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DIAGNOSTIC COMPOSITION AND METHOD

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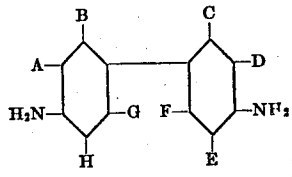
The present invention relates to diagnostic compositions and, more particularly, to diagnostic compositions suitable for use in the qualitative detection and semi-quantitative estimation of blood in body fluids, such as urine, vomitus, gastric contents, semen, cerebrospinal fluid and in feces. It has for its object to provide a simple, rapid and convenient method for performing this test with a high degree of accuracy and specificity, and one that can readily be used by the average physician without laboratory equipment, supplies of fresh chemicals or specialized analytical training.

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.

However, the methods heretofore employed for the detection of occult blood require some technical ability, experience and fresh supplies of reagents which are not always available to the average physician. Thus, the benzidine reaction which is capable of great accuracy and is one of the most delicate tests for the detection of occult blood, requires that a fresh solution of benzidine in glacial acetic acid be prepared daily. Since these solutions change rapidly, especially in contact with light, they rapidly lose their sensitivity and must be discarded. (Journal of Biological Chemistry, volume 19, page 445, 1914; Zentralblatt fur Chirurgie, volume 41, No. 28, 1914.)

Ruttan and Hardisty (Canadian Medical Association Journal, Nov. 1912; Biochemical Bulletin, volume 2, page 225, 1913) have described a modification of the benzidine reaction, using o-tolidine dissolved in glacial acetic acid. This solution is said to remain stable for one month. It has, however, been my experience that after one week, the solution of o-tolidine becomes too insensitive for use since it will no longer detect traces of occult blood that will give a definite positive reaction with a fresh o-tolidine solution.

The "benzidine" reaction for the detection of occult blood is based, in general, on the reaction of the peroxidase present in the blood with the hydrogen peroxide of the reagent to form "active" oxygen. The benzidine acts as an oxygen acceptor and is readily oxidized to a colored quinonoid form. In general, any compound of the general formula:

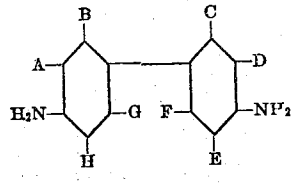


where A, B, C, D, E, F, G and H are members of the group consisting of hydrogen and alkyl radicals, may be used as an oxygen acceptor in the "benzidine" reaction. Examples of such members are benzidine, orthotolidine, metatolidine, etc.

The present invention has for its further object to provide a means whereby an unskilled person may perform a qualitative test for occult blood in body fluids or feces without the use of any equipment whatsoever. It will enable the average physician to perform such a test at the patient's bedside, by adding to the specimen to be tested a measured amount of a dry composition which remains stable and sensitive over long periods of time under all ordinary conditions of temperature and humidity.

The basis of the present invention is the finding that such compositions of high sensitivity, good specificity and great stability can be obtained by mixing, in the dry state:

(*a*) A compound of the general formula:



where A, B, C, D, E, F, G and H are members of the group consisting of hydrogen and alkyl radicals.

(*b*) A member of the group comprising the peroxides, perborates and persulfates of the alkali metals and the alkali-earth metals, and

(*c*) a member of the group of solid compounds which contain, when reacted in aqueous solution, at least one acidic hydrogen ion.

When such composition is added to an aqueous body fluid, or to a suspension of feces in water, the solid compound containing at least one acidic hydrogen atom will react with the peroxide (or perborate or persulfate) to form hydrogen peroxide. In the presence of blood peroxidase, "active" oxygen will be formed which will oxidize the

benzidine acceptor to the characteristic blue or greenish colored quinonoid derivative.

Typical members of the group of solid compounds which contain, when reacted in aqueous solution, at least one acidic hydrogen ion are citric acid, tartaric acid, oxalic acid, monobasic sodium phosphate, monobasic calcium phosphate, potassium pyrosulfate, sodium pyrophosphate, sodium bisulfate, etc., etc. Members of this group, when reacted in aqueous solution with a member of the group consisting of the peroxides, perborates and persulfates of the alkali metals and the alkali-earth metals, will form hydrogen peroxide at a slow and steady rate.

To determine qualitatively whether occult blood is present in a specimen, one drop of the material to be tested (in the case of feces—an aqueous suspension of the specimen) is deposited on a measured quantity of the dry composition, e. g. a five-grain tablet. In the presence of blood, a blue or green colored spot will form within a short time. If, after five minutes, there is no change of color, the specimen is free of occult blood. Alternatively, the dry composition may be suspended in water and mixed with the material to be tested. In the presence of occult blood, a blue or green color will be obtained within five minutes.

A semi-quantitative estimation of the amount of occult blood present in the specimen may be made by measuring the time required for the first definite blue or green coloration to be observable. A definite coloration within thirty seconds is reported as four plus, thirty seconds to one minute—three plus, one minute to two minutes—two plus, two minutes to three minutes—one plus, three minutes to five minutes—plus minus. If no definite coloration is obtained after five minutes, the test is reported as negative.

It is obvious, of course, that diluents, excipients, dispersing agents, auxiliary substances and coating materials may be incorporated into these compositions without changing the basis of the present invention. Diluents such as talc, kaolin and gypsum may be added to obtain a tablet with better keeping qualities, or a more stable product. Excipients such as lactose, starch or flour may be added to facilitate the formation of granules. Dispersing agents such as saponin, gelatin, agar, soap, alkali-metal silicates, tragacanth or gum arabic may be added to accelerate the disintegration of the tablet when placed into fluid. Auxiliary agents, such as coloring matters or perfumes may likewise be added. To improve the keeping qualities of these compositions in tablet form, they may be covered with a water-dispersible varnish or wax. These compositions may be used in the form of powder, or granules, or dispensed in capsules, or they may be pressed into tablets of such size as is convenient for the performance of a single test.

The following examples of typical compositions covered by this invention are intended to define and illustrate but in no way to limit this invention to the reagents, proportions or conditions described therein. Obvious modifications will occur to any person skilled in the art.

Example I

	Grams
Anhydrous granular citric acid	1000
Benzidine, chemically pure	100
Barium peroxide, heavy powder	250
Talc powder	250

are intimately mixed until homogeneous. The mixture is then compressed into five-grain tablets.

The sensitivity of this composition is such that it will detect one part of blood in one million parts of urine or one and a half million parts of water.

Example II

	Grams
Sodium acid pyrophosphate	1000
Orthotolidine, chemically pure	50
Sodium perborate monohydrate	200
Kaolin powder	500

are intimately mixed until homogeneous. The mixture is then compressed into five-grain tablets.

The sensitivity of this composition is such that it will detect one part of blood in eight hundred thousand parts of urine or one million parts of water.

Having described my invention, what I claim and desire to protect by Letters Patent is:

1. Diagnostic compositions comprising in dry solid form a member of the group consisting of benzidine, ortho-tolidine and metatolidine, a member of the group consisting of the peroxides, perborates and persulfates of the alkali metals and the alkali-earth metals and a solid compound which contains at least one available acidic hydrogen atom.

2. Diagnostic compositions comprising in dry solid form a member of the group consisting of benzidine, ortho-tolidine and meta-tolidine, barium peroxide and a solid compound which contains at least one available acidic hydrogen atom.

3. Diagnostic compositions comprising in dry solid form a member of the group consisting of benzidine, ortho-tolidine and meta-tolidine, barium peroxide and citric acid.

4. Diagnostic compositions comprising in dry solid form benzidine, barium peroxide and citric acid.

5. Diagonstic compositions comprising in dry solid form orth-tolidine, sodium perborate and sodium acid pyrophosphate.

6. A process for testing for blood in fluids which consists in adding a measured amount of said fluid to a measured amount of a composition comprising in dry solid form a member of the group consisting of benzidine, ortho-tolidine and meta-tolidine, a member of the group consisting of the peroxides, perborates and persulfates of the alkali metals and the alkali-earth metals, and a solid compound which contains at least one available acidic hydrogen atom, whereby a detectable color-change results.

7. A process for testing for blood in fluids which consists in mixing a measured amount of said fluid with a suspension in water of a measured amount of a composition comprising in dry solid form a member of the group consisting of benzidine, ortho-tolidine and meta-tolidine, a member of the group consisting of the peroxides, perborates and persulfates of the alkali metals and the alkali-earth metals, and a solid compound which contains at least one available acidic hydrogen atom, whereby a detectable color-change results.

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