



STOOL OCCULT BLOOD TESTS*

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Abstract

Three commonly used tests for occult blood in the stool were studied both in vitro and in vivo. The orthotolidine method (Hematest) is the most sensitive test; then the benzidine test. Although the guaiac test is the least sensitive, it also less expensive but sensitive enough to be utilized as a routine test. In contrast to previous recommendation the 3-days period of meat-free diet prior to the test is not required. Single GI bleeding of 5 ml. or more can cause positive stool guaiac test. Upper GI bleeding of 10 ml. or more is almost always associated with strongly positive stool guaiac test and melena or tarry stool in children. Oral iron medication may cause positive stool occult blood tests.

Introduction

The detection of occult blood in the stool is among one of the most frequently requested routine tests performed in the hospital laboratories. The etiologies, the frequency and the significance of the positive test varied from place to place. One of the main reasons for the varying

results is stem from the different methods employed. Most of the popular tests for the presence of blood in the stool and urine are the modifications of the technique which depends on determination of peroxidase activity as an indication of hemoglobin content. The different phenolic

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compounds used such as gum guaiac, orthotolidine, benzidine, phenolphthalein etc (1) (2) (3) are differed chiefly in their sensitivities. Orthotolidine is 1 to 10 times more sensitive than benzidine method; benzidine, 10 to 100 times more sensitive than guaiac, depending to some extent on the technique used. Some of these tests are so sensitive that for a positive report to be significant of a possible GI lesion the patient must have been on a meat-free diet for at least 3 days preceding the tests. The sensitivity of the guaiac reaction is appreciated by the fact that it is negative when normal diets are ingested.

The authors are reporting their experience with the sensitivities of the 3 commonly used tests for occult blood in the stool, the effects of the hospital diet, medicinal iron and gastrointestinal bleeding on their positivities.

MATERIAL AND METHODS.

MATERIAL

The experiments were carried out in 19 healthy children between 2 months and 12 year of age who had no history of gastrointestinal disease, GI bleeding, epis-taxis or any medication particularly iron preparation.

METHODS

The stool specimen were obtained by spontaneous defaecation or rectal swab at interval from 16 to 72 hours.

GUM GUAIAIC TEST.

REAGENTS

1. Filter paper (free of positive color reaction)
2. Glacial acetic acid
3. Saturated alcoholic gum guaiac (dissolve 1 gm. of powdered gum guaiac in 5 ml. 95 % ethyl alcohol).
4. Freshly prepared 3 volume per cent hydrogen peroxide solution, stored in refrigerator until used.

PROCEDURE

1. A small quantity of stool is smeared on a piece of filter paper with an applicator stick.
2. Two drops of glacial acetic acid is then added and mixed with stool.
3. Add 2 drops of alcoholic guaiac solution.
4. Then add 2 drops of hydrogen peroxide solution, mix.

INTERPRETATION

The appearance of the blue or blue green colors are considered a positive reaction. If the deep blue color developed within one minute it is graded as 4+. The negative result is when there is no color or only a slight trace of green color is noted within 5 minutes.

BENZIDINE TEST.

REAGENTS

1. Benzidine powder

2. Glacial acetic acid
3. Freshly prepared 3 volume per cent hydrogen peroxide solution.

PROCEDURE

1. A small quantity of stool is smeared on a clean slide with an applicator stick.
2. Equal amount of benzidine powder is then added, mix.
3. Add 2 drops of glacial acetic acid, mix.
4. Add 2 drops of hydrogen peroxide solution, mix.

INTERPRETATION

The presence of any blue, blue green or purple color at 30 to 60 seconds is considered a positive reaction. In general, if the deep blue color is noted within 30 seconds it is considered as 4+. The lesser degree of color change developed within 1 to 5 minutes are graded from 1+ to 3+ accordingly. If no color only a slight trace of green color is noted within 5 minutes it is considered as a negative reaction.

HEMATEST.

PROCEDURE

1. Make thin smear of stool on filter paper square (provided by the

manufacturer). Do not use emulsion.

2. Place HEMATEST TABLET (or, thotolidine, strontium, peroxide, tartaric acid and calcium acetate, AMES Co.) across edge of smear.
3. Flow a drop of water on top of tablet, wait 5 to 10 seconds and flow second drop on tablet so that it runs down sides on to the filter paper.
4. Observe color of filter paper around tablet exactly 2 minutes later. Filter paper around tablet turns blue within 2 minutes indicates the positive reaction.

The concentration of blood is roughly proportional to intensity of blue color and speed with which it develops.

RESULTS

I. AN IN VITRO SENSITIVITY OF DIFFERENT METHODS

The whole blood specimen was diluted serially with 0.85% NaCl solution before being tested with the '3 method described above. The results are shown in table I.

TABLE I: IN VITRO SENSITIVITY OF DIFFERENT TESTS.

Whole blood	Guaiaac	Benzidine	Hematest
undiluted	++++	++++	++++
1/8 dilution	++++	++++	++++
1/40 dilution	+++	++++	++++
1/80 dilution	++	++++	++++
1/100 dilution	+	+++	++++
1/160 dilution	+	++	++++
1/200 dilution	+	+	+++
1/400 dilution	-	+	+++
1/800 dilution	-	-	+++
1/1600 dilution	-	-	+++

Note : Hematest is at least 16 times more sensitive than Benzidine test; Benzidine test is about 2x more sensitive than guaiaac test.

II. IN VIVO SENSITIVITY OF DIFFERENT TESTS.

Varying amount of whole blood were given to subjects via nasogastric intubation as a single dose. The stool samples were collected between 16-24 hours or longer. The results represent the strongest positive reaction detected and are summarized in Table II. below.

TABLE II: IN VIVO SENSITIVITY OF DIFFERENT TESTS.

Volume of Blood (ml.)	Patients	Age (yr.)	Guaiac	Benzidine	Hematest	Comments
2.5	R.T.	2/12	-	+	+	
	J.N.	3/12	-	-	-	
	S.W.	1	-	-	-	
5.0	S.S.	2/12	-	+	+	
	B.J.	9/12	-	+++	+++	
	S.W.	1	-	+++	+++	
	B.S.	2	-	-	+	
	C.Y.	3	-	-	-	
	C.R.	9	-	++	++	
10.0	R.T.	2/12	++	++++	++++	Melena
	B.S.	2	++++	++++	++++	Melena
	C.R.	3	++++	++++	++++	Melena
	C.L.	7	++	++++	++++	Melena
	T.W.	10	++	+++	+++	Melena
15.0	C.N.	3/12	-	+	+	
	B.S.	2	++++	++++	++++	Melena
	P.D.	3	+++	++++	++++	Melena
	C.R.	9	++++	++++	++++	Melena
	T.W.	10	+	++	+++	
20.0	A.R.	9	++	++++	+++	Melena
	S.N.	11	++	+++	+++	Melena
	T.B.	12	++	+++	+++	Melena

OBSERVATIONS

1. The in vivo results are comparable with the in vitro experiment.
2. More than 5 ml. of blood is needed to produce positive stool guaiac test. Or positive stool guaiac test means the GI bleeding of more than 5 ml. at any time.
3. Melena is almost always present when 10 ml. or more of blood is given in a single dose into the GI tract (comparable to single GI hemorrhage)
4. Stool guaiac test of the melena can give positive results ranging from 2+ to 4+ (see discussion).

III. EFFECT OF HOSPITAL DIET.

The hospital diet contains rice, eggs, noodles, meats and vegetables. The stool

specimens obtained from patients who were being admitted to the Pediatric ward were tested for occult blood. Thirty children who had negative occult blood by those 3 tests were also had negative test after received hospital diet for 3 successive days. Thus indicated that the average high protein diet is not interfere with these tests and the test could be done in any patient without 3 days period of meat-free diet prior to the test.

IV. EFFECT OF MEDICINAL IRON.

Varying dose of iron medications were given to children who had negative occult blood for at least 2 successive days prior to the experiment. The tests for occult blood were done in the stool specimens collected 16-48 hours later.

TABLE III. EFFECT OF ORAL IRON MEDICATIONS ON THE TESTS FOR OCCULT BLOOD IN STOOL

Iron Medication	Patients	Age (Yr.)	Guaiac	Benzidine	Hematest	Comments
1.2 ml. Fer-in-sol	C.N.	3/12	-	+	+	dark-colored stool
2.4 ml. Fer-in-sol	S.N.	11	-	+	+	
	T.K.	11	-	+	++	
	A.R.	9	-	++	++	
	C.Y.	13	+	++	++	
	N.G.	11	+	++	+++	
200 mg. Tab. Fersolate	N.A.	12	-	-	+	dark-colored stool
	S.N.	11	-	+	++	dark-colored stool
	T.N.	9	-	++	++	dark-colored stool
	B.C.	13	-	++	++	
	T.B.	12	+	++	+++	

OBSERVATIONS

1. Several iron preparations (including Fer-in-Sol, Jectofer, Imferon, Tab. Fersolate, FeCl_3 , etc.) gave negative result with all 3 tests above in vitro because they do not contain any peroxidase activity.
2. When given by mouth, the iron medications can induce positive occult blood test (see discussion).
3. While the Hematest and Benzidine test gave positive result in almost every instances the guaiac test is positive in less than one third of cases received therapeutic dose of oral medication. Thus indicated that for the purpose of follow up the patient to insure the regularity of medication administration the Hematest and Benzidine test are preferable.
4. The etiology of positive occult blood test after oral iron medication is speculated in the discussion part.

DISCUSSION

Passage of more than 2.8 ml. of blood in a 24-hour period is taken as an important sign of gastrointestinal disease.(4) Of one group of patients with significant GI bleeding, 18 % were found to have malignant tumors and 30 % had benign peptic ulcer. (4) Drugs, particularly salicylates, steroids, rauwolfia derivatives, indomethacin, and colchicine, have been

shown to be associated with increased GI blood loss in normal subjects and even more pronounced increase in bleeding from pathological sources. This effect may even follow parenteral administration of the drugs.

Guaiac test, as well as all other tests for occult blood in the stool is only qualitative and not even semiquantitative. (4) Neither the guaiac test nor the benzidine test are specific for hemoglobin and therefore are not specific for blood. The presence of purulent material, iodides, and bromides are known to give false positive reaction. (5) Dietary meat contains hemoglobin and myoglobin as well as other enzymes which may give positive tests for as long as 4 days after ingestion. But our experience as well as the others (4) had shown at the stool guaiac reaction is negative when normal diets are ingested. For this reason the filter-paper-guaiac test which is simple but has the required range of sensitivity is recommended as a routine screening test for occult blood.

Although it is known that as much as 50 to 75 ml. of blood from the upper GI tract is required to produce a tarry stool or melena (4), we found that as little as 10 ml. of blood from the upper GI tract may cause the melena in most of our children. One investigator reported that as much as 25-50 ml. of bleeding in the GI tract might be missed with the guaiac test. (4) Our results are in agreement

with previous experiences that the guaiac reaction may become positive when only 2 to 12 ml. of blood is introduced into the stomach. (2) (3) (6) Most of the hemoglobin entering the GI tract reaches the stool in the form of hematin, which has peroxidase activity. A large amount of activity may be lost through further degradation to inactive material. It has been shown that a 80 to 120-fold decrease in peroxidase activity of blood passing through the GI tract as compared to blood added directly to the stool. (4) This may explain why the melena may give only 2+ reaction to the guaiac test.

Brayshaw et al (1963) (7) confirmed the previous experiences (2) (3) (6) (8) that oral iron therapy did not alter the incidence of guaiac positive stool results but only caused an increase in trace positive orthotolidine test. Our results are in agreement with the current opinion that the oral iron medication may cause higher incidence of positive stool occult blood test. (4) The later, resulting from the increased fecal peroxidase activity, may be due to the actual bleeding secondary to GI irritation produced by some iron compounds.

CONCLUSIONS

From the above study it is concluded that

1. The commercially available orthotolidine test, is the most sensitive technique for the detection of the occult blood in stool.

2. Filter - paper - guaiac test is the simple method with optimum sensitivity for the routine screening test of the occult blood in stool.

3. Hospital diet has no ill effect on any test for stool occult blood.

4. Melena occurs in almost every children with upper GI hemorrhage of 10 ml. or more.

5. Stool guaiac test positive indicated the presence of GI bleeding of more than 5 ml.

6. Oral iron medication may cause significant positive stool occult blood test and, occasionally dark-colored stools.

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