ENSURING VIRAL SAFETY OF EQUINE IMMUNOGLOBULINS DURING PRODUCTION

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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 (antirab immune globulin), 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 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:

1. Types of biosystems for cultivating viruses, including the method for adding them to the biosystems;
2. Virus cultivation time in the biosystems;
3. Minimum duration of virus cultivation in the biosystems;
4. Detection methods for live viruses, including the sensitivity of these methods; and
5. Biological safety of equine blood plasma/serum and drug intermediates.

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| 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 ≥ 106 TCID<sub>50</sub> | [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<sub>50</sub> 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] |
|     |                | Slec 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                               | [61, 62] |
| 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 (s/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 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] |
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, including 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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