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First-line of Protection from Severe Respiratory Viruses in Hong Kong: A Comparative Examination of Major Rapid Antigen Brands

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Abstract

Respiratory illnesses have continued to be one of the world's most pressing healthcare issues since the COVID-19 pandemic. However, since common contagious respiratory illnesses caused by SARS-CoV-2, influenza viruses, respiratory syncytial virus (RSV) and/or adenoviruses (AdV) all share similar symptoms, distinguishing them can be difficult and may lead to inaccurate treatment decisions. Additionally, according to the Centers for Disease Control and Prevention (CDC), people can be co-infected with multiple viruses simultaneously. Therefore, it is crucial to have rapid diagnostic tests that can detect and differentiate between contagious respiratory infections, allowing for early and accurate treatment to be provided for patients before symptoms worsen. PHASE Scientific has developed the INDICAID™ RESPIRATORY 5-in-1 Rapid Antigen Test (INDICAID™) for the detection and differentiation of SARS-CoV-2, Influenza A/B, RSV, and AdV viral antigens. The sensitivity of the Rapid Antigen Test (RAT) is the most important performance metric for accurate detection. This study is to evaluate the the Limit of Detection (LoD) for a total of 10 community existing strains of influenza A, B, SARS-CoV-2, RSV and AdV of four major brands of RATs in Hong Kong: 1) INDICAID™ RESPIRATORY 5-in-1 Rapid Antigen Test, 2) BioTeke™ Multiple Respiratory Multipathogen Antigen Test Kit (immunochromatographic assay) (SARS-CoV-2 | Flu A | Flu B | RSV | ADV), 3) Bandi-Check™ SARS-CoV-2/InfluenzaA&B/RSV and Adenovirus 5 in 1 Antigen Rapid Test (Colloidal Gold) and 4) REAGEN® SARS-CoV-2/RSV/ADV&FluA/B Antigen Rapid Test Kit. For Influenza A (H1N1), INDICAID™ exhibits 4 times better LoD than BioTeke™ and Bandi-Check™, while REAGEN® was not able to produce a definite positive test result in all concentrations of the virus tested. For Influenza A (H3N2), INDICAID™ exhibits 2 times better LoD than BioTeke™, Bandi-Check™ and REAGEN®. For Influenza B(Victoria) B/Colorado/06/2017 strain, INDICAID™ exhibits 2 times better LoD than BioTeke™ and Bandi-Check™. For the Influenza B (Yamagata) B/Phuket/3073/2013 strain, INDICAID™ exhibits the same LoD as Bandi-Check™, and BioTeke™. REAGEN® showed poorer performance in both Influenza B strains and was not able to produce a definite positive test result in all concentrations of the virus tested. For SARS-CoV-2 USA-WA1/2020 strain, INDICAID™ exhibits 1.6 times better LoD than Bandi-Check™ and BioTeke™. For SARS-CoV-2 USA/CA-Stanford-109_S21/2022 strain, INDICAID™ showed a similar performance to BioTeke™ while Bandi-Check™ and REAGEN® were not able to produce a definite positive test result in all concentrations of the virus tested. REAGEN® showed poorer performance in both SARS-CoV-2 strains and was not able to produce a definite positive test result in all concentrations of the virus tested. For Respiratory syncytial virus A (RSV-A) strain, INDICAID™ exhibits 4 times better LoD than Bandi-Check™ and REAGEN®, and showed similar performance as BioTeke™. For Respiratory syncytial virus B (RSV-B) strain, INDICAID™ showed 4 times and 2 times better LoD than REAGEN® and BioTeke™ respectively, while displaying similar performance with Bandi-Check™. For AdV Type 1 strain, INDICAID™ exhibits 2 times better LoD than the other 3 brands. For AdV Type 7A, INDICAID™ showed a similar performance to BioTeke™ while Bandi-Check™ and REAGEN® were not able to produce a definite positive test result in all concentrations of the virus tested. In this study, REAGEN® was unable to produce a definite positive test result for 6 out of 10 virus strains at all concentrations tested, and therefore no LoD value was determined in the presented data. The overall performance of the INDICAID™ RESPIRATORY 5-in-1 Rapid Antigen Test is better than BioTeke™, Bandi-Check™ and REAGEN® in detecting the most common strains of SARS-CoV-2, Influenza A/B, RSV, and AdV.

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 rapidly spread worldwide before the World Health Organization (WHO) declared it a global pandemic [1]. As of July 2023, over 767 million cases of COVID-19 with 6 million deaths have been reported [2]. Symptoms include coughs, fevers, chills, headaches, ageusia, anosmia, myalgia, nausea, vomiting, diarrhea, dyspnea, malaise, anorexia, and fatigue [3]. Severe cases can lead to respiratory, neurological, cardiovascular, and renal complications, ultimately resulting in death. Current drug treatments target molecules involved in the invasion of SARS-CoV-2 into the host cell or molecules associated with SARS-CoV-2 viral replication inside the host [1].

Influenza viruses are categorized into four types: influenza A, B, C, and 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 include 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 eyes, chills, malaise, and anorexia. Fatalities resulting from influenza are caused by respiratory, musculoskeletal, neurological, and cardiovascular complications [3]. Known drug treatments for influenza inhibit the structural and functional proteins of the virus or interfere with influenza virus replication within the host cell [1] and have been shown to be effective in reducing complications.

Respiratory syncytial virus (RSV) mainly causes lower respiratory tract infections such as bronchiolitis and pneumonia in infants under 6 months old and upper respiratory tract infections such as rhinitis and colds in older children and adults. RSV infection is the leading cause of hospitalization for viral respiratory tract infections in infants and young children, posing a serious health hazard, especially for premature infants, infants with congenital heart disease, or primary immune deficiency [6]. RSV infection causes minor damage to the ciliated epithelial cells of the respiratory tract but can lead to bronchiolitis, pneumonia, and other serious respiratory diseases in infants aged 2 to 6 months.

Adenovirus (AdV) can infect the respiratory tract, gastrointestinal tract, urethra and bladder, eyes, liver, etc. Typical symptoms of respiratory infections include cough, nasal congestion, and pharyngitis, accompanied by fever, chills, headache, and muscle pain. There are four different syndromes associated with AdV, including acute febrile pharyngoconjunctival fever, acute respiratory diseases, ARD, pneumonia [7].

Rapid Antigen Tests play a crucial role in early identification of the specific pathogens 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 INDICAID™ products 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™ products in accurately detecting respiratory infections at an early stage.

The INDICAID™ RESPIRATORY 5-in-1 Rapid Antigen Test developed by PHASE Scientific International Ltd, the BioTeke™ Multiple Respiratory Multipathogen Antigen Test Kit (immunochromatographic assay) (SARS-CoV-2 | Flu A | Flu B | RSV | ADV)developed by BioTeke Corporation (Wuxi) Co., Ltd., the Bandi-Check™ SARS-CoV-2/Influenza A&B/RSV and Adenovirus 5 in 1 Antigen Rapid Test (Colloidal Gold) manufactured by HENGAN PHARMACARE Co., Ltd., and the REAGEN® SARS-Cov-2/RSV/ADV&Flu A/B Antigen Rapid Test Kit manufactured by Shenzhen Reagent Technology Co., Ltd. were included in this study. The objective of the study was to compare the analytical sensitivity using commercially available virus culture fluid.

Methods

Limit of detection (LoD) determination:

INDICAID™ RESPIRATORY 5-in-1 Rapid Antigen Test LoD was determined for SARS-CoV-2, influenza A&B, RSV, and AdV using analyte from two strains of each virus. This testing processes utilized a contrived sample matrix consisting of pooled human donors' nasal wash (PNW) obtained from Lee Biosolutions, MO, USA. In this study, 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. Each strain of the virus was independently added to PNW and diluted to various concentrations using PNW. The LoD was determined by testing a 2-fold dilution series of three replicates per concentration, and the lowest concentration that gave all three positive results 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 into the buffer solution following the manufacturer's Instructions for Use (IFU). A fixed amount of buffer, according to the IFU, was dispensed onto 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, SARS-CoV-2, RSV and AdV) by comparing it to a reference line intensity chart that had 12 shades, ranging from the lightest (0) to the 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™, Bandi-Check™, and REAGEN® were tested with PNW spiked with two strains of SARS-CoV-2, Influenza A&B, RSV, and AdV, respectively. Each virus strain was tested independently at 2-4 concentrations closest to the LoD of the INDICAID™ in triplicates. Aside from the contrived sample, all materials 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
ZeptoMetrix®	0810040ACF	Respiratory syncytial virus A	$5.01x10^5$ $TCID_{50}$/mL
ZeptoMetrix®	0810040CF	Respiratory syncytial virus B	$3.16x10^6$ $TCID_{50}$/mL
ZeptoMetrix®	0810050CF	Adenovirus Type 1	$2.82x10^7$ $TCID_{50}$/mL
ZeptoMetrix®	0810021CF	Adenovirus Type 7A	$3.16x10^6$ $TCID_{50}$/mL
Lee Biosolutions	991-26-P	Pooled Human Donors Nasal Wash - Normal	Not Available

Test kits

Manufacturer	Description
PHASE Scientific	INDICAID™ RESPIRATORY 5-in-1 Rapid Antigen Test
BioTeke™	BioTeke™ Multiple Respiratory Multipathogen Antigen Test Kit (Immunochromatographic Assay) (SARS-CoV-2 | Flu A | Flu B | RSV | ADV)
Banitore	Bandi-Check™ SARS-CoV-2/InfluenzaA&B/RSV and Adenovirus 5 in 1 Antigen Rapid Test (Colloidal Gold)
REAGEN®	REAGEN® SARS-CoV-2/RSV/ADV&FluA/B Antigen Rapid Test Kit

Results

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

For Influenza A test line performance comparison (Table 1 and 2), in the two strains of Influenza A tested, INDICAID™ outperformed BioTeke™, Bandi-Check™ and REAGEN®. INDICAID™ exhibits 4 times better LoD than BioTeke™ and Bandi-Check™ while REAGEN® showing the weakest performance in detecting

Influenza A H1N1 strain and was not able to produce a definite positive test result in all concentrations of the virus tested. In Influenza A H3N2, INDICAID™ exhibits 2 times better LoD than BioTeke™, Bandi-Check™ and REAGEN®.

Table 1: Influenza A Test Results.

Sub-type	Strain	Source	Product code	Concentration tested ($TCID_{50}$/mL)	Number of devices with positive result			
					INDICAID™	Bandi-Check™	BioTeke™	REAGEN®
H1N1	A/California /7/2009	ZeptoMetrix®	0810165 CF	141	3/3	3/3	3/3	1/3
				70.5	3/3	1/3	2/3	0/3
				35.25	3/3	0/3	0/3	0/3
				17.625	0/3	0/3	0/3	0/3
H3N2	A/Victoria/ 361/11	ZeptoMetrix®	0810240 CF	38.9	3/3	3/3	3/3	3/3
				19.45	3/3	1/3	1/3	0/3
				9.725	1/3	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™	Bandi-Check™	BioTeke™	REAGEN®
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	38.9

For Influenza B test line performance comparison (Table 3 and 4), INDICAID™ outperformed Bandi-Check™, BioTeke™, and REAGEN® on Influenza B. In the Influenza B (Victoria) B/Colorado/06/2017 strain, INDICAID™ exhibits 2 times better LoD than BioTeke™, Bandi-Check™, while REAGEN® showed poorer performance and was not able to produce a definite positive test result in all concentrations of the virus tested. For the Influenza B (Yamagata) B/Phuket/3073/2013 strain, INDICAID™, Bandi-Check™, and BioTeke™ displayed consistent performance, while REAGEN® showed poorer performance and was not able to produce a definite positive test result in all concentrations of the virus tested. REAGEN® showed poorer performance in both Influenza B strains and was not able to produce a definite positive test result in all concentrations of the virus tested.

Table 3: Influenza B Test Results.

Sub-type	Strain	Source	Product code	Concen-tration tested ($TCID_{50}$/ mL)	Number of devices with positive result			
					INDICAID™	Bandi-Check™	BioTeke™	REAGEN®
Victoria	B/Colorado /06/2017	ZeptoMetrix®	0810573 CF	234	3/3	3/3	3/3	0/3
				117	3/3	0/3	0/3	0/3
				78	0/3	0/3	0/3	0/3
Yama-gata	B/Phuket/ 3073/2013	ZeptoMetrix®	0810515 CF	37.2	3/3	3/3	3/3	2/3
				18.6	0/3	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™	Bandi-Check™	BioTeke™	REAGEN®
0810573CF	Influenza B (Victoria) B/Colorado/06/2017	117	234	234	/
0810515CF	Influenza B (Yamagata) B/Phuket/3073/2013	37.2	37.2	37.2	/

For SARS-CoV-2 test line performance comparison (Table 5 and 6), INDICAID™ outperformed Bandi-Check™, BioTeke™ and REAGEN®. In SAVS-CoV-2 USA-WA1/2020 strain, INDICAID™ exhibits 1.6 times better LoD than Bandi-Check™ and BioTeke™ in detecting the USA-WA1/2020 strain, while REAGEN® showed poorer performance and was not able to produce a definite positive test result in all concentrations of the virus tested. INDICAID™ outperformed Bandi-Check™ and REAGEN® and consistent with BioTeke™ on the USA/CA-Stanford-109_S21/2022 strain. REAGEN® showed poorer performance in both SARS-CoV-2 strains and was not able to produce a definite positive test result in all concentrations of the virus tested.

Table 5: SARS-CoV-2 Test Results

Strain	Source	Product code	Concentration tested ($TCID_{50}$/mL)	Number of devices with positive result			
				INDICAID™	Bandi-Check™	BioTeke™	REAGEN®
USA-WA1/2020	ZeptoMetrix®	0810587 UV	1510	3/3	3/3	3/3	0/3
			945	3/3	0/3	0/3	0/3
			472.5	0/3	0/3	0/3	0/3
USA/CA-Stanford-109_S21/2022	ZeptoMetrix®	0810665 CFHI	11900	3/3	2/3	3/3	2/3
			5950	2/3	0/3	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™	Bandi-Check™	BioTeke™	REAGEN®
0810587UV	2019-nCoV/USA-WA1/2020	945	1510	1510	/
0810665CFHI	USA/CA-Stanford-109_S21/2022	11900	/	11900	/

For RSV test line performance comparison (Table 7 and 8) INDICAID™ has outperformed Bandi-Check™, BioTeke™ and REAGEN®. INDICAID™ exhibits 4 times better LoD than Bandi-Check™ and REAGEN®, and showed similar performance to BioTeke™ in detecting the Respiratory syncytial virus A (RSV-A) strain. For Respiratory syncytial virus B (RSV-B) detection, INDICAID™ exhibited 4 times better LoD than REAGEN® and 2 times better LoD than BioTeke™, and showed similar performance with Bandi-Check™.

Table 7: RSV Test Results.

Strain	Source	Product code	Concentration tested ($TCID_{50}$/mL)	Number of devices with positive result			
				INDICAID™	Bandi-Check™	BioTeke™	REAGEN®
Respiratory syncytial virus A	ZeptoMetrix®	0810040 ACF	501	3/3	3/3	3/3	3/3
			250.5	3/3	1/3	3/3	2/3
			125.25	3/3	0/3	3/3	0/3
			62.625	0/3	0/3	0/3	0/3
Respiratory syncytial virus B	ZeptoMetrix®	0810040 CF	527	3/3	3/3	3/3	3/3
			263.5	3/3	3/3	3/3	1/3
			131.75	3/3	3/3	1/3	0/3
			65.875	1/3	0/3	0/3	0/3

Table 8: RSV LoD Results Summary.

Product Number	Description	Limit of Detection concentration in PNW ($TCID_{50}$/mL) (Concentration before dilution in buffer)			
		INDICAID™	Bandi-Check™	BioTeke™	REAGEN®
0810040A CF	Respiratory syncytial virus A	125.25	501	125.25	501
0810040CF	Respiratory syncytial virus B	131.75	131.75	263.5	527

For AdV test line performance comparison (Table 9 and 10) INDICAID™ outperformed Bandi-Check™, BioTeke™ and REAGEN®. INDICAID™ exhibits 2 times better LoD than Bandi-Check™, BioTeke™ and REAGEN® in detecting the AdV Type 1 strain. In detecting AdV Type 7A, INDICAID™ showed a similar performance to BioTeke™ while Bandi-Check™ and REAGEN® was not able to produce a definite positive test result.

Table 9: AdV Test Results.

Strain	Source	Product code	Concentration tested ($TCID_{50}$/mL)	Number of devices with positive result			
				INDICAID™	Bandi-Check™	BioTeke™	REAGEN®
Type 1 (Species C)	ZeptoMetrix®	0810050 CF	7050	3/3	3/3	3/3	3/3
			3525	3/3	0/3	2/3	0/3
			1762.5	0/3	0/3	0/3	0/3
Type 7A (Species B)	ZeptoMetrix®	0810021 CF	790	3/3	2/3	3/3	2/3
			395	2/3	0/3	0/3	0/3

Table 10: AdV LoD Results Summary.

Product Number	Description	Limit of Detection concentration in PNW ($TCID_{50}$/mL) (Concentration before dilution in buffer)			
		INDICAID™	Bandi-Check™	BioTeke™	REAGEN®
0810050CF	Adenovirus Type 1	3525	7050	7050	7050
0810021CF	Adenovirus Type 7A	790	/	790	/

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.

INDICAID™ RESPIRATORY 5-in-1 Rapid Antigen Test exhibited superior performance in all 10 strains of Influenza A, Influenza B , SARS-CoV-2, RSV and Adenovirus tested, compared to at least 1 of the 3 commonly available Hong Kong RAT brands tested, namely BioTeke™ Multiple Respiratory Multipathogen Antigen Test Kit, Bandi-Check™ SARS-CoV-2/Influenza A&B/RSV and Adenovirus 5 in 1 Antigen Rapid Test, and REAGEN® SARS-CoV-2/RSV/ADV&Flu A/B Antigen Rapid Test Kit.

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™a and Bandi-Check™ for the B/Phuket/3073/2013 strain. For SARS-CoV-2, INDICAID™ outperformed Bandi-Check™, BioTeke™, and REAGEN® in detecting the 2019-nCoV/USA-WA1/2020 strain. INDICAID™ outperformed Bandi-Check™ and REAGEN®, and consistent with BioTeke™ on the USA/CA-Stanford-109_S21/2022 strain. For RSV, INDICAID™ outperformed Bandi-Check™ and REAGEN® and showed similar performance to BioTeke™ in detecting the Respiratory syncytial virus A (RSV-A) strain. For Respiratory syncytial virus B (RSV-B) detection, INDICAID™ outperformed REAGEN® and BioTeke™, and showed similar performance with Bandi-Check™. For AdV, INDICAID™ had outperformed BioTeke™, Bandi-Check™, and REAGEN® in detecting AdV Type 1 strain. In detecting AdV Type 7A, INDICAID™ outperformed Bandi-Check™ and REAGEN® and showed a similar performance to BioTeke™.

In conclusion, the INDICAID™ Respiratory 5-in-1 Rapid Antigen Test exhibited a better Limit of Detection (LoD) for influenza A, influenza B, SARS-CoV-2, RSV and AdV compared to commonly available RAT 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, SARS-CoV-2, RSV and AdV, surpassing the performance of other market-leading brands.

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