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CEREBROSPINAL FLUID CONTROL STANDARD

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ABSTRACT OF THE DISCLOSURE

A synthetic control standard for the determination of cerebrospinal fluid constituents is provided by diluting normal blood serum with an aqueous solution of glucose and saline to a total protein concentration of 30 to 145 mg. per 100 ml.

This invention relates to a synthetic control standard for use in the determination of cerebrospinal fluid proteins and other constituents.

Cerebrospinal fluid is a clear, colorless fluid which is contained in the subarachnoid spaces of the brain and spinal cord and the ventricles of the brain. It contains a few lymphocytes and has a protein content of about 15 to 45 mg. per 100 ml. (mg. percent). Under certain patient conditions, for example, brain damage, brain or spinal tumors, it is desired to assay the cerebrospinal fluid for total protein content and other parameters.

The normal adult total volume of cerebrospinal fluid is about 125 and 150 ml. and it is renewed about every 3 to 4 hours. Normally, a spinal cord puncture will supply a specimen of only about 5 to 10 ml. and repeat punctures are usually to be avoided. In an average 6 ml. specimen, 4 ml. is generally used up in laboratory analysis for the patient from which the sample is obtained, leaving only about 2 ml. per patient for retention for other purposes. In a 300 bed hospital, it generally takes on the average about 9 months to collect one liter of surplus human cerebrospinal fluid. Thus, cerebrospinal fluids are precious specimens.

In the determination of cerebrospinal fluid protein, as with other biological fluid materials, it is desirable to employ control standards of known composition for comparison with the patients sample. Heretofore, these control standards have comprised carefully collected and processed human cerebrospinal fluid. However, because of the relatively small amount of material available, the product is costly. Moreover, because cerebrospinal fluid is collected in such small quantities, the material tends to deteriorate over the normal storage period required for collection of a suitable quantity for sale such as a liter or more. Enzymes in the fluid will tend to react with other constituents and product break-down products. Because of the numerous small samples which make up any collection of significant volume, the risk of bacterial contamination is greater than in ordinary blood serum collection. Furthermore, the human cerebrospinal fluid material is difficult to handle in processing since it does not lyophilize readily. Because its mass is relatively small, it forms a fine powder which tends to collect on the upper walls and closure of the sample bottles with a consequent loss of material upon opening the closure and reconstitution with water prior to use.

Accordingly, it is an object of this invention to provide a synthetic control standard for use in the determination of cerebrospinal fluid.

It is another object of this invention to provide a synthetic cerebrospinal fluid control standard which has improved lyophilization handling characteristics over the naturally occurring material. Other objects and advantages of the invention will be apparent to those skilled in the art upon reading of the disclosure hereof.

In accordance with the invention, a synthetic control standard for the determination of cerebrospinal fluid is prepared from normal blood serum by dilution with a reagent comprising glucose and chloride ion in aqueous solution to a level whereby the total protein concentration ranges from about 30 to about 145 mg. percent. It is preferred that the diluted serum contains from about 40 to about 200 mg. percent glucose and from about 80 to about 130 meq. per liter of chloride ion.

The chloride ion can be provided by use of an aqueous solution of an alkali metal chloride, preferably sodium chloride. The chloride salt and the glucose preferably are analytical or reagent grade materials.

For some purposes it is desired to include in the synthetic control standard of this invention a small amount of pre-albumin, preferably from about 1 to about 3 mg. percent. Pre-albumin is a special fraction obtained from blood serum and is described, for example, by Got, et al., "Isolement et caractérisation d'une préalbumine du sérum humain," Protides of the Biological Fluids, Vol. 10, H. Peeters, ed., Amsterdam, Elsevier, 1963, pp. 125–126; Schultze and Hermans, Molecular Biology of Human Protein, Vol. 1, Amsterdam, Elsevier, 1965, pp. 184–185.

After dilution of the blood serum with aqueous glucose and saline or other chloride containing ion, with or without the added pre-albumin, the aqueous solution is lyophilized to a dry material. The lyophilized material is improved by the addition of a small amount of gum acacia prior to lyophilization, for example, on the order of about 0.1%. This improved lyophilized, dry material can be readily reconstituted with water prior to use without product loss on the container closure.

The synthetic cerebrospinal fluid control standard made as described herein has been found to produce much sharper bands in the electrophoretic pattern in actual use than obtained with the normal human cerebrospinal fluid. This is an added advantage of the invention in practice and enables the technician to improve the accuracy of the control procedures.

The following examples will further illustrate the invention, although the invention is not limited to these specific examples.

EXAMPLE 1

A synthetic control standard for use in the determination of cerebrospinal fluid is prepared as follows:

Human blood serum from which the clotting factors have been removed is diluted with an aqueous solution of glucose and sodium chloride containing 6.38 grams of NaCl and 600 mg. of glucose per liter. The serum is thereby diluted to a total protein concentration of 110 mg. per 100 ml. by admixing 65 parts by volume of the diluent solution with one part by volume of the serum. To one liter of the diluted serum is then added 10 ml. of a 10% solution of gum acacia in water. The resulting solution is then filled in 3 ml. aliquots into 5 ml. bottles and lyophilized. The lyophilized, dry material is stored at 2° to 8° C. until required for use. In use, the dry material is reconstituted by dilution with 3 ml. of distilled water per bottle. Upon reconstitution of the fore-

going material, the following assay values were determined.

Constituent:	Value
Chloride, meq. per liter	105
Glucose, mg. per 100 ml.	57
Protein, total, mg. per 100 ml.	110
Pre-albumin, percent of total	0
Albumin, percent of total	58
α_1-globulin, percent of total	6
α_2-globulin, percent of total	12
β-globulin, percent of total	13
γ-globulin, percent of total	11
Colloidal gold (Lange's test)	1111100000

EXAMPLE 2

Example 1, above, is repeated except that 1–3 mg. percent of pre-albumin is added to the solution prior to lyophilization.

The control standards prepared in Examples 1 and 2, above, are used in the same manner as an unknown cerebrospinal fluid for comparison with the patient's sample to provide a check on the analytical determination.

Various other examples and modifications of the foregoing examples can be made by the person skilled in the art after reading the foregoing description and the appended claims without departing from the spirit scope of the invention. All such further examples and modifications are included within the scope of the appended claims.

What is claimed is:

1. The method of making a synthetic control standard for use in the determination of cerebrospinal fluid constituents comprising diluting normal blood serum with an aqueous diluent containing from about 40 to about 200 mg. per 100 ml. of glucose and from about 80 to about 130 milliequivalents per liter of chloride ion to a level at which the total protein concentration ranges from about 30 to about 145 mg. per 100 ml.

2. The method of claim **1** including the additional steps of admixing the liquid control standard with about 0.1% gum acacia and lyophilizing to a dry, powdered material.

3. A synthetic control standard for use in the determination of cerebrospinal fluid comprising normal blood serum diluted with an aqueous diluent containing from about 40 to about 200 mg. per 100 ml. of glucose and from about 80 to about 130 milliequivalents per liter of chloride ion to a level at which the total protein concentration ranges from about 30 to about 145 mg. per 100 ml.

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