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Determination of FMDV 146S particle concentration by spectrometric method during viral RNA quantification

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SUMMARY
During FMD vaccine production, special attention is paid to the concentration of 146S particles bearing the critical biological features of FMDV and being the main components that have an effect on vaccine immunogenicity. For this reason, each batch of vaccine raw material is tested for 146S component concentration. The paper presents the results of the use of a spectrometric method for whole particle concentration determination during quantification of FMDV RNA extracted after immune capture. It is an inexpensive, easy-to-perform method allowing for determination of FMDV 146S particle concentration in the non-inactivated culture suspension. 146S particle concentration was found to depend on the number of RNA molecules extracted from virions after their strain-specific immune capture and quantitatively detected by the spectrometric method. The presented method allows for determination of 146S component concentration in the non-inactivated vaccine raw material using the proposed linear model. The spectrometric method showed 94.5–99.5% correlation with real-time reverse transcription polymerase chain reaction and complement fixation test based on the results of tests of 360 non-inactivated suspensions of FMDV of all types. Tests of the positive control demonstrated 99.0–99.6% compatibility of actual and expected results. FMDV genome and 146S particles were not detected in the negative control, and that was in line with expectations.

Key words: FMDV RNA, 146S component concentration, spectrometric analysis, immune capture.

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Определение концентрации 146S частиц вируса ящура спектрометрическим способом при оценке количества вирусной РНК

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РЕЗЮМЕ
В процессе промышленного производства противоящурных вакцин особое внимание уделяют концентрации 146S частиц, которые наделены важнейшими биологическими свойствами вируса ящура и являются основными компонентами, влияющими на иммуногенную активность вакцинных препаратов.

ORIGINAL ARTICLES | BIOTECHNOLOGY ОРИГИНАЛЬНЫЕ СТАТЬИ | БИОТЕХНОЛОГИЯ

По этой причине каждую серию вакцинного сырья исследуют на определение концентрации 146S компонента. В статье представлены результаты изучения применения спектрометрического метода для определения концентрации полных частиц при оценке количества РНК вируса ящура, выделенной после иммунного захвата. Данный способ является дешевым, простым в исполнении, позволяет определять концентрацию 146S частиц вируса ящура в неинактивированной культуральной суспензии. Выявлено существование зависимости между концентрацией 146S частиц и количеством молекул РНК, выделенных из вирионов после их штаммоспецифического иммунного захвата и количественно детектированных спектрометрическим методом. Представленный метод позволяет определять значение концентрации 146S компонента вируса ящура в неинактивированном сырье для вакцины с применением предложенной линейной модели. Корреляция спектрометрического способа с полимеразной цепной реакцией с обратной транскрипцией в режиме реального времени и реакцией связывания комплемента при тестировании 360 неинактивированных суспензий вируса ящура всех типов составила 94,5–99,5%. Для положительного контроля совпадение фактических и ожидаемых результатов соответствовало 99,0–99,6%. В отрицательном контрольном образце геном и 146S частицы вируса ящура не обнаружены, что соответствовало ожиданиям.

Ключевые слова: РНК вируса ящура, концентрация 146S компонента, спектрометрический анализ, иммунный захват.

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INTRODUCTION

Foot-and-mouth disease is a highly contagious viral disease characterized by fever that affects wild and domestic cloven-hoofed animals [1]. The disease agent is an RNA-virus of the order *Picornavirales*, family *Picornaviridae*, genus *Aphthovirus*. Foot-and-mouth disease virus (FMDV) is highly antigenically variable due to mutations in the capsid protein genes and encompasses A, O, C, Asia-1, SAT-1, SAT-2, SAT-3 types and multiple genetic lineages [2].

FMDV virion is about 23–25 nm in diameter. The genome is a single-stranded positive-sense RNA consisting of approximately 8,500 nucleotide bases. The virion has a molecular weight of about 8,080,000 Da [3]. When reproduced in susceptible cell lines, FMDV forms 4 variants of components: 146S particles (virions) containing one viral RNA molecule and 60 polypeptide copies, each is a complex of VP_1-VP_2-VP_3-VP_4 proteins; 75S component lacking RNA and comprising 60 copies of VP_1-VP_3-VP_0 polypeptide; 12S particles represented by VP_1, VP_2, VP_3 proteins; 3.8S subunits consisting of non-structural VP_g protein [4].

The system of measures for FMD control and prevention includes mass immunization of cattle and small ruminants, as well as post-vaccination immunity level monitoring [1, 5]. During FMD vaccine production, special attention is paid to the concentration of 146S particles bearing the critical biological features of FMDV and being the major components that have an effect on vaccine immunogenicity [4]. Quantitative complement fixation test (CFT) and real-time reverse transcription polymerase chain reaction (rtRT-PCR) are used to determine 146S component concentration in the vaccine raw material used for every batch. The first technique has several disadvantages: it is labour-intensive and time-consuming (the test lasts up to 3 days) and does not allow for simultaneous testing of a large number of samples, its cost is rather high [6]. Real-time RT-PCR reduces the test duration to 4 hours, but requires expensive equipment and reagents [7]. Besides, when a sample contains an excessive amount of ballast components, reliability of accurate determination of 146S immunogenic component concentration may be decreased due to anticomplementarity in CFT and high ballast protein content in the sorbent at the RNA extraction stage.

It is, therefore, reasonable to search for an alternative method for FMDV 146S component quantification in the non-inactivated vaccine raw material.

The aim of the study is to assess spectrometric method potential for determination of 146S particle concentration during FMDV RNA quantification.

MATERIALS AND METHODS

Virus. Non-inactivated suspensions of culture FMDV A/Turkey/2006, A/ARRIAH/2015, O/Primorsky/2012, O/Primorsky/2014, Asia-1/Tajikistan/2011, C_1/Zakarpatsky/1972, SAT-1/Akhalkalaksky/62, SAT-2/Saudi Arabia 7/2000, SAT-3/Bechuanaland 1/65 vaccine strains deposited in the FGBI "ARRIAH" Collection of Microorganism Strains were used. The virus was reproduced in the continuous suspension culture of baby hamster kidney cells (BHK-21).

Immunoassay plate coating. A 24-well plate with DNA/RNA/DNAase/RNAase-free surface was coated with strain-specific anti-FMDV polyclonal antibodies in a volume of 1.0 cm^3 with IgG concentration in suspension of 5.0 $\mu g/cm^3$ at (4 ± 2) °C during 18 hours. Open binding sites were blocked with 0.5% bovine serum albumin (BSA) suspension at (37 ± 1) °C for 1 hour. This is a preparatory process that should be carried out beforehand, prior to analysis.

Immune capture of FMDV virions. The virus suspension was added to the wells coated with antibodies to the virions of a particular FMDV strain, 3.0 cm^3 of the suspension per well, and incubated at 37 °C for 1 hour. Immune complexes were formed, with some of them being 146S particle – strain-specific antibody complexes. The suspension was removed from the wells, but the film on the bottom

was left intact. Then 1/15 M phosphate buffered saline (PBS) was added to the wells, 1.0 cm³ of PBS per well, with subsequent resuspension.

RNA extraction from FMDV 146S particles. 10 ml of a solution containing 50% of phenol (pH < 7.0) and 50% of 4 M guanidine isothiocyanate (GITC) were added to 1.0 cm³ of 146S particle – strain-specific antibody immune complex suspension and incubated for 20 minutes at 23–25 °C. The prepared lysate was centrifuged at 14,000 rpm for 8 minutes. The supernatant was transferred into a centrifuge tube, 2.5 cm³ of chloroform were added, and the content was incubated for 4 minutes with periodic mixing. After exposure, the mixture was centrifuged at 14,000 rpm for 12 minutes at 4–5 °C. As a result of precipitation, the tube content was fractionated into three phases: 1) the straw-coloured lower phase containing the complex of phenol and chloroform with bound lipids and polypeptides; 2) the white interphase comprising peptide components and DNA; 3) the upper phase representing a clear RNA extract [8, 9]. The entire upper phase was collected into an empty tube, while other fractions were left intact, and 4.5 cm³ of 100% isopropyl alcohol were added. The prepared mixture was incubated for 8 minutes at 23–25 °C; after that the tube content was centrifuged at 14,000 rpm for 8 minutes at 23–25 °C. The supernatant was removed, and FMDV RNA pellet remained in the tube. 2.0 cm³ of 80% ethanol were added to the RNA pellet. The content was mixed and pelleted at 14,000 rpm for 6 minutes at 23–25 °C. The supernatant was removed, and the RNA pellet was dried with an air stream at ambient temperature for 5 minutes. Then 0.1 cm³ of TE buffer (10 mM tris(oxymethil)aminomethane, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.0–7.2) free from RNAases and Mg^{2+} cations was added to RNA; the content was heated at 55–60 °C for 2–3 minutes to maximally dissolve FMDV RNA. Thirty-fold eluates of FMDV RNA standard dilutions and the negative control were prepared.

Assessment of FMDV RNA eluate purity using spectral analysis. FMDV RNA eluate spectral absorbance was measured at wavelengths of 205–325 nm. Using the said spectral range, the preparation was tested for residual phospholipids, polysaccharides and guanidine isothiocyanate, phenol, polypeptides, large conglomerates by optical density (OD) determination at 205, 235, 270, 280 and 320 nm, respectively [10, 11]. RNA eluate was considered to be free from protein and phenol contamination when the extinction coefficient R_1 (OD_{262}/OD_{280}) was 1.8–2.2 and its value was close to 2.0. FMDV RNA extract was considered to be uncontaminated with polysaccharides when the extinction coefficient R_2 (OD_{262}/OD_{235}) was 2.00–2.02 [12]. When 1% of RNA is replaced with polysaccharide components, the R_2 value decreases by 0.002. $R_2 > 2.02$ was indicative of nucleic acid dissociation and the presence of nucleotides in the eluate. OD_{320} tending to zero reflected the absence of large suspended particles in the extract [8].

Complement fixation test (CFT). Quantitative CFT was used for FMDV 146S particle concentration determination; the test was carried out in compliance with requirements [6].

Real-time reverse transcription polymerase chain reaction (rtRT-PCR). Real-time RT-PCR was used for FMDV genome detection and quantification in the non-inactivated vaccine raw material. The amount of components for the reaction, as well as time and temperature parameters for thermal cycling are specified in the previously described requirements [7].

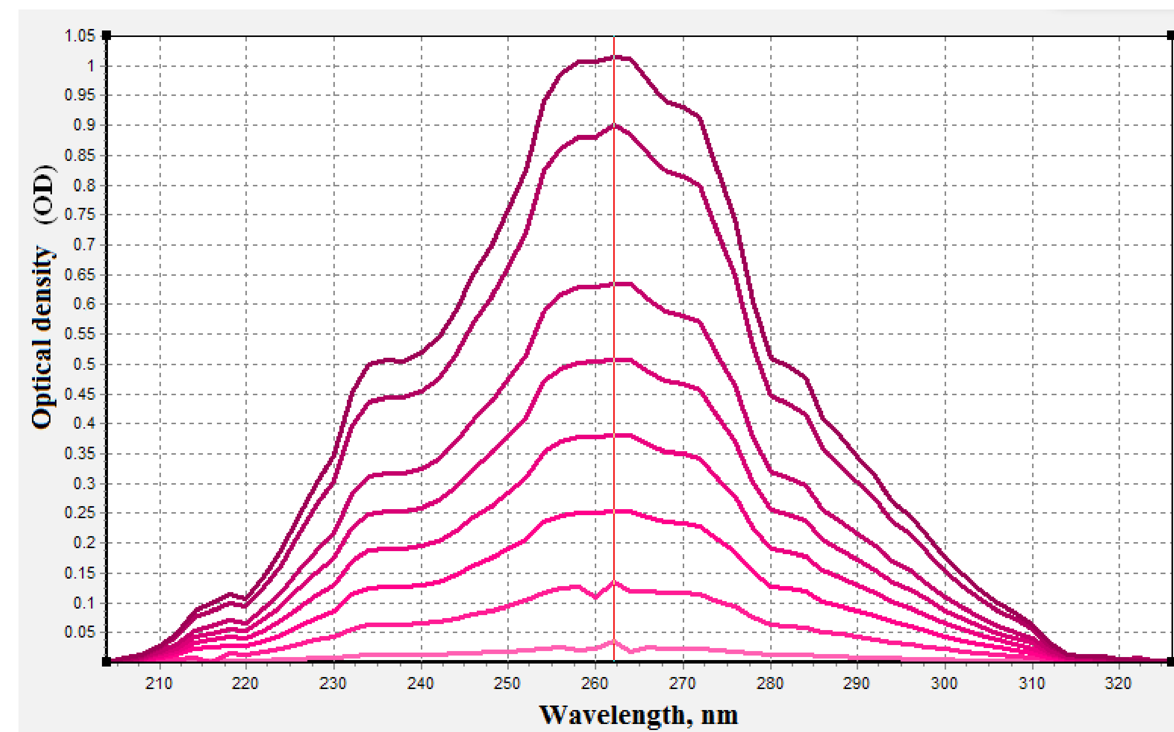

Fig. 1. Spectrograms of dilutions of standard FMDV Asia-1/Tajikistan/2011 strain RNA eluate. The figure shows (from top downwards) graphs for some dilutions of eluates corresponding to the following 146S particle concentrations: 4.0; 3.5; 3.0; 2.5; 2.0; 1.5; 1.0; 0.5; 0.1 µg/cm³ (n = 3)

Рис. 1. Спектрограммы разведений стандартного элюата РНК вируса ящура штамма Азия-1/Таджикистан/2011. Сверху вниз отражены графики для некоторых разведений элюатов, соответствующих концентрациям 146S частиц: 4,0; 3,5; 3,0; 2,5; 2,0; 1,5; 1,0; 0,5; 0,1 мкг/см³ (n = 3)

RESULTS AND DISCUSSION

During the first stage of the study, a non-inactivated suspension of culture FMDV Asia-1/Tajikistan/2011 strain with 146S particle concentration of (4.00 ± 0.11) µg/cm³ (based on CFT and rtRT-PCR data) used as a positive standard was tested. Immune capture of FMDV virions was carried out using an immunoassay plate coated with polyclonal antibodies specific to the whole virus particles of the specified strain. BHK-21 cell suspension with a concentration of cells of (3.00 ± 0.10) mln/cm³ not infected with FMDV served as a negative control. As a result of serological reaction, a suspension of FMDV 146S particles bound with strain-specific immunoglobulins G was prepared. The suspension was used for RNA extraction from the whole virus particles, and a 30-fold extract of FMDV Asia-1/Tajikistan/2011 strain RNA in a volume of 0.1 cm³ was prepared.

During the next stage of the study, the purity of FMDV Asia-1/Tajikistan/2011 strain (positive standard) RNA eluate was assessed by spectral analysis in the ultraviolet region. Extinction values were registered for every 2 nm in the range from 205 to 325 nm, and the entire absorption spectrum of RNA was recorded using Spectrum software version 5.0 (Fig. 1).

The test results for 30-fold preparation showed that $OD_{205-259}$ and $OD_{263-325}$ values did not exceed $OD_{260-262}$ values (0.001–1.006 < 1.007–1.014 and 1.013–0.004 < 1.007–1.014), thus indicating the high purity of the prepared RNA eluate. During spectral analysis, no pronounced peaks were observed on the graph at wavelengths of 205, 235, 270, 280 and 320 nm, and that was indicative of the almost total absence of contamination with phospholipids, polysaccharides and residual GITC, phenol, polypeptides, large conglomerates, respectively. The extinction coefficient R_1 was 1.988 (OD_{262}/OD_{280} = 1.014/0.510), i.e. close to the normal value of 2.000, and that showed the absence of DNA and the presence of trace amounts of protein impurities. Nucleic acid degradation and free nucleotides were not observed in the solution, since R_1 did not exceed 2.000. The extinction coefficient R_2 (OD_{262}/OD_{235} = 1.014/0.506) of 2.004 showed that the eluate of FMDV RNA (positive standard) was not contaminated with polysaccharides and GITC. Given that the R_2 value decreases by 0.002 when 1% of RNA is replaced with polysaccharide components, no carbohydrate impurities were detected in the prepared eluate.

During the following stage of the study, quantification of RNA molecules extracted from FMDV 146S particles was performed. One-stranded RNAs have their absorption maxima at 252–271 nm due to high spectral absorbance of ribonucleoside-5′-triphosphates in this range, in particular: adenosine-5′-triphosphate (λ_{ATP}) – 259 nm, uridine-5′-triphosphate (λ_{UTP}) – 262 nm, guanosine-5′-triphosphate (λ_{GTP}) – 252 nm, cytidine-5′-triphosphate (λ_{CTP}) – 271 nm [13]. Spectral studies demonstrate that maximum absorption wavelengths of whole RNA monomers are within the same range; thus, extinction is the highest in the specified wavelength range [14]. In the course of the study, nucleotide analysis of RNAs of a wide variety of isolates and strains of seven types of FMDV available in the GenBank database was carried out [3]. Based on the test results, average percentages of ribonucleoside-5′-triphosphates (W_{ATP}, W_{UTP}, W_{GTP}, W_{CTP}) in the genome were determined. The test results are presented in Table 1.

Based on the data obtained, average maximum absorption wavelengths (λ_{max}) of RNAs were calculated for each FMDV type using the following formula: $\lambda_{max} = \lambda_{ATP} \times W_{ATP} + \lambda_{UTP} \times W_{UTP} + \lambda_{GTP} \times W_{GTP} + \lambda_{CTP} \times W_{CTP}$. It was determined that λ_{max} for the genome of FMDV strains of type A was 261.64 nm, type O – 261.51 nm, type C – 261.68 nm, type Asia-1 – 261.63 nm, type SAT-1 – 261.74 nm, type SAT-2 – 261.70 nm, type SAT-3 – 261.77 nm. λ_{max} values were experimentally determined by biospectrometry of RNA eluates of all FMDV types and found to be close to empirical values – 262 nm (Table 1).

The number of one-stranded RNA molecules in the pure preparation was calculated on the basis of the Bouguer – Lambert – Beer law which states that the intensity of a parallel beam of monochromatic light decreases when it passes through the absorbing medium [10]. Using this law, it is possible to relate the amount of light absorbed to the concentration of absorbing particles. At λ = 260–262 nm, average extinction coefficient for one-stranded RNA is 0.024 (µg/cm³)⁻¹ cm⁻¹. Therefore, the optical density of a solution with nucleic acid concentration of 1.00 µg/cm³ exposed to ultraviolet light at a wavelength of 260 nm (OD_{260}) is 24.000, and, consequently, optical density of 1.000 corresponds to an eluate with RNA concentration of 41.67 µg/cm³ [13]. Measurements showed that FMDV RNA has its absorption maximum at λ = 262 nm; the OD_{262} value for a highly purified extract with RNA concentration of 41.67 µg/cm³ is 1.000.

The use of Bouguer – Lambert – Beer law is relevant for viral RNA molecule quantification in diluted eluates. However, at high analyte concentrations (> 1.00 µg/cm³), the distance between the molecules of an ultraviolet absorbing material is significantly reduced. As a result, the effect of each particle on surface charge distribution between the neighboring molecules grows, and this may lead to a change in RNA absorbance at the specified wavelength. Thus, for FMDV RNA molecule quantification in high concentration preparations, analyte dilutions should be made using TE buffer. Prior to

Table 1
Ribonucleoside-5′-triphosphate percentages and maximum absorption wavelengths for RNAs of different FMDV types

Таблица 1
Процентное содержание рибонуклеозид-5′-трифосфатов и длины волн с максимальным поглощением РНК вируса ящура разных типов

FMDV type	Average percentages of ribonucleoside-5′-triphosphates in FMDV genome (*W*), %				Maximum absorption wavelengths for RNAs, nm	
	ATP	UTP	GTP	CTP	calculated	empirical
A	25.1	20.9	25.7	28.3	261.64	262
O	25.6	21.4	25.9	27.1	261.51	262
C	25.1	21.2	25.4	28.3	261.68	262
Asia-1	24.7	21.2	25.9	28.2	261.63	262
SAT-1	25.2	20.9	25.1	28.7	261.74	262
SAT-2	25.4	21.0	25.3	28.6	261.70	262
SAT-3	25.3	20.9	24.9	28.9	261.77	262

Table 2
Relationship between 146S particle concentration and 30-fold number of RNA molecules extracted from whole particles of FMDV ($n = 3$, $p < 0.005$)

Таблица 2
Зависимость между концентрацией 146S частиц и 30-кратным количеством молекул РНК, выделенных из полных частиц вируса ящура ($n = 3$, $p < 0{,}005$)

Type of control sample	146S particle concentration, µg/ml	Average number of RNA molecules extracted from FMDV 146S particles in 30-fold eluate		
		theoretical value	based on spectrometric method data	reliability, %
positive standard	0.1	7,457,680,118	7,315,278,733	98.05
	0.2	14,915,360,236	14,349,200,591	96.05
	0.3	22,373,040,353	21,383,122,450	95.37
	0.4	29,830,720,471	28,698,401,183	96.05
	0.5	37,288,400,589	36,013,679,916	96.46
	0.6	44,746,080,707	43,328,958,649	96.73
	0.7	52,203,760,825	49,800,166,759	95.17
	0.8	59,661,440,943	57,115,445,492	95.54
	0.9	67,119,121,060	66,118,865,471	98.49
	1.0	74,576,801,178	73,715,501,078	98.83
	1.1	82,034,481,296	79,061,281,690	96.24
	1.2	89,492,161,414	88,346,058,544	98.70
	1.3	96,949,841,532	95,379,980,402	98.35
	1.4	104,407,521,649	100,163,047,266	95.76
	1.5	111,865,201,767	109,166,467,245	97.53
	1.6	119,322,881,885	114,512,247,858	95.80
	1.7	126,780,562,003	122,952,954,088	96.89
	1.8	134,238,242,121	130,830,946,570	97.40
	1.9	141,695,922,239	137,302,154,679	96.80
	2.0	149,153,602,356	147,712,359,030	99.02
	2.1	156,611,282,474	153,620,853,391	98.05
	2.2	164,068,962,592	158,403,920,255	96.42
	2.3	171,526,642,710	164,875,128,365	95.97
	2.4	178,984,322,828	170,502,265,852	95.03
	2.5	186,442,002,946	180,349,756,454	96.62
	2.6	193,899,683,063	190,197,247,056	98.05
	2.7	201,357,363,181	196,949,812,040	97.76
	2.8	208,815,043,299	203,421,020,150	97.35
	2.9	216,272,723,417	211,580,369,506	97.78
	3.0	223,730,403,535	221,146,503,234	98.83
	3.1	231,188,083,652	224,522,785,726	97.03
	3.2	238,645,763,770	228,743,138,841	95.67
	3.3	246,103,443,888	237,465,201,946	96.36
	3.4	253,561,124,006	245,061,837,553	96.53
	3.5	261,018,804,124	255,190,685,029	97.72
	3.6	268,476,484,242	261,943,250,013	97.51
	3.7	275,934,164,359	265,038,175,631	95.89
	3.8	283,391,844,477	271,790,740,615	95.73
	3.9	290,849,524,595	284,451,799,961	97.75
	4.0	298,307,204,713	287,546,725,579	96.26
negative control	0.0	0	not detected	–

measuring the absorbance of an eluate dilution, TE buffer background values should be automatically subtracted. When calculating 146S RNA concentration in the preparation, conversion factor for FMDV nucleic acid ($F_{FMDV\ RNA} = 41.67$) and dilution factor (*DF*) should be taken into account; besides, background values for samples (OD_{320}) and the negative control ($OD_{260\ C-}$) should be subtracted from OD_{262}. The following should be included when calculating the number of FMDV RNA molecules ($N_{FMDV\ RNA}$): Avogadro's number (6.022045×10^{23} mole^{-1}), the average molecular weight of a ribonucleoside ($Mw_{ribonucleoside} = 340.5$ Da), FMDV RNA length ($L = 8{,}500$ nucleobases) [13]; besides, nucleic acid weight should be converted from micrograms to grams according to the International System of Units (Le Système International d'Unités) [11] (the conversion factor is $1/10^6$), and the number of molecules for 30-fold eluate should be converted into that for 1-fold dilution (the conversion factor is $1/10^{1.48}$) (the total conversion factor is $1/10^{7.48}$).

When FMDV is reproduced in susceptible cell lines, 146S particles (virions) are formed, with each of them comprising one RNA molecule. It should be noted that FMDV suspensions contain RNA as part of virions (98–99%), and also a small amount (1–2%) of RNA in the free state [4, 15]. In other words, when calculating the number of RNA molecules extracted from the whole particles, a coefficient of 0.98 should be included.

The number of RNA molecules extracted from FMDV 146S particles ($N_{RNA\ 146S}$) should be calculated using the following formula taking into account the above mentioned parameters:

$$N_{RNA\ 146S} = 0.98 \times \frac{41.67 \times DF \times (OD_{262} - OD_{320} - OD_{260\ C-}) \times N_A}{10^{7.48} \times Mw_{ribonucleoside} \times L},$$

where *DF* is the dilution factor for the eluate of RNA extracted from FMDV 146S particles;

OD_{262} is the optical density value for the eluate of RNA extracted from FMDV 146S particles at a wavelength of 262 nm;

OD_{320} is the optical density value for the extract of RNA extracted from FMDV 146S particles at a wavelength of 320 nm;

$OD_{260\ C-}$ is the optical density value for the negative control at a wavelength of 260 nm;

N_A is the Avogadro constant (6.022045×10^{23} mole^{-1});

$Mw_{ribonucleoside}$ is the average molecular weight of a ribonucleoside (340.5 Da);

L is FMDV genome length (8,500 nucleobases);

41.67 is the factor for FMDV RNA ($F_{FMDV\ RNA}$);

$1/10^{7.48}$ is the total factor for conversion of weight from micrograms to grams and for conversion of FMDV RNA molecule number for 30-fold eluate into that for 1-fold dilution.

The next stage of the study was to search for a model for FMDV 146S particle concentration determination based on the number of molecules of the extracted viral RNA. To identify the relationship between FMDV 146S particle concentration and the number of viral RNA molecules, serial dilutions of 30-fold standard of RNA extracted from FMDV Asia-1/Tajikistan/2011 strain suspension with whole particle concentration of 4.0 µg/cm³ were made; thus, the standard samples of nucleic acid corresponding to 146S particle concentrations of 0.1 to 4.0 µg/cm³ (in increments of 0.1 µg/cm³) were prepared. Prior to measuring the absorbance of the prepared standard samples, TE buffer background values were automatically subtracted. Spectral analysis of the prepared positive standards was performed, and extinction values were determined at wavelengths of 262 nm and 320 nm. The optical density of the negative control was measured to detect the presence of non-specific RNA molecules that were found to have their absorption maxima at $\lambda = 260$ nm. Spectral analysis of some of the specified standards is presented in Figure 1. The results of measurements and calculations of the number of RNA molecules extracted from 146S particles are presented in Table 2 demonstrating that the $N_{RNA\ 146S}$ values for all dilutions of 30-fold standard with 146S particle concentrations of 0.1 to 4.0 µg/cm³ were between 7,315,278,733 and 287,546,725,579, respectively.

It is known that whole, and free RNA content in the virus suspensions is low [4]; hence, there is a direct relationship FMDV particles comprise one RNA molecule between the number of 146S particles and the number of FMDV RNA molecules quantified in the same volume of non-inactivated suspension after the virus reproduction. It is known that the molecular weight of a whole FMDV virion (Mw_{146S}) is 8,080,000–8,167,500 Da on average [3, 4, 15]. Given that the virus genome length is 8,500 nucleobases [3, 4] and the average molecular weight of a ribonucleside is 340.5 Da, the molecular weight of the viral RNA ($Mw_{FMDV\ RNA}$) is about 2,894,250 Da, and this is on average 2.79–2.82 times less than Mw_{146S}. Put differently, theoretically 146S particle concentration (µg/cm³) in FMDV suspensions is on average 2.79–2.82 times higher than viral RNA concentration (µg/cm³); hence,

$$\frac{m_{146S}}{m_{RNA\ 146S}} = 2.79 \div 2.82, \text{ or } m_{RNA146S} = \frac{m_{146S}}{2{,}79 \div 2{,}82}.$$

This expression is substituted into the equation

$$\frac{m_{146S}}{N_{RNA\ 146S}}$$

instead of $m_{RNA\ 146S}$, taking into account that

$$N_{RNA\ 146S} = \frac{m_{RNA} \times N_A}{10^6 \times L \times Mw_{ribonucleoside}},$$

where m_{RNA} is FMDV RNA weight;

N_A is the Avogadro constant (6.022045×10^{23} mole^{-1});

L is FMDV genome length (8,500 nucleobases);

$Mw_{ribonucleoside}$ is the average molecular weight of a ribonucleoside (340.5 Da);

$1/10^6$ is the factor for weight conversion from micrograms to grams.

By rearrangements, we obtain

$$\frac{N_{RNA\ 146S}}{m_{146S}} = 7.38 \times 10^{10} - 7.46 \times 10^{10}.$$

This means that theoretically the number of RNA_{146S} molecules is on average $7.38 \times 10^{10} - 7.46 \times 10^{10}$ times higher than 146S particle concentration, as was experimentally confirmed by tests of dilutions of standards with known 146S particle concentrations using the proposed method. The test results are presented in Tables 2 and 3.

The data presented in Table 2 show that reliability of quantification of RNA molecules extracted from FMDV suspensions with 146S particle concentrations of 0.1 to 4.0 µg/cm³ was between 95.03 and 99.02%.

Based on the data obtained on the number of viral RNA molecules in the dilutions of the standard

Table 3
Relationship between 146S particle concentration and the number of RNA molecules extracted from whole particles of FMDV Asia-1/Tajikistan/2011 strain ($n = 3$, $p < 0.005$)

Таблица 3
Зависимость между концентрацией 146S частиц и количеством молекул РНК, выделенных из полных частиц вируса ящура штамма Азия-1/Таджикистан/2011 ($n = 3$, $p < 0{,}005$)

146S particle concentration in a standard, µg/ml	Average optical density (OD) values			Calculated number of RNA_{146S} molecules
	standard samples		negative control	
	OD_{262}	OD_{320}*	$OD_{260\,C-}$*	
0.1	0.026 ± 0.001	0.002	0.006	7,315,278,733
0.2	0.051 ± 0.001	0.000	0.006	14,349,200,591
0.3	0.076 ± 0.001	0.001	0.006	21,383,122,450
0.4	0.102 ± 0.001	0.002	0.006	28,698,401,183
0.5	0.128 ± 0.001	0.001	0.006	36,013,679,916
0.6	0.154	0.002	0.006	43,328,958,649
0.7	0.177 ± 0.001	0.001	0.006	49,800,166,759
0.8	0.203	0.003	0.006	57,115,445,492
0.9	0.235 ± 0.001	0.002	0.006	66,118,865,471
1.0	0.262 ± 0.001	0.003	0.006	73,715,501,078
1.1	0.281 ± 0.001	0.004	0.006	79,061,281,690
1.2	0.314 ± 0.001	0.003	0.006	88,346,058,544
1.3	0.339 ± 0.001	0.003	0.006	95,379,980,402
1.4	0.356 ± 0.001	0.005	0.006	100,163,047,266
1.5	0.388	0.004	0.006	109,166,467,245
1.6	0.407	0.005	0.006	114,512,247,858
1.7	0.437 ± 0.001	0.006	0.006	122,952,954,088
1.8	0.465 ± 0.001	0.006	0.006	130,830,946,570
1.9	0.488 ± 0.001	0.004	0.006	137,302,154,679
2.0	0.525 ± 0.001	0.007	0.006	147,712,359,030
2.1	0.546 ± 0.001	0.005	0.006	153,620,853,391
2.2	0.563	0.005	0.006	158,403,920,255
2.3	0.586 ± 0.001	0.007	0.006	164,875,128,365
2.4	0.606 ± 0.001	0.005	0.006	170,502,265,852
2.5	0.641 ± 0.001	0.008	0.006	180,349,756,454
2.6	0.676 ± 0.001	0.006	0.006	190,197,247,056
2.7	0.700	0.002	0.006	196,949,812,040
2.8	0.723 ± 0.001	0.002	0.006	203,421,020,150
2.9	0.752 ± 0.002	0.003	0.006	211,580,369,506
3.0	0.786 ± 0.001	0.004	0.006	221,146,503,234
3.1	0.798 ± 0.002	0.001	0.006	224,522,785,726
3.2	0.813 ± 0.001	0.005	0.006	228,743,138,841
3.3	0.844 ± 0.001	0.001	0.006	237,465,201,946
3.4	0.871 ± 0.002	0.003	0.006	245,061,837,553
3.5	0.907 ± 0.001	0.001	0.006	255,190,685,029
3.6	0.931 ± 0.002	0.002	0.006	261,943,250,013
3.7	0.942 ± 0.001	0.002	0.006	265,038,175,631
3.8	0.966 ± 0.001	0.002	0.006	271,790,740,615
3.9	1.011 ± 0.002	0.003	0.006	284,451,799,961
4.0	1.024 ± 0.002	0.002	0.006	287,546,725,579

OD_{262} – extinction value at 262 nm; OD_{320} – extinction value at 320 nm;
$OD_{260\,C-}$ – extinction value for the negative control at 260 nm;
* values remained unchanged when measured.

OD_{262} – значение экстинкции при 262 нм; OD_{320} – значение экстинкции при 320 нм;
$OD_{260\,К-}$ – значение экстинкции для отрицательного контроля при 260 нм;
* значения при измерениях не менялись.

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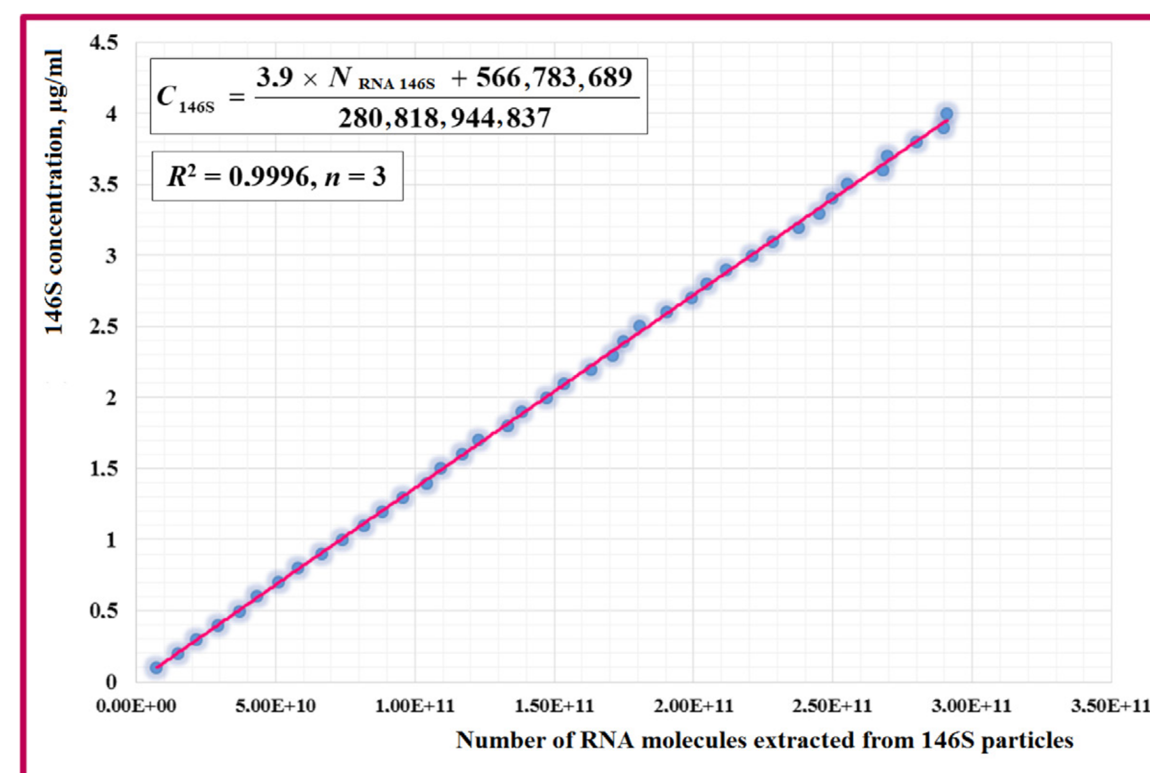

Fig. 2. Relationship between FMDV 146S particle concentration and the number of viral RNA molecules in standard solutions. The number of molecules is expressed in exponential notation (E = 10)

Рис. 2. Зависимость концентрации 146S частиц вируса ящура от количества молекул вирусной РНК в стандартных растворах. Количество молекул выражено в экспоненциальном формате (E = 10)

and corresponding 146S particle concentrations, a calibration graph of the relationship between C_{146S} and $N_{RNA\ 146S}$ was plotted represented as the model

$$C_{146S} = \frac{3.9 \times N_{RNA\ 146S} + 566{,}783{,}689}{280{,}818{,}944{,}837}$$

with the coefficient of determination R^2 of 0.9996 (Fig. 2). No FMDV was detected in the negative control.

The obtained model based on the number of FMDV RNA molecules extracted from 146S particles after immune capture of virions allows for determination of whole particle concentration in the non-inactivated culture virus suspensions.

During the final stage of the study, the presented method for 146S particle concentration determination was tested using 360 non-inactivated suspensions of the following FMDV vaccine strains: A/Turkey/2006 (40 samples), A/ARRIAH/2015 (40 samples), O/Primorsky/2012 (40 samples), O/Primorsky/2014 (40 samples), Asia-1/Tajikistan/2011 (40 samples), C_1/Zakarpatsky/1972 (40 samples), SAT-1/Akhalkalaksky/62 (40 samples), SAT-2/Saudi Arabia 7/2000 (40 samples), SAT-3/Bechuanaland 1/65 (40 samples). The samples were parallely tested with rtRT-PCR and CFT in triplicate (Table 4).

The compatibility of actual and expected (based on rtRT-PCR and CFT data) results of culture FMDV 146S component concentration determination was 96.2–99.4% for A/Turkey/2006 strain, 94.9–99.3% for A/ARRIAH/2015, 97.2–99.2% for O/Primorsky/2012, 97.0–99.3% for O/Primorsky/2014, 97.6–99.5% for Asia-1/Tajikistan/2011, 95.2–97.7% for C_1/Zakarpatsky/1972, 94.8–97.3% for SAT-1/Akhalkalaksky/62, 96.5–99.1% for SAT-2/Saudi Arabia 7/2000, 94.5–98.5% for SAT-3/Bechuanaland 1/65. Tests of the positive control demonstrated 99.0–99.6% compatibility of actual and expected results. FMDV genome and 146S particles were not detected in the negative control, and that was in line with expectations.

Thus, the spectrometric method for determination of culture FMDV 146S component concentration showed 94.5–99.5% correlation with rtRT-PCR and CFT based on the test results.

CONCLUSION

A method for spectrometric determination of 146S particle concentration during quantification of FMDV RNA extracted after immune capture is proposed. This is an inexpensive, easy-to-perform method for determination of FMDV 146S particle concentration in the non-inactivated vaccine raw material with high ballast component content.

146S particle concentration was found to depend on the number of RNA molecules extracted from virions after their strain-specific immune capture and quantitatively detected using the spectrometric method. The presented spectrometric method allows for FMDV 146S particle concentration determination in the non-inactivated vaccine raw material using the following linear model

$$C_{146S} = \frac{3.9 \times N_{RNA\ 146S} + 566{,}783{,}689}{280{,}818{,}944{,}837}.$$

The proposed method showed 94.5–99.5% correlation with rtRT-PCR and CFT. Tests of the positive control

Table 4
Compatibility of actual and expected results of 146S particle concentration determination by spectrometric method with rtRT-PCR and CFT ($n_{tests} = 3$)

Таблица 4
Степень совпадения фактических и ожидаемых результатов спектрометрического способа определения концентрации 146S частиц с ОТ-ПЦР-РВ и РСК ($n_{иссл.} = 3$)

Sample status	FMDV strain	Number of samples	Compatibility of spectrometric method results with other methods, %	
			rtRT-PCR [7]	CFT
test samples	A/Turkey/2006	40	97.0–99.4	96.2–97.1
	A/ARRIAH/2015	40	96.8–99.3	94.9–97.0
	O/Primorsky/2012	40	98.3–99.2	97.2–98.5
	O/Primorsky/2014	40	98.0–99.3	97.0–98.1
	Asia-1/Tajikistan/2011	40	98.4–99.5	97.6–98.5
	C_1/Zakarpatsky/1972	40	97.0–97.7	95.2–97.1
	SAT-1/Akhalkalaksky/62	40	96.1–97.3	94.8–96.0
	SAT-2/Saudi Arabia 7/2000	40	98.0–99.1	96.5–98.2
	SAT-3/Bechuanaland 1/65	40	97.9–98.5	94.5–97.9
positive control	Asia-1/Tajikistan/2011	40	99.2–99.6	99.0–99.3
negative control	–	40	100	100

demonstrated 99.0–99.6% compatibility of actual and expected results. FMDV genome and 146S particles were not detected in the negative control, and that was in line with expectations.

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Investigation of healing effects of Afyonkarahisar Region thermal spring water on experimentally-induced nephritis in mice

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SUMMARY

The aim of this study was to determine the efficacy of Afyokarahisar termomineral water in the treatment of nephritis. For this purpose, 40 Albino mouse at the same daily age were used. Nephritis was induced by adding adenine to their feed at a rate of 0.2% for 6 weeks. After nephritis was induced, a 21-day treatment period was started, and the mice were equally divided into two groups as control and study. While control group mice were received tap water daily and bathing in tap water, study group animals were given fresh Süreyya I hot spring water daily and bathing in this water. Clinical, hematological, blood biochemical and histopathological examinations were performed before the study, after nephritis formation, and on days of 1st, 7th, 14th and 21st of treatment period. Results of this study showed that WBC, NEUT and MCV levels increased significantly ($p < 0.05$) following nephritis formation, while RBC, HB, HCT, LYM, MCH and MCHC levels decreased significantly ($p < 0.05$). It was also determined that AST, GGT, GLU, BUN and IgG levels of blood biochemical parameters were significantly increased ($p < 0.05$) and TP, ALB levels decreased significantly ($p < 0.05$) after nephritis formation. At the end of the study, it was seen that all the measured parameters turned to the normal range in the study group animals, whereas problems still continuing with control group animals. Consequently, it was concluded that Süreyya I hot spring water was very successful in the treatment of nephritis and considered as an option in the treatment of nephritis.

Key words: Afyonkarahisar, balneotherapy, biochemistry, mouse, nephritis, treatment.

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Исследование лечебного действия термальной воды региона Афьонкарахисар на экспериментально индуцированный нефрит у мышей

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