Examples for positive and negative test results

If blue colour is seen on or at the edge of one or more of the faecal specimens within 60 seconds, the patient has tested positive for faecal occult blood.

D Bildet sich der typische blauwe Farbton innerhalb 60 Sekunden auf oder am Rand von einer oder mehreren Stuhlproben, so ist der Test auf okkultes Blut im Stuhl positiv.

F Si la coloration bleue typique apparait dans les 60 secondes sur l'échantillon fécal ou sur ses bords, le test de recherche de sang occulte est positif.

I Se questa tipica colorazione blu appare sopra o agli angoli di uno o più campioni di feci, entro 60 secondi, il paziente è positivo per il test del sangue occult.

E Si este típico color azul se observa en la zona de la muestra o en el borde del espécimen en los primeros 60 segundos, el paciente se considerará positivo para el test de sangre oculta en heces.

D, E, F, I}
Product Instructions

Intended Use

The Hemoccult® test is a rapid, convenient and qualitative method for detecting faecal occult blood, which may be indicative of gastrointestinal disease, including colorectal cancer. The Hemoccult® test is widely used internationally and is recommended for professional use to screen asymptomatic average risk persons for colorectal cancer. Bleeding from pathologies may be found in the early developmental stages while the cancer is still curable, or even in the benign adenoma (polyp) stage which may be removed, avoiding cancer. According to the world-wide accepted Adenoma Carcinoma Sequence, more than 90% of all colorectal carcinoma develops from adenomas, i.e., dysplasia. Evidence-based and well respected clinical studies have shown significantly reduced mortality from colorectal cancer in populations screened with Hemoccult®. Hemoccult® is also used as a diagnostic aid during routine physical examinations, for hospital patients, to monitor for bleeding in patients with iron deficiency anaemia or recuperating from surgery, peptic ulcer, ulcerative colitis and other conditions. Hemoccult® is not a test for colorectal cancer or any other specific diseases.

How it works

Hemoccult® test cards contain filter paper treated with guaiac resin, to which faecal specimens are applied. To develop the dried samples of faeces, an alcoholic stabilised solution of hydrogen peroxide is used. The test result is positive for occult blood in faeces if a blue colour appears on the filter paper within 60 seconds. The test reaction is based on the oxidation of guaiac by hydrogen peroxide to blue-coloured compounds, which occurs only if a peroxidase substance is present. Although haemoglobin is not an enzyme, its haem part has an oxygen transferring property and catalyses the reaction. The presence of haemoglobin released from lysed erythrocytes or haem is a prerequisite for a positive test result.

Reagents and Materials

- 40 Pouches, each containing 3 Hemoccult® test cards treated with guaiac resin (≥ 0.05 mg), 6 applicators and Patient Instructions
- 2 bottles of 15 ml Hemoccult® developer, an alcoholic/aqueous stabilised solution of hydrogen peroxide (H₂O₂: 0.45 g)

Hemoccult® test cards must be developed only with Hemoccult® developer.

Sensitivity

The sensitivity of Hemoccult® has been precisely defined. It has been proven in several prospective, controlled and mostly randomised studies in USA, England, and Continental Europe with approximately 350,000 individuals enrolled that mortality reduction of colorectal carcinoma in
the population can be achieved at about 35% with annual screening and about 20% with biannual screening. Sensitivity has been carefully adjusted to achieve a high accuracy for lower gastrointestinal bleeding associated with cancers and precancerous colorectal neoplasia, but avoiding positive results from minimal (non-pathological) content of blood. Therefore, bleeding of the upper gastrointestinal tract will give positive results only if, after the bacterial-enzymatic degradation in the alimentary canal, the stool sample still contains enough non-degraded haemoglobin or haem. More degraded products like porphyrins and bilirubin do not react. Program sensitivity for screening with Hemoccult® for colorectal cancer has been determined as 72-78%, specificity as 98% and Positive Predictive Value as 10-17%. In most of these trials, participants were asked to follow a diet absent of raw, rare or red meat products for at least 3 days prior to and during the sample collection period. Participants were also asked to restrict the use of drugs such as acetylsalicylic acid, ibuprofen, indomethacin, naproxen and other non-steroidal anti-inflammatory drugs, for 7 days prior to and during the sample collection period in order to eliminate positive test results from gastric bleeding induced by these drugs in some individuals. Additionally, in most of these trials ascorbic acid (Vitamin C) over 250 mg/day from fruits or supplements was restricted in this diet due to the potential for false-negatives.

Clinical studies using [51Cr] chromium-labeled blood cells suggest that a daily blood loss of 2-3 mL is the lower limit of blood loss that may be associated with gastrointestinal pathology. Based on in vitro studies in which fresh whole blood was added to faecal samples from asymptomatic, normal volunteers, Hemoccult® produced positive test results approximately 50% of the time at 0.3 mg Hb/g of faeces.

Dietary Measures

The dietary restrictions usually prescribed for faecal occult blood testing may be modified for organised screening programs to ensure patient compliance with the test. Reduction of dietary restrictions may influence the positivity rate, but, for broad use of Hemoccult®, varying diet restrictions are recommended in different countries, the most limiting being the U.S.A.

In France, for the National Screening Programme to detect colorectal cancer, there are no dietary restrictions.

Since 1977 Hemoccult® has been used routinely in the German Statutory Program for Early Detection of Cancer without diet restrictions to enable high patient compliance. Before the start of the program it was locally shown that normal vs. restricted diet did not change the positivity rate of the test, even an additional 200 grams of steak tartar (rare meat) did not lead to positive test results. Schwartz et al. presented the results of the first year of the screening program. More than three million individuals (2.42 million females and 1.06 million males) age 45 years or older performed a 3 day test for occult blood. The participants were informed that, despite the earlier recommendation, no special diet restrictions should be followed. Participants were told to eat a high fibre diet, to avoid raw, rare meat products and to avoid large amounts of drugs containing Vitamin C. With this advice, only 1.0% of the females and 1.5% of the males had a positive test result, i.e., the positivity
rate stayed below 3% overall. The yield of CRC in positive tested subjects was 4.7% in the females and 10.7% in the males. Consequently, only those tests that allowed a normal diet were approved for use in the national German CRC Screening Program. As a result of the balanced sensitivity of Hemoccult®, it is sufficient to advise the patient to follow a high fibre diet including whole meal bread, wheat bran or nuts, to enlarge stool volume and to shorten the gastrointestinal passage time for 3 days before and during the test. Roughage in the diet like nuts can increase test accuracy by helping uncover 'silent' lesions which may only bleed intermittently. Black pudding should be avoided, however.

High intake of Vitamin C may inhibit the positive results. In 1975 Jaffe et al. made this observation with a female patient with known blood loss who was taking 2 g of Vitamin C per day. When the intake of Vitamin C was stopped for several days the test result became positive. A few days after restarting the Vitamin C the test again became negative. The authors attempted to quantify this observation by studying additional volunteers. 4 healthy subjects with negative occult blood tests swallowed 20 ml of autologous blood. 2 to 4 days later the tests were strongly positive. When these subjects took additionally Vitamin C (750 mg up to 1500 mg per day in 3-4 equally divided doses) one hour after ingestion of blood, the intensity of the blue colour test result was progressively inhibited, with complete inhibition observed above 1500 mg/day.2

Starting at a daily intake of 750 mg, vitamin C apparently is not always absorbed completely. If the stool sample still contains remaining quantities, positive test results can be weakened or inhibited due to the reducing property of Vitamin C. The risk assessment of these results lead in the USA to a daily limit of 250 mg Vitamin C, in Europe the upper limit is usually given as 500 mg/day. This quantity can be followed easily under normal diet, if the patients do not also take drugs containing Vitamin C and avoid juices with added Vitamin C for 3 days before the test and through the test period.

Patients who are treated with drugs which may induce intestinal bleeding (e.g., acetylsalicylic acid in excess of 325 mg per day, NSAIDs, anticoagulants, etc.) should be advised by their doctor if intake can be reduced or interrupted without risk for six days or more (at least three days before and three days during the test period).

How to use

1. The patient receives a pouch with instructions for use, 3 test cards and 6 applicators. The pouch is opened by detaching the storage bag at the perforated line.
2. The patient writes his/her name and date of birth on the storage bag.
3. On 3 consecutive days with bowel movement, the patient takes 2 small samples from the stool. The samples should be collected before the stool makes contact with the toilet water, which may be covered before a bowel movement with one page of a newspaper, folded twice. Afterwards the toilet shall be flushed 2-3 times, to avoid blocking of the outlet. The patient may also place a paper plate, a container or a chamber pot into the toilet.
With the first applicator the patient takes a faecal sample – depending on its consistency lentil- to pea-sized – and spreads it onto the left rectangle (A) inside the test cards, covering it almost completely. With the second applicator another stool sample is taken from a different location and spread onto the right rectangle (B). The test card is closed, on the front side is written the patient's name and the date of the stool sample and the test card put into the storage bag in order to dry.

4. The procedure is repeated on the next days with bowel movements using the 2nd and 3rd Hemoccult® test cards.

5. The storage bag is closed and given back to the physician for interpretation. The Hemoccult® bag is made of a bacteria-proof paper. Sealed it provides for hygienic storage and transport, until the test cards are read in the lab.

Evaluation

This test should not be performed by individuals with blue colour deficiency (colour blindness). Development of the Hemoccult® test cards should not be done before the stool samples have dried. In addition, Hemoccult® test cards are best developed no sooner than 48 hours after the last stool sample has been applied to reduce the possibility of false positive results from plant peroxidases in the diet. For maximum sensitivity, test cards must be developed within 14 days of collection.

The analyst opens the back of the 3 test cards of a patient, lays them side by side, and initially applies only 1 drop of the Hemoccult® developer solution in the centre of each of the 6 test fields. In a second round another drop is added to each specimen. If then the outer ring of the moist circle did not yet leave the area of the stool specimen, a 3rd or 4th drop may follow.

Any blue on or at the edge of the specimen, irrespective of its intensity, is positive and should be followed up by the usual diagnostic procedures. The interpretation has to be performed within 60 seconds, because the colour fades with time.

Developing and Interpreting the Performance Monitor® (Quality Control)

1. Apply 1 drop of Hemoccult® Developer between the positive and negative Performance Monitor® areas.

2. Interpret results within 10 seconds. If the test card and developer are functioning correctly, a blue colour will appear in the positive Performance Monitor® area (⊙) and no blue will appear in the negative Performance Monitor® area (⊙).

3. Neither the intensity nor the shade of the blue colour from the positive Performance Monitor® area should be used as a reference for the appearance of positive test results.

4. Any blue colour originating from the positive Performance Monitor® area should be ignored when interpreting the specimen test results.
Performance Monitor® (Quality Control)

The function and stability of the test cards and developer are tested using the Performance Monitor®. The positive (⊕) Performance Monitor® area contains a haemoglobin-derived catalyst which will turn blue within 10 seconds after applying developer. The negative (⊖) Performance Monitor® area does not contain a catalyst and should not turn blue after applying developer.

If the Performance Monitor® areas do not function, the test results are invalid.

Important

• Even if only one of the 6 smears shows blue during the 60 second observation period, the patient test result is positive, since in this sample occult blood was found. Diagnostic follow-up is needed.

• In no case should a positive Hemoccult® test be repeated in order to re-examine the result, because the bleeding may be intermittent.

• A negative test result does not exclude a colorectal lesion, since gastrointestinal bleedings may be intermittent, blood is sometimes distributed in the faeces non-homogeneously and haemoglobin is degraded during the passage through the colon. In case of unclear gastrointestinal distress or other reasons for suspicion, diagnostic follow-up should be done in spite of a negative test result.

• No samples should be collected if blood is visible in the stool (e.g. menstruation, active haemorrhoids).

• Bleeding of haemorrhoids only rarely causes positive test results, since dried blood with intact erythrocytes usually does not release haemoglobin. If there is a positive test result in patients with active haemorrhoids, the test has to be repeated after treatment and cure, since the positive result may have been caused by another bleeding source in the colon.

• Trials have shown that dietary iron supplements in usual dosage do not cause false-positive test results. However, some iron supplements contain Vitamin C in excess of 250 mg.

• Drugs and excess alcohol consumption may cause a positive test result, if they induce gastrointestinal bleeding.

• The application of antiseptic preparations containing iodine to the anal area may cause false-positive results.

• Rehydration of the sample and use of gastric samples is not recommended.

• Any positive result should be followed by usual diagnostic procedures.

*The WHO recommendation is as follows:* A patient with a positive stool test should have a complete evaluation of the colon by colonoscopy or by flexible sigmoidoscopy and double-contrast barium enema. Colonoscopy is preferred.
Storage and stability
When Hemoccult® test cards and developer are stored properly, they are stable until the expiration date printed on the box and the pouches. Do not refrigerate or freeze. Protect from direct sun light, UV-irradiation, high temperature and oxidising gases or volatile chemicals (e.g., iodine, chlorine, ammonia or ozone). Hemoccult® test cards in which the filter paper is already coloured blue or bluish-green before being given to the patients must not be used.

Precautions and Warnings
The developer solution is flammable; avoid open flames. It vaporises easily and should be stored well sealed. Care should be taken to keep it from coming into contact with eyes or skin. If accidental contact with skin occurs, rinse well with plenty of water. If there is contact with eyes rinse the open eye well with plenty of water for at least 15 minutes and seek immediate medical advice.

For disposal of test cards and developer local or internal regulations have to be observed. Used test cards should be handled as potentially infectious waste.

Highly flammable
Bibliography • Literatur • Bibliographie • Bibliografia • Bibliografía • Litteraturhenvisninger • Litteraturförteckning

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