

SERATEC® HemDirect Hemoglobin Assay
REF HbF07

A visual one-step immunoassay for the rapid identification of human blood in forensic samples by the determination of hemoglobin. In-vitro diagnostic device for professional use only.

Intended use

The SERATEC® HemDirect test serves the rapid identification of human blood for forensic purposes. The detection is based on the determination of human hemoglobin in the sample by a specific antigen/antibody reaction. The result is interpreted visually by the appearance of a red test result line in hemoglobin positive samples. The test is easy to perform (no training necessary) and can be used directly at the crime scene, if required.

Introduction

The red blood pigment, hemoglobin (Hb), is located in the erythrocytes and predominantly serves the transport of oxygen and carbon dioxide within the body. Hemoglobin has a molecular weight of 64.5 kDa. It 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.

For the examination of a crime it might be essential to know if biological samples contain blood and/or if the blood is of human origin. Various confirmatory tests for the identification of blood are based on the detection of hemoglobin but do not allow to differentiate if the blood is human. In order to analyze this, additional testing is required. Frequently immunological tests or DNA-based hybridization assays are performed. As these assays are time consuming and work intense, it is generally not possible to perform them directly at the crime scene.

The use of one-step immunoassays may eliminate these problems. In these tests human hemoglobin is generally detected by a specific antibody pair. Originally developed for an early detection of colon carcinoma by the determination of occult blood in stool samples, the SERATEC® HemDirect test combines a high specificity with an excellent sensitivity and can be used for the identification of traces of human blood in forensic samples. Employing the test has the following advantages over the conventional methods:

- The generation of the result does not require any difficult manipulations of the sample and can be performed directly at the crime scene.
- The result is generated rapidly. 5 minutes after the addition of the sample the result can be read and interpreted visually without the aid of further equipment.
- The sensitivity of the test is very high. Samples containing only 40 ng/ml human hemoglobin are detected easily by the test. Fresh human blood diluted in the extraction buffer up to 10^6 shows clear positive test results. Even 10^7 dilutions are generally detected by the SERATEC® HemDirect test.
- The test is specific for human hemoglobin. A cross reactivity is observed for blood of primates. Therefore a positive test results allows the conclusion that the sample tested most probably contained blood of human origin.

Studies^{1,2} show, that immunoassays are also suited for the detection of old blood stains. Even a 31 year old blood stain, that had been extracted in the laboratory, reacted positive when tested. Dried blood stains and muscle tissue stored at different environmental conditions for one month, also gave positive test results following an extraction. In contrast, the addition of detergents like SDS or sarcosyl or bleach to the sample led to negative or only weak positive results. This was probably due to the denaturation of hemoglobin.

The test result of immunoassays will be affected in its evidence by two facts. First, hemoglobin may also be present in other body fluids (e.g. urine, stool, seminal fluid, vaginal fluid or saliva) in trace amounts yielding positive test results. However, this observation should not be a problem for the detection of human blood in the large bulk of cases. Second it must be kept in mind that very high concentrations of human hemoglobin in the sample may lead to negative test results – a phenomenon known as high dose hook effect (prozone effect). The high dose hook effect can be avoided by testing different dilutions of a sample. As the visible color caused by the hemoglobin vanishes between a 10^{-3} and a 10^{-4} dilution, the color of the sample might be a guide for the user. At these concentrations no high dose hook effect is expected.

Description of the test

Originally the SERATEC® HemDirect test was developed for the determination of occult blood in stool samples and was used for the early detection of colon cancer. In the forensic use, the test is employed for the detection of human hemoglobin with the aim to identify traces of human blood in forensic material obtained at a crime scene.

Principle of the test

The SERATEC® HemDirect test is a chromatographic immunoassay (CIA). It contains two monoclonal murine anti-hHb (human hemoglobin) antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane as a line. The upstream control region contains immobilized polyclonal goat anti-rabbit antibodies that are also fixed on the membrane as a line. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad that contains the dried and gold-labeled second monoclonal murine anti-hHb antibody that will bind the hemoglobin present in the sample. Additionally the pad contains gold-labeled rabbit antibodies.

Through the capillary effect of the membrane, the reaction mixture moves across the membrane towards the test and the control region. In any case the colored gold-labeled rabbit-antibodies will bind to the anti-rabbit-antibody at the control region resulting in the formation of the red control line in the upper part of the result well. This line indicates the correct performance of the test.

If the sample contains hHb, the hHb-gold-labeled anti-hHb-antibody complex will bind to the immobilized monoclonal antibody of the test region that recognizes another epitope on the hemoglobin molecule (sandwich complex). The binding is indicated by the formation of an additional line in the test result region.

A **high dose hook effect** can be observed, if too much free hemoglobin that is not bound to the gold-labeled antibody reaches the test result region. If the amount of hemoglobin is high, the antibody fixed at the test result region becomes saturated with free hemoglobin. This prevents the binding of the hemoglobin complexed with the gold-labeled antibody, thus repressing the formation of the test result line. The test result appears negative in spite of the presence of hemoglobin in the sample.

Materials

Materials provided: 40 individually wrapped tests, tubes with 2 ml extraction buffer for extracting the sample material

Materials required but not provided: Timer

Storage and Stability

The test is stable up to the expiry date stated on the sealed pouch. The tests can be stored at room temperature or refrigerated at +2 to +30°C (38-86 °F). The test must remain in the sealed pouch until use.

Qualitative Characteristics**Sensitivity**

The test is capable of detecting hemoglobin in a concentration range of at least 40 ng/mL to 500 µg/ml.¹ At higher concentrations a beginning high dose hook effect can be observed resulting in a continuous decrease of the color intensity of the test result line.

Reference Material

The qualitative characteristics of the test are confirmed in a final QC testing using human hemoglobin purchased from SIGMA. Using the extraction buffer as solvent the amount of hemoglobin is adjusted to the concentrations needed to confirm the performance characteristics of the test.

Performance Characteristics

For the detection of 40 ng/ml hemoglobin in sample buffer (indicated by the appearance of the test result line) the SERATEC® HemDirect test showed the following performance characteristics:

Diagnostic sensitivity:	100 %
Diagnostic specificity:	100 %
Positive predictive value:	100 %
Negative predictive value:	100 %
Reproducibility:	100 %

Specificity

The SERATEC® HemDirect Test shows no cross reactivity with bovine, dog, rabbit, cat, pig, wild boar, horse, chicken, sheep, mule, goat or red deer hemoglobin.

Blood of primates and blood of ferret reacted positive in the test. ¹

Test procedure

For your safety

Samples containing human blood and all materials coming in contact with them should be handled and disposed of as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

- Test for single *in-vitro* diagnostic use only.
- Do not use the test after the expiration date or if the pouch has been damaged.
- Do not open pouch until ready to perform the assay.
- The test and all materials coming in contact with the sample should be autoclaved before their disposal. They contain potentially infectious material.
- The test consists of potentially infectious materials (e.g. antibodies). These materials do not cause any danger if the device is used according to the instructions.

Specimen collection/specimen preparation

Hold collection tube upright down and unscrew the blue cap. Collect some sample material (e.g. dried blood, blood stained thread of clothing) with the applicator stick attached at the cap. Re-insert the stick into the tube, thus transferring the sample material into the extraction buffer. 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. If necessary the extraction can be done in the laboratory using the provided buffer (transfer buffer into a different reaction tube, add sample material, extract for two hours on a shaker, centrifuge briefly, and test supernatant).

As solvent we recommend using the extraction buffer provided in the kit. However, other buffers at a neutral pH range (e.g. Phosphate, HEPES or TRIS buffered saline) might be used as well. The addition of pure water for the extraction is not recommended since it leads to a decrease in sensitivity of the test. No detergents like SDS or Sarcosyl should be added to the extraction buffer. If you feel unsure, if an unknown buffer is suitable for the extraction, it might be useful to test a serial dilution of fresh human blood in the buffer to check, if $10^6/10^7$ dilutions show positive test results. The first dilution step should be carried out in distilled water to ensure a complete lysis of the erythrocytes. Alternatively, a solution with 40 ng/ml hemoglobin can be used.

Note!

High Dose Hook Effect

Due to their high content of hemoglobin, fresh bloodstains should be diluted prior to testing to avoid a high a dose hook effect. As a guide for a suitable dilution, you may want to use the color of the sample. The visual detectable color caused by hemoglobin vanishes between a 10^3 and a 10^4 dilution. At this concentration range there is no danger of a high dose hook effect. Samples that exhibit an obvious color due to hemoglobin might cause negative test results because of the high dose hook effect.

pH value of the Sample

If possible the pH value of the sample should be close to the neutral range. Samples with a pH less than 5 might cause false positive test results. At the alkaline range, the sensitivity of the test is slightly decreased between pH 9 and 11. At pH values of 12 or more the test is no longer functional. Generally no drastic changes in the pH are expected after the extraction of small amounts of sample material, because the extraction buffer exhibits a sufficient buffer capacity.

Influence of the temperature

The sensitivity of the test is only guaranteed if the test is carried out at room temperature. A drop of the temperature to 8 °C leads to a decrease of the sensitivity to approximately 400 ng/ml.

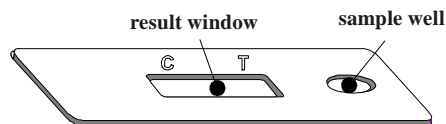
Extraction Buffer

The extraction buffer is composed of the following ingredients:

12.1 g TRIS; 8.8 g $\text{Na}_3\text{Citrate}$; 0.2 g NaN_3 → adjust pH auf 6.8 with HCl
0.5 g Tween 20; 5.0 g BSA → fill with distilled water to 1000 ml

Assay Procedure

- Bring test device and sample to room temperature prior to the testing. Remove the test from the protective pouch when ready to start the assay. Label the device for identification purposes if necessary.



- Add 3 drops of the sample (approximately 100 μl) into the round opening (sample well). If the extraction was carried out in the collection tube, remove the white conic cap at the lower end of the tube. Using a piece of paper (danger of splattering), break the seal of the tube by a twisting motion. Hold the tube upright down and dispense three drops of liquid into the round sample well by exerting a light pressure on the walls of the tube. Start the timer. Keep remaining liquid in case it might be necessary to test additional dilutions.
- Wait for 5 minutes. During this time one or two red lines appear in the result window. **Negative results should be confirmed after 10 minutes.**

Interpretation of results

Negative result (no hemoglobin in the sample or hemoglobin beyond the detection limit)

Only **one** red colored line appears in the **control region (C)**. The **absence** of a line in the test result region T indicates a negative test result.

In this case the sample most likely does not contain human blood or human hemoglobin.

Note:

Make sure that the dilution of the sample is within the detectable range. Hemoglobin concentrations that are too low e.g. due to an insufficient extraction or hemoglobin concentrations that are too high e.g. due to an unsuitable dilution (also see high dose hook effect) interfere with the formation of the test result line.

Positive result (human hemoglobin present in the sample)

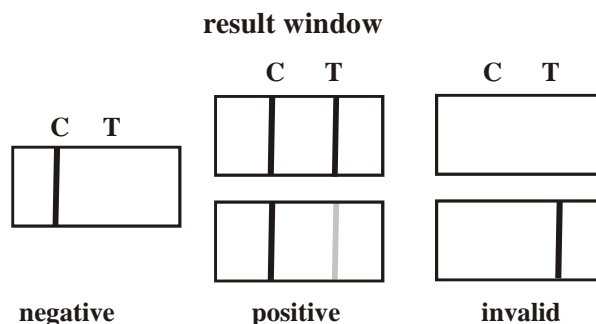
Two red colored lines appear in the result window, one at the **control region (C)** and one in the **test result region (T)**. The color intensities of the two lines may vary. Even a weak test result line indicates a positive test result.

In this case it is very likely that the sample contains human hemoglobin.

Invalid result

The control line (C) does not appear.

In this case the test is invalid and should be repeated with a new cassette.



Literature

¹Amanda Misencik1 and Dale L. Laux: Validation Study of the Seratec HemDirect Hemoglobin Assay for the Forensic Identification of Human Blood. Midwestern Association of Forensic Science, Newsletter Spring 2007, p. 25 et seq.
<http://mafcs.net/fileadmin/Research/Validation%20Study%20of%20Seratec%20HemDirect%20Hemoglobin%20Assay.pdf>

²Hochmeister et al. (1999) Validation Studies of an Immunochromatographic 1-Step Test for the Forensic Identification of Human Blood. J Forensic Sci Vol 44: 597-601.