B70 An Improved Method for the Identification of Menstrual Blood Using Real Time PCR

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Attendees will learn that it is possible to identify the menstrual origin of blood stains using real time PCR and that the question whether blood stains were produced by injury or by menstrual bleeding can be of crucial importance in forensic casework.

This presentation will impact the forensic community and/or humanity by demonstrating a reliable method is available which allows the detection of menstrual blood markers in blood stains. Compared to a previously published paper the new technique is based on real time PCR, is much more specific and senstive and therefore suitable for the use in forensic casework.

In forensic casework type and origin of stains frequently are of crucial importance. Whereas presumptive or specific tests exist e.g. for blood and semen, the question whether a stain was produced by injury or by menstrual bleeding remained unsolved over many years. Some time ago a new technique was introduced based on the detection of mRNA by reversetranscription coupled to PCR. The evaluation of potential candidate genes showed that matrix metalloproteinases were suitable markers for menstrual blood and never were positive when blood from injuries or other body fluids was examined (*Martin Bauer, Dieter Patzelt. Evaluation of mRNA markers for the identification of menstrual blood. Journal of Forensic Sciences 2002; 47:1278-1283)*.

During the last years the authors have had the opportunity to do some casework for police agencies in Germany and Great Britain. When confronted with “real” stains produced outside the laboratory it became evident that the sensitivity of the method was not sufficient because it had been only tested with artificial stains up to that time. Furthermore, an improved visualisation method with the possibility of quantifying results seemed to be helpful because it frequently was difficult to assess bands in agarose gels stained with ethidium bromide when their fluorescence was very weak. For these reasons, researcher decided to re-establish the test using real time PCR and sequence-specific probes which promised to be the perfect solution for the problems mentioned above. The use of Taqman®-probes allowed to amplify very short fragments with a size of less than 50 bp and the detction of the fluorescent dyes coupled to the probes is much more sensitive and specific than the assessment of agarose gel bands with the naked eye.

Commercially available and self-designed primers and Taqman®- probes for Matrix Metalloproteinases and house keeping genes were used with samples left over from previous studies and from the cases submitted for examination. In addition, 60 new samples from healthy volunteers have been collected. RNA isolation and reverse transcription were performed as described previously; however, as primer for reverse transcription now random hexamer primers were used instead of an oligo-(dT)-primer.

Results again confirm that the detection of mRNA specific for Matrix Metalloproteinases is a specific and sensitive indicator for the presence of menstrual blood. The use of real time PCR allowed amplification of RNA/c-DNA even in small and degraded samples using primers and probes for housekeeping genes which were negative with the conventional technique. This is particularly important because casework experience showed that it can be essential not only to prove that a given sample is menstrual blood but to state that it is not menstrual blood. With the new technique it is now possible to do this with a reasonable degree of certainty due to the sensitive and quantitative detection of housekeeping gene-mRNA. Negative result can be clearly differentiated from positive results due to the continuous monitoring of fluorescence during PCR.

In this presentation the authors want to introduce the new technique and to show that now reliable and consistent results can be obtained from stains of minimal size and high degradation as it often happens in forensic practice. To demonstrate the relevance of this test casework examples will be presented in which the identification of menstrual blood played an important role in police investigations.

Menstrual Blood, Real Time PCR, mRNA

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