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## 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*

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### Review Timeline:

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

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*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>

October 4, 2021

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

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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The ASM Journals program strives for constant improvement in our submission and publication process. Please tell us how we can improve your experience by taking this quick [Author Survey](#).

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](mailto:spectrum@asmusa.org)

Reviewer comments:

Reviewer #1 (Comments for the Author):

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Two comments/suggestions:

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This paper reports the identification of single-point mutations in the N gene of SARS-CoV-2 associated with a gene target failure by the Cepheid Xpert commercial system. To determine the mutation(s) responsible for the N gene detection failures, the genomic products from the Cepheid Xpert system were sequenced and compared to whole genomes of SARS-CoV-2 from clinical cases. However, the manuscript has some concerns.

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

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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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- Manuscript: A .DOC version of the revised manuscript
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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.

If your manuscript is accepted for publication, you will be contacted separately about payment when the proofs are issued; please follow the instructions in that e-mail. Arrangements for payment must be made before your article is published. For a complete list of **Publication Fees**, including supplemental material costs, please visit our [website](#).

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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.

October 18, 2021

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

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.

Thank you for submitting your manuscript to Microbiology Spectrum. 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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Washington, DC 20036  
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Staff Comments:

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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:

Your manuscript has been accepted, and I am forwarding it to the ASM Journals Department for publication. You will be notified when your proofs are ready to be viewed.

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Thank you for submitting your paper to Spectrum.

Sincerely,

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Journals Department  
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