Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2838041 C 2018/09/25

(11)(21) **2 838 041**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) **Date de dépôt PCT/PCT Filing Date:** 2012/05/02
(87) **Date publication PCT/PCT Publication Date:** 2012/11/08
(45) **Date de délivrance/Issue Date:** 2018/09/25
(85) **Entrée phase nationale/National Entry:** 2013/12/02
(86) **N° demande PCT/PCT Application No.:** US 2012/036103
(87) **N° publication PCT/PCT Publication No.:** 2012/151266
(30) **Priorités/Priorities:** 2011/05/03 (US61/481,991); 2011/08/29 (US61/528,493); 2011/11/30 (US61/565,225); 2012/01/04 (US61/582,878)

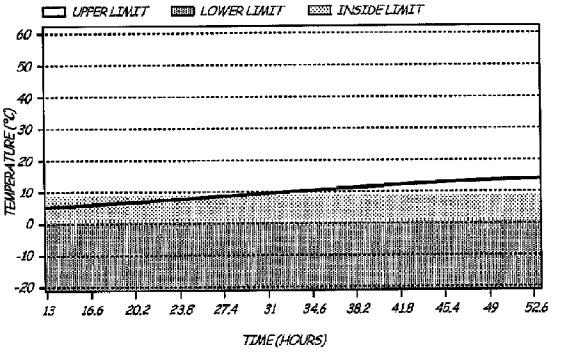
(51) **Cl.Int./Int.Cl.** ***A61K 39/00*** (2006.01), ***A61K 38/17*** (2006.01), ***A61P 35/00*** (2006.01), ***A61P 37/00*** (2006.01), ***G01N 33/53*** (2006.01)

(72) **Inventeur/Inventor:**
HAR-NOY, MICHAEL, IL

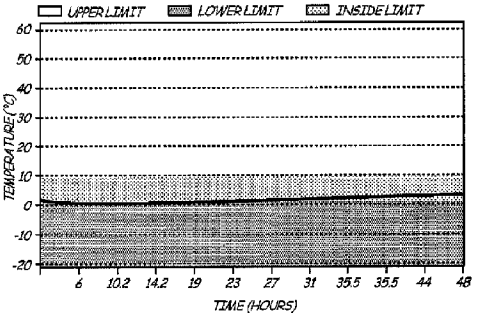
(73) **Propriétaire/Owner:**
IMMUNOVATIVE THERAPIES, LTD., IL

(74) **Agent:** MARKS & CLERK

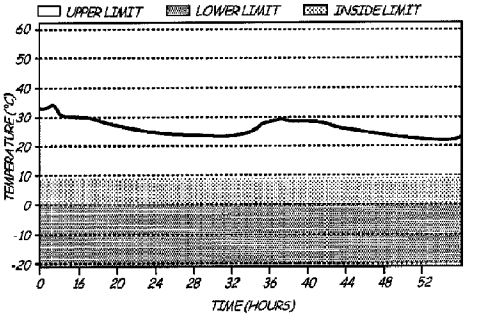
(54) **Titre : PROCEDES DE MANIPULATION DE MEDICAMENTS BIOLOGIQUES CONTENANT DES CELLULES VIVANTES**
(54) **Title: METHODS FOR HANDLING BIOLOGICAL DRUGS CONTAINING LIVING CELLS**



A



B



C

(57) **Abrégé/Abstract:**
The present invention includes methods for handling live cell compositions in non- nutritive buffer. The cells in the compositions maintain their identity and functional characteristics after being stored in non-nutrititive media up to about 72 hours. The storage

CA 2838041 C 2018/09/25

(11)(21) 2 838 041

(13) C

(57) **Abrégé(suite)/Abstract(continued):**
method enables the cells to be manufactured at a processing facility and shipped to a point of care site. The invention also includes compositions that have been stored in non-nutritive buffer at storage temperatures while maintaining the functional characteristics.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 November 2012 (08.11.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/151266 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *A61P 35/00* (2006.01)
A61K 38/17 (2006.01) *A61P 37/00* (2006.01)
G01N 33/53 (2006.01)

(21) International Application Number:
PCT/US2012/036103

(22) International Filing Date:
2 May 2012 (02.05.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/481,991	3 May 2011 (03.05.2011)	US
61/528,493	29 August 2011 (29.08.2011)	US
61/565,225	30 November 2011 (30.11.2011)	US
61/582,878	4 January 2012 (04.01.2012)	US

(72) Inventor; and
(71) Applicant : **HAR-NOY, Michael** [US/IL]; 22/1 Emek Zebulun, 71700 Modi'in, Jerusalem (IL).

(74) Agents: **SAWICKI, Z., Peter** et al.; Westman, Champlin & Kelly, P.A., 900 Second Avenue, South, Suite 1400, Minneapolis, MN 55402-3319 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) **Title**: METHODS FOR HANDLING BIOLOGICAL DRUGS CONTAINING LIVING CELLS

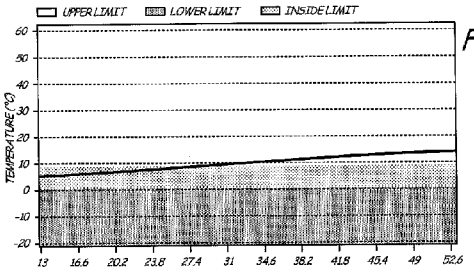


Fig. 1A

Fig. 1B

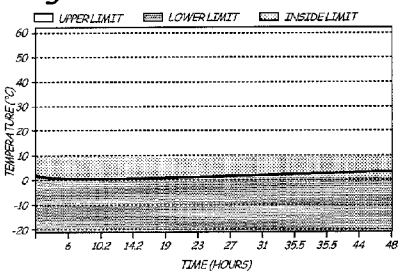
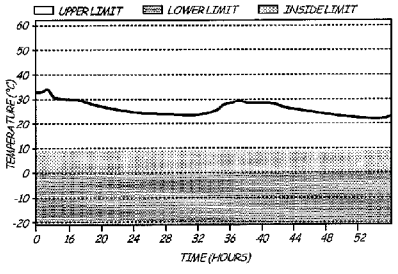


Fig. 1C



(57) **Abstract**: The present invention includes methods for handling live cell compositions in non- nutritive buffer. The cells in the compositions maintain their identity and functional characteristics after being stored in non-nutrititive media up to about 72 hours. The storage method enables the cells to be manufactured at a processing facility and shipped to a point of care site. The invention also includes compositions that have been stored in non-nutritive buffer at storage temperatures while maintaining the functional characteristics.

WO 2012/151266 A1

METHODS FOR HANDLING BIOLOGICAL DRUGS CONTAINING LIVING CELLS

FIELD

[0001] This invention relates to methods for handling biological drugs containing live cell suspensions formulated in non-nutritive buffer. More specifically, the present invention relates to the packaging, shipping, and distribution of live immune cell suspensions in non-nutritive media, whereby the cells maintain their unique identity, function and viability properties.

BACKGROUND

[0002] Cell therapy is a potentially curative therapy against tumors, viruses and bacterial pathogens. Cell therapy can also be used to treat autoimmune diseases (e.g. rheumatoid arthritis, multiple sclerosis and type I diabetes), neurological disorders (such as Alzheimer's, ALS and Parkinson's disease), as well as anti-aging treatment, wound healing and treatment of cardiovascular disorders. Harnessing the power of the immune system to treat or prevent diseases is a major goal of immunotherapy. A variety of immunotherapy methods and compositions have been developed in order to enhance or suppress the immune response in patients. Cell therapy methods often involve *ex-vivo* manipulations such as proliferation, differentiation and/or activation of cells. Cells that are more than minimally manipulated are considered to be biological drugs by the United States Food and Drug Administration (USFDA) as well as regulatory agencies in other jurisdictions. Before such biological drugs can be marketed for treatment or prevention of any disease, these products must first be investigated in human clinical trials under an Investigational New Drug Application (IND) or equivalent.

[0003] For commercial use, the processes used to manufacture biological drugs containing living cells must be standardized so that the resulting cells have pre-determined identity, functional and viability release criteria. The processes to cause the proliferation, differentiation and/or activation of cells intended for use as a biological drug generally occurs *ex-vivo* where the cells are kept in nutrient-rich culture media. However, prior to administering the cells to humans, the cells must be transferred to a non-nutrient infusion buffer. Because these buffer solutions do not contain nutrients, the cells remain viable for only short periods of time. Further, even if the cells remain viable

after being placed in non-nutrient infusion buffer, they quickly lose their unique identity and functional characteristics. Losing their unique identity and functional characteristics disqualifies the cells to be used as a biological drug. This limitation requires that cells intended for use of biological drugs must be formulated at or near the point-of-care. The requirement that cells be formulated at or near the point-of-care because of the limited shelf life of living cell products in formulation severely limits the commercial viability of this class of product.

[0004] Living cells are relatively stable in nutrient rich culture media. Examples of nutrient-rich culture media include, for example, X-Vivol 5 (BioWhittaker, Walkersville, MD), RPMI 1640, DMEM, Ham's F12, McCoys 7A and Medium 199. The medium can be supplemented with additional ingredients including serum, serum proteins, growth suppressing, and growth promoting substances, such as mitogenic monoclonal antibodies and selective agents for selecting genetically engineered or modified cells. However, transfer of the cells to non-nutritive buffer such as is required for administration to a patient can lead to rapid degradation of the cellular identity, cell viability and the functional characteristics of the cells. Examples of non-nutrient buffers include, for example, isotonic solutions such as normal saline, phosphate buffered saline, 5% dextrose, Plasma-Lyte (Baxter Scientific, Deerfield, IL) and Normasol (Abbott Laboratories, Abbott Park, IL). In addition, when cells are transferred to non-nutritive buffer, it is generally believed that reagents that provide activation and/or differentiation signals as well as other components such as stimulatory molecules or cytokines should be removed prior to transfer into non-nutritive buffer. (See U.S. Patent 6,867,041 to Berenson et al.) Therefore, cells in non-nutritive buffer generally have a limited shelf-life and can, for example, start losing their identifying properties and activity within minutes and rarely maintain their functional and identity characteristics for more than a few hours.

[0005] Currently, immunotherapeutic compositions that include living cells are generally produced in a cGMP facility close to the point of care of the patient (See US patent Publication no. 2003/0175242 to Gruenberg). Formulation of biological drugs with living cells must be performed under highly controlled and sterile conditions in cGMP facilities. The live cells are manipulated at the cGMP facility and formulated for infusion

into a patient. Once the cells are prepared for infusion, the cells are quickly transferred to the point of care site and administered to the patient. The major drawback of this process is that cGMP facilities need to be present near every point of care site. The cGMP facilities require considerable monetary capital to staff and run under the required rules and regulations. The need to establish a multiplicity of these centers at or near every point of care is cost prohibitive and a severe limitation to the commercial potential of this class of drug. This leads to a difficult choice of incurring great expense by building a large number of cGMP facilities in order to increase accessibility to patients or to providing limited accessibility for patients by building only a limited number of cGMP facilities to minimize the capital expenditures. Thus, there is a need in the field of live cell therapeutics for methods that enable cells in non-nutritive buffer to have a more extended shelf-life. Furthermore, a method is needed that would enable the packaging, shipping and mass distribution of formulated cell products suspended in non-nutrient containing infusion buffer.

[0006] Problems with maintaining the identity and function of cells used in adoptive immunotherapy after formulation are described, for example, in U.S. Patent Publication No. 2003/0175272 to Gruenberg. This publication teaches that T-cells must be reactivated just prior to patient administration (no more than 4 hours prior to infusion) to maintain functional characteristics of cytokine production. The function of the cells can be maintained up to 48 hours only if the formulation includes autologous plasma. However, collection of plasma from every intended patient is not conducive to mass distribution and commercialization.

SUMMARY

[0007] In a first aspect, this invention includes a biologic drug composition. The drug composition comprises living cells formulated in non-nutritive buffer. The living cells, after being stored for greater than about 6 hours in the non-nutritive buffer, maintain their identity and at least one functional characteristic that defined the living cells prior to formulation in the non-nutritive buffer. These living cells are useful in immunotherapy after storage in the non-nutritive buffer. They maintain their identity and at least one functional characteristic that defined the living cells prior to formulation in the non-nutrient buffer for at least 72 hours.

[0008] In another aspect, this invention includes a method of handling a biological drug composition with living cells. The method comprises formulating the living cells in a non-nutritive buffer and maintaining the living cells in the non-nutritive buffer at a storage temperature below about 20°C. The living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer. The living cells are useful for immunotherapy after being stored for greater than about 72 hours in the non-nutritive buffer. Preferably, the storage temperature is in a range between about 4°C and about 8°C and the concentration of the cells in the non-nutritive buffer is about 10^7 cells/ml or greater. In compositions of T-cells, the living cells are preferably formulated in an activated state. In order to activate the T-cells, it is preferable to use immobilized monoclonal antibodies reactive to cell surface molecules. Preferably, the cell surface molecules are a combination of first one of the following: CD3, MHCI, MHCII, CD2 and second a co-stimulatory molecule. Preferably the co-stimulatory molecule is CD28. The living cells are placed in a flexible container or syringe, wherein the flexible container or syringe is packaged in a temperature controlled device that maintains the living cells at the storage temperature. The method also includes shipping and distributing the package in the temperature controlled device to the point of care.

[0009] In yet another aspect, this invention includes a method of providing living cell compositions to a point of care facility. The method comprises formulating the living cells in a non-nutritive buffer at a processing facility and transporting the cells to a point of care facility in a package equipped to maintain a storage temperature below about 20°C. The living cells are at the storage temperature for up to about 72 hours while maintaining their identity and at least one of the functional characteristics of the living cells useful in immunotherapy.

[0010] In a further aspect, this invention includes a method of administering immunotherapy to a patient. The method comprises administering a composition that includes living cells formulated in non-nutritive buffer, wherein the composition has been stored for up to about 72 hours in the non-nutritive buffer, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that

defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful in immunotherapy.

[0011] In yet a further aspect, this invention includes another method of administering immunotherapy to a patient. The method comprises administering a composition that includes living cells formulated in non-nutritive buffer, wherein the composition was previously stored in a frozen state, for example in liquid nitrogen, for up to 2 years or more and has been thawed and formulated and then stored for up to about 72 hours in the non-nutritive buffer, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful in immunotherapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Fig. 1A is a plot of the temperature change in the container during transportation where the container was not preconditioned.

[0013] Fig. 1B is a plot of the temperature recorded inside the preconditioned aerogel insulated box.

[0014] Fig. 1C is a plot of the air temperature during transportation.

[0015] Figs. 2A-2C show the expression of CD40L for HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping.

[0016] Figs. 3A-3C show the cell viability of HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping.

[0017] Figs. 4A-4C show the secretion of IFN-γ of HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping.

[0018] Figs. 5A-5C show the secretion of IFN-γ of HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping followed by incubation at 37°C for 6 hours.

[0019] Figs 6A-6C show the expression of CD40L of HTC273, HTC264, and HTC245, respectively, cells and formulated for ID, IT and IV administration.

[0020] Figs. 7A-7C show the cell viability for HTC273, HTC264, and HTC245, respectively, cells and formulated for ID, IT and IV administration.

[0021] Figs. 8A-8C show the secretion of IFN-γ of HTC273, HTC264, and HTC245, respectively, cells and formulated for ID, IT and IV administration.

[0022] Figs 9A-9C show the expression of CD40L of HTC273, HTC264, and HTC245, respectively, cells formulated for ID, IT and IV administration and stored for 24 and 48 hours.

[0023] Figs. 10A-10C show the secretion of IFN-γ of HTC273, HTC264, and HTC245, respectively, cells formulated for ID, IT and IV administration and stored for 24 and 48 hours.

[0024] Figs. 11A-11C show the viability of HTC273, HTC264, and HTC245, respectively, cells formulated for ID, IT and IV administration and stored for 24 and 48 hours.

[0025] Figs. 12A-12C show the expression of CD40L, viability of cells, IFN-γ by the HTC264 cells after 24, 48 and 72 hours of storage.

[0026] Fig. 12D shows the secretion of IFN-γ by the HTC264 cells after 72 hours of storage and incubation at 37°C for 24 hours.

[0027] Figs. 13A-13C show the expression of CD40L, viability of cells, IFN-γ by the HTC245 cells after 24, 48 and 72 hours of storage.

[0028] Fig. 13D shows the secretion of IFN-γ by the HTC245 cells after 72 hours of storage and incubation at 37°C for 24 hours.

[0029] Figs. 14A-14C show the expression of CD40L, viability of cells, IFN-γ by the HTC273 cells after 24, 48 and 72 hours of storage.

[0030] Fig. 14D shows the secretion of IFN-γ by the HTC273 cells after 72 hours of storage and incubation at 37°C for 24 hours for 3 different batches of cells.

[0031] Fig. 15A-15C shows the CD40L expression for CAC and CFB after 48 hours for HTC245, HTC264, and HTC273, respectively.

[0032] Fig. 16A-16C shows the cell viability for CAC and CFB after 48 hours for HTC245, HTC264, and HTC273, respectively.

[0033] Fig. 17A-17C shows the secretion of IFN-γ for CAC and CFB after 48 hours HTC245, HTC273, and HTC264, respectively.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0034] This invention relates to the packaging, storage and distribution of live cell biological drug products formulated in non-nutritive buffer that can exhibit cellular characteristics useful for immunotherapy even after extended periods of time. These live

cell biological drugs can maintain viability as well as pre-determined identity and functional characteristics even after about 72 hours in the non-nutritive buffer.

[0035] In some exemplary embodiments, immune Th1 memory cells used as a biological drug product can maintain viability, retain pre-determined identity (CD4+, CD45RO+, $CD40L^{hi}$, $CD62L^{lo}$) and recover functional criteria such as secretion of IFN-gamma > $1000pg/10^6$ cells/4h. These immune Th1 memory cells can exhibit these cellular characteristics for up to at least about 72 hours when formulated in a non-nutritive buffer with CD3/C28-coated microbeads and maintained in a refrigerated state.

[0036] The biological products that include the living cells can be packaged in environmentally controlled conditions to maintain the desired storage conditions and shipped nearly anywhere in the world to points-of-care by an express courier service (e.g. Federal Express, United Parcel Service (UPS), and similar international couriers). Preferably, the package with the living cells is stored and transported under refrigeration temperatures. At the point-of-care, the formulated cells can be removed from the package and administered to a patient. The formulated cells remain stable after removing from the refrigerated packaging for up to about 6 hours. Preferably, the cells are first removed from refrigerated packaging at the point-of-care and allowed to equilibrate to room temperature from 1-2 hours prior to patient administration. The transported cells are surprisingly stable and can be used for similar methods as cells that were not stored for extended periods of time. Alternatively, the cells are stored and transported in a frozen state to a point-of care. The cells can be stored in a frozen state at a point-of-care and be formulated in an automated or semi-automated closed, sterile system and then stored on-site in a refrigerated state for up to about 72 hours before administration to a patient.

[0037] Living cells can be any cell that is more than minimally manipulated as that term is used by the FDA to determine that the cell product is a biological drug requiring evaluation in humans only under an Investigational New Drug (IND) Application or equivalent and manufactured under Good Manufacturing Practices (GMP) in accordance with 21 C.F.R. parts 211, 606 and 820 as applicable.

[0038] The living cells can be of a single type or a mixture as long as they have defined identity and functional criteria. The cells can be natural or engineered, derived from autologous, allogeneic and/or xenogeneic donors. While the living cells are the active

ingredient of the biological drug, other substances can be added to the cells, such as biologically active proteins, peptides, chemicals, nucleotides (RNA, DNA) and/or devices. The cells can be freely suspended in formulation or attached to a surface or device or encapsulated in a device or material. The cells should be intended to treat or prevent the occurrence of a disease or condition. The cells can be infused, injected or implanted in any location of the body.

[0039] By functional characteristics, it is meant to include a variety of functions, particularly immune functions and differentiation functions performed by the cells and useful in immunotherapy and stem cell therapy. These immune functions can include, for example, secretion of molecules, expression of cell surface moieties, recognition of molecules, the ability to respond to molecules and grow and/or change into a particular cell type or cause other cells in the body to grow, change, die or in some manner alter the normal or disease function as well as other immunological and cell differentiation functions known in the art. The immunological functions can be processes, or cascades of processes or production of molecules that are involved in the innate and/or the adaptive immune system response or modulation of the adaptive or innate immune response. The functions may be related to cell mediated immunological functions and/or to the humoral system, both immunostimulatory and immunosuppressive functions. The functional characteristics may be related to immunological memory or related to distinguishing between self and non-self antigens, or recognition of pathogens, such as bacteria, viral or fungus as well as tumors or other abnormal or undesired cells or tissues. Other functions may be related to surface molecules which mediate such functions as trafficking to a particular organ or tissue or location, surface molecules that block, promote or otherwise modulate immune responses or enable the differentiation to a particular cell type.

[0040] This disclosure describes biological drug products that include living cells formulated in non-nutritive buffer as the active ingredient. In some embodiments, the cells are living immune cells that can be used for immunotherapy or stem cell therapy. The compositions are stable in non-nutritive buffer for at least about 6 hours at room temperature and for at least about 24, preferably at least about 48, and more preferably at least about 72 hours at refrigeration temperatures. Surprisingly, the live cells in the

compositions can maintain their identity, viability and functional characteristics that they exhibited in nutrient containing media even after formulation into non-nutrient buffer. The compositions described herein can be packaged and advantageously be shipped and distributed using commercial couriers in containers that maintain the appropriate storage conditions from a processing facility to a point of care. Such capabilities can result in substantial savings of labor, time and money in production and administration of therapeutic compositions containing live cells. Furthermore, accessibility of live cell therapeutic compositions for patients is greatly enhanced since a processing facility can produce, package and distribute the cells to any point of care site in the world.

[0041] This disclosure also describes methods of maintaining live cell suspensions for extended periods of time in non-nutritive buffer. The methods include transferring the live cells into non-nutritive buffer and storing them at a cooler storage temperature. In some embodiments, the live cell compositions are stored under refrigeration conditions. When desired, the compositions are removed from storage and placed at room temperature for a period of time. In some embodiments, the functional characteristics of the live cells are substantially recovered after the live cell suspensions have been placed at about room temperature for a period of time. In other embodiments the functional characteristics of the live cells are substantially recovered after the live cell suspensions have been placed in physiological conditions for a period of time.

[0042] The therapeutic compositions described herein include live cells. By live cells, it is meant that >70% of the cells are viable as determined by appropriate assay techniques such as trypan blue extrusion, MTT or bioluminescent detection of the ATP levels such that the cells are capable of *ex vivo* manipulations such as expansion, differentiation, and/or activation under appropriate conditions. The compositions, however, may include some inactivated cells, radiated cells and/or non-viable cells. The live cells may be derived from a number of sources including, for example, immortalized cell lines, primary cell cultures, biological fluids, tissues, cord blood, peripheral blood, bone marrow, frozen aliquots of cells and the like. Live cells derived from other sources that are capable of *ex-vivo* manipulations as described above are also within the scope of the invention.

[0043] The cells in the therapeutic composition can be allogeneic cells. Cells derived, for example, from blood or marrow of allogeneic donors may be processed in a desired manner and then formulated for infusion into a patient. The infusion formulation placed in a syringe or transfer pack or other suitable device for holding human use products can be packaged and shipped to the point of care site for patient administration. Alternatively, the cells in the therapeutic composition may be autologous cells that have been manipulated, formulated, packaged and shipped and are to be reinfused into the same patient. The living cells may also be derived from a non-human source and have been manipulated, formulated, packaged and shipped for human administration (xenogeneic). The same therapeutic compositions described for human administration can also be used in non-human therapeutic and disease prevention settings.

[0044] In an embodiment where the live cells in the compositions are immune cells, these immune cells can include cells derived from bone marrow or cord blood, granulocytes, such as neutrophils, basophils, and eosinophils. The immune cells can also be monocytes, macrophages, dendritic cells, natural killer cells, lymphocytes including B-cells, T-cells and NKT cells. T-cells can be, for example, CD4+ cells (including Th0, Th1, Th2, Th17 and Treg cells) and/or CD8+ cells (Tc1 and Tc2).

[0045] One immunotherapy method for enhancing the cellular immune response in subjects is a type of cell therapy called adoptive immunotherapy. A cell therapy is a drug whose active ingredient is wholly or in part a living cell. Adoptive immunotherapy is a cell therapy that involves the removal of immune cells from a subject, the *ex-vivo* processing (i.e., activation, purification and/or expansion of the cells) and the subsequent infusion of the resulting cells back into the same subject (autologous therapy) or into a different subject (allogeneic therapy).

[0046] The biological drug product can include live cells that have been manipulated using a variety of *ex vivo* manipulations for adoptive immunotherapy. Live cells that have been manipulated *ex vivo* can include, for example, LAK cells (Rosenberg U.S. Pat. No. 4,690,915), TIL cells (Rosenberg U.S. Pat. No. 5,126,132), cytotoxic T-cells (Cai, et al U.S. Pat. No. 6,255,073; Celis, et al. U.S. Pat. No. 5,846,827), expanded tumor draining lymph node cells (Terman U.S. Pat. No. 6,251,385), various preparations of lymphocytes (Bell, et al U.S. Pat. No 6,194,207; Ochoa, et al. U.S. Pat. No 5,443,983;

Riddell, et al. U.S. Pat. No. 6,040,180; Babbitt, et al. U.S. Pat. No. 5,766,920; Bolton U.S. Pat. No. 6,204,058), CD8+ TIL cells (Figlin et al. (1997) Journal of Urology 158:740), CD4+ T-cells activated with anti-CD3 monoclonal antibody in the presence of IL-2 (Nishimura (1992) J. Immunol. 148:285), T-cells co-activated with anti-CD3 and anti-CD28 in the presence of IL-2 (Garlie et al. (1999) Journal of Immunotherapy 22:336) antigen-specific CD8+ CTL T-cells produced ex-vivo and expanded with anti-CD3 and anti-CD28 monoclonal antibodies (mAb) in the presence of IL-2 (Oelke et al. (2000) Clinical Cancer Research 6:1997), and injection of irradiated autologous tumor cells admixed with Bacille Calmette-Guerin (BCG) to vaccinate subjects followed seven days later by recovery of draining lymph node T-cells which are activated with anti-CD3 mAb followed by expansion in IL-2 (Chang et al. (1997) Journal of Clinical Oncology 15:796).

[0047] In one exemplary embodiment, the therapeutic composition of this disclosure includes at least some T-cells, preferably allogeneic T-cells. These T-cells are also preferably activated through cell surface activation to form activated Th1 memory cells. The T-cells may be activated in a variety of ways including by the use of immobilized monoclonal antibodies specific for T-cell surface molecules. Suitable activated T-cells are, for example, described in U.S. Patent No. 7,435,592. The cells preferably have cell surface moieties that are cross-linked by monoclonal antibodies or other binding agents. These monoclonal antibodies and/or binding agents are preferably cross-linked by, for example, immobilization on a solid surface in order to activate the T-cells. These are referred to herein as cells activated in culture (CAC). These *ex vivo* prepared CAC can be frozen for future use or formulated for infusion.

[0048] In preferred embodiments, the *ex vivo* prepared CAC are stored frozen until needed for patient administration. Prior to administration to the patient the CAC are thawed, washed and reactivated in nutrient media by cross-linking of the cell surface binding moieties such as CD3 and CD28 as described, for example in U.S. Patent No. 7,402,431. The CAC, together with the cross-linking agent, can then be washed and transferred to a non-nutritive buffer such as a formulation buffer. The reactivated cells in formulation buffer are referred to herein as

cells in formulation buffer (CFB). The CFB can be administered to the patient for therapeutic purposes. Generally, these reactivated cells, once transferred to non-nutritive buffer have a limited shelf life. Living cells can be formulated at a density of at least about 10^6 cells per ml, preferably at about 10^7 cells per ml or higher. In some embodiments, the living cells may be formulated at a density at about 10^8 cells per ml or higher. The specific concentration of the cells may be determined by the specific use of the cells and the therapy protocol.

[0049] The therapeutic composition may also include a number of other components. These components can include, for example, agents that maintain the live cells in the desired activation state. In one exemplary embodiment, the therapeutic composition can include agents that maintain the T-cells in an activated state such as Dynabeads ClinExVivo™ described below in the Examples.

[0050] The present invention includes methods of storing and handling the live cell compositions to increase the shelf life. Shelf life as used herein is defined as the amount of time after formulation that the CFB maintain viability, pre-defined identity and functional characteristics. Generally, the cells are transferred to non-nutritive buffer that is appropriate for infusion into a patient. The cells can be in a variety of non-nutritive buffers. Non-nutritive buffer, as referred to herein, is any type of media, buffer or other liquid that lacks the appropriate components to support cellular proliferation and/or expansion. The non-nutritive buffers generally are isotonic, USP sterile, pyrogen-free and contain the appropriate components and/or buffering system to maintain live cells intact and are licensed for human parenteral use. In an exemplary embodiment, the non-nutritive buffer is a formulation buffer that is Plasmalyte A (Baxter Scientific, Deerfield, IL) with 1% human serum albumin. (McKesson, San Francisco, California)

[0051] In embodiments with activated cells, particularly activated Th1 cells, the activation signals for the cells are maintained even when the cells are transferred to the non-nutritive buffer. For example, in embodiments where the cells are activated by cross-linking the cell surface binding moieties, the cross-linking is preferably maintained in the non-nutritive buffer. The maintenance of the cross-linking during storage can be critical to restoring the functional characteristics of the composition after removal from storage. Cell compositions in which the activating components are removed in non-

nutritive buffer do not recover in the same manner as the cells that have maintained the activated state.

[0052] The methods described herein also include the handling of the live cells after the cells are transferred into a non-nutritive buffer. The live cell composition can be transferred to an environment with a cooler temperature for storage in order to increase the shelf life of the compositions. The cooler temperature to which the cells in non-nutritive buffer are transferred to is referred to herein as the storage temperature. The cells are generally transferred to the storage temperature as quickly as possible after being placed in the non-nutritive buffer. The cells are preferably transferred to the storage temperature in less than about six hours after being placed in non-nutritive buffer, more preferably in less than about four hours after being placed in non-nutritive buffer. In even more preferred embodiments, the cells are transferred to the storage temperature in less than about one hour after being placed in the non-nutritive buffer.

[0053] The storage temperature at which the compositions can be held varies but is generally below physiological temperature i.e. below at about 37°C. Preferably, the cells are stored at refrigeration temperatures. Refrigeration temperatures can be between the range of about -2°C and about 12°C. More preferably, the cells are stored at a temperature between above 0°C and about 10°C. Most preferably, the cells are stored between about 4°C and about 8°C.

[0054] The compositions described herein may also be packaged, shipped and distributed from a manufacturing or processing facility to a point of care site. A manufacturing or processing facility can be a facility such as a hospital, clinic or any production facility capable of handling living cells for biological drugs in compliance with established guidelines. A point of care can be a hospital, clinic or any other site at which a patient is generally administered care. The compositions are generally packaged for shipping in a manner that maintains the compositions within the storage temperature range stated above. The cells can be stored and shipped in a variety of containers. The cells can be stored and shipped in, for example, a flexible container, syringe and the like. When shipping, the container such as a syringe can be placed in a package such as an insulated box. The package or box is, preferably, preconditioned at the desired storage temperature prior to the container with the live cells being placed in the package. The compositions,

for example, can be packaged in ice or aerogel packed boxes. The packages are preferably insulated boxes that are able to maintain the desired storage temperatures, regardless of the external temperature. The boxes are also preferably preconditioned, meaning they have been stored or set at the desired temperature prior to container with the living cells being placed inside. In preferred embodiments, the packages are preconditioned prior to placement of the biological product and packages are transported under refrigeration or freezing conditions to the point of care. Any type of shipping method may be used but in exemplary embodiments shipping is by commercial couriers.

[0055] The shelf life of the compositions described herein can be surprisingly extended when the compositions are stored within the storage temperature range. The shelf life of the live cell compositions can be extended for greater than about 6 hours. Preferably, the shelf life of the live cell compositions can be extended for greater than about 24 hours, and more preferably for greater than about 48 hours. In even more preferred embodiments, the shelf life of the compositions can be extended for up to about 72 hours. In the most preferred embodiment, the shelf life can be extended for up to about 120 hours. Shelf lives of greater than about 120 hours are also within the scope of this invention.

[0056] The cellular compositions in non-nutritive buffer stored according to the methods described herein can maintain their viability, identity and function during the storage period and after removal from storage. The viability of the cells can be determined by a variety of methods known in the art include assay techniques such as trypan blue extrusion, MTT, 7-Amino-Actinomycin D or bioluminescent detection of the ATP levels.

[0057] The identity of the cells can be confirmed by a variety of methods. The cells can be assayed for a variety of external and internal cell markers that are indicative of the particular cell type in the composition. External markers are categorized by the Cluster designation of monoclonal antibodies (cluster of differentiation (CD) designated from 1st to 8^{th} workshops on international human leukocyte differentiation antigens with total number of (247) CDs. Leukocytes express distinct assortments of molecules on their cell surfaces, many of which reflect either different stages of their lineage-specific differentiation or different states of activation or inactivation. Leukocyte cell surface molecules are routinely detected with anti-leukocyte monoclonal antibodies (mAbs).

Using different combination of mAbs, it is possible to chart the cell surface immunophenotypes of different leukocyte subpopulations, including the functionally distinct mature lymphocyte subpopulations of B-cells, helper T-cells (Th), cytotoxic T-cells (Tc), and Natural Killer (NK) cells.

[0058] Even after storage in non-nutritive media, the live cells in the composition exhibit the functional characteristics that were present prior to formulation in the non-nutritive media. Functional characteristics can include a variety of activities including, for example, expression of functional molecules such as CD40L, FasL, perforin and granzymeB, co-stimulatory molecules 4-1BBL, CD28, CTLA4, and TNF-related activation-induced cytokine (TRANCE), TWEAK, PD-1, B7 family, adhesion molecules such as the integrins, the cadherins, and the selectins and secretion of a variety of cytokines and chemokines and expression of receptors for these cytokines and chemokines. Cytokines and chemokines are redundant secreted proteins with growth, differentiation, and activation functions that regulate and determine the nature of immune responses and control immune cell trafficking and the cellular arrangement of immune organs. Cytokines can include, for example, IL2, IL3, IL4, IL5, IL6, GMCSF, IFN-gamma and the like.

[0059] In some embodiments, the functional characteristics are retained after formulation and throughout storage and the levels of the enzymes or the markers can be assayed soon after removal from storage and shipping. For example, the CD40L expression can be assayed after the compositions are removed from storage and allowed to incubate at RT for about 2 hours. The CD40L expression can be similar to the levels of CD40L expression at the time of formulation and storage. See, for example, Figs. 2A-2C. Similarly, the number of viable cells in the compositions can be determined after removal from storage and incubation at RT for about 2 hours. The number of viable cells can be similar to the cell viability levels at the time of formulation and storage. See, for example, Figs. 3A-3C.

[0060] In other embodiments, the functional characteristics can be recovered after the cells are exposed to physiological conditions. This can indicate that the cellular compositions, upon infusion into a patient, can function as intended and secrete or express components characteristic of the cells at the time of formulation. The secretion

of IFN-γ, for example, can be depressed when the cells are formulated and placed in storage. IFN-gamma may be referred to herein as IFN-γ or IFN-g. The return of the cells to room temperature does not restore the secretion of IFN-g but incubating the cells at 37°C for 24 hours increases the secretion of the IFN-γ to levels similar to the levels at the time of formulation. See, for example, Figs. 12D, 13D and 14D. Advantageously, the decrease in the IFN-γ levels during storage can prevent exhaustion of cellular resources. If the cellular resources for secretion are sufficiently preserved during storage, then the cells generally can restart the secretion of the IFN-γ under appropriate physiological conditions. Thus, administration of the composition to a patient can then still provide the patient with the IFN-γ and other inflammatory cytokines derived as a result of the administration of the therapeutic composition, even though the composition has been stored for an extended period of time prior to administration.

[0061] Extension of the shelf life of the compositions can be demonstrated in a variety of ways. As used herein, extension of shelf life can refer to the live cells in the compositions maintaining their viability, identity and their functional characteristics even after the extended storage times described above. Generally, after storage for at least 24 hours, the compositions maintain at least about 50 percent of the activity of a defining characteristic in non-nutritive buffer relative to the activity at the time of formulation. Preferably, at least about 75 percent and more preferably, at least about 85 percent and even more preferably, at least about 90 percent of the activity is maintained after storage relative to the activity at the time of formulation.

[0062] In preferred embodiments, after storage for at least 48 hours the compositions maintain at least about 50 percent of the activity of a defining characteristic in non-nutritive buffer relative to the activity at the time of formulation. Preferably, at least about 75 percent and more preferably, at least about 85 percent and even more preferably, at least about 90 percent of the activity is maintained after storage relative to the activity at the time of formulation.

[0063] In more preferred embodiments, after storage for at least 72 hours, the compositions maintain at least about 50 percent of the activity of a defining characteristic in non-nutritive buffer relative to the activity at the time of formulation. Preferably, at least about 75 percent and more preferably, at least about 85 percent and even more

preferably, at least about 90 percent of the activity is maintained after storage relative to the activity at the time of formulation.

[0064] The cellular compositions can be administered to a patient using a variety of methods. The compositions may be administered intradermally, intravenously, intrathecally, intratumorally and the like.

EXAMPLES

[0065] Materials: PE-conjugated CD40L was purchased from Beckman Coulter, Brea, CA. 7-Amino-Actinomycin D (7-AAD) (1000x) was purchased from Cayman Chemical Co., Ann Arbor, MI. PlasmaLyte A was purchased from Baxter Scientific, Deerfield, IL. Human serum albumin (HSA) was purchased from McKesson, San Francisco, California. FcR Binding Inhibitor was purchased from eBioscience, San Diego, CA. Dynabeads ClinExVivo™ was purchased from Invitrogen, Carlsbad, CA.

[0066] Preparation of Cells in Formulation Buffer (CFB) - Cells activated in culture media(CAC) were placed into cRPMI media for washing. Time was recorded to indicate the beginning of the formulation protocol. The cells in cRPMI media were centrifuged, the supernatant removed and the cells resuspended in cRPMI buffer. Cell viability was determined by using Trypan Blue assays. The total cell number and the concentration of live cells were used to determine the percentage of viable cells. If the sample had greater than 80 percent cell viability, then the procedure was continued for reactivation and formulation of cells.

[0067] The CAC cells were resuspended at a concentration of 1 x 10^7 cells/ml. Reactivation was done at a live cell concentration of 1 x 10^7 cells/ml. Reactivation was done in a 24 well plate, 6 well plate or a 75 cm^3 flask depending on the volume. Dynabeads ClinExVivo™ CD3/CD28 were added to reactivate the cells and incubated at 36-38°C and 5% CO_2 for 4 hours. After incubation for about 4 hours, then the cells were removed and transferred to a 50 ml. tube with final formulation buffer (FFB). FFB is PlasmaLyte A with 1% HSA. The reactivated cells were centrifuged, supernatant removed and resuspended in FFB. These are referred to as cells in formulation buffer (CFB).

[0068] CFB were resuspended in FFB at a concentration 10^7 cells per ml. The CFB were resuspended for ID, IT or IV administration. 1ml of the cell suspension was added to a 3ml syringe as an ID formulation. IT and IV formulation were 3 ml and 5 ml, respectively. The syringes with the appropriate formulations were stored in refrigeration with an average temperature of about 4°C.

[0069] Harvesting of samples after storage - The cells and supernatant were collected at different time points. The time points were as follows: 0(initial); 2 hours at Room Temp (RT); 48 hours at 4°C; and 48 hours at 4°C followed by 2 hours RT.

[0070] At each time point, 100ul cell suspensions were collected and the cells were spun at 400g for 5 min at 4°C. The supernatant was then transferred to another tube for IFN-γ detection later using ELISA. The cells were resuspended in 150ul staining buffer for flow cytometry. In some experiments, the cells were resuspended in 100ul cRPMI medium and cultured in the incubator at 37°C for 24 h with 5% CO_2. The supernatant was taken after 24 h incubation and the IFN-γ was detected by ELISA.

[0071] Flow cytometry (CD40L and 7-AAD) - 50ul cell suspension were transferred from above (150 ul) into 3 eppendorf tubes, labeled as unstained, CD40L and 7-AAD, respectively. The unstained tube was incubated on ice for 20 min. For the CD40L tube, the cells were pre-incubated with FcR Binding inhibitor according to the instructions of the manufacturer for 20 minutes on ice. Then 40ul staining buffer (PBS+1%FBS) and 10ul PE-CD40L antibody was added into the cell suspension and incubated for additional 20 min on ice in the dark.

[0072] Cell viability was tested by flow cytometry of 7-AAD. 7-AAD intercalates into DNA of dead or damaged cells, thus determination of 7-AAD positive cells is an indicator of cell viability. For 7-AAD tubes, the tubes were centrifuged at 400g for 5 min at 6C. After removing the supernatant, the cell pellets were resuspended in 100ul 1x 7-AAD solution. The tube was incubated on ice for 15 min in the dark. 1ml of staining buffer was added to the CD40L tube and then the 3 tubes were centrifuged together. After discarding the supernatant, the cell pellets were resuspended in 0.4ml staining buffer and FACS was run.

[0073] IFN-γ ELISA - The IFN-γ secreted in the supernatant was determined by IFN-γ sandwich ELISA kit (R&D Systems, Mpls. MN) according to the manufacturer instructions.

[0074] Example 1 - This experiment was done to determine if cells in formulation buffer (CFB) are stable at low temperatures after transportation. Batches of cell suspensions were formulated in FFB and transported through a mailing service (Federal Express). The temperature was monitored by a data logger. The temperature change inside a box that was not preconditioned and a preconditioned Aerogel insulated box was monitored. The outside temperature was also monitored. Three different batches were formulated and transported. Supernatant samples were taken of cells activated in culture media (CAC), CFB right after formulation, CFB after 2hours at Room Temperature (RT), CFB after 48hours at 4°C, and CFB after 48 hours at 4°C and 2 hours at RT. CAC was tested for expression of CD40L and the remaining cells were tested for expression of CD40L, and the viability of cells.

[0075] Fig. 1A and Fig. 1B shows the temperature that the cells were subjected to during transportation. Fig. 1A shows that the temperature varied from about 5°C to about 13.7°C within about 48 hours when the samples were not packaged in a preconditioned box. The samples were stable indicating a broader fluctuation of temperature is acceptable. Fig. 1B shows that the temperature inside the preconditioned and insulated box remains fairly stable. It varied from 0.2°C to 2.2°C. Fig. 1C shows the variation of the outside temperature during the shipping period.

[0076] Fig. 2A-2C shows that the expression of CD40L did not change much. Fig. 3A-3C shows the cell viability after the shipping process is similar to the cell viability prior to shipping. These results indicate that keeping the therapeutic compositions within a broad range like about 2°C to about 13°C within the package was not detrimental.

[0077] Example 2 - This study was performed to determine whether the low temperature can extend the expiration of CFB. The stability of different formulations of CFB at RT was performed. CFB were formulated for intradermal (ID), intratumoral (IT) or intravenous (IV) administration as described above. The stability of these formulations was tested to see if low temperature stability can be extended.

[0078] Batches HTC264, HTC245 and HTC273 were formulated for ID, IT and IV and tested for expression of CD40L, cell viability and secretion of IFN-γ for 6 hours at RT after formulation. Fig. 6A-6C, Fig. 7A-7C and Fig. 8A-8C show the results of these tests. All three of these parameters are stable after 6 hours at RT. Fig. 9A-9C shows that the expression of CD40L is stable after storage for 48h at 4ºC. Fig. 11A-11C indicates that the cell viability is stable after storage for 48h at 4ºC. Fig. 10A-Fig.10C indicates that the IFN-γ secretion is does not recover as well after 48hours at 4ºC. However, as shown below this can be recovered by transferring back to RPMI and incubating at 37ºC for 24 hours.

[0079] Three batches (HTC264, HTC245 and HTC273) were formulated as ID, IV or IT formulations. 4 total syringes of each formulation were made (1 for RT, 1 for 24h 4ºC, 1 for 48h 4ºC, 1 for 72h 4ºC) and incubated at 4ºC for different periods of time. The samples were collected after incubation back at RT for 2 hours. Table 1 below shows the timepoints, samples and tests that were performed for each batch of cells. IFN-γ levels were also determined when the cells were incubated at 37ºC for 24 hours.

TABLE 1

Time	Samples	Test
-4h	CAC	CD40L
0	CFB, supernatant	CD40L, IFN-γ, viability
2h	CFB, supernatant	CD40L, IFN-γ, viability
24h 4ºC	CFB, supernatant	CD40L, IFN-γ, viability
24h 4ºC-2h RT	CFB, supernatant	CD40L, IFN-γ, viability
48h 4ºC	CFB, supernatant	CD40L, IFN-γ, viability
48h 4ºC-2h RT	CFB, supernatant	CD40L, IFN-γ, viability
72h 4ºC	CFB, supernatant	CD40L, IFN-γ, viability
72h 4ºC-2h RT	CFB, supernatant	CD40L, IFN-γ, viability

[0080] Fig. 12A-12D, Fig. 13A-13D and Fig. 16A-14D shows the results for batches HTC264, HTC245 and HTC273, respectively. Intradermal formulations of these batches were tested as indicated. The results showed that keeping the CFB at 4ºC can maintain

the expression of CD40L on the cell surface even after 72h (Fig. 12A, Fig. 13Aand Fig. 16A). The cell viability was not affected much by low temperature storage (Fig. 12B, Fig. 13B and Fig. 16B). The IFN-γ secretion levels (Fig. 12C, Fig. 13C and Fig. 16C) are depressed when the cells are returned to RT for only 2 hours. However, the IFN-γ levels recover (Fig. 12D, Fig. 13D and Fig. 14D) when the cells are transferred back to RPMI media and incubated at physiological temperature (37ºC) for 24 hours. This indicates that the cells are still able to secret IFN-γ after keeping low temperature for 72 hours. This suggests that if these cells are administered therapeutically, the IFN-γ can be produced in the patient at similar levels to the cells that have not been subjected to lengthy storage.

[0081] Example 3 - This experiment was done to compare the stability of the CAC cells and the CFB cells. Three different batches of cells were formulated as ID syringe. One syringe for CAC and one syringe for CFB for each batch. The CAC was thawed and washed with cRPMI. After cell counting, the cell pellet was resuspended in 10^9 cells/ml with FFB and 1 ml of cell suspension was transferred into a 3 ml syringe. For CFB, the cell pellet was resuspended in 10^9 cells/ml with cRPMI and mixed with the anti-CD3/anti-CD28 beads. The cell and bead mixture were incubated for 4 hours at 37°C with 5% CO_2. The cells were washed with FFB and resuspended in 10^7 cells/ml with FFB. The cell suspension was transferred into a 3 ml syringe. At each time point, 100ul samples were obtained from the syringe for CD40L, IFN-γ, viability tests. After incubation at 4ºC for 48 hours, some samples were centrifuged at 400g for 5 min. to remove the FFB. After discarding the supernatant, the cell pellet was resuspended in 100ul cRPMI and incubated at 37ºC with 5% CO_2 for 2 hours. The supernatant was collected for IFN-γ detection. Table 2 below lists the samples that were collected and the tests that were performed.

TABLE 2

Time	Samples	Test
0	CAC, supernatant	CD40L, IFN-γ, viability
2h, RT	CAC, supernatant	CD40L, IFN-γ, viability
48h, 4°C	CAC, supernatant	CD40L, IFN-γ, viability
48h 4°C-2h RT	CAC, supernatant	CD40L, IFN-γ, viability
0	CFB, supernatant	CD40L, IFN-γ, viability
2h, RT	CFB, supernatant	CD40L, IFN-γ, viability
48h, 4°C	CFB, supernatant	CD40L, IFN-γ, viability
48h, 4°C-2h RT	CFB, supernatant	CD40L, IFN-γ, viability

[0082] The results indicated that incubation of CAC for 4°C for 48 hours decreased the CD40L expression on cell surface significantly See Fig. 15A-15C. However, incubation of CFB at 4°C for 48 hours could maintain the CD40L expression suggesting that the crosslinking of CD3 and CD28 are essential for stability of the cells. CFB are able to maintain viability and secrete high amounts of IFN-γ, even after 48hour incubation at 4°C. See Figs. 16A-C and Figs. 17A-C.

[0083] Example 4 - This study was performed to determine the stability of formulated CFB after packaging and shipment from a production facility in Jerusalem, Israel to a point-of-care. It was crucial to confirm that CFB product continues to meet pre-established identity and functional characteristics after 72 hours in transit, since at the end of the formulation process, the cells are transferred to a non-nutrient infusion buffer, in which the cells may lose their viability and unique identity and functional characteristics. It is known that low temperatures can slow down the gene expression and activity of cells and that this gene expression can be restored by returning cells back to physiological temperature. For this reason, the shipping is done using pre-validated, refrigerated, temperature-controlled containers.

[0084] The CFB cells were tested to check if their pre-defined identity and functional characteristics are kept after 72 hours in transit, by comparing the cells characteristics

prior transit (at Baseline – formulated syringes after 4h activation= FF) to those obtained after shipping to NY and back, at minimum 72 hours after FF completed.

The pre defined end point parameters were:

1. Viability test: CFB viability must be >70% live cells at all tested time points.

2. Rapid Endotoxin Test: endotoxin levels of sample collected at Baseline and after 72h at 4^0C must be <0.5Eu/ml.

3. Gram's Stain: no bacteria should be observed on the slide of samples collected at all tested time points.

4. Surface Staining – CD40L AM (CFB-CAC)≥30:

5. USP Sterility: no growth of the formulated sample in all tested mediums.

6. IFNγ secretion tested by ELISA:

   6.1 IFNγ accumulated during 4 hours activation >1000pg IFNγ per 1×10^6

   6.2 IFNγ accumulated during 24h after Baseline >6,000pg per 1×10^6 cells

   6.3 IFNγ accumulated during 24h after 72h at 2^0C - 8^0C >6,000pg per 1×10^6 cells

Results:

3 separated Final formulation processes were performed on doses from batch HTC300.

The formulated product, packaged in syringes, was shipped with Flying Cargo (FC) to NY, and back to Jerusalem Israel.

Syringes in transit were kept at 2-8°C, from formulation end time up to 72 hours as was shown by temperature logger inside the shipping package. All the results are summarized in Table 3.

TABLE 3

Formulation Number	Cell Type/Time	Cell Viability	CD40L AM CFB –CAC	IFNγ (pg/10^6cells)	Endotoxin (EU/ml)	Gram's Stain	Sterility	Pass/Fail
HTC300 T7-71+72	CAC	97.95%						Pass
	CFB Baseline	90.91%	143.60	8,027	<0.2	Pass	Pass	
	After 24 hours at 37°C in cRPMI			45,741				
	after 72h at 4°C	90.24%	192.02		<0.277	Pass		
	After 24 hours at 37°C in cRPMI			28,955				
HTC300 T7-73+74	CAC	99.40%						Pass
	CFB Baseline	98.23%	117.64	6,215	<0.219	Pass	Pass	
	After 24 hours at 37°C in cRPMI			31,155				
	after 72h at 4°C	95.65%	166.52		<0.208	Pass		
	After 24 hours at 37°C in cRPMI			13,284				
HTC300 T7-77+78	CAC	98.45%						Pass
	CFB Baseline	97.61%	165.39	8,960	<0.208	Pass	Pass	
	After 24 hours at 37°C in cRPMI			42,520				
	after 72h at 4°C	92.81%	231.50		<0.2	Pass		
	After 24 hours at 37°C in cRPMI			22,583				

[0085] As can be seen in Table 3, all three formulated batches passed all pre-defined acceptance criteria, hence demonstrating that CFB's stability in suggested distribution conditions.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of handling a biological drug composition with living cells comprising:

formulating the living cells in a non-nutritive buffer, wherein the buffer comprises a Plasmalyte buffering system and is isotonic, sterile and pyrogen-free, the living cells having been activated, cryopreserved, thawed and reactivated and wherein the living cells have been activated by cross-linking cell surface moieties by monoclonal antibodies or other binding agents; and

maintaining the living cells in the buffer at a storage temperature below 20°C, wherein the living cells are activated T-cells that maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the buffer, the living cells useful for immunotherapy after being stored for up to 72 hours in the buffer;

wherein the living cells in the composition express CD40L after storage in an amount of at least 80% relative to the expression of CD40L at the time of formulation; and

wherein the living cells secrete IFN-γ and the level of secretion is in an amount of at least 80% compared to the levels at the time of formulation.

2. The method of claim 1, wherein the storage temperature is in a range between 0°C and 10°C.

3. The method of claim 1 or 2, wherein the concentration of the cells in the buffer is about 10^6 cells/ml or greater and/or the living cells are placed in a flexible container or syringe, wherein the flexible container or syringe is packaged in a temperature controlled device that maintains the living cells at the storage temperature.

4. The method of any one of claims 1 to 3, wherein the living cells are CD4+ cells, wherein the CD4+ cells are Th1 cells when activated.

5. The method of claim 4, wherein the Th1 cells are activated by immobilized monoclonal antibodies that are cross-linked or the Th1 cells are activated by immobilized anti-CD3 and anti-CD28 monoclonal antibodies.

6. The method of any one of claims 1 to 5, wherein the secretion of IFN-γ after storage is recovered after incubation at 37°C for at least 24 hours.

7. A method of providing living cell compositions to a point of care facility comprising:

formulating the living cells with activating signals in a non-nutritive buffer at a processing facility, wherein the buffer comprises a Plasmalyte buffering system and is isotonic, sterile and pyrogen-free, the living cells having been activated, cryopreserved, thawed and reactivated and wherein the living cells have been activated by cross-linking cell surface moieties by monoclonal antibodies or other binding agents; and

transporting the cells to the point of care facility in a package equipped to maintain a storage temperature below 20°C, wherein the living cells are activated T-cells and are at the storage temperature with the activating signals for up to 72 hours while maintaining their identity and at least one of the functional characteristics of the living cells useful in immunotherapy;

wherein the live cells in the composition express CD40L after transportation and removal from storage temperature in an amount of at least 80% relative to the expression of CD40L at the time of formulation; or

wherein the cells in the composition secrete IFN-γ after transportation and removal from storage temperature in an amount of at least 80% compared to the levels at the time of formulation.

8. The method of claim 7, further comprising placing the formulated cells in a flexible container or syringe prior to transporting.

9. The method of claim 7 or 8, wherein the live cells are CD4+ cells, wherein the CD4+ cells are Th1 cells when activated.

10. The method of claim 9, wherein the Th1 cells are activated by immobilized monoclonal antibodies, wherein the monoclonal antibodies are cross-linked.

11. The method of any one of claims 7 to 10, wherein the secretion of IFN-γ after transportation and storage is recovered after incubation at 37°C for at least 24 hours.

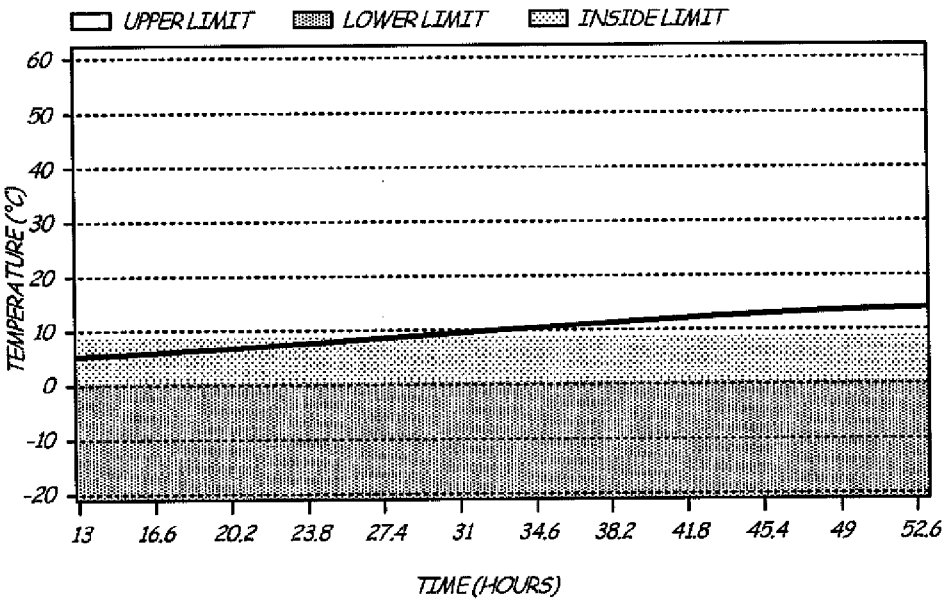

UPPER LIMIT
LOWER LIMIT
INSIDE LIMIT
60
50
40
30
20
10
0
-10
-20
TEMPERATURE (°C)
13
16.6
20.2
23.8
27.4
31
34.6
38.2
41.8
45.4
49
52.6
TIME (HOURS)

Fig. 1A

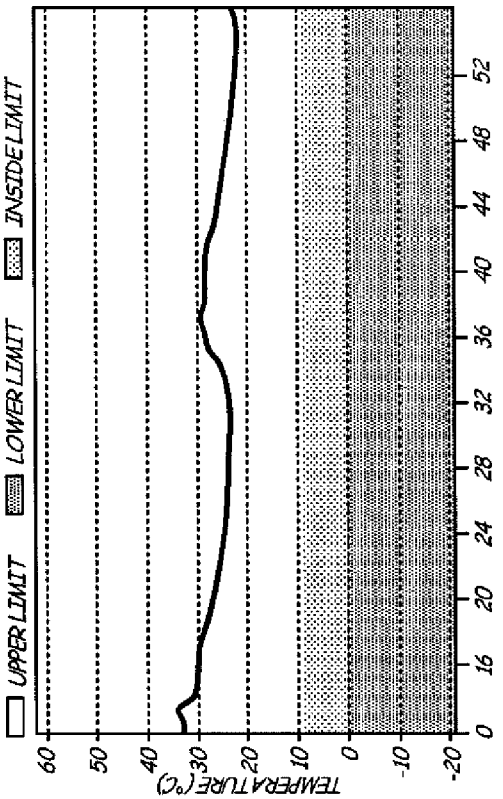

UPPER LIMIT
LOWER LIMIT
INSIDE LIMIT
TEMPERATURE (°C)
60
50
40
30
20
10
0
-10
-20
0
16
20
24
28
32
36
40
44
48
52
TIME (HOURS)

Fig. 1C

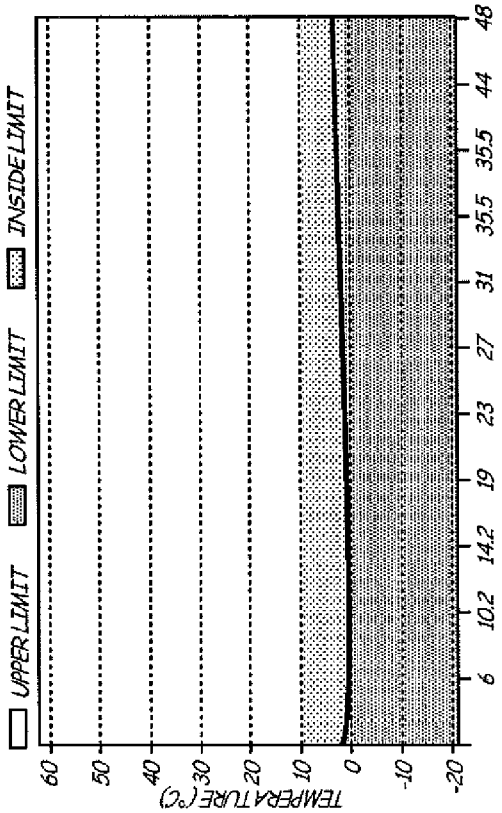

UPPER LIMIT
LOWER LIMIT
INSIDE LIMIT
TEMPERATURE (°C)
60
50
40
30
20
10
0
-10
-20
6
10.2
14.2
19
23
27
31
35.5
35.5
44
48
TIME (HOURS)

Fig. 1B

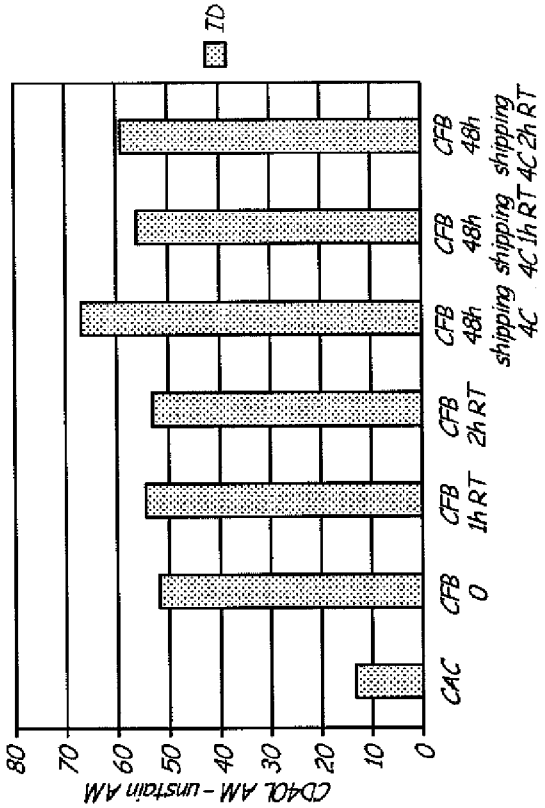


Fig. 2B

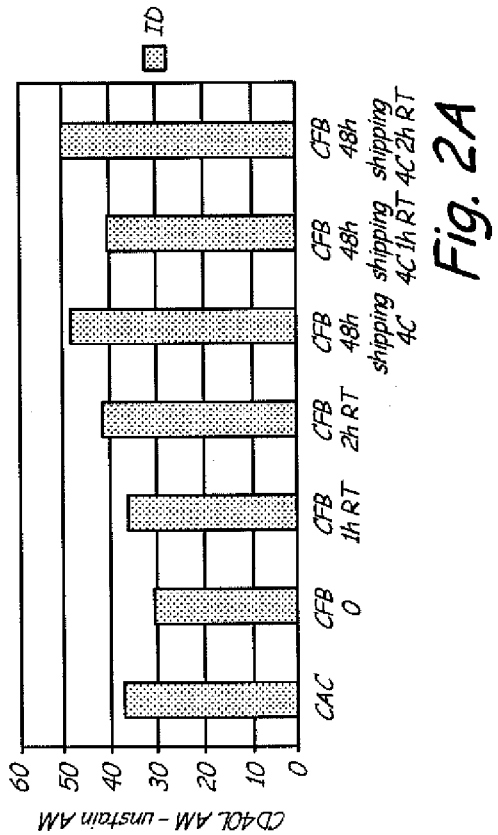


Fig. 2A

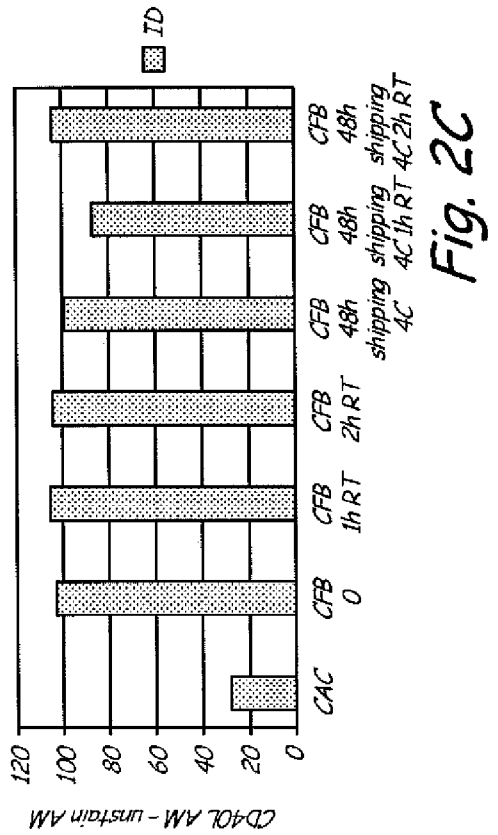


Fig. 2C

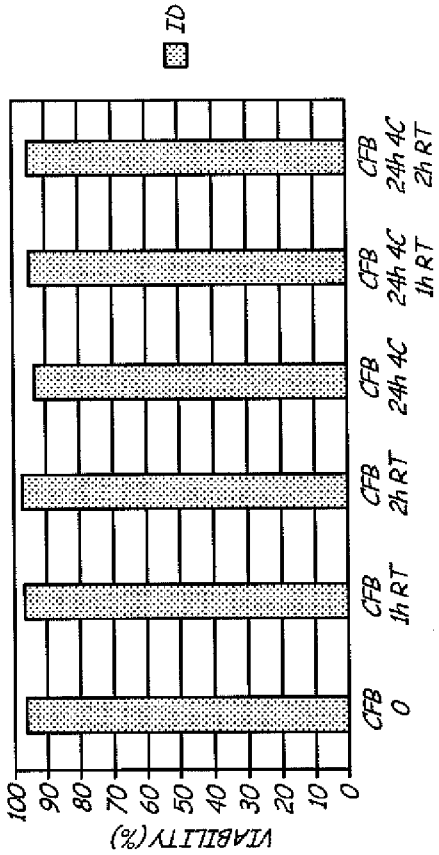


Fig. 3B

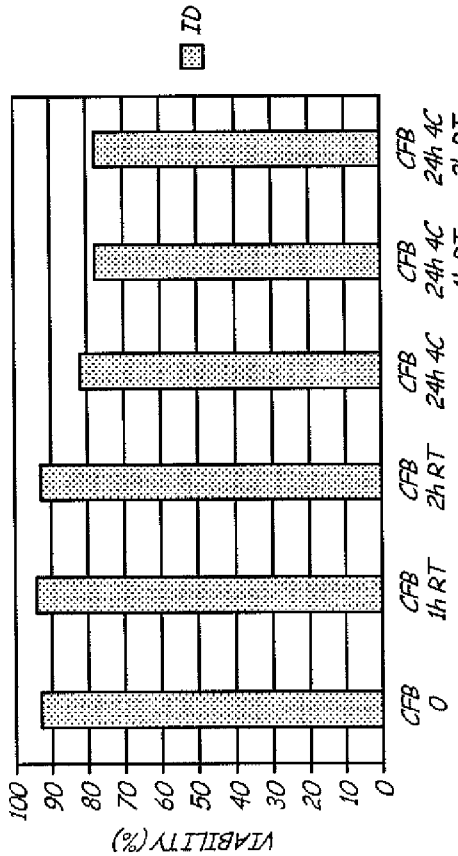


Fig. 3A

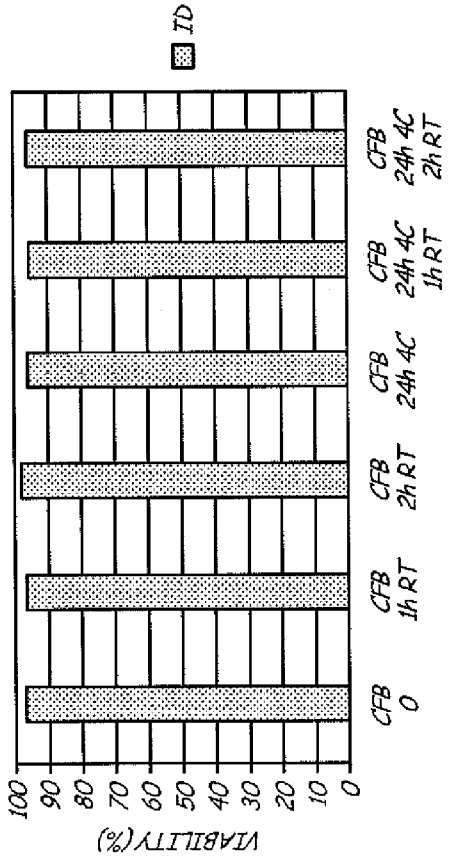


Fig. 3C

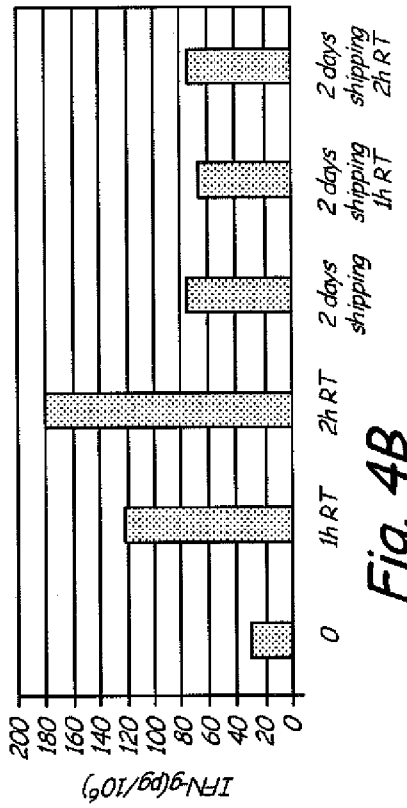


Fig. 4B

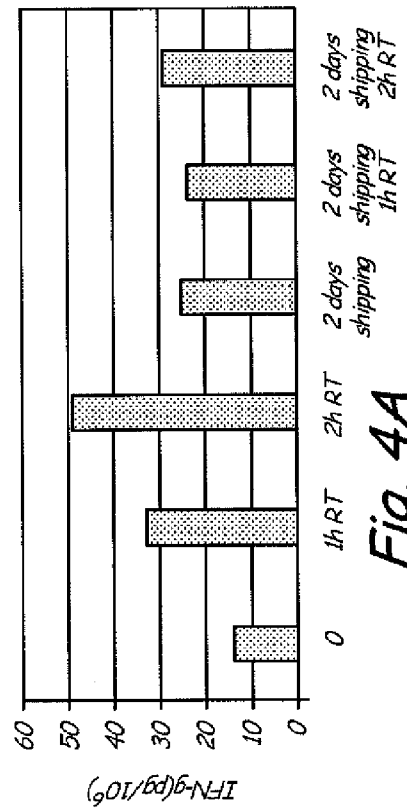


Fig. 4A

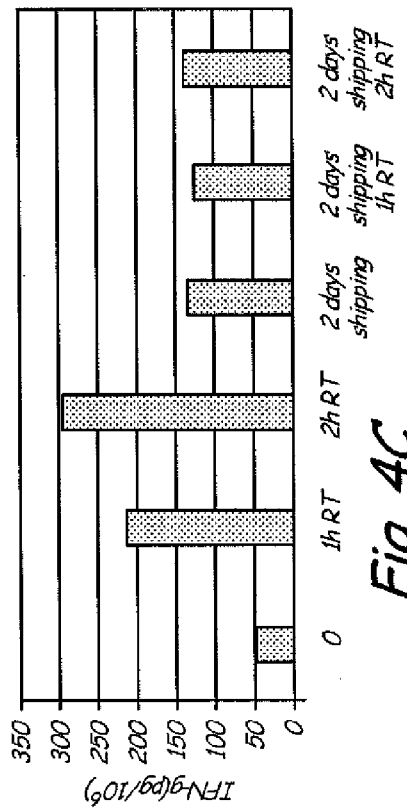


Fig. 4C

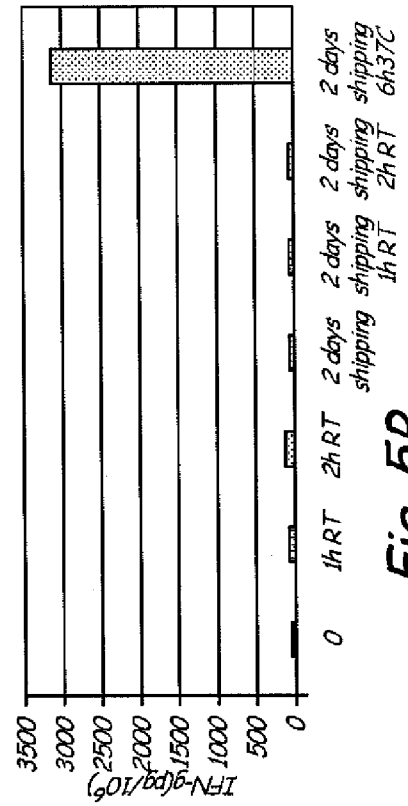


Fig. 5B

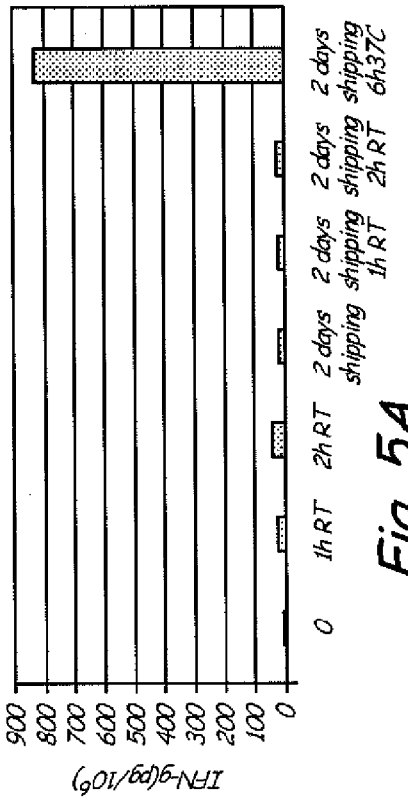


Fig. 5A

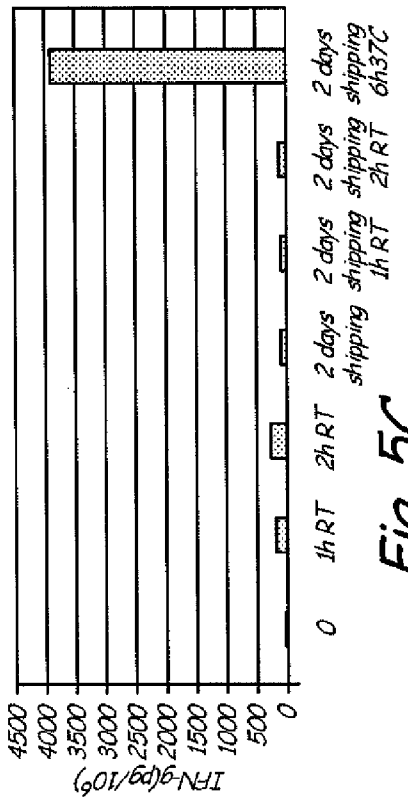


Fig. 5C

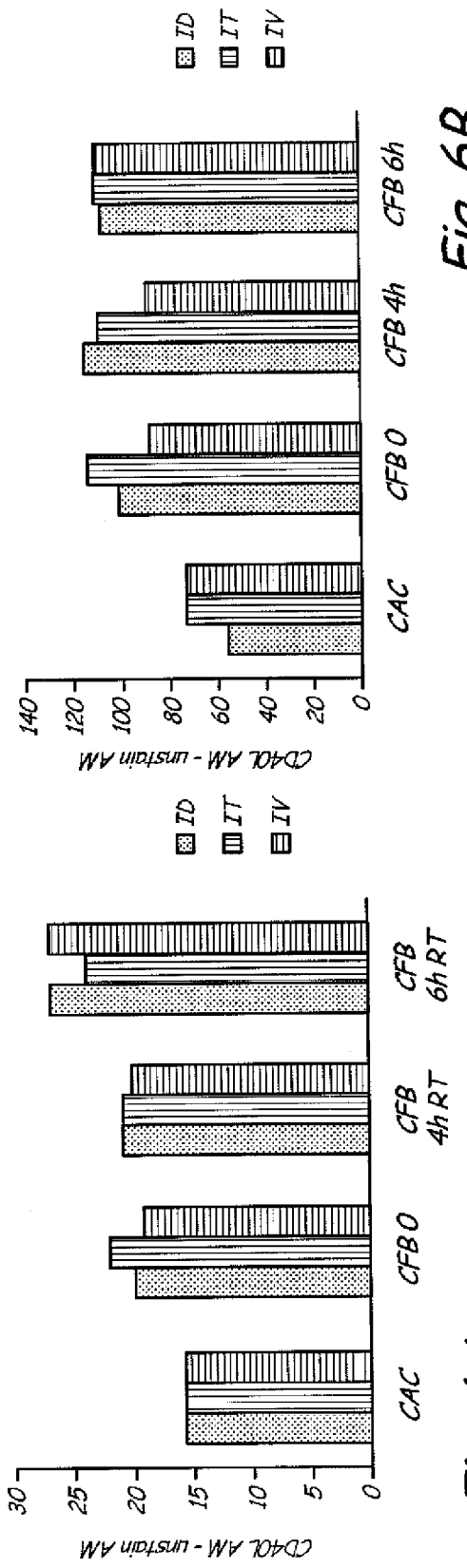


Fig. 6A

Fig. 6B

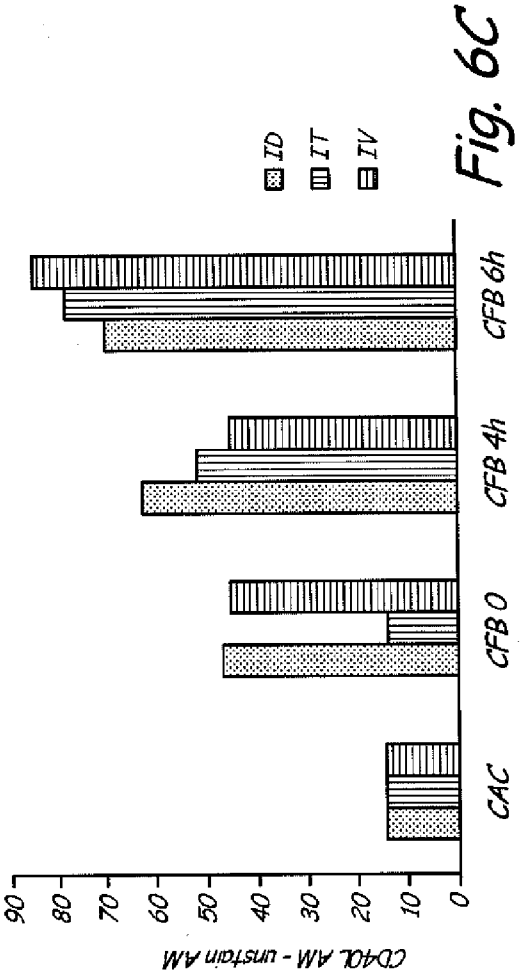


Fig. 6C

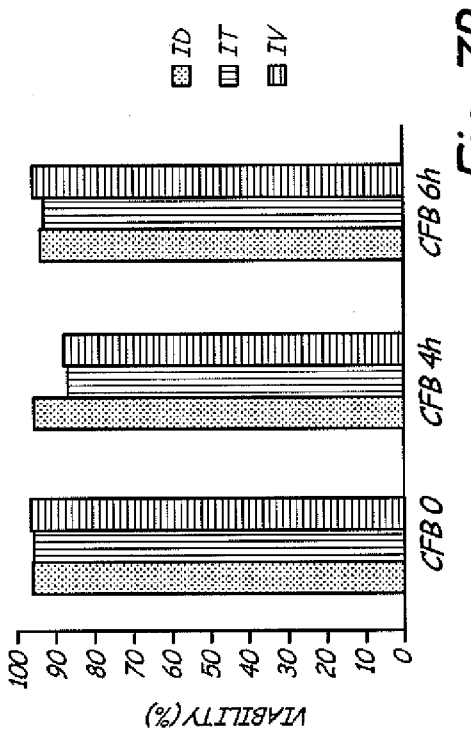


Fig. 7B

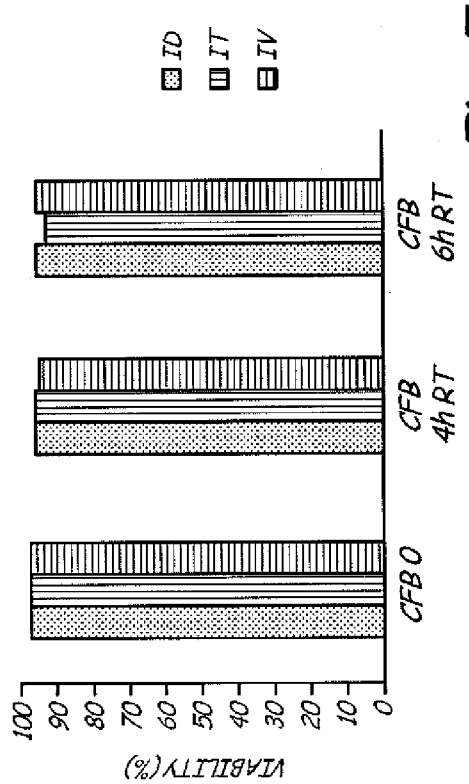


Fig. 7A

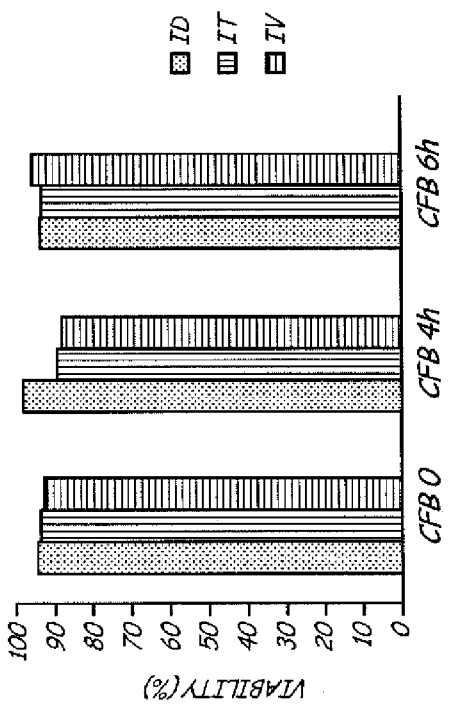


Fig. 7C

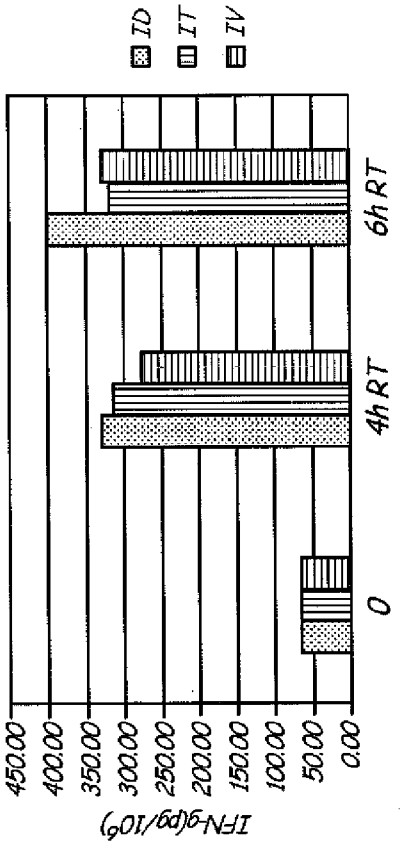


Fig. 8B

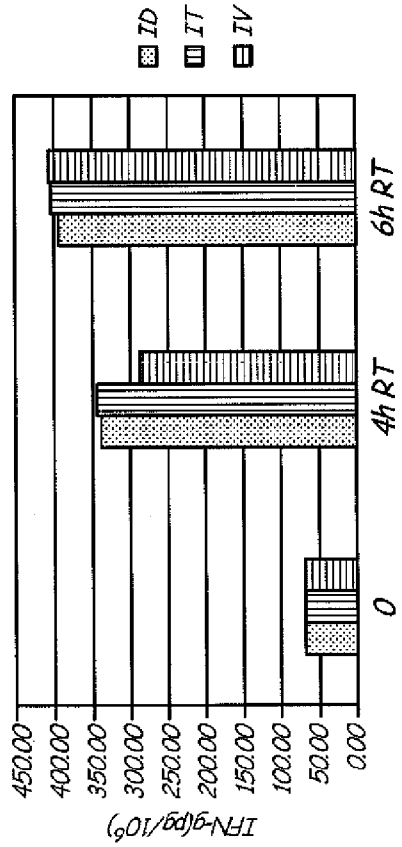


Fig. 8A

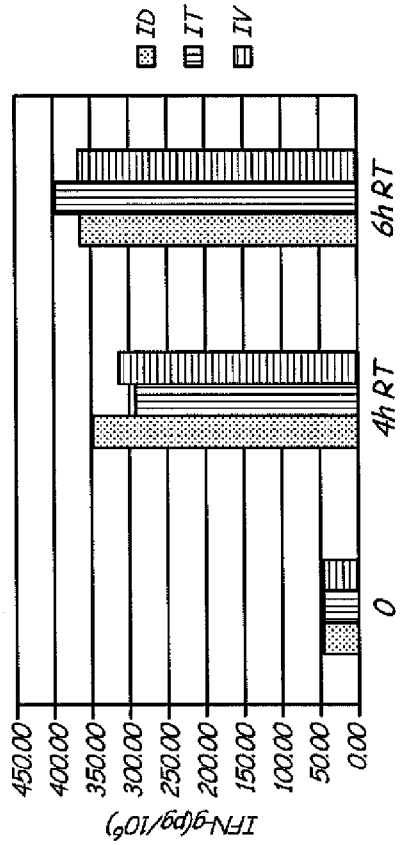


Fig. 8C

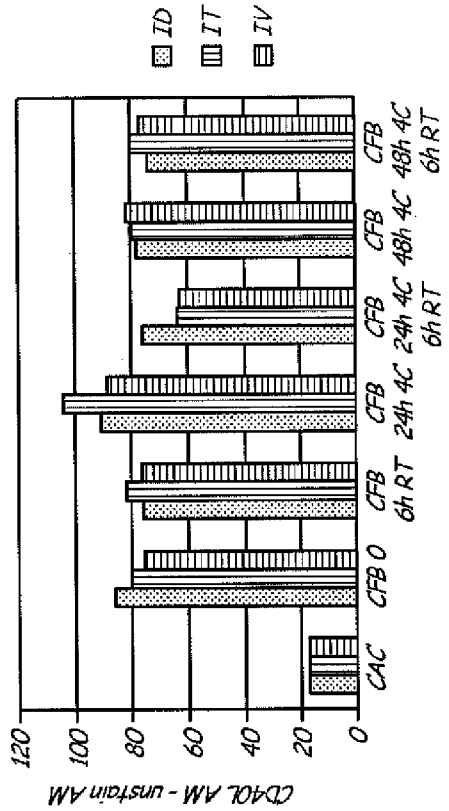


Fig. 9B

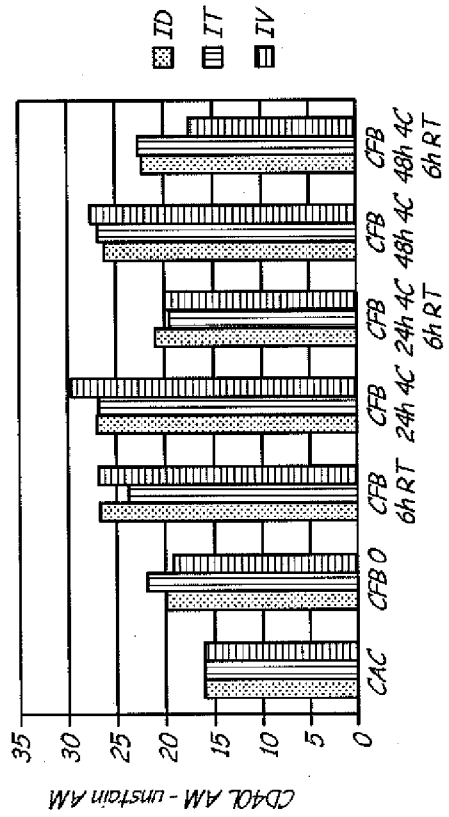


Fig. 9A

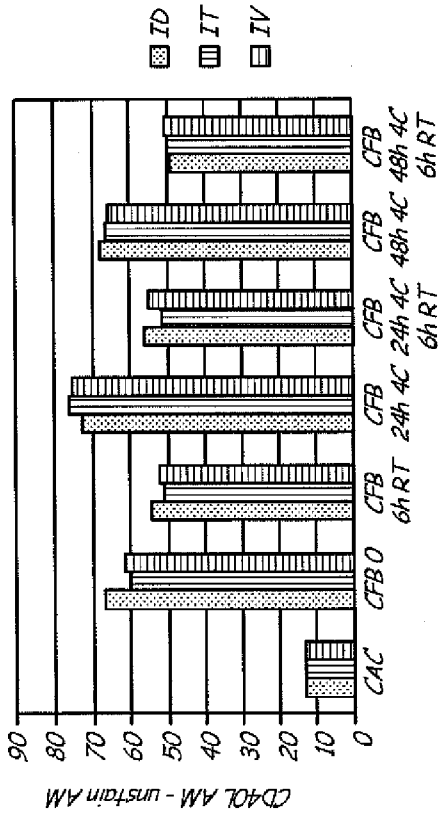


Fig. 9C

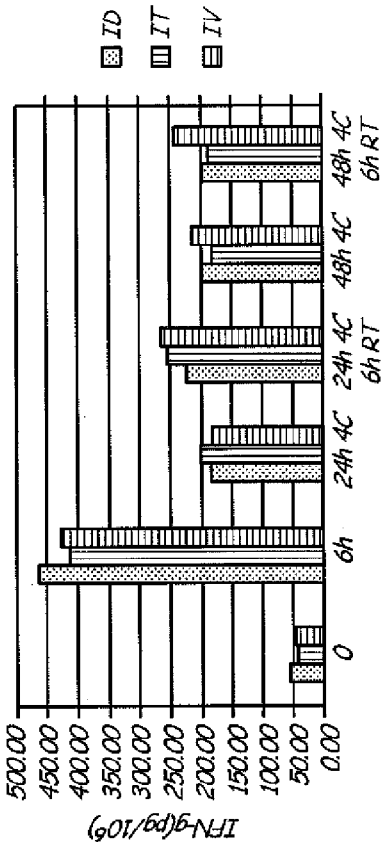


Fig. 10B

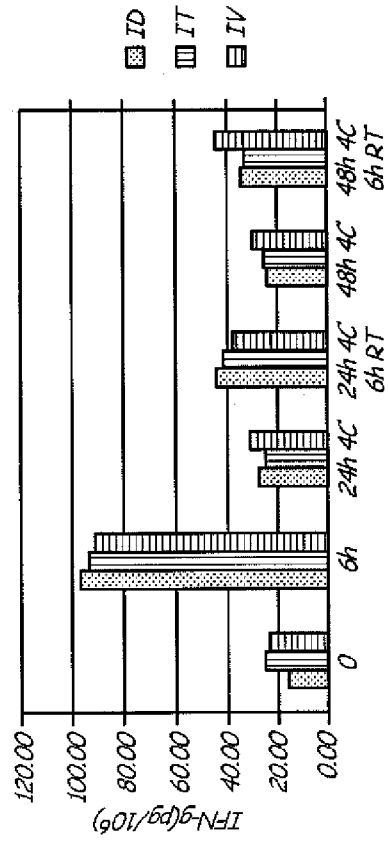


Fig. 10A

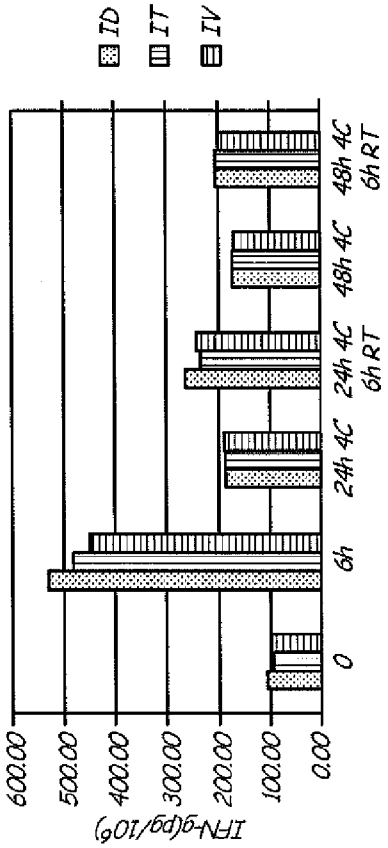


Fig. 10C

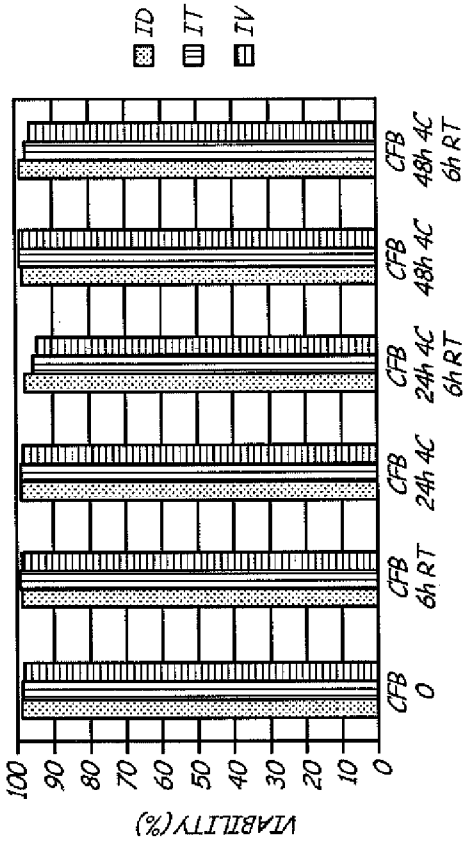


Fig. 11B

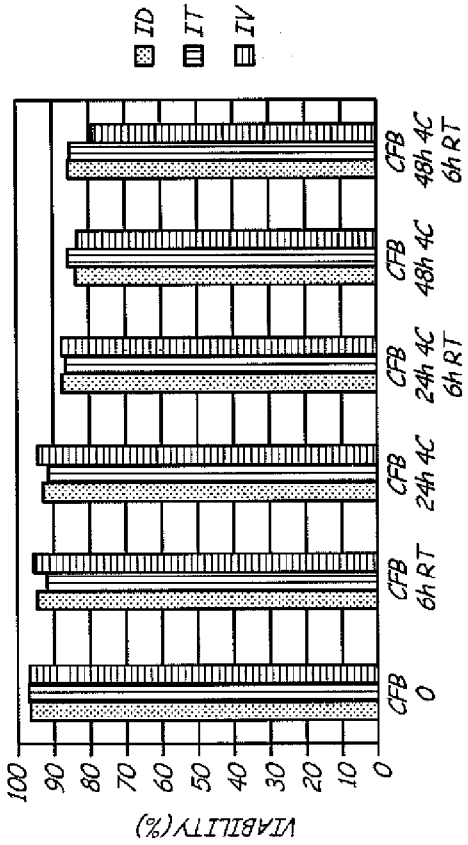


Fig. 11A

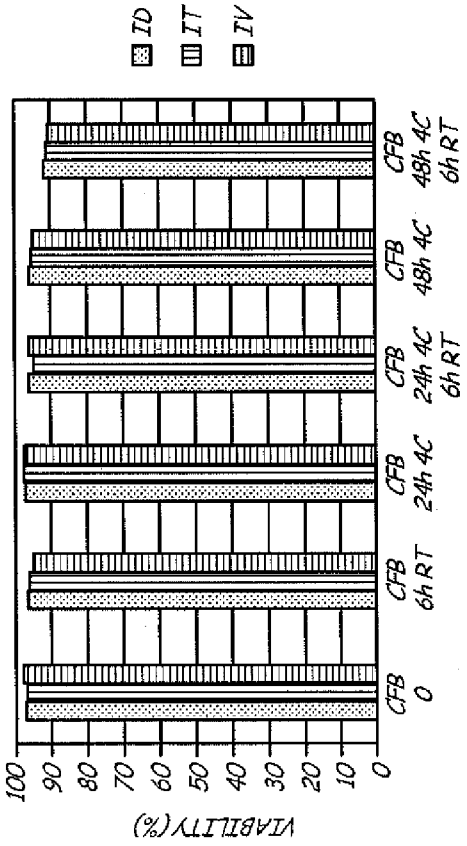


Fig. 11C

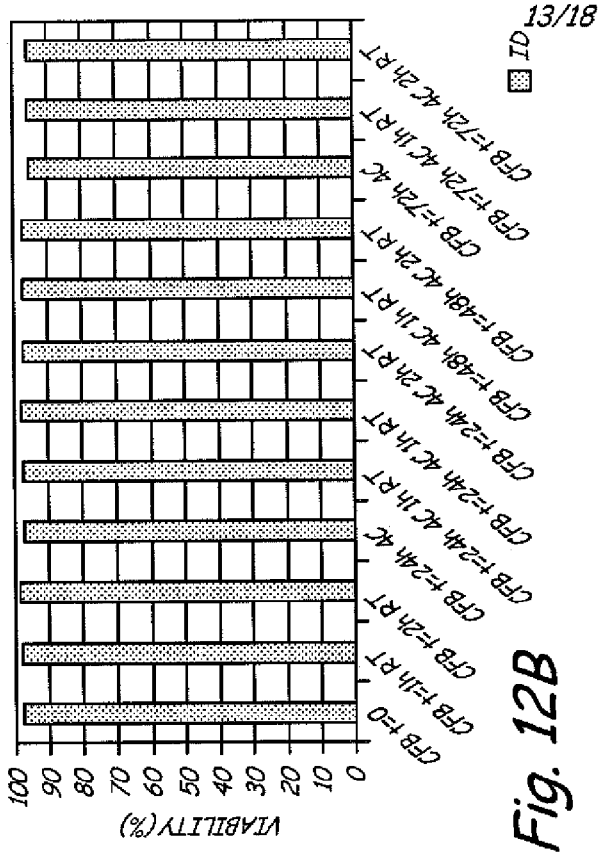


Fig. 12B

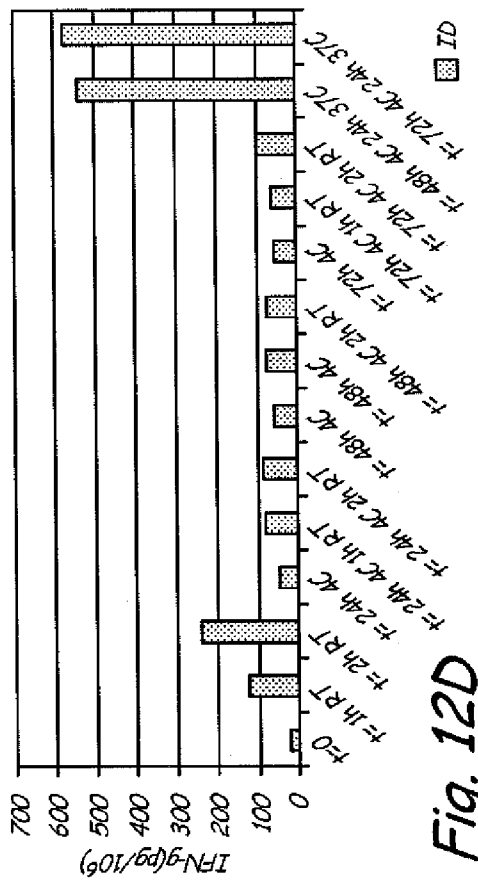


Fig. 12D

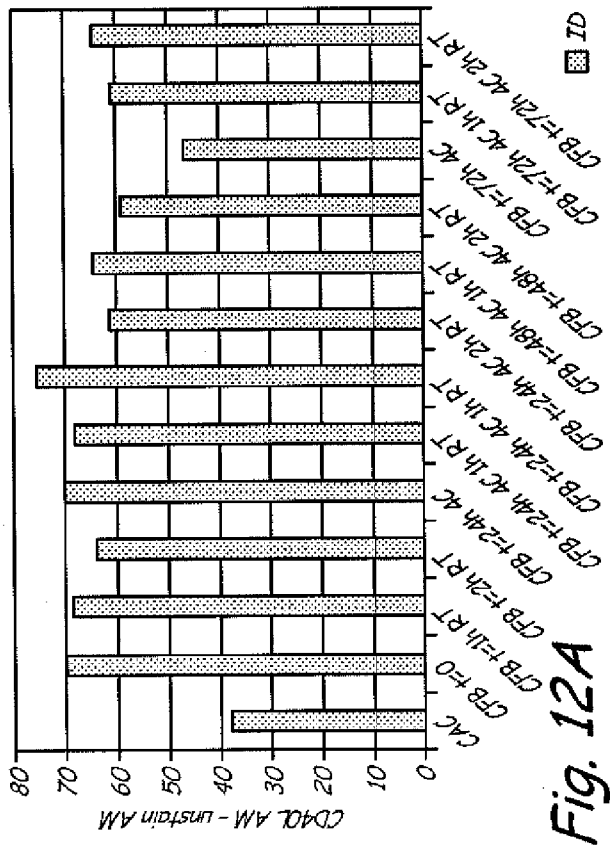


Fig. 12A

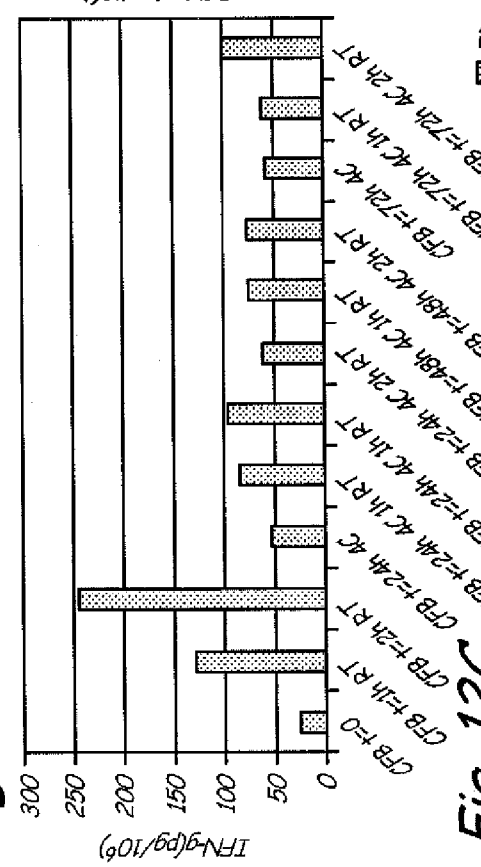


Fig. 12C

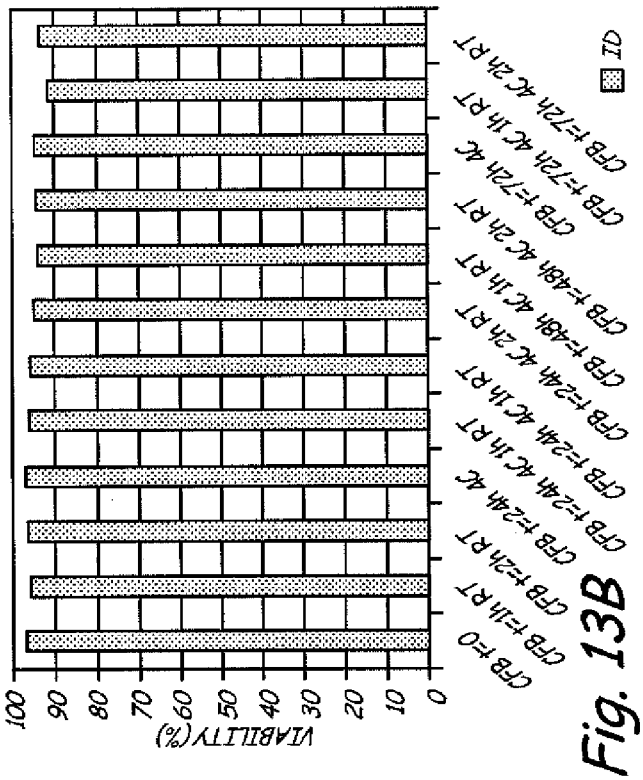


Fig. 13B

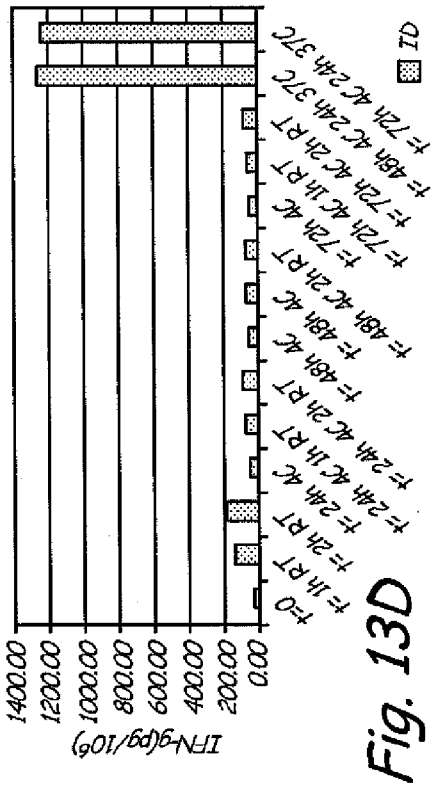


Fig. 13D

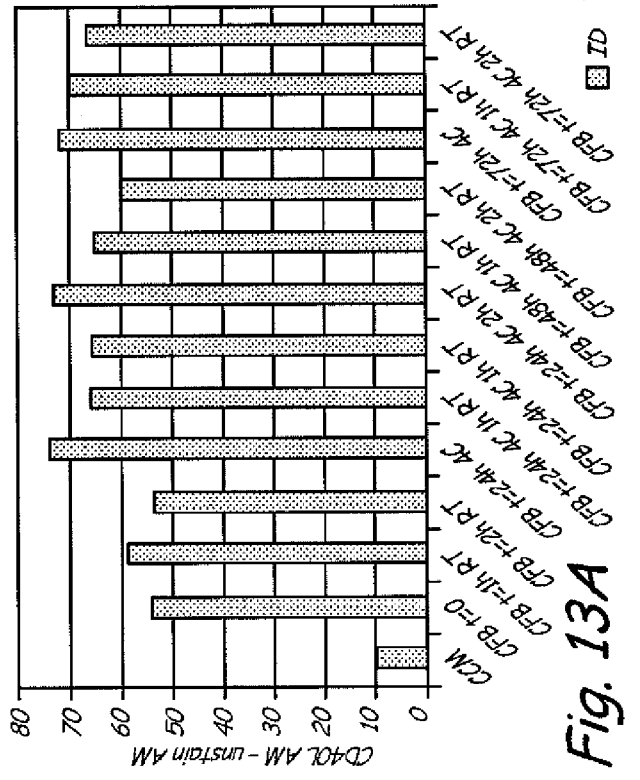


Fig. 13A

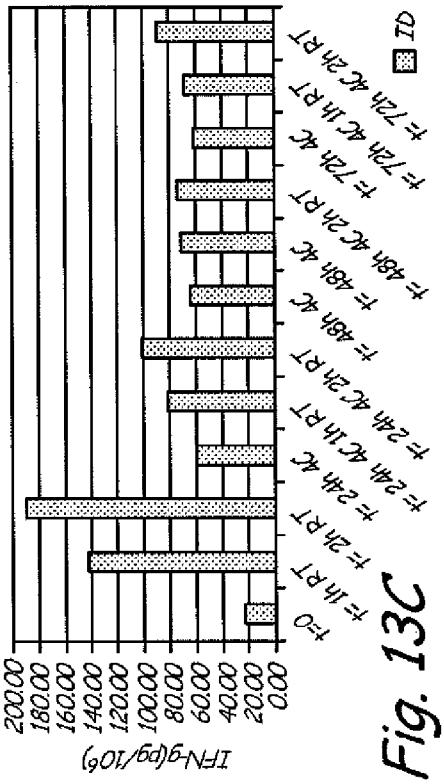


Fig. 13C

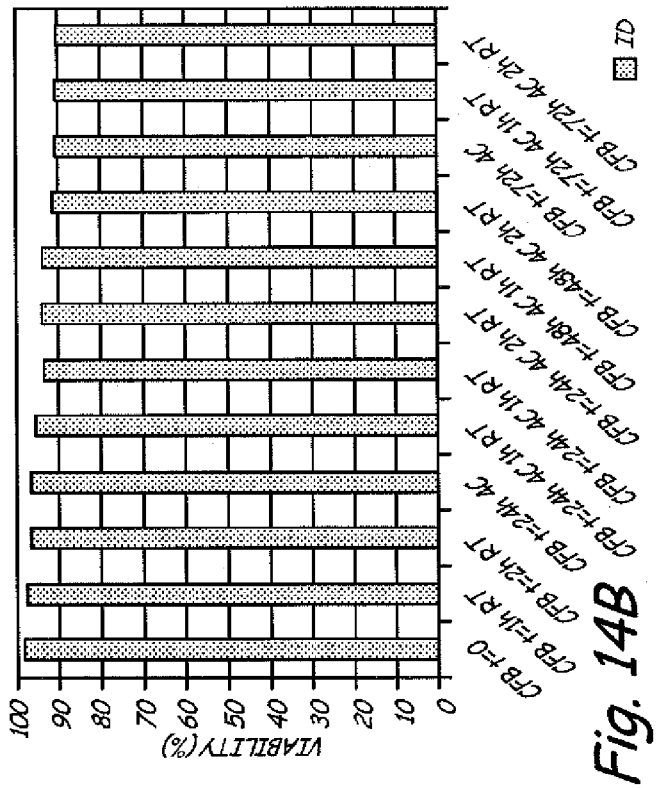


Fig. 14B

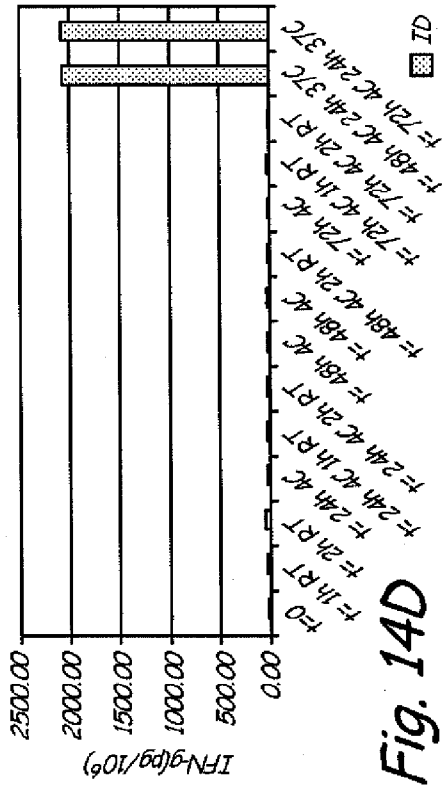


Fig. 14D

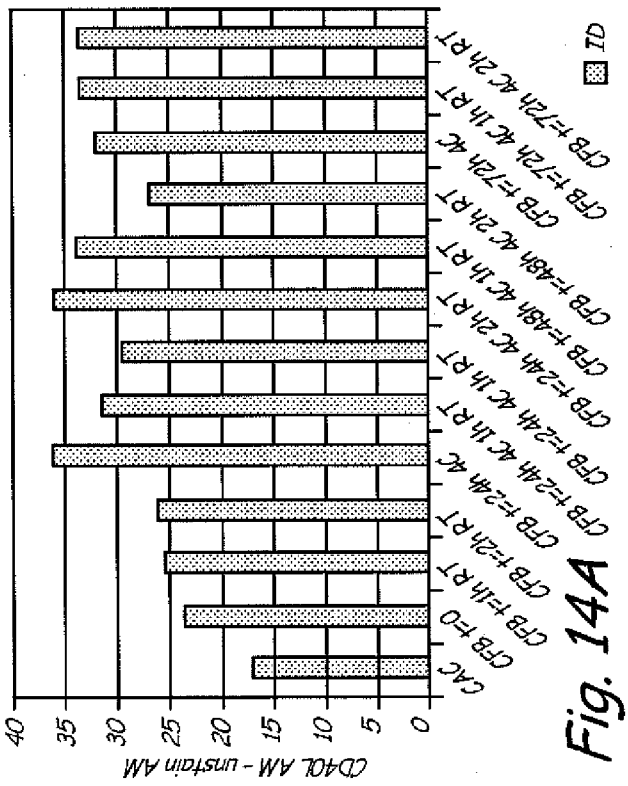


Fig. 14A

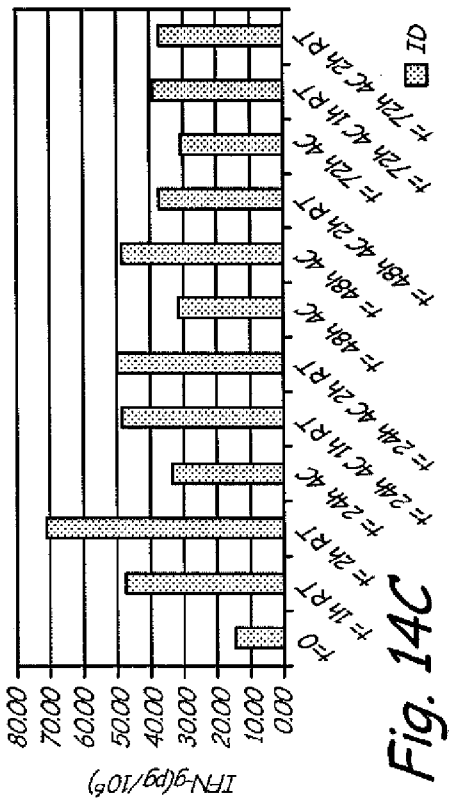


Fig. 14C

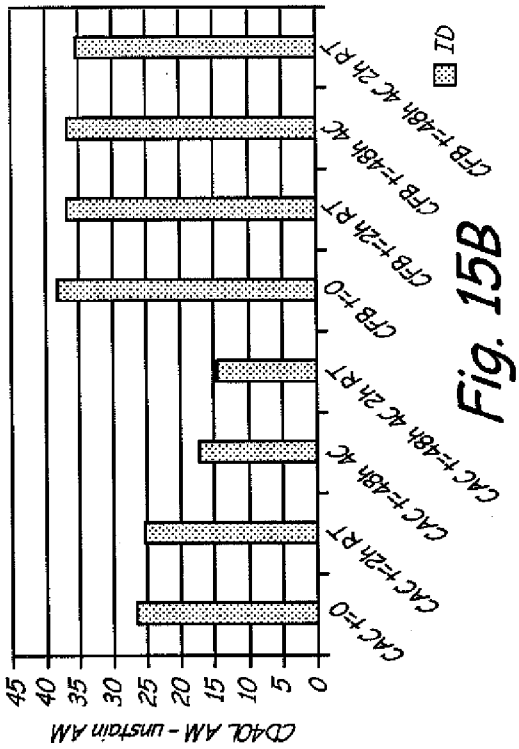


Fig. 15B

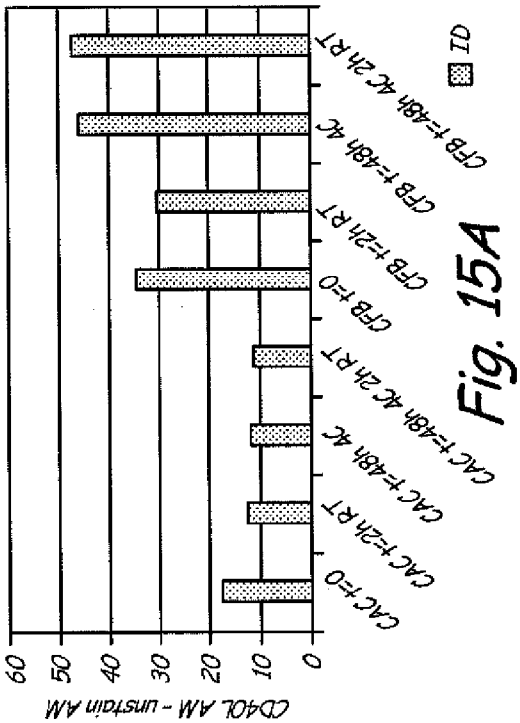


Fig. 15A

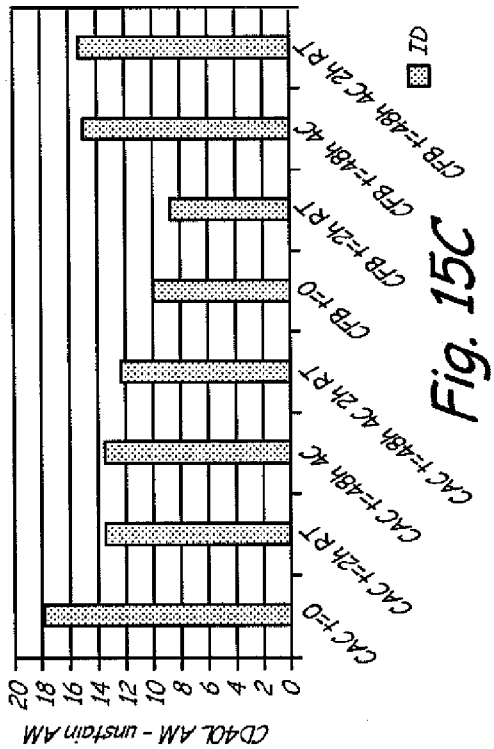


Fig. 15C

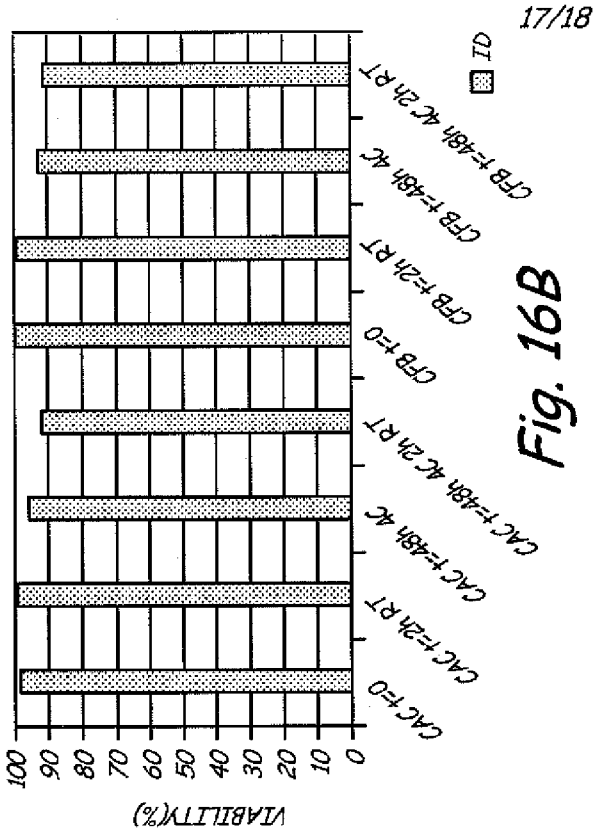


Fig. 16B

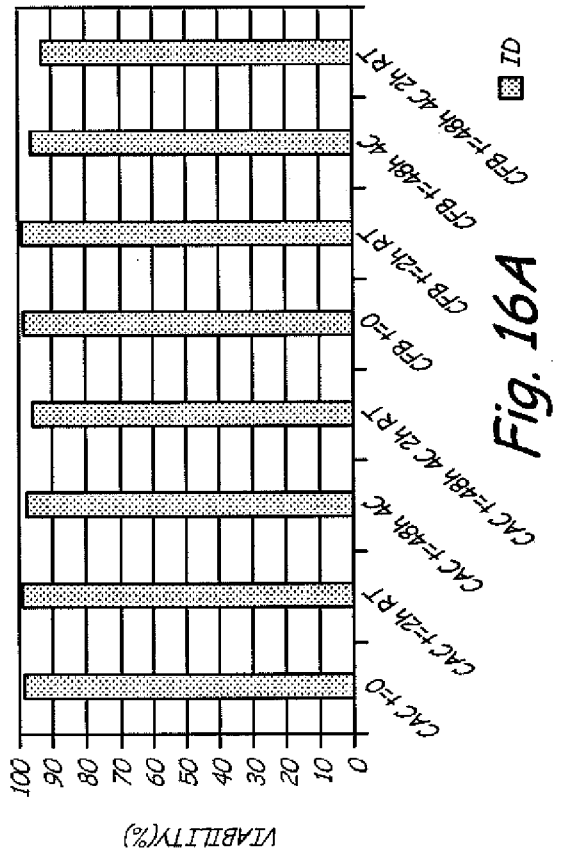


Fig. 16A

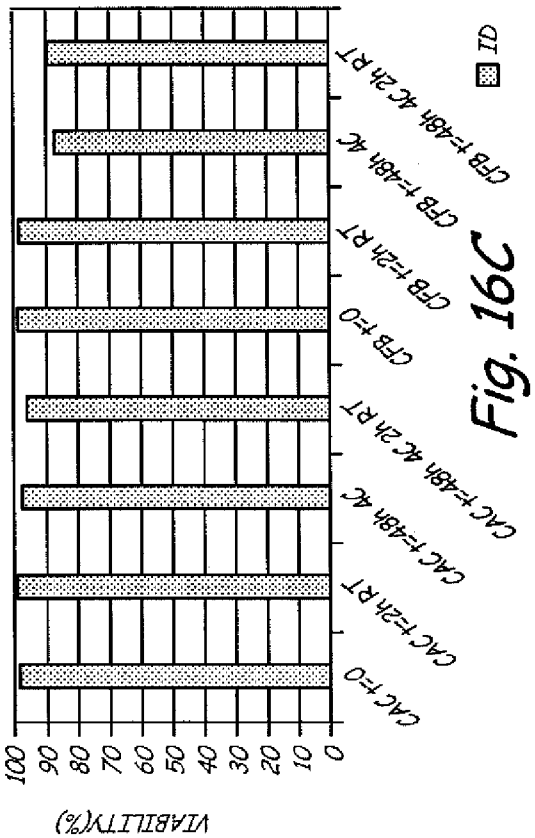


Fig. 16C

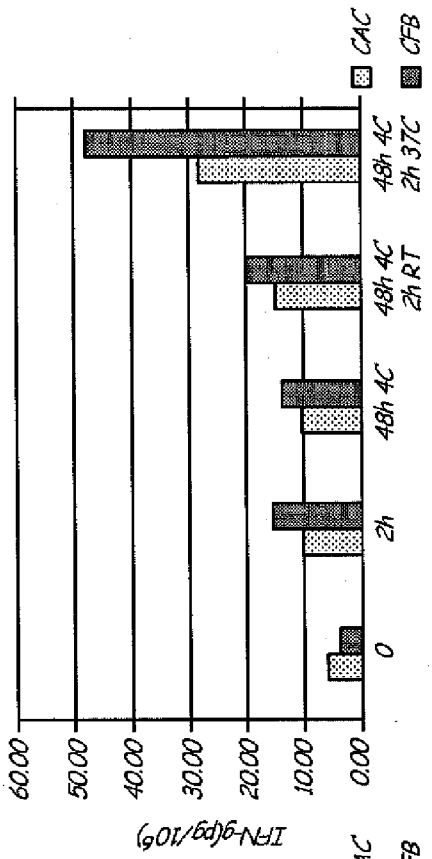


Fig. 17B

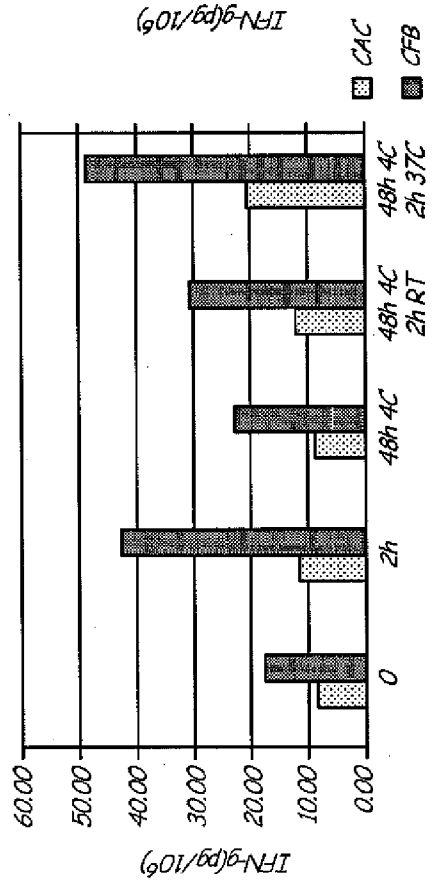


Fig. 17A

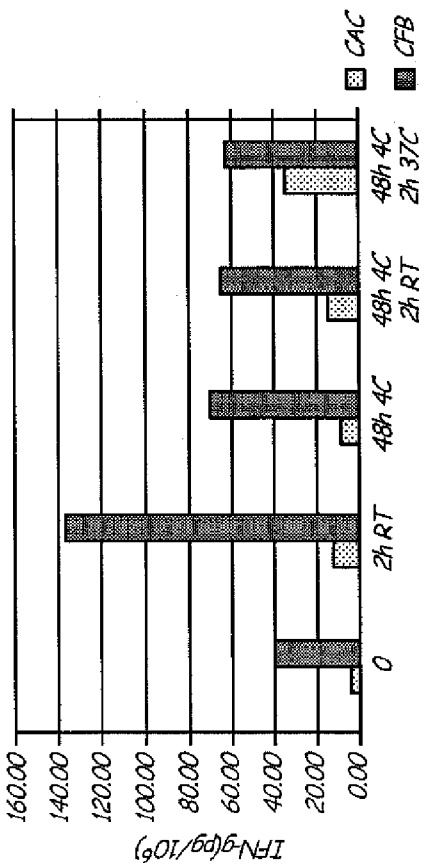
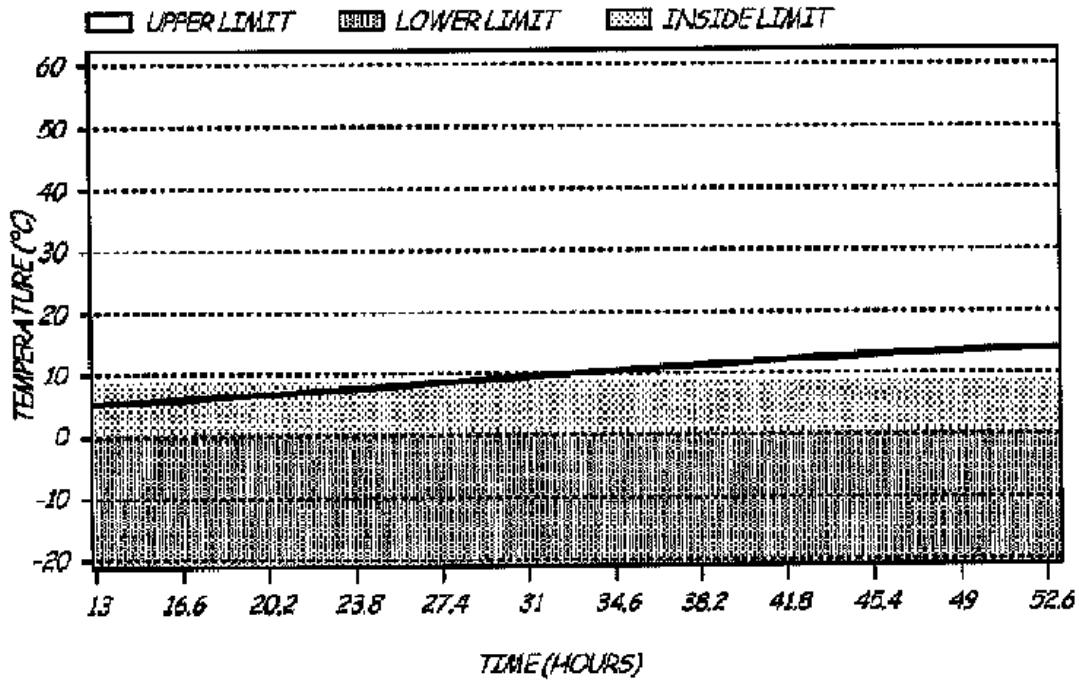
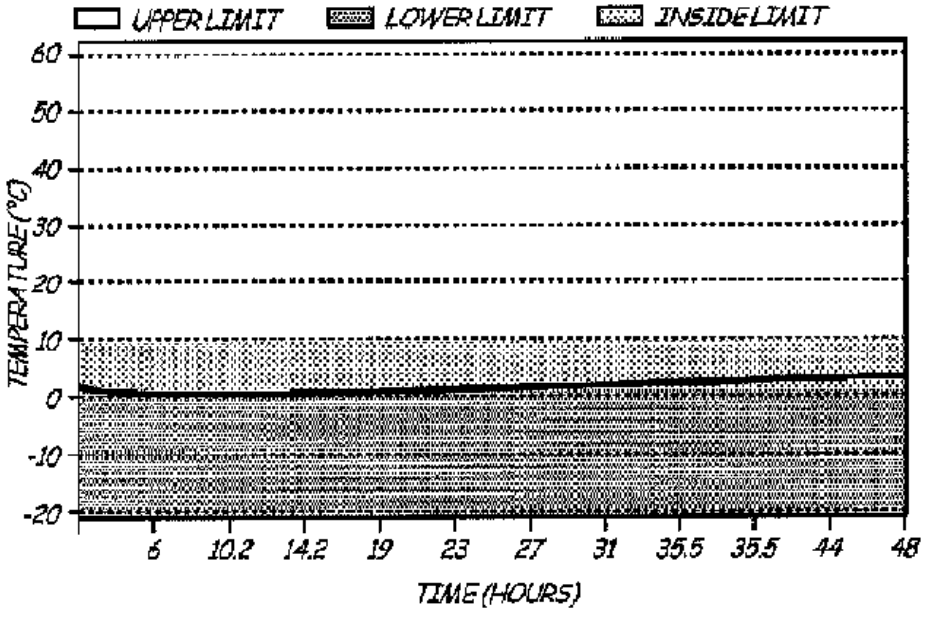


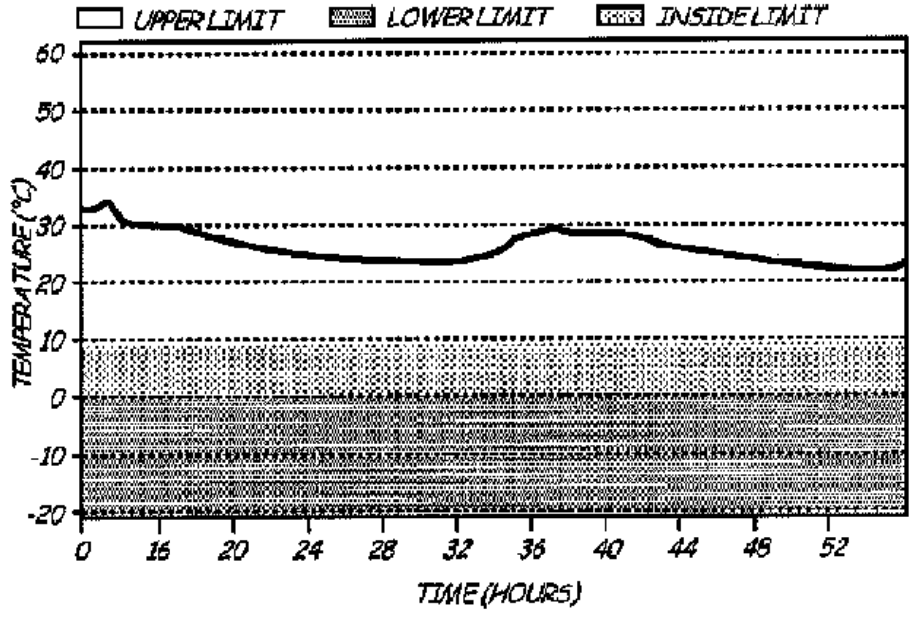
Fig. 17C


UPPER LIMIT
LOWER LIMIT
INSIDE LIMIT
60
50
40
30
20
10
0
-10
-20
TEMPERATURE (°C)
1.3
16.6
20.2
23.8
27.4
31
34.6
38.2
41.8
45.4
49
52.6
TIME (HOURS)

A


UPPER LIMIT
LOWER LIMIT
INSIDE LIMIT
60
50
40
30
20
10
0
-10
-20
TEMPERATURE (°C)
6
10.2
14.2
19
23
27
31
35.5
35.5
44
48
TIME (HOURS)

B


UPPER LIMIT
LOWER LIMIT
INSIDE LIMIT
60
50
40
30
20
10
0
-10
-20
TEMPERATURE (°C)
0
16
20
24
28
32
36
40
44
48
52
TIME (HOURS)

C