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M. C. RHEES

2,878,715

METHOD OF BLOOD PLASMA PROTHROMBIN TIME DETERMINATIONS

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2 Sheets-Sheet 1

FIG. 1.

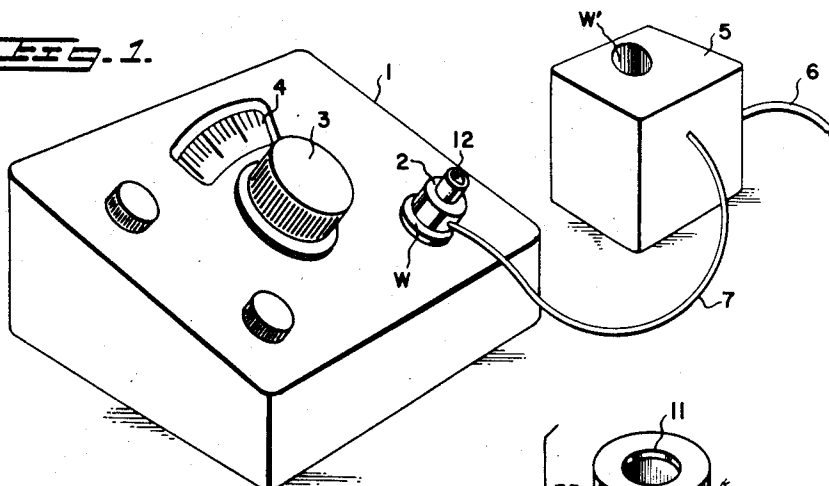


FIG. 2.

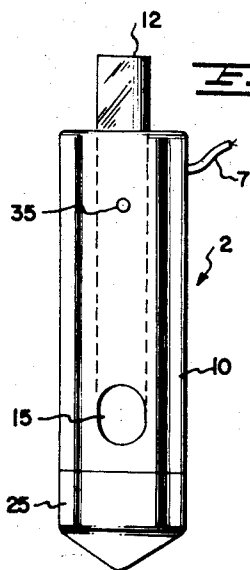


FIG. 3.

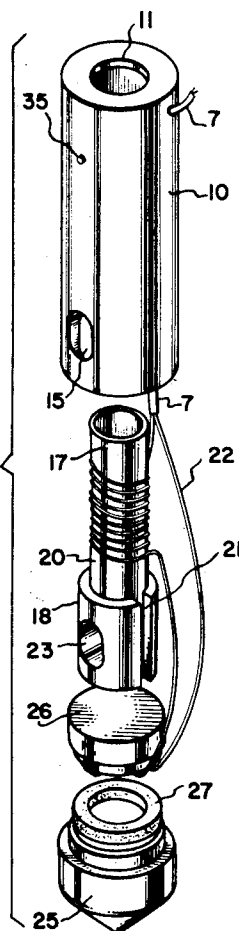
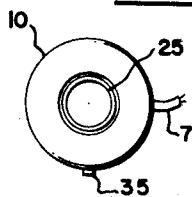


FIG. 4.



INVENTOR

Mark C. Rhee

Albert H. Kichner

ATTORNEY

BY

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M. C. RHEES

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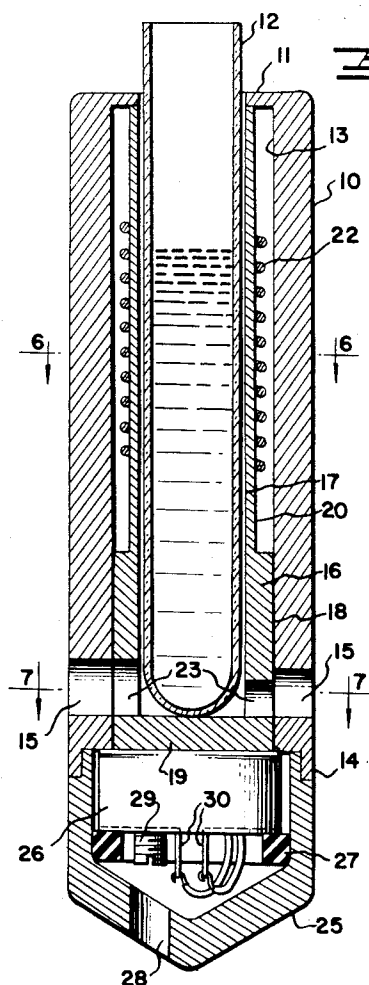


FIG. 5.

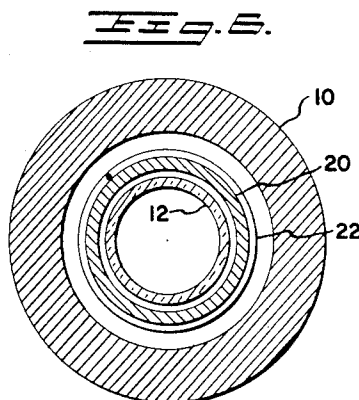


FIG. 6.

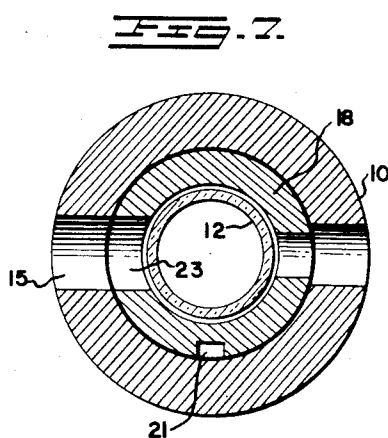


FIG. 7.

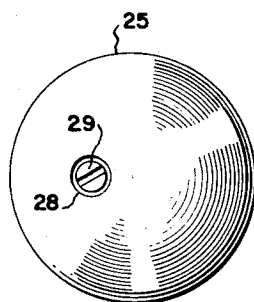


FIG. 8.

INVENTOR

Mark C. Rhee

BY

Albert H. Kirchner

ATTORNEY

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2,878,715

METHOD OF BLOOD PLASMA PROTHROMBIN TIME DETERMINATIONS

Mark C. Rhees, Seattle, Wash.

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3 Claims. (Cl. 88—14)

The present invention relates to blood plasma prothrombin time determinations and provides a method of making such determinations and an instrumentality useful in practicing the method.

Important objects are to improve the accuracy of such determinations by providing novel technique for controlling temperature and eliminating errors inherent in the prior art apparatus and methods.

The new method involves photoelectric detection of the instant of clotting by the use of a standard type of spectrophotometer in combination with a novel adapter provided by the invention for maintaining a sample of thromboplastin-plasma mixture at a constant, accurately controlled temperature and positioning the sample in the spectrophotometer.

In addition to the enhanced precision of the new procedure, an important advantage of the invention is the simplicity of the mechanical equipment and the ease with which the procedure can be learned by relatively inexperienced personnel, with the result that the human equation error is substantially eliminated so that different workers arrive at uniform determination figures in separate tests of the same material.

In the accompanying drawings, which illustrate in certain preferred forms the apparatus useful in practicing the new method,

Figure 1 is a perspective view of the complete apparatus;

Fig. 2 is a side elevational view of the new adapter or test tube holder;

Fig. 3 is an exploded perspective view of the adapter;

Fig. 4 is a top plan view of the adapter;

Fig. 5 is an axial sectional view of the adapter on a relatively enlarged scale;

Fig. 6 is a transverse sectional view taken on the line 6—6 of Fig. 5;

Fig. 7 is a transverse sectional view taken on the line 7—7 of Fig. 5; and

Fig. 8 is a bottom plan view of the adapter.

In Fig. 1 the reference numeral 1 designates a spectrophotometer of known type. This instrument has the usual well W, which in Fig. 1 is shown filled with the adapter 2 provided by the present invention, as well as an adjusting knob 3 and a galvanometer dial 4. Alternatively a colorimeter of standard type could be substituted for the spectrophotometer shown.

The power supply 5 comprises simply a casing containing a 110 volt A.C. stepdown transformer of the door bell type having an input cord 6 and an output cord 7 connected to the adapter and designed to deliver about 8 volts. The casing may conveniently be provided with a well W', similar in size to that of the well W of the spectrophotometer, for holding the adapter 2 when not in use.

The adapter 2 comprises an outer casing 10 of generally tubular form preferably made of machined aluminum, dyed black and anodized. The upper end of the casing is inwardly flanged, as shown at 11, so that the orifice,

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which in the illustrated embodiment is designed to accommodate rather snugly a 10 mm. culture tube or cuvette 12, is of slightly larger diameter than the bore 13, which is cylindrical and extends uniformly through the open bottom end of the casing, which is surrounded by an axially directed peripheral flange 14. Just above its open bottom the casing is drilled diametrically to provide two aligned transverse sight openings 15.

A core member 16, made of the same material as the casing 10, is contained within the casing and has a cylindrical bore 17 in which the tube 12 makes a loose fit. For snugly seating the core member in the casing, the lower end portion 18 of the core member has an external diameter equal to the diameter of the bore of the casing, and the length of the core member is such that its upper end engages the flange 11 at the top of the casing when the closed bottom 19 of the cord member is aligned in the same plane as the bottom of the casing (at the root of the flange 14). Most of the length of the core member is of reduced outside diameter, as shown at 20, and the lower portion 18 is slotted lengthwise, as shown at 21 in Fig. 3, to accommodate two leads of the heating wire 22, which will be hereinafter described. Just above its bottom the core member is diametrically bored to provide oppositely aligned sight openings 23, which register with the casing sight openings 15 when the core member is in place, as shown in Fig. 5.

A cap 25, made of the same material as the casing and core member, has an axially projecting internal flange complementary to the flange 14 at the bottom of the casing and makes a tight friction fit over the casing bottom, providing a hollow chamber containing a small thermostat 26.

An annular rubber gasket 27 may be interposed between the thermostat and the closed bottom of the cap 25 to cushion the thermostat and press it up against the bottom 19 of the core member 16. The cap is drilled to provide a small opening 28 for insertion of a tool, such as a small screwdriver, for effecting adjustment of the regulator screw 29 of the thermostat.

The terminals 30 of the thermostat are connected to the leads of the heating wire 22 which extend up through the slot 21 for connection with the power supply output cord 7 which enters the casing 10 through a hole in its upper side wall. The heating wire 22 in the preferred, illustrated embodiment of the invention consists of approximately 25 inches of cotton covered #34 Nichrome steel wire disposed in ten turns around the upper, smaller diameter portion of the core member, the remainder of the length of the wire being folded on itself and fitted into the slot 21. The arrangement of this wire is important, as will be explained hereinafter. For clarity of illustration the twisted condition of the wire and its position in the slot 21 are not shown in Fig. 3.

As will be seen in Fig. 3, the thermostat and heating wire turns are in series and are supplied by the cord 7 from the power supply 5. For the type of instrument shown in Fig. 1, in which the well is of plastic having good heat insulating properties, it is appropriate to supply about five watts at 8 volts. With instruments having metal wells somewhat more power should be used.

The thermostat is adjusted to maintain a temperature of 37° C. It is important that the device function with precision to maintain this temperature, and it is for this purpose that the wiring arrangement be made in the following manner:

Of the 25 inches of wire, ten turns are made around the reduced portion 20 of the core member. The remainder is twisted and fitted into the slot 21. If all of the wire were disposed in additional turns, the result would be too much lag at the thermostat and the temperature could not be maintained accurately at the set-

ting. Moreover, if part or all of the wire were disposed nearer the thermostat, the control would be too sensitive and the tube 12 would not reach the desired temperature.

Power of five watts at eight volts is preferred for a spectrophotometer of the kind selected to illustrate this disclosure of the invention because such power results in the thermostat keeping the circuit closed about half of the time, thus maintaining the temperature within a cycle range of not more than 0.5° C. Excessive power would bring the tube to too high a temperature before the thermostat would open the circuit, so that a cycle range of as much as several degrees would result.

For use with the type of instrument shown in Fig. 1 the casing has a small pin 35 projecting from the upper portion of its side wall. This serves to align the registering openings 15 and 23 with the light beam when the adapter is inserted in the well W, as shown in Fig. 1.

In use the thermostat 26 is regulated to keep the contents of the test tube 12 at a constant temperature of 37° C., which of course is normal body blood temperature, and the method invention is practiced as follows:

(1) A quantity of freshly drawn blood is mixed with sodium citrate and the plasma is separated by centrifuging, all in a well known manner. The proportion recommended is 0.5 ml. of 0.1 M sodium citrate to 4.5 ml. of blood.

(2) Into each of several 10- by 75-mm. cuvettes is measured a 0.10-ml. portion of 0.02 M calcium chloride solution and the cuvettes are placed in a 37° C. constant temperature water bath located near the spectrophotometer.

(3) Equal parts of plasma and thromboplastin reagent are thoroughly mixed in a test tube. It is convenient to use 0.40 ml. of plasma with 0.40 ml. of "Difco" thromboplastin reagent produced by Difco Laboratories, Incorporated, Detroit, Michigan, and to use 1 ml. serologic pipets for measuring these quantities.

(4) The tube containing the thromboplastin-plasma mixture and the 1-ml. pipet used in measuring the two ingredients of the mixture are placed for five minutes in the 37° C. water bath.

(5) When the mixture has come to temperature, one of the cuvettes containing the 37° C. calcium chloride solution is quickly wiped dry and placed in the warm adapter in the spectrophotometer well. The wavelength is set at near 500 mμ and the galvanometer indicator is adjusted so that it is just visible in the extreme right side of the scale opening.

(6) Two-tenths ml. of the test mixture is drawn into the tip end of the warm 1-ml. pipet and forcibly blown into the cuvette in the adapter, and at the instant of blowing a stopwatch is started. Immediately then the pipet is "palmed" in such a way that the cuvette can be removed from the adapter and mixing is completed as by striking a few sharp blows on the hand. The cuvette is then returned to the adapter, and the pipet is wiped and returned to the tube containing the thromboplastin-plasma mixture. This operation should be completed within an elapsed time of five seconds. When the cuvette is finally placed in the adapter, the galvanometer indicator will return to about the center of the scale and come to rest. The end-point of the determination is the instant at which the indicator makes a positive move toward the left. At this instant the stopwatch is stopped, and the elapsed time noted is the prothrombin time.

It is recommended that the test be repeated at least two more times and the average value taken.

It may be pointed out that if oxalated plasma is used the slow precipitation of calcium oxalate may not allow the indicator to come to rest when the cuvette containing the mixture is placed in the spectrophotometer well.

It will be appreciated by those skilled in the art that the controlled constant temperature made possible by the new apparatus, employed in practicing the new method, is productive of a high degree of uniformity in results so that errors attributable to variations in temperature, not only as between different determinations of the same plasma, but also during the making of a single determination, are effectively eliminated.

While the invention, particularly the apparatus used in practicing it, has been explained in terms of a single preferred construction, it is to be understood that variations from the disclosed embodiment may be practiced without departing from the essential principles of the invention as defined by the broader of the appended claims. Thus, for example, while aluminum is the preferred material for the casing and core member because of the high coefficient of heat-conductivity of aluminum, other materials could be substituted. So also with other features of the disclosed structure, as will be understood.

I claim:

1. A method of blood plasma prothrombin determination comprising bringing separately a quantity of plasma and a quantity of clotting solution to a temperature of substantially 37° C., positioning one of said quantities in a photoelectric device responsive to a change in turbidity, adding the other quantity, and observing the time elapsed between the time of the addition and the time of the photoelectric response to clotting, while maintaining the temperature of the quantities at substantially 37° C. throughout the entire elapsed time.

2. A method of blood plasma prothrombin determination comprising bringing separately a quantity of plasma and a quantity of clotting solution to a temperature of substantially 37° C., positioning the clotting solution in a spectrophotometer, admixing the plasma therewith, and observing the time elapsed between the time of the admixing and the time of the response of the spectrophotometer indicator to a sudden increase in turbidity resulting from clotting, while maintaining the temperature of the mixture at substantially 37° C. throughout the entire elapsed time.

3. A method of blood plasma prothrombin determination comprising bringing separately a quantity of plasma and a quantity of 0.02 M calcium chloride solution to a temperature of substantially 37° C., positioning the solution in a spectrophotometer, admixing the plasma therewith, and observing the time elapsed between the time of the admixing and the time of the response of the spectrophotometer indicator to a sudden increase in turbidity resulting from clotting, while maintaining the temperature of the mixture at substantially 37° C. throughout the entire elapsed time.

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