ENSURING VIRAL SAFETY OF EQUINE IMMUNOGLOBULINS DURING PRODUCTION

V. V. Mashin,[1] A. N. Sergeev,[1,*] N. N. Martynova,[1] M. D. Oganov,[1] A. A. Sergeev,[2] V. V. Kataeva,[1] and N. V. Zagidullin[1]

Equine blood plasma/serum and intermediates must be monitored for the presence of live viruses pathogenic in humans during production of equine immunoglobulins. Information concerning low-cost and simple methods for the detection of live horse viruses pathogenic and non-pathogenic to humans was gained using data of modern domestic and foreign literature. These methods are based on cultivation of these viruses on sensitive biosystems. The presented information can be used to set up blood plasma/serum control of horses at different stages of immunoglobulin production, i.e., when taking blood from horses during their quarantine period, when collecting blood from immunized horses, and before bottling the medicinal intermediate in the primary package.

Keywords: equine immunoglobulin, equine virus, cultivation biosystem, duration of cultivation, detection method.

Various injectable drugs, e.g., immunoglobulins and their fragments $[F(ab)_2]$, are currently produced from blood serum/plasma in many countries, including Russia [1 – 3], for medical uses such as especially hazardous viral infections (antirabic immunoglobulin), bacterial toxins [antitetanic, anti-diphtheria, and anti-botulin type A based on $F(ab)_2$ fragments, and other sera], and snake [serum against viper venom based on $F(ab)_2$ fragments] and scorpion venoms [Anascorp® based on $F(ab)_2$ fragments].

Blood plasma/serum and drug intermediates based on it should be produced and controlled considering information about existing types of equine diseases caused by viruses pathogenic for humans to minimize the risk of viral contamination. We used data taken from existing domestic and foreign literature to compile a list of critical viruses causing diseases in horses that included 36 infectious viruses, 25 of which are pathogenic for humans with 13 of the 25 being distributed not only abroad but also in Russia. Therefore, equine blood plasma/serum and drug intermediates based on it must be controlled during production of equine immunoglobulin drugs for the presence/absence of viruses pathogenic for humans. This is especially important for equine disease vectors that are found in Russia (13 viral pathogens, e.g., Getah, Japanese encephalitis, West Nile fever, tick-borne encephalitis, rabies, equine herpes types 1 – 4, equine influenza, encephalomyocarditis, foot-and-mouth disease, reoviruses types 1 – 3, equine rotavirus, equine adenovirus, and equine coronavirus vectors). Control of heterologous blood plasma/serum and drug intermediates based on it for the presence/absence of namely these viruses is required in existing pharmacopoeias of leading countries (USA, Great Britain) and the European Pharmacopoeia [4 – 6].

Considering the above, the aim of the present work was to analyze domestic and foreign scientific publications that include information on the least expensive and simplest methods for detecting live equine viruses based on cultivation of these viruses in sensitive biosystems to ensure the viral safety of the produced equine immunoglobulin drugs. Information in the following areas was gathered to achieve this aim:

types of biosystems for cultivating viruses, including the method for adding them to the biosystems;

virus cultivation time in the biosystems;

[1] Microgen Scientific Industrial Company for Immunobiological Medicines JSC, 10 2nd Volkonskii Pereulok, Moscow, 127473 Russia.

[2] AVVA Pharmaceuticals Ltd., Representative Moscow Office, Office 8, 4/3 Aviamotornaya St., Moscow, 111116 Russia.

* e-mail: a.n.sergeev@microgen.ru

TABLE 1

No.	Disease vector	Virus cultivation system (virus introduction method)	Virus growth time	Virus detection method during growth	Ref.
1	Eastern equine encephalomyelitis virus[*]	Passaged cell culture (CC): Vero, RK-13, BHK-21, primary CC of chick (CEF) and duck embryo fibroblasts (DEF) (on monolayer)	2 – 3 days	Cytopathic effect (CPE)	[7 – 9]
		10 – 11-day chick embryo (CE) (in allantoic cavity)	0.5 – 1 days	Death	
		1 – 8-week mouse, hamster, guinea pig – g/p, chick (intracerebral – i/c, subcutaneous – s/c, intraperitoneal – i/p)	2 – 9 days	Death	
2	Western equine encephalomyelitis virus[*]	Passaged CC: Vero, RK-13, BHK-21,; primary CC: CEF and DEF (on monolayer)	2 – 3 days	CPE	[7 – 9]
		10 – 11-day CE (in allantoic cavity)	0.5 – 1 days	Death	
		1 – 8-week mouse, hamster, g/p, chick (i/c)	2 – 9 days	Death	
3	Venezuelan equine encephalomyelitis virus[*]	Passaged CC: Vero, RK-13, BHK-21, primary CC: CEF and DEF (on monolayer)	2 – 3 days	CPE	[7, 8, 10, 11]
		10 – 11-day CE (in allantoic cavity)	1 – 2 days	Death	
		1 – 4-week mouse, hamster, g/p, rat, rabbit (i/c, s/c, i/p)	6 – 9 days	Death	
4	Getah virus[**]	Passaged CC: Vero, RK-13, BHK-21 (on monolayer)	4 – 9 days	CPE	[12, 13]
		Suckling mouse (i/c)	5 – 10 days	Death	
5	Ross River virus[*]	Passaged CC: Vero, HeLa, HLE (on monolayer)	4 – 6 days	CPE	[14, 15]
		Suckling mouse (i/c)	5 – 10 days	Death	
6	St. Louis encephalitis virus[*]	Passaged CC: Vero, C6/36 (on monolayer)	Vero – 7 – 10 days, C6/36 – 4 days	CPE	[16, 17]
		Pheasant, chick	4 – 10 days	Death	[18]
7	Japanese encephalitis B virus[**]	Passaged CC: Vero, BHK-21, C6/36 (on monolayer)	4 – 6 days	CPE	[19]
		Erythrocytes, pH 9.0	0.5 h	Hemagglutination	[20, 21]
		3-week mouse (intraconjunctival – i/j, i/p)	I/c – 4.8 days, i/p – 13 days	Death upon i/c administration – 100%, upon i/p – 58%	[22, 23]
8	West Nile virus[**]	Passaged CC: Vero, RK-13 (on monolayer)	3 days	CPE	[24, 25]
		Mouse (i/p)	7 – 8 days	Death	[26]
9	Tick-borne encephalitis virus[**]	Passaged CC: Vero, HeLa, HLE (on monolayer)	4 – 6 days	CPE	[27]
		Suckling mouse (i/c)	5 – 10 days	Death	
10	Dengue virus[*]	Passaged CC: C6/36, Vero, BHK-21 (on monolayer)	4 days	CPE	[28, 29]
		Suckling mouse (i/c)	5 – 10 days	Death	
11	Zika virus[*]	Passaged CC: C6/36, Vero (on monolayer)	4 – 5 days	CPE	[30, 31]
		Suckling mouse (i/c)	5 – 10 days	Death	
12	Vesicular stomatitis virus[*]	Passaged CC: Vero, BHK-21 (on monolayer)	3 days	CPE	[32, 33]
		Mouse, chick, g/p, 2 – 6-month hamster (i/p)	Hamster – 3 days	Death	[33]
		7 – 11-day CE	2 – 3 days	Death	[34]
13	Rabies virus[**]	Passaged CC: Vero, BHK-21, N2a, primary CC: CEF (on monolayer or in suspension)	Vero (3 passages) – 4 – 5 days	N2a – 2 days	CPE
		Suckling mouse (i/c)	3 – 5 weeks	Death	
14	Equine herpesvirus types 1 – 4[**]	Passaged CC: RK-13, Vero, primary CC: horse hide – equine dermis cells, ED (on monolayer)	Vero (3 passages) – 2 – 10 days, RK-13 and ED – 3 – 7 days	CPE (rounded cells, syncytia)	[36 – 38]
		11 – 13-day CE (in chorion-allantoic membrane – CAM), 5 – 7-day CE (in yolk sac)	Type 1 (3 passages) – 5 – 6 days	Plaque on CAM	[39]
		3 – 4-week mouse	Type 1 – 3 days	Death upon inoculation $\geq 10^6$ TCID$_{50}$	[37]

No.	Disease vector	Virus cultivation system (virus introduction method)	Virus growth time	Virus detection method during growth	Ref.
15	Hendra virus[*]	Passaged CC: Vero, MDBK, BKH, LLC-MK2, MRC5 (on monolayer)	Vero – 3 days, LLC-MK2, MRC5 – 12 days	CPE (syncytia)	[40, 41]
		G/p, cat (s/c 5000 $TCID_{50}$ of virus)	Cat – 6 – 7 days, g/p – 9 – 12 days	Death	[42]
16	Nipah virus[*]	Passaged CC: Vero, RK-13, BHK	3 – 5 days	CPE (syncytia)	[43, 44]
		7 – 10-day CE (in allantoic cavity)	2 – 3 days	Hemagglutination	
		Cat, ferret, hamster	6 – 8 days	Neurological and respiratory symptoms, death	
17	Equine influenza virus[**]	Passaged CC: Vero, MDCK (on monolayer)	3 – 5 days	CPE (with trypsin), hemadsorption with chick erythrocytes	[45]
		9 – 11-day CE (in allantoic cavity)	3 days	Hemagglutination	
		Balb/c mouse (i/n)	2 – 5 days	Body mass and activity loss, sleepiness	[46]
18	Borna disease virus[*]	Passaged CC: Vero, C6, MDCK (on monolayer)	C6 and Vero – 60 days	CPE (syncytia with pH lowered to 5.0)	[47, 48]
		Rabbit (i/c), 1-day rat (i/c)	60 days	Death of rats or weight gain	[49]
19	Reoviruses types 1 – 3[**]	Passaged CC: Vero and L929 (on monolayer)	6 – 11 days	CPE	[50, 51]
20	Equine rotavirus[**]	Passaged CC: MA-104 and Vero with added trypsin (on monolayer)	7 days	CPE (rounding and graininess)	[52 – 54]
		Slc suckling mouse: ddY (peroral)	3 days	Diarrhea in 80 – 100% of animals	[55]
21	Horsepox virus[*]	Passaged CC: RK-13, Vero (on monolayer)	3 – 5 days	CPE	[56]
		10 – 11-day CE (in CAM)	3 days	Pockmarks in CAM	[57]
22	Equine adenovirus[**]	Passaged CC: FEK, Vero (on monolayer)	FEK (3 passages) – 7 days, Vero – 2 days	CPE (cell rounding and fusion)	[58, 59]
23	Encephalomyocarditis virus[**]	Passaged CC: Vero, A-549, HLE (on monolayer)	2 days	CPE	[60]
		Suckling mouse (i/c)	5 – 10 days	Death	
24	Foot-and-mouth disease virus[**]	Passaged CC: BHK, Vero (on monolayer)	2 days	CPE	[61, 62]
		Mouse (s/c, i/p)	7 – 14 days	Death	
25	Equine coronavirus[**]	Passaged CC: HRT-18, Vero (on monolayer)	HRT-18, Vero (3 passages) – 5 days	CPE (cell rounding, syncytia)	[63, 64]
		Murine erythrocytes	1 h	Hemagglutination	[65]
26	Louping ill virus	Primary CC of swine kidneys (on monolayer)	3 – 5 days	CPE	[66]
		Sheep (i/c)	5 – 10 days	Encephalitis symptoms	
27	Equine hepacivirus	Did not multiply in cell lines, no animal models			[67]
28	Equine pegivirus	Did not multiply in cell lines, no animal models			[68]
29	Bovine and equine papilloma viruses	Did not multiply in cell lines, no animal models			[69]
30	Equine arteritis virus	Passaged CC: RK-13, Vero, BHK-21 (on monolayer)	2 – 4 days	CPE	[70, 71]
		Murine and chick erythrocytes treated with Tween-80	At 37° C – 1 h, at 4° C – 1 days	Hemagglutination	[72]
31	African horse sickness	Passaged CC: Vero, BHK-21 (on monolayer)	3 – 7 days	CPE	[73]
		Suckling mouse (i/c)	3 days	Death	[74]
32	Equine rhinitis virus A and B	Passaged CC: RK-13, EFK, Vero (on monolayer)	5 days	CPE	[75]

No.	Disease vector	Virus cultivation system (virus introduction method)	Virus growth time	Virus detection method during growth	Ref.
33	Equine infectious anemia virus	Passaged CC of leukocytes, kidney fibroblasts, equine vessel endothelium (on monolayer)	3 – 7 days	CPE	[69, 76]
34	Equine foamy virus	Passaged CC of stallion kidney with mononuclear peripheral equine blood (on monolayer)	10 days	CPE (vacuolization, syncytia)	[77]
35	Equine parvovirus hepatitis	Did not multiply in cell lines, no animal models			[67]
36	Cytomegalovirus	Passaged CC: human and calf embryo lung fibroblasts (on monolayer)	5 – 7 days	CPE (syncytia)	[78, 79]

Note:
* Equine virus pathogenic in humans
** Equine virus pathogenic in humans and distributed in Russia.

virus detection methods during their cultivation in the biosystems.

Table 1 lists the results of these investigations.

Table 1 shows the following:

a broad spectrum of biosystems can be used to grow equine viruses, e.g., various types of cell cultures (primary: chick and duck embryo fibroblasts, horse kidneys, etc.; passaged: Vero, BHK-21, RK-13, etc.); chick embryos of various ages (from 7 to 13-day); and various types of small laboratory animals (mouse, Syrian hamster, guinea pig, rat, rabbit);

these biosystems are inoculated (before starting to grow various types of viral agents) in various ways by adding the studied materials (for cell cultures, on a monolayer and in a suspension; for chick embryos, in chorion–allantoic membrane, in yolk sac and allantoic cavity; for laboratory animals, intracerebral, subcutaneous, intraperitoneal, etc.);

the equine virus growth times in the biosystems vary from 2 to 60 days, but are most often up to 14 days, depending on the type of virus and biosystem;

a broad spectrum of virus detection methods based on their cultivation in sensitive biosystems are used depending on the type of equine virus: visual inspection (recording external disease symptoms of laboratory animals, plaques and their appearance on chick embryo chorion–allantoic membranes and on a cell-culture monolayer), microscopic inspection (recording cytopathic effects on cell-culture monolayers such as portions of destroyed cells, syncytia and specific inclusion formation), hemagglutination and hemadsorption methods using erythrocytes of various origins (rooster, guinea pig, human, etc.).

Thus, the growth and detection of viruses that can occur in equine plasma/serum is a complex and nontrivial process.

The optimal (inexpensive) methods for minimizing the extent of studies for the presence of living viral agents pathogenic for humans in various materials during production of drugs based on equine blood plasma/serum are based on the use in all cases of 1 – 2 types of passaged cell cultures (*in vitro* experiments) and/or 1 – 2 types of laboratory animals, in-

cluding chick embryos (*in vivo* experiments). An analysis of the methods given in Table 1 showed that passaged Vero cell culture was successfully used to grow all types of equine viruses pathogenic for humans (25 pathogens). A cytopathic effect, hemagglutination, and hemadsorption were recorded after 2 – 60 days. These effects were observed within 7 d if the focus was on viruses causing diseases among horses only in Russia (the 13 viral pathogens mentioned above) and up to 21 d if three blind passages in Vero cell culture were used. Most of the listed viruses multiplied excellently in mice with the correct choice of inoculation route with recording of lethal outcomes for 5 – 16 d and in chick embryos with recording of lethal outcomes, hemagglutination, and plaques in chorion–allantoic membranes for 2 – 3 d. Such biosystems could also be used to confirm results obtained in passaged Vero cell culture.

Thus, a broad spectrum of domestic and foreign scientific literature sources was analyzed. Simple and inexpensive methods for detection of living equine viruses (potentially hazardous for humans) based on cultivation of these vectors on sensitive biosystems were proposed based on these data. The results on detection of equine viruses could be used in early production stages of equine immunoglobulin drugs:

to control equine blood plasma/serum (for their possible growth) during their quarantine (at the acquisition stage) for the presence/absence of equine viruses pathogenic for humans distributed in Russia;

to control pools of immune blood plasma/serum from immunized horses for the presence/absence of equine viruses pathogenic for humans found in Russia;

to control drug intermediates before bottling in the primary package for the presence/absence of equine viruses pathogenic for humans distributed in Russia.

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