measurable cellular DNA material. With N equaling one, there are no statistical conclusions, however, the M-Vac collected 39 times more DNA material than the swab and it collected 22 times more DNA material from the stained swatch that had already been sampled with the double swab method (see Graph 2).

Conclusions

A 0.45 µm PES filter is sufficient for concentrating DNA material. Filtering is a viable means of concentrating M-Vac samples. The filter material did not interfere or inhibit the cell lysing or qPCR processes. The M-Vac collects significantly more DNA material from cotton substrates than the swab.



Graph 1: qPCR Results – Swab and M-Vac DNA material collection of saliva from cotton



Graph 2: qPCR Results – DNA material collected with the M-Vac from the stained swatch that had already been swabbed

Sample	Average Quant	Total DNA (ng)	Sample Method
#1 Filter	2.92E-01	52.56	M-Vac of Positive
#2 Filter	2.82E-03	0.51	M-Vac of Reference
#3 Filter	1.62E-01	29.16	M-Vac of Swabbed Positive
#4	7.46E-03	1.34	Swab of Positive
#5	N/A	0.00	Swab of Reference
1A	N/A	0.00	M-Vac of Positive Filtrate
2A	N/A	0.00	M-Vac of Reference Filtrate
3A	N/A	0.00	M-Vac of Swabbed Positive Filtrate

Table 1: qPCR Data – Swab, M-Vac and filtrate