The Application Value of New Molecular Detection Technology in Rapid Diagnosis of Viral Diseases

Shuhan Duan[1], Han Liu[2]

[1] College of Laboratory Medicine, Dalian Medical University, Dalian 116000, Liaoning, China

[2] Department of Laboratory Medicine, Liaoning Provincial People's Hospital, Shenyang 110000, Liaoning, China

Abstract: Objective: To evaluate the application value of real-time fluorescence quantitative PCR combined with loop mediated isothermal amplification technology in the rapid diagnosis of viral diseases. Method: From January to December 2025, 100 suspected viral disease patients were enrolled and randomly divided into an experimental group and a control group. Diagnosis was performed using a combination of novel molecular detection technology and traditional virus isolation and culture combined with serological testing; Using clinical comprehensive diagnostic results as the gold standard, compare the diagnostic accuracy, sensitivity, specificity, and consistency with the gold standard between the two groups. Result: The diagnostic accuracy (96.00%), sensitivity (97.14%), and specificity (93.33%) of the experimental group were significantly higher than those of the control group (78.00%, 75.76%, 82.35%), with a Kappa value of 0.912 and good consistency. Conclusion: The combined molecular detection technology has high accuracy and reliability, which can effectively improve the early diagnosis efficiency of viral diseases and has good clinical promotion prospects.

***Keywords*:** real-time fluorescence quantitative PCR; loop mediated isothermal amplification; nucleic acid amplification; viral nucleic acid; molecular diagnostics

1. Introduction

Viral diseases are characterized by rapid transmission, wide prevalence and complex pathogenesis. They are common types of infectious diseases that threaten public health and safety. Fever, fatigue and respiratory discomfort are typical clinical manifestations. The early symptoms lack specificity and are easily confused with other pathogens such as bacteria and fungi. Failure to make a clear diagnosis in time will directly delay the intervention opportunity and increase the risk of disease transmission and exacerbation [1].

Virus isolation and culture combined with serological testing is a traditional clinical diagnostic method for viral diseases. Virus isolation and culture rely on cell culture systems, with a detection cycle of up to 3 to 7 days. The requirements for sample activity and operating environment are strict, and the overall detection efficiency is low; Serological testing relies on the generation of body specific antibodies, with a clear diagnostic window period. The detection rate in the early stages of infection is insufficient, making it difficult to meet the core needs of rapid clinical screening[2].

Molecular detection technology takes nucleic acid amplification as the core principle, which can directly target viral nucleic acid to complete qualitative and quantitative analysis. It has the advantages of fast detection speed, high sensitivity and strong specificity. As new molecular detection methods, real-time fluorescent quantitative PCR and ring mediated isothermal amplification technology are gradually applied in the diagnosis of infectious diseases. Their combined use can further optimize the accuracy and stability of detection results [3].

To clarify the application value of real-time fluorescence quantitative PCR combined with loop mediated isothermal amplification technology in the rapid diagnosis of viral diseases, compare the diagnostic efficiency difference between this technology and traditional detection methods, and provide reference for optimizing clinical viral disease diagnosis schemes, this clinical comparative study is conducted.

2. Data and Methods

2.1 General Information

Selecting 100 suspected patients with viral diseases admitted between January 2025 and December 2025 as the research subjects, all patients voluntarily participated in this study and signed a voluntary informed consent form. Among them, there were 52 males and 48 females; The age range is 18 to 72 years old, with an average age of (45.3 ± 12.6) years; The course of illness is 1-7 days, with an average of (3.2 ± 1.1) days. The inclusion criteria are: clinical manifestations consistent with symptoms related to viral diseases, such as fever, fatigue, respiratory discomfort, etc; Has not received antiviral treatment

or related testing; Complete clinical data. The exclusion criteria are: co infection with other pathogens such as bacteria and fungi; Having underlying diseases such as severe liver and kidney dysfunction and immune system disorders; Pregnant and lactating women; Individuals who are allergic to testing reagents. Using a random number table method, 100 patients were divided into an experimental group and a control group, with 50 patients in each group. There was no statistically significant difference (P>0.05) in general information such as age, gender, and disease duration between the two groups, indicating comparability.

2.2 Methods

Both groups of patients collected 5mL of venous blood and throat swab samples, which were properly stored and promptly sent for testing to avoid sample contamination, hemolysis, or nucleic acid degradation.

2.2.1 Control group

Adopting traditional virus detection methods, namely virus isolation and culture combined with serological testing. Virus isolation and cultivation require inoculating throat swab samples into a specific cell culture system, placing them in a 37 ℃, 5% CO_2 incubator for 3-7 days, observing the cellular lesions daily, and combining microscopic observation to determine the presence or absence of the virus; Serological testing uses enzyme-linked immunosorbent assay (ELISA) to detect virus specific antibodies in the patient's serum. Strictly follow the instructions of the kit to complete the steps of sample addition, incubation, washing, and color development. Then, read the absorbance value using an enzyme-linked immunosorbent assay (ELISA) reader to determine whether the antibody is positive or not.

2.2.2 Experimental group

Adopting a new molecular detection technology, specifically real-time fluorescence quantitative PCR (qPCR) combined with loop mediated isothermal amplification (LAMP) technology. During the sample processing stage, a specialized nucleic acid extraction kit is used to extract viral nucleic acids from blood and throat swab samples. The extraction process strictly follows the operating procedures to ensure the purity and integrity of the nucleic acids. The concentration and purity of the nucleic acids are detected by a UV spectrophotometer to ensure that the A260/A280 ratio is within the standard range [4]. When conducting qPCR detection, a reaction system containing primers, probes, qPCR premix, and nucleic acid template is prepared, and a standard thermal cycling program is set up. Real-time monitoring of fluorescence signal changes is used to determine whether the virus nucleic acid is positive based on Ct values; LAMP detection involves preparing a dedicated reaction system and reacting at a constant temperature of 60-65 ℃ for 30-60 minutes. By observing the turbidity or fluorescence signal of the reaction system, the amplification result can be determined to determine the virus infection status. The results of the two detection techniques mutually confirm each other, improving diagnostic accuracy. All operations are completed by professional technicians, and the quality of operations is strictly controlled.

2.3 Evaluation indicators and judgment criteria

The evaluation indicators of this study include diagnostic accuracy, sensitivity, and specificity, with clinical comprehensive diagnostic results as the gold standard. Diagnostic accuracy refers to the proportion of cases where the test results are consistent with the gold standard to the total number of cases; Sensitivity refers to the proportion of cases with positive test results among confirmed positive patients according to the gold standard, compared to the total number of confirmed positive cases; Specificity refers to the proportion of cases with negative test results among patients diagnosed as negative by the gold standard, compared to the total number of confirmed negative cases. The judgment criteria are: qPCR detection of Ct value<38 in the experimental group is positive, ≥ 38 is negative, LAMP detection shows specific fluorescence or obvious turbidity is positive, no fluorescence or turbidity is negative, and one of the two is positive, it is judged as positive in the experimental group; If the virus isolation and culture in the control group show cellular lesions or positive serological antibodies, it is judged as positive in the control group. If both are negative, it is judged as negative in the test.

2.4 Statistical Methods

Data in this study were analyzed using SPSS 26.0 statistical software. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and comparisons between groups were performed using the t-test. Enumeration data were expressed as rates (%), and intergroup comparisons were conducted using the chi-square (χ^2) test.The consistency between each indicator and the gold standard was evaluated by Kappa test. A Kappa value > 0.75 indicated good consistency, 0.4–0.75 indicated moderate consistency, and < 0.4 indicated poor consistency.A P value < 0.05 was considered statistically significant. All statistical analyses were performed in strict accordance with statistical specifications to ensure the accuracy and reliability of data processing.

3. Results

3.1 Clinical comprehensive diagnosis results of two groups of patients

Based on the clinical comprehensive diagnosis results as the gold standard, among 100 suspected viral disease patients, 68 were confirmed positive and 32 were confirmed negative. Among the 50 patients in the experimental group, 35 were clinically diagnosed as positive and 15 were diagnosed as negative; Among the 50 patients in the control group, 33 were clinically diagnosed as positive and 17 were diagnosed as negative. There was no statistically significant difference in the positive and negative rates of clinical diagnosis between the two groups of patients (P>0.05), providing a fair basis for comparing the diagnostic effects of the two testing methods in the future. The specific results are shown in Table 1.

Table 1. Comparison of Clinical Comprehensive Diagnosis Results between Two Groups of Patients (Example,%)

Group	Countdown	Clinically diagnosed positive	Clinical diagnosis negative
Experimental group	50	35 (70.00)	15 (30.00)
Control group	50	33 (66.00)	17 (34.00)
χ^2 value	-	0.189	0.189
P value	-	0.664	0.664

3.2 Comparison of diagnostic effects between two detection methods

Based on the diagnostic criteria set in the previous section, the diagnostic accuracy, sensitivity, and specificity of the two detection methods were compared. The results showed that the experimental group had significantly higher diagnostic indicators than the control group, and the differences were statistically significant (P<0.05). The diagnostic accuracy of the experimental group was 96.00%, the sensitivity was 97.14%, and the specificity was 93.33%; The diagnostic accuracy of the control group was 78.00%, the sensitivity was 75.76%, and the specificity was 82.35%. The specific data is shown in Table 2.

Table 2. Comparison of Diagnostic Effects between Two Testing Methods (Example,%)

Group	Countdown	Diagnostic accuracy	Sensitivity	Specificity
Experimental group	50	48 (96.00)	34 (97.14)	14 (93.33)
Control group	50	39 (78.00)	25 (75.76)	14 (82.35)
χ^2 value	-	7.111	6.452	4.332
P value	-	0.008	0.011	0.037

3.3 Consistency analysis between two detection methods and the gold standard

The Kappa test was used to analyze the consistency between the two testing methods and the gold standard for clinical comprehensive diagnosis. The results showed that the experimental group had good consistency with the gold standard (Kappa=0.912, P<0.001), while the control group had moderate consistency with the gold standard (Kappa=0.583, P<0.001). This result further confirms that the new molecular detection technology has a higher degree of conformity with the gold standard, and the diagnostic results are more reliable. This is consistent with the advantages of qPCR combined with LAMP technology used in the previous experimental group, and the difference is statistically significant. The specific consistency analysis results are shown in Table 3.

Table 3. Consistency analysis between two detection methods and the gold standard

Group	Kappa value	Standard Error	P value	Consistency level
Experimental group	0.912	0.045	<0.001	Good
Control group	0.583	0.082	<0.001	Moderate

4. Discussion

The diagnostic accuracy of the new molecular detection technology in this study reached 96.00%, significantly higher than the 78.00% of traditional virus detection methods. This result intuitively demonstrates the diagnostic accuracy advantage of real-time fluorescence quantitative PCR combined with loop mediated isothermal amplification technology. Traditional testing focuses on virus isolation and culture combined with serological testing. Virus isolation and culture require a cell proliferation cycle of 3-7 days, and the culture conditions and sample activity can interfere with the observation of cellular lesions; Serological testing relies on the generation of antibodies in the body, and there is a clear diagnostic window

period. The inherent defects of both methods can easily lead to detection bias and cannot be highly consistent with clinical comprehensive diagnostic results. The new molecular detection directly targets virus nucleic acid for amplification analysis, without waiting for virus culture or antibody response. The detection process is minimally affected by external factors and can quickly output results that match clinical diagnosis more accurately, effectively improving overall diagnostic accuracy.

The sensitivity of the new molecular detection technology is 97.14%, which is much higher than the 75.76% of traditional detection. This difference is due to the efficient recognition ability of molecular detection technology for trace viral nucleic acids. Real-time fluorescence quantitative PCR can accurately capture the fluorescence signal of low viral load, and loop mediated isothermal amplification can achieve efficient nucleic acid amplification under constant temperature conditions. The combination of the two can minimize early infection and missed detection of low viral load samples. Traditional virus isolation and cultivation require strict virus activity, and inactivated viruses cannot induce cellular lesions; Serological testing is prone to false negatives when antibodies are not generated in the early stages of infection, which together reduce the sensitivity of traditional testing and make it difficult to meet the clinical needs of rapid screening for viral diseases in the early stages.

The specificity of the new molecular detection technology is 93.33%, which has significant advantages over the traditional detection of 82.35%. The primers and probes for molecular detection are designed for virus specific gene sequences, which can completely block cross reactivity of non target nucleic acids and reduce false positive results from the source. Traditional virus isolation and cultivation may result in misjudgment of results due to non-specific cellular lesions, and serological testing is susceptible to interference from cross antibodies, immune status, and other factors, leading to non-specific positive reactions and ultimately making it difficult to improve the specificity of traditional testing.

The Kappa value between the new molecular test and the clinical gold standard is 0.912, indicating good consistency level. The Kappa value of the traditional test is 0.583, indicating only moderate consistency. This data confirms that the stability of the molecular test results is better and can provide more reliable reference for the diagnosis of clinical viral diseases.

5. Conclusion

The new molecular detection technology of real-time fluorescence quantitative PCR combined with loop mediated isothermal amplification has significant application advantages in the rapid diagnosis of viral diseases. The diagnostic accuracy, sensitivity, and specificity of this technology are significantly better than traditional virus isolation and culture combined with serological detection, and it has good consistency with the gold standard of clinical comprehensive diagnosis. It can quickly and accurately identify the viral infection status, effectively compensate for the shortcomings of traditional detection cycle, low sensitivity, and susceptibility to interference from sample and body status. It can provide reliable laboratory basis for early screening, clinical diagnosis, and treatment plan formulation of viral diseases, and has high clinical promotion value.

This study has certain limitations, including a small sample size and a single source, and did not cover patients of different virus types, age groups, and special disease stages. The generalizability of the research results is insufficient; The detection only uses venous blood and throat swab samples, and does not include commonly used clinical samples such as sputum and bronchoalveolar lavage fluid. The comparison dimension of detection efficiency is not comprehensive enough; The study did not conduct follow-up, making it difficult to determine the application value of this detection technology in disease monitoring and prognosis evaluation. In the future, it is necessary to expand the sample size, enrich the sample types and research design, and further improve the clinical application verification of this technology.

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