The Value of Immunochemical Faecal Occult Blood tests in Clinical Use
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This booklet is intended to provide healthcare practitioners with an overview of QuikRead go iFOBT and QuikRead FOB quantitative, as well as the diagnostic potential of immunochemical Faecal Occult Blood tests.

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Introduction

The purpose of this booklet is to describe how Orion Diagnostica’s QuikRead go® iFOBT and QuikRead® FOB quantitative can be used as aid in diagnostics, and explain how the user can benefit from the test features. Both tests are quantitative immunochemical tests intended for measuring Faecal Occult Blood (FOB), i.e. haemoglobin (Hb) concentrations in human faeces.

A small amount of blood is lost every day in healthy persons due to intestinal physiological bleeding \(^1\)-\(^3\). In some conditions, such as e.g. iron-deficiency anaemia (IDA) and colorectal cancer (CRC) \(^3\),\(^4\) the amount of bleeding may however be greater and a more thorough investigation, such as colonoscopy or sigmoidoscopy \(^3\),\(^5\), is required to clarify the origin of bleeding. As FOB tests measure the amount of Hb in faeces they are useful when suspecting excess bleeding in the gastrointestinal tract.

Traditionally FOB testing has been performed using qualitative chemical guaiac-based tests (gFOBTs), but the value of immunochemical FOB tests (iFOBTs) is becoming increasingly recognised \(^5\). iFOBTs have enabled a significant improvement in analytical specificity \(^6\). Besides offering objective results, quantitative iFOBTs enable the user to perform FOB testing according to case-specific parameters as well as national guidelines.

QuikRead go iFOBT and QuikRead FOB quantitative are user-friendly tests for fast and reliable measurement of occult blood in faeces. The faeces sample is collected in a QuikRead FOB Sampling vial, which is suitable for transportation and enables home sampling. The measurement is performed on an instrument that provides objective results within minutes. With QuikRead go iFOBT, operated on Orion Diagnostica’s next generation Point-of-Care test system, the users’ various needs are taken even further into account by flexible choice of result presentation: Hb concentrations can be displayed either as qualitative or as quantitative, obtained as ng/ml in buffer or as µg/g in faeces, whichever is preferred by the user.
Gastrointestinal bleeding

There are many causes of and possible clinical scenarios associated with gastrointestinal bleeding. Bleeding might be massive or small-scaled, obvious or hidden and originate from e.g. the upper or lower gastrointestinal tract. The main focus in this booklet is on occult, i.e. hidden bleeding from the lower gastrointestinal tract.

Occult gastrointestinal bleeding is defined as bleeding that is unknown to the patient. It is the most common form of gastrointestinal bleeding, and can be caused by various lesions in the gastrointestinal tract. Significant amounts of blood can be hidden in faeces; patients losing up to 100 ml of blood per day may have normal-appearing faeces 7−10. Faecal occult bleeding might be identified during routine laboratory testing, in screening of asymptomatic subjects for colon cancer, or as part of a physical examination 11. Occult bleeding itself is not a disease, but it might be a symptom of various conditions, such as CRC or chronic iron loss leading to IDA. Most people to date are not tested for FOB or IDA, and the incidence of occult bleeding is most likely underestimated 4.

Occult gastrointestinal bleeding differs significantly from obscure gastrointestinal bleeding, which is characterised by evident and recurrent bleeding. Visible blood is easily detected as melena or as bright blood in faeces 12. Obscure bleeding accounts for only approx. 5% of clinically evident gastrointestinal bleeding, and is commonly caused by bleeding from a difficult to identify source, such as the small intestine. The diagnostic and therapeutic challenges with obscure bleeding are great, and the site of haemorrhage should be identified as soon as possible 4.

Normal physiological bleeding

The human gastrointestinal tract consists of the oesophagus, stomach, small intestine, colon and rectum. These form a digestive system with the main purpose to utilise nutrients in ingested food and contain the waste until it is
The normal physiological bleeding from the gastrointestinal tract is 0.5–1.5 ml/day in healthy persons \(^1,^2,^14−^16\). Bleeding can be caused by many non-neoplastic reasons and gastric irritants \(^3,^4\), some of which are listed in table 1 below.

Table 1. Non-neoplastic factors causing gastrointestinal bleeding

- Aspirin
- Nonsteroidal anti-inflammatory drugs
- Gum disease
- Gastritis and oesophagitis
- Gastroduodenal and oesophageal ulcers
- Peptic ulcer disease (PUD)
- Vascular ecstasias
- Haemorrhoids
- Portal hypertensive gastropathy (PHG)
- Various gastrointestinal parasites

Aspirin and anticoagulant therapy have been associated with occult gastrointestinal bleeding \(^3,^17\), but the statement has been challenged \(^18,^19\). A combination of aspirin and warfarin leads to slightly increased levels of occult blood, but neither one alone seems to cause positive FOB tests. The increase is most likely minimal \(^18,^19\). The yield of gastrointestinal evaluation may, however, be small in patients with too high a degree of anticoagulation or chronic bleeding e.g. in cases of intrinsic coagulopathy \(^20\).

**Age and gender**

Recent research in various parts of Europe has shown that physiological faecal haemoglobin concentrations vary significantly with gender and age \(^21−^27\). The concentrations are higher in men than in women, and increase by age regardless of gender (figure 1). Possible explanations to difference observed between genders are higher blood Hb concentrations and a faster colonic transit time in men \(^21,^27\).
Figure 1. Faecal Hb values for the 95th percentile in Scottish men and women of various ages. Data shows that 95% of subjects have faecal Hb concentrations below the indicated values. Men have higher Hb values than women, and the concentrations increase with age regardless of gender.

Sites of bleeding and intraluminal metabolism of Hb

Gastrointestinal bleeding can theoretically originate from all parts of the gastrointestinal tract, but the site of bleeding affects the intraluminal metabolism of Hb. Therefore, Hb, and its moieties haem and globin, appear in different forms in faeces depending on the source of the bleeding.

Hb originating from the upper gastrointestinal tract is cleaved to haem and globin by gastric pepsin and pancreatic proteases when it reaches the proximal small intestine. Part of the haem that is not reabsorbed in the small intestine is converted to porphyrins and iron. Partially degraded haem can thus be present in the faeces if the site of bleeding is in the upper or middle gastrointestinal tract, whereas the haem in faeces is intact if the bleeding site is in the lower gastrointestinal tract.

The globin moiety of human Hb is degraded by pepsin as well as pancreatic and intestinal proteases in the upper gastrointestinal tract. Partially degraded globin can be present in faeces if bleeding originates from the middle gastrointestinal tract, and intact globin can be found if bleeding appears in the lower gastrointestinal tract. As globin is degraded in the upper gastrointestinal tract, it is a better marker for bleeding from the lower gastrointestinal tract than haem or porphyrins.
Colorectal Cancer (CRC)

CRC is a cancer from uncontrolled cell growth in the colon, rectum or appendix and it affects mainly elderly. It is the most common cancer and the second most common cause of cancer deaths in Europe; about 432 000 new cases are reported annually, and in 2012 approx. 212 000 deaths were reported. Worldwide CRC is the third most common cancer with approx. 1.2 million cases, and fourth most common cause of cancer death with 0.6 million deaths annually. CRC occurs with equal frequency in men and women, but some racial differences in CRC survival have been observed. CRC mortality rates have decreased in recent years, possibly due to increased awareness, more accurate diagnosis and earlier detection and removal of premalignant polyps. Although mortality rates are decreasing, the incidence of CRC continues to increase, probably due to the aging population and lifestyle choices.

Many factors and conditions that elevate the risk of CRC are known, and some of these are listed in table 2. The conditions include hereditary disorders which lead to multiple noncancerous growths in the intestines, whereas the listed other factors are mainly associated with lifestyle and diet.

Table 2. Conditions and factors elevating the risk of CRC

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Other factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Ulcerative colitis</td>
<td>• Age</td>
</tr>
<tr>
<td>• Crohn’s disease</td>
<td>• Nicotin</td>
</tr>
<tr>
<td>• Hereditary nonpolyposis colorectal cancer (HNPCC)</td>
<td>• Alcohol</td>
</tr>
<tr>
<td>• Familial adenomatous polyposis (FAP)</td>
<td>• Overweight</td>
</tr>
<tr>
<td>• Peutz–Jeghers syndrome (PJS)</td>
<td>• Inactivity</td>
</tr>
<tr>
<td>• Juvenile Polyposis Syndrome (JPS)</td>
<td>• Large consumption of red meat</td>
</tr>
<tr>
<td>• Previous CRC or polyps</td>
<td>• High calorie intake, low fibre content</td>
</tr>
<tr>
<td></td>
<td>• Diabetes mellitus type 2</td>
</tr>
</tbody>
</table>

Typical symptoms of CRC include gastric dysfunction such as stomach pain, constipation, occasional diarrhoea and difficulties with defaecation. Visible blood can appear in faeces, and patients also often suffer from anaemia. These symptoms are common in other diseases as well, and therefore only nonspecific indicators of CRC.
Progression and survival rates

CRC forms when normal cells or benign mucosal tumours (polyps or bulges) in the colorectal area become malignant. The disease is classified in stages according to its progression (Stage 0–4, Dukes A–D CRC). Survival of CRC is related to the clinical and pathological stage of the disease at the time of diagnosis. The 5-year survival rate for patients with cancer limited to the bowel wall is 83–95% \(^{34,38}\), whereas it is only 35–65% in those with involvement of lymph nodes and <10% in patients with metastatic disease \(^{34,36,39}\). Detecting CRC or its precursors early is possible \(^{13}\), which emphasises the need of proper diagnostics.

Polyps are mucosal masses in the colon and rectum. Hyperplastic polyps and mucosal tags are generally small and of no clinical importance, whereas adenomatous polyps are premalignant. Adenomatous polyps account for approx. half to two thirds of colorectal polyps and are found throughout the colon and rectum \(^{3}\). It is generally accepted that most CRCs develop from adenomatous polyps, but the transformation occurs slowly and the estimated rate of polyps progressing to cancer is only 2.5 polyps per 1000 per year \(^{40}\). The progression from normal mucosa to adenocarcinoma usually takes about 8–10 years \(^{36}\). It is associated with accumulation of genetic alterations as well as environmental factors such as fat and fibre intake \(^{3}\).

About two thirds of cancers bleed in the course of a week \(^{41}\) and approx. 90% of these will be detected with repeated testing \(^{42}\). Adenomatous polyps \(\geq 1-1.5\) cm in size are more likely, whereas hyperplastic polyps are less likely to bleed \(^{43,44}\). The bleeding caused by cancer tends to be intermittent and the blood is often unevenly distributed in faeces. Irregular bleeding and uneven distribution leads to challenges in testing, and a clean faecal sample can thus not completely rule out the possibility of cancer or benign polyps \(^{13}\).

Screening programmes

CRC screening is recommended by the Council of the European Union (2003/878/EC) and the number of European countries that are implementing national CRC screening programs is growing \(^{36}\). In 2009, 19 of the 27
European Union countries implemented screening for CRC. The population usually invited to screening programmes is asymptomatic average-risk men and women of approx. 50–74 years.

CRC screenings are performed in order to lower the burden of cancer in the population by discovering disease in its early stages. Early discovery reduces the incidence of CRC and enables more effective treatment, e.g. removal of lesions. It also affects the costs of treatment; disease detected when the cancer has spread to other organs of the body (stage 4, Dukes D CRC) is twice as expensive to treat in the first year as disease detected when malignancies are restricted on the inner mucous surface (stage 1, Dukes A CRC).

The two main types of CRC and high-risk adenoma screening strategies are bowel visualisation (e.g. colonoscopy or flexible sigmoidoscopy) and measurement of faecal markers (e.g. FOB or DNA marker). The pros and cons of different screening strategies need to be taken into account when preparing recommendations and screening. National guidelines have chosen different approaches; some countries perform visual preliminary screening by colonoscopy or flexible sigmoidoscopy, but performing FOB tests (FOBTs) is the most widely used strategy. Several studies have shown that using FOBTs in population screening can reduce mortality from colorectal neoplasia. Compared to visualisation techniques, the testing of faecal markers is safe, less expensive and non-invasive, and sampling is possible to do at home. The European Group on Tumor Markers recommends screening with FOBTs to be performed at least biennially, whereas The American Gastroenterological Association recommends that persons at average risk should be tested for FOB annually. Annual investigations and removal of polyps are also recommended for individuals who have conditions that lead to an elevated risk of CRC.

As bleeding might be caused by many physiological and disease-related factors, FOBTs are not specific for colorectal neoplasia. FOBTs provide information on excessive bleeding and are thus merely indications of the possible presence of cancers and polyps. Finding of occult blood in faeces therefore mandates further investigations of the gastrointestinal tract, starting typically...
with the colon. Investigations are needed to locate the source of bleeding. Using a FOBT in deciding on which patients are in need of further examination can aid in lowering the number of persons undergoing unnecessary further examinations, in preventing or decreasing the rates of possible complications, and therefore leading to cost savings. There are, however, significant differences between FOBTs as well as between further examination techniques. These are presented in more detail in the following chapter.

Faecal Occult Blood tests (FOBTs)

FOBTs are used for example in colorectal cancer screening programmes, in the course of clinical evaluation, and when investigating possible causes of anaemia. The concept of detecting occult bleeding in CRC screening is based on the observations that cancers bleed more than mucosa. The bleeding increases gradually with growing size of polyps and advancing stage of CRC, and FOBTs can potentially detect both CRC and its preliminary stages.

FOBT cut-off concentrations are set to detect excess bleeding and do not typically detect normal physiological bleeding. In qualitative tests the cut-off concentration set by the manufacturer dictates which individuals will get a positive respective negative result, and thus who will be sent for further investigation. The most often used cut-off concentration is 100 ng haemoglobin/ml buffer (1:200 dilution ratio), but it has been challenged by more recent studies.

The likelihood that FOBTs will detect gastrointestinal bleeding depends on many factors, such as the anatomical level of the bleeding, faecal transit time, faecal mixing and intraluminal Hb metabolism and degradation. Additionally the features of the bleeding, e.g. its irregularity, pattern and rate, as well as the sensitivity and characteristics of the chosen FOBT affect the results.
Methods

The test method traditionally most used for detecting occult blood in faeces is the guaiac-based FOB test (gFOBT)\textsuperscript{55-58}. However, an ongoing change towards favouring immunochemical FOB tests (iFOBTs, or faecal immunochemical tests [FITs]) is evident\textsuperscript{21, 36}. iFOBTs have enabled a significant improvement in analytical specificity \textsuperscript{6}, and have many advantages that speak both to patients and healthcare personnel. The fundamental differences between the two methods, as well as a review of current recommendations and further investigation techniques, are presented below.

gFOBTs

Guaiac-based tests for detection of blood in faeces measure pseudoperoxidase activity of haem in Hb \textsuperscript{7, 20}. The tests have been available for decades, and are widely evaluated\textsuperscript{48, 59, 60}. The haem component of Hb is identical across human and animal species, chemically robust and only partially degraded during its passage through the gastrointestinal tract\textsuperscript{17}. Advantages of gFOBTs include an affordable price and suitability to screening programs. The main drawbacks, in turn, concern the specificity of source of bleeding, dietary restrictions, test interpretation and performance.

During sampling a small amount of faeces is smeared onto a filter paper on a test card, incorporating the guaiac reagent. Because of intermittent or low-volume bleeding or heterogeneous sample material, obtaining many samples is preferred. The sensitivity of gFOBTs increase with the numbers of samples per faeces as well as with number of faeces sampled\textsuperscript{3}. One or two samples are usually taken from each of three separate bowel movements of every tested person\textsuperscript{3, 45}. Samples smeared on gFOBT test cards are fairly stable\textsuperscript{61}, and the test card can be returned to the healthcare provider e.g. by mail.

When a gFOBT is performed hydrogen peroxidase reagent is added on the faeces samples. A positive result is registered if a blue colour appears as a result of oxidation by peroxidases\textsuperscript{20}, in any of the sample windows after addition of the reagent. gFOBTs are visually interpreted qualitative tests. Interpretation of gFOBTs is not always unequivocal and might lead to false
negative results, especially if proper training is not given \(^3,^{62}\). The result interpretation must be done immediately as the colour may fade. Additionally, dietary iron supplements, bismuth, or other factors turning faeces dark, green or black might lead to difficulties in interpreting the blue colour change of a positive gFOBT \(^3\).

**Sources of bleeding and dietary restrictions**

Due to the intraluminal metabolism of haem, gFOBTs measure bleeding from both the upper and lower gastrointestinal tract \(^2,^9,^{20}\). The test is therefore not specific for colorectal neoplasia, and might reflect upper gastrointestinal tract bleeding caused by other reasons \(^3,^{20}\) (see table 1). Research has shown that many lesions in the upper gastrointestinal tract bleed sufficiently to produce positive gFOBT results \(^1,^9\). Use of gFOBTs in CRC screening is therefore likely to be associated with some false positive results due to bleeding in the upper gastrointestinal tract \(^9\).

The use of gFOBTs is heavily affected by dietary factors \(^3,^{20}\). Due to haem, peroxidase or pseudoperoxidase activity in other substances than human Hb, gFOBTs do not recognise human occult blood specifically. There is a risk of false positive and false negative gFOBT results if e.g. the factors in table 3 are present in faeces of persons under investigation \(^3\). Persons being tested are requested to take into consideration dietary restrictions two days prior to FOB sampling and throughout the test period \(^3,^{42}\). Some evidence do although exist that false positive samples might be avoided with a delay of over 48 hours before analysing the gFOBT \(^45\). False negative results can be avoided by ingestion of fibres that increase the transit time of faeces \(^3\).
Table 3. Factors that may cause false positive or false negative results when using gFOBTs. For more detailed information see Gnauck et al. (1984). ³

<table>
<thead>
<tr>
<th>Factors that may cause false positive results</th>
<th>Factors that may cause false negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Red meat</td>
<td>• Vitamin C</td>
</tr>
<tr>
<td>• Bacteria</td>
<td>• Citrus fruits</td>
</tr>
<tr>
<td>• Turnips</td>
<td></td>
</tr>
<tr>
<td>• Horseradish</td>
<td></td>
</tr>
<tr>
<td>• Hyeroxides in various fruits and vegetables</td>
<td></td>
</tr>
</tbody>
</table>

**Test performance**

The sensitivity and specificity for colorectal neoplasia is highly variable between different gFOBTs ⁶⁰, ⁶³. The reported sensitivities vary between 51–100%, the specificities between 90.4–97% and the positive predictive value between 2.4–17% ⁶³. The likelihood that a gFOBT will be positive is in general related to the size and location of the bleeding lesion ², ⁶⁴. gFOBTs are far less sensitive for detecting polyps than CRC, mainly due to the smaller or insignificant amount of bleeding from polyps ³ or the heterogeneity of sample material. The low clinical sensitivity and specificity of gFOBTs also leads to many normal colonoscopies ⁴⁵. Various trials have, however, shown a 16% reduction in the relative risk of CRC mortality when using gFOBTs in screening ⁶⁵.

If faecal samples collected on gFOBTs are rehydrated the sensitivity of the test is increased, on expense of specificity and positive predictive value of the test. The proportion of cancers found increases, but as does the proportion of persons who receive a false positive result and must undergo further diagnostic tests in vain ³. According to the European Group on Tumor Markers rehydration of samples should not be performed ⁴⁵.
iFOBTs

Immunochemical FOBTs exist both as qualitative and quantitative. Qualitative iFOBTs are visually interpreted. Quantitative tests in turn are often instrument-read and have thus enhanced quality while eliminating the potential for visual bias by the observer. iFOBTs use antibodies to detect the human globin epitopes present in occult blood. The antibodies are attached to latex particles, dye or an enzyme that in the presence of human globin forms a detectable complex. Detecting methods include turbidity, latex agglutination, haemagglutination, colloidal gold agglutination or coloured dye produced by an enzyme.

iFOBTs are rapidly replacing gFOBTs because of their many advantages. These include greater clinical and analytical sensitivity, collection of a single sample, simple and hygienic sampling devices, higher specificity for lower gastrointestinal tract bleeding, and no dietary restrictions. The use of iFOBTs results in improved clinical performance and higher participation rates in screenings. Compared to gFOBTs, iFOBTs might, however, require a larger initial investment and have slightly weaker sample stability after collection.

Sources of bleeding and dietary restrictions

Due to the intraluminal degradation of the Hb moiety globin, iFOBTs specifically detect gastrointestinal bleeding from the lower gastrointestinal tract. Small amounts of bleeding from the upper gastrointestinal tract remain undetected. Therefore, it can be stated that iFOBTs have a theoretical advantage over gFOBTs in localising bleeding to the lower gastrointestinal tract.

Antibodies that are used in iFOBTs are directed towards human globin epitopes. As globin is species-specific iFOBTs should not be subject to interference from dietary blood. As the potential for dietary interference is small no dietary restrictions are needed prior to or during sampling for iFOBTs.
Test performance

Even though iFOBTs are not as widely evaluated as traditional gFOBTs, adequate population-based comparative studies have been made. Overall, the sensitivity of iFOBTs for CRC is stated to be 61–91% and the specificity 91–98%. iFOBTs enable detection of Hb in faeces at lower concentrations than gFOBTs, and therefore increase clinical sensitivity by detecting small or intermittently bleeding lesions. The superiority of iFOBTs over gFOBTs has been confirmed by several recent studies, some indicating even a two or three times higher sensitivity at increased specificity. There is, however, not yet any direct evidence of a decrease in CRC mortality in prospective randomised trials.

Adequate clinical sensitivity and specificity can be obtained using a single iFOBT test per subject. Likely due to the improved clinical performance, the use of only one or two samples and simpler sample collection and handling techniques, screening programmes with iFOBTs has been shown to have a participation rate higher than gFOBTs.

Even though gFOBTs are more affordable than iFOBTs, studies have shown that use of iFOBTs is a more cost-effective strategy in CRC screening. This is likely due to the increased sensitivity of the tests as well as the higher participation rate. The higher test costs are also balanced by use of automated analysers that lead to reduced staff costs, and by the need of performing fewer tests per patient. Cost-analyses do, however, need to be made separately in each country, as e.g. test and personnel costs, logistics and preferences of screening vary.

Quantitative iFOBTs

An increasing amount of structured screening programmes use automated iFOBTs for quantitative measurements of faecal Hb concentrations. In addition to the advantages of qualitative iFOBTs, quantitative iFOBTs offer the user a numerical result of the Hb concentration in the sample, can be
automated and thus objectively interpreted. These are considerable improvements compared to qualitative tests that cannot provide objective information on the amount of bleeding. Clinically relevant advantages are described in more detail below.

- Possibility to adjust cut-off concentration according to study settings, national guidelines, available local resources, as well as age and gender of subjects

- Patient-specific follow-up and screening strategies based on additional knowledge of amount and variability of occult bleeding or underlying conditions elevating the risk of CRC

**Adjustable cut-off concentration**

The most prominent advantage of a quantitative FOB test is that the user can select the cut-off concentration in order to decide on further investigation. This means that the analytical sensitivity of the test can be adjusted according to e.g. screening settings, national guidelines or local requirements. The goal with choosing a cut-off concentration is to provide an adequate positivity rate with acceptable trade-off between detection rate and unnecessary colonoscopies performed. The choice depends on the test device, sampling, number of samples used, intended detection rate, prevalence of CRC in population, and political issues such as availability of colonoscopy.

Several studies on methods and FOB cut-off values have been performed, and some of the results have been applied to routine use on national level. Most studies recommend a cut-off concentration of 75–100 ng Hb/ml (15–20 µg Hb/g faeces) in order to reach optimum cost-effectiveness.

As described on page 9 and in figure 1, faecal Hb concentrations are not comparable between men and women of different age. The differences emphasise that one, by the manufacturer predetermined, Hb cut-off value is not appropriate in FOB testing. When using one cut-off value more men
and elderly people will get positive test results, even though their faecal Hb concentration might be completely normal. More tailored strategies for screening programs are thus needed, and it is possible that different cut-off concentrations are appropriate to use as criteria for further investigation for specific groups of individuals.

Table 4. The measuring range and result presentation units of QuikRead go iFOBT and QuikRead FOB quantitative.

<table>
<thead>
<tr>
<th>Measuring range</th>
<th>QuikRead go iFOBT</th>
<th>QuikRead FOB quantitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>75–1000 ng/ml (15–200 µg/g)</td>
<td>100–1000 ng/ml (20–200 µg/g)</td>
<td></td>
</tr>
<tr>
<td>Result unit</td>
<td>As quantitative: ng/ml and/or µg/g</td>
<td>ng/ml, convertible to µg/g</td>
</tr>
<tr>
<td>As qualitative: positive / negative</td>
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<td></td>
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</tbody>
</table>

Knowing the total amount of Hb in the faecal mass, enables comparison of FOB results obtained with different methods. Test manufacturers use various sampling devices and buffers, collect various masses of faeces and report Hb concentrations in different ways. Therefore FOB results expressed in ng Hb/ml buffer are not comparable between different methods.

A suggestion on standardisation of units for reporting faecal Hb concentrations has been made. The only unit that allows comparison of FOB results between test devices and across clinical studies is µg Hb/g faeces. The unit can be calculated if the dilution ratio of the sample is known, but sophisticated automated iFOBTs provide the user with results expressed in µg/g automatically.

**Patient-specific follow-up and screening strategies**

Occult bleeding increases gradually with growing size of polyps and advancing stage of CRC (figure 2), and FOB tests can potentially detect both CRC and its preliminary stages. Qualitative test indicating only that the result is positive, do not give any information on the amount of occult bleeding. Risk stratification based on the exact numerical Hb concentration might help clinicians in identifying subjects with alarmingly high FOB concentra-
tions that should undergo further examinations immediately. The interval between screen-detected disease and the start of definitive management is an unpleasant time for the patient and it presents the opportunity for disease progression 28, 96.

![Figure 2](image-url)

**Figure 2.** Faecal Hb concentrations and 95% Confidence Intervals according to stage of adenomas and cancer in 815 patients (mean age 57.4 years). Study performed with quantitative iFOBT and colonoscopy investigation 66.

Irregular bleeding patterns and rates 54 as well as many other factors might cause varying faecal Hb concentrations. There is a risk of false results in case a qualitative test is used and the subject’s average Hb concentration in faeces is very variable and/or close to the cut-off concentration of the FOB test. In these cases a quantitative test would clarify the situation significantly, thus eliminating guess-work, false positives and unnecessary further examinations.

Patients with FOB concentrations close to the cut-off concentration might benefit from more active follow-up even though such strategies are not yet implicated in screening programmes 97. According to Chen et al. 97 high-risk subjects (80−99 ng Hb/ml) could be offered screening with shorter intervals compared to low-risk subjects (0−19 ng Hb/ml). This approach would be cost-effective; even if reducing the screening interval of high- and very-high risk individuals from two to one years, the number of positive FOB tests and thus colonoscopies would be lower 97. The same risk categorisation has been used when comparing faecal Hb concentrations by age and gender, and could also according to McDonald et al. be useful in screening programme planning 21. The threshold for screening might additionally differ in populations with high-risk of CRC 84, 98. Clinicians could want to detect even small amounts of
bleeding and perform more aggressive screening for patients with e.g. FAP, HNPCC or family history of CRC.

Further investigations

If a FOB test is positive, an investigation is performed to identify the source of bleeding. The most common techniques for further investigations include colonoscopy, flexible sigmoidoscopy, air-contrast barium enema, molecular markers, virtual colonoscopy or colon capsule endoscopy. Colonoscopy is considered the most effective visualisation technique to detect CRC and high-risk adenomas, and the recommended method to evaluate the colon in patients with increased FOB concentrations. All diagnostic tools for examining the colon can, however, miss clinically serious lesions.

Colonoscopy directly visualises the entire colon, has high sensitivity for mucosal lesions and allows intervention. It offers the potential to find and remove lesions throughout the colon and rectum. The spread of the tumour as well as the general condition of the patient affect choice of treatment and chances of recovery. Several studies have covered the cost-effectiveness of CRC screening. As a primary screening method colonoscopy is limited by low adherence of the population, high costs and risks. Screening with FOBT alone is stated to be more cost-effective than computed-tomography-colonoscopy and optical-colonoscopy alone.

Flexible sigmoidoscopy involves direct visualisation of the distal colonic mucosa. Reaching approx. 50 cm far from the anal sphincter, it is not as broad and extensive examination as colonoscopy. Screening with sigmoidoscopy alone is less effective than combining it with FOB testing. Pre-screening for FOB combined with sigmoidoscopy leads to cancer being detected in an earlier stage, and to patients surviving longer compared to screening with sigmoidoscopy alone. Air-contrast barium enema together with flexible sigmoidoscopy accurately detects colon cancer and large adenomas but is less accurate than colonoscopy. With advances in technology, air-contrast barium enemas are being used less often, and are rarely indicated by physicians in screening.
Virtual colonoscopy or computed tomographic studies of the colon might be useful in colonic evaluation \(^{112}\), but clinical experience as of today is limited \(^{20}\). The technique does not detect small lesions or allow polypectomy \(^{36}\). Using molecular DNA markers is expensive and time-consuming; it requires skills and a suitable panel of markers. It might not suit mass population screening and has not undergone validations \(^{45}\). Capsule endoscopy in turn involves swallowing an endoscopic capsule with a built-in micro camera. The technique is at the stage of testing for use in screening, it is non-invasive and painless \(^{36}\).

Many factors are of importance when choosing a CRC examination method. These are e.g. accuracy of the procedure, costs, patient acceptance, safety and availability of diagnostic tools, and complication rates of the methods \(^{4,20}\). Due to colonoscopy being costly, invasive and scarce it should only be undertaken in subjects at increased risk of CRC \(^{28}\). Complication deaths from colonoscopies, perforations, major bleeding episodes and minor complications have been reported \(^{3,109}\). For the patient the investigation might be painful or uncomfortable \(^{13}\). It is important to ensure that subjects involved in screening are fully informed about the risks of CRC and the importance of detecting cancer early. Healthcare providers should be able to give details of the screening procedure and the risks associated with different investigations \(^{3}\).

**Guidelines and recommendations**

As indicated previously, using FOB tests in population screening can reduce mortality from colorectal neoplasia \(^{42,50-53}\). There are variations in national guidelines and practices concerning FOBTs; some still favour the established gFOBTs, whereas others are up to speed and recommend iFOBTs and quantitative tests. In the light of the previously discussed differences between various FOB testing methods, recommendations are still needed e.g. regarding which methods and cut-off concentrations to use and how many samples to obtain.

The European Guidelines for quality assurance in colorectal cancer screening and diagnosis \(^{6}\) state that an ideal biochemical test for CRC screening would use a biomarker that is specific and sensitive for cancer and advanced pre-cancer. The sample should be easily collected and could be safely and
cheaply transported for automated analysis. Both iFOBTs and gFOBTs fulfil these criteria \(^{17,28}\). iFOBTs are, however, both analytically and clinically more sensitive and specific for detection of Hb than gFOBTs. The guidelines additionally emphasise the value of automated measurement and adjustable cut-off concentration. Quantitative iFOBTs are currently the test of choice for population screening and adequate clinical sensitivity and specificity can be obtained using a single iFOBT per subject \(^{17}\).

Also the European Group on Tumor Markers recommends the use of quantitative iFOBTs with adjustable cut-off value for colorectal neoplasia screening in average-risk populations. The recommendation is based on the significant amount of advantages of iFOBTs compared to gFOBTs. For countries not yet using FOB tests, iFOBTs should be considered the standard for CRC screening \(^{45}\).

“The dilemma for a population screening programme is where to draw the line between detection rates, cost and the inconvenience and morbidity associated with colonoscopy.”

“Adaption of a test device and the selection of a cut-off concentration should follow a local pilot study to ensure that the chosen test, test algorithm and transport arrangements work together to provide a positivity rate that is clinically, logistically and financially acceptable.”

*European guidelines for quality assurance in colorectal cancer screening and diagnosis* \(^{17}\)
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