3,359,072
METHOD OF MAKING AND USING DIAGNOSTIC AID FOR DETERMINATION OF ALBUMIN IN BIOLOGICAL FLUIDS
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8 Claims. (Cl. 23—230)

ABSTRACT OF THE DISCLOSURE
A novel diagnostic acid for use in the determination of albumin in biological fluids is disclosed. This diagnostic aid comprises a paper sheet having a wettable surface prepared by first impregnating the paper with a solution of an acid buffer, drying the buffer impregnated sheet, thereafter impregnating the dried sheet with a solution of an indicator dye exhibiting the so-called "protein error," the solution of acid buffer having a pH below 7 but being so constituted as to maintain the indicator dye on the alkaline side of the acid-to-base color change point thereof and thereafter finally drying this twice impregnated sheet.

The test papers are characterized by an increased sensitivity and can be employed for the determination of albumin in biological fluids such as urine where the concentration of the albumin is as low as 0.005%. The color of the paper strip undergoes a change in color, the same corresponding to the concentration of albumin in the biological fluid, i.e., the strips which are pale yellow in color on immersion into an albumin containing fluid, take on strong blue colorations, the color intensity being a measure of the albumin concentration.

This invention relates to new articles of manufacture and to new methods of manufacturing the same. More particularly, the invention relates to a diagnostic aid for use in carrying out relatively rapid determinations for the presence of albumin in biological fluids.

The qualitative and quantitative determination of albumin in biological fluids, as for instance urine, spinal fluid, etc. is of critical importance to the physician in making a diagnosis in kidney disease, circulatory disturbances, arteriosclerosis, hypertension, diabetes, brain and spinal cord damage, etc. Methods for the detection of albumin are known, as for example, methods based on the sulfo-salicylic acid test, acetic acid-heat test, nitric acid ring test, and picric acid test. All of the aforesaid methods are based on precipitation reactions and cannot be carried out without a certain minimum amount of laboratory equipment. Therefore, these methods do not lend themselves for use as rapid diagnostic procedures. For carrying out rapid, and preferably, on the spot determinations, there is required a simple color determination which can be carried out by untrained personnel as a matter of routine. Test papers meet the requirement of providing a means for making rapid, on the spot determinations of this type.

A color reaction suitable for use in the analytical detection of albumin has been described by Feigl et al. (Mikrochim. Acta 2/1937, page 107). The reaction involved is based on the so-called "protein error" exhibited by certain indicators: If the pH value of a reagent solution is held below the characteristic acid-to-base color change of the indicator, upon the addition of albumin, a color change occurs which is independent of the pH value and which is due to the content of albumin in the solution; the color intensity varying directly with the concentration of albumin. Feigl developed the afore-mentioned albumin detection procedure for use as a spot reaction test (wet), in connection with which he used as reagent a potassium salt of tetrabromo-phenolphthalein-ethyl ester and acetic acid. For the preparation of albumin testing papers on the basis of the Feigl reaction, the acetic acid must be replaced by a non-volatile acid. According to the processes described in British Patent Nos. 814,223, 826,066, and 840,362, there is suggested as a replacement for the acetic acid an acid buffer substance, such as, for example, citrate buffer. However, the albumin testing strips, obtained in this manner, are not entirely satisfactory in that the differences in color between the negative and positive albumin reactions in albumin concentration ranges of below 0.1% are very slight. A positive test produces a more or less strongly yellowish green color, and the negative test is greenish yellow and is of a darker coloration than the dry test strip. Furthermore, the sensitivity of the test papers leaves much to be desired (detection limit about 0.03 to 0.01% of albumin). However, it is most important for the diagnostician to be able to establish the presence of albumin in the urine in the same concentrations as is possible in the acetic acid-heat test. It is accordingly necessary for a satisfactory albumin determination aid to be able to detect the presence of albumin in urine in concentrations of 0.005%.

In the process of the above-mentioned British patents, the absorbent carrier or test paper strip is impregnated with a solution which contains the buffer substance together with the color producing agent.

This invention has an object a simple and practical method for producing a diagnostic aid for use in the determination of albumin in urine in concentrations as low as 0.005%.

A further object of the invention is to produce a simple and practical method for producing a diagnostic aid for use in the determination of albumin in urine concentrations as low as 0.005% which determinations can be carried out rapidly without the use of laboratory equipment and by untrained personnel as routine matters.

A still further object of the invention is a simple and practical method for producing a diagnostic aid comprising a test paper for use in the determination of albumin in urine in concentrations as low as 0.005% which determination can be carried out rapidly without the use of laboratory equipment and by untrained personnel as routine matters.

A still further object of the present invention is a method for this purpose applicable to the treatment of preformed test paper strips.

Other objects will appear hereinafter.

In accordance with the invention, it has now surprisingly been found that test papers for the determination of albumin in urine having a considerably increased sensitivity can be obtained by impregnating a test paper with a buffering solution having a pH of below 7 and thereafter impregnating the test paper with a chemical reagent capable of changing color in the presence of albumin, the gradation of color changes being a measure of the albumin content. The test paper obtained in this way are colored a pale yellow and on immersion into an albumin-containing fluid take on strong blue colorations, the gradation of color (i.e., color intensity) being a measure of the albumin concentration, the colors produced being clearly distinguishable from the yellow coloration of a negative test. Using the test strips prepared in accordance with the invention, it is possible to positively detect
0.005% of albumin and even lower albumin concentrations being capable of measurement. The color of the paper strip (yellow) does not change in either neutral or in an alkaline buffer.

The test strip of the invention, comprising a diagnostic aid for the determination of albumin in biological fluids is composed of a reagent capable of changing color to indicate the presence of protein, an acidbuffering substance, and an absorbent carrier, which has been impregnated by immersing the absorbing carrier, first with an acid buffer solution, and subsequently with a solution of the color producing chemical reagent.

As chemical reagents or indicating agents, there may be used any indicator dye which exhibits the so-called “protein error,” that is demonstrating the presence of albumin by a color change in the acid pH range, in particular tetra-bromo-phenolphthalein-ethyl ester and -butyl ester, as well as tetra-bromo-benzazaine.

It is particularly important to select a suitable solvent for the indicator. Most advantageously a readily volatile solvent should be used in order that the test strip may be quickly followed after the impregnation. If the indicator is dissolved in methanol or acetone, this solution then being used to impregnate a paper strip which has already been impregnated with the buffering agent, the test paper obtained will demonstrate numerous blue spots. In accordance with the invention, it has been found that albumin test papers of a uniformly light color are obtained if a halogenated hydrocarbon is used as solvent for the indicator dye. Most suitable for this purpose are, for example, chloroform or carbon tetrachloride. A particularly suitable solvent is methylene chloride.

As a buffering system can be adjusted to a pH of below 7.0 can be used and preferably a citrate buffer, i.e., an aqueous solution of citric acid and tertiary sodium citrate. The optimum pH value lies at about 5.0.

This finding is to be considered as very surprising for this pH value is already on the “alkaline side” of the acid-base color-change range of the indicator dyes. In the literature, as cited above, it has expressly been pointed out that the optimum pH values for the albumin detection reaction based on the so-called “protein error” of indicator dyes are somewhat below the pH transition point of the indicator, i.e., in the “acid region” thereof (British Patent 404,362, pH 3.5-4.1). Even when the finished test paper according to the present invention is immersed into a solution without albumin, the indicator will not shift from its acid color range into the alkaline one. It has so far not been possible to find an explanation for this pH displacement caused by the simple step of the separate application of buffer substance and dye solution according to the present invention.

The preparation of the diagnostic acid according to the invention takes place otherwise in the conventional manner. The absorbent carrier, as for example, filter paper, is firstly impregnated with a buffer solution, and thereafter is dried immediately. Advantageously, there is added to the buffer solution one of the usual surface-active agents, as for example, sodium laurel sulfate or polyoxyethylene sorbitan monolaurate. This produces, on using the test paper an accelerated absorption of the fluid being analyzed and thereby the buffer can be made shorter. The plastic synthetic foils are thereby welded together and the paper strip is welded on one or both sides to the plastic sheet by the hot contact. Shallow grooves provided on the rollers assure that the portions of the plastic sheets adjacent the strips of paper are firmly welded together. The synthetic plastic bands with the paper strips are cross-wise to form the test paper strips. There are obtained by this process narrow foil strips having a length of 60 mm. and a width of 5 mm. containing a securely sealed in test paper strip of the size of about 2 x 5 mm. The test paper strip is covered on both sides with foil. Perpendicular cuts of the sealed plastic provide individual plastic strips containing the test paper. The small cut edges expose enough of the absorbing paper to enable the solutions being tested to be absorbed rapidly and reliably. Further, in the case of turbid solutions, a filtration of the solution being tested is achieved at the cut edge.

It has proven advantageous to effect the sealing-in of the test strips by including in the assembly a second, unimpregnated paper strip (filter paper Schleicher & Schüll No. 1450) in a superposed arrangement to the test paper strip in which connection the test strip is overlaid by the transparent plastic foil and the unimpregnated paper strip by the white filter paper.

It is to be understood that the procedure as set out in the above given example can be repeated in an analogous manner using other buffer and indicator solutions: In place of the above described citrate buffer solution, any other buffering system adjusted to a pH of below 7 may be used, i.e. malonic acid-malonate buffer, boric acid-borate buffer or tartaric acid-tartrate buffer. Suitable in-

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4 EXAMPLE

Composition of the buffer solution

<table>
<thead>
<tr>
<th>Grams</th>
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</thead>
<tbody>
<tr>
<td>Tertiary sodium citrate</td>
</tr>
<tr>
<td>Citric acid</td>
</tr>
<tr>
<td>Sodium laurel sulfate</td>
</tr>
</tbody>
</table>

Distilled water, Ad. 100 milliliters.

Composition of the indicator solution

<table>
<thead>
<tr>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetra bromo phenolphthalein ethyl ester</td>
</tr>
<tr>
<td>Methylene chloride</td>
</tr>
</tbody>
</table>

Filter paper (Schleicher & Schüll No. 2316) is impregnated first with a buffer solution as above set out by running such a paper band through a bath of the impregnating solution and thereafter drying the paper in the open air. Then, the dried paper is impregnated for a second time, but using the indicator solution as above set out and thereafter once more air dried. The dried paper is formed into rolls and the rolls are cross-cut to the desired width of 3 mm. The test strips thereby obtained are colored pale yellow and do not change their color on being moistened with albumin-free normal urine. The presence of albumin in the urine produces a more or less visible blue coloration in the test paper, the color intensity being used to grade the albumin content. In this manner the presence of albumin in urine can be clearly detected even when the same is present in concentrations of 0.005%.
indicator dyes are also tetra-bromo-phenolphthalein-butyl ester, tetra-bromo-benzaurine, bromo-phenol-blue, tetra-bromo-phenol-blue, bromo-cresol-green, thymol-blue, alizarine or methyl violet. These indicator dyes may be dissolved in water, methanol, ethanol, acetone, methylene chloride, chloroform or carbon tetrachloride. Instead of sodium lauryl sulfate, also polyoxyethylene sorbitane monolaurate can be used as surface-active agent which is to be added to the buffer solution.

When using the above named components in preparing the doubly impregnated test sheets according to the method set out in the above given example, the same results with respect to detection of albumin were obtained. Naturally, depending on the kind of the specific indicator dye, the colors of the test papers will vary. It is therefore advantageous to prepare a color comparison scale for each kind of test paper which shows the different colors of said papers as a function of the albumin content of the liquid to be tested. Such a scale can be prepared in the conventional manner using printed color for comparison purposes.

What is claimed is:

1. Analytical test paper for the detection of albumin in biological fluids comprising a substantially plane paper sheet, having a wettable surface prepared by first impregnating said paper sheet with a solution of an acid buffer, drying said buffer impregnated sheet and thereafter impregnating said sheet with a solution of an indicator dye exhibiting the so-called "protein error," said solution of acid buffer having a pH value below 7 and regulated for maintaining the indicator dye at a pH on the alkaline side of the acid-to-base color change point thereof and being dried after said buffer impregnated sheet.

2. The method of producing an analytical test paper for detection of albumin in biological fluids which comprises immersing an analytical test paper comprising a substantially plane paper sheet having a wettable surface which has been impregnated with an acid buffer solution, thereafter dried, said buffer impregnated sheet thereafter being impregnated with a solution of an indicator dye exhibiting the so-called "protein error," said solution of acid buffer having a pH value below 7 and regulated for maintaining the indicator dye at a pH on the alkaline side of the acid-to-base color change point thereof and being dried after said buffer impregnated sheet, said test paper thereafter being included between a superposed upper and lower sheet of a water insoluble plastic and subjecting the assembly to increased pressure and temperature to thereby bond the plastic sheets together on their longitudinal edge portions.

3. The method according to claim 2 wherein said indicator dye is a member selected from the group consisting of tetra-bromo-phenolphthalein ethyl ester, tetra-bromo-phenolphthalein butyl ester and tetra-bromo-benzaurine.

4. The method according to claim 2 wherein said indicator dye is employed in the form of its solution in a halogenated hydrocarbon.

5. The method according to claim 2 wherein said paper sheet is impregnated with a buffer solution comprising tertiary sodium citrate, citric acid, sodium lauryl sulfate, and distilled water.

6. The method according to claim 2 wherein said paper sheet is impregnated with an indicator dye solution comprising tetra-bromo-phenolphthalein ethyl ester, and methylene chloride.

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