BRIEF REPORT

PERFORMANCE OF TWO COMMERCIAL FLUORESCENCE IMMUNOASSAYS FOR DETECTION OF INFLUENZA A AND B IN REPUBLIC OF KOREA

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Abstract. Fluorescence immunoassay (FIA) has become the method of choice for rapid detection of influenza. Here, we evaluated, using a multiplex real time polymerase chain reaction (RT-PCR) assay as reference, the performance of a newly developed STANDARD F Influenza A/B FIA equipped with a digital readout with that of Sofia Influenza A+B FIA on nasopharyngeal swab specimens (n = 16,810) collected from February 2015 to November 2019. For detection of influenza A, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of STANDARD F Influenza A/B FIA was 55.2%, 99.4%, 71.1%, and 98.7%, respectively, and 63.6%, 99.8%, 36.8%, and 99.9%, respectively for influenza B. Comparable values were obtained with Sofia Influenza A+B FIA, except specificity of influenza B detection is significantly lower compared to that of STANDARD F Influenza A/B FIA (p-value <0.001). When specimens were stratified according to age of subjects, STANDARD F Influenza A/B FIA sensitivity in detecting influenza A was highest for ≤18 years of age and lowest (reduced by ~50%) for 19-64 years of age. Due to lack of adequate positive samples, no comparative data were available for influenza B detection. In conclusion, STANDARD F Influenza A/B FIA demonstrated satisfactory performance for detecting influenza A and B, and could be used as a point-of-care detection method particular in children, with the caveat that negative results for adults should be confirmed by RT-PCR (at least in the detection of influenza A).

Keywords: age stratification, fluorescence immunoassay, influenza A, influenza B, nasopharyngeal swab

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INTRODUCTION

Influenza is a contagious respiratory disease with clinical presentations ranging from asymptomatic to severe complications, resulting in a high rate of morbidity and even death (Nicholson *et al*, 2003; Vos *et al*, 2019). Thus, it is difficult to distinguish viral infection stemming from influenza A or B or from other febrile viral infections, such as adenovirus, human metapneumovirus, rhinovirus, or respiratory syncytial virus, without confirmatory tests (Call *et al*, 2005). Expeditious and accurate diagnostic procedures are critical for avoiding unnecessary antibiotic use and in implementing prompt infection control measures (Hayden *et al*, 1989; Falsey *et al*, 2007; Low, 2008).

Virus culture is the gold standard for diagnosis of influenza virus (Vemula *et al*, 2016), but this is a lengthy process and RT-PCR has become the most ideal means of diagnosis (Hazelton *et al*, 2015; Merckx *et al*, 2017; Vos *et al*, 2019). However, RT-PCR requires relatively expensive, specialized equipment and skilled personnel. Rapid influenza diagnostic tests (RIDTs) are currently playing a stellar role as point-of-care tests (POCTs) in routine clinical settings as they have high specificity (76-100%) but varying sensitivity (10-96%) (Piche-Renaud *et al*, 2016; Merckx *et al*, 2017; Yang *et al*, 2018). On the other hand, RIDTs are comparatively convenient, time efficient and cost effective, and have been widely deployed in primary care hospitals (Merckx *et al*, 2017;Vos *et al*, 2019). In order to enhance the large variations in sensitivity, fluorescence immunoassays (FIAs) with digital readouts were developed to replace RIDT strip tests that can produce equivocal colorimetry visual results (Ryu *et al*, 2016).

Here, we carried out a retrospective evaluation of the diagnostic performances of two commercial FIAs in comparison to RT-PCR assay result comparisons, in order to evaluate the clinical usefulness of rather recently commercialized STANDARD F Influenza A/B FIA in Korea.

MATERIALS AND METHODS

Data collection

We conducted a retrospective data analysis using the medical records of influenza A and B assay that had been carried out in the Department of Laboratory Medicine, Inje University

Busan Paik Hospital, Busan, Republic of Korea from February 2015 to May 2021.

The study protocols were approved by the Institutional Review Board, Inje University Busan Paik Hospital (BPIRB 2021-06-038) with participant consent exemption.

Laboratory investigations

Sofia Influenza A+B FIA (Quidel Corp, San Diego, CA) was employed for assay of influenza A and B from February 2015 to November 2019 and STANDARD F Influenza A/B FIA (SD Biosensor Inc, Suwon, Republic of Korea) from February 2018 to May 2021. All samples were tested by LG AdvanSure RV Real-Time PCR (LG Chem Ltd, Seoul, Republic of Korea). In both the STANDARD F Influenza A/B FIA and Sofia Influenza A+B FIA, the test strip was scanned and fluorescence reported in arbitrary unit.

Statistical analysis

Samples were divided into three age groups, and percent sensitivity, percent specificity, percent positive predictive value, and percent negative predictive value [plus corresponding 95% confidence interval (CI)] of STANDARD F Influenza A/B and Sofia Influenza A+B FIAs were determined for each age group, employing LG AdvanSure RV RT-PCR assay as reference. Pearson's chi-squared test was used for comparing sensitivities or specificities between kits or age groups and we interpreted it as significant when p-value <0.05.

RESULTS

Among samples (n = 16,810) collected from February 2015 to May 2021, 5,987 (35.6%) samples were assayed by STANDARD F Influenza A/B FIA resulting in 128 and 19 positives for influenza A and B respectively, and 10,823 (64.4%) samples by Sofia Influenza A+B FIA yielding 383 and 289 were positives for influenza A and B respectively (Table 1). LG AdvanSure RV RT-PCR assay confirmed 165 and 11 samples were positive for influenza A and B respectively among 5,987 samples tested for the STANDARD F Influenza A/BTM FIA and 553 and 202 respectively among 10,823 samples tested for Sofia Influenza A+B FIA.

In the detection of influenza A, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were not significantly different between both FIAs, and similar results were obtained for detection of influenza B, except percent specificity of Sofia Influenza A+B FIA was significantly lower than that of STANDARD F Influenza A/B FIA (p-value <0.001) (Table 2).

When data were stratified by age groups, namely, ≤18, 19-64 and ≥65 years of age, detection of influenza A by STANDARD F Influenza A/B FIA was most sensitive for samples from ≤18 years of age and lowest among 19-64 years of age (Table 2). As regards to the detection of influenza B, owing to the low number of positive samples

Table 1

Comparison of influenza A and B detection among two fluorescence immunoassays (FIAs) and multiplex RT-PCR assay of samples collected in the Republic of Korea (February 2015 - May 2021).

Influenza type	RT-PCR[a]	Standard F[b] FIA (Number of samples)			Sofia[c] FIA (Number of samples)		
		Positive	Negative	Total	Positive	Negative	Total
Influenza A	Positive	91	74	165	296	257	553
	Negative	37	5,785	5,822	87	10,183	10,270
	Total	128	5,859	5,987	383	10,440	10,823
Influenza B	Positive	7	4	11	98	104	202
	Negative	12	5,964	5,976	191	10,430	10,621
	Total	19	5,968	5,987	289	10,534	10,823

[a]LG AdvanSure RV Real-time PCR (LG Chem Ltd, Seoul, Republic of Korea); [b]STANDARD F Influenza A/B FIA (SD Biosensor Inc, Suwon, Korea); [c]Sofia Influenza A+B FIA (Quidel Corp, San Diego, CA)
RT-PCR: real time polymerase chain reaction

Table 2

STANDARD F and Sofia fluorescence immunoassays (FIAs) performances in detecting influenza A and B in samples from three age groups of subjects in Republic of Korea (February 2015 - May 2021)

Influenza type/FIA system	Age group, years (Number of samples)	Percent sensitivity (95% CI)	Percent specificity (95% CI)	Percent PPV (95% CI)	Percent NPV (95% CI)
Influenza A					
STANDARD F[a]	≤18 (743)	87.0 (67.9-95.5)	98.9 (97.8-99.4)	71.4 (54.7-88.2)	99.6 (99.1-100)
	19-64 (1,650)	36.7 (24.7-50.7)	99.2 (98.6-99.5)	58.1 (40.7-75.4)	98.1 (97.4-98.8)
	≥65 (3,594)	57.0 (46.9-66.6)	99.5 (99.3-99.7)	76.8 (66.9-86.8)	98.9 (98.5-99.2)
	Total (5,987)	55.2 (47.5-62.5)	99.4 (99.1-99.5)	71.1 (63.2-78.9)	98.7 (98.5-99.0)
Sofia[b]	≤18 (2,983)	71.7 (62.5-79.4)	99.4 (99.0-99.6)	81.7 (73.9-89.6)	99.0 (98.6-99.3)
	19-64 (2,552)	45.5 (36.9-54.3)	99.0 (98.5-99.3)	68.8 (58.6-78.9)	97.3 (96.7-98.0)
	≥65 (5,288)	50.6 (45.2-56.0)	99.1 (98.8-99.3)	78.6 (73.0-84.1)	96.8 (96.3-97.3)
	Total (10,823)	53.5 (47.4-57.6)	99.2 (99.0-99.3)	77.3 (73.1-81.5)	97.5 (97.2-97.8)
Influenza B					
STANDARD F[a]	≤18 (743)	-c	-c	-c	-c
	19-64 (1,650)	-c	-c	-c	-c
	≥65 (3,594)	62.5 (30.6-86.3)	99.9 (99.7-99.9)	50.0 (19.0-81.0)	99.9 (99.8-100)
	Total (5,987)	63.6 (35.4-84.8)	99.8 (99.7-99.9)	36.8 (15.2-58.5)	99.9 (99.9-100)

Table 2 (cont)

Influenza type/FIA system	Age group, years (Number of samples)	Percent sensitivity (95% CI)	Percent specificity (95% CI)	Percent PPV (95% CI)	Percent NPV (95% CI)
Sofia[b]	≤18 (2,983)	58.2 (47.2-68.5)	98.6 (98.1-99.0)	52.9 (42.4-63.4)	98.9 (98.5-99.2)
	19-64 (2,552)	39.5 (25.6-55.3)	98.1 (97.5-98.6)	24.2 (13.5-34.9)	99.1 (98.7-99.5)
	≥65 (5,288)	43.5 (33.5-54.1)	98.0 (97.6-98.4)	26.4 (19.1-33.7)	99.1 (98.8-99.3)
	Total (10,823)	48.5 (41.7-55.4)	98.2 (97.9-98.4)	33.9 (28.5-39.4)	99.0 (98.8-99.2)

[a]STANDARD F Influenza A/B™ FIA (SD Biosensor Inc, Suwon, Korea); [b]Sofia Influenza A+B™ FIA (Quidel Corp, San Diego, CA); [c]Too few positive cases to allow calculation of reliable value
CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value
Performance was compared to LG AdvanSure RV Real-time PCR (LG Chem Ltd, Seoul, Republic of Korea)

obtained from STANDARD F Influenza A/B FIA for both ≤18 and 19-64 years of age groups, no comparisons based on age stratifications could be made between the two FIAs (Table 2).

DISCUSSION

Traditional RIDTs that detect viral antigens by immunoassays have been widely deployed as a diagnostic tool for influenza diagnosis; however, the convenience is compromised by poor and varying sensitivity (Merckx *et al*, 2017). In order to improve RIDT sensitivity, FIAs such as Sofia Influenza A+B FIA, and STANDARD F Influenza A/B IFA have been developed with digital reading systems that can complement the ambiguity of visual interpretation.

A meta-analysis of the performances of existing FIAs indicated BD Veritor™ System Flu A + B FIA (BD Diagnostics, Sparks, MD) has the sensitivity between 64 and 94% for both influenza A and B detection), and Sofia Influenza A+B FIA showed a sensitivity of 41-96 and 33-98% for influenza A and B detection respectively (Merckx *et al*, 2017). In our study, the STANDARD F Influenza A/BTM FIA demonstrated sensitivity of influenza A and B detection within the literature range normalized to an RT-PCR assay.

Merckx *et al* (2017) reported pooled FIA specificity is 98.3 and 98.7% for influenza A and B respectively. Similar results were obtained with STANDARD

F Influenza A/B FIA. It is worth noting that specificity of influenza B detection by STANDARD F assay (99.8%) is slightly, but significant, higher than that by Sofia assay (98.2%) (*p*-value <0.001).

Sensitivity of RIDTs have been shown to be higher in samples from children (Cho *et al*, 2013; Merckx *et al*, 2017), a finding also observed in our study (in ≤18 years of age group compared to adults). This result is thought to be due a higher viral load in children whose immune system is still not fully developed (Frank *et al*, 1981). Our results showed that the sensitivity of STANDARD F maintained its high until 18 years although some other studies reported a decrease of sensitivity after 2 or 5 years (Cheng *et al*, 2009; Gao *et al*, 2012). STANDARD F Influenza A/B FIA could be recommended as a POCT for influenza in children. Sensitivity of STANDARD F FIA for detecting influenza A among samples from 19-64 years of age group was the lowest (36.7%) compared to samples from other subjects, suggesting that negative FIA results in this age group should be confirmed by an RT-PCR assay. The variations in FIA's diagnostic performance for influenza among different age groups highlight the need to test FIA kits across a wide age range prior to widespread adoption.

There are several limitations in our study. Firstly, we could not compare the two FIA test kits on the same specimens. Secondly, the duration of symptoms before samples were collected were not recorded, an important factor that could affect FIA performance (Cheng *et al*, 2009; Merckx *et al*, 2017). Thirdly, owing to the limited number of samples positive for influenza B determined by the STANDARD F Influenza A/B FIA, the performance of this assay could not be assessed across all subjects ages for detection of influenza B.

In conclusion, evaluation of the performances of STANDARD F Influenza A/B fluorescence immunoassays showed this fluorescence immunoassay has comparable sensitivity when compared with Sofia Influenza A+B FIA and the STANDARD F method would be more suitable for a point-of-care test particularly for children in a clinical setting. Assessing the performance of a FIA for detection of influenza across a wide range of subjects ages is recommended before being adopted for widespread use due to variations in sensitivity among the different age groups.

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CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES

Call SA, Vollenweider MA, Hornung CA, Simel DL, McKinney WP. Does this patient have influenza? *JAMA* 2005; 293: 987-97.

Cheng CK, Cowling BJ, Chan KH, *et al*. Factors affecting QuickVue Influenza A + B rapid test performance in the community setting. *Diagn Microbiol Infect Dis* 2009; 65: 35-41.

Cho CH, Woo MK, Kim JY, *et al*. Evaluation of five rapid diagnostic kits for influenza A/B virus. *J Virol Methods* 2013; 187: 51-6.

Falsey AR, Murata Y, Walsh EE. Impact of rapid diagnosis on management of adults hospitalized with influenza. *Arch Intern Med* 2007; 167: 354-60.

Frank AL, Taber LH, Wells CR, Wells JM, Glezen WP, Paredes A. Patterns of shedding of myxoviruses and paramyxoviruses in children. *J Infect Dis* 1981; 144: 433-41.

Gao F, Loring C, Laviolette M, Bolton D, Daly ER, Bean C. Detection of 2009 pandemic influenza A(H1N1) virus Infection in different age groups by using rapid influenza diagnostic tests. *Influenza Other Respir Viruses* 2012; 6: e30-4.

Hayden FG, Belshe RB, Clover RD, Hay AJ, Oakes MG, Soo W. Emergence and apparent transmission of rimantadine-resistant influenza A virus in families. *N Engl J Med* 1989; 321: 1696-702.

Hazelton B, Nedeljkovic G, Ratnamohan VM, Dwyer DE, Kok J. Evaluation of the Sofia Influenza A + B fluorescent immunoassay for the rapid diagnosis of influenza A and B. *J Med Virol* 2015; 87: 35-8.

Low D. Reducing antibiotic use in influenza: challenges and rewards. *Clin Microbiol Infect* 2008; 14: 298-306.

Merckx J, Wali R, Schiller I, *et al*. Diagnostic accuracy of novel and traditional rapid tests for influenza infection compared with reverse transcriptase polymerase chain reaction: a systematic review and meta-analysis. *Ann Intern Med* 2017; 167: 394-409.

Nicholson KG, Wood JM, Zambon M. Influenza. *Lancet* 2003; 362: 1733-45.

Piche-Renaud PP, Turcot J, Chartrand C, *et al*. Evaluation of a fluorescent immunoassay rapid test (Sofia) for detection of influenza A+B and RSV in a tertiary pediatric setting. *Diagn Microbiol Infect Dis* 2016; 84: 304-8.

Ryu SW, Lee JH, Kim J, *et al*. Comparison of two new generation influenza rapid diagnostic tests with instrument-based digital readout systems for influenza virus detection. *Br J Biomed Sci* 2016; 73: 115-20.

Vemula SV, Zhao J, Liu J, Wang X, Biswas S, Hewlett I. Current approaches for diagnosis of influenza virus infections in humans. *Viruses* 2016; 8: 96.

Vos LM, Bruning AHL, Reitsma JB, *et al*. Rapid molecular tests for influenza, respiratory syncytial virus, and other respiratory viruses: a systematic review of diagnostic accuracy and clinical impact studies. *Clin Infect Dis* 2019; 69: 1243-53.

Yang JH, Huang PY, Shie SS, *et al*. Diagnostic performance of the Sofia® influenza A+B fluorescent immunoassay in adult outpatients in Northern Taiwan. *J Med Virol* 2018; 90: 1010-8.