CLAIMS

What is claimed is:

1. A method of detecting an endotoxin in a sample using a chromogenic assay, the method comprising:
    a. contacting the sample with a reagent comprising limulus amebocyte lysate (LAL) and a chromogenic substrate;
    b. measuring a chromogenic effect resulting from a change in the chromogenic substrate in the presence of endotoxin in the sample;
    wherein the LAL is substantially free of coagulogen.

2. The method of claim 1, wherein the substrate is a p-nitroaniline covalently bonded to a greater than three amino acids.

3. The method of any one of claims 1 to 2, wherein the chromogenic substrate is Ac-Ile-Glu-Ala-Arg-pNA.

4. The method of any one of claims 1 to 3, wherein the change in the chromogenic substrate occurs due to an enzymatic reaction.

5. The method of any one of claims 1 to 4, wherein the enzymatic reaction is cleavage of a chromophore from a polypeptide.

6. The method of any one of claims 1 to 5, wherein the measuring a chromogenic effect is accomplished by measuring absorbance at 380 nm – 420 nm.

7. The method of any one of claims 1 to 6, wherein the measuring a chromogenic effect is accomplished by measuring absorbance at 405 nm.

8. The method of any one of claims 1 to 7, wherein the reagent is a liquid.

9. The method of any one of claims 1 to 8, wherein the reagent is an aqueous liquid.

10. The method of any one of claims 1 to 9, wherein the reagent is lyophilized and then reconstituted in an aqueous liquid prior to contacting with the sample.

11. The method of any one of claims 1 to 10, wherein the LAL is lyophilized, and then reconstituted prior to contacting with the sample.

12. The method of any one of claims 1 to 11, wherein the chromogenic substrate is lyophilized, and then reconstituted prior to contacting with the sample.

13. The method of any one of claims 1 to 12, wherein the sample is a biological sample.

14. The method of any one of claims 1 to 13, wherein the sample is selected from the group consisting of a parenteral dosage form, vaccine, antibiotic, therapeutic protein, therapeutic nucleic acid, therapeutic antibody, and biological product.

15. The method of any one of claims 1 to 14, wherein the LAL substantially free of coagulogen has less than 5% (wt/wt) of coagulogen relative to total protein in the LAL as measured by SDS-PAGE and protein stain.

16. The method of any one of claims 1 to 15, wherein the LAL substantially free of coagulogen has less than 2% (wt/wt) of coagulogen relative to total protein in the LAL as measured by SDS-PAGE and protein stain.

17. The method of any one of claims 1 to 16, wherein the LAL substantially free of coagulogen has less than 0.5% (wt/wt) of coagulogen relative to total protein in the LAL as measured by SDS-PAGE and protein stain.

18. The method of any one of claims 1 to 17, wherein the chromogenic assay is conducted using single cuvette spectroscopy, multiple cuvette spectroscopy, or a microplate reader

19. The method of any one of claims 1 to 18, further comprising: comparing the chromogenic effect to a standard to determine the quantity of endotoxin in the sample.

20. A method of detecting an endotoxin in a biological sample using a chromogenic assay, the method comprising:

    a. contacting the biological sample with an aqueous reagent comprising limulus amebocyte lysate (LAL) and Ac-Ile-Glu-Ala-Arg-pNA;

b. measuring the change in absorbance at 405nm resulting from the enzymatic cleavage of pNA from Ac-Ile-Glu-Ala-Arg-pNA in the presence of endotoxin in the sample;

wherein the LAL is substantially free of coagulogen.

21. The method of claim 20, wherein the method has a sensitivity of >0.001 EU/mL endotoxin.

22. A composition comprising limulus amebocyte lysate (LAL) and a chromogenic substrate, wherein the LAL is substantially free of coagulogen.

23. The composition of claim 22, wherein the chromogenic substrate is Ac-Ile-Glu-Ala-Arg-pNA.

24. The composition of any one of claims 22 to 23, wherein the LAL and chromogenic substrate are lyophilized.

25. The composition of any one of claims 22 to 23, wherein the LAL and chromogenic substrate are in an aqueous solution.

26. The composition of any one of claims 22 to 25, wherein the composition comprises 70% LAL preparation and 30% chromogenic substrate.

27. A kit comprising:

a. limulus amebocyte lysate (LAL), wherein the LAL is substantially free of coagulogen;

b. a chromogenic substrate; and

c. instructions for detecting an endotoxin using the LAL and chromogenic substrate.

28. The kit of claim 27, wherein the LAL is lyophilized.

29. The kit of any one of claims 27 to 28, wherein the LAL is in an aqueous solution.

30. The kit of any one of claims 27 to 29, wherein the LAL and the chromogenic substrate are in a single container.

~~31. The kit of claim any one of claims 27 to 30, further comprising a sterile container comprising the LAL.~~

~~32. The kit of claim 31, wherein the sterile container is a sterile vial.~~

~~33. The kit of any one of claims 27 to 32, further comprising a control standard endotoxin.~~

~~34~~1. A method of making limulus amebocyte lysate (LAL) substantially free of coagulogen, wherein the LAL substantially free of coagulogen comprises less than 5% (wt/wt) of coagulogen relative to total protein in the LAL as measured by SDS-PAGE with protein stain, the method comprising:

~~a. providing~~subjecting a composition comprising LAL, wherein the LAL comprises coagulogen~~;~~

~~b. adjusting the buffer; and~~

~~c. subjecting the composition of (b)~~ to tangential flow filtration (TFF) using a 20 kDa to 50 kDa TFF filter, wherein the TFF produces a retentate and a filtrate; and collecting the retentate, thereby ~~isolating~~obtaining the LAL substantially free of coagulogen.

2. The method of claim 1, wherein the TFF is performed at a flow rate of 300 ml/min to 600 ml/min.

3~~5~~. The method of claim ~~34~~2, wherein the TFF is performed at a flow rate of 350 ml/min to 500 ml/min.

4. The method of any one of claims 1 to 3, wherein the composition comprising LAL is in a buffer.

~~36~~5. The method of ~~any one of~~ claim~~s 34 to 35~~, wherein the buffer is a Tris buffer or MES buffer.

6. The method of claim 4 or 5, wherein the buffer has a pH of about 6.0 to about 9.0.

~~3~~7. The method of ~~any one of~~ claim~~s 34 to 3~~6, wherein the buffer has a pH of about 7.0 to 8.0.

~~38. The method of any one of claims 34 to 37, wherein the filter is tangential flow filtration (TFF).~~

8. The method of any one of claims 1 to 7, wherein the TFF filter comprises a membrane selected from modified polyethersulfone (mPES), polysulfone (PS) and polyethersulphone (PES).

~~3~~9. The method of ~~any one of~~ claim~~s 34 to 3~~8, wherein the TFF filter compris~~es~~ a ~~modified polyethersulfone (~~mPES~~)~~ membrane filter.

10. The method of any one of claims 1 to 9, wherein the LAL substantially free of coagulogen comprises less than 1% (wt/wt) of coagulogen relative to total protein in the LAL as measured by SDS-PAGE with protein stain.

11. The method of claim 10, wherein the LAL substantially free of coagulogen comprises less than 0.5% (wt/wt) of coagulogen relative to total protein in the LAL as measured by SDS-PAGE with protein stain.