

# Evaluation of Clinical Application of Chemiluminescence and Real-time, Fluorescence-based Quantitative PCR in Diagnosis of Epstein-Barr Virus Infection

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**Abstract: Objective:** To compare the effects of clinical application of chemiluminescence and real-time, fluorescence-based quantitative PCR in the detection Epstein-Barr virus (EBV). **Methods:** The data of chemiluminescence and real-time fluorescent quantitative PCR from 97 patients who were suspected of being infected with EBV from January 2016 to January 2019 in our hospital were analyzed. The specific stage of EBV infection was analyzed, and the differences in results of the two detection methods were compared. **Results:** Chemiluminescence method was used to detect EBV infection during the active phase. The sensitivity of the chemiluminescence method was 76.7% (56/73) and the real-time quantitative PCR method was 90.4% (66/73). There was a statistical difference between the two detection methods ( $P < 0.05$ ). **Conclusion:** There was no statistical difference in positive predictive values between the chemiluminescence method and the real-time, fluorescence-based quantitative PCR method in the detection of EBV infection, but the sensitivity of chemiluminescence method is slightly lower than the real-time quantitative PCR method. It is noteworthy that chemiluminescence method is convenient and fast while the real-time, fluorescence-based quantitative PCR method is more accurate, which can provide a more accurate reference for clinical treatment.

**Keywords:** Epstein-Barr virus; Chemiluminescence method; Real-time, Fluorescence-based quantitative

PCR method

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## 1 Introduction

Epstein-Barr virus (EBV) belongs to the subfamily  $\gamma$  herpesvirus containing double-stranded DNA. EBV is widely distributed in human oropharyngeal epithelial cells and B lymphocytes. It is a common pathogenic virus in clinical setting<sup>[1-2]</sup>. After EBV infection, patients may harbor the virus for life, and all organs and body systems of the infected individuals can be affected at the time of onset of infection<sup>[3-4]</sup>. Modes of EBV infection includes primary infection, reactivation, recurrent infection, and previous infection. The clinical manifestations of patients with EBV infection include mononucleosis, lymphadenopathy, fever, sore throat, and fatigue. However, due to the diversity and complexity of the manifestations after EBV infection, the rate of clinical misdiagnosis of EBV infection is high and the early diagnosis rate is low, which is detrimental to treatment planning<sup>[5-6]</sup>. Chemiluminescence and real-time, fluorescence-based quantitative PCR are the main laboratory diagnostic methods for EBV infection. In order to evaluate the specificity and sensitivity of the two methods, our hospital carried

out the detection of 97 patients with suspected EBV infection using chemiluminescence and real-time, fluorescence-based quantitative PCR methods at the same time.

## 2 Materials and methods

### 2.1 Study participants and diagnostic criteria

From January 2016 to January 2019, 97 patients who were suspected EBV infection were subject to EBV detection using by chemiluminescence and real-time, fluorescence-based quantitative PCR. These patients were included in this study. Among the selected patients, there were 53 males and 44 females who were 3 to 46 years old with an average age of  $24.16 \pm 8.73$  years. According to the final diagnosis, 73 cases had infectious mononucleosis. There were 24 healthy individuals who were recruited into control group in the present study.

According to clinical diagnostic criteria, the inclusion criteria for all EBV infection include:

#### 2.1.1 According to diagnostic criteria

- (1) A patient who had at least 3 of the following the following symptoms are considered a positive case: fever; pharyngitis, tonsillitis; neck lymphadenopathy (greater than 1 cm); liver enlargement; splenomegaly.
- (2) Hematological examination: WBC classification lymphocytes  $>50\%$  or total lymphocyte count  $\geq 5.0 \times 10^9/L$ ; Atypical lymphocytes  $\geq 10\%$  or total count  $\geq 1.0 \times 10^9/L$ .
- (3) EBV antibody test: EB nuclear antigen was negative in the acute phase, and one of the following: anti-VCA-IgM antibody was initially positive, and then becomes negative; double serum anti-VCA-IgG antibody titer was elevated by more than three times; transient increase of EA antibody; initial positive result of VCA-IgG antibody; EB virus nuclear antigen antibody converts to positive at late stage.
- (4) EBV DNA test: An EBV-infected patient was positive for EBV DNA in blood, saliva, oropharyngeal epithelial cells, urine or tissues.
- (5) Examination of EBV antigen: Nasopharyngeal swabs tested positive for antigen.

Patients who fulfill the abovementioned requirements of clinical symptoms and hematological examination and have one of items 3 to 5 can be diagnosed as a positive, EBV-infected patient.

#### 2.1.2 Other criteria

Patients and/or their guardians voluntarily participate and sign relevant documents. This study does not involve the formulation of a treatment plan, and has been approved by the ethics committee of our hospital. Exclusion criteria include

- (1) Patients with congenital heart and lung disease,
- (2) Patients with other infectious diseases,
- (3) Patients with coagulopathy, and
- (4) Patients with diseases of the blood system, immune system or abnormal functions.

### 2.2 Detection methods

Three milliliters of blood specimens from each of the patients recruited in this study were collected in blood collection tubes containing pro-gel, and 1ml of whole blood with blood collection tubes containing EDTA anticoagulant. The coagulation tube specimens were centrifuged at 3500 rpm for 15 min using a centrifuge to obtain plasma for examination.

The chemiluminescence-based detection of EBV nuclear antigen antibody was performed using LIAISON chemiluminescence immunoanalyzer:

- (1) The case was positive if EBV capsid antigen antibody IgM (VCA-IgM) concentration is equal to or higher than 40 U/ml.
- (2) The case was positive if EBV capsid antigen antibody IgG (VCA-IgG) concentration is equal to or higher than 20 U/ml.
- (3) The case was positive if EBV early antigen antibody IgG (EA-IgG) concentration is equal to or higher than 40 U/ml.
- (4) The case was positive if EBV nuclear antigen antibody IgG (EBNA-IgG) concentration is equal to or higher than 20 U/ml.

Real-time, fluorescence-based quantitative PCR was carried out using ABI stratagene fluorescence-based PCR analyzer and the EBV nucleic acid quantitative detection kit from Hunan Shengxiang Biological Technology Co., Ltd. Whole blood genome extraction reagent was used to isolate and obtain DNA which was then used in the tests. 40 ml of PCR mixed solution was added to each PCR reaction tube which was then put it into the amplifier to quantify the fluorescence resulted from DNA amplification. The result of Ct less than or equal to 39 was determined as positive for EBV.

### 2.3 Observation methods

The specificity and sensitivity of the two methods for

the detection of EBV infection were compared.

## 2.4 Statistical analysis

The comparison of count data was performed using Chi-square test, and the data were expressed in n format. The comparison of measurement data was conducted using test for homogeneity of variance and independent t test. The data were expressed in  $\bar{x} \pm s$  format.  $P < 0.05$  was considered statistically

significant.

## 3 Results

The positive predictive value of chemiluminescence detection in the detection of EBV infection was 76.7% (56/73) and PCR method was 90.4% (66/73). The statistical difference between the two detection methods ( $P < 0.05$ ) is shown in Table 1.

**Table 1.** Comparison of the results of chemiluminescence method and real-time quantitative PCR method for detecting EBV infection (n)

	Chemiluminescence method		Real-time quantitative PCR method	
	+(n)	-(n)	+(n)	-(n)
EBV infection	66	7	56	17
Non-EBV infection	0	24	4	20
<i>Total</i>	66	31	60	37

Real-time, fluorescence-based quantitative PCR method: sensitivity was 90.4%, specificity was 100%, positive predictive value was 100%, negative predictive value was 77.4%.

Chemiluminescence method: sensitivity was 76.6%, specificity was 83.3%, positive predictive value was 93%, negative predictive value was 58.5%.

## 4 Discussion

EBV is a double-stranded DNA virus, and humans are its only natural host<sup>[7-8]</sup>. If the clinical manifestations of EBV infection in human body become more complicated, the EBV infection will become more harmful. Thus, it is crucial to obtain a reliable diagnosis as soon as possible. However, symptoms of EBV infection are easy to be confused with those of other infections or causes, so clinical diagnosis has to be made in accordance with laboratory test results<sup>[9-10]</sup>. The infants and young children aged 3 years and younger are subject to initial infection of EBV, but the immune function of children is not fully developed at the time of infection, and the body's immune system produces relatively low level of antibodies after the infection especially in the early stages of infection, which could be even be lower than the lower test limit<sup>[11-13]</sup>. This problem can be effectively avoided by using the chemiluminescence method. The main underlying principle of the chemiluminescence method is that the corresponding antigen coated by the surface of the magnetic particles forms a luminescence reaction under the action of the isoluminol derivative excitation liquid. The intensity of luminescence signal reflects the concentration

of the test substance<sup>[14]</sup>. The disadvantage of the chemiluminescence method is that it is easily affected by the components of complement, rheumatoid factor, heterophilic antibodies, drugs and other components, resulting in false negative results, whereas the advantage of chemiluminescence method is that its reagent has strong anti-interference function, and jaundice, lipemia, hemolysis, etc. have a weak influence on chemiluminescence-based detection. The detection operation of this method is relatively convenient. At present, this technique has become fully automated, and the result interpretation is not affected by subjective factors of human. After EBV infection, related antibodies are produced one after another. VCA-IgM is an early infection antibody that disappears within a few weeks after the infection. It is mainly used as a determinant of acute EBV infection. Level of IgG reaches its peak around 14 days after infection, and then gradually declined, and maintained at a low level in the human body for life. VCA-IgM can only be maintained for a short period of time in the body, and its sensitivity is low. Therefore, the diagnosis of non-EBV infection cannot be made because of the negativity of VCA-IgM. A comprehensive test of the four antibodies of EBV is required to correctly confirm the detection EBV in order to accurately reflect the specific situation of the infection.

Real-time, fluorescence-based quantitative PCR technology is a detection method that involves incorporation of fluorophores in the PCR reaction and quantitative analysis of the standard curve of the PCR process and the unknown template through real-

time monitoring of the accumulation of fluorescent signals[15]. In fluorescence-based quantitative PCR detection, the quality control level of plasma and serum samples is higher. These specimens have higher specificity, and there are not many factors that can interfere with the detection results. Fluorescence-based quantitative PCR method can intuitively quantify the EBV-DNA load in patients' plasma. The quantified EBV-DNA load can provide more accurate guidance for the evaluation of clinical efficacy, but the method takes a long time and the operation is more complicated. More stringent requirements for environmental specimens are usually warranted. The results of this study show that the sensitivities of the two methods in the detection of EBV infection were statistically different, while the positive predictive values of the two detection methods for EBV infection were not statistically different. In conclusion, both chemiluminescence and real-time, fluorescence-based quantitative PCR methods can produce accurate diagnosis of EBV infection, although both detection methods have their own advantages. In clinical application, Determining the detection methods can be selected according to clinical needs and disease manifestations or combined use of multiple detection methods can be considered to further improve the accuracy of detection.

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