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A Multicenter Study on the Clinical Utility of a Rapid Antigen Combo Test for Concurrent Detection of SARS-CoV-2, Influenza A, Influenza B, and RSV

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Abstract: The SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test is a chromatographic immunoassay designed for the qualitative detection of SARS-CoV-2 nucleocapsid protein, Influenza A, Influenza B and Respiratory Syncytial Virus (RSV) antigens in human nasopharyngeal and nasal swab specimens. This study aims to systematically evaluate the performance characteristics, including sensitivity, specificity, accuracy and clinical utility, of this combo test compared to reverse transcription - polymerase chain reaction (RT-PCR), the gold standard method. A total of 2286 specimens (1369 nasopharyngeal swabs and 917 nasal swabs) from individuals with suspected respiratory viral infections were tested. The results demonstrated high relative sensitivity (ranging from 92.9% to 97.0%), specificity (ranging from 96.2% to 99.7%) and accuracy (ranging from 95.9% to 99.2%) across different viruses and specimen types. The test showed no cross-reactivity with other common respiratory viruses and bacteria and its limit of detection was determined to be 100 pg/mL for SARS-CoV-2, 1.5×10^5 $TCID_{50}$/Test for Influenza A, 1.0×10^5 $TCID_{50}$/Test for Influenza B and 1.2×10^4 $TCID_{50}$/Test for RSV. Intra-assay and inter-assay precision were excellent, with >99% correct identification of specimens. These findings indicate that the SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test is a reliable, rapid and practical tool for the simultaneous detection of multiple respiratory viral antigens, which can aid in timely clinical decision-making and infection control.

1. Introduction

Respiratory viral infections pose a significant threat to public health worldwide, causing substantial morbidity, mortality and economic burdens. Among these, infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A and B viruses and respiratory syncytial virus (RSV) are major contributors to respiratory illness, especially during seasonal outbreaks and pandemics[1]. The clinical manifestations of these infections often overlap, including

fever, cough, sore throat and fatigue, making differential diagnosis challenging based solely on symptoms [2]. Accurate and rapid identification of the causative pathogen is crucial for appropriate patient management, antiviral treatment initiation and implementation of infection control measures to prevent transmission [3].

Traditional diagnostic methods for respiratory viruses, such as virus isolation in cell culture, are time-consuming and require specialized laboratory facilities, limiting their utility in point-of-care settings. Reverse transcription-polymerase chain reaction (RT-PCR) is considered the gold standard due to its high sensitivity and specificity, but it also has drawbacks, including longer turnaround times, higher costs and the need for trained personnel and complex equipment [4]. Chromatographic immunoassays have emerged as a valuable alternative for rapid antigen detection, offering advantages such as simplicity, portability and quick results, making them suitable for use in various settings, including clinics, emergency departments and even resource-limited areas[5].

The SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test is a novel chromatographic immunoassay that enables the simultaneous qualitative detection of four major respiratory viral antigens in a single test. This combo test has the potential to address the unmet need for rapid differential diagnosis of these infections. However, comprehensive evaluation of its performance characteristics, including sensitivity, specificity, accuracy and limitations, is essential before widespread clinical application. The purpose of this study is to assess the diagnostic performance of the SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test using nasopharyngeal and nasal swab specimens, with RT-PCR as the reference method and to discuss its clinical utility and limitations.

2. Materials and Methods

2.1 Specimen Collection

A total of 2286 specimens were collected from individuals with suspected respiratory viral infections across multiple clinical sites, including locations in Poland, Sweden, Spain, China, Slovenia, etc. These specimens comprised 1369 nasopharyngeal swabs and 917 nasal swabs, targeting SARS-CoV-2, Influenza A, Influenza B, and RSV.

All specimens were confirmed by RT-PCR, with specific positive and negative distributions for each virus and specimens were collected following standard procedures. If not tested immediately, they were stored at 2-8 ℃ in dry, sterile, tightly sealed plastic tubes, with a maximum storage time of 24 hours.

2.2 Test Kit and Procedure

The SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test kits from Hangzhou AllTest Biotech Co., Ltd. were used. Each kit contains test cassettes, specimen collection swabs, extraction buffer tubes, control swabs, a workstation and instructions. The test is based on the double antibody sandwich technique, with colloidal gold-conjugated monoclonal antibodies as detectors and pre-coated antibodies on test lines (T, A, B), plus a control line (C) to verify validity.

The test procedure is conducted strictly according to the package insert. Firstly, equilibrate the kit, specimens and controls to a temperature of 15-30 ℃. Secondly, prepare the specimen by immersing a swab in the extraction buffer, rotating it, squeezing and then attaching the dropper tip. Lastly, add three drops of the extracted specimen to the test cassette well (S) and start the timer, read the results at 15 minutes, ensuring that no interpretation is made after 20 minutes. Controls are tested in the same manner.

Result interpretation is as follows: a positive result for SARS-CoV-2/RSV is indicated by two

lines (C + T) in the COVID-19/RSV window. A positive result for Influenza A shows two lines (C + A) in the FLU A+B window, while a positive result for Influenza B displays two lines (C + B) in the same window. If both Influenza A and B are positive, three lines (C + A + B) will appear in the FLU A+B window. A negative result shows one line (C) with no test lines and an invalid result occurs when there is no C line, necessitating a retest with a new kit.

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity and Specificity

266 SARS-CoV-2 positive specimens and 584 SARS-CoV-2 negative specimens confirmed by PCR, 111 Influenza A positive specimens and 704 Influenza A negative specimens confirmed with PCR, 9 Influenza B positive specimens and 722 Influenza B negative specimens confirmed with PCR, 89 RSV positive specimens and 582 RSV negative specimens confirmed by PCR and clinical symptoms were used in the clinical study. Commercial PCR kits served as the reference method for the SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test. The result shows the SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test has a high restive sensitivity and high relative specificity when tested with the 2286 specimens.

3.1.2 Cross-Reactivity and Interference

The test showed no cross-reactivity with various viral strains, including Adenovirus type 3, Adenovirus type 7, Human coronavirus OC43, Human coronavirus 229E, Human coronavirus NL63, Human coronavirus HKU1, MERS COV Florida, Human Rhinovirus 2, Human Rhinovirus 14, Human Rhinovirus 16, Measles, Mumps, Parainfluenza virus 2, Parainfluenza virus 3 at specified concentrations. Additionally, no interference was observed from common substances such as whole blood, mucin and various medications (Budesonide Nasal Spray, Dexamethasone, Flunisolide, Mupirocin, Oxymetazoline, Phenylephrine, Rebetol, Relenza, Tamiflu, Tobryamycin) at the tested concentrations.

3.1.3 Precision

Intra-assay and inter-assay precision were evaluated using negative specimens and weak and strong positive specimens for each virus. Three different lots of test kits were used with 10 replicates tested per day for 3 consecutive days. The specimens were correctly identified >99% of the time, indicating excellent precision.

3.2 Discussion

3.2.1 Performance Characteristics

The SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test demonstrated high relative sensitivity, specificity and accuracy for the detection of all four respiratory viral antigens in both nasopharyngeal and nasal swab specimens, with results comparable to those reported for other commercial antigen tests[6]. The relative sensitivity for SARS-CoV-2 was 97.0% in both nasopharyngeal and nasal swabs, which is consistent with previous studies indicating that antigen tests perform well during the acute phase of infection when viral load is high. For Influenza A and B, the sensitivity ranged from 92.9% to 95.8% and specificity was >99% in most cases, suggesting that

the test is reliable for the rapid diagnosis of influenza infections, which is crucial for timely antiviral treatment initiation, as oseltamivir is most effective when administered within 48 hours of symptom onset [7].

The RSV detection performance was also satisfactory, with sensitivity ranging from 94.3% to 96.3% and specificity from 96.2% to 99.7%. This is particularly important for pediatric populations, as RSV is a leading cause of severe respiratory illness in young children [8]. The high specificity observed indicates that the test has a low false positive rate, minimizing unnecessary interventions and anxiety.

Nasal swabs, which are easier to collect and more comfortable for patients, showed comparable or even slightly better performance than nasopharyngeal swabs for some viruses, such as RSV (96.3% sensitivity in nasal vs. 94.3% in nasopharyngeal) and SARS-CoV-2 (98.9% accuracy in nasal vs. 98.0% in nasopharyngeal). This suggests that nasal swabs could be a viable alternative to nasopharyngeal swabs for this test, potentially increasing patient acceptance and compliance, especially in pediatric and outpatient settings [9].

3.2.2 Limitations

Despite its strong performance, the SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test has several limitations that need to be considered. First, as with all antigen tests, its sensitivity is lower than that of RT-PCR, especially when viral loads are low [10]. This means that false negative results can occur, particularly in the early or late stages of infection. Therefore, negative results should be interpreted with caution, especially in individuals with strong clinical suspicion or known exposure and confirmed by RT-PCR if necessary [11].

Second, the test is qualitative and does not provide quantitative information about viral load, which may be useful for monitoring disease progression or response to treatment. However, qualitative results are sufficient for most point-of-care diagnostic purposes, where the primary goal is to identify the presence of the virus.

Third, the test performance is dependent on proper specimen collection, handling and storage. Deviations from the recommended procedures, such as using inappropriate specimen types (e.g., specimens stored in viral transport media), incorrect swab collection techniques or improper storage conditions, may lead to inaccurate results. Healthcare providers must be trained to follow the standardized protocols outlined in the package insert to ensure optimal performance.

Additionally, while the test showed no cross-reactivity with the tested viral strains and substances, it is possible that new viral variants or untested interfering substances could affect results. Continuous monitoring of test performance against emerging variants is essential to maintain its reliability [12].

3.2.3 Comparison with Other Diagnostic Methods

Compared to single-target antigen tests, the combo test offers the advantage of simultaneous detection, reducing the need for multiple tests and saving time and resources. This is particularly beneficial in settings where the differential diagnosis includes multiple respiratory viruses. In comparison to RT-PCR, the combo test is faster and more cost-effective, making it suitable for rapid screening and point-of-care applications. However, RT-PCR remains the gold standard for confirmation of negative results or in cases where high sensitivity is required, such as in asymptomatic individuals or for epidemiological surveillance [13].

4. Conclusion

The SARS-CoV-2/Influenza A+B/RSV Antigen Combo Rapid Test is a reliable and efficient

lateral flow immunoassay for the qualitative detection of SARS-CoV-2, Influenza A, Influenza B and RSV antigens in both nasopharyngeal and nasal swab specimens. It demonstrates high sensitivity, specificity and accuracy, with comparable performance between the two specimen types and nasal swabs offering a more patient-friendly alternative. The test's rapid turnaround time, ease of use, and ability to simultaneously detect multiple viral antigens make it a valuable tool for timely clinical decision-making, infection control and appropriate patient management.

While the test has limitations, including lower sensitivity compared to RT-PCR and the need for proper specimen handling, its strengths make it a practical option for point-of-care testing in various healthcare settings. Future studies should focus on evaluating its performance against emerging viral variants and in larger, diverse patient populations to further validate its clinical utility.

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