Forensic Science International: Genetics Supplement Series 7 (2019) 250–252

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Evaluation of two DNA/RNA co-extraction methods for body fluid identification in forensics

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ARTICLE INFO

Keywords:
mRNA
body fluid identification
forensic
SEGM1
PRM1

ABSTRACT

Body fluid identification has become a field of interest in forensic casework as it can add value to particular investigative scenarios. Identifying the source of the biological material is not always an upfront task using conventional methods; therefore, profiling of specific mRNA markers can provide the answer. The implementation of RNA based analyses in forensic casework must focus on the quality and sensitivity of methods, starting with nucleic acid extraction, and without loss of DNA for STR profiling. In this work, two methods for DNA and RNA co-extraction were tested and compared: a commercial kit that uses a spin, mini column methodology, and a quick, simple nucleic acid isopropanol precipitation based protocol. Both methods simultaneously extract DNA and RNA, crucial for forensic casework and were tested in semen samples. Nucleic acid quantifications as well as purity assessment ratios (OD_{260}/OD_{230} and OD_{260}/OD_{280}) were obtained by both methods to infer on the use of extracts in downstream applications such as PCR. The performance of the two tested protocols was further evaluated by analyzing two semen mRNA specific markers, PRM1 and SEMG1. When compared to the commercially developed kit, results suggest that the literature adapted protocol allowed more carryover of contaminants absorbing at 230 nm and 280 nm as the purity ratios were below the accepted standard ranges. Negative results for mRNA profiling supported the QC results obtained by spectrophotometry. On the hand, PRM1 and SEGM1 were positive in RNA samples extracted with the commercial kit.

1. Introduction

In most scenarios of forensic investigations, as for example in cases of sexual aggressions, it may be necessary to detect and identify the biological nature of fluid stains. This is because they play an extremely important role in the resolution of legal cases [1], as for example, in the reconstruction of the crime circumstances. The identification and analysis of these biological traces are often very difficult due to the limitations of the conventional methods currently used. Due to its potential advantages of molecular methods [1], namely the analysis of differential gene expression through messenger RNA (mRNA) of different tissues and, therefore, specific to each body fluid, these have been distinguished as emergent techniques. Studies have been increasing over the past years and focus on the research interest in identifying body fluid and tissue types for forensic applications [1–4]. In this work, two methods for DNA and RNA co-extraction were tested and compared: a commercial kit based on a spin, mini column methodology, and a quick, simple isopropanol nucleic acid precipitation based protocol [5]. Both methods were used to co-extract nucleic acids from semen samples for forensic applications. The performance of techniques was further evaluated by mRNA profiling of two semen specific markers, PRM1 and SEGM1, and by quality control assessment of purity ratios of sample absorbance at A260, A280 and A230 nm.

2. Material and Methods

2.1. Samples

14 semen samples were collected from healthy donors in 50 ml sterile vessels under informed consent and stored at -20 °C until further use.

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https://doi.org/10.1016/j.fsigss.2019.09.096
Received 17 September 2019; Accepted 26 September 2019
Available online 27 September 2019

2.2. Co-extractions

All samples were extracted with both methodologies using 200 µl of semen. The commercial co-extraction kit *ExtractME* RNA & DNA Kit (BLIRT S.A., Poland) was used following the manufacturer's instructions. However, slight modifications were applied to the protocol in the lysis step: addition of 20 µl of DTT at 1 mM and an incubation step of 30 min at 56 °C. As for the in-house manual method, an adapted protocol from Shojaie N. et al. [5] was used. This protocol was developed by the authors to simultaneously extract DNA and RNA from animal cells based on a modified Laemelli buffer [5]. The published protocol was followed [5]; however, some modifications were introduced to the lysis step to adapt to the type of sample (semen): 20 µl DTT 1 mM, 20 µl proteinase K and incubation of 30 min at 56 °C.

2.3. Quantifications, mRNA markers, PCR and CE

Quantifications and quality control ratios measured by absorbance at A260, A280 and A230 nm were obtained using a Nanodrop 1000 spectrophotometer (Thermofisher Scientific). PRM1 [2] and SEMG1 [3] were selected as semen-specific markers. Reverse transcription reactions were performed using the SensiFAST cDNA synthesis kit (Blirt S.A.) according to the company's recommendations. Primer concentrations were at 0.25 µM in PCR and amplified using the Multiplex PCR Mastermix (Qiagen). Thermocycling conditions used were as following: 95 °C for 15 minutes; 35 cycles of 94 °C for 30 s, 57 °C for 90 s, 72 °C for 40 s and at 72 °C for 30 minutes. DNA genotyping was performed using the autosomal short tandem repeats (STRs) included in the Global Filer PCR Amplification kit (Thermo Fisher Scientific) following the manufacturer's recommendations. Separation of DNA and RNA products was done by capillary electrophoresis (CE) using an ABI3500 platform and LIZ500 internal size standard (Applied Biosystems).

3. Results and discussion

Initial testing of the commercial kit comprised four semen samples. As low quantifications results of DNA (1.8 ± 0.5 ng/µl) and RNA (8.2 ± 4.5 ng/µl) were obtained, 20 µl of DTT 1 mM were added followed by an incubation at 56 °C for 30 minutes to improve the efficacy of the kit and recovery of nucleic acids by adapting the protocol to semen samples. The same modifications were added to the manual in-house protocol [5] as well as addition of proteinase k to the lysis buffer. Therefore, the remaining 10 samples, which were extracted under equal conditions, are used for comparison purposes.

3.1. Nucleic acid quantifications

RNA concentrations were higher for the majority of samples (90%) obtained with the adapted literature protocol (160.8 ± 82.3 ng/µl) when compared to the commercial kit (67.1 ± 49.5 ng/µl). As for the DNA samples the average concentration observed for the manual procedure was 36.8 ± 28.7 ng/µl versus 46.0 ± 22.7 ng/µl for the company kit.

3.2. QC assessment of the ExtractME RNA & DNA kit

The majority of extracts were within the range of acceptable standard purity quality assessment ratios: for OD_{260}/OD_{280}, 9 RNA and DNA samples (representing 90% of the samples) presented values of ~2.0 and of ~1.8, respectively. As for the possible presence of inhibitors detected by the OD_{260}/OD_{230} ratio, only 4 samples (out of the total 20 DNA and RNA extracts considered for comparisons) presented acceptable values of purity within the standard values (~1.8-2.2). Five samples had values between 1.3 and 1.6, and the remaining majority with $OD_{260}/OD_{230} < 1.1$. This indicates the presence of co-purified contaminants in the extracts of both DNA and RNA that are carried over during procedure.

3.3. QC assessment of the manual adapted protocol

A total of 9 DNA and RNA extracts obtained with this procedure presented values of $OD_{260}/OD_{280} < 1.3$ and < 1.0, respectively. At the OD_{260}/OD_{230} readings, only one sample had a standard value of ~2.0 while the remaining had appreciably lower values (0.2-0.6) indicating a high concentration of inhibitors (i.e., co-purified contaminants) present in the analyzed extracts. Although high concentrations of nucleic acids were estimated, a first look at the absorbance ratios of OD_{260}/OD_{280} and OD_{260}/OD_{230} suggest that the presence of inhibitors will contribute to complications in downstream applications, such as PCR, particularly for the samples obtained with the non-commercial procedure.

3.4. mRNA profiling of semen specific markers

To evaluate the performance of the manual method and of the *ExtractME* RNA & DNA kit for the co-extraction of forensic samples, end-point PCR was performed using two mRNA semen specific markers, PRM1 and SEGM1. All semen extracts (n = 14) obtained with the commercial kit had positive expression for both mRNA markers. For the adapted manual protocol, no expression of the semen markers was detected in any of the samples (n = 13). When contemplating the QC assessment ratios, the lack of detection of expression in RNA extracts from the literature-adapted protocol, could be due to a very high concentration of contaminant carryover that most probably inhibits PCR. One of the critical steps observed in this method, apart from the formulation of the extraction buffer as suggested by the authors [5], was the separation of DNA from RNA, which involves the removal of the DNA white coil (separation) after precipitation by isopropanol addition to the lysate. Also, it could be that some DNA still remains in the RNA extract which would justify the higher nucleic acid quantities absorbing at 260 nm when compared to the other methodology. A DNase treatment on the RNA extract could be included to remove any residual DNA. Short tandem repeat profiling was also performed for all DNA extracts obtained from both methods. The same tendency of results was observed: for DNA samples extracted with the commercial kit *ExtractME* RNA & DNA full (clean) profiles were obtained for each of the 14 samples (data not shown). On the other hand, only 15% of the samples (2 out of 13 samples) extracted using the manual method resulted in full male DNA profiles corresponding to the same profiles obtained with the extraction commercial kit.

The main aim of this study was to compare the efficiencies of a column-based extraction commercial kit (*ExtractM*e RNA & DNA, BLIRT S.A.) and a simple, low cost manual based procedure [5]. The objective was to test if the latter protocol would perform at the same level as a commercially developed and optimized kit. This would allow implementation of a straightforward and low cost DNA and RNA co-extraction protocol in any forensic laboratory, where many times, budget limitations can determine the implementation of RNA based methods. The commercial kit demonstrated highly satisfactory results and therefore adequate to extract DNA and RNA from semen samples. Despite the non-favorable results for the tested literature based method in the present study, it must be noted that this protocol was developed for animal cell culture extraction [5], demonstrating good results, but it was not developed or tested in body fluids. Therefore adjustments and refinements, are needed to potentially and successfully extract DNA and RNA from body fluids (or other tissue types) relevant in forensics, using a simple, low cost in-house developed method. Additionally, to remove some of the inhibitors present in the extracts, a purification protocol can be applied to further resolve inhibition. As well, a DNase treatment step should be added to clean-up any residual DNA in the RNA sample.

Role of Funding

This work was partially financed by FEDER—Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020-Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through FCT—Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Inovação in the framework of the project "Institute for Research and Innovation in Health Sciences" (POCI-01-0145-FEDER-007274). I. Gomes is funded by the "FCT Scientific Employment Stimulus - Individual Call" program (CEECIND/02609/2017).

Declaration of Competing Interest

None.

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