ABSTRACT

Detection and identification of human biological fluids, such as blood, semen, saliva, vaginal secretions, menstrual blood, urine and sweat, represent vitally important forensic biology analyses. In numerous cases, not only is the establishing a DNA profile of great significance, but also determination of DNA tissue source is substantial. The profile of a victim's DNA from the clothing of a perpetrator has an entirely different probative value when DNA originates from the epithelium (sudoral and fatty substance) as compared to the situation when it is possible to demonstrate that the source of DNA is vaginal secretions of the victim. The presence of the victim's epithelium may bear no relation whatsoever to the crime, while the presence of vaginal secretions definitely does. Similarly, the presence of peripheral blood may have a completely different significance as compared to the presence of menstrual blood. In the same way the presence of saliva has different relevance than the presence of semen. In legal proceedings, a proper and reliable determination of the source of DNA is essential and an error in identification may result in grave legal and social consequences.

With classical methods, only blood and semen can be identified specifically (100% confirmation of the human biological fluid presence), for other biological fluids there have only been presumptive tests, which are insufficient not only because of frequent lack of specificity, but also in the aspect of the high sensitivity of the methods of DNA amplification. Among classical methods there is not universal technique which identifies various biological fluids. Another problem is the destructive character of numerous preliminary tests, especially when the amount of evidentiary material is scarce.

The research carried out over the past years has resulted in considerable progress in identification of biological fluids. For example, methods based on Raman spectrometry or DNA markers of biological fluids have been investigated. However, a detection technique based on the presence of mRNA molecules specific to given tissues is of particular interest, and my dissertation is devoted to this issue.

The main aim of the research was to develop new methods for the identification of biological fluids important in forensic science, with particular emphasis on those fluids for which there have not been specific detection methods. The developed methods were evaluated in terms of their usefulness for analysis of different types of stains in forensic genetics.

There were two tests elaborated by me and described in publications concerning: the identification of vaginal secretions and menstrual blood (hexaplex) and distinguishing between peripheral and menstrual blood (heptapleks). They are based on detection of tissue-specific transcripts (mRNA) of the following genes: human beta-defensin 1 (*HBD1*), mucin 4 (*MUC4*), haemoglobin alpha locus 1 (*HBA*), erythroid delta-aminolevulinate synthase 2 (*ALAS2*), metalloproteinases 7 and 11 (*MMP7, MMP11*), glucose 6-phosphate dehydrogenase (*G6PDH*) and bacterial markers: 16S–23S rRNA intergenic spacer region (ISR) of *Lactobacillus crispatus* and *Lactobacillus gasseri/Lactobacillus johnsonii.* The research was performed on samples of various biological fluids collected from women and men, right after the collection and stored under different conditions as well as for different periods of time. Additionally, it was performed on biological material from the own resources of the Department of Forensic Medicine, Medical University of Gdańsk (DFM MUG).

Conclusions are contained in three publications[1] which constitute thematically coherent collection of articles published in indexed scientific journals with international scope:

1. Jakubowska J.*, Maciejewska A., Pawłowski R., **mRNA profiling for biological fluids identification in forensic genetics,** Problems of Forensic Sciences (2011) 87:204–215

2. Jakubowska J., Maciejewska A., Pawłowski R.*, Bielawski KP., **mRNA profiling for vaginal fluid and menstrual blood identification,** Forensic Science International: Genetics (2013) 7:272-278, IF 3.202

3. Jakubowska J., Maciejewska A., Bielawski KP., Pawłowski R.*, **mRNA heptaplex protocol for distinguishing between menstrual and peripheral blood,** Forensic Science International: Genetics 13 (2014) 53-60, IF 3.202

[1] All articles included in the dissertation as well as resulting from other projects, conference reports, and other achievements were published or presented under my maiden name (Joanna Jakubowska).

The review paper entitled: **'mRNA profiling for biological fluids identification in forensic genetics'** summarizes the current knowledge (2011) on the identification of a variety of biological fluids by mRNA profiling in forensic genetics. All the available sources and publications on the subject were used in the review paper. It contains information on different methods of biological fluids identification, methodology, known markers and housekeeping genes used in mRNA profiling, markers specificity and their stability in stains. The analysis of literature data shows that mRNA profiling is a promising technique that can be widely applied in forensic laboratories. The article was published in a Polish journal of international scope in Polish and English.

The aim of the project presented in the publication: **'mRNA profiling for vaginal fluid and menstrual blood identification'** was to develop an mRNA profiling based method for identification of vaginal secretions and menstrual blood. Due to the lack of specificity of the known markers of vaginal secretions and their unstable expression in different women, I proposed the multiplex test (hexaplex), which uses human and bacterial markers: HBD1, MUC4, MMP11, 16S-23S rRNA ISR *L.crispatus* and *L.gasseri / L.johnsonii* and G6PDH, in order to maximize its reliability.

The authors of previously described mRNA profiling tests focused on including markers of numerous biological fluids into a single reaction. However, in my opinion, the more important issue for the credibility of the method is to analyze many markers of the biological fluid of interest, especially if the expression of markers is not constant in different individuals or during the menstrual cycle.

In the course of the research there were analyzed: sixty vaginal and menstrual blood swabs obtained at different stages of a menstrual cycle from fifteen women aged 25-47, samples of semen, saliva and sweat obtained from six individuals, peripheral blood samples obtained from 12 individuals, stains of biological fluids on various substrate, as well as samples of biological material from the resources of the DFM MUG. The methodology of the assay consists of the following steps: RNA isolation from a stain, reverse transcription, amplification of markers in end-point PCR reaction, amplicons detection by capillary electrophoresis.

The assay was presented in the context of the methods which have been used so far, and the following issues were discussed: the specificity and sensitivity of the

proposed markers and the entire test, changes in distribution and expression of markers in different women and in a menstrual cycle, the stability of the markers on the basis of analysis of various biological stains, application of an assay in the case of inhibition of the PCR reaction and mixtures of vaginal secretion with blood and semen. I also suggested the rules of interpretation of the test result. Thanks to the analysis of the five markers, including two bacterial ones, the method provides high reliability of the identification. Its elaboration can significantly improve the quality of expertise, especially due to the lack of other satisfactory methods for vaginal secretions identification.

The aforementioned test is also described by me in the chapter of the book: (Jakubowska J.*, Maciejewska A., Pawłowski R., mRNA profiling for vaginal fluid and menstrual blood identification. In: Forensic DNA Typing Protocols, Editor: W. Goodwin, 2nd Edition, Springer), which is currently at the stage of preparation for printing or in print. This chapter is a detailed manual indicating step by step how to use the test in the laboratory practice.

The aim of the research presented in the manuscript entitled: **'mRNA heptaplex protocol for distinguishing between menstrual and peripheral blood'** was to create the mRNA profiling-based test useful in distinguishing between menstrual and peripheral blood. The proposed test (heptaplex) uses transcripts of the following genes: *MMP7, MMP11, HBD1, MUC4, HBA, ALAS2* and *G6PDH*. This project was raised as a response to a pressing problem in forensic genetics which was the lack of a simple, reliable method that could be used in those cases in which the origin of the tissue has been recognized as a blood, but the source of bleeding is unknown (menstrual blood or derived from injury).

The research material included: 28 samples of menstrual blood or vaginal secretions collected from 12 women, peripheral blood samples obtained from 12 individuals, samples of semen, saliva and sweat collected from 6 individuals, as well as 15 menstrual and 12 peripheral blood stains from the own resources of the DFM MUG. The test methodology involves similar steps as aforementioned hexaplex.

The publication contains: a detailed description of the test and its embedding in the context of the previously used methods, results of the specificity and sensitivity

testing, which include mixtures of biological fluids. Differences in the expression of markers in different women during the menstrual cycle were found. A detailed guidance on how to interpret the results of the analysis is also posted. The publication includes the example of a case conducted at the DFM MUG, in which the test was used in practice to identify the source of blood on underwear and trousers of the victim of sexual assault. Since May 2013 I have been working in the Laboratory of Forensic Biology and Genetics DFM MUG examining biological traces on evidence items and preparing reports in the field of forensic genetics, which allowed me to take an active part in the implementation of the methods developed in the doctoral project.

The developed test is a reliable method for identification of menstrual and peripheral blood, particularly in the absence of other specific methods for menstrual blood identification. It allows to confirm its presence thanks to the simultaneous detection of four vaginal markers, including two characteristic for the first period of the menstrual cycle, two blood markers and the housekeeping gene mRNA. Such a comprehensive approach minimizes the risk of a false negative result in case of lack or low expression of one or two markers. In addition, the high specificity of the markers (except for HBD1 and MUC4) and strict rules of interpretation allow to minimize the possibility of a false positive.

The results presented in the aforementioned publications indicate that the mRNA profiling can be a reliable method for biological fluids identification, especially in case of biological fluids which can not be identified using classical confirmatory methods. The developed tests detect several markers characteristic for one or two biological fluids in one reaction, which increases the specificity of the detection, particularly in the cases where the individual markers alone do not fully meet expectations in terms of specificity and universality (presence in all individuals).

Developing the new methods influences the credibility of expert testimonies and improves the quality of research undertaken at the request of law enforcement, especially in cases of sexual offenses. The developed tests have been implemented and have been used in casework.

OŚWIADCZENIA AUTORÓW O WKŁADZIE PRACY W POWSTANIE PUBLIKACJI SKŁADAJĄCYCH SIĘ NA ROZPRAWĘ DOKTORSKĄ