Fecal occult blood: a comparison of chemical and immunochemical tests

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ABSTRACT – Background – Colorectal bleeding is a warning sign that may be identified by fecal occult blood testing. A positive fecal occult blood test result requires a subsequent colonoscopy, a costly and invasive examination. Therefore, the use of diagnostic tests with optimal sensitivity and specificity is warranted. In this study, we evaluated four different fecal occult blood tests in 176 patients undergoing colonoscopy and compared their results.

Objective – To assess the sensitivity, specificity and predictive values of chemical and immunochemical fecal occult blood tests in patients undergoing colonoscopy and to evaluate the degree of concordance between the tests and colonoscopy.

Methods – Patients with indications for colonoscopy also underwent fecal occult blood testing by chemical (toluidine test) and immunochemical methods, employing three commercially available kits. Based on the endoscopic findings, the colonoscopy was rated as positive or negative for colorectal bleeding. The degree of concordance between the fecal occult blood tests and the colonoscopy was evaluated by the kappa index.

Results – Forty-four (25%) colonoscopies were categorized as positive for colorectal bleeding. The toluidine test presented lower concordance than the immunochemical tests, which showed moderate concordance with the colonoscopy. The toluidine test had the least sensitivity, specificity, and positive and negative predictive values.

Conclusion – The immunochemical fecal occult blood tests showed greater sensitivity, specificity and predictive values in detecting colorectal bleeding. The immunochemical tests had superior indexes of agreement with colonoscopy compared to the toluidine test.


INTRODUCTION

Gastrointestinal tract bleeding, whether evident or occult, is potentially a hazardous symptom or sign and should not be ignored. The differential diagnosis includes benign and malignant conditions. Colorectal cancer (CRC) warrants special diagnostic consideration because it is frequently lethal. Screening programs for populations at average risk for CRC have been recommended strongly by the health organizations of various nations\(^\text{1-3}\). Although the screening strategies vary among the countries in which they are implemented, they invariably include two options: a fecal occult blood test (FOBT) and lower digestive tract endoscopy (rectosigmoidoscopy and/or colonoscopy)\(^\text{2,4-6}\).

FOBTs are widely accepted as non-invasive and highly cost-effective methods of CRC screening. Initially, the guaiac test, which employs a reaction with the heme fraction of hemoglobin, was used for the detection of occult bleeding. Despite its well-documented role in the reduction of CRC mortality\(^\text{5,6}\), this method has low sensitivity and a high false-positive rate. More recently, immunochemical tests, which are specific for the identification of human hemoglobin, have attracted increased interest due to their greater sensitivity in detecting advanced adenomas and neoplasms\(^\text{7,8}\). More recently, fecal testing combining immunochemical tests and multiple DNA markers associated with CRC was introduced with the goal of increasing the sensitivity of detection not just of CRC but also of advanced adenomas\(^\text{9,12}\).

Colonoscopy is considered the “gold standard” diagnostic test for CRC detection\(^\text{10}\); however, it is an invasive procedure that involves risk\(^\text{13,14}\), high cost and requires bowel preparation with restricted diets and strong laxatives\(^\text{11,16}\). Patients with positive FOBT results must undergo a complementary investigation to identify the etiology of the bleeding. Thus, the FOBT method clearly should be sufficiently accurate to prompt further investigation only for those patients with actual colorectal bleeding.

Many laboratories in Brazil are believed to use the guaiac-based test as the principal tool for fecal occult blood detection. Developing countries may also use this “in-house” technique, also called the ortho toluidine (toluidine) test, in which the reagents are applied to the sample in the laboratory.

The purpose of this study was to evaluate the sensitivity, specificity, positive predictive value and negative predictive value of different types of FOBT, including chemical and immunochemical methods, in ambulatory patients undergoing colonoscopy. In the present study, three immunochemical tests and the ortho toluidine test were used in 176 patients who subsequently underwent colonoscopy, and the results were prospectively compared.
METHODS

Subjects

This study was approved by the local Ethics Committee. The inclusion criteria were patients older than 14 years of both genders who had indications for colonoscopy and who attended at the Clinics Hospital of the University of São Paulo Medical School, São Paulo, Brazil. The exclusion criteria were the patients who reported hematochezia or hematuria in the preceding seven days, women who were menstruating and patients who refused to follow a restricted diet (free of tomatoes, radishes, beets, deeply colored liquids and red meat) in the two days preceding the exam. All patients included in the study gave written informed consent and underwent both colonoscopy and FOBT.

FOBT

Feces were collected the day before the colonoscopy. The patients were instructed to evacuate in a dry, clean environment, store the feces sample in a universal collection flask and maintain the sample under refrigeration until delivered. All samples were analyzed using the toluidine test and three commercially available immunochemical tests: BioNexia® Hb/Hp Complex (Dima Diagnostika, Germany), Imuno Rápido Sangue Oculto® (Wama Diagnóstica, Brazil) and Feca-Cult One Step Teste® (Alamar Tecnoscientifica LTDA, Brazil).

All FOBTs were performed by the same investigator, who was blind to the colonoscopy results. The FOBT results were considered positive or negative. Hydrogen peroxide and toluidine were used for the “in-house” FOBT technique. The reaction was considered positive when the initially colorless product developed a dark greenish-blue color and negative when no color change occurred after two minutes (FIGURE 1). The interpretation of the BioNexia® Hb/Hp Complex, Imuno Rápido Sangue Oculto® and Feca-Cult One Step Teste® results were based on the appearance of colored bands provided in the test kit, according to the manufacturer recommendations, with detection of human hemoglobin at concentrations of 25 ng/mL, 50 ng/mL and 40 ng/mL, respectively (FIGURES 2, 3 and 4).
Colonoscopy
Following bowel preparation, the colonoscopies were performed under conscious sedation by experienced endoscopists blind to the FOBT results. The colonoscopy results were categorized as positive or negative according to the respective presence or absence of a colorectal bleeding source.
Colonoscopy was considered positive when the findings were consistent with colorectal bleeding, that is, active inflammatory bowel disease, diverticular disease with bleeding, vascular lesions with signs of bleeding, active mucosal inflammation, polyps ≥10 mm, and early or advanced neoplasia. Colonoscopy was considered negative when the cecum was reached under conditions of adequate bowel preparation and no conditions associated with colorectal bleeding were detected.

Statistical analysis
Statistical analysis of the diagnostic tests was performed using the kappa measure-of-agreement index and PASW Statistics 18. A P value of <0.05 was considered statistically significant.

RESULTS
The study included 114 women (64.8%) and 62 men (35.2%) with a mean age of 55.5 years. Forty-five (25%) colonoscopies were categorized as positive and 132 (75%) were negative according to the criteria regarding the colorectal bleeding source. The correlation between the FOBTs and colonoscopy and their respective kappa values are shown in TABLE 1.

Agreement between each FOBT test and the colonoscopy was evaluated using the kappa statistic. The toluidine test showed slight agreement (kappa 0.00–0.19), whereas the immunochemical tests demonstrated moderate agreement (kappa 0.40–0.59).

The sensitivity, specificity, positive predictive value and negative predictive value for each FOBT test were evaluated separately for colonoscopies, considering positive or negative for a possible source of colorectal bleeding (TABLE 2).

Seventy-two (40.9%) patients had consumed at least one foodstuff that might result in a false-positive toluidine test (TABLE 3). According to the kappa index, the agreement between

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**TABLE 1. Results of FOBTs and colonoscopy categorization.**

<table>
<thead>
<tr>
<th>FOBT</th>
<th>Colonoscopy</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Total</td>
<td>Kappa</td>
</tr>
<tr>
<td>Toluidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>95</td>
<td>24</td>
<td>119</td>
<td>67.6</td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
<td>20</td>
<td>57</td>
<td>32.4</td>
</tr>
<tr>
<td>BioNexia® Hb/Hp Complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>105</td>
<td>14</td>
<td>119</td>
<td>67.6</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>30</td>
<td>57</td>
<td>32.4</td>
</tr>
<tr>
<td>Imuno Rápido Sangue Oculto®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>112</td>
<td>15</td>
<td>127</td>
<td>72.2</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>29</td>
<td>49</td>
<td>27.8</td>
</tr>
<tr>
<td>Feca-Cult One Step Teste®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>110</td>
<td>15</td>
<td>125</td>
<td>71.0</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>29</td>
<td>51</td>
<td>29.0</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>44</td>
<td>176</td>
<td>100.0</td>
</tr>
</tbody>
</table>

FOBT: fecal occult blood test.

**TABLE 2. Sensitivity, specificity, positive predictive value and negative predictive value of the FOBTs.**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Toluidine</th>
<th>BioNexia® Hb/Hp Complex</th>
<th>Imuno Rápido Sangue Oculto®</th>
<th>Feca-Cult One Step Teste®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>45.5%</td>
<td>68.2%</td>
<td>65.9%</td>
<td>65.9%</td>
</tr>
<tr>
<td>Specificity</td>
<td>72%</td>
<td>79.5%</td>
<td>84.8%</td>
<td>83.3%</td>
</tr>
<tr>
<td>PPV</td>
<td>35.1%</td>
<td>52.6%</td>
<td>59.2%</td>
<td>56.9%</td>
</tr>
<tr>
<td>NPV</td>
<td>79.8%</td>
<td>88.2%</td>
<td>88.2%</td>
<td>88%</td>
</tr>
</tbody>
</table>

FOBT: fecal occult blood test; PPV: positive predictive value; NPV: negative predictive value.

**TABLE 3. Toluidine FOBT results in relation to colonoscopy in the group that did not follow the recommended diet.**

<table>
<thead>
<tr>
<th>FOBT</th>
<th>Colonoscopy</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Toluidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>50</td>
<td>11</td>
<td>15.3</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>18.1</td>
<td>12</td>
<td>16.7</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>68.1</td>
<td>23</td>
<td>31.9</td>
</tr>
</tbody>
</table>

FOBT: fecal occult blood test.
the test evaluated and the colonoscopy was considered weak ($P=0.03$; kappa=0.25).

In total, ten patients (5.7%) used antiplatelet drugs, oral anticoagu-
lants or nonsteroidal anti-inflammatory drugs. The interference of
these medicines was analyzed with respect to the results of the
four FOBTs, as shown in Table 4.

**TABLE 4.** Evaluation of the FOBTs in relation to colonoscopy in the

group that used interfering medications.

<table>
<thead>
<tr>
<th>FOBT</th>
<th>FOBT Results</th>
<th>Kappa</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>True-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluidine</td>
<td>6</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.429</td>
</tr>
<tr>
<td>BioNexia® Hb/</td>
<td>5</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Hp Complex</td>
<td>0</td>
<td>10</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.747</td>
</tr>
<tr>
<td>Imuno Rápido</td>
<td>6</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>Sangue Oculto®</td>
<td>0</td>
<td>10</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>Feca-Cult One</td>
<td>6</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>Stop Test®</td>
<td>0</td>
<td>10</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
</tbody>
</table>

FOBT: fecal occult blood test.

No agreement was observed ($P>0.05$) between the FOBT and
colonoscopy results in those patients who used at least one
interfering medication.

**DISCUSSION**

A recent meta-analysis\(^{(22)}\) compared chemical to immuno-
chemical FOBTs. That study included five randomized clinical
trials that enrolled more than 20,000 patients and 11 cohort studies.
Compared with these systematically reviewed data, the toluidine
test in our study showed less sensitivity (45% versus 54%) and
less specificity (72% versus 80%) than the guaiac-based tests.
In contrast, the commercially available immunochemical tests in the
current study showed sensitivity and specificity similar to that
described by Zhu et al. in the meta-analysis\(^{(21)}\). One multicenter study
on nearly 10000 patients comparing fecal DNA test (Cologuard®)
to immunochemical test using guaiac-based as the gold standard
showed that the fecal DNA test had a higher sensitivity detecting
CRC (92% vs. 74%). Fecal DNA test had lower specificity at 87%-90%
compared to immunochemical test (95%-96%)\(^{(12)}\).

Since 1971\(^{(13)}\), dietary restrictions have been recommended
on the days preceding the collection of fecal samples for analy-
sis by guaiac-based tests\(^{(10)}\). However, a systematic review\(^{(19)}\)
reported that no evidence existed to justify the recommendation
of dietary restriction prior to the collection of feces for guaiac-
based method. Regarding the toluidine test in the present study,
the ingestion of peroxidase-rich foodstuffs was associated with
better agreement between the colonoscopy and the fecal test
among those patients who did not follow the dietary restriction
instructions compared to those who did. It is possible that the
quantity ingested may have been insufficient to alter the FOBT
result. Vitamin C, present in the fruit juices used for hydration
during the bowel preparation, probably inhibited the enzymatic
action and interfered with the results. We believe that detailed
information on the daily diet and considering the quantity and
quality of the food might clarify these hypotheses.

In patients using medications with anticoagulant properties,
no agreement was observed between the FOBT and colonoscopy
results, similar to the observations of Clarke et al.\(^{(20)}\). In the present
study, these medicines possibly altered the results of the fecal tests.
This hypothesis requires careful analysis, considering the small
number of study patients using antiplatelet, oral anticoagulant or
nonsteroidal anti-inflammatory drugs.

FOBT effectiveness is well known to depend on the collect-
ion and storage of the feces, the frequency of the bleeding, the
type of test employed and the interpretation of the results. In this
study, the fecal collection method was unlikely to have interfered
with the results, thereby confounding the results of some of the
FOBTs, because all the analyses were performed using the same
fecal sample and the samples that were not adequately stored were
disregarded. Understandably, any lesion identified as a potential
source of hemorrhage may bleed intermittently. Despite the col-
lection of samples within the shortest interval possible in relation
to the colonoscopy, actively bleeding foci were not ensured at the
sample collection time. Notably, whereas the immunochemical
tests have well defined detection limits, the minimal hemoglobin
concentration detectable by the toluidine test is not known. The
interpretation of the toluidine test results may be affected by many
factors, such as the reagent preparation, dilution of the feces, and
skill of the operator reading the results, including the interpretation
of the color obtained. One study showed that considerable varia-
tions in interpretation occurred with the use of a chemical FOBT\(^{(21)}\).

Anal diseases might have influenced the results of the fecal tests.
Primarily, hemorrhoidal bleeding may be a cause of false-positive
FOBT results\(^{(22)}\). We conducted a pilot study involving proctologic
examinations to evaluate those patients with false-positive FOBT
results. However, difficulties in defining the possible sources of
orificial bleeding discouraged us from proceeding with this com-
plementary evaluation.

The main limitation of the present study was defining positive
and negative results of the colonoscopy, which is considered the
“gold standard” method for colorectal disease detection. The cat-
gorization of the colonoscopy results regarding the likely source
of colorectal bleeding is fundamental for the veracity of this study.
Moreover, most publications on FOBT are studies of population
screening, with large sample sizes for the diagnosis of invasive CRC
and advanced adenomas. The current recommendations for CRC
screening by FOBT are the evaluation of three samples for guaiac-
based tests and one or two samples for the immunochemical tests\(^{(22)}\).

**CONCLUSION**

Immunochimical FOBTs were superior in sensitivity, specificity,
positive predictive value and negative predictive value compared to
the toluidine chemical test in evaluating the source of colorectal
bleeding by the analysis of one fecal sample.

The three immunochemical tests used in this study had an ac-
ceptable degree of concordance with the colonoscopy and similar
performance to each other.

Finally, this study examined the practicality of using immuno-
chemical tests in public hospitals in developing countries, where
the likelihood of adequate refrigerated fecal conservation and the
viability of the samples following transport to the hospital are
often unknown.
ACKNOWLEDGMENTS

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Authors’ contribution


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Authors’ contribution


RESUMO – Contexto – O sangramento colorretal é considerado um sinal de alarme e não deve ser ignorado. O resultado positivo de um teste de pesquisa de sangue oculto nas fezes (PSOF) requer investigação complementar com colonoscopia, exame invasivo e de alto custo. Justifica-se, portanto, a aplicação de um teste diagnóstico mais sensível e específico. No presente estudo, foram avaliados quatro diferentes testes de PSOF em 176 pacientes submetidos à colonoscopia e seus resultados foram comparados. Objetivo – Avaliar a sensibilidade, a especificidade e os valores de predição dos testes químico e imunoquímico de PSOF em pacientes submetidos à colonoscopia e avaliar o grau de concordância entre os testes de PSOF e a colonoscopia. Métodos – Pacientes com indicação de realizar colonoscopia foram submetidos também à PSOF pelo método químico (o-toluidina) e pelo método imunoquímico, empregando três kits comerciais disponíveis no mercado. Fundamentado nos achados endoscópicos, a colonoscopia foi categorizada em positiva ou negativa, de acordo com a possível fonte de sangramento colorretal. O grau de concordância entre os testes de PSOF foi avaliado pelo índice kappa. Resultados – Quarenta e quatro (25%) colonoscopias foram categorizadas como positivas quanto à fonte de sangramento colorretal. O teste da o-toluidina revelou menor concordância que os testes imunoquímicos, os quais apresentaram moderada concordância com a colonoscopia. O teste da o-toluidina revelou menor sensibilidade, especificidade, valor preditivo positivo e valor preditivo negativo. Conclusão – Os testes imunoquímicos revelaram maior sensibilidade, especificidade e valores de predição na detecção de sangramento colorretal. Os testes imunoquímicos apresentaram melhores índices de concordância com a colonoscopia, quando comparados ao teste da o-toluidina.