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(54) **ENHANCING ENDOTOXIN DETECTION**

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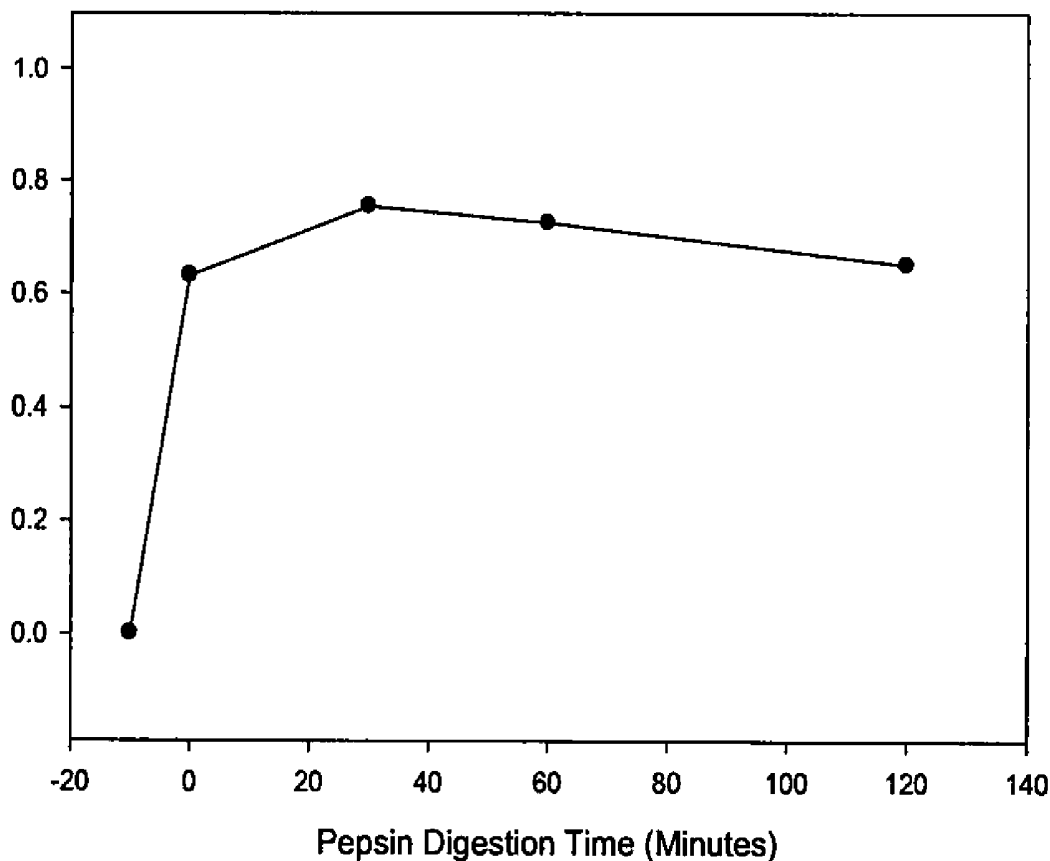
(57) **ABSTRACT**

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Provided herein are methods for detecting gram negative bacteria or lipopolysaccharide in a sample. Kits for detecting gram negative bacteria or lipopolysaccharide in a sample are provided.

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### 1 EU/ml PPC Recovery with Peptide X



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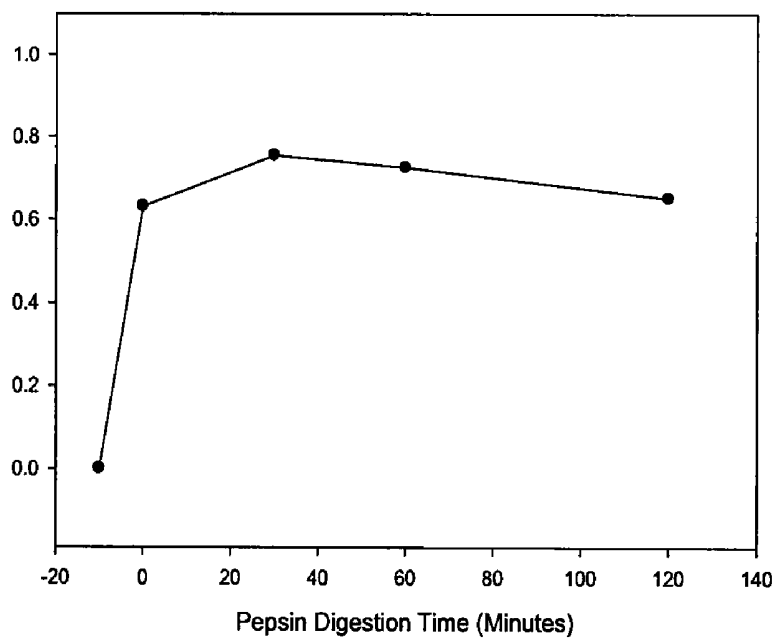


FIG. 1A

LPS Recovery in Peptide X Sample

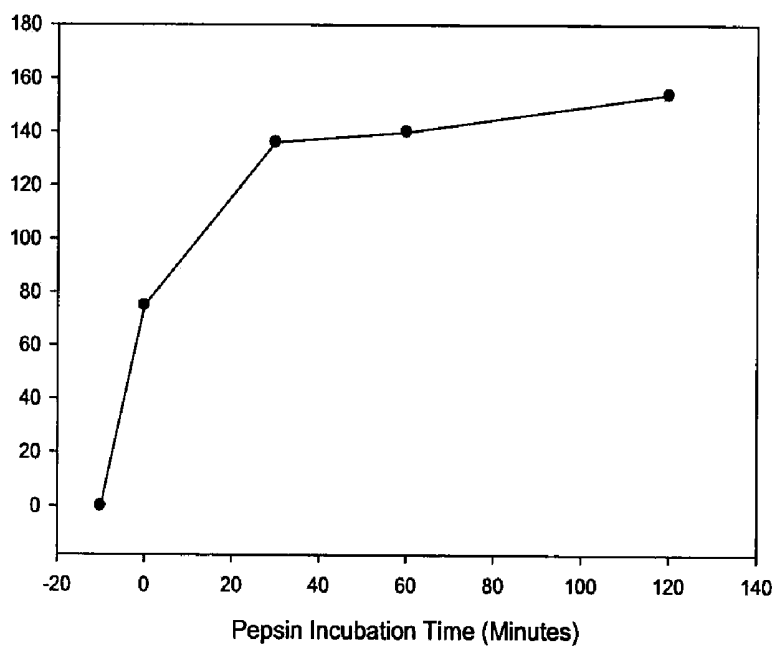


FIG. 1B

LPS Recovery in BSA Samples

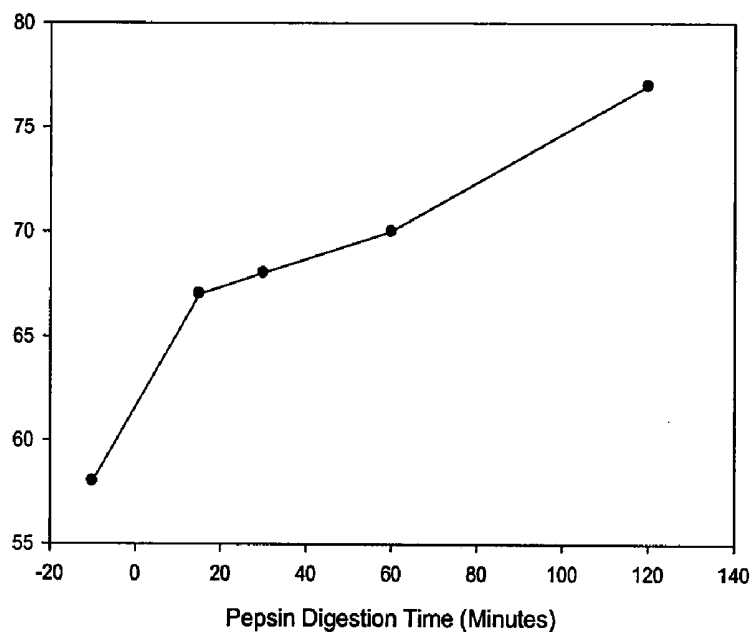


FIG. 2

Effect of Pepsin on rFC Activity

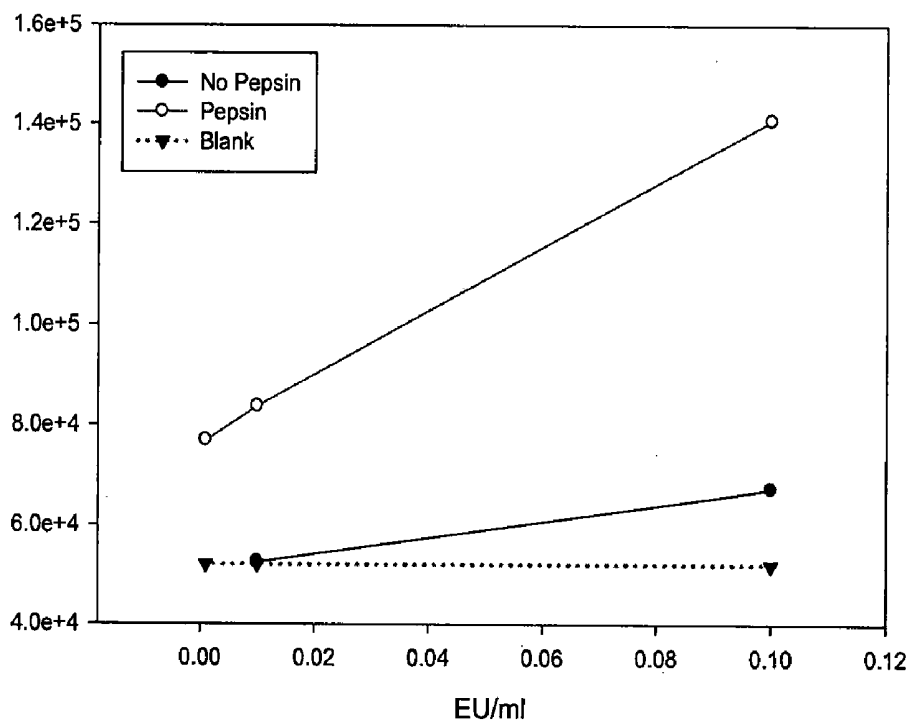


FIG. 3

## ENHANCING ENDOTOXIN DETECTION

### BACKGROUND

[0001] Endotoxin, also known as lipopolysaccharide (LPS), is an integral component of the gram-negative bacterial cell membrane and is responsible for many, if not all, of the toxic effects that occur during gram-negative bacterial sepsis. LPS is a mixture of glycolipids consisting of a variable polysaccharide domain covalently bound to a conserved glucosamine-based phospholipid known as lipid A. LPS directly stimulates host monocytes and macrophages to secrete a wide array of inflammatory cytokines that include tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukins-1 (IL-1), and interleukin-8 (IL-8). Excessive release of these cytokines by host macrophages contributes to organ failure and death that occur after episodes of gram-negative bacterial sepsis. The pro-inflammatory bioactivities exhibited by LPS typically reside in the lipid A moiety.

### SUMMARY

[0002] Provided herein are methods for detecting gram negative bacteria or lipopolysaccharide in a sample. For example, provide is a method for detecting gram negative bacteria or lipopolysaccharide in a sample comprising contacting the sample with one or more lipopolysaccharide binding polypeptides in the presence of an inactive acidic protease, and determining whether the one or more lipopolysaccharide binding polypeptides bind to the sample. Binding indicates the sample contains gram negative bacteria or lipopolysaccharide. Also provided herein are methods for detecting gram negative bacteria or lipopolysaccharide in a sample comprising contacting the sample with an active acidic protease under conditions that result in degradation of the proteins in the sample, contacting the sample with one or more lipopolysaccharide binding polypeptides in the presence of an inactive acidic protease, and determining whether the one or more lipopolysaccharide binding polypeptides bind to the sample.

[0003] Provided is a method for detecting gram negative bacteria or lipopolysaccharide in a sample comprising contacting the sample with an active acidic protease under conditions that result in degradation of the proteins in the sample, further contacting the sample with an amoebocyte lysate, and determining whether a gelation reaction occurs in the amoebocyte lysate.

[0004] Kits for detecting gram negative bacteria or lipopolysaccharide in a sample are provided. The kits comprise an acidic protease. The kits also comprise one or more amoebocyte lysates and/or one or more lipopolysaccharide binding polypeptides.

[0005] The details of one or more aspects are set forth in the accompanying drawings and description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

### DESCRIPTION OF DRAWINGS

[0006] FIGS. 1A-1B show positive product control (PPC) recovery and lipopolysaccharide (LPS) recovery in peptide x sample. Data shown is from 60 minute PyroGene® (Lonza; Basel, Switzerland) incubation. FIG. 1A is a graph representing the ability to recover 1 Endotoxin Unit (EU)/ml PPC spike before and after Pepsin digestion. Without Pepsin there is a 0% recovery indicating complete assay inhibition with intact

Peptide X. Upon addition of Pepsin, Peptide X is degraded and PPC recovery increases to as much as 75%. Industry standards dictate that a PPC recovery in the 50-200% range is acceptable. FIG. 1B is a graph representing the ability to recover the 250 EU/ml (calculated) added to the Peptide X sample prior to incubation. As with the PPC recovery in FIG. 1A, samples with intact Peptide X result in 100% assay inhibition. Addition of Pepsin is accompanied by an increase in LPS recovery to 154 EU/ml.

[0007] FIG. 2 is a graph showing LPS recovery in bovine serum albumin (BSA) sample. Data shown is from the 60 minute PyroGene® (Lonza; Basel, Switzerland) incubation. This graph represents the ability to recover the 100 EU/ml (calculated) LPS added to the BSA sample prior to incubation. With BSA, PPC recovery was sufficient in all samples, including non-digested BSA. The sample with intact BSA showed 58 EU/ml. Addition of Pepsin is accompanied by a steady increase in LPS recovery to 77 EU/ml, indicating a 33% increase in sensitivity in samples treated with Pepsin.

[0008] FIG. 3 is a graph showing the effect of pepsin on recombinant Factor C (rFC) activity. Data shown is from the 60 minute PyroGene® (Lonza; Basel, Switzerland) incubation. Solid black line with closed black circles for data points indicates results from LPS in water. Solid black line with open circles for data points indicates results from LPS in water containing 0.05% Pepsin. Dotted black line with triangles for data points indicates the value of water. Treatment with Pepsin enhances the activity of the rFC Enzyme as evidenced by the increase in fluorescence.

### DETAILED DESCRIPTION

[0009] Since endotoxin contains a negative charge, a lipid and a carbohydrate, many proteins are known to bind endotoxin and affect the ability of assays to properly measure it. Specifically, proteins binding to endotoxin can cause either inhibition or enhancement in the standard assays resulting in false positives and negatives, as well as affecting accuracy. For example, serine protease inhibitors, such as, soybean trypsin inhibitor, alpha2 macroglobulin, aprotinin, anti-plasmin, anti-thrombin III, anti-trypsin and hirudin, inhibit methods of detecting endotoxin. As described in the examples below, treatment of such samples, including serum and blood samples, with an acidic protease degrades the proteins in the sample without affecting endotoxin, thus, increasing the accuracy of endotoxin detection assays. The acidic proteases can be used in, for example, LAL, Factor C and cytokine ELISA assays. There are a variety of assays for the diagnosis of gram negative bacterial infections. The current assay used by pharmaceutical and medical industries is based on a substance called Limulus Amoebocyte Lysate (LAL). The lysate contains proteins that react in the presence of endotoxins.

[0010] Factor C is a serine protease zymogen. It is the key enzyme in the *C. rotundicauda* amoebocyte lysate (CAL) that is activated by LPS to initiate the coagulation cascade. Factor C activity is the basis of a sensitive assay for femtogram levels of endotoxin used in the quality control of pharmaceutical products. The importance of Factor C in the detection of endotoxin has led to the expression of recombinant Factor C, rFC, as an alternative source to alleviate the batch-to-batch and seasonal variation in the sensitivity of detection of endotoxin.

[0011] Inactive acidic proteases, such as inactive pepsin and inactive HIV protease, enhance the activity of Factor C by acting as a chaperone and maintaining Factor C in proper

conformation. The ability of inactive pepsin and inactive HIV protease to act as chaperones has been described (Hulko et al., *Protein Science* 16:644-53 (2007)).

**[0012]** Provided herein are improved methods and kits for detecting gram negative bacteria or lipopolysaccharide in a sample. For example, provided herein are methods for detecting gram negative bacteria or lipopolysaccharide in a sample comprising contacting the sample with one or more lipopolysaccharide binding polypeptides in the presence of an inactive acidic protease, and determining whether the one or more lipopolysaccharide binding polypeptides bind to the sample. Binding indicates the sample contains gram negative bacteria or lipopolysaccharide. Optionally, the method further comprises inactivating the acidic protease prior to contacting the sample with the one or more lipopolysaccharide binding polypeptides. Optionally, the method further comprising contacting the sample with an active acidic protease under conditions that result in degradation of the proteins in the sample prior to contacting the sample with the one or more lipopolysaccharide binding polypeptides. Provided herein are methods for detecting gram negative bacteria or lipopolysaccharide in a sample comprising contacting the sample with an active acidic protease under conditions that result in degradation of the proteins in the sample, contacting the sample with one or more lipopolysaccharide binding polypeptides in the presence of an inactive acidic protease, and determining whether the one or more lipopolysaccharide binding polypeptides bind to the sample. Binding indicates the sample contains gram negative bacteria or lipopolysaccharide. Optionally, the method further comprises inactivating the acidic protease after contacting the sample with the active acidic protease. Optionally, a digestion buffer comprises the active acidic protease. Optionally, the acidic protease is inactivated by a pH of about 7.0.

**[0013]** Also provided is a method for detecting gram negative bacteria or lipopolysaccharide in a sample comprising contacting the sample with an active acidic protease under conditions that result in degradation of the proteins in the sample, further contacting the sample with an amoebocyte lysate, and determining whether a gelation reaction occurs in the amoebocyte lysate. A gelation reaction indicates the sample contains gram negative bacteria or lipopolysaccharide. Optionally, the amoebocyte lysate is selected from the group consisting of lysates of *Limulus polyphemus*, *Tachypleus tridentatus*, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*.

**[0014]** Lipopolysaccharide binding polypeptides suitable for use in the provided methods and kits include lipopolysaccharide binding polypeptides of an amoebocyte lysate. The amoebocyte lysate is, optionally, selected from the group consisting of lysates of *Limulus polyphemus*, *Tachypleus tridentatus*, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*. Optionally, the one or more lipopolysaccharide binding polypeptides are polypeptides comprising a lipopolysaccharide binding domain of a Factor C protein. The endotoxin/lipid A-binding domain of Factor C lies within the amino terminal portion of the protein encompassed by rFCES; that is, the first 350 amino acids, numbered as in SEQ ID NO:8. The Factor C protein and its domains, including sushi domains, are described in U.S. Pat. No. 6,719,973, which is incorporated by reference herein in its entirety. U.S. Pat. No. 6,719,973 also discloses sushi peptides of the Factor C protein and methods for making and using the sushi peptides. Thus, the lipopolysaccharide binding domain of a Factor C

protein is optionally a sushi domain or peptide. The lipopolysaccharide binding domain of a Factor C protein is, for example, amino acids 1-333 of a Factor C protein (e.g., amino acids 1-333 of SEQ ID NO:8), amino acids 29-330 of SEQ ID NO:8, a sushi 1 domain of a Factor C protein (e.g., amino acids 29-201 of SEQ ID NO:8), a sushi 2 domain of a Factor C protein (e.g., amino acids 195-260 of SEQ ID NO:8), a sushi 3 domain of a Factor C protein (e.g., amino acids 264-330 of SEQ ID NO:8), a sushi 1 peptide (SEQ ID NO:1), a sushi 2 peptide (SEQ ID NO:2), a sushi 3 peptide (SEQ ID NO:3), a sushi 1Δ peptide (SEQ ID NO:4), a sushi 3Δ peptide (SEQ ID NO:5), a sushi 4 peptide (SEQ ID NO:6) or a sushi 5 peptide (SEQ ID NO:7).

**[0015]** Optionally, the step of contacting the sample with the one or more lipopolysaccharide binding polypeptides comprises contacting the sample with a dimer or a tetramer comprising a lipopolysaccharide binding domain of a Factor C protein. As used herein, the phrase Factor C dimer refers to two molecules of a Factor C protein or portion thereof linked together. The provided Factor C dimers comprise a first and a second polypeptide, wherein the first and second polypeptides each comprise a lipopolysaccharide binding domain of a Factor C protein. Optionally, the first and second polypeptide are the same or different polypeptides. Optionally, the lipopolysaccharide binding domain of a Factor C protein is a sushi peptide. The sushi peptide can be a sushi 1 peptide (SEQ ID NO:1), a sushi 2 peptide (SEQ ID NO:2), a sushi 3 peptide (SEQ ID NO:3), a sushi 1Δ peptide (SEQ ID NO:4), a sushi 3Δ peptide (SEQ ID NO:5), a sushi 4 peptide (SEQ ID NO:6) or a sushi 5 peptide (SEQ ID NO:7). Optionally, the sushi peptide is a sushi 3 peptide or a sushi 3Δ peptide. Optionally, the polypeptides of the Factor C dimer are linked by disulfide bonds. A disulfide bond is a bond between cysteine residues. Optionally, each of the first and second polypeptides that comprise the Factor C dimer consists of SEQ ID NOs:1, 2, 3, 4, 5, 6 or 7.

**[0016]** The term protein, peptide, polypeptide, or peptide portion is used broadly herein to mean two or more amino acids linked by a peptide bond. It should be recognized that the terms peptide and polypeptide are not used herein to suggest a particular size or number of amino acids comprising the molecule and that a peptide can contain up to several amino acid residues or more. The provided peptides are produced by a proteolytic reaction on a polypeptide, i.e., a peptide produced upon cleavage of a peptide bond in the polypeptide. The provided peptides are optionally produced using methods of chemical synthesis or methods of recombinant DNA technology, to produce a synthetic polypeptide. For example, the provided peptides are synthesized using step-wise solid phase peptide synthesis on 2-chlorotrityl resin using standard Fmoc protecting groups and pseudo-prolines. Alternatively, the peptides are synthesized by combining two or more smaller peptides that have been chemically synthesized.

**[0017]** Nucleic acids that encode the aforementioned peptide sequences, variants and fragments thereof are also disclosed. These sequences include all degenerate sequences related to a specific polypeptide sequence, i.e., all nucleic acids having a sequence that encodes one particular polypeptide sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the polypeptide sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact

disclosed and described herein through the disclosed protein sequence. A wide variety of expression systems are used to produce Factor C peptides as well as fragments, isoforms, and variants.

**[0018]** The nucleic acid sequences provided herein are examples of the genus of nucleic acids and are not intended to be limiting. Also provided are expression vectors comprising nucleic acids that encode the peptide sequences, variants or fragments thereof, wherein the nucleic acids are operably linked to an expression control sequence. Further provided are cultured cells comprising the expression vectors. Such expression vectors and cultured cells can be used to make the provided peptides.

**[0019]** By isolated or purified is meant a composition (e.g., a polypeptide or nucleic acid) that is substantially free from other materials, including materials with which the composition is normally associated in nature. The polypeptides of the invention, or fragments thereof, are obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding the polypeptide (e.g., in a cell or in a cell-free translation system), or by chemically synthesizing the polypeptide.

**[0020]** In the provided methods and kits, the lipopolysaccharide binding polypeptides are optionally labeled with a detectable moiety or further comprise a reporter protein or affinity tag. The lipopolysaccharide binding polypeptides are directly labeled or indirectly labeled (e.g., by a secondary or tertiary antibody that is labeled with a detectable moiety). Numerous labels are available including, but not limited to radioisotopes, fluorescent labels, and enzyme-substrate labels. Radioisotopes include, for example,  $^{35}\text{S}$ ,  $^{14}\text{C}$ ,  $^{125}\text{I}$ ,  $^3\text{H}$ , and  $^{131}\text{I}$ . Fluorescent labels include, for example, rare earth chelates (europium chelates), fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin and Texas Red. The labels are optionally conjugated to the antigen binding partner using the techniques disclosed in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al., Ed., Wiley-Interscience, New York, N.Y., Pubs., (1991), for example.

**[0021]** When using enzyme-substrate labels, the enzyme preferably catalyzes a chemical alteration of the chromogenic substrate, which can be measured using various techniques. For example, the enzyme catalyzes a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme alters the fluorescence or chemiluminescence of the substrate. The chemiluminescent substrate becomes electronically excited by a chemical reaction and then emits light which can be measured (using a chemiluminescence meter, for example), or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRP), alkaline phosphatase,  $\beta$ -galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes are described in O'Sullivan et al., Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzym. (ed J. Langone & H. Van Vunakis), Academic press, New York, 73: 147-166 (1981). Examples of enzyme-substrate combinations include, for example, horseradish peroxidase (HRP) with

hydrogen peroxidase as a substrate, alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate, and  $\beta$ -D-galactosidase ( $\beta$ -D-Gal) with a chromogenic substrate (e.g. p-nitrophenyl- $\beta$ -D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl- $\beta$ -D-galactosidase.

**[0022]** An assay is optionally employed wherein the lipopolysaccharide binding polypeptides are attached to a solid support, such as, for example, a column, chip, or plate. The solid support is optionally a mobile solid support (e.g., a bead). A sample derived from the subject or control is passed over (i.e., contacted with) the solid support. Labeled lipopolysaccharide binding polypeptides are then added to the solid support. The amount of label detected that is attached to the target is correlated to a quantity of target in the sample. For example, a lipopolysaccharide binding polypeptide is immobilized on the bottom of a microtiter plate. A sample to be tested is then added to the microtiter plate under conditions that allow binding of the lipopolysaccharide binding polypeptide to gram negative bacteria, endotoxin or lipopolysaccharide if present in the sample. A second lipopolysaccharide binding polypeptide that is labeled with, for example, biotin, is added to the microtiter plate. The labeled polypeptide is detected with avidin coupled to alkaline phosphatase. The avidin coupled to alkaline phosphatase detects bound and labeled polypeptide by liberating p-nitrophenol from a p-nitrophenylphosphate (pNA) substrate producing a yellow color that can be measured at 405 nm.

**[0023]** FACS sorting is used to detect and quantitate, for example, fluorescently labeled beads. For example, the lipopolysaccharide binding polypeptide is linked to a bead. The beads are then contacted with a sample to be tested. A second lipopolysaccharide binding polypeptide that is labeled with, for example, a fluorescent label is added to the beads that have been contacted with the test sample. The bound, labeled beads are then detected and quantitated using a FACS sorter on the basis of one or more fluorescent labels. Optionally, the beads are magnetic beads or fluorescent beads. For example, the lipopolysaccharide binding polypeptide is linked to a mobile solid support such as a bead, which can be added to a biological sample, such as whole blood or blood products like serum, and removed by centrifugation. Optionally, the lipopolysaccharide binding polypeptide is linked to a magnetic bead, such as an iron bead, and removed by a magnetic field.

**[0024]** By way of another example, the lipopolysaccharide binding polypeptide is immobilized on an immobile solid support, such as, for example, a column, filter or membrane. Thus, the lipopolysaccharide binding polypeptide is immobilized, for example, on a hemodialysis membrane or filtration system to remove bacteria and/or endotoxin from whole blood or blood products. Thus, support as used herein refers to any support matrix, such as those solid supports known in the art, which serve to immobilize the lipopolysaccharide binding polypeptide. Suitable supports include, but are not limited to, glass, agarose, plastic, silica, polyacrylamide, hydrogels, gels, membranes, filters, meshes, beads or particles comprised of or coated with cellulose, acrylates, polyacrylates, polyhydroxymethacrylates, polystyrene, dextran, agarose, polysaccharides, hydrophilic vinyl polymers, polymerized derivatives, as well as any porous or non-porous matrices.

**[0025]** Optionally, the lipopolysaccharide binding polypeptides further comprise a reported protein or affinity tag. Suitable reporter proteins include, for example, green



are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, if there is a variety of additional steps that can be performed in a method, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed. Thus, a number of aspects have been described. Nevertheless, it will be understood that various modifications may be made. Furthermore, when one characteristic or step is described, it can be combined with any other characteristic or step herein even if the combination is not explicitly stated. Accordingly, all combination of disclosed agents, steps and characteristics are provided even in the absence of explicit disclosure herein.

**[0031]** Ranges may be expressed herein as from about one particular value, and/or to about another particular value. When such a range is expressed, this includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent about, it will be understood that the particular value is disclosed.

**[0032]** Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

**[0033]** The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of the methods and materials provided herein.

## EXAMPLES

### Example 1

#### Use of Pepsin to Enhance Methods of Detecting Endotoxin in a Sample

**[0034]** The following experiments compared the recovery of native or endogenous lipopolysaccharide (LPS) in a protein sample before and after digestion with pepsin. Several proteins are known to bind LPS and affect their ability to be properly measured. Treatment of such samples with pepsin via the following protocol increases the accuracy of Limulus Amebocyte Lysate (LAL) and recombinant Factor C (rFC) assays as well as cytokine ELISA assays. The protocol outlines the procedure used to evaluate a peptide sample (Peptide X) and a protein sample (bovine serum albumin (BSA)). Both Peptide X and BSA contained negligible amounts of endogenous LPS, so a defined amount of *E. coli* O55:B5 LPS was added.

**[0035]** Pepsin Stock Preparation. To create a 5% pepsin stock solution, 250 mg of pepsin was added to 5 ml of digestion buffer (20 mM sodium acetate, pH 4.5). This mixture was vortexed for several minutes until all of the pepsin went into solution. The pepsin was then stored at 4° C.

**[0036]** LPS Stock Preparation. To create different concentrations of LPS stock solutions, 1 ml of endotoxin-free water was added to a 5 mg vial of *E. coli* O55:B5 LPS to create a 5 mg/ml (50,000,000 Endotoxin Units (EU)/ml) stock solution. This mixture was vortexed for several minutes until all of the LPS went into solution. To create a 500,000 EU/ml solution,

10 µl of the 5 mg/ml LPS solution was added to 990 µl of endotoxin-free water. To create a 50,000 EU/ml solution, 100 µl of the 5 mg/ml LPS solution was added to 900 µl of endotoxin-free water. To create a 10,000 EU/ml solution, 200 µl of the 5 mg/ml LPS solution was added to 800 µl of endotoxin-free water.

**[0037]** Peptide X and Lipopolysaccharide (LPS) Sample Preparation. Since Peptide X contained a negligible amount of endogenous LPS, a defined amount of *E. coli* O55:B5 LPS was added to carry out the experiment. Peptide X was obtained as a 20 mg/ml stock solution. A 0.1 mg/ml Peptide X, 250 EU/mL LPS solution was made by adding 10 µl of the 20 mg/ml Peptide X solution and 10 µl of the 50,000 EU/ml LPS stock solution to 1980 µl of digestion buffer (20 mM sodium acetate, pH 4.5).

**[0038]** Pepsin digestion, preparation for PyroGene® assay (Lonza; Basel, Switzerland) and PyroGene® assay (Lonza; Basel, Switzerland). Pepsin digestion occurs at 37° C. To perform these experiments, a water bath was set to 37° C. To assay different time points, 270 µl of the Peptide X and LPS solution was aliquoted into six endotoxin-free glass dilution tubes. 30 µl of 5% pepsin were added to each sample, and the sample was vortexed. The samples were incubated at 37° C. for 0, 15, 30, 60, and 120 minutes. A control sample was treated with 30 µl of digestion buffer instead of pepsin and was not incubated at 37° C. The 0 minute time point sample was mixed with pepsin but was not incubated at 37° C.

**[0039]** The samples were then diluted 100-fold in endotoxin-free water. Briefly, 50 µl of each sample was diluted in 450 µl of endotoxin-free water for a 1:10 dilution, and 50 µl of the 1:10 dilution was diluted in 450 µl of endotoxin-free water for a 1:100 dilution. Thus the final concentrations of the samples was 1:100.

**[0040]** The PyroGene® assay (Lonza; Basel, Switzerland) was carried out according to manufacturer's protocols. LPS standards were created according to manufacturer's protocols. Each sample was tested both with and without a 1 EU/ml Positive Product Control (PPC) LPS spike. Samples with a 1 EU/ml PPC LPS spike were created by taking 45 µl of the sample and adding 5 µl of 10 EU/ml LPS. Each sample was added to 50 µl of PyroGene® working reagent (20 µl of recombinant Factor C (rFC) assay buffer, 5 µl rFC enzyme solution, and 25 µl fluorogenic substrate). The sample and working reagent were added to a single well of a Corning® (Corning Incorporated Life Sciences; Lowell, Mass.) 3604 Assay Plate and incubated in a BioTek Synergy 2 Plate Reader (BioTek; Winooski, Vt.) at 37° C. Fluorescence readings were made at 15, 30, 45, and 60 minute time points. Results are shown in Table 1 and FIG. 1.

TABLE 1

Fluorescence data at 60 minute PyroGene® (Lonza; Basel, Switzerland) incubation.			
[Pepsin]	Digestion Time	Measured LPS Content	1 EU/ml PPC Recovery
0%	0'	0 EU/ml	0 EU/ml
0.5%	0'	75 EU/ml	0.631 EU/ml
0.5%	30'	136 EU/ml	0.755 EU/ml
0.5%	60'	140 EU/ml	0.726 EU/ml
0.5%	120'	154 EU/ml	0.652 EU/ml

Data were gathered for Peptide X and LPS samples tested with and without a 1 EU/ml PPC LPS spike. Without pepsin there is a 0% recovery indicating a complete assay inhibition with intact Peptide X. Upon addition of pepsin, Peptide X is degraded and PPC recovery increases to as much as 75%.

**[0041]** BSA and LPS Sample Preparation. Since BSA contains a negligible amount of endogenous LPS, a defined amount of *E. coli* O55:B5 LPS was added to carry out the experiment. A 1 mg/ml BSA, 100 EU/ml LPS solution was made by adding 20  $\mu$ l of a 100 mg/ml BSA solution and 20  $\mu$ l of a 10,000 EU/ml LPS stock solution to 1960  $\mu$ l of digestion buffer (20 mM sodium acetate, pH 4.5).

**[0042]** Pepsin digestion, preparation for PyroGene<sup>®</sup> assay (Lonza; Basel Switzerland), and PyroGene<sup>®</sup> assay (Lonza; Basel, Switzerland). The pepsin digestion, preparation for PyroGene<sup>®</sup> assay (Lonza; Basel, Switzerland), and the PyroGene<sup>®</sup> assay (Lonza; Basel, Switzerland) were carried out as described above for the Peptide X and LPS sample. Results are shown in Table 2 and FIG. 2.

TABLE 2

Fluorescence data at 60 minute PyroGene <sup>®</sup> (Lonza; Basel, Switzerland) incubation.		
[Pepsin]	Digestion Time	Measured LPS Content
0%	0'	58 EU/ml
0.5%	15'	67 EU/ml
0.5%	30'	68 EU/ml
0.5%	60'	70 EU/ml
0.5%	120'	77 EU/ml

Data were gathered for BSA and LPS samples treated with pepsin. The sample with untreated BSA and LPS showed 58 EU/ml. Addition of pepsin increased LPS recovery to 77 EU/ml, indicating a 33% increase in sensitivity.

## Example 2

Use of Pepsin to Increase Sensitivity of Methods of  
Detecting Endotoxin

**[0043]** The following experiments compare a series of LPS dilutions in water to a similar series containing a defined amount of pepsin. This data shows pepsin enhances the activity of the rFC Enzyme in the Lonza PyroGene<sup>®</sup> assay and results in an increase in sensitivity. This allows LPS detection below the current 0.01 EU/ml LPS concentration.

**[0044]** Pepsin Stock Preparation. To create a 5% stock solution of pepsin, 250 mg of pepsin was dissolved in 5 ml of digestion buffer (20 mM sodium acetate, pH 4.5). The mixture was vortexed until all the pepsin went into solution. The 5% pepsin solution was stored at 4° C.

**[0045]** LPS Sample Preparation. To create a series of LPS dilutions, a Lonza<sup>®</sup> (Lonza; Basel, Switzerland) LPS stock (20 EU/ml) was serially diluted. A 10 EU/ml LPS solution was created by diluting 200  $\mu$ l of water with 200  $\mu$ l of Lonza<sup>®</sup> stock (Lonza; Basel, Switzerland) for a final volume of 400  $\mu$ l. A 1 EU/ml LPS solution was created by adding 50  $\mu$ l of the 10 EU/ml LPS solution to 450  $\mu$ l of water for a final volume of 500  $\mu$ l. A 0.1 EU/ml LPS solution was created by adding 50  $\mu$ l of the 1 EU/ml LPS solution to 450  $\mu$ l of water for a final volume of 500  $\mu$ l. A 0.01 EU/ml LPS solution was created by adding 50  $\mu$ l of a 0.1 EU/ml LPS solution to 450  $\mu$ l of water for a final volume of 500  $\mu$ l.

**[0046]** LPS and Pepsin Sample Preparation. To create the same series of LPS dilutions that also contain 0.05% pepsin, two stock solutions of pepsin were made. A 0.1% stock solution of pepsin was made by adding 196  $\mu$ l of water to 4  $\mu$ l of 5% pepsin for a final volume of 200  $\mu$ l. A 0.05% stock solution of pepsin was made by adding 1782  $\mu$ l of water with 18  $\mu$ l of 5% pepsin for a final volume of 1800  $\mu$ l. 200  $\mu$ l of the 0.1% pepsin solution was added to 200  $\mu$ l of Lonzag (Lonza; Basel, Switzerland) LPS stock (20 EU/ml) to give 400  $\mu$ l of a 10 EU/ml LPS, 0.05% pepsin solution. The 0.05% stock solution of pepsin was divided equally (450  $\mu$ l) into 4 tubes. A 1 EU/ml LPS, 0.05% pepsin solution was created by adding 50  $\mu$ l of the 10 EU/ml LPS, 0.05% pepsin solution to 450  $\mu$ l of a 0.05% pepsin solution. A 0.1 EU/ml LPS, 0.05% pepsin solution was created by adding 50  $\mu$ l of the 1 EU/ml LPS, 0.05% pepsin solution to 450  $\mu$ l of a 0.05% pepsin solution. A 0.01 EU/ml LPS, 0.05% pepsin solution was created by adding 50  $\mu$ l of the 0.1 EU/ml LPS, 0.05% pepsin solution to 450  $\mu$ l of a 0.05% pepsin solution. A 0.001 EU/ml LPS, 0.05% pepsin solution was created by adding 50  $\mu$ l of the 0.01 EU/ml LPS, 0.05% pepsin solution to 450  $\mu$ l of a 0.05% pepsin solution.

**[0047]** PyroGene<sup>®</sup> assay (Lonza; Basel, Switzerland). To test the endotoxin content of the samples prepared above, the PyroGene<sup>®</sup> assay (Lonza; Basel, Switzerland) was performed according to the manufacturer's specifications. Briefly, 50  $\mu$ l of each sample was mixed with 50  $\mu$ l of PyroGene<sup>®</sup> (Lonza; Basel, Switzerland) working reagent (20  $\mu$ l rFC assay buffer, 5  $\mu$ l rFC enzyme solution, and 25  $\mu$ l fluorogenic substrate) and was added to a single well of a Corning<sup>®</sup> (Corning Incorporated Life Sciences; Lowell, Mass.) 3604 Assay Plate. The plate was incubated in a BioTek Synergy 2 Plate Reader (BioTek; Winooski, Vt.) at 37° C. and fluorescence readings were taken at 15, 30, 45, and 60 minute time points. Results are shown in Table 3 and in FIG. 3.

TABLE 3

Fluorescence data at 60 minute PyroGene <sup>®</sup> (Lonza; Basel, Switzerland) incubation.	
Sample	Fluorescence
10 EU/ml in water	1,012,941
1 EU/ml in water	214,209
0.1 EU/ml in water	67,374
0.01 EU/ml in water	52,604
10 EU/ml in water with 0.05% Pepsin	3,161,479
1 EU/ml in water with 0.05% Pepsin	586,139
0.1 EU/ml in water with 0.05% Pepsin	140,712
0.01 EU/ml in water with 0.05% Pepsin	83,678
0.001 EU/ml in water with 0.05% Pepsin	76,873
Water	52,096

Data were gathered for serial dilutions of untreated and treated LPS samples. Samples were treated with 0.05% pepsin. Treated samples were more sensitive to recombinant Factor C as evidenced by the increase in fluorescence.

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 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
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 <223> OTHER INFORMATION: Sushi 1 Peptide

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 1                   5                   10                   15  
 Gln Trp Ser Asn Phe Pro Pro Lys Cys Ile Arg Glu Cys Ala Met Val  
                   20                   25                   30  
 Ser Ser

<210> SEQ ID NO 2  
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Glu Cys Ala Met Val Ser Ser Pro Glu His Gly Lys Val Asn Ala Leu  
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 Ser Gly Asp Met Ile Glu Gly Ala Thr Leu Arg Phe Ser Cys Asp Ser  
                   20                   25                   30  
 Pro Tyr Tyr Leu Ile Gly Gln Glu Thr Leu Thr Cys Gln Gly Asn Gly  
                   35                   40                   45  
 Gln Trp Asn Gly Gln Ile Pro Gln Cys Lys Asn  
                   50                   55

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His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln  
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                   20                   25                   30  
 Leu Met

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 1                   5                   10                   15  
 Gln Trp Ser Asn Phe Pro Pro Lys Cys Ile Arg Glu Cys Ala Met Val

-continued

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20                      25                      30  
 Ser Ser  
  
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 1                      5                      10                      15  
  
 Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe  
                     20                      25                      30  
  
 Leu Met  
  
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 1                      5                      10                      15  
  
 Phe Arg Gln Leu Thr Arg Val Thr Arg Thr Cys Ser Arg Phe Leu Arg  
                     20                      25                      30  
  
 Arg Met  
  
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                     20  
  
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 1                      5                      10                      15  
  
 Ala Gln Lys Met Arg Pro Val Gln Ser Lys Gly Val Asp Leu Gly Leu  
                     20                      25                      30  
  
 Cys Asp Glu Thr Arg Phe Glu Cys Lys Cys Gly Asp Pro Gly Tyr Val  
                     35                      40                      45  
  
 Phe Asp Ile Pro Val Lys Gln Cys Thr Tyr Phe Tyr Arg Trp Arg Pro  
                     50                      55                      60

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Tyr Cys Lys Pro Cys Asp Asp Leu Glu Ala Lys Asp Ile Cys Pro Lys  
 65 70 75 80  
 Tyr Lys Arg Cys Gln Glu Cys Lys Ala Gly Leu Asp Ser Cys Val Thr  
 85 90 95  
 Cys Pro Pro Asn Lys Tyr Gly Thr Trp Cys Ser Gly Glu Cys Gln Cys  
 100 105 110  
 Lys Asn Gly Gly Ile Cys Asp Gln Arg Thr Gly Ala Cys Ala Cys Ala  
 115 120 125  
 Asp Arg Tyr Glu Gly Val His Cys Glu Ile Leu Lys Gly Cys Pro Leu  
 130 135 140  
 Leu Pro Ser Asp Ser Gln Val Gln Glu Val Arg Asn Pro Pro Asp Asn  
 145 150 155 160  
 Pro Gln Thr Ile Asp Tyr Ser Cys Ser Pro Gly Phe Lys Leu Lys Gly  
 165 170 175  
 Met Ala Arg Ile Ser Cys Leu Pro Asn Gly Gln Trp Ser Asn Phe Pro  
 180 185 190  
 Pro Lys Cys Ile Arg Glu Cys Ala Met Val Ser Ser Pro Glu His Gly  
 195 200 205  
 Lys Val Asn Ala Leu Ser Gly Asp Met Ile Glu Gly Ala Thr Leu Arg  
 210 215 220  
 Phe Ser Cys Asp Ser Pro Tyr Tyr Leu Ile Gly Gln Glu Thr Leu Thr  
 225 230 235 240  
 Cys Gln Gly Asn Gly Gln Trp Asn Gly Gln Ile Pro Gln Cys Lys Asn  
 245 250 255  
 Leu Val Phe Cys Pro Asp Leu Asp Pro Val Asn His Ala Glu His Lys  
 260 265 270  
 Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr  
 275 280 285  
 Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Gly Phe Asp  
 290 295 300  
 Thr Leu Lys Cys Asn Pro Asp Gly Ser Trp Ser Gly Ser Gln Pro Ser  
 305 310 315 320  
 Cys Val Lys Val Ala Asp Arg Glu Val Asp Cys Asp Ser Lys Ala Val  
 325 330 335  
 Asp Phe Leu Asp Asp Val Gly Glu Pro Val Arg Ile His Cys Pro Ala  
 340 345 350  
 Gly Cys Ser Leu Thr Ala Gly Thr Val Trp Gly Thr Ala Ile Tyr His  
 355 360 365  
 Glu Leu Ser Ser Val Cys Arg Ala Ala Ile His Ala Gly Lys Leu Pro  
 370 375 380  
 Asn Ser Gly Gly Ala Val His Val Val Asn Asn Gly Pro Tyr Ser Asp  
 385 390 395 400  
 Phe Leu Gly Ser Asp Leu Asn Gly Ile Lys Ser Glu Glu Leu Lys Ser  
 405 410 415  
 Leu Ala Arg Ser Phe Arg Phe Asp Tyr Val Arg Ser Ser Thr Ala Gly  
 420 425 430  
 Lys Ser Gly Cys Pro Asp Gly Trp Phe Glu Val Asp Glu Asn Cys Val  
 435 440 445  
 Tyr Val Thr Ser Lys Gln Arg Ala Trp Glu Arg Ala Gln Gly Val Cys  
 450 455 460  
 Thr Asn Met Ala Ala Arg Leu Ala Val Leu Asp Lys Asp Val Ile Pro



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Val	Gln	Pro	Ile	Cys	Leu	Pro	Thr	Asp	Ile	Thr	Thr	Arg	Glu	His	Leu
				885					890					895	
Lys	Glu	Gly	Thr	Leu	Ala	Val	Val	Thr	Gly	Trp	Gly	Leu	Asn	Glu	Asn
			900					905					910		
Asn	Thr	Tyr	Ser	Glu	Thr	Ile	Gln	Gln	Ala	Val	Leu	Pro	Val	Val	Ala
		915					920					925			
Ala	Ser	Thr	Cys	Glu	Glu	Gly	Tyr	Lys	Glu	Ala	Asp	Leu	Pro	Leu	Thr
	930					935					940				
Val	Thr	Glu	Asn	Met	Phe	Cys	Ala	Gly	Tyr	Lys	Lys	Gly	Arg	Tyr	Asp
945					950					955					960
Ala	Cys	Ser	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Phe	Ala	Asp	Asp	Ser
				965					970					975	
Arg	Thr	Glu	Arg	Arg	Trp	Val	Leu	Glu	Gly	Ile	Val	Ser	Trp	Gly	Ser
			980					985						990	
Pro	Ser	Gly	Cys	Gly	Lys	Ala	Asn	Gln	Tyr	Gly	Gly	Phe	Thr	Lys	Val
		995					1000					1005			
Asn	Val	Phe	Leu	Ser	Trp	Ile	Arg	Gln	Phe	Ile					
	1010						1015								

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What is claimed is:

1. A method for detecting gram negative bacteria or lipopolysaccharide in a sample comprising the steps of:

- (a) contacting the sample with one or more lipopolysaccharide binding polypeptides in the presence of an inactive acidic protease; and
- (b) determining whether the one or more lipopolysaccharide binding polypeptides bind to the sample, binding indicating the sample contains gram negative bacteria or lipopolysaccharide.

2. The method of claim 1, wherein the acidic protease is pepsin.

3. The method of claim 1, wherein the acidic protease is selected from the group consisting of a fungal acidic protease, a microbial acidic protease and an animal acidic protease.

4. The method of claim 1, wherein the acidic protease is selected from the group consisting of rennin, chymosin, plasmepepsin, cathepsin D, cathepsin E, human urinary acid protease, HIV protease, *Neurospora oryzae* protease, *Mucor pusillus* protease, *Mucor miehei* protease, *Aspergillus niger* protease, *Rhizopus chinensis* protease, *Endothia parasitica* protease, yeast proteinase A, aspergillopepsinogen, rhizopuspepsin, penicillopepsin, and endothiapepsin.

5. The method of claim 1, further comprising inactivating the acidic protease prior to step (a).

6. The method of claim 1, further comprising contacting the sample with an active acidic protease under conditions that result in degradation of the proteins in the sample prior to step (a).

7. The method of claim 6, wherein a digestion buffer comprises the active acidic protease.

8. The method of claim 6, further comprising inactivating the acidic protease prior to step (a) but after contacting the sample with active acidic protease.

9. The method of claim 8, wherein the acidic protease is inactivated by a pH of about 7.0.

10. The method of claim 1, wherein the one or more lipopolysaccharide binding polypeptides are lipopolysaccharide binding polypeptides of an amebocyte lysate.

11. The method of claim 10, wherein the amebocyte lysate is selected from the group consisting of lysates of *Limulus polyphemus*, *Tachypleus tridentatus*, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*.

12. The method of claim 1, wherein the one or more lipopolysaccharide binding polypeptides are polypeptides comprising a lipopolysaccharide binding domain of a Factor C protein.

13. The method of claim 12, wherein the polypeptides comprising a lipopolysaccharide binding domain of a Factor C protein are selected from the group consisting of amino acids 1-333 of a Factor C protein, a sushi-1 peptide, a sushi-1Δ peptide, a sushi-3 peptide, a sushi-3Δ peptide, a sushi 4 peptide, and a sushi 5 peptide.

14. The method of claim 1, wherein the one or more lipopolysaccharide binding polypeptides further comprise a reporter protein or an affinity tag.

15. The method of claim 14, wherein the reporter protein is selected from the group consisting of green fluorescent protein (GFP), alkaline phosphatase, peroxidase, and luciferase.

16. The method of claim 14, wherein the affinity tag is polyhistidine, avidin, streptavidin or biotin.

17. The method of claim 1, wherein the one or more lipopolysaccharide binding polypeptides are detectably labeled.

18. The method of claim 17, wherein the detectable label is a fluorescent label.

19. The method of claim 1, wherein contacting the sample with the one or more lipopolysaccharide binding polypeptides comprises contacting the sample with a dimer or a tetramer comprising a lipopolysaccharide binding domain of a Factor C protein.

20. The method of claim 19, wherein the dimer or tetramer comprises a sushi peptide.

21. The method of claim 20, wherein the sushi peptide is selected from the group consisting of a sushi 1 peptide, a sushi 2 peptide, a sushi 3 peptide, a sushi 1Δ peptide, a sushi 4 peptide, a sushi 5 peptide and a sushi 3Δ peptide.

22. The method of claim 1, wherein the one or more lipopolysaccharide binding polypeptides are coupled to a solid support.

23. The method of claim 22, wherein the solid support is a column or plate.

24. The method of claim 22, wherein the solid support is a mobile solid support.

25. The method of claim 24, wherein the mobile solid support is a magnetic bead.

26. The method of claim 24, wherein the mobile solid support is a fluorescent bead.

27. The method of claim 1, wherein the sample is a biological sample.

28. The method of claim 27, wherein the biological sample is selected from the group consisting of plasma, blood, serum, urine and saliva.

29. A method for detecting gram negative bacteria or lipopolysaccharide in a sample comprising the steps of:

(a) contacting the sample with an active acidic protease under conditions that result in degradation of the proteins in the sample;

(b) further contacting the sample with an amebocyte lysate; and

(c) determining whether a gelation reaction occurs in the amebocyte lysate, a gelation reaction indicating the sample contains gram negative bacteria or lipopolysaccharide.

30. The method of claim 29, wherein the amebocyte lysate is selected from the group consisting of lysates of *Limulus polyphemus*, *Tachypleus tridentatus*, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*.

31. A kit for detecting gram negative bacteria or lipopolysaccharide in a sample comprising an amebocyte lysate and an acidic protease.

32. The kit of claim 31, wherein the amebocyte lysate is selected from the group consisting of lysates of *Limulus polyphemus*, *Tachypleus tridentatus*, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*.

33. The kit of claim 31, wherein the acidic protease is pepsin.

34. The kit of claim 31, wherein the acidic protease is selected from the group consisting of rennin, chymosin, plasmeprin, cathepsin D, cathepsin E, human urinary acid protease, HIV protease, *Neurospora oryzae* protease, *Mucor pusillus* protease, *Mucor miehei* protease, *Aspergillus niger* protease, *Rhizopus chinensis* protease, *Endothia parasitica* protease, yeast proteinase A, aspergillopepsinogen, rhizopuspepsin, penicillopepsin, and endothiasepsin.

35. A kit for detecting gram negative bacteria or lipopolysaccharide in a sample comprising one or more lipopolysaccharide binding polypeptides and an acidic protease.

36. The kit of claim 35, wherein the one or more lipopolysaccharide binding polypeptides are lipopolysaccharide binding polypeptides of an amebocyte lysate.

37. The kit of claim 35, wherein the amebocyte lysate is selected from the group consisting of lysates of *Limulus polyphemus*, *Tachypleus tridentatus*, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*.

38. The kit of claim 35, wherein the acidic protease is pepsin.

39. The kit of claim 35, wherein the acidic protease is selected from the group consisting of rennin, chymosin, plasmeprin, cathepsin D, cathepsin E, human urinary acid protease, HIV protease, *Neurospora oryzae* protease, *Mucor pusillus* protease, *Mucor miehei* protease, *Aspergillus niger* protease, *Rhizopus chinensis* protease, *Endothia parasitica* protease, yeast proteinase A, aspergillopepsinogen, rhizopuspepsin, penicillopepsin, and endothiasepsin.

40. The kit of claim 35, wherein the one or more lipopolysaccharide binding polypeptides are polypeptides comprising a lipopolysaccharide binding domain of a Factor C protein.

41. The kit of claim 40, wherein the polypeptides comprising a lipopolysaccharide binding domain of a Factor C protein are selected from the group consisting of a sushi-1 peptide, a sushi-1Δ peptide, a sushi-3 peptide, a sushi-3Δ peptide, a sushi 4 peptide, and a sushi 5 peptide.

42. The kit of claim 40, wherein the one or more lipopolysaccharide binding polypeptides further comprise a reporter protein or an affinity tag.

43. The kit of claim 42, wherein the reporter protein is selected from the group consisting of green fluorescent protein (GFP), alkaline phosphatase, peroxidase, and luciferase.

44. The kit of claim 42, wherein the affinity tag is polyhistidine or biotin.

45. The kit of claim 35, wherein the lipopolysaccharide binding polypeptides are detectably labeled.

46. The kit of claim 45, wherein the detectable label is a fluorescent label.

47. The kit of claim 35, wherein the lipopolysaccharide binding polypeptides are a dimer or a tetramer comprising the lipopolysaccharide binding domain of a Factor C protein.

48. The kit of claim 47, wherein the dimer or tetramer comprises a sushi peptide.

49. The kit of claim 48, wherein the sushi peptide is selected from the group consisting of a sushi 1 peptide, a sushi 2 peptide, a sushi 3 peptide, a sushi 1Δ peptide, a sushi 4 peptide, a sushi 5 peptide and a sushi 3Δ peptide.

50. The kit of claim 35, wherein the one or more lipopolysaccharide binding polypeptides are coupled to a solid support.

51. The kit of claim 50, wherein the solid support is a column or plate.

52. The kit of claim 50, wherein the solid support is a mobile solid support.

53. The kit of claim 52, wherein the mobile solid support is a magnetic bead.

54. The kit of claim 52, wherein the mobile solid support is a fluorescent bead.

55. The kit of claim 35, further comprising one or more buffers.

56. The kit of claim 55, wherein the buffer is a digestion buffer.

57. The kit of claim 35, wherein the one or more lipopolysaccharide binding polypeptides and the acidic protease are in separate containers.

58. The kit of claim 35, wherein the one or more lipopolysaccharide binding polypeptides and the acidic protease are in the same container.

59. The kit of claim 35, wherein the acidic protease is and inactive acidic protease.

60. The kit of claim 35, further comprising an inactive acidic protease.

专利名称(译)	增强内毒素检测		
公开(公告)号	<a href="#">US20100041061A1</a>	公开(公告)日	2010-02-18
申请号	US12/193169	申请日	2008-08-18
[标]申请(专利权)人(译)	拜奥泰克公司		
申请(专利权)人(译)	BIODTECH INC.		
当前申请(专利权)人(译)	BIODTECH INC.		
[标]发明人	PEPE MICHAEL G CHAMPION MILTON KEITH		
发明人	PEPE, MICHAEL G. CHAMPION, MILTON KEITH		
IPC分类号	G01N33/53 C12Q1/37		
CPC分类号	G01N33/56911 G01N2400/50 G01N2333/96477		
其他公开文献	US7846678		
外部链接	<a href="#">Espacenet</a> <a href="#">USPTO</a>		

摘要(译)

本文提供了用于检测样品中的革兰氏阴性细菌或脂多糖的方法。提供了用于检测样品中的革兰氏阴性细菌或脂多糖的试剂盒。

