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Using spectrometric analysis for indirect estimation of 146S component concentration while measuring FMDV RNA amount

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SUMMARY
Foot and mouth disease has a negative impact on economy due to the high cost of eradication campaigns and stringent measures imposed on domestic and international trade in animal products. Prevention and control measures include mass vaccination of susceptible animals and control of post-vaccination immunity level. Concentration of 146S particles, which are the main component affecting the vaccine immunogenicity, is determined during commercial scale production of FMD vaccines. The paper assesses feasibility of spectrometric analysis for indirect determination of 146S component concentration while measuring amount of FMDV RNA isolated after serological binding. This method is cheap, easy-to-use and makes it possible to determine indirectly concentration of FMDV 146S particles in inactivated vaccine raw materials within 3–4 hours. Study of cultural FMDV suspensions shows that the linear model $C_{146S} = (3.9 \times N_{RNA\ 146S} + 566{,}783{,}689)/280{,}818{,}944{,}837$ makes it possible to estimate FMDV 146S component concentration in the vaccine raw materials with the help of a spectrometric analysis. The actual results obtained in real-time reverse transcription – polymerase chain reaction (rtRT-PCR) were 97.0–99.9% consistent with the expected results of the spectrometric analysis used to determine cultural FMDV 146S component concentration. When compared to a complement fixation test, the actual results were 94.5–99.5% in line with the expected ones. The actual results for positive control were 99.0–99.6% in line with the expected ones. As expected, no FMDV genome or 146S particles were detected in the negative control sample.

Keywords: Foot and mouth disease virus (FMDV) RNA, 146S component concentration, spectrometric analysis.

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Применение спектрометрического способа опосредованной оценки концентрации 146S компонента при определении количества РНК вируса ящура

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РЕЗЮМЕ
Ящур причиняет серьезный экономический ущерб, который выражается в существенных затратах на ликвидацию болезни, введение строгих ограничений, налагаемых на внутреннюю и международную торговлю продукцией животноводства. Комплекс мер для борьбы и профилактики заболевания

предполагает массовую иммунизацию восприимчивых животных, а также контроль уровня напряженности поствакцинального иммунитета. При промышленном изготовлении противоящурных вакцинных препаратов определяют концентрацию 146S частиц, которые являются основным компонентом, влияющим на иммуногенную активность вакцины. В статье представлены результаты оценки возможности применения спектрометрического способа для опосредованного определения концентрации 146S компонента при определении количества РНК вируса ящура, выделенной после серологического связывания. Данный способ является дешевым, простым в исполнении, позволяет опосредованно определять концентрацию 146S частиц вируса ящура в неинактивированном сырье для вакцины в течение 3–4 ч. При исследовании суспензий культурального вируса ящура доказано, что линейная модель вида $C_{146S} = (3{,}9 \times N_{РНК\ 146S} + 566\,783\,689)/280\,818\,944\,837$ с помощью спектрального исследования позволяет оценивать концентрацию 146S компонента вируса ящура в сырье для вакцины. Совпадение фактических результатов полимеразной цепной реакции с обратной транскрипцией в режиме реального времени и ожидаемых результатов по определению концентрации 146S компонента культурального вируса ящура спектрометрическим способом составило 97,0–99,9%. При сравнении с данными, полученными в реакции связывания комплемента, совпадение фактических и ожидаемых результатов соответствовало значениям 94,5–99,5%. Для положительного контроля совпадение фактических и ожидаемых результатов составило 99,0–99,6%. В отрицательном контрольном образце геном и 146S частицы вируса ящура не обнаружены, что также соответствовало ожиданиям.

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

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INTRODUCTION

Foot and mouth disease is a highly infectious viral disease primarily affecting wild and domestic cloven-hooved [1]. FMD virus is characterized by high antigenic variability due to changes in surface protein genes; there are 7 serotypes: A, O, C, Asia-1, SAT-1, SAT-2, SAT-3, including many subtypes [2].

The genome is represented by a single-stranded, positive-sense RNA of 8,500 bp. The virion molecular weight ranges between 8,080,000 and 8,167,500 Da [2, 3]. Following infection of biological systems, FMDV forms four types of particles with different sedimentation coefficients: 146S component (whole virus particles) consisting of one viral RNA molecule and 60 copies of VP_1-VP_2-VP_3-VP_4 polypeptide;75S component lacking RNA and including 60 copies of VP_1-VP_3-VP_0 polypeptide; 12S particles represented by proteins VP_1, VP_2, VP_3; 3.8S subunits consisting of functional protein VP_g [4].

FMD causes huge economic losses associated with the costs of the disease eradication and stringent measures imposed on domestic and international trade in animal products. Complex measures for FMD prevention and control include stamping-out, mass immunization of susceptible animals as well as control of postvaccinal immunity level [1, 5].

Concentration of 146S component, which directly affects the vaccine immunogenicity, is determined during commercial scale production of FMD vaccines [2]. A quantitative complement fixation test (CFT) is traditionally used for this purpose; afterwards the results are assessed pursuant to the methodical instructions [6]. However, the test has a number of disadvantages: it is labour-intensive and time-consuming (up to 3 days); it is impossible to simultaneously test a great number of samples, despite it is required by the production process; a high cost of the procedure. A real-time reverse transcription – polymerase chain reaction (rtRT-PCR) has been used in recent years to determine indirectly concentration of FMDV 146S component in non-inactivated suspension [7]. The represented method is highly sensitive, specific and cost-effective; is rapidly performed, provides rapid results and enables to simultaneously test dozens of samples of non-inactivated virus-containing material. However, rtRT-PCR requires expensive chemicals and equipment.

A spectrometric analysis has been developed to indirectly determine concentration of FMDV 146S component in non-inactivated suspensions while measuring amount of the viral RNA isolated after serological binding of the whole particles; with linear model $C_{146S} = (3{,}9 \times N_{RNA\ 146S} + 566{,}783{,}689)/280{,}818{,}944{,}837$ used. The proposed method is cheap, easy-to-use and makes it possible to determine concentration of FMDV 146S particles in the non-inactivated raw materials for vaccine within 3–4 hours [8].

The purpose of the research is to assess the feasibility of the spectrometric analysis for indirect determination of 146S component concentration while measuring amount of FMDV RNA in suspensions.

MATERIALS AND METHODS

Virus. We used in the research non-inactivated cultural FMDV suspensions of the following vaccine production strains: A/Turkey/2006, A/ARRIAH/2015, O/Primorsky/2012, O/Primorsky/2014, Asia-1/Tajikistan/2011, SAT-2/ Saudi Arabia 7/2000. The virus was cultivated in suspension continuous baby hamster kidney cell line (BHK-21/SUSP/ARRIAH) at a concentration of $(3.0–3.5) \times 10^6$ million cells/cm^3.

Plate immobilization and serological binding of FMDV with strain-specific immunoglobulin G (IgG) were carried out in accordance with the requirements provided earlier [8].

RNA extraction from the whole FMDV particles. RNA elution from the FMDV 146S component bound with strain-specific IgG was carried out in accordance with P. Chomczynski's method [9, 10]. As a result, 30-fold viral RNA extracts were obtained (0.1 cm^3 each). These eluates were either immediately used in further activities or were frozen at the temperature of –20 °C.

Using spectrum analysis to measure purity of FMDV RNA eluate. The spectral absorption capacity of RNA extracts was measured in the UV spectrum at wavelength from 205 to 325 nm. The residues of phospholipids, polysaccharides and guanidine isothiocyanate, phenol, polypeptides and large conglomerates were measured by the optical density (OD) values at wavelength of 205, 235, 270, 280 and 320 nm, respectively [11]. The RNA eluate was considered free from protein and carbolic acid impurities if the R_1 extinction coefficient (OD_{262}/OD_{280}) was within the range of 1.8–2.2 and was tended to 2.0. The exceeded value suggested nucleic acid degradation into oligonucleotides and free nucleotides. FMDV RNA extract was considered free from carbohydrates, if R_2 (OD_{262}/OD_{235}) extinction coefficient ranged between 2.00–2.02. If 1% RNA is substituted with polysaccharide components, the R_2 coefficient decreases by 0.002. If $R_2 > 2.02$, dissociation of nucleic acid is observed and free nucleotides or oligonucleotides in the tested sample eluate are reported. The absence of large suspended particles in the FMDV RNA extract was confirmed, if OD_{320} tended to zero [12].

Estimating the number of FMDV RNA molecules. The number of RNA molecules extracted from the whole FMDV particles ($N_{RNA\ 146S}$) was estimated using the following formula:

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

where K – dilution factor of the RNA eluate extracted from the whole FMDV particles;

OD_{262} – optical density of the RNA extract isolated from FMDV 146S component at wavelength of 262 nm;

OD_{320} – optical density of the RNA extract isolated from FMDV 146S component at wavelength of 320 nm;

$OD_{260\,C-}$ – optical density of the negative control at wavelength of 260 nm;

N_A – Avogadro's constant (in the SI system (SI), according to redefinition of the SI base units exactly equals to $6.02214076 \times 10^{23}$ mol^{-1});

$Mw_{ribonucleoside}$ – ribonucleoside molecular-weight average (340.5 Da);

L – length of FMDV RNA (about 8,500 bp, pursuant to the data provided by the National Center for Biotechnology Information);

41.67 – FMDV RNA factor ($F_{FMDV\ RNA}$);

$1/10^{7.48}$ – cumulative coefficient for mass conversion from [µg] to [g] and recalculation of FMDV RNA molecule number from the 30-fold eluate into one-fold extract [8].

Determination of 146S particle concentration using the number of FMDV RNA molecules. Based on the data obtained on the number of viral RNA molecules ($N_{RNA\ 146S}$), concentration of FMDV 146S particles (C_{146S}) was determined with the help of the math expression given below: $C_{146S} = (3.9 \times N_{RNA\ 146S} + 566{,}783{,}689)/280{,}818{,}944{,}837$ [8].

In accordance with the relevant requirements, a qualitative CFT was simultaneously carried out to determined concentration of the whole FMDV particles (in µg/cm^3) [6].

Real-time reverse transcription – polymerase chain reaction (rtRT-PCR). FMDV RNA in non-inactivated suspension used for vaccine production was quantified in rtRT-PCR, in accordance with the relevant requirements. Amplification cycle threshold was determined in the test, thus making it possible to estimate indirectly the amount of the virus nucleic acid; and the data obtained were interpreted to determine concentration of FMDV 146S particles [7].

Control samples. Non-inactivated suspension of Asia-1/Tadjikistan/2011 FMDV strain with 146S component at a concentration of 3.7 µg/cm^3 was used as a positive control cultivated in suspension continuous baby hamster kidney cell line (BHK-21/SUSP/ARRIAH). Suspension of BHK-21/SUSP/ARRIAH cell culture at a concentration of $(3.0–3.5) \times 10^6$ million cells/cm^3 was used as a negative control.

Statistical data processing included calculation of arithmetical means, confidence level for statistical difference between mean values determined with differential Student's – Fischer test; calculation of standard deviations in concentration of RNA molecule numbers and the number of the whole FMDV particles. The data were processed and diagrams were drawn using StatSoft (version 6.0) and Microsoft Excel 2010 software application package.

RESULTS AND DISCUSSION

A spectrometric analysis was checked in this research as a way to determine indirectly concentration of 146S particles while FMDV RNA amount is estimated in the strains indicated in "Materials and Methods". Each test included the use of positive and negative control samples indicated above.

At the first stage, concentration of FMDV 146S component of Asia-1/Tajikistan/2011 strain (positive control) was determined in spectrometric analysis while estimating amount of the viral RNA isolated after serological binding with the help of IgG; and in quantitative CFT and rtRT-PCR variants. Immunobinding of the whole virus particles from non-inactivated virus suspension was carried out as well as extraction of RNA from FMDV 146S component bound by strain-specific antibodies. Summary data on purity of the obtained eluates for experimental and control samples are given in the table below.

The spectrum analysis of the 30-fold FMDV RNA eluate of Asia-1/Tajikistan /2011 strain demonstrates that the mean extinction values at wavelengths of 205–259 nm ($OD_{205-259}$) and 263–325 nm ($OD_{263-325}$) did not exceed $OD_{260-262}$ (0.001–0.948 < 0.951–0.952 and 0.947–0.002 < 0.951–0.952), that means the preparation contained mainly ribonucleic acid. The eluate did not contain phospholipids, polysaccharides and guanidine isothiocyanate residues, carbolic acid, proteins and large conglomerates, as there were no marked peaks in the graphs at wavelengths of 205, 235, 270, 280 and 320 nm, correspondingly. The R_1 extinction coefficient was 1.996 (OD_{262}/OD_{280} = 0.952/0.477) which is close to norm of 2.000 and demonstrates high purity of the eluate as well as almost total absence of polypeptide components and residues of carbolic acid after RNA extraction. The R_2 extinction coefficient was 2.000 (OD_{262}/OD_{235} = = 0.952/0.476) which corresponded to norm and accounted for high purity of the control preparation, thus, increasing reliability of the carried out test [12].

The spectrometric analysis of FMDV RNA demonstrated the following mean optical density values:

OD_{262} = 0.952 ± 0.001, OD_{320} = 0.002, OD_{260C-} = 0.006 (for OD_{320} and OD_{260}). Arithmetic mean of the RNA molecules (number of structural particles) isolated from FMDV 146S component of Asia-1/Tajikistan/2011 strain was 265,600,889,380 ± 331,586,628. Using the developed linear bond model C_{146S} and $N_{RNA\ 146S}$ [8], concentration of the analyte was indirectly determined in the positive control (3.691 ± 0.004 μg/cm³) and it had 99.43% and 99.84% correlation with the results of quantitative CFT (3.710 ± 0.170 μg/cm³) and rtRT-PCR (3.700 ± 0.030 μg/cm³), correspondingly. Thus, the positive control met the requirements for purity and for the declared amount of the 146S component. None of the used methods detected RNA or FMDV 146S particles in the negative control.

Concentration of FMDV 146S component was simultaneously determined in experimental samples of the following strains: A/Turkey/2006, A/ARRIAH/2015, O/Primorsky/2012, O/Primorsky/2014, Asia-1/Tajikistan/2011, SAT-2/Saudi Arabia 7/2000 with the help of a spectrometric analysis while estimating the amount of FMDV RNA eluated after serological binding and with the help of quantitative CFT and rtRT-PCR. The test stages were carried out in accordance with procedures described above. The figure and the table provide results of the purity estimation for the RNA eluates of the abovementioned FMDV strains.

The spectrum analysis of the 30-fold FMDV RNA eluate of A/Turkey/2006 strain demonstrates that mean extinction values at wavelengths of 205–259 and 263–325 nm did not exceed $OD_{260\text{-}262}$ (0.001–0.482 < 0.483–0.488 and 0.487–0.002 < 0.483–0.488), which means the preparation contained mainly RNA molecules. The eluate was not contaminated with phospholipids, polysaccharides or guanidine isothiocyanate residues, phenol, protein components or large conglomerates, as there were no marked peaks in the graphs at wavelengths of 205, 235, 270, 280 and 320 nm, correspondingly. The R_1 extinction coefficient was 1.984 (OD_{262}/OD_{280} = 0.488/0.246) which is close to norm of 2.000 and meant almost total absence of polypeptide components and residues of carbolic acid after RNA extraction. The R_2 extinction coefficient was 1.992 (OD_{262}/OD_{235} = 0.488/0.245) which is close to 2.000 and accounts for high purity of the eluate. When 1% RNA was substituted with polysaccharides, the R_2 coefficient decreased by 0.002, i.e. polysaccharide level in the obtained extract did not exceed 4% and it is admissible (not more than 10%) [12].

The spectrum analysis of the FMDV RNA extract of A/Turkey/2006 strain demonstrated the following mean values of optical density: OD_{262} = 0.488 ± 0.001, OD_{320} = 0.002, OD_{260C-} = 0.006 (for OD_{320} and OD_{260}). Arithmetic mean of the structural particles (RNA) isolated from the whole FMDV particles was 135,051,299,685 ± 281,356,875. Using the developed linear bond model C_{146S} and $N_{RNA\ 146S}$ [8] concentration of the 146S component was calculated and it was 1.878 ± 0.004 μg/cm³ and it had 98.84% and 99.89% correlation with the results of quantitative CFT (1.900 ± 0.180 μg/cm³) and rtRT-PCR (1.895 ± 0.032 μg/cm³), correspondingly. Thus, the previously developed linear model makes it possible (with the help of a spectrum analysis) to determined concentration of FMDV 146S component in the non-inactivated suspension for vaccine production.

The spectrum analysis of the 30-fold FMDV RNA eluate of A/ARRIAH/2015 strain demonstrated that mean extinction values at wavelengths of 205–259 and 263–325 nm did not exceed $OD_{260\text{-}262}$ (0.001–0.403 < 0.404–0.407 and 0.403–0.001 < 0.404–0.407), which means that the obtained preparation contained mainly ribonucleic acid. The extract did not contain phospholipids, polysaccharides or guanidine isothiocyanate residues, phenol, polypeptides or large suspended particles, as there were no marked peaks in the graphs at wavelengths of 205, 235, 270, 280 and 320 nm, correspondingly. The R_1 extinction coefficient was 1.995 (OD_{262}/OD_{235} = 0.407/0.204) which is close to norm of 2.000 and meant almost total absence of protein and carbolic acid impurities. The R_2 extinction coefficient was 1.995 (OD_{262}/OD_{280} = 0.407/0.204) which is close to 2.000 and accounts for high purity of the extract. When 1% RNA was substituted with polysaccharide components, the R_2 coefficient decreased by 0.002, i.e. polysaccharide components in the obtained preparation did not exceed 2.5% and it is admissible [12].

The spectrum analysis of the FMDV RNA eluate of A/ARRIAH/2015 strain demonstrated the following mean values of optical density: OD_{262} = 0.407 ± 0.001, OD_{320} = 0.003, OD_{260C-} = 0.006 (for OD_{320} and OD_{260}). Arithmetic mean of the RNA molecules eluated from FMDV 146S particles after strain-specific serological binding resulting from neutralization test was 111,698,679,114 ± 281,356,870. Concentration of FMDV 146S component in the obtained sample was 1.553 ± 0.004 μg/cm³ and it had 98.29% and 99.94% correlation with the results of quantitative CFT (1.58 ± 0.17 μg/cm³) and rtRT-PCR (1.555 ± 0.041 μg/cm³), correspondingly. Thus, the above-mentioned linear mathematical function makes it possible (with the help of a spectrum analysis) to determined concentration of the whole FMDV particles in the non-inactivated suspension for production of a large range of vaccines [8].

The spectrum analysis of the FMDV RNA eluate of A/Primorsky/2014 strain demonstrated that mean values of $OD_{205\text{-}259}$ and $OD_{263\text{-}325}$ did not exceed $OD_{260\text{-}262}$ (0.001–0.311 < 0.312–0.314 and 0.311–0.001 < 0.312–0.314), which means that the obtained extract contained mainly ribonucleic acid molecules. The eluate was not contaminated with phospholipids, polysaccharides or guanidine isothiocyanate residues, carbolic acid, polypeptides or large aggregated particles, as there were no marked peaks in the graphs at the wavelengths of 205, 235, 270, 280 and 320 nm, respectively. The R_1 extinction coefficient was 1.987 (OD_{262}/OD_{280} = 0.314/0.158) which is close to norm of 2.000 and means almost total absence of peptides and residues of carbolic acid in the extracted RNA. The R_2 extinction coefficient was 2.000 (OD_{262}/OD_{235} = 0.314/0.157) which is close to norm and accounts for the absence of polysacciride impurities and signs of viral RNA degradation [12].

The spectrum analysis of the FMDV RNA eluate of A/Primorsky/2014 strain demonstrated the following mean values of optical density: OD_{262} = 0.314 ± 0.002, OD_{320} = 0.002, OD_{260C-} = 0.006 (for OD_{320} and OD_{260}). The average number of RNA structural particles in the extract was 86,095,203,549 ± 562,713,749, concentration of viral 146S component was 1.198 ± 0.008 μg/cm³ which had 99.01% and 99.50% correlation with the results of quantitative CFT (1.21 ± 0.15 μg/cm³) and rtRT-PCR (1.999 ± 0.045 μg/cm³), correspondingly. Thus, the earlier developed linear mathematical model makes it possible (with the help of a spectrum analysis) to determined concentration of FMDV 146S component in the non-inactivated raw materials for production of FMD vaccines [8].

Table
Correlation between results of indirect 146S concentration determination in the tested samples based on the number of FMDV RNA molecules and correlation with CFT and rtRT-PCR data ($n = 3, p < 0.005$)

Таблица
Соотношение результатов опосредованного определения концентрации 146S частиц исследуемых образцов по количеству молекул РНК вируса ящура и корреляции с данными РСК и ОТ-ПЦР-РВ ($n = 3, p < 0,005$)

FMDV strain	Spectrum analysis of RNA eluate						$OD_{260C\text{-}mean}$	$N_{RNA\ 146Smean}$	$C_{146Smean}$, μg/cm³		
	$OD_{262mean}$	$OD_{235mean}$	$OD_{280mean}$	$OD_{320mean}$	R_1	R_2			Spectrum analysis*	CFT [6]	rtRT-PCR [7]
Asia-1/Tajikistan/2011	0.810 ± 0.001	0.407 ± 0.001	0.406 ± 0.001	0.003 ± 0.000	1.995	2.000	0.006 ± 0.000	225,366,856,349 ± 281,356,874	3.132 ± 0.004	3.150 ± 0.170	3.137 ± 0.040
A/Turkey/2006	0.488 ± 0.001	0.245 ± 0.002	0.246 ± 0.001	0.002 ± 0.000	1.984	1.992	0.006 ± 0.000	135,051,299,685 ± 281,356,871	1.878 ± 0.004	1.900 ± 0.180	1.876 ± 0.040
O/Primorsky/2014	0.314 ± 0.002	0.157 ± 0.001	0.158 ± 0.001	0.002 ± 0.000	1.987	2.000	0.006 ± 0.000	86,095,203,549 ± 562,713,749	1.198 ± 0.008	1.210 ± 0.150	1.204 ± 0.050
A/ARRIAH/2015	0.407 ± 0.001	0.204 ± 0.001	0.204 ± 0.001	0.003 ± 0.000	1.995	1.995	0.006 ± 0.000	111,698,679,114 ± 281,356,870	1.553 ± 0.004	1.580 ± 0.170	1.554 ± 0.041
O/Primorsky/2012	0.339 ± 0.002	0.170 ± 0.001	0.171 ± 0.001	0.001 ± 0.000	1.982	1.994	0.006 ± 0.000	93,410,482,282 ± 562,713,747	1.299 ± 0.008	1.330 ± 0.160	1.314 ± 0.050
SAT-2/Saudi Arabia 7/2000	0.203 ± 0.002	0.102 ± 0.001	0.103 ± 0.001	0.001 ± 0.000	1.971	1.990	0.006 ± 0.000	54,864,590,497 ± 562,713,752	0.764 ± 0.008	0.750 ± 0.140	0.768 ± 0.040
Asia-1/Tajikistan/2011 (positive control)	0.952 ± 0.001	0.476 ± 0.001	0.477 ± 0.001	0.002 ± 0.000	1.996	2.000	0.006 ± 0.000	265,600,889,380 ± 331,586,628	3.691 ± 0.004	3.710 ± 0.170	3.700 ± 0.030

$OD_{262mean}$ – mean extinction for 262 nm; $OD_{235mean}$ – mean extinction for 235 nm; $OD_{280mean}$ – mean extinction for 280 nm;
$OD_{320mean}$ – mean extinction for 320 nm; $OD_{260C\text{-}mean}$ – mean extinction of negative control for 260 nm;
R_1 – extinction coefficient (OD_{262}/OD_{280}), calculated based on OD mean values at the wavelengths of 262 and 280 nm;
R_2 – extinction coefficient (OD_{262}/OD_{235}), calculated based on OD mean values at the wavelengths of 262 and 235 nm;
$N_{RNA\ 146Smean}$ – average number of RNA molecules, isolated from FMDV 146S particles; $C_{146Smean}$ – mean concentration of FMDV 146S particles;
* The proposed spectrometric method to indirectly determine concentration of the whole FMDV particles [8].

$OD_{262ср.}$ – среднее значение экстинкции при 262 нм; $OD_{235ср.}$ – среднее значение экстинкции при 235 нм; $OD_{280ср.}$ – среднее значение экстинкции при 280 нм;
$OD_{320ср.}$ – среднее значение экстинкции при 320 нм; $OD_{260К\text{-}ср.}$ – среднее значение экстинкции отрицательного контроля при 260 нм;
R_1 – коэффициент экстинкции (OD_{262}/OD_{280}), рассчитанный исходя из средних значений OD при длинах волны 262 и 280 нм;
R_2 – коэффициент экстинкции (OD_{262}/OD_{235}), рассчитанный исходя из средних значений OD при длинах волны 262 и 235 нм;
$N_{РНК\ 146Sср.}$ – среднее количество молекул РНК, выделенных из 146S частиц вируса ящура; $C_{146Sср.}$ – среднее значение концентрации 146S частиц вируса ящура;
* Предложенный спектрометрический способ опосредованного определения концентрации полных частиц вируса ящура [8].

The spectrum analysis used for indirect determination of 146S particles concentration when number of FMDV RNA molecules was calculated in A/Primorsky/2012 strain demonstrated that mean values of $OD_{205-259}$ and $OD_{263-325}$ did not exceed $OD_{260-262}$ (0.002–0.335 < 0.336–0.339 and 0.334–0.001 < 0.336–0.339) which means the extract contained mainly RNA molecules. The eluate was not contaminated with phospholipids, carbohydrate components or guanidine isothiocyanate residues, hydroxybenzene, proteins or conglomerates, as there were no marked peaks in the graphs at wavelengths of 205, 235, 270, 280 and 320 nm, correspondingly. The R_1 extinction coefficient was 1.982 (OD_{262}/OD_{280} = 0.339/0.171) which is close to norm of 2.000 and means almost total absence of polypeptides, peptides or residues of carbolic acid in the analyzed extract. The R_2 extinction coefficient was 1.994 (OD_{262}/OD_{235} = 0.339/0.170) which is close to norm of 2.000. The data obtained explain the absence of signs of viral RNA degradation and the amount of polysacciride impurities not exceeding 3% (which is admissible) [12].

Based on the results of the spectrum analysis of the RNA eluate, the following mean values of optical density were obtained: OD_{262} = 0.339 ± 0.002, OD_{320} = 0.001, OD_{260C-} = 0.006 (for OD_{320} and OD_{260}). The average number of RNA molecules in the sample was 93,410,482,282 ± 562,713,747, and concentration of the whole FMDV particles in A/Primorsky/2012 strain was 1.299 ± 0.008 μg/cm³ which had 97.67% and 98.66% correlation with the results of quantitative CFT (1.33 ± 0.16 μg/cm³) and rtRT-PCR (1.302 ± 0.052 μg/cm³), correspondingly. Thus, the mathematical expression used in the research (with the help of a spectrum analysis) makes it possible to determined concentration of FMDV component with 146S sedimentation coefficient in the non-inactivated suspension for FMD vaccines [8].

The spectrum analysis of the 30-fold FMDV RNA eluate of Asia-1/Tajikistan /2011 strain demonstrates that the mean $OD_{205-259}$ and $OD_{263-325}$ values did not exceed $OD_{260-262}$ (0.001–0.801 < 0.802–0.810 and 0.801–0.003 < < 0.802–0.810) that means the eluate contained mainly ribonucleic acid. The extract did not contain phospholipids, carbohydrates or residues of guanidine isothiocyanate detergent, hydroxybenzene, polypeptides or suspended conglomerates, as there were no marked peaks in the graphs at wavelengths of 205, 235, 270, 280 and 320 nm, correspondingly. The R_1 extinction coefficient was 1.995 (OD_{262}/OD_{280} = 0.810/0.406) which is close to norm of 2.000 and means high purity of the RNA eluate and almost total absence of polypeptide components or residues of carbolic acid. The R_2 extinction coefficient was 1.990 (OD_{262}/OD_{235} = 0.810/0.407) which is also close to 2.000 and accounts for high purity of the sample. When 1% RNA was substituted with polysaccharide components, the R_2 coefficient decreased by 0.002, therefore, the obtained extract contained not more than 5% of carbohydrate components, which is admissible [12]. Thus, the RNA eluate isolated from FMDV Asia-1/Tajikistan/2011 strain was highly pure, which made it possible to determine

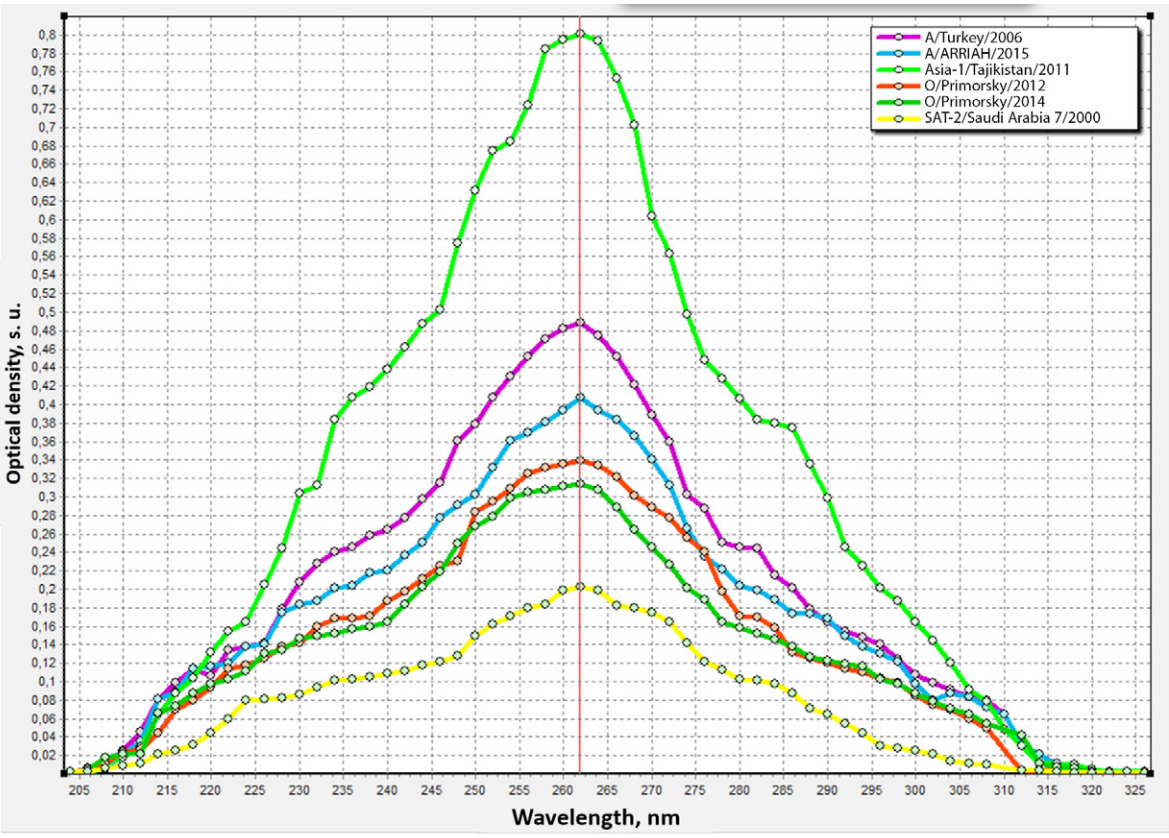

Fig. Spectrograms of the FMDV RNA extracts of the studied strains to assess purity and determine concentration of 146S particles in the original suspensions

Рис. Спектрограммы экстрактов РНК вируса ящура исследуемых штаммов для оценки чистоты и определения концентрации 146S частиц в исходных суспензиях

the number of structural particles (RNA molecules) with a high degree of reliability.

Based on the results of the spectrum analysis, the following mean values of optical density OD_{262} were obtained: 0.810 ± 0.001, OD_{320} = 0.003, OD_{260C-} – 0.006 (for OD_{320} and OD_{260}). Arithmetic mean of the structural particles (RNA) isolated from the whole FMDV particles was 225,366,856,349 ± 281,356,874, concentration of FMDV 146S component of Asia-1/Tajikistan/2011 strain was 3.132 ± 0.004 μg/cm^3 and it had 99.49 and 99.76% correlation with the results of CFT (3.150 ± 0.170 μg/cm^3) and rtRT-PCR (3.136 ± 0.040 μg/cm^3), correspondingly. Thus, the linear algebraic bond function of C_{146S} and $N_{RNA\ 146S}$ developed on the basis of spectrum analysis data makes it possible to determined concentration of the whole particles in the non-inactivated suspension for FMD vaccines [8].

The spectrum analysis of the 30-fold FMDV RNA eluate of SAT-2/Saudi Arabia 7/2000 strain demonstrated that mean extinction values at wavelengths of 205–259 and 263–325 nm did not exceed $OD_{260\text{-}262}$ (0.002–0.199 < < 0.200–0.203 and 0.198–0.001 < 0.200–0.203), which means that the eluate contained mainly ribonucleic acid. The extract was not contaminated with phospholipid impurities, carbohydrates or guanidine isothiocyanate residues, hydroxybenzene, polypeptides and suspended conglomerates, as there were no marked peaks in the graphs at wavelengths of 205, 235, 270, 280 and 320 nm, correspondingly. The R_1 extinction coefficient was 1.971 (OD_{262}/OD_{280} = 0.203/0.102) which is close to norm of 2.000 and means almost total absence of polypeptides and residues of carbolic acid in the extract. The R_2 extinction coefficient was 1.990 (OD_{262}/OD_{235} = 0.203/0.102) which is close to 2.000 and accounts for high purity of the extract. When 1% RNA was substituted with carbohydrates, the R_2 coefficient decreased by 0.002, i.e. polysaccharide content in the obtained preparation did not exceed 2.5% and it is admissible [12].

Based on the results of the spectrum analysis of the RNA extract, the following mean values of extinction were obtained: OD_{262} = 0.203 ± 0.002, OD_{320} = 0.002, OD_{260C-} = 0.006 (for OD_{320} and OD_{260}). The average number of RNA molecules isolated from FMDV particles of SAT-2/ Saudi Arabia 7/2000 strain (with 146S sedimentation coefficient) was 54,864,590,497 ± 562,713,752. Concentration of FMDV 146S component was 0.764 ± 0.008 μg/cm^3 which had 98.10 and 99.48% correlation with the results of quantitative CFT (0.75 ± 0.14 μg/cm^3) and rtRT-PCR (0.765 ± 0.044 μg/cm^3), correspondingly. Thus, the previously developed linear mathematical model makes it possible (with the help of a spectrum analysis) to determine concentration of the whole FMDV particles in the non-inactivated suspension for vaccine preparations [8].

At the end of the research, the spectrometric method was tested for its ability to determine concentration of 146S particles in 410 non-inactivated suspensions of FMDV vaccine strains A/Turkey/2006, A/ARRIAH/2015, O/Primorsky/2012, O/Primorsky/2014, Asia-1/Tajikistan/2011, SAT-2/ Saudi Arabia 7/2000. Quantitative rtRT-PCR and CFT tests were simultaneously used in three replications. The actual results obtained in real-time reverse transcription – polymerase chain reaction (rtRT-PCR) were 97.0–99.9% consistent with the expected results of the spectrometric analysis used to determine cultural FMDV 146S component concentration. When compared to a complement fixation test, the actual results were 94.5–99.5% in line with the expected ones. Actual results for positive control were 99.0–99.6% in line with the expected ones. As expected, no FMDV RNA or 146S particles were detected in the negative control sample. Thus, the carried out research demonstrated that the spectrum analysis used to determine concentration of 146S component (when estimating the number of FMDV RNA isolated after serological binding of the whole particles with the help of strain-specific IgG) had 94.5–99.9% correlation with quantitative variants of CFT and rt RT-PCR.

CONCLUSION

We assessed the feasibility of a spectrum analysis to determine indirectly concentration of 146S component based on the number of FMDV RNA molecules isolated after serological binding of the whole virus particles with the help of IgG. This method is cheap, easy-to-use and makes it possible to determine concentration of FMDV 146S particles in non-inactivated suspencion for FMD vaccine within 3–4 hours.

Tests of non-inactivated suspensions of cultural FMDV strains A/Turkey/2006, A/ARRIAH/2015, O/Primorsky/2012, O/Primorsky/2014, Asia-1/Tadjikistan/2011, SAT-2/Saudi Arabia 7/2000 proved that the linear algebraic model C_{146S} = (3.9 × $N_{RNA\ 146S}$ + 566,783,689)/280,818,944,837 makes it possible (with the help of a spectrum analysis) to assess indirectly concentration of FMDV 146S component in the raw materials for a wide range of vaccines.

It was found, that the spectrum analysis used to measure the whole virus particles (when estimating the number of FMDV RNA isolated after serological binding) had 94.5–99.9% correlation with quantitative CFT and rtRT-PCR.

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