Dear Mr. Schuit,

Thank you for sending us your paper. We sent it for external peer review and discussed it at our manuscript committee meeting. We recognise its potential importance and relevance to general medical readers, but I am afraid that we have not yet been able to reach a final decision on it because several important aspects of the work still need clarifying.

We hope very much that you will be willing and able to revise your paper as explained below in the report from the manuscript meeting, so that we will be in a better position to understand your study and decide whether the BMJ is the right journal for it. We are looking forward to reading the revised version and, we hope, reaching a decision.

Please remember that the author list and order were finalised upon initial submission, and reviewers and editors judged the paper in light of this information, particularly regarding any competing interests. If authors are later added to a paper this process is subverted. In that case, we reserve the right to rescind any previous decision or return the paper to the review process. Please also remember that we reserve the right to require formation of an authorship group when there are a large number of authors.

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**Report from The BMJ's manuscript committee meeting**

These comments are an attempt to summarise the discussions at the manuscript meeting. They are not an exact transcript.

Members of the committee were: Joseph Ross (chair), Julie Morris (statistician), Tim Feeney, Naz Islam, Elizabeth Loder, Tiago Villanueva, Di Wang

Decision: Put points

Detailed comments from the meeting:
First, please revise your paper to respond to all of the comments by the reviewers. Their reports are available at the end of this letter, below.

Please also respond to these additional comments by the committee:

Thank you for submitting your paper. We feel it is topical and interesting, however we do have some additional queries. Please make sure to address all questions and comments, and add or modify the text of the paper if necessary to make things clear for readers.

- The text says, “the analysis only included those with symptoms at time of sampling” but only 60% said they were testing due to symptoms. This seems inconsistent. Can the authors clarify this?

- Why did the authors have people go home and take the test instead of in the office?

- Can the authors comment on why sensitivity and specificity was different in different groups even though in theory they should be invariant to disease prevalence?

- We are interested to know more about the much higher sensitivity (~15%) in the confirmatory testers. Is it mainly attributed to the generally higher viral load, or the proficiency of the testers? If it’s the latter, are these assays ready for self application in general population?

- Is this a select group of patients? It seems those included here might have done more testing in the past and be more likely to obtain an adequate sample for testing. Is this still making these tests look better than they should?

- Please make sure the manuscript reports the exact time window of the study—-to us it is unclear.

- Can the authors include a box with information about where these tests are used and how commonly used they are?

- Please share how you will disseminate or share the evidence in addition to publication, blogs, specific groups, social media, opinions written with an end-user etc. This is mandatory for The BMJ, and should be placed in the endmatter.

- Do the authors think performing a self test after having just been tested by healthcare workers would have increased the quality of self testing since the user would have an example to go off of?

In your response please provide, point by point, your replies to the comments made by the reviewers and the editors, explaining how you have dealt with them in the paper.

Comments from Reviewers

Reviewer: 1

Comments:
The study performed by E Schuit et al. evaluate whether accuracies of Ag-RDTs with nasal self-sampling changed over time and to quantify whether addition of OP to nasal self test sampling provide better sensitivities
Authors used 3 different rapid tests, Flowflex, MPbio and Siemens Clinitest and were compared to the RT-PCR as reference standard test using the Cobas SARS-CoV-2 test on either Cobas 6800 or 8800 platform.
They used a viral load cut-off as a proxy of infectiousness of >= 5.2 log10 SARS-CoV-2 E gene-copies/mL, based on a previous study performed by the same team and published in the BMJ, which
indicate that under this level viral culture did not grow and they conclude that under this level the patient is not contagious.

Main remarks:
- Flowflex is a rapid test commercialized by Acon, a company based in San Diego, California, but linked with Chinese manufacturers. Siemens Clinitest is the not alone distributor of the Chinese Orient Gene test, which is available on other brands all around Europe. Acon and Siemens are only distributors, as SD Biosensor is a South Korean test and Roche did not manufacture the test they sell.
- The viral cut-off chosen do not meet the MDCG (Medical Device Coordination Group) which have defined the quality criteria of a rapid test based on Ct level. The viral load was determined according to standard curves to transform the Ct in quantified viral loads. In that case, before each PCR run a standardized quantified sample of 5.2 log10 copies/mL has to be done at each PCR series to be sure about the concordance of the CT level given by the RT-PCR corresponding to the quantified Viral load. It is not mentioned that it has been done in each center in the study.
- When a patient initiates a COVID infection, Ct level is high during the first or second day then decrease and then increase again over a week (less or more). When the Ct is over 30 for example, on one day, it could decrease on the day after and the patient considered by this hypothesis not contagious become contagious. The principle of a positive rapid test is to isolate the patient form other people to prevent the spread of the infection. Using this cut-off has no public health implication. The patient has really a SARS-CoV-2 and need to be isolated as it is not possible to forecast what will be the evolution of the viral load in the next days
- Bekliz et al. published a study on retrospective samples to evaluate the sensitivity of 7 rapid tests to detect delta and omicron. It was based on a limited number of samples but Abbott Panbio for example showed a decrease from 67.7% to 36.1%. In that study the Flowflex was quite good with a sensitivity of 91.2% for delta and 88.9% for omicron. In a study we have performed (not published) on SD biosensor, the sensitivity was as low as 15%.
- Using this cut-off has another limit: the specificity of the test will decrease at 90%, but recommendations for rapid test specificity need to be more than 99%

Minor remarks
- Participants who did not complete the RDT within 3 hours of their test-site visit were contacted with the request to perform the self test. How many of participants delayed to perform the rapid test. What were the range of the delay? If the rapid test was performed even 24 hours later, it is difficult to compare with a virus load measured 24h before as the VL will change form one day to another
- How the variant have been determined. Did mutation panel kit used for every patient and which mutation were included in the panel mutation kit. What it the Roche mutation panel kit?
- Page 10: sensitivities of all three Ag-RDT was 28.6%. I think that, according to the next number it should be 82.6%

Finally, in the context of omicron first generation RDT sensitivities decrease and second generation ultrasensitive tests are arriving on the market (Hotgene, Fosun, Intec, Autobio, Bioperfectus...). Using this cut off and to the traditional CT levels is confusing, and the study appears to make more a promotion of some distributors to help these companies to stay on the market. The cut-off increase artificially the sensitivity of each test and do not help to select the best rapid test in the present context

Additional Questions:
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Dear Editor,

First of all, thank you for inviting me to review this interesting paper, by Schuit et al., entitled “Accuracy of COVID-19 self-tests with unsupervised nasal or nasal plus oropharyngeal self-sampling in symptomatic individuals in the Omicron period”.

The authors aimed to assess the performances of rapid antigen diagnostic tests (Ag-RDTs) with nasal self-sampling, and oropharyngeal plus nasal (OP-N) self-sampling, which show useful results for researchers working in a similar field of work.

There are a few issues that need to be addressed including some further clarifications to enhance the overall clarity of the manuscript and potential impact on routine care.

TITLE:
- maybe better to mention that this is a diagnostic accuracy study (instead of prognostic accuracy for example)

WHAT THIS STUDY ADDS:
- Some of the percentages are shown with 1 decimal figure, others are not. Best to be consistent here.
- the statements are still very verbatim what the results show. It would be better to mention here what these results mean for clinical practice and further research in a few statements.

ABSTRACT:
- interventions: There is a different timeframe defined for the different test methods, how is this taken into account in the analyses and how comparable are these periods?
- Some of the percentages are shown with 1 decimal figure, others are not. Best to be consistent here.
- conclusions: please mention the clinical implications of your findings for current practice.

INTRODUCTION:
- line 121: suggest to add "diagnostic" to "accuracy"
- line 124: " yields higher diagnostic accuracies": please specify which diagnostic accuracies you are focussing on here (both sensitivity and specificity, or just sensitivity?). This is important to assess the clinical relevance of your findings.

METHODS:
- specimen collection and testing: the different tests were not performed simultaneously, thus a head-to-head comparison is possible. Furthermore, the order of tests was not randomised, so the presence of viral material over time could have influenced the results.
- the description of the methods for sampling demonstrates large heterogeneity in recruitment and sampling, which further complicates the analyses and the generalisability of these findings.
- apart from the comparison between sensitivities ("Sensitivities in the first and last week were compared by Chi-square tests"), I cannot find the methodology of the time-trend analyses mentioned in the study design-section.
- Please also specify the software used for your analyses.

RESULTS:
- the time-trend analysis description is a bit vague and it is difficult to assess its relevance.
- the heterogeneity inherent to the data is addressed by performing subgroup analyses, however further diluting the overall message.

DISCUSSION:
- The first section contains a lot of results, which are already shown earlier. Consider focusing on the main findings only to further set the stage of your discussion section.
- The heterogeneity is, in my view, not sufficiently highlighted in the discussion section. It would be useful for the readers to learn more about the potential impact of your choice of design/methods on the study results and how this could feed into future research.
- "We found trends towards lower sensitivities": not sure what is meant here, but unless you have shown a statistical significant trend, it might be better to avoid such statements.

Additional Questions:
<br>
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