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2,799,660

BLOOD TEST COMPOSITION AND METHOD

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10 Claims. (Cl. 252-408)

This invention which relates to a diagnostic composition for detecting occult blood in animal fluids is a continuation-in-part of co-pending application, Serial No. 232,666, filed June 20, 1951, now abandoned.

The importance of occult blood detection in fluids of animal origin, such as urine, for example, is well established. However, the presently available methods for determining such blood are not entirely satisfactory in that either the sensitivity of the diagnostic compositions used is not great enough, or the means used are not reproducible as closely as would be desirable, or the materials used for determinations are not sufficiently stable for general use by a physician, for example.

The present invention is directed to a tablet which is adapted for use in determining occult blood in such materials as urine and the like or in fluids containing urine which tablet is superior to the tableted compositions now available in several important respects, namely, in that with the present tablet the time of reaction is much more uniform for any given concentration of blood, and furthermore, the sensitivity of the tablet within a given time is substantially and unexpectedly greater.

Generally, the tablet of our invention is formed of a mixture of a blood indicator such as, for example, o-tolidine, o-toluidine, benzidine, o-tolidine-dihydrochloride and similar compounds; an oxidizing agent of the peroxide type such as sodium carbonate peroxide, sodium perbor silicate, strontium peroxide, sodium pyrophosphate peroxide, barium peroxide, magnesium peroxide, and urea peroxide; an acetate such as calcium acetate, sodium acetate, potassium acetate, lithium acetate and the like; a water insoluble pigment or dye; and a water-soluble solid acid which is stronger than acetic acid so that it can react with the aforementioned acetate salt to produce acetic acid. Examples of such acids are citric, fumaric, itaconic, maleic, malic, malonic, and mandelic acids. Additionally, there must be present an effervescent couple comprising, for example, an acid in solid dry form and a carbonate or bicarbonate which is preferably any alkaline earth or alkali metal carbonate or bicarbonate such as sodium, potassium, and calcium carbonates and bicarbonates. Diluents and excipients of the kind conventionally used in preparation of tablets are likewise used in conjunction with the aforementioned materials.

The above composition is prepared in tablet form, each tablet containing conveniently 2 grains of material and being conveniently 1/8 inch thick, and about 1/4 inch in diameter. In determining the presence or absence of occult blood in a sample of urine a drop of the urine specimen is placed on a piece of dry filter paper, and when the drop has soaked into the paper a tablet prepared as above described is placed in the center of the drop and then two drops of water added to the tablet. With a positive test a ring of color appears on the filter paper surrounding the tablet, the color ranging from a very faint to a very deep blue, depending upon the concentration of blood in the sample. The higher the blood concentration, the deeper the color, and vice versa. The afore-

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said method of determining occult blood is found superior to the methods taught by the prior art, not only in the degree of sensitivity reached but also in the greater extent of reproducibility.

In connection with the composition and method of the present invention, it is absolutely essential that the composition contain, in addition to the blood indicator and an oxidizing agent, an acetate, a water soluble organic acid such as tartaric acid which is capable of releasing acetic acid when contacted with an acetate, and an effervescent couple. Without the co-presence of these materials neither the degree of sensitivity nor the degree of reproducibility of test results will be adequate.

While the formulation of our diagnostic composition may be varied in accordance with the procedures known to those skilled in the art, suitable formulations are as follows:

EXAMPLE I

Components of reagent mixture:	Parts by weight
o-Tolidine.....	From 0.5 to 4, preferably 1.
Strontium peroxide.....	From 5 to 15, preferably 9.
Tartaric acid.....	From 20 to 60, preferably 30.
Calcium acetate.....	From 20 to 75, preferably 60.
Corn starch.....	From 1 to 10, preferably 5.
Effervescent couple.....	From 5 to 20, preferably 10.

EXAMPLE II

Components of reagent mixture:	Parts by weight
o-Tolidine.....	From 0.5-4, preferably 1.
Strontium peroxide.....	From 5-15, preferably 7.5.
Succinic acid.....	From 20-75, preferably 40.
Calcium acetate.....	From 20-75, preferably 60.
Corn starch.....	From 1-10, preferably 5.
Effervescent couple.....	From 5-20, preferably 10.

EXAMPLE III

Components of reagent mixture:	Parts by weight
o-Tolidine.....	From 1-6, preferably 4.
Sodium perbor silicate.....	From 8-20, preferably 10.
Succinic acid.....	From 20-75, preferably 40.
Calcium acetate.....	From 20-75, preferably 60.
Corn starch.....	From 1-10, preferably 5.
Effervescent couple.....	From 5-20, preferably 10.

EXAMPLE IV

Components of reagent mixture:	Parts by weight
o-Tolidine.....	From 0.5-4, preferably 1.
Strontium peroxide.....	From 5-15, preferably 7.5.
Itaconic acid.....	From 20-75, preferably 40.
Calcium acetate.....	From 20-75, preferably 60.
Corn starch.....	From 1-10, preferably 5.
Effervescent couple.....	From 5-20, preferably 10.

EXAMPLE V

Components of reagent mixture:	Parts by weight
Benzidine hydrochloride.....	From 1-5, preferably 1.
Strontium peroxide.....	From 5-15, preferably 7.5.
Tartaric acid.....	From 20-60, preferably 30.
Calcium acetate.....	From 20-75, preferably 60.
Corn starch.....	From 1-10, preferably 5.
Effervescent couple.....	From 5-20, preferably 10.

EXAMPLE VI

Components of reagent mixture:	Parts by weight
o-Tolidine-dihydrochloride.....	From 1-5, preferably 1.
Strontium peroxide.....	From 5-15, preferably 7.5.
Tartaric acid.....	From 20-60, preferably 30.
Calcium acetate.....	From 20-75, preferably 60.
Corn starch.....	From 1-10, preferably 5.
Effervescent couple.....	From 5-20, preferably 10.

EXAMPLE VII

Components of reagent mixture:	Parts by weight
o-Tolidine.....	From 0.5-4, preferably 1.
Strontium peroxide.....	From 5-15, preferably 7.5.
Itaconic acid.....	From 20-75, preferably 30.
Potassium acetate.....	From 20-75, preferably 60.
Corn starch.....	From 1-10, preferably 5.
Effervescent couple.....	From 5-20, preferably 10.

The present invention is particularly useful in the determination of occult blood in the presence of urine and, in sharp contrast with diagnostic compositions of the prior art, is sensitive to the extent of detecting of one part blood in 100,000 of uncentrifuged urine.

The net effect of the effervescent couple and the acetate components of our formulation is to increase substan-

tially the sensitivity, reproducibility and the speed of the test procedure. This is illustrated by tests performed with the following four (4) compositions, each being prepared as a 2 grain tablet:

Tablet A

	Parts by weight
o-Tolidine	1
Strontium peroxide	9
Tartaric acid	30
Starch	5
Talcum	5

Tablet B

	Parts by weight
o-Tolidine	1
Strontium peroxide	9
Tartaric acid	30
Calcium acetate	60
Starch	5
Talcum	5

Tablet C

	Parts by weight
o-Tolidine	1
Strontium peroxide	9
Tartaric acid	30
Calcium acetate	60
Starch	5
Talcum	5
Effervescent couple (tartaric acid and sodium bicarbonate)	10

only in trace amounts sufficient to establish a color tint in the tablet. Thus for the water insoluble coloring material D and C Red No. 35 it has been found that approximately one part in 1,000 parts of the remaining composition is adequate. Other suitable colors are D and C Yellow No. 11 and D and C Orange No. 17. However, almost any insoluble pigment or dye which contrasts strongly with blue would be satisfactory unless it should in some other way interfere with the test as by reacting with another ingredient of the tablet. It is to the general formulations of Examples I to VII above as represented by tablets C and D that this application is related, the peculiar merits of such formulations being made especially evident by comparison with the performance of the other compositions of tablets A and B.

These tablets were tested as follows:

Blood was diluted with normal urine to prepare samples which contained blood in concentrations of 1:100,000, 1:20,000, and 1:40,000 parts of urine. To make the test one drop of the specimen was placed on a filter paper square laid out on a white tile. When the drop had soaked into the paper a tablet was placed in the center of the wetted area and then two drops of water added so that they fell on the tablet and ran over onto the paper. The examination of the test was made at the end of 2, 3, 4 and 5 minutes, the time being determined by a stop watch.

Each type table was tested a large number of times with each of the 3 concentrations of blood. The results are recorded in Table I.

TABLE I

Concentration of blood added to urine	Tablet A					Tablet B					Tablet C				
	No. Tests	Percent positive in—				No. Tests	Percent positive in—				No. Tests	Percent positive in—			
		2 min.	3 min.	4 min.	5 min.		2 min.	3 min.	4 min.	5 min.		2 min.	3 min.	4 min.	5 min.
1=10,000	100	90	99	100	-----	155	60	81	89	98	100	100	-----	-----	-----
1=20,000	100	7	29	43	50	230	63	80	88	95	100	100	-----	-----	-----
1=40,000	100	0	0	0	0	280	30	47	68	82	100	98	100	-----	-----

Tablet D

	Parts by weight
o-Tolidine	1
Strontium peroxide	9
Tartaric acid	30
Calcium acetate	60
Starch	5
Talcum	5
Effervescent couple	10
Water-insoluble pigment or dye	0.1

It will be noticed that tablet A is of the general type of prior art compositions such as are described, for example, in U. S. Patent 2,290,436, that tablet B is the composition of tablet A to which calcium acetate has been added and that tablet C is of the same composition as tablet A to which both calcium acetate and effervescent couple have been added.

Tablet D is the same composition as tablet C so modified as to include a water-insoluble pigment or dye. To incorporate the pigment or dye in the tablet it is simply necessary that it is dry and finely divided and it is then mixed intimately with the remainder of the composition prior to compressing the material into tablets. The amount of pigment required would obviously vary depending on its hiding power and opacity, therefore the amount considered most satisfactory for any given pigment must be determined by trial. However, the necessary amount is generally not very much, being present

The superior performance of tablet B over tablet A shows that the addition of calcium acetate increases considerably the sensitivity of the test. Further it is obvious that tablet C is more uniform in reaction time than tablet B and in addition the sensitivity of the tablet within a given time period is very substantially improved.

In the practice of our invention we have found that our test procedure which employs filter paper provides a much more sensitive test than other methods such as placing a drop of the sample directly on the tablet or dissolving the reagent mixture in water and adding it to the specimen. Under these latter conditions tablet A gives a negative test for blood at 1:0,000 and indeed only a very slowly formed positive with 1 part of blood in 1,000 parts of urine. The sensitivity of any of the compositions to blood dissolved in water is much higher than it is to blood in urine. Urine appears to contain a factor which inhibits the formation of color with o-tolidine, benzidine, etc., in the presence of blood and a peroxide. The use of the filter paper seems to a large extent to get around this difficulty and brings about a marked increase in sensitivity.

The tablets B and C were both compared by testing them with the sediments obtained by centrifuging samples of urine containing very small quantities of blood. In this case blood was diluted with normal urine to produce blood concentrations of 1 part in 50,000; 1 part in 100,000; 1 part in 300,000 and 1 part in 1,000,000 parts of urine. In making the test 15 cc. of one of the specimens

was centrifuged in a conical tube at moderate speed. The supernatant liquid was drawn off and the sediment which remained tested by placing 1 drop of it on filter paper and then proceeding with the test as previously described. The results of a series of tests are recorded in the following table.

TABLE II
[Tests on sediment after centrifuging.]

Concentration of blood added to urine	Tablet B					Tablet C				
	No. Tests	Percent positive in—				No. Tests	Percent positive in—			
		2 min.	3 min.	4 min.	5 min.		2 min.	3 min.	4 min.	5 min.
1:50,000.....	20	70	85	95	100	20	100	-----	-----	-----
1:100,000.....	50	72	96	98	100	20	100	-----	-----	-----
1:300,000.....	50	38	52	72	92	20	100	-----	-----	-----
1:1,000,000.....	75	32	39	49	61	40	92	95	95	95

It is again seen from the above results that with tablet C positive readings were obtained more consistently and reliably than when tablet B was used.

Notwithstanding the very meritorious results obtained by the use of tablet C as described above, there is some slight difficulty in deciding whether the test employing tablet C reads positive or negative when there is extremely little or no blood in the test sample. In these situations the subjective viewpoint of the person reading the result has considerable effect since he is not guided by any clearly defined color change.

When using such a tablet as represented by tablet C in the manner described above, the test is considered to be positive when blue color appears on the paper surrounding the reacting tablet. However, it is a fact that as the effervescent tablet reacts, the material of the tablet sloughs off onto the paper in the area immediately around the tablet. In places where the amount on the paper is relatively little it is sometimes difficult to decide whether the paper should be considered as having turned blue or whether the blue color which can be seen is some spilled over tablet material since there is a tendency for the test material to take on a little gray or very pale blue color. Further than this, even when there is no question as to which is material and which is clear paper there can be confusion from the shadows which the tablet throws on the paper because these often have a light grayish cast and are irregular in outline since the tablet on effervescing has lost its usual outline.

We have found that these difficulties can be eliminated and the use of the test very greatly improved by the device of coloring the material of the test tablet with a coloring material which contrasts with the blue color of a positive test in such a way that the contrasting color remains with the reagent material and does not diffuse out on its own into the paper and so hide the appearance of a possible positive reaction. To achieve these objects the added coloring material should be a water-insoluble pigment or dye so that it will not leach away from the reagent material with which it is combined in the tablet when the whole mass is wetted as described in the test. In addition, in order that the coloring material should perform its function of defining clearly where the reagent material is at the completion of the test, it is important that its color contrast strongly with the blue color which forms on the paper when the test is positive and for this reason yellow, orange or red pigments are the most satisfactory. Further, the coloring materials should have the property of good opacity and hiding power so that they shall stand out well in contrast to blue and further they shall cover up any tendency of the material of the test itself to turn blue.

The advantage of coloring the tableted composition in

the manner described can be illustrated by the following record.

A number of urines were obtained which were judged by microscopic examination of the sediment to contain no red blood cells and could therefore be expected to give negative tests, that is there should be no blue color-

ing on the paper surrounding the tablet material. A series of tests were carried out in which a number of operators tested the two tablet compositions with each of the urines but in such a manner that they did not run the tests with the two tablets concurrently and so were unable to compare their findings with one composition against their findings with another on the same urine sample. Altogether 600 tests were carried out with each composition, the readings being taken at the end of 2 minutes. With tablet C, the tests on these negative urines were considered to show at least a faintly positive test in 75 cases or 12.5% of the tests run, whereas in the series of tests performed with the composition containing a red pigment, tablet D, in the manner described above, only 9 tests or 1.5% of the total were considered as showing anything but a clear cut negative test.

The tablet of the present invention thus provides an improved means for detecting blood in fluids such as urine by which means the virtual elimination of false positive readings is achieved in the difficulty detectable areas of extremely low concentration or complete absence of blood with resulting marked improvement in test reliability.

Having thus described our invention, we claim:

1. A blood-detecting diagnostic composition in tablet form comprising a compound selected from the group consisting of o-tolidine, o-toluidine, benzidine and o-tolidine dihydrochloride, a peroxide which will liberate hydrogen peroxide, a water soluble solid acid stronger than acetic acid, an acetate salt which will react with the said solid acid to produce acetic acid, an effervescent couple, and a water-insoluble material contrasting in color with the color formed by said color responsive compound in the presence of blood.

2. A diagnostic composition in tablet form comprising a member of the group consisting of o-tolidine, o-toluidine, benzidine and o-toluidine dihydrochloride, a peroxide which will liberate hydrogen peroxide, water soluble solid acid stronger than acetic acid an acetate salt which will react with the last said acid to produce acetic acid, an effervescent couple, and a water-soluble material contrasting in color with the color formed in combination with blood by the selected member of said group.

3. A diagnostic composition in tablet form for detecting blood in the presence of urine comprising by weight, from 0.5 to 4 parts o-tolidine, 5 to 15 parts strontium peroxide, 20 to 60 parts tartaric acid, 20 to 75 parts calcium acetate, 1 to 10 parts corn starch, 5 to 20 parts of a solid effervescent couple, and a minor amount of a water-insoluble material contrasting in color with the color formed in combination with blood by said o-tolidine.

4. A diagnostic composition in tablet form for detecting blood in the presence of urine comprising 1 part o-tolidine, 9 parts strontium peroxide, 30 parts tartaric acid, 60 parts calcium acetate, 5 parts corn starch, 10 parts of a solid effervescent couple, and a minor amount of a water-insoluble material contrasting in color with the color formed in combination with blood by said o-tolidine.

5. A blood detecting diagnostic composition in tablet form comprising a compound selected from the group consisting of o-tolidine, o-toluidine, benzidine and o-tolidine dihydrochloride, a peroxide which will liberate hydrogen peroxide, an acetate selected from the group consisting of calcium acetate and alkali metal acetates, a water soluble acid stronger than acetic acid, and an effervescent couple.

6. A diagnostic composition in tablet form for detecting blood in the presence of urine comprising by weight from 0.5 to 4 parts o-tolidine, 5 to 15 parts of strontium peroxide, 20 to 60 parts of tartaric acid, 20 to 75 parts of calcium acetate, 1 to 10 parts cornstarch, and 5 to 20 parts of a solid effervescent couple, said tablet effecting a color change on urine wetted filter paper when placed on such paper and the tablet contacted with a small amount of water.

7. A diagnostic composition in tablet form for detecting blood in the presence of urine comprising 1 part of o-tolidine, 9 parts of strontium peroxide, 30 parts of tartaric acid, 60 parts of calcium acetate, 5 parts of cornstarch, and 10 parts of a solid effervescent couple, the tablet effecting a color change on urine-wetted filter paper upon which said tablet is placed, after a small amount of water is placed on said tablet.

8. A diagnostic composition in tablet form comprising a member of the group consisting of o-tolidine, o-toluidine, benzidine, o-tolidine dihydrochloride, a peroxide for liberating hydrogen peroxide, a water soluble solid acid stronger than acetic acid and an effervescent couple, an acetic salt which will react with the said solid acid to produce acetic acid, said tablet effecting a color change on urine wetted filter paper when placed thereon, and said tablet is contacted with a small amount of water, when said urine contains blood.

9. A method of determining occult blood in urine which comprises wetting a portion of dry filter paper with urine, placing on the urine wetted portion of said paper a tablet composed of a mixture comprising by weight from about 0.5 to 6 parts of a compound selected from the group consisting of o-tolidine, o-toluidine, benzidine and o-tolidine dihydrochloride, a peroxide which liberates hydrogen peroxide, an acetate selected from the group consisting of calcium acetate and alkali metal acetates, about 20 to 75 parts by weight of a water soluble solid acid stronger than acetic acid, and about 5 to 20 parts of an effervescent couple and then adding a small amount of water to said tablet.

10. The composition of claim 2 wherein the said water-insoluble material is selected from the group consisting of red pigments, yellow pigments, and orange pigments.

References Cited in the file of this patent

UNITED STATES PATENTS

2,290,436	Kamlet	July 21, 1942
2,387,244	Compton et al.	Oct. 23, 1945

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 2,799,660

July 16, 1957

Richard S. Nicholls et al.

It is hereby certified that error appears in the printed specification of the above numbered patent requiring correction and that the said Letters Patent should read as corrected below.

Column 6, line 60, before "water" insert -- a --; line 63, after "acetic" strike out the comma; same line, for "water-soluble" read -- water-insoluble --; column 8, line 6, for "acetic" read -- acetate --.

Signed and sealed this 8th day of October 1957.

(SEAL)

Attest:

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Commissioner of Patents