iLite® Type I IFN
ASSAY READY CELLS

***iLite* Type I IFN Assay Ready Cells are type I IFN responsive reporter gene cells designed for use in assays measuring the functional activity of type I Interferon (IFN) or the presence of neutralizing antibodies against type I IFN.**

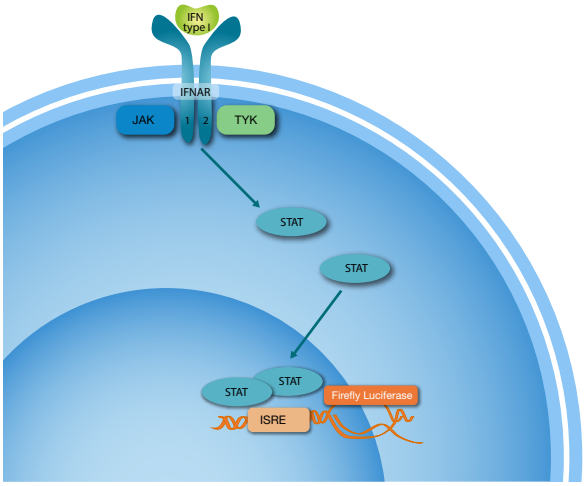
Type I IFNs are cytokines mainly produced by immune cells, their main functions including activation of immune cells and involvement in the innate immune response against viral infections. As pharmaceuticals, IFN-alpha is widely used for treating a range of hematological cancers while IFN-beta is an established therapy for treatment of remitting multiple sclerosis. In both types of treatment, development of neutralizing antibodies (NAbs) has shown to be correlated with loss or reduction of response, demonstrating the need for Type I IFN NAb assays.

Although Type I IFN drugs have been on the market for a long time, the immune activating nature of Type I IFN has recently sparked interest in new drug development, with several new inhibitory agents currently in the pipeline for inflammatory diseases such as systemic lupus erythematosus (SLE).

With the *iLite* Type I IFN Assay Ready Cells, unique on the market, the functional activity of Type I Interferon can be measured, along with the presence of NAbs against IFN-alpha or IFN-beta. Due to the functional nature of the assay, both Type I IFN, and any inhibitory agents against Type I IFN or its receptor can be measured using the same assay.

The assay is run in a standard 96 well format, and is as easy as running an ELISA.

- Highly sensitive assay with large dynamic range
- Unique Assay Ready Cell format
- Highly reproducible assay results



***iLite*® Type I IFN Assay Ready Cells**	
Product code	BM3049
Format	Assay Ready Cells

Related Products		Complementry Products	
BM3044	*iLite*® TNF-alpha Assay Ready Cells	BM3249	*iLite*® IFN beta 1a
BM4050	*iLite*® GM-CSF Assay Ready Cells	BM3251	*iLite*® IFN beta 1a NAb positive control
BM4012	*iLite*® IL-12 Assay Ready Cells	BM3250	*iLite*® Diluent D
BM4023	*iLite*® IL-23 Assay Ready Cells	BM3134	*iLite*® Diluent B

Application	The *iLite*® Type I IFN Assay Ready Cells can be used for quantification of IFN α or β and for measurement of both anti-IFNα antibodies and anti-IFNβ antibodies. Application Notes for the following assays are available: • Quantification of IFN α or β • Determination of neutralizing antibodies to Type I IFN
Incubation time	Type I Interferon: 30min + 18 hours NAbs to IFN alpha or beta 1a: 30min + 18 hours
Detection system	Luminescence
Availability	Research Use Only (RUO)*

**These products are intended for professional research use only. The data and results originating from using the products, should not be used either in diagnostic procedures or in human therapeutic applications.*

In accepting delivery of iLite® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third party, and recipient is only to use them directly in assays. The iLite® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered iLite® Assay Ready Cells would constitute an infringement.

Doc No: E-252-GB03, December 2020

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Answers in Life Science

Comparison of methods for measurement of
TYPE I INTERFERON NEUTRALIZING ANTIBODIES

INTRODUCTION

Human Interferon (huIFN) beta is a member of the Type I Interferon family. Type I Interferons are endogenous glycoproteins with immunomodulatory, antiviral and anti-proliferative effects (1). In the clinic, huIFN beta is used to treat patients with relapsing, remitting multiple sclerosis (MS) and other diseases. A number of studies have demonstrated that induction of anti-IFN beta antibodies with neutralizing capacity (NAb) during recombinant IFN-beta treatment leads to reduced efficacy of treatment (2, 3). Today, medical authorities require that anti-drug antibodies (ADA) are monitored during drug development, and recommend monitoring during clinical use (4, 5).

Here, a comparison is presented between the traditional "gold standard" assay for measurement of NAbs against IFN beta; the cytopathic effect reduction (CPE) assay, and a newer technology, the reporter gene assay (RGA).

The CPE and RGA Assays

In the case of the CPE assay, the anti-viral effect of IFN beta is employed to measure the presence of NAbs. In brief, serum samples with suspected NAbs are pre-incubated with a known amount of IFN beta and subsequently added to cells, in this case A549 cells. After incubation, virus is added to the cells to allow infection (EMCV in this study), which ultimately leads to cell death. In the serum samples where IFN beta activity is high, the cells will be protected from the virus, resulting in less cell death. In the serum samples where NAbs are present, IFN beta activity will be lower, thus leading to higher degrees of cell death (6).

In this comparison, the *iLite* Type I IFN Assay Ready Cells were used as the RGA. These cells are based on human promonocytic U937 cells, which have been stably transfected with a luciferase reporter gene under the control of a Type I IFN responsive chimeric promoter. Stimulation of the cells with IFN beta will lead to activation of the reporter gene and a proportional luminescent readout. If NAbs are present in the serum, the IFN beta activity will be neutralized resulting in a decrease of the luminescent signal (7). Further accounts of the performance of the *iLite* Type I IFN Assay Ready Cells can be found in papers by Hermanrud et al (8) and Wadhwa et al (9).

RESULTS

Comparisons between the two NAb assays were done on two different MS patient cohorts, at two different laboratories. In the first screening cohort, serum samples from 74 patients were analysed using both methods. As seen in Table 1, the two methods were in complete agreement, resulting in an accuracy of 100%.

***iLite* IFN assay**	**CPE assay with A549 cells**		**Total**
	Positive	**Negative**	
Positive	15	0	15
Negative	0	59	59
Total	15	59	74
Sensitivity	100% (95% CI*: 82%-100%)		
Specificity	100% (95% CI*: 95%-100%)		
Accuracy	100% (95% CI*: 96%-100%)		

** Binomial confidence interval.*

Table 1. *A first cohort of serum samples from 74 MS patients was analyzed for presence of NAbs using the CPE and iLite methods.*

In a second cohort, serum samples from 235 MS patients were analysed for the presence of NAbs and compared between the two methods. In this larger cohort, the accuracy was found to be 95%, as seen in the table below.

***iLite* IFN assay**	**CPE assay with A549 cells**		**Total**
	Positive	Negative	
Positive	93	3	96
Negative	8	131	139
Total	101	134	235
Sensitivity	92% (95% CI*: 85%-97%)		
Specificity	98% (95% CI*: 94%-100%)		
Accuracy	95% (95% CI*: 92%-98%)		

** Binomial confidence interval.*

Table 2. *A second cohort of serum samples from 235 MS patients was analyzed for presence of NAbs using the CPE and iLite methods.*

Taken together, these results indicate that the RGA *iLite* Type I IFN Assay Ready Cells performs as well as the current gold standard, the CPE assay.

CONCLUSION

In this study, two different assays for measurement of NAbs in sera from MS patients treated with IFN beta were compared. Using a CPE assay with A549 cells and a reporter gene assay, *iLite* Type I IFN Assay Ready Cells, the results showed confidence intervals between 0.82-1.0 for the sensitivity, specificity and accuracy, thus demonstrating that no significant difference in performance could be found between the two assays. However, although similar results were obtained, it is noteworthy that the CPE assay was more cumbersome to perform and the assay turnaround time was more than twice as long as the *iLite* assay. The CPE assay relies on many complex factors, e.g. the metabolic state of the cells, virus replication, the ability of the IFN to protect the cells, and the manual determination of cell death, which all lead to an inherent variability of the assay (10, 11). In this study, the reproducibility of the *iLite* assay was found to be in the range of 20-28%, for inter lot, day, assay and operator. In addition, similar results were obtained from two different laboratories, further demonstrating the robustness of the assay. In conclusion, *iLite* Type I IFN Assay Ready Cells was found to be an attractive option for measurement of NAbs against IFN beta, being equal in performance to the current gold standard and superior in terms of turnaround time and workload.

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