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(54) Title: A CELL-MEDIATED IMMUNE RESPONSE ASSAY AND KITS THEREFOR

(57) Abstract: The present invention provides methods and kits for measuring a cell-mediated immune (CMI) in a small volume of whole undiluted blood collected from a subject. In particular, the methods are for measuring responses in undiluted whole blood samples having a volume of, for example, 50µl to 500µl. Thus, capillary sampling and rapid testing of subjects including paediatric, adult or geriatric human subjects are facilitated.

A CELL-MEDIATED IMMUNE RESPONSE ASSAY AND KITS THEREFOR

FIELD

The present invention relates generally to methods and kits for use in diagnosis, monitoring or treatment that measure cellular responsiveness to an agent *in vitro*. In particular, the present invention provides a system for measuring a cell-mediated immune (CMI) response to an antigen in a small sample of whole blood collected from a subject. The methods and kits will find broad application in the analysis of whole blood samples having a range of different volumes including those from infants and children or other subjects where sample volume is limiting or where small sample volumes are desirable.

BACKGROUND

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

The function of the immune response is to disarm invading pathogens or toxins. The immune response can in some circumstances be very destructive to an organism and survival depends upon the ability of the immune system to distinguish self from non-self. Autoimmune diseases, for example, develop when the immune system over responds to self. Some immune responses are against non-self molecules that are relatively harmless. Asthma and hayfever, for example, involve immune responses to non-self where the immune response is more debilitating than the causative agent. Generally, the innate immune system screens out responses to non-pathogenic organisms and helps to prevent

adaptive immune responses to such harmless agents.

Adaptive immune responses are carried out by lymphocytes such as B lymphocytes that carry out antibody responses or T lymphocytes which carry out cell mediated responses. B lymphocytes produce immunoglobulins which help to deactivate pathogens and toxins. T cells react directly with non-self molecules (antigens) that are presented on the surface of host cells in association with major histocompatibility (MHC) molecules that provide a repertoire of "self" molecules. In both cases, a cellular response is generated that is specific to particular epitopes of the non-self molecule and provides a network of immune responses and immune effector molecules.

Accordingly, one method for diagnosing or monitoring an infection or evaluating the ability of a subject to mount an immune response to non-self is to determine whether the subject has mounted an immune response to antigen stimulation. As the T cell response comprises the production of effector T cells that are capable of responding to an antigen or can be stimulated to respond to the antigen by producing immune effector molecules, one can measure the production of these molecules *in vitro* in response to specific antigens as a measure of a cell mediated immune response. However, as non-self antigenic molecules are presented to T-cells by antigen presenting cells there is a complex interaction of molecules and cells that must take place successfully *in vitro* in order to produce sufficient immune effector molecule for detection.

Most *in vitro* methods for detecting cell mediated immune responses involve the purification of peripheral blood mononuclear cells from whole blood using various separation techniques. Such assays include chromium release assays, cytotoxicity assays, MHC class I tetramer assays, assays for IFN-γ or other cytokines, of which ELISPOT provides a good example. The ELISPOT method immobilizes antigen presenting cells and has been used to detect the number of T-cells producing certain cytokines in response to antigenic stimulation.

If whole blood is used, it is generally diluted in a culture medium in order to dilute red

blood cells, which are considered to reduce the sensitivity of the assays. An in-tube cell-mediated immune response assay which uses undiluted whole blood is described in International Publication No. W0 2004/042396 in the name of Cellestis Limited incorporated herein in its entirety by reference. International Publication No. W0 2004/042396 discloses the use of blood collection tubes for the incubation of sample with antigen and a simple sugar and shows enhanced sensitivity using the tube system compared to assays where blood is transferred to and incubated in 24-well microtitre plates.

For whole blood assays in humans and livestock animals, at least about three millilitres of blood is taken from the subject in order to provide sufficient material to perform cell-mediated immune response assays. This amount is generally taken by venous blood sampling, *via* needle into a collection vessel, often under vacuum.

Various methods of detecting immune effector molecules, such as enzyme-linked immunosorbent assay (ELISA), radio-immuno assay (RIA), or cytommetric methods can use small volumes, however, there is a need in the art for improved systems for conducting the antigenic stimulation phases of *in vitro* cell-mediated immune response assays. In particular, methods that allow whole blood testing in small volumes of blood such as those obtained by peripheral capillary sampling are needed. The ability to screen small samples of blood would greatly facilitate sampling of children or other subjects where blood may be limited or difficult to obtain, and allows blood sampling without venous blood sampling by using capillary blood such as that obtained by prick testing of the thumb, heel, ear-lobe or other convenient site, and for testing multiple or a range of antigen including mitogen and hapten stimulants in a single blood draw of low volume.

SUMMARY OF THE BROAD EMBODIMENTS

The present invention is predicated, in part, upon the surprising discovery that it is possible to generate and detect a cellular immune response in a very small volume of whole blood from a subject, and that this does not have to be venous or arterial blood. This means that collection of blood for the conduct of cell-mediated immune response assays can be achieved using, for example, prick sampling of peripheral capillary blood which generally yields volumes of about one millilitre or less. Further, that very small samples of whole blood can be tested for their ability to produce immune effector molecules, facilitating multiple testing from small samples.

In one broad embodiment, the present invention provides a method for measuring a cell-mediated immune response in a sample from a subject, wherein the sample comprises cells that secrete an immune effector molecule following stimulation by an agent such as an antigen. In one particular embodiment, the present invention provides a method for measuring a cell-mediated immune (CMI) response in a sample of whole blood collected from a subject wherein said whole blood sample comprises cells of the immune system which are capable of producing immune effector molecules following stimulation by an antigen, the method comprising: (i) incubating a whole blood sample from a peripheral capillary or less than 0.5mL whole blood from a artery or vein of a subject with an antigen in an incubation container substantially without dilution of the sample; and (ii) detecting or measuring the presence of an immune effector molecule or of a nucleic acid molecule capable of producing an effector molecule indicative of the capacity of the subject to mount a cell-mediated response.

In another embodiment, the method comprises: (i) collecting a whole blood sample from a peripheral capillary or less than 0.5mL whole blood from an artery or vein of a subject into a container; (ii) incubating whole blood with an antigen and anti-coagulant; and (iii) detecting or measuring the presence of an immune effector molecule or of a nucleic acid molecule capable of producing an immune effector molecule indicative of the capacity of the subject to mount a cell-mediated response.

The method will find broad application in selecting a suitable therapeutic protocol for the treatment of a subject having, for example, an inflammatory disease condition, a pathogenic infection such as one caused by a bacterial, viral, parasite or fungal pathogen, an autoimmune disorder, immuno-incompetence, allergy or cancer or a propensity for developing such a disorder.

In a preferred embodiment, the methods comprise collecting and/or incubating a capillary blood sample or collecting a sample from the subject with a capillary sampling device.

The method comprises, in some embodiments, incubating the sample with an agent under conditions in which the shape of the sample comprises a dimension which has been optimised. In some embodiments, the dimension has been optimised for a particular subject or subject population. In other embodiments, the dimension has been optimised for a particular cellular sample. In some embodiments, the dimension is the height of the sample. In another embodiment, the volume is optimised. In a further embodiment, the concentration of peripheral blood mononuclear cells (PBMC) or other immune cells is also optimised. By "optimised" is meant that the selected dimension value or range provides the optimal cellular response compared to other values or ranges tested. Thus, in some embodiments, the sample comprises a dimension that has been pre-selected using the methods disclosed herein to provide an optimal cellular response.

As illustrated in Example 1, standard conjugate-linked immunoassay testing demonstrated that IFN-γ is produced in total volumes of blood incubation as small as 0.5mL, 0.4mL, 0.3mL, 0.2mL and 0.1mL. Further experiments, described in Examples 2 to 4 show that incubation of blood samples as small as 20μl and/or having a sample height of 4mm can generate sufficient immune effector molecules to be useful in a diagnostic assay. Optimum results are obtained in sample heights of about 6mm to about 12mm or about 5mm to about 18mm and intermediate values, independent of the volume of sample collected or incubated.

Accordingly, in some embodiments, the incubation container is suitable for maintaining an optimal shape of the sample, wherein the shape has one or two or more dimensions selected from: (i) a maximum circular diameter of less than about 6mm; (ii) a height of at least about 4mm to 6mm to a maximum height of about 12mm to 20mm; or (iii) a volume of less than 0.5mL and optionally less than about 400μl.

In another aspect, the present invention provides a kit for measuring a cell mediated response to an agent in a whole blood sample from a subject, the kit comprising: a collection vessel housed separately or together with an agent capable of stimulating an immune cell to secrete an immune effector molecule, and further optionally comprising instructions for use. In some embodiments, the sample is transferred from a collection container to one or more containers for incubation with antigen and the kit comprises one or more collection container and one or more incubation container. Conveniently, in some embodiments, the collection container comprises anticoagulant. In other embodiments, the incubation container comprises antigen and optionally a simple sugar such as dextrose.

Accordingly, the present invention provides kits for measuring a cell-mediated immune response in a whole blood sample collected from a subject, the kits comprising in multicomponent form: (i) one or more collection and/or incubation containers suitable for holding or incubating a whole peripheral capillary blood sample or less than 0.5mL of whole venous or arterial blood; (ii) one or more test antigens for analysis of *in vitro* responses thereto and optionally a control antigen; (iii) reagents for measuring the presence or elevation in the level of an immune effector molecule; and (iv) optionally a set of instructions comprising any of the herein disclosed methods.

In some embodiments, the incubation container is suitable for maintaining an optimal shape of the sample, wherein the shape has one or two or more dimensions selected from: (i) a maximum circular diameter of less than about 6mm; (ii) a height of at least about 4mm to 6mm to a maximum height of about 12mm to 20mm; or (iii) a volume of less than 0.5mL and optionally less than 400μl.

Instructions, for example, may comprise instructions to collect whole blood and mix blood in collection/incubation container in order to mix anticoagulant with the blood. In other embodiments, the instructions include instructions to incubate the whole blood sample with an antigen and optionally with a control antigen or mitogen. In other embodiments, the instructions comprise instructions to centrifuge the incubation container and collect plasma. In some embodiments, the instructions comprise instructions to detect an immune effector molecule in plasma.

In some embodiments, the collection vessel is marked to identify a sample height of about 12mm. In some embodiments, the kit comprises a plurality of marked collection vessels of the same and/or different dimensions. In some embodiments, the kit comprises a capillary sampling device. In another embodiment, the kit comprises one or more test antigens for diagnosis and optionally a mitogen as a control antigen for the analysis of *in vitro* responses thereto. Optionally, the kit further comprises reagents appropriate for measuring the presence or elevation in the level of an immune effector molecule or their encoding molecules, including positive and negative controls. In some embodiments, the kit further comprises reagents appropriate for the conduct of an assay for immune effector detection. In one embodiment, the assay is an assay for IFN-γ, or a downstream effector molecule. In a preferred embodiment, the reagent comprises an antibody conjugate for detecting IFN-γ, TNF or GM-CSF. In an exemplary embodiment the antibody conjugate detects IFN-γ. Such assays include, for example, an ELISA or ELISPOT based assay or similar assays known in the art. In another embodiment, the assay is a reverse transcription-amplification assay for RNA encoding the immune effector molecule, such as IFN-γ. Such assays are known in the art and are described for example, Sambrook, *Molecular Cloning: A Laboratory Manual*, 3rd Edition, CSHLP, CSH, NY, 2001 and Ausubel (Ed) *Current Protocols in Molecular Biology*, 5th Edition, John Wiley & Sons, Inc, NY, 2002.

In another aspect, the present invention contemplates methods which may be automated or semi-automated, computer programs, computer products, computers for facilitating the interpretation of output from the subject assays.

The above summary is not and should not been seen in any way as an exhaustive recitation of all embodiments of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation of a system used to carry out the instructions encoded by the storage medium.

Figure 2 is a diagrammatic representation of a cross-section of a magnetic storage medium.

Figure 3 is a diagrammatic representation of a cross-section of an optically readable data storage system.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

The term "about" provides for some variation or correction in the numerical value of the term which it precedes. It pertains to a quantity, volume, level, value, percentage, dimension, size or amount that varies by as much as 35%, 30%, 25%, 20%, 15%, 10%, 5%, 4% or 3% to a recited term. Thus, "at least about 6mm" includes 4mm or 5mm as well as 6mm and heights greater than 6mm, while "about 12mm" includes 13mm, 14mm, 15mm or 16mm and heights smaller than 12mm. In addition, the term covers parts of unit numerical values, such as 6.5mm or 6.9mm etc. In a preferred embodiment, the variation is minor and is limited to a 10% or 15% variation in the numerical value.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an antigen" means one antigen or more than one antigen, "an immune effector molecule" means one or more immune effector molecules.

The term "antigen" as used herein includes any molecule or agent that stimulates an immune response, and particularly a cellular immune response and includes an antisense protein or peptide, a hapten, mitogen, allergen or toxin or any naturally occurring or synthetic molecule or parts thereof having this activity. In some embodiments, the antigen comprises one or more full length or part length polypeptides. In other embodiments, the antigen comprises a peptide or a set of peptides from one or more different full length or part length polypeptides. In some embodiments, antigens are employed which mimic one or more of the effects of antigens presented to the immune system *in vivo*. Generally, test

antigens are selected for optimum selectivity and sensitivity in a given population or subject. In one illustrative embodiment, the antigen is an antigen from *Mycobacterium tuberculosis*. In some embodiments, the antigen is a tuberculosis (TB)-specific antigen. In other embodiments, the antigen is purified protein derivative from *Mycobacterium ruberculosis* or *M. avium*. In some embodiments, the antigen simulates mycobacterial proteins such as ESAT-6 (Skjot *et al.*, *Infection and Immunity*, *68*(1):214-20, 2000), CFP-10 and TB7 (Brock *et al.*, *Int. J. Tuberc. Lung. Dis*, *5*(5):462-467, 2001). A mitogen may be used as a positive control or to detect the ability of cells in the sample to mount an antigen non-specific immune response. In other embodiments, the agent is a mitogen. In other embodiments, the antigen is selected from a self-antigen, an antigen from a pathogenic organism, a metal or inorganic molecule stimulating immune response, or a tumor antigen. In some embodiments, the agent (antigen) is a phospholipid, phosphoprotein or phospholipoprotein. In another illustrative embodiment, the antigen is from cytomegalovirus (CMV). In some embodiments, the antigen from a pathogenic organism is a bacterial, viral, parasite or fungal antigen or analog thereof.

Unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Each embodiment described herein is to be applied *mutatis mutandis* to each and every other embodiment unless specifically stated otherwise.

Preferably, the "subject" is human. The present invention contemplates, however, primates, livestock animals, companion animals and avian species as well as non-mammalian animals such as reptiles and amphibians. The assay has applications, therefore, in human, livestock, veterinary and wild life therapy, diagnosis and monitoring. In some embodiments, the human subject is selected from a group exhibiting a particular attribute or condition. In some embodiments, the subject is a paediatric, adult or geriatric subject. In some embodiments, the subject has or has had a pathogenic infection, an autoimmune

disorder, or cancer, or is undergoing treatment for cancer, or has a propensity for developing such a condition, is immunocompromised or undergoing an inflammatory response. Once the subject has been evaluated including using the present methods and/or kits, they may then be treated, and, accordingly, methods encompassing diagnosis and treatment are also specifically contemplated.

Accordingly, the present invention provides any of the herein disclosed methods wherein the subject is human, including a paediatric, adult or geriatric subject. In other embodiments, the subject is an animal or bird, such as a livestock, racing, exotic, migratory animal or bird.

As stated above, one of the significant advantages of the present invention is the facility to conduct CMI assays using undiluted whole blood from a peripheral capillary. Accordingly, in some embodiments, the method comprises collecting a sample from the subject with a capillary sampling device. The device may be a prick device suitable for capillary sampling any peripheral capillary such as those of the thumb, finger, heel, toe, ear lobe etc. In some embodiments, the device comprises a capillary tube. A capillary tube or other narrow or conical container is useful to form a sample shape of optimum height with very small samples, such as those between about 20μl to 50μl and about 200 μl to 250μl. In some embodiments, the incubation container is suitable for maintaining an optimal shape of the sample, wherein the shape has one or two or more dimensions selected from: (i) a circular diameter of less than 6mm; (ii) a height of at least about 4mm to 6mm to a maximum height of about 12mm to 20mm; or (iii) a volume of less than 0.5mL and optionally less than 400μl. In some embodiments, the sample in the container has a height of at least about 6mm to a maximum of about 12mm. In some embodiments, the sample has a height of 4mm, 5mm, 6mm, 7mm, 8mm, 9mm, 10mm, 11mm, 12mm, 13mm, 14mm, 15mm, 16mm, 17mm, 18mm, 19mm or 20mm or an intervening height. In other embodiments, the sample has a height of 6mm, 7mm, 8mm, 9mm, 10mm, 11mm or 12mm or an intermediate height. Regarding the volume of the sample, in some embodiments, the total sample volume incubated is less than 500μl, less than 400μl, less than 300μl, less than 200μl, less than 100μl, or less than 50μl. Where the sample is capillary blood the total

sample volume incubated is selected from about 2000μl, 1500μl, 1400μl, 1300μl, 1200μl, 1100μl, 900μl, 800μl, 700μl, 600μl, 500μl, 400μl, 300μl, 200μl, 100μl, 50μl or 40μl or an intermediate volume. In some embodiments, the sample is collected into an about 3-4mm diameter capillary tube.

Reference to "immune cells" includes cells such as lymphocytes including natural killer (NK) cells, T-cells, ($CD4^+$ and/or $CD8^+$ cells), B-cells, macrophages and monocytes, dendritic cells or any other cell which is capable of producing an effector molecule in response to direct or indirect antigen stimulation. Conveniently, the immune cells are lymphocytes and more particularly T-lymphocytes.

Accordingly, the present invention contemplates the methods as herein disclosed wherein the immune cells are selected from a natural killer (NK) cell, T-cell, B-cell, macrophage or monocyte. In a preferred embodiment the cells are T-cells.

The immune effector molecules may be any of a range of molecules which are produced in response to cell activation or stimulation by an antigen. Although an interferon (IFN) such as IFN-γ is a particularly useful immune effector molecule, others include a range of cytokines such as interleukins (IL), e.g. IL-2, IL-3 IL-4, IL-5, IL-10 or IL-12, tumor necrosis factor alpha (TNF-α, TNF-β), a colony stimulating factor (CSF) such as granulocyte (G)-CSF or granulocyte macrophage (GM)-CSF amongst many others such as complement or components in the complement pathway, perforins, defensins, cathelicidins, granzymes, Fas ligand, CD-40 ligand, exotaxins, cytotoxins, chemokines and monokines.

Accordingly, in some embodiments the present invention provides methods wherein the immune effector molecule is a cytokine, component of the complement system, perforin, defensin, cathelicidin, granzyme, Fas ligand, CD-40 ligand, exotaxin, cytotoxin, chemokine or monokine. In a preferred embodiment, the cytokine is IFN-γ, TNFα or GM-CSF.

By "whole blood" is meant blood from a subject that has not been substantially diluted or fractionated, maintaining the ambient environment of blood for the cells as close to natural plasma conditions as practical. Thus the addition of small volumes or dried amounts of, for example antigen, sugar or anticoagulant does not constitute dilution in accordance with the present invention, whereas addition of culture medium in excess of the blood volume constitutes dilution. Notwithstanding that whole undiluted blood is the preferred and most convenient sample, the present invention extends to other samples containing immune cells such as lymph fluid, cerebral, fluid, tissue fluid (such as bone marrow or thymus fluid) and respiratory fluid including nasal and pulmonary fluid. Derivatives of these samples may also be obtained by processing. For example, buffy coat cells or peripheral blood mononuclear cells or antigen processing cells are obtained by methods known in the art. Whole blood may also be treated to remove components such as red blood cells and/or platelets by methods known in the art. Substantial dilution would occur by the addition to the sample of more than about 40% to 50% of the original volume.

Accordingly, in some embodiments, the method comprises detecting the presence of an effector molecule or a nucleic acid molecule capable of producing an effector molecule. In this embodiment, the presence or an elevation in the level of a effector molecule or a nucleic acid molecule capable of producing the effector molecule is indicative of the capacity of the subject to mount a cell-mediated response.

In an illustrative embodiment, the shape of the sample comprises a height which has been optimised. In one example of this embodiment, the cellular sample comprises a height of at least 6 millimetres (mm) to a maximum of about 12mm or any intervening height. As the skilled artisan will appreciate, the presently disclosed requirement for an optimised height for the sample permits considerable variation or choice concerning the volume of sample employed and the shape of the container in which the sample is incubated. Thus, in some embodiments, a large volume of sample, say 10 millilitres (mL) of blood is incubated in a container of appropriate dimensions to ensure that the height of the sample during incubation does not exceed about 12mm. At the other end of scale, a 50 microlitre (μl) sample could be incubated, for example, in a 3-4mm diameter capillary tube in order

to provide a sample height of at least about 6mm to a maximum of about 12mm or any intervening height. The present invention is not necessarily limited to any particular volume of sample to be incubated or to any particular dimension or shape of sample containing vessel. However, it is a preferred aspect that the present invention facilitates the use of small volumes of blood (including capillary sampling volumes) and therefore avoids the need for venous blood sampling. Further, that there is no need to dilute blood, requiring further handling steps and maintains the blood in its optional state for measuring an immune response. The above optimum heights have been determined using whole blood from human donors. Other subjects or subject populations or subgroups or cellular sample types have different features and exhibit some variation in the optimum height for sample incubation. In these groups some further minor variation in the minimum and maximum height for sample incubation through optimisation is contemplated. Once the present invention is appreciated, such optimisation is well within the skill of the addressee.

In some embodiments, the container in which sample and antigen are co-incubated is also the collection container used to collect sample from the subject. Any one of a large number of different available containers may be used provided that they provide suitable sample dimensions. A number of different tubes are described in the Examples for the purpose of illustration and the present invention is in no way restricted to these containers. In some embodiments, the container is a tube which comprises a vacuum to facilitate the collection of blood from a subject. In other embodiments, the container is a capillary tube. In some embodiments, a capillary tube is used to collect blood from the surface of the skin by capillary action. In some embodiments, the sample is collected from a subject into a collection container containing antigen or to which antigen is subsequently added. In some embodiments, the blood is sampled using a capillary sampling device such as a pin prick device and blood is collected into a heparinised collecting container and subsequently transferred into an appropriate container for co-incubation with agent.

In some embodiments, the sample is a blood sample. Generally, blood is maintained in the presence of an anticoagulant such as heparin which may be in the container when blood is added or is added subsequently. Optionally, a simple sugar such as dextrose is contained

in the container or added to the incubation mixture. In some preferred embodiments, the blood sample is a whole blood sample. In some embodiments, whole blood from a subject is collected into a container containing antigen and/or anti-coagulant, in other embodiments, antigen and/or anti-coagulant are added to the blood thereafter.

In one embodiment, the method comprises: collecting a blood sample from a subject using a capillary sampling device and introducing blood into a suitable collection vessel. In some embodiments, the capillary sampling device comprises an anticoagulant and the antigen. In other embodiments, the collection vessel or subsequent vessel comprises the antigen. In other embodiments, the collection vessel comprises a simple sugar such as dextrose or other agent that maintains the ability of the sample cells to mount a CMI response. By whatever route, the method comprises contacting the antigen with the blood sample substantially without dilution of the sample and incubating the sample with the antigen under conditions in which the shape of the sample comprises a height that has been optimised for a particular subject or subject population or sample type. In another embodiment, the method comprises incubating the sample with the agent and detecting the presence of an effector molecule or a nucleic acid molecule capable of producing an effector molecule. In an illustrative embodiment, the immune effector molecule is a cytokine such as IFN-γ.

In other embodiments, blood is collected by standard procedures into a collection vessel and transferred to sample (testing) vessels of pre-determined dimensions to ensure that a defined volume of blood is incubated with the antigen under conditions in which the shape of the sample comprises a height or volume that has been optimised for a particular subject or subject population or sample type.

The use of blood collection tubes as collection vessels and testing vessels is disclosed in International Publication No. WO 2004/042396 in the name of Cellestis Limited the content of which is incorporated herein in its entirety by reference.

In some embodiments, the blood sample incubated with an antigen comprises a volume of

less than about 1mL of blood or more than about 2mL of blood. In other embodiments, the 1mL blood sample or about 1mL blood sample during incubation does not comprise a breadth of 13mm.

In some embodiments, the capillary sampling device is a prick device, such as, but by no means limited to those described in US Patent No. 4,469,110.

In another embodiment, the method comprises evaluating a cell-mediated immune response in a blood sample from one or more subject groups wherein samples from each subject group are evaluated to determine a minimum volume of sample to be assessed from each subject group. Optionally, the method comprises sampling the amount of blood appropriate to each subject or subject group wherein each sample comprises, during incubation with antigens, a shape comprising a height that has been optimised for a particular subject or subject population or sample type. In this way, for example, the results from the analysis of subject samples comprising small sample volumes (less than about one millilitre) can readily be compared with the results from larger samples comprising, for example, several millilitres of blood. Thus, the present invention, by characterising and controlling a variable in the cell-mediated response assay, enhances the diagnostic value of the output from the assay.

The present invention is also predicated, in part, upon the observation that the height of the sample during incubation, determined by the shape of the incubation vessel, can be used to modulate the sensitivity of cell-mediated immune response assays. In one embodiment, the present invention provides a method of measuring a cell-mediated immune response in a cellular sample, said method comprising: incubating the sample with an antigen under conditions in which the shape of the sample comprises a height of at least 6 millimetres (mm) to about 12mm. In a particularly useful application of this observation, the present invention provides a method of assaying samples from subjects where sample volume is limiting or where low sample volumes are desirable. In accordance with one embodiment of the present invention practised with blood samples, blood samples as small as about 20μl to about 200μl are employed, wherein the shape of the sample during incubation

comprises a height of at least about 6 millimetres (mm) at its highest point to a maximum height of about 12mm at its highest point.

Accordingly, in one aspect the present invention provides a method of performing a cell mediated immune response assay on a sample from a subject wherein said method avoids the use of needles, the method comprising collecting blood using a capillary sampling device to take small volumes of blood. In another related aspect, the invention encompasses the practise of the herein described assays including the use of small volumes of sample such as one or more samples of about 20μl to less than but about 1mL. In other embodiments, standard blood sampling for cellular assay techniques are employed and larger sample volumes are used, typically 1mL to 5mL but encompassing volumes as great as about 10 to 200mLs or more. In a preferred embodiment, the total incubation volume of whole blood is within the range of about 50μl to less than about 500 μl.

The present invention provides a method for measuring a cell mediated immune (CMI) response in a subject sample comprising incubating the sample with an agent under conditions in which the shape of the sample comprises a dimension which has been optimised. In some embodiments, the cellular sample is incubated with the antigen for from about 4 or 5 to about 50 hours.

In some embodiments, the method is based upon measuring immune effector molecule production by cells of the immune system in response to antigenic stimulation. In other embodiments, immune effector molecule is the immediate effector molecule produced by effector T cells in response to antigen stimulation. In other embodiments, a downstream effector is measured. For example, IFN-γ or other immediate effector molecules elicit the production of further effector molecules whose production is measured. In another embodiment, the production of immune effectors is measured by measuring the level or presence of nucleic acid molecules capable of producing immune effectors. Accordingly, in some embodiments, immune effectors may be detected using ligands or binding molecules such as antibodies specific for the effectors or by measuring the level of expression of genes encoding the effectors. The present invention provides, therefore, a

means to determine the cellular responsiveness of a subject and, in turn, provides a means for the diagnosis of infectious diseases, pathological conditions, immune status, level of immunocompetence and a marker of T-cell responsiveness to endogenous or exogenous antigens.

Accordingly, in another embodiment, the present invention contemplates a method for measuring a CMI response in a subject, said method comprising i) collecting a fluid sample from the subject into a collection vessel wherein said sample comprises cells of the immune system which produce immune effector molecules following stimulation by an agent. In some embodiments, the collection vessel comprises an anticoagulant, such as heparin. In other embodiments, the collection vessel comprises the agent. In some embodiments, the method further comprises contacting the agent with the sample in the collection vessel. The method further comprises iii) incubating said sample with an antigen under conditions in which the shape of the sample comprises a dimension which has been optimised. In some embodiments, the method optionally comprises iv) detecting the presence of an immune effector molecule or a nucleic acid molecule capable of producing either of these, wherein presence or elevation in the level of a effector molecule or a nucleic acid molecule capable of producing the effector molecule is indicative of the capacity of the subject to mount a cell-mediated response. In other embodiments, the immune effector is a cytokine, cytotoxin or chemokine. In an illustrative embodiment, the immune effector is IFN-γ.

In some embodiments, the shape of the sample is optimised by measuring effector cell function in samples having a range of dimensions and selecting the shape that is associated with the most sensitive measurement of effector cell function. In a preferred embodiment, the height of the sample is varied. In an illustrative embodiment, the height of the sample is varied about 12mm for a maximum height and about 6mm for a minimum height.

According to a preferred embodiment the present invention provides a method for measuring a CMI response in a human subject, said method comprising collecting a sample from said human subject using a capillary sampling device into a collection vessel.

In some embodiments, the sample comprises cells of the immune system which are capable of producing immune effector molecules following stimulation by an antigen, mitogen or hapten. In some embodiments, the method comprises incubating said sample with an antigen and then measuring the presence of or elevation in the level of an immune effector molecule wherein the presence or level of said immune effector molecule is indicative of the capacity of said human subject to mount a cell-mediated immune response.

Accordingly, in another preferred embodiment, the present invention provides a method for measuring a CMI response in a subject, said method comprising collecting a sample from said subject into a collection vessel wherein said sample comprises cells of the immune system which are capable of producing IFN-γ molecules following stimulation by an antigen, incubating said sample with an antigen and then measuring the presence of or elevation in the level of an IFN-γ molecule wherein the presence or level of said IFN-γ molecule is indicative of the capacity of said subject to mount a cell-mediated immune response.

The sample collected from the subject is generally deposited into a blood collection vessel. Notwithstanding that whole undiluted blood is the preferred and most convenient sample, the present invention extends to other samples containing immune cells such as lymph fluid, cerebral, fluid, tissue fluid and respiratory fluid including nasal and pulmonary fluid.

The cells of the CMI system lose the capacity to mount a CMI response in whole blood after extended periods following blood draw from the subject, and responses without intervention are often severely reduced or absent by 24 hours following blood draw. The reduction of labor and need for specialized equipment in the present invention allows CMI stimulation with antigens to be performed at the point of care locations such as physicians' offices, clinics, outpatient facilities and veterinary clinics or on farms. Once antigen stimulation is complete, the requirement for fresh and active cells no longer exists. IFN-γ and other cytokines or immune effector molecules are stable in plasma and, thus, the sample can be stored, or shipped without special conditions or rapid time requirements in a

similar fashion to standard serum samples used for other infectious disease or other disease diagnosis.

The incubation step may be from about 4 or 5 hours to 50 hours, more preferably about 5 hours to 40 hours and even more preferably about 8 to 24 or about 16 to 24 hours or a time period in between. In some embodiments, after an optional initial mixing step to distribute antigens throughout the sample, the sample incubating is carried out without mixing further.

Accordingly, another preferred embodiment of the present invention contemplates a method for measuring a CMI response in a subject including a human subject, said method comprising collecting a sample of whole blood from said subject by capillary sampling, incubating said whole blood sample with an antigen and then measuring the presence or elevation in level of an immune effector molecule such as IFN-γ wherein the presence or level of said immune effector molecule is indicative of the capacity of said subject to mount a cell-mediated immune response.

The ability to measure CMI is important for assessing a subject's ability to respond to an infection by an pathogenic agent such as a microorganism or virus or parasite, to mount an autoimmune response such as in diabetes to protect against cancers or other oncological conditions or to test for sensitivity to environmental antigens (allergy testing). Consequently, reference to "measuring a CMI response in a subject" includes and encompasses immune diagnosis of infectious and autoimmune diseases, a marker for immunocompetence and the detection of T-cell responses to endogenous and/or exogenous antigens (including a measure of the efficacy of a vaccine) as well as a marker for allergies, inflammatory diseases and cancer.

The ability to perform this test in small volumes of blood is important for pediatric and other samples where blood may be limiting. The absence of any handling steps in purifying lymphocytes adds an advantage in small blood volumes as purification and enumeration of lymphocytes from small volumes has practical difficulties, as does the

addition of warmed sterile media and reagents in a sterile environment. The ability to obtain an optimal CMI response in a small volume by adjusting the relative proportions (such as, shape, width and height) of the incubating vessel or sample provides valuable advantages. The terms vessel, container, compartment are used interchangeably and include any receptacle that holds any volume, such as a well, dip, tube, eppendorf and the like.

Autoimmune diseases contemplated herein include *inter alia* Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Autoimmune Addison's Disease Multiple Sclerosis, Autoimmune disease of the adrenal gland, Autoimmune Hemolytic Anemia, Autoimmune Hepatitis, Autoimmune oophoritis and orchitis, Behcet's Disease, Bullous Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Syndrome (CFIDS), Chronic Inflam. Demyelinating, Chronic Inflam. Polyneuropathy, Churg-Strauss Syndrome, Cicatricial Pemphigoid, CREST Syndrome, Cold Agglutinin Disease, Crohn's Disease, Dermatitis herpetiformis, Discoid Lupus, Essential Mixed Cryoglobulinemia, Fibromyalgia, Glomerulonephritis, Grave's Disease, Guillain-Barre, Hashimoto's Thyroiditis, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy Insulin Dependent Diabetes (Type I), Lichen Planus, Lupus, Meniere's Disease, Mixed Connective Tissue Disease, Multiple sclerosis, Myasthenia Gravis, Myocarditis, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglancular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjogren's Syndrome, Stiff-Man Syndrome, Systemic lupus erythematosus, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis and Vitiligo.

It is generally important to assess the potential or actual CMI responsiveness in these individuals.

Other disease conditions contemplated include inflammatory disease conditions.

Examples of inflammatory disease conditions contemplated by the present invention include but are not limited to those disease and disorders which result in a response of redness, swelling, pain, and a feeling of heat in certain areas that is meant to protect tissues affected by injury or disease. Inflammatory diseases which can be treated using the methods of the present invention, include, without being limited to, acne, angina, arthritis, aspiration pneumonia, disease, empyema, gastroenteritis, inflammation, intestinal flu, NEC, necrotizing enterocolitis, pelvic inflammatory disease, pharyngitis, PID, pleurisy, raw throat, redness, rubor, sore throat, stomach flu and urinary tract infections, Chronic Inflammatory Demyelinating Polyneuropathy, Chronic Inflammatory Demyelinating Polyradiculoneuropathy, Chronic Inflammatory Demyelinating Polyneuropathy, Chronic Inflammatory Demyelinating Polyradiculoneuropathy.

Cancer therapy also is somewhat dependent on CMI. Cancers contemplated herein include: a group of diseases and disorders that are characterized by uncontrolled cellular growth (e.g. formation of tumor) without any differentiation of those cells into specialized and different cells. Such diseases and disorders include ABL1 protooncogene, AIDS Related Cancers, Acoustic Neuroma, Acute Lymphocytic Leukaemia, Acute Myeloid Leukaemia, Adenocystic carcinoma, Adrenocortical Cancer, Agnogenic myeloid metaplasia, Alopecia, Alveolar soft-part sarcoma, Anal cancer, Angiosarcoma, Aplastic Anaemia, Astrocytoma, Ataxia-telangiectasia, Basal Cell Carcinoma (Skin), Bladder Cancer, Bone Cancers, Bowel cancer, Brain Stem Glioma, Brain and CNS Tumours, Breast Cancer, CNS tumours, Carcinoid Tumours, Cervical Cancer, Childhood Brain Tumours, Childhood Cancer, Childhood Leukaemia, Childhood Soft Tissue Sarcoma, Chondrosarcoma, Choriocarcinoma, Chronic Lymphocytic Leukaemia, Chronic Myeloid Leukaemia, Colorectal Cancers, Cutaneous T-Cell Lymphoma, Dermatofibrosarcoma-provesselrans, Desmoplastic-Small-Round-Cell-Tumour, Ductal Carcinoma, Endocrine Cancers, Endometrial Cancer, Ependymoma, Esophageal Cancer, Ewing's Sarcoma, Extra-Hepatic Bile Duct Cancer, Eye Cancer, Eye: Melanoma, Retinoblastoma, Fallopian Vessel cancer, Fanconi Anaemia, Fibrosarcoma, Gall Bladder Cancer, Gastric Cancer, Gastrointestinal Cancers, Gastrointestinal-Carcinoid-Tumour, Genitourinary Cancers,

Germ Cell Tumours, Gestational-Trophoblastic-Disease, Glioma, Gynaecological Cancers, Haematological Malignancies, Hairy Cell Leukaemia, Head and Neck Cancer, Hepatocellular Cancer, Hereditary Breast Cancer, Histiocytosis, Hodgkin's Disease, Human Papillomavirus, Hydatidiform mole, Hypercalcemia, Hypopharynx Cancer, IntraOcular Melanoma, Islet cell cancer, Kaposi's sarcoma, Kidney Cancer, Langerhan's-Cell-Histiocytosis, Laryngeal Cancer, Leiomyosarcoma, Leukaemia, Li-Fraumeni Syndrome, Lip Cancer, Liposarcoma, Liver Cancer, Lung Cancer, Lymphedema, Lymphoma, Hodgkin's Lymphoma, Non-Hodgkin's Lymphoma, Male Breast Cancer, Malignant-Rhabdoid-Tumour-of-Kidney, Medulloblastoma, Melanoma, Merkel Cell Cancer, Mesothelioma, Metastatic Cancer, Mouth Cancer, Multiple Endocrine Neoplasia, Mycosis Fungoides, Myelodysplastic Syndromes, Myeloma, Myeloproliferative Disorders, Nasal Cancer, Nasopharyngeal Cancer, Nephroblastoma, Neuroblastoma, Neurofibromatosis, Nijmegen Breakage Syndrome, Non-Melanoma Skin Cancer, Non-Small-Cell-Lung-Cancer-(NSCLC), Ocular Cancers, Oesophageal Cancer, Oral cavity Cancer, Oropharynx Cancer, Osteosarcoma, Ostomy Ovarian Cancer, Pancreas Cancer, Paranasal Cancer, Parathyroid Cancer, Parotid Gland Cancer, Penile Cancer, Peripheral-Neuroectodermal-Tumours, Pituitary Cancer, Polycythemia vera, Prostate Cancer, Rare-cancers-and-associated-disorders, Renal Cell Carcinoma, Retinoblastoma, Rhabdomyosarcoma, Rothmund-Thomson Syndrome, Salivary Gland Cancer, Sarcoma, Schwannoma, Sezary syndrome, Skin Cancer, Small Cell Lung Cancer (SCLC), Small Intestine Cancer, Soft Tissue Sarcoma, Spinal Cord Tumours, Squamous-Cell-Carcinoma-(skin), Stomach Cancer, Synovial sarcoma, Testicular Cancer, Thymus Cancer, Thyroid Cancer, Transitional-Cell-Cancer-(bladder), Transitional-Cell-Cancer-(renal-pelvis-/-ureter), Trophoblastic Cancer, Urethral Cancer, Urinary System Cancer, Uroplakins, Uterine sarcoma, Uterus Cancer, Vaginal Cancer, Vulva Cancer, Waldenstrom's-Macroglobulinemia, Wilms' Tumour.

Any of a range of antigens may be tested such as those specific for a particular organism, virus, autoantigen or cancer cell. Alternatively, more general agents may be used to test generic capacity of a cell-mediated immune response. Examples of the latter include PPD from *M. tuberculosis* and tetanus toxoid. Any peptide, polypeptide or protein,

carbohydrate, glycoprotein, phospholipid, phosphoprotein or phospholipoprotein or non-protein chemical agent may be used in the present assay system.

As stated above, detection of the immune effector molecules may be made at the protein or nucleic acid levels. Consequently, reference to "presence or level of said immune effector molecule" includes direct and indirect data. For example, high levels of IFN-γ mRNA is indirect data showing increased levels of IFN-γ. Assays known in the art for assessing RNA are described for example in Sambrook, *Molecular Cloning: A Laboratory Manual*, 3rd Edition, CSHLP, CSH, NY, 2001 and Ausubel (Ed) *Current Protocols in Molecular Biology*, 5th Edition, John Wiley & Sons, Inc, NY, 2002.

Ligands to the immune effectors are particularly useful in detecting and/or quantitating these molecules. Antibodies to the immune effector molecules are particularly useful. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays, ELISA and ELISSPOT. Rapid point of care immunochromatographic devices are also included. Reference to "antibodies" includes parts of antibodies, mammalianized (e.g. humanized) antibodies, recombinant or synthetic antibodies and hybrid and single chain antibodies.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the immune effectors or antigenic fragments thereof and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of the immune effector, or antigenic part thereof, collecting serum from the animal and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The

preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates, therefore, a method for detecting an immune effector in a sample comprising immune cells from a subject, said method comprising contacting said sample or an aliquot of said sample with an antibody specific for said immune effector or antigenic fragment thereof for a time and under conditions sufficient for an antibody-effector complex to form, and then detecting said complex.

A sample includes whole blood. This method includes micro-arrays and macro-arrays on planar or spherical solid supports.

A wide range of immunoassay techniques are available as can be seen by reference to U.S. Patent Nos. 4,016,043, 4,424,279 and 4,018,653.

The following is a description of one type of assay. An unlabeled antibody is immobilized on a solid substrate and the sample to be tested for the immune effectors (e.g. antigens) brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labeled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of antigen. This generalized technique is well known to those skilled in the art as would be any of a number of variations.

In these assays, a first antibody having specificity for the instant immune effectors is either covalently or passively bound to a solid surface. The solid surface is typically glass or a

polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of vessels, beads, spheres, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-120 minutes or where more convenient, overnight) and under suitable conditions (e.g. for about 20°C to about 40°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the antigen. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

There are many variations to this assay. One particularly useful variation is a simultaneous assay where all or many of the components are admixed substantially simultaneously.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules. Examples of suitable fluorophores are provided in Table 2. In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic

substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labeled antibody is added to the first antibody-antigen complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of antigen which was present in the sample. Again, the present invention extends to a substantially simultaneous assay.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. The fluorescent labeled antibody is allowed to bind to the first antibody-antigen complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the antigen of interest. Immunofluorescene and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

There are a range of other detection systems which may be employed including colloidal gold and all such detection systems are encompassed by the present invention.

The present invention also contemplates genetic assays such as involving RT-PCR analysis or other amplification based strategies known in the art to detect RNA expression products of a genetic sequence encoding an immune effector.

In one embodiment, PCR is conducted using pairs of primers, one or both of which are generally labeled with the same or a different reporter molecule capable of giving a

distinguishable signal. The use of fluorophores is particularly useful in the practice of the present invention. Examples of suitable fluorophores may be selected from the list given in Table 2. Other labels include luminescence and phosphorescence as well as infrared dyes. These dyes or fluorophores may also be used as reporter molecules for antibodies.

Any suitable method of analyzing fluorescence emission is encompassed by the present invention. In this regard, the invention contemplates techniques including but not restricted to 2-photon and 3-photon time resolved fluorescence spectroscopy as, for example, disclosed by Lakowicz *et al., Biophys. J., 72*: 567, 1997, fluorescence lifetime imaging as, for example, disclosed by Eriksson *et al., Biophys. J., 2*:64, 1993 and fluorescence resonance energy transfer as, for example, disclosed by Youvan *et al., Biotechnology. 3*:1-18, 1997.

Luminescence and phosphorescence may result respectively from a suitable luminescent or phosphorescent label as is known in the art. Any optical means of identifying such label may be used in this regard.

Infrared radiation may result from a suitable infrared dye. Exemplary infrared dyes that may be employed in the invention include but are not limited to those disclosed in Lewis *et al., Dyes Pigm., 42*(2):197, 1999, Tawa *et al., Mater. Res. Soc. Symp. Proc., 488* [Electrical, Optical and Magnetic Properties of Organic Solid-State Materials IV], 885-890, Daneshvar *et al., J. Immunol. Methods, 226*(1-2):119-128, 1999, Rapaport *et al., Appl. Phys. Lett., 74*(3):329-331, 1999 and Durig *et al., J. Raman Spectrosc., 24*(5):281-285, 1993. Any suitable infrared spectroscopic method may be employed to interrogate the infrared dye. For instance, fourier transform infrared spectroscopy as, for example, described by Rahman *et al., J. Org. Chem., 63*:6196, 1998 may be used in this regard.

Suitably, electromagnetic scattering may result from diffraction, reflection, polarization or refraction of the incident electromagnetic radiation including light and X-rays. Such scattering can be used to quantitate the level of mRNA or level of protein.

Flow cytometry is particularly useful in analyzing fluorophore emission.

As is known in the art, flow cytometry is a high throughput technique which involves rapidly analyzing the physical and chemical characteristics of particles (e.g. labeled mRNA, DNA or proteins) as they pass through the path of one or more laser beams while suspended in a fluid stream. As each particle intercepts the laser beam, the scattered light and fluorescent light emitted by each cell or particle is detected and recorded using any suitable tracking algorithm as, for example, described hereunder.

A modern flow cytometer is able to perform these tasks up to 100,000 cells/particles s^{-1}. Through the use of an optical array of filters and dichroic mirrors, different wavelengths of fluorescent light can be separated and simultaneously detected. In addition, a number of lasers with different excitation wavelengths may be used. Hence, a variety of fluorophores can be used to target and examine, for example, different immune effectors within a sample or immune effectors from multiple subjects.

Suitable flow cytometers which may be used in the methods of the present invention include those which measure five to nine optical parameters (see Table 3) using a single excitation laser, commonly an argon ion air-cooled laser operating at 15 mW on its 488 nm spectral line. More advanced flow cytometers are capable of using multiple excitation lasers such as a HeNe laser (633 nm) or a HeCd laser (325 nm) in addition to the argon ion laser (488 or 514 nm).

For example, Biggs *et al., Cytometry, 36:*36-45, 1999 have constructed an 11-parameter flow cytometer using three excitation lasers and have demonstrated the use of nine distinguishable fluorophores in addition to forward and side scatter measurements for purposes of immunophenotyping (i.e. classifying) particles. The maximum number of parameters commercially available currently is 17: forward scatter, side scatter and three excitation lasers each with five fluorescence detectors. Whether all of the parameters can be adequately used depends heavily on the extinction coefficients, quantum yields and amount of spectral overlap between all fluorophores (Malemed *et al., "Flow cytometry and*

sorting", 2nd Ed., New York, Wiley-Liss, 1990). However, it will be understood that the present invention is not restricted to any particular flow cytometer or any particular set of parameters. In this regard, the invention also contemplates use in place of a conventional flow cytometer, a microfabricated flow cytometer as, for example, disclosed by Fu *et al.*, *Nature Biotechnology, 17*: 1109-1111, 1999.

The assay of the present invention may be automated or semi-automated for high throughput screening or for screening for a number of immune effectors from the one subject. The automation is conveniently controlled by computer software.

The present invention contemplates a computer program product, therefore, for assessing the presence or absence or the level of one or more immune effectors, said product comprising:-

(1) code that receives, as input values, the identity of a reporter molecule associated with a labeled mRNA or antibody:

(2) code that compares said input values with reference values to determine the level of reporter molecules and/or the identity of the molecule to which the reporter molecule is attached; and

(3) a computer readable medium that stores the codes.

In another embodiment, the program product further comprises code that receives as input information concerning the height of the sample in the test tube. In some embodiments, the information identifies a test vessel comprising a sample which has a shape that falls outside one or more pre-determined dimensions. In some embodiments, information may be in the form of a signal that reports the detection of a sample (and the corresponding test vessel) in which the height of the sample exceeds at least about 12mm or other value corrected for different samples. In another embodiment, information may be in the form of

a signal that reports the detection of a sample in which the height of the sample is less than about 6mm or other value corrected for different samples.

Still another aspect of the present invention extends to a computer for assessing the presence or absence or level of one or more immune effectors, said computer comprises:-

(1) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said machine-readable data comprise input values which identify a reporter molecule associated with a labeled mRNA or antibody;

(2) a working memory for storing instructions for processing said machine-readable data;

(3) a central-processing unit coupled to said working memory and to said machine-readable data storage medium, for processing said machine readable data to compare said values to provide an assessment of the identity or level of reporter molecules or of molecules to which they are attached; and

(4) an output hardware coupled to said central processing unit, for receiving the results of the comparison.

A version of these embodiments is presented in Figure 1, which shows a system 10 including a computer 11 comprising a central processing unit ("CPU") 20, a working memory 22 which may be, e.g. RAM (random-access memory) or "core" memory, mass storage memory 24 (such as one or more disk drives or CD-ROM drives), one or more cathode-ray vessel ("CRT") display terminals 26, one or more keyboards 28, one or more input lines 30, and one or more output lines 40, all of which are interconnected by a conventional bidirectional system bus 50.

Input hardware 36, coupled to computer 11 by input lines 30, may be implemented in a variety of ways. For example, machine-readable data of this invention may be inputted *via* the use of a modem or modems 32 connected by a telephone line or dedicated data line 34. Alternatively or additionally, the input hardware 36 may comprise CD. Alternatively, ROM drives or disk drives 24 in conjunction with display terminal 26, keyboard 28 may also be used as an input device.

Output hardware 46, coupled to computer 11 by output lines 40, may similarly be implemented by conventional devices. By way of example, output hardware 46 may include CRT display terminal 26 for displaying a synthetic polynucleotide sequence or a synthetic polypeptide sequence as described herein. Output hardware might also include a printer 42, so that hard copy output may be produced, or a disk drive 24, to store system output for later use.

In operation, CPU 20 coordinates the use of the various input and output devices 36,46 coordinates data accesses from mass storage 24 and accesses to and from working memory 22, and determines the sequence of data processing steps. A number of programs may be used to process the machine readable data of this invention. Exemplary programs may use, for example, the following steps:-

(1) inputting input values which identifies a reporter molecule associated with a labeled mRNA or antibody;

(2) assessing including comparing said input values with reference values to determine the level of reporter molecule and/or the identity of the molecule to which the reporter molecule is attached; and

(3) outputting the results of the assessment.

Figure 2 shows a cross section of a magnetic data storage medium 100 which can be encoded with machine readable data, or set of instructions, for assessing the level of an

immune effector which can be carried out by a system such as system 10 of Figure 1. Medium 100 can be a conventional floppy diskette or hard disk, having a suitable substrate 101, which may be conventional, and a suitable coating 102, which may be conventional, on one or both sides, containing magnetic domains (not visible) whose polarity or orientation can be altered magnetically. Medium 100 may also have an opening for receiving the spindle of a disk drive or other data storage device 24. The magnetic domains of coating 102 of medium 100 are polarized or oriented so as to encode in manner which may be conventional, machine readable data for execution by a system such as system 10 of Figure 1.

Figure 3 shows a cross section of an optically readable data storage medium 110 which also can be encoded with such a machine-readable data, or set of instructions, for designing a synthetic molecule of the invention, which can be carried out by a system such as system 10 of Figure 1. Medium 110 can be a conventional compact disk read only memory (CD-ROM) or a rewritable medium such as a magneto-optical disk, which is optically readable and magneto-optically writable. Medium 100 preferably has a suitable substrate 111, which may be conventional, and a suitable coating 112, which may be conventional, usually of one side of substrate 111.

In the case of CD-ROM, as is well known, coating 112 is reflective and is impressed with a plurality of pits 113 to encode the machine-readable data. The arrangement of pits is read by reflecting laser light off the surface of coating 112. A protective coating 114, which preferably is substantially transparent, is provided on top of coating 112.

The present invention further contemplates kits for assessing the capacity of a subject to mount a cell mediated response according to the methods described herein. The kit is conveniently in compartmental form with one or more compartments adapted to receive a sample from a subject such as whole blood preferably collected by capillary sampling, such as by a prick device. Thus in some embodiments, the kit comprises a device suitable for capillary sampling such as a device that punctures or perforates the skin to allow bleeding from peripheral capillaries. Containers for receiving a sample may have the same

uniform dimensions or they may comprise a plurality of dimensions. In some embodiments, the containers for receiving samples are marked or otherwise arranged such that the height of the sample in the containers may be assessed. Containers may also be adapted to contain an anticoagulant where the sample is whole blood with or without a simple sugar, such as dextrose, to maintain the effective functional capacity of the immune cells.

Generally, the kit is in a form which is packaged for sale with a set of instructions. The instructions would generally be in the form of a method for measuring a CMI response in a subject, said method comprising collecting a sample from said subject wherein said sample comprises cells of the immune system which are capable of producing immune effector molecules following stimulation by an antigen, incubating said sample with an antigen under conditions in which the shape and the sample comprises a dimensions which has been optimised and then optionally measuring the presence or elevation in level of an immune effector molecule wherein the presence or level of said immune effector molecule is indicative of the capacity of said subject to mount a cell-mediated immune response.

Conveniently, the kit further comprises a capillary sampling device and/or an incubator. In some embodiments blood is collected using an about 3-4mm diameter capillary tube.

In some embodiments, the subject from whom the sample is derived is a human subject such as a pediatric, adult or geriatric subject. Any animal or bird may be a subject.

Although the illustrated immune effector molecule is IFN-γ, other cytokines such as TNFα and GM-CSF a readily assayed, as are components of the complement system, perforins, defensins, cathelicidins, granzymes, Fas ligand, CD-40 ligand, exotaxin, cytotoxins, chemokines or monokines. In some embodiments the immune cells tested are selected from a natural killer (NK) cell, T-cell, B-cell, macrophage or monocyte.

In some embodiments the kit comprises an antigen is selected from a self-antigen, an antigen from a pathogenic organism, a metal or inorganic antigen, or a tumour antigen or

an analog thereof. In some embodiments, the antigen is from M*ycobacterium* such as but in no way limited *to* ESAT-6, CFP-10 and TB7. In other embodiments, the antigen tested is tetanus toxoid (TT) or purified protein derivative (PPD) from *M. tuberculosis* or *M. avium.*

As described in relation to the method, the container is selected to provide the optimum sample shape during the incubation step. In some embodiments, the incubation container forms a sample height of at least about 4mm to 6mm to a maximum height of about 12mm to 20mm. In other embodiments, a sample height of at least about 6mm to a maximum of about 12mm is preferred. In some embodiments, the incubation container forms a sample height of 4mm, 5mm, 6mm, 7mm, 8mm, 9mm, 10mm, 11mm, 12mm, 13mm, 14mm, 15mm, 16mm, 17mm, 18mm, 19mm or 20mm or an intervening height. In other embodiments, the incubation container forms a sample height of 6mm, 7mm, 8mm, 9mm, 10mm, 11mm or 12mm or an intermediate height. In relation to the volume of sample in the incubation container, in some embodiments, the incubated sample has a volume of less than 500μl, less than 400μl, less than 300μl, less than 200μl, less than 100μl, or less than 50μl. In other embodiments, the sample is capillary blood and the incubated sample has a volume of about 2000μl, 1500μl, 1400μl, 1300μl, 1200μl, 1100μl, 900μl, 800μl, 700μl, 600μl, 500μl, 400μl, 300μl, 200μl, 100μl, 50μl or 40μl or an intermediate volume.

In some embodiments, the kit comprises reagents for detecting IFN-γ and these include an antibody conjugate for detecting IFN-γ.

The present invention further provides a method of treatment of a subject having a pathogenic infection, an autoimmune disorder or cancer or a propensity for developing such a disorder, said method comprising assessing the ability of said subject to mount a cell mediated immune response by the method of measuring a CMI response in a subject, said method comprising collecting a sample from said subject optionally by capillary sampling wherein said sample comprises cells of the immune system which are capable of producing immune effector molecules following stimulation by an antigen, incubating said sample in an incubation container with an antigen under conditions in which the shape of

the sample comprises a dimension which has been optimised and then measuring the presence of or elevation in the level of an immune effector molecule wherein the presence or level of said immune effector molecule is indicative of the capacity of said subject to mount a cell-mediated immune response and then selecting a suitable therapeutic protocol. In some embodiments, the sample is a blood sample. In some embodiments, the shape of the sample comprises a height of at least 6mm to a maximum of about 12mm. In other embodiments, the sample volume is less than 1mL, even preferably less than 0.5mL including 0.01mL samples. In some embodiments, the subject is a livestock animal or human or avian subject. In further embodiments, the incubation container is suitable for maintaining an optimal shape of the sample, wherein the shape has one or two or more dimensions selected from: (i) a maximum circular diameter of less than 6mm; (ii) a height of at least about 4mm to 6mm to a maximum height of about 12mm to 20mm; or (iii) a volume of less than 0.5mL and optionally less than 400μl.

In another aspect, the present invention provides a method for optimising an *in vitro* cellular immune response, the method comprising: i) incubating a plurality of cellular samples having a range of different heights or other dimensions in incubation containers wherein the cellular sample comprises cells that secrete an immune effector molecule following stimulation by an agent (such as an antigen, hapten or mitogen) with the agent *in vitro* for a time and under conditions sufficient for the cells to secrete the immune effector molecule; and ii) measuring the presence or level of the immune effector molecule from each sample; and iii) identifying the sample dimension that provides the optimal cellular response. In some embodiments, the dimension is the height of the sample in the incubating vessel. In another embodiment, the dimension is the volume of the sample in the incubating vessel. In other embodiments, the dimension is the maximum circular diameter of the sample in the incubation container.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Detection of immune effector molecule in small volumes of sample incubated with antigen

Whole blood from healthy donors (four donors) was collected into 9mL Vacuette Li-heparin tubes having a cylindrical shape with 6.6mm diameter and a U-shaped base. Aliquots of 0.1, 0.2, 0.3, 0.4 and 0.5mL of blood were stimulated in Vacuette Mini-Collect tubes (no-additive). Blood was stimulated using Tetanus Toxoid and Phytohaemagglutinin-P (Mitogen). The volume of antigen added to each tube was proportional to the blood volume e.g., 0.1mL blood was stimulated with 0.01mL antigen; 0.4mL of blood was stimulated with 0.04mL antigen.

The IFN-γ responses generated in small volumes of blood were compared with responses generated using 1mL of blood in a 13/75 Vacuette blood collection tube (Control).

Blood was incubated with antigen for between 16 and 24 hours at 37°C before the plasma was removed for IFN-γ detection (QuantiFERON-TB Gold ELISA). Only 25μL of plasma was assayed for the 0.1mL samples due to insufficient sample being obtained.

ELISA testing demonstrated that IFN-γ was produced in volumes of blood as little as 0.1mL (see Table 3). Within the 6.6mm diameter vessel results for 0.3-0.4mL blood in response to antigen were similar to the 1mL control for each subject in a 11mm diameter round vessel. Mitogen results were less affected by volumes and were similar from 0.1mL to 0.5mL indicating less need to optimise the volume with the container proportions.

In accordance with the present invention optimal cellular responses as measured by cytokine production or further effector mechanisms can be achieved in small volumes (such as those obtainable by prick sampling) as long as pre-optimisation of the vessel is performed; once it is realised this is possible, the optimisation can be done by one skilled in the art.

EXAMPLE 2

Detection of immune effector molecule in small volume of whole blood incubated in containers having different internal shapes

Heparinised blood was dispensed into 3x5mL aliquots in polypropylene tubes. Antigen, either human cytomegalovirus (CMV) or tetanus toxoid (tetanus) was added to the blood at appropriate concentrations. Each tube was mixed thoroughly and the blood (50μl) dispensed into various containers where the volume of blood assumed different heights. Specifically, PCR tubes (conical to 10mm height with maximum diameter 5mm, u-shaped base), minicollect vessels (cylindrical with 6.5mm diameter, u-shaped based), 96-well plate (cylindrical with 6.5mm, diameter flat base) and 48-well plate (cylindrical with 11mm diameter, flat base). The containers were incubated for 20 hours at 37°C prior to removing plasma (20μl) for testing by QFT-ELISA which detects the production of IFN-γ using a labelled antibody. In a control experiment, 1mL of blood was incubated in a cylindrical container (internal diameter of 10.5mm, flat base) and 20μl of plasma tested by QFT-ELISA. The results are shown in Table 4 where IFN-γ in IU/mL are shown for nil antigen (Nil), tetanus toxoid (TT) and cytomegalovirus (CMV). Responses of 0.20 IU/mL and above Nil are significant. The height of the blood volume and the maximum circular diameter in the different containers is also shown in the Table 4. Strong signal are detected in subjects 1, 3, 4 and 5 with CMV and/or TT for sample heights of 11.5mm and 6mm. The signal drops off for CMV as the height goes below 6mm to 3mm and then 1.5mm. Accordingly, a 15μl whole blood sample provides a strong signal provided this volume of blood is incubated in an appropriate container providing a sample height of at least about 4mm.

EXAMPLE 3

Detection of immune effector molecule in peripheral capillary blood

Capillary blood was collected (150μl) by finger prick into a lithium heparin minicollect tube. Small volume (50μl) were transferred into three PCR tubes. The PCR tubes were conical having a cylindrical upper portion tapering over 10mm to a conical base. Antigen

being CMV or tetanus toxoid (Tetanus) or no antigen (Nil) were added to the tubes which were incubated for 20 hours at 37°C. Thereafter, plasma (20μl) was removed and tested using QFT-ELISA. As shown in Table 5, the positive CMV control subject (TR) sample generated a significant signal compared to negative controls indicating that the assay can be conducted with volumes as small as 50μl of capillary blood.

EXAMPLE 4

Detection of immune effector molecules down to 6mm height and up to 18mm height

Heparinised human blood of different volumes was incubated with antigen (no antigen (Nil), tetanus toxoid (TT) or cytomegalovirus (CMV)) in three types of container providing a blood height of 11.5mm, 18mm and 6mm. Plasma was tested in QFT-ELISA. The results (see Table 6) again show efficacy down to 6mm. The results also show positive results with the 18mm conical tube indicating that immune effector molecule are produced at sample heights of above 18mm however optimum results are achieved between 5 and 18mm.

The results shown in Figure 3 were retabulated in Table 8 also providing the heights of the various blood samples. Here it can be seen that a reduced signal is produced as sample height increases from 16mm upwards. The mitogen results have been removed from Table 3 in Table 8 because PHA-P is a non-specific stimulant whereas tetanus toxoid requires cellular antigen processing and presentation.

EXAMPLE 5

Detection of immune effector molecule down to 4mm and 20μl of whole blood

Heparinised human blood of different volumes: 50μl, 40μl, 30μl, 20μl and 10μl were tested in small PCR tubes providing sample heights of 6mm, 5.5mm, 5mm, 4mm and 2.5mm, respectively. Antigens were: no antigen (N) tetanus toxoid (TT) and cytomegalovirus (CMV) at appropriate concentration. Blood was incubated at 37°C for 20 hours and plasma removed for testing by QFT-ELISA. A control experiment (Table 7 Continued)

using 1mL cultures used small volumes of plasma (50μl, 40μl, 30μl, 20μl, 15μl, 10μl and 5μl) to test by QFT-ELISA. The results shown in Table 7 show a positive signal down to 20μl of blood with a height of 4mm although optimum results were found at 5.5 and 6mm.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 1

List of suitable fluorophores

Probe	Ex[1] (nm)	Em[2] (nm)
Reactive and conjugated probes		
Hydroxycoumarin	325	386
Aminocoumarin	350	455
Methoxycoumarin	360	410
Cascade Blue	375; 400	423
Lucifer Yellow	425	528
NBD	466	539
R-Phycoerythrin (PE)	480; 565	578
PE-Cy5 conjugates	480; 565; 650	670
PE-Cy7 conjugates	480; 565; 743	767
APC-Cy7 conjugates	650; 755	767
Red 613	480; 565	613
Fluorescein	495	519
FluorX	494	520
BODIPY-FL	503	512
TRITC	547	574
X-Rhodamine	570	576
Lissamine Rhodamine B	570	590
PerCP	490	675
Texas Red	589	615
Allophycocyanin (APC)	650	660
TruRed	490, 675	695
Alexa Fluor 350	346	445
Alexa Fluor 430	430	545
Alexa Fluor 488	494	517
Alexa Fluor 532	530	555
Alexa Fluor 546	556	573
Alexa Fluor 555	556	573
Alexa Fluor 568	578	603
Alexa Fluor 594	590	617
Alexa Fluor 633	621	639
Alexa Fluor 647	650	688
Alexa Fluor 660	663	690
Alexa Fluor 680	679	702
Alexa Fluor 700	696	719
Alexa Fluor 750	752	779
Cy2	489	506
Cy3	(512); 550	570; (615)
Cy3,5	581	596; (640)

Probe	Ex[1] (nm)	Em[2] (nm)
Cy5	(625); 650	670
Cy5,5	675	694
Cy7	743	767
Nucleic acid probes		
Hoeschst 33342	343	483
DAPI	345	455
Hoechst 33258	345	478
SYTOX Blue	431	480
Chromomycin A3	445	575
Mithramycin	445	575
YOYO-1	491	509
SYTOX Green	504	523
SYTOX Orange	547	570
Ethidium Bormide	493	620
7-AAD	546	647
Acridine Orange	503	530/640
TOTO-1, TO-PRO-1	509	533
Thiazole Orange	510	530
Propidium Iodide (PI)	536	617
TOTO-3, TO-PRO-3	642	661
LDS 751	543; *590*	712; *607*
Fluorescent Proteins		
Y66F	360	508
Y66H	360	442
EBFP	380	440
Wild-type	396, 475	50, 503
GFPuv	385	508
ECFP	434	477
Y66W	436	485
S65A	471	504
S65C	479	507
S65L	484	510
S65T	488	511
EGFP	489	508
EYFP	514	527
DsRed	558	583
Other probes		
Monochlorobimane	380	461
Calcein	496	517

1 Ex: Peak excitation wavelength (nm)

2 Em: Peak emission wavelength (nm)

TABLE 2

Exemplary optical parameters which may be measured by a flow cytometer.

Parameter	Acronym	Detection angle form incident laser beam	Wavelength (nm)
Forward scattered light	FS	2-5°	488*
Side scattered light	SS	90°	488*
"Green" fluorescence	FL1	90°	510-540†
"Yellow" fluorescence	FL2	90°	560-580†
"Red" fluorescence	FL3	90°	>650#

* using a 488 nm excitation laser
† width of bandpass filter
\# longpass filter

TABLE 3

Optimisation of small vessel for blood stimulation (6.6mm internal diameter)

Blood Volume (mL)	Subject 1			Subject 2			Subject 3			Subject 4		
	Nil	TT	Mit	Nil	TT	Mit	Nil	TT	Mit	Nil	TT	Mit
0.1	0.11#	1.31#	2.44#	0.37#	4.2#	42.57*#	0.21#	0.17#	13.93#	0.08#	0.99#	40.39*#
0.2	0.16	4.88	3.82	0.06	24.03	42.57*	0.14	1.14	17.02	0.07	6.1	40.39*
0.3	0.16	10.13	2.72	0.07	42.57*	42.57*	0.15	1.72	8.74	0.08	14.37	40.39*
0.4	0.15	10.45	3.29	0.05	42.57*	42.57*	0.12	4.01	8.82	0.08	12.11	40.39*
0.5	0.19	3.29	3.11	0.18	42.57*	42.57*	0.11	3.15	12.21	0.08	6.36	40.39*
Control	0.32	11.51	4.91	1.51	42.57*	42.57*	0.24	4.37	19.93	0.09	9.01	40.39*

Nil = PBS alone, TT = Tetanus Toxoid, Mit = Mitogen (PHA-P)

* = Off-scale result

= Only 25μL of plasma was available for testing (corrected result 2X shown)

TABLE 4

Subject	Antigen	PCR Tube (50μl culture, 20μl in EIA)	MiniCollect (50μl culture, 20μl in EIA)	96 well plate (50μl culture, 20μl in EIA)	48 well plate (50μl culture, 20μl in EIA)	Control (1mL culture, 50μl in EIA)	Control (1mL culture, 20μl in EIA)
		Conical to 10mm height with maximum diameter of 5mm, u-shaped base	Cylindrical with 6.5mm diameter, u-shaped base	Cylindrical with 6.5mm diameter, flat base	Cylindrical with 11mm diameter, flat base	Cylindrical with 10.5mm diameter, flat base (gel)	
		Blood Height = 6mm	Blood Height = 3mm	Blood Height = 1.5mm	Blood Height = 0.5mm	Blood height = 11.5mm	
1	Nil	0.13	0.03	0.87	0.06	0.02	0.02
	TT	5.82	4.26	0.39	0.39	12.16	12.16
	CMV	3.72	1.99	1.07	0.97	6.24	3.92
2	Nil	0.07	0.02	0.05	0.04	0.02	0.02
	TT	0.34	0.96	0.15	0.27	1.30	0.81
	CMV	0.05	0.02	0.05	0.05	0.02	0.02
3	Nil	0.09	0.02	0.05	0.05	0.03	0.02
	TT	12.16	12.16	12.16	12.16	12.16	12.16
	CMV	7.38	1.31	0.69	0.56	12.16	7.83
4	Nil	0.14	0.05	0.10	0.07	0.05	0.04
	TT	0.86	0.44	0.37	0.21	5.31	3.26
	CMV	0.07	0.05	0.12	0.06	0.05	0.04
5	Nil	0.08	0.07	5.50	0.11	0.03	0.04
	TT	2.34	0.58	0.18	0.15	7.87	5.00
	CMV	0.22	0.10	0.11	0.12	0.12	0.11

Comparison is with 1mL using 20μl of plasma.

IFN-gamma in IU/mL in response to Nil, CMV Antigen or Tetanus Toxoid results shown.

TABLE 5

Capillary Blood

Subject	IFN-gamma (IU/mL)		
	Nil	**CMV**	**Tetanus**
TR	0.22	27.07	-
JH	0.12	-	0.4

TABLE 6

Varied Volume of Culture in PCR Tubes

IFN-gamma in IU/mL in response to Nil, CMV Antigen or Tetanus Toxoid results shown

Subject	Antigen	1mL control Cylindrical 11.5mm blood height	300µl Conical* 18mm blood height	50µl Conical* 6mm blood height
1	N	0.10	0.11	0.17
	TT	5.51	3.99	4.66
	CMV	2.68	2.98	1.84
2	N	0.11	0.12	0.06
	TT	0.35	0.49	0.70
	CMV	0.11	0.10	0.12
3	N	0.07	0.10	0.08
	TT	13.81	13.07	9.28
	CMV	3.00	3.93	1.79
4	N	0.05	0.10	0.08
	TT	1.09	1.16	0.60
	CMV	0.08	0.10	0.10
5	N	0.04	0.04	0.06
	TT	0.54	0.21	0.14
	CMV	0.07	0.06	0.07

20µl plasma assayed in each case

Shows efficacy down to 6mm height and 50µl

* Tubes taper up to 10mm height and then are cylindrical to 20mm height. Diameter of tube at cyclindrical section is 5mm.

TABLE 7

Varied Low Volume Culture

IFN-gamma in IU/mL in response to Nil, CMV Antigen or Tetanus Toxoid results shown

Subject	Antigen	PCR Tubes (conical to 10mm height with maximum diameter of 5mm)				
		50µl culture (25µl Plasma in EIA)	40µl culture (20µl Plasma in EIA)	30µl culture (15µl Plasma in EIA)	20µl culture (10µl Plasma in EIA)	10µl culture(5 µl Plasma in EIA)
		Blood height = 6mm	Blood height = 5.5mm	Blood height = 5mm	Blood height = 4mm	Blood height = 2.5mm
1	N	0.14	0.07	0.04	0.05	0.05
	TT	6.05	5.96	3.62	1.98	0.08
	CMV	3.37	3.59	1.79	1.22	1.11
2	N	0.04	0.05	0.12	0.05	0.13
	TT	1.82	0.25	0.30	0.23	0.13
	CMV	0.04	0.05	0.06	0.05	0.07
3	N	0.05	0.05	0.06	0.02	2.52
	TT	14.28	14.28	9.97	2.04	0.41
	CMV	2.39	2.75	0.39	0.74	0.26
4	N	0.09	0.07	0.07	0.06	0.06
	TT	0.25	0.47	0.36	0.08	0.09
	CMV	0.07	0.07	0.08	0.07	0.10
5	N	0.06	0.04	0.06	0.03	0.05
	TT	0.49	0.48	0.16	0.09	0.04
	CMV	0.04	0.06	0.04	0.02	0.04

TABLE 7 continued

Subject	Antigen	1mL Control (cylindrical with 10.5mm diameter)							
		1mL culture (50μl Plasma in EIA)	1mL culture (40μl Plasma in EIA)	1mL culture (30μl Plasma in EIA)	1mL culture (25μl Plasma in EIA)	1mL culture (20μl Plasma in EIA)	1mL culture (15μl Plasma in EIA)	1mL culture (10μl Plasma in EIA)	1mL culture (5μl Plasma in EIA)
		Blood height = 11.5mm							
1	N	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03
	TT	13.48	13.48	13.48	13.48	13.48	8.05	6.04	3.40
	CMV	8.04	7.40	6.59	5.72	4.64	4.19	2.82	1.99
2	N	0.02	0.02	0.02	0.02	0.03	0.02	0.03	0.04
	TT	0.95	0.80	0.67	0.58	0.52	0.45	0.35	0.33
	CMV	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.04
3	N	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03
	TT	14.28	14.28	14.28	14.28	14.28	14.28	14.28	14.28
	CMV	9.16	6.97	6.36	6	5.3	4.84	3.77	2.13
4	N	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04
	TT	3.98	3.49	3.02	2.43	2.07	1.69	1.17	0.08
	CMV	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.04
5	N	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.02
	TT	0.98	0.9	0.79	0.69	0.59	0.47	0.37	0.22
	CMV	0.08	0.08	0.07	0.07	0.07	0.06	0.05	0.04

TABLE 8

Varied Volume of Culture in Minicollect (R)Tubes (Greiner Bio-One)

Blood height		4mm	8mm	12mm	16mm	20mm	
Subject	Antigen	100µl (25µl assayed)	200µl	300µl	400µl	500µl	1mL control (50µl assayed)
1	N	0.11	0.16	0.16	0.15	0.19	0.32
	TT	1.31	4.88	10.13	10.45	3.29	11.51
2	N	0.37	0.06	0.07	0.05	0.18	1.51
	TT	4.20	24.03	42.57*	42.57	42.57	42.57
3	N	0.21	0.14	0.15	0.12	0.11	0.24
	TT	0.17	1.14	1.72	4.01	3.15	4.37
4	N	0.08	0.07	0.08	0.08	0.11	0.05
	TT	0.99	6.10	14.37	12.11	6.36	1.09

* indicates maximum for that run, real value is >

Indicates maximum values obtained with protein antigen(excluding control)

N= Nil, TT = tetanus toxoid added

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Claims:

1. A method for measuring a cell-mediated immune (CMI) response in a sample of whole blood collected from a subject wherein said whole blood sample comprises cells of the immune system which are capable of producing immune effector molecules following stimulation by an antigen, the method comprising:
   i) incubating a whole blood sample from a peripheral capillary or less than 0.5mL whole blood from an artery or vein of a subject with an antigen in an incubation container substantially without dilution of the sample; and
   ii) detecting or measuring the presence or elevation in the level of an immune effector molecule or of a nucleic acid molecule capable of producing an effector molecule indicative of the capacity of the subject to mount a cell-mediated response.

2. The method of claim 1, comprising:
   i) collecting a whole blood sample from a peripheral capillary or less than 0.5mL whole blood from an artery or vein of a subject into a container.

3. The method of claim 1 or 2 for use in selecting a suitable therapeutic protocol for the treatment of a subject having symptoms of an inflammatory disease condition, a pathogenic infection, an autoimmune disorder, immuno-incompetence, allergy or cancer or a propensity for developing such a disorder.

4. The method of claim 1 or 2 wherein the method comprises incubating and/or collecting a capillary blood sample.

5. The method of claim 2 wherein the method comprises collecting a sample from the subject with a capillary sampling device.

6. The method of claim 2 or 3 or 4 wherein whole blood from a subject is collected into a container containing antigen and/or anti-coagulant or wherein antigen and/or

anti-coagulant are added to the blood thereafter.

7. The method of claim 6 wherein the anti-coagulant is heparin.

8. The method of claim 1 or 2 wherein the incubation step is conducted in the presence of a simple sugar, such as dextrose.

9. The method of any one of claims 1 to 8 where the subject is human.

10. The method of any one of claims 1 to 8 wherein the subject is an animal or bird.

11. The method of claim 9 wherein the human is a pediatric, adult or geriatric subject.

12. The method of any one of claims 1 to 11 wherein the whole blood sample is incubated with the antigen for from about 4 hours to about 50 hours.

13. The method of claim 1 or 2 wherein the immune effector molecule in part ii) is a cytokine, component of the complement system, perforin, defensin, cathelicidin, granzyme, Fas ligand, CD-40 ligand, exotaxin, cytotoxin, chemokine or monokine.

14. The method of claim 13 wherein the cytokine is IFN-γ, TNFα or GM-CSF.

15. The method of claim 1 or 2 wherein the immune cells are selected from a natural killer (NK) cell, T-cell, B-cell, macrophage or monocyte.

16. The method of claim 15 wherein the cells are T-cells.

17. The method of claim 1 or 2 wherein the antigen is selected from a self-antigen, an antigen from a pathogenic organism, a metal or inorganic antigen, or a tumour antigen or an analog thereof.

18. The method of claim 17 wherein the pathogenic organism is a bacterial, viral, fungal or parasitic organism.

19. The method of claim 17 wherein the antigen is from M*ycobacterium.*

20. The method of claim 19 wherein the antigen is a mycobacterial protein selected from ESAT-6, CFP-10 and TB7.

21. The method of claim 17 wherein the antigen is tetanus toxoid (TT) or purified protein derivative (PPD) from *M. tuberculosis* or *M. avium.*

22. The method of claim 1 or 2 wherein the sample in the container has a height of at least about 4mm to about 6mm to a maximum height of about 12mm to about 20mm.

23. The method of claim 22 wherein the sample in the container has a height of at least about 6mm to a maximum of about 12mm.

24. The method of claim 1 wherein the sample has a height of 4mm, 5mm, 6mm, 7mm, 8mm, 9mm, 10mm, 11mm, 12mm, 13mm, 14mm, 15mm, 16mm, 17mm, 18mm, 19mm or 20mm or an intervening height.

25. The method of claim 23 wherein the sample has a height of 6mm, 7mm, 8mm, 9mm, 10mm, 11mm or 12mm or an intermediate height.

26. The method of any one of claim 1 to 25 wherein the total sample volume incubated is less than 500μl, less than 400μl, less than 300μl, less than 200μl, less than 100μl, or less than 50μl.

27. The method of any one of claim 1 to 25 wherein the sample is capillary blood and the total volume incubated is about 2000μl, 1500μl, 1400μl, 1300μl, 1200μl,

1100μl, 900μl, 800μl, 700μl, 600μl, 500μl, 400μl, 300μl, 200μl, 100μl, 50μl or 40μl or an intermediate volume.

28. The method of any one of claims 1 to 27 wherein the sample is a capillary blood sample.

29. The method of claim 2 wherein the sample is collected into an about 3-4mm diameter capillary tube.

30. The method of any one of claim 1 to 29 wherein the incubation container is suitable for maintaining an optimal shape of the sample and wherein the shape has one or two or more dimensions selected from: (i) a maximum circular diameter of less than about 6mm; (ii) a height of at least about 4mm to 6mm to a maximum height of about 12mm to 20mm; or (iii) a volume of less than 0.5mL and optionally less than 400 μl.

31. A kit for measuring a cell-mediated immune (CMI) response in a sample of whole blood collected from a subject wherein said whole blood sample comprises cells of the immune system which produce immune effector molecules following stimulation by an antigen, the kit comprising in multicomponent form: (i) one or more collection and/or incubation containers suitable for holding or incubating a peripheral capillary whole blood sample or less than 0.5mL of whole venous or arterial blood; (ii) one or more test antigens for analysis of *in vitro* responses thereto and optionally a control antigen; (iii) reagents for measuring the presence or elevation in the level of an immune effector molecule; and (iv) optionally a set of instructions comprising any of the herein disclosed methods.

32. The kit of claim 31 wherein the incubation container is suitable for forming an optimal shape of the sample wherein the shape has one or two or more dimensions selected from: (i) a maximum circular diameter of less than about 6mm; (ii) a height of at least about 4mm to 6mm to a maximum height of about 12mm to

20mm; or (iii) a volume of less than 0.5mL and optionally less than 400 µl.

33. The kit of claim 31 wherein the instructions comprise instructions: (i) to collect whole blood and mix blood in collection/incubation container; (ii) to incubate the whole blood sample with an antigen and optionally with a control antigen or mitogen; (iii) to centrifuge the incubation container and collect plasma; and (iv) to detect an immune effector molecule in plasma.

34. The kit of claim 31, 32 or 33 further comprising a capillary sampling device.

35. The kit of claim 31, 32 or 33 wherein the subject is human.

36. The kit of claim 31, 32 or 33 wherein the subject is an animal or bird.

37. The kit of claim 36 wherein the human is a pediatric, adult or geriatric subject.

38. The kit of claim 31 further comprising an incubator wherein the whole blood sample is incubated with the antigen for from about 4 hours to about 50 hours.

39. The kit of claim 31 wherein the immune effector molecule in part (iii) is a cytokine, component of the complement system, perforin, defensin, cathelicidin, granzyme, Fas ligand, CD-40 ligand, exotaxin, cytotoxin, chemokine or monokine.

40. The kit of claim 39 wherein the cytokine is IFN-γ, TNFα or GM-CSF.

41. The kit of claim 31 wherein the immune cells are selected from a natural killer (NK) cell, T-cell, B-cell, macrophage or monocyte.

42. The kit of claim 41 wherein the cells are T-cells.

43. The kit of claim 31 wherein the antigen is selected from a self-antigen, an antigen

from a pathogenic organism, a metal or inorganic antigen, or a tumour antigen or an analog thereof.

44. The kit of claim 43 wherein the antigen is from *Mycobacterium.*

45. The kit of claim 43 wherein the antigen is a mycobacterial protein selected from ESAT-6, CFP-10 and TB7.

46. The kit of claim 43 wherein the antigen is tetanus toxoid (TT) or purified protein derivative (PPD) from *M. tuberculosis* or *M. avium.*

47. The kit of claim 31 or 32 wherein the incubation container forms a sample height of at least about 4mm to 6mm to a maximum height of about 12mm to 20mm.

48. The kit of claim 47 wherein the incubation container forms a sample height of at least about 6mm to a maximum of about 12mm.

49. The kit of claim 31 or 32 wherein the incubation container forms a sample height of 4mm, 5mm, 6mm, 7mm, 8mm, 9mm, 10mm, 11mm, 12mm, 13mm, 14mm, 15mm, 16mm, 17mm, 18mm, 19mm or 20mm or an intervening height.

50. The kit of claim 31 or 32 wherein the incubation container forms a sample height of 6mm, 7mm, 8mm, 9mm, 10mm, 11mm or 12mm or an intermediate height.

51. The kit of claim 32 wherein the incubated sample has a volume less than 500μl, less than 400μl, less than 300μl, less than 200μl, less than 100μl, or less than 50μl.

52. The kit of claim 32 wherein the sample is capillary blood and the incubated sample has a volume of about 2000μl, 1500μl, 1400μl, 1300μl, 1200μl, 1100μl, 900μl, 800μl, 700μl, 600μl, 500μl, 400μl, 300μl, 200μl, 100μl, 50μl or 40μl or an intermediate volume.

53. The kit of any one of claims 31 to 52 wherein the sample is a capillary blood sample.

54. The kit of claim 31 wherein the reagents comprise an antibody conjugate for detecting IFN-γ.

55. The kit of any one of claim 31 to 54 comprising an about 3-4mm diameter capillary tube.

****

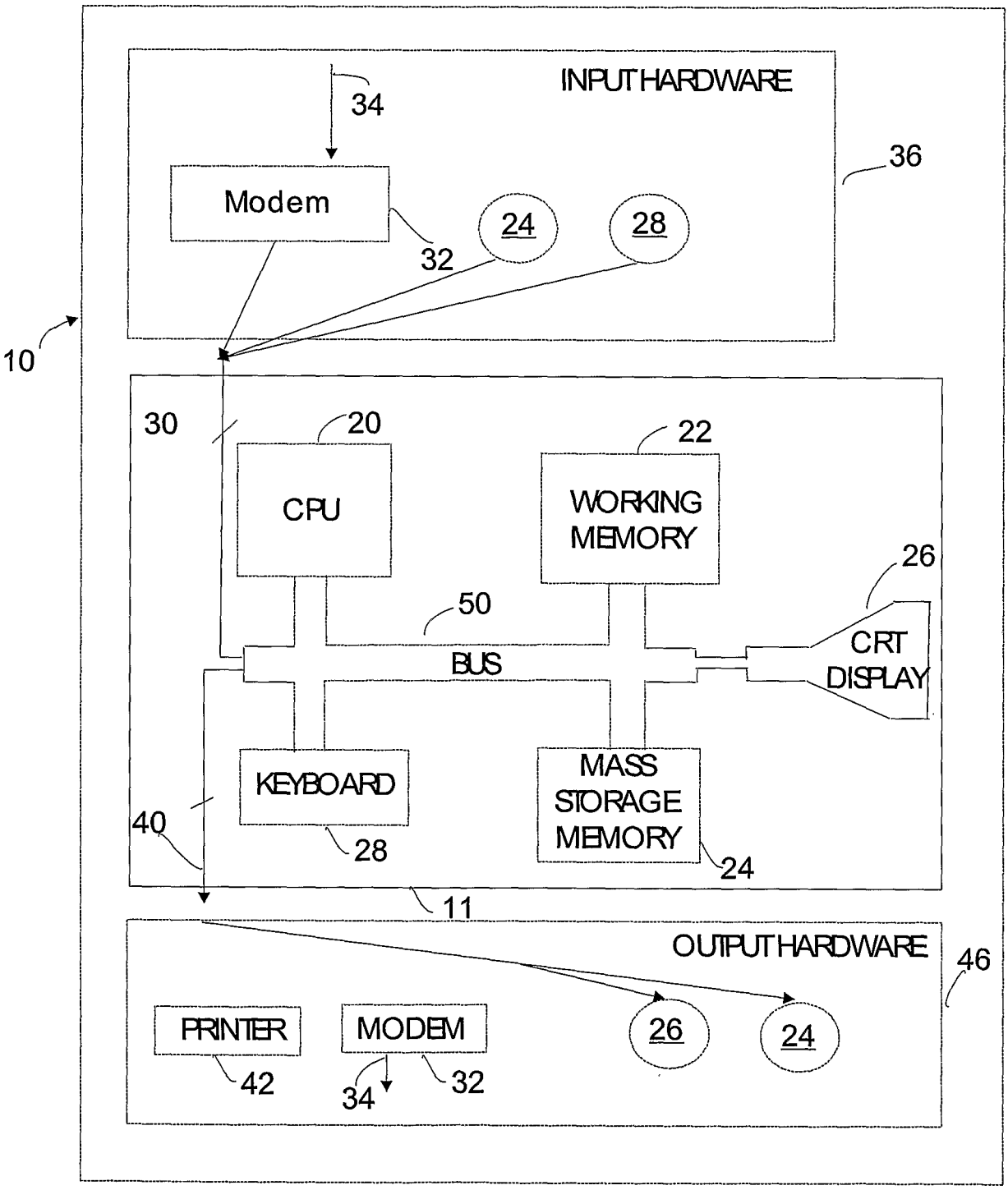
INPUT HARDWARE
34
36
Modem
32
24
28
10
30
20
CPU
22
WORKING MEMORY
26
50
BUS
CRT DISPLAY
KEYBOARD
28
40
MASS STORAGE MEMORY
24
11
OUTPUT HARDWARE
46
PRINTER
42
MODEM
34
32
26
24

Figure 1

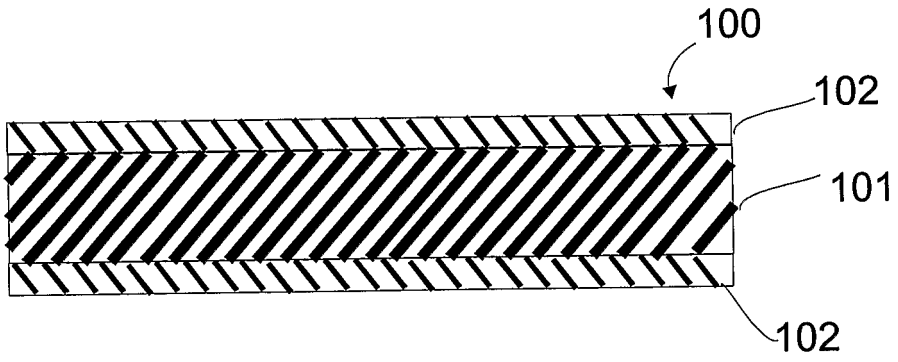

Figure 2

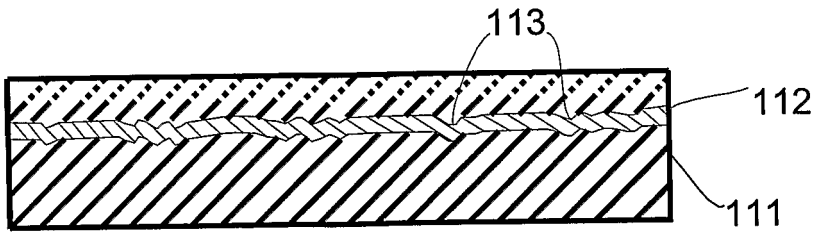

Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000377

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

G01N 33/53 (2006.01) ***G01N 33/49*** (2006.01) ***G01N 33/50*** (2006.01) ***C12Q 1/68*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FILES

WPI, MEDLINE, EPODOC, CA PLUS and Keywords used: (Cellular w immunity) or (Cell+s mediat) or (Cell+ p immune+), Assay or Immunoassay or Diagnos or method or detect, Antigen or tt or tetanus toxoid or esat-6 or cfp-10 or TB7 or CMV or cytomegalovirus or phytohaemagglutinin or PPD or Purified Protein Derivative or mycobacterium, Interferon gamma or IFN or CD40 ligand or CD40 L or Fas ligand or Fas L or exotaxin or cytotoxin or chemokine or monokine or perforin or defensin or cathelicidin or granzyme or GM CSF or TNF alpha, Whole blood, ml or mm or microliter

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Benyoucef S et al. An Interferon-γ (IFN-γ) based whole blood assay to detect T cell response to antigens in HIV-1 infected patients. *Pathologie Biologie*, 1997, Vol. 45, No. 5, Pages 400-403. (Page 400, Summary, lines 8-12) (Page 400, Summary, lines 16-18)	1-4, 6-7, 9-18, 26, 30-32, 35, 37-44, 51, 54 19-21, 33, 36, 45-46
Y	(Page 400, Summary, lines 8-12) (Page 400, Summary, lines 16-18)	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
30 April 2008

Date of mailing of the international search report
02 JUN 2008

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000377

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,494,799 (Wood Paul R. and Corner Leigh A) February 27 1996 (Abstract) (Column 1, lines 44-46) (Column 1, lines 39-43) (Column 4, lines 3-4) (Column 2, lines 30-49) (Column 2, lines 53-56)	19, 33, 36
Y	Brock I et al. Performance of whole blood IFN-γ test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. *The International Journal of Tuberculosis and Lung Disease*, 2001, Vol. 5, No. 5, Pages 462-467. (Page 462, Column 1, lines 18 – Column 2, line 3) (Abstract) (Page 462, Column 1, lines 38-42)	20, 45
Y	Streeton J.A et al. Sensitivity and Specificity of a gamma interferon blood test for tuberculosis infection. *The International Journal of Tuberculosis and Lung Disease*, 1998, Vol. 2, No. 6, Pages 443-450. (Abstract) (Page 443, Column 2, lines 9-10, 17)	21, 46
Y	Baird M.A et al. Dendritic cell presentation of PPD and 19 kDa protein of *Mycobacterium tuberculosis* and emergent T helper cell phenotype. *Immunology and Cell Biology*, 1995, Vol. 73, Pages 537-543. (Abstract) (Page 538, Column 2, lines 40-41)	21, 46
Y	Van Crevel R et al. Disease-specific ex vivo stimulation of whole blood for cytokine production: applications in the study of tuberculosis. *Journal of Immunological Methods*, 1999, Vol. 222, Pages 145-153. (Abstract) (Page 147, Column 1, line 17)	21, 46
Y	Katial R.K et al. Cell-Mediated Immune Response to Tuberculosis Antigens: Comparison of Skin Testing and Measurement of In Vitro Gamma Interferon Production in Whole-Blood Culture. *Clinical and Diagnostic Laboratory Immunology*, 2001, Vol. 8, No. 2, Pages 339-345. (Abstract) (Page 340, lines 24-26) (Page 340, lines 31-32)	21, 46
Y	Antas P.R.Z et al. Whole Blood Assay to Access T Cell-immune Responses to *Mycobacterium tuberculosis* Antigens in Healthy Brazilian Individuals. *Memorias Instituto Oswaldo Cruz*, 2004, Vol. 99, No. 1, Pages 53-55 (Abstract (Page 53, Column 2, lines 6-7)	21, 46
Y	Kampmann B et al. Evaluation of Human Antimycobacterial Immunity Using Recombinant Reporter Mycobacteria. *The Journal of Infectious Diseases*, 2000, Vol. 182, Pages 895-901. (Abstract) (Page 896, Column 2, lines 6-12) (Page 896, Column 2, lines 37-38)	21, 46

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000377

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Whist S.K et al. The use of interlukin-2 receptor expression as a marker of cell-mediated immunity in goats experimentally infected with *Mycobacterium avium ssp. paratuberculosis*. *Veterinary Immunology and Immunopathology*, 2000, Vol. 73, Pages 207-218. (Title) (Page 209, lines 3-4) (Abstract) (Page 209, line 20)	21, 46

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2008/000377

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	5494799	AU	71659/87	EP	0296158	US	5334504
		WO	8705400				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

专利名称(译)	细胞介导的免疫应答测定法及其试剂盒		
公开(公告)号	EP2132569A1	公开(公告)日	2009-12-16
申请号	EP2008714423	申请日	2008-03-14
[标]申请(专利权)人(译)	塞尔雷斯蒂斯有限公司		
申请(专利权)人(译)	CELLESTIS有限公司		
当前申请(专利权)人(译)	CELLESTIS有限公司		
[标]发明人	RADFORD ANTHONY J JONES STEPHEN L HOWARD JENNY L		
发明人	RADFORD, ANTHONY, J. JONES, STEPHEN, L. HOWARD, JENNY, L.		
IPC分类号	G01N33/53 G01N33/49 G01N33/50 C12Q1/68 G01N33/569 G01N33/68		
CPC分类号	G01N33/56972 G01N33/6863 G01N2333/35 G01N2333/57		
优先权	2007901385 2007-03-16 AU		
其他公开文献	EP2132569A4 EP2132569B1		
外部链接	Espacenet		

摘要(译)

本发明提供了用于测量从受试者收集的少量全部未稀释血液中的细胞介导免疫（CMI）的方法和试剂盒。特别地，该方法用于测量体积为例如50μl至500μl的未稀释全血样品中的反应。因此，促进了包括儿科，成人或老年人受试者的受试者的毛细血管取样和快速测试。