



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.

## The arrival of SARS-CoV-2-neutralizing antibodies in a currently available commercial immunoglobulin



To the Editor:

The coronavirus disease 2019 (COVID-19) pandemic has heightened awareness of the primary immunodeficiency/inborn errors of immunity community, and its impact on those with immunodeficiency diseases has been reported recently.<sup>1</sup> Management of these patients often involves administration of therapeutic immunoglobulin (IgG); however, the formation of antibodies to novel pathogens, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lags behind plasma donor convalescence or vaccination. Confirming the presence of neutralizing antibodies has value for future immunoglobulin-based modalities and is of clear benefit to this patient population.

To evaluate the presence of SARS-CoV-2 antibodies in currently available preparations of Hizentra (20% liquid for subcutaneous infusion), 10 recent lots representing expiration dates encompassing 1 year were analyzed by using a SARS-CoV-2 ELISA. Our customized ELISA provided broad SARS-CoV-2 antibody detection using whole cell antigen generated from VeroE6 cells infected with the SARS-CoV-2 strain USA-WA1/2020, thus allowing for the greatest breadth of epitope coverage recognizing both natural and vaccine-elicited antibodies.<sup>2</sup> For analysis, Hizentra samples were serially diluted to empiric ranges for detection. Positive detection was based on an OD<sub>405 nm</sub> value of 0.2 or higher. The first sequential sample to cross this threshold was lot 5 (expiration date July 20, 2023 [Fig 1, A]). To better quantify titers, we compared OD results for the 1:1600 dilution, which showed a steady increase in titers culminating in a 10.7-fold elevation (sample 10 vs sample 5) by sample lot 10 (expiration date December 22, 2023). Interestingly, the titer for lot 10 was 2.2-fold higher than that for SARS-CoV-2 convalescent serum, which served as a positive control (Fig 1, A).

Neutralization studies were carried out by using our mNeonGreen SARS-CoV-2 fluorescent reporter system, which is equivalent to well-established plaque reduction assays.<sup>3</sup> Hizentra lots 1 through 5 were devoid of detectable neutralization (defined as neutralization negative at a dilution of 1:20). However, by sample lot 6 (expiration date August 8, 2023), neutralization activity was detected, and it escalated in each subsequent lot from titers of 41 to 2523, culminating in a 61.5-fold increase by lot 10 (a 50% neutralization titer [NT<sub>50</sub>] of 2523 for sample 10 [expiration date December 22, 2023] vs an NT<sub>50</sub> of 41 for sample 6 [expiration date August 8, 2023] [Fig 1, B]).

Collectively, these data confirmed the chronologic availability of SARS-CoV-2-neutralizing antibodies in Hizentra. Detection was correlated with neutralization and increased temporally, ultimately reaching impressive titer increases of 10.7-fold and 61.5-fold, respectively (Fig 1, A and B). The NTs in sample lot 10 (NT<sub>50</sub> = 2523) were 4.2-fold higher than the reported average

titer observed in 64 patient sera collected 1 month after natural infection (NT<sub>50</sub> = 601).<sup>4</sup>

Although our study is limited in terms of both product brand and scale, we believe that it is important to expedite findings that may directly affect patients undergoing IgG replacement therapy. "Does my immunoglobulin contain SARS-CoV-2 antibodies?" is a question of intense interest that is frequently asked by both patients with primary immunodeficiency and the health care provider community. According to a recent publication devoted to current manufacturer-directed inquiries, 65% of patients and 45% of health care providers have inquired about the presence of SARS-CoV-2 antibodies in their immunoglobulin products.<sup>5</sup> This level of inquiry warrants experimental investigation and timely reporting to answer the question. From the results of our study, the initial detection of SARS-CoV-2-neutralizing antibodies has been observed in current patient-accessible lots of the IgG therapeutic Hizentra.

We thank L.M. for supplying essential assay reagents.

Aaron L. Miller, MS<sup>a,b</sup>

Nicholas L. Rider, DO<sup>c</sup>

Richard B. Pyles, PhD<sup>a,b</sup>

Barbara Judy, PhD<sup>d</sup>

Xuping Xie, PhD<sup>e</sup>

Pei-Yong Shi, PhD<sup>e</sup>

Thomas G. Ksiazek, DVM, PhD<sup>d</sup>

From <sup>a</sup>the Assay Development Service Division, Galveston National Laboratory and <sup>d</sup>the Department of Pathology, <sup>b</sup>the Department of Pediatrics and <sup>c</sup>the Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Tex, and <sup>e</sup>the Division of Immunology, Allergy and Retrovirology, William T. Shearer Center for Immunobiology, Texas Children's Hospital, Houston, Tex. E-mail: [aamiller@utmb.edu](mailto:aamiller@utmb.edu).

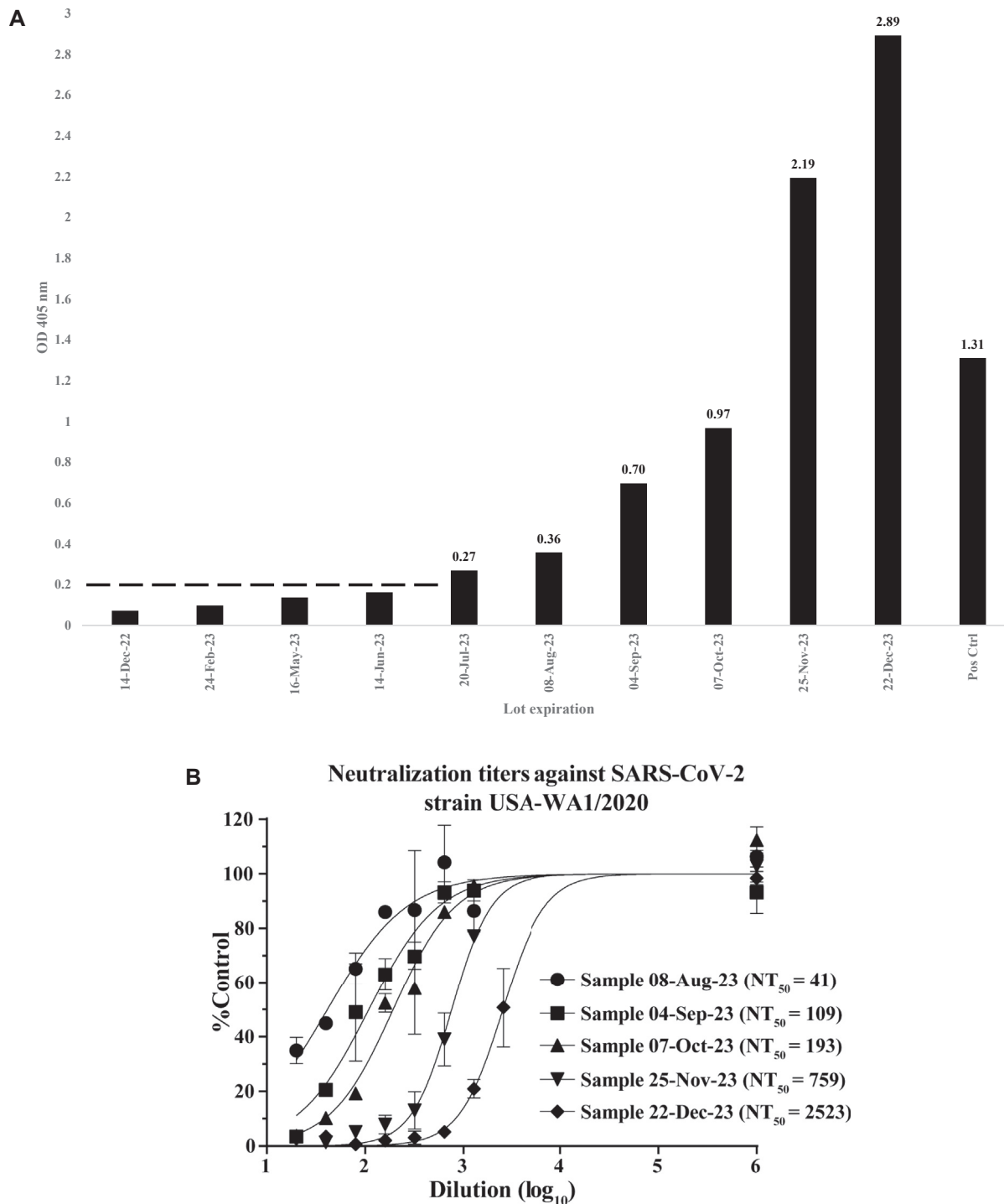
Supported by the National Institutes of Health (grants HHSN272201600013C, AI134907, AI145617, and UL1TR001439 [to P.-Y.S.]) and awards from the Sealy & Smith Foundation, the Kleberg Foundation, the John S. Dunn Foundation, the Amon G. Carter Foundation, the Gillson-Longenbaugh Foundation, and the Summerfield Roberts Foundation (to P.-Y.S.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest. Hizentra samples were obtained and evaluated with no input or affiliation from the manufacturer, CSL Behring.

### REFERENCES

1. Meyts I, Buccioli G, Quinti I, Neven B, Fischer A, Seoane E, et al. Coronavirus disease 2019 in patients with inborn errors of immunity: an international study. *J Allergy Clin Immunol* 2021;147:520-31.
2. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003; 348:1953-66.
3. Muruato AE, Fontes-Garfias CR, Ren P, Garcia-Blanco MA, Menachery VD, Xie X, et al. A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. *Nat Commun* 2020;11:4059.
4. Zou J, Xia H, Xie X, Kurhade C, Machado RRG, Weaver SC, et al. Neutralization against Omicron SARS-CoV-2 from previous non-Omicron infection. *Nat Commun* 2022;13:852.
5. Stinca S, Barnes TW, Vogel P, Meyers W, Schulte-Pelkum J, Filchtinski D, et al. Modelling the concentration of anti-SARS-CoV-2 immunoglobulin G in intravenous immunoglobulin product batches. *PLoS One* 2021;16:e0259731.

<https://doi.org/10.1016/j.jaci.2022.03.026>



**FIG 1.** SARS-CoV-2 antibody assessment of Hizentra lots. **A**, Ten temporal lots of Hizentra were diluted for detection within the linear range of a SARS-CoV-2–specific ELISA. Lot 1 (expiration date December 14, 2022) is the leftmost sample, and lot 10 (expiration date December 22, 2023) is the rightmost sample, followed by the positive control (de-identified SARS-CoV-2 convalescent patient serum). The results for the 1:1600 dilution are depicted, indicating relative antibody levels (OD<sub>405 nm</sub>) at lot expiration dates. The threshold for ELISA positivity is shown as a dashed line (OD<sub>405 nm</sub> ≥ 0.2) established by a 1:400 dilution (not shown). **B**, All 10 sample lots (lots 6 through 10 are shown) were measured for neutralizing activities against SARS-CoV-2 engineered with an mNeonGreen (mNG) fluorescent reporter virus on VeroE6 cells by fluorescent focus reduction NT (FFRNT) as described previously.<sup>3,4</sup> Error bars indicate SDs from duplicates. The nonlinear regression curves of the relative infectivity versus the Hizentra dilutions (log<sub>10</sub> values) were created with Prism 9 (GraphPad Software, San Diego, Calif) and used to determine the fold dilution that neutralized 50% of mNG SARS-CoV-2 infectivity (defined as FFRNT<sub>50</sub>). The calculated NT<sub>50</sub> values are shown for sample lots 6 to 10 (expiration dates Aug 8 through December 22, 2023).