Analytical sensitivity of seven SARS-CoV-2 antigen-detecting rapid tests for Omicron variant

Meriem Bekliz[1], Kenneth Adea[1], Catia Alvarez[1], Manel Essaidi-Laziosi[1], Camille Escadafal[2], Laurent Kaiser[3,4,5], Isabella Eckerle[1,3,5*]

[1]Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland.

[2]FIND, Geneva, Switzerland

[3]Division of Infectious Diseases, Geneva University Hospitals, 1205 Geneva, Switzerland.

[4]Laboratory of Virology, Division of Infectious Diseases and Division of Laboratory Medicine, University Hospitals of Geneva & Faculty of Medicine, University of Geneva, 1205 Geneva, Switzerland.

[5]Geneva Centre for Emerging Viral Diseases, University Hospitals Geneva, and University of Geneva, Switzerland.

[*]Corresponding author: Email: Isabella.Eckerle@hcuge.ch

Keywords

SARS-CoV-2; COVID19; Antigen-detecting rapid diagnostic tests; variants of concern; Omicron variant

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 Omicron is in principle detectable by Ag-RDTs, no published data are a yet available for the newly emerged Omicron variant. Here, we have performed an analytical sensitivity testing with cultured virus in seven Ag-RDTs for their sensitivity to 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. Importantly, while analytical 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 in Omicron-infected individuals.

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 no published data on Ag-RDT sensitivity for Omicron is available so far.

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 1**), 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.

Of note, while in this analysis 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.

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. If the lower sensitivity towards Omicron that we observed here is confirmed by findings from clinical validations, 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 testing with cultured virus may be a proxy for clinical sensitivity, is not a replacement for clinical evaluations and has several limitations, e.g. ratio between infectious virus, viral protein and RNA copies might differ between patient specimens and cultured virus isolates. In addition, other factors, such as *in vivo* shedding of infectious virus and overall viral loads could further influence clinical test performance. Therefore, further studies on diagnostic accuracy of Ag-RDTs for the newly emerged VOC Omicron are urgently needed to guide public health responses.

Funding

This work was supported by the Swiss National Science Foundation (grant number 196383), the Fondation Ancrage Bienfaisance du Groupe Pictet, and FIND, the global alliance for diagnostics. The Swiss National Science Foundation and the Fondation Ancrage Bienfaisance du Groupe Pictet had no role in data collection, analysis, or interpretation. Antigen rapid diagnostic tests were provided by FIND and FIND was involved in methodology, data analysis and interpretation. CE is an employee of FIND.

Acknowledgments

We thank Silvio Steiner, Jenna Kelly and Volker Thiel for sequencing of the Omicron isolate.

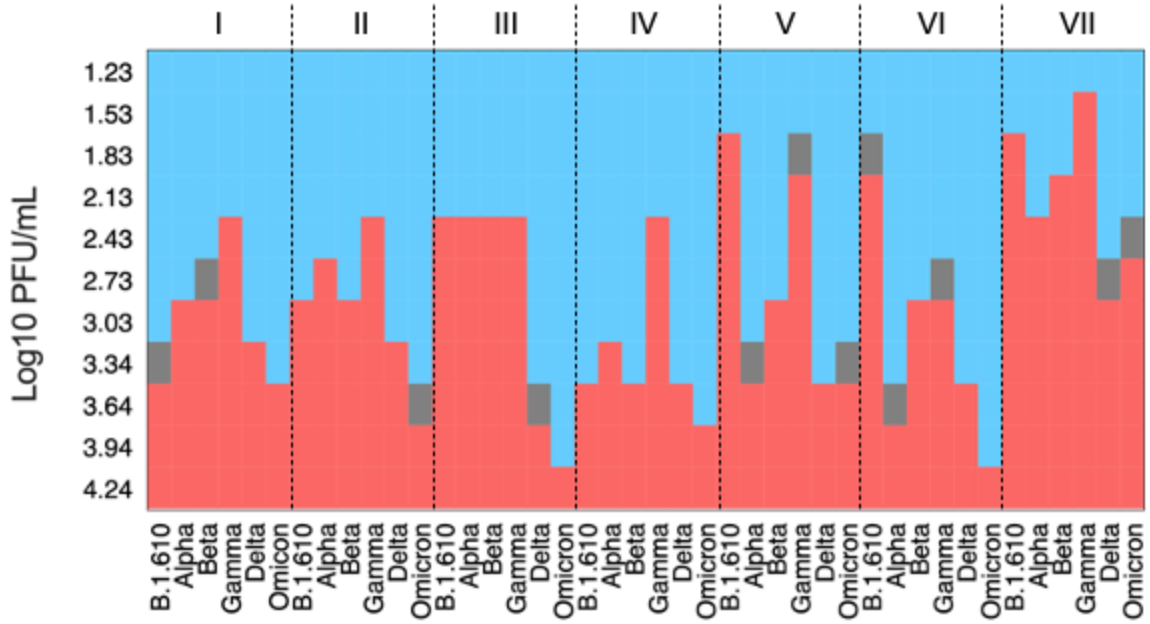
Conflicts of Interest

The authors declare no competing interests.

References

1. Nordgren J, Sharma S, Olsson H, et al. SARS-CoV-2 rapid antigen test: High sensitivity to detect infectious virus. *J Clin Virol* 2021; **140**: 104846.
2. World Health Organization (WHO) Antigen-Detection in the Diagnosis of SARS-CoV-2 Infection Using Rapid Immunoassays. [(accessed on 28 April 2021)];Available online: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays.
3. Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 Test Sensitivity - A Strategy for Containment. *N Engl J Med* 2020; **383**(22): e120.
4. WHO Weekly epidemiological update on COVID-19. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---10-august-2021 Date: August, 10 2021.
5. World Health Organization. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/ (accessed 08.12.2021).
6. Wilhelm A, Widera M, Grikscheit K, et al. Reduced Neutralization of SARS-CoV-2 Omicron Variant by Vaccine Sera and monoclonal antibodies. *medRxiv* 2021: 2021.12.07.21267432.
7. Cele S, Jackson L, Khan K, et al. SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection. *medRxiv* 2021: 2021.12.08.21267417.
8. Rössler A, Riepler L, Bante D, Laer Dv, Kimpel J. SARS-CoV-2 B.1.1.529 variant (Omicron) evades neutralization by sera from vaccinated and convalescent individuals. *medRxiv* 2021: 2021.12.08.21267491.
9. Dejnirattisai W, Shaw RH, Supasa P, et al. Reduced neutralisation of SARS-COV-2 Omicron-B.1.1.529 variant by post-immunisation serum. *medRxiv* 2021: 2021.12.10.21267534.
10. Gardner BJ, Kilpatrick AM. Estimates of reduced vaccine effectiveness against hospitalization, infection, transmission and symptomatic disease of a new SARS-CoV-2 variant, Omicron (B.1.1.529), using neutralizing antibody titers. *medRxiv* 2021: 2021.12.10.21267594.
11. Pulliam JRC, van Schalkwyk C, Govender N, et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. *medRxiv* 2021: 2021.11.11.21266068.
12. Covariants. 2021. https://covariants.org/per-country (accessed 09.12.2021.
13. Leary S, Gaudieri S, Parker MD, et al. Generation of a novel SARS-CoV-2 sub-genomic RNA due to the R203K/G204R variant in nucleocapsid. *bioRxiv* 2021: 2020.04.10.029454.
14. Mourier T, Shuaib M, Hala S, et al. Saudi Arabian SARS-CoV-2 genomes implicate a mutant Nucleocapsid protein in modulating host interactions and increased viral load in COVID-19 patients. *medRxiv* 2021: 2021.05.06.21256706.
15. FIND. 2021. https://www.finddx.org/sarscov2-eval-antigen/ (accessed 17.12.2021.
16. Bekliz M, Adea K, Essaidi-Laziosi M, et al. SARS-CoV-2 antigen-detecting rapid tests for the delta variant. *Lancet Microbe* 2021.
17. Bekliz M, Adea K, Essaidi-Laziosi M, et al. SARS-CoV-2 rapid diagnostic tests for emerging variants. *Lancet Microbe* 2021; **2**(8): e351.
18. World Health Organization. 2021. https://extranet.who.int/pqweb/sites/default/files/documents/211125_EUL_SARS-CoV-2_products_list.pdf.

Figure 1



Heat map based on Log_{10} PFU/mL 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 Antigen test (Wondfo); V) Beijng Tigsun Diagnostics Co. Ltd (Tigsun); VI) Onsite COVID-19 Ag Rapid Test (CTK Biotech); VII) Flowflex (ACON Biotech).

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