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DIAGNOSTIC COMPOSITION

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The present invention relates to new and improved diagnostic compositions useful for the qualitative detection and quantitative estimation of ketone bodies in body fluids, particularly acetone bodies in the urine.

More specifically, the invention relates to diagnostic compositions in solid dry form, preferably tableted in suitable sized tablets, which composition can readily be used, even by unskilled persons, rapidly to detect the presence of acetone in urine without evolvement of ammonia, with ready distinction between positive and negative tests, and without the use of equipment or apparatus other than some means of obtaining a drop of test fluid.

In the metabolism of fat, acetone bodies or ketone bodies are regarded as normal intermediate compounds which are subsequently oxidized to carbon dioxide and water. The ketone bodies include acetone, acetoacetic acid (beta-ketobutyric acid or diacetic acid) and beta-hydroxybutyric acid. Under normal circumstances, no significant quantity of these ketone substances appears in the urine. However, if there is an excessive metabolism of fat, the intermediate acetone bodies accumulate in the blood and are excreted in the urine in variable amounts. In diabetes mellitus such an excessive fat metabolism occurs and many of the symptoms of this disease can be ascribed to the toxic effects of the acetone bodies. The medical profession is well aware of the usefulness in diagnosis of tests for acetone bodies in the urine in diabetes mellitus cases. Acetone bodies also occur in the urine in other well recognized disturbances of the metabolism, and in such cases it is also important to carry out tests for detection of these substances.

A variety of reagents and techniques have been used or proposed in the past for the detection of acetone bodies in urine. A number of such reagents and techniques have involved the use of a water soluble nitroprusside as a reactive ingredient or agent. In one particular reagent formulation, the nitroprusside reaction is carried out in the presence of ammonia in order to develop particular colorations (see United States Patent No. 2,186,992 to Fortune). An improvement over the Fortune type formulation is disclosed in copending application Serial No. 12,699, filed March 2, 1948, now Patent No. 2,509,140 issued on May 23, 1950, by Alfred H. Free, assigned to the assignee of the present application. Application Serial No. 12,699 discloses formulations for detection of acetone bodies in the urine which contain water soluble nitroprusside, an aliphatic amino acid and an alkaline material.

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It was found, according to application Serial No. 12,699, that when the soluble nitroprusside is present in alkaline solution with an aliphatic amino acid (e. g. glycine), a diagnostic composition is provided which is particularly adapted for the detection of acetone bodies in urine without evolvement of ammonia.

According to the present invention, it has been discovered that the addition of lactose to the diagnostic formulation set forth in application Serial No. 12,699 greatly enhances the usefulness and reliability of that type of diagnostic formulation.

The object of the present invention, generally stated, is the provision of improved diagnostic compositions in stable dry form, preferably as tablets, which can be used even by an unskilled person conveniently to give an accurate qualitative test for, and a quantitative estimation of, the presence of acetone bodies in urine, which test clearly distinguishes between positive and negative specimens, even when the quantity of acetone is small, so as to give only what is known as a "trace positive."

An important object of the invention is the provision of a stable dry diagnostic composition for the detection of acetone bodies in urine which contains a water-soluble nitroprusside, an aliphatic amino acid, and an alkaline material, as active ingredients, and in addition contains lactose which serves to prevent color change in the case of acetone-negatives and to keep the colors in the whole range of positives very truly and characteristically lavender, so that there will be no chance for confusion between positive and negative specimens, even where there are only trace amounts of acetone bodies in the positives.

Other objects of the invention will in part be obvious and will in part appear hereinafter.

The following example discloses a presently preferred embodiment of the invention.

Example I

The following formulation is uniformly composed by known blending and mixing techniques, and is then tableted in known manner:

	Parts by weight
Glycine	4.5
Sodium nitroprusside	0.5
Disodium phosphate (anhydrous)	47.0
Sodium borate	36.5
Lactose	10.0
Corn starch	1.25
Magnesium stearate	0.25
	100.00

In use, a drop of the urine specimen to be tested is dropped onto a tablet made from the above formulation. If the specimen contains acetone bodies, a characteristic and definite lavender coloration is quickly produced which is highly characteristic and specific to acetone positive specimens. Trace positives (i. e. positive specimens with only a trace amount of acetone bodies), will also give this characteristic lavender coloration.

The depth of the shade or hue of the lavender coloration can be used as a basis for estimating quantitatively the amount of acetone bodies present in the specimen. Thus, a color chart may be supplied which is graduated into different shades or hues of lavender corresponding to known concentrations of acetone bodies. By using such a chart in connection with the testing of a specimen, not only can a determination be made as to whether or not a specimen is negative or positive, but further, positive specimens can be compared with the standard color chart to obtain a fairly accurate estimation of the quantity of acetone bodies present.

The application of a negative specimen to a lactose-free test tablet or composition causes the tablet, or composition, to take on a gold coloration which progressively changes a brown shade as time passes. A positive specimen applied to a lactose-free test tablet produces a lavender coloration which, with the passage of time becomes muddied with brown.

Not only do the lactose containing tablets result in the production of a stable cream color when negative specimens are applied thereto, but in the case of positive reactions a true lavender color is obtained which remains constant for 15 to 30 minutes or more, after the maximum color intensity is reached. Thus an important advantage of a lactose-containing composition, as hereinbefore described, lies in the fact that the time within which a test must be read is substantially extended. Furthermore, since the hue of the final color is stable, the use of a color chart is made much less difficult and more accurate than heretobefore.

The fact that no color changes are obtained with negative specimens when the formulations of the present invention are used is an important feature of particular significance in litigation cases where the reliability of an acetone test is brought under close scrutiny. Obviously, the reliability and probative value of any test procedure is greatly enhanced if it can be unequivocally testified that no coloration is obtained with a negative specimen on the one hand, whereas all positive specimens give a characteristic lavender coloration on the other hand. Such testimony is much more forceful and influential than testimony in which it would be necessary to state that coloration is obtained even with negative specimens, but such coloration is different from that obtained with positive specimens.

Example 2

By omitting the sodium borate, corn starch and magnesium stearate from the formulation in Example 1, a dry powder formulation may be prepared which, as such, is suitable for use in the detection of acetone bodies in urine. However, it is not adapted to be tableted.

Although the formulation set forth in Example 1 is the presently preferred one for the preparation of reaction test tablets, and the formulation set forth in Example 2 is the preferred one for reaction test powder, it will be understood that those skilled in the art will be able to prepare a large number of other specific formulations embodying our present invention, but which may be somewhat different in respect to specific proportions than the formulations set forth in Examples 1 and 2.

In our formulations, the reactive ingredients may be considered to be the water-soluble nitroprusside, and the aliphatic amino acid. For practical purposes and availability, sodium nitroprusside is the preferred soluble nitroprusside and glycine is the preferred aliphatic amino acid. However, all of the alkali metal nitroprusides are water soluble, and any one of them may be used such, for example, as the potassium nitroprusside. Thus, potassium nitroprusside in a molecularly equivalent amount may be substituted for the sodium nitroprusside in Examples 1 and 2. The glycine may be replaced with another aliphatic amino acid such as alanine, glutamic acid, arginine, aspartic acid and lysine.

In addition to the amino acid and the water-soluble nitroprusside, two other ingredients are essential in our formulations. One is an alkaline material which is necessary in order to have the test reaction carried out under alkaline conditions. Generally, any alkaline material which will produce this result may be used, it of course being necessary that the alkaline material be one that permits the preparation and storage of a stable diagnositic composition, which does not interfere with the test reaction, and which is not reactive with either the glycine or the soluble nitroprusside. Although anhydrous disodium phosphate is the preferred alkaline material for use in our reagent test compositions, other dry alkaline solids which may be used include, the alkalimetal carbonates and hydroxides, trisodium phosphate, dipotassium phosphate, and the like. Instead of using a single alkaline ingredient, it is possible to use a mixture of the ingredients, or the alkaline material may be provided in combination with the aliphatic amino acid. For example, the alkali metal salts of the amino acids, such as potassium or sodium glycinate, may be used both for providing the amino acid and for providing the alkalinity.

The other essential ingredient for our preferred test reagent composition is lactose. Other stabilizing materials such as sucrose or dextrose may be used; however, we have found that generally they are not as desirable as lactose because they appear to have an inhibiting effect on color formation. That is to say, while these less desirable materials may prevent the negatives from turning gold and the positives from turning brown, the intensity of the lavender is reduced.

In addition to the four essential ingredients, diluents may be added as desired, in order to obtain better tableting properties, improved color (i. e. whiteness), improved free-flowing properties, etc.

The exact proportions of the amino acid, soluble nitroprusside, alkaline material and lactose, are not particularly critical within broad limits, and the ingredients may be employed in a rather wide range of proportions. However, as the proportions of the ingredients in the diagnostic formulations are varied, it has been noted that the properties of the formulations are altered in one or more of the following respects:

1. The speed of reaction of the reagent composition with acetone positive urines.

2. The sensitivity of the reagent detecting small quantities of acetone bodies.

3. The stability of the reagent formulations when stored under adverse environmental conditions.

When sodium nitroprusside is used as the water soluble nitroprusside in our diagnostic compositions, this ingredient should not comprise over approximately 5% of the total bulk of the mixture and should represent at least 0.04% by weight of the total bulk. The preferred range of concentration for this ingredient is from 0.5 to 1%. Other soluble nitroprussides are preferably used in molecularly equivalent concentration.

The relative proportions of disodium phosphates and glycine or their equivalents as indicated above, in the reagent compositions can vary quite widely and still the reagent compositions will give satisfactory results with both acetone positive and acetone negative specimens. For instance, mixtures where the amount of glycine was about half that of the disodium phosphate gave clear cut tests. On the other hand, good tests were also obtained when the amount of glycine was only 1% of that of the disodium phosphate.

The concentration of the lactose is not particularly critical. Enough of the lactose should be used so that the beneficial results contributed by it are fully obtained. On the other hand, if too much lactose is employed, there will be a tendency to slow the reaction somewhat. Desirably, enough lactose is used in any particular formulation to obtain the advantages contributed by it while not using enough appreciably to slow the test reaction.

In general the optimum ranges of concentration of the four preferred ingredients are as follows:

	Percent by weight
Sodium nitroprusside	0.04– 5.0
Glycine	1.00–20.0
Disodium phosphate	20.00–80.0
Lactose	5 –25

When diluents or other inactive ingredients are employed, they do not serve to affect the above preferred concentrations of the required components except that the sodium nitroprusside or its equivalent should represent at least 0.04% by weight of the total bulk. It will of course be understood that in any formulation equivalent components or ingredients may be substituted in equivalent concentrations, as pointed out above.

Having thus fully described our invention and set forth formulations representing the preferred embodiments thereof, what is claimed as new is:

1. A diagnostic composition in solid dry form for detecting acetone bodies in urine, comprising from 0.04 to 5.0% of an alkali metal nitroprusside, an alkali metal glycinate and lactose.

2. A diagnostic composition for detecting acetone bodies in urine, comprising from 0.04 to 5.0% of a water-soluble nitroprusside, an aliphatic amino acid, an alkaline material, and a sugar.

3. A diagnostic composition for detecting acetone bodies in urine, comprising from 0.04 to 5.0% of a water-soluble nitroprusside, an aliphatic amino acid, an alkaline material, and a sugar selected from the group consisting of lactose, dextrose and sucrose.

4. A diagnostic composition in solid dry form for detecting acetone bodies in urine, comprising by weight, from 0.04–5.0% of sodium nitroprusside, from 1–20% of glycine, from 20–80% of disodium phosphate, and from 5 to 25% of lactose.

5. A diagnostic composition in solid dry form for detecting acetone bodies in urine, comprising by weight, approximately 4.5 parts of glycine, approximately 0.5 part of sodium nitroprusside, approximately 47 parts of anhydrous disodium phosphate, and approximately 10 parts of lactose.

6. The composition of claim 5 containing in addition, approximately 36.5 parts of sodium borate, approximately 1.25 parts of corn starch, and approximately 0.25 parts of magnesium stearate.

7. Diagnostic tablets tableted from the composition called for in claim 4.

8. A diagnostic composition for detecting acetone bodies in urine, comprising from 0.04 to 5.0% of a water-soluble nitroprusside, lactose and a component selected from the group consisting of (*a*) a mixture of an alkaline material and an aliphatic amino acid and (*b*) an alkaline alkali metal salt of an aliphatic amino acid.

9. A diagnostic composition for detecting acetone bodies in urine comprising from 0.04 to 5.0% of a water-soluble nitroprusside, a member of the group consisting of (*a*) an alkaline material plus an aliphatic amino acid and (*b*) an alkaline alkali metal salt of an aliphatic amino acid, and a sugar in quantity sufficient to enhance the stability of color developed by said composition in the presence of acetone bodies.

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