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Short communication

Quantification of anti-SARS-CoV-2 antibodies in human serum with LC-QTOF-MS

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ABSTRACT

The aim of this study was to develop the first quantitative serological test for anti-SARS-CoV-2 antibodies in human serum with liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Other assays, mostly immunoassays, are only qualitative or semi-quantitative, and hence, actual antibody concentrations after SARS-CoV-2 infection are unknown. In our assay, anti-SARS-CoV-2 antibodies were isolated with spike protein subunit 1 (S1) coupled to magnetic beads. IgG1 signature peptide GPSVFLAPSSK was selected for quantification using ipilimumab calibration standards and SILuMAb K1 as the stable-isotope labeled internal standard. The anti-SARS-CoV-2 IgG1 calibration range was from 1.35 to 135 nM. Inter-assay accuracies were between 98.8%−107% with inter-assay precisions between 8.37%−13.5% measured at 3 concentration levels on three separate occasions. Anti-SARS-CoV-2 IgG1 antibodies were quantified in PCR-positive patients with mild to severe symptoms. IgM signature peptide DGFFGVPVR was detected in patients that recently recovered from COVID-19. A unique and quantitative LC-QTOF-MS method to quantify anti-SARS-CoV-2 IgG1 in serum was successfully developed and its clinical applicability has been demonstrated.

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1. Introduction

Antibody response after infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently one of the most researched topics in the coronavirus disease 2019 (COVID-19) pandemic. It is still unclear what kind of immunity profile protects individuals from re-infection, and an understanding of the immune reaction after SARS-CoV-2 infection is of pivotal importance to control the pandemic. Vaccination programs against COVID-19 have been started. Many vaccines that have been authorized by governmental agencies contain genetic material (mRNA or DNA) encoding for the SARS-CoV-2 membrane spike protein (S-protein). The coronavirus needs the S-protein to invade cells and this protein is highly immunogenic [1,2]. Measurement of the antibody response against S-protein will add to the knowledge about protection against COVID-19 in the population or certain subsets of the population that are not investigated widely yet. There is a high demand for a better understanding of antibody responses following SARS-CoV-2 infection or vaccination, and to support this research reliable quantitative serological assays are urgently needed [3,4]. Thus far, detection of anti-SARS-CoV-2 antibodies has been described with the use of enzyme-linked immunosorbent assays (ELISA), chemiluminescence, immunofluorescence, and lateral flow platforms [5,6]. Of these tests, ELISA is used most to demonstrate if anti-SARS-CoV-2 antibodies are present in the blood of a subject or not. Instead of absolute quantitative results, anti-SARS-CoV-2 antibody ELISAs only give relative titers (optical density/cut-off ratios) as the best indication of the amount of antibodies present. The comparison of results between studies is therefore not possible. Furthermore, the results of these assays mostly indicate the total amount of anti-SARS-CoV-2 antibodies present in the sample, which is a mix of several isotypes of immunoglobulins (e.g. IgG, IgM, IgA). When a distinction between these different antibody types is desired, separate ELISAs runs have to be conducted. Another disadvantage of ligand binding assays is the high percentage of false-
positive results originating from the blocking or coating matrix (apart from the specific antigen used) [3]. The availability of quantitative, specific, multiplexed assays that can discriminate between antibody isotypes will support further research on the immune response after a SARS-CoV-2 infection. Therefore, we decided to develop and validate a Liquid Chromatography—Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) assay for the quantification of anti-SARS-CoV-2 antibodies in human serum. The most specific and highly immunogenic SARS-CoV-2 protein, the S1-subunit of the S-protein, was coupled to magnetic beads, was used to catch the anti-SARS-CoV-2 antibodies from serum [6]. Comparative studies show that the S1-subunit of the S-protein is more sensitive and specific than N-protein or the full S-protein for the detection of anti-SARS-CoV-2 antibodies [3,7–10]. Furthermore, immune responses to the vaccines that have been authorized by the EMA thus far are based on the specific antibody response against the S-protein. After the specific isolation of anti-SARS-CoV-2 antibodies, a digestion procedure with trypsin was executed and, as a proof of concept, the IgG1 signature peptide GPSVFPLAPSSK in the constant part of the IgG1 anti-SARS-CoV-2 antibodies was selected for the quantification (Fig. 1). Of the four IgG subclasses, IgG1 is the most abundant (60%) followed by IgG2 (32%). While IgG2 is mostly involved in bacterial immunity, IgG1 recognizes foreign antigens and membrane proteins like N- and S-protein [11]. The selected IgG1 peptide is also present in ipilimumab, a humanized monoclonal antibody of the subtype IgG1 used for the treatment of advanced melanoma. This biotherapeutic protein was spiked to control human serum to prepare calibration standards for the quantification of anti-SARS-CoV-2 IgG1 antibodies and SILuMAb K1 was added as the internal standard. The application of the method was demonstrated in serum samples of several patients who were tested PCR positive for SARS-CoV-2. The sample pretreatment of the method is specific and the accurate mass LC-QTOF-MS data provides highly selective data. In the full scan mode, a fingerprint of a sample is obtained and the data can be mined afterwards, without re-analyzing the sample. The ability to multiplex through the detection of IgM signature peptides have been shown.

2. Materials & methods

2.1. Materials

Acetonitrile, water, and formic acid 99% (all UPLC-MS grade) were purchased from Biosolve (Valkenswaard, The Netherlands). Pierce trypsin protease (MS grade) and phosphate buffered saline (PBS) originated from Thermo Scientific (Rockford, IL, USA). SILu™MAB K1 Stable-Isotope Labeled Universal Monoclonal Antibody, Trizma® Tris base, glycine hydrochloride, DL-dithiothreitol (DTT), and Emsure® glacial acetic acid were from Sigma Aldrich (Saint Louis, MO, USA). Streptavidin magnetic beads 20 mg (2 mL) were from Roche (Mannheim, Germany) and ipilimumab (IPM, Yervoy®) 5 mg/mL was from Bristol-Myers Squibb (Dublin, Ireland). Biotinylated S1 protein was purchased from Bios Antibodies (Woburn, MA, USA). For all experiments, Protein LoBind vials (Eppendorf, Hamburg, Germany) were used. Incubations under continuous mixing were performed on an Eppendorf™ ThermoMixer C. A PureProteome magnetic stand (Merck, Darmstadt, Germany) was used for the extraction of magnetic beads.

2.2. Preparation of biotinylated S1 - streptavidin magnetic beads

A volume of 2000 µL (sufficient to process 50 patient samples) of biotinylated S1 protein coupled to streptavidin magnetic beads (b-S1-beads) was prepared for the isolation of anti-SARS-CoV-2 antibodies. Streptavidin magnetic beads (10 mg/mL) were vortexed, and 1750 µL was transferred into a Protein LoBind 2.0 mL vial. The vial was placed into the magnetic stand, and the supernatant was removed. The beads were washed three times with 1500 µL phosphate buffered saline (PBS) and were resuspended in 1000 µL PBS. The 1000 µL bead suspension was added to 250 pmol of biotinylated S1 (b-S1) protein in PBS solution (1 nM) followed by a 30 min incubation under continuous mixing (1000 rpm) at room temperature. The vial was placed into the magnetic stand, and the supernatant was discarded. The beads were washed three times with 1000 µL PBS and re-suspended in 2000 µL PBS.

2.3. Isolation of anti-SARS-CoV-2 antibodies

A volume of 40 µL serum of each patient sample was diluted with 160 µL PBS in Protein LoBind 1.5 mL vials. To each diluted serum sample, 40 µL of b-S1-magnetic bead suspension was added followed by 30 min incubation under continuous mixing (1000 rpm) at room temperature to allow binding of the anti-SARS-CoV-2 antibodies to S1-protein on the magnetic beads. The vial was then placed into the magnetic stand, and the supernatant was discarded. The b-S1-beads with captured anti-SARS-CoV-2 antibodies were washed three times with 500 µL PBS to wash away unbound serum compounds, and they were re-suspended in 250 µL PBS. The beads were transferred into a new Protein LoBind 1.5 mL vial to prevent digestion of vial-surface-adsorbed proteins. The vial was placed into the magnetic stand, and the supernatant was discarded. Next, the anti-SARS-CoV-2 antibodies were eluted from the magnetic beads by the addition of 25 µL 0.2 M glycine in water (pH 2.5) followed 5 min of incubation under continuous mixing (1000 rpm) at room temperature to allow dissociation of the antibodies from S1-protein. The eluate containing anti-SARS-CoV-2 antibodies was transferred into a new Protein LoBind 1.5 mL vial. The elution was repeated yielding a total of 50 µL eluate. The pH was neutralized with 5 µL 1 M Tris buffer (pH 8.5).

Fig. 1. Chemical structure of the IgG1 signature peptide GPSVFPLAPSSK and the y7 ion that was selected for the LC-QTOF-MS quantification of anti-SARS-CoV-2 IgG1 antibody concentrations.
2.4. Digestion procedure

To the neutralized eluate, 50 µL of SILuMAb K1 solution (5 µg/mL) was added. Reduction was achieved by the addition of 25 µL DTT 100 mM solution followed by 30 min incubation under continuous mixing (1000 rpm) at 60 °C. An amount of 10 µL trypsin solution (25 µg/mL) was added, and the samples were digested for 16 h at 37 °C under continuous mixing (1000 rpm). The digestion was quenched with 10 µL of 10% (v/v) formic acid in acetonitrile. The samples were centrifuged for 5 min at 4000 x g, and an aliquot of 20 µL of the supernatant was injected into the LC-QTOF-MS system.

2.5. Calibration standards

A solution of IPM 5 mg/mL was diluted in glycine 0.2 M: Tris 1 M (10:1, v/v) solution to mimic the bead-elution conditions after the isolation of anti-SARS-CoV-2 antibodies. Concentrations of 1.35, 6.76, 33.8, 67.6, and 135 nM IPM (0.2, 1, 5, 10, and 20 µg/mL respectively) were subsequently prepared in duplicate from an IPM formulation containing 5 mg/mL (33.8 µM). Volumes of 50 µL were processed as described under ‘2.4 Digestion procedure’.

2.6. LC-QTOF-MS conditions and settings

All measurements were performed on a Nexera X2 LC-30 CE (Shimadzu, Tokyo, Japan) coupled to a TripleTOF 6600, SCIEX (Framingham, MA, USA). The analytical column was an AdvanceBio Peptide plus reversed-phase (150 x 2.1 mm ID, 2.7 µm and 300 Å pore size), and the guard column was an AdvanceBio Peptide Mapping (5 x 2.1 mm ID, 2.7 µm, both Agilent, Santa Clara, CA, USA). The column was maintained at 55 °C during the analysis. The mobile phases were (A) 0.1% (v/v) formic acid in water, and (B) 0.1% (v/v) formic acid in acetonitrile. The LC gradients in minutes per percentage of mobile phase B were 0.0 (min)/6.5 (% B), 7.0/10, 7.5/85, 8.5/85, 9.0/6.5, and 10.0/6.5. The flow rate was 0.5 mL/min and the run time 10 min. The mass spectrometer was operated in positive enhanced sensitivity mode. Curtain gas, nebulizing gas, and drying gas were set to 20 psi, 50 psi, and 50 psi, respectively. The source temperature was 550 °C, and the turbo ion spray, collision energy, and declustering potential were 5500 V, 30 V, and 30 V, respectively. A full scan was conducted as well as product ion scans for GPSVFPLAPSSK (593.8), GPSVFPLAPS[K]-SIL (597.8), and DGFFGVPVR (455.2). The selected product ions for quantification were 699.4036, 707.4036, and 590.3046, respectively. Quantification of the product ions was performed using a 0.1 Da mass range for optimal selectivity.

2.7. Sample collection

From 15 volunteers who had mild symptoms during infection, serum samples were collected 9–12 weeks after they were tested PCR-positive for the SARS-CoV-2 (A1–A12) or 2–5 weeks after start of symptoms (A13–A15). Furthermore, blank serum of volunteers from the pre-COVID-19 era was used as a negative control and for specificity and interference studies (B1–B31). Serum samples (C1–CS) were collected from a SARS-CoV-2 PCR positive volunteer who had been admitted to the hospital due to severe respiratory distress. Whole blood was collected by a finger prick using a Hem-Col kit (Labonovum, Limmen, the Netherlands). Serum samples were stored at −20 °C until analysis. Ethical approval was provided by the Institutional Review Board of the Netherlands Cancer Institute (reference number IRBb21–129).

Fig. 2. Mass spectrometric detection of the IgG1 signature peptide GPSVFPLAPSSK. Representative extracted ion chromatograms of transition m/z 593.8 → 699.4 in serum of a PCR-positive tested patient A9 (panel a, representing an anti-SARS-CoV-2 IgG1 concentration of 63 nM) and blank sample B5 (panel b). GPSVFPLAPSSK elutes after approximately 6.5 min. MS spectrum and MS² spectrum of precursor ion m/z 593.8 are presented in panels c and d, respectively.
3. Results

3.1. Signature peptide detection

When a patient sample was processed and analyzed, both the extracted ion chromatogram (XIC) of the precursor m/z 593.8 (GPSVFPLAPSSK²⁺) from the total ion current and the XIC of transition m/z 593.8 → 699.4036 (y₂) for the product ion resulted in a single peak at 6.5 min (Fig. 2a). The precursor ion (m/z 593.8273) was detected with −2.19 ppm mass deviation (Fig. 2c). The y₄, y₇, y₉, y₁₀, and y₁₁ ions were detected with −0.88, −3.30, +1.87, −0.78, and −5.22 ppm mass deviation, respectively, from the monoisotopic masses confirming the chemical structure of the selected peptide (Fig. 2d). In processed IPM samples, identical precursor and product ions were detected as observed when the anti-SARS-CoV-2 antibodies were digested.

3.2. Stable isotope-labeled internal standard

Stable Isotope-Labeled Universal Monoclonal Antibody (StLuMab) K1 is a human monoclonal IgG1 antibody labeled with ¹³C₆ ¹⁵N₄ arginine and ¹³C₆ ¹⁵N₂ lysine residues. After digestion of StLuMab K1 GPSVFPLAPSS[K]-SIL is formed, and this peptide was used as the internal standard. GPSVFPLAPSS[K]-SIL was detected with the transition m/z 597.8 → 707.4036 (y₂) at 6.5 min.

3.3. Validation experiments

For the quantification, IPM calibration standards were prepared in duplicate containing five different concentration levels (1.35–135 nM) of IPM. Calibration standards were processed in duplicate in a calibration range of 1.35 – 135 nM and linear regression was applied with a weighting factor of 1/x². The LLOQ was 1.35 nM, and the limit of detection was 0.8 nM. The intra- and inter-assay accuracy and precision were measured by the analysis of quality control samples containing 1.35 nM, 67.6 nM, and 135 nM of IPM. The intra-assay accuracy and precision ranged from 96.3% to 107% and from 13.9% to 15.7%, respectively, and the inter-assay accuracy and precision ranged from 98.8% to 107% and from 8.37% to 13.5%, respectively (Table 1). To evaluate the reproducibility of isolation and digestion of anti-SARS-CoV-2 IgG1 antibodies a sample was processed in triplicate. The relative standard deviation between the three samples was 10.2% showing that no significant additional variation was measured due to the isolation of the antibodies since the variation was comparable to the variation after digestion only (Table 1). Stability of anti-SARS-CoV-2 IgG1 antibodies in serum was evaluated after one freeze/thaw cycle and after 1–6 months of storage at −20 °C (Fig. 3). The measured concentration after the freeze/thaw cycle was 93.1% of the initially measured concentration. After six months of storage at −20 °C, 93.4% of the initially measured concentration was recovered. Samples were considered stable when 85–115% of the initial measured concentration was recovered. These results demonstrated that the IgG1 antibodies in serum were stable after one freeze/thaw cycle and after storage for at least 6 months at −20 °C. The specificity of the method was evaluated by the analysis of 31 blank samples. Results are presented in Fig. 4a. Carry-over was examined by the injection of a high concentration patient sample (63.4 nM) followed by a blank sample, and no carry-over was observed.

3.4. Application of the method

To test the clinical applicability of our assay, serum samples were collected from 12 volunteers (A1-A12) with mild COVID-19 symptoms, 9–12 weeks after a positive SARS-CoV-2 PCR test, and three serum samples from volunteers (A13–15) within 2–5 weeks after start of symptoms. As shown in Fig. 4b, our LC-QTOF-MS assay was able to successfully quantify anti-SARS-CoV-2 IgG1 antibodies in the range from 3.8 to 63.4 nM. All concentrations were within the calibration range. A signal at the transition of the GPSVFPLAPSSK peptide was also detected in blank samples (B1–B31) due to non-specific binding of other endogenous IgGs. However, blanks and patient samples could effectively be distinguished (p < 1.0e4, unpaired, two-sided Mann–Whitney-Wilcoxon test). In another example, a patient with severe symptoms of COVID-19 was followed in time up until 36 weeks after being tested PCR-positive for SARS-CoV-2. Blood was drawn by a finger prick at 10, 15, 22, 31, and 44 weeks (C1, C2, C3, C4, and C5, respectively) after PCR diagnosis. We did not observe a decline in anti-SARS-CoV-2 IgG1 concentration during a period of 6 months of storage at −20°C.

![Fig. 3.](image-url) Long term stability of anti-SARS-CoV-2 IgG1 antibodies in serum stored at −20°C.

Table 1

| Nominal concentration (nM) | Number of replicates in one run | Mean measured concentration (nM) | Standard deviation (nM) | Intra-assay accuracy (%) | Intra-assay precision (%) |
|---------------------------|--------------------------------|-------------------------------|------------------------|------------------------|--------------------------|
| 1.35                      | 3                              | 1.30                          | 0.193                  | 96.3                   | 14.8                     |
| 67.6                      | 3                              | 72.1                          | 1.13                   | 107                    | 15.7                     |
| 135                       | 3                              | 144                           | 20.0                   | 107                    | 13.9                     |

| Inter-assay validation parameters | Number of replicates in three runs | Mean measured concentration (nM) | Standard deviation (nM) | Inter-assay accuracy (%) | Inter-assay precision (%) |
|----------------------------------|------------------------------------|-------------------------------|------------------------|------------------------|--------------------------|
| 1.35                            | 6                                  | 1.45                          | 0.195                  | 107                    | 13.5                     |
| 67.6                            | 6                                  | 69.2                          | 5.79                   | 103                    | 8.37                     |
| 135                             | 6                                  | 133                           | 17.8                   | 98.8                   | 13.3                     |

Table 1 Intra-assay and inter-assay performance data for the quantification of the IgG1 signature peptide GPSVFPLAPSSK based on the analysis of calibration standards analyzed in duplicate on 3 separate occasions.
almost 6 months (Fig. 4c). This conservation of antibody concentrations in time corresponds to earlier findings of a study performed in the Icelandic community [15].

The ability of IgM anti-SARS-CoV-2 antibody detection with the LC-QTOF-MS assay was evaluated. TIC chromatograms of the patient samples were searched for the presence of doubly charged IgM signature peptide DGFFGVP [13]. This peptide was not detected in serum samples A1-A12. However, in serum samples A13-A15 we did observe a signal for the DGFFGVP transition 455.2 → 590.3046 (m/z) at a retention time of 3.4 min while this signal was only minimally present in blank samples [Fig. 5]. The DGFFGVP y4, y5, y6 ions were detected with +0.41, +10.2, +0.84 ppm mass deviation, respectively, from the monoisotopic masses confirming the chemical structure of the selected peptide.

4. Discussion

SARS-CoV-2 contains several immunogenic parts and proteins, with N- and S-protein being most immunogenic. However unlikely, patients may obtain immunity without developing antibodies

Fig. 4. Panel a: Anti-SARS-CoV-2 IgG1 antibodies in 31 blank serum samples (B1-B31) measured with LC-QTOF-MS. Panel b: Anti-SARS-CoV-2 IgG1 antibodies in PCR-positive COVID-19 patients (A1-A15) with mild symptoms, and Panel c: A severe case of COVID-19 (C1-C5). The number on top of the bars represents the number of weeks after the positive PCR test. The dotted line in all panels represents the LLOQ of 1.35 nM.

Fig. 5. Areas of IgM signature peptide DGFFGVP at transition 455.2 → 590.3046 (m/z) detected in 3 patient serum samples (A13-A15), and for comparison the areas that were measured in two blank serum samples (B1 and B2). The number on top of the bar represents the number of days after start of the COVID-19 symptoms.
against S1-protein [10], and this assay will not detect antibodies against SARS-CoV-2 parts other than S1-protein. The addition of biotinylated N-protein to this assay will probably result in higher sensitivity, however, specificity will likely be reduced because of cross-reactivity [8,10]. Therefore we chose to use a subunit of the S1 protein for a specific extraction of the anti-SARS-CoV-2 antibodies from human serum.

The amino acid sequence of the (hyper) variable regions of anti-SARS-CoV-2 antibodies to S1 is unknown and probably diverse. Therefore, a signature peptide from the constant region of IgG antibodies (GPSVFLAPSSK) was chosen for LC-QTOF-MS quantification (Fig. 1) [12–14]. Notably, this peak was also detected in blank samples (Fig. 2b), however, signals were low and clearly distinguishable from the responses detected in the PCR-positive COVID-19 patients. This low signal in blank samples is probably the result of non-specific binding of IgG1 antibodies during sample pretreatment.

Ideally, a polyclonal anti-SARS-CoV-2 S1 antibody product should be used as a reference standard, but such a product was not commercially available at the time of this study. However, a monoclonal anti-SARS-CoV-2 S1 antibody product is now available. Monoclonal antibodies uniformly target only one epitope and thus, such a product does not adequately represent the binding characteristics of the largely diverse pool of anti-SARS-CoV-2 S1 antibodies present in clinical samples. Thus, it was not possible at this point to quantitatively examine the recovery of anti-SARS-CoV-2 S1 antibodies from serum using bS1-beads and to use a polyclonal antibody as reference standard. As an alternative, the widely available monoclonal antibody IPM was used for the quantification. IPM is a fully human IgG1 monoclonal antibody and contains the same constant sequences as endogenous human IgG1 antibodies. The average molecular weight of IgG1 antibodies is 146 kDa [11], while IPM has a molecular weight of 148 kDa. Therefore, the quantification error based on the differences between IPM and anti-SARS-CoV-2 antibodies was expected to be around 1.4%.

The addition of a digested SIluMab K1 peptide solution to the digested patient samples was compared to GPSVFLAPSSK area without internal standard corrections. Results showed that the accuracy and precision of the assay were not improved. Hence, it can be concluded that variation does not derive from QTOF-MS detection but rather from the sample pretreatment procedure. When intact SIluMab K1 is introduced into the patient samples before digestion, accuracy and precision were improved from around 60–10% when compared to absolute areas of GPSVFLAPSSK. However, although the addition of SIluMab K1 before digestion is able to correct for variation in digestion recoveries, it is not capable to correct for the anti-SARS-CoV-2 antibody isolation.

The application of our assay to clinical cases showed that IgG concentrations in patients with mild COVID-19 symptoms differed profoundly from blank serum and that IgG concentrations remained stable between 10 and 22 weeks after infection in a subject with severe COVID-19 symptoms. Furthermore, it has been shown that the median day of seroconversion for IgM was 13 days after start of symptoms, and in some cases IgM seroconversion does not occur at all [9,16]. Subsequently, IgM production ceases as IgG seroconversion occurs. A decline in anti-SARS-CoV-2 IgG concentrations was observed after three weeks [16]. Serum samples (A1-A12) were taken 9–12 weeks after infection indicating the possibility that IgM concentrations had already waned, and as predicted, in these samples no IgM signature peptide peaks were detected. However, serum samples A13-A15 were taken within 35 days after start of symptoms, and in these samples, we did observe a signal. These results show that our assay could be used as a multiplexed assay of IgG and IgM quantification, however this application should be investigated further with multiple patient samples collected days up to a month after the SARS-CoV-2 infection.

5. Conclusions

Here, we describe the first LC-QTOF-MS assay for the quantification of anti-S1 SARS-CoV-2 IgG1 antibodies in human serum. We show that we could identify IgG1 signature peptide GPSVFLAPSSK and IgM signature peptide DGFFGVPR based on MS and MS2 spectra with high mass accuracy, and that anti-SARS-CoV-2 IgG1 antibodies could successfully be quantified using IPM calibration standards. Until now, it is unknown what the antibody concentrations after SARS-CoV-2 infection are. This is the first assay to quantify the concentrations of anti-SARS-CoV-2 IgG1 antibodies.

The described LC-QTOF-MS assay can be applied to various clinical research questions regarding COVID-19. ELISA assays are suitable as qualitative tests in which no quantitation is needed and no distinction is made between different antibodies. However, to answer quantitative questions about, for example, the kinetics and seroconversion of different anti-SARS-CoV-2 antibodies, this LC-QTOF-MS assay can be used. Direct detection of the IgG1 peptide structure will overcome specificity problems that have been observed in ELISA assays, and the possibility to multiplex will enable detection of IgG1 and IgM within the same sample. The possibility to gain concentrations of antibodies instead of a surrogate measure (titers) will substantiate the knowledge of anti-SARS-CoV-2 antibody behavior and will facilitate comparison of antibody quantities between different studies.

CRediT authorship contribution statement

Karen de Jong: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualisation. Hilde Rosing: Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Supervision. Marit Vermunt: Resources. Alwin Huijtema: Supervision, Writing – review & editing. Jos Beijnen: Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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