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Measurement of blood coagulation time

The invention is concerned with a method for measuring the coagulation time of a blood sample, in which a sample-reagent mixture is formed by introducing the sample and at least one reagent into a cuvette, and an apparatus suitable for carrying out the method.

There are known a method and an apparatus of the type mentioned above (German Utility Model GM 7707 546), in which the coagulation of the sample-reagent mixture is brought about by stirring the mixture with a stirring rod which must be introduced into the cuvette before the measurement and which is driven by a magnetic stirring arrangement situated outside the cuvette.

When a large number of measurements is to be carried out, the expenditure of work for the introduction of the stirring rod into the cuvette is found to be a disadvantage of the known method mentioned above. Since the magnetic stirring arrangement represents a considerable part of the total material cost of the known apparatus, it would moreover be desirable to have an apparatus which operates without such an arrangement.

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The problem underlying the invention is therefore to provide a method and an apparatus of the type described above which requires a low operating and material cost.

In accordance with the invention this problem has been solved by a method of the type referred to earlier, which comprises moving a sample-reagent mixture in a stationary cuvette in such a way that the mixture flows back and forth around a sharp edge formed by faces of a partition of the cuvette intersecting at an acute angle, the edge projecting into a stationary cuvette, whereby a clot forms on the sharp edge.

A further object of the invention is an apparatus for measuring the coagulation time of a blood sample with a cuvette for receiving the sample and the reagent. In the apparatus in accordance with the invention the upper part of the cuvette is divided into two chambers by a partition and the partition has a sharp edge which is situated at a distance from the bottom of the cuvette and which is formed by the intersection at an acute angle of faces of the partition.

In comparison with the above-mentioned, known method and the corresponding apparatus, the solution provided by the invention has the advantage that no stirring rod need be introduced into the cuvette and in that accordingly also no drive for such a stirrer is required. The present invention therefore makes it possible to achieve a substantial reduction in the operating and material costs.

A working example of the invention is described hereinafter on the basis of the accompanying drawings.

Fig. 1 shows the cuvette of an apparatus in accordance with the invention and the electro-optical arrangement provided therein.

Fig. 2 shows a cross-section of the cuvette of the apparatus in accordance with the invention and the arrangement provided therein for the production of a variable air pressure in the cuvette.

Fig. 3 shows steps which are carried out in a method in accordance with the invention.

Fig. 4a to 6b show an alternative form of cuvette and its use in an apparatus in accordance with the invention.

Fig. 7 shows schematically a cross-section of a preferred embodiment of the arrangement 2) shown in Fig. 2 for the production of a variable air pressure.

Fig. 8 shows schematically a typical variation with time of the air pressure which can be produced with the arrangement in accordance with Fig. 7.

Fig. 1 shows a cuvette 1 used in an apparatus in accordance with the invention. This cuvette is made of a material which enables optical measurements to be carried out on the content of the cuvette. This cuvette need not necessarily have the form shown in Fig. 1. However, it is essential that it has a partition 2 with a sharp edge 3. This partition separates the upper part of the cuvette into two chambers 7 and 8.

The apparatus also contains a light source 5 (e.g. a light-emitting diode) and a light receiver 6 (e.g. a photodiode) in the arrangement shown in Fig. 1. With these components there is produced and, respectively, received a beam of light 4 which passes through the cuvette, whereby it passes close to and below the edge 3, in order to enable the measurement of the absorbance of the cuvette content traversed by the beam of light 4. The path of the beam of light 4 is so chosen that a clot 33 formed on the edge 3 is traversed by the beam of light 4.

Moreover, the apparatus contains a light source 62 (e.g. a light-emitting diode 62) and a light receiver 63

(e.g. a photodiode 63) in the arrangement shown in Fig. 1. With these components there is produced and, respectively, received a beam of light 61 which passes through the cuvette, whereby it passes above the edge 3, in order to enable a measurement of the absorbance of the cuvette content which is traversed by the beam of light 61. The path of the beam of light 61 is so chosen that the maximum amount of sample provided, which is introduced into the cuvette, is not detected by the beam of light 61, but that after addition of a reagent even in the case of the minimum volume chosen for the sample-reagent mixture, this mixture is traversed by the beam of light 61.

In order to reduce the influence of the ambient light on the absorbance measurements with the beams of light 4 and 61, it is preferable to use photodiodes with a built-in infra-red filter as light receivers. The filter range of the latter filter preferably goes from about 900 to 1000 nm and has a minimum attenuation at about 940 nm. By means of these filters, the sensitivity of the measurements to an ambient illumination with incandescent light is made about 5 times smaller.

As shown in Fig. 2, the chamber 7 in the upper part of the cuvette 1 is connected by means of an opening 9 with an arrangement 21 with which a variable air pressure acting on the content of the cuvette can be produced. By means of

this pressure the sample-reagent mixture in the apparatus in accordance with the invention is moved in the manner which is illustrated by Fig. 3 (3). As described hereinafter with reference to Fig. 7 and Fig. 8. the arrangement 21 can contain, for example, a vibrating membrane 22 as the pump element. The air pressure produced therewith passes into the cuvette 1 through a conduit 23 and through the opening 9. The cuvette has an opening 11 which remains open in operation.

As shown in Fig. 3, the following steps are carried out in the performance of the method in accordance with the invention with the apparatus described above:

(1) A predetermined volume of the blood or plasma sample 31 is introduced into the cuvette and incubated therein. As shown in Fig. 3 as an example, the amount of sample is preferably chosen so small that it does not reach the edge 3 and thus air can flow freely between the surface of the sample and the edge 3. The air pressure produced by the arrangement 21 can thus cause no movement of the sample which remains stationary as long as a predetermined amount of reagent is not yet indroduced into the cuvette.

(2) A predetermined volume of reagent is introduced into the cuvette. The respective amounts of sample and reagent are chosen so that the surface of the sample-reagent mixture lies in each case above the edge 3 and the beam of light 61. Thereby, the following effects are achieved as soon as the reagent is introduced into the cuvette :

(3) The sample-reagent mixture is detected with the beam of light 61. This point in time marks the beginning of the measurement of the coagulation time. This point in time is registered with electronic signal processing means known per se. By the action of the above-mentioned air pressure, which is produced with the means shown in Fig. 2, the sample-reagent mixture now flows and thereby moves back and forth between the positions (a) and (b) shown in Fig. 3 (3). Thereby the sample is mixed well together with the reagent and a flowing of the mixture around the sharp edge 3 of the cuvette is brought about.

(4) By the relative movement of the liquid in relation to the edge 3, shown in (3), a clot 33 forms on the edge 3 at the moment of the clotting. Since this clot is detected with the beam of light 4, a sudden and perceptible change in the absorbance measured with this beam of light results. By determining

this change the point in time of the coagulation and at the same time the coagulation time are clearly and precisely measured. For this determination and for the recording of the values ascertained there are used electronic signal processing or data recording means known per se.

In a variant of the method described above, and insofar as the measurement is not impaired thereby, the amount of sample can be chosen so large that also when the sample alone is situated in the cuvette its surface lies above the edge 3. In this case it is traversed by the beam of light 4 before the addition of reagent to the sample and moves by the action of the air pressure mentioned above, analogously as shown in Fig. 3 (3) for the sample-reagent mixture.

As shown in Fig. 4a to 6b, the apparatus in accordance with the invention can also operate with a different form of cuvette.

Fig. 4a shows a cross-section of a cuvette 41 shown in side-elevation in Fig. 4b. As in the case of cuvette 1 in Fig. 1 the cuvette 41 also has a partition 44 with a sharp edge 43. The top 45 of the cuvette 41 is open. As shown in Fig. 5, the variable air pressure is supplied to the cuvette 41 through a lateral opening 42 which is

connected via a conduit 77 with the arrangement which produces the air pressure. In contrast to the cuvette in accordance with Fig. 1 the cuvette 41 in Fig. 4a - 6b has the advantage that it enables a simpler operation, since it can be easily placed in the measuring device.

Fig. 6a shows the cuvette 41 when it contains only the sample. Fig. 6b shows the cuvette 41 when it contains the sample-reagent mixture. In Fig. 6a and 6b the position of the beams of light 4 and 61, respectively, are each marked with a small circle.

The mode of operation of the apparatus in accordance with the invention with the cuvette 41 in accordance with Fig. 4a and 4b is the same as described above on the basis of Fig. 3.

Fig. 7 shows schematically a cross-section of a preferred embodiment of the arrangement 21 (in Fig. 1) for the production of the variable air pressure. This arrangement is contained in a housing 71. A membrane 72 which has a core of sheet steel 73 is arranged in this housing. An oscillation of the membrane 72 is brought about with an electromagnetic drive assembly which contains a magnetic core 74 and a magnetic coil 75 and to which is applied a suitable alternating current. Thereby there results in the housing 71 a variable air pressure which

is applied to two cuvettes of the apparatus in accordance with the invention via conduits 77 and 78, respectively.

Fig. 8 shows schematically a typical variation with time of the air pressure which can be produced with the arrangement shown in Fig. 7. The air pressure varies between +2 and -2 millibar, and its variation with time has approximately the form of a sine wave with a frequency of about 40 Hz.

The working example described above operates, for example, with a sample volume of 100 or 200 µl and with a fixed reagent volume of 200 µl.

CLAIMS:

1. A method for measuring the coagulation time of a blood sample, which method comprises moving a sample-reagent mixture in a stationary cuvette in such a way that the mixture flows back and forth around a sharp edge formed by faces of a partition of the cuvette intersecting at an acute angle, the edge projecting into the cuvette, whereby a clot forms on the sharp edge.

2. A method according to Claim 1, wherein the mixture is moved by air pressure.

3. A method according to Claim 1, wherein the beginning of the measurement of the coagulation time is detected by determining a change in the absorbance which occurs as a result of the addition of the reagent to the sample, and which is measured with a first beam of light which passes above the edge.

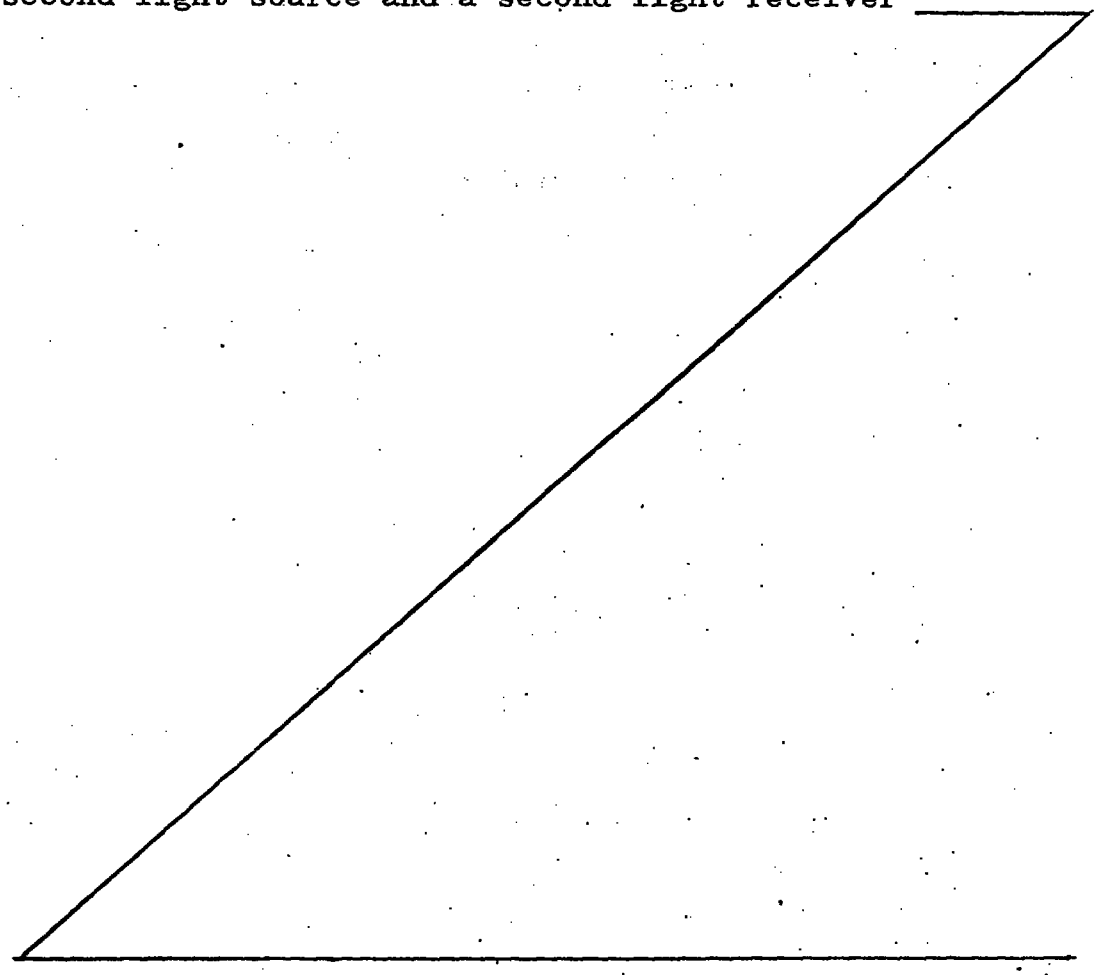
4. A method according to Claim 1, wherein the formation of a clot which forms on the edge is detected by measuring the absorbance of the sample-reagent mixture with a second beam of light which passes through the cuvette, said beam of light passing close to and below the edge.

5. An apparatus for measuring the coagulation time of a blood sample, with a cuvette for receiving the sample and at least one reagent, wherein the upper part of the cuvette is divided into two chambers by a partition and wherein the partition has a sharp edge which is situated at a distance from the bottom of the cuvette and which is formed by the intersection at an acute angle of faces of the partition.

6. An apparatus according to Claim 5, wherein one of the chambers in the upper part of the cuvette is connected via an opening with an arrangement with which there can be produced a variable air pressure with which a liquid contained in the cuvette can be moved when the level of the liquid is so high that no air can flow freely between it and the edge.

7. An apparatus according to Claim 5, which contains a first light source and a first light receiver which are arranged in relation to the cuvette so that a first beam of light emanating from the first light source and received by the first light receiver passes through the cuvette, said first beam of light passing above the edge.

8. An apparatus according to Claim 5, which contains a second light source and a second light receiver



which are arranged in relation to the cuvette so that a second beam of light emanating from the second light source and received by the second light receiver passes through the cuvette, said second beam of light passing close to and below the edge.

9. An apparatus according to claim 8, wherein the path of the second beam of light is chosen so that a clot formed on the edge is detected by this beam of light.

10. A method for measuring the coagulation time of a blood sample substantially as hereinbefore described with reference to any of the Figures of the accompanying drawings.

11. An apparatus for measuring the coagulation time of a blood sample, substantially as hereinbefore described with reference to, and as shown in, any of Figures 1, 2, 3, 7 and 8 of the accompanying drawings.

12. An apparatus for measuring the coagulation time of a blood sample, substantially as hereinbefore described with reference to, and as shown in, Figures 4a to 6b of the accompanying drawings.