Interpretation of Hemoccult®
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♦ Is this positive?

♦ In the German Programme for Early Detection of Colorectal Cancer only 1% of women and 1.5% of men of the millions screened each year have a positive Hemoccult® result, because this test has an enormous specificity, which is a must for a Screening of healthy asymptomatic individuals. But the seldom event of a positive test result has to be recognised with high personal security. This training session shall enable you to differentiate easily between positive and negative test results!
Interpretation of Hemoccult®

- The test for occult blood is positive if the blue colour, which is typical for Hemoccult®, develops on one or more of the six test fields within 60 seconds. If you intend to interpret this test, you should not suffer from blue colour deficiency!

- If you use Hemoccult® for Screening - i.e. for the early detection of bowel cancer in healthy individuals - allow the faecal samples to dry for 48 hours or more before developing the test! By this measure you avoid the possibility of false-positive results due to non-digested plant peroxidases from the nutrition. Otherwise, such false-positive results may happen seldom with individuals, who eat high amounts of uncooked vegetables or raw fruits.

- In the dried faecal samples haemoglobin is very stable. From the sample application you have 14 days time to develop the test.

- If Hemoccult® becomes positive, the guaiac resin within the reagent paper is oxidised by the hydrogenperoxide (H₂O₂) of the developer solution and changes to blue. To make this happen, a haemoglobin molecule must support this chemical reaction as a catalysing agent, which transfers an oxygen atom from H₂O₂ to the guaiac molecule! Each haemoglobin molecule will repeat this support as long guaiac resin and H₂O₂ is present in the neighbourhood.

- The blue colour can be created only there, where haemoglobin is present, this means inside of the faecal sample. But the blue dye migrates together with the developer solution outside in radial streaks and can then easily be recognised.
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- **Check the Pouch!**
  - Can the patient be identified without doubt?
  - Prepare your records to document the result

- **Check the Test Cards!**
  - Were all 3 Test Cards returned?
  - Name of the patient (again)
  - Dates of stool samples (3 different days, 48 hours or older?)

- **Interpret the Test Cards!**
  - Open the flaps on the back of the 3 Test Cards and place the cards side by side
  - Apply only 1 drop of developer to the centre of each test field
  - Continue by applying a second drop of developer to each test field
  - For larger stool samples add more drops of developer
  - Observe the test fields for up to 60 seconds
  - Apply one drop of developer to the Performance Monitor. - Read within 10 sec.

- **Record the Result!**
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How does a negative test result look like?

- No blue colour on or at the edge of the stool sample
- No blue streaks

Performance Monitor: Apply 1 drop of developer between the 2 white circular areas - read within 10 seconds
How does a positive test result look like?

- Blue colour on or at the edge of the stool sample
- Blue radial streaks starting at the stool sample
Interpretation of Hemoccult®

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  - Blue colour on or at the edge of the stool sample
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How does a positive test result look like?

- Blue colour on or at the edge of the stool sample
- Blue radial streaks starting at the stool sample

These samples made of artificial stool do not penetrate the reagent paper as natural stool. This allows to recognise that the blue colour develops inside the sample area.
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Positive Test Result (right)

- Blue colour on or at the edge of the stool sample
- Blue radial streaks starting at the stool sample

With these samples of artificial stool you can easily see that the blue colour develops inside the sample area.
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♦ Positive Test Result (right)
  • Blue colour on or at the edge of the stool sample
  • Blue radial streaks starting at the stool sample

With these samples of artificial stool you can easily see that the blue colour develops inside the sample area.
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Positive Test Result (right)

- Blue colour on or at the edge of the stool sample
- Blue radial streaks starting at the stool sample
The Blue Colour Fades:

- after 30 seconds
- 2 minutes later

Because the blue colour is not stable, the interpretation of the test result has to be performed within 60 seconds! If a test becomes positive, most often you will see develop the blue colour already after 10 - 20 seconds. Then you can exactly observe, how this dye migrates out of the sample together with the developer solution. When you read test cards, you should avoid any disturbance, you should not leave, but look exactly, if and how the test field becomes positive.
The interpretation of the test result shall be done within 60 seconds, because the blue colour fades rather quickly. Where the intensity of blue is high (as here tight to the circle front of the developer) it usually stays for many weeks. But already within 2 minutes its shade turns from the typical Hemoccult® blue (with a clearly visible content of red) to a darker “dirty” blue. You will also recognise that areas of less intensive blue colour have already disappeared after 2 minutes, and then would not be detected.
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♦ Small Samples
  • Negative Test Result

In the test instruction the patients are asked to cover the whole test field with a thin smear of a faecal sample. The greater the contact area is between the sample and the guaiac paper, the more probable haemoglobin will be in contact to the reagent paper, even if distributed non-homogeneously in the sample. In the above shown example with artificial stool both samples are negative, no blue colour is visible! You will see also that the developer circle has further grown between 30 and 60 seconds.
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Small Samples

- Positive Test Result (right)

after 30 seconds after 60 seconds

Of course, even small samples are judged as positive, if the typical blue colour appears. This is the proof that haemoglobin (or haem as the first breakdown product, when the protein chain of globin often is already digested and therefore became non-detectable for immunochemical testing methods) catalysed the oxidation of guaiac resin. You will see again that the developer circle grew further between 30 and 60 seconds: The blue colour migrated with the developer and therefore was somewhat “diluted”.
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Small Samples

- Positive Test Result (left)

after 30 seconds  after 60 seconds

Here is a further example for small samples, prepared from artificial stool. Again, there is no doubt that the left test field is positive. This means for the patient that his/her test for occult blood has a positive result, even if this is the only positive test field in all three test cards. The bleeding source has to be identified by further investigation, which usually will be done by colonoscopy.
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So far the “Must“ -

...and now the “Optional“!
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- Light Blue Discoloration, e.g. by Sun Light, UV, Ozone
  - Unused Test Cards

normal colour  bluished Guaiac paper

On the right test card you will recognise an even, light blue discoloration of the reagent paper. This is caused by an oxidation of the guaiac resin by light or gas.
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♦ Light Blue Discoloration, e.g. by Sun Light, UV, Ozone
  • Unused Test Cards

If developer is applied to the reagent paper (without any stool sample), a light brown circle is formed, where the earlier, almost non-visible guaiac resin concentrates, when migrating with the front of the developer. But if the reagent paper was bluished before, this blue dye moves also together with the front of the developer solution, where it becomes clearly visible as mixture with the brown colour of normal guaiac. Inside the circle the area becomes almost white.
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- Light Blue Discoloration, e.g. by Sun Light, UV, Ozone
  - Negative Test Result

Now you will have no problem to determine that the typical blue colour of a positive Hemoccult®-reaction cannot be seen on the left (normal) test card, nor on the right (bluished) test card. Although the right test card shows a bluish discoloration outside of the circle area wetted by the developer, you can clearly state: The test result is negative!
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- Light Blue Discoloration, e.g. by Sun Light, UV, Ozone
  - Positive Test Result

A different, but also a clear result: Not disturbed by the fact that the reagent paper of the right test card shows a light blue discoloration, on both right test fields the typical blue of a positive Hemoccult®-reaction is seen. The blue colour migrates in radial streaks with the developer away from the sample versus the circle! Both left test fields are negative. (Again we took artificial stool to prepare these samples)
**Interpretation of Hemoccult®**

- **Dried Whole Blood**
  - if visible blood is present in the stool, e.g. due to haemorrhoids, the test result is negative,
  - if the erythrocytes are dried and intact. This increases the specificity for the target lesions!

  **on each left test field:**
  - dried whole blood

  **on each right test field:**
  - haemolysed blood *(ca. 200 times diluted)*

On the left test card you see how fresh blood looks like after 48 hours drying **before** the developer is added. *(The small drop of water with lysed blood on the right test field you may detect only in a darkened room.)* The right test card is the same as left **after** adding the developer to the left test field with dried blood: By getting wet, the red shades somewhat versus black, but the typical blue of a positive Hemoccult®-reaction is not detectable.
Interpretation of Hemoccult®

- Dried Whole Blood
  - if visible blood is present in the stool, e.g. due to haemorrhoids, the test result is negative, if the erythrocytes are dried and intact.
  - on each left test field: dried whole blood
  - on each right test field: haemolysed blood (ca. 200 times diluted)

Again the left test card, before any developer was applied. - Look at the right side: Only after the developer was added also to the right test field, you will get an impression of the unbelievable high sensitivity of Hemoccult® for blood, which is based on the fact that each single molecule of haemoglobin or haem will catalyse the creation of the blue dye several hundred times - as long as guaiac and H₂O₂ molecules are still in its neighbourhood. By this repeated action there is formed such amount of blue dye that you can recognise it easily with your eyes.
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Thank you for your attention!