

To: BLA STN 103628/5008 (NAB supplement)
BLA STN 103628/5021 (HuSA-free formulation)
From: Gary Kikuchi
Through: Elizabeth Shores, Chief LI, DTP, Amy Rosenberg, Director, DTP
Date: August 21, 2002

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ASR 11/16/02

Review of assay validation for Avonex immunogenicity (interferon beta-1a, for MS)

Table of Contents

Item	Page
I. Administrative	3
II. Summary	3
A. Product Summary	3
B. Timetable of Regulatory Submissions	3
C. Implications of antibodies to interferon beta	3
D. Structure of Review	4
E. Issues with Assay methods and validation	6
II. Biogen screening ELISA assay	7
A. Description of Assay methods	7
1. Overview	7
2. Detailed methods	7
3. Definitions and calculations	7
4. Problems/caveats/comments	7
a. Original FDA Comments	7
b. Sponsor CR response	8
c. FDA comment on information	8
B. Elements of validation of screening ELISA	10
1. Definition of cutoff value	10
a. Method	10
b. Problems/caveats/comments	10
2. Assay precision	10
a. Method	10
b. Outcome	10
b. Problems/caveats/comments	10
3. Assay sensitivity	11
a. Method	11
b. Outcome	11
c. Problems/caveats/comments	11
1) Original FDA comments	11
2) Sponsor CR response	11
3) FDA comment on information	12
4. Assay specificity	12
a. Method	12
b. Problems/caveats/comments	12
1) Original FDA comments	12
2) Sponsor CR response	13
3) FDA comment on information	13
5. Assay robustness	13
a. Problems/caveats/comments	13
b. Sponsor CR response	13

c. FDA comment on information	14
III. Biogen neutralizing antibody assay	15
A. Description of Assay methods	15
1. Overview	15
2. Detailed Methods	15
3. Definitions/Calculations	16
4. Problems/caveats/comments	17
a. sample dilution	17
b. Definition of CPE	17
c. Automated method for data analysis	18
B. Elements of validation of neutralizing antibody assay	19
1. Definition of cutoff value	19
a. Method	19
b. Outcome	19
c. Problems/caveats/comments	19
1) Original FDA comment	19
2) Sponsor CR response	19
3) FDA comment on information	19
2. Assay precision	20
Experiment 1	20
Experiment 2	20
Experiment 3	20
Original FDA comment	21
Sponsor CR response	21
FDA comment on information	21
3. Assay sensitivity	21
a. Comparison with WHO standards	22
b. Sensitivity based on monkey study	22
c. Comparison of NAB with ELISA	23
d. Sensitivity based on concordance	24
4. Assay specificity	24
a. Problems/caveats/comments	24
Original FDA comment	24
Sponsor CR response	24
FDA comment on information	24
5. Assay robustness	25
Original FDA comment	25
Sponsor CR response	25
FDA comment on information	26
C. Comparison between Biogen and Assays	27
1. analysis	27
A. Method	27
B. Outcome	28
C. Problems/caveats/comments	28
2. Tables of concordance	28
Sponsor CR response	28
FDA comment on information	29
Comments to Sponsor	30

I. Administrative

Immunogenicity Reviewer: Gary Kikuchi
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 CSO: Karen Winestock

Milestones: BLA STN 103628/5021 Action due date September 8, 2002
 BLA STN 103628/5008 Action due date November 12, 2002

II. Summary**A. Product Summary:**

Avonex (BG9418) is interferon beta-1a licensed by Biogen, for multiple sclerosis. The following table summarizes the status of commercial preparations of interferon-beta:

Company	Trade Name	Generic name	Host cell	1 α sequence	Glycosylated	Route	US license
Biogen	Avonex	IFN-beta-1a	CHO	Native	Yes	IM	Approved
Berlex/Chiron	Betaseron	IFN-beta-1b	E.Coli	Ser17Cys	No	SubQ	Approved
Serono	Rebif	IFN-beta-1a	CHO	Native	Yes	Sub Q	Approved

The interferon-beta material designated BG9015, which was used for the pivotal phase III trials to support this license application, was originally manufactured as a joint venture between Biogen Inc. and Rentschler AG. Avonex (BG9418) is the material licensed by Biogen; the license was approved based on the phase 3 clinical data using BG9015.

B. Timetable of regulatory submissions

4/6/01 Submission of 103628/5008, the NAB supplement
 2/5/02 CR letter issued for 103628/5008
 5/9/02 CR response received for 103628/5008
 5/9/02 Submission of 103628/5021, the HuSA-free supplement
 9/8/02 Action due date for HuSA-free supplement
 11/12/02 Action due date for NAB supplement

C. Implications of antibodies to interferon-beta 1a

The implications of neutralizing antibodies to interferon-beta that cross-react with endogenous interferon in MS patients are briefly mentioned within the supplement. Neutralizing antibodies (NAB) reduce pharmacodynamic parameters such as induction of neopterin or beta 2 microglobulin. In addition, NAB reduce the clinical efficacy as assessed by a reduction in gadolinium positive (Gd+) MRI lesions. The sponsor states that there is no correlation between NAB status and disability progression or relapse rate on interferon-beta 1a treatment in the phase 3 pivotal study, although some effect on

these outcomes is briefly mentioned. Knockout studies indicate that mice with targeted disruptions in the type I interferon receptor are more susceptible to viral infections. However, perhaps in part due to the redundancy in the type I interferons (there are at least 12 known forms of interferon alpha, as well as interferon beta), novel clinical syndromes due to presence of patient antibodies to interferon beta have not yet been described.

D. Structure of Review:

This review covers assay methods and validation for the Biogen ELISA and neutralizing antibody assay, provided in the NAB supplement (103628/5008). The review incorporates new information, dated 5/9/02, provided by Biogen in response to the CR letter of 2/5/02. The assay methods and validation are also essential for review of the HuSA-free supplement (103528/5021.)

The pivotal phase 3 clinical trial used to support licensure of Avonex (BG9418) was based on BG9015 material, and used the _____ assay to monitor immunogenicity. Detailed methods and validation regarding the _____ assay are not available. The sponsor states that the _____ assay was a CPE neutralization assay, based on the _____ The _____ assay used _____

The NAB supplement proposes a new assay, the Biogen assay, to monitor immunogenicity. The Biogen assay consists of an ELISA screening assay and a neutralization assay for ELISA-positive samples:

1. The screening ELISA is []
2. The neutralizing antibody assay is based on the protective effect of interferon beta to inhibit viral cytopathic effect (CPE). In this assay, []

With regard to assay methods and validation, the NAB supplement contains two general types of information:

- Laboratory methods and validation information regarding the ELISA and the neutralizing antibody assay
- Comparison of the Biogen assay with the _____ assay based on clinical data

This review focuses on laboratory methods, validation, and elements of comparison between the _____ and Biogen assays presented by the sponsor. Additional analysis of the clinical data, using statistical analysis of the _____ transport file, is outside the scope of this document.

Biogen also requests a labeling change in the NAB supplement. The sponsor states that BG9418 has a reduced incidence of immunogenicity, approximately 5%, versus approximately 24% observed in phase 3 trials with BG9015. The sponsor states that the immunogenicity assays are comparable and that manufacturing changes for the bulk drug substance and drug product are the reason for reduced immunogenicity of BG9418.

The HuSA-free supplement (103628/5021) contains antibody incidence and adverse event data from clinical trial C98-844. This was a multi-center, single-arm, open label study of 153 MS patients (intent-to-treat basis) treated with HuSA-free Avonex at 30 mcg weekly IM. Presence of antibodies was determined using the assays described in 103628/5008 (NAB supplement.) The sponsor stated that the incidence of immunogenicity observed in this clinical trial with the liquid formulation was similar to the incidence found in previous clinical trials found with the lyophilized formulation. The incidence rate is quoted as 24%, which is the rate based on clinical trials with BG9015 available at approval. There were no revisions to the immunogenicity section of the label submitted to the HuSA-free supplement.

E. Issues with Assay methods and validation:

The assays appear to be, for the most part, appropriate in design and validation.

For the ELISA assay, the key issue is whether the _____
 _____ Biogen has amassed considerable
 information regarding interferon beta structure and the _____
 _____ based on this information, a more structural approach to the
 concern regarding _____ is needed. This information could be provided as a
 post-marketing commitment if data is not available. Validation of the ELISA assay
 appears appropriate.

For the neutralizing antibody assay, some clarification is needed regarding several minor
 points regarding validation:

- Clarification regarding how _____

- A quantitative definition is needed for _____ CPE that ensures operator uniformity.
- More detail is needed comparing the _____ of reading CPE with the

- Information is needed regarding establishment of cutoff based on appropriate
 statistical analysis of sera from healthy patients
- The potential for inter-operator variability needs to be quantified.

With regard to comparison of the clinical results of the Biogen assay with the _____
 _____ assay, this comparison is not complete, because review of the statistical _____
 transport database has not been completed. However, the following information may be
 helpful in establishing the validity of the Biogen assay:

- More in depth regression analysis, including plotting data of low titer and
 calculation of a true regression curve.
- Reports comparing the Biogen assay with the _____
 _____ neutralizing antibody assays described in the supplement.

III. Biogen screening ELISA assay

A. Description of assay methods

1. Overview: As mentioned above, the screening ELISA is a

[Redacted content]

4. Problems/Caveats/Comments:

a. **Original FDA comments in CR letter of 2/5/02:** The key concern stemming from assay design is whether the _____ The text of this comment (5a) from the CR letter was: *The ELISA screening assay for antibodies to interferon beta-1a has not been fully validated. Please provide ...clarification to determine whether there is potential for _____ that could significantly affect the screening ability of the ELISA for patient antibodies.*

c. **Sponsor CR response (dated 5/9/02):** To address this question, the sponsor provided empirical data regarding whether serum samples that are negative for antibodies in the screening ELISA are also negative in the neutralizing assay.

The sponsor described 122 ELISA negative serum samples were tested in the neutralizing antibody bioassay. Of these, 60 samples were collected at time points 9-12 months post-treatment. Only 3/122 ELISA negative samples were shown to have neutralizing activity and all returned titers \leq below the proposed biological cutoff of the assay.

The sponsor also described other datasets analyzed in collaboration with outside laboratories: _____ (583 samples); _____ (128 samples); _____ (20 samples); and _____ (36 samples.) In all cases, the collaborating laboratory only performed a neutralizing assay, whereas Biogen performed the ELISA screen and then the neutralizing assay. Of 699 samples, 114 were discordant overall: 100 of those were positive in the Biogen ELISA and neutralizing assays, but negative in the neutralizing assays run in an outside laboratory, and only 14 (2%) were positive in neutralizing assays and negative in the Biogen assay.

The sponsor concludes that these data provide evidence that the _____ does not significantly _____

There are several possible explanations for this:

[]

c. **FDA comment on information to date:** This response is not acceptable because it does not address the FDA concern using available data regarding interferon beta-1a structure. Biogen scientists are authors on at least three papers describing in detail the secondary and tertiary fine structure of interferon beta-1a, and the location of the binding site of the molecule _____

[]

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3. Assay sensitivity:

a. **Method:** Pooled monkey samples, which were titered in the antibody ELISA assay, were also titered in the neutralizing assay. The data for this experiment are shown below:

	NAB titer SOP160a.48 issue 2	NAB titer SOP 160a.48 issue 1	ELISA Assay titer
Monkey A (#QCB950090)	178	Not done	8000
Monkey B (#QCB950205)	533	Not done	8000
Monkey C (#87-120)	8538	4000	16000

(Note: In SOP 160a.48 Issue 1, the samples were _____ resulting in a detection limit of \sim and in method SOP 160a.48 Issue 2, the samples were _____ resulting in a detection limit of \sim Issues regarding this are discussed further below.)

b. **Outcome:** The sponsor states that these that these results show that the antibody ELISA assay will pick up antibodies at a higher dilution than in the neutralizing assay, making the ELISA an appropriate IFN-beta antibody screen.

c. Problems/Caveats/Comments:

1) **Original FDA comments in CR letter of 2/5/02:** Additional validation of assay sensitivity is needed as described in comment 5b: *Please provide...information [regarding] ... the sensitivity of the ELISA expressed either in mass units or in terms of WHO international standards for antibodies to interferon beta-1a.*

2) Sponsor CR response (dated 5/9/02):

The sponsor stated that determination of sensitivity in terms of mass units was not done, as expressing sensitivity in mass units is problematic because of epitope specificity and differences in affinity. Furthermore, the sponsor cited the ICH Guidelines for antigenicity assays (published in 1997, Section 3.6, Docket No. 97D-0113, Federal Register Vol 62 No. 222, p61517, attached) as evidence that results from antibody testing should be reported as titers. Therefore the sponsor expressed sensitivity in terms of dilution and titers.

To address the FDA comment, the sponsor provided information regarding the titer of WHO international reference preparation of antibodies to Interferon beta-1a (G038-501-572) as shown in the table below.

Comparison of Titers Obtained in the Biogen ELISA Titration Assay to Reported NAB Titers		
Reference Preparation	Biogen NAB Result Log Titer	Biogen ELISA Result Log Titer
G038-501-572 Human Anti-IFNbeta	—————	—————

The log titer of the international standard, using the Biogen ELISA ——— is higher than the neutralizing antibody titer. The ELISA titer is higher than the neutralizing antibody bioassay, which makes the ELISA an appropriate screen. These results also compare favorably to the published result of 3.24 ± 0.34 from the international collaborative study.

3) **FDA comment on information provided to date:** Sensitivity expressed in terms of WHO international standards is acceptable.

4. **Specificity**

a. **Method:** The original validation report, Bioassay report BA18-003, described the specificity of the ELISA for Avonex in the presence of BG9588 ———

b. **Problems/Caveats/comments:**

1) **Original FDA comment in CR letter of 02/05/02:** These studies do not examine the specificity of the ELISA for reagents that resemble the product more closely than ———, such as interferon-alpha (which is known also to be reactive in the antiviral assay) interferon gamma, other cytokines, or other antibodies. The text of comment (5c) is: *Please provide...information validating the specificity of the ELISA in terms of the ability of this assay to distinguish between antibodies to interferon alpha, beta, and a negative control cytokine.*

2) **Sponsor CR response (dated 5/9/02):** The sponsor tested specificity using

Screening ELISA and NAB Assay Results Using Various Anti-IFN Reference Antisera

Reference Preparation	Screening ELISA Result	NAB Assay Result
[]	[]	[]

The sponsor asserted that specificity was demonstrated, because the ELISA did not recognize _____ and the neutralizing antibody assay did not recognize either _____
 _____ The sponsor stated that the cross-reactivity of the : _____
 _____ in the ELISA is most likely due to the sequence homology (~30%) between _____
 _____ and IFN-beta.

3) **FDA comment on information provided to date:** Validation of assay specificity is acceptable.

5. **Robustness**

a. **Problems/Caveats/Comments:** Assay robustness, or stability of assay with regard to freeze-thaw and long-term storage was not validated in the original assay. The text of comment 5d is: *Please provide...Data demonstrating that the ELISA has been optimized with regard to variation in all key parameters, including reagent concentration, sample freeze-thaw and stability of serum samples over long-term storage.*

b. **Sponsor CR response, dated 5/9/02:**

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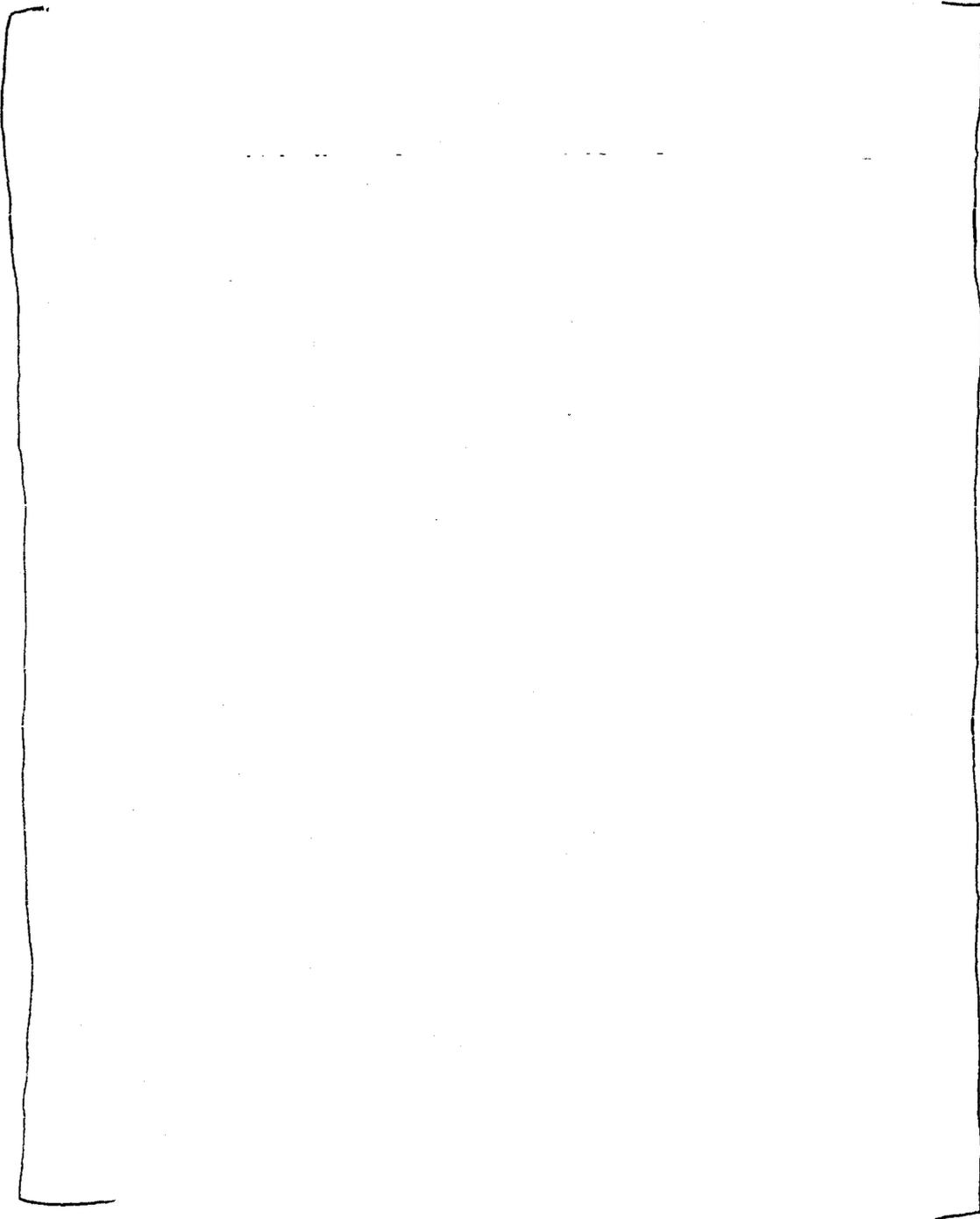
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IV. Biogen Neutralizing Antibody Assay

A. Description of assay methods

1. Overview: Neutralizing antibody is detected by its ability to neutralize the protective effect of interferon beta in a cytopathic effect (CPE) assay. In this assay,



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4. Problems/Caveats/comments:

The calculation method is that of _____ which is accepted in the literature. There are three issues stemming directly from these methods, regarding

- a. Sample dilution
- b. Definition of CPE
- c. Use of automated methods for data analysis

a. **FDA comment regarding sample _____ in CR letter of 02/05/02:** There are two versions of the Biogen neutralizing antibody assay described in SOPs 160a.48 issue 1 and issue 2. In SOP 160a.48 Issue 1, the samples were _____ resulting in a detection limit of _____ and in method SOP 160a.48 Issue 2, the samples were _____ resulting in a detection limit of _____

	Monkey A (#9)		Monkey B (#20)		Monkey C (#13)	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
Day 1	<4	<10	<4	<10	<4	<10
Day 2	<4	<10	4	<10	4	30
Day 3	4	30	12	30	108	270
Day 4	12	30	36	30	108	810

Two alternative methods are described, a _____ and a _____. In the SOP and validation protocol, only a _____ is mentioned. However, in the concordance database, neutralization titers as low as _____ are reported. The text of this comment (6a) is: *Please provide ...clarification regarding whether the _____ used during clinical testing is the same as that described in the validation report. If not, please provide a justification for use of the unvalidated _____*

Sponsor CR response (dated 5/9/02): A sample _____ is used for clinical sample testing which was also used during assay validation. Section 4.4.5.1 of the Neutralizing Antibody Assay SOP (#160a.48, submitted May 14, 2001, AVONEX.001) states that the samples must be _____ which results in a _____ final on-plate _____. On page 4 of the Validation Report (BA18-041 submitted May 14, 2001, AVONEX.001) in section 3.2, it is stated that the final serum concentration is _____ serum/well.

FDA comment regarding information provided to date: The sponsor has clarified that the final sample _____. However, the sponsor needs to clarify how neutralization titers as low as _____ are reported if the sample _____

b. **FDA comment regarding definition of _____ CPE":** The SOP and validation protocol do not explain how terms like _____ CPE," are defined. This allows for some subjectivity in interpretation of results, which may be reflected in operator variability. The text of this comment (6f) is: *Please provide...a definition of _____ CPE.*

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5. Specificity

a. **Problems/caveats/comments.** In the original information provided May 2001, information regarding specificity of the neutralizing antibody assay was not provided. **FDA Comment in CR letter of 02/05/02: *Please provide...information validating the specificity of the neutralization assay in terms of the ability of the assay to distinguish between antibodies to interferon alpha, beta and a negative control cytokine.***

Sponsor CR response of 5/9/02: To determine specificity of the NAB bioassay,

_____ were tested in the anti-viral assay. As shown below, in the NAB bioassay, only the anti-IFN beta antiserum was able to neutralize the ability of IFN-beta to protect against _____ virus infection. Please also refer to our response to 5c.

NAB Assay Results Using Various Anti-IFN Reference Antisera

Reference Preparation	NAB Assay Result
[]	[]

FDA comment on information to date: Information regarding assay specificity is acceptable.

6. Robustness

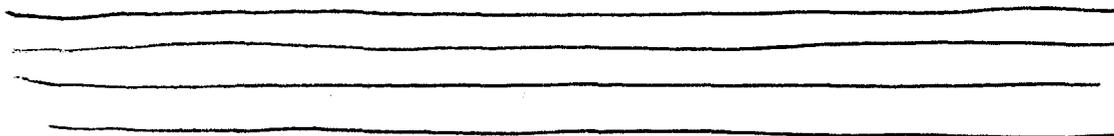
a. **Problems/Caveats/Comments:** In the original information provided May 2001, information regarding assay robustness was not provided. **FDA Comment in CR letter of 02/05/02: *Please provide...evidence that the neutralization assay has been optimized with regard to variation in all key parameters, including reagent concentration, sample freeze-thaw and stability of sample over long-term storage.***

Sponsor CR response of 5/09/02: To address issues of assay robustness and optimization, the sponsor provides Method Development Report BA18-008. In this report, the assay was optimized with respect to



Information regarding optimization and preparation of EMC virus is provided in SOP#160a.65.

The effects of freeze-thaw cycles of patients samples were also provided by the sponsor.



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Comments to Sponsor:

1. For the screening ELISA assay, please commit to providing additional information regarding whether the _____
 _____ You have provided data suggesting that some positive human serum samples may be completely _____
 _____ Please assess the extent of _____
 _____ serum samples, and also assess the incidence of patient samples with that are significantly _____

2. Regarding validation of the neutralizing antibody assay, please provide:
 - a. Clarification regarding how neutralization titers as low as _____ are derived based on sample _____
 - b. A quantitative definition of _____ CPE that ensures operator uniformity.
 - c. Assurance that the _____, gives equivalent results to the _____ for which validation information has been supplied.
 - d. Information establishing the cutoff based on appropriate statistical analyses of sera from healthy patients.
 - e. Quantification of the potential for inter-operator variability.

3. Regarding comparison of the clinical data derived using the Biogen immunogenicity assay, please provide:
 - a. Revised _____ analysis, comparing the Biogen and the _____ assay at all titers (including those _____) and statistical calculation of the _____ based on these data.
 - b. Please provide summary reports of comparison between the Biogen assay and the _____ neutralizing antibody assays described in the supplement.