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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
10 March 2022 (10.03.2022)

(54) Title: SARS-COV-2 RAPID SEQUENCE-BASED DIAGNOSTIC ASSAY WITH SENSORS OF IMMUNE ACTIVATION AND THE VIRAL MICROBIOTA

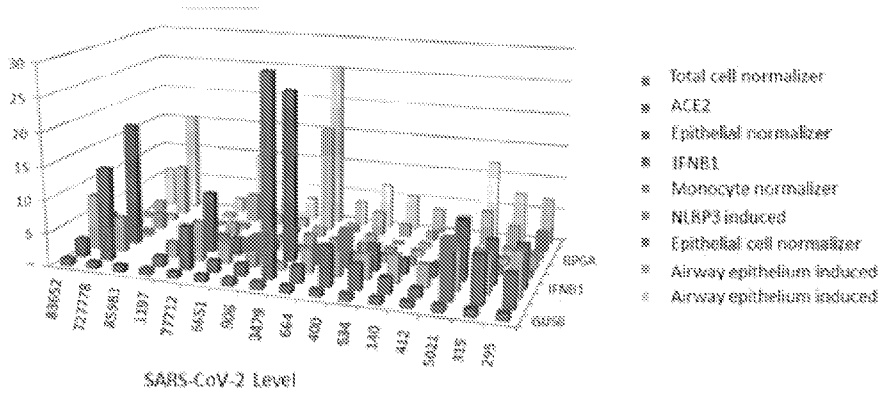

FIG. 2

(57) Abstract: Disclosed are assays, kits, primers, and methods for detecting SARS-CoV-2. The assays, kits, primers, and methods can also include sensors of immune activation and viral microbiota.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/44915

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/70, C12Q 1/6869, C12N 15/11 (2022.01)

CPC - C12Q 1/6883, C12Q 2600/118, C07K 14/165 C12Q2600/16, C12Q 2600/158

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PARK et al. Optimization of primer sets and detection protocols for SARS-CoV-2 of coronavirus disease 2019 (COVID-19) using PCR and real-time PCR., Experimental & Molecular Medicine, 16 June 2020, vol 52, pp 963-977; abstract, pg 966, col 1, para 4 - col 2	1-3, 11, 13-24
A	BLANCO-MELO et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19., Cell. 28 May 2020; vol 181, no 5, pp 1036-1045; abstract,pg 1307, col 2, para 1, pg e2, key resources table	1-3, 11, 13-24
A	CN111440896 A (GUANGXI YUANYUAN MEDICAL LABORATORY CO LTD) 24 July 2020 (24.07.2020), abstract, SEQ ID NO: 90	1-3, 11, 13-24
A	CN111424119 A (MICRO ROCK MEDICAL TECHNOLOGY BEIJING CO LTD) 17 July 2020 (17.07.2020) abstract, claim 1, SEQ ID NO: 3	1-3, 11, 13-24
A	US 2018/0086807 A1 (MODERNA TX, INC) 29 March 2018 (29.03.2018) abstract, para [1459], SEQ ID NOs: 632, 634	1-3, 11, 13-24

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2022	FEB 09 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/44915

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/44915

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

------ see extr sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
claims 1-3, 11 and 13-24, limited to SEQ ID NOs: 1-2, and 31-32

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/44915

Continuation of Box No. III. Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-11 and 13-24 directed to an assay composition and kit, said assay comprising at least one primer (set) for detection of SARS-CoV-2 and at least one primer (set)for detection of a host gene The detection assay (and kit comprising said assay) will be searched to the extent that the SARS-CoV-2 primer set comprises SEQ ID NOs: 1 and 2, and the host gene detection primer set comprises SEQ ID NOs: 31-32 (inflammasome signature gene). It is believed that claims 1-3, 11 and 13-24, limited to said SARS-CoV-2 detection primer sets encompass this first named invention, and thus these claims will be searched without fee to the extent that the SARS-CoV-2 detection assay/kit comprises said primers. Additional SARS-CoV-2 detection assay/kits will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected SARS-CoV-2 detection assay/kit. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a SARS-CoV-2 detection assay/kit comprising two sets of primers used to detect SARS-CoV-2 comprising SEQ ID NOs: 1-2 and 3-4, and a set of primers to detect an epithelial marker comprising SEQ ID NOs: 41-42 (claims 1, 3-4, 9-10 and 13-24).

Group II+: Claims 12 and 26, directed to method of simultaneously detecting SARS-CoV-2 with at least one primer pair and at least one host gene with a second primer pair, and treating a subject based on the results of the method. Group II+ will be searched upon payment of additional fees. The detection/treatment method may be searched, for example, to the extent that the SARS-CoV-2 primer set comprises SEQ ID NOs: 1 and 2, and the host gene detection primer set comprises SEQ ID NOs: 31-32. It is believed that claims 12 and 26, limited to said primer detection/treatment method, read on this exemplary invention. Additional primer detection/treatment methods will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected primer detection/treatment methods. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a SARS-CoV-2 detection/treatment method comprising two sets of primers used to detect SARS-CoV-2 comprising SEQ ID NOs: 1-2 and 3-4, and a set of primers to detect an epithelial marker comprising SEQ ID NOs: 41-42 (claims 12 and 26).

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

Special Technical Features

No technical features are shared between the primer nucleic acid sequences of Groups I+ and II+, accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ and or Group II+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Groups I+ requires an isolated assay/kit composition of matter, not required by Group II+.

Group II+ requires a method of detecting and treating a subject having SARS-CoV-2, not required by Group I+.

Common Technical Features

The inventions of Group I+ and II+ share the technical feature of at least one primer (set) for detection of SARS-CoV-2 and at least one primer (set) for detection of a host gene associated with SARS-CoV-2, for use together in diagnosing subjects infected with SARS-CoV-2.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by the article entitled "Optimization of primer sets and detection protocols for SARS-CoV-2 of coronavirus disease 2019 (COVID -19) using PCR and real-time PCR" by Park et al. (hereinafter 'Park') (Experimental & Molecular Medicine, 16 June 2020, vol 52, pp 963-977) in view of the article entitled "Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19" by Blanco-Melo et al. (hereinafter 'Blanco')(Cell. 28 May 2020; vol 181, no 5, pp 1036-1045).

********** See Next Extra Sheet to continue **********

continuation of previous extra sheet:

Park teaches multiplex PCR detection of SARS-CoV-2 using primer sets (abstract "we provide three-step guidelines for the design and optimization of specific primer sets. The three steps include (1) the selection of primer sets for target genes (RdRP, N, E, and S) in the genome of interest (SARS-CoV-2), (2) the in silico validation of primer and amplicon sequences, and (3) the optimization of PCR conditions (i.e., primer concentrations and annealing temperatures) for specific hybridization between the primers and target genes, and the elimination of spurious primer dimers. Furthermore, we ...applied a multiplex PCR-based protocol that allows the simultaneous testing of primer sets for RdRP, N, E, and S all in one reaction."), further including human genes as internal positive controls (IPC) (pg 966, col 1, para 4 - col 2, para 1 "Traditional PCR for SARS-CoV-2 detection The SARS-CoV-2_IBS_E2, SARS-CoV-2_IBS_RdRP2, SARS-CoV-2_IBS_S2, and SARS-CoV-2_IBS_N1 primer sets were used for the detection of SARS-CoV-2 transcripts. In addition, the GAPDH primer set was used for the human IPC sample"). Park does not expressly teach use of human (host) genes indicative od inflammation due to viral infection, however, Blanco teaches monitoring host cell genes in covid infected samples abstract "we offer an in-depth analysis of the transcriptional response to SARS-CoV-2
compared with other respiratory viruses. Cell and animal models of SARS-CoV-2 infection, in addition to transcriptional and serum profiling of COVID-19 patients, consistently revealed a unique and inappropriate inflammatory response. This response is defined by low levels of type I and III interferons juxtaposed to elevated chemokines and high expression of IL-6. W", pg 1307, col 2, para 1 ". In low-MOI infections (MOI, 0.2), exogenous expression of ACE2 enabled SARSCoV-2 to replicate and comprise ~54% of the total reads mapping more than 300x coverage across the ~30-kb genome (Figures 1A and 1B). ...Furthermore, qPCR analyses of these cells demonstrated that the levels of Envelope (E) and non-structural protein 14 (nsp14) were more than three orders of magnitude higher in the presence of ACE2 (Figure 1D). It is noteworthy that, despite this dramatic increase in viral load, we observed neither activation of TBK1, the kinase responsible for IFN-I and IFN-III expression, nor induction of STAT1 and MX1, IFN-I-stimulated genes" pg e2, key resources table, shows primer pairs from SARSCoV-2 and a host gene. Since PArk teaches mutiplex testing of samples for expression of multiple SARSCoV-2 genes and Blanco teaches monitoring transcritption of host genes to evaluate SARSCoV-2 infection, it would have been obvious to an artisan of ordinary skill in the art to experiment with primers pairs from both SARSCoV-2 and specific host genes, because said combination would allow evaluation of the host response to SARSCoV-2 infection, which would beuseful for diagnosis and treatment of the viral infection.

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.