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(54) **AN ASSAY FOR MEASURING CELL-MEDIATED IMMUNORESPONSIVENESS**

TEST ZUR MESSUNG VON ZELLVERMITTELTER IMMUNANSPRECHBARKEIT

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**Description****FIELD**

5 **[0001]** The present invention relates to a method for measuring cell-mediated immune response activity in a subject and use of one or more agents which potentiate the adaptive system and one or more agents which potentiate the innate immune system in the manufacture of a diagnostic assay of cell-mediated immune responsiveness. The present invention enables determination of the immunosuppressive effects of disease conditions, therapeutic agents and environmental contaminants. The assay is also capable of integration into pathology architecture to provide a diagnostic reporting system and to facilitate point of care clinical management.

**BACKGROUND**

15 **[0002]** Immunological-based diagnostics provides an important tool in detecting a variety of disease conditions. This is especially the case given the specificity of components within the immune system. Notwithstanding this specificity, immunological-based diagnostics are not necessarily always sensitive enough to detect low levels of adaptive and/or innate immune activity, such as in response to a low grade infection or in the presence of a persistent low level infection or in subjects exhibiting immunodeficiency or any form of immunosuppression. There is a need to develop diagnostic assays with enhanced sensitivity in relation to cell-mediated immunoresponsiveness due to adaptive and innate immune potential. This is particularly the case in subjects exposed to or exhibiting disease conditions and agents which induce or are associated with immunosuppression.

20 **[0003]** One form of immunological-based diagnostic assays involves the stimulation of T-cells with antigens or mitogens in either isolated cell culture or whole blood culture followed by the detection of effector molecules such as cytokines produced by the activated T-cells (also referred to as effector T-cells). The effector molecules are generally detected using techniques such as enzyme immunoassays, multiplex bead analysis, ELISpot and flow cytometry. Such assays are useful for detecting disease-specific T-cell responses.

25 **[0004]** The ability to assess cell-mediated immune responsiveness of a subject is particularly important in managing immunodeficiency and immunosuppression. Immunodeficiency is characterized by a reduced ability to effectively mount an immune response. This compromised or absent response can result from a primary or acquired (secondary) immunodeficiency.

30 **[0005]** Primary immunodeficiencies (PIDs) are genetically inherited and characterized by deficiencies of distinct components of the adaptive or innate immune system (Hu and Gatti, Curr Opin Allergy Clin Immunol. 8(6):540-546, 2008). Nonetheless, most immunodeficiencies are acquired (secondary) and can be induced by a pathogenic agent, as occurs with HIV infection; induced by drugs, as in immunosuppressive treatment following organ transplantation; induced by disease conditions, as can occur in cancer (e.g. leukemia, lymphoma); or induced by environmental contaminants and pollutants.

35 **[0006]** The molecular basis of immunodeficiency is diverse, however, cell-mediated immunity plays a key role in mediating many of the observed clinical manifestations. At present, immunodeficiency syndromes are diagnosed and managed in an *ad hoc* manner depending on the causal agent.

40 **[0007]** For example, the primary hallmark of HIV infection is progressive loss of CD4<sup>+</sup> T-cells and impaired global immune function including an impaired antigen-specific adaptive immune response to pathogens such as cytomegalovirus (CMV) and Hepatitis B virus (HBV) [Douek et al, Annu. Rev. Med. 60:471-84, 2009 and Chang et al, J. Virol. 83:7649-58, 2009]. Hence, CD4<sup>+</sup> T-cell count and viral load remain the key surrogate markers of HIV infection and have been extensively validated as predictive of both immune dysfunction and with progressive infection and immune reconstitution following prophylaxis (Hengel and Kovacs, J. Infect. Dis. 188(12):1791-3, 2003). However, many instances exist in which clinical disease does not correlate with the level of immune function as predicted by these surrogate markers particularly when opportunistic infections develop in the presence of normal CD4<sup>+</sup> T-cell counts and low viral loads (Solomon et al, J. Infect. Dis. 187:1915-23, 2003).

45 **[0008]** Similarly, monitoring the cellular immunodeficiency status of patients that have undergone solid organ transplants (SOTs) and are receiving medications to suppress their immune system (immunosuppressants) as an anti-rejection measure, are also managed in an *ad hoc* manner. Typically, patient immunosuppressant drug levels are monitored as are regular blood counts and incidence of graft, nosocomial or community-acquired infections (Schrem et al, Dtsch Arztebl Int. 106(9):148-156, 2009).

50 **[0009]** The inherent problem associated with many of the current markers used to monitor and define immunodeficiency associated with disease states is the absence of cell-mediated immunity. A number of T-cell function tests have been developed that measure lymphoproliferative responses to mitogens such as phytohemagglutinin (PHA), pokeweed mitogen and concanavalinA (ConA). However, these only measure the functional ability of T-cells; a subset of cells involved in cell-mediated immunity. Importantly, it has become increasingly evident that innate immune mechanisms

contribute greatly to host defence, either through acting alone or by enhancing specific T-cell responses (Cooper et al, Hematology:314-30, 2003). Therefore, the functional responses of innate (natural killer [NK] cell) and adaptive (T-cell) immune cells together form a more comprehensive analysis of cell-mediated immunity.

**[0010]** The ability to assess cell-mediated immunity is of clinical importance. For example, in excess of 33 million adults and children are infected with HIV (2008 Report on the Global AIDS Epidemic, UNAIDS; ISBN 978 92 9 173711 6) and currently around 100,000 solid organ transplantations are performed per year worldwide (Matesanz et al, Transplant Proc. 41(6):2297-301, 2009).

**[0011]** Innate immune dysregulation has been implicated in HIV infection with a whole blood IFN- $\gamma$  assay described for the monitoring of innate immune responsiveness to the TLR-7/8 agonist compound (a imidazoquinoline compound, R848) [Nowroozalizadeh et al, Cytokine 46:325-31, 2009]. Furthermore, a diagnostic device that measures functional T-cell responses (Cylex ImmuKnow (Registered Trade Mark), as described in US 2003/0199006 A1) measures ATP production from PHA-stimulated CD4<sup>+</sup> cells (Kowalski et al, J Immunotoxicol. 4(3):225-32, 2007). Specifically, the Cylex assay has demonstrated clinical utility in the SOT setting such that adverse events including infection and rejection can be adequately managed.

**[0012]** Deetz CO et al. (Infection and Immunity, Aug. 2006, pp- 4505-4511) report on gamma-interferon secretion by human V $\gamma$ 2V $\delta$ 2 T cells after stimulation with antibody against the T-cell receptor plus the toll-like receptor 2 agonist Pam<sub>3</sub>Cys.

**[0013]** Llewellyn-Smith N et al. (Neurology 1997; 48; pp. 810-816) report on the effects of anti-CD3 antibody treatment on lymphocyte subsets and stimulated tumor necrosis factor alpha production.

**[0014]** Caron G et al. (The Journal of Immunology, 2005, 175: 1551-1557) report on direct stimulation of human T cells via TLR5 and TLR7/8, whereby flagellin and R-484 up-regulate proliferation and IFN- $\gamma$  production by memory CD4<sup>+</sup> T cells.

**[0015]** International patent application WO 2008/113119 A1 is related to methods and kits for measuring a cell-mediated immune response in an undiluted whole blood sample.

**[0016]** International patent application WO 2010/009494 A1 which is a document under Art. 54(3) EPC 2000, is related to a method for measuring cell-mediated immune response activity.

**[0017]** There is a need to develop an assay which can assess the combined functional status of innate and adaptive cell-mediated immunoresponsiveness in a subject and to determine disease conditions and agents which induce immunosuppression.

## SUMMARY

**[0018]** The problem underlying the present invention is solved by the subject matter of the attached independent claims; preferred embodiments may be taken from the attached dependent claims.

**[0019]** More specifically, the problem underlying the present invention is solved in a first aspect by a method for measuring cell-mediated immune response activity in a subject, said method comprising contacting a sample source of lymphocytes from the subject with one or more agents which potentiate the adaptive immune system and one or more agents which potentiate the innate immune system, wherein the one or more agents potentiating the adaptive immune system are selected from the group consisting of anti-CD3 antibodies, antibodies to the T-cell receptor complex, CpG oligonucleotides, staphylococcal enterotoxin B (SEB), phytohemagglutinin (PHA), phorbol myristate acetate (PMA), concanavalin A (ConA) and pokeweed mitogen and wherein the one or more agents potentiating the innate immune system are selected from the group consisting of a TLR-3 ligand, a TLR-9 ligand and lipomannan, and measuring the presence or elevation in the level of an immune effector molecule from immune cells, wherein the presence or level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject.

**[0020]** More specifically, the problem underlying the present invention is solved in a second aspect by a method for measuring cell-mediated immune response activity in a subject, said method comprising contacting a sample source of lymphocytes from the subject with one or more agents which potentiate the adaptive immune system and one or more agents which potentiate the innate immune system and measuring the presence or elevation in the level of an immune effector molecule from immune cells, wherein the presence or level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject, wherein the one or more agent which potentiates the adaptive immune system stimulates *via* T-cell receptor-independent means and is selected from the group consisting of phorbol myristate acetate (PMA), concanavalin A (ConA) and pokeweed mitogen.

**[0021]** In an embodiment of the first and the second aspect, the subject is suffering from a disease or condition.

**[0022]** In an embodiment of the first and the second aspect, the level of the immune effector molecule is indicative of the extent of immunosuppression induced or associated with the disease condition

**[0023]** In an embodiment of the first and the second aspect, the sample is whole blood undiluted and wherein the sample is whole blood which comprises from about 10% to 100% by volume of the sample to be assayed, preferably the whole blood comprises from about 50% to 100% by volume of the sample to be assayed and more preferably the

whole blood comprises from about 80% to 100% by volume of the sample to be assayed.

**[0024]** In an embodiment of the first and the second aspect, the whole blood is further incubated with an antigen.

**[0025]** In an embodiment of the first and the second aspect, the agent which potentiates the adaptive immune system stimulates *via* T-cell receptor-independent means.

**[0026]** In an embodiment of the first and the second aspect, the immune effector molecule is a cytokine.

**[0027]** In an embodiment of the first and the second aspect, the immune effectors are detected with antibodies specific for same.

**[0028]** In an embodiment of the first and the second aspect, the subject has an infection by a pathogenic agent selected from *Mycobacterium* species, *Staphylococcus* species, *Streptococcus* species, *Borrelia* species, *Escherichia coli*, *Salmonella* species, *Clostridium* species, *Shigella* species, *Proteus* species, *Bacillus* species, Herpes virus, Hepatitis B or C virus and Human immune deficiency virus (HIV) or a disease resulting therefrom.

**[0029]** In an embodiment of the first and the second aspect, the subject has a disease condition selected from 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 inflammatory demyelinating, chronic inflammatory 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, polyglanular 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, vitiligo and inflammatory bowel disease.

**[0030]** In an embodiment of the first and the second aspect, the subject has a cancer selected from 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 tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous T-Cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, hematological 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, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-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, 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-tumors, 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 tumors, 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 and Wilms' tumor.

**[0031]** In an embodiment of the first and the second aspect, the TLR-9 ligand is CpG oligodeoxynucleotide.

**[0032]** In an embodiment of the first and the second aspect, the TLR-3 ligand is poly (I:C).

**[0033]** More specifically, the problem underlying the present invention is solved in a third aspect by the use of one or more agents which potentiate the adaptive system and one or more agents which potentiate the innate immune

system in the manufacture of a diagnostic assay of cell-mediated immune responsiveness by the method of incubating innate and adaptive stimulants with a source comprising T-cells and NK cells and detecting the presence or elevation in effector molecules, wherein the one or more agents potentiating the adaptive immune system are selected from the group consisting of anti-CD3 antibodies, antibodies to the T-cell receptor complex, CpG oligonucleotides, staphylococcal enterotoxin B (SEB), phytohemagglutinin (PHA), phorbol myristate acetate (PMA), concanavalin A (ConA) and pokeweed mitogen, wherein the diagnostic assay is for the diagnosis of

an infection by a pathogenic agent selected from *Mycobacterium* species, *Staphylococcus* species, *Streptococcus* species, *Borrelia* species, *Escherichia coli*, *Salmonella* species, *Clostridium* species, *Shigella* species, *Proteus* species, *Bacillus* species, Herpes virus, Hepatitis B or C virus and Human immune deficiency virus (HIV) or a disease resulting therefrom;

a disease condition selected from 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 inflammatory demyelinating, chronic inflammatory 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, polyglanular 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, vitiligo and inflammatory bowel disease; or

a cancer selected from 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 tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous T-Cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, hematological 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, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-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-tumors, 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 tumors, 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 and Wilms' tumor.

**[0034]** The present invention as defined in the claims thus provides a method for assessing the status and potential of cell-mediated immunity in a subject. The method of the invention as defined in the claims is predicated on the co-stimulation of adaptive and innate immune responses. This provides a predictive measure of cell-mediated immunity potential including the extent of any immunosuppression in a target subject. In an embodiment of the invention as defined in the claims, cells in whole blood or a fraction thereof are contacted with one or more agents which stimulate both the adaptive and innate immune responses in lymphocytes. The level of cell-mediated immune responsiveness is determined

by measuring the presence or level of an effector molecule produced by immune cells. In a particular embodiment of the invention as defined in the claims, the whole blood or a fraction thereof comprises T-cells and NK cells. The one or more agents stimulate or potentiate an adaptive immune response *via* T-cells and innate immunity *via* NK cells. The assay, therefore, measures the combined adaptive and innate cell-mediated immune potential.

**[0035]** The combined immune response provides a greater measure of cell-mediated immune reactivity compared to adaptive or innate immune reactivity measured singularly. Furthermore, the assay can be conducted using small blood volumes such as from 1 $\mu$ l to 1000 $\mu$ l and hence the assay is amenable for finger prick and capillary based sampling. Large volume assays such as from 1.5ml to 200ml including 5ml, 10ml and 20ml volumes are also contemplated herein.

**[0036]** The ability to assess combined adaptive and innate immunity is important, for example, to identify disease conditions and therapeutic and environmental agents which induce immunosuppression which could lead to a greater risk of secondary infections.

**[0037]** Accordingly the present invention as defined in the claims method for measuring cell-mediated immune response activity in a subject comprises contacting a sample source of lymphocytes from the subject with one or more agents which potentiates adaptive and innate immunity and measuring the level of an immune effector molecule produced by immune cells wherein the level of the immune effector molecule is indicative of the level of cell-mediated immunoresponsiveness of the subject.

**[0038]** According to the invention as defined in the claims, the level of the immune effector molecule is indicative of the level of cell-mediated immunoresponsiveness of the subject wherein the level of immunoresponsiveness is indicative of the presence or absence or level or stage of a disease or condition selected from the list comprising an infection by a pathogenic agent, an autoimmune disease, a cancer, an inflammatory condition, exposure to a toxic agent, exposure to a therapeutic agent and an immunodeficiency.

**[0039]** Also disclosed is an assay to detect the presence, absence, level or stage of a disease or condition in a subject, the method comprising contacting a sample source of lymphocytes from the subject with one or more agents which potentiate adaptive and innate immunity and measuring the elevation in the level of an immune effector molecule from immune cells wherein the level of the immune effector molecule is indicative of the disease or condition.

**[0040]** Also disclosed is a method for monitoring a response to a therapeutic protocol for a disease or condition in a subject, the method comprising contacting a source of lymphocytes from the subject with an agent used in the therapeutic protocol and measuring the presence or elevation in the level of an immune effector molecule from immune cells in the presence of one or more molecules which potentiate adaptive and innate immunity wherein the level of the immune effector molecule is indicative of the efficacy of the therapeutic protocol.

**[0041]** The instant disclosure also relates to a method for determining whether an agent induces immunosuppression in a subject, the method comprising contacting a source of lymphocytes from the subject after exposure to the agent with one or more potentiators which stimulate adaptive and innate immunity and measuring the presence and level of an effector molecule from the lymphocytes wherein the level of the effector molecule determines the level of immunosuppression induced by the agent.

**[0042]** The method of the present invention as defined in the claims may also be referred to as an "assay". The assay herein is useful *inter alia* in assessing the general immune responsiveness of a subject or for detecting the immunoresponsiveness to specific disease conditions such as autoimmune disease, Celiac's disease, cancer, infection by a pathogenic organism or agent, exposure to a toxic agent or medicament and immunodeficiency or immunosuppression conditions such as induced by a disease condition. The source of lymphocytes is conveniently whole blood but the present invention contemplates the use of any source of lymphocytes including fractionated samples comprising lymphocytes as well as samples having undergone cell isolation and/or depletion. Micro-samples of blood such as derived from pin pricks and capillary methods also form part of the present invention. The samples comprise at least T-cells (T-lymphocytes) and NK cells (NK-lymphocytes). Reference to "immune cells" includes T-cells.

**[0043]** Optionally, a simple sugar such as dextrose or glucose is added to the reaction mixture. Reference to "whole blood" includes whole blood without dilution as well as whole blood used in an assay at a volume of from about 10% to about 100% of the total sample assay volume (i.e. reaction mixture), including from about 50% to about 100% and 80% to about 100%. Assay volumes may range from 1 $\mu$ l to greater than 1000 $\mu$ l including from 1.5ml to 200ml such as 5ml to 20ml.

**[0044]** One type of agent may be used to potentiate adaptive and innate immunity or two or more types of agents may be employed together. An example includes co-stimulation with a TCR agonist and a TLR agonist. The lymphocytes may also be exposed to a particular antigen to measure the combined antigen-induced adaptive and innate immune response capability of a subject.

**[0045]** The subjects may be human or non-human animals. Hence, the present invention has human medical, veterinary and livestock applications. Humans represent a particularly useful subject in the practice of the present invention.

**[0046]** The instant disclosure is also related to a method for detecting whether a disease condition is inducing immunosuppression in a subject the method comprising contacting a source of lymphocytes from the subject with a disease condition with one or more agents which potentiate adaptive and innate immunity and measuring the presence or level

of an immune effector molecule from the lymphocytes wherein the level of the immune effector molecule is indicative of the extent of immunosuppression induced or associated with the disease condition.

[0047] Kits and skin tests are also disclosed.

[0048] In an embodiment of the invention as defined in the claims, the sample is whole blood which is collected in collection tubes containing the agents or to which the agents are added for incubation. Generally, blood is maintained in the presence of heparin. Heparin may be in the tube when blood is added or is subsequently added. The use of blood collection tubes is compatible with standard automated laboratory systems and these are amenable to analysis in large-scale and random access sampling. Blood collection tubes also minimize handling costs and reduce laboratory exposure to whole blood and plasma and, hence, reduce the risk of laboratory personnel from contracting a pathogenic agent such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV). Furthermore, use of the collection tubes to conduct the incubation renders the assay more sensitive than the previously used 24 well culture well plates.

[0049] The present invention as defined in the claims therefore provides an enhanced cell-mediated immune assay comprising in one embodiment the use of a collection tube, optionally a simple sugar such as dextrose and the incubation step with one or more agents which potentiate adaptive and innate immunity. The incubation step is generally from about 5 to about 50 hours such as 24 hours. An antigen may optionally also be added.

[0050] The immune effector molecules are generally a cytokine such as but not limited to IFN- $\gamma$ , an interleukin (e.g. IL-2, IL-4, IL-6, IL-10, IL-12 or IL-13), Transforming Growth Factor beta (TGF $\beta$ ) or a Granulocyte or Granulocyte Macrophage Colony Stimulating Factor (G-CSF and GM-CSF, respectively). Other effector molecules are listed in Table 6. The presence or level of the immune effector molecule may be determined at the level of the molecule itself or to the extent to which a gene is expressed encoding the molecule.

[0051] The assay of the present invention as defined in the claims may also be used in a personalized medicine approach in the management of therapeutic protocols and/or as part of a pathology architecture platform. Such a platform may be web-based or non-web-based. A business method is therefore also provided whereby blood is collected in transportable tubes which is then analyzed for cell-mediated immunoresponsiveness at a defined location and the results then sent in the form of an electronic report *via* appropriate architecture to a clinical care provider.

[0052] In addition, the ability to enable the assay to be conducted in small blood volumes is useful for the pediatric, elderly and infirmed populations.

## BRIEF DESCRIPTION OF THE FIGURES

[0053] Some figures contain color representations or entities. Color photographs are available from the Patentee upon request or from an appropriate Patent Office. A fee may be imposed if obtained from a Patent Office.

**Figures 1A through C** are graphical representations showing single stimulant IFN- $\gamma$  responses from whole blood cultures. Normal healthy donor population spread using TCR stimulants mitogen (A) and anti-CD3 antibody (B) or TLR stimulant R848 (C).

**Figures 2A through C** are graphical representations showing synergy of anti-CD3 antibody (TCR agonist) with various TLR agonists. (A) TLR-2; Pam3CSK4 and lipomannan, (B) TLR-4; LPS (lipopolysaccharide) and (C) TLR-7/8; R848 (imidazoquinoline) compound. Numbers above the graph indicate the fold increase in the IFN- $\gamma$  response of cultures co-stimulated with a TLR/anti-CD3 antibody *versus* cultures stimulated with anti-CD3 antibody alone.

**Figure 3** is a graphical representation of using the synergy of TCR/TLR co-stimulation to set a cut-off (threshold) IFN- $\gamma$  response in a normal healthy individual. This allows a healthy population reference range to be set resulting in the ability to observe a drop in the IFN- $\gamma$  response from an immunodeficient individual. Therefore, a measure of immunosuppression due to pharmacological treatment or disease can be assumed if the response falls below the threshold (cut-off) of the healthy population,

**Figure 4** is a graphical representation showing detection of multiple analytes in human plasma from treated (TCR + TLR agonists) and untreated (NIL) whole blood. Human whole blood was treated by stimulating with a combination of anti-CD3 antibody (TCR agonist) and the TLR-7/8 agonist; R848 (imidazoquinoline). Several chemokines, cytokines and extracellular signaling molecules are only found in the plasma of whole blood stimulated with a TCR and TLR agonist simultaneously (Blue bars; TCR + TLR). Several other molecules are up-regulated as compared with untreated whole blood (red bars; NIL).

**Figure 5** is a graphical representation of the synergy observed between dual stimulant (TCR + TLR) IFN- $\gamma$  responses from whole blood cultures as compared with stimulation of whole blood with either a TCR agonist or TLR agonist

alone in an HIV infected and normal healthy cohort. Study populations include (i) untreated HIV positive (sourced from South Africa); and (ii) normal healthy donors (sourced from Australia). Population medians are shown in Table 7.

**Figure 6** is a graphical representation of the correlation observed between the IFN- $\gamma$  response level and CD4 T-cell count observed in combined agonist (TCR + TLR) stimulated whole blood of an HIV infected cohort.

**Figure 7** is a graphical representation showing the stimulation of whole blood with a combination of TCR and TLR agonists which allows the determination of immunosuppressant inhibitory concentration in a given donor.

## DETAILED DESCRIPTION

**[0054]** Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

**[0055]** As used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a T-cell" includes a single T-cell, as well as two or more T-cells; reference to "an agent" includes a single agent, as well as two or more agents; reference to "the invention" includes single or multiple aspects of an invention; and so forth. The terms "T-cells" and "T-lymphocytes" are used interchangeably herein. An "immune cell" includes a T-cell and cells of the innate immune system such as NK cells.

**[0056]** Reference to an "agent", "reagent", "molecule" and "compound" includes single entities and combinations of two or more of such entities. A "combination" also includes multi-part such as a two-part composition where the agents are provided separately and used or dispensed separately or admixed together prior to dispensation. For example, a multi-part assay pack may have two or more agents which potentiate adaptive and innate immunity separately maintained. In an embodiment, the two agents are a TCR agonist and a TLR agonist. Hence, this aspect of the present invention includes agents dried and loose or immobilized to a compartment wall or solid support in an assay pack. A "combination" also includes a fusion or chimeric molecule comprising an adaptive immunity stimulant and an innate immunity stimulant. The adaptive immunity stimulant may modulate adaptive immunity *via* T-cell receptor-dependent or -independent means. An example of a TCR agonist is anti-CD3 antibody. A TLR agonist includes a TLR-7/8 agonist such as R848 (imidazoquinoline).

**[0057]** The present invention as defined in the claims is predicated in part by the augmentation of production of effector molecules from lymphocytes exposed to adaptive and innate immunity potentiation. This enables a more sensitive assay to assess the cell-mediated immune responsiveness of a subject. The present invention provides, therefore, an assay to detect, assess or otherwise monitor a cell-mediated response in a subject by measuring the presence or level of effector molecules from T-cells stimulated by an adaptive and innate immunity potentiator. Lymphocytes are contacted with an agent which potentiates adaptive and innate immunity. According to the invention as defined in the claims, at least two agents are employed wherein at least one potentiates adaptive immunity (*via* T-cells) and at least one potentiates innate immunity (e.g. *via* NK cells). In an alternative, a chimeric molecule is used comprising an adaptive immunity stimulant and an innate immunity stimulant. The adaptive immunity stimulant may be *via* T-cell receptor dependent or independent means. The present invention as defined in the claims provides, therefore, a means to determine the responsiveness of cell-mediated immunity in a subject and, in turn, provides a means for determining whether a disease condition or an agent induces or is associated with immunosuppression. The method also enables diagnosis of infectious diseases, pathological conditions, determination of the level of immunocompetence and assessing of adaptive and innate immune cell responsiveness to endogenous or exogenous agents as well as assessing exposure to a toxic agent such as beryllium or other toxicants.

**[0058]** Accordingly, the present invention as defined in the claims contemplates a method for measuring cell-mediated immune response activity in a subject, the method comprising contacting a source of lymphocytes from the subject with one or more agents which potentiates adaptive and innate immunity and measuring the level of an immune effector molecule produced by immune cells wherein the level of the immune effector molecule is indicative of the level of cell-mediated immunoresponsiveness of the subject.

**[0059]** In an embodiment of the invention as defined in the claims, the method for measuring cell-mediated immune response activity in a subject comprises contacting a source of lymphocytes from the subject with one or more agents which potentiate adaptive and innate immunity and measuring the elevation in the level of an immune effector molecule from immune cells wherein the level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject wherein the level of responsiveness is indicative of the presence or absence or level or stage of a disease or condition selected from the list comprising an infection by a pathogenic agent, an autoimmune disease, a cancer, an inflammatory condition and exposure to a toxic agent.

**[0060]** In yet another embodiment of the invention as defined in the claims, the method comprises contacting a source of lymphocytes from the subject with one or more agents which potentiate adaptive and innate immunity and measuring

the elevation in the level of an immune effector molecule from immune cells wherein the level of the immune effector molecule is indicative of the level of cell-mediated responsiveness and is indicative of the presence or absence or level or stage of a disease or condition selected from the list comprising an infection by a pathogenic agent, an autoimmune disease, a cancer, an inflammatory condition and exposure to a toxic agent.

**[0061]** Disclosed is an assay to detect the presence, absence, level or stage of a disease or condition in a subject, the method comprising contacting a source of lymphocytes from the subject with one or more agents which potentiate adaptive and innate immunity and measuring the elevation in the level of an immune effector molecule from immune cells wherein the level of the immune effector molecule is indicative of the disease or condition.

**[0062]** The present disclosure further contemplates a method for determining whether an agent induces immunosuppression in a subject, the method comprising contacting a source of lymphocytes from the subject after exposure to the agent with one or more potentiators which stimulate adaptive and innate immunity and measuring the presence and level of an effector molecule from the lymphocytes wherein the level of the effector molecule determines the level of immunosuppression induced by the agent.

**[0063]** In accordance therewith, the agent may be a medicament or an environmental toxicant.

**[0064]** In an embodiment of the present invention, the blood sample is co-stimulated with an anti-CD3 antibody as a TCR agonist and a TLR-7/8 agonist such as imidazoquinoline [R848]).

**[0065]** Hence, the present invention as defined in the claims contemplates a method for measuring cell-mediated immune response activity in a subject, the method comprising contacting a source of lymphocytes from the subject with a TCR agonist and a TLR agonist to potentiate the adaptive and innate immunity and measuring the level of an immune effector molecule produced by immune cells wherein the level of the immune effector molecule is indicative of the level of cell-mediated immunoresponsiveness of the subject.

**[0066]** In an embodiment of the present invention as defined in the claims the method is a method for measuring cell-mediated immune response activity in a subject, comprising contacting a source of lymphocytes from the subject with a TCR agonist and a TLR agonist which potentiate adaptive and innate immunity and measuring the elevation in the level of an immune effector molecule from immune cells wherein the level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject wherein the level of responsiveness is indicative of the presence or absence or level or stage of a disease or condition selected from the list comprising an infection by a pathogenic agent, an autoimmune disease, a cancer, an inflammatory condition and exposure to a toxic agent.

**[0067]** In another embodiment of the invention as defined in the claim, the method is suitable for detecting whether a disease condition is inducing immunosuppression in a subject the method comprising contacting a source of lymphocytes from the subject with a disease condition with the one or more agents which potentiate adaptive and innate immunity and measuring the presence or level of an immune effector molecule from the lymphocytes wherein the level of the immune effector molecule is indicative of the extent of immunosuppression induced or associated with the disease condition.

**[0068]** A use as defined in the claims is also provided for one or more agents which potentiate adaptive and innate immunity in the manufacture of a diagnostic assay of cell-mediated immune responsiveness by the method of incubating lymphocytes with one or more molecules which potentiate the adaptive and innate immune system and detecting the presence or elevation in an effector molecule.

**[0069]** This use includes the use for detecting or monitoring the presence, absence, level or stage of a disease or condition such as an infection by a pathogenic agent, an autoimmune disease, a cancer, an inflammatory condition and/or exposure to a medicament or a toxic agent such as a beryllium or other environmental toxicant. Measuring "an immune effector molecule" includes measuring one or more different types of molecules.

**[0070]** Reference to the potentiation of adaptive and innate immunity includes stimulating T-cell receptor-dependent and/or -independent immunity and Toll-like receptor (TLR)-dependent immunity such as mediated by NK cells. By "immune cells" is meant to include "T-cells". An additional agent may also be added to modulate regulatory T-cell (T-reg cells) activity. The latter encompasses inhibiting the suppressor function of T-reg cells. Agents which modulate T-reg cells encompassed herein include a CD25 ligand; a sense or antisense oligonucleotide to genetic material encoding JAK1 or TYK2; a neutralizing antibody; a CpG containing oligonucleotide; an oligonucleotide acting as a TLR modulating agent; and other TLR modulating agents.

**[0071]** In a particular embodiment of the invention as defined in the claims, the T-reg cells are immune response suppressor cells the activity of which is inhibited.

**[0072]** Accordingly, the present invention as defined in the claims further encompasses a method for measuring cell-mediated immune response activity in a subject, the method comprising contacting a source of regulatory T-cells from the subject with an agent selected from (i) an inhibitor of suppressor regulatory T-cells; and (ii) an activator of immune augmenting cells or a subset thereof; and further contacting T-cells with one or more agents which potentiate adaptive and innate immunity and measuring the elevation in the level of an immune effector molecule from immune cells wherein the level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject.

**[0073]** Examples of inhibitors or modulators of T-reg function include CD25 ligands such as but not limited to a polyclonal

or monoclonal antibody to CD25 or an antigen-binding fragment thereof, humanized or deimmunized polyclonal or monoclonal antibodies to CD25 or a recombinant or synthetic form of the polyclonal or monoclonal antibodies. Other examples of agents include sense or antisense nucleic acid molecules directed to the mRNA or DNA (i.e. genetic material) encoding Janus Tyrosine Kinase 1 (JAK1) or Tyrosine Kinase 2 (TYK2) or small molecule inhibitors of JAK1 or TYK2 proteins. Reference to "small molecules" includes immunoglobulin new antigen receptors (IgNARs) as described in International Patent Publication No. WO 2005/118629. Yet still further examples of suitable agents stimulating agents such as CpG molecules which act *via* Toll-like receptors (TLRs) and/or other mechanisms. Hence, CpG containing oligonucleotides and an oligonucleotide acting as a TLR modulating agent also form part of the present invention.

**[0074]** A single type of agent may be used or two or more types of agents may be employed to modulate T-reg cells. For example, the assay may be conducted with a CD25 ligand and a JAK1/TYK2 sense or antisense oligonucleotide; a CD25 ligand and a TLR modulating agent; a JAK1/TYK2 sense or antisense oligonucleotide and a TLR modulating agent; or a CD25 ligand, a JAK1/TYK2 sense or antisense oligonucleotide and a TLR modulating agent. Alternatively, just one type of agent is employed. In another alternative, a CpG comprising oligonucleotide and a TLR modulating agent is used.

**[0075]** In accordance with the various aspects of the present invention, the lymphocyte/immune cells may also be exposed to an antigen to measure antigen-induced adaptive and innate immunoresponsiveness.

**[0076]** Reference to a "subject" includes a human or non-human species including primates, livestock animals (e.g. sheep, cows, pigs, horses, donkey, goats), laboratory test animals (e.g. mice, rats, rabbits, guinea pigs, hamsters), companion animals (e.g. dogs, cats), avian species (e.g. poultry birds, aviary birds), reptiles and amphibians. The present invention has applicability, therefore, in human medicine as well as having livestock and veterinary and wild-life applications which includes the horse, dog and camel racing industries. For example, the assay of the present invention may be routinely carried out on horses before and/or after heavy exertion (such as a race) to screen for evidence of exercise-induced pulmonary hemorrhage (EIPH). All horses exhibit some form of EIPH to some degree during exercise. However, sub-clinical forms of EIPH can be hard to detect.

**[0077]** Reference to a human includes particular populations of humans such as pediatric, elderly and infirmed populations as well as particular cohorts or populations of a particular ethnicity.

**[0078]** In another embodiment of the invention as defined in the claims, the subject is a human and the cell-mediated immune response assay is used in screening for responsiveness to pathogenic microorganisms, viruses and parasites, potential for development or monitoring autoimmune conditions, Celiac's disease, monitoring a subject's response to oncological challenge and for determining the presence of any immunodeficiency or immunosuppression. The latter may occur, for example, due to certain medicaments including various chemotherapeutic agents. Alternatively, exposure to environmental toxicants and pollutants.

**[0079]** 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- $\gamma$  is a particularly useful immune effector molecule, others include a range of cytokines such as interleukins (IL), e.g. IL-2, IL-4, IL-6, IL-10, IL-12 or IL-13, Tumor Necrosis Factor alpha (TNF- $\alpha$ ), Transforming Growth Factor beta (TGF- $\beta$ ), 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. Other effector molecules which may be profiled include those listed in Table 6.

**[0080]** Examples of T-cell receptor agonists include PHA, staphylococcal enterotoxin B (SEB), CpG oligonucleotides, antibodies to the T-cell receptor complex and anti-CD3 antibodies. Examples of T-cell receptor-independent adaptive immune stimulants include PMA and ConA. TLR agonists include Pam3CSK4 (TLR-2 ligand), Lipomannan (TLR-2 ligand), poly(I:C)- (TLR-3 ligand), Lipopolysaccharide (TLR-4 ligand), an imidazoquinoline compound such as R848 (TLR-7/8 ligand) and CpG oligodeoxynucleotides (TLR-9 ligand). In terms of selecting a TLR agonist, in decreasing order, agonists are selected from agonists for TLR-7/8 > TLR-4 > TLR-3 > TLR-2.

**[0081]** A "CpG molecule" means an oligonucleotide comprising a CpG sequence or motif. The present invention extends to any modulator of Toll-like receptor (TLR) function or other aspect of the immune system.

**[0082]** The assay of the present invention as defined in the claims enables detection of the presence or absence or level or stage of a disease or condition in a subject such as infection by a pathogenic agent, an autoimmune disease, cancer, exposure to an inflammatory agent exposure to a medicament, exposure to a toxic agent such as beryllium or other toxicant or pollutant and immunodeficiency or immunosuppression such as induced by a disease condition.

**[0083]** The adaptive and innate immunity agent(s) may be free standing in a reactive vessel or may be immobilized to a solid support such as a bead or a side or bottom of a reaction vessel. The agent(s) may also be in dried form which is re-constituted prior to or during use. Similarly, if an antigen is included, the antigen may be free standing or immobilized in a reactive vessel such as to the vessel itself or a bead or other solid support.

**[0084]** In one embodiment of the invention as defined in the claims, the sample collected from the subject is generally deposited into a blood collection tube. A blood collection tube includes a blood draw tube or other similar vessel. Conveniently, when the sample is whole blood, the blood collection tube is heparinized. Alternatively, heparin is added to the tube after the blood is collected. Notwithstanding that whole blood is particularly contemplated and a 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 as well as samples having undergone cell depletion. Reference to "whole blood" includes whole blood which has not been diluted such as with tissue culture, medium, reagents, excipients, etc. In one embodiment, the term "whole blood" includes an assay sample (i.e. reaction mixture) comprising at least 10% by volume whole blood. The term "at least 10% by volume" includes blood volumes of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and 100% by volume of total assay volume of the reaction mixture. Additional agents may be added such as culture media, enzymes, excipients antigen and the like without departing from the sample comprising "whole blood".

**[0085]** Small blood volumes may also be used such as for 1 $\mu$ l to 1000 $\mu$ l. Examples include 5 $\mu$ l, 10 $\mu$ l, 20 $\mu$ l, 50 $\mu$ l, 100 $\mu$ l and 500 $\mu$ l as well as fractions in between. Hence, sample volumes include from about 1 $\mu$ l to about 200ml including 1ml, 5ml, 10ml and 20ml.

**[0086]** The use of blood collection tubes is compatible with standard automated laboratory systems and these are amenable to analysis in large-scale and random access sampling. Blood collection tubes also minimize handling costs and reduce laboratory exposure to whole blood and plasma and, hence, reduce the risk of laboratory personnel from contracting a pathogenic agent such as HIV or hepatitis B virus (HBV) or hepatitis C virus (HCV).

**[0087]** Combining the incubation step with the collection tube is particularly efficacious and enhances the sensitivity of the assay as does the optional feature of incubating the cells in the presence of a simple sugar such as dextrose or glucose.

**[0088]** The cells of the cell-mediated immune system lose the capacity to mount an immune response in whole blood after extended periods following blood draw from the subject, and responses without intervention are often severely reduced or absent 24 hours following blood draw. The reduction of labor and need for specialized plastic ware allows cell-mediated immune 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- $\gamma$  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.

**[0089]** The incubation step may be from 1 to 50 hours, such as 1 to 40 hours or 8 to 24 hours or a time period in between including 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 hours. A period of 24 hours is particularly convenient.

**[0090]** The ability to measure cell-mediated immunity is important for assessing a subject's ability to respond to an infection by a pathogenic agent such as a microorganism or virus or parasite, to mount an autoimmune response such as in autoimmune diabetes or to protect against cancers or other oncological conditions or to detect an inflammatory condition or to detect exposure or sensitivity of a subject to a toxic agent such as beryllium. The assay described herein also enables detection of disease conditions which lead to immunosuppression or immunosuppression induced by medicaments. Consequently, reference to "measuring a cell-mediated immune response in a subject" includes and encompasses immune diagnosis of infectious and autoimmune diseases, a marker for immunocompetence as well as a marker for inflammatory diseases, cancer and toxic agents. Importantly, the combined adaptive and innate immune responsiveness is determined. Furthermore, the ability to use small blood volumes enables assays to be readily conducted on, for example, the pediatric, elderly and infirmed populations.

**[0091]** In an embodiment of the invention as defined in the claims, disease conditions leading to immunosuppression include chronic infection and cancer. Medicaments which can lead to immunosuppression include those used to treat rheumatoid arthritis, cancer and inflammatory bowel disease.

**[0092]** Pathogenic or infectious agents include bacteria, parasites and viruses. Examples of bacteria include Gram-positive and Gram-negative microorganisms such as *Mycobacterium* species, *Staphylococcus* species, *Streptococcus* species, *Escherichia coli*, *Salmonella* species, *Clostridium* species, *Shigella* species, *Proteus* species, *Bacillus* species, *Hemophilus* species, *Borrelia* species amongst others. *Mycobacterium tuberculosis* is a particularly useful target as well as conditions arising from infection by *M. tuberculosis* such as tuberculosis (TB). Examples of viruses include Hepatitis virus (Hepatitis B virus and Hepatitis C virus), Herpes virus and Human immune deficiency virus (HIV) as well as diseases resulting therefrom. Parasites include *Plasmodium* species, ringworm, liver parasites and the like. Other pathogenic agents include eukaryotic cells such as yeasts and fungi.

**[0093]** Autoimmune diseases contemplated herein for detection 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 inflammatory demyelinating, chronic inflammatory 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, polyglanular 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.

**[0094]** It is generally important to assess the potential or actual cell-mediated responsiveness in subjects exposed to these infectious entities. The method of the present invention as defined in the claims can also be used to detect the presence or absence of these conditions as well as the level or stage of disease process.

**[0095]** Other disease conditions which can lead to immunosuppression include inflammatory disease conditions.

**[0096]** 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. In terms of non-human applications, the present invention extends to detecting EIPH in horses and various conditions in animals such as facial tumor disease in the Tasmanian Devil.

**[0097]** Cancer therapy also is somewhat dependent on cell-mediated immunity and the cancer itself or drugs used to treat cancer can lead to immunosuppression. 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 tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous T-Cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, hematological 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, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-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, 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-tumors, 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 tumors, 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 and Wilms' tumor.

**[0098]** As indicated above, the lymphocyte immune cells may also be exposed to an antigen to measure antigen-induced combined adaptive and innate immunity.

**[0099]** The detection of the immune effector molecules may be measured at the protein or nucleic acid levels. Consequently, reference to "presence or level" of the immune effector molecule includes direct and indirect data. For example,

high levels of cytokine mRNA are indirect data showing increased levels of the cytokine.

**[0100]** Ligands to the immune effectors are particularly useful in detecting and/or quantitating these molecules. Antibodies to the immune effectors are particularly useful. Techniques for the assays contemplated herein are known in the art and include, for example, radioimmunoassay, sandwich assays, ELISA and ELISpot. Reference to "antibodies" includes parts of antibodies, mammalianized (e.g. humanized) antibodies, deimmunized antibodies, recombinant or synthetic antibodies and hybrid and single chain antibodies. For skin tests, in humans, humanized or deimmunized antibodies are particularly contemplated herein to detect effector molecules.

**[0101]** Both polyclonal and monoclonal antibodies are obtainable by immunization with the immune effector molecules or antigenic fragments thereof and either type is utilizable for immunoassays. 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 immunoabsorbent 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.

**[0102]** The use of monoclonal antibodies in an immunoassay is particularly useful 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.

**[0103]** Another aspect of the present disclosure contemplates, therefore, a method for detecting an immune effector molecule in a sample comprising lymphocytes from a subject, the method comprising contacting the sample or an aliquot of the sample with an antibody specific for the immune effector molecule or an antigenic fragment thereof for a time and under conditions sufficient for an antibody-effector complex to form, and then detecting the complex wherein the immune effector molecule is generated after incubation of the lymphocytes with one or more agents which potentiate the adaptive and innate immune systems.

**[0104]** A "sample" includes whole blood or a fraction thereof comprising lymphocytes. This method includes micro-arrays, macro-arrays and nano-arrays on planar or spherical solid supports. A micro- or macro-array is useful. A "sample" also includes a small volume sample of from about 1  $\mu$ l to 1000  $\mu$ l including 5  $\mu$ l, 10  $\mu$ l, 20  $\mu$ l, 50  $\mu$ l and 100  $\mu$ l.

**[0105]** 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.

**[0106]** 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 effector molecules (e.g. cytokines) 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-immune effector molecule complex, a second antibody specific to the effector molecule, 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-effector-labeled antibody. Any unreacted material is washed away, and the presence of the effector molecule 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.

**[0107]** 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 tubes, 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 effector molecule. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the effector molecule.

**[0108]** 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. Furthermore, binding of an antibody to a cytokine may be determined by binding of a labeled antibody directed to the first mentioned antibody.

**[0109]** 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 1. 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 color 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.

**[0110]** 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 of excitability in the molecule, followed by emission of the light at a characteristic color 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. Immunofluorescence and enzyme immunoassay 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.

**[0111]** 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.

**[0112]** The present disclosure also contemplates genetic assays such as involving PCR analysis to detect RNA expression products of a genetic sequence encoding an immune effector.

**[0113]** In one example, 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 1. Other labels include luminescence and phosphorescence as well as infrared dyes. These dyes or fluorophores may also be used as reporter molecules for antibodies.

Table 1

| <i>List of suitable fluorophores</i> |                      |                      |
|--------------------------------------|----------------------|----------------------|
| Probe                                | Ex <sup>1</sup> (nm) | Em <sup>2</sup> (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                  |

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(continued)

| <i>List of suitable fluorophores</i>  |                      |                      |
|---------------------------------------|----------------------|----------------------|
| Probe                                 | Ex <sup>1</sup> (nm) | Em <sup>2</sup> (nm) |
| <b>Reactive and conjugated probes</b> |                      |                      |
| 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)           |
| Cy5                                   | (625); 650           | 670                  |
| Cy5,5                                 | 675                  | 694                  |
| Cy7                                   | 743                  | 767                  |
| <b>Nucleic acid probes</b>            |                      |                      |
| 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                  |

(continued)

| Nucleic acid probes                                                                                |          |          |
|----------------------------------------------------------------------------------------------------|----------|----------|
| 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      |
| <sup>1</sup> Ex: Peak excitation wavelength (nm)<br><sup>2</sup> Em: Peak emission wavelength (nm) |          |          |

**[0114]** Any suitable method of analyzing fluorescence emission is encompassed by the present invention. In this regard, the present 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 et elia 3:1-18, 1997.

**[0115]** 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.

**[0116]** Infrared radiation may result from a suitable infrared dye. Exemplary infrared dyes that may be employed in the present 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.

[0117] 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.

[0118] Flow cytometry is particularly useful in analyzing fluorophore emission.

[0119] 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.

[0120] A modern flow cytometer is able to perform these tasks up to 100,000 cells/particles s<sup>-1</sup>. 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.

[0121] 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 2) 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).

Table 2

| <i>Exemplary optical parameters which may be measured by a flow cytometer.</i> |         |                                          |                 |
|--------------------------------------------------------------------------------|---------|------------------------------------------|-----------------|
| 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                                                              |         |                                          |                 |

[0122] 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. Selection of 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). 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.

[0123] The assay of the present invention as defined in the claims 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.

[0124] The present invention as defined in the claims further contemplates therefore web-based and non-web-based systems where data on the cell-mediated immunoresponsiveness of a subject are provided by a client server or other architecture platform to a central processor which analyses and compares to a control and optionally considers other information such as patient age, sex, weight and other medical conditions and then provides a report, such as, for example, a risk factor for disease severity or progression or status or an index of probability of disease development. A business method is therefore also provided whereby blood is collected in transportable tubes which is then analyzed for cell-mediated immunoresponsiveness at a defined location and the results then sent in the form of an electronic report via a client server or other architecture platform to a clinical care provider.

[0125] Hence, knowledge-based computer software and hardware also form part of the present invention. This facilitate clinical care to ascertain whether a disease condition including infection, cancer or inflammation or a medicament or toxicant is inducing or is associated with immunosuppression.

**[0126]** In particular, the assays of the present invention as defined in the claims may be used in existing or newly developed knowledge-based architecture or platforms associated with pathology services. For example, results from the assays are transmitted *via* a communications network (e.g. the internet) or telephone connection to a processing system in which an algorithm is stored and used to generate a predicted posterior probability value which translates to the index of cell-mediated immunoresponsiveness or immunosuppression which is then forwarded to an end user in the form of a diagnostic or predictive report. This report may also form the basis of clinical care management and personalized medicine.

**[0127]** The assay may, therefore, be in the form of a kit or computer-based system which comprises the reagents necessary to detect the concentration of the immune effector molecule following exposure of lymphocytes to one or more agents which potentiate adaptive and innate immunity and the computer hardware and/or software to facilitate determination and transmission of reports to a clinician.

**[0128]** In an embodiment of the present invention as defined in the claims a method is contemplated allowing a user to determine the status of cell-mediated immunoresponsiveness of a subject, the method including:

- (a) receiving data in the form of levels or concentrations of an immune effector molecule which relative to a control provide a correlation to the state of cell-mediated immunoresponsiveness from the user *via* a communications network, the immune effector molecule measured after exposure of lymphocytes to one or more agents which potentiate adaptive and innate immune responses;
- (b) processing the subject data *via* univariate or multivariate analysis to provide an immunoresponsiveness value;
- (c) determining the status of the subject in accordance with the results of the immunoresponsiveness value in comparison with predetermined values; and
- (d) transferring an indication of the status of the subject to the user *via* the communications network.

**[0129]** Reference to the "univariate" or "multivariate" analysis includes an algorithm which performs the univariate or multivariate analysis function.

**[0130]** Conveniently, the method generally further includes:

- (a) having the user determine the data using a remote end station; and
- (b) transferring the data from the end station to the base station *via* the communications network.

**[0131]** The base station can include first and second processing systems, in which case the method can include:

- (a) transferring the data to the first processing system;
- (b) transferring the data to the second processing system; and
- (c) causing the first processing system to perform the univariate or multivariate analysis function to generate the cell-mediated immunoresponsiveness value.

**[0132]** The method may also include:

- (a) transferring the results of the univariate or multivariate analysis function to the first processing system; and
- (b) causing the first processing system to determine the status of the subject.

**[0133]** In this case, the method also includes at least one of:

- (a) transferring the data between the communications network and the first processing system through a first firewall; and
- (b) transferring the data between the first and the second processing systems through a second firewall.

**[0134]** The second processing system may be coupled to a database adapted to store predetermined data and/or the univariate or multivariate analysis function, the method including:

- (a) querying the database to obtain at least selected predetermined data or access to the univariate or multivariate analysis function from the database; and
- (b) comparing the selected predetermined data to the subject data or generating a predicted probability index of a level of cellular immunoresponsiveness or immunosuppression.

**[0135]** The second processing system can be coupled to a database, the method including storing the data in the database.

**[0136]** The method can also include having the user determine the data using a secure array, the secure array of elements capable of determining the level of immune effector molecule and having a number of features each located at respective position(s) on the respective code. In this case, the method typically includes causing the base station to:

- (a) determine the code from the data;
- (b) determine a layout indicating the position of each feature on the array; and
- (c) determine the parameter values in accordance with the determined layout, and the data.

**[0137]** The method can also include causing the base station to:

- (a) determine payment information, the payment information representing the provision of payment by the user; and
- (b) perform the comparison in response to the determination of the payment information.

**[0138]** In an embodiment of the present invention as defined in the claims also a base station is provided for determining the status of a subject with respect to cell-mediated immunoresponsiveness or immunosuppression, the base station including:

- (a) a store method;
- (b) a processing system, the processing system being adapted to:
- (c) receive subject data from the user *via* a communications network, the data including levels of immune effector molecule wherein the level of the effector molecule relative to a control provides a correlation to the state of cell-mediated immunoresponsiveness wherein the immune effector molecule is determined after exposure of lymphocytes to one or more agents which potentiate the adaptive and innate immune system;
- (d) performing an algorithmic function including comparing the data to predetermined data;
- (e) determining the status of the subject in accordance with the results of the algorithmic function including the comparison; and
- (c) output an indication of the status of the subject to the user *via* the communications network.

**[0139]** The processing system can be adapted to receive data from a remote end station adapted to determine the data.

**[0140]** The processing system may include:

- (a) a first processing system adapted to:
  - (i) receive the data; and
  - (ii) determine the status of the subject in accordance with the results of the univariate or multivariate analysis function including comparing the data; and
- (b) a second processing system adapted to:
  - (i) receive the data from the processing system;
  - (ii) perform the univariate or multivariate analysis function including the comparison; and
  - (iii) transfer the results to the first processing system.

**[0141]** The base station typically includes:

- (a) a first firewall for coupling the first processing system to the communications network; and
- (b) a second firewall for coupling the first and the second processing systems.

**[0142]** The processing system can be coupled to a database, the processing system being adapted to store the data in the database.

**[0143]** In accordance with this embodiment, levels of the immune effector molecule may be screened alone or in combination with other biomarkers or disease indicators. An "altered" level means an increase or elevation or a decrease or reduction in the concentrations of the immune effector molecule.

**[0144]** The determination of the concentrations or levels of the immune effector molecule enables establishment of a diagnostic rule based on the concentrations relative to controls. Alternatively, the diagnostic rule is based on the application of a statistical and machine learning algorithm. Such an algorithm uses relationships between effector molecule and disease status observed in training data (with known disease or cell-mediated immunoresponsiveness status) to infer relationships which are then used to predict the status of subjects with unknown status. An algorithm can be

employed which provides an index of probability that a subject has a certain level of cell-mediated immunoresponsiveness and/or a disease condition. The algorithm performs a univariate or multivariate analysis function.

**[0145]** Hence, the present invention as defined in the claims encompasses, in an embodiment, a diagnostic rule based on the application of statistical and machine learning algorithms. Such an algorithm uses the relationships between immune effector molecule and level of cell-mediated immunoresponsiveness or immunosuppression observed in training data (with known immune status) to infer relationships which are then used to predict the status of patients with unknown immune status. Practitioners skilled in the art of data analysis recognize that many different forms of inferring relationships in the training data may be used without materially changing the present invention.

**[0146]** The present invention as defined in the claims, in an embodiment, further contemplates the use of a knowledge base of training data comprising levels of immune effector molecule from a subject with a known cell-mediated immunoresponsiveness level to generate an algorithm which, upon input of a second knowledge base of data comprising levels of the same immune effector molecule from a subject with an unknown immunoresponsiveness level, provides an index of probability that predicts the nature of the cell-mediated immunoresponsiveness.

**[0147]** The term "training data" includes knowledge of levels of immune effector molecule relative to a control wherein the immune effector molecule is determined after exposure of lymphocytes to one or more agents which potentiate the adaptive and innate immune system. A "control" includes a comparison to levels of immune effector molecule in a subject with "normal" immunoresponsiveness or may be a statistically determined level based on trials.

**[0148]** Hence, the term "training data" includes levels of an immune effector molecule.

**[0149]** The levels or concentrations of the immune effector molecule provide the input test data referred to herein as a "second knowledge base of data". The second knowledge base of data either is considered relative to a control or is fed into an algorithm generated by a "first knowledge base of data" which comprise information of the levels of an immune effector in a subject with a known immunological status. The second knowledge base of data is from a subject of unknown status with respect to cell mediated immunoresponsiveness. The output of the algorithm or the comparison to a control is a probability or risk factor, referred to herein as "an index of probability", of a subject having a certain level of immunoresponsiveness or immunosuppressive.

**[0150]** Data generated from the levels of immune effector molecule are input data. The input of data comprising the immune effector levels is compared with a control or is put into the algorithm which provides a risk value of the likelihood that the subject has, for example, an immunosuppressive condition. A treatment regime can also be monitored to ascertain the presence of any immunosuppression. A level of immunosuppression may increase the risk of a subject getting a secondary infection or having a relapse (e.g. during cancer therapy or treatment of a pathogenic infection).

**[0151]** As described above, methods for diagnosing an immunoresponsiveness or immunosuppressive condition by determining the extent to which a subject can mount an adaptive and innate immune response *via* a level of an immune effector molecule provides a second knowledge base data in an algorithm generated with first knowledge base data or levels of the same effector molecule in subjects with a known immune status. Also provided are methods of detecting immunoresponsiveness comprising determining the presence and/or velocity of an immune effector molecule following stimulation of the adaptive and innate immune system in a subject's sample. By "velocity" it is meant the change in the concentration of the effector molecule in a subject's sample over time.

**[0152]** As indicated above, the term "sample" as used herein means any sample containing an effector molecule following stimulation of adaptive and innate immune process including, but not limited to, biological fluids (including whole blood, plasma, serum, ascites), tissue extracts, freshly harvested cells, and lysates of cells which have been incubated in cell cultures.

**[0153]** The method of the invention as defined in the claims may be used in the diagnosis and staging of a disease. The present invention may also be used to monitor the progression of a condition and to monitor whether a particular treatment is effective or not. In particular, the method can be used to monitor immunosuppression following surgery, cancer therapy or other or medication or exposure to toxicants.

**[0154]** In an embodiment of the invention as defined in the claims, the method may comprises a method for monitoring for immunosuppression in a subject, comprising:

(a) providing a sample from a subject;

(b) determining the level of an immune effector molecule following stimulation of adaptive and innate immune processes;

wherein the level of the immune effector relative to a control provides a correlation to the state of cell-mediated immunoresponsiveness and subjecting the levels to an algorithm to provide an index of probability of the subject having a certain level of immunoresponsiveness; and

(c) repeating steps (a) and (b) at a later point in time and comparing the result of step (b) with the result of step (c) wherein a difference in the index of probability is indicative of the progression of the condition in the subject.

**[0155]** Reference to an "algorithm" or "algorithmic functions" as outlined above includes the performance of a univariate

or multivariate analysis function. A range of different architectures and platforms may be implemented in addition to those described above. It will be appreciated that any form of architecture suitable for implementing the present invention may be used. However, one beneficial technique is the use of distributed architectures. In particular, a number of end stations may be provided at respective geographical locations. This can increase the efficiency of the system by reducing data bandwidth costs and requirements, as well as ensuring that if one base station becomes congested or a fault occurs, other end stations could take over. This also allows load sharing or the like, to ensure access to the system is available at all times.

**[0156]** In this case, it would be necessary to ensure that the base station contains the same information and signature such that different end stations can be used.

**[0157]** It will also be appreciated that in one example, the end stations can be handheld devices, such as PDAs, mobile phones, or the like, which are capable of transferring the subject data to the base station *via* a communications network such as the Internet, and receiving the reports.

**[0158]** In the above aspects, the term "data" means the levels or concentrations of the immune effector following potentiation of adaptive and innate immune processes. The "communications network" includes the internet and mobile telephone network and telephone land line. When a server is used, it is generally a client server or more particularly a simple object application protocol (SOAP).

**[0159]** Included are experiments that demonstrate the cell-mediated immune responsiveness of a subject by measuring responsiveness to particular innate and adaptive immune stimulants. In an embodiment of the invention as defined in the claims, one or more samples such as a sample of peripheral blood, of enriched white cell fraction of blood or bronchoalveolar lavage may be obtained from a subject having or suspected of development of a particular disease (e.g. autoimmune disease, infection to a pathogenic agent or exposure to beryllium) and the immune responsiveness measured by determination of effector molecules from effector T-cells (e.g. CD4<sup>+</sup> T-cells). The assay is conducted in the presence of one or more agents which potentiate the adaptive and innate immune responses as defined in the claims.

**[0160]** The immunobinding methods include methods for detecting or quantifying the amount of a reactive component in a sample, which methods require the detection or quantitation of any immune complexes formed during the binding process. Here, one would obtain a sample suspected of containing a cytokine following the co-stimulation of the adaptive and innate immune processes as defined in the claims and contacting the sample with an antibody and then detecting or quantifying the amount of immune complexes formed under the specific conditions.

**[0161]** Contacting the chosen biological sample with the antibody under conditions effective and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of adding the composition to the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, i.e. to bind to, any antigens present. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, ELISpot, dot blot or Western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

**[0162]** In a particular embodiment of the invention as defined in the claims, the method contemplated detecting the presence, absence, level or stage of a disease or condition in a human subject, the method comprising contacting whole blood, which comprises at least 10% of the total volume in a reaction mixture, with one or more agents which potentiate the adaptive and innate immune system and measuring the presence or elevation in the level of an immune effector molecule from T-cells wherein the presence or level of the immune effector molecule is indicative of the disease or condition.

**[0163]** The present disclosure concerns kits for use with the methods described above. In one example, an immuno-detection kit is contemplated. In another example, a kit for analysis of a sample from a subject having or suspected of developing a metal or chemically-induced disease is contemplated. In a more particular example, a kit for analysis of a sample from a subject having or suspected of developing a disease is contemplated. In a more particular example, a kit is for assessing the cell-mediated immune responsiveness of a subject before or after a disease state has developed or before or after a subject is given a medicament or is exposed to a toxicant or pollutant. If an antigen is also employed, the kit may also comprise a particular antigen.

**[0164]** The immunodetection reagents of the kit may take any one of a variety of forms, including those detectable labels that are associated with or linked to the given antibody or antigen, and detectable labels that are associated with or attached to a secondary binding ligand. Exemplary secondary ligands are those secondary antibodies that have binding affinity for the first antibody or antigen, and secondary antibodies that have binding affinity for a human antibody.

**[0165]** Further suitable immunodetection reagents for use in the kits include the two-component reagent that comprises a secondary antibody that has binding affinity for the first antibody or antigen, along with a third antibody that has binding affinity for the second antibody, the third antibody being linked to a detectable label.

**[0166]** The kits may further comprise a suitably aliquoted composition of antigen or effector molecule, whether labeled or unlabeled, as may be used to prepare a standard curve for a detection assay.

**[0167]** The kits may contain antibody-label conjugates either in fully conjugated form, in the form of intermediates, or as separate moieties to be conjugated by the user of the kit. The components of the kits may be packaged either in

aqueous media or in lyophilized form.

**[0168]** The container means of any of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the testing agent, the antibody or antigen may be placed, and preferably, suitably aliquoted. Where a second or third binding ligand or additional component is provided, the kit will also generally contain a second, third or other additional container into which this ligand or component may be placed. The kits of the present invention will also typically include a means for containing the antibody, antigen, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

**[0169]** The present disclosure further contemplates an improved assay to detect a cell-mediated immune response or the level thereof in a subject, the assay comprising incubating a source of lymphocytes from the subject and detecting for the presence or elevation in effector molecules, the improvement comprising further incubating the lymphocytes with one or more agents which potentiate the adaptive and innate immune systems.

**[0170]** The present disclosure 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 condition or disorder, the method comprising contacting a source of lymphocytes from the subject with one or more agents which potentiate the adaptive and innate immune systems and measuring the presence or elevation in the level of an immune effector molecule from T-cells wherein the presence or level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject which is indicative of the presence, absence, level or state of the condition or disorder and then treating the condition or disorder.

**[0171]** The present invention is further described by the following Examples.

## EXAMPLE 1

### *Development of Assay*

**[0172]** Heparinized blood samples were collected into Li-Hep Vacuette [Registered Trade Mark] tubes (Greiner Bio-one, Germany).

**[0173]** Aliquots of the blood samples were incubated with various concentrations of T-cell receptor agonists: phytohemagglutinin (Cellistis Limited, Australia) anti-human CD3 $\epsilon$  antibody (mouse IgG<sub>1</sub> clone UCHT1; eBioscience, San Diego), and antibodies to T-cell receptor complex; and Toll-like receptor agonists: Lipomannan TLR-2 ligand (InvivoGen, San Diego), Pam3CSK4 TLR-2 ligand (InvivoGen, San Diego), Poly (I:C) TLR-3 ligand (InvivoGen, San Diego), Lipopolysaccharide TLR-4 ligand (Sigma, Australia), Imidazoquinoline compound - TLR-7/8 ligand, R848 (InvivoGen, San Diego) and CpG oligodeoxynucleotides TLR-9 ligand (Hycult Biotechnology, Netherlands) or saline control in a number of different sized blood collection tubes recommended by the manufacturers of the human Quantiferon [Registered Trade Mark] test (Cellestis Limited, Australia). T-cell receptor-independent stimulants include phorbol myristate acetate (PMA), concanavalinA (ConA) and pokeweed mitogen. Aliquots may be small volumes such as 1 $\mu$ l to 1000 $\mu$ l or larger volumes such as 1.5ml to 200ml.

**[0174]** In some experiments, glucose was added at various concentrations to the blood before initiation of incubation. In other experiments, two or more stimulants consisting of at least one TCR stimulant and one TLR stimulant were added to blood together in combination.

**[0175]** Stimulated blood samples were incubated for 1 to 48 hours at 37°C, after which plasma was harvested from above the settled blood cells. The amount of IFN- $\gamma$  present in each plasma sample was then quantified using the Quantiferon-TB [Registered Trade Mark] ELISA (Cellestis Limited, Australia) as per the manufacturer's instructions. Sample IFN- $\gamma$  was alternatively quantified using the more sensitive Quantiferon-TB Gold [Registered Trade Mark] ELISA (Cellestis Limited, Australia) as per the manufacturer's instructions.

**[0176]** ELISA optical density values for IFN- $\gamma$  standards run on each ELISA plate were used to construct a standard curve from which the amount of IFN- $\gamma$  present in each of the test plasma samples was converted to IU/mL values.

## EXAMPLE 2

### *Combinations of T-cell receptor (TCR)-dependent agonists and Toll-like receptor (TLR) agonists*

**[0177]** Potential combinations of TCR and TLR agonists used in the assay of Example 1 are shown in Table 3.

Table 3

| Combinations of TCR and TLR agonists used by the assay |                    |                       |                        |                                 |                                       |                                |
|--------------------------------------------------------|--------------------|-----------------------|------------------------|---------------------------------|---------------------------------------|--------------------------------|
| TCR agonist                                            | TLR agonist        |                       |                        |                                 |                                       |                                |
|                                                        | Pam 3CSK4<br>TLR-2 | Lipomannan<br>(TLR-2) | Poly (I:<br>C)-(TLR-3) | Lipopoly-<br>saccharide (TLR-4) | Imidazo-quinoline<br>(R848) (TLR-7/8) | CpG oligonucleotide<br>(TLR-9) |
| PHA (phytohemagglutinin)                               | ✓                  | ✓                     | ✓                      | ✓                               | ✓                                     | ✓                              |
| anti-CD3 <sub>ε</sub> antibody                         | ✓                  | ✓                     | ✓                      | ✓                               | ✓                                     | ✓                              |
| Staphylococcal enterotoxinB<br>(SEB)                   | ✓                  | ✓                     | ✓                      | ✓                               | ✓                                     | ✓                              |
| Antibodies to T-cell receptor<br>complex               | ✓                  | ✓                     | ✓                      | ✓                               | ✓                                     | ✓                              |

**EXAMPLE 3****Combination of T-cell receptor (TCR)-independent agonists and Toll-like receptor (TLR) agonists**

**[0178]** Potential combinations of TCR-independent agonists and TLR agonists used in the assay of Example 1 are shown in Table 4.

Table 4

| <b>Combinations of TCR and TLR agonists used by the assay</b>        |                        |                           |                            |                                    |                                           |                                    |
|----------------------------------------------------------------------|------------------------|---------------------------|----------------------------|------------------------------------|-------------------------------------------|------------------------------------|
| <b>TCR-independent agonists</b>                                      | <b>TLR agonist</b>     |                           |                            |                                    |                                           |                                    |
|                                                                      | <b>Pam 3CSK4 TLR-2</b> | <b>Lipomannan (TLR-2)</b> | <b>Poly (I: C)-(TLR-3)</b> | <b>Lipopoly-saccharide (TLR-4)</b> | <b>Imidazo-quinoline (R848) (TLR-7/8)</b> | <b>CpG oligonucleotide (TLR-9)</b> |
| <b>ConA<sup>1</sup></b>                                              | ✓                      | ✓                         | ✓                          | ✓                                  | ✓                                         | ✓                                  |
| <b>PMA<sup>2</sup></b>                                               | ✓                      | ✓                         | ✓                          | ✓                                  | ✓                                         | ✓                                  |
| <b>Pokeweed mitogen</b>                                              | ✓                      | ✓                         | ✓                          | ✓                                  | ✓                                         | ✓                                  |
| <sup>1</sup> concanavalinA<br><sup>2</sup> phorbol myristate acetate |                        |                           |                            |                                    |                                           |                                    |

**EXAMPLE 4****Assessment of assay**

**[0179]** The assay involves co-stimulation of T-cell receptor (TCR) and Toll-like receptor (TLR) to assess the combined functional innate and adaptive immunity of an individual. Stimulation of whole blood *in vitro* with a single stimulant towards TCR or TLR in isolation produces a variable response across healthy donors from extremely high IFN- $\gamma$  levels to undetectable levels in certain healthy individuals (Figure 1). For the purposes of setting a healthy reference range and observing a drop in function due to immunodeficiency, a synergy was found that occurs upon combining innate and adaptive immune responses (Figure 2). Dual stimulation potentiates responses elevating the normal healthy population mean allowing a cut-off level to be determined (Figure 3). An elevated normal healthy population mean also allows one to observe a drop in the IFN- $\gamma$  response from an immunodeficient individual. Therefore, a measure of immunosuppression due to pharmacological treatment or disease can be assumed if the response falls below the threshold (cut-off) of the healthy population.

**EXAMPLE 5****Use of T-cell receptor agonist**

**[0180]** This Example provides a method of assessing combined innate and adaptive immunity *in vitro* using a toll-like receptor (TLR) agonist (imidazoquinoline compound, R848 that is specific for NK cells) and a T-cell receptor (TCR) agonist for co-stimulation of immune cells. This *in vitro* diagnostic is for use with whole blood, peripheral blood mononuclear cells (PBMCs) and purified cell populations with feeder layers. However, other TLR agonists specific for different cell types of the immune system could also be included.

**[0181]** The QFN in-tube technology Cellestis provides a method for measuring cell mediated immunity (CMI) following stimulation of whole blood with a combination of stimulants that specifically activate distinct immune cells. The stimulation is carried out in a closed vessel that is incubated for 24 hours at 37°C without humidification. Plasma is harvested from the whole blood and IFN- $\gamma$  is detected using a one-step ELISA, providing a measure of target cell function. Specifically, the assay measures the functional immune status of individuals by assessing the combinatorial innate (NK cell) and adaptive (T cell) immune response. The test is used to monitor the general immune status of an individual for *in vitro* diagnostic and prognostic applications.

**[0182]** The device measures the total IFN- output produced in response to a combination of two stimulants provided simultaneously. The resulting "value" refers to the net functional immune state of an individual at a given point in time. This allows for longitudinal and latitudinal comparison of immune state.

**[0183]** Data indicate that the following order, TLR-7/8 > TLR-4 > TLR-3 > TLR-2 represents the decreasing effectiveness of TLR class of agonists that exert a synergistic response when used in combination with anti-CD3 antibody (TCR agonist) for the purpose of developing a diagnostic assay with clinical utility. In this Example, therefore, TLR-7/8 agonist is used such as an imidazoquinoline compound (R848) to obtain a maximal synergistic response. However, different applications may require the use of sub-maximal synergy or indeed the use of two or more combinations of TLR classes with a TCR agonist such as anti-CD3 antibody or mitogen (phytohemagglutinin; PHA). It is shown that in the diagnostic assay, mitogen exerts its function *via* the TCR located on T-cells. This is evidenced by the ability of anti-CD3 antibody to abrogate the mitogen response when both stimulants are used in combination.

#### EXAMPLE 6

##### *Determination of immunosuppression in subjects on medicaments*

**[0184]** The assay is conducted on subjects exposed to medicaments such as chemotherapeutic agents or agents used to treat rheumatoid arthritis, cancer or inflammatory bowel disease. In subjects where immunosuppression is identified, care is taken to avoid secondary infections. For example, antibiotics may be provided or immuno stimulants.

#### EXAMPLE 7

##### *Stimulation of whole blood with TCR agonist and TLR agonist*

**[0185]** Whole blood was stimulated with a combination of anti-CD3 antibody (TCR agonist) and the TLR-7/8 agonist; R848 (imidazoquinoline). The observed levels of Monokine induced by Gamma interferon (MIG; CXCL9) and IFN- $\gamma$  are shown in Table 5. Values are shown with the corresponding background control subtracted (NIL). The data show that MIG and IFN- $\gamma$  correspond as indicators of stimulation.

**Table 5**

| Subject | MIG (CXCL9) pg/mL | IFN- $\gamma$ pg/mL |
|---------|-------------------|---------------------|
| 1       | >2000             | 34280               |
| 2       | 1567              | 20040               |
| 3       | 1494              | 20000               |
| 4       | >2000             | 31680               |
| 5       | >2000             | 35320               |
| 6       | >2000             | 73760               |
| 7       | 1744              | 25400               |
| 8       | >2000             | 24840               |

**[0186]** Whole blood was also stimulated with or without a combination of anti-CD3 antibody (TCR agonist) and the TLR-7/8 agonist; R848 (imidazoquinoline). The resulting plasma was used to profile known cytokines, chemokines and extracellular signaling molecules. Targets detected could be used singularly or in combination with other effectors as reporter molecules. This is particularly useful for multiplexing. The targets detected are shown in Table 6.

**Table 6**

| Targets                       |
|-------------------------------|
| Complement Component 5a (C5a) |
| Gro $\alpha$ (CXCL1)          |
| sICAM-1 (CD54)                |
| IFN- $\gamma$ (Type II IFN)   |
| IL-1 $\alpha$ (IL-1F1)        |
| IL-1 $\beta$ (IL-1F2)         |

(continued)

| Targets                |
|------------------------|
| IL-1ra (IL-1F3)        |
| IL-6                   |
| IL-8 (CXCL8)           |
| IL-10                  |
| IL-16 (LCF)            |
| IL-17                  |
| IP-10 (CXCL10)         |
| I-TAC (CXCL11)         |
| MCP-1 (CCL2)           |
| MIF (GIF)              |
| MIP-1 $\alpha$ (CCL3)  |
| MIP-1 $\beta$ (CCL4)   |
| Serpin E1 (PAI-1)      |
| RANTES (CCL5)          |
| TNF- $\alpha$ (TNFSF2) |
| MIG (CXCL9)            |

Table 7

|                     | HIV+ve<br>Untreated<br>(TCR) | HIV+ve<br>Untreated<br>(TLR) | HIV+ve<br>Untreated<br>(TCR+TL R) | Healthy<br>Normal<br>(TCR) | Healthy<br>Normal<br>(TLR) | Healthy Normal<br>(TCR+TLR) |
|---------------------|------------------------------|------------------------------|-----------------------------------|----------------------------|----------------------------|-----------------------------|
| Number of<br>values | 24                           | 24                           | 24                                | 50                         | 49                         | 50                          |
| Minimum             | 0.935                        | 0                            | 2.65                              | 0.67                       | 7.78                       | 34.17                       |
| 25%<br>Percentile   | 26.63                        | 1.601                        | 54.35                             | 7.068                      | 24.74                      | 232.1                       |
| Median              | 77.45                        | 6.675                        | 91.28                             | 21.76                      | 52.43                      | 554                         |
| 75%<br>Percentile   | 108.6                        | 22.41                        | 291.5                             | 42.39                      | 87.33                      | 882.5                       |
| Maximum             | 331                          | 122                          | 1349                              | 154.4                      | 439.1                      | 1989                        |

**[0187]** The resulting profile of cytokines, chemokines and extracellular signaling molecules is represented graphically in Figure 4. Several cytokines, chemokines and extracellular signaling molecules were only found in plasma of whole blood stimulated with a TCR and TLR agonist simultaneously (blue bars, TCR + TLR). Several others were up-regulated as compared with untreated whole blood (red bars, NIL).

**[0188]** The synergy observed between dual stimulation with TCR and TLR agonists on IFN- $\gamma$  responses from whole blood cultures compared to stimulation of whole blood with either a TCR agonist or TLR agonist alone is shown in Figure 5.

**[0189]** The population tested were an HIV infected cohort and a normal healthy cohort. Study populations include (i) untreated HIV positive (sourced from South Africa); and (ii) normal healthy donors (sourced from Australia). Population medians are shown in Table 7.

**[0190]** A correlation between IFN- $\gamma$  response level and CD4 T-cell count following TCR + TLR co-stimulation is shown

in Figure 6. Whole blood from an HIV infected cohort was stimulated.

**[0191]** The stimulation of whole blood with a combination of TCR and TLR agonists also allows determination of immunosuppressant inhibitory concentration in a given donor (Figure 7).

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## Claims

1. A method for measuring cell-mediated immune response activity in a subject, said method comprising contacting a sample source of lymphocytes from the subject with one or more agents which potentiate the adaptive immune

system and one or more agents which potentiate the innate immune system, wherein the one or more agents potentiating the adaptive immune system are selected from the group consisting of anti-CD3 antibodies, antibodies to the T-cell receptor complex, CpG oligonucleotides, staphylococcal enterotoxin B (SEB), phytohemagglutinin (PHA), phorbol myristate acetate (PMA), concanavalin A (ConA) and pokeweed mitogen and wherein the one or more agents potentiating the innate immune system are selected from the group consisting of a TLR-3 ligand, a TLR-9 ligand and lipomannan, and measuring the presence or elevation in the level of an immune effector molecule from immune cells, wherein the presence or level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject.

2. A method for measuring cell-mediated immune response activity in a subject, said method comprising contacting a sample source of lymphocytes from the subject with one or more agents which potentiate the adaptive immune system and one or more agents which potentiate the innate immune system and measuring the presence or elevation in the level of an immune effector molecule from immune cells, wherein the presence or level of the immune effector molecule is indicative of the level of cell-mediated responsiveness of the subject, wherein the one or more agent which potentiates the adaptive immune system stimulates *via* T-cell receptor-independent means and is selected from the group consisting of phorbol myristate acetate (PMA), concanavalin A (ConA) and pokeweed mitogen.
3. The method of Claim 1 or 2, wherein the subject is suffering from a disease or condition.
4. The method of Claim 3, wherein the level of the immune effector molecule is indicative of the extent of immunosuppression induced or associated with the disease condition.
5. The method of Claim 1 or 2 or 3 or 4, wherein the sample is whole blood undiluted and wherein the sample is whole blood which comprises from about 10% to 100% by volume of the sample to be assayed, preferably the whole blood comprises from about 50% to 100% by volume of the sample to be assayed and more preferably the whole blood comprises from about 80% to 100% by volume of the sample to be assayed.
6. The method of Claim 1 or 2 or 3 or 4, wherein the whole blood is further incubated with an antigen.
7. The method of Claim 1 or 3 or 4, wherein the agent which potentiates the adaptive immune system stimulates *via* T-cell receptor-independent means.
8. The method of Claim 1 or 2 or 3 or 4, wherein the immune effector molecule is a cytokine.
9. The method of Claim 1 or 2 or 3 or 4, wherein the immune effectors are detected with antibodies specific for same.
10. The method of Claim 1 or 2 or 3 or 4, wherein the subject has an infection by a pathogenic agent selected from *Mycobacterium* species, *Staphylococcus* species, *Streptococcus* species, *Borrelia* species, *Escherichia coli*, *Salmonella* species, *Clostridium* species, *Shigella* species, *Proteus* species, *Bacillus* species, Herpes virus, Hepatitis B or C virus and Human immune deficiency virus (HIV) or a disease resulting therefrom.
11. The method of Claim 10 wherein the subject has a disease condition selected from 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 inflammatory demyelinating, chronic inflammatory 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, polyglanular 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, vitiligo and inflammatory bowel disease.
12. The method of Claim 1 or 2 or 3 or 4, wherein the subject has a cancer selected from 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 tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous T-Cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, hematological 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, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-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-tumors, 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 tumors, 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 and Wilms' tumor.

13. Use of one or more agents which potentiate the adaptive system and one or more agents which potentiate the innate immune system in the manufacture of a diagnostic assay of cell-mediated immune responsiveness by the method of incubating innate and adaptive stimulants with a source comprising T-cells and NK cells and detecting the presence or elevation in effector molecules, wherein the one or more agents potentiating the adaptive immune system are selected from the group consisting of anti-CD3 antibodies, antibodies to the T-cell receptor complex, CpG oligonucleotides, staphylococcal enterotoxin B (SEB), phytohemagglutinin (PHA), phorbol myristate acetate (PMA), concanavalin A (ConA) and pokeweed mitogen, wherein the diagnostic assay is for the diagnosis of an infection by a pathogenic agent selected from *Mycobacterium* species, *Staphylococcus* species, *Streptococcus* species, *Borrelia* species, *Escherichia coli*, *Salmonella* species, *Clostridium* species, *Shigella* species, *Proteus* species, *Bacillus* species, Herpes virus, Hepatitis B or C virus and Human immune deficiency virus (HIV) or a disease resulting therefrom; a disease condition selected from 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 inflammatory demyelinating, chronic inflammatory 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, vitiligo and inflammatory bowel disease; or a cancer selected from 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 tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous T-Cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, hematological 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, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-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-tumors, 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 tumors, 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 and Wilms' tumor.

14. The method of Claim 1, wherein the TLR-9 ligand is CpG oligodeoxynucleotide.

15. The method of Claim 1, wherein the TLR-3 ligand is poly (I:C).

## Patentansprüche

1. Verfahren zum Messen von Zell-vermittelter Immunantwortaktivität in einem Lebewesen, wobei das Verfahren umfasst Kontaktieren einer Proben-Lymphozytenquelle von dem Lebewesen mit einem oder mehreren Agenzien, die das adaptive Immunsystem potenzieren, und einem oder mehreren Agenzien, die das angeborene Immunsystem potenzieren, wobei das eine oder die mehreren Agenzien, das/die das adaptive Immunsystem potenziert/potenzieren, ausgewählt ist/sind aus der Gruppe bestehend aus anti-CD3-Antikörpern, Antikörper gegen den T-Zellrezeptorkomplex, CpG-Oligonukleotiden, Staphylokokken-Enterotoxin B (SEB), Phytohämagglutinin (PHA), Phorbolmyristat-Acetat (PMA), Concanavalin A (ConA) und Pokeweed-Mitogen, und wobei das eine oder die mehreren Agenzien, das/die das angeborene Immunsystem potenziert/potenzieren, ausgewählt ist/sind aus der Gruppe bestehend aus einem TLR-3-Liganden, einem TLR-9-Liganden und Lipomannan, und Messen der Anwesenheit oder der Erhöhung des Titers eines Immuneffektormoleküls von Immunzellen, wobei die Anwesenheit oder der Titer des Immuneffektormoleküls das Ausmaß an Zell-vermittelter Reaktionsfähigkeit des Lebewesens anzeigt.
2. Verfahren zum Messen von Zell-vermittelter Immunantwortaktivität in einem Lebewesen, wobei das Verfahren umfasst Kontaktieren einer Proben-Lymphozytenquelle von den Lebewesen mit einem oder mehreren Agenzien, das/die das adaptive Immunsystem potenziert/potenzieren, und einem oder mehreren Agenzien, das/die das angeborene Immunsystem potenziert/potenzieren, und Messen der Anwesenheit oder der Erhöhung des Titers eines Immuneffektormoleküls von Immunzellen, wobei die Anwesenheit oder der Titer des Immuneffektormoleküls das Ausmaß von Zell-vermittelter Reaktionsfähigkeit des Lebewesens anzeigt, wobei das eine oder die mehreren Agenzien, das/die das adaptive Immunsystem potenziert/potenzieren, vermittels eines T-Zellrezeptor-unabhängigen Mittels stimuliert/stimulieren, und ausgewählt ist/sind aus der Gruppe bestehend aus Phorbolmyristat-Acetat (PMA), Concanavalin A (ConA) und Pokeweed-Mitogen.
3. Verfahren nach Anspruch 1 oder 2, wobei das Lebewesen an einer Krankheit oder einem Zustand leidet.

4. Verfahren nach Anspruch 3, wobei der Titer des Immuneffektormoleküls das Ausmaß von Immunsuppression anzeigt, die durch die Krankheit oder den Zustand induziert oder damit verbunden ist.
5. Verfahren nach Anspruch 1 oder 2 oder 3 oder 4, wobei die Probe unverdünntes Vollblut ist und wobei die Probe Vollblut ist, das von etwa 10 Vol.-% bis etwa 100 Vol.-% der zu testenden Probe umfasst, bevorzugterweise umfasst das Vollblut von etwa 50 Vol.-% bis 100 Vol.-% der zu testenden Probe und bevorzugterweise umfasst das Vollblut von etwa 80 Vol.-% bis 100 Vol.-% der zu testenden Probe.
6. Verfahren nach Anspruch 1 oder 2 oder 3 oder 4, wobei das Vollblut weiter mit einem Antigen inkubiert wird.
7. Verfahren nach Anspruch 1 oder 3 oder 4, wobei das Agens, das das adaptive Immunsystem potenziert, vermittels eines T-Zellrezeptor-unabhängig Mittels stimuliert.
8. Verfahren nach Anspruch 1 oder 2 oder 3 oder 4, wobei das Immuneffektormolekül ein Zytokin ist.
9. Verfahren nach Anspruch 1 oder 2 oder 3 oder 4, wobei die Immuneffektoren mit dafür spezifischen Antikörpern nachgewiesen werden.
10. Verfahren nach Anspruch 1 oder 2 oder 3 oder 4, wobei das Lebewesen eine Infektion aufweist durch ein pathogenes Agens, das ausgewählt ist aus *Mycobacterium*-Arten, *Staphylococcus*-Arten, *Streptococcus*-Arten, *Borrelia*-Arten, *Escherichia coli*, *Salmonella*-Arten, *Clostridium*-Arten, *Shigella*-Arten, *Proteus*-Arten, *Bacillus*-Arten, Herpesvirus, Hepatitis B- oder C-Virus and menschlichem Immundefizienzvirus (HIV) oder einer sich daraus ergebenden Krankheit.
11. Verfahren nach Anspruch 1, wobei das Lebewesen eine Krankheit aufweist, die ausgewählt ist aus Alopecia Areata, Spondylarthritis, anti-Phospholipid-Syndrom, multipler Sklerose bei Addison-Syndrom, Autoimmunerkrankung der Nebenniere, autoimmuner hämolytischer Anämie, autoimmuner Hepatitis, autoimmuner Oophoritis and Orchitis, Behcet'scher Krankheit, bullösem Pemphigoid, Kardiomyopathie, zöliaker Spru-Dermatitis, chronischem Ermüdungssyndrom (CFIDS), chronischer inflammatorischer Demyelinisation, chronischer inflammatorischer Polyneuropathie, Churg-Strauss-Syndrom, Narbenpemphigoid, CREST-Syndrom, Kälteagglutininkrankheit, Crohn'scher Krankheit, herpetiformer Dermatitis, Lupus discoides, essentieller gemischter Kryoglobulinämie, Fibromyalgie, Glomerulonephritis, Basedow-Krankheit, Guillain-Barre, Hashimoto-Syndrom, idiopathischer Lungenfibrose, idiopathischer thrombozytopenischer Purpura (ITP), IgA-Nephropathie, Insulin-abhängigem Diabetes (Type I), Lichen Planus, Lupus, Morbus Meniere, gemischter Bindegewebskrankheit, multipler Sklerose, Myasthenia gravis, Myocarditis, Pemphigus vulgaris, perniziöser Anämie, Polyarteritis nodosa, Polychondritis, polyglanculären Syndromen, rheumatischer Polymyalgie, Polymyositis und Dermatomyositis, primärer Agammaglobulinämie, primärer biliärer Leberzirrhose, Psoriasis, Raynaud'schem Phänomen, Reiter'schem Syndrom, rheumatischem Fieber, rheumatoider Arthritis, Sarkoidose, Sklerodermie, Sjogren'schem Syndrom, Stiff-man-Syndrom, systemischem Lupus erythematosus, Takayasu-Syndrom, Riesenzellenarteriitis, Colitis ulcerosa, Uveitis, Vasculitis, Vitiligo und entzündlicher Darmerkrankung.
12. Verfahren nach Anspruch 1 oder 2 oder 3 oder 4, wobei das Lebewesen einen Krebs aufweist, der ausgewählt ist aus ABL1 Protooncogen, mit AIDS im Zusammenhang stehenden Krebserkrankungen, Akustikusneurinom, akuter lymphozytärer Leukämie, akuter myeloischer Leukämie, Zylindrom, Nebennierenkarzinom, agnogener myeloischer Metaplasie, Alopezie, alveolärem Weichteilsarkom, Anuskarzins, Angiosarkom, aplastischer Anämie, Astrozytom, Ataxia teleangiectatica, Basalzellenkarzinom (Haut), Blasenkarzins, Knochenkarzins, Darmkarzins, Hirnstammgliom, Gehirn- und ZNS-Tumoren, Brustkarzins, ZNS-Tumoren, karzinoiden Tumoren, Zervixkarzinom, Kindergehirntumoren, Kinderkarzins, Kinderleukämie, Kinderweichteilsarkom, Chondrosarkom, Choriokarzinom, chronischer lymphozytärer Leukämie, chronischer myeloischer Leukämie, kolorektalen Krebserkrankungen, Haut-T-Zelllymphom, Dermatofibrosarkom-Protuberanz, desmoplastischem Small-Round-Cell-Tumor, Duktuskarzinom, endokrinen Krebserkrankungen, Endometriumkarzinom, Ependyom, Ösophaguskarzinom, Ewing-Sarkom, extrahepatischem Gallengangkarzinom, Augenkarzins, Auge: Melanom, Retinoblastom, Eileiterkarzins, Fanconi'scher Anämie, Fibrosarkom, Gallenblasenkarzins, Magenkarzins, gastrointestinalen Krebserkrankungen, gastrointestinalem-karzinoidem Tumor, urogenitalen Krebserkrankungen, Keimzellentumoren, Gestations-Trophoblasten-Krankheit, Gliom, gynäkologischen Krebserkrankungen, hämatologischen Malignitäten, Haarzell-Leukämie, Kopf- und Halskarzins, hepatozellulärem Karzinom, erblichem Brustkarzins, Histiozytose, Hodgkin'scher Krankheit, menschlichem Papillomavirus, Blasenmole, Hyperkalziämie, Hypopharynx-Krebs, intraokularem Melanom, Inselzellenkarzinom, Kaposi-Sarkom, Nierenkarzinom, Langerhans'scher Zellen-Histiozytose, Larynxkarzinom, Leiomyosarkom, Leukämie, Li-Fraumeni-

Syndrom, Lippenkrebs, Liposarkom, Leberkrebs, Lungenkrebs, Lymphödem, Lymphom, Hodgkin-Lymphom, Non-Hodgkin-Lymphom, Brustkrebs beim Mann, malignem stabförmigen Tumor der Niere, Medulloblastom, Melanom, Merkel-Karzinom, Mesotheliom, metastatischem Krebs, Mundkrebs, multipler endokriner Neoplasie, Mycosis fungoides, myelodysplastischen Syndromen, Myelom, myeloproliferativen Erkrankungen, Nasenkrebs, Nasopharynxkarzinom, Nephroblastom, Neuroblastom, Neurofibromatose, Nijmegen-Breakage-Syndrom, Hautkrebs vom nicht-Melanom-Typ, nicht kleinzelligem Lungenkarzinom (NSCLC), Augenkrebserkrankungen, Ösophaguskarzinom, Mundhöhlenkrebs, Mundrachenraumkrebs, Osteosarkom, Stoma-Ovarialkarzinom, Bauchspeicheldrüsenkrebs, Nasennebenhöhlenkrebs, Nebenschilddrüsenkrebs, Ohrspeicheldrüsenkrebs, Peniskrebs, peripher-neuroektodermalen Tumoren, Hypophysenkarzinom, Polycythaemia vera, Prostatakrebs, seltenen Krebserkrankungen und damit verbundenen Erkrankungen, Grawitz-Tumor, Retinoblastom, Rhabdomyosarkom, Rothmund-Thomson-Syndrom, Speicheldrüsenkarzinom, Sarkom, Schwannom, Sezary-Syndrom, Hautkrebs, kleinzelligem Lungenkrebs (SCLC), Dünndarmkrebs, Weichteilsarkom, Rückenmarktumoren, Plattenepithelkarzinom (der Haut), Magenkrebs, Synovialkarzinom, Hodenkrebs, Thymuskarzinom, Schilddrüsenkrebs, Übergangsepithelzellenkrebs (der Blase), Übergangsepithelzellenkrebs (der Niere, des Beckens/des Harnleiters), trophoblastischem Karzinom, Harnröhrenkarzinom, Krebserkrankung des Hamsystems, Uroplakine, Uterussarkom, Uteruskrebs, Vaginalkarzinom, Vulvakarzinom, Waldenström'scher Makroglobulinämie und Wilms-Tumor.

13. Verwendung von einem oder mehreren Agenzien, das/die das adaptive System potenziert/potenzieren, und einem oder mehreren Agenzien, das/die das angeborene Immunsystem potenziert/potenzieren, bei der Herstellung eines diagnostischen Tests auf Zellvermittelte Immunreaktionsfähigkeit durch das Verfahren von Inkubieren von angeborenen und adaptiven stimulierenden Agenzien mit einer Quelle umfassend T-Zellen und NK-Zellen und Nachweisen der Anwesenheit oder der Erhöhung von Effektormolekülen, wobei das eine oder die mehreren Agenzien, das /die das adaptive Immunsystem potenziert/potenzieren, ausgewählt ist/sind aus der Gruppe bestehend aus anti-CD3-Antikörpern, Antikörpern gegen den T-Zell-Rezeptorkomplex, CpG-Oligonukleotiden, Staphylokokken-Enterotoxin B (SEB), Phytohämagglutinin (PHA), Phorbolmyristat-Acetat (PMA), Concanavalin A (ConA) und Pokeweed-Mitogen, wobei der diagnostische Test ist für die Diagnose von einer Infektion durch ein pathogenes Agens, das ausgewählt ist aus *Mycobacterium*-Arten, *Staphylococcus*-Arten, *Streptococcus*-Arten, *Borrelia*-Arten, *Escherichia coli*, *Salmonella*-Arten, *Clostridium*-Arten, *Shigella*-Arten, *Proteus*-Arten, *Bacillus*-Arten, Herpesvirus, Hepatitis B- oder C-Virus and menschlichem Immundefizienzvirus (HIV) oder einer sich daraus ergebenden Krankheit; einer Krankheit oder einem Zustand, die/der ausgewählt ist aus Alopecia Areata, Spondylarthritis, anti-Phospholipid-Syndrom, multipler Sklerose bei Addison-Syndrom, Autoimmunerkrankung der Nebenniere, autoimmuner hämolytischer Anämie, autoimmuner Hepatitis, autoimmuner Oophoritis and Orchitis, Behcet'scher Krankheit, bullösem Pemphigoid, Kardiomyopathie, zöliaker Spru-Dermatitis, chronischem Ermüdungssyndrom (CFIDS), chronischer inflammatorischer Demyelinisation, chronischer inflammatorischer Polyneuropathie, Churg-Strauss-Syndrom, Narbenpemphigoid, CREST-Syndrom, Kälteagglutininkrankheit, Crohn'scher Krankheit, herpetiformer Dermatitis, Lupus discoides, essentieller gemischter Kryoglobulinämie, Fibromyalgie, Glomerulonephritis, Basedow-Krankheit, Guillain-Barre, Hashimoto-Syndrom, idiopathischer Lungenfibrose, idiopathischer thrombozytopenischer Purpura (ITP), IgA-Nephropathie, Insulin-abhängigem Diabetes (Type I), Lichen Planus, Lupus, Morbus Meniere, gemischter Bindegewebskrankheit, multipler Sklerose, Myasthenia gravis, Myocarditis, Pemphigus vulgaris, perniziöser Anämie, Polyarteritis nodosa, Polychondritis, polyglandulären Syndromen, rheumatischer Polymyalgie, Polymyositis und Dermatomyositis, primärer Agammaglobulinämie, primärer biliärer Leberzirrhose, Psoriasis, Raynaud'schem Phänomen, Reiter'schem Syndrom, rheumatischem Fieber, rheumatoider Arthritis, Sarkoidose, Sklerodermie, Sjögren'schem Syndrom, Stiff-man-Syndrom, systemischem Lupus erythematosus, Takayasu-Syndrom, Riesenzellenarteriitis, Colitis ulcerosa, Uveitis, Vasculitis, Vitiligo und entzündlicher Darmerkrankung; oder einem Krebs, der ausgewählt ist aus ABL1 Protooncogen, mit AIDS im Zusammenhang stehenden Krebserkrankungen, Akustikusneurinom, akuter lymphozytärer Leukämie, akuter myeloischer Leukämie, Zylindrom, Nebennierenkarzinom, agnogener myeloischer Metaplasie, Alopezie, alvelolärem Weichteilsarkom, Anuskarbs, Angiosarkom, aplastischer Anämie, Astrozytom, Ataxia teleangiectatica, Basalzellenkarzinom (Haut), Blasenkrebs, Knochenkrebs, Darmkrebs, Hirnstammgliom, Gehirn- und ZNS-Tumoren, Brustkrebs, ZNS-Tumoren, karzinoiden Tumoren, Zervixkarzinom, Kindergehirntumoren, Kinderkrebs, Kinderleukämie, Kinderweichteilsarkom, Chondrosarkom, Choriokarzinom, chronischer lymphozytärer Leukämie, chronischer myeloischer Leukämie, kolorektalen Krebserkrankungen, Haut-T-Zelllymphom, Dermatofibrosarkom-Protuberanz, desmoplastischem Small-Round-Cell-Tumor, Duktuskarzinom, endokrinen Krebserkrankungen, Endometriumkarzinom, Ependymom, Ösophaguskarzinom, Ewing-Sarkom, extrahepatischem Gallengangkarzinom, Augenkrebs, Auge: Melanom, Retinoblastom, Eileiterkrebs, Fanconi'scher Anämie, Fibrosarkom, Gallenblasenkrebs, Magenkrebs, gastrointestinalen Krebserkrankungen, gastrointestinalen-karzinoidem Tumor, urogenitalen Krebserkrankungen, Keimzelltumoren, Gestations-Trophoblasten-Krankheit, Gliom, gynäkologischen Krebserkrankungen, hämatologischen Malignitäten, Haarzell-Leukämie,

Kopf- und Halskrebs, hepatozellulärem Karzinom, erblichem Brustkrebs, Histiozytose, Hodgkin'scher Krankheit, menschlichem Papillomavirus, Blasenmole, Hyperkalziämie, Hypopharynx-Krebs, intraokularem Melanom, Inselzellenkarzinom, Kaposi-Sarkom, Nierenkarzinom, Langerhans'scher Zellen-Histiozytose, Larynxkarzinom, Leiomyosarkom, Leukämie, Li-Fraumeni-Syndrom, Lippenkrebs, Liposarkom, Leberkrebs, Lungenkrebs, Lymphödem, Lymphom, Hodgkin-Lymphom, Non-Hodgkin-Lymphom, Brustkrebs beim Mann, malignem stabförmigen Tumor der Niere, Medulloblastom, Melanom, Merkel-Karzinom, Mesotheliom, metastatischem Krebs, Mundkrebs, multipler endokriner Neoplasie, Mycosis fungoides, myelodysplastischen Syndromen, Myelom, myeloproliferativen Erkrankungen, Nasenkrebs, Nasopharynxkarzinom, Nephroblastom, Neuroblastom, Neurofibromatose, Nijmegen-Breakage-Syndrom, Hautkrebs vom nicht-Melanom-Typ, nicht kleinzelligem Lungenkarzinom (NSCLC), Augenerkrankungen, Ösophaguskarzinom, Mundhöhlenkrebs, Mundrachenraumkrebs, Osteosarkom, Stoma-Ovarialkarzinom, Bauchspeicheldrüsenkrebs, Nasennebenhöhlenkrebs, Nebenschilddrüsenkrebs, Ohrspeicheldrüsenkrebs, Peniskrebs, peripher-neuroektodermalen Tumoren, Hypophysenkarzinom, Polycythaemia vera, Prostatakrebs, seltenen Krebserkrankungen und damit verbundenen Erkrankungen, Grawitz-Tumor, Retinoblastom, Rhabdomyosarkom, Rothmund-Thomson-Syndrom, Speicheldrüsenkarzinom, Sarkom, Schwannom, Sezary-Syndrom, Hautkrebs, kleinzelligem Lungenkrebs (SCLC), Dünndarmkrebs, Weichteilsarkom, Rückenmarktumoren, Plattenepithelkarzinom (der Haut), Magenkrebs, Synovialkarzinom, Hodenkrebs, Thymuskarzinom, Schilddrüsenkrebs, Übergangsepithelzellenkrebs (der Blase), Übergangsepithelzellenkrebs (der Niere, des Beckens/des Harnleiters), trophoblastischem Karzinom, Harnröhrenkarzinom, Krebserkrankung des Harnsystems, Uroplakine, Uterussarkom, Uteruskrebs, Vaginalkarzinom, Vulvakarzinom, Waldenström'scher Makroglobulinämie und Wilms-Tumor.

14. Verfahren nach Anspruch 1, wobei der TLR-9-Ligand CpG-Oligodesoxynukleotid ist.

15. Verfahren nach Anspruch 1, wobei der TLR-3-Ligand Poly(I:C) ist.

## Revendications

1. Méthode permettant de mesurer l'activité de réponse immunitaire à médiation cellulaire chez un sujet, ladite méthode comprenant la mise en contact d'une source d'échantillon de lymphocytes du sujet avec un ou plusieurs agents potentialisant le système immunitaire adaptatif et un ou plusieurs agents potentialisant le système immunitaire inné, dans laquelle l'un ou les plusieurs agents potentialisant le système immunitaire adaptatif sont choisis dans le groupe constitué des anticorps anti-CD3, des anticorps contre le complexe récepteur de la cellule T, des oligonucléotides CpG, de l'entérotoxine B staphylococcique (SEB), de la phytohémagglutinine (PHA), de l'acétate de myristate de phorbol (PMA), de la concanavaline A (ConA) et du mitogène de phytolaque, et dans laquelle l'un ou les plusieurs agents potentialisant le système immunitaire inné sont choisis dans le groupe constitué d'un ligand TLR-3, d'un ligand TLR-9 et d'un lipomannane, et la mesure de la présence ou de l'élévation du niveau d'une molécule effectrice immunitaire provenant de cellules immunitaires, dans laquelle la présence ou le niveau de la molécule effectrice immunitaire indique le niveau de la réactivité à médiation cellulaire du sujet.
2. Méthode permettant de mesurer l'activité de réponse immunitaire à médiation cellulaire chez un sujet, ladite méthode comprenant la mise en contact d'une source d'échantillon de lymphocytes du sujet avec un ou plusieurs agents potentialisant le système immunitaire adaptatif et un ou plusieurs agents potentialisant le système immunitaire inné, et la mesure de la présence ou de l'élévation du niveau d'une molécule effectrice immunitaire provenant de cellules immunitaires, dans laquelle la présence ou le niveau de la molécule effectrice immunitaire indique le niveau de réactivité à médiation cellulaire du sujet, dans laquelle l'un ou les plusieurs agents potentialisant le système immunitaire adaptatif stimule par des moyens indépendants du récepteur de la cellule T et est sélectionné dans le groupe constitué de l'acétate de myristate de phorbol (PMA), de la concanavaline A (ConA) et du mitogène de phytolaque.
3. Méthode selon la revendication 1 ou 2, dans laquelle le sujet est atteint d'une maladie ou d'un état pathologique.
4. Méthode selon la revendication 3, dans laquelle le niveau de la molécule effectrice immunitaire indique l'ampleur de l'immunosuppression induite ou associée à l'état pathologique.
5. Méthode selon la revendication 1 ou 2 ou 3 ou 4, dans laquelle l'échantillon est du sang total non dilué et dans laquelle l'échantillon est du sang total qui comprend environ 10 % à 100 % en volume de l'échantillon à tester, de préférence le sang total comprend environ 50 % à 100 % en volume de l'échantillon à tester et, plus préférablement, le sang total comprend environ 80 % à 100 % en volume de l'échantillon à tester.

6. Méthode selon la revendication 1 ou 2 ou 3 ou 4, dans laquelle le sang total est en outre incubé avec un antigène.
7. Méthode selon la revendication 1 ou 3 ou 4, dans laquelle l'agent qui potentialise le système immunitaire adaptatif stimule par des moyens indépendants du récepteur de la cellule T.
8. Méthode selon la revendication 1 ou 2 ou 3 ou 4, dans laquelle la molécule effectrice immunitaire est une cytokine.
9. Méthode selon la revendication 1 ou 2 ou 3 ou 4, dans laquelle les effecteurs immunitaires sont détectés avec des anticorps spécifiques à ceux-ci.
10. Méthode selon la revendication 1 ou 2 ou 3 ou 4, dans laquelle le sujet est infecté par un agent pathogène choisi parmi les espèces *Mycobacterium*, les espèces *Staphylococcus*, les espèces *Streptococcus*, les espèces *Borrelia*, *Escherichia coli*, les espèces *Salmonella*, les espèces *Clostridium*, les espèces *Shigella*, les espèces *Proteus*, les espèces *Bacillus*, le virus de l'herpès, le virus de l'hépatite B ou C et le virus de l'immunodéficience humaine (VIH) ou une maladie qui en résulte.
11. Méthode selon la revendication 10, dans laquelle le sujet présente un état pathologique choisi parmi l'alopécie en aires, la spondylarthrite ankylosante, le syndrome des antiphospholipides, la maladie d'Addison auto-immune, la sclérose en plaques, la maladie auto-immune de la glande surrénale, l'anémie hémolytique auto-immune, l'hépatite auto-immune, l'oophorite et l'orchite auto-immune, la maladie de Behcet, la pemphigoïde bulleuse, la cardiomyopathie, la dermatite coeliaque, le syndrome de fatigue chronique (CFIDS), la démyélinisation inflammatoire chronique, la polyneuropathie inflammatoire chronique, le syndrome de Churg-Strauss, la pemphigoïde cicatricielle, le syndrome de la crête, la maladie de l'agglutinine froide, la maladie de Crohn, la dermatite herpétiforme, le lupus discoïde, la cryoglobulinémie mixte essentielle, la fibromyalgie, la glomérulonéphrite, la maladie de Grave, le syndrome de Guillain-Barré, la thyroïdite de Hashimoto, la fibrose pulmonaire idiopathique, la purpura thrombopénique idiopathique (ITP), la néphropathie IgA, le diabète insulino-dépendant (type I), le lichen plan, le lupus, la maladie de Menière, la maladie mixte du tissu conjonctif, la sclérose en plaques, la myasthénie grave, la myocardite, le pemphigus vulgaris, l'anémie pernicieuse, la polyartérite noueuse, la polychondrite, les syndromes polyglandulaires, la pseudo-polyarthrite rhizomélisque, la polymyosite et la dermatomyosite, l'agammaglobulinémie primaire, la cirrhose biliaire primaire, le psoriasis, le phénomène de Raynaud, le syndrome de Reiter, la fièvre rhumatique, l'arthrite rhumatoïde, la sarcoïdose, la sclérodermie, le syndrome de Sjogren, le syndrome de l'homme raide, le lupus érythémateux systémique, l'artérite de Takayasu, l'artérite temporale/l'artérite à cellules géantes, la rectocolite hémorragique, l'uvéite, la vascularite, le vitiligo et la maladie inflammatoire de l'intestin.
12. Méthode selon la revendication 1 ou 2 ou 3 ou 4, dans lequel le sujet présente un cancer choisi parmi le protooncogène ABL1, les cancers liés au SIDA, le neurome acoustique, la leucémie lymphocytaire aiguë, la leucémie myéloïde aiguë, le carcinome adénocystique, le cancer de la surrenalence, la métaplasie agnogène myéloïde, l'alopécie, le sarcome alvéolaire des parties molles, le cancer de l'anus, l'angiosarcome, l'anémie aplasique, l'astrocytome, l'ataxie-télangiectasie, le carcinome basocellulaire (peau), le cancer de la vessie, les cancers de l'os, le cancer de l'intestin, le gliome du tronc cérébral, les tumeurs cérébrales et du système nerveux central (SNC), le cancer du sein, les tumeurs du SNC, les tumeurs carcinoïdes, le cancer du col de l'utérus, les tumeurs cérébrales infantiles, le cancer infantile, la leucémie infantile, le sarcome des tissus mous de l'enfant, le chondrosarcome, le choriocarcinome, la leucémie lymphoïde chronique, la leucémie myéloïde chronique, les cancers colorectaux, le lymphome cutané à cellules T, le dermatofibrosarcome protuberans, la tumeur desmoplastique à petites cellules rondes, le carcinome canalaire, les cancers du système endocrinien, le cancer de l'endomètre, l'épendymome, le cancer de l'oesophage, le sarcome d'Ewing, le cancer des canaux de bile extra-hépatique, le cancer des yeux, le mélanome oculaire, le rétinoblastome, le cancer de la trompe de Fallope, l'anémie de Fanconi, le fibrosarcome, le cancer de la vésicule biliaire, le cancer de l'estomac, les cancers gastro-intestinaux, la tumeur carcinoïde gastro-intestinale, les cancers de l'appareil génito-urinaire, les tumeurs des cellules germinales, la maladie trophoblastique gravidique, le gliome, les cancers gynécologiques, les hémopathies malignes, la leucémie à tricholeucocytes, le cancer de la tête et du cou, le cancer hépatocellulaire, le cancer du sein héréditaire, l'histiocytose, la maladie de Hodgkin, le papillomavirus humain, la mole hydatiforme, l'hypercalcémie, le cancer de l'hypopharynx, le mélanome intraoculaire, le cancer des cellules des îlots pancréatiques, le sarcome de Kaposi, le cancer du rein, l'histiocytose des cellules de Langerhan, le cancer du larynx, le léiomyosarcome, la leucémie, le syndrome de Li-Fraumeni, le cancer de la lèvre, le liposarcome, le cancer du foie, le cancer du poumon, le lymphoedème, le lymphome, le lymphome de Hodgkin, le lymphome non-hodgkinien, le cancer du sein chez l'homme, la tumeur du rein rhabdoïde maligne, le médulloblastome, le mélanome, le cancer des cellules de Merkel, le mésothéliome, le cancer métastatique, le cancer de la bouche, l'adénomatose polyendocrinienne, la mycose fongicoïde, les syndromes myélodysplasiques, le myé-

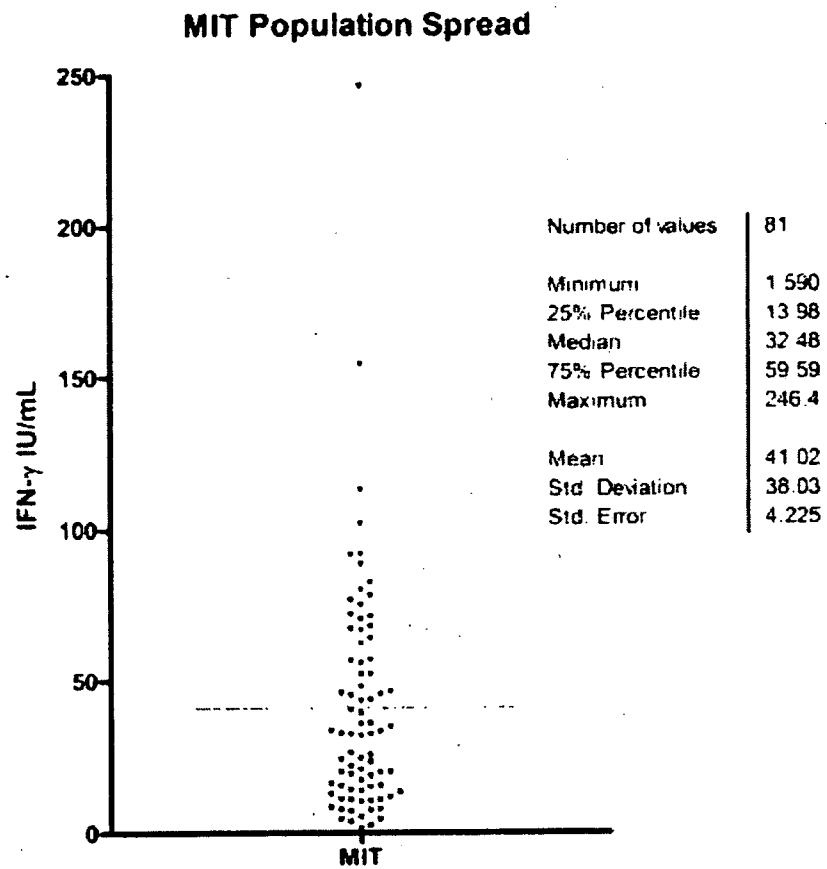
lome, les troubles myéloprolifératifs, le cancer du nez, le cancer du nasopharynx, le néphroblastome, le neuroblastome, la neurofibromatose, le syndrome de Nimègue, le cancer de la peau sans présence de mélanome, le cancer du poumon non à petites cellules (NSCLC), les cancers oculaires, le cancer de l'oesophage, le cancer de la cavité buccale, le cancer de l'oropharynx, l'ostéosarcome, le cancer de l'ovaire d'ostomie, le cancer du pancréas, le cancer paranasal, le cancer de la parathyroïde, le cancer de la glande parotide, le cancer du pénis, les tumeurs neuroectodermiques périphériques, le cancer de l'hypophyse, la maladie de Vaquez, le cancer de la prostate, les cancers rares et troubles associés, le carcinome des cellules rénales, le rétinoblastome, le rhabdomyosarcome, le syndrome de Rothmund-Thomson, le cancer des glandes salivaires, le sarcome, le schwannome, le syndrome de Sézary, le cancer de la peau, le cancer du poumon à petites cellules (SCLC), le cancer de l'intestin grêle, le sarcome des tissus mous, les tumeurs de la moelle épinière, le carcinome épidermoïde de la peau, le cancer de l'estomac, le sarcome synovial, le cancer des testicules, le cancer du thymus, le cancer de la thyroïde, le cancer des cellules transitionnelles de la vessie, le cancer des cellules transitionnelles rhéno-pelviens/de l'urètre, le cancer trophoblastique, le cancer de l'urètre, le cancer du système urinaire, l'uroplakins, le sarcome utérin, le cancer de l'utérus, le cancer vaginal, le cancer de la vulve, la macroglobulinémie de Waldenström et la tumeur de Wilms.

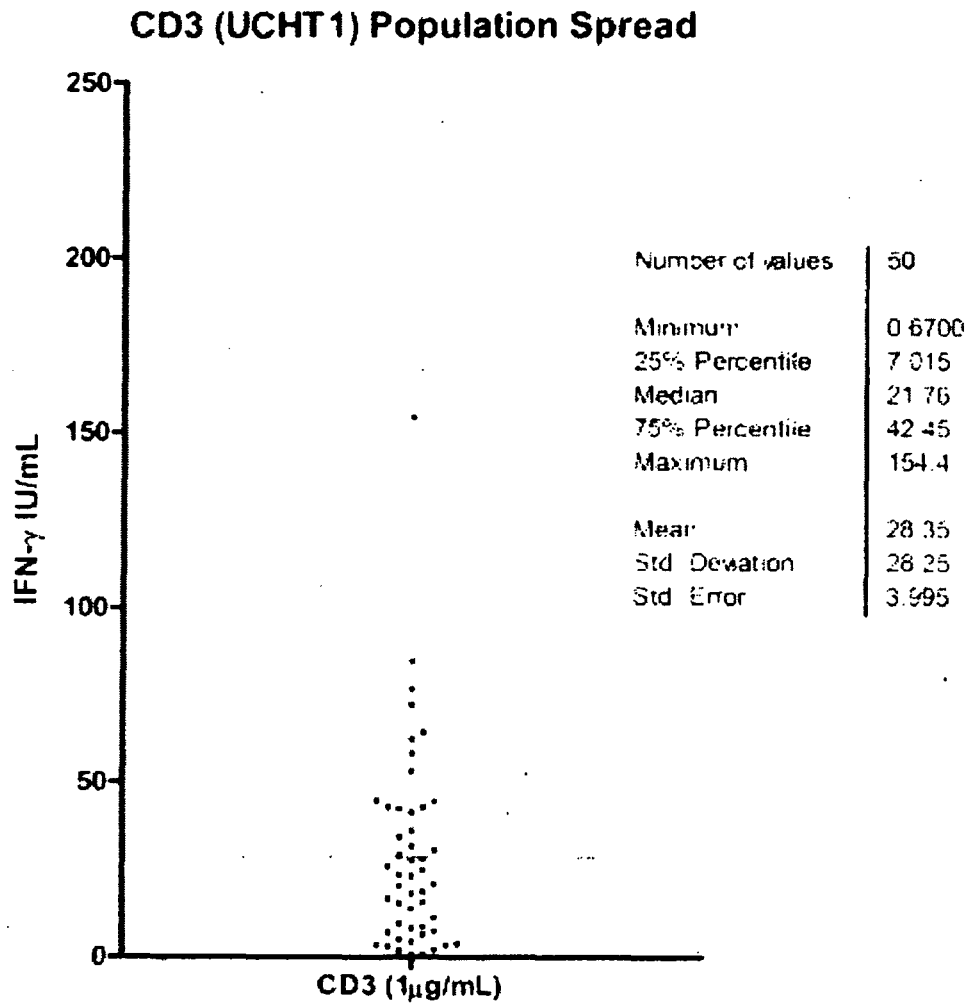
13. Utilisation d'un ou de plusieurs agents potentialisant le système adaptatif et d'un ou de plusieurs agents potentialisant le système immunitaire inné dans la fabrication d'un test de diagnostic de la réactivité immunitaire à médiation cellulaire par le procédé d'incubation de stimulants innés et adaptatifs avec une source comprenant des cellules T et des cellules NK, et de détection de la présence ou de l'élévation dans des molécules effectrices, dans laquelle les un ou plusieurs agents potentialisant le système immunitaire adaptatif sont choisis dans le groupe constitué des anticorps anti-CD3, des anticorps contre le complexe récepteur de la cellule T, des oligonucléotides CpG, de l'entérotoxine B staphylococcique (SEB), de la phytohémagglutinine (PHA), de l'acétate de myristate de phorbol (PMA), de la concanavaline A (ConA) et du mitogène de phytolaque, dans laquelle le test de diagnostic sert au diagnostic d'une infection par un agent pathogène choisi parmi les espèces *Mycobacterium*, les espèces *Staphylococcus*, les espèces *Streptococcus*, les espèces *Borrelia*, *Escherichia coli*, les espèces *Salmonella*, les espèces *Clostridium*, les espèces *Shigella*, les espèces *Proteus*, les espèces *Bacillus*, le virus de l'herpès, le virus de l'hépatite B ou C et le virus de l'immunodéficience humaine (VIH) ou une maladie qui en résulte ;
- d'un état pathologique choisi parmi l'alopécie en aires, la spondylarthrite ankylosante, le syndrome des antiphospholipides, la maladie d'Addison auto-immune, la sclérose en plaques, la maladie auto-immune de la glande surrénale, l'anémie hémolytique auto-immune, l'hépatite auto-immune, l'oophorite et l'orchite auto-immune, la maladie de Behçet, la pemphigoïde bulleuse, la cardiomyopathie, la dermatite coeliaque, le syndrome de fatigue chronique (CFIDS), la démyélinisation inflammatoire chronique, la polyneuropathie inflammatoire chronique, le syndrome de Churg-Strauss, la pemphigoïde cicatricielle, le syndrome de la crête, la maladie de l'agglutinine froide, la maladie de Crohn, la dermatite herpétiforme, le lupus discoïde, la cryoglobulinémie mixte essentielle, la fibromyalgie, la glomérulonéphrite, la maladie de Grave, le syndrome de Guillain-Barré, la thyroïdite de Hashimoto, la fibrose pulmonaire idiopathique, la purpura thrombopénique idiopathique (ITP), la néphropathie IgA, le diabète insulino-dépendant (type I), le lichen plan, le lupus, la maladie de Ménière, la maladie mixte du tissu conjonctif, la sclérose en plaques, la myasthénie grave, la myocardite, le pemphigus vulgaris, l'anémie pernicieuse, la polyartérite noueuse, la polychondrite, les syndromes polyglandulaires, la pseudo-polyarthrite rhizomélisque, la polymyosite et la dermatomyosite, l'agammaglobulinémie primaire, la cirrhose biliaire primaire, le psoriasis, le phénomène de Raynaud, le syndrome de Reiter, la fièvre rhumatique, l'arthrite rhumatoïde, la sarcoïdose, la sclérodermie, le syndrome de Sjögren, le syndrome de l'homme raide, le lupus érythémateux systémique, l'artérite de Takayasu, l'artérite temporelle/l'artérite à cellules géantes, la rectocolite hémorragique, l'uvéite, la vascularite, le vitiligo et la maladie inflammatoire de l'intestin ; ou
- d'un cancer choisi parmi le protooncogène ABL1, les cancers liés au SIDA, le neurome acoustique, la leucémie lymphocytaire aiguë, la leucémie myéloïde aiguë, le carcinome adénocystique, le cancer de la surrenalence, la métaplasie agnogène myéloïde, l'alopécie, le sarcome alvéolaire des parties molles, le cancer de l'anus, l'angiosarcome, l'anémie aplasique, l'astrocytome, l'ataxie-télangiectasie, le carcinome basocellulaire (peau), le cancer de la vessie, les cancers de l'os, le cancer de l'intestin, le gliome du tronc cérébral, les tumeurs cérébrales et du système nerveux central (SNC), le cancer du sein, les tumeurs du SNC, les tumeurs carcinoïdes, le cancer du col de l'utérus, les tumeurs cérébrales infantiles, le cancer infantile, la leucémie infantile, le sarcome des tissus mous de l'enfant, le chondrosarcome, le choriocarcinome, la leucémie lymphoïde chronique, la leucémie myéloïde chronique, les cancers colorectaux, le lymphome cutané à cellules T, le dermatofibrosarcome protuberans, la tumeur desmoplastique à petites cellules rondes, le carcinome canalaire, les cancers du système endocrinien, le cancer de l'endomètre, l'épendymome, le cancer de l'oesophage, le sarcome d'Ewing, le cancer des canaux de bile extra-hépatique, le cancer des yeux, le mélanome oculaire, le rétinoblastome, le cancer de la trompe de Fallope, l'anémie de Fanconi, le fibrosarcome, le cancer de la vésicule biliaire, le cancer de l'estomac, les cancers gastro-intestinaux, la tumeur carcinoïde gastro-intestinale, les cancers de l'appareil génito-urinaire, les tumeurs des cellules germinales,

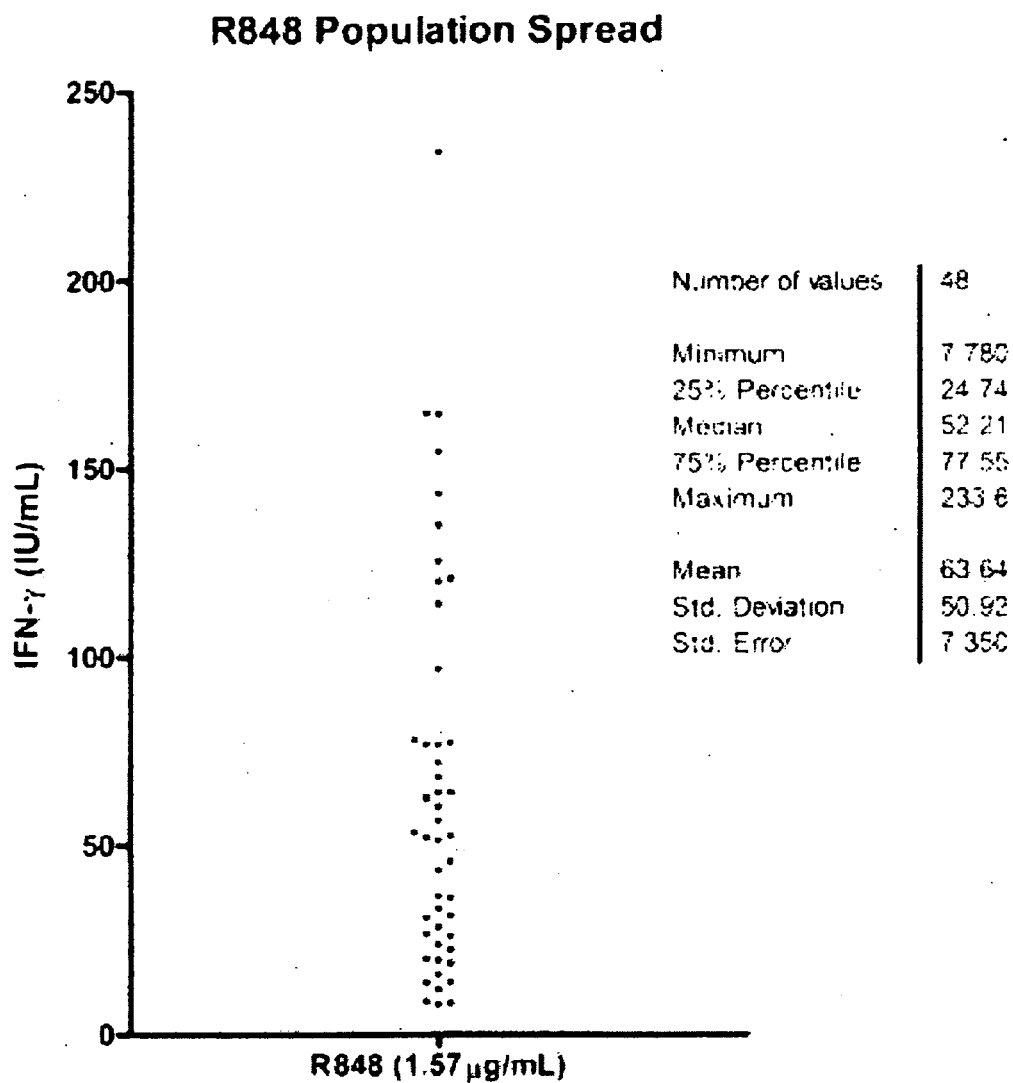
la maladie trophoblastique gravidique, le gliome, les cancers gynécologiques, les hémopathies malignes, la leucémie à tricholeucocytes, le cancer de la tête et du cou, le cancer hépatocellulaire, le cancer du sein héréditaire, l'histiocytose, la maladie de Hodgkin, le papillomavirus humain, la mole hydatiforme, l'hypercalcémie, le cancer de l'hypopharynx, le mélanome intraoculaire, le cancer des cellules des îlots pancréatiques, le sarcome de Kaposi, le cancer du rein, l'histiocytose des cellules de Langerhan, le cancer du larynx, le léiomyosarcome, la leucémie, le syndrome de Li-Fraumeni, le cancer de la lèvre, le liposarcome, le cancer du foie, le cancer du poumon, le lymphoedème, le lymphome, le lymphome de Hodgkin, le lymphome non-hodgkinien, le cancer du sein chez l'homme, la tumeur du rein rhabdoïde maligne, le médulloblastome, le mélanome, le cancer des cellules de Merkel, le mésothéliome, le cancer métastatique, le cancer de la bouche, l'adénomatose polyendocrinienne, la mycose fongoïde, les syndromes myélodysplasiques, le myélome, les troubles myéloprolifératifs, le cancer du nez, le cancer du nasopharynx, le néphroblastome, le neuroblastome, la neurofibromatose, le syndrome de Nimègue, le cancer de la peau sans présence de mélanome, le cancer du poumon non à petites cellules (NSCLC), les cancers oculaires, le cancer de l'oesophage, le cancer de la cavité buccale, le cancer de l'oropharynx, l'ostéosarcome, le cancer de l'ovaire d'ostomie, le cancer du pancréas, le cancer paranasal, le cancer de la parathyroïde, le cancer de la glande parotïde, le cancer du pénis, les tumeurs neuroectodennales périphériques, le cancer de l'hypophyse, la maladie de Vaquez, le cancer de la prostate, les cancers rares et troubles associés, le carcinome des cellules rénales, le rétinoblastome, le rhabdomyosarcome, le syndrome de Rothmund-Thomson, le cancer des glandes salivaires, le sarcome, le schwannome, le syndrome de Sézary, le cancer de la peau, le cancer du poumon à petites cellules (SCLC), le cancer de l'intestin grêle, le sarcome des tissus mous, les tumeurs de la moelle épinière, le carcinome épidermoïde de la peau, le cancer de l'estomac, le sarcome synovial, le cancer des testicules, le cancer du thymus, le cancer de la thyroïde, le cancer des cellules transitionnelles de la vessie, le cancer des cellules transitionnelles rhéro-pelviens/de l'urètre, le cancer trophoblastique, le cancer de l'urètre, le cancer du système urinaire, l'urolakins, le sarcome utérin, le cancer de l'utérus, le cancer vaginal, le cancer de la vulve, la macroglobulinémie de Waldenström et la tumeur de Wilms.

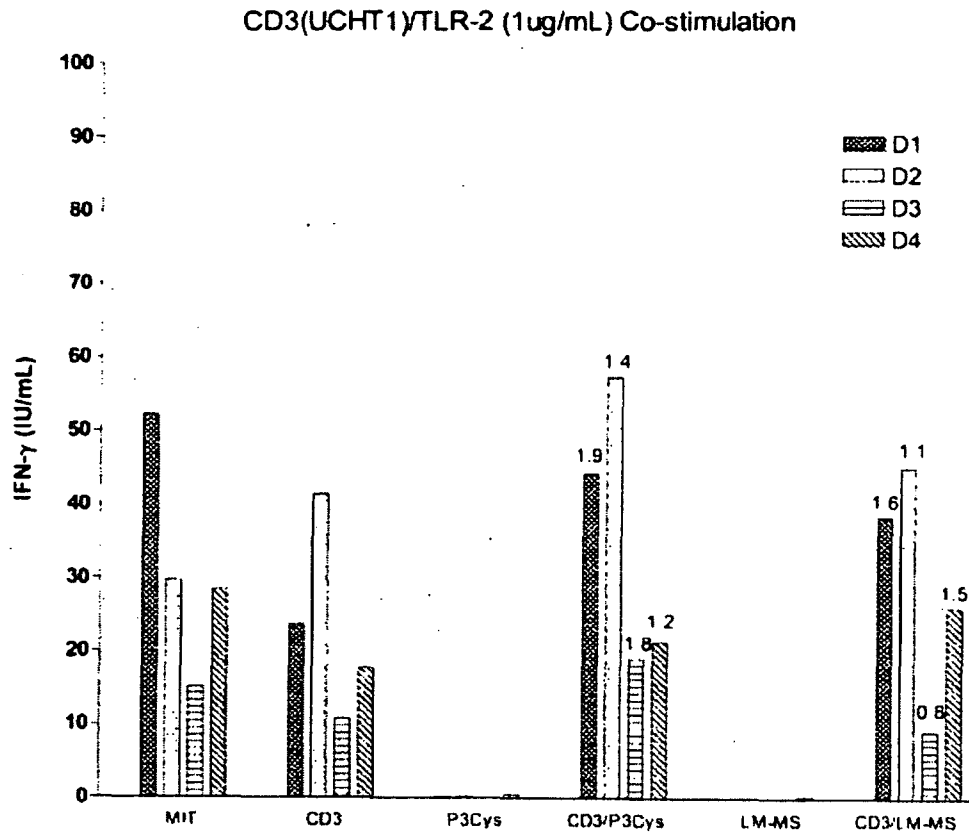
**14.** Procédé selon la revendication 1, dans lequel le ligand TLR-9 est un oligodésoxynucléotide CpG.

**15.** Procédé selon la revendication 1, dans lequel le ligand TLR-3 est un poly (I:C).

**Figure 1A**

**Figure 1B**

**Figure 1C**



**Figure 2A**

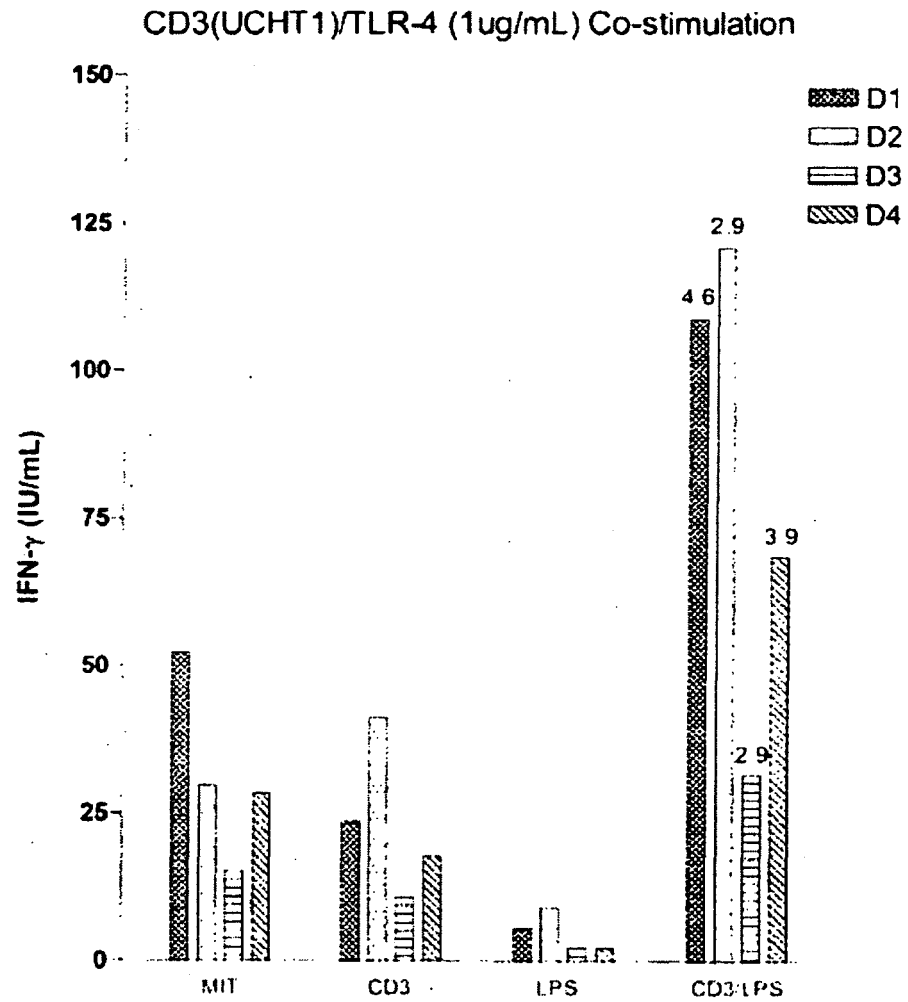


Figure 2B

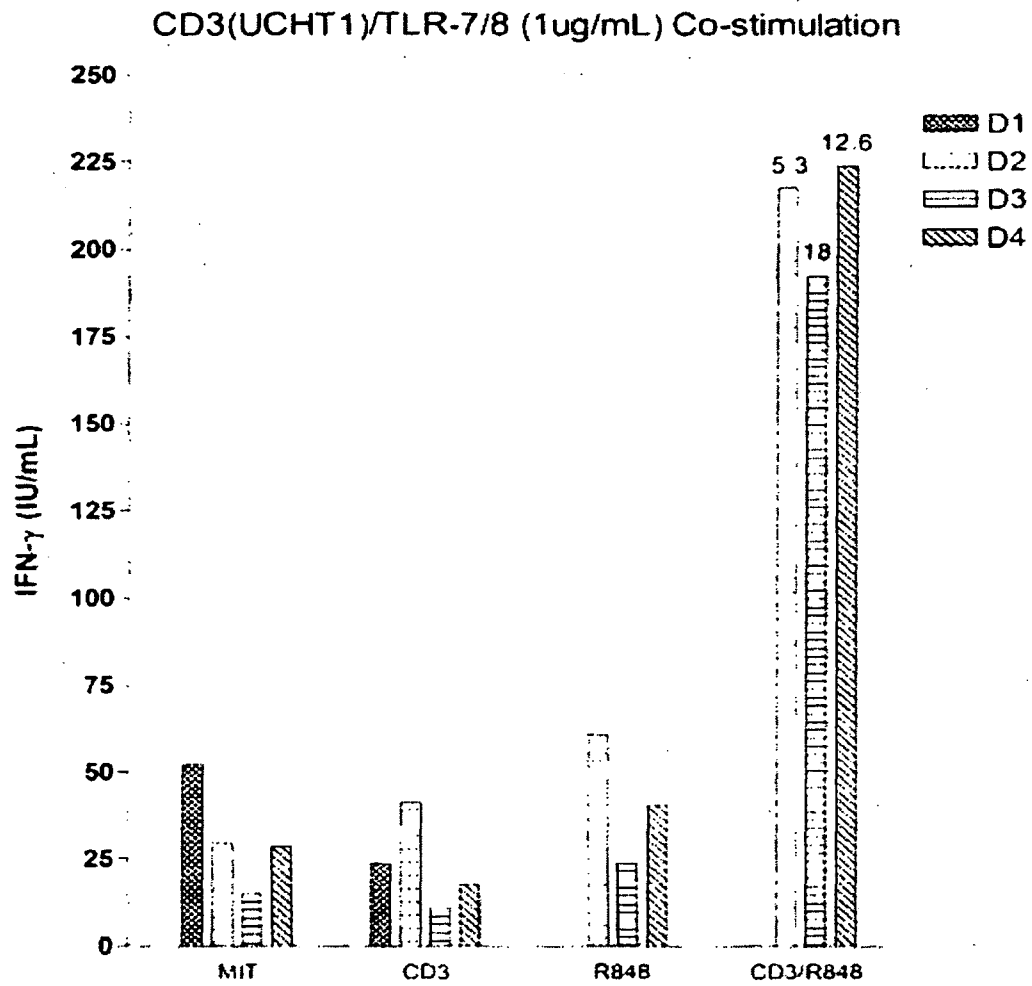
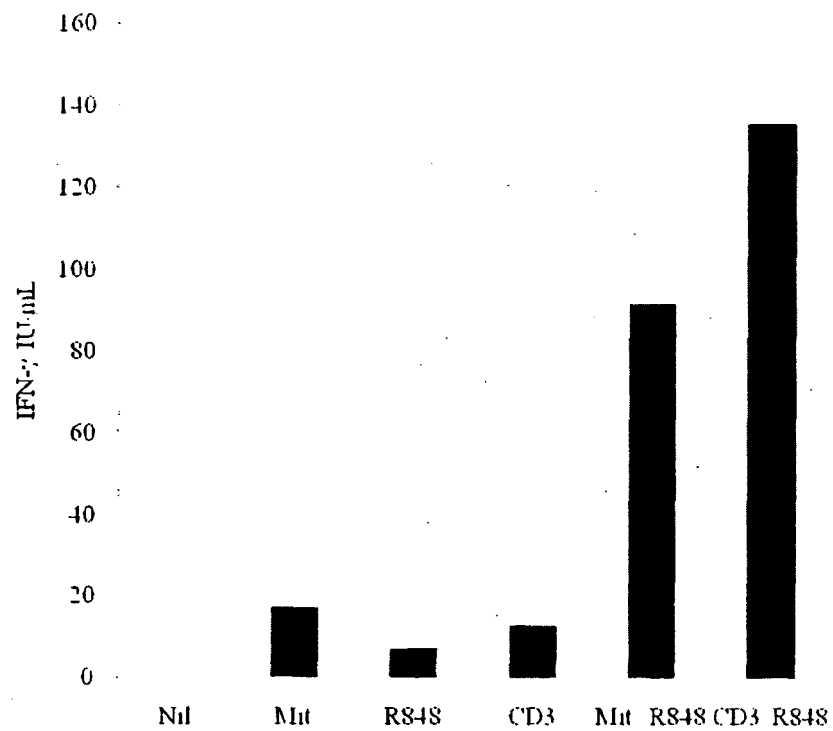
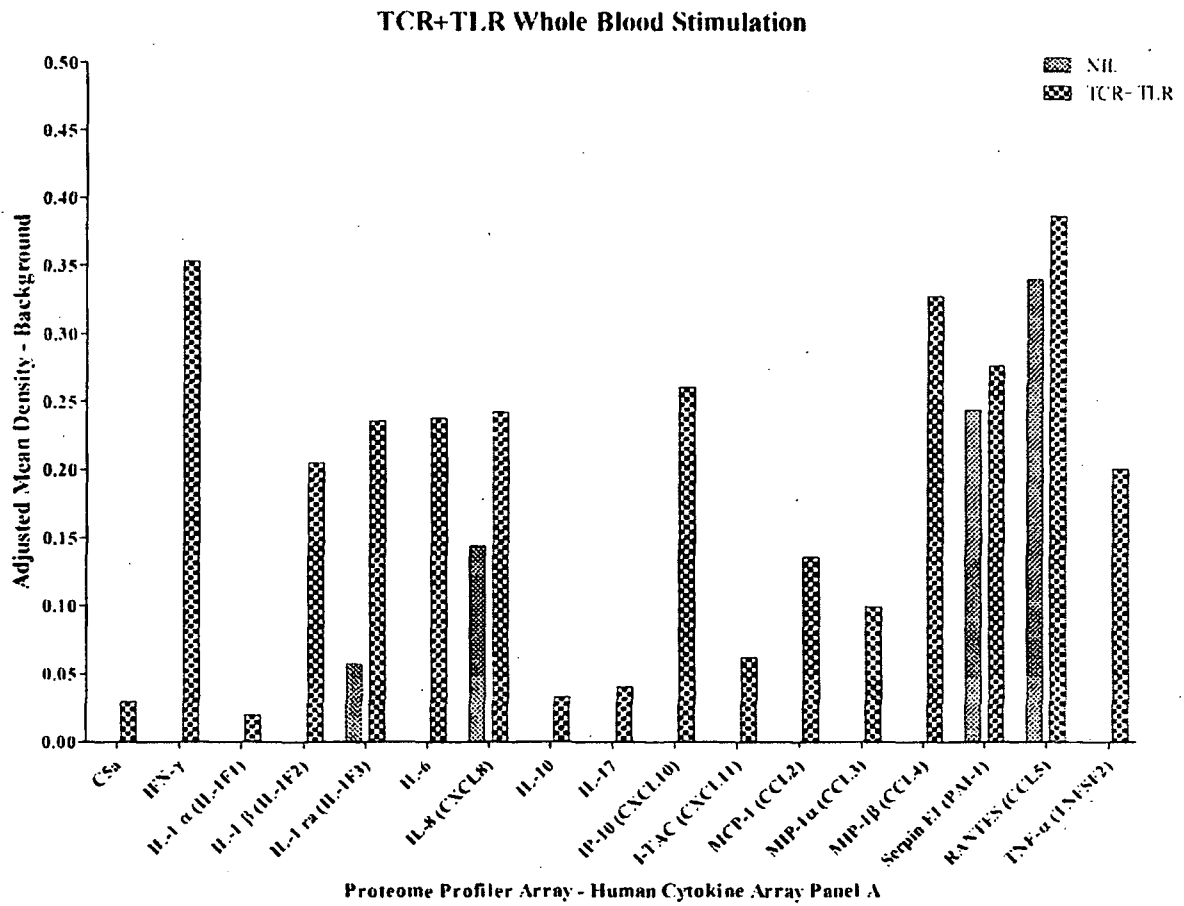


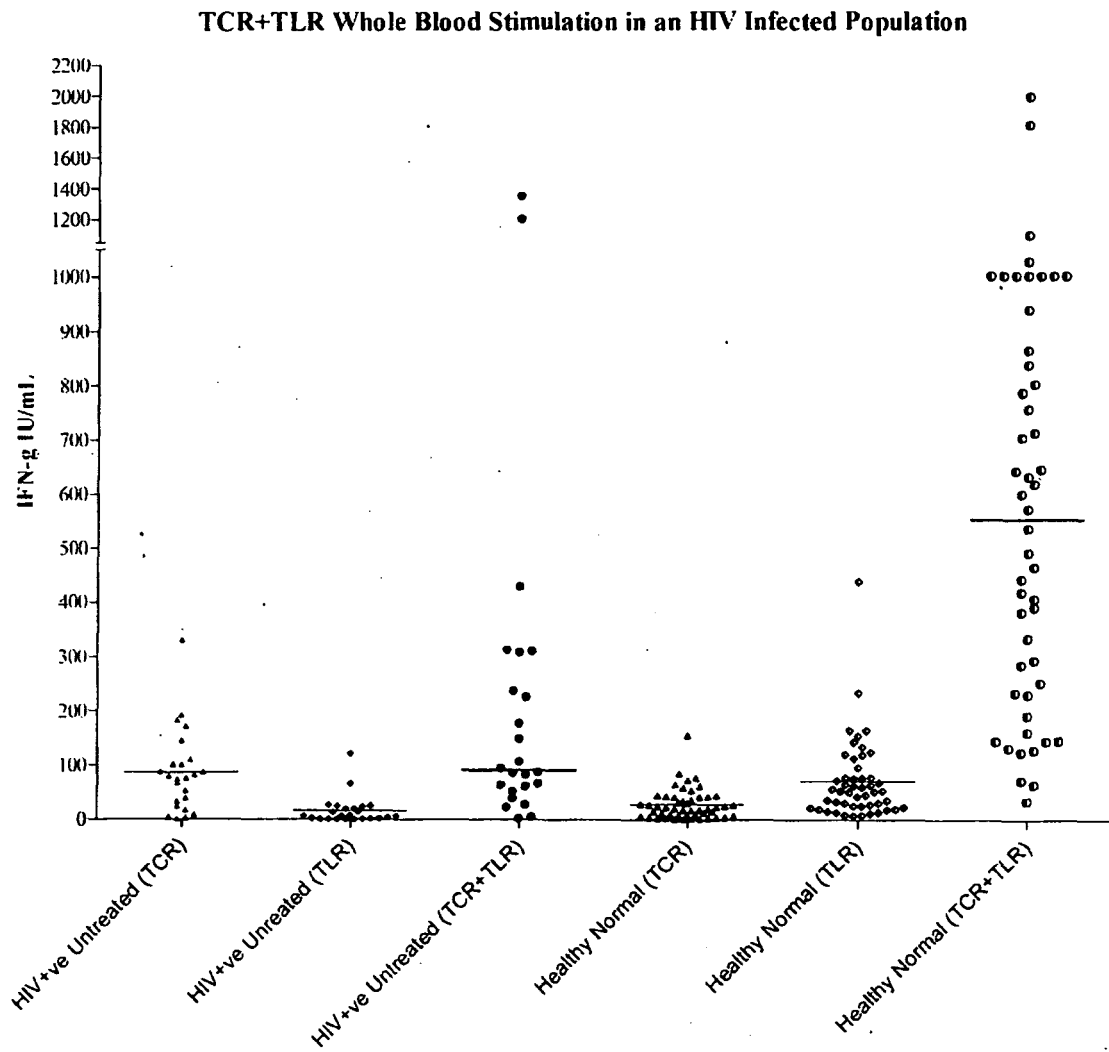
Figure 2C

### Representative Example



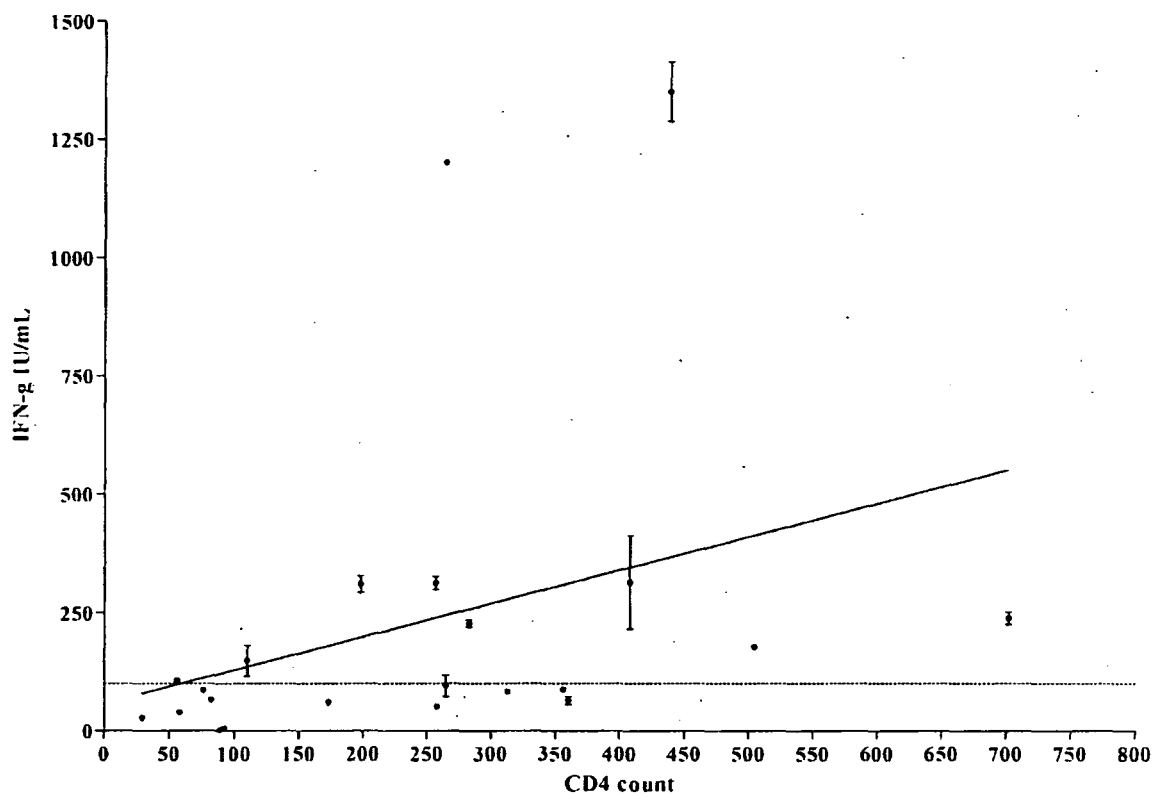
**Figure 3**

**Figure 4**



**Figure 5**

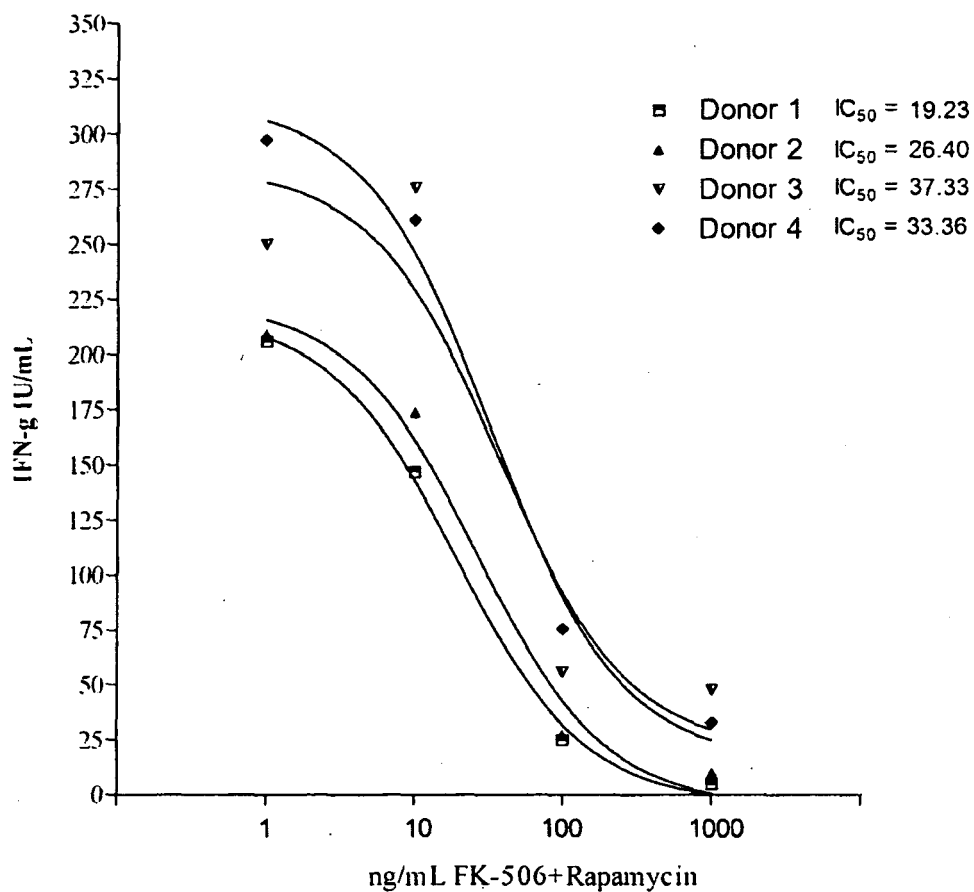
## TCR+TLR Stimulated INF-g Correlation with CD4 Count in an HIV Infected Population



|                                              |                        |
|----------------------------------------------|------------------------|
| Number of XY Pairs                           | 22                     |
| Spearman r                                   | 0.5626                 |
| 95% confidence interval                      | 0.1718 to 0.8004       |
| P value (two-tailed)                         | 0.0064                 |
| P value summary                              | **                     |
| Exact or approximate P value?                | Gaussian Approximation |
| Is the correlation significant? (alpha=0.05) | Yes                    |

Figure 6

Immunosuppressant (FK-506+Rapamycin) Treated Whole Blood  
Stimulated with TCR+TLR agonists



**Figure 7**

## REFERENCES CITED IN THE DESCRIPTION

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

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|----------------|------------------------------------------------------------------|---------|------------|
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#### 摘要(译)

本发明总体上涉及基于免疫学的诊断测定法领域。更特别地，本发明涉及一种用于测量细胞介导的免疫应答性的方法。本发明还使得能够确定疾病状况，治疗剂和环境污染物的免疫抑制作用。本发明的测定法还能够整合到病理学体系中以提供诊断报告系统并促进护理点临床管理。