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[54] **DIAGNOSTIC COMPOSITIONS FOR GLUTAMIC OXALIC TRANSAMINASE (GOT) AND GLUTAMIC PYRUVIC TRANSAMINASE (GPT) DETERMINATIONS**

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[51] **Int. Cl.** **G01n 31/14**

[58] **Field of Search** ..... 195/103.5 R, 150

[56] **References Cited**

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[57] **ABSTRACT**

Stable, lyophilized diagnostic compositions are disclosed effective in determining glutamic oxalic transaminase (GOT) and glutamic pyruvic transaminase (GPT) comprising malate dehydrogenase (for GOT) or lactate dehydrogenase (for GPT), a pyridine nucleotide and a buffer having a sodium content of less than about 600 meq./1. capable of regulating the pH of the composition in reconstituted form. Also disclosed are the aqueous reconstituted products, the method of determining the activity of GOT and GPT in a sample using the subject compositions and the process for making the disclosed diagnostic compositions.

46 Claims, No Drawings

DIAGNOSTIC COMPOSITIONS FOR GLUTAMIC OXALIC TRANSAMINASE (GOT) AND GLUTAMIC PYRUVIC TRANSAMINASE (GPT) DETERMINATIONS

BACKGROUND OF THE INVENTION

This invention relates generally to stable, lyophilized diagnostic compositions. More particularly, it relates to unusually stable novel diagnostic compositions useful in determining the activity of glutamic oxalic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in samples containing said enzymes.

GOT and GPT are enzymes whose elevated presence in the bloodstream of humans and animals is indicative of certain malfunctions and/or disorders. For example, elevated levels of glutamic oxalic transaminase is an important indication of the status of patients with severe heart disease. Accordingly, the determination of the activity of this enzyme in the body is also a useful diagnostic tool for doctors and hospital personnel.

Generally, if any heart muscle damage has taken place or is occurring, this will manifest itself as an increase in GOT levels. In addition to signaling heart disease, elevated GOT levels also indicate the possibility of severe liver disfunctions such as cirrhosis, cancer of the liver or hepatitis.

Similarly, the elevated presence of GPT is indicative of liver disfunctions of the kind just named. Consequently, the prognosis and cause of liver disease can be determined by an accurate GPT determination.

It is readily apparent that certain characteristics are essential for a material to be an effective diagnostic reagent. These include, among others, good stability, ease of manufacture and consistency in composition. Diagnostic reagents are commonly prepared and used in the reconstituted state i.e., as an aqueous solution. It is unusual to observe any substantial degree of stability in diagnostic reagents in this form.

For this reason, the operator will generally prepare the reagent just prior to use by simply combining the necessary components. The drawbacks of this manipulation are glaring: it is subject to human error; it is time consuming; standardization of the reagent is required; and most importantly, production of a consistent reagent is virtually impossible.

The presently disclosed compositions overcome these severe handicaps, the most crucial being the stability aspect. When preparing a reagent as described above, it is imperative that the reagent be used within a relatively short period of time. If not, decomposition which takes place renders the material totally useless and it must be discarded.

It is one object of this invention to prepare stable, lyophilized diagnostic compositions which can be stored for indefinite periods of time. In addition, once a batch of material is prepared and a sample is standardized, that step is no longer necessary and one need only add the proper amount of water for reconstitution. Accordingly, the possibility of human error is minimized, the time consuming steps of preparation just prior to use and standardization are eliminated, and most importantly, a stable product capable of prolonged storage is available for use at any time requiring only a simple reconstitution step prior to use.

The prior art has not disclosed stable, lyophilized diagnostic reagents which are effective in determining GOT and GPT in samples containing these enzymes which are free of the abovedescribed disadvantages.

The present invention does disclose such compositions, their effective use and the means for preparing same.

SUMMARY OF THE INVENTION

Accordingly, this invention relates to two stable, lyophilized diagnostic compositions. The first is highly effective in determining glutamic oxalic transaminase (GOT) and comprises malate dehydrogenase (MDH); a pyridine nucleotide such as reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH); and a buffer having a sodium content of less than about 600 meq./1. capable of regulating the pH of the reconstituted product.

The second is highly effective in determining glutamic pyruvic transaminase (GPT) and comprises lactate dehydrogenase (LDH); a pyridine nucleotide such as reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH); and a buffer having a sodium content of less than about 600 meq./1. capable of regulating the pH of the reconstituted product.

The reconstituted form of each of these compositions is preferred and is prepared simply by adding a predetermined amount of water resulting in a solution having a pH preferably in the range from 6.0 to 8.0. It is possible and within the purview of this invention to prepare an intermediate reconstituted product outside the pH range of 6 to 8 which can then be adjusted subsequently by adding more buffer thereto. Although the dry reagent can be used in the analytical determination, it is preferred to use the reconstituted form.

Another preferred embodiment of both compositions concerns the use of NADH as the pyridine nucleotide component.

The reconstituted forms of these compositions are characterized by critical properties and limitations which must be present in order to have a diagnostic reagent having the highly desirable advantages described earlier. For instance, for the GOT reagent, the MDH must be present in an amount to provide an activity of from about 0.5 units/ml. to about 10 units/ml., with a 3 units/ml. activity being the most preferred.

For the GPT reagent, the corresponding LDH activity upon reconstitution must be present in an amount to provide activity of from 8 units/ml. to about 80 units/ml., with a 40 units/ml. activity being most preferred.

The amount of pyridine nucleotide, preferable NADH, is present in both reconstituted compositions in an amount to obtain an optical density in the range of from 2.1 to 1.0 spectrophotometrically determined at 340 nm using a 10 mm. light path. Most preferred is an optical density of 1.8.

The buffer in both reconstituted compositions has a sodium content less than 600 meq./1. and is capable of regulating the pH of the reconstituted product. The preferred pH range regulated by the buffer is between 6 and 8. To regulate within this range certain buffers are highly preferred. These include potassium phosphate, sodium phosphate and mixtures of the two. The terms potassium and sodium phosphate encompass all three possible salts, the di-potassium or di-sodium

forms most preferred. If the particular sodium or potassium salt is not specified, the di-salt is intended.

If sodium phosphate alone is used as the buffer, the amount used is dictated by the sodium content in the reconstituted product. It should not surpass a concentration of 600 meq./1. and is preferably about 400 meq./1.

When potassium phosphate is the buffer, it is added in an amount to provide a concentration of from 0.05 M to 0.4 M, with a 0.1 M concentration most preferred.

The type of buffer which is of choice for purposes of this invention are salts derived from strong bases and weak acids. Sodium and potassium phosphate are typical examples. Others of this type include ammonium acetate, lithium phosphate and sodium borate.

However, other buffers which are not within this category are equally applicable. For instance, representative useful illustrations include albumin and tris buffer tris(hydroxymethyl)aminomethane.

Another preferred embodiment of both compositions concerns the inclusion of albumin. The preferred concentration upon reconstitution is about 0.15 g./100 ml.

Yet another preferred aspect of this invention concerns the use of these compositions in the quantitative procedures for determining the particular enzyme.

In both techniques, the usual procedure consists of mixing the sample with a substrate and subsequently analyzing for enzyme. This is usually effected in an instrument devised for that purpose. The inventive embodiment comprises combining the herein disclosed diagnostic composition with the reaction product and photometrically measuring the decrease in NADH concentration.

In the GOT determination, the substrate is preferably an aspartic acid-α-ketoglutaric acid mixture whereas in the GPT determination, the substrate is preferably an alanine-α-ketoglutaric acid mixture.

The unknown enzyme concentration in both procedures is directly proportional to the decrease in NADH concentration photometrically determined.

Another important embodiment of this invention deals with the preparation of the stable, lyophilized diagnostic compositions. The process is the same for both and comprises the commingling of MDH or LDH with pyridine nucleotide and buffer. The resulting aqueous solution is lyophilized. The reconstituted products having a pH in the range from 6.0 to 8.0 are characterized by the limiting properties described above.

A preferred aspect of both processes concerns the use of potassium phosphate as buffer and the inclusion of albumin as a reagent.

DETAILED DESCRIPTION OF THE INVENTION

In both the GOT and GPT diagnostic compositions, the essential components comprise:

1. an enzyme
2. a pyridine nucleotide, and
3. a buffer

For the GOT diagnostic reagent, the enzyme component is malate dehydrogenase (MDH) whereas lactate dehydrogenase (LDH) is the enzyme component in the GPT diagnostic composition. Both enzymes are commercially available and in such preparation, are usually derived from animal sources. They are commonly used components for reagents used in GOT and GPT determinations.

The amount of MDH which one adds to the GOT diagnostic reagent is calculated by determining the activity of a reconstituted product. For purposes of this invention, it is found out the reconstituted product should possess an MDH activity of from 0.5 units/ml. to about 20 units/ml. in order to provide an efficient diagnostic reagent. An activity of 3 units/ml. is preferred.

The amount of LDH which one adds to the GPT diagnostic reagent is calculated by determining the activity of a reconstituted product. For purposes of this invention, it is found that the reconstituted product should possess an LDH activity of 8 units/ml. to about 80 units/ml. An activity of 40 units/ml is preferred.

The pyridine nucleotide components of the herein disclosed invention are also commercially available. In preparing the diagnostic compositions, it is mandatory that the NADH or NADPH is in the reduced state since the ultimate determination of enzyme in the sample is directly proportional to the decrease in NADH in the reduced state to the oxidized state, namely, NAD. Similarly, if NADPH is used, the corresponding decrease in reduced state to oxidized state (NADP) is measured.

NADH and NADPH are well known co-factors and are particularly useful in enzymatic reactions requiring oxidation.

The amount of pyridine nucleotide in the diagnostic reagent (for both GOT and GPT) is determined by measuring the optical density of the reconstituted product. It is found that an optical density in the range from 2.1 to 1.0 spectrophotometrically determined at 340 nm using a 10 mm. light path is necessary to provide the effective diagnostic reagents of this invention.

The third component of the subject compositions, namely, the buffer, is the most important. It appears to play a critical role in the stabilization of the lyophilized material as well as the reconstituted product. The manner which brings this about is not fully understood at this time.

With both the GOT and GPT reagents, the type of buffer required for a satisfactory product should have a sodium content of less than 600 meq./1. capable of regulating the pH of the composition, preferably in the range from 6.0 to 8.0. The preferred buffer is potassium phosphate added in an amount to provide from 0.05 M to 0.4 M based on reconstituted material. Most preferred is a concentration of 0.1 M. Other buffers of preference include sodium phosphate-potassium phosphate mixtures and sodium phosphate by itself.

It is apparent from the above discussion, that the diagnostic compositions of this invention are characterized by properties exhibited in the reconstituted product. Accordingly, the processes for preparing such compositions are not critical with respect to initial reagent concentrations so long as the final product upon reconstitution contains the well defined properties clearly delineated above.

The process for preparing the subject diagnostic compositions comprises admixing MDH or LDH, pyridine nucleotide and buffer material. The resulting aqueous solution is then lyophilized to provide a dry, stable diagnostic reagent.

If the diagnostic reagent is to contain albumin, it is included as a reagent during the mixing step. Although albumin, preferably bovine albumin, is not an essential

component, it serves a highly useful purpose. It prevents the settling of solid materials to avoid base-line drift during the analyzing procedure. However, it plays no role in the enzymatic reaction mechanism. When incorporated into the diagnostic composition, it should be present in an amount to provide a concentration upon reconstitution of about 0.15/100 ml. This applies to both diagnostic compositions, i.e., the GOT and GPT reagents.

At this point, it should be mentioned that the reconstituted diagnostic reagents disclosed herein characterized in the matter described above will preferably have a pH in the range of 6.0 to 8.0. If the reconstituted product is outside this range, adjustment thereof will be effected prior to use in the determination.

In the process for preparing the GOT or GPT reagent, it is preferred to use a 0.5 potassium phosphate buffer solution, a 90 percent by weight aqueous solution of NADH (or NADPH) and an ammonium sulfate suspension of MDH or LDH.

When bovine albumin is included, it is supplied in the solid state.

The lyophilized compositions of this disclosure are highly stable. For instance, they can be stored indefinitely at a temperature of 4°C and they are stable at 45°C for at least 2 days.

Even the reconstituted product is relatively stable. It is stable for up to 12 hours at 4°C without change in optical density and at 25°C, the optical density changes less than .2 percent in 3 hours.

The subject diagnostic compositions are suitably used in automatic analyses of samples for GOT and GPT activity. It is known in determining for GOT and GPT to mix the sample (under investigation) with a substrate and analyze for enzyme activity. The mixing step may simultaneously be heated. Moreover, it is generally preferred to pass the reaction mixture through a dialyzing membrane. The dialysis product is then analyzed for enzyme activity.

The present invention relates in part to the improved modification of combining the dialysis product with the GOT or GPT diagnostic composition, usually in reconstituted form, and photometrically measuring the decrease in NADH concentration.

The concentration of enzyme in question is directly proportional to the decrease in NADH concentration.

The sample used in the GOT determination can be a serum sample (SGOT), a plasma sample, or it can be an aqueous solution, suspension or dispersion. An example of the latter type is an albumin sample. In the GOT analyses, the preferred substrate is an aspartic -α-ketoglutaric acid mixture. For the GPT test, an alanine -α-ketoglutaric acid mixture is the substrate of choice.

EXAMPLE I

Preparation of Glutamic Oxalic Transaminase (GOT) Diagnostic Composition

To 800 ml. of an 0.5 M potassium phosphate aqueous solution is added 1.25 g. of NADH (90 percent by weight aqueous solution) followed by the addition of 15,000 units of MDH as an ammonium sulfate suspension. The resulting solution is brought to 1 liter volume by the addition of a supplemental amount of 0.5 M aq. potassium phosphate buffer and then dispersed into vials in 5 ml. portions.

The vials are then subjected to lyophilizing conditions and the resulting dry, stable compositions stored at 4°C.

RECONSTITUTION

The dry compositions prepared above are reconstituted by the addition of 25 ml. of distilled water. The reconstituted diagnostic reagent exhibits the following properties:

pH	7.4
Optical Density	1.75 ± 0.05
MDH Activity	3 units/ml.
Potassium phosphate conc.	0.1 M

The dry, lyophilized product can be stored indefinitely at a temperature of 4°C and is stable for approximately 2 days at 45°C.

The reconstituted product is stable for up to 12 hours at 4°C without change in optical density, and at 25°C the optical density changes less than 0.2 percent in 3 hours.

EXAMPLE II

The procedure of Example I is repeated except 7.5 g. of bovine albumin is included in the 0.5 M potassium phosphate buffer solution.

The properties of the reconstituted product are unchanged and the lyophilized material has comparable stability.

EXAMPLE III

To 800 ml. of an 0.5 M potassium phosphate aq. solution is added 1.25 g. of NADH (90 percent by weight aqueous solution) followed by the addition of 200,000 units of LDH as an ammonium sulfate suspension. The resulting solution is brought to 1 liter volume by the addition of a supplemental amount 0.5 M aq. potassium phosphate buffer and then dispersed into vials in 5 ml. portions.

The vials are then subjected to lyophilizing conditions and the resulting dry, stable compositions stored at 4°C.

RECONSTITUTION

The dry compositions prepared above are reconstituted by the addition of 25 ml. of distilled water. The reconstituted diagnostic reagent is characterized by the following properties:

pH	7.4
Optical Density	1.75 ± 0.05
LDH Activity	40 units/ml.
Potassium phosphate conc.	0.1 M

The dry, lyophilized product can be stored indefinitely at a temperature of 4°C and is stable for at least 2 days at 45°C.

The reconstituted product is stable for up to 12 hours at 4°C without change in optical density, and at 25°C the optical density changes less than 0.2 percent in 3 hours.

EXAMPLE IV

The procedure of Example III is repeated except 1.5 g. of bovine albumin is included in the 800 ml. portion of 0.5 M potassium phosphate buffer solution.

The properties of the resulting reconstituted product are unchanged and the lyophilized material has comparable stability.

EXAMPLE V

The reconstituted diagnostic reagent prepared according to Example II is used in determining the GOT activity of an unknown sample in the following manner:

Using AutoAnalyzer II, SMA-12/60, and SMA-12 MICRO (all registered trademarks of Technicon Instruments Corporation, Tarrytown, N.Y.), the sample (about 0.3 ml.) is mixed with a substrate comprising an aspartic acid α- ketoglutaric acid mixture (75:1 by weight; about 0.4 ml.) dissolved in a phosphate buffer. The products of the reaction dialyze through a dialyzer and are reacted with the reconstituted reagent as prepared according to Example II (about 0.3 ml.). The change in optical density is determined in a photometer at 340 nm.

The activity of GOT is directly proportional to the decrease in the optical density of the NADH.

EXAMPLE VI

The procedure of Example V is repeated for the determination of GPT activity in a sample except the reconstituted diagnostic reagent of Example IV is used in place of the reagent of Example II and the substrate is an alanine-α-ketoglutaric acid mixture.

EXAMPLE VII

The procedure of Example I is repeated to prepare reconstituted diagnostic reagents having the following properties:

	A	B	C
pH	6	8	7
Optical Density	1.0	2.1	1.5
MDH Activity (units/ml.)	0.5	10	5
Potassium phosphate conc.	0.05M	0.4M	0.3M

EXAMPLE VIII

The procedure of Example III is repeated to prepare reconstituted diagnostic reagents having the following properties:

	A	B	C
pH	6	8	7
Optical Density	1.0	2.1	1.5
LDH Activity (units/ml.)	8	80	60
Potassium phosphate conc.	0.05M	0.4M	0.3M

EXAMPLE IX

The procedure of Example I is repeated except that instead of potassium phosphate, a buffer consisting of a mixture of potassium phosphate and sodium phosphate is used. The reconstituted product has a sodium content of 400 meq./1. and exhibits comparable stability.

EXAMPLE X

The procedure of Example I is repeated except that sodium phosphate buffer is used in place of potassium phosphate. The reconstituted product has a sodium content of 400 meq./1. and exhibits comparable activity.

EXAMPLE XI

The procedure of Example V is repeated for the determination of GPT activity of an unknown sample wherein instead of the diagnostic reagent prepared according to Example II, the one described in Example IV is used instead.

What is claimed is:

1. A stable, lyophilized diagnostic composition effective in determining glutamic oxalic transaminase (GOT) in a sample containing said enzyme which consists of malate dehydrogenase (MDH) present in an amount to provide an activity upon reconstitution of from about 0.5 units/ml to about 10 units/ml; a pyridine nucleotide selected from the group consisting of reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), said pyridine nucleotide present in an amount to obtain an optical density upon reconstitution in the range of from 2.1 to 1.0 spectrophotometrically determined at 340 nm using a 10 mm light path; and a buffer selected from the group consisting of potassium phosphate and a mixture of potassium phosphate and sodium phosphate, said mixture having a sodium content less than 600 meq./1 upon reconstitution, which regulates the pH of the reconstituted product in the range from 6 to 8.

2. The composition as claimed in claim **1** wherein said buffer is potassium phosphate present in an amount to provide a concentration of from about 0.05 M to 0.4 M upon reconstitution.

3. The composition as claimed in claim **1** wherein said pyridine nucleotide is NADH.

4. The composition as claimed in claim **1** wherein said activity of MDH is about 3 units/ml.

5. The composition as claimed in claim **1** wherein said optical density is 1.8.

6. The composition as claimed in claim **2** wherein said concentration is 0.1 M.

7. The composition as claimed in claim **1** wherein said buffer is a mixture of potassium phosphate and sodium phosphate and albumin is included in an amount to provide a concentration upon reconstitution of about 0.15 g./100 ml.

8. A stable aqueous solution having a pH in the range from 6.0 to 8.0 which comprises the composition of claim **1** dissolved in water.

9. A stable, lyophilized diagnostic composition effective in determining glutamic pyruvic transaminase (GPT) in a sample containing said enzyme which consists of lactate dehydrogenase (LDH) present in an amount to provide an activity upon reconstitution of from 8 units/ml to about 80 units/ml; a pyridine nucleotide selected from the group consisting of reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) said pyridine nucleotide present in an amount to obtain an optical density upon reconstitution in the range of from 2.1 to 1.0 spectrophotometrically determined at 340 nm using a 10 mm light path; and a buffer selected from the group consisting of potassium phosphate and a mixture of potassium phosphate and sodium phosphate, said mixture having a sodium content less than 600 meq./1 upon reconstitution, which regulates the pH of the reconstituted product in the range from 6 to 8.

10. The composition as claimed in claim 9 wherein said buffer is potassium phosphate present in an amount to provide a concentration of from about 0.05 M to 0.4 M upon reconstitution.

11. The composition as claimed in claim 9 wherein said pyridine nucleotide is NADH.

12. The composition as claimed in claim 9 wherein said activity of LDH is about 40 units/ml.

13. The composition as claimed in claim 9 wherein said optical density is 1.8.

14. The composition as claimed in claim 10 wherein said concentration is 0.1 M.

15. The composition as claimed in claim 9 wherein said buffer is a mixture of potassium phosphate and sodium phosphate and albumin is included in an amount to provide a concentration upon reconstitution of about 0.15 g./100 ml.

16. A stable aqueous solution having a pH in the range from 6.0 to 8.0 which comprises the composition of claim 9 dissolved in water.

17. In a method for quantitatively determining the activity of glutamic oxalic transaminase (GOT) in a sample by mixing said sample and a substrate and analyzing for GOT; the improvement which comprises combining the stabilized composition as claimed in claim 1 with the reaction product from said mixing step and photometrically measuring the decrease in NADH concentration.

18. The method of claim 17 including the further step of passing the reaction product through a dialysis membrane prior to combining with said composition.

19. The method of claim 17 including the further step of heating the reaction mixture of said sample and substrate.

20. The method of claim 17 wherein said substrate is an aspartic acid - α-ketoglutaric acid mixture.

21. The method of claim 17 wherein said composition buffer regulates the reconstituted product in the range from 6 to 8.

22. The method of claim 21 wherein said buffer is potassium phosphate.

23. The method of claim 21 wherein said buffer is a mixture of potassium phosphate and sodium phosphate.

24. The method of claim 22 wherein said composition comprises malate dehydrogenase, NADH and potassium phosphate.

25. The method of claim 17 wherein said composition is in the form of an aqueous solution having a pH in the range from 6.0 to 8.0.

26. In a method for quantitatively determining the activity of glutamic pyruvic transaminase (GPT) in a sample by mixing said sample and a substrate and analyzing for GPT, the improvement which comprises combining the stabilized composition as claimed in claim 9 with the reaction product from said mixing step and photometrically measuring the decrease in NADH concentration.

27. The method of claim 26 including the further step of passing the reaction product of said sample and said substrate through a dialysis membrane prior to combining with said composition.

28. The method of claim 26 including the further step of heating the mixture of said sample and substrate.

29. The method of claim 26 wherein said substrate is an alanine -α-ketoglutaric acid mixture.

30. The method of claim 26 wherein said composition buffer regulates the reconstituted product in the range from 6 to 8.

31. The method of claim 30 wherein said buffer is potassium phosphate.

32. The method of claim 30 wherein said buffer is a mixture of potassium phosphate and sodium phosphate.

33. The method of claim 31 wherein said composition comprises lactate dehydrogenase, NADH and potassium phosphate.

34. The method of claim 26 wherein said composition is in the form of an aqueous solution having a pH in the range from 6.0 to 8.0.

35. A process for the preparation of a stable, lyophilized diagnostic composition as claimed in claim 1 which comprises admixing malate dehydrogenase, a pyridine nucleotide selected from the group consisting of reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH); and a buffer selected from the group consisting of potassium phosphate and a mixture of potassium phosphate and sodium phosphate which regulates the pH of the reconstituted product in the range from 6 to 8; and lyophilizing the resulting aqueous product which upon reconstitution is characterized by an optical density within the range from 2.1 to 1.0 and an MDH activity of from 0.5 units/ml. to about 10 units/ml.

36. The process of claim 35 wherein said buffer is potassium phosphate added as an 0.5 M aqueous solution whose concentration upon reconstitution is from 0.05 to 0.4 M.

37. The process of claim 35 wherein said pyridine nucleotide is NADH and the reconstituted lyophilized product is characterized by an optical density of 1.8 and an MDH activity of 3 units/ml.

38. The process of claim 36 wherein said potassium phosphate concentration is about 0.1 M.

39. The process of claim 35 wherein said buffer is a mixture of potassium phosphate and sodium phosphate.

40. The process of claim 39 wherein albumin is added in an amount to provide a concentration of about 0.15 g./100 ml of reconstituted lyophilized product.

41. A process for the preparation of a stable, lyophilized diagnostic composition as claimed in claim 9 which comprises admixing lactate dehydrogenase; a pyridine nucleotide selected from the group consisting of reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH); and a buffer selected from the group consisting of potassium phosphate and a mixture of potassium phosphate and sodium phosphate which regulates the pH of the reconstituted product in the range from 6 to 8; and lyophilizing the resulting aqueous product which upon reconstitution is characterized by an optical density within the range from 2.1 to 1.0 acid and an LDH activity of from 8 units/ml to about 80 units/ml.

42. The process of claim 41 wherein said buffer is potassium phosphate added as an 0.5 M aqueous solution whose concentration upon reconstitution is from 0.05 M to 0.4 M.

43. The process of claim 41 wherein said pyridine nucleotide is NADH and the reconstituted lyophilized

product is characterized by an optical density of 1.8 and an LDH activity of 40 units/ml.

44. The process of claim **41** wherein said buffer is potassium phosphate and said potassium phosphate concentration is about 0.1 M.

45. The process of claim **41** wherein said buffer is a mixture of potassium phosphate and sodium phosphate.

46. The process of claim **45** wherein albumin is added in an amount to provide a concentration of about 0.15 g./100 ml of reconstituted lyophilized product.

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,819,488 Dated June 25, 1974

Inventor(s) Robert L. Rush, Luis P. Leon and Anne C. De Lea

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 7, line 14, change "75:1" to -- 7.5:1 --

Column 8, line 6, change "Example IV" to

-- Example III --.

Signed and sealed this 29th day of April 1975.

(SEAL)
Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents
and Trademarks