Review 2: "Hitting the diagnostic sweet spot: Point-of-care SARS-CoV-2 salivary antigen testing with an off-the-shelf glucometer"

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

Review:

The authors developed a novel antigen assay for detecting SARS-CoV-2 virus. This review was divided into two parts. The first part was the review of principles and validation of the assay, the second part was the clinical application of the assay.

1. Principles and validation of the assay*1.1 Principles of the assay*

The assay was based on an aptamer-based competitive approach using a glucometer as a device to read the signals. Aptamer refers to the molecules that bind to a specific target molecule. The SARS-CoV-2 specific aptamer was utilized in this assay. This aptamer was conjugated to magnetic bead while the aptamer was also pre-hybridized with an oligonucleotide strand. This oligonucleotide strand was covalently linked to an invertase enzyme.

In the presence of SARS-CoV-2 antigen/protein, a complex was formed between the SARS-CoV-2 antigen/protein and the aptamer. The low affinity oligonucleotide strand will be released. This released strand will convert sucrose to glucose and the results will be read by the glucometer.

In the absence of SARS-CoV-2 antigen/protein, the aptamer remains hybridized with the oligonucleotide strand, sucrose will not convert to glucose. No glucose signals were read by the glucometer.

A series of experiments were performed to establish the assay and confirmed the above principles.

1.2 Validation of the assay

Two aptamers were evaluated, they were SARS-CoV-2 specific S protein aptamer and N protein aptamer.

The assay specificity was confirmed by testing against influenza A (H1N1) and MERS-CoV proteins. The signals for these two proteins were <300% less than the SARS-CoV-2 specific proteins.

The assay sensitivity was confirmed by testing seven saliva samples, three of them were SARS-CoV-2 patients confirmed by RT-PCR. Although the glucose signals for all of the seven samples were read by the glucometer, the signals from the three SARS-CoV-2 patients (positive signals) were higher than the four non-SARS-CoV-2 patients (noise signals). When comparing between S protein aptamer and N protein aptamer, the ratio between the two signals (positive and noise) were higher for the former one. It means that the S protein aptamer had a higher signal to noise ratio than the N protein aptamer.

The S protein aptamer was chosen for evaluating another set of 24 saliva samples which comprised 16 SARS-CoV-2 patients and 8 healthy individuals. When the cut-off 52 mg/dl was set, the assay was capable of distinguishing between positive and negative samples. Positive samples ranged 68-404 mg/dl while negative samples ranged 14-37 mg/dl.

2. Clinical application of the assay

2.1 Technical requirements

The aptamer-based assay required more technical steps when comparing with lateral flow antigen detection assays. The aptamer-based assay was not easy to execute. It is recommended that a pre-training course should be provided for individuals to perform the assay.

The turnaround time for the aptamer-based assay was 60 minutes which was longer than the lateral flow antigen detection assays. For lateral flow antigen detection assays, results were read by the operator within 10 to 30 minutes [1].

2.2. Sensitivity

In the 'Introduction' section, the authors mixed up the lateral flow antigen detection assays with lateral flow antibody detection assays. For the lateral flow antigen detection assays, data on the specificity were consistently reported to be high (>97%) while the sensitivity were highly variable between different studies and different brands [1].

In the 'Discussion' section, the authors compared the aptamer-based assay with other assays. The information for the test 'Respi-Strip (Coris Bio)' listed in 'Table 1' was incorrect. The turnaround time should be 15 minutes but not 30 minutes.

'Table 1' showed that the 'Respi-Strip (Coris Bio)' test shared similar limit of detection with the aptamer-based assay. Different studies evaluated the performance of the 'Respi-Strip (Coris Bio)' test

[2-5], the authors are recommended to compare the clinical sensitivity between the 'Respi-Strip (Coris Bio)' test and the aptamer-based assay.

'Table 1' showed that the sensitivity of the aptamer-based assay was 100%. Recently, numerous lateral flow antigen detection tests for diagnosing COVID-19 patients are available in the market and evaluated in different studies. Irrespective of the days after symptom onset, the clinical sensitivity of these tests ranged from 70.6% to 76.3% [6-8]. Some of them were placed in the list of the 'WHO Emergency Use Listing for In vitro diagnostics (IVDs) Detecting SARS-CoV-2' [9]. The authors are recommended to comment the clinical sensitivity between these tests with the aptamer-based assay.

2.3 Specificity

False positive issue has been reported for the antigen assay in Japan. This antigen assay is a quantitative test which required setting cutoff value to distinguish positive and negative cases. The dilemma for this test is the occurrence of false positive cases and false negative cases by adjusting the cutoff value. High cutoff value can prevent false positive cases, however, sensitivity is lower. Low cutoff value can increase sensitivity, however, false positive cases will be increased [10].

The aptamer-based assay was also a quantitative test and required setting a cutoff value. The authors are recommended to comment on this issue.

3. Conflicts of interest

None.

4. References

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