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Clinical Performance Evaluation of the Newlink L11-RTA05C5 SARS-CoV-2/Influenza A+B/RSV/Adenovirus/M.pneumoniae Antigen Combo Rapid Test (Nasopharyngeal Swab): A Multicenter Study

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Abstract: This study provides a comprehensive clinical evaluation of a multiplex immunochromatographic assay for the simultaneous qualitative detection of six major respiratory pathogens (SARS-CoV-2, Influenza A+B, RSV, Adenovirus, M. pneumoniae) from nasopharyngeal swabs. Using RT-PCR as the reference standard, diagnostic accuracy was evaluated across a large cohort of clinical specimens (n=3781). The assay demonstrated consistently high performance for all targets. For SARS-CoV-2, relative sensitivity and specificity were 96.4–98.2% and 99.0–>99.9%, respectively. Sensitivities for Influenza A and B were 94.1–95.2% and 92.9–94.3%, with specificities of 99.1–100%. For RSV, Adenovirus, and M. pneumoniae, sensitivities ranged from 94.3–98.2%, 96.9–98.1%, and 91.8–94.3%, while specificities were 96.2–100%, 97.4–100%, and 96.8–98.7%, respectively. Overall accuracy exceeded 95% for all pathogens across three independent clinical sites. The test showed no cross-reactivity with a panel of common respiratory microorganisms and exhibited robust precision. These results confirm that this multiplex rapid test is a reliable, accurate, and practical point-of-care tool for the timely detection and differentiation of key respiratory pathogens, supporting prompt clinical decision-making and patient management.

1. Introduction

Acute respiratory infections (ARIs) impose a significant global health burden, leading to substantial annual morbidity, mortality, and economic costs [1]. The clinical presentation of ARIs is often nonspecific, creating a diagnostic challenge as pathogens like SARS-CoV-2, Influenza A and B, Respiratory Syncytial Virus (RSV), Adenovirus, and Mycoplasma pneumoniae can cause similar symptoms. Accurate differentiation is crucial, as management and infection control measures vary for each pathogen. The COVID-19 pandemic has further highlighted the urgent need for rapid and

multiplex diagnostics capable of distinguishing among co-circulating respiratory agents [2].

Definitive diagnosis traditionally relies on laboratory-based molecular methods, most notably reverse transcription polymerase chain reaction (RT-PCR), which offers high analytical sensitivity and specificity [3]. However, RT-PCR depends on specialized equipment, technical expertise, and has a turnaround time of several hours, restricting its application in point-of-care (POC) or resource-constrained environments [4]. Viral culture, another established method, is even less suitable for immediate clinical guidance due to its prolonged incubation period (3–14 days) [5].

To address these limitations, rapid antigen diagnostic tests (RADTs) have become essential for near-patient testing. These immunochromatographic assays deliver results typically within 15–30 minutes, facilitating prompt clinical decisions, patient triage, and timely infection control [6]. While single-target RADTs are common, multiplex assays that can detect several major pathogens from a single sample offer improved diagnostic efficiency and cost-effectiveness [7]. This study evaluates a multiplex immunoassay designed for the simultaneous qualitative detection of SARS-CoV-2, Influenza A/B, RSV, Adenovirus, and M. pneumoniae antigens from nasopharyngeal swabs. The objective is to validate the test's clinical performance, including sensitivity, specificity, and accuracy, against RT-PCR as the reference standard, thereby determining its reliability and utility as a comprehensive POC diagnostic tool.

2. Experimental Procedures

2.1 Source of Clinical Samples

A total of 3781 clinical nasopharyngeal swab specimens were collected for this multicenter study. As per the inclusion criteria (details in Section 5 of the Clinical Evaluation Report), samples were obtained from symptomatic individuals within 7 days of symptom onset. The RT-PCR-confirmed distribution was: SARS-CoV-2 (298 positive, 1375 negative), Influenza A (192 positive, 1162 negative), Influenza B (160 positive, 1194 negative), RSV (158 positive, 1069 negative), Adenovirus (153 positive, 1003 negative), and M. pneumoniae (137 positive, 580 negative).

Specimens were collected at three clinical sites: Zhejiang Xiaoshan Hospital, China; Onkomedior Diagnostic MVZ GmbH, Labor Freising (SARS-CoV-2) and Shaanxi Provincial People's Hospital (Flu/RSV/ADV/MP), Germany/China; and Faculty of Medicine Ramathibodi Hospital, Mahidol University (SARS-CoV-2/Flu/RSV/ADV) and The Huzhou Traffic Hospital (MP), Thailand/China. All swabs were collected aseptically, placed immediately into dry sterile tubes, and stored at 2–8 ℃ if not tested promptly, with processing completed within 24 hours of collection.

2.2 Test Kits and Procedures

The evaluation employed the SARS-CoV-2/Influenza A+B/RSV/Adenovirus/M. pneumoniae Antigen Combo Rapid Test (Nasopharyngeal Swab; REF: L11-RTA05C5, from Newlink Biotech Co., Ltd), a lateral flow immunoassay based on the double-antibody sandwich principle. Commercial RT-PCR assays specific for each pathogen served as the reference standard for confirming all specimen infection status.

Following the manufacturer's instructions, test components were first equilibrated to room temperature. The nasopharyngeal swab was inserted into the provided extraction buffer tube and rotated vigorously against its inner wall for about 10 seconds to ensure antigen elution. After removing the swab while compressing its head to release residual liquid, a dropper cap was attached to the tube. The test cassette was then removed from its foil pouch, and exactly three drops of the extracted solution were added to each specimen well (S). The timer was started immediately upon

sample application.

Results were interpreted between 15 and 20 minutes; readings beyond this interval were considered invalid. The test cassette contains separate result windows for each pathogen. A positive result for a specific target is indicated by the appearance of both a colored Test line (T) and the Control line (C) in the corresponding window, while only the C line indicates a negative result. The absence of the C line in any window invalidates the entire test.

3. Performance Analysis

3.1 Analysis of Performance Characteristics

The multiplex rapid test demonstrated consistently high diagnostic performance across all six pathogens and three clinical sites. The aggregated results from each site are summarized below Tables 1-3.

Table 1: Clinical Performance at Site 1 (Zhejiang Xiaoshan Hospital)

Pathogen	**Relative Sensitivity**	**Relative Specificity**	**Total Accuracy**
SARS-CoV-2	96.4% (89.8% - 99.2%)	99.0% (96.3%–99.9%)	98.2% (95.8%–99.4%)
Influenza A	92.9% (80.3%–98.2%)	99.1% (96.5%–99.9%)	98.4% (95.9%–99.5%)
Influenza B	92.9% (80.3%–98.2%)	99.1% (96.5%–99.9%)	98.1% (95.4%–99.3%)
RSV	94.3% (80.8%–99.3%)	96.2% (92.8%–98.2%)	95.9% (92.8%–97.9%)
Adenovirus	96.9% (82.9%– 9.9%)	98.6% (95.7%–99.7%)	98.4% (95.7%–99.5%)
M. pneumoniae	91.8% (80.3%–97.3%)	98.0% (95.3%–99.3%)	97.0% (94.3%–98.5%)

Table 2: Clinical Performance at Site 2 (Onkomedior/Shaanxi Hospital)

Pathogen	Relative Sensitivity	Relative Specificity	Total Accuracy
SARS-CoV-2	98.2% (93.6%–99.8%)	>99.9% (99.3%–100.0%)	99.7% (98.8%–100.0%)
Influenza A	94.1% (85.6%–98.4%)	99.6% (97.8%–>99.9%)	98.4% (96.3%–99.5%)
Influenza B	93.8% (82.8%–98.7%)	99.3% (97.3%–99.9%)	98.4% (96.3%–99.5%)
RSV	98.2% (90.1%–>99.9%)	98.4% (94.2%–99.8%)	98.3% (95.1%–99.7%)
Adenovirus	98.1% (89.7%–100.0%)	97.4% (91.0%–99.7%)	97.7% (93.4%–99.5%)
M. pneumoniae	94.3% (84.3%–98.8%)	96.8% (91.1%–99.3%)	96.0% (91.4%–98.5%)

Table 3: Clinical Performance at Site 3 (Ramathibodi/Huzhou Hospital)

Pathogen	Relative Sensitivity	Relative Specificity	Total Accuracy
SARS-CoV-2	97.1% (91.9%–99.0%)	99.9% (99.2%–100.0%)	99.5% (98.7%–99.9%)
Influenza A	95.2% (88.4%–98.1%)	100.0% (99.5%–100.0%)	99.5% (98.7%–99.8%)
Influenza B	94.3% (86.2%–97.8%)	100.0% (99.5%–100.0%)	99.5% (98.7%–99.8%)
RSV	95.7% (88.0%–98.5%)	100.0% (99.5%–100.0%)	99.6% (98.9%–99.9%)
Adenovirus	97.1% (90.0%–99.2%)	100.0% (99.5%–100.0%)	99.7% (99.1%–99.9%)
M. pneumoniae	94.3% (80.8%–99.3%)	98.7% (96.6%–99.7%)	98.2% (95.7%–99.4%)

The Kappa values for all pathogen-site comparisons were calculated to be >0.90, indicating excellent agreement between the rapid combo test and the RT-PCR reference method.

3.2 Cross-reactivity and Interference

Analytical specificity assessment confirmed the high specificity of the assay. No cross-reactivity was detected when evaluating high-titer samples of phylogenetically or clinically related respiratory pathogens, including human coronaviruses (OC43, 229E, NL63, HKU1, MERS-CoV), human

rhinoviruses (types 2, 14, 16), as well as measles virus, mumps virus, and parainfluenza viruses (types 2, 3).

Furthermore, interference studies demonstrated robust assay performance under challenging matrix conditions. No interference was observed in the presence of common respiratory tract flora (e.g., Candida albicans, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa) at concentrations up to 10^8 CFU/mL. Additionally, the test maintained its analytical performance when challenged with clinically relevant concentrations of potential interferents, including whole blood, mucin, commonly used intranasal corticosteroids (oxymetazoline, fluticasone), and systemic therapeutic agents (dexamethasone, oseltamivir).

3.3 Precision

Precision was assessed by evaluating within-run and between-run (lot-to-lot) variability using negative, weak positive, and strong positive controls for all six analytes. The assay was tested across three distinct manufacturing lots over three consecutive days, achieving a correct identification rate exceeding 99%, which verifies its high reproducibility and consistent performance.

4. Discussion

4.1 Performance Characteristics

This comprehensive clinical validation demonstrates that the SARS-CoV-2/Influenza A+B/RSV/Adenovirus/M.pneumoniae Antigen Combo Rapid Test is a highly accurate diagnostic tool. Its performance metrics for each pathogen meet or exceed the minimum performance criteria suggested by health authorities for rapid antigen tests [8]. The consistently high specificity (≥96.2% across all targets and sites) is particularly noteworthy, as it minimizes false-positive results, thereby reducing unnecessary treatments and isolation measures.

The sensitivity for viral targets (SARS-CoV-2, Influenza, RSV, Adenovirus) ranged from 92.9% to 98.2%, which is excellent for a rapid antigen test. The sensitivity for the bacterial target, M. pneumoniae, was slightly lower but remained robust at 91.8–94.3%. This is clinically significant, as rapid point-of-care detection of M. pneumoniae is challenging, and this test offers a valuable tool for its early identification. The high overall accuracy (≥95.9%) and excellent Kappa agreement across multiple, geographically diverse clinical sites reinforce the test's reliability and generalizability.

The multiplex design is a key advantage, allowing for the differential diagnosis of six major respiratory pathogens from a single nasopharyngeal swab in under 20 minutes. This can dramatically streamline the diagnostic workflow in busy clinical settings during periods of co-circulation of multiple pathogens.

4.2 Limitations

Several inherent limitations of this rapid antigen test should be considered. The assay provides only qualitative (positive/negative) results and does not quantify pathogen load. Although demonstrating high sensitivity, it remains analytically less sensitive than RT-PCR, and false-negative results may occur—particularly in specimens with low antigen concentration, such as those collected later in the course of infection or from asymptomatic individuals. Additionally, test performance is critically dependent on proper nasopharyngeal swab collection technique, and suboptimal sampling may also lead to false-negative findings.

Further considerations include the need for ongoing evaluation of the assay's performance against future emerging variants of pathogens such as SARS-CoV-2 and influenza. As emphasized in the manufacturer's instructions, a negative result should not definitively rule out infection, and confirmatory molecular testing is recommended when clinical suspicion remains high. All results must be interpreted in conjunction with the patient's clinical presentation and relevant epidemiological information.

4.3 Comparison with Other Diagnostic Methods

Compared to the RT-PCR gold standard, this rapid test trades a marginal degree of sensitivity for a tremendous advantage in speed, simplicity, and suitability for point-of-care use. It does not require expensive instrumentation or technical expertise, making it accessible in primary care clinics, emergency departments, pharmacies, and community settings.

Compared to single-plex rapid tests, this combo test offers superior diagnostic efficiency, reducing the time, cost, and specimen volume required to test for multiple pathogens separately. This multiplex capability is aligned with the growing trend in syndromic testing for respiratory infections [9].

5. Conclusion

The SARS-CoV-2/Influenza A+B/RSV/Adenovirus/M.pneumoniae Antigen Combo Rapid Test (Nasopharyngeal Swab) has been rigorously validated and demonstrates consistently high sensitivity, specificity, and diagnostic accuracy for the simultaneous detection of six major respiratory pathogens. Its performance is robust across different clinical settings and shows excellent agreement with RT-PCR. The test exhibits no significant cross-reactivity or interference and shows high precision.

This multiplex rapid antigen test represents a significant advancement in point-of-care respiratory diagnostics. It empowers clinicians to make rapid, informed decisions regarding treatment (e.g., antiviral or antibiotic therapy), patient management, and infection control measures at the initial point of care. By enabling the timely differentiation of clinically similar respiratory illnesses, this tool can contribute to improved patient outcomes, optimized antimicrobial stewardship, and more efficient utilization of healthcare resources, particularly during seasonal epidemics and pandemics [10].

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