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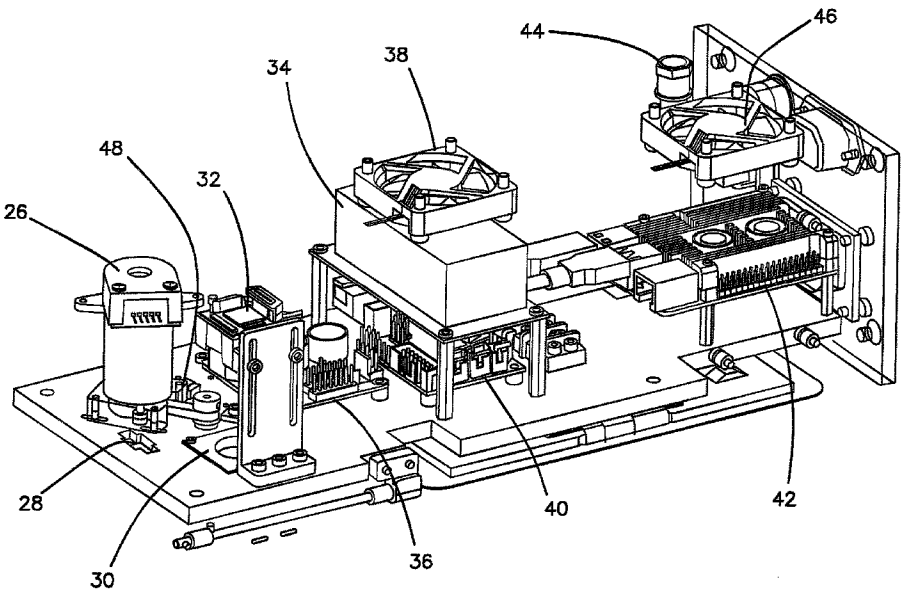

(57) Abrégé/Abstract:
An automated system communicated to a remote server for diagnostically field testing a sample taken from a patient using an automated portable handheld instrument to determine the presence of Covid-19 and/or antibodies thereto includes microfluidic

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(57) Abrégé(suite)/Abstract(continued):
circuits defined in a rotatable disk for performing a bioassay using a microarray to generate an electrical signal indicative of a bioassay measurement; the microarray operationally positioned in the microfluidic circuit; one or more lasers; one or more positionable valves in the microfluidic circuit; and a backbone unit for rotating the disk according to a protocol to perform the bioassay, for controlling the lasers to selectively open the positionable valves in the microfluidic disk, for operating the microarray to generate a digital image as a bioassay measurement; for communicating the bioassay measurement to the remote server, and for associating the performed bioassay and its corresponding bioassay measurement to the patient.

Abstract

An automated system communicated to a remote server for diagnostically field testing a sample taken from a patient using an automated portable handheld instrument to determine the presence of Covid-**19** and/or antibodies thereto includes microfluidic circuits defined in a rotatable disk for performing a bioassay using a microarray to generate an electrical signal indicative of a bioassay measurement; the microarray operationally positioned in the microfluidic circuit; one or more lasers; one or more positionable valves in the microfluidic circuit; and a backbone unit for rotating the disk according to a protocol to perform the bioassay, for controlling the lasers to selectively open the positionable valves in the microfluidic disk, for operating the microarray to generate a digital image as a bioassay measurement; for communicating the bioassay measurement to the remote server, and for associating the performed bioassay and its corresponding bioassay measurement to the patient.

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APPARATUS AND METHOD FOR POINT-OF-CARE, RAPID, FIELD-DEPLOYABLE DIAGNOSTIC TESTING OF COVID-19, VIRUSES, ANTIBODIES AND MARKERS

Field

The embodiments described herein relate to the field of point-of-care (POC) pathogen and multiplexed pathogen and antibody array detection platforms and methods, such as in CPC C**40**B **60/12**.

Background

COVID-**19** testing involves analyzing samples to assess the current or past presence of SARS-CoV-**2**. The two main branches detect either the presence of the virus or of antibodies produced in response to infection. Tests for viral presence are used to diagnose individual cases and to allow public health authorities to trace and contain outbreaks. Antibody tests instead show whether someone once had the disease. They are less useful for diagnosing current infections because antibodies may not develop for weeks after infection. They are used to assess disease prevalence, which aids the estimation of the infection fatality rate. Individual jurisdictions have adopted varied testing protocols, including whom to test, how often to test, analysis protocols, sample collection and the uses of test results. This variation has likely significantly impacted reported statistics, including case and test numbers, case fatality rates and case demographics.

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Test analysis is often performed in automated, high-throughput, medical laboratories by medical laboratory scientists. Alternatively, point-of-care testing can be done in physician's offices, workplaces, institutional settings or transit hubs. Positive viral tests indicate a current infection, while positive antibody tests indicate a prior infection. Other techniques include a chest CT scan, checking for elevated body temperature or checking for low blood oxygen level.

Detection of the virus

Reverse transcription polymerase chain reaction

Polymerase chain reaction (PCR) is a process that amplifies or replicates a small, well-defined segment of DNA many hundreds of thousands of times, creating enough of it for analysis. Test samples are treated with certain chemicals that allow DNA to be extracted. Reverse transcription converts RNA into DNA. Reverse transcription polymerase chain reaction (RT-PCR) first uses reverse transcription to obtain DNA, followed by PCR to amplify that DNA, creating enough to be analyzed. RT-PCR can thereby detect SARS-CoV-**2**, which contains only RNA. The RT-PCR process generally requires a few hours.

Real-time PCR (qPCR) provides advantages including automation, higher-throughput, and more reliable instrumentation. It has become the preferred method. The combined technique has been described as real-time RT-PCR or quantitative RT-PCR and is sometimes abbreviated qRT-PCR, rRT-PCR, or RT-qPCR, although sometimes RT-PCR or PCR are used. The Minimum Information for Publication of Quantitative Real-

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Time PCR Experiments (MIQE) guidelines propose the term RT-qPCR, but not all authors adhere to this.

Samples can be obtained by various methods, including a nasopharyngeal swab, sputum (coughed up material), throat swabs, deep airway material collected via suction catheter or saliva. It has been remarked that for **2003** SARS, "from a diagnostic point of view, it is important to note that nasal and throat swabs seem less suitable for diagnosis, since these materials contain considerably less viral RNA than sputum, and the virus may escape detection if only these materials are tested." The likelihood of detecting the virus depends on collection method and how much time has passed since infection. Some have found that tests performed with throat swabs are reliable only in the first week. Thereafter the virus may abandon the throat and multiply in the lungs. In the second week, sputum or deep airways collection is preferred. Collecting saliva may be as effective as nasal and throat swabs, although this is not certain. Sampling saliva may reduce the risk for health care professionals by eliminating close physical interaction. It is also more comfortable for the patient. Quarantined people can collect their own samples. A saliva test's diagnostic value depends on sample site (deep throat, oral cavity, or salivary glands). One study found that saliva yielded greater sensitivity and consistency when compared with swab samples. On **15** August **2020**, the US FDA authorized a saliva test developed at Yale University, which gives results in hours. Viral burden measured in upper respiratory specimens declines after symptom onset.

Isothermal amplification assays

Isothermal nucleic acid amplification tests also amplify the virus's genome. They are

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faster than PCR because they don't involve repeated heating and cooling cycles. These tests typically detect DNA using fluorescent tags, which are read out with specialized machines. CRISPR gene editing technology was modified to perform the detection: if the CRISPR enzyme attaches to the sequence, it colors a paper strip. The researchers expect the resulting test to be cheap and easy to use in point-of-care settings. The test amplifies RNA directly, without the RNA-to-DNA conversion step of RT-PCR.

Antigens

An antigen is the part of a pathogen that elicits an immune response. Antigen tests look for antigen proteins from the viral surface. In the case of a coronavirus, these are usually proteins from the surface spikes.**[40]** One of the challenges is to find a target unique to SARS-CoV-**2**. Isothermal nucleic acid amplification tests can process only one sample at a time per machine. RT-PCR tests are accurate but require too much time, energy, and trained personnel to run the tests. Using these current methods, it is generally believed that there will never be the ability on a [PCR] test to do **300** million tests a day or to test everybody before they go to work or school.

Samples may be collected via nasopharyngeal swab, a swab of the anterior nares, or from saliva. The sample is then exposed to paper strips containing artificial antibodies designed to bind to coronavirus antigens. Antigens bind to the strips and give a visual readout. The process takes less than **30** minutes, can deliver results at point-of-care, and does not require expensive equipment or extensive training. Swabs of respiratory viruses often lack enough antigen material to be detectable. This is especially true for asymptomatic patients who have little if any nasal discharge. Viral proteins are not

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amplified in an antigen test. According to the World Health Organization (WHO) the sensitivity of similar antigen tests for respiratory diseases like the flu ranges between **34**% and **80**%. Based on this information, half or more of COVID-**19** infected patients might be missed by such tests, depending on the group of patients tested. While some doubt whether an antigen test can be useful against COVID-**19**, others have argued that antigen tests are highly sensitive when viral load is high and people are contagious, making them suitable for public health screening. Routine antigen tests can quickly identify when asymptomatic people are contagious, while follow-up PCR can be used if confirmatory diagnosis is needed.

Imaging

Typical visible features on chest CT initially include bilateral multilobar ground-glass opacities with a peripheral or posterior distribution. Subpleural dominance, crazy paving, and consolidation may develop as the disease evolves. Chest CT scans and chest x-rays are not recommended for diagnosing COVID-**19**. Radiologic findings in COVID-**19** lack specificity.

Antibody tests

The body responds to a viral infection by producing antibodies that help neutralize the virus. Blood tests (serology tests) can detect the presence of such antibodies. Antibody tests can be used to assess what fraction of a population has once been infected, which can then be used to calculate the disease's mortality rate. SARS-CoV-**2** antibodies' potency and protective period have not been established. Therefore, a positive

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antibody test may not imply immunity to a future infection. Further, whether mild or asymptomatic infections produce sufficient antibodies for a test to detect has not been established. Antibodies for some diseases persist in the bloodstream for many years, while others fade away. The most notable antibody classes are IgM and IgG. IgM antibodies are generally detectable several days after initial infection, although levels over the course of infection and beyond are not well characterized. IgG antibodies generally become detectable **10–14** days after infection and normally peak around **28** days after infection. Genetic tests verify infection earlier than antibody tests. Only **30**% of those with a positive genetic test produced a positive antibody test on day **7** of their infection.

Types of Tests

Rapid diagnostic test (RDT)

RDTs typically use a small, portable, positive/negative lateral flow assay that can be executed at point-of-care. RDTs may process blood samples, saliva samples, or nasal swab fluids. RDTs produce colored lines to indicate positive or negative results.

Enzyme-linked immunosorbent assay (ELISA)

ELISAs can be qualitative or quantitative and generally require a lab. These tests usually use whole blood, plasma, or serum samples. A plate is coated with a viral protein, such as a SARS-CoV-**2** spike protein. Samples are incubated with the protein, allowing any antibodies to bind to it. The antibody-protein complex can then be detected with another wash of antibodies that produce a color/fluorescent readout.

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Neutralization assay

Neutralization assays assess whether sample antibodies prevent viral infection in test cells. These tests sample blood, plasma, or serum. The test cultures cells that allow viral reproduction (e.g., VeroE**6** cells). By varying antibody concentrations, researchers can visualize and quantify how many test antibodies block virus replication.

Chemiluminescent immunoassay

Chemiluminescent immunoassays are quantitative lab tests. They sample blood, plasma, or serum. Samples are mixed with a known viral protein, buffer reagents and specific, enzyme-labeled antibodies. The result is luminescent. A chemiluminescent microparticle immunoassay uses magnetic, protein-coated microparticles. Antibodies react to the viral protein, forming a complex. Secondary enzyme-labeled antibodies are added and bind to these complexes. The resulting chemical reaction produces light. The radiance is used to calculate the number of antibodies. This test can identify multiple types of antibodies, including IgG, IgM, and IgA.

Neutralizing vs. binding antibodies

Most, if not all, large scale COVID-**19** antibody testing looks for binding antibodies only and does not measure the more important neutralizing antibodies (NAb). A NAb is an antibody that defends a cell from an infectious particle by neutralizing its biological effects. Neutralization renders the particle no longer infectious or pathogenic. A binding antibody binds to the pathogen but the pathogen remains infective; the purpose can be to flag the pathogen for destruction by the immune system. It may even enhance

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infectivity by interacting with receptors on macrophages. Since most COVID-**19** antibody tests return a positive result if they find only binding antibodies, these tests cannot indicate that the subject has generated protective NAbs that protect against re-infection.

It is expected that binding antibodies imply the presence of NAbs and for many viral diseases total antibody responses correlate somewhat with NAb responses, but this is not established for COVID-**19**. A study of **175** recovered patients in China who experienced mild symptoms reported that **10** individuals had no detectable NAbs at discharge, or thereafter. How these patients recovered without the help of NAbs and whether they were at risk of re-infection was not addressed. An additional source of uncertainty is that even if NAbs are present, viruses such as HIV can evade NAb responses. Studies have indicated that NAbs to the original SARS virus (the predecessor to the current SARS-CoV-**2**) can remain active for two years and are gone after six years. Nevertheless, memory cells including Memory B cells and Memory T cells can last much longer and may have the ability to reduce reinfection severity.

Other tests

Following recovery, many patients no longer have detectable viral RNA in upper respiratory specimens. Among those who do, RNA concentrations three days following recovery are generally below the range in which replication-competent virus has been reliably isolated. No clear correlation has been described between length of illness and duration of post-recovery shedding of viral RNA in upper respiratory specimens.

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Infectivity

Infectivity is indicated by the basic reproduction number (R**0**, pronounced "R naught") of the disease. SARS-CoV-**2** is estimated to have an R**0** of **2.2** to **2.5**. This means that in a population where all individuals are susceptible to infection, each infected person is expected to infect **2.2** to **2.5** others in the absence of interventions. R**0** can vary according factors such as geography, population demographics and density. In New York state R**0** was estimated to be **3.4** to **3.8** during its epidemic. On average, an infected person begins showing symptoms five days after infection (the "incubation period") and can infect others beginning two to three days before that. One study reported that **44**% of viral transmissions occur within this period. According to CDC, a significant number of infected people who never show symptoms are nevertheless contagious. In vitro studies have not found replication-competent virus after **9** days from infection. The statistically estimated likelihood of recovering replication-competent virus approaches zero by **10** days. Infectious virus has not been cultured from urine or reliably cultured from feces; these potential sources pose minimal if any risk of transmitting infection and any risk can be sufficiently mitigated by good hand hygiene.

Patterns and duration of illness and infectivity have not been fully described. However, available data indicate that SARS-CoV-**2** RNA shedding in upper respiratory specimens declines after symptom onset. At **10** days recovery of replication-competent virus in viral culture (as a proxy of the presence of infectious virus) approaches zero. Although patients may produce PCR-positive specimens for up to six weeks, it remains unknown whether these samples hold infectious virus. After clinical recovery, many patients do not continue to shed. Among recovered patients with detectable RNA in upper

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respiratory specimens, concentrations after three days are generally below levels where virus has been reliably cultured. These data were generated from adults across a variety of age groups and with varying severity of illness. Data from children and infants were not available.

Nucleic acid tests

Tests developed in China, France, Germany, Hong Kong, Japan, the United Kingdom, and the US targeted different parts of the viral genome. WHO adopted the German system for manufacturing kits sent to low-income countries without the resources to develop their own tests.

Abbott Laboratories' ID Now nucleic acid test uses isothermal amplification technology. The assay amplifies a unique region of the virus's RdRp gene; the resulting copies are then detected with "fluorescently-labeled molecular beacons". The test kit uses the company's "toaster-size" ID Now device, which is widely deployed in the US. The device can be used in laboratories or in point-of-care settings and provides results in **13** minutes or less.

Primerdesign offers its Genesig Real-Time PCR Coronavirus (COVID-**19**). Cobas SARS-CoV-**2** Qualitative assay runs on the Cobas® **6800/8800** Systems by Roche Molecular Systems. They are offered by the United Nations and other procurement agencies.

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Antigen tests

Quidel's "Sofia **2** SARS Antigen FIA"**[160][46]** is a lateral flow test that uses monoclonal antibodies to detect the virus's nucleocapsid (N) protein. The result is read out by the company's Sofia **2** device using immunofluorescence. The test is simpler and cheaper but less accurate than nucleic acid tests. It can be deployed in laboratories or at point-of-care and gives results in **15** minutes. A false negative result occurs if the sample's antigen level is positive but below the test's detection limit, requiring confirmation with a nucleic acid test.

Serology (antibody) tests

Antibodies are usually detectable **14** days after the onset of the infection. Multiple jurisdictions survey their populations using these tests. The test requires a blood draw. Private US labs including Quest Diagnostics and LabCorp offer antibody testing upon request. Antibody tests are available in various European countries. Quotient Limited developed a CE marked COVID-**19** antibody test. Roche offers a selective ELISA serology test.

Sensitivity and specificity

Sensitivity indicates whether the test accurately identifies whether the virus is present. Each test requires a minimum level of viral load in order to produce a positive result. A **90**% sensitive test will correctly identify **90**% of infections, missing the other **10**% (a false negative). Even relatively high sensitivity rates can produce high rates of false negatives in populations with low incidence rates.

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Specificity indicates how well-targeted the test is to the virus in question. Highly specific tests pick up only the virus in question. Non-selective tests pick up other viruses as well. A **90**% specific test will correctly identify **90**% of those who are uninfected, leaving **10**% with a false positive result. Low-specificity tests have a low positive predictive value (PPV) when prevalence is low. For example, suppose incidence is **5**%. **100** people selected at random would contain **95** people who are negative and **5** people who are positive. Using a test that has a specificity of **95**% would yield on average **4.75** people who are actually negative who would incorrectly test positive. If the test has a sensitivity of **100**%, all five positive people would also test positive, totaling **9.75** positive results. Thus, the PPV is **51.3**%, an outcome comparable to a coin toss. In this situation retesting those with a positive result increases the PPV to **95.5**%, meaning that only **4.5**% of the second tests would return the incorrect result, on average less than **1** incorrect result.

Causes of test error

Improper sample collection is exemplified by failure to acquire enough sample and failure to insert a swab deep into the nose. This results in insufficient viral load, one cause of low clinical sensitivity. The time course of infection also affects accuracy. Samples may be collected before the virus has had a chance to establish itself or after the body has stopped its progress and begun to eliminate it. Improper storage for too long a time can cause RNA breakdown and lead to wrong results as viral particles disintegrate. Improper design and manufacturing can yield inaccurate results. Millions of tests made in China were rejected by various countries throughout the period of

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March **2020** through May **2020**. Test makers typically report the accuracy levels of their tests when seeking approval from authorities. In some jurisdictions, these results are cross-validated by additional assessments. Reported results may not be achieved in clinical settings due to such operational inconsistencies.

PCR-based test

RT-PCR is the most accurate diagnostic test. It typically has high sensitivity and specificity in a laboratory setting: however, in one study sensitivity dropped to **66**-**88**% clinically. In one study sensitivity was highest at week one (**100**%), followed by **89**.**3**%, **66**.**1**%, **32**.**1**%, **5**.**4**% and zero by week six. A Dutch CDC-led laboratory investigation compared **7** PCR kits. Test kits made by BGI, R-Biopharm AG, BGI, KH Medical and Seegene showed high sensitivity. High sensitivity kits are recommended to assess people without symptoms, while lower sensitivity tests are adequate when diagnosing symptomatic patients.

Isothermal nucleic amplification test

One study reported that the ID Now COVID-**19** test showed sensitivity of **85**.**2**%. Abbott responded that the issue could have been caused by analysis delays. Another study rejected the test in their clinical setting because of this low sensitivity.

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What is needed is an apparatus and method for point-of-care, rapid, field-deployable diagnostic testing of Covid-**19**, viruses, antibodies, and markers, which can be used by unskilled health workers, which is sensitive and specific, and which gives diagnostic results in **30** minutes or less with highly developed diagnostic data processing in the Cloud.

Brief Summary

In one embodiment, there is provided an automated system operable to communicate with a remote server for diagnostically field testing a sample taken from a subject using an automated portable handheld instrument to determine the presence of antibodies against at least one of viral antigens in the sample. The system comprises one or more types of microfluidic circuits defined in a rotatable disk, each type of microfluidic circuit for performing a bioassay using an immunofluorescence microarray based on immunofluorescence to generate an electrical signal indicative of a bioassay measurement, wherein the immunofluorescence microarray is operationally positioned in at least one of the microfluidic circuits and wherein the rotatable disk further comprises at least one positionable valve in at least one of the microfluidic circuits. The system further includes a backbone unit for rotating the rotatable disk according to a predetermined protocol to perform the bioassay wherein the backbone unit is configured to operate the immunofluorescence microarray in order to generate the electrical signal indicative of the bioassay measurement, communicate the bioassay measurement to the remote server, and to associate the performed bioassay and its corresponding bioassay measurement to the subject. The backbone unit comprises at least one laser

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configured to ablate the at least one positionable valve, a scanner disposed on the external portion of the backbone unit to scan a barcode on the disk to associate the disk with the bioassay measurement, and a camera configured to capture a digital image of the bioassay measurement, wherein the digital image includes microarray antigen spots and wherein the electrical signal indicative of the bioassay measurement includes a representation of the digital image.

The bioassay may be a serology test, testing for at least one of (a) immunoglobulin-G (IgG); and (b) immunoglobulin-M (IgM).

The bioassay may be at least one of (a) a respiratory antibody test and (b) an antigen test.

The serology test may test for Covid-**19**.

The rotatable disk may have a center and may comprise: a sample inlet; a blood-plasma separation chamber in communication with the sample inlet and positioned on the rotatable disk radially farther from the center of the rotatable disk than the sample inlet; a mixing chamber in communication with the blood-plasma separation chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the blood-plasma separation chamber; a first wash chamber in communication with to the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; an antibody chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; a second wash chamber in communication with

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the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; a microarray chamber in communication with the mixing chamber, the immunofluorescence microarray being disposed in the microarray chamber; and the microarray chamber positioned on the rotatable disk radially farther from the center of the rotatable disk than the mixing chamber; and a waste chamber in communication with the microarray chamber by a siphon and by a corresponding selectively openable spin-dry valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the microarray chamber.

The digital image of the bioassay measurement may include a digital image of at least one microarray antigen spot which has been fluorescently labeled by a secondary antibody binding to subject antibodies from the sample. The remote server may be a Cloud server. The backbone unit may include network circuitry which communicates the digital image to the Cloud server and a corresponding schema file associating the subject to the performed bioassay and its corresponding bioassay measurement. The Cloud server, operating in an automated and modular protocol, may align the at least one microarray antigen spot of the digital image, detect the aligned at least one microarray antigen spot of the immunofluorescence microarray and analyze each of the aligned at least one microarray antigen spots of the digital image to assign a scalar value to the at least one microarray antigen spot to produce a processed microarray measurement set of data. The Cloud server, operating in an automated protocol, may analyze the processed microarray measurement set of data to produce a diagnosis of

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the bioassay measurement. The Cloud server, operating in an automated protocol, may report the results to the subject as determined by the schema file.

The Cloud server may include a cloud-based module for automatically determining under automated control whether corresponding Z-scores of the processed microarray measurement set of data are of at least one of positive and negative indications are indicative of Covid-**19** rather than Z-scores of the plurality of viral infections sharing the at least one of Covid-**19** antigens and antibodies.

The Cloud server may include means for identifying a least one of positive and negative indications of the digital image of the at least one microarray antigen spot for a plurality of acute respiratory infections selected from the group including SARS-CoV-**2**, SARS-CoV, MERS-CoV, common cold coronaviruses, and multiple subtypes of influenza, adenovirus, metapneumovirus, parainfluenza, and respiratory syncytial virus.

The Cloud server may include a cloud-based module for automatically evaluating antigens to discriminate output data of a positive group of antigens from a negative group of antigens across a range of assay cutoff values using receiver-operating-characteristic (ROC) curves for which an area-under curve (AUC) is measured to determine high performing antigens to diagnose Covid-**19**.

The Cloud server may include a cloud-based module for automatically determining under automated control an optimal sensitivity and specificity for Covid-**19** from a combination of a plurality of high performing antigens based on a corresponding Youden Index calculated for the combination of the plurality of high-performing antigens.

In another embodiment, there is provided an automated system operable to communicate with a cloud-based server for diagnostically field testing a sample taken

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from a subject using an automated portable handheld instrument to determine the presence of antibodies against at least one viral antigen thereto in a serology test to detect Covid-**19**. The system includes: a microfluidic circuit defined in a rotatable disk for performing a bioassay using an immunofluorescence microassay based on immunofluorescence to generate a digital image indicative of a bioassay measurement; and a backbone unit for rotating the rotatable disk according to a predetermined protocol to perform the bioassay, wherein the backbone unit is configured to operate the immunofluorescence microarray to generate the digital image indicative of a bioassay measurement, communicate the digital image to the cloud-based server, and to associate the performed bioassay and its corresponding bioassay measurement to the subject. The rotatable disk has a center and comprises: a sample inlet; at least one positionable valve in the microfluidic circuit; a blood-plasma separation chamber in communication with the sample inlet and positioned on the rotatable disk radially farther from the center of the rotatable disk than the sample inlet; a mixing chamber in communication with the blood-plasma separation chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the blood-plasma separation chamber; a first wash chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; an antibody chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; a second wash chamber in communication with the

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mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; a microarray chamber in communication with the mixing chamber, the immunofluorescence microarray being disposed in the microarray chamber, and the microarray chamber positioned on the rotatable disk radially farther from the center of the rotatable disk than the mixing chamber; and a waste chamber in communication with the microarray chamber by a siphon and by a corresponding selectively openable spin-dry valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the microarray chamber. The backbone unit includes network circuitry which communicates the digital image to the Cloud server and a corresponding schema file associating the subject to the performed bioassay and its corresponding bioassay measurement, at least one laser configured to ablate the at least one positionable valve; a scanner disposed on the external portion of the backbone unit; and a camera configured to capture the digital image indicative of the bioassay measurement. The Cloud server, operating in an automated and modular protocol, may align at least one microarray antigen spot of the digital image, detect the aligned at least one microarray antigen spot of the immunofluorescence microarray and analyze the aligned at least one microarray antigen spot of the digital image to assign a scalar value to the at least one microarray antigen spot to produce a processed microarray measurement set of data. The Cloud server, operating in an automated protocol, may analyze the processed microarray measurement set of data to produce a diagnosis of the bioassay measurement. The Cloud server, operating in an automated protocol, may report the results to the subject as determined by the schema file.

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The Cloud server may include a cloud-based module for automatically determining under automated control whether corresponding Z-scores of the processed microarray measurement set of data of at least one of (a) positive and (b) negative indications are indicative of Covid-**19** rather than Z-scores of the plurality of viral infections sharing atat least one of Covid-**19** antigens and antibodies.

The Cloud server may include means for identifying at least one of positive and negative indications of the digital image of microarray antigen spots for a plurality of acute respiratory infections selected from the group including SARS-CoV-**2**, SARS-CoV, MERS-CoV, common cold coronaviruses, and multiple subtypes of influenza, adenovirus, metapneumovirus, parainfluenza, and respiratory syncytial virus.

The Cloud server may include a cloud-based module for automatically evaluating antigens to discriminate output data of a positive group of antigens from a negative group of antigens across a range of assay cutoff values using receiver-operating-characteristic (ROC) curves for which an area-under curve (AUC) is measured to determine high performing antigens to diagnose Covid-**19**.

The Cloud server may include a cloud-based module for automatically determining under automated control an optimal sensitivity and specificity for Covid-**19** from a combination of a plurality of high performing antigens based on a corresponding Youden Index calculated for the combination of the plurality of high-performing antigens.

In another embodiment, there is provided a method for operating the automated system described above for diagnostically field testing a sample taken from a subject using an automated portable handheld instrument to determine the presence of at least one viral antigen in the sample. The method involves introducing the sample into a sample inlet

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disposed on the rotatable disk; scanning the rotatable disk with a scanner disposed in a backbone unit and associating the rotatable disk with the subject; inserting the rotatable disk into the backbone unit; transferring the sample to a blood-plasma separation chamber in communication with the sample inlet and positioned on the rotatable disk radially farther from the center of the rotatable disk than the sample inlet; separating the blood from the plasma by spinning the rotatable disk at a first rotational speed for a first period of time; opening a first valve including a laser-meltable plug using at least one laser disposed in the backbone unit, the first valve being disposed in a conduit in the rotatable disk between the blood-plasma chamber and a mixing chamber in communication with the blood-plasma separation chamber through the selectively openable first valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the blood-plasma separation chamber; transferring the sample to the mixing chamber and to a microarray chamber in communication with the mixing chamber, an immunofluorescence microarray being disposed in the microarray chamber; and the microarray chamber positioned on the rotatable disk radially farther from the center of the rotatable disk than the mixing chamber; reciprocating the sample in the microarray chamber for a plurality of cycles within a first rotational speed range, followed by priming the chamber at a second rotational speed and evacuating the chamber at a third rotational speed for the first period of time to a waste chamber in communication with the microarray chamber by a siphon and by a corresponding selectively openable spin-dry valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the microarray chamber; opening a second valve including a laser-meltable plug using the at least one laser disposed in the

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backbone unit, the second valve being disposed in a conduit in the rotatable disk between the mixing chamber and a first wash chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; transferring a first wash from the first wash chamber through the mixing chamber to the microarray chamber; reciprocating the first wash in the microarray chamber for a plurality of cycles within the first rotational speed range, followed by priming the chamber at the second rotational speed and evacuating the chamber at the third rotational speed for a second period of time to the waste chamber; opening a third valve including a laser-meltable plug using the at least one laser disposed in the backbone unit, the third valve being disposed in a conduit in the rotatable disk between the mixing chamber and an antibody chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; transferring the secondary antibody from the antibody chamber through the mixing chamber to the microarray chamber; reciprocating the secondary antibody in the microarray chamber for a plurality of cycles within the first rotational speed range, followed by priming the chamber at the second rotational speed and evacuating the chamber at the third rotational speed for the second period of time to the waste chamber; opening a fourth valve including a laser-meltable plug using the at least one laser disposed in the backbone unit, the fourth valve being disposed in a conduit in the rotatable disk between the mixing chamber and a second wash chamber in communication with the mixing chamber through a corresponding selectively openable

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valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber; transferring a second wash from the second wash chamber through the mixing chamber to the microarray chamber; reciprocating the second wash in the microarray chamber for a plurality of cycles within the first rotational speed range, followed by priming the chamber at the second rotational speed and evacuating the chamber at the third rotational speed for the second period of time to the waste chamber; opening a fifth valve including a laser-meltable plug using the at least one laser disposed in the backbone unit, the fifth valve being disposed in a conduit in the rotatable disk between the microarray chamber and the waste chamber; spin drying the microarray chamber by spinning the rotatable disk at **5500**rpm for one minute; moving the microarray chamber to a position wherein a digital image can be taken of the immunofluorescence microarray; and generating the digital image of the immunofluorescence microarray with a camera disposed in the backbone unit, the digital image including depictions of microarray antigen spots.

The method may further involve: communicating the digital image using the backbone unit including network circuitry which communicates the digital image to a Cloud server and communicates a corresponding schema file associating the subject to the performed bioassay and its corresponding bioassay measurement; aligning the microarray antigen spots of the digital image in the Cloud server, operating in an automated and modular protocol; detecting each of the aligned microarray antigen spots of the immunofluorescence microarray in the Cloud server, operating in an automated and modular protocol; analyzing each of the microarray antigen spots of the digital image in the Cloud server, operating in an automated and modular protocol to assign a

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scalar value to each microarray antigen spot to produce a processed microarray measurement set of data; analyzing the processed microarray measurement set of data to produce a diagnosis of the bioassay measurement in the Cloud server, operating in an automated protocol; and reporting the results to the subject as determined by the schema file using the Cloud server, operating in an automated protocol.

The step of analyzing the processed microarray measurement set of data may involve identifying at least one of positive and negative indications of the digital image of microarray antigen spots for a plurality of acute respiratory infections selected from the group including SARS-CoV-**2**, SARS-CoV, MERS-CoV, common cold coronaviruses, and multiple subtypes of influenza, adenovirus, metapneumovirus, parainfluenza, and respiratory syncytial virus.

Reciprocating the sample in the microarray chamber for a plurality of cycles within a first rotational speed range may involve reciprocating the sample at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the first period of time comprises evacuating the chamber at **1000**rpm for **5** minutes.

Reciprocating the first wash in the microarray chamber for a plurality of cycles within the first rotational speed range may involve reciprocating the first wash at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the first period of time comprises evacuating the chamber at **1000**rpm for **2** minutes.

Reciprocating the secondary antibody in the microarray chamber for a plurality of cycles within the first rotational speed range may involve reciprocating the secondary antibody

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at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the second period of time comprises evacuating the chamber at **1000**rpm for **2** minutes.

Reciprocating the second wash in the microarray chamber for a plurality of cycles within the first rotational speed range comprises reciprocating the second wash at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the second period of time comprises evacuating the chamber at **1000**rpm for **2** minutes.

The disclosure can be better understood by turning now to the following drawings wherein like elements are referenced by like numerals.

Brief Description of the Drawings

Fig. **1** is a front three-quarter perspective of the backbone unit.

Fig. **2** is the view of Fig. **1** with the disk lid opened.

Fig. **3** is an end plan view of the backbone unit showing the external connections.

Fig. **4** is a front three-quarter perspective of the backbone unit with the cover removed showing the major components included in the backbone unit.

Fig. **5** is a block diagram of the circuitry and elements in the backbone unit.

Fig. **6** is a diagram of an LED ring board to provide even IR activation illumination to the sample in the microarray chamber.

Fig. **7** is a top plan view of the microfluidic disk carrying a microarray.

Fig. **8** is a flow diagram of the operation of the disk of Fig. **7**.

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Fig. **9** is flow diagram of the workflow implemented by the backbone unit.

Fig. **10** is a block diagram of the software architecture used to implement the workflow of Fig. **9**.

Fig. **11** is a screenshot of the operator interface.

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Fig. **12** is a screenshot of the operator interface when Scan QR Code is chosen in Fig. **11**.

Fig. **13** is a block diagram of the backend operation architecture of the software operating in the backbone unit.

Fig. **14** is a flow diagram of the digital image analysis performed by the Cloud server.

Fig. **15** is a block diagram of the software organization used to implement the flow diagram of Fig. **14**.

Figs. **16**a and **16**b are graphs showing the IgG seroreactivity as measured by means of the fluorescence intensity of serum specimens on the coronavirus antigen microarray. Fig. **16**a is a graph of fluorescence values from antigen spots specific to a plurality of viruses, shown side by side. Fig. **16**b is an enlargement of SARS-CoV-**2**, SARS-CoV and MERS-CoV antigen spots.

Fig. **17** is a graph of normalized IgG reactivity of positive and negative sera on coronavirus antigen microarray. The plot shows IgG reactivity against each antigen measured as mean fluorescence intensity (MFI) with full range (bars) and interquartile range (boxes) for convalescent sera from PCR-positive individuals (positive, red) and sera from nine individuals prior to pandemic (negative, blue). Below the plot, the heatmap shows average reactivity for each group (white::: low, black::: mid, red::: high). The antigen labels are color coded for respiratory virus group.

Figs. **18**a -**18**d are graphs of an individual patient's fluorescence values for IgG and IgM detection in a sample using a microarray, and the corresponding Z-score statistics. The red line is an average positive result used to assess whether a measure is positive. The blue line is an average of negative results. The red corresponds to an average

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seropositive result which is additionally confirmed via PCR. The blue line corresponds to an average seronegative result which is confirmed via PCR. If a patient's IgG bar graph looks like the red line, they test positive, if it looks like the blue line, they test negative.

Fig. **18**a is a graph of the fluorescence values for IgG for several viruses, namely SARS-CoV**2**, SARS, MERS, CommonCoV, Influenza, ADV, MPV, PIV and RSV as a function of the antigen spots on the microarray as seen as listed on the x-axis in Fig. **18**c.

Fig. **18**b is a graph of the fluorescence values for IgM for several viruses, namely SARS-CoV**2**, SARS, MERS, CommonCoV, Influenza, ADV, MPV, PIV and RSV as a function of the antigen spots on the microarray as seen as listed on the x-axis in Fig. **18**d.

Fig. **18**c is a bar graph of the Z-score statistics of the IgG readings for several viruses, namely SARS-CoV**2**, SARS, MERS, CommonCoV, Influenza, ADV, MPV, PIV and RSV as a function of the antigen spots on the microarray as listed on the x-axis.

Fig. **18**d is a bar graph of the Z -score statistics of the IgM readings for several viruses, namely SARS-CoV**2**, SARS, MERS, CommonCoV, Influenza, ADV, MPV, PIV and RSV as a function of the antigen spots on the microarray as listed on the x-axis.

Fig. **19** is a diagrammatic depiction of the microarray of the embodiment for testing for Covid-**19**.

Fig. **20** is a bar graph which reports the value of each control spot, and mean value and standard deviation for each antigen.

Figs. **21**a- **21**d is a diagram of the user flow or interaction with the system.

Fig. **22** is a tree graph of the data chain identification used to maintain data accountability for the tests and all involved components.

Fig. **22**a is a diagram of an image of the microarray where after potential fiducials

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are identified, the program compares the distance ratios between all sets (combinations) of three contours, looking for ratios that match the theoretical fiducial spacing ratios given in the schema file identified in Fig. **22**a as dashed circles.

Fig. **22**b is a diagram of an image of the microarray where after determining the fiducials, a minimum fit rectangle is drawn around the three fiducials identified in Fig. **22**b as a dashed rectangle.

Fig. **22**c is a diagram of an image of the microarray where the minimum fit rectangle is then cropped and rotated so that the fiducials are located in the top left, bottom left, and top right as depicted in Fig. **22**c.

Fig. **23** is a diagram of an image of a spot of the microarray the foreground median intensity is calculated and subtracted from the background mean intensity. Each spot is individually masked, and the median of each spot is calculated. Likewise, the mean of each background annulus is calculated and subtracted from the spot median as depicted in Fig. **23**.

The disclosure and its various embodiments can now be better understood by turning to the following detailed description of the preferred embodiments which are presented as illustrated examples of the concepts described herein. It is expressly understood that the concepts described herein may be broader than the illustrated embodiments described below.

Detailed Description of the Preferred Embodiments

The apparatus of the illustrated embodiments include a backbone unit which includes the electronics, camera, optics, digital data gathering and communication via the

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internet to Cloud-based expert diagnostic servers, and electromechanical elements needed to provide field portable diagnostic testing of Covid-**19** and other viral or bacterial infections. The same backbone unit supports at least three different microfluidic compact disks **68** (CDs) used for diagnostic assays or testing, namely for virology detection using a surface acoustical wave (SAW) detector, for microarray serology detectors for antibodies like IgG and IgM, and RT-PCR assays for nucleic acid targets using fluorescence detectors, which are denoted by Autonomous Medical Devices Inc. as its A**10**, A**20** and A**30** CD's respectively. The unit and its corresponding CDs are measurement or assay devices and do not perform high level diagnosis analysis, but provide the data needed to do so to fully developed diagnostic databases and expert systems resident in the Cloud in internet communication with the backbone unit.

The Backbone Unit

The backbone unit **10** shown in Fig. **1** is a desktop rectangular chassis with a color touch screen **12** on its top surface with a closeable lid **14** under which the microfluidic compact disks **68** (CDs) are placed on a spindle **16** shown in Fig. **2** for operation. More will be described below about the corresponding microfluidic disks **68**. As shown in Fig. **3** one end of unit **10** is provided with a plurality of data and power connectors, such as external AC power receptacle **24**, power switch **22**, external USB port **20** and external Ethernet port **18**. The primary electrical circuits, digital circuits, photonic elements, and electromechanical elements are depicted in perspective view of Fig. **4** which shows the interior layout of unit **10**. Included among the pictured elements are CD motor **26**

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which spins the CDs, a CD index **28**, a camera illumination subsystem **30**, a camera **32**, a power supply **34** with cooling fan **38**, a motor controller **36** and a control board **40**. To the side of motor **26** is a laser **48** used in CD operations, e.g. for opening selected valves in the CD. Also included is a CPU or Raspberry Pi **42**, an electrical fuse **44** and a second cooling fan **46**. On one of the long sides of unit **10**, a quick response (QR) scanner (not shown) is also provided wherein patient information is integrated into the data output.

The operation of unit **10** is now better understood by referring to the block diagram of Fig. **5** which shows the supporting circuitry and photonics. A photonics control board **40**, a CPU board **42** which is supported and coupled to power supply **34**, provides a plurality of DC voltages (e.g. **5** and **24** VDC) and ground connections. CPU board **42** carries a raspberry pi **43** CPU, which is the main control circuit for unit **10** and handles all high-level programming commands, communications, and data handling. CPU **41** on photonics board **40** is a state machine and provides the needed drive and command signals to motor **26** and various LEDs **56** and lasers **48**. CPU **41** controls the speed and rotation provided by motor **26** through direction-enable-break motor commands communicated to translated to brushless motor driver **52**. Driver **52** also communicates a tachometer signal, TACHO to CPU **41**, and receives a reference signal, VREF, from OpAMP **53** which in turn communicates to CPU **41** through an onboard digital-to-analog converter.

CPU **43** is an ARM-based (Advanced RISC machine) processor with a Linux operating system. CPU **43** is coupled to and drives camera **32** and provides raw image processing though a USB link to generate a transmissible digital data image through

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a wireless module ultimately to Cloud **134**. CPU **43** is associated with a fan **55**, clock **35**, RAM memory **37** and an eMMC (embedded multimedia controller) flash memory **39**, a micro-secure digital memory card (SD) **61**, an audio amplifier **65** with headphone speakers **63**, power management circuit **71** and a power connector **69**. Memory card **61** is used to capture copies of the test results that are additionally transmitted on the cloud **134**. The audio amplifier **65** is to be used with the speaker **63** which will transmit the health or status of the device to the user (test status, errors, etc). CPU **43** is coupled to display **12**, both through HDMI and USB connections. Display **12** optionally drives a pair of stereo speakers **13** for communication to the user. CPU **43** is optionally communicated through a seven port USB hub **91** with a **6**-degrees of freedom inertial measurement unit (IMU) **93**, microphone **95**, global navigation satellite system (GNSS) **97** with antenna, mouse/keyboard **99**, barcode reader **89** allowing for location tracking, handing history, and user interaction and developer programming in the field.

A microcontroller with CPU **41** with its memory **43** and external oscillator/clock **43** in photonics board **40** is coupled to CPU **42** and provides the controls for motor **26** according to the protocol shown in the flow diagram of Fig. **7** and various LEDs, lasers and sensors operationally associated with the RT-PCR process performed on disk **68**. Using an on-chip digital-to-analog converter CPU **41** is coupled through an operational amplifier **53** to the reference and tachometer input/outputs of driver **52** as well as directly providing directional, enable and break motor commands to driver **52**. CPU **41** commands brushless motor driver **52** to drive spindle or CD motor **26**. Motor **26** includes an encoder whose signal is feedback to CPU **41** so that its speed, and direction of rotation is controlled in a closed loop servo mode. Driver **52** supplies **3**-phase

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driving signals to motor **26**, which includes a Hall Effect sensor returning an rpm feedback signal to buffer **52** indicative of the motor rpm. CPU **41** is also coupled to an encoder feedback signal from motor **26**.

As shown in Fig. **5** photonic control board **40** is coupled to power supply **34** and includes a voltage boost circuit **80** increasing the **5**V supply to **6**V and a low dropout regulator (LDO) **82** (**3.3**V, **1**A). CPU **41** is clocked by oscillator **43**, and includes memory **45**, a temperature/humidity sensor **47**, an in circuit serial programming (*ICSP*) and debug interface **49**, a source of a reference voltage VREF coupled to CPU **41** through an onboard analog-to-digital converter and a limit switch **51** built into unit **10**'s lid so that the motor **26** and all other photonics is shut down whenever the lid is lifted.

The operation of photonics board **40** with respect to disk **68** can now be understood. The movement and position of disk **68** is tracked by a disk mounted magnet **66** sensed by magnetic and optical index driver **64** coupled to CPU **41** by which the angular orientation or position of disk **68** is determined. The test sample is disposed into sample inlet **94** of Fig. **7** in step **93** of Fig. **8**. The treated sample is transferred at step **95** to a blood-plasma separation chamber **72**, served by sample inlet **94**. After separation as described below in connection with Figs. **7** and **8**, the separated plasma is transferred to receiving chamber **98** and then to microarray chamber **74** in which microarray **92** is disposed, where it is activated by **593**nm LEDs **268** on an LED ring board **269** shown in Fig. **6**, which are driven by LED driver **86** coupled to CPU **41**. In the embodiment of Figs. **5** and **6**, **10** LEDs, each operating at **593**nm are providing in a ring surrounding the detection chamber **209** to provide a field of substantially even IR illumination to activate the fluorescent readout. Camera **32** takes a digital image of the fluorescently tagged

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sample through low pass filter **76** and lens **78**, which image is communicated to CPU **42** from which it is transmitted to Cloud **134**. The prepared sample can then be disposed in waste chamber **114**. The bio-readout is the data the camera **32** captures fluoroscopically activated microarray **92**. The fluorescence intensity corresponds to the concentration of the sample. The camera **32** detects the fluorescence of the microarray **92**. Camera **32** is focused on the microarray chamber **72** through lens **76** and a low pass filter **78** for fluorescence imaging. An LED driver **86** is included in photonic controller **40** which drives the **593**nm LEDs to activate the fluorescence of the tags in chamber **74**.

A**20** – Disk Operation

Before discussing diagnostic methods for Covid-**19** on a microarray, turn now and consider the general operation of disk **68** when a microarray detector **92** is employed as depicted in the top plan view of Fig. **7**. The elements described below on disk **68**, which has a diameter of **70** mm and **4.5** mm thickness, are provided in duplicate to allow either redundant measurements to be made or two separate antibody tests using different microarrays **92** to be run simultaneously on the same patient. Disk **68** is made of clear plastic and has multiple chambers, channels and valves numerically machined therein as described in detail in the following. Disk **68** may be sealed on its top and bottom surfaces by a thin laminate layer of plastic. The method begins at step **193** with insertion of the sample taken from the patient at the point-of-care into the sample inlet **94**. Disk **68** is spun at **5500**rpm for **1** min at step **193** as depicted in the flow diagram of Fig. **8** to drive the sample into a blood- plasma separation chamber **72** where the

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centrifuging action separates the heavier blood constituents from the plasma. A first laser valve **62** is opened by positioning disk **68** so that laser valve **96**, which is a meltable plug, is aligned with an underlying laser **48** in unit **10**. The laser **48** is fired and valve **96** is opened and in about **0.5** min the plasma or serum flows from separation chamber **72** through receiving chamber **98** to microarray chamber **74** wherein microarray **92** is disposed. The transferred serum is reciprocated in microarray chamber **74** to react with the antibody dots of microarray **92** for about **5** min at step **197** for **40** cycles at **2700-5428** rpm followed by priming chamber **74** at **170**rpm and then evacuating chamber **74** by rotation at 1**000**rpm through the primed siphon **93** into waste chamber **114**.

At **199** laser valve **106** is aligned with a laser **48** in unit **10** and opened with a **0.5**min exposure. Thereafter, a wash buffer **#1** stored in chamber **100** is transferred to microarray chamber **74** by reciprocation at step **201** for about **5** min at step **197** for **20** cycles at **2700-5428** rpm followed by priming chamber **100** at **170**rpm and then evacuating chamber **74** by rotation at **1000**rpm for about **2**min.

At step**203** laser valve **108** is aligned with a laser **48** in unit **10** and opened with a **0.5**min exposure. A secondary antibody stored in chamber **102** is transferred to microarray chamber **74** by reciprocation for about **5** min for **20** cycles at **2700-5428** rpm followed by priming chamber **102** at **170**rpm and then evacuating chamber **74** by rotation at **1000**rpm at step **205** for about **2** min. The secondary antibody is an anti-antibody. The antibody in blood binds to the antigen. The secondary antibody is an antibody that specifically binds to the tail of the antibody in the blood sample. This secondary antibody carries the fluorescent tag.

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At step **207** laser valve **110** is aligned with a laser **48** in unit **10** and opened with a **0.5**min exposure. Thereafter, a wash buffer **#2** stored in chamber **104** is transferred to microarray chamber **74** by reciprocation at step **209** for about **5** min at step **197** for **20** cycles at **2700-5428** rpm followed by priming chamber **104** at **170**rpm and then evacuating chamber **74** by rotation at **1000**rpm for about **2**min.

At step **211** valve **112** is aligned with a laser **48** in unit **10** and opened with a **0.5**min exposure. At step **213** disk **68** is spun at **5500**rpm for about **1** min to spin dry chamber **74** with wash **#2** being evacuated to waste chamber **114**. Chamber **74** and microarray **92** are then moved to align with camera **32** in unit **10**. One or more grayscale images using induced fluorescence are taken by camera **32**, stored and transmitted at step **215** in about **1** min by CPU **42** to the Cloud for data processing and diagnostic analysis as described below.

The total time needed to run the assay is about **16.5** minutes.

Cloud Processing and Diagnosis

Unit **10** performs the physical assay test using disk **68** and the detector provided in disk **68**. What results in raw data in some form. Unit **10** does not further process the data nor analyze it to derive a diagnosis of the patient, but transmits the raw data to the Cloud, where remote servers provide processing and diagnostic analysis of the data. Using information associated with or in the patient's scanned QR code, the test results are then stored in a database and transmitted back to the patient's computer, smartphone or other electronic address of a health provider associated with the patient without further involvement with unit **10**.

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Fig. **9** is a diagram of data processing in the A**20** at a high level. The patient's QR scan assigned to him or her by the health providers is scanned at step **216** associating a person with a test and at step **218** the disk barcode is scanned to associate a disk with the same test. The assay is run at step **220** as described above in connection with Figs. **7** and **8** ending with a captured fluorescently stimulated image of microarray **92** at step **222**. Unit **10** then sends it captured grayscale image or images to the Cloud at step **224**. At this point, unit **10**'s role in the test is ended.

Prior to transmission of the captured data, unit **10** operates under software control as depicted in Fig. **10**. A quality assurance test of the harness or wiring assemblies of unit **10** can be initiated by activating a QA Test Harness button **116** on touchscreen display **12** or a menu for operator interface (human-machine interface HMI) **118** activated, both of which operate with Java Script Object Notation (JSON). JSON is a type of data file that contains a human readable element. JSON is used because it is operating system agnostic, secure, and lossless (no data loss from the original data that comes off the camera sensor). Fig. **11** depicts a screenshot of touchscreen **12** when operator interface **118** is activated by a power on switch activation to display a Scan QR Code button and a Run Test button to scan the patient's QR code to associate the patient with the test and then to run the assay as described above respectively. Upon activation of the Scan QR Code button the operator then sees the screen of Fig. **12** and has access to the gear icon to allow setting the WiFI via a QR code. The gear icon is an icon on the human interface (GUI) which when touched, allows the user to enter the WIFI information for the device either manually or via a QR code.

Unit **10** operates autonomously under client/Python module **122**, which includes

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responsive action to exterior communications as well as operating according to the onboard stored Linux Oracle programming protocol. The operator interface **118** communicates with the autonomously running backend software **120**, which controls all operations of unit **10** through device control module **124**. Major functions include Cloud bidirectional communication by Cloud module **126** hardware control module **128** and database module **130**.

Fig. **13** illustrated the backend operation architecture. Database **130** is a SQLite device database module. SQLite is a widely used C-language library that implements a small, fast, self-contained, high-reliability, full-featured, SQL database engine. SQLite is an embedded SQL database engine. Unlike most other SQL databases, SQLite does not have a separate server process. SQLite reads and writes directly to ordinary disk files. A complete SQL database with multiple tables, indices, triggers, and views, is contained in a single disk file. SQLite is a compact library. Python device control **124** bidirectionally communicates with database module **130** and includes as an operating submodule Python hardware control **128**, which controls CD motor **26**, camera **32**, lasers **48** and the other electronic and electromechanical devices of unit **10**. Device control **124** is communicated via JSON and first-in-first-out (FIFO) with light and versatile graphics library (LVGL/C - HMI) **118**, which is an open-source graphics library providing the tools needed to create an embedded graphic user interface (GUI) with graphical elements, visual effects and a low memory footprint made available to touchscreen **12**. Both device control **124** and LVGL/C - HMI **118** are supported by a library of C executables library **132**, which bidirectionally communicates with QR reader **50**. Oracle or Linux based Cloud communication through module **126** to Cloud **134** with a

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red hat package manager (RPM) protocol which is used to store installation packages on Linux operating systems. C executables library **132** communicates with the Cloud **134** using a hypertext transfer protocol secure (HTTPS) encryption of JSON code.

Image Processing in the Cloud

As described above unit **10** generates raw digital images taken by camera **32** and transmits them unprocessed to Cloud **134**. The object is to convert the scanned microarray images into a scalar value for each microarray dot or site. The image data processing proceeds by the steps of alignment **136**, spot detection **138** and spot analysis **140** as depicted in Fig. **14**. Microarray spot image analysis is described in detail in Bell et.al. "An Integrated Digital Imaging System and Microarray Mapping Software for Rapid Multiplexed Quantitation of Protein Microarray Immunoassays," Grace Bio Labs, Bend, Oregon. The program structure of Fig. **15** has been written to keep each phase of the analysis modular. Each phase is passed an image **142**, and a JSON information file **144**. Each phase performs its work, and hands off the results for downstream processing.

The primary goal of the alignment step **236** is to correct for image inconsistencies, including angle of rotation, scale, and background noise. The alignment algorithm filters through all the shapes in an image, looking for objects that would qualify for spots or fiducials. After finding any potential spot or fiducial, the program looks for spacing ratios between all the potential fiducials that match the fiducial pattern indicated in the JSON schema file. Once the fiducials have been found, the image is rotated and cropped at

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step **246** to include only the region of interest. All processing is done on grayscale images.

Original or raw grayscale images **142** are imported into the program. The image **142** contains background information or noise that is not relevant to the processing of the image **142**. The alignment phase aims to remove this region of noninterest (nROI) information by identifying the three bright fiducial spots at the corners of the microarray. A bilateral filter is applied to the image to reduce noise, but to keep sharp edges for downstream processing. Next, the image **142** is processed through an adaptive threshold filter to obtain a binary image of contours. Each contour is then filtered for a range of sizes or pixel areas. The size ranges are known beforehand and scale with the dimension of the image. Contours that are too large or too small are ignored. The remaining contours have a minimum fit circle drawn around their perimeter; the area of this circle is compared to the area of the contour to determine how 'circular' the contour is. Contours that have an area similar to the area of the bounding circle are retained. After potential fiducials are identified, the program compares the distance ratios between all sets (combinations) of three contours, looking for ratios that match the theoretical fiducial spacing ratios given in the schema file (Fig. **22**a, dashed circles). The matching set of three contours are defined as the fiducials. After determining the fiducials, a minimum fit rectangle is drawn around the three fiducials (Fig. **22**b, dashed rectangle). The minimum fit rectangle is then cropped and rotated so that the fiducials are located in the top left, bottom left, and top right (Fig. **22**c). The location of each fiducial, and general information about the alignment routine is added to the JSON schema returned with the cropped image to be used by the next downstream application.

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In the spot detection step **238** the primary purpose is to determine where each microarray spot is located within the region of interest image. This will be used downstream to determine each spot value. Using the fiducial locations and known size of the microarray, the cropped image is subdivided into a grid, where each square should contain a spot. Adaptive thresholding is applied within each square of the grid. The adaptive threshold image of each square is used to calculate the image moment, which is used to determine centroids for spots:

$$C = \frac{\sum_{p=1}^{N} I_p(\overline{X_p} + \overline{Y_p})}{\sum_{p=1}^{N} I_p}$$

Where I_p is the pixel intensity at the pixel p, $\overline{X_p}$ and $\overline{Y_p}$ are the distance vectors to the pixel p relative to a reference point, and N is the total number of pixels in the grid region. Spot diameters are measured in each square if detected. If no visible spot is detected, each spot is assigned the average diameter of found spots.

The purpose of the spot analysis phase is to assign a single scalar value to each spot in the grid. Currently this is done by calculating the foreground median intensity and subtracting it from the background mean intensity. Each spot is individually masked, and the median of each spot is calculated. Likewise, the mean of each background annulus is calculated and subtracted from the spot median (Fig. **23**). The analysis output value is packed into the JSON structure for each spot and returned as a final result.

Diagnostic Processing the Cloud

Before considering the details of diagnostic processing of the processed image data in the Cloud, turn first and consider the microarrays used in the illustrated

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embodiments. The "multiplexed antibody array" in disk **68** provides an individual's virus "exposure fingerprint", the "legacy antibody profile" reflecting past exposure and vaccination history. This array analysis approach is significantly more data rich (e.g. **67** antigens with **4** replicates per array) and is more quantitative than lateral flow assays in current use for measuring antibodies against the virus. To appreciate this point turn to Figs. **16**a and **16**b where we show both positive and negative **2019** nCOV Array Sensitivity IgG results obtained on blood samples from the COVID-**19** Washington State **2020** outbreak

High throughput cloning and constructing microarrays have previously been developed that contain human and animal antibodies with antigens from more than **35** medically important pathogens, including bacteria, parasites, fungi and viruses such as vaccinia, monkey pox, Herpes **1** & **2**, Varicella zoster, HPV, HIV, Dengue, influenza, West Nile, Chikungunya, adenovirus, and coronaviruses. A DNA microarray (also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. DNA microarrays are used to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. Each DNA spot contains picomoles (10^{-12} moles) of a specific DNA sequence, known as *probes* (or *reporters* or *oligos*).

These can be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA, also called anti-sense RNA, sample, called *target*, under high-stringency conditions. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. The original

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nucleic acid arrays were macro arrays approximately **9** cm × **12** cm and the first computerized image-based analysis was published in **1981**. We have probed over **25000** samples from humans and animals infected with pathogens and identified over **1000** immunodominant and candidate vaccine antigens against these pathogens. We have shown that the individual proteins/antibodies printed on these arrays **92** capture antibodies and/or antigens present in serum from infected individuals and the amount of captured antibody can be quantified using fluorescent secondary antibody.

In this way a comprehensive profile of antibodies that result after infection or exposure can be determined that is characteristic of the type of infection and the stage of diseases. Arrays **92** can be produced and probed in large numbers (>**500** serum or plasma specimens per day) while consuming <2µl of each sample. This microarray approach allows investigators to assess the antibody repertoire in large collections of samples not possible with other technologies.

A coronavirus antigen microarray **92** (COVAM) was constructed containing **67** antigens that are causes of acute respiratory infections. The viral antigens printed on this array **92** are from epidemic coronaviruses including SARS-CoV-**2**, SARS-CoV, MERS-CoV, common cold coronaviruses (HKU**1**, OC**43**, NL**63**, **229**E), and multiple subtypes of influenza, adenovirus, metapneumovirus, parainfluenza, and respiratory syncytial virus. The SARS-CoV-**2** antigens on this array **92** include the spike protein (S), the receptor-binding (RBD), S**1**, and S**2** domains, the whole protein (S**1**+S**2**), and the nucleocapsid protein (NP) as shown in the graph of Fig. **17**. There is a similar set of antigens represented on the array from SARS-CoV, MERS-CoV, and the four common cold corona viruses.

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To determine the antibody profile of SARS-CoV-**2** infection, the differential reactivity to these antigens was evaluated for SARS-CoV-**2** convalescent blood specimens from PCR-positive individuals (positive group) and sera collected prior to the COVID-**19** pandemic from naïve individuals (negative control group). As shown in the heatmaps of Figs. **17**a and **17**b, the positive group is highly reactive against SARS-CoV-**2** antigens. This is more evident for the IgG than for IgA. The negative controls do not react to SARS-CoV-**2**, SARS-CoV or MERS-CoV antigens despite showing high reactivity to the common cold coronavirus antigens. Positive group displays high IgG reactivity to SARS-CoV-**2** NP, S**2**, and S**1**+S**2** antigens and to a lesser degree SARS-CoV-**2** S**1** shown in Figs.**17**a and **17**b. The positive group also demonstrates high IgG cross-reactivity against SARS-CoV NP, MERS-CoV S**2** and S**1**+S**2** antigens, while the negative group demonstrates low cross-reactivity with S**1**+S**2** and S**2** antigens from SARS-CoV-**2** and MERS-CoV and no cross-reactivity against other SARS-CoV-**2** antigens.

Table **1** contains the fluorescence intensity results for IgG shown in Fig. **18**a, the Z-score statistics for the fluorescence results in Fig. **18**c, the fluorescence intensity results for IgM shown in Fig. **18**b, and the Z-score statistics for the fluorescence results of Fig. **18**d. The Z-score shows how many standard deviations above (positive) or below (negative) the mean negative results a confirmed positive IgG or IgM sample is. Statistically significant Z-scores (**5** or greater) have shaded numerals.

Antigens were then evaluated to discriminate the positive group from the negative group across a full range of assay cutoff values using receiver-operating-characteristic (ROC) curves for which an area-under curve (AUC) was measured. High-performing antigens for detection of IgG are defined by ROC AUC >**0.85** as shown in Table **1**. Four

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antigens are ranked as high-performing antigens: SARS-CoV-**2** NP, SARS-CoV NP, SARS-CoV-**2**S**1**+S**2**, and SARS-CoV-**2**_S**2**. Additional high-performing antigens included SARS-CoV-**2** S**1** (with mouse Fc tag) and RBD, and MERS-CoV S**2**. The optimal sensitivity and specificity were also estimated for the seven high-performing antigens based on the Youden Index. Youden's J statistic (also called Youden's Index) is a single statistic that captures the performance of a dichotomous diagnostic test. Informedness is its generalization to the multiclass case and estimates the probability of an informed decision. The lowest sensitivity was seen for SARS-CoV-**2** S**1**, which correlates with the relatively lower reactivity to this antigen in the positive group. The lowest specificity was seen for SARS-CoV-**2** S**2**, which correlates with the cross-reactivity for this antigen seen in a subset of the negative group. To estimate the gain in performance by combining antigens, all possible combinations of up to four of the seven high-performing antigens were tested in silico for performance in discriminating the positive and negative groups. The ROC curve with AUC, sensitivity, and specificity was calculated for each combination. There is a clear gain in performance by combining two or three antigens. For IgG, the best discrimination was achieved with the two-antigen combination of SARS-CoV-**2**S**2** and SARS-CoV NP, with similar performance upon the addition of SARS-CoV-**2**S**1** with mouse Fc tag (AUC = **0.994**, specificity = **1**, sensitivity = **0.944**). The addition of a fourth antigen decreased the performance.

Table **2** shows the performance data for combinations of high-performing antigens. ROC, AUC values and sensitivity and specificity based on Youden index for discrimination of positive and negative sera were derived for each individual antigen

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ranked, and high-performing antigens with ROC AUC > **0.86** are indicated above the lines.

Figs. **18**a-d show an example of a single confirmed positive patient results. Fig. **18**a shows the normalized fluorescence intensity for various IgG antibodies in a serum, with the two lines showing the average results for a confirmed positive (top) and confirmed negative (bottom). Fig. **18**b shows the normalized fluorescence intensity for various IgM antibodies in a serum, with the two lines showing the average results for a confirmed positive (top) and confirmed negative (bottom). Fig. **18**c shows the plotted Z-scores for the IgG antibodies between a positive and negative result, with the three dotted lines representing the various Z-score thresholds for mild, moderate, and significant response. Fig. **18**d shows the plotted Z-scores for the IgM antibodies between a positive and negative result, with the three dotted lines representing the various Z-scores.

More particularly, the A**20** serology test is an optical microarray test that performs an indirect immunofluorescence assay for qualitative detection of IgM and IgG antibodies to SARS-CoV-**2** in human blood. The serology test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-**2**, indicating recent or prior infection. The serology test currently produces an image of the microarray and a graph of the intensities of the spots on the array. To develop a diagnostic standard known RT-PCR positive and negative samples are tested on the apparatus described above. This establishes cutoff thresholds for reactivity to each of the three SARS-CoV-**2** antigens in the microarray, which enables the apparatus to autonomously provide a qualitative "yes" (reactive) or "no" (non-reactive) result.

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Microarray Description

The serology test contains two identical microarrays on disk **68**, one for testing IgG presence and the other for IgM presence. The two classes of antibodies are probed separately by using IgG or IgM reporter antibodies. Each of the two microarrays has the form diagrammatically depicted in Fig. **19**. The microarray spots are characterized as:

Negative Controls: BUFFER (**10** spots): Phosphate-buffered saline (PBS) with **0.001**% Tween-**20** (Polyethylene glycol sorbitan monolaurate, Polyoxyethylenesorbitan monolaurate). These spots are printing buffers and serve as a negative control to determine the baseline fluorescence of the array.

Positive Controls **1**: HuIgG (**5** spots): Human IgG printed in concentrations of eight dilutions from **0.3** to **0.001** mg/ml for a total of **40** spots. These spots serve as a positive control to indicate that the reporter antibody for IgG is performing appropriately to accurately determine cutoff values of the array when testing on serum samples. The concentration ladder can serve as a rough guide to interpret the microarray's fluorescence.

HuIgM (**5** spots): Human IgM printed in concentrations of eight dilutions from **0.3** to **0.001** mg/ml for a total of **40** spots. These spots serve as a positive control to indicate that the reporter antibody for IgM is performing appropriately to accurately determine cutoff values of the array when testing on serum samples. The concentration ladder can serve as a rough guide to interpret the microarray's fluorescence.

Positive Controls **2**: a.HuIgG (**3** spots): anti-Human IgG printed in concentrations of **0.3**, **0.1**, and **0.03** mg/ml. These spots serve as a positive control to indicate that there are human IgG antibodies in the sample. a.HuIgM (**3** spots): anti-Human IgM printed

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in concentrations of **0.3**, **0.1**, and **0.03** mg/ml. These spots serve as a positive control to indicate that there are human IgM antibodies in the sample.

Antigens: SGC-SPIKE**19200701** (**8** spots): SARS-Cov-**2** Spike Protein (University of Oxford). Printed at **0.2** mg/ml. SARS-CoV**2**.NP (**8** spots): SARS-Cov-**2** Nucleocapsid Protein (Sinobiological). Printed at **0.2** mg/ml. SARS-CoV**2**.RBD.mFc (**8** spots): SARS-Cov-**2** Spike Protein (RBD, mFc Tag) (Sinobiological). Printed at **0.2** mg/ml.

Fiducial (**3** spots): Streptavidin, Alexa Fluor **647** conjugate. These spots are designed to be the brightest spots on the array and are used to locate and orient the array.

PBSTwash (**21** spots): PBS + **0.05**% tween**20** used for washing pins.

Blank (**2** spots): Unused microarray locations.

Microarray Results

The images of each microarray in an A**20** serology test are uploaded to a server on the Oracle Cloud for analysis. After the corner fiducials are used to locate and orient the microarrays, the images are analyzed to produce scalar values for each spot in the microarray. These measurements are the median fluorescence intensity of each spot, minus the mean fluorescence intensity of the surrounding annulus. These measurements will be available to the user online in a file in JSON format, along with a plot summarizing the values of the three SARS-CoV-**2** antigens printed on the microarray. The JSON file is a hierarchical file with the following top-level structure:

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Top-Level Overview of JSON

```
{
   "diskTypeID": "1234-02",
   "spots": [
      {...},
      {...}
   ],
   // information about the grid analysis
   "gridInfo": {
      "info": "Grid Detect",
      "version": "0.1",
      "avg_spot_dia": 83
   }
}
```

The measurements for each spot are contained in a list in the “spots” entry, with thorough details of each spot:

JSON Details

```
{
   // The QR code on the disk.
   "diskTypeID": "1234-02",
   // Array of all 'spots' on the microarray  image
```


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```
"spots": [
  {
    // spot row
    "row": "2",
    // spot col
    "column": "5",
    // group name if multiple virus-specific antigens are used
    "group": "",
    // name of the spot
    "id": "SGC-SPIKE19200701",
    // (x,y) pixel position of the spot
    "position": [
      329,
      146
    ],
    // Array of different types of analyses
    "analysis": [
      {
        // Name of the analysis method
        "name": "Spot Mean - Donut Median",
        // Version of this analysis method
        "version": "0.0",
        // Final value of this analysis  method
```


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```
            "value": 1.2824578790882057
          }
        ]
      },
      // {...} many more spots //
    ]
}
```


The accompanying summary figure of each microarray is a bar chart, which reports the value of each control spot, and mean value and standard deviation for each antigen such as shown in an example in Fig. **20**. From these results a conventional statistical model to distinguish blood samples with and without anti-SARS-CoV-**2** antibodies is established.

Overall System Usage

The overall user flow or user interaction with the system is illustrated in Figs. **21**a – **21**e. In Fig. **21**a the action of the patient, unit **10**, Cloud **134** and the test operator running unit **10** are each identified in four horizontal rows. At step **400** the patient logs into a portal on the internet to schedule a diagnostic test at an available test location. The remote server in Cloud **134** communicated to the portal schedules the patient's test at step **402** and generates a unique QR code which has: **1**) the test time and location; and **2**) which test to run, i.e. whether a disk **68** associated with A**10**, A**20** or A**30** is to be run. The QR code is how the patient controls the use of the testing information and its privacy. The QR code is sent to the patient at step **404**, which the patient downloads into his or her

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smartphone, laptop, or computer. Meanwhile at step **406** the remote server in Cloud **134** transmits the appointment information for the patient and inserts it into the testing schedule. At this point the procedure there may be a pause of one or more days before further action is taken.

On the day of the appointment at step **408** in Fig. **21**b the patient goes to the testing location and scan his or her QR code into unit **10** in response to a screen prompt at step **410**. The validity of the QR code is checked at step **412** in Cloud **134** and the remote server communicates to the testing site which of the disks **68** is to be used for the test, authorizing unit **10** to use a specific type of disk **68**. The test operator, after checking at step **414** the humidity and temperature levels on the disk packaging to verify the integrity of disk **68**, scans the disk's QR code at step **416** in response to a screen prompt from unit **10** at step **418**. Cloud **134** communicates the disk's metadata to unit **10** at step **422**, which metadata includes the status of the disk batch, a JSON file for the spin protocol, and grayscale TIF files of the microarrays **92** generated during quality control testing for the disk **68**. Unit **10** downloads the disk's metadata from Cloud **134** at step **420** and the authority to use disk **68** in the test is determined.

If it is determined at step **420** that the authority to use disk **68** is denied, the operator is advised to reject disk **68** and replace it with another at step **424**, after which the procedure returns to step **414**. If use of disk **68** is authorized, then a blood sample, such as a finger prick, is taken from the patient by the test operator at step **426**, loaded by the test operator into disk **68** at step **428**, and disk **68** then loaded into unit **10** at step **430**. Unit **10** displays a screen prompt to the test operator to begin the test at step **432** in Fig.

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21c. In response the test operator touches the start button on the screen display at step **434**.

Pretest diagnostic data is gathered in step **436**, this includes checking the optical system at step **438** with both microarrays **92** in disk **68** by verifying that: **1**) the three fiducial spots in each array are visible; **2**) the fiducial intensity is within **20**% of the original images of the microarray; and **3**) the fiducial spots are in focus. Similarly, a watchdog routine in COU **43** at step **440** outputs diagnostic data from camera **32**, the LEDs **56**, motor **26**, and lasers **48**. Thereafter, unit **10** runs a spin protocol on disk **68** at step **442** as described above and takes a grayscale image of each microarray **92** at the end of the assay. The watchdog routine in CPU **43** at step **444** continues to monitor unit **10** during the assay procedure and generates an error message display in the event of a fault and stops the test or assay if needed.

The grayscale TIF image taken by camera **32** of each microarray **92** is uploaded to Cloud **134** at step **446** in Fig. **21**d both of each microarray before the test to provide background data, after the test to provide test data, and files including the diagnostic data taken before and after the test. At step **448** an image processing algorithm as described above processes the digital images of microarrays **92** to generate a JSON file listing each spot name, spot location and fluorescence intensity, from which a diagnosis is made.

From the JSON output file the test processing is determined as being passed or failed at step **452** in Fig. **21**e. If the test passed, a predictive diagnosis is made of the diagnosis and a confidence interval calculated. The predictive diagnosis is provided from a statistical model such as logistic regression or random forest, using

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fluorescence intensity or calculated Z-scores. At the same time at step **454** a list of antigens with mean fluorescent intensity values and standard deviations is plotted. These results are communicated from Cloud **134** to the patient's smartphone, laptop, or computer at step **456**, and depending on the access level granted, the patient can see the results, graphs and/or raw data. At step **458** the patient then has the choice to forward the test data to his or her doctor, healthcare technician, research facility, governmental authority or wherever the patient deems necessary.

Data Chain Identification

Control of the data sent to the remote server in Cloud **134** is realized utilizing the identification chain **300** of Fig. **22**. This identification chain **300** can be viewed as a tree graph as shown in Fig. **22**, where each box is a node in the tree that can be traversed in either direction, and where each node corresponds to a manufactured component. Each node in the chain can be queried recursively to yield information of its corresponding components, or its own details of manufacture catalog number, date, origin, and batch. Each test **302** is encoded in a transmitted image analysis data file **304**, which includes a TIFF package **306**, which in turn is tied to a unique patient/test code **308**, a unique machine ID **310**, a unique cartridge code **312**, and a UTC timestamp of the performed test. Connecting the test code to the patient/test code **308**, machine ID **310**, and timestamp **314** guarantees that no two test results can be misidentified, as two tests cannot be performed on the same machine at the same time.

Attaching a unique cartridge code **312** further guarantees the uniqueness of each test and its results, but also creates a complete identification chain to connect a particular

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test **302** and its results to every relevant assembly component involved in that test **302**. This provides full traceability, allowing one to identify all component lot numbers used in a particular disc **68**, or all discs **68** utilizing a particular component lot number. This allows one to acquire data from compromised tests and determine a faulty component lot or recall all discs that utilize a faulty component lot.

The machine ID **310** is uniquely defined by its camera serial number **316** and on-board computer (pi raspberry) serial number **318**. The machine ID **310** can then provide the hierarchy of all sub-assemblies of all its mechanical and electrical components.

The cartridge code **312** is traced to the cartridge assembly batch **320**, which details the date of assembly **328**, microarray information **322**, disc information **324**, and reagent catalog and lot number **326** stored on the cartridge. The disc information **324** contains details of the disc design **330** and disc injection batch **332**. The microarray information **322** contains details of the printing date **334**, the microarray layout **336**, the glass slide etching batch **338**, the printing protein catalog and lot number **340**, the nitrocellulose lot **342** used in the microarray. The glass slide etching batch **338** refers in turn to the glass slide lot **344**.

Many alterations and modifications may be made by those having ordinary skill in the art without departing from the spirit and scope of the embodiments. Therefore, it must be understood that the illustrated embodiment has been set forth only for the purposes of example and that it should not be taken as limiting the embodiments as defined by the following embodiments and its various embodiments.

Therefore, it must be understood that the illustrated embodiment has been set forth only for the purposes of example and that it should not be taken as limiting the

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concepts described herein. For example, notwithstanding the fact that the elements of the embodiment are set forth below in a certain combination, it must be expressly understood that the embodiment may include other combinations of fewer, more or different elements, which may be disclosed above.. A teaching that two elements are combined in a described combination is further to be understood as also allowing for a combination in which the two elements are not combined with each other but may be used alone or combined in other combinations. The excision of any disclosed element of the embodiments is explicitly contemplated as within the scope of the teachings herein.

The words used in this specification to describe the various embodiments are to be understood not only in the sense of their commonly defined meanings, but to include by special definition in this specification structure, material or acts beyond the scope of the commonly defined meanings. Thus, if an element can be understood in the context of this specification as including more than one meaning, then its use herein must be understood as being generic to all possible meanings supported by the specification and by the word itself.

The definitions of the words or elements described herein include not only the combination of elements which are literally set forth, but all equivalent structure, material or acts for performing substantially the same function in substantially the same way to obtain substantially the same result. In this sense it is therefore contemplated that an equivalent substitution of two or more elements may be made for any one of the elements in a combination or that a single element may be substituted for two or more

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elements in a combination. Although elements may be described above as acting in certain combinations and even initially described as such, it is to be expressly understood that one or more elements from a described combination can in some cases be excised from the combination and that the described combination may include a subcombination or variation of a subcombination.

Insubstantial changes from the teachings herein as viewed by a person with ordinary skill in the art, now known or later devised, are expressly contemplated as being equivalently within the scope of the teachings herein. Therefore, obvious substitutions now or later known to one with ordinary skill in the art are defined to be within the scope of the defined elements.

The concepts described herein are thus to be understood to include what is specifically illustrated and described above, what is conceptionally equivalent, what can be obviously substituted and also what essentially incorporates the essential idea of the embodiments.

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TABLE 1

name	value IgG	ref. IgG	z.score IgG	value IgM	ref. IgM	z.score IgM
SARS.CoV.2.NP	14561.00	1374.91	11.15	1729.80	1420.89	0.17
SARS.CoV.2.Pl.pro	403.50	685.28	−0.40	180.72	153.55	0.12
SARS.CoV.2.S1	2553.60	1695.89	0.81	1379.55	825.83	2.66
SARS.CoV.2.S1.HisTag	3673.05	250.83	15.86	1568.15	67.92	11.26
SARS.CoV.2.S1.mFcTag	7448.25	545.47	13.16	5666.20	659.51	9.57
SARS.CoV.2.S1.RBD	7467.90	570.75	12.91	6846.95	1273.64	10.58
SARS.CoV.2.S1+S2	7518.00	2233.85	2.86	3435.40	969.59	5.86
SARS.CoV.2.S2	2452.55	1278.24	0.97	1178.90	583.27	3.11
SARS.CoV.2.Spike.RBD.Bac	2254.20	888.46	3.34	2099.50	520.37	4.72
SARS.CoV.2.Spike.RBD.His.HEK	2699.60	278.67	9.42	2110.35	115.50	14.17
SARS.CoV.2.Spike.RBD.rFc	4981.15	839.19	9.79	2974.90	618.89	8.03
SARS.CoV_NP	14529.50	2759.59	10.41	3772.35	2415.02	0.66
SARS.CoV_PLpro	1069.70	583.84	1.49	292.55	423.26	−0.51
SARS.CoV_S1.HisTag	679.55	1418.36	−0.85	276.60	856.47	−1.87
SARS.CoV_S1.RBD.HisTag	502.40	573.32	−0.87	227.70	448.94	−0.94
SARS.CoV_S1.RBD.rFcTag	853.15	1669.10	−1.39	637.10	925.24	−0.59
MERS.CoV_NP	501.15	1878.35	−0.55	1208.60	1569.42	−0.13
MERS.CoV_S1.AA1.725.His.HEK	137.65	303.19	−0.85	21.85	111.41	−0.81
MERS.CoV_S1.RBD.367.606.rFcTag	1141.75	3405.61	−1.78	775.00	1934.58	−0.47
MERS.CoV_S1.RBD.383.502.mFcTag	373.45	1266.92	−0.99	1247.55	2310.08	−0.82
MERS.CoV_S2	4564.35	2780.55	0.83	455.75	948.89	−0.77
OcCoV.HKU23.NP	917.55	2571.47	−0.97	352.05	588.45	−0.51
hCoV.229E.S1	3155.40	5439.22	−1.01	167.12	372.36	−0.82
hCoV.229E.S1_S2	7544.50	10038.24	−1.03	615.45	1927.26	−0.53
hCoV.HKU1.HE	3360.50	6284.33	−0.88	1380.10	4284.79	−1.02
hCoV.HKU1.S1_AA1.760	1648.75	2920.27	−0.78	53.15	180.58	−1.23
hCoV.HKU1.S1_AA13.756	998.35	3012.07	−0.94	251.50	422.75	−1.05
hCoV.HKU1.S1_S2	6798.20	4890.55	0.95	538.55	1013.67	−1.37
hCoV.NL63.S1	1281.60	1659.04	−0.52	124.70	253.78	−0.85
hCoV.NL63.S1_S2	2030.60	3302.70	−1.17	394.60	1028.94	−0.77
hCoV.OC43.HE	1283.10	2992.66	−1.11	153.25	447.21	−1.01
hCoV.OC43.S1	212.90	383.06	−0.74	67.05	223.34	−1.17
hCoV.OC43.S1_S2	12497.90	7958.39	2.01	841.28	1169.18	−0.61
Flu.B_Mal.HA1	7884.25	8556.08	−0.23	184.90	438.99	−0.45
Flu.B_Mal.HA1+HA2	11362.85	11918.07	−0.24	446.90	515.63	−0.12
Flu.B_Phu.HA1	7333.90	6356.85	0.32	176.35	332.97	−0.52
Flu.B_Phu.HA1+HA2	10142.05	11923.46	−0.67	656.40	1997.51	−0.55
Flu.H1N1.HA1	1731.10	4421.82	−1.03	252.75	489.13	−1.12
Flu.H1N1.HA1+HA2	10997.75	10597.92	0.10	1197.55	576.35	0.86
Flu.H3N2.HA1	13223.85	9003.17	1.17	260.70	336.37	−0.24
Flu.H3N2.HA1+HA2	13491.35	11237.43	0.78	696.30	1184.50	−0.56
Flu.H5N1.HA1	1045.55	3725.37	−0.99	931.65	1504.46	−0.82
Flu.H5N1.HA1+HA2	7265.50	9349.40	−0.63	1856.95	1730.49	0.15
Flu.H7N9.HA1	654.45	1138.46	−0.59	82.55	117.95	−1.23
Flu.H7N9.HA1+HA2	838.35	1501.44	−0.59	16.00	103.03	−2.19
hAdV3.Fiber	3108.70	5882.67	−0.62	820.25	857.55	−0.10
hAdV3.Penton	2758.50	3406.34	−0.35	796.30	624.49	0.13
hAdV4.Fiber	4197.65	3940.21	0.09	519.55	640.59	−0.33
hAdV4.Penton	1466.70	2241.25	−0.44	490.85	640.63	−0.45
hMPV.A_G.52N.228N	603.80	1741.45	−1.59	496.10	616.97	−0.39
hMPV.B_F.280D.490G	275.70	746.49	−0.91	309.00	462.87	−0.47
hMPV.B_G.52D.238S	560.40	1016.13	−0.44	197.10	474.71	−0.75
hPIV.1.12D3_F	5352.15	7393.48	−0.83	796.92	1315.02	−0.93
hPIV.1.12D3_H	4208.50	7259.00	−1.50	1091.15	2833.11	−1.15
hPIV.3.2010_H	5143.55	7617.24	−0.75	840.20	1376.41	−0.90
hPIV.4.b.2016_H	1839.00	4052.51	−1.16	811.40	1267.62	−0.90
RSV.A.F	6134.12	9708.27	−1.86	473.80	1159.34	−1.17
RSV.A.G	4018.20	9280.15	−1.93	855.55	829.59	0.57
RSV.B.F	10774.30	11656.42	−0.49	1036.20	1529.28	−0.39
RSV.B.G	8945.12	11369.46	−0.87	1356.35	782.25	0.89

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TABLE 2

N	Antigen Combination	IgG AUC	IgG Spec	IgG Sens
1	SARS-CoV-2_S1+S2	0.975	0.987	0.889
1	SARS-CoV-2_NP	0.975	0.961	0.889
1	SARS-CoV-2_S2	0.951	0.921	0.833
1	SARS-CoV_NP	0.957	0.974	0.833
1	SARS-CoV-2_S1 (mFcTag)	0.88	0.987	0.667
1	MERS-CoV_S2	0.873	0.763	0.889
1	SARS-CoV-2_S1-RBD	0.849	0.947	0.833
2	SARS-CoV-2_NP ; MERS-CoV_S2	0.988	0.934	1
2	SARS-CoV-2_S1+S2 ; SARS-CoV-2_NP	0.988	0.963	0.947
2	**SARS-CoV-2_S1+S2 ; SARS-CoV_NP**	0.975	0.974	0.889
2	**SARS-CoV-2_S2 ; SARS-CoV_NP**	**0.994**	**1**	**0.944**
3	SARS-CoV-2_NP ; SARS-CoV-2_S2 ; SARS-CoV_NP	0.988	1	0.944
3	SARS-CoV-2_S1+S2 ; SARS-CoV-2_NP ; SARS-CoV_NP	0.981	1	0.889
3	SARS-CoV-2_S1+S2 ; SARS-CoV-2_S2 ; SARS-CoV_NP	0.975	1	0.889
3	**SARS-CoV-2_S1+S2 ; SARS-CoV_NP ; SARS-CoV-2_S1, (mFcTag)**	0.969	0.961	0.889
3	SARS-CoV-2_S2 ; SARS-CoV_NP ; MERS-CoV_S2	0.988	1	0.944
3	**SARS-CoV-2_S2 ; SARS-CoV_NP ; SARS-CoV-2_S1, (mFcTag)**	**0.994**	**1**	**0.944**
4	SARS-CoV-2_S1+S2 ; SARS-CoV-2_NP ; SARS-CoV-2_S2 ; SARS-CoV_NP	0.981	1	0.944
4	SARS-CoV-2_S1+S2 ; SARS-CoV-2_NP ; SARS-CoV_NP ; SARS-CoV-2_S1-RBD	0.975	1	0.833
4	SARS-CoV-2_S1+S2 ; SARS-CoV-2_S2 ; SARS-CoV_NP ; SARS-CoV-2_S1, (mFcTag)	0.981	0.987	0.944
4	SARS-CoV-2_S1+S2 ; SARS-CoV_NP ; MERS-CoV_S2 ; SARS-CoV-2_S1-RBD	0.975	1	0.944
4	SARS-CoV-2_S2 ; SARS-CoV_NP ; SARS-CoV-2_S1, (mFcTag) ; SARS-CoV-2_S1-RBD	0.988	1	0.944

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EMBODIMENTS IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An automated system operable to communicate with a remote server for diagnostically field testing a sample taken from a subject using an automated portable handheld instrument to determine the presence of antibodies against at least one viral antigen in the sample, the system comprising:

one or more types of microfluidic circuits defined in a rotatable disk, each type of microfluidic circuit for performing a bioassay using an immunofluorescence microarray based on immunofluorescence to generate an electrical signal indicative of a bioassay measurement, wherein the immunofluorescence microarray is operationally positioned in at least one of the microfluidic circuits and wherein the rotatable disk further comprises at least one positionable valve in at least one of the microfluidic circuits; and

a backbone unit for rotating the rotatable disk according to a predetermined protocol to perform the bioassay wherein the backbone unit is configured to operate the immunofluorescence microarray in order to generate the electrical signal indicative of the bioassay measurement, communicate the bioassay measurement to the remote server, and to associate the performed bioassay and its corresponding bioassay measurement to the subject and wherein the backbone unit comprises:

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at least one laser configured to ablate the at least one positionable valve;

a scanner disposed on the external portion of the backbone unit to scan a barcode on the disk to associate the disk with the bioassay measurement; and

a camera configured to capture a digital image of the bioassay measurement, wherein the digital image includes microarray antigen spots and wherein the electrical signal indicative of the bioassay measurement includes a representation of the digital image.

2. The system of claim **1** wherein the bioassay is a serology test for testing for at least one of (a) immunoglobulin-G (IgG) and (b) immunoglobulin-M (IgM).

3. The system of claim **1** where the bioassay is at least one of (a) a respiratory antibody test and (b) an antigen test.

4. The system of claim **2** where the serology test tests for Covid-**19**.

5. The system of claim **1** where the rotatable disk has a center and comprises:

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a sample inlet;

a blood-plasma separation chamber in communication with the sample inlet and positioned on the rotatable disk radially farther from the center of the rotatable disk than the sample inlet;

a mixing chamber in communication with the blood-plasma separation chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the blood-plasma separation chamber;

a first wash chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

an antibody chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

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a second wash chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

a microarray chamber in communication with the mixing chamber, the immunofluorescence microarray being disposed in the microarray chamber and the microarray chamber positioned on the rotatable disk radially farther from the center of the rotatable disk than the mixing chamber; and

a waste chamber in communication with the microarray chamber by a siphon and by a corresponding selectively openable spin-dry valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the microarray chamber.

6. The system of claim **3** where the digital image of the bioassay measurement includes a digital image of at least one microarray antigen spot which has been fluorescently labeled by a secondary antibody binding to subject antibodies from the sample,

where the remote server is a Cloud server,

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where the backbone unit includes network circuitry which communicates the digital image to the Cloud server and a corresponding schema file associating the subject to the performed bioassay and its corresponding bioassay measurement;

where the Cloud server, operating in an automated and modular protocol, aligns the at least one microarray antigen spot of the digital image, detects the aligned at least one microarray antigen spot of the immunofluorescence microarray and analyzes the aligned at least one microarray antigen spot of the digital image to assign a scalar value to the at least one microarray antigen spot to produce a processed microarray measurement set of data;

where the Cloud server, operating in an automated protocol, analyzes the processed microarray measurement set of data to produce a diagnosis of the bioassay measurement; and

where the Cloud server, operating in an automated protocol, reports the results to the subject as determined by the schema file.

7. The system of claim **6** where the Cloud server comprises a cloud-based module for automatically determining under automated control whether corresponding Z-scores of the processed microarray measurement set of data are of at least one of (a) positive and (b) negative indications are indicative of Covid-**19** rather than

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Z-scores of the plurality of viral infections sharing at least one of the Covid-**19** antigens and antibodies.

8. The system of claim **6** where the Cloud server comprises means for identifying at least one of (a) positive and (b) negative indications of the digital image of the at least one microarray antigen spots for a plurality of acute respiratory infections selected from the group including SARS-CoV-**2**, SARS-CoV, MERS-CoV, common cold coronaviruses, and multiple subtypes of influenza, adenovirus, metapneumovirus, parainfluenza, and respiratory syncytial virus.

9. The system of claim **6** where the Cloud server comprises a cloud-based module for automatically evaluating antigens to discriminate output data of a positive group of antigens from a negative group of antigens across a range of assay cutoff values using receiver-operating-characteristic (ROC) curves for which an area-under curve (AUC) is measured to determine high performing antigens to diagnose Covid-**19**.

10. The system of claim **6** where the Cloud server comprises a cloud-based module for automatically determining under automated control an optimal sensitivity and specificity for Covid-**19** from a combination of a plurality of high performing antigens based on a corresponding Youden Index calculated for the combination of the plurality of high-performing antigens.

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11. An automated system operable to communicate with a cloud-based server for diagnostically field testing a sample taken from a subject using an automated portable handheld instrument to determine the presence of antibodies against at least one viral antigen thereto in a serology test to detect Covid-**19** comprising:

a microfluidic circuit defined in a rotatable disk for performing a bioassay using an immunofluorescence microarray based on immunofluorescence to generate a digital image indicative of a bioassay measurement; and

a backbone unit for rotating the rotatable disk according to a predetermined protocol to perform the bioassay, wherein the backbone unit is configured to operate the immunofluorescence microarray to generate the digital image indicative of a bioassay measurement, communicate the digital image to the cloud-based server, and to associate the performed bioassay and its corresponding bioassay measurement to the subject,

where the rotatable disk has a center and comprises:

a sample inlet;

at least one positionable valve in the microfluidic circuit;

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a blood-plasma separation chamber in communication with the sample inlet and positioned on the rotatable disk radially farther from the center of the rotatable disk than the sample inlet;

a mixing chamber in communication with the blood-plasma separation chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the blood-plasma separation chamber;

a first wash chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

an antibody chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

a second wash chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

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a microarray chamber in communication with the mixing chamber, the immunofluorescence microarray being disposed in the microarray chamber, and the microarray chamber positioned on the rotatable disk radially farther from the center of the rotatable disk than the mixing chamber; and

a waste chamber in communication with the microarray chamber by a siphon and by a corresponding selectively openable spin-dry valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the microarray chamber;

where the backbone unit comprises:

network circuitry which communicates the digital image to the Cloud server and a corresponding schema file associating the subject to the performed bioassay and its corresponding bioassay measurement;

at least one laser configured to ablate the at least one positionable valve;

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a scanner disposed on the external portion of the backbone unit; and

a camera configured to capture the digital image indicative of the bioassay measurement,

where the Cloud server, operating in an automated and modular protocol, aligns at least one microarray antigen spot of the digital image, detects the aligned at least one microarray antigen spot of the immunofluorescence microarray and analyzes the aligned at least one microarray antigen spot of the digital image to assign a scalar value to the at least one microarray antigen spot to produce a processed microarray measurement set of data;

where the Cloud server, operating in an automated protocol, analyzes the processed microarray measurement set of data to produce a diagnosis of the bioassay measurement; and

where the Cloud server, operating in an automated protocol, reports the results to the subject as determined by the schema file.

12. The system of claim **11** where the Cloud server comprises a cloud-based module for automatically determining under automated control whether corresponding Z-scores of the processed microarray measurement set of data of at least one of (a)

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positive and (b) negative indications are indicative of Covid-**19** rather than Z-scores of the plurality of viral infections sharing at least one of Covid-**19** antigens and antibodies.

13. The system of claim **11** where the Cloud server comprises means for identifying at least one of (a) positive and (b) negative indications of the digital image of microarray antigen spots for a plurality of acute respiratory infections selected from the group including SARS-CoV-**2**, SARS-CoV, MERS-CoV, common cold coronaviruses, and multiple subtypes of influenza, adenovirus, metapneumovirus, parainfluenza, and respiratory syncytial virus.

14. The system of claim **11** where the Cloud server comprises a cloud-based module for automatically evaluating antigens to discriminate output data of a positive group of antigens from a negative group of antigens across a range of assay cutoff values using receiver-operating-characteristic (ROC) curves for which an area-under curve (AUC) is measured to determine high performing antigens to diagnose Covid-**19**.

15. The system of claim **11** where the Cloud server comprises a cloud-based module for automatically determining under automated control an optimal sensitivity and specificity for Covid-**19** from a combination of a plurality of high performing antigens based on a corresponding Youden Index calculated for the combination of the plurality of high-performing antigens.

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16. A method for operating the automated system of claim **1** for diagnostically field testing a sample taken from a subject using an automated portable handheld instrument to determine the presence of antibodies against at least one viral antigen in the sample, the method comprising:

introducing the sample into a sample inlet disposed on the rotatable disk;

scanning the rotatable disk with a scanner disposed in a backbone unit and associating the rotatable disk with the subject;

inserting the rotatable disk into the backbone unit;

transferring the sample to a blood-plasma separation chamber in communication with the sample inlet and positioned on the rotatable disk radially farther from the center of the rotatable disk than the sample inlet;

separating the blood from the plasma by spinning the rotatable disk at a first rotational speed for a first period of time;

opening a first valve comprising a laser-meltable plug using at least one laser disposed in the backbone unit, the first valve being disposed in a conduit in the rotatable disk between the blood-plasma chamber and a mixing chamber

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in communication with the blood-plasma separation chamber through the selectively openable first valve and positioned on the rotatable disk radially farther from the center of the rotatable disk than the blood-plasma separation chamber;

transferring the sample to the mixing chamber and to a microarray chamber in communication with the mixing chamber, an immunofluorescence microarray being disposed in the microarray chamber and the microarray chamber positioned on the rotatable disk radially farther from the center of the rotatable disk than the mixing chamber;

reciprocating the sample in the microarray chamber for a plurality of cycles within a first rotational speed range, followed by priming the chamber at a second rotational speed and evacuating the chamber at a third rotational speed for the first period of time to a waste chamber in communication with the microarray chamber by a siphon and by a corresponding selectively openable spin-dry valve positioned on the rotatable disk radially farther from the center of the rotatable disk than the microarray chamber;

opening a second valve comprising a laser-meltable plug using the at least one laser disposed in the backbone unit, the second valve being disposed in a conduit in the rotatable disk between the mixing chamber and a first wash chamber in communication with the mixing chamber through a corresponding

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selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

transferring a first wash from the first wash chamber through the mixing chamber to the microarray chamber;

reciprocating the first wash in the microarray chamber for a plurality of cycles within the first rotational speed range, followed by priming the chamber at the second rotational speed and evacuating the chamber at the third rotational speed for a second period of time to the waste chamber;

opening a third valve comprising a laser-meltable plug using the at least one laser disposed in the backbone unit, the third valve being disposed in a conduit in the rotatable disk between the mixing chamber and an antibody chamber in communication with the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

transferring the secondary antibody from the antibody chamber through the mixing chamber to the microarray chamber;

reciprocating the secondary antibody in the microarray chamber for a plurality of cycles within the first rotational speed range, followed by priming

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the chamber at the second rotational speed and evacuating the chamber at the third rotational speed for the second period of time to the waste chamber;

opening a fourth valve comprising a laser-meltable plug using the at least one laser disposed in the backbone unit, the fourth valve being disposed in a conduit in the rotatable disk between the mixing chamber and a second wash chamber communicated to the mixing chamber through a corresponding selectively openable valve and positioned on the rotatable disk radially closer to the center of the rotatable disk than the mixing chamber;

transferring a second wash from the second wash chamber through the mixing chamber to the microarray chamber;

reciprocating the second wash in the microarray chamber for a plurality of cycles at within the first rotational speed range, followed by priming the chamber at the second rotational speed and evacuating the chamber at the third rotational speed for the second period of time to the waste chamber;

opening a fifth valve comprising a laser-meltable plug using the at least one laser disposed in the backbone unit, the fifth valve being disposed in a conduit in the rotatable disk between the microarray chamber and the waste chamber;

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spin drying the microarray chamber by spinning the rotatable disk at **5500** rpm for one minute;

moving the microarray chamber to a position wherein a digital image can be taken of the immunofluorescence microarray; and

generating the digital image of the immunofluorescence microarray with a camera disposed in the backbone unit, the digital image including depictions of microarray antigen spots.

17. The method of claim **16** further comprising:

communicating the digital image using the backbone unit including network circuitry which communicates the digital image to a Cloud server and communicates a corresponding schema file associating the subject to the performed bioassay and its corresponding bioassay measurement;

aligning the microarray antigen spots of the digital image in the Cloud server, operating in an automated and modular protocol;

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detecting each of the aligned microarray antigen spots of the immunofluorescence microarray in the Cloud server, operating in an automated and modular protocol;

analyzing each of the microarray antigen spots of the digital image the Cloud server, operating in an automated and modular protocol to assign a scalar value to each microarray antigen spot to produce a processed microarray measurement set of data;

analyzing the processed microarray measurement set of data to produce a diagnosis of the bioassay measurement in the Cloud server, operating in an automated protocol; and

reporting the results to the subject as determined by the schema file using the Cloud server, operating in an automated protocol.

18. The method of claim **17** where analyzing the processed microarray measurement set of data comprises identifying at least one of (a) positive and (b) negative indications of the digital image of microarray antigen spots for a plurality of acute respiratory infections selected from the group including SARS-CoV-**2**, SARS-CoV, MERS-CoV, common cold coronaviruses, and multiple subtypes of influenza, adenovirus, metapneumovirus, parainfluenza, and respiratory syncytial virus.

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19. The method of claim **16** where reciprocating the sample in the microarray chamber for a plurality of cycles within a first rotational speed range comprises reciprocating the sample at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the first period of time comprises evacuating the chamber at **1000** rpm for **5** minutes.

20. The method of claim **16** where reciprocating the first wash in the microarray chamber for a plurality of cycles within the first rotational speed range comprises reciprocating the first wash at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the first period of time comprises evacuating the chamber at **1000** rpm for **2** minutes.

21. The method of claim **16** where reciprocating the secondary antibody in the microarray chamber for a plurality of cycles within the first rotational speed range comprises reciprocating the secondary antibody at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the second period of time comprises evacuating the chamber at **1000** rpm for **2** minutes.

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22. The method of claim **16** where reciprocating the second wash in the microarray chamber for a plurality of cycles within the first rotational speed range comprises reciprocating the second wash at **2700-5428** rpm, where priming the chamber at the second rotational speed comprises priming the chamber at **170** rpm, and where evacuating the chamber at the third rotational speed for the second period of time comprises evacuating the chamber at **1000** rpm for **2** minutes.

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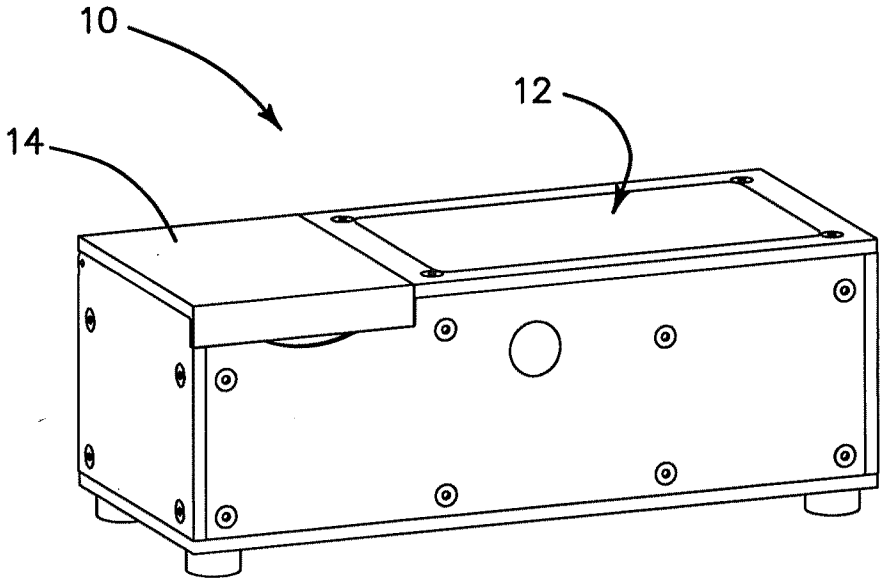

FIG. 1

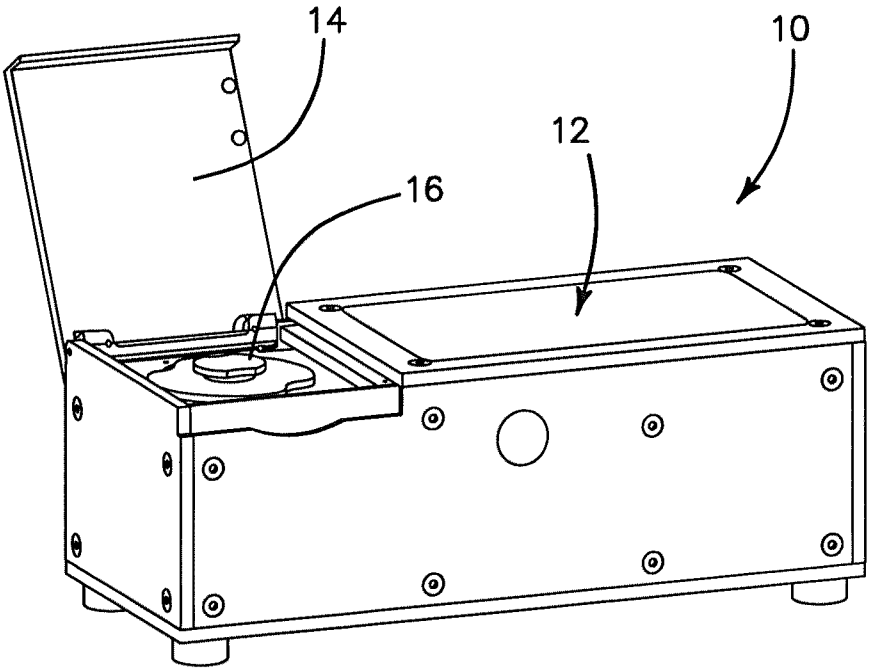

FIG. 2

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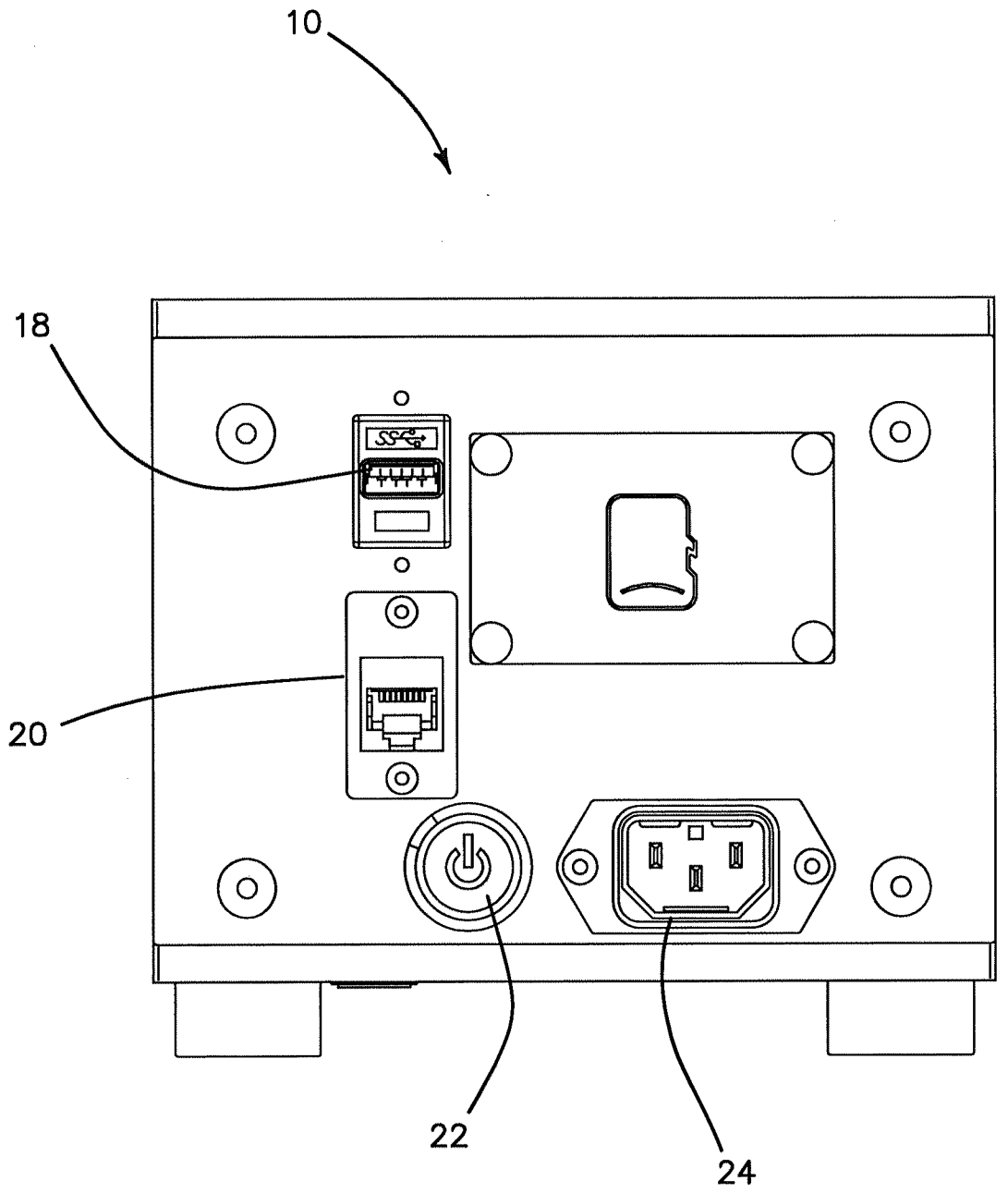

FIG. 3

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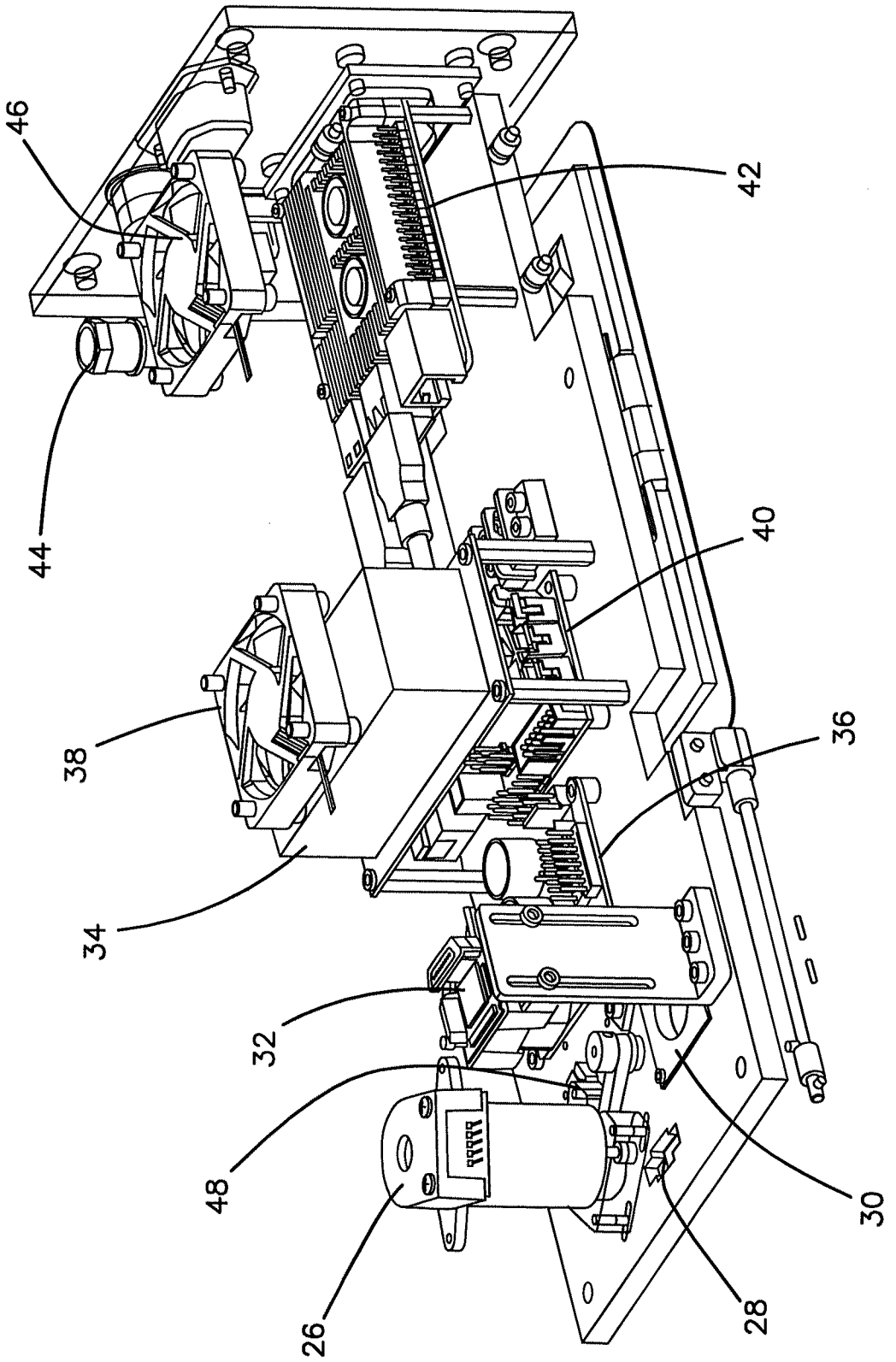

FIG. 4

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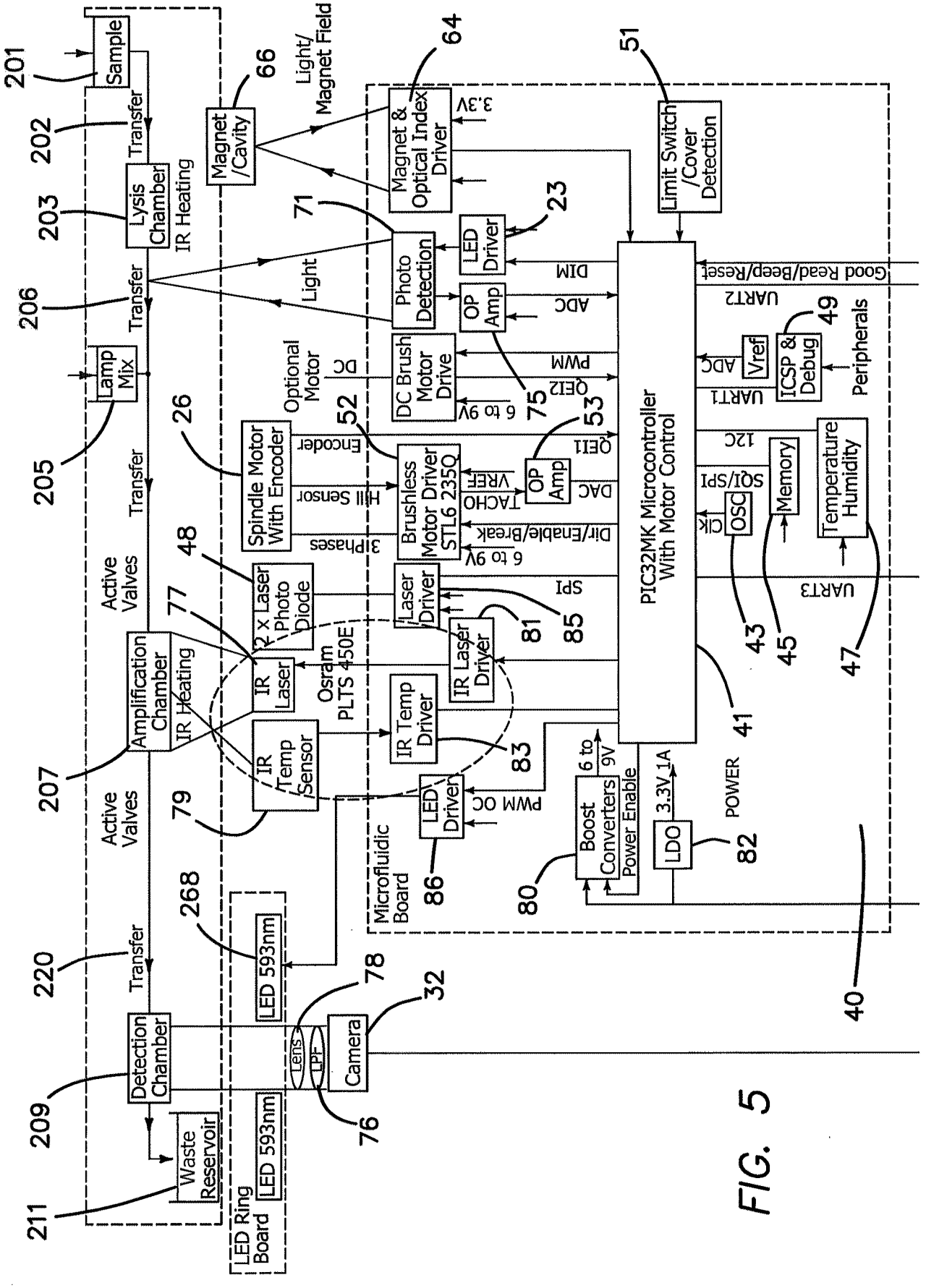

FIG. 5

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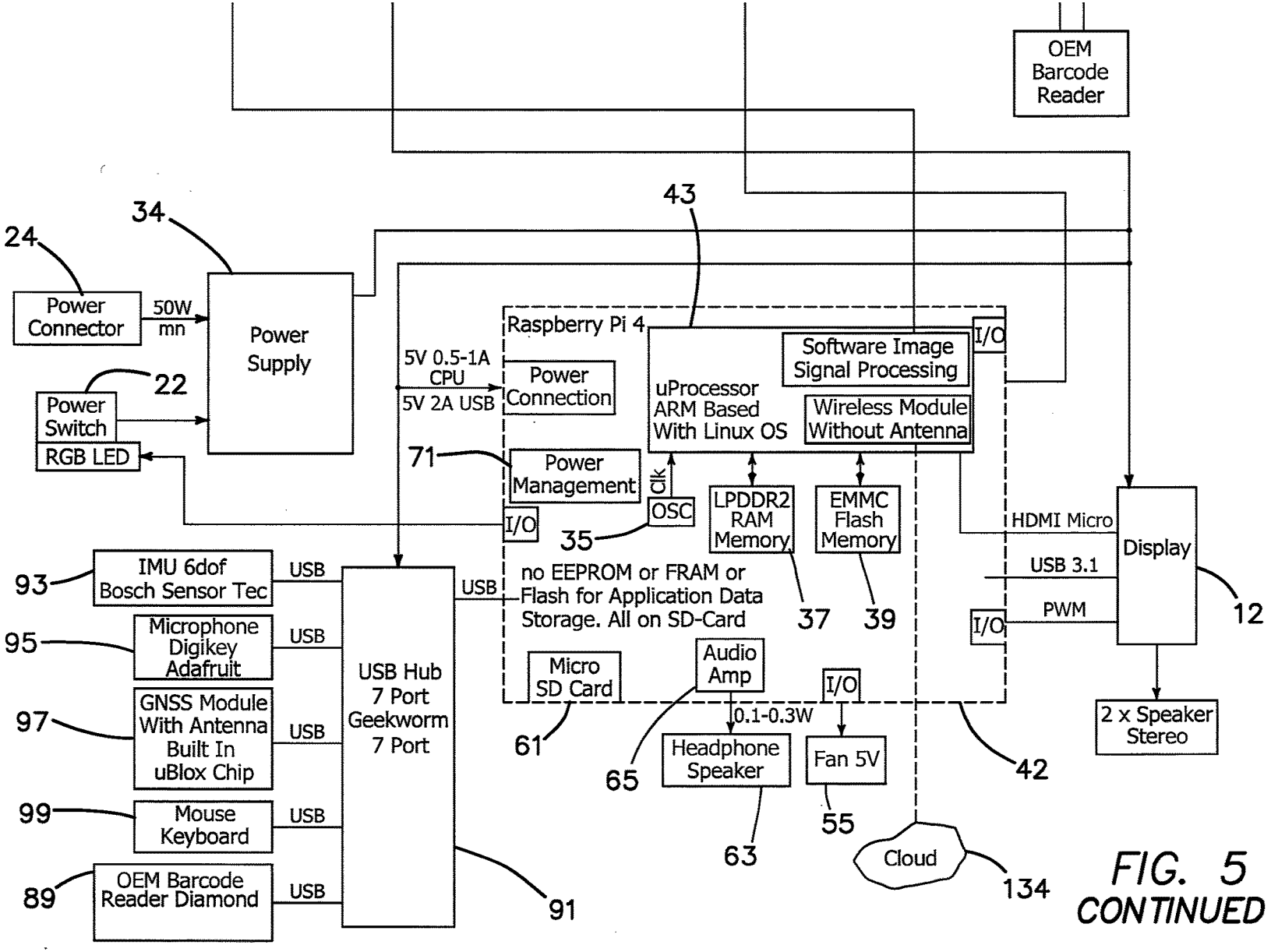

FIG. 5
CONTINUED

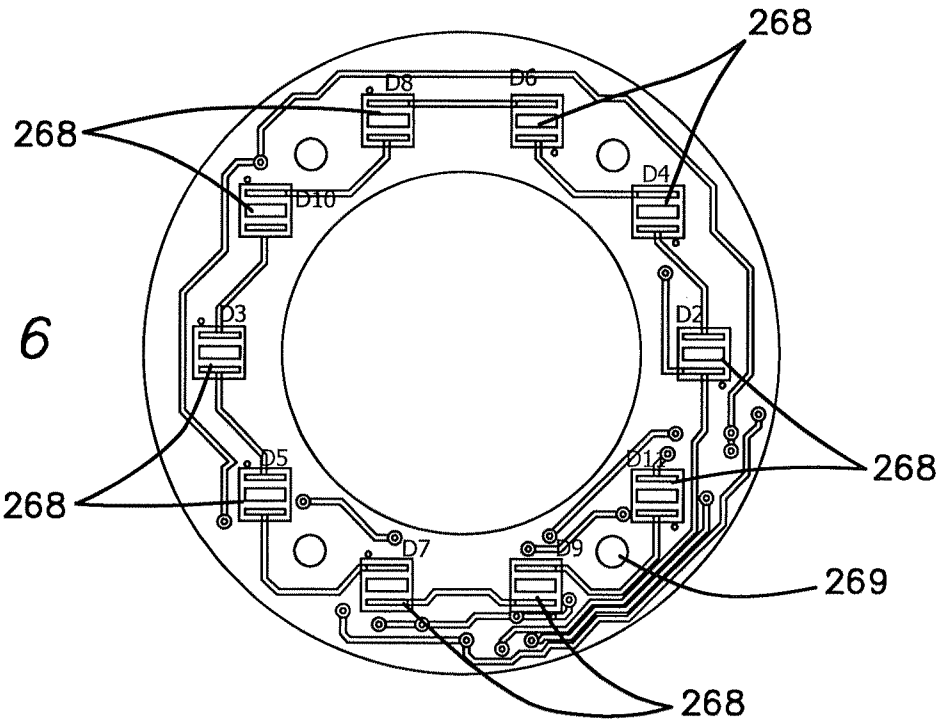

FIG. 6

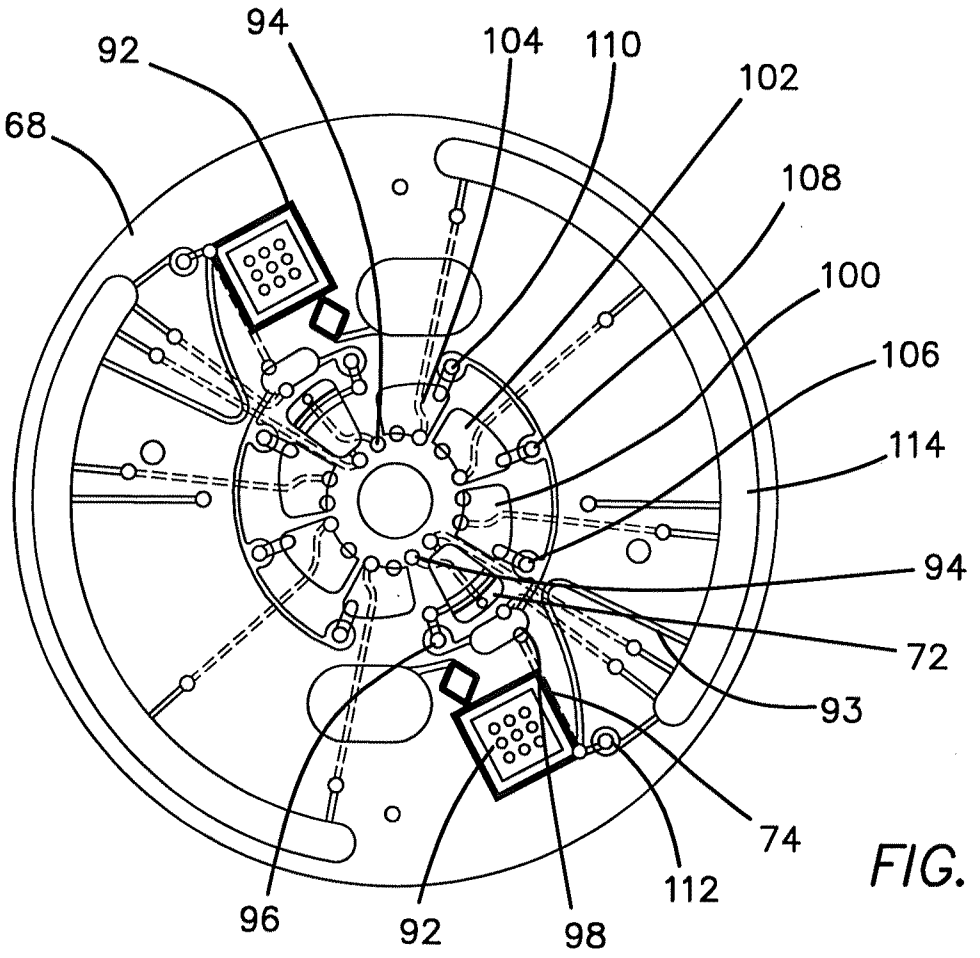

FIG. 7

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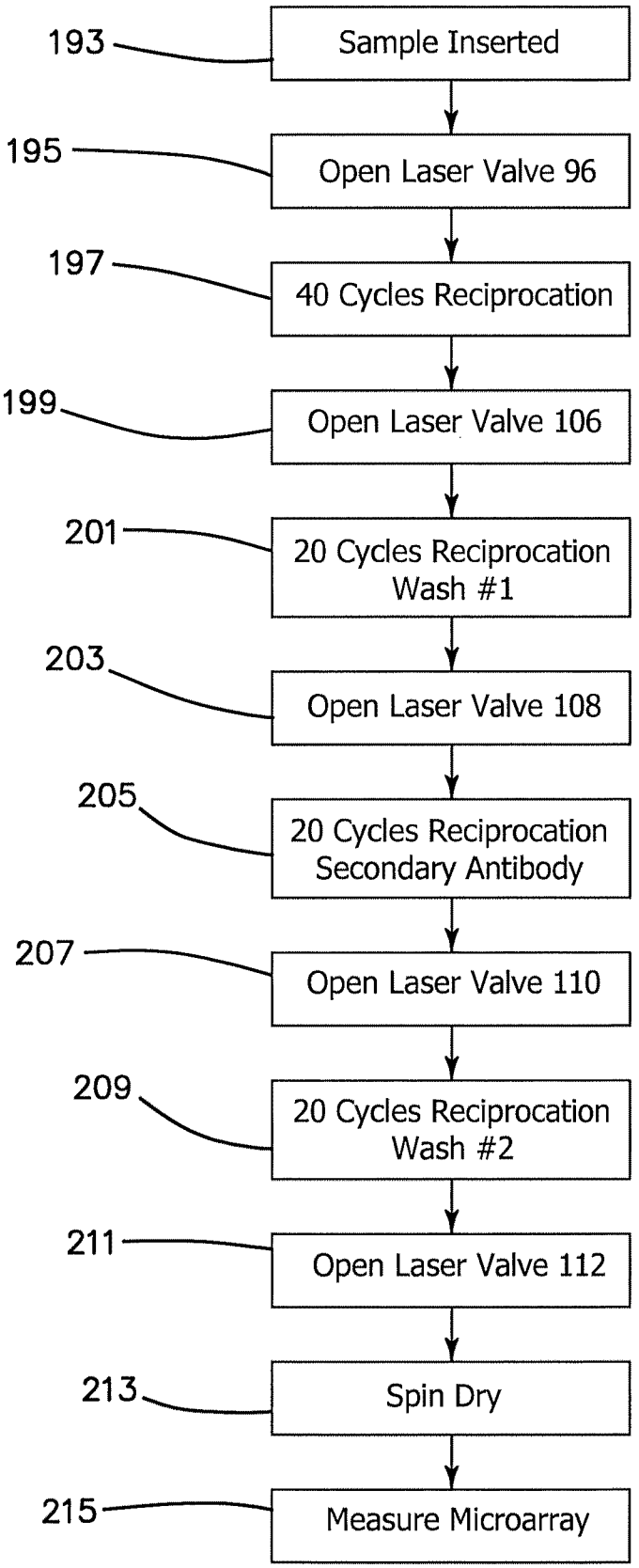

193
Sample Inserted
195
Open Laser Valve 96
197
40 Cycles Reciprocation
199
Open Laser Valve 106
201
20 Cycles Reciprocation
Wash #1
203
Open Laser Valve 108
205
20 Cycles Reciprocation
Secondary Antibody
207
Open Laser Valve 110
209
20 Cycles Reciprocation
Wash #2
211
Open Laser Valve 112
213
Spin Dry
215
Measure Microarray

FIG. 8

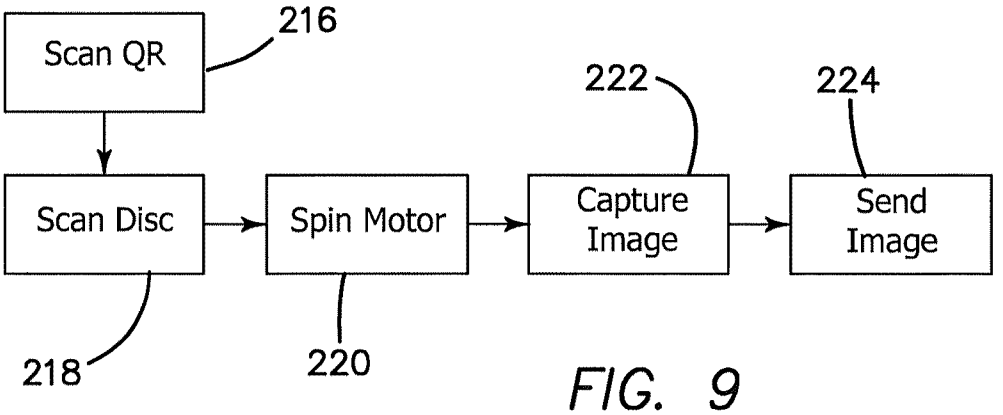


FIG. 9

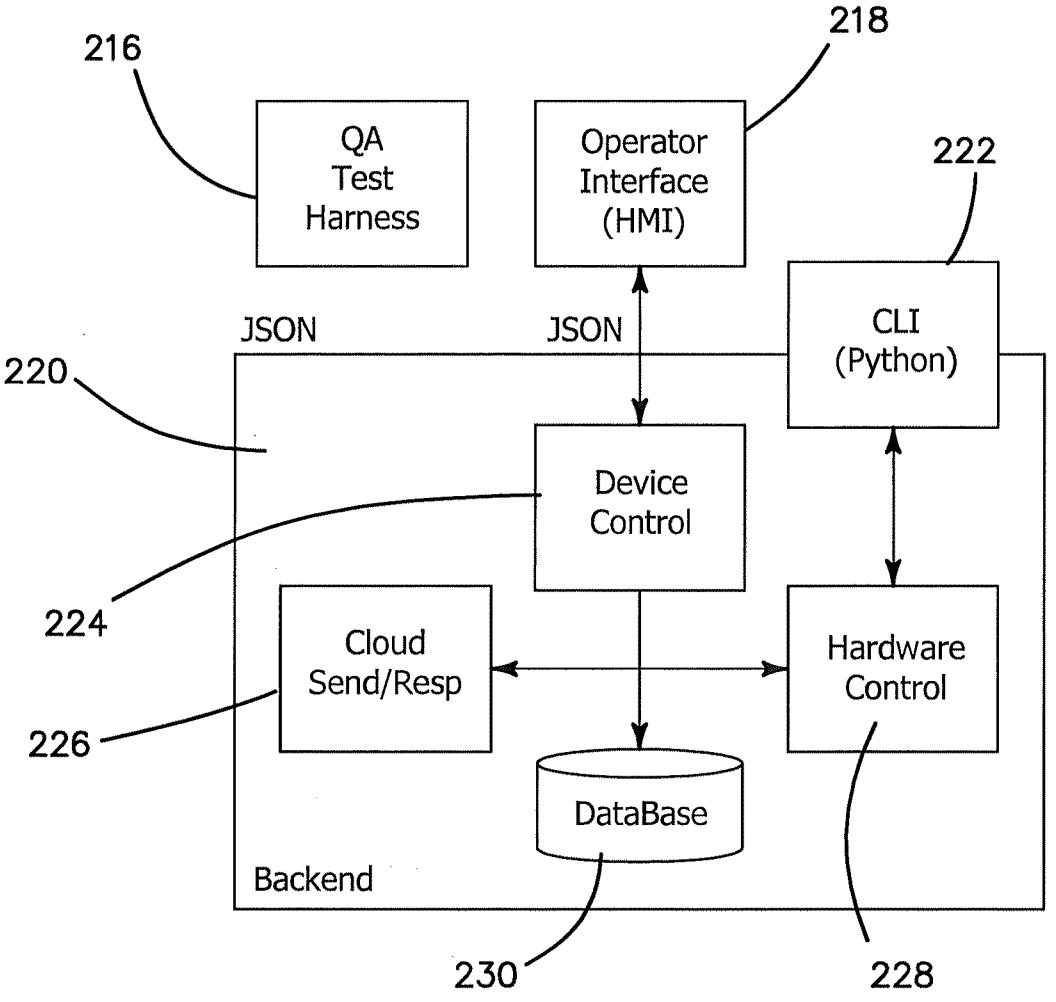


FIG. 10

Date Reçue/Date Received 2020-11-04

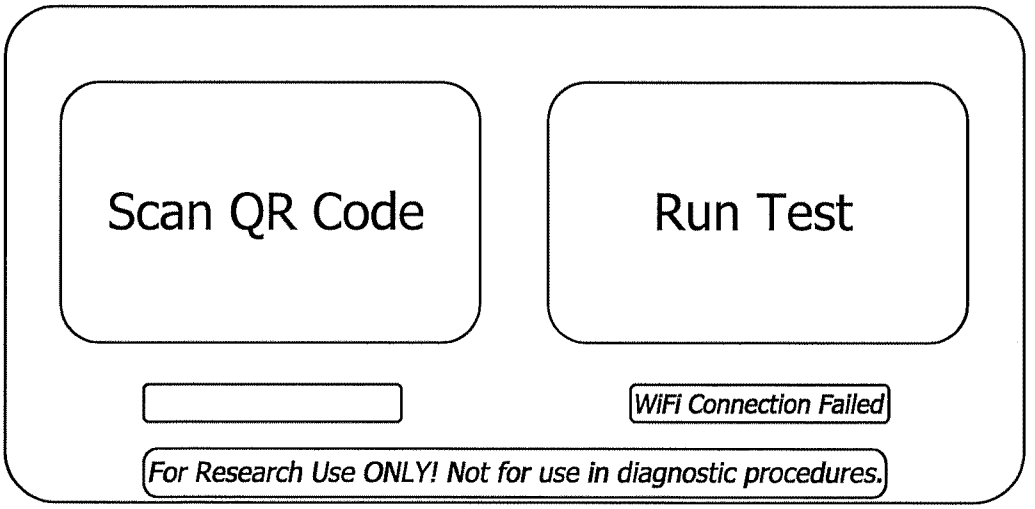


FIG. 11

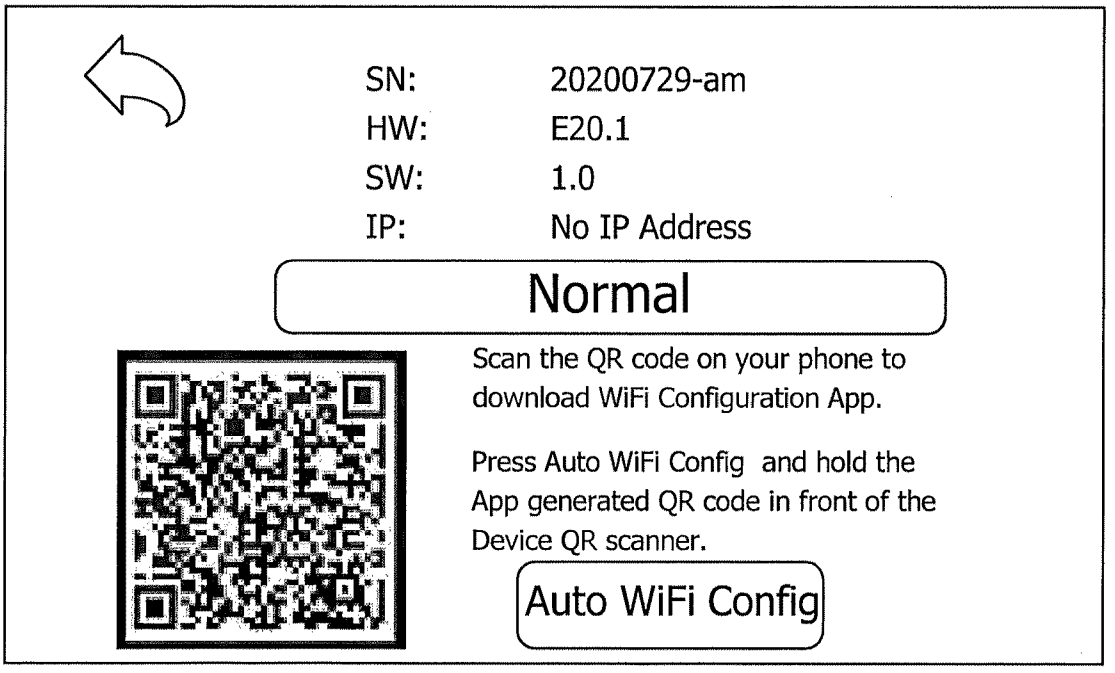


FIG. 12

Date Reçue/Date Received 2020-11-04

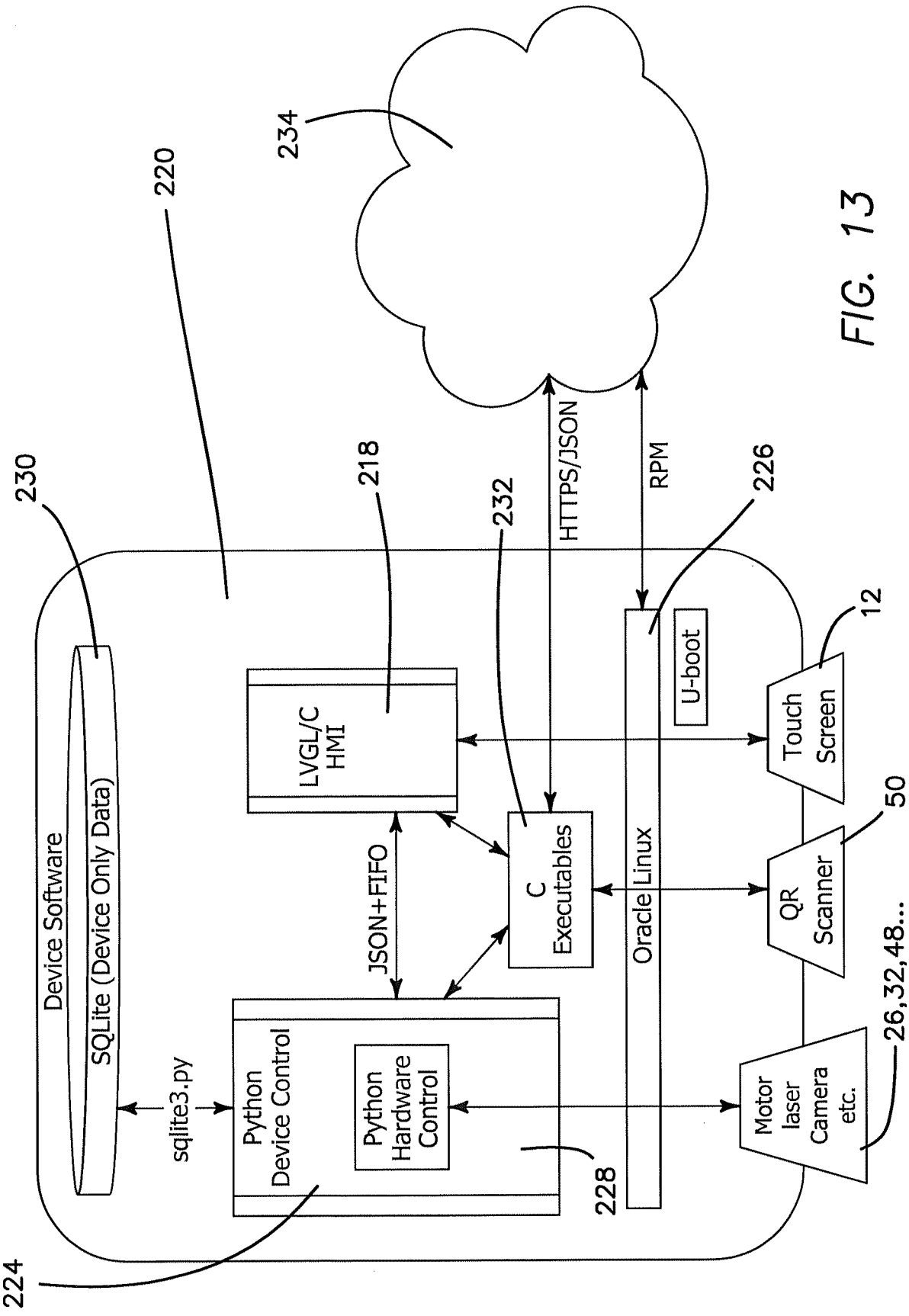

234
220
230
218
232
HTTPS/JSON
RPM
226
12
Device Software
SQLite (Device Only Data)
LVGL/C HMI
U-boot
Touch Screen
JSON+FIFO
C Executables
Oracle Linux
QR Scanner
50
sqlite3.py
Python Device Control
Python Hardware Control
Motor laser Camera etc.
26,32,48...
228
224

FIG. 13

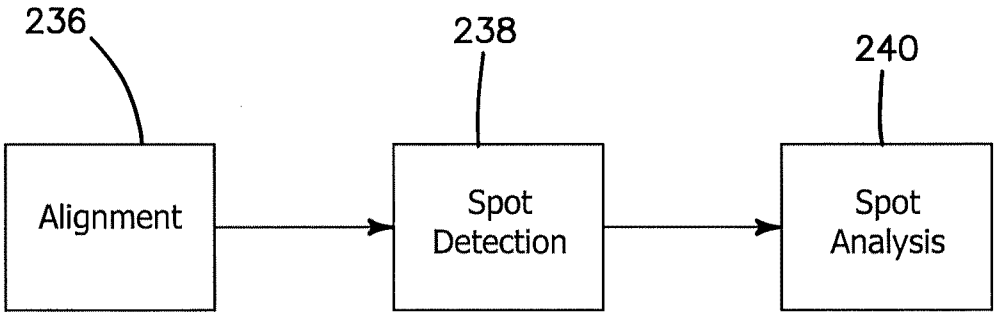

236
238
240
Alignment
Spot Detection
Spot Analysis

FIG. 14

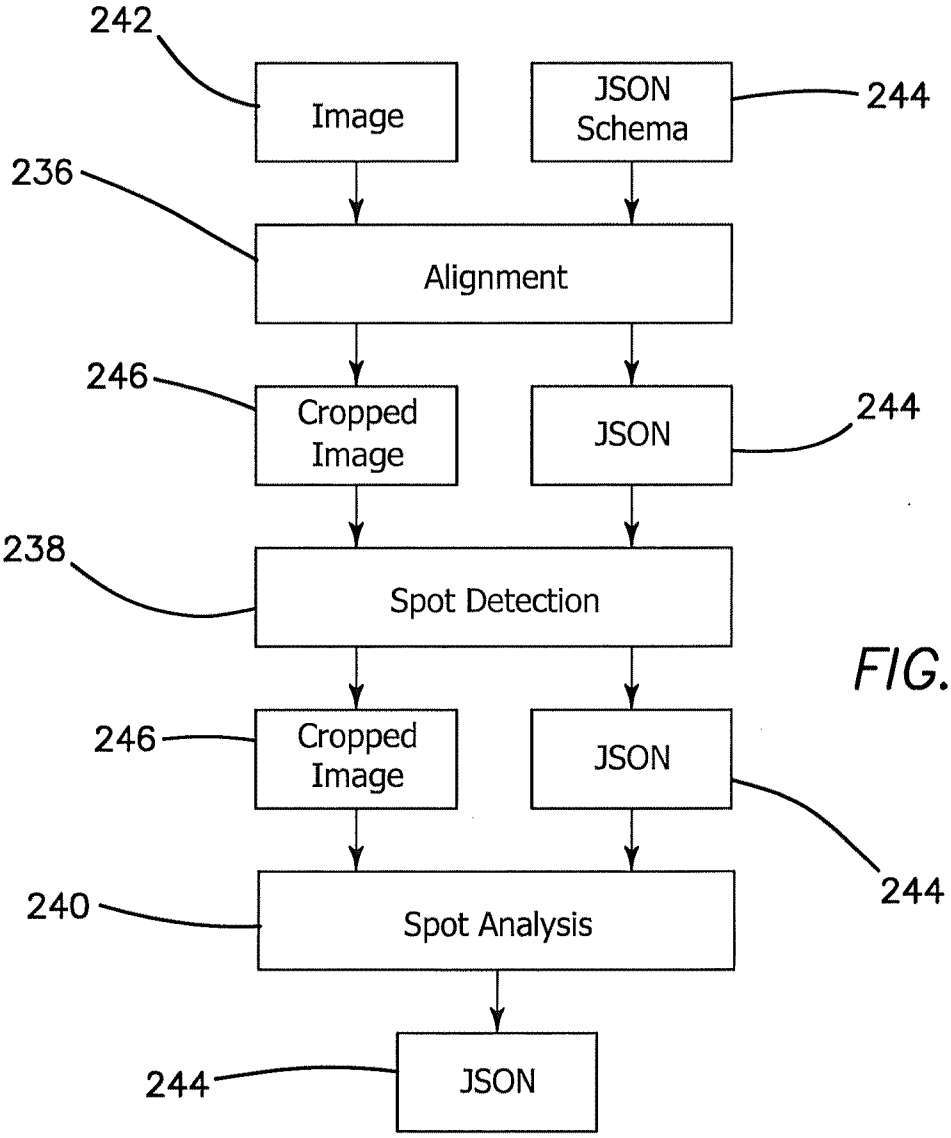

242
Image
JSON Schema
244
236
Alignment
246
Cropped Image
JSON
244
238
Spot Detection
246
Cropped Image
JSON
244
240
Spot Analysis
244
JSON

FIG. 15

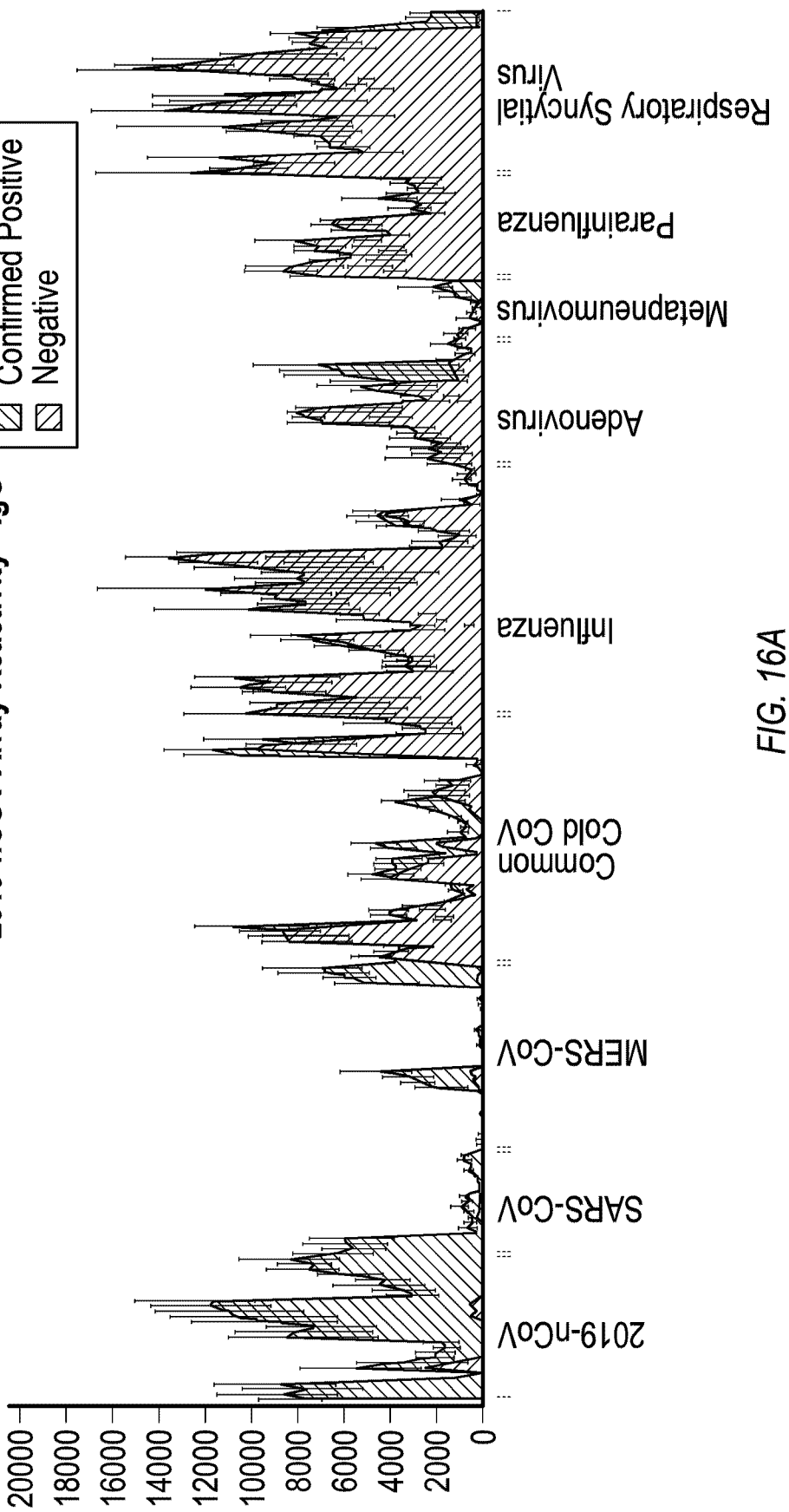

2019-nCOV Array Reactivity - IgG
Confirmed Positive
Negative
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
2019-nCoV
SARS-CoV
MERS-CoV
Common Cold CoV
Influenza
Adenovirus
Metapneumovirus
Parainfluenza
Respiratory Syncytial Virus

FIG. 16A

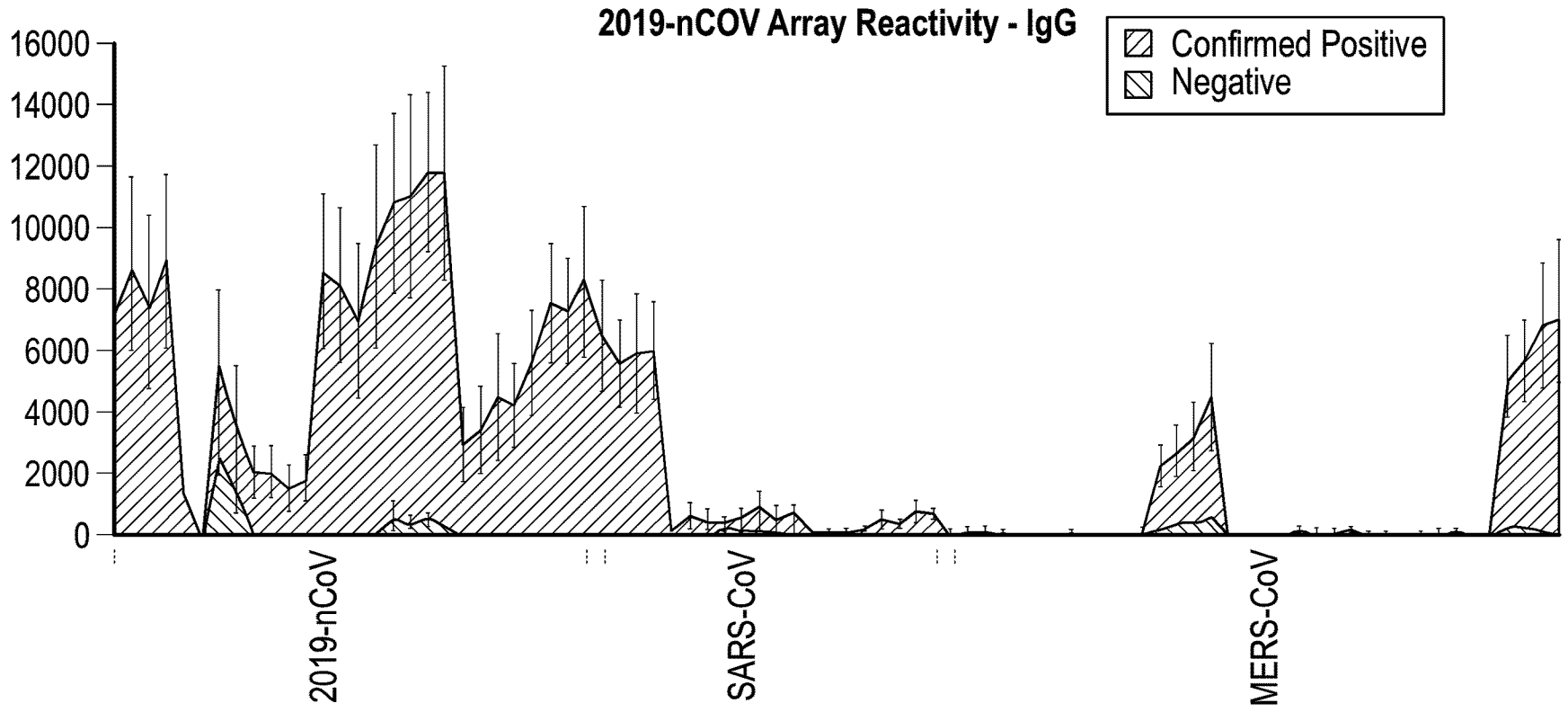


FIG. 16B

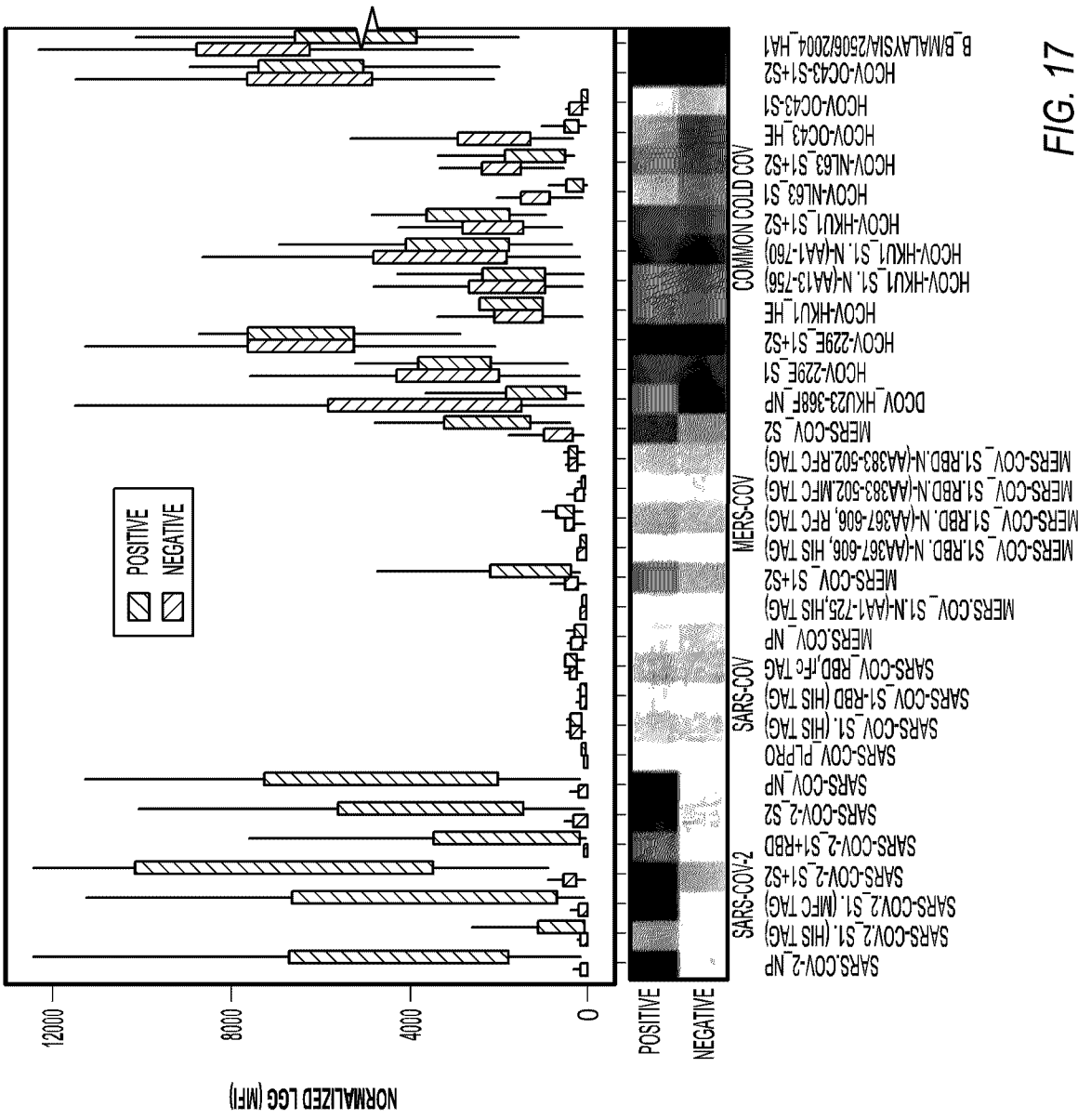

POSITIVE
NEGATIVE
12000
8000
4000
0
NORMALIZED LOG (MFI)
POSITIVE
NEGATIVE
SARS-COV-2
SARS-COV
MERS-COV
COMMON COLD COV
SARS.COV-2_NP
SARS-COV.2_S1. (HIS TAG)
SARS-COV.2_S1. (MFC TAG)
SARS-COV-2_S1+S2
SARS-COV-2_S1+RBD
SARS-COV-2_S2
SARS-COV_NP
SARS-COV_PLPRO
SARS-COV_S1. (HIS TAG)
SARS-COV_S1-RBD (HIS TAG)
SARS-COV_RBD,rFc TAG
MERS.COV_NP
MERS.COV_S1.N-(AA1-725,HIS TAG)
MERS-COV_S1+S2
MERS-COV_S1.RBD. N-(AA367-606, HIS TAG)
MERS-COV_S1.RBD. N-(AA367-606, RFC TAG)
MERS-COV_S1.RBD.N-(AA383-502.MFC TAG)
MERS-COV_S1.RBD.N-(AA383-502.RFC TAG)
MERS-COV_S2
DCOV_HKU23-368F_NP
HCOV-229E_S1
HCOV-229E_S1+S2
HCOV-HKU1_HE
HCOV-HKU1_S1. N-(AA13-756)
HCOV-HKU1_S1. N-(AA1-760)
HCOV-HKU1_S1+S2
HCOV-NL63_S1
HCOV-NL63_S1+S2
HCOV-OC43_HE
HCOV-OC43-S1
HCOV-OC43-S1+S2
B_B/MALAYSIA/2506/2004_HA1

FIG. 17

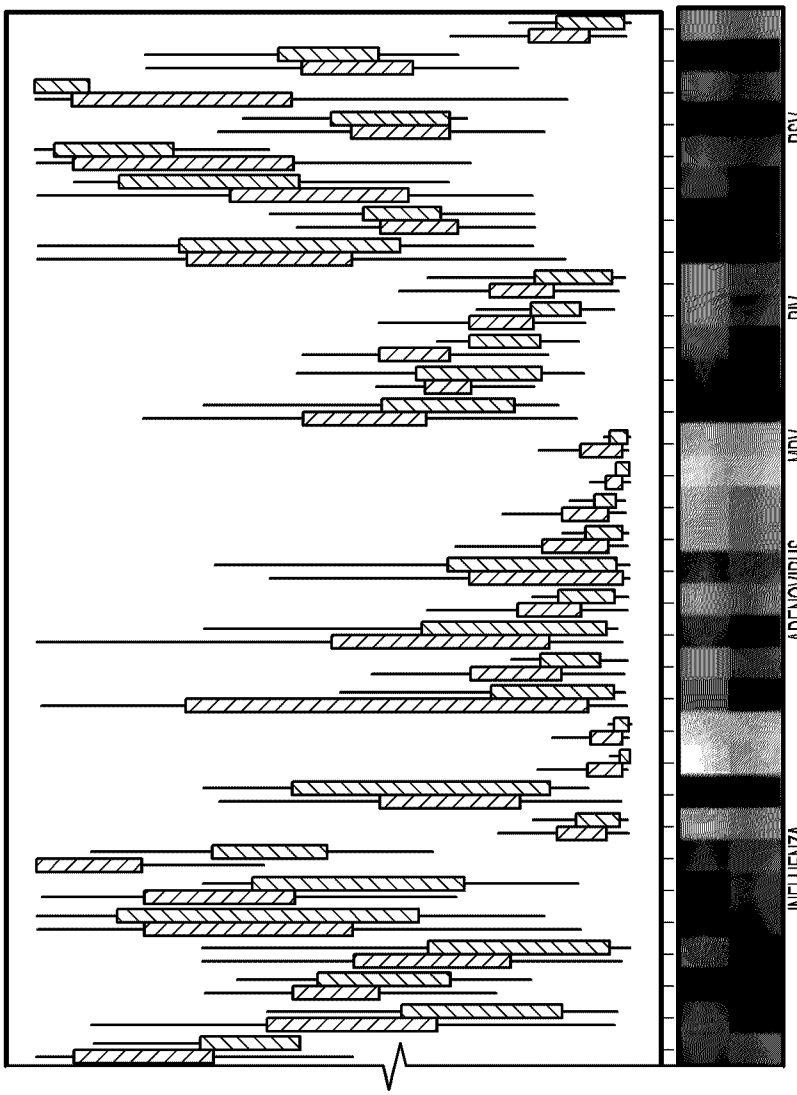


FIG. 17
(Continued)

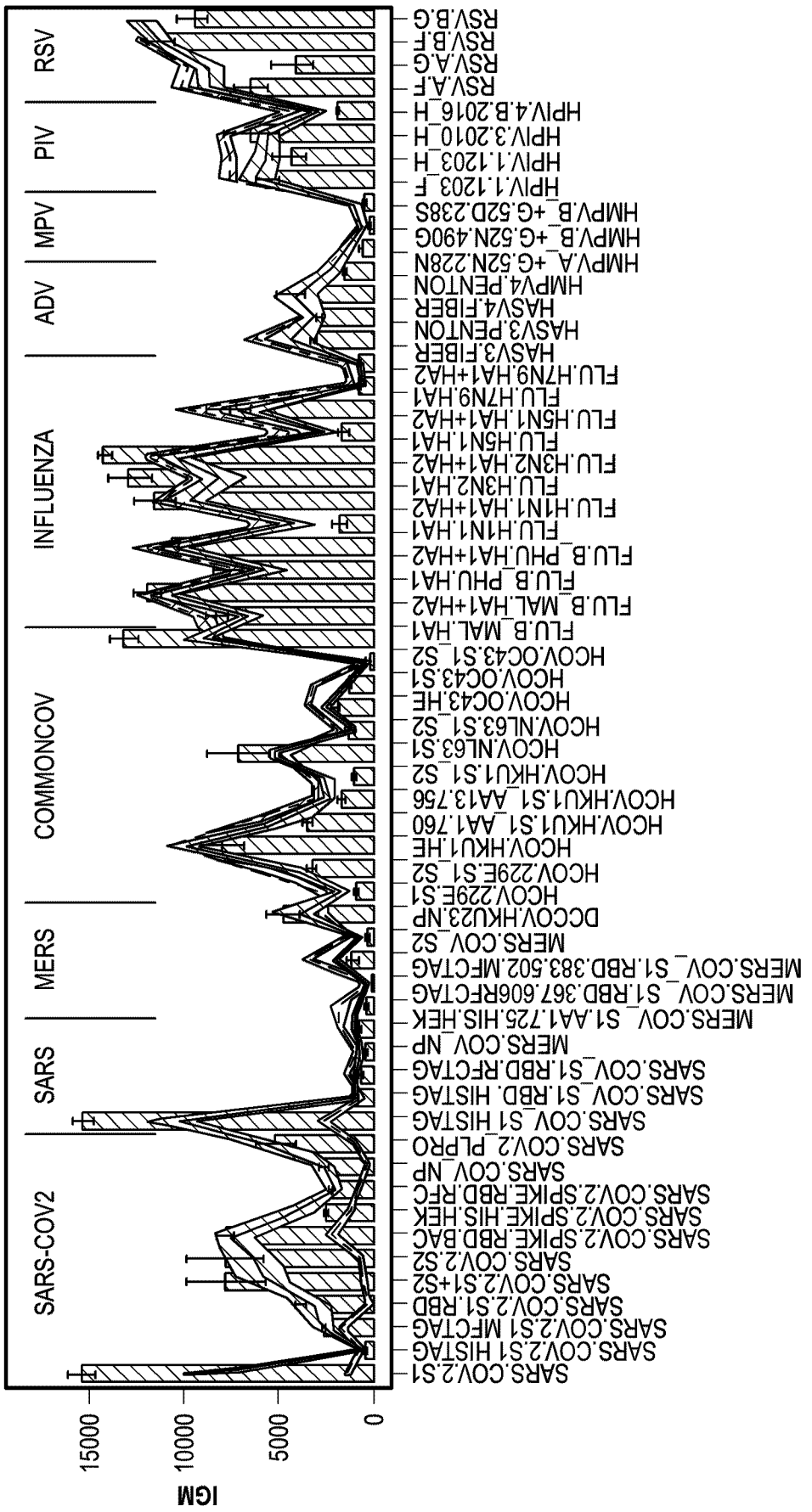

IGM
15000
10000
5000
0
SARS-COV2
SARS
MERS
COMMONCOV
INFLUENZA
ADV
MPV
PIV
RSV
SARS.COV.2.S1
SARS.COV.2.S1 HISTAG
SARS.COV.2.S1 MFCTAG
SARS.COV.2.S1.RBD
SARS.COV.2.S1+S2
SARS.COV.2.S2
SARS.COV.2.SPIKE.RBD.BAC
SARS.COV.2.SPIKE.HIS.HEK
SARS.COV.2.SPIKE.RBD.RFC
SARS.COV_NP
SARS.COV.2_PLPRO
SARS.COV_S1 HISTAG
SARS.COV_S1.RBD. HISTAG
SARS.COV_S1.RBD.RFCTAG
MERS.COV_NP
MERS.COV_S1.AA1.725.HIS.HEK
MERS.COV_S1.RBD.367.606RFCTAG
MERS.COV_S1.RBD.383.502.MFCTAG
MERS.COV_S2
DCCOV.HKU23.NP
HCOV.229E.S1
HCOV.229E.S1_S2
HCOV.HKU1_HE
HCOV.HKU1.S1_AA1.760
HCOV.HKU1.S1_AA13.756
HCOV.HKU1.S1_S2
HCOV.NL63.S1
HCOV.NL63.S1_S2
HCOV.OC43_HE
HCOV.OC43.S1
HCOV.OC43.S1_S2
FLU.B_MAL.HA1
FLU.B_MAL.HA1+HA2
FLU.B_PHU.HA1
FLU.B_PHU.HA1+HA2
FLU.H1N1.HA1
FLU.H1N1.HA1+HA2
FLU.H3N2.HA1
FLU.H3N2.HA1+HA2
FLU.H5N1.HA1
FLU.H5N1.HA1+HA2
FLU.H7N9.HA1
FLU.H7N9.HA1+HA2
HASV3.FIBER
HASV3.PENTON
HASV4.FIBER
HMPV4.PENTON
HMPV.A_+G.52N.228N
HMPV.B_+G.52N.490G
HMPV.B_+G.52D.238S
HPIV.1.1203_F
HPIV.1.1203_H
HPIV.3.2010_H
HPIV.4.B.2016_H
RSV.A.F
RSV.A.G
RSV.B.F
RSV.B.G

FIG. 18A

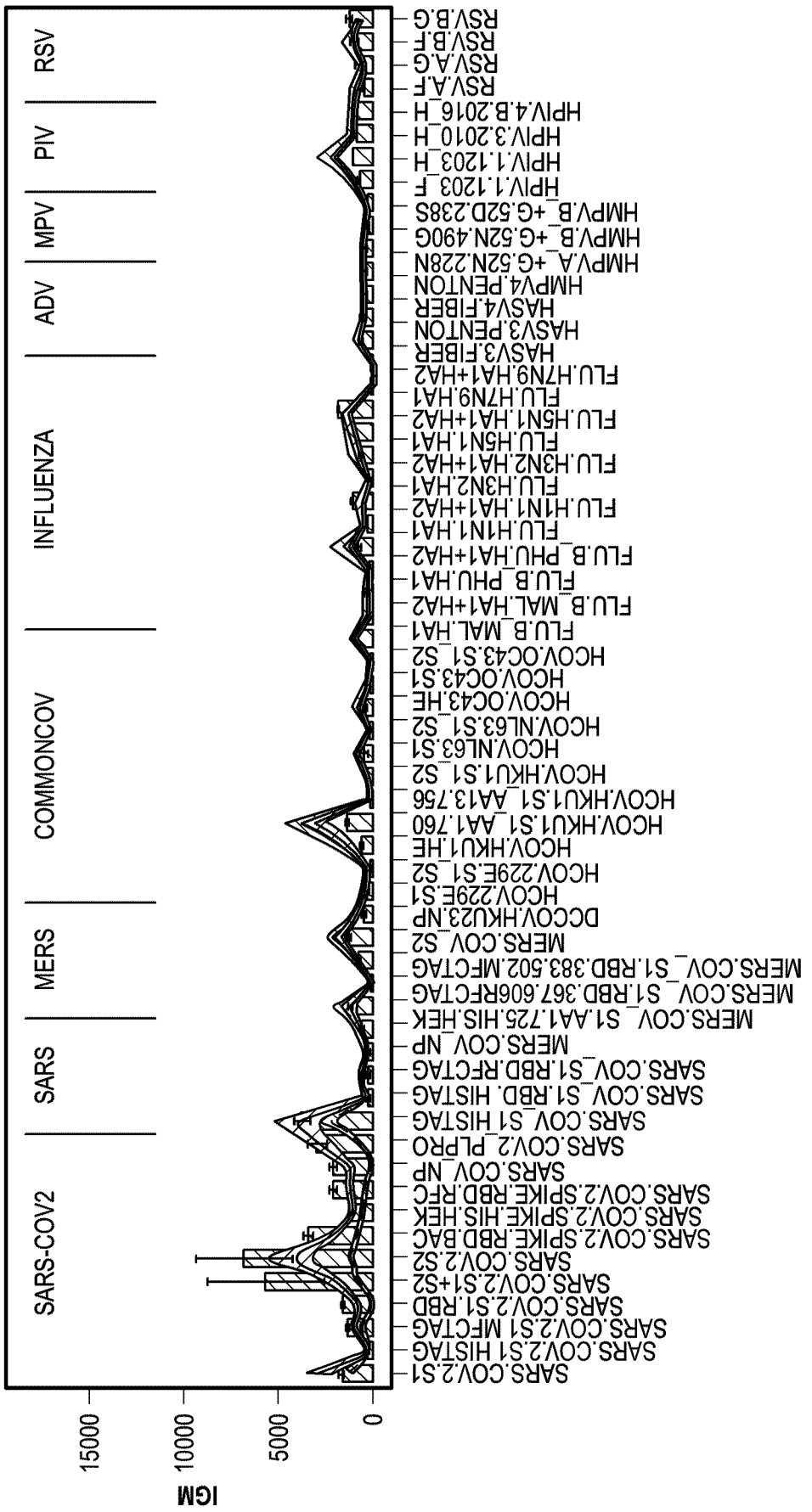

IGM
15000
10000
5000
0
SARS-COV2
SARS
MERS
COMMONCOV
INFLUENZA
ADV
MPV
PIV
RSV
SARS.COV.2.S1
SARS.COV.2.S1 HISTAG
SARS.COV.2.S1 MFCTAG
SARS.COV.2.S1.RBD
SARS.COV.2.S1+S2
SARS.COV.2.S2
SARS.COV.2.SPIKE.RBD.BAC
SARS.COV.2.SPIKE.HIS.HEK
SARS.COV.2.SPIKE.RBD.RFC
SARS.COV_NP
SARS.COV.2_PLPRO
SARS.COV_S1 HISTAG
SARS.COV_S1.RBD. HISTAG
SARS.COV_S1.RBD.RFCTAG
MERS.COV_NP
MERS.COV_S1.AA1.725.HIS.HEK
MERS.COV_S1.RBD.367.606RFCTAG
MERS.COV_S1.RBD.383.502.MFCTAG
MERS.COV_S2
DCCOV.HKU23.NP
HCOV.229E.S1
HCOV.229E.S1_S2
HCOV.HKU1.HE
HCOV.HKU1.S1_AA1.760
HCOV.HKU1.S1_AA13.756
HCOV.HKU1.S1_S2
HCOV.NL63.S1
HCOV.NL63.S1_S2
HCOV.OC43.HE
HCOV.OC43.S1
HCOV.OC43.S1_S2
FLU.B_MAL.HA1
FLU.B_MAL.HA1+HA2
FLU.B_PHU.HA1
FLU.B_PHU.HA1+HA2
FLU.H1N1.HA1
FLU.H1N1.HA1+HA2
FLU.H3N2.HA1
FLU.H3N2.HA1+HA2
FLU.H5N1.HA1
FLU.H5N1.HA1+HA2
FLU.H7N9.HA1
FLU.H7N9.HA1+HA2
HASV3.FIBER
HASV3.PENTON
HASV4.FIBER
HMPV4.PENTON
HMPV.A_+G.52N.228N
HMPV.B_+G.52N.490G
HMPV.B_+G.52D.238S
HPIV.1.1203_F
HPIV.1.1203_H
HPIV.3.2010_H
HPIV.4.B.2016_H
RSV.A.F
RSV.A.G
RSV.B.F
RSV.B.G

FIG. 18B

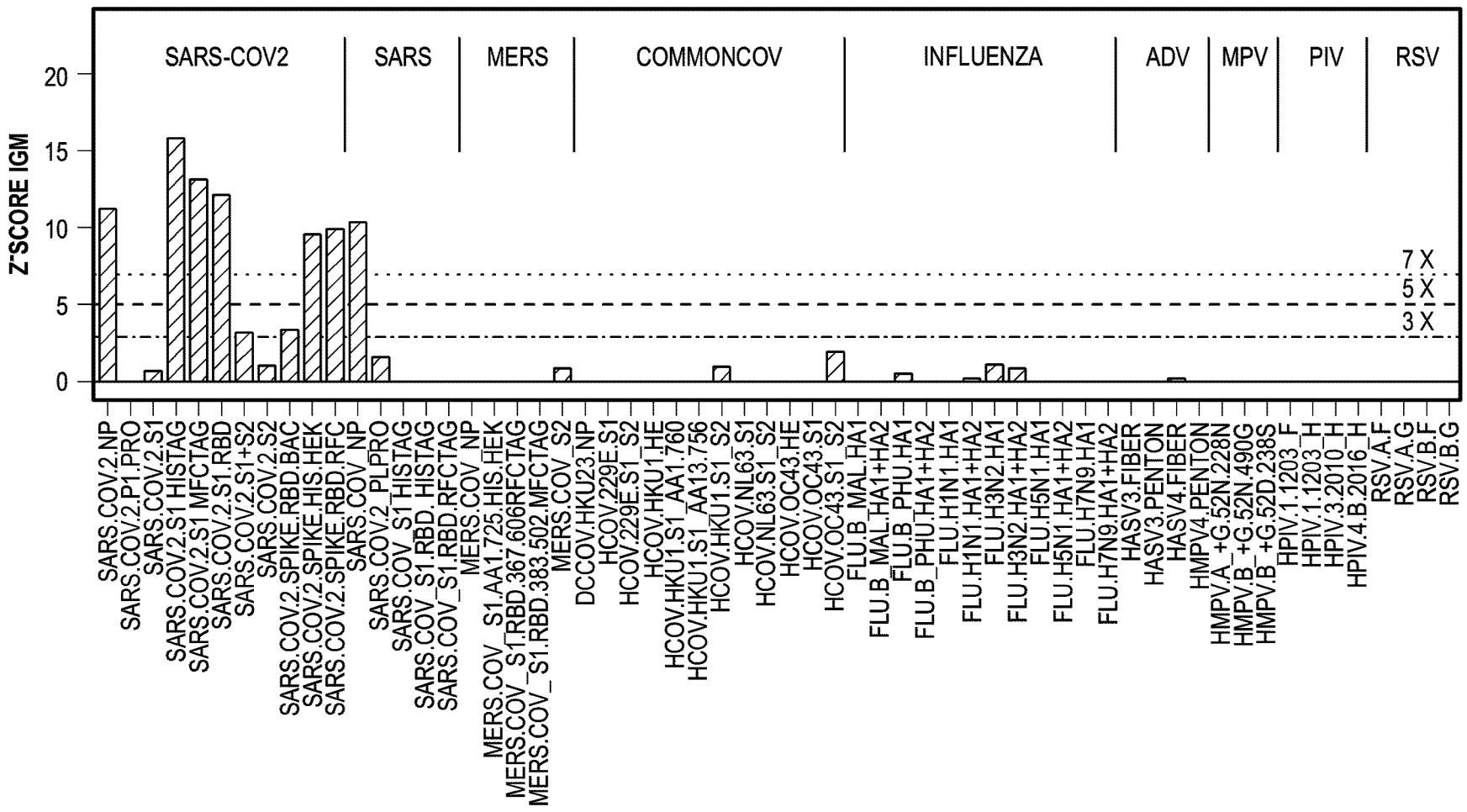


FIG. 18C

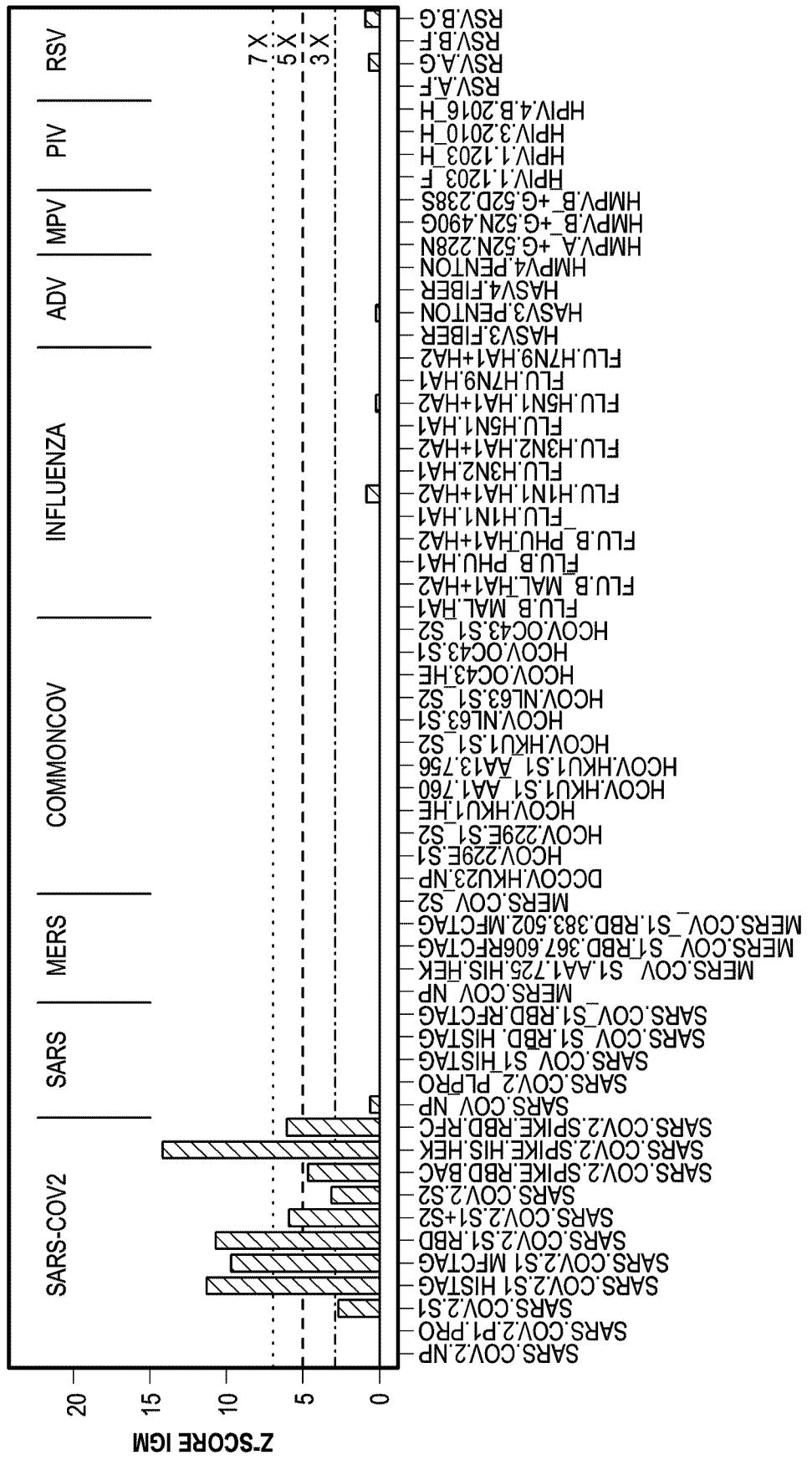

Z-SCORE IGM
0
5
10
15
20
3 X
5 X
7 X
SARS-COV2
SARS
MERS
COMMONCOV
INFLUENZA
ADV
MPV
PIV
RSV
SARS.COV2.NP
SARS.COV2.P1.PRO
SARS.COV2.S1
SARS.COV2.S1 HISTAG
SARS.COV2.S1 MFCTAG
SARS.COV2.S1.RBD
SARS.COV2.S1+S2
SARS.COV2.S2
SARS.COV2.SPIKE.RBD.BAC
SARS.COV2.SPIKE.HIS.HEK
SARS.COV2.SPIKE.RBD.RFC
SARS.COV_NP
SARS.COV2_PLPRO
SARS.COV_S1 HISTAG
SARS.COV_S1.RBD. HISTAG
SARS.COV_S1.RBD.RFCTAG
MERS.COV_NP
MERS.COV_S1.AA1.725.HIS.HEK
MERS.COV_S1.RBD.367.606RFCTAG
MERS.COV_S1.RBD.383.502.MFCTAG
MERS.COV_S2
DCCOV.HKU23.NP
HCOV.229E.S1
HCOV.229E.S1_S2
HCOV.HKU1_HE
HCOV.HKU1.S1_AA1.760
HCOV.HKU1.S1_AA13.756
HCOV.HKU1.S1_S2
HCOV.NL63.S1
HCOV.NL63.S1_S2
HCOV.OC43_HE
HCOV.OC43.S1
HCOV.OC43.S1_S2
FLU.B_MAL.HA1
FLU.B_MAL.HA1+HA2
FLU.B_PHU.HA1
FLU.B_PHU.HA1+HA2
FLU_H1N1.HA1
FLU.H1N1.HA1+HA2
FLU.H3N2.HA1
FLU.H3N2.HA1+HA2
FLU.H5N1.HA1
FLU.H5N1.HA1+HA2
FLU.H7N9.HA1
FLU.H7N9.HA1+HA2
HASV3.FIBER
HASV3.PENTON
HASV4.FIBER
HMPV4.PENTON
HMPV.A_+G.52N.228N
HMPV.B_+G.52N.490G
HMPV.B_+G.52D.238S
HPIV.1.1203_F
HPIV.1.1203_H
HPIV.3.2010_H
HPIV.4.B.2016_H
RSV.A.F
RSV.A.G
RSV.B.F
RSV.B.G

FIG. 18D

	1	2	3	4	5	6
1	Fiducial	BUFFER	BUFFER	BUFFER	BUFFER	BUFFER
2	PBSTwash	PBSTwash	a.HulgG_0.3	a.HulgG_0.1	a.HulgG_0.03	HulgG_0.3
3	PBSTwash	HulgM_0.3	HulgM_0.14	HulgM_0.06	HulgM_0.028	HulgM_0.0128
4	PBSTwash	SGC-SPIKE19200701	PBSTwash	SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP
5	SGC-SPIKE19200701	PBSTwash	HulgG_0.3	HulgG_0.14	HulgG_0.06	HulgG_0.028
6	HulgM_0.06	HulgM_0.028	HulgM_0.0128	HulgM_0.006	HulgM_0.0026	HulgM_0.001
7	SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP	PBSTwash	SGC-SPIKE19200701	PBSTwash
8	HulgG_0.14	HulgG_0.06	HulgG_0.028	HulgG_0.0128	HulgG_0.006	HulgG_0.0026
9	HulgM_0.006	HulgM_0.0026	HulgM_0.001	PBSTwash	HulgG_0.3	HulgG_0.14
10	HulgM_0.3	HulgM_0.14	HulgM_0.06	HulgM_0.028	HulgM_0.0128	HulgM_0.006
11	HulgG_0.0128	HulgG_0.006	HulgG_0.0026	HulgG_0.001	PBSTwash	HulgM_0.3
12	PBSTwash	SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP	PBSTwash	SGC-SPIKE19200701
13	Fiducial	PBSTwash	PBSTwash	a.HulgM_0.03	a.HulgM_0.1	a.HulgM_0.3

FIG. 19

7	8	9	10	11	12	13
BUFFER	BUFFER	BUFFER	BUFFER	BUFFER	BUFFER	Fiducial
HulgG_0.14	HulgG_0.06	HulgG_0.028	HulgG_0.0128	HulgG_0.006	HulgG_0.0026	HulgG_0.001
HulgM_0.006	HulgG_0.0026	HulgM_0.001	PBSTwash	SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP
PBSTwash	SGC-SPIKE19200701	PBSTwash	SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP	PBSTwash
HulgG_0.0128	HulgG_0.006	HulgG_0.0026	HulgG_0.001	PBSTwash	HulgM_0.3	HulgM_0.14
PBSTwash	SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP	PBSTwash	SGC-SPIKE19200701	PBSTwash
SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP	PBSTwash	SGC-SPIKE19200701	PBSTwash	HulgG_0.3
HulgG_0.001	PBSTwash	HulgM_0.3	HulgM_0.14	HulgM_0.06	HulgM_0.028	HulgM_0.0128
HulgG_0.06	HulgG_0.028	HulgG_0.0128	HulgG_0.006	HulgG_0.0026	HulgG_0.001	PBSTwash
HulgM_0.0026	HulgM_0.001	PBSTwash	HulgG_0.3	HulgG_0.14	HulgG_0.06	HulgG_0.028
HulgM_0.14	HulgM_0.06	HulgM_0.028	HulgM_0.0128	HulgM_0.006	HulgM_0.0026	HulgM_0.001
PBSTwash	SARS-CoV2.RBD.mFc	PBSTwash	SARS-CoV2.NP	PBSTwash	SGC-SPIKE19200701	PBSTwash
BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK

FIG. 19
CONTINUED

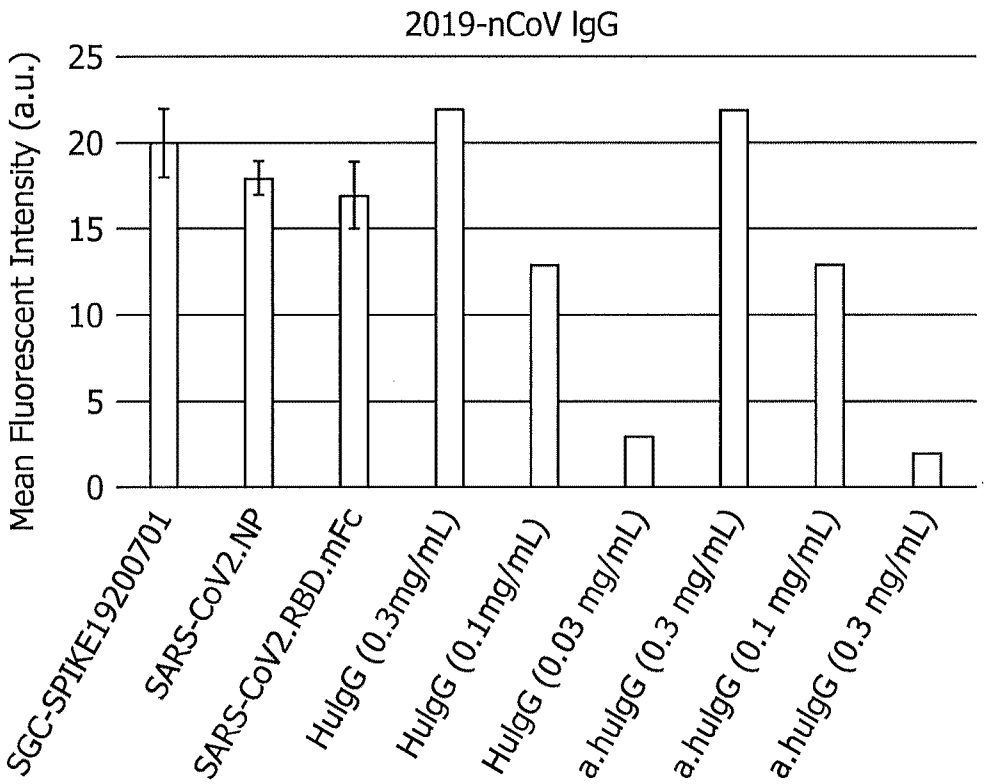


FIG. 20

Date Reçue/Date Received 2020-11-04

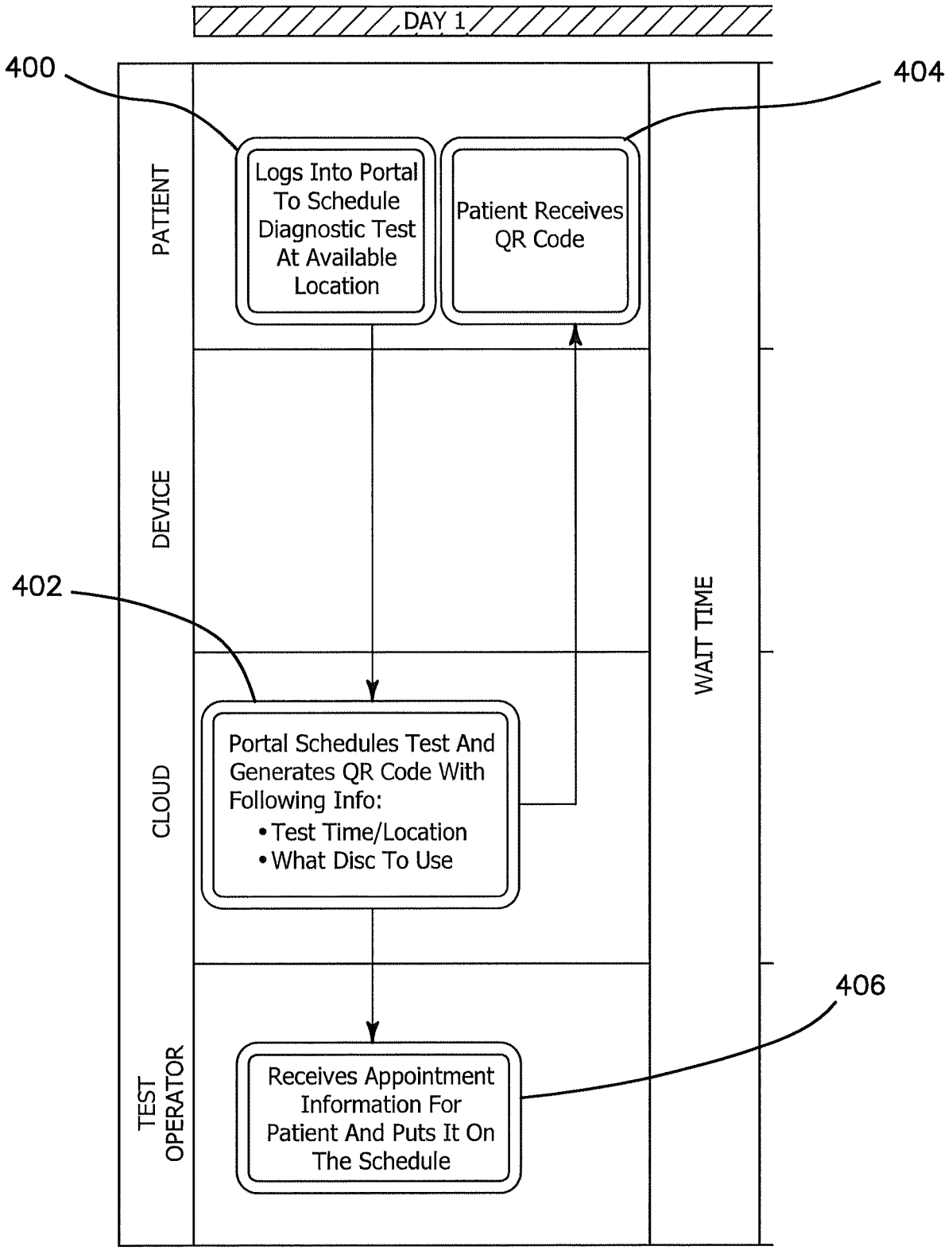


FIG. 21A

Date Reçue/Date Received 2020-11-04

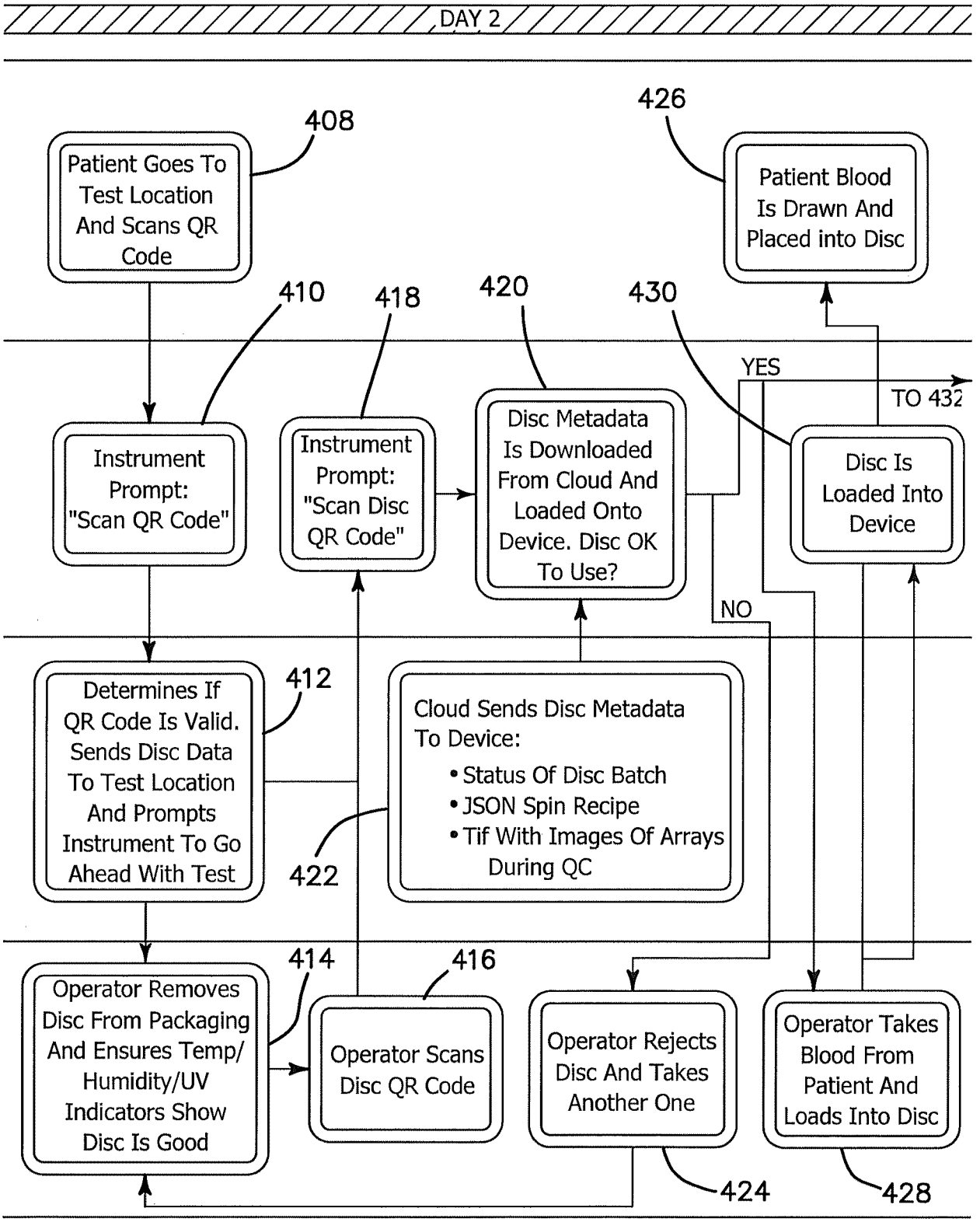


FIG. 21B

Date Reçue/Date Received 2020-11-04

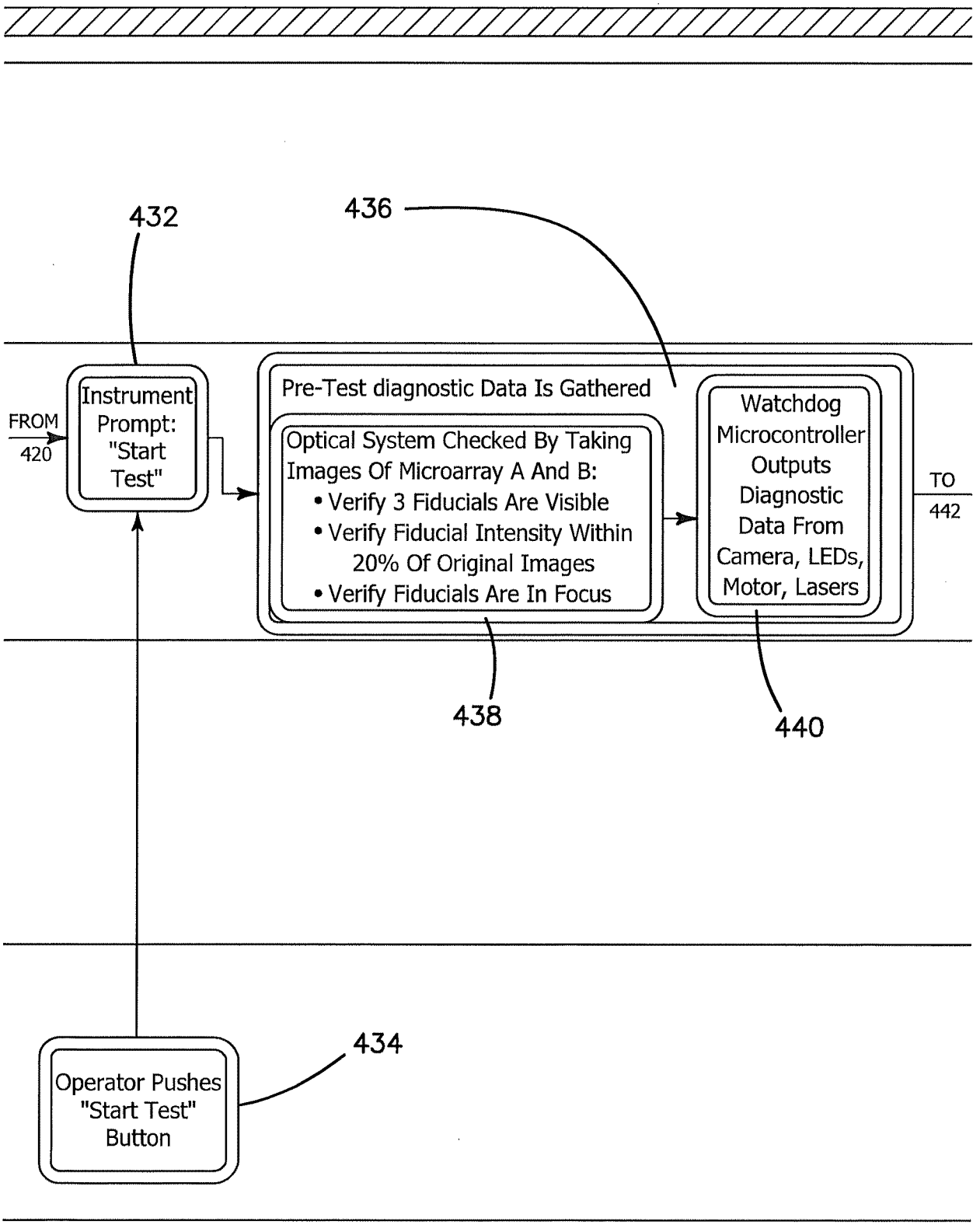


FIG. 21C

Date Reçue/Date Received 2020-11-04

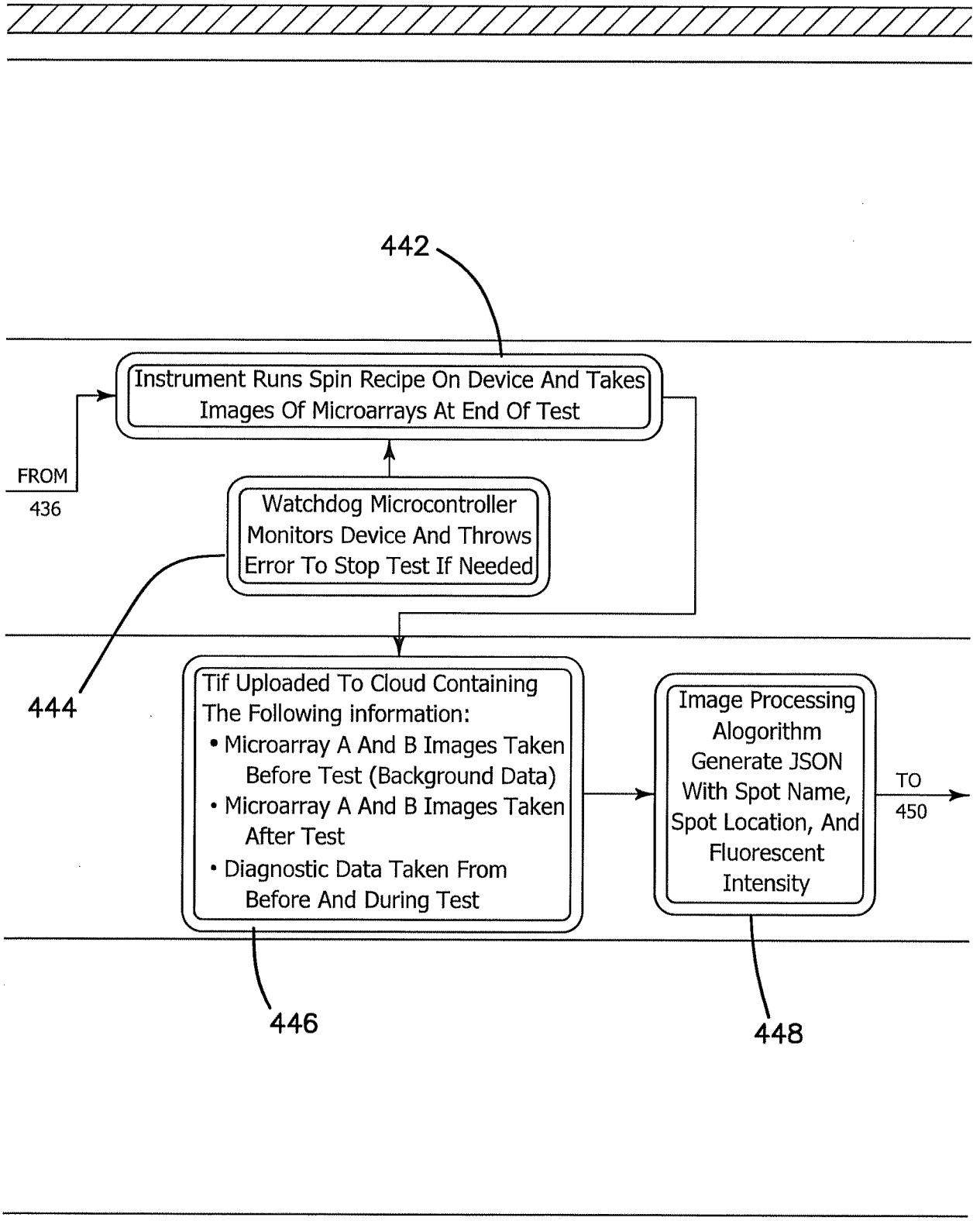


FIG. 21D

Date Reçue/Date Received 2020-11-04

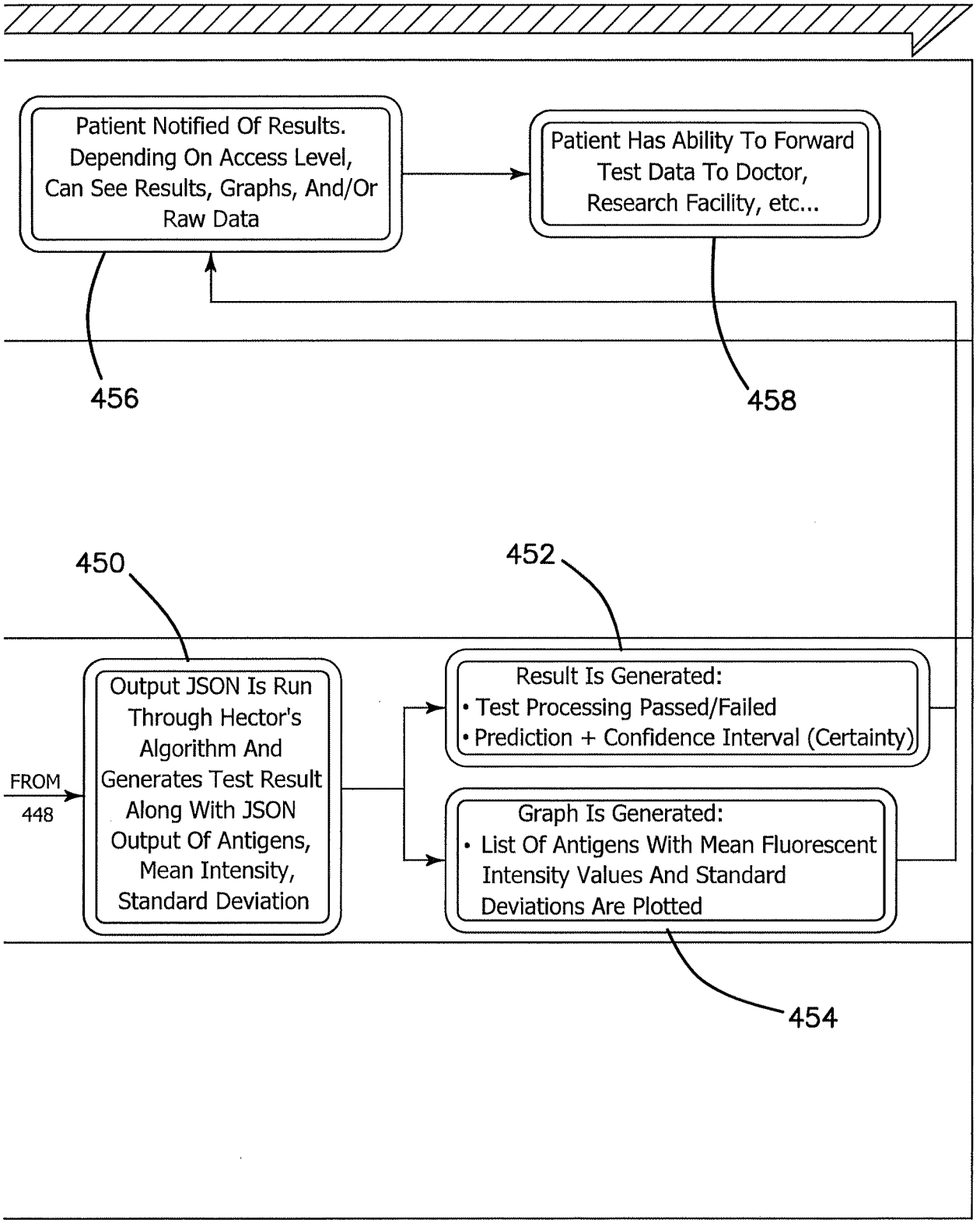


FIG. 21E

Date Reçue/Date Received 2020-11-04

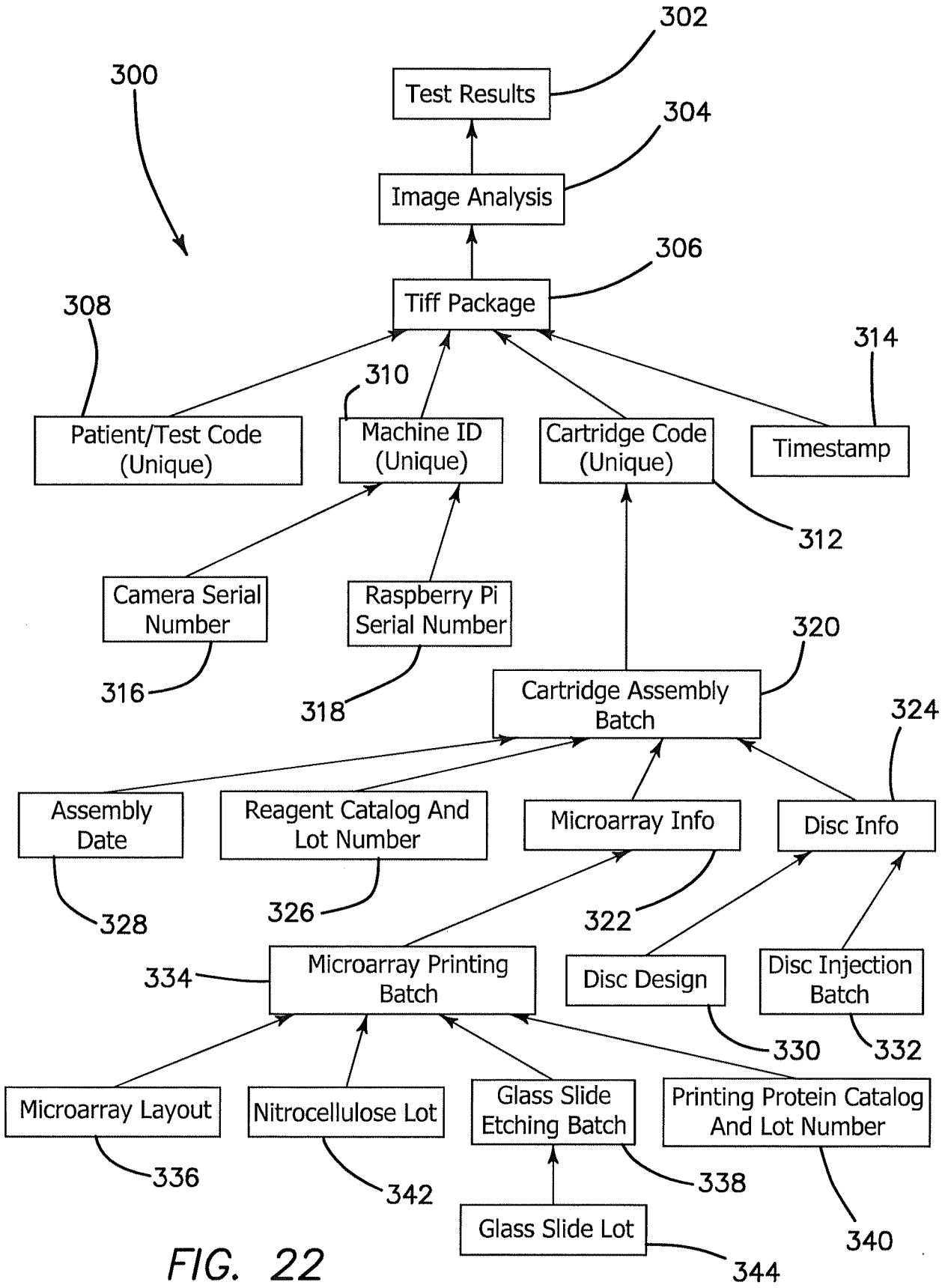

300
302
Test Results
304
Image Analysis
306
Tiff Package
308
Patient/Test Code (Unique)
310
Machine ID (Unique)
Cartridge Code (Unique)
312
314
Timestamp
Camera Serial Number
316
Raspberry Pi Serial Number
318
320
Cartridge Assembly Batch
324
Assembly Date
328
Reagent Catalog And Lot Number
326
Microarray Info
322
Disc Info
334
Microarray Printing Batch
Disc Design
330
Disc Injection Batch
332
Microarray Layout
336
Nitrocellulose Lot
342
Glass Slide Etching Batch
338
Printing Protein Catalog And Lot Number
340
Glass Slide Lot
344

FIG. 22

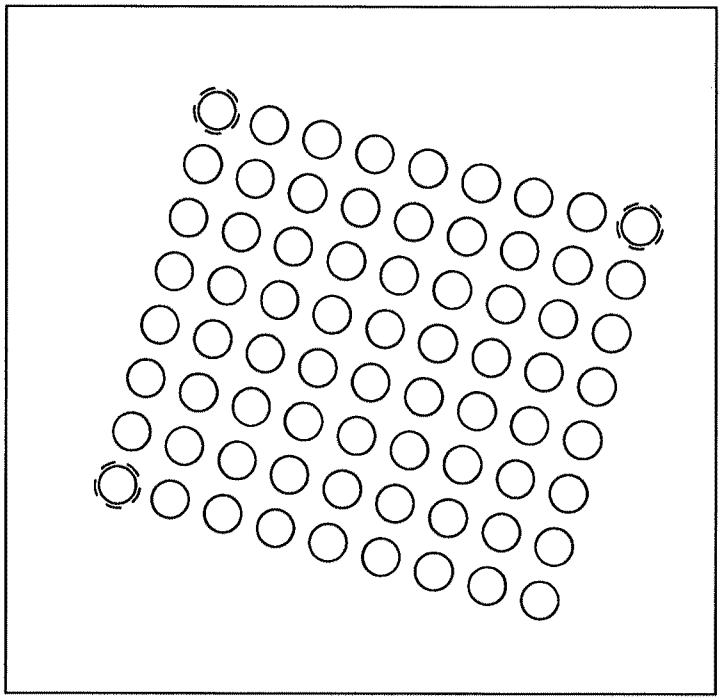


FIG. 22A

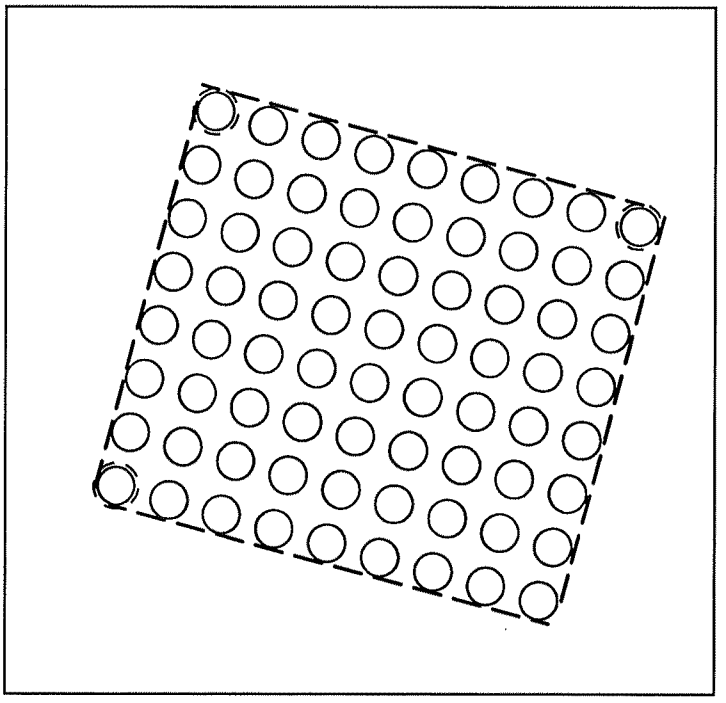


FIG. 22B

Date Reçue/Date Received 2020-11-04

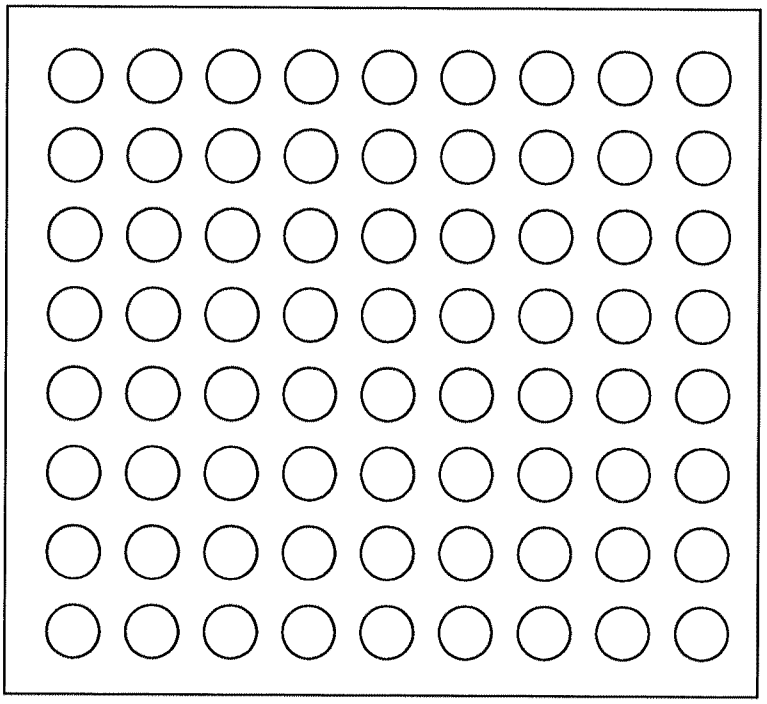


FIG. 22C

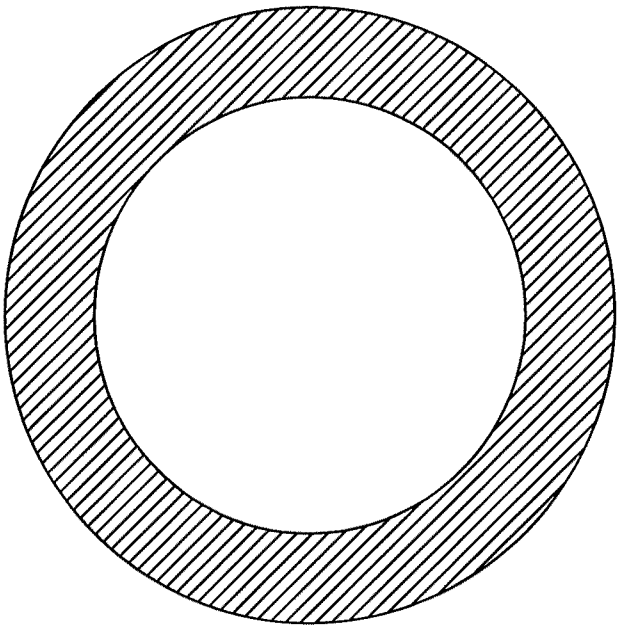
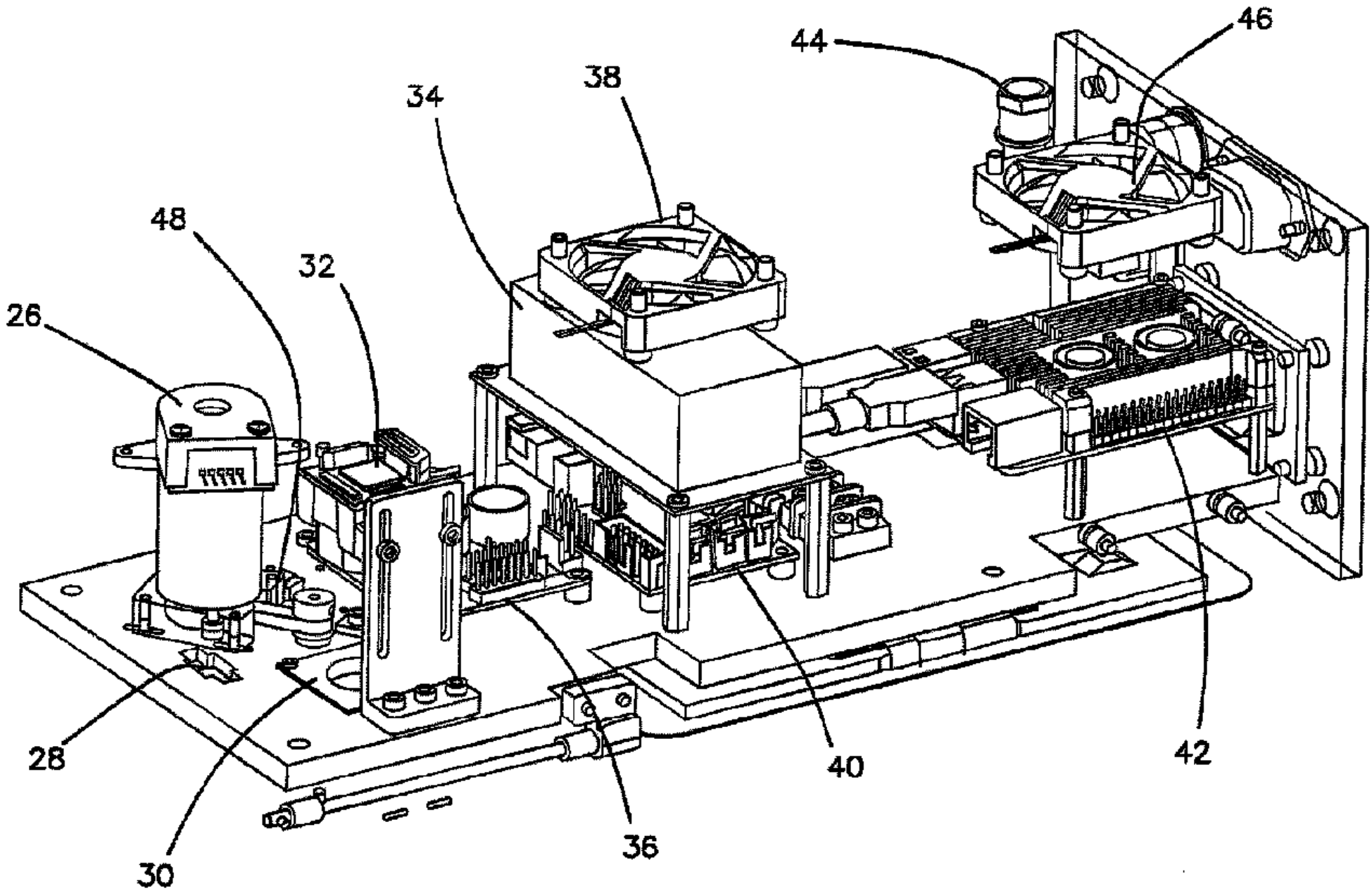


FIG. 23

Date Reçue/Date Received 2020-11-04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