(54) Title: DEVICE AND PROCESS FOR BINDING ASSAYS

(57) Abstract: A device for performing quantitative affinity-chromatographical quick tests with photometric evaluation including at least one filter element (2) formed from a porous filler material (5) on which receptors are immobilized and which is provided in a container (1) having an inlet (3) and an outlet (4), said filter element (2) having a reflecting inner surface, characterized in that: -the porous filler material (5) is provided between two liquid-permeable separator elements (6) to form a column-shaped segment (8); -at least two column-shaped segments (8) are provided in the device; -the receptors are in a specific concentration within a range of from 10ng/ml to 200ng/ml of filter volume; -the separator elements (6) have reflecting properties on the surface adjacent to the porous filler material; -the device has such a design as to allow the device to be flowed through by a solution containing analytes that can be bound to receptors immobilized at the surface of the porous filler materials at a rate within a range of from 1.5ml/min to 0.01ml/min; -the device has a means (10) with analytes bound to receptors immobilized at the surface of the porous filler materials that is quantified on one or more segments (8) by light transmittance measurement or/and light scattering measurement at an angle of between 180° and 90° either directly upon addition of one or more secondary reagents undergoing specific binding with the analytes to produce a color directly or indirectly; -wherein the device has an evaluation unit that allows the amount of analyte to be assayed by means of a calibration curve taking into account the transmittance or values derived therefrom of at least two segments.
before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
Quantitative Heterogeneous Fast Binding Assay with Broad Dynamic Range and Simple Photometric Detection

The present invention relates to a process and a device for performing quantitative affinity-chromatographical quick tests with photometric evaluation with a porous filler material having a reflecting inner surface and receptors immobilized thereon and being provided in a container having an inlet and outlet.

Affinity-chromatographical quick tests are based on biospecific interactions of ligands and receptors. Typically, the receptor is immobilized on a surface of a solid while the ligand to be assayed is supplied via a liquid phase and is specifically bound to the surface. A usual construction form for quick tests is described in U.S. Patent No. 4,943,522. Such quick tests are employed, for example, as pregnancy test strips. They are prepared on the basis of nitrocellulose strips which are capped by a glass fiber membrane at the upper portion thereof. The sample is applied to the upper portion of the glass fiber membrane and reacts, while it migrates through the non-woven material, with the conjugate incorporated therein. As a rule, these are antibody-gold particles. The immunocomplex formed migrates further through the nitrocellulose membrane and is bound to capture antibodies immobilized thereon. As a result, a stained band is formed. In the past, there have been numerous attempts to read out such test strips quantitatively by either reflectometry or transmittance measurement. However, the sensitivity and precision that can be achieved thereby is relatively poor, and the dynamic range of such measurements is limited to 2-2.5 orders of magnitude.

Another quick test method is described in EP-A-0 557 288, EP-A-0 634 015 or PCT/EP94/00086. It is based on a flow column containing porous material with an immobilized receptor. The binding of an analyte is effected while flowing through
the column. The bound analyte can subsequently be visualized by adding a second, labeled, receptor. The use of an enzyme-antibody conjugate and subsequent addition of precipitating tetramethylbenzidine has proven advantageous. In this case, a blue precipitate forms that can be detected quantitatively by analyzing the transmitted light directly by transmittance measurement on the porous material. A particularly high sensitivity and low detection limits are achieved thereby if a measuring cuvette according to DE 102004 006470A1 is employed. Such a procedure is described in Gessler et al., 2005. This method is clearly superior to the above described membrane method in terms of sensitivity, but likewise it also exhibits a very narrow dynamic range of 2.5 orders of magnitude at most. In the method described, scattered light measurements show a behavior that is comparable in principle.

Alternative reading-out techniques, such as fluorometry and luminometry, have a better dynamic range of up to 4 orders of magnitude. Generally, however, the necessary measuring technology is relatively tedious and thus expensive in these methods. In addition, interference may occur from quenching and bleaching effects. Therefore, in contrast to photometry, these techniques are not suitable for the preparation of simple and inexpensive measuring systems.

With analytes occurring in a broader range of concentrations, the narrow dynamic range of the photometric methods in turn has the result that several dilutions of the solution to be analyzed would have to be prepared and examined for one analysis. This procedure is not user-friendly and has the consequence that the quick test method cannot be employed.

It was the object of the invention to provide a process and a corresponding device which allow analytes to be assayed quickly and precisely by affinity chromatography in a broad dynamic range on the basis of a simple, inexpensive optical transmission technique.

The above object is achieved by a device according to claim 1 and a process according to claim 10. The dependent claims relate to particular embodiments of the device according to the invention.
The device according to the invention is suitable for performing quantitative affinity-chromatographical quick tests with photometric evaluation. The device includes at least one filter element 2 formed from a porous filler material 5 on which receptors are immobilized and which is provided in a container 1 having an inlet 3 and an outlet 4. The filter element 2 has a reflecting inner surface. The device according to the invention is characterized in that:

- the porous filler material 5 is provided between two liquid-permeable separator elements 6 to form a column-shaped segment 8;

- at least two column-shaped segments 8 are provided in the device;

- the receptors are in a specific concentration within a range of from 1 ng/ml to 200 g/ml of filter volume;

- the separator elements 6 have reflecting properties on the surface adjacent to the porous filler material;

- the device has such a design as to allow the device to be flowed through by a solution containing analytes that can be bound to receptors immobilized at the surface of the porous filler materials at a rate within a range of from 1.5 ml/min to 0.01 ml/min;

- the device has a means 10 with analytes bound to receptors immobilized at the surface of the porous filler materials that is quantified on one or more segments 8 by light transmittance measurement or/and light scattering measurement at an angle of between 180° and 90° either directly of upon addition of one or more secondary reagents undergoing specific binding with the analytes to produce a color directly or indirectly;

- wherein the device has an evaluation unit that allows the amount of analyte to be assayed by means of a calibration curve taking into account the transmittance or values derived therefrom of at least two segments.
In one embodiment, the segments 8 containing the porous filler material have a length of from 0.5 to 10 mm and a diameter of from 0.5 to 5 mm.

Figure 1 shows a schematic representation of the device according to the invention.

It may be advantageous if at least two containers are connected with one another (Figure 2). The outlet of one container may be connected to the inlet of said at least second container detachably, for example, by insertion.

In particular, the separator element 6 in the device according to the invention is a porous filter element 2 having a mean pore diameter of from 1 to 100 µm and a pore volume fraction of from 20% to 80%.

In a further embodiment of the device according to the invention, the evaluation unit has a photodiode/light-emitting diode assembly for establishing the transmittance that can be designed as light-emitting diodes and a CCD line or CCD array.

A spectrometric evaluation of transmittance can also be performed.

For detecting the analyte, staining by a receptor/particle conjugate and/or receptor/enzyme conjugate with subsequent addition of an enzyme substrate may be effected.

In another embodiment of the device according to the invention, the receptor concentration in the segments increases from the inlet 3 to the outlet 4.

The present invention also relates to a process for performing quantitative affinity-chromatographical quick tests for the presence of analytes using photometric evaluation wherein a solution containing or suspected to contain an analyte is introduced through the inlet 3 of container 1 of the device according to the invention and contacted with the filler material in segments 8 and, following adsorption of the analyte to receptors having affinity for said analyte, the presence of the analyte bound to the receptors is assayed quantitatively and/or qualitatively.
The invention is described in more detail in the following.

We explicitly proceed from the process described above on the basis of a photometric measuring cuvette with a heterogeneous porous filler material. The porous filler material is preferably employed in the form of filter segments on the inner surface of which receptors are immobilized. According to the invention, at least two of these elements may be arranged in succession in one tube and separated by liquid-permeable separator elements having reflecting properties on the interface towards the filter element (Figure 1). The linear arrangement is flowed through successively by the solution containing the analyte (ligand) to be determined.

Figure 1 shows a separator disk 6 having one or two reflecting major surfaces.

In particular, the inserted filter elements 2 (Figure 1) have a reflecting inner surface. The mean pore diameters should be within a range of from 1 µm to 100 µm, and the pore volume fraction is to be from 20% to 80%.

In one embodiment according to the invention, column-shaped receptor-containing filter elements 2 having a length within a range of from 0.5 to 5 mm and a diameter within a range of from 0.5 to 5 mm are employed. The separator elements 6 have the same diameters, but a lower thickness within a range of from 10 µm to 2.5 mm. Generally, each receptor-containing filter element 2 is bounded by two separator elements 6 on the top and bottom thereof. In a simple embodiment according to the invention, the following sequence is obtained:


In a further embodiment of the device according to the invention in the form of an analytical tube, a substantially higher number of filter elements 2 and separator elements 6 may be arranged in succession. Through the linear arrangement that can be flowed through, the solution containing the ligand to be assayed can flow at a rate of from 1.5 ml/min to 0.01 ml/min with a correspondingly sized metering device or by hydrostatic pressure alone, wherein the specific concentration of the
receptor on the active receptor-containing filter elements should be selected within a range of from 2 µg/ml to 200 mg/ml of filter volume.

The quantification of the bound ligand is effected separately on the successively arranged filter elements 2 in a per se known manner. For this purpose, one or more per se known marker reagents, such as immuno, gold, antibody, stain conjugates, antibody-enzyme conjugates etc. that react with the ligand and form a readily detectable, especially by optical detection, complex or catalyze the formation of a detectable colored matter may usually be additionally added before the measurement. According to the invention, the change in transmittance of or the light scattered at a defined angle from the individual filter elements is recorded. Optical densities can be calculated therefrom on the basis of the Lambert-Beer law. Namely:

\[ \ln \left( \frac{I_0}{I} \right) = OD = \varepsilon \cdot d_{eff} \cdot c \]

where:  
OD = optical density  
\( \varepsilon \) = extinction coefficient  
\( d_{eff} \) = effective layer thickness, which depends on the light-scattering properties of the filters;  
c = concentration of the analyte to be assayed;  
\( I_0 \) = transmittance before the filter element is stained with the labeling substance;  
\( I \) = transmittance after the filter element has been stained with the labeling substance.

According to the invention, the intensity values of the transmitted or scattered light of values derived therefrom, especially the optical densities calculated according to the stated equation, of at least two successive filter segments 2 are determined for calibration. Subsequently, derived measuring values, which are employed for establishing the calibration, are produced by combining these values on the basis of a suitable mathematical function. A simple form of combination is the summation or conversion to mean values of the OD values from the two filters.
It has been found that a calibration function having a broader dynamic range than that obtained from the evaluation of a single filter under comparable reaction conditions can be generated by plotting these derived values against the concentration.

Thus, an extension of the measuring range of up to 4 powers of ten, i.e., dynamic ranges of up to 6 powers of ten, in concentration can be realized. To achieve this, more than two filter segments should usually be provided in succession, wherein such filter segments can have a very thin design. Typical measuring set-ups for the quantification of the transmitted or scattered light are photodiodes/light-emitting diodes assemblies or light-emitting diode cells in combination with CCD lines or CCD arrays.

The procedure described is illustrated in Figure 4 taking a three-layered filter set-up as an example in comparison with a one-layered conventional set-up. In the example shown, the volume of the individual reference filter corresponds to the sum of the filter volumes of the three smaller filters in the multifilter set-up. The Figure is based on the data from Example 1.

Example 2 shows for an analogous three-layered set-up that very good coefficients of variation and thus a precision that is clearly superior to that of the conventional set-up can also be achieved with this process.

In the case of Example 3, the optical detection was effected with a CCD array. The Example shows at the same time that it is possible to arrange several segment assemblies having different specificities in succession and thus to perform multiplex analyses.

In the Example of Figure 4, a set-up is shown in which the receptor concentration on the filter segments increases from the inlet to the outlet. In this case, a relatively uniform color along the segment column whose length correlates with the analyte concentration is to be noted.
Figure 4: Photometric calibration curves for the immunological determination of *F. tularensis* on a porous filter element with immobilized anti-*F. tularensis* antibody and peroxidase/TMB detection (heterogeneous immunoassay according to Example I).

Top: Calibration curve and optical set-up of a conventional measuring assembly based on a 5 x 5 mm polyethylene filter element and a single-beam photometer.

Middle: Calibration curves and optical set-up for an assembly proposed according to the invention of three filter elements of 1.6 x 5 mm separated by separator elements and the corresponding photometric three-layer set-up; the calibration curves were respectively established on the filter elements and may be used singly or on the basis of suitable mathematical combinations for evaluating the measurement.

Bottom: Summed calibration curve calculated from the 3 calibration curves for the *F. tularensis* determination on the set-up proposed according to the invention.

Example 1. Detection of LPS from *Franciscella tularensis*:

Sintered parts consisting of HDPE with a mean pore diameter of 40 µm and a pore volume fraction of 50% in the dimensions (diameter x length) 5 x 5 mm (type 1) or 5 x 1.65 mm (type 2) on which 9 µg or 3 µg each, respectively, of monoclonal anti-*Franciscella tularensis* antibodies was immobilized were employed. Analogous filter elements in the dimensions 5 x 0.5 mm (type 3) without binding activity (block filters) were employed as porous separator elements.

By inserting a type 1 filter element into microcolumns (d = 5 mm, V = 750 µl), an assembly corresponding to the scheme on the top of Figure 2 was prepared. This one-layered assembly corresponds to the above described prior art and served as a reference system.
The preparation of the multilayered assembly proposed according to the invention in accordance with the middle and bottom of Figure 2 was effected by sequential insertion according to the scheme:


5 F. tularensis extracted lipopolysaccharide was used as the control material. The concentration was stated on the basis of the germ numbers in cfu/ml before the extraction. The assays were performed in pipetting racks with a zero control and 6 calibrating samples each in duplicate. The addition of the sample and of all the subsequent reagents was effected directly into the inlet of the microcolumn. The flow rates were within a range of from 250 to 300 µl/min. After the binding of the sample (500 µl), an immune complex was formed on the column by adding anti-F. tularensis-POD conjugate and detected by precipitating TMB and subsequently quantified by transmittance measurement. The assay scheme is as follows:

• 500 µl sample in PBS buffer
• incubation for 4 min
• 500 µl of anti-F. tularensis/poly-HRP conjugate (8 µg/ml)
• incubation for 4 min
• 2 x 750 µl PBS buffer
• determination of transmittance
• 500 µl of TMB substrate solution precipitating
• incubation for 6 min
• 500 µl of PBS buffer
• determination of transmittance

The optical read-out of the reference columns was effected with a miniphotometer (SENOVA Cat. No. 30010001RDR). It is capable of realizing transmittance measurements on one-layered columns at a wavelength of 532 nm and an aperture diameter of 3.6 mm. The multilayer columns were measured with a modified photometer of the same fundamental design. It had a modified measuring head with 3 beam paths (aperture diameter 1.2 mm), each on the level of the active filter elements.
From the measured transmittance values, the OD values were calculated on the basis of formula 1. These OD values and the OD mean values calculated therefrom were shown as a function of the LPS concentration in Figure 4. The upper part shows the calibration curve of the one-layered system, the middle part shows the calibration of the individual filter layers, and the bottom part shows the calibration on the basis of the summed OD values.

**Example 2: Detection of *Streptococcus mutans***

Sintered parts consisting of HDPE with a mean pore diameter of 40 µm and a pore volume fraction of 50% in the dimensions (diameter x length) 5 x 5 mm (type 1) or 5 x 1.65 mm (type 2) on which 15 µg or 5 µg each, respectively, of polyclonal affinity-purified serum from rabbit against *Streptococcus mutans* was immobilized were employed.

Disks according to Figure 3 were employed as separator elements. They had a metallic surface with partially reflecting properties. In addition, block filters according to type 3 in Example 1 were employed.

By inserting a type 1 filter element into microcolumns (d = 5 mm, V = 750 µl), an assembly corresponding to the scheme of Figure 4 (top) was prepared. This one-layered assembly corresponds to the above described prior art and served as a reference system. The preparation of the multilayered assembly proposed according to the invention in accordance with the middle and bottom of Figure 4 was effected by sequential insertion according to the scheme:

Type 3/type 2/disk/type 2'/disk/type 2/type 3'.

Cell culture material of *S. mutans* was used as the control material. The concentration in cfu/ml was determined by plating. The assays were performed in pipetting racks with a zero control and 6 calibrating samples each in duplicate. The addition of the sample and of all the subsequent reagents was effected directly into the inlet of the microcolumn. The flow rates were within a range of from 180 to 200 µl/min. After the binding of the sample (500 µl), an immune complex was formed
on the column by adding affinity-purified biotinylated anti-S. *mutans* serum (rabbit). Subsequently, streptavidin-magnetic particles (0.5 to 1 µm, Chemagen GmbH, Baesweiler, Germany) were added for optical detection. The magnet particles can be well detected on the filters by photometry by transmittance measurement at 525 nm. The assay scheme is as follows:

- 500 µl sample
- incubation for 4 min
- 500 µl of anti-S. mutans-biotin (IgG fraction 8 µg/ml)
- incubation for 4 min
- determination of transmittance
- 500 µl of streptavidin-magnetic particles (Chemagen)
- 750 µl of PBS buffer
- determination of transmittance

The determination of the transmittance values was effected as described in Example 1. The following Table 1 shows the obtained calibration data as mean values.

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<th>cfu/ml</th>
<th>OD one layer</th>
<th>OD filter 1</th>
<th>OD filter 2</th>
<th>OD filter 3</th>
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<th>mean value</th>
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<td>0.238</td>
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<td>1.177</td>
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<td>1.722</td>
<td>1.239</td>
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Table 1: Calibration values (mean values from duplicate determinations) for the S. mutans test on a one-layered filter (type 1) and the three-layered filter assembly and the sums and mean values calculated therefrom
Example 3: Simultaneous assay of *F. tularensis* and *Y. pestis*

Sintered parts consisting of HDPE with a mean pore diameter of 12 µm and a pore volume fraction of 40% in the dimensions (diameter x length) 2.5 x 1.6 mm were employed. Some of the filters were coated with 2 µg of monoclonal antibody against *F. tularensis* (type 1"), and some of the filters were coated with 2 µg of monoclonal antibody against the Fl antigen of *Y. pestis* (type 2"). The separator elements were prepared from 0.15 mm thick aluminum foil that was provided with 3 holes each (d = 0.2 mm). In addition, block filters in the dimensions 2.5 mm x 1.6 mm were employed.

A multilayer assembly corresponding to Figure 5 and based on 2 parallel tubes connected through a channel was prepared. The sample (500 µl) was preincubated with 4 µg of anti-*F. tularensis-POD* conjugate and 3 µg of anti-*V. pestis-POD* conjugate for 6 minutes. Subsequently, metering with a syringe pump was effected at a flow rate of 1.2 ml/min. Subsequently, 1.5 ml of PBS buffer was metered through a second channel of the syringe pump in 5 seconds, followed by metering 500 µl of precipitating TMB through a third channel in 3 seconds. After an incubation time of 4 minutes, PBS buffer (500 µl) was again metered through channel 2. Subsequently, the optical evaluation was performed in the CCD array assembly as shown in Figure 5.

In an exemplary manner, Figure 5 shows the result in a schematic form for an analysis with a high (5000 cfu/ml) *F. tularensis* concentration and low *Y. pestis* concentration (0.2 ng/ml) and the converse case of a low *F. tularensis* (425 cfu/ml) and high *Y. pestis* concentration (10 ng/ml). The calibrations obtained for the two analytes on the basis of the sums of the calculated OD values of the 6 filters are shown in Figure 6.

Example 4: Quantification of *Botulinus* toxin A by the number of stained segments

Eleven sintered parts consisting of HDPE with a mean pore diameter of 12 µm and a pore volume fraction of 40% in the dimensions (diameter x length) 1 x 1.6 mm were employed.
The filters were coated with 7.5 µg of monoclonal antibody against *Botulinus* toxin A. The separator elements were prepared from 0.15 mm thick aluminum foil that was provided with 3 holes each (d = 0.2 mm).

A multilayer assembly corresponding to Figure 6 was prepared.

The sample (900 µl) was preincubated with 4 µg of biotinylated anti-BotA antibody for 6 minutes. Subsequently, the metering was effected by feeding onto the column at a flow rate of about 0.4 ml/min. Subsequently, 500 µl of SA-polyHRP was pipetted, the column was washed twice with 1.5 ml of PBS buffer after 6 minutes, followed by metering 500 µl of precipitating TMBN in about 1.25 minutes. After an incubation time of 6 minutes, 1 ml of PBS buffer was again metered. Subsequently, the optical evaluation was performed by a CCD array assembly.

In an exemplary manner, Figure 6 shows the result in a schematic form for an analysis with a low (0.5 ng/ml) and high (5 ng/ml) concentration. The number of stained filters directly indicates the detected concentration in the sample.
CLAIMS:

1. A device for performing quantitative affinity-chromatographical quick tests with photometric evaluation including at least one filter element (2) formed from a porous filler material (5) on which receptors are immobilized and which is provided in a container (1) having an inlet (3) and an outlet (4), said filter element (2) having a reflecting inner surface, characterized in that:

- the porous filler material (5) is provided between two liquid-permeable separator elements (6) to form a column-shaped segment (8);

- at least two column-shaped segments (8) are provided in the device;

- the receptors are in a specific concentration within a range of from 1 ng/ml to 200 g/ml of filter volume;

- the separator elements (6) have reflecting properties on the surface adjacent to the porous filler material;

- the device has such a design as to allow the device to be flowed through by a solution containing analytes that can be bound to receptors immobilized at the surface of the porous filler materials at a rate within a range of from 1.5 ml/min to 0.01 ml/min;

- the device has a means (10) with analytes bound to receptors immobilized at the surface of the porous filler materials that is quantified on one or more segments (8) by light transmittance measurement or/and light scattering measurement at an angle of between 180° and 90° either directly or upon addition of one or more secondary reagents undergoing specific binding with the analytes to produce a color directly or indirectly;
wherein the device has an evaluation unit that allows the amount of analyte to be assayed by means of a calibration curve taking into account the transmittance or values derived therefrom of at least two segments.

2. The device according to claim 1, characterized in that the segments (8) containing the porous filler material have a length of from 0.5 to 10 mm and a diameter of from 0.5 to 5 mm.

3. The device according to claim 1 and/or 2, characterized in that at least two devices are connected with one another.

4. The device according to at least one of claims 1 to 3, characterized in that the separator element is a porous filter element having a mean pore diameter of from 1 to 100 µm and a pore volume fraction of from 20% to 80%.

5. The device according to at least one of claims 1 to 4, characterized in that the evaluation unit has a photodiode/light-emitting diode assembly for establishing the transmittance.

6. The device according to at least one of claims 1 to 5, characterized in that the evaluation unit has an assembly of light-emitting diodes and a CCD line or CCD array for establishing the transmittance.

7. The device according to at least one of claims 1 to 6, characterized in that the staining is effected by a receptor-particle conjugate.

8. The device according to at least one of claims 1 to 7, characterized in that the staining is effected by a receptor-enzyme conjugate followed by adding an enzyme substrate.

9. The device according to claims 1 to 8, characterized in that the receptor concentration in the segments increases from the inlet (3) to the outlet (4).
10. A process for performing quantitative affinity-chromatographical quick tests for the presence of analytes using photometric evaluation wherein a solution containing or suspected to contain an analyte is introduced through the inlet of the device according to any of claims 1 to 9 and contacted with the porous filler material in segments (8) and, following adsorption of the analyte to receptors having affinity for said analyte, the presence of the analyte bound to the receptors is assayed quantitatively and/or qualitatively.
**INTERNATIONAL SEARCH REPORT**

**INTERNATIONAL APPLICATION N°**

PCT/EP2008/056675

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**A. CLASSIFICATION OF SUBJECT MATTER**

**INV. G01N33/543 G01N33/538**

According to International Patent Classification (IPC) as well as both national classification and IPC.

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**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

G01N

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

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>A</td>
<td>EP 0 021 214 A (AMES YISSUM LTD [IL]) 7 January 1981 (1981-01-07) page 18, line 5 - line 7; figure 4</td>
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<td>A</td>
<td>DE 10 2004 006470 A1 (SENNOVA GESELLSCHAFT FUR BIOWISSENSCHAFT [DE]) 1 September 2005 (2005-09-01) cited in the application claim 1</td>
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Date of the actual completion of the international search: 10 September 2008

Date of mailing of the international search report: 22/09/2008

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Authorized officer: Van Bohemen, Charles

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<td>20-07-1995</td>
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<td>EP 0739487 A1</td>
<td>30-10-1996</td>
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<td></td>
<td>JP 9507577 T</td>
<td>29-07-1997</td>
</tr>
<tr>
<td>WO 8707384</td>
<td>03-12-1987</td>
<td>AU 7033787 A</td>
<td>22-12-1987</td>
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<td></td>
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<td>AU 5881780 A</td>
<td>12-03-1981</td>
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