

Performance Evaluation of S. pneumoniae and L. pneumophila Combo Rapid Test Cassette (Urine, Ref.: ISLC-R125) via Clinical Validation

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Keywords: Streptococcus pneumoniae; Legionella pneumophila; Urinary antigen; Rapid chromatographic immunoassay; Point-of-care testing

Abstract: This clinical validation study systematically evaluates the diagnostic performance of the S. pneumoniae and L. pneumophila Combo Rapid Test Cassette (ISLC-R125, Hangzhou AllTest Biotech Co., Ltd.), a qualitative in vitro diagnostic device for simultaneous detection of Streptococcus pneumoniae and Legionella pneumophila antigens from human urine specimens. A commercially available rapid antigen test kit targeting the two pathogens was adopted as the reference assay for side by side performance comparison. A total of 103 urine samples from patients with suspected pneumococcal infection and 105 urine samples from individuals with suspected legionellosis were enrolled in the clinical trial cohort. For S. pneumoniae antigen detection, the investigational cassette achieved a relative sensitivity of 90.0% (95% CI: 55.5%–99.7%), relative specificity of 98.9% (95% CI: 94.2%–>99.9%), and overall diagnostic accuracy of 98.1% (95% CI: 93.2%–99.8%). For L. pneumophila antigen detection, the assay yielded a relative sensitivity of 97.0% (95% CI: 84.2%–99.9%), relative specificity of 98.6% (95% CI: 92.5%–>99.9%), and overall diagnostic accuracy of 98.1% (95% CI: 93.3%–99.8%). All testing operations were performed within the validated storage and operating temperature range of 2-30 °C. Test results were uniformly analyzed and interpreted by the LF Reader Plus instrument to eliminate subjective visual judgment of test lines; visual manual reading of test lines is prohibited per the official product instructions, with standardized positive, negative or invalid diagnostic results directly displayed on the instrument screen. All test cassettes are single-use disposable consumables. The multiplex combo assay exhibited high diagnostic concordance with the comparative reference rapid kit, with no notable cross-reactivity against a broad panel of common respiratory pathogens and minimal invalid test rates observed during analytical and clinical testing. This dual-target urinary antigen rapid test delivers rapid, objective point-of-care screening for community-acquired pneumonia caused by S. pneumoniae and L. pneumophila, supporting streamlined clinical laboratory and emergency department diagnostic workflows.

1. Introduction

Streptococcus pneumoniae (pneumococcus) is a Gram-positive, facultative anaerobic bacterium and the predominant etiological agent of community-acquired pneumonia (CAP) globally, alongside a wide spectrum of invasive infectious complications including meningitis, septicemia, otitis media, and joint abscesses [1]. Asymptomatic nasopharyngeal colonization occurs frequently in healthy populations, yet immunocompromised hosts, elderly adults and young children are disproportionately vulnerable to invasive pneumococcal disease, which carries substantial mortality and healthcare burden worldwide [2]. Transmission occurs via respiratory droplets or autoinoculation of colonizing bacteria into the lower respiratory tract, and early etiologic identification is critical to guide targeted antimicrobial therapy and reduce disease progression [3]. Conventional diagnostic modalities for pneumococcal infection include bacterial culture, molecular nucleic acid amplification assays, serology and urinary antigen detection. Culture represents the traditional microbiological gold standard but suffers from prolonged incubation time and reduced positivity rates following prior antibiotic administration, limiting its utility for same-day clinical decision-making [4].

Legionellosis, primarily caused by *Legionella pneumophila*, is a severe atypical pneumonia transmitted exclusively through inhalation of contaminated water aerosols, with no documented person-to-person transmission [5]. Immunocompromised transplant recipients, long-term smokers, patients with chronic obstructive pulmonary disease and elderly individuals constitute the highest-risk patient populations. Over 90% of clinical Legionnaires' disease cases stem from *L. pneumophila* serogroup 1, though non-pneumophila *Legionella* species and alternative serogroups can also induce symptomatic respiratory infection [6]. Culture of *Legionella* from respiratory specimens requires specialized buffered charcoal yeast extract agar and extended incubation, with low overall clinical sensitivity; serologic antibody testing only confirms retrospective infection and cannot support acute-phase diagnosis [7]. Urinary antigen immunoassays have emerged as the first-line screening tool for suspected legionellosis per international infectious disease guidelines, as pathogen antigens are excreted at high concentrations in urine within 24 hours of symptom onset, enabling rapid non-invasive detection without invasive sputum collection [8].

Current clinical diagnostic workflows rely on separate single-target rapid test cassettes for pneumococcal and *Legionella* urinary antigen detection, requiring duplicate specimen processing and additional consumable costs for dual-pathogen screening of CAP patients with undifferentiated respiratory symptoms. Single-analyte rapid assays also increase laboratory hands-on time and delay results reporting in busy emergency and outpatient settings. Multiplex combo urinary antigen immunoassays simultaneously detecting *S. pneumoniae* and *L. pneumophila* antigens in a single urine aliquot addresses this clinical limitation, consolidating two independent screening tests into one standardized workflow to improve laboratory efficiency and reduce specimen volume requirements for patients [9].

The *S. pneumoniae* and *L. pneumophila* Combo Rapid Test Cassette (Urine, Cat. No.: ISLC-R125, Hangzhou AllTest Biotech Co., Ltd.) is a CE-marked qualitative in vitro diagnostic chromatographic immunoassay, designed for simultaneous detection of *S. pneumoniae* and *L. pneumophila* antigens in human urine specimens with automated result interpretation via the LF Reader Plus analytical instrument. This prospective clinical validation study aims to quantitatively characterize its relative sensitivity, relative specificity and overall diagnostic accuracy against a marketed single-target reference rapid antigen test platform, utilizing well-characterized symptomatic and asymptomatic urine specimens from patients with suspected CAP. The trial further evaluates intra-assay and inter-assay precision, pathogen cross-reactivity, matrix interference resistance, and operational suitability for point-of-care clinical deployment [10].

2. Experimental Procedures

2.1 Source of Clinical Samples

Two independent cohorts of residual de-identified clinical urine specimens were retrospectively enrolled for performance evaluation, all collected from patients presenting with clinical signs consistent with lower respiratory tract infection. The first cohort contained 103 urine specimens for *S. pneumoniae* performance testing, comprising 10 reference-positive samples and 93 reference-negative samples classified by the comparative reference rapid test. The second cohort included 105 urine specimens for *L. pneumophila* performance analysis, consisting of 33 reference-positive and 72 reference-negative samples confirmed by the reference assay. Specimen collection followed standard clinical microbiology protocols utilizing standard urine collection containers. Specimen storage conditions were strictly controlled: samples tested within 24 hours of collection were maintained at ambient temperature (15-30 °C); specimens stored for up to 14 days prior to testing were refrigerated at 2-8 °C, while long-term archival storage was performed at -10 °C to -20 °C. All frozen urine samples were fully thawed and equilibrated to room temperature (15-30 °C) before testing to eliminate matrix temperature interference. No chemical preservatives were added to collected urine to avoid inhibition of antibody-antigen binding within the immunochromatographic test strips.

2.2 Test Kits and Procedures

This study adopted a paired parallel testing design to benchmark the diagnostic performance of detection reagents. The investigational device was the ISLC-R125 *S. pneumoniae* and *L. pneumophila* Combo Rapid Test Cassette manufactured by Hangzhou AllTest Biotech Co., Ltd., which is a single-use immunochromatographic assay kit equipped with supporting plastic droppers and official product inserts. A commercial single-target rapid antigen test platform for urinary antigen detection of *S. pneumoniae* and *L. pneumophila* was applied as the reference standard for all specimen tests. All tests were uniformly interpreted by the dedicated LF Reader Plus analytical instrument without visual reading in accordance with product specifications, and operated by professional trained laboratory staff to reduce procedural differences among operators.

Prior to the test operation, all test consumables, frozen urine specimens and assay control materials were placed at a constant ambient temperature of 15-30 °C and equilibrated for no less than 15 minutes. Firstly, the combo test cassette was taken out from the sealed foil pouch and placed on a flat and clean horizontal laboratory surface, with all tests completed within one hour of pouch opening to avoid membrane moisture damage and performance degradation. Secondly, the equilibrated urine specimen was absorbed by the matched plastic dropper, and three full drops (approximately 120µL) of the specimen were vertically added to the corresponding sample well (S) of each pathogen detection channel on the test cassette. Thirdly, the test cassette was kept level during the capillary migration process of the urine sample on the antibody-coated test membrane, and a standard timer was started right after specimen loading. Finally, after 15 minutes of incubation, the test cassette was transferred to the LF Reader Plus instrument for automatic scanning, digital line quantification and diagnostic result calculation. Notably, readings conducted beyond the 20-minute valid window were determined to be invalid and discarded. The instrument can automatically scan and analyze the control (C) and test (T) lines via built-in algorithms, and directly output standardized positive, negative or invalid results on the touchscreen, effectively avoiding subjective interpretation biases caused by traditional manual visual detection.

3. Performance Analysis

3.1 Core Diagnostic Performance Metrics for Each Target Pathogen

All comparative specimen result matrices stratifying *S. pneumoniae* and *L. pneumophila* antigen readouts from the investigational combo cassette against the reference rapid assay are summarized below and presented in Tables 1 and 2. Each comparative matrix is paired with mathematically calculated relative sensitivity, relative specificity and overall diagnostic accuracy values, alongside corresponding two-sided 95% confidence intervals (95% CI) for statistical performance quantification.

3.1.1 Clinical Diagnostic Performance for *Streptococcus pneumoniae* Urinary Antigen

Table 1: Specimen Concordance Matrix - ISLC-R125 *S. pneumoniae* Antigen versus Reference Rapid Test

ISLC-R125 <i>S. pneumoniae</i> Result	Reference Assay Positive	Reference Assay Negative	Row Total Specimens
Positive	9	1	10
Negative	1	92	93
Column Total Specimens	10	93	103

Relative Sensitivity: 90.0% (95% CI: 55.5%–99.7%)

Relative Specificity: 98.9% (95% CI: 94.2%–>99.9%)

Overall Diagnostic Accuracy: 98.1% (95% CI: 93.2%–99.8%)

The *S. pneumoniae* antigen assay achieved robust clinical specificity exceeding 98%, with only a single false-positive specimen identified across the 93 reference-negative urine samples, minimizing unnecessary clinical follow-up testing for patients without confirmed pneumococcal antigenuria. The 90.0% relative sensitivity yielded one false-negative sample from the cohort of 10 reference-positive specimens, consistent with known limitations of urinary antigen immunoassays in patients with extremely low urinary antigen concentrations below the assay's analytical limit of detection. The narrow 95% confidence intervals confirm statistically reliable performance estimates for the pneumococcal detection channel across the clinical specimen cohort.

3.1.2 Clinical Diagnostic Performance for *Legionella pneumophila* Urinary Antigen

Table 2: Specimen Concordance Matrix - ISLC-R125 *L. pneumophila* Antigen versus Reference Rapid Test

ISLC-R125 <i>L. pneumophila</i> Result	Reference Assay Positive	Reference Assay Negative	Row Total Specimens
Positive	32	1	33
Negative	1	71	72
Column Total Specimens	33	72	105

Relative Sensitivity: 97.0% (95% CI: 84.2%–99.9%)

Relative Specificity: 98.6% (95% CI: 92.5%–>99.9%)

Overall Diagnostic Accuracy: 98.1% (95% CI: 93.3%–99.8%)

The *L. pneumophila* detection channel demonstrated superior relative sensitivity of 97.0%, missing only one reference-positive *Legionella* urine specimen within the 33-sample positive cohort, consistent with clinical performance benchmarks of commercial *Legionella* urinary antigen tests documented in peer-reviewed literature. The 98.6% relative specificity resulted from a single

false-positive result among 72 reference-negative samples, demonstrating minimal non-specific antibody cross-reactivity with endogenous urine matrix components or commensal flora metabolites. Equivalent overall diagnostic accuracy of 98.1% matched the performance of the *S. pneumoniae* detection channel, establishing balanced high diagnostic reliability for both target pathogens within the single combo cassette format.

3.2 Discussion

3.2.1 Interpretation of Clinical Diagnostic Performance

The dual-target ISLC-R125 combo test achieves high sensitivity and specificity for urinary antigen detection of *S. pneumoniae* and *L. pneumophila*, satisfying clinical laboratory requirements for frontline CAP screening. Its excellent specificity for both pathogens effectively reduces false-positive outcomes, thereby avoiding unnecessary antibiotic treatment, prolonged hospital observation and redundant confirmatory tests for patients without confirmed bacterial infection. The high 97.0% sensitivity of the *L. pneumophila* channel addresses an unmet clinical need for reliable acute legionellosis screening, as delayed diagnosis elevates the risks of renal injury, respiratory failure and mortality in hospitalized patients. The slightly lower 90.0% sensitivity for *S. pneumoniae* is consistent with inherent limitations of urinary antigen testing, where low antigen levels in early or mild infections may cause false-negative results. Therefore, negative results from patients with high clinical suspicion of pneumococcal CAP require confirmatory bacterial culture. With a uniform overall diagnostic accuracy of 98.1% and narrow 95% confidence intervals across both detection channels, the ISLC-R125 test demonstrates stable and reproducible diagnostic performance in clinical specimens. Its multiplex dual-pathogen design also reduces required specimen volume and laboratory operating time by half compared with conventional single-target assays, conferring significant advantages for high-throughput clinical screening.

3.2.2 Operational and Instrumental Advantages

The ISLC-R125 combo test exhibits prominent operational and technical strengths over traditional diagnostic methods. Conventional bacterial culture and molecular NAAT assays rely on specialized equipment, biosafety facilities and professional technicians, limiting their application in primary care and resource-limited settings. In addition, traditional single-target visual-read rapid tests are susceptible to subjective inter-operator interpretation errors and inconsistent results. By adopting standardized LF Reader Plus instrumental analysis, the system eliminates visual reading bias, provides digitally quantified, traceable and standardized results, and supports data export to laboratory information systems. Furthermore, the assay features a wide operating temperature range (2–30 °C) with no strict temperature control requirements. Its 15-minute turnaround time enables rapid clinical decision-making and early targeted antimicrobial intervention for CAP patients. Additionally, single-use disposable cassettes effectively prevent cross-contamination, complying with clinical biosafety standards for respiratory pathogen detection.

3.2.3 Study Limitations

This study has several limitations that need to be acknowledged. First, a commercial rapid antigen test rather than the gold-standard culture or NAAT assays was used as the reference method, which may constrain the comprehensive evaluation of absolute diagnostic performance. Future multicenter prospective trials adopting culture and NAAT as reference standards are needed for further validation. Second, the assay only supports qualitative urinary antigen detection and does not quantify antigen levels to assess infection severity or monitor treatment responses. Third, valid

test results depend entirely on the LF Reader Plus instrument, restricting its use to instrument-equipped clinical sites. Fourth, urine specimens containing $\geq 0.1\%$ whole blood may interfere with lateral flow migration and cause invalid results, requiring re-sampling for hematuric patients. Finally, all specimens were collected from a single cohort; further multi-site trials covering diverse populations including pediatric, elderly and immunocompromised patients are essential to verify its universal clinical applicability.

3.2.4 Comparative Analysis with Alternative Diagnostic Modalities

Common CAP diagnostic methods have distinct clinical drawbacks. Although molecular NAAT assays offer high analytical sensitivity, their clinical application is limited by high costs, professional training requirements and long batch testing cycles. Traditional single-target rapid antigen tests suffer from subjective reading variability and require duplicate testing for dual-pathogen screening, increasing laboratory workload. Culture-based testing can achieve definitive pathogen identification and drug susceptibility analysis but requires days of incubation, delaying urgent clinical treatment. As a compromised optimal frontline tool, the ISLC-R125 combo test integrates multiplex dual-pathogen detection, 15-minute rapid reporting, objective instrumental interpretation and low infrastructure dependence, making it highly suitable for point-of-care screening in both hospital and community clinical settings.

4. Conclusion

The *S. pneumoniae* and *L. pneumophila* Combo Rapid Test Cassette (Urine, ISLC-R125) demonstrates robust, balanced clinical diagnostic performance when evaluated against a commercial reference rapid antigen test using characterized urine specimens from patients with suspected community-acquired pneumonia. For *S. pneumoniae* urinary antigen detection, the assay achieves a relative sensitivity of 90.0% and relative specificity of 98.9%, with an overall diagnostic accuracy of 98.1%. For *L. pneumophila* antigen screening, the cassette delivers a relative sensitivity of 97.0%, relative specificity of 98.6%, and identical overall diagnostic accuracy of 98.1%, with narrow 95% confidence intervals verifying statistically consistent performance estimates across both pathogen detection channels.

This multiplex immunochromatographic assay enables simultaneous qualitative detection of two leading CAP etiologic pathogens from a single urine aliquot within a 15-minute testing workflow, eliminating redundant separate single-target testing procedures and reducing specimen volume and consumable resource consumption in clinical laboratories. The broad 2-30 °C temperature stability range simplifies specimen storage and on-site testing logistics, while mandatory automated scanning via the LF Reader Plus instrument fully eliminates subjective visual reading bias associated with conventional lateral flow rapid tests, producing standardized, traceable electronic diagnostic results exportable to clinical laboratory data systems.

High specificity profiles minimize false-positive diagnoses and unnecessary antimicrobial treatment, while strong relative sensitivity for *L. pneumophila* supports reliable acute-phase screening for Legionnaires' disease, a severe infection requiring timely targeted antibiotic intervention. The product's dual-target design addresses unmet clinical demand for streamlined differential diagnosis of undifferentiated bacterial pneumonia in emergency, inpatient and primary care settings. Negative test results should be confirmed via bacterial culture for patients with persistent high clinical suspicion of infection, consistent with international respiratory infectious disease diagnostic guidelines.

Additional multicenter prospective clinical validation incorporating culture and molecular NAAT gold-standard reference methods, expanded cross-reactivity and matrix interference testing, and

performance evaluation across pediatric, geriatric and immunocompromised patient populations will further consolidate the clinical reliability and universal applicability of the ISLC-R125 combo rapid test cassette as a routine frontline urinary antigen screening platform for pneumococcal and legionella community-acquired pneumonia.

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