(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
09 June 2022 (09.06.2022)

(10) International Publication Number
WO 2022/119896 A1

(51) International Patent Classification:
C12Q 1/04 (2006.01) *G01N 33/569* (2006.01)
C12Q 1/70 (2006.01) *C07K 14/01* (2006.01)
G01N 33/543 (2006.01) *C07K 14/165* (2006.01)

(21) International Application Number: PCT/US2021/061358

(22) International Filing Date: 01 December 2021 (01.12.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/119,721 01 December 2020 (01.12.2020) US

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(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2022/119896 A1

(54) Title: METHODS FOR DETECTING AIRBORNE VIRUSES IN AIR SAMPLES

(57) Abstract: Provided herein are methods for analyzing or detecting airborne viruses for example airborne respiratory viruses by obtaining an air sample, isolating particulate matter therefrom and mixing the particulate matter with a suspension of labeled magnetic beads to produce a virus complex. The virus complex is bound to one or more labeled antibodies specific for viral proteins where the antibodies are linked to a protein microarray containing a magnet array. The microarray is imaged to detect signals from the bound virus complex and from the labeled antibodies from which signals the airborne viruses may be identified.

METHODS FOR DETECTING AIRBORNE VIRUSES IN AIR SAMPLES

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Cross-Reference to Related Application

This international application claims benefit of priority under 35 U.S.C. §119(e) of provisional application U.S. Serial No. 63/119,721, filed December 1, 2020, the entirety of which is hereby incorporated by reference.

10

BACKGROUND OF THE INVENTION

Field of the Invention

15 The invention relates to the fields of microarray technology and virology. More specifically, the present invention is directed to magnetic field enhanced protein microarray technology to analyze a panel of multiple airborne viruses.

Description of the Related Art

20 Presently, respiratory virus testing of air samples is performed via ordinary air filter-based collection followed by rinsing of the filters, sample concentration, RNA purification and a nucleic acid based viral test, such as isothermal amplification or q-rtPCR. Air filtration has a low recovery of the viral sample and cannot distinguish intact viral particles from viral debris. The subsequent concentration and purification 25 reactions require multiple steps and thus take several hours and require significant manual processing or mechanical automation. The subsequent terminal nucleic acid tests are sensitive and specific but are difficult to multiplex. Thus, in the aggregate the airborne testing of today requires several hours and cannot support rapid analysis of multiple pathogens.

30 Moreover, although “fast” protein analyte tests used to analyze human samples are done in 10 minutes, they are not sensitive enough to accommodate the low viral load seen in air samples. Also, physical methods such as light scatter can detect individual viral particles, but cannot resolve closely related viruses, such as,

COVID-19 virus versus coronaviruses that cause the common cold or animal diseases.

Thus, there is a deficiency in the art for microarray technology that provides a quick analysis of a panel of multiple airborne viruses to enable large-scale collection thereof for analysis. The present invention fulfills this longstanding need and desire in the art.

SUMMARY OF THE INVENTION

The present invention is directed to a method for analyzing the viral complement of an air sample. In the method the air sample is obtained and particulate matter is collected from the air sample. The particulate matter is mixed with a suspension of first fluorescently labeled magnetic beads that bind to intact viruses therein to produce a complex. The complex binds to second fluorescently labeled capture antibodies specific for a viral protein to form a bound virus complex, where the second fluorescently labeled capture antibodies are linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array. The protein microarray is imaged to detect simultaneously signals from the bound virus complex and from the second fluorescently labeled capture antibodies, thereby analyzing the viral complement of the air sample. The present invention is direct to a related method further comprising, prior to the imaging step, removing unbound virus complex via the magnet array.

The present invention also is directed to a method for detecting at least one airborne respiratory virus. In the method an air sample is obtained and particles are collected from the air sample. The particles are added to a suspension of magnetic beads labeled with a first detectable label where the magnetic beads bind to intact respiratory viruses therein to produce a respiratory virus complex. The respiratory virus complex binds to capture antibodies labeled with a second detectable label specific for at least one respiratory viral protein to form a bound respiratory virus complex, where the capture antibodies are linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array. The protein microarray is imaged to detect simultaneously signals from the bound respiratory virus complex and from the capture antibodies, thereby detecting the at least one airborne

respiratory virus. The present invention is direct to a related method further comprising, prior to the imaging step, removing unbound respiratory virus complex via the magnet array.

The present invention is directed further to a method for detecting Respiratory
5 Syndrome Coronavirus 2 (SARS-CoV-2) present in air. In the method a sample of air is obtained and particulate matter is collected from the sample of air. The particulate matter is mixed with a suspension of magnetic beads labeled with a first fluorophore that bind to intact SARS-CoV-2 viruses therein to form a SARS-CoV-2 complex. The SARS-CoV-2 complex binds to capture antibodies labeled with a
10 second fluorophore or other label specific for a SARS-CoV-2 protein, where the capture antibodies are linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array and the unbound SARS-CoV-2 complex is removed via the magnet array. The protein microarray is imaged to detect simultaneously signals from bound SARS-CoV-2 complex and from the capture
15 antibodies, thereby detecting SARS-CoV-2 virus present in the air.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

20

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and
25 can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered
30 limiting in their scope.

FIG. 1 illustrates the magnetic bead-based viral capture and fluorescent dye based detection on a protein microarray.

FIGS. 2A-2C illustrate the magnetic field induced binding, mixing, and imaging of the magnet array. FIG. 2A illustrates binding and shows the binding position A. FIG. 2B illustrates washing to the side and shows the mixing/imaging position B. FIG. 2C is an alternative imaging position C to that in FIG. 2B and illustrates moving the magnet array above the plate and moving the bead complex to the edge of the meniscus.

FIG. 3 illustrates the structure of a typical 3-dimensional microarray probe “spot”.

DETAILED DESCRIPTION OF THE INVENTION

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are collected herein. These definitions should be read in light of the remainder of the disclosure and understood as by a person of skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art.

The articles "a" and "an" when used in conjunction with the term “comprising” in the claims and/or the specification, may refer to “one”, but it is also consistent with the meaning of “one or more”, “at least one”, and “one or more than one”. Some embodiments of the invention may consist of or consist essentially of one or more elements, components, method steps, and/or methods of the invention. It is contemplated that any composition, component or method described herein can be implemented with respect to any other composition, component or method described herein.

The term “or” in the claims refers to “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or”.

The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included.

The term "including" is used herein to mean "including, but not limited to". "Including" and "including, but not limited to" are used interchangeably.

As used herein, the term “about” refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly

indicated. The term "about" generally refers to a range of numerical values (e.g., ± 5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure. For example, a sample of about 1 cubic meter of air encompasses an air sample of 0.9 cubic meters of air to 1.1 cubic meters of air.

As used herein, the ordinal adjectives "first" and "second", unless otherwise specified are used to describe a common object, merely indicate that different instances of like objects are being referred to, and are not intended to imply that the objects so described must be in a given sequence, either temporally, spatially, in ranking, or in any other manner.

In one embodiment of the present invention, there is provided a method for analyzing the viral complement of an air sample, comprising obtaining the air sample; collecting particulate matter from the air sample; mixing the particulate matter with a suspension of first fluorescently labeled magnetic beads that bind to intact viruses therein to produce a complex; binding the complex to second fluorescently labeled capture antibodies specific for a viral protein to form a bound virus complex, said second fluorescently labeled capture antibodies linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array; and imaging the protein microarray to detect simultaneously signals from bound virus complex and from the second fluorescently labeled capture antibodies, thereby analyzing the viral complement of the air sample. Further to this embodiment the method comprises, prior to the imaging step, removing unbound virus complex via the magnet array.

In both embodiments the first fluorescently labeled magnetic beads may be coated with a hydrogel or with biotin. Also in both embodiments the first fluorescently labeled magnetic beads may be labeled with a green fluorescent dye or a strepavidin-linked fluorescent dye. In addition the second fluorescently labeled capture antibodies may be labeled with a red fluorescent dye. Alternatively, the second fluorescently labeled capture antibodies are labeled with a red fluorescent dye linked to an oligothymidine. Furthermore the second fluorescently labeled capture antibodies may be specific for different surface proteins on a virus or variants thereof. Alternatively, the second fluorescently labeled capture antibodies

may be specific for viral surface proteins from a panel of different viruses or variants thereof.

In both embodiments the virus in the complex may be a coronavirus, a respiratory syncytial virus A (RSV A), a respiratory syncytial virus B (RSV B) or a variant of each or a combination thereof. Particularly, the coronavirus may be a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or variant thereof. Also, the second labeled capture antibodies may be specific for a spike protein or a G protein or an F protein on the virus. In addition the virus in the complex may be an influenza A virus or an influenza B virus or a subtype of each or a combination thereof. Furthermore, the second fluorescently labeled capture antibodies may be specific for a surface protein on the virus. Representative examples of the surface protein are neuraminidase or haemagglutinin or a combination thereof.

In another embodiment of the present invention, there is provided a method for detecting at least one airborne respiratory virus, comprising obtaining an air sample; collecting particles from the air sample; adding the particles to a suspension of magnetic beads labeled with a first detectable label, said magnetic beads binding to intact respiratory viruses therein to produce a respiratory virus complex; binding the respiratory virus complex to capture antibodies labeled with a second detectable label specific for at least one respiratory viral protein to form a bound respiratory virus complex, said capture antibodies linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array; and imaging the protein microarray to detect simultaneously signals from the bound respiratory virus complex and from the capture antibodies, thereby detecting the at least one airborne respiratory virus. Further to this embodiment the method comprises, prior to the imaging step, removing unbound complex via the magnet array.

In both embodiments the magnetic beads may be coated with a hydrogel or with biotin. Also in this embodiment the first detectable label may be a green fluorescent dye or a strepavidin-linked fluorescent dye. In addition the second detectable label may be a red fluorescent dye. Alternatively, the second detectable label may be a red fluorescent dye linked to an oligothymidine.

In both embodiments the capture antibodies may be specific for different viral proteins on a single virus or variants or subtypes thereof or may be specific for viral

proteins from a panel of respiratory viruses or variants or subtypes thereof. Particularly, the capture antibodies may be specific for a spike protein or a G protein or an F protein or neuraminidase or haemagglutinin or a combination thereof. Also the respiratory virus may be at least one of a coronavirus, a respiratory syncytial virus A (RSV A), a respiratory syncytial virus B (RSV B) or a variant of each, an influenza A virus or an influenza B virus or a subtype of each or a combination of the viruses. A representative example of a coronavirus is a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or variant thereof.

In yet another embodiment of the present invention, there is provided a method for detecting Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) present in air, comprising obtaining a sample of air; collecting particulate matter from the sample of air; mixing the particulate matter with a suspension of magnetic beads labeled with a first fluorophore that bind to intact SARS-CoV-2 viruses therein to produce a SARS-CoV-2complex; binding the SARS-CoV-2 complex to capture antibodies labeled with a second fluorophore or other label specific for a SARS-CoV-2protein, said capture antibodies linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array; removing unbound SARS-CoV-2 complex via the magnet array; and imaging the protein microarray to detect simultaneously signals from bound complex and from the capture antibodies, thereby detecting SARS-CoV-2 virus present in the air.

In this embodiment the magnetic beads may be coated with a hydrogel or with biotin. Also in this embodiment the first fluorophore may be a green fluorescent dye or a strepavidin-linked fluorescent dye. In addition, in one aspect the second fluorophore may be a red fluorescent dye. In another aspect the second fluorophore may be a red fluorescent dye linked to an oligothymidine. Furthermore, the capture antibodies may be specific for a spike protein on the SARS-CoV-2 virus.

Provided herein are methods for analyzing, detecting or identifying at least one airborne virus, preferably multiple airborne viruses, present in an air sample. These methods provide a fast analyte test for viral proteins in about ten minutes. This enables large-scale collection as required for monitoring airborne viral load in environments such as, but not limited to, a military base, a business, an airport, or a hotel.

Generally, the methods analyze respiratory viral pathogens in the air via a

vortex based air sample collection, followed by magnetic bead based collection of intact viral particles and the by analysis of their surface protein complement while still an intact particle on a microarray. This enables analysis of about 100 air samples (or less) in parallel. More particularly, the method may comprise the steps
5 of:

1. Direct air to fluid vortex transfer (e.g. Bertin Coriolis);
2. Magnetic bead capture of fluid phase virus particles (e.g. Ceres Nanotrap);
3. Adsorptive dye labeling of resulting [Virus+Bead] complex;
4. Magnetic field induced binding of [Virus+Bead+Dye] complexes to a protein
10 or antibody microarray, for example, a 144 element microarray;
5. Magnetic field induced removal of non-specifically bound [Virus+Bead+Dye] complexes within the protein or antibody microarray; and
6. Imaging of up to 96 of these arrays in parallel at 10 seconds/array.

The magnetic beads may be coated with a hydrogel and labeled with a first
15 fluorescent label or a first detectable label or a first fluorophore, such as a green fluorescent dye, for example, but not limited to, the cyanine dye CY-3. Alternatively, the magnetic beads may be coated with biotin and labeled with a strepavidin (SA)-linked fluorophore, such as, but not limited to, SA-phycoerythrin.

The antibodies may be labeled with a second fluorescent label or a second
20 detectable label or second fluorophore, such as a red fluorescent dye, for example, but not limited to, the cyanine dye CY-5. The antibodies also may be labeled with a red fluorescent dye linked to an oligonucleotide such as the oligothymidine Oligo-T. Alternatively, particularly when the magnetic beads are coated with biotin, the antibodies may be labeled with strepavidin. One of ordinary skill in the art is well-
25 able to determine a set of labels that enable two-color signaling during microarray imaging.

The viruses or airborne viruses detectable by the methods described herein may be a coronavirus, for example, but not limited to, Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which causes the respiratory infection coronavirus
30 disease 2019 (COVID-19), a respiratory syncytial virus A (RSV A), a respiratory syncytial virus B (RSV B) or a variant of each, an influenza A virus or an influenza B virus or a subtype of each or any combination of these. The antibodies comprising the microarray are specific for at least one viral protein on each virus, for example,

the spike protein on the coronaviruses or the G or F proteins of RSV viruses or a surface protein on the influenza viruses, for example, neuraminidase or haemagglutinin. The antibodies may be specific for or directed to different proteins on a single virus or may be specific for at least one protein on each of multiple
5 viruses thereby enabling detection of one or more viruses.

The microarray comprises a substrate to which the antibodies are surface-linked by UV crosslinking of a polymer, such as an oligonucleotide comprising about 60 nucleotides, for example, but not limited to, an oligothymidine (Oligo-T). The microarray comprises a magnet array disposed over or under the microarray plate
10 for removal of unbound [Virus+Bead+Dye] complexes. A representative example of a magnetic array is an 8x12 array of neodymium magnets. When disposed under the microarray plate the magnet array is effective to wash the unbound complexes to the side of each well. When disposed over the microarray plate the magnet array is effective to move the unbound complexes to the top of each well

15 The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

20 Method

The method entails the following eight steps requiring about 10 min. These method steps may be performed by any adult operator and requires minimal training and no fluid handling other than with a disposable eye dropper.

1. Add 4 drops of Magnetic Bead Suspension to the Bertin Coriolis Sample
25 Collection Cup and swirl to generate a stable [Virus+Bead+nDye] complex.

2. Place each Collection Cup in its position in a magnetic chuck. Let sit 1min.

3. Use the same eye dropper to remove the collection fluid from the cup.

4. Use a fresh eye dropper to add 6 of drops binding/labeling buffer, swirl.

5. Use the same eye dropper to transfer the entire [Virus+Bead+nDye]
30 complex into one well of the 96-well protein microarray plate

6. Place the filled plate at Position A of the 8 x 12 magnetic chuck. Sit at room temperature (RT) for 1min to allow magnetically enhanced microarray binding (FIG. 2A)

7. Move the filled plate to position b of the magnetic chuck. Sit at RT for 1 minute to remove unbound or weakly bound [Virus+Bead+nDye] to the periphery of each well, to allow magnetically-enhanced 2-dimensional bead mixing/washing (FIG. 2B or, alternatively, FIG. 2C)

5 8. Take the 96-well plate and immediately image from the bottom for 0.5min/array. Total elapsed time for manual processing and imaging of 1 sample = 10min, 96 samples = 30min footprint = all equipment in a single large suitcase.

EXAMPLE 2

10 Analysis of the viral complement captured from 1 cubic meter (1 m^3) of air

A 1 cubic meter air sample is collected via a representative air sample collector (Bertin Coriolis Micro-Microbial Air Sampler (1)). The particulate complement of a 1 m^3 air sample can be collected into 2mls of an aqueous collection buffer. A representative buffer of that type is phosphate buffered saline (PBS) with 15 0.1% added TWEEN 20 as a wetting agent, but other neutral saline buffers, e.g.150 mM NaCl or NaCitrate, with a small amount of a non-ionic wetting agent, e.g., TWEEN 20 or NP40 or an equivalent, may be substituted. The particulate matter of the air, including viruses, thus collected as a suspension in the collection buffer are presented for subsequent analysis in the collection cup of the Bertin device.

20 The suspended virus is then treated with 4 drops (about 200 µL) of a magnetic bead suspension that comprises in this example a suspension of ferrite magnetic beads (about 2nm) coated by the manufacturer with a hydrogel, i.e., Nanotrap particles, that is designed to bind to enveloped respiratory viruses (Ceres Nanotrap (2)). These Nanotrap particles also stably bind partially hydrophobic dyes 25 such as, but not limited to, CY-3. In that way, upon adding about 100 dye equivalents per Ceres particle, a fluorescently labeled [Bead+nDye] complex is generated on mixing and is then ready to serve as the magnetic bead suspension (FIG. 1). The magnetic bead suspension is diluted with PBS to about 10,000 magnetic beads/ml, so that, upon addition of 4 drops of that suspension to 2 ml of 30 collection buffer, the resulting [Bead+nDye] complex has been diluted to about 1,000 Beads/ml. Upon mixing of the magnetic bead suspension with a SARS-CoV2 (or equivalent) enveloped viral suspension in the collection buffer, the enveloped virus (e.g. SARS-CoV-2) adsorbs to the [Bead+nDye] complex (FIG. 2A) to produce a

[Virus+ Bead+nDye] complex and thus be made available for binding to a microarray substrate (FIG. 1).

The microarray substrate comprises a protein microarray fabricated such that each spot in the microarray is about 100μM in diameter and spaced about 250**μ**m on center. Each spot of the microarray contains a capture antibody specific for the spike protein of SARS-CoV2 or for another representative respiratory virus such as influenza an antibody specific for an influenza surface protein such as neuraminidase or haemagglutinin. In the specific case of SARS-CoV2 analysis, a representative spike protein specific antibody is represented by Recombinant SARS-CoV-2 Spike RBD Protein with His tag (RP01258) from Abclonal. Representative printing conditions include formulation of the anti-spike antibody at 1 mg/ml in a print solution containing PBS, 1mg/ml of bovine serum albumin and 0.1 mg/ml Oligo-dT (60 bases long) and 0.01mg/ml Oligo-dT (25 bases long modified at its 5' terminus with CY5, to confer a red fluorescent marker signal to the microarray spot containing the antibody.

Microarray fabrication printing of the above mentioned antibody print solution is performed by contact or ink jet methods to deliver about 0.3 nL (300 pL) per spot onto a glass or plastic substrate. Upon printing, the resulting air-dried spots are crosslinked at about 300 mJ to induce thymidine crosslinking, thereby entrapping the antibody in the microarray spot, linking the antibody to the red fluorescent marker and in turn fixing the spot onto the underlying glass or plastic surface to be made available for binding to a cognate solution state [Virus+ Bead+nDye] complex (FIG. 3).

Upon addition of the [Virus+ Bead+nDye] complex in the collection buffer to the microarray surface, if needed, a binding buffer is added to raise the ionic strength and the complex binds to surface bound antibody. Unbound [Virus+ Bead+nDye] is removed from the center of the microarray by the action of a cognate array of permanent magnets as described in FIG. 2B or, alternatively, FIG. 2C. Having been pulled to the edge of the microarray via the action of the permanent magnets, the remaining dye-labelled [Virus+ Bead+nDye] complex is imaged via two color fluorescence detection using an imager, such as the Sensovation Sensospot imager. The imager detects the location of the protein spots by analysis of CY-5 fluorescence in each microarray spot and detection of the bound [Virus+ Bead+nDye]

complex being obtained via simultaneous imaging herein of the CY-3 dye, or equivalent dye to the Ceres Magnetic bead carrier.

At a minimum, such protein microarrays may be fabricated to possess a number of different antibodies specific for SARS-CoV2 variants (a different antibody per microarray spot) or to resolve mixtures of respiratory pathogens, with each spot containing antibodies specific for a panel of respiratory viruses, for example, antibodies against SARS-CoV2 and/or SARS-CoV2 variants in some spots, antibodies against Influenza A and/or Influenza A variants in some spots, antibodies against Influenza B and/or Influenza B variants in some spots, and antibodies against RSV A and/or RSV B variants in some spots. The resulting protein microarray resolves a mixture of SARS-CoV2, Influenza A, Influenza B, RSV A, RSV B and subtypes of each for which well-defined surface protein specific antibodies may be obtained in a pure form.

The following references are relevant to the instant method.

1. bertin-instruments.com/product/air-samplers/coriolis-micro-air-sampler.
2. f1dcaaa6-ae11-4fe9-9f94e8797af93d5b.filesusr.com/ugd/f7710c_399c19f862ff 4fba8b4312c1f0c59809.pdf
3. Hogan *et al*. Methods for Detecting Low Levels of COVID-19 Virus, U.S. Serial No. 16/950,171, November 17, 2020.
4. Hogan, M.E. Compositions and Methods for Entrapping Protein on a Surface, U.S. Patent No. 10,105,674, October 23, 2018.
5. Hogan, M.E. Compositions and Methods for Entrapping Protein on a Surface, U.S. Patent No. 9,751,069, September 5, 2017.
6. Hogan *et al*. Microarray Based Multiplex Pathogen Analysis and Uses Thereof, U.S. Patent No. 10,612,075, April 7, 2020.

WHAT IS CLAIMED IS:

1. A method for analyzing the viral complement of an air sample, comprising:

5 obtaining the air sample;

collecting particulate matter from the air sample;

mixing the particulate matter with a suspension of first fluorescently labeled magnetic beads that bind to intact viruses therein to produce a complex;

binding the complex to second fluorescently labeled capture antibodies 10 specific for a viral protein to form a bound virus complex, said second fluorescently labeled capture antibodies linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array; and

imaging the protein microarray to detect simultaneously signals from the bound virus complex and from the second fluorescently labeled capture antibodies, 15 thereby analyzing the viral complement of the air sample.

2. The method of claim 1, further comprising, prior to the imaging step, removing unbound virus complex via the magnet array.

20 3. The method of claim1, wherein the first fluorescently labeled magnetic beads are coated with a hydrogel or with biotin.

4. The method of claim 1, wherein the first fluorescently labeled magnetic beads are labeled with a green fluorescent dye or a strepavidin-linked fluorescent 25 dye.

5. The method of claim 1, wherein the second fluorescently labeled capture antibodies are labeled with a red fluorescent dye.

30 6. The method of claim 1, wherein the second fluorescently labeled capture antibodies are labeled with a red fluorescent dye linked to an oligothymidine.

7. The method of claim 1, wherein the second fluorescently labeled capture antibodies are specific for different surface proteins on a virus or variants thereof.

5 8. The method of claim 1, wherein the second fluorescently labeled capture antibodies are specific for viral surface proteins from a panel of different viruses or variants thereof.

9. The method of claim 1, wherein the virus in the complex is a 10 coronavirus, a respiratory syncytial virus A (RSV A), a respiratory syncytial virus B (RSV B) or a variant of each or a combination thereof.

10. The method of claim 9, wherein the coronavirus is a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or variant thereof.

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11. The method of claim 10, wherein the second fluorescently labeled capture antibodies are specific for a spike protein or a G protein or an F protein on the virus.

20 12. The method of claim 1, wherein the virus in the complex is an influenza A virus or an influenza B virus or a subtype of each or a combination thereof.

13. The method of claim 12, wherein the second fluorescently labeled capture antibodies are specific for a surface protein on the virus.

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14. The method of claim 13, wherein the surface protein is neuraminidase or haemagglutinin or a combination thereof.

15. A method for detecting at least one airborne respiratory virus, 30 comprising:

obtaining an air sample;

collecting particles from the air sample;

adding the particles to a suspension of magnetic beads labeled with a first detectable label, said magnetic beads binding to intact respiratory viruses therein to produce a respiratory virus complex;

binding the respiratory virus complex to capture antibodies labeled with a second detectable label specific for at least one respiratory viral protein to form a bound respiratory virus complex, said capture antibodies linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array; and

imaging the protein microarray to detect simultaneously signals from the bound respiratory virus complex and from the capture antibodies, thereby detecting the at least one airborne respiratory virus.

16. The method of claim 15, further comprising, prior to the imaging step, removing unbound respiratory virus complex via the magnet array.

17. The method of claim 15, wherein the magnetic beads are coated with a hydrogel or with biotin.

18. The method of claim 15, wherein the first detectable label is a green fluorescent dye or a strepavidin-linked fluorescent dye.

19. The method of claim 15, wherein the second detectable detectable label is a red fluorescent dye ~~or strepavidin~~.

20. The method of claim 15, wherein the second detectable label is a red fluorescent dye linked to an oligothymidine.

21. The method of claim 15, wherein the capture antibodies are specific for different viral proteins on a single virus or variants or subtypes thereof or are specific for viral proteins from a panel of respiratory viruses or variants or subtypes thereof.

22. The method of claim 21, wherein the capture antibodies are specific for a spike protein or a G protein or an F protein or neuraminidase or haemagglutinin or a combination thereof.

23. The method of claim 22, wherein the respiratory virus is at least one of a coronavirus, a respiratory syncytial virus A (RSV A), a respiratory syncytial virus B (RSV B) or a variant of each, an influenza A virus or an influenza B virus or a subtype of each or a combination of said viruses.

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24. The method of claim 23, wherein the coronavirus is a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or variant thereof.

25. A method for detecting Respiratory Syndrome Coronavirus 2 (SARS-
10 CoV-2) present in air, comprising:

obtaining a sample of air;

collecting particulate matter from the sample of air;

mixing the particulate matter with a suspension of magnetic beads labeled with a first fluorophore that bind to intact SARS-CoV-2 viruses therein to produce a
15 SARS-CoV-2 complex;

binding the SARS-CoV-2 complex to capture antibodies labeled with a second fluorophore or other label specific for a SARS-CoV-2protein to form a bound SARS-CoV-2 complex, said capture antibodies linked via an oligonucleotide to a surface of a protein microarray comprising a magnet array;

20 removing unbound SARS-CoV-2 complex via the magnet array; and

imaging the protein microarray to detect simultaneously signals from the bound SARS-CoV-2 complex and from the capture antibodies, thereby detecting SARS-CoV-2 virus present in the air.

25 26. The method of claim 25, wherein the magnetic beads are coated with a hydrogel or with biotin.

27. The method of claim 25, wherein the first fluorophore is a green fluorescent dye or a strepavidin-linked fluorescent dye.

30

28. The method of claim 25, wherein the second fluorophore is a red fluorescent dye.

29. The method of claim 25, wherein the second fluorophore is a red fluorescent dye linked to an oligothymidine.

30. The method of claim 25, wherein the capture antibodies are specific for
5 a spike protein on the SARS-CoV-2 virus.

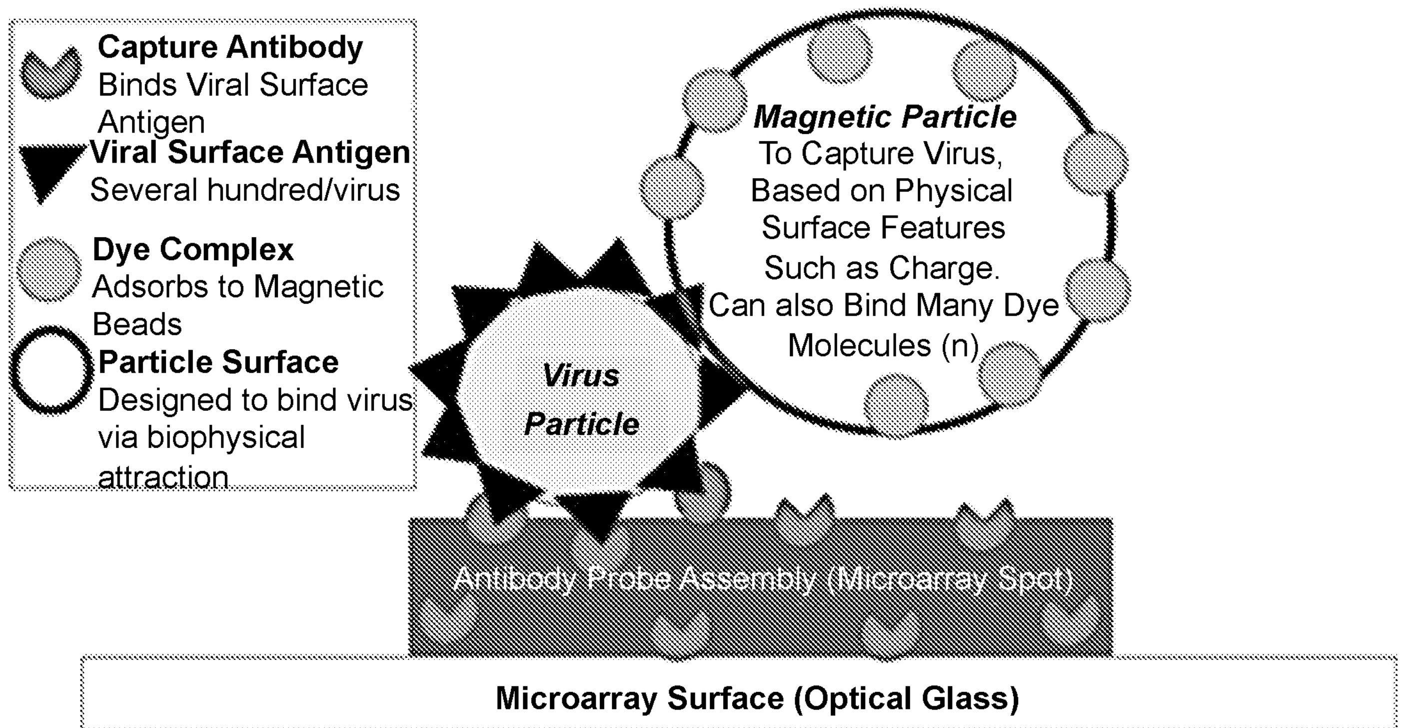

FIG. 1

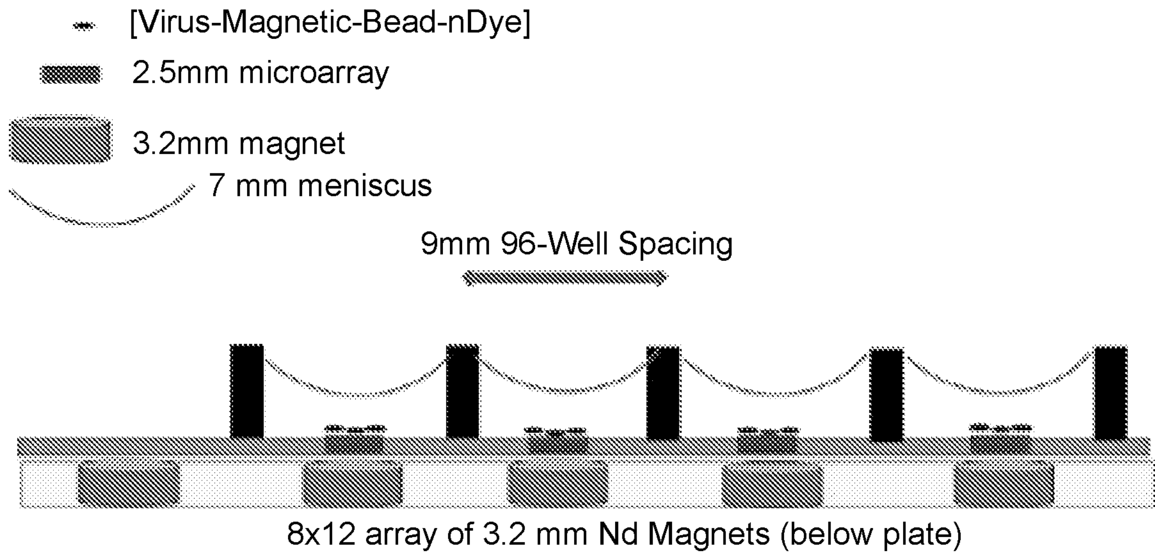

FIG. 2A

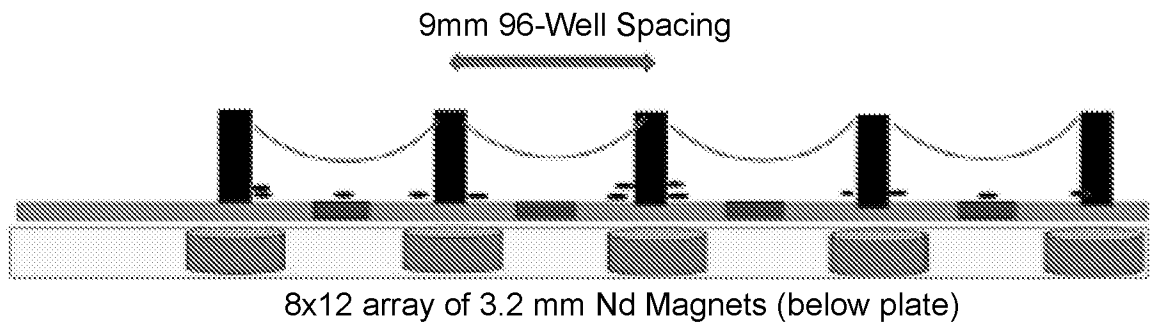

FIG. 2B

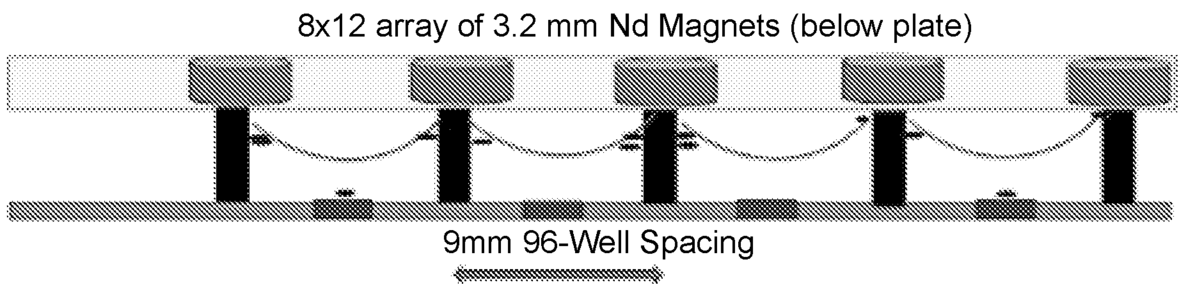

FIG. 2C

Protein (Antibody) Microarray Probe

Polymer formulated with the Protein (Antibody)
To link, post-printing, by UV crosslinking the Antibody molecules to the surface whie retaining flexibility of antibody orientation to the fluid surface during binding. Also used as a spacer to separate antibodies snd to modify surface charge within the Spot Domain

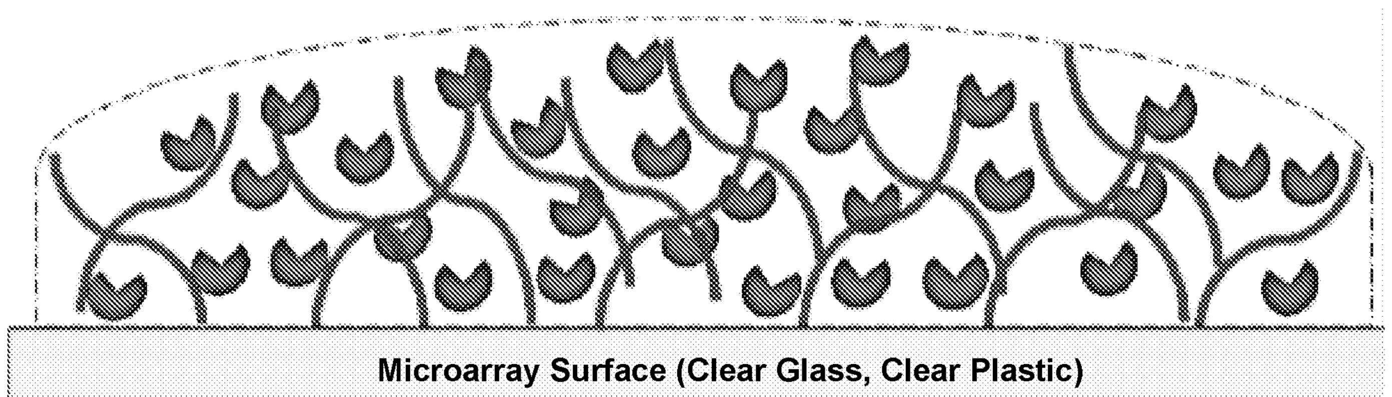

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2021/061358

A. CLASSIFICATION OF SUBJECT MATTER

(see extra sheet)

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)

C12Q, G01N, C07K

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

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

E-Library, Espacenet, PatSearch, PATENTSCOPE, RUPTO, NCBI, EMBL-EBI, Google, Google Scholar, PubMed, USPTO, ScienceDirect

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2005/085849 A2 (U S. GENOMICS, INC.), 15.09.2005, claims 48, 51, 54, 57, 58, 61, 64, p.8 lines 8-12, p.10 lines 5-20, p.11lines 4-5, p.15 lines 12-15, p.16 lines 20-30, p.16 line 32 - p.17 line 2, p.26 lines 19-29, p.31 lines 4-5	1-30
Y	OTIENO BRUNAH A et al. Cancer Diagnostics via Ultrasensitive Multiplexed Detection of Parathyroid Hormone-Related Peptides with a Microfluidic Immunoarray. Analytical chemistry, 2016, v.88, 18, p.9269-75. doi:10.1021/acs.analchem.6b02637, abstract, p.9274 left column, para.4	1-30
Y	WOLD E. D. et al. Antibody microarrays utilizing site-specific antibody-oligonucleotide conjugates. Bioconjugate chemistry, 2015, v.26, no.5, p.1-14. doi:10.1021/acs.bioconjchem.5b00111, [online], [retrieved on 08.03.2022]. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4787600/#SD1> abstract, Figure S1 and 1, Chapter “Conclusion”	1-30

[X] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents:

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

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“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	Date of mailing of the international search report
10 March 2022 (10.03.2022)	17 March 2022 (17.03.2022)

Name and mailing address of the ISA/RU:	Authorized officer
Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	S. Ilchenko Telephone No. (8-499) 240-25-91

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FABIANI L. et al. Magnetic beads combined with carbon black-based screen-printed electrodes for COVID-19: A reliable and miniaturized electrochemical immunosensor for SARS-CoV-2 detection in saliva. Biosensors & bioelectronics, Available online 3 October 2020, v.171, 112686, p.1-9. doi:10.1016/j.bios.2020.112686, abstract	1-30
Y	US 8007999 B2 (THERANOS, INC.), 30.08.2011, claims	1-30
Y	US 6627748 B1 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK), 30.09.2003, column 6 lines 28-31, column 4 lines 43-45, column 13 line 35	6, 20, 29

C12Q 1/04 (2006.01)
C12Q 1/70 (2006.01)
G01N 33/543 (2006.01)
G01N 33/569 (2006.01)
C07K 14/01(2006.01)
C07K 14/165(2006.01)