ORIGINAL ARTICLE

Comparison of Rapid Influenza Diagnostic Tests With Digital Readout Systems and Conventional Rapid Influenza Diagnostic Test for Influenza Virus Detection

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ABSTRACT

Introduction: Rapid diagnosis for influenza virus infection is essential for proper patient management, delivering prompt treatment and reducing unnecessary antiviral therapy. Early diagnosis helps in disease prevention and control. Real-time reverse transcription-polymerase chain reaction (RT-PCR) assay yields high sensitivity and specificity in detecting influenza virus infection. However, it is relatively expensive and requires trained personnel and special equipment. In this study, we compared two rapid influenza diagnostic tests (RIDTs): digital readout systems (STANDARD™ F Influenza A/B FIA, fluorescence immunoassay) and conventional visual confirmation (QuickNavi™-Flu2, chromatography immunoassay) with the real-time RT-PCR assay. **Methods:** Two hundred ninety-eight respiratory samples were obtained from patients suspected of influenza infection at Siriraj Hospital from December 2018 to December 2019. **Results:** Real-time RT-PCR results showed the detection of influenza A virus in 99 samples (60%), influenza B virus in 61 samples (37%) and co-infection by both viruses in 5 samples (3%) by the real-time RT-PCR assay. The QuickNavi™-Flu2 sensitivity for detecting influenza A and B viruses were 81.73% and 84.85%, and the specificity was 100%. The STANDARD™ F Influenza A/B FIA sensitivity for detecting influenza A and B viruses were 84.62% and 83.33%, respectively. The specificity for influenza A virus detection was 99.25% and 94.74% for influenza B virus. **Conclusion:** The STANDARD™ F Influenza A/B FIA and the QuickNavi™-Flu2 showed acceptable and comparable sensitivity and specificity. Both RIDTs are potential alternative methods of real-time RT-PCR for rapid screening of influenza virus infection.

Keywords: Influenza viruses, Rapid influenza diagnostic test, Fluoroimmunoassay, Chromatography immunoassay, Real-time RT-PCR

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INTRODUCTION

Influenza is an acute respiratory disease caused by influenza type A, type B, and type C viruses. Influenza infection causes significant morbidity and mortality, especially in young children, elderly and immunocompromised individuals (1). The signs and symptoms of influenza virus infection include fever greater than or equal to 38°C, myalgia, headache, sore throat, and dry cough (2,3). Clinical manifestations of influenza virus are hardly differentiated from other respiratory viruses, such as respiratory syncytial virus, adenovirus, parainfluenza virus and others that can present as "influenza-like illness" (4,5).

Influenza has high morbidity and mortality rates; therefore, rapid and accurate diagnostic tests for influenza virus infection are crucial for patient management and disease prevention and control (6). Laboratory diagnostic tests for influenza virus infection include viral isolation in cell culture and embryonated eggs, real-time reverse transcription-polymerase chain reaction (RT-PCR), rapid molecular assays, immunofluorescence assays for viral antigen detection, and rapid antigen tests (7). Viral isolation is the gold standard for influenza diagnosis; however, it generally takes days to weeks for the result, which will exceed the therapeutic window. Currently, RT-PCR is a diagnostic method that has replaced viral isolation due to its high sensitivity and short turn-around time. However, it is an expensive method requiring special equipment and still takes greater than or equal to 4 hours of operating time (8-11). Thus, rapid influenza diagnostic test (RIDT) is an alternative method for the first-line screening, given its simple procedure with no need for special equipment, low cost, and quick result (10 to 30 minutes) (12,13). At present, there are several commercially available rapid influenza diagnostic tests, which are easy to use, quick and increasingly sensitive. Each RIDT kit has different advantages and

disadvantages. In this study, we compared two RIDTs: digital readout systems and conventional rapid influenza diagnostic tests using clinical respiratory samples, which were sent to the Virology laboratory unit, Department of Microbiology, Siriraj Hospital, Bangkok from December 2018 to December 2019. The study's benefits include selecting a convenient and simple RIDT kit, which provides more accuracy and higher sensitivity and specificity. This can further improve the efficacy of infectious disease control, provide early treatment for infected individuals and reduce unnecessary treatment for uninfected individuals.

MATERIALS AND METHODS

Ethical issues

This study was approved by the Institutional Review Board of the Faculty of Medicine Siriraj Hospital, Mahidol University [SIRB protocol 733/2561 (IRB4); COA: Si 733/2018].

Clinical specimens

Respiratory samples from patients suspected of influenza virus infection were sent to the Virology Laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital for rapid antigen testing for influenza virus from December 2018 to December 2019. Specimen types included nasal swab (NS), nasopharyngeal swab (NPS), throat swab (TS), nasopharyngeal wash/aspirate (NPW/NPA), and sputum (SPT). Samples were collected in 2 mL viral transport media (VTM) and transported at 2-8°C to the Virology Laboratory. We randomly selected approximately 10% of samples each month and recruited a total of 298 respiratory samples to this study.

Clinical specimens were tested by the QuickNavi™-Flu2 as a laboratory routine practice and compared the results with the STANDARD™ F Influenza A/B FIA at the same time. All samples were tested for influenza virus nucleic acid detection by the real-time RT-PCR assay to confirm the diagnosis.

Rapid influenza diagnostic tests (RIDTs)

The QuickNavi™-Flu2 is a chromatographic immunoassay detecting influenza A and B antigens. Monoclonal antibodies against the nucleoprotein antigens of influenza A and B viruses are separately coated on a nitrocellulose membrane. The immune complexes are captured by antibodies to influenza A or B viruses. To perform the assay, 150 µL of samples were added to the specimen buffer tube and mixed. Three drops of the extracted sample were applied into the sample well of the test device. The test result was read by visual confirmation within 5 minutes after adding the specimen.

STANDARD™ F Influenza A/B FIA is a fluorescence immunoassay using fluorescence signal detection (europium) to detect influenza virus nucleoproteins with STANDARD™ F Analyzer. In the presence of influenza A or B nucleoproteins, the europium conjugated monoclonal antibodies against an antigen of influenza A or B viruses will react and form immune fluorescence particle complexes. The intensity of fluorescence light is detected by the STANDARD™ F Analyzer (SD Biosensor, Republic of Korea). To perform the assay, 300 µL of samples were added to the specimen buffer tube and mixed. Four drops of extracted samples were applied into the sample well of the test device, followed by the signal detection according to the manufacturer's recommended procedures of STANDARD™ F200 Analyzer. The cut off index (COI) of 1.00 is interpreted as positive.

The characteristics of these two RIDTs: conventional visual confirmation and digital readout systems are demonstrated in Table I.

Table I: Comparison of technical and laboratory characteristics of the two rapid diagnostic kits for influenza

	QuickNavi™-Flu2	STANDARD™ F Influenza A/B FIA
Principle	Immunochromatographic assay	Fluorescent immunoassay
Assay volume	150 µL	300 µL
Assay time	5 min	10 min
Discrimination of influenza A/B	Yes	Yes
Recommended specimen	NPW, NPA, NPS, NS, TS	NPW, NPA, NPS, NS
Instrument	No/ Quick Navi Reader	STANDARD™ F Analyzer
Interpretation	Naked eyes/ Immunochromato reader	Digital reader only

Note. NPW: nasopharyngeal wash, NPA: nasopharyngeal aspirate, NPS: nasopharyngeal swab, NS: nasal swab, TS: throat swab

Viral RNA extraction and real-time RT-PCR assay for influenza virus RNA detection

Total RNA was extracted from 200 µL of the specimen in 200 µL lysis buffer using Magtration® Reagent MagDEA® DxSV (PSS, Japan) and was eluted with 100 µL elution buffer. Influenza virus RNA was detected by the real-time one-step RT-PCR based on multiple detection temperature (MuDT) technology (Allplex™ Respiratory Panel 1, Seegene, Korea). The RT-PCR assay was performed according to the manufacturer's recommended procedures on CFX96 Touch™ real-time PCR detection system (Bio-Rad, USA). The conditions consisted of 1 cycle of 20 min at 50°C and 15 min at 95°C and followed by 45 cycles of 10 sec at 95°C, 1 min at 60°C, 10 sec at 72°C (fluorescence was detected at 60°C and 72°C). Specimen that showed the cycle threshold before 42 cycles were considered RT-PCR positive, and more than 42 cycle were considered RT-PCR negative for influenza virus.

Statistical analysis

Descriptive statistics were used to describe general information of patients. Continuous data were presented

in median and range. Categorical data were presented in numbers, percentages, and 95% confidence interval (95%CI). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) were calculated using an online statistical tool (MedCalc's Diagnostic test evaluation calculator).

RESULTS

Characteristics of positive influenza cases determined by real-time RT-PCR

Of 298 respiratory samples, there were 133 (44.6%) samples from males and 165 (55.4%) from females. Respiratory specimens included nasal swab (n=131, 44%), throat swab (n=3, 1%), nasopharyngeal swab (n=2, 0.7%), nasopharyngeal wash (n=129, 43.3%), nasopharyngeal aspirate (n=2, 0.7%) and sputum (n=31, 10.4%). We used the real-time RT-PCR assay (Allplex™ Respiratory Panel 1, Seegene, Korea) as a reference method. Of 298 specimens, 99 samples (60%) were positive for influenza A virus only; 61 samples (37%) were positive for influenza B virus only; five samples (3%) were positive for both influenza A and B viruses. Patients with positive influenza A virus were mainly from the age group 19-59 years old (44.44%), followed by ≥60 years old (40.4%). In contrast, patients with positive influenza B virus were mainly from the age group 0-18 years old (45.9%), followed by 19-59 years old (37.7%). Patients with positive influenza A virus and influenza B virus were primarily female (56.57% and 60.66%, respectively) (Table II).

Performance of QuickNavi™-Flu2 and STANDARD™ F influenza A/B FIA rapid antigen assays compared with the real-time RT-PCR

We evaluated the two RIDTs' performance using the real-time RT-PCR as the standard test. The QuickNavi™-Flu2 correctly identified 85 samples out of 104 positive influenza A samples, 56 samples out of 66 positive influenza B samples, and all 133 samples with negative results for influenza A and B viruses (Table III). The QuickNavi™-Flu2 sensitivity for influenza A and B in all respiratory specimens were 81.73% (95%CI, 72.95 to 88.63%) and 84.85% (95%CI, 73.90 to 92.49%), respectively (Tables III and V). The assay yielded the highest sensitivity in the nasal swab/nasopharyngeal swab/throat swab (NS/NPS/TS) group, which were 90.57% for influenza A virus detection and 96.67% for influenza B virus detection. Detection of influenza viruses in sputum showed the lowest sensitivity: 71.43% for influenza A virus and 66.67% for influenza B virus (Table III). The QuickNavi™-Flu2's specificity for both influenza A and B virus detection in all respiratory specimens was 100% (95%CI, 97.26 to 100%) (Tables III and V).

On the other hand, the STANDARD™ F Influenza A/B FIA correctly identified 88 samples out of 104 positive influenza A samples. It accurately identified 55 samples of positive influenza B virus. The STANDARD™ F Influenza A/B FIA sensitivity for influenza A and B in all respiratory specimens was 84.62% (95%CI, 76.22 to 90.94%) and 83.33% (95%CI, 72.13 to

Table II: Characteristics of positive influenza cases determined by real-time RT-PCR

N (%)		Positive influenza A only	Positive influenza B only	Positive influenza A and B (Co-infection A&B)
		99 (60.00)	61 (36.97)	5 (3.03)
Age (median)		51Y (min=6M, max=99Y)	15Y (min=8M, max=92Y)	13Y (min=2Y, max=92Y)
	0-18	15 (15.15)	28 (45.90)	3 (60.00)
	19-59	44 (44.44)	23 (37.70)	0 (0.00)
	≥60	40 (40.40)	10 (16.40)	2 (40.00)
Gender	Male	43 (43.43)	24 (39.34)	2 (40.00)
	Female	56 (56.57)	37 (60.66)	3 (60.00)

Note. M: months, Y: years

Table III: Performance of the QuickNavi™-Flu2 rapid antigen assay compared with the real-time RT-PCR in different respiratory specimen types

		% (No./Total samples)			
		NS/NPS/TS	NPW/NPA	SPT	All specimen
Influenza A	Sensitivity	90.57 (48/53)	72.73 (32/44)	71.43 (5/7)	81.73 (85/104)
	Specificity	100 (54/54)	100 (61/61)	100 (18/18)	100 (133/133)
	PPV	100 (48/48)	100 (32/32)	100 (5/5)	100 (85/85)
	NPV	91.53 (54/59)	83.56 (61/73)	90.00 (18/20)	87.50 (133/152)
Influenza B	Sensitivity	96.67 (29/30)	76.67 (23/30)	66.67 (4/6)	84.85 (56/66)
	Specificity	100 (54/54)	100 (61/61)	100 (18/18)	100 (133/133)
	PPV	100 (29/29)	100 (23/23)	100 (4/4)	100 (56/66)
	NPV	98.18 (54/55)	89.71 (61/68)	90.00 (18/20)	93.01 (133/143)

Note. Samples which show co-infection with influenza A and B viruses were included for result analysis.
NS: nasal swab, NPS: nasopharyngeal swab, TS: throat swab, NPW: nasopharyngeal wash, NPA: nasopharyngeal aspirate, SPT: sputum

Table IV: Performance of the Standard™ F influenza A/B FIA rapid antigen assay compared with the real-time RT-PCR in different respiratory specimen types

		% (No./Total samples)							
		NS/NPS/TS		NPW/NPA		SPT		All specimen	
Influenza A	Sensitivity	90.57	(48/53)	79.55	(35/44)	71.43	(5/7)	84.62	(88/104)
	Specificity	100	(54/54)	100	(61/61)	94.44	(17/18)	99.25	(132/133)
	PPV	100	(48/48)	100	(35/35)	83.33	(5/6)	98.88	(88/89)
	NPV	91.53	(54/59)	87.14	(61/70)	89.47	(17/19)	89.19	(132/148)
Influenza B	Sensitivity	96.67	(29/30)	73.33	(22/30)	66.67	(4/6)	83.33	(55/66)
	Specificity	98.15	(53/54)	98.36	(60/61)	73.68	(14/19)	94.74	(126/133)
	PPV	96.67	(29/30)	95.65	(22/23)	44.44	(4/9)	88.71	(55/62)
	NPV	98.18	(53/54)	88.24	(60/68)	87.50	(14/16)	91.97	(126/137)

Note. Samples which show co-infection with influenza A and B viruses were included for result analysis.
NS: nasal swab, NPS: nasopharyngeal swab, TS: throat swab, NPW: nasopharyngeal wash, NPA: nasopharyngeal aspirate, SPT: sputum

Table V: Summary of the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the QuickNavi™-Flu2 and Standard™ F influenza A/B FIA rapid antigen assays in all respiratory specimens

	Number of cases							
	Rapid + PCR +	Rapid + PCR -	Rapid - PCR +	Rapid - PCR -	**Sensitivity(%), 95%CI**	**Specificity(%), 95%CI**	**PPV(%), 95%CI**	**NPV(%), 95%CI**
Influenza A								
QuickNavi-Flu2	85	0	19	133	81.73, 72.95 – 88.63	100, 97.26 – 100	100	96.32, 94.57 – 97.52
Standard F	88	1	16	132	84.26, 76.22 – 90.94	99.25, 95.88 – 99.98	95.93, 76.93 – 99.40	96.86, 95.16 – 97.98
Influenza B								
QuickNavi-Flu2	56	0	10	133	84.85, 73.90 – 92.49	100, 97.26 – 100	100	96.93, 94.69 – 98.24
Standard F	55	7	11	126	83.33, 72.13 – 91.38	94.74, 89.46 – 97.86	76.81, 61.50 – 87.29	96.45, 94.05 – 97.90

91.38%), respectively (Tables IV and V). Similar to the QuickNavi™-Flu2, the STANDARD™ F Influenza A/B FIA yielded the highest sensitivity in NS/NPS/TS samples and the lowest sensitivity in sputum for detection of both influenza A and B viruses (Table IV). The specificity of the STANDARD™ F Influenza A/B FIA in all respiratory samples for influenza A detection was 99.25% (95%CI, 95.88 to 99.98%) and 94.74% (95%CI, 89.46 to 97.86%) for influenza B detection (Tables IV and V). The false positive result for influenza A virus was found in one sputum sample. There were seven samples (one nasopharyngeal wash, one nasal swab, five sputum samples) in which the STANDARD™ F Influenza A/B FIA showed false positive results for influenza B virus detection (Tables IV).

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of both QuickNavi™-Flu2 and STANDARD™ F Influenza A/B FIA were summarized in Table V. As previously mentioned, both RIDTs showed the lowest sensitivity in sputum samples (Tables II and III), which are not the recommended specimen type for both assays. Therefore, we reanalyzed the sensitivity and specificity of both rapid antigen assays after excluding 31 sputum samples. The QuickNavi™-Flu2 sensitivity for influenza A and B in respiratory specimens, excluding sputum, was 82.47% (95%CI, 73.43 to 89.45%) and 86.67% (95%CI, 75.41 to 94.06%), respectively. The QuickNavi™ -Flu2 specificity for influenza A and B in respiratory specimens, excluding sputum, were both 100% (95%CI, 96.84 to 100%). The STANDARD™ F Influenza A/B FIA sensitivity for influenza A and B in respiratory specimens, excluding sputum, was 85.57% (95%CI, 76.97 to 91.88%) and 85.00% (95%CI, 73.43 to 92.90%), respectively. The STANDARD™ F Influenza A/B FIA specificity for influenza A and B in respiratory specimens, excluding sputum, was 100% (95%CI, 96.84 to 100%) and 98.26% (95%CI, 93.86 to 99.79%), respectively.

DISCUSSION

The rapid influenza diagnostic tests (RIDTs) have become widely used in many diagnostic laboratories for the first-line screening of influenza infection according to their simple procedure and fast turnaround time. However, the sensitivity and specificity of RIDTs are considered lower when compared to the real-time RT-PCR assay. In this study, we evaluated the performance of two rapid influenza diagnostic tests (RIDTs): the conventional chromatographic immunoassay (QuickNavi™-Flu2) and the digital lateral flow immunoassay with fluorescence labeling (STANDARD™ F Influenza A/B FIA) with the real-time RT-PCR assay using 298 respiratory samples.

The STANDARD™ F Influenza A/B FIA yielded slightly higher sensitivity for influenza A virus detection than the QuickNavi™-Flu2 but comparable sensitivity for influenza B virus detection (Table V). Among the samples with only the STANDARD™ F Influenza A/B FIA but not the QuickNavi™-Flu2 correctly identified

the positive results, the cycle threshold value varies from 26.46-39.91 (Table S1). Our study demonstrated the higher sensitivity of the STANDARD™ F Influenza A/B FIA (84.26% for influenza A, 83.33% for influenza B), compared to other studies previously reported (72-78% for influenza A, 57-61% for influenza B) (14,15). There were 16 false-negative samples for influenza A and 10 false-negative samples for influenza B virus detection, respectively. Most false-negative results for influenza A virus detection by the STANDARD™ F Influenza A/B FIA were from samples with high cycle threshold value (Ct-value: 35.18 – 41.98). Of note, one sample with co-infection of influenza A and B viruses as determined by real-time RT-PCR (Ct-value: 24.79 for influenza A, Ct-value: 24.85 for influenza B), the STANDARD™ F Influenza A/B FIA correctly detect only influenza B virus antigen. Our results suggested a limitation of RIDTs in identification of influenza A and B virus co-infection, which is in concordance with other studies (16,17). The STANDARD™ F Influenza A/B FIA yielded lower specificity for detecting influenza B virus than the QuickNavi™-Flu2 but comparable specificity for detecting influenza A virus.

STANDARD™ F Influenza A/B FIA showed false positive results for influenza B virus detection. Most samples with the false positive results for influenza B yielded relatively low cut-off value (COI), which were 1.35 – 2.69. The one sample showing a false positive result for influenza A had a COI of 2.30. Therefore, obtaining low COI from the digital readout of the STANDARD™ F Influenza A/B FIA should be carefully interpreted. The false-positive influenza B virus detection should be aware of those with low COI.

Our study also indicate that specimen types may affect the sensitivity and specificity of the RIDTs. We compared each specimen type's sensitivity and specificity and found that the nasal and nasopharyngeal swabs had the highest sensitivity, while sputum had the lowest sensitivity detected by both RIDTs. Moreover, sputum samples are not the recommended specimen type for RIDTs, which could be due to a lower yield for detection of influenza viruses (18-21). The specimen type that showed many false negative results were nasopharyngeal washes (n=15), nasal swabs (n=6), and sputum samples (n=4). Almost 70% of false negative samples had cycle threshold value more than 35 by real-time RT-PCR. When excluding thirty-one sputum samples, which were not recommended by the assay kits, the sensitivity and specificity provided better specificity. The specificity of the STANDARD™ F went up to 100% (Table VI). However, sputum is one of the specimen types usually sent to the laboratory for influenza virus detection. Therefore, the rapid antigen test using sputum should be aware of the possibility of false positive and false negative results. Apart from the specimen types, other factors including how specimens were collected and processed, and the patient information could affect the detection rate. Most specimens in this study were obtained from adults. Some studies reported higher sensitivity of the RIDTs in specimens collected from children than adults, possibly due to a better yield for influenza virus detection from children who usually had more prolonged periods of symptoms than adults (19,22,23). The clinical information of symptom onset and treatment intervention of the patients would affect the efficiency of virus detection, and thus are useful for result interpretation.

Overall, both RIDTs had good sensitivity (81.73-84.62%) and specificity (99.25-100%) in influenza A virus detection. The sensitivity of influenza B virus detection was 83.33-84.85%, while the specificity was 94.74-100% (Table V). Our findings demonstrated that both RIDTs are potential alternative methods for rapid screening of influenza virus infection. The conventional chromatographic immunoassay has a workflow advantage over the digital lateral flow immunoassay with fluorescence labeling when several samples were tested simultaneously. The assay time of the conventional chromatographic immunoassay can even be shortened if the samples show strongly positive. On the other hand, the digital readout immunoassay provided the cut-off value, which will be less subjective for result interpretation. The Standard F200 model allows several strips to react outside the reader. However, signal measurement still requires one by one operation using the strip reader, thus taking more time. To overcome this limitation, the Standard F2400 model allows processing up to 24 strips at once for detection and measurements simultaneously. Accordingly, the number of specimens

Table VI: Summary of the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the QuickNavi™-Flu2 and Standard™ F influenza A/B FIA rapid antigen assays in respiratory specimens, excluding sputum samples

	Number of cases				Sensitivity(%), 95%CI	Specificity(%), 95%CI	PPV(%), 95%CI	NPV(%), 95%CI
	Rapid + PCR +	Rapid + PCR -	Rapid - PCR +	Rapid - PCR -				
Influenza A								
QuickNavi-Flu2	80	0	17	115	82.47, 73.43 – 89.45	100, 96.84 – 100	100	96.46, 94.66 – 97.67
Standard F	83	0	14	115	85.57, 76.97 – 91.88	100, 96.84 – 100	100	97.07, 95.33 – 98.17
Influenza B								
QuickNavi-Flu2	52	0	8	115	86.67, 75.41 – 94.06	100, 96.84 – 100	100	97.29, 94.95 – 98.56
Standard F	51	2	9	113	85.00, 73.43 – 92.90	98.26, 93.86 – 99.79	91.09, 72.05 – 97.59	96.91, 94.49 – 98.28

Note. Thirty-one sputum samples were excluded for the analysis.

and laboratory workflow and conditions should also be considered along with the assays' sensitivity and specificity when selecting the suitable diagnostic assays for each diagnostic laboratory.

CONCLUSION

The STANDARD™ F Influenza A/B FIA and the QuickNavi™-Flu2 showed comparable sensitivity and specificity for rapid influenza antigen detection. Compared with the real-time RT-PCR, the sensitivity in detecting influenza A and B viruses were more than 80%; the specificity in detecting influenza A virus was 99-100% for both RIDTs. The specificity in detecting influenza B virus of QuickNavi™ -Flu2 and the STANDARD™ F Influenza A/B FIA was 100% and 94.74%, respectively. Apart from the sensitivity and specificity, the laboratory workflow should be considered when selecting the suitable assays (digital readout-fluorescence or conventional chromatographic immunoassays) for each diagnostic laboratory.

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