



(Un)Reliable detection of menstrual blood in forensic casework — evaluation of the Seratec® PMB test with mock samples

Helen Konrad¹ · Benno Hartung¹ · Micaela Poetsch¹

Received: 27 October 2023 / Accepted: 20 November 2023
© The Author(s) 2023

Abstract

The identification of the type of body fluid in crime scene evidence may be crucial, so that the efforts are high to reduce the complexity of these analyses and to minimize time and costs. Reliable immunochromatographic rapid tests for specific and sensitive identification of blood, saliva, urine and sperm secretions are already routinely used in forensic genetics. The recently introduced Seratec® PMB test is said to detect not only hemoglobin, but also differentiate menstrual blood from other secretions containing blood (cells) by detecting D-dimers. In our experimental set-up, menstrual blood could be reliably detected in mock forensic samples. Here, the result was independent of sample age and extraction buffer volume. It was also successfully demonstrated that all secretions without blood cells were negative for both, hemoglobin (P) and D-dimer (M). However, several blood cell-containing secretions/tissues comprising blood (injury), nasal blood, postmortem blood and wound crust also demonstrated positive results for D-dimer (M) and were therefore false positives. For blood (injury) and nasal blood, this result was reproduced for different extraction buffer volumes. The results of this study clearly demonstrate that the Seratec® PMB test is neither useful nor suitable for use in forensic genetics because of the great risk of false positive results which can lead to false conclusions, especially in sexual offense or violent acts.

Keywords Rapid test · Immunochromatographic lateral flow strip test · Stain identification · Body fluids · Menstrual blood

Introduction

In forensic genetics, the use of immunochromatographic lateral flow strip test for the determination of body fluids such as blood, sperm secretion, saliva and urine has been established in routine casework for many years [1–4]. The principle of these tests is based on a sandwich ELISA, the enzymatic or immunological detection of specific proteins [5]. They have a separation section with detection range and control range. Different antibodies, monoclonal or polyclonal, immobilize the target structures and indicate a positive result by a color detection, usually a red line.

Commercially available strip tests for blood provide highly specific and sensitive detection for human blood, e.g. by detecting hemoglobin (OBTI Test Bluestar, Bluestar Forensics, Monaco) or glycophorin A (RSID Blood, Galantos Geneticity, Germany). However, these tests cannot

further differentiate peripheral blood from veins or arteries from nasal blood or menstrual blood [1]. However, this differentiation may be essential in a forensic genetic context.

Recently, a strip test specific for menstrual blood has been developed by Seratec® (Göttingen, Germany), the Seratec® PMB test [6, 7]. It is based on the detection of human hemoglobin for the blood component and the detection of D-dimers for the differentiation of menstrual blood. The detection limits are 20 ng/ml for hemoglobin and 400 ng/ml for D-dimers. Hemoglobin is an oxygen-carrying protein in red blood cells [8], and D-dimers are formed during fibrinolysis, which acts as an antagonist to blood clotting [9]. Identification is based on a higher D-dimer concentration in menstrual blood compared to other secretions containing blood cells [10].

This study aims to assess the suitability of this strip test for use in routine casework.

✉ Micaela Poetsch
micaela.poetsch@uk-essen.de

¹ Institute of Legal Medicine, University Hospital Essen, Hufelandstr. 55, D-45122 Essen, Germany

Material and methods

Samples

The study comprised blood from different origins (EDTA buffered and injury), menstrual blood, nasal blood, post-mortem blood, nasal secretion, saliva, sperm secretion, vaginal secretion, urine and wound crust (male donor) from different individuals of different ages. Overall, 34 samples from 7 different donors (n: five females, n: two males; healthy volunteers) were collected using predominantly forensic swabs in 2023 in the Institute of Legal Medicine, University Hospital Essen, Germany. Nose blood and blood from an injury was collected with sterile, absorbent paper tissue.

Compliance with ethical standards

All samples were obtained after informed consent and with approval of the Medical Ethics Committee at the University of Duisburg-Essen in accordance with the Declaration of Helsinki and national laws (ethic vote numbers: 16–7113-BO, 21–9843-BO).

Sample preparation and menstrual blood testing

Sample preparation was performed according to the manufacturer's instructions for the Seratec® PMB Test (Seratec®, Göttingen, Germany) [10] using different volumes of the extraction buffer (300 µl, 500 µl and 2 ml) provided in the kit and using a quantity of sample material typical for routine analysis. Regardless of the starting material, an incubation time of 45 min was chosen. Three drops (about 120 µl) of each eluate were added to the test cassette. After a reaction time of 10 min at room temperature, the result was evaluated.

DNA quantification, amplification and STR — analysis

According to the manual of the kit, no DNA extraction is needed for downstream analysis [10]. Therefore, sample solution was directly applied to real-time PCR using the PowerQuant™ System (Promega) for DNA concentration measurement. This kit provides a reproducible and reliable detection threshold at least down to 25 pg DNA [11]. Samples were analyzed in duplicate using 2 µl of each. DNA amplification was done using multiplex PCR Kit Powerplex® ESX17fast, evaluation was performed on

an ABI3500 Genetic Analyzer (Applied Biosystems) with GeneMapper® ID-X Software.

Experimental setup

Setup No. 1

Two samples of menstrual blood in addition to negative reference samples (two samples each of blood (EDTA), blood (injury), nasal blood, postmortem blood, vaginal secretion, nasal secretion, saliva, urine, sperm secretion and wound crust) were extracted in 500 µl. The wound crust was divided into two parts, each approximately 11 mm × 1.5 mm in size, and both samples were also extracted with 500 µl of buffer. Additionally, five negative controls were performed using sterile water and the kit's extraction buffer on the strip tests. All samples were applied directly to the test cassette after the incubation phase and read after the specified time.

Setup No. 2 – evaluation of buffer volume impact

Two samples each of blood (injury), menstrual blood, nasal blood, and postmortem blood were extracted in three different volumes (300 µl, 500 µl, and 2 ml) of extraction buffer.

Setup No. 3 — evaluation of time frame

Menstrual blood samples of one female were collected, dried and stored on a forensic cotton swab (nerbe plus GmbH & Co. KG, Winsen, Germany). Using these samples, two Seratec® PMB tests were performed on collection day and once a week over a period of 12 weeks resulting in 26 tests.

Results and discussion

Reliability of data, DNA concentrations and STR typing

All tests were successful and showed a positive control line, all but one (postmortem blood) blood positive secretions showed a second line for hemoglobin. Each fluid was tested as a duplicate, and the results were always identical. All samples included in this study showed a DNA concentration between 0.56 pg/µl (urine) and 7.72 ng/µl (wound crust) (Table S1). A complete DNA profile could be established for every sample containing at least 7 pg/µl DNA. With less DNA, at least a partial profile (> 1.5 pg/µl) could be generated, below this only single alleles were detected (Table S1).

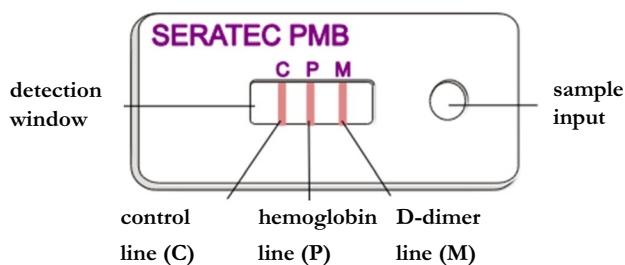


Fig. 1 Detection window of the Seratec® PMB test with its separation section and the positions of the three red lines (C), (P) and (M)

Evaluation

Test results are displayed in the detection window and were read after 10 min. The manufacturer emphasizes that even weak lines should be considered as a positive result [10]. There are three different scenarios for a positive result (Fig. 1):

Scenario 1

Hemoglobin and D-dimers are positive. Three red lines can be seen, the hemoglobin line (P), the D-dimer line (M) and the control line (C).

Scenario 2

Hemoglobin is positive. Two red lines can be seen, the control line (C) and the hemoglobin line (P).

Scenario 3

D-dimers are positive. There are also two red lines, the control line (C) and the D-dimer line (M). Since this is an unrealistic result, it can be assumed that the sample is too highly concentrated with regard to the hemoglobin content. This phenomenon is referred to as the *high dose hook effect* [12].

A negative result is indicated by only one red line, the control line (C). If no line is visible, not even the control line (C), the test result is invalid (Fig. 1).

Mock case samples

Setup No. 1

The negative controls using sterile water and the kit’s extraction buffer showed true negative results. Both menstrual blood samples demonstrated true positive results. Regarding negative reference samples, vaginal secretion, nasal secretion, saliva, urine and sperm secretion showed the expected negative results, i.e. only the control

Table 1 Results of the Seratec PMB test for different reference secretions

Number of tests	Reference secretions									
	Blood (EDTA)	Blood (injury)	Nasal blood	Post mortem blood	Vaginal secretion	Nasal secretion	Saliva	Urine	Sperm secretion	Wound crust
#1	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check
	P: positive	P: positive	P: positive	P: negative*	P: negative	P: negative	P: negative	P: negative	P: negative	P: positive
	M: negative	M: positive	M: positive	M: positive*	M: negative	M: negative	M: negative	M: negative	M: negative	M: positive
#2	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check
	P: positive	P: positive	P: positive	P: positive	P: negative	P: negative	P: negative	P: negative	P: negative	P: positive
	M: negative	M: positive	M: positive	M: positive	M: negative	M: negative	M: negative	M: negative	M: negative	M: positive

False positives and false negatives are shown in bold. *high dose effect [12]

C control line, P hemoglobin line, and M d-dimer line

line (Table 1). EDTA blood also demonstrated a reliable result as described in scenario 2 (Table 1). In contrast, in samples containing blood (taken out of an injury), nasal blood, postmortem blood, and wound crust not only the hemoglobin line (P), but also a positive D-dimer line (M) was found in every sample and every analysis (Tables 1 and 2). This phenomenon was also described in a study by Tsai et al. (2022) [13]. Here, testing of artificially degraded blood samples and postmortem blood resulted in false positive detection of menstrual blood.

Moreover, in one of our samples (one replicate of post-mortem blood), the high dose effect, i.e. the absence of the hemoglobin line (P) due to excessive hemoglobin concentration, was observed (Table 1) [12].

D-dimers are basically formed during fibrinolysis, which occurs as a counter mechanism to coagulation [9]. If coagulation is increased locally or globally due to injury, disease, or blunt trauma, fibrinolysis is increased, too. Because of this, D-dimers have been used as biomarkers in diagnostics for many years in various applications, e.g., for the detection of thrombosis. An overview is given in the review by Weitz et al. (2017) [14].

The risk that samples containing blood secured in the context of forensic genetic investigations were caused by sharp force or blunt trauma, i.e., injury-related, or originated from cadavers is very high. An increased D-dimer concentration due to previous increased blood coagulation and fibrinolysis cannot be excluded for this sample material, as demonstrated by the results of this study.

In contrast, EDTA buffered blood, as expected, showed a positive result for hemoglobin (P) and a negative result for D-dimer (M), but this preserved blood does not correspond to the typical blood in forensic genetic sample material. The EDTA in the tubes prevents coagulation of the blood and thus processes such as fibrinolysis. Accordingly, EDTA buffered blood does not reflect real conditions of the typical sample material in forensic genetics.

Setup No. 2

Regardless of the amount of used buffer (300 µl, 500 µl and 2 ml), menstrual blood samples demonstrated unambiguously results as described by scenario 1 with all three lines clearly visible after the specified diffusion time of 10 min (Table 2), which is in line with a description in the manual [10]. However, contrary to the description in the manual, blood (injury), nasal blood, and postmortem blood also displayed three lines for all buffer volumes (scenario 1, Table 2). Therefore, these samples showed false-positive results regardless of the buffer volume.

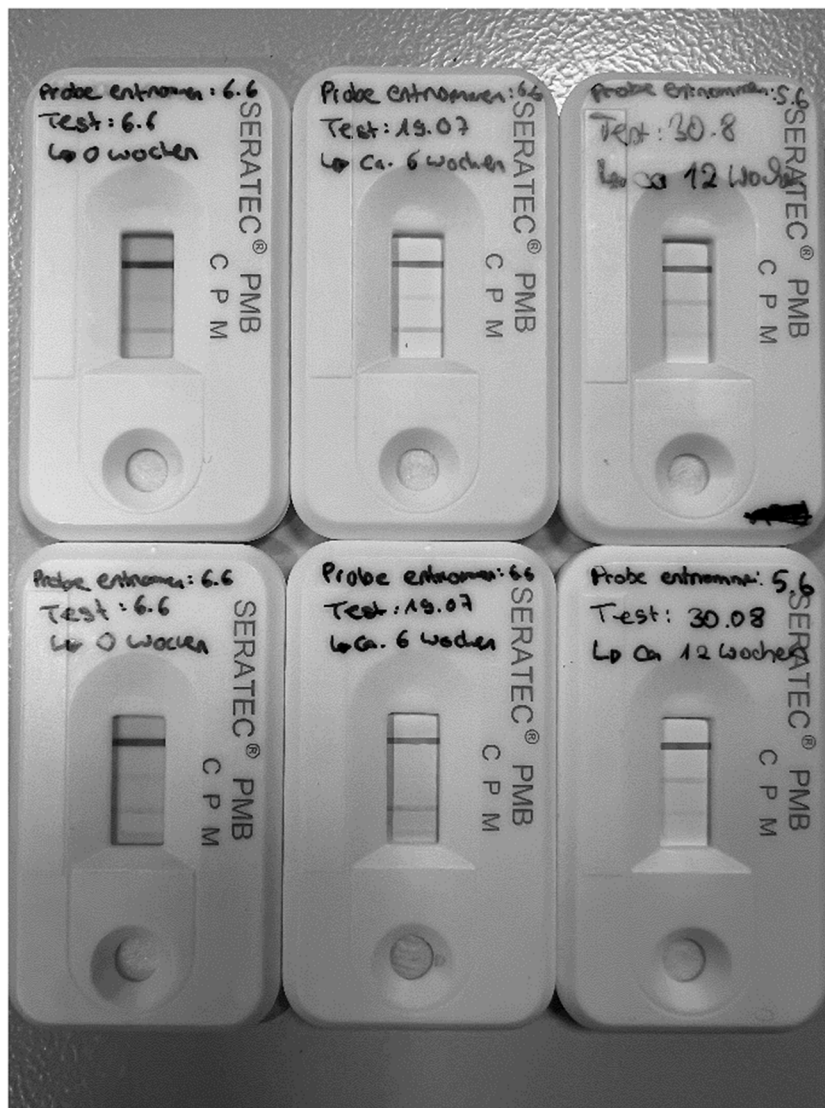
Table 2 Results of the Seratec® PMB test for three secretions extracted in different buffer volumes

Num- ber of tests	300 µl			500 µl			2 ml					
	Menstrual blood	Blood (injury)	Nasal blood	Post mortem blood	Menstrual blood	Blood (injury)	Nasal blood	Post mortem blood	Menstrual blood	Blood (injury)	Nasal blood	Post mortem blood
#1	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check
	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive
	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive
#2	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check	C: check
	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive	P: positive
	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive	M: positive

False positives are shown in bold

C control line, P hemoglobin line, and M d-dimer line

Fig. 2 Menstrual blood testing over a period of 12 weeks. The results at start time, after 6 weeks and after 12 weeks are shown as examples



Setup No. 3

The weekly tests with dried menstrual blood provided reliable results over a period of 12 weeks. Regardless of the age of the menstrual blood, it was clearly identified by scenario 1, three red lines for hemoglobin (P), D-dimer (M) and the control line (C), as such. However, a slight decrease in the intensity of the D-dimer (M) lines with increasing time was observed (Fig. 2). It should be mentioned that one of the samples dried for 6 weeks showed an extremely weak, but still recognizable hemoglobin line (P). In general, the intensity of this band varied over time period, but was basically always positive.

Conclusion

The results of this study clearly demonstrate that the Seratec® PMB test can identify menstrual blood independent of the sample age and buffer volume. However, it is neither useful nor suitable for use in forensic genetics, since there is a great risk of false positive results, which can lead to false conclusions, especially in sexual offenses or homicides. Thus, the false positive identification of menstrual blood in blood, nasal blood or postmortem blood results in a false assessment of the trace. However,

since false negative results are not obtained, at least the presence of menstrual blood can be ruled out. Reliable determination of secretion in suspected menstrual blood should rather be performed with proven methods, such as RNA expression or DNA methylation [15, 16].

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00414-023-03138-3>.

Author contribution Data collection: Helen Konrad.

Data analysis, writing and review: Helen Konrad.

Responsibility, editing and review: Micaela Poetsch.

Critical revision: Benno Hartung.

Funding Open Access funding enabled and organized by Projekt DEAL.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Hochmeister MN, Budowle B, Sparkes R, Rudin O, Gehrig C, Thali M, Schmidt L, Cordier A, Dirnhofer R (1999) Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. *J Forensic Sci* 44(3):597–602
- Old JB, Schweers BA, Boonlayangoor PW, Reich KA (2009) Developmental validation of RSID-saliva: a lateral flow immunochromatographic strip test for the forensic detection of saliva. *J Forensic Sci* 54(4):866–873
- Old J, Schweers BA, Boonlayangoor PW, Fischer B, Miller KW, Reich K (2012) Developmental validation of RSID™-Semen: a lateral flow immunochromatographic strip test for the forensic detection of human semen. *J Forensic Sci* 57(2):489–499
- Akutsu T, Watanabe K, Sakurada K (2012) Specificity, sensitivity, and operability of RSID™-urine for forensic identification of urine: comparison with ELISA for Tamm-Horsfall protein. *J Forensic Sci* 57(6):1570–1573
- Wobst J (2011) Identifizierung von Körperflüssigkeiten und Haut mittels RNA-Markern für den forensischen Anwendungsbereich Diplomarbeit. Albert-Ludwigs-Universität Freiburg, Freiburg im Breisgau
- Holtkötter H, Dierig L, Schürenkamp M, Sibbing U, Pfeiffer H, Vennemann M (2015) Validation of an immunochromatographic D-dimer test to striply identify menstrual fluid in forensic exhibits. *Int J Legal Med* 129(1):37–41
- Holtkötter H, Filho C, Schwender K, Stadler C, Vennemann M, Pacheco A, Roca G (2018) Forensic differentiation between peripheral and menstrual blood in cases of alleged sexual assault—validating an immunochromatographic multiplex assay for simultaneous detection of human hemoglobin and D-dimer. *Int J Legal Med* 132(3):683–690
- Hünefeld FL (1840) *Der Chemismus in der thierischen Organisation*. F.A. Brockhaus, Leipzig
- Cesarman-Maus G, Hajjar KA (2005) Molecular mechanisms of fibrinolysis. *Br J Haematol* 129(3):307–321
- Seratec® PMB Test User Manual: http://www.Seratec.com/docs/user_instructions/PMB_de.pdf. Seratec® GmbH. Göttingen. Germany. Accessed 15.11.2023
- Poetsch M, Konrad H, Helmus J, Bajanowski T, von Wurmb-Schwark N (2016) Does zero really mean nothing?—first experiences with the new PowerQuant(TM) system in comparison to established real-time quantification kits. *Int J Legal Med* 130(4):935–940
- Rodbard D, Feldman Y, Jaffe ML, Miles LE (1978) Kinetics of two-site immunoradiometric ('sandwich') assays-II. Studies on the nature of the 'high-dose hook effect'. *Immunochemistry* 15(2):77–82
- Tsai LC, Liu KL, Lin WY, Lin YC, Huang NE, Lee JC, Linacre A, Hsieh HM (2022) Evaluation of three commercial kits effective identification of menstrual blood based on the D-dimer. *Forensic Sci Int* 338:111389
- Weitz JI, Fredenburgh JC, Eikelboom JW (2017) A test in context: D-Dimer. *J Am Coll Cardiol* 70(19):2411–2420
- Sauer E, Extra A, Cachee P, Courts C (2017) Identification of organ tissue types and skin from forensic samples by microRNA expression analysis. *Forensic Sci Int Genet* 28:99–110
- Konrad H, Jürgens L, Hartung B, Poetsch M (2023) More than just blood, saliva or sperm – setup of a workflow for body fluid identification by DNA methylation analysis. *Int J Legal Med* 137(6):1683–1692 (**Online first**)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.