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Comparative Study of Two Semi-automated Forensic DNA Extraction Methods: Automate Express™ & Hamilton Microlab STAR™ System

دراسة مقارنة بين طريقتين شبه آليتين لاستخلاص الحمض النووي الجنائي: نظام Automate Express™ ونظام Hamilton Microlab STAR™

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Abstract

Automation in forensic DNA analysis is crucial for analysts to reduce time, improve results, and decrease risk of contamination. With the variety of commercially available automated DNA extraction systems, comes the need for end-users to be informed of what they provide and what they might lack. Thus, this study aimed to evaluate the efficiency of two semi-automated DNA extraction systems used for forensic DNA analysis: Automate Express™ and Hamilton Microlab STAR™ system, for four parameters; reproducibility, stability, sensitivity and contamination. Overall, the results indicated that both semi-automated systems performed similarly in providing robust and reproducible DNA results while maintaining good capability to overcome PCR inhibition with low risk of contamination. The two semi-automated systems showed higher DNA recovery than organic extraction using phenol-chloroform by 22% for semen and 7% for blood samples. In addition, three sample types, blood, saliva, semen were tested to compare the two systems (total samples n=100).

Keywords: Forensic science; Hamilton Microlab STAR™; Automate Express™; DNA extractions; Investigator Quantiplex Pro™.

المستخلص

التحول الآلي في فحوصات وتحليل الحمض النووي في المجال الجنائي يسهم في تقليص الوقت، تحسين النتائج والحد من فرص تلوث العينات. التنوع في أنظمة استخلاص الحمض النووي الآلية التجارية يتطلب تقييم فاحصي الحمض النووي لمعرفة منافع وسلبيات هذه الأنظمة. تهدف هذه الدراسة إلى تقييم مدى فعالية وكفاءة نظامين من أنظمة استخلاص الحمض النووي الشبه آليه والمستخدمة في مجال الحمض النووي الجنائي وهي نظام Automate Express™ ونظام Hamilton Microlab STAR™ system في أربعة معايير مختلفة: 1- قابلية التكرار، 2- الاستقرار، 3 - الحساسية، 4- مخاطر التلوث. بصوة عامة أشارت النتائج إلى أن كلاً من النظامين الشبه آليين يؤديان أداءً متشابهًا في توفير نتائج قوية قابلة لتكرار استخلاص الحمض النووي، مع المحافظة على قدرة جيدة للتغلب على مثبطات تفاعل البلمرة المتسلسل مع مخاطر تلوث منخفضة. أظهر النظامان الشبه آليان معدل استعادة أعلى للحمض النووي مقارنة بنظام الاستخلاص العضوي باستخدام الفينول كلوروفورم بنسبة 22٪ لعينات السائل المنوي و 7٪ لعينات الدم. بالإضافة إلى ذلك، تم فحص ثلاث أنواع من العينات للمقارنة بين كلا النظامين الشبه آليين (مجموع العينات المفحوصة - 100 = n). بشكل عام، أظهرت النتائج

الكلمات المفتاحية: علوم الأدلة الجنائية، هاملتون مايكرولاب ستار، اكسبريس الآلية، استخلاص الحمض النووي، كوانتيبليكس برو.

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Overall, the data showed the average DNA recovery for Hamilton was higher than the DNA recovery by Automate Express™ for the blood and semen sample types indicating better performance of the Hamilton Microlab STAR™ in terms of recovery and sensitivity level.

أن متوسط استعادة الحمض النووي بواسطة نظام Hamilton Microlab STAR™ system أعلى من متوسط استعادة الحمض النووي بواسطة نظام Automate Express™ لعينات الدم والسائل المنوي مما يشير إلى أن أداء نظام Hamilton Microlab STAR™ system أفضل من حيث الإستعادة للحمض النووي ومستوى الحساسية.

1. Introduction

Successful DNA profiling relies on the efficiency of DNA isolation, purification, and extraction [1]. Nowadays, DNA extraction methods have evolved from using traditional labor-intensive manual extraction methods, such as organic extraction using Phenol-chloroform, to more sophisticated types of automated bench-top instrumentation. Nonetheless, the widespread availability of different commercial automated DNA extraction systems demands a need from users to study their benefits and limitations. Forensic DNA analysts seek DNA extraction methods that effectively produce good yield and high recovery of allelic information [1, 2, 3]. Different studies published in the literature demonstrate the performance of manual extractions such as organic phenol-extraction [4, 5], and semi-automated extractions such as Automate Express™ [2, 6, 7], and Hamilton Microlab STAR™ System [8, 9]. In a crime scene, the amount of biological samples present may be limited [6]. Therefore, ideally, automation for DNA extraction should be sensitive to lower amounts of biological samples and be able to produce high DNA yield at low quantities [1,2]. Furthermore, a challenge commonly encountered in crime scene samples is the presence of PCR inhibitors that can disrupt the DNA amplification process [2]. Some known PCR inhibitors include heme from hemoglobin in blood, fabric dyes such as indigo dye in denim, melanin in hair and humic acid found in soil [2]. Hence, the ability of automation to overcome PCR inhibition is critical for successful downstream DNA analysis [2]. Another important factor is the ability to carry out analysis in such instrumentation without the risk of contamination or carry-over between samples as resampling is almost never possible in forensic biological sampling. Finally, a critical feature of automated DNA extraction systems is their robustness and ability to reproduce results to lend validity to the method performed in courtrooms. Examples of DNA extraction systems used in forensic facilities are the Automate Express™ and Hamilton Microlab STAR™ systems. Automate Express™ is a small benchtop DNA extraction instrument that serves as a liquid handler with ready-to-use cartridges that allow the purification and extraction of DNA in a short amount of time [2, 6, 7,10]. One of its limitations is the number of samples it can handle; only 13 samples per run, but it remains a favorable option for smaller and urgent casework. The Hamilton Microlab STAR™ system is a larger highly sophisticated instrument with a built-in centrifuge, thermal cycler, shaker and liquid handling apparatus can take up to 96 samples per run [8, 9, 11]; however, with longer run times compared to the Automate Express™. Both systems operate using the Applied Biosystems™ Prepfiler™ Forensic DNA Extraction Kits chemistries (Prepfiler™ and Prepfiler Express™) which rely on the same basic principle for DNA extraction employing the use of magnetic particles and multicomponent surface chemistry to extract, purify, and elute DNA efficiently [1, 6, 12, 13]. In this study, our focus is comparing two semi-automated DNA extraction systems, Automate Express™ and Hamilton Microlab STAR™

System, by evaluating different parameters including: 1-reproducibility, 2-sensitivity, 3- stability and 4-contamination.

2. Materials and Methods

Sample details are mentioned later in each criterion being investigated. All biological samples used in this study were obtained using informed consent and with the approval of the General Department of Forensic Science and Criminology, Dubai police. The extraction reagents, settings and details are mentioned for each method below:

2.1 Automate Express™ DNA Extraction System

Following the manufacturer's recommendation [10], the swab cuttings were placed into labelled Automate Express™ LySep column assembly for extraction. After sample preparation, 500 µl of PrepFiler Lysis buffer and 5 µl of DTT were added to the samples and were set for incubation at 70.0°C for 40 minutes at 750 rpm in a thermocycler. After that, the samples were centrifuged at 10,000 xg for 2 minutes in a LySep column that was subsequently removed and the sample tube containing the centrifuged sample was loaded into the instrument with the appropriate consumables required for the run. The investigator card, PrepFiler Express™ Forensic DNA Extraction Kit and its protocol, were used with a final elution volume of 50µl. The eluted purified DNA extract is transferred to an elution tube at the end of the run.

2.2 Hamilton Microlab STAR™ extraction System

Similarly, the samples prepared for the Hamilton Microlab STAR™ extraction were cut into appropriately labelled tubes following the manufacturer's recommendations [11]. Prior to extraction, incubation was done on the Hamilton Microlab STARlet™ system. The PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems™) was used. The amounts of reagent were determined by the instrument and the default incubation settings were applied, producing lysate in a 24-deep-well plate.

This process is followed up by a purification/extraction process on the Hamilton Microlab STAR™ System. The final DNA extract is transferred to a 96-well PCR plate at the end of the run.

2.3 Organic Extraction

Organic Extraction [4, 5, 14] was performed manually by adding 450 µl of stain extraction buffer (SEB) and 20µl of DTT (Dithiothreitol) and 20 µl of proteinase K to a labelled Eppendorf tube containing the sample swab/cutting. The samples were incubated at 56.0°C and mixed at 350 rpm overnight. The purification and the concentration steps were followed using phenol-chloroform and Microcon® Centrifugal Filters. The final elution volume was 50 µl using TE buffer (Tris-EDTA Buffer).

2.4 Realtime PCR

Realtime PCR setup for both extraction systems was performed using the QIAgility pipetting robot by QIAGEN with the Investigator Quantiplex Pro Kit [15,16]. Important features of this kit are the ability to quantify DNA amounts, determine mixture proportions, and detect both degradation and PCR inhibition [15, 16]. Both Realtime PCR 7500 instrument and Quantstudio5 were used.

2.5 PCR and Capillary Electrophoresis (CE)

PCR was performed for selective samples using the GlobalFiler™ PCR Amplification Kit (Applied Biosystems™) [17, 18] followed by electrophoresis using 3500 Genetic Analyzer. In the CE parameter, a 5-seconds injection time and 1.2 kV were used during the run. Profiles were analyzed using Genemapper IDX 1.6v software and analytical threshold was set to 85 RFU.

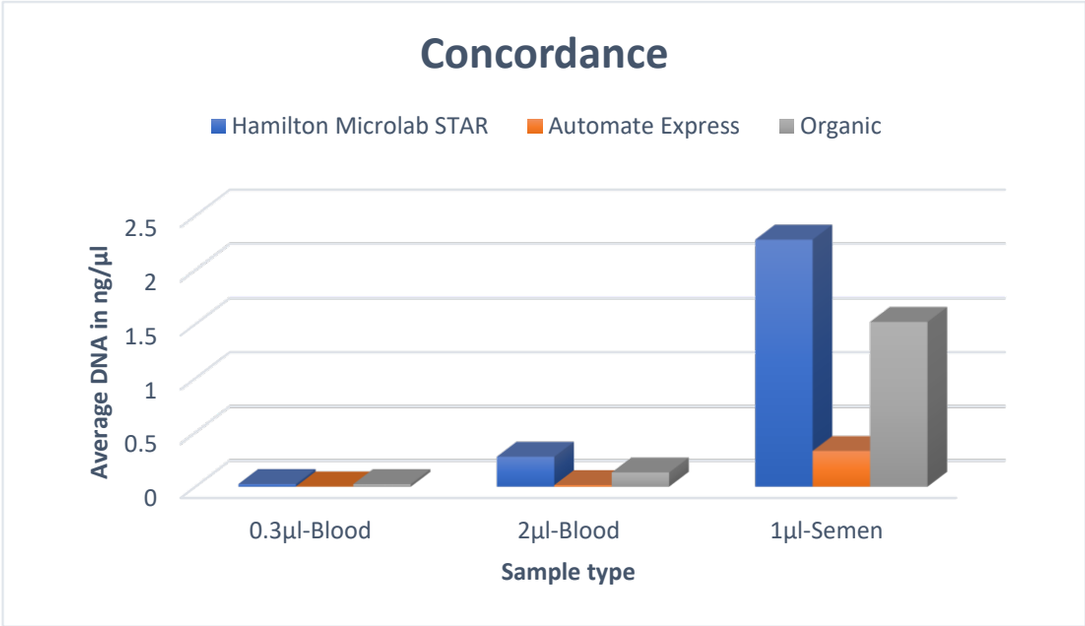

Figure 1 - *Results of concordance study: quantitation results across three DNA extraction methods for 0.3µl blood, 1µl blood, and 1µl semen*

3. Results and Discussion:

3.1 Benchmarking

As a first step, benchmarking of the automated DNA extraction method was done against organic manual extraction method by organic phenol-chloroform [4, 5, 14]. A concordance was done with the three DNA extraction methods: the Automate Express™ and Hamilton Microlab STAR™ System (semi-automated), and a manual extraction method using organic extraction (phenol-chloroform). Samples tested were liquid human blood, 2µl, and human semen, 1µl, used in triplicate each. The volumes of each sample type were directly pipetted onto a cotton swab, in triplicate, for each of the three extraction methods. The DNA extract was quantified using Quantiplex Pro kit on Realtime PCR 7500 instrument (Applied Biosystems™) by using the QIAgility pipetting robot by QIAGEN (Fig-1 shows the results of concordance study quantitation results across three DNA extraction methods for 0.3µl blood, 1µl blood, and 1µl semen).

Downstream DNA profiles were obtained using Globafiler™ PCR amplification kit followed by capillary electrophoresis using 3500 Genetic Analyzer. The average percentage of alleles recovered for the three triplicates were calculated for DNA profiles obtained in each DNA extraction method. It was shown that both semi-automated DNA extraction systems were able to achieve nearly 100% allelic information recovery, for both sample types, human blood and human semen. In contrast, for the case of organic extraction, DNA profiles revealed a reduced average recovery for human semen at 78% alleles recovered. In conclusion, the two semi-automated extraction methods showed high recovery of allelic information for both sample types, human and blood, even surpassing organic extraction by phenol-chloroform for human semen by 22% (Fig-2 summarizes the downstream DNA results of the concordance study by plotting average percentage of alleles recovered using Globalfiler™ PCR kit for two sample types across the three extraction methods). Howev-

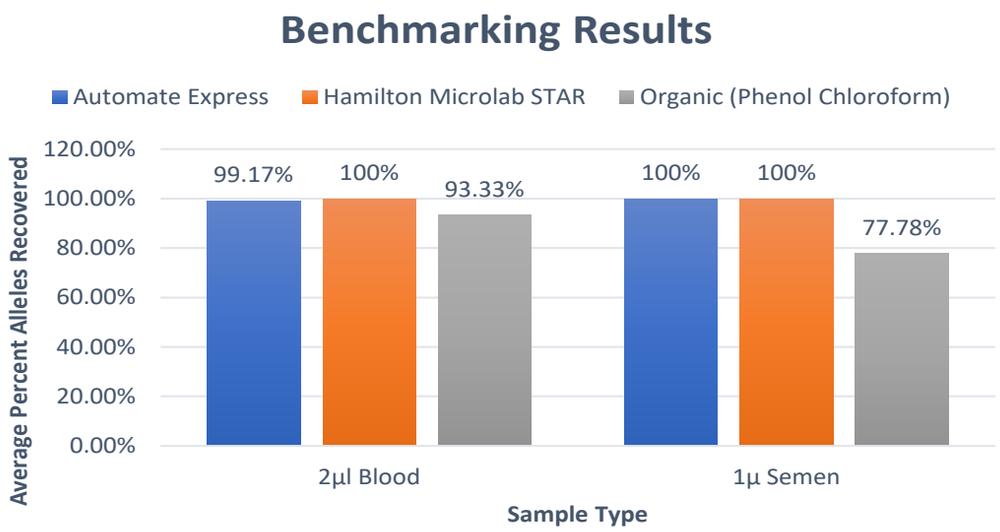

Figure 2 - *Downstream DNA analysis results as average percentage of alleles recovered using Globalfiler PCR kit for 2 µl blood and 1µl semen for Automate Express, Hamilton Microlab STAR, and organic (Phenol-chloroform) extraction*

er, the Hamilton Microlab STAR™ performed slightly better than the Automate Express™ with no drop out and higher DNA extract recovery as observed in Fig 2; while both performed collectively better than organic phenol-chloroform extraction. Similar results were observed in the literature where automated extraction methods have shown equal or better results for DNA yield than organic phenol-chloroform extraction with faster, lesser-intervention and safer approach [6, 19].

3. 2 Reproducibility

To test the reproducibility of the Automate Express™ samples of human blood and human semen of both 1*µ*l and 2*µ*l each from four donors, were analyzed in triplicate, separately over two days. A total of 96 samples were analyzed and quantified using Investigator Quantiplex Pro Kit. The results of both human blood quantities (1*µ*l and 2*µ*l) from both donors were well above our validated in-house quantitative DNA threshold of 0.0035 ng/*µ*l for Investigator Quantiplex Pro Kit by approximately 35-fold or greater. For the human semen quantities (1*µ*l and 2*µ*l) from both donors, higher DNA recovery was observed with a minimum DNA quantity 343-fold greater. These quantities are plotted for visual representation in Fig 3 (Fig 3 displays average DNA quantities from day 1 and day 2 on both Automate Express™ and Hamilton Microlab STAR™ for both blood and semen in different volumes). These samples were then processed downstream for DNA analysis using Globalfiler™ PCR chemistry. The results showed 100% allele recovery of all concentrations of human blood and human semen for all 96 samples using both semi-automated systems, the Hamilton Microlab STAR™ as well as the Automate Express™.

3.3 Sensitivity

A sensitivity study was carried out on the two semi-automated DNA extraction platforms. Serial dilution was performed starting with 1*µ*l of input volume for human blood and human semen samples directly dispensed onto a cotton swab. The initial buccal cell sample was prepared by collecting a buccal swab

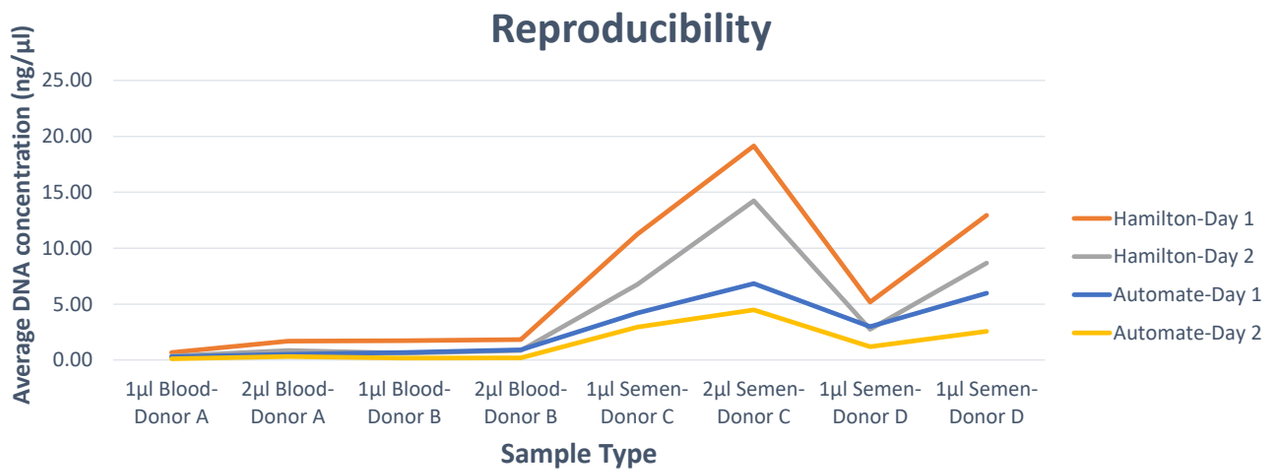

Figure 3 - *Reproducibility results displayed as mean DNA quantities from day 1 and day 2 on both Automate Express and Hamilton Microlab STAR for both blood and semen of different volumes*

which was subsequently agitated in 180µl of distilled water. Different volumes of human blood and human semen were analyzed in triplicates on both systems. In addition, buccal samples were analyzed in quadruplets. (Total number of samples amounts to 100). A dilution series of the three sample types was prepared with the following volumes: 1µl, 0.5µl, 0.25µl, 0.125 µl and 0.0625µl. Quantitation was done using Investigator Quantiplex Pro Kit on Quantsudio5. A student t-test was performed on the results using the Excel built-in function to find whether there was a significant difference in the means (Table 1- shows the average DNA quantities, in ng/µl, for two automated extraction systems for each sample type and t-test of the means). A two-tailed distribution was used, and the data was assumed to be homoscedastic - assuming equal variances. Results are visually summarized in Fig 4 (Fig 4- plots the average DNA quantities, in ng/µl, for two automated extraction systems for each sample type for five different volumes, A- Semen, B-Blood, C-Buccal. Error bars represent the standard deviation from the mean). For human semen extraction, average DNA recovery of all volumes was higher in Hamilton than Automate Express. In all except one volume, 0.125µl, the results were significantly higher for Hamilton than Automate Express. As for blood extraction, average DNA recovery by Hamilton was higher than Automate Express in all except one volume, 0.125 µl. A notably high standard deviation from the mean of the extracted DNA by Automate Express at 0.125µl was observed and the result was not significantly different than Hamilton. The DNA quantities recovered by Hamilton were significantly higher for the larger volumes (1µl and 0.5µl) than Automate Express. Furthermore, for buccal samples extraction, t-test indicates no significant difference between the DNA quantification results except at 0.0625µl and 0.25µl. Overall, the total average DNA quantities recovered from Hamilton Microlab STAR™ were more than those from Automate Express™. Notably, DNA from semen recovered by Hamilton had a high statistical strength. In general, sensitivity of Hamilton Microlab STAR™ to recover DNA material was relatively better than Automate Express™ for semen and blood samples and slightly better for buccal cells.

3.4 Stability

In this study, DNA extraction of human blood and two PCR inhibitors were tested on both Automate

Table 1 – *Sensitivity data as average DNA quantities for Automate Express and Hamilton Microlab STAR extraction systems for each sample with student-t test P-values*

Sensitivity Quantitation Results				
Sample type	**Volume of Sample**	**Average DNA Quantity (ng/µ) for Automate Express**	**Average DNA Quantity (ng/µ) for Hamilton**	**t-test (pvalue)**
Human Semen (n=3each)	1 µl	1.60	5.05	0.023
	0.5 µl	1.17	2.72	0.002
	0.25 µl	0.44	1.58	0.012
	0.125 µl	0.54	0.80	0.271
	0.0625 µl	0.18	0.47	0.022
Uuman Blood (n=3 each)	1 µl	0.39	0.76	0.015
	0.5 µl	0.14	0.30	0.021
	0.25 µl	0.10	0.14	0.084
	0.125 µl	0.13	0.11	0.874
	0.0625 µl	0.02	0.04	0.256
Buccal (n=4 each)	1 µl	0.0178	0.0317	0.220
	0.5 µl	0.0032	0.0301	0.136
	0.25 µl	0.0014	0.0071	0.014
	0.125 µl	0.0018	0.0023	0.726
	0.0625 µl	0.0012	0.0002	0.007

Express™ DNA extraction system and the Hamilton Microlab STAR™ System. A blood sample of 1μl was used with humic acid 2.5mg/μl (1μl) only, with denim material as a substrate and with both humic acid on denim. These tests were performed in triplicate as follows: blood on denim, blood on cotton swab with the addition of humic acid, and blood on denim with the addition of humic acid. Quantitation was done the 7500 Realtime-PCR using the Investigator Quantiplex Pro Kit (Fig 5 shows quantitation results for two automated DNA extraction systems with 3 different combinations of PCR inhibitors). In addition to quantitation, the Investigator Quantiplex Pro Kit has quality indicators including an inhibition index that can detect potential PCR inhibition [12,13]. For all samples on both systems, the inhibition index was 'below the threshold'; hence, no PCR inhibition was detected. DNA extraction efficiency for both systems was high as they were able to extract quantifiable amounts of DNA irrespective of the presence of different inhibitors. Even with the addition of humic acid and the use of denim substrate, the average amounts of DNA yield exceed our validated in-house quantitative DNA threshold of 0.0035 ng/μl and are therefore considered sufficient for DNA profiling purposes.

3.5 Contamination

To identify the possibility of contamination within both semi-automated extraction systems, different volumes of blood samples were used on the instrument with blanks in between.

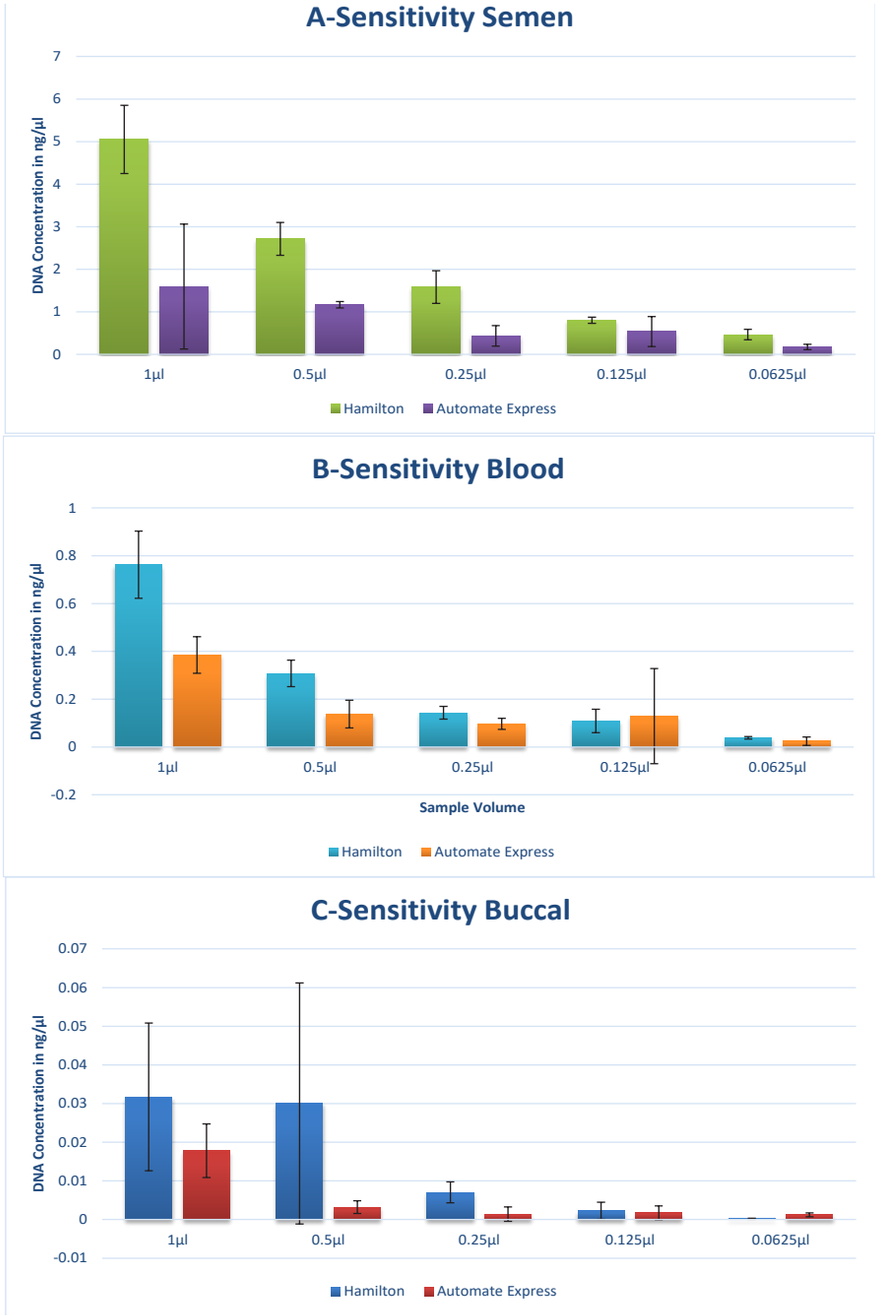

Figure 4- *Sensitivity data plotted as average DNA quantities, in ng/µl, for two automated extraction systems for each sample volume – A-Blood B-Semen C-Buccal, Error bars are the standard deviation from the mean*

The layout of the Automate Express™ instrument, which uses 13 samples per run, was done in alteration. No DNA was detected in all blanks and the instrument reagents blank for the Automate Express™. No contamination was detected.

For the Hamilton Microlab STAR™ system, which uses 24 samples per deep-well incubation plate in a 4 by 6 grid layout, the samples were placed alternatingly in a checkerboard pattern. However, minor amounts of DNA were detected in the Real-

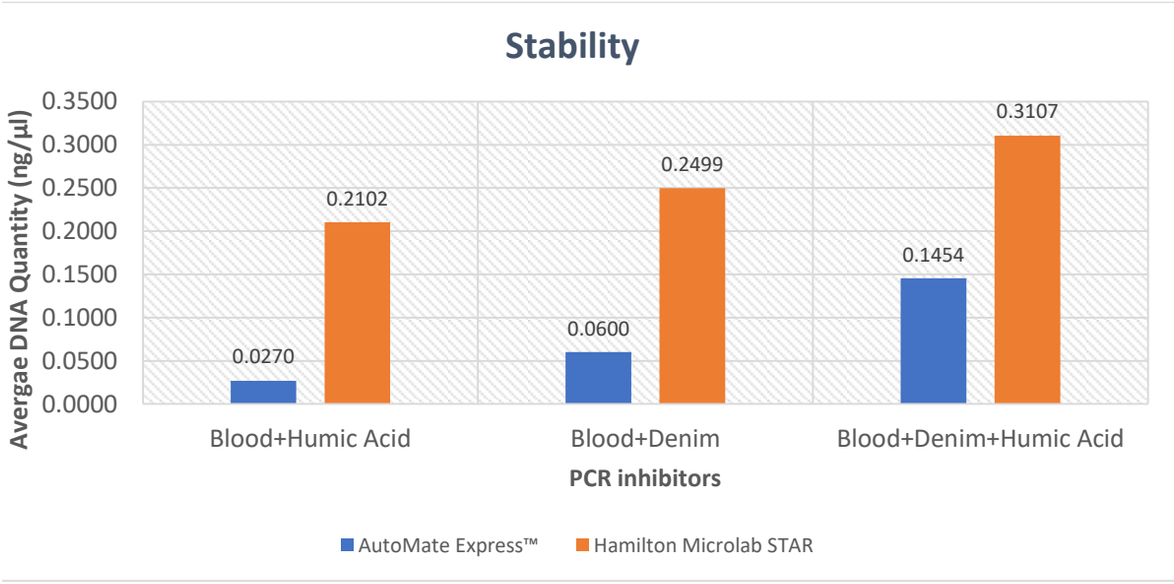

Figure 5- *Stability results for two automated DNA extraction systems with different PCR inhibitors*

time PCR results for only one of the Hamilton Micro STAR™ incubation plate blanks; 0.000206 ng/µl and the instrument reagent blank 0.000117 ng/µl, well below our DNA quantitation threshold of 0.0035 ng/µl. Nevertheless, these samples were amplified and analyzed to check for detectable peaks in the EPG. The results showed no detectable peaks at the analytical threshold of 85 RFU.

4. Conclusion

In this study, the goal was to compare two different automated extraction methods in terms of reproducibility, sensitivity, contamination, and capability to overcome inhibitors in casework samples. Benchmarking with organic phenol chloroform showed that automation was better in achieving allelic information for DNA profiles with Hamilton Microlab STAR™ ranking top of the three extraction methods for three different volumes of human blood.

For the performance evaluation of the two semi-automated forensic DNA extraction methods, mock samples of human blood, human semen, and human buccal cell samples in known amounts were prepared in the laboratory for use in the experiments carried out in this study. However, some potential limitations of the sampling method may arise when handling real-life casework samples in forensic investigations. Factors not considered in this study include but are not limited to: environmental circumstances at the crime scene such as heat, indoor or outdoor location of the biological samples, and the effect of other PCR inhibitors not discussed in this paper. These factors may or may not have any bearing on the results. Furthermore, it should be noted that the sampling method used was deemed appropriate for this study for the purpose of overall performance evaluation.

Reproducibility between Hamilton Microlab STAR™ and Automate Express™ was assessed using human blood and human semen in different volumes from different donors analyzed, in triplicate, separately over two days and quantified using Realtime-PCR with Investigator Quantiplex Pro Kit, totaling 96 samples analyzed. Globalfiler PCR and subsequent CE analysis indicate 100% allelic data recovery in all EPGs for both systems, estab-

lishing equal reproducible DNA profiling capability of the two automated DNA extraction systems for the given sample dataset. These results support the reliability of using Automate Express and Hamilton Microlab STAR™ systems to extract DNA from human blood and human semen, typical forensic biological samples encountered in physical and sexual assault cases in any crime laboratory.

Moreover, sensitivity was studied using a dilution series of three sample types of varying concentrations for both semi-automated extraction methods. In general, results reveal higher sensitivity in DNA extraction for semen and blood types by the Hamilton Microlab STAR™™ system versus Automate Express™ with t-test result supporting significantly higher DNA quantities extracted as per the Real-time-PCR results.

For stability, PCR inhibition of DNA was tested using human blood and known PCR inhibitors, such as humic acid and denim substrate as well as blood with a combination of humic acid and denim. Using Investigator Quantiplex Pro Realtime-PCR, quantifiable DNA amounts, well above the inhouse DNA quantity threshold, were successfully detected on both systems. Furthermore, Investigator Quantiplex Pro uses a quality indicator, inhibition index, to detect potential PCR inhibition. In this stability parameter dataset, no sign of PCR inhibition was detected. This indicates high efficiency for isolation and purification of DNA using both automated DNA extraction systems.

Finally, contamination risk of both systems was assessed using blanks. During the contamination study, low amounts of DNA were detected in two blanks from the Hamilton™ extraction, but no peaks were present in the EPG of these samples as the amount of DNA found was negligible. Thus, it was deemed that contamination does not pose an issue for the Hamilton™ Microlab STAR™ system.

Overall, both instruments showed highly reproducible, stable, and sensitive results with low risk of contamination suitable for use in forensic casework analysis. Although, Hamilton Microlab STAR™ platform performed slightly better than the Automate express in term of sensitivity to DNA extract recovered. Overall, automated systems are more reliable as they reduce the incidence of human errors. In addition, the automated system is more efficient and saves time and effort.

Conflict of interest

The authors declare no conflicts of interest.

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