**Epidemiology and Infection**

**Cambridge University Press**

**Improved diagnosis of SARS-CoV-2 by using Nucleoprotein and Spike protein fragment 2 in quantitative dual ELISA tests**

Carolina De M. Verissimo1\*; Carol O’Brien2; Jesús López Corrales1; Amber Dorey1; Krystyna Cwiklinski1; Richard Lalor1; Jack M. Doyle2; Stephen Field3; Claire Masterson4; Eduardo Ribes Martinez4; Gerry Hughes5,6; Colm Bergin5,6; Kieran Walshe2; Bairbre McNicholas7; John G. Laffey4; John P. Dalton1; Colm Kerr5,6;Sean Doyle2

**SUPPLEMENTARY FILE**

C:\Users\0123744S\Desktop\COVID\paper\Submitted\Fig S1.tif

**Supplementary Figure S1.** Mass spectrometry analysis (MS/MS) to confirm the SARS-CoV-2 protein sequences recombinantly expressed. A: Nucleocapsid protein (Npro). B: Subunit 2 of Spike protein (S2Frag). The tryptic peptides that match the known sequence of amino acids for the SARS-CoV-2 identified by MS/MS are highlighted in yellow, and cover 53% and 68% of the Npro and S2Frag sequence, respectively.

**C:\Users\0123744S\Desktop\COVID\paper\Submitted\Fig S2.tif**

**Supplementary Figure S2. The variability of the antibody response to viral proteins of individuals positive for SARS-CoV-2.** A, Recombinant proteins resolved in a 4-12% SDS-PAGE and stained with Coomassie-blue. B – D, western-blots showing the antibody response of different SARS-CoV-2 infected individuals to different viral protein. M, molecular weight in kDa. Spike protein (Spro), Spike protein fragment 1 (S1Frag), Spike protein fragment 2 (S2Frag), S2Prime protein (S2Pri) and Receptor binding domain (RBD).

C:\Users\0123744S\Desktop\COVID\paper\Submitted\Fig S3.tif

**Supplementary Figure S3. Correlation between antibody response to SARS-CoV-2 antigens and age in 42 RT-PCR-confirmed COVID-19 individuals.** Correlation analysis (Spearman test) with 95% confidence interval. P values are shown in the figure.

C:\Users\0123744S\Desktop\COVID\paper\Submitted\Fig S4a.tif

**Supplementary Figure S4A. Western blots analyses of serum samples from COVID-19 confirmed cases.** The recombinant proteins were resolved in a SDS-PAGE gel and stained with Coomassie blue as a control for the western blots. A, Spike protein (Spro); B, Spike protein fragment 1 (S1Frag); C, Nucleocapsid protein (Npro); D, 3Clike protease (Main protease) (internal control); E, Spike protein fragment 2 (S2Frag); F, S2Prime protein (S2Pri); G, Receptor binding domain (RBD). MW, Molecular weight marker in kDa. C1-C34, C85-C86, HCW10, HCW19, HCW30, HCW32, Im1-3 and Im5, individual study code for serum samples used in the Western blot analyses.

C:\Users\0123744S\Desktop\COVID\paper\Submitted\Fig S4b.tif

**Supplementary Figure S4B. Western blots analyses of serum samples from COVID-19 confirmed cases.** The recombinant proteins were resolved in a SDS-PAGE gel and stained with Coomassie blue as a control for the western blot, according Supplementary Fig S4A. A, Spike protein (Spro); B, Spike protein fragment 1 (S1Frag); C, Nucleocapsid protein (Npro); D, 3Clike protease (Main protease) (internal control); E, Spike protein fragment 2 (S2Frag); F, S2Prime protein (S2Pri); G, Receptor binding domain (RBD). MW, Molecular weight marker in kDa. C1-C34, C85-C86, HCW10, HCW19, HCW30, HCW32, Im1-3 and Im5, individual study code for serum samples used in the Western blot analyses.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Supplementary Table S1. Antibody levels to Npro and S2Frag in COVID-19 hospitalised patients | | | | | | | | | | |
| Sample | **1st sample**  **(DAO)** | **Npro ELISA** | **S2Frag ELISA** | **2nd sample**  **(DAO)** | **Npro ELISA** | **S2Frag ELISA** | **RT-PCR** | **Sex** | **Age** | **ICU** | |
| OD/CO | OD/CO | OD/CO | OD/CO |
| 009 | 17 | **12.86** | **3.89** | 54 | **11.70** | **2.17** | Positive | M | 71 | Yes | |
| 015 | 11 | **3.69** | **1.67** | 58 | **10.59** | **2.73** | Positive | M | 69 | Yes\* | |
| 018 | 22 | **12.24** | **3.26** | 31 | **9.86** | **1.19** | Positive | M | 66 | Yes\* | |
| 021 | 16 | **11.75** | **1.78** | 22 | **11.34** | **1.53** | Positive | M | 65 | Yes\* | |
| 023 | 18 | **13.19** | **2.39** | 32 | **11.41** | **2.06** | Positive | F | 56 | Yes\* | |
| 024 | 33 | **12.28** | **1.37** | 65 | **8.27** | **1.02** | Positive | F | 66 | Yes\* | |
| 029 | 11 | **11.67** | **1.42** | 21 | **11.02** | **2.09** | Positive | M | 42 | No\* | |
| 041 | 20 | **12.76** | **6.52** | 33 | **11.56** | **2.14** | Positive | M | 80 | No | |
| 070 | 7 | **3.97** | 0.58 | 13 | **11.68** | **1.39** | Positive | M | 53 | Yes | |
| 077 | 24 | **12.03** | **1.55** | 39 | **10.87** | **1.81** | Positive | M | 62 | No | |
| 078 | 9 | **1.28** | 0.60 | 28 | **11.20** | **1.93** | Positive | F | 35 | No\* | |
| 085 | 0 | 0.85 | 0.35 | 40 | **5.81** | 0.70 | Positive | F | 71 | No | |
| 086 | 0 | 0.76 | 0.60 | 40 | **3.01** | **1.59** | Positive | M | 79 | No | |
| 090 | 7 | 0.97 | 0.67 | 9 | **1.76** | **2.47** | Positive | F | 44 | No | |
| 091 | 12 | **5.79** | 0.85 | 20 | **7.72** | **10.26** | Positive | F | 70 | No | |
| 4-024 | - | - | - | - | 0.35 | 0.30 | Negative | F | 78 | Ni | |
| 4-028 | - | - | - | - | 0.36 | 0.47 | Negative | M | Ni | Ni | |
| 4-043 | - | - | - | - | 0.54 | 0.91 | Negative | M | Ni | Ni | |
| 4-064 | - | - | - | - | 0.88 | 0.84 | Negative | F | Ni | Ni | |
| 4-074 | - | - | - | - | 0.83 | **3.08** | Negative | F | Ni | Ni | |
| 4-089 | - | - | - | - | **1.30** | 0.80 | Negative | M | Ni | Ni | |
| 4-090 | - | - | - | - | **8.84** | 0.65 | Negative | F | Ni | Ni | |
| 4-118 | - | - | - | - | 0.55 | 0.41 | Negative | F | Ni | Ni | |
| 4-120 | - | - | - | - | 0.52 | 0.74 | Negative | F | Ni | Ni | |
| 4-231 | - | - | - | - | **1.32** | 0.93 | Negative | F | 89 | Ni | |
| DAO: number of days after the onset of symptoms. OD: Optical density at 450 nm. CO: Cut-off of the specific ELISA. OD/CO >1: Positive for ELISA; OD/CO <1: Negative for ELISA. ICU: Intensive care unit. (\*): Patients that required invasive ventilation. Ni. Not informed. | | | | | | | | | | | |