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RESEARCH ARTICLE

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Comparison of three rapid influenza diagnostic tests with digital readout systems and one conventional rapid influenza diagnostic test

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Background: Rapid influenza diagnostic tests (RIDTs) show variable sensitivities in clinical settings. We aimed to compare three digital RIDTs and one conventional RIDT.

Methods: We assessed 218 nasopharyngeal swabs from patients between neonates and 90 years old in 2016. Three digital RIDTs were BUDDI, Sofia Influenza A+B Fluorescence Immunoassay, Veritor System Flu A+B assay. One conventional test was the SD Bioline Influenza Ag A/B/A(H1N1/2009). All test results were compared with those from the Anyplex Flu A/B Typing Real-time Detection real-time PCR. The four RIDTs were tested with diluted solutions from the National Institute for Biological Standards and Control (NIBSC) to compare lower detection limit. Cross-reactivity of four RIDTs within other respiratory viruses was identified.

Results: For influenza A, BUDDI, Sofia, Veritor, and Bioline showed 87.7%, 94.5%, 87.7%, and 72.6% sensitivity, and 100%, 97.7%, 96.5%, and 100% specificity. For influenza B, BUDDI, Sofia, Veritor, and Bioline showed 81.7%, 91.7%, 81.7%, and 78.3% sensitivity, and 100%, 95.3%, 100%, and 100% specificity, respectively. Each RIDT could detect diluted NIBSC solution, according to the level of dilution and specific influenza subtypes. Cross-reactivity of four RIDTs with other respiratory viruses was not noted.

Conclusions: Sofia showed the highest sensitivity for influenza A and B detection. BUDDI and Veritor showed higher detection sensitivity than a conventional RIDT for influenza A detection, but similar results for influenza B detection. Further study is needed to compare the test performance of RIDTs according to specific, prevalent influenza subtypes.

KEYWORDS

digital readout system, influenza, rapid influenza diagnostic test, sensitivity, specificity

1 | INTRODUCTION

Seasonal influenza infection can be a burden on public health authorities. Rapid diagnosis is an important initial step in the appropriate management of influenza disease. Traditionally, viral cultures, serological tests, rapid antigen tests, and molecular methods have been used to diagnose influenza infection.[1] Rapid influenza diagnostic tests (RIDTs) have been used widely in the clinical setting because they can be handled readily and at relatively low cost, although they have low and variable detection sensitivities. Thus, a negative RIDT result does not confirm a status free of influenza virus infection. The clinical sensitivities of these tests have been reported to range from 20% to 90%, according to sample collection method, storage and transport, specimen type, swab and transport media used, and degree of adherence

to manufacturers' recommendations for test procedures.[1] The performance of RIDTs is also dependent on the prevalence of the influenza viruses circulating in the population.[2,3] Furthermore, clinicians should be cautious about using RIDTs in certain patient populations and with respect to how the results should be interpreted.[3-5] Thus, further validation of RIDTs should be provided before routine clinical use. In this study, we compared three RIDTs with digital readout systems, and one conventional RIDT, with respect to the detection sensitivity and relative limit of detection according to influenza subtype.

2 | MATERIALS AND METHODS

We included 218 left-over nasopharyngeal swab specimens from non-duplicated patients who visited the hospital with suspicion of influenza infection. A total of 218 specimens were sum of 90 adults and 128 children (0-17 years old). All specimens were deeply frozen at −70°C or lower before the comparison tests. Nasopharyngeal swab specimens were collected from patients, between neonates and 90 years old, from February 2016 to March 2016 and stored in universal transport medium (UTM; Asan Pharmaceutical, Seoul, Republic of Korea). The study was approved by the Institutional Review Board (IRB) of Kangwon National University School of Medicine (IRB No. KNUH-2016-02-002).

We compared three RIDTs with digital readout systems: BUDDI (NanoEnTek, Seoul, Korea), the Sofia Influenza A+B Fluorescence Immunoassay (Quidel, San Diego, CA, USA), and the BD Veritor System Flu A+B assay (BD Diagnostics, Sparks, MD, USA). A conventional rapid test (SD Bioline Influenza Ag A/B/A(H1N1/2009), Standard Diagnostics, Yongin, Korea) system for influenza detection was included for comparison. All RIDT results were compared with the results of real-time PCR results obtained with the Anyplex FluA/B Typing Real-time Detection system (Seegene, Seoul, Korea).

2.1 | Three digital rapid influenza antigen detection tests

2.1.1 | BUDDI Influenza A and B test

BUDDI (NanoEnTek) is a newly developed digital readout system to diagnose influenza virus. The method is based on immunochromatography and a digital readout system. Briefly, an antibody colloidal gold probe was applied on the conjugate pad, and influenza antibody was immobilized to a nitrocellulose membrane as the capture reagent to prepare the RIDT strip test. Equal volumes of specimen in UTM (75 μL) and reagent buffer (75 μL) were mixed thoroughly and five drops (100 μL) of mixed sample was loaded into the test device. In 15 minutes, the BUDDI device reads the intensity of the reaction band. If the band intensity is higher than a cut-off level, BUDDI reports a positive result of influenza infection. This test required less than 30 minutes to accomplish the qualitative detection of influenza antigen. The results of the BUDDI system can be transferred to a central laboratory or the Centers for Disease Control (CDC) via an appropriate wireless network.

2.1.2 | Sofia Influenza A+B Fluorescence Immunoassay

The Sofia Influenza A+B Fluorescence Immunoassay (Quidel) is a rapid diagnostic kit that uses immunofluorescence technology to enhance its sensitivity. It detects the nucleoprotein of the influenza virus and can discriminate strain A from B in nasal swabs, aspirates, and nasopharyngeal swab specimens. This test uses a lateral flow design with location-dependent lines and zones. The Sofia Analyzer scans the test strip and displays the results after using method-specific algorithms. This assay can be completed within 15 minutes, and results are reported as positive or negative for influenza virus A or B, without providing the numerical value assigned for each specimen. Briefly, equal volumes of specimen in UTM (250 μL) and reagent buffer (prepared by reconstituting lyophilized buffer with detergent and reducing agent, 250 μL) were mixed in a reaction tube. A final volume of 120 μL of sample was placed in a Sofia reaction cassette using a premeasured pipette, incubated for 15 minutes, and analyzed using the Sofia fluorescent reader. The results also can be interfaced directly using a local area network (LAN) to a laboratory information system.

2.1.3 | BD Veritor System Flu A+B assay

BD Veritor System Flu A+B assay (BD Diagnostics) is a rapid qualitative chromatographic immunoassay for the detection of influenza A and B viral nucleoprotein antigens from nasal and nasopharyngeal swabs of patients with a time-to-result of 10 minutes. The system comprises a unique reagent tube containing mucolytic agents and a detergent solution, a test device, and a digital reader. When nasal or nasopharyngeal swab specimens are processed with the reagent tube and added to the test device, influenza A or B viral antigens bind to anti-influenza antibodies conjugated to detector particles in the test strip. The antigen-conjugate complex migrates across the test strip to the reaction area, and is captured by antibodies against influenza A, influenza B, and a control antibody on three separate membranes.[6] For this study, 75 μL of specimen in UTM was mixed with an equal volume of lysis buffer (75 μL). Three drops (80 μL) of the mixed sample were placed into a Veritor test device cassette and allowed 10 minutes for incubation. Then, the cassette was placed into the colorimetric reader for analysis.

2.2 | Conventional rapid influenza antigen detection test

The SD Bioline Influenza Ag A/B/A(H1N1/2009) (Standard Diagnostics) is an immunochromatographic assay for the qualitative detection of influenza virus types A and type B using embedded mouse monoclonal anti-influenza A and anti-influenza B antibodies on the test strip.[7] Briefly, 75 μL of nasopharyngeal specimen in UTM was mixed with the same volume of reagent solution. The test strip was inserted into a tube containing the total volume of 150 μL of the reaction mixture. Test results were examined visually and interpreted after 10-15 minutes.

2.3 | Real-time reverse transcription PCR (rRT-PCR) analysis

Viral RNAs were extracted from 530 μL of the nasopharyngeal specimen using the SeePrep12 Viral NA kit (Seegene) and the SeePrep12 Viral NA instrument (Seegene). This is an automated nucleic acid (NA) extraction system for downstream detection, for extracting and purifying NAs using a magnetic bead method. An internal control (10 μL) was added to each specimen before the extraction step to confirm the entire process, from NA extraction to PCR.

The Anyplex FluA/B Typing Real-time Detection (Seegene)[8] was used to determine the presence or absence of the influenza A and B viruses and 2009 pandemic H1 (pdm09, 2009 pandemic strain of swine origin). The assay detects the M1 gene of influenza A, the PB1 gene of influenza B, and the H1 gene of the A (H1N1) pdm09 virus. Real-time reverse transcription PCR was performed according to the manufacturer's instructions. Briefly, 5 μL of RNA was added to 20 μL one-step RT-PCR master mix solution containing 5× FluA/B OM, 5× Anyplex RT-PCR buffer, Anyplex RT-PCR enzyme mix, and RNase-free water. Positive and negative controls were included in each run. The CFX96 real-time thermocycler (Bio-Rad, Hercules, CA, USA) was used for amplification and detection of the signal. Conditions were as follows: initial incubation at 50°C for 20 minutes and 95°C for 15 minutes, followed by 45 cycles of 95°C for 30 seconds and 55°C for 60 seconds.

2.4 | Comparison of influenza subtype detection limits

A comparison study was performed to compare the influenza subtype detection capacity among four different RIDT assays using 20 different influenza antigen reagents from the National Institute for Biological Standards and Control (NIBSC). First, the stock solution of each subtype reagent was diluted in the recommended buffer to 512 ng/mL. Then, the 512 ng/mL NIBCS influenza A subtypes were diluted serially to 128, 64, 16, 8, 4, 2, and 1 ng/mL. The 512 ng/mL NIBCS influenza B subtypes were diluted serially to 128, 64, 16, and 8 ng/mL. The diluted subtype reagents were measured to determine and compare the detection limit of each RIDT.

2.5 | Cross-reactivity with other respiratory viruses

The nonspecific positive results of four kinds of RIDTs with other respiratory viruses excluding influenza viruses were identified. All of 29 respiratory swab specimens were confirmed by real-time PCR (Seegene RV16 real-time PCR, Seegene Seoul, Korea) using manufacturer's recommended real-time PCR protocol. The RV16 real-time PCR has been updated from previous reports.[9–12] Those swab specimens had five adenovirus (AdV), one parainfluenza virus 1 (PIV 1), one parainfluenza virus 3 (PIV 3), eight rhinovirus A/B/C (HRV), one respiratory syncytial virus A (RSV A), one respiratory syncytial virus B (RSV B), three bocavirus 1/2/3/4 (HBoV), three metapneumovirus (MPV), one coronavirus NL63 (CoV NL63), one coronavirus OC43 (CoV OC43). Also, compound viral infection specimens such as 1 AdV+PIV 2+ HRV+Enterovirus (HEV), 1 HRV+HEV, 1 HRV+CoV NL63, 1 HRV+HBoV were tested for cross-reactivity of four RIDTs.

3 | RESULTS

3.1 | Comparison of RIDT specification

The four RIDTs were compared according to sample volume, assay time, discrimination of influenza A and B, recommended specimen types, and test principles. We briefly describe the specifications of each RIDT in Table 1. Also, the 218 enrolled samples were classified as 73 cases of influenza A, 60 of influenza B, and 85 negatives (Table 2).

3.2 | Comparison of sensitivity and specificity

For influenza A, BUDDI, Sofia, Veritor, and Bioline showed 87.7%, 94.5%, 87.7%, and 72.6% detection sensitivity and 100%, 97.7%, 96.5%, and 100% detection specificity, respectively.

TABLE 1 Characteristics of each rapid diagnostic test for influenza virus detection

Rapid test kits	Assay volume (μL)	Assay time (min)	Discrimination of influenza A/B	Recommended specimen	Interpretation
BUDDI influenza A/B	100	15	Yes	NPW, NPA, NPS, LNS, TS	Optical reader
Sofia Influenza A+B Fluorescence Immunoassay	120	15	Yes	NPW, NPA, NPS	Fluorescence reader
BD Veritor System Flu A+B	80	10	Yes	NPW, NPA, NPS	Optical reader
SD Bioline Influenza Ag A/B/A(H1N1/2009)	150	10-15	Yes	NPW, NPA, NPS, LNS, TS, BAL	Visual

NPW, nasopharyngeal wash; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; LNS, lower nasal swab; TS, throat swab; BAL, bronchoalveolar lavage.

TABLE 2 Characteristics of enrolled patients

Characteristics	Total	Influenza A	Influenza B	Negative
Number of patients	218	73	60	85
Mean age (y range)	23.8 (2 mo-90)	25.3 (5 mo-87)	10 (2 mo-73)	32.2 (2 mo-90)
Male/Female ratio	0.8 (99:119)	0.8 (33:40)	0.9 (29:31)	0.8 (37:48)

TABLE 3 Performance characteristics of four rapid influenza diagnostic tests for the detection of influenza A/B compared to RT-PCR

Influenza type	Rapid test	Sensitivity % (n, 95% CI)	Specificity % (n, 95% CI)	PPV % (n, 95% CI)	NPV % (n, 95% CI)
Influenza A (n=73)	BUDDI	87.7 (64/73, 77.9-94.2)	100 (85/85, 95.8-100)	100 (64/64, NA)	90.4 (85/94, 83.7-94.6)
	Sofia	94.5 (69/73, 86.6-98.5)	97.7 (83/85, 91.8-99.7)	97.2 (69/71, 89.8-99.3)	95.4 (83/87, 88.9-98.2)
	Veritor	87.7 (64/73, 77.9-94.2)	96.5 (82/85, 90-99.3)	95.5 (64/67, 87.5-98.5)	90.1 (82/91, 83.2-94.4)
	Bioline	72.6 (53/73, 60.9-82.4)	100 (85/85, 95.8-100)	100 (53/53, NA)	81 (85/105, 74.5-86.1)
Influenza B (n=60)	BUDDI	81.7 (49/60, 69.6-90.5)	100 (85/85, 95.8-100)	100 (49/49, NA)	88.5 (85/96, 81.9-93)
	Sofia	91.7 (55/60, 81.6-97.2)	95.3 (81/85, 88.4-98.7)	93.2 (55/59, 84-97.3)	94.2 (81/86, 87.5-97.4)
	Veritor	81.7 (49/60, 69.6-90.5)	100 (85/85, 95.8-100)	100 (49/49, NA)	88.5 (85/96, 81.9-93)
	Bioline	78.3 (47/60, 65.8-87.9)	100 (85/85, 95.8-100)	100 (47/47, NA)	86.7 (85/96, 80.2-91.4)

BUDDI, BUDDI Influenza A/B test; Sofia, Sofia Influenza A+B Fluorescence Immunoassay; Veritor, BD Veritor System Flu A+B; Bioline SD Bioline Influenza Ag A/B/A(H1N1/2009); PPV, positive predictive value; NPP, negative predictive value; NA, not applicable.

The positive predictive value (PPV) and negative predictive value (NPV) were 100%, 97.2%, 95.5%, 100% and 90.4%, 95.4%, 90.1%, 81%, respectively.

For influenza B, BUDDI, Sofia, Veritor, and Bioline showed 81.7%, 91.7%, 81.7%, and 78.3% detection sensitivity and 100%, 95.3%, 100%, and 100% detection specificity, respectively.

The positive predictive value (PPV) and negative predictive value (NPV) were 100%, 93.2%, 100%, 100% and 88.5%, 94.2%, 88.5%, 86.7%, respectively (Table 3).

3.3 | Comparison of sensitivity and specificity according to age

In children (birth to 17), for influenza A, BUDDI, Sofia, Veritor, and Bioline showed 92.1%, 97.4%, 94.7%, and 76.3% detection sensitivity and 100%, 100%, 97.4%, and 100% detection specificity, respectively. For influenza B, BUDDI, Sofia, Veritor, and Bioline showed 84.3%, 94.1%, 86.3%, and 82.4% detection sensitivity and 100%, 94.9%, 100%, and 100% detection specificity, respectively (Table 4).

In adults (over 17), for influenza A, BUDDI, Sofia, Veritor, and Bioline showed 82.9%, 91.4%, 80.0%, and 68.6% detection sensitivity and 100%, 95.7%, 95.7%, and 100% detection specificity, respectively. For influenza B, BUDDI, Sofia, Veritor, and Bioline showed 66.7%, 77.8%, 55.6%, and 55.6% detection sensitivity and 100%, 95.7%, 100%, and 100% detection specificity, respectively (Table 4).

3.4 | Comparison of RIDT detection limits

Serially diluted NIBSC solutions showed some differences in low-level detection power between the RIDTs. Overall, the digitalized influenza detection tests had a higher detection power than those of the conventional RIDT. Also, BUDDI showed high detection sensitivity for the A/Texas/36/91, A/Beijing/262/95, and A/Sydney/5/97 influenza subtypes. Sofia showed high detection sensitivity for A/Texas/36/91, A/Singapore/1/57, A/Vietnam/1194/2004, and A/Cambodia/RO 405050/2007. Veritor showed high detection sensitivity for A/Sydney/5/97, A/New York/55/2004, A/Vietnam/1194/2004, and A/Cambodia/RO405050/2007. Specific subtypes of influenza A, such as A/Equine/Newmarket/1/93 (H3N8), could not be detected by the Sofia or Bioline in 512 ng/mL NIBSC stock solution. For the influenza B subtype, Sofia had better detection sensitivity than the other RIDTs (Table 5).

3.5 | Cross-reactivity for other respiratory viruses

We identified that four RIDTs did not show cross-reactivity results in 29 specimens which had other respiratory viruses excluding influenza viruses.

4 | DISCUSSION

Influenza can be a major public health burden. Both influenza A and influenza B have caused over 200 000 hospitalizations and 30 000-50 000 deaths.[13–17] Diagnosis of influenza infections include viral isolation in cell culture, immunofluorescence assays, nucleic acid amplification tests, immunochromatography-based rapid diagnostic tests, etc.[18]

The rapid diagnosis of influenza may help in managing patients and lowering overall treatment costs. The available rapid diagnostic assays are relatively simple to perform and can produce results within less than 30 minutes, but they suffer from inaccuracy, with widely varying diagnostic sensitivities and specificities.[3,19] Indeed, RIDTs may have inconsistent accuracy, with reported sensitivities ranging from 10% to 80%,[3,5,20,21] whereas specificity usually exceeds 90%.

In this study, we evaluated a new RIDT, BUDDI, for the first time. BUDDI is underpinned by an immunochromatographic method with a digital readout system. The reaction band of BUDDI is digitalized according to reaction intensity. Final results of BUDDI can be transferred through a network to another place to help rapid diagnosis. The sensitivities were higher in the digital readout systems than in the "conventional" RIDT, as we expected. Sofia showed the highest sensitivity for influenza A and B detection. BUDDI showed the same sensitivity as Veritor. The cause of the difference may be related to the immunofluorescence technology of Sofia, which enhances its sensitivity. In this study, we identified 66 influenza H1N1 subtype among 73 influenza

TABLE 4 Performance characteristics of four rapid influenza diagnostic tests for the detection of influenza A/B compared to RT-PCR in children (birth to 17) and adults (>17)

Influenza type	Rapid test	Sensitivity % (value, 95% CI)	Specificity % (value, 95% CI)
Children Influenza A (n=38)	BUDDI	92.1 (35/38, 78.6-98.3)	100 (39/39, 91.0-100)
	Sofia	97.4 (37/38, 86.2-99.9)	100 (39/39, 91.0-100)
	Veritor	94.7 (36/38, 82.3-99.4)	97.4 (38/39, 86.5-99.9)
	Bioline	76.3 (29/38, 59.8-88.6)	100 (39/39, 91.0-100)
Children Influenza B (n=51)	BUDDI	84.3 (43/51, 71.4-93.0)	100 (39/39, 91.0-100)
	Sofia	94.1 (48/51, 83.8-98.8)	94.9 (37/39, 82.7-99.4)
	Veritor	86.3 (44/51, 73.7-94.3)	100 (39/39, 91.0-100)
	Bioline	82.4 (42/51, 69.1-91.6)	100 (39/39, 91.0-100)
Adult Influenza A (n=35)	BUDDI	82.9 (29/35, 66.4-93.4)	100 (46/46, 92.3-100)
	Sofia	91.4 (32/35, 76.9-98.2)	95.7 (44/46, 85.2-99.5)
	Veritor	80 (28/35, 63.1-91.6)	95.7 (44/46, 85.2-99.5)
	Bioline	68.6 (24/35, 50.7-83.2)	100 (46/46, 92.3-100)
Adult Influenza B (n=9)	BUDDI	66.7 (6/9, 29.9-92.5)	100 (46/46, 92.3-100)
	Sofia	77.8 (7/9, 40-97.2)	95.7 (44/46, 85.2-99.5)
	Veritor	55.6 (5/9, 21.2-86.3)	100 (46/46, 92.3-100)
	Bioline	55.6 (5/9, 21.2-86.3)	100 (46/46, 92.3-100)

BUDDI, BUDDI Influenza A/B test; Sofia, Sofia Influenza A+B Fluorescence Immunoassay; Veritor, BD Veritor System Flu A+B; Bioline SD Bioline Influenza Ag A/B/A(H1N1/2009).

TABLE 5 Comparison of the lower limit of detection level of four kinds of rapid diagnostic test for influenza virus detection

NIBSC code	Influenza A strain	Subtype	BUDDI (ng/mL)	Sofia (ng/mL)	Veritor (ng/mL)	Bioline (ng/mL)
92/530	A/Texas/36/91	H1N1	<1	<1	2-4	4-8
97/760	A/Beijing/262/95	H1N1	<1	2-4	2-4	16-64
01/614	A/New Caledonia/20/99	H1N1	16-64	16-64	16-64	>512
99/714	A/Singapore/1/57	H2N2	1-2	<1	4-8	16-64
80/517	A/Bangkok/1/79	H3N2	4-8	4-8	4-8	16-64
93/500	A/Beijing/32/92	H3N2	128-512	128-512	128-512	>512
97/596	A/Equine/Newmarket/1/93	H3N8	16-64	>512	16-64	>512
99/624	A/Sydney/5/97	H3N2	1-2	4-8	1-2	16-64
00/552	A/Duck/Singapore-Q. F119-3/97	H5N3	4-8	4-8	4-8	64-128
07/336	A/mallard/ Netherlands/12/2000	H7N3	64-128	64-128	16-64	128-512
04/264	A/New York/55/2004	H3N2	4-8	4-8	1-2	64-128
09/184	A/Vietnam/1194/2004	H5N1	1-2	<1	<1	16-64
05/234	A/Hiroshima/52/2005	H3N2	4-8	4-8	4-8	64-128
06/120	A/Wisconsin/67/2005	H3N2	8-16	4-8	4-8	128-512
08/216	A/Cambodia/RO405050/2007	H5N1	4-8	1-2	1-2	16-64
NIBSC code	**Influenza B strain**		**BUDDI (ng/mL)**	**Sofia (ng/mL)**	**Veritor (ng/mL)**	**Bioline (ng/mL)**
04/110	B/jiangsu/10/2003		8-16	<8	<8	16-64
92/628	B/Yamagata/16/668		128-512	64-128	128-512	128-512
06/126	B/Malaysia/2506/2004		>512	128-512	>512	>512
14/252	B/Phuket/3073/2013		>512	64-128	>512	>512
16/118	B/Brisbane/60/2008		>512	64-128	>512	>512

NIBSC, National Institute for Biological Standards and Control; BUDDI, BUDDI Influenza A/B test; Sofia, Sofia Influenza A+B Fluorescence Immunoassay; Veritor, BD Veritor System Flu A+B; Bioline SD Bioline Influenza Ag A/B/A(H1N1/2009).

A type persons (90.4%) through real-time PCR. It is important to understand the detection power of RIDT according to the influenza subtypes. Because influenza shows seasonal variations, specific influenza subtypes should be considered in upgrade of RIDTS.

We also compared the four RIDTs according to the NIBSC subtype of influenza to evaluate the detection power of each test. There were some differences in detection power according to NIBSC subtype. BUDDI showed high detection sensitivity for A/Texas/36/91, A/Beijing/262/95, and A/Sydney/5/97. Sofia showed high detection sensitivity for A/Texas/36/91,A/Singapore/1/57,A/Vietnam/1194/2004, and A/Cambodia/RO405050/2007. Veritor showed high detection sensitivity for A/Sydney/5/97, A/New York/55/2004, A/Vietnam/1194/2004, and A/Cambodia/RO405050/2007. However, specific subtypes, such as A/Beijing/32/92 (Bioline) and A/Equine/Newmarket/1/93 (Sofia and Bioline), B/Malaysia/2506/2004 (BUDDI, Veritor and Bioline), B/Phuket/3073/2013 (BUDDI, Veritor and Bioline), B/Brisbane/60/2008 (BUDDI, Veritor and Bioline), could not be detected at the level of the 512 ng/mL NIBSC stock solution. These differences could be caused by different coverage levels of monoclonal antibodies to specific influenza subtypes. RIDTs should be updated to target prevalent influenza subtypes and to identify many influenza subtypes (to increase detection sensitivity).

This study had some limitations. First, we did not include large number of samples and various influenza subtypes. Second, the mean age of the enrolled individuals was relatively young, at 10-32.2 years, for a random selection within a specific period. Cruz et al.[2] reported that the performance of RIDTs have been known to be better in children compared with adults (approximately 13% higher), maybe due to higher viral loads and longer viral shedding in children compared with adults. We also identified the higher sensitivity of four RIDTs in children group than those of adults in our study. Third, test results were compared only with those of real-time PCR, which could have false positives and negatives. Also, only nasopharyngeal swab specimens were included, without considering specimen variations. However, for the first time, this study presented the detection sensitivity of a newly developed digital readout system, BUDDI. The BUDDI had the same detection sensitivity as a previously launched RIDT (Veritor), and higher sensitivity than a conventional RIDT. However, BUDDI showed less sensitivity than Sofia. Also, BUDDI could detect more than other RIDTs in terms of specific influenza biotypes, such as influenza A A/Texas/36/91, A/Beijing/262/95, and A/Sydney/5/97.

In conclusion, RIDTs with digital readout systems showed higher detection sensitivity than a conventional rapid test. The fluorescence technique of Sofia gave it the highest detection sensitivity, but there were differences in low-level detection power according to influenza subtype. Further well-designed prospective studies are needed for additional assessment of the value of updated RIDTs according to specific influenza subtypes.

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