

# THE LANCET

## Infectious Diseases

### Supplementary appendix

This appendix formed part of the original submission. We post it as supplied by the authors.

Supplement to: Romero C, Díez J-M, Gajardo R. Anti-SARS-CoV-2 antibodies in healthy donor plasma pools and IVIG products—an update. *Lancet Infect Dis* 2021; **22**: 10.

## **Anti-SARS-CoV-2 antibodies in healthy donor plasma pools and IVIG products— an update**

Figure 1. Anti-SARS-CoV-2 antibody titres in plasma pools collected in Spain (A), the USA (B; the polynomial trend is shown in the figure: thick red line), and central European countries (C), and in IVIG batches manufactured from the plasma collected in these countries (D). Gamunex products were manufactured at Grifols Biologicals and Grifols Therapeutics in Los Angeles, CA, USA, and Clayton, NC, USA, respectively, and Flebogamma DIF was manufactured in Parets Del Vallès (Spain). The thin red line shows the cutoff value for a positive response. Cross-reactivity with pre-existing antibodies to common seasonal coronaviruses has been demonstrated,<sup>1,2</sup> therefore a cutoff value was necessary to determine specific anti-SARS-CoV-2 antibodies. The cutoff was calculated as the mean value of pre-COVID-19 batches of IVIG plus three standard deviations. Values above the cutoff were considered positive for specific anti-SARS-CoV-2 antibodies. Values below the cutoff were considered a result of cross-reactivity with seasonal coronavirus antibodies.

Figure 1A.

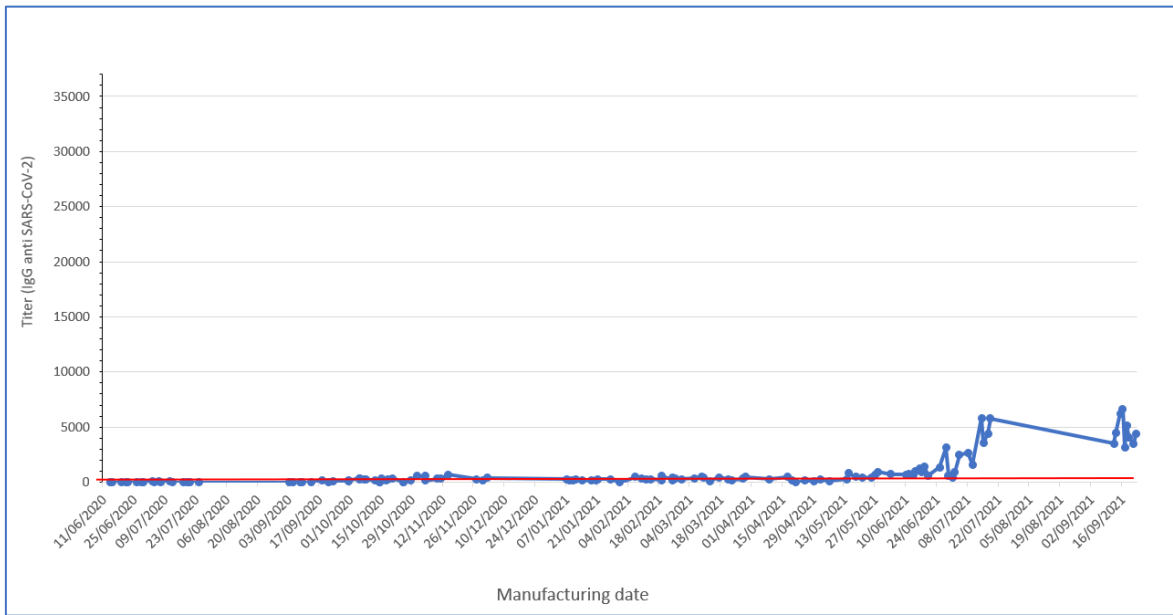


Figure 1B.

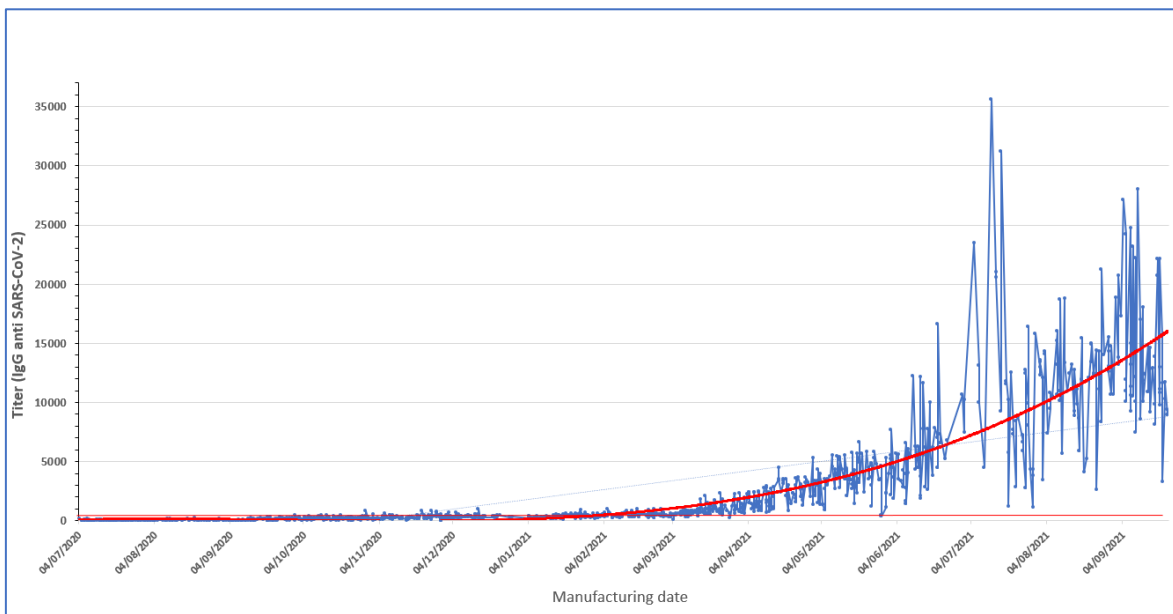


Figure 1C.

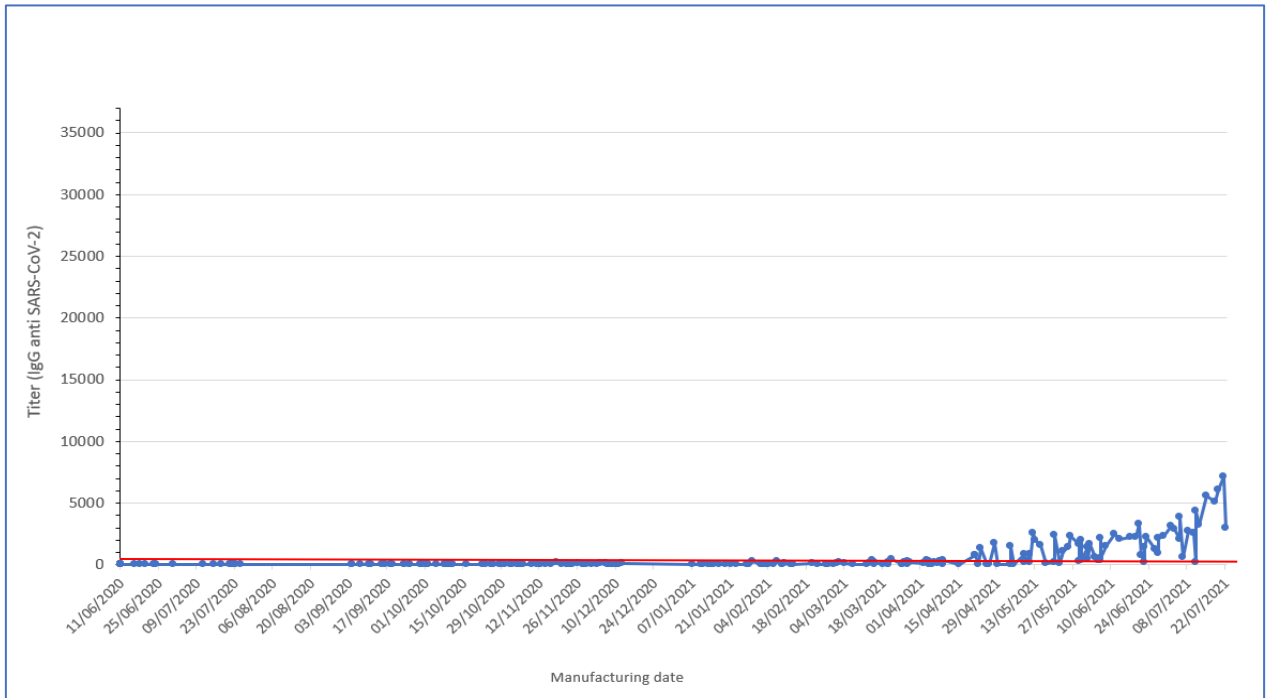
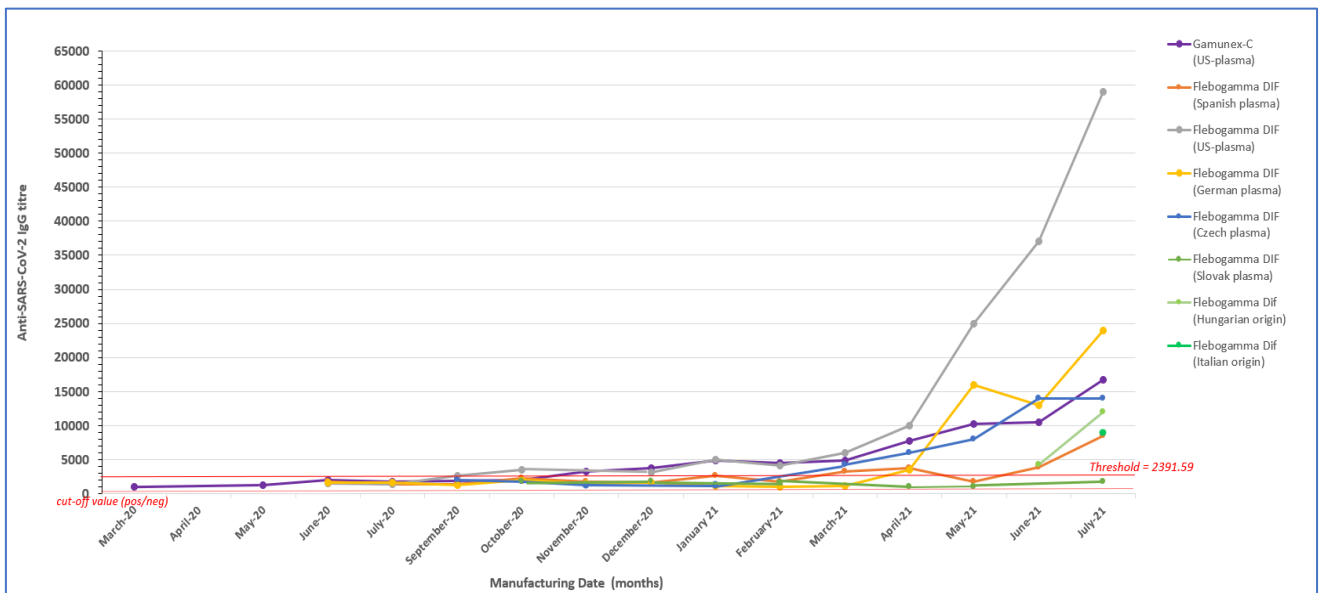


Figure 1D.



## Supplementary appendix

**Supplementary Table 1. SARS-CoV-2 neutralisation measured in immunoglobulin (IgG) final products.**

IgG final product (Country-Production date)	SARS-CoV-2 VIRUS ID: EPI_ISL_510689 ID <sub>50</sub>
Gamunex-C 10% (USA-12/20)	< LOD
Gamunex-C 10% (USA-12/20)	< LOD
Gamunex-C 10% (USA-1/21)	133·0
Gamunex-C 10% (USA-3/21)	64·2
Gamunex-C 10% (USA-4/21)	74·4
Flebogamma-DIF 5% (USA-3/21)	0·5
Flebogamma-DIF 10% (Czechia-3/21)	33·5
Flebogamma-DIF 5% (Spain-5/21)	375·1

ID<sub>50</sub> = dilution producing 50% neutralisation; LOD = limit of detection. EPI\_ISL\_510689 was the first SARS-CoV-2 virus strain. Neutralisation assays were performed as described in Diez et al.<sup>3</sup>

**Supplementary Table 2. SARS-CoV-2 neutralisation activity (ID<sub>50</sub>) by immunoglobulin (IgG) final products tested against pseudoviruses expressing the S-proteins of the major variants of concern.**

IgG final product (Country-Production date)	WH1 (original)	D614G (2nd Wave)	B.1.1.7 <i>alpha</i>	B.1.351 <i>beta</i>	P.1 <i>gamma</i>	B.1.617.2 <i>delta</i>
Gamunex-C 10% (USA-5/21)	422	259	129	117	115	243
Flebogamma-DIF 10% (Germany-5/21)	875	383	385	139	184	444

Generation of pseudoviruses and neutralisation assays were performed as described in Diez et al.<sup>3</sup>

### Acknowledgements

The authors acknowledge the expert technical assistance of the personnel of the Quality Control Departments of Grifols Biologicals (Los Angeles, CA, USA), Grifols Therapeutics (Clayton, NC, USA), Instituto Grifols SA (Barcelona, Spain) and the Immunotherapies Unit, Grifols Global Research & Development Bioscience Industrial Group (Barcelona, Spain). In addition, the authors acknowledge IRSI-Caixa (Badalona, Spain) and CReSA-IRTA (Bellaterra, Spain) for performing the pseudovirus and the SARS-CoV-2 neutralisation experiments, respectively. Writing and editorial assistance from the Grifols Scientific Publications Department is also acknowledged.

### References

- 1 Díez JM, Romero C, Gajardo R. Currently available intravenous immunoglobulin contains antibodies reacting against severe acute respiratory syndrome coronavirus 2 antigens. *Immunotherapy* 2020; **12**: 571–76.

- 2 Ng KW, Faulkner N, Cornish GH, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science* 2020; **370**: 1339–43.
- 3 Díez JM, Romero C, Cruz M, Vandenberg P, Merritt WK, Pradenas E, Trinité B, Blanco J, Clotet B, Willis T, Gajardo R. Anti-SARS-CoV-2 hyperimmune globulin demonstrates potent neutralization and antibody-dependent cellular cytotoxicity and phagocytosis through N and S proteins. *J Inf Dis* 2021; jiab540.