Sorenson Forensics

M-Vac Forensic Evaluation Study--Summary Report

Introduction:

The purpose of this study was to evaluate how much biological material can be recovered for forensic DNA profiling using the M-Vac, as compared to traditional methods of sampling used for casework (either swabbing the entire area and consuming ~half of each wet/dry swab or consuming a cutting of the stain). A total of three sets of DNA extractions/comparisons were processed to obtain evaluation data for the M-Vac. Blood and saliva samples were spotted and allowed to dry on white cotton, blue denim, polyester and nylon (figure 1.) DNA profiles were developed from biological material recovered from all types of cloth material. In order to minimize variables in the comparison, an evaluation of the Millipore Amicon filtration device for concentrating liquid samples from the M-Vac was first necessary, to get an estimate of the amount of DNA loss from this filter.

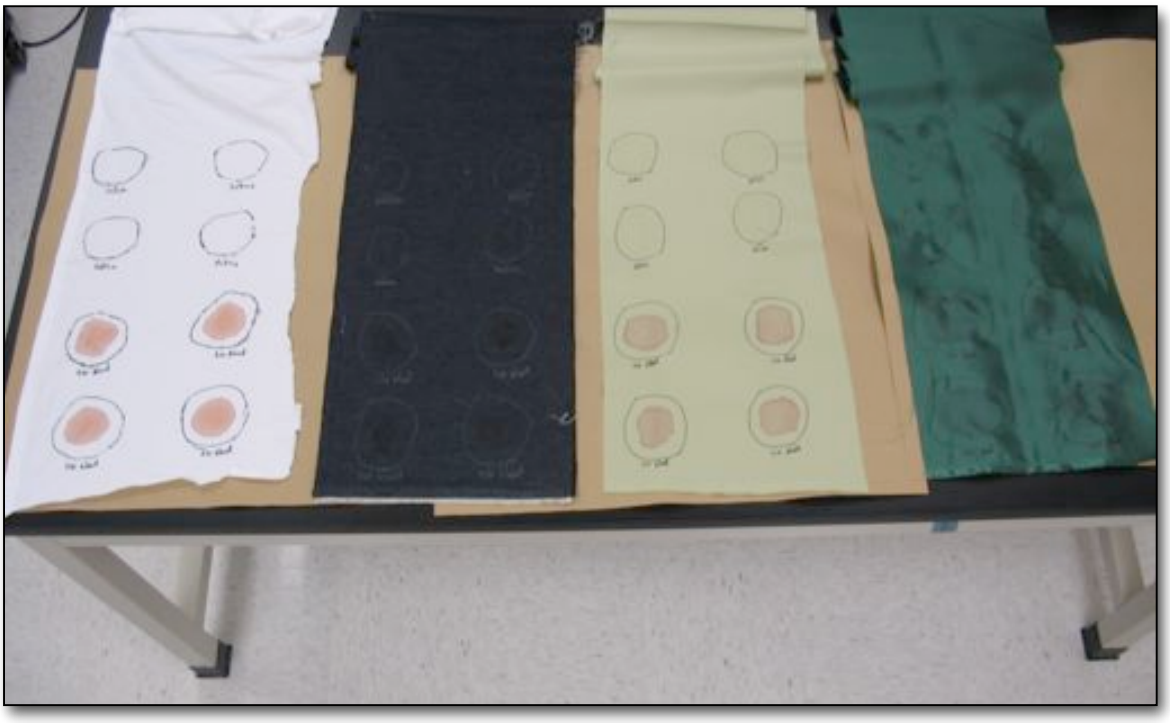

Figure 1. Blood and Saliva Stains Spotted on Cotton, Blue Denim, Polyester and Nylon

For the Millipore Amicon filter assessment, a sample consisting of 3 uL of the 200 ng/uL Quantifiler DNA Standard added to 15 mL TE-4, pH 8.0 was prepared. The sample was mixed, then 10 uL was removed in order to quant the pre-spun sample. The remaining sample was then added to an Amicon filter device. The sample was spun at 2000 rpm, checking every 5 minutes to see how far the sample was spun down, for a total of ~ 20 minutes. The remaining volume was ~240 uL.

The DNA quantity in the samples was then estimated by QPCR (quantified in replicate) and setup on the Biomek FXP robot; the AB 7900 was used to generate QPCR data. The quantification (quant) results are as follows:

Sample	Replicate 1 Quant value (ng/uL)	Replicate 2 Quant value (ng/uL)	Average Quant value (ng/uL)	Sample Volume (uL)	Total DNA (ng)
1AQ (pre-spin)	0.033	0.037	0.035	~15000	525
1BQ (post-spin)	0.993	1.215	1.104	~240	264.96

The above data indicated that up to half the DNA added to the Amicon filter could be lost. Even with this amount of DNA loss, it was still possible to use the filter concentration device, as long as the sampling methods incorporated use of the Amicon filter in an identical manner.

First Run Set:

For the first run set comparing sampling methods, 500 uL of a 1:2 dilution of saliva was spotted on several areas on white cotton material. The stains were allowed to dry overnight. The M-Vac instrument was used to collect two entire saliva stains (~50 mL liquid). One wet and one dry swabbing from two additional entire saliva stains were collected as well. The swabs were soaked in ~50 mL TE-4 while the M-Vac samples spun through the Amicon filters. Before the first, second, and third spins, ~15mL of each sample was added to the Amicon filter devices. Before the fourth spin, the remaining volume (~10mL) of each sample was added to the Amicon filter devices. The length of each spin and the final volume of each sample are shown in the table below.

Sample	1st Amicon spin	2nd Amicon spin	3rd Amicon spin	4th Amicon spin	Final Volume (uL)
Swabs #1	15 min	15 min	15 min	15 min	240
Swabs #2	15 min	15 min	15 min	15 min	240
M-Vac #1	15 min	15 min	15 min	10 min	200
M-Vac #2	15 min	15 min	15 min	10 min	200

Due to the amount of swab material, the swabs were split into two 1.5mL tubes each and the tubes were labeled them Swabs1A, Swabs 1B, Swabs 2A, and Swabs 2B. The final volume of samples Swabs #1 and Swabs #2 were then split and half were placed in the “A” tubes and half in the “B” tubes. The final volume of samples M-Vac #1 and M-Vac #2 were also split between tubes labeled M-Vac 1A, M-Vac 1B, M-Vac 2A, and M-Vac 2B. 20uL of neat saliva were placed in two tubes labeled Neat saliva 1 and Neat saliva 2.

These samples (Swabs1A, Swabs 1B, Swabs 2A, Swabs 2B, M-Vac 1A, M-Vac 1B, M-Vac 2A, M-Vac 2B, Neat saliva 1, and Neat saliva 2) were extracted, as well as a reagent blank using our usual procedure. The samples were digested overnight, and extracted the next day. The Vivacon filters were pre-wet with ~ 500 uL of TE-4 and spun before adding sample. 500uL of TE-4 was added to each Vivacon filter, then the appropriate samples were added (Note: For those samples that had previously been split to two tubes, the “A’s” and “B’s” were combined into one Vivacon filter. Enough TE-4 was added to bring the total volume in the filter to 2mL. The samples were spun in the Jouan centrifuge at 2500 x g. After the first spin, an additional 1mL TE-4 was added to each filter and spun again. Finally the samples were inverted, the retentate volume of each was measured, and the samples were placed in new 1.5mL tubes.

The quant was set up on the Biomek FX robot. The table below shows the length of the spins, the retentate volume, quant values, and total DNA of each sample. Significantly more DNA was obtained by the M-Vac procedure. These results also verified the sampling approach was suitable for the remainder of the study.

Sample	Vivacon 1st spin (minutes)	Vivacon 2nd spin (minutes)	Retentate Volume (uL)	Replicate 1 Quant value (ng/uL)	Replicate 2 Quant value (ng/uL)	Average Quant value (ng/uL)	Total DNA (ng)
RB090413JJP	30	25	150	0	0	0	0
Swabs #1	35	30	210	0	0	0	0
Swabs #2	35	25	160	0.022	0	0.022	3.52
M-Vac #1	50	30	190	1.584	1.279	1.432	272.08
M-Vac #2	50	30	240	0.911	0.781	0.846	203.04
Neat saliva #1	30	25	180	0.213	0.202	0.208	37.44
Neat saliva #2	30	25	165	0.831	0.526	0.678	111.87

Second Run Set:

A 1:10 dilution of the blood was made using 1 mL of neat blood and 9 mL of TE-4. 500 uL of the 1:10 blood dilution was spotted on each of four types of material: white cotton, blue denim, polyester, and nylon. 500 uL of neat saliva was spotted on the same four types of material (see pictures of the stains, which are circled and noted). The stains were allowed to dry overnight. The next day, samples from the blood stains on each material were collected. The material containing the saliva stains were placed in a bag for later use. The M-Vac instrument was used to collect one entire blood stain from each type of material. One ~1 cm2 cutting from a second blood stain from each type of material was taken. Since the centrifuge being used for the Amicon filter devices only holds four tubes, the cuttings were allowed to soak in ~50 mL of TE-4 while the M-Vac samples were spun.

For the M-Vac samples, before the first, second, and third spins, ~15mL of each sample was added to the Amicon filter devices. For the cutting samples, before the first, second, and third spins, ~15mL of each sample was added to the Amicon filter devices. Before the fourth spin, the remaining volume (~5mL) of each sample was added to the Amicon filter devices. The length of each spin and the volume of each sample after each spin are shown in the tables below. After the Amicon spinning, each cutting was added to one of the appropriate 1.5 mL tubes. There ended up being 3 samples each for the M-Vac samples (12 total), and 4 samples each for the cutting samples (16 total).

Sample	1st Amicon spin	Volume after 1st spin (uL)	2nd Amicon spin	Volume after 2nd spin (uL)	3rd Amicon spin	Volume after 3rd spin (uL)
M-Vac-blood – white cotton	17 min	175	17 min	175	15 min	150
M-Vac-blood – blue denim	17 min	160	17 min	150	15 min	150
M-Vac-blood - polyester	17 min	160	17 min	160	15 min	130
M-Vac-blood - nylon	17 min	150	17 min	130	15 min	125

Sample	1st Amicon spin	Volume after 1st spin (uL)	2nd Amicon spin	Volume after 2nd spin (uL)	3rd Amicon spin	Volume after 3rd spin (uL)	4th Amicon spin	Volume after 4th spin (uL)
Cutting-blood - white cotton	17 min	150	15 min	150	15 min	100	8 min	165
Cutting-blood - blue denim	17 min	160	15 min	160	15 min	160	8 min	185
Cutting-blood - polyester	17 min	130	15 min	160	15 min	160	8 min	180
Cutting-blood - nylon	17 min	130	15 min	160	15 min	140	8 min	130

These samples were extracted, as well as a reagent blank and 20 uL of 1:10 blood dilution, using our usual procedure. The samples were digested overnight, and extracted the next day. The Vivacon filters were pre-wet with ~500 uL of TE-4 and spun before adding sample. 500uL of TE-4 was added to each Vivacon filter, then approximately half of the appropriate samples was added (for example, for the samples that started in four tubes, the top layer of two tubes were combined in a Vivacon filter, then the top layer of the other two tubes were combined in a 1.5 mL microcentrifuge tube to spin later). Enough TE-4 was added to bring the total volume in the filter to 2mL. The samples were spun in the Jouan centrifuge at 2500 x g. After the first spin, the remaining volume of samples was added to the Vivacon filters and the samples were spun again at 2500 x g. After the second spin, an additional 1mL TE-4 was added to each filter and spun again. Finally the samples were inverted, the retentate volume of each was measured, and the samples were placed in new 1.5mL tubes. Therefore, what started out as 30 samples ended up as 10 samples.

The quant was set up on the robot. The table below shows the length of the spins, the retentate volume, quant values, and total DNA of each sample.

Sample	1st spin (minutes)	2nd spin (minutes)	3rd spin (minutes)	Retentate Volume (uL)	Replicate 1 Quant value (ng/uL)	Replicate 2 Quant value (ng/uL)	Mean Quant value (ng/uL)	Total DNA (ng)
RBQ090423-JJP1	30	25	20	75	0	0	0	0
1:10 blood dilution	30	25	20	75	0.371	0.668	0.519	38.925
Cutting-blood - white cotton	30	60	50	300	0.007	0.022	0.014	4.2
Cutting-blood - blue denim	30	25	20	50	0.581	0.448	0.515	25.75
Cutting-blood - polyester	30	25	20	50	0	0.006	0.006	0.3
Cutting-blood - nylon	60	50	40	110	0.098	0.139	0.119	13.09
M-Vac-blood – white cotton	60	60	50	300	0.133	0.208	0.171	51.3
M-Vac-blood – blue denim	30	50	40	130	0.235	0.293	0.264	34.32
M-Vac-blood - polyester	30	50	40	100	0.013	0.051	0.032	3.2
M-Vac-blood - nylon	30	50	40	140	0.039	0.035	0.037	5.18

A QPCR assay was also set up manually using the same samples.

Results from the manual QPCR setup:

Sample	Retentate Volume (uL)	Replicate 1 Quant value (ng/uL)	Replicate 2 Quant value (ng/uL)	Mean Quant value (ng/uL)	Total DNA (ng)
RBQ090423JJP	75	0	0	0	0
1:10 blood dilution	75	0.371	0.399	0.385	28.875
Cutting-blood - white cotton	300	0.019	0.007	0.013	3.9
Cutting-blood - blue denim	50	0.618	0.612	0.615	30.75
Cutting-blood - polyester	50	0	0	0	0
Cutting-blood - nylon	110	0.095	0.128	0.112	12.32
M-Vac-blood – white cotton	300	0.061	0.088	0.074	22.2
M-Vac-blood – blue denim	130	0.186	0.170	0.178	23.14
M-Vac-blood - polyester	100	0.028	0.012	0.020	2
M-Vac-blood - nylon	140	0.023	0.011	0.017	2.38

Third Run Set:

The material on which 500 uL of neat saliva had been previously spotted was used for this set. However, since saliva had previously been tested on white cotton, only the blue denim, polyester, and nylon material were used. The M-Vac instrument was used to collect one entire saliva stain from each type of material (~45 ml total liquid from each stain). One wet and one dry swabbing from a second entire saliva stain was taken from each type of material. Given the quant and DNA profile results from the previous test of blood on nylon, an M-Vac sampling, as well as one wet and dry swabbing, was taken from additional blood stains on nylon. The swabs were allowed to dry. The swab material was removed from the sticks, and soaked in ~45 mL of TE-4 while the M-Vac samples were spun through the Amicon filter devices.

For the M-Vac and swab samples, before the first, second, and third spins, ~15mL of each sample was added to the Amicon filter devices. The length of each spin and the volume of each sample after each spin are shown in the tables below. After the Amicon spinning, the swab material was added to two of the appropriate 1.5 mL tubes. There ended up being 3 samples each for the M-Vac samples (12 total), and 3 samples each for the swab samples (12 total).

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Sample	1st Amicon spin	Volume after 1st spin (uL)	2nd Amicon spin	Volume after 2nd spin (uL)	3rd Amicon spin	Volume after 3rd spin (uL)
M-Vac-blood - nylon	17 min	150	15 min	150	15 min	160
M-Vac-saliva - blue denim	17 min	160	15 min	200	15 min	250
M-Vac-saliva - polyester	22 min	300	22 min	280	22 min	190
M-Vac-saliva - nylon	17 min	250	15 min	240	15 min	250

Sample	1st Amicon spin	Volume after 1st spin (uL)	2nd Amicon spin	Volume after 2nd spin (uL)	3rd Amicon spin	Volume after 3rd spin (uL)
Swabs-blood - nylon	17 min	175	15 min	160	15 min	160
Swabs-saliva - blue denim	17 min	170	15 min	150	15 min	160
Swabs-saliva - polyester	17 min	170	15 min	170	15 min	170
Swabs-saliva - nylon	17 min	165	15 min	150	15 min	160

These samples were extracted, as well as a reagent blank and 20 uL of neat saliva, using our usual procedure. The samples were digested overnight, and extracted the next day. The Vivacon filters were pre-wet with 500 uL of TE-4 and spun for 10 minutes before adding sample. 500uL of TE-4 was added to each Vivacon filter, then approximately half of the appropriate samples were added to the Vivacon filters. Enough TE-4 was added to bring the total volume in the filter to 2mL. The samples were spun in the Jouan centrifuge at 2500 x g. After the first spin, the remaining volume of samples was added to the Vivacon filters, then enough TE-4 to bring the total volume in the filter to 2mL was added. The samples were spun again at 2500 x g. After the second spin, an additional 1mL TE-4 was added to each filter and spun again. Finally the samples were inverted, the retentate volume of each was measured, and the samples were placed in new 1.5mL tubes. Therefore, what started out as 26 samples ended up as 10 samples.

The quant was set up on the robot. The table below shows the length of the spins, the retentate volume, quant values, and total DNA of each sample.

Sample	1st spin (min)	2nd spin (min)	3rd spin (min)	Retentate Volume (uL)	Replicate 1 Quant value (ng/uL)	Replicate 2 Quant value (ng/uL)	Mean Quant value (ng/uL)	Total DNA (ng)
RBQ090428-JJP1	30	25	15	40	0	0	0	0
Neat saliva	30	25	15	55	0.936	0.737	0.837	46.035
M-Vac-blood - nylon	30	25	15	50	0.137	0.145	0.141	7.05
M-Vac-saliva - blue denim	30	25	15	40	2.472	2.321	2.397	95.88
M-Vac-saliva - polyester	30	25	15	40	3.687	3.772	3.729	149.16
M-Vac-saliva - nylon	30	25	15	45	2.940	2.057	2.499	112.455
Swabs-blood - nylon	30	25	15	80	0.036	0.059	0.047	3.76
Swabs-saliva - blue denim	30	25	20	130	0.362	0.503	0.433	56.29
Swabs-saliva - polyester	30	25	20	90	0.061	0.018	0.040	3.6
Swabs-saliva - nylon	30	25	20	130	0.009	0.000		1.17?

Quality of DNA Profile Results:

The M-Vac procedure provided DNA of quality suitable to obtain excellent DNA profile results using a commonly used STR multiplex kit (AB Identifiler). No inhibitors or sample degradation attributable to the M-Vac was detected. No DNA inhibition was detected in any of the M-Vac samples quantified by QPCR. Amplification failures of samples that were expected to produce DNA profiles were most likely due to PCR master mix preparation, or possibly thermal-cycler problems (this was verified by repeating the procedure, which obtained DNA profiles of excellent quality.) The vivacon DNA concentration devices were excluded as a possibility of the amplification failures. The results obtained from traditional sampling methods often did not yield sufficient DNA to obtain profile results of equivalent quality from those obtained from the M-Vac samplings (please see the electronic binder which contains all printouts of DNA profile results.)