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Evaluation of the DiaSorin LIAISON SARS-CoV-2 antigen assay on nasopharyngeal swabs in two different SARS-CoV-2 pandemic waves in Switzerland: The impact of the Omicron variant on its performance

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ABSTRACT

Background: SARS-CoV-2 antigen tests reliably detect individuals with high viral loads and provide an efficient diagnostic tool to manage the current SARS-CoV-2 pandemic. However, mutations in SARS-CoV-2 variants of concern that appeared after validation of most antigen tests might impact their diagnostic performance.

Objectives: To assess the impact of the Omicron variant on the performance of the DiaSorin LIAISON SARS-CoV-2 antigen test, we evaluated its sensitivity and specificity on nasopharyngeal swabs (NPS) compared to rRT-PCR in the second and the Omicron pandemic wave in Switzerland.

Study design: A random selection of NPS from patients undergoing SARS-CoV-2 diagnostics by rRT-PCR were collected during the second and the Omicron pandemic wave and further analyzed by the LIAISON antigen test. Sensitivity and specificity compared to rRT-PCR were calculated.

Results: Test performance did not change in the two investigated periods. The overall sensitivity of 75.8% in the second and 76.5% in the Omicron wave increased to 87.1% and 88.4%, excluding samples with rRT-PCR Ct-value >30. By lowering the cut-off from 200 TCID₅₀/ml to 62 TCID₅₀/ml to discriminate between negative and positive samples using a ROC-curve, the sensitivity resulted in 88.8% for the second and 93.3% for the Omicron pandemic wave. The specificity of the LIAISON antigen test was 100% in both collectives.

Conclusion: Omicron variant does not seem to affect the performance of the LIAISON antigen test. The WHO recommended sensitivity of ≥80% for antigen testing was fulfilled during both pandemic periods in samples with Ct-value <30 or by optimizing the assay cut-off.

1. Background

In the current pandemic of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an early and accurate diagnosis is crucial to support medical decisions and help prevent the spread of infection. In SARS-CoV-2 diagnostics, the detection of SARS-CoV-2 specific RNA by real-time reverse transcription polymerase chain reaction (rRT-PCR) is considered the gold standard [1,2]. However, molecular-based methods are expensive, and the theoretically technical turnaround time of only a few hours often exceeds 24 h due to high sample volume and the laboratory capacity [3]. Furthermore, the method's high sensitivity might cause prolonged positivity, although the individuals are no longer considered infectious [4].

Compared to rRT-PCR, the less expensive SARS-CoV-2 antigen detecting assays, commercially available either as rapid or automated high-throughput tests, have a lower sensitivity. However, they have been shown to correlate with a high viral load and the ability to replicate in cell cultures, which seem to indicate contagiousness [4,5]. Therefore,

by rapidly and reliably identifying individuals with high viral load, antigen testing has been established to be an easy and efficient diagnostic tool for preventing the spread of infection. However, in published reports, diagnostic performance and reliability of the several antigen tests on the market may vary considerably due to different methodologies and test settings [6]. To address this issue, WHO has established criteria for diagnostic performance of SARS-CoV-2 antigen assays, which state that tests should have a minimum sensitivity of ≥80% and a minimum specificity of ≥97% [6].

The emergence of new mutations in SARS-CoV-2 variants of concern (VOCs), which potentially affect the detection of the target of most antigen tests - the nucleocapsid - added to the complexity of evaluations of these antigen detecting assays since most of them were validated before said emergence. Most notably, the newest variant Omicron, first reported in South Africa in November 2021, revealed, in addition to several mutations in the spike protein, new mutations in the nucleocapsid domain [7,8]. Furthermore, newly published data showed reduced analytical and clinical sensitivity of different SARS-CoV-2 commercial

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antigen tests in Omicron isolates and Omicron patient samples [9–11]. This is in contrast to previous VOCs that did not seem to affect the sensitivity of various SARS-CoV-2 antigen tests [12,13]. To our knowledge, no study has investigated the Omicron variant's impact on the diagnostic performance of the DiaSorin LIAISON SARS-CoV-2 antigen test, an automated quantitative chemiluminescence assay to detect the nucleocapsid of SARS-CoV-2, which enables rapid, large-scale and high-throughput SARS-CoV-2 diagnostics.

2. Objectives

This study aimed to assess the impact of the newly emerged Omicron variant on the diagnostic performance of the quantitative, automated DiaSorin LIAISON SARS-CoV-2 antigen test (LIAISON antigen test). For this purpose, we evaluated the sensitivity and specificity of the LIAISON antigen test on nasopharyngeal swabs in comparison to rRT-PCR in the second and the Omicron SARS-CoV-2 pandemic wave in Switzerland.

3. Study design

3.1. Periods of sample collection

Nasopharyngeal swabs (NPS) were first collected between October 4th, 2020, and February 17th, 2021. During this time, the second SARS-CoV-2 pandemic wave was ongoing in Switzerland. Therefore, we named this period "the second pandemic wave". The different circulating SARS-CoV-2 strains/variants at this time were characterized according to the federal SARS-CoV-2 sequencing surveillance data: In autumn 2020, pre-VOC strains (EU-strains and others) were gradually replaced by the first VOC, the Alpha variant (B.1.1.7), which appeared at the beginning of January 2021, and increased to 50–55% by mid-February [14,15]. The second period of sample collection spanned from January 23rd to March 2nd, 2022. During this period, here called "the Omicron pandemic wave", the VOC Omicron almost exclusively circulated in Switzerland with an abundance of 99–100% [14,15].

3.2. rRT-PCR and sample collection

NPS collected from patients undergoing SARS-CoV-2 diagnostics during the second and Omicron pandemic wave were first analyzed by SARS-CoV-2 rRT-PCR on the Cobas® 6800 system (Cobas® rRT-PCR) at the institute of infectious disease (IFIK) in Bern, Switzerland according to the manufacturer's instructions (Roche). For logistical reasons, it was not possible to further analyze rRT-PCR positive samples fresh with the LIAISON antigen test. Therefore, a random selection of samples tested positive by Cobas® rRT-PCR (leftover Viral Transport Medium >1 ml, storage at 4°C for a maximum of 48 h after collection, homogeneous representation of different E-gene Ct-values groups with Ct-values ranging from ct <20, ct 20–25, ct 25–30 to ct >30) were frozen at –80°C and included in the study to assess the sensitivity of the LIAISON antigen test in the two pandemic waves.

A minimal proportion of NPS collected from patients who needed urgent diagnostics (often a rule out diagnostics before ICU admission or urgent surgery) were analyzed at IFIK by SARS-CoV-2 rRT-PCR on the Roche's Liat system (Liat rRT-PCR) according to the manufacturer's instructions. Liat rRT-PCR provides results in only 20–30 min although it allows the analysis of a single sample at a time. NPS tested by Liat rRT-PCR, being promptly sent and tested by rRT-PCR were ideal to be analyzed by LIAISON antigen test freshly and within 12 h after their collection, as recommended by DiaSorin. Therefore, 47 NPS of the second wave and 18 NPS of the Omicron wave resulted negative by Liat rRT-PCR were used to assess the specificity of the LIAISON antigen test.

3.3. DiaSorin LIAISON SARS-CoV-2 antigen test

Frozen samples tested positive by Cobas® rRT-PCR were first thawed at room temperature. 1 ml of each specimen was then added to a tube

containing 1 ml inactivation buffer provided in the Kit, vortexed, and incubated for 120 min (virus inactivation). The inactivated samples were finally processed with the quantitative chemiluminescence LIAISON SARS-CoV-2 antigen test (LIAISON antigen test) on a LIAISON® XL Analyzer (DiaSorin). Negative SARS-CoV-2 Liat rRT-PCR samples were inactivated and analyzed by the LIAISON antigen test directly after the PCR result. Except for the use of frozen positive samples, which is not recommended by DiaSorin, LIAISON antigen tests were performed according to the manufacturer's instructions, including the use of a cut-off of 200 TCID₅₀/ml to discriminate between positive and negative samples.

3.4. Statistical analysis

Sensitivity and specificity were calculated with a 95%CI in relation to rRT-PCR using the software package MedCalc for Windows, version 20.008 (MedCalc Software, Ostend, Belgium). A ROC curve was plotted using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, CA, USA).

4. Results

The emergence of new mutations in VOCs might impact the diagnostic performance of SARS-CoV-2 antigen tests. In this line, this study focused on the newest VOC Omicron: sensitivity and specificity of the quantitative automated LIAISON antigen test were evaluated in patient samples from the second and the Omicron pandemic wave in Switzerland.

Based on the selection criteria, 161 rRT-PCR positive NPS collected during the second pandemic wave and 119 rRT-PCR positive NPS collected during the Omicron pandemic were further tested by the LIAISON antigen test. rRT-PCR positive NPS collected during the two pandemic waves were homogeneously represented and distributed in both collectives by their E-gene rRT-PCR Ct-values (considered an expression of viral load) (Suppl. Table 1).

4.1. Sensitivity and specificity of the LIAISON SARS-CoV-2 antigen test compared to rRT-PCR in the second and the Omicron SARS-CoV-2 pandemic wave

Out of the 161 positive Cobas® rRT-PCR samples collected during the second pandemic wave, 122 samples were tested positive and 39 samples negative by the LIAISON antigen test with an overall sensitivity of 75.8% [95%CI: 68.4–82.2%]. Out of the 119 positive Cobas® rRT-PCR samples collected during the Omicron pandemic wave, 91 samples were tested positive and 28 samples negative by the LIAISON antigen test with an overall sensitivity of 76.5% [95%CI: 67.8–83.8%]. The specificity of the LIAISON antigen test evaluated on negative Liat rRT-PCR samples (N=47 and N=18) was 100% in both groups (Table 1).

Table 1

Sensitivity and specificity of the LIAISON antigen test compared to rRT-PCR in NPS collected in the second and the Omicron SARS-CoV-2 pandemic wave in Switzerland.

Sensitivity	Second wave (N=161):	75.8% [95%CI: 68.4–82.2%]
	Omicron wave (N=119):	76.5% [95%CI: 67.8–83.8%]
Specificity	Second wave (N=47):	100% [95%CI: 92.5–100%]
	Omicron wave (N=18):	100% [95%CI: 81.5–100%]

Sensitivity and specificity are calculated using the DiaSorin recommended cut-off of 200 TCID₅₀/ml.

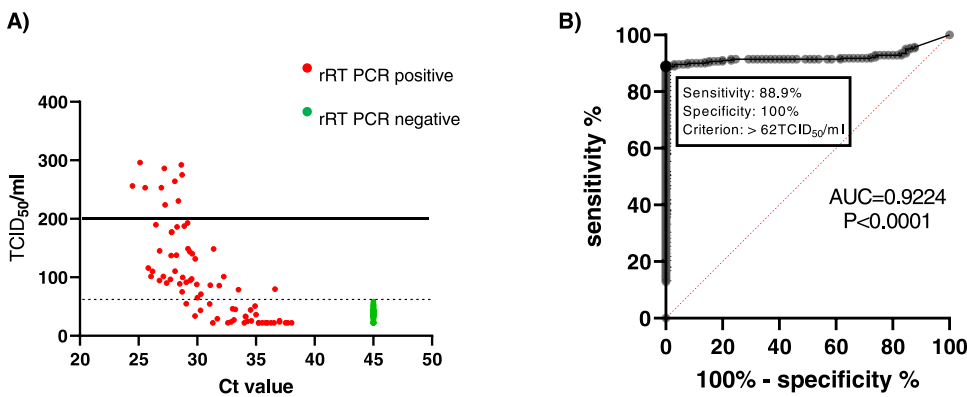


Fig. 1. A: Positive rRT-PCR results with TCID₅₀/ml <300 and negative rRT-PCR results from the whole study are plotted to their rRT-PCR Ct-values and shown in red and green, respectively. Ct-value of the rRT-PCR negative samples is arbitrarily set to 45. The straight line indicates the cut-off of 200 TCID₅₀/ml recommended by DiaSorin. The dotted line indicates the highest value in TCID₅₀/ml (58.33) of the rRT-PCR negative samples. B: ROC analysis for the LIAISON SARS-CoV-2 antigen test. Respective AUC is depicted.

Table 2

Sensitivity of the LIAISON antigen test compared to rRT-PCR according to different rRT-PCR E-gene Ct-value in NPS collected in the second and the Omicron SARS-CoV-2 pandemic wave in Switzerland.

Ct-value	SARS-CoV-2 Pandemic wave	Sensitivity [95%CI]
>30	Second wave (N=22)	4.5% [95%CI: 0.1–22.8%]
	Omicron wave (N=16)	0.0% [95%CI: 0–20.6%]
<30	Second wave (N=139)	87.1% [95%CI: 80.3–92.1%]
	Omicron wave (N=103)	88.4% [95%CI: 80.5–93.8%]
<28	Second wave (N=120)	95.0% [95%CI: 89.4–98.1%]
	Omicron wave (N=93)	93.5% [95%CI: 86.5–97.6%]
<25	Second wave (N=93)	100% [95%CI: 96.1–100%]
	Omicron wave (N=68)	100% [95%CI: 94.7–100%]

Sensitivity and specificity are calculated using the DiaSorin recommended cut-off of 200 TCID₅₀/ml.

4.2. Sensitivity of the LIAISON SARS-CoV-2 antigen test compared to rRT-PCR according to E-gene Ct-values in the second and the Omicron SARS-CoV-2 pandemic wave

The performance of antigen tests depends strongly on the viral load of the samples investigated. Therefore, we additionally calculated the sensitivity of the LIAISON antigen test according to different Ct-values for both pandemic waves. Excluding NPS with higher Ct-values increased sensitivity in both groups. Considering samples with Ct-value <30, the sensitivity of the LIAISON antigen test showed no difference in the two collectives, ranging from 87.1 [95%CI: 80.3–92.1] to 100% [95%CI: 96.1–100] in the second and from 88.4 [95%CI: 80.5–93.8] to 100.0% [95%CI: 94.7–100] in the Omicron pandemic wave, respectively. Only one sample with Ct-value >30 was positive by the LIAISON antigen test during the second pandemic wave, while all samples with Ct-value >30 resulted negative during the Omicron pandemic wave (Table 2).

4.3. Impact of TCID₅₀/ml cut-off on the sensitivity of the LIAISON SARS-CoV-2 antigen test in the second and the Omicron SARS-CoV-2 pandemic wave

Quantification of SARS-CoV-2 antigen by the LIAISON antigen test is provided by two calibrators and indicated in TCID₅₀/ml. DiaSorin recommends a 200 TCID₅₀/ml cut-off to discriminate between negative and positive samples. In our dataset, TCID₅₀/ml values of PCR negative samples from both groups were far below the recommended manufacturer's cut-off (Fig. 1A). Therefore, we calculated the sensitivity of the LIAISON antigen test compared to rRT-PCR in both pandemic waves using the cut-off value that provides optimal sensitivity without compromising 100% specificity based on a ROC curve calculation (Fig. 1B). With a cut-off of 62 TCID₅₀/ml, the sensitivity increased to 88.8% [95%CI: 82.9–93.2%] and 93.3% [95%CI: 87.2–97.1] in the second and the Omicron pandemic wave, respectively (Table 3).

Table 3

Sensitivity and specificity of the LIAISON antigen test compared to rRT-PCR by using a cut-off of 62 TCID₅₀/ml in NPS collected in the second and the Omicron SARS-CoV-2 pandemic wave in Switzerland.

Sensitivity using a cut-off of 62 TCID ₅₀ /ml	Second wave (N=161):	88.8% [95%CI: 82.9–93.2%]
	Omicron wave (N=119):	93.3% [95%CI: 87.2–97.1%]
Specificity using a cut-off of 62 TCID ₅₀ /ml	Second wave (N=47):	100% [95%CI: 92.5–100%]
	Omicron wave (N=18):	100% [95%CI: 81.5–100%]

5. Discussion

Although SARS-CoV-2 rRT-PCR is still considered the gold standard in the diagnostics of the current SARS-CoV-2 pandemic, SARS-CoV-2 antigen testing has become increasingly important as it provides an inexpensive, reliable, and time-efficient way to isolate infectious individuals. The emergence of VOCs, such as Omicron, led to further investigations of how new mutations in the target of most antigen tests, the nucleocapsid, might impact their diagnostic performance [10,16–18]. This could be the case for the DiaSorin LIAISON SARS-CoV-2 antigen test, an automated quantitative chemiluminescence assay that detects the SARS-CoV-2 nucleocapsid and enables rapid, large-scale, and high-throughput diagnostics.

Therefore, in the current study, we evaluated the LIAISON antigen test from DiaSorin in NPS collected during the second and Omicron SARS-CoV-2 pandemic wave to assess the impact of the Omicron mutations on its performance.

The overall sensitivities of the LIAISON antigen test for clinical samples collected during the second and the Omicron pandemic wave (75.8 vs. 76.5%) were very similar. Identical results were obtained by calculating the sensitivity in relation to Ct-value (87.1 vs. 88.4% for Ct-values <30; 95 vs. 93.5.0% for Ct-value <28 and 100% in both groups for Ct-values <25), which shows that circulating of VOC Omicron did not affect the sensitivity of the LIAISON antigen test. These data can be considered robust since samples with different Ct-values are homogeneously represented in the two collectives investigated. Our results on the LIAISON antigen test contrast with recently published data that showed a decreased sensitivity of the Roche rapid antigen test and other antigen rapid tests in Omicron virus isolates and Omicron patient samples compared to previously circulating virus variants [9–11]. These published results seem to be confirmed by a short evaluation of the Roche SARS-CoV-2 rapid antigen test we performed in a small subset of NPS collected in the same periods as the present study. Indeed, our data showed a reduction of sensitivity of the Roche SARS-CoV-2 rapid antigen test in the Omicron wave compared to the second pandemic wave (70.3% [95%CI: 53–84%] vs. 81.8% [95%CI: 67–92%]) (data not shown). As addressed by Osterman et al. [13], the observed discrepancy between the LIAISON and other antigen tests might be based on the polyclonal antibodies used in the LIAISON antigen assay, which assumes a more reliable binding to

multiple epitopes on the nucleocapsid protein, compared to monoclonal antibody assays.

Despite an excellent specificity of 100%, the overall sensitivity of the LIAISON antigen test did not fulfill, neither during the second pandemic wave nor during the Omicron pandemic wave, the performance criteria of WHO, requiring a sensitivity of at least 80% for antigen tests. However, considering only samples from patients with higher viral RNA (Ct <30), sensitivity increased from 75.8 to 87.1% in the second pandemic wave and from 76.5 to 88.4% in the Omicron pandemic wave. These results once again emphasize that the antigen test is particularly reliable in samples from patients with high viral load.

The sensitivity values reported in our study were calculated using the cut-off of 200 TCID₅₀/ml as recommended by DiaSorin to discriminate between negative and positive samples. However, since the LIAISON antigen test is a quantitative assay, some authors suggested the possibility of reducing the cut-off to improve the sensitivity [13,19,20]. Therefore, by using ROC analysis, we lowered the threshold of the LIAISON antigen test to 62 TCID₅₀/ml, which led to an increase in sensitivity up to 88.8% in the second wave and 93.3% in the Omicron wave without affecting specificity. Again, no difference was noted between the two periods considered.

One limitation of our study is the use of frozen rRT-PCR positive samples, which is not in accordance with the manufacturer's instructions. Compared to the analysis of freshly collected samples, the additional freeze-thaw cycle may have underestimated the assay's sensitivity. However, since the same procedure was uniformly used for both periods of sample collections, this did not affect the comparability of the considered pandemic waves addressed in our study.

A further limitation of our study derives from not having sequenced the samples included. This was due to the high financial and laboratory burden required for sequencing. Therefore, the viral characteristics of our collectives are based on Switzerland surveillance data for the corresponding periods. However, in the "Omicron wave," practically only Omicron variants were circulating in Switzerland (73% BA1/BA1.1 and up to 27% BA.2). Moreover, a retrospective analysis of the sequencing data of our institution's NGS team being part of the Swiss surveillance showed that 58 out of 119 samples of the Omicron wave collective had been sequenced, resulting in 100% Omicron genotype (86% BA.1/BA.1.1, 14% BA.2). When writing this paper, the BA.2 variant, still underrepresented in our study, replaced almost completely the BA.1/BA.1.1. It is unclear whether this increased circulation of the BA.2 variant, carrying an additional mutation in the nucleocapsid gene [7,8], will affect the performance of the LIAISON antigen test and other rapid tests. Further studies will be needed in this regard. No sample of the second pandemic wave included in the study was sequenced. Based on the Swiss surveillance data, this collective is presumably inhomogeneous, containing SARS-CoV-2 pre-VOC strains (EU-strain and others) as well as the first VOC, the Alpha variant (B.1.1.7) [14,15]. Nevertheless, various publications showed that the Alpha variant (B.1.1.7) did not impact the diagnostic of antigen tests in comparison to the previous circulating SARS-CoV-2 strains [12,13].

In summary, our data showed that, in contrast to recent data raising concerns about the performance of antigen tests in Omicron samples, mutations of Omicron do not seem to affect the sensitivity of the automated quantitative DiaSorin LIAISON SARS-CoV-2 antigen test. WHO criterion of a sensitivity $\geq 80\%$ was fulfilled in both periods of pandemic considered by optimizing the assay cut-off or when patient samples with low viral load were excluded. Our data highlight once again the importance of using antigen tests primarily in individuals expected to have a high viral load.

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Declaration of Competing Interest

The authors have no conflict of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2022.100095.

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