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(54) Title: DETERMINATION OF CORRECTED DERIVED FIBRINOGEN RESULTS

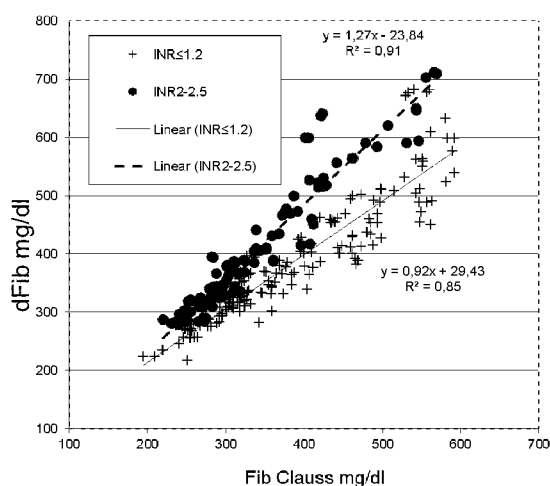


Fig. 1

(57) Abstract: The present invention relates to a method for determining a corrected derived plasma fibrinogen concentration in a blood-derived sample of a subject suffering from or suspected to suffer from prolonged coagulation time, comprising a) determining a first value of a transmission-related parameter in said sample before coagulation and a second value of said transmission-related parameter after coagulation, b) providing a value of a coagulation time-related parameter for said sample, c) providing a correction based on values of reference samples for a coagulation time-related parameter, for a transmission-related parameter, and for a fibrinogen concentration-related parameter, d) based on the values of the parameters of steps b) and c), correcting said value of said transmission-related parameter or a value of a parameter derived therefrom; and e) determining a corrected derived plasma fibrinogen concentration, and to a database, a device, uses and methods related thereto.



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Determination of corrected derived Fibrinogen results

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Field of the invention

The present invention relates to a method for determining a corrected derived plasma fibrinogen concentration in a blood-derived sample of a subject suffering from or suspected to suffer from prolonged coagulation time, comprising a) determining a first value of a transmission-related parameter in said sample before coagulation and a second value of said transmission-related parameter after coagulation, b) providing a value of a coagulation time-related parameter for said sample, c) providing a correction based on values of reference samples for a coagulation time-related parameter, for a transmission-related parameter, and for a fibrinogen concentration-related parameter, d) based on the values of the parameters of steps b) and c), correcting said value of said transmission-related parameter or a value of a parameter derived therefrom; and e) determining a corrected derived plasma fibrinogen concentration; and to a database, a device, uses and methods related thereto.

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Related art

The absorbance change measured in a prothrombin time (PT) coagulation assay can be calibrated to report the fibrinogen concentration. This is referred to as derived fibrinogen concentration or "derived Fibrinogen" (dFib). Since dFib is calculated from the identical measurement as the PT test, it provides an inexpensive estimate of the fibrinogen concentration.

For dFib, it is known from literature that a prolongation of the prothrombin time (PT) may lead to an overestimation of the fibrinogen concentration (see e.g. Chitolie et al, Blood Coagulation and Fibrinolysis 1994, 5; De Cristofaro et al, Blood Coagulation and Fibrinolysis 1998, 9). Due to the described overestimation, current dFib assays do not provide reliable results for patient samples with prolonged PT times, which is typically outlined in the package insert as a limitation of the test. On the other hand, prolonged PT times have a high medical relevance, as they are e.g. found in patients treated with oral vitamin K antagonists, liver disease, or coagulation abnormalities of different etiology.

Thus, an accurate estimation of the fibrinogen concentration by means of the dFib method is currently limited to samples with a normal PT time. Samples with a prolonged PT time

instead need to be tested with the Fibrinogen Clauss method, causing additional costs for the customer.

Problem to be solved

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It is therefore an objective of the present invention to provide means and methods to avoid the aforementioned drawbacks of the prior art.

Summary of the invention

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This problem is solved by a method for determining a corrected derived plasma fibrinogen concentration in a blood-derived sample of a subject, a database and a data carrier comprising relevant data and a device adapted for being used in said method, with the features of the independent claims. Preferred embodiments, which may be realized in an isolated fashion or in any arbitrary combination are listed in the dependent claims.

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Accordingly, the present invention relates to a method for determining a corrected derived plasma fibrinogen concentration in a blood-derived sample of a subject suffering from or suspected to suffer from prolonged coagulation time, comprising

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a) determining a first value of a transmission-related parameter in said sample before coagulation and a second value of said transmission-related parameter after coagulation,

b) providing a value of a coagulation time-related parameter for said sample,

c) providing a correction based on values of reference samples for a coagulation time-related parameter, for a transmission-related parameter, and for a fibrinogen concentration-related parameter,

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d) based on the values of the parameters of steps b) and c), correcting said value of said transmission-related parameter or a value of a parameter derived therefrom; and

e) determining a corrected derived plasma fibrinogen concentration.

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As used in the following, the terms “have”, “comprise” or “include” or any arbitrary grammatical variations thereof are used in a non-exclusive way. Thus, these terms may both refer to a situation in which, besides the feature introduced by these terms, no further features are present in the entity described in this context and to a situation in which one or more further features are present. As an example, the expressions “A has B”, “A comprises B” and “A includes B” may both refer to a situation in which, besides B, no other element is present in A (i.e. a situation in which a solely and exclusively consists of B) and to a situation in which, besides B, one or more further elements are present in entity A, such as element C, elements C and D or even further elements.

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Further, as used in the following, the terms "preferably", "more preferably", "most preferably", "particularly", "more particularly", "specifically", "more specifically" or similar terms are used in conjunction with optional features, without restricting alternative possibilities. Thus, features introduced by these terms are optional features and are not intended to restrict the scope of the claims in any way. The invention may, as the skilled person will recognize, be performed by using alternative features. Similarly, features introduced by "in an embodiment of the invention" or similar expressions are intended to be optional features, without any restriction regarding alternative embodiments of the invention, without any restrictions regarding the scope of the invention and without any restriction regarding the possibility of combining the features introduced in such way with other optional or non-optional features of the invention.

The method for determining a corrected derived plasma fibrinogen concentration of the present invention, preferably, is an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate, e.g., to providing a blood sample and/or obtaining a blood-derived sample derived therefrom for step a), or calculating parameters derived from the actual measurement values. Moreover, one or more of said steps may be performed by automated equipment.

As used herein, the term "plasma fibrinogen concentration" relates to the concentration of the clotting factor fibrinogen in the plasma of a subject. Methods for determining the plasma fibrinogen concentration are known to the skilled person. In an embodiment, plasma fibrinogen concentration is determined by mass spectrometry, by an immunological method, e.g. ELISA, by the Clauss method, by the fibrinogen kinetic method using batroxabin, or by the clot recovery method. In a further embodiment, plasma fibrinogen concentration is determined as a derived plasma fibrinogen concentration

As used herein, the term "derived plasma fibrinogen concentration" relates to a value of a plasma fibrinogen concentration determined by measuring a change in a transmission-related parameter of a plasma sample before and after coagulation, and by determining the plasma fibrinogen concentration by comparing said change to a set of calibration values obtained from samples of known plasma fibrinogen concentration or obtained from a sample of known plasma fibrinogen concentration and from one or more dilutions obtained from said sample of known plasma fibrinogen concentration. Accordingly, the term "corrected derived plasma fibrinogen concentration" relates to a derived plasma fibrinogen concentration which was corrected for systematic errors causing false values to be obtained for certain kinds of samples as specified herein.

The term "blood-derived sample", as used herein, relates to a sample of blood or a sample derived therefrom comprising the clotting factors comprised in blood. In an embodiment, the sample is a plasma sample, in a further embodiment an EDTA plasma or citrate plasma sample. Methods for obtaining blood derived samples from a subject are known to the skilled person and include, in an embodiment, arterial or venous puncture, and puncture of the skin.

The term "subject", as used herein, relates to a vertebrate animal, in an embodiment a mammal, in a further embodiment a human. In an embodiment, the subject is a subject suffering from or suspected to suffer from prolonged coagulation time. Prolonged coagulation time is frequently associated with specific diseases or physiological states; accordingly, in an embodiment, the subject is a subject suffering from liver disease, in particular progressive liver disease, suffering from a consumptive coagulopathy, receiving lysis therapy, receiving blood expanders, suffering from congenital fibrinogen deficiency, suffering from disseminated intravascular coagulation and/or receiving anticoagulation therapy as specified elsewhere herein. In a further embodiment, the subject is a subject receiving anticoagulation therapy as specified elsewhere herein. In a further embodiment, the subject is a subject suffering from an inflammatory condition and/or a condition involving tissue damage as specified elsewhere herein. It is further envisaged that the suspicion of a subject to suffer from prolonged coagulation time may also be based on a different underlying disease, e.g. acute or subacute liver intoxication, and the like.

In an embodiment, the subject is a subject receiving anticoagulation therapy, in an embodiment oral anticoagulation therapy. The term "anticoagulation therapy", as used herein, relates to heparin therapy, in an embodiment unfractionated heparin therapy or low molecular weight heparin therapy; factor Xa inhibitor therapy, in an embodiment synthetic pentasaccharide or direct factor Xa inhibitor therapy; direct thrombin inhibitor therapy; or vitamin K antagonist therapy. In an embodiment, anticoagulant therapy is a therapy comprising administration of a 4-hydroxycoumarin, in an embodiment a therapy comprising administration of acenocoumarol, dicumarol, ethyl biscoumacetate, phenprocoumon, warfarin, coumatetralyl, difenacoum, flocoumafen, bromadiolone, tiocloamarol, brodifacoum, and/or phenprocoumon (marcumar, (RS)-4-Hydroxy-3-(1-phenylpropyl)coumarin); in an embodiment comprising administration of warfarin ((RS)-4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one); in a further embodiment comprising administration of (-)-warfarin ((S)-4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one), in a further embodiment comprising administration of phenprocoumon (marcumar, (RS)-4-Hydroxy-3-(1-phenylpropyl)coumarin). In a further embodiment, anticoagulant therapy is a therapy comprising administration of Phenindione, Clorindione, and/or Diphenadione. In an embodi-

ment, oral anticoagulation therapy is direct oral anticoagulation therapy, in a further embodiment includes or is Dabigatran (3-({2-[(4-Carbamimidoylphenylamino)methyl]-1-methyl-1H-benzimidazole-5-carbonyl}pyridine-2-ylamino)propionic acid) therapy or Rivaroxaban ((S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]oxazolidin-5-yl]methyl} thiophene-2-carboxamide) therapy.

As used herein, the term "prolonged coagulation time" relates to an increase in time required for a blood-derived sample to clot as compared to a healthy reference, i.e. a sample from at least one apparently healthy subject. In an embodiment, the healthy reference is an average value obtained from of a population of apparently healthy subjects. In an embodiment, a prolonged coagulation time is a coagulation time prolonged by at least a factor of 1.1, by at least a factor of 1.5, by at least a factor of 1.9, or by at least a factor of 2 as compared to a healthy reference. In an embodiment, a prolonged coagulation time is a coagulation time prolonged by a factor of from 1.1 to 8, in an embodiment of from 1.5 to 6, in a further embodiment of from 1.9 to 5, in a further embodiment of from 2 to 3 as compared to a healthy reference. Methods for determining coagulation time in a sample are known to the skilled person and include the well known methods of determining prothrombin time, determining an activated partial Thromboplastin time, determining a Quick value, or determining an international normalized ratio (INR).

The term "transmission-related parameter" relates to a parameter indicating or correlating with the ratio of transmitted light versus incident light of a sample or to a parameter derived therefrom. In an embodiment, a transmission-related parameter is derived from the aforesaid ratio by standard operations of mathematics, physics and/or chemistry. Accordingly, in an embodiment, the transmission-related parameter is a transmission coefficient, an extinction coefficient, a transmittance, an absorbance, or an absorption. Moreover, in an embodiment, the transmission-related parameter is a value derived from one of the aforesaid parameters by a standard mathematical operation, e.g. to correct for a dilution applied to a sample, for a calibration factor, and the like. In an embodiment, an uncorrected derived plasma fibrinogen concentration is determined from a first value of a transmission-related parameter determined before coagulation and a second value of said transmission-related parameter determined after coagulation in a sample as specified elsewhere herein. Thus, in an embodiment, step a) of the method for determining a corrected derived plasma fibrinogen concentration of the present invention further comprises, based on said first and second value of a transmission-related parameter, determining an uncorrected derived plasma fibrinogen concentration. Also, in an embodiment, step a) of the method for determining a corrected derived plasma fibrinogen concentration of the present invention comprises the further step of calculating a difference from said second and first value of a

transmission-related parameter and, based on the value of said difference, determining an uncorrected derived plasma fibrinogen concentration. Accordingly, in an embodiment, an uncorrected derived plasma fibrinogen concentration is a parameter derived from a transmission-related parameter, in an embodiment a parameter derived from a first and second transmission-related parameter as specified elsewhere herein.

Methods for determining a transmission-related parameter in a blood sample are known to the skilled person and are, in an embodiment, transmission measurement, nephelometry, or turbidimetry. In an embodiment, transmission-related parameters are determined by turbidimetry. It is understood by the skilled person that a transmission-related parameter may be determined at a specific wavelength; thus, in an embodiment, said transmission-related parameter is determined at a wavelength of visible light, in an embodiment at a wavelength of from 300 nm to 700 nm, in an embodiment of from 300 nm to 600 nm or of from 500 nm to 700 nm. In an embodiment, the transmission-related parameter is determined at a wavelength of from 550 nm to 650 nm, in a further embodiment of from 600 nm to 650 nm, in a further embodiment at a wavelength of 625 ± 10 nm, in a further embodiment at a wavelength of 625 nm.

Means and methods for determining a time point before start of coagulation and a time point after coagulation, i.e. a time point after coagulation has reached an endpoint, are also known to the skilled person. In an embodiment, coagulation is started by adding at least one inducer of coagulation to a sample, whereafter measurement of a transmission-related parameter is started, in an embodiment is started immediately; in a typical coagulation, transmission will stay constant for some time (initial plateau), after which there is a continuous increase in transmission, approaching a second, lower plateau of transmission when coagulation has come to an end. As is understood by the skilled person, the second plateau will formally be approached tangentially; accordingly, in an embodiment, the second plateau is considered to have been reached if the absolute absorption change, in particular an absorption increase, over time is below a pre-specified value, in embodiment is below 0.01/s, in a further embodiment is below 0.005/s, in a further embodiment is below 0.005/s; in a further embodiment, the second plateau is considered to have been reached if the relative absorption change, in particular an absorption increase, over time is below a pre-specified value, in an embodiment in case the value at time x is less than the value at time $x-1s$ minus 20%, in a further embodiment in case the value at time x is less than the value at time $x-1s$ minus 10%, in a further embodiment in case the value at time x is less than the value at time $x-1s$ minus 5%. In a further embodiment, the end point is calculated by a simple equation such as $y = a x + b$ where y = endpoint in seconds, x is the clotting time and a and b are pre-determined variables. The skilled person is aware that samples

may comprise interferants, e.g. from hemolysis having occurred in the sample, and that determination of a time point after coagulation may require special measures; accordingly, the methods for determining a time point after coagulation as specified herein, in an embodiment, refer in particular to standard samples, comprising negligible amounts of interferants. The skilled person is aware of methods usable to compensate for deviations in the measured values caused by said interferants; in particular, fitting the measured data into a mathematical model as specified herein below may be used.

Moreover, in an embodiment, the first and/or second plateau are determined by fitting the measured data into a mathematical model of a coagulation curve. In an embodiment, the transmission-related parameter is determined at regular intervals, e.g. every 30 s, in an embodiment every 15 s, in an embodiment every 10 s, in a further embodiment every 5 s or every second; it is, however, also envisaged that the transmission-related parameter is determined continuously at least until said second plateau is approached. Thus, in an embodiment, a value or an average of values of the first plateau are chosen as a basis to provide a first transmission-related parameter in said sample before coagulation, and a value or an average of values of the second plateau are chosen as a basis to provide a second transmission-related parameter in said sample after coagulation. In a further embodiment, the first and second plateau are determined by fitting the measured data into a mathematical model of a coagulation curve and the first and the second values of the transmission-related parameter are calculated on the basis of said mathematical model; in an embodiment, a value lying before the first inflection point of the graph corresponding to the change of the transmission-related parameter over time is selected as the first value of the transmission-related parameter and a value lying after the second inflection point of the graph corresponding to the change of the transmission-related parameter over time is selected as the second value of the transmission-related parameter.

In an embodiment, a difference of the first value of said transmission-related parameter and the second value of said transmission-related parameter is calculated according to the method of the present invention, i.e. in an embodiment, the second value of said transmission-related parameter is subtracted from the first value of said transmission-related parameter, or, in an embodiment, vice versa. It is, however, also envisaged that a difference is not calculated; in an embodiment, a correlation matrix may be used for correction, which may, e.g. be a three-dimensional correlation matrix.

It is also known to the skilled person that the aforesaid transmission-related parameters may be used to determine a coagulation time-related parameter, e.g. by determining when

said second plateau is reached and/or by extrapolating from the slope of a graph representing the change of said transmission-related parameter over time.

5 The term "coagulation time" relates to a parameter indicating time required for a blood-derived sample to clot. Accordingly, the term "coagulation-time related parameter" relates to a parameter indicating or correlating with the time required for a blood-derived sample to clot or to a parameter derived therefrom. In an embodiment, a coagulation-time related parameter is derived from the aforesaid parameter by standard operations of mathematics, physics and/or chemistry. Accordingly, in an embodiment, the coagulation-time related
10 parameter is a prothrombin time, an activated partial Thromboplastin time, a Quick value, or an international normalized ratio (INR). In an embodiment, the coagulation-time related parameter is an INR. Methods for determining coagulation time are well-known in the art; in an embodiment, coagulation time is determined by determining at least one transmission-related parameter over time as specified elsewhere herein, by determining coagulation
15 clot mass over time, or by determining a viscosity-related parameter over time, e.g. oscillation of a steel bead embedded in a coagulation reaction (Hubbuck et al. (1996), Clin. Lab. 42: 637-640). In an embodiment, coagulation time is determined by determining at least one transmission-related parameter over time as specified elsewhere herein.

20 The term "fibrinogen concentration-related parameter", as used herein, relates to a parameter indicating or correlating with the concentration of fibrinogen concentration in a sample or to a parameter derived therefrom. In an embodiment, a fibrinogen concentration-related parameter is derived from the aforesaid parameter by standard operations of mathematics, physics and/or chemistry. In an embodiment, the fibrinogen concentration-related parameter
25 is the plasma fibrinogen concentration as specified elsewhere herein.

The term "reference sample", as used herein, relates to a sample for which at least one coagulation time-related parameter, at least one transmission-related parameter, and at least one fibrinogen concentration-related parameter is known. In an embodiment, said parameters
30 are available from (an) earlier determination(s), i.e. do not have to be determined at the time of determining the parameters of a sample of interest. In a further embodiment, at least the transmission-related parameter or parameters of the reference samples are available from earlier determinations, or at least the transmission-related parameter or parameters of the reference samples are determined concomitantly to a sample of interest. In a further
35 embodiment, at least the transmission-related parameter or parameters of the reference samples are available from earlier determinations. According to the present invention, a multitude of reference samples is used, wherein a multitude refers to at least five, in an embodiment at least ten, in a further embodiment at least 20, in a further embodiment at

least 25 reference samples. Also according to the invention, the multitude of samples comprises at least two, in an embodiment at least five, in an embodiment at least ten, in a further embodiment at least 20 samples of subjects known to suffer from a prolonged coagulation time. In an embodiment, at least 20%, in a further embodiment at least 30%, in a further embodiment at least 40% of the reference samples in the multitude of reference samples are samples of subjects known to suffer from a prolonged coagulation time. In an embodiment, of from 10% to 90%, in a further embodiment of from 20% to 80%, in a further embodiment of from 30% to 70% of the reference samples in the multitude of reference samples are samples of subjects known to suffer from a prolonged coagulation time. In a further embodiment, at least 20%, in a further embodiment at least 30%, in a further embodiment at least 40% of said reference samples of subjects known to suffer from a prolonged coagulation time are reference samples of subjects suffering from a coagulation time prolonged by the prolongation expected for a sample of a subject -60% to +60%, in an embodiment -30% to +30%. Thus, e.g., in case for a subject receiving anticoagulation therapy a prolongation of coagulation time by a factor of approx. 2.5 is expected, the above-indicated fraction of reference samples, in an embodiment, is from subjects suffering from a coagulation time prolonged by a factor of 1.0 to 4.0, in an embodiment 1.75 to 3.25

The term "correction", as used herein, relates to a modification of the value of a derived plasma fibrinogen concentration, in an embodiment an uncorrected derived plasma fibrinogen concentration as specified elsewhere herein, determined in a sample of a subject. As will be understood, in an embodiment, said correction is a compensation for systematic deviations from the actual fibrinogen concentration present in a sample occurring in a subject suffering from prolonged coagulation time.

According to the present invention, the correction is based on values of reference samples for a coagulation time-related parameter, for a transmission-related parameter, and for a fibrinogen concentration-related parameter as specified elsewhere herein; thus, in an embodiment, the correction is an empirically defined correction. In an embodiment, the correction is specific for a specific method of determining the transmission-related parameter, for a specific method of determining the fibrinogen concentration-related parameter and/or for a specific method of determining the coagulation time-related parameter. Accordingly, in an embodiment, the correction is based on coagulation time-related parameters and transmission-related parameters determined for the reference samples by the same method as is used for the samples of interest, in a further embodiment, determined for the reference samples prior to and by the same method as is used for the samples of interest, in a further embodiment, determined for the reference samples prior to and by the same method and using the same reagents as are used for the samples of interest.

The correction can, in principle be based on any kind of procedure reflecting the correlation between a coagulation time-related parameter, a transmission-related parameter, and a fibrinogen concentration-related parameter in the reference samples known to the skilled person. Accordingly, the correction, in embodiments, may comprise: (i) providing one or more correction factors for a transmission-related parameter, a fibrinogen concentration-related parameter, and/or an uncorrected derived fibrinogen concentration for a sample having a coagulation time-related parameter within a pre-specified range, (ii) providing correction factors for a transmission-related parameter, a fibrinogen concentration-related parameter, and/or an uncorrected derived fibrinogen concentration in dependence of a coagulation time-related parameter; (iii) or may comprise establishing a 2- or 3-dimensional representation of the values determined for the coagulation time-related parameter, for the transmission-related parameter and for the fibrinogen concentration-related parameter or the uncorrected derived fibrinogen concentration provided for the reference samples, from which the corrected derived plasma fibrinogen concentration can be read graphically or can be calculated. Accordingly, a correction factor, a correction curve, a calibration curve, a correction area, and/or a calibration area may be provided. As will be understood by the skilled person, correction, in an embodiment, further comprises applying said correction factor to the first and/or second transmission-related parameter and/or to said coagulation time-related parameter or to a value derived therefrom, in particular the uncorrected derived plasma fibrinogen concentration, to obtain a corrected derived plasma fibrinogen concentration. It will be further understood by the skilled person that, in the context of the correction of the present invention, a correction factor may be a factor, a summand, a minuend, a subtrahend, a multiplicand, a dividend, a divisor, an exponent or any other factor suitable in correction. In an embodiment, the correction factor comprises a multiplicand and a summand or a subtrahend; in a further embodiment, the correction factor is a multiplicand. As will be understood by the skilled person, in some embodiments, correcting a value of a transmission-related parameter may be replacing uncorrected derived plasma fibrinogen concentration with a corrected derived plasma fibrinogen concentration, e.g. in cases where the corrected derived plasma fibrinogen concentration can be directly read from e.g. a calibration curve or a calibration area.

Advantageously, it was found in the scientific work underlying the present invention that there is a consistent deviation of a derived plasma fibrinogen concentration from the actual plasma fibrinogen concentration in samples of subjects under vitamin K antagonist therapy and suffering from prolonged coagulation time and that, therefore, the derived plasma fibrinogen concentration can be corrected to better reflect the actual plasma fibrinogen concentration in the patient. Moreover, it was found that the deviation of the derived plasma fibrinogen concentration from the actual plasma fibrinogen concentration correlates with

the degree of prolongation of coagulation time and that a correction function can be provided on the basis of this correlation. Also, it was established that for subjects suffering from moderately prolonged coagulation time, a single multiplicand can be sufficient to provide corrected derived plasma fibrinogen concentrations of reasonable accuracy. The correction according to the present invention compensates for the overestimation of the fibrinogen concentration in samples with prolonged coagulation times. In case of a software-based implementation of the correction, the customer can directly receive a corrected and thus reliable result even for a sample having prolonged coagulation time. Thus, using the method of the present invention, the dFib assay can also be used for fibrinogen screening of patients with prolonged coagulation time. A basis for the correction, e.g. the PT time, may be extracted from the identical measurement as the dFib result itself.

The definitions made above apply mutatis mutandis to the following. Additional definitions and explanations made further below also apply for all embodiments described in this specification mutatis mutandis.

The present invention further relates to a method for determining a derived plasma fibrinogen concentration in a blood-derived sample of a subject, wherein said method comprises

- a) determining a coagulation time-related parameter,
- b) in case said coagulation time-related parameter indicates prolonged coagulation, performing the method for determining a corrected derived plasma fibrinogen concentration of present invention, and, thereby,
- c) determining a derived plasma fibrinogen concentration.

The method for determining a derived plasma fibrinogen concentration of the present invention, preferably, is an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate, e.g., to providing a blood sample and/or obtaining a blood-derived sample derived therefrom for step a), or calculating parameters derived from the actual measurement values. Moreover, one or more of said steps may be performed by automated equipment.

Moreover, the present invention relates to a database, in an embodiment a database comprised on a data carrier, comprising at least fibrinogen concentration-related parameters of reference samples and/or one or more parameters derived therefrom, and identifiers of said reference samples.

A "database" in accordance with the present invention is a collection of information (i.e. data) which is stored on a suitable medium, e.g. a data carrier, in a systematic way. Said collection of information may be stored on a single storage medium or on physically sepa-

rated separate storage media operatively linked to each other. If the information is stored on physically separate storage media, e.g., due to fragmentation of the information, it is envisaged that the partial information stored on each of said media can be reassembled so that the collection of information in its entirety can be investigated, e.g., by a query search.

5 Suitable storage media for information include entire computers, data processing devices or isolated magnetic, optical or other storage media such as solid state memory, hard disks, CDs, CD-ROMs, and the like. The database may further comprise a database management system. Database management systems may be on the basis of a network model, a hierarchical model, a relational model or an object-oriented database model. An alternative data-
10 base management system may be based on the so-called fuzzy logic. It is envisaged that the database referred to in accordance with the present invention has a structure which allows data analysis by a suitable analyzing tool, e.g., a computer implemented search algorithm, in order to answer queries regarding the collection of information stored in the said database.

15 As used herein, an "identifier" is a unique sign allowing unambiguous allocation of a reference sample to the values of one or more parameters as specified elsewhere herein stored in a database. In an embodiment, said value is a value of a fibrinogen concentration-related parameter. In a further embodiment, said values are the values of a fibrinogen concentra-
20 tion-related parameter and of a coagulation time-related parameter. In a further embodiment, said values are the values of a fibrinogen concentration-related parameter and of a transmission-related parameter. In a further embodiment, said values are the values of a fibrinogen concentration-related parameter, of a coagulation time-related parameter, and of a transmission-related parameter. In an embodiment, the identifier is a series of numbers,
25 letters, and/or symbols; in an embodiment, the identifier is an identifier code.

In an embodiment, the database comprises at least fibrinogen concentration-related parameters of reference samples and/or one or more parameters derived therefrom, and identifiers of said reference samples. Thus, in case transmission-related parameters and coagulation time-related parameters are determined in reference samples identifiable by said identifier, a correction factor according to the present invention may be provided based the
30 values of said parameters.

In a further embodiment, the database comprises at least fibrinogen concentration-related
35 parameters and coagulation time-related parameters of reference samples and/or one or more parameters derived therefrom, and identifiers of said reference samples. Thus, in case transmission-related parameters are determined in reference samples identifiable by said

identifier, a correction factor according to the present invention may be provided based the values of said parameters.

In a further embodiment, the database comprises fibrinogen concentration-related parameters, coagulation time-related parameters, and transmission-related parameters of reference samples and/or one or more parameters derived therefrom, and, in an embodiment, identifiers of said reference samples. Thus, from the data comprised in the database of this embodiment, a correction factor according to the present invention may be provided based the values of said parameters. It will be understood that providing identifiers for the reference samples is not mandatory in such case. It will be understood that it may be sufficient to provide one or more parameters derived from the aforesaid parameters in said database, e.g., a correction factor, a correction curve, a calibration curve, a correction area, and/or a calibration area, as specified elsewhere herein.

The present invention further relates to a data carrier, comprising coagulation time-related parameters, transmission-related parameters, and fibrinogen concentration-related parameters of reference samples and/or a correction parameter, a correction curve, a calibration curve, a correction area, and/or a calibration area derived therefrom.

Further, the present invention relates to a device for determining a corrected derived fibrinogen value of a blood-derived sample, comprising an analysis unit and an evaluation unit, wherein

(i) said analysis unit is adapted for determining at least a first transmission-related parameter of said sample before coagulation, a second transmission-related parameter after coagulation of said sample, and

(ii) said evaluation unit comprises a memory unit comprising a database according to the present invention and means for providing a corrected derived fibrinogen value.

The term “device”, as used herein, relates to a system of means comprising at least the means described operatively linked to each other as to allow the determination. Typical means for determining a transmission-related parameter and for determining a time point before and after coagulation are well known in the art and are for example described in Hubbuch et al. (1996), Clin. Lab. 42: 637-640. How to link the means of the device in an operating manner will depend on the type of means included into the device. In an embodiment, the means are comprised by a single device. However, it is also contemplated that the means of the current invention, in an embodiment, may appear as separate devices and are, in a further embodiment, packaged together as a kit. The person skilled in the art will

realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized technician.

In an embodiment, the device includes an analysis unit for determining transmission-related parameters and a computer unit for processing data received from the analysis unit and/or from the database for providing a corrected derived fibrinogen concentration. In an embodiment, providing a corrected derived fibrinogen concentration comprises determining a coagulation time-related parameter based on transmission-related parameters determined by the analysis unit as specified elsewhere herein. Accordingly, in an embodiment, the analysis unit is adapted to measure a multitude of values of said transmission-related parameter of the same sample at time intervals, in an embodiment at regular time intervals, in a further embodiment as specified elsewhere herein.

In an embodiment, the memory unit of the device comprises a correction parameter and the evaluation unit additionally comprises a tangibly embedded algorithm for deciding whether said correction parameter shall be applied to the first and/or to the second transmission-related parameter or to a parameter derived therefrom. In a further embodiment, the memory unit comprises a correction curve, and/or a calibration curve and a tangibly embedded algorithm for applying said correction curve, and/or calibration curve to the first and/or to the second transmission-related parameter or to a parameter derived therefrom. Thus, in an embodiment, the tangibly embedded algorithm implements the correction according to the method of the present invention. In an embodiment, the evaluation unit is adapted to determine a value of a coagulation-related parameter and an uncorrected derived plasma fibrinogen concentration from said multitude of values of said transmission-related parameter.

The present invention also relates to a method for assisting (i) in diagnosing a fibrinogen deficiency in a subject, (ii) in monitoring a diagnosed fibrinogen deficiency in a subject, (iii) in evaluating treatment of a fibrinogen deficiency in a subject and/or (iv) in deciding whether a subject should undergo an invasive measure, comprising

a) determining a corrected derived plasma fibrinogen concentration in a sample of said subject according to the method according to the present invention, and

b) diagnosing prolonged coagulation time with a fibrinogen deficiency, a severe progression of a diagnosed fibrinogen deficiency, inadequacy of fibrinogen deficiency treatment and/or advising against said invasive measure in case the corrected derived plasma fibrinogen concentration determined is low, in particular is less than 200 mg/dL, in an embodiment is less than 150 mg/dL; and thereby assisting in at least one of diagnosing of (i), monitoring of (ii), evaluating of (iii), and deciding of (iv).

Moreover, the present invention relates to a method for assisting in diagnosing an inflammatory condition and/or a condition involving tissue damage in a subject, comprising

a) determining a corrected derived plasma fibrinogen concentration in a sample of said subject according to the method according of the present invention, and

b) indicating an increased risk of suffering from an inflammatory condition and/or a condition involving tissue damage if the corrected derived plasma fibrinogen concentration determined is higher than 300 mg/dL, in an embodiment higher than 350 mg/dL, in a further embodiment higher than 400 mg/dL; thereby assisting in diagnosing an inflammatory condition and/or a condition involving tissue damage.

The methods for assisting of the present invention, preferably, are in vitro methods. Moreover, they may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate, e.g., to providing a blood sample and/or obtaining a blood-derived sample derived therefrom for step a), or calculating parameters derived from the actual measurement values. Moreover, one or more of said steps may be performed by automated equipment. In embodiments of the methods for assisting of the present invention, the subject is suffering from prolonged coagulation.

A prolonged coagulation may be the consequence of various diseases and physiological states and may or may not be associated with a decreased or increased plasma fibrinogen concentration; further, a decreased plasma fibrinogen concentration is a major contraindication for surgical measures. On the other hand, an increased plasma fibrinogen concentration may be an indicator of inflammatory condition and/or a condition involving tissue damage. Accordingly, it is important for the practitioner to be able to determine correct plasma fibrinogen concentrations even in cases where a subject is concomitantly suffering from prolonged coagulation. Thus, the method for determining a corrected derived plasma fibrinogen concentration of the present invention may be used in the aforesaid methods for assisting in diagnosis and monitoring.

In particular, the method may be used to assist in diagnosing a subject suffering from prolonged coagulation time with a fibrinogen deficiency. Causes of fibrinogen deficiency are known to the skilled person and include progressive liver disease, e.g. cirrhosis or chronic hepatitis; disseminated intravascular coagulation, e.g. caused by sepsis, cancer, allergic reaction, trauma, and/or surgery; and congenital fibrinogen deficiency, e.g. afibrinogenemia, hypofibrinogenemia, or dysfibrinogenemia. Moreover, the method may be used to assist in the monitoring of a diagnosed fibrinogen deficiency in a subject; i.e. surveilling progression of a pre-diagnosed fibrinogen deficiency and, in an embodiment, evaluation whether fibrinogen substitution therapy shall be administered. Also, the method may be

used to evaluate the treatment of a fibrinogen deficiency in a subject, in an embodiment to determine adequacy of fibrinogen substitution therapy. Or the method may be used to assist in the decision whether a subject should undergo an invasive measure, in particular surgery. In an embodiment, plasma fibrinogen concentrations are considered a reason to advise against or a contraindication to said invasive measure in case a corrected derived plasma fibrinogen concentration determined is less than 200 mg/dL, in an embodiment is less than 150 mg/dL.

In a further embodiment, the method may be used to assist in diagnosing an inflammatory condition and/or a condition involving tissue damage in a subject. As is known to the skilled person, there is an increased risk of suffering from an inflammatory condition and/or a condition involving tissue damage in case plasma fibrinogen concentrations are inappropriately high. Thus, the method of the present invention, in an embodiment, comprises indicating an increased risk of suffering from an inflammatory condition and/or a condition involving tissue damage if the corrected derived plasma fibrinogen concentration determined is higher than 300 mg/dL, in an embodiment higher than 350 mg/dL, in a further embodiment higher than 400 mg/dL. In an embodiment, the inflammatory condition and/or condition involving tissue damage comprises cardiovascular disease; cancer; inflammatory disorder, in an embodiment rheumatoid disorder; infection, in an embodiment acute infection; and/or trauma.

The term “diagnosing”, as used herein, refers to assessing the probability according to which a subject is suffering or will suffer from a disease or condition referred to in this specification. As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for 100% of the subjects to be diagnosed. The term, however, requires that, in an embodiment, a statistically significant portion of subjects can be correctly diagnosed to suffer from the disease or condition. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student’s t-test, Mann-Whitney test etc. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the probability envisaged by the present invention allows that the diagnosis will be correct for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

The term "assisting in diagnosing", as used herein, relates to providing information relevant for establishing, but, in an embodiment not anticipating, diagnosis. As will be understood by the skilled person, establishing a diagnosis typically requires collecting and evaluating additional parameters, symptoms, and/or further information of a subject's medical history. The same applies mutatis mutandis to assisting in evaluating, deciding, and the like.

Further, the present invention relates to a use of a device according to the present invention, of a database of the present invention, or of a data carrier of the present invention for determining a corrected derived plasma fibrinogen concentration in a sample; and to a use of the device according to the present invention, the database of the present invention, or the data carrier of the present invention for the manufacture of a device for determining a corrected derived plasma fibrinogen concentration in a sample.

The invention further discloses and proposes a computer program including computer-executable instructions for performing at least one of the methods according to the present invention in one or more of the embodiments enclosed herein when the program is executed on a computer or computer network. Specifically, the computer program may be stored on a computer-readable data carrier. Thus, specifically, one, more than one or even all of method steps a) to d) as indicated above may be performed by using a computer or a computer network, preferably by using a computer program.

The invention further discloses and proposes a computer program product having program code means, in order to perform the method according to the present invention in one or more of the embodiments enclosed herein when the program is executed on a computer or computer network. Specifically, the program code means may be stored on a computer-readable data carrier.

Further, the invention discloses and proposes a data carrier having a data structure stored thereon, which, after loading into a computer or computer network, such as into a working memory or main memory of the computer or computer network, may execute the method according to one or more of the embodiments disclosed herein.

The invention further proposes and discloses a computer program product with program code means stored on a machine-readable carrier, in order to perform the method according to one or more of the embodiments disclosed herein, when the program is executed on a computer or computer network. As used herein, a computer program product refers to the program as a tradable product. The product may generally exist in an arbitrary format, such

as in a paper format, or on a computer-readable data carrier. Specifically, the computer program product may be distributed over a data network.

5 Finally, the invention proposes and discloses a modulated data signal which contains instructions readable by a computer system or computer network, for performing the method according to one or more of the embodiments disclosed herein.

10 Preferably, referring to the computer-implemented aspects of the invention, one or more of the method steps or even all of the method steps of the method according to one or more of the embodiments disclosed herein may be performed by using a computer or computer network. Thus, generally, any of the method steps including provision and/or manipulation of data may be performed by using a computer or computer network. Generally, these method steps may include any of the method steps, typically except for method steps requiring manual work, such as providing the samples and/or certain aspects of performing
15 the actual measurements.

Specifically, the present invention further discloses:

- 20 - A computer or computer network comprising at least one processor, wherein the processor is adapted to perform the method according to one of the embodiments described in this description,
- a computer loadable data structure that is adapted to perform the method according to one of the embodiments described in this description while the data structure is being executed on a computer,
- 25 - a computer program, wherein the computer program is adapted to perform the method according to one of the embodiments described in this description while the program is being executed on a computer,
- a computer program comprising program means for performing the method according to one of the embodiments described in this description while the computer program is being executed on a computer or on a computer network,
- 30 - a computer program comprising program means according to the preceding embodiment, wherein the program means are stored on a storage medium readable to a computer,
- a storage medium, wherein a data structure is stored on the storage medium and wherein the data structure is adapted to perform the method according to one of the
35 embodiments described in this description after having been loaded into a main and/or working storage of a computer or of a computer network, and
- a computer program product having program code means, wherein the program code means can be stored or are stored on a storage medium, for performing the

method according to one of the embodiments described in this description, if the program code means are executed on a computer or on a computer network.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

Summarizing the findings of the present invention, the following embodiments are particularly envisaged:

1. A method for determining a corrected derived plasma fibrinogen concentration in a blood-derived sample of a subject suffering from or suspected to suffer from prolonged coagulation time, comprising
 - a) determining a first value of a transmission-related parameter in said sample before coagulation and a second value of said transmission-related parameter after coagulation,
 - b) providing a value of a coagulation time-related parameter for said sample,
 - c) providing a correction based on values of reference samples for a coagulation time-related parameter, for a transmission-related parameter, and for a fibrinogen concentration-related parameter,
 - d) based on the values of the parameters of steps b) and c), correcting said value of said transmission-related parameter or a value of a parameter derived therefrom; and
 - e) determining a corrected derived plasma fibrinogen concentration.
2. The method of embodiment 1, wherein said subject suffering from prolonged coagulation time is a subject suffering from liver disease, suffering from consumptive coagulopathy, receiving lysis therapy, receiving blood expanders, suffering from congenital fibrinogen deficiency, suffering from disseminated intravascular coagulation, and/or receiving anticoagulation therapy.
3. The method of embodiment 1 or 2, wherein said subject suffering from prolonged coagulation time is a subject receiving anticoagulation therapy, in an embodiment oral anticoagulation therapy.
4. The method of embodiment 1 to 3, wherein said anticoagulation therapy is a heparin therapy, in an embodiment unfractionated heparin therapy or low molecular weight heparin therapy; factor Xa inhibitor therapy, in an embodiment synthetic pentasaccharide or direct factor Xa inhibitor therapy; direct thrombin inhibitor therapy; or vitamin K antagonist therapy.

5. The method of any one of embodiments 1 to 4, wherein said anticoagulant therapy is a therapy comprising administration of a 4-hydroxycoumarin, in an embodiment a therapy comprising administration of acenocoumarol, dicumarol, ethyl biscoumacetate, warfarin, and/or phenprocoumon (marcumar, (RS)-4-Hydroxy-3-(1-phenylpropyl)cumarin); in an embodiment comprising administration of warfarin ((RS)-4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one); in a further embodiment comprising administration of (-)-warfarin ((S)-4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one), in a further embodiment comprising administration of phenprocoumon (marcumar, (RS)-4-Hydroxy-3-(1-phenylpropyl)cumarin).

6. The method of any one of embodiments 1 to 4, wherein said anticoagulant therapy is a therapy comprising administration of 1,3-indandione, in an embodiment, Phenindione (2-phenylindene-1,3-dione), Clorindione (2-(4-chlorophenyl)indene-1,3-dione), and/or Diphenadione (2-(2,2-diphenylacetyl)indene-1,3-dione).

7. The method of any one of embodiments 1 to 6, wherein said blood-derived sample is a plasma sample.

8. The method of any one of embodiments 1 to 7, wherein said blood-derived sample of a subject suffering from prolonged coagulation time is a sample wherein coagulation is prolonged by a factor of from 1.1 to 8, in an embodiment of from 1.5 to 6, in a further embodiment of from 1.9 to 5, in a further embodiment of from 2 to 3 as compared to a healthy reference.

9. The method of any one of embodiments 1 to 8, wherein said coagulation time-related parameter is (i) a prothrombin time or a value derived therefrom, in an embodiment a Quick value or an INR value, or (ii) an activated partial Thromboplastin time.

10. The method of any one of embodiments 1 to 9, wherein the coagulation time related parameter of said sample is increased by a factor of from 1.1 to 8, in an embodiment of from 1.5 to 6, in a further embodiment of from 1.9 to 5, in a further embodiment of from 2 to 3 as compared to a healthy reference.

11. The method of any one of embodiments 1 to 10, wherein said transmission-related parameter is determined by a photo-optical method, in an embodiment by nephelometry or by turbidimetry, in a further embodiment by turbidimetry.

12. The method of any one of embodiments 1 to 11, wherein said transmission-related parameter is a transmission or an absorbance, in an embodiment measured at 405 ± 10 nm or at 625 ± 10 nm, in an embodiment at 625 ± 10 nm.

5 13. The method of any one of embodiments 1 to 12, wherein said transmission-related parameter is a transmission, in an embodiment a transmission measured at 625 nm, in a further embodiment an absorption calculated from a transmission measured at 625 nm.

10 14. The method of any one of embodiments 1 to 13, wherein said method comprises the further step of calculating a difference from the first and the second value of the transmission-related parameter.

15 15. The method of any one of embodiments 1 to 14, wherein said first and second values of a transmission-related parameter are a first and a second absorbance value, and wherein said difference is an absorbance difference (Δ abs).

20 16. The method of any one of embodiments 1 to 15, wherein said sample is a sample with an uncorrected derived plasma fibrinogen concentration of at least 200 mg/dL, in an embodiment at least 250 mg/dL, in a further embodiment at least 300 mg/dL.

25 17. The method of any one of embodiments 1 to 16, wherein said method comprises the further step of providing the value of said coagulation time-related parameter in said sample, in an embodiment, wherein step b) is determining a value of a coagulation time-related parameter for said sample.

17. The method of any one of embodiments 1 to 16, wherein said reference samples comprise samples from subjects having normal coagulation and samples from subjects having prolonged coagulation.

30 18. The method of any one of embodiments 1 to 17, wherein said fibrinogen concentration-related parameters of reference samples are determined by a method different from the derived plasma fibrinogen concentration method.

35 19. The method of any one of embodiments 1 to 18, wherein said values of a fibrinogen concentration-related parameter of reference samples are determined by mass spectrometry, by an immunological method, by the Clauss method, by the fibrinogen kinetic method using batroxabin, or by the clot recovery method and/or wherein said fibrinogen concentra-

tion-related parameter determined is the fibrinogen concentration in said sample, in an embodiment is the fibrinogen mass concentration in said sample.

20. A method for determining a derived plasma fibrinogen concentration in a blood-derived sample of a subject, wherein said method comprises

- a) determining a coagulation time-related parameter,
- b) in case said coagulation time-related parameter indicates prolonged coagulation, performing the method for determining a corrected derived plasma fibrinogen concentration of any one of embodiments 1 to 19, and, thereby,
- c) determining a derived plasma fibrinogen concentration.

21. A database, in an embodiment a database comprised on a data carrier, comprising values of fibrinogen concentration-related parameters of reference samples and/or one or more parameters derived therefrom, and identifiers of said reference samples.

22. The database of embodiment 21, wherein said database further comprises values of coagulation time-related parameters and/or values of transmission-related parameters of said reference samples.

23. A database, in an embodiment a database comprised on a data carrier, comprising fibrinogen concentration-related parameters, coagulation time-related parameters, and transmission-related parameters of reference samples and/or one or more parameters derived therefrom.

24. The database of any one of embodiments 21 to 23, wherein said parameter derived therefrom is a correction factor, a correction curve, a calibration curve, a correction area, and/or a calibration area.

25. A data carrier, comprising coagulation time-related parameters, transmission-related parameters, and fibrinogen concentration-related parameters of reference samples and/or a correction parameter, a correction curve, a calibration curve, a correction area, and/or a calibration area derived therefrom.

26. A device for determining a corrected derived fibrinogen value of a blood-derived sample, comprising an analysis unit and an evaluation unit, wherein

- (i) said analysis unit is adapted for determining at least a first transmission-related parameter of said sample before coagulation, a second transmission-related parameter after coagulation of said sample, and

(ii) said evaluation unit comprises a memory unit comprising a database according to any one of embodiments 21 to 24 and means for providing a corrected derived fibrinogen value.

5 27. The device of embodiment 26, wherein said memory unit comprises a correction parameter and wherein said evaluation unit additionally comprises a tangibly embedded algorithm for deciding whether said correction parameter shall be applied to the first and/or to the second transmission-related parameter or to a parameter derived therefrom.

10 28. The device of embodiment 26 or 27, wherein said memory unit comprises a correction curve, and/or a calibration curve and a tangibly embedded algorithm for applying said correction curve, and/or calibration curve to the first and/or to the second transmission-related parameter or to a parameter derived therefrom.

15 29. The device of any one of embodiments 26 to 28, wherein said algorithm implements the correction according to the method of any one of embodiments 1 to 19.

30. The device of any one of embodiments 26 to 29, wherein said analysis unit is adapted to measure a multitude of values of said transmission-related parameter of the
20 same sample at time intervals, in an embodiment at regular time intervals.

31. The device of embodiment 30, wherein said evaluation unit is adapted to determine a value of a coagulation-related parameter and an uncorrected derived plasma fibrinogen concentration from said multitude of values of said transmission-related parameter.

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32. A method for assisting (i) in diagnosing a fibrinogen deficiency in a subject, (ii) in monitoring a diagnosed fibrinogen deficiency in a subject, (iii) in evaluating treatment of a fibrinogen deficiency in a subject and/or (iv) in deciding whether a subject should undergo an invasive measure, comprising

30 a) determining a corrected derived plasma fibrinogen concentration in a sample of said subject according to the method according to any one of embodiments 1 to 19, and

b) diagnosing prolonged coagulation time with a fibrinogen deficiency, a severe progression of a diagnosed fibrinogen deficiency, inadequacy of fibrinogen deficiency treatment and/or advising against said invasive measure in case the corrected derived plasma
35 fibrinogen concentration determined is less than 200 mg/dL, in an embodiment is less than 150 mg/dL; thereby assisting in at least one of diagnosing of (i), monitoring of (ii), evaluating of (iii), and deciding of (iv).

33. The method of 32, wherein said fibrinogen deficiency is caused by congenital fibrinogen deficiency, progressive liver disease, and/or disseminated intravascular coagulation.

5 34. The method of 32 or 33, wherein said disseminated intravascular coagulation is caused by sepsis, cancer, allergic reaction, trauma, and/or surgery.

35. The method of any one of 32 to 34, wherein said subject is a subject suffering from prolonged coagulation time

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36. A method for assisting in diagnosing an inflammatory condition and/or a condition involving tissue damage in a subject, comprising

a) determining a corrected derived plasma fibrinogen concentration in a sample of said subject according to the method according to any one of embodiments 1 to 19, and

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b) indicating an increased risk of suffering from an inflammatory condition and/or a condition involving tissue damage if the corrected derived plasma fibrinogen concentration determined is higher than 300 mg/dL, in an embodiment higher than 350 mg/dL, in a further embodiment higher than 400 mg/dL; thereby assisting in diagnosing an inflammatory condition and/or a condition involving tissue damage.

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37. The method of embodiment 36, wherein said inflammatory condition and/or condition involving tissue damage comprises cardiovascular disease; cancer; inflammatory disorder, in an embodiment rheumatoid disorder; infection, in an embodiment acute infection; and/or trauma .

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38. The method of embodiment 36 or 37, wherein said subject is a subject suffering from prolonged coagulation time.

39. Use of the device according to any one of embodiments 26 to 31, the database of
30 embodiment 21, or the data carrier of embodiment 25 for determining a corrected derived plasma fibrinogen concentration in a sample.

40. The use of embodiment 39, wherein said corrected derived plasma fibrinogen concentration is determined according to the method according to any one of embodiments 1
35 to 19.

41. Use of the device according to any one of embodiments 26 to 31, the database of embodiment 21, or the data carrier of embodiment 25 for the manufacture of a device for determining a corrected derived plasma fibrinogen concentration in a sample.

- 5 42. The use of embodiment 41, wherein said corrected derived plasma fibrinogen concentration is determined according to the method according to any one of embodiments 1 to 19.

Short description of the Figures

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Further optional features and embodiments of the invention will be disclosed in more detail in the subsequent description of preferred embodiments, preferably in conjunction with the dependent claims. Therein, the respective optional features may be realized in an isolated fashion as well as in any arbitrary feasible combination, as the skilled person will realize.

- 15 The scope of the invention is not restricted by the preferred embodiments. The embodiments are schematically depicted in the Figures. Therein, identical reference numbers in these Figures refer to identical or functionally comparable elements.

In the Figures:

20

Figure 1: Correlation of the derived fibrinogen concentration (dFib, Y-axis) with the fibrinogen concentration determined according to the Clauss method (Fib Clauss, x-axis) for samples with normal INR (crosses) and high-INR samples (dots)(Example 1). Linear regressions for both sample types are shown as well.

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Figure 2: Data and representation as in Fig. 1, but with dFib values of high-INR samples corrected by multiplication with a correction factor of 0.835.

Figure 3: Data and representation as in Fig. 1, but with dFib values of high-INR samples corrected by a correcting function as described in Example 3.

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Figure 4: Correlation of the INR with the difference of the absorption of a sample after coagulation and the absorption of a sample before coagulation (Δabs), in samples having the same fibrinogen concentration, but different coagulation times.

- 35 Figure 5: as in Fig. 1, but with values from Example 5 (high-INR samples).

Figure 6: Correlation between the INR values (x-axis) of samples and their dFib/Fib Clauss ratios (y-axis) in the samples of Example 5.

Figure 7: Data and representation as in Fig. 5, but with dFib values of all samples corrected as described in Example 5.

Figure 8: Correlation of the derived fibrinogen concentration (dFib, Y-axis) with the fibrinogen concentration determined according to the Clauss method (Fib Clauss, x-axis) for samples of Example 5 before (crosses) and after (dots) correction was applied to all samples.

Figure 9: Correlation between the PT values (x-axis, in [s]) of samples and their dFib/Fib Clauss ratios (y-axis) in the presence of oral anticoagulants (Example 6); A) Dabigatran, B) Rivaroxaban.

Examples

Example 1

Two types of fresh patient samples (citrate plasma) were used: samples with a normal PT time ($\text{INR} \leq 1.2$, $N=171$) and samples from patients with elongated PT times due to Anti Vitamin K therapy ($\text{INR} 2-2.8$; $N=109$). For all samples, PT and dFib were determined and fibrinogen concentration was measured according to the Clauss method, using commercially available reagents, on an optical coagulation analyzer.

Without correction, the dFib overestimation of the samples with $\text{INR} 2-2.8$ leads to a segregation from the normal sample collective. This is also characterized by the difference in slope when fitting the two data sets to a simple linear regression (normal INR ($\text{INR} \leq 1.2$): slope 0.92; high INR ($2 \leq \text{INR} \leq 2.8$): slope 1.27).

Example 2: applying a simple correction factor

To determine a dFib correction factor, the ratio of the Fibrinogen Clauss and the dFib result was calculated for each of the samples from the method comparison, e.g.:

- Fib Clauss result: 288 mg/dl
- Derived Fib result: 345 mg/dl
- Ratio: $288/345 = 0.8348$.

The mean over the ratios of all samples was determined as 0.835. This value was used as a multiplicative correction factor for each of the high-INR dFib samples, e.g.:

- $345 \text{ mg/dl} * 0.835 = 288 \text{ mg/dl}$.

This very simple correction already lead to a significant change in the method comparison: the two sample collectives now show a very good overlap and more similar slopes of 0.92 (normal INR) and 1.07 (high INR) (Fig. 2).

5

Example 3: improved correction

For improved correction, best fit lines were established for normal INR ($\text{INR} \leq 1.2$: $y = 0.9234x + 29.4344$ (eq.1)) and high INR values ($\text{INR} 2-2.8$: $y = 1.2722x - 23.8409$ (eq. 2)). From these, a correcting function was derived as follows:

10

$$y(\text{corr}) = 0.9234 / 1.2722 * y + 29.4344 - (-23.8409) = 0.7258 * y + 53.2753 \text{ (eq.3)}$$

Using eq. 3, the dFib values measured for high INR samples were corrected. The result is shown in Fig. 3.

15

Example 4

Commercially available samples having essentially the same fibrinogen concentration, but different INR values, were analyzed as specified in example 1. For the samples, difference of the absorption of a sample after coagulation and the absorption of a sample before coagulation (Δabs) was calculated and was correlated to the respective INR as a measure of coagulation time (Fig. 4).

20

Surprisingly, it was found that Δabs , and as a consequence the dFib calculated on the basis of Δabs , is directly proportional to the INR, although the actual fibrinogen concentrations were the same; thus, correction factors may be provided in dependence on specific INR ranges, e.g. by providing a table of INR specific correction factors.

25

Example 5

Experiment 1 was repeated using samples with a normal PT time ($\text{INR} \leq 1.2$, $N=7$) and samples from patients with elongated PT times due to Anti Vitamin K therapy, said samples covering a broader range of INR values ($\text{INR} 1.3-4.7$; $N=75$). Uncorrected results are shown in Fig. 5. In the cohort having increased INR values, the Fibrinogen concentration is overestimated as compared to subjects with normal INR.

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To apply a further improved correction, the ratio of dFib value to the Fib Clauss value was calculated for each sample, and said ratio was correlated with the INR value of the respective sample (Fig. 6). As shown, dFib measurement increasingly overestimates the fibrinogen concentration with increasing INR. The correlation was linear even for INR values of more than 4, the regression line having a slope of 0.13. This slope was used as a correction factor according to the following formula:

$$\text{dFib}_{\text{corr}} = \text{dFib}_m / ((\text{INR}-1) * \text{correction factor} + 1) = \text{dFib}_m / ((\text{INR}-1) * 0.13 + 1), (\text{eq. 4})$$

with $\text{dFib}_{\text{corr}}$ = corrected derived Fibrinogen value, and dFib_m = derived Fibrinogen value measured.

After correction, the two sample collectives now show a very good overlap and more similar slopes of 0.98 (normal INR) and 1.19 (high INR) (Fig. 7). Moreover, it was found that it is not required to distinguish between samples with high INR and samples with normal INR for applying the correction, since applying the correction to all samples, i.e. irrespective of whether the INR of the sample is normal or increased, provides for an improved correlation between Fibrinogen concentrations determined by the dFib method (including correction) and the Clauss method (Fig. 8).

Example 6

Samples from two commercially available normal plasma pools were mixed with increasing quantities of oral anticoagulants, i.e. Dabigatran (five concentration steps from 0 µg/ml to 0.63 µg/ml) or Rivaroxaban (five concentration steps from 0 µg/ml to 2 µg/ml), respectively. For all samples, PT values in [s], dFib and Fib Clauss values were determined as specified above. For each sample, the ratio of dFib value to the Fib Clauss value was calculated and said ratio was correlated with the PT value of the respective sample (Fig. 9). As in Examples 4 and 5, a linear correlation was found between the PT value and the dFib/Fib Clauss ratio. Thus, also with oral anticoagulants Dabigatran and Rivaroxaban, the principle of correction described above is applicable.

Claims

1. A method for determining a corrected derived plasma fibrinogen concentration in a blood-derived sample of a subject suffering from or suspected to suffer from prolonged coagulation time, comprising
 - a) determining a first value of a transmission-related parameter in said sample before coagulation and a second value of said transmission-related parameter after coagulation,
 - b) providing a value of a coagulation time-related parameter for said sample,
 - c) providing a correction based on values of reference samples for a coagulation time-related parameter, for a transmission-related parameter, and for a fibrinogen concentration-related parameter,
 - d) based on the values of the parameters of steps b) and c), correcting said value of said transmission-related parameter or a value of a parameter derived therefrom; and
 - e) determining a corrected derived plasma fibrinogen concentration.
2. The method of claim 1, wherein said subject suffering from prolonged coagulation time is a subject receiving anticoagulation therapy, in an embodiment oral anticoagulation therapy.
3. The method of claim 1 or 2, wherein said anticoagulation therapy is a heparin therapy, in an embodiment unfractionated heparin therapy or low molecular weight heparin therapy; factor Xa inhibitor therapy, in an embodiment synthetic pentasaccharide or direct factor Xa inhibitor therapy; direct thrombin inhibitor therapy; or vitamin K antagonist therapy; in an embodiment wherein said anticoagulant therapy is a therapy comprising administration of a 4-hydroxycoumarin, in an embodiment a therapy comprising administration of acenocoumarol, dicumarol, ethyl biscoumacetate, warfarin, and/or phenprocoumon (marcumar, (RS)-4-Hydroxy-3-(1-phenylpropyl)cumarin); in an embodiment comprising administration of warfarin ((RS)-4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one); in a further embodiment comprising administration of (-)-warfarin ((S)-4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one), in a further embodiment comprising administration of phenprocoumon (marcumar, (RS)-4-Hydroxy-3-(1-phenylpropyl)cumarin).
4. The method of any one of claims 1 to 3, wherein said blood-derived sample of a subject suffering from prolonged coagulation time is a sample wherein coagulation is prolonged by a factor of from 1.1 to 8, in an embodiment of from 1.5 to 6, in a

further embodiment of from 1.9 to 5, in a further embodiment of from 2 to 3 as compared to a healthy reference.

- 5 5. The method of any one of claims 1 to 4, wherein said coagulation time-related parameter is (i) a prothrombin time or a value derived therefrom, in an embodiment a Quick value or an INR value, or (ii) an activated partial Thromboplastin time.
- 10 6. The method of any one of claims 1 to 5, wherein said transmission-related parameter is determined by a photo-optical method, in an embodiment by nephelometry or by turbidimetry, in a further embodiment by turbidimetry.
7. The method of any one of claims 1 to 6, wherein step b) is determining a value of a coagulation time-related parameter for said sample.
- 15 8. A database comprised on a data carrier, comprising values of fibrinogen concentration-related parameters of reference samples and/or one or more parameters derived therefrom, and identifiers of said reference samples; in an embodiment further comprising values of coagulation time-related parameters and/or values of transmission-related parameters of said reference samples.
- 20 9. A device for determining a corrected derived fibrinogen value of a blood-derived sample, comprising an analysis unit and an evaluation unit, wherein
(i) said analysis unit is adapted for determining at least a first transmission-related parameter of said sample before coagulation, a second transmission-related parameter after coagulation of said sample, and
25 (ii) said evaluation unit comprises a memory unit comprising a database according to claim 8 and means for providing a corrected derived fibrinogen value.
- 30 10. The device of claim 9, wherein said memory unit comprises a correction parameter and wherein said evaluation unit additionally comprises a tangibly embedded algorithm for deciding whether said correction parameter shall be applied to the first and/or to the second transmission-related parameter or to a parameter derived therefrom;
or wherein said memory unit comprises a correction curve, and/or a calibration curve and a tangibly embedded algorithm for applying said correction curve, and/or
35 calibration curve to the first and/or to the second transmission-related parameter or to a parameter derived therefrom.

11. The device of claims 9 or 10, wherein said analysis unit is adapted to measure a multitude of values of said transmission-related parameter of the same sample at time intervals, in an embodiment at regular time intervals.
- 5 12. The device of claim 11, wherein said evaluation unit is adapted to determine a value of a coagulation-related parameter and an uncorrected derived plasma fibrinogen concentration from said multitude of values of said transmission-related parameter.
- 10 13. A method for assisting (i) in diagnosing a fibrinogen deficiency in a subject, (ii) in monitoring a diagnosed fibrinogen deficiency in a subject, (iii) in evaluating treatment of a fibrinogen deficiency in a subject and/or (iv) in deciding whether a subject should undergo an invasive measure, comprising
- 15 a) determining a corrected derived plasma fibrinogen concentration in a sample of said subject according to the method according to any one of claims 1 to 7, and
- 20 b) diagnosing prolonged coagulation time with a fibrinogen deficiency, a severe progression of a diagnosed fibrinogen deficiency, inadequacy of fibrinogen deficiency treatment and/or advising against said invasive measure in case the corrected derived plasma fibrinogen concentration determined is less than 200 mg/dL, in an embodiment is less than 150 mg/dL; thereby assisting in at least one of diagnosing of (i), monitoring of (ii), evaluating of (iii), and deciding of (iv).
14. A method for assisting in diagnosing an inflammatory condition and/or a condition involving tissue damage in a subject, comprising
- 25 a) determining a corrected derived plasma fibrinogen concentration in a sample of said subject according to the method according to any one of claims 1 to 7, and
- 30 b) indicating an increased risk of suffering from an inflammatory condition and/or a condition involving tissue damage if the corrected derived plasma fibrinogen concentration determined is higher than 300 mg/dL, in an embodiment higher than 350 mg/dL, in a further claim higher than 400 mg/dL; thereby assisting in diagnosing an inflammatory condition and/or a condition involving tissue damage.
15. Use of the device according to any one of claims 9 to 12 or the database of claim 8
- 35 for determining a corrected derived plasma fibrinogen concentration in a sample.
16. A data carrier, comprising coagulation time-related parameters, transmission-related parameters, and fibrinogen concentration-related parameters of reference

samples and/or a correction parameter, a correction curve, a calibration curve, a correction area, and/or a calibration area derived therefrom.

- 5 17. Use of the device according to any one of claims 9 to 12, the database of claim 8, or the data carrier of claim 16 for the manufacture of a device for determining a corrected derived plasma fibrinogen concentration in a sample.

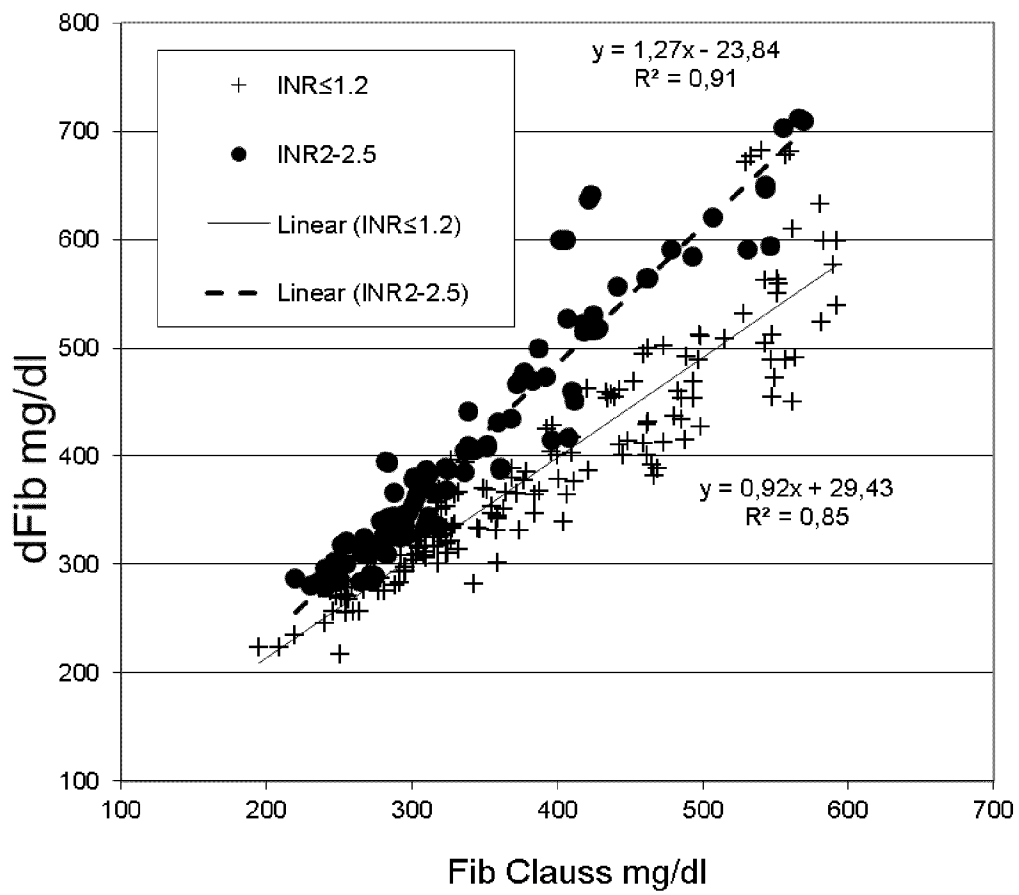


Fig. 1

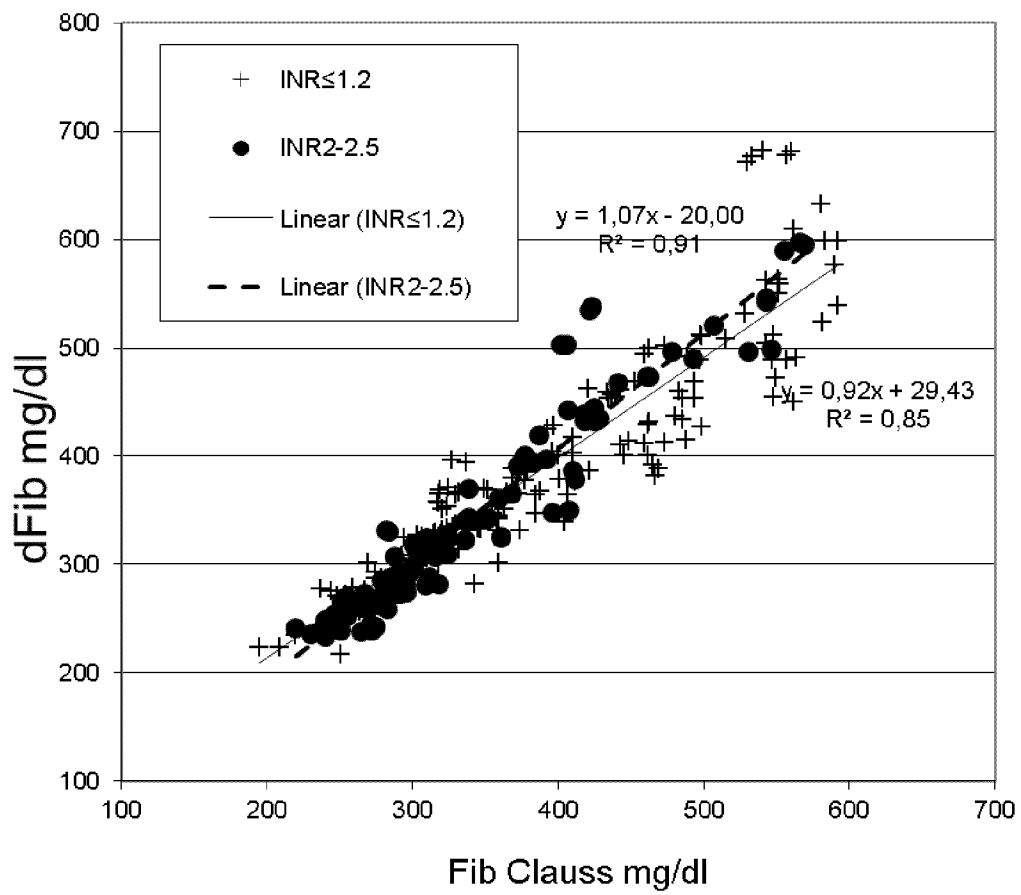


Fig. 2

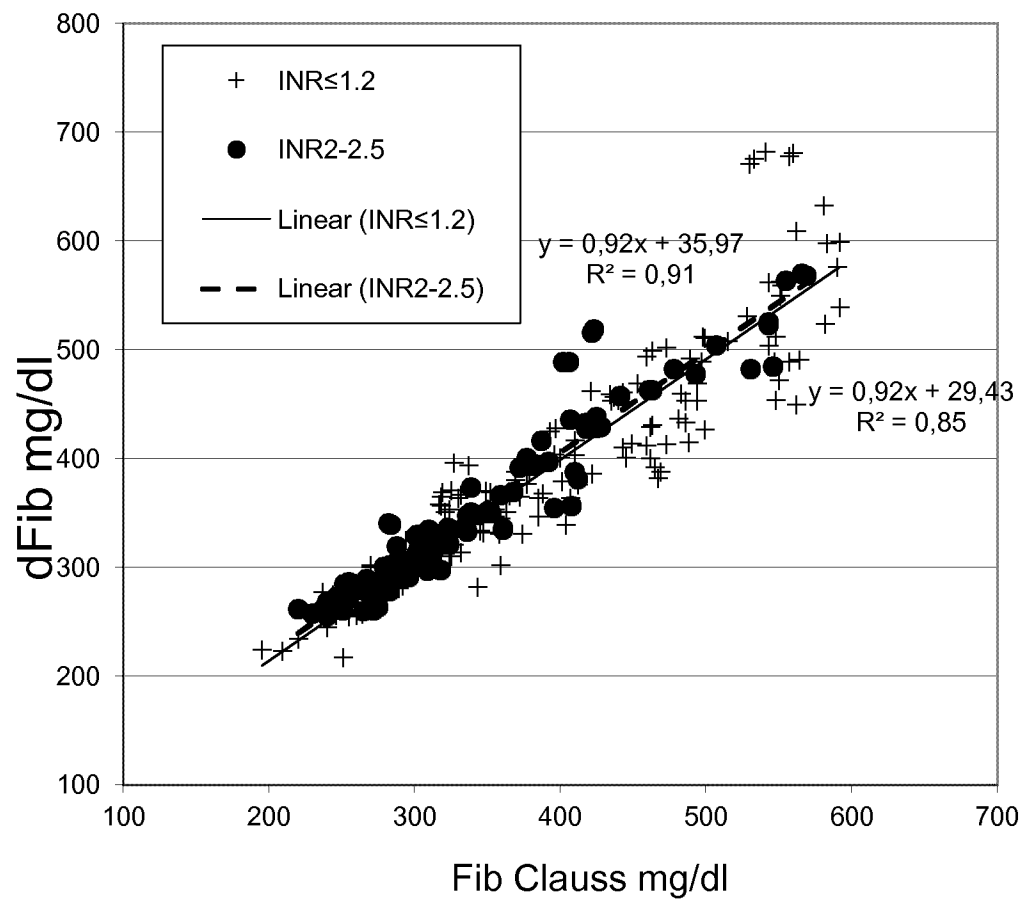


Fig. 3

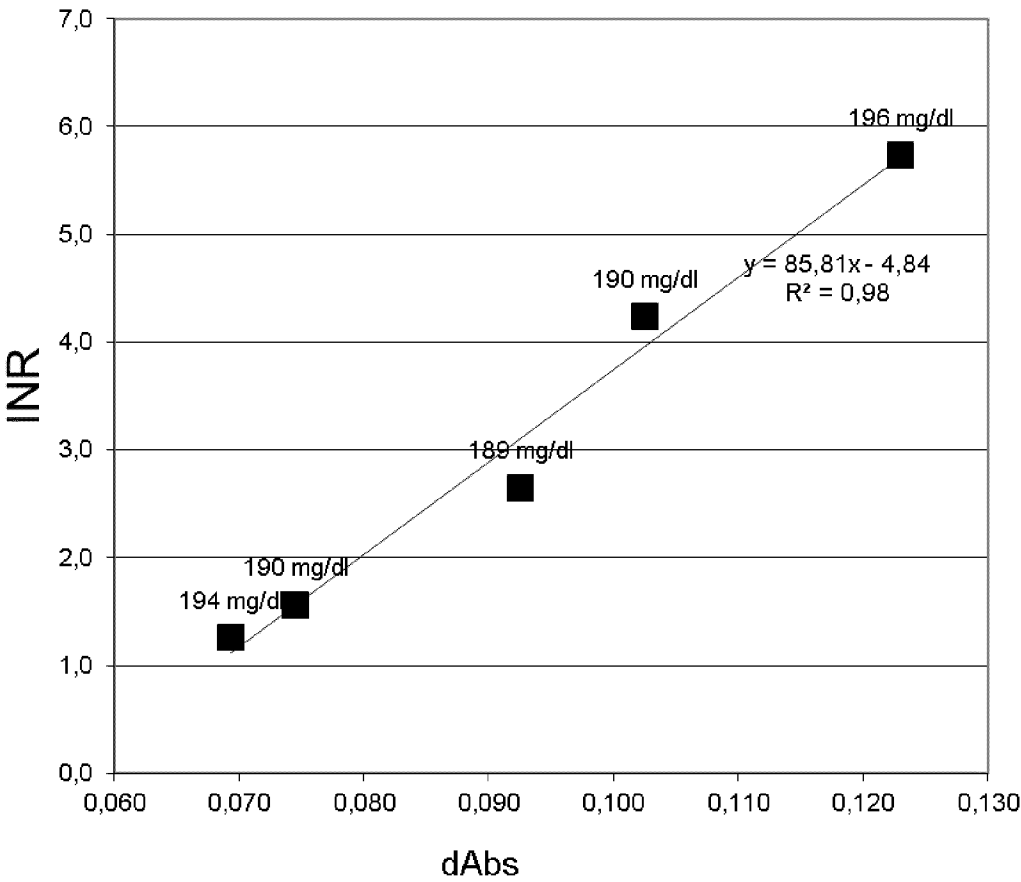


Fig. 4

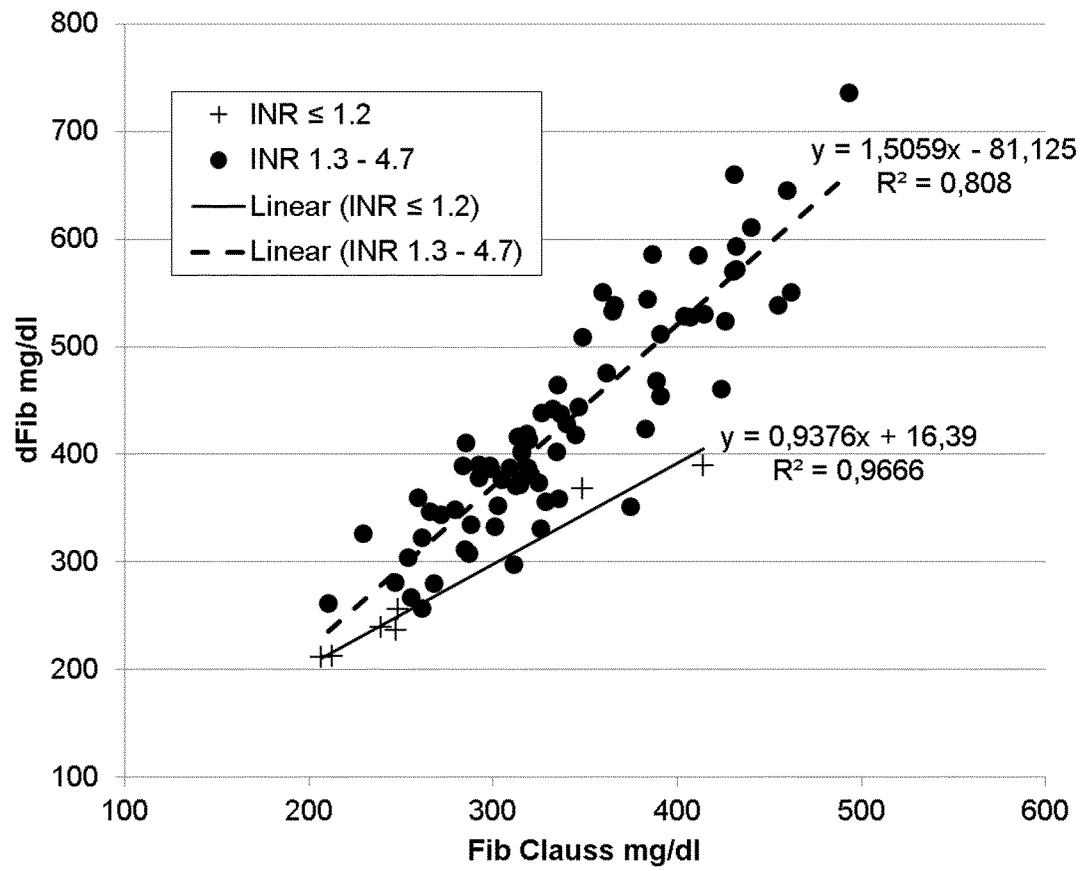


Fig. 5

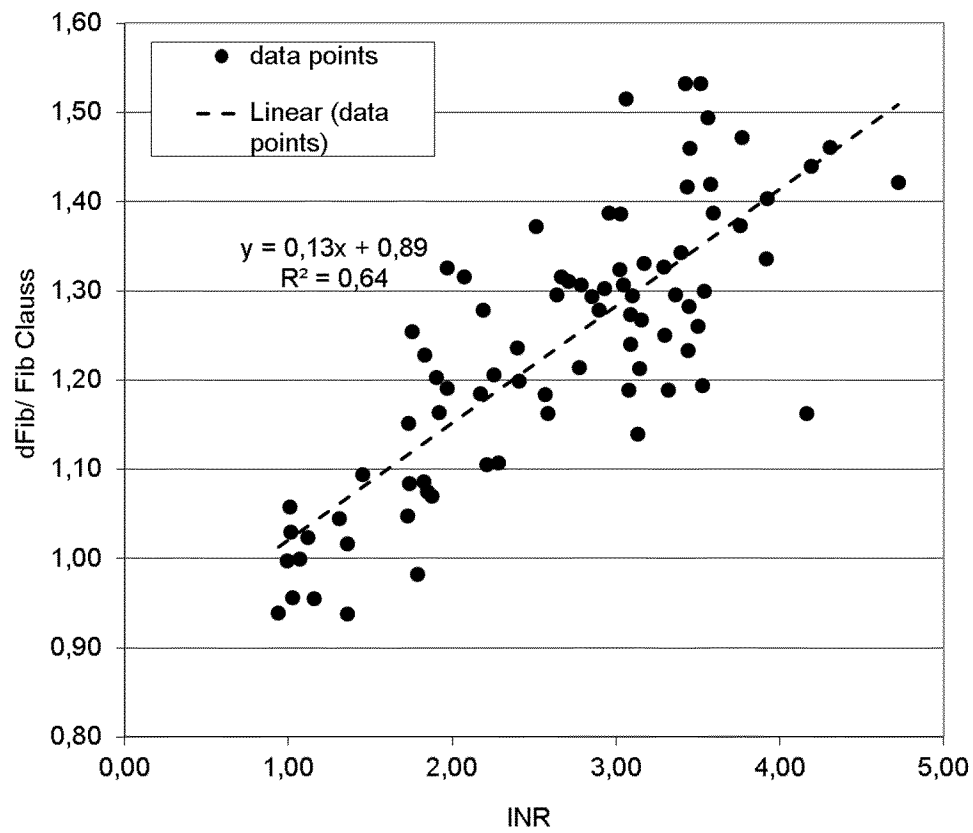


Fig. 6

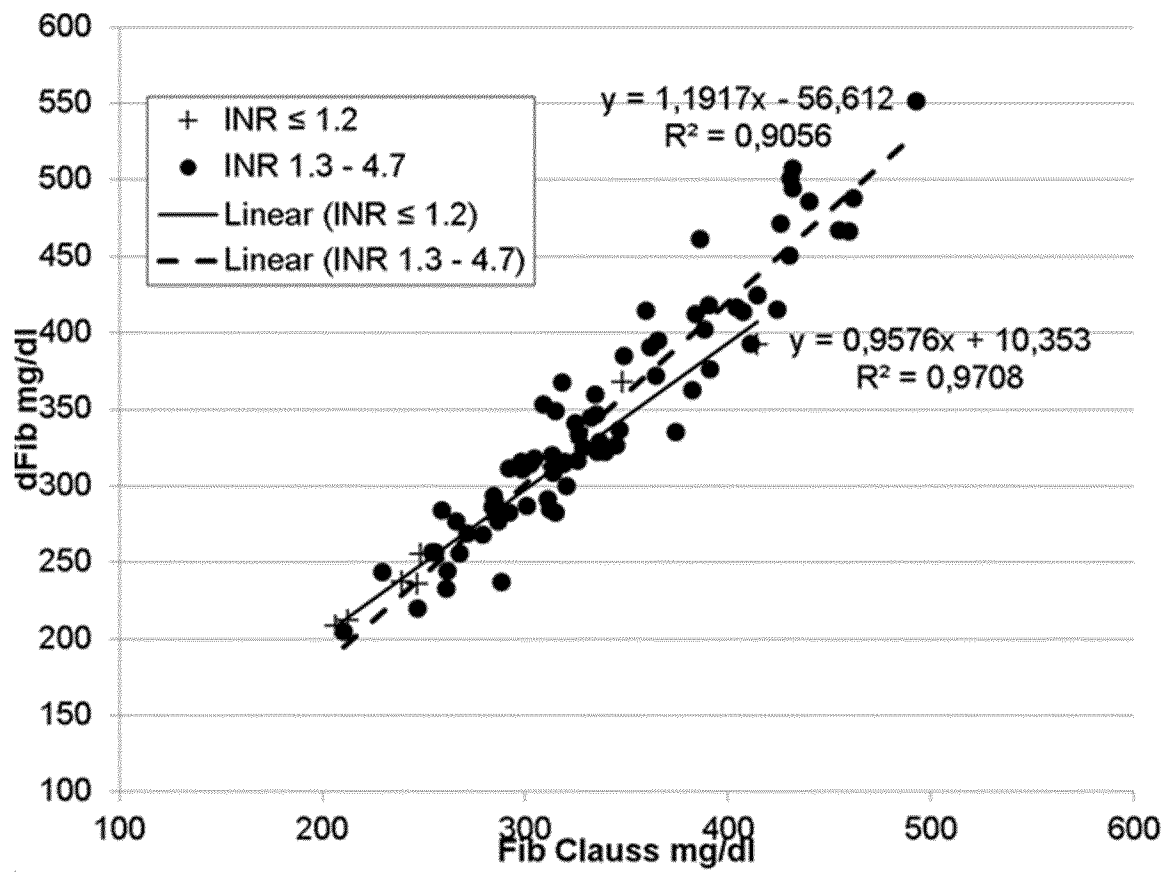


Fig.7

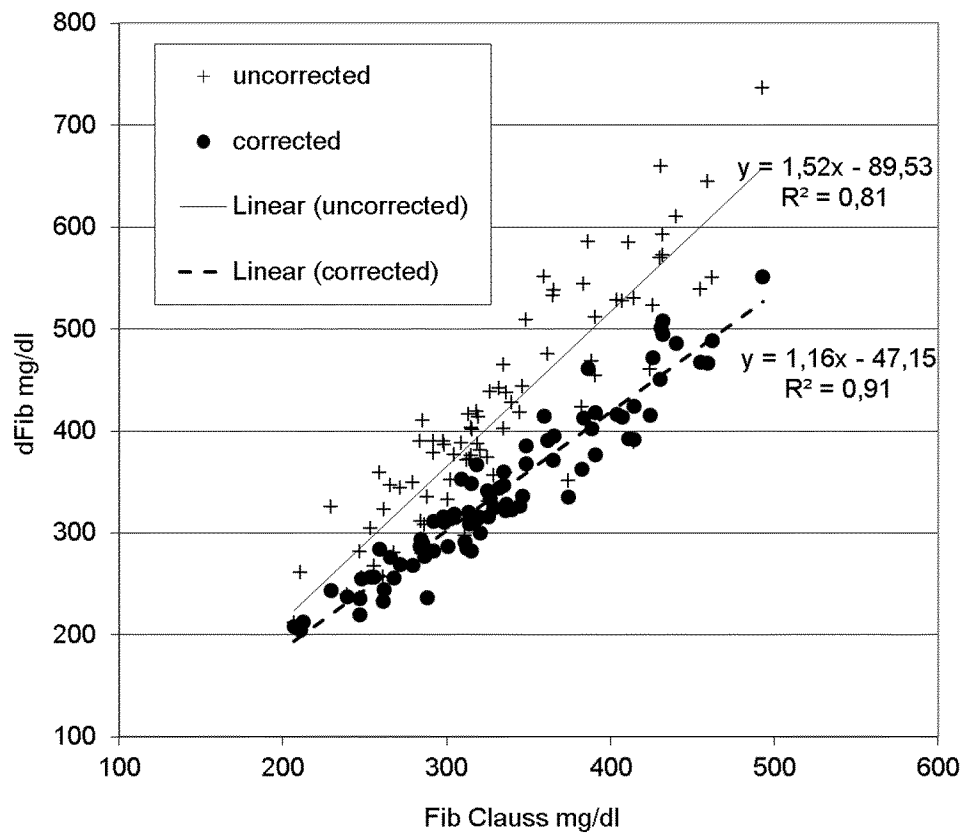
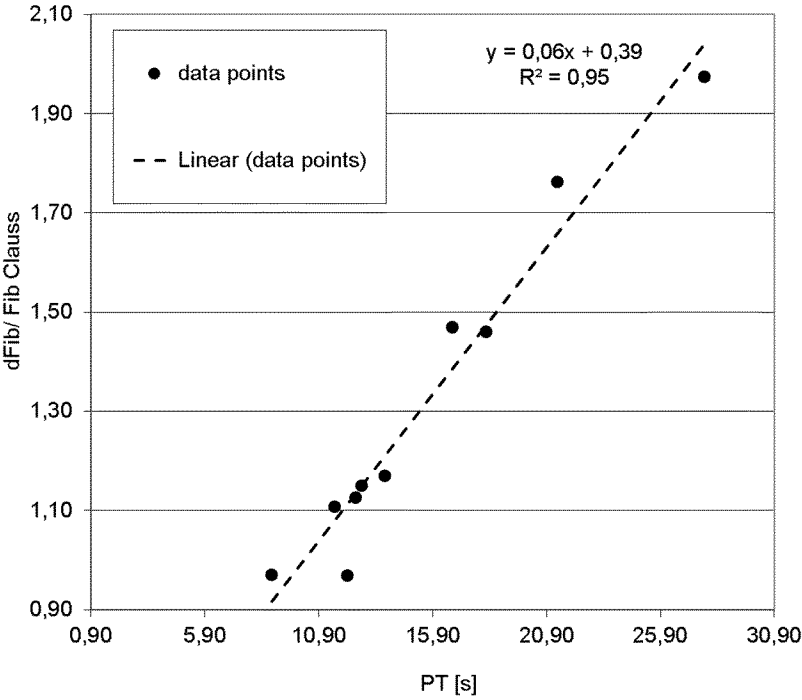


Fig. 8

A



B

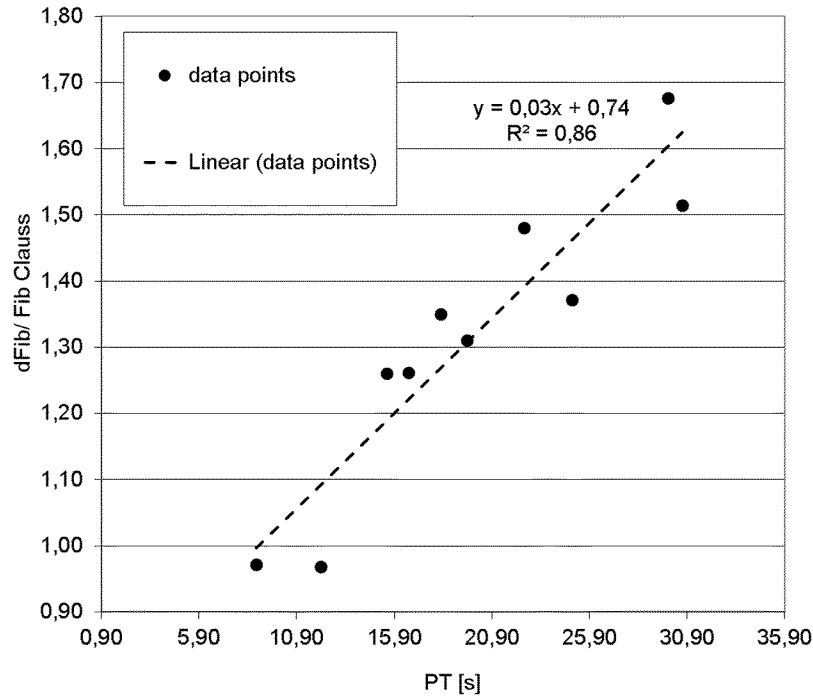


Fig. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/053610A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/86
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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A. S. LAWRIE ET AL: "Prothrombin time derived fibrinogen determination on Sysmex CA-6000", JOURNAL OF CLINICAL PATHOLOGY, vol. 51, no. 6, 1 June 1998 (1998-06-01), pages 462-466, XP055274375, GB ISSN: 0021-9746, DOI: 10.1136/jcp.51.6.462 the whole document abstract figure 2 last paragraph of discussion ----- -/--	1-17



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

3 March 2017

Date of mailing of the international search report

13/03/2017

Name and mailing address of the ISA/

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Authorized officer

Jenkins, Gareth

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/053610

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHITOLIE A ET AL: "Inaccuracy of the 'derived' fibrinogen measurement", BLOOD COAGULATION & FIBRINOLYSIS, RAPID COMMUNICATIONS, OXFORD, OXFORD, GB, vol. 5, no. 6, 1 December 1994 (1994-12-01), pages 955-957, XP009097640, ISSN: 0957-5235, DOI: 10.1097/00001721-199412000-00012	8,16,17
A	the whole document abstract	1-7,9-15
X	----- W. MIESBACH ET AL: "Comparison of the fibrinogen Clauss assay and the fibrinogen PT derived method in patients with dysfibrinogenemia", THROMBOSIS RESEARCH, vol. 126, no. 6, 1 December 2010 (2010-12-01), pages e428-e433, XP055187558, ISSN: 0049-3848, DOI: 10.1016/j.thromres.2010.09.004	8,16,17
A	the whole document abstract	1-7,9-15
X	----- I. J. MACKIE ET AL: "A Performance Evaluation of Commercial Fibrinogen Reference Preparations and Assays for Clauss and PT-derived Fibrinogen", THROMBOSIS AND HAEMOSTASIS, vol. 87, no. 6, 1 June 2002 (2002-06-01), pages 997-1005, XP055274378, DE	8,16,17
A	ISSN: 0340-6245 the whole document abstract	1-7,9-15
