Utility and Application of the *VeriKine-HS™* Human IFN-β Serum ELISA Kit

A pbl interferonsource White Paper

Abstract

The induction of type I interferons (IFNs) is a first line of defense against myriad pathogens encountered by the body. One member of this family, IFN-β, has long been known to function as an inhibitor of viral replication as part of the body's innate antiviral response. IFN-β is also widely used as a treatment for Multiple Sclerosis patients, either as a single agent or as part of a combinatorial approach. In addition, with the resurgence of interest in antitumor immunity as a promising treatment approach for several types of cancer, there has been renewed focus on applying IFN-β as a therapy due to the anti-tumor and immunomodulatory properties of the protein. Furthermore, ligation of Toll-Like Receptors (TLRs) leads to IFN-β production and, as novel TLR agonists and antagonists are being developed as therapeutics for several diseases, this warrants the monitoring of IFN-β as a downstream readout for compound effectiveness. A phenomenon often observed with cytokines is their ability to exert opposing effects in different contexts. Therefore, during clinical trials it is crucial to monitor IFN-β levels in order to ensure a desirable outcome is obtained. Inarguably, there is a necessity for a relatively simple, cost effective, and highly reproducible assay for detecting IFN-β. Available from PBL InterferonSource, a trusted leader in the interferon assay field, the *VeriKine-HS™* Human IFN-β Serum enzyme linked immuno-sorbent assay (ELISA) kit (PBL InterferonSource catalog # 41415) stands out for ease of use, sensitivity, and reproducibility. The applications of this ELISA in a variety of R&D and clinical settings are described below, followed by an overview of the unique methodological benefits of this assay.

Multiple Sclerosis and IFN-β

Although IFN-β administration is currently the standard of care for patients with multiple sclerosis (MS), nearly a third of patients fail to respond to this therapy. Efforts to improve the efficacy of IFN-β therapy for MS patients continue[1]. New formulations and derivatives of IFN-β with improved pharmacokinetics are being developed to achieve both heightened therapeutic efficacy and decreased frequencies of patient injections. Recent evaluation of the immune response in MS patients has suggested that a failure to respond to IFN-β therapy in some patients may be linked to high pre-treatment serum levels of IL-17 and IFN-β, as individuals with this phenotype have been shown to respond poorly to IFN-β therapy[2]. Additionally, many patients develop neutralizing antibodies to the IFN-β therapeutic protein, thereby decreasing biologic activity and effectiveness[3]. Implications of both of these phenomena are significant, as having the ability to accurately predict the likelihood that a given patient may respond positively to IFN-β therapy will not only be of enormous clinical benefit, but will also aid in the development of novel therapeutics by allowing researchers to differentiate patient susceptibilities. To promote continued discovery in this field, researchers and clinicians must be able to rapidly and reproducibly determine the levels of IFN-β present in patient samples. In response to these needs, PBL InterferonSource has developed the *VeriKine-HS™* Human IFN-β-specific colorimetric sandwich ELISA, as well as a biological assay to analyze neutralizing antibodies against IFN-β.

IFN-β and Pharmacokinetics (PK)

Currently, there are several IFN-β therapeutics on the market that are approved for the treatment of MS. Both IFN-β biogenerics and next generation molecules, including hyperglycosylated and pegylated versions, are in clinical trials (www.clinicaltrials.gov). Pegylation of IFN-β alters the pharmacokinetics of the molecule such that sustained serum levels of IFN-β can be achieved after a single injection[4, 5]. This, in turn, would decrease the required frequency of injections for patients, thereby providing an advance in care and better compliance. Many ongoing studies utilize co-administration of IFN-β and other drugs to examine the net result on patient responses to therapy[6]. In these situations, both pharmacokinetic and pharmacodynamic evaluations must be performed to identify optimal doses of each agent, as these may differ from when either drug is administered as a single agent. These types of clinical evaluations require a means for both rapid and accurate detection of IFN-β in patient samples. Along with native human IFN-β, the *VeriKine-HS™* assay detects several versions of therapeutic IFN-β including IFN-β expressed in mammalian cells and in E. coli. In addition, this assay may also be useful in quantifying IFN-β molecules which have been covalently linked to polyethylene glycols (PEG) to enhance serum half-life.

IFN-β as an Anti-Tumor Immunotherapeutic

In addition to the use of IFN-β as a treatment for MS, several trials have been conducted to ascertain the possible benefits of the molecule in a variety of cancers. Numerous groups have reported the ability of IFN-β to mediate complex antitumor effects, including direct induction of cancer cell apoptosis and potent enhancement of antitumor immune responses through stimulation of macrophages, dendritic cells, natural killer cells, and T cells[4, 7-9]. Several studies have examined IFN-β proteins for anti-tumor efficacy and potency, and IFN-β gene therapy has recently been applied to several types of cancer including glioma and neuroblastoma[7,10,11]. Monitoring the effectiveness of gene transfer methods requires the use of a sensitive assay to detect human IFN-β, expressed as a result of gene transfer, in complex matrices such as serum.

IFN-β and TLR Therapeutics

Some of the most exciting new drugs currently in the pipeline are small molecule agonists and antagonists of TLRs (Toll-Like Receptors). TLRs are highly conserved receptors of the innate immune system that recognize pathogen–associated molecular patterns (PAMPs). These short recognition sequences are found in bacterial and viral components ranging from bacterial lipopolysaccharide (LPS) and flagellin to double-stranded RNA and unmethylated CpG DNA motifs. Ligation of those TLR receptors that bind dsRNA, LPS, ssRNA , or CpG DNA (TLRs 3, 4, 7, 8, and 9, respectively) initiates signaling cascades that culminate in the production of type I IFNs such as IFN-β, proinflammatory cytokines, and chemokines (Figure 1)[12,13]. One well known, FDA-approved TLR agonist is *Aldara™* (imiquimod), which targets TLR-7 and is used for the treatment of several skin conditions including actinic keratosis and superficial basal cell carcinoma. Current studies are focused on the use of TLR agonists as adjuvants in vaccines for cancers and infectious diseases, and TLR antagonists for treatment of SLE14-16. Many TLR agonists and antagonists have progressed to clinical trials for examination of safety and efficacy (www.clinicaltrials.gov).

Because IFN-β production is a major downstream readout for TLR activation, it is frequently utilized in the research setting and in clinical trials as an indicator of TLR agonist potency in vitro (tissue culture media) or in vivo (serum or plasma). This is particularly true for studies on novel TLR-based therapies that employ or inhibit IFN-β production as an intended component of their mechanisms of action. Of interest are several preclinical studies and clinical trials involving novel therapeutic TLR-4 antagonists aimed at treating sepsis, as well as TLR-3 agonists for treating H1N1 influenza and melanoma, among others[17-19].

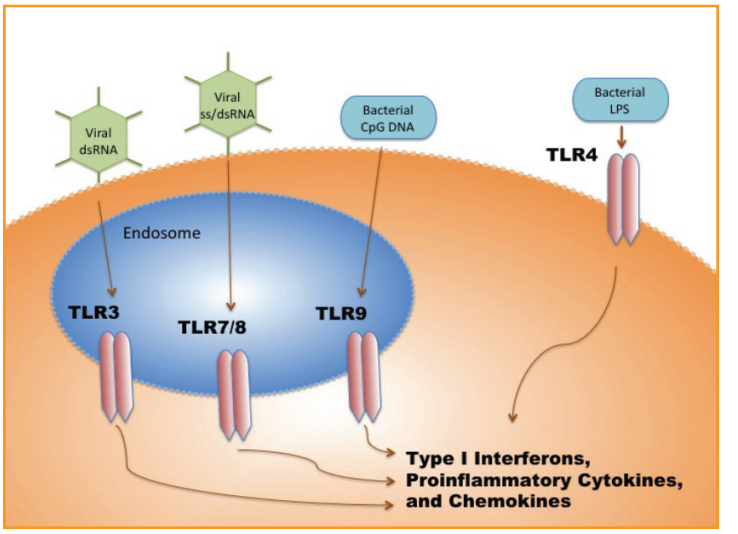

Figure 1. TLR signal transduction. TLR signaling in response to pathogens results in activation of the innate immune system.

IFN-β and Virology

The *VeriKine-HS™* IFN-β ELISA is also a powerful tool for basic virology research. The assay can be used to evaluate and compare differences in immune responses, allowing the user to detect changes in kinetics or strength of interferon production following infection of cells with different viruses or unique viral variants. For some viruses, including those for which highly sensitive virus particle detection proves difficult of costly, the *VeriKine-HS™* assay may be used to quantify increases in host IFN-β production as an early indicator of viral load. Numerous mechanistic questions can be studied with this assay. The ELISA is suitable for use with tissue culture supernatants, as well as complex including human serum and plasma.

IFN-β as a Marker of Toxicology

During pharmaceutical development, drug candidates with unintended off-target effects must be rapidly identified and discarded. Certainly, any drug that induces expression of copious amounts of IFN-β could be detrimental, as unchecked IFN-β production can be deleterious due to the molecule's potent anti-proliferative activity on cells. Therefore, the ability to identify early in the development process those drug candidates that may spuriously result in induction of IFN-β is crucial to expediting delivery of the right lead molecule to the clinic. If identified late in the development process, such off-target effects of a drug may cause dose-limiting side effects in patients resulting in a narrow therapeutic index, marginal clinical applicability, and costly detours in the drug approval processes.

VeriKine-HS™ Human IFN-β Serum ELISA Performance

The *VeriKine-HS™* ELISA is a valuable tool for researchers and clinicians alike facilitating both pre-clinical drug development studies and patient evaluations in clinical trials. ELISA is a powerful technique that combines the specificity of antibodies with the sensitivity of a simple enzyme assay for the rapid detection and quantification of biological substances. The *VeriKine-HS™* assay uses the 'sandwich' strategy in which antigen is captured by a microplate-bound antibody and detected by second antibody, each of which recognizes particular epitopes in the IFN-β molecule. Because both antibodies are specific for the target protein, this method can increase the assay specificity two to five times over other direct or indirect detection methods, thereby eliminating the need for the sample purification prior to analysis. Thus, the *VeriKine-HS™* IFN-β ELISA is suitable for analyzing samples in tissue culture medium, serum, plasma, and cerebrospinal fluid. Because this assay also relies upon two monoclonal antibodies, greater long term consistency of results is achieved vs. assays containing polyclonal antibodies. Using human serum or tissue culture media, inter- and intra-assay CVs are each ≤10%, and the IFN-β1a standard curve is run from 2.3 to 150 pg/ml.

ELISA Performance in Complex Matrices

The utility of the *VeriKine-HS™* ELISA for analysis of plasma samples was demonstrated during a spike recovery assay. As seen in Table 1, plasma and serum components did not interfere substantially with the ELISA's ability to quantify IFN-β levels. This study was performed through addition of a known concentration of purified recombinant IFN-β1a (spike) to IFN-free plasma, then calculating the difference between the known IFN-β spike and that detected by the ELISA (recovery). The percent difference between the spike and that recovered in the ELISA (#41415-1) is less ≤11% in each matrix.

IFN-β Spike Recovery *Matrix*	*% Recovery from Low Spike (5 pg/ml)*	*% Recovery from Medium Spike (25 pg/ml)*	*% Recovery from High Spike (100 pg/ml)*
Serum	91%	100%	95%
Plasma (Na-Citrate)	89%	92%	94%
Plasma (Na-Heparin)	97%	95%	100%
Plasma (Na-EDTA)	89%	95%	93%

Table 1. Spike recovery/matrix compatibility of VeriKine-HS™ Human IFN-β Serum ELISA in biological sample matrices.

Sera from a subset of MS patients have been shown to contain IFN-β neutralizing antibodies. Therefore, it is important to consider possible interference of these antibodies when using any ELISA.

ELISA Sensitivity

In a study of over 100 normal human serum samples, 11% of the samples exhibited greater than 1 pg/ml of IFN-β while the assay detected an average of 0.38 pg/ml (Figure 2) across the entire group. This detection of baseline IFN-β in a number of patient sera by the *VeriKine-HS™* ELISA demonstrates the sensitivity and utility of this assay as previous assays have been insufficiently sensitive to quantify IFN-β under such circumstances.

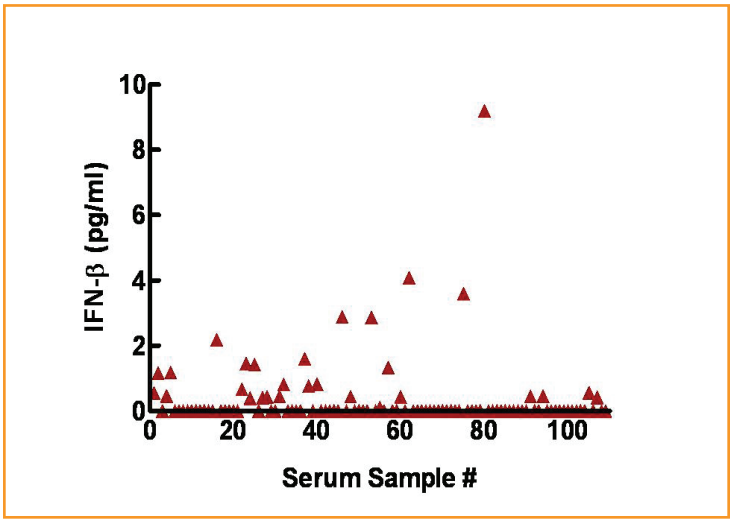

Figure 2. IFN-β quantified (pg/ml) in (normal human) serum samples obtained from 109 individuals. IFN-β was quantified using VeriKine-HS™ IFN-β Serum ELISA.

The *VeriKine-HS™* ELISA has been used to analyze large numbers of patient samples from clinical trials. This single-analyte colorimetric ELISA remains among the simplest, most affordable, and reproducible of assays by which to monitor expression of IFN-β in serum and plasma.

Clearly, IFN-β plays a pivotal role not only in the protective response to many infections and diseases, but also in the generation of side effects and pathological sequelae when produced unchecked. The ability to rapidly and precisely quantify the production of this crucial member of the IFN family of cytokines is required not only in the clinical setting, but also for drug discovery and basic research. The *VeriKine-HS™* Human IFN-β Serum ELISA is gaining rapid recognition as the benchmark method for reliable quantification of endogenous IFN-β protein and IFN-β-based therapeutics.

References

1. Yen, J.H. & Ganea, D. Interferon beta induces mature dendritic cell apoptosis through caspase-11/caspase-3 activation. *Blood* 114, 1344-1354 (2009) PMCID# 19531658.
2. Axtell, R.C., de Jong, B.A., Boniface, K., van der Voort, L.F., Bhat, R., De Sarno, P., Naves, R., Han, M., Zhong, F., Castellanos, J.G., Mair, R., Christakos, A., Kolkowitz, I., Katz, L., Killestein, J., Polman, C.H., de Waal Malefyt, R., Steinman, L. & Raman, C. T helper type 1 and 17 cells determine efficacy of interferon-beta in multiple sclerosis and experimental encephalomyelitis. *Nat Med* 16, 406-412 (2010) PMCID# 20348925.
3. Petkau, A.J., White, R.A., Ebers, G.C., Reder, A.T., Sibley, W.A., Lublin, F.D. & Paty, D.W. Longitudinal analyses of the effects of neutralizing antibodies on interferon beta-1b in relapsing-remitting multiple sclerosis. *Mult Scler* 10, 126-138 (2004) PMCID# 15124756.
4. Baker, D.P., Lin, E.Y., Lin, K., Pellegrini, M., Petter, R.C., Chen, L.L., Arduini, R.M., Brickelmaier, M., Wen, D., Hess, D.M., Chen, L., Grant, D., Whitty, A., Gill, A., Lindner, D.J. & Pepinsky, R.B. N-terminally PEGylated human interferon-beta-1a with improved pharmacokinetic properties and in vivo efficacy in a melanoma angiogenesis model. *Bioconjug Chem* 17, 179-188 (2006) PMCID# 16417267.
5. Daud, A.I., Xu, C., Hwu, W.J., Urbas, P., Andrews, S., Papadopoulos, N.E., Floren, L.C., Yver, A., Deconti, R.C. & Sondak, V.K. Pharmacokinetic/pharmacodynamic analysis of adjuvant pegylated interferon alpha-2b in patients with resected high-risk melanoma. *Cancer Chemother Pharmacol* PMCID# 20509027.
6. Chan, H.L., Ren, H., Chow, W.C. & Wee, T. Randomized trial of interferon beta-1a with or without ribavirin in Asian patients with chronic hepatitis C. *Hepatology* 46, 315-323 (2007) PMCID# 17654600.
7. Carpi, A., Nicolini, A., Antonelli, A., Ferrari, P. & Rossi, G. Cytokines in the management of high risk or advanced breast cancer: an update and expectation. *Curr Cancer Drug Targets* 9, 888-903 (2009) PMCID# 20025599.
8. Sterman, D.H., Gillespie, C.T., Carroll, R.G., Coughlin, C.M., Lord, E.M., Sun, J., Haas, A., Recio, A., Kaiser, L.R., Coukos, G., June, C.H., Albelda, S.M. & Vonderheide, R.H. Interferon beta adenoviral gene therapy in a patient with ovarian cancer. *Nat Clin Pract Oncol* 3, 633-639 (2006) PMCID# 17080181.
9. Swann, J.B., Hayakawa, Y., Zerafa, N., Sheehan, K.C., Scott, B., Schreiber, R.D., Hertzog, P. & Smyth, M.J. Type I IFN contributes to NK cell homeostasis, activation, and antitumor function. *J Immunol* 178, 7540-7549 (2007) PMCID# 17548588.
10. Aoki, T., Takahashi, J.A., Ueba, T., Oya, N., Hiraoka, M., Matsui, K., Fukui, T., Nakashima, Y., Ishikawa, M. & Hashimoto, N. Phase II study of nimustine, carboplatin, vincristine, and interferon-beta with radiotherapy for glioblastoma multiforme: experience of the Kyoto Neuro-Oncology Group. *J Neurosurg* 105, 385-391 (2006) PMCID# 16961130.
11. Rosati, S.F., Williams, R.F., Nunnally, L.C., McGee, M.C., Sims, T.L., Tracey, L., Zhou, J., Fan, M., Ng, C.Y., Nathwani, A.C., Stewart, C.F., Pfeffer, L.M. & Davidoff, A.M. IFN-beta sensitizes neuroblastoma to the antitumor activity of temozolomide by modulating O6-methylguanine DNA methyltransferase expression. *Mol Cancer Ther* 7, 3852-3858 (2008) PMCID# 19056675.
12. Petzke, M.M., Brooks, A., Krupna, M.A., Mordue, D. & Schwartz, I. Recognition of Borrelia burgdorferi, the Lyme disease spirochete, by TLR7 and TLR9 induces a type I IFN response by human immune cells. *J Immunol* 183, 5279-5292 (2009) PMCID# 19794067.
13. Noppert, S.J., Fitzgerald, K.A. & Hertzog, P.J. The role of type I interferons in TLR responses. *Immunol Cell Biol* 85, 446-457 (2007) PMCID# 17667935.
14. Pulendran, B. Tolls and beyond--many roads to vaccine immunity. *N Engl J Med* 356, 1776-1778 (2007) PMCID# 17460233.
15. Wang, D., Bhagat, L., Yu, D., Zhu, F.G., Tang, J.X., Kandimalla, E.R. & Agrawal, S. Oligodeoxyribonucleotide-based antagonists for Toll-like receptors 7 and 9. *J Med Chem* 52, 551-558 (2009) PMCID# 19102653.
16. Lenert, P.S. Classification, mechanisms of action, and therapeutic applications of inhibitory oligonucleotides for Toll-like receptors (TLR) 7 and 9. *Mediators Inflamm* 2010, 986596 (2010) PMCID# 20490286.
17. Gearing, A.J. Targeting toll-like receptors for drug development: a summary of commercial approaches. *Immunol Cell Biol* 85, 490-494 (2007) PMCID# 17667933.
18. Conforti, R., Ma, Y., Morel, Y., Paturel, C., Terme, M., Viaud, S., Ryffel, B., Ferrantini, M., Uppaluri, R., Schreiber, R., Combadiere, C., Chaput, N., Andre, F., Kroemer, G. & Zitvogel, L. Opposing effects of toll-like receptor (TLR3) signaling in tumors can be therapeutically uncoupled to optimize the anticancer efficacy of TLR3 ligands. *Cancer Res* 70, 490-500 (2010) PMCID# 20068181.
19. Wong, J.P., Christopher, M.E., Viswanathan, S., Karpoff, N., Dai, X., Das, D., Sun, L.Q., Wang, M. & Salazar, A.M. Activation of toll-like receptor signaling pathway for protection against influenza virus infection. *Vaccine* 27, 3481-3483 (2009) PMCID# 19200852.