

1

2,990,253

DIAGNOSTIC COMPOSITION

Robert R. Smeby, Elkhart, Ind., assignor to Miles Laboratories, Inc., Elkhart, Ind., a corporation of Indiana
 No Drawing. Filed May 21, 1959, Ser. No. 814,666
 3 Claims. (Cl. 23-253)

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

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 either in the presence or absence of abnormal carbohydrate metabolism, the intermediate ketone 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 the disease can be ascribed to the toxic effects of the ketone bodies. The medical practitioner is well aware of the usefulness of tests for ketone bodies in the urine in diabetes mellitus. These bodies occur in the urine in other well recognized disturbances of metabolism, and in such cases it is also important to carry out tests for these substances.

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 presence of ammonia in order to develop particular colorations (see United States Patent No. 2,186,902 to Fortune). An improvement over the Fortune type formulation is disclosed in United States Patent No. 2,509,140, issued May 23, 1950, 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 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 ketone bodies in urine without evolution of ammonia.

An improvement of this test composition is described and claimed in U.S. Patent No. 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 No. 2,509,140, greatly enhanced the utility and reliability of the diagnostic composition.

The above-mentioned patents have contributed greatly to the art of diagnosing for ketonuria and the advances made have been worthwhile. Commercial diagnostics made in accordance with the disclosures thereof have aided the physician and clinician in the diagnosis and control of ketonuria.

Prior art, in summary, then teaches the use of a water soluble nitroprusside, an aliphatic amino acid and an alka-

2

line phosphate buffer as essential ingredients of a test for ketone bodies. The test device itself comprises the reagent composition in the form of a tablet.

However, from a commercial point of view embodiment of an invention of a diagnostic composition in the form of bibulous strips or "sticks" is highly preferred since this provides for diagnosticians simple "dip and read" tests. The advantages of a diagnostic in this form are obvious: ease and simplicity of test procedure, absence of cumbersome equipment, ease of disposal of test device, rapidity of test procedure, etc.

But if an aqueous solution of the above basic constituents were used to impregnate a bibulous strip, no practical diagnostic product would be obtained. Sodium nitroprusside is very unstable in an aqueous, alkaline medium, i.e., most of it is destroyed so that no perceptible reaction with acetoacetic acid (acetoacetate) is obtained with such a strip. Attempts to place the aqueous alkaline material separately on the sticks or strips failed when upon exposure to air the reagent composition rapidly deteriorated.

As mentioned above, sodium nitroprusside is very unstable in an aqueous, alkaline medium, but it is a medium of this type that is required to achieve the desired reaction between ketone (sodium acetoacetate) and sodium nitroprusside. Until now, it has thus not been possible to achieve a soluble, alkaline impregnating mixture to prepare test strips which will preserve the sodium nitroprusside required for the chromogenic reaction.

I have now discovered a process whereby the required alkaline nitroprusside is obtained on test strips without being prematurely exposed to the unfavorable medium of aqueous alkali.

More specifically, I have found that a ketone diagnostic in "stick" form which is not subject to instability can be prepared in a two-step procedure by first applying the sodium nitroprusside to the bibulous carriers in an acidic aqueous medium which will preserve this compound of precarious stability and, after drying, dipping these carriers, to achieve the necessary alkalinity, into a non-aqueous solution of organic bases, such as various amines or aminoalcohols or mixtures thereof.

In addition, I have also found that incorporation of such substances as sodium chloride and sodium alginate further helps to stabilize these compositions appreciably. Their function as stabilizers, however, is not yet fully understood.

A diagnostic composition according to the present invention then comprises as essential constituents sodium nitroprusside, glycine, mono- and disodium phosphate, sodium chloride or alginate and one or two amines or aminoalcohols and is thus patentably distinct from prior art compositions.

The invention will now be illustrated, but not limited, by the following examples:

EXAMPLE I

Formulation of the impregnating solutions

	Gm.
A. Glycine -----	10
Sodium nitroprusside -----	0.5
Sodium chloride -----	10
Monosodium phosphate-monohydrate -----	23.8
Disodium phosphate-anhydrous -----	1.25
Water, to make 100 ml.	
B. 1,3-diamino-2-propanol -----	10
2-amino-2-methyl-1,3-propanediol -----	20
Anhydrous ethanol, to make 100 ml.	

Preparation of impregnating solutions

Solution A.—Ten grams of glycine and 0.5 gm. of sodium nitroprusside were mixed together and 50 ml. of water added thereto. Similarly, 23.8 gm. of mono-