SUMMARY OF THE INVENTION

[0015] Applicants have developed an in-vitro pyrogen test that is sensitive and detects pyrogenic contaminants present in medical products. Generally, the present invention is directed to a method of detecting non-endotoxin pyrogens in a sample by combining monocytes, i.e., in the form of a monocyte-containing reagent, and the sample to be tested in a first assay system comprising at least one surface comprising polypropylene, such that the monocytes are in contact with the surface. The monocytes and the sample are incubated so that the monocytes produce a cytokine or an endogenous mediator of the inflammatory response during incubation. The contents of the first assay system are transferred to a second assay system comprising at least one surface treated with an antibody to a cytokine or an endogenous mediator or marker of the inflammatory response. The second assay system is assayed for the presence of cytokine or endogenous mediator bound to the antibody on the surface. When the monocytes used in this method are PBMCs or monocytic cell line cells, the monocytes are present in the assay systems at a high cell density.

[0015a] The present invention is directed to 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.

[0015b] The present invention is directed to 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.

[0015c] The present invention is directed to 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.

[0015d] The present invention is directed to 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.

[0015e] The present invention is directed to 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.

[0015f] The present invention is directed to 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.

[0015g] The present invention is directed to a diagnostic kit for use in the *in vitro* pyrogen test as defined above, 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.

[0015h] The present invention is directed to 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.

[0016] The present invention is also generally directed to a method of detecting non-endotoxin pyrogens in a parenterally-administered medical product by combining whole blood, as the monocyte-containing reagent, and the medical product to be tested in a first assay system comprising at least one surface comprising polypropylene, such that the blood is in contact with the surface. The blood and the medical product are incubated such that the blood produces the cytokine IL-6 during incubation. The contents of the first assay system are transferred to a second assay system comprising at least one surface treated with an antibody to IL-6. The second assay system is assayed for the presence of IL-6 bound to the antibody on the surface.

[0017] The present invention is further drawn to a method of culturing monocytes, i.e., in the form of a monocyte-containing reagent, for use in a non-endotoxin pyrogen test by combining the monocytes and a sample to be tested in an assay system comprising at least one surface comprising polypropylene, such that the monocytes are in contact with the surface.

[0018] The invention is also drawn to a method of culturing monocytes as part of a non-endotoxin pyrogen test by combining the monocytes and a sample in an assay system comprising at least one microtiter well shaped such that the monocyte-containing reagent is concentrated as compared to a flat-bottomed microtiter well, a surface of the well comprising polypropylene, such that the monocytes are in contact