



# Development of a Biological Assay to Analyze Neutralizing Antibodies Directed against Human IFN- $\beta$ .

Jessica J. Esposito<sup>1,2</sup>, Sara Crisafulli<sup>2</sup>, Thomas B. Lavoie Ph.D.<sup>2</sup> and Ronald G. Jubin, Ph.D.<sup>2</sup>  
 Kean University 1000 Morris Avenue Union NJ 07083<sup>1</sup>  
 PBL Biomedical Laboratories, 131 Ethel Road West, Suite 6, Piscataway, NJ 08854<sup>2</sup>

## Abstract

Interferon-beta (IFN- $\beta$ ) is the most commonly prescribed treatment for Multiple Sclerosis. Although not a cure, it does modify the disease course and reduce progression. IFN- $\beta$  therapy is a life-long commitment therapy with a consequential formation of antibodies (Abs) that can bind to the therapeutic agent. Therapeutic formulations of IFN are sold based on international units determined via antiviral assays. These assays represent a good basis for the development of an assay to screen the neutralizing capacity of anti-IFN- $\beta$  antibodies (NABs) that may develop in patients undergoing IFN- $\beta$  treatment for MS. In assay development, sensitivity, precision and robustness are important parameters; however, the most challenging aspect of NAb assay development is preparation of a potent NAb to serve as a positive control. Since Ab activity cannot be determined *a priori*, the only way to evaluate candidates is through rigorous screening.

## Introduction

Interferon-beta (IFN- $\beta$ ) is the most commonly prescribed treatment for Multiple Sclerosis. By an unknown mechanism, this treatment reduces the damaging effects to the nervous system in relapsing-remitting and progressive MS and thus slows the progression of disease. Treatment is essentially for life since IFN- $\beta$  is not a cure.

As seen with other biotherapeutics, a certain percentage of the patients develop antibodies toward IFN- $\beta$ . The two types of antibodies produced are defined in functional terms. Binding antibodies (BAbs) are any antibodies that can bind to the IFN- $\beta$ . Neutralizing antibodies (NABs) are a subset of BAbs which have the ability to inhibit the biological activity of the IFN- $\beta$ . NABs are of particular concern since they may interfere with the therapeutic efficacy of the IFN- $\beta$ .

Interferon was originally identified as an antiviral protein and inhibition of viral-mediated lysis is still the standard assay used for IFN. When cells in culture are challenged with a virus they may be subject to death and lysis, otherwise known as Cytopathic Effect (CPE). By preventing viral killing, the interferon blocks the CPE. The assay we use is based on the inhibition of the CPE. When there are NABs in a particular sample, the effectiveness of the interferon is blocked and CPE is observed.

## Results

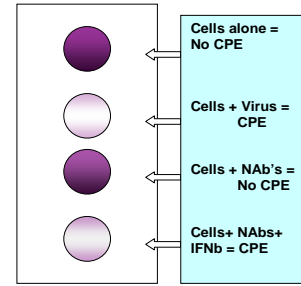


Figure 1a. CPE Representation.

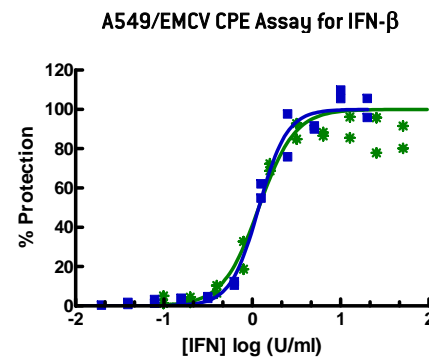


Figure 1b. CPE Assay.

Interferon activity was measured using a cytopathic effect assay (CPE). The assay is based on the ability of encephalomyocarditis virus to kill the human lung carcinoma cell line A549. Cell density and EMC virus dilution were optimized to yield a good differential between the cell control (no virus) and the virus control (no IFN). Under these conditions, IFN- $\beta$  protects the cells in a dose dependent manner. Shown above are duplicates from the titration of IFN- $\beta$  in the A549/EMCV CPE assay. The EC50 for interferon generally ranges from 0.5 to 2 unit/ml.

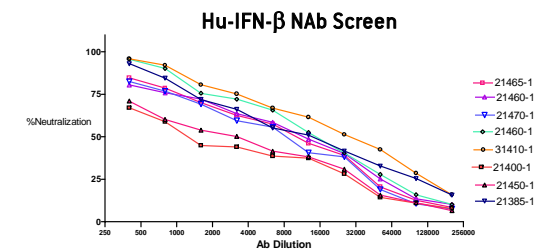


Figure 2a. IFN- $\beta$  1a Dose-dependency on the neutralizing effects of anti-IFN- $\beta$  Abs (Part I).

A series of Monoclonal (MAb) and Polyclonal (PAb) antibodies were examined for neutralization potential. In one format, antibodies were serially diluted and screened against a constant concentration of IFN- $\beta$ -1a at 10 IU/mL. Several candidate antibodies were identified by this approach. All Abs displayed neutralizing activities in a dose-dependent manner, with the PAb 31410-1 displaying best fit neutralization.

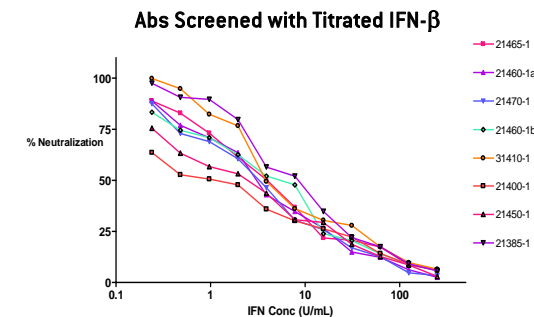


Figure 2b. IFN- $\beta$ -1a dose-dependency on the neutralizing effects anti-IFN- $\beta$  Abs (Part II).

Clearly to have utility as a standard the antibody chosen should work in multiple assay formats. In order to examine this potential we diluted the antibodies (1:100) and incubated them with varying concentrations of IFN- $\beta$ -1a. Again, PAb 31410-1 displayed most potent activity and low CV rate with MAb 21460-1 exhibiting good NAB activity and a low CV rate.

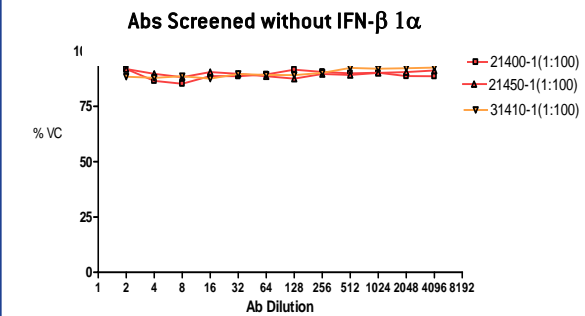


Figure 3a. Specificity of NAb assay: Abs screened in AVA without IFN- $\beta$  1a.

To examine the presence of any off-target effects associated with a NAb assay, we examined the Abs without IFN- $\beta$  (Figure 3a) in an AV assay not displaying a dose-dependent alteration of AV activity. To further determine whether the Abs might affect type I IFN signalling, we performed a study using IFN- $\alpha$  in place of IFN- $\beta$  (Figure 3b) with Abs not exhibiting any dose-dependent inhibition of IFN- $\alpha$  in place of the IFN- $\beta$ -1a.

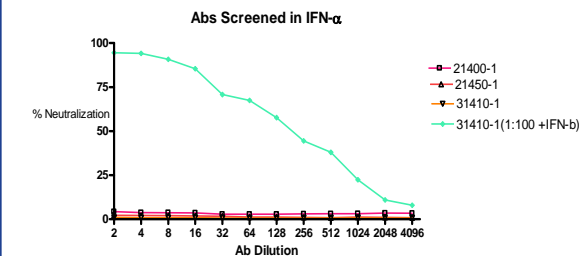


Figure 3b. Specificity of NAb assay: Abs screened in AVA with IFN- $\alpha$ .

Inter-Operator Performance	Ab	Neutralization Titer (U/mL)
Operator 1 (JE)	31410-1 (1:10)	2.13 x 10e4
	31410-1 (1:100)	2.5 x 10e4
Operator 2 (SC)	31410-1 (1:10)	2.0 x 10e4
	31410-1 (1:100)	2.56 x 10e4

Table 1. Inter-operator Study.

To establish a robust assay it is critical that different operators obtain similar results. The table above shows the results of one such study. As can be seen the results are very comparable.

Ab	Neutralization Titer (U/mL)	Neutralization Titer (U/mL)	Intra-Assay Precision (CV%) Ab at 50% CPE	Inter-Assay Precision (CV%) Ab Screen
21460-1	4.19 x 10e4	5.79 x 10e4	18.3	10.8
21470-1	4.94 x 10e4	9.89 x 10e4	11.72	9.67
21465-1	4.19 x 10e4	2.47 x 10e4	8.23	6.48
21400-1	2.12 x 10e4	5.79 x 10e4	9.18	8.26
31410-1	2.2 x 10e4	1.62 x 10e5	8.05	5.09
21450-1	4.09 x 10e4	1.61 x 10e4	23.6	14.87

Table 2. NAb activity and assay precision.

Next we calculated the neutralizing activity of each Ab to directly compare efficacy (Table 2) with PAb 31410-1 displaying highest neutralization capacity. In addition, inter- and intra-assay precisions were evaluated for each Ab. Again, PAb 31410-1 displayed most potent activity and low CV rate with MAb 21460-1 exhibiting good NAB activity and a low CV rate. Inter-operator performance (Table 1) was evaluated with PAb 31410-1, showing little variation in final neutralization titers.

## Conclusion

The results reported in this study have shown that a panel of anti-IFN- $\beta$  antibodies displayed varying neutralizing capacities. PAb 31410-1 displayed the most potent activity; however, PAb activity can shift over time as the immune response to the antigen changes in the animal. Separately we identified at least one MAb 21460-1 that has potential use in these assays as well. Being a MAb, it targets a single epitope, whereas PAB's have a complex mixture. Therefore, we cannot be certain of the actual anti-IFN- $\beta$  antibody concentration within the total protein levels. Calculation and comparison of neutralization titers further demonstrate that PAb 31410-1 has the most potent neutralization and MAb 21460-1 displays good NAB activity, both with low CV rates. Comparison of the assay with inter-operator performance confirms a stable NAB assay with little variation in final neutralization titers. The initial studies are promising and warrant further expanded studies with MS patient serum samples to better determine the assay sensitivity, precision and false positive rate.