United States Patent Office 3,497,319
Patented Feb. 24, 1970

3,497,319

DIAGNOSTIC REAGENT

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No Drawing. Filed Nov. 2, 1966, Ser. No. 591,409
Int. Cl. G01n *31/00*; C09k *3/00*; A61k *27/00*
U.S. Cl. 23—230 4 Claims

ABSTRACT OF THE DISCLOSURE

Qualitative one-step screening test for fibrinogen is provided by a diagnostic reagent consisting of thrombin dissolved in saline and lyophilized.

This invention relates to a method of preparing a useful diagnostic reagent, and more particularly, a product useful as a screening reagent for hypofibrinogenemia.

Hypofibrinogenemia is a blood disorder which is evidenced by abnormally low concentrations of fibrinogen in blood plasma. A means therefor of establishing such a deficiency by way of a general screening reagent is highly desirable especially in obstetrics where complications during pregnancy may occur particularly in patients who have severe premature separation of the placenta, longstanding intrauterine fetal death or an amniotic fluid embolus.

The reagent prepared by the process disclosed herein is particularly advantageous since the test can be accomplished within a matter of minutes and further for the reason that the technician running the test has but one simple physical step to follow leaving very little chance of error. Accordingly, the process of this invention comprises:

(a) Dissolving thrombin in saline; and

(b) Lyophilizing an aliquot of said resulting solution that reconstitution with a pre-determined amount of water provides a reagent for hypofibrinogenemia testing with a thrombin content of from about 40 to 60 units/ml.

The initial step consists of dissolving thrombin in saline. Thrombin is an enzyme occurring in blood cerum and for purposes of this invention it is preferred to use thrombin isolated from bovine blood serum. It is possible, however, and within the scope of this invention to use human thrombin. The desirability of using bovine thrombin resides in the fact that such material is commercially available and relatively inexpensive. Since reference is made to thrombin content in terms of units per ml., it should be pointed out that a standard of one unit in that amount of thrombin which is necessary to coagulate 1 ml. of standard fibrinogen solution in 15 seconds. Further in this regard, it is a purpose of this invention to prepare a diagnostic product with a thrombin content of from about 40 to 60 units/ml.

With regard to the term "saline," it is meant to indicate throughout a standard physiological saline solution and, in this instance, an 0.85% by weight aqueous sodium chloride solution.

The second step of the above described process consists of lyophilizing or freeze-drying aliquots of the resulting solution with that reconstitution with calculated amounts of water results in a reagent available for testing purposes having a thrombin content of from about 40 to 60 units/ml.

Since it will be most appropriate or preferred to pipette automatically 0.5 ml. aliquots so that after lyophilization, reconstitution with 0.2 ml. of water provides a reagent having a thrombin content of about 10 units, then of necessity the initial thrombin-saline solution must contain about 20 units of said thrombin per ml. of saline. It is

obvious therefore that the aliquot amounts and reconstitution values will depend on the thrombin amount in the original solution. Hence, if the thrombin content in the original solution is 40 units/ml., then 0.25 ml. aliquots which are lyophilized, are reconstituted with 0.2 ml. of water to provide similar reagents.

The one-step diagnostic method for hypofibrinogenemia consists of the following: a sample of reagent (0.2 ml.; 50 units/ml.) is combined with 0.2 ml. of a suspect plasma specimen. The container holding the resulting mixture is agitated by a slow tilting movement twice. After 2 minutes, the nature of the clot formed is observed by allowing it to run down the side of the tube.

If normal levels of fibrinogen exist in the specimen, the clot at 2 minutes is firm and extrudes little or no fluid.

If sub-normal levels of fibrinogen (hypofibrinogenemia) exist in the specimen, the clot is weak and represents less volume than the fluid present.

The following examples are given to more fully illustrate the present invention. It is to be understood that these examples are for illustrative purposes only and that the invention is not meant to be limited to the specific details of the examples.

EXAMPLE I

Bovine thrombin is dissolved in 100 ml. of saline (0.85%) to provide a solution having a thrombin content of 200 units/ml. By means of an automatic pipetting machine, vials are filled with 0.5 ml. of the above solution. The vials are then stored in a freezer overnight and lyophilized the next day. Each vial is then reconstituted with 0.2 ml. of distilled water as needed to provide a reagent having a thrombin content of about 50 units/ml.

EXAMPLE II

The procedure of Example I is repeated wherein said vials are filled with 0.4 ml. portions instead of 0.5 ml. portions in order to provide a reagent having a thrombin content of about 40 units/ml.

EXAMPLE III

The procedure of Example I is repeated wherein said vials are filled with 0.6 ml. portions instead of 0.5 ml. portions in order to provide a reagent having a thrombin content of about 60 units/ml.

EXAMPLE IV

Test procedure

To a sample of reagent (0.2 ml.) as prepared in Example I is added 0.2 ml. of a suspect plasma specimen. The container holding the resulting mixture is agitated by a slow tilting movement twice. After 2 minutes, the nature of the clot formed is observed by allowing it to run down the side of the tube.

If normal levels of fibrinogen exist in the specimen, the clot at 2 minutes is firm and extrudes little or no fluid.

If sub-normal levels of fibrinogen (hypofibrinogenemia) exist in the specimen, the clot is weak and represents less volume than the fluid present.

EXAMPLE V

The procedure of Example I is repeated wherein an equivalent amount of human thrombin is used in lieu of bovine thrombin with comparable results.

What is claimed is:

1. A process of preparing a one-step diagnostic product useful as a screening reagent for hypofibrinogenemia, which comprises:

(a) dissolving thrombin in saline to a predetermined concentration; and

(b) lyophilizing aliquots of said resulting solution.

2. A process of preparing a one-step diagnostic product useful as a screening reagent for hypofibrinogenemia which comprises:

(a) dissolving thrombin in saline in an amount to provide about 20 units of said thrombin per ml. of saline; and

(b) lyophilizing approximately a 0.5 ml. of aliquot of said resulting solution such that reconstitution with approximately 0.2 ml. of water provides a suitable reagent for hypofibrinogenemia testing having a thrombin content of about 10 units.

3. The product as prepared by the process of claim 2.

4. A one-step diagnostic method for determining hypofibrinogenemia which comprises: commingling approximately 0.2 ml. of suspect human plasma with the diagnostic reagent as prepared by the process of claim 2 and observing the mixture for the nature of the clot which forms.

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U.S. Cl. X.R.

195—103.5; 252—408; 424—2