Forensic Science International 203 (2010) 71–75

Contents lists available at ScienceDirect

Forensic Science International

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

mRNA profiling in forensic genetics I: Possibilities and limitations☆

Marielle Vennemann [a,b,*], Antje Koppelkamm [a]

[a] Institute of Legal Medicine, University of Freiburg, Albertstr. 9, 79104 Freiburg, Germany
[b] Centre for Forensic Science, Strathclyde University, Royal College, 204 George Stree, Glasgow G1 1XW, Scotland, UK

ARTICLE INFO

Article history:
Available online 17 August 2010

Keywords:
mRNA profiling
Post mortem tissue
Degradation
Body fluid identification
Cause of death

ABSTRACT

Molecular investigations gain increasing interest in forensic medicine. Examination of gene expression levels at the time point of death might have the power to become a complementing tool to the current methods for the determination of cause and circumstances of death. This includes pathophysiological conditions of disease and injury as well as the duration of agony or other premortem factors. Additionally, recent developments in forensic genetics revealed that tissue specific mRNAs can be used to determine the type of body fluid present in a crime scene stain.

Although RNA is known to be rather instable, RNA could be extracted in adequate quality from tissue samples collected during medico-legal autopsy. Nevertheless, working with human postmortem tissue means to deal with highly variable RNA integrities. This review aims to give a brief overview of the possible advantages of postmortem mRNA profiling and to shed further light into the limitations of this method arising from reduced RNA integrities.

1. Introduction

1.1. RNA as the intermediate template of protein biosynthesis

When James D. Watson and Francis Crick [1] described the three dimensional structure of DNA in 1953 this was the first idea on how genetic information is encoded and how it is transferred from one generation to the next. Now we know that the main precondition for the transfer of genetic information from one cell to its daughter cells as well as from one generation to the next is the high stability of genomic DNA [2,3]. Even though the structure of RNA is similar to DNA, its function is time limited resulting in relatively short half-lifes. One central assignment of RNA is the conversion of genetic information into proteins and the regulation of this process. During this, the two strands of DNA are separated and RNA is synthesised by RNA polymerases complementary to the coding strand. To ensure the possibility of regulation of gene expression it is crucial to avoid the accumulation of certain mRNAs. Thus, RNA needs to be degraded in the cell by omnipresent, highly reactive ribonucleases [4]. This is essential for the regulation of translation and therefore for the control of the amount of a gene product [5].

1.2. Different RNA families

By now, many different forms of RNA molecules are known which can be differentiated not only by their specific configuration and secondary structure but mainly by their function and half-life (Table 1). On the one hand there are RNAs, like *messenger* RNA (mRNA), *transfer* RNA (tRNA) and *ribosomal* RNA (rRNA) which are directly involved in the biosynthesis of proteins [6]. On the other hand there are numerous functional RNAs which for example are involved in the regulation of the gene expression process or which have a catalytic character, like ribozymes.

In the first step of protein biosynthesis, genetic information is transcribed into mRNA which is then transferred to the ribosome. The proportion of mRNA is only 3–5% of the total amount of RNA in the cell while the 28S, 18S and 5.8S rRNAs form the largest RNA family [7]. The rRNAs build the two ribosomal subunits. During translation they catalyse the binding of peptides. The tRNA is also involved in the process of translation and helps transferring the correct amino acids from the cytoplasm to the ribosomes [8]. Beside the initiation of transcription and translation the half-life of different RNA families is a crucial point in gene expression regulation. rRNA as well as tRNA remain stable for several days while different mRNAs show quite varying half-lives. There are rather instable transcripts which are degraded after minutes but also transcripts that remain stable for several days.

Now an additional group of relatively short RNA molecules is known. A number of small RNAs are also involved in the regulation of gene expression [9]. MicroRNAs (miRNAs) and anti-sense RNAs (asRNAs) are directly synthesised as single stranded molecules

☆ This paper is part of the special issue entitled: Molecular Pathology in Forensic Medicine, Guest-edited by Burkhard Madea and Pekka Saukko.

* Corresponding author.
E-mail address: Marielle.heinrich@uniklinik-freiburg.de (M. Vennemann).

0379-0738/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2010.07.006

Table 1
Overview over different RNA families with their specific functions and half-lives.

Type of RNA	Abbreviation	Main function	Half-life
Messenger RNA	mRNA	Template for translation	Minutes to days
Transfer RNA	tRNA	Transport of amino acids	Days
Ribosomal RNA	rRNA	Ribosomal structure	Days
Small nuclear RNA	snRNA	mRNA processing	Days
Small interfering RNA	siRNA	Regulation of gene expression	Days to weeks
microRNA	miRNA	Regulation of gene expression	Days to weeks
PIWI interacting RNA	piRNA	Regulation of gene expression	Days to weeks
Anti-sense RNA	asRNA	Regulation of gene expression	Days to weeks
Riboswitches	–	Regulation of gene expression	Days to weeks
Ribozymes	–	Enzymatic function	Days

while small interfering RNAs (siRNAs) are cut from double stranded pre molecules with the help of dicer [10]. Mature miRNAs and siRNAs usually have a length of only 21–26 nucleotides. Similar to asRNAs, they are involved in post-transcriptional mRNA degradation and the control of translation activities [11,12]. The expression of miRNAs is mainly tissue specific [13] and they act as marker for post-transcriptional gene silencing: miRNA binds to the 3′-untranslated region (3′-UTR) of the mRNAs which then are either destroyed by nucleases or their translation is blocked [14]. Current developments in biochemistry revealed that the expression of up to 30% of all genes is regulated by miRNAs [8].

A rather specific group of small RNAs is called PIWI interacting RNAs (piRNAs). They are involved in gene silencing of transposons during spermatogenesis [15,16]. To fulfil the function of gene regulation, the family of small RNAs needs to show a comparably long half-life with up to two weeks.

A further family of relatively short functional RNAs comprise small nuclear RNAs (snRNA) which usually have a length between 90 and 190 nucleotides. They are responsible for processing of pre mRNA into mature mRNA within the splicosome [17].

2. Possible applications in forensic genetics

Many cellular decisions, including survival, growth and differentiation are regulated by specific gene expression patterns [18]. Thus, the possibility to quantify gene expression levels of specific genes currently gains increasing importance in a wide range of scientific disciplines.

mRNA as the intermediate template in protein synthesis is a target for the analysis of gene expression patterns because it reveals the activities of genes within a certain tissue type at a certain point of time. Of course, the question of correlations between changes in the normal expression pattern and pathological changes of the tissue arises immediately. From a medico-legal point of view, the analysis of gene expression patterns might give hints on pathological states [19] or the circumstances leading to death [20]. Some authors hope to use specific mRNA degradation patterns as a new tool for the accurate determination of the time since death [21].

2.1. Identification of body fluids

Blood and saliva are considered the most common types of body fluid that form a common source for genetic information used in forensic science. But also ejaculate, vaginal secretion, lacrimal fluid and sweat are common subjects. The determination of the type of body fluid is important to insure the correct handling of samples, e.g. in cases of mixtures from different sources like ejaculate and vaginal secretion [22]. Current tests for the identification of body fluids use chemo luminescence and the detection of specific proteins (e.g. [23]). Due to the fact that gene expression patterns are tissue specific, a determination of the type of body fluid based on mRNA profiling may be possible. First experiments show that RNA can be isolated in suitable quality and quantity from menstrual blood [24,25], blood, saliva and vaginal secretion [26–28].

Recently, a number of articles concentrating on the practical considerations connected to mRNA profiling using crime scene stains were published by several authors. These works address the challenge of isolating mRNA and DNA in suitable quality and quantity from limited amounts of stains [29,21] as well as the validation of methods to analyse certain transcripts, e.g. by end point PCR or real time PCR [23,30] and suitable sets of stable mRNA markers [31,28].

One problem when working with crime scene stains is that due to degradation, false negative results are possible. Additionally, many transcripts are not completely tissue specific but may show high expression levels in one body fluid and very low expression levels in another. Thus, a qualitative analysis, e.g. using end point PCR, might not be appropriate but rather quantitative analyses are suitable. Nussbaumer et al. [32] showed that kalikrein 3 (KLK) transcripts are detectable in ejaculate but not in blood, saliva and vaginal secretion. Thus, in this case analysis via end point PCR is sufficient. This group also showed that other transcripts, like hemoglobin-alpha-1 (HBA) and mucein (MUC) show high expression levels in ejaculate and vaginal secretion, respectively, but are also detectable in other body fluids. This condition requires a quantitative approach. In quantitative analyses, the validation of a suitable normalisation strategy plays a central role [33]. Without normalisation, analyses might lead to false negative or false positive results. But also in cases of qualitative analyses, it seems reasonable to include a quality marker to exclude the risk of false negative results. A useful quality marker might be the transcript of a low expressed gene which degrades similar or even faster than the gene of interest. Another approach for this problem was described by Juusola and Ballantyne [27] by using not one marker for each body fluid but a set of three markers.

Since gene expression patterns are tissue specific it has to be expected that also the presence of certain regulatory elements, like microRNAs, are highly tissue dependent. Due to their high stability and comparably long half-lives, microRNAs gained increasing interest for their use in body fluid identification recently [34,35].

2.2. Molecular determination of the cause and circumstances of death

The composition of different RNA transcripts within the cells of a tissue, the RNA pool, is not considered stable, but changes according to the specific needs of the cell in response to external conditions. Thus, each event, may it be a molecular event or some external impact influencing the whole organism, leaves a molecular mark in terms of shifted proportions of different

transcripts within the mRNA pool. Since one has to expect that changes in gene expression activities can happen at least up to 30 min postmortem [20], there might be a chance to use mRNA profiling in postmortem human tissue to obtain hints on events that happened at the time point of death. Thus, a molecular approach to the analysis of cause and circumstances of death seems reasonable and promising.

Ikematsu et al. [20] presented a first example for the identification of possible biomarkers indicating certain causes of death: They examined gene expression changes in mice with respect to two different causes of death, slow strangulation and fast execution using a guillotine. Four genes were identified that showed significantly different expression patterns between the two groups. Thus, these genes are candidate genes as possible biomarkers for strangulation. Recently, further candidate genes for the identification of methamphetamine related deaths [36] and hypoxia related deaths [37–39] were described. Additionally, works on contusion stress [40] and mechanical asphyxiation [41] were published recently.

When working with human postmortem tissue, one has to deal with impaired RNA. Nevertheless, several studies could show that it is very well possible to isolate RNA from postmortem material in suitable quantity and quality to perform gene expression studies [42–46]. Thus, it is possible to indeed use these tissues to obtain information on gene expression activities. Beside the chance to validate molecular methods to complement the current morphological techniques for the determination of cause and circumstances of death, it might also be interesting to provide gene expression data from healthy human tissue. When analysing the molecular cause of human diseases it is crucial to have a set of normal and healthy tissue samples to be able to distinguish between normal and pathological gene expression patterns [47].

But even though the first experiences in working with post mortem tissues appear to be quite promising [45–50] one has to keep in mind that a certain bias in the mRNA pool might occur due to degradation. Thus, it is crucial to obtain as much information on degradation processes and parameters influencing RNA integrity as possible.

3. mRNA stability

3.1. mRNA stability in living cells

Once the mature mRNA transcripts are transported into the cytoplasm, the half-life of different RNA families is determined by specific sequence elements. Short-lived mRNAs show sequence segments with a high number of uracil and adenine nucleotides. These AU rich elements (ARE) are located in the 3′-UTR and target the mRNA for rapid degradation. Polyadenylate ribonucleases break down the mRNA in 3′–5′-direction (reviewed in [51]). Besides degradation in 3′–5′-orientation, RNA breakdown starting from the 5′-end is also known after enzymatic removal of the methylguanosine cap [52]. The type of mRNA determines the preferred way of degradation in the course of mRNA turnover [3].

Due to the fact that gene expression is a complex process that requires numerous steps including transcription, mRNA processing, transport of mature mRNAs into the cytoplasm and the whole complex of translation, errors might occur that lead to a termination of translation. Additionally, non-sense mutation might occur during DNA replication and mRNA synthesis. Thus, numerous control mechanisms are needed to distinguish between intact and impaired transcripts. Once an impaired mRNA is detected, its rapid degradation is insured to avoid translation of these transcripts (e.g. [3]). The controlled degradation of mRNA is a method of regulating translation and thus, the amount of gene product obtained. Thereby RNases play an important role. They are ubiquitously present, highly reactive enzymes [4].

3.2. mRNA stability in biological stains

Zubakov et al. [31] were able to identify blood and saliva specific mRNA markers that showed stable expression patterns in stains after up to 180 days of storage. These finding raise hope that even in stains with a long period of time passing before discovery and analysis accurate determination of the type of body fluid is still possible.

On the other hand, Bauer et al. [5] and Bauer and Patzelt [53] found highly varying transcript quantities of β-actin and cyclophilin A in up to 15 years old blood stains. Thus, they postulated that there might be a possibility of using transcripts with constant degradation rates for the determination of the age of a blood stain.

A more comprehensive study on the influence of various storage conditions on the analysis of different transcripts [54] showed that detection of mRNA from samples stored indoors (at room temperature) was successful even after 547 days. If samples were kept outdoors, but protected from precipitation, tissue specific mRNAs could still be analysed in saliva (up to 7 days), blood (up to 30 days) and vaginal secretion (up to 180 days). Rain was found to have a negative impact on the stability of transcripts with a detection period of a few days only.

3.3. mRNA stability in postmortem tissue

The RNase activated degradation of RNA is essential for the regulation of gene expression within the living cell. For a long time it was common sense that ubiquitous RNases would destroy gene transcripts shortly after death (e.g. [4]). But recent studies could show that isolation of intact RNA is possible even several days postmortem [55–58,43,42,45].

Beside enzymatic degradation RNA decay might also be influenced by external factors like sun light, humidity or high temperatures during the postmortem interval (PMI). The PMI describes the delay between the time point of death and the collection of samples. In the forensic context a PMI of at least several hours cannot be avoided. The influence of the duration of PMI on the integrity of RNA was subject of several works. Catts [42] could show that prolonged PMI leads to degradation in transcripts with certain sequence motifs isolated from mouse brain tissue at different time points after death. Using the same animal model it was shown that the intensity of 28S and 18S rRNA bands in a denaturing agarose gel decreases with increasing PMI, while different tissue types showed different degradation rates [43].

From human and animal post mortem material we know that the influence of prolonged PMIs is different between tissues [45,50]. In human postmortem heart tissue, a highly significant correlation between PMI and the RNA quality, described as RNA integrity number (RIN) [59] was observed while in human skeletal muscle tissue, no correlation was found (Fig. 1).

Several parameters might influence the postmortem stability of RNA. It is already known that the integrity of RNA may differ between tissue types as well as between donors [44,60]. The latter might mainly be due to the cause of death and thus, to antemortem internal factors [61]. For example, RNA degradation in human brain tissue seems to be accelerated if the deceased received intensive care and/or showed brain acidosis [62]. Additionally, the following factors are currently discussed to influence the integrity of RNA: gender, age at death, certain medication, terminal coma, hypoxia, pyrexia, and dehydration [63]. Drug and alcohol abuse [19] as well as various types of stress [61] were also found to be correlated with low RNA integrities. In postmortem human skeletal muscle tissue

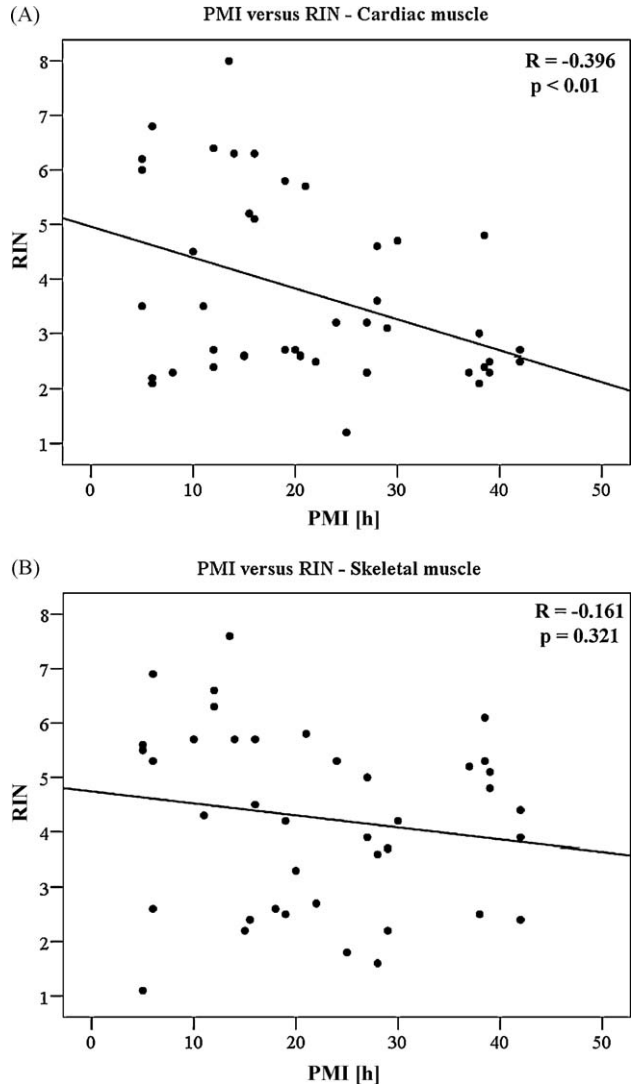

Fig. 1. Correlation between the postmortem interval (PMI) and the quality of total RNA isolated from cardiac muscle (A) and skeletal muscle (B). RNA quality was determined using Agilent Bioanalyzer 2000 and a RNA integrity number (RIN) was achieved [as described in 49]. The higher the RIN value, the higher the overall RNA integrity [59]. The Pearson correlation (R) was found to be highly significant (p) in cardiac muscle but not significant in skeletal muscle.

(M. iliopsoas), we observed that obesity is correlated with impaired RNA (data not published yet). Since it was shown that there is a temperature gradient between body core and shell [64] the anatomical location of this tissue and the reduced cooling rate of a body with a higher BMI during storage of 4 °C might be an explanation for this finding. Current studies using microarray based gene expression profiling found that in human postmortem brain tissue the duration of agony with brain acidosis seems to be the most critical factor for RNA preservation while the postmortem interval has no or only a weak impact [65–69].

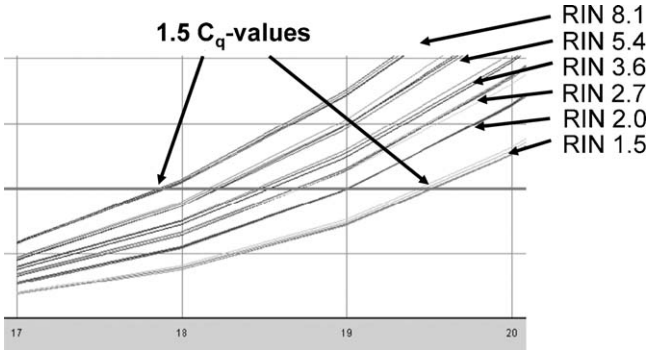

Fig. 2. Reverse transcription real time PCR results for ACTB obtained from an artificially degraded RNA sample (human heart). A shift of 1.5 cycle of quantification (Cq) values was observed between the highest and the lowest RNA integrity number (RIN).

4. Considerations on real time quantitative PCR

Even though further RNA degradation can be reduced by correct storage of samples, working with postmortem human tissue means working with impaired RNA which may influence the results obtained by quantitative gene expression analysis [70]. We examined this effect using an artificially degraded mRNA from total human heart. The reverse transcription real time PCR assay to detect the transcript of β-actin (ACTB) showed a shift of cycle of quantification (Cq) values of 1.5 in correlation to the decreasing RIN (Fig. 2). Thus, when observing rather small differences in transcript amounts they might not be true differences in terms of gene expression but have to be explained by partial degradation of samples. Small expression differences might not be detectable in postmortem tissue whereas huge changes can be analysed with the necessary reliability.

Another problem concerning human tissue samples in general is the fact that one has to deal with rather heterogeneous sample populations because ante- and postmortem parameters cannot be influenced and sometimes might even not be entirely known. Consequently, some unknown parameters might have an impact on the expression of the genes of interest and might even mask the influence of the parameters investigated.

Thus, a series of precautions are necessary to ensure that data indeed are biologically meaningful. These precautions include the accurate collection of samples used as biological replicates, a careful validation of the appropriate normalisation strategy, investigation of the influence of degradation on the transcript amount of the genes of interest and finally a careful and deliberate data interpretation [71].

References

[1] J. Watson, F. Crick, Molecular structure of nucleic acids, Nature 4356 (1953) 737–738.
[2] C.J. Wilusz, W. Wang, S.W. Peltz, Curbing the nonsense: the activation and regulation of mRNA surveillance, Genes Dev. 15 (2001) 2781–2785.
[3] J.W. Wilusz, M. Wormington, S. Petltz, The cap-to-tail guide to mRNA turnover, Nat. Rev. Mol. Cell Biol. 2 (2001) 237–246.
[4] T.W. Phang, C.Y. Shi, J.N. Chia, C.N. Ong, Amplification of cDNA via RT-PCR using RNA extracted from post-mortem tissue, J. Forensic Sci. 39 (1994) 1275–1279.
[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 Sci. Int. 138 (2003) 94–103.
[6] H. Matthaei, M.W. Nirenberg, The dependence of cell-free protein synthesis in *E. coli* upon RNA prepared from ribosomes, Biochem. Biophys. Res. Commun. 28 (1961) 404–408.
[7] J.S. Mattick, G.J. Michael, The evolution of controlled multitasked gene networks: the role of introns and other noncoding RNAs in the development of complex organisms, Mol. Biol. Evol. 18 (2001) 1611–1630.
[8] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are micro RNA targets, Cell 120 (2005) 15–20.
[9] D. Voet, J. Voet, C. Pratt, U. Hahn, Lehrbuch der Biochemie, 1. Auflage, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2002.
[10] J. Rossi, Mammalian Dicer finds a partner, EMBO Rep. 6 (2005) 927–929.
[11] M.A. Valencia-Sanchez, J. Liu, G.J. Hannon, R. Parker, Control of translation and mRNA degradation by miRNAs and siRNAs, Genes Dev. 20 (2006) 515–524.
[12] L. Wu, J.G. Belasco, Let me count the ways: mechanisms of gene regulation by miRNAs and siRNAs, Mol. Cell 29 (2008) 1–7.
[13] I. Beuvink, F.A. Kolb, W. Budach, A. Garnier, J. Lange, F. Natt, U. Dengler, J. Hall, W. Filipowicz, J. Weiler, A novel microarray approach reveals new tissue specific signatures of known and predicted mammalian microRNAs, Nucleic Acids Res. 35 (2007) e52.
[14] S. Chang, S. Wen, D. Chen, P. Jin, Small regulatory RNAs in neurodevelopmental disorders, Hum. Mol. Genet. 18 (2009) R18–R26.

[15] A.G. Seto, R.E. Kingston, N.C. Lau, The coming of age for Piwi proteins, Mol. Cell. 26 (2007) 603–609.
[16] G. Wang, V. Reinke, *C. elegans* Piwi, PRG-1, regulates 21U-RNAs during spermatogenesis, Curr. Biol. 18 (2008) 861–867.
[17] S. Egloff, D. O'Reilly, S. Murphy, Expression of human snRNA genes from beginning to end, Biochem. Soc. Trans. (Pt) (2008) 590–594.
[18] S. Bustin, Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays, J. Mol. Endocrin. 25 (2000) 169–193.
[19] J. Liu, J.M. Lewohl, R.P. Dodd, P.K. Randall, R.A. Harris, R.D. Mayfield, Gene expression profiling of individual cases reveals consistent transcriptional changes in alcoholism human brain, J. Neurochem. 90 (2004) 1050–1058.
[20] K. Ikematsu, R. Tsuda, I. Nakasono, Gene response of mouse skin to pressure injury in the neck region, Legal Med. 8 (2005) 128–131.
[21] M. Bauer, I. Gramlich, S. Polzin, D. Patzelt, Quantification of mRNA degradation as possible indicator of postmortem interval—a pilot study, Legal Med. (Tokyo) 5 (2003) 220–227.
[22] P. Gill, A. Jeffreys, D. Werrett, Forensic application of DNA 'fingerprints', Nature 318 (1985) 577–579.
[23] 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 Sci. Int. 188 (2009) 1–17.
[24] M. Bauer, D. Patzelt, Evaluation of mRNA markers for the identification of menstrual blood, J. Forensic Sci. 6 (2002) 1278–1282.
[25] M. Bauer, D. Patzelt, Identification of menstrual blood by real time RT-PCR: technical improvements and the practical value of negative test results, Forensic Sci. Int. 174 (2007) 54–58.
[26] J. Juusola, J. Ballantyne, Messenger RNA profiling: a proper method to supplant conventional methods for body fluid identification, Forensic Sci. Int. 135 (2003) 85–96.
[27] J. Juusola, J. Ballantyne, Multiplex mRNA profiling for the identification of body fluids, Forensic Sci. Int. 152 (2005) 1–12.
[28] J. Juusola, J. Ballantyne, mRNA profiling for body fluid identification by multiplex quantitative RT-PCR, J. Forensic Sci. 52 (2007) 1252–1262.
[29] M. Alvarez, J. Juusola, J. Ballantyne, An mRNA and DNA co-isolation method for forensic casework samples, Anal. Biochem. 335 (2004) 289–298.
[30] C. Haas, B. Klesser, C. Maake, W. Bär, A. Kratzer, mRNA profiling for body fluid identification by reverse transcription endpoint PCR and realtime PCR, For. Sci. Int. : Genet. 3 (2009) 80–88.
[31] D. Zubakov, E. Hanekamp, M. Kokshoorn, W. van Ijcken, M. Kayser, Stable RNA markers for identification of blood and saliva stains revealed from whole genome expression analysis of time-wise degraded samples, Int. J. Legal Med. 122 (2008) 135–142.
[32] C. Nussbaumer, E. Gharehbaghi-Schnell, I. Korschineck, Messenger RNA profiling: a novel method for body fluid identification by real-time PCR, Forensic Sci. Int. 157 (2006) 181–186.
[33] J. Vandesompele, K. DePreter, F. Pattyn, B. Poppe, N. VanRoy, A. DePappe, F. Speleman, Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes, Genome Biol. 3 (2002) (Research0034, Epub2002).
[34] D. Zubakov, A.W. Boersma, Y. Choi, P.F. van Kuijk, E.A. Wiemer, M. Kayser, MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation, Int. J. Legal Med. (2010) (2010 February 10. Epub ahead of print).
[35] E.K. Hanson, H. Lubenow, J. Ballantyne, Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs, Anal. Biochem. 387 (2009) 303–314.
[36] A. Matsuo, K. Ikematsu, I. Nakasono, C-fos, fos-B, c-jun and dusp-1 expression in the mouse heart after single and repeated methamphetamine administration, Legal Med. (Tokyo) 11 (2009) 285–290.
[37] B. Zhu, S. Tanaka, T. Ishikawa, et al., Forensic pathological investigation of myocardial hypoxia-inducible factor-1α, erythropoietin and vascular endothelial growth factor in cardiac death, Legal Med. (Tokyo) 10 (2008) 11–19.
[38] D. Zhao, B. Zhu, T. Ishikawa, D. Li, T. Michiue, H. Maeda, Quantitative RT-PCR assays of hypoxia-inducible factor-1α, erythropoietin and vascular endothelial growth factor mRNA transcripts in the kidneys with regard to the cause of death in medicolegal autopsy, Legal Med. 8 (2006) 258–263.
[39] D. Zhao, T. Ishikawa, L. Quan, et al., Tissue-specific differences in mRNA quantification of glucose transporter 1 and vascular endothelial growth factor with special regard to death investigations of fatal injuries, Forensic Sci. Int. 177 (2008) 176–183.
[40] S. Takahashi, Expression levels of mRNAs for catecholamine biosynthetic enzymes as markers of acute response to contusion stress during the early postmortem period, Tohoku J. Exp. Med. 216 (2008) 239–248.
[41] K. Ikematsu, H. Takahashi, T. Kondo, R. Tsuda, I. Nakasono, Temporal expression of immediate early gene mRNA during the supravital reaction in mouse brain and lung after mechanical asphyxiation, Forensic Sci. Int. 179 (2008) 152–156.
[42] V.S. Catts, A microarray study of post-mortem mRNA degradation in mouse brain tissue, Mol. Brain Res. 138 (2005) 164–177.
[43] H. Inoue, A. Kimura, T. Tuji, Degradation profile of mRNA in a dead rat body: basic semi-quantification study, Forensic Sci. Int. 130 (2002) 127–132.
[44] J. Lee, A. Hever, D. Willhite, A. Zlotnik, P. Hevezi, Effects of RNA degradation on gene expression analysis of human post-mortem tissues, FASEB J. 19 (2005) 1356–1358.
[45] M. Heinrich, K. Matt, S. Lutz-Bonengel, Successful RNA extraction from various human postmortem tissue, Int. J. Legal Med. 121 (2007) 136–142.
[46] M. Heinrich, S. Lutz-Bonengel, K. Matt, U. Schmidt, Real time PCR detection of five different "endogenous control gene" transcripts in forensic autopsy material, Forensic Sci. Int. 27 (2007) 163–169.
[47] L. Hsiao, F. Dangond, T. Joshida, et al., A compendium of gene expression in normal human tissues, Physiol. Genomics 7 (2001) 97–104.
[48] T. Fracasso, M. Heinrich, C. Hohoff, B. Brinkmann, H. Pfeiffer, Ultrasound-accelerated formalin fixation improves the preservation of nucleic acids extraction in histological sections, Int. J. Legal Med. 123 (2009) 521–525.
[49] A. Koppelkamm, B. Vennemann, T. Fracasso, S. Lutz-Bonengel, U. Schmidt, M. Heinrich, Validation of adequate endogenous reference genes for the normalisation of qPCR gene expression data in human post mortem tissue, Int. J. Legal Med. (2010) 20300940 (Epub ahead of print, PMID).
[50] B. Bahar, F.J. Monahan, A.P. Moloney, O. Schmidt, D.E. MacHugh, T. Sweeney, Long-term stability ofRNA in post-mortem bovine skeletal muscle, liver and subcutaneous adipose tissues, BMC Mol. Biol. 8 (2007) 108.
[51] T.J. Gingerich, J.J. Feige, J. LaMarre, AU-rich elements and the control of gene expression through regulated mRNA stability, Anim. Health Res. Rev. 5 (2004) 49–63.
[52] T.E. LaGrandeur, R. Parker, mRNA decapping activities and their biological roles, Biochimie 78 (11–12) (1996) 1049–1055.
[53] M. Bauer, D. Patzelt, A method for simultaneous RNA and DNA isolation from dried blood and semen stains, Forensic Sci. Int. 136 (2003) 76–78.
[54] M. Setzer, J. Juusola, J. Ballantyne, Recovery and stability of RNA in vaginal swabs and blood, semen, and saliva stains, J. Forensic Sci. 53 (2008) 296–305.
[55] S.A. Johnson, D.G. Morgan, C.E. Finch, Extensive post-mortem stability of RNA from rat and human brain, J. Neurosci. Res. 16 (1986) 267–280.
[56] L. Marchuk, P. Sciore, C. Reno, C.B. Frank, D.A. Hart, Postmortem stability of total RNA isolated from rabbit ligament, tendon, cartilage, Biochim. Biophys. Acta 1379 (1998) 171–177.
[57] R. Fitzpatrick, O.M. Casey, D. Morris, T. Smith, R. Powell, J.M. Sreenan, Postmortem stability of RNA isolated from bovine reproductive tissue, Biochim. Biophys. Acta 1574 (2002) 10–14.
[58] P. Preece, An optimistic view for quantifying mRNA in post-mortem human brain, Mol. Brain Res. 116 (2003) 7–16.
[59] A. Schroeder, O. Mueller, S. Stocker, et al., The RIN: an RNA integrity number for assigning integrity values to RNA measurements, BMC Mol. Biol. 7 (2006) 3–16.
[60] M.R. Hynd, J.M. Lewohl, H.L. Scott, P.R. Dodd, Biochemical and molecular studies using human autopsy brain tissue, Int. Sci. Neurochem. 85 (2003) 543–562.
[61] A.J.L. Barton, R.C.A. Pearson, A. Najlerahim, P.J. Harrison, Pre- and post-mortem influences on brain RNA, J. Sci. Neurochem. 61 (1993) 1–11.
[62] A. Thorsell, S. Gruber, A. Mathé, M. Heilig, Neuropeptide Y (NPY) mRNA in rat brain tissue: effects of decapitation and high-energy microwave irradiation on post mortem stability, Neuropeptides 35 (2001) 168–173.
[63] P. Preece, N.J. Cairns, Quantifying mRNA in post-mortem human brain: influence of gender, age of death, post-mortem interval, brain pH, agonal state and interlobe mRNA variance, Mol. Brain Res. 118 (2003) 60–71.
[64] G. Mall, W. Eisenmenger, Estimation of time since death by heat-flow Finite-Element model. Part I: method, model, calibration and validation, Legal Med. (Tokyo) 7 (2005) 1–14.
[65] T. Popova, D. Mennerich, A. Weith, K. Quast, Effect of RNA quality on transcript intensity levels in microarray analysis of human post-mortem brain tissues, BMC Genomics (2008) 91–115.
[66] A. Stan, S. Ghose, X. Gao, et al., Human postmortem tissue: what quality markers matter? Brain Res. 1123 (2006) 1–11.
[67] S. Weis, I. Llenos, J. Dulay, M. Elashoff, F. Martinez-Murillo, C. Miller, Quality control for microarray analysis of human brain samples: the impact of post-mortem factors, RNA characteristics, and histopathology, J. Neurosci. Methods 165 (2007) 198–209.
[68] H. Tomita, M. Vawter, D. Walsh, et al., Effect of agonal and postmortem factors on gene expression profile: quality control in microarray analysis of postmortem human brain, Biol. Psychiatry 55 (2004) 346–352.
[69] M. Atz, D. Wals, P. Cartagena, et al., Methodological considerations for gene expression profiling of human brain, J. Neurosci. Method 163 (2007) 295–309.
[70] C. Buesa, T. Maes, F. Subirada, M. Barrachina, I. Ferrer, DNA chip technology in brain banks: confronting a degrading world, J. Neuropathol. Exp. Neurol. 63 (2004) 1003–1014.
[71] M. Vennemann, A. Koppelkamm, mRNA profiling in forensic genetics. II: practical considerations, this issue.