



## 22 **Abstract**

23 The emergence of novel SARS-CoV-2 variants of concern (VOCs) requires investigation of a  
24 potential impact on diagnostic performance, such as Antigen-detecting rapid diagnostic tests  
25 (Ag-RDT). Although anecdotal reports have been circulating that Omicron is in principle  
26 detectable by Ag-RDTs, no published data are yet available for the newly emerged Omicron  
27 variant. Here, we have performed an analytical sensitivity testing with cultured virus in seven  
28 Ag-RDTs for their sensitivity to Omicron compared to data earlier obtained on VOCs Alpha,  
29 Beta, Gamma and Delta and a pre-VOC isolate of SARS-CoV-2. Overall, we have found a  
30 tendency towards lower sensitivity for Omicron compared to pre-VOC SARS-CoV-2 and the  
31 other VOCs across tests. Importantly, while analytical testing with cultured virus may be a  
32 proxy for clinical sensitivity, is not a replacement for clinical evaluations which are urgently  
33 needed for Ag-RDT performance in Omicron-infected individuals.

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

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

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

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

68 Seven Ag-RDTs were used: I) Panbio COVID-19 Ag Rapid test device (Abbott); II) Standard  
69 Q COVID-19 Ag (SD Biosensor/Roche); III) Sure Status (Premier Medical Corporation), the  
70 three latter being WHO-EUL approved and thus of high global public health relevance,<sup>18</sup> IV)  
71 2019-nCoV Antigen test (Wondfo); V) Beijing Tigsun Diagnostics Co. Ltd (Tigsun); VI) Onsite  
72 COVID-19 Ag Rapid Test (CTK Biotech); VII) ACON biotech (Flowflex), several of them  
73 being on the waiting list for WHO-EUL approval.

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

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

81 (ACON biotech) showed the highest overall sensitivity for all SARS-CoV-2 isolates used  
82 compared to the others, and here, Omicron was detected with even slightly higher sensitivity  
83 than Delta but still lower than Alpha, Beta, Gamma and pre-VOC SARS-CoV-2.

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

89 Differences in analytical sensitivity between Ag-RDTs might be explained by the different  
90 epitopes used in each test, potentially affected by the mutations in the nucleocapsid. If the lower  
91 sensitivity towards Omicron that we observed here is confirmed by findings from clinical  
92 validations, the use of Ag-RDTs in the early symptomatic period of an Omicron infection or in  
93 asymptomatic patients could be less reliable, with important implications for public health  
94 measures.

95 Importantly, while analytical testing with cultured virus may be a proxy for clinical sensitivity,  
96 is not a replacement for clinical evaluations and has several limitations, e.g. ratio between  
97 infectious virus, viral protein and RNA copies might differ between patient specimens and  
98 cultured virus isolates. In addition, other factors, such as *in vivo* shedding of infectious virus  
99 and overall viral loads could further influence clinical test performance. Therefore, further  
100 studies on diagnostic accuracy of Ag-RDTs for the newly emerged VOC Omicron are urgently  
101 needed to guide public health responses.

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109

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112

113 **Conflicts of Interest**

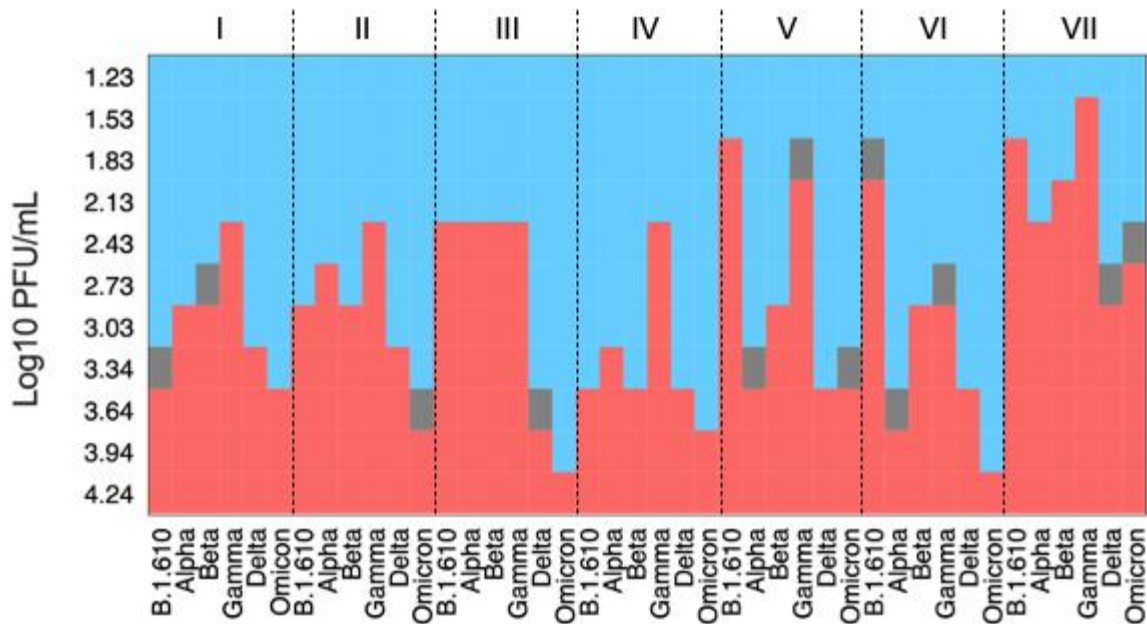
114 The authors declare no competing interests.

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158 [2\\_products\\_list.pdf](https://extranet.who.int/pqweb/sites/default/files/documents/211125_EUL_SARS-CoV-2_products_list.pdf).

159

160 **Figure 1**



161

162 Heat map based on Log<sub>10</sub> PFU/mL for analytical sensitivity of seven Ag-RDTs assays with an  
 163 early-pandemic SARS-CoV-2 isolate (B.1.610), the VOCs Alpha, Beta, Gamma and Delta in  
 164 comparison Omicron.

165 Ag-RDTs used: I) Panbio COVID-19 Ag Rapid test device (Abbott); II) Standard Q COVID-  
 166 19 Ag (SD Biosensor/Roche); III) Sure Status (Premier Medical Corporation); IV) 2019-nCoV  
 167 Antigen test (Wondfo); V) Beijing Tigsun Diagnostics Co. Ltd (Tigsun); VI) Onsite COVID-  
 168 19 Ag Rapid Test (CTK Biotech); VII) Flowflex (ACON Biotech).

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