Rapid Immunochemical Detection of Fecal Occult Blood by Use of a Latex-Agglutination Test

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A novel immunochemical test, "Hemoxel," is characterized and compared with three guaiac tests: "Hemoccult" (Smith-Kline, U.S.A.), "Hemofec" (Boehringer Mannheim, F.R.G.), and "Fecatwin Sensitive" (Labsystems, Finland). We tested 191 stool specimens from patients, 144 from healthy persons. The sensitivity of Hemoxel was 0.6 mL of blood per 100 g of stool. It reacts with neither animal hemoglobin nor human myoglobin, unlike the guaiac tests, nor does it react with the stool of healthy persons under no dietary restrictions, while the guaiac tests react variably. As a rule, Hemoxel is insensitive to bleeding in the upper gastrointestinal tract, unlike the guaiac tests. However, Hemoxel seems to be more sensitive to bleeding in the lower gastrointestinal canal than are the other tests, and we show it to be suitable for screening for occult blood originating there. It is unaffected by diet. It is simple to perform, requires no special equipment, and stool specimens, stored in the test diluent, are stable for testing for two weeks.

Additional Keyphrases: colorectal cancer; screening; guaiac tests compared; gastrointestinal bleeding; "kit" methods

Detection of increased fecal hemoglobin is important in the diagnostic evaluation of patients with suspected gastrointestinal bleeding, and is the only practical method for population screening for colorectal cancer—the second commonest cancer after lung cancer in the western world (1, 2). Detection of fecal occult blood has been based on qualitative leuco-dye tests, with either guaiac (3, 4) or o-tolidine (5) as an oxidizable substance; on immunochemical tests (6–9); or on quantification of fecal porphyrin compounds (2, 10). The most widely used leuco-dye tests are based on oxidation of colorless guaiac to a colored compound in the presence of hydrogen peroxide and hemoglobin.

However, interpretation of the guaiac-based tests is complicated, for several reasons. The amount of blood required to yield a positive guaiac-reaction varies, owing to fecal hydration. Moreover, blood from dietary red meat may trigger oxidation of the indicator dye and give a positive result in the quantitative assay of fecal blood based on the fluorescence of heme-derived porphyrins (10–12). In addition, non-heme peroxidases, vitamin C, and elemental iron interfere with the guaiac tests (5, 10, 13–15). On the other hand, bacteria or enzymes may convert more than 80% of heme entering the gastrointestinal tract to porphyrins, which do not react in guaiac tests. Therefore, in advance of the chemical screening tests, the patient must go on a controlled special diet for several days, if results are to be reliable.

These triggering or inhibiting factors can be circumvented by using immunochemical detection of human blood in the feces (6–9). Owing to hydrolysis of the globin chains by digestive enzymes, however, these tests seem to be more sensitive to blood originating from more-distal lesions of the gastrointestinal tract than to blood coming from the stomach.

In this study we compare an immunochemical occult blood test, Hemoxel, with three guaiac tests: Hemofec, Hemoccult, and Fecatwin Sensitive.

Materials and Methods

The Tests

The Hemoxel test (Orion Diagnostica, Espoo, Finland) is a latex-agglutination test kit, which includes a latex reagent consisting of polystyrene beads coated with antibodies (16) against human hemoglobin. These agglutinate when hemoglobin is present in the specimen in nondigested form. The Hemoxel kit also includes control reagent (polystyrene beads coated with immunoglobulins of a non-immunized New Zealand White rabbit) and a specimen-dilution vial containing a proprietary physiological-saline-based diluent (Orion Diagnostica) to free the hemoglobin from the fecal material and to preserve the hemoglobin undegraded for several days.

The latex-agglutination test is performed as follows: Add about 0.1 g wet weight of stool sample, taken from the patient's stool specimen with a fixed-size sampling scoop provided in the specimen vial, to the Hemoxel sampling vial and mix thoroughly with the diluent included in the vial, to give a 1/250 dilution of the stool. Squeeze 10 to 15 drops from the suspension through the vial's filter cap and discard. Apply the succeeding two drops to the Hemoxel test card, on separate test areas. Deliver one drop of the Hemoxel reagent onto one of the test areas and one drop of the Hemoxel control reagent onto the other test area. After mixing both reagents with the corresponding sample drop, tilt the test card and rock it by hand for 3 min. Any agglutination visible on the test area with the Hemoxel reagent with simultaneous non-agglutination in the test area with the Hemoxel control reagent is to be regarded as a positive result.

Hemoccult (SmithKline Diagnostica, Sunnyvale, CA 94086), Hemofec (Boehringer Mannheim, Mannheim, F.R.G.), and Fecatwin Sensitive (Labeystems, Helsinki, Finland) were used as the comparative guaiac-based tests according to the instructions given by the manufacturers. A positive result was seen as a bluish coloring of the paper.

Stool Specimens

All stool specimens in this study were from patients with no need for a bed pan or from healthy persons, and were not contaminated with urine. The sample for testing was taken with a fixed-size scoop by laboratory technicians from the stool specimens after homogenization.

All clinical stool specimens were obtained from patients with a diet low in peroxidases (no red meat, no uncooked peroxidase-rich vegetables) of the Helsinki University Central Hospital Clinics of Internal Medicine. To avoid too many negative samples, only those positive in the initial Hematest (Amer Division of Miles Labs., Elkhart, IN 46514)
were included in the study, with about the same number of negative specimens. Thus 191 specimens with clinical information were obtained.

Stool specimens from 144 healthy persons (no special diet) were obtained as the normal-population control specimens.

The analytical sensitivity and specificity studies were based on adding hemolyzed human blood to stool from a single individual.

All stool specimens were processed within the same day by placing a sample from each specimen in the Hemolex diluent and taking smears for the other tests.

**Blood Specimens**

The human blood added to stool specimens was venous blood from a healthy individual. The samples of different normal human hemoglobins, hemoglobin F (48% Hb F) and hemoglobin A₂ (29% Hb A₂), as well as certain human hemoglobin variants—hemoglobin Helsinki [β82 (EF6) Lys → Met], hemoglobin Vaasa [β39 (C5) Gln → Glu] (17), and hemoglobin Meilahti [β36 Pro → Met], reported at the 9th International Mass Spectrometry Conference, Vienna, August 30—September 3, 1982, by Y. Wada, E. Ikkala, K. Imai, et al.—were from erythrocyte concentrates (by courtesy of Ms. M. Hirvonen, Finnish Red Cross, Central Laboratory, Helsinki, Finland). Blood from a patient with sickle cell anemia (Hb SS) was obtained by courtesy of Ms. Julie De Marco, Medical Technology Corp., Somerset, NJ. The animal blood samples were obtained from a municipal slaughterhouse.

**Results**

**Analytical Sensitivity of Hemolex**

Analytical sensitivity was determined by using hemolyzed human citrated blood (hemoglobin concentration 150 g/L) diluted in physiological saline (Table 1). Also, hemolyzed human erythrocyte concentrates, including the different above-mentioned human hemoglobins and human hemoglobin variants, were diluted in physiological saline to give a concentration of 150 g/L and used for testing the analytical sensitivity of Hemolex.

The results demonstrate the dependence of the agglutination reaction on the hemoglobin concentration. They also suggest the so-called prozone phenomenon (commonly seen in immunological reactions in antigen excess) with higher concentrations of hemoglobin. In the absence of feces the detection limit (for pure normal human hemoglobin) is about 1 mg of Hb per liter, corresponding to 0.13 mL of blood per 100 mL of saline (Hb 150 g/L). The results with different human hemoglobins show no dependence of Hemolex on the type of human hemoglobin, because both Hb S, Hb F, and Hb A₂ as well as all the variants showed detection limits similar to the limits for normal human hemoglobin when measured with Hemolex in different dilutions.

The analytical sensitivity of Hemolex was further studied by using normal human stool to which hemolyzed human blood was added (corresponding to 1.5, 1.3, 1.0, 0.6, 0.4, and 0.3 mL of blood per 100 g of stool). The stool samples with the different amounts of added hemoglobin were tested with Hemolex. The smallest amount of hemoglobin detected was taken as the analytical sensitivity of the test (Table 2). Obviously, the analytical sensitivity of Hemolex in detecting hemoglobin in feces is 90 mg of Hb per 100 g of stool, corresponding to 0.6 mL of blood per 100 g of stool (Hb 150 g/L in blood). This is approximately fivefold less than the sensitivity when hemolyzed blood was tested in saline (Table 1). The reproducibility of the Hemolex test was good (100%) in all experiments where the blood concentration in stool was 0.6 mL/100 g or more. Only when borderline concentrations were measured, possibly owing to uneven distribution of blood in the stool (in spite of homogenization) and subjective interpretation of weak agglutination, were there some fluctuations as seen in the example in Table 2.

**Specificity**

*Human hemoglobin.* Hemolex, an immunological test, is sensitive to undigested hemoglobin. It was supposed that blood originating from the stomach, as in the case of a bleeding ulcer, would be digested and degraded and thus would not be detected as a positive Hemolex test. To establish this, two volunteers ingested their own blood, 2 mL and 10 mL, respectively. Four stool specimens were collected from each individual during two days and tested with Hemolex and Fecatwin. Hemolex was negative in both cases of blood ingestion during the whole stool-collection period. Furthermore, even though the sensitivity of the Hemolex test was increased 25-fold by diluting 1 g of stool with 10 mL of diluent (instead of 0.1 g with 25 mL) the result was negative. On the other hand, Fecatwin gave a positive test as early as 36 h after ingestion and remained positive until the end of the test period in the case of the 10-M L blood dose; the smaller dose resulted in a negative reaction with Fecatwin throughout the test period.

*Human myoglobin.* We tested human urine containing myoglobin in a concentration of 25 mg/L with Hemolex, using a 25-μL drop of urine (not mixed with the diluent) mixed with the latex reagent. We saw no agglutination.

*Animal hemoglobin.* We studied the sensitivity of Hemolex to different animal hemoglobins, using 10-fold serial dilutions, with the Hemolex sample diluent, of blood from various animals: cattle (Hb 104 g/L), chicken (Hb 131 g/L), fish (Hb 101 g/L), goat (Hb 131 g/L), horse (Hb 200 g/L), pig (Hb 116 g/L), rabbit (Hb 91 g/L), and sheep (Hb 88 g/L). Hemolex did not react with any of the animal hemoglobins tested, but both Hemocult and Hemofec reacted with all, even when the sample was 1000-fold diluted. Fecatwin was also reactive with all animal hemoglobins, although the

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**Table 1. Sensitivity of Hemolex in Measuring Hemolyzed Human Citrated Blood Hemoglobin (Hb 150 g/L) Diluted in Physiological Saline**

<table>
<thead>
<tr>
<th>Hb, mg/L</th>
<th>750</th>
<th>375</th>
<th>150</th>
<th>37.5</th>
<th>9.4</th>
<th>3.8</th>
<th>1.5</th>
<th>0.9</th>
<th>0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolex</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>

*25 μL of each dilution was pipetted onto the test area followed by 25 μL of the latex reagent.*

**Table 2. Analytical Sensitivity of Hemolex**

<table>
<thead>
<tr>
<th>Blood, mL/100 g stool</th>
<th>1.5</th>
<th>1.3</th>
<th>1.0</th>
<th>0.6</th>
<th>0.4</th>
<th>0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, mg/100 g stool</td>
<td>225</td>
<td>195</td>
<td>150</td>
<td>90</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>Hemolex</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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</tr>
</tbody>
</table>

*Normal human stool spiked with different amounts of hemolyzed human blood (Hb 150 g/L). Four separate samplings from the same stool specimens were tested in this example.*
The smallest concentration of cattle hemoglobin we tested (Hb 104 mg/L) was negative.

**Healthy persons.** The reactivity of Hemolex with stools from healthy persons was studied in two stages. Four successive daily stool specimens from 44 healthy adults (uncontrolled diet) were collected and tested with Hemolex, Hemofec, Hemocult, and Fecatwin. For the latter part of the study we used three successive stool specimens from 100 healthy persons over 40 years of age (84 persons 40 to 60 years and 16 older than 50 years). This group of specimens was tested with Hemocult and Hemolex only. To discover if any hemoglobin could be detected in the stool of healthy persons, we also used the Hemolex test as sensitized to different detection levels, from 4 mg of Hb per 100 g of stool to 100 mg/100 g, by testing the specimens so that up to 25 times more stool was mixed with the same amount of diluent (1 g of stool in 10 mL of diluent). For all 144 persons (476 specimens) results were negative with Hemolex, six persons (eight specimens) were positive with Hemocult, eight persons (10 specimens) were positive with Hemofec, and 16 persons (30 specimens) were positive with Fecatwin. Furthermore, eight persons (11 specimens) were found positive with Hemocult in the latter part of the study.

The specimens of the 100 healthy persons mentioned were also tested with Hemolex at different levels of reactivity by suspending increased amounts of stool in the diluent. As a result, eight specimens (six persons) were positive with Hemolex at the sensitivity level of 4 mg of Hb per 100 g of stool (corresponding to 30 μL of blood per 100 g of stool), while at the sensitivity level of 32 mg/100 g (corresponding to 0.24 mL of blood per 100 g of stool) we saw no positive results. The specimens that were found positive in the sensitized Hemolex tests were all negative with Hemocult, while all those positive with Hemocult were negative with Hemolex, even at the most sensitive levels.

All these experiments clearly demonstrate the nonreactiveness of stool from healthy non-diet-restricted persons with the Hemolex test, whereas the guaiac tests react randomly.

### Comparison of Hemolex with Hemofec, Hemocult, and Fecatwin in Clinical Stool Specimens

We tested 191 clinical stool specimens with the immunochromatographic test Hemolex and the three guaiac tests, Hemofec, Hemocult, and Fecatwin. Table 3 shows the distribution of different result combinations.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Hemolex</th>
<th>Hemofec</th>
<th>Hemocult</th>
<th>Fecatwin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. specimens</td>
<td>29</td>
<td>66</td>
<td>26</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specimen</th>
<th>+</th>
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</table>

The specimens of the 100 healthy persons were, in six cases, from patients with no verified bleeding in the lower gastrointestinal tract and, in one case, from a patient with numerous diverticulae and carcinoma of rectum.

When the test results from such patients who had verified bleeding in the upper or lower gastrointestinal tract were compared, the following results were obtained: of 11 specimens from patients with bleeding in the upper gastrointestinal tract, Hemolex was positive in three, Hemofec in nine, Hemocult in 10, and Fecatwin in 11. On the other hand, when 15 specimens from patients with bleeding in the lower gastrointestinal tract were tested, Hemolex was positive in 14 cases, Hemofec in five, Hemocult in 10, and Fecatwin in 12.

### Stability of Stool Specimens

We examined the stability of the stool specimen in the diluent, using 25 100-mg stool specimens from healthy adults. Hemolyzed blood (hemoglobin 158 g/L) was diluted 100-fold with isotonic saline, and 60 μL was added to each specimen, to correspond to the detection limit of Hemolex (0.6 mL/100 g). After mixing thoroughly, we mixed each specimen with 25 mL of the Hemolex sample diluent and allowed the mixture to stand at room temperature (20–22 °C). Each specimen was tested with Hemolex at two-day intervals for a fortnight. We found all specimens were still positive by Hemolex after being preserved for 14 days in the sample diluent.

### Discussion

There is some physiological loss of blood from the gastrointestinal tract, resulting in the presence of less than 2 mg of hemoglobin per gram wet weight of stool, corresponding to a loss of about 2 mL of blood per day (10). This has also been confirmed by measuring normal enteral blood loss with isotope-labeled erythrocytes (18, 19). To detect enteral bleeding reliably, a fecal occult-blood test must measure human blood. Leuco-dye tests based either on the use of guaiac (3) or a-tolidine (20) detect hemoglobin compounds on the basis of their peroxidase activity. Thus they give a false-positive reaction when there are sufficient amounts of exogenous (i.e., dietary) animal hemoglobins or vegetables rich in peroxidases (11) in stool. Moreover, false-negative reactions have been attributed to reducing substances such as ascobic acid (6), antacids (15), defective guaiac or peroxidase developer (21), and hemoglobin degradation during fecal storage (10).

The use of aspirin and some other drugs can cause slightly increased enteral bleeding as side effects (14, 19, 22), which can be augmented by the simultaneous use of alcohol (23, 24). Long-distance running may also be a cause of enteral bleeding in healthy persons (25). Finally, fecal hemoglobin, like other fecal constituents in general, becomes more concentrated in dry stool, which may lead to a positive reaction.
The specificity of Hemolex for human hemoglobin excludes any need for specific pre-test diets such as are needed in non-immunological tests. Hemoglobin and heme compounds in general tend to be degraded by enzymes and bacteria during their intestinal passage (6). Naturally, this happens more the higher the bleeding site is in the gastrointestinal tract, as is supported by our observations on ingested human blood. In contrast to Hemolex, positive reactions in the guaiac tests proved the presence of heme-derived fecal peroxidase-activity in these cases.

In its present form, Hemolex will detect 0.6 mL of blood per 100 g of stool, which is essentially less than that of Hemoccult (2 mL/100 g), Hemofec (1–2 mL/100 g), and Fecatwin Sensitive (1–4 mL blood/100 g). The test results with patients having verified bleeding in the upper or lower gastrointestinal canal suggest that Hemolex is less sensitive to bleeding in the upper gastrointestinal tract than the guaiac tests but more sensitive to bleeding in the lower part. However, it may also detect bleeding in the upper part of the gastrointestinal tract when the extent of hemorrhage is sufficient. Our study suggests that, of the physiological blood loss of 2 mL per 100 g of stool into the gastrointestinal tract, <0.6 mL/100 g originates from the lower part. The analytical sensitivity of Hemolex (Table 1) has to be considered when bloody stools are tested. In cases of extensively bloody feces it would be advisable to further dilute the stool suspension in the case of a negative result, to avoid the prozone effect. However, no occult blood test is necessary in such cases.

The results for stools from healthy persons when Hemolex was used in sensitized reaction levels also suggest the assurance with which Hemolex can be used in screening, because overdosing of the stool specimen does not reveal more positives except in very high overdoses. The lack of reactivity of Hemolex to animal hemoglobins and, on the other hand, its independence from reactivity to different human hemoglobins clearly support the suitability of Hemolex for screening purposes; further, it is not affected by diet, as shown in the experiments with healthy persons. Further advantages of Hemolex are its simple latex test format—a technique that is familiar in clinical laboratories—its rapidity, and no requirement for special equipment. Furthermore, the specimens are stable in the sample diluent, making it possible to collect specimens over a period of time and test them together.

We are grateful to Mrs. Aila Åkerlund and Mrs. Marita Winter for their technical assistance.

References