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(56) Documents Cited:
EP 1249703 A1 **WO 2010/057318 A1**
WO 2008/007270 A1 **WO 2003/015923 A1**
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US 20100189604 A1 **US 20060133954 A1**

(58) Field of Search:
INT CL **B01F, B01L, G01N**
Other: **EPODOC, WPI**

(54) Title of the Invention: **Laboratory apparatus for research and diagnosis**
Abstract Title: **Bioanalytical device with mixing device**

(57) An analytical device for performing immunoassay and polymerase chain reactions (PCR) where the reactants are mixed by repeated propulsion around a circuit (1) by an inert bead containing a magnetic core (3), driven by a rotating magnet 6. Other means of propelling the reactants such as a pump, turbine, pressure change chemical reaction or the effect of gravity are also disclosed. In the case of immunoassay the rapid recirculation allows increased rates of collision between reactants without the need for physical shaking of the device, increasing the rate of reaction. In the case of PCR, the recirculation of the PCR mix passes through zones situated along the circuit (1) each zone exhibiting a different temperatures to the preceding zone thereby allowing the amplification of DNA by PCR.

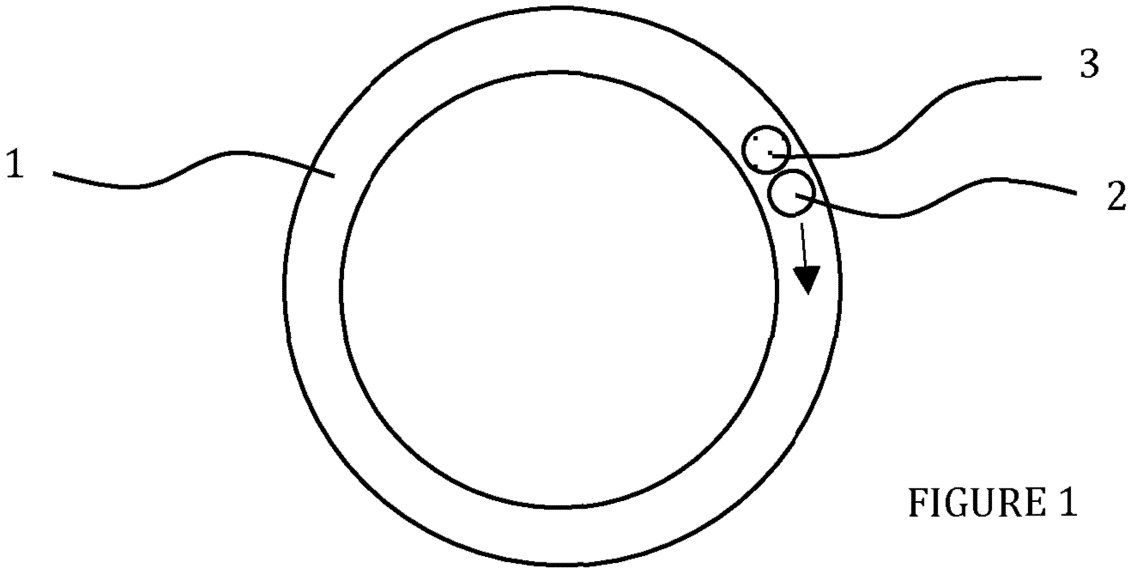

FIGURE 1

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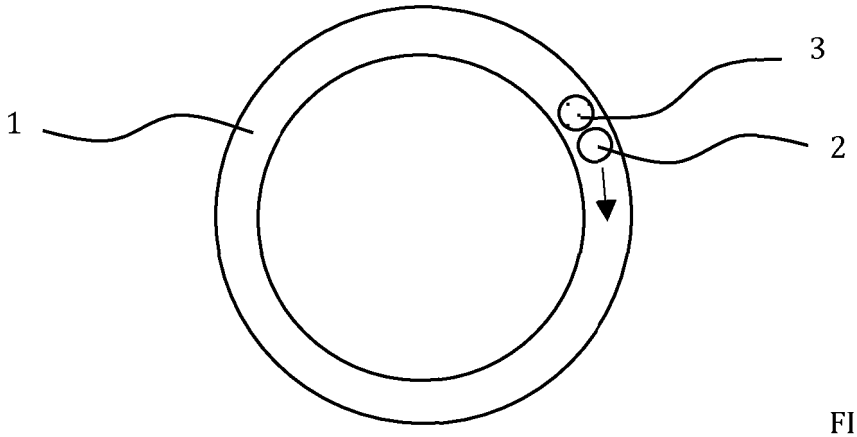

FIGURE 1

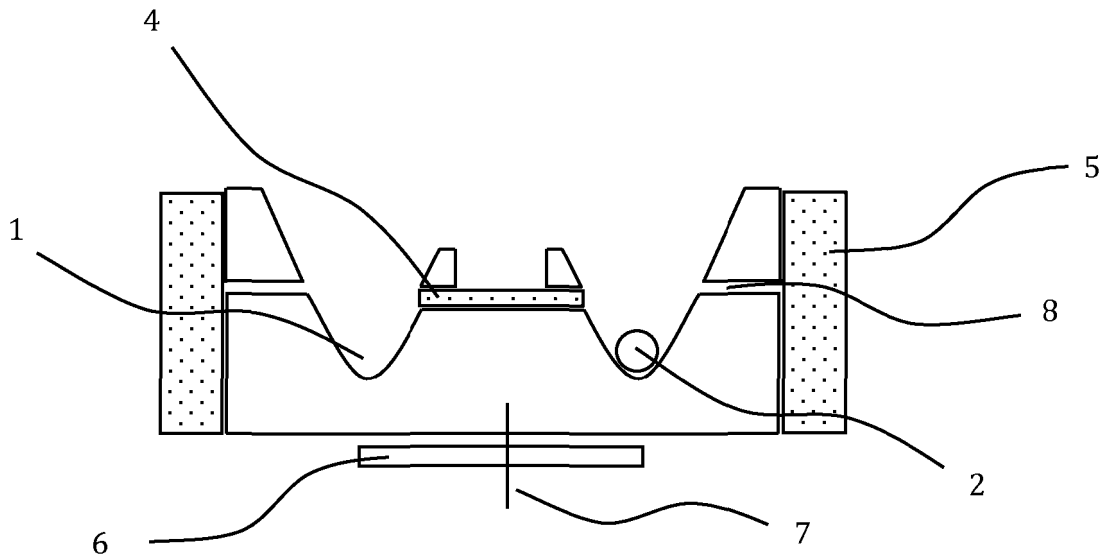

FIGURE 2

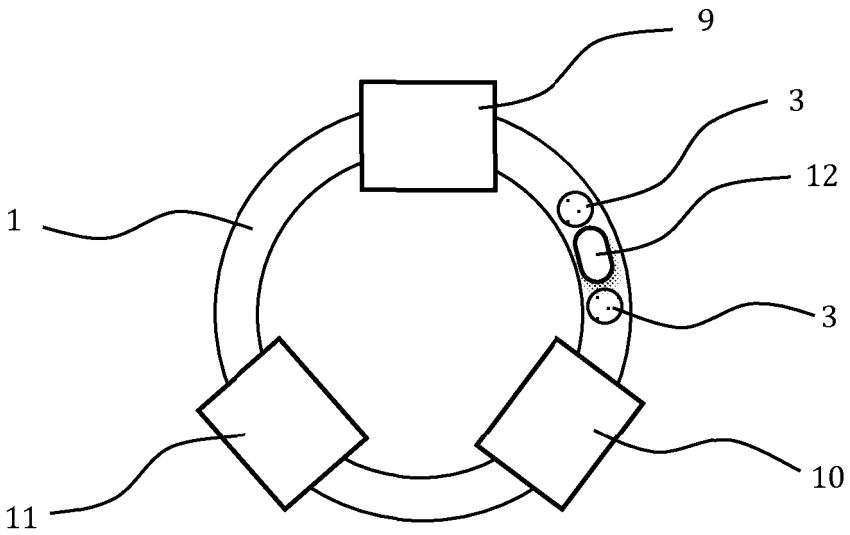

FIGURE 3

LABORATORY APPARATUS FOR RESEARCH AND DIAGNOSIS

The present invention relates to apparatus for the diagnosis, monitoring and research of clinical disease.

In particular, the present invention relates to immunoassay, nucleic acid detection and polymerase chain reaction (PCR) apparatus suited for both point-of-care (POC) and laboratory situations.

In order for reactions to reach equilibrium in a short period of time most medical laboratory analytical techniques such as ELISA and automated commercial enzyme immunoassay systems employ a means of agitation of the reactants by physical shaking of the reaction vessel such as a test tube or microtitre plate. In the case of PCR, there is a need to sequentially expose reactants to different temperatures in order for amplification of target DNA to take place.

Some immunoassay formats, such as the immunochromatographic strips commonly found in POC tests, do not employ agitation of the reactants. High lateral flow rates are employed in immunochromatographic techniques to provide rapid results. However, high lateral flow rates along these thin membranes gives inferior analytical sensitivity due to the rapid passage of analyte through the detector zone without binding to the immunoadsorbent. Similarly, particulate and viscous fluids, such as plasma or serum, flow slower through the membranes than aqueous fluids such as urine with consequential reduced sensitivity of the device, longer times to result and the need for a centrifugation or filtration step to remove blood cells which can block the flow of reactants through the membrane giving an erroneous result.

To overcome these limitations, the present invention proposes a circuit through which immunoreactants can rapidly and repeatedly circulate allowing interactions between reactants to reach equilibrium quicker, resulting in improved analytical sensitivity and faster result times. Similarly, with PCR, the circuit containing sample DNA, primers, polymerase enzymes and bases circulate through zones along the circuit of different

temperatures allowing PCR to take place at high speed eliminating the lag due to heating and cooling times.

In the present invention, all the analyte remains available to the immunoreactants during the assay procedure. Reaction rates are increased due to the rapid recirculation of the analyte through the immunoreactants which increases the probability of interaction between analytes and immunoreactants without the need for physical shaking of the device. The immunoreactants could also comprise of intact or organism parts that take part in the adsorption or detection of the analyte.

High dose hook effects associated with non-sequential immunometric assays, particulate and or viscous samples, slow reaction rates and non-specific binding may be overcome by incorporating wash steps and permitting the analyte, reactants and wash solutions to repeatedly circulate over the immunoadsorbent in sequence.

The immunoassay analytical test apparatus according to the invention comprises:

(a) a zone for receiving a sample containing an analyte;
(b) a zone for receiving a mobile phase, which zone may be the same as the sample receiving zone, or different thereto;
(c) a zone for absorbing excess reactants and wash solutions;
(d) a detection means for permitting detection of said analyte by immunoreaction; and
(e) a means for circulating immunoreactants through the mobile phase containing the reactants.

The immunoadsorbent according to the present invention comprises of macroscopic particles on to which materials which bind to the analyte are adsorbed and circulated through the immunoreactants during the test procedure.

It is a preferred feature of the present invention that the immunoadsorbent is repeatedly circulated through a mobile phase containing the analyte and immunoreactants.

According to a first embodiment of the present invention this may be achieved by providing a means of repeated circulation of the immunoadsorbent and analyte through a mobile phase containing the immunoreactants.

Therefore, according to a first embodiment of the present invention the test apparatus comprises:

(a) a zone for receiving a sample containing an analyte;
(b) a zone for receiving a mobile phase, which zone may be the same as the sample receiving zone, or different thereto;
(c) a means of circulating the immunoadsorbent through the mobile phase containing the immunoreactants, analyte and wash solutions; and
(d) a means of delivering the immunoadsorbent to the detection zone.

To operate the apparatus according to the present invention, a user provides a sample containing the analyte, for example, a sample of blood. The blood or other sample is then allowed to be taken up by the sample receiving zone. The sample receiving zone preferably comprises of a suitable blood filter to aid with the separation of plasma from the whole blood sample. Mobile phase may then be applied preferably upstream or directly on top of the blood sample. Aliquots of mobile phase, which may also contain one or more immunoreactants that allow detection of the analyte, may be applied directly or indirectly to the mobile phase receiving zone, which may be the same or different area to the sample receiving zone, from a separate container holding mobile phase, for example, from one or more dropper bottles. Typical means for repeated circulation of reactants within the circuit include the action of one or more applications of a magnetic field, pump, turbine, gravity, electric motor, friction power, potential energy, temperature gradient, pressure gradient, chemical reaction, osmotic pressure, electromagnetic radiation, electric current or sound waves.

An example of the invention will now be described by referring to the accompanying drawings:

Figure 1 shows a circuit within the apparatus suitable for immunoassay

Figure 2 shows a cross section of an apparatus suitable for immunoassay

Figure 3 shows a circuit within an apparatus suitable for PCR

Example 1

An immunoadsorbent (2), comprised of house dust mite allergens adsorbed to one or more macroscopic polystyrene beads 1 to 5mm in diameter arranged as a 'train' which may be separated from each other by inert beads to aid identification of the immunoadsorbent beads. The train of beads are rapidly and continuously propelled around a continuous "track" (1) containing serum from a patient and gold labelled anti-human IgE antibodies for a fixed period of time. The rapid circulation of the train of immunoadsorbent beads through the mobile phase containing the immunoreactants and analyte increasing the rate of reaction. During the rapid recirculation of immuoreactants and analyte, human IgE antibodies specific for house dust mite allergens will bind to the allergen adsorbed to the polystyrene bead. Simultaeneously, the gold labelled anti-human IgE antibodies will bind to the bound human IgE antibodies. The quantity of house dust mite allergen specific IgE antibodies will be proportional to the intensity of the pink colouration of the bead due to the binding of the gold labelled antibodies. Similarly, enzyme, fluorescent and other detection systems common to those skilled in the art of immunoassays may be substituted for the gold label.

In a preferred embodiment of the invention a "chaser bead" (3) containing a core of metal, such as iron, which can be forced to travel around the circuit track by a rotating magnet (6) about an axis (7) causing the immunoadsorbent beads to be propelled around the track containing the immunoreactants and analyte. Absorbent pads (5) encase the apparatus which absorb wash solutions and used reactants ejected through pores in the apparatus (8).

In a further preferred embodiment of the invention the train of beads comprises multiple beads each with differing specificity allowing different analytes to be detected simultaneously from the same sample.

In a further embodiment the train of beads comprises of additional beads that are adsorbed with variable amounts of reagents to provide a calibration curve and 'on board' controls.

A further embodiment of the present invention includes a filter (4) for the removal of unwanted particulate components such as red blood cells from the patient sample.

According to a further embodiment of the present invention the immunoreactants repeatedly circulate over a stationary immunoadsorbent. Said immunoadsorbent comprises of a stationary surface on to which materials which bind to the analyte are adsorbed. Analyte and immunoreactants for producing a detectable signal are circulated over the immunoadsorbent propelled by one or more "chaser beads" or a turbine.

Therefore, according to said further embodiment the test apparatus comprises:

(a) a zone for receiving a sample containing an analyte;
(b) a zone for receiving a mobile phase, which zone may be the same as the sample receiving zone, or different thereto;
(c) a means of circulating the immunoreactants in the mobile phase over the stationary immunoadsorbent; and
(d) a detection means for permitting detection of said analyte by immunoreaction.

Reaction kinetics may be further enhanced by increasing the temperature of the immunoreactants with a source of heat, surface modification of the beads such as dimples or protuberances to enhance physical mixing and baffles along the circuit to provide further agitation to the flow of immunoreactants in the apparatus.

Example 2

PCR requires the repeated sequential heating and cooling of the PCR mix to promote amplification of the DNA sequence of interest. In a further embodiment of the invention, the circuit containing the typical PCR mix of buffers, enzymes, bases, primer sequence and sample DNA is flanked with one or more distinct and separate zones of differing temperatures typical for PCR. For example, temperature zone 1

(9), temperature zone 2 (10), temperature zone 3 (11). A bolus (12) of the PCR mix constructed previous to the introduction to the circulation channel (1) or with the reagents added sequentially, is propelled by one or more "chaser beads" (3), proximal or proximal and distal to the PCR mix bolus, to repeatedly circulate within the apparatus causing the reactants to be sequentially exposed to regions of differing temperature promoting amplification of the desired DNA sequence. The duration of exposure of the bolus of reaction mix to the different temperatures being determined by the zone length and length of time within the zone. Where the bolus does not completely fill the circuit an additional gaseous or immiscible liquid spacer such as an oil can help maintain the PCR reaction mix as a discrete bolus.

Therefore, according to said further embodiment of the present invention the apparatus comprises:

(a) a means for receiving a bolus of reaction mix for PCR;
(b) a series of zones along the path of the circuit of with defined temperatures which may be different or similar to other zones along the path;
(c) a means of circulating the bolus of reaction mix through the temperature zones; and
(d) a means of retrieval of amplification products at the conclusion of the PCR.

Claims:

1. Immunoassay analytical test apparatus, which apparatus comprises:
    a. a zone for receiving a sample containing an analyte;
    b. a zone for receiving a mobile phase, which zone may be the same as the sample receiving zone, or different thereto;
    c. a zone for receiving immunoreactants which zone may be the same as the sample receiving zone, mobile phase receiving zone or different thereto;
    d. detection means for permitting detection of said analyte by immunoreaction; and
    e. a means for propelling repeated circulation of immunoadsorbent and or immunoreactants.

2. Apparatus according to claim1, wherein said immunoadsorbent is circulated through a mobile phase containing immunoreactants.

3. Apparatus according to claim1, wherein said immunoadsorbent is able to rotate about an axis.

4. Apparatus according to claim1, wherein said immunoadsorbent has surface modifications to enhance physical interaction with the said mobile phase.

5. Apparatus according to claim 1, wherein said mobile phase containing immunoreactants is circulated over a stationary immunoadsorbent.

6. Apparatus according to claim 1, wherein said mobile phase containing immunoreactants and immunoadsorbent are both moving in opposing directions.

7. Apparatus according to claim 1, wherein said mobile phase containing immunoreactants and immunoadsorbent are both moving in the same directions.

8. Apparatus according to claim 1, wherein said mobile phase containing immunoreactants and immunoadsorbent are both moving in random directions.

9. PCR apparatus, which apparatus comprises:
   (a) a means for receiving a reaction mix for PCR;
   (b) a series of zones along the path of the circuit of with defined temperatures which may be different or similar to other zones along the path;
   (c) a means of moving the reaction mix through the temperature zones; and
   (d) a means of retrieval of amplification products at the conclusion of the PCR;

10. Apparatus according to any preceding claim, wherein said movement is due to application of a magnetic field.

11. Apparatus according to any preceding claim, wherein said movement is due to the action of a pump.

12. Apparatus according to any preceding claim, wherein said movement is due to the action of a turbine.

13. Apparatus according to any preceding claim, wherein said movement is due to the effect of gravity.

14. Apparatus according to any preceding claim, wherein said movement is due to an electric motor.

15. Apparatus according to any preceding claim, wherein said movement is due to friction power.

16. Apparatus according to any preceding claim, wherein said movement is due to the release of potential energy.

17. Apparatus according to any preceding claim, wherein said movement is due to the effect of a change in temperature.

18. Apparatus according to any preceding claim, wherein said movement is due to the effect of a change in pressure.

19. Apparatus according to any preceding claim, wherein said movement is due to the effect of chemical reactions.

20. Apparatus according to any preceding claim, wherein said movement is due to the effect of an electric current.

21. Apparatus according to any preceding claim, wherein said movement is due to the flow of liquid.

22. Apparatus according to any preceding claim, wherein mixing action is enhanced by the inclusion of baffles.

23. Apparatus according to any preceding claim for monitoring allergen specific IgE.

24. Apparatus according to any preceding claim, wherein blood samples are separated by a filter before reaching the immunoadsorbent.

25. Apparatus according to any preceding claim where the immunoreactants are released from a dried form during the immunoassay procedure by the application of sample.

26. Apparatus according to any preceding claim where the immunoreactants are released from a dried form during the immunoassay procedure by the application of sample containing the analyte.

27. Apparatus according to any preceding claim where the immunoreactants are released from a dried form during the immunoassay procedure by the application of a diluent.

28. Apparatus according to any preceding claim, wherein analyte and immunoreactants are in contact with the immunoadsorbent simultaneously.

29. Apparatus according to any preceding claim, wherein analyte and immunoreactants are in contact with the immunoadsorbent sequentially.

30. Apparatus according to any preceding claim where part of the device is disposable.

31. Apparatus according to any preceding claim, wherein said immunoadsorbent and immunoreactants are exchanged for reagents for the detection of nucleic acids.

32. Apparatus according to any preceding claim, wherein said immunoadsorbent is comprised of one or more organisms or parts thereof.

33. Apparatus according to any preceding claim, wherein said immunoreactants is comprised of one or more organisms or parts thereof.

34. Apparatus according to any preceding claim, wherein said immunoadsorbent and immunoreactants are comprised of one or more organisms or parts thereof.

35. Apparatus according to any preceding claim where one or more said components form part of an electronic chip.

36. Apparatus according to any preceding claim, wherein said immunoadsorbent and or immunoreactants are put in motion by the effects of sub-atomic particles.

37. Apparatus according to any preceding claim, wherein said immunoadsorbent and or immunoreactants are put in motion by the effects of electromagnetic radiation.

38. Apparatus according to any preceding claim, wherein said immunoadsorbent and or immunoreactants are put in motion by the effects of sound waves.

39. Apparatus according to any preceding claim, wherein said immunoadsorbent and or immunoreactants are put in motion by the effects of gravitational fields.

40. Apparatus according to any preceding claim, wherein said immunoadsorbent and or immunoreactants are put in motion by the effects of thermal energy.

41. An analytical test method or PCR method utilising apparatus according to any preceding claim.

Amendments to the claims have been filed as follows

Claims:

1. An analytical test apparatus, which apparatus comprises:
    a. a zone for receiving a sample containing an analyte;
    b. a zone for receiving a mobile phase, which zone may be the same as the sample receiving zone, or different thereto;
    c. a zone for receiving reactants which zone may be the same as the sample receiving zone, mobile phase receiving zone or different thereto;
    d. a detection means for permitting detection of said analyte; and
    e. a means for rapidly propelling repeated circulation of reactants around a continuous track circuit by the action of an external rotating magnetic field on one or more inert magnetic beads within said continuous track circuit.

2. Apparatus according to claim 1, wherein said inert magnetic beads have a core that is attracted by a magnetic field.

3. Apparatus according to claim 1, wherein said inert magnetic beads have a core that is itself a magnet.

4. Apparatus according to any preceding claim, wherein the temperatures at discreet locations along the said continuous track circuit are controlled.

5. Apparatus according to any preceding claim that contains an immunoadsorbent.

6. Apparatus according to any preceding claim, wherein said rotating magnetic field forces said inert magnetic beads in motion around a continuous track circuit which in turn forces liquid reactants around said circuit.

7. Apparatus according to any preceding claim, wherein said rotating magnetic field forces said inert magnetic beads in motion around a continuous track

circuit which in turn pushes one or more non-magnetic immunoadsorbent beads around said circuit.

8. Apparatus according to any preceding claim, wherein said reactants comprise of one or more immunoadsorbent beads which are circulated through a mobile phase containing immunoreactants and analyte.

9. Apparatus according to any preceding claim, wherein said reactants comprise of immunoadsorbent beads separated by inert non magnetic beads.

10. Apparatus according to any preceding claim, wherein said immunoadsorbent is able to rotate about an axis.

11. Apparatus according to any preceding claim, wherein said mobile phase containing said immunoreactants and analyte is circulated over stationary said immunoadsorbent.

12. Apparatus according to any preceding claim, wherein said mobile phase containing said immunoreactants and said immunoadsorbent are both moving in relatively opposing directions.

13. Apparatus according to any preceding claim, wherein said immunoadsorbent has surface modifications to enhance physical interaction with the said mobile phase.

14. Apparatus according to any preceding claim, wherein mixing action is enhanced by the inclusion of baffles.

15. Apparatus according to any preceding claim for monitoring allergen specific IgE.

16. Apparatus according to any preceding claim, wherein plasma is separated from a blood samples by an integrated filter before reaching said immunoadsorbent.

17. Apparatus according to any preceding claim, wherein analyte and said immunoreactants are in contact with said immunoadsorbent simultaneously.

18. Apparatus according to any preceding claim, wherein analyte and said immunoreactants are in contact with said immunoadsorbent sequentially.

19. Apparatus according to any preceding claim wherein part of the device is disposable.

20. Apparatus according to any preceding claim, wherein said immunoadsorbent is comprised of one or more organisms or parts thereof.

21. Apparatus according to any preceding claim, wherein said immunoreactants are comprised of one or more organisms or parts thereof.

22. Apparatus according to any preceding claim, wherein said immunoadsorbent and said immunoreactants are comprised of one or more organisms or parts thereof.

23. Apparatus according to claim 1, wherein said reactants are reagents for the detection of nucleic acids.

24. An analytical test device utilising apparatus according to any single or combination of preceding claims.

Application No: GB1117736.7 **Examiner:** Dr J.P. Bellia

Claims searched: 1-8 and 10-41 in part **Date of search:** 10 July 2012

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1, 3, 5, 7, 10, 11, 17-21, 23-35, 40	WO02/40874 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) See paragraphs 144-147, 157-160, 314-315
X	1-3, 7, 10 23-35	WO2008/007270 A1 (SPINOMIX) See Figure 11 paragraphs 87-97
X	1-3, 7, 10, 23-35	US2010/189604 A1 (GUASCH) See paragraphs 31 and 32
X	1, 2, 11, 23-35	WO2010/057318 A1 (EARLY WARNING) See page 23 line 27-page 26 line 10 and Figures
X	1, 2, 3, 7, 10, 23-34	US2006/133954 A1 (SCHROEDER et al) See paragraphs 39-43 and Figures
X	1, 2, 3, 7, 14, 23-34	EP1249703 A1 (HITACHI) See Embodiments and Figures
X	1, 5, 11, 18, 21, 23-35	WO03/015923 A1 (BIOMICRO SYSTEMS) See page 3 line 20-page 4 line 10 and Figures
X	1, 5, 10, 23-35	US2011/076777 A1 (SANDHU) See Figures and Examples

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC
B01F; B01L; G01N
The following online and other databases have been used in the preparation of this search report
EPODOC, WPI

International Classification:

Subclass	Subgroup	Valid From
G01N	0033/543	01/01/2006
B01F	0005/10	01/01/2006