THE EMBO JOURNAL

A functional assay for SARS-CoV-2 N-antibodies

Anna Albecka, Dean Clift, Marina Vaysburd, Tyler Rhinesmith, Sarah Caddy, Helen Baxendale, David Favara, and Leo James
DOI: 10.15252/embj.2021108588

Corresponding author(s): Leo James (lcj@mrc-lmb.cam.ac.uk)

Review Timeline:

Submission Date:	27th Apr 21
Editorial Decision:	4th Jun 21
Revision Received:	17th Jun 21
Editorial Decision:	25th Jun 21
Revision Received:	29th Jun 21
Accepted:	2nd Jul 21

Editor: Karin Dumstrei

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 4th Jun 2021

Dear Leo,

Thank you for submitting your manuscript to The EMBO Journal.

Your study has now been seen by two referees and their comments are provided below. As you can see below both referees appreciate the analysis and find it suitable for publication in The EMBO Journal. They raise relative minor concerns that should be fairly easy to sort out. Let me know if we need to discuss anything further - happy to do so via email or video call.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Please also take a look at the attached revision guideline with helpful tips for how to prepare the revision.

I thank you for the opportunity to consider your work for publication.

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 2nd Sep 2021.

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

In this manuscript the authors develop a new assay to measuring the potential neutralizing activity of N-specific antibodies generated following SARS-CoV-2 infection. The assay involves electroporating the antibodies into a cell and then challenging the cell with virus. This assay allowed the authors to show that when N-specific IgG is electroporated into cells, it is able to prevent viral replication on SARS-CoV-2 in a TRIM21 dependent manner. This gives a functional activity for the high levels of N-IgG observed in sera. They were further able to show that the neutralizing activity correlated with the level of N-IgG in patient sera and that the neutralization was facilitated by N-IgG and not S-IgG. Overall, this is a very interesting study that will be of broad interest as well as having specific relevance to COVID-19 vaccination. The manuscript is clearly written, and the

experiments are carried out to a high standard.

How was it determined that the electroporation has led to the IgG entering the cell?

Is there any evidence that vaccines containing N have better protective capacity that Spike based vaccines? E.g. vaccines based on inactivated viruses such as sinovac?

Are there any known correlations between N IgG levels and disease severity and/or progression following SARS-CoV-2 infection? This would give biological significance to the functional activity measured.

Do the authors anticipate that all epitopes on N will facilitate neutralization?

Stats for the correlation on Figure 4C are missing.

Referee #2:

In this work, the authors developed an artificial in vitro method called EDNA, by electroporating antibodies into cells, to provide quantitative measurement of N-antibody activity. The author further proved that the N-antibodies neutralize SARS-CoV-2 intracellularly by recruiting the cytosolic Fc-receptor TRIM21. As claimed by the authors, the function of antibodies to intracellular antigens has been shown to prevent infection by arenaviruses ebolavirus, HCMV, HIV and influenza virus. However, comparing with Spike-induced neutralizing antibodies, Nucleoprotein-antibodies are expected for more in-depth investigation, I recommend acceptance with minor revisions.

Specific comments:
1. The authors are suggested to add an abstract graphic as Figure1A or Figure S1A, to better summarize their design of this new method.
2. In Figure 1A, the titrations of anti-MHV polyclonal serum added either extracellular or intracellular lack data from 16h to 24h, is there any difficulty quantifying cell area?
3. Line 94, "electroporated L929 cells with serial dilutions of an anti-N monoclonal antibody". Is there any difference among N-antibodies with different epitopes?
4. In Figure 1I. The authors utilized the CHX model to test N-protein levels, and should also present the protein level without electroporation to rule out other perturbations and be a positive control.
5. The "N-antibody" mentioned in the manuscript is ambiguous. It is important to address neutralizing and non-neutralizing functions. Line 50, some references the authors provide are involved non-neutralizing antibodies, are the mechanisms underlying via ADIN pathways?
6. A cohort of four SARS-CoV-2 convalescents is relatively small and inadequate to draw a distinct conclusion of antibody portfolios. The authors may indicate more details in Figure 3A and 3B explanation.
7. Line 141, "... to make it permissive for SARS CoV-2 entry", should it be "sensitive"? not "permissive".
8. According to previous studies and common sense, the antibodies are not efficiently taken up by cells in vitro, however, in this artificial method, this process is bypassed by using electroporation. Since the situation is much complicated in COVID-19 convalescent patients, the authors are suggested adding some sentences to address the limitations of this method in the discussion section.

Referee #1:

Q1. How was it determined that the electroporation has led to the IgG entering the cell?

A1. We established conditions for efficient electroporation of IgGs into cells using Alexa488-labelled antibodies and a combination of flow cytometry and confocal microscopy. In Figure S1H, we show dose-dependent delivery of Alexa488-IgGs by quantifying the mean fluorescence intensity of electroporated cells. In Figure S1I, confocal microscopy images show that Alexa488-IgGs are distributed diffusely throughout the cell 6 hours post-electroporation.

Q2. Is there any evidence that vaccines containing N have better protective capacity that Spike based vaccines? E.g. vaccines based on inactivated viruses such as sinovac?

A2. This is an important question that needs to be addressed but as yet there is very limited data. It is hard to compare existing vaccines, even those just using Spike, because different platforms and delivery modalities are being used. However, all vaccines that have achieved WHO Emergency Use Listing are effective in preventing disease and hospitalization due to COVID-19 (https://www.who.int/news-room/feature-stories/detail/the-sinovac-covid-19-vaccine-what-you-need-to-know). Whether there are differences in longevity of protection or efficacy against the continuing emergence of variants remains to be determined.

Q3. Are there any known correlations between N IgG levels and disease severity and/or progression following SARS-CoV-2 infection? This would give biological significance to the functional activity measured.

A3. This is also a crucial question that we are actively seeking to address. We are currently participating in a multi-centre study to address what aspects of humoral immunity correlate with protection. Our first pilot data set has recently been deposited on MedRxiv (https://www.medrxiv.org/content/10.1101/2021.05.21.21257572v1). In this dataset, we observed that severe COVID-19 patients had significantly higher N IgG levels and intracellular neutralizing titres than mild or asymptomatic seropositive participants. This is consistent with the high and persistent viral replication and antigen expression in COVID-19 patients. This suggests that N antibody levels are biologically significant and we are seeking to test this further in a larger patient group. Whether this increased antibody response contributes to antibody-mediated immunopathology will also be important to determine.

Q4. Do the authors anticipate that all epitopes on N will facilitate neutralization?

A4. Our previous work on the mechanism of TRIM21-mediated neutralization suggests that the principle factors required for activity are antibody:antigen (https://pubmed.ncbi.nlm.nih.gov/27881870/) and antibody:TRIM21 affinity (https://pubmed.ncbi.nlm.nih.gov/26962230/). More specifically, off-rate is crucial. We don't anticipate that different epitopes per se will elicit different neutralization activity, unless upon antibody binding the Fc is not accessible for TRIM21 binding. This may be interesting to test in future using a panel of monoclonal antibodies against defined epitopes. We have added a sentence to the discussion to address this question (Lines 267-269).

Q5. Stats for the correlation on Figure 4C are missing.

A5. We have added an inset panel to Figure 4C showing a nonlinear fit to the log transformed data and the R^2 value.

Referee #2:

Q1.The authors are suggested to add an abstract graphic as Figure1A or Figure S1A, to better summarize their design of this new method.

A1. We are grateful for this suggestion and have added a graphical abstract giving an overview of the new method.

Q2. In Figure 1A, the titrations of anti-MHV polyclonal serum added either extracellular or intracellular lack data from 16h to 24h, is there any difficulty quantifying cell area?

A2. No scans were taken during that time due to instrument availability. We apologise that this is not ideal but are confident in the trajectory of the collected data.

Q3. Line 94, "electroporated L929 cells with serial dilutions of an anti-N monoclonal antibody". Is there any difference among N-antibodies with different epitopes?

A3. Unfortunately, we were not able to source additional monoclonal antibodies. Most described MHV anti-N antibodies were made in the 1980s and have been difficult to get hold of. We are grateful to Dr. Peter Rottier who was able to help us obtain the MHV antiserum we used in our study. However, we believe that different epitopes will be less important than the antibody-antigen off-rate (please see response A4 to Referee 1 above).

Q4. In Figure 1I. The authors utilized the CHX model to test N-protein levels, and should also present the protein level without electroporation to rule out other perturbations and be a positive control.

A4. As requested, we have now included data showing electroporation does not alter N-protein levels.

Q5. The "N-antibody" mentioned in the manuscript is ambiguous. It is important to address neutralizing and non-neutralizing functions. Line 50, some references the authors provide are involved non-neutralizing antibodies, are the mechanisms underlying via ADIN pathways?

A5. We have added the following to the introduction:

Lines X: "Crucially however, because internal antigens are usually hidden inside the virion, antibodies against them do not bind infectious viral particles. Consequently, N-antibodies and similar typically do not block infectious entry of viruses into cells in standard in vitro assays and are described as 'non-neutralizing'. The mechanisms behind the immune protection provided by non-neutralizing antibodies like N-antibodies remain largely unknown."

And to the discussion:

Lines 273-278: "Infection with most enveloped viruses results in high titre antibodies against internal antigens and while these are typically non-neutralizing in vitro (at least as characterized in standard assays) they are often highly protective in vivo[31]. Based on our results here, and recently published{Caddy, 2020 #2507}, we propose that ADIN may provide one underlying mechanism by which non-neutralizing antibodies mediate protection. However, ADIN does not explain how and where non-neutralizing antibodies like those against N protein meet their antigen."

Q6. A cohort of four SARS-CoV-2 convalescents is relatively small and inadequate to draw a distinct conclusion of antibody portfolios. The authors may indicate more details in Figure 3A and 3B explanation.

A6. We have added the following to the results:

Lines 167-169: "The data shows that a good dynamic range in antigen reactivity is capable of being measured but a larger cohort would be required to draw any conclusions about antibody portfolios."

Analysis of the data for correlations in antigen reactivity is performed only on the larger cohort in Figure 4. We are also involved in studies attempting to correlate antibody portfolios with disease severity and progression (see answer A3 to Review 1).

Q7. Line 141, "... to make it permissive for SARS CoV-2 entry", should it be "sensitive"? not "permissive".

A7. We have changed the text as suggested.

Q8. According to previous studies and common sense, the antibodies are not efficiently taken up by cells in vitro, however, in this artificial method, this process is bypassed by using electroporation. Since the situation is much complicated in COVID-19 convalescent patients, the authors are suggested adding some sentences to address the limitations of this method in the discussion section.

A8. We have expanded the discussion section as follows:

Lines 277-281: "However, ADIN does not explain how and where non-neutralizing antibodies like those against N protein meet their antigen. EDNA is an in vitro assay in which antibodies are artificially delivered directly into the cytosol. This is a limitation of the method and does not address the issue of cellular uptake and cytosolic import during natural SARS-CoV-2 infection in vivo."

1st Revision - Editorial Decision 25th Jun 2021

Dear Leo,

Thank you for submitting your revised manuscript to The EMBO Journal. I have looked at your response and all looks good. I am therefore very pleased to let you know that we will accept the manuscript for publication here. Before sending you the formal acceptance letter there are just a few formatting issues that we need to resolve.

- Please upload individual figures files.

- Please add 3-5 keywords

- Please re-label "Data and materials availability" as "Data Availability" and place this section after Structured Methods. As far as I can see no data is generated that needs to be deposited in a database. If this is correct please state: This study includes no data deposited in external repositories.

- Competing interests should be re-labeled as Conflict of Interest

- The reference format should be alphabetical, 10 et al.

- The movie should be called "Movie EV1". Please also correct callout in text. The legend should be removed from appendix and zipped to the movie file.

- The appendix needs a ToC. Figures in the appendix should be labeled as "Appendix Figure S1". Please also correct callouts in text. Alternatively, you can also upload the appendix figures as Expanded view figures. Please see guide to authors https://www.embopress.org/page/journal/14602075/authorguide#expandedview

- We encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.

- We include a synopsis of the paper (see http://emboj.embopress.org/). Please provide me with a general summary statement and 3-5 bullet points that capture the key findings of the paper.

- We also need a summary figure for the synopsis. The size should be 550 wide by [200-400] high (pixels). You can also use something from the figures if that is easier.

- Our publisher have done their pre-publication checks on the manuscript and made comments in the figure legends. Please take a look at the "Data Edited Manuscript file" word document to see their marked changes.

- Remove Reagents & Tools Table from MS file and upld as a separate file.

- Rename Methods & Protocols => should be Structured Methods

- Please merge acknowledgements it is now in two parts.

That should be all.

You can use the link below to upload the revised version

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 23rd Sep 2021.

https://emboj.msubmit.net/cgi-bin/main.plex

2nd Revision - Editorial Decision 2nd Jul 2021

Dear Leo,

Thank you for submitting your revised manuscript to The EMB Journal. I have now had a chance to take a careful look at it and all looks good.

I am therefore very pleased to accept the manuscript for publication here.

Congratulations on a nice study

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here: https://emboj.embopress.org/about#Transparent_Process

Your manuscript will be processed for publication in the journal by EMBO Press. Manuscripts in the PDF and electronic editions of The EMBO Journal will be copy edited, and you will be provided with page proofs prior to publication. Please note that supplementary information is not included in the proofs.

Should you be planning a Press Release on your article, please get in contact with embojournal@wiley.com as early as possible, in order to coordinate publication and release dates.

If you have any questions, please do not hesitate to call or email the Editorial Office. Thank you for your contribution to The EMBO Journal.

** Click here to be directed to your login page: https://emboj.msubmit.net

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Leo James
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2021-108588

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Power analysis
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We performed a power analysis in order to determine sample sizes for each group.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	We did not exclude any animals from our data sets.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Different investigators dosed and monitored the animals than those who performed the tissue analysis.
For animal studies, include a statement about randomization even if no randomization was used.	We did not use randomization.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	We did not use blinding
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes
Is there an estimate of variation within each group of data?	There is a measured determination of error
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	We used the anti-nucleoprotein antibody KL53, as described in: Zeller, W., Bruns, M., & Lehmann-Grube, F. (1988). Lymphocytic choriomeningitis virus X. Demonstration of nucleoprotein on the surface of infected cells. Virology, 162(1), 90–97. https://doi.org/10.1016/0042-6822(88)90397-2
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Sources of all cells lines are ATCC

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	We used C57BL/6 mice of both genders. Animals were between 8 to 12 weeks of age. Animals were housed at the MRC ARES facility and husbandry was performed according to the Home Office Animals (Scientific Procedures) Act (1986) and approved by the Medical Research Council Animal Welfare and Ethical Review Body. Animals were bred within the facility.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Experiments were conducted in accordance with the 19.b.7 moderate severity limit protocol and Home Office Animals (Scientific Procedures) Act (1986) and approved by the Medical Research Council Animal Welfare and Ethical Review Body.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We did not use ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Not applicable
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not applicable
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not applicable
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Not applicable
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not applicable
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not applicable
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not applicable

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Provided
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	All data is contained within the work.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	Not applicable
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Checked

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No