

Performance Evaluation of the Chlamydia Rapid Test for Qualitative Detection of Chlamydial Antigens in Clinical Diagnosis

Zhang Lei^{1,*}, Yang Feng², Zhu Junzhe³

¹Zhejiang Gongshang University, Hangzhou, Zhejiang, 310018, China

²Community Health Service Center, Yipeng Street, Qiantang District, Hangzhou, Zhejiang, 310000, China

³Wenzhou Medical University, Wenzhou, Zhejiang, 310000, China

*Corresponding author: zhanglei@zjgsu.edu.cn

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Abstract: In this study, the objective was to evaluate the diagnostic performance of the Chlamydia Rapid Test, a rapid chromatographic immunoassay specifically designed for the qualitative detection of Chlamydia trachomatis in various specimen types. The aim was to improve the diagnosis of Chlamydia infection, considering its global prevalence as the leading cause of sexually transmitted venereal infection. The material and methods involved evaluating the sensitivity, specificity, and accuracy of the Chlamydia rapid test using specimens obtained from patients at STD clinics. The Chlamydia Rapid Test Cassette (Swab/Urine) was compared to PCR, which served as the reference method for the evaluation. The research findings demonstrated the high accuracy of the Chlamydia Rapid Test in detecting the microorganism across different specimen types. For female cervical swab specimens, the test exhibited a relative sensitivity of 93.3% and a relative specificity of 97.5%, resulting in an overall accuracy of 96.6%. In the case of male urethral swab specimens, the relative sensitivity was 86.2% and the relative specificity was 95.8%, with an overall accuracy of 92.7%. Additionally, for male urine specimens, the test displayed a relative sensitivity of 94.6% and a relative specificity of >99.9%, leading to an overall accuracy of 97.9%. These results highlight the effectiveness of the test in precisely identifying the presence or absence of the microorganism in diverse specimen types. In conclusion, the Chlamydia Rapid Test developed by Hangzhou AllTest Biotech Co., Ltd has demonstrated excellent performance in detecting Chlamydia antigen in various specimen types, including female cervical swabs, male urethral swabs, and male urine samples. Due to its high accuracy and reliability, this test is suitable for clinical application as an auxiliary diagnostic tool. Furthermore, its quick and accurate results make it particularly valuable in facilitating timely diagnosis and treatment, especially in resource-limited areas.

1. Introduction

Chlamydia, an infectious disease transmitted through sexual contact, is caused by the bacterium *Chlamydia trachomatis* (CT). The World Health Organization (WHO) reports an estimated annual incidence of 127.2 million new cases of CT, with a substantial number originating from resource-limited countries^[1].

Most cases are detected in individuals below the age of 25. The initial infection often does not present with symptoms, although it may involve the presence of vaginal discharge or burning during urination^[2]. Asymptomatic infection can only be detected through proactive testing. Untreated CT infection in women can result in serious complications, including pelvic inflammatory disease (PID), ectopic pregnancy, tubal infertility, and chronic pelvic pain^[3]. Men with symptomatic *Chlamydia trachomatis* (CT) infection typically experience urethritis, which is characterized by the presence of a mucoid or watery discharge from the urethra and discomfort while urinating (dysuria)^[4]. In addition, genital CT infection significantly increases the risk of HIV transmission and the development of cervical carcinoma associated with human papillomavirus (HPV)^[5]. Thus, it is vital to timely detect and diagnose chlamydia infections in order to prevent their widespread occurrence and the resulting serious complications.

Regular screening is recommended for at-risk sexually active individuals since most chlamydial infections are asymptomatic. This approach allows for the effective management of the infections by promptly identifying and treating the infected cases.^[6] Chlamydia screening is rare in developing countries, despite the fact that they have the highest incidence of new chlamydial infections^[7]. Laboratory-based nucleic acid amplification tests (NAATs) offer a precise diagnostic method, with most utilizing polymerase chain reaction (PCR) and incorporating fluorescently labeled probes to detect amplified products in real time. However, these tests are often unaffordable and inaccessible in developing countries. This is mainly due to the requirements of laboratory infrastructure, trained personnel, and the high associated costs^[8]. In addition, the time delay of 7-10 days between obtaining laboratory results and initiating treatment may render timely intervention for patients impossible^[9]. Therefore, a point-of-care (POC) test with proven diagnostic accuracy may well help limit the spread of CT and improve prognosis. Utilizing rapid point-of-care (POC) tests can serve as a cost-effective approach to enhance the effectiveness of interventions for STI screening. The primary benefit lies in their ability to provide results during the patient's initial consultation, eliminating the need for subsequent patient follow-up visits.^[10]

The aim of this study was to assess the diagnostic utility of the immunochromatographic-based Chlamydia Rapid Test, developed by Hangzhou AllTest Biotech Co., Ltd, in identifying *C. trachomatis*. Biological samples obtained from male and female subjects undergoing specific analyses were evaluated using the rapid test in a diagnostic laboratory. Subsequently, the obtained results were compared with those derived from the PCR test to establish the test's efficacy.

2. Materials and Methods

2.1. Sample Collection

The Chlamydia Rapid Test Cassette (Swab/Urine) can be performed using a female cervical swab, male urethral swab or male urine specimens. Clinical specimens were collected in this study for experimental purposes.

To collect specimens from the female cervical swabs, the provided swab in the kit or any plastic-shaft swab can be used. To prevent sample contamination, it is crucial to remove excess mucus from the endocervical area using a cotton ball before specimen collection. When collecting a specimen, the user should insert the swab into the endocervical canal, going beyond the

squamocolumnar junction until most of the tip is no longer visible. This ensures the acquisition of columnar or cuboidal epithelial cells, which serve as the primary reservoir for the Chlamydia organism. The user should rotate the swab firmly in one direction (clockwise or counterclockwise) for 360°, allow it to stand for 15 seconds, and then withdraw the swab. Care should be taken to prevent contamination from exocervical or vaginal cells. It is advised not to treat swabs with 0.9% sodium chloride before specimen collection. In case the test is to be conducted immediately, the swab should be placed into the extraction tube.

To collect Male Urethral Swab Specimens, it is recommended to use standard plastic or wire-shaft sterile swabs. Prior to specimen collection, patients should be instructed to refrain from urinating for at least one hour. To collect a specimen, the user should insert the swab into the urethra approximately 2-4 cm, and rotate it 360° in one direction (clockwise or counterclockwise). The user should allow the swab to stand for 10 seconds, and then withdraw it. It is advised not to treat the swabs with 0.9% sodium chloride before specimen collection. If the test is to be conducted immediately, the swab should be placed into the extraction tube.

For the collection of Male Urine Specimens, it is recommended to obtain 15-30ml of clean first morning urine in a sterile urine cup. This is because first morning urine samples yield the highest concentrations of Chlamydia antigen. To prepare the specimen, mix it by inverting the container and transfer 10ml of the urine into a centrifuge tube. The user should add 10ml of distilled water to the tube and centrifuge it at 3,000 rpm for 15 minutes. After centrifugation, carefully discard the supernatant while keeping the tube inverted. Any remaining supernatant on the rim of the tube can be removed by blotting it onto an absorbent pad. If the test is to be conducted immediately, the urine pellet should be treated according to the provided Directions for Use.

The collected samples were divided into two portions, with half of them allocated for immunochromatographic analysis (rapid testing), and the remaining half for biomolecular analysis (real-time quantitative PCR). The samples designated for rapid testing were stored at 2-8 °C and analyzed within 72 hours after collection, while those intended for real-time PCR analysis were stored in a freezer at -15 °C to -30 °C until further processing. Prior to initiating the immunochromatographic and biomolecular analyses, the samples were kept at room temperature (15 °C - 25 °C).

2.2. Screen Test

Before performing the analysis, it is crucial to confirm that the assay, reagents, sample, and/or standards have attained ambient conditions (15-25 °C). To start the testing procedure, the user should remove the examination cassette from the foil bag and use it within one hour for the most accurate outcomes. Next, the user should extract the Chlamydia antigen based on the specific type of specimen provided. Optimal results are obtained by carefully following the procedure steps without delay from initiation.

The immunoassay utilizes an antibody specifically targeting the Chlamydia antigen which is immobilized on the test strip's detection zone. During the process, the extracted antigen solution interacts with an antibody directed against Chlamydia that has been conjugated to microparticles. This immune complex migrates via capillary action to the membrane where it reacts with the bound antibody matching Chlamydia, thereby generating a colored line in the test region. The visualization of this colored band within the detection zone indicates a positive result, whereas its absence denotes a negative result. To function as a procedural control, a colored line will consistently manifest in the control zone, signifying that an adequate specimen volume was added and fluid flow occurred as expected via membrane wicking.

It is recommended that sufficient time be allotted for chromogenesis to occur. For optimal

interpretation, results should be read at the designated 10-minute time point. The visual color intensity may continue developing slightly after this mark. However, for most accurate assessment, conclusions should not be derived beyond the 20-minute incubation period, as signal strength can diminish and potentially result in an erroneous determination if analyzed later than the recommended interpretation window.

In the PCR detection of Chlamydia, clinical specimens are collected from patients and processed to extract and purify Chlamydia DNA. A PCR reaction mixture, containing specific primers for Chlamydia, enzymes, and buffers for DNA amplification, is prepared. The Chlamydia DNA undergoes cyclic amplification in a PCR machine through heating and cooling steps to facilitate DNA replication. Subsequently, the PCR amplification products are analyzed, often using gel electrophoresis or other methods, to identify specific Chlamydia DNA sequences. This PCR analysis allows for determining the presence and extent of Chlamydia infection in the sample.

3. Results and Discussion

3.1. Results

The Chlamydia Rapid Test Cassette was assessed using specimens obtained from patients at STD clinics, with PCR employed as the reference method. Specimens are categorized as positive if the PCR results yield a positive outcome, and as negative if the PCR results indicate a negative result.

In the first set of experiments (Table 1), a total of 205 Female Cervical Swab Specimens were subjected to *C. trachomatis* detection to compare the performance characteristics of the rapid test and PCR test. Among the 45 samples that showed positive results in the PCR test, the rapid test identified 42 as positive (with a relative sensitivity of 93.3%). Regarding the 160 samples that tested negative in the PCR test, the rapid test correctly identified 156 as negative (with a relative specificity of 97.5%). The overall accuracy was determined to be 96.6%.

Table 1: Performance Characteristics For Female Cervical Swab Specimens

Method		PCR		Total Results
Chlamydia Rapid Test Cassette (Swab/Urine)	Results	Positive	Negative	
	Positive	42	4	46
	Negative	3	156	159
Total Results		45	160	205

Relative Sensitivity: 93.3% (81.7%-98.6%)*

Relative Specificity: 97.5% (93.7%-99.3%)*

Overall Accuracy: 96.6% (93.1%-98.6%)*

*: 95% Confidence Intervals

Table 2: Performance Characteristics For Male Urethral Swab Specimens

Method		PCR		Total Results
Chlamydia Rapid Test Cassette (Swab/Urine)	Results	Positive	Negative	
	Positive	50	5	55
	Negative	8	115	123
Total Results		58	120	178

Relative Sensitivity: 86.2% (74.6%-93.9%)*

Relative Specificity: 95.8% (90.5%-98.6%)*

Overall Accuracy: 92.7% (87.8%-96.1%)*

*: 95% Confidence Intervals

The second set of experiments (Table 2) involved testing a total of 178 Male Urethral Swab Specimens for *C. trachomatis* detection. Of the 58 samples that yielded positive results in the PCR test, the rapid test detected 50 as positive (representing a relative sensitivity of 86.2%). For the 120 samples that tested negative in the PCR test, the rapid test accurately identified 115 as negative (resulting in a relative specificity of 95.8%). The overall accuracy was determined to be 92.7%.

In the third set of experiments (Table 3), a total of 97 specimens obtained from Male Urine Specimens were evaluated for the detection of *C. trachomatis*. Among the 37 samples that displayed positive outcomes in the PCR test, the rapid test accurately identified 35 as positive, achieving a relative sensitivity of 94.6%. Regarding the 60 samples that tested negative in the PCR test, the rapid test exhibited no positive detection results, demonstrating a relative specificity exceeding 99.9%. The rapid test's overall accuracy was determined to be 97.9%.

Table 3: Performance Characteristics For Male Urine Specimens

Method		PCR		Total Results
Chlamydia Rapid Test Cassette (Swab/Urine)	Results	Positive	Negative	
	Positive	35	0	35
	Negative	2	60	62
Total Results		37	60	97

Relative Sensitivity: 94.6% (81.8%-99.3%)*

Relative Specificity: >99.9% (95.1%-100%)*

Overall Accuracy: 97.9% (92.7%-99.7%)*

*: 95% Confidence Intervals

3.2. Discussion

The research findings above indicate the high accuracy of the test in detecting the microorganism across various specimen types. It demonstrated a relative sensitivity of 93.3% and a relative specificity of 97.5% for female cervical swab specimens, resulting in an overall accuracy of 96.6%. In the case of male urethral swab specimens, the relative sensitivity was 86.2% and the relative specificity was 95.8%, with an overall accuracy of 92.7%. Moreover, for male urine specimens, the test exhibited a relative sensitivity of 94.6% and a relative specificity of over 99.9%, leading to an overall accuracy of 97.9%. Furthermore, the results can be obtained within 10 minutes. Based on the aforementioned data results, the Chlamydia Rapid Test demonstrates outstanding accuracy, specificity, and sensitivity. This test kit provides dependable and precise results, empowering healthcare professionals to make well-informed decisions, support timely interventions, and contribute to public health and safety.

However, it is essential to consider the limitations associated with the Chlamydia Rapid Test Cassette (Swab/Urine). These limitations include the incapability to determine quantitative values or the rate of increase, the restricted evaluation with specimens other than those specified, the impact of specimen collection methods and patient factors on detection, the potential persistence of antigen following antimicrobial therapy, and the potential occurrence of false positive results due to excessive blood on the swab. Healthcare professionals should be cognizant of these limitations and employ appropriate interpretation and specimen collection techniques to ensure accurate diagnosis and informed treatment decisions.

4. Conclusion

The Chlamydia Rapid Test Cassette, manufactured by Hangzhou AllTest Biotech Co., Ltd,

exhibits excellent sensitivity and specificity, delivering results within a span of 10 minutes. As a result, it proves to be an ideal primary diagnostic tool, particularly in scenarios where patients necessitate testing and treatment during a single consultation. Additionally, it can serve as a screening instrument in regions characterized by elevated infection rates or in settings where resources are limited. Nonetheless, additional investigations are warranted to ascertain the practicality and cost-effectiveness of The Chlamydia Rapid Test in these particular applications.

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