Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

To:	File for STN 125462
From:	Robert W. Fisher, DH, OBRR, HFM-345
Through:	Michael Kennedy, Team Leader LPD, DH, OBRR, HFM-345
CC:	Nannette Cagungun, RPM, RPMB, DBA, OBRR, HFM-380
Applicant:	Cangene Corporation
Product:	Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-(Equine)
Subject:	CMC midcycle review, STN 125462/0 adventitious agents safety evaluation

Recommendation:

Convey the following letter ready comments to the sponsor:

1. Table 2 and Table 6 in "3.2.A.2 Adventitious Agents Safety Evaluation" indicate a reduction factor of -(b)(4)- log for BVDV, however your viral reduction table in the prescribing information does not include this data. Moreover, PV-194-04-002 does not include validation of S/D inactivation of BVDV. Please clarify whether S/D inactivation of BVDV was validated, and if so, please submit the validation report.
2. Please provide a description and a list of specifications for the -----(b)(4)----- filter.
3. Please clarify the geometry of your ---(b)(4)--- virus filtration system. Are --(b)(4)-- filters fed by a ---------(b)(4)---------?
4. ---(b)(4)--- has calculated reduction factors for Ad2 and EMCV using a -------(b)(4)------- ----------, while the PV.194.04.004 process validation report and the viral clearance table in your package insert apparently used approximately half this volume. Please explain the discrepancy.
5. Table 1 in ---(b)(4)--- report AB00AV.022271.BSV specifies a volume of -----(b)(4)------ ---------- samples, while your viral load calculations are based on -------(b)(4)------- volume samples. Likewise, the volumes listed in table 6-8 for PPV, WNV, and XMuLV clearance do not appear to reflect those in the applicable ---(b)(4)---- reports. Please explain the discrepancies in how Cangene and ----(b)(4)--- have calculated log reduction factors.
6. Please provide a table summarizing your viral filtration validation studies as follows: ----------(b)(4)---------- ---------- -

(b)(4)-. For each of the table entries, please provide a copy of the original source documentation (e.g. lab notebook page/pages).

The following questions and requests have already been submitted to the sponsor:

Who owns the horses in your donor herd?

Who is responsible for the immunization program

Are there quality agreements in place for horse herd maintenance and the immunization program?

Please submit ----(b)(4)---- reports AB32EU.034090.BSV, AB32EZ.034090.BSV, and AB47CR.034090.BSV.

Please submit a validation report or a cross-reference to a master file supporting use of the West Nile Virus -(b)(4)- test on equine plasma performed at the ----------(b)(4)----------.

Executive Summary:

Cangene Corporation (Cangene) has submitted a Biologics License Application for Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-Equine with an indication for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotypes A, B, C, D, E, F, or G. The application includes vaccination information for the horse herd, an overview of plasma testing procedures, and validation reports for viral clearance at two steps in the manufacturing process. The primary issue identified to date is a lack of validation data for plasma testing procedures (9 CFR adventitious agent tests and West Nile Virus ------(b)(4)------), and discrepancies between Cangene and ---(b)(4)---- reduction factor calculations for the nanofiltration step.

Submission Review Summary:

1. STN 125462/0 is an eCTD format original Biologics License Application (BLA) for Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-Equine (HBAT) submitted by Cangene Corporation.
    a. This submission was received at DCC on 20 September 2012 and a chair assigned on 21 September 2012.
    b. The Action Due Date is 22 March 2013.
2. HBAT is a polyclonal antibody preparation manufactured from hyperimmune horse plasma.
    a. The initial lots of HBAT were produced from a herd of 42 horses immunized and plasmapheresed at BioWhittaker Inc, Walkersville, MD.
        i. The facility was certified by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC).
        ii. Horses were tested for equine infectious anemia prior to acquisition and annually thereafter.
        iii. Horses received vaccinations for rabies, tetanus, equine influenza, Eastern and Western equine encephalitis, and Potomac horse fever.
    b. Between 2005 and 2011, horse immunization and plasma collection was performed at two facilities: Auburn University College of Veterinary Medicine, Auburn, AL, and Lake Immunogenics, Inc., Ontario, NY. Since 2011

immunization and plasma collection have only been performed at the Auburn University facility.

i. Horses added to the herd were subject to a quarantine period of at least 21 days and were physically and procedurally separated from the production animals. Cross over procedures to include containment procedures were specified in standard operating procedures. During the quarantine period the horse or horses were examined daily by a veterinarian or a trained designate.

ii. Horses were vaccinated against rabies, tetanus, eastern and western equine encephalitis, equine influenza, West Nile virus, equine herpes, and *Streptococcus equi*. Except for a modified live vaccine used for *Streptococcus equi* only killed vaccine preparations were used. Horses were dewormed during quarantine and every 3-4 months thereafter.

iii. Horses at the Lake Immunogenics facility were vaccinated against Potomac Horse Fever; this vaccine is not utilized at the Auburn facility.

iv. Horses were deferred from plasma donation for 14 days after the *Streptococcus* vaccine, and 7 days for killed vaccines, dewormers, or the botulinum neurotoxin immunogens.

3. Collected plasma is quarantined and subjected to testing for adventitious agents.
   a. Sampling and pooling procedures
      i. A single plasma collection from the donor horse is aliquoted into approximately -----(b)(4)----. Each bottle has a maximum fill of approximately -(b)(4)- and is considered a 'unit' of plasma.
      ii. Each plasma unit is tested for West Nile Virus (WNV).
         1. Cangene utilizes a ----------(b)(4)---------- service provided by the ----------(b)(4)----------. They have indicated a sensitivity of -------(b)(4)------, but did not provide a validation report. A validation report was requested on 28 November 2012.
      iii. A -(b)(4)- hold time is placed on each plasma collection and is enforced by Cangene's Quality Control unit.
      iv. 9 CFR testing is performed on a 'virtual plasma pool' composed of representative samples of all plasma units in a manufacturing run.
   b. 9 CFR testing
      i. In lieu of the extensive plasma testing that is typical of human plasma derived products, Cangene utilizes the test procedures specified in 9 CFR 113 designed to identify adventitious agents in animal serum samples. These tests include immunofluorescent antibody (IFA) staining per 9 CFR 113.47 as well as detection of cytopathology effects (CPE) and hemadsorbing agents per 9 CFR 113.46. These tests are performed at ----------(b)(4)----------.
      ii. The 9 CFR test procedure has been modified by -------(b)(4)---------
      ----

      ----------.
      iii. Cangene indicates that the sensitivity of the 9 CFR testing is at least --(b)(4)- for bovine viral diarrhea virus, equine herpesvirus, bovine parainfluenza virus, equine arteritis virus, sindbis virus, and reovirus type 3. This is based on a spiking study performed with each of the 6 viruses in

an equine plasma matrix.

1. Validation reports to support this claim were requested on 28 November 2012.

4. Validation of manufacturing procedure
    a. Virus model systems
        i. Cangene evaluated their manufacturing process for the removal or inactivation of a panel of viruses selected to represent potential contaminants and/or different virus families.
            1. Xenotropic Murine Leukemia Virus (XMuLV) is a large (80-110 nm) enveloped, single-stranded RNA virus of the *Retroviridae* family and is a specific model for Equine Infectious Anemia virus and a nonspecific model for similarly sized enveloped RNA viruses such as the *Rhabdoviridae*. ----------(b)(4)---------- ---------- ---------- The viral clearance studies used XMuLV strain -------(b)(4)---------- ----------.
            2. West Nile Virus (WNV) is 40-70 nm enveloped single-stranded RNA virus of the *Flaviviridae* family that has a demonstrated potential for blood-borne transmission (Pealer et al., 2003). The viral inactivation studies used WNV strain ---------(b)(4)---------- ----------, while the viral clearance studies used WNV strain ----------(b)(4)---------- ----------
            3. Bovine Viral Diarrhea Virus (BVDV) is 50-70 nm enveloped single-stranded RNA virus of the *Flaviviridae* family used as a specific model for other flaviviruses and for togaviruses such as Eastern equine encephalitis virus, Western equine encephalitis virus, and Venezuelan equine encephalitis virus. The viral clearance study used BVDV strain ----------(b)(4)---------- ----------
            4. Pseudorabies Virus (PRV; Suid herpesvirus 1) is a large (150-200 nm) enveloped double-stranded DNA virus of the *Herpesviridae* family that is used as a specific model for equine herpesviruses and as a nonspecific model for other large, enveloped DNA viruses such as the *Poxviridae*. The viral inactivation studies used PRV strain ----------(b)(4)----------
            5. Adenovirus Type 2 (Ad2) is a 70-90 nm non-enveloped double-stranded DNA virus of the *Adenoviridae* family. Adenoviruses have a relatively high resistance to chemical inactivation, and serve as a specific model for Equine adenovirus. Ad2 strain ----(b)(4)---------- was used for the viral clearance studies with an -------(b)(4)---------
            6. Parainfluenza III Virus (PI3) is a large (100-200 nm) single-stranded segmented RNA virus of the *Paramyxoviridae* family. It serves as a specific model for influenza viruses (including Equine influenza virus). The viral inactivation studies used PI3 strain ---------- ----------(b)(4)----------

7. Encephalomyocarditis Virus (EMCV) is a small (25-30 nm) non-enveloped RNA virus of the *Picornaviridae* family. It has a high resistance to chemical inactivation and is an accepted model for hepatitis A virus. ----------(b)(4)----------

8. Porcine Parvovirus (PPV) is a small (18-24 nm) non-enveloped DNA virus of the *Parvoviridae* family used as a nonspecific model of small, high-titer viruses that are difficult to remove from process intermediates due to their small size and high resistance to chemical inactivation. It is also a specific model for Equine Parvovirus and Adeno-Associated Viruses. ----------(b)(4)----------

ii. The choice of model viruses appears adequate considering the equine donor source.

b. Determination of viral titer
   i. All studies used a ----------(b)(4)---------- inoculum per sample dilution. The $TCID_{50}$ endpoint was calculated according to the Spearman Kärber method.
   ii. For samples with no virus detected at the highest dilution, ------(b)(4)------ was used. The assay infected ----------(b)(4)---------- and also used a Poisson distribution to estimate the virus titer at the 95% confidence limit.

c. Manufacturing steps validated for viral inactivation (process validation report PV.194.04.002)
   i. Cangene employs a solvent/detergent step for inactivation of any viruses found in the plasma pool.

      The inactivation studies utilized worst-case scenarios ----(b)(4)----

      PI3 inactivation was validated in study ------(b)(4)-----
      ----------(b)(4)----------

-----------------------------------(b)(4)----------------------------------
----

----

ii. PRV inactivation was validated in study --------(b)(4)-------.
--------------------------------------(b)(4)------------------------------------
----

----

iii. WNV inactivation was validated in study --------(b)(4)--------
---------------------------------------(b)(4)------------------------------------
----

----

iv. XMuLV inactivation was validated in study --------(b)(4)-------.
--(b)(4)------------------------------------

--- ----

---------------------------------(b)(4)-------------------------------------
----

----

vii. The viral inactivation studies appear to support the clearances indicated in Table 3. The level of clearance demonstrated appears to be adequately robust for inactivation of enveloped viruses in the plasma pool.
--(b)(4)------------------------------

---.

d. Manufacturing steps validated for virus removal (process validation report PV.194.04.004)

   i. The botulinum antitoxin manufacturing process includes a nanofilter for removal of viruses from process intermediates ----(b)(4)---
----
------------------------------------(b)(4)--

The capacity of the manufacturing process for removal of viruses was demonstrated at --------------------------(b)(4)--------------------------------------
----

----

ii. Removal of Ad2 was validated in study----------(b)(4)----------.

---------(b)(4)---------

iii. Removal of BVDV was validated in report ----------(b)(4)----------.

---------(b)(4)---------

--(b)(4)----------------------------------

----

iv. Removal of EMCV was validated in report ----------(b)(4)-----------.
--(b)(4)------------------------------------

----

v. Removal of PPV was validated in report----------(b)(4)------------.
---------------------------------------(b)(4)--

----

vi. WNV-removal was validated in report -----------(b)(4)----------
---(b)(4)--------------------------------------

-------(b)(4)-------

----

ix. XMuLV removal was validated in report ----------(b)(4)----------
--------------------------------------(b)(4)-------------------------------------
----

----

e. In general, the viral inactivation and inactivation studies appear to support robust viral clearance for BAT manufacture. The actual values claimed in the prescribing information need to be checked against the actual ----(b)(4)---- studies, since there is a discrepancy between the reduction factors claimed by Cangene and those calculated by -----(b)(4)----.

5. Cangene has not carried out any formal prion clearance studies, but considers the risk to be minimal.
    a. Transmission is typically associated with neural tissue; blood components and plasma fractions are less of a risk.
    b. Horses are not a species considered susceptible to TSE (unlike ovine and bovine species).
    c. Materials used in production are derived from non-ruminant source materials. In particular, the pepsin used for manufacturing is -(b)(4)--derived, is sourced from USDA and/or Agriculture Canada inspected slaughterhouses, and is considered suitable for human use.

Two (2) Pages Determined to be Non-Releasable: (b)(4)

[--(b)(4)--]

Table 1. Current label claims for viral inactivation and clearance.

	Enveloped					Non-enveloped		
Genome	RNA	RNA	RNA	DNA	RNA	DNA	RNA	RNA
Virus	XmuLV	WNV	BVDV	PRV	PI3	Ad2	EMC	Porcine Parvovirus
Family	Retro	Flavi	Flavi	Herpes	Paramyxo	Adeno	Picorna	Parvo
Size (nm)	80-110	40-70	50-70	150-200	100-200	70-90	25-30	18-24
(b)(4)Nanofiltration (log_{10})	≥ 2.7	≥ 2.1	(b)(4)			≥ 4.7	≥ 4.5	(b)(4)
S/D (log_{10})	≥ 4.3	≥ 5.1		≥ 5.1	≥ 5.5			
Total Reduction (log_{10})	≥ 7.0	≥ 7.2	≥ 4.1	≥ 5.1	≥ 5.5	≥ 4.7	≥ 4.5	(b)(4)

References:

Käsermann, F., & Kempf, C. (2003). Sodium hydroxide renders the prion protein PrPSc sensitive to proteinase K. *Journal of General Virology*, *84*(11), 3173–3176. doi:10.1099/vir.0.19355-0

Lemmer, K., Mielke, M., Kratzel, C., Joncic, M., Oezel, M., Pauli, G., & Beekes, M. (2008).

Decontamination of surgical instruments from prions. II. In vivo findings with a model system for testing the removal of scrapie infectivity from steel surfaces. *Journal of General Virology*, *89*(Pt 1), 348–358. doi:10.1099/vir.0.83396-0

Pealer, L. N., Marfin, A. A., Petersen, L. R., Lanciotti, R. S., Page, P. L., Stramer, S. L., … Chamberland, M. E. (2003). Transmission of West Nile Virus through Blood Transfusion in the United States in 2002. *New England Journal of Medicine*, *349*(13), 1236–1245. doi:10.1056/NEJMoa030969