

# **Performace of two faecal immunochemical tests in detecting advanced neoplasia at different positivity thresholds: a cross sectional study of the Dutch national colorectal cancer screening programe**

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## ABSTRACT

### Background

Faecal immunochemical tests (FITs) are recommended for colorectal cancer screening. Two frequently used FIT methods (FOB-Gold, Sentinel Diagnostics, Milan, Italy and OC-Sensor, Eiken Chemical, Tokyo, Japan) perform similarly in detecting advanced neoplasia (ie, colorectal cancer and advanced adenoma) at a fixed positivity cutoff for haemoglobin concentration. It is unclear whether the performance of the two methods is comparable at other thresholds. We compared the accuracy of the two assays in detecting advanced neoplasia across various thresholds.

### Methods

In a cross-sectional study in the Dutch national screening programme, individuals who were screening naïve (age 55-75 years) living in the southwest region of the Netherlands were invited to use two different FIT assays on the same bowel movement. Eligible participants were randomly selected from municipal registers. Participants were referred for colonoscopy if either FIT assay result met the predefined positivity threshold ( $\geq 15$   $\mu\text{g}$  haemoglobin per g faeces). We compared the respective distributions of reported hemoglobin concentration and positivity rates with various FIT positivity thresholds. The performance of each FIT for identifying advanced neoplasia at colonoscopy in FIT-positive assays was compared with the area under the receiver operating characteristic curve.

### Results

21,078 (50.0%) of 42,179 invitees completed both FIT assays. The distribution of haemoglobin concentrations differed significantly between the two FITs ( $p < 0.0001$ ), with higher positivity rates for OC-Sensor at FIT thresholds of 5 and 10  $\mu\text{g}$  haemoglobin per g faeces, similar positivity rates at 15 and 20  $\mu\text{g}$  haemoglobin per g faeces, and higher rates for FOB-Gold at FIT thresholds of 25-150  $\mu\text{g}$  haemoglobin per g faeces. 2046 (9.7%) of 21,078 participants had at least one FIT assay that was positive and of these, 1724 (84.3%) attended colonoscopy. The accuracy of results in individuals undergoing colonoscopy did not significantly differ between the FITs, with an area under the receiver operating characteristic curve of 0.675 (95% CI: 0.649 to 0.702) for FOB-Gold and 0.686 (95% CI: 0.661 to 0.712) for OC-Sensor ( $p = 0.40$ ). At identical positivity rates, the positive predictive value of the two FIT assays was similar (difference varying from 0.5% [95% CI -2.6 to 3.7] at a positivity rate of 3.5% to 2.4% [-2.5 to 7.3] at a positivity rate of 2.0%).

### Conclusion

The two widely used FITs have significantly different distributions of reported hemoglobin concentration and yield different positivity rates at equal thresholds. However, they perform similarly in detecting advanced neoplasia at a preset positivity rate. When implementing

either FIT in a screening programme, the desired positivity rate that identifies participants to be referred for colonoscopy should first be set, guided by available resources and feasibility.

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## INTRODUCTION

Faecal immunochemical tests (FITs) are the recommended non-invasive test of choice for population-based colorectal cancer screening in Europe.<sup>1, 2</sup> Compared with the guaiac faecal occult blood test, FITs have a higher sensitivity in detecting colorectal cancer and advanced adenoma (ie, advanced neoplasia), especially at low haemoglobin concentrations, and provides a quantitative measurement of the faecal haemoglobin concentration.<sup>2, 3</sup> The quantitative measurement enables screening programmes to choose a positivity threshold that balances a high diagnostic yield (minimising the number of false-negative FIT results) while limiting the proportion of negative colonoscopies (minimising the number of false-positive FIT results), taking resource constraints, capacity, and costs into account.<sup>4</sup>

At least four quantitative FIT assays are now available for colorectal cancer screening and evidence for their diagnostic performance is increasing.<sup>5</sup> A large paired comparative study showed that two of the most widely used FITs, FOB Gold (Sentinel Diagnostics, Milan, Italy) and OC-Sensor (Eiken Chemical, Tokyo, Japan), had a similar yield in detecting advanced neoplasia at a fixed threshold of 15 µg hemoglobin per g faeces.<sup>6</sup> It remains unknown whether this equivalence in yield extends to other positivity thresholds. This information could be useful to enable the appropriate FIT threshold to be adopted in a screening programme that uses a different positivity threshold than previously studied, or in screening programmes in which a different threshold is considered.

In a previous trial (n=12 054), in which participants were randomly allocated to either a FOB-Gold or an OC-Sensor test, different positivity rates for the two tests were found at a threshold of 10 µg haemoglobin per g faeces. However, positive predictive values for the detection of advanced neoplasia were similar when the FITs were compared at identical positivity rates.<sup>7</sup> We aimed to evaluate this finding in a substantially larger study with a paired design, and to explain the probable reasons for differences in positivity rates. We compared the full haemoglobin distributions of the two FIT assays, the positivity rates at equal thresholds, the performance in detecting advanced neoplasia, and the positive predictive values at identical positivity rates.

## METHODS

### Study population and design

This large, cross-sectional, cohort study was done between May 10, 2016 and March 1, 2017 within the Dutch national colorectal cancer screening programme. Our study was designed to compare the diagnostic yield of advanced neoplasia in two FIT assays at a fixed faecal haemoglobin concentration threshold. The structure of the Dutch national colorectal cancer screening programme and the design of this study have previously been

described in detail.<sup>6,8</sup> In short, a random selection was made of screening naive individuals, eligible for screening (aged 55 to 75 years) in 2016 and living in the southwest region of the Netherlands including the provinces of Zuid-Holland and Zeeland. Individuals were eligible based on their home address and age. The random selection was made with use of a computer run algorithm (SPSS version 23). All selected individuals were sent an invitation, a consent form and two FIT assays (FOB-Gold and OC-Sensor) by post. Invitees were asked to provide a sample for both FIT assays in the same bowel movement. Participants sent the used FITs and the consent form, including sampling date, to one accredited centralized laboratory (Starlab-MDC, Rotterdam,

Netherlands) in a sealed pre-paid envelope. The study was approved by the Dutch National Health Council (Population Screening Act; publication no. 2015/09) and registered in the Dutch Trial Registry (NTR5874). All study participants gave written informed consent.

### Procedures

The FOB-Gold tests were measured using a Bio Majesty JCA-BM6010/C analyser and the OC-Sensor tests by the OC-Sensor Diana analyser. Details of the analysis have been previously described.<sup>6</sup> Quantitative results for both tests were provided in ng haemoglobin per mL buffer and converted into µg haemoglobin per g of faeces for the purpose of this study.<sup>9</sup> All OC-Sensor FIT concentrations greater than 200 µg haemoglobin per g faeces were reported as more than 200 µg haemoglobin per g faeces, but FOB-Gold FIT concentrations were reported without an upper limit.

Participants were informed about their FIT results (negative or positive) by post and were invited for a precolonoscopy interview if either of both of the FIT concentrations exceeded the preset positivity threshold of 15 µg haemoglobin per g faeces. During the precolonoscopy interview, participants were excluded from colonoscopy if they had a life expectancy of 5 years or less, a proctocolectomy in the past, were under current treatment for colorectal cancer, had a history of inflammatory bowel disease, or had undergone a complete colonoscopy in the past 5 years.<sup>10</sup>

Colonoscopies were done in one of the population screening certified colonoscopy centers by accredited endoscopists who do at least 200 colonoscopies a year with an adenoma detection rate of 30% or more. All endoscopically identified lesions were reported using an automated structured colonoscopy reporting system. Removed lesions were sent for pathology review in separate containers.<sup>11</sup> Adenomas of 10 mm or more, with 25% or more villous component, or high-grade dysplasia, or a combination, were defined as advanced adenoma. Advanced neoplasia was defined as advanced adenoma or colorectal cancer.

Each participant was classified according to the most advanced lesion detected. Logistics were done in accordance with the Dutch colorectal cancer screening quality guidelines.<sup>8</sup>

### Statistical analysis

Socioeconomic status was assessed with the Dutch area social status score and was grouped into quintiles. These scores are a composite measure, including education, income and employment status developed by the Netherlands Institute of Social Research.<sup>12</sup> Participation rate was calculated as the number of participants who returned at least one FIT relative to the number of invitees. Not included in this paired analysis were participants who returned one or two FITs that were not analysable (due to faecal overload, buffer loss, missing barcode or another technical problem), who had one or two unreliable test results (if the return date was more than 6 days after sampling or if the sampling date was missing), or participants in whom one test was missing.

The reported faecal haemoglobin concentration of each FIT assay was reflected in a cumulative distribution function. We evaluated the difference between the two curves with a two-sample Kolmogorov-Smirnov test statistic, using a permutation test to calculate the p value while accounting for the paired nature of the data.<sup>13</sup>

To further evaluate the positivity rates of each FIT at different thresholds, the reported haemoglobin concentrations were examined with use of potential thresholds with 5 µg haemoglobin per g faeces increments (5, 10, 15, and so on, up to 150 µg haemoglobin per g faeces). Positivity rates were calculated by dividing the number of positive tests by the total number of participants who completed two tests. Absolute differences in positivity rate between FOB-Gold and OC-Sensor were calculated with corresponding 95% CIs using a Wald interval with Bonett-Price adjustment.<sup>14</sup>

The performance of the two FITs in detecting advanced neoplasia was evaluated in all participants who attended colonoscopy after one or two positive FIT results by calculating the area under the receiver operating characteristic curve (AUC). We compared the AUC using the method of DeLong and colleagues.<sup>15</sup>

The positive predictive value was defined as the proportion of participants with a positive FIT result in whom advanced neoplasia was identified at colonoscopy. Absolute differences in positive predictive values between FOB-Gold and OC-Sensor were also compared at equal positivity rates (2-6%); 95% CIs were calculated with use of a Wald interval with Bonett-Price adjustment. Calculated p values were two-sided and differences were considered significant if p values were less than 0.05. The study sample size was based on the comparison of the diagnostic yield for the two FITs, the initial objective of the study.<sup>6</sup>

SPSS (version 23) was used for statistical analyses, except for the permutation test, which was run in R (version 3.5.1).

### **Role of funding source**

The Netherlands Organization for Health Research and Development of the Dutch Ministry of Health (ZonMw) funded this study but was not involved in the study design; in the data collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. CMdK, EW, IL-V, PMB and ED had access to the raw data and ED had final responsibility for the decision to submit for publication.

## **RESULTS**

### **Study participants**

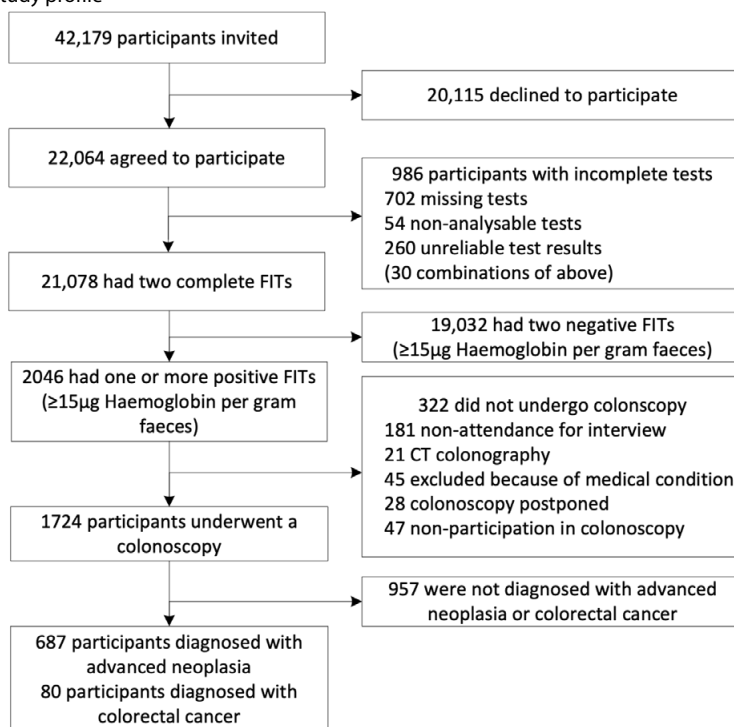
Of 42,179 invitees, 22,064 (52.3%) participated in the study and 21,078 (50.0%) completed both FITs (Figure 1). The 986 (4.5%) participants with incomplete FIT results had either returned only one test ( $n=702$ ), one or two non-analysable tests ( $n=54$ ), had one or two unreliable test results ( $n=260$ ); some participants also had a combination of these ( $n=30$ ), and were excluded from the paired analyses. The proportion of participants with complete tests was equal between women and men (10,489 [49.8%] vs 10,589 [50.2%]). The median age of participants was 60 years (IQR 58 to 62) and socioeconomic status was classified as very low in 15.9%, low in 23.3%, average in 18.4%, high in 22.1%, very high in 20.0% and missing in 0.2% of the participants.

### **Distributions of Hb concentrations**

Figure 2 shows the cumulative distributions of reported haemoglobin concentration for FOB-Gold and OC-Sensor, including the FIT results of all 21,078 participants that completed two tests. The two distributions differed significantly ( $p<0.0001$ ), with the difference most marked at lower concentrations. In 18,438 (87.5%) of the FOB-Gold tests, no haemoglobin was detected, resulting in a median haemoglobin concentration of 0.0  $\mu\text{g}$  haemoglobin per g faeces (IQR 0.0 to 0.0). With the OC-sensor test, 8070 (38.3%) test results reporting 0.0  $\mu\text{g}$  haemoglobin per g faeces with a median haemoglobin concentration of 0.5  $\mu\text{g}$  haemoglobin per g faeces (IQR 0.0 to 2.2). The maximum reported haemoglobin concentration with FOB-Gold was 285  $\mu\text{g}$  haemoglobin per g faeces. OC-Sensor test results were capped at 200  $\mu\text{g}$  haemoglobin per g faeces; 282 participants (1.3%) had an OC-Sensor test result exceeding this cap.

### **Positivity rates of both FIT at potential thresholds**

As a direct consequence of the difference in haemoglobin distribution, positivity rates at each threshold was different for FOB-Gold and OC-Sensor (Figure 3, Supplementary Table 1). The positivity rate of FOB-Gold was 4.8% lower than that of OC-Sensor at a haemoglobin

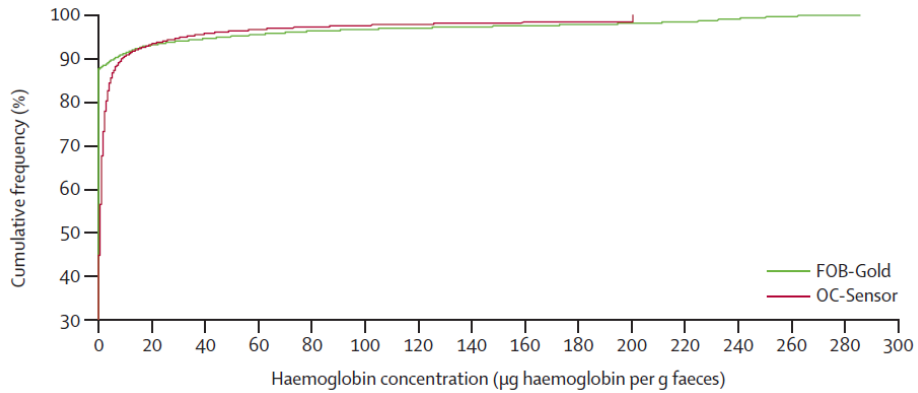
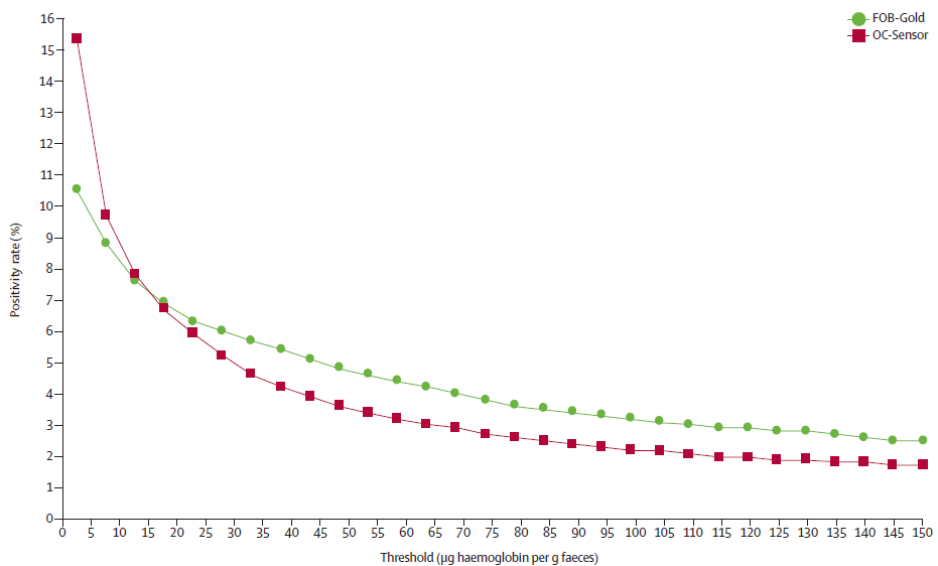
**Figure 1** Study profile

concentration threshold of 5 µg haemoglobin per g faeces (95%CI: -5.2% to -4.4% ) and 0.9% lower at threshold of 10 µg haemoglobin per g faeces (95%CI: -1.2% to -0.6% ). Similar positivity rates were found at thresholds of 15 µg haemoglobin per g faeces (difference -0.3%; 95%CI: -0.5% to 0.0%) and 20 µg haemoglobin per g faeces (0.2%; 95%CI: -0.1% to 0.4%). A higher positivity rate of FOB-Gold than OC-Sensor was found at thresholds 25 µg haemoglobin per g faeces (0.4%; 95%CI: 0.2% to 0.7%) up to 150 µg haemoglobin per g faeces (0.8%; 95%CI: 0.6% to 0.9%; Figure 3).

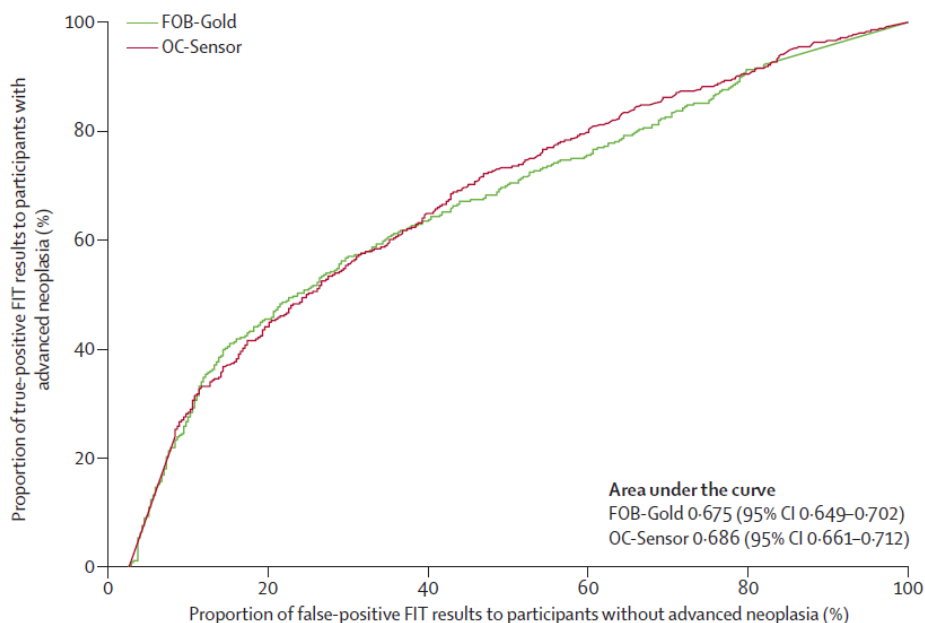
### Performance of both FIT in detecting advanced neoplasia at equal positivity rates

2046 (9.7%) of 21,078 participants had at least one positive FIT, of whom 1724 (84.3%) attended colonoscopy. Discrimination in participants with one or two true-positive FIT results (at 15 µg haemoglobin per g faeces) was not significantly different for FOB-Gold and OC-Sensor with an AUC of 0.675 (95%CI: 0.649 to 0.702) for FOB-Gold and 0.686 (95%CI: 0.661 to 0.712) for OC-Sensor ( $p=0.40$ ; Figure 4). This finding is reflected in the similar positive predictive value to detect advanced neoplasia at equal positivity rates for both FIT assays (Figure 5, Supplementary Table 2). The difference in positive predictive value between FOB-Gold and OC-Sensor varied from 0.5% (95%CI: -2.6% to 3.7%) at a



**Figure 2** Cumulative distributions of reported haemoglobin concentrations with FOB-Gold and OC-Sensor**Figure 3** Positivity rates of FOB-Gold and OC-Sensor at potential thresholds

positivity rate of 3.5%, to a maximum difference of 2.4% at a positivity rate of 2.0% (95%CI: -2.5% to 7.3%).

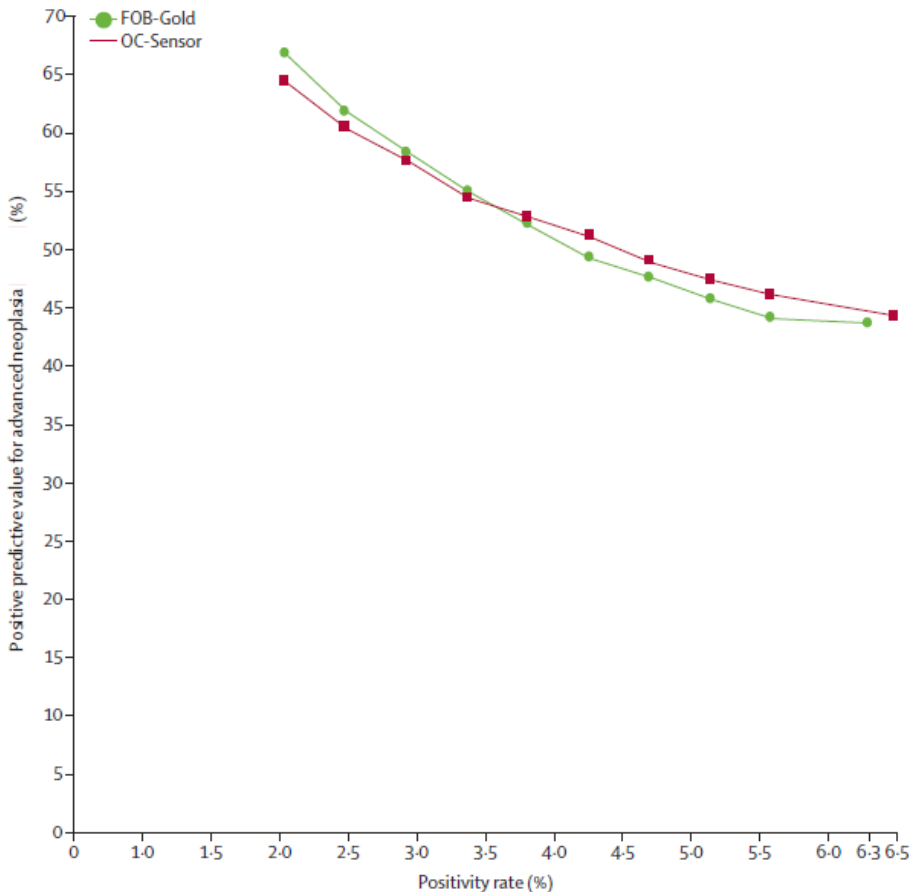
**Figure 4** Accuracy of FOB-Gold and OC-Sensor in detecting advanced neoplasia at colonoscopy

## DISCUSSION

We compared reported haemoglobin concentrations with two FITs in over 20,000 participants undergoing colorectal cancer screening who completed both tests in the same bowel movement. Although the haemoglobin concentrations and related positivity rates per haemoglobin threshold within paired samples of FOB-Gold and OC-Sensor tests differed, their capacity to identify participants with advanced neoplasia at colonoscopy was similar when compared at identical positivity rates. This finding implies that FOB-Gold and OC-Sensor are equally accurate in the detection of advanced neoplasia in organised colorectal cancer screening, if the haemoglobin positivity threshold is standardised to yield the same positivity rate.

When interpreting our results, some limitations have to be considered. Because we did not have informed consent for invitees who did not participate in the study, no demographic data were available for these individuals. Participants with two FIT results of less than 15 µg haemoglobin per g faeces were not invited for colonoscopy, so we could not evaluate the false negative rates and the discriminatory performance of FOB-Gold and OC-Sensor in the total study group. We could not calculate estimates of sensitivity and specificity and our estimates of AUC do not reflect the full accuracy of the two tests. About half of the invitees agreed to participate and to complete two tests in this study. This participation is lower than the participation rate of 73% in the national programme, with one test.<sup>8</sup> Although the

**Figure 5** Positive predictive value for advanced neoplasia at fixed positivity rates for FOB-Gold and OC-Sensor



study group represents an unselected sample of the intended use population, we cannot exclude selection bias. However, this bias is unlikely to influence our results regarding the comparative performance of the two tests. Although this investigation was a large, paired study, producing valid comparisons, the nature of the Dutch FIT-based colorectal cancer screening programme is such that the positivity rates and predictive values reported here are not unconditionally applicable to other screening settings, populations, and individuals who are not screening naïve. We are aware that manufacturers might adjust their products in the future, which could affect the transferability of our results over time.<sup>16</sup>

This study found a significant difference in reported haemoglobin concentration between two types of FIT, even though participants were requested to sample the two FITs on the same day and in the same bowel movement. The exact reasons for this difference are

not entirely clear. A different method of FIT analysis and calibration, a different buffer composition, antibody specificity, or the settings of the analyser, such as maximum concentration reported (maximal 200 µg haemoglobin per g faeces for OC-Sensor and no maximum for FOB-Gold), might have affected the precise differences in haemoglobin distributions. These differences and the fact that the precision of FIT is limited at low haemoglobin concentrations could also explain the wider range of haemoglobin concentrations in the lower limits of OC-Sensor in contrast to FOB-Gold. The limit of detection for FOB-Gold is reported by the manufacturer to be 15 ng haemoglobin per mL buffer (approximately 2.5 µg haemoglobin per g faeces) versus 20 ng haemoglobin per mL buffer (approximately 4 µg haemoglobin per g faeces) for OC-Sensor.<sup>2</sup> As recently discussed by Fraser and colleagues, statements about the accuracy of FITs at low faecal haemoglobin concentrations should therefore be interpreted with caution, as well as statements based on very detailed FIT haemoglobin concentration results.<sup>17</sup>

The difference in haemoglobin concentrations between FITs could also be attributed to the different sampling devices and techniques of the FIT. As instructed by the manufacturer, the FOB-Gold probe should be inserted into the faeces at four different places, whereas the OC-sensor should be scraped through the faeces. Blood, if present, is usually not evenly distributed through the faeces, which might influence the amount of haemoglobin measured by each FIT. Therefore, differences in haemoglobin distribution and positivity rate are also probable if two of the same FIT assays are used to sample the same faeces sample.

The selected positivity rate varies across countries and even across screening regions, typically aiming at a high diagnostic yield while limiting the proportion of negative colonoscopies, and taking resource constraints, capacity, and costs into account.<sup>18</sup> Several studies have evaluated FIT positivity thresholds and reported on the corresponding positivity rate for in different screening programmes. In the USA, 20 µg haemoglobin per g faeces is mostly used;<sup>19</sup> in France 20-30 µg haemoglobin per g faeces was selected;<sup>20</sup> in Germany the selected threshold ranges between 9 and 25 µg haemoglobin per g faeces;<sup>21</sup> and in Thailand, 25 µg haemoglobin per g faeces was recommended because colonoscopy capacity is limited.<sup>22</sup> In Scotland, because of scarce colonoscopy capacity, a threshold of 80 µg haemoglobin per g faeces was chosen, resulting in a low FIT positivity rate of 2.4%, similar to the positivity rate with the previously used guaiac faecal occult blood test.<sup>3</sup> In England, a threshold of 120 µg haemoglobin per g faeces is considered for implementation. whereas Wales is expected to launch its screening programme with a threshold of 150 µg haemoglobin per g faeces.<sup>23, 24</sup>

The accuracy of these two widely used FITs has previously been shown to be similar at a positivity threshold of 15 µg haemoglobin per g faeces.<sup>6</sup> Our study supports this equal accuracy for other thresholds, provided the positivity rate is adjusted for. The positivity rate was similar at 15 µg haemoglobin per g faeces but would have differed if a higher or a lower positivity threshold had been selected for the two FITs. These results suggest a stepwise process for selecting the FIT positivity threshold in a screening programme. The developers of such a programme could first select the desired positivity rate or predictive value. Then, data from this study or from similar studies that reflect the intended setting and population could be used to select a preliminary positivity threshold. In a pilot study, the developers could invite screening participants for colonoscopy on the basis of a slightly lower threshold. Then the positivity rate and positive predictive value could be evaluated for the preliminary threshold. The threshold could then be modified, if necessary, on the basis of the desired positivity rate or predictive value. The opposite approach, starting with a higher threshold and increasing the sensitivity from there, could be an alternative approach. Participation rates in the pilot study could be used to further evaluate the burden on colonoscopy services, potentially leading to further modifications of the threshold. In the Netherlands, for example, the threshold was raised from 15 µg to 47 µg haemoglobin per g faeces 6 months after the implementation of the national programme, because the positivity rate was higher than expected, as the oldest screenees were invited first.<sup>8</sup>

At present, more stratified or personalised screening strategies are being explored with positivity thresholds that differ across population subgroups.<sup>25-28</sup> A higher incidence of advanced neoplasia in men than in women and in individuals eligible for screening of an older age than of a younger age, for example, leads to higher positivity rates but also to higher proportion of missed diagnosis in these subgroups, if the same threshold is used for all individuals. Changing the threshold for specific subgroups could then lead to positive predictive values that are similar across subgroups, if so desired. However, before implementation of sex or age specific thresholds is considered, the actual consequences should first be evaluated in the intended screening setting and population.

This large trial, implemented within an organised colorectal cancer screening programme, showed that despite the significant differences in the distributions of reported haemoglobin, FOB-Gold and OC-Sensor can be considered exchangeable based on their performance in detecting advanced neoplasia if they are standardized at the same positivity threshold.

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**Supplementary Table 1** Differences in positivity rates of FOB-Gold and OC-Sensor at potential thresholds

Threshold (µg Hb/g)	FOB-Gold positive (n)	FOB-Gold positive (%)	OC-Sensor positive (n)	OC-Sensor positive (%)	Absolute difference (%)	95%CI
5	2210	10.5	3219	15.3	-4.8	-5.2 to -4.4
10	1864	8.8	2051	9.7	-0.9	-1.2 to -0.6
15	1597	7.6	1651	7.8	-0.3	-0.5 to 0.0
20	1452	6.9	1418	6.7	0.2	-0.1 to 0.4
25	1336	6.3	1250	5.9	0.4	0.2 to 0.7
30	1261	6.0	1096	5.2	0.8	0.6 to 1.0
35	1194	5.7	974	4.6	1.0	0.8 to 1.3
40	1135	5.4	893	4.2	1.2	0.9 to 1.4
45	1075	5.1	821	3.9	1.2	1.0 to 1.4
50	1017	4.8	763	3.6	1.2	1.0 to 1.4
55	975	4.6	721	3.4	1.2	1.0 to 1.4
60	921	4.4	679	3.2	1.2	0.9 to 1.4
65	877	4.2	640	3.0	1.1	0.9 to 1.3
70	834	4.0	607	2.9	1.1	0.9 to 1.3
75	798	3.8	578	2.7	1.9	1.7 to 2.1
80	758	3.6	553	2.6	1.0	0.8 to 1.2
85	738	3.5	523	2.5	1.0	0.8 to 1.2
90	716	3.4	511	2.4	1.0	0.8 to 1.2
95	690	3.3	490	2.3	1.0	0.8 to 1.1
100	668	3.2	472	2.2	0.9	0.8 to 1.1
105	650	3.1	454	2.2	0.9	0.8 to 1.1
110	633	3.0	439	2.1	0.9	0.8 to 1.1
115	619	2.9	426	2.0	0.9	0.7 to 1.1
120	603	2.9	412	2.0	0.9	0.7 to 1.1
125	589	2.8	403	1.9	0.9	0.7 to 1.1
130	580	2.8	392	1.9	0.9	0.7 to 1.1
135	566	2.7	387	1.8	0.9	0.7 to 1.0
140	551	2.6	376	1.8	0.8	0.7 to 1.0
145	535	2.5	365	1.7	0.8	0.7 to 1.0
150	518	2.5	357	1.7	0.8	0.6 to 0.9

**Supplementary Table 2** Differences in positive predictive value for advanced neoplasia at fixed positivity rates for FOB-Gold and OC-Sensor

Positivity rate (%)	FIT positive (n)	AN FOB-Gold (n)	(%)	AN OC-Sensor (n)	(%)	Absolute difference (%)	95% CI
2.0	422	283	67.1	273	64.7	2.4	-2.5 to 7.3
2.5	527	327	62.1	320	60.7	1.3	-2.8 to 5.4
3.0	632	370	58.5	365	57.8	0.8	-2.8 to 4.3
3.5	738	407	55.1	403	54.6	0.5	-2.6 to 3.7
4.0	843	440	52.2	446	52.9	-0.7	-3.4 to 2.0
4.5	949	469	49.4	487	51.3	-1.9	-4.4 to 0.6
5.0	1056	503	47.6	517	49.0	-1.3	-3.7 to 1.0
5.5	1156	529	45.8	548	47.4	-1.6	-3.9 to 0.6
6.0	1265	559	44.2	583	46.1	-1.9	-4.0 to 0.2
6.3	1331	583	43.8	-	-	-	-
6.5	1363	-	-	604	44.5	-	-