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

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

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

23 **ABSTRACT**

24 There are concerns about neutralizing antibodies (NAbs) potency against SARS-CoV-2  
25 variants. Despite decreased NAb titers elicited by BNT162b2-vaccine against VOC202012/01  
26 and 501Y.V2 (SA) strains, 28/29 healthcare workers (HCW) had a NAb titer  $\geq 1:10$ . In contrast,  
27 six months after COVID-19 mild forms, only 9/15 (60%) of HCW displayed detectable NAbs  
28 against SA strain.

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## 45 INTRODUCTION

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

54 These variants harbor a specific pattern of deletion and mutations including amino-acid  
55 replacements at key sites in the S Receptor Binding Domain (RBD) (K417N, E484K, N501Y  
56 for the SA strain and only N501Y for the UK strain) and in the N-terminal domain ( $\Delta$ 69/70 and  
57  $\Delta$ Y144 deletions for the UK strain and L18F, D80A, D215G and  $\Delta$ 242-244 for the SA strain).

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

75

## 76 **MATERIALS AND METHODS**

### 77 **Study population and serum specimen**

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

### 85 **Virus neutralization test**

86 The neutralizing activity of the various serum specimen was assessed with a whole virus  
87 replication assay as previously described (9) using three SARS-CoV-2 clinical isolates D614G,  
88 UK and SA (GenBank accession number MW322968, MW633280 and MW580244  
89 respectively). Microscopy examination was performed on day 4 to assess the cytopathic effect  
90 (CPE). Neutralizing antibody (NAb) titers are expressed as the highest serum dilution  
91 displaying 100% (NT<sub>100</sub>), 90% (NT<sub>90</sub>) or 50% (NT<sub>50</sub>) inhibition of the CPE. A same known  
92 positive control serum was added to each experiment to assess the repeatability.

### 93 **Statistical analysis**

94 NT<sub>50</sub> or NT<sub>90</sub> were inferred by non-linear regression using a four-parameter variable slope  
95 model using GraphPad Prism 8.0.2 software. Geometric mean titer (GMT) with 95%  
96 confidence interval (95%CI) were calculated for NT<sub>100</sub>, NT<sub>50</sub> and NT<sub>90</sub> (Figure 1 and Table S1).  
97 Difference in distribution of NT<sub>100</sub> between UK strain or SA strain with the D614G strain was  
98 performed with a two-tailed Mann-Whitney-U test. A probability value of  $p < 0.05$  was  
99 considered statistically significant. No statistical comparisons were made between post  
100 vaccines and mild COVID-19 groups.

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## 102 RESULTS

103 We studied two sets of serum samples from HCW: a convalescent group of 15 participants with  
104 SARS-CoV-2 proven infection on March 2020 and a vaccinated group of 29 participants  
105 without history of clinical COVID-19. The median [IQR] age was 50 [32 – 66] years and 40%  
106 (6/15) were male for the convalescent group. The median age was 55 [38 – 65] and 31% (9/29)  
107 were male for the vaccinated group. Convalescent sera were collected 6 months after the  
108 symptom's onset (184 [182 – 189] days). Three weeks after the first injection of the BNT162b2  
109 vaccine, 52% (13/25) of HCW harbored NT<sub>100</sub>  $\geq$  1:5 against the D614G strain, 24% (6/25) were  
110 neutralizing against the UK strain and only two (8%) had detectable NAbs against the SA strain  
111 (Figure 1A). Seven days after the booster immunization, all but one HCW displayed  
112 ~~neutralizing activity~~ NT<sub>100</sub> against the three SARS-CoV-2 clinical strains with a GMT of 117.3  
113 (95%CI, 90.4 to 152.0) against the D614G strain, 45.1 (95%CI, 34.3 to 59.3) against the UK  
114 strain and 22.9 (95%CI, 16.6 to 31.6) against the SA strain. The NT<sub>100</sub> against UK and SA  
115 strains were significantly reduced compared to NT<sub>100</sub> against the D614G strain 7 days after the  
116 second injection of BNT162b2 vaccine (respectively,  $p < 0.0001$  and  $p < 0.0001$ ) (Figure 1B).  
117 Six months after the symptom's onset, all the 15 HCW of the convalescent group harbored  
118 NT<sub>100</sub> against the D614G strain (GMT of 21.0; 95%CI, 11.8 to 37.1) and the UK strain (GMT

119 of 14.5; 95%CI, 8.8 to 23.9) without statistical difference between the respective NT<sub>100</sub> (p =  
120 0.40). However, only 60% (9/15) serum samples of these HCW displayed a neutralizing activity  
121 against the SA strain with a NT<sub>100</sub> GMT of 3.3 (95%CI, 1.8 to 6.1; p < 0.0001) (Figure 1C).

122

## 123 **DISCUSSION**

124 In this work we assessed the neutralizing activity of sera from 15 convalescent COVID-19 or  
125 29 BNT162b2-vaccinated HCW against the two rapidly spreading SARS-CoV-2 variants of  
126 concern VOC202012/01 and 501Y.V2 and the globally circulating variant D614G using a VNT  
127 with whole replicating clinical strains. Based on a very strict criterion of 100% inhibition of  
128 CPE to determine NT<sub>100</sub>, we show that, three weeks after a single dose of BNT162b2, these  
129 NT<sub>100</sub> remain inexistent or low among HCW especially against the UK and SA variants and  
130 could questioned the extend of the dosing interval of BNT162b2 in some countries in order to  
131 vaccinate as many people as possible. This observation was confirmed with less strict/restrictive  
132 criteria of NT<sub>90</sub> or NT<sub>50</sub> (Table S1). However, we were not able to follow participants more  
133 than three weeks after the first injection because all of them received a second dose of  
134 BNT162b2 according to the French guidelines. Nevertheless, seven days after the booster  
135 immunization all but one vaccinated HCW develop NAbs against the three strains with a highest  
136 neutralizing activity against the strain closely related to the Wuhan ancestral strain, the D614G  
137 strain. Despite a 2.60-fold reduction of NT<sub>100</sub> GMT against the UK and a 5.12-fold reduction  
138 against the SA strains in comparison with the D614G most of the participants have displayed a  
139 neutralizing activity  $\geq$  1:10 which could be at least indicative of a potential protection against  
140 severe COVID-19 even with these variants. Although the correlates of protection are not  
141 already known, there is probably a certain degree of protection before the NAbs are detectable.  
142 Based on NT<sub>100</sub>, NT<sub>90</sub> and NT<sub>50</sub> values, we also demonstrate a lack of serum neutralizing  
143 activity against SA strain in up to 40% of HCW recovered from mild form of COVID-19 six

144 months after the symptom's onset associated with a 6.32 to 7.17-fold reduction of GMT in the  
145 HCW with detectable NAb. This finding, and the recent report describing a severe case of  
146 reinfection by the SA variant four months after a first COVID-19 infection[9], highlights the  
147 need of vaccination even in people who had recovered from a previous COVID-19, especially  
148 during the increased circulation of the SARS-CoV-2 variants. Nevertheless, correlates of  
149 immunity to the SARS-CoV-2 are not well defined, only few studies have tried to assess these  
150 correlates in other human coronaviruses with experimental challenges on volunteers. They  
151 showed an association between serum NAb titers pre-exposure and viral excretion[10]. Further  
152 studies are required to determine the SARS-CoV-2 correlates of vaccine-induced protection  
153 based on NAb and T cell responses. A limitation of our work is that we were not able to assess  
154 potential cellular response differences against the three strains in the vaccinated or convalescent  
155 groups although it has been described generation of a robust CD4+ and CD8+ responses against  
156 the Wuhan ancestral strain[11]. The long-term evaluation regarding the lasting of NAb induced  
157 by vaccination is needed to assess the durability of protection against SARS-CoV-2 variants.

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## 159 **CONCLUSION**

160 In conclusion, in BNT162b2-vaccinated participants with two dose regimen, despite  
161 heterogeneity neutralizing capacity against the three SARS-CoV-2 variants, most of the sera  
162 harbored at least a NAb titer  $\geq 1:10$ . Although immune protection correlates need to be defined,  
163 our findings suggests a certain humoral protection activity either on UK or SA variants after  
164 two doses of mRNA-vaccine. We also show that six months after SARS-CoV-2 infection  
165 leading to mild forms of COVID-19, an important proportion of HCW displayed no neutralizing  
166 activity against SA strain. This result supports a strong recommendation for SARS-CoV-2  
167 vaccination of previously infected subjects.



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174 UK and SA strains.

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

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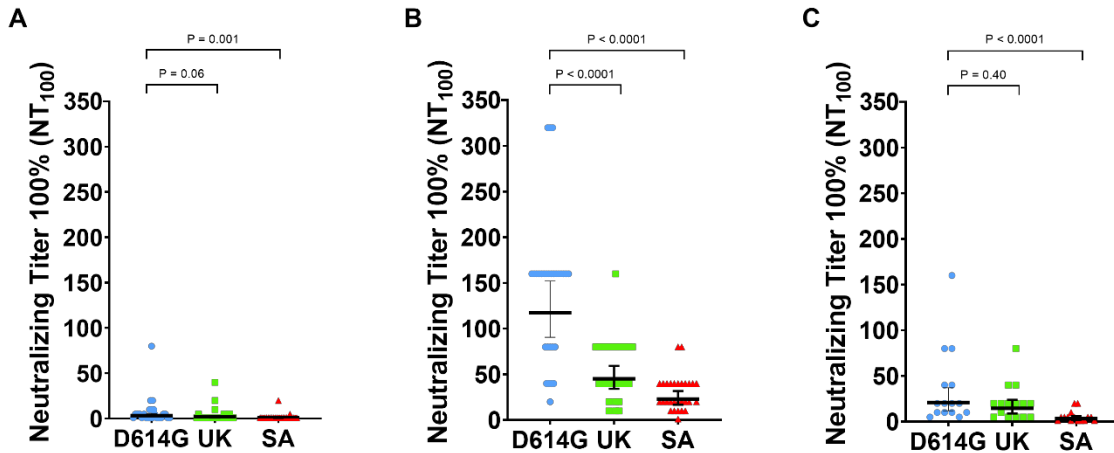
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257 **Fig. 1. Neutralizing antibody (NAb) titer with 100% inhibition (NT<sub>100</sub>) against clinical**  
 258 **strains of D614G, United-Kingdom (UK) and South African (SA) SARS-CoV-2 variants**  
 259 **of 29 BNT162b2-vaccine elicited sera and 15 convalescent sera recovered from healthcare**  
 260 **workers (HCW). (A) NT<sub>100</sub> against the three clinical isolates of BNT162b2-vaccine elicited**  
 261 **HCW sera recovered three weeks after first injection. (B) NT<sub>100</sub> against the three clinical isolates**  
 262 **of BNT162b2-vaccine elicited HCW sera recovered seven days after second injection. (C)**  
 263 **NT<sub>100</sub> against the three clinical isolates of convalescent COVID-19 HCW sera recovered 6**  
 264 **months after the symptom's onset. NT<sub>100</sub> against D614G strain are in blue dot, NT<sub>100</sub> against**  
 265 **UK strain are in green square and NAb titer against SA strain are in red triangle. Black**  
 266 **horizontal lines indicate geometric median titer (GMT) of NT<sub>100</sub>. Whiskers indicate 95%**  
 267 **confidence interval. Two-tailed P values were determined using the Mann-Whitney test and are**  
 268 **reported on each panel.**

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272 **Table S1. Geometric median titer (GMT) of neutralizing antibody (NAb) with 50% (NT<sub>50</sub>) or 90% (NT<sub>90</sub>) inhibition against clinical strains**  
 273 **of D614G, United-Kingdom (UK) and South African (SA) SARS-CoV-2 variants of 29 BNT162b2-vaccine elicited sera and 15 convalescent**  
 274 **sera recovered from healthcare workers (HCW). 95% confidence interval were written between parentheses.**

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	D614G strain		UK strain		SA strain		GMT ratio D614G/UK		GMT ratio D614G/SA	
	NT50	NT90	NT50	NT90	NT50	NT90	NT50	NT90	NT50	NT90
BNT162b2 - 1 <sup>st</sup> injection	5.38 (3.0 to 9.6)	4.90 (2.8 to 8.7)	3.46 (2.0 to 5.7)	2.89 (1.8 to 4.7)	1.47 (1.0 to 2.2)	1.39 (1.0 to 2.0)	1.56	1.70	3.66	3.53
BNT1622 - 2 <sup>nd</sup> injection	174.2 (133.5 to 227.2)	157.9 (122.4 to 203.8)	72.34 (52.8 to 99.1)	55.89 (39.2 to 79.6)	40.55 (28.5 to 57.7)	31.99 (22.4 to 45.8)	2.41	2.83	4.30	4.94
Convalescent	35.40 (20.2 to 62.1)	30.88 (17.1 to 55.7)	28.11 (16.5 to 47.8)	20.04 (12.1 to 33.2)	4.94 (2.4 to 10.1)	4.43 (2.3 to 8.5)	1.26	1.54	7.17	6.97

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