since many samples have ESRs as low as 10 mm/hour. Inflammatory factors which increase ESR include fibrinogen, C-Reactive Protein (CRP) and some immunoglobulins, which can increase ESR to as high as 100 mm/hour.

[0005] Traditional techniques of performing sedimentation tests have various limitations. For instance as discussed, Westergren sedimentation tests require a substantially high volume of blood to be withdrawn. Additionally, traditional sedimentation test techniques take a substantial period of time and may result in time lags in obtaining test results that could lead to delays in diagnoses and treatments which can have a deleterious effect on a patient's health.

INCORPORATION BY REFERENCE

[0006] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY

[0007] It may be desirable to have sedimentation rate test that can be completed in a very short time, such as but not limited to being on the order of seconds to a few minutes. For distributed test settings, it may also be desirable to have sedimentation rate measurements that use only small blood volumes, such as can be obtained by alternate site, non-venous blood draws or minimal venous draws. It may be further desirable to make the sedimentation measurement in an automated fashion (no human observation required) and to create an objective record of the measurement. Additionally, further information useful in optimizing management of patients may be obtained by performing and/or maximizing the speed of multiplexed measurement of other analytical parameters in parallel with sedimentation rate measurement. Thus, in a first aspect, the present invention refers to a method comprising: using an accelerated blood component separation technique on a blood sample for a period of time to separate formed blood components from plasma; determining a sedimentation rate of the formed blood component based on at least the following: a time-related compaction curve and a hematocrit correction factor, wherein the time-related compaction curve for at least one formed blood component in said blood sample is determined after accelerated blood component separation has begun, said compaction curve having an initial approximately linear portion and a non-linear portion after the linear portion. In a second aspect, the present invention refers to a method comprising: using an accelerated blood component separation technique on a blood sample for a period of time to separate formed blood components from plasma; establishing a time-related compaction curve

for at least one formed blood component in said blood sample after accelerated blood component separation has begun, said compaction curve having an initial approximately linear portion; determining sedimentation rate of the formed blood component based on at least the following: the compaction curve and a hematocrit correction factor. In a third aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; using imaging of the vessel in a single state condition to establish sedimentation rate; correcting for hematocrit effect on sedimentation rate of the formed blood component by using a hematocrit correction factor. In a third aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; using imaging of the vessel in a single state condition to establish sedimentation rate; correcting for hematocrit effect on sedimentation rate of the formed blood component by using a hematocrit correction factor. In a fourth aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; correcting for hematocrit effect on sedimentation rate of the formed blood component by using a hematocrit correction factor on the approximately linear portion of said compaction curve. In a fourth aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; establishing a time-related compaction curve for at least one formed blood component in said blood sample after centrifuging has begun; correcting for impact of hematocrit on sedimentation rate of the formed blood component using a hematocrit correction factor based on the formula: $U_{corr} = \frac{U_{uncorr}}{\left(1-\frac{\varphi}{\varphi_{max}}\right)^{\gamma}}$ where U_{uncorr} and U_{corr} are the uncorrected (raw) and corrected sedimentation rates, φ is the volume fraction of cells (hematocrit), and φ_{max} and γ are empirical parameters obtained by curve fitting. In a fifth aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; establishing a time-related compaction curve for at least one formed blood component in said blood sample after centrifuging has begun; correcting for impact of hematocrit on sedimentation rate of the formed blood component using a hematocrit correction factor based on the formula $U_{corr} = \frac{U_{uncorr}}{\left(1-\frac{\varphi}{\varphi_{max}}\right)^{\gamma}}$ where U_{uncorr} and U_{corr} are the uncorrected (raw) and corrected sedimentation rates, φ is the volume fraction of cells (hematocrit), and φ_{max} and γ are empirical parameters obtained by curve fitting. In a sixth aspect, the present invention refers to a method comprising: collecting a plurality of images of formed blood component and plasma interface positions over time from an accelerated blood sample compaction process; performing image transformation on

said plurality of images to transform images with curved interfaces into corrected images with straight line interfaces; establishing a time-related compaction curve based on interface positions in said corrected images, for at least one formed blood component in said blood sample. In a seventh aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; collecting a plurality of images of formed blood component and plasma interface positions over time; performing image transformation on said images to transform images with curved interfaces into corrected images with straight line interfaces; establishing a time-related compaction curve based on interface positions in said corrected images, for at least one formed blood component in said blood sample after centrifuging has begun. In an eighth aspect, the present invention refers to a method comprising: using a programmable processor-controlled system to transfer at least a portion of a blood sample from a blood sample location into a centrifugation vessel; using a sample handling system under programmable processor control to transfer said vessel from a first addressable position to a centrifuge with a second addressable position; centrifuging the blood sample in the vessel for a period of time; collecting a plurality of images of formed blood component and plasma interface positions over time; establishing a time-related compaction curve based on interface positions in said corrected images, for at least one formed blood component in said blood sample after centrifuging has begun. In a ninth aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; varying centrifuging speed to establishing a linear compaction curve of at least one formed blood component over the period of time until compacting has completed; monitoring centrifuging speed profile for at least a portion of the time period; and determining blood component sedimentation rate based on the centrifuging speed profile. In a tenth aspect, the present invention refers to a method comprising: centrifuging a blood sample in a vessel for a period of time; collecting at least a first single image of formed blood component and plasma interface positions at an initial time; collecting at least a second single image of formed blood component and plasma interface positions at a second time while rate of sedimentation is still linear; calculating sedimentation rate for at least one formed blood component in said blood sample based on linear sedimentation rate calculated and a hematocrit correction factor. In an eleventh aspect, the present invention refers to a device for use with a sample, the device comprising: a centrifuge having a centrifuge vessel holder configured to allow for detection of blood component interface position in the vessel holder during centrifugation. In a twelve aspect, the present invention refers to a system comprising: a centrifuge having a centrifuge vessel holder configured to allow for detection of blood

component interface position in the vessel holder in the vessel holder during centrifugation; a sample handling system for transporting a blood sample from a first location to a location on the centrifuge; and a processor programmed to record interface position during a least a portion of centrifugation.

[0008] In one ~~embodiment~~example described herein, the sedimentation rate measurement method may use (1) centrifugal techniques for separating red blood cells from plasma and (2) video and/or still imaging capability. Both may be used alone or in combination to accelerate erythrocyte sedimentation and to measure its rate. Of course, techniques other than centrifugation for accelerating sedimentation may be used in place of or in combination with centrifugation to separate blood components.

[0009] In one non-limiting example, the method may advantageously enable (1) rapid measurement of ESR (seconds) with small blood sample volumes such as about 20-25 microliters ("uL" or "μL") or less, (2) use of automated image analysis to determine both red blood cell sedimentation rate and hematocrit, and/or (3) automated techniques to compensate for

effects of hematocrit on uncorrected ESR so as to provide a value corresponding to the traditional Westergren method. Of course, alternative ~~embodiments~~examples using large volumes of blood are not excluded. Because of the ability to correct for hematocrit, some ~~embodiments~~examples of sedimentation measurement techniques described herein is more robust than traditional Westergren techniques and can be used on sample with fibrinogen and/or hematocrit levels outside the narrow range required by Westergren testing.

[0010] Using an ~~embodiment~~example herein, corrected ESR can be acquired in a matter of seconds using a small blood volume and which compensates for effects of hematocrit ESR. The results acquired in a matter of seconds during initial centrifugation can accelerate deliver of a diagnosis to the patient.

[0011] Moreover, in the context of multiplexed assay procedures, a common pre-processing step already involves separating red and white cells from plasma or serum prior to measurements of cellular markers and of analytes present in plasma/serum. Thus, it is convenient to incorporate an ESR measurement along with such a pre-processing procedure that will already be performed during the course of assay preparation. The ESR measurement will not create significant burden in terms of additional processing time or use of limited quantities of blood available from non-venous collection methods. By way of non-limiting example, it should be understood that assay processing, including pre-processing step(s), may occur in a single instrumented system. Optionally, some ~~embodiments~~examples may perform one or more steps in one instrument and another one or more steps in another instrument.

[0012] It should also be understood that ~~embodiments~~examples described herein may be adapted to have one or more of the features described below. In one non-limiting example, a typical protocol may take 20 uL of blood in a centrifuge vessel and spin in a swing-out centrifuge rotor at 4000 rpm (580*g) for about 10 s. During this time, the interface between the portion of the sample containing the red blood cells and that cleared of red blood cells is observed by video imaging. Although other time periods are not excluded, it can be advantageous to obtain the ESR measurement in this short period of time. Optionally, some ~~embodiments~~examples may correct these "raw" ESR values for the effects of hematocrit. Hematocrit may be measured in the same operation as that used for measurement of raw ESR. In one non-limiting example, following a relatively low speed spin during centrifugation to measure ESR, the spin speed is increased to pack the red blood cells. Hematocrit is determined by image analysis of the packed red blood cells and the supernatant plasma volumes. Optionally, other techniques for measuring hematocrit may also be used to correct "raw" ESR values.

[0013] At least some of the ~~embodiments~~examples herein may have ESR corrected without using calculations of the slope of an essentially linear transform of the non-linear (exponential) portion of the sedimentation curve.

[0014] At least some of the ~~embodiments~~examples herein may have ESR corrected without calculating a mathematical function for a plurality of the erythrocyte/plasma interface positions occurring in a non-linear portion of the sedimentation curve.

[0015] At least some of the ~~embodiments~~examples herein may have ESR corrected without selecting a segment of the sedimentation curve which lies in said non-linear portion of the sedimentation curve.

[0016] At least some of the ~~embodiments~~examples herein may have ESR corrected based only on measurements of linear portion(s) of the sedimentation curve.

[0017] At least some of the ~~embodiments~~examples herein may have ESR corrected based on measurements which consists essentially of linear portion(s) of the sedimentation curve. By "consists essentially of", we mean at least 90% or more of the measurement is based on the linear portion(s).

[0018] At least some of the ~~embodiments~~examples herein may have ESR corrected without determining a mathematical function for a non-linear segment of the sedimentation curve representative of the magnitude of intercellular erythrocyte repulsion in the blood sample.

[0019] At least some of the ~~embodiments~~examples herein may have ESR corrected without negating the time period during the centrifugation of the sample during which a linear portion of the sedimentation curve is formed.

[0020] At least some of the ~~embodiments~~examples herein may have ESR corrected for hematocrit using hematocrit measurements not derived from centrifugal techniques, such as for example, lysis of red cells with detergent and mixing with ferricyanide and cyanide followed by measurement of the absorbance of the cyan-met-hemoglobin formed.

[0021] At least some of the ~~embodiments~~examples herein may have the blood sample adjusted so that it is at a known hematocrit level for the sedimentation measurement.

[0022] In at least one ~~embodiment~~example described herein, a method is provided comprising: using an accelerated blood component separation technique on a blood sample for a period of time to separate formed blood components from plasma; determining a sedimentation rate of the formed blood component based on at least the following: a time-related compaction curve and a hematocrit correction factor, wherein the time-related compaction curve for at least one formed blood component in said blood sample is determined after accelerated blood component

separation has begun, said compaction curve having an initial approximately linear portion and a non-linear portion after the linear portion.

[0023] In at least one ~~embodiment~~example described herein, a method is provided comprising: centrifuging a blood sample in a vessel for a period of time; establishing a time-related compaction curve for at least one formed blood component in said blood sample after centrifuging has begun, said compaction curve having an initial approximately linear portion; correcting for hematocrit effect on sedimentation rate of the formed blood component by using a hematocrit correction factor on the approximately linear portion of said compaction curve.

[0024] It should be understood that ~~embodiments~~examples in this disclosure may be adapted to have one or more of the features described below. In one non-limiting example, the method comprises calibrating sedimentation rates from centrifuge based technique with sedimentation rates from a reference technique. Optionally, the reference technique is the Westergren technique. Optionally, the sample is about 25 uL or less. Optionally, centrifuging occurs at a first speed for a first period of time and then at a second, faster speed for a second period of time. Optionally, centrifuging comprises using a centrifuge configured to allow the blood sample to be visually observed during centrifugation to establish interface positions of one or more formed blood components in the blood sample. Optionally, centrifuging comprises using a centrifuge having a window thereon to enable visual observation of the blood sample to establish erythrocyte/plasma interface positions over time. Optionally, centrifuging comprises using a centrifuge, a light source, and an image capture device to enable visual observation of the blood sample to establish formed blood component/plasma interface positions over time. Optionally, compaction curve data is collected by capturing a plurality of images of interface positions of one or more formed blood components in a centrifuge vessel over the time period. Optionally, pixel positions in the plurality of images are used to accurately determine interface position. Optionally, compaction curve data is collected by capturing a single image of an interface position of one or more formed blood components in the centrifuge vessel after a time period, wherein sedimentation rate is calculated based on position of the meniscus of the supernatant liquid and the interface position. Optionally, compaction curve data is collected while the sample is being centrifuged. Optionally, centrifugation is used to obtain hematocrit measurement and to correct for hematocrit impact on sedimentation rate measurement. Optionally, correcting for hematocrit comprises calculating a mathematical function for a plurality of formed blood component interface positions occurring in said curve, said function being operative to correct for sedimentation rate variations due to hematocrit. Optionally, hematocrit measurement in the sample is derived from a technique separate from centrifugation

Optionally, image transformation is used for conversion of a curved interface to a flat interface. Optionally, image transformation parameters are selected, video of formed blood component interface position is put through image transformation, and then a region of interest is chosen that covers both the whole range of positions for both air/plasma interface and erythrocyte interface. Optionally, each timepoint in the video, pixel intensity values for each row across a sample vessel containing the sample, within the region of interest are averaged to produce a single column representing the intensity radially down the sample vessel. Optionally, a linear region of a sedimentation profile is used to extract a sedimentation rate. Optionally, the formed blood component is white blood cells. Optionally, the formed blood component is platelets.

[0025] It should be understood that ~~embodiments~~examples in this disclosure may be adapted to have one or more of the features described below. In one non-limiting example, the method comprises performing image transformation on said images to transform images with curved interfaces into corrected images with straight line interfaces; establishing a time-related compaction curve based on interface positions in said corrected images, for at least one formed blood component in said blood sample after centrifuging has begun. Optionally, the method comprises: using a programmable processor-controlled system to transfer at least a portion of a blood sample from a blood sample location into a centrifugation vessel; using a sample handling system under programmable processor control to transfer said vessel from a first addressable position to a centrifuge with a second addressable position; centrifuging the blood sample in the vessel for a period of time; collecting at least one image of formed blood component and plasma interface position after centrifuging; establishing a time-related compaction curve based on interface position(s) in the image, for at least one formed blood component in said blood sample after centrifuging has begun. Optionally, the vessel is removed from the centrifuge to obtain said image. Optionally, the vessel is returned to the centrifuge after said image is obtained. Optionally, the method comprises varying centrifuging speed to establishing a linear compaction curve of at least one formed blood component over the period of time until compacting has completed; monitoring centrifuging speed profile for at least a portion of the time period; and determining blood component sedimentation rate based on the centrifuging speed profile. Optionally, the method comprises collecting at least a first single image of formed blood component and plasma interface positions at an initial time; collecting at least a second single image of formed blood component and plasma interface positions at a second time while rate of sedimentation is still linear; calculating sedimentation rate for at least one formed blood component in said blood sample based on linear sedimentation rate calculated and a hematocrit correction factor.

[0026] In yet another ~~embodiment~~example described herein, a method is provided comprising: centrifuging a blood sample in a vessel for a period of time; using imaging of the vessel in a single state condition to establish sedimentation rate; and correcting for hematocrit effect on sedimentation rate of the formed blood component by using a hematocrit correction factor. As used herein, using imaging can comprise using a single image of the vessel to determine sedimentation rate. By of non-limiting example, when using a single image of the vessel, a meniscus of supernatant liquid in the vessel shows an initial level and an interface position of formed components with the supernatant liquid shows current position, from which sedimentation rate is calculated. Optionally, some ~~embodiment~~example may use a plurality of images for sedimentation rate calculation, but all of the images are of the vessel while the vessel is in a single state condition. Optionally, all of the images are of the vessel at a single point in time. Optionally, all of the images are of the vessel while the formed component interface position is not changing in the vessel.

[0027] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Figure 1 shows a graph of red cell sedimentation of high, medium, and low ESR blood samples in Westergen-Katz tubes.

[0029] Figures 2A-2B are images of blood samples in transparent centrifugation vessels.

[0030] Figure 3 shows a schematic of a centrifuge with one embodiment of a detection system.

[0031] Figures 4-5 show images captured using one embodiment of a detection system.

[0032] Figures 6A-7C show a series of corrected and uncorrected images of interfaces in a blood sample undergoing centrifugation.

[0033] Figures 8A-8B show various kymographs for one test sample.

[0034] Figure 9 shows a sedimentation graph for one test sample

[0035] Figures 10A-10B show sedimentation graphs with various fitted functions fitted to the data of Figure 9 plotted thereon.

[0036] Figures 11-14 are graphs showing various sample sedimentation characteristics for samples with various levels of added fibrinogen.