Date: December 17, 2014

From: Miriam Ngundi, OVRR/DBPAP/LRSP
                   Consult Reviewer – TNA Assays

To: STN 125562/0
                   Anthrasil™, Anthrax Immune Globulin Intravenous (Human) [AIGIV]
                   Biologics License Application – BL 125562

Through: Mike Schmitt, Ph.D., Chief LRSP, OVRR/DBPAP

Applicant: Cangene

Subject: Complete Review and Approval Memorandum

Executive Summary

Cangene submitted this original Biological License Application (BLA), Submission Tracking Number (STN) #: 125562/0, seeking United States licensure of Anthrasil™, Anthrax Immune Globulin Intravenous (Human) [AIGIV]. AIGIV is indicated for the treatment of adult and pediatric patients with toxemia associated with inhalational anthrax in combination with appropriate antibacterial drugs. AIGIV is a sterile gamma globulin (IgG) fraction of human plasma containing antibodies to Bacillus anthracis. It is prepared from Source Plasma obtained from selected healthy donors who have been immunized with BioThrax® (Anthrax Vaccine Adsorbed). AIGIV is a clear or slightly opalescent colorless liquid essentially free of foreign particles that is formulated in 10 g% maltose and 0.03% polysorbate 80. It contains no preservatives and is intended for single use by intravenous (IV) administration.

Clinical trials in a population exposed to anthrax are not feasible due to the small number of naturally occurring anthrax cases, and the ethical concerns of deliberately exposing healthy individuals to a serious life-threatening pathogen. As a result, the efficacy of AIGIV was assessed in two animal models (rabbit and cynomolgus macaque). To support licensure of AIGIV, studies included:
- Non-clinical model development studies to characterize the disease and identify appropriate real time clinical markers that could be used as triggers for therapeutic intervention
- Pharmacokinetics (PK) in rabbit and non-human primate (NHP) animal models
- Post-exposure prophylaxis (rabbit model) and therapeutic efficacy studies (rabbit and NHP)

- Clinical study to provide information on the PK and safety of AIGIV in healthy human volunteers
- Dose modeling and simulation.

Scope of Review:
The scope of my review focused on the validation of Toxin Neutralizing Assays (TNAs) used for product release (potency by TNA) and in the pharmacokinetics (PK) studies (rabbit, non-human primate, and human).

Material Reviewed
The following module sections of the original BLA, submitted July 25, 2014, were reviewed for information relevant to the TNAs:
3. Quality
   3.2.S.4.1.   Specification
   3.2.S.4.2.   Analytical Procedures
   3.2.S.4.3.   Validation of Analytical Procedures
   3.2.S.4.5.   Justification of Specification
   3.2.S.5.   Reference Standards or Materials
   3.2.S.7.   Stability (TNA data)
   3.2.P.5.6.   Justification of Specifications
   3.2.P.8.   Stability (TNA data)
5. Clinical Study Reports
   5.3.1.4.   Reports of Bioanalytical and Analytical Methods for Human Studies (TNA methods)
After review of the above documents, subsequent information requests were sent to the sponsor on October 6, 2014 and November 11, 2014. The information requests are available in the EDR. The sponsor submitted the following amendments as responses to CBRE's information request:
Amendment 0.7: Submitted October 17, 2014
Amendment 0.16: Submitted December 8, 2014

Recommendation
The TNA for product release was characterized and validated for product in the range of
(b) (4)
      This range covers the proposed specification of
(b) (4)   , final target potency of (b) (4)   and stability specification of ≥60 Units/vial (maximum extractable volume of (b) (4)). The assay seems adequate to detect meaningful changes to the (b) (4)   and final Drug products, and therefor is suitable as a release and stability test method of the product.

TNAs for Pharmacokinetic Studies in non-clinical rabbit serum samples, non-clinical monkey serum samples and clinical human serum samples were characterized and validated. Based on the Validation Reports the quantitation ranges for the three assays are (b) (4)   for rabbit, monkey and human samples, respectively. The assays seem adequate to measure AIGIV in uninfected
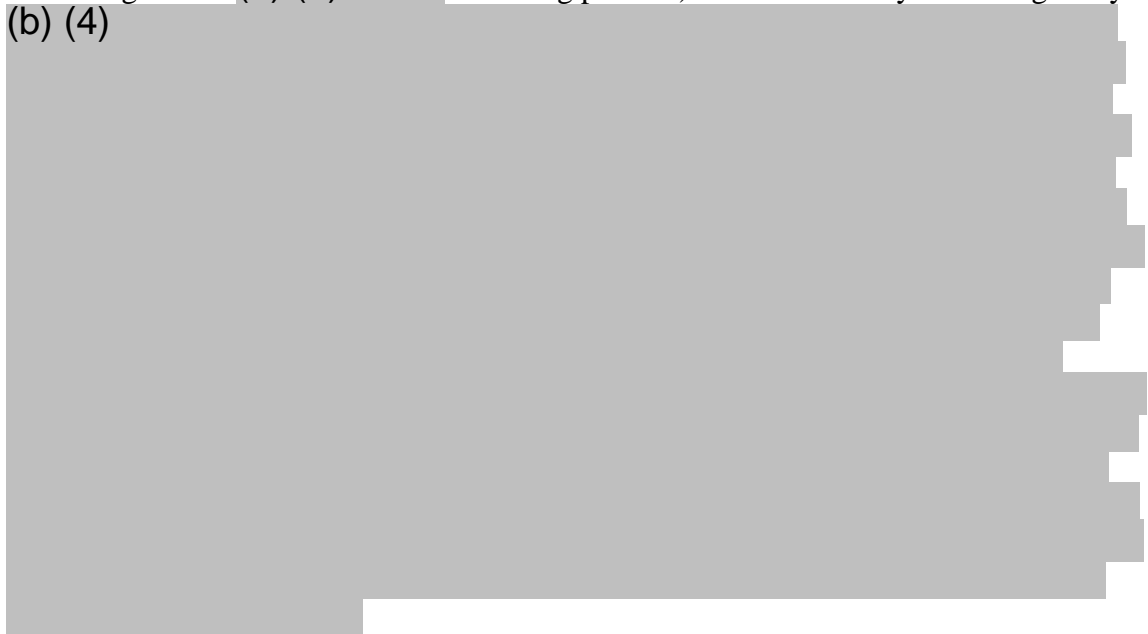
subjects. The method is limited in measuring AIGIV in infected model studies since PA in serum of infected animals binds to AIGIV, reducing the effective or "free" neutralizing capacity of AIGIV measured in the TNA. Cangene stated that during assay validation this effect was communicated to study directors and Cangene pre-clinical scientists to assist in interpretation of TNA results in infected model studies.

Based on the data I reviewed, TNA assays for product release and pharmacokinetic studies seem adequate for their intended use and, therefore I recommend the approval of the BLA.

Review
Validation of Toxin Neutralization Assay for product release
The Toxin Neutralization Assay (TNA) is used to assess the potency of the purified immune globulin (b) (4)   and Drug product) and as a stability indicating assay
(b) (4)

The following documents were submitted in support of validation of TNA for product release.

   i)   Report B55 (sign of date 2003-10-22):
        Original validation for (b) (4)
        immunoglobulin finished product

   ii) B55-B (sign off date 2005-11-16):
        (a) Validation of new liquid formulation
        (b) Qualification of alternate culture/assay media components
        (c) (b) (4)

   iii) B55-F (sign off date 2006-10-23):

(a) Change in (b) (4)   component concentrations
(b) Extension of validated range of method for liquid samples

iv) AL_MV_B55_rep_v8 (sign off date 2008-05-26):
(a) Qualification of a new in- house internal control (b) (4)
(b) Evaluation of (b) (4)   stability for liquid samples

v) VAL_MV_B55_rep_v8_ADD002 (sign off date 2009-12-18):
Summary report

vi) MV_B55_rep_v8_AD03 (sign off date 2011-12-02):
Qualification of procedure for testing effectiveness of new lethal factor lot

vii) SOP #87.040.0016.RR (effective date 09-05-25)

viii) SOP #87.040.0016.16 (effective date 2014-08-27)

The initial validation (Report B55) assessed accuracy, precision, linearity, specificity, robustness and stability using (b) (4)

Reports B55-B, B55-F and VAL MV B55 rep v8 assessed accuracy, precision, and stability in the range (b) (4) liquid formulated AIGIV (b) (4) finished product. The test samples were assayed alongside in-house qualified negative control and internal control standards. Changes to the initial assay method (Report B55) were made including (b) (4)   ,
(b) (4)   . CBER asked that the sponsor justify how validation for samples ranging (b) (4)   were bridged to the re-optimized levels of lethal toxin used in the later validation studies. Cangene stated that the range of concentrations of (b) (4)   included in the validation study with the re-optimized toxin levels covered the full range of expected potencies based on the current knowledge of the AIGIV process at the time of the validation study. The firm also stated that a bridging study for concentrations from (b) (4)   was not necessary based on the updated knowledge of the expected sample range. The sponsor's response is adequate because the (b) (4)   concentration range covers the proposed potency specification of (b) (4)   , target potency of (b) (4)   and the set stability specification of ≥ 60 U/vial.

Accuracy was assessed for each concentration range (b) (4)

acceptance criteria of percent recovery within (b) (4) of expected potency was met for all samples in both sample ranges.

Precision was evaluated by testing the (b) (4)

The acceptance criteria coefficient of variation (%CV) (b) (4) for both intra-assay and inter-assay precision were met for all samples in both concentration ranges. At

the request of CBER the precision data were re-evaluated using log-transformed data for (b) (4)   sample range; the precision estimated using this analysis met the acceptance criteria.

Linearity was assessed from accuracy data by linear regression analysis of the expected versus observed concentration within the assay range. An acceptance criterion of correlation coefficient (R^2) (b) (4) was met for (b) (4)   concentration range. At the request of CBER, dilutional linearity for samples in (b) (4)   concentration range was re-evaluated using log transformed data of relative potency. This analysis resulted in a R^2 of 0.8966 and did not meet the acceptance criteria set using un-transformed data. However, based on the trend in the linearity plot, the data appear acceptable to support dilutional linearity of the assay for the intended use.

Specificity was assessed using spiked purified product, AIGIV placebo and a non-specific immune globulin product. Acceptance criteria of percent recovery of (b) (4) for spiked validation sample and no significant different in (b) (4) responses for placebo and non-specific IgG versus lethal toxin control wells were all met.

Robustness was assessed by testing (b) (4)

The acceptance criteria for accuracy and precision were met under all conditions tested.

Short-term and (b) (4)   stability were assessed. (b) (4)

The percent recovery of all test samples met the acceptance criteria of (b) (4)   of untreated test sample and (b) (4)   test sample for short-term and (b) (4)   stability, respectively.

CBER requested Cangene provide data to support the stability of the assay from the time of validation to the analysis of AIGIV for lot release and stability testing. The firm stated that no trend in either reference standard or internal control standard (ICS) tracking data has been seen, which supports stability of the method throughout its life cycle. However, Cangene provided system suitability tracking data (log-transformed) for Internal Control Standard (b) (4)   from 2008 to 2014. The geometric CV% for the log transformed potency values generated in six years was 11.2%. While Cangene did not provide data to demonstrate the assay stability from validation to five years following the validation, data from the most recent six years of routine use of the assay suggest that the assay is stable.

New lots of all critical reagents (b) (4)

with the exception of rPA are qualified prior to use in the assay. CBER requested that Cangene develop a protocol to be used to qualify new lots of (b) (4). In response the sponsor stated (b) (4)

control new lots of (b) (4) in the assay. The assay design has adequate controls that monitor the activity of lethal toxin, therefore the assay is adequate for intended use.

(b) (4)

Validation of TNA for Quantification of Anthrax Immune Globulin (AIG) in Non-clinical Rabbit Serum Samples
The following documents were submitted in support of validation of TNA for rabbit serum samples

    i) MV.120 (sign off date 2007-08-24):
       Validation of SOP #87.040.008.RR, Toxin Neutralization Assay (TNA) for Quantitation of Anthrax Immune Globulin (AIG) in Non-Clinical Rabbit Serum Samples

    ii) VAL_MV_120_rep_v2_AD01 (sign off date 2001-09-12):
       a) Specificity/Selectivity for AIG in the presence and absence of levoflovacin spiked into anthrax-infected rabbit sera
       b) Long-term stability of AIG in normal rabbit serum
       c) Freeze-thaw stability of AIG in anthrax-infected rabbit serum with and without levofloxacin

    iii) Val_MV_120_rep_v2_AD02 (sign off date 2012-11-29):
       Long-term stability of AIG in anthrax-infected rabbit serum ±levofloxacin

    iv) SOP # 87.040.0008.00 (effective date 2007-06-27)

Accuracy and precision were assessed using ICS samples at (b) (4)

(b) (4)

　　　Appropriate system suitability criteria were incorporated and single factor ANOVA model was used to estimate the precision of the assay.

All individual replicates of all test samples had percent recoveries (b) (4) and met the accuracy acceptance criteria of percent recovery of each test sample. In addition, the replicates met the requirement that the 95% confidence interval of each mean percent recovery value must be (b) (4)   of its expected value. Precision of the assay was demonstrated: all test samples had %CV <14% and therefore met the acceptance criteria of overall %CV (b) (4) at each concentration. The LLOQ and ULOQ of the assay were established at (b) (4)   based on the acceptance criteria of the lowest and highest test samples, respectively, that have acceptable accuracy and precision.

Dilutional linearity was assessed by assaying a (b) (4)

           Accuracy acceptance criterion was met with %CV <7% for each sample across all dilutions and therefore meeting the precision acceptance criterion of %CV (b) (4) as well.

Specificity was assessed by assaying an AIG sample at (b) (4)

mean % recovery for all samples was between 80 and 120%. The results met the acceptance criterion that the mean % recovery at low (b) (4)   and high (b) (4)   concentrations must be within (b) (4)

Freeze-thaw stability was assessed by (b) (4)

           All samples with the exception of (b) (4)   for 500 mU/ml met the acceptance criterion that the freeze-thawed samples must have a value (b) (4) of the zero freeze-thaw control samples.

CBER requested that Cangene justify why incurred samples were not included in the validation. The sponsor responded that the TNA method for testing rabbit serum samples was validated before animal studies were performed; therefore, incurred samples were not available for inclusion in the validation. The firm further stated that incurred sample reanalysis (ISR) has been performed for multiple rabbit studies in the AIGIV non-clinical program to demonstrate suitability of the method for incurred sample testing. The ISR was deemed successful if (b) (4)

           The three studies had percent difference values of 96%, 95% and 100% passing the accuracy limit, which indicated that the ISR demonstrated that incurred samples supported the precision and accuracy of the validated assay.

To demonstrate the long-term stability of the assay, CBER requested that Cangene provide data, from tracking of reference standard or internal control standard, that support the stability of the assay from validation to the analysis of the rabbit serum samples used

in the PK studies. Cangene did not provide such data, but explained that three internal control standards at (b) (4)

respectively. The variability is within the expected assay precision and supports assay stability.

New lots of all critical reagents (b) (4) were qualified against set acceptance criteria prior to use in the assay.

Cangene assessed the specificity/selectivity, long term stability and freeze-thaw stability of AIG in presence and absence of levofloxacin spiked into anthrax-infected or uninfected rabbit sera. Based on the acceptance criteria, the validation demonstrated that:
- The TNA method could selectively and specifically measure free neutralizing AIG in the presence and absence of levofloxacin in both normal and anthrax-infected rabbit serum. However, the presence of circulating rPA in infected animals interfered with the performance of the assay.
- An inverse relationship existed between free neutralizing TNA activity and anthrax toxin (protective antigen) concentration in infected serum.
- Stability of AIG in normal rabbit serum was acceptable after a time period of (b) (4)
- AIG was stable in anthrax-infected rabbit serum with and without levofloxacin for (b) (4)
- Stability of AIG in anthrax-infected rabbit serum ± levofloxacin was acceptable after a time period of 536 days (b) (4)

While Cangene claims that the TNA method can selectively and specifically quantify free neutralizing AIG in the presence and absence of levofloxacin in anthrax-infected rabbit serum, no pre-defined acceptance criteria were set. Data for the assessment of selectivity in infected sera was evaluated based on an inverse relationship between TNA results and toxin concentration in infected samples. This does not limit the intended use of the assay since data from uninfected animals suppo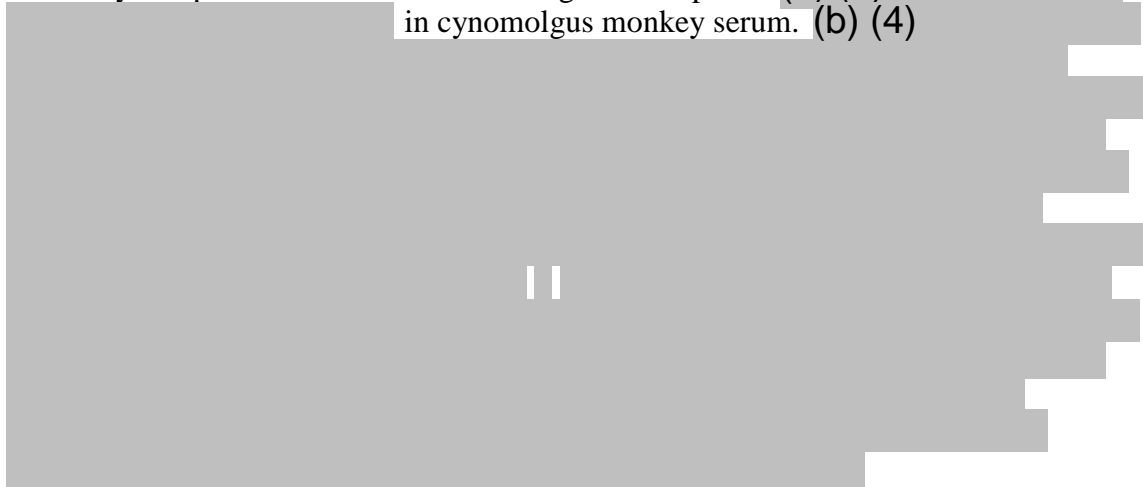rt selectivity and specificity of the assay. Cangene acknowledged that protective antigen (PA) in serum of infected animals would bind to AIGIV, reducing the effective or "free" neutralizing capacity of AIGIV that would be measured in the TNA. The sponsor explained that no criterion was assigned since the extent of toxin interference in the assay could not be predicted prior to the validation experiments. The firm further stated that this relationship was communicated to study directors and Cangene pre-clinical scientists to assist in interpretation of TNA results in infected model studies.

Validation of TNA for Quantification of Anthrax Immune Globulin (AIG) in Non-clinical Monkey Serum Samples

The following documents were submitted in support of validation of TNA for non-clinical monkey serum samples:

    i) VAL_MV_134_rep_v1 (sign off date 2008-08-28):
        Validation of SOP # 87.040.0048.00 – Toxin Neutralization Assay (TNA) for Quantification of Anthrax Immune Globulin (AIG) in Non-Clinical Monkey Serum Samples

    ii) VAL_MV_134_rep_v1_ADD001 (sign off date 2009-06-29):
        Specificity/Selectivity for AIG in anthrax-infected cynomolgus serum

    iii) VAL_MV_134_rep_v1_ADD002 (sign off date 2010-07-15):
        a) Specificity/Selectivity for AIG in combination with Ciprofloxacin spiked into normal and anthrax-infected cynomolgus monkey serum
        b) Long-Term Stability of AIG in normal cynomolgus monkey serum (based on Accuracy criteria)
        c) Freeze-Thaw, Bench-Top and Short-Term -20°C Stability of AIG in anthrax-infected cynomolgus monkey serum containing Ciprofloxacin

    iv) VAL_MV_134_rep_v1_AD03 (sign off date 2012-11-29):
        Long-term stability of AIG in anthrax-infected monkey serum ± ciprofloxacin

    v) SOP#87.040.0048.00 (effective date 2008-05-12)

Accuracy and precision were assessed using ICS samples at (b) (4) in cynomolgus monkey serum. (b) (4)

Dilutional linearity was assessed by assaying (b) (4) Linearity was evaluated by standard curve regression analysis. All dilutions met the acceptance criteria of (b) (4) for all assays and %CV (b) (4) for each replicate.

Specificity was assessed by (b) (4)

100% of all individual samples passed the acceptable recovery criteria of (b) (4) for high samples and (b) (4) for low/LLOQ samples.

The freeze-thaw/bench-top stability was assessed by (b) (4)

All samples had %recovery within (b) (4) and therefore met the validation acceptance criteria for accuracy.

CBER requested that Cangene justify why incurred samples were not included in the validation. The sponsor responded that the TNA method for testing NHP samples was validated before animal studies were performed; therefore, incurred samples were not available for inclusion in the validation. The firm further stated that incurred sample reanalysis (ISR) was performed for NHP GLP study (b) (4) 695-G005780 to demonstrate suitability of the method for incurred sample testing. The set acceptance criteria was ISR was deemed successful if (b) (4) from the TNA method validation. All the samples in this study met accuracy acceptance criteria. ISR resulted in 100% percent difference values passing the accuracy limit which supported the precision and accuracy of the validated assay.

To demonstrate the long-term stability of the assay, CBER requested that Cangene provide data from reference standard or internal control standard tracking that support the stability of the assay from validation to the analysis of the NHP serum samples used in the PK studies. Cangene did not provide such data, but explained that three internal control standards at (b) (4)

. The variability is within the assay precision and confirms assay stability of the assay throughout the duration of the GLP testing.

New lots of all critical reagents (b) (4) were qualified against set acceptance criteria prior to use in the assay.

Cangene assessed the specificity/selectivity, long-term stability and freeze-thaw stability of AIG in presence and absence of ciprofloxacin (cipro) spiked into anthrax-infected or uninfected rabbit sera. Based on the set acceptance criteria, the validation demonstrated that:

- The assay accurately and precisely quantified free neutralizing AIG in normal cynomolgus monkey serum in the presence of cipro

- The TNA method could selectively and specifically measured AIG in the presence of cipro in both normal and anthrax-infected cynomolgus monkey serum based on the data displaying a good inverse correlation between TNA results and toxin concentrations in infected animals
- Stability of AIG in normal cynomolgus monkey serum was acceptable after a time period of (b) (4)
- AIG was stable in anthrax-infected cynomolgus monkey serum containing Cipro for up to (b) (4)
- AIG was stable in anthrax-infected cynomolgus monkey serum containing Cipro when stored at (b) (4)
- Stability of AIG in anthrax-infected monkey serum was shown to be acceptable after a time period of (b) (4)
- Stability of AIG in anthrax-infected monkey serum + cipro was shown to be acceptable after a time period of (b) (4)

Cangene did not defined acceptance criteria for the assessment of specificity/selectivity in anthrax-infected serum due to the interference from anthrax toxin in the samples. The specificity/selectivity assessment was based on the data displaying a good inverse correlation between TNA results and toxin concentrations in the samples. These data were limited and therefore selectivity/specificity in infected monkey was not adequately demonstrated. However, the assay is adequate for intended use based on data from uninfected animals.

<u>Validation of TNA for Quantification of Anthrax Immune Globulin (AIG) in Clinical Human Serum Samples</u>
The following documents were submitted in support of validation of TNA for clinical human serum samples:
- i) VAL_MV_143_rep_v1 (sign off date 2008-06-29:
   Validation of SOP #87.040.0050.00 – Toxin Neutralization Assay (TNA) for Quantification of Anthrax Immune Globulin (AIG) in Clinical Human Serum Samples

- ii) SOP # 87.040.4450.00 (effective date 2008-06-25)

Accuracy and precision were assessed using ICS samples at (b) (4)

determined to be (b) (4)   , based on the lowest and highest concentration samples, respectively, to achieve acceptable accuracy and precision.

Linearity of sample dilutions was evaluated by testing samples at (b) (4)
                                            Both initial dilutions gave results within acceptable accuracy limits of (b) (4) absolute mean bias. The R^2 value for 4-parameter curve fit of all assays was ≥0.990 for all valid assays which demonstrated linearity of the standard curve.

Specificity/selectivity of the method was assessed by (b) (4)

                                            The assessment met the set acceptance criteria that the |Mean %RE| of spiked samples must be (b) (4) for high samples and (b) (4) for low/LLOQ samples, with (b) (4) of matrices (individuals) meeting the criteria. In addition the potency for unspiked serum samples had to be (b) (4). The data provided are adequate to support specificity/selectivity of the assay for its intended use. However, the criterion as stated could have been met even if all the failing samples were from a given donor group, indicating a lack of specificity in that matrix. Future studies should increase the individuals per matrix or require (b) (4) of matrices (individuals) to meet the absolute mean relative error criteria.

The ability of AIG in serum samples to withstand degradation after a series of freeze-thaw/bench-top transitions was established using (b) (4)

                                            All samples met the recovery acceptance criterion of being within (b) (4) .

New lots of all critical reagents (b) (4)
                                    were qualified against set acceptance criteria prior to use in the assay.

CBER requested that Cangene justify why incurred human samples were not included in the validation. The sponsor responded that the TNA method for testing clinical serum samples was validated before clinical Study AX-001was performed; therefore, incurred samples were not available for inclusion in the validation. The firm further stated that incurred sample reanalysis (ISR) was performed for StudyAX-001 samples to demonstrate suitability of the method for incurred sample testing. The acceptance criteria was that ISR was deemed successful if (b) (4)
                    of the mean of the original and repeat results based on validation accuracy

limits. The ISR evaluation resulted in 98% of repeat results meeting the accuracy limits and therefore demonstrated suitability of the method for incurred samples.

To demonstrate long-term stability of the assay, Cangene provided the trended results for (b) (4)   used in the human TNA during the original method validation study and subsequently during routine use in the TNA to support clinical study AX-001. The %CV for the combined data were (b) (4)   The variability was within the limits for assay precision and therefore demonstrated assay stability from validation to sample analysis.

Conclusion
The TNA for product release appears to be appropriate for use as a bioanalytical method for control of the drug substances and drug product. The assay appears to be performing adequately for its intended purposes. The specifications for product release are within the range of the assay. TNAs for Pharmacokinetic Studies in rabbit serum samples, monkey serum samples and human serum samples appear appropriate for use to support non-clinical and clinical studies of AIGIV. The assays appear to perform adequately for their intended use.

Based on the information I reviewed, I recommend approval of the product.