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Journal of Infection and Chemotherapy xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: www.elsevier.com/locate/jic

Original Article

Analytical sensitivity of six lateral flow antigen test kits for variant strains of SARS-CoV-2

Yoshitomo Morinaga [a,c,*], Hiroshi Yamada [a], Yoshihiro Yoshida [a], Hitoshi Kawasuji [b], Yoshihiro Yamamoto [b,c]

[a] Department of Microbiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan
[b] Department of Clinical Infectious Diseases, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan
[c] Clinical and Research Center for Infectious Diseases, Toyama University Hospital, Toyama, Japan

ARTICLE INFO

Keywords:
Omicron
Antigen tests
Lateral flow tests
Immunochromatography
Point-of-care testing

ABSTRACT

Introduction: The lateral flow antigen test is a useful tool for rapid diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The analytical sensitivity of six lateral flow antigen test kits was compared.

Methods: The limit of detection (LoD) and time to positive results were evaluated for six lateral flow tests including ImmunoArrow®, ESPLINE® SARS-CoV-2, QuickNavi™ COVID19 Ag, ImmunoAce® SARS-CoV-2, Panbio™ COVID-19 Ag Rapid Test Device, and SARS-CoV-2 Rapid Antigen Test using the heat-inactivated virus. The LoD of ImmunoArrow® against the Omicron variants was compared with that against the wild-type using recombinant proteins.

Results: ImmunoArrow® and ESPLINE® showed the lowest LoD. The time to positive results of all tests except for ESPLINE® was within 200 s in the evaluation at high dose of antigens (2.5×10^5 $TCID_{50}$/mL) and 500 s in the evaluation at low dose of antigens (2.5×10^4 $TCID_{50}$/mL). The LoD of ImmunoArrow® against the Omicron variants was the same concentration against the wild-type antigen.

Conclusions: ImmunoArrow® detected SARS-CoV-2 antigens including the Omicron variants with good sensitivity among the six lateral flow antigen tests. These finding support that it can support the rapid diagnosis of COVID-19 with the good sensitivity.

1. Introduction

The emergence of the coronavirus disease 2019 (COVID-19) pandemic has impacted our health and social activities. The lateral flow test is based on the immunochromatography and used as a method to detect antigens and antibodies for diagnosis of a variety of infectious diseases. Because they are convenient and cost-saving, it is also used for the rapid diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [1,2].

Most SARS-CoV-2 antigen tests detects the viral nucleocapsid protein [3]. In contrast to the spike protein in which variants have amino acid alterations, it is known that the sequence of the nucleocapsid protein is relatively stable even in variants [4,5]. However, it is also true that there is slightly different in the amino acid sequence between variants. Therefore, it is important to compare the performance of tests between wild type and variants.

ImmunoArrow® SARS-CoV-2 (ImmunoArrow®) is a lateral flow antigen test which was authorized as an in vitro diagnostic in 2021 in Japan. Here, we report the performance of this test using heat-inactivated virus with comparing 5 lateral flow antigen tests. In addition, in response to the appearance of the Omicron variant, the limit of detection (LoD) of ImmunoArrow® against this variant was evaluated using recombinant proteins.

2. Materials and methods

2.1. Evaluation of the LoD

ImmunoArrow® SARS-CoV-2 (ImmunoArrow®, TOYOBO Co., Ltd., Osaka, Japan) was provided by TOYOBO Co. Ltd. In addition to

* Corresponding author. Department of Microbiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama, 930-0194, Japan.
E-mail address: morinaga@med.u-toyama.ac.jp (Y. Morinaga).

https://doi.org/10.1016/j.jiac.2022.10.004
Received 2 August 2022; Received in revised form 9 September 2022; Accepted 3 October 2022
Available online 17 October 2022

ImmunoArrow®, ESPLINE® SARS-CoV-2 (ESPLINE®, Lot K4B1I020, Fujirebio Inc., Tokyo, Japan), QuickNavi™-COVID19 Ag (QuickNavi™, Lot 1041081, Denka Co., Ltd., Tokyo, Japan), ImmunoAce® SARS-CoV-2 (ImmunoAce®, Lot 191211098, TAUNS Laboratories, Inc., Izunokuni, Japan), Panbio™ COVID-19 Ag Rapid Test Device (Panbio™, Lot 41ADG542A, Abbott Diagnostics Medical Co., Ltd., Tokyo, Japan), and SARS-CoV-2 Rapid Antigen Test (Rapid Antigen Test, Lot QC0391058I, Roche Diagnostics K.K., Tokyo, Japan), were evaluated for their LoDs. To prepare the testing solution, heat-inactivated wild-type SARS-CoV-2 (USA-WA1/2020) (ZeptoMetrix, NY, USA), and its variants including the Alpha (B.1.1.7) (ZeptoMetrix), Beta (B.1.351) (ZeptoMetrix), Gamma (P.1) (ZeptoMetrix), Delta (B.1.617.2) (ZeptoMetrix), Kappa (B.1.617.1) (ZeptoMetrix). To make testing solutions, the heat-inactivated viruses were dissolved by D-PBS and serially diluted at the concentration of 2.5×10^5 to 7.9×10^1 $TCID_{50}$/mL by a half-logarithmic dilution. Then, 50 μL of the testing solution were introduced into the recommended volume of lysis reagent for each test (sample-reagent mixture). The indicated volume of the sample-reagent mixture was applied into each test cartridge according to each manufacturer's protocol. The reaction was judged by two judges and repeated additional one experiment. The LoD was defined as the minimum concentration of the sample solution in which at least one judge judged positive. The confirmation of the control line was requested for all judgement to ensure testing.

2.2. Evaluation of the time to positive test results

The time to positive test results of each test was measured from applying the sample-reagent mixture to the recognition of positive test results. This evaluation was performed only for wild type and the Delta variant at high dose (2.5×10^5 $TCID_{50}$/mL) and low dose (2.5×10^4 $TCID_{50}$/mL). Three judges examined duplicates independently and the results were blinded to each judge. The confirmation of the control line was requested for all judgement to ensure testing.

2.3. Evaluation of the Omicron variant

The recombinant SARS-CoV-2 nucleocapsid antigens of the wild type (RayBiotech, GA), the Omicron variant B.1.1.529 (Acro BIOSYSTEMS, DE), and the Omicron variant BA.2 (Acro BIOSYSTEMS) were used for the evaluation of the LoD of ImmunoArrow® to compare its performance to detect them. The reason for using recombinant proteins for this

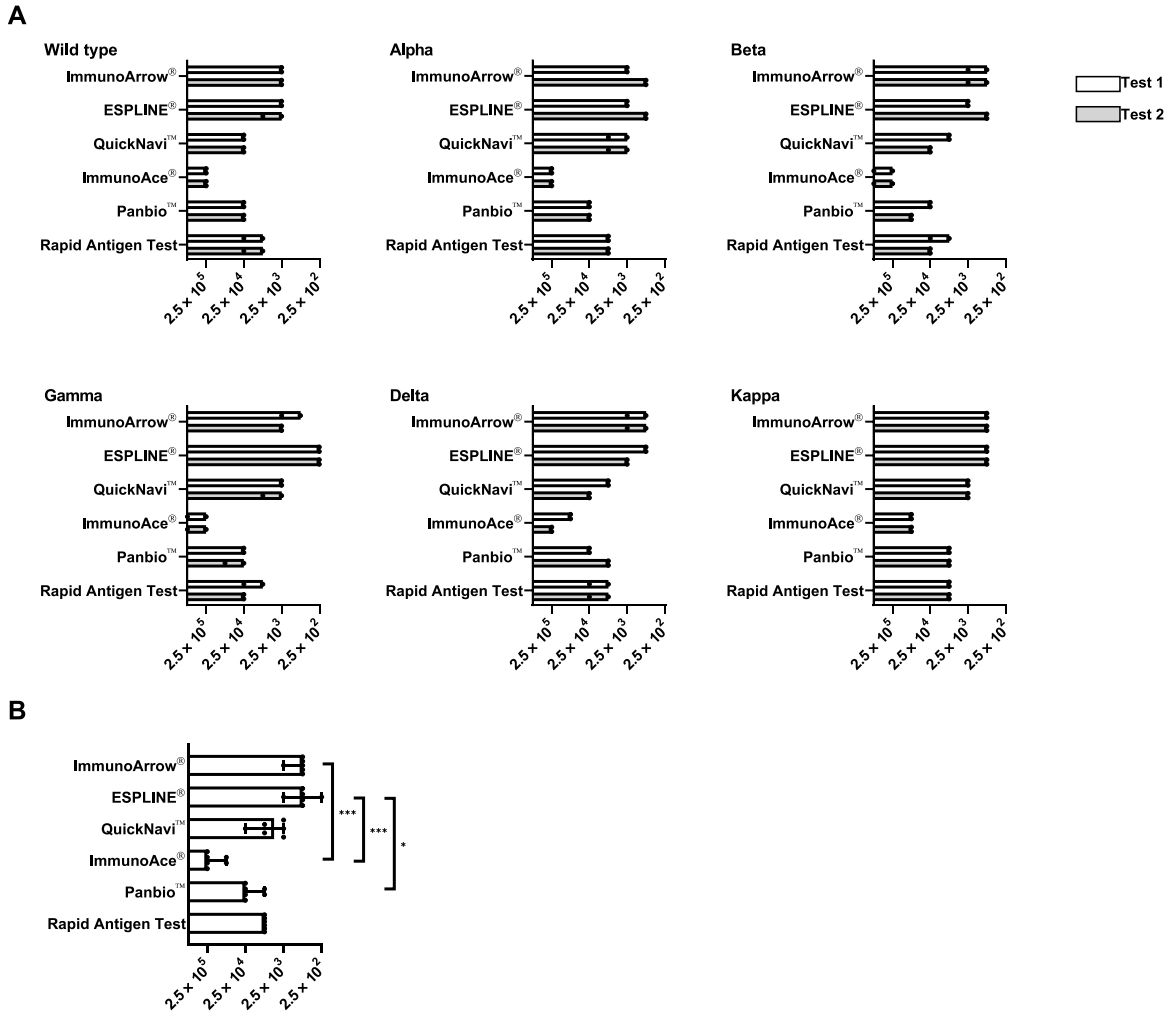

Fig. 1. LoDs of lateral flow antigen tests,
(A) Six lateral flow antigen tests were evaluated by two judges twice. The evaluated range of the heat-inactivated virus was 2.5×10^5–7.9×10^1 $TCID_{50}$/mL. Bars represent the lowest LoD among the judges. Each dot represents the result of each judge. (B) Median of the LoDs for 6 viruses is shown. Each dot represents the LoD of each virus. Error bar represents the range.

evaluation is that the inactivated viruses of Omicron variants were not available because they appeared during the period of the evaluation.

Each recombinant antigen was diluted by the lysis reagent. The level at which two individuals judged positive in triplicate was defined as the 1 × LoD of the wild type. Three judges examined single test independently. The confirmation of the control line was requested for all judgement to ensure testing.

2.4. Statistical analysis

The Friedman test with Dunn's test was used for multiple comparisons among the three paired groups in the experiment of the LoD. For the experiment of the time to positive test results, one-way ANOVA with Holm-Sidak's multiple comparisons test was used.

3. Results

3.1. Evaluation of the LoD

The LoD was evaluated using the heat-inactivated viruses of the SARS-CoV-2 wild strain and 5 variants (Fig. 1A). For the wild type, ImmunoArrow® and ESPLINE® showed the lowest LoD. They detected 2.5×10^3 $TCID_{50}$/mL, followed by Rapid Antigen Test (7.9×10^3 $TCID_{50}$/mL), Panbio™ and QuickNavi™ (2.5×10^4 $TCID_{50}$/mL), and ImmunoAce® (2.5×10^5 $TCID_{50}$/mL). For the variants, all tests detected each variant virus. The limits of detection against the variants did not show significant differences compared with those against the wild type. For all the 6 SARS-CoV-2 viruses, ImmunoArrow® and ESPLINE® were most sensitive (median, 7.9×10^2 $TCID_{50}$/mL) and showed significantly lower detection limits compared to ImmunoAce® (median, 2.5×10^5 $TCID_{50}$/mL, $p < 0.001$, Fig. 1B). There was also a significance difference between ESPLINE® and Panbio™ (median, 2.5×10^4 $TCID_{50}$/mL, $p < 0.05$, Fig. 1B).

3.2. Evaluation of the time to positive test results

The time from applying the solution to the reaction became positive was evaluated for the wild type (Fig. 2A) and the Delta variant (Fig. 2B).

For high dose (2.5×10^5 $TCID_{50}$/mL) of the wild type, QuickNavi™ (mean ± SD, 64.3 ± 2.9 s) and Rapid Antigen Test (79.2 ± 5.8 s) detected antigens the most rapidly, followed by ImmunoArrow® (138.3 ± 2.9 s), ImmunoAce® (186.5 ± 18.2 s), Panbio™ (192.5 ± 13.0 s), and ESPLINE® (906.7 ± 10.1 s). There were significant differences between the tests ($p < 0.05$) excluding the two comparisons: QuickNavi™-Rapid Antigen Test, and ImmunoAce®-Panbio™. For low dose (2.5×10^4 $TCID_{50}$/mL) of the wild type, Rapid Antigen Test was the fastest (255.8 ± 24.0 s), followed by ImmunoArrow® (382.5 ± 26.5 s), QuickNavi™ (456.2 ± 13.2 s), Panbio™ (481.7 ± 27.5 s), and ESPLINE® (790.8 ± 10.1 s). ImmunoAce™ was not evaluated because the viral concentration was out of its LoD. There were significant differences ($p < 0.01$) except for between QuickNavi™ and Panbio™.

For high dose of the Delta variant, QuickNavi™ (50.7 ± 1.1 s) and Rapid Antigen Test (61.7 ± 5.8 s) were the fastest, followed by ImmunoArrow® (108.3 ± 5.2 s), ImmunoAce® (98.3 ± 9.5 s), Panbio™ (125.8 ± 5.2 s), and ESPLINE® (726.7 ± 26.7 s). For low dose of the Delta variant, Rapid Antigen Test (140.0 ± 10.9 s) and QuickNavi™ (152.8 ± 4.0 s) were the fastest, followed by ImmunoArrow® (301.7 ± 21.0 s), Panbio™ (339.2 ± 5.2 s), and ESPLINE® (885.0 ± 26.1 s).

3.3. Evaluation of the Omicron variant

Finally, the LoD of ImmunoArrow® against the Omicron variant was evaluated (Table 1). Compared with the LoD of wild type, ImmunoArrow® detected 100% of the same concentration of Omicron antigens. At half concentration of Omicron B.1.1.529, 33% was positive for ImmunoArrow®.

4. Discussion

ImmnoArrow® showed the lowest LoD and there was maximum 100-fold or more difference in the LoD between the lateral flow antigen tests. The difference in the test LoDs like our study was observed in other reports. For example, a comparative study for the Delta variant reported from Japan showed quite similar findings [6]. While the most sensitive test was ESPLINE® and ImmunoArrow® in this report, LoDs of the QuickNavi™, Panbio™ and Rapid Antigen Test were 10-fold-higher, and that of ImmunoAce® was 100-fold higher. In another study for antigen tests mainly marketed outside Japan [7], there was a 50-fold difference between the tests.

The SARS-CoV-2 variants have variations in their amino acids of the nucleocapsid protein; however, their variations are less frequent than those of the spike proteins. In the present study, each test showed no difference in the detection sensitivity between the wild type and the variants. Moreover, the ImmunoArrow® detected the Omicron variant antigen as little as the wild-type antigen. These results suggested that the lateral flow antigen test can be used for the detection of SARS-CoV-2 like the wild-type and the previous variants. Similar findings have been observed in the major previous variants and the Omicron variant [8,9]. However, some reports also indicated the reduced clinical sensitivity of antigen tests for diagnosis of infection with the Omicron variant compared with the previous variants or the wild-type [10,11]. Therefore, more sensitive tests can be better for preventing the increase in false negatives.

The ImmunoArrow® showed positive results relatively rapidly among the tests. When the positive line appears within the reaction time, a judgement can be made before completing the reaction time. Because the time to positive results can be viral load-dependent [12], the rapid clinical response to those results can lead to the appropriate infection control and treatment.

Traditionally, the lateral flow devices have been used in the clinical settings as point-of-care testing. However, the COVID-19 pandemic has expanded the situations for using lateral flow antigen tests out of the healthcare settings. They have been also used as a self-testing in many countries such as Japan, the United States [13], the United Kingdom [14], and Australia [8]. In these situations, it is difficult to standardize the sampling procedures and the device handling. Therefore, the improved test performance can contribute to minimizing the error among self-testing.

The findings in the present study does not directly indicate the difference in clinical efficacy, because the present study does not include the evaluation of the pre-test process. In the clinical settings, test results can be affected by the disease conditions of the subject, the skill levels of the sample collector, and the sampling tools including swabs.

In conclusion, ImmunoArrow® showed the highest sensitivity among six lateral flow antigen tests and its LoD against the Omicron variant was the same concentration of the wild-type. It can support the diagnosis of COVID-19 with the good sensitivity among known lateral flow antigen tests.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Funding

This study was supported by the funding from TOYOBO Co., Ltd.

Authorship statement

Conceptualization: YM; Methodology: YM HY, and YYo; Validation: YM HY, and YYo; Formal Analysis: YM HY, and YYo; Investigation: YM HY, and YYo; Data Curation: YM; Writing–Original Draft Preparation:

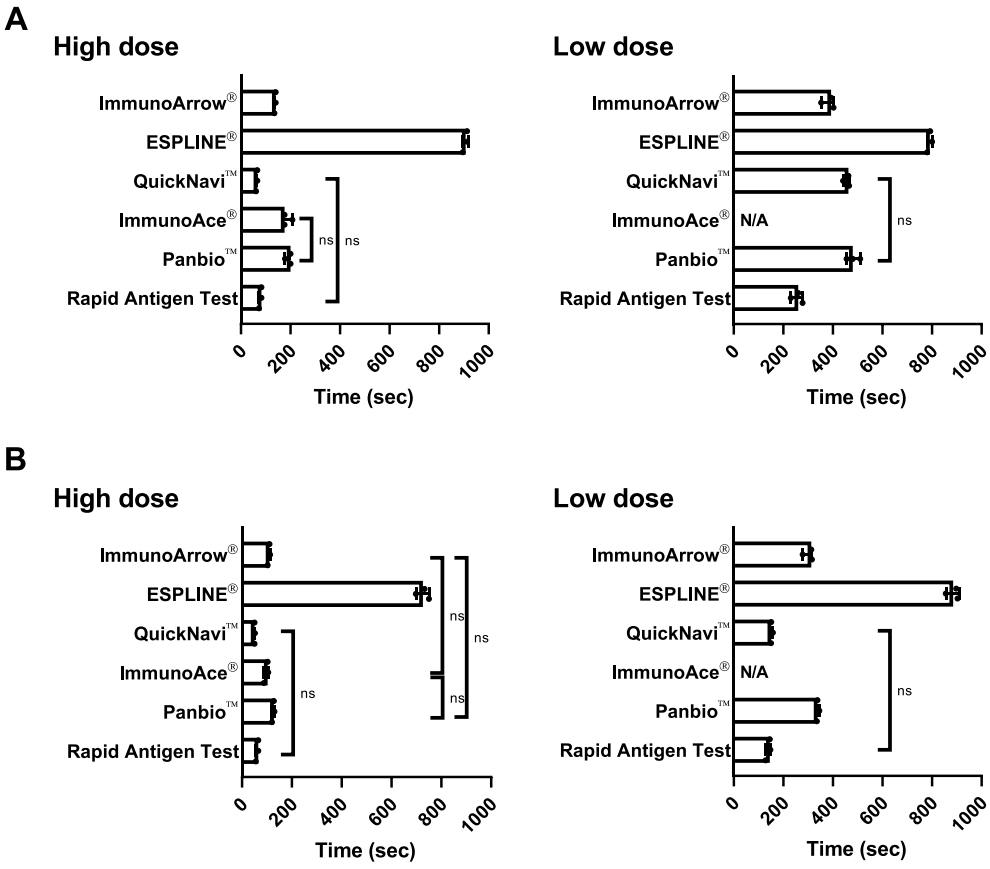

Fig. 2. Time to positive test results of lateral flow antigen tests,
Mean time to positive test results for detection of the wild-type (A) and the Delta variant (B) is shown. The evaluation was performed at the condition of high dose (2.5×10^5 $TCID_{50}$/mL) and low dose (2.5×10^4 $TCID_{50}$/mL) of heat-inactivated virus. Three judges evaluated the time and each dot represents the result of each judge. Error bar represents the standard deviation. ImmunoAce™ was not evaluated at low dose because the viral concentration was out of its LoD. Significant differences were observed between groups except for antigen tests marked with ns (not significance). N/A, not applicable.

Table 1
The LoD of ImmunoArrow® against the Omicron variant.

Antigen	Judge	Result		
		0.5 × LoD	1 × LoD	2 × LoD
Wild-type	1	−	+	+
	2	−	+	+
	3	−	+	+
Omicron B.1.1.529	1	−	+	+
	2	+	+	+
	3	−	+	+
Omicron BA.2	1	−	+	+
	2	−	+	+
	3	−	+	+

YM; Writing–Review and Editing: YM, HK, and YYa; Visualization: YM; Supervision: YM; Project Administration: YM; Funding acquisition: YM and YYa.

All authors meet the ICMJE authorship criteria.[1]

Declaration of competing interest

The authors have no conflicts of interest to declare.

[1] SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, COVID-19: coronavirus disease 2019, LoD: limit of detection.

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