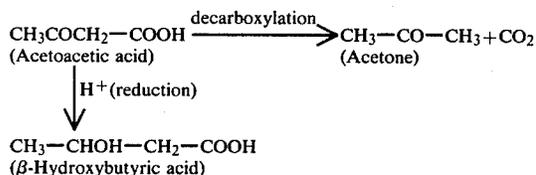


TEST MEANS AND METHOD FOR DETECTING KETONE BODIES

BACKGROUND OF THE INVENTION

The present invention relates generally to the field of diagnostic compositions, and more particularly, to diagnostic tests useful in qualitative and quantitative determination of ketone bodies in body fluids, especially acetoacetic acid in urine.

As used herein, ketone bodies includes compounds such as acetone, acetoacetic acid and β -hydroxybutyric acid (though the latter is not a ketone). The chemical relationship among these compounds is indicated below.



Acetone is a decomposition product of acetoacetic acid and is probably not otherwise produced as such in humans, although it is invariably found in urine when the other two compounds are present.

Acetoacetic and β -hydroxybutyric acids appear to be intermediate products in the breakdown of fatty acid chains (via acetoacetyl coenzyme A). Under normal conditions fatty acids are oxidized to carbon dioxide and water and intermediate products do not appear to any great extent in blood or urine.

When the body metabolizes inadequate amounts of carbohydrate because of metabolic disorder or inadequate diet, increased fatty acid metabolism gives rise to the appearance of ketone bodies in the blood (ketonemia) and urine (ketonuria). Clinical conditions, most especially diabetes mellitus, require detection and surveillance of ketonuria for management. Thus, efforts have been reported toward simplifying production and increasing stability of sodium nitroprusside tests which have been widely used for such detection.

The use of soluble nitroprussides in detection of ketone bodies, known as the Legal test, has long been recognized. Swinehart, *Coordination Chem. Rev.*, 2(4), 386-403 (December, 1967) presents a summary of the work concerning the reaction of sodium nitroprusside with acetone and acetoacetic acid. This work by Swinehart delineates the mechanism of reaction of sodium nitroprusside at the site of the acidic hydrogen as well as the action of the nitrosyl moiety of sodium nitroprusside.

Fortune U.S. Pat. No. 2,186,902 was among the first to disclose a formulation wherein the nitroprusside reaction is carried out in the presence of ammonia in order to develop particular colorations.

Free U.S. Pat. No. 2,509,140 later disclosed formulations for detection of ketone bodies in urine which contain water soluble nitroprusside, an aliphatic amino acid (glycine) and an alkaline material. Nicholls U.S. Pat. No. 2,577,978 discloses the addition of lactose or similar sugars to the Free composition.

Smeby U.S. Pat. No. 2,990,253 provides a test composition incorporated into a bibulous strip wherein, because of the instability of nitroprusside in an alkaline aqueous medium, the nitroprusside is kept separate. Separation is achieved by a two-step device preparation

method wherein the nitroprusside is first applied to the carrier in an acid aqueous medium, thus preserving the stability of the compound, and, after drying, the carrier is dipped into a non-aqueous solution of organic bases such as various amines or amino alcohols to achieve the necessary alkalinity.

Mast U.S. Pat. No. 3,212,855 discloses an improved two-dip preparation wherein a bibulous carrier is first impregnated with an alkaline buffer and an amino acid and, after drying, is then impregnated with alkali metal nitroprusside, an organic film-forming compound of acid pH and an organic solvent.

Ogawa U.S. Pat. No. 3,880,590 discloses a ketone test strip prepared by a single-dip method. The composition is a nitroprusside salt in combination with a salt of a heavy metal of specific gravity more than 5, such as nickel, copper, cobalt, manganese, chromium and zinc, for determinations at acid pH. Other metals having a specific gravity greater than 5 include arsenic (5.7), lead (11.34) and mercury (13.6). Substances, such as buffers, used to keep the composition at alkaline pH are excluded.

Concern has been expressed about the use of ketone detection procedures involving heavy metals. High density metals, especially nickel, cobalt and chromium, have been the subject of extensive government toxicity studies. Notably, Christensen, H. E., ed., *Registry of Toxic Effects of Chemical Substances*, U.S. Department of Health, Education and Welfare, Rockville, Maryland (1975) has reported the toxicity of these metals and many of their salts. The HEW study reports, for example, a lethal dose 50 (LD50) of 26 mg/kg for the compound nickel chloride when injected intraperitoneally in the mouse. A lowest reported lethal dose (LDLo) of 10 mg/kg is reported for intravenous administration in a dog system. The LDLo is the lowest dose of a substance introduced by any route other than inhalation over any given period of time and reported to have caused death. A LD50 of 140 mg/kg is reported for copper(II)chloride (cupric chloride) when orally administered to the rat. A LD50 for intraperitoneal introduction to the mouse is recorded at 7400 micrograms per kilogram. The toxicity of cobalt(II)chloride is reported as a LD50 of 80 mg/kg (oral) and 20 mg/kg (intravenous) in the rat.

Additionally, in 1976, the National Cancer Institute, in its *Survey of Compounds Which Have Been Tested for Carcinogenic Activity*, reported cobalt chloride, copper, nickel and many of its salts, zinc and a number of its salts, lead, and other heavy metals as giving rise to animal tumors. Searle, C. D., *Chemical Carcinogens ACS Monograph 193*, Washington, American Chemical Society (1976) at pages 327-329 has specifically identified nickel as a particularly aggressive carcinogen.

Thus, in summary, certain amino acids and heavy metal salts have been used in attempts to improve the reaction of acetoacetic acid with sodium nitroprusside. Improvements have been realized through these efforts, however, these have been somewhat limited and, as can be readily seen, the use of many of these materials is accompanied by significant health hazards.

Also, it has been an accepted fact that use of the sodium nitroprusside test at alkaline pH (in contrast to acid pH) required a "two-dip" manufacturing procedure, this being a significant economic factor, in test strip production.