Analytical and Clinical Performance of Kroma iT, A Compact Fully-automated Immunochemistry Analyzer for Fecal Occult Hemoglobin

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Abstract. Background: We performed a laboratory and clinical evaluation of Kroma iT (Linear Chemicals S.L), an immunoturbidimetric analyzer for the detection of fecal occult blood. Materials and Methods: After a familiarization period, the imprecision, linearity of dilution and carry-over were determined and a clinical evaluation was performed on 210 patients. Results: Within-run imprecision ranged between 1.06% and 8.04%. Between-run imprecision ranged between 3.11% and 13.09%. Linearity of dilution revealed a mean recovery of all dilutions of 95.24%, with a standard deviation of 7.47%. No carry-over was detected. The clinical evaluation demonstrated that the mean hemoglobin levels of the fecal immunochemical test values from patients with advanced neoplasms (colorectal cancer plus advanced adenoma) were significantly higher than those of cases with a normal colonoscopy examination. Sensitivity for advanced neoplasms at cut-off values between 80 and 300 ng/ml (6.4-24 mg Hb/Kg feces) ranged from 45.5% to 36.4% and the specificity ranged from 86.8% to 92.3%. The positive predictive values for detecting colorectal cancer and advanced adenomas were 5.4-6.4% and 27-34% respectively and the negative predictive value ranged from 92.5% to 91.7%. Using two samples per patient, a substantial increase of sensitivity was observed, with only a slight decrease in specificity. Conclusion: Kroma iT analyzer is easy to handle and safe for personnel to use. Its analytical and clinical performance makes it suitable for use in a clinical chemistry laboratory for the early detection of advanced neoplasms.

Worldwide, one million people each year will develop colorectal cancer (CRC) and the incidence of this tumor is increasing. In industrialized countries, CRC is the third most common malignancy in men and the second in women (1). Screening for CRC and its pre-malignant lesions can identify the disease at an earlier stage. Fecal occult blood tests (FOBT) have been developed to detect otherwise undetectable bleeding from colorectal neoplasms before there are any clinical signs or symptoms. Different trials have proved the effectiveness of FOBT screening, demonstrating a reduction in mortality of 15-33% (2-4) and several screening trials have confirmed the superiority of fecal immunochemical test (FIT) screening over the more traditionally used guaiac-based FOBT, both with respect to attendance, as well as the detection rate of advanced neoplasms (ACRN) (5-10). The latest generation FIT provides a quantitative measurement of microscopic blood loss in stool that allows for selection of an optimal cut-off (6, 11). In the present study, a new FIT assay for the detection of fecal hemoglobin was evaluated for its analytical and clinical performance.

Materials and Methods

Patients. A total of 210 consecutive patients at Hospital Clinic (Barcelona) who required colonoscopy for the investigation of gastrointestinal symptoms or colonic polyp surveillance were recruited for this study. Patients with a previously positive FOBT, history of known gastrointestinal bleeding, active rectal bleeding, menstruation, hematuria, and known ulcerative colitis were excluded. Patients were asked to begin fecal sampling for the FIT five days before colonoscopy to ensure that two samples were collected before bowel preparation commenced. No dietary restriction was required. Medications such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) were withdrawn one week before preparation for colonoscopy. The study was approved by the Hospital Clinic Ethics Committee (2013/8431), and all examinees provided written informed consent. All participants received an oral or telephone explanation of the tests and written instructions on preparing the FIT. Patients were asked to prepare fecal samples from two consecutive stool specimens using the collection kit provided by the manufacturer (Linear Chemicals S.L. Spain).
Samples. Three sample sources were used: Samples collected by the patients, stabilizing buffer spike with human capillary blood, and control material provided by the manufacturer. To collect the sample, the patient inserts a probe into several different areas of the stool and then re-inserts it firmly into the test tube container to seal it. The probe tip with the fecal sample (approximately 20 mg) is suspended in a standard volume of hemoglobin-stabilizing buffer (approximately 1.6 ml). Samples were stored in double ziplock bags at 4°C until analysis within a maximum of five days.

Analyzer. The instrument used for quantification of the FIT is Kroma i't (Linear Chemicals S.L., Spain, distributed in Spain by Laboratorios LETI, S.L. Unipersonal, Spain) a desktop instrument based on immunoturbidimetry, performing up to 150 tests/h. It is self-contained with reagent, buffer, washing and fluid-disposal bottles, and requires access to a standard power supply. Twenty-seven of the patient-prepared fecal sample tubes are loaded into the sample tray. The instrument automatically mixes the fecal buffer solution with the latex–anti-human hemoglobin antibody reagent. The latex particles coated with anti-human hemoglobin are agglutinated when they react with feces samples containing human hemoglobin. Following the development cycle, there is automatic flushing of the system. Agglutination of the latex particles is proportional to the concentration of the hemoglobin in the sample and can be measured by turbidimetry and compared to that of a standard calibration curve. By applying a conversion factor of 0.08, the concentration of hemoglobin in buffer (ng/ml) is transformed to the concentration of hemoglobin in feces (mg/kg). The range of measurements is 50-1000 ng Hb/ml, approximately equivalent to 4-80 mg Hb/kg feces. All events were performed following the maintenance tasks, calibration and quality controls as recommended by the manufacturer.

Protocol design. An evaluation protocol was designed, including a familiarization phase, and a test phase in which imprecision, linearity of dilution and carry-over were determined. Furthermore, a clinical evaluation was performed to compare the hemoglobin levels for the different colonoscopy findings, including receiver operating characteristic (ROC) curves. The sensitivity, the specificity for different colonoscopy findings, including receiver operating characteristic (ROC) curves. The sensitivity=true positive/(true positive + false negative) and the specificity=true negatives/(true negatives + false positives), and the predictive values for ACRN, CRC and AA at different hemoglobin levels were calculated. ROC curves for one test and two tests were compared using the Delong method (13) and differences in specificity using one or two samples at the same cut-off point were calculated. We calculated Pearson correlation coefficients between each pair of measures and Cohen’s kappa coefficient was calculated to measure the agreement between the first and the second FIT. Statistical analysis was performed using PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA, USA). A significance level of \( p<0.05 \) was regarded as a statistically significant difference between two results.

Results

Analytical Performance.

Test reproducibility. Within-run imprecision of the low control (111.82±1.19 ng/ml), high control (417.00±8.89 ng/ml), spiked buffers (low 69.06±3.68 ng/ml, high 684.64±55.05 ng/ml) and patient samples (263.64±15.77 ng/ml, 718.13±8.67 ng/mL) ranged between 1.06% and 8.04%. Between-run imprecision of the low control (113.92±4.31 ng/ml), high control (412.63±25.30 ng/ml), spiked buffers (low 273.14±8.49 ng/ml, high 798.96±31.07 ng/ml) and patient samples (255.85±15.92 ng/mL, 578.57±75.72 ng/ml) ranged between 3.11% and 13.09%.

Linearity of dilution. Linearity of dilution revealed a mean recovery of all dilutions of 95.24%, with a standard deviation of 7.47% (minimum 85%, maximum 111.4%) (Figure 1).
The mean and standard deviation after low measurements and high measurements was 2.6±3.6 ng/ml and 3±4.5 ng/ml respectively, therefore the intra-assay carry-over met the set requirements.

**Clinical Performance.**

**Patient and colonoscopy results.** Out of 210 patients (103 males, 107 females) with a mean age of 59±15 years colonoscopy detected advanced neoplasms in 25 patients (11.9%). These included three CRCs and 22 AAs. NAAs were found in 39 (18.6%) patients and other lesions such as hyperplastic or inflammatory polyps (n=20), haemorrhoids (n=41), angiodisplasia (n=2), inflammatory bowel disease (n=1) and minor irrelevant lesions (n=5) were found in 85 (40.5%) patients; in 61 (29%) patients, no findings were detected and the colonoscopy was reported as normal.

**FOBT results.** For the entire population undergoing colonoscopy, the mean, standard deviation and median FIT measure of all analyzed samples were 231.8±950.7 and 1.0 (interquartile range: 1.0-14.8) ng/ml respectively. The FIT results according to colonoscopy and pathology diagnosis are provided in Table I. The FIT values from patients with advanced neoplasms (CRC plus AA) were significantly higher than in cases those with a normal colonoscopy examination (Figure 2). The positivity rate at 80, 100, 150, 200, 250 and 300 ng/ml was 17.6%, 15.5%, 15%, 12.6%, 11.6% and 11.4% respectively.

**Sensitivity, specificity and predictive values for advanced neoplasms.** Figure 3 displays the ROC curve for advanced neoplasms obtained with the FIT measurements for each participant. We measured the sensitivity and specificity of the FIT results at different hemoglobin thresholds (Table II). At the 80 ng/ml fecal hemoglobin threshold, the sensitivity and specificity for detecting all advanced neoplasms were 48.0% (95% confidence interval CI=33.7-62.6%) and 86.8% (95%
CI=82.9-90.1%). At the 300 ng/ml fecal hemoglobin threshold, the sensitivity and specificity for detecting all advanced neoplasms were 38.0% (95% CI=24.7-52.8%) and 92.5% (95% CI=89.3-94.9%), respectively. The 80 ng/ml fecal hemoglobin threshold increased sensitivity but reduced specificity. The sensitivity for detecting cancer was considerably higher than that for detecting all clinically-advanced neoplasms. In the studied group, the positive predictive value for detecting CRC and AA was 5.4-6.4% and 27-34%, respectively, and the negative predictive value was 92.5-91.7%.

Diagnostic yield using one or two samples. The correlation coefficient of the first and second FIT was 0.479. These moderate correlations most probably reproduce daily variations in blood loss, otherwise the kappa coefficient showed a substantial agreement between the first and the second FIT. We measured the hemoglobin content of each of two consecutive fecal samples but considered them to represent one test, to which we assigned the highest of the two FIT results. Figure 4 displays the ROC curves for advanced neoplasms obtained with the first, second and the highest of both FIT measurements for each participant. No statistical differences were observed among them. Table III shows the sensitivity and specificity for advanced neoplasms of first, second, the highest and the mean of both FITs at different cut-off values, as well as the kappa coefficient between first and second sample.

Discussion

This colonoscopy-controlled study allowed for a detailed evaluation of an automated desktop instrument for quantitative, immunochemical determination of fecal occult hemoglobin. The test used in the current study is easy to perform, and the results are independent of operator experience. The technical evaluation included study of reproducibility, linearity of dilution and carry-over.

The clinical evaluation in a mixed group of individuals demonstrated at a threshold of 80-300 ng/ml (6.4-24 mg Hb/kg feces) adequate sensitivity for advanced neoplasms of 45.5%-36.4% and acceptable specificity of 86.8%-92.3%. The positivity rate at different cut-offs was quite high compared to the median in risk screening (14, 15) and would lead to an excessive colonoscopy rate. However, the studied population was not a true screening population because all the patients had symptoms or were indicated...
for colonoscopy. From this study, we confirmed that a FIT threshold of 200 ng Hb/mL (16 mg Hb/kg feces) allowed detection of the majority of the carcinomas and 38.6% of AAs, giving together a sensitivity of 41%. It provided an acceptable specificity of 91% and using two samples for each patient and choosing the highest result, the sensitivity for advanced neoplasm increased to 52%, providing a specificity of 87.6%. These results are suitable for screening and are consistent with results from other studies that used immunochemical tests to screen larger populations (16-19).

In conclusion, the current study provides useful data regarding the potential application of automated FIT in screening the population for CRC. The compact fully-automated immunochemistry analyzer evaluated for fecal hemoglobin measurement demonstrates an adequate analytical and clinical performance. We have demonstrated that the Kroma iT assay (Linear Chemicals S.L.) is a robust, specific, and accurate tool for the detection of advanced neoplasm and provides the basis for a large-scale screening program.

Conflicts of Interest

Laboratorios LETI, S.L. Unipersonal and Linear Chemicals, S.L. provided instruments, reagents and technical support.

Potential Financial Conflicts of Interest

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References


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