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SPECIFIC TEST COMPOSITION FOR OCCULT BLOOD

Ernest C. Adams, Jr., and James A. Peterson, Elkhart, Ind., assignors to Miles Laboratories, Inc., Elkhart, Ind., a corporation of Indiana

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This invention relates to a composition which has utility in the detection of blood. Particularly the invention relates to compositions which are suitable for use in the qualitative detection and quantitative estimation of blood in body fluids such as urine, vomitus, gastric contents, cerebral spinal fluids and in feces.

The detection of occult blood in body fluids and feces has become an invaluable aid to the medical practitioner in the correct diagnosis of a great number of disorders. Blood is found in the gastric contents and in vomitus in conditions associated with erosion of the mucous membranes, in ulcers and in carcinomas. In the feces, the regular and frequent occurrence of occult blood is suggestive of gastro intestinal cancer, gastric or duodenal ulcers or hemorrhoids. In these conditions, the hemorrhage is often so slight that it is not possible to detect blood by microscopic identifications of the erythrocytes (red blood cells) and a sensitive and specific chemical test for occult blood becomes invaluable. In the urine, blood cells (hematuria) or blood pigment (hemoglobinuria) is found in typhus, scurvy, purpura, pyemia, nephritis, renal calculi, as the result of a burn covering a large part of the body, by the action of various hemolytic toxins, etc.

The prior art has recognized the need for a simple, reliable test for occult blood. U.S. Patent No. 2,290,436, issued July 21, 1942, to Kamlet, U.S. Patent No. 2,799,660, issued July 16, 1957 to Nicholls and Fonner and U.S. Patent No. 2,838,377, issued June 10, 1958 to Fonner (all assigned to the instant assignee) illustrate various test compositions which have been supplied to meet this need.

The instant inventive concept, like those of the prior art, are based on the catalytic activity of the prosthetic groups present in blood. These catalytically active substances identified in hemoglobin belong to the general class of hemoproteins, conjugate proteins all of which have the same prosthetic group, iron protoporphyrin or haem. This prosthetic group has the ability to catalyze the transfer of oxygen from an oxygen source to an acceptor which in turn becomes oxidized. If the acceptor is a dye percursor, colorless until it becomes oxidized and colored in its oxidized form, then the presence of the catalytic activity is indicated by color formation.

Thus the composition of this invention comprises an indicator or dye precursor plus an oxygen source. In the presence of the hemoglobin of blood, which contains the catalytic prosthetic group, the transfer of oxygen from the oxygen source to the acceptor or dye precursor will occur and the indicator will become oxidized and colored. The presence of color, then, is an indication of the presence of blood, and the rapidity of color formation and the depth or density of the color, when compared to a set of standards, is a means of the quantitative estimation of the blood present.

In the diagnostic compositions of the prior art the oxygen source has uniformly been of an inorganic nature such as the peroxides, perborates or persulfates of the alkali or alkaline earth metals. Representative examples include barium peroxide, strontium peroxide, magnesium peroxide, sodium perbosilicate, sodium perborate and the like.

It has been found in the past that the test compositions of the prior art utilizing an inorganic peroxide as the oxygen source must be very carefully controlled as to sensitivity to avoid the possibility of obtaining false positive reactions due to the presence of substances demonstrating peroxidative activity other than the prosthetic groups of hemoglobin of blood. The presence of ingested materials such as vegetable peroxidase in human feces has been found to give a positive reaction with the blood tests of the prior art. Other peroxidatively active materials such as lacto-peroxidase from milk and verdo-peroxidase will also give false positive reactions with an overly sensitive prior art blood test.

It has now been found, and forms the object of this invention, that certain organic hydroperoxides have a specificity for the prosthetic groups of the hemoglobin of blood. Concentrations of horseradish peroxidase which give an intense reaction with orthotolidine as an indicator and an inorganic peroxide give no reaction with orthotolidine and the hydroperoxides of this invention. Using the organic hydroperoxides it is therefore possible to design a test that is extremely sensitive for the detection of occult blood and yet which will give no false positive reactions in the presence of vegetable peroxidases.

The instant inventive concept will be more clearly explained by reference to the following illustrative examples.

Example 1

Filter paper sticks prepared by cutting E and D No. 627 filter paper into 1 cm. x 10 cm. strips were impregnated with 4 N citrate buffer solution (pH 4.8) and dried in an oven. The impregnated portion was overlaid with a chloroform solution containing 0.05 ml. cumene hydroperoxide and 20 mg. o-tolidine base per ml. The sticks were then dried a few minutes in an oven.

These sticks reacted with 30 seconds to give a blue color with dilutions of blood in water as high as 1:100,000. They were less sensitive in urine, reacting at a dilution of 1:20,000 to give the same color.

The specificity of these sticks can be seen by comparing the reactions with blood and horseradish peroxidase with similar reactions of tablets made with strontium peroxide and also of sticks made with 1-hydroxycyclohexylhydroperoxide-1, and ethyl hydrogen peroxide. The following table illustrates this.

	Minimum concentration of peroxidase in water giving reaction, percent	Minimum concentration of hemoglobin in water giving reaction, percent
Cumene hydroperoxide	0.00015	0.00015
1-hydroxycyclohexylhydroperoxide-1	0.0000015	0.00075
Ethyl hydrogen peroxide	0.0000015	0.0015
Strontium peroxide	0.0000015	0.0003

It can be seen that based on the minimum concentrations giving reactions that cumene hydroperoxide has 5 times the activity with hemoglobin as the non-specific 1-hydroxycyclohexylhydroperoxide-1, but only 0.01 of the 1-hydroxycyclohexylhydroperoxide-1 activity with horseradish peroxidase. If the intensity of the reaction is considered, it can be shown that cumene hydroperoxide has only 0.001 of the activity of 1-hydroxycyclohexylhydroperoxide-1 with peroxidase while retaining the same 5 times activity with blood. Simple comparisons can be made with the other two nonspecific peroxides, ethyl hydrogen peroxide and strontium peroxide.

Example 2

Sticks were prepared using diisopropylbenzene hydroperoxide in place of the cumene hydroperoxide of Example 1.

These sticks also possessed the same specificity for the blood catalyzed reaction as noted for the cumene hydroperoxide sticks. They reacted within 30 seconds to give a blue color with dilutions of blood in water as high as 1:100,000. They were less sensitive in urine, reacting at a dilution of 1:20,000.

Example 3

Sticks were prepared using the para-menthane hydroperoxide in place of the cumene hydroperoxide in Example 1.

These sticks reacted within 30 seconds to give a blue color with dilutions of blood in water as high as 1:100,000. They were less sensitive in urine, reacting at a dilution of 1:20,000. The sticks appeared to have the same stability as sticks made with cumene hydroperoxide. They also possessed the same specificity for blood as noted for the cumene hydroperoxide preparations.

Example 4

A powder was prepared with the following compositions:

o-Tolidine base	milligrams	100
Tartaric acid	do	350
Calcium acetate	do	6000
Sodium bicarbonate	do	500
D and C Red No. 35	do	10
Cumene hydroperoxide	do	180

This was used as a powder and also was compressed into tablets.

These materials were used as follows:

One drop of a urine or water solution containing blood was placed on a filter paper, the tablet or powder was placed over the spot, and 2 drops of water was added. A blue color developed within 30 seconds with a dilution of 1:100,000 blood in water and with 1:20,000 blood in urine. For detection of occult blood in feces, either a smear of the feces was made on the filter paper, or an emulsion of the faces was treated as the urine. The test would detect 1:2,000 blood in the emulsion and 1:200 in the smear.

Example 5

In this example diisopropylbenzene hydroperoxide was used in place of the cumene hydroperoxide of Example 4 and tablets were prepared.

One drop of a urine or water solution containing blood was placed on a filter paper, the tablet or powder was placed over the spot, and 2 drops of water was added. A blue color developed within 30 seconds with a dilution of 1:100,000 blood in water and with 1:20,000 blood in urine. For detection of occult blood in feces, either a smear of the feces was made on the filter paper, or an emulsion of the feces was treated as the urine. The test would detect 1:2,000 blood in the emulsion and 1:200 in the smear.

Example 6

In this example para-menthane hydroperoxide was used in place of cumene hydroperoxide of Example 4.

One drop of a urine or water solution containing blood was placed on a filter paper, the tablet was placed over the spot, and 2 drops of water was added. A blue color developed within 30 seconds with a dilution of 1:100,000 blood in water and with 1:20,000 blood in urine. For detection of occult blood in feces, either a smear of the feces was made on the filter paper, or an emulsion of the feces was treated as the urine. The test would detect 1:2,000 blood in the emulsion and 1:200 in the smear.

Example 7

A powder was prepared with the following composition:

o-Tolidine base	milligrams	100
Sodium citrate (dihydrate)	do	163
Citric acid (anhydrous)	do	37
Lactose	do	2000
Sodium bicarbonate	do	25
D and C Red No. 35	do	5
Cumene hydroperoxide	do	200

This material was used as a powder and also was compressed into tablets.

One drop of urine or water solution containing blood was placed on a filter paper, the tablet or powder was placed over the spot, and 2 drops of water was added. A blue color developed within 30 seconds with a dilution of 1:100,000 blood in water and with 1:20,000 blood in urine. For detection of occult blood in feces, either a smear of the feces was made on the filter paper, or an emulsion of the feces was treated as the urine. The test would detect 1:2,000 blood in the emulsion and 1:200 in the smear.

Example 8

In this example diisopropylbenzene hydroperoxide was used in place of the cumene hydroperoxide of Example 7.

These tablets were used as follows:

One drop of a urine or water solution containing blood was placed on a filter paper, the tablet was placed over the spot, and 2 drops of water was added. A blue color developed within 30 seconds with a dilution of 1:100,000 blood in water and with 1:20,000 blood in urine. For detection of occult blood in feces, either a smear of the feces was made on the filter paper, or an emulsion of the feces was treated as the urine. The test would detect 1:2,000 blood in the emulsion and 1:200 in the smear.

Example 9

In this example para-menthane hydroperoxide was used in place of the cumene hydroperoxide of Example 7.

These tablets were used as follows:

One drop of a urine or water solution containing blood was placed on a filter paper, the tablet was placed over the spot, and 2 drops of water was added. A blue color developed within 30 seconds with a dilution of 1:100,000 blood in water and with 1:20,000 blood in urine. For detection of occult blood in feces, either a smear of the feces was made on the filter paper, or an emulsion of the feces was treated as the urine. The test would detect 1:2,000 blood in the emulsion and 1:200 in the smear.

It will be seen by consideration of the examples above that the test compositions to which the instant invention is directed may be made either in the form of sticks by impregnating the reactants on a strip of a bibulous material such as filter paper strips or the test compositions may be prepared in the form of a powder or compressed into tablets. In either event, the dye precursor or indicator material may be selected from that group of indicators which will accept the catalyzed transfer of oxygen from the organic hydroperoxide and thereby become oxidized into their colored form. Such dye precursors include o-tolidine, o-toluidine, p-toluidine, o-phenylenediamine, N,N'-dimethyl-p-phenylenediamine, N,N'-diethyl-p-phenylenediamine, benzidine, p-anisidine, o-cresol, m-cresol, p-cresol, alphanaphthol, betanaphthol, catechol, guaiacol, pyrogallol, etc.

The preferred organic hydroperoxides are those of the isopropylbenzene tertiary hydroperoxide type and include cumene hydroperoxide, diisopropylbenzene and para-menthane hydroperoxide. It has been found that other organic hydroperoxides such as ethyl hydroperoxide, and 1-hydroxycyclohexylhydroperoxide-1, do not exhibit this specificity for the prosthetic group of hemoglobin.

In the test compositions of this invention it is essential

that there be present a buffer material in order to maintain the pH of that portion of the substance being tested within a pH range suitable for the oxidation of the indicator. Normally this pH range will be from about 4 to 7 but will depend upon the indicator used. Buffering materials such as citrate, tartrate, phosphate, phthalate, acetate and mixtures of these may be used.

In the tablet compositions it has been found advantageous to include excipients such as lactose, starch, flour and the like to facilitate the formation of granules. It has also been found advantageous to include in the tablet formulations a background dye of a water insoluble coloring material. This insoluble pigment or dye should contrast strongly with the positive color of the indicator such as the blue of the orthotolidine. It has been found that, in addition to the D and C Red No. 35, described above, that said compositions may be made with D and C Yellow No. 11 and D and C Orange No. 15.

The tablet compositions of this invention may also include as a buffer an acetate such as calcium, sodium, potassium, lithium acetate and the like and a water soluble solid acid which is stronger than acetic acid so that upon hydrolysis it can react with the aforementioned acetate salt to produce acetic acid. Examples of such acid are citric, fumaric, itaconic, maleic, malic, malonic and mandelic.

To summarize with respect to the tablet embodiment of this invention, the test composition will comprise the following:

Constituent	Preferred range (parts by weight)
Indicator	50-150
Organic hydroperoxide	150-300
Buffer	150-300
Excipient	2,000-5,000
Water insoluble dye	5-10

With respect to the preferred embodiment, that embodiment wherein the active components are impregnated upon a bibulous carrier such as a filter paper strip the test composition will comprise the following:

Constituent	Preferred range (parts by weight)
Indicator dye	15-25
Organic hydroperoxide	20-70
Buffer	50-400
Stabilizer	5-10

To summarize briefly, the instant invention relates to an improved test composition for the detection of blood which comprises an organic hydroperoxide, an indicator material capable of accepting oxygen from the hydroperoxide which is released catalytically by the prosthetic group of hemoglobin in blood and a buffer for maintaining the pH of the material being tested to within a pH of about 4 to 7. The test compositions of this invention may be in the form of a strip of bibulous material such as a paper strip upon which the active ingredients are impregnated or the active ingredients may be combined with suitable excipients and an acetic acid forming combination.

What is claimed is:

1. A test composition in dry solid form for the detection of blood which comprises an organic hydroperoxide selected from the group consisting of cumene hydroperoxide, diisopropylbenzene hydroperoxide and paramenthane hydroperoxide, an indiactor capable of accepting the catalytic transfer of oxygen from the hydroperoxide by means of the prosthetic group of hemoglobin and becoming oxidized to its colored form, and a buffer maintining the pH of material being tested to one within a range of from 4 to 7.

2. A test composition for the detection of blood which comprises a compression of an organic hydroperoxide selected from the group consisting of cumene hydroperoxide, diisopropylbenzene hydroperoxide and paramenthane hydroperoxide, an indicator capable of accepting the catalytic transfer of oxygen from the hydroperoxide by means of the prosthetic group of hemoglobin and becoming oxidized to its colored form, a buffer maintaining the pH of material being tested to one within a range of from 4 to 7, an excipient and a combination of an acetate salt and a water soluble acid capable of combining with said acetate salt upon hydrolysis to form acetic acid.

3. A test composition in dry solid form for the detection of blood which comprises an organic hydroperoxide selected from the group consisting of cumene hydroperoxide, diisopropylbenzene hydroperoxide and paramenthane hydroperoxide, an indicator selected from the group consisting of the derivatives of aniline which are capable of accepting the catalytic transfer of oxygen from the said hydroperoxide by means of the prosthetic group of hemoglobin and thereby becoming oxidized to its colored form, and a buffer for maintaining the pH of the material being tested to one within a range of from 4 to 7.

4. A test composition in accordance with claim 3 wherein said organic hydroperoxide is present in an amount between about 20 and 70 parts by wt., wherein said indicator is present in amounts between about 15 and 25 parts by wt., and wherein said buffer is present in amounts between about 50 and 400 parts by wt.

5. A test composition in accordance with claim 3 wherein said organic hydroperoxide is cumene hydroperoxide.

6. A test composition in accordance with claim 3 wherein said organic hydroperoxide is diisopropylbenzene hydroperoxide.

7. A test composition in accordance with claim 3 wherein said organic hydroperoxide is para-menthane hydroperoxide.

8. A test composition in accordance with claim 3 wherein said indicator material is orthotolidine.

References Cited in the file of this patent

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