WHITE PAPER

Rapid Antigen Showdown: A Comparative Study of Rapid Antigen Tests for Hong Kong's Top Viral Threats

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Abstract

With the ongoing COVID-19 pandemic, and seasonal influenza (Flu), respiratory illnesses continue to be among the world's most pressing healthcare issues. Although COVID-19 and influenza are contagious respiratory illnesses and share similar symptoms, they are caused by different viruses. According to CDC, people may be co-infected with multiple viruses simultaneously. There is an urgent need for rapid COVID-19 and Influenza A/B diagnostic tests so that early treatment can be provided to patients before their symptoms worsen. PHASE Scientific has developed the INDICAID™ COVID-19/FLU A&B Rapid Antigen Test (RAT) that can detect and differentiate the SARS-CoV-2 and Influenza viral antigen present in upper respiratory specimens. The sensitivity of the RAT is the most important performance metrics for accurate detection. This study is to evaluate the Limit of Detection (LoD) of total of 6 community existing strains of influenza A, B and SARS-CoV-2 for three major brands in HK market: 1) INDICAID™ COVID-19/FLU A&B Rapid Antigen Test, 2) BioTeke™ Multiple Respiratory Multipathogen Antigen Test Kit and 3) Bandi-Check™ Influenza A/B/Corona Virus (COVID-19) Antigen Rapid Test (Colloidal Gold). For influenza A, INDICAID™ exhibits 4 times (H1N1) and 2 times (H3N2) better LoD than the other two brands. For influenza B (Victoria), INDICAID™ shows 3 to 1.5 times better LoD than BioTeke™ and Bandi-Check™, respectively. For influenza B (Yamagata), INDICAID™ exhibits same LoD as the other two brands. For SARS-CoV-2 standard strain (2019-nCoV/USA-WA1/2020), INDICAID™ is 1.6 times better LoD than BioTeke™ and same LoD as Bandi-Check™. On current most prevalence Omicron XXB strain, INDICAID™ shows same LoD as the other two brands. The overall performance of INDICAID™ COVID-19/FLU A&B Rapid Antigen Test is better than BioTeke™ and Bandi-Check™ in detection of most popular strains of influenza A/B and SARS-CoV-2.

Introduction

Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in December 2019 and then rapidly spread world-wide, before World Health Organization (WHO) declared it as a global pandemic [1]. As of July 2023, over 767 million cases of COVID-19 with 6 million deaths were reported [2]. Symptoms include coughs, fevers, chills, headache, ageusia, anosmia, myalgia, nausea, vomiting, diarrhea, dyspnea, malaise, anorexia and fatigue [3]. Severe cases can lead to respiratory, neurological, cardiovascular, and renal complications, which eventually result in death. Current drug treatment target molecules that are involved in the invasion of SARS-CoV-2 to the host cell or molecules that associate with the SARS-CoV-2 viral replication inside the host [1].

Influenza viruses are categorized into 4 types, influenza A, B, C, D. Influenza A and B infect humans and cause seasonal flu. Influenza A is further divided into several subtypes based on the antigenicity and the combination of hemagglutinin (HA) and neuraminidase (NA) present on the virus, currently circulating subtypes includes H1N1 and H3N2. Influenza B is classified into two lineages: B/Yamagata and B/Victoria [4]. Clinical presentation of influenza includes dry cough, sneezing, nasal discharge, fever, headache, myalgia, lacrimation, burning sensation in the eye, chills, malaise and anorexia. Fatalities resulting from influenza are caused by respiratory, musculoskeletal, neurological and cardiovascular complications [3]. Known drug treatments are available for influenza that inhibit the structural and functional proteins of influenza or interfere with the influenza virus replication within the host cell [1] and were shown to be effective in reducing complications. RAT plays a crucial role in early identification of the specific pathogen causing respiratory infections, enabling timely administration of appropriate medication and effective measures to protect others. RATs with higher sensitivity provide enhanced capabilities for detecting diseases in their early stages. Our study aims to assess the disparity in detection sensitivity between the INDICAID product and other widely available RATs in the market. By conducting this evaluation, we seek to determine the performance differences and potential advantages of the INDICAID™ product in accurately detecting respiratory infections at an early stage.

The INDICAID™ COVID-19/FLU A&B Rapid Antigen Test developed by PHASE Scientific International Ltd, the BioTeke™ Multiple Respiratory Multipathogen Antigen Test Kit (Immunochromatographic Assay) developed by BioTeke™ Corporation (Wuxi) Co., Ltd, and the Bandi-Check™ Influenza A/B/Corona Virus (COVID-19) Antigen Rapid Test (Colloidal Gold) manufactured by Diasia Biomedical Technology Co., Ltd, were included in this study. The objective of the study was to compare the analytical sensitivity of the assays using commercially available virus culture fluid.

Methods

Limit of detection (LoD) determination:

INDICAID™ COVID-19/FLU A&B Rapid Antigen Test LoD was determined for influenza A, influenza B, and SARS-CoV-2 viruses using analyte from two strains of each virus. The contrived sample matrix utilized in the testing process consisted of pooled

human donors' nasal wash (PNW) obtained from Lee Biosolutions, MO, USA. The INDICAID™ kit was used to test the stock pooled negative nasal wash (PNW) samples in three replicates. All three replicates tested negative for all viruses, indicating that the stock PNW was classified as negative. The virus culture fluid, obtained from Zeptometrix, NY, USA, had a predetermined concentration of the virus as provided by the supplier. The 6 strains of virus were independently added into PNW and diluted to various concentrations using PNW. LoD was determined by testing a 2-fold dilution series of 3 replicates per concentration, the lowest concentration that gave all 3 positive result was deemed as the LoD. Fifty (50) µL of the spiked samples were dispensed onto the sterile disposable swab provided in each test kit and eluted to the buffer solution following the manufacturer's Instructions for Use (IFU). A fixed amount of buffer according to the IFU was dispensed on to the test cassette. Results were interpreted at a fixed time after sample addition according to the IFU. A line intensity score was given to each line (control, influenza A, influenza B and SARS-CoV-2) through comparison to a reference line intensity chart that has 12 shades, from lightest (0) to darkest (12). A line intensity of 3 or higher was defined as positive. The LoD was reported as the $TCID_{50}$/mL concentration in the PNW sample.

Parallel Comparison of assays:
The forementioned kits from INDICAID™, BioTeke™ and Bandi-Check™ were tested with PNW spiked with various virus strains independently at a range of concentrations in triplicates. Only the 3 concentrations closest to LoD of INDICAID™ kit was shown in the following tables. Aside from the contrived sample, all material used in the test were provided in the respective kit. The test method and result interpretation were the same as those described in the LoD determination section.

Testing Materials

Manufacturer	Product Number	Description	Stock Concentration (from Certificate of Analysis from supplier)
ZeptoMetrix®	0810165CF	Influenza A (H1N1) A/California/7/2009	1.41×10^5 $TCID_{50}$/mL
ZeptoMetrix®	0810240CF	Influenza A (H3N2) A/Victoria/361/2011	3.89×10^4 $TCID_{50}$/mL
ZeptoMetrix®	0810573CF	Influenza B (Victoria) B/Colorado/06/2017	1.17×10^5 $TCID_{50}$/mL
ZeptoMetrix®	0810515CF	Influenza B (Yamagata) B/Phuket/3073/2013	1.86×10^4 $TCID_{50}$/mL
ZeptoMetrix®	0810587UV	SARS-CoV-2 USA-WA1/2020	1.51×10^6 $TCID_{50}$/mL
ZeptoMetrix®	0810665CFHI	SARS-CoV-2 Lineage XBB; Omicron Var. USA/CA-Stanford-109_S21/2022	5.95×10^6 $TCID_{50}$/mL
Lee Biosolutions	991-26-P	Pooled Human Donors Nasal Wash - Normal	Not Available

Test kits

Manufacturer	Description
PHASE Scientific	INDICAID™ COVID-19/FLU A&B Rapid Antigen Test
BioTeke™	Multiple Respiratory Multipathogen Antigen Test Kit (Immunochromatographic Assay)
Banitore	Bandi-Check™ Influenza A/B/Corona Virus (COVID-19) Antigen Rapid Test (Colloidal Gold)

Results
A total of 2 influenza A, 2 influenza B and 2 SARS-CoV-2 strains were evaluated.

For Influenza A test line performance (Table 1 and 2), in the two strains of Influenza A tested, INDICAID™ outperformed BioTeke™ and Bandi-Check™.

Table 1: Influenza A Test Results.

Sub-type	Strain	Source	Product code	Lot	Concentration tested ($TCID_{50}$/mL)	Number of devices with positive result		
						INDICAID™	BioTeke™	Bandi-Check™
H1N1	A/California/7/2009	ZeptoMetrix®	0810165CF	329126	141	3/3	3/3	3/3
					35.25	3/3	0/3	0/3
					17.625	0/3	0/3	0/3
H3N2	A/Victoria/361/11	ZeptoMetrix®	0810240CF	328059	38.9	3/3	3/3	3/3
					19.45	3/3	0/3	0/3
					9.725	0/3	0/3	0/3

Table 2: Influenza A LOD Results Summary.

Product Number	Description	Limit of Detection concentration in PNW ($TCID_{50}$/mL) (Concentration before dilution in buffer)		
		INDICAID™	BioTeke™	Bandi-Check™
0810165CF	Influenza A (H1N1) A/California/7/2009	35.25	141	141
0810240CF	Influenza A (H3N2) A/Victoria/361/2011	19.45	38.9	38.9

For Influenza B test line performance (Table 3 and 4), INDICAID™ outperformed BioTeke™ and Bandi-Check™ on Influenza B (Victoria) B/Colorado/06/2017, and for the Influenza B (Yamagata) B/Phuket/3073/2013, INDICAID™, BioTeke™ and Bandi-Check™ displayed equivalent performance.

Table 3: Influenza B Test Results.

Sub-type	Strain	Source	Product code	Lot	Concentrat-ion tested ($TCID_{50}$/mL)	Number of devices with positive result		
						INDICAID™	BioTeke™	Bandi-Check™
Victoria	B/Colorado/06/2017	ZeptoMetrix®	0810573 CF	326 890	234	3/3	3/3	3/3
					117	3/3	0/3	3/3
					78	3/3	0/3	0/3
					39	0/3	0/3	0/3
Yamagata	B/Phuket/3073/2013	ZeptoMetrix®	0810515 CF	329 592	37.2	3/3	3/3	3/3
					18.6	0/3	0/3	0/3

Table 4: Influenza B LOD Results Summary.

Product Number	Description	Limit of Detection concentration in PNW ($TCID_{50}$/mL) (Concentration before dilution in buffer)		
		INDICAID™	BioTeke™	Bandi-Check™
0810573CF	Influenza B (Victoria) B/Colorado/06/2017	78	234	117
0810515CF	Influenza B (Yamagata) B/Phuket/3073/2013	37.2	37.2	37.2

For SARS-CoV-2 test line performance (Table 5 and 6). For the COVID-19 strain 2019-nCoV/USA-WA1/2020, INDICAID™ outperformed BioTeke™ and consistent with Bandi-Check™; for the USA/CA-Stanford-109_S21/2022, INDICAID™, BioTeke™ and Bandi-Check™ displayed equivalent performance.

Table 5: SARS-CoV-2 Test Results

Strain	Source	Product code	Lot	Concentration tested ($TCID_{50}$/mL)	Number of devices with positive result		
					INDICAID™	BioTeke™	Bandi-Check™
USA-WA1/2020	ZeptoMetrix®	0810587 UV	328677	1510	3/3	3/3	3/3
				945	3/3	0/3	3/3
				472.5	0/3	0/3	0/3
USA/CA-Stanford-109_S21/2022	ZeptoMetrix®	0810665 CFHI	331181	11900	3/3	3/3	3/3
				5950	2/3	0/3	0/3

Table 6: SARS-CoV-2 LOD Results Summary

Product Number	Description	Limit of Detection concentration in PNW ($TCID_{50}$/mL) (Concentration before dilution in buffer)		
		INDICAID™	BioTeke™	Bandi-Check™
0810587UV	2019-nCoV/USA-WA1/2020	945	1510	945
0810665CFHI	USA/CA-Stanford-109_S21/2022	11900	11900	11900

Conclusion

The accuracy of a self-test assay is influenced by two key factors: the sampling technique employed by the lay user and the sensitivity and specificity of the device itself [5]. Although manufacturers provide comprehensive instructions on sampling techniques through instructional materials or videos, it is challenging to ensure that lay users interpret and implement these instructions correctly. Therefore, it is crucial for manufacturers to enhance the sensitivity and specificity of their devices to improve the overall accuracy of the test results.

The INDICAID™ COVID-19/FLU A&B Rapid Antigen Test exhibited overall superior performance in 4 of the 6 strains of Influenza A, Influenza B, and SARS-CoV-2 tested, compared to the BioTeke™ Multiple Respiratory Multipathogen Antigen Test Kit and Bandi-Check™ Influenza A/B/Corona Virus (COVID-19) Antigen Rapid Test (Colloidal Gold). Specifically, INDICAID™ consistently outperformed the other tests for the evaluated strains of Influenza A, demonstrating better sensitivity. For Influenza B, INDICAID™ showed superior performance for the B/Colorado/06/2017 strain, while exhibiting comparable performance to BioTeke™ and Bandi-Check™ for the B/Phuket/3073/2013 strain. Moreover, INDICAID™ outperformed BioTeke™ for the tested COVID-19 strain 2019-nCoV/USA-WA1/2020 and demonstrated equivalent performance to both BioTeke™ and Bandi-Check™ for the USA/CA-Stanford-109_S21/2022 strain.

In conclusion, the INDICAID™ COVID-19/FLU A&B Rapid Antigen Test exhibited a better Limit of Detection (LoD) for influenza A, influenza B and SARS-CoV-2 compared to common available brands in Hong Kong. A better LoD indicates that the test can detect infections at an earlier stage when the viral load in patients is still low. Early detection enables individuals to seek prompt treatment and self-isolation, thereby reducing the risk of transmitting the virus to others. These findings highlight the reliability and efficiency of INDICAID™ as a diagnostic tool for detecting multiple strains of influenza A, influenza B, and SARS-CoV-2, surpassing the performance of the market-leading brand.

References

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