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OPTIMIZATION OF THE QUANTITATIVE PCR FOR DETECTION HHV-6 INFECTION IN PATIENTS WITH NEUROLOGICAL DISORDERS

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Abstract

Human herpesvirus 6 (HHV-6) may cause some serious neurological disorders: multiple sclerosis, meningoencephalitis, mesial temporal lobe epilepsy, etc. Detecting this virus and providing early diagnosis lead to improved outcomes. A rapid method based on real-time quantitative polymerase chain reaction (qPCR) has been optimised to screen and quantify r HHV-6.

HHV-6 viral load could be a potential marker of prevention, diagnosis and treatment in patients with epilepsy and other neurological disorders. The aim of our study is to optimize of HHV-6 infection detection in patients with epilepsy and meningoencephalitis using qPCR "in house" system.

The 18 patients with epilepsy (10 children aged 5-14 and 8 adults 18-30) and 20 patients with meningoencephalitis (14 females, 6 males; mean age 37 [range: 19-49] were included in the study. Randomly selected 20 gender-and age-matched healthy blood donors as controls were checked for evidence of HHV-6.

We found HHV-6 in 6/18 (33%) of serum samples (SS) and in 8/18 (44%) of peripheral blood lymphocytes (PBL) among the patients with epilepsy. For patients with meningoencephalitis: 9/20 (45%) in SS and 10/20 (50%) in PBL. For healthy blood donors: 0/20 in SS and 1/20 (5%) in PBL.

Optimized "in house" qPCR system provides higher sensitivity in comparison to reference commercial kits. HHV-6 probably affects the development of mental health disorders and have to be monitored.

Key words: Human herpesvirus 6, qPCR, neurological disorders

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Introduction

Human herpesvirus 6 (HHV-6A and HHV-6B) is a widespread virus which is genetically related to human cytomegalovirus (HCMV) and HHV-7. HHV6 infection exhibits a wide cell tropism in vivo and, like other herpesviruses, induces a life long latent infection in humans, and there are many clinical manifestations, mainly chronic ones[1] Previously, it was believed that HHV-6 A and B are variants of HHV-6, but now there is evidence that they are two different viruses [2] Primary HHV-6B infection usually occurs in early childhood and can cause exantema subitum and occurs approximately in 30% of children. After the primary HHV-6B infection a latency form can develop which is life long and reactivates usually under immunosuppression or in immunocompromised individuals [3]. Experimental data suggests that HHV6 has been found in some serious neurological disorders: multiple sclerosis meningoencephalitis, and mesial temporal lobe epilepsy (MTLE). HHV-6 has been shown to have tropism for glioblastoma and neuroblastoma lines and is capable of infecting the central nervous system and causing encephalitis and meningitis. [4–11].

The forms of HHV-6 infection can be different: acute, persistent, latent, or chromosomally integrated. The detection of viral sequences in peripheral blood leukocytes (PBL) is a marker of latent/persistent infection, in serum virus DNA it is a marker of active infection. At the present time molecular assays are most sensitive for diagnosing HHV-6 infection they provide the ability to determine the active form of infection and the amount of virus DNA in serum. It has also been demonstrated that HHV-6 DNA in PBL does not correlate with the amount of virus replicating in an active form. Quantitative determination of viral DNA in serum is a more sensitive method in the diagnosis of the active infection The virus can be isolated from blood serum during the acute stage of infection, CD4+ T-lymphocytes CD34+, CD8+, monocytes/macrophages are targets for permissive replication HHV-6 virus in vivo[12,13,14,15].

There are different virology methods for diagnostics of HHV-6 infection. One of them is a method for isolating the virus in lymphoid cell lines. This is a specific and sensitive method, but it is time consuming, laborious and requires a sensitive cell culture. Clinical material should be taken in the early stages of the disease, when the probability of finding the virus is high. The antigen of the virus is determined by the methods of immunofluorescence, by monoclonal antibodies etc., but these are qualitative methods. Molecular assays such as polymerase chain reaction (PCR) are currently the most sensitive means of diagnosing an HHV-6 infection [16,17, 18,19,20]

At present, there are many different approaches to HHV6 detection, including viral load estimation. Some examples are multiplex droplet digital PCR detection, Nested PCR and solid phase real time PCR [3,16,19,20]. A lot of different commercial kits are used to detect DNA HHV-6. It is well known that the HHV6 viral load testing of clinical samples (whole blood, PBL, saliva, and cerebrospinal fluid) is very important step for virus mediated disease role understanding and is generally applied to different patients with both acute and chronic lesions. Using PCR in real time during the examination of clinical materials of patients with different pathologies provides a rapid diagnosis of HHV -6 infection and allows a timely start of etiotropic treatment.

The main goal of our study is the optimization of the quantitative real time PCR for detection of HHV-6 infection in patients with epilepsy and meningoencephalitis

Materials and methods

2.1.Patients and specimens.

This study was performed at the Republican Research and Practical Center for Epidemiology and Microbiology (Belarus). The 18 patients with epilepsy (10 children aged 5-14 and 8 adults 18-30) and 20 patients with meningoencephalitis (14 females, 6 males; mean age 37 years [range: 19-49]) were included in the study. All the clinical specimens were obtained from Republican Mental Health Center (Minsk, Belarus). Randomly selected 20 gender- and age-matched healthy blood donors as controls were checked for evidence of HHV-6

infection. These clinical specimens were obtained from Republican Scientific and Practical Center for Transfusiology and Medical Biotechnology, Minsk, Belarus.

We established the cohorts with the approval of the local Ethics Committee Ministry for Health of Belarus - №.02040/2257 from 03.04.2004. All the participants gave informed consent before the study.

2.2. Preparation of DNA from biological samples

Sample collection, preparation and storage. Blood and EDTA blood samples were collected by venipuncture. Serum and plasma aliquotes were stored at -20 ° C. The PBL fraction purified from 500 `l EDTA blood using "Hemolytic" (AmpliSense, Russia) according to the manufacturer‘s instructions. Purified PBL was stored at -20 ° C.

DNA purification. Viral DNA was purified from 100 ul of both blood serum and PBL fraction using "RIBO--prep" commercial kit (AmpliSense, Russia) according to the manufacturer‘s instructions. Purified DNA was dissolved and stored before analysis in 50 `l of 1xTE buffer at -20o C.

HHV6 detection and viral load estimation using commercial kits. Polymerase chain reaction (PCR) was used for the detection of viral sequences in DNA isolated from peripheral blood leukocytes and plasma (markers of latent/persistent and active infection, respectively).

The primary detection of HHV-6 genomic sequence was performed by classic PCR using "AmpliSens® HHV VI-Eph" kit (AmpliSens, Russia) and MJ Mini (Bio-Rad, USA) instrument, data not provided. Viral load in positive samples was estimated by Rotor-Gene 6000 (Corbett Research, Australia) Real Time PCR system using AmpliSens® HHV6-screentitre-FRT" kit (AmpliSense, Russia).

Statistical analysis

Statistical analyses were performed using SPSS 23.0 software (SPSS, Inc., Chicago, IL, USA). The Pearson‘s correlation was used to compare results between domestic and commercial kits. The p-value (two-tiled) below 0.01 was received. Statistical difference in the prevalence of latent/persistent and active HHV-6 infection between tested groups was performed using Fisher‘s test. Student‘s t-test was used to assess the analysis of continuous variable values, with a value of $p<0.05$ considered as significant.[21].

Results

3.1. Primer and probe design

There are enough genes for simultaneous quantitation of HHV6A and HHV6B with maximum accuracy. There are enough HHV6 protein coding genes with high identity for both HHV6A and HHV6B subtypes. Some of them were chosen for subsequent pre-primer design analysis (see Table 1).

Tab. 1: Candidate protein coding genes for HHV6A and HHV6B primers and probes design.

№	Gene symbol	Identity,%	Reference sequence
1	U4	98.1	HHV6A NC_001664.2 HHV6B NC_000898.1
2	U7	97.3	
3	U25	98.1	
4	U33	98.3	
5	U38	97.5	
6	U56	97.6	
7	U60	100.0	
8	U63	98.6	
9	U94	97.6	

Based on a preliminary analysis of the nucleotide sequences we chose the most suitable gene – U25 because the reaction efficiency was higher when compared with other genes.
Specific oligonucleotide primers and probes for HHV6 U25 (target gene), GAPDH (internal control) qPCR amplification designed using Gene Bank nucleotide sequences, and UGENE v.1.22.0 Primer3 integrated algorithm (UniPro, Russia). We used conservative sequence of U25 to exact cover both HHV6A and HHV6B genomes. The best oligonucleotides set for HHV6 was found within the +36 484 bp and +36 440 on HHV6 complete genome sequence. It corresponds to +312 and +395 bp on the U25 gene sequence. At the same time, the best set of oligonucleotides for internal control amplification was located within +3015 and +3144 bp on the GAPDH genomic sequence (Gene Bank accession number NC 000012). Graphical representation of designed oligonucleotides localization is shown in Fig.1. Subsequent detailed oligonucleotides sequences analysis using mfold (http://unafold.rna.albany.edu/?q=mfold/dna-folding-form) and IDT OligoAnalyser v.3.1 (https://www.idtdna.com/calc/analyzer) showed the absence of stable secondary structures within the experimental conditions. The specificity of selected oligonucleotides and amplicons was confirmed by free online tool NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All of real time PCR primers and probes sequences are shown in Table 2.

Fig. 1: Graphical representation of designed oligonucleotides localization for both target U25 HHV6 gene and internal control human GAPDH gene.

Tab. 2: Specific real time PCR primers and probes sequences for - target U25 HHV6 gene and internal control human GAPDH gene.

Gene name	Real time PCR primers and probes (5›- 3›)
U25	GTTGACCGCAGAGGATCTGT
	GCCGCCCAGAGAACACTAT
	FAM-GCGGCTTACGATTTTGTGAC-BHQ1
GAPDH	CTCCCACCTTTCTCATCCAAG
	ACATCACCCCTCTACCTCC
	ROX-AAAGCCAGTCCCCAGAACCCC-BHQ2

Because one of the most important quality criterion for virus detection and load estimation is an appropriate positive and internal controls set, we obtained each by recombinant DNA technology. The received pDNA samples contained the plasmid vector and insertion of the specific target sequence - amplicon. The positive control samples for the target gene U25 HHV6 and internal control for gene GAPDH were produced, using TOPOff TA Cloningff Kit (Invitrogen, USA, pGlow-TOPO vector (see fig.2). The presence of specific inserts target amplicons and internal control was confirmed by sequencing and real time PCR.

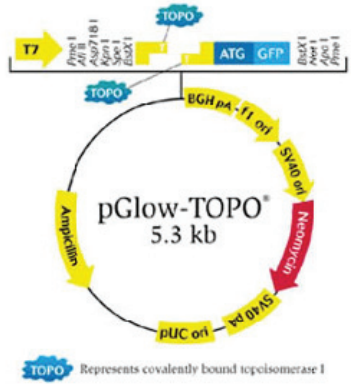

Fig. 2: The structure of commercial bacterial vector. HHV6 U25 positive control.

Optimization of the reaction conditions

Real time PCR optimization as well as basic test system parameters evaluation include the exact determination of reaction mixture composition; the optimal cycling and annealing/elongation temperature; quantities of primers and probes, analytical sensitivity and specificity determination.

The total volume of the reaction mix was 25 `l. We used 50 ng of total DNA (in the case of clinical samples), 200 nM of each primer and 100 nM probe for HHV6 U25 target sequence detection as well as for internal control - GAPDH; 0.2 mM of each dNTP; 2 mM of MgCl2 and 1.25 IU Hot Start Taq DNA-polymerase (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) as a start reaction protocol. Real Time PCR conditions were as follows: 2 min at 50 ˚ C, 3 min at 95 ˚ C, followed by 45 cycles for 10 sec at 95 ˚ C and 60 sec at 60 ˚ C. (two step reaction). Each clinical

DNA sample used in the present study was confirmed by AmpliSens HHV6-skrin- FL kit as a reference test system. The amplification was carried out in Bio-Rad CFX96touch System. Fluorescence detection was performed by FAM/ ROX channels at the end of each amplification cycle.

Optimization of the ratio of oligonucleotide primers and probes has been carried out (Table 3).The oligonucleotides, including TaqMan probes for PCR synthesized by «Primetech», Belarus. The concentration of primers and probes varied within the 100-1000 nM and 50-500 nM respectively. The submaximal concentration of oligonucleotides (primers and probe) provides no results in Real Time PCR. Approximately the same results were received with medium concentrations. We think that this was due to both secondary structures forming rate and other unspecified reasons. On the other hand, too low concentrations provides non-optimal reaction effectiveness. Thus, the optimal ratio of oligonucleotides according to experiment results were 1:2 - one part of the probe and 2 of each oligonucleotide primer. The quantitation cycle was equal to 35.9 in the reaction using 200 nM of primer and 100 nM of the probe. The gradient of temperature was used for the next stage of optimization the reaction conditions (Table 4). Three positive samples of HHV-6 DNA were used to choose the optimum temperature.

Tab. 3: Determination of the optimal ratio and amounts of the oligonucleotides in the reaction mix for real time PCR HHV6

The variants of combination primers and probe	oligonucleotide concentration in the reaction mix			Cq
	HHV6-F	HHV6-P	HHV6-R	
№1 (nM)	1000	500	1000	-
№2(nM)	500	250	500	-
№3(nM)	200	100	200	35,9*
№4(nM)	100	50	100	38,7

Tab. 4: Amplification HHV-6 DNA at the temperature gradient

Temperature annealing / elongation, to C	Cq DNA HHV-6		
	Sample1	Sample2	Sample3
65.5	-	-	-
64.3	-	-	-
62.4	35	37	35
59.9**	34	36	36
58.1	38	40	40
56.8	-	-	-

The optimal temperature profile was found based on real-time PCR data of three separate positive samples with annealing / elongation temperature gradient. The criterion for the optimal temperature was the smallest value of the amplification cycle at which the results were detected. As shown in table 4 the most effectively annealing/elongation temperature range was between 59.9-62.4. Thus, amplification curves cross the threshold line at the 34 - 36 cycles. Variations of 1 cycle or less were considered an experimental bias and were considered as one value.

3.3. Analytical sensitivity, specificity "in-house" qPCR assay

The analytical sensitivity and linear detection range of the "in-house" HHV-6 qPCR assay were determined using a tenfold dilution series (from 5*109 to 5*104 copies per reaction) and used as a set of standards. Positive and negative controls were included in each run. Optimized real time PCR protocol with 100% effectiveness and R2 = 0.996 was determined (fig.3).

HHV6 LOD. Limit of detection estimated using:10-fold dilutions of plasmid DNA contained HHV6 U25 amplicon. DNA samples received from HHV6 HHV6-positive patients; DNA samples received from HHV6-negative patients; sterile PCR-grade water as a negative reaction control (see fig.4).

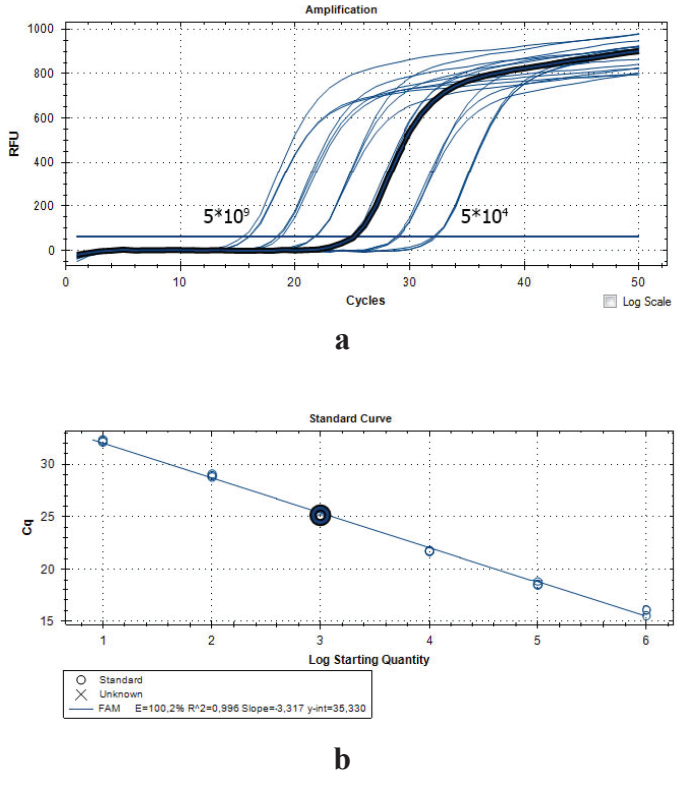

Fig. 3: The results of amplification HHV6 10-fold serial dilutions (a) and standard curve (b) under optimized real time PCR reaction conditions.

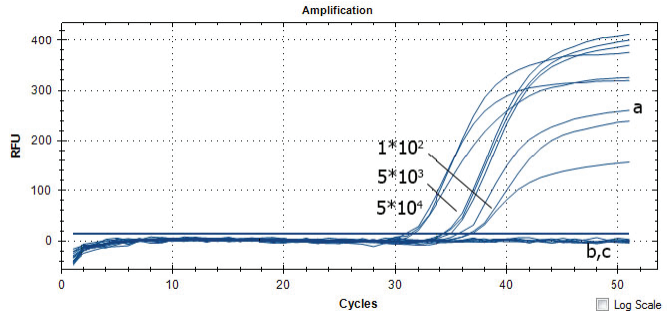

Fig. 4: The results of HHV6 real time PCR detection system LOD estimation. Presented the results of 10-fold plasmid DNA dilutions in triplets (1*102, 5*103 and 5*104 per reaction), HHV6-positive clinical sample (a), HHV6-negative clinical sample (b) and negative reaction control - PCR-grade water (c).

The "in-house" kit sensitivity analysis was performed using the plasmid DNA dilutions as a positive reaction control, and PCR-grade water as a negative reaction control

The analytical sensitivity of the "in- house" kit was determined by tenfold dilutions of pDNA with a concentration from 106 to 10 copies / reaction. Limiting dilution pDNA, in which recorded positive signal contained 1000 copies DNA / mL or 10 copies pDNA / reaction.

3.4.Sensitivity and specificity of the qPCR assays on clinical specimens

To determine of viral DNA isolated from PBL and blood serum as markers of latent/persistent and active infection, respectively used the qualitative "in house" HHV-6 PCR test. The samples from 20 patients with meningoencephalitis and 18 with epilepsy were investigated by the qualitative "in house" HHV-6 PCR test (Table 5). HHV-6 genomic sequences were found in serum of 8 patients and in PBL DNA samples from 9 patients with epilepsy. Active infection in 7 patients and latent/persistent in 5 patients with meningoencephalitis were detected. DNA in PBL was detected from 1 patient from blood donors.

Tab. 5: Detection of HHV-6 DNA infection in blood serum and PBL by the qualitative "in house" HHV-6 PCR test.

Diagnosis	n	HHV-6 DNA (positive patients)			
		Serum (%)	P-value	PBL	P-value
Epilepsy	18	6 (33.3)	<0.05	8 (44,4)	>0.05
Meningoencephalitis	20	9 (45.0)	>0.05	10 (50,0)	>0.05
Blood donors(BD)	20	0		1	

DNA was detected in serum and in PBL of patients with epilepsy in 33.3% and 44,4% respectively. The patients with meningoencephalitis had DNA HHV-6 in serum and in PBL in 45.0% and 50,0% respectively. The results of the occurrence DNA in blood serum (active form) in patients with epilepsy compared with the control group were not significant ($p < 0.05$). There was an increase DNA in PBL of the patients with latent / persistent infections (>0.05). The active and latent/persistent forms infection were registered in 9 and 10 patients with meningoencephalitis respectively at a statistically significant level in comparison with BD patients.

Comparative determination of viral load in positive samples was carried out by "in-house" quantitative HHV-6 and HHV6-screentitre-FRTkit (AmpliSense, Russia).

The samples from 8 patients with epilepsy and 7 patients with meningoencephalitis were examined by making a tenfold dilution series of serum blood samples (Tab.6).

The samples from four patients with epilepsy had viral load 1000 -1500 copies/ml by using in-house test systems, it was approximately ten copies of target DNA/ reaction. Three patients had a low viral load 220 - 450 copies/ml.

All samples from 8 patients with epilepsy were tested by HHV6-screentitre-FRT kit and four patients were positive.

The test system "in-house" quantitative HHV-6 and HHV6-screentitre-FRT kit were used to determine the viral load in patients with meningoencephalitis A high viral load was detected in 5 patients 1000 -1500 copies/ml and 1 patient had <200 copies/ml. Almost the same results were obtained by HHV6-screentitre-FRT kit. The samples that were inconsistent between the quantitative assays contained low levels of HHV-6 DNA <200 copies/ml

Tab. 6: The results of DNA detection viral load in blood serum of positive patients by two PCR-kits

"Clinical specimens from patients with	"in-house" qualitative HHV-6	HHV-6 "in-house" quantitative HHV6- screen FL. Belarus) result (copies/ml)	HHV6 -screentitre-FRTkit (AmpliSense, Russia). result (copies/ml)
epilepsy	Positive Positive Positive Positive Positive Positive Positive Positive	Positive (<1000) Positive (<250) Positive (1500) Positive (< 1000) Negative Positive(1250) Positive (<220) Positive (<450)	Positive (<250) Negative Positive (<1000) Positive (<250) Negative Positive (<1000) Negative Positive (<250)
meningoencephalitis	Positive Positive Positive Positive Positive Positive Positive	Positive (<200) Positive (<1500) Positive (<1500) Positive (<1000) Negative Positive (<1250) Positive (<1000)	Negative Positive (<1000) Positive (1000) Positive (<1000) Negative Positive (<1000) Positive (<1000)

The specificity for the qualitative HHV-6 "in- house" assay was confirmed by testing samples from HHV-6 patients with epilepsy. DNA HHV-6, varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) were investigated.

Tab. 7: The specificity of the "in- house" kit compared with commercial HHV6- FRT kit (AmpliSense)

Kits	DNA viruses			
	DNA HV-6 positive/ total amount	Varicella zoster	Epstein-Barr	Cytomegalovirus
"in-house" qualitative HHV-6	8/18	no	no	no
HHV6- FRT kit (Ampli-Sense, Russia)	7/18	no	no	no

Comparative analysis of the "in house" kit (HHV6- screen FL. Belarus) and the commercial one (AmpliSens, Russia) showed similar results in the determination of HHV-6 DNA in clinical specimens of patients with epilepsy, but one sample was inconsistent between the assays due to contained low levels of HHV-6 DNA (¡200 copies/ reaction). No signal for amplification was found for samples containing DNA viruses: varicella zoster virus (VZV), Epstein-Barr (VEB), nor cytomegalovirus (CMV).

Discussion

There are some neurological disorders, such as meningoencephalitis and mesial temporal lobe epilepsy which may be caused by HHV-6 as etiological factor in these diseases or may beassociated with neurological disorders HHV-6 infection can be reactivated under various triggering factors, such as surgery, immunosuppression, and others. In such clinical situations specific HHV-6 diagnostics are needed (Akinsoji et.al 2020, Säll O et.al 2019).

There are various modifications of real time PCR systems for HHV-6 DNA detection. in clinical specimens such as whole blood, serum, PBLs or cerebrospinal fluid. Qualitative detection of the virus from clinical specimens by real time PCR has a limited value compared to quantitative ones for diagnosing patients with HHV-6 infection (Karlsson et.al.2012; Flamand et.al.2010; Pyöriä et.al.2020); the determination of HHV-6makes it possible to provide precise, rapid and early diagnosis of neurological disorders in patients and use timely essential therapy that has been measured to improve the disease outcome.

The aim of this study was the optimization of quantitative PCR for detection HHV-6 infection in patients with epilepsy and meningoencephalitis and evaluate a new "in-house" kit by quantitative HHV-6 PCR assay on clinical specimens.

The real time PCR optimization was carried out. The basic test system parameters including exact determination of reaction mixture composition; the optimal cycling and annealing/elongation temperature; suitable quantities of primers and probes were studied.

The concentration of primers and probes varied within the 100-1000 nM and 50-500 nM respectively. The quantitation cycle was equal to 35.9 in the reaction using 200 nM of primer and 100 nM of the probe. The most effectively annealing/ elongation temperature range was between 59.9 ° -62.4 degrees, the threshold line was at the 34 - 36 cycles.

The results using of primers and probe for test optimisation were 200 nM of each primer and 300 nM of probe concentrations, initially showed a 35.9 Ct reaction. The concentrations of 100 nM of each primer and 50 nM of probe showed less efficiency 38.7 Ct reaction. The optimal effectively annealing/elongation temperature range was between 59.9o-62.4 degrees.

The high sensitivity of TaqMan associated PCR systems can lead to false positive or false negative results. Therefore, real time PCR analysis optimization is the one of the most important condition of HHV6 diagnostics in clinics.

The PCR test system must include a set of positive and negative control samples aimed at preventing false-negative and false-positive results, as well as for the technical quality control of components test systems. Typically, for positive control samples PCR diagnostic test systems recombinant DNA technology is used. DNA samples from the plasmid vector and insertion of the target sequence were obtained by this technology. The HHV6 U25 gene contained plasmid DNA and enabled a quantitative analysis of the samples by using dilution series between 10,000 and 10 copies / reaction (calibrators) to be carried out. It should also be considered that the virus tends to change DNA sequence as a result of mutation, because we chose the targets for the design conservative region of gene U25, and it is specific to the basic genome of HHV-6 and absent from HHV -7.

The PCR test system was obtained with 100% efficiency, if the number of copies in 10 ul of sample, is at least 10 copies/reaction. We received optimized real time PCR protocol with 100% effectiveness. The curve of amplification crossed the threshold line on the 32-38 cycles. All the results obtained after 38 cycles, should be evaluated carefully.

Detection of the virus-specific antigens in PBS is a sensitive and reliable method of diagnosing HHV-6 infection. But this method is more qualitative than quantitative. At the present time quantitative PCR methods are used to evaluate HHV-6 viral loads. The applicability of the qPCR methods for qualitative determination of HHV-6 DNA in samples was also studied in our laboratory and provides diagnostic services for neurological complications associated with HHV-6 infection. HHV-6 viremia was found in serum of 6 patients with epilepsy, which indicate an active infection. There were samples from 8 patients with epilepsy with DNA in PBL -- latent/persistent infection. The active infections and latent/persistent infections were detected in 9 patients and in 10 patients with meningoencephalitis respectively.

The sensitivity of quantitative real time PCR methods was estimated by making a tenfold dilution series 10^2-10^6 HHV-6 genome copies/reaction of a blood serum sample with a high

HHV-6 DNA viral load (1000 copies/reaction). Comparative studies of the analytical sensitivity of two test systems "in--house" HHV6- screen FL (Belarus)" and HHV6-screentitre-FRT kit AmpliSense (Russia) showed almost similar results, one sample was inconsistent because it contained very low level of HHV-6 DNA (¡200 copies/ml). The new "in-house" HHV-6 qPCR could detect 10-10^5 copies of the HHV-6 genome per reaction and the dynamic quantitative range of the assay for whole blood specimens was 500-5 10^7 copies/ml.

In this way the test system may be used for the diagnosis of infection caused by HHV-6. Of course, we clearly understand that HHV-6 DNA in EDTA blood samples indicates the presence of HHV-6 DNA in blood cells rather than cell-free circulating virus particles, and thus HHV-6 DNA in the plasma does not necessarily reflect the amount of active virus. This work was carried out to obtain suitable real-time PCR test system for detection of HHV6 DNA viral load in clinical specimens.

In conclusion, the qPCR developed was simple, rapid and sensitive, allowing the detection of a wide range of HHV-6 loads. The optimized "in house" qPCR system provides higher sensitivity in comparison to reference commercial kit. HHV-6 probably takes part in the development of mental health disorders and have to be monitored. Additional studies are needed to elucidate molecular and cell mechanisms of HHV-6 impact.

Conflict of interest

The authors declare no conflict of interest.

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