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Project Title: Utility of Fecal Immunochemical Test vs. Guaiac Fecal Occult Blood Test for Assessment of Gastrointestinal Bleeding Among Hospitalized Patients

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SUMMARY: (no more than 250 words single spaced)

Background: Guaiac fecal occult blood testing (gFOBT), is currently available for "off-label" use in hospitalized patients. We hypothesized that a newer version of the FOBT (Fecal Immunochemical Test, FIT) which has been replacing gFOBT in the outpatient setting, can be used to replace gFOBT in the inpatient setting as well.

Methods: FIT was introduced and performed alongside gFOBT on all inpatient stool specimens at Health Sciences Centre (HSC) sent for occult blood testing to clinical biochemistry laboratory from February 2014 to February 2015. Retrospective chart review was performed.

Results: 134 patient charts were reviewed, with 155 Day 1 gFOBT results, 184 Day 3 gFOBT results and 180 FIT results. Positivity rates were greater for Day 1 and Day 3 gFOBT (28% and 25% respectively), than for FIT (18%). FIT was found to have high concordance with gFOBT for negative test results (96% and 94%), but lower concordance for positive test results (56% and 56%). Agreement between FIT and gFOBT was lower than agreement between the two times of gFOBT, and was lowest for the indication of Melena (79%). 30% of patients with a positive Day 3 gFOBT received endoscopy. Of the 20 patients that received endoscopy, 16 patients (80%) had endoscopy performed prior to the release of the FOBT results to clinicians.

Conclusions: FIT cannot be used interchangeably with gFOBT in the inpatient setting. However, overall FOBT has limited utility for inpatients, and should only be used for colon cancer screening.

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Introduction

Fecal occult blood testing (FOBT) is used as a screening test for Colorectal Cancer (CRC), which has led to a decrease in incidence and mortality of CRC when used in conjunction with additional testing strategies (such as colonoscopy) (1–3). However, FOBT is often used "off-label" in the inpatient setting to detect the presence of a gastrointestinal (GI) bleed in symptomatic patients (4,5). In this case, FOBT is used to assist clinical judgement, in order to determine whether additional diagnostic testing/interventions (such as gastrointestinal endoscopy) should be performed. However, the criteria used for FOBT in the outpatient screening setting may not be suitable for the inpatient population, as the inpatient population often requires both emergency ordering of the test, as well as rapid test result availability (5–9). As it stands, the current standards for FOBT in place in Manitoba laboratories may not meet these criteria.

Today there are many types of FOBT on the market. Hospital laboratories in Manitoba currently employ the Guaiac FOBT (gFOBT; Hemocult Sensa II), which detects the peroxidase-like activity of heme within stool (2,10). However, this property is also present in non-human sources of hemoglobin as well as plant peroxidases, which can lead to false positive test results (10). As such, gFOBT requires that the patient undergoes dietary restriction of foods containing these factors (such as red meats, and certain raw fruits and vegetables) for 3 days prior to stool specimen collection. As an extra precaution or alternatively, development of the gFOBT is held for 3 days after arrival at the laboratory, to allow for degradation of the plant peroxidases. In addition, certain medications have been shown to interfere with the gFOBT, such as high doses of vitamin C, which can lead to a false negative test result (11). Also, as occult GI bleeding (such as bleeding due to CRC) may be intermittent, the sensitivity of an individual gFOBT is relatively poor. As a result, the gFOBT requires multiple sampling (3 stool specimens over 3 consecutive days) to improve sensitivity. However, multiple sampling, patient dietary and medication restriction, and time lag until test result availability reduce the utility of gFOBT in the inpatient setting.

The Fecal Immunochemical Test (FIT) is a relatively new version of FOBT. FIT uses antibodies specific for human-globin chain content in stool, and therefore is less likely to be affected by plant peroxidases, non-human sources of hemoglobin, and medications (2,4,12). FIT has been found to have greater sensitivity than gFOBT for CRC screening, and therefore does not require multiple sampling to accurately detect GI blood loss (13). However, FIT is a more expensive assay than gFOBT, and is subject to extremes in temperature. In addition, the utility of FIT in assessing an upper GI bleed has been questioned, as hemoglobin is degraded by stomach acid during its passage along the GI tract (2,14). Despite these issues, FIT is thought to be of greater utility than gFOBT in the outpatient setting, and has been adopted by CRC screening programs in several Canadian provinces, including a pilot project in Manitoba's ColonCheck program (3,15–17).

The aim of this study was to compare the utility of the FIT and gFOBT assays in hospitalized patients. Due to the apparent strengths of FIT over gFOBT (one time sampling, reduced

requirement for dietary/medication restriction, and reduced time until test result availability), we hypothesized that FIT would be of equal or greater utility than gFOBT for the inpatient population, which could lead to potential replacement of gFOBT in all settings by FIT.

Materials & Methods

Study Design

In order to compare FIT to gFOBT (Hemoccult Sensa II, Beckman Coulter Inc.), a commercial quantitative FIT (OC-Auto Micro 80 by Eiken Chemical Company Ltd., distributed by Polymedco/Somagen Diagnostics Inc.) was introduced to the Diagnostic Services of Manitoba (DSM) laboratory at Health Sciences Centre. During the study period from February 2014 to February 2015, all inpatient stool specimens collected at HSC that were sent for occult blood were automatically assigned a FIT assay in addition to the usual gFOBT. For these specimens, the gFOBT was performed alongside FIT on Day 1 (ie. the day that the specimen arrived to the DSM lab). The stool specimen was also applied to a second gFOBT card on Day 1, which was held until development on Day 3, to allow for the degradation of plant peroxidases from the specimen (as per DSM laboratory guidelines). The FIT and Day 3 gFOBT results were then entered into the laboratory information system and released to the ordering physician simultaneously after the Day 3 gFOBT results became available. The Day 1 gFOBT results were recorded in a study binder in the DSM laboratory, and were not released to the ordering physician. FIT results were recorded as a quantitative result in ng hemoglobin (hb)/mL, while gFOBT results were recorded as a qualitative (positive/negative) result. In cases where the FIT result exceeded the manufacturer's recommendation for the upper limit of accurate hemoglobin detection (>1000 ng hb/mL), and where the numerical value was not available, the FIT result was recorded as >1000 ng hb/mL.

Retrospective chart review was performed on patients that had at least one FIT and one Day 3 gFOBT result available. Study data collected during chart review included: patient demographic information, details and indications related to the FOBT order, patient medication status, patient laboratory values prior to FOBT specimen collection, details related to patient management, and patient endoscopy details. The indications for the FOBT order were determined using information in the progress notes in the patient chart, as well as the patient's most recent clinical laboratory values within 48 hours prior to stool specimen collection. The indication of anemia included patients that had hemoglobin values below the lower limit of the normal range, or where anemia was documented in the patient progress notes. The indication of overt GI bleeding included patients that experienced coffee ground emesis, hemeatemesis, hematochezia, or bright red blood per rectum prior to stool specimen collection. The indication of GI symptoms included patients that experienced abdominal pain, diarrhea, dyspepsia, nausea, or vomiting prior to stool specimen collection. The indication of melena included patients that experienced a black stool prior to or during stool specimen collection. All other indications involved information explicitly documented in the progress notes in the patient chart.

Data Analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at The University of Manitoba (18). Standard descriptive statistical analysis was performed to describe the data. The positivity rates and concordance (performance of the assay) between Day 1 gFOBT, Day 3 gFOBT, and FIT results were compared using contingency tables. The percent agreement between each type of FOBT, including the percent agreement based on the indications for FOBT order, was determined. Cohen's kappa coefficient was calculated as a measure of the agreement between each type of FOBT. In patients that received an endoscopy, only endoscopies performed within 7 days prior to or up to 90 days post stool specimen collection were included in analysis, in order to relate the FOBT results to the endoscopy findings. Patient endoscopies that identified findings considered to be high-risk for gastrointestinal bleeding (such as gastrointestinal malignancy, arteriovenous malformation, inflammatory bowel disease, mass, stricture, ulcer, esophagitis, varices, and active bleeding) were considered positive for further analysis. For patients that submitted multiple stool specimens for fecal occult blood testing, each specimen was compared to endoscopy results and included in analysis. As well, patients with multiple endoscopy results were considered to have an overall positive endoscopy result if they contained at least one finding suggestive of GI bleeding. The patient endoscopy data was compared between the Day 1 gFOBT, Day 3 gFOBT, and FIT results using contingency tables. The True Positive Rate (TPR) and False Positive Rate (FPR) for Day 1 gFOBT, Day 3 gFOBT, and FIT were determined in patients that received an endoscopy. All of the above analyses utilized the manufacturer's recommended value of ≥ 100 ng hb/mL as the cut-off for a positive FIT outcome, which corresponds to ± 20 µg hb/g feces (19). In addition, the outcomes for FIT were also evaluated at cut-off concentrations less than or greater than the manufacturer's recommended value using Receiver Operating Characteristic (ROC) analysis. ROC curves were generated by plotting sensitivity against 1-specificity of FIT results, using gFOBT results as a "gold standard" outcome.

All statistical and graphical analysis was performed using Stata version 11.2MP software (20).

The research study was approved by the University of Manitoba Health Research Ethics Board on November 27, 2013 (Ethics # *H2013:398*).

Results

Patient Demographics and Management

During the study period, 134 patient records were reviewed. Each patient was unique (ie. only had one hospital admission included in the study) and had at least one Day 3 gFOBT and at least one FIT result available. Of all patients included in analysis, 104 patients (77.6%) had one stool specimen collection sent for FOBT, 11 patients (8.2%) had two stool specimen collections sent for FOBT, 18 patients (13.4%) had three stool specimen collections sent for FOBT, and 1 patient (0.8%) had four stool specimen collections sent for FOBT throughout the course of their hospital encounter. This yielded 155 Day 1 gFOBT results, 184 Day 3 gFOBT results, and 180 FIT results for analysis. 14 patients (10.5%) did not have any Day 1 gFOBT results

available. Overall, the patient cohort was 58.2% male, and patient age on admission ranged from 0 (newborn) to 94.5 years old (median age of 49.8 years). While 21 patients (15.7%) were found to be NPO (nothing by mouth) status at the time of stool specimen collection, 0 patients (0%) were found to have a diet restriction (of substances known to cause a false positive or negative result) initiated 72 hours prior to at least one stool specimen collection for FOBT. In addition, 2 patients (1.5%) were found to be receiving medications (vitamin C) that could have caused a false negative gFOBT result, at the time of stool specimen collection.

Of the 134 patients included in analysis, 28 patients (20.9%) were found to have at least one positive FIT result, 39 patients (29.1%) were found to have at least one positive Day 1 gFOBT result, and 37 patients (27.6%) were found to have at least one positive Day 3 gFOBT result. Of the patients with at least one positive FIT result, 7 (25.0%) underwent endoscopy within 7 days prior to or up to 90 days post stool specimen collection. Similarly, of the patients with at least one positive Day 1 or Day 3 gFOBT result, 11 (28.2% and 29.7% for Day 1 and Day 3 gFOBT respectively) underwent endoscopy within the same time frame. Of all patients that received an endoscopy (20), 4 patients (20.0%) had endoscopy performed after the release of the FIT and Day 3 gFOBT results. The remaining 16 patients (80.0%) had endoscopy performed prior to FOBT stool specimen collection, or following stool specimen collection but prior to Day 3 gFOBT result availability.

Positivity and Concordance (Performance of the assay) of Day 1 gFOBT, Day 3 gFOBT and FIT

Table 1 illustrates the outcomes of the Day 1 gFOBT, Day 3 gFOBT, and FIT assays performed. Of the FOBTs performed, 43 of all Day 1 gFOBTs were positive (27.7% positivity), 45 of all Day 3 gFOBTs were positive (24.5% positivity), and 33 of all FITs were positive (18.3% positivity).

Figure 1 illustrates the concordance between the Day 1 gFOBT, Day 3 gFOBT and FIT results. Comparison of FIT and gFOBT results show a high degree of concordance for negative results (96.4% and 94.1% for Day 1 and Day 3 gFOBT respectively), with a lesser degree of concordance for positive results (55.8% and 55.6%). Comparison of Day 1 and Day 3 gFOBT results show high degree of concordance for negative results (91.5%), as well as positive results (86.8%).

Table 2 illustrates the agreement between the Day 1 gFOBT, Day 3 gFOBT, and FIT results. Agreement between FIT and gFOBT was found to be 85.0% ($\kappa = 0.5838$, $p<0.001$) and 84.4% ($\kappa = 0.5447$, $p<0.001$) (for Day 1 and Day 3 gFOBT respectively), while agreement between Day 1 and Day 3 gFOBT was found to be 90.3% ($\kappa = 0.7497$, $p<0.001$).

Indications for FOBT Order and Stool Specimen Collection

Table 3 illustrates the indications for FOBT ordering. The most common indications for the ordering of FOBT included anemia (41.3%), overt GI bleeding (16.1%), melena (14.3%), GI symptoms (13.6%), and non-bloody diarrhea (6.3%). Less common indications for the ordering of FOBT included iron deficiency (1.8%), CRC screening (1.8%), and FOBT ordering prior to initiating anticoagulants (0.4%).

Also indicated in *Table 3* are the positivity rates for Day 1 gFOBT, Day 3 gFOBT, and FIT based on the indications for FOBT ordering. The indications that yielded the highest positivity rates for Day 1 gFOBT included: melena (46.7%), overt GI bleeding (34.1%), anemia (33.7%), iron deficiency (20.0%), and GI symptoms (18.5%). The indications that yielded the highest positivity rates for Day 3 gFOBT included: melena (46.3%), anemia (29.7%), overt GI bleeding (26.1%), GI symptoms (20.5%), and iron deficiency (20.0%). Finally, the indications that yielded the highest positivity rates for FIT included: melena (34.2%), overt GI bleeding (24.4%), anemia (20.2%), and GI symptoms (15.8%).

Table 4 indicates the agreement between FOBT results based on the indications for FOBT ordering. Comparison of FIT to both Day 1 and Day 3 gFOBT yielded similar rates of agreement, with the indications for FOBT showing the greatest agreement between the tests being: GI symptoms (88.9% and 89.5% for Day 1 and Day 3 gFOBT respectively), overt GI bleeding (86.9% and 88.9%), anemia (80.7% and 84.2%), and melena (79.3% and 79.0%) ($p<0.001$ for each comparison). In addition, agreement between FIT and both Day 1 and Day 3 gFOBT was 80.0% for the indication of iron deficiency, although this was not statistically significant. In contrast, comparison of Day 1 and Day 3 gFOBT by indication for FOBT order showed the highest rates of agreement for the indications of: iron deficiency (100%, $p<0.05$), melena (96.7%, $p<0.001$), GI symptoms (96.3%, $p<0.001$), anemia (91.6%, $p<0.001$), and overt GI bleeding (88.6%, $p<0.001$).

Figure 2 illustrates the concordance between the Day 1 gFOBT, Day 3 gFOBT and FIT results, for stool specimens sent for occult blood testing based on the indication of melena. Comparison of FIT and gFOBT results show a high degree of concordance for negative results (100% and 94.7% for Day 1 and Day 3 gFOBT respectively), with a lesser degree of concordance for positive results (57.1% and 63.2%). However, comparison of Day 1 and Day 3 gFOBT results show high degree of concordance for negative results (94.1%), as well as positive results (100%).

Receiver Operating Characteristics Analysis

Figure 3 illustrates the ROC curves of FIT compared to Day 1 and Day 3 gFOBT. The overall agreement between FIT and gFOBT, using all FIT result values as a cut-off for a positive FIT outcome, was greatest between FIT and Day 1 gFOBT (Area under the curve = 0.8358), compared to FIT and Day 3 gFOBT (Area under the curve = 0.7824). The individual FIT cut-offs that showed the greatest agreement between FIT and Day 1 gFOBT (85.6% agreement) occurred at values of 98, 137, and 358 ng hb/mL. In contrast, the individual FIT cut-offs that showed the greatest agreement between FIT and Day 3 gFOBT (85.0% agreement) occurred at values of 156, 216, 295, 358, 584 ng hb/mL.

Endoscopy Findings

Of the 134 patients included in analysis, 20 patients (14.9%) received an endoscopy within 7 days prior to or up to 90 days following stool specimen collection for FOBT. Of these, 2 patients (10.0%) had 2 different types of endoscopy performed on the same day (gastroscopy/colonoscopy and gastroscopy/flexible sigmoidoscopy), the findings of which were

combined into a single endoscopy result for each patient. Of the 20 patients that received an endoscopy, 9 (45.0%) were considered to have a positive endoscopy result, due to findings that were considered high-risk for GI bleeding. Of the 20 patients that received an endoscopy, 11 (55.0%) had a positive Day 1 gFOBT, 11 (55.0%) had a positive Day 3 gFOBT, and 7 (35.0%) had a positive FIT result.

Table 5 illustrates the FOBT results and endoscopy findings for patients with a positive endoscopy result. The most common findings identified in positive endoscopies include: ulcer (33.3%), stricture (13.3%), and esophagitis (13.3%). Less common findings identified in positive endoscopies include: active bleeding (6.7%), mass (6.7%), gastroesophageal cancer (6.7%), esophageal varices (6.7%), Dieulafoy's lesion (6.7%), and granularity, friability, loss of vascular pattern secondary to Mycobacterium Avium-Intracellulare infection (6.7%).

Figure 4 illustrates the comparison of Day 1 gFOBT, Day 3 gFOBT, and FIT results with the endoscopy results. Day 1 gFOBT was determined to have a True Positive Rate (TPR) of 90.0% and a False Positive Rate (FPR) of 30.8%. Day 3 gFOBT was determined to have a TPR of 58.3% and a FPR of 31.8%. Finally, FIT was determined to have a TPR of 30.0% and a FPR of 25.0%.

Discussion

Current guidelines recommend that FOBT should be used as a screening test for CRC in the outpatient population (21). The goal of FOBT in this setting is to identify asymptomatic people who are at risk of developing CRC, in the hopes of intervening in the disease process to ultimately reduce the risk of morbidity and mortality. An effective screening test (such as FOBT) would therefore be expected to be sensitive to the disease being screened for, be easy to administer, and be of low cost burden to the health care system. Sensitivity is of particular importance in this case, in order to ensure that all individuals that are suspected of having the disease are identified, as patients that receive a false negative test result may go unrecognized and may not receive the necessary intervention. In contrast, the goal of FOBT performed in the inpatient population is to identify patients with acute or significant GI bleeding, which may be life threatening. In this case, the results from the FOBT may play a key role in determining the clinical course for the patient, and may influence many aspects of patient care, such as the ordering of follow-up testing and interventions, as well as changes in medication orders (4). Due to the favourable performance of FIT over gFOBT in the outpatient screening setting, FIT is becoming the screening test of choice for CRC. However, the use of FIT in the inpatient setting has yet to be explored.

The aim of our study was to compare the utility of FIT and gFOBT in the inpatient setting. We found that the positivity rates for both gFOBT (27.7% and 24.5% for Day 1 and Day 3 gFOBT respectively) and FIT (18.3%) were similar to positivity rates reported by others for gFOBT and FIT (similar to the assays used in the study), but greater than the positivity rates found in the outpatient screening setting (2,22). This is expected, as FOBT performed in the

inpatient setting is used to assess patients with suspected GI bleeding who are more likely to have a positive FOBT result.

FIT and gFOBT show high concordance for negative results (96.4% and 94.1% for Day 1 and Day 3 gFOBT respectively), but much lower concordance for positive results (55.8% and 55.6%). In addition, there are a significant number of cases in which there is a positive gFOBT result, but a negative FIT result (44.2% and 44.4%). This could be due to false positive gFOBT results, due to the higher specificity of FIT. However, comparative study with a gold standard investigation, such as endoscopy will need to be performed to determine if that indeed is the case.

As previous studies have shown that FIT may be of questionable utility in assessing upper GI bleeding, we examined the results for FOBTs performed for the indication of melena (14). We found that the positivity rates of gFOBT (46.7% and 46.3% for Day 1 and Day 3 gFOBT respectively) were greater than the positivity rates of FIT (34.2%) in these cases. As well, FIT and gFOBT show high concordance for negative results (100% and 94.7%), but much lower concordance for positive results (57.1% and 63.2%), in FOBTs performed for the indication of melena. These findings support the previous concerns on using FIT to assess an upper GI bleed.

Gastrointestinal endoscopy was considered the gold standard investigation to determine whether GI bleeding was present in the study population. Overall, 20 patients (14.9%) were found to have received an endoscopy within 7 days prior to or up to 90 days post stool specimen collection for FOBT. This date range for endoscopy was chosen in order to relate the FOBT results with endoscopy results for a given event of suspected GI bleeding. Patients that underwent endoscopy were found to have a positivity rate of 45.0%. The True Positive Rate of gFOBT (90.0% and 58.3% for Day 1 and Day 3 gFOBT respectively) was found to be greater than the TPR of FIT (30.0%). Likewise the False Positive Rate of gFOBT (30.8% and 31.8% Day 1 and Day 3 gFOBT respectively) was found to be greater than the FPR of FIT (25.0%). However, it should be noted that the TPR and FPR determined in this study are based only on patients who received an endoscopy. However, in this study, the majority of patients (85.1%) did not receive an endoscopy. Furthermore, the decision to pursue endoscopy in a given patient was left to the judgment of the clinician overseeing patient care, which was likely influenced by other factors of the patient's clinical presentation. Therefore, the TPR and FPR of gFOBT and FIT determined in this study are not reflective of the study patient population.

Some studies have suggested that diet may not have a significant impact on gFOBT, and have questioned whether the gFOBT requires a 72 hour hold prior to development. However, prior to our study there was no data comparing Day 1 and Day 3 gFOBT for the Hemoccult Sensa II assay. In order to assess this, gFOBT was performed on Day 1 and Day 3 after laboratory arrival for each stool specimen sent for occult blood testing. We found that Day 1 and Day 3 gFOBT perform similarly, and show high concordance for negative and positive test results (91.5% and 86.8% for negative and positive results respectively). As such, if DSM is to continue

using gFOBT, it may be reasonable to remove the requirement for a 3 day hold prior to gFOBT development.

Our data indicates that FOBT may not be useful for symptomatic inpatients. Previous studies have shown that FOBT is being ordered inappropriately in the inpatient setting (5,7–9). Sharma et al recommended that FOBT only be used in hospital on patients that are suitable candidates for CRC screening, who should be identified at discharge and instructed on the proper performance of FOBT at home (5). However, Ip et al have shown that some physicians surveyed in Winnipeg hospitals believe that gFOBT is beneficial when ordered for the indications of anemia or melena (23). We found that of the 20 patients who received endoscopy, 16 patients (80%) received endoscopy prior to the release of the FOBT results. This indicates that the FOBT results are not the main factor in determining whether endoscopy should be pursued in symptomatic patients and are not impacting positively on patient management.

In our study, FIT results were not released to the ordering physicians prior to the Day 3 gFOBT results. Therefore, we cannot determine accurately if and how the FIT results affected the management of the study patient population.

Conclusions

Although FIT provides practical aspects that favour its introduction into hospital settings (lack of requirement of patient dietary restriction, one-time sampling, shorter time until test result availability, and improved specificity for human hemoglobin) it may not offer any further benefit than what is currently offered by gFOBT. This is supported by the fact that FIT is more expensive than gFOBT, and that FIT is not as sensitive in detecting upper GI bleeding. Our study suggests there is no benefit in holding development of the gFOBT Hemoccult Sensa II assay. Importantly, since FOBT does not seem to positively impact symptomatic inpatient management in our and prior studies, it should not be available for use outside of the current guidelines for CRC screening.

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Appendix - Tables and Figures

Table 1: Positivity rates of Day 1 gFOBT, Day 3 gFOBT, and FIT assays.

FIT assay is considered positive for values ≥ 100 ng hb/mL buffer.

Test	Positive Results	Negative Results	Total
Day 1 gFOBT	43 (27.7%)	112 (72.3%)	155 (100%)
Day 3 gFOBT	45 (24.5%)	139 (75.5%)	184 (100%)
FIT	33 (18.3%)	147 (81.7%)	180 (100%)

Fig. 1A.

FIT Result	Day 1 gFOBT Result		
	Positive	Negative	Total
Positive	24 (55.8%)	4 (3.6%)	28 (18.3%)
Negative	19 (44.2%)	106 (96.4%)	125 (81.7%)
Total	43 (100%)	110 (100%)	153 (100%)

Fig. 1B.

FIT Result	Day 3 gFOBT Result		
	Positive	Negative	Total
Positive	25 (55.6%)	8 (5.9%)	33 (18.3%)
Negative	20 (44.4%)	127 (94.1%)	147 (81.7%)
Total	45 (100%)	135 (100%)	180 (100%)

Fig. 1C.

Day 1 gFOBT Result	Day 3 gFOBT Result		
	Positive	Negative	Total
Positive	33 (86.8%)	10 (8.6%)	43 (27.7%)
Negative	5 (13.2%)	107 (91.5%)	112 (72.3%)
Total	38 (100%)	117 (100%)	155 (100%)

Figure 1: Comparison of FOBT results.

FIT assay is considered positive for values ≥ 100 ng hb/mL buffer.

A) FIT vs Day 1 gFOBT

B) FIT vs Day 3 gFOBT

C) Day 1 gFOBT vs Day 3 gFOBT

Table 2: Agreement between FOBT results.

FIT assay is considered positive for values ≥ 100 ng hb/mL buffer.

Test	Agreement	Expected Agreement	Kappa Coefficient	P-value
FIT vs Day 1 gFOBT	85.0%	63.9%	0.5838	0.0000
FIT vs Day 3 gFOBT	84.4%	65.8%	0.5447	0.0000
Day 1 gFOBT vs Day 3 gFOBT	90.3%	61.3%	0.7497	0.0000

Table 3: Comparison of indications for FOBT order and corresponding test results for Day 1 gFOBT, Day 3 gFOBT, and FIT.

FIT assay is considered positive for values ≥ 100 ng hb/mL buffer.

Indication	Day 1 gFOBT Results			Day 3 gFOBT Results			FIT Results		
	Pos	Neg	Total	Pos	Neg	Total	Pos	Neg	Total
Anemia[1]	32 (43.8%) [33.7%]	63 (39.4%) [66.3%]	95 (40.8%) [100%]	35 (42.7%) [29.7%]	83 (40.7%) [70.3%]	118 (41.3%) [100%]	23 (40.4%) [20.2%]	91 (41.4%) [79.8%]	114 (41.2%) [100%]
Iron Deficiency	1 (1.4%) [20.0%]	4 (2.5%) [80.0%]	5 (2.2%) [100%]	1 (1.2%) [20.0%]	4 (2.0%) [80.0%]	5 (1.8%) [100%]	0 (0.0%) [0.0%]	5 (2.3%) [100%]	5 (1.8%) [100%]
Non-Bloody Diarrhea	0 (0.0%) [0.0%]	15 (9.4%) [100%]	15 (6.4%) [100%]	0 (0.0%) [0.0%]	18 (8.8%) [100%]	18 (6.3%) [100%]	0 (0.0%) [0.0%]	18 (8.2%) [100%]	18 (6.5%) [100%]
CRC Screening	0 (0.0%) [0.0%]	5 (3.1%) 100%]	5 (2.2%) 100%]	0 (0.0%) [0.0%]	5 (2.5%) [100%]	5 (1.8%) [100%]	0 (0.0%) [0.0%]	5 (2.3%) [100%]	5 (1.8%) [100%]
Melena	14 (19.2%) [46.7%]	16 (10.0%) [53.3%]	30 (12.9%) [100%]	19 (23.2%) [46.3%]	22 (10.8%) [53.7%]	41 (14.3%) [100%]	13 (22.8%) [34.2%]	25 (11.4%) [65.8%]	38 (13.7%) [100%]
Prior to Initiating Anticoagulants	0 (0.0%) [N/A]	0 (0.0%) [N/A]	0 (0.0%) [N/A]	0 (0.0%) [0.0%]	1 (0.5%) [100%]	1 (0.40%) [100%]	0 (0.0%) [0.0%]	1 (0.5%) [100%]	1 (0.4%) [100%]
Overt GI Bleeding[2]	15 (20.6%) [34.1%]	29 (18.1%) [65.9%]	44 (18.9%) [100%]	12 (14.6%) [26.1%]	34 (16.7%) [73.9%]	46 (16.1%) [100%]	11 (19.3%) [24.4%]	34 (15.5%) [75.6%]	45 (16.3%) [100%]
GI Symptoms[3]	5 (6.9%) [18.5%]	22 (13.8%) [81.5%]	27 (11.6%) [100%]	8 (9.8%) [20.5%]	31 (15.2%) [79.5%]	39 (13.6%) [100%]	6 (10.5%) [15.8%]	32 (14.6%) [84.2%]	38 (13.7%) [100%]
Other[4]	6 (8.2%) [50.0%]	6 (3.8%) [50.0%]	12 (5.2%) [100%]	7 (8.5%) [53.8%]	6 (2.9%) [46.2%]	13 (4.6%) [100%]	4 (7.0%) [30.8%]	9 (4.1%) [69.2%]	13 (4.7%) [100%]
Total	73 (100%) [31.3%]	160 (100%) [68.7%]	233 (100%) [100%]	82 (100%) [28.7%]	204 (100%) [71.3%]	286 (100%) [100%]	57 (100%) [20.6%]	220 (100%) [79.4%]	277 (100%) [100%]

The proportion of FOBTs ordered for each indication and the corresponding result is presented as (%) running in columns.
The proportion of each FOBT result out of the total tests performed for a particular indication is presented as [%] running in rows.

[1] Includes patients that had hemoglobin values below the normal range within 48 hours prior to FOBT stool specimen collection.

[2] Includes patients that experienced: Coffee ground emesis, Hemeatemesis, Hematochezia, and Bright Red Blood Per Rectum.

[3] Includes patients that experienced: Abdominal pain, Diarrhea, Dyspepsia, and Nausea/Vomiting.

[4] Includes the following indications:

- Differentiate between menstrual blood or GI bleed
- Patient had previous positive gFOBT result but negative FIT result from the same sample - 2 more FOBT samples were sent for FOBT
- Previous history of upper GI bleed
- Occasional blood on peri-anal area
- Stool contained frothy mucus
- Patient experienced "red jelly-like" stool
- Patient experienced BM with "red flecks"
- Newborn experienced ?bloody BM - unsure if bloody stool or blood from the mother during delivery

N/A - Indicates scenario where proportion could not be calculated.

Table 4: Percent agreement between FOBT results based on indication for FOBT order.

FIT assay is considered positive for values ≥ 100 ng hb/mL buffer.

Indication	FIT and Day 1 gFOBT Results			FIT and Day 3 gFOBT Results			Day 1 and Day 3 gFOBT Results		
	% Agreement	Expected agreement	P-value	% Agreement	Expected agreement	P-value	% Agreement	Expected agreement	P-value
Anemia [1]	80.7%	59.6%	0.0000	84.2%	61.5%	0.0000	91.6%	56.7%	0.0000
Iron Deficiency	80.0%	80.0%	N/A	80.0%	80.0%	N/A	100.0%	68.0%	0.0127
Non-Bloody Diarrhea	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CRC Screening	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Indication (continued)	FIT and Day 1 gFOBT Results (continued)			FIT and Day 3 gFOBT Results (continued)			Day 1 and Day 3 gFOBT Results (continued)		
	% Agreement	Expected agreement	P-value	% Agreement	Expected agreement	P-value	% Agreement	Expected agreement	P-value
Melena	79.3%	50.8%	0.0003	79.0%	50.0%	0.0001	96.7%	50.4%	0.0000
Prior to Initiating Anticoagulants	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Overt GI Bleeding[2]	86.1%	57.4%	0.0000	88.9%	61.9%	0.0000	88.6%	57.2%	0.0000
GI Symptoms[3]	88.9%	72.2%	0.0008	89.5%	69.8%	0.0000	96.3%	67.5%	0.0000
Other[4]	83.3%	50.0%	0.0072	61.5%	48.5%	0.1539	75.0%	50.0%	0.0395

[1-4] See legend of Table 3 for details.

N/A - Indicates scenario where percent agreement, expected agreement, or p-value could not be calculated.

Fig. 2A.

FIT Result	Day 1 gFOBT Result		
	Positive	Negative	Total
Positive	8 (57.1)	0 (0.0%)	8 (27.6%)
Negative	6 (42.9%)	15 (100%)	21 (72.4%)
Total	14 (100%)	15 (100%)	29 (100%)

Fig. 2B.

FIT Result	Day 3 gFOBT Result		
	Positive	Negative	Total
Positive	12 (63.2%)	1 (5.3%)	13 (34.2%)
Negative	7 (36.8%)	18 (94.7%)	25 (65.8%)
Total	19 (100%)	19 (100%)	38 (100%)

Fig. 2C.

Day 1 gFOBT Result	Day 3 gFOBT Result		
	Positive	Negative	Total
Positive	13 (100%)	1 (5.9%)	14 (46.7%)
Negative	0 (0.0%)	16 (94.1%)	16 (53.3%)
Total	13 (100%)	17 (100%)	30 (100%)

Figure 2: Comparison of results of FOBT ordered for Melena.

FIT assay is considered positive for values ≥ 100 ng hb/mL buffer.

A) FIT vs Day 1 gFOBT

B) FIT vs Day 3 gFOBT

C) Day 1 gFOBT vs Day 3 gFOBT

Fig. 3A.

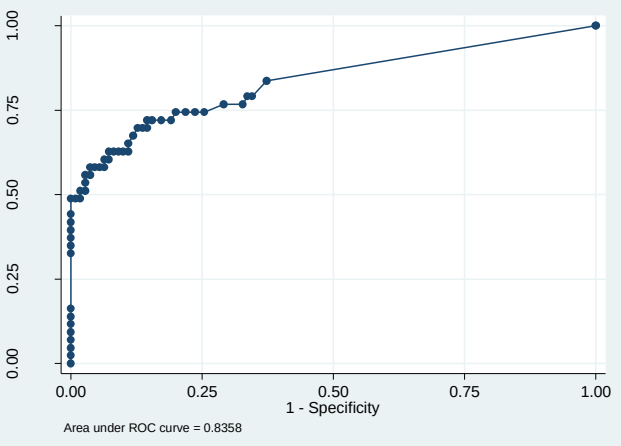

Fig. 3B.

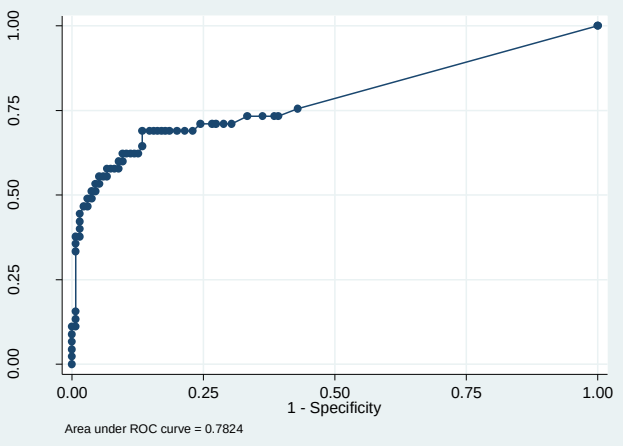

Figure 3: Receiver Operating Characteristics plot of FIT results vs gFOBT results.

A) FIT vs Day 1 gFOBT B) FIT vs Day 3 gFOBT

Table 5: Summary of FOBT Results and Endoscopy Findings in Patients with a Positive Endoscopy Result.

Patient ID	Day 1 gFOBT Result	Day 3 gFOBT Result	FIT Result (ng hb/mL)	Days Between Stool Specimen Collection and Endoscopy[1]	Endoscopy Findings
53	Positive	Positive	2209	1	Esophageal varices at GE junction
33	Negative	Negative	0	-4	Esophagitis
116	Positive	Positive	0	-1	Active bleeding Ulcer Esophagitis
27	Positive	Positive	0	-6	Mass Stricture, Gastroesophageal cancer
79	Positive	Positive	216	5	Ulcer Dieulafoy's lesion
9	Positive	Positive	0	-5	Ulcer
56	Positive	Positive	941	2	Granularity, friability, loss of vascular pattern secondary to Mycobacterium Avium Intracellulare infection
42	Positive	Negative	26	1	Stricture Ulcer
136	N/A N/A	Negative Negative	0 0	4 3	Ulcer

[1] A positive result indicates the number of days following stool specimen collection until endoscopy was performed.
A negative result indicates the number of days that endoscopy preceded stool specimen collection.
N/A - Indicates Day 1 gFOBT results not available.

Fig. 4A.

Day 1 gFOBT Result	Endoscopy Result: Positive	Negative	Total
Positive	9 (90.0%)	4 (30.8%)	13 (56.5%)
Negative	1 (10.0%)	9 (69.2%)	10 (43.5%)
Total	10 (100%)	13 (100%)	23 (100%)

Fig. 4B.

Day 3 gFOBT Result	Endoscopy Result: Positive	Negative	Total
Positive	7 (58.3%)	7 (31.8%)	14 (41.2%)
Negative	5 (41.7%)	15 (68.2%)	20 (58.8%)
Total	12 (100%)	22 (100%)	34 (100%)

Fig. 4C.

FIT Result	Endoscopy Result: Positive	Negative	Total
Positive	3 (30.0%)	5 (25.0%)	8 (26.7%)
Negative	7 (70.0%)	15 (75.0%)	22 (73.3%)
Total	10 (100%)	20 (100%)	30 (100%)

Figure 4: Comparison of endoscopy and FOBT results.

FIT assay is considered positive for values ≥ 100 ng hb/mL buffer.

A) Day 1 gFOBT
B) Day 3 gFOBT
C) FIT