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DIAGNOSTIC DEVICE

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This invention relates to an improved diagnostic composition and to a method for its preparation. In particular, this invention is concerned with a diagnostic test useful in qualitative detection and quantitative determination of ketone bodies in body fluids, especially acetoacetic acid (beta-ketobutyric acid) in urine. More particularly, this invention is concerned with a diagnostic test reagent composition which is incorporated upon a bibulous carrier.

The fats utilized by the body normally undergo complete oxidation with formation of carbon dioxide and water. Since the fatty acid molecules have long chains of carbon atoms, it is theorized that a large number of intermediate products are formed during the oxidation and under normal circumstances these are rapidly further oxidized. Findings in the field of fat metabolism suggest that during the course of oxidation of the fatty acid chain the carbon atoms are split off in pairs, or in other words, oxidation takes place at the β-carbon atom. In certain abnormal physical conditions the oxidation of fat is incomplete and certain products such as acetoacetic acid appear in the urine. These substances are called "ketone bodies" and their appearance creates a condition which is called ketosis. Ketosis occurs typically in diabetes mellitus, but it also occurs as a result of other abnormal conditions, e.g., fasting, hyperpituitary activity, etc. Under these abnormal conditions ketone bodies tend to accumulate in the blood and, because the rental threshold for them is low, they appear in the urine. The healing arts have long recognized the usefulness of tests for ketone bodies in the urine, hence it is considered extremely desirable to provide a simple and economical test for the qualitative and quantitative determination of ketone bodies in the urine which may be advantageously used by the laboratory technician as well as the physician.

A variety of reagents and techniques have been used or proposed in the past for the detection of ketone 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 present of ammonia in order to develop particular colorations (see, for example, U.S. Patent 2,186,902 to Fortune). An improvement over the Fortune-type formulation is disclosed in U.S. Patent 2,509,140 to Alfred H. Free, and assigned to the assignee of the present application. This patent discloses formulations for the detection of ketone bodies in urine which contain water soluble nitroprusside, an aliphatic amino acid and an alkaline material. It was found, according to the patent, that when a 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 ketone bodies in urine without evolution of ammonia.

An improvement of the foregoing test composition is described and claimed in U.S. Patent 2,577,978, issued December 11, 1951, to Nicholls and Fonner and assigned to the assignee of the present application. It was discovered by these patentees that incorporation of lactose or similar sugars into the diagnostic composition of U.S. Patent 2,509,140 greatly enchanced the utility and reliability of the diagnostic composition.

A still further improved test composition is described in U.S. Patent 2,990,253, issued June 27, 1961, to Robert R. Smeby and assigned to the assignee of the present application, which provides a test composition in the form of bibulous strips or sticks. However, because of the instability of nitroprusside in an aqueous alkaline medium, the nitroprusside must be kept separated not only during the impregnation of the carrier but until such time as the test is ready for use. A method was discovered by the patentee of U.S. 2,990,253 of achieving the necessary separation, which separation was effected by first applying the nitroprusside to the carrier in an acidic, aqueous medium thus preserving the stability of the compound and, after drying, dipping the carrier into a nonaqueous solution of organic bases such as various amines or aminoalcohols to achieve the necessary alkalinity.

The volatility and hygroscopicity of the amine constituents of the prior art formulations, however, are undesirable features of that test. Further, the selection of amines or aminoalcohols or mixtures thereof is rendered difficult in that all amines and aminoalcohols are not operable. In addition, in all of the prior compositions and methods, it has been difficult if not substantially impossible to protect the nitroprusside ingredients from the deleterious effects of moisture and alkaline sodium phosphates during storage.

While the foregoing discussed patents have contributed greatly to the advancement of the art of diagnosing for ketonuria and other disturbances of metabolism evidenced by the presence of ketone bodies in the urine and the advances made have been worthwhile, none have completely solved the problem of the instability of sodium nitroprusside in an aqueous alkaline medium. Nitroprusside is stable only at a pH below 7 and is operable only in an alkaline medium at a pH over 8. In other words, most of the nitroprusside is destroyed so that no perceptible reaction with acetoacetic acid can be obtained under those circumstances. The commercial diagnostic methods made available in accordance with the disclosures thereof have, however, aided the physicians and clinicians in the diagnoses and control of the causes of ketonuria.

To summarize, the prior art teaches the use of a water soluble nitroprusside, an aliphatic amino acid and an alkaline phosphate buffer as essential ingredients of a test for ketone bodies and teaches the preparation of such diagnostic compositions in the form of bibulous strips or sticks and a method of preparing same.

From a commercial point of view the test compositions in the form of bibulous strips or sticks are highly preferred for the reason that such provide the diagnostician with a simple "dip and read" test. Such simple "dip and read" tests provide many advantages over prior known liquid or tabletted reagent compositions from the standpoint of absence of cumbersome equipment, ease and simplicity of test procedure, ease of disposal of test devices and rapidity of test procedure, to mention a few of the advantages.

In accordance with this invention, we have discovered an improved diagnostic composition and method of preparing such ketone diagnostic composition in "strip or stick" form which successfully overcomes the hereinabove enumerated disadvantages of the prior known compositions.

More specifically, we have discovered an improved composition for a ketone diagnostic in "stick" form which is tremendously stable, and resistant to the deteriorative effects of moisture and the alkaline sodium phosphates. In addition, we have discovered an improved method for preparing an improved diagnostic in "strip or stick" form comprising a two-step procedure which involves initially treating the bibulous carrier with

an aqueous phosphate-containing formulation and, secondly, impregnating the thusly treated carrier with a novel formulation comprising sodium nitroprusside and an organic film-forming polymer.

Among the numerous advantages provided by this invention, one is that the first step of the preparation may be carried out well ahead of the second step, i.e., the carrier impregnated with the phosphate-containing composition may be prepared and stored for periods of time prior to impregnation with the second formulation.

Broadly, the initial treating formulation comprises a buffer, providing a pH range of about 8–10, and an amino acid. By way of example of buffering systems useful in the compositions of this invention are tri- and disodium phosphates, borates, citrates, carbonates, ethylene diamine tetraacetate (sodium salt), etc. While any water soluble amino acid may be used in the compositions of this invention, in the preferred embodiment the amino acid is selected from the group of glycine and alanine.

The second treatment formulation comprises alkali metal nitroprusside, an organic film-forming compound and an organic solvent. The organic film-forming compounds called for in the compositions of this invention may be any organic film-forming compound which is soluble in the commonly used organic solvents, does not exhibit strong buffering capacity, and has a pH on the acid side, for example, polyvinylpyrrolidone-vinyl acetate copolymers; vinyl pyrrolidone-styrene copolymers; water solutions of acrylic copolymers; coploymers of methyl vinyl ether and maleic anhydride; polyethylene glycol; polyvinyl acetate; and interpolymers of methyl vinyl ether and maleic anhydride. From an economic standpoint and ease of handling, however, copolymers of polyvinylpyrrolidone-vinyl acetate are preferred. It is readily seen that the selection of an organic film-forming compound meeting the requirements of this invention is dictated solely by economic considerations.

Among the organic solvents found suitable for use in the compositions and method of this invention are dimethyl sulfoxide, methanol, ethanol and dimethyl formamide and mixtures thereof. In addition to the foregoing ingredients, we have found that it is desirable but not essential to include such diluent substances as chloroform, carbon tetrachloride, benzene, etc. and a wetting agent, for example, aerosol, diglycol laurate and organic phosphate esters of anionic detergents in ethanol which are known commercially as Gafac RE610 and Gafac RE510, and mixtures thereof. The diluent substances are useful to reduce hygroscopicity of the testing reagents, while the wetting agent aids in producing an even diffusion of color on the diagnostic stick.

The following examples will illustrate the improved diagnostic composition of the present invention, the scope of the invention not, however, being limited to the specific details of these examples:

Example 1.—Formulation of the impregnating solutions

A

$Na_3PO_4.12H_2O$	g.	210
Disodium phosphate, anhydrous	g.	90
Glycine	g.	187
Distilled water to	ml.	1000

B

Sodium nitroprusside, anhydrous	g.	8
Polyvinyl pyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml.	65
Dimethyl sulfoxide	ml.	380
Anhydrous ethanol	ml.	185
Chloroform	ml.	350
Organic phosphate ester of anionic detergent	ml.	17

PREPARATION OF IMPREGNATING SOLUTIONS

Solution A.—210 grams of trisodium phosphate, 90 grams of disodium phosphate and 187 grams of glycine were mixed together in the dry state. 750 ml. of boiling hot distilled water were added to the dry mixture and stirred until solution occurred.

Solution B.—8 grams of sodium nitroprusside were measured into a one liter volumetric flask. To this was added 65 ml. of polyvinylpyrrolidone/vinyl acetate copolymer and 185 ml. of anhydrous ethanol and the solution mixed thoroughly. 380 ml. of dimethyl sulfoxide were then added to the mixture with stirring until the nitroprusside was solubilized. 350 ml. of chloroform, 17 ml. of a 10% anionic detergent (organic phosphate ester) in anhydrous ethanol were then added to the solution.

PREPARATION OF REAGENT STRIPS

Bibulous "sticks," that is, absorbent paper cut into narrow strips having dimensions of about 3" x 1/5" x 0.029", imprinted with a water impervious barrier portion of about 7/16" from the tip were dipped into impregnating Solution A, followed by drying in a drying tunnel at a temperature of about 100° C., for 13 minutes. After drying, the strips were similarly dipped into Solution B and dried at about 85° C. for 11 minutes in a forced draft oven. The finished impregnated strips are light buff in color.

In preparing the formulations for use in the diagnostic strips of this invention, we have found the optimum ranges of essential ingredients to be about 0.5–25 grams sodium nitroprusside; 11.7–585.0 grams amino acid; 18.8–940.0 grams buffer, comprising trisodium phosphate in the range of about 13.2–657.0 grams and a range of about 5.6–282.5 grams disodium phosphate; and 4.1–202.5 grams organic film-forming material.

The following examples are illustrative of other formulations prepared in accordance with this invention:

Example 2

Solution A:		
Na_2HPO_4, anhydrous	gm.	58.4
Glycine	gm.	20.0
Distilled water	ml.	180.0
Solution B:		
Sodium nitroprusside	gm.	1.0
Anhydrous methanol	ml.	100.0
Diglycol laurate	ml.	1.0
Polyvinylpyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml.	20.0

Example 3

Solution A:		
Sodium borate	gm.	5.0
Glycine	gm.	10.0
Distilled water	ml.	100.0
Solution B:		
Sodium nitroprusside	gm.	0.5
Anhydrous methanol	ml.	9.0
Anhydrous ethanol	ml.	40.0
Polyvinylpyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml.	3.0
Diglycol laurate	ml.	2.0

Example 4

Solution A:		
Glycine	gm.	25.0
Na_2CO_3	gm.	30.0
Distilled water	ml.	100.0
Solution B:		
Sodium nitroprusside	gm.	0.5
Anhydrous methanol	ml.	9.0
Anhydrous ethanol	ml.	40.0
Polyvinylpyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml.	3.0
Diglycol laurate	ml.	2.0

Example 5

Solution A:

Glycine	gm.	25.0
Ethylene diamine tetraacetate (sodium salt)	gm.	36.3
Distilled water	ml.	100.0

Solution B:

Sodium nitroprusside	gm.	0.8
Polyvinylpyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml.	6.5
Anhydrous ethanol	ml.	18.5
Dimethylsulfoxide	ml.	39.0
Chloroform	ml.	34.0
Aerosol (25%) in ethanol	ml.	0.4
Organic phosphate ester of anionic detergent (10%) in ethanol	ml.	0.7

Example 6

Solution A:

Glycine	gm.	25.0
$Na_3PO_4.12H_2O$	gm.	28.0
Na_2HPO_4, anhydrous	gm.	12.0
Distilled water	ml.	100.0

Solution B:

Sodium nitroprusside	gm.	0.8
Anhydrous ethanol	ml.	18.5
Dimethylsulfoxide	ml.	39.0
Chloroform	ml.	34.0
Polyvinyl acetate	ml.	6.5
Organic phosphate ester of anionic detergent (10%) in ethanol	ml.	0.7
Aerosol (25%) in ethanol	ml.	0.4

Example 7

Solution A:

Glycine	gm.	25.0
$Na_3PO_4.12H_2O$	gm.	28.0
Na_2HPO_4, anhydrous	gm.	12.0
Distilled water	ml.	100.0

Solution B:

Sodium nitroprusside	gm.	0.8
Anhydrous ethanol	ml.	18.5
Dimethylsulfoxide	ml.	39.0
Chloroform	ml.	34.0
Organic phosphate ester of anionic detergent (10%) in ethanol	ml.	0.7
Aerosol (25%) in ethanol	ml.	0.4
Interpolymer of methyl vinyl ether and maleic anhydride	gm.	1.0

Example 8

Solution A:

Glycine	gm.	25.0
$Na_3PO_4.12H_2O$	gm.	28.0
Na_2HPO_4, anhydrous	gm.	12.0
Distilled water	ml.	100.0

Solution B:

Sodium nitroprusside	gm.	0.5
Anhydrous methanol	ml.	40.0
Aerosol (25%) in ethanol	ml.	1.0
Ethyl cellulose	gm.	0.1
Anhydrous ethanol	ml.	10.0

Example 9

Solution A:

Glycine	gm.	25.0
$Na_3PO_4.12H_2O$	gm.	28.0
Na_2HPO_4	gm.	12.0
Distilled water	ml.	100.0

Solution B:

Sodium nitroprusside	gm.	0.4
Polyvinylpyrrolidone/vinyl acetate copolymer	ml.	3.25
Anhydrous ethanol	gm.	9.25
Ethyl lactate	ml.	19.0
Chloroform	ml.	17.5
Organic phosphate ester of anionic detergent (10%) in ethanol	ml.	0.35
Aerosol (25%) in ethanol	ml.	0.2

The preparation of the impregnating solutions and reagent strips based on the foregoing Examples 2 through 9 are carried out in the manner described in Example 1.

In use, an impregnated strip prepared as described above is dipped in the liquid specimen to be tested. When contacted with a fluid specimen containing ketone bodies, the test strip will give a positive color reaction. The color resulting on the strip is then compared with a precalibrated color chart for determination of the quantitative amount of ketone bodies contained on the specimen tested. The color developed on the strips in the presence of ketone bodies varies in intensity according to the amount of ketone bodies present in the specimen, i.e., from very light purple indicating the presence of 10–20 mg. percent of ketone bodies to a very dark purple indicating over 100 mg. percent. Utilizing the diagnostic strips of this invention, a positive color reaction will develop within 15 to 30 seconds in the presence of ketone bodies.

It is to be understood that other bibulous materials, e.g., small sticks of wood, etc., as well as other methods for applying the impregnating solutions to the test strips and for drying the thus impregnated strips may also be employed.

It is obvious that certain changes may be made in the above compositions and methods without departing from the spirit and scope of the invention and it is intended that all matter contained in the foregoing description shall be interpreted as illustrative and not in a limiting sense. It is also understood that other modifications may be made without departing from the spirit and scope of the appended claims.

We claim:

1. A process for the preparation of a test device for the detection of ketone bodies in body fluids which comprises:

(A) impregnating a bibulous carrier with an aqueous solution of a buffer providing a pH range of from about 8 to about 10 and a water soluble amino acid,

(B) drying the impregnated bibulous carrier;

(C) further impregnating the bibulous carrier in the area previously impregnated with the buffer and amino acid with a solution, in an organic solvent, of

(1) an alkali metal nitroprusside, and

(2) a polymeric substance selected from the group consisting of polyvinylpyrrolidone-vinyl acetate copolymers, methyl vinyl ether-maleic anhydride copolymers, polyethylene glycol, polyvinyl acetate, methyl vinyl ether-maleic anhydride interpolymers, vinyl pyrrolidone-styrene copolymers and water soluble acrylic copolymers; and

(D) removing the solvent from the further impregnated bibulous carrier.

2. A process as in claim **1** wherein the amino acid is selected from the group consisting of glycine and alanine.

3. A process as in claim **1** wherein the buffer is a mixture of disodium phosphate and trisodium phosphate.

4. A process as in claim **1** wherein the solvent is selected from the group consisting of dimethyl sulfoxide, methanol, ethanol, dimethyl formamide and mixtures thereof.

5. A process as in claim **1** wherein the organic film-forming polymeric substance has a pH on the acid side.

6. A test device for the detection of ketone bodies in body fluids prepared by a process which comprises:

(A) impregnating a bibulous carrier with an aqueous solution of a buffer providing a pH range of from about 8 to about 10 and a water soluble amino acid;

(B) drying the impregnated bibulous carrier;

(C) further impregnating the bibulous carrier in the area previously impregnated with the buffer and amino acid with a solution, in an organic solvent, of

(1) an alkali metal nitroprusside, and

(2) a polymeric substance selected from the group consisting of polyvinylpyrrolidone-vinyl acetate copolymers, methyl vinyl ether-maleic anhydride copolymers, polyethylene glycol, polyvinyl acetate, methyl vinyl ether-maleic anhydride interpolymers, vinyl pyrrolidone-styrene copolymers and water soluble acrylic copolymers; and,

(D) removing the solvent from the further impregnated bibulous carrier.

7. A test device as in claim 6 wherein the amino acid is selected from the group consisting of glycine and alanine.

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