

SERATEC® PMB Test

REF: PMB, PMB/8, PMB/30

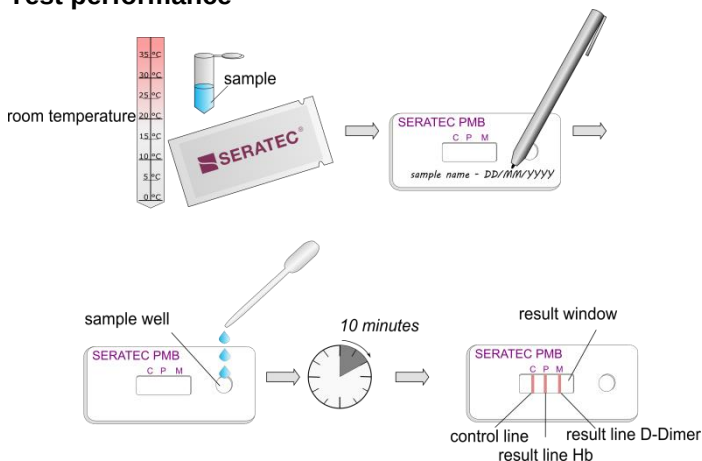
Application

The SERATEC® PMB Test is a chromatographic immunoassay for rapid detection of human hemoglobin (Hb) and human D-dimer to identify peripheral blood and/or menstrual blood in forensic samples. The product contains four monoclonal anti-human antibodies as active components.

Materials

- 8 or 30 (PMB/8, PMB/30) individually packaged PMB Tests in cassette format with one plastic pipette each
 - 8 or 30 (PMB/8, PMB/30) vials with 1.5 ml extraction buffer
 - Instructions for use
- Additionally required: stopwatch or timer

Test performance



1. Bring all test components to room temperature before performing the test. Low temperatures can lead to a decrease in sensitivity.
2. Remove the test cassette from the pouch and label it for identification.
3. Add 3 drops of the sample (approx. 120 µl) to the sample well with the enclosed plastic pipette and start the time measurement.
4. Read the test result after 10 minutes at room temperature. The liquid in the sample well should have been completely absorbed.
5. Keep the remaining sample material to perform further testing if necessary.

Interpretation of results

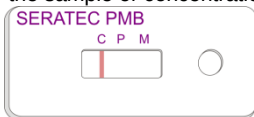
After 10 minutes, up to three lines may be visible in the result window:

Hb result line (P): only visible when the sample is Hb-positive; the colour intensity of the line may vary and depends on the Hb concentration of the sample.

D-dimer result line (M): only visible when the sample is D-dimer-positive; the colour intensity of the line may vary and depends on the D-dimer concentration of the sample.

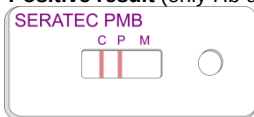
Control line (C): Control for potential application errors and for the integrity of the test components. This line is always visible after successful performance of the test.

Negative result (Hb and D-dimer are not detectable; no Hb/D-dimer in the sample or concentration below the limit of detection):



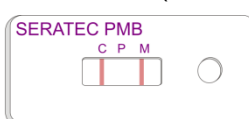
One line visible in the result window. The result lines (P and M) are not visible. The appearing control line (C) confirms that the test has been performed correctly.

Positive result (only Hb detectable):



Two lines visible in the result window: the Hb result line (P) and the control line (C). Any visible P-line (with strong or weak intensity) is to be considered a positive result.

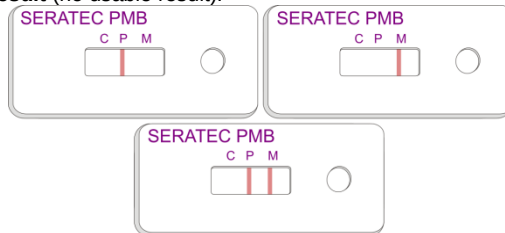
Positive result (D-dimer detectable):



Two lines visible in the result window: the D-dimer result line (M) and the control line (C). Any visible M-line (with strong or weak intensity) is to be considered a positive result.

Note: In the majority of cases, a positive detection of D-dimer also means that Hb can be detected. In this case, three lines are visible in the result window: the D-dimer result line (M), the Hb result line (P) and the control line (C).

Invalid result (no usable result):



No control line (C) visible. In this case, the test is invalid and should be repeated with a new test cassette.

Sample preparation

In order to obtain optimal test results, follow these instructions:

- It is not recommended to use unknown samples undiluted. Liquid samples should be diluted at least 1:50 prior to testing.
- Viscous samples should be diluted until the sample flows smoothly on the test membrane.
- Use the buffer solution included in the scope of supply, as it has been developed specifically for the PMB Test. Other buffer solutions or the use of water may result in reduced sensitivity or fluctuating line intensities.
- Do not use liquids with a pH below 3 or above 12, as this may cause incorrect or invalid results.
- Adding detergents such as SDS, sarcosyl or bleach to the sample material may cause incorrect or invalid results. This is probably caused by the denaturation of Hb and D-dimers.
- Tissue particles do not affect the test result.
- Cotton swabs, cloth or condom pieces should be extracted in a sufficient amount of buffer. The cut piece should be between 0.25 and 1 cm² in size and can be given directly into the buffer vial. Alternatively the samples can be collected with the applicator attached to the lid of the buffer tube.
- An extraction time of approx. 10 minutes is recommended. You should however follow the rule that the older or smaller the stain, the longer the recommended extraction time. [1,2]
- Extracted samples are stable at room temperature for about 2 days. Samples kept for longer periods should be stored dry and cold (2 – 8 °C). Liquid samples may be frozen.

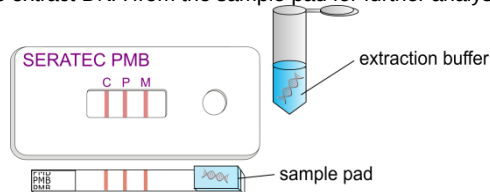
Extraction buffer

The supplied extraction buffer contains the following constituents (in 1 l distilled H₂O):
12,1 g Tris; 8,8 g Na₃Citrat; 0,2 g NaN₃; 0,5 g Tween 20; 5 g BSA; pH 6,8.

DNA profiling

The extracted samples can be stored for further analyses (e.g. DNA profiling; see Sample preparation).

The extracted sample is compatible with DNA analyses. It is also possible to extract DNA from the sample pad for further analysis.[3,4]



Safety information

Forensic samples are potentially infectious material which should be examined with the appropriate care and only when suitable protective measures (e.g. gloves, laboratory clothing) are applied. Materials used to perform the test should be autoclaved before disposal, as they contain potentially infectious material. Observe the following instructions:

- Do not use the product if damaged.
- Only remove the test cassette from the pouch immediately before use.
- Do not use the product after the expiration date.
- The materials used in the test (e.g. antibodies) are potentially infectious materials. When used and disposed of properly, however, there is no danger to the user or others.
- Do not freeze the test cassette.

Background

The red blood pigment hemoglobin (Hb) is a protein complex which occurs in red blood cells and primarily serves to transport gas in the body. It has a molecular weight of 64.5 kDa and consists of 4 subunits (amino acid chains), two of which are identical. Each subunit is associated with a haem group, an iron complex responsible for oxygen binding. At concentrations of 120-160 mg/ml (women) and 140-180 mg/ml (men), Hb is one of the most common proteins in blood.

D-dimers are proteins which are produced as fibrin breakdown products during fibrinolysis (the dissolution of a blood clot by the body). It contains two cross-linked D-fragments of fibrin and is therefore called a D-dimer. Menstruation involves increased fibrinolysis, resulting in an increase in the proportion of D-dimers in menstrual blood. However, the concentration in peripheral blood is not increased because the coagulation process is mainly extravascular. For this reason, D-dimers are suitable as markers for the identification of menstrual blood.[4–7]

The SERATEC® PMB Test **combines the detection of human hemoglobin and D-dimer** and enables **identification** as well as **differentiation of peripheral blood and menstrual blood**. The test offers the following benefits in forensic applications:

- Easy handling without additional equipment – directly at the crime scene or in the laboratory.
- A quick and reliable result after 10 minutes.
- Very high specificity through direct detection of human Hb and/or D-dimer (see Specificity).

Sensitivity

The SERATEC® PMB Test can be used to detect quantities of min. 20 ng/ml human Hb or 400 ng/ml human D-dimer. In very high Hb concentrations, the **high dose hook effect** can cause reduced line intensity (P line); therefore, it is recommended to always dilute fresh liquid samples (see sample preparation). Human blood diluted in the range between 1:50 and 1:10⁷ is positively detected in the recommended extraction buffer.

Specificity

Hemoglobin: The SERATEC® PMB Test does not show any cross-reactivity with other proteins in blood. No cross-reactivity has been observed with the blood of various animal species (dog, rabbit, cat, cattle, pig, wild boar, horse, chicken, sheep, mule, goat, red deer and others).[1] Primate and ferret blood can cause positive results.

D-dimer: In case of an increased D-dimer concentration in peripheral blood, the SERATEC® PMB Test can show a false-positive result for menstrual blood. This can e.g. occur due to the existence of thrombosis, postoperative wound healing, malignant tumour diseases, liver cirrhosis. The test-specific limits of detection as well as the recommended sample dilution (see Sample preparation) reduce the probability that samples not containing any menstrual blood show a positive test result.

Storage and shelf life

- Store test cassettes and buffer solution at +2 to +30 °C (38 to 86 °F).
- Keep test cassettes in the pouch until use.
- Do not use after the specified expiration date.

Quality features

Our products are manufactured according to the quality standards of European standard ISO 9001. The performance characteristics are confirmed during final quality control in application of the following standard: *human hemoglobin* (Sigma Aldrich, H7379); Liquicheck™ D-Dimer Control (Bio-Rad).

Please contact us if you have any questions or require more information.

Literature

- [1] A. Misencik, D.L. Laux, Validation Study of the Seratec HemDirect Hemoglobin Assay for the Forensic Identification of Human Blood, in: 2007.
- [2] M.N. Hochmeister, B. Budowle, R. Sparkes, O. Rudin, C. Gehrig, M. Thali, L. Schmidt, A. Cordier, R. Dirnhofer, Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood, *J. Forensic Sci.* 44 (1999) 597–602.
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- [5] H.H. Chan, J.A. Johnson, A. Panju, C.A. Bradley, D-Dimer Assay during Menstrual Period., *Blood*. 104 (2004) 4035–4035. <http://www.bloodjournal.org/content/104/11/4035> (accessed June 26, 2019).
- [6] H. Holtkötter, L. Dierig, M. Schürenkamp, U. Sibbing, H. Pfeiffer, M. Vennemann, Validation of an immunochromatographic D-dimer test to presumptively identify menstrual fluid in forensic exhibits, *Int. J. Legal Med.* 129 (2015) 37–41. doi:10.1007/s00414-014-1097-7.
- [7] D.J. Baker, E.A. Grimes, A.J. Hopwood, D-dimer assays for the identification of menstrual blood, *Forensic Sci. Int.* 212 (2011) 210–214. doi:10.1016/j.forsciint.2011.06.013.

Symbols

