Sensitivity of SARS-CoV-2 antigen-detecting rapid tests for Omicron variant

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Abstract

The emergence of novel SARS-CoV-2 variants of concern (VOCs) requires investigation of a potential impact on diagnostic performance, such as Antigen-detecting rapid diagnostic tests (Ag-RDT). Although anecdotal reports have been circulating that the newly emerged Omicron variant is in principle detectable by Ag-RDTs, few data on sensitivity are available. Here, we have performed an analytical sensitivity testing with cultured virus in seven Ag-RDTs for Omicron compared to data earlier obtained on VOCs Alpha, Beta, Gamma and Delta and a pre-VOC isolate of SARS-CoV-2. Overall, we have found a tendency towards lower sensitivity for Omicron compared to pre-VOC SARS-CoV-2 and the other VOCs across tests. In addition, we have retrospectively tested original nasopharyngeal specimens from Omicron-infected individuals on five Ag-RDTs, with considerable failure to detect specimens with high viral load and infectious virus presence in the majority of tests. Importantly, while analytical and retrospective testing with cultured virus may be a proxy for clinical sensitivity, is not a replacement for clinical evaluations which are urgently needed for Ag-RDT performance at the point of care in Omicron-infected individuals.

Main text

The emergence of novel SARS-CoV-2 variants of concern (VOCs) requires investigation of a potential impact on diagnostic performance. SARS-CoV-2 antigen-detecting rapid diagnostic tests (Ag-RDT) offer quick, cheap and laboratory-independent results at the point of care.[1] Although sensitivity is lower compared to RT-PCR, they enable reliable detection of high viral loads associated with infectious virus presence, making them important public health tools.[2,3] However, the majority of Ag-RDT validation studies were performed prior to the emergence of SARS-CoV-2 variants of concern (VOC).[4]

The VOC Omicron was first reported at the end of November from South Africa and is characterized by a high number of mutations compared to earlier circulating SARS-CoV-2.[5] The majority of mutations are located in the Spike protein, that are, according to preliminary data, associated with considerable escape from neutralization by both disease- and vaccine derived antibodies, and probably also associated lower vaccine effectiveness.[6,7,8,9,10] Current epidemiological data show that Omicron circulation is associated with a steep increase in case numbers as well as an increased risk of reinfections.[11]

Beyond the Spike mutations, Omicron has also mutations in the nucleocapsid, which is the target of almost all Ag-RDTs. Two mutations found in Omicron are R203K and G204R that have been described already before Omicron in some SARS-CoV-2 sequences, were linked to increased sub-genomic RNA and increased viral loads.[12-14] In addition, a deletion (Del31-33) is found in the nucleocapsid of Omicron, as well as another mutation P13L, which is present in some, but not all Omicron sequences. No information on a potential impact of these mutations on Ag-RDTs performance is available so far. Anecdotal reports were circulating on positive detection of Omicron-confirmed patient samples by Ag-RDTs but experimental data on Ag-RDT sensitivity for Omicron are missing.

Here, we have evaluated test analytical sensitivity using cultured SARS-CoV-2 Omicron variant, in comparison with earlier data on isolates of the other VOCs (Alpha, Beta, Gamma and Delta) and an early-pandemic (pre-VOC) SARS-CoV-2 isolate (B.1.610) in seven Ag-RDTs, three of them WHO-EUL approved.[15-17] All viruses were isolated from clinical samples. Isolates were grown in Vero-E6 cells as described previously.[16] The Omicron variant which was initially isolated on Vero-TMPRSS cells, then further passaged with a stock passage (p2) prepared on VeroE6. Vero TMPRSS were kindly received from National Institute for Biological Standards and Controls (NIBSC, Cat. Nr. 100978). The following mutations and deletion in the nucleocapsid were present in the original patients' sequence as well as in the virus isolate of the passage used in this study: R203K, G204R, P13L, Del31-33. The starting dilution of infectious titers for all viruses used in this study was 4.24 $\log_{10}$ PFU/mL.

Seven Ag-RDTs were used: I) Panbio COVID-19 Ag Rapid test device (Abbott); II) Standard Q COVID-19 Ag (SD Biosensor/Roche); III) Sure Status (Premier Medical Corporation), the three latter being WHO-EUL approved and thus of high global public health relevance,[18] IV) 2019-nCoV Antigen test (Wondfo); V) Beijng Tigsun Diagnostics Co. Ltd (Tigsun); VI) Onsite COVID-19 Ag Rapid Test (CTK Biotech); VII) ACON biotech (Flowflex), several of them being on the waiting list for WHO-EUL approval.

All Ag-RDT assays were performed according to the manufacturers' instructions with the exception that 5 µL of virus dilution was directly added to the proprietary buffer, and then applied to the Ag-RDT in duplicates under BSL3 conditions.[17] Ag-RDT buffer without virus was used as a negative control. Any visible test band in the presence of a visible control band was considered as positive.

When assessing by infectious virus titers (PFU/mL) (**Fig 1A**), analytical sensitivity to detect Omicron was lower than for the other VOCs in most of the tests evaluated. One test, Flowflex

(ACON biotech) showed the highest overall sensitivity for all SARS-CoV-2 isolates used compared to the others, and here, Omicron was detected with even slightly higher sensitivity than Delta but still lower than Alpha, Beta, Gamma and pre-VOC SARS-CoV-2. When assessing by Ct values as a proxy for viral RNA copies, sensitivity for Omicron was at least comparable to Delta. Here, other VOCs, such as Alpha or Gamma were detected with the lowest sensitivity (**Fig. 1B**).

Differences in the assessment between PFU and Ct values are most likely due to different ratios between infectious particles and RNA copies for each variant. However, since the main public health benefit of Ag-RDTs are the detection individuals with infectious virus shedding and not just presence of viral RNA, assessment of infectious viral particles is of higher relevance in this context.

Of note, while in the analysis for infectious virus, the previous VOCs Alpha, Beta, Gamma and Delta were mainly detected with comparable or even higher sensitivity compared to pre-VOC SARS-CoV-2, here Omicron is the first VOC which showed a tendency towards lower analytical sensitivity across assays. However, we have also observed considerable heterogeneity in sensitivity patterns across variants and between individual assays in this analytical testing using cultured virus.

In addition, we have tested five Ag-RDTs with original patient specimens as a retrospective sensitivity study with 10 nasopharyngeal specimens of confirmed Omicron patients collected in viral transport medium during the first 5 days post-symptom onset (**Table 1**). All specimens had one freeze-thaw cycle and were in parallel assessed for presence of infectious virus by inoculation on Vero-TMPRSS cells. Of note, all specimens available for this testing were collected from vaccinated individuals experiencing an Omicron break-through infection.

Here, we have seen considerable failure in four tests to detect patient specimens with Ct values ranging between 19.4 and 22.6 in the early symptomatic period (day 0-3 days post symptom onset) and with presence of infectious virus. Three samples with Ct values ranging between 22.6 and 25.9 did not yield any virus isolate in cell culture, and none of these samples yielded a positive result in any test. Of note, one test, 2019-nCoV Antigen test (Wondfo) correctly showed a positive result in all samples with infectious virus in contrast to the other tests where failure of several specimens with infectious virus was observed. On the contrary, the same test showed rather low sensitivity compared to other tests when assessing cultured virus (**Fig. 1A**).

Differences in analytical sensitivity between Ag-RDTs might be explained by the different epitopes used in each test, potentially affected by the mutations in the nucleocapsid, but could also be related to other properties of Omicron, such as the ratio of infectious virus particles/viral protein or infectious virus particles/viral RNA. If the lower sensitivity towards Omicron that we observed here is confirmed by findings from clinical validations at the point of care, the use of Ag-RDTs in the early symptomatic period of an Omicron infection or in asymptomatic patients could be less reliable, with important implications for public health measures.

Importantly, while analytical and retrospective testing may be a proxy for clinical sensitivity, is not a replacement for clinical evaluations at the point of case and has several limitations, e.g. infectious virus, viral protein and RNA copies might differ considerably between patient specimens and cultured virus isolates. Furthermore, since many Omicron infections are currently observed in vaccinated individuals, it remains unclear if virus shedding and test performance differs between unvaccinated and vaccinated individuals. To date, most validation studies of Ag-RDTs were done in the first year of the pandemic, before circulation of VOC and in mostly immune-naïve individuals experiencing their primary SARS-CoC-2 infection. Other factors, such as *in vivo* shedding of infectious virus and overall viral loads of Omicron could further influence clinical test performance. The discrepancies in our results between

testing with cultured virus and retrospective patient samples highlights the need for proper clinical studies in well-defined patient cohorts. Therefore, further studies on diagnostic accuracy of Ag-RDTs performed at the point of care for the newly emerged VOC Omicron are urgently needed to guide public health responses.

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Conflicts of Interest

The authors declare no competing interests.

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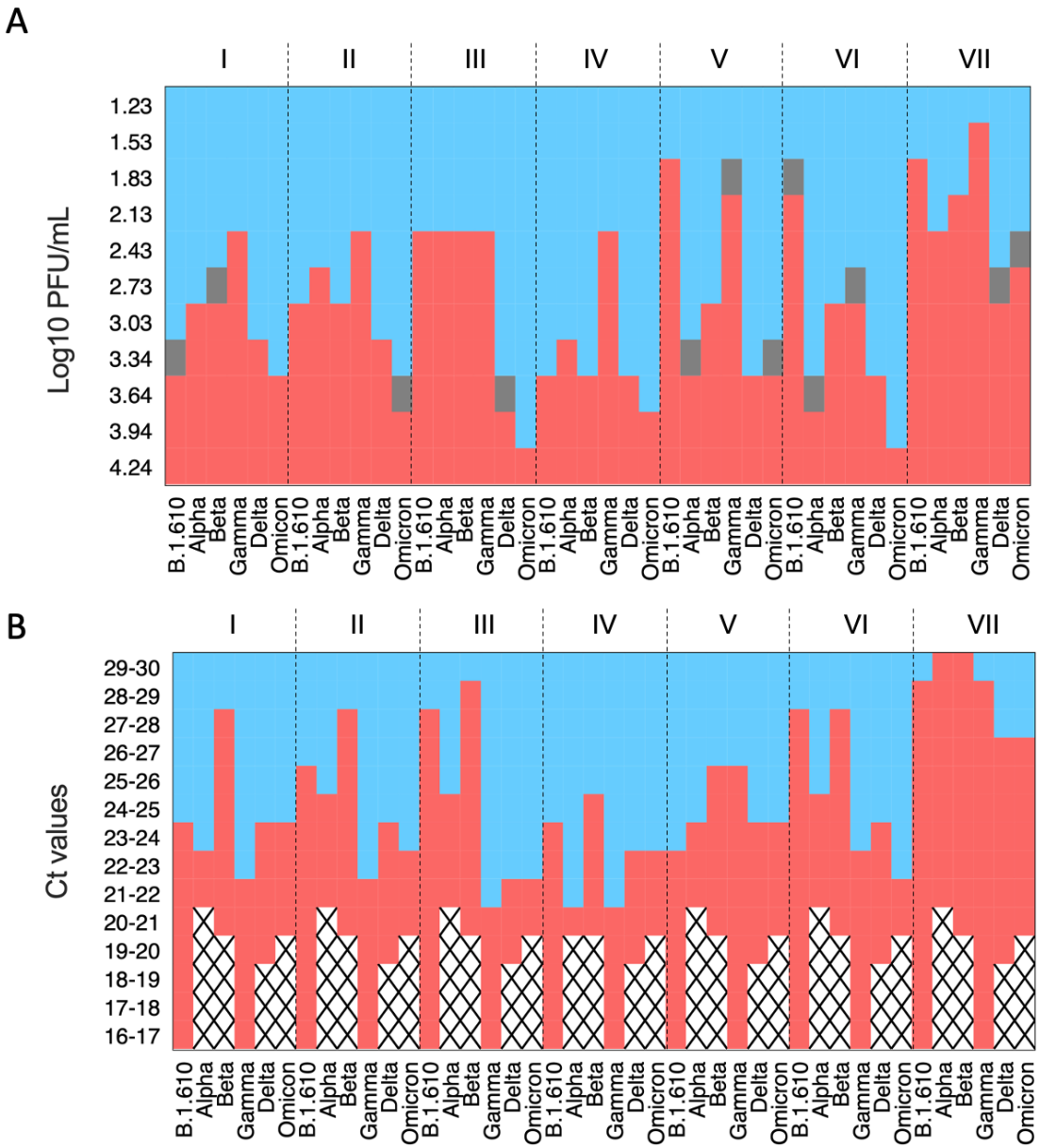
Figure 1



Heat map based on Log_{10} PFU/mL (**Fig 1A**) and on Ct values (**Fig 1B**) for analytical sensitivity of seven Ag-RDTs assays with an early-pandemic SARS-CoV-2 isolate (B.1.610), the VOCs Alpha, Beta, Gamma and Delta in comparison Omicron.

Ag-RDTs used: I) Panbio COVID-19 Ag Rapid test device (Abbott); II) Standard Q COVID-19 Ag (SD Biosensor/Roche); III) Sure Status (Premier Medical Corporation); IV) 2019-nCoV

202 Antigen test (Wondfo); V) Beijng Tigsun Diagnostics Co. Ltd (Tigsun); VI) Onsite COVID-
203 19 Ag Rapid Test (CTK Biotech); VII) Flowflex (ACON Biotech).

204 Analytical sensitivity for early-pandemic SARS-CoV-2 B.1.610, Alpha, Beta, Gamma and
205 Delta have already been published before but were added here for consistency reasons and
206 better interpretability of the data on Omicron.[15,16]

				Panbio COVID-19 Ag Rapid test device (Abbott)		Standard Q COVID-19 Ag (SD Biosensor/Roche)		Sure Status (Premier Medical Corporation)		Onsite COVID-19 Ag Rapid Test (CTK Biotech)		2019-nCoV Antigen test (Wondfo)	
Sample ID	**CT value**	**Infectious virus**	**DPOS**	A	B	A	B	A	B	A	B	A	B
1	19.4	+	0	-	-	+	-	-	-	+	-	+	+
2	19.6	+	2	+	+	+	+	+	+	+	+	+	+
3	20.1	+	2	+	+	-	+	+	-	+	+	+	+
4	21.3	+	2	-	-	-	-	-	-	-	-	+	+
5	21.6	+	3	+	-	-	-	-	-	-	-	+	+
6	22.2	+	3	+	+	-	-	-	+	+	+	+	+
7	22.6	+	2	-	+	-	-	-	-	+	+	+	+
8	22.7	-	5	-	-	-	-	-	-	-	-	-	-
9	24.5	-	4	-	-	-	-	-	-	-	-	-	-
10	25.9	-	1	-	-	-	-	-	-	-	-	-	-

Table 1. Retrospective testing of nasopharyngeal patient specimens with confirmed Omicron infection. Ct values refer to E-Gene assay (Cobas 6800, Roche) of the initial diagnostic RT-PCR. DPOS; days post symptom onset. Infectious virus presence was assessed by inoculating original patient specimens on Vero-TMPRSS cells. All tests were performed in duplicates (A/B) by adding 5 µL of original viral transport medium to the proprietary buffer, then as per manufacturers recommendation. Any visible test band in the presence of a visible control band was considered as positive.