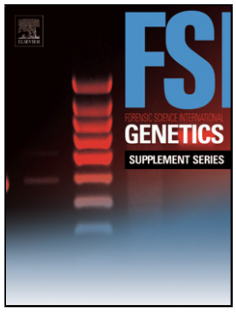

Time since deposition of biological fluids using RNA degradation

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1. **Title:** Time since deposition of biological fluids using RNA degradation

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3. **Abstract**

In criminal casework where sexual intercourse has taken place, body fluids are usually left behind. Body fluid identification (BFID) of the type and origin can provide important information regarding what has occurred and who from, however BFID struggles to determine time since deposition (TSD) of biological stains. According to the Crime Survey in England and Wales in 2017, it shows that the majority of sexual offences that occur are committed by people already known to the victims, roughly around 90%, with 56% of these being committed by partners or ex partners. In these cases, the defence is that their DNA will be present because they are in a relationship or were in a prior relationship. To challenge this defence, the degradation of RNA in semen stains could potentially provide information regarding when a stain was deposited. The degradation pattern of a few semen specific mRNA genes and housekeeping genes were evaluated over a 1 month period. These semen stains were extracted periodically and quantified using qPCR generating real-time levels of RNA which can then be compared to the levels of fresh semen. This study shows that different semen specific primers will degrade at different rates over a period of time and therefore could be used to determine the time since deposition of biological fluids.

4. **Keywords**

Body fluid identification; Time since deposition mRNA Semen specific

5. **Introduction**

Body fluid identification (BFID) of type, whether that be blood, saliva, semen or vaginal material and origin provide important links between a crime scene and individuals [1]. However, BFID provides no information of when the biological material was deposited [2]. Originally, studies looked into the possibility of retrieving RNA from samples after long term storage such as Karlsson, *et al* (2003) before trying to determine the time those samples were deposited. Karlsson, *et al* showed that reference genes Beta-Actin and GAPDH can be extracted after storage of 1 month, 21 years and 27 years [3]. Although these types of studies show that old samples can provide the ability to retrieved RNA, it does not show when those samples were deposited. To date there have been a number of studies looking into stain age prediction of biological stains. RNA is used over DNA in these experiments to determine the time since deposition as DNA is much more stable than RNA, and therefore RNA molecules are susceptible to degradation [4]. Being susceptible to degradation allows the analysis of the levels of RNA markers after a period of time [2]. Over the last couple of years, the number of techniques using RNA has increased exponentially. Several approaches have been used to determine the ages of stains. Bauer et al, 2003 [5] developed a semi-quantitative duplex reverse transcription-polymerase chain reaction assay and investigated 106 bloodstains that were up to 15 years old. Anderson et al, 2005 [2] used real-time reverse transcriptase PCR to show the ratios of mRNA and rRNA genes over time from human blood that had been dried for 150 days. However, neither of these studies used body fluid specific markers, therefore mixed stains i.e. multiple body fluids would cause an issue to their findings, if those fluids were deposited at different times.

In order to counteract the issue of mixed stains, a series of semen specific markers were analysed over a period of a month, periodically throughout. The aim of the study was to determine whether there is a change in the levels of the semen specific markers from a fresh sample to that of a sample that was a month old.

6. **Materials and Methods**

Sample collection

Semen samples were deposited into sterile 1.5ml microcentrifuge tubes. Aliquots were recovered at 5 time points, fresh, week 1, week 2, week 3 and week 4.

Extraction

Samples were extracted using the Qiagen miRNeasy ® Mini kit, using the semen extraction protocol as per manufacturer's instructions.

cDNA synthesis and Quantitative PCR

cDNA synthesis was conducted using the iScript™ Reverse Transcription Supermix (company and country). Absolute quantification of the markers was carried out using SYBR Green chemistry (company and country) on the OneStep Plus qPCR machine (Applied Biosystems, UK).

7. Results and discussion

As seen in Figure 1, PRM 1 and KLK 3 has relatively low expression in the fresh sample and although there does appear to be a decrease over time, the low initial expression makes it difficult to observe a clear degradation profile. Semen specific markers, PRM 2 and TGM 4 exhibit a significant degradation from the fresh samples and samples that have been stored for a week dropping from a concentration of 42.728ng/µL and 26.465ng/µL in the fresh sample to 13.862ng/µL and 7.689ng/µL respectively a week later. Both markers then continue to degrade at a similar rate for the remaining of the study. The reference gene GADPH follows a relatively linear decrease, in that the concentrations shown in the qPCR reaction start at around 7ng/µL in a fresh sample and then begins to decrease in concentration around a half very week that the sample is stored. These different degradation rates could potentially be used to estimate the time since deposition of body fluids, especially for samples that are earlier than 3 weeks old. However, it is clear that multiple primers will have to be characterised from a sample for stain age prediction.

8. Conclusion

In conclusion, this study shows that different semen specific primers will degrade at different rates over a period of time and therefore a semen-age prediction model is possible.

9. Acknowledgements

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10. Conflict of interest

None

11. Role of funding

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12. References

[1] J.H. An, K.J. Shin, W.I. Yang, H.Y. Lee, Body fluid identification in forensics. *The Korean Society for Biochemistry and Molecular Biology.* (2012) 545-553.
[2] S. Anderson, B. Howard, G.R. Hobbs, C.P. Bishop, A method for determining the age of a bloodstain. *Forensic Science International.* 148 (2005) 37-45.
[3] H. Karlsson, C. Guthenberg, U. Von Döbeln, K. Kristenssson, Extraction of RNA from dried blood on filter papers after long-term storage. *Clinical chemistry*. *49*. 6. (2003) 979-981.
[4] S. Alshehhi, N.A. Mccallum, P.R. Haddrill, Quantification of RNA degradation of blood-specific markers to indicate the age of bloodstains. *Forensic Science International: Genetics Supplement Series 6.* (2017) 453-455.
[5] M. Bauer, S. Polzin, D. Patzelt, Quantification of RNA degradation by semi-quantitative duplex and competitive RT-PCR: A possible indicator of the age of bloodstains? *Forensic Science International.* 138 (2003) 94-103.
[6] E.M. Glare, M. Divjak, M.J. Bailey, E.H. Walters, Beta-Actin and GAPDH housekeeping gene expression in asthmatic airways is variable and not suitable for normalising mRNA levels. *Thorax.* 57 (2002) 765-770.
[7] M. Jodar, S. Kalko, J. Castillo, J.L. Ballesca, R. Oliva, Differential RNAs in the sperm cells of asthenozoospermic patients. *Human reproduction.* 27. 5 (2012) 1431-1438.
[8] G.C. Ostermeier, R.J. Goodrich, M.P. Diamond, D.J. Dix, S.A. Krawetz, Toward using stable spermatozoal RNAs for prognostic assessment of male factor fertility. *Fertility and Sterility.* 83. 6. (2005) 1687-1694.
[9] R.I. Fleming, S. Harbison, The development of a mRNA multiplex RT-PCR assay for the definitive identification of body fluids. *Forensic Science International: Genetics.* 4. (2010) 244-256.
[10] J. Meola, L.R. Goulart, J.D.D. Oliveira, A.F. Neves, W.P. Oliveira Jr, A.C.M. Saraiva, A.C. Capaneli, A.M. Cardoso, L.D. Prado, S.A. Borba, H.D. Silva, Differential expression of KLK 2 and KLK 3 genes in peripheral blood and tissues of patients with prostate cancer. *Genetics and Molecular Biology.* 29. 2. (2006) 193-199.

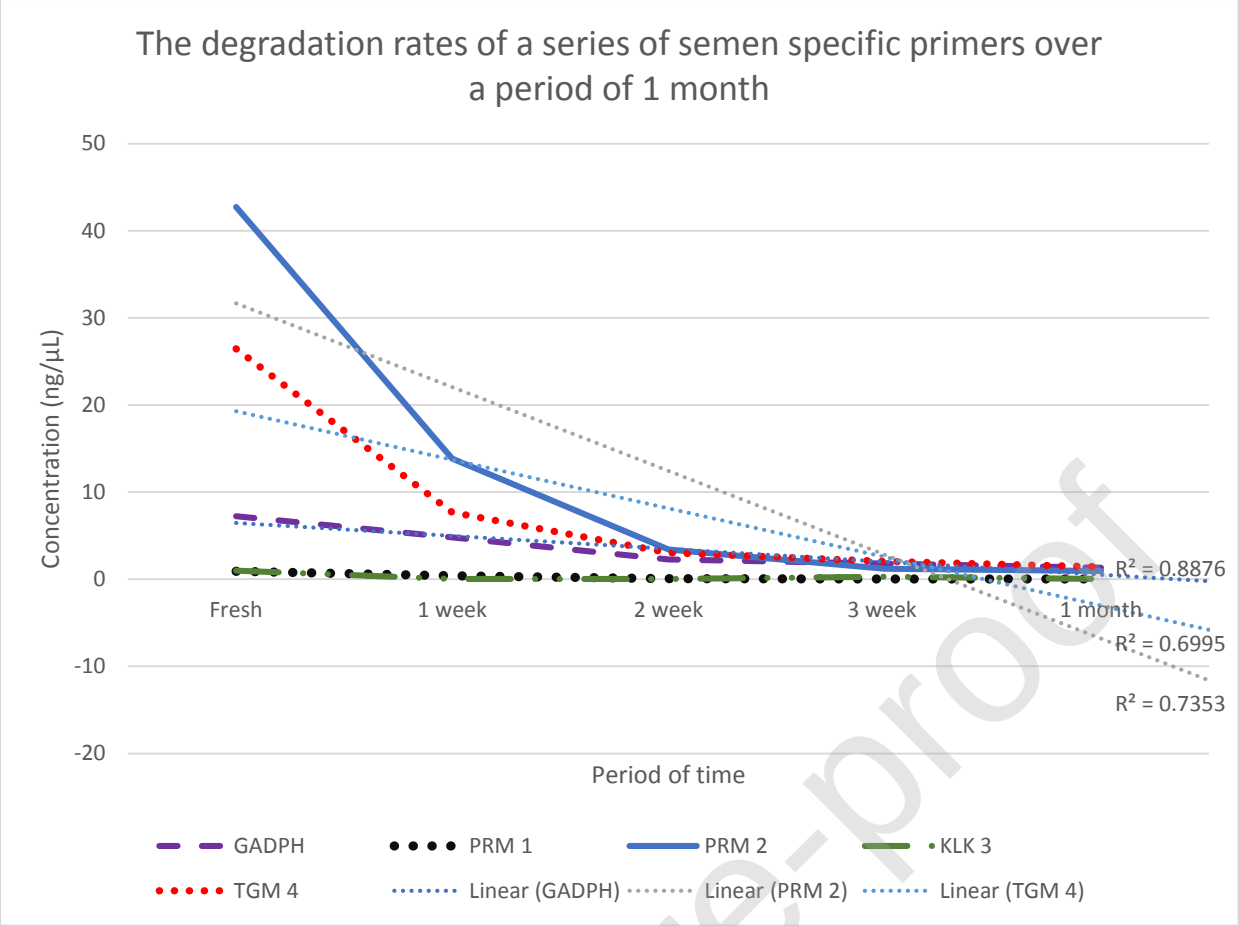

Figure 1: *The graph shows the degradation levels of semen specific primers over a period of time ranging from a fresh sample to a sample that has been stored for a month at room temperature. R^2 value of 0.6995 corresponds to primer TGM 4, R^2 value of 0.0.7353 corresponds to primer PRM 2 and R^2 value of 0.8876 corresponds to primer GADPH. Error bars were omitted for clarity.*