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3,212,855

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 renal 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 presence 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 enhanced the utility and reliability of the diagnostic composition.

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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 tableted 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