EUROPEAN PATENT SPECIFICATION

Method for the determination of analyte concentration in a lateral flow sandwich immunoassay exhibiting high-dose hook effect

Verfahren zur Bestimmung von Analytkonzentration in ein Hochdosis Hook-Effekt aufweisendes Querfluss-Sandwichimmunoassay

Méthode pour la détermination de la concentration d’un analyte dans un immunoessai sandwich à flux latéral présentant un hook effect à haute dose

Designated Contracting States:
BE CH DE ES FI FR GB IE IT LI NL SE

Priority: 27.07.1998 US 122736

Date of publication of application: 22.03.2000 Bulletin 2000/12

Divisional application: 08165985.6

Proprietor: Siemens Healthcare Diagnostics Inc. Tarrytown, NY 10591 (US)

Inventors:
- Kuo, Hai-Hang Granger, Indiana 46530 (US)
- Meritt, Lisa A. Goshen, Indiana 46526 (US)

Representative: Maier, Daniel Oliver et al
Siemens AG
CT IP Com E
Postfach 22 16 34
80506 München (DE)

References cited:
EP-A- 0 462 376
EP-A- 0 895 084
WO-A-98/22800

- PRODUCT DESCRIPTION: “Semi-quantitative rapid test for the determination of C-reactive protein (CRP) in plasma or serum” RAPID CRP TEST, 25 May 1997 (1997-05-25),

Remarks:
The file contains technical information submitted after the application was filed and not included in this specification

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).
Immunochromatographic strip formats have become increasingly popular for quantitative and semi-quantitative assays which use visual detection schemes. This type of immunoassay involves the application of a liquid test sample suspected of containing an analyte to be detected to an application zone of an immunochromatographic test strip. The strip is comprised of a matrix material through which the fluid test medium and analyte suspended or dissolved therein can flow by capillarity from the application zone to a capture zone where a detectable signal, or the absence of such, reveals the presence of the analyte. Typically, the strip will include means for immunospecifically binding the analyte to be detected with its specific binding partner which bears the detectable label. In one such scheme, as disclosed in U.S. Patent 4,446,232; the strip contains an enzyme labeled, mobile binding partner for the analyte which is in a zone of the strip downstream from the sample application zone. If analyte is present in the test sample, it will combine with its labeled binding partner to form a complex which will flow along the strip to a detection zone which contains a substrate for the enzyme label which is capable of providing a colored response in the presence of the enzyme label. The strip contains another zone in which analyte is immobilized, so that the labeled binding partner which does not combine with analyte, due to the absence of sufficient analyte in the sample, will be captured and thereby inhibited from reaching the detection zone. There have been published various modifications of this technique, all of which involve competitive specific binding systems in which the presence or absence of analyte in the test sample is determined by the detection or lack thereof of labeled binding partner in the capture zone.

An alternative to the above described immunometric assay which detects the free labeled antibody is the so-called sandwich format in which the capture zone contains immobilized antibodies against an epitope of the analyte which is different than the epitope to which the labeled antibody is specific. In this format, there is formed a sandwich of the analyte between the immobilized and labeled antibodies and it is therefore an immunometric assay which detects the bound labeled antibody species. This type of immunostrip format works well in connection with the analysis of relatively low concentrations of analyte, but can be of limited utility in the analysis of fluids containing high analyte concentration. This adverse effect is caused by the presence of excessive free analyte in the sample that competes for binding with the immobilized antibody in the strip’s capture band with the analyte which has become bound to the labeled antibody by interaction therewith in a portion of the strip upstream from the capture zone. This competition can result in less of the analyte/labeled antibody conjugate being captured by the capture antibody and consequently less signal being detected in the capture zone than would be in the case if there were less analyte in the test sample. A dose-response curve prepared using this type of test strip will show increasing signal with increasing analyte up to the point where the analyte concentration begins to block the interaction between the immobilized capture antibody and the labeled antibody/labeled antibody complex. Beyond this point, increasing analyte in the test fluid results in a decrease in the signal, so that the dose-response curve indicates decreasing signal with increasing analyte. The slope of this sort of dose-response curve somewhat resembles a hook which accounts for this phenomena being known as the hook effect. Traditionally, when the hook effect is observed or suspected, the fluid sample is diluted to several dilutions to ensure the validity of the results. The high dose hook effect may not occur if sufficient labeled or capture antibody is present in the assay medium. A complete dose-response curve (low to high analyte concentration) is usually needed to verify the existence of this effect. Accordingly, sample dilution is generally carried out whenever there is reason to expect that the assay might exhibit the hook effect. It is an object of the present invention to provide a sandwich type assay method using an immunochromatographic strip whose efficacy is not affected by high analyte concentrations in the test sample and, accordingly, does not require sample dilution or reassaying of samples containing high analyte concentrations. This method involves providing a strip with at least two capture bands and optionally a collection band in which there is immobilized a binding partner for labeled antibody which will bind labeled antibody which has not formed a complex with analyte to thereby facilitate its capture in one of the capture bands. The collection band is optional since it is not needed for the assay method to work in the sandwich format. However, by using a strip which contains a collection band, each sample measurement will provide more information thereby improving the assay’s sensitivity and/or precision.

In EP 0 462 376 A2 there is disclosed a procedure in which signal at the capture site and conjugate collection site of an immunochromatographic strip are detected and the analyte concentration is determined by the intensity of the signal at the capture site relative to the signal at the recovery site. Also of interest in this regard is U.S. Patent 5,569,608. In co-pending application [Serial No. 08/900,586] there is disclosed an assay using an immunochromatographic strip having multiple capture and/or collection sites in which the signal from the detectable label in the capture zone(s) and collection zone(s) is determined whereupon a final response signal is determined using an algorithm and a number of signals which are chosen in a manner suited for a particular assay to provide a value for analyte concentration.
Summary of the Invention

[0005] The present invention is a method according to claim 1 for determining the concentration of an analyte in a fluid test medium. The method comprises the steps of:

a) Providing a strip of a porous material through which a test fluid suspected of containing the analyte can flow by capillarity;

the strip has at least two distinct capture regions in which there are immobilized antibodies specific to a first epitope of the analyte. There are also provided antibodies specific to a second epitope of the analyte which bear a detectable label and are capable of flowing through the strip along with the fluid test medium upon applying it to the strip upstream from the first of the at least two distinct capture zones wherein the strip has one collection region in which there is immobilized a collection means for the labeled antibody;

b) Applying the fluid test medium to the strip and allowing it to flow along the strip carrying labeled antibodies along with it to thereby contact the immobilized antibodies in the distinct capture regions. When sufficient analyte is present in the fluid test medium to partially block binding of the immobilized antibody with the first epitope of the analyte in at least the first distinct capture region with which the fluid test medium comes into contact as it flows along the strip, there is formed a sandwich of the immobilized antibody, the analyte and labeled antibody in the distinct capture regions through which the fluid test medium carries analyte the quantity of which sandwich is limited by the partial blocking of the immobilized antibody;

c) Detecting, in a quantitative manner, the signal emitted from the label on the labeled antibody in each of the distinct capture regions in which the sandwich has formed. This provides a pattern of signals which is unique to the concentration of analyte in the fluid test medium; and

d) Mathematically combining the unique set of signals to create a monotonous dose-response curve to factor out the blocking of the binding between the immobilized antibody and the first epitope of the analyte.

Brief Description of the Drawings

[0006]

Fig. 1 is a representation of an assay device useful in the present invention.

Fig. 2 is a dose response curve of the reflectance change of an immunostrip having only one capture band.

Fig. 3 represents a complete dose response curve of the reflectance change of an immunostrip having 3 capture bands and one collection band which have been combined according to the present invention.

Fig. 4 and 5 are partial dose-response curves of the reflectance change of an immunostrip having 3 capture bands and one collection band which have been combined by multiple mathematical methods.

Description of the Invention

[0007] The invention is practiced by first providing the test matrix through which the fluid test sample can flow by capillarity. Typically, the matrix will be in the form of a strip through which the test fluid flows horizontally. While the matrix could be assembled in a layered format through which the test fluid could flow vertically from top to bottom or vice-versa, the following discussion is focused on the preferred strip format.

[0008] The strip can be prepared from any matrix material through which the test fluid and an analyte contained therein can flow by capillarity and can be of a material which is capable of supporting non-bibulous lateral flow. This type of flow is described in U.S. Patent 4,943,522 as liquid flow in which all of the dissolved or dispersed components of the liquid are carried through the matrix at substantially equal rates and with relatively unimpaired flow, as contrasted to preferential retention of one or more components as would be the case if the matrix material were capable of absorbing or imbibing one or more of the components. An example of such a material is the high density or ultra high molecular weight polyethylene sheet material obtainable from Porex Technologies. Equally suitable for use as the matrix from which the chromatographic strips can be fabricated are bibulous materials such as paper, nitrocellulose and nylon.

[0009] In a preferred embodiment of the present invention there is provided a test device comprising a strip of nitrocellulose having a region 2 (Fig. 1) which contains mobile specific binding partner for the analyte which bears a detectable
label and can react with analyte present in the fluid test sample applied to wicking pad 1 to form an analyte/labeled binding partner complex upon applying the test sample to the wicking pad and allowing it to flow up the strip to region 2 where analyte in the test sample combines with labeled antibody specific to the analyte and flows to area 3 which contains two or more capture bands 3' which contain immobilized antibodies specific for an epitope of the analyte separate from that to which the labeled antibodies are specific to form an immobilized binding partner/analyte/labeled binding partner sandwich in one or more of capture bands. When the analyte level in the test fluid is low, the sandwich is formed in the first distinct capture region without any interference, and the signal from the labeled antibody can be read without additional steps. However, when excessive analyte is present, blocking of some binding sites on the immobilized antibody occurs thereby reducing sandwich formation in the first capture region. In this situation, unbound analyte-labeled antibody conjugate flows through the first capture region and is captured in the second capture region. When the analyte concentration is sufficiently high to block binding sites in the second capture region, capture of the analyte-labeled antibody conjugate takes place in a third or possible subsequent capture regions.

[0010] The number of capture bands is generally determined by the analyte concentration range, the amount of immobilized antibody in each band and the levels of differentiation required. Theoretically, there is no limitation on the number of capture bands as long as they are needed and there is enough space available on the test strip. Typically, a maximum of 3 capture bands 3' will be incorporated into capture region 3 of the strip since this will provide enough capacity for most assays. The labeled anti-analyte antibody is typically added to region 2 but not in excess of that which is necessary to form conjugates with all of the analyte which would be expected to be present in the test sample. Excess labeled antibody/analyte conjugate is captured in the collection band of area 4 by a collection means for labeled antibody such as immobilized IgG or it may all bind to the capture bands at medium or high analyte concentration with no conjugate reaching the collection band. [The collection band may function as an internal control for the assay or participate in the calculation of analyte concentration.] In the latter embodiment, it is possible to have two or more collection bands on the strip for better analyte measurement to improve the assay performance. The strip may optionally contain a desiccated absorbant pad 5. This pad serves as a liquid sink to facilitate the capillary flow of test fluid through the strip. The desiccant in the pad enhances the process by effectively absorbing the liquid which reaches the top of the strip.

[0011] Upon development of the strip by application thereto of fluid test sample containing analyte, the signal produced by the label in each of the capture bands and optional collection bands is quantitatively detected, such as by use of a reflectance spectrometer, to obtain a pattern of signals which is unique to the concentration of analyte in the fluid test medium. This pattern of signals is then mathematically combined to create a monotonous (continuous increase with analyte concentration) dose-response curve. The curve is constructed to factor out the hook effect blocking of the binding between the immobilized antibody and the first epitope of the analyte which is accomplished by providing a one-to-one relationship between the assay response and the analyte concentration. The problem caused by the hook effect is that the same assay response can result from more than one analyte concentration. A monotonous dose-response curve alleviates this problem, so that unambiguous results can be obtained by a single sample measurement. An immunostrip for the detection of C-reactive protein (CRP) having multiple capture bands is disclosed in Labmedica, April/May 1990, but there is no suggestion that the signals from the capture bands be mathematically combined. It appears that the multiple capture bands in the strip described in this reference are used to indicate the analyte concentration level which is proportional to the number of visible bands on the strip. These multiple bands are not designed to measure the analyte in the range where hook effect occurs because this assay is based on sequential saturation of analyte-labeled antibody complex in the capture zones without the blocking of free analyte. This is in contrast to the mathematical treatment of the pattern of signals in the present invention. Unlike the immunostrip described in this reference, the present assay system is designed to directly measure the analyte concentration above the level where the hook effect occurs. The mathematical treatment of the signal patterns generated by the assay provides a unique revenue (a monotonous dose-response curve) to evaluate the analyte concentration beyond the limit imposed by the hook effect.

[0012] In a preferred method of practicing the present invention the antibody label is capable of reflecting light at a predetermined wavelength and there is provided a reflectance spectrometer having a detector of reflectance intensity with means for moving the developed strip and detector relative to each other such as a specimen table on which the strip is placed which can be moved laterally under the read head of the detector. This technique assists in providing accurate quantitation since the location of the strip relative to the detector can be under microprocessor control, so that the reflectance of the desired regions can be determined and then combined via the use of preprogrammed software to provide the monotonous dose-response curve. Other labels, such as radio isotopes and enzymes are suitable, since the only essential property of the label is that it be capable of being quantitatively detected.

[0013] In the preferred embodiment of the present invention in which the test strip contains 3 capture regions and a single collection region, the unique pattern of signals is combined by:

i) ratioing the signals of the second and third capture regions against the signal of the first capture region;

ii) multiplying the two ratios by numbers that are within the same range of magnitude as the signal from the collection
The method of practicing the present invention is further illustrated by the following examples:

**Example I**

The multiple band immunostrip format of the present invention was demonstrated using a lateral flow nitrocellulose strip containing three test bands of monoclonal mouse anti-C reactive protein (CRP) antibody and one control band of polyclonal donkey antigoat antibody (IgG). The strip was prepared as follows:

The assay was performed by mixing CRP calibrator containing affinity purified CRP of known concentrations in buffer at pH 7.0 with an aqueous assay solution 0.2% (w/v) BSA; 0.05% (w/v) Triton X-100; 0.75% (w/v) glycine; 5.85% (w/v) NaCl and 0.2% (w/v) NaN₃ at pH 8.2 containing 0.04% (w/v) of a polyclonal goat anti-CRP antibody labeled with blue latex particles which was then pipetted onto a cassette containing the nitrocellulose strip. After five minutes the reflectance changes in the capture and collection bands were measured using a CLINITEK® 50 reflectance spectrometer. By using different decode calculations including $\Delta R$ where $\Delta R$ = absolute background reflectance reading near the capture (test) or collection (control) bands minus absolute reflectance reading of the test or control band. Both reflectance readings were obtained 5 minutes after the start of the assay. Two dose response curves were obtained as set out in Figs. 2 and 3. The first curve was derived from the reflectance change of the first test band, i.e. Decode 1 = $\Delta R$ of first test band. The curve of Fig. 3 was generated by a method of data computation utilizing the change in reflectance from all 3 capture bands and the collection band (the assay response) to calculate the analyte concentration, i.e.:

$$\text{Decode 2} = 100 \cdot \frac{T2}{T1} + 50 \cdot \frac{T3}{T1} - CL$$

where:

- $T1 = \Delta R$ of first capture band
- $T2 = \Delta R$ of second capture band
- $T3 = \Delta R$ of third capture band
- $CL = \Delta R$ of collection band

The calculated decode values (overall assay responses) were plotted versus the known analyte concentrations and a dose-response curve (Fig. 3) was constructed by curve fitting. This curve is represented by the equation:

$$100 \cdot \frac{T2}{T1} + 50 \cdot \frac{T3}{T1} - CL = 31.005 \cdot \text{ln(Analyte concentration)} - 170.29$$

By solving this equation with assay responses obtained from the capture and collection bands using test fluid containing unknown concentrations of analyte, the unknown analyte concentration was calculated as:

$$\text{Analyte concentration} = \text{Exp}([100 \cdot \frac{T2}{T1} + 50 \cdot \frac{T3}{T1} - CL + 170.29]/31.005]$$

As shown by the first dose response curve of Fig. 2, the dynamic range of the assay in which only a single band is read is limited by the high dose hook effect, i.e. the analyte concentration above a certain threshold cannot be measured without sample dilution because of a decrease in the response signal. In contrast to the single band test, the multiple band assay gives more than one result per test and displays a unique pattern of band signals for each analyte concentration level. These patterns can be used directly for assessment or represented numerically, such as in the dose response curve of Fig. 3, for quantitative or semi-quantitative evaluation. Dynamic range is the range between the maximum and minimum assay responses. In Fig. 3, the dynamic range is approximately -60-70.

**Example II**

In addition to analyte measurement with a single dose-response curve, the multiple band assay provides the option of multi-curve analyte calculation. This calculation method divides the entire analyte concentration range into a
few sections which are governed by dose-response curves derived from various signal combinations. It is designed to utilize the most sensitive portion of each dose-response curve in a specified concentration region so that any level of analyte (high, medium or low) can be estimated with minimum error. An algorithm based on the signal from each band is used to direct each obtained assay response to the correct concentration region for data processing.

**[0020]** An example of a multi-curve analyte calculation using two dose-response curves is illustrated by the following algorithm:

```
T1, T2, T3, CL
(inputs)

CL>80?
Yes → Decode 3
No

|T1-T2|<3CL?
Yes → Decode 4
No

T2>3CL?
Yes → Decode 4
No

Decode 3
```

**[0021]** This algorithm was derived empirically by comparing the experimental data. The algorithm is designed to demonstrate how a multicurve analyte calculation can be carried out in accordance with the present invention. The decision routine, including the step CL>80?, |T1-T2|<3CL?, T2>3CL?, is one method of determining whether the analyte concentration of the test sample was above or below 250 ng/mL which was the borderline between the two regions governed by the dose-response curves shown in Figs. 4 and 5. The decodes represent values derived from the reflectance changes of capture and collection bands to calculate the analyte concentration based on a dose response curve. Decode is a number representing the reflectance of color from the reagent as measured by the CLINITEK® instrument.

**[0022]** This experiment was also carried out using calibrators of known analyte concentrations. The assay responses were divided into two groups based on their corresponding analyte concentrations. For the group having low analyte concentrations (0 \sim 250 mg/mL), the assay responses were mathematically combined by the defined decode 3 equation decode 3 = Cl. The dose response curve for decode 3 = CL is set out in Fig. 4. This equation includes only the response from the collection band because the capture bands did not exhibit a significant level of differentiation at the low end of the concentration range. The decode values were then plotted against the known analyte concentrations and used to generate a dose-response curve by curve fitting (Fig. 4). The mathematical equation for the curve was represented by the equation:

\[
\text{Decode 3} = -0.4675\times(\text{Analyte concentration}) + 134.08
\]

**[0023]** The dose response curve for decode 4 = T1/CL + 10 * T2/T1 + 100 * T3/T1 is set out in Fig. 5. The result and decode values were plotted against the known analyte concentrations to obtain a dose-response curve by curve fitting. Several signal combinations (decode calculations) were then used to generate a group of dose response curves (such as Figs. 4 and 5) that are sensitive (significant change in assay response as the analyte concentration varies) in different analyte concentration regions.
which was combined with the defined Decode 4 equation to give

\[ \frac{T_1}{CL} + 10^*T_2/T_1 + 100^*T_3/T_1 = 16.302^*\text{Exp}[0.0008^*\text{Analyte concentration}] \]

This equation was then transformed into

\[ \text{Analyte concentration} = \ln\left(\frac{\frac{T_1}{CL} + 10^*T_2/T_1 + 100^*T_3/T_1}{16.302}\right)/0.0008 \]

which was used to determine the unknown analyte concentration of test sample.

This is a generalized example in which the sandwich assay for an analyte is carried out using an immunoformat having a single capture band and using an immunoformat with multiple capture bands. For a typical sandwich immunoformat with a single capture band, the dose-response curve is established as follows:
where the equation $Y = f(X)$ is the best fitted dose-response curve obtained by curve fitting.

The present invention involves the use of a sandwich immunoformat with multiple capture bands. More than one dose response curve (one for each capture band) is prepared by performing the assay with calibrators of known analyte concentration. The analyte concentration of the test sample is calculated by simultaneously solving multiple mathematical equations, each of which represents an individual dose-response curve. One way to simplify this complex procedure is to mathematically combine the responses of capture and collection bands by a defined equation (referred to herein as a decode equation) to form an overall assay response (referred to herein as decode) which is similar to that of an immunoformat with a single capture band. The data processing steps are illustrated as follows:

1) Establishment of dose-response curve

Calibrators with known analyte concentrations $X$

Perform the assay

Responses from capture band $Y$

Plot $Y$ against $X$

Obtain dose-response curve by curve fitting

Mathematical equation for the dose-response curve (best fitted) is expressed as $Y = f(X)$

e.g., $Y = -0.3X^2 + 4X - 2$

The analyte concentration $x$ is calculated by solving the equation $y = f(x)$

e.g., $y = -0.3x^2 + 4x - 2$

Overall assay responses (or decodes) calculated by the decode equation $x = g(Y_1, Y_2, Y_3, ...)$

e.g., $x = Y_1 + Y_2 - Y_3 + ...$

Plot $x$ against $X$
where \( g \) is a mathematical function describing how \( Y_1, Y_2, Y_3 \ldots \) are combined, and

\[
\text{Obtain dose-response curve by curve fitting}
\]

\[
\text{Mathematical equation for the dose-response curve (best fitted) is expressed as } \chi = h(x)
\]

\[\text{e.g., } \chi = -x^2 + 8x - 5\]

where \( h = \) the best fitted dose-response curve which is obtained by curve fitting.

2) Calculation of unknown analyte concentration

\[\text{[0029]}\]

\[
\text{Test sample with unknown concentration } x
\]

\[
\text{Perform the assay}
\]

\[
\text{Responses from capture and collection bands } y_1, y_2, y_3, \ldots
\]

\[
\text{Mathematically combine } y_1, y_2, y_3, \ldots
\]

\[
\text{Overall assay response (or decode) calculated by the decode equation }
\]

\[\chi = g(y_1, y_2, y_3, \ldots)\]

\[\text{e.g., } \chi = y_1 + y_2 - y_3 + \ldots\]

\[
\text{Use } \chi \text{ and the dose-response curve to estimate } x
\]

\[
\text{The analyte concentration } x \text{ is calculated by solving the equation }
\]

\[\chi = h(x)\]

\[\text{e.g., } \chi = -x^2 + 8x - 5\]

\[\text{[0030]} \quad \text{The decode equation } \chi = g(y_1, y_2, y_3 \ldots) \text{ and the dose-response curve equation } y = h(x) \text{ can be combined to give:}
\]

\[g(y_1, y_2, y_3 \ldots) = h(x) \text{ and the calculation of unknown analyte concentration therefore becomes:} \]
These data processing steps, including the establishment of the dose-response curve and calculation of the unknown analyte concentration, were followed in both Examples I and II.

Claims

1. A method for determining the concentration of an analyte in a fluid test medium which comprises:

   a) providing a strip of a porous material through which the test fluid suspected of containing the analyte can flow by capillarity which strip has 3 capture regions in which are immobilized antibodies specific to a first epitope of the analyte; and also providing labeled antibodies specific to a second epitope of the analyte which are able to flow through the strip along with the fluid test medium upon its application to the strip; and the stripe having one collection region in which there is immobilized a collection means for the labeled antibody;

   b) applying the fluid test medium to the strip and allowing it to flow along the strip carrying the labeled antibodies with it to thereby contact the immobilized antibodies in the distinct capture regions and, when sufficient analyte is present in the fluid test medium to partially block binding of the immobilized antibody with the first epitope of the analyte in at least the first distinct capture region with which the fluid comes into contact as it flows along the strip, forming a sandwich of the immobilized antibody, analyte and labeled antibody in the distinct capture regions through which the fluid test medium carries analyte, the quantity of the sandwich formation being limited by the partial blocking of the immobilized antibody;

   c) quantitatively detecting the signal from the label on the labeled antibody in each of the distinct capture regions in which the sandwich has formed and in the collection region to obtain a pattern of signals which pattern is unique to the concentration of analyte in the fluid test medium; and

   d) mathematically combining the unique pattern of signals to create a monotonous dose response curve to factor out the blocking of the binding between the immobilized antibody and the first epitope of the analyte.

2. The method of Claim 1 wherein the collection means is IgG.

3. The method of Claim 1 wherein the labeled antibody is labeled with a material capable of reflecting light at a predetermined wavelength and the signal from the labeled antibody is quantitatively detected by measuring the change in reflectance (ΔR) from the capture and collection regions by means of a reflectance spectrometer after application of the test sample.

4. The method of Claim 1 wherein the analyte concentration is determined by a multi-curve calculation in which the entire analyte concentration range is divided into sections which are governed by dose response curves derived from various signal combinations.

5. The method of Claim 1 wherein the fluid test sample comprises urine.
Patentansprüche

1. Verfahren zur Bestimmung der Konzentration eines Analyts in einem flüssigen Probenmedium, welches Folgendes umfasst:

   a) einen Streifen eines porösen Materials bereitzustellen, durch den das flüssige Probenmedium, das vermutlich das Analyt enthält, aufgrund des Kapillareffekts hindurchfließen kann, wobei der Streifen porösen Materials drei (3) Aufnahmebereiche aufweist, in denen immobilisierte Antikörper vorhanden sind, die für ein erstes Epitop des Analyts spezifisch sind; und außerdem gelabelte Antikörper bereitzustellen, die für ein zweites Epitop des Analyts spezifisch sind und die in der Lage sind, zusammen mit dem flüssigen Probenmedium durch den Streifen porösen Materials zu fließen, wenn dieses auf den Streifen aufgebracht wird; und wobei der Streifen porösen Materials einen Sammelbereich besitzt, in dem ein Sammelmittel für den gelabelten Antikörper immobilisiert ist;

   b) das flüssige Probenmedium auf den Streifen porösen Materials aufzubringen und an dem Streifen porösen Materials entlang fließen zu lassen, wobei es die gelabelten Antikörper mit sich führt, um dadurch die immobilisierten Antikörper in den verschiedenen Aufnahmebereichen zu kontaktieren und, wenn in dem flüssigen Probenmedium ausreichend viel Analyt vorhanden ist, die Bindung der immobilisierten Antikörper mit dem ersten Epitop des Analyts in mindestens dem ersten separaten Aufnahmebereich, mit dem das flüssige Probenmedium in Kontakt kommt, wenn es an dem Streifen porösen Materials entlang fließt, teilweise zu blockieren, sodass in den verschiedenen Aufnahmebereichen, durch die das flüssige Probenmedium Analyt transportiert, ein Sandwich aus dem immobilisierten Antikörper, dem Analyt und dem gelabelten Antikörper gebildet wird, wobei die Quantität der Sandwichbildung durch das teilweise Blockieren des immobilisierten Antikörpers begrenzt wird;

   c) quantitativ das Signal von dem Label des gelabelten Antikörpers in jedem der verschiedenen Aufnahmebereiche, in denen sich das Sandwich gebildet hat, und in dem Sammelbereich zu erkennen, um ein Signalmuster zu erhalten, wobei das Muster eindeutig für die Konzentration von Analyt in dem flüssigen Probenmedium ist; und

   d) das eindeutige Signalmuster mathematisch zu kombinieren, um eine monotone Dosis-Wirkungs-Kurve zu erhalten und das Blockieren der Bindung zwischen dem immobilisierten Antikörper und dem ersten Epitop des Analyts auszuklammern.

2. Verfahren gemäß Anspruch 1, wobei das Sammelmittel IgG ist.


4. Verfahren gemäß Anspruch 1, wobei die Analytkonzentration anhand einer Mehrkurven-Berechnung bestimmt wird, bei der der gesamte Bereich der Analytkonzentrationen in Abschnitte untergliedert wird, die von Dosis-Wirkungs-Kurven bestimmt werden werden, welche von verschiedenen Signalkombinationen abgeleitet wurden.

5. Verfahren gemäß Anspruch 1, wobei das flüssige Probenmedium Urin umfasst.

Revendications

1. Procédé de détermination de la concentration d’un analyte dans un milieu fluide de test, dans lequel

   a) on se procure une bande d’une matière poreuse à travers laquelle le fluide de test suspecté de contenir l’analyte peut passer par capillarité, cette bande ayant 3 régions de capture, dans lesquelles sont immobilisés des anticorps spécifiques à un premier épitope de l’analyte ; et on se procure aussi des anticorps marqués spécifiques à un deuxième épitope de l’analyte, qui peuvent passer à travers la bande en même temps que le milieu fluide de test après son application à la bande ; la bande ayant une région de recueil dans laquelle est immobilisé un moyen de recueil de l’anticorps marqué ;

   b) on applique le milieu fluide de test à la bande et on le fait s’écouler le long de la bande en transportant les anticorps marqués avec lui pour venir ainsi en contact avec les anticorps immobilisés dans les régions de capture distinctes et, lorsque suffisamment d’analyte est présent dans le milieu fluide de test pour la fixation partielle par blocage de l’anticorps immobilisé par le premier épitope de l’analyte dans au moins la première région de capture distincte avec laquelle le fluide vient en contact alors qu’il s’écoute le long de la bande, on
forme un sandwich de l'anticorps immobilisé, de l'analyte et de l'anticorps marqué dans les régions de capture distinctes, à travers lequel le milieu fluide de test transporte de l'analyte, la quantité de formation de sandwich étant limitée par le blocage partiel de l'anticorps immobilisé ;
c) on détecte quantitativement le signal provenant du marqueur sur l'anticorps marqué dans chacune des régions de capture distinctes, dans lesquelles le sandwich a été formé et dans la région de recueil pour obtenir une séquence de signaux, cette séquence étant univoque à la concentration d'analyte dans le milieu fluide de test ; et
d) on combine mathématiquement la séquence univoque de signaux pour créer une courbe monotone de réaction à la dose pour factoriser le blocage de la fixation entre l'anticorps immobilisé et le premier épitope de l'analyte.

2. Procédé suivant la revendication 1, dans lequel le moyen de recueil est une IgG.

3. Procédé suivant la revendication 1, dans lequel l'anticorps marqué est marqué par une substance apte à réfléchir de la lumière à une longueur d'onde déterminée à l'avance et on détecte quantitativement le signal provenant de l'anticorps marqué en mesurant la variation du facteur de réflexion (\( \Delta R \)) des régions de capture et de recueil au moyen d'un spectromètre de réflexion après application de l'échantillon de test.

4. Procédé suivant la revendication 1, dans lequel on détermine la concentration de l'analyte par un calcul à courbes multiples, dans lequel toute la plage de concentration de l'analyte est subdivisée en des sections, qui sont régies par des courbes de réaction à la dose déduites de diverses combinaisons de signal.

5. Procédé suivant la revendication 1, dans lequel l'échantillon fluide de test comprend de l'urine.
REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader’s convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

• US 4446232 A [0001]
• US 08900586 B [0004]
• US 4943522 A [0008]

Non-patent literature cited in the description

• Labmedica, April 1990 [0011]