Rapid SARS-CoV-2 Variants Enzymatic Detection (SAVED) by CRISPR-Cas12a

Jun Yang, Nilakshi Barua, Md Nannur Rahman, Carmen Li, Norman Wai-Sing Lo, Kai Yan Yeong, Tsz Fung Tsang, Xiao Yang, Yuk-Yam Cheung, Alan Tsang, Rick Jason W. Chan, Eddie Leung, Paul Chan, and Margaret Ip

Corresponding Author(s): Margaret Ip, Chinese University of Hong Kong

Review Timeline:

Submission Date: August 18, 2022
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Editor: Maria Grazia Cusi

Reviewer(s): Disclosure of reviewer identity is with reference to reviewer comments included in decision letter(s). The following individuals involved in review of your submission have agreed to reveal their identity: Kaan Çeylan (Reviewer #2)

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.)

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September 18, 2022

Prof. Margaret Ip
Chinese University of Hong Kong
Microbiology
Prince of Wales Hospital,
Ngan Shing Street
Shatin
Hong Kong

Re: Spectrum03260-22 (Rapid SARS-CoV-2 Variants Enzymatic Detection (SAVED) by CRISPR-Cas12a)

Dear Prof. Margaret Ip:

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 instructions on submitting your revised paper are below.

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Sincerely,

Maria Grazia Cusi
Editor, Microbiology Spectrum

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

Reviewer comments:

Reviewer #1 (Comments for the Author):

Dear authors,

This manuscript (Ms#Spectrum03260-22) entitled "Rapid SARS-CoV-2 Variants Enzymatic Detection (SAVED) by CRISPR-Cas12a" by Yang et al. describes Cas-12a can be used for the rapid
differentiation of SARS-CoV-2 variants. Indeed, the specificity and accuracy of the present assay using blind clinical samples would be applicable to clinical settings. I'll provide you with a few comments below.

Comment-1 (Table 1): Too busy to compare the difference between mutation sites. Please modify Table 1 in a reader-friendly style.

Comment-2 (Figures 1o, 1u, S15, S21): As the authors mentioned in Legend for Figure1, the assay for T478K and N501Y was conducted using RNA templates because the use of DNA templates resulted in non-specific amplification. Why did this inappropriate reaction occur?

Comment-3: Please describe the reason why you set the fluorescence threshold as 5000 AU.

Comment-4 (Figure 4): Some mutation sites such as T478K, E484A, and L452R showed no dose-dependency. The authors should discuss why the present assay resulted in the tendency.

Reviewer #2 (Comments for the Author):

Dear author,

Your work titled Rapid SARS-CoV-2 Variants Enzymatic Detection (SAVED) by CRISPR-Cas12a has been reviewed by me. Congratulations for the hard work you put into it. While the text is generally acceptable, some points may be challenging for the reader.

First of all, since the tables are very long, it will be difficult for the reader to follow. It becomes difficult to understand the subject, as repetitive expressions are included in the findings and discussion part. In addition, since two different molecular techniques are compared, it would be appropriate to mention the cost difference between them in the study.

I would appreciate it if you would consider these issues.

Good work.

Staff Comments:

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• 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.

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Dear Reviewers,

Thank you very much for your kind comments. Your suggestions are highly appreciated, and we have modified the manuscript according to your comments. Please check the following point-by-point response.

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 instructions on submitting your revised paper are below.

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**Response:** None.
Reviewer comments:

Reviewer #1 (Comments for the Author):

Dear authors,

This manuscript (Ms#Spectrum03260-22) entitled "Rapid SARS-CoV-2 Variants Enzymatic Detection (SAVED) by CRISPR-Cas12a" by Yang et al. describes Cas-12a can be used for the rapid differentiation of SARS-CoV-2 variants. Indeed, the specificity and accuracy of the present assay using blind clinical samples would be applicable to clinical settings. I'll provide you with a few comments below.

Comment-1 (Table 1): Too busy to compare the difference between mutation sites. Please modify Table 1 in a reader-friendly style.

Response: Many thanks for your comments. We have replaced the original Table 1 with the original Figure S1. Kindly check Figure 1.

Comment-2 (Figures 1o, 1u, S15, S21): As the authors mentioned in Legend for Figure 1, the assay for T478K and N501Y was conducted using RNA templates because the use of DNA templates resulted in non-specific amplification. Why did this inappropriate reaction occur?

Response: Many thanks for your comments. The primers of T478K and N501Y have mismatch(es) for the Omicron variant containing S477N+T478K and Q498R+N501Y. RT-RPA by T478K and N501Y primers using RNA samples decreased amplification...
compared RPA using DNA samples. The fluorescence signal between T478K and S477N+T478K, N501Y and Q498R+N501Y already showed obvious difference by RPA following CRISPR-Cas12a detection, with the help of reverse transcription, the signal of S477N+T478K and Q498R+N501Y lower than the threshold, which enables these mutation sites differentiation. Kindly check lines 508-512 of the marked-up manuscript or lines 399-403 of the revised manuscript.

Comment-3: Please describe the reason why you set the fluorescence threshold as 5000 AU.

Response: We used 5000 AU as the threshold and validated against spectrophotometry because below 5000 AU the signal was not visible under the UV light. Kindly check lines 282-283 of the marked-up manuscript or lines 174-175 of the revised manuscript.

Comment-4 (Figure 4): Some mutation sites such as T478K, E484A, and L452R showed no dose-dependency. The authors should discuss why the present assay resulted in the tendency.

Response: Many thanks for your critical comment. Though there were some fluctuations on the fluorescence value compared to the Ct value, our platform's clinical samples were positive. As long as it was positive, there was an agreement between our platform and RT-qPCR. The fluctuations has been also observed in the experiments conducted by de Puig et al., 2021; Patchsung et al., 2020; Broughton et al., 2020; Liang et al., 2021 (The reference details and figures are mentioned in the following table for your kind reference).

| Figure No. | References |
|------------|------------|
| Figure 4   | de Puig H, Lee RA, Najjar. Minimally instrumented SHERLOCK (miSHERLOCK) for CRISPR-based point-of-care diagnosis of SARS-CoV-2 and emerging variants. Sci Adv, 2021;7. DOI: 10.1126/sciadv.abh2944 |
| Figure 4   | Patchsung M, Jantarug K, Pattama A, et al. Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. Nat Biomed Eng, 2020;4:1140–1149. DOI: 10.1038/s41551-020-00603-x. |
| Figure 2e  | Broughton JP, Deng X, Yu G, et al. CRISPR–Cas12-based detection of SARS-CoV-2. Nat Biotechnol, 2020;38:870– |
Reviewer #2 (Comments for the Author):

Dear author,
Your work titled Rapid SARS-CoV-2 Variants Enzymatic Detection (SAVED) by CRISPR-Cas12a has been reviewed by me. Congratulations for the hard work you put into it. While the text is generally acceptable, some points may be challenging for the reader.

First of all, since the tables are very long, it will be difficult for the reader to follow.

Response: Thank you for your comments. We have replaced the Table 1 with Figure 1, which is much convenient for readers to follow. For the original Table 2 and Table 3, now they are Table 1 and Table 2 in the marked-up manuscript. As so many mutation sites need to be mentioned, it will lose important information if shortened.

It becomes difficult to understand the subject, as repetitive expressions are included in the findings and discussion part.

Response: We have deleted the respective repetitive expressions in following lines:
Lines 347-348: “The result of 30 min incubation was the same as 1.5 h, thus, here only shows 30 min incubation.”
Lines 379-382: “we used 69 clinical samples and 10 no template controls (NTC); The samples included Wild type (n=5), Alpha (n=10), Beta (n=10), Delta (n=20), Omicron BA.1 (n=5), and BA.2 (n=19) variants of SARS-CoV-2.”
Lines 387-389: “The sequences, variant types, and Ct value of clinical samples were unknown to the researcher until the completion of analyzing SAVED CRISPR-Cas12a-based detection data to minimize interpretation bias.”
Lines 453-457: “We opted for the four strategies to optimize crRNAs for detection. In this study, all selected crRNAs targeting SNP belong to chimeric or short crRNAs strategies (Figs. 1-2 and Table 1). Mutation sites with deletions or insertions can be differentiated by regular length spacer, otherwise, at least 18-nt can be used as many mismatches were present (Fig. 1d,e,f,h,i).”
Lines 463-465: “As we reported previously, when we used 24-nt spacer, 20-nt spacer, and chimeric crRNAs, the specificity of chimeric crRNA was the strongest, whereas the signal was the weakest (Figs. S11-17,21) [32]).”

In addition, since two different molecular techniques are compared, it would be appropriate to mention the cost difference between them in the study.

Response: We added the cost difference between our platform and RT-qPCR in Table S3 and lines 429-430 of the marked-up manuscript or lines 320-321 of the revised manuscript.

I would appreciate it if you would consider these issues.

Good work.

Staff Comments:

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• 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

Response: The files have been prepared according to your requirements.

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Thank you for submitting your paper to Spectrum.

Sincerely,

Maria Grazia Cusi
Editor, Microbiology Spectrum

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

Supplemental file 1: Accept