

New Screening Method for Occult Gastrointestinal Bleeding: Immunologic and Guaiac Slide Tests

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A valid mass screening method for occult, bleeding gastrointestinal pathology including colorectal cancer should be (1) monospecific for human hemoglobin, (2) sensitive for approximately 3 mg of human blood per 1 g of stool, (3) capable of differentiating upper and lower gastrointestinal bleeding, (4) cost effective, (5) uncomplicated, and (6) acceptable to patients.

Hemoccult II, a guaiac peroxidase detection test, is non-specific for human blood and cannot differentiate between upper and lower gastrointestinal bleeding. A radial immunodiffusion slide test for detecting human hemoglobin was compared with a guaiac test over a four-year period in 211 patients.

In gastrointestinal problems diagnosed by endoscopy, roentgenographic rays, and other procedures, the Hemoccult II was positive in 9 of 41 cases of upper gastrointestinal tract origin (21 percent detection rate), whereas the radial immunodiffusion method, expected to be negative as a result of action of gastrointestinal proteases, was positive in only 3 and negative in 38 of the 41 samples (92 percent accuracy). The two tests were equally effective in detecting lower gastrointestinal bleeding (14 of 37 samples, 37 percent accuracy).

The findings of this study indicate that the immunologic test may remedy the deficiencies of the guaiac test. The concomitant use of the immunologic and appropriately sensitive guaiac test appears to fulfill screening test requisites.

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A large number of patients suffer from hidden gastrointestinal (GI) blood loss. Bleeding occurs in over 80 percent of the more than 125,000 people with colorectal cancer every year.¹⁻⁵ A valid mass screening method is needed for the early detection of occult pathological GI bleeding.

since at least 80 percent of colorectal cancers can be cured if detected early enough.⁶⁻¹⁰ Identifying occult blood in a patient's stool is important in the detection of colorectal cancer and other major GI diseases.^{11,12}

The currently used Hemoccult II guaiac peroxidase activity detection test by itself is of limited value as a mass screening test for pathological occult blood in the stool.^{6,13-16} It is not monospecific for human blood^{13,6,12,17,18}; it is, instead, an indirect assay for what may be human blood, and its sensitivity is uncertain.¹⁹⁻²¹ Studies using chromium 51 elimination rate^{22,23} indicate that the normal GI blood loss per day is 1.3 to 3 mL (equivalent to 1.3 mg to 3 mg/day for healthy control).^{19,24-27} The Hemoccult II test is 37 percent positive for 2 to 5 mg of hemoglobin per g of stool, 60 percent positive for 5 to 20 mg/g and 90 percent positive for 20 mg/g of stool.^{23,27} The guaiac test is unable to define the site of GI bleeding, whether upper or lower.¹⁰ Although the Hemoccult II fecal sample slide kit may be inexpensive, the test is not cost effective because the subsequent diagnostic workup for the many false positives becomes very expensive.²⁸ A positive guaiac test may indicate bleeding from anywhere in the GI tract, presenting the dilemma of whether to begin an expensive investigation in the upper or lower GI tract.

The guaiac test also poses compliance difficulties.²⁹ Fecal matter containing products other than human hemoglobin and animal blood (eg, uncooked vegetables, fresh fruits, bacteria, vitamin C, cimetidine), cause false reactions with the guaiac test.^{3,6,10,12,27,30,31}

An analysis of the Sloan-Kettering mass screening study and the Minnesota prospective trial of Hemoccult screening by Applegate and Spector¹³ emphasizes the serious weaknesses of the Hemoccult II test. The American Cancer Society and the National Cancer Institute have stated that they do not recommend the Hemoccult II as a mass screening test.²⁸ The Food and Drug Administration also has expressed concern about information disseminated with regard to the guaiac test as a screening test for large bowel cancer (*American Medical News*, December 24, 1982).

A number of investigators acknowledge the inadequacies of the guaiac test⁶ and suggest using immunologic testing to detect fecal occult human blood.^{7,17,32} It has been found that occult human

hemoglobin in feces can be detected by several immunological tests (eg, radial immunodiffusion (RID), enzyme-linked immunosorbent assay (ELISA), and counterimmunoelectrophoresis (CIE)).^{3,17,18,33} The immunologic test is specific for human hemoglobin^{10,17} and the sensitivity can be controlled.¹⁰ The immunologic test is uncomplicated¹⁰ and identifies human hemoglobin material, not peroxidase-positive material in the GI tract.^{3,7,33,34} Samples for the immunologic test can be collected on a treated filter-paper slide packet similar to that used for the guaiac test, and the test thereby conforms to patient acceptability. Special dietary compliance by the patient is unnecessary,¹⁵ and the immunologic test can be performed either immediately after sample collection or weeks later.¹⁰

The basic cost of the immunologic test in its present small-scale production is more than the guaiac, but mass production would reduce the price, and unnecessary extensive workups of false positives would further minimize costs. Moreover, it might be more cost effective to perform the immunologic test only to verify suspected GI bleeding or positive guaiac results.

During a four-year period the effectiveness of the guaiac Hemoccult II test was compared with that of radial immunodiffusion in detecting occult blood in diseases in which bleeding occurred in either the upper or lower GI tract. The purpose of this study has been to find a valid screening method with the following necessary criteria: (1) monospecificity for human hemoglobin only, (2) appropriate sensitivity for pathological occult bleeding (ie, approximately 3 mg of human blood per g of stool), (3) detection of only lower GI human blood, and (4) differentiation of the site of upper or lower GI tract bleeding (ie, a combination of an immunologic test with an appropriately sensitive guaiac peroxidase detection test).

Methods

The Hemoccult II and the radial immunodiffusion (RID) test were conjointly done on fresh stool specimens. Barrows et al¹⁷ have previously described the preparation of their RID plates used

for the detection of occult blood.*

Patient fecal material was spread evenly onto specially treated filter paper (filter paper soaked in a pH-balanced solution of dextran, streptomycin, penicillin, amphotericin B, and sodium azine) as provided by Barrows. Following drying of the fecal material on the filter paper, a 1/8-inch disc was cut and removed using a commercial conductor's punch. These discs reflecting patient specimens, as well as appropriate control discs containing known amounts of hemoglobin, were placed on the RID plates. The disc was subsequently rehydrated with 10 μ L of phosphate-buffered saline, and the plates were then incubated for 18 to 24 hours at room temperature. Perpendicular diameters of precipitation were measured following this incubation period utilizing the data from the hemoglobin control discs. A standard RID curve could then be constructed.

Specifically measured punch discs from fecal smear samples were made from 211 patients randomly visiting offices of Wright State University gastroenterologists and family physicians. History, physical examination stool specimens for Hemoccult II and RID tests, and signed consent were minimum prerequisites for patients entering this investigation. A data-gathering card and diagnostic flow sheet were designed and followed in making appropriate diagnoses. The workup and diagnosis in each case were thorough and reliable. Specimens were processed for the Hemoccult II and RID tests as described.

Results

As predicted by Barrows et al¹⁷ and Songster et al,¹⁰ occult blood was detected in stool specimens using the RID test.³⁵⁻³⁷ There is a significant difference between the accuracy of the Hemoccult II test and the RID test, particularly in differentiating upper and lower GI tract bleeding. The accuracy of the combined guaiac and immunologic testing depends on what each of the tests is designed to do. The guaiac peroxidase detection test is an indi-

rect test for human blood that may arise anywhere in the GI tract.^{21,34,38,39} The immunologic test is specific for human blood associated only with the large bowel^{8,17,18} because pancreatic carboxypeptidase^{40,41} usually degrades upper GI tract blood before it reaches the large intestine.^{10,26,42} Consequently, the RID test should be positive for lower GI bleeding and negative for upper GI bleeding. The Hemoccult II test should be positive for both upper and lower GI tract bleeding.

The Hemoccult II detected peroxidase activity in only nine of the 41 cases of upper GI pathology (21.9 percent). In 38 of 41 cases of upper GI pathology (92.3 percent), the RID test results were negative, as would be expected, since this test primarily detects lower GI bleeding. The RID was positive in three of 41 instances (7 percent false positives) (Table 1), which may reflect a false-positive reaction or, more likely, the detection of blood from the upper GI region that escaped degradation by proteolytic enzymes.

The two tests were equally effective in detecting lower GI bleeding in that both detected fecal hemoglobin in 37.8 percent of the sample tests. Songster et al,¹⁵ in their study involving 150 consecutive colorectal carcinoma cases, found that occult bleeding was detected by Hemoccult II in 40 percent of the overall cases, whereas the immunologic test was positive in 65 percent.

When used simultaneously, the RID and Hemoccult II tests detect lower GI bleeding more accurately than either test alone. Using both the RID and the Hemoccult II tests, four of five colorectal cancers were identified. In addition, the RID test was positive in the one case of metastatic cancer of the ileum for which the Hemoccult II test was negative.

Comment

From this investigation and the studies of others, it can be determined that (1) occult human blood in stool specimens can be detected by immunologically specific assays (ie, RID); (2) this immunologic assay seems to be monospecific for human hemoglobin, whereas the guaiac test is nonspecific and detects only peroxidase activity; (3) the guaiac test may provide an indirect clue

*Radial immunodiffusion plates containing goat anti-hemoglobin sera were supplied by Dr. George Barrows, University of Louisville School of Medicine, Louisville, Kentucky.

Table 1. Patients (n = 211) With Radial Immunodiffusion (RID) or Guaiac (Hemoccult II) Positive Tests Categorized by Diagnoses and Correlated Results

Diagnosis	Total	RID and/or Hemoccult Positive	Hemoccult Positive	RID Positive
Diseases that could specifically be correlated with site of bleeding				
Lower gastrointestinal tract				
Carcinoma of large bowel	5	4	3	3
Metastatic cancer of ileum	1	1	0	1
Polyps in large bowel	13	3	3	2
Ulcerative colitis	8	8	6	5
Diverticula of colon (bleeding)	1	1	1	1
Crohn's disease of ileum	7	1	0	1
Ulcerative proctosigmoiditis	2	1	1	1
Upper gastrointestinal tract				
Gastric carcinoma	2	2	1	1
Upper gastrointestinal ulcer	25	5	5	1
Gastritis	12	2	2	0
Gastric Crohn's disease	1	1	1	0
Hiatal hernia, esophagitis	1	1	0	1
Total	78	30	23	17
Diseases that could not be correlated specifically with site of bleeding				
Old inactive Crohn's disease	1	1	1	0
Hemorrhoids (not bleeding)	12	1	1	0
Anemia, hepatomegaly	1	1	1	0
Diabetes	2	1	0	1
Anal irritation, spastic colon	1	1	1	1
Reflux esophagitis, hemorrhoids	3	1	0	1
Diarrhea	8	1	0	1
Liver disease	1	1	0	1
Arteriosclerotic cardiovascular disease, constipation	4	1	1	0
No pathology found	11	0	1	0
Esophageal spasm	4	1	1	0
Proctitis (no bleeding)	4	3	3	1
Total	52	14	10	6

that occult bleeding has occurred somewhere in the GI tract; and (4) upper and lower GI bleeding can be differentiated by use of both tests together, the guaiac for upper and lower GI bleeding and the immunologic for lower GI bleeding only.

Continuing studies are anticipated to examine the accuracy of this immunologic test and to investigate the efficacy of other immunologic assays (eg, ELISA, CIE) for the detection of occult blood.

The earlier recognition of human pathological fecal blood resulting from cancer, polyps, drug ingestion, gastritis, peptic ulcer disease, ulcerative colitis, Crohn's disease, and other GI disorders by an uncomplicated, cost-effective, valid method appears near realization. The combined use of an appropriately sensitive guaiac test to detect more upper and lower GI pathology while confirming its accuracy with an improved and refined immunologic test would be the best method. After com-

pletion of the next phase of the study and development of a valid stool test screening method for pathological amounts of human blood in fecal specimens, a large-scale screening trial in asymptomatic patients should be undertaken.

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