ANALYTICAL COMPARISON OF THREE QUANTITATIVE IMMUNOCHEMICAL FECAL OCCULT BLOOD TESTS FOR COLORECTAL CANCER SCREENING

Running title: Analytical comparison of immunochemical FOBTs

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Abbreviations:
Hb: hemoglobin
G-FOBT: guaiac faecal occult blood test
I-FOBT: immunochemical fecal occult blood tests
CRC: Colorectal Cancers
ABSTRACT

**Background:** The superiority of several immunochemical fecal occult blood tests (I-FOBT) over guaiac-based tests in colorectal cancer screening is now established. The aim of this study was to compare the analytical performance of three quantitative I-FOBTs.

**Methods:** Stool samples from ten healthy volunteers, initially I-FOBT negative, supplemented with human blood, were used to compare reproducibility and stability of measurement at varying storage temperatures (4°C, 10°C, 20°C, 30°C) and durations before test analysis (1 to 10 days) for three I-FOBTs (New Hemtube / Magstream HT, OC-Auto sampling bottle3 / OC-Sensor DIANA and FOB Gold / SENTiFOB). Concentrations ranging from 0 to 350μg Hb/g of feces were evaluated.

**Results:** The measurement reproducibility of OC-Sensor was superior to Magstream, and far superior to FOB Gold. For all tests, variability was essentially related to sampling. Detected hemoglobin levels were substantially lower for all tests at temperatures above 20°C. At 20°C this loss in concentration was less important with OC-Sensor (significant 1.7% daily decrease vs 7.4% for Magstream and 7.8% for FOB Gold). At 30°C, daily loss was 8.6% with OC-Sensor, whereas after 24 hours, only 30% of the original hemoglobin was detected with FOB Gold, compared to 70% with Magstream. No hemoglobin was detected on day 5 for the latter two tests.

**Conclusion:** Concerning reproducibility and temperature stability, OC-Sensor performed better than Magstream, and far better that FOB Gold.

**Impact:** Independently of the chosen test, the delay between sampling and test processing should be reduced, the maximal admissible delay depending on ambient temperature

Word count: 249
INTRODUCTION

Colorectal cancer is a major public health issue in all industrialized countries. Screening using guaiac fecal occult blood tests (G-FOBT) reduces specific mortality related to colorectal cancer (1). Several studies have concluded that both the Magstream (Fujirebio, Japan) and OC-Sensor (Eiken Chemical Co, Japan) automated immunochemical (I-) FOBTs offer a gain in sensitivity in the detection of advanced neoplasias, compared to G-FOBT, at a cost of lower specificity (2-4). For both tests, ideal balance between sensitivity and specificity can be reached by variation in hemoglobin (Hb) concentration cut-off and number of samples (5-8). For both tests, a gain in both sensitivity and specificity for the detection of advanced neoplasias was possible (4,7-8).

Since it has been established that I-FOBTs perform better than G-FOBTs, these tests are expected to be used in all national screening programs using FOBT. Accordingly, the use of a fecal immunochemical test has been included in US guidelines for colorectal cancer screening (9). However, several I-FOBT are available and their performance is difficult to compare since the cut-off provided in studies is expressed in concentration of hemoglobin in the collecting tube depending on the concentration of hemoglobin in the feces, but also on the volume of buffer in the tube, and on the amount of feces introduced in the tube. Therefore, optimal test, optimal number of samples, or optimal hemoglobin concentration cut-off are for the moment indeterminate (10). Moreover seasonal variations in positivity rates of screening programs using OC Sensor or Magstream I-FOBT has raised the question of the sensibility to temperature (11-13), and laboratory analyses have established a decrease in hemoglobin concentration in OC Sensor I-FOBT with increasing delay in the sample (11,14).

As a summary, the performance of I-FOBT depends mainly on the test's sensitivity to hemoglobin, on its reproducibility of sampling and measurement, and on the stability of
hemoglobin in the collecting tube, in particular with regard to temperature variations and delay from fecal sampling to test analysis.

Our study aimed to compare measurement precision and reproducibility, together with hemoglobin measurement stability at varying storage temperatures and varying delays between sampling and analysis of three I-FOBTs previously used in colorectal cancer screening programs: Magstream (New Hemtube) analyzed using a Magstream HT automated instrument (Fujirebio, Japan), OC-Sensor (OC-Auto sampling bottle3) analyzed using an OC-Sensor Diana instrument (Eiken Chemical Co, Japan; distributed by Mast Diagnosis), and FOB Gold (distributed by SKD, France) analyzed using a SENTiFOB instrument (Sentinel diagnostics, Italy).

MATERIAL AND METHODS

Immunochemical fecal occult blood tests

All three tests use polyclonal rabbit antibodies directed against human hemoglobin HbA. All tests are fully automated (Table 1). Analysis of OC-Sensor and FOB Gold is based on immunoturbidimetry, which involves a measurement of the absorbance of light through the tube, which increases with the importance of hemoglobin-antibodies complexes. Analysis of Magstream involves the use of an automated visual measurement of migration of agglutinated magnetic particles. In routine use, the crude pixel value generated by Magstream is converted into MSR units, an arbitrary unit proposed by the manufacturer. For the present study, we asked Fujirebio to provide software (not routinely integrated in the machine) to allow for the collection of the measurements (pixel values). Although Magstream is commercialized by Fujirebio as a qualitative test, a quantitative measure is provided by the instrument, so we considered the test as quantitative in the analysis.
Fecal sampling method

Firstly, freshly collected stools, obtained from ten healthy subjects aged less than 50 years, were tested using all three tests to confirm the initial absence of hemoglobin. These stools were mixed and homogenized, then divided into containers. In each of these containers, a volume of human whole blood lysate, the hemoglobin content of which had previously been measured (Advia 2120, Siemens), was added to obtain all pre-specified hemoglobin concentrations in the stool. Each container was vigorously shaken after adjunction of blood. For each of the concentrations, sampling of all three tests was performed using the same containers, ensuring that concentration was identical for all tests. Finally collecting tubes were shaken after sampling, and before analysis. This procedure was repeated two times leading to two distinct stool mixtures, one being analyzed to evaluate reproducibility, the other to evaluate stability to storage.

Experimental plan

Two distinct experiments were performed: the first one to evaluate and compare reproducibility of the tests (experiment 1), the other one to evaluate and compare their sensitivity to temperature and delay of storage (experiment 2). For each of these two experiments, a distinct stool mixture was performed, as described above.

Experiment 1 (stool mixture n°1)

In order to compare tests for a given hemoglobin concentration, we initially explored, for each test, the relationship between concentration in the feces and the value provided by the instrument, for a total of ten values of hemoglobin concentration in feces, varying from 0 to 350 μg Hb/g of feces. This range of concentrations was selected to: 1/ cover the range of usual or proposed hemoglobin positivity cut-offs of all tests, and 2/ cover the range of physiological bleeding of colorectal lesions (15). To assess measurement reproducibility, ten tubes were
collected for each instrument and for each concentration, and all tubes were repeatedly analyzed five times leading to a total of fifty readings per concentration and test. In this experiment, all prepared I-FOBTs were stored for 3 days at 10°C before being analyzed, approximating to the conditions of a screening program involving mailed samples.

Experiment 2 (stool mixture n°2)

A second experimental plan was developed to assess the influence of storage temperature and delay between sampling and analysis for different hemoglobin concentrations in feces. For each I-FOBT, four storage temperatures: 4°C, 10°C, 20°C and 30°C, and five delays between sampling and analysis: 1 day, 3 days, 5 days, 7 days and 10 days were tested for six hemoglobin concentrations in feces [0, 20, 75, 100, 150, 250] µg Hb/g of feces. Due to the large number of combinations of the three aforementioned parameters in an exhaustive plan (360 combinations), and to the relatively slow operating rate of one of the machines (SENTiFOB, Sentinel Diagnostics) we used an optimal experience plan, determined using the ADX (Analysis and Design of eXperiments) tool developed by SAS software. Four factors were introduced in this experimental plan (I-FOBT, storage temperature, delay and concentration), as well as their two-level interactions. Optimization of the design was achieved by D-optimal optimization (maximization of the determinant of the information matrix), in order to perform a linear regression evaluating the mean daily decrease in standardized concentrations (to avoid the impact of the slope of the relationship between fecal concentration and buffer concentration of blood) in the collecting tubes. However due to the non-linear relationship between concentration of blood in the feces, and in the collecting tube (roughly logarithmic) and to the semi-quantitative nature of the Magstream test (see results), each test was assessed individually and no overall analysis was conducted (see statistical methods below).
In our experimental plan, 32 combinations of storage temperature, delay between sampling and analysis, and fecal hemoglobin concentration were used for each test. The experimental plan is presented in annex 1, or could be deducted from table 2 which presents mean measurement obtained for each test, according to temperature, concentration or delay, and therefore in which non-empty cells correspond to evaluated combinations of temperature*concentration*time*test. Ten tubes were collected for each of the included situations, each tube being analyzed 5 times.

Temperature of storage was monitored using a system of electronic measurement of temperature every 5 minutes, and radiofrequency recording (AOIP instrument). All tubes, whatever test or concentration, were stored in the same conditions for each temperature (dedicated fridge for 4°C and 10°C temperatures with a range of variations of temperature of +/- 2°C, air-conditioned room for 20°C temperature with a range of variations from 20°C to 22°C, and a dedicated incubator for 30°C temperature with a range of variations of +/- 1°C).

Statistical analysis

Reproducibility of I-FOBTs (Experiment 1)

For each test, and for each of the experimental concentrations, the inter-tube and intra-tube variances were determined using a random effect model (SAS proc mixed). This procedure enabled us to compute the variation coefficients due to sampling (inter-tube) and reading (intra-tube).

Stability to temperature and duration of storage (Experiment 2)

In an exploratory analysis we computed the mean measurement obtained for each test, according to temperature, concentration or delay (Table 2). Then for each temperature and processing delay, the mean measurement was standardized on the initial fecal concentration to
allow comparison of variations in concentration due to storage duration and temperature between tests. For FOB Gold and OC-Sensor, the buffer concentration was directly proportional to fecal concentration (Figure 1). The theoretical relationship between fecal hemoglobin concentration \( C_f \) (expressed in \( \mu g/g \)) and concentration of hemoglobin in the buffer \( C_b \) (expressed in ng/mL), is given by the following formula: \( C_b = \left( C_f \times q_s \right) / v_b \) where \( q_s \) is the quantity of stools introduced in the tube (in mg), and \( v_b \) is the volume of buffer (in mL). However as we did not check independently the values of \( q_s \) and \( v_b \) provided by the manufacturers, the buffer concentration provided by the automated analyzer was simply divided by the initial theoretical fecal concentration, in order to standardize data. For Magstream, the pixel value provided by the automated analyzer had an inverse logarithm relationship to fecal hemoglobin concentration, and the mean pixel value was 330 in the absence of hemoglobin in the sample (Figure 1). The following transformation was therefore applied to data: \( \left( 330 - \text{pixel} \right) / \log \text{(initial fecal concentration)} \). However this transformation was less adapted to smaller concentrations. For each test we evaluated the mean daily decrease in standardized hemoglobin concentration according to temperature using mixed effect linear models.

Statistical analysis was performed using SAS software, version 9.1 (SAS Institute, Cary, USA).

RESULTS

Experiment 1

Relationship between measurements and fecal concentration of hemoglobin

Firstly, the relationship of the values provided by each automated analyzer (3 days' storage at 10°C) was assessed according to the initial fecal hemoglobin concentration. A linear
relationship between measurement and fecal hemoglobin content was observed for OC-Sensor and FOB Gold, but not for Magstream (Figure 1).

For Magstream, the pixel values were bounded in the range 130-330 pixels. Above a concentration in the tested stools of 250μg Hb/g, the pixel measurements in the tube did not change with concentration (the mean pixel measurement was 130 pixels). Therefore, the test could only be considered as quantitative within a fecal hemoglobin concentration range of 20-200μg Hb/g of feces.

Instrument measurements have been plotted on figure 1 and the expected value is represented as a dotted line according to linear modeling for FOB Gold and OC Sensor, and loess (local polynomial fitting) modeling for Magstream. We defined overlap between concentrations if no cut-off could perfectly separate instrument measurements between two successive fecal concentrations. For hemoglobin concentrations up to 150μg Hb/g of feces, there was no overlap in the range of measurements obtained with the different hemoglobin concentrations in feces selected in our protocol, for OC-Sensor. For both Magstream and FOB Gold, overlap was observed between concentrations in the entire range of these tested concentrations.

**Reproducibility** (Figure 2)

Reproducibility could be explored in the entire concentration range for OC-Sensor and FOB Gold. However it was only explored for concentrations below 250μg Hb/g of feces for Magstream, since for higher concentrations, the absence of measurement variation between concentrations using this test would lead to artificially good reproducibility.

The best reproducibility (smaller total variation coefficient) was observed with OC-Sensor, with the exception of concentrations below 75μg Hb/g of feces, for which Magstream offered better reproducibility. However, variability for small concentrations using Magstream was reduced, due to lacking quantitative measurements above 330 pixels. The worst
reproducibility was observed with the FOB Gold test. Indeed, the mean total variation coefficient observed between 75 and 250μg Hb/g of feces was 0.07 for OC-Sensor, 0.10 for Magstream and 0.18 for FOB Gold.

For all tests, measurement variability involved inter-tube variability, rather than intra-tube variability (the variation coefficients associated with sampling were far greater than those associated with reading). Intra-tube variability was low for all tests (the mean coefficient of variation due to reading between 75 and 250μg Hb/g of feces was 0.025, 0.016 and 0.060 for FOB Gold, OC-Sensor and Magstream respectively).

The difference between these sources of variation was the highest for FOB Gold, for which the inter-tube variability was particularly important compared to the other tests. The mean coefficient of variation due to sampling between 75 and 250μg Hb/g of feces was 0.15 for FOB Gold, whereas it was only 0.10 for OC-Sensor and 0.07 for Magstream.

Inter-tube variability associated with FOB gold tended to decrease as the hemoglobin concentration in feces increased. Inter-tube variability associated with OC-Sensor was stable between 75 and 250μg Hb/g of feces, but was high for concentrations below 75, or above 250μg Hb/g of feces. The latter concentrations were higher than the upper limits of good performance recommended by the manufacturer. Inter-tube variability using Magstream increased as did fecal hemoglobin concentration up to 150μg Hb/g of feces, then decreased, due to a non-linear relationship between pixels and concentration.

**Experiment 2**

**Stability**

Mean measurements according to test, temperature, concentration and time are provided in Table 2. For all three tests, measurement was stable over time, independently of storage temperature when no hemoglobin was added to feces. Relationship between instrument
measurements (pixel or buffer concentration) and fecal hemoglobin concentration was verified (data not shown). Figure 3 and 4 plots mean measurement per machine according to storage duration or temperature. A decrease in measurement was observed for all tests at increased storage durations, and at temperatures of 20°C and 30°C, but not 4°C or 10°C.

Figure 5 provides the mean of ratio of measurements standardized on the experimental fecal concentration for each combination of storage duration and temperature and for each concentration (see methods). The stability of the hemoglobin measurement provided by all three tests was good at 4°C and 10°C for OC-Sensor and Magstream. However, an increase in concentration provided by FOB Gold was observed within the first days, particularly at a storage temperature of 4°C. A similar (yet smaller) increase in concentration detected by OC-Sensor and Magstream over the first days cannot be excluded, based on our data.

At a storage temperature of 20°C, a substantial decrease in hemoglobin concentration was observed over time using all three tests. The best stability was observed using OC-Sensor, with a significant daily decrease in measurement of 1.7% (p<0.01) at 20°C, compared with a daily decrease of 7.4% and 7.8% with Magstream and FOB Gold respectively (p<10^{-3}). However, the decrease observed with Magstream depended on the initial hemoglobin concentration. It was greater with the smaller concentration of 20μg Hb/g of feces.

At 30°C, the performance of OC-Sensor was far better than that of FOB Gold and Magstream. Indeed, the mean daily decrease in measurement observed with OC-Sensor was 8.6% (p<10^{-3}), whereas the mean decrease in measurement observed on the first day was 30% for Magstream, and 70% for FOB Gold. The decrease observed with OC-Sensor at 30°C depended on the initial concentration, being more important for small concentrations. On day 7, 38% of an initial concentration of 150μg Hb/g of stool was still detected with OC-Sensor.

On day 10, 25% of an initial concentration of 250μg Hb/g of stool was still detected by the
same test. On the contrary, from day 5 on, no hemoglobin content was detected by FOB Gold or Magstream in the samples.

**DISCUSSION**

Our results show that the precision and the reproducibility of hemoglobin measurements in feces are better with OC-Sensor than with Magstream, and better with Magstream (in the range of concentrations where the test can be considered as quantitative) than with FOB Gold. Concerning stability at varying temperatures, our results show that independently of the test used, there is a substantial loss in hemoglobin measurement as from 20°C. This loss is more important for FOB Gold than for the other tests. At 20°C and 30°C, denaturation of haemoglobin is less important and occurs less quickly in the buffers used with OC-Sensor than with Magstream devices.

Independently of the test used, intra-tube variability was lower than inter-tube variability. Similar findings were found in an experimental study conducted in UK, although intra-tube variability was assessed in solution of hemoglobin, and inter-tube in artificially positive stools (16). The inter-tube variability estimated in our experiment was a consequence of both the tube characteristics and the biologist's reproducibility in using the test (sampling of feces using the immunochemical tests probes). In real screening settings, such a tube effect would probably be greater since a patient is certainly less reproducible in his sampling technique than a biologist, and patients do not mix their stool to homogenize their blood content before performing the test. Moreover the tube effect measured in screening programs including several tubes per patient also includes the effect of the intermittency of the bleeding associated with colonic lesions. To the contrary, the intra-tube variability measured in our
experiment should be similar to that which occurs in real screening settings, since it is solely an effect of the automated analyzer itself.

The lower inter-tube variability when using OC-Sensor could be explained by a more accurately calibrated quantity of stool incorporated in the sample (not measurable in our study). In addition more overlap occurred between the evaluated concentrations with FOB Gold and Magstream than with OC Sensor. Accordingly the loss in precision of these tests (including inter-tube and intra-tube variability) could have consequences on positivity rates in real screening settings.

Temperature-related hemoglobin degradation is expected; however, it can be delayed by the use of a suitable stabilizing agent in the buffer. Such stabilizing agents are included in all three tests. Nevertheless, the stability of hemoglobin measurement at varying temperatures and over time was better with OC Sensor than with Magstream and far better than FOB Gold. The superiority of OC Sensor was also observed in the NHS Evaluation report (16). At a temperature of 20°C, the decrease observed with FOB Gold and Magstream was at least twice that observed with OC-Sensor. At 30°C, the performance of Magstream and FOB Gold was very poor, whereas the OC Sensor was much better and remained reliable, even at this high temperature. I-FOBT sensitivity variations related to storage duration have previously been described in both laboratory experiments and genuine screening settings (11-14). The interaction between storage duration and temperature has been quantified for the OC Sensor test in a laboratory experiment: the daily decrease in fecal hemoglobin measurement was 0.3% at 4°C, 2.2% at 20°C and 3.7% at 28°C (14). Our findings (2% daily decrease at 20°C and 9% daily decrease at 30°C) are consistent with this observation, although the decrease observed at 30°C appeared more important. This could be explained by a difference in initial hemoglobin concentration in feces, the relative decrease being higher for small initial concentrations. Such differences in sensitivity related to storage temperature and duration,
and affecting the reliability of colorectal screening test programs, could have an important impact on screening organization and test choice, particularly in countries where ambient temperatures are high. It would appear that, whenever possible, the delay between sampling and test processing should be reduced to three days, or CRC screening programs should be stopped during the summer in countries with long period of very high temperatures (>30°C). In addition patients should be advised to store fecal samples in the refrigerator at home before forwarding them by post. Acceptability of such a recommendation needs further investigations.

Our study has several limitations associated with experimental conditions and data analysis constraints. Since it was based on laboratory preparation of positive fecal samples, it is possible that these artificial positive samples behave differently than native positive samples, despite the fact that we used human feces. However decrease in hemoglobin from real positive samples has also been demonstrated with OC Sensor (11). On the one hand, mixed or non-mixed stool samples could behave differently in the application of I-FOBT. On the other hand the mixture of stool samples from several subjects done in our protocol allowed us to avoid differential bias between concentrations and tests in case of undetected hemoglobin previously present in one or several fecal samples. Additionally, sampling error was probably underestimated since the tests were performed by trained biologists, rather than by subjects from the general population. For example, no miss-manipulation of tubes occurred, such as opening the wrong side of the FOB Gold test or spilling the OC Sensor or FOB Gold buffer. Targeted concentrations were not checked in the samples by independent hemoglobin measurement methods. Nevertheless, within each of the experiments (for example storage evaluation) calibrated stool samples were obtained from the same negative-FOB tested stools. Finally the non-linear relationship between pixel value and concentration of hemoglobin in...
the buffer for the Magstream test implied transformation of crude data. However we selected
the transformation which minimized underlying assumptions and independently of the results.
However, the fact that this study was entirely laboratory-performed enabled every parameter
to be controlled, as from the introduction of blood into the stool. This is not the case for
studies exploring the stability of fecal samples sent to the laboratory after having been
performed by the patient at home, measurement of initial hemoglobin concentration in the
stool, and storage duration or temperature from home to laboratory being unknown (11,14).
This also enabled the three tests to be compared on the same stools. Moreover, the use of
blood lysate guaranteed the best possible conditions for the antibody-antigen reaction. Finally,
our laboratory study was designed to fit as most as possible real screening settings with the
choice of a range of concentrations adapted to physiological bleeding of lesions and discussed
cut-offs, temperatures close to usual weather conditions, and delays compatibles with mailing
of samples by post to a central analysis center, as it is the case in FOBT-based screening
programs.

Our results demonstrate better analytical and stability performances of OC Sensor or
Magstream compared to FOB Gold. Comparison between OC Sensor and Magstream was
made more complex since: 1/ the area of optimal performance was different for both tests,
OC Sensor detecting smaller hemoglobin concentrations, 2/ the relationship between
measurement of OC Sensor and Magstream was not linear. The improved stability offered by
OC Sensor compared to Magstream, and the semi-quantitative nature of the Magstream offer
strong arguments in favor of OC Sensor. Nevertheless, several studies have demonstrated the
good performance of Magstream in population surveys, even using only one sample at the
manufacturer’s threshold (17-18). Costs of the tests should also be considered, together with
adaptation of screening programs to limit the effect of sensitivity of tests to temperature on
their performances (12). Further studies and cost-effectiveness analysis are needed to compare
Magstream and OC Sensor in real screening settings, appropriately taking into account temperature and duration of storage.

ACKNOWLEDGEMENTS

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REFERENCES


**Table 1**  Technical comparison of the immunochemical fecal occult blood tests

<table>
<thead>
<tr>
<th></th>
<th>OC Sensor</th>
<th>FOB Gold</th>
<th>Magstream</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Buffer volume</strong>*</td>
<td>2ml</td>
<td>1.7ml</td>
<td>1ml</td>
</tr>
<tr>
<td><strong>Fecal sampling volume</strong>*</td>
<td>10mg</td>
<td>10mg</td>
<td>0.3mg</td>
</tr>
<tr>
<td><strong>Antibodies used</strong></td>
<td>Rabbit anti-human HbA (polyclonal)</td>
<td>Rabbit anti-human HbA (polyclonal)</td>
<td>Rabbit anti-human HbA (polyclonal)</td>
</tr>
<tr>
<td><strong>Proven analytical range</strong></td>
<td>50 to 1,000ng/ml (equivalent to 10 to 200 μg/g)</td>
<td>50 to 1,000ng/ml</td>
<td>from 20 ng/ml (MSR=1) (commercialized as a qualitative test)</td>
</tr>
<tr>
<td><strong>Reading technique</strong></td>
<td>Anti-human Hb antibodies are adsorbed on latex particles. In the presence of blood in stools they provoke the antigen-antibody reaction and agglutination, consequently referred to as the latex agglutination test. Changes in sample turbidity by latex agglutination is measured optically.</td>
<td></td>
<td>Magnetic gelatin particles are attached to anti-human Hb antibodies. Collecting tubes are titled 60° from the horizontal position, enabling free magnetic particles to slide down the slope of the well, thus forming a measurable line. The higher the presence of human Hb, the shorter the line is. A digital picture of the line is taken by the machine.</td>
</tr>
<tr>
<td>Absorbance 570 nm</td>
<td>Absorbance 660 nm</td>
<td></td>
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<td>-------------------</td>
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<td></td>
<td></td>
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<tr>
<td>Collecting tube</td>
<td>FOB Gold</td>
<td>OC-Auto sampling bottle3</td>
<td></td>
</tr>
<tr>
<td>Automated analyzer</td>
<td>SENTiFOB</td>
<td>Diana</td>
<td></td>
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<tr>
<td>Rates of reading</td>
<td>75 tubes / hour</td>
<td>280 tubes / hour</td>
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<tr>
<td>Usual threshold</td>
<td>175 ng Hb / ml in the buffer</td>
<td>100 ng Hb / ml in the buffer</td>
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</table>

- Adjustable threshold due to quantitative nature of the test.
- Theoretically non adjustable threshold since the test is commercialized as a qualitative test by the manufacturers.

* taken from documents provided by the manufacturer
Table 2  Mean measurement according to test*concentration*temperature*delay

<table>
<thead>
<tr>
<th>Test Concentration of haemo-globin in the feces (µg/g)</th>
<th>OC-Sensor</th>
<th>FOB Gold</th>
<th>Magstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC-Sensor</td>
<td>FOB Gold</td>
<td>Magstream</td>
<td></td>
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<tr>
<td>Concentration of haemo-globin in the feces (µg/g)</td>
<td>0  20  75  100  150  250</td>
<td>0  20  75  100  150  250</td>
<td>0  20  75  100  150  250</td>
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<tr>
<td>Experiment</td>
<td>Temperature</td>
<td>Days</td>
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<tr>
<td>1</td>
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<tr>
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TITLES AND FIGURE LEGENDS:

Figure 1: Value returned by the automated analyzers according to test and fecal hemoglobin concentration

Legend:

Manufacturer’s thresholds are presented as a continuous line (175 ng/ml for FOB Gold; 100 ng/ml for OC Sensor; 211 Pixels for Magstream)

Data modeling is presented as a dotted line (linear modeling: FOB Gold, OCsensor; loess modeling: Magstream)

Figure 2: Variation coefficients due to sampling or reading according to fecal hemoglobin concentration

Legend:

x Variation coefficients due to sampling

◊ Variation coefficients due to reading

• Variation coefficients total

* : Quantification of hemoglobin implied dilution during reading

**: Not calculable

Figure 3: Comparison of mean measurements and standard deviation according to test:

duration of storage
**Figure 4:** Comparison of mean measurements and standard deviation according to test:
temperature of storage

**Figure 5:** Comparison of the evolution of the measured concentration in the buffer
grouping according to storage temperature and duration

**Legend:**
- ○ mean ratio Temperature = 4°C; modelization (— ) linear (OC-Sensor); polynomial (FOB Gold, Magstream)
- Δ mean ratio Temperature = 10°C; modelization (----- ) linear (OC-Sensor); polynomial (FOB Gold, Magstream)
- + mean ratio Temperature = 20°C; modelization (….. ) linear (OC-Sensor); polynomial (FOB Gold, Magstream)
- × mean ratio Temperature = 30°C; modelization ( -.. .) linear (OC-Sensor); polynomial (FOB Gold, Magstream)
ANALYTICAL COMPARISON OF THREE QUANTITATIVE IMMUNOCHEMICAL FECAL OCCULT BLOOD TESTS FOR COLORECTAL CANCER SCREENING

Lydia Guittet, Elodie Guillaume, Romuald Levillain, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst May 16, 2011.

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