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DIAGNOSTIC PREPARATION AND PROCESS FOR THE DETERMINATION OF SERUM ALKALINE PHOSPHATASE

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This invention relates to a diagnostic preparation and relates more particularly to a stable composition for use in a simplified, semiquantitative determination of alkaline phosphatase in serum. The determination of serum alkaline phosphatase is of considerable value in the diagnosis of such conditions as bone cancer, liver damage, bile obstruction, and gallstone disorders.

An object of this invention is the preparation of a stable, diagnostic composition in tablet form suitable for use in the determination of serum alkaline phosphatase.

A further object of this invention is to provide a method for determining serum alkaline phosphatase where the use of solutions and the necessity for precise and time-consuming measurements and reactions is eliminated. Other objects of this invention will appear from the following detailed description.

One of the present tests for determining serum alkaline phosphatase was developed by Huggins and Talalay (J. Biol. Chem. 159, 399 (1945)). In this assay, a buffered solution of sodium phenolphthalein phosphate containing 5% serum is incubated at 37° C. for one hour. The incubation is then halted and the reaction mixture is made alkaline with a solution of sodium pyrophosphate in glycine buffer. The amount of phenolphthalein released by the alkaline phosphatase present is measured in a colorimeter, and the alkaline phosphatase concentration in the serum is determined from a standard curve.

In accordance with the present invention and in accordance with the novel method which has now been developed, serum whose alkaline phosphatase content is to be determined is incubated with a solid preparation containing sodium phenolphthalein phosphate and a buffer and the reaction mixture thus formed is then made alkaline. The depth of color which develops and which is due to the free phenolphthalein formed by the action of the enzyme present is then compared with a standard color chart from which one is readily able to obtain a semiquantitative reading of the serum alkaline phosphatase concentration. The buffer employed should be present in sufficient quantity to overcome the buffering action of the serum and to maintain the serum pH in a range of about 9 to 11, the range in which the serum alkaline phosphatase enzyme is active.

The incubation period employed varies with temperature. At a temperature of 67° F. the incubation period should be about thirty minutes, at 80° F. about twenty minutes and at 86.5° F. about sixteen minutes. The time and temperature relationship within this range is essentially a straight-line relationship. At 93° F. the incubation time should be thirteen minutes, and at 98.6° F. twelve minutes. The incubation is preferably carried out at temperatures within the range given which are the usual room temperatures.

In addition to the sodium phenolphthalein phosphate and buffer, I preferably include an activator for the enzyme such as a magnesium salt, for example magnesium sulfate. The activator is employed to provide sufficient magnesium ions in the event that the magnesium concentration in the serum tested is abnormally low and insufficient to activate the enzyme.

While tris (hydroxymethyl)-aminomethane has proven to be a satisfactory buffer in my novel compositions, other buffers of similar solubility and basicity may also be used

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in order to maintain the pH within the preferred range during the incubation period. Examples of other buffers which may also be employed are the sodium salt of 5,5-diethyl barbituric acid, sodium carbonate and guanidine carbonate.

The solid substrate tablet preparation of this invention preferably contains the active components in a ratio of about 1.5 parts by weight of sodium phenolphthalein phosphate to 0.1 part of magnesium sulfate and 98.4 parts of the buffer, e.g.: tris (hydroxymethyl)-aminomethane. Although inert binding materials may be used as an aid in forming these tablets, this is generally unnecessary.

This new and novel substrate tablet is convenient and stable. In use, no measurement of substrate volume is required since the tablet volume is essentially zero, the entire tablet being of the order of only about 20 mg. in weight. Since the test is run in whole serum in the presence of an excess of the substrate rather than in 5% buffered serum solution as in the prior testing method, the volume of serum used need not be measured accurately. Since the concentration of alkaline phosphatase in the reaction mixture determines the amount of color produced and the serum is essentially the whole volume of the reaction mixture, the concentration of alkaline phosphatase in the reaction mixture is essentially independent of the volume of serum used. Thus, a semiquantitative measure of serum alkaline phosphate is obtained, and no accurate measurements are necessary.

The present technique is simple and rapid requiring an incubation time of only twenty minutes at 80° F. instead of the one hour needed in the older method. Further, only small volumes of serum are required in the test, the preferred volume range being 0.1 to 0.3 ml. With such small serum samples, volumes of 0.5N aqueous sodium hydroxide of the order of 0.04 ml. are sufficient to stop the incubation and develop the full phenolphthalein color. The simplicity of the method of this invention obviates the need for expensive equipment and highly trained technical personnel to perform the test. The simplicity and rapidity of the present method makes it readily available for the screening of serum alkaline phosphatase levels in large population groups. In view of the known value of this diagnostic test, this convenient method of determining serum alkaline phosphatase levels could be of great value for the early diagnosis of cancer, liver disfunction, and gall bladder disfunction.

It is to be understood that the proportion of the active ingredients set forth above is the preferred one, but it may be subjected to some variations without affecting its use and value as mentioned herein. Thus, for example, a 20 mg. tablet may contain from about 0.2–0.6 mg. of sodium phenolphthalein phosphate, corresponding to about 1 to about 3 percent by weight of the tablet, up to about 0.02 mg. of magnesium sulfate that is, up to about 0.1 percent by weight of the tablet, with the remainder of the active components being the buffer.

It is to be understood that other salts of phenolphthalein phosphate, such as other alkali metal and alkaline earth metal salts may be used. Thus, in place of the sodium salt, the calcium and barium salts of phenolphthalein phosphate may be employed.

The following example will serve to illustrate my invention and sets forth the detailed method for the determination of serum alkaline phosphatase following my method.

Example

To approximately 0.2 ml. of serum is added 10 to 30 mg. of a solid composition, in either powder or tablet form, containing approximately 1.5 parts sodium phenolphthalein phosphate, approximately 0.1 part magnesium sulfate, and approximately 98.4 parts tris (hydroxy-

methyl)-aminomethane. The mixture is incubated for about twenty minutes at 80° F. and to the incubated mixture is then added one drop (about 0.04 ml.) of 0.5 N aqueous sodium hydroxide to make the mixture alkaline. The developed color is then matched against a standard color chart, as described. The said color chart is developed by matching the color densities of quantitatively determined serum alkaline phosphatase values against the color densities produced in the method of this invention. Thus, a direct reading of serum alkaline phosphatase concentration is had.

It is to be understood that the foregoing detailed description is given merely by way of illustration and that many variations may be made therein without departing from the spirit of my invention.

Having described my invention, what I desire to secure by Letters Patent is:

1. A dry preparation for use in the determination of serum alkaline phosphatase which comprises about 1 to about 3 percent by weight of sodium phenolphthalein phosphate, up to about 0.1 percent by weight of a magnesium salt and tris(hydroxymethyl)-aminomethane in an amount sufficient to maintain the serum pH in a range of about 9 to about 11.

2. A dry preparation according to claim 1 wherein said magnesium salt is magnesium sulfate.

3. A dry preparation according to claim 2 in the form of a tablet weighing about 10 to about 30 milligrams.

4. A tablet for the determination of serum alkaline phosphatase consisting of about 0.3 mg. sodium phenolphthalein phosphate, about 0.02 mg. of magnesium sulfate, and about 19.68 mg. tris(hydroxymethyl)-amino-methane.

5. A method for the determination of serum alkaline phosphatase, which comprises adding to a sample volume of serum about 10 to about 30 milligrams of a dry preparation comprising about 1 to about 3 percent of the weight of the preparation of sodium phenolphthalein phosphate, up to about 0.1 percent of the weight of the preparation of a magnesium salt and tris(hydroxymethyl)-aminomethane in an amount sufficient to maintain the

serum pH in a range of from about 9 to 11, incubating the mixture to permit the enzyme present to hydrolyze the phenolphthalein compound, making the incubated mixture alkaline, and comparing the developed color to the color developed in the presence of a known concentration of serum alkaline phosphatase.

6. A method for the determination of serum alkaline phosphatase which comprises adding to approximately 0.2 ml. of serum a 10 to 30 mg. tablet containing about 1.5% by weight of sodium phenolphthalein phosphate, about 0.1% by weight of magnesium sulfate, and about 98.4% by weight of tris(hydroxymethyl)-amino-methane, incubating the mixture at a temperature of from 67 to 98.6° F. from 30 to 12 minutes, adding from 0.02 to 0.10 cc. of 0.5 N aqueous alkali metal hydroxide, and comparing the developed color to the color developed in the presence of a known concentration of serum alkaline phosphatase.

7. A method for the determination of serum alkaline phosphatase which comprises adding to approximately 0.2 ml. of serum a 20 mg. tablet containing about 0.3 mg. sodium phenolphthalein phosphate, about 0.02 mg. of magnesium sulfate, and about 19.68 mg. tris(hydroxymethyl)-amino-methane, incubating the mixture, making the mixture alkaline by adding about 0.4 cc. of a 0.5 N aqueous sodium hydroxide to the incubated mixture, and comparing the developed color to the color developed in the presence of a known concentration of serum alkaline phosphatase.

References Cited in the file of this patent

UNITED STATES PATENTS

2,359,052 Scharer ----- Sept. 26, 1944

OTHER REFERENCES

- J. Biol. Chem., 159 (399-410) (1945).
 Practical Physiological Chemistry by Hawk et al., 12th ed., The Blakiston Company (1947) page 584.
 Sumner et al.: "The Enzymes," vol. II, part 2, published by Academic Press, Inc., New York, 1952, pp. 1161 and 1162.