

## Peer Review File

**Manuscript Title:** SARS-CoV-2 B.1.1.7 sensitivity to mRNA vaccine-elicited antibodies

**Reviewer Comments & Author Rebuttals****Reviewer Reports on the Initial Version:**

Referees' comments:

Referee #1 (Remarks to the Author):

In their manuscript, Collier and colleagues describe a reduction of 'neutralization' (it is all pseudotyped entry inhibition data) of the B.1.1.7 variant by sera from elderly BNT162b2 vaccinees after the first vaccine dose. It also tests neutralization of B.1.1.7 plus E484 – which is now becoming prevalent in the UK. Initially, I assumed this paper would talk about homemade British problems (immunity after one shot) only, but it has many different angles (one shot, elderly vaccinees, B.1.1.7+E484K) which make it really interesting. Compliments to the authors for checking so many boxes. Of course, there are several points that need the authors' attention.

**Major points**

- 1) I think it would be important to include in the introduction that the Pfizer trials showed that most individuals have NO neutralizing antibodies after the prime and if they are detected, they are low. This is nothing unusual, this is consistent with available data. This aspect is also missing from the discussion.
- 2) Another point that needs to be made in the intro is, that the vaccine rollout in the UK is done by giving one shot and then waiting a long time, which is against the recommendation of the manufacturers and against the recommendations of FDA and EMA (and other regulatory agencies). And it is not based on any supportive data. This sets the stage, because readers outside of the UK cannot be expected to understand what is ongoing on the national level there. This aspect is also missing from the discussion.
- 3) Negative controls are missing in many panels, including Fig. 1c, d, e, f, g, h, and many other panels. Figure 4b is missing a limit of detection.
- 4) Looking at the actual curves in Extended Data Figure 1, there is only a real difference in two sera.
- 5) Neutralization should not be used in the title since bona fide neutralization against authentic SARS-CoV-2 was not measured.

**Minor points**

- 1) Many abbreviations are not defined including SARS-CoV-2, ACE2, COVID-19, FPLC, TMPRSS2, SFU, DMEM, FBS, BirA, SEC
- 2) Line 40: Please update the number of countries.

- 3) Line 45 and throughout the manuscript, including the methods part: Midsentence, this should be 'spike', not 'Spike'
- 4) Line 50 and lines 377-381: It is not clear why words start with capitalized letters mid-sentence.
- 5) Line 50: 'out of'
- 6) Line 55: It already did, its not just likely.
- 7) Line 59 and 61: 'SARS-CoV-2', not 'SARS-COV-2'
- 8) Line 97: Is 'N' ever defined?
- 9) 103: There are two typos in this line.
- 10) Line 119: This is consistent with findings in <https://www.medrxiv.org/content/10.1101/2021.01.19.21249592v1>
- 11) Line 190: 'ELISpot', not 'ELISPOT'
- 12) Line 195: Given the low proportion of neutralizers and the low titers it is really hard to judge what this 'escape' actually means. Yes, it is bad for the people who do have neutralizing antibodies after the first shot but most don't. And during Phase III protection was significant, even in the absence of neutralizing antibodies.
- 13) Line 227: Nice point!
- 14) Line 231: 'neutralizing'
- 15) Line 238: It is not a virus, it is a pseudotyped particle.
- 16) Line 256: 'mutations'
- 17) Line 260-261: Please check the grammar of this sentence.
- 18) Line 303: 'COVID-19', not 'Covid-19'
- 19) Line 396: What was the actual antibiotics concentration?
- 20) Figure 1e and f: On the x-axis it should be B.1.1.7
- 20) Figure 1f: The legend is not needed and the colors of the error bars are confusing.

Referee #2 (Remarks to the Author):

This paper looks at the effects of mutations in the B.1.1.7 viral lineage on recognition by serum antibodies. Although the title is very exciting, the paper seemed rushed and incomplete. I found the actual quality of the data and the way it was described quite disappointing given that I have typically associated some of the authors of this study with high-quality and rigorous scientific work.

The actual scientific findings are as follows:

1) They show the B.1.1.7 spike binds ACE2 with higher affinity than the “wildtype” spike. As far as I know, this is the first time that this fact has been reported and this part of the study appears to be well done, accurately described, and a valuable contribution to the field.

2) Second, they show that the mutations in B.1.1.7 moderately reduce recognition by sera from individuals who have received one dose of the vaccine. This finding is presented very dramatically, but it seems overhyped and described in a way that doesn’t adequately convey some serious limitations. First, the individuals had just received ONE dose of the vaccine, whereas the actual vaccine regimen has two doses. Second, the decreases in titer are real but modest, and don’t seem to justify the hyperbolically worded “escape.” Finally, as mentioned below, another more systematic and less hyperbolic study has already shown that B.1.1.7 is recognized only slightly less well by individuals who have received both vaccine doses. So the alarming implication of the way the result is spun in this paper doesn’t really capture these realities.

3) They show the NTD deletion in B.1.1.7 disrupts binding by NTD-binding antibodies. This is a solid finding, although similar findings on NTD deletions and monoclonal antibodies have been reported elsewhere.

4) They show that adding E484K to B.1.1.7 does greatly reduce serum binding. But the large effect of E484K has already been reported by others in recent studies not cited here.

So overall, there is some good work here (like the ACE2 affinity) and the NTD deletion disrupting NTD binding antibodies. But the top-line claims about effect of B.1.1.7 on Pfizer vaccine sera that presumably have led Nature to rush this paper to review so quickly seem to gloss over a lot of important caveats and limitations.

#### MAJOR COMMENTS:

- A lack of effect of the B.1.1.7 mutations on fully Pfizer vaccinated (primed + boost) individuals has already been reported: <https://www.biorxiv.org/content/10.1101/2021.01.18.426984v1>

- Title is hyperbolic: the main finding is that B.1.1.7 mutations have at most mild effects on neutralizing titer of vaccine sera, and the study doesn’t even study the full two-dose of vaccine, so a title suggesting “escape” from vaccine-elicited antibodies is highly misleading.

- Highly unusual study population: median age 82.

- Lines 123-124: suggest an allosteric effect of del69-70 mutation, but provide no evidence. Is this a suggestion supported by data, or pure speculation? Should be clarified.

- There have been better studies of NTD deletion effects on anti-NTD monoclonal antibodies: <https://www.biorxiv.org/content/10.1101/2021.01.14.426475v1.full> and <https://www.biorxiv.org/content/10.1101/2021.01.19.427324v1>.

- A number of studies of other studies have already shown that E484K has a major effect on serum antibody binding, including: <https://www.biorxiv.org/content/10.1101/2020.12.31.425021v1> and <https://pubmed.ncbi.nlm.nih.gov/33501451/>

- The text says that stronger ACE2 binding is associated with better transmission in SARS-CoV-1. Should be pointed out that it’s unclear how well that extrapolates to SARS-CoV-2 as SARS-CoV-1 was initially only poorly adapted to humans, whereas SARS-CoV-2 is now pretty well adapted.

## MINOR COMMENTS:

- Line 51: a space is missing out "out of"
- Line 104: misspelled word and missing space.

## Referee #3 (Remarks to the Author):

In this manuscript, Collier et al. analyzed the susceptibility of the SARS-CoV-2 variant B.1.1.7 to sera from vaccinees and convalescent individuals, as well as to SARS-CoV-2 specific monoclonal antibodies. A particular focus is placed on the investigation of sera from a real life-cohort of 23 mostly elderly individuals having received the BNT162b2 mRNA vaccine. All of these samples were obtained three weeks after the first dose of vaccination and before the required second dose.

The study is timely and adds to the body of mostly non peer-reviewed literature investigating the impact of SARS-CoV-2 mutations on viral resistance to vaccine-induced serum activity as well as monoclonal antibodies. The authors focus on the B.1.1.7 variant that is rapidly spreading across the globe. The novelty is the assessment of the impact of the recently observed B.1.1.7 variant carrying the E484K mutation. This mutation has been demonstrated to be associated with immune escape and is also present in the variants 501Y.V2 and 501Y.V3.

While acknowledging the efforts by the authors to generate very timely data on a highly relevant question, the manuscript in its current form lacks coherency and bases its main conclusions on a very limited and not necessarily representative set of data. Given the high number of individuals vaccinated by now, it would appear more appropriate to investigate and conclude on the impact of the B.1.1.7 variant in a much higher number of individuals including subjects having received the full vaccination course.

Despite appreciating the short time during which the manuscript was finalized, readability and accuracy of the figures is subpar. Moreover, while the question addressed is of immediate interest, the small number and selection of samples limits the validity of the conclusions and the overall significance of the study.

## Major and minor points

- The data presented does not support the selected title. In the abstract the authors write "The vaccine sera exhibited a broad range of neutralizing titres against the wild-type pseudoviruses that were modestly reduced against B.1.1.7 variant". The title also suggests that the monoclonal antibodies tested were derived from vaccinees.
- The authors investigated the neutralization sensitivity of pseudoviruses carrying the mutations del69/70, N501Y and A570D. It is unclear why this combination of mutations was investigated.
- The difference in neutralization between WT virus and B.1.1.7 virus appears to be higher than what has been reported for recipients of the mRNA-1273 vaccine (full course) as well as of the BNT162b2 vaccine (Muik et al., Science 2021). In the discussion, the authors point out that the low baseline titers after the single prime may contribute to the observed results. Additional samples with low, middle and high levels of SARS-CoV-2 neutralizing serum activity from vaccinees and convalescent COVID-19 patients would be helpful to better interpret the observed results.
- The number of convalescent individuals is low. Only 7 samples were tested against B.1.1.7. This

should be extended with sets of samples carrying low, middle and high levels of SARS-CoV-2 neutralizing antibodies.

- The authors speculate that N501Y may be a key mutation for the loss of polyclonal neutralizing activity from vaccinees because of resistance of some RBM antibodies. This is, however, inconsistent with the observed increased neutralizing sensitivity of the N501Y-containing pseudovirus shown in Extended Data Fig. 2 (as well as other data from BNT162b2 vaccinees; Xie et al. bioRxiv).
- The source/reference for several antibodies is missing, and it is not clear why some antibodies are labeled as "RBD (?)".
- Figure 3a is of limited value in its current form. Highlighted sequences are essentially invisible.
- Text, figure label, and figure legend are inconsistent in the number of B.1.1.7 viruses bearing the E484K mutation (15, 18, or 16 sequences are mentioned).
- Figure 4a is of limited value and does seem not to correspond to participant IDs in Table 1 (e.g. ID 5; ID50 for WT in figure is approximately 400; ID50 for WT in table is 27).
- Only 12 sera were tested for B.1.1.7 + E484K but are compared to 24 sera for WT and B.1.1.7. To enable comparison, all sera should be tested against all variants.
- 
- According to Table 1, Figure 1, and the reporting summary, a cohort of 23 participants was investigated. Figure 4 now includes 24 individuals. Are these the same samples?
- There is no information on statistics in the method section, p values are not indicated, error bars are only partially indicated, and there is no description of the statistical methods applied in the figure legends. This is in contrast to what is stated in the reporting summary. As such, it is not possible to assess the statistical methodology.
- While the observations on ACE-2 affinity to B.1.1.7 are of interest, they feel out of place and do not contribute much to the main message of the manuscript.
- Several figures lack axis labels (e.g., 1c-d; Fig. 4b; Extended Data Fig. 1), show incorrect labels (e.g., B.1.1.7 in 1c-d; 1e-f; ID500 in Fig 1e-f), are cut-off and do not clearly display results of apparent statistical analyses (e.g., Fig. 1c-d, Fig. 4b, Extended Data Fig. 1), or are of very low quality making them difficult (e.g., Fig. 1a; all of Figure 2) or impossible (Extended Data Fig. 5) to read.
- The authors speculate that increased vaccinee serum activity may be related to the 69/70 deletion. This could be addressed experimentally.
- Fig. 2c indicates antibody 4A8 as neutralizing, Extended Data Table 1 indicates it as non-neutralizing
- Figure 3: A summary of Ext. Data Figure 3 would be helpful (i.e., as in Ext Data Fig 2)
- Figure 4: Sera 3008 and 3048 of Ext. Data Fig. 4 are already shown in main figure
- Line 76: P.1 should be referred to as N501Y.V3 (not .V2).
- Lines 145-146: According to the data presented in Extended Data Table 1, there seem to be 7 and not 6 RBM-targeting mAbs with a 2-to-10-fold IC50 reduction.

- Line 194: Space missing between “with” and “almost”.
- There are a lot of neutralization curves for convalescent serum samples, but none for the vaccinee samples. However, these curves appear particularly important to understand impact on maximum percentages of inhibition.
- Figure 1c, d, e, f, 4b, Extended Figure 2: Adding the x-fold change in neutralization titer would be helpful.

#### Author Rebuttals to Initial Comments:

Dear Editor

Please see our responses to reviewers below

Sincerely

Ravi Gupta

Referee #1:

In their manuscript, Collier and colleagues describe a reduction of ‘neutralization’ (it is all pseudotyped entry inhibition data) of the B.1.1.7 variant by sera from elderly BNT162b2 vaccinees after the first vaccine dose. It also tests neutralization of B.1.1.7 plus E484 – which is now becoming prevalent in the UK. Initially, I assumed this paper would talk about homemade British problems (immunity after one shot) only, but it has many different angles (one shot, elderly vaccinees, B.1.1.7+E848K) which make it really interesting. Compliments to the authors for checking so many boxes. Of course, there are several points that need the authors’ attention.

Major points

1) I think it would be important to include in the introduction that the Pfizer trials showed that most individuals have NO neutralizing antibodies after the prime and if they are detected, they are low. This is nothing unusual, this is consistent with available data. This aspect is also missing from the discussion.

response: we have now added this information to the paper and thank the reviewer for this important point.

2) Another point that needs to be made in the intro is, that the vaccine rollout in the UK is done by giving one shot and then waiting a long time, which is against the recommendation of the manufacturers and against the recommendations of FDA and EMA (and other regulatory agencies). And it is not based on any supportive data. This sets the stage, because readers outside of the UK cannot be expected to understand what is ongoing on the national

level there. This aspect is also missing from the discussion.

response: we agree this is vital and have now added this information to the paper

3) Negative controls are missing in many panels, including Fig. 1c, d, e, f, g, h, and many other panels. Figure 4b is missing a limit of detection.

response: we have negative control wells in our experiments but felt that including them in these multi point graphs would require an additional colour, impeding readability. We have provided negative control data in figure 1 and can provide the underlying data including negative control wells. For a limit of detection we set a dilution of 1:10 for 50% neutralisation. We have added this information to the legends.

4) Looking at the actual curves in Extended Data Figure 1, there is only a real difference in two sera.

response: yes we agree and this illustrates heterogeneity by individual. Therefore summary measures have limited value and we have presented fold changes as individual data points so that the heterogeneity can be seen.

5) Neutralization should not be used in the title since bona fide neutralization against authentic SARS-CoV-2 was not measured.

response: we have modified the title accordingly to:

**SARS-CoV-2 B.1.1.7 sensitivity to mRNA vaccine-elicited, convalescent and monoclonal antibodies**

Minor points

1) Many abbreviations are not defined including SARS-CoV-2, ACE2, COVID-19, FPLC, TMPRSS2, SFU, DMEM, FBS, BirA, SEC

response: we have now defined these

2) Line 40: Please update the number of countries.

response: we have now updated

3) Line 45 and throughout the manuscript, including the methods part: Midsentence, this should be 'spike', not 'Spike'

response: we have now updated thank you

4) Line 50 and lines 377-381: It is not clear why words start with capitalized letters mid-sentence.

response: this has been fixed thank you

5) Line 50: 'out of'

response: this has been fixed thank you

6) Line 55: It already did, its not just likely.

response: this has been fixed

7) Line 59 and 61: ‘SARS-CoV-2’, not ‘SARS-COV-2’

response: this has been fixed

8) Line 97: Is ‘N’ ever defined?

response: we have now defined it in line 101

9) 103: There are two typos in this line.

response: these have been fixed

10) Line 119: This is consistent with findings

in <https://www.medrxiv.org/content/10.1101/2021.01.19.21249592v1>

response: thank you we have now added this reference

11) Line 190: ‘ELISpot’, not ‘ELISPOT’

response: We have modified this, in fact it is a FluoroSpot assay– apologies for the confusion.

12) Line 195: Given the low proportion of neutralizers and the low titers it is really hard to judge what this ‘escape’ actually means. Yes, it is bad for the people who do have neutralizing antibodies after the first shot but most don’t. And during Phase III protection was significant, even in the absence of neutralizing antibodies.

Response: we removed the word escape and agree it is a complex area.

13) Line 227: Nice point!

response: thank you

14) Line 231: ‘neutralizing’

response: We have modified this

15) Line 238: It is not a virus, it is a pseudotyped particle.

response: We have modified this thank you for pointing this inconsistency

16) Line 256: ‘mutations’

response: We have modified this thank you

17) Line 260-261: Please check the grammar of this sentence.

response: We have checked the sentence and modified

18) Line 303: 'COVID-19', not 'Covid-19'

response: We have modified this

19) Line 396: What was the actual antibiotics concentration?

Response: penicillin 100IU/mL and strep at 100ug/mL, this has now been amended

20) Figure 1e and f: On the x-axis it should be B.1.1.7

response: We have modified this

20) Figure 1f: The legend is not needed and the colors of the error bars are confusing.

response: We have modified this thank you

Referee #2:

This paper looks at the effects of mutations in the B.1.1.7 viral lineage on recognition by serum antibodies. Although the title is very exciting, the paper seemed rushed and incomplete. I found the actual quality of the data and the way it was described quite disappointing given that I have typically associated some of the authors of this study with high-quality and rigorous scientific work.

The actual scientific findings are as follows:

1) They show the B.1.1.7 spike binds ACE2 with higher affinity than the "wildtype" spike. As far as I know, this is the first time that this fact has been reported and this part of the study appears to be well done, accurately described, and a valuable contribution to the field.

Response: we thank the reviewer for this comment.

2) Second, they show that the mutations in B.1.1.7 moderately reduce recognition by sera from individuals who have received one dose of the vaccine. This finding is presented very dramatically, but it seems overhyped and described in a way that doesn't adequately convey some serious limitations. First, the individuals had just received ONE dose of the vaccine, whereas the actual vaccine regimen has two doses. Second, the decreases in titer are real but modest, and don't seem to justify the hyperbolically worded "escape." Finally, as mentioned below, another more systematic and less hyperbolic study has already shown that B.1.1.7 is recognized only slightly less well by individuals who have received both vaccine doses. So

the alarming implication of the of the way the result is spun in this paper doesn't really capture these realities.

Response: we did not intend for the data to be presented dramatically and have re-phrased throughout to take this into account. The word escape has been removed as it only applies to the mAb and responses to the 484K virus in some participants.

3) They show the NTD deletion in B.1.1.7 disrupts binding by NTD-binding antibodies. This is a solid finding, although similar findings on NTD deletions and monoclonal antibodies have been reported elsewhere.

Response: we thank the reviewer for this comment. In this study we also show the lack of neutralizing activity and our data are consistent with the pre-print study by The David Ho lab that was preprinted contemporaneously with submission.

4) They show that adding E484K to B.1.1.7 does greatly reduce serum binding. But the large effect of E484K has already been reported by others in recent studies not cited here.

Response: although other studies (now cited) have tested E484K, we feel it is vital to study all eight mutations together with E484K to understand the overall impact on neutralisation sensitivity, especially as there may be interactions between mutations that we do not yet understand. We thank the reviewer for pointing out the additional studies that need to be cited.

So overall, there is some good work here (like the ACE2 affinity) and the NTD deletion disrupting NTD binding antibodies. But the top-line claims about effect of B.1.1.7 on Pfizer vaccine sera that presumably have led Nature to rush this paper to review so quickly seem to gloss over a lot of important caveats and limitations.

Response: We agree that the title should not have used the word escape for vaccine elicited antibodies towards B.1.1.7, but our data on the E484K alongside B.1.1.7 are of concern given this virus is transmitting. We aimed to provide a comprehensive and up to date analysis with mAbs, vaccine and convalescent sera.

We have now addressed the limitations on first and second dose including samples from younger individuals. We show that the second dose sera are higher titre as expected, but are similar in their neutralisation profiles for the mutant Spike proteins tested.

#### MAJOR COMMENTS:

- A lack of effect of the B.1.1.7 mutations on fully Pfizer vaccinated (primed + boost) individuals has already been reported: <https://www.biorxiv.org/content/10.1101/2021.01.18.426984v1>

Response: yes there are other preprints posted contemporaneously (Jan 19/20th) with ours that show similar data, though the paper cited has few details and no data on sera or mAb and B.1.1.7, or on the newly expanding B.1.1.7 with E484K. We have now cited this paper and added data from the second vaccination, allowing direct comparisons within individual.

- Title is hyperbolic: the main finding is that B.1.1.7 mutations have at most mild effects on neutralizing titer of vaccine sera, and the study doesn't even study the full two-dose of vaccine, so a title suggesting "escape" from vaccine-elicited antibodies is highly misleading.

Response: we agree it appears (unintentionally) misleading and we have now changed the title. We have also now tested sera after the second dose.

- Highly unusual study population: median age 82.

Response: we now added younger participants and our findings are similar to other studies.

- Lines 123-124: suggest an allosteric effect of del69-70 mutation, but provide no evidence. Is this a suggestion supported by data, or pure speculation? Should be clarified.

Response: this is speculation and we have therefore removed it from the results section, particularly given length limitations

- There have been better studies of NTD deletion effects on anti-NTD monoclonal antibodies: <https://www.biorxiv.org/content/10.1101/2021.01.14.426475v1.full> and <https://www.biorxiv.org/content/10.1101/2021.01.19.427324v1>.

Response: We had already cited the first one (David Veessler is a co-author here). We have cited the paper from the Crowe lab now.

- A number of studies of other studies have already shown that E484K has a major effect on serum antibody binding, including: <https://www.biorxiv.org/content/10.1101/2020.12.31.425021v1> and <https://pubmed.ncbi.nlm.nih.gov/33501451/>

Response: we thank the reviewer for this and we have now added these preprint references. No other study has investigated the mutations in the new UK variant of concern that is transmitting and under intensive public health investigation.

- The text says that stronger ACE2 binding is associated with better transmission in SARS-CoV-1. Should be pointed out that it's unclear how well that extrapolates to SARS-CoV-2 as SARS-CoV-1 was initially only poorly adapted to humans, whereas SARS-CoV-2 is now pretty well adapted.

Response: we have added text to clarify

#### MINOR COMMENTS:

- Line 51: a space is missing out "out of"

Response: we have resolved this

- Line 104: misspelled word and missing space.

Response: we have resolved this

Referee #3:

In this manuscript, Collier et al. analyzed the susceptibility of the SARS-CoV-2 variant B.1.1.7 to sera from vaccinees and convalescent individuals, as well as to SARS-CoV-2 specific monoclonal antibodies. A particular focus is placed on the investigation of sera from a real life-cohort of 23 mostly elderly individuals having received the BNT162b2 mRNA vaccine. All of these samples were obtained three weeks after the first dose of vaccination and before the required second dose.

The study is timely and adds to the body of mostly non peer-reviewed literature investigating the impact of SARS-CoV-2 mutations on viral resistance to vaccine-induced serum activity as well as monoclonal antibodies. The authors focus on the B.1.1.7 variant that is rapidly spreading across the globe. The novelty is the assessment of the impact of the recently observed B.1.1.7 variant carrying the E484K mutation. This mutation has been demonstrated to be associated with immune escape and is also present in the variants 501Y.V2 and 501Y.V3.

While acknowledging the efforts by the authors to generate very timely data on a highly relevant question, the manuscript in its current form lacks coherency and bases its main conclusions on a very limited and not necessarily representative set of data. Given the high number of individuals vaccinated by now, it would appear more appropriate to investigate and conclude on the impact of the B.1.1.7 variant in a much higher number of individuals including subjects having received the full vaccination course.

Despite appreciating the short time during which the manuscript was finalized, readability and accuracy of the figures is subpar. Moreover, while the question addressed is of immediate interest, the small number and selection of samples limits the validity of the conclusions and the overall significance of the study.

#### Major and minor points

- The data presented does not support the selected title. In the abstract the authors write “The vaccine sera exhibited a broad range of neutralizing titres against the wild-type pseudoviruses that were modestly reduced against B.1.1.7 variant”. The title also suggests that the monoclonal antibodies tested were derived from vaccinees.

response: we have now modified the title and thank the reviewer for this point.

- The authors investigated the neutralization sensitivity of pseudoviruses carrying the mutations del69/70, N501Y and A570D. It is unclear why this combination of mutations was investigated.

response: we used these as they were intermediates during sequential mutagenesis of S1. We think the data are useful in that they show N501Y's effect on neutralisation is not evident with this mutation combination. We have kept as supplementary data and hope the reviewer agrees.

- The difference in neutralization between WT virus and B.1.1.7 virus appears to be higher than what has been reported for recipients of the mRNA-1273 vaccine (full course) as well as of the BNT162b2 vaccine (Muik et al., Science 2021). In the discussion, the authors point out

that the low baseline titers after the single prime may contribute to the observed results. Additional samples with low, middle and high levels of SARS-CoV-2 neutralizing serum activity from vaccinees and convalescent COVID-19 patients would be helpful to better interpret the observed results.

**Response: we have now increased numbers of both vaccinees with a range of neutralising serum activity as requested. The second dose indeed has resulted in higher titres and lower fold change (1.9) that is similar to other studies. We also increased the number of convalescent individuals substantially and found slightly higher FC compared to vaccinees for B.1.1.7 virus.**

- The number of convalescent individuals is low. Only 7 samples were tested against B.1.1.7. This should be extended with sets of samples carrying low, middle and high levels of SARS-CoV-2 neutralizing antibodies.

**Response: we have now increased numbers of both vaccinees and convalescent COVID-19 patients in the study.**

- The authors speculate that N501Y may be a key mutation for the loss of polyclonal neutralizing activity from vaccinees because of resistance of some RBM antibodies. This is, however, inconsistent with the observed increased neutralizing sensitivity of the N501Y-containing pseudovirus shown in Extended Data Fig. 2 (as well as other data from BNT162b2 vaccinees; Xie et al. bioRxiv).

**Response: we have altered text to reflect this point and also included the Xie reference.**

- The source/reference for several antibodies is missing, and it is not clear why some antibodies are labeled as “RBD (?)”.

**Response: this has now been rectified. For most mAbs in Extended Data Table 1 there is a Ref to these mAbs. For those where Ref was not available, we indicated them as presented for the first time in this study (and added a Method paragraph on their isolation).**

- Figure 3a is of limited value in its current form. Highlighted sequences are essentially invisible.

**Response: This was a resolution problem during the submission process. We apologize.**

- Text, figure label, and figure legend are inconsistent in the number of B.1.1.7 viruses bearing the E484K mutation (15, 18, or 16 sequences are mentioned).

**Response: We have now finalised the numbers**

- Figure 4a is of limited value and does seem not to correspond to participant IDs in Table 1 (e.g. ID 5; ID50 for WT in figure is approximately 400; ID50 for WT in table is 27).

**Response: The figures now reflect much larger numbers of participants and the table repeats data – we have therefore removed it.**

- Only 12 sera were tested for B.1.1.7 + E484K but are compared to 24 sera for WT and B.1.1.7. To enable comparison, all sera should be tested against all variants.

Response: This comparison has now been done where sera quantities permitted

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- According to Table 1, Figure 1, and the reporting summary, a cohort of 23 participants was investigated. Figure 4 now includes 24 individuals. Are these the same samples?

Response: yes they are, we have harmonised the reporting summary now with the updated vaccinee numbers

- There is no information on statistics in the method section, p values are not indicated, error bars are only partially indicated, and there is no description of the statistical methods applied in the figure legends. This is in contrast to what is stated in the reporting summary. As such, it is not possible to assess the statistical methodology.

Response: we have now added a statistics section to the methods and described the statistics used in the figure legends.

- While the observations on ACE-2 affinity to B.1.1.7 are of interest, they feel out of place and do not contribute much to the main message of the manuscript.

Response: we have kept these data (shown as supplementary), based on comments from one of the other reviewers, but will leave to the discretion of the editor.

- Several figures lack axis labels (e.g., 1c-d; Fig. 4b; Extended Data Fig. 1), show incorrect labels (e.g., B.1.1.7 in 1c-d; 1e-f; ID500 in Fig 1e-f), are cut-off and do not clearly display results of apparent statistical analyses (e.g., Fig. 1c-d, Fig. 4b, Extended Data Fig. 1), or are of very low quality making them difficult (e.g., Fig. 1a; all of Figure 2) or impossible (Extended Data Fig. 5) to read.

Response: we apologise for this. The image quality may have resulted from the difficulties we had in uploading the images when we had updated data on the new VOC in the UK. We have resolved the issues now and thank the reviewer for pointing them out.

- The authors speculate that increased vaccinee serum activity may be related to the 69/70 deletion. This could be addressed experimentally.

Response: we are working on a separate report on the impact of 69/70 on susceptibility to sera and feel it would be out of place here without doing mutagenesis on other residues

- Fig. 2c indicates antibody 4A8 as neutralizing, Extended Data Table 1 indicates it as non-neutralizing

Response: The minus in the ED Table 1 indicates that this antibody is not neutralizing B.1.1.7, but (as shown) it neutralises WT virus.

- Figure 3: A summary of Ext. Data Figure 3 would be helpful (i.e., as in Ext Data Fig 2)

Response: ext data Fig 2 are example curves. The summary data are in the main figure 1.

- Figure 4: Sera 3008 and 3048 of Ext. Data Fig. 4 are already shown in main figure

Response: We have now moved the curves from main fig into Ext. Data.

- Line 76: P.1 should be referred to as N501Y.V3 (not .V2).

Response: we have resolved this thank you for pointing this out

- Lines 145-146: According to the data presented in Extended Data Table 1, there seem to be 7 and not 6 RBM-targeting mAbs with a 2-to-10-fold IC50 reduction.

Response: There are 6 antibodies with an IC50 in this range. A 7th mAb with 1.4 fold change was not counted.

- Line 194: Space missing between “with” and “almost”.

Response: we have resolved this

- There are a lot of neutralization curves for convalescent serum samples, but none for the vaccinee samples. However, these curves appear particularly important to understand impact on maximum percentages of inhibition.

Response: we fully agree and have now included example curves as extended data Fig 3.

- Figure 1c, d, e, f, 4b, Extended Figure 2: Adding the x-fold change in neutralization titer would be helpful.

Response: We have now added these numbers

#### Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors addressed the reviewers comments well.

Referee #2 (Remarks to the Author):

The authors have addressed all of the technical comments. I am still underwhelmed by the papers impact for Nature, in that it seems to mostly confirm results that have now been well established for a while (B.1.1.7 doesn't have a huge antigenic effect, but E484K does, mutations to NTD supersite ablate binding of NTD specific antibodies). If someone were to ask me for a good reference on any of these points, I'd point them to a different paper than this one. But the results do seem valid. And the B.1.1.7 ACE2 binding is valuable.

## Referee #3 (Remarks to the Author):

In their revised manuscript, Collier et al. include additional serum neutralization analyses using samples obtained from individuals receiving the first and the second vaccination dose of BNT162b2. While the authors addressed most of the reviewer's comments, the manuscript still contains numerous errors to an extent that is rather unusual (see comments below). In contrast to the initial statement by the authors that 'B.1.1.7 escapes from mRNA vaccine-elicited neutralizing antibodies', the data and conclusions presented now are in line with findings of other groups showing a rather small decrease in the susceptibility of B.1.1.7 to BNT162b2-induced SARS-CoV-2 neutralizing antibodies. The main novelty of the manuscript remains in the analysis of measuring serum activity against the B.1.1.7 variant carrying an additional E484K mutation which previously has been shown to affect antibody neutralization.

## Specific comments:

- The introduction of the abstract has not been revised. It should, however, now state that activity was measured after both first and second vaccine dose.
- Line 93-95: In contrast to what is stated, monoclonal antibodies were not tested against B.1.1.7 with the E484K mutation, but only against pseudoviruses with the mutations K417N, E484K, and N501Y.
- Extended Data Fig. 1A is misleading: The authors state to have performed analyses using serum samples of 37 participants (see line 98) versus 23 in the original manuscript. However, this figure and the figure legend seem identical to the previous version and therefore do not include any new samples. The number of individuals studied should be clearly stated.
- Extended Data Fig. 1B: The numbers of the y-axis do not appear to match the Spike total IgG MFI that is shown in Extended Data Fig. 1A. In 1A, there seem to be 5 samples with an MFI <10,000 against S protein; in 1B, there are > 15. Also, Figure 1B shows 25 measurements. Why? It should be clearly stated how many samples were included for the analyses (also for Extended Data Fig. 1C and 1D). Finally, the ID50 titers do not seem to match with samples shown in Figure 1B.
- Line 115: The difference between 318 and 77 is 0.6 log, not "one order of magnitude".
- Figures 1C and 1D are identical.
- Figures 1B-1G: The labeling of the y-axis is incorrect. It is not showing (log10). This is also the case in the Extended Data.
- Line 131-132: If the "variation" (what is meant by that?) were due to low neutralizing titers after the first dose, wouldn't one expect to see that the convalescent serum samples tested here (with high titers) were even less affected by the B.1.1.7?
- Figure 3b-g: y-axis labelling is incorrect (as for Figs. 1B-G)
- To fully appreciate the impact of the E484K mutation, it would be helpful to not only show a fold-change in serum neutralization compared to wild-type, but to also show actual serum titers (as is done in Figure 1).
- Figure 3b, 3d, and 3f (referred to in lines 154-157): According to the legend, the lines indicate the mean fold-change compared to the wild-type virus, and this is also what is pointed out in the text. However, these numbers do not match. For example, in 3f, the line indicates a mean change for B.1.1.7+E484K variant of below 10, but the text in figure and manuscript indicate an 11.4-fold

change.

Minor points:

- Line 55 should be modified to make clear that maintained neutralization was observed in RBD-targeting nAbs outside of the RBM. The way it is currently written is imprecise.
- Line 70: "Vaccine efficacy" may be a more appropriate phrase than "protection rate"
- Line 79: For P.1, the dot is missing
- Line 114: This should reference Fig. 1b-c instead of 1c-d
- Figure 1C-1G: The GMT and/or fold-change between the groups should be indicated in the figure itself.
- Line 122: No reduction in neutralizing activity against WT would be expected, so this should be re-phrased.
- Line 144: This is referring to number of sequences of B.1.1.7 with the E484K mutation, not B.1.1.7.
- Line 254: This combination of mutations actually made the virus more sensitive to neutralization, so it did make an impact (although not negative)

#### **Author Rebuttals to First Revision:**

Dear Editor

Please find below responses to reviewer comments

Yours sincerely

Ravi Gupta

Collier et al. include additional serum neutralization manuscript, analyses using samples obtained from individuals receiving the first and the second vaccination dose of BNT162b2. While the authors addressed most of the reviewer's comments, the manuscript still contains numerous errors to an extent that is rather unusual (see comments below). In contrast to the initial statement by the authors that 'B.1.1.7 escapes from mRNA vaccine-elicited neutralizing antibodies', the data and conclusions presented now are in line with findings of other groups showing a rather small decrease in the susceptibility of B.1.1.7 to BNT162b2-induced SARS-CoV-2 neutralizing antibodies. The main novelty of the manuscript remains in the analysis of measuring serum activity against the B.1.1.7 variant carrying an additional E484K mutation which previously has been shown to affect antibody neutralization.

Specific comments:

- The introduction of the abstract has not been revised. It should, however, now state that activity was measured after both first and second vaccine dose.

Response: we have revised the introduction as suggested and stated that activity was measured after both first and second vaccine dose.

- Line 93-95: In contrast to what is stated, monoclonal antibodies were not tested against B.1.1.7 with the E484K mutation, but only against pseudoviruses with the mutations K417N, E484K, and N501Y.

Response: we have corrected this.

- Extended Data Fig. 1A is misleading: The authors state to have performed analyses using serum samples of 37 participants (see line 98) versus 23 in the original manuscript. However, this figure and the figure legend seem identical to the previous version and therefore do not include any new samples. The number of individuals studied should be clearly stated.

Response: We apologize for the inconsistency. Serum neutralisation was determined with sera from 37 participants after vaccine dose 1 and 21 participants after vaccine dose 2. Antibody levels were determined on 25 participants after vaccine dose 1 and 21 participants after vaccine dose 2. T cell responses against SARS-COV-2 were determined on 24 participants after vaccine dose 1 and 21 participants after vaccine dose 2. In the revised figure legend we indicate the number included in each analysis for clarity. Extended Data Fig. 1A has been revised to include the Spike IgG at 3 dilutions of sera.

- Extended Data Fig. 1B: The numbers of the y-axis do not appear to match the Spike total IgG MFI that is shown in Extended Data Fig. 1A. In 1A, there seem to be 5 samples with an MFI <10,000 against S protein; in 1B, there are > 15. Also, Figure 1B shows 25 measurements. Why? It should be clearly stated how many samples were included for the analyses (also for Extended Data Fig. 1C and 1D). Finally, the ID50 titers do not seem to match with samples shown in Figure 1B.

Response: The numbers included in each analysis have now been included in the figure legend. In Extended Data Fig. 1B, Spike IgG levels in the correlation are at sera dilution 1:1000. The y-axis in Fig. 1B now matches with Fig 1A. We were only able to do serum antibody levels on 25 out of 37 participants. For Extended Data Fig. 1C and 1D the numbers included have now been indicated in the legend.

- Line 115: The difference between 318 and 77 is 0.6 log, not "one order of magnitude".

Response: We have corrected this now, and thank the reviewer for this comment.

- Figures 1C and 1D are identical.

Response: we thank the reviewer for pointing this out, this is now corrected

- Figures 1B-1G: The labeling of the y-axis is incorrect. It is not showing (log10). This is also the case in the Extended Data.

Response: we have now amended the labelling and thank the reviewer.

- Line 131-132: If the "variation" (what is meant by that?) were due to low neutralizing titers after the first dose, wouldn't one expect to see that the convalescent serum samples tested here (with high titers) were even less affected by the B.1.1.7?

Response: we have removed the sentence as it was superfluous as pointed out.

- Figure 3b-g: y-axis labelling is incorrect (as for Figs. 1B-G)

Response: we have now amended the labelling and thank the reviewer.

- To fully appreciate the impact of the E484K mutation, it would be helpful to not only show a fold-change in serum neutralization compared to wild-type, but to also show actual serum titers (as is done in Figure 1).

Response: we have now shown actual serum titres in the new Extended Data Fig 5.

- Figure 3b, 3d, and 3f (referred to in lines 154-157): According to the legend, the lines indicate the mean fold-change compared to the wild-type virus, and this is also what is pointed out in the text. However, these numbers do not match. For example, in 3f, the line indicates a mean change for B.1.1.7+E484K variant of below 10, but the text in figure and manuscript indicate an 11.4-fold change.

Response: Thank you for pointing this out. Median and 95% CI intervals were plotted on the graphs in figures 3b, d and f. This has now been revised to mean and SD and the statistical test amended accordingly.

Minor points:

- Line 55 should be modified to make clear that maintained neutralization was observed in RBD-targeting nAbs outside of the RBM. The way it is currently written is imprecise.

Response: we have modified the text accordingly.

- Line 70: "Vaccine efficacy" may be a more appropriate phrase than "protection rate"

Response: we agree thank you for this. The wording is now amended in the new version of the shortened introduction. Now Line 66.

- Line 79: For P.1, the dot is missing

Response: we have now added the dot, thank you for pointing this out. Now Line 71.

- Line 114: This should reference Fig. 1b-c instead of 1c-d

Response: we have now amended the text accordingly. Now Line 107.

- Figure 1C-1G: The GMT and/or fold-change between the groups should be indicated in the figure itself.

Response: we have now amended the figure accordingly

- Line 122: No reduction in neutralizing activity against WT would be expected, so this should be re-phrased.

Response: one might have expected reduced activity due to N501Y so we have left this as it is.

- Line 144: This is referring to number of sequences of B.1.1.7 with the E484K mutation, not B.1.1.7.

Response: we have now amended the text accordingly

- Line 254: This combination of mutations actually made the virus more sensitive to neutralization, so it did make an impact (although not negative)

Response: we have now amended the text accordingly. Now Line 114-115.