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Proceeding Paper

Performance of ID NOW Influenza A&B 2 †

Susana Bandarra [1], Célia Neto [1], Lurdes Monteiro [1,*] and Laura Brum [2]

[1] Molecular Pathology Laboratory, SYNLAB Lisboa, Av. Columbano Bordalo Pinheiro, 75A, 1070-061 Lisbon, Portugal; susana.bandarra@synlab.pt (S.B.); celia.neto@synlab.pt (C.N.)
[2] Clinical Laboratories SYNLAB Portugal, Av. Columbano Bordalo Pinheiro, 75A, 1070-061 Lisbon, Portugal; laura.brum@synlab.pt
* Correspondence: lurdes.monteiro@synlab.pt; Tel.: +351-217-216-060
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Abstract: ID NOW™ INFLUENZA A&B 2 is a point-of-care assay for rapid molecular diagnosis of Influenza A and B. The present study aims to evaluate the performance of ID NOW™ INFLUENZA A&B 2 compared to a reference RT-PCR assay. A total of 67 nasopharyngeal swabs from 67 patients for screening of Influenza A and B by RT-PCR (Allplex™ RP1) were also tested with ID NOW assay. Of the seventeen positive Influenza A and five positive Influenza B, fifteen (88%) and five (100%), respectively, were also detected by the ID NOW assay. The overall agreement with the reference test was 95.5%. The sensitivity was 88.2% for Influenza type A and 100% for Influenza type B, and the specificity was equal to or higher than 96% for both.

Keywords: Influenza A; Influenza B; point-of-care; molecular detection

1. Introduction

The Influenza viruses can cause significant seasonal morbidity and mortality, particularly in young children and patients with underlying chronic diseases. Influenza diagnosis is mainly based on clinical symptoms; however, a robust microbiological diagnosis is required for hospitalized patients or those at risk of developing complications, such as pneumonia. In these cases, Influenza diagnosis based on clinical observation alone can be difficult, as a variety of other respiratory viruses with similar symptoms are often circulating at the same time [1]. Rapid and early identification of the viral cause of acute respiratory infection has a critical role in clinical decision making, particularly for patients at risk for severe complications of Influenza who should be treated early in the course of infection. The implications for patient management include hospital admission or discharge, reduction in diagnostic investigations, and administration of antibiotics and antiviral therapy [2]. This not only improves patient monitoring and care and infection control, but also reduces healthcare costs.

Currently, there are several methods available to test for Influenza, divided into antigen detection-based assays, viral culture, and molecular detection. Real-time polymerase chain reaction (RT-PCR) is the gold standard for identifying Influenza viruses but requires specialized technology and skilled molecular technicians.

The ID NOW™ INFLUENZA A&B 2 assay, performed on the ID NOW instrument (Abbott Molecular Inc.), is a point-of-care detection system for Influenza viruses. ID NOW uses isothermal nucleic acid amplification technology, using primers and fluorescent probes specific for the amplification of RNA targets without the need for a thermal cycler. Like other platforms, ID NOW uses disposable test units containing target-specific reagents where extraction, amplification, and detection take place. The ID NOW™ INFLUENZA A&B 2 assay has been described as an easy-to-use device that provides robust and accurate results within 15 min for the detection of Influenza A/B [3,4]. In the present study, we aim

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to evaluate the performance of the ID NOW™ INFLUENZA A&B 2 assay compared to a reference RT-PCR commonly used in our routine diagnosis of Influenza.

2. Materials and Methods

Nasopharyngeal swabs (NPS) from 67 individuals from hospital centers were tested between January and February 2020. Samples were processed simultaneously with ID NOW™ INFLUENZA A&B 2 assay (Abbott) and the reference method Allplex™ Respiratory Panel (RP) 1 (Seegene) within 24 h of reception at the laboratory. Previously to amplification with Allplex™ RP1, RNA was extracted from 200 µL of a sample using QIACube-QIAamp viral RNA mini kit (Qiagen). The real-time RT-PCR was conducted on the CFX96 (Bio-Rad), and subsequently interpreted by Seegene's Viewer software.

ID Now platform is an automated assay that utilizes molecular isothermal nucleic acid amplification technology and uses as targets polymerase basic gene 2 for Influenza A virus and polymerase acidic gene for Influenza B virus detection. Specificity, sensitivity, negative and positive predictive values (NPV and PPV), and agreement between both tests were analyzed with SPSS software, v.28.

3. Results

A total of 67 NPS from subjects aged between 3 weeks and 96 years (median age = 65 years; 25.4% < 1–18 years, 50.7% > 65 years, and 23.9% [18–64 years]) were tested, including 26 females (39%) and 41 males (61%). Using the reference method, a total of 21 (31.3%) NPS were positive, while 46 (68.7%) samples were negative for both viruses. Using the molecular platform ID NOW, a total of 22 (32.8%) NPS were positive, while 45 (67.2%) were negative for both viruses. The results obtained are detailed in Table 1.

Table 1. Agreement of ID NOW™ INFLUENZA A&B 2 with the reference Allplex™ RP1 method.

	Allplex RP1		Total	Percent Agreement (Influenza A/B)	95% CI [1]
	Detected	Not Detected			
ID NOW					
Positive	20 *	2	22	95.2%	80.7–99.7
Negative	1	44	45	95.7%	87.2–99.3
Total	21	46	67	95.5%	

[1] CI—Confidence interval; * one sample was only positive for Influenza B.

Detailed evaluation of the results showed that among the positive results by the reference method, sixteen samples were positive for Influenza A, four were positive for Influenza B, and one was positive for both Influenza A and Influenza B. By the ID NOW platform, fifteen (from the sixteen RT-PCR positive) were positive for Influenza A and four (from the four RT-PCR positive) for Influenza B, showing a false-negative for Influenza A. Regarding the positive sample for both viruses, only the Influenza B was detected by ID NOW, resulting in an Influenza A false-negative sample, but being a positive sample in global (Table 1). Of the 46 negative samples for both viruses by the reference method, only forty-four samples were negative using ID NOW, two samples positive for Influenza A representing two false-positive results. All negative Influenza B samples on RT-PCR were also negative with ID NOW.

The results comparison between ID NOW and the reference method showed a negative agreement of 96.0% for Influenza A, 100% for B (Table 2), and 95.7% for both viruses (Table 1) and a positive agreement of 88.2% for Influenza A, 100% for B (Table 2), and 95.2% for both viruses (Table 1). The overall agreement of the ID NOW and the reference multiplex RT-PCR was 94.0%, 100%, and 95.5% for Influenza A, Influenza B, and altogether (Influenza A and B), respectively.

Table 2. Estimated diagnostic performance of ID NOW™ INFLUENZA A&B 2.

Measurement	Influenza A	Influenza B
Sensitivity	88.2% (15/17); CI: 67.9–97.9	100% (5/5)
Specificity	96.0% (48/50); CI: 88.2–99.3	100% (62/62)
PPV	88.2% (15/17); CI: 67.9–97.9	100% (5/5)
NPV	96.0% (48/50); CI: 88.2–99.3	100% (62/62)

CI—95% confidence interval; PPV—positive predictive value; NPV—negative predictive value.

The sensitivity for the ID NOW assay was 88.2% (95% CI: 67.9–97.9) for Influenza type A, 100% for Influenza type B, and 95.2% (95% CI: 80.7–99.7) for both. The specificity was equal to or higher than 96% (95% CI: 88.2–99.3) for both. Overall, the positive predictive value (PPV) was 90.9 (95% CI: 74.5–98.4), and the negative predictive value (NPV) was 97.8 (95% CI: 90.6–99.9). In Table 2, all clinical performance parameters are detailed per type of Influenza. The Kappa value was 0.866 (86.6%).

4. Discussion

The main advantage of ID NOW™ INFLUENZA A&B 2 is a shorter turnaround time compared to the RT-PCR Allplex™ RP1 assay or any other currently available molecular assay for Influenza A/B detection. To evaluate the capacity of detecting the Influenza virus in this point-of-care system, this study was conducted by mimicking the conditions of routine diagnostic testing, and the results indicate that the ID NOW™ Influenza A&B 2 has good overall performance and a high degree of concordance with the reference method.

The published results for ID NOW™ INFLUENZA A&B 2 show sensitivities and specificities between 90 and 100% when compared to various RT-PCR assays [3–6]. These high levels of sensitivity and specificity were also found in this study, with a sensitivity > 88% and 100% specificity in the detection of Influenza A and B viruses when compared with RT-PCR Allplex™ RP1 assay. When comparing performance by Influenza type, as in other studies, Influenza B showed the highest sensitivity. However, the number of positive cases for Influenza B was always lower than for Influenza A.

In the case of Influenza A, the two false negatives obtained using the ID NOW platform had a Ct > 37.0 using the reference method (median Ct value of 38.7 $\pm$ 1.8). Thus, since the Ct was close to the limit of detection of the reference method, this indicated that negative results obtained with ID NOW occurred probably in samples with low viral load. Other studies have also reported false negative results for samples with Ct > 37.0 (using the same reference RT-PCR assay) [3] or lower Ct > 31.0 (CDC FLU A/B PCR assay) [4]. In addition, it was verified that positive samples for other respiratory viruses than Influenza A or B were correctly detected as negative for Influenza with ID NOW assay, indicating the absence of cross-reactions with other respiratory viruses.

The rate of invalid results, which can have a negative impact on the cost of the test, the time-to-result, and consequently on patient care, has previously been reported to be relatively low, ranging from 0 to 7.5% [5–7]. In accordance with this, no invalid results were obtained in this study.

In conclusion, the results of this study suggest that the ID NOW™ INFLUENZA A&B 2 assay can be used as a test for rapid and accurate diagnosis of Influenza in clinical practice. ID NOW assay combines high speed, sensitivity, and accuracy. However, it should be emphasized that point-of-care systems cannot replace reference methodology in a transversal way.

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Institutional Review Board Statement: Ethical review and approval were waived for this study since this study was carried out on surplus samples that were sent to the laboratory as part of the molecular diagnosis of Influenza A/B, and under no circumstances shall the use of samples for this study jeopardize the diagnosis.

Informed Consent Statement: Patient consent was not required as surplus samples that arrived at the laboratory with a clinician's request to detect Influenza A/B were used.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest. The manufacturer had no role in the design of this study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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