Single-point mutations in the N gene of SARS-CoV-2 adversely impact detection by a commercial dual target diagnostic assay

Sharon Miller, Terence Lee, Adam Merritt, Todd Pryce, Avram Levy, and David Speers

Corresponding Author(s): Sharon Miller, PathWest Laboratory Medicine

Review Timeline:

| Event                  | Date           |
|------------------------|----------------|
| Submission Date        | September 5, 2021 |
| Editorial Decision     | October 4, 2021 |
| Revision Received      | October 12, 2021 |
| Editorial Decision     | October 18, 2021 |
| Revision Received      | October 21, 2021 |
| Accepted               | October 25, 2021 |

Editor: Yun Young Go

Reviewer(s): The reviewers have opted to remain anonymous.

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. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1128/Spectrum.01494-21
Re: Spectrum01494-21 (Single-point mutations in the N gene of SARS-CoV-2 adversely impact detection by a commercial dual target diagnostic assay)

Dear Dr. Sharon Anne Miller:

Thank you for submitting your manuscript to Microbiology Spectrum. Your manuscript has been reviewed by two experts in the field. As their comments indicate, the manuscript needs modifications before it could be considered acceptable for publication. When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed information on submitting your revised paper are below.

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Sincerely,

Yun Young Go

Editor, Microbiology Spectrum

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org

Reviewer comments:

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Staff Comments:

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• Point-by-point responses to the issues raised by the reviewers in a file named "Response to Reviewers," NOT IN YOUR COVER LETTER.
• Upload a compare copy of the manuscript (without figures) as a "Marked-Up Manuscript" file.
• Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file.
• Manuscript: A .DOC version of the revised manuscript
• Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at https://journals.asm.org/journal/Spectrum/submission-review-process. Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript."

Please return the manuscript within 60 days; if you cannot complete the modification within this time period, please contact me. If you do not wish to modify the manuscript and prefer to submit it to another journal, please notify me of your decision immediately so that the manuscript may be formally withdrawn from consideration by Microbiology Spectrum.

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1. Note the location of the patients from whom these specimens were derived and compare to previously publications. The emergence of these mutations on another continent adds to the genomics analyses and the hypothesis that these mutations are independently emerging.

In this study, the five isolates containing the C29197T mutation were from a cluster of related cases. Analysis of these sequences using the GISAID database showed them to be closely related to sequences isolated in Colorado, USA. Rhoads et al. (9) and Leelawong et al. (8) also identified the C29197T mutation in cases from Ohio and New York, respectively.

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Yes, these samples were confirmed with an in-house RT-PCR system.

Comment 4: It would be helpful for readers if the sequences were deposited in GISAID or GenBank and mentioned it in data availability section.

A data availability section consisting of the GISAID Accession numbers has been added.

Comment 5: Laboratory researchers would be interested to know if the described mutation could possibly affect (in silico experiment) any of the commonly used commercial/in-house diagnostic assays?

Yes, the described mutation could possibly affect the ability of the gene Xpert kit in detecting the N gene target of SARS-CoV-2.
Re: Spectrum01494-21R1 (Single-point mutations in the N gene of SARS-CoV-2 adversely impact detection by a commercial dual target diagnostic assay)

Dear Dr. Sharon Anne Miller:

In the revised version, the authors have made an effort to improve the manuscript following the reviewers' comments. However, comments 3 and 5 from Reviewer #2 still need additional clarification.

Specific comments:
1. Page 7, lines 152-153. Please provide specifics of the in-house RT-PCR performed in the study (e.g., target gene) and the reference material.
2. The possible impact of the described mutation in commonly used commercial or in-house diagnostic assays (other than Xpert assay), as suggested by reviewer #2 should be discussed thoroughly.

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Staff Comments:

Preparing Revision Guidelines
To submit your modified manuscript, log onto the eJP submission site at https://spectrum.msubmit.net/cgi-bin/main.plex. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:
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Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

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The samples were confirmed positive for SARS-CoV-2 by an in-house RT-PCR using SARS-CoV-2 specific targets in the E gene (16) and the spike protein (unpublished data).

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The following paragraph has been added to the discussion

Our results show that the N2 gene target region used by the Xpert is consistent with the CDC 2019_nCoV_N2 probe sequence. This is significant as other commercial or in-house diagnostic assays designed using this sequence may encounter similar issues. The C29197T and C29200T mutations, located within the CDC probe sequence, are likely responsible for the failed detection of the N gene target in the Xpert assay and have the potential to negatively impact detection in other assays which also use this probe sequence.
October 25, 2021

Dr. Sharon Anne Miller
PathWest Laboratory Medicine
Microbiology
Queen Elizabeth II Medical Centre
Nedlands
Perth, WA 6009
Australia

Re: Spectrum01494-21R2 (Single-point mutations in the N gene of SARS-CoV-2 adversely impact detection by a commercial dual target diagnostic assay)

Dear Dr. Sharon Anne Miller:

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