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(54) Title: RADIAL FLOW IMMUNOASSAY AND METHODS OF PRODUCTION AND USE THEREOF

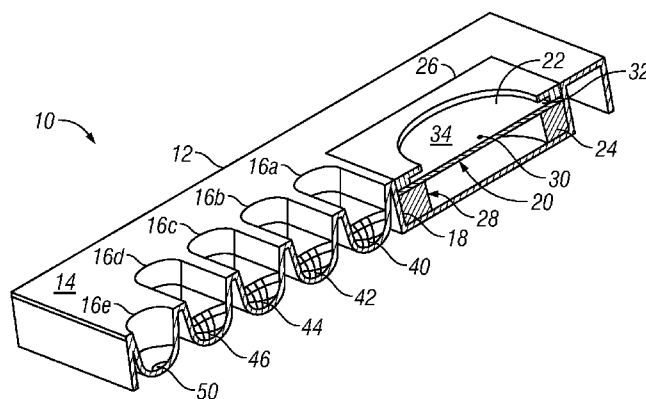


FIG. 1

(57) Abstract: A radial flow immunoassay includes a membrane having a plurality of immobilized distinct antigens disposed thereon. Methods of producing the immunoassay, and methods of utilizing same to detect immunoglobulin present in a biological sample, are also disclosed.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/28265

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/53, G01N 33/543 (2012.01)

USPC - 435/7.1; 435/7.92, 436/518

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): G01N 33/53, G01N 33/543 (2012.01)

USPC: 435/7.1; 435/7.92, 436/518

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

USPC: 436/524, 436/527, 436/528, 436/530

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

PubWEST (DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ), Google Scholar, Google Patents

Search Terms Used: radial immunodiffusion, radial immunoassay, radial diffusion, radial immune diffusion, streptavidin, dendrimer, IgG, IgE, biotinylated, nitrocellulose, glass fiber, silica, SAM, Promega, biotin, capture

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/088055 A1 (BABSON) 5 August 2010 (05.08.2010) esp: paras [0006], [0007], [0008] [0009], [0010],[0014], [0015].	1-24
Y	US 2003/0134286 A1 (EDWARDS et al.) 17 July 2003 (17.07.2003) para [0010], [0032]	1-24
Y	US 2011/0045606 A1 (KENNEDY) 24 February 2011 (24.02.2011) entire document.	1-24
Y	US 2002/0123074 A1 (SELF et al.) 5 September 2002 (05.09.2002) entire document.	1-24
Y	US 2010/0330706 A1 (WEI et al.) 30 December 2010 (30.12.2010) entire document.	1-24
Y	US 5,006,464 A (CHU et al.) 9 April 1991 (09.04.1991) entire document.	1-24

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



* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 August 2012 (07.08.2012)

Date of mailing of the international search report

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Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/28265

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

Group I claims 1-24 are directed to a method of detecting an immunoglobulin present in a biological sample and a method of diagnosing allergy in a patient in vitro.

Group II claims 25-27 are directed to a microarray.

Group III claims 28-35 are directed to an immunoassay kit.

Group IV claims 36-48 are directed to an immunoassay device.

-continued on extra sheet---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-24

Remark on Protest

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

Continuation of Box II Lack of Unity of Invention:

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

The special technical features of the Groups are delineated above. The shared technical feature of all the Groups is a membrane having a plurality of immobilized distinct antigens disposed thereon. However, this is not an improvement over the prior art of WO 2010/088055 A1 to Babson (5 August 2010) (hereinafter 'Babson') that specifically teaches a method of detecting an immunoglobulin present in a biological sample (abstract; para [0001]) comprising a membrane having a plurality of immobilized distinct antigens disposed thereon (para [0006] - "Known allergens are attached to a membrane and then a sample taken from the patient is applied, so that allergen-specific IgE antibodies in the sample may react with the known allergens."; para [0007] - "binding biotinylated known allergens as discrete microspots to a streptavidin-linked membrane"; para [0015]). Further, as to Group I, Babson teaches the steps of disposing a biological sample on a membrane (para [0006] - "a sample taken from the patient is applied [to a membrane]"; para [0015] - "blood or other liquid biological sample would be deposited on the membrane"), wherein the membrane is provided with a plurality of immobilized distinct antigens disposed thereon (para [0006] - "Known allergens are attached to the membrane"; para [0007] - "binding biotinylated known allergens as discrete microspots to a streptavidin-linked membrane"), wherein the biological sample such that the biological sample is brought into contact with each of the plurality of immobilized distinct antigens (para [0015] - "blood or other liquid biological sample would be deposited on the membrane containing the known allergens and incubated for about 30 minutes"), and wherein any immunoglobulin present in the biological sample that is specific for one or more of the plurality of immobilized distinct antigens binds to said immobilized distinct antigens (para [0006] - "Known allergens are attached to a membrane and then a sample taken from the patient is applied, so that allergen-specific IgE antibodies in the sample may react with the known allergens."; applying a wash solution to the membrane to wash the biological sample across the membrane (para [0007] - "Excess sample is then washed away"; para [0015] - "After incubation, the membrane would be washed with a non-ionic detergent to remove sample residue"); disposing a labeled reagent specific for the immunoglobulin on the membrane and wherein the labeled reagent binds to any immunoglobulin bound to an immobilized distinct antigen (para [0006] - "The IgE antibodies are identified by applying labeled anti-IgE, which binds to the IgE antibodies"; para [0007] - "the membrane is coated with alkaline phosphatase-labeled anti-IgE which binds to any spots containing allergen-specific IgE"; applying a wash solution to the membrane to wash unbound labeled reagent (para [0007] - "a second wash the membrane is coated with a chemiluminescence substrate for alkaline phosphatase"; detecting the labeled reagent bound to the membrane; and determining that the biological sample contains an immunoglobulin specific for the distinct antigen to which the labeled reagent is bound (para [0006] - "The IgE antibodies are identified by applying labeled anti-IgE, which binds to the IgE antibodies, and then measuring the bound anti-IgE by the response produced when the label is contacted with an appropriate substrate (e.g. chemiluminescence"; para [0007] - "The light emitted by alkaline phosphatase is measured and correlated with the amount of allergen-specific IgE in the sample.")). Babson does not disclose specific geometric designs of the membrane (i.e. specific placement of the sample on the center of the membrane, direction of a radially diffusing reaction and/or wash, etc.). However the geometric specification of the instant application are indicative of radial immunodiffusion. Radial immunodiffusion was a well known and widely used method of performing immunoreactive analyses long in use with membrane-based detection systems. Therefore, specific limitations claiming radial diffusion or the like such as "disposing a biological sample on a center of a membrane", "wherein the biological sample moves across the membrane to a periphery of the membrane", "applying a wash solution to the center of the membrane to wash the biological sample across the membrane to the periphery thereof via radial diffusion", etc. would have been obvious to one of ordinary skill in the art without undue experimentation as appropriate and within the scope and intent of the prior art of Babson as above.

Group II further requires that the surface of the membrane comprises streptavidin-labeled dendrimer thereon, and wherein the plurality of distinct antigens are biotinylated for immobilization on the membrane. This special technical feature is not shared by any of Groups I, III, and IV. Nevertheless, Babson teaches wherein a surface of the membrane comprises streptavidin-labeled dendrimer thereon (para [0015]), and wherein the plurality of distinct antigens are biotinylated for immobilization on the membrane (para [0015]).

Group III further requires a labeled reagent for detecting immunoglobulin bound to at least one of the plurality of immobilized distinct antigens. This special technical feature is not shared by any of Groups I, II, and IV. Nevertheless, Babson teaches a labeled reagent for detecting immunoglobulin bound to at least one of the plurality of immobilized distinct antigens (para [0008]).

Group IV further requires a housing, a first well formed in the housing and containing a volume of labeled reagent for detecting immunoglobulin bound to at least one of the plurality of immobilized distinct antigens, a second well formed in the housing and containing a volume of wash solution; and a third well formed in the housing and containing a volume of rinse solution. None of these further limitations are required by any of Groups I-III.

Therefore the inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. In order for all inventions to be examined, the appropriate examination fees must be paid.