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Declarations under Rule 4.17:
— *as to the identity of the inventor (Rule 4.17(i))*
— *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))*
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22 June 2023 (22.06.2023)

(54) Title: METHOD FOR CONCENTRATION AND AVIDITY DETECTION OF ANTIBODIES AGAINST INFECTIOUS DISEASES MEASURED WITH EVANESCENT FIELD BASED BIOSENSORS USING A CONTINUOUS GRADIENT OF LIGAND DENSITIES

(57) Abstract: The core of this patent application is about the invention of a plug & play method and device for concentration (titer) and avidity parameters using continuous gradients of ligand densities applied on the biosensor surface. In a previous study, humoral antibody responses and strength of binding against specific SARS-CoV-2 proteins was investigated. Surface Plasmon Resonance imaging (SPRi) was used to determine the strength of binding of IgG, IgM and IgA against the Receptor Binding Domain and Nucleocapsid (RBD/NCP) of SARS-CoV-2 in sera of 119 COVID-19 patients. Interestingly, in contrast to the titer of antibodies, the binding strength (off-rate increased) went down with increasing disease severity. The invention is a measure of concentration and affinity/avidity parameters in a single experiment in a plug & play manner. The ligand density is a critical parameter with a huge effect on the value of the off-rate. A factor 10 mismatch can easily be obtained. Besides off-rate also the on-rate at a fixed ligand density should be measured, but then the effective concentration should be known too. The core of the invention is to apply a continuous gradient on the sensor surface for determining the titer and avidity of the antibodies and measure the avidity parameter at constant but low R_{max} value and the concentration of the antibodies at high R_{max} values. In this way, generated antibodies to infectious disease can be determined for each patient in particular for determining and prediction of COVID19 severity. A double gradient method can be applied to measure on two targets simultaneously.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2022/080871

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.:
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 additional 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 an 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.:

1-13

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/EP2022/080871

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/543 G01N33/557
ADD.

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)
G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHASFOORT RICHARD B M ET AL: "Presence and strength of binding of IgM, IgG and IgA antibodies against SARS-CoV-2 during CoViD-19 infection", BIOSENSORS AND BIOELECTRONICS, ELSEVIER SCIENCE LTD, UK, AMSTERDAM , NL, vol. 183, 16 March 2021 (2021-03-16), XP086577819, ISSN: 0956-5663, DOI: 10.1016/J.BIOS.2021.113165 [retrieved on 2021-03-16] cited in the application abstract figures 1-3 page 3, column 2, paragraph 4 - paragraph 6 -/--	1-13

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

[x] 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

"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
25 March 2023	26/05/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schwachtgen, J

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/080871

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	& SCHASFOORT RICHARD B.M. ET AL: "High throughput surface plasmon resonance imaging method for clinical detection of presence and strength of binding of IgM, IgG and IgA antibodies against SARS-CoV-2 during CoViD-19 infection", METHODSX, vol. 8, 1 January 2021 (2021-01-01), page 101432, XP093031281, NL ISSN: 2215-0161, DOI: 10.1016/j.mex.2021.101432 Introduction; page 2, paragraph 1 page 4, paragraph 3; figure 8 page 9, paragraph 3 - paragraph 4; figure 11	1-13
Y	SCHASFOORT RICHARD B M ET AL: "Interpolation method for accurate affinity ranking of arrayed ligand-analyte interactions", ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, AMSTERDAM, NL, vol. 500, 13 February 2016 (2016-02-13), pages 21-23, XP029488683, ISSN: 0003-2697, DOI: 10.1016/J.AB.2016.01.023 cited in the application abstract page 1, column 2, line 10 - line 16	1-13
Y	WO 2012/152941 A1 (SSENS B V [NL]; SCHASFOORT RICHARD BERNARDUS MARIA [NL] ET AL.) 15 November 2012 (2012-11-15) claims 1-9,13-15 page 9, line 4 - page 12, line 20 page 17, paragraph 3; figure 5	1-13
Y	JP 2013 061179 A (KONICA MINOLTA HOLDINGS INC) 4 April 2013 (2013-04-04) claims 1-6 paragraph [0012] paragraph [0032]	1-13
Y	EP 3 677 901 A1 (FURUNO ELECTRIC CO [JP]) 8 July 2020 (2020-07-08) column 11, line 53 - column 12, line 5; figure 11	1-13

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/080871

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012152941 A1	15-11-2012	CN 103748467 A	23-04-2014
		EP 2707715 A1	19-03-2014
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		US 2023097717 A1	30-03-2023
		WO 2019044418 A1	07-03-2019

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13

Method for predicting infectious disease severity comprising exposing a sample of a patient's body fluid having an infectious disease to an immunogenic antigen immobilized in a gradient on a label-free and real-time imaging biosensor, wherein the ratio of levels and affinity of at least two immunogenic proteins predicts the severity of the infectious disease in the patient.

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2. claims: 14-17

Device comprising a SPR imager and a line printer suitable for creating a gradient in a track

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