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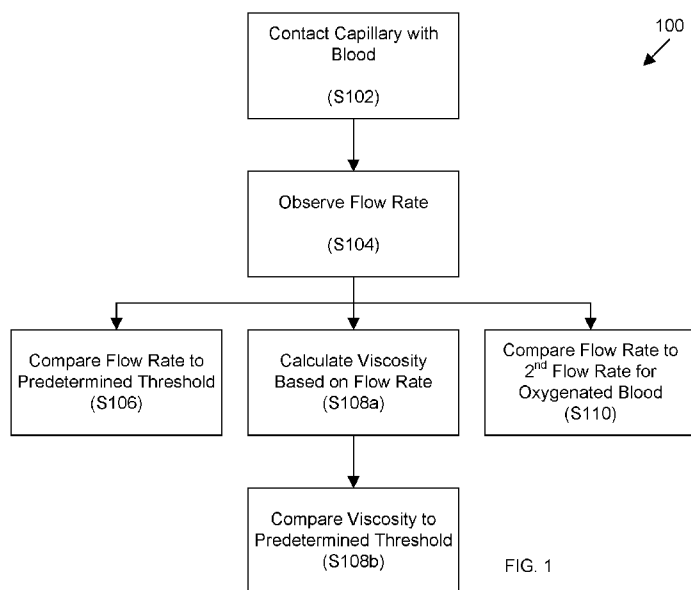
(54) **Title:** METHODS, KITS AND SYSTEMS FOR SCREENING FOR SICKLE-CELL DISEASE

FIG. 1

(57) **Abstract:** One aspect of the invention provides a method of screening for sickle-cell disease. The method includes: contacting an end of a capillary tube with deoxygenated blood; observing a flow rate of the deoxygenated blood within the capillary tube; and performing one or more steps selected from the group consisting of: comparing the flow rate to a predetermined flow-rate threshold for the capillary tube indicative of sickle-cell disease; calculating viscosity based on the flow rate and comparing the viscosity to a predetermined viscosity threshold for the capillary tube indicative of sickle-cell disease; and comparing the flow rate to a second flow rate for oxygenated blood from a same subject within a substantially identical capillary tube.

METHODS, KITS, AND SYSTEMS FOR SCREENING FOR SICKLE-CELL DISEASE**CROSS-REFERENCE TO RELATED APPLICATION**

This application claims priority to US Provisional Patent Application Serial No. 62/269,571, filed December 18, 2015. The entire content of this application is hereby
5 incorporated by reference herein.

BACKGROUND OF THE INVENTION

Sickle cell disease is a genetic condition that causes deoxygenated hemoglobin in red blood cells to assemble into rigid polymers. The flexibility of cells—essential for cell traversal of the capillary bed—is thus destroyed.

10 Due to its nature as a genetic disorder, sickle cell disease continues to be present in significant numbers of people throughout the world. In the US, life-span has been extended thanks to early and regular care, despite the fact that only one drug is available. While median life span for the 100,000 sickle patients in the US is around 40 years, it is less than 5 years in Africa, where it is estimated by the WHO that 300,000 new cases arise yearly.

SUMMARY OF THE INVENTION

15 One aspect of the invention provides a method of screening for sickle-cell disease. The method includes: contacting an end of a capillary tube with deoxygenated blood; observing a flow rate of the deoxygenated blood within the capillary tube; and performing one or more steps selected from the group consisting of: comparing the flow rate to a predetermined flow-rate
20 threshold for the capillary tube indicative of sickle-cell disease; calculating viscosity based on the flow rate and comparing the viscosity to a predetermined viscosity threshold for the capillary tube indicative of sickle-cell disease; and comparing the flow rate to a second flow rate for oxygenated blood from a same subject within a substantially identical capillary tube.

25 This aspect of the invention can have a variety of embodiments. The capillary tube can be unobstructed. The capillary tube and the substantially identical capillary tube can be unobstructed.

The blood can have been deoxygenated through the use of a chemical reductant.

The flow rate of deoxygenated sickle-cell blood can be slower than that of oxygenated sickle-cell blood. Deoxygenated sickle-cell blood can be more viscous than oxygenated sickle-

cell blood. The flow rate of deoxygenated healthy blood can be equal to that of oxygenated healthy blood. Deoxygenated healthy blood can have the same viscosity as oxygenated healthy blood. The flow rate of deoxygenated sickle trait blood can resemble the flow rate of deoxygenated healthy blood immediately after deoxygenation and more closely resemble the flow rate of deoxygenated sickle-cell blood as time after deoxygenation increases.

The capillary tube can have a volume of about 0.25 μ L.

Another aspect of the invention provides a method of screening for sickle-cell disease. The method includes: contacting an end of a capillary tube with oxygenated blood, wherein the capillary tube is adapted and configured to deoxygenate the oxygenated blood to produce deoxygenated blood; observing a flow rate of the deoxygenated blood within the capillary tube; and performing one or more steps selected from the group consisting of: comparing the flow rate to a predetermined flow-rate threshold for the capillary tube indicative of sickle-cell disease; calculating viscosity based on the flow rate and comparing the viscosity to a predetermined viscosity threshold for the capillary tube indicative of sickle-cell disease; and comparing the flow rate to a second flow rate for oxygenated blood from a same subject within a substantially identical capillary.

This aspect of the invention can have a variety of embodiments. The capillary tube can be unobstructed. The capillary tube and the substantially identical capillary tube can be unobstructed.

The flow rate of deoxygenated sickle-cell blood can be slower than that of oxygenated sickle-cell blood. Deoxygenated sickle-cell blood can be more viscous than oxygenated sickle-cell blood. The flow rate of deoxygenated healthy blood can be equal to that of oxygenated healthy blood. Deoxygenated healthy blood can have the same viscosity as oxygenated healthy blood. The flow rate of deoxygenated sickle trait blood can resemble the flow rate of deoxygenated healthy blood immediately after deoxygenation and more closely resemble the flow rate of deoxygenated sickle-cell blood as time after deoxygenation increases.

The capillary tube can have a volume of about 0.25 μ L. The capillary tube can be glass capillary tube.

Another aspect of the invention provides a system for rapidly screening for sickle-cell disease. The system includes: a capillary assembly including two or more capillary tubes having identical composition and dimensions and each having two open ends; and a connecting body

coupled to the two or more capillary tubes. The connecting body holds the two or more capillary tubes parallel to each other in an array wherein each tube is a defined distance from each neighboring tube.

This aspect of the invention can have a variety of embodiments. The system can further
5 include a handle coupled to the connecting body.

The system can further include a sample container adapted and configured to hold two or more liquid samples in designated locations such that the designated locations are oriented in an array complementarily aligned with the array of the two or more capillary tubes, such that the two or more capillaries can simultaneously contact the two or more liquid samples. The sample
10 container can be a flat surface. The designated locations can be markings on the flat surface. The sample container can be a piece of glass. The designated locations can be wells adapted and configured to hold the two or more liquid samples.

The sample container can further include a strong reducing agent capable of deoxygenating one or more of the two or more liquid samples. The sample container can further
15 include one or more oxygen permeable materials. The sample container can further include one or more materials selected from the group consisting of polydimethylsiloxane, poly(methyl methacrylate), silicone rubber, polyurethane, polyethylene, amorphous fluoropolymers, and liquid crystal polymers.

The system can further include one or more pipettes for transferring the two or more
20 liquid samples to the sample container. The one or more pipettes can include one or more oxygen permeable materials. The one or more pipettes can include one or more materials selected from the group consisting of polydimethylsiloxane, poly(methyl methacrylate), silicone rubber, polyurethane, polyethylene, amorphous fluoropolymers, and liquid crystal polymers. The one or more of the pipettes can include a strong reducing agent capable of deoxygenating
25 one or more of the two or more liquid samples.

The system can further include a robotic arm.

The two or more capillary tubes can be unobstructed.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the nature and desired objects of the present invention,
30 reference is made to the following detailed description taken in conjunction with the

accompanying drawing figures wherein like reference characters denote corresponding parts throughout the several views.

FIG. 1 depicts a method of screening for sickle-cell disease according to an embodiment of the invention.

5 FIGS. 2A-2F depict capillaries and systems according to embodiments of the invention.

FIG. 3A depicts the relationship between viscosity of a blood sample and the hematocrit (fraction of red blood cells) of the blood sample.

FIG. 3B depicts the rate of ascent of blood samples up a capillary as a function of time for both oxygenated and deoxygenated blood samples comprising a 95:5 SS : AA blood ratio.

10 FIG. 4A depicts the rise times of oxygenated vs. deoxygenated blood samples having varying traits using a vertical capillary system.

FIG. 4B depicts ratios of deoxygenated rise times to oxygenated rise times using a vertical capillary system.

15 FIGS. 5A and 5B depict displacement over time for oxygenated vs. deoxygenated blood samples having varying traits.

FIG. 6 depicts the effect of sodium dithionite on blood flow.

FIG. 7 depicts the delay in rigidification of blood cells in an AS blood sample in comparison to an SS blood sample.

20 FIGS. 8A-8C depict the rise times of oxygenated vs. deoxygenated blood samples for sickle cell (8A), healthy (8B) and sickle trait (8C) blood.

FIGS. 9A-9J depict the run-through times of oxygenated vs. deoxygenated blood samples having varying traits using a horizontal capillary system.

DEFINITIONS

25 The instant invention is most clearly understood with reference to the following definitions.

As used herein, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard
30 deviations of the mean. “About” can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%,

3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

As used in the specification and claims, the terms “comprises,” “comprising,” “containing,” “having,” and the like can have the meaning ascribed to them in U.S. patent law and can mean “includes,” “including,” and the like.

Unless specifically stated or obvious from context, the term “or,” as used herein, is understood to be inclusive.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 (as well as fractions thereof unless the context clearly dictates otherwise).

The following abbreviations are used herein:

AA	phenotypically normal blood
AS	sickle trait blood
PDMS	polydimethylsiloxane
PMMA	poly(methyl methacrylate)
SC	blood heterozygous for hemoglobin S (HbS) and hemoglobin C (HbC)
SS	sickle cell disease blood

DETAILED DESCRIPTION OF THE INVENTION

Aspects of the invention provide methods for screening for sickle-cell disease. Without being bound by theory, it is believed that deoxygenated sickle cells are more rigid than oxygenated sickle cells, which influences the viscosity of deoxygenated sickle blood relative to oxygenated sickle blood. In contrast, there should be little or no difference in the viscosity of between deoxygenated non-sickle blood relative to oxygenated non-sickle blood. Advantageously, aspects of the invention facilitate detection of sickle-cell disease without the use of obstructed capillaries.

Referring now to FIG. 1, one aspect of the invention provides a method 100 of screening for sickle-cell disease.

In step S102, an end of a capillary tube is contacted with blood. The capillary tube can be unobstructed so that pressure does not increase above the blood drawn into the capillary. For example, the capillary can be free from the beads disclosed in International Publication No. WO 2015/054378.

5 In some embodiments, the blood was previously deoxygenated. For example, a reducing agent such as a dithionite, *e.g.*, sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) can be added to the blood prior to contact with the capillary tube. In another example, the blood is placed on an oxygen-permeable substrate or within an oxygen-permeable vessel. Suitable oxygen-permeable materials include soft polymers such as poly(methyl methacrylate) (PMMA), polydimethylsiloxane (PDMS),
10 silicone rubber, polyurethane, polyethylene, amorphous fluoropolymers (available, *e.g.*, under the TEFLON® AF trademark from The Chemours Company of Wilmington, Delaware), and liquid crystal polymers available, *e.g.*, under the VECTRA® trademark from CNA Holdings LLC of Irving, Texas).

In other embodiments, the blood is oxygenated blood that is subsequently deoxygenated
15 within the capillary tube. The capillary tube can include a variety of features to facilitate deoxygenation.

In one embodiment, the capillary tube includes a reducing agent. For example, the reducing agent can be dried to an interior wall of the capillary tube such as in U.S. Patent No. 4,308,029. All or a portion of the capillary tube can be modified to increase the reducing-
20 agent-carrying capacity. For example, all or a portion of the capillary tube can be etched with an acid. In another example, all or a portion of the capillary tube can have a non-cylindrical cross-section (*e.g.*, triangular, square, *n*-gon, and the like) to provide increased surface area for the dried reducing agent to attach.

In another embodiment, the capillary includes at least an oxygen-permeable portion that
25 will draw oxygen from the blood. A comparison can be made between flow of blood that is deoxygenated by the oxygen-permeable portion and a control tube that has the same structure, but is saturated with oxygen prior to introduction of the sample (*e.g.*, by flowing oxygen through the capillary prior to introduction of the sample).

In step S104, a flow rate of the deoxygenated blood within the capillary tube is measured.
30 The flow rate can be measured optically, *e.g.*, by calculating the time required for the blood to travel a distance (*e.g.*, between a first point and a second point) within the capillary tube. The

measurements can be made by eye or can be automated using a camera (e.g., a digital camera) and software including instructions to detect movement of blood within the capillary across a plurality of images or frames within a video.

In steps S106, S108, and S110, the measurement of the flow rate of the deoxygenated blood can be interpreted using one or more approaches.

In step S106, the calculated flow rate of the deoxygenated blood can be compared to a predetermined flow-rate threshold for the capillary tube indicative of sickle-cell disease. For example, clinical data can be used to establish a range of flow rates indicative of sickle-cell disease for a particular capillary tube having a defined material and cross-section.

In step S108, the viscosity of the deoxygenated blood can be calculated. The rise of the blood through the capillary tube can be modeled by deriving an equation from Poiseuille's equation governing the flow of viscous liquids through small tubes. Applicant derived the following equation for the height of the liquid, denoted L , at time t after the blood is introduced at the bottom of the tube:

$$-L - Z \ln \left(1 - \frac{L}{Z} \right) = \frac{t}{\tau}$$

where Z is the Jurin length and τ is the characteristic time. The Jurin length Z is defined by $Z = \frac{2\sigma \cos \theta}{\rho g r}$ in which σ is the surface tension, θ is the contact angle, ρ is the blood density, g is the acceleration of gravity, and r is the radius of the capillary. The characteristic time includes the viscosity as well, and is given by $\tau = \frac{8\eta}{\rho g r^2}$ in which η is the viscosity and the other symbols are as defined above. Therefore the characteristic time is proportional to the viscosity. This viscosity can then be compared to a predetermined viscosity threshold for the capillary tube indicative of sickle-cell disease.

In step S110, the flow rate can be compared to a second flow rate for oxygenated blood from a same subject within a substantially identical capillary. The comparison can be under a race condition in which both two capillaries are contacted blood samples at the same time and a user or a device detects which blood sample reaches a defined point first. In other embodiment, the flow rate is separately measured and then numerically compared to determine whether a difference exists. For example, the two flow rates can be compared to determine if one flow rate is higher than the other, one flow rate can be subtracted from the other, or one flow rate can be divided by the other.

FIGS. 2A-2C depict cross-sections of various embodiments of capillaries.

The capillary tube 202a-c can be a cylinder having a sufficiently small cross-section to induce capillary or wicking flow of a fluid into the capillary tube 202a-c. For example, the cylinder can have a largest cross-sectional dimension of about 5 mm, about 4 mm, about 3 mm, about 2 mm, about 1 mm, and the like. For example, capillary tube 202a-c can have a circular, rectangular, or square cross-section. Capillary tubes fabricated from materials such as borosilicate, clear fused quartz, and synthetic fused silica are commercially available from sources such as VitroCom of Mountain Lake, New Jersey and Drummond Scientific Company of Broomall, Pennsylvania. For example, VitroCom offers capillary tubes having circular cross-sections with internal diameters (IDs) of 0.05 mm, 0.10 mm, 0.15 mm, 0.20 mm, 0.30 mm, 0.40 mm, 0.50 mm, 0.60 mm, 0.70 mm, 0.80 mm, 0.90 mm, 1.00 mm, 1.50 mm, 2.00 mm, and the like and lengths of 100 mm, 300 mm, 600 mm, and the like. Capillary tubes 202a can be selected so that the anticipated blood samples will not wick through to the other end of the capillary tube 202a-c or can be selected so that the exit of the blood sample at an opposite end of the capillary tube 202a-c marks an end point of the measurement.

FIG. 2A depicts a capillary that can be fabricated from a variety of materials such as glass or soft polymers such as PMMA.

FIG. 2B depicts a capillary 202b including a dried reducing agent 204 at a first end of the capillary. The dried reducing agent can optionally extend the entire length of the capillary 202b, although partial coverage may preferred so that movement of the blood sample within the capillary 202b can be more clearly visualized and will reflect blood-capillary interactions instead of blood-reducing-agent interactions.

The capillary 202b of FIG. 2B can be provided in a control version that includes an equivalent (*e.g.*, by mass, volume, molar volume, and the like) amount of a non-reducing salt to facilitate control comparisons of the blood samples that are not deoxygenated.

FIG. 2C depicts a capillary 202c including at least one oxygen-permeable portion 206. The oxygen-permeable portion 206 can be mechanically or chemically bonded to an oxygen-impermeable portion 208 of the capillary 202c.

Embodiments of the invention can be implemented in systems and/or kits.

For example, kits can be provided including capillaries and/or reducing agents (*e.g.*, reducing agents dried within capillaries). The kits can also include instructions for performing the methods described herein.

5 Systems can include devices for holding the capillaries and/or measuring blood flow within the capillary, calculating flow rates within the capillary, comparing blood flow and/or viscosity between samples, and/or conveying a diagnosis.

FIGS. 2D and 2E depict a capillary system of the invention 210, demonstrating the main principle of the invention, wherein deoxygenated sickle cell disease or sickle cell trait blood has a slower flow rate through a capillary than oxygenated sickle cell disease or sickle cell trait
10 blood.

Referring now to FIG. 2D, the system of the invention can include a sample slide 212 adapted and configured for holding one or more blood samples 214, 216.

The substrate 212 can be configured to configured to complement the capillary assembly.

For example, the substrate can include one or more markings corresponding to the spatial
15 arrangement of the capillaries 202. These markings can direct the user to approximate targets for depositing blood samples. Likewise, the substrate 212 can define a plurality of wells having a complementary special relationship to the capillaries. (In some embodiments, the capillary assembly can be configured for compatibility with various commercially available well plates.)

In another example, the substrate 212 can be configured to facilitate deoxygenation of
20 one of the blood samples (*e.g.*, through the use of salts or materials as described herein in the context of capillary design).

The one or more blood samples 214, 216 can include an oxygenated blood sample 214 and/or a deoxygenated blood sample 216.

A capillary assembly 218 including a plurality of capillaries 202a-c can facilitate the
25 handling of the one or more capillaries 202a-c for simultaneous or substantially simultaneous contact with blood samples 214, 216. The apparatus 218 can include a handle 220 or other structure to facilitate handling of the capillary assembly 218 (*e.g.*, by a technician's hands, with tools such as forceps, by a robotic device, and the like).

Referring now to FIGS. 2D and 2E, system 210 can facilitate rapid qualitative
30 comparison between oxygenated blood samples and deoxygenated blood samples. The system 210 can enable two identical capillaries 202a to be individually dipped into an

oxygenated blood sample 214 and a deoxygenated blood sample 216 on a sample slide 212 simultaneously, such that the sample slide 212 is substantially horizontal and the capillaries 202a are dipped into the blood samples such that they are in a substantially vertical orientation, perpendicular to the sample slide 212. A comparison can then be made between the flow rate of the oxygenated blood sample 214 and the deoxygenated blood sample 216 by comparing the oxygenated blood level 222 and the deoxygenated blood level 224 through the respective capillaries 202a at a given time point.

Referring now to FIG. 2F, other embodiments of the invention provide assemblies 226 including one or more sample wells 228 adapted and configured to hold a blood sample and one or more capillaries 202a-c. The capillaries 202 and sample wells 226 can lie horizontally. The assembly 226 can be a plastic mold defining and/or housing the sample wells 228 and the capillaries 202. The assembly 226 can include a capillary 202c including at least one oxygen-permeable portion 206, which allows for *in situ* deoxygenation of a blood sample. In other embodiments, an oxygen reducing agent can be present in one or more sample wells 226 or deoxygenation can occur before introduction of a blood sample into the well(s) 226.

WORKING EXAMPLES

Procedure

Typical Single Capillary Experiments

0.25uL glass DRUMMOND® capillaries were marked with a permanent marker at 1 cm from the top end of the capillary. This was the “Start” mark and the “Finish” was at the top of the capillary. Two vials of blood were prepared: one vial contained oxygenated blood, while the other contained deoxygenated blood with an appropriate amount of sodium-dithionite-water solution (usually 1-2 × the amount calculated to strip all the oxygen so as to safeguard against partially compromised dithionite). The spectrum of each vial sample was obtained and recorded. The capillary was fitted into an eye-drop holder so as to better handle it. 2-5 μL of blood from the oxygenated blood vial was pipetted onto a glass slide coverslip. The capillary was then held at a 50-60° angle relative to the surface, and dipped into the blood droplet. A stopwatch was used to measure the time required for the blood to travel the distance from the first mark to the top. This time was recorded. The trial was repeated four times with a fresh capillary and droplet

of blood for a total of five trials. The process was repeated for blood from the deoxygenated vial, measuring the capillaries' spectra after each trial to verify deoxygenation.

Parallel Capillary Experiment

Two capillaries were prepared as above and placed into a pair of eye-droppers that were
5 attached together. The marks were lined up relative to one another. A 5 μ L drop of oxygenated blood was placed onto a glass slide coverslip, and, after changing the glass pipette, a 5 μ L drop of deoxygenated blood was placed next to the oxygenated drop such that one capillary contacted the oxygenated blood sample and the other capillary contacted the deoxygenated blood sample. The capillaries were then dipped simultaneously into the drops. Visual inspection determined
10 which capillary ran faster. With a stopwatch, the deoxygenated capillary was timed between marks. Spectra of each capillary was then measured and recorded.

Capillary-Based Deoxygenation

A 1 M sodium dithionite solution was prepared, pulled into a capillary and then expelled, and the tube dried by passing nitrogen gas through the capillary tube. Once dry, the tube was
15 used to aspirate a few μ L of blood, and expel it back onto a slide. In doing so, the blood deoxygenates in the capillary (as verified by direct measurement). The drops of blood on a slide can be then used like the blood in the vial, above.

Results

Referring to FIGS. 4A-4E, the method differentiates strongly between oxygenated and
20 deoxygenated samples in SS and sickle variants. Tested were SS (pure sickle blood), AS (sickle trait), SC (a patient heterozygous for hemoglobin S (HbS) and hemoglobin C (HbC)), and S β^0 thalassemia. Also shown are normal (AA) blood, and sickle blood from a patient that was exchange-transfused, which could have 30% or more normal blood from the exchange. In AA and SS blood that was exchange-transfused (marked T), there was statistically no difference
25 between oxygenated and deoxygenated samples. Error bars reported here are one standard deviation using the Student-T distribution.

Capillary Displacement Curves

Referring to FIGS. 5A and 5B, Applicant used video capture to examine in detail the dynamics of sample mixtures of SS and AA blood rising up a 0.25 μ L capillary. The goal of this
30 mixing experiment was to study the sensitivity of the method and its potential use as a severity diagnostic. Because the AA and SS blood had different hematocrits, Applicant created mixtures

that increased the overall hematocrit of the mixture, but decreased the percentage of sickling cells as the mixtures progress.

Sodium dithionite causes a change in the behavior of the cells. Whereas one would expect exact parity for 0% SS between oxygenated and deoxygenated samples, the deoxygenated samples proceed faster. This is not because the red blood cells are deoxygenated, but because of the dithionite, as shown below in FIG. 6.

Blood exposed to oxygen or CO behaves identically as shown above in red and blue legends. Dithionite, while it reduces oxygen, does not affect CO. The green curves are replicates of blood with CO and dithionite, which runs faster than the CO sample without dithionite.

Differentiation of SS and AS Blood

The kinetics of the polymerization of hemoglobin generate a time delay between deoxygenation and the rigidification/sickling of the cell for SS and AS blood. Because this delay time for AS is much greater than that of SS, a second measurement allows a significant portion of AS cells to sickle and rigidify, increasing its viscosity. This feature allows for the distinction between an AS sample and an AA sample. The time delay can be seen in the results reported in FIG. 7, wherein the number of sickled cells is measured as a function of scattering intensity.

Referring to FIGS. 8A-8C, this principle allows for distinction between AA, AS and SS samples by comparing the flow rate of an oxygenated sample with two deoxygenated samples taken at different time points. As can be seen in FIG. 8A, the change in flow rate of oxygenated and deoxygenated SS blood is apparent at both early and later time points. FIG. 8B demonstrates that there is no change in the flow rate of oxygenated and deoxygenated AA blood, regardless of the time the deoxygenated blood was tested. FIG. 8C, however, shows that while there is no significant difference in the flow rate of oxygenated and deoxygenated AS blood for early deoxygenation measurements, similar to AA blood, later deoxygenation measurements, after the AS blood has been given time to undergo rigidification, exhibits a much slower flow rate.

Results

Referring to FIGS. 9A-9J, the horizontal capillary device also differentiates strongly between oxygenated and deoxygenated samples in SS and sickle variants. Shown are results from normal (AA) blood (9A, 9B), AS (sickle trait) (9C, 9D), SC (a patient heterozygous for HbS and HbC) (9E, 9F), SS (pure sickle blood) (9G, 9H), SS(HU) (pure sickle blood from a

patient treated with hydroxyurea) (9I), and SS(transfused) (pure sickle blood which has been exchange transfused) (9J). Blood was deoxygenated using dithionite and was added to the horizontal capillary device quickly in all tests barring the experiment reported in FIG. 9G. AA blood showed no statistical difference between oxygenated and deoxygenated samples while AS, SC and SS blood showed varying degrees of difference between oxygenated and deoxygenated samples. Additionally, it was found that SS blood which had been deoxygenated using dithionite and used quickly exhibited similar flow rates with SS blood which had been allowed to stir with dithionite for an extended period of time (FIGS. 9G, 9H).

EQUIVALENTS

Although preferred embodiments of the invention have been described using specific terms, such description is for illustrative purposes only, and it is to be understood that changes and variations may be made without departing from the spirit or scope of the following claims.

INCORPORATION BY REFERENCE

The entire contents of all patents, published patent applications, and other references cited herein are hereby expressly incorporated herein in their entireties by reference.

CLAIMS

1. A method of screening for sickle-cell disease, the method comprising:
contacting an end of a capillary tube with deoxygenated blood;
observing a flow rate of the deoxygenated blood within the capillary tube; and
performing one or more steps selected from the group consisting of:
 comparing the flow rate to a predetermined flow-rate threshold for the capillary tube indicative of sickle-cell disease;
 calculating viscosity based on the flow rate and comparing the viscosity to a predetermined viscosity threshold for the capillary tube indicative of sickle-cell disease;
and
 comparing the flow rate to a second flow rate for oxygenated blood from a same subject within a substantially identical capillary tube.
2. The method of claim 1, wherein the capillary tube is unobstructed.
3. The method of claim 1, wherein the capillary tube and the substantially identical capillary tube are unobstructed.
4. The method of claim 1, wherein the blood has been deoxygenated through the use of a chemical reductant.
5. The method of claim 1, wherein the flow rate of deoxygenated sickle-cell blood is slower than that of oxygenated sickle-cell blood.
6. The method of claim 1, wherein deoxygenated sickle-cell blood is more viscous than oxygenated sickle-cell blood.
7. The method of claim 1, wherein the flow rate of deoxygenated healthy blood is equal to that of oxygenated healthy blood.
8. The method of claim 1, wherein deoxygenated healthy blood has the same viscosity as oxygenated healthy blood.

9. The method of claim 1, wherein the flow rate of deoxygenated sickle trait blood resembles the flow rate of deoxygenated healthy blood immediately after deoxygenation and more closely resembles the flow rate of deoxygenated sickle-cell blood as time after deoxygenation increases.
10. The method of claim 1, The method of claim 1, wherein the capillary tube has a volume of about 0.25 μL .
11. A method of screening for sickle-cell disease, the method comprising:
contacting an end of a capillary tube with oxygenated blood, wherein the capillary tube is adapted and configured to deoxygenate the oxygenated blood to produce deoxygenated blood;
observing a flow rate of the deoxygenated blood within the capillary tube; and
performing one or more steps selected from the group consisting of:
 comparing the flow rate to a predetermined flow-rate threshold for the capillary tube indicative of sickle-cell disease;
 calculating viscosity based on the flow rate and comparing the viscosity to a predetermined viscosity threshold for the capillary tube indicative of sickle-cell disease;
and
 comparing the flow rate to a second flow rate for oxygenated blood from a same subject within a substantially identical capillary.
12. The method of claim 11, wherein the capillary tube is unobstructed.
13. The method of claim 11, wherein the capillary tube and the substantially identical capillary tube are unobstructed.
14. The method of claim 11, wherein the flow rate of deoxygenated sickle-cell blood is slower than that of oxygenated sickle-cell blood.
15. The method of claim 11, wherein deoxygenated sickle-cell blood is more viscous than oxygenated sickle-cell blood.

16. The method of claim 11, wherein the flow rate of deoxygenated healthy blood is equal to that of oxygenated healthy blood.
17. The method of claim 11, wherein deoxygenated healthy blood has the same viscosity as oxygenated healthy blood.
18. The method of claim 11, wherein the flow rate of deoxygenated sickle trait blood resembles the flow rate of deoxygenated healthy blood immediately after deoxygenation and more closely resembles the flow rate of deoxygenated sickle-cell blood as time after deoxygenation increases.
19. The method of claim 11, wherein the capillary tube has a volume of about 0.25 μL .
20. The method of claim 11, wherein the capillary tube is glass capillary tube.
21. A system for rapidly screening for sickle-cell disease, the system comprising:
a capillary assembly comprising two or more capillary tubes having identical composition and dimensions and each having two open ends; and
a connecting body coupled to the two or more capillary tubes, the connecting body holding the two or more capillary tubes parallel to each other in an array wherein each tube is a defined distance from each neighboring tube.
22. The system of claim 21, further comprising:
a handle coupled to the connecting body.
23. The system of claim 21, further comprising:
a sample container adapted and configured to hold two or more liquid samples in designated locations such that the designated locations are oriented in an array complementarily aligned with the array of the two or more capillary tubes, such that the two or more capillaries can simultaneously contact the two or more liquid samples.
24. The system of claim 23, wherein the sample container is a flat surface.

25. The system of claim 24, wherein the designated locations are markings on the flat surface.
26. The system of claim 24, wherein the sample container is a piece of glass.
27. The system of claim 23, wherein the designated locations are wells adapted and configured to hold the two or more liquid samples.
28. The system of claim 23, wherein the sample container comprises a strong reducing agent capable of deoxygenating one or more of the two or more liquid samples.
29. The system of claim 23, wherein the sample container comprises one or more oxygen permeable materials.
30. The system of claim 29, wherein the sample container comprises one or more materials selected from the group consisting of polydimethylsiloxane, poly(methyl methacrylate), silicone rubber, polyurethane, polyethylene, amorphous fluoropolymers, and liquid crystal polymers.
31. The system of claim 23, further comprising one or more pipettes for transferring the two or more liquid samples to the sample container.
32. The system of claim 21, wherein the one or more pipettes comprise one or more oxygen permeable materials.
33. The system of claim 32, wherein the one or more pipettes comprises one or more materials selected from the group consisting of polydimethylsiloxane, poly(methyl methacrylate), silicone rubber, polyurethane, polyethylene, amorphous fluoropolymers, and liquid crystal polymers.
34. The system of claim 21, wherein the one or more of the pipettes comprises a strong reducing agent capable of deoxygenating one or more of the two or more liquid samples.

35. The system of any one of claims 21-34, further comprising:
a robotic arm.
36. The system of any one of claims 21-34, wherein the two or more capillary tubes are unobstructed.

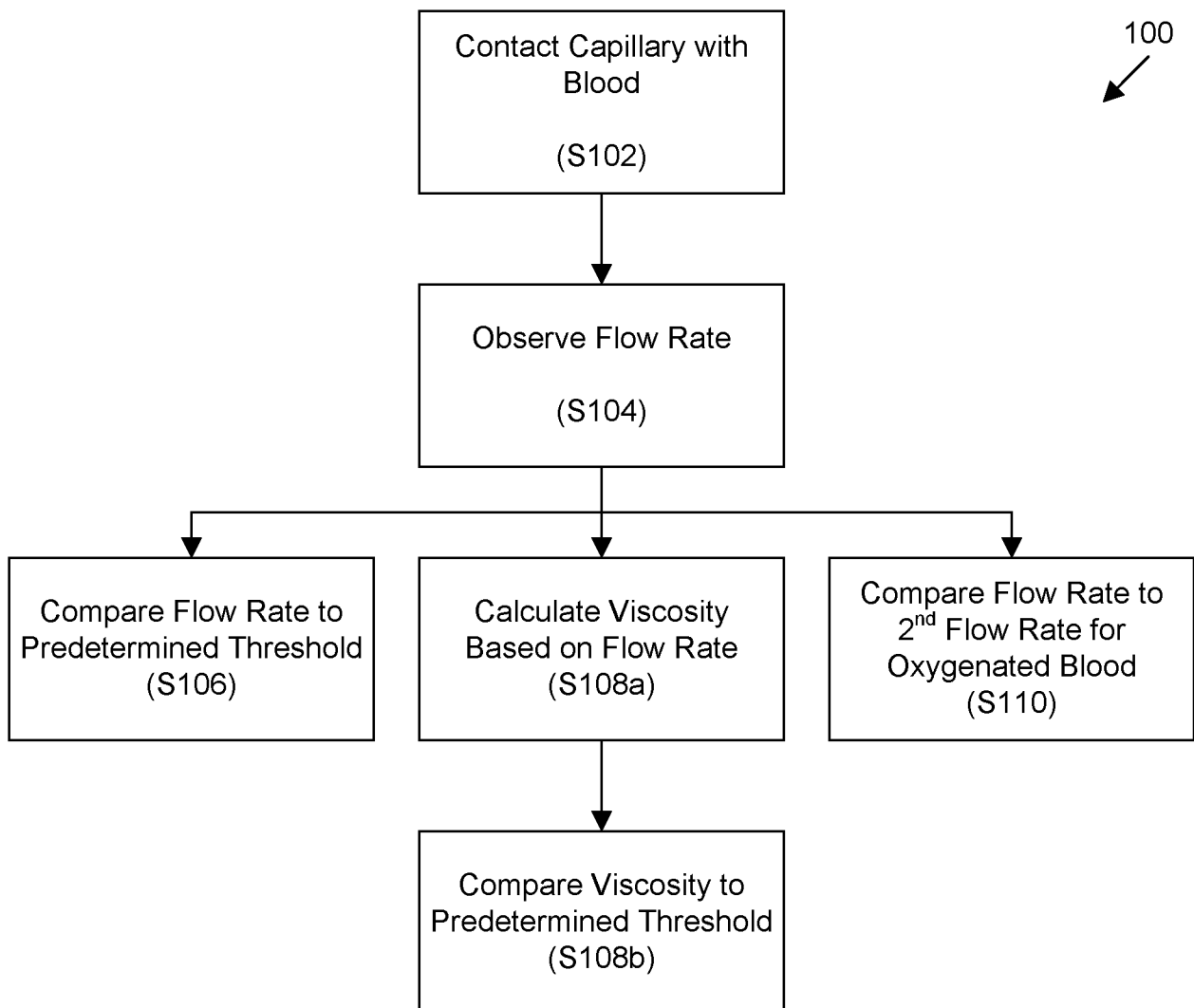


FIG. 1

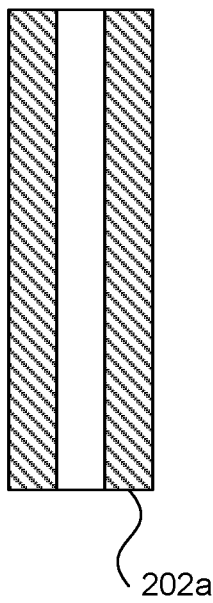


FIG. 2A

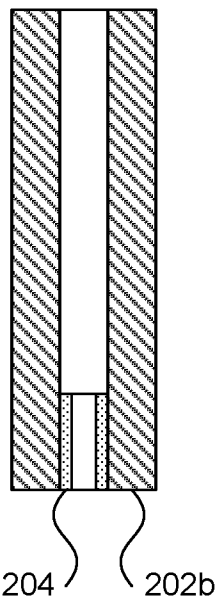


FIG. 2B

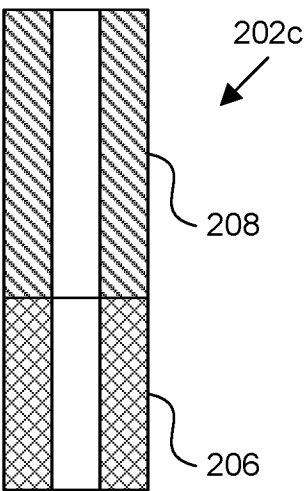


FIG. 2C

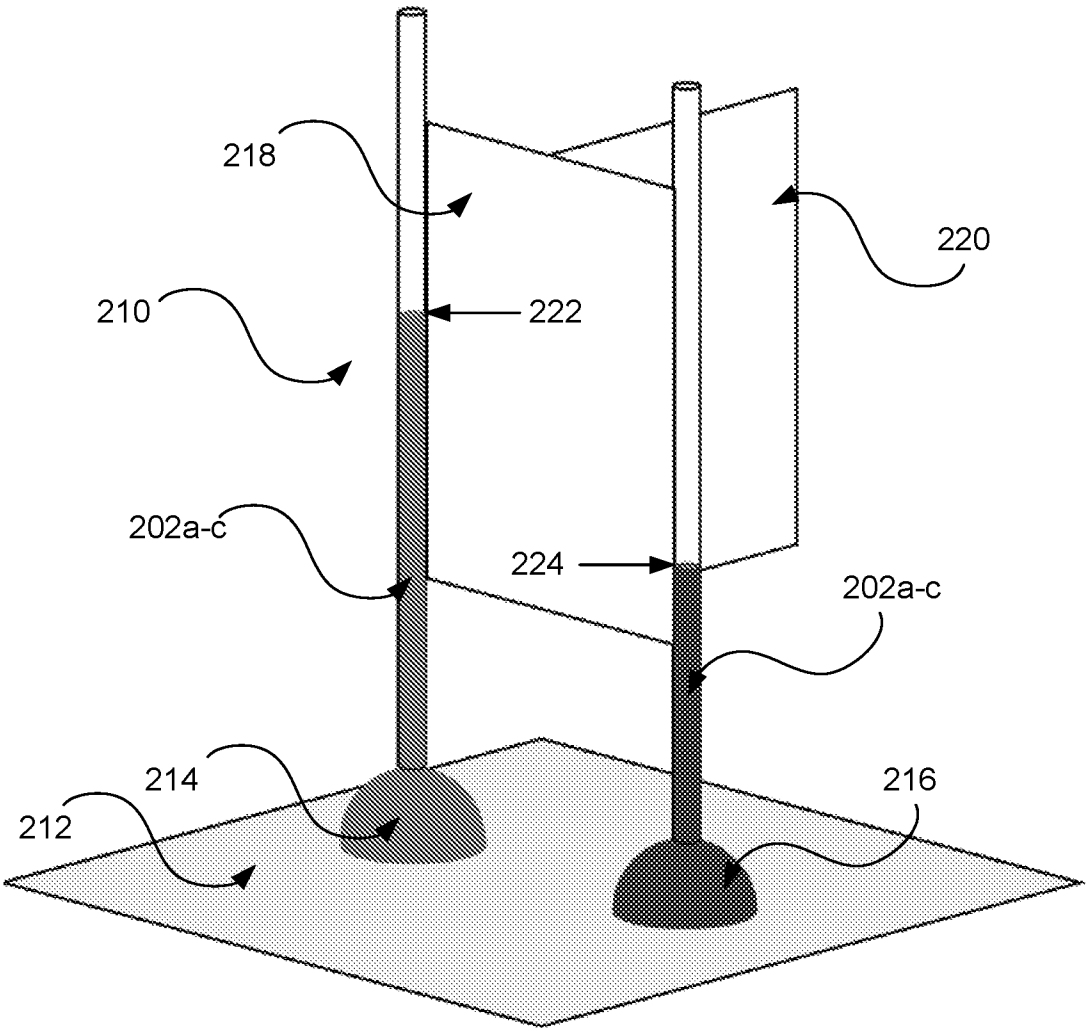


FIG. 2D

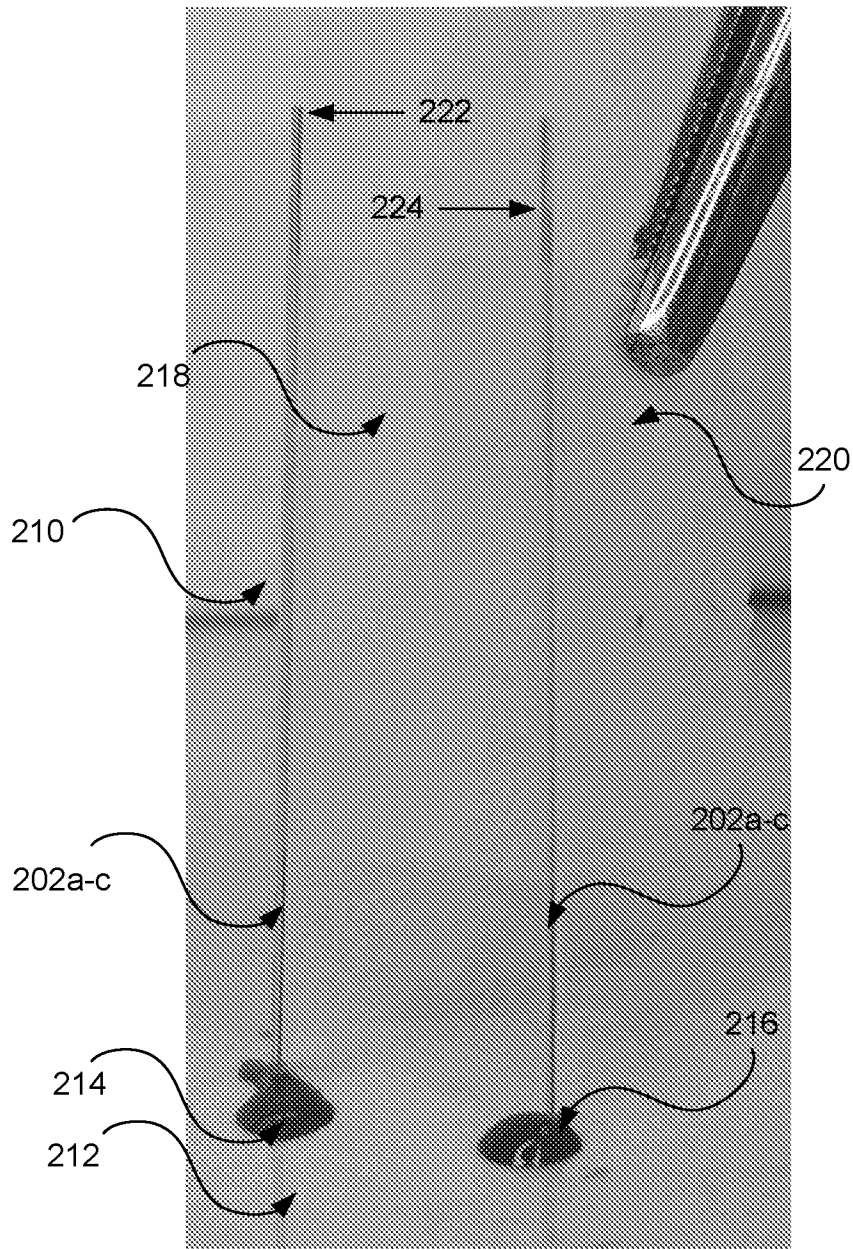


FIG. 2E

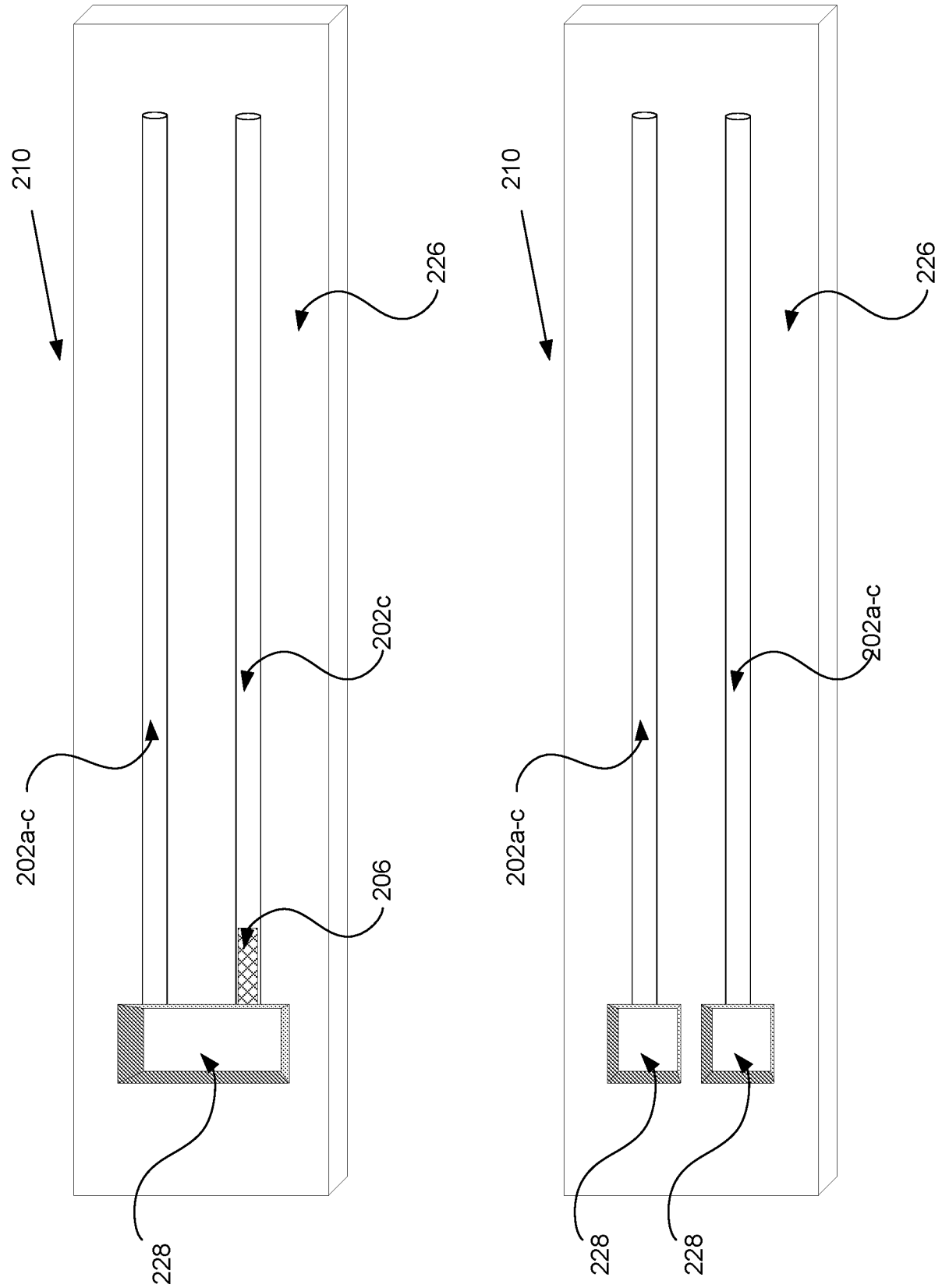


FIG. 2F

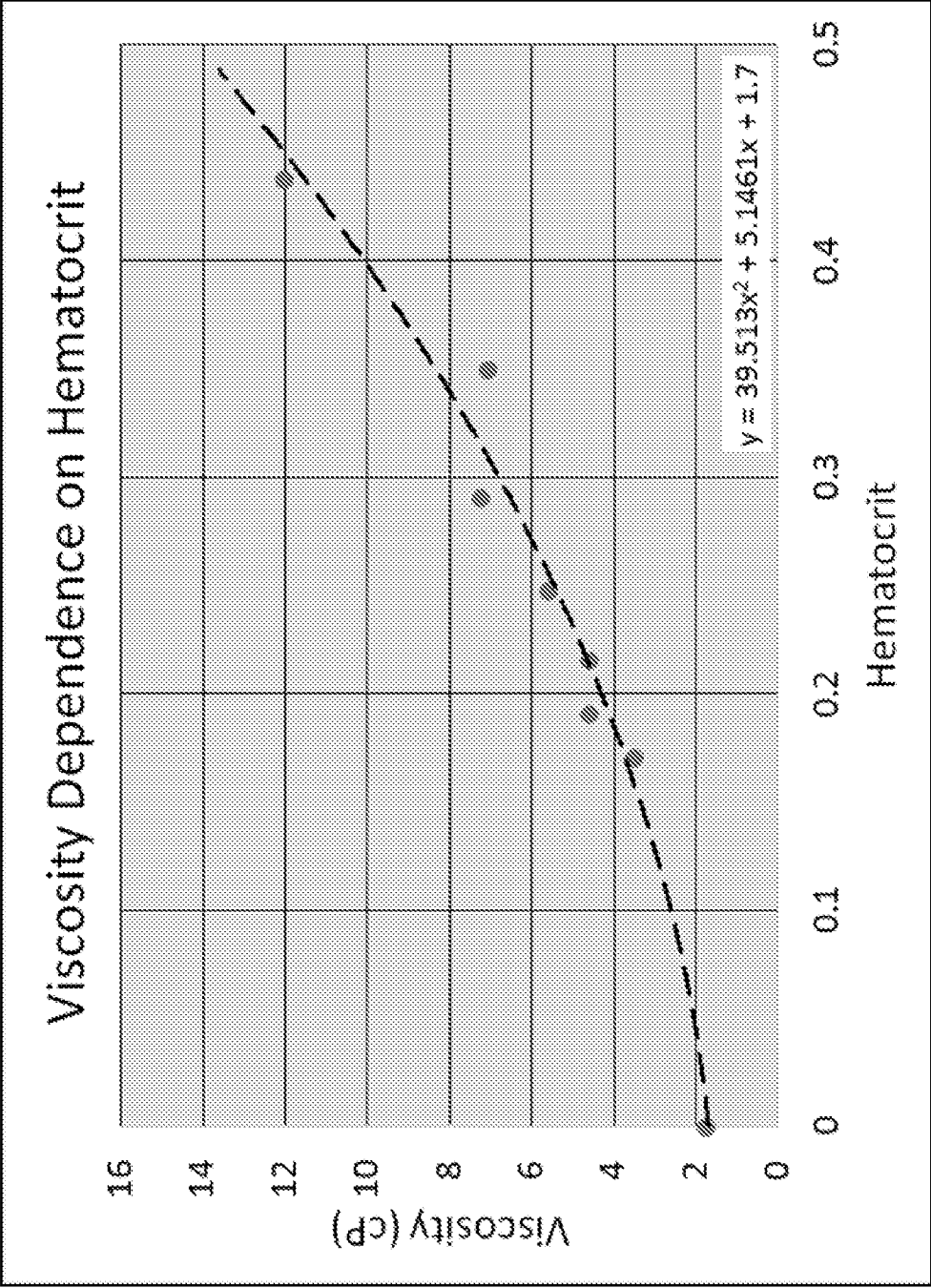


FIG. 3A

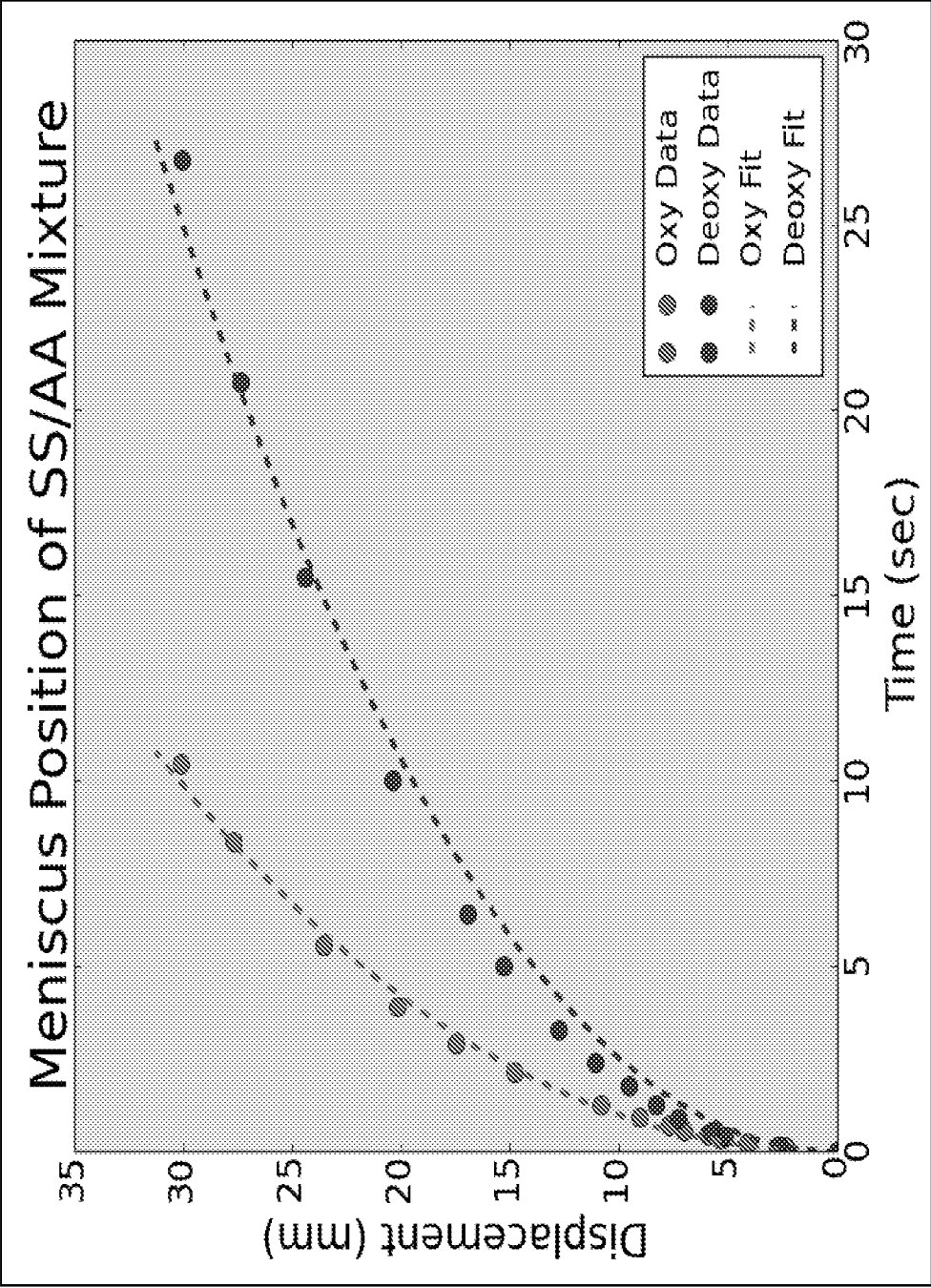


FIG. 3B

Summary of Deoxy-In-a-Vial Capillary Test

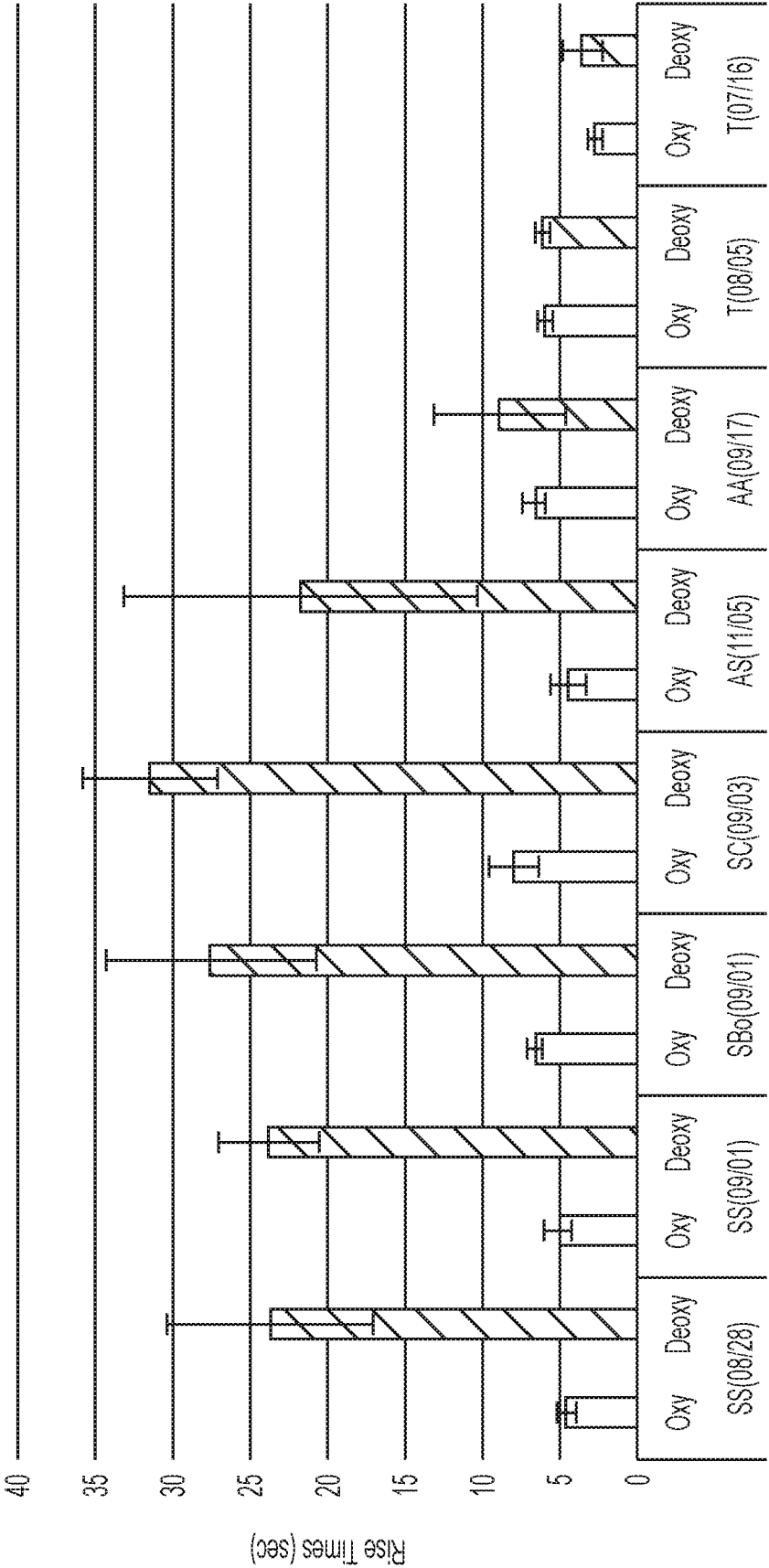


FIG. 4A

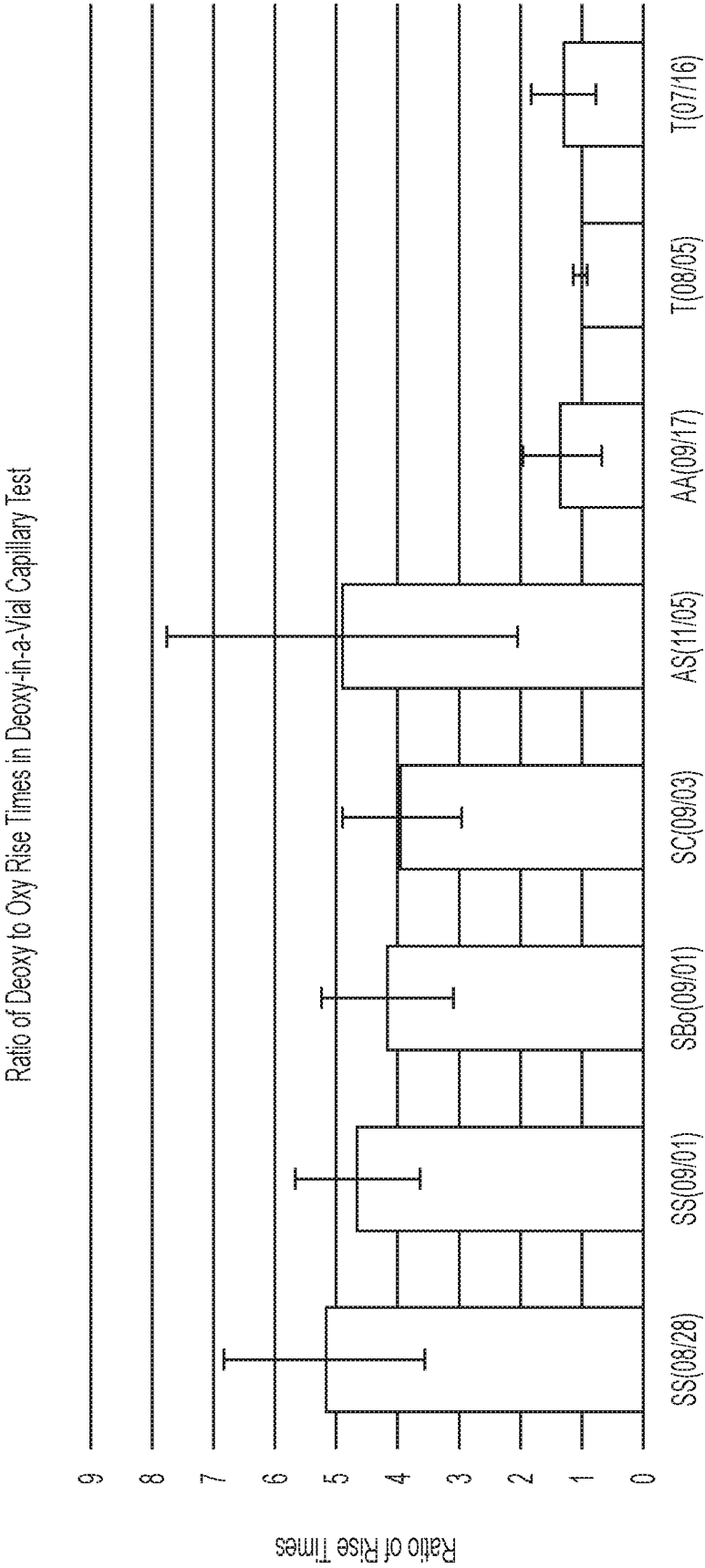


FIG. 4B

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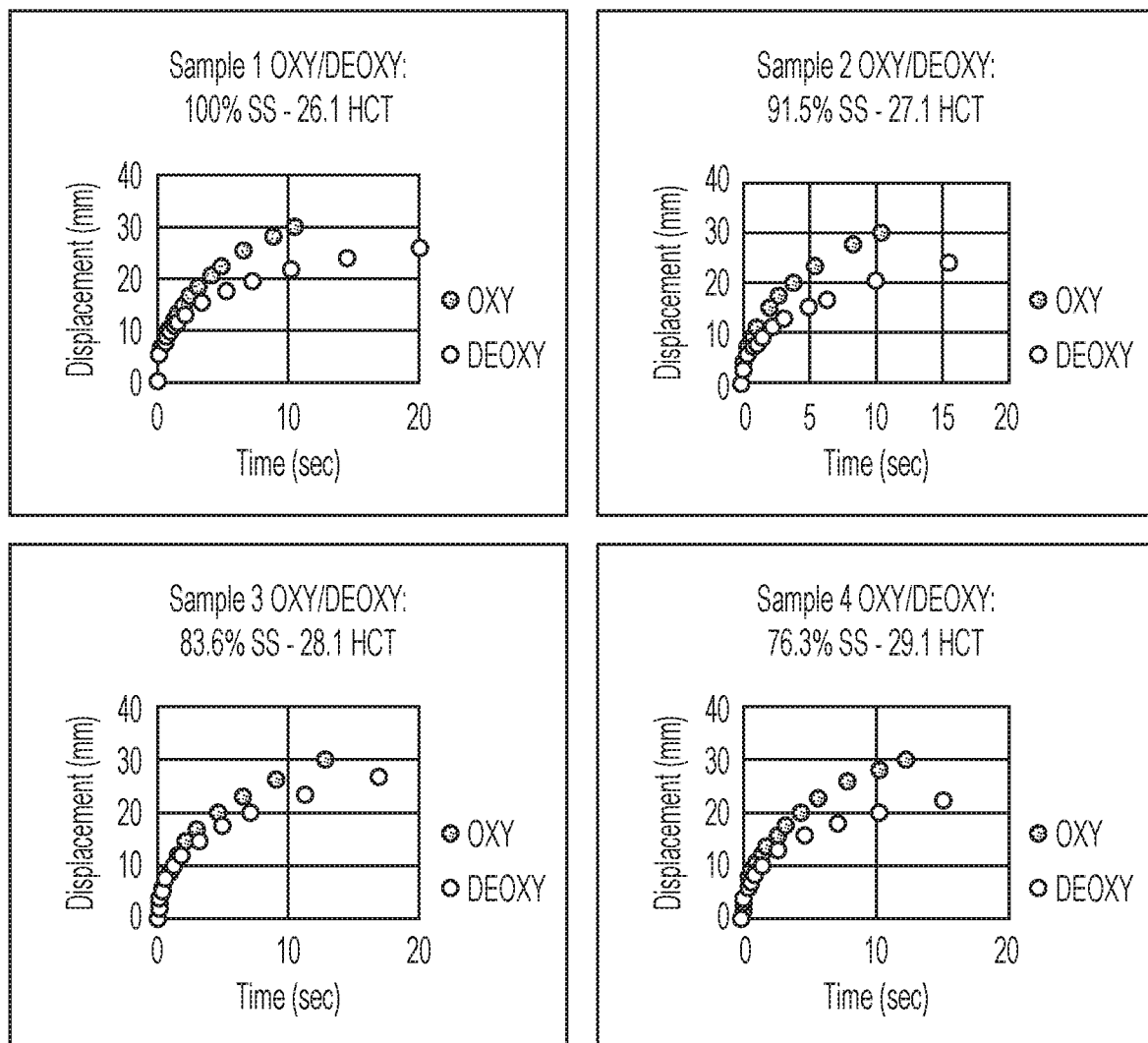


FIG. 5A

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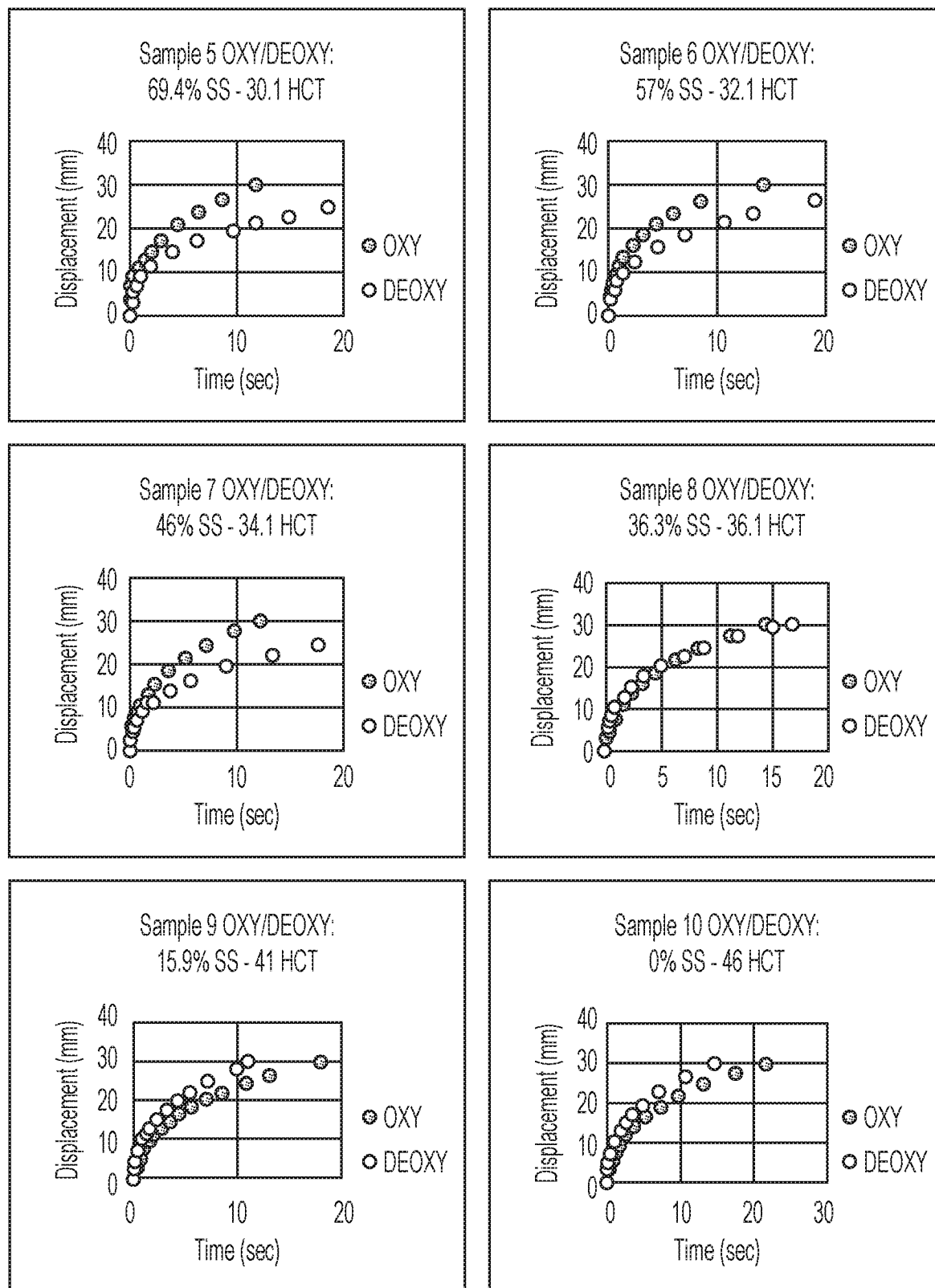


FIG. 5B

12/26

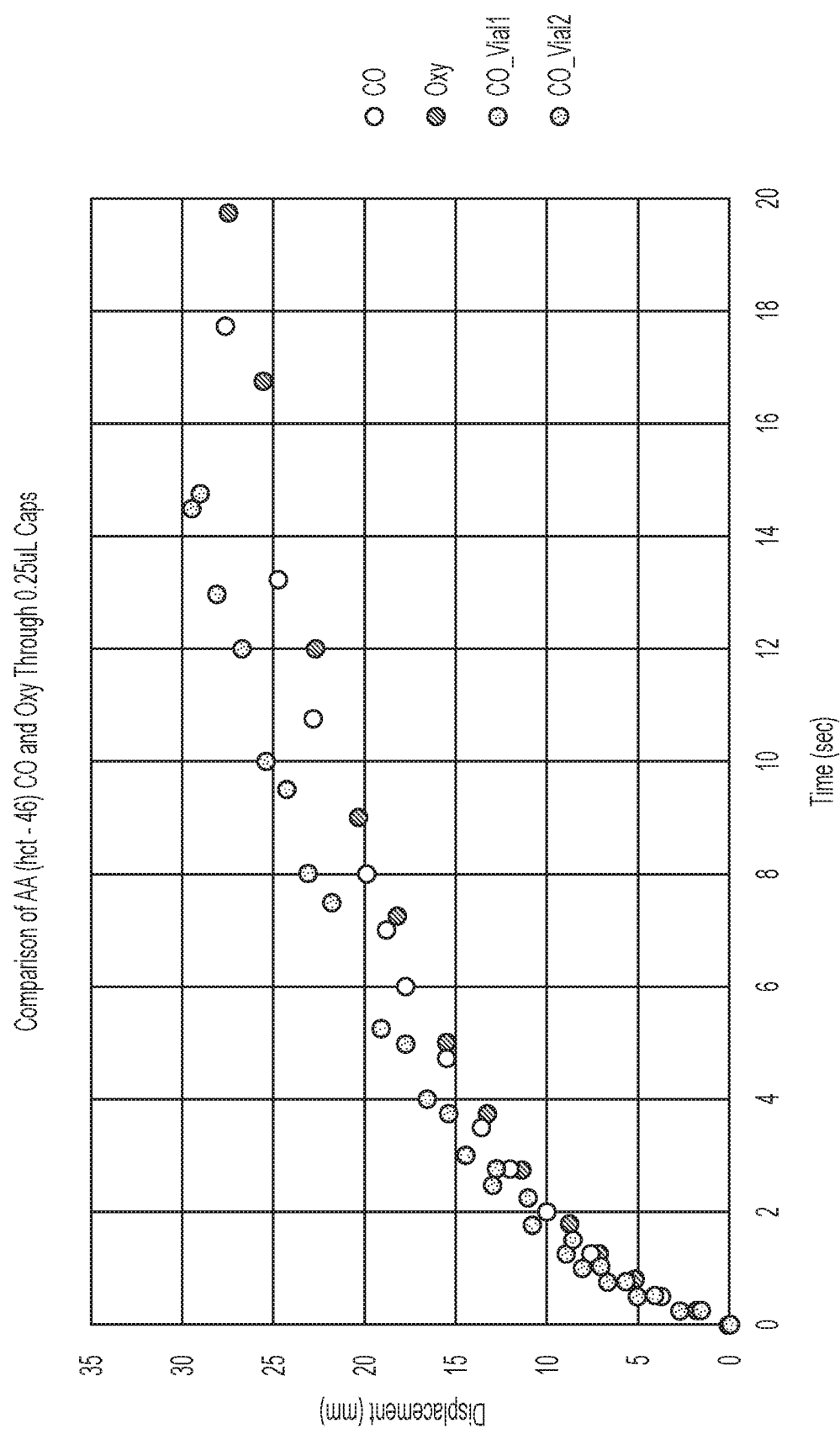


FIG. 6

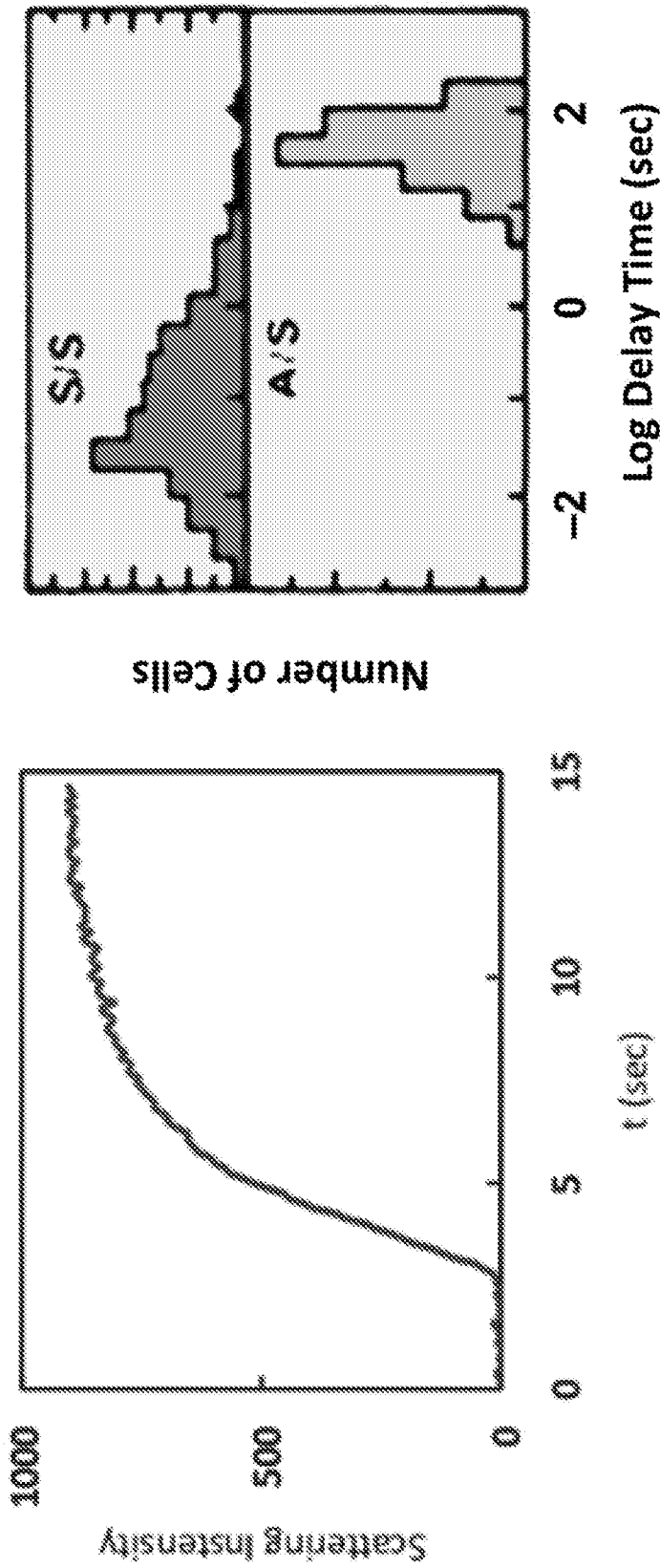


FIG. 7

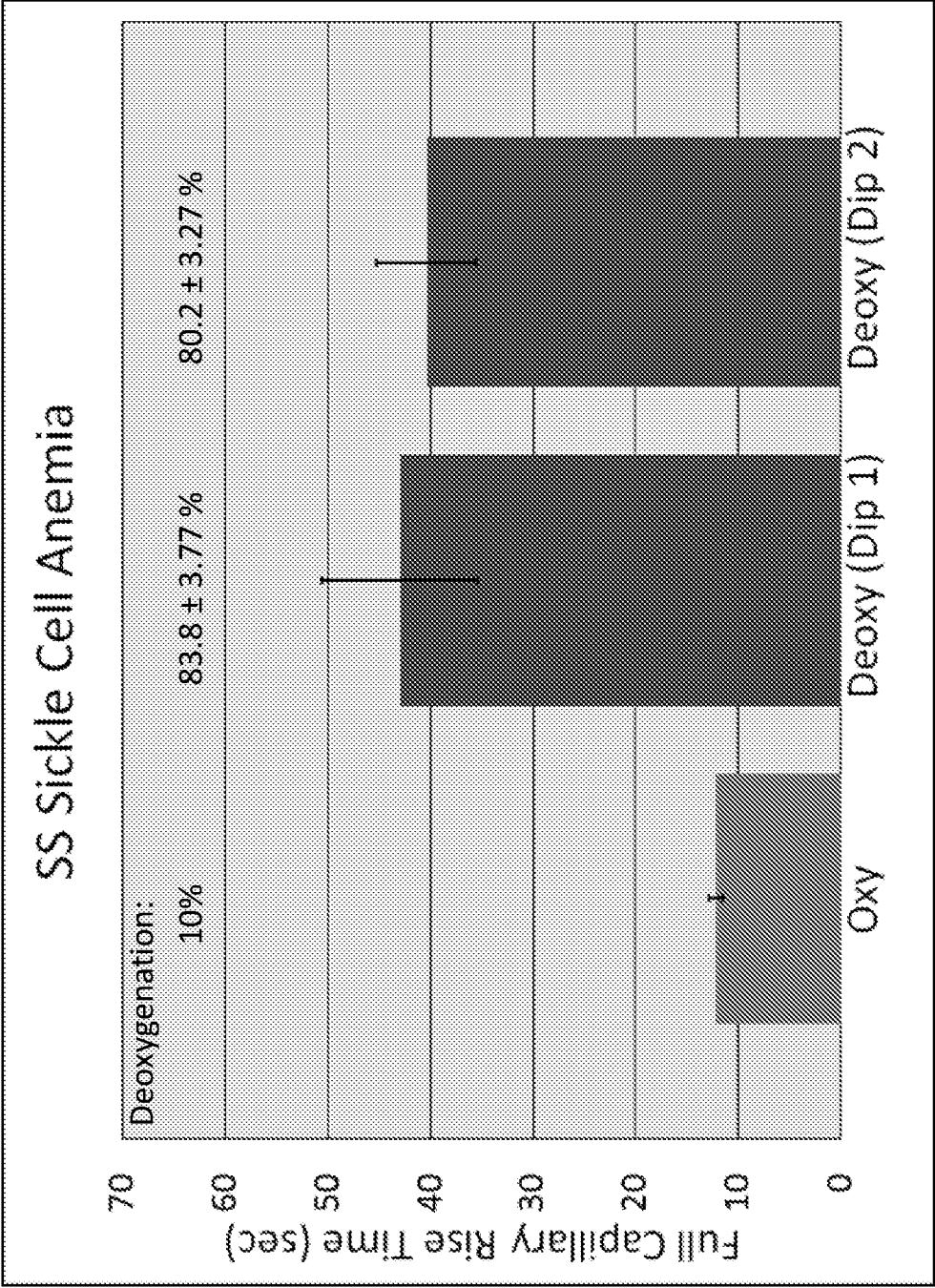


FIG. 8A

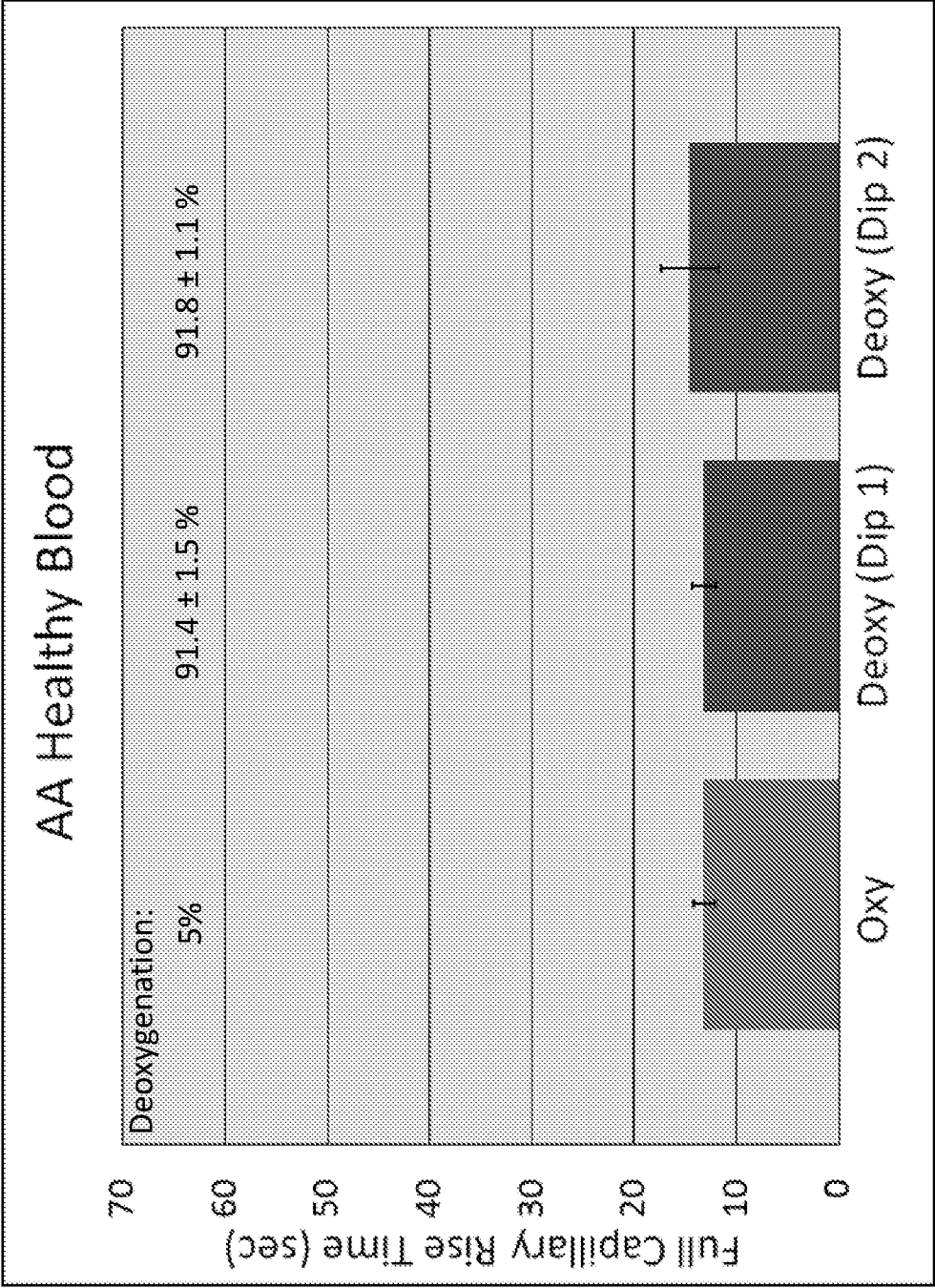


FIG. 8B

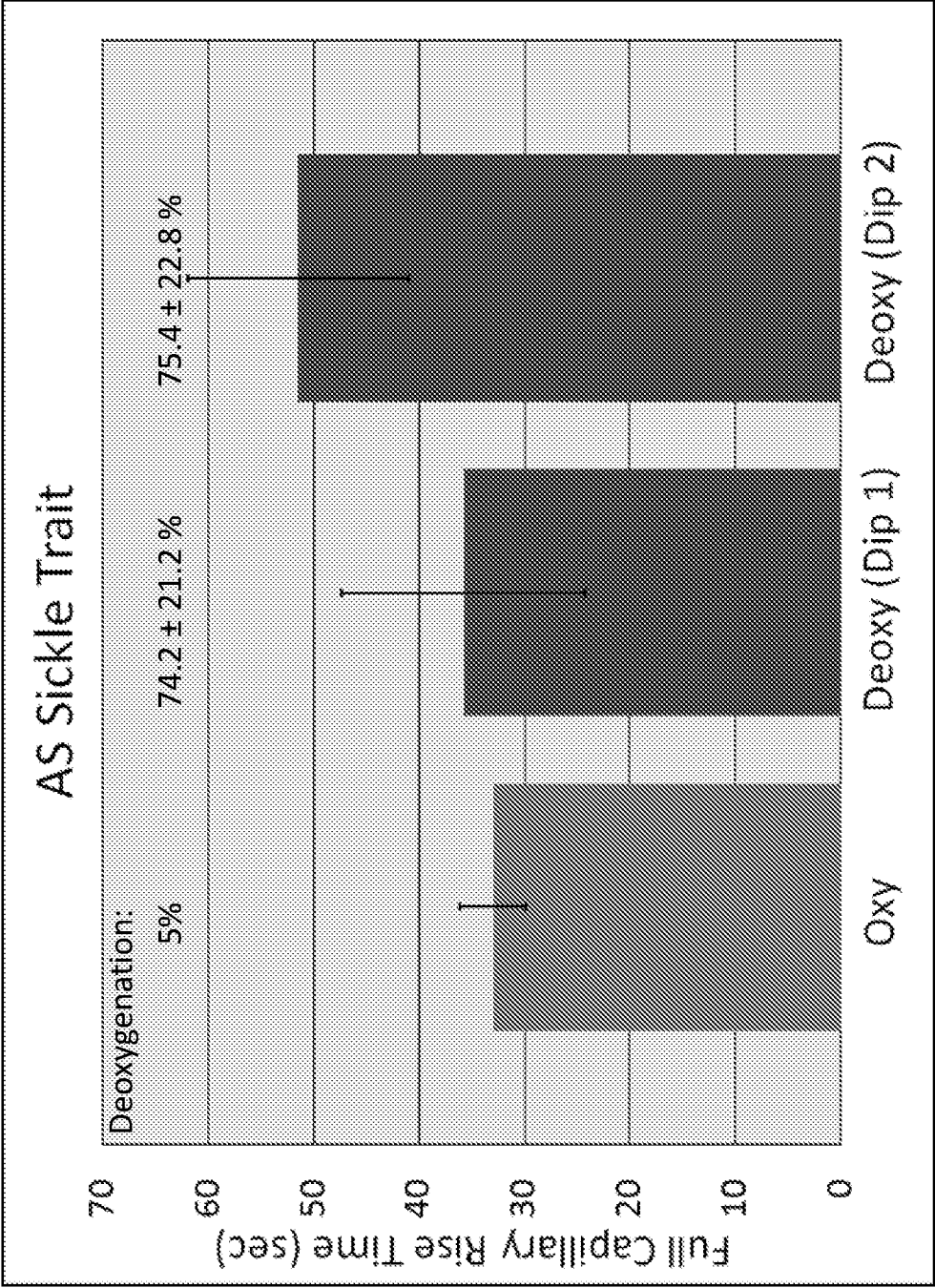


FIG. 8C

AA Blood Through 0.25 μ L Horizontal Capillaries - Deoxy-in-a-Vial Trials

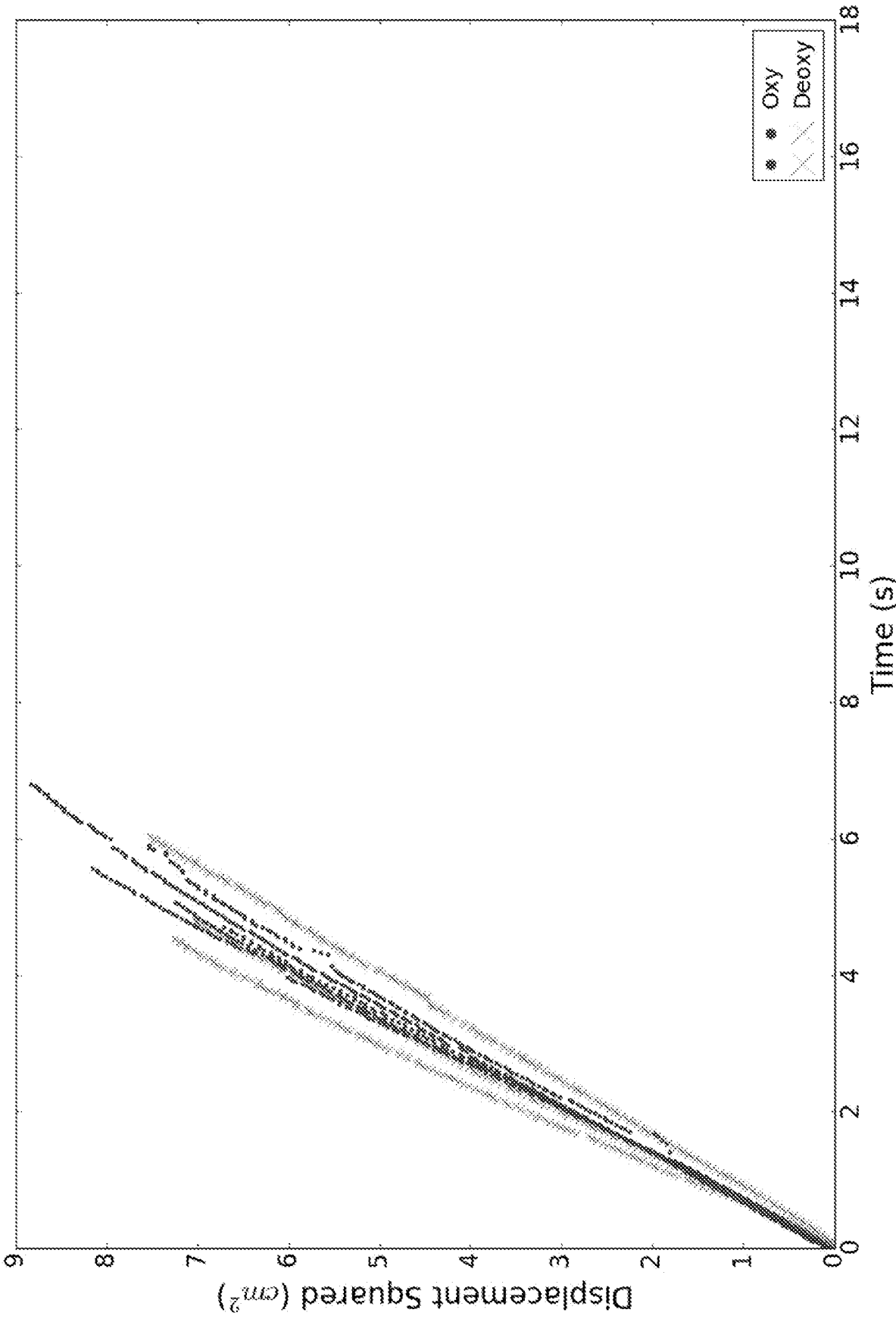


FIG. 9A

AA Blood Through 0.25 μ L Horizontal Capillaries - Quick-Deoxy

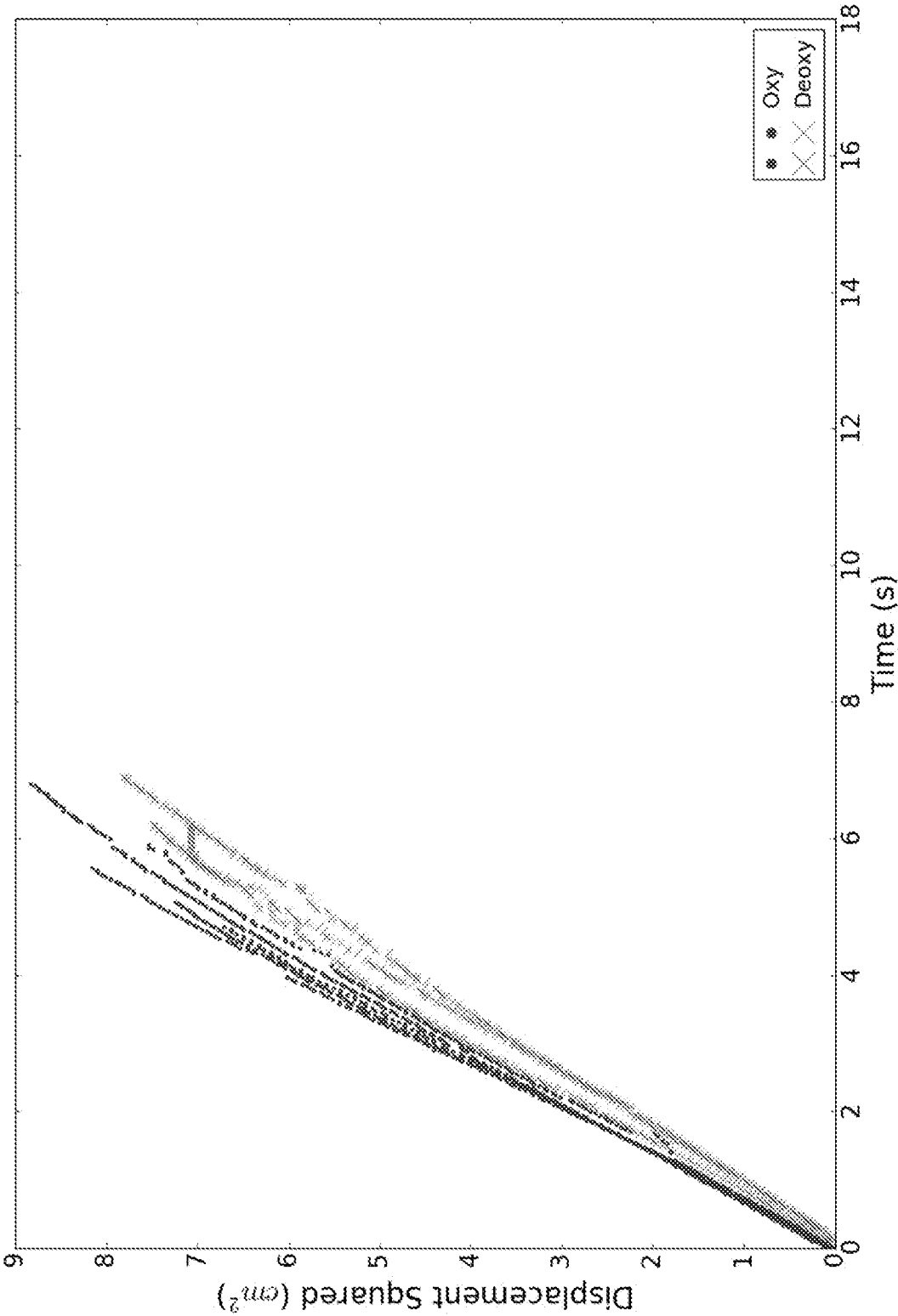


FIG. 9B

AS Blood Through 0.25 μ L Horizontal Capillaries - Quick Deoxy Trials

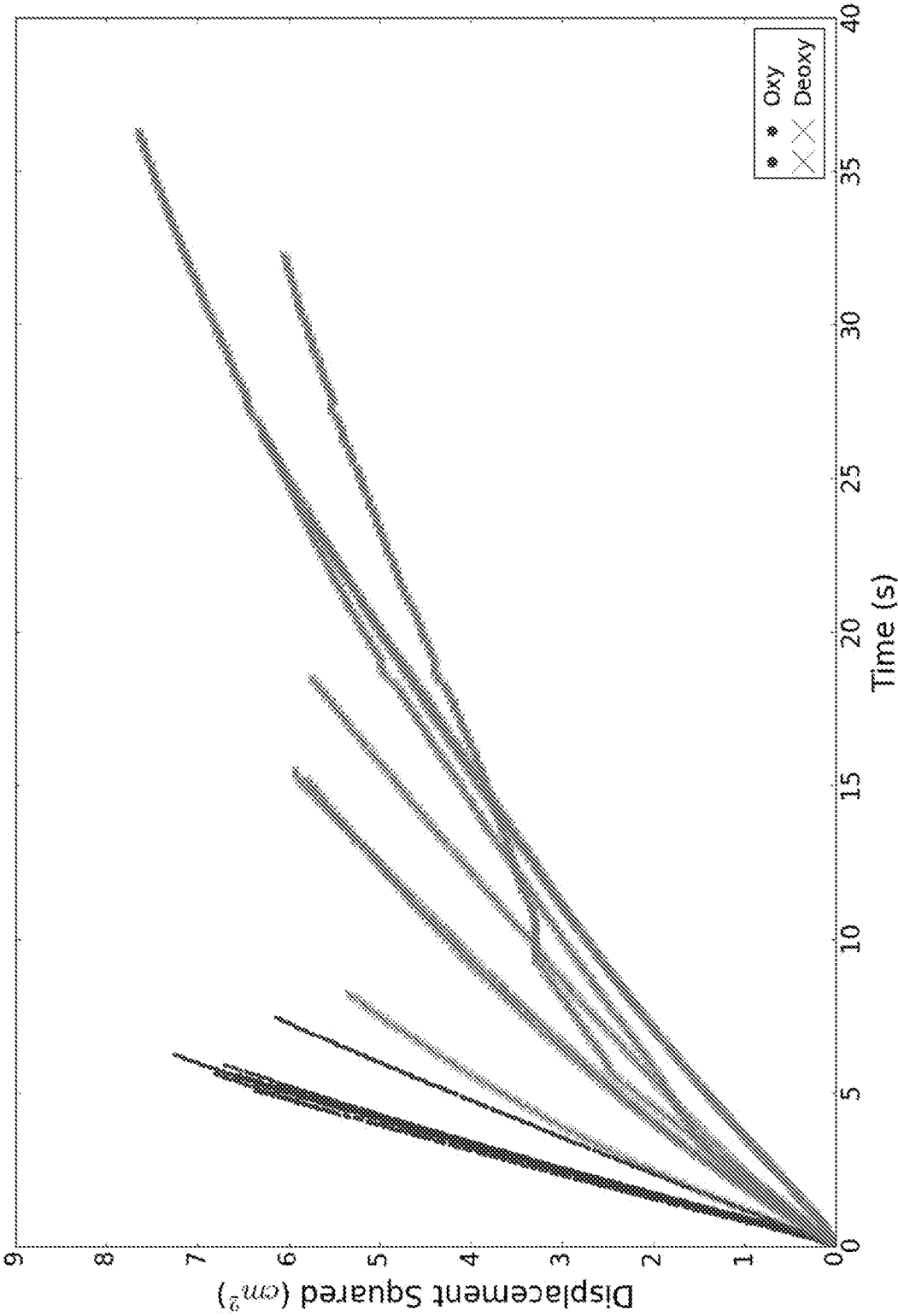


FIG. 9C

AS Blood Through 0.25 μ L Horizontal Capillaries - Quick Deoxy Trials

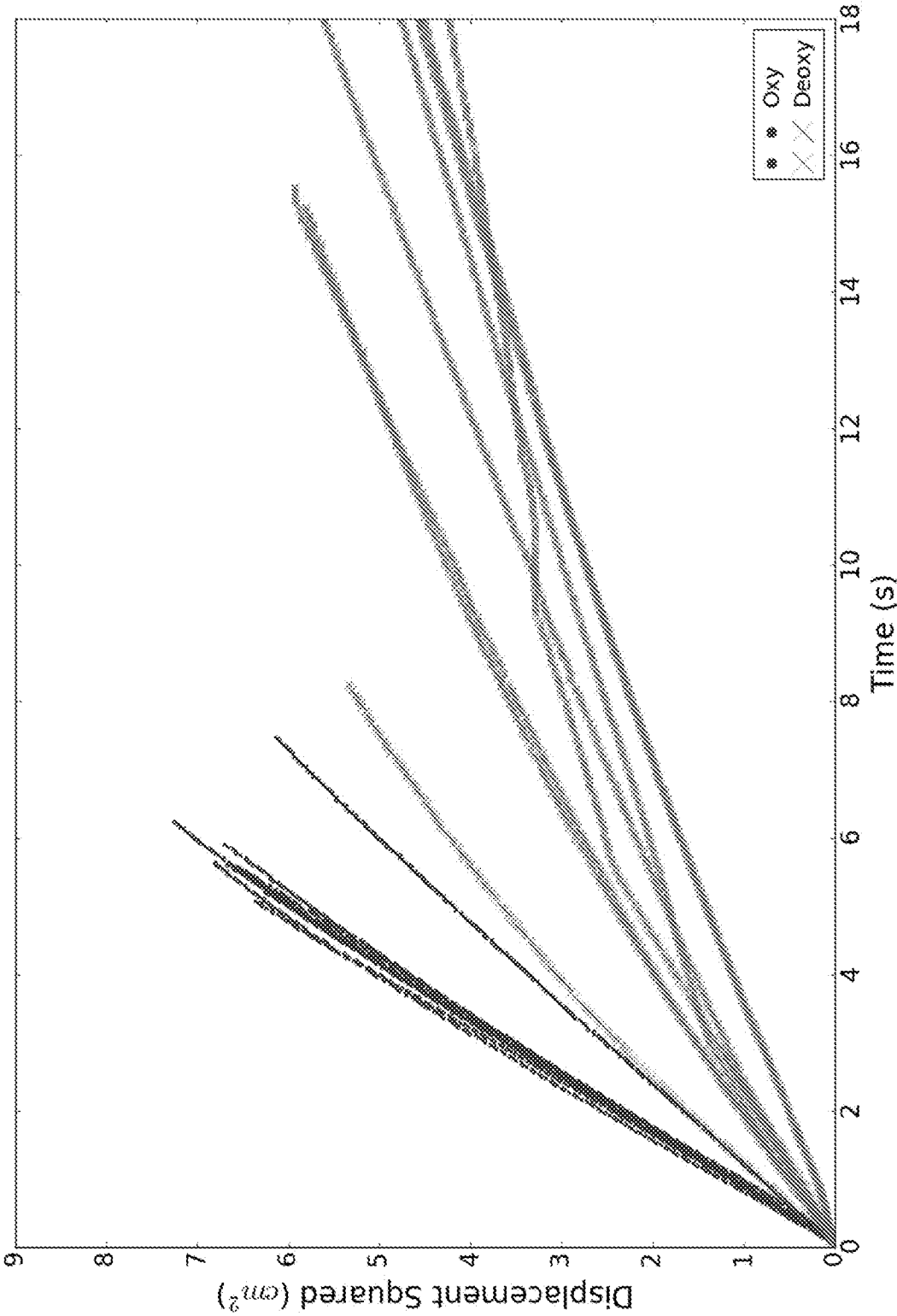


FIG. 9D

SC Blood Through 0.25 μ L Horizontal Capillaries - Quick Deoxy Trials

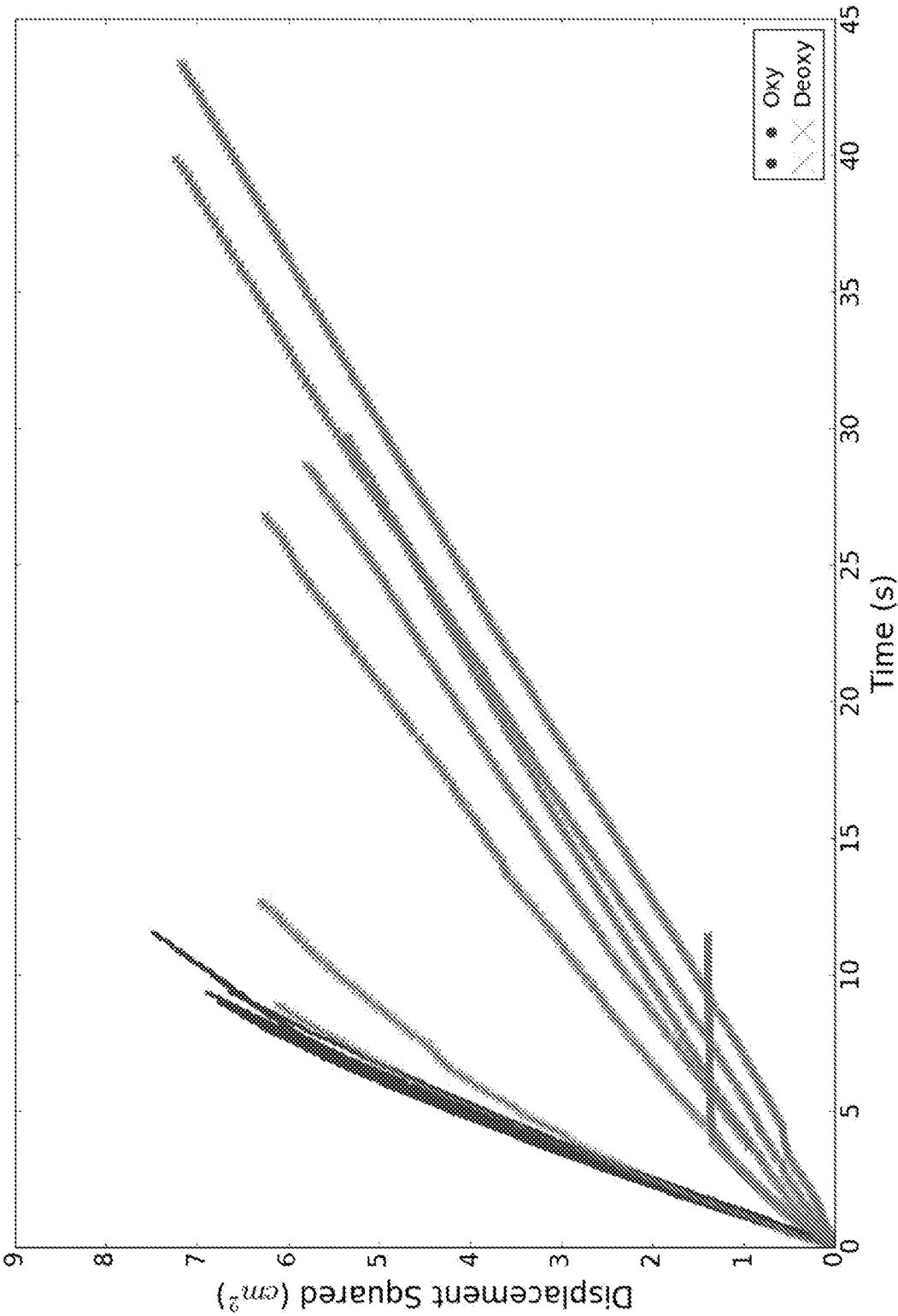


FIG. 9E

SC Blood Through 0.25 μ L Horizontal Capillaries - Quick Deoxy Trials

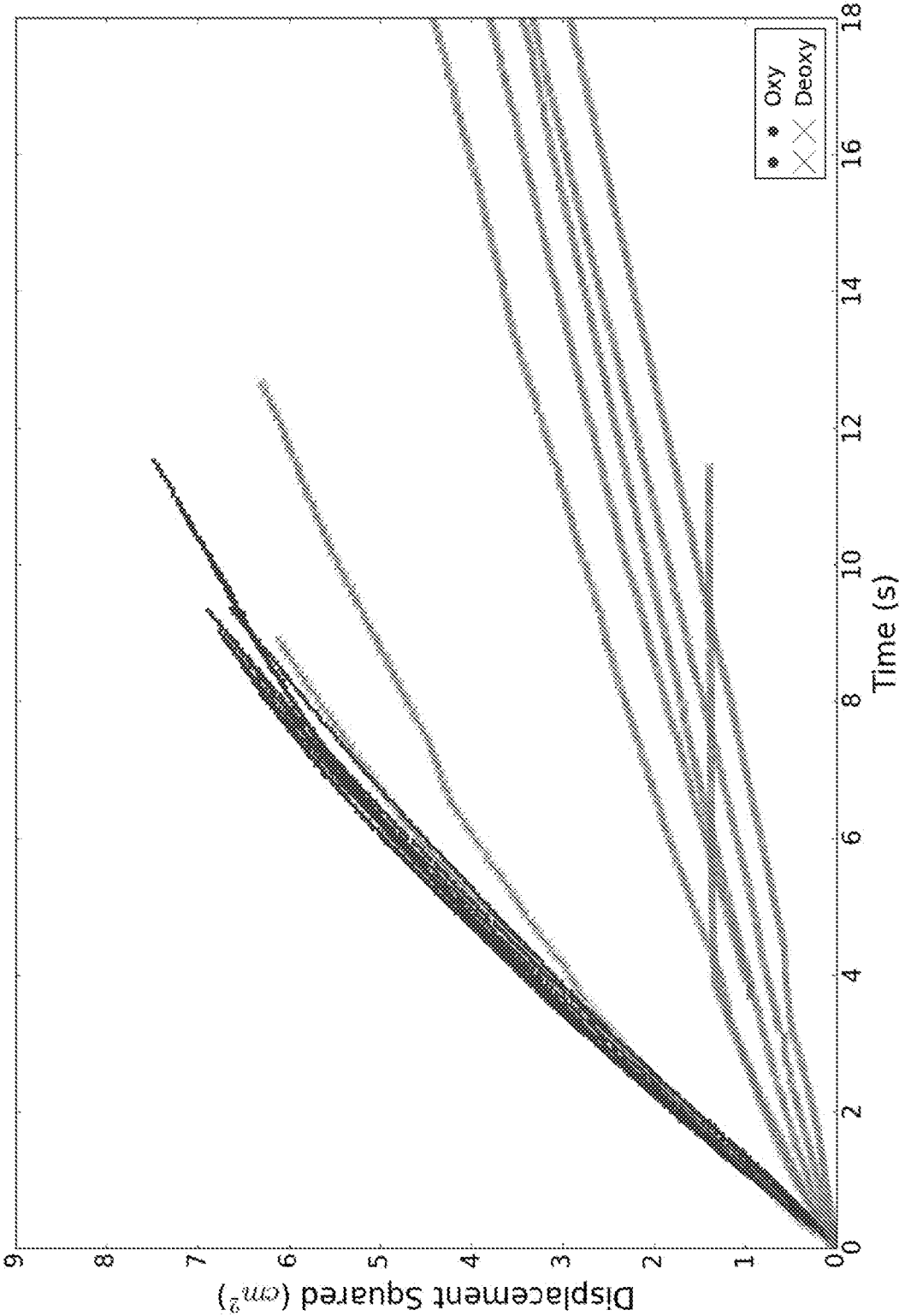


FIG. 9F

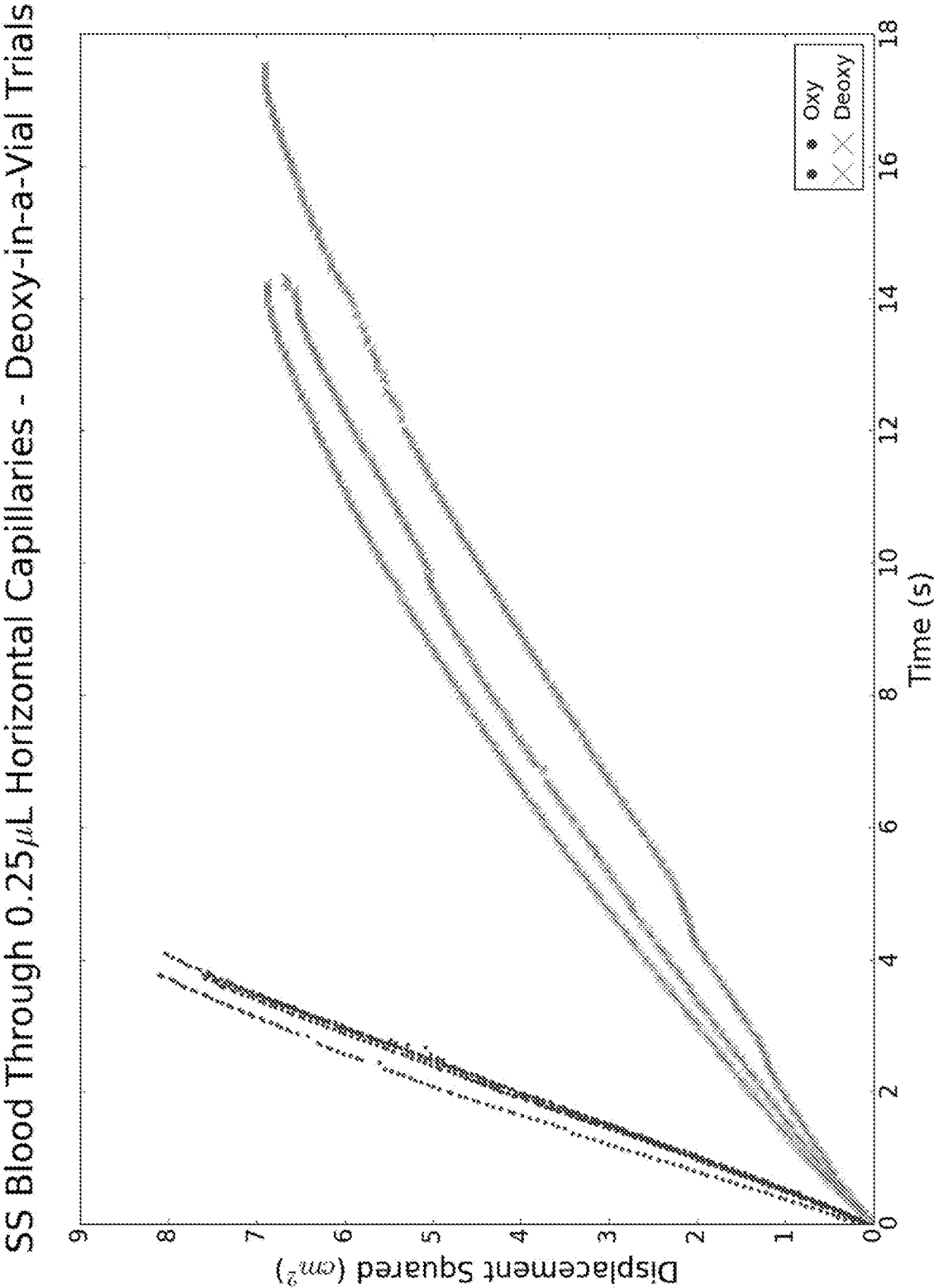


FIG. 9G

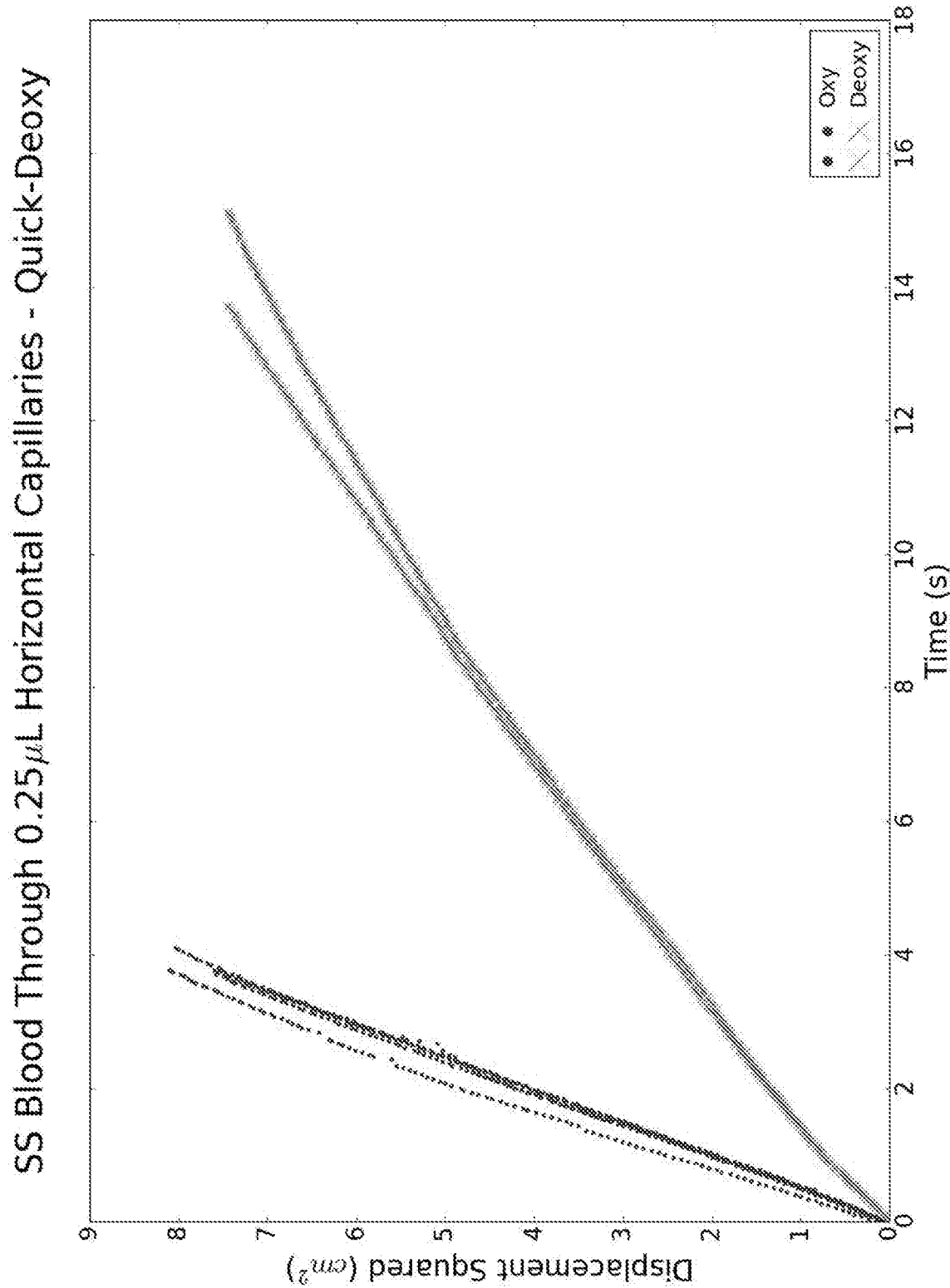


FIG. 9H

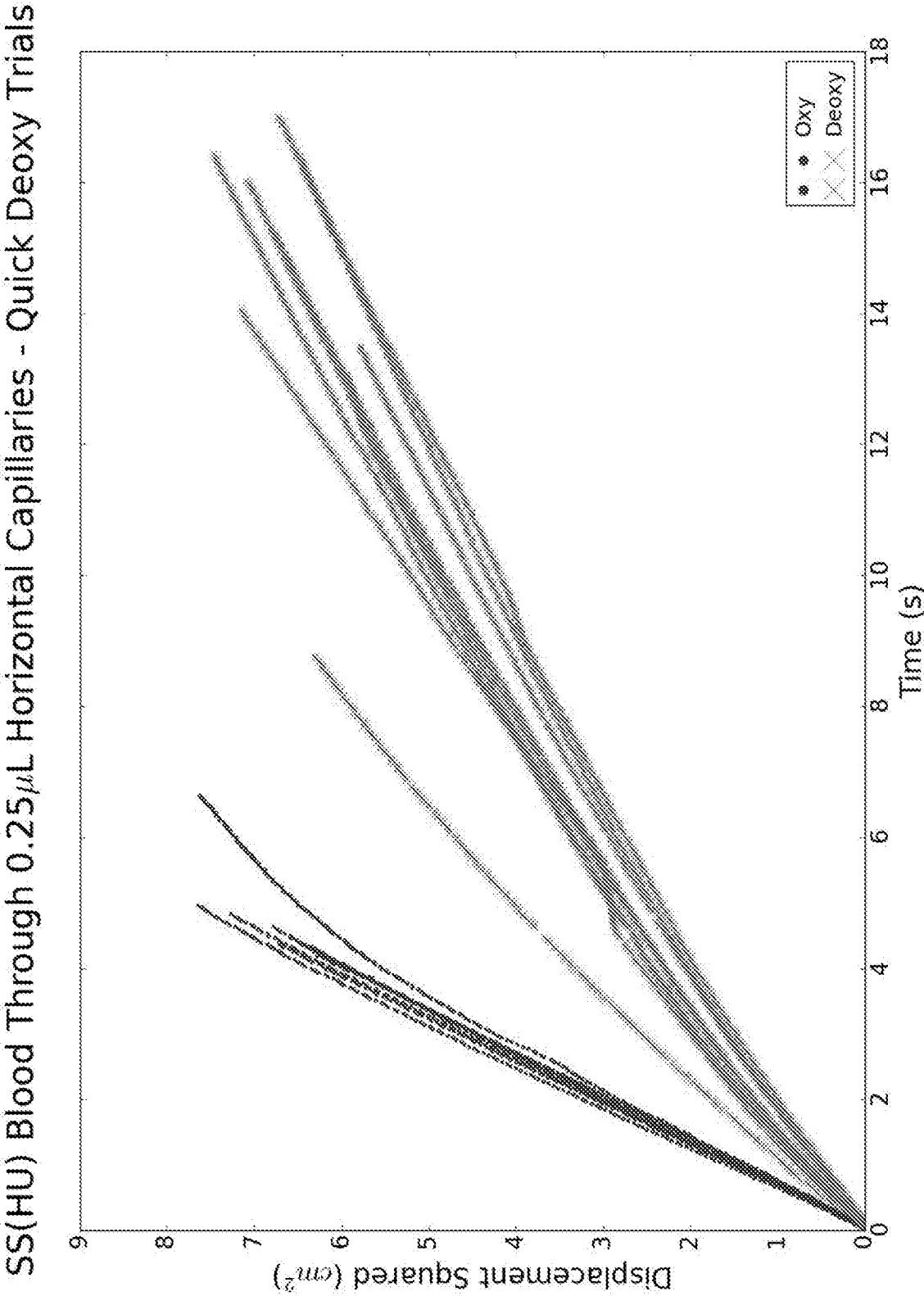


FIG. 9I

SS(Transfused) Blood Through 0.25 μ L Horizontal Capillaries - Quick Deoxy Trials

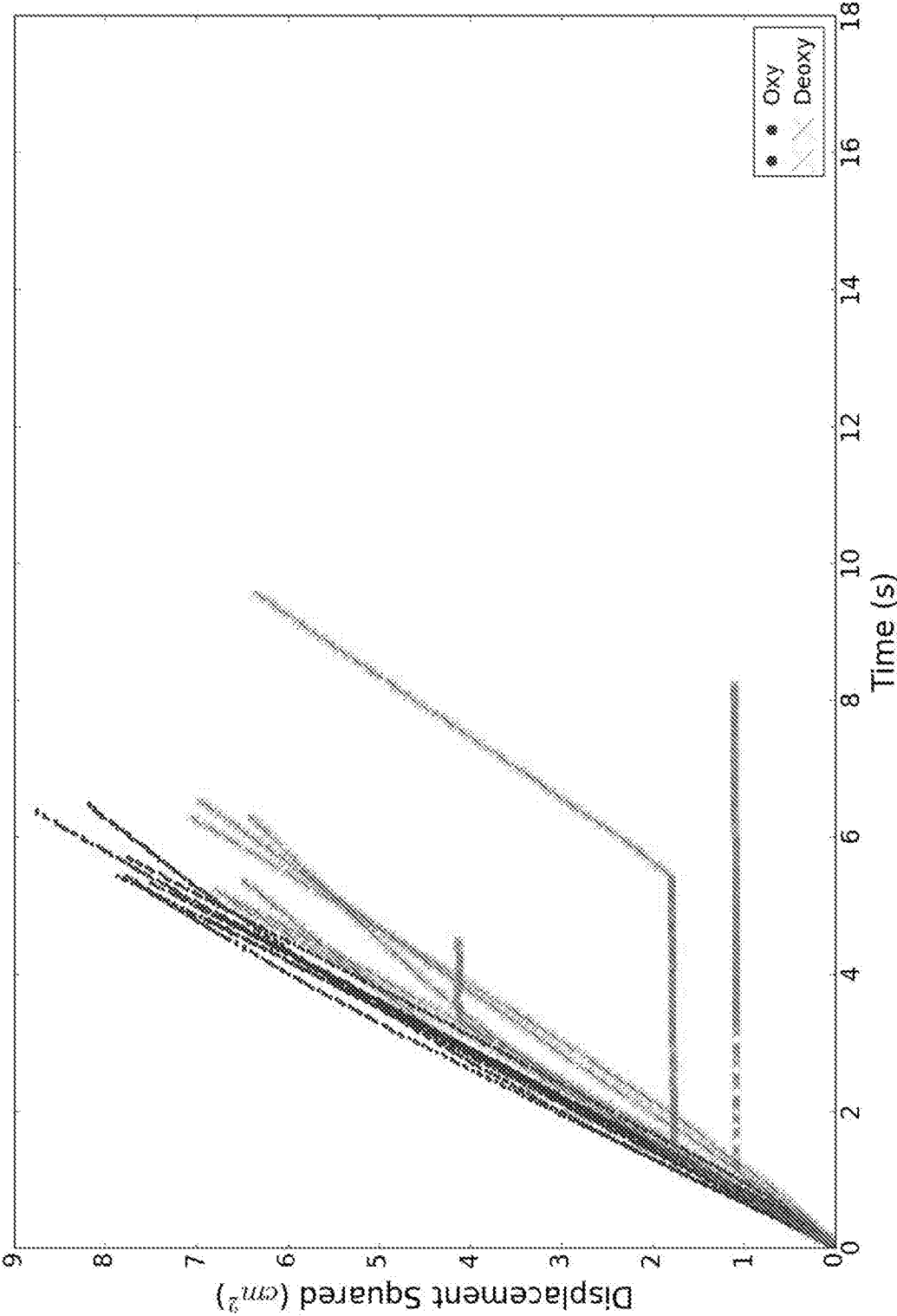


FIG. 9J

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/66641

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-20, directed to a method of screening for sickle-cell disease.

Group II, claims 21-36, directed to a system comprising a capillary assembly and a connecting body.

---Please see continuation in next supplemental box-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/66641

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/49 (2017.01)

CPC - G01N 33/4915

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	WO 2015/054378 A1 (DREXEL UNIVERSITY) 16 April 2015 (16.04.2015) Especially Abstract; Claim 1; Claim 9; Claim 11; p5, ln 19-20; p9, ln 7-10; p12, ln 7-11; p13, ln 3-8; p13, ln 9-15; p13, ln 25-27	1-8, 10-17, 19, 20 ----- 9, 18
Y	CHARACHE et al. Rate of Sickling of Red Cells during Deoxygenation of Blood from Persons with Various Sickling Disorders. Blood July 1964, Vol. 24, No. 1, pp 25-48. Especially Figure 2A; p28, para 1	9, 18

☐ Further documents are listed in the continuation of Box C.

* 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

11 February 2017

Date of mailing of the international search report

13 APR 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/66641

Continuation of Box No. III Lack of Unity

The inventions listed as Groups I and II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I has the special technical feature of contacting a capillary tube with deoxygenated blood, observing a flow rate, and determining a value indicative of sickle-cell disease, that is not required by Group II.

Group II has the special technical feature of a system comprising a capillary assembly of two or more capillary tubes held in parallel to each other by a connecting body, that is not required by Group I.

Common technical features:

Groups I-II share the common technical feature of use of capillary tubes for screening for sickle-cell disease. However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is anticipated by WO 2015/054378 A1 to Drexel University (hereinafter 'Drexel').

Drexel discloses a device for screening for sickle-cell disease (Abstract - 'In certain embodiments, the device of the present invention is used to determine if the individual suffers from a red blood cell abnormality, such as sickle cell disease'), the device comprising a capillary tube (Claim 1 - 'A device comprising a central tube with two open extremities, wherein each extremity of the central tube is attached to a terminal open capillary tube').

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.