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SERATEC® PMB Test

REF: PMB, PMB/8, PMB/30

Intended use

The SERATEC® PMB test is a chromatographic immunoassay for the rapid detection of human blood and/or menstrual blood in forensic samples via the determination of human hemoglobin and D-dimer. It contains monoclonal antibodies as active compounds.

Introduction

Hemoglobin (Hb) is located in the erythrocytes and pre-dominantly serves to carry oxygen and carbon dioxide within the body. Hemoglobin has a molecular weight of 64.5 kDa and is composed of 4 amino acid chains, two of which are identical. Each chain is associated with a hem group that contributes to around 4% of the total weight of the molecule. With concentrations between 120-160 mg/ml and 140-180 mg/ml for women and men, respectively, hemoglobin is one of the most abundant proteins in blood.
D-dimer is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It contains two crosslinked D fragments of the fibrin protein, from which the name D-dimer is derived.
Whereas menstruation is associated with activation of coagulation and fibrinolytic pathways, D-dimer is suitable for the detection of menstrual blood.[1,4,5,6]
The D-dimer level in peripheral blood does not show significant change during the menstruation period. The clotting process during menstruation may mainly be extravascular and, therefore, not affect the D-dimer level.[1]
The **SERATEC® PMB Test** combines the detection of human hemoglobin and D-dimer. Thus, the product is suitable for the differentiation between human peripheral blood and human menstrual blood. The test shows the following benefits for the forensic application:

- Easy in use, directly at crime scenes or in the laboratory
- Quick and reliable result after only 5 -10 minutes
- High sensitivity. Samples containing only 20 ng/mL of human hemoglobin react positively. Fresh human blood diluted in the extraction buffer down to 10^{-7} shows a clearly visible positive result.
- Optimized sensitivity of D-dimer is 400 ng/mL.
- The test is specific to human hemoglobin and D-dimer. Cross reactivity may occur with primate and ferret blood.

High Dose Hook Effect: Fresh bloodstains should be diluted prior to testing to avoid false negative results. With the provided buffer, dilutions in the range from 1:50 to $1:10^7$ are tested positively. For this reason, a dilution of 1:50 of fresh blood samples is strongly recommended. For cuttings or swabs an extraction in 0.5 mL of buffer is recommended. The color of the sample could be used as guidance for the dilution factor: diluted at 10^{-3} - 10^{-4}, the sample becomes colorless.

Materials provided

- 8 (PMB/8) or 30 (PMB/30) individually sealed PMB cassettes, with one plastic pipette each
- 8 (PMB/8) or 30 (PMB/30) buffer tubes containing 1.5mL of the standard buffer solution
- user instruction leaflet

Materials required but not provided: Timer

Storage and Stability

Both cassettes and buffer are stable up to the batch expiration dates stated on the sealed pouch and the buffer tube. Cassettes and buffer can be stored at room temperature (+2-+30°C resp. 38-86°F). The cassette must remain in the sealed pouch until use.

Sensitivity

Hemoglobin: 20 ng/mL
D-Dimer: 400 ng/mL

Specificity

Hemoglobin: the test does not cross-react with bovine, dog, rabbit, cat, pig, wild boar, horse, chicken, sheep, mule, goat and red deer hemoglobin. The complete cross-reactivity table is shown in (2). Primate and ferret blood may react positively.

D-Dimer: Thrombosis, postoperative wound healing, malign tumor disease or hepatic cirrhosis may be the cause of the elevated concentration of D-dimer in peripheral blood.
The cut-off value of 400 ng/mL was carefully considered and set to avoid positive results when testing samples which do not contain menstrual blood.

Reference Material

The qualitative characteristics of the test are confirmed in a final quality control using human hemoglobin supplied by Sigma and human D-Dimer supplied by Hytest. The extraction buffer is used as solvent.

Precautions

Forensic samples and all materials coming in contact with it should be handled and disposed of as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire. The product itself and all materials coming in contact with forensic samples should be autoclaved before disposal.

- Do not use cassettes or buffer after expiration date.
- Do not use cassettes if the pouch has been damaged.
- The product consists of potentially infectious materials, e.g. antibodies. These materials do not pose any danger if the product is used properly.
- Do not open the pouch until ready to perform the assay.
- Do not freeze the test cassette.

Specimen collection and handling

- It is strongly recommended to use the provided buffer. It is adapted for obtaining optimal results. Other buffers may lead to varying line intensities. Using water as buffer may be the cause of decreased test sensitivity.
- Detergents like SDS or Sarcosyl should not be added to the extraction buffer as they may cause denaturation of hemoglobin.
- Do not use liquids with a pH value below 4 or above 12 for testing. It can cause false or invalid results. The provided buffer ensures the optimal pH value for testing.
- Tissue particles do not interfere with the test result.
- Samples being subject to storage of two days or longer should be kept dry and cold (2-8°C). Liquid samples may be frozen.
- Very old or small stains should be extracted longer than the fresh ones and large stains.[2]
- Fabric cuttings from dried stains should have an area between 0.25 and 1 cm^2 for extraction in the buffer.

Specimen preparation

Hold the collection tube upright and unscrew the purple cap. Collect the sample material (e.g. fresh or dried blood, blood stained thread of clothing) with the applicator stick attached at the cap or with other appropriate tools. Samples (e.g. cotton swabs, fabric cuttings) can be placed directly in the tube. Screw the cap tightly and extract the blood by shaking thoroughly. At this stage the specimen is stable at room temperature for at least 2 days.
The extraction of old bloodstains may be difficult.[2] If necessary, the extraction can be done in the laboratory with the help of a shaker.
As solvent we recommend using the extraction buffer provided with the test kit. Also, other buffers with a neutral pH range (e.g. Phosphate, HEPES or TRIS buffered saline) may be used.
If necessary, the extraction buffer can be prepared as follows:
12.1g TRIS; 8.8g Na_3Citrate; 0.2g NaN_3 →adjust pH to 6.8 with HCl, 0.5g Tween 20; 5.0g BSA → fill with distilled water to 1000 mL.

Assay Procedure

- Allow all test components to warm up to room temperature before starting the test. Testing samples at a decreased temperature of 8°C will lead to a decrease in sensitivity of hemoglobin down to 200 ng/mL.
- Remove the cassette out of the foil pouch and tag the cassette with a marker, if necessary.

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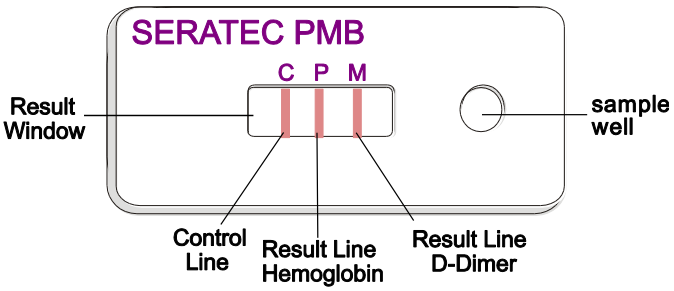
- Add about 120 µL (three drops) into the sample well of the cassette by using the plastic pipette. Alternatively, if the extraction has taken place in the buffer tube, break the seal of the tube using a piece of paper (risk of splashing!) and carefully press three drops of the extract out of the tube. Keep remaining sample in a sealable vial for further testing.
- Read result after 10 minutes of incubation time at room temperature.



Interpretation of results

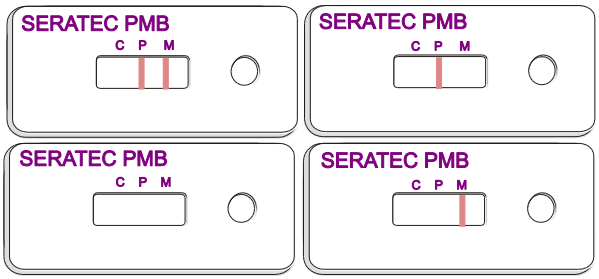
Negative result (no hemoglobin and no D-dimer in the sample or hemoglobin/D-dimer below the detection limit)
Only **one** red colored line appears in the **control region (C)** after 10 minutes. The **absence** of the **P and M lines** indicates a negative test result. **Note:** Make sure that the dilution of the sample is within the detectable range of Hemoglobin and D-dimer concentrations!

Positive results
Version 1 (human hemoglobin and D-dimer present in the sample)
Three red colored lines appear in the result window, one in the **control region (C)**, one in the **test region (P)** and one in the **test region (M)**. The color intensities of the three lines may vary. Even a weak test result line after 10 minutes indicates a positive test result.
Interpretation: Hemoglobin and D-dimer concentration above the detection limit. This shall be considered a strong indication for menstrual blood or a mixture of menstrual and peripheral blood.

Version 2 (human hemoglobin present in the sample)
Two red colored lines appear in the result window, one at the **control region (C)** and one in the **test region (P)**. No line appears in the **test region (M)**. The color intensities of the two lines may vary.
Interpretation: Hemoglobin concentration is above the detection limit. This is a strong indication of human peripheral blood.

Version 3 (human D-dimer present in the sample)
Two red colored lines appear in the result window, one at the **control region (C)** and one in the **test region (M)**. No line appears in the **test region (P)**. The color intensities of the two lines may vary.
Interpretation: D-dimer concentration is above the detection limit. It is unlikely that D-dimer is positive and hemoglobin is negative with the same sample. However, this may occur if the sample is high concentrated. In this case the hemoglobin value may be negative due to the Hook Effect. Please dilute the sample, e.g. 1:10 using the provided buffer and run a new test.

Invalid result
The control line (C) does not appear. In this case the test is invalid and should be repeated using a new cassette.



Literature

(1) Chan, H. H., Johnson J. A., Panju, A., Bradley, C.A. 2004. D-Dimer Assay during Menstrual Period, **Blood**, 104:4035.

(2) Misenick, A., Laux D. L. 2007. Validation Study of the *Seratec HemDirect Hemoglobin Assay* for the Forensic Identification of Human Blood. **Midwestern Association of Forensic Science**, **Newsletter Spring** , p. 25 et seq.
http://mafs.net/fileadmin/Research/Validation%20Study%20of%20Seratec%20HemDirect%20Hemoglobin%20Assay.pdf

(3) Hochmeister et al. 1999. Validation Studies of an Immunochromatographic 1-Step Test for the Forensic Identification of Human Blood. **J Forensic Sci**. 44: 597-601.

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(5) Holtkoetter H., Stadler C.; Dias Filho C.R., Roca M.G. 2016. Development and validation of a simple preliminary test for menstrual blood detection, 5th ENQFor and 2nd Brazilian Society of Forensic Sciences Meeting Book of abstract, p36.
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(6) Baker D.J., Grimes E.A., Hopwood A.J. 2011.
D-dimer assays for the identification of menstrual blood. **Forensic Science International**, 212(1-3):210-4.
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Symbols

	Expiration Date
	Storage Temperature
LOT	Lot Number