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RESEARCH ARTICLE

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Simultaneous detection of Marburg virus and Ebola virus with TaqMan-based multiplex real-time PCR method

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Abstract

Background: Marburg virus (MARV) and Ebola virus (EBOV) are acute infections with high case fatality rates. It is of great significance for epidemic monitoring and prevention and control of infectious diseases by the development of a rapid, specific, and sensitive quantitative PCR method to detect two pathogens simultaneously.

Methods: Primers and TaqMan probes were designed according to highly conserved sequences of these viruses. Sensitivity, specificity, linear range, limit of detection, and the effects of hemolysis and lipid on real-time qPCR were evaluated.

Results: The linearity of the curve allowed quantification of nucleic acid concentrations in range from 10^3 to 10^9 copies/ml per reaction (MARV and EBOV). The limit of detection of EBOV was 40 copies/ml, and MARV was 100 copies/ml. It has no cross-reaction with other pathogens such as hepatitis b virus (HBV), hepatitis c virus (HCV), human papillomavirus (HPV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV). Repeatability analysis of the two viruses showed that their coefficient of variation (CV) was less than 5.0%. The above results indicated that fluorescence quantitative PCR could detect EBOV and MARV sensitively and specifically.

Conclusions: The TaqMan probe-based multiplex fluorescence quantitative PCR assays could detect EBOV and MARV sensitively specifically and simultaneously.

KEYWORDS
Ebola virus, Marburg virus, polymerase chain reaction, simultaneous detection

Yu and Wu contributed equally to this work.

1 | INTRODUCTION

Filoviruses such as Marburg virus (MARV) and Ebola virus (EBOV) can cause severe hemorrhagic fevers in humans and primates, and as one of the most dangerous pathogens for humans listed by the World Health Organization (WHO).[1] MARV and EBOV are mainly transmitted by direct contact and aerosol,[2,3] the incubation period is commonly 3–9 days, the elderly can be more than 2 weeks. A variety of body fluids, such as secretions, excretions, contaminants, and environments from patients with hemorrhagic fever and animals infected with MARV or EBOV can infect a variety of immune cells through damaged skin, eyeballs, nasal passages, and oral mucosa.[4] Patients can present with serious bleedings and hemorrhagic shock syndrome.[5] It has high pathogenicity and fatality rate.

MARV was first discovered in Marburg, Germany in 1967 and was named Marburg virus according to the location of this disease. It was the first filovirus found in human.[6] MARV is a member of the filoviruses family, has caused outbreaks in sub-Saharan Africa, and can cause severe disease with a high case fatality rate. Human-to-human transmission may occur in a home or hospital setting.[7] Although MARV was discovered more than 50 years ago, there is no effective treatment has yet been developed,[8] except the vaccine has a preventive effect.[9] The first outbreak of the EBOV occurred in the Ebola River region in southern Sudan and Zaire (is now the democratic republic of Congo region) in 1976, patient mortality rates as high as 90%.[10] In the outbreak in West Africa between 2013 and 2016, there were about 28,000 cases were confirmed and 11,000 deaths were reported, indicating the high mortality rate of this disease.[11]

Although the viruses have only been endemic in some African countries,[12] and no large-scale virus-infected patients have been reported in other regions, there is a potential risk of virus transmission owing to countries around the world interact more and more with each other and the movement of people and goods increases. Moreover, MARV and EBOV can be used as a potential biological terrorist weapons or biological agents to use.[13] Therefore, it is necessary to establish a rapid and specific laboratory test method for the early diagnosis and prevention of MARV and EBOV infection. TaqMan-based real-time fluorescence quantitative polymerase chain reaction (PCR) has the characteristics of rapid, sensitive, specific, and high throughput, and has been used in the detection of a variety of viruses.[14–16]

MARV and EBOV has a negative-strand RNA of approximately 19 kb, respectively. This RNA genome contains seven genes, include nucleoprotein (NP), virion protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24, and the RNA-dependent RNA polymerase L.[2,17] Each one of these genes encodes a single protein product.[18,19] We aimed to establish a rapid, sensitive TaqMan-based real-time fluorescence quantitative PCR detection assays according to the genetic sequence of the viruses, to provide technical support for laboratory testing of prevention and control of these severe infectious diseases.

2 | MATERIALS AND METHODS

2.1 | Identification of sequences and design of primers and probes

According to the whole genome sequence of 42 strains of MARV published by GenBank, DNASTAR software was used for multiple sequence alignment (MSA) to screen the highly conserved NP gene of MARV nuclear protein as the target gene. According to the whole genome sequence of EBOV published by GenBank, DNASTAR software was used for multiple sequence alignment to screen out the highly conserved NP gene of EBOV as the target gene.

Primer Premier 6.0 software was used to design specific primers and probes that met multiple response conditions. Multiple primers were designed with the following default settings: primer melting temperature (T_m) set at 60°C approximately. TaqMan probes for EBOV and MARV were labeled at the 5′-end with the reporter molecule: hexachloro-6-carboxy-fluorescein (HEX), pentamethine cyanine (CY5), respectively. And the TaqMan probes were labeled at the corresponding 3′-end with the quenchers: 6-carboxy-tret ramethyl-rhodamine (TAMRA), 8-Bromo-7-hydroxyquinoline 2 (BHQ2) (Sangon Biotech Co., Ltd.), respectively. And the size of PCR product of EBOV and MARV were 163 and 180 bp, respectively (Table 1).

TABLE 1 A real-time qPCR assay were designed for the detection of viruses

Species	Primer/probe	Sequence (5′–3′)	Probe type	Fluorescent reporter dye at the 5′ ends	Quenching group at the 3′ ends	Product size (bp)
Ebola virus	EBL-FP	GAGAAAAGGCTTGCCTTGAG				
	EBL-RP	CATGTGCATCCCTTGGTGTA	TaqMan			163
	EBL-Prb	CCAACAGCTTGGCAATCAGTAGG		HEX	TAMRA	
Marburg virus	MRB-FP	CACAGTTTGTTGGAGTTGGGT				
	MRB-RP	ATGCTCAACACACAACGTCA	TaqMan			180
	MRB-Prb	ACTGCCCCTCATGTTCGTAATAA		CY5	BHQ2	

Abbreviations: BHQ2, 8-Bromo-7-hydroxyquinoline 2; CY5, pentamethine cyanine; HEX, hexachloro-6-carboxy-fluorescein; TAMRA, 6-carboxy-tretramethyl-rhodamine.

2.2 | Gene synthesis and preparation of standard substance positive template

The highly conserved sequences of MARV NP gene and EBOV NP gene were synthesized artificially as the target genes and cloned into pUC57 vector through gene synthesis. The gene synthesis was completed by Sangon Co., Ltd.

The cloned strain of pUC57 was cultured in *E. coli* and plasmid pUC57 was extracted. The absorbance (A) of plasmid at wavelengths of 260 and 280 nm was measured by spectrophotometer, and purity was determined according to the ratio of A260/A280. The plasmid concentration was determined and converted to copy number of plasmids according to the following formula. Dissolve a certain amount of plasmid in the blood and calculate the template concentration and copy number in the blood according to the following formula.

$$\text{copy number (copies/ml)} = \text{plasmid concentration (g/ml)} \times 6.02 \times 10^{23} / (\text{total length of plasmid} \times 660)$$

$$\text{or: copy number (copies/}\mu\text{l)} = \text{plasmid concentration (ng/}\mu\text{l)} \times 6.02 \times 10^{23} \times 10^{-9} / (\text{total length of plasmid} \times 660)$$

(6.02×10^{23} was Avogadro's constant, 660 was the average molecular weight of each base).

Finally, the template was stored at −20°C for reserve.

2.3 | Optimization of fluorescence quantitative PCR reaction system and reaction conditions

TaqMan-based real-time PCR was performed on the Lightcycler 480 fluorescence quantitative PCR system (Roche Diagnostics). First, a single system qualitative reaction was performed for each pathogen. On this basis, the primer concentration, probe concentration, and T_m of the multiple fluorescence quantitative PCR reaction were optimized to establish the optimal reaction system for simultaneous detection of two viruses.

A master mix reaction was prepared and dispensed in 23 μl aliquots into the PCR reaction tubes. Then 2 μl of template nucleic acid was added to each tube. The final reaction mixture contained 120 nmol/L of each primer and probe for MARV, 200 nmol/L of each primer, and probe for EBOV. The mixture was heated for reverse transcription at 50°C for 10 min, followed by one cycle of denaturation at 95°C for 5 min. PCR amplification was carried out for 45 cycles at 95°C for 15 s and 58°C for 40 s. The fluorescence was read at the end of this second step allowing a continuous monitoring of the amount of template nucleic acid.

2.4 | Verification of method performance

2.4.1 | Preparation of standard curve

The plasmid standards of 1.0×10^8, 1.0×10^7, 1.0×10^6, 1.0×10^5, 1.0×10^4, 1.0×10^3, 1.0×10^2, and 1.0×10^1 copies/ml were repeated in batches, and each template was tested three times. The threshold cycle (Ct) values obtained and the accuracy and stability of the detection system were calculated by SPSS 21.0 software.

2.4.2 | Sensitivity test

The plasmid of EBOV and MARV nucleic acid concentrations of 1.0×10^9, 1.0×10^8, 1.0×10^7, 1.0×10^6, 1.0×10^5, 1.0×10^4, 1.0×10^3, 4.0×10^2, 2.0×10^2, 1.0×10^2, 4.0×10^1, 2.0×10^1, 1.0×10^1, and 1.0×10^0 copies/ml were prepared in batches and each template was tested three times. Real-time fluorescence quantitative PCR was used for detection and statistical analysis to determine its sensitivity.

2.4.3 | Specificity test

The specificity of the assays was verified by the existence or absence of amplification using MARV template nucleic acid corresponding to EBOV primers and probe or vice versa. In addition, potential cross-reaction was assessed using serum samples of viral nucleic acid from seven viral species (such as hepatitis b virus (HBV), hepatitis c virus (HCV), human papillomavirus (HPV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), cytomegalovirus (CMV), and human immunodeficiency virus 1 (HIV-1)), which are a part of the collection samples of Center for Precision Medicine, Meizhou People's Hospital.

2.4.4 | The effect of lipid and hemolysis on the detection of the assay

The influence of lipid and hemolysis on the amplification performance of the assay was investigated. The 50 serum samples with heavy, moderate, and mild blood lipid and hemolysis were collected, respectively, each separate serum 2 ml, stored at −20°C. Lipid and hemolysis samples of EBOV and MARV nucleic acid templates with final concentrations of 2.0×10^8 copies/ml (I level), 3.0×10^6 copies/ml (II level), and 4.0×10^4 copies/ml (III level) were prepared with standard plasmid. Real-time fluorescence quantitative PCR was used for detection and statistical analysis to study whether lipid and hemolysis affected the detection results.

3 | RESULTS

3.1 | Preparation of standard curve

A 10-fold series of diluted plasmids were used as templates for amplification, and the corresponding standard curve was established. The standard curve range was 4.0×10^1–1.0×10^9 copies/ml. The results showed that the copy number had a good correlation with the corresponding Ct value, and the correlation coefficient $R^2 > 0.99$,

indicating that the standard sample was quantified and the correlation between each concentration group was reliable (Figure 1).

3.2 | The results of only MARV detection, simultaneous detection, and only EBOV virus detection of two viruses with various mixtures of synthesized two plasmids

The two viruses mixtures of synthesized plasmids with EBOV and MARV nucleic acid templates final concentrations of 5.0×10^8, 5.0×10^6, and 5.0×10^4 copies/ml were prepared, respectively. PCR reaction performances were determined by the existence or absence of amplification when only MARV detection, simultaneous detection, and only EBOV detection, respectively. In the samples with two plasmid templates, if only MARV primers and probes were added into the reaction system, the CY5-labeled MARV probe showed fluorescence signal while the HEX-labeled EBOV probe showed no fluorescence signal, indicating amplification of MARV template, but EBOV was not amplified. Similarly, EBOV has similar results. In the samples with two plasmid templates, if MARV and EBOV were detected at the same time, both Cy5 and HEX-labeled probes showed fluorescence signals, indicating that both MARV and EBOV templates were amplified (Figure 2).

3.3 | Determination of linear range and limit of fluorescence quantitative PCR

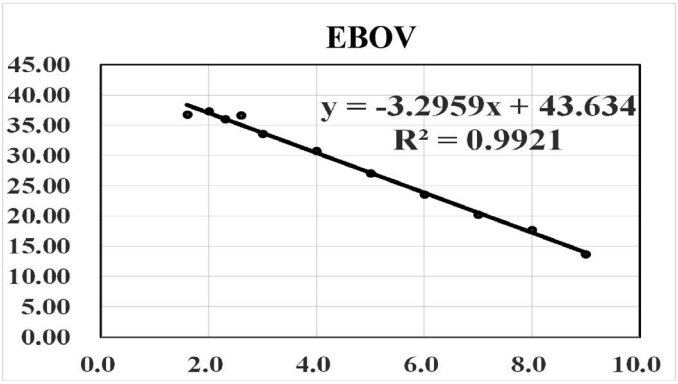
The sensitivity of the MARV and EBOV TaqMan-based fluorescence quantitative PCR detection assay were evaluated by using the serial dilutions of the synthesized two plasmids. A Ct value was calculated from the amplification plot covering this range of dilutions and the standard curves were drawn. The linearity of the curve allowed quantification of nucleic acid molecules in range from 10^3 to 10^9 copies/ml per reaction (both MARV and EBOV). The limit of detection of EBOV and MARV was 40 and 100 copies/ml, respectively.

3.4 | Repeatability analysis of fluorescence quantitative PCR

Three concentrations (1.0×10^6, 1.0×10^5, and 1.0×10^4 copies/ml) of EBOV and MARV plasmids were determined repeatedly ($n = 3$). According to statistical analysis, the final measured values of EBOV were 1.081×10^6, 1.118×10^5, and 1.016×10^4 copies/ml, with coefficients of variation (CV) of 3.03%, 3.72%, and 2.18%, respectively. And the final measured values were 1.219×10^6, 1.261×10^5, and 1.204×10^4 copies/ml for MARV, with CVs of 3.42%, 4.15%, and 4.38%, respectively. Repeatability analysis showed that their CV

(A)

Concentration (copies/ml)	logC	Ct
4.00E+01	1.6	36.79
1.00E+02	2.0	37.35
2.00E+02	2.3	36.01
4.00E+02	2.6	36.68
1.00E+03	3.0	33.59
1.00E+04	4.0	30.81
1.00E+05	5.0	27.05
1.00E+06	6.0	23.59
1.00E+07	7.0	20.24
1.00E+08	8.0	17.69
1.00E+09	9.0	13.73



(B)

Concentration (copies/ml)	logC	Ct
4.00E+01	1.6	36.83
1.00E+02	2.0	36.69
2.00E+02	2.3	36.16
4.00E+02	2.6	33.74
1.00E+03	3.0	32.82
1.00E+04	4.0	29.51
1.00E+05	5.0	25.88
1.00E+06	6.0	22.85
1.00E+07	7.0	18.03
1.00E+08	8.0	16.39
1.00E+09	9.0	12.58

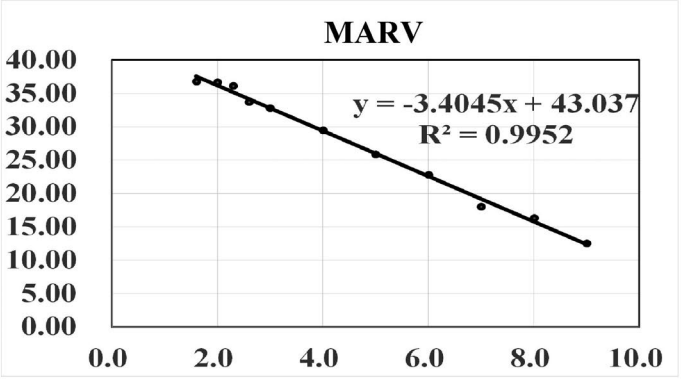


FIGURE 1 Standard curves obtained with serial dilutions of EBOV and MARV in vitro from PCR products. Ct values calculated from results in fluorescence quantitative PCR are plotted against the log of the initial starting quantity of nucleic acid (copies/ml). The results showed that the set copy number had a good correlation with the corresponding Ct value, and the correlation coefficient $R^2 > 0.99$

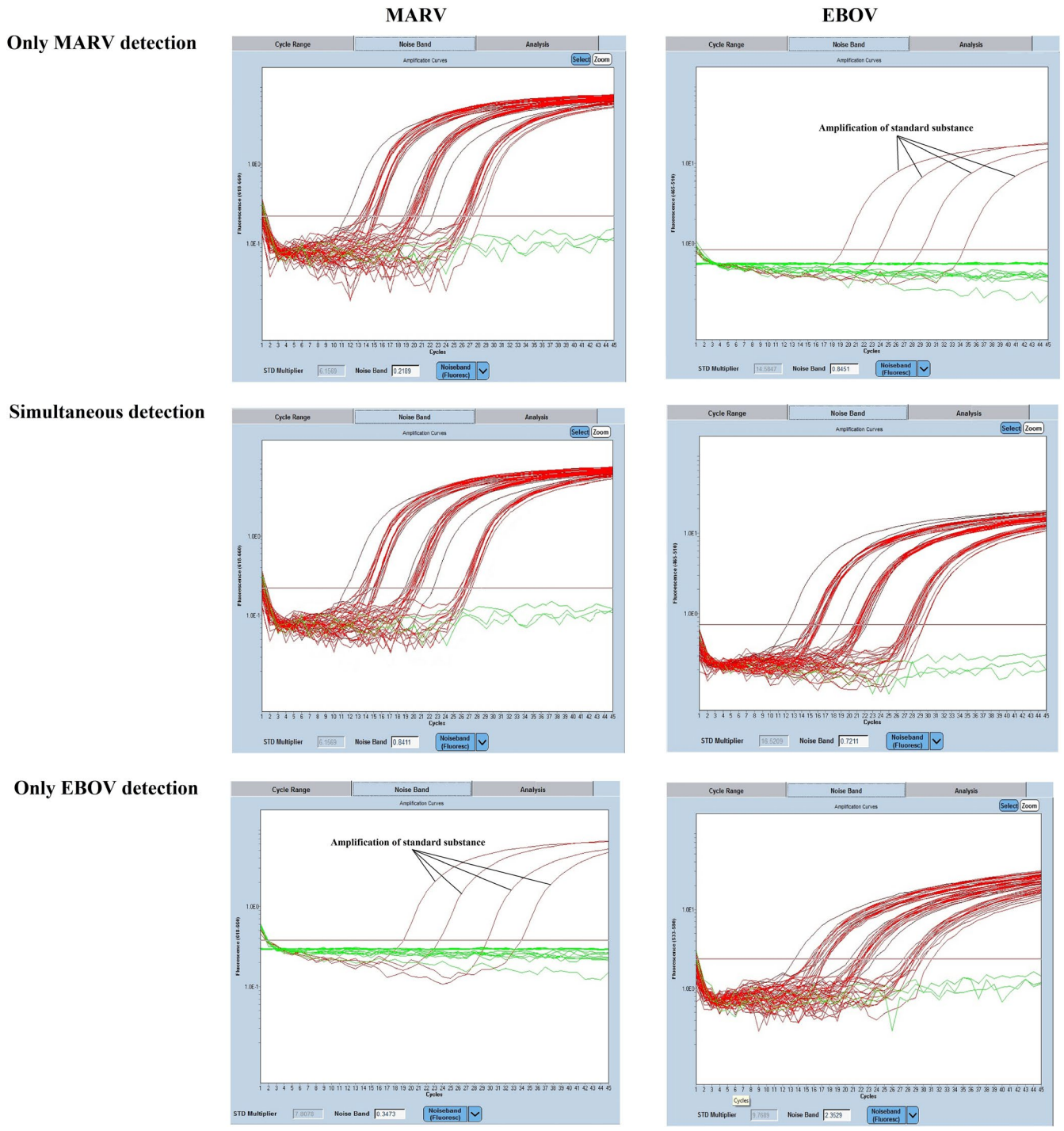


FIGURE 2 The results of only MARV detection, simultaneous detection, and only EBOV virus detection of two viruses with various mixtures of synthesized two plasmids

was less than 5.0%. These indicating that this method has good accuracy and repeatability.

3.5 | Determination of specificity

The specificity of the assays was verified by the existence or absence of amplification using MARV template nucleic acid corresponding to EBOV primers and probe or vice versa. In addition, potential cross-reactions were assessed using viral nucleic acid from seven viral species samples, which are a part of the collected samples of Center for Precision Medicine, Meizhou People's Hospital. It has no cross-reaction with other pathogens such as hepatitis b virus (HBV), hepatitis c virus (HCV), human papillomavirus (HPV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), cytomegalovirus (CMV), and human immunodeficiency

virus 1 (HIV-1). The results showed no cross-reaction and good specificity of the assay.

3.6 | The effect of lipid and hemolysis on the performance of the assay

There were significant differences of final measured values in the I level of EBOV ($p = 0.002$) and MARV ($p < 0.001$) affected by different degrees of hemolysis (Table 2). There were significant differences of final measured values in the I level of EBOV ($p = 0.001$) and MARV ($p < 0.001$) affected by different lipid turbidity (Table 3). There were no significant differences of final measured values in the level II, III of EBOV and MARV affected by different lipid turbidity and the degree of hemolysis (Tables 2 and 3; Figure 3). Hemolytic specimens can be properly diluted to avoid interference. For lipid blood specimens, it is better to use high-speed centrifugation to extract the clear solution.

4 | DISCUSSION

Common laboratory detection methods for virus include isolation and culture,[20] transmission electron microscopy (TEM),[21] antigen capture ELISA,[22,23] immunoglobulin IgM and IgG antibody ELISA,[24] reverse transcription PCR (RT-PCR),[25,26] real-time quantitative PCR (RT-qPCR) detection[27,28] and immunohistochemical staining.[29,30] Isolation and culture of the virus is very sensitive, but it must be performed in a biosafety level 4 laboratory (BSL-4). Transmission electron microscopy (TEM) is a rapid detection technology, which usually requires very advanced equipment and laboratory. Therefore, these two methods are rarely used in laboratory detection. Currently, there are three detection methods commonly used in the process of outbreak: (1) antibody ELISA; (2) antigen-capture ELISA; (3) molecular biological detection technology based on real-time PCR.[31]

The commonly used real-time fluorescence PCR techniques include TaqMan probe-based real-time quantitative PCR and dye-based real-time quantitative PCR. The advantages of TaqMan probe method lies in its high specificity and low risk of contamination of samples, but the disadvantage is the high cost of probe synthesis. The method of SYBRGreen dye is more general but less specific than that of fluorescent probe.[32] In recent years, with the development of fluorescence PCR technology, a variety of real-time fluorescence PCR methods for hemorrhagic fever viruses have been established. TaqMan probe-based fluorescence PCR detection method for 28 hemorrhagic fever viruses has been established by Pang et al., 45–150 copies of synthetic viral RNA can be detected.[33]

Currently, most laboratories generally use PCR as their preferred and fastest diagnostic method for emergency detection and clinical samples.[34] Real-time fluorescent quantitative PCR based

TABLE 2 Effects of different hemolysis on TaqMan probe real-time quantitative PCR detection

	Group				
	Severe hemolysis	Moderate hemolysis	Mild hemolysis	Normal sample	*p* Value
Ebola virus					
I level ($n = 3$)	$9.413 \times 10^7 \pm 2.868 \times 10^7$	$7.567 \times 10^7 \pm 7.508 \times 10^6$	$9.413 \times 10^7 \pm 2.868 \times 10^7$	$1.907 \times 10^8 \pm 2.994 \times 10^7$	0.002
II level ($n = 3$)	$2.320 \times 10^6 \pm 1.019 \times 10^6$	$1.937 \times 10^6 \pm 3.800 \times 10^5$	$2.153 \times 10^6 \pm 2.739 \times 10^5$	$3.317 \times 10^6 \pm 2.122 \times 10^5$	0.072
III level ($n = 3$)	$3.253 \times 10^4 \pm 6.901 \times 10^3$	$2.523 \times 10^4 \pm 4.008 \times 10^3$	$2.569 \times 10^4 \pm 3.991 \times 10^3$	$3.347 \times 10^4 \pm 5.601 \times 10^3$	0.183
Marburg virus					
I level ($n = 3$)	$8.937 \times 10^7 \pm 7.671 \times 10^6$	$1.283 \times 10^8 \pm 8.737 \times 10^6$	$1.227 \times 10^8 \pm 1.193 \times 10^7$	$2.147 \times 10^8 \pm 2.376 \times 10^7$	<0.001
II level ($n = 3$)	$3.453 \times 10^6 \pm 4.061 \times 10^5$	$3.437 \times 10^6 \pm 5.463 \times 10^5$	$2.683 \times 10^6 \pm 3.513 \times 10^5$	$3.513 \times 10^6 \pm 1.531 \times 10^5$	0.092
III level ($n = 3$)	$4.140 \times 10^4 \pm 9.823 \times 10^3$	$3.850 \times 10^4 \pm 3.300 \times 10^3$	$2.867 \times 10^4 \pm 1.457 \times 10^3$	$3.563 \times 10^4 \pm 7.784 \times 10^3$	0.179

TABLE 3 Effects of different lipid turbidity on TaqMan probe real-time quantitative PCR detection

	Group				
	Severe lipid turbidity	Moderate lipid turbidity	Mild lipid turbidity	Normal sample	*p* Value
Ebola virus					
I level ($n = 3$)	$9.477 \times 10^7 \pm 2.911 \times 10^7$	$2.677 \times 10^8 \pm 3.331 \times 10^7$	$1.937 \times 10^8 \pm 2.994 \times 10^7$	$1.577 \times 10^8 \pm 2.639 \times 10^7$	0.001
II level ($n = 3$)	$2.877 \times 10^6 \pm 8.373 \times 10^5$	$3.647 \times 10^6 \pm 2.511 \times 10^5$	$3.473 \times 10^6 \pm 2.139 \times 10^5$	$3.683 \times 10^6 \pm 1.976 \times 10^5$	0.197
III level ($n = 3$)	$4.880 \times 10^4 \pm 8.448 \times 10^3$	$7.503 \times 10^4 \pm 2.547 \times 10^4$	$5.717 \times 10^4 \pm 1.339 \times 10^4$	$4.330 \times 10^4 \pm 3.869 \times 10^3$	0.131
Marburg virus					
I level ($n = 3$)	$1.227 \times 10^8 \pm 1.193 \times 10^7$	$2.737 \times 10^8 \pm 1.365 \times 10^7$	$2.147 \times 10^8 \pm 2.376 \times 10^7$	$2.247 \times 10^8 \pm 2.627 \times 10^7$	<0.001
II level ($n = 3$)	$5.017 \times 10^6 \pm 2.710 \times 10^5$	$5.103 \times 10^6 \pm 3.086 \times 10^5$	$4.513 \times 10^6 \pm 1.531 \times 10^5$	$5.010 \times 10^6 \pm 2.536 \times 10^5$	0.075
III level ($n = 3$)	$3.850 \times 10^4 \pm 3.300 \times 10^3$	$3.563 \times 10^4 \pm 2.363 \times 10^3$	$3.423 \times 10^4 \pm 3.101 \times 10^3$	$3.517 \times 10^4 \pm 3.296 \times 10^3$	0.404

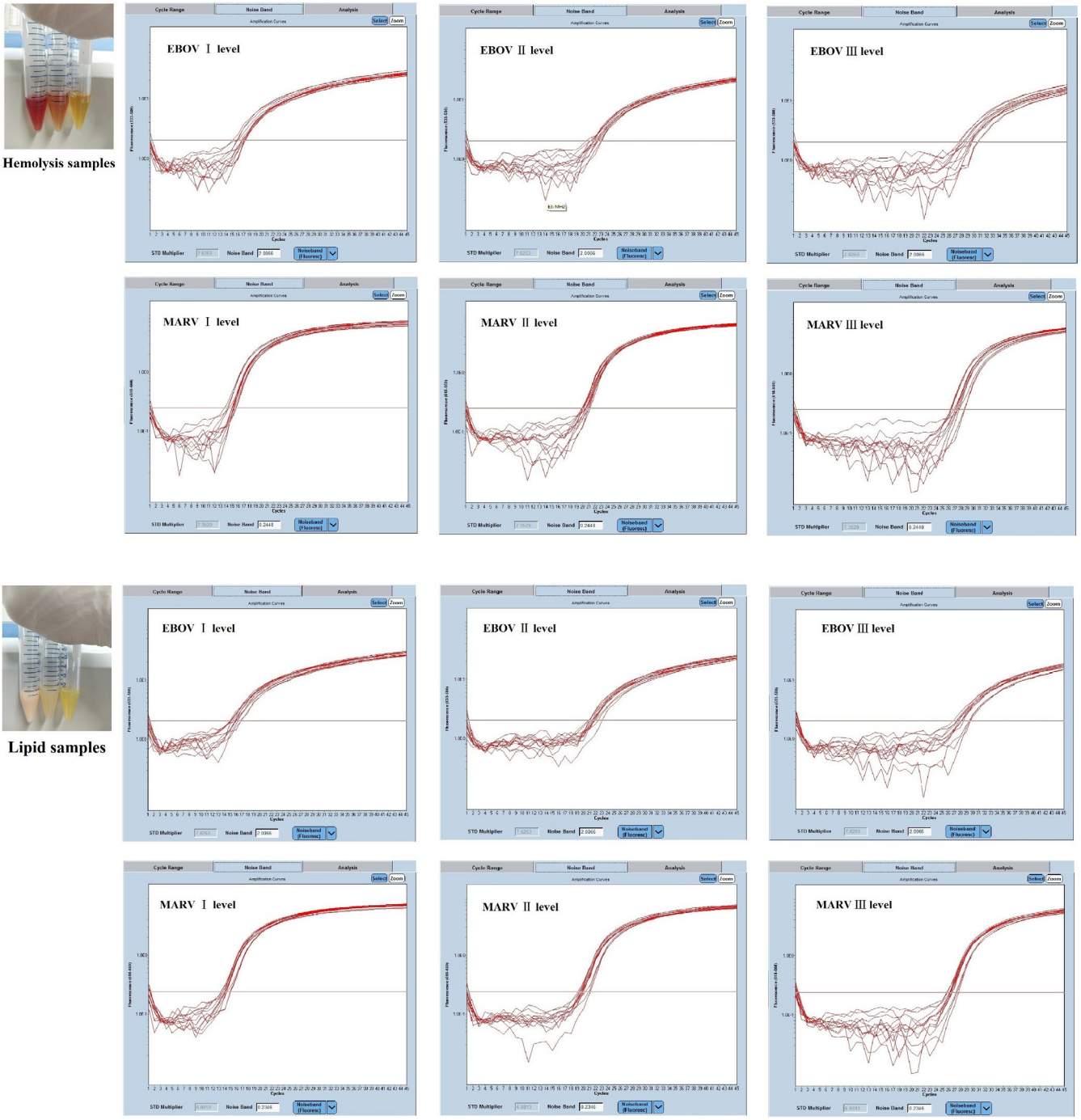


FIGURE 3 Quantitation of EBOV and MARV in lipid and hemolysis samples using a Roche LightCycle system, which final concentration of 2.0×10^8 (I level), 3.0×10^6 (II level), and 4.0×10^4 copies/ml (III level) prepared with standard plasmid

on fluorescent probe can simultaneously detect EBOV and MARV. The single-tube multiplex PCR method adopted in this study can simultaneously detect EBOV and MARV in one reaction tube. The linearity of the curve allowed quantification of nucleic acid molecules in a range from 10^3 to 10^9 copies/ml per reaction in the assay. The limit of detection for EBOV and MARV was 40 and 100 copies/ml, respectively. It has no cross-reaction with other pathogens such as HBV, HCV, HPV, EBV, HSV, CMV, and HIV-1. In addition, repeatability analysis of the two viruses showed that their coefficient of variation (CV) was less than 5%, indicating that the method has good repeatability. The above results indicated that fluorescence quantitative PCR could detect EBOV and MARV sensitively and specifically. Multiplex fluorescent quantitative PCR has advantages in the rapid diagnosis of mixed infection. Multiple real-time fluorescent PCR can simultaneously detect multiple target sequences in one reaction, so it has the advantages of large detection flux, fast detection speed, simple operation, and low detection cost. It is superior to the single-virus real-time PCR assay.

Both EBOV and MARV are virulent viruses, we could not test the virus or positive clinical samples to verify the detection assay, which is the biggest shortcoming of this study. In addition, although relatively conservative fragments are selected for amplification and detection in the design of the assay, there is the possibility of mutation of pathogen genes. Although the probability of mutation in the conservative region selected for amplification detection is very small, this possibility cannot be completely avoided theoretically. Moreover, PCR-based detection has shortcomings and cannot produce absolutely reliable results under any circumstances. PCR-based technologies are susceptible to contamination and lead to false-positive results, and the consequences of false-positive and false negative results of qPCR tests can have serious implications for outbreak management, especially in the early stages of disease and the early rehabilitation of survivors.[35,36]

At present, the research on these viruses is still relatively few and the depth of research is not enough in China. Although the natural epidemics of these viruses occur only in individual countries and regions, they are likely to become invasive due to countries around the world interact more and more with each other and the movement of people and goods increases. And they have the potential to become a means of bioterrorism or war. Accordingly, we must pay close attention to virulent virus, and the detection method for virulent virus continues to study. In this study, we established a TaqMan probe-based multiplex fluorescence quantitative PCR detection assay to detect EBOV and MARV, to provide technical support for laboratory testing of prevention and control of these severe infectious diseases.

The emergence of complex global public health crises such as climate change and extremes, biodiversity loss, and the global rise of resistant antibiotics has resulted in an unprecedented rise in direct and indirect mortality and morbidity. Public health emergencies occur frequently.[37] The risk of mixed infection with multiple pathogens continues to increase. Real-time fluorescence PCR technology is a method that adds fluorescence molecules to the PCR reaction system, monitors the whole PCR process by real-time detection of the accumulation of fluorescent signals. The technology has the characteristics of high sensitivity and specificity, multiple reactions, high degree of automation, no pollution, real-time, and accuracy.[38] It provides technical support for rapid diagnosis of mixed infection. It is feasible to develop multiplex real-time assayable a greater number of dangerous viruses based on TaqMan-based fluorescence PCR.

5 | CONCLUSION

The TaqMan probe-based multiplex fluorescence quantitative PCR assays could detect EBOV and MARV sensitively, specifically, and simultaneously. This study might provide technical support for laboratory testing of prevention and control of these severe infectious diseases. The further validation of this detection assay and developing multiplex real-time PCR assayable a greater number of dangerous viruses will be the focus of our next work.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Heming Wu and Zhixiong Zhong designed the study. Heming Wu and Zhikang Yu performed the experiments. Qingyan Huang helped to analyze the data. Heming Wu prepared the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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