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(54) Title: A METHOD FOR ANALYZING BLOOD SAMPLES FOR DETECTION OF PATHOLOGIES

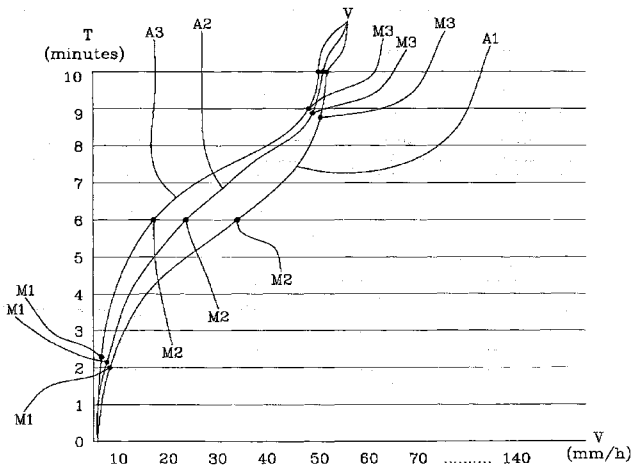

Fig.2

(57) Abstract: A method for analyzing blood samples, comprising the steps of: preparing a blood sample (1) within a respective container (2) provided with an anticoagulant substance; measuring the rate (V) at which the corpuscular components (1a) contained in the blood sample (1) sediment on the bottom of said container (2), said rate (V) being measured over a predetermined time period (T); detecting, within said time period (T), at least one sedimentation trend (A1, A2, A3) representative of steps of aggregation of the corpuscular components (1 a); and comparing the detected sedimentation trend (A1, A2, A3) with at least one reference parameter (P1, P2, P3) representative of at least one given pathology.

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A METHOD FOR ANALYZING BLOOD SAMPLES FOR DETECTION OF PATHOLOGIES

Technical Field

The present invention relates to a method for analyzing blood samples.

In particular, the present invention relates to a method for evaluating the presence of pathologies in a subject from whom a blood sample is taken for analysis.

As is well known, blood samples can be examined and analyzed using different methods, all aimed at evaluating the health conditions of the subject concerned.

Such methods can be of a specific type, in which the pathology a subject may be affected by is determined with good precision, or else of a non-specific type, in which only an indication is given as to the possible presence of a pathology in the subject.

In the former case, specific tests are generally conducted in a laboratory using equipment and processors capable of evaluating and examining the composition of the blood in order then to determine any factors in the blood sample indicating problems or pathologies. In this case a specialized physician examines the blood sample and subsequently derives a clinical picture.

This type of analysis, though capable of precisely determining the presence of a specific pathology, has major drawbacks due mostly to the complexity of the analyses.

In fact, the blood sample is analyzed by means of particular equipment that is structurally complicated and costly and used only by specialized personnel in a laboratory setting. It is therefore impossible to have an evaluation of the blood sample within a short time and at a low cost.

For this reason, especially if it is not determined whether or not a pathology is present, this type of analysis proves to be disadvantageous in terms of the times and costs of performing it.

To overcome this drawback, use is made of non-specific tests able to

provide an estimate, within a limited time and at a low cost, concerning the presence of an abnormal condition in the subject.

The most common and widely used non-specific test is the erythrocyte sedimentation rate (ESR) test, which is a measure of the speed at which red blood cells separate from plasma and settle on the bottom of a blood sample container.

The erythrocyte sedimentation rate is essentially conditioned by the characteristics of the plasma (in particular its protein composition) and the characteristics of the red blood cells (shape, number, tendency to aggregate, etc.).

This test is easy to perform, inexpensive and fast and, despite its non-specificity, it can indicate whether or not a pathology is present; the latter can be precisely identified by performing further tests of a specific type.

In particular, the erythrocyte sedimentation rate is measured by means of suitable devices, either manual or automatic, such as, for example, the devices described in international patent application WO 2001/23864, which are capable of measuring the sedimentation rate of the corpuscular components of blood (red and white blood cells and platelets).

The measured rate is then compared with a reference parameter to establish whether the subject is healthy or may be affected by any current pathologies.

Advantageously, the test is performed in a short time with simple devices that can also be used by non-specialized personnel as a first screening to determine whether it is necessary to proceed with the performance of other diagnostic tests.

The method generally used to analyze the erythrocyte sedimentation rate is the Westergren method, in which the blood sample is rendered non-coagulable by adding sodium citrate and allowed to sediment in a glass tube graduated in millimetres at a controlled temperature.

The glass container is then placed in special housing compartments fashioned in the above-mentioned known devices. Such devices are

equipped with optical sensors capable of reading the speed at which the corpuscular components fall over a pre-determined time and of providing the values of the sedimentation rate via suitable management software.

The test is based on the tendency of erythrocytes (red blood cells) to remain in suspension if they remain separated from one another. In this case there is very little (slow) sedimentation, indicating that the blood sample belongs to a healthy subject.

If, on the other hand, the erythrocytes aggregate and form cellular clumps (called rouleaux), they will fall more rapidly to the bottom of the glass tube, thus indicating the presence of a pathology in the blood sample.

This phenomenon is due to the negative electrical charges present on the outer membrane of the erythrocytes which repel one another, tending to block the phenomenon of agglomeration (rouleaux formation).

The ability of the red blood cells to repel one another is reduced if, for example, there is a current inflammation (presence of proteins, particularly C-reactive protein, *fibrinogen* IgM etc..) which reduces the negative charge on the membrane.

In this case, the red blood cells tend to adhere to one another, forming agglomerates (clumps of red blood cells) which deposit more quickly on the bottom of the test tube, as they are heavier.

This test method, too, however, poses major drawbacks.

In fact, as specified above, the erythrocyte sedimentation rate test is not able to provide any information in a specific manner as to the ongoing pathology.

For this reason, utilization of this method remains very limited and it is of little use in the case of an in-depth evaluation of the health conditions of a subject.

Moreover, if the erythrocyte sedimentation rate test indicates the presence of a pathology, specific examinations will in any case have to be carried out, with a consequent increase in the times and costs of performing a complete analysis which can provide sufficient information about the

subject's health conditions.

In this context, the technical task at the basis of the present invention is to propose a method for analyzing blood samples which overcomes the aforementioned drawbacks of the prior art.

In particular, it is an object of the present invention to provide a method for analyzing blood samples that is capable of furnishing specific information as to the presence of pathologies in a blood sample within a limited time and at a limited cost.

In particular, it is an object of the present invention to provide a method for analyzing blood samples capable of specifying types of pathologies without there being any need to examine the blood sample diagnostically.

A further object of the present invention is to provide a method for analyzing blood samples that can be implemented in a simple manner, also by non-specialized personnel.

Finally, it is an object of the present invention to provide a method for analyzing blood samples that can be implemented without setting up any specific equipment.

The stated technical task and the specified objects are substantially achieved by a method for analyzing blood samples, comprising the technical features set forth in one or more of the appended claims.

Additional features and advantages of the present invention will become more apparent from the approximate, and hence non-limiting, description of a preferred but non-exclusive embodiment of a method for analyzing blood samples, as schematically illustrated in the appended figures, in which:

- figure 1 shows a perspective view of blood samples contained in respective test tubes and in respective sedimentation conditions;
- figure 2 shows diagrams which represent the sedimentation trends of different blood samples;
- figure 3 shows diagrams of different reference sedimentation trends corresponding to specific pathologies; and

figure 4 shows a diagram that represents the way in which the sedimentation trend of a respective blood sample is drawn.

The method of the present invention is carried out, as illustrated in figure 1, by preparing a blood sample 1 inside a respective container 2. The container 2 preferably consists in a test tube graduated in millimetres, made of glass or another transparent material, in which an anticoagulant substance, preferably sodium citrate, has been previously added inside the container 2.

The blood sample 1 is then allowed to sediment, with the test tube set in a predetermined position, in order to be able to measure the rate V at which the corpuscular components 1a contained in the blood sample 1 settle on the bottle of the container 2.

The rate V is measured over a predetermined period of time T, which can be, for example, 10 minutes.

Advantageously, the rate V is measured by means of an electronic device for measuring the erythrocyte sedimentation rate - which is not described or illustrated herein, as it is of a known type and not part of the present invention - equipped with suitable optical sensors capable of measuring the speed at which the corpuscular components 1a (red and white blood cells and platelets) fall, that is, the time it takes them to separate from the plasma 1b. The test tube is thus inserted in a respective test seat of the device for measuring the erythrocyte sedimentation rate, in which the optical screening is carried out to determine the rate V over the predetermined time T.

The measured rate V is then compared with a reference rate indicative of whether or not a pathology is present.

In other words, the reference value determines the limit below which the measured rate V must fall in order to determine that the blood sample 1 belongs to a healthy subject.

In this case, in fact, the red blood cells remain in suspension for a longer time, lengthening the sedimentation process (fall toward the bottom of the

container 2). This behaviour is due to the presence of negative charges on the surface membrane of the red blood cells, which tend to repel one another, avoiding the agglomeration thereof.

In contrast, in the event that the measured rate V is above the reference value, the presence of a pathology is determined. In this situation, any inflammations present in the blood favour the agglomeration of the red blood cells (as a result of the loss of the negative charge), which fall quickly toward the bottom of the test tube, thus reducing the sedimentation times.

In general, the reference value is established as 20mm/h in women and 15mm/h in men.

The management software of the device thus estimates whether the blood sample 1 reveals pathologies or not.

In this case, a sedimentation trend A1, A2, A3 representative of steps of aggregation of the corpuscular components 1a is measured within the time period T.

This step is carried out by reading, in at least one moment M1, M2 or M3 of the time period T, the formation of agglomerates of the corpuscular components 1a and the fall thereof in the plasma 1b contained in the blood sample 1.

In particular, the sedimentation trend A1, A2, A3 is represented by a curve (figures 2 and 4) obtained by reading the formation of agglomerates and the fall thereof in the plasma for each moment that occurs sequentially along the whole time period T.

As is illustrated in figure 4, the reading moments are represented by predetermined time intervals, generally one reading every two seconds, in which the rate values are plotted. Taken together, these moments, which are graphically represented by means of mutually parallel segments extending as far as the measured rate, thus write the aforesaid curve representative of the sedimentation trend A1, A2, A3.

It should be noted in particular that in figure 2 three curves A1, A2, A3 are

represented by way of non-limiting example, each curve belonging to a sedimentation trend of respective blood samples.

It is illustrated, again by way of example, that each blood sample has the same rate V over the unit of time T (about 50 mm/h).

However, the sedimentation trends are different from one another, since each sedimentation trend A1, A2, A3 exhibits a specific curve.

In this respect, it should be specified that the sedimentation occurs in three steps: the first step is determined by the formation of aggregates; in the second step, a further aggregation of the cellular clumps (rouleaux) takes place; finally, in the third step an acceleration of sedimentation occurs, so that the aggregates accumulate on the bottom of the container 2.

These steps, which can take place differently depending on the various pathologies present in the blood sample, thus determine the difference in the respective sedimentation curves.

In fact, for each pathology, the corpuscular bodies 1a exhibit a specific behaviour (deriving from the loss of the negative charge of the red blood cells) in their ability to aggregate and therefore the way in which the sedimentation occurs.

For this reason, the sedimentation diagrams can be different from one another, though with identical sedimentation rates, or else they can have similar reaction times but different final values. Furthermore, the curves can provide indications about the presence of different points of aggregation or a different curve amplitude, the rates V being equal (as in the case illustrated in figure 2).

The plasma 1b, whose components are more numerous than in the corpuscular body 1a, also tends to vary the profile of the curve representative of the sedimentation trend if affected by pathologies.

In this regard, it shall be underscored that at specific reading moments M1, M2, M3, the individual sedimentation trends A1, A2, A3 follow profiles that are different from one another.

In such moments M1, M2, M3, therefore, a different behaviour is recorded with respect to the steps of aggregation and falling of the corpuscular components 1a.

The curve of each blood sample 1 is plotted by means of suitable management software, which can be integrated into an erythrocyte sedimentation rate measuring device of the known type as summarily described above.

Advantageously, the sedimentation trend A1, A2, A3 detected for each blood sample is compared with at least one reference parameter representative of at least a given pathology. This reference parameter is represented by a reference sedimentation trend P1, P2, P3 of one or more pathologies.

The comparison is made by detecting whether the sedimentation trend A1, A2, A3 has peculiar characteristics that are likenable (sometimes equal) to the reference sedimentation trend P1, P2, P3. In other words, the moments M1, M2 and M3 (representative of the peculiar characteristics) are compared with the moments present in the reference curves.

Preferably, the comparison step is carried out with a plurality of reference parameters, each of which represented by a reference sedimentation trend P1, P2, P3 of a specific pathology or specific group of pathologies.

In this case as well, in order to simplify the comparison step, the reference sedimentation trends P1, P2, P3 are represented by reference curves. Consequently, the step of comparing the sedimentation trends A1, A2, A3 is carried out by comparing each curve associated with the sedimentation trend A1, A2, A3 with a plurality of reference curves.

In this manner, by verifying the similarity of such curves (measured curve and reference curve) it is possible to establish a specific pathology or a specific group of pathologies for each sedimentation trend A1, A2, A3. A specific result is thus given with respect to the pathology of each blood sample 1 that is analyzed.

Advantageously, the comparison can also be made by observing only

specific moments M1, M2, M3 (figure 2) that occur in the curve. If such moments M1, M2, M3 correspond to respective reference moments, it will be possible to determine the specific pathology of the blood sample 1. This situation is given by the fact that, as specified above, at the moments M1, M2, M3 the curves exhibit a very different behaviour in the steps of aggregation of the corpuscular components 1a.

Preferably, the comparison between the sedimentation trend A1, A2, A3 and at least one reference parameter is made by an electronic processing unit integrated with the device for measuring the sedimentation rate.

Furthermore, it should be specified that the above-described method of analysis of the present invention has advantageous application if repeated with different blood samples of the same subject. In this case, it will be possible to monitor a predefined pathology, the course thereof during a treatment therapy, or the health conditions of the subject in general.

In this situation, a series of samples are taken at different times within a predefined period, which is determined based on the pathology or health conditions to be monitored.

For example, to verify whether a therapy is working properly, it may be provided for blood samples (which are analyzed according to the above-described method) to be taken at given intervals of time falling within the period in which the treatment is implemented. The performance and effectiveness of the treatment can thus be kept monitored and timely intervention can be undertaken to correct the treatment if necessary.

The present invention thus solves the problems of the prior art and has numerous advantages.

First, it should be noted that the above-described method enables specific indications to be provided as to the presence of a given pathology or group of pathologies, in a simple, fast manner and at very modest costs.

This advantage is given by the fact that use is not made of complicated and costly laboratory instruments used only by specialized personnel in a laboratory setting.

The method of the present invention can be carried out with very simple machinery, also usable by non-specialized personnel, since it is based on a direct comparison between measured values (curves representing the sedimentation trend) and reference values.

Such machinery, e.g. devices for measuring the erythrocyte sedimentation rate (ESR), is known to be simple, low-cost, and capable of providing results in a very short time.

In other words, the above-described method is implemented with a test (ESR) that is typically non-specific but which enables specific information to be given, based on a comparison of sedimentation trends, as to the presence and type of pathologies in the blood sample.

Consequently, the method provides such specific information without there being any need to analyze the composition of the blood and thus with considerable savings in the costs of carrying out laboratory analyses.

CLAIMS

1. A method for analyzing blood samples, comprising the steps of:

- preparing a blood sample (1) within a respective container (2);
- preventing the coagulation of said blood sample (1); and
- measuring the rate (V) at which the corpuscular components (1a) contained in the blood sample (1) sediment on the bottom of said container (2), said rate (V) being measured over a predetermined time period (T);

characterized in that it further comprises the steps of:

- detecting, within said time period (T), a sedimentation trend (A1, A2, A3) representative of steps of aggregation of the corpuscular components (1a); and
- comparing the detected sedimentation trend (A1, A2, A3) with at least on reference parameter of representative of at least one given pathology.

2. The method according to the preceding claim, characterized in that said reference parameter is represented by a reference sedimentation trend (P1, P2, P3) for one or more pathologies, said step of comparing the sedimentation trend (A1, A2, A3) being implemented by determining whether said sedimentation trend (A1, A2, A3) has characteristics identical to those of said reference sedimentation trend (P1, P2, P3).

3. The method according to any one of the preceding claims, characterized in that the detected sedimentation trend (A1, A2, A3) is compared with a plurality of reference parameters, each of which represented by a reference sedimentation trend (P1, P2, P3) for a specific pathology or a specific group of pathologies.

4. The method according to any one of the preceding claims, characterized in that the sedimentation trend (A1, A2, A3) is determined by reading, in at least one moment (M1, M2, M3) of the time period, the formation of agglomerates of corpuscular components (1a) and the fall thereof in the plasma contained in the blood sample (1).

5. The method according to the preceding claim, characterized in that said

sedimentation trend (A1, A2, A3) is represented by a curve obtained by reading the formation of agglomerates and the fall thereof in the plasma (1b) for each moment that elapses sequentially along the whole time period (T); said moments being predetermined time intervals.

6. The method according to the preceding claim, characterized in that said reference parameter is represented by a reference curve; said step of comparing the sedimentation trend (A1, A2, A3) being carried out by comparing the curve of said sedimentation trend (A1, A2, A3) with a plurality of reference curves.

7. The method according to any one of the preceding claims, characterized in that it further comprises the step of comparing the measured sedimentation rate (V) with a rate indicative of whether or not a pathology is present.

8. The method according to any one of the preceding claims, characterized in that at least said step of comparing the sedimentation trend (A1, A2, A3) with at least one reference parameter is carried out by an electronic processing unit.

9. The method according to any one of the preceding claims, characterized in that said steps of measuring the sedimentation rate (V) and of determining the sedimentation trend (A1, A2, A3) are carried out by an electronic device for measuring the erythrocyte sedimentation rate .

Fig.1

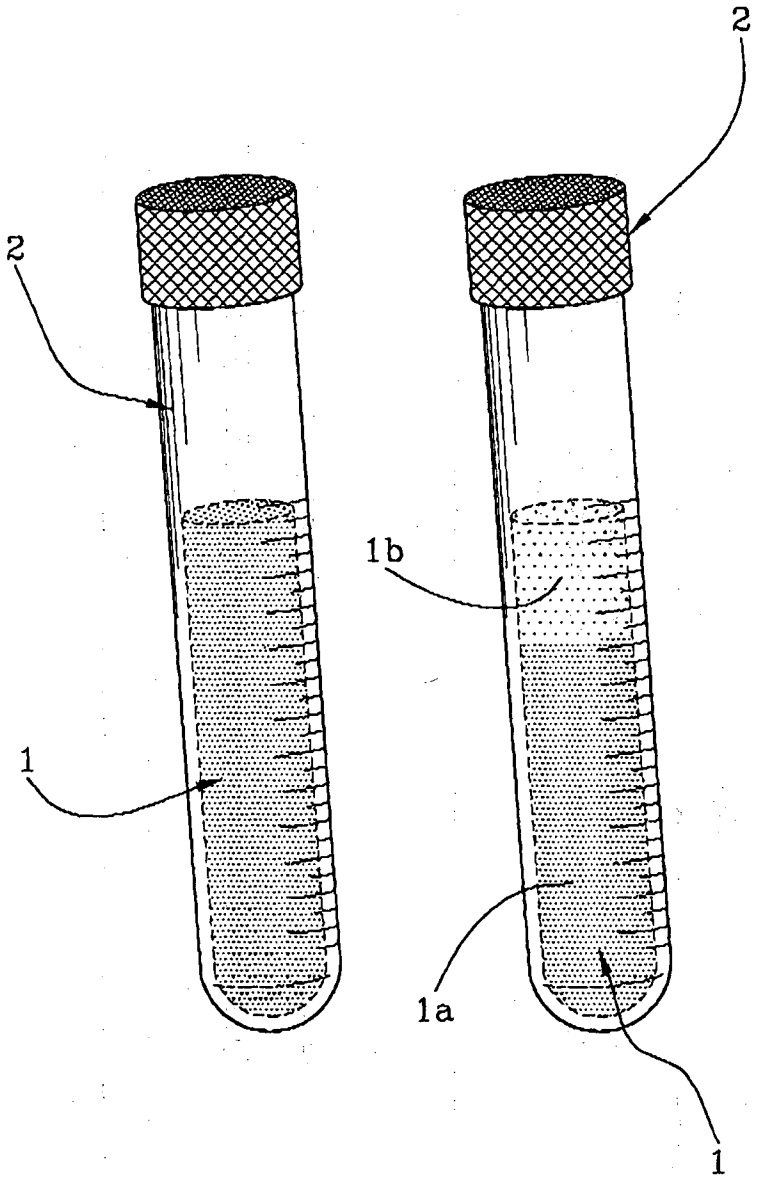
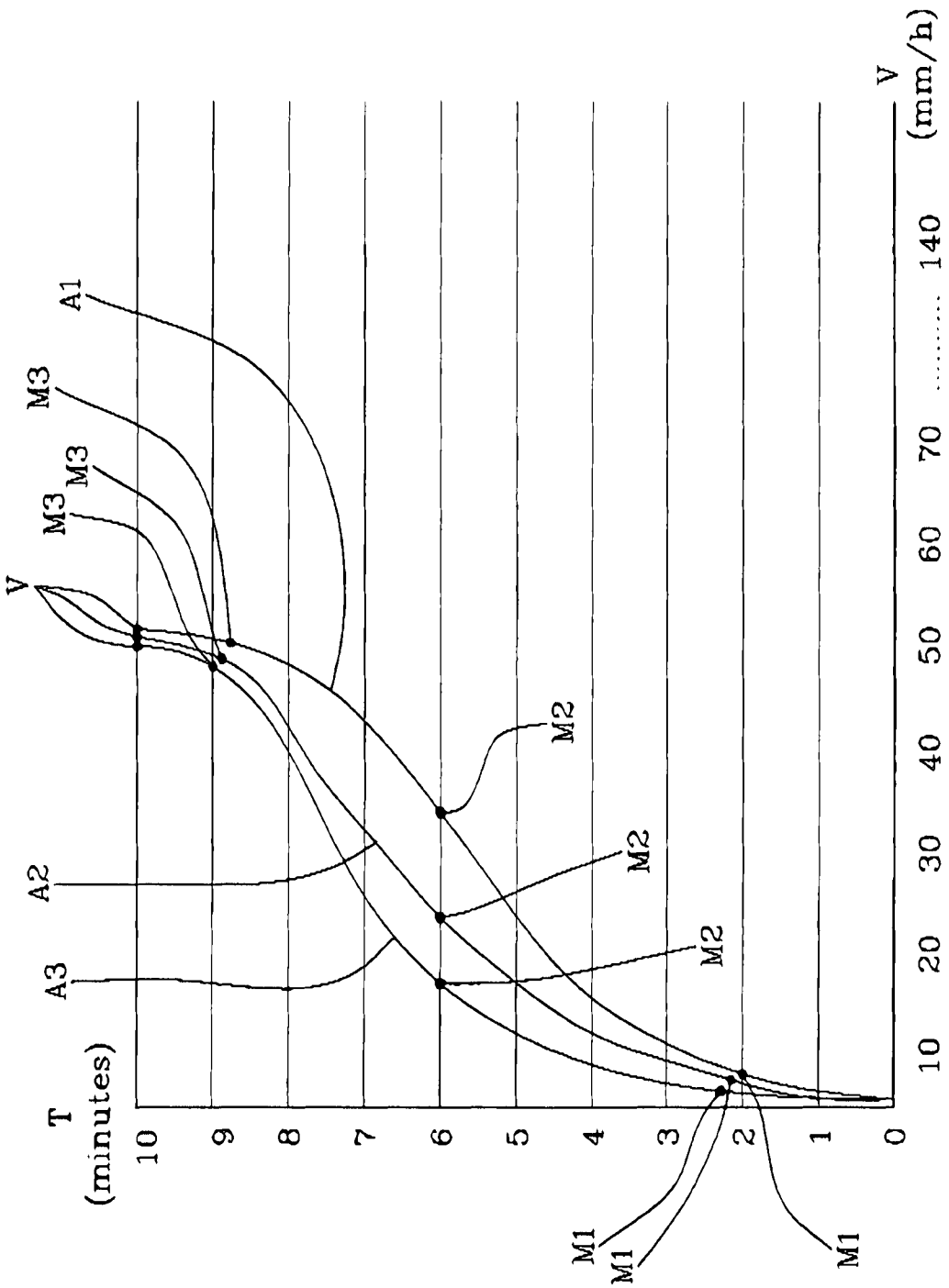


Fig.2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A2
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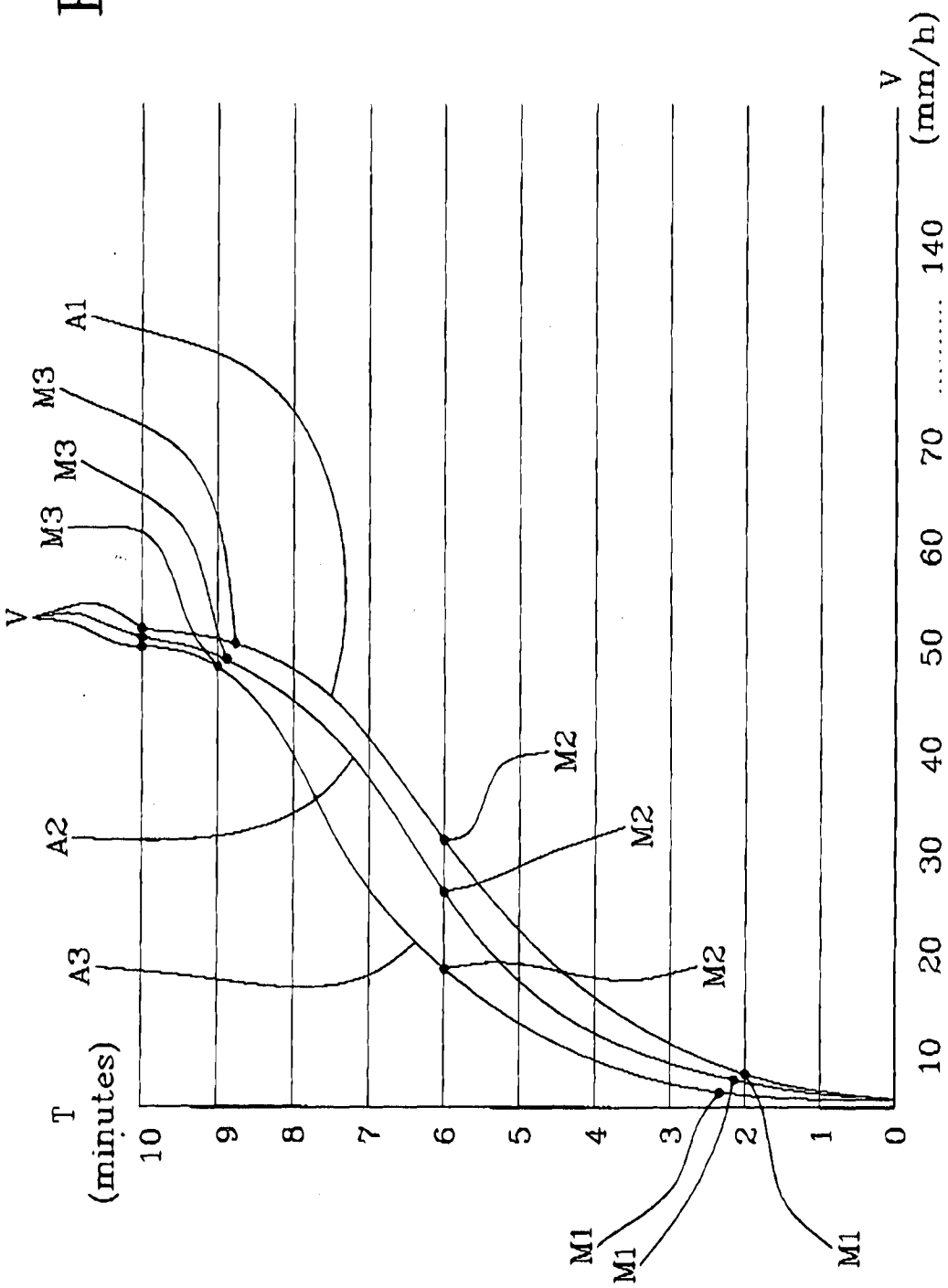
M1
M2
M3

Fig.3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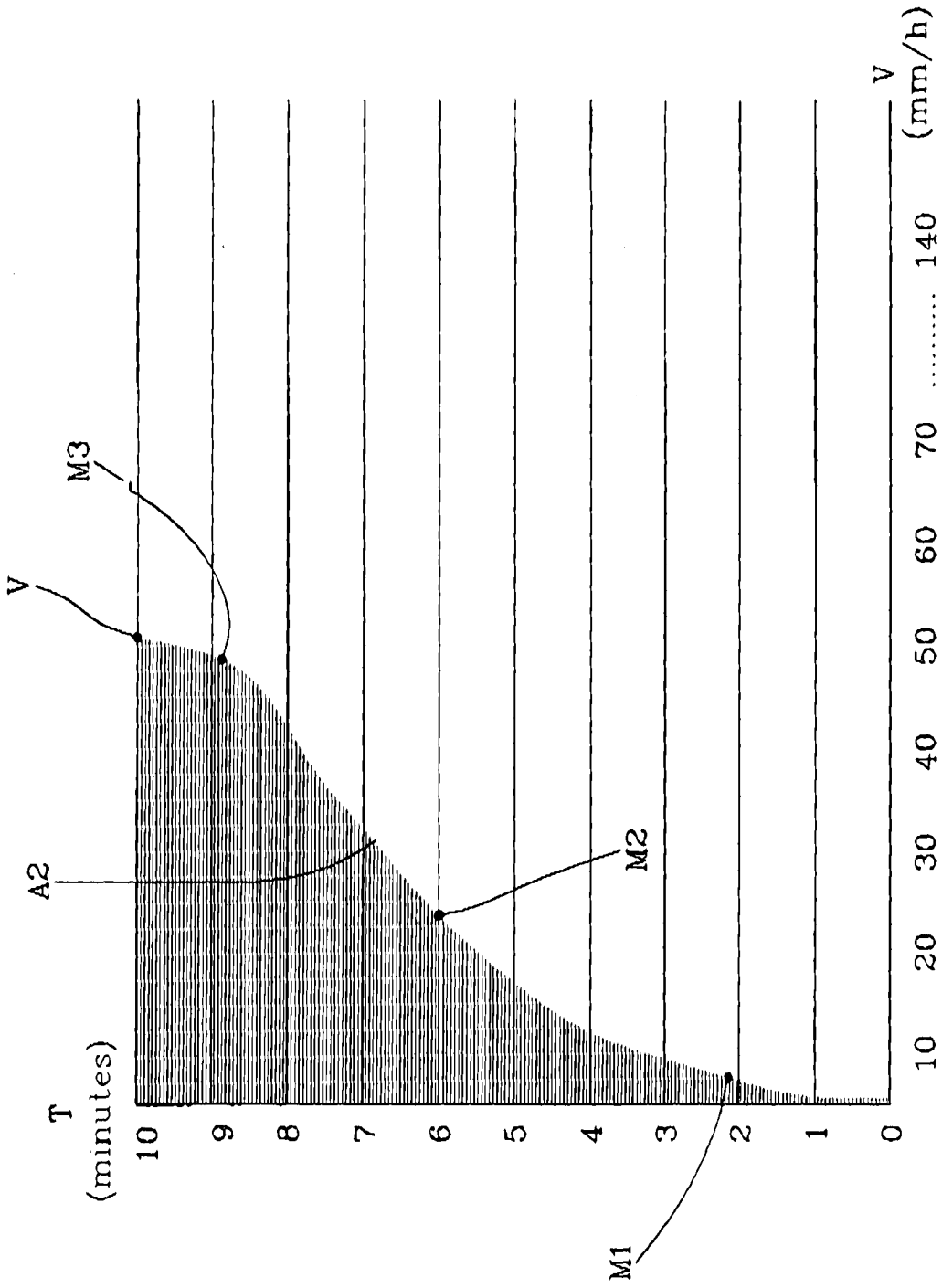
M3

Fig.4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M2
M1

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/050812

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/49 G01N15/05
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VOEIKOV V L ET AL: "Blood as an active colloidal system: The nonlinear nature of erythrocyte sedimentation in whole blood revealed by video recording with high spatial-temporal resolution", MOSCOW UNIVERSITY CHEMISTRY BULLETIN, ALLERTON PRESS, INC, HEIDELBERG, vol. 66, no. 4, 6 October 2011 (2011-10-06), pages 259-264, XP019961393, ISSN: 1935-0260, DOI: 10.3103/S0027131411040092 page 260, left-hand column, paragraph 7 - paragraph 10 page 260, right-hand column, paragraph 7 page 261, left-hand column, paragraph 4 - right-hand column, paragraph 1 figure 3 -----	1-9

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 April 2015	08/05/2015

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/050812

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VLADIMIR L. VOEIKOV ET AL: "Computerized video-enhanced high temporal resolution of erythrocytes sedimentation rate (ESR-graphy) reveals complex dynamic and self-organizing properties of whole blood", PROCEEDINGS OF SPIE, vol. 3923, 31 May 2000 (2000-05-31), pages 32-43, XP055112474, ISSN: 0277-786X, DOI: 10.1117/12.387143 page 33 - page 37 figure 4	1-9
X	HOLLEY L ET AL: "Influence of fibrinogen and haematocrit on erythrocyte sedimentation kinetics", BIORHEOLOGY, ELSEVIER SCIENCE LTD., OXFORD, GB, vol. 36, no. 4, 1 January 1999 (1999-01-01), pages 287-297, XP009175126, ISSN: 0006-355X page 288, paragraph 3 - page 289, paragraph 4	1-9
X	Tl Fabry: "Mechanism of erythrocyte aggregation and sedimentation", Blood, 1 November 1987 (1987-11-01), pages 1572-1576, XP055112465, UNITED STATES Retrieved from the Internet: URL:http://bloodjournal.hematologylibrary.org/cgi/content/abstract/70/5/1572 [retrieved on 2014-04-07] table 1 page 1575	1-9
X	CAB-CAUICH CESAR ET AL: "Monitoring of blood sedimentation by a multiplexed light transmission method", REVIEW OF SCIENTIFIC INSTRUMENTS, AIP, MELVILLE, NY, US, vol. 77, no. 4, 3 April 2006 (2006-04-03), pages 44301-044301, XP012093002, ISSN: 0034-6748, DOI: 10.1063/1.2188847 pages 044301-3, right-hand column, paragraph 2	1-9