

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
4 September 2003 (04.09.2003)

PCT

(10) International Publication Number  
**WO 03/073099 A1**

- (51) International Patent Classification<sup>7</sup>: **G01N 33/53**
- (21) International Application Number: PCT/US03/05980
- (22) International Filing Date: 27 February 2003 (27.02.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/359,932 27 February 2002 (27.02.2002) US  
60/363,073 11 March 2002 (11.03.2002) US  
60/396,392 17 July 2002 (17.07.2002) US  
60/404,652 20 August 2002 (20.08.2002) US
- (71) Applicant (for all designated States except US):  
**BIOMERIEUX, INC.** [US/US]; 100 Rodolphe Street,  
Durham, NC 27712 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **TOH, Cheng, Hock** [MY/GB]; 35 Beaconsfield Road, Liverpool L25 6EQ (GB). **TEJIDOR, Liliana** [US/US]; 10440 Leslie Drive, Raleigh, NC 27615 (US). **NEISHEIM, Mike** [US/CA]; 70-1 Place D'Armes, Kingston, Ontario K7K-6S4 (CA). **JONES, Gregory** [US/US]; 2525 Comanche Trail, Hillsborough, NC 27278 (US).
- (74) Agents: **ROESLER, Judith, Ann** et al.; BioMerieux, Inc., 100 Rodolphe Street, Durham, NC 27712 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**  
— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



**WO 03/073099 A1**

(54) Title: METHOD FOR DIAGNOSING AND MONITORING HEMOSTATIC DYSFUNCTION, SEVERE INFECTION AND SYSTEMATIC INFLAMMATORY RESPONSE SYNDROME

(57) Abstract: A method for diagnosing and monitoring subjects for hemostatic dysfunction, severe infection and systematic inflammatory response syndrome is provided whereby lipoproteins are examined for abnormalities, particularly for prothrombinase enhancement, through quantitative and qualitative analysis.

Method for Diagnosing and Monitoring Hemostatic Dysfunction,  
Severe Infection and Systematic Inflammatory Response Syndrome

5

Field of Invention

This application claims priority from U.S. Provisional Patent Appls. SN 60/359,932 (filed February 27, 2002), SN 60/363,073 (filed March 11, 2002), SN 60/396,392 (filed July 17, 2002) and SN 60/404,652 (filed August 20, 2002).

10

Background of the Invention:

Severe infection and sepsis are common causes of morbidity and mortality. Sepsis is a vast clinical entity that takes many forms. The pathophysiology of a host response to infection is complex and the signs and symptoms of systemic inflammation may have an infectious or non-infectious etiology and are not specific. Patients with systemic infection are often difficult to distinguish from patients with similar clinical signs and laboratory findings without infection. Infection has multiple causes including that caused by bacterium, fungi, parasites and viruses.

15

Bacteriological evidence of infection may not develop at the same time as clinical signs of distress. Further, it requires time to grow a culture of organism from a blood sample to confirm the presence of infective bacteria and the results may be incorrect due to contamination, etc. As used herein, severe infection may include a diagnosis of sepsis, severe sepsis, septicaemia, and septic shock as well as disseminated intravascular coagulation ("DIC"). Also included in the definition of infection is systemic inflammatory response syndrome "SIRS" although it may have infectious as well as non-infectious origin (both of which are encompassed herein).

20

25

SIRS may exhibit or develop into systemic inflammation that ultimately leads to multiple organ dysfunction syndrome. Patients with SIRS may develop the syndrome from infection, trauma, burns, pancreatitis, etc.

As used herein hemostatic dysfunction may be defined as an error in  
5 coagulation. For both DIC and sepsis, there is increasing recognition of common and overlapping pathophysiological pathways that link inflammation and coagulation. The recent therapeutic success of recombinant human activated protein C (APC) in severe sepsis especially after a myriad of unsuccessful strategies would support this further. APC suppresses thrombin generation via the inactivation of coagulation co-factors,  
10 Va and VIIIa and is also thought to have anti-inflammatory properties.

There is a continuing need to find early indicators or markers of infection, SIRS and hemostatic dysfunction due to lack of specificity of current methods of diagnosis. An early diagnosis may greatly increase recovery of the patient and reduce the morbidity and mortality rates associated with this population. Further a diagnostic  
15 marker or test to monitor the efficacy of treatment of the host response to infection, SIRS and hemostatic dysfunction is needed as well.

The time dependent measurement profiles of coagulation screening assays have been associated with predicting congenital, acquired imbalances and hemostatic dysfunction as described in Givens et al. WO 96/41291 and Toh et al. WO 00/46603.  
20 Once such profile is that of an activated partial thromboplastin time (“APTT”) assay having a decrease in plasma light transmittance before clot formation, now commonly referred to as a biphasic waveform (also referred to herein as BPW). This BPW has

been associated with critically ill patients having DIC which is common in many primary diseases including sepsis. The biphasic waveform on coagulation instruments offers a simple and rapid test for early diagnosis of hemostatic dysfunction, including DIC.

5           As described in WO 01/96864 (Dec. 20, 2001), a calcium-dependent complex between C reactive protein (CRP) and lipoprotein (particularly very low density lipoprotein (VLDL)) has been identified as the molecular mechanism underlying the biphasic waveform. The complex may be used to identify patients with sepsis, SIRS and septicaemia in addition to patients with other hemostatic dysfunction that can lead  
10 to bleeding or thrombosis including DIC. Further, WO 01/96864 describes detecting the complex by a clotting assay, latex agglutination or gold sol assay, and immunoassay whereby the precipitate is formed prior to or in the absence of clot formation, depending on the reagent used.

          While the biphasic waveform and the CRP-lipoprotein complex provide  
15 advances in the early diagnosis of different kinds of severe infection and haemostatic dysfunction (including DIC and sepsis), there is a continued need to further identify early diagnostic means, particular markers severe infection, SIRS and hemostatic dysfunction.

#### Summary Of The Invention

20           It has been discovered that diagnosis and monitoring of a host response to severe infection, SIRS and hemostatic dysfunction may be accomplished by detecting

qualitative and quantitative differences between lipoproteins of this population as compared with lipoproteins found in normal, healthy samples.

In one preferred embodiment, a method for diagnosis and monitoring severe infection, SIRS and hemostatic dysfunction has been found said method comprising

5 (a) obtaining a patient sample; (b) measuring a lipoprotein fraction from said sample for an abnormality; and (c) correlating said lipoprotein measurement to an abnormality found in patients having severe infection, SIRS or hemostatic dysfunction. Further, said method can be utilized to predict an increased likelihood of system failure or mortality in said patient.

10 Another aspect of the invention is a method for predicting an increased likelihood of infection (particularly sepsis) in a patient, comprising: (a) obtaining a sample of very large density lipoproteins (VLDLs) from a patient; and (b) determining the activity of VLDLs in said sample for activating prothrombin; a greater activity of said VLDLs for activating prothrombin indicating an increased likelihood of infection

15 in said patient. The determining step may be carried out directly or indirectly by any suitable means, such as by measuring rate of thrombin generation.

In yet another aspect of the invention, the method for diagnosing severe infection, SIRS or hemostatic dysfunction is accomplished by steps comprising (a) obtaining a patient sample; (b) subjecting the patient sample to a biphasic waveform

20 screening test to obtain a normal or biphasic waveform result; (c) subjecting said patient samples demonstrating said biphasic waveform result to a lipoprotein analysis;

and (d) comparing said lipoprotein analysis with that of a normal sample to diagnosis of severe infection, SIRS or hemostatic dysfunction.

In yet another aspect of the invention, a method for diagnosing severe infection is accomplished by the steps comprising (a) obtaining a sample from a patient; (b) measuring said sample lipoprotein fraction for binding specificity with Annexin V; and (c) correlating said binding specificity prothrombin activation with the diagnosis and/or monitoring of a host response to severe infection, including SIRS, DIC and sepsis. Further, said method can be utilized to predict an increased likelihood of system failure or mortality in said patient.

10 Brief Description of the Drawings:

FIG. 1 illustrates a normal waveform at 580 nm (panel A) and a decrease in light transmission prior to formal clot formation to produce a biphasic waveform profile (panel B) where the dashed line denotes transmittance level at 18 seconds (TL 18) as the quantitative index, in A, TL18 = 100 and in B, TL18 = 63.

15 FIG. 2 shows logistic regression evaluation of the TL 18 data showing mortality fraction increasing from 0.26 in patients with a normal waveform to 0.61 when TL18 values reduced by 25-35%, with open circles = observed fractions; dashed lines = 95% confidence limits.

FIG. 3 shows the logistic regression evaluation of the TL18 data in the same cohort of patients for the association with sepsis with open circles = observed fractions, dashed lines = 95% confidence limits.

20

FIGs. 4A-C illustrate logistic models for DIC prediction from (A) APTT-TL18 values, (B) CRP and (C) triglyceride measurements. Open circles are observed fractions; dashed lines indicate 95% confidence limit.

FIGs. 5A-C illustrate size-exclusion chromatography of serum from (A) normal individual, (B) intensive care patient without a biphasic waveform, and (C) intensive care patient with a biphasic waveform. Fractions were measured for CRP (thick line), apo B (thin line) and apo E (dotted line).

FIGs. 6A-B illustrate prothrombinase supporting activity. (A) shows the comparative activity generated by PCPS (●), VLDL from normal individuals (□) and from patients with the biphasic waveform (◆). (B) shows the differences in 81 samples from normal VLDL (□), intensive care patients with (◆) and without (Δ) the biphasic waveform.

FIGs. 7A-C illustrate total triglyceride levels where (A) shows the difference in plasma triglyceride concentrations between normal individuals (x), patients with normal waveforms (+) and biphasic waveforms at different APTT-TL18 values (◇). (B) and (C) shows time courses from 2 patients who recover and die from sepsis, respectively, in terms of total prothrombinase activity (◆), as calculated by the product of plasma triglyceride and the specific prothrombinase activity/ mM triglyceride, and APTT-TL18 values (●).

FIGs. 8A-C illustrate Annexin V studies. (A) shows the FACS plot comparing VLDL from normal and patient with the biphasic waveform (arrowed) following addition of fluorescent conjugated annexin V. (B) illustrates the effect on

prothrombinase activity by incubating increasing concentrations of annexin V with VLDL from normal individuals (open bars) and patients with the biphasic waveform (shaded bars) is shown. Data are mean  $\pm$  SEM; n=4 in each group (\* p< 0.01, \*\* p < 0.002). (C) demonstrates the comparative effect of increasing annexin V (single line) or 9D2 antibody (dashed line) on prothrombinase activity in VLDL from normal ( $\square$ ) versus patient with the biphasic waveform ( $\blacklozenge$ ).

FIGs. 9A-B illustrate two dimensional thin layer chromatography comparison of (A) normal and (B) biphasic waveform patient VLDL with PC denoting phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; Cer, cerebrosides.

FIG. 10 illustrates prothrombinase activity of VLDL from biphasic waveform patients (n=3) before and after controlled, apo B and apo E immunoadsorption expressed as mean  $\pm$  SEM (\* p< 0.01, \*\* p < 0.002).

FIG. 11 illustrates comparative procoagulant cofactor activity of VLDL from 7 normal versus 7 patients with biphasic waveforms in a modified APTT clotting assay. The box plot represents the 25<sup>th</sup> to 75<sup>th</sup> percentile interquartile ranges of the clot times (in seconds) generated. The thickened line represents the median and the extreme upper and lower data points are represented outside the interquartile range.

FIGs. 12A-B provide a serial sample profile of slope-1 values for a representative patient (A) and the corresponding agarose gel electrophoresis of patient's plasma and isolated VLDL (B).

#### Detailed Description

Although the lipoprotein-acute phase protein complex (typically referred to as CRP-VLDL) formed *in-vivo* has been described in patients having infection and sepsis, the measurement of quantitatively and qualitatively differentiating the lipoprotein subfraction separate from the complex substrate not been previously  
5 associated with infection, SIRS, sepsis or hemostatic dysfunction. It has been discovered that the lipoprotein fractions of patients with severe infection, SIRS and hemostatic dysfunction are distinguishable from those of normal patients and thus the lipoprotein itself may be used as a biomarker to diagnose and monitor these disease states. The differences in the lipoprotein of this population is not limited to just that  
10 lipoprotein fraction that forms the complex. Disturbances in these lipoprotein subclasses influence complex formation and may also be determinants of clinical outcome.

The step of obtaining lipoproteins from patient samples may be carried out by any suitable means, such as by collecting a blood sample from a patient and then  
15 utilizing a means to examine (quantitative or qualitative) of the abnormal lipoproteins found in patients with severe infection, SIRS and hemostatic dysfunction.

Still further, separating the abnormal lipoproteins may not be required if a specific binding assay is utilized, wherein said assay may be competitive or non-competitive. In such assays, a component may be designed that binds directly or  
20 indirectly to a part or all of the lipoprotein anomalies found in patients with severe infection, SIRS and hemostatic dysfunction, particularly sepsis and DIC, thus a patient sample (sample is defined herein to include plasma, whole blood sample,

serum, etc.) may be directly analysed for the anomaly relating to the lipoprotein.

More preferably, the assay may be a clotting assay, latex agglutination or gold sol assay, ligand assay, protein binding assay or immunoassay. More preferably serum

or plasma is utilized as the patient sample, most preferably plasma. In one preferred

5 assay, surface changes of the abnormal lipoprotein are measured by the lipoprotein

binding to Annexin 5 (or another specific ligands that recognize anionic

phospholipids), either directly or indirectly. If preferred, the lipoprotein may be

subfractionated into VLDL, IDL and LDL. Further, antibodies (or fragments thereof)

specific to Annexin 5 may be prepared and utilized in a specific binding assay such

10 that the abnormal lipoproteins are detected and thereafter correlated with patients

having severe infection, SIRS or hemostatic dysfunction, particularly those with DIC or sepsis.

More particularly, according to the invention, provided is a diagnostic method

for host response to infection, SIRS or hemostatic dysfunction, more preferably for

15 sepsis and DIC, whereby an *in-vivo* circulating complex of lipoproteins is identified as

abnormal. Preferably the method is specific to beta lipoproteins (apoB containing

lipoproteins), particularly those of the same buoyant density including very low

density lipoprotein (VLDL), low density lipoprotein (LDL), and intermediate density

lipoprotein (IDL). More preferably anionic phospholipids are examined for

20 abnormalities and most preferably VLDL is examined. The method may be used for

predicting the prognosis of a patient as well as monitor the patient once therapy is

initiated. The testing of the lipoproteins may be manual or automated and may

include NMR analysis. More particularly, current chemistry tests for measuring cholesterol and triglycerides (TG) are independent of particle concentrations. By use of NMR technology, the core lipids (TG or cholesterol esters) do not impact particle concentration measurements. NMR gives a total number of particles of a certain size, thus allowing for calculation of total surface area whereas current chemistry tests assume that the TG are found solely in the VLDL size particles and LDL size particles are assumed to contain mainly cholesterol esters. Traditional chemistry tests for lipoproteins do not give information on the particle numbers and surface areas. According to one of the embodiments of this invention, these differences of particle numbers and surface areas of lipoproteins may be associated with severe infection including DIC and sepsis.

More particularly, it has been discovered that patients with severe infection, SIRS and hemostatic dysfunction have pro-coagulant lipoproteins referred to as "abnormal" lipoproteins herein. The changes in the lipoproteins are believed to be abnormal due to their metabolism. According to the invention, the lipoprotein changes may manifest in changes in physical properties as well as qualitative functional changes including an increase in procoagulant activity and enhanced prothrombinase activity. Lipoprotein changes that may be measured to develop a relationship between abnormal patient samples (from those having severe infection, SIRS or hemostatic dysfunction, particularly sepsis and DIC) and normal patient samples include the following: a shift in VLDL, IDL, LDL or high density lipoprotein (HDL) protein levels; increased surface exposed of anionic phospholipids

(more preferably phospholipid phosphatidylserine (PS)); shift in VLDL size; shift in VLDL lipids (core and surface); shift in VLDL charge; appearance of procoagulant lipoproteins; indication of lipoprotein cascade disruption (drop in all lipoprotein concentrations), mildly elevated or depressed VLDL levels; depressed LDL levels; appearance of SAA-HDL; post-translational changes; lipoprotein assembly constituent changes; lipoprotein mobility changes; secretion changes; perfusion; lipoprotein enzyme changes (CETP, LCAT, etc.); lipoprotein receptor changes, indicator of lipoprotein oxidation, and endotoxin incorporation. In one preferred embodiment the abnormal lipoprotein detected and associated with severe infection, SIRS and hemostatic dysfunction, more preferably sepsis and DIC, is the lipoproteins with enhanced ability to support prothrombinase activity (particularly VLDL). Additionally, in one preferred embodiment, the abnormal lipoproteins of patients with severe infection, SIRS and hemostatic dysfunction (more preferably with sepsis or DIC) may be identified by using various methods including measuring specific apolipoproteins, particle sizes, numbers or lipids. More particularly, it involves the total surface area of lipoproteins relative to normals.

Thrombin generation *in vivo* is considered to be a pivotal process in DIC and markers of its generation increase during disease progression. For the enzymatic conversion of prothrombin to thrombin to occur at physiologically relevant rates, the components must be localised to appropriate surfaces. For *in vivo*, this is presumed to be supplied by activated platelets, mononuclear and perturbed endothelial cells. VLDL, at physiological levels, can also support relevant rates of thrombin generation

and this is thought to be of relevance to the significant prediction of triglycerides to cardiovascular events. According to the invention, it has been discovered that CRP-VLDL is more than just a marker or predictor of DIC and that it exists *in vivo* with a possible pathogenic role through the ability to enhance and sustain the generation of thrombin.

Thrombin, as the main effector protease of the coagulation cascade, is of pivotal importance in the pathogenesis of DIC. Its procoagulant effect in converting fibrinogen to fibrin and its anticoagulant facilitation from binding to endothelial thrombomodulin to activate the protein C can be disregulated in DIC. According to the invention, it has been discovered that this enhanced procoagulant aspect of the VLDL from patients with the biphasic waveform may demonstrated through the significant shortening of clot time in a modified APTT assay. It has also been found that the added normal or patient VLDL provided the only differentiating source of phospholipid surface provision for the coagulation reaction. While not wishing to be bound by theory, this demonstration may also be one explanation for the finding that shorter APTT clot times are associated with adverse outcomes in a general hospital setting.

Still further, the invention may be practiced utilizing APTT parameters of waveform technology such as rate and/or acceleration. Also, the global coagulability assays and methods as taught in WO 02/34110 may be utilized to capture lipoprotein abnormalities in patients having severe infection, SIRS and hemostatic dysfunction. For example the detection of abnormal lipoprotein step may be carried out by

detecting an enhanced procoagulant aspect of the VLDL from patients with the biphasic waveform by measuring a shortening of clot time in a modified APTT assay or measuring clot formation or measuring increased rate of acceleration of clot formation in a dilute tissue factor based assay.

5           In one preferred embodiment of the invention, it has been discovered that patients with hemostatic dysfunction, SIRS and a host response to severe infection have a VLDL that can significantly enhance thrombin generation. Moreover, the calculation of total thrombin generating capacity from the quantitative and qualitative changes in VLDL within serial samples of patients with sepsis and DIC show a direct  
10           positive correlation with clinical progression. This supports the relevance of thrombin as a major player in the pathophysiology of sepsis. While its primary role may have been as part of the acute phase protective initial response, the protracted or enhanced response fuelled by VLDL procoagulant surfaces may lead to deleterious consequences.

15           According to the invention a biomarker is provided, said biomarker of increased prothrombinase potential to better target anticoagulant based therapies such as recombinant human APC in severe sepsis. Further provided is insight into mechanisms other than by way of microparticles and cell surfaces that can enhance and abnormally sustain thrombin generation *in vivo* in disease states. Although not  
20           wishing to be bound by theory, it is believed that the qualitative increase in thrombin generation is associated with a lipoprotein compositional change in response to severe infection, SIRS and hemostatic dysfunction, particularly sepsis and inflammation.

In one preferred embodiment, the ability to support prothrombinase activity of the VLDL is measured. As used herein the ability to support prothrombinase activity also refers to and is used interchangeably with prothrombinase activity (PTase) and have been found to be altered in patients who have a biphasic waveform. Biphasic waveforms may be identified and measured by appropriate methods known to those skilled in the art utilizing coagulation assays and means for measuring the pre-coagulation phase (slope-1) over a time to provide a profile with determined normal and abnormal parameters. Preferably, an activated partial thromboplastin time (APTT) assay may be used with an optical transmittance coagulation analyzer, such as the MDA 180<sup>®</sup>(bioMerieux). For example, a normal waveform at 580 nm having a sigmoidal waveform pattern is characterized by an initial 100% light transmittance phase prior to formal clot formation as is illustrated in panel A of FIG.1. In contrast, patients with a biphasic waveform (BPW) have an immediate, progressive fall in light transmittance that occurs even in the pre-clotting phase that affects the early part of the curve to produce a biphasic profile as is illustrated in panel B, FIG. 1. Patient populations with biphasic waveforms have been associated with higher mortality and sepsis incidence as depicted in FIGs 2 and 3, respectively.

Accordingly to the invention, patients with a biphasic waveform have been observed to have at least about a two fold increase in specific PTase activity in lipoproteins (particularly VLDL) from biphasic waveform patients (e.g. patient in acute phase host response). The range of abnormality or increase in specific PTase activity (thrombin generating potentiation) of abnormal lipoprotein (particularly VLDL)

of patients with severe infection, SIRS and hemostatic dysfunction may range from about two to about eight fold, more preferably from three to four fold, as compared with PTase activity of lipoproteins (particularly VLDL) from the normal, healthy population. It has been found that the total PTase mirrors APTT waveform (WF) changes. While not wishing to be bound by theory, it is believed that the enhanced PTase activity is due to increased anionic phospholipid exposure. Further the enhanced PTase is not believed to be due to platelet or endothelial microparticles. apoB presence on VLDL is believed to be important to PTase activity. Preferably, the PTase activity may be detected by a modified , more preferably with a modified coagulation assay such as APTT or modified tissue based assay. Preferably the assay to detect lipoprotein abnormality is carried out without forming a CRP-lipoprotein complex, more preferably a CRP-VLDL complex.

In another embodiment of the invention, it has been discovered that patients with severe infection, SIRS and sepsis (most preferably DIC) may be diagnosed by identification of an increased surface of negatively charged (anionic) phospholipid surface, particularly that of phospholipid phosphatidylserine (PS). While not wishing to be bound by theory, it is believed that the anionic phospholipid may be released from platelet microparticles which have demonstrable procoagulant activity in patients with sepsis. Similarly, the anionic phospholipid may be hepatically derived from membrane anionic phospholipid translocation or shedding in early endothelial dysfunction or apoptosis associated with multi-organ failure and IDC. Further, low expression of apolipoprotein E within the VLDL particles may substantially affect the

uptake of remnant particle composition and may have relevance to their atherogenicity and their ability to support thrombin generation.

As known, the cell membrane PS is translocated to the outer layer of the membrane as one of the initial steps in apoptosis which mediates macrophage recognition and phagocytosis. PS is also crucial for coagulation reaction such as prothrombinase assembly. Therefore the increased thrombin generating potential of VLDL particles from patients with the CRP-VLDL complex is believed to be caused by an increase in exposed anionic phospholipid (including PS) sites on the particle surface, potentially absorbed onto the VLDL surface from apoptotic cells or by conformational change within the particles.

Annexin A5 (A5) is a calcium dependent phospholipid binding protein that has a high affinity for negatively charged phospholipid surfaces, with a higher specificity for PS than other anionic phospholipids. As previously described, specific binding assays may be prepared that take advantage of this binding affinity such that the abnormal lipoprotein of patients with severe infection, SIRS and hemostatic dysfunction may be measure utilizing A 5 as a labelled binding protein, antibodies to A5, etc., to detect the abnormal lipoproteins either directly or indirectly.

Accordingly to another embodiment of the present invention, it has been found that the A5 binding capacity may identify exposed anionic phospholipid sites and block the anionic phospholipid dependent reactions. As demonstrated in FIG. 8B, A5 inhibits prothombinase assembly. As depicted in FIG. 8B, the first bar shows normal patients treated with A5 and the second bar shows biphasic patients treated

with A5 at varying concentrations of A5. Preferably the A5 is administered in an effective amount (preferably at least about 100 ng/ml, more preferably within the range of 100 to 5000 ng/ml) to re-equilibrate the hemostatic system of a biphasic patient with severe infection, DIC or sepsis. A5 has anticoagulant properties

5 demonstrable *in-vitro*, and thus it is thought that to form an anti-thrombotic shield around exposed PS, prohibiting the formation of functional procoagulant complexes occurs. Anti-phospholipid antibodies putatively disrupt this shield thus explaining the prothrombotic tendency observed in these patients. Accordingly to the invention, A5 may be provided as a therapy for patients with clinical conditions associated with DIC

10 because the therapeutic intervention could re-equilibrate the hemostatic system to impact upon the high mortality of this group.

The following non-limiting examples illustrate this invention.

### EXAMPLES

#### Example 1

15 Methods

*Clinical study*

In a 24-month prospective study, APTT waveform analysis was performed on a daily basis in all consecutive adult patient admissions to the ITU of the Royal Liverpool University Hospital. The study was conducted to gain pathophysiological

20 and mechanistic insight on clinical disease from when CRP-VLDL complexation is maximal. As such, the association of interest is between the lowest TL18 value; i.e. the most abnormal waveform in detecting maximal complex formation, as compared

with its individual components (CRP or VLDL) for each individual patient episode with the diagnosis of DIC. DIC was defined according to the International Society of Thrombosis and Haemostasis Standardization Sub-Committee cumulative score of 5 or above, derived from changes in the platelet count, prothrombin time (PT), fibrinogen and D-dimer levels (Taylor, F.B., Toh, C.H., Hoots, W.K., Wada, H., Levi, M. 2001. Towards definition, clinical and laboratory criteria and a scoring system for disseminated intravascular coagulation. *Thromb. Haemost.* **86**:1327-1330). PT, fibrinogen and D-dimers were measured using Simplastin S, Fibriquik and MDA<sup>®</sup> D-dimer latex particle based immunoassay, respectively on plasma collected into 0.105M trisodium citrate (one part plus 9 parts blood) on the MDA 180<sup>®</sup> (bioMerieux, Inc. Durham, NC USA), an automated photo-optical-based coagulation analyser. The DiaMed AG CRP ELISA (Cressier sur Morat, Switzerland) was used and plasma triglyceride (TG) as marker of VLDL was measured using the Sigma Infinity Reagent system. The diagnosis of DIC was performed blinded to the results of the waveform analysis.

*APTT Waveform Analysis.*

Waveform analysis, on the MDA 180<sup>®</sup> at 580 nm, using Platelin LS reagent on fresh citrated plasma has been well described (for example, Downey, C., Kazmi, R., Toh, C.H. 1997. Novel and diagnostically applicable information from optical waveform analysis of blood coagulation in disseminated intravascular coagulation. *Br. J. Haem.* **98**:68-73 and Downey, C., Kazmi, R., Toh, C.H. 1998. Early identification and prognostic implications in disseminated intravascular coagulation

through transmittance waveform analysis. *Thrombos. Haemostas.* **80**:65-69).

Quantitation of the degree of biphasic waveform abnormality was by the light transmission level at 18 seconds (TL18) into the APTT reaction with normal waveforms having a mean TL18 of 100% (99.43-100.69) and a mean coefficient of  
5 variation of 0.15%.

### *Materials*

Human factors X (FX), V (FV) and prothrombin (FII) were purified from plasma and converted to FXa and FVa, as previously described. The thrombin chromogenic substrate, S-2238 was obtained from Chromogenix (Milan, Italy).  
10 Human recombinant CRP was obtained from Calbiochem (Nottingham,UK) and annexin V from BD Biosciences (San Diego, CA) with its fluorescein labelled form from Boehringer Mannheim (Werk Penzberg, Germany). Apolipoprotein (apo) B and E standards, Infinity cholesterol/ triglyceride reagents, the respective standards and Silica, fumed, were from Sigma (St Louis, MO). Goat anti-human apo B-100 and goat  
15 anti-human apo E were from Abcam (Cambridge, UK). Rabbit anti-human apo B-100, rabbit anti-human apo E and monoclonal mouse anti-human glycoprotein IB were from Dako (Glostrup, Denmark). Mouse anti- $\alpha_v\beta_3$  integrin complex and mouse anti-IgG<sub>1</sub>/G<sub>2a</sub> were from BD Pharmingen (San Diego, CA). Goat-anti-rabbit IgG conjugated to HRP was from Santa Cruz Biotechnology (California, US). Protein G  
20 sepharose was from Zymed laboratories Inc. (San Francisco, CA). Phospholipid standards were acquired from Sigma, St Louis, MO. All other reagents were of analytical grade.

*Chromatography studies*

Size exclusion chromatography was undertaken with a HiPrep Sephacryl S-300 (Amersham Pharmacia, Amersham, UK) column (180 cm<sup>3</sup>) equilibrated with tris-buffered saline (TBS) pH 8, 2.2 mM CaCl<sub>2</sub> at room temperature (RT). 5ml test serum  
5 was applied to the column and fractions were collected every 2 minutes for 90 min at a peristaltic pump speed of 1.5ml/min. The column was washed with approximately 500 ml 0.2 M sodium hydroxide between runs. The different sera tested were from 2 normal individuals without the biphasic waveform (CRP = 5 and 8 µg/ml respectively), 3 ITU patients without the biphasic waveform (CRP = 125, 150 and 150  
10 µg/ml) and 4 ITU patients with the biphasic waveform (CRP = 120, 130, 145, and 165 µg/ml). In 2 from the last group, the same serum underwent repeat chromatography but in the presence of 10 mM EDTA to disrupt any divalent cation-dependent complex. Fractions were kept at 4°C and quantified for CRP, apo B and E by ELISA. For apo B/E determinations, 96-well plates were coated overnight at 4°C with 100µl  
15 goat-anti-human apo B/E at 5 µg/ml in 50 mM sodium hydrogen carbonate pH 9.5, per well. Following washes with 2% BSA/ HEPES buffered saline (HBS)/ Tween 20, plates were blocked for 1h at RT with PBS-2% BSA. 100µl standard (A4183 for apo B and A2673 - A2456 for apo E) /sample fractions were applied and incubated for 2 h at RT. Detection was with 100 µl rabbit-α-human apo B/E at 2 µg/ml followed by  
20 100µl goat-anti-rabbit-HRP conjugate at 1:20000 in washing buffer. The signal was generated with 100 µl 2.9% O-phenylenediamine dihydrochloride in 6ml deionised water with 2.5 µl 30% hydrogen peroxide (Sigma-Aldrich Company Ltd., St. Louis,

MO, US) and the reaction stopped by 50  $\mu$ l 0.5M Sulphuric acid. Plates were read on Spectramax Plus (Molecular Devices Corp., Stanford, CA) at 490 nm.

Two-dimensional thin layer chromatography (TLC) was performed by the method of Vitiello and Zanetta (Vitiello, F., Zanetta, J.P. 1978. Thin-layer chromatography of phospholipids. *J. Chromatogr.* **166**:637-40). In brief, TLC plates (Whatman LabSales, Hillsboro, OR) were activated for 30 min at 125°C. The sample was spotted 15 mm from the edge of the plate and run for 140 mm in methyl acetate: n-propanol: chloroform: methanol: 0.25% potassium chloride (25:25:25:10:9) using a well-saturated tank. The plate was then dried under nitrogen before running in the perpendicular direction in chloroform: methanol: acetone: acetic acid: water (75:15:30:15:7.5), using the same saturation conditions. The plates were then sprayed with ninhydrin (95 ml 0.2% ninhydrin in methanol plus 5 ml 10% aqueous acetic acid) then with 50% aqueous sulphuric acid before heating to 120°C for 15 min. Standards used were phosphatidyl-choline (PC), -serine (PS), -ethanolamine (PE), sphingomyelin (SM), and cerebroside.

*VLDL isolation and quantitation.*

This was as previously described after removal of chylomicrons through centrifugation of test plasma at 14000 rpm for 10 min at 10°C (1). The VLDL fraction (density < 1.019 g/mL) was stored at 4°C and used fresh within 4 days of isolation.

20 *Prothrombinase supporting activity*

Isolated VLDL (100, 300, and 500  $\mu$ M TG) or PC/PS vesicles (75:25) at 50  $\mu$ mol/L/phosphate, prepared as previously described, were first diluted in TBS (pH

7.4), 5 mmol/L CaCl<sub>2</sub> and incubated with 15 nM FVa, 1 μM II. The reaction was initiated with the addition of 0.1 nmol/L FXa. At timed intervals, 10 μl aliquots were taken into 90 μl TBS, 2 mmol/L EDTA. 10 μl was then aliquoted into a 96 well plate, to which 190 μl (400 μmol/L final conc.) of S-2238 was added. Chromogenic  
5 liberation at 405 nm was determined on the Spectramax plate reader. Rates of thrombin generation were established by comparison to a calibration curve constructed from a known human thrombin standard. In experiments assessing the contribution of CRP to prothrombinase assembly, 500 μM TG VLDL was incubated with 100 μg/ml CRP for 15 min at RT prior to the introduction of the coagulation  
10 proteins into the reaction.

The oxidation status of VLDL was investigated by the method based on thiobarbituric acid reacting substances (Wallin, B., Rosengren, B., Shertzer, H.G., Camejo, G. 1993. Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: its use for evaluation of  
15 antioxidants. *Anal. Biochem.* **208**:10-5). 75 μl 1.3% thiobarbituric acid (in 0.3% NaOH), 50 μl trichloroacetic acid, 40 μl TBS buffer and 25 μl of VLDL was incubated at 60°C for 40 min and then cooled on ice. 10 μl 20% SDS was then added prior to absorbance monitoring at A530-A600 nm on the Spectramax reader.

To delineate the contribution of phospholipid surfaces, FACS was performed  
20 with fluorescein labelled annexin V incubated with VLDL (final concentration 500 μM TG) suspended in HEPES binding buffer, 50 mM calcium on a Becton Dickinson flow cytometer using Cellquest software. The effect of annexin V addition on

prothrombinase generation was assessed by prior incubation of increasing annexin V concentrations (0-100  $\mu\text{g/ml}$  at RT for 15min) with 300  $\mu\text{M}$  TG VLDL in TBS/ 5 mM  $\text{CaCl}_2$ . In separate experiments, prothrombinase activity was compared after 500  $\mu\text{mol/l}$  TG VLDL in TBS/ 5mM  $\text{CaCl}_2$  was pre-incubated with increasing concentrations (0, 10, 100, 1000 ng/ml) of either annexin V or the phospholipid specific antibody 9D2 (S Ran, University of Texas) at RT for 15min.

The possibility of platelet or endothelial microparticle contamination in the isolated VLDL was determined by FACS using established platelet and microparticle gates. VLDL (final concentration of 500  $\mu\text{M}$  TG) was incubated with 100  $\mu\text{g/ml}$  fluorescein-conjugated monoclonal antibodies specific for platelet glycoprotein 1b and endothelial cell- $\alpha_v\beta_3$  integrin complex (10  $\mu\text{l}$ ). The respective isotype controls were used concurrently. Assessment for bound coagulation proteins in the VLDL was by using specific reaction mixes deficient in factors Va, II or Xa for the standard chromogenic prothrombinase assays. The absence of thrombin generation indicated deficiency of the coagulation factor assessed.

#### *Immunoabsorption analysis*

Rabbit anti-human apo B/E (1mg) were coupled to 150  $\mu\text{l}$  protein G sepharose beads. Control sepharose, without antibody addition, was prepared similarly. 250 $\mu\text{l}$  VLDL standardised for TG was mixed by end over end rotation and incubated overnight at 4°C. Harvested supernatant was analysed for prothrombinase-supporting assembly and apoB/E quantitation, as described above, and by Western blotting. The latter was by way of SDS-PAGE using a 4% stacking gel, and a 5% or 15% running

gel for apoE and apoB respectively. 25  $\mu$ l VLDL in double strength sample buffer was loaded and run at 45mA for approximately 1h. Transfer onto Immobilon membrane (Millipore, Billerica, Massachusetts) was at 400 mA for 1 h and overnight blocking in 5% milk-TBS-0.1% Tween. Incubation was initially with 1mg/ml antibody apoB/E in 5 3% milk TBS-0.1% Tween for 1 h at RT and then with anti-rabbit HRP (1 in 6000) in 3% milk TBS-0.1% Tween for 40 min at RT after in-between washing. Following further washing in TBS-0.1% Tween, detection was with chemiluminescence (ECL, Amersham Pharmacia, Amersham, UK) and exposure to x-ray film.

#### *Procoagulant cofactor clotting assay*

10 To determine procoagulant activity of VLDL in the plasma milieu, a modified APTT was performed on the Spectramax microtitre plate reader. 25  $\mu$ l platelet-poor plasma from normal, overnight-fasted individuals was incubated with 25  $\mu$ l of 0.175% silica in imidazole buffer at 37°C. After 180 seconds, 50  $\mu$ l of a 50:50 mix between VLDL (from normal individuals or patients with biphasic waveform abnormalities 15 with equivalent TG levels) and 25 mM  $\text{CaCl}_2$  was added and the time to clot formation was recorded. This was defined as the time at half-maximum absorbance change. Each run, with all samples performed in duplicates, compared VLDL from a normal versus VLDL from a patient with a biphasic waveform added to the same normal plasma.

#### 20 *Statistics*

In the clinical study, non-linear regression analysis with a three-parameter logistic model was fitted to the data in the association between the most abnormal

waveform result, the corresponding CRP and TG levels respectively and the diagnosis of DIC (Toh, C.H., Ticknor, L.O., Downey, C., Giles, A.R., Paton, R., Wenstone, R. 2003. Early identification of sepsis and mortality risks through simple, rapid clot-waveform analysis. *Intensive Care Med.* **29**:55-61 and Ratkowsky, D.A.1983. *Nonlinear Regression Modeling*. Marcel Dekker Inc., New York). All these calculations were done using S-PLUS 2000 (MathSoft, Seattle, WA). The results from the experimental work were calculated as means +/- SEM. The means were compared using either a univariate ANOVA (prothrombinase) or the analysis of variance by students t-test. Non-parametric testing was also used to supplement the t-test to make no assumption about data distribution. Calculations were performed with the use of SPSS software. A value of  $p < 0.05$  was used to define statistical significance.

## Results

### *Extent of correlation to DIC*

Of a total of 1187 patients admitted consecutively to the ITU over the study period of 24 months, 758 (64%) had a biphasic waveform; i.e. a TL18 < 99% at some time during the course of their intensive care stay. The data shown in Fig 4A demonstrates the increasing association between the degrees of waveform abnormality, in reflecting the extent of CRP-VLDL formation, with the diagnosis of DIC. In those with normal waveforms, 10-15% fulfilled the criterion for DIC and this likelihood increased to 60% when the TL18 was reduced by 20%. In those patients with TL18 values of 60% or less, a plateau was reached in the virtual 100% association with DIC. In comparison, the individual components to the complex

underlying the biphasic waveform were less strongly associated with DIC as shown in figures 4B and C respectively. The highest levels of CRP and triglyceride, as a marker of VLDL, were associated with DIC in approximately 60% and 70% of cases respectively. Although there were several relatively large triglyceride readings that were highly influential in fitting the logistic curve, as compared to the majority of values, these are shown to reflect the trend of increasing DIC at these large values. These results link high levels of CRP-VLDL complex formation with certainty as to the presence of clinical DIC and that this is as a function of the complex and/or each of its individual components.

10 *Chromatographic evidence of LCCRP in vivo*

Figure 5A shows the chromatographic profile from serum of a normal, fasted individual with apo B broadly eluting over early fractions, as a result of its presence in most classes of lipoproteins. Apo E, a marker of the less dense lipoproteins, would generally be detected later on although there would be individual variation. A consistent finding however would be the appearance of CRP as a single peak towards the middle of the elution profile that is greatly amplified, as part of the acute phase response, in intensive care patients. In patients without the biphasic waveform (Fig 5B), this remains a single peak but a second, smaller and earlier CRP peak would also be detected in patients with the biphasic waveform, which co elutes with apoB and E (Fig 5C). This earlier CRP peak in a “heavy” form within the void volume also contains peak concentrations of apoB. Its profile is calcium dependent and disappears in the presence of EDTA into the volume of the single CRP peak.

*Qualitative and quantitative VLDL changes in patients with the biphasic waveform enhance prothrombinase activity.*

The kinetic contribution of VLDL to thrombin generation was compared to PCPS vesicles. Fig. 6A demonstrates the immediate response with PCPS vesicles with  
5 half maximal response within 30 seconds. By comparison, the half maximal response for biphasic VLDL is 8 min and 25 min for normal VLDL. This demonstration of variation in the ability to support thrombin generation was further assessed in a large sample set that also looked at differences between VLDL in different patient groups. Rates of thrombin formation were determined at numerous concentrations of normal  
10 VLDL (n=24) and VLDL from ICU patients with (n= 25) and without the biphasic waveform (n=22). Slopes of the relationships depicted in Fig. 6B indicate that the biphasic waveform patients were 2.5 fold more potent than normal VLDL and VLDL from ITU patients without the waveform abnormality ( $p<0.0001$ ). There was no difference between VLDL from normal and patients without the biphasic waveform in  
15 the rate of thrombin generation ( $p=0.23$ ). The inclusion of increasing concentrations of CRP did not significantly vary the rates of thrombin generation from the different groups of VLDL tested.

In the same samples, the total triglyceride content had been quantified prior to VLDL separation. Figure 7A shows that the total triglyceride level is frequently  
20 elevated in patients with the biphasic waveform only and there is a positive correlative trend with increasing waveform abnormality. As both qualitative and quantitative changes in VLDL are relevant in terms of thrombin generating potential, the product

of total plasma triglyceride and the specific prothrombinase activity [IIa (sec-1)/mM TG] was ascertained. This relationship is demonstrated in individual patient series from samples over several days in Figs. 7B and C, which respectively illustrate examples of severe sepsis treated to resolution and where there is terminal decline.

5 *VLDL surface enhancement of thrombin generation in patients with the biphasic waveform patients.*

As phospholipid surfaces are integral to coagulation reactions, we assessed if increased availability on VLDL surfaces could account for the enhanced prothrombinase activity. Utilising fluorescent-labelled annexin V, a calcium-  
10 dependent binding protein specific for coagulant-active phospholipids (Meers, P., Mealy, T. 1993. Calcium-dependent annexin V binding to phospholipids: stoichiometry, specificity, and the role of negative charge. *Biochemistry*. 32:11711-21 and Tait, J.F., Gibson, D. 1992. Phospholipid binding of annexin V: effects of calcium and membrane phosphatidylserine content. *Arch. Biochem. Biophys*.  
15 298:187-91), we compared VLDL from 16 patients with the biphasic waveform to a corresponding number from normal individuals by FACs. The more intense signal from VLDL isolated from the biphasic patients (geo. mean fl 58.02 vs. 19.25) suggests increased coagulant-active lipid exposure. Fig. 8A illustrates typical experimental findings. This was unaffected by incubation with CRP (data not  
20 shown). The ability of annexin V to inhibit prothrombinase activity is demonstrated in Fig. 8B with data of VLDL from 4 normals and 4 patients with biphasic waveforms. There was significant inhibition of thrombin generation for biphasic VLDL at 100

ng/ml annexin V ( $p=0.003$ ). The degree of inhibition of prothrombinase activity is far greater on the VLDL derived from patients with the CRP-VLDL complex when compared to normal thereby suggesting that the increased thrombin generation was due to increased coagulant-active phospholipid exposure in VLDL from biphasic waveform patients.

This enhanced procoagulant potential could be explained by either *in vivo* and/or *ex vivo* contamination with platelet or endothelial microparticles. This was excluded as flow cytometry of lipoprotein microparticles failed to identify fragments specific for endothelial cells and platelets. In addition, the possible contribution of bound prothrombinase proteins to the harvested VLDL was also investigated. No significant thrombin was generated in the absence of purified factors Va, II or Xa by means of the prothrombinase assay, indicating absence of relevant coagulation protein association with the VLDL.

To assess if this increased coagulant activity was due to increased PS exposure in VLDL from patients with the biphasic waveform, the 9D2 monoclonal antibody (Dr Ran, Univ. of Texas) was used in 3 separate experiments. This has well characterised specificity for PS, phosphatidic acid, cardiolipin, phosphatidyl-inositol and -glycerol with no reactivity to PC, PE and SM (Ran, S., Downes, A., Thorpe, P.E. 2002. Increased exposure of anionic phospholipids on the surface of tumor blood vessels. *Cancer Res.* **62**:6132-6140). Figure 8B shows that it has no significant inhibitory effect on the surface ability of VLDL to support prothrombinase activity.

This suggests that changes in PS are not responsible for the prothrombinase enhancement.

*VLDL from biphasic waveform patients lack PE but similarly contain PC, SM and Cerebrosides.*

5 To validate the above findings and further investigate the phospholipid moieties involved, TLC was performed. The method, in being sensitive down to detection limits of 0.25 µg of amino-containing phospholipid, confirmed the lack of PS in VLDL from both normal individuals (n=5) and patients (n=10). PC, SM and cerebrosides were present in all VLDL, thus explaining the relative lack of 9D2  
10 antibody abrogation of thrombin generation (Fig. 9A). The only compositional difference was a lack of PE in biphasic waveform patients only (Fig 9B). This was a reproducible finding that was specific for patients with the biphasic waveform as ICU patients without the waveform abnormality had TLC findings similar to that of normal.

15 *Immunoabsorption of apolipoprotein B from VLDL disrupts the particle structure and ability to support thrombin generation*

To define the contribution of VLDL proteins, immobilised antibodies against apo B-100 or apo-E were tested for their ability to adsorb prothrombinase activity. Anti-apo B IgG adsorbed 80% of the prothrombinase activity when compared with  
20 control sepharose beads. Based on SDS-PAGE and densitometric analyses, immobilised anti-apoB antibody adsorbed the target antigen; i.e. the stained apo B protein band in adsorbed supernatant was 5% of that from control Sepharose beads.

The adsorption process itself leads to loss of prothrombinase potential but specific apo B removal leads to significant loss of activity ( $p= 0.0002$ ) in 3 different starting VLDL (Fig. 10). Further flow cytometric analysis to assess VLDL structural integrity after apo B immunoadsorption showed no identifiable particles. This suggests that apo B contributes to the enhanced thrombin generation of VLDL from biphasic waveform patients by structurally supporting the necessary conformation of lipids to enhance prothrombinase assembly.

By contrast, immunoadsorption of apo E down to levels less than 5% compared with controlled adsorption did not diminish prothrombinase activity (Fig. 10) or its structural integrity by FACs. Apo E is therefore not essential in the surface configuration that optimises thrombin enhancement.

*VLDL from biphasic waveform patients show procoagulant activity in clotting assays of normal plasma*

Following on from the above prothrombinase findings, we examined for procoagulant cofactor activity of VLDL in the plasma milieu using a modified APTT clotting assay. As seen in Fig. 10, the addition into normal plasma of isolated VLDL from biphasic waveform patients showed significant shortening of clot time when compared with addition into the same plasma of equivolume VLDL from normal individuals ( $p=0.000$ ). The mean clot time was 203 seconds (SEM 0.95) for VLDL from 7 normal unrelated donors and this decreased to 178 seconds (SEM 3.02) for VLDL from 7 patients with biphasic waveforms (TL18 77-85). The box plot in Fig. 10 shows the interquartile ranges from 25<sup>th</sup> to 75<sup>th</sup> percentile and median clot times of

205 and 179 seconds respectively for normal or biphasic VLDL addition. This clear difference could not be accounted for by differences in total TG content with considerable overlap between normal (range of 0.4 to 1.5 mM) and biphasic waveform patients (range 0.5 to 1.8 mM). Thus, VLDL from patients who exhibit the  
5 biphasic waveform show significant procoagulant cofactor activity.

In this study, we were able to demonstrate that VLDL from patients with the biphasic waveform had at least twice the thrombin generating potential of normal VLDL and that this was not due to lipid oxidation or contamination with microparticles. While previous studies have established that normal VLDL can  
10 generate physiological levels of thrombin albeit with considerable donor variability (Moyer, M.P., Tracy, R.P., Tracy, P.B., van't Veer, C., Sparks, C.E., Mann, K.G. 1998. Plasma lipoproteins support prothrombinase and other procoagulant enzymatic complexes. *Arterioscler. Thromb. Vasc. Biol.* 18:458-465 and Rota, S., McWilliam, N.A., Baglin, T.P., Byrne, C.D. 1998). Atherogenic lipoproteins support assembly of  
15 the prothrombinase complex and thrombin generation: modulation by oxidation and vitamin E. *Blood.* 91:508-15), this differential increase was consistent in patients with the biphasic waveform within the large sample set investigated and could play a significant role supporting thrombin generation *in vivo*.

Although we initially hypothesised that the increased thrombin generating  
20 potential in VLDL from patients with the biphasic waveform, was due to increased anionic phospholipid availability, we were unable to show that this was due to increased phosphatidylserine. This was on the basis of two separate lines of evidence.

Firstly, the data from the 9D2 blocking antibody with well-characterised specificity for PS showed no inhibition of prothrombinase activity. Secondly, the TLC studies which would have been sensitive to detecting 0.25µg of PS showed no evidence even in VLDL from biphasic waveform patients with enhanced prothrombinase supporting activity. Deguchi *et al* have found a small amount of PS (0.4%) in normal VLDL (Deguchi, H., Fernandez, J.A., Hackeng, T.M., Banka, C.L., Griffin, J.H. 2000 Cardiolipin is a normal component of human plasma lipoproteins. *Proc. Natl. Acad. Sci. USA.* 97:1743-1748) and it may be that trace amounts could support prothrombinase activity (Moyer, M.P., Tracy, R.P., Tracy, P.B., van't Veer, C., Sparks, C.E., Mann, K.G. 1998. Plasma lipoproteins support prothrombinase and other procoagulant enzymatic complexes. *Arterioscler. Thromb. Vasc. Biol.* 18:458-465). However, there could be alternative explanations especially as the most appropriate composition of phospholipids for activating the prothrombin complex *in vivo* is still a matter of debate. The importance of PS stems from *in vitro* studies under static conditions where compositional PC: PS ratios of 3:1 appear most similar to the procoagulant effect of activated platelets (Deguchi, H., Fernandez, J.A., Hackeng, T.M., Banka, C.L., Griffin, J.H. 2000 Cardiolipin is a normal component of human plasma lipoproteins. *Proc. Natl. Acad. Sci. USA.* 97:1743-1748). By contrast, vesicles containing PC alone can cause the highest increase in procoagulant activity, as measured by fibrin deposition (Higgins, D.L., Callahan, P.J., Prendergast, F.G., Nesheim, M.E., Mann, K.G. 1985. Lipid mobility in the assembly and expression of the activity of the prothrombinase complex. *J. Biol. Chem.* 260:3604-3612). PC may

be particularly relevant in interpreting our findings and Rosing *et al* have also shown the ability of positively charged membranes to enhance prothrombin activation (Galan, A.M., Hernandez, M.R., Bozzo, J., Reverter, J.C., Estelrich, J., Roy, T., Mazzara, R., Ordinas, A., Escolar, G. 1998. Preparations of synthetic phospholipids promote procoagulant activity on damaged vessels: studies under flow conditions. *Transfusion*. 38:1004-1010). The findings that PE was absent specifically from VLDL of patients with the biphasic waveform may also be important especially in the effect of shortening plasma clot times. PE is largely considered to promote anticoagulant pathways and its blockade by anti-phospholipid antibodies promotes the procoagulant activity of thrombin (Rosing, J., Speijer, H., Zwaal, R.F. 1988. Prothrombin activation on phospholipid membranes with positive electrostatic potential. *Biochemistry*. 27:8-11). However, in addition to compositional differences, conformation in the presentation of appropriate surfaces for coagulation reactions also needs to be considered. The loss of prothrombinase activity through apoB immunoadsorption in disrupting lipoprotein integrity highlights this particular aspect.

As shown, VLDL from patients manifesting the biphasic waveform can significantly enhance thrombin generation. Moreover, the calculation of total thrombin generating capacity from the quantitative and qualitative changes in VLDL within serial samples of patients with sepsis and DIC show a direct positive correlation with clinical progression. This supports the relevance of thrombin as a major player in the pathophysiology of sepsis. Whilst its primary role may have been as part of the acute phase protective initial response, the protracted or enhanced

response fuelled by VLDL procoagulant surfaces may lead to deleterious consequences.

### Example 2

*Measurements of CRP and VLDL.* In fifteen patient plasmas of patients  
5 exhibiting a biphasic waveform, VLDL measurements were obtained by initial centrifugation at 356,000 x g at 10°C for 2 h in polycarbonate centrifuge tubes (11x 34mm, Beckman Instruments, Palo Alto, CA) in a Beckman TL-100 tabletop ultracentrifuge. The upper VLDL fraction was collected and the volume recorded. LDL, isolated as described Gabel, B.R., et al. *Biochemistry*, 1998,37:7892-7898 and  
10 Wang, X. et al. *Arterioscler. Thromb. Vasc. Biol.*, 200, 20:1301-1308, was used as the standard for the ApoB-100 ELISA. The cholesterol concentration was determined colorimetrically at 500nm in the isolated VLDL fractions from biphasic patient and normal plasma samples using the Cholesterol Infinity Reagent and Cholesterol Calibrator according to the manufacturer's protocol. The total protein concentrations  
15 in the recovered VLDL fractions were determined with the Bradford assay using BSA as a standard. CRP levels were determined by ELISA using a rabbit anti-human CRP IgG for capture with detection by the same antibody, conjugated to HRP in the method described by Tijssen, et al. *Analytical Biochemistry*, 1984, 136: 451-457. CRP was also determined in the 1187 clinical study patients with levels cross-checked  
20 using the Eurogenetics kit. As a marker of VLDL levels, triglyceride concentrations were also determined in the same cohort.

*CRP detection in isolated VLDL Fractions from Patient Sera.*

Serum from four patients with varying degrees of the BPW was obtained. Each of these was divided into three 0.9 ml aliquots. The first aliquot was left as collected. One-tenth ml of 0.1M EDTA was added to the second aliquot to chelate and disrupt any complex that might be present. One-tenth ml of 0.25M CaCl<sub>2</sub> was added to the third aliquot to optimize complex formation. All samples were then centrifuged as described above for VLDL isolation. The isolated fractions were then measured for CRP and triglyceride (Sigma Diagnostics Infinity Reagent) with values then expressed as CRP per mM VLDL triglyceride measured.

*Prothrombinase supporting activity of isolated VLDL.*

The isolated VLDL of 3 patients with a BPW, along with a sample isolated from a pool of 20 healthy volunteers, were analyzed for their ability to replace the phospholipid component in prothrombin activation. The VLDL concentrations were adjusted to 100, 200 and 300μM (cholesterol) and initial rates of thrombin formation were measured by fluorescence in a fluorescence microtitre plate reader by a procedure generally described by Nesheim, M.E., et al., *J. Biol. Chem.* 1979, 254: 10952-10956.

*Evidence for the existence of the CRP/VLDL complex in the sera of patients with the biphasic waveform.*

Although the inference could be drawn that CRP/VLDL complex would exist in circulation due to the presence of plasma Ca<sup>2+</sup>, the possibility that the complex might form due to the initial exposure to the anticoagulant used to prepare the plasma samples prior to recalcification was investigated. Experiments were performed in an

effort to identify the complex in the sera of blood not exposed to the anticoagulant. CRP was detected by ELISA in VLDL isolated from untreated sera of patients with the BPW as shown in Table I, below.

**Table I**

CRP in VLDL fractions from Untreated, EDTA and Calcium Treated  
Samples of Serum from Patients with Positive Biphasic Waveforms

Patient No.	Serum CRP ( $\mu\text{g/ml}$ )	VLDL fraction		VLDL fraction plus EDTA		VLDL fraction plus $\text{Ca}^{2+}$	
		<u>Total</u>	<u>CRP mM</u>	<u>Total</u>	<u>CRP mM</u>	<u>Total</u>	<u>CRP mM</u>
		<u>CRP</u>	<u>Trig</u>	<u>CRP</u>	<u>Trig</u>	<u>CRP</u>	<u>Trig</u>
1	323	14.3	1.29	1.3	0.08	160	21.92
2	281	18.6	4.04	1.1	0.15	172	40.95
3	264	4.2	1.17	0.9	0.12	157	50.70
4	218	21.5	9.77	2.2	0.85	157	112.14

As the interaction between CRP and VLDL is  $\text{Ca}^{2+}$ -dependent, prior chelation of the sample led to a loss of detectable CRP in all patients examined. Likewise, prior incubation with additional  $\text{Ca}^{2+}$  to promote complex formation between CRP and VLDL led to enhanced detection of CRP within the VLDL fraction. VLDL from normal patients did not have detectable CRP and patients with high CRP without a BPW had no recoverable VLDL. From these observations, it was concluded that the complex does exist to a measurable extent in the blood of patients whose plasmas exhibit a BPW.

### Example 3

Twenty samples from patients experiencing an acute phase response as evidenced by elevated levels of IL-6, CRP, fibrinogen and/or SAA were examined.

All twenty patients showed some degree of activated coagulation and displayed marked disruptions in their lipoprotein profile, with all patients experiencing hypolipidemia in the form of low cholesterol and often low triglyceride levels. Sixteen of the 20 patients were adjudicated as having been infected during or prior to  
5 ICU admission. The lipoprotein profiles and VLDL properties were studied in the manner described below.

#### *Lipoprotein Profiles*

A portion of each plasma was sent for a lipoprotein profile measured by NMR analysis to obtain additional information not found in the classic lipid chemistries  
10 currently in common use by clinical laboratories. NMR analysis provided a quantitative analysis of the number of lipoprotein particles at a given size, regardless of the lipid chemistry of the particle. From this analysis, two observations were common for all subjects. The HDL sized particles decreased in quantity by over 50% from normal particle concentrations. VLDL sized particles also decreased in all  
15 subjects during the protocol blood work. The drop in VLDL quantities varied depending on the day of blood work.

The quantity of LDL sized particles showed some variation depending on the day of blood work, but was less variable than seen with VLDL and HDL sized particles. Four of the samples displayed very low LDL levels for a period of time  
20 during the ICU study.

To get an idea of the overall changes in all lipoprotein classes, a calculation was performed to calculate the total surface area from all of the lipoprotein classes.

By performing this calculation, it is an indirect indicator of the amount of total core lipids that the lipoproteins could potentially carry. Six of the 20 subjects showed total drops in lipoprotein levels (via surface area) of greater than 67% with 13/20 showing drops of greater than 50%. This is summarized in Table II below.

Table II Lipoprotein Surface Area	
Patent Number	Total Lipoprotein Surface Area % Drop from Normal
1	58
2	44
3	60
4	68
5	53
6	76
7	53
8	38
9	68
10	55
11	41
12	53
13	38
14	50
15	49
16	79
17	70
18	44
19	37
20	74
	<p>Average nanoMolar Normal Pool Area  <math>9.13 \times 10^6 \text{ nm}^2/\text{L}</math></p> <p>To calculate surface area:                      Average diameter of VLDL = calculated by NMR (Range 33 to 140 nm).                      Average diameter of LDL = calculated b NMR (Range 19-22 nm).                      Average diameter of HDL = calculated by NMR (8-11 nm).                      All lipoprotein concentrations give by NMR and nMolar.</p> <p>Formula would then be <math>4 * 3.1416 * (\text{average diameter}/2)^2 * (\text{lipoprotein concentration in nMolar})</math>.</p>

*VLDL Properties*

The VLDL of the 20 patient samples was isolated for each specimen by ultra centrifugation at the density of 1.006 g/cc (unadjusted density). This buoyant density subclass was then analyzed by agarose electrophoresis side by side with the initial plasma. Isolated from normal sera, the lipoproteins from this buoyant density (termed VLDL) usually showed pre-beta migration patterns upon electrophoresis in non-denaturing agarose gels. A typical pattern emerged upon isolation and fraction, all patients experienced the electrophoretic shift from pre-beta migration to some degree of beta migration. Often there was observed an evidence in the VLDL of the complete shift to beta migration on at least one day of the ICU blood work or upwards to an entire week.

From running the total plasma on the agarose gel, changes in the HDL migration patterns were also noted. In normal donors, HDL migrates at the alpha region. For all ICU patients (twenty), the HDL changed migration to a post-alpha/pre-beta which is indicative of the serum amyloid A (SAA) incorporation.

A portion of the isolated VLDL was run on reducing SDS polyacrylamide gels. VLDL is a lipoprotein which usually contains five plus proteins: apo-B, apo-E, apo-CI, apo-CII, apo-CIII are the most commonly found proteins in normal VLDL. By SDS-page analysis, there appeared to be changes in the proteins as analyzed within a patient's stay in the ICU. Apo-B was always present. The apo-C's were difficult to analyze due to their small size, but there did appear to be some changes occurring

during the patient's stay in ICU. Apo-E changes were the most common and obvious change seen with the 20 ICU patients. At times the apo-E band disappeared during the patient's stay. FIG. 12A shows the serial sample biphasic slope-1 data from a patient identified as Patient 20. FIG. 12B shows the corresponding agarose gels that

5 illustrate a shift in mobility of VLDL for the patient FIG. 12A. The shifts in lipoproteins corresponded with the appearance of the abnormal waveform patterns .

**That which is claimed is:**

1. A method for diagnosis and monitoring a host response to infection, SIRS and sepsis, said method comprising (a) obtaining a patient sample; (b) measuring a lipoprotein fraction from said sample for an abnormality; and (c) correlating said lipoprotein measurement to an abnormality found in patients having severe infection, SIRS or hemostatic dysfunction.

2. A method according to claim 1 wherein said lipoprotein abnormality is enhanced ability to support prothrombinase activity for patients with severe infection, SIRS and/or sepsis.

3. The method according to claim 1, wherein said lipoprotein fraction comprises beta lipoproteins and said enhanced ability to support prothrombinase activity demonstrates at least a two-fold increase in prothrombin activity as compared with normal samples. .

4. The method according to claim 1, wherein said step (b) is carried out by measuring rate of thrombin generation and said beta lipoprotein comprises an apoB containing lipoprotein.

5. The method according to claim 1, wherein said determining step is carried out in the absence of forming a complex between said betalipoprotein and C-reactive protein (CRP).

6. A method to predict an increased likelihood of system failure or mortality in said patient, said method comprising (a) obtaining a patient sample; (b) measuring from said sample a lipoprotein fraction for PTase; (c) comparing said prothrombin

activation of said sample to a standard of normal patient samples or abnormal patient samples; and (d) utilizing said comparison to predict the mortality of said patient wherein said lipoprotein for supporting PTase for patients with increased likelihood of system failure or mortality demonstrates at least a two-fold increase in PTase activity  
5 as compared with normal samples.

7. A method for predicting an increased likelihood of infection in a patient, said method comprising: (a) obtaining a sample of very large density lipoproteins (VLDLs) from a patient; and (b) determining the activity of VLDLs in said plasma or serum sample for activating prothrombin; a greater activity of said VLDLs for  
10 activating prothrombin indicating an increased likelihood of infection in said patient.

8. A method according to claim 7 wherein said patient has a biphasic waveform.

9. A method according to claim 7 wherein a measurement of the activity of VLDL is carried out directly or indirectly.

15 10. A method according to claim 7 wherein said activity of VLDL is carried out by a method comprising measuring a rate of thrombin generation.

11. A method for diagnosing severe infection, SIRS or sepsis, said method comprising (a) obtaining a patient sample; (b) subjecting the patient sample to a biphasic waveform screening test to obtain a normal or biphasic waveform result; (c)  
20 subjecting said patient samples demonstrating said biphasic waveform result to a to determine PTase of the lipoproteins; and (d) determining a relationship with those

patients exhibiting increased PTase activities with a diagnosis of severe infection, SIRS or sepsis.

12. The method according to claim 12, wherein said infection is sepsis.

13. The method according to claim 11 wherein said relationship indicates that  
5 the patient has DIC.

14. A method for diagnosis and monitoring a host response to infection, SIRS and sepsis, said method comprising (a) obtaining a blood sample or serum sample from a patient; (b) measuring said sample lipoprotein fraction for qualitative or quantitative properties of lipoproteins utilizing an analysis comprising NMR; (c)  
10 comparing said properties of lipoproteins of said sample to a standard of normal patient samples or abnormal patient samples; and (d) utilizing said comparison to diagnose and/or monitor a host response to infection, SIRS and sepsis.

15. A specific binding assay diagnosing and monitoring patients with severe infection, SIRS and sepsis said method comprising (a) obtaining a sample from a  
15 patient; (b) contacting said sample with a specific binding agent to capture a lipoprotein containing an abnormality; (c) quantitatively or qualitatively analyzing said captured abnormal lipoprotein to obtain a result; and (d) correlating said result with abnormal lipoproteins found in patients with severe infection, SIRS and hemostatic dysfunction.

20 16. An assay according to claim 15 wherein said specific binding agent is Annexin 5.

17. An assay according to claim 16 wherein said specific binding agent is an antibody specific to Annexin 5.

18. A method for treating a patient for severe infection, SIRS or sepsis, said method comprising administering an effective amount of Annexin 5 to block  
5 prothrombinase assembly.

19. A method according to claim 18 wherein said patient has DIC.

20. A method for predicting the existence of sepsis in a patient, said method comprising: a) obtaining a sample of lipoproteins from a patients, (b) determining whether the lipoproteins are abnormal as compared to lipoproteins from a  
10 normal patient sample, and (c) predicting the existence of sepsis in the patient based on detected abnormal lipoproteins.

21. A method according to claim 18 wherein the abnormal lipoproteins are selected from the group consisting of VLDL, IDL and LDL.

22. A method for predicting the hemostatic dysfunction in a patient, said  
15 method comprising: a) obtaining a sample of lipoproteins from a patients, (b) determining whether the lipoproteins are abnormal as compared to lipoproteins from a normal patient sample, and (c) predicting the existence of hemostatic dysfunction in the patient based on detected abnormal lipoproteins.

23. A method according to claim 23 wherein said step (b) is carried out by  
20 detecting an enhanced procoagulant aspect of the VLDL from patients with the biphasic waveform by measuring a shortening of clot time in a modified APTT assay

or measuring clot formation or measuring increased rate of acceleration of clot formation in a dilute tissue factor based assay.

24. A method for diagnosis and monitoring a host response to infection, SIRS and sepsis, said method comprising (a) obtaining a patient sample; (b) measuring a lipoprotein fraction from said sample for total surface area; and (c) correlating said lipoprotein measurement to increased surface area lipoprotein abnormality found in patients having severe infection, SIRS and hemostatic dysfunction.

25. A method according to claim 24 wherein said lipoprotein surface area is increased at least about 45% as compared with normal patient samples.

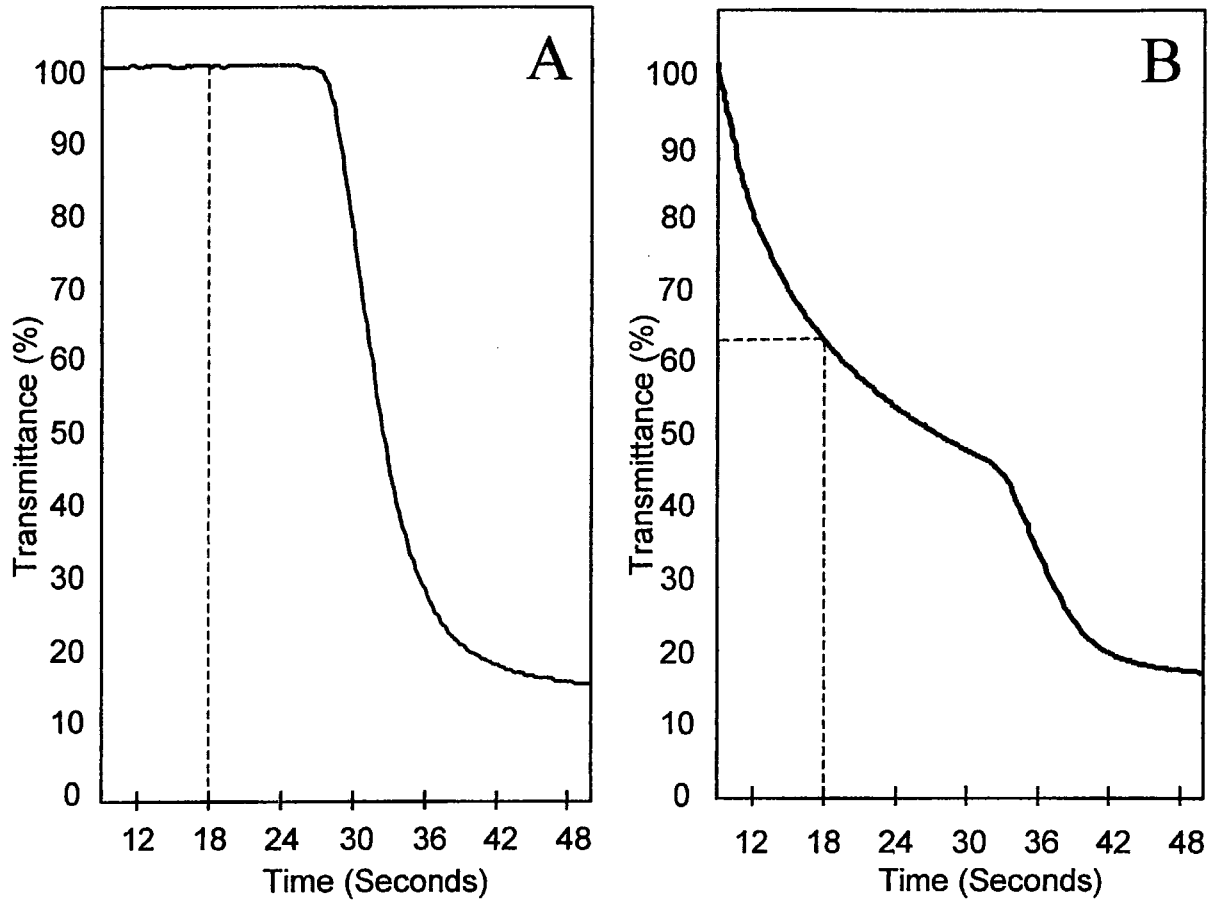
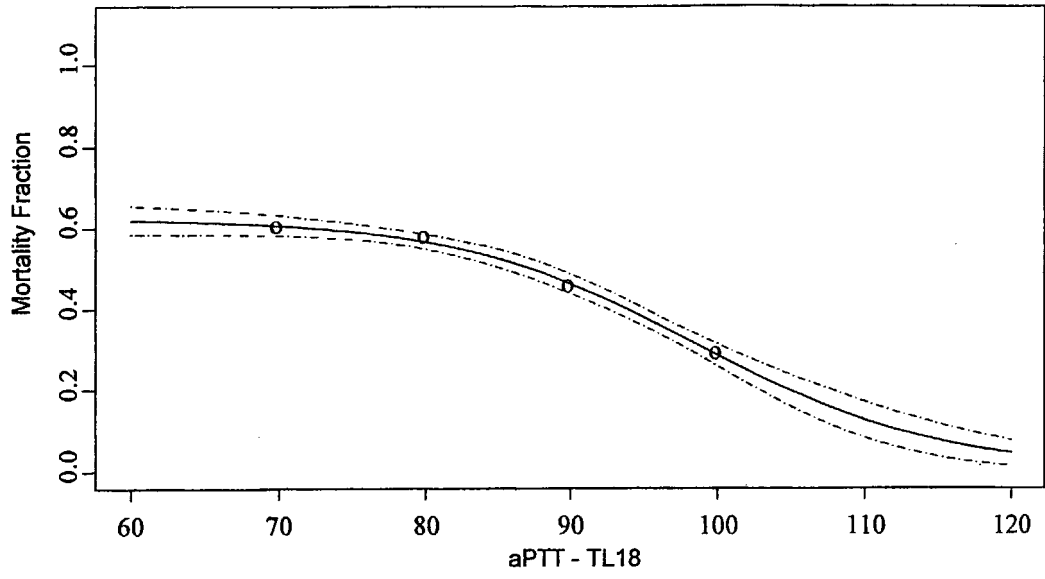
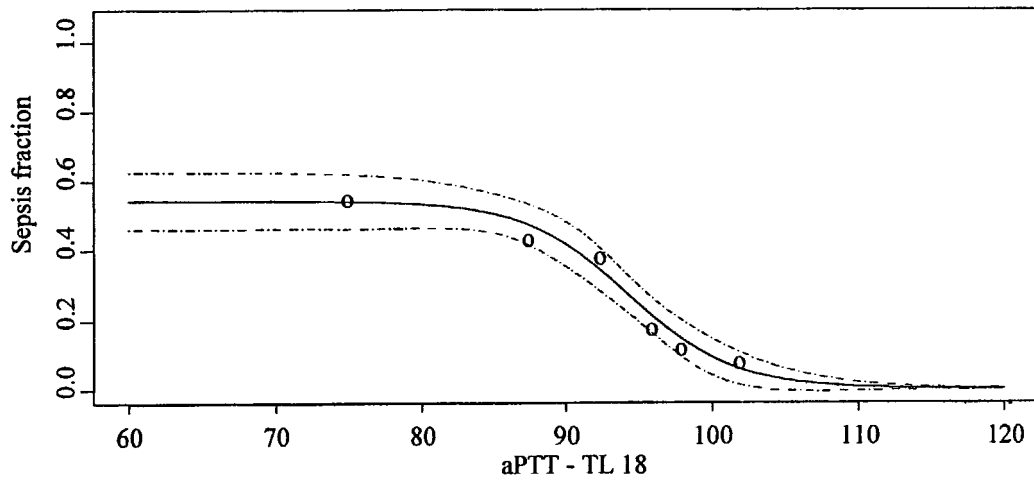


Fig. 1



**FIG. 2**



**FIG. 3**

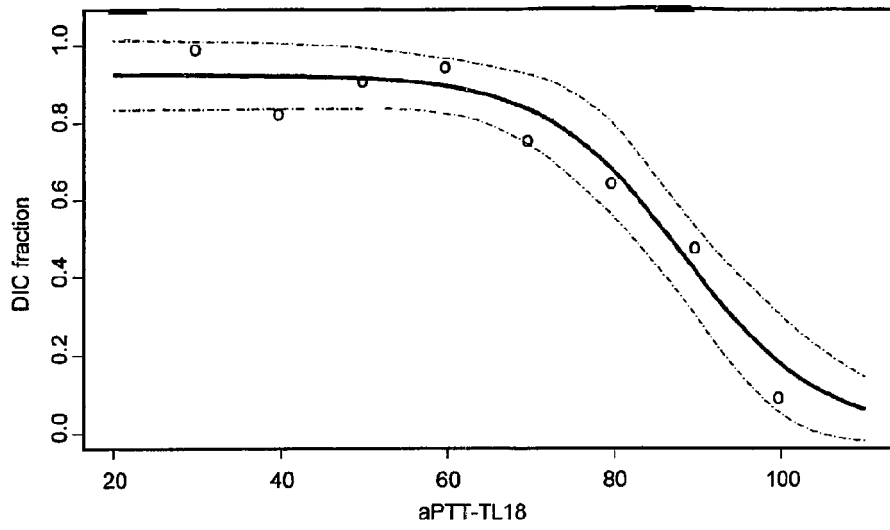


Fig. 4A

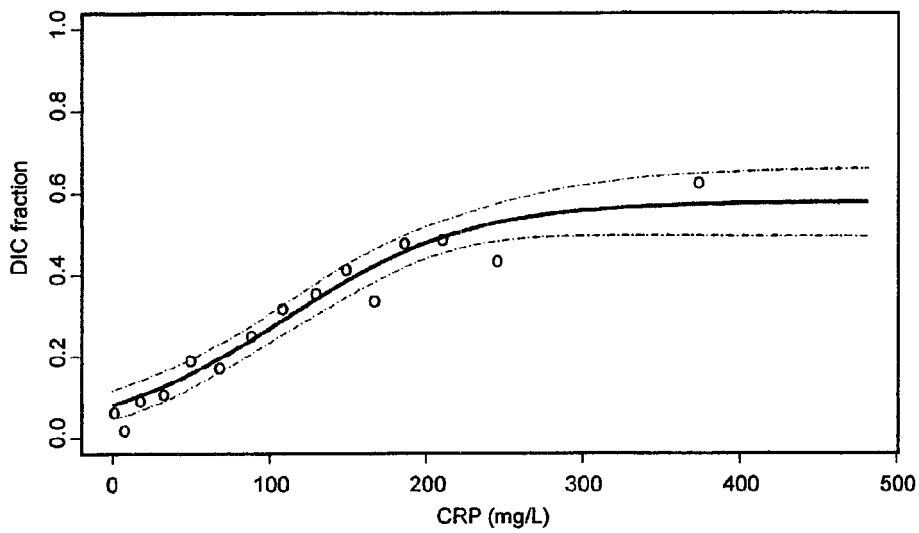


Fig. 4B

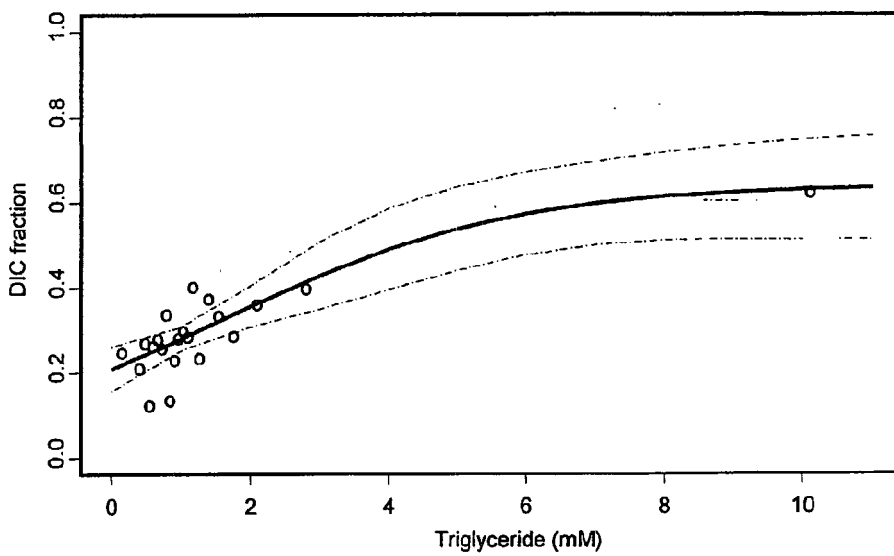


Fig. 4C

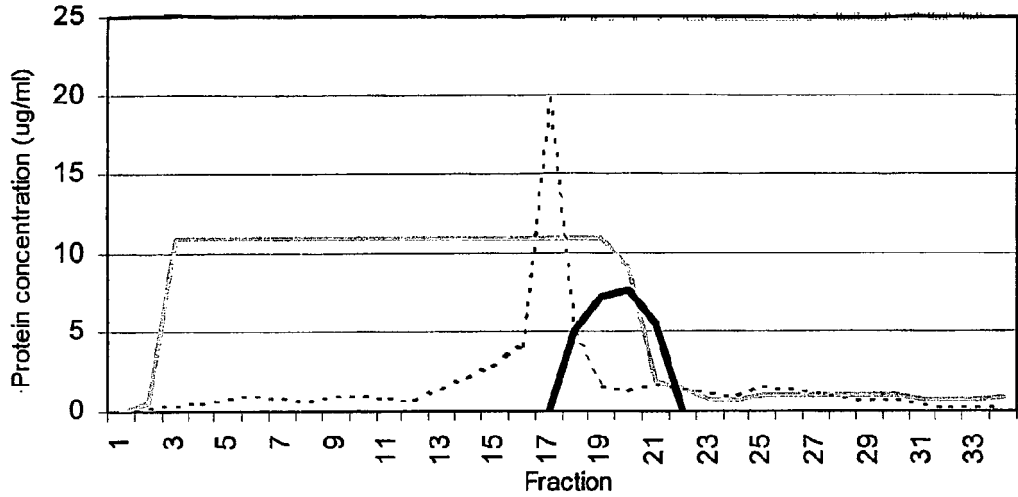


Fig. 5A

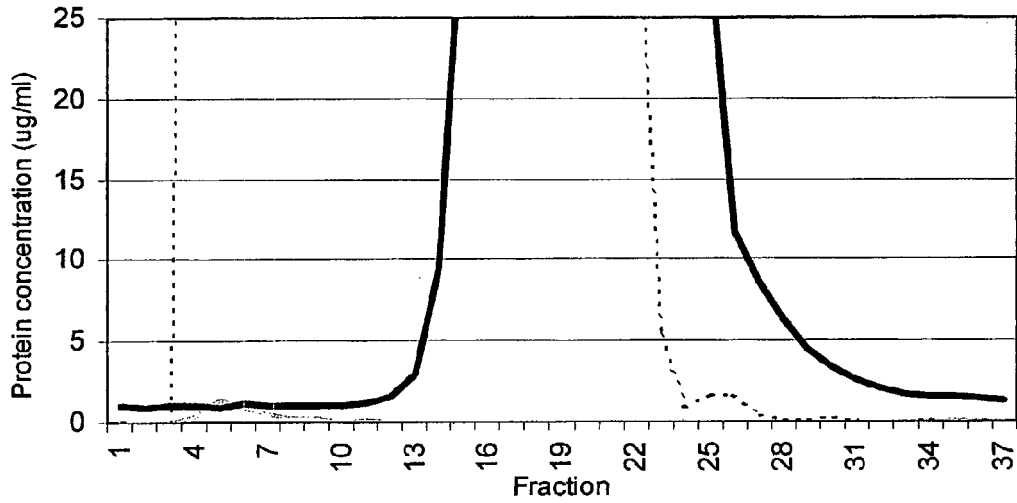


Fig. 5B

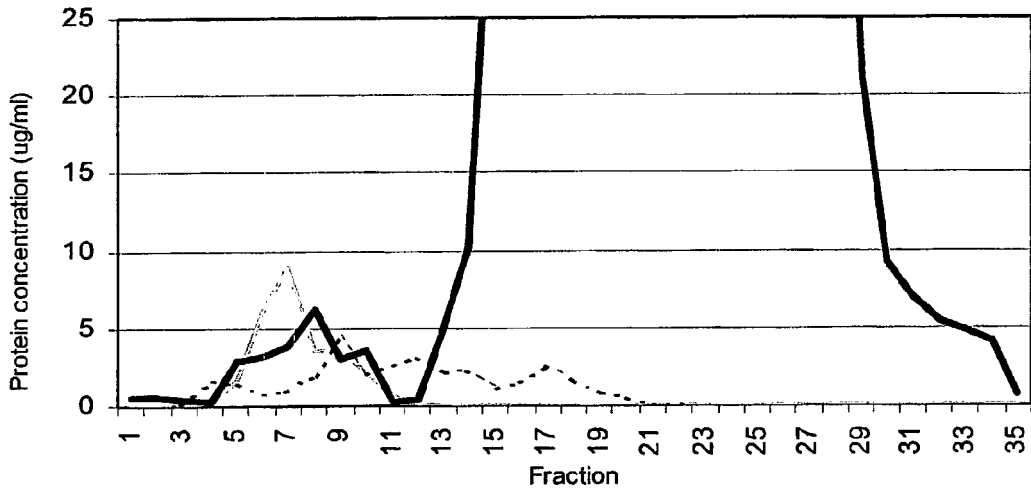


Fig. 5C

Fig. 6A

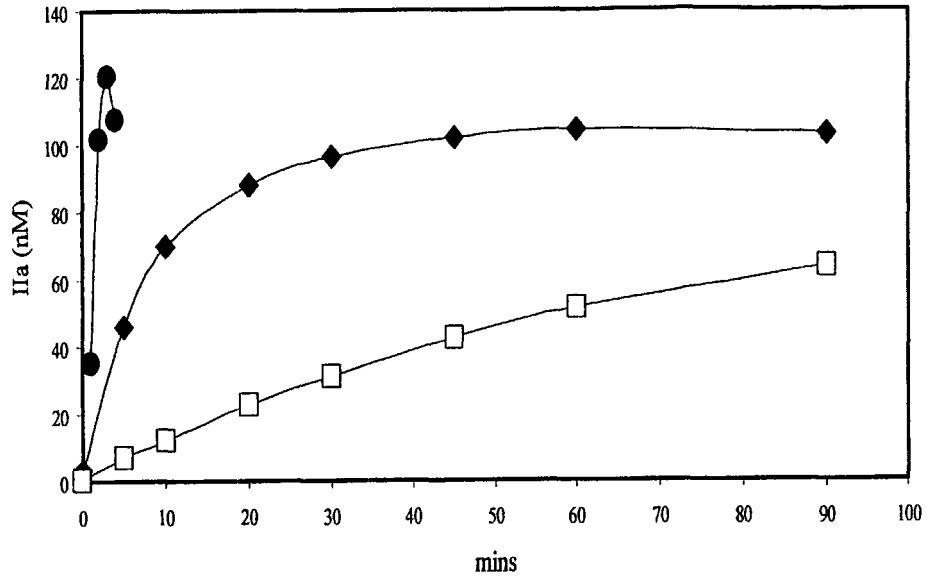
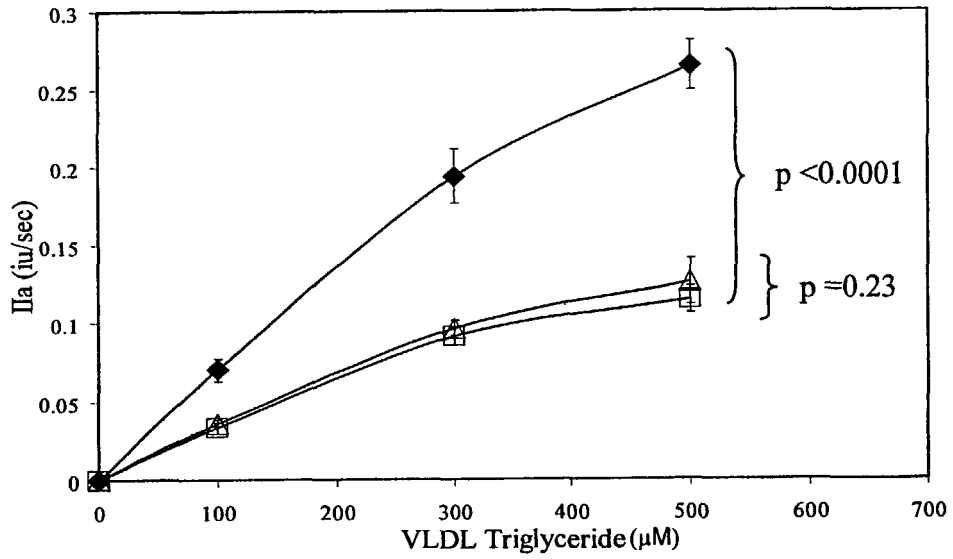


Fig. 6B



6/12

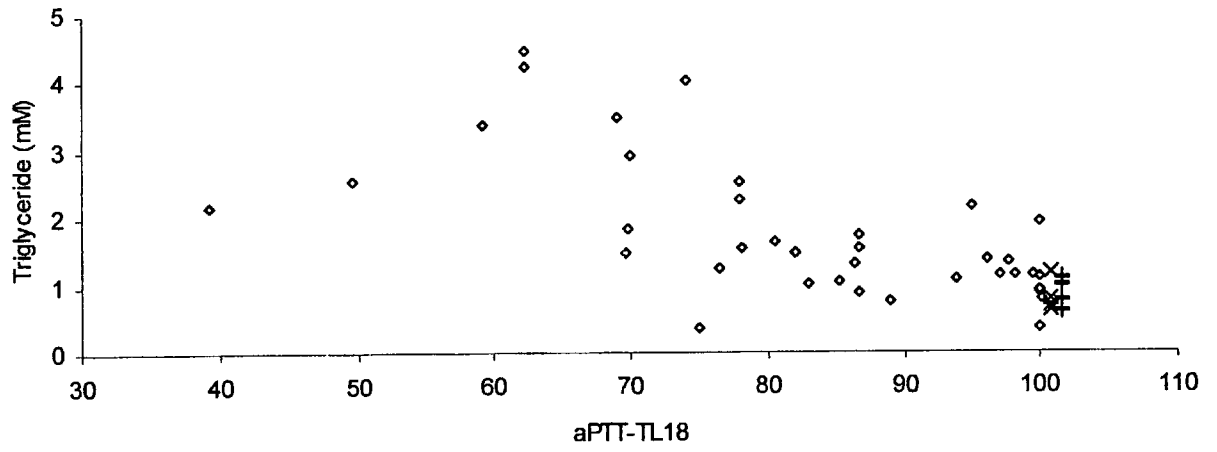


Fig. 7B

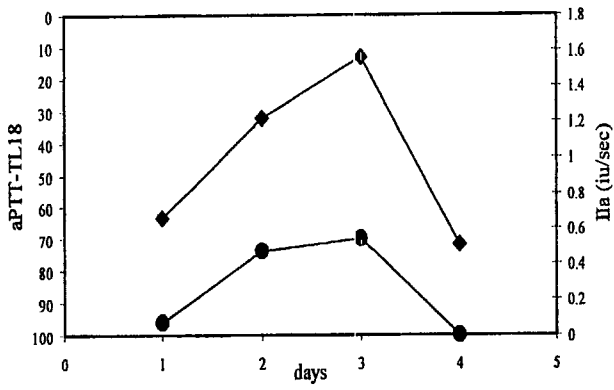
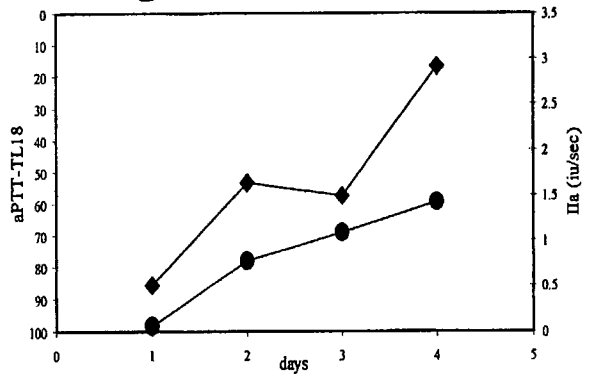


Fig. 7C



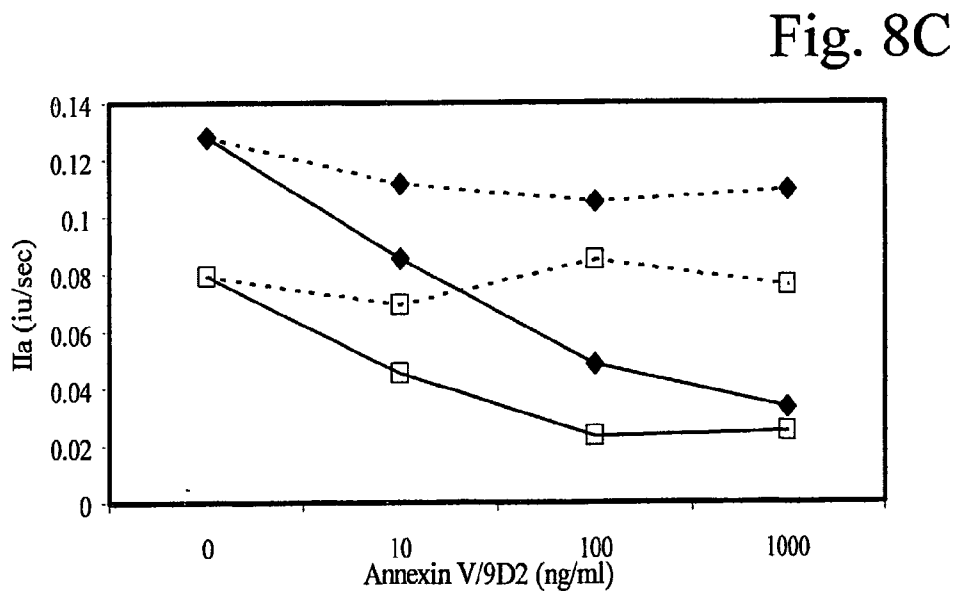
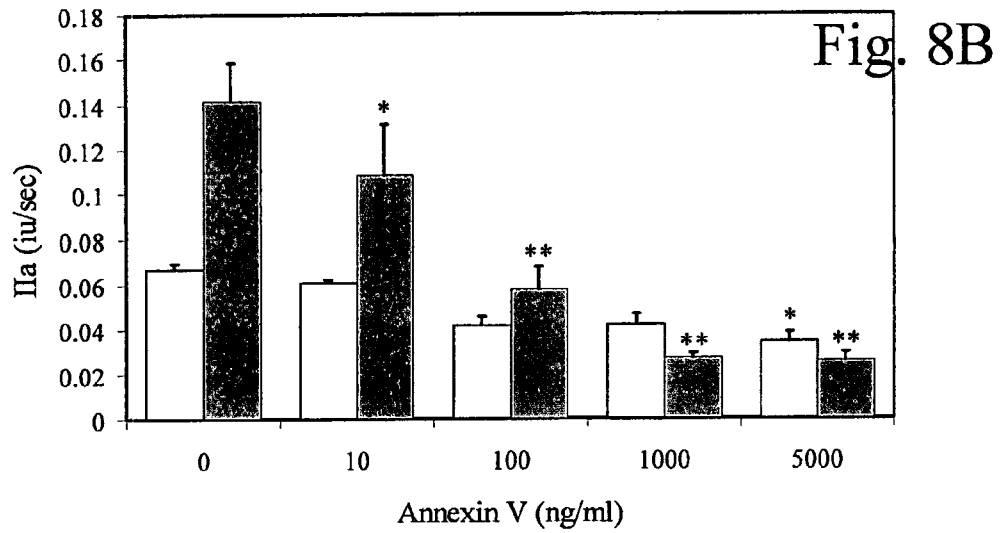
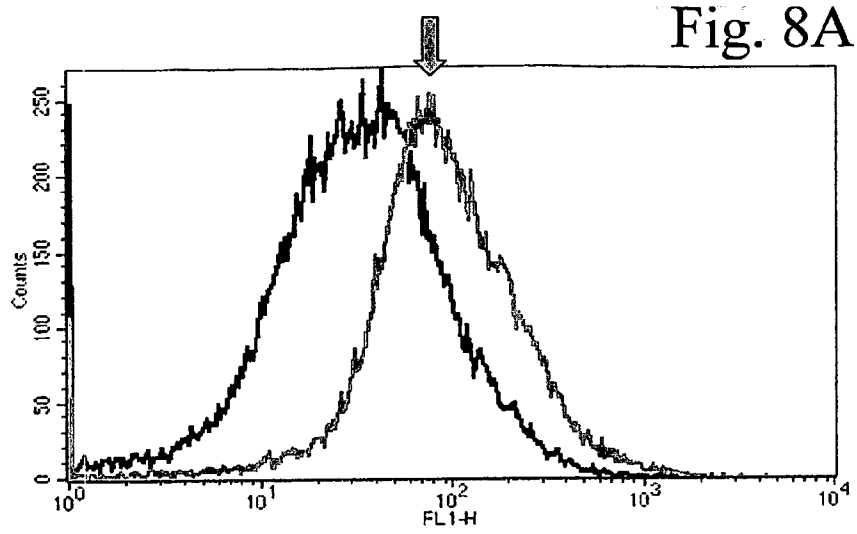


Fig. 9A

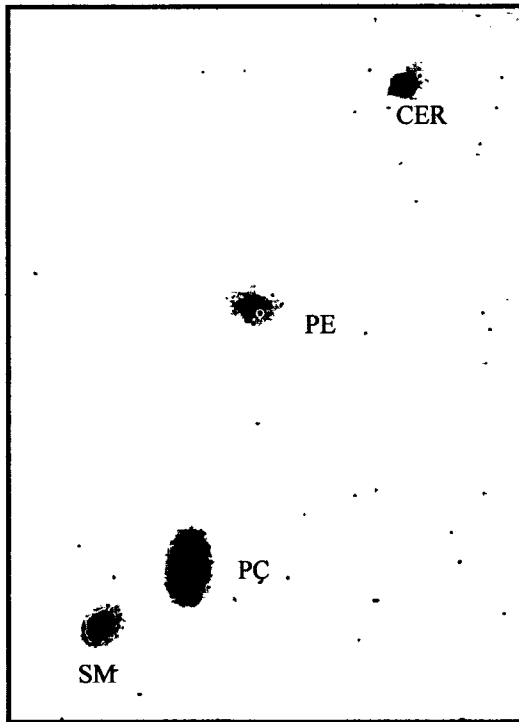
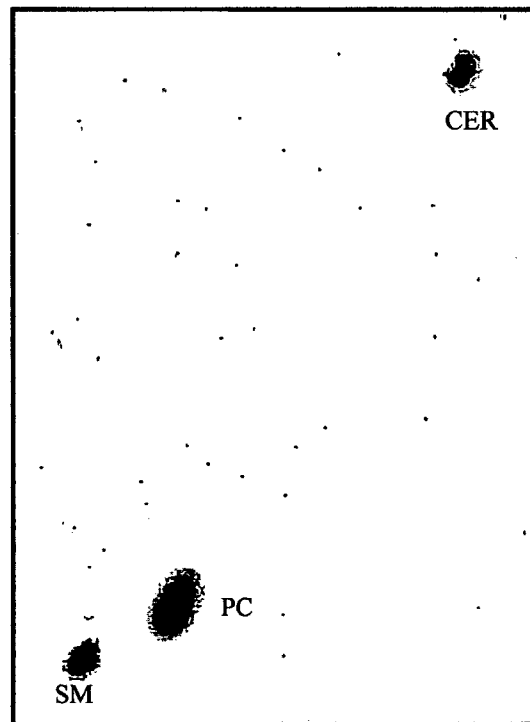


Fig. 9B



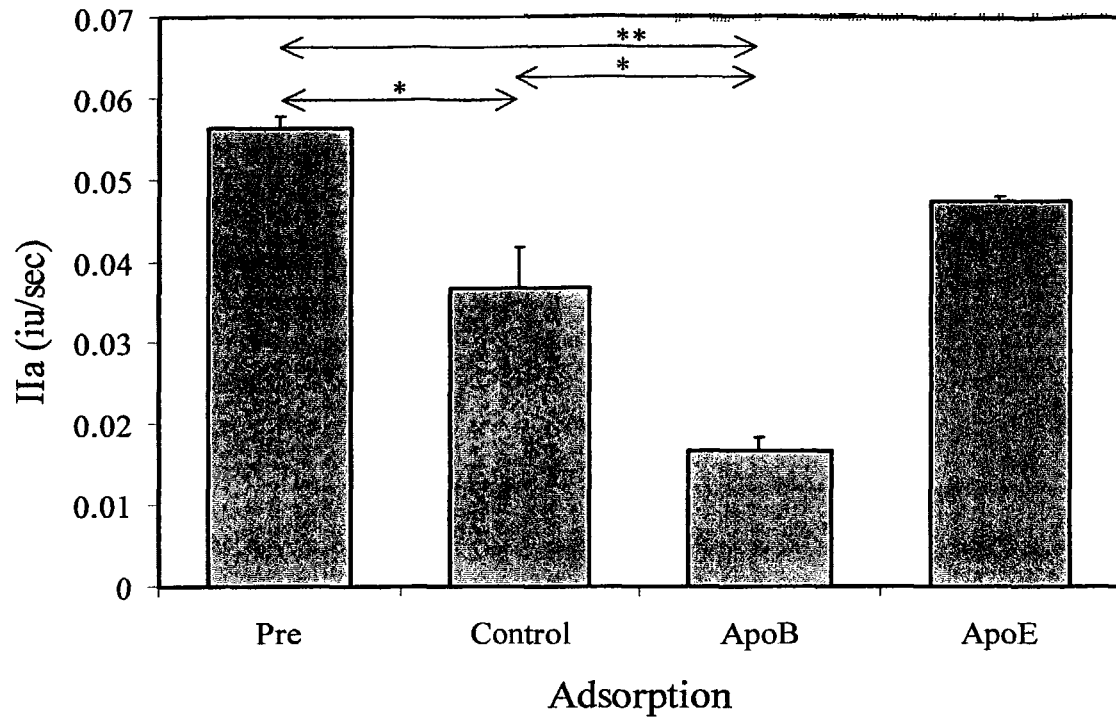
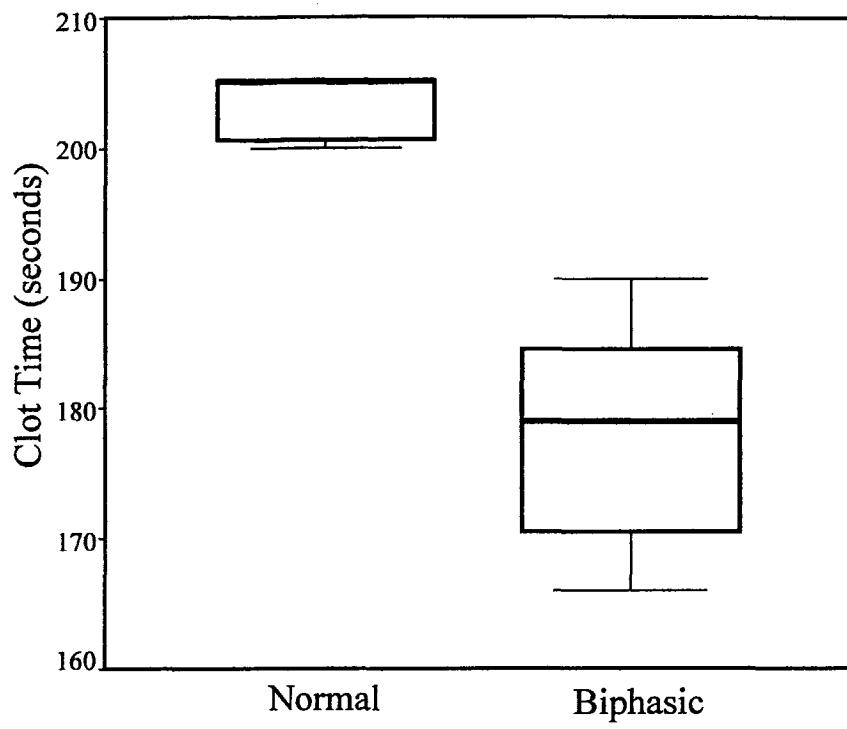


Fig. 10

Fig. 11



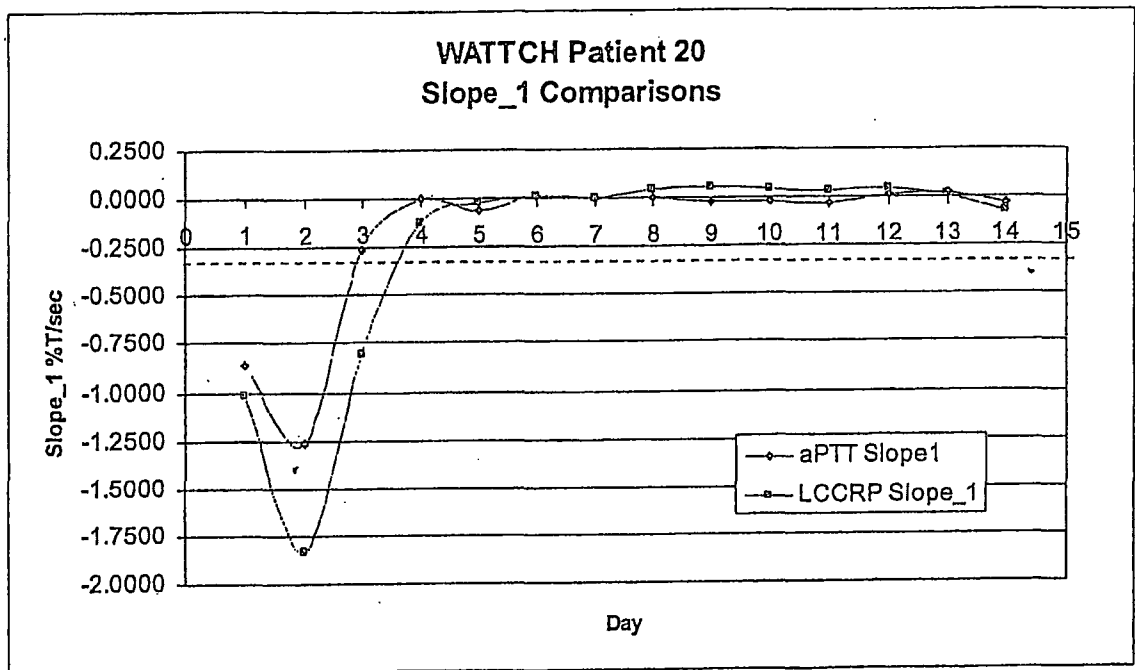


Fig. 12A

### PATIENT 20

#### Agarose Gel Electrophoresis of

#### Patient Plasma and Isolated VLDL

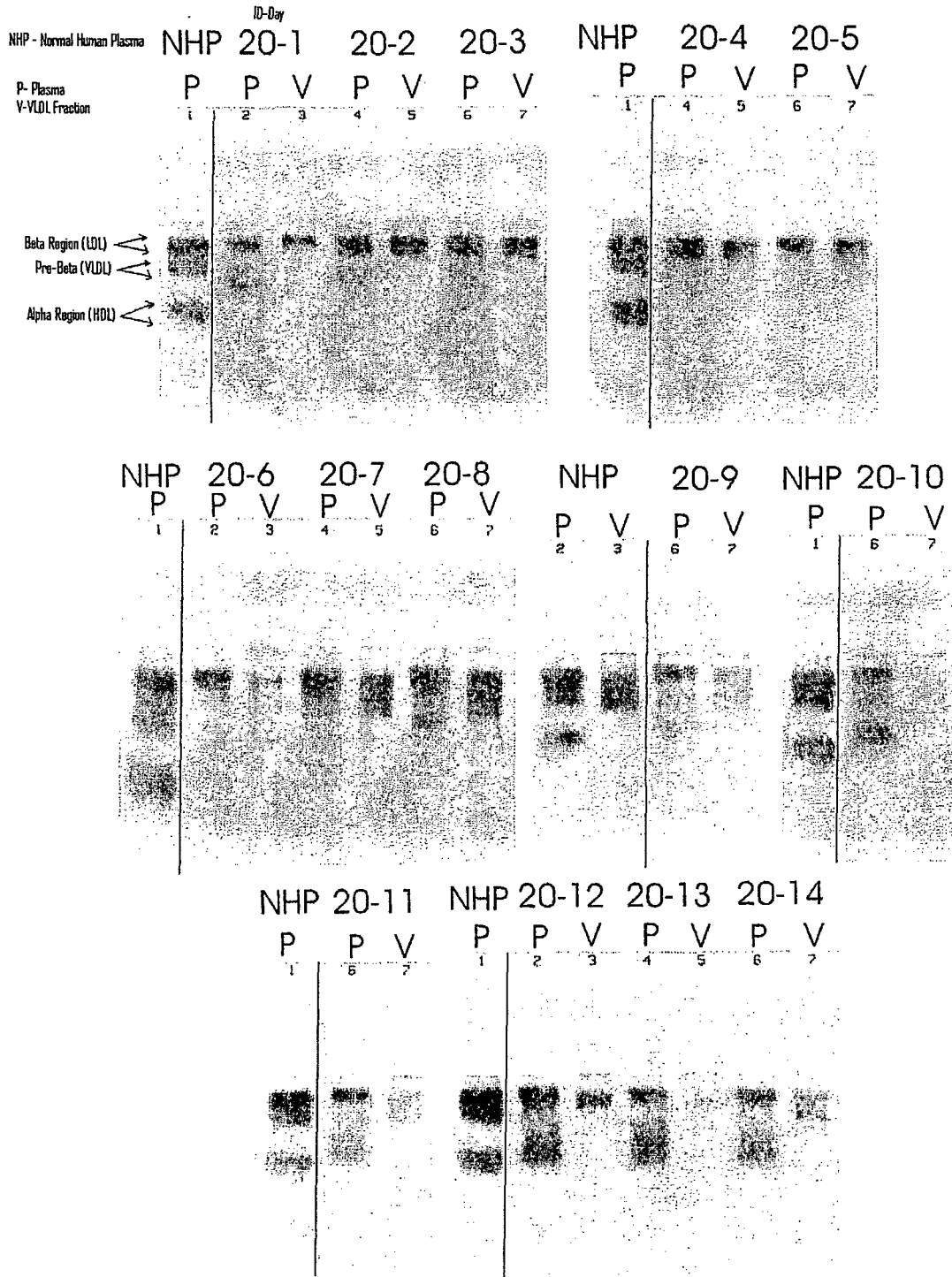


Fig. 12B

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US03/05980

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>				
IPC(7) : G01N 33/53 US CL : 435/7.1				
According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>				
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/7.1				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
A,P	US 2002/0150534 A1 (YU et al.) 17 October 2002 (17.10.02), see the entire document.	1-25		
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.				
* Special categories of cited documents: <table border="0" style="width:100%"> <tr> <td style="width:50%">           "A" document defining the general state of the art which is not considered to be of particular relevance            "E" earlier application or patent published on or after the international filing date            "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)            "O" document referring to an oral disclosure, use, exhibition or other means            "P" document published prior to the international filing date but later than the priority date claimed         </td> <td style="width:50%">           "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention            "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone            "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art            "&amp;" document member of the same patent family         </td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family			
Date of the actual completion of the international search 23 May 2003 (23.05.2003)		Date of mailing of the international search report <b>12 JUN 2003</b>		
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230		Authorized officer <i>Valerie Bell-Harris for</i> Raymond J. Henley III Telephone No. 703-308-1235		

专利名称(译)	诊断和监测止血功能障碍，严重感染和系统性炎症反应综合征的方法		
公开(公告)号	<a href="#">EP1476752A1</a>	公开(公告)日	2004-11-17
申请号	EP2003711279	申请日	2003-02-27
[标]申请(专利权)人(译)	生物梅里埃公司		
申请(专利权)人(译)	BIOMERIEUX INC.		
当前申请(专利权)人(译)	BIOMERIEUX INC.		
[标]发明人	TOH CHENG HOCK TEJIDOR LILIANA NEISHEIM MIKE JONES GREGORY		
发明人	TOH, CHENG, HOCK TEJIDOR, LILIANA NEISHEIM, MIKE JONES, GREGORY		
IPC分类号	G01R33/465 A61K38/00 A61P1/18 A61P7/00 A61P7/02 A61P7/04 A61P17/02 A61P29/00 A61P31/04 A61P31/10 A61P31/12 A61P33/00 A61P43/00 C12Q1/37 C12Q1/56 G01N33/53 G01N33/86 G01N33 /92		
CPC分类号	A61P1/18 A61P17/02 A61P29/00 G01N33/92		
代理机构(译)	FROUD , CLIVE		
优先权	60/359932 2002-02-27 US 60/363073 2002-03-11 US 60/396392 2002-07-17 US 60/404652 2002-08-20 US		
其他公开文献	EP1476752A4		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

提供了用于诊断和监测止血功能障碍，严重感染和系统性炎症反应综合征的受试者的方法，其中通过定量和定性分析检查脂蛋白的异常，特别是对于促凝血酶原增强。