

1 **TITLE:** Neutralization heterogeneity of United Kingdom and South-African SARS-CoV-2
2 variants in BNT162b2-vaccinated or convalescent COVID-19 healthcare workers

3 **AUTHORS:**

4 Stéphane Marot^{1*}, Isabelle Malet¹, Aude Jary¹, Valentin Leducq¹, Basma Abdi¹, Elisa Teyssou¹,
5 Cathia Soulie¹, Marc Wirден¹, Christophe Rodriguez^{2,3}, Slim Fourati^{2,3}, Jean-Michel
6 Pawlotsky^{2,3}, David Boutolleau¹, Sonia Burrel¹, Vincent Calvez¹, Anne-Geneviève Marcelin¹.

7 Affiliations:

8 ¹Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique
9 (iPLESP), Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié Salpêtrière Hospital,
10 Department of Virology, Paris, France.

11 ²Department of Virology, Hôpitaux Universitaires Henri Mondor, AP-HP, Créteil, France.

12 ³Team “Viruses, Hepatology, Cancer”, Institut Mondor de Recherche Biomédicale, INSERM
13 U955, Université Paris-Est, Créteil, France.

14 *Corresponding author. Stéphane Marot, Department of Virology, Pitié Salpêtrière Hospital,
15 AP-HP, CERVI, 83 Boulevard de l'Hôpital, 75013, Paris, France. Email:
16 stephanesylvain.marot@aphp.fr

17 Alternate corresponding author: Anne-Geneviève Marcelin, , Department of Virology, Pitié
18 Salpêtrière Hospital, AP-HP, CERVI, 83 Boulevard de l'Hôpital, 75013, Paris, France. Email:
19 anne-genevieve.marcelin@aphp.fr

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22 **Running title:** COVID-19 infection or vaccine-elicited NABs

23 **ABSTRACT**

24 There are concerns about neutralizing antibodies (NAb) potency against the newly emerged
25 VOC202012/01 (UK) and 501Y.V2 (SA) SARS-CoV-2 variants in mRNA-vaccinated subjects
26 and in recovered COVID-19 patients. We used a viral neutralization test with a strict 100%
27 neutralizing criterion on UK and SA clinical isolates in comparison with a globally distributed
28 D614G SARS-CoV-2 strain. In two doses BNT162b2-vaccinated healthcare workers (HCW),
29 despite heterogeneity in neutralizing capacity against the three SARS-CoV-2 strains, most of
30 the sera harbored at least a NAb titer $\geq 1:10$ suggesting a certain humoral protection activity
31 either on UK or SA variants. However, six months after mild forms of COVID-19, an important
32 proportion of HCW displayed no neutralizing activity against SA strain. This result supports
33 strong recommendations for vaccination of previously infected subjects.

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46 INTRODUCTION

47 In the gene encoding the Spike (S) protein of SARS-CoV-2, various mutations have been
48 reported[1,2] and recently, the United Kingdom (UK) and South Africa (SA) have faced a rapid
49 increase in COVID-19 cases mediated by the emergence of new variants (VOC-202012/01 for
50 UK and 501Y.V2 for SA)[3,4]. The spreading of these variants has increased rapidly in other
51 countries and recent observations suggests that they are significantly more transmissible than
52 previously circulating variants. It is still not fully known if the pathogenicity is either increased,
53 although some elements have been recently released with likely enhanced disease severity for
54 the UK strain[5].

55 These variants harbor a specific pattern of deletion and mutations including amino-acid
56 replacements at key sites in the S Receptor Binding Domain (RBD) (K417N, E484K, N501Y
57 for the SA strain and only N501Y for the UK strain). In the era of the COVID-19 vaccination,
58 the question remained whether these variants could escape the neutralizing response elicited by
59 mRNA-vaccine. Two recent studies performed on engineered SARS-CoV-2 viruses containing
60 only some mutations from the newly emerged UK and SA variants showed weaker
61 neutralization capacity of vaccine-elicited sera[6,7]. Another study tested SARS-CoV-2-S
62 pseudoviruses bearing either the Wuhan reference strain or the UK spike protein with
63 BNT162b2 vaccine-elicited sera showed a slightly reduced but overall largely preserved
64 neutralizing titers against the UK pseudovirus[8]. However, none of these studies was
65 performed on clinical isolates harboring the full genomic mutations background of UK and SA
66 strains. Thus, the question remained whether a replicating virus with the full set of S mutations,
67 which may potentially interfere with antibody binding would be neutralized efficiently by
68 convalescent COVID-19 or BNT162b2-immune sera, especially in the healthcare workers
69 (HCW), a particularly exposed population to SARS-CoV-2 infection.

70 To answer this question, we performed a virus neutralization test (VNT), with a strict 100%
71 inhibition criterion, on sera from HCW with either previous mild forms of COVID-19 or
72 BNT162b2 immunization using three clinical isolates of SARS-CoV-2 variants: a D614G strain
73 (D614G) which became the dominant form of the virus circulating globally in the second part
74 of 2020[2], a UK strain (UK, lineage B.1.1.7) and a SA strain (SA, lineage B.1.351).

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76 **MATERIALS AND METHODS**

77 **Study population and serum specimen**

78 Convalescent sera were recovered six months after symptom's onset from symptomatic
79 healthcare workers with a positive RT-PCR result. BNT162b2-vaccine elicited sera were
80 recovered three weeks after the first injection and seven days after the booster immunization.
81 This retrospective study was carried out in accordance with the Declaration of Helsinki without
82 addition to standard of care procedures. Data collection were declared to the Sorbonne
83 Université Data Protection Committee under number 2020-025 in accordance with French law.
84 Written informed consent for participation in this study was obtained from all participants.

85 **SARS-CoV-2 IgG immunoassay**

86 SARS-CoV-2 anti-nucleocapsid (N) IgG were determined using a commercially available
87 immunoassay kit (Alinity SARS-CoV-2 IgG assay, Abbott Laboratories) according to the
88 manufacturer's instructions.

89 **SARS-CoV-2 strains**

90 SARS-CoV-2 clinical isolates D614G, UK and SA (GenBank accession number MW322968,
91 MW633280 and MW580244 respectively) were isolated from SARS-CoV-2 RT-PCR
92 confirmed patients. Viral stocks were generated and titrated by the limiting dilution assay

93 allowing calculation of tissue culture infective dose 50% (TCID₅₀) after one passage of isolates
94 on Vero cells.

95 **Virus neutralization test**

96 The neutralizing activity of the various serum specimen was assessed with a whole virus
97 replication assay as previously describe (9). Microscopy examination on day 4 to assess the
98 cytopathic effect (CPE). Neutralization antibody (NAb) titers are expressed as the highest
99 serum dilution displaying 100% inhibition of the CPE. A same known positive control serum
100 was added to each experiment to assess the reproductivity.

101 **Statistical analysis**

102 Difference in distribution of NAb titer between UK strain or SA strain with the D614G strain
103 was performed with a two-tailed Mann-Whitney-U test in GraphPad Prism 6.0. A probability
104 value of $p < 0.05$ was considered statistically significant.

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106 **RESULTS**

107 We studied two sets of serum samples from HCW: a convalescent group of 15 participants with
108 SARS-CoV-2 proven infection on March 2020 and a vaccinated group of 29 participants
109 without history of clinical COVID-19 and which were negative for SARS-CoV-2 anti-
110 nucleocapsid (N) IgG. The median [IQR] age was 50 [40 – 58] years and 40% (6/15) were male
111 for the convalescent group. The median age was 55 [49 – 58] and 32% (9/29) were male for the
112 vaccinated group. Convalescent sera were collected 6 months after the symptom's onset (184
113 [182 – 189] days). BNT162b2 vaccine-elicited sera were collected 3 weeks after the first
114 injection (except for four HCW) and 7 days after the second injection of BNT162b2-vaccine.
115 All the serum samples were tested for their neutralizing activity against SARS-CoV-2 D614G,
116 UK and SA clinical strains. Three weeks after the first injection of the BNT162b2 vaccine, 52%

117 (13/25) of HCW harbored neutralizing activity with a NAb titer $\geq 1:5$ against the D614G strain,
118 24% (6/25) were neutralizing against the UK strain and only two (8%) had detectable NAb
119 against the SA strain (Figure 1 A, Table S1). Seven days after the booster immunization, all but
120 one HCW displayed neutralizing activity against the three SARS-CoV-2 clinical strains with a
121 median neutralizing titer of 1:160 [80 – 160] against the D614G strain, 1:40 [40 –80] against
122 the UK strain and 1:20 [20 – 40] against the SA strain. The median neutralizing titers against
123 UK and SA strains were significantly reduced compared to median NAb titer against the D614G
124 strain 7 days after the second injection of BNT162b2 vaccine (respectively, $p < 0.0001$ and $p <$
125 0.0001) (Figure 1 B, Table S1). Six months after the symptom's onset, all the 15 HCW of the
126 convalescent group harbored neutralizing activity against the D614G strain (median
127 neutralizing titer of 1:20 [1:10 – 1:40]) and the UK strain (median neutralizing titer 1:20 [1:5 –
128 1:20]) without statistical difference between the respective NAb titers ($p = 0.40$). However,
129 only 60% (9/15) serum samples of these HCW displayed a neutralizing activity against the SA
130 strain with a median titer of 1:5 (Figure 1 C, Table S2).

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132 **DISCUSSION**

133 In this work we assessed the neutralizing activity of sera from 15 convalescent COVID-19 or
134 29 BNT162b2-vaccinated HCW against the two rapidly spreading SARS-CoV-2 variants of
135 concern VOC202012/01 and 501Y.V2 and the globally circulating variant D614G using a VNT
136 with whole replicating clinical strains. Based on a very strict criterion of 100% inhibition of
137 CPE to determine NAb titer, we show that, three weeks after a single dose of BNT162b2, these
138 NAb titers remain low or absent among HCW especially against the UK and SA variants and
139 could questioned the extend of the dosing interval of BNT162b2 in some countries in order to
140 vaccinate as many people as possible. However, we were not able to follow participants more
141 than three weeks after the first injection because all of them received a second dose of

142 BNT162b2 according to the French guidelines. Nevertheless, seven days after the booster
143 immunization all but one vaccinated HCW develop NAb against the three strains with a highest
144 neutralizing activity against the strain closely related to the Wuhan ancestral strain, the D614G
145 strain. Despite lower NAb titers against the UK and the SA strains most of the participants have
146 displayed a neutralizing activity $\geq 1:10$ which could be at least indicative of a potential
147 protection against severe COVID-19 even with these variants. We also demonstrate a lack of
148 serum neutralizing activity against SA strain in up to 40% of HCW recovered from mild form
149 of COVID-19 six months after the symptom's onset. This finding, and the recent report
150 describing a severe case of reinfection by the SA variant four months after a first COVID-19
151 infection[9], highlights the need of vaccination even in people who had recovered from a
152 previous COVID-19, especially during the increased circulation of the SARS-CoV-2 variants.
153 Nevertheless, correlates of immunity to the SARS-CoV-2 are not well defined, only few studies
154 have tried to assess these correlates in other human coronaviruses (HCoV) with experimental
155 challenges on volunteers. They showed an association between serum NAb titer pre-exposure
156 and viral excretion[10]. Further studies are required to determine the SARS-CoV-2 correlates
157 of vaccine-induced protection based on NAb and T cell responses. A limitation of our work is
158 that we were not able to assess potential cellular response differences against the three strains
159 in the vaccinated or convalescent groups although it has been described generation of a robust
160 CD4+ and CD8+ responses against the Wuhan ancestral strain[11]. The long-term evaluation
161 regarding the lasting of NAb induced by vaccination is needed to assess the durability of
162 protection against SARS-CoV-2 variants.

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164 **CONCLUSION**

165 In conclusion, in BNT162b2-vaccinated participants with two dose regimen, despite
166 heterogeneity neutralizing capacity against the three SARS-CoV-2 variants, most of the sera

167 harbored at least a NAb titer $\geq 1:10$. Although immune protection correlates need to be defined,
168 our findings suggests a certain humoral protection activity either on UK or SA variants after
169 two doses of mRNA-vaccine. However, we show that six months after SARS-CoV-2 infection
170 leading to mild forms of COVID-19, an important proportion of HCW displayed no neutralizing
171 activity against SA strain. This result supports a strong recommendation for SARS-CoV-2
172 vaccination of previously infected subjects.

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181 UK and SA strains.

182 **Competing interests:** Authors declare that they have no competing interests.

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255 **Fig. 1. Neutralization antibody (NAb) titer against clinical strains of D614G, UK and SA**
256 **SARS-CoV-2 variants of 29 BNT162b2-vaccine elicited sera and 15 convalescent sera**
257 **recovered from healthcare workers (HCW).** (A) NAb titer against the three clinical isolates
258 of BNT162b2-vaccine elicited HCW sera recovered three weeks after first injection. (B) NAb
259 titer against the three clinical isolates of BNT162b2-vaccine elicited HCW sera recovered
260 seven days after second injection. (C) NAb titer against the three clinical isolates of
261 convalescent COVID-19 HCW sera recovered 6 months after the symptom's onset. NAb titer
262 against D614G strain are in blue, NAb titer against UK strain are in red and NAb titer against
263 SA strain are in green. Black horizontal lines indicate median values. Two-tailed P values
264 were determined using the Mann-Whitney test and are reported on each panel.

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277 **Table S1.**

278 **Serum neutralizing antibody (NAb) titer against three clinical strain of 29 vaccinated**
 279 **healthcare workers three weeks after the first dose of 30 µg BNT162b2 and seven days**
 280 **after the second dose.** D614G: globally circulating D614G variant, UK: VOC202012/01
 281 variant and SA: 501Y.V2 variant. N/A: non-applicable, four serums were not available for
 282 testing three weeks after the first injection of BNT162b2.

Serum ID	3 weeks after first BNT162b2 injection (NAb titer)			7 days after second BNT162b2 injection (NAb titer)		
	D614G strain	UK strain	ZA strain	D614G strain	UK strain	ZA strain
V1	10	0	0	160	40	20
V2	5	0	0	160	40	40
V3	5	0	0	160	40	20
V4	20	40	5	160	80	40
V5	0	0	0	80	40	20
V6	0	0	0	40	10	10
V7	0	0	0	160	40	10
V8	0	0	0	40	20	10
V9	0	5	0	40	20	10
V10	0	0	0	160	80	20
V11	0	0	0	160	40	40
V12	0	0	0	160	80	40
V13	5	0	0	160	40	20
V14	5	0	0	80	40	20
V15	5	5	0	160	80	40
V16	5	0	0	160	40	40
V17	N/A	N/A	N/A	160	80	80
V18	5	5	0	80	20	40
V19	0	0	0	80	80	20
V20	0	0	0	160	80	40
V21	10	20	0	320	160	20
V22	0	0	0	160	80	40
V23	80	10	20	320	80	80
V24	10	0	0	160	80	20
V25	20	0	0	160	80	20
V26	0	0	0	20	10	0
V27	N/A	N/A	N/A	80	40	40
V28	N/A	N/A	N/A	40	10	10
V29	N/A	N/A	N/A	320	80	40

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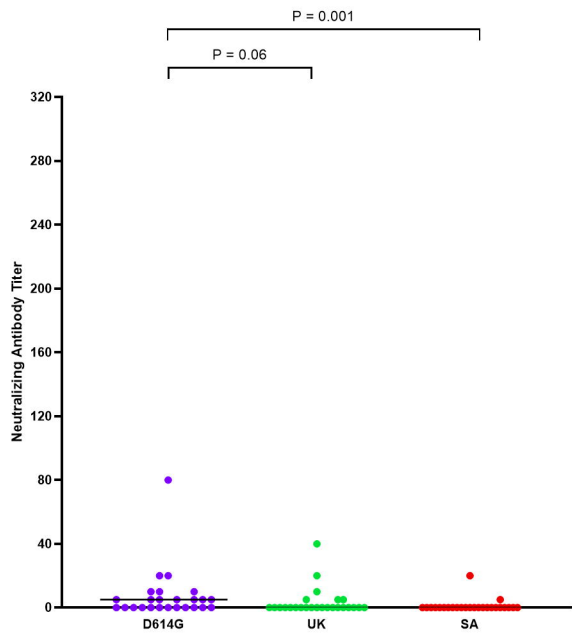
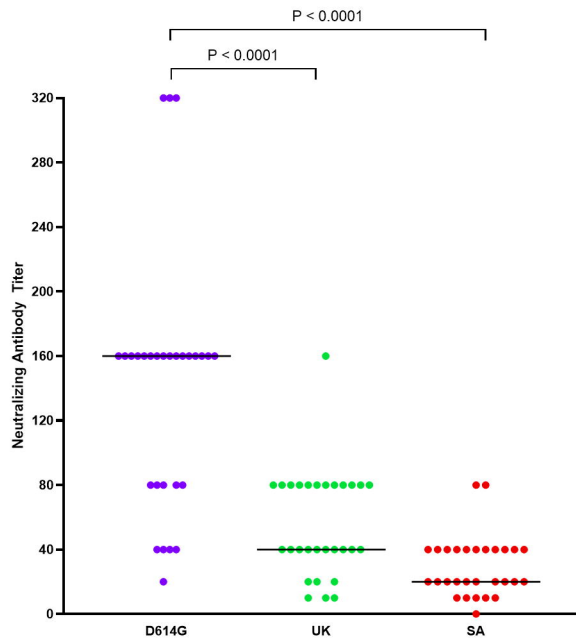
285

286 **Table S2.**

287 **Serum neutralizing antibody (NAb) titer against three clinical strains of 15 COVID-19**
288 **recovered healthcare workers six months after symptom's onset. D614G: globally**
289 **circulating D614G variant, UK: VOC202012/01 variant and SA: 501Y.V2 variant.**

Serum ID	D614G strain	UK strain	ZA strain
I1	20	10	5
I2	5	5	0
I3	5	5	0
I4	10	5	0
I5	10	20	0
I6	20	5	5
I7	10	5	0
I8	10	20	5
I9	20	20	5
I10	40	20	5
I11	80	80	20
I12	40	40	0
I13	20	20	5
I14	80	20	10
I15	160	40	20

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A**B****C**