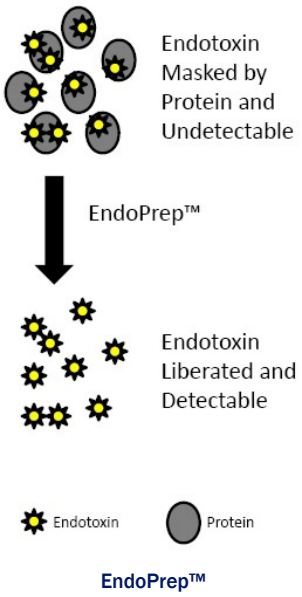
Predictive Oncology®

EndoPrep™

Improved Endotoxin Detection in Protein/Peptide and Antibody Samples using EndoPrep™



EndoPrep™

- Increases detection accuracy
- Removes inhibitory effect of peptides and proteins on endotoxin
- Works with LAL and Recombinant Factor C Assays
- Requires less than 60 minutes for most samples
- Easy to use

Limulus amoebocyte lysate (LAL) and recombinant Factor C (rFC) assays have become the standard for detecting endotoxin in biological samples. However, many sample components, including proteins, have been shown to artificially enhance or inhibit detection. Here we describe a sample treatment system which neutralizes the effect of proteins and results in more accurate endotoxin determinations.

Introduction

Accurate quantitation of endotoxin is crucial for production of samples for biological use. The FDA has established strict guidelines for allowable endotoxin content of injectible drugs [1]. In addition, endotoxin affects a number of cell types in tissue culture [2]. Currently, LAL and rFC assays comprise the majority of endotoxin testing. These assays rely on enzymatic reactions and are thus susceptible to a litany of factors that can both inhibit and enhance enzyme performance. One accepted practice for confronting such issues is diluting the samples until the inhibitory/enhancement factor has no effect on assay performance. However, each sample dilution step results in a correlated loss of assay sensitivity. In addition to problems with enzymatic reactions, many molecules are known to bind and alter the activity of endotoxin. For example, cationic proteins tightly bind to the negative endotoxin molecule and cannot be dissociated by simple dilution. In these situations strategies such as perchloric acid treatment [3], phenol extraction [4], and the dilution-heating method [5] must be used. However, these have been shown to be inadequate and cumbersome. Using salts or detergents to dissociate protein-endotoxin complexes is not acceptable because these materials also tend to inhibit enzymatic assays. Because of these reasons, a method to treat samples and directly remove the inhibitory/enhancement effect would result in more accurate assay results.

Several attempts have been made to digest protein solutions with proteases prior to LAL or rFC assays but with limited success. Factor C, the LAL enzyme that

initiates the clotting cascade, is a serine protease as is the downstream enzyme Factor B and the proclotting enzyme. Therefore, using common serine proteases to digest the protein can activate the LAL cascade. This has been seen in difficulties using trypsin, chymotrypsin [6], and pronase [7]. Proteinase K treatments have shown some success but require 12-18 hour digestions and may also activate the LAL cascade, resulting in the appearance of low level endotoxin contamination [6]. Here a sample treatment kit is described which sufficiently degrades most proteins and peptides in under 60 minutes and will not interfere with LAL and rFC assays.

Materials and Methods

Supplies. Endotoxin detection and quantitation was performed with the PyroGene® assay from Lonza (Walkersville, MD) according to manufacturer's specifications with and without 1 EU/ml positive product controls (PPC) to validate assay reliability. The assay has a range of detection from 0.01 to 10 EU/ml. Hemoglobin (bovine erythrocytes) was purchased from Calbiochem (La Jolla, CA) and contained endogenous endotoxin at a level of 300 EU/mg. Rabbit IgG (from plasma) was also from Calbiochem and contained approximately 2 EU/mg. Bovine serum albumin was purchased from Sigma (St. Louis, MO) and contained <1 EU/mg. Sushi 3 peptide was chemically synthesized by American Peptide (Sunnyvale, CA) and contained no detectable endotoxin. Peptide X was supplied by a proprietary source and contained <1 EU/mg. Endotoxin-free water was from Predictive Oncology (Birmingham, AL). When sample endotoxin was low, endogenous endotoxin was added to the levels indicated. Endotoxin was purchased from List Biological Laboratories, Inc. (Campbell, CA) in the form of *Escherichia coli O55:B5* lipopolysaccharide.

EndoPrep™ Protocol. The EndoPrep™ kit consists of SB™ Digestion Buffer and SB™ Protease Solution. All experiments were performed with the following protocol. The protein sample was diluted to a suitable working level using SB™ Digestion Buffer. Aliquots of 270 µl of this solution were mixed with 30 µl of the SB™ Protease Solution and vortexed for 10 seconds. A sample containing 30 µl of SB™ Digestion Buffer instead of the SB™ Protease Solution was included in each set of experiments to determine baseline endotoxin without digestion. After mixing, the tubes were covered with Parafilm and incubated in a 37°C water bath for the indicated times. After treatment, the samples were diluted 1:100 in endotoxin-free water and subjected to PyroGene® testing. The 10% sample dilution resulting from addition of SB™ Protease Solution was taken into account and calculated into the results given.

Polyacrylamide Gel Electrophoresis (PAGE). PAGE analysis was performed with each sample treatment to monitor correlation of endotoxin detection with protein degradation. For PAGE analysis, 20 µl of the undiluted digestion sample was added to a mixture containing 45 µl endotoxin-free water, 10 µl 5 mM DTT, and 25 µl CBS Scientific (Del Mar, CA) ClearPAGE™ 4x Sample Buffer (additional water replaced DTT for non-reducing electrophoresis). This sample was heated in a 70°C water bath for 10 minutes and 17 µl was loaded into a CBS Scientific ClearPAGE 10-20% TEO-CI SDS Gel submerged in CBS Scientific ClearPAGE 1x Tris-Tricine-SDS Run Buffer (Reducing or Non-Reducing) and electrophoresed at 200 Volts for 45 minutes with a current gradient from 60 to 30 mA. All gels were silver stained using Sigma (St. Louis, MO) ProteoSilver™ Silver Stain Kit according to manufacturer's specifications.

PPC Inhibition Assay. PPC inhibition assays were performed with cationic peptides to determine the extent of inhibition on PPC recovery. Serial 10-fold dilutions of peptide were made in endotoxin-free water. Aliquots of 270 µl of each dilution were added to an endotoxin-free glass tube containing 30 µl of 10 EU/ml LPS for a final endotoxin concentration of 1 EU/ml. 50 µl of each sample was tested in the Lonza PyroGene® assay. A sample without peptide was included as control and reference.

PyroGent® Gel Clot Assay. To verify the applicability of EndoPrep™ to gel clot assays, digested samples were tested using the Lonza PyroGent® system. For the assay, 100 µl samples were mixed with 100 µl of PyroGent® assay lysate in endotoxin-free glass vials and vortexed for 10 seconds. The samples were then incubated in a 37°C water bath without agitation for 60 minutes. After incubation the samples were tested for clot formation by visual inspection. Along with the samples a series of standards ranging from 0.03 to 0.12 EU/ml and blanks were tested in triplicate to verify assay reliability.

Results

Peptide X

Peptide X (proprietary) is a mixture of short cationic peptides shown to be extremely effective in a therapeutic setting. This product has a molecular weight range from 2.0 to 6.5 kDa with an average size of 3.5 kDa. In LAL and rFC assays, Peptide X completely inhibits endotoxin detection, even preventing recovery of defined endotoxin spikes. Preliminary experiments determined that the inhibitory effect is caused by tight binding of the peptide to endotoxin. This binding could not be displaced by salt, detergent, ultrafiltration, or heating. This mechanism of inhibition also causes extensive dilution to be insufficient. To demonstrate the extent of endotoxin inhibition by Peptide X, dilutions of the peptide in water were spiked with 1 EU/ml endotoxin and tested with the PyroGene® assay for spike recovery (Figure 1*A*). Starting with a concentration of 0.1 mg/ml, the first several 10-fold dilutions still showed inhibition at or near 100%. Dilution to 0.1 µg/ml was required to detect over 50% of endotoxin. For full endotoxin recovery, the peptide had to be diluted to a concentration of 10 ng/ml, which represents a 2,000,000-fold dilution of the therapeutic dose. This type of dilution prevents the detection of endogenous endotoxin.

Protein X was treated with the **EndoPrep™** sample preparation kit to remove this inhibitory activity. A 0.1 mg/ml sample of Peptide X was prepared in SB™ Digestion Buffer and tested for PPC recovery with and without **EndoPrep™** treatment. Consistent with the above results, the PPC of the untreated sample was 100% inhibited (Figure 1*B*). Treatment with **EndoPrep™** increased PPC recovery to over 63% without incubation in the 37°C water bath. Proper incubation further increased the recovery to over 75%. This level of PPC recovery is in excess of the 50% required by the FDA to be considered an acceptable assay. To test this further, the ability of **EndoPrep™** to allow detection of contaminating endotoxin was examined. New samples of 0.1 mg/ml Peptide X were prepared in SB™ Digestion Buffer contain-

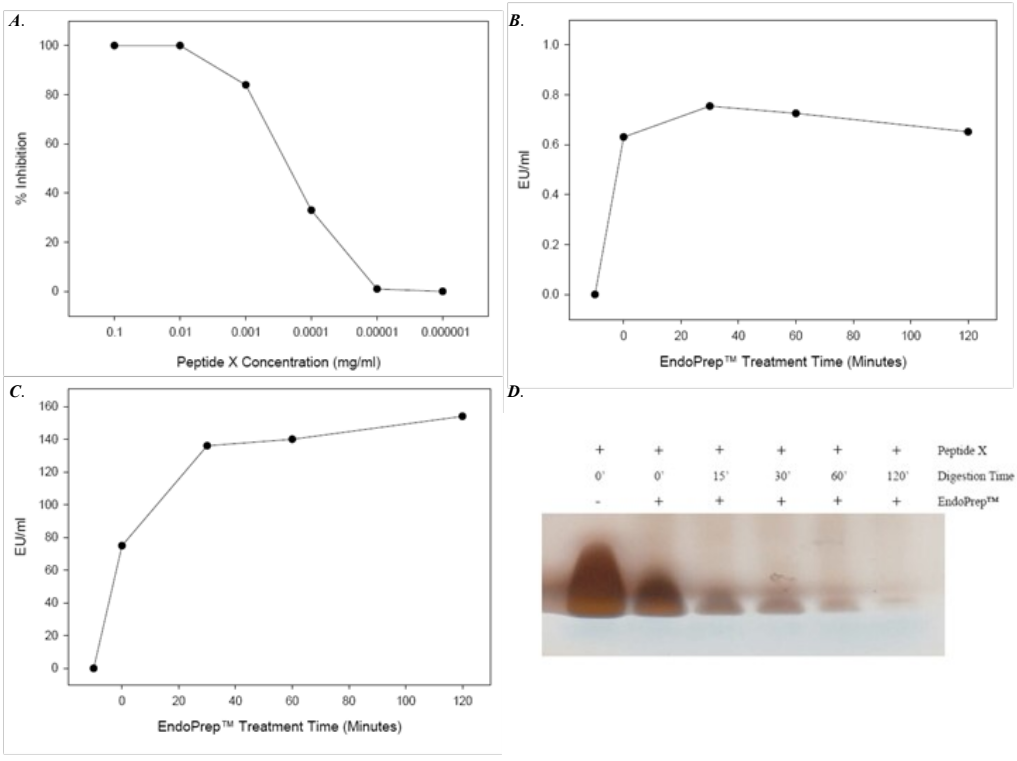


Figure 1. Treatment of Peptide X with EndoPrep™. (*A*) The PPC Inhibition Assay shows extent of peptide dilution required to overcome inhibitory effect. (*B*) Recovery of a 1 EU/ml PPC in samples of Peptide X before and after treatment with EndoPrep™. (*C*) Recovery of defined endotoxin contamination in a sample of Peptide X. (*D*) PAGE data showing Peptide X degradation with EndoPrep™ treatment.

ing approximately 250 EU/mg endotoxin. Similar to the PPC results, treatment with **EndoPrep™** increased detection from 0 EU/ml to more than 150 EU/ml over the course of treatment (Figure 1*C*). This represents the detection of over 60% of the calculated value of initial endotoxin added. PAGE analysis showed that the increase in endotoxin detection corresponded to the degradation of Peptide X (Figure 1*D*). **EndoPrep™** addition without incubation reduced the amount of peptide by approximately 25-30%. Proper incubation further reduced the amount of native peptide to less than 10% after 30 minutes of treatment and almost negligible amounts after 120 minutes.

In addition to testing Peptide X digestions with the PyroGene® assay, they were also tested with the PyroGent® Gel Clot Assay from Lonza (Walkersville, MD) to demonstrate that **EndoPrep™** was applicable to more than one type of endotoxin detection system. The assay had a sensitivity of 0.06 EU/ml and was performed on untreated and treated 0.1 mg/ml peptide samples both with and without a 0.1 EU/ml PPC spike. All of the control reactions tested as expected (Data Not Shown). The 0.1 mg/ml peptide sample did not cause a clot formation either with or without **EndoPrep™** treatment indicating low endogenous endotoxin (Table 1). When the untreated peptide sample contained a 0.1 EU/ml PPC spike, which is in excess of the sensitivity level of the assay, there was no clot formation indicating that the endotoxin was masked by the peptide. When the treated peptide sample was spiked with a 0.1 EU/ml PPC there was clot formation indicating that the inhibitory factor was removed by digestion and the assay was detecting an endotoxin level in excess of 0.06 EU/ml.

Peptide (mg/ml)	EndoPrep™ Treatment	Spike (EU/ml)	Clot Formation?
0.1	No	-	No
0.1	No	0.1	No
0.1	Yes	-	No
0.1	Yes	0.1	Yes

Table 1. EndoPrep™ Treated Samples in PyroGent® Gel Clot Assay

Sushi 3 Peptide

Factor C is the enzyme which initiates the enzymatic cascade in LAL and rFC assays. The endotoxin-binding site of Factor C has been isolated to a 34 amino acid peptide termed Sushi 3 (S3). S3 is known to bind tightly to endotoxin [8] via both electrostatic and hydrophobic interactions [9, 10]. This binding is so strong it has been developed and is commercially available for use in endotoxin removal media [11, 12]. Much like Peptide X, the tight interaction of S3 with endotoxin masks activity as detected in commonly used assays. Results of PPC inhibition tests (Figure 2*A*) demonstrate the requirement for extensive dilution of the peptide to prevent masking of 1 EU/ml. For complete PPC recovery, the peptide must be diluted to a concentration of 0.1 µg/ml. As with Peptide X, dilution of this extent prevents accurate endotoxin detection or quantitation.

To test the ability of **EndoPrep™** to alleviate the inhibitory effect of S3, a 0.1 mg/ml sample was prepared in SB™ Digestion Buffer from a 1 mg/ml stock solution. Previous experiments showed that the peptide contained no detectable endotoxin so exogenous endotoxin was added to the sample to a final

concentration of 70 EU/mg. The control sample that received no SB™ Protease Solution tested at 17 EU/ml indicating over 75% inhibition (Figure 2*B*). Treatment with the **EndoPrep™** system recovered 29 EU/ml of endotoxin activity after a short incubation and 51 EU/ml after 120 minutes of incubation. This final sample represents a 300% increase in detection accuracy. The PAGE analysis corresponds to endotoxin recovery (Figure 2*C*). There is significant degradation after 15-30 minutes of incubation followed by further degradation with the 60 minute sample. After 120 minutes of treatment the peptide is almost undetectable.

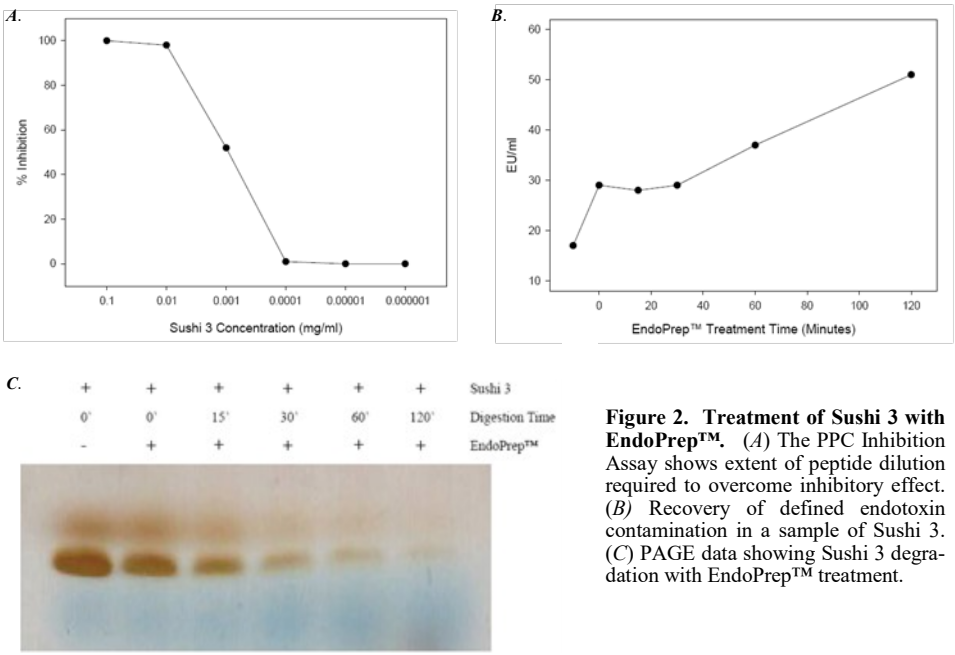


Figure 2. Treatment of Sushi 3 with EndoPrep™. (*A*) The PPC Inhibition Assay shows extent of peptide dilution required to overcome inhibitory effect. (*B*) Recovery of defined endotoxin contamination in a sample of Sushi 3. (*C*) PAGE data showing Sushi 3 degradation with EndoPrep™ treatment.

Hemoglobin

The previous examples involved cationic peptides which caused complete or near complete endotoxin masking. However, the **EndoPrep™** system can also be used to increase the accuracy of endotoxin detection in protein samples that do not exhibit such dramatic effects.

Hemoglobin is the metalloprotein in erythrocytes responsible for delivering oxygen throughout the body. It has a moderate affinity for endotoxin (k_D 3.1 x 10^{-8}) as shown with ultrafiltration, density centrifugation, ethanol precipitation, and non-denaturing PAGE [13, 14]. Given its importance in biology and its potential role in artificial blood development, accurate endotoxin detection in hemoglobin is of great importance.

To test the effect of **EndoPrep™** treatment on hemoglobin (bovine erythrocyte), a 1 mg/ml sample was prepared in POAI™ Digestion Buffer from a 50 mg/ml stock solution and treated as previously described. The untreated sample, containing no SB™ Protease Solution, gave a value of 330 EU/mg (Figure 3*A*). After treatment with **EndoPrep™** for 30 minutes, the measured endotoxin value increased nearly 70% to 560 EU/mg. This increase in activity continued over the 120 minute time course with results of 650 EU/mg at 60 minutes and 850 EU/mg after 120 minutes. This final data point represents an increase in measured endotoxin of over 150%. Accompanying the graph are the PAGE results of the samples indicating the extent of hemoglobin digestion over the time course (Figure 3*B*). In addition to the 16 kDa monomer, hemoglobin forms 32 kDa dimers and 64 kDa tetramers that can be visualized in reducing conditions. The extent of protein digestion can be seen by comparing the untreated sample in the first lane to the sam-

ple in the second lane which contains both hemoglobin and **EndoPrep™** but was not incubated in the water bath. Here, there is already significant reduction in all three hemoglobin populations. After 30 minutes of incubation at 37°C both the dimer and tetramer populations are completely digested. In addition, the monomer population is decreased by over 75%. After treatment for 120 minutes the monomer population is further reduced to less than 10% of the starting material.

These results show that as hemoglobin is digested, there is an accompanying increase in endotoxin activity. This pattern indicates that hemoglobin inhibits the detection of endotoxin. This is in contrast to some reports in the literature which claim an enhancing effect from hemoglobin [13, 14, 15, 16]. One explanation given for the enhancing effect is that hemoglobin has a detergent-like activity which reduces LPS aggregate size and allows the LPS monomers to be more biologically available. However, most endotoxin de-aggregating molecules, including melittin, lysozyme, complement proteins, BPI, and polymyxin B, cause a decrease in activity. It has also been suggested that enhancement may be due to phospholipid peroxidation, which would only be possible under certain assay criteria. This type of modification is not possible with our assay and may explain why some reports indicate inhibition while other publications report enhancement. Also, protein concentration and assay methods have been shown to be contributing factors in conflicting results involving protein effect on endotoxin [14]. Regardless of the direction of effect, the above protocol would remove the hemoglobin from the sample and allow a more accurate determination of endotoxin content.

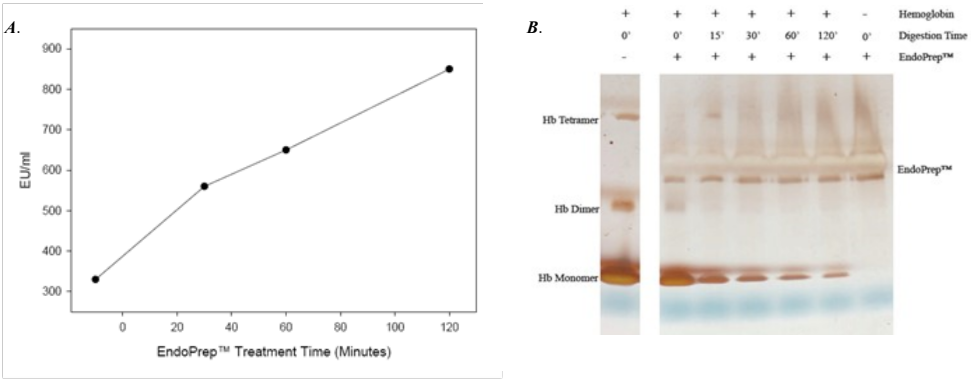


Figure 3. Treatment of Hemoglobin with EndoPrep™. (*A*) Recovery of endogenous endotoxin contamination in a sample of hemoglobin from bovine erythrocytes. (*B*) PAGE data showing hemoglobin degradation with EndoPrep™ treatment. The gel was run under reducing conditions resulting in three distinct hemoglobin populations. Locations of each hemoglobin population and the EndoPrep™ protease are indicated.

Bovine Serum Albumin

Albumin is the most abundant circulatory protein at a concentration of approximately 50 mg/ml (0.75 mM). It is involved in the delivery of fatty acids, hormones, bilirubin, bile salts, metals, and drugs throughout the body [17]. It consists of three homologous domains, two of which have an exposed hydrophobic pocket thought to bind the Lipid A portion of the endotoxin molecule [18]. This is consistent with other reports suggesting that human serum albumin binds Lipid A via a non-electrostatic interaction [19].

To test the effect of albumin on endotoxin detection a 100 mg/ml stock solution of bovine serum albumin (BSA) was prepared in endotoxin-free water. From this stock a 1 mg/ml sample was prepared in POAI™ Digestion Buffer. Previous experiments determined that the endogenous endotoxin contamination of the BSA stock was very low (about 0.05 EU/mg) so exogenous endotoxin was added to a final concentration

of 100 EU/mg. This sample was allowed to sit at room temperature for 30 minutes before the assay to allow potential interactions between the contents to be established. The untreated sample, containing no SB™ Protease Solution, gave a value of 58 EU/ml (Figure 4*A*), over 40% less than the calculated amount added. After treatment with SB™ Protease Solution for 15 minutes, the measured endotoxin value increased to 67 EU/ml. At this same time point the PAGE data indicates extensive BSA digestion (Figure 4*B*). Increases in digestion time increased detectable endotoxin levels to as much as 77 EU/ml. This indicates recovery of nearly 80% of the known contaminating endotoxin and an increase in accuracy of over 30% compared to untreated sample.

There is no consensus on the effects of albumin on endotoxin detection in the literature. Studies have shown that albumin contributes to the solubility of the Lipid A portion of endotoxin to increase pyrogenicity and gelation in an LAL assay [20]. Studies with recombinant human serum albumin (rHSA) show that the protein causes a change in micelle formation from a cubic form, which is typically moderately toxic, to a non-lamellar structure, indicating an increase in activity. However, this change in micelle structure caused minimal change in cytokine induction. In addition, tests with rHSA have shown complex changes in LAL coagulation assays without any discernible pattern [19]. Similar reports with purified HSA have indicated enhancement activity with chromogenic LAL tests but no effect in gelation LAL assays [14]. Other groups have found no effect of HSA on endotoxin activity as determined by either colorimetric or kinetic turbidimetric LAL assays or with the rabbit pyrogen test [21]. The current tests indicate a slight inhibitory effect of BSA on endotoxin detection. Regardless, as with hemoglobin, these tests show complete degradation of the protein and increased detection accuracy without negative effects on the assay system.

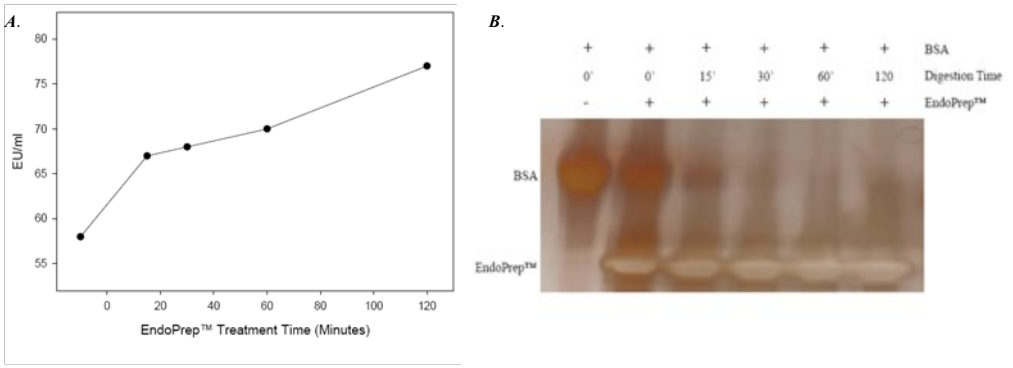


Figure 4. Treatment of BSA with EndoPrep™. (*A*) Recovery of defined endotoxin contamination in a sample of BSA. (*B*) PAGE data showing BSA degradation with EndoPrep™ treatment. Location of BSA and the EndoPrep™ protease are indicated.

IgG

IgG is the most abundant immunoglobulin accounting for approximately 75% of all serum antibodies. It is a tetrameric molecule consisting of two g heavy chains (about 50 kDa each) and two light chains (about 23 kDa each). Each heavy chain is linked to a light chain through disulfide bonding. Likewise, the heavy chains are linked together with disulfide bonds giving the molecule the signature "Y" structure. The number of antibody therapies has risen greatly in the last decade and account for more than 400 ongoing pre-approval clinical studies [22]. Indications include arthritis, Crohn's disease, transplantation technology, psoriasis, autoimmunity, RSV, multiple sclerosis, and asthma. This is in addition to the field of cancer

therapeutics which currently has promising antibody candidates for leukemia, mesothelioma, lymphoma, and colon, breast, kidney, lung, uterine, and pancreatic cancers [22, 23, 24, 25]. One reason for the increase in such therapies is the advent of recombinant antibodies. While this is a promising technology it demands a greater emphasis and sensitivity to endotoxin removal and detection.

The literature contains several reports indicating that IgG binds and neutralizes endotoxin. TNFα expression in response to endotoxin is reduced in the presence of IgG with a corresponding decrease in mortality in rats [26, 27]. IgG has also been shown to neutralize free endotoxin in a dose-dependent method as assayed with a chromogenic LAL assay [28]. To test the application of **EndoPrep™** on the inhibitory activity of IgG a 0.5 mg/ml sample of rabbit IgG (from plasma) was prepared in SB™ Digestion Buffer from a 12.5 mg/ml stock solution. Previous studies showed low endotoxin contamination so exogenous endotoxin was added to a final concentration of approximately 100 EU/ml. This solution was allowed to sit at room temperature for 30 minutes before the assay to allow potential interactions between IgG and endotoxin to form. Assay results from the untreated IgG sample showed the recovery of 81 EU/ml (Figure 5*A*). Treatment with **EndoPrep™** increased endotoxin activity to as much as 123 EU/ml, an increase of over 50%. PAGE analysis showed a corresponding change in IgG structure (Figure 5*B*). In reduced conditions, the heavy and light chains run as distinct bands at approximately 53 and 23 kDa respectively. With the addition of the SB™ Protease Solution there is a decrease in the heavy chain population and corresponding appearances of a large fragment at 25-30 kDa and small Fc fragments near the bottom of the gel. In agreement with most reports in the literature, these results indicate that IgG has an inhibitory effect on endotoxin activity. This effect is removed with **EndoPrep™** treatment.

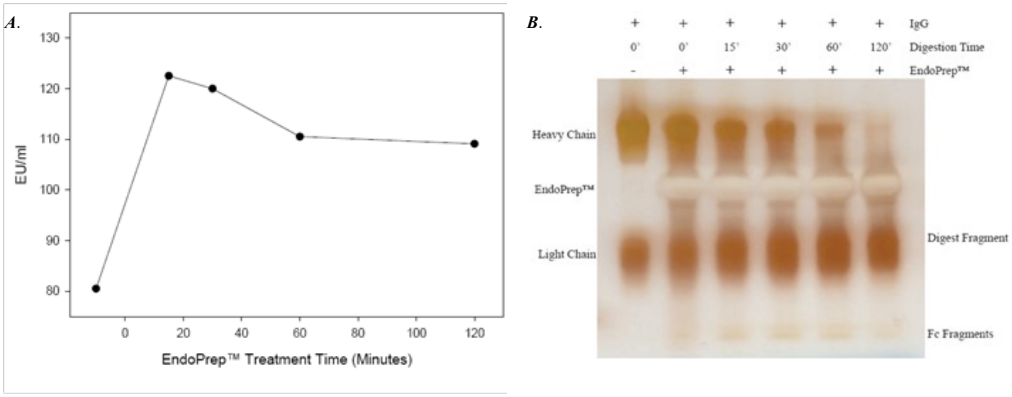


Figure 5. Treatment of IgG with EndoPrep™. (*A*) Recovery of defined endotoxin contamination in a sample of rabbit IgG from plasma. (*B*) PAGE data showing IgG degradation with EndoPrep™ treatment. The gel was run under reducing conditions resulting in the separation of the light and heavy chains. Digestion results in the cleavage of the heavy chain into a large fragment and small Fc fragments. Locations of the heavy and light chains, the EndoPrep™ protease, and the digestion products are indicated.

Discussion

Each endotoxin molecule contains long hydrophilic sugar chains, hydrophobic fatty acid chains, and negatively charged phosphate groups. Because of these properties, endotoxin is known to bind a wide variety of proteins. This binding is known to change the micelle and/or aggregate structure and result in changes in activity. Because accurate endotoxin detection is of the utmost importance in the clinical setting, sensitive methods of detection are paramount. Here we have described the use of **EndoPrep™** sample preparation system to remove the effects of proteins from samples containing endotoxin. Included have been examples of whole proteins and small peptides. In addition, the detection of both endogenous and exogenous endotoxin has been exhibited. In all examples shown, treatment with **EndoPrep™** increased the accuracy of endotoxin quantitation using either classical LAL or rFC assays (Table 2). The extent of change with **EndoPrep™** was dependent on the protein being tested. For example, the recovery of a large endotoxin spike was completely inhibited by untreated Peptide X but was recoverable following **EndoPrep™** treatment. Conversely, BSA only had a small inhibitory effect on endotoxin recovery. However, this effect was also removed with **EndoPrep™** treatment.

Sample	EU/ml Before EndoPrep™	EU/ml After EndoPrep™	Increase
Peptide X	<0.01	154	>15,000%
Sushi 3	17	54	200%
Hemoglobin	330	850	158%
BSA	58	77	33%
IgG	81	123	52%

Table 2. Summary of Results. Summary of the effects of EndoPrep™ treatment on various proteins and peptides. The table includes the amount of endotoxin determined experimentally in the samples before treatment and the peak recovery after treatment. The increase in detection is given as percent increase in endotoxin recovery.

EndoPrep™
Kit Includes:

1. POAI™ Digestion Buffer
2. POAI™ Protease Solution

References

1. U.S. Department of Health and Human Services, Food and Drug Administration. 1985. Bacterial Endotoxins/Pyrogens
2. Ryan, J. 2008. Endotoxins and Cell Culture: Technical Bulletin. Corning, Inc.
3. Obayashi, T. (1984) Addition of perchloric acid to blood samples for colorimetric limulus test using chromogenic substrate: comparison with conventional procedures and clinical applications. J. Lab. Clin. Med. 104(3): 321-330.
4. Westphal, O., O. Luderitz, and F. Blister. (1952) Extraction of bacteria with phenol/water. Naturforsch. B: Anorg. Chem. Org. Chem. Biochem. Biophys. Biol. 7B: 148-155.
5. Roth, R.I., F.C. Levin, and J. Levin (1990) Optimization of detection of bacterial endotoxin in plasma with the Limulus test. J. Lab. Clin. Med. 116(2): 153-161.
6. Petsch, D., W.D. Deckwer, and F.B. Anspach (1998) Proteinase K digestion of proteins improves detection of bacterial endotoxins by the Limulus amoebocyte lysate assay: application for endotoxin removal from cationic proteins. Anal. Biochem. 259(1): 42-47.
7. Rudbach, J.A. and A.G. Johnson (1964) Restoration of endotoxin activity following alteration by plasma. Nature 202: 811-812.
8. Tan, N.S., M.L.P. Ng, Y.H. Yau, P.K.W. Chong, B. Ho, and J.L. Ding (2000) Definition of endotoxin binding sites in horseshoe crab factor C recombinant sushi proteins and neutralization of endotoxin by sushi peptides. FASEB J. 14(12): 1801-1813.
9. Li, P., M. Sun, T. Wohland, D. Yang, B. Ho, and J.L. Ding (2006) Molecular mechanisms that govern the specificity of sushi peptides for gram-negative bacterial membrane lipids. Biochemistry. 45(35): 10554-10562.
10. Li, P., M. Sun, B. Ho, and J.L. Ding (2006) The specificity of sushi peptides for endotoxin and anionic phospholipids: potential application of POPG as an adjuvant for anti-LPS strategies. Biochem. Soc. Trans. 34(2): 270-272.
11. Ding, J.L., Y. Zhu, and B. Ho (2001) High-performance affinity capture-removal of bacterial pyrogen from solutions. J. Chromatogr. B Biomed. Sci. Appl. 759(2): 237-246.
12. www.biodtechinc.com
13. Roth, R.I. and W. Kaca (1994) Toxicity of hemoglobin solutions: hemoglobin is a lipopolysaccharide (LPS) binding protein which enhances LPS biological activity. Artif. Cells Blood Substit. Immobil. Biotechnol. 22(3): 387-398.
14. Kaca, W., R.I. Roth, and J. Levin (1994) Hemoglobin, a newly recognized lipopolysaccharide (LPS)-binding protein that enhances LPS biological activity. J. Biol. Chem. 269 (4): 25078-25084.
15. Roth, R.I. (1994) Hemoglobin enhances the production of tissue factor by endothelial cells in response to bacterial endotoxin. Blood 83(10): 2860-2865.
16. Roth, R.I., J. Levin, K.W. Chapman, M. Schmeizl, and F.R. Rickles (1993) Production of modified crosslinked cell-free hemoglobin for human use: the role of quantitative determination of endotoxin contamination. Transfusion 33(11): 919-924.
17. Peters, Jr. T. Albumin: An Overview and Bibliography. 2d ed. Kankakee, IL: Miles Inc. Diagnostic Division, 1992.
18. David, S.A. The Interaction of Lipid A and Lipopolysaccharide with Human Serum Albumin. In: Brade, H., ed. Endotoxin in Health and Disease. New York: Marcel-Dekker, Inc., 1999: 413-422.
19. Jurgens, G., M. Muller, P. Garidel, M.H. Koch, H. Nakakubo, A. Blume, and K. Brandenburg (2002) Investigation into the interaction of recombinant human serum albumin with Re-lipopolysaccharide and lipid A. J. Endotoxin. Res. 8: 115-126.
20. Rietschel, E.T., Y.B. Kim, D.W. Watson, C. Galanos, O. Luderitz, and O. Westphal (1973) Pyrogenicity and immunogenicity of lipid A complexed with bovine serum albumin or human serum albumin. Infect. Immun. 8(2): 173-177.
21. Asakawa, S., H. Fujiwara, S. Naito, R. Homma, S. Ishida, F. Chino, M. Tsuchiya, S. Matsuura, S. Tanaka, and M. Ohki (1994) Application of the Limulus test for practical quality control on endotoxin content in commercial human serum albumin (HSA) products. In comparison with the rabbit pyrogen test. Yakugaku. Zasshi. 114(11): 888-893.
22. Dubel, S. (2007) Recombinant therapeutic antibodies. Appl. Microbiol. Biotechnol. 74(4): 723-729.
23. Creput, C., A. Durrbach, B. Deroure, and B. Charpentier (2007) New therapeutic targets for antibodies and recombinant proteins in organ transplantation. Curr. Opin. Mol. Ther. 9(2): 153-159.
24. Rasmussen, S.K., L.K. Rasmussen, D. Weilguny, and A.B. Tolstrup (2007) Manufacture of recombinant polyclonal antibodies. Biotechnol. Lett. 29(6): 845-852.
25. Nayeem, M.S. and R.H. Khan (2006) Recombinant antibodies in cancer therapy. Curr. Protein Pept. Sci. 7(2): 165-170.
26. Yentis, S.M., R.P. Gooding, and P.G. Riches (1994) The effects of IgG and immune complexes on the endotoxin-induced cytokine response. Cytokine. 6(3): 247-254.
27. Xuan, D., D.P. Nicolau, C.H. Nightingale, and R. Quintiliani (2001) Circulating tumor necrosis factor-alpha production during the progression of rat endotoxic sepsis. Chemotherapy. 47(3): 194-202.
28. Xuan, D., D.P. Nicolau, P.R. Tessier, L. Bow, R. Quintiliani, and C.H. Nightingale (1997) In vitro reduction of endotoxin concentrations with the 5S fragment of immunoglobulin G. Antimicrob. Agents Chemother. 41(7): 1512-1516.

Predictive Oncology®

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Endotoxin detection products:

EndoPrep™	*20 reactions*	*EDP-4001.01*
ESP™	*20 reactions*	*ESP-9001.01*

Endotoxin removal products:

EndoBind-R™	*1 ml column*	*EBR-3001.01*
EndoBind-R™	*5 ml column*	*EBR-3005.01*
EndoBind-R™	*Bulk resin*	*Inquire*
EndoBind-R™ Single-Use		*EBR-3001.11*