Tests for occult blood in stools of children

A. E. A. FORD-JONES* and J. J. COGSWELL

From the Department of Paediatrics, Guy's Hospital, London

Ford-Jones, A. E. A., and Cogswell, J. J. (1975). *Archives of Disease in Childhood*, 50, 238. **Tests for occult blood in stools of children.** Three tests for the presence of occult blood in the stools of children were examined; the orthotolidine test, the guaiacum resin test, and a modified reduced phenolphthalein test. The stools of 71 neonates, 33 normal children, and 8 children with suspected gastrointestinal pathology were examined. The 1% orthotolidine test and the guaiacum resin test produced a high number of false positive results in healthy children eating a meat-containing diet. It is concluded that a positive result by these tests is of little diagnostic value in children eating a normal diet.

There is no general agreement on the most satisfactory test to detect occult blood in the stools of children, and there is little standardization of the methods used. After warnings about the carcinogenic properties of orthotolidine (Department of Health and Social Security, 1969), a convenient commercially available test† was withdrawn from the market. Uncertainties about the sensitivities and practicalities of alternative tests for occult blood in the stools of children prompted this study. Three methods which could be carried out in the side room of a paediatric ward were examined: the orthotolidine test, the guaiacum gum test, and a modified reduced phenolphthalein test.

Material

Group 1. The stools of 71 healthy newborn babies were examined. All the infants were less than 1 week of age, and 25 were entirely breast fed.

Group 2. The stools of 33 normal children aged 6 months to 13 years were examined. These children had been admitted to hospital for minor surgical procedures not involving the gastrointestinal tract. All were eating a meat-containing ward diet. The stools were collected before the child was submitted to anaesthesia.

Group 3. The stools of 8 children with systemic or gastrointestinal disease were examined. The clinical details of this group are included in Table IV. None

of these ill children had overt melaena, but on clinical grounds occult gastrointestinal bleeding was thought to be present.

Methods

Orthotolidine test. This test depends on the peroxidase activity of the red cell and its iron-containing derivatives. Oxygen liberated from hydrogen peroxide by this action is accepted by the orthotolidine to produce a blue compound. The sensitivity of the orthotolidine test varies with the concentration used, but a 1% solution is claimed to detect gastrointestinal bleeding in adults with a high degree of certainty (Varley, 1967).

In this study the perborate test of Abbott was used as a simpler modification of the 1% orthotolidine test (Huntsman and Liddell, 1961). The technique was as follows. 0.2 g powder containing equal quantities of sodium perborate and *o*-toluidine was added to 10 ml 50% acetic acid immediately before use. A thin smear of faeces was made in the center of a piece of filter paper and 6 drops of the mixture added to the centre of the smear. After 2 minutes the colour change was noted and a blue/green colour reported as positive. Quantitation of the response was reported as strongly positive, weakly positive, or negative.

Guaiac test. The guaiacum resin test was performed as follows (Barnet, 1952). One drop of a solution containing 2 g guaiacum resin dissolved in 100 ml glacial acetic acid was placed on a faecal smear on a filter paper. This was followed by one drop of freshly prepared hydrogen peroxide solution ('10-volumes'). A green-blue colour appearing within 30 seconds was interpreted as strongly positive, a faint blue colour was weakly positive, and no colour was a negative result. Colour changes occurring after 30 seconds were ignored. The guaiacum solution was

Received 19 August 1974.

*Present address: Kingston General Hospital, Kingston, Ontario, Canada.

†Haematest, Ames Co. Ltd.

made up before each study, and the peroxide solution renewed every 4 weeks.

Modified phenolphthalein test. A suspension of faeces was dissolved in dilute acetic acid without boiling. One drop of this suspension was spotted onto filter paper and followed by one drop of reduced phenolphthalein reagent and a drop of hydrogen peroxide solution. A pink colour developing within 30 seconds was interpreted as positive (Benson, 1968).

Results

The tests selected for this study were chosen for their suitability as side room techniques. The orthotolidine test was the quickest to perform and easiest to interpret, but gloves were worn because of the reagent's potential carcinogenicity. The guaiacum solution had to be made up before each study, since the shelf life in a dark bottle is short. The reduced phenolphthalein test was the most laborious to perform, though the stage of boiling the faeces in acetic acid was omitted. This test was not carried out on the stools of the normal babies.

The comparative sensitivities of the 3 tests are shown in Table I. The 1% orthotolidine test was

TABLE I

Sensitivity of the 3 tests assessed by diluted solutions of blood

Test	Blood solution		
	1:5 000	1:10 000	1:20 000
1% o-tolidine	Strong positive	Weak positive	Negative
Guaiacum resin	Weak positive	Negative	Negative
Reduced phenolphthalein	Negative	Negative	Negative

the most sensitive and was standardized to a 1 in 10 000 dilution of blood (Huntsman and Liddell, 1961). The guaiacum resin test was less sensitive than the 1% orthotolidine test. The reduced phenolphthalein test, when the boiling stage was omitted, was insensitive to blood dilutions weaker than 1 in 5000.

The stools of the 71 normal infants were examined by two tests only—the 1% orthotolidine test and the guaiacum resin test (Table II). Less than 8% of the healthy babies had a negative orthotolidine test when their stools were examined in the first week of life, and 30% were negative by the guaiacum resin test. 33 of the 71 stools examined were collected from infants under 48 hours of age, and all these specimens showed a weakly or strongly

TABLE II

Stools of 71 normal infants (group 1) tested by two methods

Test	Strong positive	Weak positive	Negative
1% o-tolidine	12	53	6
Guaiacum resin	7	42	22

positive result on both tests. There was no significant difference ($P > 0.05$) in the number of positive tests between breast-fed babies and bottle-fed infants.

The results of 33 normal children tested while eating the ward diet are shown in Table III. 12%

TABLE III

Stools of 33 normal children (group 2) tested by three methods

Test	Strong positive	Weak positive	Negative
1% o-tolidine	11	18	4
Guaiacum resin	8	16	9
Reduced phenolphthalein	0	0	33

had a negative orthotolidine test, and 27% had a negative test to guaiacum resin. Strong positive results to the orthotolidine test was found in 33% and to the guaiacum test in 24%. Results in the 8 abnormal children with their age and diagnosis are given in Table IV.

Discussion

Tests for occult blood in the faeces of children are frequently ordered though the limitations and diagnostic value of such tests are not widely known.

In this study it was found that healthy children on a normal ward diet gave a high number of false positive results for occult blood by the orthotolidine and guaiacum tests. This finding contrasts with the results in adults in whom meat eating was not found to be responsible for false positive reactions (Needham and Simpson, 1952). Paper orthotolidine tests and paper guaiac tests have also been reported to produce a high number of false positive results in children (Huntsman and Liddell, 1961). These authors speculated that the relative immaturity of the digestive tract in children may account for the increased faecal peroxidase activity. The stools of normal infants during the first week of life also produced a high number of positive results by

TABLE IV

Results of occult blood tests carried out on the stools of 8 children in whom gastrointestinal bleeding was anticipated (group 3)

Subject	Age (yr)	Diagnosis	1% Toluidine	Guaiacum	Phenolphthalein
1	2	Hirschsprung's disease	++	++	0
2	5	Henoch-Schönlein purpura	0	0	0
3	3	Aplastic anaemia	++	+	0
4	4	Hirschsprung's disease	++	++	0
5	8	Chronic renal failure	+	+	0
6	5	Acute renal failure	++	++	+
7	6	Glomerulonephritis	++	++	+
8	7	Asthma (steroid therapy)	++	++	0

++ , strongly positive;

+, weakly positive;

0, negative.

the orthotolidine and and guaiacum tests, probably due to swallowed blood or haemorrhagic disease of the newborn.

The phenolphthalein tests did not produce positive results in the stools of normal children, but its sensitivity was such that it only produced two weakly positive results in the stools of 8 children in whom gastrointestinal bleeding was likely to be present.

The ideal test of occult blood in the stools of children would be sensitive, easy and safe to use, give no false positives, and be unaffected by diet. None of the reagents tested fulfilled these requirements. It is suggested that a positive occult blood by the 1% orthotolidine test, or the guaiacum gum test, is of little diagnostic value in infants or children eating a normal diet.

We thank Dr. J. Liddell for his help in the preparation of this manuscript.

REFERENCES

- Barnett, R. N. (1952). The guaiac test; correlation with clinical findings. *Gastroenterology*, **21**, 540.
- Benson, S. (1968). Determination of blood glucose using commercially available enzyme-dye reagent and the detection of faecal occult blood. *Proceedings of the Association of Clinical Biochemists*, **5**, 131.
- Department of Health and Social Security (1969). Hospital Memorandum, No. 57.
- Huntsman, R. G., and Liddell, J. (1961). Paper tests for occult blood in faeces and some observations on the fate of swallowed red cells. *Journal of Clinical Pathology*, **14**, 436.
- Needham, C. D., and Simpson, R. G. (1952). The benzidine test for occult blood in faeces. *Quarterly Journal of Medicine*, **21**, 123.
- Varley, H. (1967). *Practical Clinical Biochemistry*, 4th ed., p. 344. Heinemann, London.

Correspondence to Dr. J. J. Cogswell, Department of Paediatrics, Guy's Hospital, London SE1 9RT.