(54) Title: A METHOD AND AN APPARATUS FOR DETECTING CELLS

The invention relates to a method and an apparatus for detecting cells, including viruses. The sample to be tested is filtered to separate the cells, and the components employed in the cell detection reaction are released and separated from the cells with the same filter that is employed for filtering the sample, and the released component is determined from the filtrate to indicate the presence of cells in the original sample. The invention further relates to a kit suitable for use in the method of the invention.
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A METHOD AND AN APPARATUS FOR DETECTING CELLS

Field of the Invention

The present invention relates to a method and an apparatus for detecting cells, including viruses. The invention further relates to a kit suitable for use in the method of the invention. More specifically, the invention relates to a method for detecting cells, wherein the sample to be tested is filtered to separate the cells and the cells separated by the filter are treated to release therefrom the components employed in the cell detection reaction.

The detection of cells relates to applications in many scientific and technical fields. Such fields of application include for instance clinical and diagnostic testing, biotechnology and molecular biology as well as food and environmental analyses.

Prior Art

Cells may be detected in a variety of ways both directly and indirectly. One indirect way is to determine certain cell components on the basis of their chemical, biological, immunological or physical properties, for instance.

Prior to the cell detection the sample must often be filtered, for example for the reason that the sample volume is so great and the cell content so low that the sensitivity of the methods employed in the detection reaction is not sufficient, or for the reason that it is desired to wash the cells on a filter prior to the actual determination. After the cells have been concentrated onto the filter and possibly washed, they are treated to release therefrom the com-
ponents employed in the cell detection reaction, said components being determined on the filter (WO patent application 88/08037 and European Patent 101 398).

In cell detection methods of this kind, problems are presented by a number of impurities that impede the determination. Such impurities include e.g. the remaining cells and cell fragments and other solid particles present in the sample. Also the filter itself may provide an interfering background. Further, the method is laborious and the loss is great, particularly with samples having a low cell content.

Summary of the Invention

The object of the invention was to develop a method and an apparatus wherewith filtered cells can be reliably and conveniently detected. The method of the invention is characterized in that the components released from the cells are separated from the cells with the same filter that is employed for the filtering of the sample, and said components are determined from the filtrate to indicate the presence of cells in the original sample.

By detection of the cell components from the filtrate and not from the filter, impurities and the background provided by the filter as set forth above are avoided. Further, the possible pathogens in the sample may be inactivated, and thus they have no access to the filtrate to be analyzed. By separating the cell components that have been released from the cells and that are significant to the detection with the same filter that is employed for the concentration of the sample, labor costs and material costs as well as losses in the content of cells or cell components are minimized. Further, it is possible to simul-
taneously determine several cell components that may be derived from a variety of cell types.

Brief Description of the Drawing

The Drawing depicts an apparatus suitable for the method of the invention.

Detailed Description of the Invention

The method of the invention may be applied to a wide variety of sample types. The method is well suited to samples having a large volume and a low cell content. The method is advantageously applied to liquid samples, but also other samples are possible, such as gas which may possibly first be extracted with a liquid. Samples that are initially in solid state may be dissolved or suspended in a liquid and possibly pre-filtered or the interfering substances removed by other means, by centrifugation for instance, prior to the detection. The method of the invention may be implemented for example in the food and beverage industry, in water, gas and air analyses, and in hygienic control in general as well as in clinical samples.

The sample to be tested is passed through a filter the pore size of which is selected on the basis of the size of the cells to be separated. In the present application, the term "cell" also encompasses viruses, even though viruses are not cells in the actual sense of the word but infection particles having an ability to penetrate a cell. Likewise, a cell component also denotes a virus component. The method of the invention is well suitable for the detection of bacteria and viruses, for instance. With bacteria, a filter having
a pore size of 0.2 to 0.5 μm is generally used. With
eucaryotic cells, a larger pore size is used, and with
viruses, a smaller pore size - an ultrafilter - is
used, respectively. For example suction or pressure
may be employed in the filtration.

The filtration may be performed in conventional
filtration apparatus wherein the filter is disposed on
the bottom of a funnel, the funnel is placed on the
opening of a suction bottle, and a vacuum is drawn
into the suction bottle e.g. with a water pump. Alter-
natively, for instance an injection syringe may be
employed, in which case the sample is drawn into the
syringe, a filter is disposed at the end of the
syringe, and the sample is pressed through the filter.

It is naturally possible to employ other filtering
systems as well. With viruses, it is practicable to
perform ultrafiltration, wherein the sample is dis-
posed on the filter of an ultrafiltration tube and is
passed through the filter by centrifuging.

After the cells in the sample have been col-
llected on the filter, it is often practicable to per-
form washing by passing a washing solution through the
filter. Distilled water or a buffer, for instance, may
be employed as the washing solution. The filtrates ob-
tained thus far are discarded. After the possible
washing, the cells are treated to release therefrom
the cell components significant to the detection of
the cells. The treatment may be carried out chemically
or physically, and preferably in such a way that the
cells or the majority of their texture remain on the
filter. The chemical treatment may be enzymatic treat-
ment, detergent treatment, extraction e.g. with an
acid or base or some other suitable solvent, or flush-
ing with water or a buffer solution, or an osmotic
shock, for instance. The desired cell components may
be physically detached from the cells for example by means of an ice press, by sonication, electroporation, shaking, or some other mechanical means.

The cell surface layer is preferably removed, said surface layer possibly in this connection also including extensions of cells, and specifically the bacterial flagella are detached and broken down and flagellin-containing structures or similar are determined. For example, the bacterial surface layer with its extensions may be removed by mild acid treatment. Thereby the flagella are detached and in that connection broken down into flagellin molecules. In the case of viruses, the antigens that are significant to the detection can be detached therefrom.

The detached components that are significant to the detection are flushed through the same filter that is employed for the filtration of the sample. The filtrate is collected, and the presence of cells in the original sample is detected from the flushing solution by means of reactions of the components that were passed through the filter on the basis of the chemical, biological, physical or immunological properties of the cell components. There are numerous methods of detecting cell components, including biochemical, immunological, and radiochemical analyses. The reagents necessary for the detection can also be passed through the filter, or they can be added directly to the filtrate.

The method of the invention can be employed both as a universal method for detecting cells and for the identification of a certain cell type. If the presence of cells in general is of interest, without any concern in the identification, the most practicable way is to define a component universally present in cells, such as LPS or structural parts thereof. Alter-
natively, the method may be employed to detect a certain cell type or strain that is of interest, in which case a cell component that is only present in the cell that is of interest is selected as the cell component to be determined. A very specific determination method is achieved by using immunological methods. It is possible to determine several cell components simultaneously from the same filtrate, and this is advantageous. In other words, it is possible to determine several cell types simultaneously by selecting the cell components to be determined in such a way that one component is only present in one cell type and another in another cell type, etc.

The method of the invention is very well suitable for determining a surface component in a cell, such as cell wall or cell membrane proteins, LPS, capsule polysaccharides, coat proteins (e.g. RS proteins, i.e. S-layer proteins), flagella or other extensions of cells or similar structures or their structural parts, etc. The released cell components are determined subsequent to separation from the main parts of the cells with a filter by conventional methods, such as a protein determination method or an immunological determination method. In principle, any protein detached from the cells can be employed to indicate the presence of cells in the original sample. For instance, gel electrophoresis, immunoblot analysis and immuno-PCR (immuno-polymerase chain reaction) technique (Sano, T., Smith, C. and Cantor, C., 1992, Science 258, pp. 120-122) are suitable for this purpose.

The method of the invention can also be applied to the detection of viruses both from impure samples and from cultures. The sample is placed on an ultra-filter through which it is passed by centrifugation, whereafter the filter is treated to detach the com-
components employed in the detection, such as antigens, from the viruses that remain in the filter. At the same time, the advantage is achieved that pathogenic viruses can be inactivated. The detached antigens are passed through the same filter by recentrifugation, and the antigens are determined from the filtrate for instance by the immuno-PCR method, and hence very small antigen concentrations can be determined. In other words, the method of the invention is suitable for the concentration and purification of antigens and the pretreatment of samples to be tested by the immuno-PCR technique.

The scope of the invention also comprises an apparatus suitable for use in the method described above. The apparatus of the invention is characterized in that it comprises means for filtering the sample to be tested so as to separate the cells; means for treating the cells separated by the filter to release therefrom the components employed in the cell detection reaction; means for separating the released components from the cells, and means for determining said component from the filtrate to indicate the presence of cells in the original sample.

The means for filtering the sample to be tested so as to separate the cells can comprise for instance one or more vessels connected to a filter that is further connected to a waste container, for passing the sample and the possible washing liquid through the filter, and possibly means for passing the sample through the filter, such as a pump, a press or a centrifuge. The pore size of the filter is selected on the basis of the size of the cells to be separated.

The means for treating the cells separated by the filter to release therefrom the components employed in the cell detection reaction can comprise a
vessel connected to the filter for passing the reagents that release cell components through the filter, or means for detaching cell components physically and means for passing a flushing solution through the filter.

The means for separating the released components from the cells consist of the same filter that is employed for filtering the sample, and possibly an intermediate part, such as transfer means enabling the use of the same filter. They may further comprise vessels that are connected to the filter for passing reagents through said filter on to said means for determining said cell component.

The means for determining the cell component comprise a measuring station, which may comprise an assay unit, a detector unit and an electronic unit for detecting the component released from the cells that is to be determined. The assay unit may comprise for instance different types of analysis systems, a light source etc. for the detection of components released from the cells on the basis of their chemical, biological, immunological or physical properties. The detector unit may comprise for instance light measuring means, radioactivity measuring means, etc. The measuring station normally further comprises an electronic unit for receiving the signal produced. The electronic unit is preferably connected to a data processing unit and a control unit. The measuring station is preferably also connected to vessels for conveying the running solutions and eluting solutions to the measuring station, the other end of which may be connected to a waste container. The measuring station is preferably such that several cell components may be determined therewith at the same time.

The apparatus of the invention usually comprises
valves or other transfer means wherewith the reagents employed, the filter itself or the filtrate to be tested can be conveyed to the desired site.

The apparatus preferably comprises a sampling vat and a vat for the washing solution, which are connected to the filter, said filter being further connected to a waste container; transfer means for transferring the filter to another station where to a third vat for the extracting solution is connected and which has a connection with the transferred filter, said filter being further connected to the measuring station, the data processing unit and the control unit; and vessels for the running solution and the eluting solution, said vessels also being connected to the measuring station.

With the apparatus of the invention, the method of the invention can be largely automated. The invention also relates to the use of the apparatus in the method of the invention. Preferably the apparatus is employed to detect several cell components simultaneously from the same sample.

The drawing shows an example of the apparatus of the invention. It is evident that the illustrated embodiment is not the only possible within the scope of the invention.

The drawing shows a sampling vat 1 that is connected via a valve 7 to a filter 10 and via a valve 14 to a waste container 6 for passing the sample through the filter into the waste container. Further, another vat 2 is connected to the filter via a valve 8 for passing a washing liquid through the filter. The apparatus further comprises transfer means 13 for transferring the filter to stations 11 and 12. A vat 3 is connected to station 11 via a valve 9 for supplying an extracting solution to the filter. The filter at
station 11 is connected to a measuring station 18 via a valve 15 for supplying the extracting solution to the measuring station where to a vat 4 is connected via a filter 16 for supplying a running solution thereto. The measuring station is connected to a data processing unit 19 for the interpretation and output of the signal. The apparatus also comprises a control unit 20 for controlling the operation of the valves and other mechanical actuators of the apparatus and for supplying a current. Further, a vat 5 is connected to the measuring station via a valve 17 for supplying the eluting solution to said measuring station for regeneration. Station 12 is a filter exchange station.

The apparatus described above is used as follows: The sample to be tested is supplied to the sampling vat 1 wherefrom it is passed by means of pressure via valve 7 through filter 10 and valve 14 into the waste container 6. The cells remaining on the filter are washed with a washing solution supplied through valve 8 from vat 2. Hereafter the filter is transferred with the transfer means 13 to station 11 at which the cells remaining on the filter are extracted with a solution from vat 3 regulated by valve 9. (Instead of transferring the filter, it is naturally possible to convey the extracting solution to a stationary filter wherefrom the filtrate is conveyed to the measuring station for instance via appropriate valves.) The extract is driven via valve 15 to the measuring station 18 through which it is passed by means of the running solution from vat 4, supplied through filter 16. The signal derived from the measuring station is interpreted by means of the data processing unit 19. The data processing unit outputs the interpreted signal and assesses the presence and amount of cell components to be determined. The
control unit 20 controls the operation of the valves and other mechanical actuators in the apparatus and supplies current to the system. After the measuring, the measuring station is regenerated with eluting solution from vat 5, supplied via valve 17. At station 12, the spent filter is replaced by a new filter.

The invention further relates to a kit comprising the means employed in the method. The kit is characterized in that it comprises means for filtering the sample to be tested to separate the cells; means for treating the cells separated by the filter to release therefrom the components employed in the cell detection reaction; means for separating the released components from the cells, and means for determining said component from the filtrate to indicate the presence of cells in the original sample.

The means for filtering the sample to be tested to separate the cells preferably comprise filters and appertaining vessels suitable for filtering, and possibly the necessary washing solutions. The means for treating the cells preferably comprise reagents for releasing the components employed in the cell detection reaction, and the means wherewith the components are separated from the cells consist of the same filter that is employed for the filtration of the sample. The means for determining the component from the filtrate comprise the reagents employed in the determination. The apparatus employed in the determination is normally not included in the kit.

The filters in the kit of the invention are selected on the basis of the size of the cells to be separated. The vessels to be connected to the filter that are suitable for filtering may be funnels connected to a suction bottle, injection syringes, or ultrafiltration tubes. The reagent to be employed for releas-
ing the cell components may be for example an enzyme, a detergent, an acid or a base solution or some other solvent, water or a buffer. The reagents needed for the detection of components released from the cells may be any reagents necessary in a conventional reaction for the detection of cell components, such as reagents employed in protein determination or immunoassays.

The kit of the invention preferably contains sample and reagent vessels, filters, reagents for releasing cell components employed in the detection reaction, and reagents for determining the released components.

The invention will be explained in more detail by means of the following non-limiting examples.

Example 1

Bacterial cultures of 1.5 ml containing the Pectinatus frisingiensis strain VTT-E-82165 (Hakalehto, E. and Finne, J., 1990, FEMS Microbiology Letters 67, pp. 307-312) were drawn into a 2 ml injection syringe and pressed through a filter disposed at the end of the syringe, the filter having a pore size of 0.45 μm (Millipore Millex HA). The cells were flushed with distilled water. The filtrates obtained were discarded. About 0.2 ml of 0.05 M HCl was then passed through the same filter using another syringe, and the filtrate was collected in an Eppendorff tube and neutralized with NaOH.

Thereafter a sample buffer employed in SDS polyacrylamide gel electrophoresis (SDS-PAGE) was added to the filtrates, and SDS-PAGE was performed on them, as described in Laemmli, U.K., 1970, Nature (London) 227, pp. 680-685, using a 8% gel and commercial molecular weight standards (Pharmacia). Some of the samples were boiled prior to the run. The gels were stained with
the protein dye Coomassie Brilliant Blue (CBB) and/or immunoblotting was performed using an antibody raised in a rabbit immunized with cells of said bacterial strain (Hakalehto, E. and Finne, J., 1990, FEMS Microbiology Letters 67, pp. 307-312). Both in direct staining and in immunoblot analysis, a distinct band was found at about 63 kDa, indicating the presence of flagellin protein of the size of about 63 kDa derived from the Pectinatus frisingiensis strain. This again is an indication of the presence of the Pectinatus frisingiensis strain in the original sample.

**Example 2**

A sample containing the bacterium Bacillus polymyxa (ATCC 842) was treated as in Example 1, and SDS-PAGE was run on the collected filtrate using a 10% gel which was subsequently stained with CBB. A distinct band was to be seen at 120-130 kDa, corresponding to the RS (Regularly Structured) protein present in the coat of these bacteria, said RS protein having a molecular weight of about 120-130 kDa.

**Example 3**

A sample containing the bacterium Enterobacter aerogenes (ATCC 13048) was treated as in Example 1. SDS-PAGE analysis was performed on the collected filtrate with a 8% gel, to give a distinct protein band slightly below the 63 kDa protein of the Pectinatus strain described in Example 1. After purification and sequential analysis, it was found that the protein band corresponds to the flagellin of the Enterobacter strain.

**Example 4**

A sample containing all of the three bacteria mentioned in Examples 1 to 3, i.e. P. frisingiensis VTT-E-82165, B. polymyxa ATCC 842 and E. aerogenes ATCC 13048, was treated as in Example 1, and SDS-PAGE
was run on the collected filtrate using a 8% gel. Distinct bands were obtained at the locus of the bands obtained in Examples 1 to 3, which indicated the presence of all three bacteria in the original sample.

Example 5

400 ml of a sample containing 0.2 ml of a culture of the bacterium *E. aerogenes* (ATCC 13048) was filtrated using a Millipore HA filter with a pore size of 0.45 μm and a diameter of 25 mm. The filtered culture was washed with distilled water or a buffer. The filtrates were discarded. Thereafter 200 μl of 0.05 M HCl was added to the filter, and the filtrate was drawn into an Eppendorff tube. NaOH was added for neutralizing purposes, and finally flushing with distilled water was performed employing suction. Thereafter SDS-PAGE was run on the sample collected in the Eppendorff tube, as in Example 1, using a 12% gel.

Silver staining was performed on the gel as follows: The gel was first shaken in 50% methanol, whereafter it was transferred to a silver solution prepared by adding dropwise 0.8 g of AgNO₃ in 4 ml of distilled water to a solution into which 0.36% of NaOH (21 ml) and ammonia (1.4 ml) had been mixed. Subsequent to the silver solution, the gel was washed with distilled water and transferred to a solution containing 1.25 ml of 1% citric acid and 0.125 ml of 30% formaldehyde in an aqueous solution (250 ml). After the stained protein bands had appeared, the gel was washed with water and transferred to a solution containing 45% of methanol and 1% of acetic acid. The most distinct protein bands were obtained at about 21 kDa and 29 kDa. Also other components detached from cells were detected, but the bands were weaker.
Example 6

A Coxsackie B 6 virus, belonging to the Picorna viruses, obtained from a patient sample was cultivated in a monkey kidney cell culture, whereafter the viruses were released from the cells by freezing and thawing the culture several times. 500 µl of this virus suspension was mixed in 500 µl of deionized water and laid on the filter of an ultrafiltration tube (Microsep 10K, Filtron, USA) and centrifuged for half an hour, 4000 - 5000 rpm. No washing had to be performed. 100 µl of 0.05 M HCl was added to the filter and was allowed to react for one hour at 37°C, whereafter centrifugation was carried out as above. 100 µl of 0.05 M NaOH was added to the filter and re-centrifuged as above. SDS-PAGE was run on the filtrate (in 10% gel) as in Example 1. Prior to the run, the samples were boiled for 5 minutes in SDS-PAGE sample buffer. The gel was stained by silver staining as in Example 5, or an immunoblot analysis was performed as in Example 1. An anti-Coxsackie B 6 antibody raised in a monkey (diluted 1:50) was employed as the first antibody, and 1:1000 diluted anti-human antibody labeled with alkaline phosphatase (Dako, Denmark) was employed as the second antibody, of the immunoblot. The anti-human antibody cross-reacted with the monkey antibody.

The silver-stained SDS gel had several bands that were partly different from the bands obtained with boiled whole viruses, respectively. In the immunoblot method, four bands were highlighted in the range 25 - 32 kDa. According to the literature, the most significant structural proteins of Picorna viruses are normally in the range 9-35 kDa (Andrews, C., Pereira, H.G. and Wildy, P., 1978. Viruses of Vertebrates. Bailliere Tindall, London).
Claims:

1. A method for detecting cells, comprising
   a) filtering the sample to be tested so as to
   separate the cells,
   b) treating the cells separated by the filter to
   release therefrom the components employed in the cell
   detection reaction,
   c) separating from the cells the components rele-
   eased therefrom with the same filter that is employed
   for the filtering of the sample, and
   d) determining said components from the filtrate
   to indicate the presence of cells in the original
   sample.

2. A method as claimed in claim 1, which is
   applied to the detection of bacteria.

3. A method as claimed in claim 1, in which a
   surface component of the cells is determined.

4. A method as claimed in claim 3, in which the
   flagella of the bacteria are detached and broken and
   the flagellin-containing structures or similar struc-
   tures are determined.

5. A method as claimed in claim 3, in which the
   RS-protein of the bacteria is determined.

6. A method as claimed in claim 1, in which a
   component present in the extensions, coat or cell wall
   of the bacteria is determined.

7. A method as claimed in claim 1, in which the
   released cell components are determined by an immuno-
   assay.

8. A method as claimed in claim 1, in which sev-
   eral cell components are determined from the filtrate
   simultaneously to detect the presence of several cell
   types in the same sample.

9. A method as claimed in claim 1, in which an
antigen to be determined by the immuno-PCR method is released from the cell.

10. A method for detecting viruses, comprising
   a) filtering the sample to be tested so as to separate the viruses,
   b) treating the viruses separated by the filter to release therefrom the components employed in the virus detection reaction,
   c) separating from the viruses the components released therefrom with the same filter that is employed for the filtering of the sample, and
   d) determining said components from the filtrate to indicate the presence of viruses in the original sample.

11. An apparatus suitable for the detection of cells, comprising
   a) means for filtering the sample to be tested so as to separate the cells;
   b) means for treating the cells separated by the filter to release therefrom the components employed in the cell detection reaction;
   c) means for separating the released components from the cells, and
   d) means for determining said component from the filtrate to indicate the presence of cells in the original sample.

12. An apparatus as claimed in claim 11, in which the means for separating the released components from the cells consist of the same filter that is employed for filtering the sample.


14. A method as claimed in claim 13, in which the apparatus is used to determine several cell components simultaneously.
15. A kit suitable for use in the method as claimed in claim 1, comprising
   a) means for filtering the sample to be tested so as to separate the cells;
   b) means for treating the cells separated by the filter to release therefrom the components employed in the cell detection reaction;
   c) means for separating the released components from the cells, and
   d) means for determining said component from the filtrate to indicate the presence of cells in the original sample.

16. A kit as claimed in claim 15, wherein the means for separating the released components from the cells consist of the same filter that is employed for filtering the sample.

17. A kit as claimed in claim 16, which contains sample and reagent vessels, filters, reagents for releasing cell components, and reagents for determining the released components.
1. A method for detecting cells, comprising
   a) filtering a sample to be tested so as to separate the cells,
   b) treating the cells on the filter to release therefrom surface components employed in the cell detection reaction,
   c) separating from the cells the surface components released therefrom with the same filter that is employed for the filtering of the sample, and
   d) determining said surface components from the filtrate to indicate the presence of cells in the original sample.

2. A method as claimed in claim 1, which is applied to the detection of bacteria.

3. A method as claimed in claim 2, in which the flagella of the bacteria are detached and broken and flagellin-containing structures or similar structures are determined.

4. A method as claimed in claim 2, in which RS-protein of the bacteria is determined.

5. A method as claimed in claim 1, in which a surface component present in extensions, coat or cell wall of the bacteria is determined.

6. A method as claimed in claim 1, in which the released cell surface components are determined by an immuno-assay.

7. A method as claimed in claim 1, in which several cell surface components are determined from the filtrate simultaneously to detect the presence of several cell types in the same sample.

8. A method as claimed in claim 1, in which an antigen to be determined by the immuno-PCR method is released from the cell surface.
9. A method for detecting viruses, comprising
   a) filtering a sample to be tested so as to separate the viruses,
   b) treating the viruses on the filter to release therefrom the components employed in the virus detection reaction,
   c) separating from the viruses the components released therefrom with the same filter that is employed for the filtering of the sample, and
   d) determining said components from the filtrate to indicate the presence of viruses in the original sample.

10. An apparatus for use in the method of claim 1 for the detection of cells, comprising
    a) means for filtering the sample to be tested so as to separate the cells;
    b) means for treating the cells separated by the filter to release therefrom the surface components employed in the cell detection reaction;
    c) means for separating the released surface components from the cells, said means consisting of the same filter that is employed for filtering the sample; and
    d) means for determining said surface component from the filtrate to indicate the presence of cells in the original sample.

11. A method for using an apparatus as claimed in claim 10 in the method as claimed in claim 1.

12. A method as claimed in claim 11, in which the apparatus is used to determine several cell surface components simultaneously.

13. A kit for use in the method as claimed in claim 1, comprising
    a) means for filtering a sample to be tested so as to separate the cells;
    b) means for treating the cells separated by the filter to release therefrom the surface components em-
ployed in the cell detection reaction;

c) means for separating the released surface components from the cells, said means consisting of the same filter that is employed for filtering the sample; and

d) means for determining said surface component from the filtrate to indicate the presence of cells in the original sample.

14. A kit as claimed in claim 13, which contains sample and reagent vessels, filters, reagents for releasing cell surface components, and reagents for determining the released surface components.

15. An apparatus for use in the method of claim 9 for the detection of viruses, comprising

a) means for filtering a sample to be tested so as to separate the viruses;

b) means for treating the viruses separated by the filter to release therefrom cell surface components employed in a virus detection reaction;

c) means for separating the released surface components from the viruses, said means consisting of the same filter that is employed for filtering the sample; and

d) means for determining said surface components from the filtrate to indicate the presence of viruses in the sample.

16. A kit for use in the method as claimed in claim 9 for the detection of viruses, comprising

a) means for filtering a sample to be tested so as to separate the viruses;

b) means for treating the viruses separated by the filter to release therefrom virus surface components employed in a virus detection reaction;

c) means for separating the released surface components from the viruses said means consisting of the same filter that is employed for filtering the sample; and
22

d) means for determining said surface components from the filtrate to indicate the presence of viruses in the sample.
# INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/FI 93/00418

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC5:** C12Q 1/00, G01N 33/543, G01N 33/569

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)

**IPC5:** C12Q, G01N

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

SE, DK, FI, NO classes as above

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

## MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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* Further documents are listed in the continuation of Box C.  
* See patent family annex.

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**Date of the actual completion of the international search**

31 January 1994

**Date of mailing of the international search report**

01 -02- 1994

**Name and mailing address of the ISA/Swedish Patent Office**

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