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# United States Patent [19]

Shuler et al.

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[54] **DIRECT READ LATERAL FLOW ASSAY FOR SMALL ANALYTES**

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[52] **U.S. Cl.** ..... **436/514**; 436/501; 436/507; 436/510; 436/512; 436/513; 435/7.5; 435/7.93

[58] **Field of Search** ..... 436/514, 501, 436/507, 510, 512, 513; 435/7.5, 7.93

[56] **References Cited**

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[57] **ABSTRACT**

The present invention relates to a method and assay device for detecting small analytes. The results of the assay can be directly read from the device, which is a lateral flow device.

**32 Claims, 3 Drawing Sheets**

ALERE EX. 1010

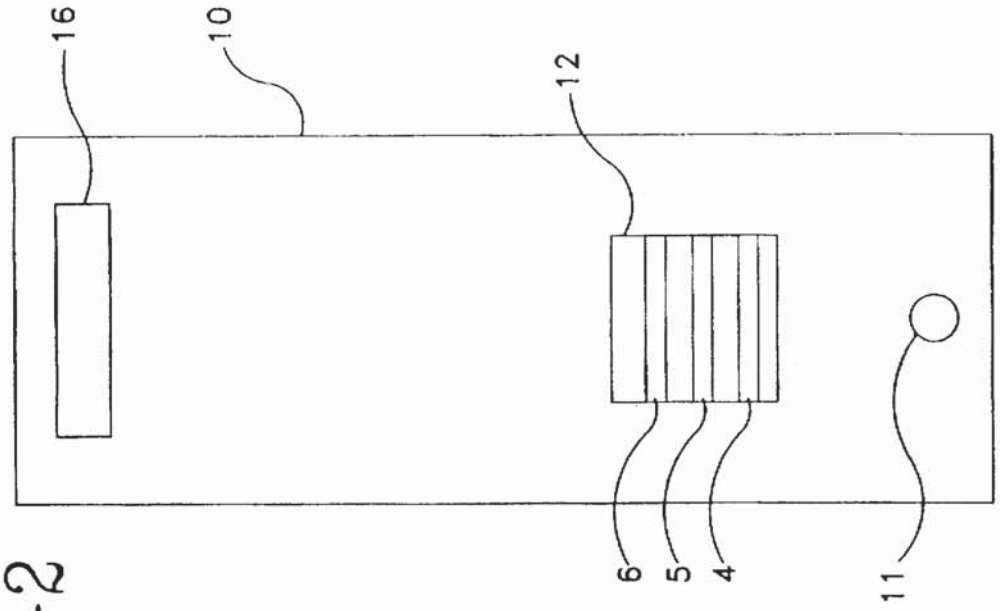



FIG-2

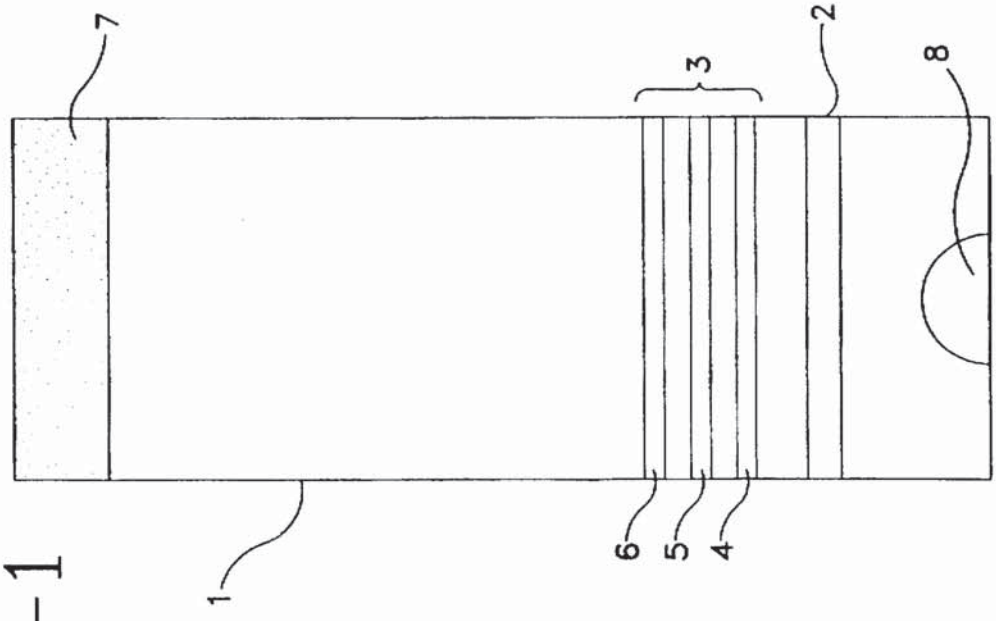
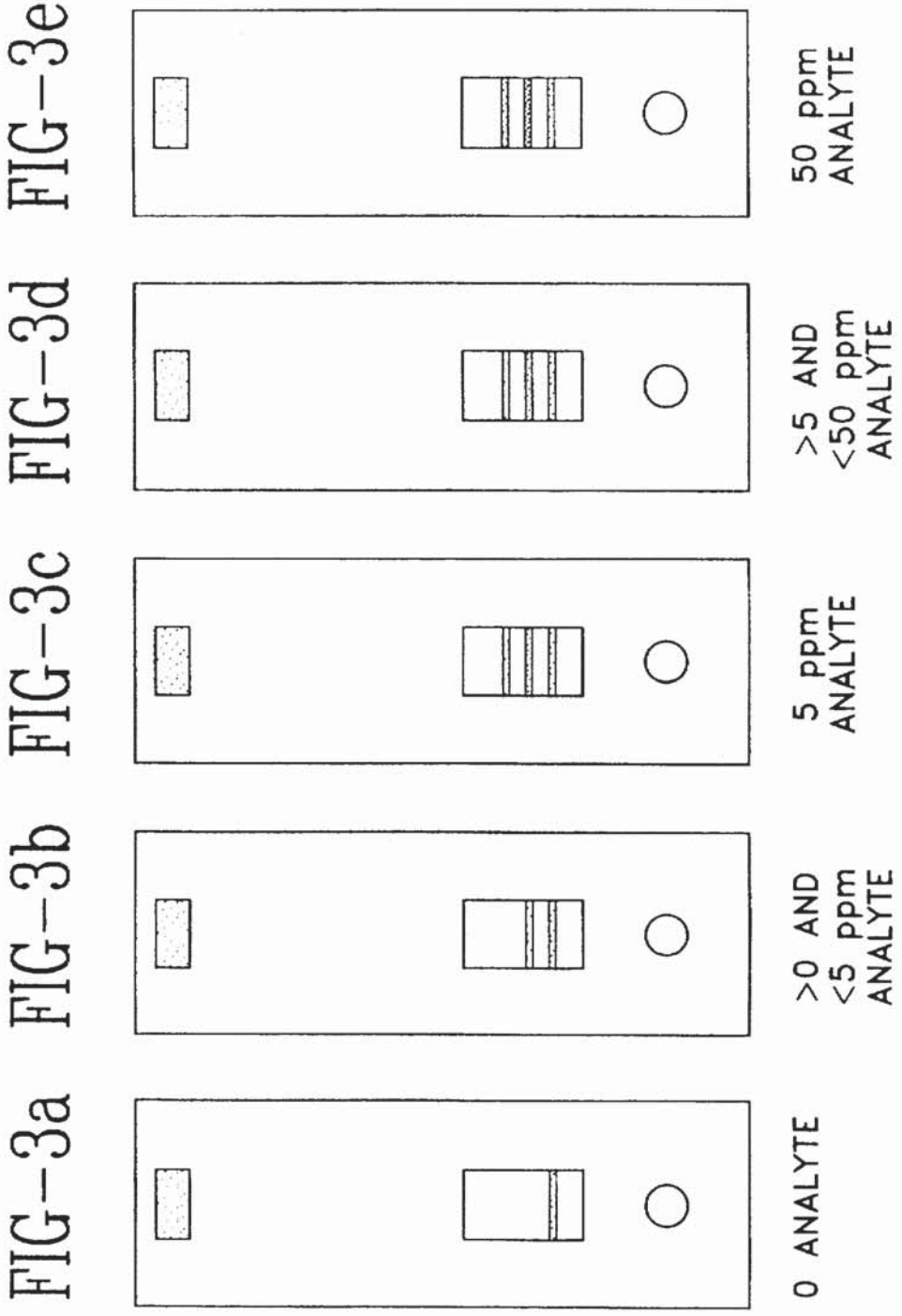



FIG-1



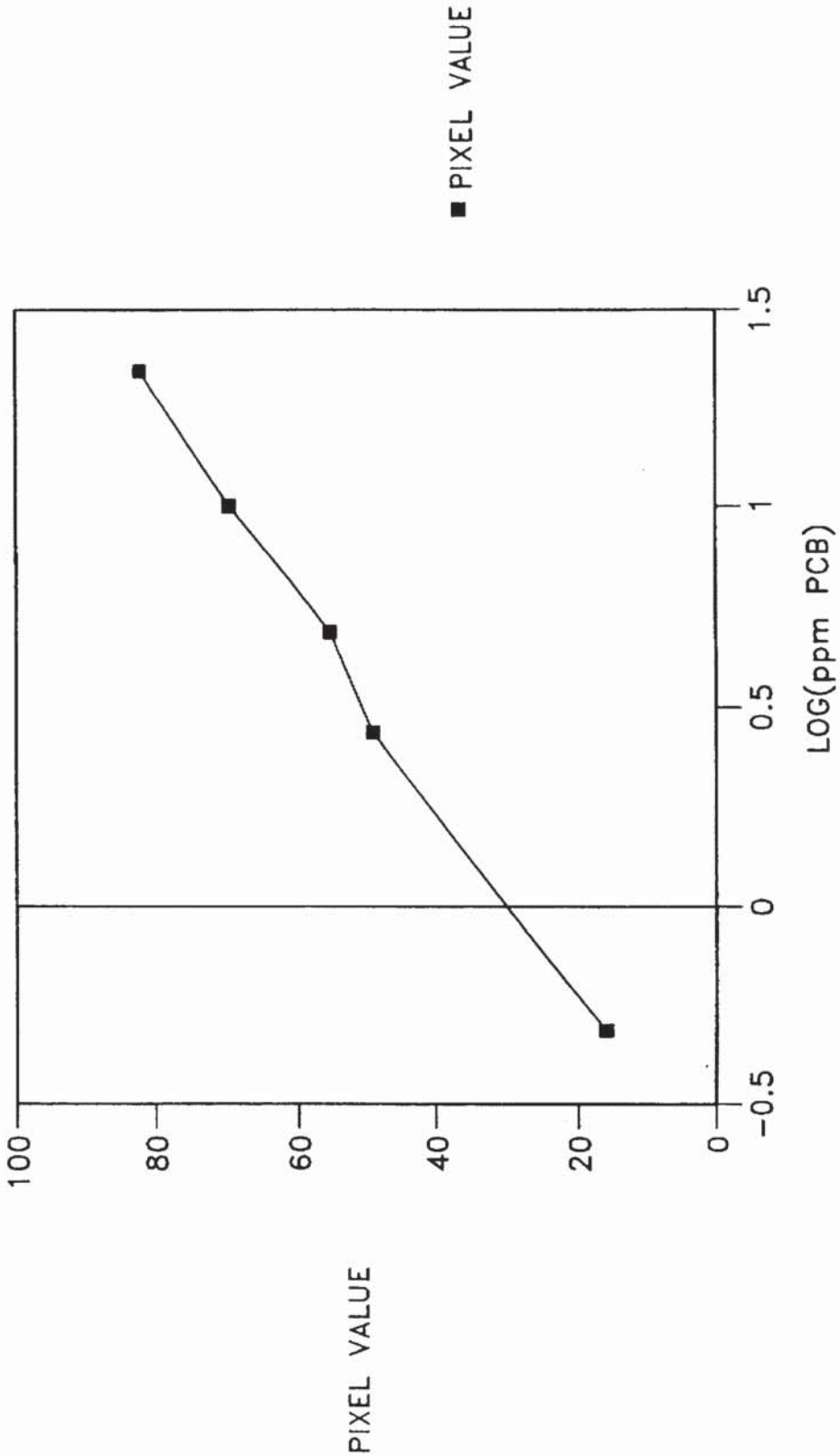



FIG-4

# DIRECT READ LATERAL FLOW ASSAY FOR SMALL ANALYTES

## FIELD OF THE INVENTION

The present invention relates to a novel lateral flow assay and method for detecting small analytes. Results can be directly read from the assay. Small analytes for medical diagnostics can be detected by utilization of the device and method of the present invention.

## BACKGROUND OF THE INVENTION

Although there are many immunoassays which exist for detection of small analytes, currently existing products which are commercially available yield "typical" competitive inhibition results, meaning, reduction of signal with increasing analyte concentration. However, the present assay, by the method and device to be described herein, yields increased signal with increasing analyte concentration.

Furthermore, presently existing products which are commercially available incorporate a read-out zone which requires the user to compare the result to a color chart. The present invention describes an assay which provides a multiple read-out: additional line(s) appear at discrete analyte concentrations.

The present invention provides an assay and method which is capable of providing a direct reading of the results of a competitive inhibition assay for small analytes.

## SUMMARY OF THE INVENTION

The present invention relates to a lateral flow assay and method for detecting small analytes. In particular, this assay is typically a competitive inhibition assay. The results of this assay can be read directly from the assay device. The device is contemplated to be used to detect small analytes useful in various types of medical diagnostic tests.

## BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** is a schematic representation of a device of the present invention.

FIG. **2** is a schematic representation of another embodiment of the device of the present invention as set forth in FIG. **1**.

FIGS. **3***a*–**3***e* are a schematic representation of the results of a direct read lateral flow assay for PCB with:

a) 0 ppm analyte; b) >0 and <5 ppm analyte; c) 5 ppm analyte; d) >5 and <50 ppm analyte; and e) 50 ppm analyte.

FIG. **4** is a graphic representation of the instrumented read-out line **1** in pixel intensity vs. Log [PCB].

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a method and device for detecting small analytes. In a preferred embodiment of the present invention, a sample (i.e., an extract or solution) suspected of containing specific analyte is added to a preparation of anti-analyte antibody which, with analyte constitutes a specific binding pair. To this mixture is added a reagent containing a tracer such as colored particles which are coated with analyte or analyte analog. Analyte or analyte analog may be attached to colored particles directly or through a carrier molecule. The colored particles will also



contain an additional label which is one member of a second specific binding pair. This label may, for example, be biotin. The mixture of sample, anti-analyte antibody solution and reagent containing colored particles is applied to a lateral flow device containing a solid support (such as for example, a nitrocellulose membrane) which contains three specific areas:

1. A sample addition area;
2. A capture area containing analyte or analyte analog immobilized onto the capture area;
3. A read-out area which contains one or more zones, each zone in the read-out area containing one or more of the following: immobilized anti-analyte antibody, immobilized complementary binding partner to the label on the colored particle (e.g., antibiotin or avidin), and immobilized analyte or analyte analog.

In the case where a sample does not contain specific analyte, a fraction of the antibody binds to the tracer such as colored particles which contain analyte or analyte analog. Only a small population of particles migrate past the capture area. The minimum number of colored particles would be available for travel to and binding to materials in the zone(s) of the read-out area.

In the case where sample contains a specific analyte to be determined in the present assay, some antibody would bind to analyte and less would be available for binding to the analyte or analyte analog containing colored particles. A larger population of colored particles migrate past the capture area to bind to one or more of the zones in the read-out area. As the sample contains increasingly larger amounts of analyte, greater amounts of unbound analyte particles are free to bind to more zone(s) in the read-out area resulting in a stronger signal or the appearance of additional lines or symbols on the assay device. Results can thus be determined in both instrumented and most significantly, non-instrumented fashion.

The assay system of the present invention can detect small analytes for medical diagnostics such as nutrients (vitamins), hormones such as estrogen and progesterone, drugs of abuse, and peptides, as well as small analytes of environmental and agricultural interest such as PCB and aflatoxin. Other small analytes of interest can include, but are not limited to, trace metals and poisons such as for example, household toxins and therapeutic drugs.

A preferred embodiment of the device of the present invention is set forth in FIG. **1**. A solid support **1**, which can be, for example, a nitrocellulose membrane, has a sample addition area **8**; a capture area **2** having analyte or analyte analog immobilized thereon; and a read-out area **3**, which contains, in this embodiment, three zones. However, it should be understood that this read-out area according to the present invention, contains at least one or more zones to provide the desired results and can contain more than three zones or less than three zones if so desired. In the embodiment set forth in FIG. **1**, zone **4** is a control zone having an irrelevant anti-analyte antibody immobilized thereon wherein this is anti-analyte antibody to a second irrelevant analyte which is different than the first analyte to be determined, and wherein this irrelevant analyte is attached to a tracer which can be, for example, a colored particle. The irrelevant analyte and tracer are added to the solution/sample to be applied to the present device prior to application of that solution/sample mixture to area **8**. The other zones **5** and **6** in the read-out area will have immobilized thereon complementary binding partner to the label on the colored particle. This complementary binding partner may, for example, be neutravidin. The area **7** indicates the distal end of the solid

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