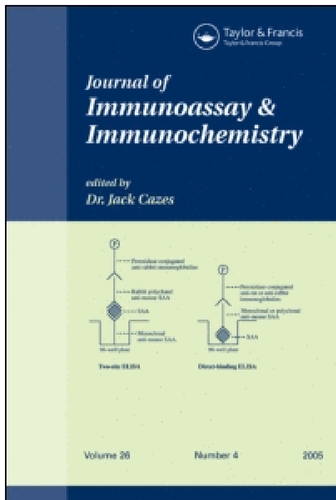
This article was downloaded by:
On: *16 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Development of an Enzyme-Linked Immunoassay for Sensitive Detection of Native and Recombinant Human Interferon-γ Using Whole IgG Fraction as Polyclonal Tracer

Cemalettin Aybay[a]
[a] Department of Immunology, Faculty of Medicine, Gazi University, Besevler, Ankara, Turkey

Online publication date: 11 October 2004

To cite this Article Aybay, Cemalettin(2005) 'Development of an Enzyme-Linked Immunoassay for Sensitive Detection of Native and Recombinant Human Interferon-γ Using Whole IgG Fraction as Polyclonal Tracer', Journal of Immunoassay and Immunochemistry, 25: 4, 321 — 334

To link to this Article: DOI: 10.1081/IAS-200033828

URL: http://dx.doi.org/10.1081/IAS-200033828

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF IMMUNOASSAY & IMMUNOCHEMISTRY
Vol. 25, No. 4, pp. 321–334, 2004

Development of an Enzyme-Linked Immunoassay for Sensitive Detection of Native and Recombinant Human Interferon-γ Using Whole IgG Fraction as Polyclonal Tracer

Downloaded At: 10:21 16 January 2011

Cemalettin Aybay*

Department of Immunology, Faculty of Medicine, Gazi University, Besevler, Ankara, Turkey

ABSTRACT

Monoclonal antibodies (mAbs) and polyclonal antibodies (pAbs) against human interferon gamma (IFN-γ) were produced and used for development of a sensitive enzyme-linked immunosorbent assay (ELISA) for the detection and quantitation of native and recombinant human IFN-γ in tissue culture fluid and human sera. The human IFN-γ ELISA was constructed using mAb CAy-IFNg111 as the capture antibody (Ab) and biotinylated polyclonal mouse immunoglobulin G (IgG) as the tracer Ab. The assay is completed within 4 hr at room temperature (RT). The human IFN-γ ELISA worked in tissue culture medium and human

*Correspondence: Cemalettin Aybay, Department of Immunology, Faculty of Medicine, Gazi University, 06500 Besevler, Ankara, Turkey; E-mail: aybayc@tr.net.

DOI: 10.1081/IAS-200033828 1532-1819 (Print); 1532-4230 (Online)
Copyright © 2004 by Marcel Dekker, Inc. www.dekker.com

Request Permissions / Order Reprints
powered by RIGHTSLINK
COPYRIGHT CLEARANCE CENTER, INC.

serum and was capable of detecting both recombinant and native human IFN-γ. The assay dynamic range extended from 16 to 1000 pg/mL and the sensitivity level was less than 3 pg/mL of human IFN-γ with averaged intra- and inter-assay variation coefficients less than 8% for both. The results demonstrated that without the need of an antigen-affinity purification, biotinylation of protein G-purified pAb, obtained from 1 mL of mouse blood, was sufficient for constructing the tracer reagent for the establishment of a highly sensitive ELISA (40,000 test) for the quantitative detection of native and recombinant human IFN-γ in culture supernatant and human sera.

Key Words: Human interferon gamma; Monoclonal antibody; Polyclonal antibody ELISA.

Downloaded At: 10:21 16 January 2011

INTRODUCTION

Cytokines play a fundamental regulatory and signaling role in immune responses. A growing number of cytokines can now be measured with bioassays using cytokine-dependent cell lines.[1–3] However, such bioassays are laborious, time-consuming and difficult to standardize.[4] Furthermore, they might be sensitive to buffers or other cytokines present in the supernatants. The development of an enzyme-linked immunosorbent assay (ELISA) for the measurement of cytokines would have numerous advantages. In addition to being rapid and easy to perform, allowing large-scale use, ELISAs are highly sensitive and specific. Therefore, the ELISA has been used extensively in laboratories for research and diagnostic purposes and is described in detail in many laboratory technique textbooks.[5]

The sensitivity and specificity of the assay are of critical importance and are controlled by the antigen-specific antibodies (Abs) used in the assay.[6] The analytical sensitivity of the assay is a limitation that controls the level of our understanding of the biological system being investigated. Consequently, many laboratories have sought to improve the sensitivity of the ELISA, especially for those used for the measurement of cytokines which are present in culture supernatants at extremely low concentrations and are, therefore, very difficult to detect.

ELISA was frequently established with a monoclonal antibody (mAb) as the capture Ab and a secondary mAb or polyclonal antibody (pAb) as the tracer. Unlabeled pAb is used in conjunction with an enzyme-conjugated Ab.[7] However, pAbs are often used in a labeled form as well. The need to purify pAbs is largely determined by the intended application of the Ab. The key determinants, when choosing an Ab purification procedure, will not

only be influenced by what is the intended use of the Ab, but also what are the available laboratory resources.

Because there is specific antigen recognition by Abs, immunoaffinity purification with immobilized antigen as affinity ligand is widely used for the preparation of pAb for labeling.[8,9] However, the antigen affinity purification of Abs might present several problems: first, there is a possibility that very high affinity Abs stick onto the column and may not be eluted from the matrix under conventional elution conditions and may, therefore, be lost during the purification process.[10] Different Abs might be eluted from affinity columns under different conditions. This may cause an established purification procedure to fail when applied to a certain Ab. In addition, the elution conditions might be too harsh and irreversible inactivation of the Ab could occur. Second, a large quantity of antigen (several milligrams) must be available to construct immunoaffinity columns[11] for Ab purification and this might be relatively very expensive. Third, care should be taken to choose a well-suited Sepharose allowing for a specific coupling of the peptide antigen to the matrix in order to avoid losses of peptide-specific Ab. Since if the conjugation is done at a different amino acid, there is substantial risk that atleast part of the peptide-specific Abs will not bind due to the change in the epitope resulting from this conjugation reaction to the Sepharose.[12] In addition, antigen must be able to withstand harsh elution conditions and it should have the ability to renature after equilibration so that it could be reused as an immunoaffinity matrix. A good example for the last situation would be the human interferon gamma (IFN-γ) that is very labile at pH 2.[13] It could not resist harsh elution conditions at low pH ranges. Therefore, an immunoaffinity column constructed with hIFN-γ seemed to be inconvenient for the purification of IFN-γ specific pAbs.

Usage of the whole immunoglobulin G (IgG) fraction instead of antigen-affinity purified IgG seemed to be more advantageous, atleast in some situations as mentioned above. Therefore, this study was planned to investigate the performance characteristics of the whole IgG fraction from immune serum and whether it would be sufficient for constructing the tracer Ab as a component of a highly sensitive hIFN-γ ELISA.

Downloaded At: 10:21 16 January 2011

EXPERIMENTAL

mAb

mAb, specific for native and recombinant human IFN-γ, was purified from the supernatant of CAy-IFNg111 hybridoma cells[14] that were cultured in RPMI-1640 (Sigma, St. Louis, MO) supplemented with 10% IgG-depleted

fetal calf serum (FCS) (FCS-gold, PAA Laboratories GmbH, Linz, Austria) using a Hi-Trap Protein G column (1 mL MAbTrap G II Kit, Supelco Bellefonte, PA).[15] The peak fraction was passed through Sephadex G-25 Fine-packed 1 × 30 cm column. Protein concentration was measured spectrophotometrically and then used as the capture Ab.

Production of pAb

Mouse antiserum to human IFN-γ was prepared by immunizing a 10 week-old BALB/c mouse four times at 4-week intervals. A 10 μg of rhIFN-γ (PeproTech, Inc. Rocky Hill, NJ) was used for each injection. Human IFN-γ was emulsified with complete Freund's adjuvant (Sigma) at 1 : 1 ratio (v/v) and injected intraperitoneally plus subcutaneously at four different sites in the abdominal region. Second and third injections of the antigen were given after mixing with incomplete Freund's adjuvant (Sigma) via the same route. Five days after the last injection of antigen in PBS only, the mouse was driven into general anesthesia with ketamine/xylazine cocktail and then 1 mL of blood was collected and the serum was recovered. Whole IgG fraction containing pAbs was purified from serum using Hi-Trap Protein G column attached to a high-pressure liquid chromatography system (Agilent 1100 Series HPLC system, Agilent Technologies, GmbH, Waldbronn, Germany).

Downloaded At: 10:21 16 January 2011

Biotinylation of Polyclonal Mouse IgG

Biotinylations of anti-human IFN-γ and anti-human tumor necrosis factor alpha (TNFα) pAbs were performed using EZ-Link Sulfo-NHS-LC-Biotin (100 mg, Cat No: 21335, Pierce Biotechnology, Inc., Rockford, IL 61105) according to the manufacturer instructions. Free biotin was chromatographically eliminated using Sephadex G-25 Fine-packed 1 × 30 cm column.

Standard Antigen

The standard component of a commercially available hIFN-γ ELISA kit (Human IFN-γ ELISA, BioSource International, Camarillo, CA) was used as the standard antigen. The standard antigen was dissolved in 20 mM phosphate buffer (pH 7.2) containing 1% bovine serum albumin (BSA) (Fatty acid free, Cat No: K41-002-500, PAA Laboratories GmbH, Linz, Austria) and 150 mM

NaCl (PBS-1% BSA). Twofold serial dilutions from 1000 to 1 pg/mL of rhIFN-γ were prepared in PBS-1% BSA.

Production of Native Human IFN-γ

Heparinized human peripheral blood was collected. Peripheral blood mononuclear cells (PBMCs) were separated using Ficoll-Hypaque centrifugation. Cells were washed three times with RPMI-1640 and then used for experiments. These cells were cultured at 1×10^6 cells/mL per well using 24-well tissue culture plates (Costar, Corning Incorporated, Corning, NY) in RPMI-1640 medium supplemented with 10% FCS and with or without the presence of staphylococcal enterotoxin B (SEB) (Sigma) at the concentration of 1 ng/mL at 37°C and 5% CO_2 atmosphere. Supernatant samples were taken from cell cultures 24 hr later and used as the source of native hIFN-γ.

Downloaded At: 10:21 16 January 2011

Human Sera

Human sera were obtained from routine laboratory and kept at −75°C until the day of study.

ELISA

High binding capacity ELISA plates (Costar, No: 3590, Corning Incorporated, Corning, NY) were coated with 50 μL of CAy-IFNg111 mAb at 5 μg/mL in 0.05 M carbonate–bicarbonate buffer (CBB) pH 9.6 by incubating overnight at +4°C. In order to block, 200 μL of PBS-1% BSA was added to each well and then incubated at room temperature (RT) for 2 hr. The plates were then washed five times with washing buffer consisting of PBS plus 0.04% Tween 20 (PBS-T). A 100 μL of the standards or the samples were added into the wells containing 50 μL of sample diluent which consisted of 1% BSA, 1% FBS, and 1% heat-inactivated mouse serum. Plates were incubated for 2 hr at RT. After washing three times with PBS-T, 100 μL of biotinylated pAb at 1 μg/mL was pipetted into each well and then incubated for 60 min at RT. After washing three times with PBS-T, to each well was added 100 μL of streptavidine–horseradish peroxidase (Vector Laboratories Inc., Burlingame, CA 94010), followed by incubation for 30 min at RT. After washing three times with PBS-T, the reaction was revealed with 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) solution for 30 min at RT. After stopping the reaction with 50 μL of 1 M

H_2SO_4, the optical density (OD) of each well was measured with an ELISA reader (LP400, Diagnostics Pasteur, France) at 450 nm.

Statistical Analysis

Regression–correlation analysis was performed. Inter- or intra-assay coefficients of variation (CV) were calculated and used for the quality control of the human IFN-γ ELISA. Calculations were performed with SPSS for Windows, version 10.0.

Downloaded At: 10:21 16 January 2011

RESULTS

Effectiveness and Specificity of Biotinylated Polyclonal IgG Fraction

A total of 4 mg IgG fraction containing anti-hIFN-γ pAb was purified from 1 mL of blood. After eliminating free biotin using Sephadex G-25 Fine column, BSA was added to a final concentration of 2 mg/mL. As shown in Fig. 1, biotinylated pAbs were specifically reacted with the antigen-coated solid phase in a concentration dependent manner. Dilution experiments demonstrated that biotinylated anti-hIFN-γ pAb conjugate could be used at a concentration of 1 μg/mL. In preliminary experiments, it was determined that 100 μL of conjugate per well, at a final concentration of 1 μg/mL, produced optimum performance (data not shown).

Standard Curve

Figure 2 shows the comparison of the standard curves obtained from the in-house ELISA and a commercially available ELISA. The standard antigen used in this assay was the standard component of the commercial ELISA. Standard recombinant hIFN-γ was prepared at various concentrations, ranging from 1 upto 1000 pg/mL. Although both of the standard curves produced a significant ($p < 0.05$) correlation, the OD levels obtained from the in-house ELISA were higher than the commercial ELISA. The detection limit defined as the minimal concentration of IFN-γ that produces a signal equal to the nonspecific background signal +2SD, of the in-house ELISA, was measured to be less than 3 pg/mL. The in-house hIFN-γ ELISA did not demonstrate cross-reaction with recombinant mouse IFN-γ (Sigma) up to

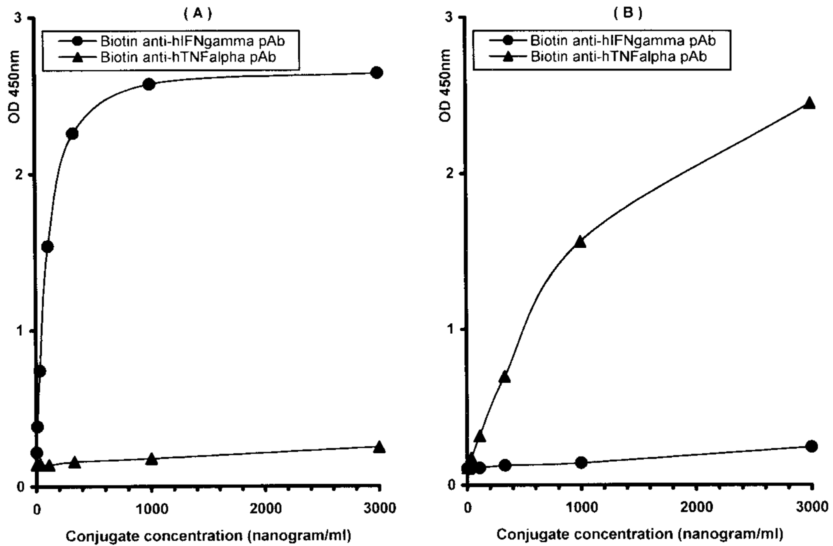


Figure 1. Specificity of biotinylated anti-hIFN-γ polyclonal mouse IgG. Serial threefold dilutions of biotinylated anti-hIFN-γ (biotin anti-hIFNgamma pAb) and anti-hTNFα polyclonal mouse antibodies (biotin anti-hTNFalpha pAb) were performed in sample diluent and then added to wells (0.1 mL/well) of the microtitre plates coated with either 50 μL of rhIFN-γ at 0.5μg/mL (A) or rhTNFα (PeproTech, Inc. Rocky Hill, NJ) at 0.5μg/mL (B) in PBS. Each dilution was assayed in duplicate, and the data reflect the mean values. Results demonstrated that biotinylated polyclonal IgG conjugates specifically reacted with antigen.

Downloaded At: 10:21 16 January 2011

100 ng/mL (data not shown). The inter- and intra-assay CV values of the in-house ELISA at different concentrations of rhIFN-γ were determined to be less than 8% (data not shown).

Serum Dilution Study

When the recombinant hIFN-γ (PeproTech, Inc. Rocky Hill, NJ) was added into pooled human serum at 1 ng/mL and then threefold serially diluted within the same serum, it was observed that the dilution curve was very similar to those produced by the same antigen added into either PBS-1% BSA or RPMI-1640 containing 10% FCS (Fig. 3). These results demonstrated that the in-house hIFN-γ ELISA is convenient for measuring hIFN-γ, not only in culture medium, but also in human serum samples.

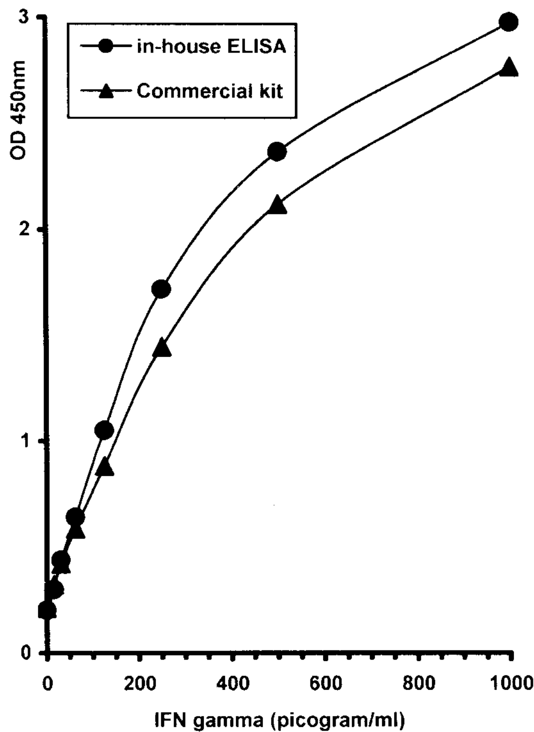


Figure 2. Standard curves of rhIFN-γ as determined by the in-house hIFN-γ ELISA and a commercial hIFN-γ ELISA kit. Standard antigen used in this assay was the standard reagent included in the commercially available hIFN-γ ELISA kit. In the in-house ELISA, CAy-IFNg111 was used as capture mAb and the detection reagent was the biotinylated anti-hIFN-γ mouse polyclonal IgG at 1 μg/mL. In the commercial ELISA antigen was captured via a mAb and the detection reagent was a biotinylated anti-hIFN-γ mouse monoclonal antibody. Each datum point represents the average value of duplicate determinations in a single microtitre plate and the equation of the regression lines in the concentrations range from 16 to 1000 pg/mL was $y = 0.0028x + 0.5119$, $r^2 = 0.8939$ for the in-house ELISA and $y = 0.0026x + 0.4523$, $r^2 = 0.9276$ for the commercial ELISA.

Downloaded At: 10:21 16 January 2011

Measurement of Native hIFN-γ

As shown in Fig. 4, native human IFN-γ could also be effectively measured with the in-house hIFN-γ ELISA. Supernatant of PBMCs stimulated with SEB at 1 ng/mL for 24 hr produced high amounts of hIFN-γ (nearly 8 ng/mL).

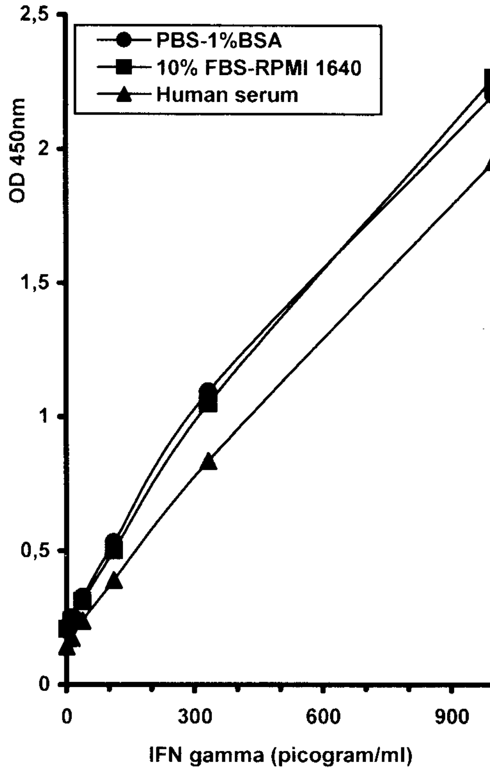


Figure 3. Measurement of hIFN-γ in various samples with the in-house ELISA. Recombinant hIFN-γ (PeproTech) was serially diluted in various samples and then 100 μL was added into CAy-IFNg111-coated wells containing 50 μL of sample diluent. Further steps of the assay were performed as described in Experimental section. Similar dilution curves were obtained with various samples containing rhIFN-γ. Each datum point represents the average value of duplicate determinations in a single microtitre plate.

Downloaded At: 10:21 16 January 2011

DISCUSSION

An increasing number of modern diagnostic and therapeutic technologies are based on the interaction between Abs with high affinity and their specific antigens. In vitro diagnostic procedures, like radioimmunoassay (RIA), immunoradiometric assay (IRMA), ELISA, or special blot techniques with radiolabeled antigens or Abs linked to radioactive isotopes, enzymes, fluorescence markers, or even to other immunoglobulins allow the detection of

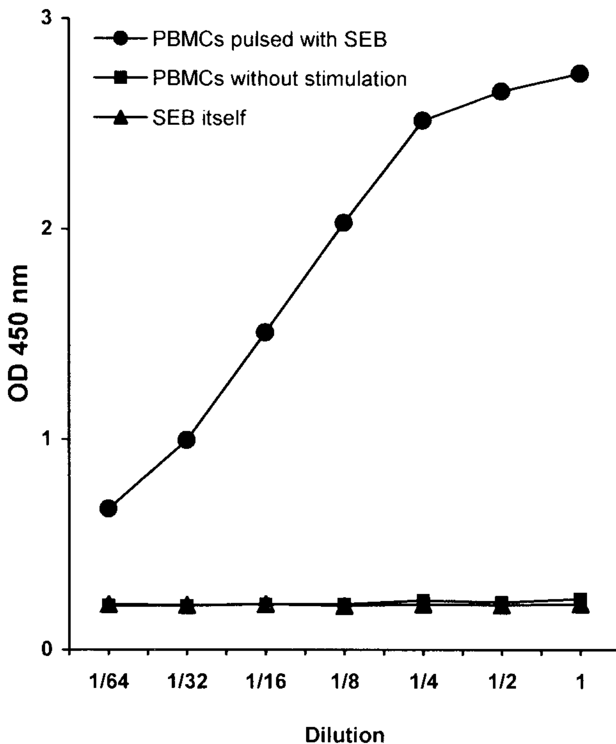


Figure 4. Measurement of native IFN-γ with the in-house ELISA. Twofold serial dilutions of culture supernatants from PBMCs stimulated with 1 ng/mL of SEB or without SEB and SEB itself at 1 ng/mL prepared in the same culture medium without PBMCs and then measured for their native IFN-γ contents as described in Experimental section. Stimulation of PBMCs with SEB at 1 ng/mL for 24 hr resulted in the production of large amounts of native IFN-γ and could be specifically measured with the in-house ELISA. Each datum point represents the average value of duplicate determinations in a single microtitre plate.

Downloaded At: 10:21 16 January 2011

minimal amounts of proteins, nucleic acids, and complexes of carbohydrates and lipids with a high degree of specificity in various media. Often, purity of the immunoglobulins is critical because other substances in the source material may interfere with the detection process. The first step is to describe the basic scenario for the purification. General considerations answer questions such as: what is the intended use of the product? What kind of starting material is available and how should it be handled? What are the purity issues in relation to the source material and intended use of the final product?

What has to be removed? What must be removed completely? What will be the final scale of purification? If there is a need for scale-up, what consequences will this have on the chosen purification technique? What are the economic constrains and what resources and equipment are available? Most purification protocols require more than one step to achieve the desired level of product purity.

Because there is specific antigen recognition by Abs, immunoaffinity purification with immobilized antigen as affinity ligand is widely used. This method is of advantage if a specific Ab must be obtained from a mixture of immunoglobulins with different specificities. However, immunoaffinity purification of Abs might present several problems as well: first, because the humoral immune system is incredibly diverse and able to produce an unlimited number of different immunoglobulins, every Ab is unique. This may cause an established purification procedure to fail when applied to a certain Ab. Second, high affinity Abs might be eliminated from the specific Ab pool when a milder condition for the elution of bound Ab was chosen. However, high affinity Abs reactive against various epitopes have important merit in the ELISA system. When they are included in the preparation of conjugate, the sensitivity and the specific signal to background ratio might be significantly increased. Consequently, the analytical sensitivity of the assay system would be increased. Third, the antigen must also be able to withstand harsh elution conditions and it should have the ability to renature after equilibration so that it could be reused as an immunoaffinity matrix.

Downloaded At: 10:21 16 January 2011

The human IFN-γ is very labile at low pH.[13] It could not resist harsh elution conditions at low pH ranges. Therefore, an immunoaffinity column constructed with hIFN-γ seemed to be inconvenient for purification of antigen-specific pAbs.

On the basis of an idea that, whether without the need of an antigen-affinity purification, the Protein G-purified polyclonal murine IgG containing high affinity Abs obtained from high-quality immune serum could be directly labeled and used as a detecting reagent and would be sufficiently enough to construct a highly sensitive hIFN-γ ELISA that is comparable with its commercial counterparts.

The data presented here show that a reliable and sensitive immunoassay for hIFN-γ has been developed. By using 100 μL of sample, the detection limit was less than 3 pg/mL and the assay dynamic range extends from 16 to 1000 pg/mL of hIFN-γ. Sample dilution experiments (Figs. 2 and 4) showed that both recombinant and native IFN-γ are recognized by the assay. When the standard curves of the in-house ELISA and a commercially available ELISA (BioSource) were analyzed, comparable values were obtained. In order to assess the quality, i.e., reproducibility, of the in-house ELISA, both intra- and inter-assay CV values were determined. Both were

measured to be less than 8%, which indicated that the in-house ELISA is highly reproducible at significant levels.

The proposed method in this study is highly sensitive, simple and rapid, and can reliably measure hIFN-γ in the picogram range in cell culture supernatants and human serum. Consequently, the in-house ELISA can be used for research studies for human IFN-γ in various test samples.

Several studies in the literature concerning construction of IFN-γ were reported. Gallati et al.[16] described a hIFN-γ ELISA with a detection limit of 300 and 60 pg/mL when performed at 2 and 16 hr assay formats, respectively. In another study, the sensitivity of hIFN-γ ELISA was reported as 1 ng/mL of recombinant human IFN-γ.[17] With an enhanced ELISA protocol, the detection levels attainable after double amplification were reported to be 20 pg/mL for human IFN-γ.[18] However, a very sensitive method was also described. When chemiluminescence instead of peroxidase was used, the sensitivity of the assay increased by a factor of 75, and 0.2 pg/mL of hIFN-γ could be measured.[19] The in-house ELISA performed using total IgG fraction as a tracer Ab demonstrated that the sensitivity levels were much better than many of those reported in the literature and as precise as that of a commercial counterpart.

Because each step in the purification process will cause some loss of product in quantity and also possibly in quality, total IgG obtained from a high quality immune serum with single step purification method should be given a chance of priority for analyzing its performance characteristics. This kind of approach presents another advantage concerning the cost-effectiveness.

These data demonstrated that, for a basic research laboratory, it seemed to be possible to establish a research ELISA using total IgG fraction as the tracer component. The methodology described in this study has a low investment cost and meets all criteria for a research kit.

ABBREVIATIONS

ELISA	Enzyme-linked immunosorbent assay
mAb	Monoclonal antibody
pAb	Polyclonal antibody
IFN-γ	Interferon gamma
CAy-IFNg111	Anti-human IFN-γ IgG1 mAb producing hybridoma cell
FCS	Fetal calf serum
BSA	Bovine serum albumin
SEB	Staphylococcal enterotoxin B
PBMC	Peripheral blood mononuclear cells
HPLC	High performance liquid chromatography

Downloaded At: 10:21 16 January 2011

REFERENCES

1. Meager, A. Biological assays for interferons. J. Immunol. Meth. **2002**, *261* (1–2), 21–36.
2. Kwack, K.; Lynch, R.G. A new non-radioactive method for IL-2 bioassay. Mol. Cells **2000**, *10* (5), 575–578.
3. Krakauer, T. A sensitive, specific immunobioassay for quantitation of human interleukin 6. J. Immunoassay **1993**, *14* (4), 267–277.
4. Kim, M.S.; Ahn, S.M.; Moon, A. In vitro bioassay for transforming growth factor-beta using XTT method. Arch. Pharm. Res. **2002**, *25* (6), 903–909.
5. Tijssen, P. Practice and theory of enzyme immunoassay. In *Laboratory Techniques in Biochemistry and Molecular Biology*; Burdon, R.H., Knippenberg, P.H., Eds.; Elsevier Science Publishers B.V.: Amsterdam, 1985; Vol. 15, 329.
6. O'Connor, T.; Gosling, J.P. The dependence of radioimmunoassay detection limits on antibody affinity. J. Immunol. Meth. **1997**, *208* (2), 181–189.
7. Graham, E.M.; Jarrett, O.; Flynn, J.N. Development of antibodies to feline IFN-gamma as tools to elucidate the cellular immune responses to FeLV. J. Immunol. Meth. **2003**, *279* (1–2), 69–78.
8. Unson, M.D.; Newton, G.L.; Arnold, K.F.; Davis, C.E.; Fahey, R.C. Improved methods for immunoassay of mycothiol. J. Clin. Microbiol. **1999**, *37* (7), 2153–2157.
9. Amuy, E.; Alape-Giron, A.; Lomonte, B.; Thelestam, M.; Gutierrez, J.M. Development of immunoassays for determination of circulating venom antigens during envenomations by coral snakes (*Micrurus* species). Toxicon **1997**, *35* (11), 1605–1616.
10. Caughey, D.J.; Narhi, L.O.; Kita, Y.; Meng, S.Y.; Wen, D.; Chen, W.; Ratzkin, B.J.; Fujimoto, J.; Iwahara, T.; Yamamoto, T.; Arakawa, T. Fractionation of polyclonal antibodies to fragments of a neuroreceptor using three increasingly chaotropic solvents. J. Chromatogr. B. Biomed. Sci. Appl. **1999**, *728* (1), 49–57.
11. Verdoliva, A.; Pannone, F.; Rossi, M.; Catello, S.; Manfredi, V. Affinity purification of polyclonal antibodies using a new all-*D* synthetic peptide ligand: comparison with protein A and protein G. J. Immunol. Meth. **2002**, *271* (1–2), 77–88.
12. Price, M.R.; Sekowski, M.; Tendler, S.J. Purification of anti-epithelial mucin monoclonal antibodies by epitope affinity chromatography. J. Immunol. Meth. **1991**, *139* (1), 83–90.
13. Kontsek, P.; Waschutza, G.; Kontsekova, E.; Otto, B. Engineered acid-stabile human interferon gamma. Cytokine **2000**, *12* (6), 708–710.

Downloaded At: 10:21 16 January 2011

14. Aybay, C. Differential binding characteristics of protein G and protein A for Fc fragments of papain-digested mouse IgG. Immunol. Lett. **2003**, *85* (3), 231–235.
15. Aybay, C.; İmir, T. Development of a rapid, single-step procedure using protein G affinity chromatography to deplete fetal calf serum (FCS) of its IgG and to isolate murine IgG1 monoclonal antibodies from supernatants of hybridoma cells. J. Immunol. Meth. **2000**, *233* (1–2), 77–81.
16. Gallati, H.; Pracht, I.; Schmidt, J.; Haring, P.; Garotta, G. A simple, rapid and large capacity ELISA for biologically active native and recombinant human IFN gamma. J. Biol. Reg. Homeost. Agents **1987**, *1* (3), 109–118.
17. Jitsukawa, T.; Nakajima, S.; Sugawara, I.; Mori, S.; DeLey, M. Characterization of murine monoclonal antibodies to human interferon-gamma (IFN-gamma) and their application for sandwich enzyme-linked immunosorbent assay (ELISA). Microbiol. Immunol. **1987**, *31* (8), 809–820.
18. O'Connor, E.; Roberts, E.M.; Davies, J.D. Amplification of cytokine-specific ELISAs increases the sensitivity of detection to 5–20 picograms per milliliter. J. Immunol. Meth. **1999**, *229*, 155.
19. Alkan, S.; Akdis, C.; Towbin, H. Chemiluminescent and enzyme-linked immuno assays for sensitive detection of human IFN-gamma. J. Immunoassay **1994**, *15* (3), 217–238.

Received April 22, 2004
Accepted May 30, 2004
Manuscript 3136

Downloaded At: 10:21 16 January 2011