Characterising degradation profiles of RNA molecules within blood stains: Pilot studies

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Introduction

One of the capability gaps in a forensic investigation is the estimation or determination of the age of the stain. For example, was the blood stain deposited today or two months ago; was the semen stain deposited yesterday or five days ago, and was the balaclava worn by the suspect at the time of the robbery? A number of strategies have been proposed, one of which is the characterisation of RNA molecules as they degrade over time. Preliminary work targeting housekeeping genes have demonstrated a proof of principle [1]. Other strategies have included chemical options, for example Raman spectroscopy [2, 3]; however, to maximise the usefulness of the data, a genetic strategy would be preferred – in order that closer links with DNA profiles can be made. Consequently, characterising the pattern of RNA expression over time since deposition should produce some insights in the degradation profile. Such insights may lead to being able to offer an opinion as to the age of the stain in criminal case work. Different time scales were explored, including a 2 year study (collection at 2 year and 10 months), a 22 days study (with a collection everyday) and a 12 hour study (with a collection every hour). The data was brought together from three different studies.

Sample collection and storage

Blood samples were collected using the finger prick method from volunteers with informed consent. The blood was deposited on to filter paper and stored at room temperature in the dark. Once the appropriate period has passed, a 3mm^2 section of filter paper was removed and stored into RNA Later (Qiagen, UK) and then stored at -20oC for the 2 year study and 3 week study. For the 12 hour study, the samples were stored at 4oC (as the RNA Later protocol calls for a 24 hour incubation period prior to storing).

Extraction and cDNA synthesis

Once all samples have been prepared and stored, they all underwent extraction at the same time, using the blood protocol of the Total RNeasy extraction kit (Qiagen, UK).

All extracted samples then underwent reverse transcription using an MMLV reverse transcription kit (Life Technologies, UK) along with random decamers as per manufacturer's instructions.

qPCR analysis

The samples then underwent qPCR analysis on a 7500 Fast Real-Time PCR machine (Life Technologies, UK) targeting GAPDH, β-Actin, 18s rRNA, 28s rRNA, and HBB; with the latter being a blood specific marker, using Taqman chemistry.

Cq values were obtained from qPCR. The formula CqMAX-Cq was used to obtain ΔCq values. The ΔCq values were used for data analysis. Paired sample t-tests were used to assess significance with a threshold of 0.05.

2 Year Study

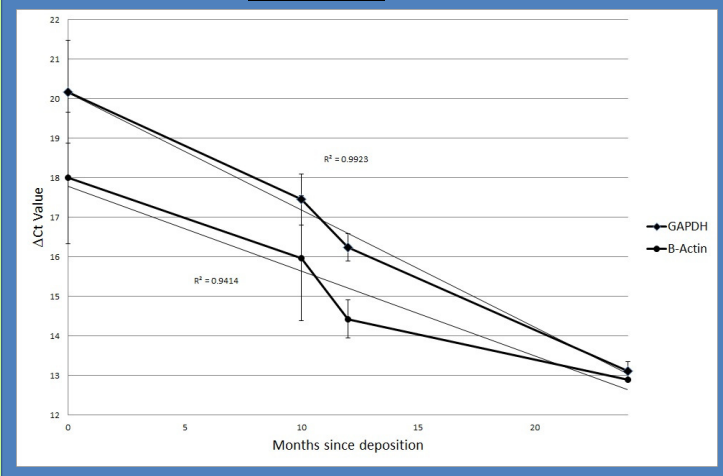

Figure 1: A scatter graph exhibiting the change in expression over a period of two years. Error bars are present as 1sd

22 Days Study

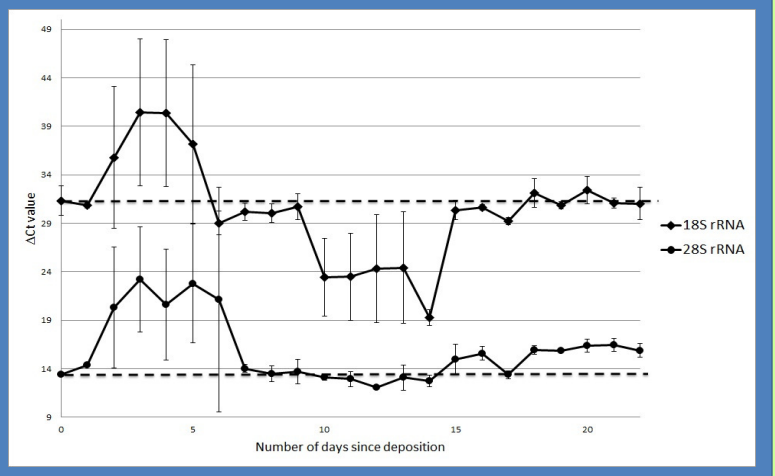

Figure 2: A scatter chart showing the expression of 18s rRNA and 28s rRNA over a 22 day period. The dashed lines represent the level of expression in fresh samples for reference purposes. Error bars are present as 1sd

11 Hours Study

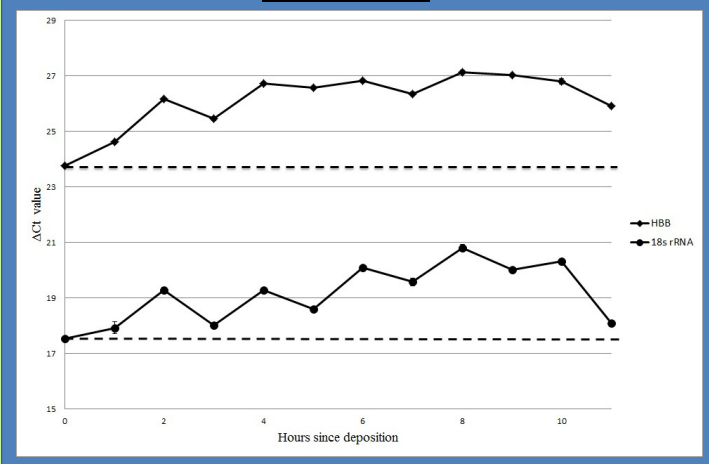

Figure 3: A scatter chart indication the expression of HBB and 18s rRNA over an 11 hour period. Error bars are present as 1sd.

Discussion

When considering the expression of 18s rRNA in both the 22 day study and the 11 hour study; it appears that there could be considerable fluctuation of 18s rRNA over a period of time. For example, the 11 hour study shows a higher expression at 11 hours, although the trend is going down; but when considering in light of the 22 day study, the expression of 18s rRNA at 1 day since deposition is lower than the fresh sample. The 22 day study then indicates that after 1 day, the expression increases again, somewhat considerably.

Whilst these studies are preliminary, they do indicate that mRNA and rRNA markers may not be suitable for stage determination in short periods of time; i.e. ~3 weeks or less. The 2 year study backed up the Anderson study in that there is a linear change over time, but only in the longer time frame. Considerable further work is needed; further markers can be explored as well as various strategies for interpretation building on the work of Weyermann *et al* [4].

In conclusion: Longer time studies do demonstrate a decrease in expression of at least two endogenous controls in a broadly linear fashion, suggesting great potential for stain age determination. However, studies of shorter time frames indicate a great deal of fluctuation, thus suggesting that the use of some RNA molecules may be unsuitable for determining the age of blood in shorter time frames.

References

[1]. S. Anderson, B. Howard, G.R. Hobbs, C.P. Bishop, A method for determining the age of a bloodstain. Forensic Science International. 148(1) (2005) 37-45. [2]. K. Virkler, I.K. Lednev, Raman spectroscopy offers great potential for the non-destructive confirmatory identification of body fluids. Forensic Science International. 181(1-3) (2008) e1-e5. [3]. K. Virkler, I.K. Lednev, Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic Science International. 188(1-3) (2009) 1-17. [4]. C. Weyermann, O. Ribaux, Situating forensic traces in time. Science & Justice. 52 (2) (2011) 68-75.