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Rapid Detection of Influenza A & B: A Comparison of New and Existing Rapid Antigen Tests with Chip-based Real-Time PCR

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Abstract

Influenza, caused by the influenza A and B viruses, is a highly communicable respiratory disease with clinical manifestations ranging from mild, asymptomatic cases to severe complications, such as viral pneumonia and death. These complications are more common in individuals with underlying health conditions, weakened immune systems, and those at the extremes of age. Accurate detection of these viruses is vital for patient management and controlling the spread of infection. Several diagnostic tests, including rapid diagnostic tests (RDTs) and molecular assays, are used to identify these viruses, with varying sensitivity and specificity based on the type of specimen and the time of symptom onset. This study aimed to compare the diagnostic performance of a new Influenza A&B rapid antigen test with the current commercially available Influenza A&B rapid antigen test and chip-based H1N1 RT-PCR. This retrospective cohort study was conducted from May to October 2023, using nasopharyngeal swab samples that were positive for H1N1 via RT-PCR. Results from both rapid tests were in 100% agreement. The performance of the evaluated Influenza A&B rapid antigen test performed better in samples with higher viral load. In instances where samples exhibit high viral loads (Ct<20) and medium viral loads (20≤Ct<25) as indicated by the equipment, the sensitivity was found to be 100%. Findings from the study suggest that while the rapid antigen test is a useful diagnostic tool for Influenza A, confirmatory testing with RT-PCR may still be necessary in cases of high clinical suspicion.

Keywords**:** *Diagnostic Kit, H1N1, Influenza A, Influenza B, Rapid Testing*.

Introduction

Influenza is an infectious respiratory disease caused by influenza viruses A and B in humans. The clinical presentation of the illness varies from asymptomatic infection to severe complications, including viral pneumonia and death, especially in patients with underlying comorbidities, immunocompromised patients, and those at the extremes of age. Influenza A and B are the types of influenza viruses that cause seasonal epidemics of disease. Influenza A viruses are of two subtypes based on the proteins present on the surface of the virus: hemagglutinin (H) and neuraminidase (N). Influenza B viruses can be further grouped into lineages and strains. Both influenza A and B are associated with significant morbidity and mortality, leading to hospital admissions and deaths annually [1]. Even though the general effects of influenza A and B are similar, there are differences in their detection and response to treatment. The sensitivity and specificity of different rapid influenza detection tests (RIDTs) and molecular assays can vary while identifying these viruses [2, 3]. The performance of these diagnostic tests can also be influenced by the type of specimen collected and the time since symptom onset [4]. Due to

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their potential as causatives for epidemics, influenza A and B viruses are significant public health concerns. Multiple diagnostic tests for these viruses have been introduced, with different assays demonstrating varying levels of sensitivity and specificity. Rapid and accurate detection of pathogens is crucial for effective patient management and control of the spread of infection [1–4].

The current testing methods for Influenza A and B include rapid diagnostic tests (RDTs), reverse transcription-polymerase chain reaction (RT-PCR), viral culture, and newer assays such as digital readout systems and neuraminidase detection assays. RDTs provide the advantage of ease of use and rapid results, with various tests presenting different sensitivities and specificities for Influenza A and B detection [5–7]. Traditional tests like RT-PCR and viral culture are more sensitive methods and are often used as confirmatory tests [8, 9]. However, these methods have limitations. RDTs, while rapid, may vary in sensitivity and specificity, with some tests performing better for certain strains or subtypes of influenza [5–7]. Digital readout systems have demonstrated higher sensitivity and specificity compared to conventional RDTs, but their clinical application needs further validation [7]. Neuraminidase detection assays have shown high sensitivity and specificity, but chances of false positives demand confirmatory testing [10]. The performance of RDTs can be influenced by factors like viral load in the specimen and the time since symptom onset [5–7]. The choice of testing method should rely on the clinical setting, the prevalence of influenza, and the need for rapid diagnosis [5–7,10].

Rapid and accurate diagnosis of influenza A and B is critical for successful patient management and epidemic control. Rapid diagnostic tests enable timely initiation of antiviral therapy, which is most useful when started early in the course of the illness and assists in patient triage to prevent nosocomial transmission [1]. Distinguishing between influenza and other respiratory pathogens, such as SARS-CoV-2, is crucial owing to their similar clinical presentations and varying treatment protocols [11]. The significance of early and accurate diagnosis of influenza A and B is underscored by the need for prompt treatment and infection control measures. Advances in diagnostic technologies are enhancing the sensitivity, specificity, and convenience of influenza testing, contributing to better patient outcomes and public health management.

In this study, the diagnostic performance of a new Influenza A&B rapid antigen test is compared to the current commercially available Influenza A&B rapid antigen test and H1N1 RT-PCR.

Methods

This is a retrospective cohort study performed between May and October 2023. Anonymous samples that were positive for H1N1 by a chip-based RT-PCR, were then tested for the new Influenza A&B rapid antigen test (RIAT-2) and the current commercially available rapid antigen test (RIAT-1). Testing occurred at the Microbiology and Molecular Biology departments of a reference laboratory. Samples were collected from the emergency department and wards of different hospitals. As this study did not involve patient demographics, and all the data except the H1N1 detected report was anonymized, ethical committee approval was not taken as per the guidelines.

Specimen Collection

Nasopharyngeal swabs: A sterile swab was inserted into the nostrils of the nasopharyngeal area. The swab is allowed to remain in the nostrils for a few seconds to absorb the secretions, gently rotated and withdrawn. One set of swabs underwent RT-PCR and RIAT-1 and the other underwent RIAT-2 as per manufacturer guidelines.

Chip-Based RT-PCR

Upon receiving the samples in the Microbiology and Molecular Biology department, viral RNA was isolated using an automated sample preparation device provided by the manufacturer. The extracted viral nucleic acid was then transferred into a microtube containing freeze-dried PCR reagents, including reverse transcriptase. After a brief 20-second incubation, the entire mixture was pipetted into an H1N1 testing chip. The chip was subsequently placed into a Real-time micro-PCR analyzer, where the RNA was first converted into complementary DNA, followed by thermal cycling. Conserved sequences of H1N1 swine influenza A virus (swH1) haemagglutinin gene and swine influenza A virus (swInfA) nucleocapsid genes were used as targeted sequences (as per manufacturer kit insert) and human RNaseP as full process internal positive control. The test results, indicating whether H1N1 was detected or not detected, were displayed at the end of the run. The entire procedure takes approximately one hour.

RIAT-1

The test was conducted according to the manufacturer's protocol. The swabs were dipped in the Antigen extraction buffer and extracted by swirling 10-15 times. Add 2-3 drops of mucus-free antigen extraction buffer into the sample well of the test device. Results are read between 15-20 minutes.

RIAT-2

The nasal swab is inserted into the extraction buffer vial, and rotated, and the specimen-extraction buffer mix thoroughly (by shaking). 2 drops (50 μL) of specimen-extraction buffer mix are added to the sample well of the device. Wait for the coloured line(s) to appear. Read results for 10 minutes.

Results

A total of 72 H1N1 RT-PCR positive samples were included, of which 48 (66.67%) were from the emergency departments and 24 (33.33%) were from different wards (Table 1). Of these 72 H1N1 RT-PCR positive, only 53 (73.61%) were positive by the evaluated Influenza A&B rapid antigen test and current commercially available Influenza A&B rapid antigen test (Table 2). Overall agreement between both the rapid antigen tests were 100%. The performance of the evaluated Influenza A&B rapid antigen test performed better in samples with higher viral load. In instances where samples exhibit high viral loads (Ct<20) and medium viral loads (20≤Ct<25) as indicated by the equipment, the sensitivity was found to be 100%.

Table 1. Number of Cases from Different Sources

Source	**Number of cases (%)**
Emergency Departments	66.67
Wards	33.33

Table 2. Number of Cases Tested Positive for Different Test Methods

Test Method		No of cases

Chip-based H1N1 RT PCR		72
RIAT-1	Influenza A	53
	Influenza B	0
RIAT-2	Influenza A	53
	Influenza B	0

Discussion

Influenza is a respiratory infection that can cause significant morbidity and mortality, particularly in the young, elderly, or immunocompromised [12]. The diagnostic performance of RIAT-2 was compared with RIAT-1, which is the existing, commercially available test kit. The results from RIAT-2 were in 100 per cent agreement with the current commercially available test kit. This study reported that RIAT-2 has a sensitivity of 74%. Several studies have been published on the performance of the Influenza A+B rapid test in detecting influenza A H1N1, with reported sensitivities ranging from 51 to 75% [13–16]. Our sensitivity estimate of 74% falls within the range of previously reported sensitivities and is very similar to the estimate of the sensitivity of 62.7% from the Suntarattiwong et al. study in Thai children [13]. In another study, the rapid test was found to have a sensitivity of 65.2% (95% CI 58.5, 71.4) and a specificity of 99.1% (95% CI 98.3, 99.6) which is very similar to the results found for H1N1pdm in this study [17].

The rapid tests provided positive results when the viral load was higher. Cheng et al showed that the performance of diagnostic tests for the detection of the influenza A (H1N1) virus is correlated with the time after symptom onset and viral load [18]. The highest viral loads in the samples were associated with a better rate of detection [19]. Factors like, the type and quality of the specimen, and time of specimen collection post-infection can also influence the performance of the rapid tests [12].

Rapid diagnosis of influenza is important during epidemics to allow treatment initiation and patient isolation. Rapid influenza diagnostic tests (RIDTs) offer a fair alternative to viral culture and RT PCR, which are sensitive but with higher turnaround times. These tests based on the principle of immunochromatography are fast and easy to perform, yielding the result in 10–15 minutes. They can detect even non-viable viruses as they are targeted at their nucleoprotein. Rapid influenza tests are very specific [20]. The significance of H1N1 rapid tests for Influenza A and B lies in their ability to provide timely diagnosis, which is crucial for effective clinical management and controlling outbreaks.

Conclusions

The Influenza A&B rapid antigen test demonstrates high specificity and sensitivity in individuals with a high viral load, establishing its suitability as a first-line test for detecting Influenza A&B. However, given the assay's measured sensitivity, a negative result on the Influenza A&B rapid antigen test may warrant additional testing with more sensitive tests, such as the RT-PCR. This is particularly the case with high clinical suspicion for an active Influenza A&B infection even after a negative Influenza A&B rapid antigen test result.

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Conflict of Interest

There is no conflict of interest between the authors.

References

[1]. Akaishi, Y., Matsumoto, T., Harada, Y., & Hirayama, Y, 2016, Evaluation of the rapid influenza detection tests GOLD SIGN FLU and Quick Navi-Flu for the detection of influenza A and B virus antigens in adults during the influenza season. *International Journal of Infectious Diseases*, *52*, 55–58. https://doi.org/10.1016/j.ijid.2016.10.002

[2]. Busson, L., Hallin, M., Thomas, I., De Foor, M., & Vandenberg, O, 2014, Evaluation of 3 rapid influenza diagnostic tests during the 2012–2013 epidemic: Influences of subtype and viral load. *Diagnostic Microbiology and Infectious Disease*, *80*(4), 287–291. https://doi.org/10.1016/j.diagmicrobio.2014.08.015

[3]. Cheng, P. K. C., Wong, K. K. Y., Mak, G. C., Wong, A. H., Ng, A. Y. Y., Chow, S. Y. K., Lam, R. K. H., Lau, C. S., Ng, K. C., & Lim, W, 2010, Performance of laboratory diagnostics for the detection of influenza A(H1N1)v virus as correlated with the time after symptom onset and viral load. *Journal of Clinical Virology*, *47*(2), 182–185. https://doi.org/10.1016/j.jcv.2009.11.022

[4]. Cho, C. H., Woo, M. K., Kim, J. Y., Cheong, S., Lee, C.-K., An, S. A., Lim, C. S., & Kim, W. J, 2013, Evaluation of five rapid diagnostic kits for influenza A/B virus. *Journal of Virological Methods*, *187*(1), 51–56. https://doi.org/10.1016/j.jviromet.2012.09.003

[5]. DiMaio, M. A., Sahoo, M. K., Waggoner, J., & Pinsky, B. A, 2012, Comparison of Xpert Flu rapid nucleic acid testing with rapid antigen testing for the diagnosis of influenza A and B. *Journal of Virological Methods*, *186*(1), 137–140. https://doi.org/10.1016/j.jviromet.2012.07.023

[6]. Ellis, J. E., Guest, P., Lawson, V., Loecherbach, J., Lindner, N., & McCulloch, A, 2022, Performance Evaluation of the Microfluidic Antigen LumiraDx SARS-CoV-2 and Flu A/B Test in Diagnosing COVID-19 and Influenza in Patients with Respiratory Symptoms. *Infectious Diseases and Therapy*, *11*(6), 2099–2109. https://doi.org/10.1007/s40121-022-00696-8

[7]. Gordon, A., Videa, E., Saborio, S., López, R., Kuan, G., Reingold, A., Balmaseda, A., & Harris, E, 2009, Performance of an Influenza Rapid Test in Children in a Primary Healthcare Setting in Nicaragua. *PLOS ONE*, *4*(11), e7907. https://doi.org/10.1371/journal.pone.0007907

[8]. Hurt, A. C., Alexander, R., Hibbert, J., Deed, N., & Barr, I. G, 2007, Performance of six influenza rapid tests in detecting human influenza in clinical specimens. *Journal of Clinical Virology*, *39*(2), 132–135. https://doi.org/10.1016/j.jcv.2007.03.002

[9]. Ko, S.-Y., Jang, J. W., Song, D. J., Lim, C. S., & Kim, W. J, 2013, Evaluation of the Simplexa Flu A/B and RSV test for the rapid detection of influenza viruses. *Journal of Medical Virology*, *85*(12), 2160–2164. https://doi.org/10.1002/jmv.23712

[10]. Kok, J., Blyth, C. C., Foo, H., Patterson, J., Taylor, J., McPhie, K., Ratnamohan, V. M., Iredell, J. R., & Dwyer, D. E., 2010, Comparison of a rapid antigen test with nucleic acid testing during cocirculation of pandemic influenza A/H1N1 2009 and seasonal influenza A/H3N2. *Journal of Clinical Microbiology*,

48(1), 290–291. https://doi.org/10.1128/JCM.01465-09

[11]. Kumar, S., & Henrickson, K. J, 2012, Update on Influenza Diagnostics: Lessons from the Novel H1N1 Influenza A Pandemic. *Clinical Microbiology Reviews*, *25*(2), 344–361. https://doi.org/10.1128/CMR.05016-11

[12]. Mitamura, K., Kawakami, C., Shimizu, H., Abe, T., Konomi, Y., Yasumi, Y., Yamazaki, M., Ichikawa, M., & Sugaya, N, 2013, Evaluation of a new immunochromatographic assay for rapid identification of influenza A, B, and A(H1N1)2009 viruses. *Journal of Infection and Chemotherapy*, *19*(4), 633–638. https://doi.org/10.1007/s10156-012-0533-1

[13]. Noyola, D. E., Paredes, A. J., Clark, B., & Demmler, G. J, 2000, Evaluation of a neuraminidase detection assay for the rapid detection of influenza A and B virus in children. *Pediatric and Developmental Pathology: The Official Journal of the Society for Pediatric Pathology and the Paediatric Pathology Society*, *3*(2), 162–167. https://doi.org/10.1007/s100240050020

[14]. Peters, T. R., Blakeney, E., Vannoy, L., & Poehling, K. A, 2013, Evaluation of the limit of detection of the BD Veritor™ system flu A+B test and two rapid influenza detection tests for influenza virus. *Diagnostic Microbiology and Infectious Disease*, *75*(2), 200–202. https://doi.org/10.1016/j.diagmicrobio.2012.11.004

[15]. Ryu, S.-W., Lee, J.-H., Kim, J., Jang, M.-A., Nam, J.-H., Byoun, M.-S., & Lim, C. S, 2016, Comparison of two new generation influenza rapid diagnostic tests with instrument-based digital readout systems for influenza virus detection. *British Journal of Biomedical Science*, *73*(3), 115–120. https://doi.org/10.1080/09674845.2016.1189026

[16]. Suntarattiwong, P., Jarman, R. G., Levy, J., Baggett, H. C., Gibbons, R. V., Chotpitayasunondh, T., & Simmerman, J. M, 2010, Clinical performance of a rapid influenza test and comparison of nasal versus throat swabs to detect 2009 pandemic influenza A (H1N1) infection in Thai children. *The Pediatric Infectious Disease Journal*, *29*(4), 366–367. https://doi.org/10.1097/INF.0b013e3181c6f05c

[17]. Takeuchi, Y., & Shimizu, H, 2000, Evaluation of influenza virus type A detection kit]. *Kansenshogaku Zasshi. The Journal of the Japanese Association for Infectious Diseases*, *74*(10), 841–845. https://doi.org/10.11150/kansenshogakuzasshi1970.74.841

[18]. Vasoo, S., Stevens, J., & Singh, K, 2009, Rapid Antigen Tests for Diagnosis of Pandemic (Swine) Influenza A/H1N1. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 49(7), 1090–1093. https://doi.org/10.1086/644743

[19]. Watcharananan, S., Kiertiburanakul, S., & Chantratita, W, 2010, Rapid influenza diagnostic test during the outbreak of the novel influenza A/H1N1 2009 in Thailand: An experience with better test performance in resource limited setting. *The Journal of Infection*, *60*(1), 86–87. https://doi.org/10.1016/j.jinf.2009.10.049

[20]. Woźniak-Kosek, A, 2015, Detection of Influenza and Other Respiratory Viruses Carried Out in the Influenza Project—Monitoring Vaccine Effectiveness (I-MOVE). *Advances in Experimental Medicine and Biology*, *836*, 29–33. https://doi.org/10.1007/5584_2014_31