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Evaluation of a multiplex bead assay against single-target assays for detection of IgG antibodies to SARS-CoV-2

Kaitlin Mitchell, Christina Carlson, Doug Nace, Brian Wakeman, Jan Drobeniuc, Glenn Niemeyer, Bonnie Werner, Alex Hoffmaster, Panayampalli Satheshkumar, Amy Schuh, Venkatachalam Udhaykumar, and Eric Rogier

Corresponding Author(s): Eric Rogier, CDC

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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.01054-22>

April 20, 2022

Dr. Eric Rogier
CDC
Atlanta

Re: Spectrum01054-22 (Evaluation of a multiplex bead assay against single-target assays for detection of IgG antibodies to SARS-CoV-2)

Dear Dr. Eric Rogier:

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,

Kileen Shier

Editor, Microbiology Spectrum

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Reviewer comments:

Reviewer #1 (Comments for the Author):

Thank you for allowing me to review this interesting paper.
I have included some comments below.

Minor

Page 3 line 59 "induvial" should be "individual"

Page 5 line 75- suggest past tense when describing number of assays in Oct 2021.

References- suggest adding dois for references 1, 5, 24, 35, 37, 39

Major
Methods Page 5- how were handled before testing and receipt by your lab? Were separate aliquots made and tested or were individual tubes run on both the Abbott and Ortho systems?
Methods Data analysis page 6- Were there rules established to call a specimen "false-positive" or "false-negative"?
Discussion- Page 11 line 247- there are multiple reasons why immunity testing is not recommended after vaccination. One is that there is no agreed upon "correlate of protection". This could be expanded and noted here
Discussion Page 14- line 304- other options for a "false-positive anti-S"; was the individual with a negative NAT swabbed to late? Was the specimen collected from a part of the respiratory tract where the virus was not present? Is the anti-S identifying a response to seasonal beta coronavirus?

Reviewer #2 (Comments for the Author):

Line 173 "The 12 specimens with variation had a high degree of "equivocal" calls for one or more antigen targets, indicating a quantitative near the assay seropositivity threshold."
Comment/Question: Were these 12 samples included in the analysis of sensitivity and specificity? In the discussion you mention a resolution protocol when there is an equivocal result, but it is not clear whether you used this to calculate sensitivity and specificity. Strictly speaking if you followed the IFU, shouldn't the data from all or most of these 12 samples be included providing RT-PCR results were available? Table 2 has an N of 300. Is this correct or is it 312 after eliminating the 11 specimens that did not have RT-PCR results? Please clarify which samples in addition to those 11 were not included and why in calculating sensitivity and specificity.
Also, I tried to review the Tetracore IFU listed in reference 16, but the link, <https://tetracore.com/product/fleximmarray-sars-cov-2-human-igg-antibody-test/>. did not work.

Line 187: "Among all specimens (n=323), 12 188 (3.7%) had positive calls for only one or two targets and were therefore called SARS CoV-2 IgG seronegative. Of these 12, one was positive only to NP, nine were positive to both RBD and NP, and two were positive to NP and the RBD/NP fusion."

Comment: Here we are back to a total of 323 samples. Are these 12 specimens included in the sensitivity and specificity calculations?

Line 208: "The MBA was compared against two commercially available SARS-CoV-2 209 serological assays..."

Comment/Question: Was the sample size 323,312 or 300?

Although the figures presenting the r squared data is interesting, the assays aren't truly quantitative, for which this type of analysis has more meaning.

Lines 223-235. "For these 25 discrepant specimens..."

Are you determining a false positive or negative based on RT-PCR? Please state that to be clear. I find this paragraph a bit confusing. There are 25 discrepant results which include 18 falsely negative, one falsely positive on Abbot and 6 on MBA that are negative using IFU interpretation regardless of whether one or two positive antigens, but you are calling them falsely positive, which I don't understand. I really think a table might be helpful here or a clearer explanation of the discrepant results for each assay. For the MBA, I would consider using the overall result interpretation and leaving out the individual antigen results.

Line 256: "Against this serum panel of 323 specimens, the MBA demonstrated high diagnostic sensitivity (89.8%) and specificity (100.0%)."

Comment: There is an N of 300 for Table 2 data from which the sensitivity and specificity is calculated.

Staff Comments:

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Thank you for submitting your paper to Microbiology Spectrum.

Line 173 “The 12 specimens with variation had a high degree of “equivocal” calls for one or more antigen targets, indicating a quantitative near the assay seropositivity threshold.”

Comment/Question: Were these 12 samples included in the analysis of sensitivity and specificity? In the discussion you mention a resolution protocol when there is an equivocal result, but it is not clear whether you used this to calculate sensitivity and specificity. Strictly speaking if you followed the IFU, shouldn't the data from all or most of these 12 samples be included providing RT-PCR results were available? Table 2 has an N of 300. Is this correct or is it 312 after eliminating the 11 specimens that did not have RT-PCR results? Please clarify which samples in addition to those 11 were not included and why in calculating sensitivity and specificity.

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Comment: There is an N of 300 for Table 2 data from which the sensitivity and specificity is calculated.

Spectrum01054-22 (Evaluation of a multiplex bead assay against single-target assays for detection of IgG antibodies to SARS-CoV-2)

Response to Reviewers

The authors thank the reviewers for their helpful comments, and for the opportunity to resubmit our revised manuscript. We address the individual reviewer comments below in bold text.

Reviewer #1 (Comments for the Author):

Thank you for allowing me to review this interesting paper.

I have included some comments below.

Minor

Page 3 line 59 "induvial" should be "individual"

Changed

Page 5 line 75- suggest past tense when describing number of assays in Oct 2021.

Changed

References- suggest adding dois for references 1, 5, 24, 35, 37, 39

The authors thank the reviewer for this suggestion and will defer to the journal copy editors regarding formatting references.

Major

Methods Page 5- how were handled before testing and receipt by your lab? Were separate aliquots made and tested or were individual tubes run on both the Abbott and Ortho systems?

This additional information has been added on line 111: "Upon receipt at the CDC in Atlanta, GA, USA, each specimen was thawed and aliquoted into multiple vials for storage at -80°C and later distribution.

Methods Data analysis page 6- Were there rules established to call a specimen "false-positive" or "false-negative"?

Yes, rules for determining a sample's 'true' category (CoV2 exposed/non-exposed) are provided on Page 5 from lines 108-111. Using this known category for a sample, an individual assays' IgG +/- call for that sample was then able to be classified as a TP/FP/TN/FN.

Discussion- Page 11 line 247- there are multiple reasons why immunity testing is not recommended after vaccination. One is that there is no agreed upon "correlate of protection". This could be expanded and noted here

The reviewer brings up an important point here in that this is a multi-factorial issue for caution regarding SARS-CoV-2 serology interpretation, and the fact there's currently no 'level of antibodies' that are known to correlate with protection. We have added an additional sentence here: "Additionally, there is currently no level of IgG antibodies known to have a protective association

against SARS-CoV-2 infection, so serosurveillance could be utilized to estimate exposure to either natural infection or vaccination, but not presently employed to estimate individual or population protection against the virus.”

Discussion Page 14- line 304- other options for a "false-positive anti-S"; was the individual with a negative NAT swabbed to late? Was the specimen collected from a part of the respiratory tract where the virus was not present? Is the anti-S identifying a response to seasonal beta coronavirus?

The reviewer provides some other reasonable explanations for a patient’s blood sample being IgG positive against coronaviral antigens, but their swab RT-PCR negative. For clarification, this sample collected in May 2020 was IgG positive only for N protein, but additional text has been added to this section to further discuss this finding: “Of the seven specimens with false-positive results (or partial false positivity on the MBA), six had been collected in September 2019 and one had been collected in May 2020, but the latter patient had a negative SARS-CoV-2 RT-PCR result, and was IgG positive for the NP only by MBA. It is possible this individual had a viral load below the limit of detection of the RT-PCR, or more likely, a positive NP IgG signal due to previous exposure to other human coronaviruses and IgG cross-binding to similar epitopes as has been observed previously (6, 10).”

Reviewer #2 (Comments for the Author):

Line 173 "The 12 specimens with variation had a high degree of "equivocal" calls for one or more antigen targets, indicating a quantitative near the assay seropositivity threshold."

Comment/Question: Were these 12 samples included in the analysis of sensitivity and specificity? In the discussion you mention a resolution protocol when there is an equivocal result, but it is not clear whether you used this to calculate sensitivity and specificity. Strictly speaking if you followed the IFU, shouldn't the data from all or most of these 12 samples be included providing RT-PCR results were available? Table 2 has an N of 300. Is this correct or is it 312 after eliminating the 11 specimens that did not have RT-PCR results? Please clarify which samples in addition to those 11 were not included and why in calculating sensitivity and specificity.

The authors thank the reviewer for bringing up this point here, and the confusion is the fault of the authors alone as the number of samples provided in Table 2 was incorrect. Instead of the N of 300, the correct N is 312. So to answer the reviewer’s question above, all 12 samples were included for analysis that had an equivocal call - meaning an overall IgG negative call (per the IFU), though individual antigen targets may be IgG positive.

Also, I tried to review the Tetracore IFU listed in reference 16, but the link,

<https://tetracore.com/product/fleximmarray-sars-cov-2-human-igg-antibody-test/>. did not work.

Thank you for bringing this to our attention. Tetracore Inc. has actually removed this current product listing from their website and is revising the listing, so the link currently leads nowhere. For the updated Reference list for this revised manuscript, we have now removed the URL

Line 187: "Among all specimens (n=323), 12 188 (3.7%) had positive calls for only one or two targets and were therefore called SARS CoV-2 IgG seronegative. Of these 12, one was positive only to NP, nine were positive to both RBD and NP, and two were positive to NP and the RBD/NP fusion."

Comment: Here we are back to a total of 323 samples. Are these 12 specimens included in the sensitivity and specificity calculations?

The authors apologize for this confusion in this section. Among all specimens tested (n=323), there were 12 with the profiles listed above. However, as this paragraph is discussing results only for specimens with corresponding RT-PCR results (n=312) this is the more appropriate denominator for consistency, and has been changed to read: " Among all specimens with corresponding RT-PCR results (n=312), 12 (3.8%) had positive calls for only one or two targets and were therefore called SARS-CoV-2 IgG seronegative. Of these 12, one was positive only to NP, nine were positive to both RBD and NP, and two were positive to NP and the RBD/NP fusion."

Line 208: "The MBA was compared against two commercially available SARS-CoV-2 209 serological assays..."

Comment/Question: Was the sample size 323,312 or 300?

For the comparison of results among these three serological assays, the sample size is provided on line 217: "Of the 323 specimens, ..."

Although the figures presenting the r squared data is interesting, the assays aren't truly quantitative, for which this type of analysis has more meaning.

The authors agree with the reviewer's assessment in that these test results are meant to be interpreted in a qualitative manner. We emphasize these qualitative findings throughout the manuscript, and even denote the positivity thresholds in Fig 1 to read more as a 2x2 table rather than only a scatterplot. The authors feel inclusion of these quantitative assay signals in Fig 1 provides further context to the concordance in results among these assays.

Lines 223-235. "For these 25 discrepant specimens..."

Are you determining a false positive or negative based on RT-PCR? Please state that to be clear. I find this paragraph a bit confusing. There are 25 discrepant results which include 18 falsely negative, one falsely positive on Abbot and 6 on MBA that are negative using IFU interpretation regardless of whether one or two positive antigens, but you are calling them falsely positive, which I don't understand. I really think a table might be helpful here or a clearer explanation of the discrepant results for each assay. For the MBA, I would consider using the overall result interpretation and leaving out the individual antigen results.

The authors thank the reviewer for the suggestions above and apologize for any confusion in the text. As our analyses here are for diagnostic sensitivity (and specificity), we are utilizing a previous positive RT-PCR test as the standard for CoV2 exposure, as we describe in Methods. So if a specimen was obtained pre-pandemic, or had the negative RT-PCR from these early 2020 samples, we are considering that a true negative for CoV2 exposure here. As the MBA includes three antigen targets, we feel it is important most importantly outline the overall test call, but also to mention the seropositivity calls for the individual targets as this would be a common issue among multiplex-based sero assays. We provide Supplemental Table 2 in order to provide full information for all of these 'discrepant' samples. We have revised the text at the beginning of this paragraph in attempt to make the results clearer: "Based on RT-PCR determination of SARS-CoV-2 exposure, for these 25 discrepant specimens, most (18, 72%) were falsely negative for SARS-CoV-2 IgG (type 2 error), one specimen (4.0%) was falsely positive by the Abbott assay (type 1 error), and six (33.3%) were falsely positive on

the Tetracore MBA for only one or two antigens, but the overall assay call was negative and therefore correct (Table S2)."

Line 256: "Against this serum panel of 323 specimens, the MBA demonstrated high diagnostic sensitivity (89.8%) and specificity (100.0%)."

Comment: There is an N of 300 for Table 2 data from which the sensitivity and specificity is calculated.

The authors thank the reviewer for pointing this out again. Per the previous comment, the N for Table 2 has been corrected (changed 300 to 312). A clarifier has been added to this sentence above to detail that only samples with RT-PCR results were used to estimate diagnostic sensitivity and specificity:

"Against this serum panel of 312 specimens with RT-PCR results, the MBA demonstrated high diagnostic sensitivity (89.8%) and specificity (100.0%)."

May 10, 2022

Dr. Eric Rogier
CDC
Atlanta

Re: Spectrum01054-22R1 (Evaluation of a multiplex bead assay against single-target assays for detection of IgG antibodies to SARS-CoV-2)

Dear Dr. Eric Rogier:

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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Sincerely,

Kileen Shier
Editor, Microbiology Spectrum

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Supplemental Material: Accept