WHAT IS CLAIMED IS:

1. A method of detecting non-endotoxin pyrogens in a sample comprising the steps of:

combining a monocyte-containing reagent and the sample to be tested in a first assay system which comprises at least one surface comprising polypropylene, wherein the monocyte-containing reagent comprises peripheral blood mononuclear cells or monocytic cell line cells, is in contact with said surface, and is present in said assay system at a high cell density;

incubating the monocyte-containing reagent and the sample, wherein the monocyte-containing reagent produces a cytokine or an endogenous mediator of the inflammatory response;

transferring the contents of the first assay system to a second assay system which comprises at least one surface treated with an antibody to a cytokine or an endogenous mediator of the inflammatory response; and

assaying the second assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface; whereby an elevated level of cytokine or endogenous mediator bound to the surface indicates the presence of pyrogens in the sample tested.

2. A method of detecting non-endotoxin pyrogens in a parenterally administered medical product comprising the steps of:

combining a monocyte-containing reagent and the medical product to be tested in a first assay system which comprises at least one surface comprising polypropylene, wherein the monocyte-containing reagent comprises whole blood and is in contact with said surface;

incubating the monocyte-containing reagent and the medical product, wherein the monocyte-containing reagent produces IL-6;

transferring the contents of the first assay system to a second assay system which comprises at least one surface treated with an antibody to IL-6; and

assaying the second assay system for the presence of IL-6 bound to the antibody on the surface; whereby an elevated level of IL-6 bound to the surface indicates the presence of pyrogens in the medical product tested.

3. A method of detecting non-endotoxin pyrogens in a parenterally administered medical product comprising the steps of:

combining a monocyte-containing reagent and the medical product to be tested in an assay system which comprises at least one surface comprising polypropylene and at least one surface treated with an antibody to IL-6, wherein the monocyte-containing reagent comprises whole blood and is in contact with said surface comprising polypropylene; and

assaying the assay system for the presence of IL-6 bound to the antibody on the surface; whereby an elevated level of IL-6 bound to the surface indicates the presence of pyrogens in the medical product tested.

4. A method of culturing cells contained within a monocyte-containing reagent for use in a non-endotoxin pyrogen assay comprising the step of:

combining the monocyte-containing reagent and a sample to be tested in an assay system which comprises at least one surface comprising polypropylene, wherein the monocyte-containing reagent comprises peripheral blood mononuclear cells or monocytic cell line cells, is in contact with said surface, and is present in said assay system at a high cell density.

5. The method of claim 4 wherein the assay system comprises at least one microtiter well shaped such that the monocyte-containing reagent is concentrated as compared to a flat-bottomed microtiter well, at least one surface of said well comprises polypropylene, and the monocyte-containing reagent is present in such well at a high cell density.

6. The method of claim 1 or 2 wherein at least one of the first and second assay system includes at least one microtiter well, and the surface(s) is at least a portion of the interior of the microtiter well.

7. The method of claim 3 or 4 wherein the assay system includes at least one microtiter well, and the surface(s) is at least a portion of the interior of the microtiter well.

8. The method of any one of claims 5-7 wherein the well is shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well.

9. The method of any one of claims 5-7 wherein the microtiter well comprises an open top, an upper region extending downwardly from the open top, and a bottom wall tapering in diameter from a location above the lowest point to the lowest point.

10. The method of any one of claims 5-7 wherein the microtiter well comprises an open top, an upper region extending downwardly from the open top having a top end and a bottom end, and a bottom region having a top end and a lowest point, the bottom region extending from the bottom end of the upper region and tapering in diameter more rapidly than the upper region from the top end of the bottom region toward the lowest point.

11. The method of claim 9 wherein the bottom wall of said microtiter well is non-planar, curved, parabolic or downwardly extending.

12. The method of any one of claims 5-7 wherein the sides of said microtiter well are sloped inward.

13. The method of claim 5 wherein the assay system comprises at least one surface treated with an antibody to a cytokine, and the surface on which the antibody is applied is at least a portion of the interior of said microtiter well.

14. The method of claim 13 wherein the antibody is an antibody to an endogenous mediator of the inflammatory response.

15. The method of claim 14 wherein the endogenous mediator is endothelin.

16. The method of claim 5 further comprising the step of assaying the assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface; whereby an elevated level of cytokine or endogenous mediator bound to the surface indicates the presence of pyrogens in the sample tested.

17. The method of any one of claims 1, 13 or 16 wherein the cytokine is selected from the group consisting of interleukin-1 (IL-1), interleukin-1ra (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α).

18. The method of claim 17 wherein the cytokine is IL-6.

19. The method of any one of claims 1-18 wherein the antibody is a polyclonal antibody.

20. The method of any one of claims 1-18 wherein the antibody is a monoclonal antibody.

21. The method of any one of claim 1-20 wherein the assay system(s) is a colorimetric enzyme-linked immunosorbent assay (ELISA), a radio-labeled immunoassay, or a fluorescence-labeled immunoassay.

22. The method of any one of claims 1 or 4-21 wherein the monocytic cell line comprises a human monocytic cell line.

23. The method of claim 22 wherein the monocytic cell line is MONOMAC-6, THP-1, or 28SC.

24. The method of any one of claims 1 and 4-21 wherein the monocytic cell line comprises a cell line having endogenous CD14 and Toll-like receptors or which has been transfected with CD14 and/or Toll-like receptors and/or reporter genes for inflammatory or pyrogenic mediators.

25. The method of any one of claims 1 and 4-24 wherein the sample is a parenterally administered medical product.

26. The method of any one of claims 2, 3 and 25 wherein the parenterally administered medical product is selected form the group consisting of a blood product, dialysis solution, vaccine, and plasma expander.

27. The method of any one of claims 1 and 4-26 wherein the monocyte-containing reagent is present in said assay system at a cell density of at least about 250,000, 500,000, 600,000, 700,000, 800,000, 900,000, 1,000,000, 1,100,000, 1,200,000, 1,300,000, 1,400,000, 1,500,000, 1,600,000, 1,700,000, 1,800,000, 1,900,000 or 2,000,000 cells per well.

28. The method of any one of claims 1 and 4-27 wherein the peripheral blood mononuclear cells comprise human peripheral blood mononuclear cells.

29. The method of any one of claims 2, 3, 6-12, 19-21 and 26 wherein the monocyte-containing reagent further comprises a diluent.

30. The method of claim 29 wherein the diluent is RPMI media.

31. The method of any one of claims 2, 3, 6-12, 19-21, 26, 29 and 30 wherein the monocyte-containing reagent further comprises an anticoagulant .

32. The method of any one of claims 2, 3, 6-12, 19-21, 26 and 29-31 wherein the whole blood comprises human whole blood.

33. The method of any one of claims 2, 3, 6-12, 19-21, 26 and 29-31 wherein the monocyte-containing reagent is present in said assay system at a cell density of greater than about 100,000, 200,000, 210,000, 220,000, 230,000, 240,000, 250,000, 260,000, 270,000, 280,000, 290,000, 300,000, 310,000, 320,000, 330,000, 340,000, 350,000, 360,000, 370,000, 380,000, 390,000 or 400,000 peripheral blood mononuclear cells per well.

34. A diagnostic kit comprising:

a microtiter plate comprising a plurality of microtiter wells shaped such that cells contained in each well are concentrated as compared to a flat-bottomed microtiter well, a surface of said well comprising polypropylene; and

a cryopreserved monocyte-containing reagent which is contained in the wells of said plate, wherein the monocyte-containing reagent comprises cryopreserved peripheral blood mononuclear cells or cryopreserved monocytic cell line cells, is in

contact with the surfaces of said wells, and is present in said well at a high cell density.

35. A diagnostic kit comprising:

an assay system for detecting non-endotoxin pyrogens in a parenterally administered medical product, said assay system comprising a microtiter plate comprising a plurality of microtiter wells shaped such that cells contained in each well are concentrated as compared to a flat-bottomed microtiter well, a surface of said well comprising polypropylene;

a cryopreserved monocyte-containing reagent which is contained in the wells of said plate, wherein the monocyte-containing reagent comprises cryopreserved whole blood and is in contact with the surfaces of said wells; and

an antibody to IL-6.

36. The kit of claim 34 or 35 wherein said well further comprises a barrier which is impervious to monocytes.

37. The kit of claim 36 wherein said barrier comprises a membrane, a grid, a sieve, or a sifter.

WHAT IS CLAIMED IS:

1. A method of detecting non-endotoxin pyrogens in a sample comprising the steps of:

(A) combining a monocyte-containing reagent and the sample to be tested in a first assay system which includes at least one microtiter well shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well, wherein the surface of the microtiter well comprises a polypropylene coating or the entire microtiter well is composed of polypropylene;

(B) incubating the monocyte-containing reagent and the sample, wherein, when the sample comprises a pyrogen, the monocyte-containing reagent produces a cytokine or an endogenous mediator of the inflammatory response;

(C) transferring the contents of the first assay system to a second assay system which comprises at least one surface treated with an antibody to the cytokine or endogenous mediator of the inflammatory response; and

(D) assaying the second assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface;

whereby an elevated level of cytokine or endogenous mediator bound to the surface indicates the presence of the pyrogens in the sample tested.

2. A method of detecting non-endotoxin pyrogens in a sample comprising the steps of:

(A) combining a monocyte-containing reagent and the sample to be tested in an assay system comprising (i) at least one microtiter well shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well, wherein the surface of the microtiter well comprises a polypropylene coating or the entire microtiter well is composed of polypropylene, and (ii) at least one surface treated with an antibody to a cytokine or an endogenous mediator of the inflammatory response;

(B) incubating the monocyte-containing reagent and the sample, wherein, when the sample comprises a pyrogen, the monocyte-containing reagent produces a cytokine or an endogenous mediator of the inflammatory response, and

(C) assaying the assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface;

whereby an elevated level of cytokine or endogenous mediator bound to the surface indicates the presence of the pyrogens in the sample tested.

3. The method of claim 1 or 2 wherein the cytokine is selected from the group consisting of interleukin-1 (IL-1), interleukin-1ra (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α).

4. The method of claim 3 wherein the cytokine is IL-6.

5. The method of claim 1 or 2 wherein the endogenous mediator is endothelin.

6. The method of any one of claims 1 to 5 wherein the sample is a medical product for parenteral administration.

7. The method of claim 6 wherein the medical product is a blood product, dialysis solution, vaccine, or plasma expander.

8. The method of any one of claims 1 to 7 wherein the microtiter well comprises an open top, an upper region extending downwardly from the open top, and a bottom wall tapering in diameter from a location above the lowest point to the lowest point.

9. The method of any one of claims 1 to 7 wherein the microtiter well comprises an open top, an upper region extending downwardly from the open top having a top end and a bottom end, and a bottom region having a top end and a lowest point, the bottom region extending from the bottom end of the upper region and tapering in diameter more rapidly than the upper region from the top end of the bottom region toward the lowest point.

10. The method of claim 9 wherein the bottom wall of said microtiter well is non-planar, curved, parabolic or downwardly extending, or the sides of said microtiter well are sloped inward.

11. The method of any one of claims 1 to 10 wherein the assay system(s) is a colorimetric

enzyme-linked immunosorbent assay (ELISA), a radio-labeled immunoassay, or a fluorescence-labeled immunoassay.

12. The method of any one of claims 1 to 11, wherein the monocyte-containing reagent comprises peripheral blood mononuclear cells (PBMCs) or monocytic cell line cells.

13. The method of claim 12, wherein the monocytic cell line comprises a human monocytic cell line.

14. The method of claim 13, wherein the monocytic cell line is MONOMAC-6, THP-1, or 28SC.

15. The method of claim 12, wherein the monocytic cell line comprises a cell line having endogenous CD14 and Toll-like receptors or which has been transfected with CD14 and/or Toll-like receptors and/or reporter genes for inflammatory or pyrogenic mediators.

16. The method of claim 12, wherein the PBMCs are human PBMCs.

17. The method of claim 12, wherein the monocyte-containing reagent is present in said assay system at a cell density which is higher than the cell density of monocytes in whole blood.

18. The method of claim 12, wherein the monocyte-containing reagent is present in said assay system at a cell density of at least or about 250,000 cells per well, at least or about 500,000 cells per well, at least or about 600,000 cells per well, at least or about 700,000 cells per well, at least or about 800,000 cells per well, at least or about 900,000 cells per well, at least or about 1,000,000 cells per well, at least or about 1,100,000 cells per well, at least or about 1,200,000 cells per well, at least or about 1,300,000 cells per well, at least or about 1,400,000 cells per well, at least or about 1,500,000 cells per well, at least or about 1,600,000 cells per well, at least or about 1,700,000 cells per well, at least or about 1,800,000 cells per well, at least or about 1,900,000 cells per well, at least or about 2,000,000 cells per well, wherein the cell density does not exceed a maximum cell density at which insufficient cell nutrients exist in the volume of reagent within the well to properly maintain the cells in the well.

19. The method of any one of claims 1 to 18, wherein the monocyte-containing reagent comprises whole blood.

20. The method of claim 19, wherein the whole blood comprises human whole blood.

21. A method of testing a sample, comprising the steps of: combining a monocyte-containing reagent and a sample to be tested in a first assay system which includes at least one microtiter well shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well, wherein the surface of the microtiter well comprises a polypropylene coating or the entire microtiter well is composed of polypropylene; incubating the monocyte-containing reagent and the sample; transferring the contents of the first assay system to a second assay system which comprises at least one surface treated with an antibody to the cytokine or endogenous mediator of the inflammatory response; and assaying the second assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface, whereby the sample is tested.

22. A method of testing a sample, comprising the steps of: combining a monocyte-containing reagent and a sample to be tested in a first assay system comprising (i) at least one microtiter well shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well, wherein the surface of the microtiter well comprises a polypropylene coating or the entire microtiter well is composed of polypropylene and (ii) at least one surface treated with an antibody to the cytokine or endogenous mediator of the inflammatory response; incubating the monocyte-containing reagent and the sample; and assaying the assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface, whereby the sample is tested.

23. The method of claim 21 or 22, wherein the sample is a pharmaceutical, nutrient, or medical product.

24. The method of any one of claims 21 to 23, wherein the cytokine or endogenous mediator of the inflammatory response is a proinflammatory cytokine or a Th1 cytokine.

25. The method of claim 24, wherein cytokine or endogenous mediator of the inflammatory response is TNF-alpha, IL-2, IFN, or IL-8.

26. The method of any one of claims 21 to 25, wherein the microtiter well comprises an open top, an upper region extending downwardly from the open top, and a bottom wall tapering in diameter from a location above the lowest point to the lowest point.

27. The method of any one of claims 21 to 25, wherein the microtiter well comprises an open top, an upper region extending downwardly from the open top having a top end and a bottom end, and a bottom region having a top end and a lowest point, the bottom region extending from the bottom end of the upper region and tapering in diameter more rapidly than the upper region from the top end of the bottom region toward the lowest point.

28. The method of claim 26, wherein the bottom wall of said microtiter well is non-planar, curved, parabolic or downwardly extending, or the sides of said microtiter well are sloped inward.

29. The method of any one of claims 21 to 28, wherein the monocyte-containing reagent comprises peripheral blood mononuclear cells or monocytic cell line cells.

30. The method of claim 29, wherein the monocytic cell line comprises a human monocytic cell line selected from the group consisting of: MONOMAC-6, THP-1, 28SC, and a cell line having endogenous CD14 and Toll-like receptors or which has been transfected with CD14 and/or Toll-like receptors and/or reporter genes for inflammatory or pyrogenic mediators.

31. The method of any one of claims 21 to 30, wherein the monocyte-containing reagent comprises a number of cells sufficient to provide at least or about 125,000 cells per well of the first assay system.

32. An *in vitro* pyrogen test, comprising the steps of:

(a) combining a monocyte-containing reagent and a sample to be tested in a first assay system which includes a microtiter plate comprising a plurality of microtiter wells, shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well wherein the bottom wall of each microtiter well is non-planar, curved, parabolic or

downwardly extending and wherein the surface of each microtiter well comprises a polypropylene coating or the entire microtiter well is composed of polypropylene;

(b) incubating the monocyte-containing reagent and the sample, wherein, when the sample comprises a pyrogen, the monocyte-containing reagent produces a cytokine or an endogenous mediator of the inflammatory response;

(c) transferring the contents of the first assay system to a second assay system which comprises at least one surface treated with an antibody to the cytokine or endogenous mediator of the inflammatory response; and

(d) assaying the second assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface whereby an elevated level of the cytokine or endogenous mediator bound to the surface indicates the presence of pyrogens in the sample tested.

33. An *in vitro* pyrogen test, comprising the steps of:

(a) combining a monocyte-containing reagent and a sample to be tested in a first assay system comprising (i) a microtiter plate comprising a plurality of microtiter wells, shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well, wherein the bottom wall of each microtiter well is non-planar, curved, parabolic or downwardly extending and wherein the surface of each microtiter well comprises a polypropylene coating or the entire microtiter well is composed of polypropylene and wherein, when the sample comprises a pyrogen, the monocyte-containing reagent produces a cytokine or an endogenous mediator of the inflammatory response, and (ii) at least one surface treated with an antibody to the cytokine or endogenous mediator of the inflammatory response;

(b) incubating the monocyte-containing reagent and the sample; and

(c) assaying the assay system for the presence of cytokine or endogenous mediator bound to the antibody on the surface whereby an elevated level of the cytokine or endogenous mediator bound to the surface indicates the presence of pyrogens in the sample tested.

34. The *in vitro* pyrogen test of claim 32 or 33, wherein the sample is a pharmaceutical, nutrient, or medical product.

35. The *in vitro* pyrogen test of any one of claims 32 to 34, wherein the cytokine or endogenous mediator of the inflammatory response is a proinflammatory cytokine or a Th1 cytokine.

36. The *in vitro* pyrogen test of any one of claims 32 to 34, wherein the cytokine or endogenous mediator of the inflammatory response is IL-2, IFN, IL-1, IL-1ra, IL-6, IL-8, TNF- α, or endothelin.

37. The *in vitro* pyrogen test of claim 32 or 33, wherein the sides of said microtiter well are sloped inward.

38. The *in vitro* pyrogen test of any one of claims 32 to 37, wherein the monocyte-containing reagent comprises peripheral blood mononuclear cells or monocytic cell line cells, optionally, wherein the monocytic cell line comprises a human monocytic cell line selected from the group consisting of: MONOMAC-6, THP-1, 28SC, and a cell line having endogenous CD14 and Toll-like receptors or which has been transfected with CD14 and/or Toll-like receptors and/or reporter genes for inflammatory or pyrogenic mediators.

39. The *in vitro* pyrogen test of any one of claims 1 to 7, wherein the monocyte-containing reagent comprises a number of cells sufficient to provide at least or about 125,000 cells per well of the first assay system.

40. A diagnostic kit for use in the *in vitro* pyrogen test of any of the preceding claims, comprising (i) an assay system comprising a microtiter plate comprising a plurality of microtiter wells, each well shaped such that, when a monocyte containing reagent is present in the microtiter wells, the monocyte-containing reagent is concentrated to provide greater cell to cell contact, as compared to a flat-bottomed well, wherein the bottom wall of each microtiter well is non-planar, curved, parabolic or downwardly extending and wherein the surface of each well comprises a polypropylene coating or the entire well is composed of polypropylene, and (ii) a cryopreserved monocyte containing reagent comprising cryopreserved whole blood, cryopreserved peripheral blood mononuclear cells (PBMCs), or cryopreserved monocytic cell

line cells; and, wherein either the assay system further comprises at least one surface treated with an antibody to a cytokine or endogenous mediator of an inflammatory response, or the kit comprises a second assay system comprising at least one surface treated with an antibody to a cytokine or endogenous mediator of an inflammatory response, wherein the cytokine or endogenous mediator is one that is produced by the monocyte-containing reagent.

41. The diagnostic kit of claim 40, wherein the cytokine or endogenous mediator of an inflammatory response is IL-2, IFN, IL-1, IL-1ra, IL-6, IL-8, TNF- α, or endothelin.

42. The diagnostic kit of claim 40, wherein each microtiter well of the microtiter plate comprises cryopreserved monocytes, optionally at the optimum number of cells per well.

43. The diagnostic kit of claim 40, wherein the cryopreserved peripheral blood mononuclear cells or cryopreserved monocytic cell line cells are present in a well of the microtiter plate at a cell density of at least about 250,000 cells per well.

44. The diagnostic kit of claim 40, wherein the cryopreserved whole blood is present in a well of the microtiter plate at a cell density of at least about 100,000 PBMCs per well.

45. A diagnostic kit comprising: a cryopreserved monocyte-containing reagent a microtiter plate comprising a plurality of microtiter wells shaped such that the monocyte-containing reagent is concentrated to provide greater cell to cell contact as compared to a flat-bottomed microtiter well, wherein each microtiter well comprises a bottom wall that is non-planar, curved, parabolic or downwardly extending, wherein the surface of the microtiter well comprises a polypropylene coating or the entire microtiter well is composed of polypropylene and either the assay system further comprises at least one surface treated with an antibody to a cytokine or an endogenous mediator of an inflammatory response, or the kit comprises a second assay system comprising at least one surface treated with an antibody to a cytokine or an endogenous mediator of an inflammatory response, wherein the cytokine or endogenous mediator of an inflammatory response is a cytokine or endogenous mediator of the inflammatory response that is produced by the monocyte-containing reagent when exposed to a sample that comprises a pyrogen.