

Performance Evaluation of Influenza A+B Rapid Test Cassette (Swab/Nasal Aspirate) via Clinical Validation

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Abstract: This study assessed the clinical performance of the CITEST DIAGNOSTICS INC. Influenza A+B Rapid Test Cassette, an immunochromatographic assay designed for the qualitative detection of Influenza A and B viral antigens. Using reverse transcription polymerase chain reaction (RT-PCR) as the gold standard, the test's accuracy was evaluated across a range of human respiratory specimens, including nasopharyngeal swabs, throat swabs, and nasal aspirates. The evaluation involved a substantial cohort of samples: nasopharyngeal swabs (283 for Influenza A, 289 for Influenza B), throat swabs (212 for Influenza A, 232 for Influenza B), and nasal aspirates (289 for Influenza A, 255 for Influenza B). The rapid test demonstrated consistently high performance metrics regardless of the sample type. Sensitivity ranged from 94.2% to 100%, while specificity was maintained between 98.9% and 99.4%. Overall accuracy was calculated at 97.8% to 99.3%. The high level of agreement with the reference RT-PCR method was further confirmed by Kappa values of 0.95 to 0.98. Additional analyses confirmed that the test is highly specific, showing no cross-reactivity with other common respiratory pathogens. The device also proved to be stable when stored at the recommended conditions of 2–30 °C. In conclusion, the findings substantiate that this rapid test cassette is a robust, accurate, and practical diagnostic tool for the timely detection and differentiation of influenza A and B viruses, facilitating prompt clinical decision-making.

1. Introduction

Influenza, a widespread acute respiratory viral infection, imposes a substantial global health burden characterized by elevated rates of illness and mortality, especially in vulnerable populations such as young children, older adults, and immunocompromised persons.^[1] Each year, seasonal outbreaks account for as many as one billion infections globally, including millions of severe cases and several hundred thousand fatalities.^[2] Timely identification and initiation of treatment within the first 48 hours after symptoms appear are essential to mitigate complications, decrease the duration of illness, and enhance patient prognosis.^[3]

The traditional gold standard for influenza diagnosis has historically been viral culture in specialized cell lines, but this method typically requires 3–14 days for results and is not suitable for rapid clinical decision-making in acute care settings ^[4]. Reverse transcription polymerase chain reaction (RT-PCR) has emerged as a highly sensitive and specific molecular method that offers improved detection rates over culture by 2–23%, but requires sophisticated laboratory infrastructure, specialized equipment, trained technical personnel and several hours to deliver results, thereby limiting its widespread implementation in resource-limited or point-of-care settings ^[5]. Rapid influenza diagnostic tests (RIDTs), particularly immunochromatographic assays, have gained popularity as they provide qualitative results within 15–30 minutes and are widely used in various healthcare settings, including physician offices, emergency departments, and outpatient clinics. However, concerns regarding variable sensitivity and potential false-negative results have been raised in some clinical evaluations ^[6].

The Influenza A+B Rapid Test Cassette (Swab/Nasal Aspirate) developed by CITEST DIAGNOSTICS INC. is designed to detect Influenza A and B nucleoproteins in nasopharyngeal swab, throat swab, or nasal aspirate specimens. This study evaluates its performance against RT-PCR in terms of sensitivity, specificity, accuracy and consistency, aiming to validate its clinical utility as a rapid and reliable diagnostic tool.

2. Experimental Procedures

2.1 Source of Clinical Samples

A cohort of 1,560 clinical specimens was obtained for this study. These samples were derived from both symptomatic and asymptomatic individuals:

Nasopharyngeal swabs: 283 for Influenza A, 289 for Influenza B

Throat swabs: 212 for Influenza A, 232 for Influenza B

Nasal aspirates: 289 for Influenza A, 255 for Influenza B

Sample collection and handling followed standardized clinical laboratory protocols. For nasopharyngeal sampling, sterile swabs were inserted nasally until reaching the nasopharyngeal resistance point, then rotated against the mucosal surface. Throat specimens were obtained from posterior pharyngeal and tonsillar regions, while nasal aspirates were collected using sterile catheter-based aspiration systems. All samples were immediately transferred to sterile containers with viral transport media, with storage at 2–8 °C for testing within 48 hours or preservation at –20 °C for extended storage. Prior to analysis, frozen specimens were completely thawed, acclimated to ambient temperature (15–30 °C), and vortex-mixed to achieve uniform antigen distribution.

2.2 Test Kits and Procedures

The evaluation employed CITEST DIAGNOSTICS INC.'s Influenza A+B Rapid Test Cassette (IIN-502) as the investigational device, with reverse transcription polymerase chain reaction serving as the reference standard. The RT-PCR analysis was conducted using influenza-specific nucleic acid detection kits from Shanghai ZJ Bio-Tech Co., Ltd. on an ABI7500 quantitative PCR system.

The testing protocol required all components to reach ambient temperature before initiation. The extraction tube was positioned in the workstation and received approximately 10 drops ($\approx 400 \mu\text{L}$) of extraction reagent. The swab specimen was immersed in the solution, rotated for 10 seconds while being pressed against the tube wall to facilitate antigen release. After squeezing the swab head during removal to recover residual liquid, a dropper tip was affixed to the tube to dispense exactly three drops ($\approx 120 \mu\text{L}$) of the processed solution into the test cassette's sample well. Results were interpreted at the 15-minute time point, with any readings beyond 20 minutes considered invalid.

The test results were defined as follows: positive for Influenza A (C and A lines visible), positive for Influenza B (C and B lines visible), positive for both (C, A, and B lines visible), negative (only C line visible), and invalid (no C line visible).

3. Performance Analysis

3.1 Analysis of Performance Characteristics

3.1.1 Key Diagnostic Metrics

The Influenza A+B Rapid Test Cassette demonstrated high performance across all specimen types when compared with RT-PCR. The experimental outcomes are shown in Tables 1-3.

Table 1: Nasopharyngeal Swab Specimen.

		Type A			Type B		
		RT-PCR		Total	RT-PCR		Total
		Positive	Negative		Positive	Negative	
	Positive	100	2	102	85	2	87
	Negative	1	180	181	2	200	202
	Total	101	182	283	87	202	289
Sensitivity Agreement		99.0%			97.7%		
Specificity Agreement		98.9%			99.0%		
Overall Accuracy		98.9%			98.6%		
Kappa		0.98			0.97		

Nasopharyngeal swabs, as the optimal specimen for respiratory virus detection, demonstrated excellent performance. For Influenza A, sensitivity reached 99.0%, specificity 98.9%, and overall accuracy 98.9%, with a Kappa value of 0.98 indicating almost perfect agreement. For Influenza B, sensitivity was 97.7%, specificity 99.0%, and accuracy 98.6%, supported by a Kappa value of 0.97. These findings confirm that nasopharyngeal swabs represent the most reliable specimen type for influenza detection with this rapid test.

Table 2: Throat Swab Specimen.

		Type A			Type B		
		RT-PCR		Total	RT-PCR		Total
		Positive	Negative		Positive	Negative	
	Positive	58	1	59	65	1	66
	Negative	3	150	153	4	162	166
	Total	61	151	212	69	163	232
Sensitivity Agreement		95.1%			94.2%		
Specificity Agreement		99.3%			99.4%		
Overall Accuracy		98.1%			97.8%		
Kappa		0.95			0.95		

Throat swabs demonstrated excellent performance despite slightly lower sensitivity compared to nasopharyngeal specimens. For Influenza A, sensitivity was 95.1%, specificity 99.3%, and overall accuracy 98.1% (Kappa = 0.95). Similarly, Influenza B detection showed 94.2% sensitivity, 99.4% specificity, and 97.8% accuracy (Kappa = 0.95). The modest reduction in sensitivity may reflect the typically lower viral load in throat specimens, while the consistently high specificity confirms the test's reliability with this more convenient sampling method.

Nasal aspirates demonstrated excellent performance, particularly for Influenza A detection, achieving 100% sensitivity, 99.2% specificity, and 99.3% overall accuracy (Kappa = 0.97). For Influenza B, performance remained strong with 97.9% sensitivity, 99.4% specificity, and 98.8% accuracy (Kappa = 0.97). These results indicate that nasal aspirates represent a highly reliable specimen type, especially valuable in pediatric populations where nasopharyngeal swabbing may be challenging.

Table 3: Nasal Aspirate Specimen.

		Type A			Type B		
		RT-PCR		Total	RT-PCR		Total
		Positive	Negative		Positive	Negative	
	Positive	46	2	48	94	1	95
	Negative	0	141	141	2	158	160
Total		46	143	189	96	159	255
Sensitivity Agreement		100%			97.9%		
Specificity Agreement		99.2%			99.4%		
Overall Accuracy		99.3%			98.8%		
Kappa		0.97			0.97		

3.1.2 Cross-reactivity and Interference

The test showed no cross-reactivity with a panel of common respiratory viruses and bacteria, including adenovirus, coronavirus, respiratory syncytial virus, parainfluenza virus and others, at high concentrations (up to 10^8 TCID₅₀/mL). No false positives were observed, confirming the test's high specificity.

3.1.3 Precision

Intra-assay and inter-assay precision were evaluated using negative, weak positive and strong positive specimens. The test demonstrated >99% correct identification across three different lots and multiple replicates, indicating high reproducibility and lot-to-lot consistency.

3.2 Discussion

3.2.1 Performance Characteristics

With its high sensitivity and specificity, this rapid test presents a viable alternative to other RIDTs, enhanced by its flexibility in accepting multiple sample sources. Its reliability is confirmed by near-perfect agreement with the reference method (Kappa values: 0.95-0.98), supporting its integration into routine clinical workflows.

3.2.2 Limitations

As a qualitative test, it does not provide viral load quantification. False negatives may occur if viral antigen levels are below the detection threshold. Proper specimen collection and handling are critical to avoid invalid results. Additionally, the test may not detect novel influenza strains not included in the validation panel.

3.2.3 Comparison with Other Diagnostic Methods

Compared to RT-PCR, the rapid test offers speed and ease of use, making it ideal for

point-of-care settings. While RT-PCR remains the gold standard for sensitivity and specificity, the rapid test provides a practical alternative for early screening and triage.

4. Conclusion

The Influenza A + B Rapid Test Cassette (Swab/Nasal Aspirate) demonstrates consistently high sensitivity, specificity, and overall diagnostic accuracy for the detection of both Influenza A and B viruses across multiple specimen types, including nasopharyngeal swabs, throat swabs, and nasal aspirates. Its excellent statistical agreement with the RT-PCR reference standard (Kappa values: 0.95–0.98), combined with its demonstrated lack of cross-reactivity with common respiratory pathogens and maintained stability under recommended storage conditions, establishes it as a reliable and robust diagnostic tool for rapid influenza detection.

In diverse clinical settings, particularly during seasonal influenza epidemics, this rapid test can significantly enhance patient care by facilitating timely clinical decision-making, enabling appropriate antiviral therapy initiation, supporting effective patient management strategies and implementing appropriate infection control measures. The test's flexibility in accepting multiple specimen types increases its utility across different patient populations and clinical scenarios. When used as part of a comprehensive diagnostic strategy that incorporates clinical symptoms, epidemiological data, and follow-up testing when indicated, this rapid influenza test substantially enhances the overall approach to influenza diagnosis and management, ultimately contributing to improved patient outcomes and more efficient healthcare resource utilization.

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