Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Ultra-fast, high throughput and inexpensive detection of SARS-CoV-2 seroconversion using Ni\textsuperscript{2+} magnetic beads

Marcelo S. Conzentino\textsuperscript{a}, Tatielle P.C. Santos\textsuperscript{a}, Khaled A. Selim\textsuperscript{b}, Berenike Wagner\textsuperscript{b}, Janette T. Alford\textsuperscript{b}, Nelli Deobald\textsuperscript{b}, Nigela M. Paula\textsuperscript{a}, Fabiane G.M. Rego\textsuperscript{c}, Dalila L. Zanette\textsuperscript{d}, Mateus N. Aoki\textsuperscript{d}, Jeanine M. Nardin\textsuperscript{e}, Maria C.C. Huergo\textsuperscript{a}, Rodrigo A. Reis\textsuperscript{f}, Luciano F. Huergo\textsuperscript{a,*}

\textsuperscript{a} Setor Litoral, UFPFR Matinhos, PR, Brazil
\textsuperscript{b} Organismic Interactions Department, Interfaculty Institute for Microbiology and Infection Medicine, Cluster of Excellence ‘Controlling Microbes to Fight Infections’, Tübingen University, Auf der Morgenstelle 28, 72076, Tübingen, Germany
\textsuperscript{c} Post-Graduation Program in Pharmaceutical Sciences, UFPFR, Curitiba, PR, Brazil
\textsuperscript{d} Instituto Carlos Chagas, FioCruz, PR, Brazil
\textsuperscript{e} Hospital Erasto Gaertner, Curitiba, PR, Brazil
\textsuperscript{f} Department of Cell Biology, UFPFR, Curitiba, PR, Brazil

ARTICLE INFO

Keywords:
COVID-19
Magnetic ELISA
SARS-CoV-2
High throughput
Magnetic beads

ABSTRACT

To monitor the levels of protecting antibodies raised in the population in response to infection and/or to immunization with SARS-CoV-2, we need a technique that allows high throughput and low-cost quantitative analysis of human IgG antibodies reactive against viral antigens. Here we describe an ultra-fast, high throughput and inexpensive assay to detect SARS-CoV-2 seroconversion in humans. The assay is based on Ni\textsuperscript{2+} magnetic particles coated with His tagged SARS-CoV-2 antigens. A simple and inexpensive 96 well plate magnetic extraction/homogenization process is described which allows the simultaneous analysis of 96 samples and delivers results in 7 min with high accuracy.

The COVID-19 outbreak, caused by the novel beta coronavirus SARS-CoV-2, has posed an extraordinary threat to human health with ~3,000,000 deaths reported worldwide by April 2021. No effective medication to treat the disease is yet available. Despite the successful development of efficient vaccines by different laboratories, vaccination is still limited to developed countries and only a small fraction of the world’s population has been immunized [1]. COVID-19 vaccination is extremely limited, if any, in developing countries, and social distancing is the only effective measure to reduce the spread of the disease.

Tracking COVID-19 is of key importance to understand and mitigate the spread of the disease. COVID-19 testing can be performed by a variety of methods with the most common being molecular or antigen tests which detect active SARS-CoV-2 infections. Alternatively, an immunological assay can be employed to detect human antibodies reacting against different SARS-CoV-2 antigens [2]. One advantage of immunological COVID-19 tests is that antibodies remain detectable for months after convalescence and thus can be used for epidemiological surveillance studies [3]. Furthermore, with the advance of COVID-19 vaccination, these immunoassays can be applied to identify the fraction of the population that developed IgG antibodies reacting against SARS-CoV-2 antigens, which are likely to reflect the protection after immunization. Since there is no knowledge on how long IgG antibodies raised against SARS-CoV-2 in the context of a natural infection or vaccination will last, large immune surveillance studies will be necessary in near future to depict that issue [4]. Hence, the development of simple, fast and inexpensive COVID-19 immunoassays that can provide quantitative data in high throughput format in response to different antigens will be key to enable such large cohort studies in the population.

We previously described a fast, simple, and inexpensive Ni\textsuperscript{2+} magnetic bead immunoassay which allows detection of human antibodies reactive against the SARS-CoV-2 nucleocapsid protein using a minimal amount of serum or blood [5]. Here we show that such system is amendable to high throughput and can deliver ultrafast results in less...
than 7 min with different SARS-CoV-2 antigens while maintaining low analytical cost.

1. Experimental section

Human samples were collected at Hospital Erasto Gaertner in Curitiba and Secretaria Municipal de Saúde in Guaratuba and Federal University of Paraná in Matinhos. Samples for serological analysis comprised both serum and plasma-EDTA. COVID-19 positive cases were confirmed by the detection of SARS-CoV-2 RNA via real-time RT-PCR from nasopharyngeal sample swabs. The time point of sampling of serum ranged from 1 to 100 days after PCR detection. Among the 63 COVID-positive cases there were 12 convalescents including 2 asymptomatic and 10 mild non-hospitalized cases. All remaining samples were collected within the first 14 days of the hospitalization period. The cohort of 204 negative controls consisted of pre pandemic samples collected in 2018. For the work done in Germany, pre pandemic samples were purchased from Central BioHub GmbH (Henningsdorf, Germany). COVID-19 positive samples were collected from convalescent donors post quarantine and were self-reported PCR-positive for SARS-CoV-2. The Institutional Ethics Review Board CEP/HEG (n# 31592620.4.1001.0098) and CEP/UFPR (n#43948621.7.0000.0102) approved this study. Informed consent was obtained from all participants in this study. All methods were performed in accordance with the relevant guidelines and regulations.

Magnetic beads-based immunoassay. The magnetic bead-based immunoassay was developed using Ni²⁺ magnetic beads as described previously [5]. The recombinant N-terminal 6x His-tagged Nucleocapsid protein of SARS-CoV-2 was expressed from the pLHSARSCoV2-N plasmid using E. coli BL21 (λDE3) as host [6]. The cells were grown in 100 ml LB medium at 120 rpm at 37°C to OD₆₀₀nm of 0.4. The incubator temperature was lowered to 16°C, after 30 min, IPTG was added to a final concentration 0.3 mM and the culture was kept at 120 rpm at 16°C overnight. Cells were collected by centrifugation at 3000×g for 5 min. The cell pellet was resuspended in 25 ml of buffer 1 (Tris-HCl pH 8 50 mM and KCl 100 mM). Cells were disrupted by sonication in an ice bath. The soluble fraction was recovered after centrifugation at 20,000×g for 10 min and incubated for 5 min on ice with gentle mixing with 10 ml of Ni²⁺ magnetic particles (Promega cat number V8550) pre equilibrated in buffer 1. The beads were washed 2 times with 25 ml of buffer 1 and 2 times with 25 ml of buffer 1 containing 100 mM imidazole. Beads were resuspended in 25 ml of TBST and stored in 0.8 ml aliquots at 4°C. The magnetic bead immunoassay was performed using the 96-sample format with flat bottom plates (Crplast). The 0.8 ml aliquots of antigen loaded beads were resuspended in 10 ml of TBST containing 1%
was recorded using a TECAN M Nano plate reader (TECAN) mono
was recorded using a TECAN M Nano plate reader (TECAN) mono
current under curve (AUC) of 0.996. A sensitivity of 97% could
be achieved at a cost of 99.5% specificity. It is important to mention that
the 96-sample format maintained high intra assay/inter assay repro-
ducibility (Table 1).
Of note, the assay was performed by directly loading soluble E. coli
extracts containing the 6x His tagged Nucleocapsid protein of SARS-
CoV-2 onto the Ni²⁺ magnetic beads. By skipping the step of protein
purification prior to loading, we were able to considerably increase the
analytical throughput. The entire process took only 12 min saving time
and costs for protein purification (Fig. 1a).
We anticipated that the high throughput chromogenic magnetic
beads immunoassay could be easily adapted to various His tagged
antigens. As a proof of concept, we immobilized a His tagged version of the
full-length SARS-CoV-2 Spike protein, which was expressed in eukary-
ocytic cells, onto the Ni²⁺ magnetic beads. The Spike coated beads were
then used to determine the presence of reactive IgG in the same cohort of samples. The chromogenic analysis using Spike antigen operated with
AUC of 0.99, and a sensitivity of 97% could be achieved at a cost of
99.5% specificity (Table 1 and Fig. 2a). Again, the levels of reproduc-
ibility were high within and between different assays (Table 1). A team
of operators in Germany independently performed all the steps of the
immunoassay, from antigen preparation to testing, and was able to
validate the ability of the chromogenic magnetic system using His-
tagged versions of either Spike or Spike RBD as antigens to discrimi-
nate COVID-19 cases (Fig. S5a). These findings suggest that this simple
and easy to implement magnetic particle immunoassay may be univer-
sally used with other His tagged antigens to track cases of diseases other
than COVID-19.
Even though the chromogenic assay generated data in a short time, we
speculated that the overall reaction time could be decreased by
changing the detection mode from chromogenic to fluorescent, thus
skipping the 5 min incubation step necessary to build up oxidized TMB
(3,3′,5,5′-Tetramethylbenzidine). As a proof of concept, we changed the
secondary anti-human IgG HPR (horseradish peroxidase)-conjugate to
phycoerythrin-conjugate. These changes allowed an ultrafast (7 min)
high throughput process which was able to discriminate COVID-19 cases
using either Nucleocapsid or Spike as antigens (Fig. 2a and Table 1). The data obtained using the chromogenic and fluorescent system showed
excellent correlation using serum (Fig. 2b and c) or blood (Fig. S5b). The
AUC, sensitivity, specificity, and reproducibility parameters of the

| Table 1 | Performance of the assay in different formats. |
|---------|------------------------------------------------|
| Assay   | AUC¹ | % Sensitivity² | Intra-assay CV%³ | Intra-assay CV%⁴ |
| S Chromogenic | 0.990 | 96.8% (95% CI 89.0-99.6) | 1.7 | 5.8 |
| S Fluorescent | 0.993 | 95.2% (95% CI 86.7-99.0) | 1.2 | 2.4 |
| N Chromogenic | 0.996 | 96.8% (95% CI 89.0-99.6) | 1.8 | 8.9 |
| N Fluorescent | 0.991 | 95.2% (95% CI 86.7-99.0) | 2.9 | 7.4 |

¹ - AUC indicates the value obtained for the area under the ROC curve.
² - Specificity was set to 99.5% (95% CI, 97.7-99.9%) for all assays.
³ - Intra-assay CV% data obtained running the same sample in four wells of the same plate.
⁴ - Inter-assay CV% data of same sample measured in duplicate in 3 different plates.

2. Results and discussion
We have previously described a magnetic particle immunoassay which was successfully applied to track SARS-CoV-2 seroconversion in humans [5]. The assay principle is based on the use of commercially available Ni²⁺ magnetic particles which are coated with the purified, 6x His-tagged Nucleocapsid protein of SARS-CoV-2. The coated beads were used for a process resembling an indirect ELISA. Beads are incubated with serum or blood, washed, incubated with anti-human IgG-HPR, washed, and finally immersed on TMB, a chromogenic HPR substrate. The whole process is performed in 12 min. This immunoassay requires extraction and homogenization of the magnetic beads in different solutions. A major drawback of the system is that it only processes 12 samples at a time. Here we describe a manually operating, inexpensive 96 well plate magnetic extraction/homogenization process which allows high throughput immunological analysis of COVID-19 cases. A 96-sample format magnetic extractor device (Fig. 1b) was built using a plastic piece fabricated on a 3D printer (Figs. S1 and S2). Inox nails were fixed to this base and a set of neodymium magnets were
 excerpts containing the 6x His tagged Nucleocapsid protein of SARS-
CoV-2 onto the Ni²⁺ magnetic beads. By skipping the step of protein
purification prior to loading, we were able to considerably increase the
analytical throughput. The entire process took only 12 min saving time
and costs for protein purification (Fig. 1a).
We anticipated that the high throughput chromogenic magnetic
beads immunoassay could be easily adapted to various His tagged
antigens. As a proof of concept, we immobilized a His tagged version of the
full-length SARS-CoV-2 Spike protein, which was expressed in eukary-
ocytic cells, onto the Ni²⁺ magnetic beads. The Spike coated beads were
then used to determine the presence of reactive IgG in the same cohort of samples. The chromogenic analysis using Spike antigen operated with
AUC of 0.99, and a sensitivity of 97% could be achieved at a cost of
99.5% specificity (Table 1 and Fig. 2a). Again, the levels of reproduc-
ibility were high within and between different assays (Table 1). A team
of operators in Germany independently performed all the steps of the
immunoassay, from antigen preparation to testing, and was able to
validate the ability of the chromogenic magnetic system using His-
tagged versions of either Spike or Spike RBD as antigens to discrimi-
nate COVID-19 cases (Fig. S5a). These findings suggest that this simple
and easy to implement magnetic particle immunoassay may be univer-
sally used with other His tagged antigens to track cases of diseases other
than COVID-19.
Even though the chromogenic assay generated data in a short time, we
speculated that the overall reaction time could be decreased by
changing the detection mode from chromogenic to fluorescent, thus
skipping the 5 min incubation step necessary to build up oxidized TMB
(3,3′,5,5′-Tetramethylbenzidine). As a proof of concept, we changed the
secondary anti-human IgG HPR (horseradish peroxidase)-conjugate to
phycoerythrin-conjugate. These changes allowed an ultrafast (7 min)
high throughput process which was able to discriminate COVID-19 cases
using either Nucleocapsid or Spike as antigens (Fig. 2a and Table 1). The data obtained using the chromogenic and fluorescent system showed
excellent correlation using serum (Fig. 2b and c) or blood (Fig. S5b). The
AUC, sensitivity, specificity, and reproducibility parameters of the
fluorescent assay were in the same range as those obtained with the chromogenic method (Table 1). The cost of the consumables per assay was below $15 independently of the detection mode used.

There are several debates in the literature with respect to the best antigen to use to detect COVID-19 cases with high sensitivity and specificity [3,9,10]. Among the cohort of 204 pre-pandemic samples examined here, one sample showed significant cross reaction to each antigen (S - full length Spike or N - Nucleocapsid) and detection mode. The data was expressed as % of a reference control. b) Correlation between % signal obtained using Spike as antigen with Chromogenic vs fluorescence detection. c) Correlation between % signal obtained using Nucleocapsid as antigen with Chromogenic vs fluorescence detection. d) Correlation between raw signal obtained using Spike vs Nucleocapsid as antigen in chromogenic and fluorescent formats (e). Samples labeled in blue (in d and e) were collected at the day of hospitalization. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Conclusions

Here we describe significant improvements to our Ni\textsuperscript{2+} magnetic beads immunoassay. Firstly, we show that the system is amendable to high throughput analysis by employing a remarkably simple and low-cost magnetic extractor device and bead homogenization process (Figs. S1–S4 and supporting video). Even though automated magnetic extractor and bead homogenization beads are commercially available, they are unaffordable to most laboratories. Furthermore, they usually require specialized plastics (deep well plates and chip combs) which significantly increases the costs of the analysis. The process described here can be performed in regular 96-well plates and PCR tube strips (Figs. S1–S4). The magnetic extraction/homogenization process device described may be applied to other processes such as purification of SARS-CoV-2 RNA from swab samples, which routinely uses magnetic bead extraction [11]. Secondly, we show that antigen can be purified on the fly by loading the cell extracts directly onto the Ni\textsuperscript{2+} magnetic beads in such way that 6x His tag antigen purification and bead loading occur simultaneously in a 12 min process (Fig. 1a). Thirdly, the magnetic bead immunoassay can be performed in an ultrafast 7 min format just by using an anti-human IgG PE conjugate. Fourthly, we show that the magnetic bead immunoassay can be applied to Nucleocapsid, Spike or Spike RBD carrying a His tag as antigens (Fig. 2a). The use of Spike RBD is of particular interest as it will enables quantification of IgG with potential neutralization activity against SARS-CoV-2.

To the best of our knowledge the method described here is the only COVID-19 immunoassay that uses the principles of the well-established indirect ELISA and delivers ultrafast results in a high throughput and inexpensive format. We believe that the technique described here will be an important tool to understand the levels of immunization to previous infections and/or vaccination in large immunological surveillance studies and accordingly mitigate the enforcement of infection prevention strategies such as social distancing.

Acknowledgment

The authors gratefully acknowledge Karl Forchhammer and Ulrich Rothbauer (Tübingen University) for the continues support. We thank Libera Lo-Presti for critical linguistic editing of the manuscript, Ulrich Rothbauer and Leda Castilho (Cell Culture Engineering Laboratory of COPPE/UFRJ) for the aliquots of Spike protein. We thank the infrastructural support by the Cluster of Excellence ‘Controlling Microbes to

Fig. 2. Performance of the Ni\textsuperscript{2+} magnetic immunoassay in different formats. a) The serum from negative pre-pandemic controls (green) and RT-qPCR COVID-19 positive cases (red) were analyzed using the indicated antigen (S - full length Spike or N - Nucleocapsid) and detection mode. The data was expressed as % of a reference control. b) Correlation between % signal obtained using Spike as antigen with Chromogenic vs fluorescence detection. c) Correlation between % signal obtained using Nucleocapsid as antigen with Chromogenic vs fluorescence detection. d) Correlation between raw signal obtained using Spike vs Nucleocapsid as antigen in chromogenic and fluorescent formats (e). Samples labeled in blue (in d and e) were collected at the day of hospitalization. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jab.2021.114360.

Author information

Notes

Federal University of Paraná UFPR has filed for patent protections for: the magnetic immunopossess assay, magnetic COVID-19 immunological test product and magnetic bead extractor device and processing method. All designed product and processes will be freely available for academic and noncommercial users.

References

[1] B. Hu, H. Guo, P. Zhou, Z.L. Shi, Characteristics of SARS-CoV-2 and COVID-19, Nat. Rev. Microbiol. (2021), https://doi.org/10.1038/s41579-020-00459-7.
[2] L.J. Carter, L.V. Garner, J.W. Smoot, Y. Li, Q. Zhou, C.J. Savelson, J.M. Sasso, A. C. Gregg, D.J. Soares, T.R. Beskid, S.R. Jervey, C. Liu, Assay techniques and test development for COVID-19 diagnosis, ACS Cent. Sci. (2020), https://doi.org/10.1021/acscentsci.0c00501.
[3] M. Ainsworth, M. Anderson, J. Auckland, J.K. Baille, E. Barnes, S. Beer, A. Beveridge, S. Bihli, L. Blackwell, M. Borak, J. Brooks, N.A. Burgess-Rowe, A. Butterfield, J. Cornall, S. Cox, D. Crawford-Jones, J.W. Crook, S. Davenport, S. D. Guest, C. Gower, T. Hughes, J. Kavanagh, D.S. Kim, R. Kirton, P. Klenerman, J.C. Knight, J. Hill, S.J. Hobbs, L. Houkoufi, A. Huan, T. Jeffery, E. Jones, A. Justice, F. Karpe, K. Karch, P. Kelemen, J.C. Knight, L. Koukouli, L. Kwok, U. Leuschner, R. Levin, A. Linder, T. Lockett, S.F. Lumley, A. Marchini, K. Berger, H.-G. Rammensee, K. Schenke-Layland, A. Nelde, M. Maerklin, J.S. Heitmann, J.S. Walz, M.F. Templin, T.O. Joos, U. Rothbauer, G.G. Krause, N. Schneiderhan-Marra, Going beyond clinical routine in SARS-CoV-2 antibody testing - a multiplex corona virus antibody test for the evaluation of cross-reactivity to endemic coronavirus antigens, medRxiv (2020), https://doi.org/10.1101/2020.07.07.20156000.
[4] R.G.F. Alvim, T.M. Lima, D.A.S. Rodrigues, F.F. Marsili, V.B.T. Bozza, L.M. Higa, F. L. Monteiro, D.P.B. Albreu, I.C. Leitao, R.S. Carvalho, R.M. Galioli, T.M.P. Castineiras, A. Nobrega, J.H. Traversos, A. Tanuri, O.C. Ferreira, A.M. Vale, L. R. Castillo, An affordable anti-SARS-CoV-2 Spike protein ELISA test for early detection of IgG seroconversion suited for large-scale surveillance studies in low-income countries, medRxiv (2020), https://doi.org/10.1101/2020.07.13.20156000.
[5] N. Okha, M. Müller, W. Li, C. Wang, C. Geerts.vanKessel, V. Corman, M. Lamers, R. Sikkmek, E. de Bruijn, F. Challand, J. Vandenpapahan, Q. Le Hingrat, D. Descamps, B. D. Solomon, L. Trimmer-Smith, M. Rattigan, B.A. Borgert, C.A. Moreno, B.D. Solomon, L. Thrasher-Smith, V. Etienne, I. Rodriguez-Barraquer, J. Lessler, H. Salje, D.S. Burke, A. Wesolowski, D.A.T. Cummings, A systematic Review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity, Nat. Commun. (2020), https://doi.org/10.1038/s41467-020-18450-4.
[6] M.S. Cozentino, M. Raboni, F.G.M. Rego, D.L. Zanette, M.N. Aoki, J.M. Nardin, B. Fornazari, H.M. Raboni, D. Souto, F.G.M. Rego, D.L. Zanette, M.N. Aoki, J.M. Nardin, F. B. Wagner, J.T. Alford, N. Deobald, F.O. Pedrosa, E.M. De Souza, M.B. Nogueira, C.S. Rollier, Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison, Lancet Infect. Dis. 20 (12) (2020), https://doi.org/10.1016/S1473-3099(20)30634-4.
[7] M.S. Conzentino et al. (EXC 2124) of the German research foundation (DFG).
[8] L.F. Huergo, K.A. Selim, M.S. Conzentino, E.C.M. Gerhardt, A.R.S. Santos, B. Wagner, J.T. Alford, N. Deobald, F.O. Pedrosa, E.M. De Souza, M.B. Nogueira, S. M. Raboni, D. Souto, F.G.M. Rego, D.L. Zanette, M.N. Aoki, J.M. Nardin, B. Fornazari, H.M.P. Morales, V.A. Borge, A. Nelde, J.S. Walz, M. Becker, N. Schneiderhan-Marra, U. Rottbauer, R.A. Reis, F. Forkhammer, Magnetic bead-based immunosassays allow rapid, inexpensive, and quantitative detection of human SARS-CoV-2 antibodies, ACS Sens. (2021), https://doi.org/10.1021/acssens.0c02544.
[9] M.S. Cozentino, F. Forkhammer, E.M. Souza, F.O. Pedrosa, M.B. Nogueira, S. M. Raboni, F.G.M. Rego, D.L. Zanette, M.N. Aoki, J.M. Nardin, B. Fornazari, H.M. Raboni, D. Souto, F.G.M. Rego, D.L. Zanette, M.N. Aoki, J.M. Nardin, B. Fornazari, H.M.P. Morales, V.A. Borge, A. Nelde, J.S. Walz, M. Becker, N. Schneiderhan-Marra, U. Rottbauer, R.A. Reis, F. Forkhammer, Magnetic bead-based immunosassays allow rapid, inexpensive, and quantitative detection of human SARS-CoV-2 antibodies, ACS Sens. (2021), https://doi.org/10.1021/acssens.0c02544.