COVID 19 Antibody Detection using Lateral Flow Assay Tests in a Cohort of Convalescent Plasma Donors

Brett Ragnesola  
New York Blood Center

Daniel Jin  
New York Blood Center

Chris C. Lamb (chrislamb@biosolutionsservices.com)  
BioSolutions Services LLC  https://orcid.org/0000-0003-3398-6382

Beth H. Shaz  
New York Blood Center

Christopher D. Hillyer  
New York Blood Center

Larry L. Luchsinger  
New York Blood Center

Research note

Keywords: Covid-19, antibody testing, convalescent plasma

DOI: https://doi.org/10.21203/rs.3.rs-38858/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Objective:

COVID19 has caused a global and ongoing pandemic. The need for population seroconversion data is apparent to monitor and respond to the pandemic. Using a lateral flow assay (LFA) testing platform, the seropositivity in 63 New York Blood Center (NYBC) Convalescent Plasma (CP) donor samples were evaluated for the presence of COVID19 specific IgG and IgM.

Results:

CP donors showed diverse antibody result. Convalescent donor plasma contains SARS-CoV-2 specific antibodies. Weak antibody bands may identify low titer CP donors. LFA tests can identify antibody positive individuals that have recovered from COVID19. Confirming suspected cases using antibody detection could help inform the patient and the community as to the relative risk to future exposure and a better understanding of disease exposure.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused over 330,000 infections and >23,000 deaths in New York State alone [1]. Due to delay in testing and asymptomatic infections the true number of cases are unknown. Few reports have characterized the prevalence of seroconversion in community populations [2, 3]. Seroconversion, the process in which a patient accumulates antigen-specific antibodies against an epitope, is the first step towards the development of adaptive immunity against pathogens. Although it is not an assurance of protection against future infections, positive seroconversion is an informative measure of previous viral infectivity within the population. To assess the seroconversion of a community, antibody testing with high sensitivity and specificity that is also easily available is necessary.

However, a crucial step in understanding the test characteristics is to ensure the assay detects antibodies in individuals with a previous documented disease. One study suggests that 75% of patients with a confirmed PCR test had a positive antibody IgG and 20% were weakly positive [4]. Another study showed 100% seroconversion in COVID19 patients and three patterns of IgM and IgG responses: synchronous seroconversion of IgG and IgM, IgM seroconversion earlier than that of IgG, and IgM seroconversion later than that of IgG [3]. In addition, assay characteristics such as antigen target (nucleocapsid and/or spike glycoprotein), total (IgG and IgM) versus IgG only, and their sensitivity and specificity are important in defining seroconversion rates [5]. Thus, more studies with various antibody tests are needed to understand seroconversion of an infected population.

In response to this need for antibody testing, a lateral flow assay (LFA) was developed to provide rapid point of care diagnostic testing of COVID19 antibodies. The LFA test is able to detect specific SARS-CoV-2 antibodies and differentiate between IgG and IgM immunoglobulin classes in a rapid, point of care test.
using either whole blood, plasma or serum. Thus, LFAs are potentially a useful assay that requires low sample input and minimum processivity. In this study, we report the sensitivity and specificity of Clungene SARS-CoV-2 IgG/IgM Rapid Test cassettes in determining the presence of binding antibodies in convalescent plasma (CP) donor samples with previously documented COVID19.

**Methods**

Convalescent donor plasma was collected by the New York Blood Center (NYBC) with written consent from patients in accordance with NYBC Institutional Review Board protocols. All donors had documented COVID19 disease by positive SARS-CoV-2 RT-PCR test, had complete resolution of symptoms at least 14 days prior to donation, and otherwise met all criteria for donating blood consistent with FDA's policy on the Collection of COVID-19 Convalescent Plasma [1]. As a negative control, fresh frozen plasma was used that was collected prior to the beginning of the epidemic.

CLUNGENE SARS-COV-2 VIRUS (COVID-19) IgG/IgM Rapid Test Cassettes were used to determine the presence of SARS-CoV-2-specific IgG and IgM. The manufacturer of the Cassette validated this immunoassay for the qualitative detection of IgG and IgM antibodies to SARS-CoV-2 and these data were submitted to FDA as part of their Emergency Use Authorization [2].

To perform assays, 20 mL of human plasma was applied to the sample pad followed by two drops of proprietary running buffer. Tests were analyzed after 15 minutes. Following incubation, high resolution images were taken of detection zone and saved as JPEG for reference and analysis. Positive and negative IgG/IgM band determinations were made by visual inspection with accordance to manufacturer instructions (Fig. 1A, 1B). All tests were performed under a NYBC IRB approved protocol using four independently trained operators.

**Results**

Convalescent donor plasma contains SARS-CoV-2 specific antibodies. Using CP donors as a prospectively positive population, we tested 63 NYBC CP donor samples for the presence of SARS-CoV-2 specific IgG and IgM. CP donors showed diverse antibody result profiles with the LFA test, including strong and weak bands as compared to FFP negative control (Fig. 1C, Table 1). All samples yielded an interpretable result with no invalid results. Overall, 88.9% (56 /63) of CP donors were considered positive. 87.3% (55/63) of CP donors were positive for IgG and 50.8% (32/63) of CP donors were positive for IgM (Fig. 2A, 2B).
| Sample# | Original/ Duplicated | Experimenter | Sample ID | IgG Result | IgM Result |
|---------|----------------------|--------------|-----------|-------------|------------|
| 1       | original             | A            | 73573     | Weak+       | Negative   |
| 2       | original             | B            | 96138     | Negative    | Negative   |
| 3       | original             | C            | 96245     | Strong+     | Negative   |
| 4       | original             | B            | 110766    | Strong+     | Strong+    |
| 5       | original             | A            | 110773    | Strong+     | Negative   |
| 6       | original             | B            | 110781    | Strong+     | Strong+    |
| 7       | original             | B            | 110782    | Strong+     | Negative   |
| 8       | original             | A            | 110788    | Strong+     | Negative   |
| 9       | original             | A            | 110790    | Strong+     | Weak+      |
| 10      | original             | A            | 110802    | Strong+     | Weak+      |
| 11      | original             | A            | 110810    | Strong+     | Weak+      |
| 12      | original             | A            | 110811    | Weak+       | Weak+      |
| 13      | original             | C            | 110958    | Strong+     | Weak+      |
| 14      | original             | C            | 110973    | Strong+     | Strong+    |
| 15      | original             | C            | 110984    | Strong+     | Negative   |
| 16      | original             | B            | 110988    | Strong+     | Negative   |
| 17      | original             | C            | 111846    | Strong+     | Strong+    |
| 18      | original             | B            | 111847    | Strong+     | Weak+      |
| 19      | original             | C            | 111848    | Strong+     | Strong+    |
| 20      | original             | C            | 111857    | Strong+     | Negative   |
| 21      | original             | C            | 116229    | Strong+     | Strong+    |
| 22      | original             | B            | 117031    | Strong+     | Weak+      |
| 23      | original             | B            | 117032    | Strong+     | Strong+    |
| 24      | original             | B            | 117055    | Negative    | Negative   |
| 25      | original             | B            | 117072    | Weak+       | Negative   |
| 26      | original             | A            | 117102    | Strong+     | Weak+      |
| 27      | original             | B            | 117131    | Negative    | Negative   |
| Sample# | Original/ Duplicated | Experimenter | Sample ID | IgG Result | IgM Result |
|---------|----------------------|--------------|-----------|------------|------------|
| 28      | original             | C            | 117707    | Strong+    | Negative   |
| 29      | original             | C            | 127010    | Strong+    | Negative   |
| 30      | original             | C            | 127161    | Negative   | Negative   |
| 31      | original             | C            | 127168    | Negative   | Negative   |
| 32      | original             | C            | 127171    | Strong+    | Negative   |
| 33      | original             | C            | 127179    | Strong+    | Negative   |
| 34      | original             | D            | 129402    | Strong+    | Strong+    |
| 35      | original             | A            | 129404    | Strong+    | Strong+    |
| 36      | original             | D            | 129405    | Strong+    | Negative   |
| 37      | original             | A            | 129408    | Negative   | Negative   |
| 38      | original             | B            | 129412    | Strong+    | Weak+      |
| 39      | original             | B            | 129414    | Strong+    | Weak+      |
| 40      | original             | B            | 129416    | Strong+    | Strong+    |
| 41      | original             | A            | 129420    | Strong+    | Strong+    |
| 42      | original             | D            | 129427    | Strong+    | Negative   |
| 43      | original             | A            | 129437    | Weak+      | Strong+    |
| 44      | original             | A            | 129455    | Strong+    | Strong+    |
| 45      | original             | A            | 129466    | Weak+      | Strong+    |
| 46      | original             | A            | 129471    | Strong+    | Strong+    |
| 47      | original             | A            | 129483    | Strong+    | Weak+      |
| 48      | original             | B            | 129491    | Strong+    | Strong+    |
| 49      | original             | B            | 129790    | Strong+    | Negative   |
| 50      | original             | A            | 129845    | Weak+      | Negative   |
| 51      | original             | B            | 129857    | Strong+    | Strong+    |
| 52      | original             | C            | 129884    | Strong+    | Negative   |
| 53      | original             | C            | 129900    | Strong+    | Negative   |
| 54      | original             | C            | 97591     | Strong+    | Negative   |
| 55      | original             | B            | 97594     | Strong+    | Weak+      |
| Sample# | Original/ Duplicated | Experimenter | Sample ID   | IgG Result | IgM Result |
|---------|---------------------|--------------|-------------|------------|------------|
| 56      | original            | C            | 97595       | Strong+    | Strong+    |
| 57      | original            | C            | 97643       | Strong+    | Negative   |
| 58      | original            | B            | 97723       | Strong+    | Weak+      |
| 59      | original            | B            | 111538      | Strong+    | Negative   |
| 60      | original            | B            | 111584      | Negative   | Negative   |
| 61      | original            | C            | 117001      | Strong+    | Negative   |
| 62      | original            | C            | 129298      | Strong+    | Negative   |
| 63      | original            | B            | 129349      | Negative   | Weak+      |
| FFP 1   | original            | C            | FFP-181484  | Negative   | Strong+    |
| FFP 2   | original            | A            | FFP-203529  | Negative   | Weak+      |
| FFP 3   | original            | A            | FFP-222235  | Negative   | Negative   |
| FFP 4   | original            | A            | FFP-222252  | Negative   | Negative   |
| FFP 5   | original            | A            | FFP-222353  | Negative   | Negative   |
| FFP 6   | original            | A            | FFP-222427  | Negative   | Negative   |
| FFP 7   | original            | A            | FFP-222604  | Negative   | Negative   |
| FFP 8   | original            | A            | FFP-222633  | Negative   | Negative   |
| FFP 9   | original            | A            | FFP-900220  | Negative   | Negative   |
| FFP 10  | original            | A            | FFP-906227  | Negative   | Negative   |
| 4       | duplicated          | D            | 110766      | Strong+    | Strong+    |
| 4       | duplicated          | B            | 110766      | Strong+    | Strong+    |
| 6       | duplicated          | D            | 110781      | Strong+    | Weak+      |
| 6       | duplicated          | B            | 110781      | Strong+    | Strong+    |
| 7       | duplicated          | D            | 110782      | Strong+    | Negative   |
| 7       | duplicated          | B            | 110782      | Strong+    | Negative   |
| 22      | duplicated          | D            | 117031      | Negative   | Negative   |
| 22      | duplicated          | B            | 117031      | Strong+    | Weak+      |
| 23      | duplicated          | D            | 117032      | Strong+    | Negative   |
| 23      | duplicated          | B            | 117032      | Strong+    | Strong+    |
| Sample# | Original/ Duplicated | Experimenter | Sample ID | IgG Result | IgM Result |
|---------|---------------------|--------------|-----------|------------|------------|
| 24      | duplicated          | D            | 117055    | Negative   | Negative   |
| 24      | duplicated          | B            | 117055    | Negative   | Negative   |
| 25      | duplicated          | D            | 117072    | Negative   | Negative   |
| 25      | duplicated          | B            | 117072    | Weak+      | Negative   |
| 34      | duplicated          | D            | 129402    | Strong+    | Strong+    |
| 34      | duplicated          | B            | 129402    | Strong+    | Weak+      |
| 36      | duplicated          | D            | 129405    | Strong+    | Negative   |
| 36      | duplicated          | B            | 129405    | Strong+    | Negative   |
| 38      | duplicated          | D            | 129412    | Strong+    | Negative   |
| 38      | duplicated          | B            | 129412    | Strong+    | Weak+      |
| 39      | duplicated          | D            | 129414    | Strong+    | Negative   |
| 39      | duplicated          | B            | 129414    | Strong+    | Weak+      |
| 40      | duplicated          | D            | 129416    | Strong+    | Strong+    |
| 40      | duplicated          | B            | 129416    | Strong+    | Strong+    |
| 42      | duplicated          | D            | 129427    | Strong+    | Negative   |
| 42      | duplicated          | B            | 129427    | Negative   | Negative   |
| 48      | duplicated          | D            | 129491    | Strong+    | Weak+      |
| 48      | duplicated          | B            | 129491    | Strong+    | Strong+    |
| 49      | duplicated          | D            | 129790    | Strong+    | Negative   |
| 49      | duplicated          | B            | 129790    | Strong+    | Negative   |
| 51      | duplicated          | D            | 129857    | Strong+    | Strong+    |
| 51      | duplicated          | B            | 129857    | Strong+    | Strong+    |

With regard to negative samples, 11.1% (7/63) of CP donors were IgG\textsuperscript{Neg} and IgM\textsuperscript{Neg}, 1.6% (1/63) were IgG\textsuperscript{Neg} and IgM\textsuperscript{Pos} and 38.1% (24/63) were IgG\textsuperscript{Pos} and IgM\textsuperscript{Neg} (Fig. 2C). In contrast, all FFP samples were IgG\textsuperscript{Neg} and 80% (8/10) were IgM\textsuperscript{Neg}. These data suggest that LFA tests possess a high degree of sensitivity (87.3% IgG, 50.8% IgM) and specificity (100.0% IgG, 80.0% IgM) for detecting SARS-CoV-2 specific antibodies.

Weak antibody bands may identify low titer CP donors. Recent studies suggest that a significant percentage of convalescent individuals may have low SARS-CoV-2 IgG or IgM titers [4, 6]. We also inferred
from conducting LFA assays that potential differences in antibody levels may occur in the CP donor population. However, LFA tests are designed to perform qualitative, and not quantitative, analysis as stated in the manufacturer's instructions. Nevertheless, to document this phenomenon, trained experimenters subjectively delineated positive results as ‘strong’ or ‘weak’ relative to the band intensity produced by each CP donor sample (Fig. 1C).

To confirm reproducibility, we re-tested random samples (n = 16) to explore whether CP donor samples could provide reproducible results (Fig. 2D, Table 1). Between replicates of paired results, 56.25% (9/16) of samples were consistently positive, 6.25% (1/16) was consistently negative, and 37.5% (6/16) were inconsistent. With regard to inconsistency, these bands were almost always visually weak (4/6). These data suggest that certain CP donors may have low levels of SARS-CoV-2 antibodies and may produce inconsistent results.

Discussion

Our study analyzed blood samples from COVID19 convalescent plasma donors to determine whether antibodies are detected using a LFA in this population. We found that the CLUNGENE SARS-COV-2 VIRUS (COVID-19) IgG/IgM LFA test possesses high sensitivity and specificity for COVID19 antibodies. The LFA test was easy to use with properly trained staff. Results were interpretable within 15 minutes and the internal procedural control confirmed that sufficient specimen volume, adequate membrane wicking and correct procedural technique were used. Since documented positive PCR tests or comparison to other antibody testing platforms were unavailable, we cannot state that the 7 negative donors in fact were infected or if they have antibody. Even if CP donor infection data were available, it may also be possible, and is probable, that some CP donors produced low amounts of antibodies that is specific to the immunological response unique to each individual, thus, below the detection limit of the LFA. The IgG results are consistent with the manufacturer's 97.4% clinical performance data which showed positive IgG agreement with known positive RT-PCR test. The IgM results are consistent with recently published data which shows that IgM can persist more than 23 days after symptom onset and can be earlier, synchronous or later than IgG.

Conclusions

Most (90%) COVID19 convalescent donors seroconverted, demonstrating the potential of LFA tests to identify antibody positive individuals that have recovered from COVID19. Confirming suspected SARS-CoV-2 cases using antibody detection at the point of care could help inform the patient and the community as to the relative risk to future SARS-CoV2- exposure and a better understanding of disease exposure. However, a coherent description of the immunological response and antiviral antibody activity (i.e. neutralizing activity) is warranted to definitively use antibody presence to prognose future disease potential. This study highlights the relevance of serological testing to support accurate estimates of the extent of the COVID-19 pandemic and the potential to assess patient immunity by antibody detection.
Limitations

Our study has several limitations, including but not limited to:

- Samples were not tested for virus neutralization; therefore neutralizing activities of the detected IgG antibodies are not known.
- The small sample size of patients and the absence of documented PCR test results makes it difficult to determine the relationship between antibody response and clinical course.

Abbreviations

- lateral flow assay (LFA)
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) aka COVID19
- convalescent plasma (CP)
- Immunoglobulin G (IgG)
- Immunoglobulin M (IgM)
- New York Blood Center (NYBC)
- polymerase chain reaction (PCR)

Declarations

Ethics approval and consent to participate

All tests were performed under a NYBC IRB approved protocol. Convalescent donor plasma was collected by the New York Blood Center (NYBC) with written consent from patients in accordance with NYBC Institutional Review Board protocols.

Consent for publication

All authors agree to submit for consideration for publication in the journal.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding authors on reasonable request.

Competing interests

CL worked with the LFD manufacturer on the Emergency Use Authorization submission to the US FDA.

Funding
The LFD used in the testing were provided by CL/BioSolutions services LLC.

Authors’ contributions

LL and CDH conceived this study. CL provided financial and administrative support. BR and DJ performed most of the data analysis and collected the data using the CP cohort. CL provided the devices for this study. All authors discussed the results, explained them further and prepared the tables and panels for these figures. CL, LL, CDH, BS and BR wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thanks Ryan Dagenais for his editorial support with the manuscript; Ryan's services were funded by BioSolutions Services LLC.

Author details

1 New York Blood Center Lindsley F. Kimball Research Institute, New York, NY 10065 USA

2 BioSolutions Services, Englewood Cliffs, New Jersey, United States; Department of Management and Entrepreneurship, Silberman College of Business, Fairleigh Dickinson University, Teaneck, New Jersey, United States; Weatherhead School of Management, Case Western Reserve University, Cleveland, Ohio, USA

References

1. DOH N. New York State Department of Health COVID-19 Tracker. 2020.
2. Ni L, Ye F, Cheng ML, Feng Y, Deng YQ, Zhao H, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. Immunity. 2020.
3. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020.
4. Ania Wajnberg MM, Emily Leven, Nicole M. Bouvier, Gopi Patel, Adolfo Firpo, Rao Mendu, Jeffrey Jhang, Suzanne Arinsburg, DO, Melissa Gitman, Jane Houldsworth, Ian Baine, Viviana Simon, Judith Aberg, Florian Krammer, David Reich, Carlos Cordon-Cardo Humoral immune response and prolonged PCR positivity in a cohort of 13432 SARS-CoV 2 patients in the New York City region. 2020.
5. CDC. Interim Guidelines for COVID-19 Antibody Testing: Centers for Disease Control and Prevention; 2020 [Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html.

6. Davide F. Robbiani CG, Frauke Muecksch, Julio C. C. Lorenzi1, Zijun Wang, Alice Cho, Marianna Agudelo, Christopher O. Barnes, Anna Gazumyan, Shlomo Finkin, Thomas Hagglof, Thiago Y. Oliveira, Charlotte Viant, Arlene Hurley, HansHeinrich Hoffmann, Katrina G. Millard, Rhonda G. Kost, Melissa Cipolla, Kristie Gordon, Filippo Bianchini1 STC, Victor Ramos, Roshni Patel, Juan Dizon, IrinaShimeliovich1, Pilar Mendoza, Harald Hartweger, Lilian Nogueira, Maggi Pack, Jill Horowitz, Fabian Schmidt, Yiska Weisblum, Eleftherios Michailidis, Alison W. Ashbrook, Eric Waltari, John E. Pak, Kathryn E. Huey-Tubman, Nicholas Koranda, Pauline R. Hoffman, Anthony P. West, Jr., Charles M. Rice, Theodora Hatzioannou, Pamela J. Bjorkman, Paul D. Bieniasz, Marina Caskey, Michel C. Nussenzweig. Convergent Antibody Responses to SARS-CoV-2 Infection in Convalescent Individuals. 2020.

Figures
Figure 1

(A) Procedural schematic for CLUNGENE Immunoassay. One drop is equal to ~20 uL. (B) Visual interpretation guide for assays. (C) Representative convalescent donor plasma (CP) or frozen fresh plasma (FFP) assay result images.
(A) Frequency of IgG assay results from CP donor samples. (B) Frequency of IgM assay results from CP donor samples. (C) Overall CP donor test result. (D) Frequency of assay result duplication using identical CP donor samples.