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**(54) METHOD AND DEVICE FOR DETECTING ANALYTES IN FLUIDS**

VORRICHTUNG UND VERFAHREN ZUR DETEKTION VON ANALYTEN IN FLÜSSIGKEITEN  
PROCEDE ET DISPOSITIF DE DETECTION D'ANALYTES DANS DES FLUIDES

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**Description**FIELD OF THE INVENTION

**[0001]** The present invention relates to test devices and methods for the determination of analytes which may be present in liquids.

BACKGROUND OF THE INVENTION

**[0002]** The quantification of chemical and biological components in aqueous sample solutions, such as whole blood, plasma, serum and urine, is important for the timely and correct diagnosis of various diseases, as well as for monitoring the progress of the medical treatment of diseases. In many cases the analytes being measured are present in only tiny amounts and are often mixed with much larger amounts of irrelevant or interfering components. Some components, such as red blood cells, prevent the analysis of the sample if they are present. Also problematic are the reagents and indicators used to detect and measure the analytes, which are often highly colored and closely resemble the reaction products in terms of their absorbance spectra. In addition, the measurement of analytes often requires multiple, incompatible reagents that must be stored separately and added sequentially. Any of these factors may complicate the detection and quantification of analytes in fluid samples.

**[0003]** These problems and issues have been addressed in a variety of ways. Analytical methods employed in clinical chemistry testing and other applications used can be divided into two broad categories of assay formats: liquid chemistry formats and dry chemistry formats. Liquid chemistry systems require that sample and liquid reagents be dispensed into reaction chambers in a timed, sequential order. Samples must often be diluted with special buffers to reduce or eliminate interfering compounds and are then added to reagents designed to react with specific analytes. In some cases, multiple reagents must be premixed immediately before use due to stability problems. In other cases additional reagents may be needed to provide color-producing, readable reactions. The results may be obtained by measuring the absorption of light by the fluid sample. Reactions involving decreases in reaction color or minor differences in color changes may further require separate tubes of reagents to standardize the results or serve as controls.

**[0004]** Dry chemistry systems utilize reagents dried onto absorbent surfaces. Most commercially available products have multiple layers of reactants sandwiched together. Some are arranged vertically and some combine vertical and horizontal arrangements. In all cases, dry chemistry systems using chromogenic reactions rely on measuring light reflected off of either the top or bottom surface of the final reagent pad. The assaying of whole blood presents additional problems since it requires a separate method for separating red blood cells from the sample such as centrifugation or the use of blood sepa-

rating filter(s) which separate the plasma for analysis. The essence of dry chemistry analysis is to contain a liquid reaction so that colored reaction products can be visualized. This is done with gels and polymers, e.g., Vitros (Johnson & Johnson) or fibrous paper-like materials, e.g., Seralyzer (Bayer Diagnostics). In all cases the reaction must be observed among a mixture of sample, diluent, reactants and product which can result in difficulties distinguishing product from non-product. In addition, since all or part of the original reactant is consumed, it can be impossible to reference back to the starting material, such as to establish a reagent baseline. In contrast, the present invention retains all components for further evaluations.

**[0005]** In methods that require precise reaction timing, such as those requiring rapid reactions or measuring a rate of change, it is often difficult to determine the exact start time of the reactions. In most cases the performance of the assay, and therefore the reliability of the results, is dependent on the ability of the test system to evenly deliver a certain amount of liquid (usually blood plasma or serum) to a final reactant material. This material must absorb a known quantity of liquid with extreme accuracy and reproducibility in order for the results to be useful. The precise volume measurements required to obtain accurate results with these types of assays present particular challenges and make them difficult to work with.

**[0006]** Both liquid and dry chemistry systems are limited in the concentrations of reactants that can be used. These concentration limits are often due to the presence of highly colored reactants that absorb or reflect light at wavelengths that interfere with or obscure the detection of reaction product which may absorb or reflect light at similar wavelengths. Various methods have been employed in attempts to solve this problem. Hochstrasser, U.S. Patent No. 3,964,871, describe a disposable indicator for measuring substances which registers the concentration of a substance in a given biological fluid with indicia which are directly readable in a convenient notation thereby reducing reliance on comparison with a color intensity scale. Kim et al., U.S. Patent No. 4,303,408, describe elements with interferent-reducing zones which remove interferents prior to the reaction zone. Despite these attempts, only marginal improvements are possible due to the physical limitations which are inherent in the methods.

**[0007]** U.S. Patent No. 5,716,852, issued to Yager et al., teaches a channel-cell system for detecting the presence and/or measuring the presence of analyte particles in a sample stream comprising a laminar flow channel, two inlets in fluid connection with the laminar flow channel for respectively conducting into the laminar flow channel an indicator stream which may comprise an indicator substance which indicates the presence of said analyte particles by a detectable change in property when contacted with said analyte particles, and the sample stream. The laminar flow channel has a depth sufficiently small to allow laminar flow of streams and a length sufficient to allow

particles of the analyte to diffuse into the indicator stream to the substantial exclusion of the larger particles in the sample stream to form a detection area. An outlet conducts the streams out of the laminar flow channel to form a single mixed stream. Yager discloses the formation of a reaction interface that forms between two fluids moving through a capillary tube in the same direction. We disclose the invention of a stable interface that forms when two liquids meet and stop in a flow matrix after conveying from opposite directions. The Yager patent is predicated on the principle of liquid laminar flow, which was known in the art. In contrast, the present invention employs bibulous material to physically contain the liquid interface.

**[0008]** U.S. Patent No. 5,187,100, issued to Matzinger et al., discusses a control solution for use with a porous reagent strip, and comprises a flexible semisolid polymer dispersed in water, such as polyvinyl acetate in distilled water, with appropriate control glucose concentration levels. This solution is useful in mimicking whole blood in conjunction with porous reagent strips to determine compliance of the strips and meters to established measurement and performance criteria.

**[0009]** U.S. Patent No. 5,147,606, issued to Charlton et al., teaches a diagnostic device that detects blood analytes with a sample volume as low as 2 microliters in the hematocrit range of 0% to 60%, or higher. This is accomplished by employing a housing with various chambers and compartments for processing the blood. A sample application port in the housing is used to introduce blood into a metering chamber. From the metering chamber, the blood flows to a reaction chamber for analyzing blood analytes. Blood entering the metering chamber flows into a fluid capillary which indicates that an adequate amount of blood has been received in the metering chamber. The reaction compartment includes a reagent and a filter, the latter of which is disposed between the metering chamber and the reagent so that the reagent reacts with the filtered blood.

**[0010]** U.S. Patent No. 4,839,297, issued to Freitag et al., teaches a test apparatus for the analytical determination of a component of a body fluid with a base layer and at least two planar test layers which, in the initial state of the test carrier, before carrying out the determination, are separate from one another but can be brought into contact with one another by external manipulation. A first test layer and a second test layer are arranged on the base layer essentially next to one another but separated in the initial state by a gap, a contact element being provided which consists of a capillary-active material which is so dimensioned that it can bridge the gap and which is so mounted and arranged that, in a first position, it cannot contact at least one of the test layers but, by external pressure, it can be brought into a second position in which it contacts both test layers in such a manner that a liquid exchange between the test layers is possible.

**[0011]** U.S. Patent No. 4,637,978, issued to Dappen, discloses an assay useful for the determination of an analyte in whole blood. In particular, this assay is useful for

the quantitative determination of peroxide-generating analytes, such as glucose or cholesterol, in whole blood. This assay utilizes a multizone element consisting essentially of a support having thereon, in order and in fluid contact, a registration zone and a reagent/spreading zone. The reagent/spreading zone has a void volume and average pore size effective to accommodate whole blood, and contains an interactive composition necessary for the analysis. Such composition is capable of providing, upon interaction with the analyte, a dye which can be spectrophotometrically detected at a wavelength greater than about 600 nm.

**[0012]** U.S. Patent No. 5,408,535, issued to Howard, III et al., discloses a video test strip reader which can simultaneously locate, color analyze and time-track multiple reagent test strips, such as those used in solid-based clinical assays. The reader includes a video imager that produces an analog signal which is converted into a digital signal representing the image. The digital signal is stored in the form of arrays of pixels containing color information. The digital signal is then processed to calculate the desired test results, such as the concentration of a constituent or other measurable properties.

**[0013]** U.S. Patent No. 4,160,008, issued to Fenocketti et al., teaches a test device for determining the presence of a liquid sample constituent. The device comprises a base support member having attached to it an indicator member which produces a detectable response, such as a color change, in the presence of the sample constituent. The indicator member comprises an upper reagent layer, a lower absorbent layer and a substantially sample-impermeable barrier layer between the upper and lower layers. The indicator member is attached to the base member along the lower side of the absorbent layer.

**[0014]** U.S. Patent No. 4,042,335, issued to Clement, discloses a multilayer element for the analysis of liquids such as biochemical and biological liquids. The invention includes a reagent layer including a composition that is interactive in the presence of a predetermined substance to be analyzed (analyte) to provide a diffusible, detectable species, *e.g.*, a dye, can be detected. Preferably between the reagent layer and the registration layer, there can be a radiation-blocking layer, such as an opaque reflecting layer, to enhance detection of the diffusible species within the registration layer. A spreading layer is separated from the registration layer by a reagent layer. In operation, a sample of liquid under analysis is applied to the reagent layer or, if present, to a spreading layer. If the sample contains analyte, a chemical reaction or other interaction within the reagent layer provides a detectable species that diffuses, via any intervening layers such as a radiation-blocking layer, into the registration layer for detection there, such as by radiometric techniques like reflection spectrophotometry.

**[0015]** U.S. Patent No. 3,992,158, issued to Przybyłowicz et al., discloses an integral analytical element capable of use in the analysis of liquids, the element having at least two superposed layers including a spreading lay-

er and a reagent layer, in fluid contact. The spreading layer, which can be an isotropically porous layer, spreads within itself at least a component of a liquid sample applied to the element, or a reaction product of such component, to obtain a uniform concentration of at least one such spread substance at the surface of the spreading layer which faces the reagent layer. The reagent layer, which preferably is uniformly permeable to at least one dissolved or dispersed component of the liquid sample or a reaction product of such a component, can include a matrix in which is distributed a material that can interact with, for example, an analyte or analyte reaction product to produce a detectable change in the element, such as one detectable by measurement of electromagnetic radiation. In a preferred embodiment, the interactive material can chemically react with an analyte or analyte reaction product to produce a color change in the element. In another preferred embodiment, the sample spreading layer can filter out chemically interfering or other undesirable materials and obtain selective spreading of sample components and/or it can provide a reflective background, often useful in obtaining analytical results.

**[0016]** U.S. Patent No. 3,811,840, issued to Bauer et al., teaches a test device for detecting low concentrations of substances in test fluids which includes an absorbent wick having a substantially flat surface portion enclosed in a fluid impervious sheath having an aperture of predetermined limited area formed therein. The aperture is contiguous to and exposes a predetermined limited area of the flat surface portion of the wick, which is incorporated with a reagent specifically reactable with the substance being detected. In use the device is immersed into the test fluid so that the aperture is submerged and the device is allowed to remain therein while the test fluid contacts the reagent area adjacent to the aperture and migrates into the remainder of the wick. The reagent is immobilized with respect to the liquid.

**[0017]** U.S. Patent No. 4,061,468, issued to Lange et al., discloses a test strip for the detecting of components in liquids, especially in body fluids. The test strip includes a holder and at least one indicator layer containing detection reagents. One surface of the indicator layer is attached to the holder and the other surface is covered with a fine meshwork.

**[0018]** Note, however, that the analytical devices of the above prior art employ fluid movement in only a single direction. Because no reaction interface is created by a movement of two fluids in opposite directions, the above prior art references cannot be employed to measure the reaction intensity or reaction rate at a reaction interface, as disclosed by the present invention.

**[0019]** The present invention provides a solution to the problems and deficiencies of current systems discussed above. Specifically, the present invention provides devices which contain all reactants necessary for sample preparation and analyte detection and methods for their use. The present invention provides devices and methods which eliminate the extreme precision in volume

measurement which is required by some methods. The results of assays conducted with the present invention are read in a generic reading area of the device, and a wide variety and versatility in reagent chemistry and concentrations is offered.

## SUMMARY OF THE INVENTION

**[0020]** The present invention provides a device for detecting and quantifying at least one analyte in a fluid sample suspected of containing the analyte by employing a liquid reactant capable of reacting with the analyte to form a detectable soluble reaction product. The device comprises a fluid transport material having a first zone ("fluid sample zone") for the application of the fluid sample at a fluid sample application site and a second zone ("liquid reactant zone") for the application of a liquid reactant at a liquid reactant application site, wherein when the fluid sample is added to the first zone, and the liquid reactant is added to the second zone, the fluid sample flows in a first direction from a fluid sample edge toward the second zone, and the liquid reactant flows in a second direction opposite to that of the first direction and toward the first zone from a liquid reactant edge. When the flowing fluid sample and the flowing liquid reactant meet, flow stops, the reactants diffuse toward one another, and the detectable reaction product is formed by a reaction between the liquid reactant and the analyte at a stable reaction interface formed at a juncture between and visually distinct from the fluid sample and the liquid reactant, and further comprising a means for calibrating a concentration of said liquid reagent, said means comprising a calibration zone comprising an amount of said analyte, said calibration zone being located between said first zone and said reaction interface, whereby in use, said analyte in said amount of analyte flows in a first direction toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge; whereby when said flowing analyte in said amount of said analyte and said flowing liquid reactant meet, flow stops and a detectable calibration product is formed by a reaction between said analyte in said amount of said analyte and said liquid reactant, and a stable calibration reaction interface is formed at a juncture between and visually distinct from said analyte in said amount of said analyte and said liquid reactant.

**[0021]** In another embodiment, the present invention also provides a device for detecting and quantifying at least one analyte in a fluid sample suspected of containing the analyte, comprising a fluid transport material having a first zone ("fluid sample zone") for the application of the fluid sample at a fluid sample application site and a second zone ("liquid reactant zone") for the application of a liquid reactant at a liquid reactant application site, wherein the reagent and the liquid form a liquid reagent containing a reactant reagent capable of reacting with the analyte present in the fluid sample to form a detect-

able reaction product, wherein when the fluid sample is added to the first zone, and the liquid reactant is added to the second zone, the fluid sample flows in a first direction from a fluid sample edge toward the second zone, the reagent is reconstituted and the reagent and the liquid form a liquid reagent containing a liquid reactant capable of reacting with the analyte to form a detectable reaction product, and the liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge. When the flowing fluid sample and the flowing liquid reactant meet, flow stops, the reactants diffuse toward one another, and the detectable reaction product is formed by a reaction between the liquid reactant and the analyte at a stable reaction interface formed at a juncture between and visually distinct from the fluid, sample and the liquid reactant, and further comprising a calibration zone comprising an amount of said analyte, said calibration zone being located between said first zone and said reaction interface, whereby in use, said analyte in said amount of analyte flows in a first direction toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge; whereby when said flowing analyte in said amount of said analyte and said flowing liquid reactant meet, flow stops and a detectable calibration product is formed by a reaction between said analyte in said amount of said analyte and said liquid reactant, and a stable calibration reaction interface is formed at a juncture between and visually distinct from said analyte in said amount of said analyte and said liquid reactant, said calibration interface further being adjacent to said reaction interface. Preferred features of the device are set out in claims 3 to 22.

**[0022]** Also described is a device for detecting and quantifying an analyte in a fluid sample suspected of containing the analyte by employing a reagent, capable of binding to the analyte and forming a detectable reaction product from a substrate, to relate an amount of the analyte to an amount of the detectable reaction product. The device comprises a fluid transport material capable of absorbing a liquid and causing capillary flow of the liquid. The fluid transport material has a first zone for the application of the fluid sample containing the reagent to a first pad to which the same analyte being detected and/or quantified is substantially irreversibly bound, and a second zone for the application of a liquid to a second pad containing a reconstitutable substrate. When the fluid sample containing the reagent is added to the first pad, the fluid sample containing the reagent that is not bound by the analyte bound substantially irreversibly to the first pad flows in a first direction from a fluid sample edge toward the second zone. When the liquid is added to the second pad, the substrate in the second pad is reconstituted by the liquid to form a liquid reactant capable of reacting with the reagent, and the liquid reactant flows in a second direction opposite to that of the first direction and toward the first zone from a liquid reactant edge. As

a result, when the flowing fluid sample containing the reagent not bound by the analyte on the first pad and the flowing liquid reactant meet, flow stops and the detectable reaction product is formed by a reaction between the liquid reactant and the reagent, and a stable reaction interface is formed at a juncture between and visually distinct from the fluid sample and the liquid reactant.

**[0023]** The fluid transport material used in the present invention is preferably capable of transporting the fluid sample and the liquid reagent by supporting capillary action and thereby facilitating the movement of fluid sample through the material. Further, the fluid transport material is preferably capable of maintaining a defined interface with little mixing of the opposing liquids while allowing diffusion to occur. In a particularly preferred embodiment, the fluid transport material comprises a nitrocellulose material cast onto a polyvinylchloride or polyester (i.e., Mylar™) backing material.

**[0024]** One or more reconstitutable reagents may be contained on the bibulous material in one or more reagent zones. Reagents to be reconstituted by the fluid sample may be located on the fluid transport material at a site which is closer to the application site for the fluid sample than to the application site for the diluent solution. Conversely, reagents which are to be reconstituted by the diluent solution may be located on the fluid transport material at a site which is closer to the application site for the diluent solution than to the application site for the fluid sample. Fluid flow through these reagent zones reconstitutes the reagents, effectively pretreating the sample or mixing and/or reacting one reagent with another. In other embodiments, the reagent or reagents may be contained in an absorbent pad placed in contact with the fluid transport material at the liquid reagent zone. The absorbent pad may be selected from the group consisting of cellulose, glass fiber, polyester, or any absorbent polymer. An absorbent pad may be placed onto the fluid sample application site to pretreat the fluid sample before it enters the absorbent material, e.g., to remove red blood cells from a red blood cell-containing sample. The reagent or reagents may be present in a plurality of locations on the fluid transport material.

**[0025]** In another embodiment, the fluid transport material may be capable of separating red blood cells from whole blood as the fluid sample travels through the bibulous material. In various embodiments the fluid transport material may be a HEMASEP L® membrane, a HEMASEP V® membrane, or a SUPOR® membrane (each available from Pall-Gelman, Port Washington, NY), a CYTOSEPP® membrane (Allstrom Filtration, Mount Holly Springs, PA), or a nitrocellulose membrane.

**[0026]** The amount of analyte present in the sample is determined by measuring the amount of detectable reaction product, and determining from the measured amount of reaction product the amount of analyte. The detectable reaction product may be measured by any appropriate means known to those in the art. For example, if the reaction product absorbs light at a particular

wavelength, the absorbance at that wavelength may be measured and related to the amount of analyte. The reaction product may alternatively, as appropriate, be measured by transmission, reflectance, fluorescence, luminescence, or by electrochemical methods, e.g., electrical conductance.

**[0027]** The concentration of unreacted reagents on the fluid transport material may provide a reference value, control, or blank for the assay. The concentration of unreacted sample on the bibulous material may also provide a reference value, control, or blank for the sample, e.g., a red blood cell-containing sample can be checked for hemolysis. The device of the present invention may further contain a means for calibrating a concentration of the liquid reagent, by adding an amount of analyte at a point adjacent to but distinct from the point in the first zone at which the fluid sample is added, such that the analyte and the liquid reactant meet and produce a detectable calibration product. The device of the present invention may further comprise a means for simultaneously applying the fluid sample to the first zone and the liquid reactant to the second zone and a sensor effective to detect the detectable reaction product. The sensor may be, for example, a CCD imaging camera or a optical imaging device.

**[0028]** The present invention also provides a method for detecting and quantifying at least one analyte in a fluid sample suspected of containing the analyte by employing a liquid reactant capable of reacting with the analyte to form a detectable soluble reaction product. The method includes the steps of providing a fluid transport material having a first zone ("fluid sample zone") for the application of the fluid sample at a fluid sample application site, and a second zone ("liquid reactant site") for the application of the liquid reactant at a liquid reactant application site, adding the fluid sample to the first zone and the liquid reactant to the second zone, whereupon the fluid sample flows in a first direction from a fluid sample edge toward the second zone, and the liquid reactant flows in a second direction opposite to that of the first direction and toward the first zone from a liquid reactant edge. When the flowing fluid sample and the flowing liquid reactant meet, flow stops, the reactants diffuse toward one another, and the detectable reaction product is formed by a reaction between the liquid reactant and the analyte at a stable reaction interface formed at a juncture between and distinct from the fluid sample and the liquid reactant. The detectable reaction product is then detected and optionally measured. The detectable reaction product may be detected and optionally measured by any appropriate method, such as, for example, absorbance, fluorescence, luminescence, transmission, or electrochemical parameters, such as changes in conductance.

**[0029]** In another embodiment, the present invention also provides a method for detecting and quantifying an analyte in a fluid sample suspected of containing the analyte by employing a reagent, capable of binding to the

analyte and forming a detectable reaction product from a substrate, to relate an amount of the analyte to an amount of the detectable reaction product. The method comprises providing a fluid transport material capable of absorbing a liquid and causing capillary flow of the liquid, the fluid transport material having a first zone for the application of the fluid sample containing the reagent to a first pad containing the analyte bound substantially irreversibly to the first pad, and a second zone for the application of a liquid to a second pad containing a reconstitutable substrate. The fluid sample containing the reagent is added to the first pad, and the liquid to the second pad, wherein the fluid sample thereafter flows in a first direction from a fluid sample edge toward the second zone, and the substrate is reconstituted by the liquid to form a liquid reactant capable of reacting with the reagent, and the liquid reactant flows in a second direction opposite to that of the first direction and toward the first zone from a liquid reactant edge. As a result, when the flowing fluid sample containing the reagent not bound by the analyte on the first pad and the flowing liquid reactant meet, flow stops and the detectable reaction product is formed by a reaction between the liquid reactant and the reagent, and a stable reaction interface is formed at a juncture between and visually distinct from the fluid sample and the liquid reactant. Thereafter, the detectable reaction product is detected and may further be quantified.

**[0030]** The reagent may be a plurality of reagents in a plurality of locations on the fluid transport material. The fluid sample may be whole blood, blood plasma, blood serum, urine, or any body fluid. In addition, the invention is useful for the detection of analytes in a broad variety of materials such as milk, environmental samples, and other samples containing target analytes. The reagents may further be added to either or both of the fluid sample and diluent solution prior to contact with the fluid transport material. In this aspect, the methods include providing a fluid transport material as described above, and the bibulous material may contain no reagents or may contain additional reagents, either dried into the fluid transport material or into a pad contacting the fluid transport material.

**[0031]** The above and other objects, features and advantages of the present invention will become apparent from the following description read in conjunction with the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0032]**

Figure 1 is an isometric view of the test strip of the present invention showing locations of application sites for fluid sample and diluent solution, direction of diluent solution and fluid sample flow, and reaction product location after completion of the test.

Figure 2 is an isometric view of another embodiment of the present invention, in which a dried reagent pad

containing one or more reagents is attached to one end of the test strip, and the diluent solution application site is located on a portion of the dried reagent pad.

Figure 3 is a schematic representation of the concentrations of analyte, reagent, and reaction product at locations along the bibulous material and illustrates that at the interface, the concentration of unreacted analyte and reagents falls sharply as they react and form reaction product, and that the concentration of reaction product correspondingly rises sharply at the interface.

Figures 4A - 4F are photographs showing results over time of a glucose assay performed with the present invention.

Figures 5A - 5I are photographs showing results over time of three concentrations of calcium assays performed with the present invention.

Figure 6 is a graph of color intensity versus time for six independently read reflective values of a reaction interface from a sample having an albumin concentration of 1.2 g/dl based on an assay run with the present invention.

Figure 7 is a numerical tabulation of color intensity versus time for six independently read reflective values of a reaction interface, and the average value and slope of sample having an albumin concentration of 1.2 g/dl based on an assay run with the present invention.

Figure 8 is a graph of color intensity versus time for six independently read reflective values of a reaction interface from a sample having an albumin concentration of 1.9 g/dl based on an assay run with the present invention.

Figure 9 is a numerical tabulation of color intensity versus time for six independently read reflective values of a reaction interface, and the average value and slope of a sample having an albumin concentration of 1.9 g/dl based on an assay run with the present invention.

Figure 10 is a graph of color intensity versus time for the averaged red reflective values of samples having albumin concentrations of 1.2 g/dl and 1.9 g/dl based on an assay run with the present invention and read over 15 seconds.

Figure 11 is a digital color image of an assay run with the present invention of a sample having an albumin concentration of 1.2 g/dl within 200 mseconds of interface formation and 2.0 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image.

Figure 12 is a digital color image of an assay run with the present invention of a sample having an albumin concentration of 1.2 g/dl at three (3) seconds after interface formation and five (5) seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image.

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Figure 13 is a digital color image of an assay run with the present invention of a sample having an albumin concentration of 1.9 g/dl within 200 mseconds of interface formation and 2.0 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image.

Figure 14 is a digital color image of an assay run with the present invention of a sample having an albumin concentration of 1.9 g/dl at three (3) seconds after interface formation and five (5) seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image.

Figure 15 is a graph of color intensity versus time for three different devices reading a sample having a glucose concentration of 50 mg/dl based on an assay run with the present invention.

Figure 16 is a graph of color intensity versus time for three different devices reading a sample having a glucose concentration of 150 mg/dl based on an assay run with the present invention.

Figure 17 is a graph of color intensity versus time for three different devices reading a sample having a glucose concentration of 250 mg/dl based on an assay run with the present invention.

Figure 18 is a graph of color intensity versus time for the average value of samples having a glucose concentration of 50 mg/dl, 150 mg/dl and 250 mg/dl based on an assay run with the present invention and read over 4.5 minutes.

Figure 19 is a graph of color intensity versus time for samples having calcium concentrations of 9.3 mg/dl and 13 mg/dl based on an assay run with the present invention.

Figure 20 is a graph of interface width versus time for samples having calcium concentrations of 9.3 mg/dl and 13 mg/dl based on an assay run with the present invention.

Figure 21 is a schematic representation of the various parameters that can be measured in the methods and devices of the present invention.

Figure 22 is a schematic representation of the measurement of a portion of the area of the product interface region as a measurement of bioactivity in an assay of glucose concentration.

Figure 23 is a schematic representation of the internal calibration procedure used in the methods and devices of the present invention, showing the application of the excess analyte near the sample pad in a strip format, and the subsequent formation of the analyte spot at the reaction product interface.

Figure 24 is a digital color image of a glucose assay that demonstrates the internal calibration procedure of the present invention. The calibrated analyte (glucose) was applied at an excess concentration of about 100 mg/dl, and the glucose assay was run in accordance with the method of the present invention. The product at the reaction interface was measured

at 20 seconds and 60 seconds after sample application.

Figure 25 is a digital color image of an alkaline phosphatase assay run in accordance with the method of the present invention at 15 seconds and 120 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 26 is a digital color image of an assay for total bilirubin run in accordance with the method of the present invention at a concentration of 4.73 mg/dl total bilirubin, and measured at 15 seconds and 275 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 27 is a digital color image of an assay for uric acid run in accordance with the method of the present invention at a concentration of 0.3 mg/dl uric acid, and measured at 5 seconds and 295 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 28 is a digital color image of an assay for  $\gamma$ -glutamyl transferase activity run in accordance with the method of the present invention at a concentration of 380 U/l of  $\gamma$ -glutamyl transferase activity, and measured at 15 seconds and 285 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 29 is a digital color image of an assay for amylase run in accordance with the method of the present invention at a concentration of 16,800 U/l amylase, and measured at 35 seconds and 275 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 30 is a digital color image of an assay for creatinine run in accordance with the method of the present invention at a concentration of 150 mg/dl creatinine, and measured at 5 seconds and 295 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 31 is a digital color image of an assay for cholesterol run in accordance with the method of the present invention at a concentration of 96 mg/dl cholesterol, and measured at 10 seconds and 300 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 32 is a digital color image of an assay for total protein run in accordance with the method of the present invention at a concentration of 6,200 mg/dl total protein, and measured at 5 seconds and 295 seconds after sample application, as well as the associated color absorption graph for a selected portion of the digital color image at each time point.

Figure 33 is a digital color absorption image of an assay for magnesium run in accordance with the method of the present invention at a concentration of 4.7 mg/dl magnesium, and measured at 5 seconds and 295 seconds after sample application, as well as the associated UV absorption graph for a selected portion of the digital UV absorption image at each time point.

Figure 34 is a digital color image of an immunoassay for T4 run in accordance with the method of the present invention, and measured at 5 minutes after sample application, as well as the associated color absorption graph for a selected portion of the digital color image.

#### DETAILED DESCRIPTIONS OF THE INVENTION

**[0033]** The present invention provides devices for detecting analytes which may be present in a fluid sample, by reacting a liquid reactant with the fluid sample and forming a detectable reaction product. The device of the present invention comprises an absorbent strip made of a fluid transport material ("bibulous material") which is capable of absorbing and transporting fluid sample, reagents, and diluent liquids via capillary action. Analytical reagents capable of being dissolved and distributed within the absorbent strip may be dried into the absorbent strip or into reagent pads in contact with the absorbent strip. The device of the present invention is particularly useful for clinical chemistry applications.

**[0034]** We have observed the unexpected result that two liquids converging from opposite ends of a bibulous material meet at a very sharply defined interface, with little mixing of the two opposing solutions over periods of time amounting to several minutes. This allows the creation of a stable reaction interface where the two opposing liquids containing reagents or analytes capable of reacting with each other meet, diffuse, and form a detectable product. The resulting product is localized at the interface for a significant period of time, allowing analysis of the reaction rate and the reaction product band intensity without the need to measure starting reagent volumes. Since the reaction product is visually distinct from the reactants, high concentrations of reactants may be used. This allows for the use of large amounts of certain reagents which can counteract endogenous interfering compounds without obscuring results due to closely related absorbency spectra or density of unreacted reagents. The present invention comprises a significant improvement over prior art disposable, analytical reagent systems in that the detectable reactant zone is distinct and separate from the unreacted reagents, allowing for the use of reaction indicators exhibiting only minor changes as well as extremely high concentrations of reactants.

**[0035]** Because the reactants, i.e., the fluid sample and the liquid reactants, are visually distinct from the reaction product, the system contains a built-in sample of both the unreacted fluid sample and the unreacted liquid re-

actant solution. These may be used as references for certain types of analytical reactions, such as those involving density changes and not spectral changes, or those requiring a starting reagent or reference value.

**[0036]** The devices of the present invention may be made of any material which is capable of absorbing liquids and causing capillary flow from areas of high concentration to areas of low concentration on the device, while maintaining a defined reaction product interface with little mixing of the opposing liquids. The materials of the device may be made of nitrocellulose membranes, cellulose sheets, porous polyethylene, polyethersulfone, or membranes of a variety of other materials. Porous plastics made from a variety of polymers may also be used, such as polyethylene, polystyrene, or polypropylene. Most preferable are bibulous materials made from nitrocellulose cast onto a backing material, such as PVC or Mylar™ (or another polyester film). An example of this type of bibulous material is that available from Schleicher and Schuell, catalog no. FF-170. When using nitrocellulose, the nitrocellulose materials should be first pretreated to render the membranes hydrophilic. The fluid transport material may be adhered to a solid backing, such as PVC or polystyrene, in order to provide durability in handling. These materials are provided by way of example, and are not intended to be limiting. The person of ordinary skill will realize that a variety of materials may be successfully utilized in the present invention, so long as the material has the properties of being able to absorb the sample, support capillary action and thereby facilitate the movement of fluid sample through the material, and maintain a defined interface with little mixing of the opposing liquids.

**[0037]** Regarding hydrophilic absorbent materials, two factors affect usefulness in the present invention. First, the pore size affects the integrity of the reaction interface. As pore size increases, the reaction interface spreads and becomes more diffuse. Second, as the percent solids (v/v) in the membrane increases, the signal intensity of the reaction interface decreases, presumably due to the lower volume of the reaction liquid at the interface. Preferably, the pore size is on the order of 5 μm, based on currently available materials. It is conceivable that a material of small pore size and low solid may be an excellent material.

**[0038]** In preferred embodiments for assaying samples containing whole blood or blood products containing unwanted red blood cells, the fluid transport material may be a material which separates plasma or the blood product from red blood cells. In these embodiments, the fluid transport material may be a HEMOSEP V® or HEMOSEP L® membrane (available from Pall-Gelman Inc., Port Washington, NY) or a CYTOSEP® membrane (available from Allstrom Filtration, Mount Holly Springs, PA).

**[0039]** The methods and devices of the present invention provide for sample preparation and analysis to be performed in one step on the same device. The device may be a strip in the form of a generally rectangular

shape. Alternatively, the bibulous material may also be a circular or linear array or star-shaped configuration which has the additional advantage of allowing multiple analytes to be analyzed in a single assay. Of course, the person of ordinary skill will realize that the bibulous material may be of any shape advantageous under the particular circumstances.

**[0040]** The present invention can employ reagent systems capable of performing chemical evaluation of analytes which are commonly present in biological fluids. Such reagent systems are widely known in the art. Of particular interest are those reagent systems utilizing a single component, i.e., where the reaction occurs in a single solution, rather than those requiring more than one timed incubation step. The present invention provides single-component reagent systems composed of several different chemical reagents where the reagents are dried in spatially separated zones on the strips. As shown in Figures 1 and 2, dried reagent strips of the present invention may be used by applying a diluent solution ("diluent solution" is defined as any liquid capable of dissolving a reagent. Any reagents which are added to the diluent solution or reconstituted by the diluent solution, are meant to be included in the definition as part of the diluent solution) to one end of the strip and a fluid sample ("fluid sample" is defined as any fluid containing an analyte to be assayed for. Any reagents which are added to the fluid sample, or reconstituted by the fluid sample, are meant to be included in the definition as part of the fluid sample) to be assayed to the other end. Referring to Figure 1, the fluid sample may be applied to the fluid sample application site 1 in the fluid sample zone ("fluid sample zone" is defined as any area on the bibulous material which is closer to the fluid sample application site than to the liquid reactant application site) and the liquid reactant may be applied to the liquid reactant application site 2 in the liquid reactant zone ("liquid reactant zone" is defined as any area on the bibulous material which is closer to the liquid reactant application site than to the fluid sample application site). As the two liquids move toward each other through the strips the sample may separate into components. For example, when cell separating strips are used, the fluid sample may separate into plasma and red blood cells. The fluid sample may also reconstitute one or more reagents as it moves through the fluid transport material, thereby pre-treating the sample before the final reactions occur. The liquid reactant simultaneously travels from the opposite end of the strip, and may optionally dissolve in sequence one or more dried reagents which may be present in the liquid reactant zone of the strip. When the fluid sample and the liquid reactant meet, a reaction interface forms between the conveying liquids. The reaction products occupy a very narrow band at the interface and are measured for rate and intensity of formation. Usually, the increase in the amount of product formed (or of decrease in the amount of reagent and/or analyte supplied) will be measured as a function of time. Alternatively, the amount of

the product, reagent and/or analyte may be measured at one or more fixed time points. The change in the amount of product formed is typically measured by measuring the increase or decrease in absorbance over time at a wavelength at which the maximal absorbance is detected. In a preferred embodiment, the reaction products will be measured using reflection. However, the person of ordinary skill will realize that other means of measuring the reaction products may also be utilized, such as through the use of electrochemical methods (e.g., measuring changes in conductance), fluorescence, luminescence, transmission or other methods known in the art which provide a detectable signal related to the presence and/or the amount of a reaction product. The resulting reactant band is stable over several minutes, with little product diffusion into the surrounding area, which allows adequate time for the determination of rate and intensity of color development.

**[0041]** Figure 2 illustrates another embodiment of the invention where one or more reagents which may be present on the bibulous material may be present in the form of a dried reagent pad 3. In this embodiment, dried reagent pad 3 is located on a portion of the diluent solution application site 2.

**[0042]** The fluid sample application site may further contain one or more pads for processing the fluid sample prior to its exposure to the reagent. As an example, the fluid sample application site may contain a blood sample-processing pad for pretreatment of whole blood samples or whole blood-containing samples. Examples of such pads include Hemasep-L or, more preferably, Hemasep-V (Pall Corporation) or glass fiber. In addition, the reagent application site furthermore may incorporate a pad containing a dried reagent capable of being reconstituted by addition of a diluent. This replaces the requirement for application of a liquid reagent with the far simpler reconstitution of a dried reagent using a diluent.

**[0043]** In some cases, it may be preferable to measure the disappearance of a reagent or analyte, rather than the appearance of a product. For example, reduced nicotinamide adenine dinucleotide (NADH), a cofactor for many enzymes, absorbs light much more strongly at 340 nm than when in its oxidized form, NAD. Accordingly, when measuring the activity of an enzyme that uses NADH as a cofactor, it may be advantageous to measure the reduction in absorbance at 340 nm. Alternatively, the formation of NADH may be measured as an increase in absorbance at 340 nm.

**[0044]** Figure 3 is a graphical illustration showing hypothetical relative concentrations of analyte to be assayed, reaction product, and reactant at locations along the bibulous material. The relative concentrations illustrated may be typical of those found on the bibulous material during an assay. Figure 3 is illustrative of four types of assays which may occur on the bibulous material. Line 70 represents product formed. Lines 50 and 60 signify "consumed analyte" and "consumed reagent", respectively, and may illustrate a reaction where the reagent or

the analyte is consumed, such as a dye-binding assay where dye is bound to an analyte and both dye and analyte are "consumed" in the complex when the reaction occurs. At the reaction interface, concentrations of unconsumed analyte and unconsumed reagent fall sharply as the concentration of reaction product rises sharply. In other types of reactions, reagent is not consumed in the reaction. For example, in enzymatic assays the enzyme is not consumed but simply converts an analyte (or substrate) to a detectable product. In this case the non-consumed reagent is represented by line 80 and the consumed analyte by line 50. In another type of assay, reagent may be consumed and analyte not consumed such as for a serum enzyme (represented by line 90). There are thus four types of assays which may occur and which are illustrated by Figure 3: those where analyte is consumed and reagent not consumed, those where analyte is not consumed and reagent is consumed, and those where analyte and reagent are both consumed or not consumed.

**[0045]** Because the reaction interface remains visually distinct from the fluid sample and reagent solution on the bibulous material, the device incorporates a built-in methodology for determining both reagent and fluid sample concentrations and dilutions. Where dried reagents are reconstituted, the device also provides for the internal calculation of reference values which take into account the extent of dilution of reagents in the diluent solution and in the fluid sample (i.e., reflecting the extent to which the dried reagents which may be present have dissolved in the diluent solution or the fluid sample), and for the measurement of background signal, all of which may be useful in certain types of analytical chemistry formats. For example, certain fluid samples may contain material that reflects light at a wavelength at or close to that of the reaction product and this signal must therefore be subtracted from the final reaction product color intensity. In other formats, e.g., where results are directly related to reagent concentration, for example in rate reactions where the analytes are in excess, a simultaneous measurement of both starting reagent concentration and product concentration by reflected light intensity at different wavelengths allows adjustment of the resulting analyte concentration values based on variability in the extent of reagent dissolution. A person of ordinary skill in the art would recognize that the method of the present invention permits the measurement of numerous other parameters that may be useful in measurements of operational stability, as well as calibration or calculation of background or reference values. Example of such parameters include, without limitation:

1. the distance between a sample application site edge and the reaction interface, and the change of the distance over time (A in Fig. 21);
2. the distance between a reagent application site edge and the reaction interface, and the change of the distance over time (B in Fig. 21);

3. the distance between a sample application site edge and the liquid interface, and the change of the distance over time (C in Fig. 21);
4. the distance between a reagent application site edge and the liquid interface, and the change of the distance over time (D in Fig. 21);
5. the distance between the reaction interface and the liquid interface, and the rate of change in the distance over time (E in Fig. 21);
6. the area of the liquid interface (I in Fig. 21);
7. the absorbance or reflectance of the sample background (J in Fig. 21);
8. the absorbance or reflectance of the reagent background (K in Fig. 21); and
9. the absorbance or reflectance of the liquid interface, and the rate of change in the same parameter over time (L in Fig. 21).

**[0046]** Clinical chemistry assays, whether they are liquid- or solid-based, can be read by kinetic analysis (rate of product formation; G in Fig. 21) or end-point analysis (amount of production at one or more given time points, F in Fig. 21). Preferably, the detectable reaction product is measured by determining the rate of product formation (G in Fig. 21). With respect to solid-based clinical chemistry assays, the present invention offers the only way to measure rate from a true time zero point since one can visualize and time the start of the reaction within milliseconds. This allows one to measure rates within the first few seconds of the assay, which is unique and useful. In addition, the width of the reaction interface band can be measured which can correlate with analyte concentration. Finally, all or a portion of the area of the interface band within a region of interest can be measured as a determination of total product formation (H in Fig. 21; Fig. 22). These parameters can be followed over time, or the same region can be compared among different samples to provide a relative measure of product development and thereby, analyte concentration.

**[0047]** The desired parameter may be measured by using any appropriate imaging device. Especially preferred are imaging devices that permit quantitation of the measured parameter by e.g., conversion of the measured values for the parameter into data suitable for processing by a computer. Examples of such devices include three-color CCD imaging cameras or three-chip imagers that may employ optics and a rotating filter wheel.

**[0048]** In another embodiment, a non-interfering component may be dried onto the fluid sample side of the bibulous material and be assayed at several points along the bibulous material as it is dissolved in the advancing sample fluid front to determine the presence of the sample at the interface. For example, a dye with an absorption spectra distinct from the product may be dried at the fluid sample side of the pad. Measurement of that dye at the fluid sample side of the reaction interface may be performed to determine the presence of fluid sample at the

reaction site.

**[0049]** In another embodiment, an amount of the analyte to be detected and/or measured may be dried at a spot on the bibulous material that is between the fluid sample application site and the liquid reagent application site. The analyte is reconstituted with a diluent, and the liquid reagent and the analyte flow toward one another as described above. The liquid reagent and the analyte meet and form a detectable calibration product. The calculation of the amount of the reaction product is assisted by a determination of the amount of reagent present at the reaction interface, which may be a function of the amount of the detectable calibration product. Generally, in the methods of the present invention directed to measurement of the concentration of an analyte, there is a great excess of reagent over analyte. However, to calibrate the amount of reagent at the reaction interface, the situation is reversed to produce an excess of analyte relative to reagent by spotting a relatively large amount of analyte onto the bibulous material, at a position in line with, but distinct from, the fluid sample application site (Fig. 23). The analyte may be applied in the form of a single high-concentration spot, but is more preferably applied as a stripe or slot of analyte (Fig. 23), since this permits the application of the same amount of analyte at a lower concentration, avoiding the possibility of problems associated with high concentrations of analyte, such as precipitation or crystallization of the analyte. The thus-applied analyte migrates with the sample toward the reagent application site and forms a large product spot at the reaction interface (Fig. 24). This spot may be measured in the same manner as the reaction product that is produced by the analyte sample. Deviations from expected values in the measurement of the calibration spot may signal the occurrence of any of a number of items that may influence reagent concentration, such as, for example, reagent degradation, reagent delivery difficulties, lack of reagent dissolution, precipitation of the reagent, and the like.

**[0050]** The above-described calibration method may also be used as a positive control method, to confirm the activity of the assay where a negative result is often expected. In this embodiment, a small amount of the analyte is applied to the bibulous material, and migrates toward the reagent application site. Formation of a detectable calibration product at the calibration interface provides evidence that the assay is working properly, and that any negative result is due to the absence of the analyte in the simultaneously-run liquid sample.

**[0051]** The following is an exemplary, non-comprehensive list of the analytes that can be identified with the present invention: alanine amino transferase (ALT) (enzyme substrate), albumin (dye binding), alkaline phosphatase (enzyme substrate), ammonia (enzymatic), amylase (enzyme substrate), aspartate amino transferase (AST) (enzyme substrate), total bilirubin (dye binding), calcium (dye binding), cholesterol (total) (enzymatic), creatine kinase (CK) (enzyme substrate), creatinine

(dye binding), 2-glutamyl transferase (GGT) (enzyme substrate), glucose (enzymatic), lactate dehydrogenase (enzyme substrate), lipase (enzyme substrate), magnesium (dye binding), phosphorus (dye binding), protein (total) (dye binding), triglyceride (enzymatic), urea nitrogen (BUN) (enzymatic), and uric acid (enzymatic). The above referenced reaction types, enzyme substrate, dye bindings and enzymatic, can result in a chromogenic and/or ultraviolet reaction to identify the above listed analytes.

**[0052]** The present invention may also employ, in place of the liquid reactant that is capable of reacting with the analyte, a reagent that is capable of binding to the analyte. The reagent may be any molecule that is capable of binding to an analyte of interest, such as an antibody, a receptor, a receptor body, an antibody fragment, an aptide, or the like. The reagent further contains (i.e., is conjugated or linked) to an enzyme or enzyme fragment that produces a detectable reaction product upon incubation of the reagent with an appropriate substrate. There are numerous methods known to detect the binding of an analyte to such a reagent. In one embodiment, illustrated herein in Fig. 34, a defined quantity of the reagent is mixed with the liquid sample that is suspected to contain the analyte of interest. Upon mixing, the reagent binds to any analyte present in the liquid sample, forming a tightly-associated complex between the analyte and the reagent. The mixture then is applied to a pad located at one end of the bibulous fluid transport material. The pad contains a defined amount of the analyte which is substantially irreversibly bound to the material that makes up the pad. By substantially irreversibly linked, it is meant that over the time course of the assay, no detectable amount of the analyte dissociates from the pad. The unbound reagent in the liquid sample binds to the analyte that is affixed to the pad, while the reagent in the liquid sample that has previously bound analyte present in the liquid sample does not bind to the analyte on the pad. Rather, this reagent, in the form of a reagent/analyte complex, then moves through the fluid transport material.

**[0053]** At the same time, a substrate present at the other end of the bibulous fluid transport material is made to flow toward the liquid sample. At the point where the substrate and the liquid sample meet, a detectable reaction product is formed by the action of the enzyme portion of the reagent with the substrate. As before, the resulting product is localized at the reaction interface for a significant period of time, allowing the reaction rate and reaction product intensity to be easily analyzed. The reaction rate and/or the product concentration may be related to the concentration or amount of the analyte in the liquid sample by any appropriate means, such as the use of a calibration reaction or a standard curve.

**[0054]** The reagent may be any molecule that is capable of binding to the analyte of interest. Such molecules may be a monoclonal antibody or a portion thereof, a receptor protein or a portion thereof, or an aptide or a portion thereof. Examples of known antibody binding por-

tions include, without limitation, an F(ab) fragment, an F(ab') fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, an scFv fragment, and the like. An example of a receptor portion that may be used in the present invention is a receptor body. The procedures for generating such antibody binding portions and receptor bodies, and for using them in binding reactions, are well known to those in the art. The reagent, in whatever form, must be able to specifically bind to the analyte of interest in the liquid sample at a sufficient affinity for the complex to pass through the pad located at one end of the bibulous fluid transport material without dissociation of a significant portion of the complex. If such dissociation occurs, then the reagent could bind to the analyte affixed to the pad, and not pass through the pad to react with the substrate in the bibulous fluid transport material.

**[0055]** The amount of reagent added to the liquid sample should fall within a range dictated by the amount of the analyte in the pad and by the anticipated amount of the analyte in the liquid sample. The amount should be at least that amount required to bind all the analyte present in the liquid sample. If the amount of reagent added is less than the amount of analyte present in the sample, the result reported will be lower than the true concentration of analyte in the liquid sample. However, the amount of the reagent added to the liquid sample should not be greater than that amount of analyte bound to the pad. If the amount of reagent added to the liquid sample is greater than the amount of analyte present in the pad, then the result reported will be greater than the true concentration of analyte in the liquid sample. Furthermore, the amount of analyte in the pad should be far in excess of that amount of analyte expected to be present in the liquid sample. If the amount of analyte present in the pad is insufficient to bind the unbound reagent in the liquid sample, then the result reported will again be greater than the true concentration of analyte in the liquid sample.

**[0056]** The reagent is linked to an enzyme that can react with the substrate that migrates from the other end of the fluid transport material. The reagent and the enzyme may be linked to one another through any means generally known to one of skill in the art, such as covalent bonding, disulfide bridging, and the like, and are commercially available from a wide variety of vendors (e.g., Sigma Chemical Co., St. Louis, MO, USA). The substrate and the enzyme are matched to one another, such that the enzyme acts on the substrate to produce a detectable reaction product. Examples of such enzymes include alkaline phosphatase,  $\beta$ -galactosidase, and peroxidase. For alkaline phosphatase, acceptable substrates may be, without limitation, *p*-nitrophenol phosphate; 4-methylumbelliferyl phosphate; or BCIP/NBT(5-bromo-4-chloro-indolyl phosphate/nitroblue tetrazolium). The alkaline phosphatase acts on *p*-nitrophenol phosphate substrate to produce the detectable reaction product *p*-nitrophenol. Alkaline phosphatase acts on 4-methylumbelliferyl phosphate to produce the fluorescent product methylumbel-

liferone. For  $\beta$ -galactosidase, acceptable substrates may include, without limitation, *o*-nitrophenyl  $\beta$ -D-galactoside or 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside ("X-gal"). The substrate *o*-nitrophenyl  $\beta$ -D-galactoside is cleaved by  $\beta$ -galactosidase to form D-galactoside and the detectable reaction product *o*-nitrophenol. For peroxidase, acceptable substrates include 3-amino-9-ethyl-carbazole ("AEC"); *o*-phenylenediamine dihydrochloride ("OPD"); 4-chloro-1-naphthol; 3,3'-diaminobenzidine tetrahydrochloride ("DAB"), and the like. The concentrations of substrate required to produce acceptable levels of detectable product for use in the present invention may be altered as required for the selected sample, assay, and conditions, and as such are easily ascertained by those of skill in the art. Other substrates for these enzymes are widely known to those in the art and may be substituted as appropriate. In addition, other enzyme/substrate pairs known to those in the art may also be employed in the methods and devices of the present invention, as appropriate.

[0057] The following examples illustrate the use of the present invention to detect and quantify particular components in a fluid sample. These examples are provided for illustration, and are not intended to be limiting. The person of skill will realize that the principles and techniques illustrated may be applied to detect a variety of analytes in a variety of fluid samples.

#### Example 1

[0058] This example illustrates how a device of the present invention was used to determine the presence and concentration (246 mg/dl) of glucose in a sample of whole blood.

[0059] A HEMOSEP L<sup>®</sup> membrane was affixed to an adhesive plastic backing and cut into 4 mm x 25 mm strips. Trinder reagent (15  $\mu$ l) (Sigma Chemical Co., St. Louis, MO) containing glucose oxidase (15,000 u/L), 4-aminoantipyrene (0.5 mM), *p*-hydroxybenzene sulfonate (20 mM) and peroxidase (10,000 u/L) at pH approximately 7.0 was dried onto one end of each test strip, comprising about one-half of the total strip area. This was accomplished by simultaneously dispensing 10  $\mu$ l of water to one end of the strip (the fluid sample side) and 10  $\mu$ l of Trinder reagent (at 5x concentration) to the other end of the strip (the diluent solution side). The strip was then air dried for 1 hour at room temperature.

[0060] After drying, 15  $\mu$ l of water was added to the outer end of the diluent solution side of the strip at the diluent solution application site and 15  $\mu$ l of whole blood was added simultaneously to the outer end of the fluid sample side of the strip at the fluid sample application site. The two liquids flowed towards each other, eventually yielding four distinct bands on the strip: a red blood cell band, a plasma band, a red/brown quinoneimine dye reaction product band (the reaction interface), and a band of unreacted Trinder reagent. As shown in Figures 4A-4F, the quinoneimine dye colored product interface band

continued to develop over several minutes, with its rate of development and final color intensity being proportional to the starting concentration of glucose in the sample.

#### 5 Example 2

[0061] This example illustrates how the present invention was applied to detect and quantify calcium in fetal calf serum.

10 [0062] Three polyethersulfone membranes were prepared as described in Example 1 and washed with 50  $\mu$ l of dilute HCl at pH 2.0. An example of a suitable polyethersulfone membrane for use in the present invention is a SUPOR<sup>®</sup> membrane (Pall-Gelman, Port Washington, NY). The person of ordinary skill will realize that other porous membranes with similar characteristics may also be applied to the present invention. Fifteen microliters of fetal calf serum containing 6.8, 13 and 18.6 mg/dl of calcium was added to the fluid sample side of each strip at the fluid sample application site while simultaneously adding 15  $\mu$ l of acidified Arsenazo III red/purple dye solution (Sigma Chemical Co., St. Louis, MO; catalog no. 588, prepared according to the manufacturer's instructions) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The blue reaction product, calcium-arsenazo III, developed at the product interface. As shown in Figures 5A-5I, both the time for color development and final intensity of blue color were proportional to the starting concentration of calcium in the fluid samples.

#### 35 Example 3

[0063] In this example, 1.2 g/dl and 1.9 g/dl concentrations of albumin in fetal calf serum were tested with the present invention using bromocresol green dye after adjustment to pH 5.5.

40 [0064] A HEMOSEP L<sup>®</sup> membrane was affixed to an adhesive plastic backing and cut into 4 mm x 25 mm strips. BCG reagent (15  $\mu$ l; Sigma Chemical Co., St. Louis, MO; catalog no. 631, prepared according to the manufacturer's instructions, except that a 10x concentrated solution of the reagent was used) was added to one end of each test strip, comprising about one-half of the total strip area. Serum (15  $\mu$ l) was added simultaneously to the outer end of the fluid sample side of the strip at the fluid sample application site. The two liquids flowed towards each other, eventually yielding three distinct bands on the strip: a serum band, a reaction product band (the reaction interface), including a dye/albumin complex, and a band of unreacted BCG reagent. Figure 5, 7 and 10 are graphical representations, and Figures 5 and 9 are numerical representations, of the color intensity of the reaction product versus time for both of the 1.2 g/dl and 1.9 g/dl samples. These graphs show that the assay performed in accordance with the subject invention produc-

es a substantial color intensity within five (5) seconds from application of the sample to the test strip of the present invention which is proportional to the original analyte concentration based on slope and final color intensity.

**[0065]** Figures 11 through 14 are digital photographs of the 1.2 g/dl and 1.9 g/dl test strips at 200 milliseconds (2.0 seconds after sample application to the test strip) and at 3.0 seconds after interface formation (5.0 seconds after sample application to the test strip). These digital images were recorded with a Sony Progressive 3CCD camera and the digital data was loaded to a Gateway PC, where a selected row of pixels of the images was analyzed using the INSPECTOR color absorption software by Matrox. Comparing Figure 11 with Figure 12 for the 1.2 g/dl sample and Figure 13 with Figure 14 for the 1.9 g/dl sample, a large increase in red absorption (green color) occurred in under three (3) seconds, as shown by line "R" in Figures 11 through 14, which represents the increase in red absorption (higher green color) across the reaction interface. In general, throughout this application an increase in absorption at a given color is reflected as a downward deflection in the color graph. Thus, the test protocol of the present invention produces an ascertainable reaction at a reaction rate such that the test can be performed in five (5) seconds from sample addition and in three (3) seconds after interface formation.

#### Example 4

**[0066]** This is another example using the protocol of Example 1, and illustrating how a device of the present invention was used to determine the presence and concentration of glucose in a sample of whole blood. In this example, 50 mg/dl, 150 mg/dl and 250 mg/dl samples were used.

**[0067]** A HEMOSEP L<sup>®</sup> membrane was affixed to an adhesive plastic backing and cut into 4 mm x 25 mm strips. 15  $\mu$ l Trinder reagent (Sigma Chemical Co., St. Louis, MO, catalog number 315, prepared according the manufacturer's instructions except a 5x concentration reagent solution was used) containing glucose oxidase (15,000 u/L), 4-aminoantipyrene (0.5 mM), p-hydroxybenzene sulfonate (20 mM) and peroxidase (10,000 u/L) at pH approximately 7.0 was added to one end of each test strip, comprising about one-half of the total strip area. Serum (15  $\mu$ l) was added simultaneously to the outer end of the fluid sample side of the strip at the fluid sample application site. The two liquids flowed towards each other, eventually yielding three distinct bands on the strip: a serum band, a red/brown quinoneimine dye reaction product band (the reaction interface), and a band of unreacted Trinder reagent. Figures 15 through 18 show the rate of development of the quinoneimine dye product. These graphs show that the assay performed in accordance with the subject invention produces a substantial color intensity within 2-4 minutes from application of the

sample to the test strip of the present invention.

#### Example 5

5 **[0068]** This is another example using the protocol of Example 2, and illustrating how the present invention was applied to detect and quantify calcium in fetal calf serum. In this example, 9.3 mg/dl and 13 mg/dl samples were used.

10 **[0069]** Polyethersulfone membranes were prepared as described in Example 1 and washed with 50  $\mu$ l of dilute HCl at pH 2.0. Fifteen microliters of fetal calf serum containing 9.3 mg/dl and 13 mg/dl of calcium was added to the fluid sample side of each strip at the fluid sample application site while simultaneously adding 15  $\mu$ l of acidified Arsenazo III red/purple dye solution to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The blue reaction product, calcium-arsenazo III, developed at the interface. Referring to Figures 19 and 20, which show graphical and numerical representations of this example, Figure 19 shows that the assay performed in accordance with the subject invention produces a substantial color intensity within one minute from application of the sample to the test strip of the present invention.

25 **[0070]** Figure 20 shows the relative stability of the interface width over time. The interface width approximately doubles from 100 seconds to 250+ seconds.

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#### Example 6

35 **[0071]** This example illustrates how the present invention was applied to detect and quantify total alkaline phosphatase in fetal calf serum. In this example, serial dilutions of alkaline phosphatase over a 10-fold range in concentrations were used.

40 **[0072]** Nitrocellulose membrane strips were washed with dilute NaOH at pH 10. Fifteen microliters of fetal calf serum containing various dilutions of alkaline phosphatase was applied to the fluid sample side of the membrane strip at the fluid sample application site while simultaneously adding 15  $\mu$ l of alkaline phosphatase reagent (Sigma Catalog No. 245, prepared according to the manufacturer's instructions except a 2x concentrated reagent solution was used) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, p-nitrophenol, developed at the product interface. Figure 25 is a digital photograph of one of the test strips, showing product formation at 15 seconds and 120 seconds after sample application to the test strip. These digital images were recorded as described for Example 3.

45 Figure 25 shows that the assay performed in accordance with the subject invention produces a detectable color intensity within 15 seconds from application of the sample to the test strip of the present invention, and a substantial

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color intensity by 120 seconds.

#### Example 7

[0073] This example shows how the present invention was applied to detect and quantify total bilirubin in fetal calf serum. In this example, 0.65 mg/dl, 2.55 mg/dl, 4.73 mg/dl, 9.39 mg/dl, and 19.57 mg/dl samples were used.

[0074] Polyethersulfone membranes were prepared as described in Example 1 and washed with water at pH 7.0. Fifteen microliters of a commercial standard solution containing 0.65 mg/dl, 2.55 mg/dl, 4.73 mg/dl, 9.39 mg/dl, or 19.57 mg/dl of bilirubin was added to the fluid sample side of each strip at the fluid sample application site while simultaneously adding 15  $\mu$ l of total bilirubin reagent (Sigma Catalog No. 550-4, reconstituted with sodium nitrite as directed by the manufacturer except a 20x concentrated reagent solution was used) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, azobilirubin, developed at the product interface. Figure 26 is a digital photograph of the 4.73 mg/dl. Figure 26 shows product formation at 15 seconds and 275 seconds after sample application to the test strip. These digital images were recorded as described for Example 3. Figure 26 shows that the assay performed in accordance with the subject invention produces a substantial color intensity within 15 seconds from application of the sample to the test strip of the present invention.

#### Example 8

[0075] This example illustrates how the present invention was applied to detect and quantify uric acid in fetal calf serum. In this example, samples ranging between 0.15 mg/dl and 15.5 mg/dl samples were used.

[0076] Polyethersulfone membranes were prepared as described in Example 1 and washed with water at pH 7.0. Fifteen microliters of fetal calf serum containing 0.15 mg/dl, 0.3 mg/dl, 0.6 mg/dl, 1.0 mg/dl, 3.7 mg/dl, 7.3 mg/dl, and 15.5 mg/dl of uric acid was added to the fluid sample side of each strip at the fluid sample application site while simultaneously adding 15  $\mu$ l of uric acid reagent (Sigma Catalog No. 685 except a 3.3x concentrated reagent solution was used) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, a quinoneimine dye having an maximal absorption at 520 nm, developed at the product interface. Figure 27 is a digital photograph of the 0.3 mg/dl test strip showing product formation within 5 seconds and 295 seconds after sample application to the test strip. These digital images were recorded as described for Example 3, and show a substantial product intensity developed after 295 seconds.

#### Example 9

[0077] This example illustrates how the present invention was applied to detect and quantify  $\gamma$ -glutamyl transferase in fetal calf serum. In this example, samples ranging between 8 Units/liter (U/L) and 725 U/L samples were used.

[0078] Nitrocellulose membranes were washed with water at pH 7.0. Fifteen microliters of fetal calf serum containing 8, 37, 107, 380, and 725 U/L of  $\gamma$ -glutamyl transferase was added to the fluid sample application site while simultaneously adding 15  $\mu$ l of  $\gamma$ -glutamyl transferase reagent (Sigma Catalog No. 419, prepared according to the manufacturer's instructions except a 2x concentrated reagent solution was used) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, 5-amino-2-nitrobenzoate, having an maximal absorption at 405 nm, developed at the product interface. Figure 28 is a digital photograph of the 380 U/L test strip showing product formation 5 seconds and 285 seconds after sample application to the test strip. These digital images were recorded as described for Example 3, and show that a substantial product development occurred after 285 seconds.

#### Example 10

[0079] This example illustrates how the present invention was applied to detect and quantify amylase in fetal calf serum. In this example, samples ranging between 88 Units/liter (U/L) and 16,800 U/L samples were used.

[0080] Nitrocellulose membranes were washed with water at pH 7.0. Fifteen microliters of fetal calf serum containing 88, 362, 1,680, and 16,800 U/L of amylase was added to the fluid sample application site, while simultaneously adding 15  $\mu$ l of amylase reagent (Sigma Catalog No. 419, prepared according to the manufacturer's instructions except a 2x concentrated reagent solution was used) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, p-nitrophenol, having an maximal absorption at 405 nm, developed at the product interface. Figure 29 is a digital photograph of the 16,800 U/L test strip showing product formation 35 seconds and 275 seconds after sample application to the test strip. These digital images were recorded as described for Example 3. A significant level of product development occurred after 275 seconds.

#### Example 11

[0081] This example illustrates how the present invention was applied to detect and quantify creatinine in fetal calf serum. In this example, samples ranging between 75 mg/dl and 300 mg/dl were used.

[0082] Nitrocellulose membranes were washed with water at pH 7.0. Fifteen microliters of fetal calf serum containing 75 mg/dl, 150 mg/dl, and 300 mg/dl of creatinine was added to the fluid sample application site, while simultaneously adding 15  $\mu$ l of creatinine reagent (Sigma Catalog No. 557-A and 557-B, prepared as instructed by the manufacturer except a 2x concentrated reagent solution was used) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, a Janovski complex, having an maximal absorption between 480 and 520 nm, developed at the interface. Figure 30 is a digital photograph of the 150 mg/dl test strip showing product formation 5 seconds and 295 seconds after sample application to the test strip. These digital images were recorded as described for Example 3, and show a significant level of the product formed after 295 seconds.

#### Example 12

[0083] This example illustrates how the present invention was applied to detect and quantify cholesterol. In this example, samples ranging between 61 mg/dl and 183 mg/dl were used.

[0084] Nitrocellulose membranes were washed with water at pH 7.0. Fifteen microliters of a commercial cholesterol standard solution containing 61 mg/dl, 96 mg/dl, 160 mg/dl, or 183 mg/dl of cholesterol was added to the fluid sample application site, while simultaneously adding 15  $\mu$ l of cholesterol reagent (Sigma Catalog No. 352) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, a quinoneimine dye having an maximal absorption at 500 nm, developed at the product interface. Figure 31 is a digital photograph of the 96 mg/dl test strip showing product formation 10 seconds and 300 seconds after sample application to the test strip. These digital images were recorded as described for Example 3. A detectable level of product was formed within 10 seconds after sample application to the test strip, and had increased substantially after 300 seconds.

#### Example 13

[0085] This example shows how the present invention was applied to detect and quantify total protein. In this example, samples ranging between 2.1 g/dl and 8.2 g/dl were used.

[0086] Nitrocellulose membranes were washed with water at pH 7.0. Fifteen microliters of fetal calf or dog serum containing 2.1 g/dl, 4.1 g/dl, 6.2 g/dl and 8.2 g/dl total protein was added to the fluid sample application site, while simultaneously adding 15  $\mu$ l of alkaline biuret reagent (Sigma Catalog No. 541, prepared according to the manufacturer's instructions except a 15x concentrated reagent solution was used) to the diluent solution side

of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, a purple copper-protein complex having an maximal absorption at 540 nm, developed at the product interface. Figure 32 is a digital photograph of the 6.2 g/dl test strip showing product formation 5 seconds and 295 seconds after sample application to the test strip. These digital images were recorded as described for Example 3. A significant level of product was detectable after 5 seconds, and a substantial amount of product was formed after 295 seconds.

#### Example 14

[0087] This example illustrating how the present invention was applied to detect and quantify magnesium in fetal calf serum. In this example, a sample containing 4.7 mg/dl magnesium was used.

[0088] Nitrocellulose membranes were washed with dilute NaOH at pH 10.0. Fifteen microliters of fetal calf serum containing 4.7 mg/dl of magnesium was added to the fluid sample application site, while simultaneously adding 15  $\mu$ l of magnesium reagent (Sigma Catalog No. 595, prepared as described by the manufacturer except a large excess of magnesium reagent ( $\approx$  100x) was used) to the diluent solution side of the strip at the diluent solution application site. In each case, the two liquids flowed towards each other and upon meeting formed a discrete interface. The reaction product, a calmagite-magnesium complex having an maximal absorption at 520 nm, developed at the product interface. Figure 33 is a digital photograph of the 4.7 mg/dl test strip showing product formation 5 seconds and 295 seconds after sample application to the test strip. These digital images were recorded as described for Example 3. The product was detectable after 5 seconds, and was substantial after 295 seconds.

#### Example 15

[0089] This example illustrating how the present invention was applied to detect and quantify a hapten, thyroxine ("T4"), bound to BSA as the analyte. The T4-BSA was bound to mouse anti-T4 conjugated to alkaline phosphatase. In this example, anti-T4-BSA at concentrations of 5  $\mu$ g/ml and 50  $\mu$ g/ml was used.

[0090] Nitrocellulose membranes were washed in Triton X-100 and rinsed with water. T4-BSA was bound to a pad on one side of the fluid transport material, while a substrate for alkaline phosphatase, *p*-nitrophenol phosphate, was dried into a pad at the opposite end of the fluid transport material. Mouse anti-T4-alkaline phosphatase conjugate was added to the pad containing the bound T4-BSA, while water was simultaneously added to the other pad containing the substrate. The liquids flowed toward each other and formed a discrete interface. The reaction product, *p*-nitrophenol, developed at the

product interface and was measured at 405 nm. Figure 34 is a digital photograph of the product formed from the reaction of mouse anti-T4-alkaline phosphatase conjugate with the substrate.

### Claims

1. A device for detecting and quantifying at least one analyte in a fluid sample suspected of containing said analyte by employing a liquid reactant capable of reacting with said analyte to form a detectable soluble reaction product, by relating said analyte to the detectable soluble reaction product of analyte and liquid reactant comprising:

a fluid transport material capable of absorbing a liquid and causing capillary flow of said liquid, said fluid transport material having a first zone for the application of said fluid sample suspected of containing said analyte, and a second zone for the application of said liquid reactant, arranged such that when said fluid sample is added to said first zone, and said liquid reactant is added to said second zone, said fluid sample flows in a first direction from a fluid sample edge toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge; and

a stable reaction interface formed at a juncture between and visually distinct from said fluid sample and said liquid reactant, whereby when said flowing fluid sample and said flowing liquid reactant meet, flow stops and said detectable reaction product is formed by a reaction between said liquid reactant and said analyte; and further comprising a means for calibrating a concentration of said liquid reagent, said means comprising a calibration zone comprising an amount of said analyte, said calibration zone being located between said first zone and said reaction interface, whereby in use, said analyte in said amount of analyte in the calibration zone flows in a first direction toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge;

whereby when said flowing analyte in said amount of said analyte and said flowing liquid reactant meet, flow stops and a detectable calibration product is formed by a reaction between said analyte in said amount of said analyte and said liquid reactant, and a stable calibration reaction interface is formed at a juncture between and visually distinct from said analyte in said amount of said analyte and said liquid reactant.

2. A device for detecting and quantifying at least one analyte in a fluid sample suspected of containing said analyte by employing a liquid reactant capable of reacting with said analyte to form a detectable soluble reaction product, by relating said analyte to the detectable soluble reaction product of analyte and liquid reactant comprising:

a fluid transport material capable of absorbing a liquid and causing capillary flow of said liquid, said fluid transport material having a first zone for the application of said fluid sample suspected of containing said analyte, and a second zone for the application of a liquid to a pad containing a reconstitutable reagent, arranged such that when said fluid sample is added to said first zone, and said liquid is added to said second zone, said fluid sample flows in a first direction from a fluid sample edge toward said second zone, said reagent is reconstituted and said reagent and said liquid form a liquid reagent containing a liquid reactant capable of reacting with said analyte to form a detectable reaction product, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge; and

a stable reaction interface formed at a juncture between and visually distinct from said fluid sample and said liquid reactant, whereby when said flowing fluid sample and said flowing liquid reactant meet, flow stops and said detectable reaction product is formed by a reaction between said liquid reactant and said analyte;

and further comprising a calibration zone comprising an amount of said analyte, said calibration zone being located between said first zone and said reaction interface, whereby in use, said analyte in said amount of analyte in the calibration zone flows in a first direction toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge;

whereby when said flowing analyte in said amount of said analyte and said flowing liquid reactant meet, flow stops and a detectable calibration product is formed by a reaction between said analyte in said amount of said analyte and said liquid reactant, and a stable calibration reaction interface is formed at a juncture between and visually distinct from said analyte in said amount of said analyte and said liquid reactant, said calibration interface further being adjacent to said reaction interface.

3. The device of claim 1 or claim 2, wherein said amount of said analyte added to said calibration zone is in

- excess of said amount of said analyte in said fluid sample, such that said amount of said analyte added to said calibration zone is sufficient to form an amount of said calibration product that enables determination of an amount of said liquid reactant.
4. The device of any of claims 1 to 3, wherein at least one of said first zone and said second zone contains a reagent.
  5. The device of any of claims 1 to 4, further comprising a pad located on said first zone.
  6. The device of claim 5, wherein said pad is effective to remove red blood cells from said fluid sample.
  7. The device of any of claims 1 to 6, wherein said detectable calibration product is formed at a rate sufficient for it to be detected within about fifteen seconds after application of said fluid sample to said fluid sample zone.
  8. The device of any of claims 1 to 7, wherein said fluid transport material is a nitrocellulose material cast on a backing material.
  9. The device of claim 8, wherein said backing material is one of polyvinylchloride or a polyester film (Mylar™).
  10. The device of any of claims 1 to 9, further comprising a means for detecting and optionally measuring an amount of said detectable reaction product.
  11. The device of claim 10, wherein said means for detecting and optionally measuring an amount of said detectable reaction product includes a member selected from the group consisting of absorbance, reflectance, transmission, fluorescence, luminescence, and conductance.
  12. The device of any of claims 1 to 11, further comprising:
    - a means for simultaneously applying said fluid sample to said first zone and said liquid reactant to said second zone; and
    - a sensor, said sensor being effective to detect said detectable reaction product.
  13. The device of claim 12, wherein said sensor is effective to detect said detectable reaction product having at least one desired wavelength of reflectance or absorption of  $\geq 250$  nanometers.
  14. The device of claim 12, wherein said sensor is effective to detect and measure an amount of said detectable reaction product that is proportional to a concentration of said analyte based on a peak intensity of reflectance or absorption of said detectable reaction product.
  15. The device of claim 12, wherein said sensor is effective to detect and measure a rate of formation of said detectable reaction product that is proportional to a concentration of said analyte.
  16. The device of claim 15, wherein said rate is calculated as a change over time of a peak intensity of reflectance or absorption of said detectable reaction product.
  17. The device of claim 16, wherein said peak intensity of reflectance or absorption is measured across a line substantially perpendicular to said reaction interface.
  18. The device of claim 16, wherein said peak intensity of reflectance or absorption is measured within a polygonal region encompassing all or a portion of said reaction interface.
  19. The device of claim 12, wherein said sensor is effective to detect and measure an amount of said detectable reaction product that is greater than or equal to a predetermined threshold intensity of reflectance or absorption indicative of the presence of said analyte.
  20. The device of any of claims 1 to 19, wherein said fluid transport material is capable of separating red blood cells from the whole blood.
  21. A method for detecting and quantifying at least one analyte in a fluid sample suspected of containing said analyte by employing a liquid reactant capable of reacting with said analyte to form a detectable soluble reaction product, by relating said analyte to the detectable soluble reaction product of analyte and liquid reactant comprising:
    - providing a fluid transport material capable of absorbing a liquid and causing capillary flow of said liquid, said fluid transport material having a first zone for the application of said fluid sample suspected of containing said analyte, and a second zone for the application of said liquid reactant;
    - adding said fluid sample to said first zone, and said liquid reactant to said second zone, wherein said fluid sample thereafter flows in a first direction from a fluid sample edge toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge;

whereby when said flowing fluid sample and said flowing liquid reactant meet, flow stops and said detectable reaction product is formed by a reaction between said liquid reactant and said analyte, and a stable reaction interface is formed at a juncture between and visually distinct from said fluid sample and said liquid reactant; and detecting said detectable reaction product.

- 22.** The method of claim 21, further comprising calibrating a concentration of said liquid reagent by applying an amount of said analyte to a calibration zone, said calibration zone being located between said first zone and said reaction interface, whereby when said amount of said analyte is added to said calibration zone, said analyte in said amount of analyte flows in a first direction toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge;

whereby said flowing analyte in said amount of said analyte and said flowing liquid reactant meet, flow stops and a detectable calibration product is formed by a reaction between said analyte in said amount of said analyte and said liquid reactant, and a stable calibration reaction interface is formed at a juncture between and visually distinct from said analyte in said excess amount of said analyte and said liquid reactant; and detecting said detectable calibration product.

- 23.** A method for detecting and quantifying at least one analyte in a fluid sample suspected of containing said analyte by employing a liquid reactant capable of reacting with said analyte to form a detectable soluble reaction product, by relating said analyte to the detectable soluble reaction product of analyte and liquid reactant comprising:

providing a fluid transport material capable of absorbing a liquid and causing capillary flow of said liquid, said fluid transport material having a first zone for the application of said fluid sample suspected of containing said analyte, and a second zone for the application of a liquid to a pad containing a reconstitutable reagent, wherein when said fluid sample is added to said first zone, and said liquid is added to said second zone, said fluid sample flows in a first direction from a fluid sample edge toward said second zone, said reagent is reconstituted and said reagent and said liquid form a liquid reagent containing a liquid reactant capable of reacting with said analyte to form a detectable reaction product, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge;

whereby when said flowing fluid sample and said flowing liquid reactant meet, flow stops and said detectable reaction product is formed by a reaction between said liquid reactant and said analyte, and a stable reaction interface is formed at a juncture between and visually distinct from said fluid sample and said liquid reactant; and detecting said detectable reaction product.

- 24.** The method of claim 23, further comprising calibrating a concentration of said liquid reagent by applying an amount of said analyte to a calibration zone, said calibration zone being located between said first zone and said reaction interface, whereby when said amount of said analyte is added to said calibration zone, said analyte in said amount of analyte flows in a first direction toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge;

whereby said flowing analyte in said amount of said analyte and said flowing liquid reactant meet, flow stops and a detectable calibration product is formed by a reaction between said analyte in said amount of said analyte and said liquid reactant, and a stable calibration reaction interface is formed at a juncture between and visually distinct from said analyte in said amount of said analyte and said liquid reactant, said calibration interface further being adjacent to said reaction interface; and measuring said calibration product at the same as at least one desired wavelength of reflectance or absorbance as said reaction product.

- 25.** The method of any of claims 21 to 24, wherein at least one of said first zone and said second zone contains a reagent.

- 26.** The method of any of claims 21 to 25, further comprising providing a pad located on said first zone.

- 27.** The method of claim 26, wherein said pad is effective to remove red blood cells from said fluid sample.

- 28.** The method of claim 21 or claim 23, wherein said detectable reaction product is formed at a rate sufficient for it to be detected within about fifteen seconds after application of said fluid sample to said fluid sample zone.

- 29.** The method of any of claims 21 to 28, wherein said at least one analyte includes a member selected from the group consisting of glucose, calcium, albumin, alkaline phosphatase, ammonia, bilirubin, uric acid, 2-glutamyl transferase, amylase, creatine kinase, creatinine, cholesterol, total protein, magnesium, lactate dehydrogenase, lipase, phosphorus, triglyceride, alanine aminotransferase, aspartate

aminotransferase, and blood urea nitrogen.

- 30.** The method of claim 22 or claim 24, wherein said amount of said analyte added to said calibration zone is in excess of said amount of said analyte in said fluid sample, such that said amount of said analyte added to said calibration zone is sufficient to form an amount of said calibration product that enables determination of an amount of said liquid reactant.
- 31.** The method of claim 22 or claim 24, wherein said detectable calibration product is formed at a rate that is proportional to a concentration of said analyte.
- 32.** The method of any of claims 21 to 29, wherein said detectable reaction product is formed in an amount greater than or equal to a predetermined threshold intensity of reflectance or absorption indicative of the presence of said analyte.
- 33.** A method for detecting and quantifying at least one analyte in a fluid sample suspected of containing said analyte by employing a reagent, capable of binding to said analyte and forming a detectable reaction product from a substrate, to relate an amount of said analyte to an amount of said detectable reaction product, comprising:

providing a fluid transport material capable of absorbing a liquid and causing capillary flow of said liquid, said fluid transport material having a first zone for the application of said fluid sample containing said reagent to a first pad containing said analyte bound substantially irreversibly to said first pad, and a second zone for the application of a liquid to a second pad containing a reconstitutable substrate;

adding said fluid sample containing said reagent to said first pad, and said liquid to said second pad, wherein said fluid sample thereafter flows in a first direction from a fluid sample edge toward said second zone, and said substrate is reconstituted by said liquid to form a liquid reactant capable of reacting with said reagent, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge;

whereby when said flowing fluid sample containing said reagent not bound by said analyte bound substantially irreversibly to said first pad and said flowing liquid reactant meet, flow stops and said detectable reaction product is formed by a reaction between said liquid reactant and said reagent, and a stable reaction interface is formed at a juncture between and visually distinct from said fluid sample and said liquid reactant; and

detecting said detectable reaction product.

**34.** The method of any of claims 21 to 33, wherein said fluid transport material is capable of separating red blood cells from the whole blood.

**35.** The method of any of claims 21 to 34, wherein said fluid transport material is a nitrocellulose material cast on a backing material.

**36.** The method of claim 35, wherein said backing material is one of polyvinylchloride or a polyester film (Mylar™).

**37.** The method of any of claims 21 to 36, further comprising measuring an amount of said detectable reaction product.

**38.** The method of any of claims 21 to 37, wherein said amount of said detectable reaction product is proportional to a concentration of said analyte based on a peak intensity of reflectance or absorption of said detectable reaction product.

**39.** The method of claim 37 or claim 38, wherein said step of measuring an amount of said detectable reaction product includes measuring a member selected from the group consisting of absorbance, reflectance, transmission, fluorescence, luminescence, and conductance.

**40.** The method of claims 39, wherein said detectable reaction product is measured by one of absorbance and reflectance.

**41.** The method of claim 40, wherein said one of absorbance and reflectance is measured at one or more wavelengths of  $\geq 250$  nanometers.

**42.** The method of any of claims 21 to 36, wherein said detectable reaction product is formed at a rate that is proportional to a concentration of said analyte.

**43.** The method of claim 42, wherein said rate is calculated as a change over time of a peak intensity of reflectance or absorption of said detectable reaction product.

**44.** The method of claim 43, wherein said peak intensity of reflectance or absorption is measured across a line substantially perpendicular to said reaction interface.

**45.** The method of claim 43, wherein said peak intensity of reflectance or absorption is measured within a polygonal region encompassing all or a portion of said reaction interface.

**46.** The method of any of claims 21 to 36, wherein said detectable reaction product is formed in an amount

- greater than or equal to a predetermined threshold intensity of reflectance or absorption indicative of the presence of said analyte.
47. The method of any of claims 21 to 46, further comprising measuring a member selected from the group consisting of: a distance between said fluid sample edge and a midpoint of said stable reaction interface, a distance between said liquid reactant edge and a midpoint of said stable reaction interface, a distance between said fluid sample edge and a midpoint of a stable liquid interface, a distance between said liquid reactant edge and a midpoint of a stable liquid interface, a distance between said midpoint of said stable reaction interface and said midpoint of a stable liquid interface, a reflectance or absorption of said detectable reaction product at a plurality of fixed time points, a reflectance or absorption of said stable liquid interface at a plurality of fixed time points, a rate of change of said reflectance or absorption of said detectable reaction product, an area of said stable reaction interface, an area of said stable liquid interface, a background level of said fluid sample, and a background level of said liquid reactant.
48. The method of claim 33, further comprising calibrating a concentration of said liquid reagent by applying an amount of said reagent to a calibration zone, said calibration zone being located between said first zone and said reaction interface, whereby when said reagent is added to said calibration zone, said reagent flows in a first direction toward said second zone, and said liquid reactant flows in a second direction opposite to that of said first direction and toward said first zone from a liquid reactant edge; whereby said flowing reagent and said flowing liquid reactant meet, flow stops and a detectable calibration product is formed by a reaction between said reagent and said liquid reactant, and a stable calibration reaction interface is formed at a juncture between and visually distinct from said reagent in said excess amount of said reagent and said liquid reactant; and detecting said detectable calibration product.
49. The method of claim 48, wherein said amount of said reagent added to said calibration zone is in excess of said amount of said reagent in said fluid sample, such that said amount of said reagent added to said calibration zone is sufficient to form an amount of said detectable calibration product that enables determination of an amount of said liquid reactant.
50. The method of claim 48, wherein said detectable calibration product is formed at a rate that is proportional to a concentration of said reagent.
51. The method of claims 31 and 50, wherein said rate
- is calculated as a change over time of a peak intensity of reflectance or absorption of said detectable calibration product.
52. The method of claim 51, wherein said peak intensity of reflectance or absorption is measured across a line substantially perpendicular to said calibration interface.
53. The method of claim 51, wherein said peak intensity of reflectance or absorption is measured within a polygonal region encompassing all or a portion of said calibration interface.
54. The method of claim 51, wherein said peak intensity of reflectance or absorption is measured across a line substantially perpendicular to said reaction interface.
55. The method of claim 51, wherein said peak intensity of reflectance or absorption is measured within a polygonal region encompassing all or a portion of said reaction interface.
56. The method of claim 48, wherein said detectable calibration product is formed in an amount greater than or equal to a predetermined threshold intensity of reflectance or absorption indicative of the presence of said reagent.
57. The method of claim 33, wherein said reagent comprises an enzyme or a portion thereof conjugated to a monoclonal antibody or a portion thereof; said enzyme or a portion thereof being capable of forming said detectable reaction product from said substrate; said monoclonal antibody or a portion thereof being capable of binding to said analyte.
58. The method of claim 57, wherein said enzyme is a member selected from the group consisting of alkaline phosphatase,  $\beta$ -galactosidase, and peroxidase.
59. The method of claim 57, wherein said portion of a monoclonal antibody is selected from the group consisting of an F(ab) fragment, an F(ab') fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, and an scFv fragment.
60. The method of claim 33, wherein said reagent comprises an enzyme or a portion thereof conjugated to a receptor protein or a portion thereof; said enzyme or a portion thereof being capable of forming said detectable reaction product from said substrate; said receptor protein or a portion thereof being capable of binding to said analyte.
61. The method of claim 33, wherein said reagent com-

prises an enzyme or a portion thereof conjugated to an antibody or a portion thereof;  
 said enzyme or a portion thereof being capable of forming said detectable reaction product from said substrate;  
 said antibody or a portion thereof being capable of binding to said analyte.

## Patentansprüche

1. Vorrichtung zum Nachweisen und quantitativen Bestimmen mindestens eines Analyten in einer flüssigen Probe, von der angenommen wird, dass sie den Analyten enthält, durch Einsetzen eines flüssigen Reaktanten, der zur Reaktion mit dem Analyten unter Bildung eines nachweisbaren löslichen Reaktionsproduktes in der Lage ist, durch Inbeziehungsetzen des Analyten zu dem nachweisbaren löslichen Reaktionsprodukt des Analyten und des flüssigen Reaktanten, umfassend:

ein flüssiges Transportmaterial, das zum Absorbieren einer Flüssigkeit und zum Verursachen einer Kapillarströmung dieser Flüssigkeit in der Lage ist, wobei das flüssige Transportmaterial eine erste Zone zum Aufbringen der flüssigen Probe, von der angenommen wird, dass sie den genannten Analyten enthält, und eine zweite Zone zum Aufbringen des flüssigen Reaktanten aufweist, die derart angeordnet sind, dass bei Zugabe der flüssigen Probe zu der ersten Zone und des flüssigen Reaktanten zu der zweiten Zone die flüssige Probe von einer Flüssigproben-Kante in einer ersten Richtung zu der zweiten Zone hin fließt und der flüssige Reaktant von einer Flüssigreaktanten-Kante aus in einer zweiten Richtung entgegengesetzt zu der ersten Richtung und zu der ersten Zone hin fließt, und  
 eine stabile Reaktions-Grenzfläche an einem Übergang zwischen der flüssigen Probe und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist, wobei beim Treffen der fließenden flüssigen Probe und des fließenden flüssigen Reaktanten das Fließen endet und das nachweisbare Reaktionsprodukt durch eine Reaktion zwischen dem flüssigen Reaktanten und dem Analyten gebildet wird, und weiter umfassend ein Mittel zum Kalibrieren einer Konzentration des flüssigen Reaktanten, wobei das Mittel eine Kalibrierungszone umfasst, die eine Menge des genannten Analyten umfasst, wobei die Kalibrierungszone zwischen der ersten Zone und der Reaktions-Grenzfläche angeordnet ist, wobei im Gebrauch der Analyt in der genannten Menge des Analyten in der Kalibrierungszone in einer ersten Richtung zu der zweiten Zone hin

fließt und der flüssige Reaktant von einer Flüssigreaktanten-Kante aus in einer zweiten Richtung, die der ersten Richtung entgegengesetzt ist und zu der ersten Zone hin fließt;  
 wobei beim Treffen des fließenden Analyten in der genannten Menge des Analyten und des fließenden flüssigen Reaktanten das Fließen endet und ein nachweisbares Kalibrierungsprodukt durch eine Reaktion zwischen dem Analyten in der genannten Menge des Analyten und dem flüssigen Reaktanten gebildet wird und eine stabile Kalibrierungsreaktions-Grenzfläche an einem Übergang zwischen dem genannten Analyten in der genannten Menge des Analyten und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist.

2. Vorrichtung zum Nachweisen und quantitativen Bestimmen mindestens eines Analyten in einer flüssigen Probe, von der angenommen wird, dass sie den Analyten enthält, durch Benutzen eines flüssigen Reaktanten, der zur einer Reaktion mit dem Analyten unter Bildung eines nachweisbaren löslichen Reaktionsproduktes in der Lage ist, durch Inbeziehungsetzen des Analyten zu dem nachweisbaren löslichen Reaktionsprodukt des Analyten und flüssigen Reaktanten, umfassend:

ein flüssiges Transportmaterial, das zum Absorbieren einer Flüssigkeit und zum Verursachen einer Kapillarströmung der genannten Flüssigkeit in der Lage ist, wobei das flüssige Transportmaterial eine erste Zone zum Aufbringen der genannten flüssigen Probe, von der angenommen wird, dass sie den Analyten enthält, und eine zweite Zone zum Aufbringen einer Flüssigkeit auf ein Kissen aufweist, das einen rekonstituierbaren Reagenten enthält, die derart angeordnet sind, dass bei der Zugabe der genannten flüssigen Probe zu der ersten Zone und der genannten Flüssigkeit zu der zweiten Zone die flüssige Probe von einer Flüssigkeitsproben-Kante in einer ersten Richtung zu der zweiten Zone hin fließt und das Reagenz rekonstituiert wird und das Reagenz und die Flüssigkeit ein flüssiges Reagenz bilden, das einen flüssigen Reaktanten enthält, der zur Reaktion mit dem genannten Analyten unter Bildung eines nachweisbaren Reaktionsproduktes in der Lage ist und dieser flüssige Reaktant von einer Flüssigreaktanten-Kante aus in einer zweiten Richtung, die der ersten Richtung entgegengesetzt ist und zu der ersten Zone hin fließt, und eine stabile Reaktions-Grenzfläche an einem Übergang zwischen der flüssigen Probe und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist, wobei beim Treffen der fließenden flüssigen Probe und des fließ-

- ßenden flüssigen Reaktanten das Fließen endet und das nachweisbare Reaktionsprodukt durch eine Reaktion zwischen dem Reaktanten und dem Analyten gebildet wird, und weiter umfassend eine Kalibrierungszone mit einer Menge des genannten Analyten, wobei die Kalibrierungszone zwischen der ersten Zone und der Reaktions-Grenzfläche angeordnet ist, wobei im Gebrauch der Analyt in der genannten Menge des Analyten in der Kalibrierungszone in einer ersten Richtung zur zweiten Zone hin fließt und der flüssige Reaktant von einer Flüssigreaktanten-Kante aus in einer zweiten Richtung, die der ersten Richtung entgegengesetzt ist und zu der ersten Zone hin fließt; wobei beim Treffen des fließenden Analyten in der genannten Menge des Analyten und des fließenden flüssigen Reaktanten das Fließen endet und ein nachweisbares Kalibrierungsprodukt durch eine Reaktion zwischen dem Analyten in der genannten Menge des Analyten und dem flüssigen Reaktanten gebildet wird und eine stabile Kalibrierungsreaktions-Grenzfläche an einem Übergang zwischen dem Analyten in der genannten Menge des Analyten und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist, wobei die Kalibrierungs-Grenzfläche weiter benachbart der Reaktions-Grenzfläche liegt.
3. Vorrichtung nach Anspruch 1 oder Anspruch 2, worin die genannte Menge des Analyten, die zu der Kalibrierungszone hinzugegeben wird, im Überschuss zu der Menge des Analyten in der genannten flüssigen Probe ist, sodass die genannte Menge des Analyten, die zu der Kalibrierungszone hinzugegeben wird, genügt, um eine Menge des Kalibrierungsproduktes zu bilden, die die Bestimmung einer Menge des flüssigen Reaktanten gestattet,
4. Vorrichtung nach irgendeinem der Ansprüche 1 bis 3, worin mindestens eine von der ersten Zone und der zweiten Zone ein Reagenz enthält.
5. Vorrichtung nach irgendeinem der Ansprüche 1 bis 4, weiter umfassend ein Kissen, das auf der ersten Zone angeordnet ist.
6. Vorrichtung nach Anspruch 5, worin das genannte Kissen wirksam ist zum Entfernen roter Blutzellen aus der Flüssigkeitsprobe.
7. Vorrichtung nach irgendeinem der Ansprüche 1 bis 6, worin das nachweisbare Kalibrierungsprodukt mit einer Rate gebildet wird, die genügt, um seinen Nachweis innerhalb von etwa 15 Sekunden nach dem Aufbringen der genannten flüssigen Probe auf die Zone der flüssigen Probe zu gestatten.
8. Vorrichtung nach irgendeinem der Ansprüche 1 bis 7, worin das flüssige Transportmaterial ein Nitrocellulosematerial ist, das auf ein rückwärtiges Material gegossen ist.
9. Vorrichtung nach Anspruch 8, worin das rückwärtige Material eines aus Polyvinylchlorid oder einem Polyesterfilm (Mylar™) ist.
10. Vorrichtung nach irgendeinem der Ansprüche 1 bis 9, weiter umfassend ein Mittel zum Nachweisen und wahlweisen Messen einer Menge des genannten nachweisbaren Reaktionsproduktes.
11. Vorrichtung nach Anspruch 10, worin das Mittel zum Nachweisen und wahlweisen Messen einer Menge des nachweisbaren Reaktionsproduktes eines einschließt, ausgewählt aus der Gruppe bestehend aus Absorption, Reflexion, Durchlässigkeit, Fluoreszenz, Lumineszenz und Leitfähigkeit.
12. Vorrichtung nach irgendeinem der Ansprüche 1 bis 11, weiter umfassend:
- ein Mittel zum gleichzeitigen Aufbringen der Flüssigkeitsprobe auf die erste Zone und des flüssigen Reaktanten auf die zweite Zone und einen Sensor, der wirksam ist, das nachweisbare Reaktionsprodukt nachzuweisen.
13. Vorrichtung nach Anspruch 12, worin der Sensor wirksam ist, das nachweisbare Reaktionsprodukt, das mindestens eine erwünschte Wellenlänge der Reflexion oder Absorption von  $\geq 250$  Nanometern hat, nachzuweisen.
14. Vorrichtung nach Anspruch 12, worin der Sensor wirksam ist, eine Menge des nachweisbaren Reaktionsproduktes nachzuweisen und zu messen, die proportional einer Konzentration des genannten Analyten ist, basierend auf einer Peakintensität der Reflexion oder Absorption des nachweisbaren Reaktionsproduktes.
15. Vorrichtung nach Anspruch 12, worin der genannte Sensor wirksam ist, eine Bildungsrate des nachweisbaren Reaktionsproduktes nachzuweisen und zu messen, die proportional einer Konzentration des genannten Analyten ist.
16. Vorrichtung nach Anspruch 15, worin die genannte Rate als eine Änderung einer Peakintensität der Reflexion oder Absorption des nachweisbaren Reaktionsproduktes über die Zeit errechnet ist.
17. Vorrichtung nach Anspruch 16, worin die Peakintensität der Reflexion oder Absorption über eine Linie gemessen ist, die im Wesentlichen senkrecht zu der

Reaktions-Grenzfläche liegt.

18. Vorrichtung nach Anspruch 16, worin die Peakintensität der Reflexion oder Absorption innerhalb einer polygonalen Region gemessen ist, die die gesamte oder einen Teil der Reaktions-Grenzfläche umfasst. 5
19. Vorrichtung nach Anspruch 12, worin der Sensor wirksam ist, eine Menge des nachweisbaren Reaktionsproduktes nachzuweisen und zu messen, die größer ist oder gleich einer vorbestimmten Schwellenintensität der Reflexion oder Absorption, das die Anwesenheit des genannten Analyten anzeigt. 10
20. Vorrichtung nach irgendeinem der Ansprüche 1 bis 19, worin das flüssige Transportmaterial in der Lage ist, rote Blutzellen aus dem gesamten Blut abzutrennen. 15
21. Verfahren zum Nachweisen und quantitativen Bestimmen mindestens eines Analyten in einer flüssigen Probe, von der angenommen wird, dass sie diesen Analyten enthält, durch Anwenden eines flüssigen Reaktanten, der zum Reagieren mit dem Analyten unter Bildung eines nachweisbaren löslichen Reaktionsproduktes in der Lage ist, durch Inbeziehungsetzen des Analyten zu dem nachweisbaren löslichen Reaktionsprodukt aus Analyten und flüssigem Reaktanten, umfassend: 20

Bereitstellen eines flüssigen Transportmaterials, das zum Absorbieren einer Flüssigkeit und zum Verursachen eines Kapillarflusses der genannten Flüssigkeit in der Lage ist, wobei das flüssige Transportmaterial eine erste Zone für das Aufbringen der flüssigen Probe, von der angenommen wird, dass sie den Analyten enthält, und eine zweite Zone für das Aufbringen des flüssigen Reaktanten aufweist; 25

Hinzugeben der flüssigen Probe zu der ersten Zone und des flüssigen Reaktanten zu der zweiten Zone, wobei die flüssige Probe danach von einer Flüssigproben-Kante in einer ersten Richtung zur zweiten Zone hin fließt und der flüssige Reaktant von einer Flüssigreaktanten-Kante in einer zweiten Richtung, entgegengesetzt zu der ersten Richtung, und zu der ersten Zone hin fließt; 30

wobei beim Treffen der fließenden flüssigen Probe und des fließenden flüssigen Reaktanten das Fließen endet und das nachweisbare Reaktionsprodukt durch Reaktion zwischen dem flüssigen Reaktanten und dem Analyten und eine stabile Reaktions-Grenzfläche an einem Übergang zwischen der flüssigen Probe und dem flüssigen Reaktanten und visuell davon unterscheidbar gebildet wird, und 35

Nachweisen des nachweisbaren Reaktionspro-

duktes.

22. Verfahren nach Anspruch 21, weiter umfassend das Kalibrieren einer Konzentration des flüssigen Reagenz durch Aufbringen einer Menge des Analyten auf eine Kalibrierungszone, wobei diese Kalibrierungszone zwischen der genannten ersten Zone und der Reaktions-Grenzfläche angeordnet ist, wobei bei Zugabe der Menge des Analyten zu der Kalibrierungszone der Analyt in der genannten Menge des Analyten in einer ersten Richtung zur zweiten Zone hin fließt und der flüssige Reaktant von einer Flüssigreaktanten-Kante in einer zweiten Richtung entgegengesetzt zu der ersten Richtung und zu der ersten Zone hin fließt; 40
- wobei beim Treffen des fließenden Analyten in der genannten Menge des Analyten und des fließenden flüssigen Reaktanten das Fließen endet und ein nachweisbares Kalibrierungsprodukt durch eine Reaktion zwischen dem Analyten in der genannten Menge des Analyten und dem flüssigen Reaktanten und eine stabile Kalibrierungsreaktions-Grenzfläche an einem Übergang zwischen dem Analyten in der überschüssigen Menge des Analyten und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist, und 45
- Nachweisen des nachweisbaren Kalibrierungsproduktes.
23. Verfahren zum Nachweisen und quantitativen Bestimmen mindestens eines Analyten in einer flüssigen Probe, von der angenommen wird, dass sie den Analyten enthält, durch Einsetzen eines flüssigen Reaktanten, der zum Reagieren mit dem Analyten zur Bildung eines nachweisbaren löslichen Reaktionsproduktes in der Lage ist, durch Inbeziehungsetzen des Analyten zu dem nachweisbaren löslichen Reaktionsprodukt des Analyten und des flüssigem Reaktanten, umfassend: 50
- Bereitstellen eines flüssigen Transportmaterials, das zum Absorbieren einer Flüssigkeit und zum Verursachen einer Kapillarströmung der Flüssigkeit in der Lage ist, wobei das flüssige Transportmaterial eine erste Zone zum Aufbringen der flüssigen Probe, von der angenommen wird, dass sie den Analyten enthält, und eine zweite Zone zum Aufbringen einer Flüssigkeit auf ein Kissen, das ein rekonstituierbares Reagenz enthält, aufweist, wobei bei der Zugabe der flüssigen Probe zu der ersten Zone und der Zugabe der Flüssigkeit zu der zweiten Zone die flüssige Probe von einer Flüssigproben-Kante in einer ersten Richtung zur zweiten Zone hin fließt, das Reagenz rekonstituiert wird und das Reagenz und die Flüssigkeit ein flüssiges Reagenz bilden, das einen flüssigen Reaktanten enthält, der zum Reagieren mit dem Analyten 55

- zur Bildung eines nachweisbaren Reaktionsproduktes in der Lage ist, und der flüssige Reaktant von einer Flüssigreaktanten-Kante in einer zweiten Richtung entgegengesetzt zu der ersten Richtung und zu der ersten Zone hin fließt; wobei beim Treffen der fließenden flüssigen Probe und des fließenden flüssigen Reaktanten das Fließen endet und das nachweisbare Reaktionsprodukt durch Reaktion zwischen dem flüssigen Reaktanten und dem Analyten und eine stabile Reaktions-Grenzfläche an einem Übergang zwischen der flüssigen Probe und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist, und Nachweisen des nachweisbaren Reaktionsproduktes.
24. Verfahren nach Anspruch 23, weiter umfassend das Kalibrieren einer Konzentration des flüssigen Reagenz durch Aufbringen einer Menge des Analyten auf eine Kalibrierungszone, wobei die Kalibrierungszone zwischen der ersten Zone und der Reaktions-Grenzfläche angeordnet ist, wobei bei der Zugabe der Menge des Analyten zu der Kalibrierungszone der Analyt in der genannten Menge des Analyten in einer ersten Richtung zur zweiten Zone hin fließt und der flüssige Reaktant von einer Flüssigreaktanten-Kante in einer zweiten Richtung entgegengesetzt der ersten Richtung und zu der ersten Zone hin fließt; wobei beim Treffen des fließenden Analyten in der genannten Menge des Analyten und des fließenden flüssigen Reaktanten das Fließen endet und ein nachweisbares Kalibrierungsprodukt durch eine Reaktion zwischen dem Analyten in der genannten Menge des Analyten und dem flüssigen Reaktanten sowie eine stabile Kalibrierungsreaktions-Grenzfläche an einem Übergang zwischen dem Analyten in der genannten Menge des Analyten und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist, wobei die Kalibrierungs-Grenzfläche benachbart der Reaktions-Grenzfläche angeordnet ist, und Messen des Kalibrierungsproduktes bei der gleichen mindestens einen erwünschten Wellenlänge der Reflexion oder Absorption des genannten Reaktionsproduktes.
25. Verfahren nach irgendeinem der Ansprüche 21 bis 24, worin mindestens eine von der ersten Zone und der zweiten Zone ein Reagenz enthält.
26. Verfahren nach irgendeinem der Ansprüche 21 bis 25, weiter umfassend das Bereitstellen eines Kissens, das auf der ersten Zone angeordnet ist.
27. Verfahren nach Anspruch 26, worin das Kissen wirksam ist, rote Blutzellen aus der flüssigen Probe zu entfernen.
28. Verfahren nach Anspruch 21 oder Anspruch 23, worin das nachweisbare Reaktionsprodukt in einer Rate gebildet wird, die genügt, um es innerhalb von etwa 15 Sekunden nach dem Aufbringen der flüssigen Probe auf die Flüssigproben-Zone nachzuweisen.
29. Verfahren nach irgendeinem der Ansprüche 21 bis 28, wobei der mindestens eine Analyt ein Glied einschließt, ausgewählt aus der Gruppe bestehend aus Glukose, Calcium, Albumin, alkalischer Phosphatase, Ammoniak, Bilirubin, Harnsäure, 2-Glutamyl-Transferase, Amylase, Kreatin-Kinase, Kreatinin, Cholesterin, Gesamtprotein, Magnesium, Lactat-Dehydrogenase, Lipase, Phosphor, Triglycerid, Alanin-Aminotransferase, Aspartat-Aminotransferase und Blutharnstoff-Stickstoff.
30. Verfahren nach Anspruch 22 oder Anspruch 24, worin die genannte Menge des genannten Analyten, die zu der Kalibrierungszone hinzugegeben wird, im Überschuss zu der Menge des Analyten in der flüssigen Probe ist, sodass die genannte Menge des genannten Analyten, die zu der Kalibrierungszone hinzugegeben wird, genügt, um eine Menge des Kalibrierungsproduktes zu bilden, das die Bestimmung einer Menge des flüssigen Reaktanten ermöglicht.
31. Verfahren nach Anspruch 22 oder Anspruch 24, wobei das nachweisbare Kalibrierungsprodukt mit einer Rate gebildet wird, die proportional zu einer Konzentration des Analyten ist.
32. Verfahren nach irgendeinem der Ansprüche 21 bis 29, worin das nachweisbare Reaktionsprodukt in einer Menge gebildet wird, die größer als oder gleich einer vorbestimmten Schwellenintensität der Reflexion oder Absorption ist, die die Anwesenheit des Analyten anzeigt.
33. Verfahren zum Nachweisen und quantitativen Bestimmen mindestens eines Analyten in einer flüssigen Probe, von der angenommen wird, dass sie den Analyten enthält, durch Einsetzen eines Reagenz, das zum Binden an den Analyten und zum Bilden eines nachweisbaren Reaktionsproduktes von einem Substrat in der Lage ist, um eine Menge des Analyten mit einer Menge des nachweisbaren Reaktionsproduktes in Beziehung zu setzen, umfassend:
- Bereitstellen eines flüssigen Transportmaterials, das zum Absorbieren einer Flüssigkeit und zum Verursachen eines Kapillarflusses dieser Flüssigkeit in der Lage ist, wobei das flüssige Transportmaterial eine erste Zone zum Aufbringen der flüssigen Probe, die das Reagenz enthält, auf ein Kissen, das den Analyten enthält, im Wesentlichen irreversibel an das Kissen ge-

- bunden, und eine zweite Zone zum Aufbringen einer Flüssigkeit auf ein zweites Kissen, das ein rekonstituierbares Substrat enthält, aufweist; Hinzugeben der flüssigen Probe, die das Reagenz enthält, auf das erste Kissen und der Flüssigkeit auf das zweite Kissen, wobei die flüssige Probe danach von einer Flüssigproben-Kante in einer ersten Richtung zur zweiten Zone hin fließt und das Substrat durch die Flüssigkeit zur Bildung eines flüssigen Reaktanten rekonstituiert wird, der in der Lage ist, mit dem Reagenz zu reagieren, und der flüssige Reaktant von einer Flüssigreaktanten-Kante in einer zweiten Richtung entgegengesetzt zu der ersten Richtung und zur ersten Zone hin fließt; wobei, wenn sich die fließende flüssige Probe, die das Reagenz enthält, das nicht durch den Analyten gebunden ist, der im Wesentlichen irreversibel an das erste Kissen gebunden ist, und der fließende flüssige Reaktant treffen, das Fließen endet und das nachweisbare Reaktionsprodukt durch eine Reaktion zwischen dem flüssigen Reaktanten und dem Reagenz und eine stabile Reaktions-Grenzfläche an einem Übergang zwischen der flüssigen Probe und dem flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar ist, und Nachweisen des nachweisbaren Reaktionsproduktes.
- 34.** Verfahren nach irgendeinem der Ansprüche 21 bis 33, worin das flüssige Transportmaterial in der Lage ist, rote Blutzellen vom ganzen Blut abzutrennen.
- 35.** Verfahren nach irgendeinem der Ansprüche 21 bis 34, worin das flüssige Transportmaterial ein Nitrocellulosematerial ist, das auf ein rückwärtiges Material gegossen ist.
- 36.** Verfahren nach Anspruch 35, worin das rückwärtige Material eines von Polyvinylchlorid oder einem Polyesterfilm (Mylar™) ist.
- 37.** Verfahren nach irgendeinem der Ansprüche 21 bis 36, weiter umfassend das Messen einer Menge des nachweisbaren Reaktionsproduktes.
- 38.** Verfahren nach irgendeinem der Ansprüche 21 bis 37, worin die genannte Menge des nachweisbaren Reaktionsproduktes proportional einer Konzentration des Analyten ist, auf der Grundlage einer Peakintensität der Reflexion oder Absorption des nachweisbaren Reaktionsproduktes.
- 39.** Verfahren nach Anspruch 37 oder Anspruch 38, worin die Stufe des Messens einer Menge des nachweisbaren Reaktionsproduktes das Messen einer Eigenschaft einschließt, ausgewählt aus der Gruppe
- bestehend aus Absorption, Reflexion, Durchlässigkeit, Fluoreszenz, Lumineszenz und Leitfähigkeit.
- 40.** Verfahren nach Anspruch 39, worin das nachweisbare Reaktionsprodukt durch eines von Absorption und Reflexion gemessen wird.
- 41.** Verfahren nach Anspruch 40, worin das eine von Absorption und Reflexion bei einer oder mehreren Wellenlängen von  $\geq 250$  Nanometern gemessen wird.
- 42.** Verfahren nach irgendeinem der Ansprüche 21 bis 36, worin das nachweisbare Reaktionsprodukt in einer Rate gebildet wird, die proportional einer Konzentration des Analyten ist.
- 43.** Verfahren nach Anspruch 42, worin die Rate als eine Änderung einer Peakintensität der Reflexion oder der Absorption des nachweisbaren Reaktionsproduktes über die Zeit errechnet wird.
- 44.** Verfahren nach Anspruch 43, worin die Peakintensität der Reflexion oder Absorption über eine Linie gemessen wird, die im Wesentlichen senkrecht zur Reaktions-Grenzfläche verläuft.
- 45.** Verfahren nach Anspruch 43, worin die Peakintensität der Reflexion oder Absorption innerhalb einer polygonalen Region gemessen wird, die die gesamte oder einen Teil der Reaktions-Grenzfläche umfasst.
- 46.** Verfahren nach irgendeinem der Ansprüche 21 bis 36, worin das nachweisbare Reaktionsprodukt in einer Menge größer als oder gleich einer vorbestimmten Schwellenintensität der Reflexion oder Absorption gebildet wird, die die Anwesenheit des Analyten anzeigt.
- 47.** Verfahren nach irgendeinem der Ansprüche 21 bis 46, weiter umfassend das Messen eines Gliedes, ausgewählt aus der Gruppe bestehend aus: einem Abstand zwischen der Flüssigproben-Kante und einem Mittelpunkt der stabilen Reaktions-Grenzfläche, einem Abstand zwischen der Flüssigreaktanten-Kante und einem Mittelpunkt der stabilen Reaktions-Grenzfläche, einem Abstand zwischen der Flüssigproben-Kante und einem Mittelpunkt einer stabilen Flüssigkeits-Grenzfläche, einem Abstand zwischen der Flüssigreaktanten-Kante und einem Mittelpunkt einer stabilen Flüssigkeits-Grenzfläche, einem Abstand zwischen dem Mittelpunkt der stabilen Reaktions-Grenzfläche und dem Mittelpunkt einer stabilen Flüssigkeits-Grenzfläche, einer Reflexion oder Absorption des nachweisbaren Reaktionsproduktes an einer Vielzahl festgelegter Zeitpunkte, einer Reflexion oder Absorption der stabilen Flüssig-

- sigkeits-Grenzfläche an einer Vielzahl festgelegter Zeitpunkte, einer Rate der Änderung der Reflexion oder Absorption des nachweisbaren Reaktionsproduktes, einer Fläche der stabilen Reaktions-Grenzfläche, einer Fläche der stabilen Flüssigkeits-Grenzfläche, einem Hintergrundniveau der flüssigen Probe und einem Hintergrundniveau des flüssigen Reaktanten.
- 48.** Verfahren nach Anspruch 33, weiter umfassend das Kalibrieren einer Konzentration des flüssigen Reagenz durch Aufbringen einer Menge des Reagenz auf eine Kalibrierungszone, wobei die Kalibrierungszone zwischen der ersten Zone und der Reaktions-Grenzfläche angeordnet ist, wobei bei Zugabe des Reagenz zu der Kalibrierungszone das Reagenz in einer ersten Richtung zu der zweiten Zone hin fließt und der flüssige Reaktant von einer Flüssigreaktanten-Kante in einer zweiten Richtung entgegengesetzt zu der ersten Richtung und zu der ersten Zone hin fließt;  
wobei beim Treffen des fließenden Reagenz und des fließenden flüssigen Reaktanten das Fließen endet und ein nachweisbares Kalibrierungsprodukt durch eine Reaktion zwischen dem Reagenz und dem flüssigen Reaktanten und eine stabile Kalibrierungsreaktions-Grenzfläche an einem Übergang zwischen dem Reagenz in der Überschussmenge des Reagenz und des flüssigen Reaktanten gebildet wird und visuell davon unterscheidbar davon ist, und Nachweisen des nachweisbaren Kalibrierungsproduktes.
- 49.** Verfahren nach Anspruch 48, worin die Menge des Reagenz, die zu der Kalibrierungszone hinzugegeben wird, im Überschuss zu der Menge des Reagenz in der flüssigen Probe ist, sodass die genannte Menge des Reagenz, die zu der Kalibrierungszone hinzugegeben wird, zur Bildung einer Menge des nachweisbaren Kalibrierungsproduktes genügt, die die Bestimmung einer Menge des flüssigen Reaktanten ermöglicht.
- 50.** Verfahren nach Anspruch 48, worin das nachweisbare Kalibrierungsprodukt in einer Rate gebildet wird, die proportional einer Konzentration des Reagenz ist.
- 51.** Verfahren nach Anspruch 31 und 50, worin diese Rate als eine Änderung einer Peakintensität der Reflexion oder Absorption des nachweisbaren Kalibrierungsproduktes über die Zeit errechnet wird.
- 52.** Verfahren nach Anspruch 51, dass die Peakintensität der Reflexion oder Absorption über eine Linie gemessen wird, die im Wesentlichen senkrecht zur Kalibrierungs-Grenzfläche verläuft.
- 53.** Verfahren nach Anspruch 51, worin die Peakintensität der Reflexion oder Absorption innerhalb einer polygonalen Region gemessen wird, die die gesamte oder einen Teil der Kalibrierungs-Grenzfläche umfasst.
- 54.** Verfahren nach Anspruch 51, worin die Peakintensität der Reflexion oder Absorption über eine Linie gemessen wird, die im Wesentlichen senkrecht zur Reaktions-Grenzfläche verläuft.
- 55.** Verfahren nach Anspruch 51, wobei die Peakintensität der Reflexion oder Absorption innerhalb einer polygonalen Region gemessen wird, die die gesamte oder einen Teil der Reaktions-Grenzfläche umfasst.
- 56.** Verfahren nach Anspruch 48, wobei das nachweisbare Kalibrierungsprodukt in einer Menge gebildet wird, die größer ist als oder gleich einer vorbestimmten Schwellenintensität der Reflexion oder Absorption, die die Anwesenheit des Reagenz anzeigt.
- 57.** Verfahren nach Anspruch 33, worin das Reagenz ein Enzym oder einen Teil davon umfasst, der mit einem monoklonalen Antikörper oder einem Teil davon konjugiert ist;  
wobei das Enzym oder ein Teil davon in der Lage ist, das nachweisbare Reaktionsprodukt aus dem Substrat zu bilden; der monoklonale Antikörper oder ein Teil davon in der Lage ist, an den Analyten zu binden.
- 58.** Verfahren nach Anspruch 57, worin das Enzym ein Glied ist ausgewählt aus der Gruppe bestehend aus alkalischer Phosphatase,  $\beta$ -Galactosidase und Peroxidase.
- 59.** Verfahren nach Anspruch 57, worin der Teil eines monoklonalen Antikörpers ausgewählt aus der Gruppe bestehend aus einem F(ab)-Fragment, einem F(ab')-Fragment, einem F(ab')<sub>2</sub>-Fragment, einem Fv-Fragment und einem scFv-Fragment.
- 60.** Verfahren nach Anspruch 33, worin das Reagenz ein Enzym oder einen Teil davon umfasst, der mit einem Rezeptorprotein oder einem Teil davon konjugiert ist;  
wobei das Enzym oder ein Teil davon in der Lage ist, das nachweisbare Reaktionsprodukt von dem Substrat zu bilden;  
wobei das Rezeptorprotein oder ein Teil davon in der Lage ist, an den Analyten zu binden.
- 61.** Verfahren nach Anspruch 33, worin das Reagenz ein Enzym oder einen Teil davon umfasst, der an ein Abtid oder ein Teil davon gebunden ist;  
wobei das Enzym oder ein Teil davon in der Lage

ist, das nachweisbare Reaktionsprodukt von dem Substrat zu bilden;  
das genannte Abtid oder ein Teil davon in der Lage ist, an den Analyten zu binden.

## Revendications

1. Dispositif pour détecter et quantifier au moins un analyte dans un échantillon de fluide suspecté de contenir ledit analyte, par l'emploi d'un réactant liquide capable de réagir avec ledit analyte pour former un produit réactionnel soluble détectable, par mise en relation dudit analyte avec le produit réactionnel soluble détectable de l'analyte et du réactant liquide, comprenant :

un matériau de transport de fluide capable d'absorber un liquide et de provoquer un écoulement capillaire dudit liquide, ledit matériau de transport de fluide ayant une première zone pour l'application dudit échantillon de fluide suspecté de contenir ledit analyte, et une deuxième zone pour l'application dudit réactant liquide, agencées de façon que, lorsque ledit échantillon de fluide est ajouté à ladite première zone, et ledit réactant liquide est ajouté à ladite deuxième zone, l'échantillon de fluide s'écoule dans une première direction allant d'un bord d'échantillon de fluide vers ladite deuxième zone, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ; et

une interface réactionnelle stable formée à une jonction entre ledit échantillon de fluide et ledit réactant liquide et visuellement distincte dudit échantillon de fluide et dudit réactant liquide, grâce à quoi, quand ledit échantillon de fluide s'écoulant et ledit réactant liquide s'écoulant se rencontrent, l'écoulement s'arrête et ledit produit réactionnel détectable est formé par une réaction entre ledit réactant liquide et ledit analyte ;

et comprenant en outre un moyen pour étalonner une concentration dudit réactif liquide, ledit moyen comprenant une zone d'étalonnage comprenant une quantité dudit analyte, ladite zone d'étalonnage étant localisée entre ladite première zone et ladite interface réactionnelle, grâce à quoi, lors de l'utilisation, ledit analyte en ladite quantité d'analyte dans la zone d'étalonnage s'écoule dans une première direction vers ladite deuxième zone, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ; grâce à quoi, quand ledit analyte s'écoulant en

ladite quantité dudit analyte et ledit réactant liquide s'écoulant se rencontrent, l'écoulement s'arrête et un produit d'étalonnage détectable est formé par une réaction entre ledit analyte en ladite quantité dudit analyte et ledit réactant liquide, et une interface réactionnelle d'étalonnage stable est formée à une jonction entre ledit analyte en ladite quantité dudit analyte et ledit réactant liquide, et visuellement distincte dudit analyte et dudit réactant liquide.

2. Dispositif pour détecter et quantifier au moins un analyte dans un échantillon de fluide suspecté de contenir ledit analyte, par l'emploi d'un réactant liquide capable de réagir avec ledit analyte pour former un produit réactionnel soluble détectable, par mise en relation dudit analyte avec le produit réactionnel soluble détectable de l'analyte et du réactant liquide, comprenant :

un matériau de transport de fluide capable d'absorber un liquide et de provoquer un écoulement capillaire dudit liquide, ledit matériau de transport de fluide ayant une première zone pour l'application dudit échantillon de fluide suspecté de contenir ledit analyte, et une deuxième zone pour l'application d'un liquide à un tampon contenant un réactif pouvant être reconstitué, agencées de façon que, lorsque ledit échantillon de fluide est ajouté à ladite première zone, et ledit liquide est ajouté à ladite deuxième zone, ledit échantillon de fluide s'écoule dans une première direction allant d'un bord d'échantillon de fluide vers ladite deuxième zone, ledit réactif soit reconstitué et ledit réactif et ledit liquide forment un réactif liquide contenant un réactant liquide capable de réagir avec ledit analyte pour former un produit réactionnel détectable, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ; et

une interface réactionnelle stable formée à une jonction entre ledit échantillon de fluide et ledit réactant liquide et visuellement distincte de ceux-ci, grâce à quoi, quand ledit échantillon de fluide s'écoulant et ledit réactant liquide s'écoulant se rencontrent, l'écoulement s'arrête et ledit produit réactionnel détectable est formé par une réaction entre ledit réactant liquide et ledit analyte ;

et comprenant en outre une zone d'étalonnage comprenant une quantité dudit analyte, ladite zone d'étalonnage étant localisée entre ladite première zone et ladite interface réactionnelle, grâce à quoi, lors de l'utilisation, ledit analyte en ladite quantité d'analyte dans la zone d'étalonnage s'écoule dans une première direction vers

- ladite deuxième zone, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ; grâce à quoi, quand ledit analyte s'écoulant en ladite quantité dudit analyte et ledit réactant liquide s'écoulant se rencontrent, l'écoulement s'arrête et un produit d'étalonnage détectable est formé par une réaction entre ledit analyte en ladite quantité dudit analyte et ledit réactant liquide, et une interface réactionnelle d'étalonnage stable est formée à une jonction entre ledit analyte en ladite quantité dudit analyte et ledit réactant liquide, et visuellement distincte dudit analyte et dudit réactant liquide, ladite interface d'étalonnage étant en outre adjacente à ladite interface réactionnelle.
3. Dispositif selon la revendication 1 ou la revendication 2, dans lequel ladite quantité dudit analyte ajoutée à ladite zone d'étalonnage est supérieure à ladite quantité dudit analyte dans ledit échantillon de fluide, de façon que ladite quantité dudit analyte ajoutée à ladite zone d'étalonnage soit suffisante pour former une quantité dudit produit d'étalonnage permettant la détermination d'une quantité dudit réactant liquide.
  4. Dispositif selon l'une quelconque des revendications 1 à 3, dans lequel au moins l'une parmi ladite première zone et ladite deuxième zone contient un réactif.
  5. Dispositif selon l'une quelconque des revendications 1 à 4, comprenant en outre un tampon localisé sur ladite première zone.
  6. Dispositif selon la revendication 5, dans lequel ledit tampon est efficace pour éliminer les globules rouges dans ledit échantillon de fluide.
  7. Dispositif selon l'une quelconque des revendications 1 à 6, dans lequel ledit produit d'étalonnage détectable est formé à une vitesse suffisante pour qu'il soit détecté environ dans les quinze secondes suivant l'application dudit échantillon de fluide à ladite zone d'échantillon de fluide.
  8. Dispositif selon l'une quelconque des revendications 1 à 7, dans lequel ledit matériau de transport de fluide est un matériau à base de nitrocellulose coulé sur un matériau de support.
  9. Dispositif selon la revendication 8, dans lequel ledit matériau de support est l'un parmi le polychlorure de vinyle ou un film de polyester (Mylar™).
  10. Dispositif selon l'une quelconque des revendications 1 à 9, comprenant en outre un moyen pour détecter et éventuellement mesurer une quantité dudit produit réactionnel détectable.
  11. Dispositif selon la revendication 10, dans lequel ledit moyen pour détecter et éventuellement mesurer une quantité dudit produit réactionnel détectable comprend un élément choisi dans le groupe constitué par l'absorbance, la réflectance, la transmission, la fluorescence, la luminescence, et la conductance.
  12. Dispositif selon l'une quelconque des revendications 1 à 11, comprenant en outre :
    - un moyen pour appliquer simultanément ledit échantillon de fluide à ladite première zone et ledit réactant liquide à ladite deuxième zone ; et un capteur, ledit capteur étant efficace pour détecter ledit produit réactionnel détectable.
  13. Dispositif selon la revendication 12, dans lequel ledit capteur est efficace pour détecter ledit produit réactionnel détectable ayant au moins une longueur d'onde souhaitée de réflectance ou d'absorption  $\geq 250$  nanomètres.
  14. Dispositif selon la revendication 12, dans lequel ledit capteur est efficace pour détecter et mesurer une quantité dudit produit réactionnel détectable qui est proportionnelle à une concentration dudit analyte sur la base d'une intensité de pic de réflectance ou d'absorption dudit produit réactionnel détectable.
  15. Dispositif selon la revendication 12, dans lequel ledit capteur est efficace pour détecter et mesurer une vitesse de formation dudit produit réactionnel détectable qui est proportionnelle à une concentration dudit analyte.
  16. Dispositif selon la revendication 15, dans lequel ladite vitesse est calculée par un changement au cours du temps d'une intensité de pic de réflectance ou d'absorption dudit produit réactionnel détectable.
  17. Dispositif selon la revendication 16, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée sur une ligne pratiquement perpendiculaire à ladite interface réactionnelle.
  18. Dispositif selon la revendication 16, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée à l'intérieur d'une région polygonale englobant tout ou une partie de ladite interface réactionnelle.
  19. Dispositif selon la revendication 12, dans lequel ledit capteur est efficace pour détecter et mesurer une quantité dudit produit réactionnel détectable qui est

supérieure ou égale à une intensité de seuil prédéterminée de réflectance ou d'absorption indicative de la présence dudit analyte.

20. Dispositif selon l'une quelconque des revendications 1 à 19, dans lequel ledit matériau de transport de fluide est capable de séparer les globules rouges à partir du sang entier.
21. Procédé pour détecter et quantifier au moins un analyte dans un échantillon de fluide suspecté de contenir ledit analyte, par l'emploi d'un réactant liquide capable de réagir avec ledit analyte pour former un produit réactionnel soluble détectable, par mise en relation dudit analyte avec le produit réactionnel soluble détectable de l'analyte et du réactant liquide, consistant à :

fournir un matériau de transport de fluide capable d'absorber un liquide et de provoquer un écoulement capillaire dudit liquide, ledit matériau de transport de fluide ayant une première zone pour l'application dudit échantillon de fluide suspecté de contenir ledit analyte, et une deuxième zone pour l'application dudit réactant liquide ;

ajouter ledit échantillon de fluide à ladite première zone, et ledit réactant liquide à ladite deuxième zone, où ledit échantillon de fluide s'écoule ensuite dans une première direction allant d'un bord d'échantillon de fluide vers ladite deuxième zone, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ;

grâce à quoi, quand ledit échantillon de fluide s'écoulant et ledit réactant liquide s'écoulant se rencontrent, l'écoulement s'arrête et ledit produit réactionnel détectable est formé par une réaction entre ledit réactant liquide et ledit analyte, et une interface réactionnelle stable est formée à une jonction entre ledit échantillon de fluide et ledit réactant liquide et visuellement distincte dudit échantillon de fluide et dudit réactant liquide ; et

détecter ledit produit réactionnel détectable.

22. Procédé selon la revendication 21, consistant en outre à étalonner une concentration dudit réactif liquide par application d'une quantité dudit analyte à une zone d'étalonnage, ladite zone d'étalonnage étant localisée entre ladite première zone et ladite interface réactionnelle, grâce à quoi, quand ladite quantité dudit analyte est ajoutée à ladite zone d'étalonnage, ledit analyte en ladite quantité d'analyte s'écoule dans une première direction vers ladite deuxième zone, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite pre-

mière direction et vers ladite première zone à partir d'un bord de réactant liquide ;

grâce à quoi ledit analyte circulant en ladite quantité dudit analyte et ledit réactif liquide circulant se rencontrent, l'écoulement s'arrête et un produit d'étalonnage détectable est formé par une réaction entre ledit analyte en ladite quantité dudit analyte et ledit réactant liquide, et une interface réactionnelle d'étalonnage stable est formée à une jonction entre ledit analyte en ladite quantité en excès dudit analyte et ledit réactant liquide, et visuellement distincte dudit analyte et dudit réactant liquide ; et détecter ledit produit réactionnel détectable.

23. Procédé pour détecter et quantifier au moins un analyte dans un échantillon de fluide suspecté de contenir ledit analyte, par l'emploi d'un réactant liquide capable de réagir avec ledit analyte pour former un produit réactionnel soluble détectable, par mise en relation dudit analyte avec le produit réactionnel soluble détectable de l'analyte et du réactant liquide, consistant à :

fournir un matériau de transport de fluide capable d'absorber un liquide et de provoquer un écoulement capillaire dudit liquide, ledit matériau de transport de fluide ayant une première zone pour l'application dudit échantillon de fluide suspecté de contenir ledit analyte, et une deuxième zone pour l'application d'un liquide à un tampon contenant un réactif pouvant être reconstitué, dans lesquelles, lorsque ledit échantillon de fluide est ajouté à ladite première zone, et ledit liquide est ajouté à ladite deuxième zone, ledit échantillon de fluide s'écoule dans une première direction allant d'un bord d'échantillon de fluide vers ladite deuxième zone, ledit réactif est reconstitué et ledit réactif et ledit liquide forment un réactif liquide contenant un réactant liquide capable de réagir avec ledit analyte pour former un produit réactionnel détectable, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ;

grâce à quoi, quand ledit échantillon de fluide s'écoulant et ledit réactant liquide s'écoulant se rencontrent, l'écoulement s'arrête et ledit produit réactionnel détectable est formé par une réaction entre ledit réactant liquide et ledit analyte, et une interface réactionnelle stable est formée à une jonction entre ledit échantillon de fluide et ledit réactant liquide et visuellement distincte dudit échantillon de fluide et dudit réactant liquide ; et détecter ledit produit réactionnel détectable.

24. Procédé selon la revendication 23, consistant en

- outre à étalonner une concentration dudit réactif liquide par application d'une quantité dudit analyte à une zone d'étalonnage, ladite zone d'étalonnage étant localisée entre ladite première zone et ladite interface réactionnelle, grâce à quoi, quand ladite quantité dudit analyte est ajoutée à ladite zone d'étalonnage, ledit analyte en ladite quantité d'analyte s'écoule dans une première direction vers ladite deuxième zone, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ;  
grâce à quoi ledit analyte circulant en ladite quantité dudit analyte et ledit réactif liquide circulant se rencontrent, l'écoulement s'arrête et un produit d'étalonnage détectable est formé par une réaction entre ledit analyte en ladite quantité dudit analyte et ledit réactant liquide, et une interface réactionnelle d'étalonnage stable est formée à une jonction entre ledit analyte en ladite quantité en excès dudit analyte et ledit réactant liquide, et visuellement distincte de ceux-ci ; ladite interface d'étalonnage étant en outre adjacente à ladite interface réactionnelle ; et mesurer ledit produit d'étalonnage à la même au moins une longueur d'onde souhaitée de réflectance ou d'absorbance que celle dudit produit réactionnel.
25. Procédé selon l'une quelconque des revendications 21 à 24, dans lequel au moins l'une parmi ladite première zone et ladite deuxième zone contient un réactif.
26. Procédé selon l'une quelconque des revendications 21 à 25, consistant en outre à disposer un tampon localisé sur ladite première zone.
27. Procédé selon la revendication 26, dans lequel ledit tampon est efficace pour éliminer les globules rouges dudit échantillon de fluide.
28. Procédé selon la revendication 21 ou la revendication 23, dans lequel ledit produit réactionnel détectable est formé à une vitesse suffisante pour qu'il soit détecté environ dans les quinze secondes suivant l'application dudit échantillon de fluide à ladite zone d'échantillon de fluide.
29. Procédé selon l'une quelconque des revendications 21 à 28, dans lequel l'au moins un analyte comprend un élément choisi dans le groupe constitué par le glucose, le calcium, l'albumine, la phosphatase alcaline, l'ammoniac, la bilirubine, l'acide urique, la 2-glutamyl transférase, l'amylase, la créatine kinase, la créatinine, le cholestérol, la protéine totale, le magnésium, la lactate déshydrogénase, la lipase, le phosphore, les triglycérides, l'alanine aminotransférase, l'aspartate aminotransférase, et l'azote uréique du sang.
30. Procédé selon la revendication 22 ou la revendication 24, dans lequel ladite quantité dudit analyte ajoutée à ladite zone d'étalonnage est supérieure à ladite quantité dudit analyte dans ledit échantillon de fluide, de façon que ladite quantité dudit analyte ajoutée à ladite zone d'étalonnage soit suffisante pour former une quantité dudit produit d'étalonnage permettant la détermination d'une quantité dudit réactant liquide.
31. Procédé selon la revendication 22 ou la revendication 24, dans lequel ledit produit d'étalonnage détectable est formé à une vitesse qui est proportionnelle à une concentration dudit analyte.
32. Procédé selon l'une quelconque des revendications 21 à 29, dans lequel ledit produit réactionnel détectable est formé en une quantité supérieure ou égale à une intensité de seuil prédéterminée de réflectance ou d'absorption, indicative de la présence dudit analyte.
33. Procédé pour détecter et quantifier au moins un analyte dans un échantillon de fluide suspecté de contenir ledit analyte, par l'emploi d'un réactant, capable de se fixer audit analyte et de former un produit réactionnel détectable à partir d'un substrat, pour mettre en relation une quantité dudit analyte avec une quantité dudit produit réactionnel détectable, comprenant :
- fournir un matériau de transport de fluide capable d'absorber un liquide et de provoquer un écoulement capillaire dudit liquide, ledit matériau de transport de fluide ayant une première zone pour l'application dudit échantillon de fluide contenant ledit réactif à un premier tampon contenant ledit analyte fixé de manière pratiquement irréversible audit premier tampon, et une deuxième zone pour l'application d'un liquide à un deuxième tampon contenant un substrat pouvant être reconstitué ;  
ajouter ledit échantillon de fluide contenant ledit réactif audit premier tampon, et ledit liquide audit deuxième tampon, où ledit échantillon de fluide s'écoule ensuite dans une première direction allant d'un bord d'échantillon de fluide vers ladite deuxième zone, et ledit substrat est reconstitué par ledit liquide pour former un réactant liquide capable de réagir avec ledit réactif, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir d'un bord de réactant liquide ;  
grâce à quoi, quand ledit échantillon de fluide s'écoulant, contenant ledit réactif non fixé par ledit analyte fixé de manière pratiquement irréversible audit premier tampon, et ledit réactant

- liquide s'écoulant se rencontrent, l'écoulement s'arrête et ledit produit réactionnel détectable est formé par une réaction entre ledit réactant liquide et ledit réactif, et une interface réactionnelle stable est formée à une jonction entre ledit échantillon de fluide et ledit réactant liquide et visuellement distincte dudit échantillon de fluide et dudit réactant liquide ; et détecter ledit produit réactionnel détectable.
- 34.** Procédé selon l'une quelconque des revendications 21 à 33, dans lequel ledit matériau de transport de fluide est capable de séparer les globules rouges du sang entier.
- 35.** Procédé selon l'une quelconque des revendications 21 à 34, dans lequel ledit matériau de transport de fluide est un matériau à base de nitrocellulose coulé sur un matériau de support.
- 36.** Procédé selon la revendication 35, dans lequel ledit matériau de support est l'un parmi le poly(chlorure de vinyle) ou un film de polyester (Mylar™).
- 37.** Procédé selon l'une quelconque des revendications 21 à 36, consistant en outre à mesurer une quantité dudit produit réactionnel détectable.
- 38.** Procédé selon l'une quelconque des revendications 21 à 37, dans lequel ladite quantité dudit produit réactionnel détectable est proportionnelle à une concentration dudit analyte sur la base d'une intensité de pic de réflectance ou d'absorption dudit produit réactionnel détectable.
- 39.** Procédé selon la revendication 37 ou la revendication 38, dans lequel ladite étape consistant à mesurer une quantité dudit produit réactionnel détectable comprend la mesure d'un élément choisi dans le groupe constitué par l'absorbance, la réflectance, la transmission, la fluorescence, la luminescence, et la conductance.
- 40.** Procédé selon la revendication 39, dans lequel ledit produit réactionnel détectable est mesuré par l'une parmi l'absorbance et la réflectance.
- 41.** Procédé selon la revendication 40, dans lequel au moins l'une parmi l'absorbance et la réflectance est mesurée à une ou plusieurs longueurs d'onde  $\geq 250$  nanomètres.
- 42.** Procédé selon l'une quelconque des revendications 21 à 36, dans lequel ledit produit réactionnel détectable est formé à une vitesse qui est proportionnelle à une concentration dudit analyte.
- 43.** Procédé selon la revendication 42, dans lequel ladite
- vitesse est calculée par un changement du cours du temps d'une intensité de pic de réflectance ou d'absorption dudit produit réactionnel détectable.
- 44.** Procédé selon la revendication 43, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée sur une ligne pratiquement perpendiculaire à ladite interface réactionnelle.
- 45.** Dispositif selon la revendication 43, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée à l'intérieur d'une région polygonale englobant tout ou une partie de ladite interface réactionnelle.
- 46.** Procédé selon l'une quelconque des revendications 21 à 36, dans lequel ledit produit réactionnel détectable est formé en une quantité supérieure ou égale à une intensité de seuil prédéterminée de réflectance ou d'absorption indicative de la présence dudit analyte.
- 47.** Procédé selon l'une quelconque des revendications 21 à 46, consistant en outre à mesurer un élément choisi dans le groupe constitué par : une distance entre ledit bord d'échantillon de fluide et un point médian de ladite interface réactionnelle stable, une distance entre ledit bord de réactant liquide et un point médian de ladite interface réactionnelle stable, une distance entre ledit bord d'échantillon de fluide et un point médian d'une interface liquide stable, une distance entre ledit bord de réactant liquide et un point médian d'une interface de liquide stable, une distance entre ledit point médian de ladite interface réactionnelle stable et ledit point médian d'une interface de liquide stable, une réflectance ou absorption dudit produit réactionnel détectable en une pluralité de points de temps fixes, une réflectance ou absorption de ladite interface liquide stable en une pluralité de points de temps fixes, une vitesse de changement de ladite réflectance ou absorption dudit produit réactionnel détectable, une surface de ladite interface réactionnelle stable, une surface de ladite interface liquide stable, un niveau de base dudit échantillon de fluide, et un niveau de base dudit réactant liquide.
- 48.** Procédé selon la revendication 33, comprenant en outre l'étalonnage une concentration dudit réactif liquide par application d'une quantité dudit réactif à une zone d'étalonnage, ladite zone d'étalonnage étant localisée entre ladite première zone et ladite interface réactionnelle, grâce à quoi, quand ledit agent est ajouté à ladite zone d'étalonnage, ledit réactif s'écoule dans une première direction vers ladite deuxième zone, et ledit réactant liquide s'écoule dans une deuxième direction opposée à ladite première direction et vers ladite première zone à partir

- d'un bord de réactant liquide ;  
grâce à quoi ledit réactif circulant et ledit réactant liquide circulant se rencontrent, l'écoulement s'arrête et un produit d'étalonnage détectable est formé par une réaction entre ledit réactif et ledit réactant liquide, et une interface réactionnelle d'étalonnage stable est formée à une jonction entre ledit réactif en ladite quantité en excès dudit réactif et ledit réactant liquide, et visuellement distincte dudit réactif et dudit réactant liquide ; et détecter ledit produit d'étalonnage détectable.
49. Procédé selon la revendication 48, dans lequel ladite quantité dudit réactif ajouté à ladite zone d'étalonnage est supérieure à ladite quantité dudit réactif dans ledit échantillon de fluide, de façon que ladite quantité dudit réactif ajouté à ladite zone d'étalonnage soit suffisante pour former une quantité dudit produit d'étalonnage détectable permettant la détermination d'une quantité dudit réactant liquide.
50. Procédé selon la revendication 48, dans lequel ledit produit d'étalonnage détectable est formé à une vitesse qui est proportionnelle à une concentration dudit réactif.
51. Procédé selon les revendications 31 et 50, dans lequel ladite vitesse est calculée par un changement au cours du temps d'une intensité de pic de réflectance ou d'absorption dudit produit d'étalonnage détectable.
52. Procédé selon la revendication 51, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée sur une ligne pratiquement perpendiculaire à ladite interface d'étalonnage.
53. Procédé selon la revendication 51, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée à l'intérieur d'une région polygonale englobant tout ou une partie de ladite interface d'étalonnage.
54. Procédé selon la revendication 51, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée sur une ligne pratiquement perpendiculaire à ladite interface réactionnelle.
55. Procédé selon la revendication 51, dans lequel ladite intensité de pic de réflectance ou d'absorption est mesurée à l'intérieur d'une région polygonale englobant tout ou une partie de ladite interface réactionnelle.
56. Procédé selon la revendication 48, dans lequel ledit produit d'étalonnage détectable est formé en une quantité supérieure ou égale à une intensité de seuil prédéterminée de réflectance ou d'absorption indicative de la présence dudit réactif.
57. Procédé selon la revendication 33, dans lequel ledit réactif comprend une enzyme ou une partie de celle-ci conjuguée à un anticorps monoclonal ou une partie de celui-ci ; ladite enzyme ou une partie de celle-ci étant capable de former ledit produit réactionnel détectable à partir dudit substrat ; ledit anticorps monoclonal ou une partie de celui-ci étant capable de se fixer audit analyte.
58. Procédé selon la revendication 57, dans lequel ladite enzyme est un élément choisi dans le groupe consistant en la phosphatase alcaline, la  $\beta$ -galactosidase, et la peroxydase.
59. Procédé selon la revendication 57, dans lequel ladite partie d'un anticorps monoclonal est choisi dans le groupe constitué par un fragment F(ab), un fragment F(ab'), un fragment F(ab')<sub>2</sub>, un fragment Fv, et un fragment scFv.
60. Procédé selon la revendication 33, dans lequel ledit réactif comprend une enzyme ou une partie de celle-ci conjuguée à une protéine de récepteur ou une partie de celle-ci ; ladite enzyme ou une partie de celle-ci étant capable de former ledit produit réactionnel détectable à partir dudit substrat ; ladite protéine récepteur ou une partie de celle-ci étant capable de se fixer audit analyte.
61. Procédé selon la revendication 33, dans lequel ledit réactif comprend une enzyme ou une partie de celle-ci conjuguée à un aptide ou une partie de celui-ci ; ladite enzyme ou une partie de celle-ci étant capable de former ledit produit réactionnel détectable à partir dudit substrat ; ledit aptide ou une partie de celui-ci étant capable de se fixer audit analyte.

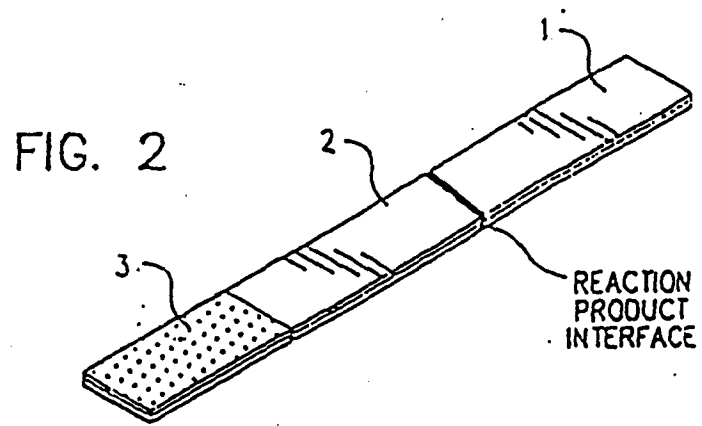
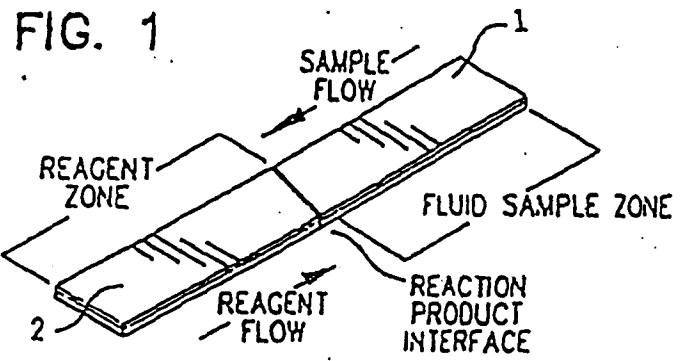
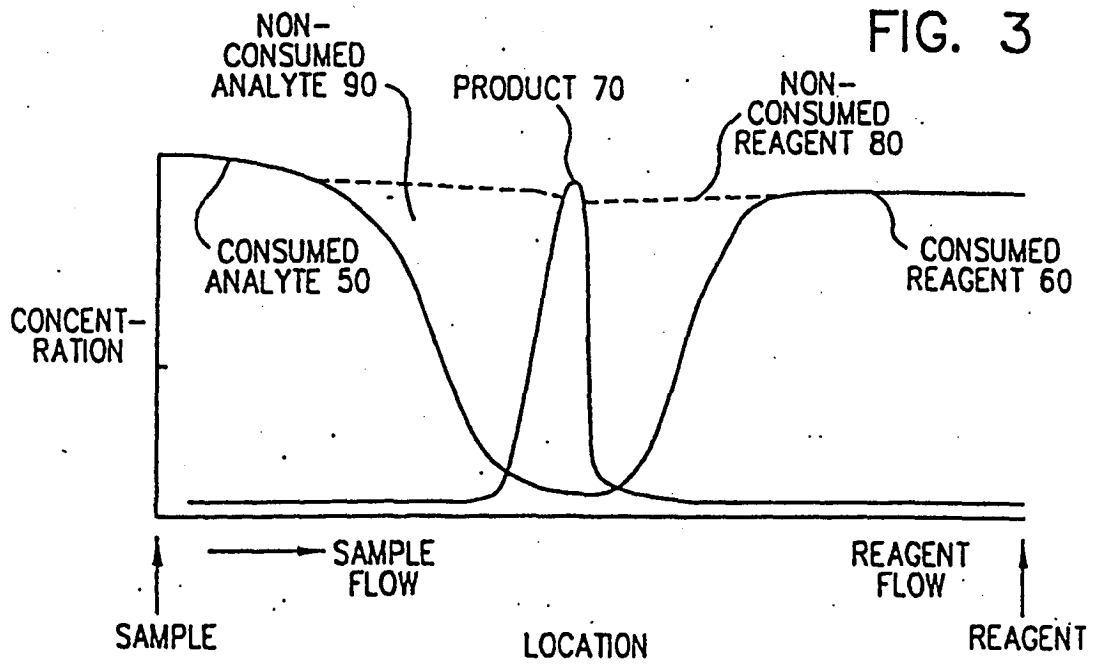


FIG. 3



**Glucose Assay (Indicator Reagent Dried on Membrane)**  
2436:72

**FIG. 4A**

Dried reagent streaking at reconstitution



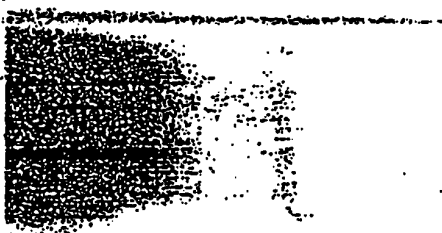
246 mg/dL Glucose, T=0

**FIG. 4D**



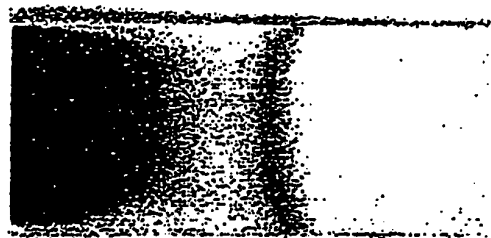
T=20 Seconds

**FIG. 4B**



T=5 Seconds

**FIG. 4E**



T=30 Seconds

**FIG. 4C**



T=10 Seconds

**FIG. 4F**



T=2 Minutes

**Calcium Assay (Fetal Calf Serum with Arsenazo III)**  
Normal Canine Range 9.7 - 12.2 mg/dL

18.6 mg/dL

FIG. 5G

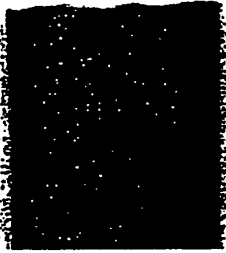


FIG. 5H

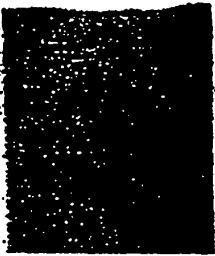


FIG. 5I

13 mg/dL

FIG. 5D

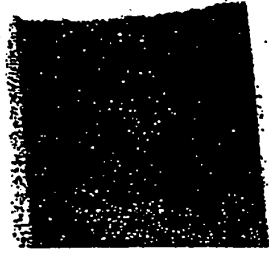


FIG. 5E

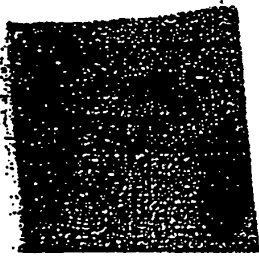


FIG. 5F

6.8 mg/dL

FIG. 5A

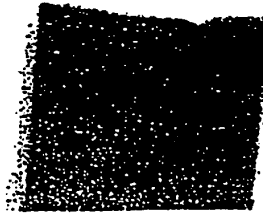


FIG. 5B

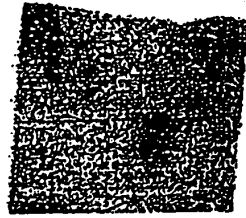


FIG. 5C

5 Seconds

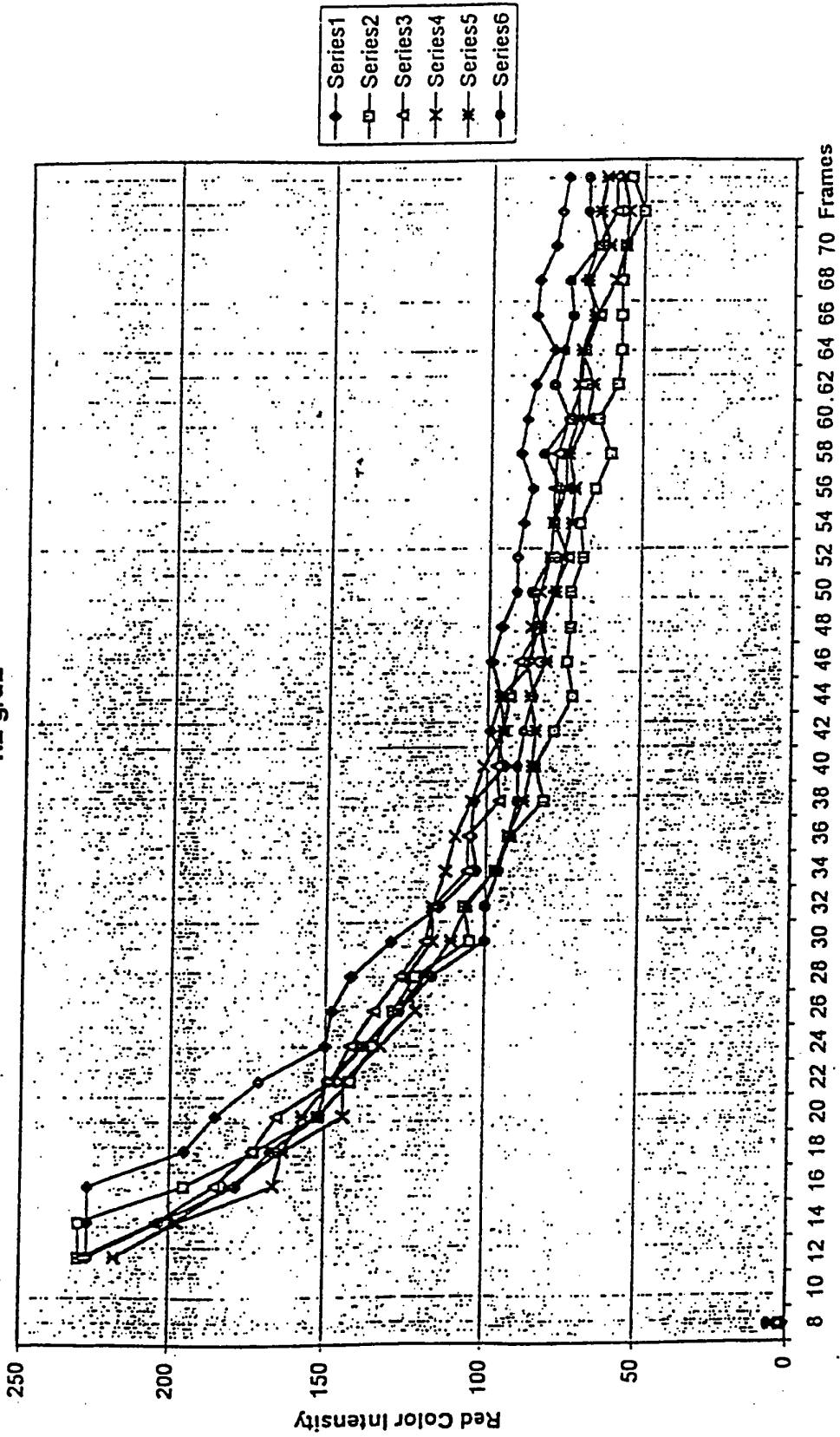
30 Seconds

2 Minutes

FIG. 6

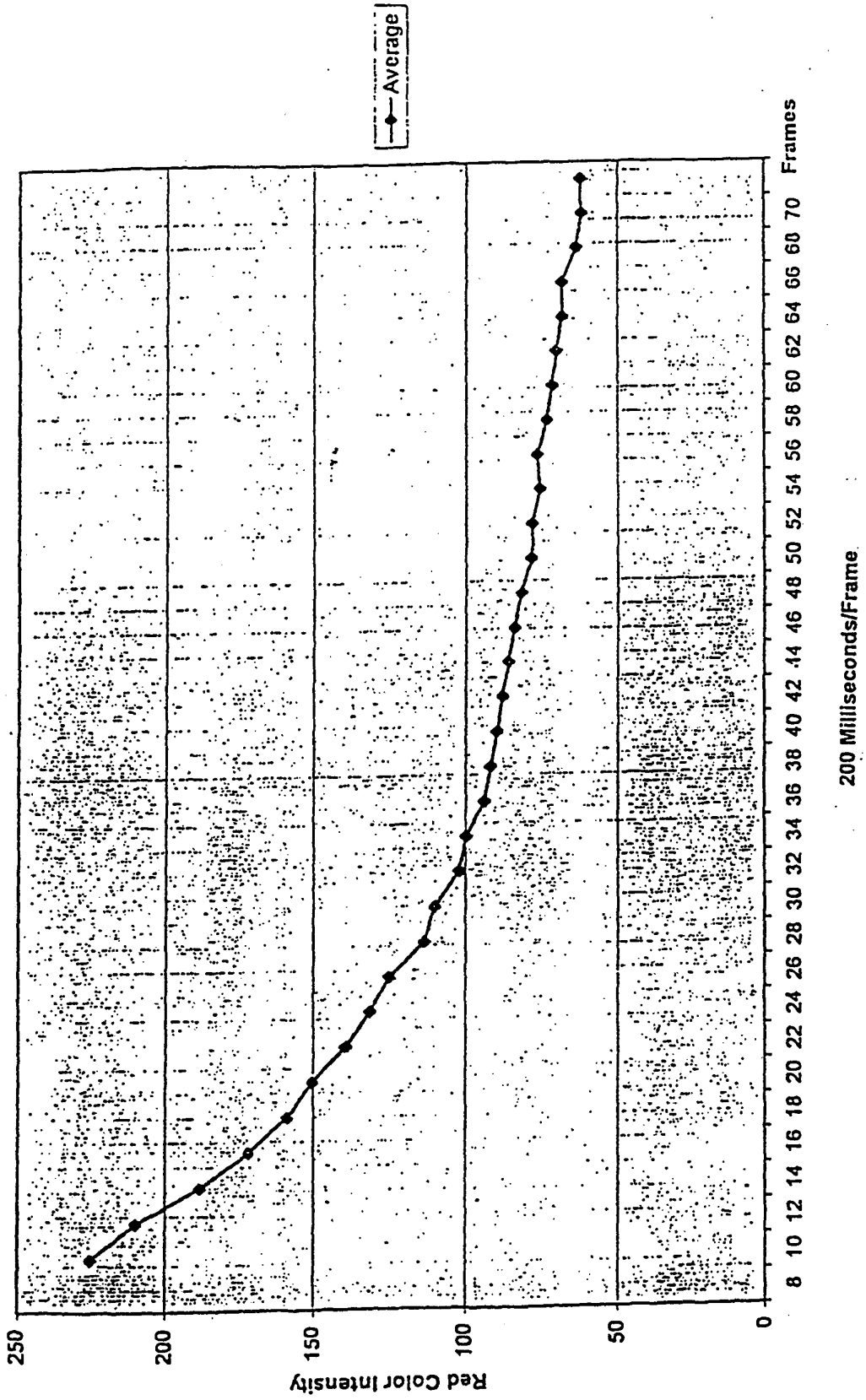
Albumin Low #3 Strip Map

1.2 g/dL



200 Milliseconds/Frame

FIG. 7  
Albumin Low #3 Strip Map Average  
1.2 g/dL



200 Milliseconds/Frame

FIG. 8  
Albumin High #3 Strip Map  
1.9 g/dL

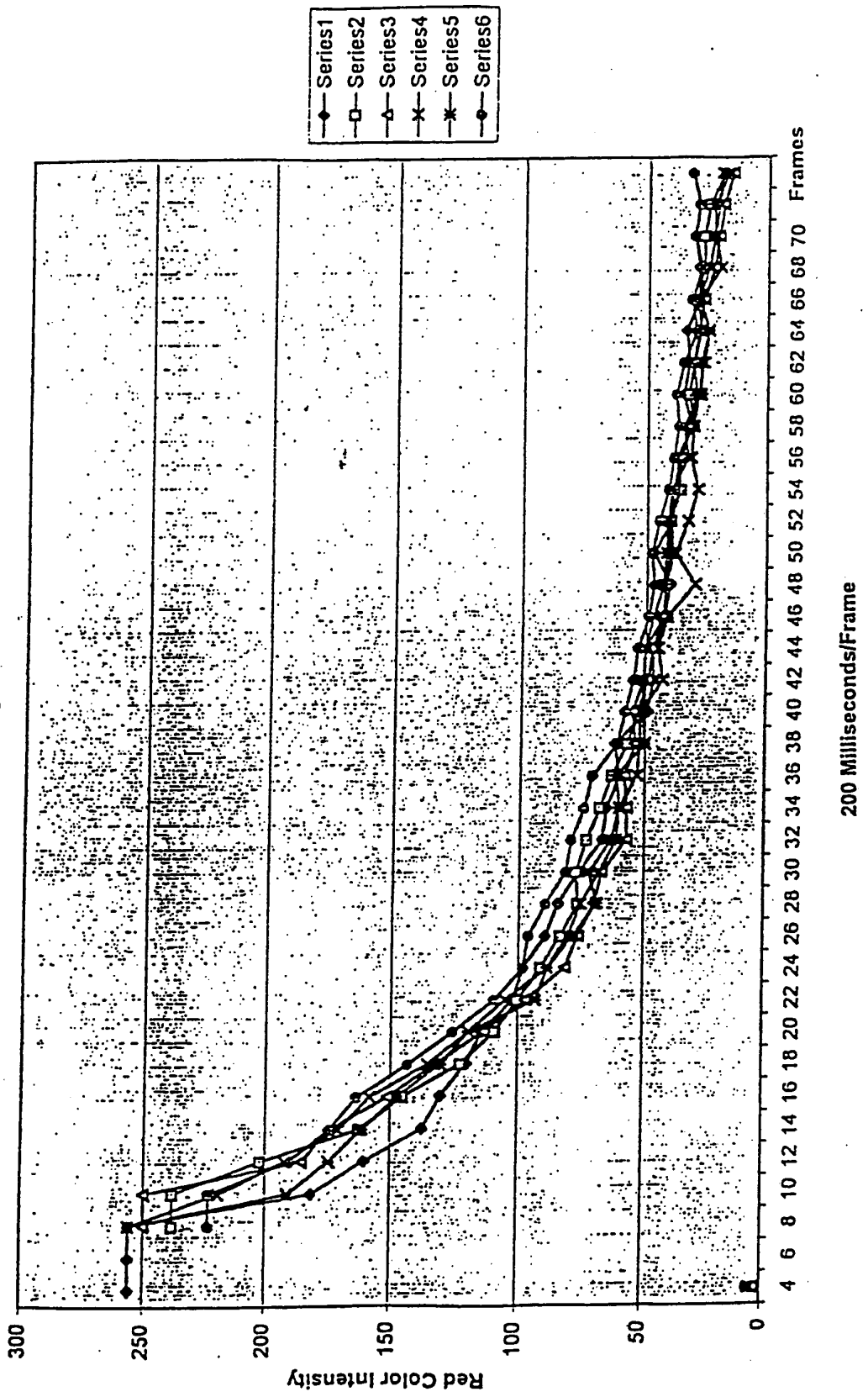


FIG. 9  
Albumin High #3 Average  
1.9 g/dL

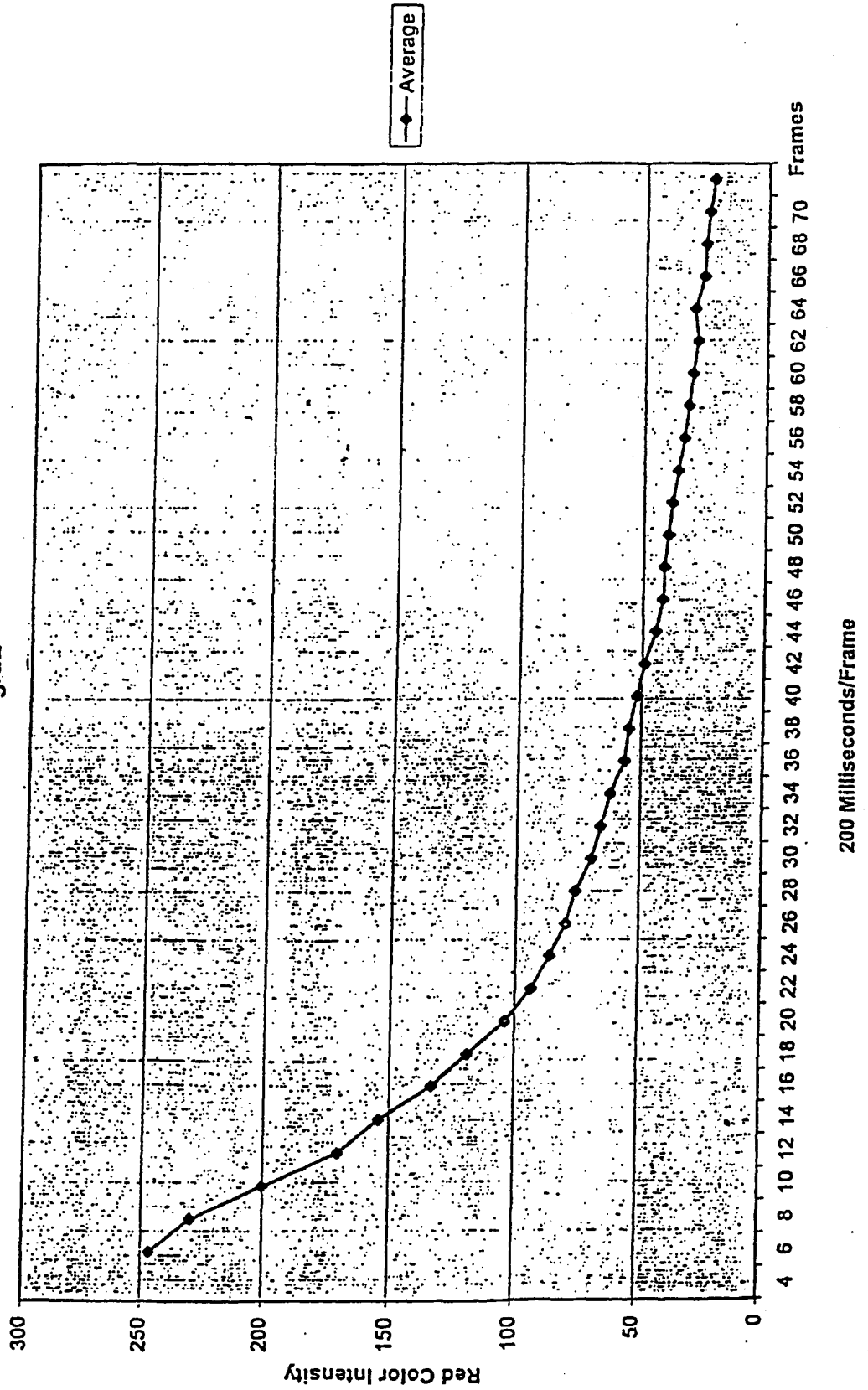
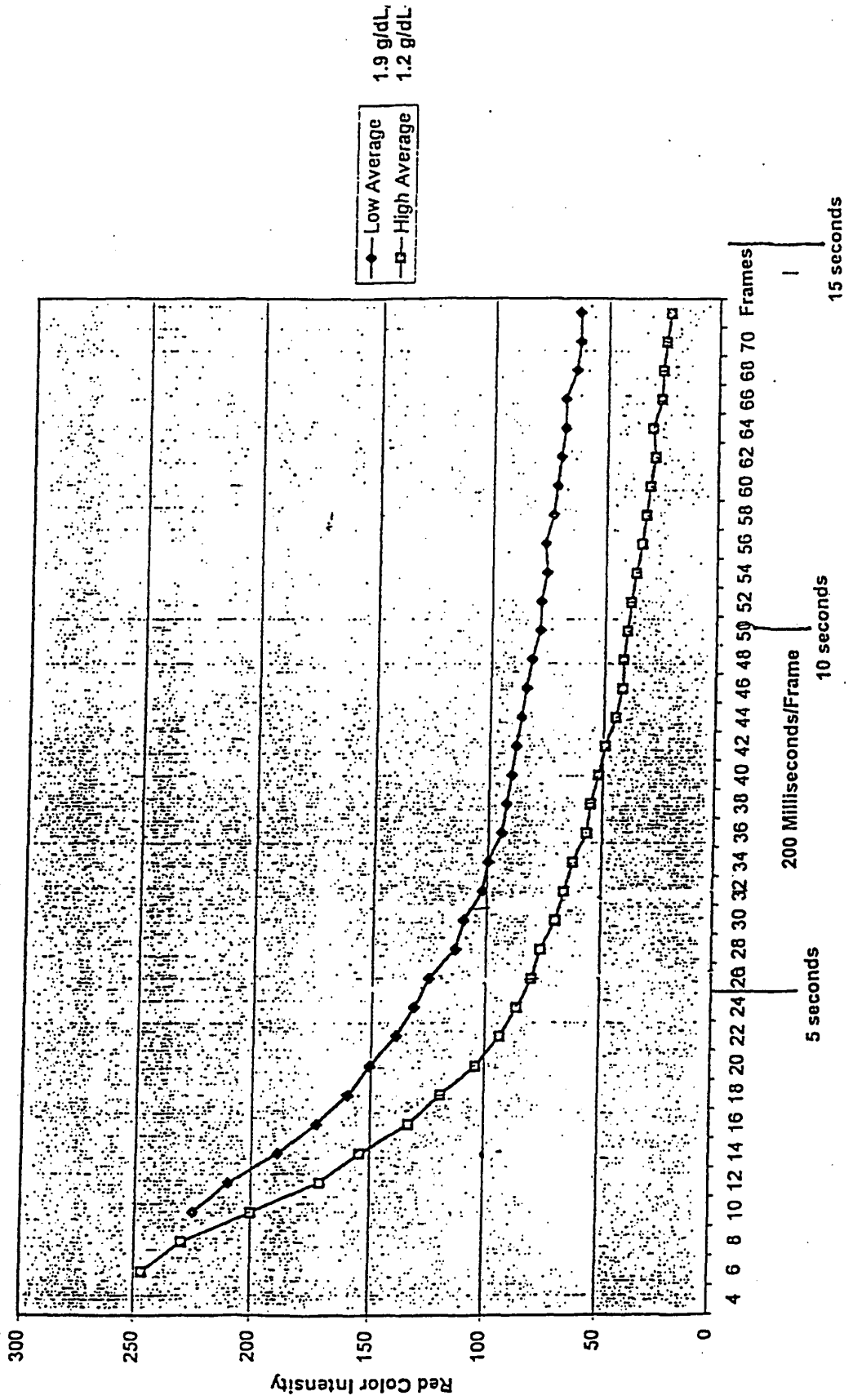


FIG. 10  
Albumin Assay Average 6 reads High vs. Low



Albumin = 1.2 g/dL

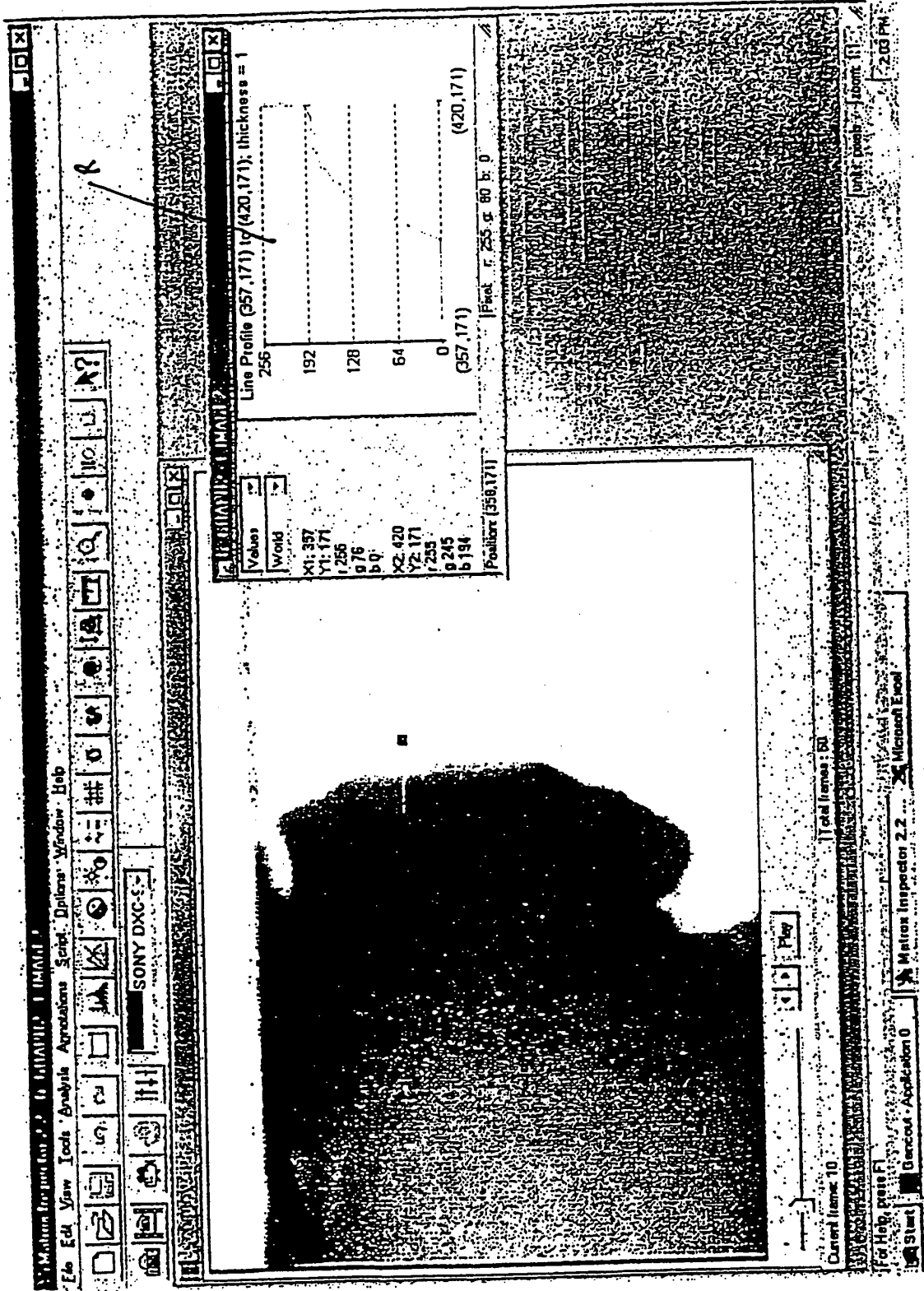


FIG. 11

Total Time=2.0 seconds  
200 msec post interface

Albumin = 1.2 g/dL

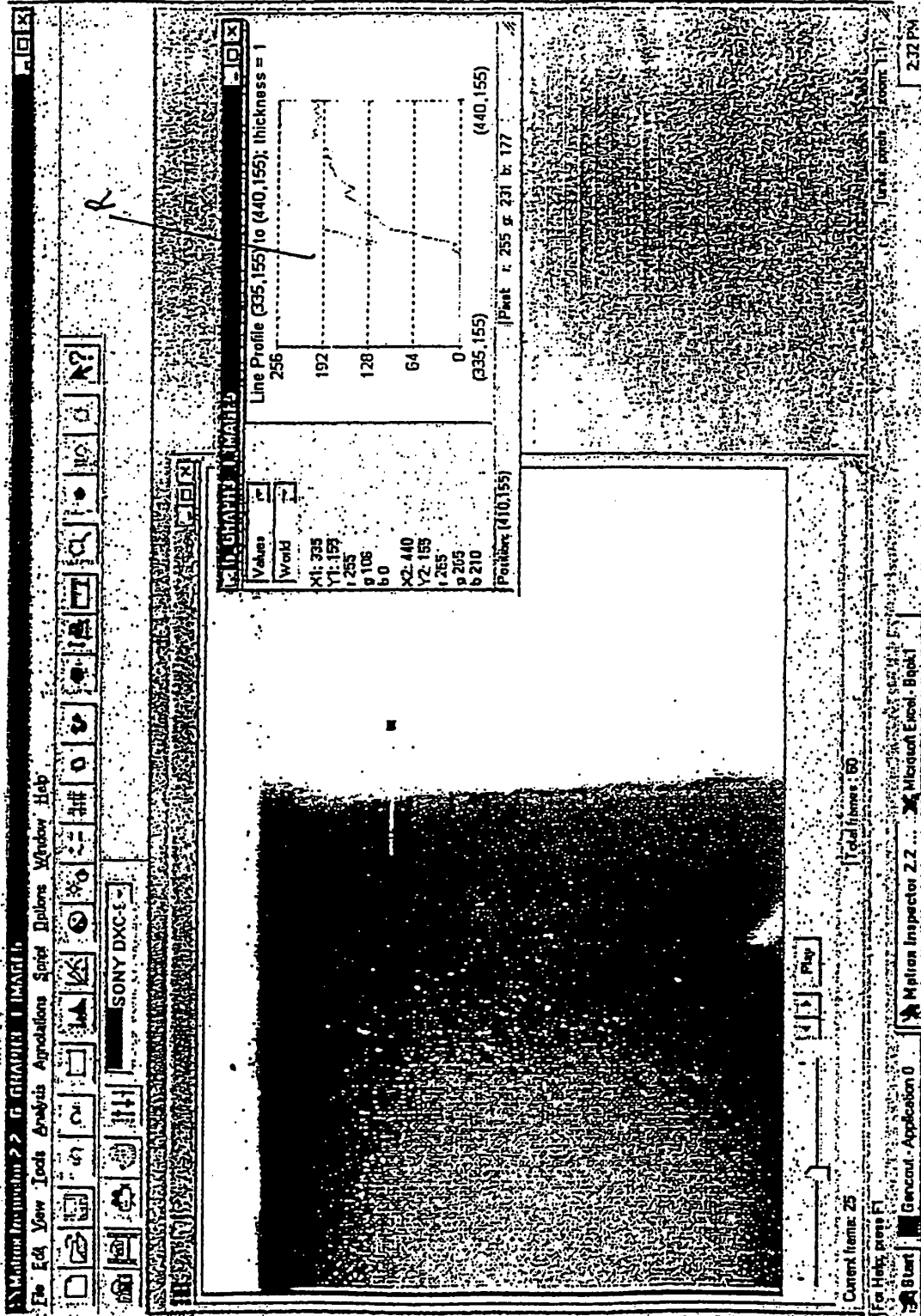


FIG. 12

Total Time=5.0 seconds  
3 sec post interface

Albumin = 1.9 g/dL

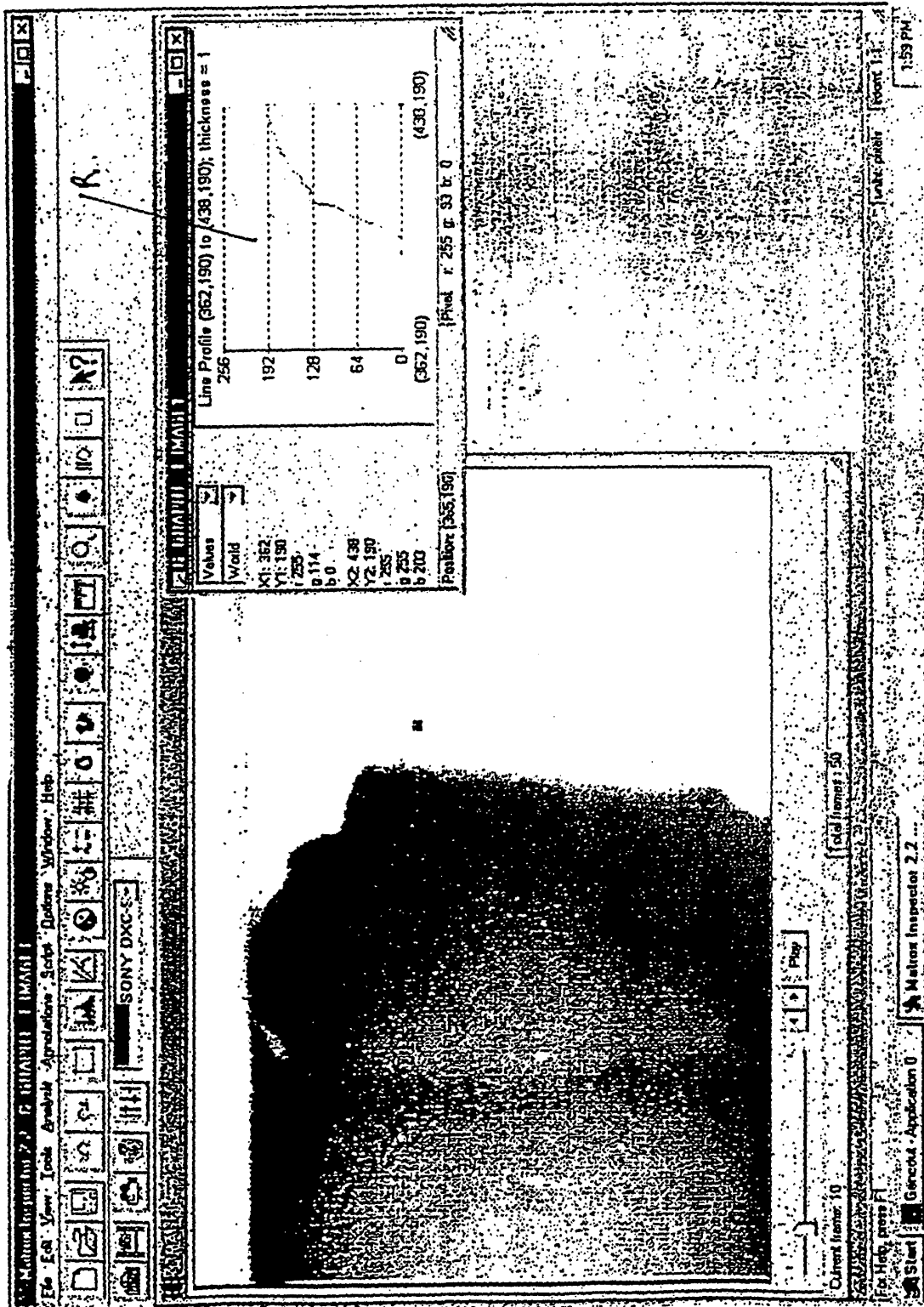


FIG. 13

Total Time=2.0 seconds  
200 msec post interface

Albumin = 1.9 g/dL

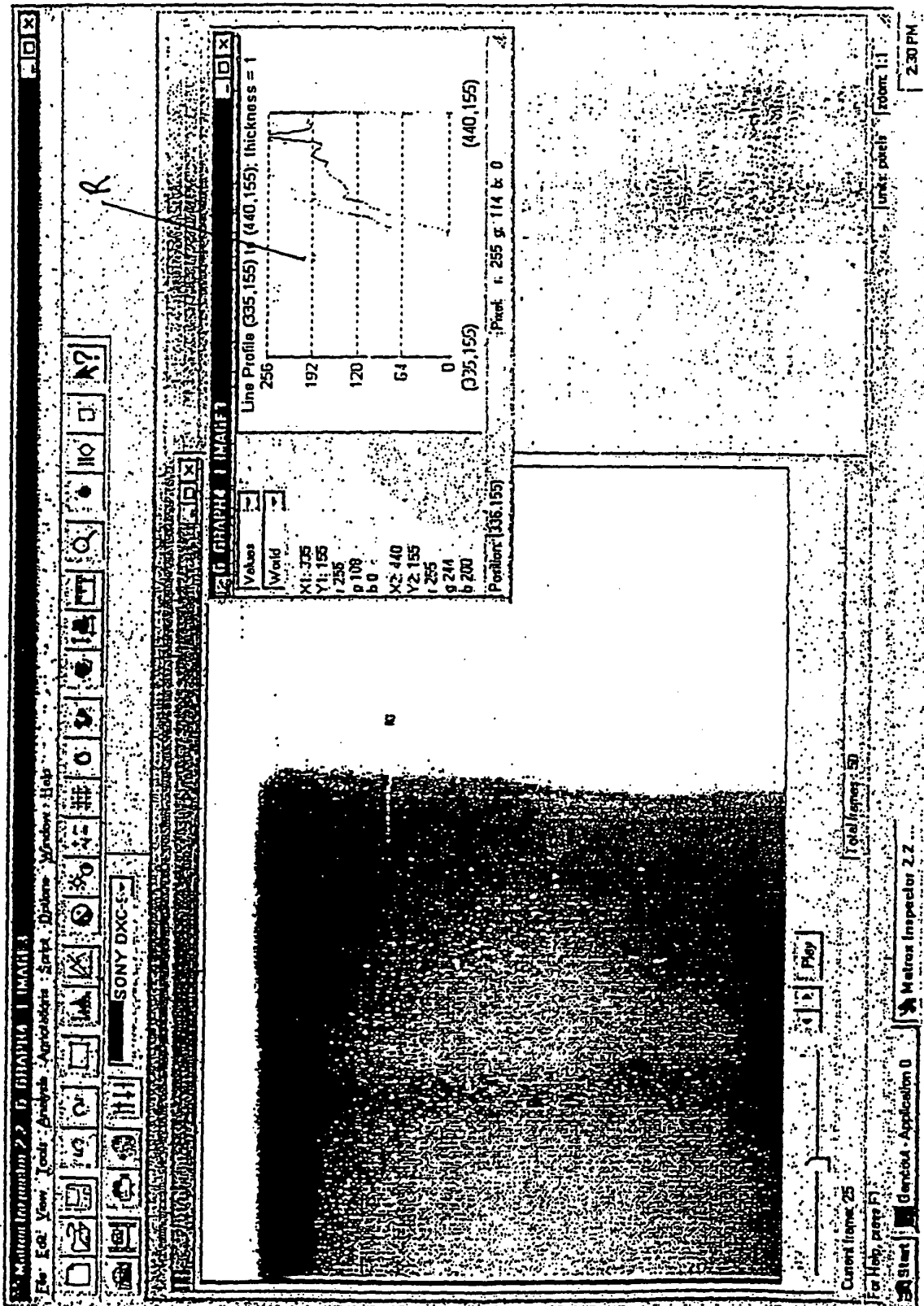


FIG. 14

Total Time=5.0 seconds  
3 sec post interface

FIG. 15

Glucose 50 mg/dl

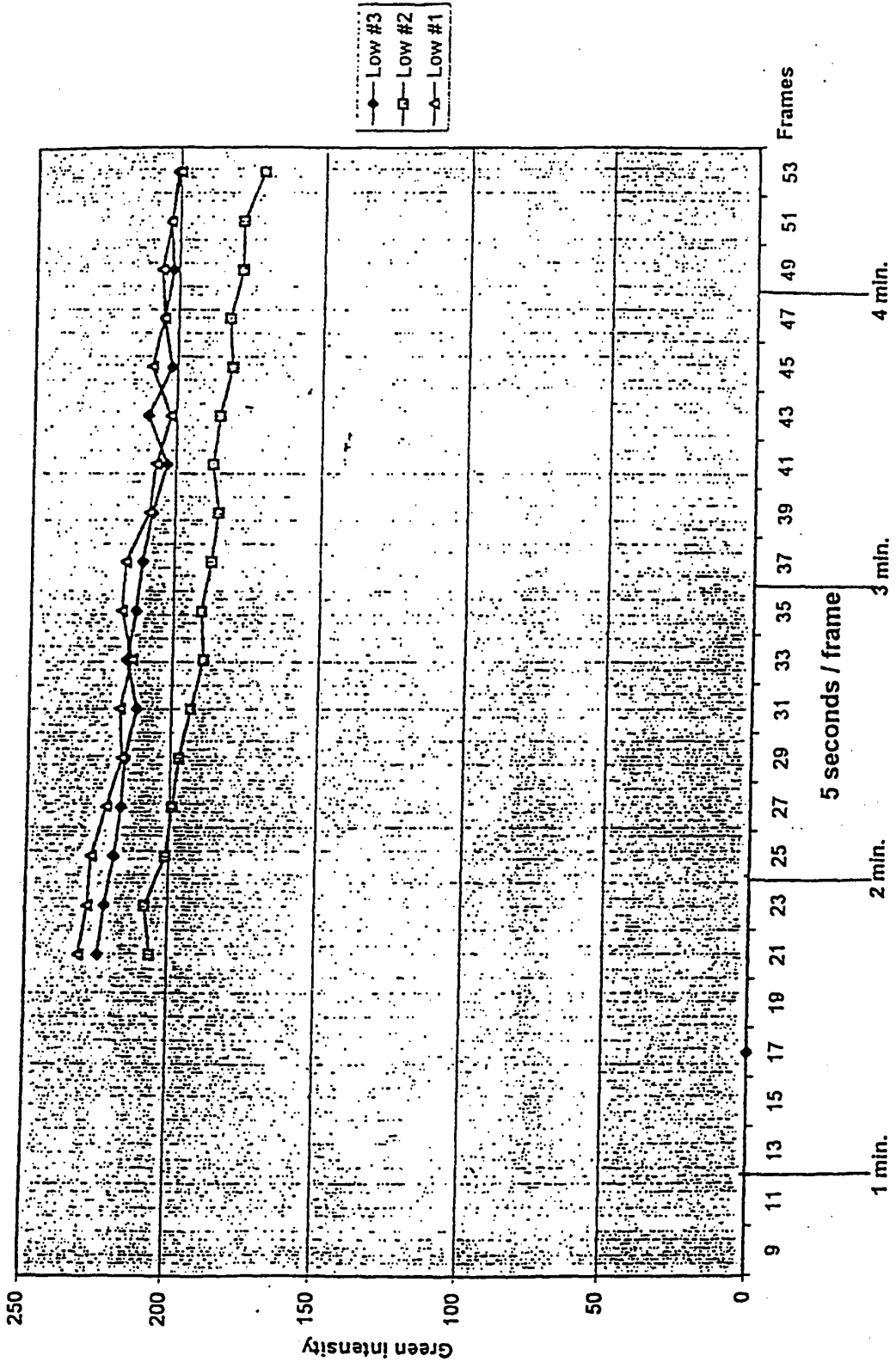


FIG. 16

Glucose 150 mg/dl

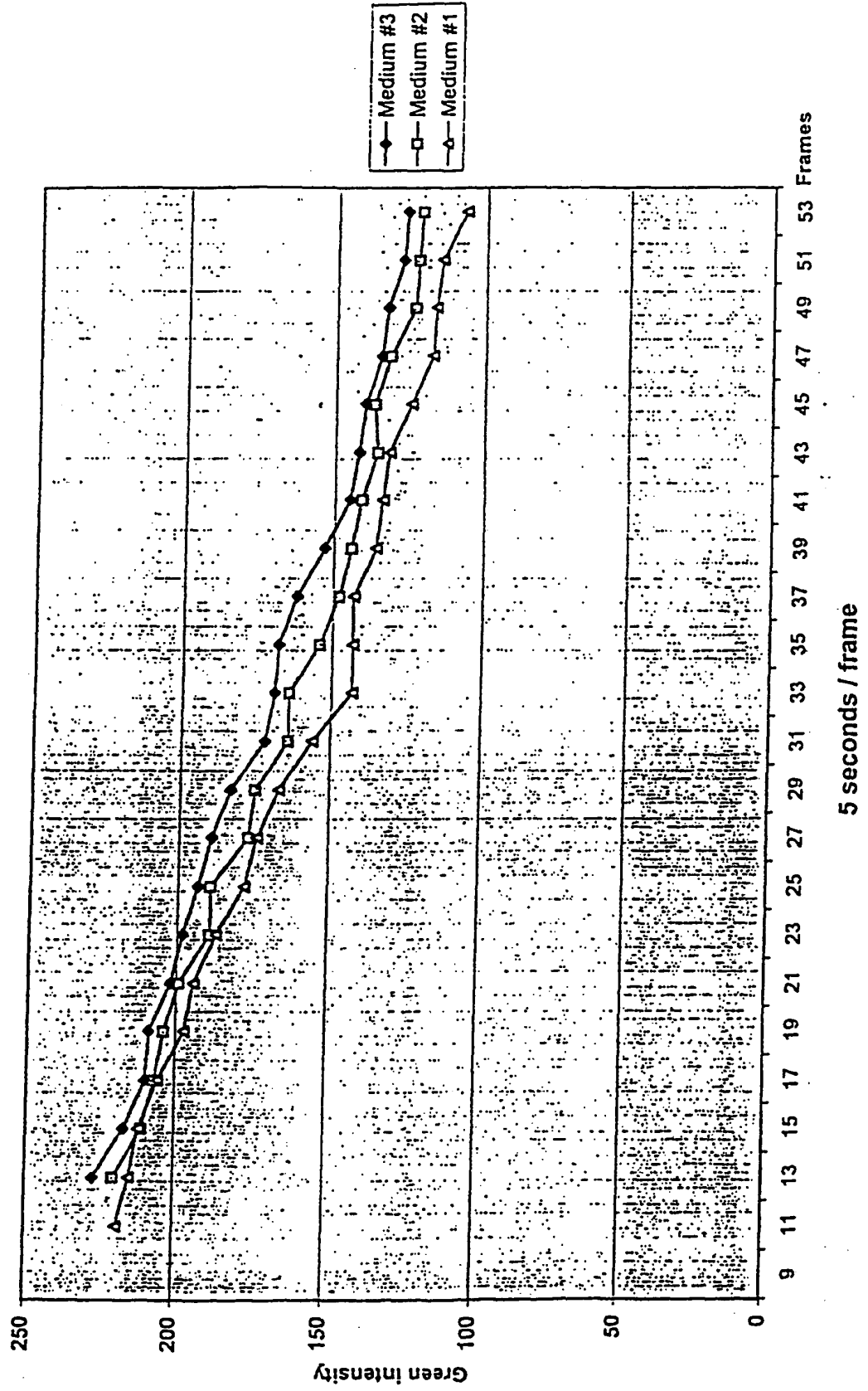
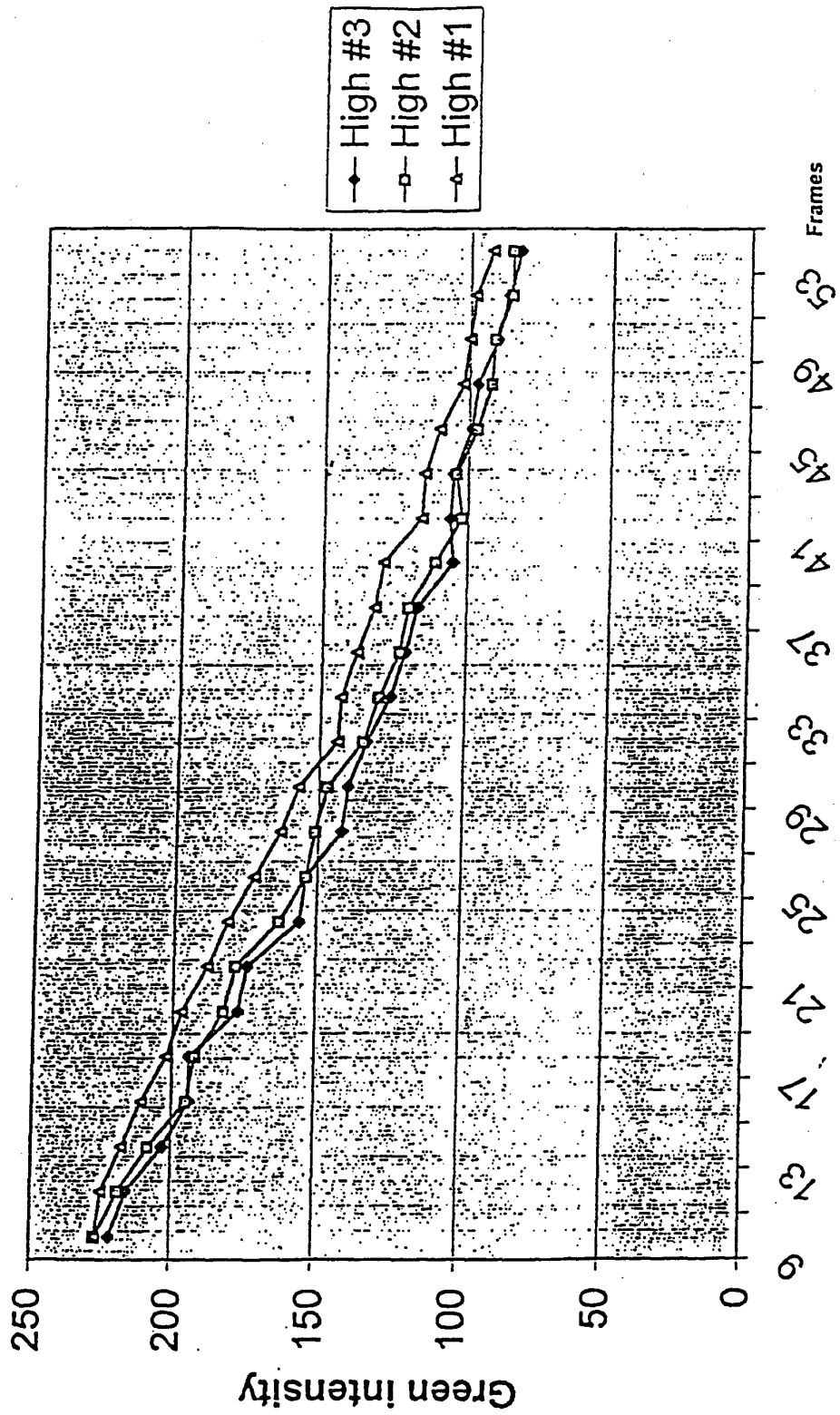


FIG. 17

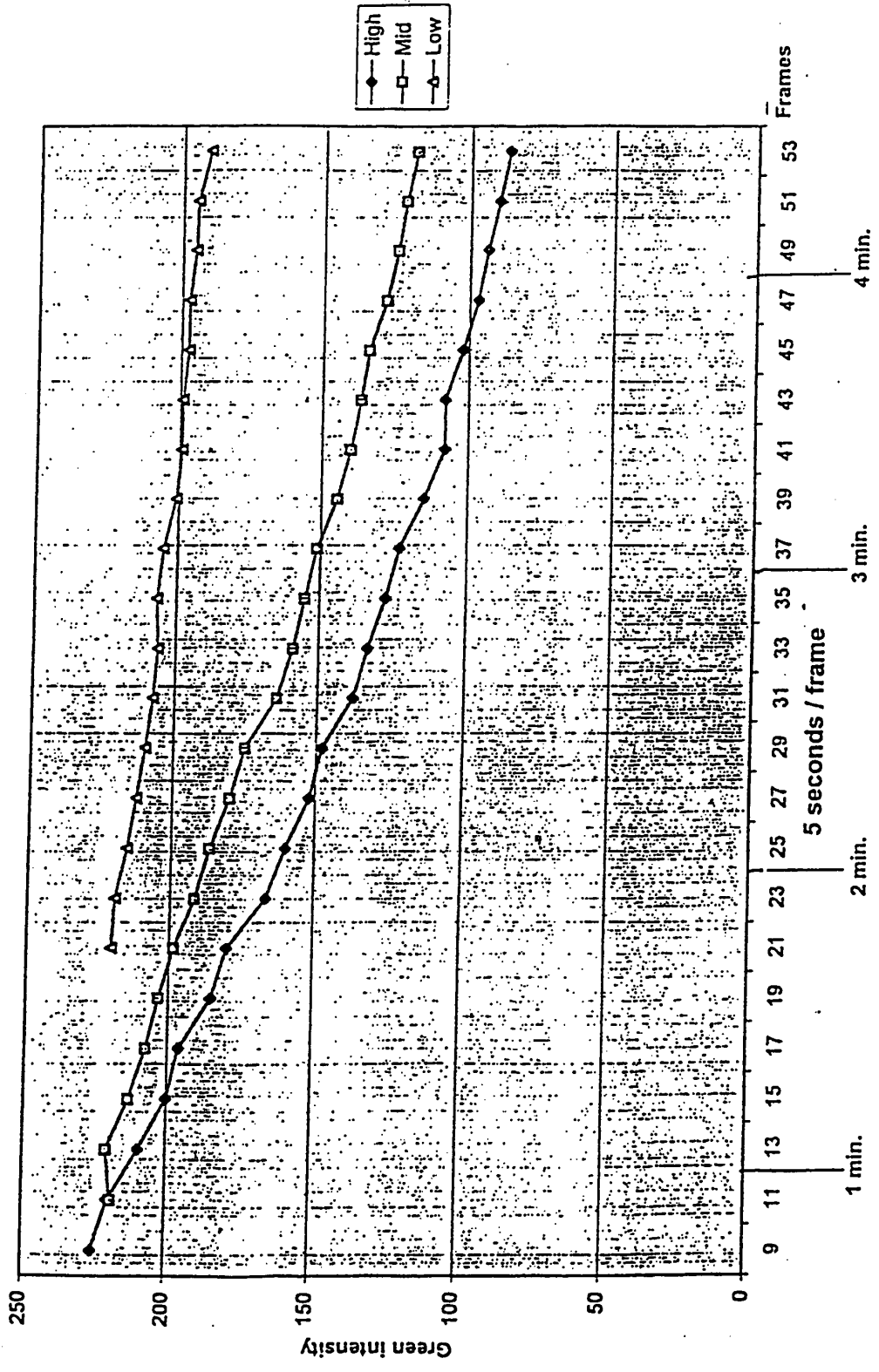
Glucose 250 mg/dl



5 seconds / frame

FIG. 18

Triplicate average 250, 150, 50 mg/dl



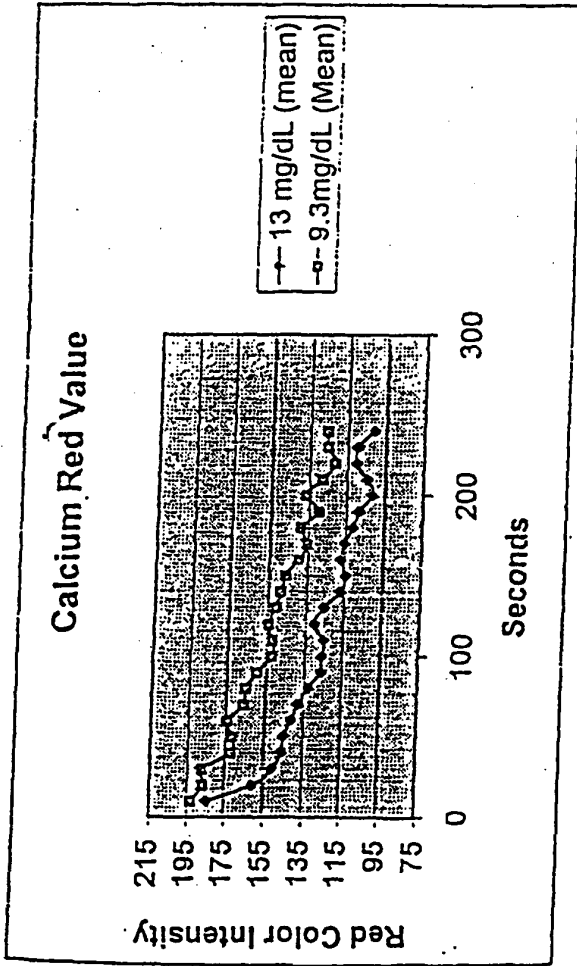


FIG. 19

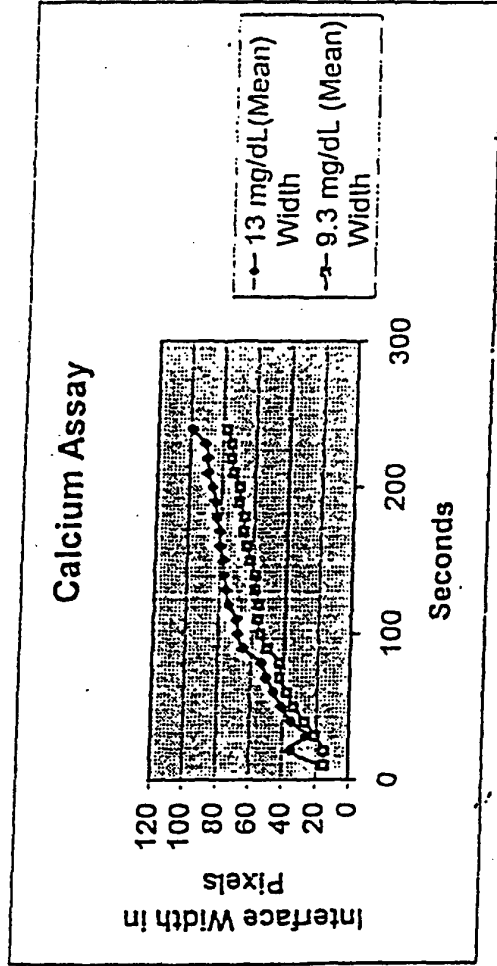
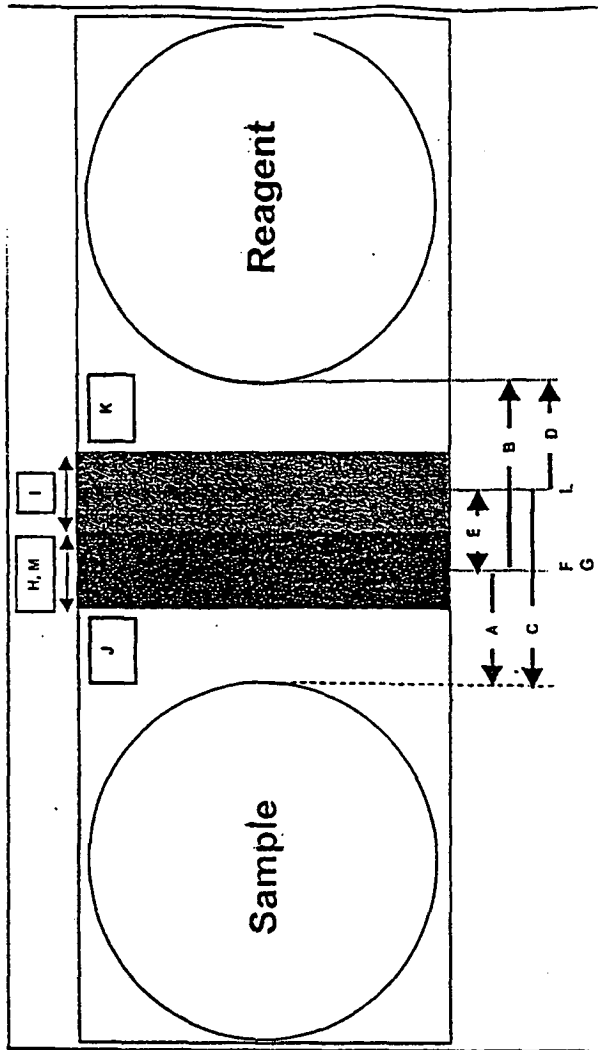
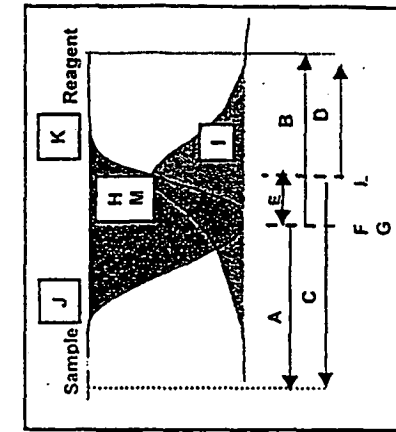


FIG. 20



- A. Reaction Interface Max distance to sample edge and  $\Delta/t$
- B. Reaction Interface Max distance to reagent edge and  $\Delta/t$
- C. Liquid-Interface Max distance to sample edge and  $\Delta/t$
- D. Liquid Interface Max distance to reagent edge and  $\Delta/t$
- E.  $\Delta$  Liquid vs Reaction interface max and  $\Delta/t$
- F. Reaction Interface Max at fixed time points
- G. Reaction Interface Max  $\Delta/t$  (slope)



- H. Reaction Interface area
- I. Liquid Interface area
- J. Sample background
- K. Reagent background
- L. Liquid Interface Max  $\Delta/t$
- M. Reaction Interface, shape of curve

Figure 21

# Glucose Total Product Analysis

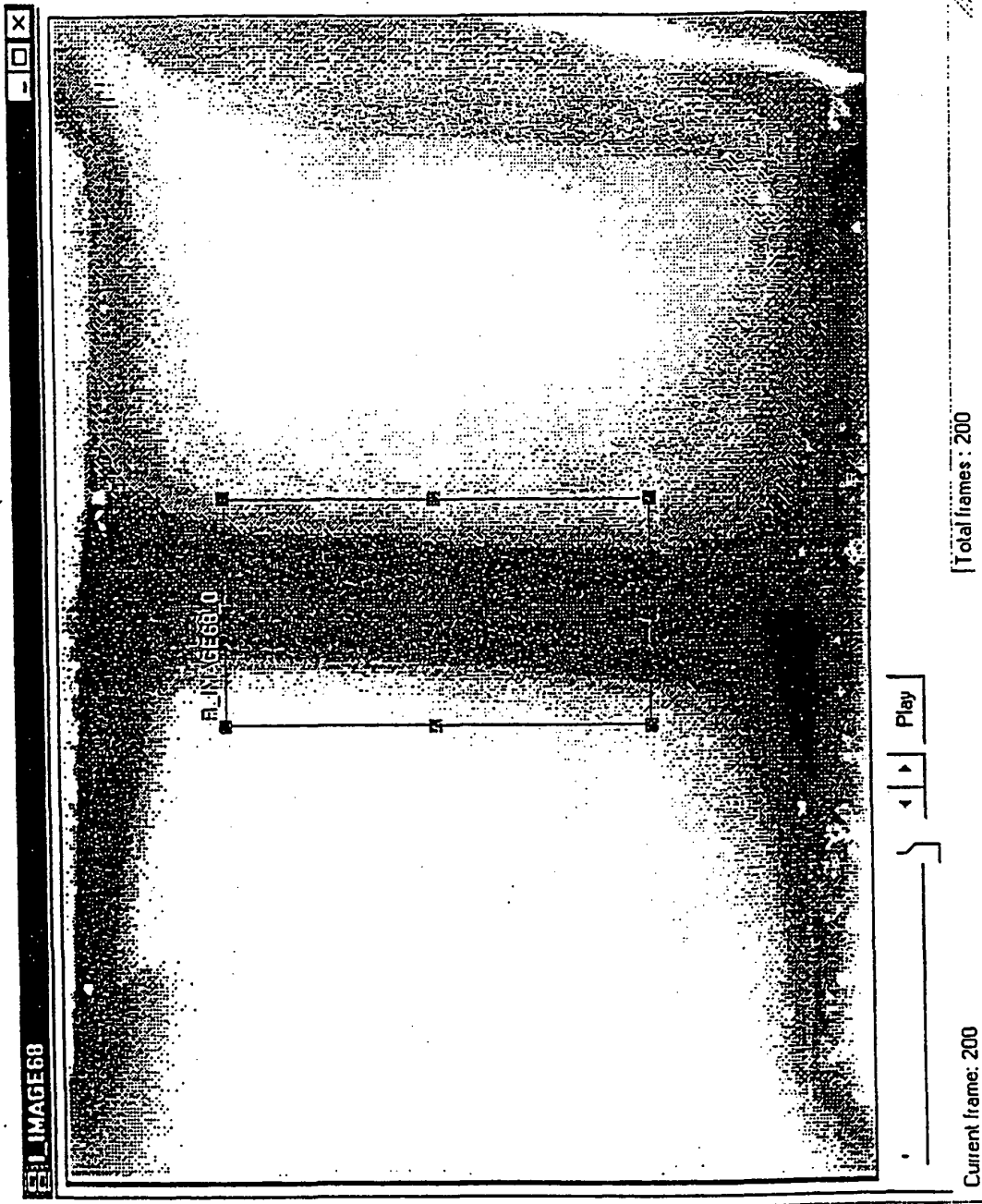


Figure 22

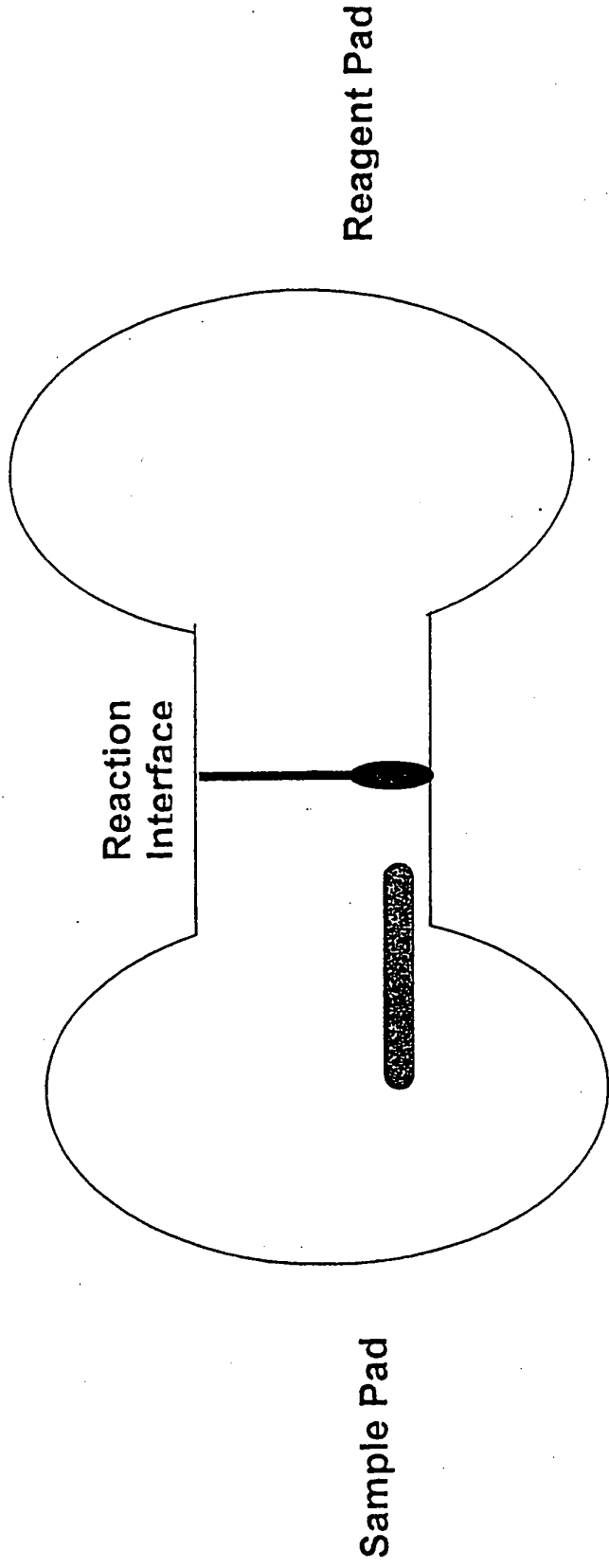


Figure 23

Calibration Spot of Excess Glucose  
(10,000mg/dL of GLU)  
Sample: FBS (~100mg/dL GLU)  
20 Seconds to 60 Seconds

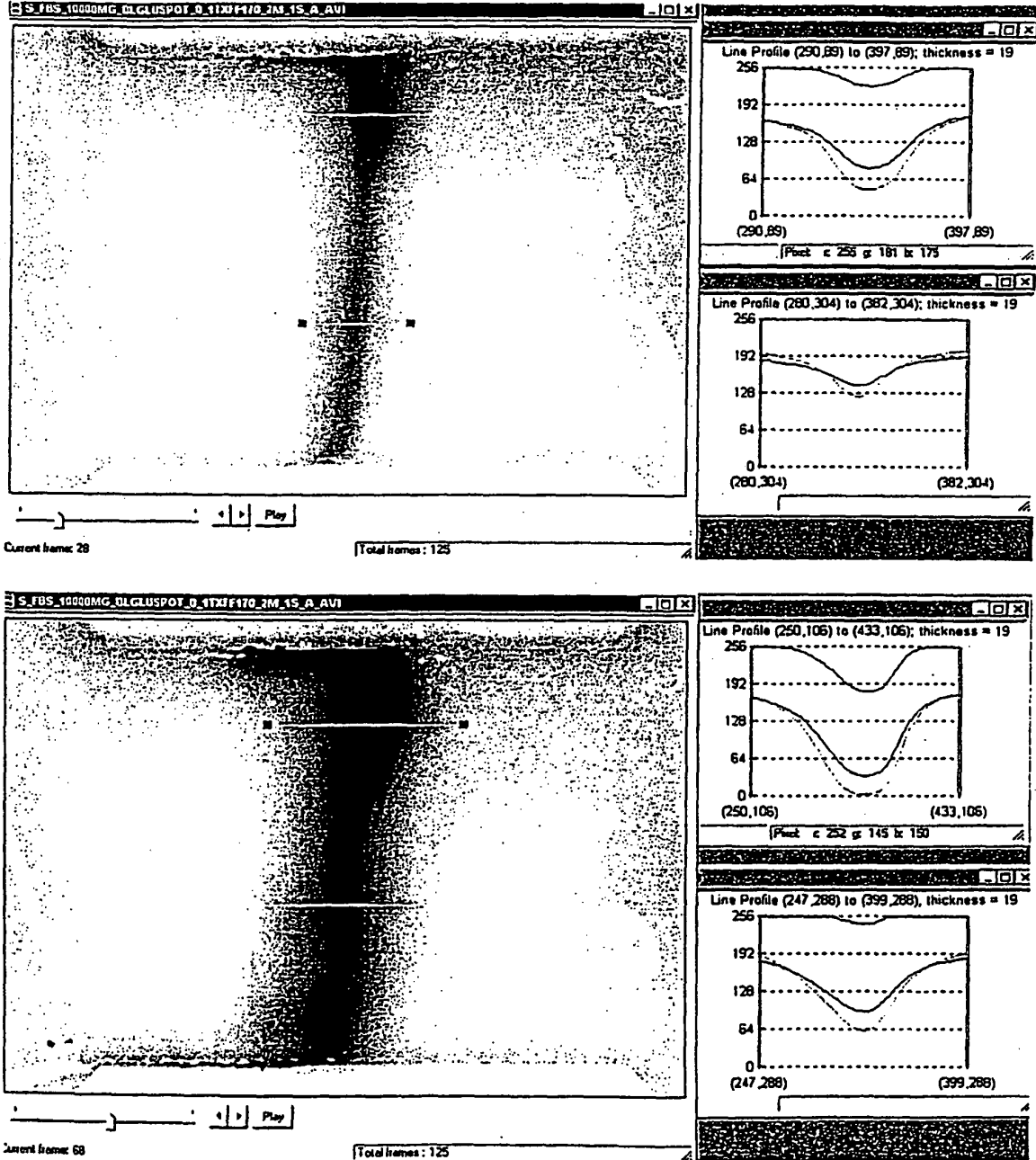


Figure 24

Alk Phos pH10 Nitro (1180)  
Time (15sec/120 sec)

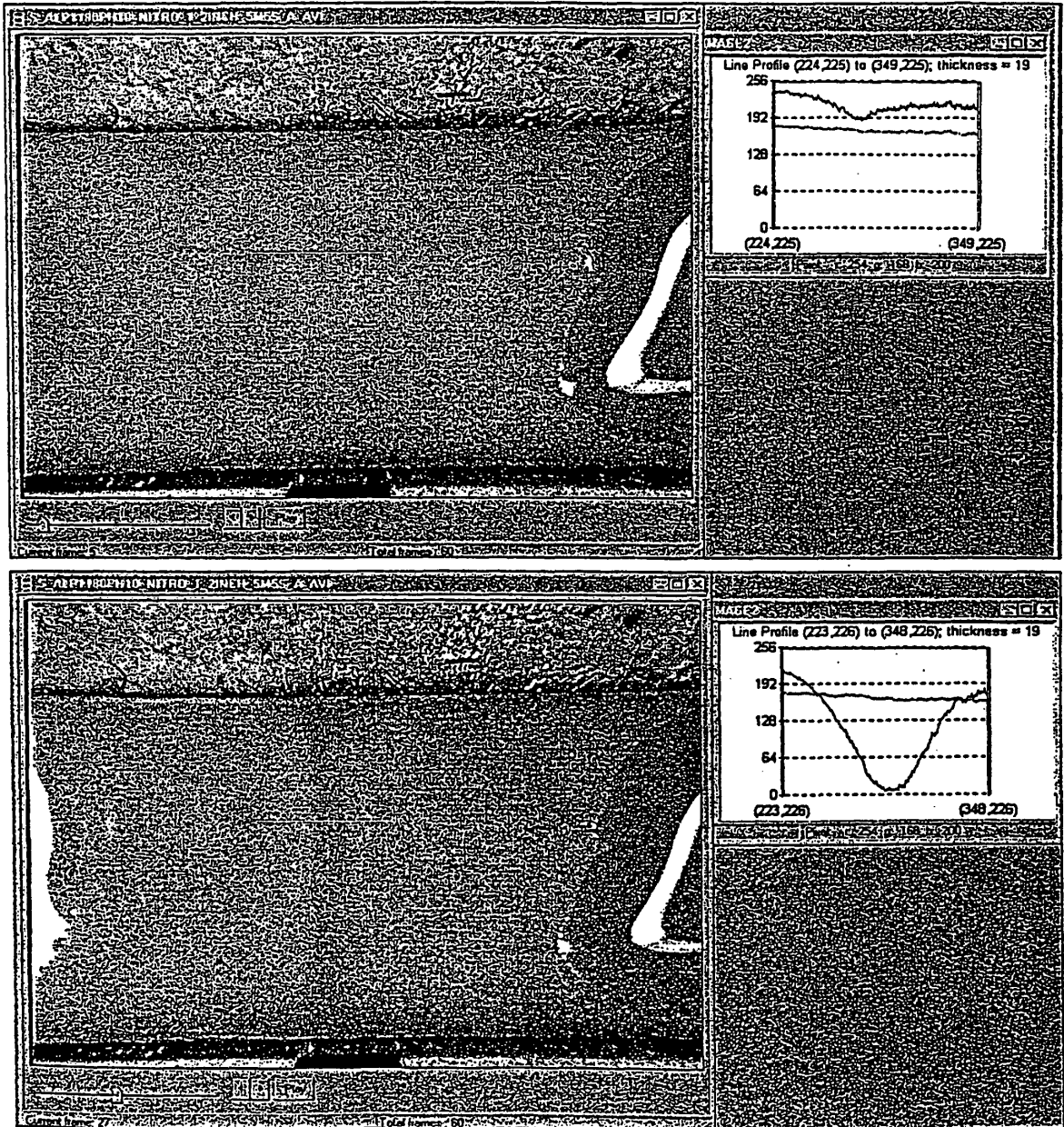


Figure 25

Total Bilirubin (4.73 mg/dL)  
Time (15 sec/275 sec)

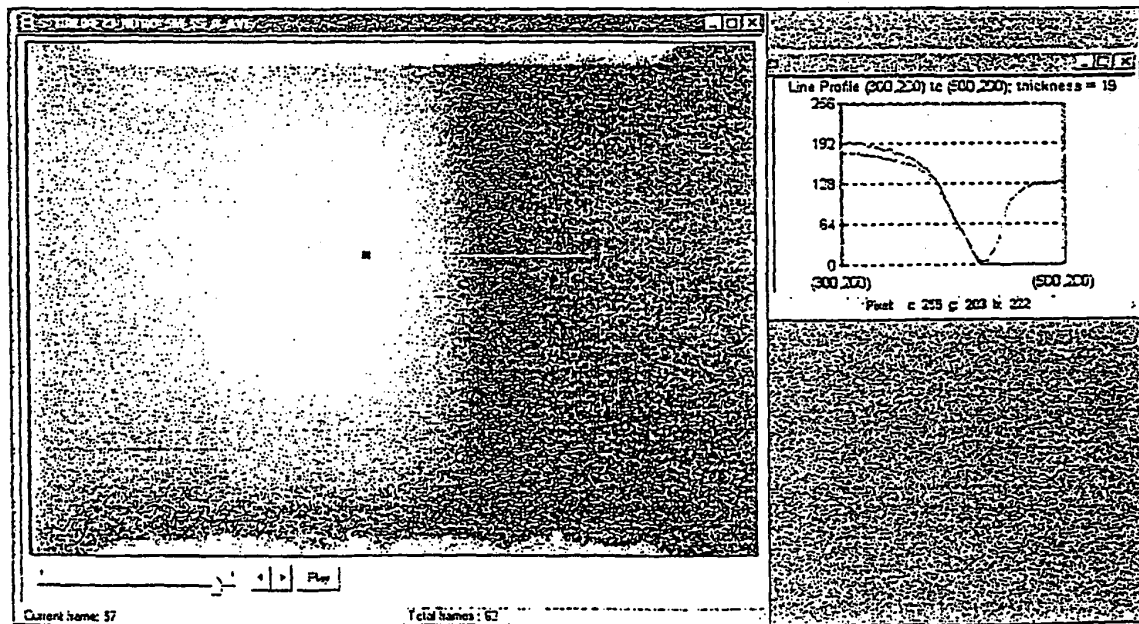
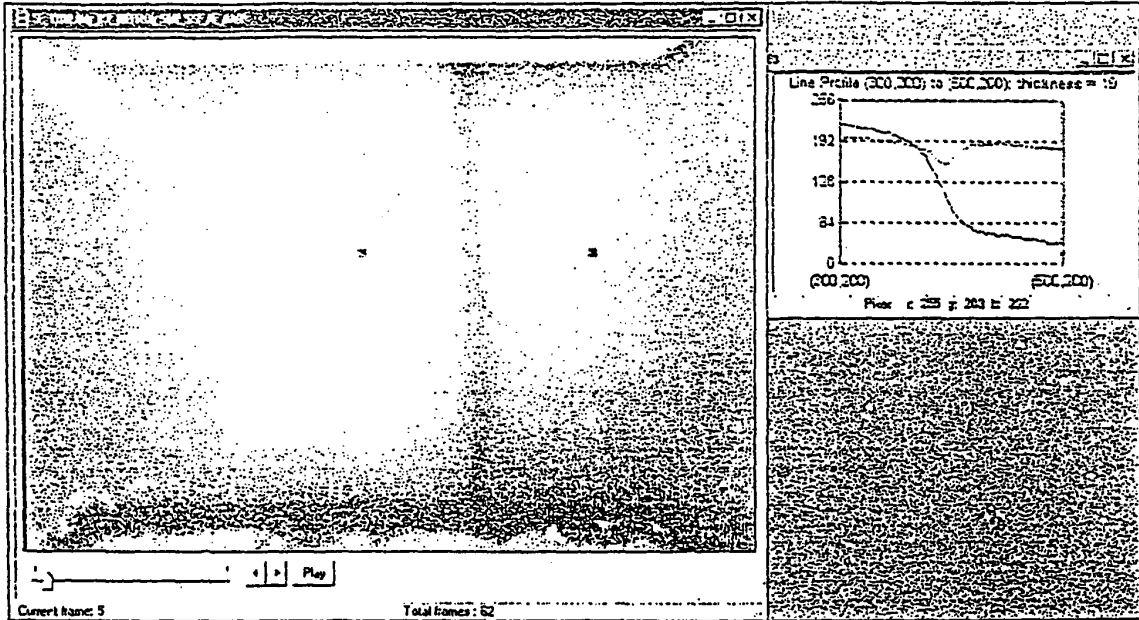


Figure 26

# Uric Acid Supor (0.3mg/dL) Time (5 sec/295 sec)

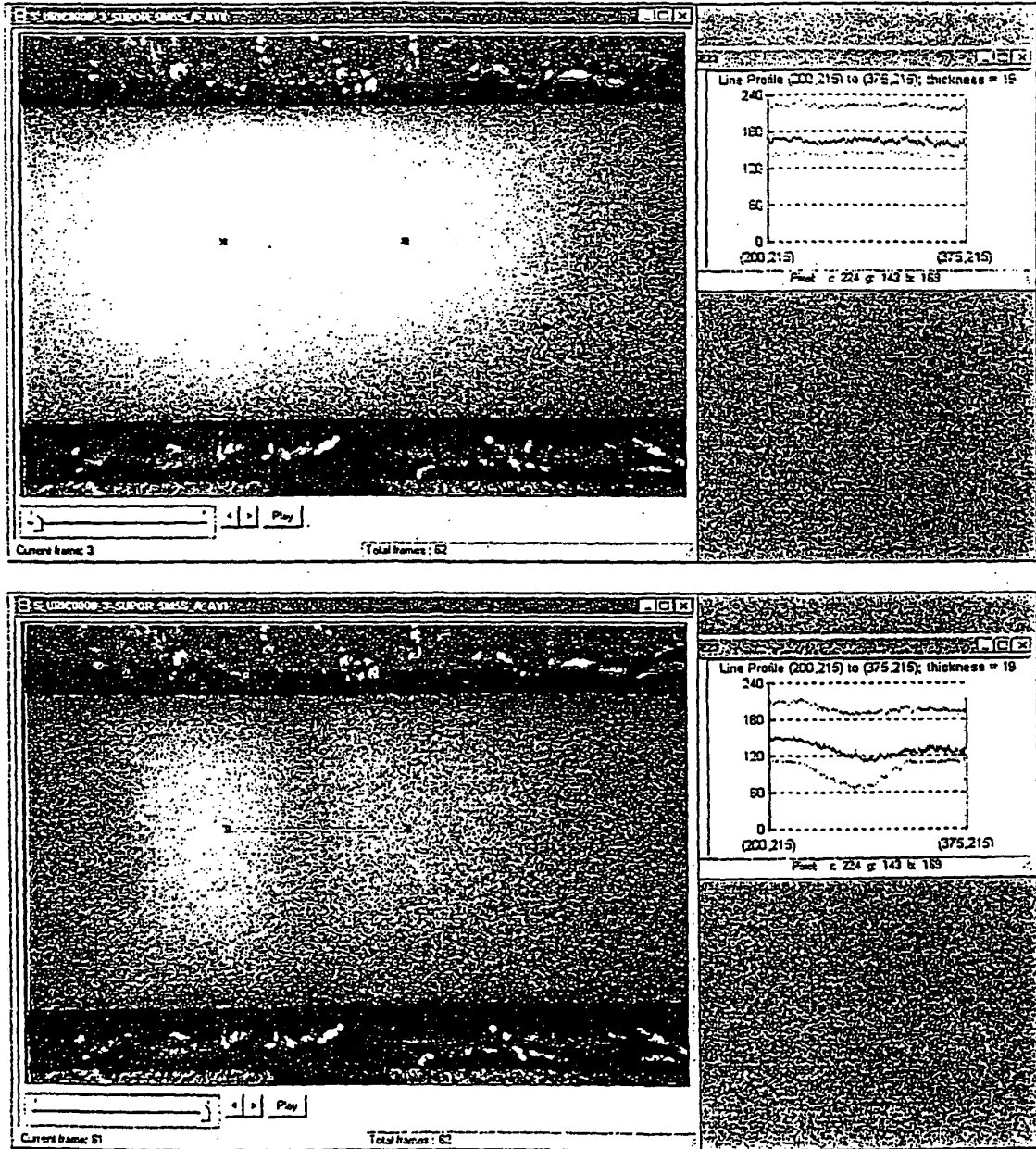


Figure 27

GGT pH7 Nitro (380 U/L)  
Time (5 sec/285 sec)

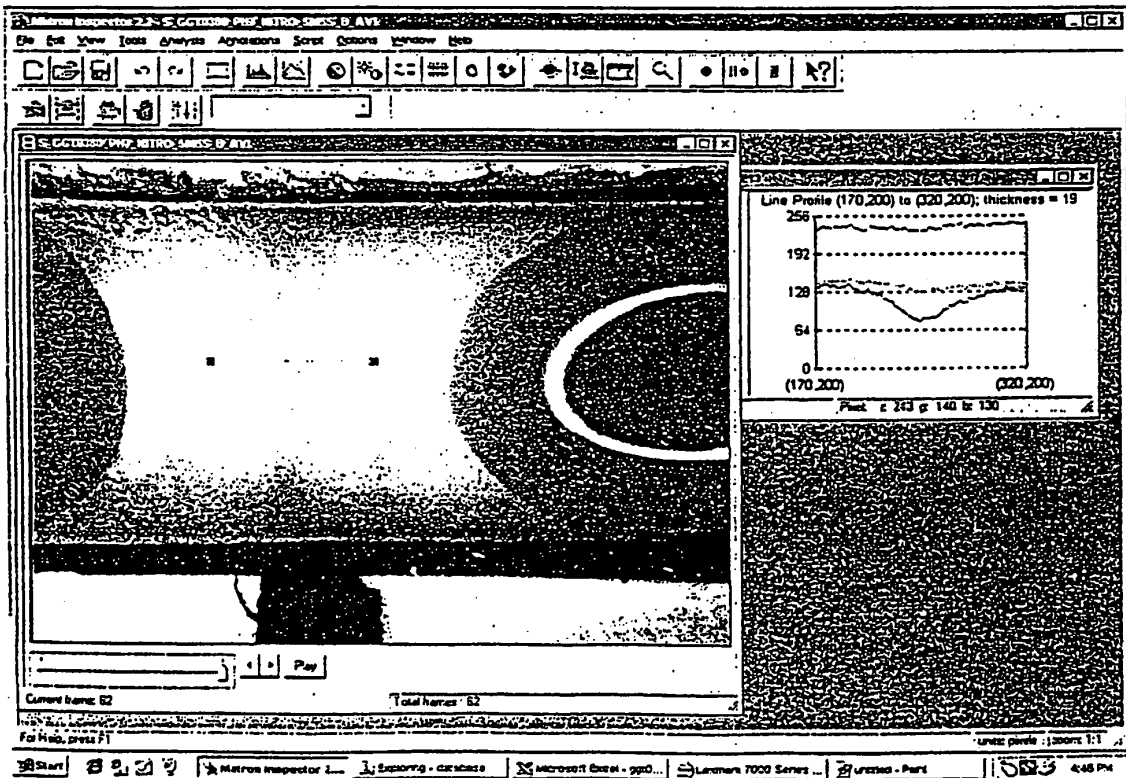
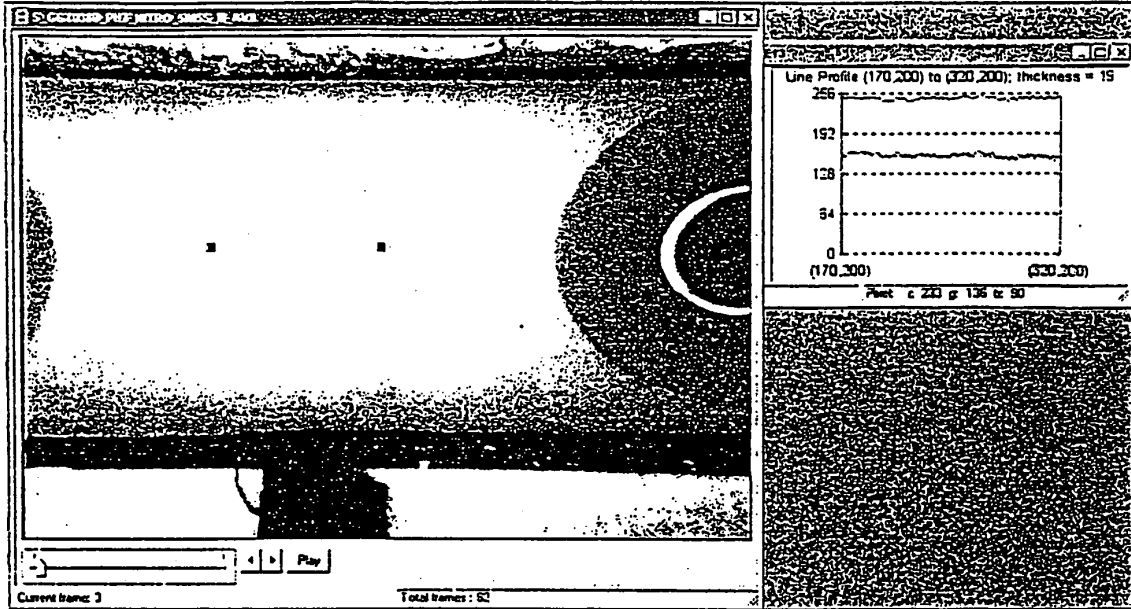


Figure 28

# Amylase (16800 U/L) pH7 Time (35 sec/275 sec)

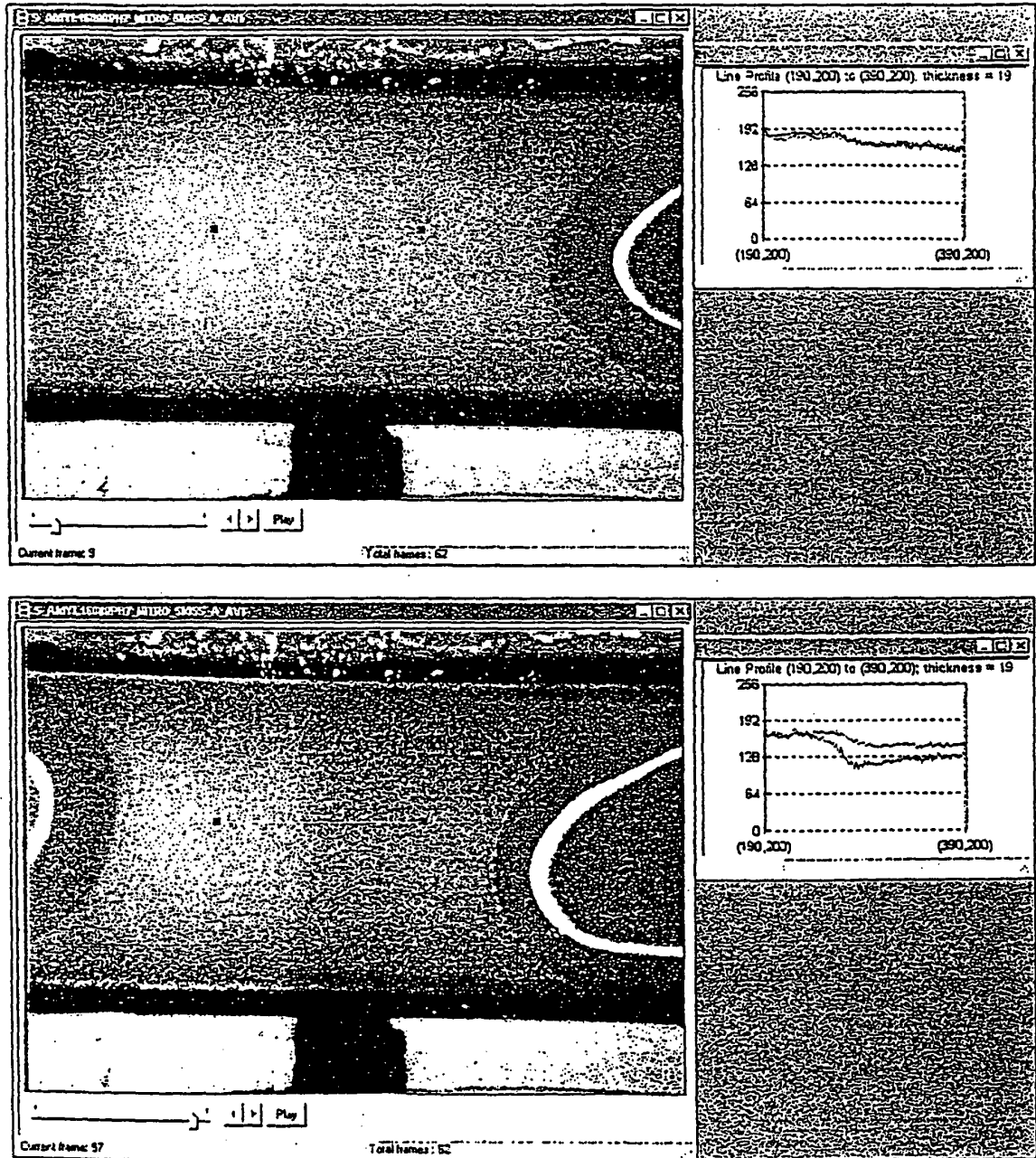


Figure 29

# Creatinine pH 7 (150 mg/dL) Time (5sec/295sec)

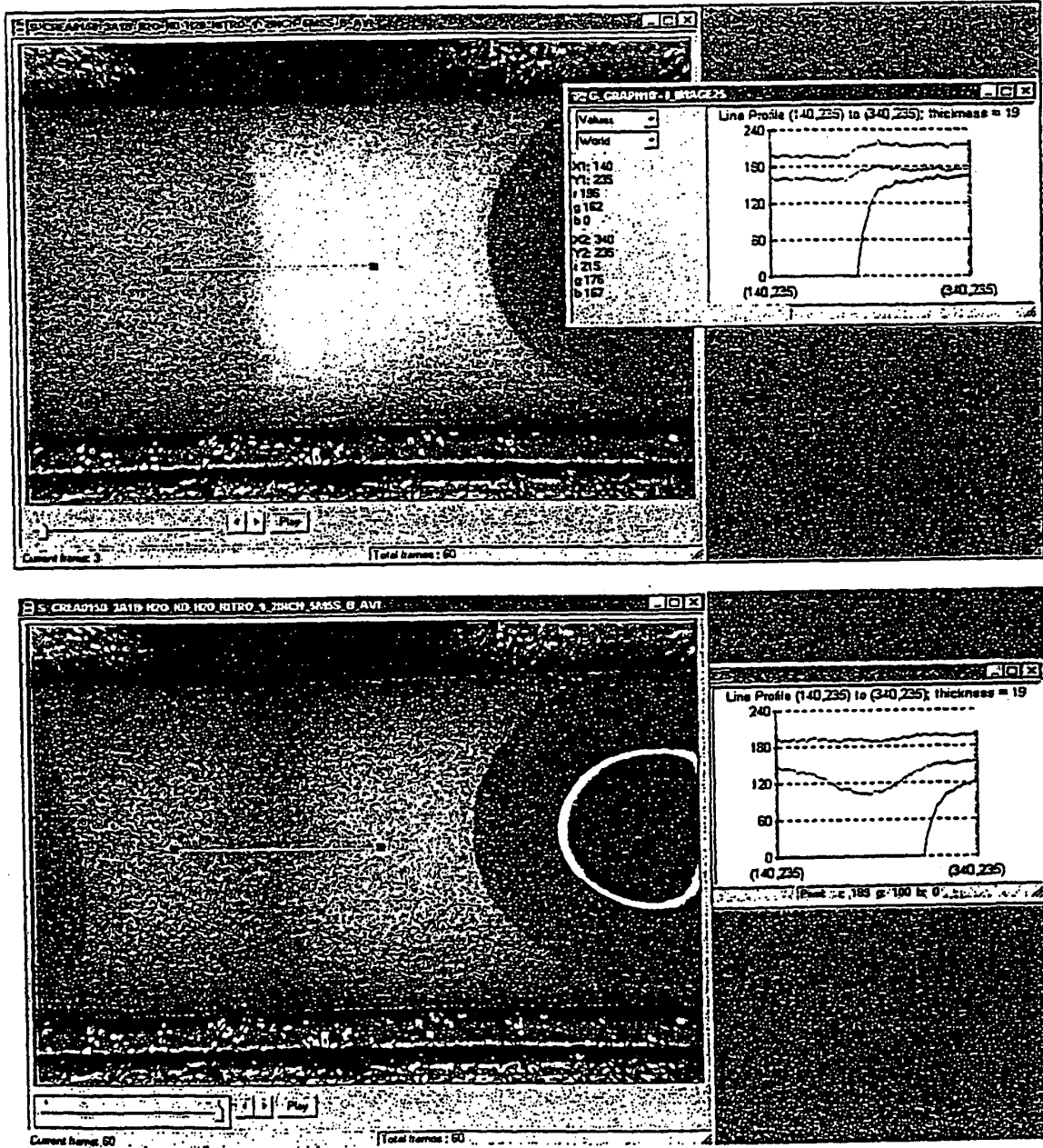


Figure 30

# Cholesterol (96 mg/dL) pH7 Time (10secs/300secs)

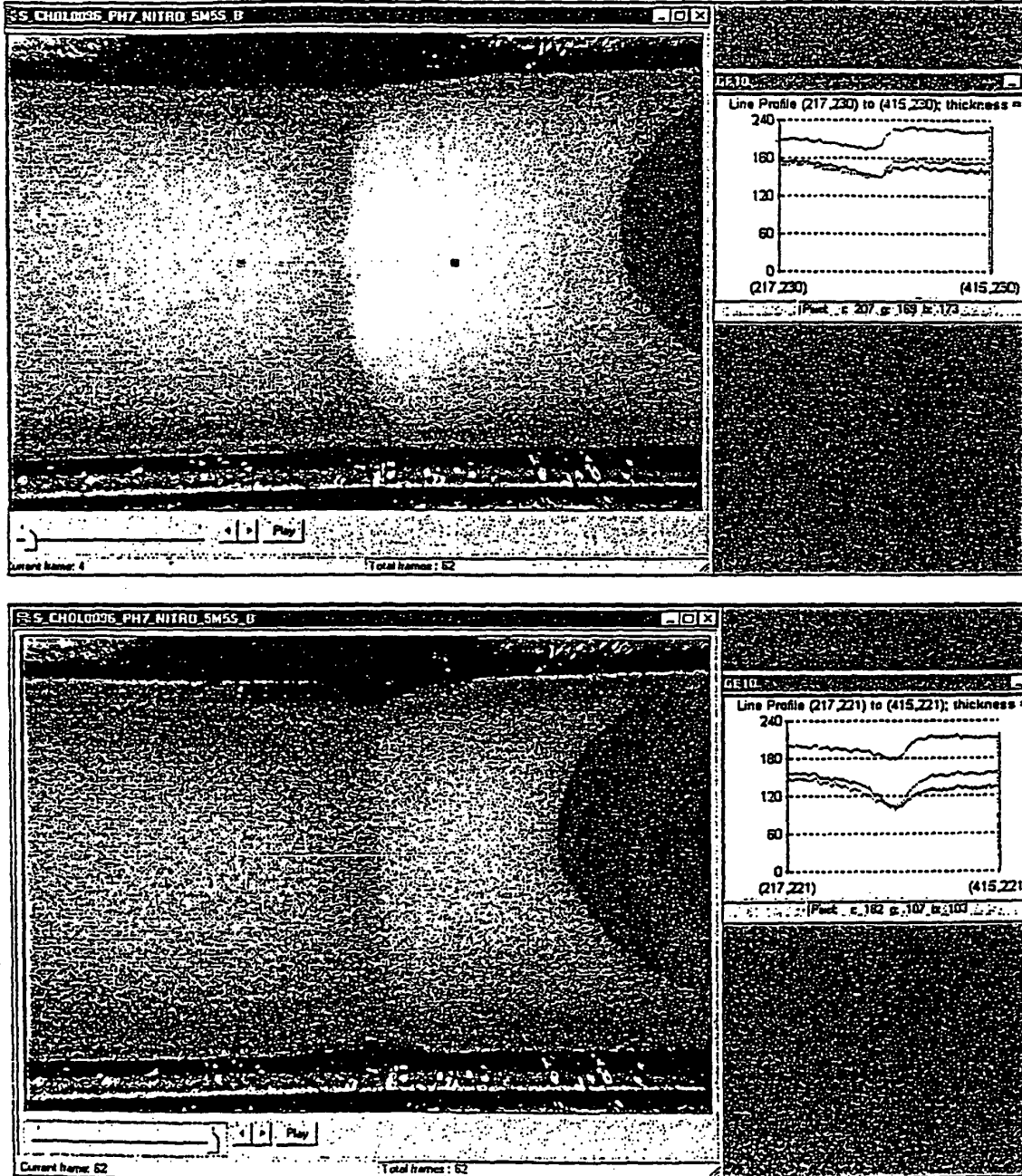


Figure 31

# Total Protein (6200mg/dL) Time (5 sec/295sec)

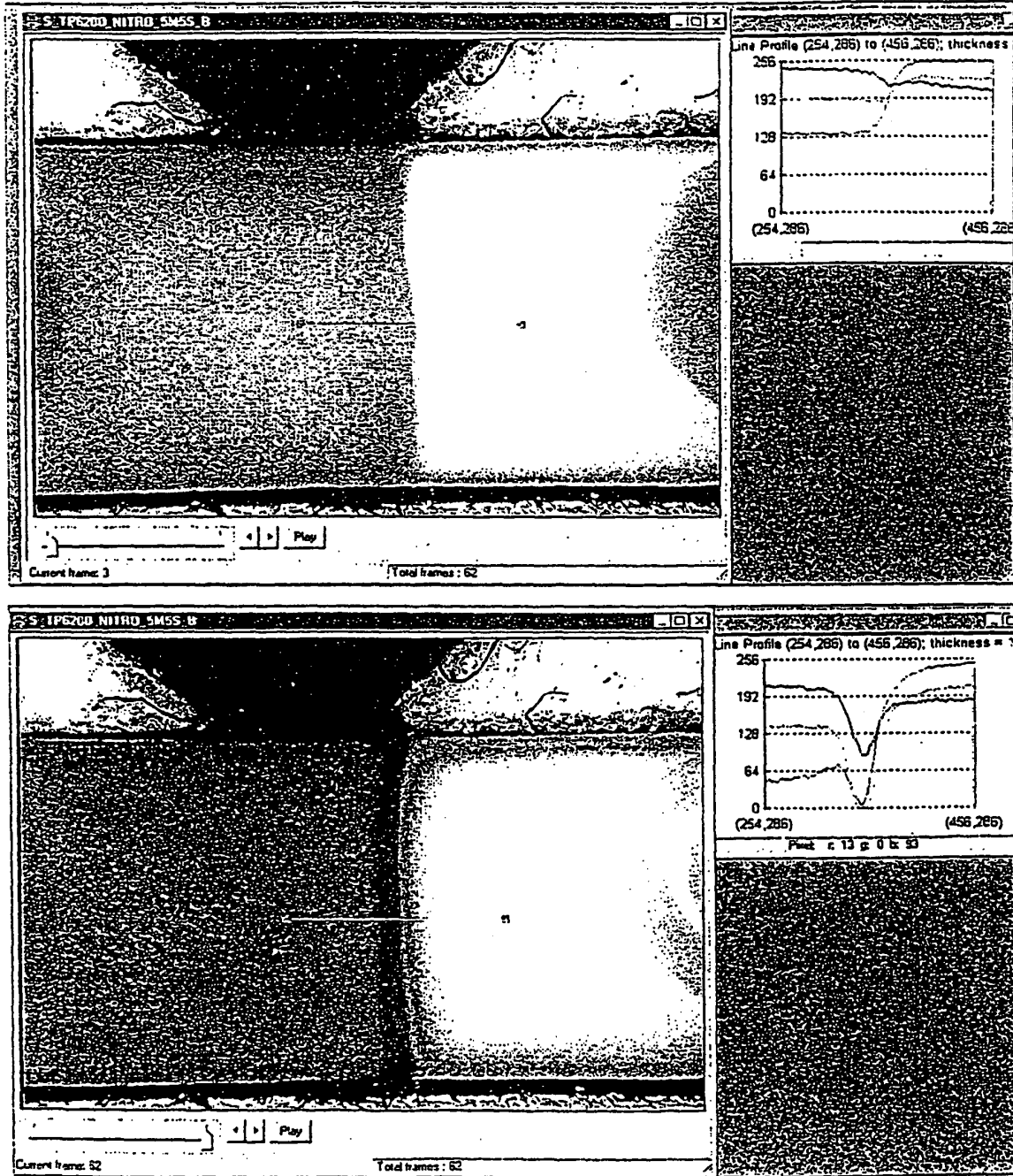


Figure 32

ip  
Magnesium (4.7 mg/dL) pH10  
Time (5sec/295sec)

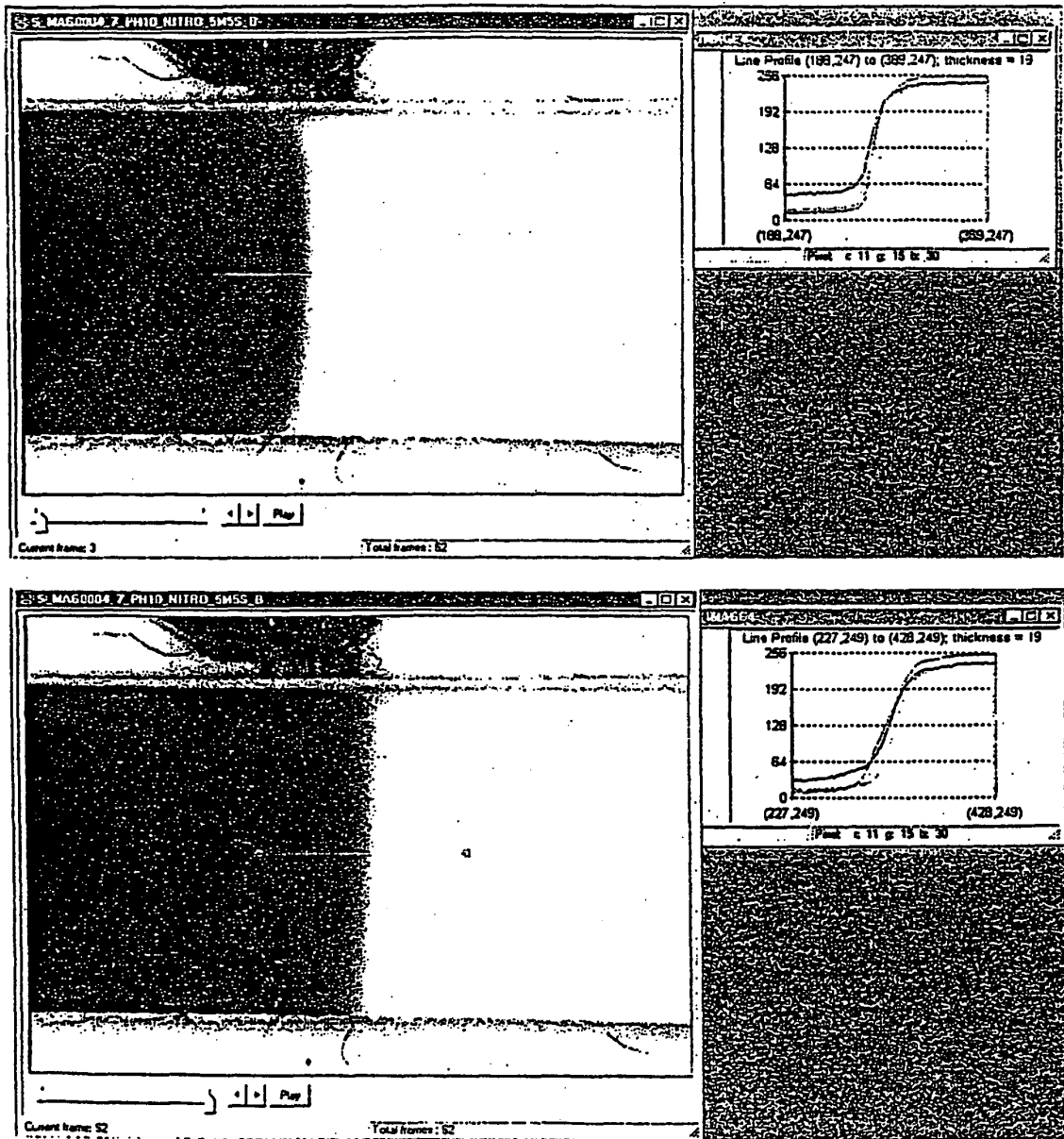


Figure 33

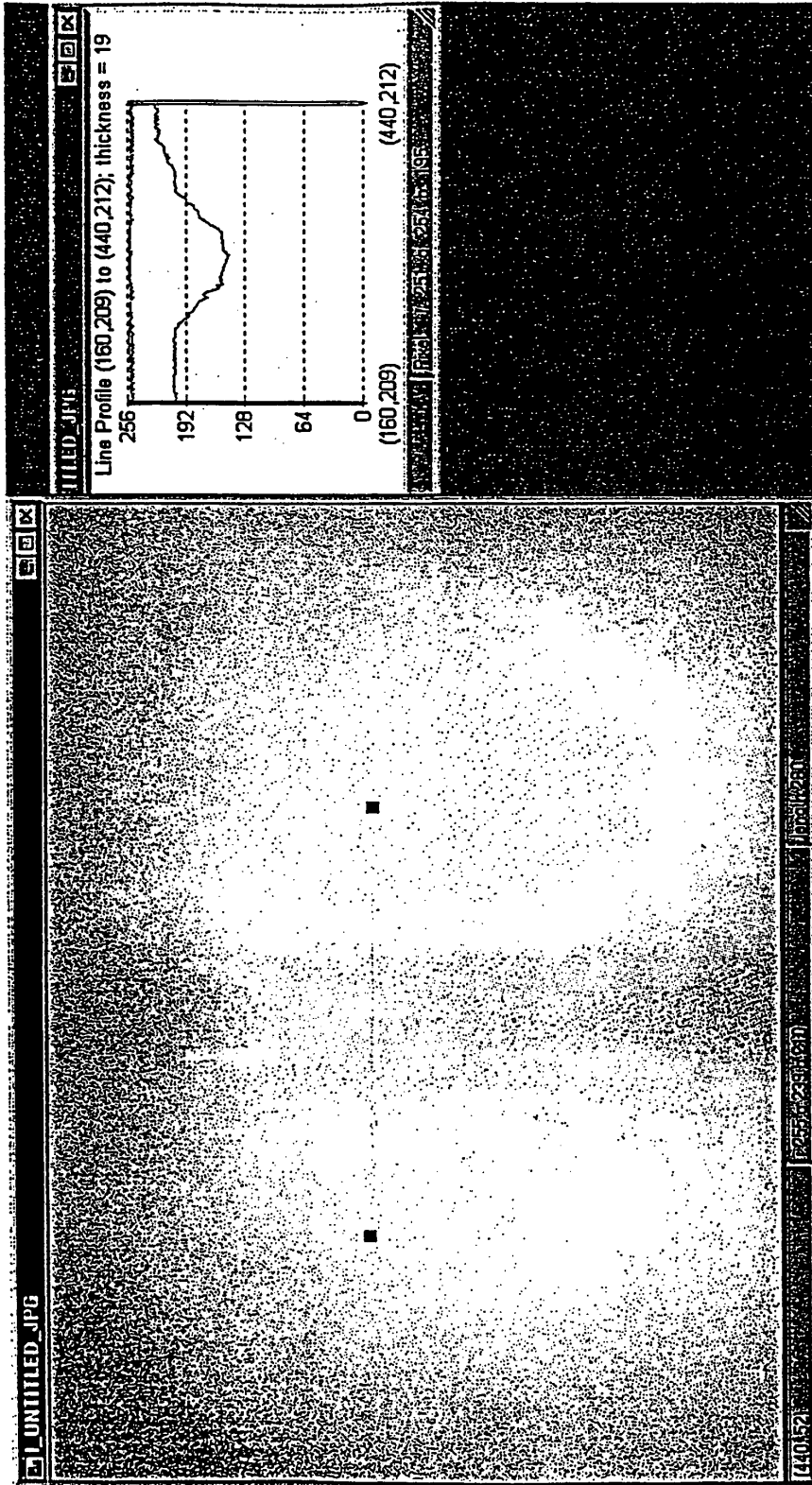


Figure 34

# Anti-T4 Alkaline Phosphatase Hapten Assay

**REFERENCES CITED IN THE DESCRIPTION**

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专利名称(译)	用于检测流体中的分析物的方法和装置		
公开(公告)号	<a href="#">EP1082614B1</a>	公开(公告)日	2007-06-27
申请号	EP2000919643	申请日	2000-03-24
[标]申请(专利权)人(译)	艾德克斯实验室公司		
申请(专利权)人(译)	IDEXX实验室, INC.		
当前申请(专利权)人(译)	IDEXX实验室, INC.		
[标]发明人	CARPENTER CHARLES R		
发明人	CARPENTER, CHARLES, R.		
IPC分类号	G01N33/558 G01N33/543 C12M1/34 C12Q1/25 G01N21/78 G01N33/483 G01N33/533 G01N33/536 G01N33/577		
CPC分类号	G01N33/558 Y10S435/81 Y10S435/91		
优先权	09/277715 1999-03-26 US 09/439024 1999-11-12 US 09/525151 2000-03-14 US		
其他公开文献	EP1082614A1		
外部链接	<a href="#">Espacenet</a>		

摘要(译)

公开了一种一次性干化学分析系统，其广泛用于检测生物流体中存在的各种分析物，例如全血，血清，血浆，尿液和脑脊液。本发明公开了在吸水材料内从两个相反方向会聚的液体之间形成的反应界面的用途。该发现包括对现有技术的一次性分析试剂系统的显著改进，因为可检测的反应物区在视觉上是不同的并且与未反应的试剂分开，允许使用仅表现出微小变化的反应指示剂以及极高浓度的反应物。此外，可以加入分阶段的多种试剂。全血可以用作样品而无需单独的细胞分离材料。最后，本发明可用于检测多种材料中的分析物，例如牛奶，环境样品和含有目标分析物的其他样品。

