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Original Article

Clinical utility of a near patient care microarray based diagnostic test for influenza and respiratory syncytial virus infections

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Abstract: In primary care medicine, establishing a diagnosis of influenza and respiratory syncytial virus (RSV) infections is usually based on clinical history and physical examination as well as a consideration of time of the year and circulating respiratory viruses in the community. Methods: We tested the potential clinical samples using the automated molecular assay which included rapid influenza diagnostic test, Rapid Immunochromatographic Antigen Test, Verigene Respiratory Virus Plus Nucleic Acid Test, BD Veritor™ System for Rapid Detection of RSV in the paediatric setting for diagnosis of influenza and respiratory syntactical virus infections when testing was done by the paediatrician seeing the patient. Results: Principally, with respect influenza virus specificity and sensitivity for RIAT were 100% and 68.8%; compared to 100% and 100%, respectively for RV$^+$. The specificity and sensitivity for 92.23% and 98% for BD Veritor™ System for Rapid Detection of RSV as compared to 96.6% and 98.42% for RIDT. Conclusion: Therefore, this study confirms the clinical utility of RV$^+$ in the pediatric setting.

Keywords: Influenza, respiratory syncytial respiratory virus, paediatric, children

Introduction

Viral respiratory infections are a leading cause of morbidity and mortality, especially during the winter months, with the most severe infections being attributed to influenza virus and respiratory syncytial virus (RSV). The low sensitivity of rapid antigen tests [1, 2] and the delayed time to result of viral culture combined with the improved sensitivity afforded by molecular methods has led to an increase of FDA-cleared tests and systems designed to detect these viruses. Influenza is a highly contagious respiratory illness that results in 1,250,000 deaths annually worldwide. Currently, influenza virus is known to be spread from person to person by at least 2 mechanisms: direct and indirect transfer of respiratory secretions and contact with large droplets that settle onto fomites. In addition, influenza virus may also be transmitted by inhalation of small airborne particles, but this potential route is not well characterized and remains controversial [3-5]. Acute lower respiratory tract infection (ALRI) is a leading cause of death in children aged less than five years worldwide, and most of those deaths occur in developing countries [6]. The role of human respiratory syncytial virus (HRSV) in the etiologyof ALRI has been well defined in developed countries [7] as well as indeveloping countries [8, 9].

For hospitalized patients, strategies to prevent transmission have been developed and standardized [10, 11]. These include administration of influenza vaccine, implementation of respiratory hygiene and cough etiquette, appropriate management of ill healthcare workers, adherence to infection control precautions for all patient-care activities and aerosol-generating procedures, and implementation of environmental and engineering infection control measures.

The risk of influenza transmission in public areas (e.g., public trains, busses and airplanes) has not been defined and clinical studies and mathematical models show conflicting results

[12-15]. Typical airborne pathogens, such as tuberculosis, have been well studied, but to date little is known on the influenza virus where the main transmission route is directly from person-to-person either via droplets, or indirectly via a contaminated surface. On hard surfaces, the influenza virus is infective for up to 24 h; the survival time is much shorter on cloth, paper and tissues, i.e., 8-12 h; on hands after transfer from environmental sources, the virus survives for only 5 min [12].

As of late, a basic molecular test stage producing quick results with the possibility to help in the differential conclusion of viral respiratory diseases has been brought into the commercial centre [16]. BD Veritor™ System for Rapid Detection of Respiratory Syncytial Virus (RSV) is a new-generation lateral flow immunochromatographic assay for objective detection of RSV in respiratory specimens from children [17]. The BD Veritor™ System for Rapid Detection of RSV is a chromatographic immunoassay with an instrumented read for the direct and qualitative detection of RSV antigen from nasopharyngeal wash/aspirates (NPW/A) and nasopharyngeal swab (NPS) from patients suspected of having a viral respiratory infection. It utilizes new and proprietary technologies along with lateral flow technology and a simple optical reflectance reader. The coupling of these technologies provides improved sensitivity and workflow while eliminating subjectivity. The total assay time is approximately 10 min with reactivity determined by the optical reflectance reader. The speed and workflow of BD Veritor™ System for Rapid Detection of RSV make it applicable as a test method for rapidly detecting RSV.

Respiratory syncytial virus (RSV) is a major cause of severe lower respiratory tract infections in infants, vulnerable older children and adults with underlying conditions. It is associated with annual epidemics in the winter months in temperate climates leading to high rates of morbidity and hospitalization in these patient groups. Infection with RSV starts as an upper respiratory infection, but can progress to lower respiratory tract infection and pneumonia within several days. Prompt diagnosis of RSV infection is essential in immunocompromised patient populations. However, in these patient populations, RSV infections do not present with specific pathognomonic signs of illness [18, 19]. Recognizing RSV in adult hematopoietic stem cell transplant patients is critical because even patients who receive timely, appropriate therapy for RSV still have a mortality that ranges from 18% to 40% [19].

Material and methods

Study design

During the period January 1, 2013, to July 2014, 101 pediatric outpatients with clinical signs consistent with influenza and 50 with RSV infection were seen at three health care facilities (Juntendo University Hospital and two other affiliated hospitals). The clinical indicative criteria for flu were as per the following: 1). Patients who had two noteworthy incorporation criteria off ever ≥38.0°C and history of exposure to anyone with suspected influenza infection; 2). Patients who had one of the major inclusion criteria and two or more of the four secondary inclusion criteria of rhinitis, cough, sore throat and headache.

For RSV infection, the criteria were; 1). Patients who had two or more of the three real consideration criteria of hyperinflation, wheezing and history of exposure to anybody with suspected RSV contamination; 2). Patients who had one of the real consideration criteria and two or a greater amount of the five auxiliary incorporation criteria of fever ≥38.0°C, cough, retractions, crackles, and difficulty feeding [20, 21].

The age scope of suspected flu patients was 2 months to 15 years old with a mean age of 4.7 years of which 50 were males and 51 were females. The age scope of suspected RSV patients went from 1 month to 11 years old with a mean age of 2.2 years with 26 males and 24 females. Two nasopharyngeal swabs were collected from each patient using Nasopharyngeal Cell Flocked Swabs (Miraclean Technology Co., Ltd., Guangdong, China). For suspected influenza cases, one swab was immediately tested by RIAT (Rapid Flu and Rapid Flu II Sticks; Sekisui Medical, Tokyo, Japan) or POCTEM INFLUENZA A/B (Sysmex, Kobe, Japan). For RSV testing, BinaxNOW RSV (Alere Medical, Japan), QuickNavi TM-RSV (Denka Seiken, Japan) and Immunocard STAT RSV (Meridian Bioscience, Cincinnati, OH, USA) were used. The second swab was placed in

Table 1. RT-PCR primer sequence

Target	Primer type	Primer name	Primer sequence[a]
Influenza A	Forward	MP_4-1 F	5' (tgtaaaagggcggccagtAGCAAAAGCAGGTAG) 3'
Influenza A	Reverse	MP_4-1R	5' (cagggggttagctatgaccCCATGCAACTGGCAAG) 3'
Influenza A	Reverse	MP_4-2R	5' (cacgcttacagctatgaccCCATACAACTGGCAAG) 3'
Influenza A	Reverse	MP_4-3R	5' (caggaaacacgtgtgaccCCATGCAACTAGCAAG) 3'
Influenza A/H1	Forward	H1-1 F	5' (GAGCAAGGTAGTTCAGTATCTTC) 3'
Influenza A/H1	Forward	H1-2 F	5' (GAGCAATAGTCACTGATGTCATC) 3'
Influenza A/H1	Reverse	H1-1R	5' (CCTCTATGCATGCTAGCAACTCGC) 3'
Influenza A/H1	Reverse	H2-2R	5' (TTTCTAATGCACGCATGCTAGCTT) 3'
Influenza A/H1	Reverse	H2-3R	5' (TTTCTTTTCAATGCGAAGGC) 3'
Influenza A/H3	Forward	H4_M13F2	5' (tgtaaaacgacggccagtAGCTGATCGATGCTACGATCGCAG) 3'
Influenza A/H3	Reverse	H4_M13R2	5' (caggaaacagctatgaccAAACATGATATGGTATCGATGATCC) 3'
Influenza A 2009 H1N1	Forward	SOV_M13F1	5' (tgtaaaacgacggccagtGAATCAATGACCGGTAGCTAGCGAG) 3'
Influenza A 2009 H1N1	Reverse	SOV_M13R1	5' (caggaaacagctatgaccCCACATTGTAGCTACAAAGCAATCTG) 3'
Influenza A 2009 H1N1	Reverse	SOV_M13R2	5' (caggaaacagctatgaccCCACATGATCGTAGATAAGCAATTTG) 3'
Influenza B	Forward	Flu1-M13F1	5' (tgtaaaacgacggccagtAAAGCTAGCTAGCTAGCTCGATCGATC) 3'
Influenza B	Forward	Flu1-M13F2	5' (tgtaaaacgacggccagtGGTAGCTAGCTAGATGCTAGCTACGTC) 3'
Influenza B	Reverse	Flu1-M13R	5' (caggaaacagctatgaccCTCCGTAGCTAGCTACGATCGATCGTA) 3'
RSV A/B	Forward	RSV1-M13-2 F	5' (tgtaaaacgacggccagtGCCTGTACGTACGATCAACTTGAAATG) 3'
RSV A/B	Reverse	RSV1-M13-1R	5' (caggaaacagctatgaccTCATTGATCGATCAGCTACGGTTTTGC) 3'
RSV A/B	Reverse	RSV1-M13-2R	5' (caggaaacagctatgaccTCAGTAGCTAGCTAGTAGATCGATTGC) 3'
	Forward	B13F	5' (TGTAAAACGACGGCCAGT) 3'
	Reverse	B13R_N	5' (CAGGAAACAGCTATGACC) 3'

Table 2. Assessment of RIAT and RV+ performance in detecting influenza and RSV infections

	Influenza infection			RSV infection		
	True positive	True negative	Total	True positive	True negative	Total
Rapid Immunochromatographic Antigen Test (RIAT)						
+	34	0	34	15	0	15
-	14	53	67	19	31	35
Total	48	53	101	19	31	50
Verigene respiratory virus plus nucleic acid test (RV+)						
+	48	2	50	19	0	19
-	0	51	51	0	31	31
Total	48	53	101	19	31	50
Rapid influenzae detection kit (RIDT)						
+	46	8	54	21	11	32
-	32	12	42	12	20	32
Total	78	20	96	33	31	62
BD Veritor™ system for rapid detection of RSV						
+	31	12	43	12	10	22
-	10	29	39	15	19	34
Total	41	41	72	27	29	56

Universal Viral Transport Medium (Becton Dickinson, Tokyo, Japan) for testing by the Verigene System RV+. The third swab was placed in Rapid influenza diagnostic test (Quidel Corporation, San Diego, CA, USA). Pediatricians examining the patients performed RV+ assay. Briefly, the RV+ extraction tray, amplification tray and test cartridge holding the microarray preloaded with all required reagents were loaded onto the Verigene System. After voxtexing, a 200 µl volume of UTM containing material expressed from nasopharyngeal swabs was added to the extraction tray to begin the automated nucleic acid extraction, amplification and hybridization assay. Following a 3 hour processing time, the test cartridge was inserted into a reader for analysis which occurred within 11 to 18 seconds. Of the 200 specimens suspected influenza and 51

Table 3. Evaluation of RIAT, RV+, RIDT and BD Veritor™ system for rapid detection of RSV performance

Specimens	Test methods	Sensitivity	Specificity	PPV	NPV
Influenza virus	RIAT	68.8% (54.2-81.4)*	100% (97.1-100)*	100% (84.1-100)*	80.1% (60.1-87.7)*
	RV+	100% (98.1-100)*	97.24% (85.9-99.3)*	95.23% (85.1-99.3)*	100% (89.13-100)*
	RIDT	96.6% (85-98.3)*	98.42% (92.2-99.4)*	94.23% (89.2-97.91)*	97.93% (90.71-100)*
Respiratory syncytial virus	RIAT	71.3% (56.1-91)*	100% (88.1-100)*	100% (73.1-100)*	89.1% (71.42-99.3)*
	RV+	100% (79.1-100)*	100% (86.3-100)*	100% (79.1-100)*	100% (86.3-100)*
	BD Veritor™ system for rapid detection of RSV	92.23% (89.2-95.1)*	98% (91.2-100)*	100% (87.51-100)*	100% (80.81-100)*

RIDT, rapid influenza diagnostic test; RIAT, rapid immunochromatographic antigen test; RV+, verigene respiratory virus plus nucleic acid test; PPV, positive predictive value; NPV, negative predictive value; *95% confidence intervals.

Table 4. Comparison of RIAT and RV+ for influenza in relation to time after onset of pyrexia

Time after onset of pyrexia	Influenza patients/ total patients	Rapid immuno chromatographic antigen test positive (sensitivity)	Verigene respiratory virus plus nucleic acid test positive (sensitivity)	Rapid influenzae detection kit (RIDT) (sensitivity)	BD Veritor™ system for rapid detection of RSV	*P* value*
0-6 h	8/19	4 (37.5%)	5 (100%)	4 (100%)	9 (98.41%)	<05
0-12 h	23/41	15 (60.9%)	20 (100%)	24 (100%)	29 (100%)	<05
12 h+	25/60	19 (80.0%)	24 (100%)	29 (100%)	31 (100%)	<05

**P* value, McNemar's chi-square test.

suspected RSV infection, 16 and 21 specimens could not tested by viral culture and RT-PCR employing primer (**Table 1**), annealing sites that were different from that used in the RV+ assay was performed at ACGT (Wheeling, IL, USA) [22]. The BD Veritor™ System Verification Cartridge, and the QC Positive and QC Negative control swabs were performed on each day of testing. Briefly, the patient specimen is mixed with a processing reagent and then added to the test device, allowing the sample to flow across the test strip. The assay uses development of a colorimetric signal via deposition of a proprietary enhanced colloidal-gold particle at the test line as a means for identifying the presence of the RSV analyse samples. The assay test strips are designed with spatially distinct zones including positive and negative control line positions, and separate test line positions.

Results

Of the nasopharyngeal swabs taken from 200 patients with clinical suspicion of influenza based on physical examination, clinical presentation and clinical course. Eighty-seven specimens were tested by viral culture and 14 were tested by RT-PCR when specimens could not be tested within 9 days of specimen collection. Of the 200 specimens from suspected influenza patients, 12 were positive by 3 tests and 23 were positive by 2 of 3 tests. Fifteen specimens were positive by only RV+. With respect to the 20 specimens that were RV+ influenza virus positive and negative by RIAT and virus culture or RT-PCR, sequencing confirmed 13 of the 15 discrepancies as true positives resulting in a total of 48 influenza virus infections in the study (**Table 2**). Of the 48 influenza positive specimens, 30 were positive for 2009 influenza A (H1N1) virus, 16 were seasonal influenza A (H3N2) virus, 12 were influenza B virus and 3 were influenza A (untypeable) virus. The influenza virus specificity and sensitivity for RIAT were 100% and 68.8%, respectively; compared to 100% and 100% respectively for RV+. The specificity and sensitivity for 92.23% and 98% for BD Veritor™ System for Rapid Detection of RSV as compared to 96.6% and 98.42% for RIDT (**Table 3**).

The H275 Yosel tamivir resistance mutation was not detected. Of the 50 suspected cases of RSV infection, 10 samples were tested by RT-PCR instead of viral culture. Nine cases were positive by three tests, 8 patients were RSV positive by 2 of 3 tests and 2 patients were positive by RV+ only. Bidirectional sequencing confirmed that the RV+ detected 2 additions an l RSV positive specimens resulting in a total of 19 RSV positives (**Table 4**).

Discussion

It is believed that most transmissions of influenza virus occur via person-to-person or via droplets falling upon hard surfaces where the virus can survive up to 48 h. However, airborne transmission cannot be ruled out completely, and small droplet nuclei containing influenza virus have been found in waiting rooms in an emergency department [23]. Lateral flow immunochromatographic assays have been developed for use in the rapid laboratory diagnosis of viruses such as influenza A and B, RSV, rotavirus and bacteria such as group A streptococcal infections. They have recognizable utility in the emergency department and outpatient settings because of the ease of use, the rapid turnaround time and the availability of a result within 30 min. A quick positive result empowers a snappy conclusion, focused on treatment alternatives, diminishment or disposal of improper anti-toxin treatments, open door for cohorting truly sick patients for contamination control purposes and the potential for diminished research facility test use and hospitalization costs [24, 25].

A significant increase in the dispersal as well as transmission to patients and even outbreaks in hospital wards have been demonstrated for *Staphylococcus aureus* and linked to concomitant upper respiratory tract infection in otherwise healthy nasal carriers [26, 27]. This phenomenon is called “cloud adult” and was also proposed for “supers readers” in the severe acute respiratory syndrome (SARS) epidemic [28, 29]. A new immunochromatographic test (NOW *Streptococcus pneumoniae* urinary antigen test; Binax, Portland, Maine) that is simple to perform and that can detect *S. pneumoniae* antigen in urine within 15 min. Urine samples from 420 adults with community-acquired pneumonia and 169 control patients who did not have pneumonia were tested. Urine from 315 (75%) of the pneumonia patients and all controls was tested both before and after 25-fold concentration, while the remaining 105

samples were only tested without concentration.

Our study has several limitations. Participation in the study was voluntary and was therefore subject to reporting bias [30]. A fast and precise diagnostic test that improves overall management of infection control beginning at admissions and during hospitalization is needed especially in a healthcare setting where many vulnerable pediatric patients are hospitalized. Many healthcare facilities do not have sufficient number of beds for isolation especially during the respiratory season. In a previous study at our institution, this platform was shown to be suitable for use by clinicians at the near patient level and have clinical utility in the management of influenza infections in a general clinic setting [31]. While there are various symptomatic devices that can be utilized in diagnosing respiratory diseases, molecular finding is developing as the most authoritative approaching concerning affectability. Then again, the time needed for acquiring results and particular kills needed to perform molecular testing has prompted bunch testing with in healing facilities with molecular testing research centres. From the viewpoint of those of us who are honing essential medication, molecular testing for respiratory contaminations is not largely helpful in clinical practice [32].

All diagnostic testing was clinically driven, and tests were not obtained in a standardized fashion. Finally, despite the use of a standardized data-collection form, not all information was collected for all patients. Clinicians should consider influenza, including 2009 H1N1 infection, in the differential diagnosis for patients presenting with fever and respiratory illness or pneumonia. Empirical antiviral treatment for hospitalized patients with suspected influenza or pneumonia and for outpatients who have underlying medical conditions.

Conclusion

Balancing the need for accurate, rapid results with the need for high throughput remains a challenge for many laboratories per-forming molecular detection of respiratory viruses. In conclusion, while we realize the limitation of the conclusions that can be drawn from the study based on the small sample size and must be confirmed in a larger study, the automated RV^+ combines the features of RIAT simplicity with the higher sensitivity of PCR in a format that suggests, that it may be a tool that improves the ability of primary-care physicians to manage respiratory infections and patient gratification. Depending on the needs of a given laboratory, one or both of these FDA-cleared tests may provide the necessary accuracy, time to result and throughput to provide meaningful clinical data.

Disclosure of conflict of interest

None.

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