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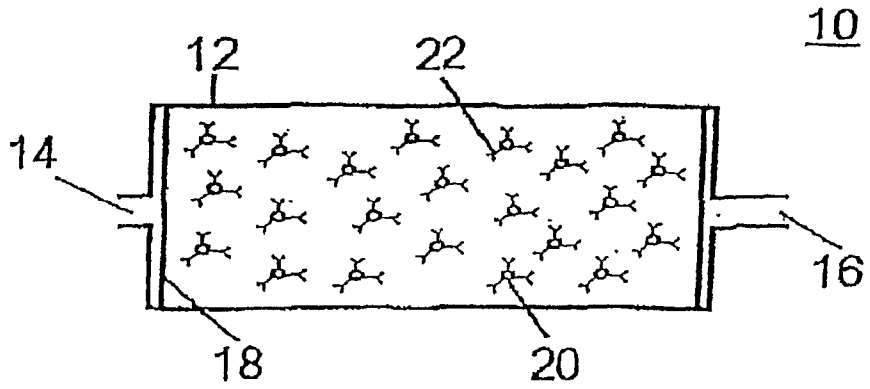
(54) **Title:** METHODS AND DEVICES FOR REMOVING PATHOGENS AND DETRIMENTAL SUBSTANCES FROM BODILY FLUIDS



(57) **Abstract:** The present invention relates to novel methods and devices for removing detrimental entities from a bodily fluid by passing the fluid through an extracorporeal adsorption container. The device has a binding means that is confined within the container, and the binding means is specific for affixing the entity to be removed, which may be a pathogen, toxic substance, prion, aberrant cell, or endogenous substance present in undesirably high levels. By passing the bodily fluid through the container, at least a portion of the detrimental substance is removed. The treated fluid is then returned to the patient.

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METHODS AND DEVICES FOR REMOVING PATHOGENS AND DETRIMENTAL SUBSTANCES FROM BODILY FLUIDS

Cross Reference to Related Applications

[0001] This provisional application claims priority to Provisional Patent Application Serial Number 60/692,086 filed June 20, 2005 under 35 U.S.C. § 119(e). The disclosures of the above-referenced applications are incorporated in their entirety by reference.

Technical Field

[0002] The present invention relates to novel methods and devices for treating viral, fungal, parasitic and bacterial infections, including septicemia, fungemia, and bacteremia, using an extracorporeal adsorption container. The devices and methods are also useful for treating other conditions where a detrimental chemical, biochemical or biological entity is present in a bodily fluid and where at least partial removal of the detrimental entity would be beneficial to a patient. The devices of the invention comprise binding means confined within a container, where the binding means is specific for affixing an infecting pathogen or a toxin produced by a pathogen, or another detrimental entity present in the patient's bodily fluid. By passing the patient's bodily fluid through the container, at least a portion of the pathogen or other detrimental entity is removed. The bodily fluid is substantially returned to the patient after it has been treated.

Background Art

[0003] Bacterial infections are becoming a greater danger. Certain bacteria have become resistant to antibiotic treatment, in some cases to a number of antibiotics, either naturally or through genetic manipulation. Septicemia is now among the most common causes of death in the United States of America (13th as of the year 2000), accounting for over ten billion dollars annually in health care costs. Fatality rates for septicemia are around 20%, totaling over 50,000 deaths annually.

[0004] In some cases a bacterium infects a person in a manner that makes any infection dangerous. Inhalation anthrax *(bacillus anthracis)* infection can be such a case. If inhaled, anthrax spores can cause a set of non-specific symptoms (malaise,

fatigue, myalgia, and fever) that do not lead to a clinical diagnosis of anthrax infection, absent actual knowledge of an anthrax exposure having taken place. The spores are deposited in the alveolar spaces and transported to mediastinial lymph nodes by lymphatic action. Once in the nodes, the spores can transform to vegetative cells. With germination, disease follows rapidly into a severe peripheral bacterial infection.

[0005] Replicating bacteria can release toxins that lead to necrosis, edema, and hemorrhage. (For the purposes of the present invention, toxins can also refer to any factors that lead to an actual toxin, such as anthrax edema factor (EF a 89kD adenylate cyclase protein) that leads to edema toxin (ET) if combined with anthrax protective antigen (PA, a 83kD cell binding component) or anthrax lethal factor (LF, a 90kD metalloprotease) which leads to lethal toxin (LT) if combined with PA.) At this point, diagnosis typically does not save the patient. In fact, antibiotic treatment may actually cause a crisis in the blood known as the Herxheimer effect that leads to death, by killing the infecting bacteria, and thereby releasing a flood of toxins to the peripheral system, a toxin overload.

[0006] In addition to external sources, bacterial infections can come from inside the patient. *Clostridium difficile* (*C. difficile*) is a bacterium that is often present in the gastrointestinal tract of healthy individuals and is typically harmless; however, it can become pathogenic when the normal populations of bacteria are substantially destroyed as by treatment of the patient with an antibiotic regime. It can also be contracted by ingestion of spores by individuals who do not already harbor the microbe; thus it is infectious as well as opportunistic. When it gets out of control, *C. difficile* causes diarrhea and can cause more severe consequences including life-threatening colitis, especially in frail patients. Because of the increased usage of antibiotics and the prevalence of this organism, *C. difficile* infections have become increasingly common in recent years: they now account for about 20% of antibiotic-related diarrhea cases, and the number of cases increases annually. M.S. Schroeder, *American Family Physician*, March 1, 2005. The effects of *C. difficile* are due to two toxins it produces, referred to as toxins A and B: toxin A is an enterotoxin, and toxin B is cytotoxic.

[0007] Similarly, many individuals carry various *Staphyloccocus* species including *S. aureus* with no ill effects, but under certain conditions may nevertheless contract

virulent 'Staph' infections from these same bacteria. These infections are increasingly dangerous because antibiotic resistance has become more frequent, thus the infections are less likely to respond to standard treatments. One example of this is methicillin-resistant *Staphylococcus aureus* (MRSA). Such multi-drug resistance (MDR) has been a significant problem in hospital settings, and is increasingly prevalent among other populations. The MDR strains are typically resistant to many different antibiotics, not just methicillin and drugs like it that are structurally related to penicillin; MDR strains, including MRSA, have been referred to as 'superbugs' for this reason. T.J. Foster, "The *Staphyloccus aureus* 'Superbug'", J. Clinical Investigation, 114(12), 1693-96 (2004). Like *C. difficile*, *S. aureus* also produces toxins that account for many of its pathogenic effects: it has multiple protective proteins on its surface that enable it to adhere to surfaces and resist attack, and it produces multiple hemolytic toxins including α toxin which depolarizes the plasma membrane of host cells; β toxin, which is a sphingomyelinase and thus degrades cell membranes; and hemolytic toxins including γ-hemolysin and leukocidin, which lyse erythrocytes and leukocytes, respectively.

[0008] New methods for the treatment of such bacterial infections are thus needed to replace or supplement conventional therapies. Other infections such as those due to virus, prions, and fungi are often more difficult to treat than bacterial infections. Even in the absence of drug resistance problems, these entities are often insensitive to available chemotherapeutic agents. Thus methods for their elimination are also needed.

[0009] Extracorporeal devices have been used in the past to remove undesirable substances from the blood of a patient. For example, U.S. 6,039,946 to Strahilevitz discloses an extracorporeal affinity adsorption device for providing therapeutic intervention. The container contains a chelant for binding metal ions in the blood and an antibody specifically binding to either an anti-cancer drug or a combined anti-cancer drug/targeting antibody. Published U.S. Patent Application No. 20020019603, U.S. Patent Application No. 20040220508, U.S. 6,676,622, US 6,569,112, US 6,264,623, US 6,039,346, and US 5,753,227 disclose extracorporeal affinity treatment devices to remove compounds that are "etiological in the pathogenesis of diseases in man"; they describe removal of lipids associated with atherosclerosis, for example, using chelation and antibody binders and similar

methods for the removal of enhancing antibodies, metals, and therapeutic agents. Some of these methods and devices use a semi-permeable membrane.

[0010] Extracorporeal devices have also been disclosed for use in the treatment of retroviral diseases such as HIV infection; U.S. 4,824,432 teaches about a container that has a means for removing interferon or HIV virus; it also mentions removal from an AIDS patient other microorganisms that cause opportunistic infections, but does not describe any method for such removal. U.S. 6,528,057, licensed to Aethlon Medical, discloses an extracorporeal system for removing viral components by passing the patient's blood through hollow fibers having immobilized affinity molecules attached to the exterior surfaces of those fibers.

[0011] Despite these, there remains a need for new, improved methods to remove various detrimental substances such as ones mentioned above, and methods to remove other viral pathogens as well as fungi, prions, and various detrimental biological and/or chemical entities not only from the blood of a patient but also from other bodily fluids.

Disclosure of the Invention

[0012] The present invention relates to novel methods and devices for treating patients having certain pathogenic infections such as bacterial, fungal, or viral infections, by using an extracorporeal adsorption container to remove the pathogen or its harmful entities from the patient's blood or other bodily fluid. It also relates to the removal of other detrimental entities, which may be exogenous or endogenous substances or cell types, whereby removal of the substance or cell type contributes to achieving an improved or desired physiological status. Bodily fluids to be treated include blood and plasma as well as cerebrospinal fluid, amniotic fluid, and the like, which are fluids that the subject needs for health reasons and yet may contain detrimental entities whose removal would provide a beneficial effect. Besides pathogens, the detrimental entities to be removed include, but are not limited to, toxins from foods, pesticides or other toxic substances accidentally ingested, chemical warfare agents such as ricin or mustard gas, excessive amounts of endogenous substances that create or exacerbate harm such as leptins, Alzheimer plaque-forming proteins, prions, cytokines, and abnormal cells such as those present in the blood of patients having myeloma or leukemia.

[0013] In one aspect, the invention provides devices that alleviate the adverse effects of pathogens or detrimental entities by treating a bodily fluid of the patient to reduce the level of a pathogen, cell, or substance prior to returning the fluid to the patient's body, thereby reducing the load of the pathogen or detrimental entity in the patient's body. This may reduce an infected patient's bacterial load to the point that antibiotic treatment can be administered without inducing a fatal bacteremia or Herxheimer effect, for example; or it may rescue a poisoning victim by removing enough of a toxic substance to allow the victim to survive, such as where a person has accidentally ingested a toxin from food, e.g. tetrodotoxin, ciguatoxin, aflatoxins, or other alkaloid or marine toxins, or has been exposed to a biological, biochemical or chemical weapon such as anthrax, ricin, or certain mustard gases.

[0014] Other aspects of the invention provide systems comprising the devices described herein, and methods of using the devices described herein to treat infections, toxin ingestion, metabolic dysfunction and other disorders wherein the reduction in the level of a detrimental endogenous or xenobiotic entity that resides in a bodily fluid provides a medical benefit to the treated patient. Still other aspects provide adsorption containers for use in the above methods, methods to use the device in combination with soluble binding means that can be admixed with the fluid to be treated, and methods to use the devices in conjunction with other medical treatments. Other aspects and embodiments of the invention will be apparent from the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The invention provides methods and devices for removing specific types of cells and/or substances from a bodily fluid of a patient to provide a medical benefit to the patient. The substances or cells to be removed include, but are not limited to, pathogens, xenobiotic substances such as toxins, carcinogens, and poisons, endogenous substances that cause detrimental effects to the patient, and aberrant cell types. 'Pathogens' as used herein includes harmful bacteria, fungi and viruses as well as parasites. Xenobiotic substances include toxins produced by pathogens, prions, and toxic compounds present in the patient's body due to ingestion, inhalation or absorption, such as toxic chemicals derived from food or from biochemical or chemical weapons. They also include substances such as a drug, imaging agent,

radiotherapeutic, or other substance that may have been deliberately administered to the patient but might then be beneficially removed. Endogenous substances include harmful or detrimental substances naturally present in the patient's body that may be injurious because they are overproduced, or that have adverse effects under particular circumstances; they also include substances such as immune components that are produced due to a dysfunction or misregulation within the body, and which are directed against a part of the patient's own body. Examples of these include autoantibodies, hormones, cytokines, ferritin, plaque-forming lipids or proteins and the like. The aberrant cell types to be removed include cancerous cells, infected cells, and malformed cells that reside at least partly in a bodily fluid. Examples of these include myeloma and leukemia cells that circulate in the blood, cells infected with a pathogenic virus, bacterium or fungus, and sickle cell red blood cells. Thus the invention provides methods to remove a wide array of detrimental entities from a bodily fluid.

[0016] The methods and devices of the invention are typically used by treating at least a portion of a subject's bodily fluid that has been removed from the subject's body. Bodily fluids suitable for use in the invention include blood, plasma, cerebrospinal fluid, and amniotic fluid; blood and cerebrospinal fluid are most often used. The bodily fluid is treated by being passed through a container referred to as an adsorption container. Confined inside the adsorption container is at least one binding means that selectively binds to the pathogen or other detrimental entity to be removed.

[0017] Because of the affinity of the binding means for the detrimental entity, a bodily fluid that is circulated through the adsorption container exits the container with a lower level of the entity than was present when the fluid was collected from the subject, and it can then be returned to the subject. Depending on the rate of flow of the fluid, the concentration of the detrimental entity, and the amount of the binding means in the container, it may be desirable to circulate the fluid through the adsorption container more than once, or to circulate it through more than one adsorption container, to further decrease the level of the pathogen or detrimental entity to be removed from the treated fluid.

[0018] In some embodiments of the invention, a bodily fluid such as blood or plasma is treated until at least about 20% or at least about 40% or at least about 60% or more than 75% of a detrimental entity is removed. In certain embodiments, the physiological load of the pathogen or other detrimental entity is monitored during treatment, and the treatment is continued, or repeated, until the load of the detrimental entity is reduced to the point that the user determines that the treated patient has benefited from the treatment.

[0019] In some embodiments, the invention is applied to the removal of pathogens or detrimental entities from bodily fluids such as cerebrospinal fluid or amniotic fluid. In these embodiments, the fluid is similarly circulated through an adsorption container which comprises a specific binding means for the pathogen or substance to be removed, and is then returned to the treated subject. Typically, in these embodiments, the device and methods are used to remove an infecting pathogen such as one causing meningitis, which may be bacterial, viral or fungal, or one causing encephalitis or myelitis; or to remove prions, which may cross the blood-brain barrier to inflict their harm in cerebrospinal fluid.

[0020] In one aspect, the invention provides a device that allows a user to remove at least some of a detrimental entity from the body of a subject, typically a human patient but optionally including other mammals as well. The removal of a pathogen or detrimental substance is accomplished by extracting a bodily fluid from the patient via means known in the art, and circulating the fluid through an adsorption container before returning it to the treated subject. Typically, the fluid is blood, plasma, cerebrospinal fluid, or amniotic fluid. In preferred embodiments, blood or cerebrospinal fluid is often treated with the device; blood may be treated while whole or it may be separated, in which case the plasma or blood cells or both may be treated by methods of the invention.

[0021] The device is capable of removing a wide array of detrimental entities from a patient's blood. Examples include pathogens such as bacteria, fungi and viruses; parasites that inhabit body fluids during at least one stage of their life cycle; toxins such as those produced by certain infectious pathogens and those ingested in contaminated food; exogenous substances such as prions; and endogenous substances present in undesirably high levels, such as excessive amounts of autoantibodies, hormones, cytokines, and the like. In some embodiments, the device is used to

remove more than one material; example when it may be used to remove an infecting pathogen and one or more toxins produced by the pathogen. Among the pathogens for which the device is useful, drug resistant bacteria and viruses are sometimes preferred. Some embodiments of the methods and devices of the invention are particularly useful to remove pathogens or toxins delivered to the subject in the form of a bioweapon or chemical weapon.

[0022] In some embodiments, the adsorption container may contain more than one binding means, such as antibodies specific for different epitopes presented by a particular bacterium; or antibodies specific for more than one strain of a pathogen; or binding means such as an immunoglobulin specific for a bacterium in addition to an antibody specific for one or more toxins produced by the bacterium. The methods of the invention include use of two or more binding means in combination, and such combinations include configurations in which a single adsorption container contains more than one such binding means as well as configurations in which separate adsorption containers are provided for different binding means.

[0023] Typically, a device of the invention is used to treat blood or plasma or cerebrospinal fluid, which is subsequently returned to the subject, and the subject benefits from the reduction in the level of pathogenic organism or detrimental substance in the treated bodily fluid.

[0024] When blood is treated, it is also possible to separate at least most of the blood cells from the plasma prior to treatment of the plasma or of the cells, thus the methods and devices are amenable to the treatment of plasma, or plasma plus leukocytes, as well as whole blood. Methods for separating blood cells from plasma are known in the art, and include the use of centrifuge devices as well as membrane-based separations. Such methods may separate bacteria out of plasma along with the blood cells, or they may leave most of the bacteria in the plasma along with any toxins produced by the bacteria. The separated plasma may be brought into contact with the binding means by passing it through an adsorption container in which the binding means is disposed. The separated blood cells may be treated as well; for example, they may be suspended in an isotonic solution and circulated through an adsorption container of the invention. In embodiments employing this feature, the blood cells and plasma may both be returned to the patient after either or each has been treated to reduce the level of pathogens and/or detrimental substances present;

alternatively, either plasma or blood cells may be returned, according to the judgment of the treating physician.

[0025] In one embodiment, the invention comprises a device or system for removing a pathogen or other detrimental entity from a bodily fluid. The device or system comprises an extracorporeal adsorption container having an inlet means and an outlet means for circulating fluid through the container. A solid support is disposed and confined within the container; it may be retained in the container by a suitable membrane or mesh barrier that is positioned and sized to permit fluids to pass into and out of the container while retaining the solid support; in some embodiments, the solid support is itself sufficiently large to be retained in the adsorption container without special structures to retain it. For example, a sheet of material such as nitrocellulose may be used as the solid support; binding means such as an antibody can readily be adsorbed to the support by known methods, and the sheet can then be rolled up and inserted into a column or adsorption container of the invention. Optionally, the solid support may also be treated with other binding means, or it may be treated with blocking reagents so that the binding capacity of the solid support is substantially occupied before a fluid is passed through the adsorption container, reducing non-specific binding that could deplete desirable components in the bodily fluid being treated.

[0026] In other embodiments, the solid support comprises fibers or hollow fibers which may be lined or coated, with a binding means, which may be inside or outside the hollow fibers depending on the configuration of the device; and the container may merely be a housing to contain these fibers. In one configuration, hollow fibers may comprise a channel that confines the fluid flowing through the container; the binding means is then typically inside the container but outside the hollow fibers, and the hollow fibers comprise pores sized to permit the detrimental entity to escape from the hollow fiber but small enough to prevent blood cells from escaping the hollow fibers. The binding means is then typically associated with an additional solid support, or it may be associated with a polymer or other relatively large chemical entity that is too large to enter the pores of the hollow fibers even though it may not be a solid.

[0027] In some embodiments, the amount of material to be removed by the device is sufficiently small that a useful quantity of binding means can be adhered or adsorbed or bonded to the interior surfaces of a container or even a tubing, in which

case the interior surfaces of the container or tubing is itself a solid support for the purposes of the invention. In other embodiments, the solid support comprises beads, rolled membranes, or gels with the binding means immobilized or coating one or more surfaces of the support.

[0028] A binding means is often associated with a solid support: the binding means employed is specific for affixing a detrimental entity such as an infecting pathogen, thereby allowing for the removal of at least a portion of the infecting pathogen and the return of the treated fluid to the patient when a bodily fluid is circulated through the container so that the detrimental entity contacts the binding means.

[0029] The detrimental entity to be removed must contact the binding means of the device for the device to remove the detrimental entity; therefore, at least a portion of the bodily fluid to be treated will contact the binding means. In some configurations of the invention, the bodily fluid and all of its components can directly contact the binding means, as when the binding means is associated with beads and the bodily fluid is passed through a bed of these beads. However, in some configurations a porous or semi-permeable membrane is employed in the device so that a bodily fluid such as blood, which contains vital cells, can be circulated through the device without having the cells contact the binding means. Thus blood may be circulated through an adsorption container inside a portion of the container such as channels or tubes defined by the semi-permeable membrane, while the binding means is in the portion of the container outside the channels or tubes defined by the semi-permeable membrane. In these configurations, the blood cells are protected from direct contact with the binding means. By selection of a semi-permeable membrane having suitable pore sizes, the detrimental entity to be removed can nevertheless directly contact the binding means, as long as the detrimental entity is smaller than a red blood cell.

[0030] In some embodiments, the binding means may be specific for binding or affixing a detrimental chemical or biochemical substance that is dissolved or suspended in a bodily fluid, or a detrimental cell type that is present in the fluid. The specificity of the binding means must be sufficient to selectively remove at least a portion of the detrimental substance or cell type from the fluid to be treated.

[0031] The methods and devices of the invention may also be useful for the treatment of certain viral infections, especially acute infections or those where symptom onset is sudden and viral replication is rapid, necessitating rapid treatment. They also may be used to treat infections with virus that are resistant to other treatment methods, and are particularly useful for the treatment of mutated or engineered viruses that may be resistant to antivirals and may be employed as bioweapons. As long as at least a portion of the virus is present in a bodily fluid, the methods and devices of the invention may be used to reduce the load of virus in a patient's body.

[0032] Similarly, the devices and methods are useful for the removal of parasites and fungi from bodily fluids, provided that the parasite or fungus spends at least a portion of its life cycle in a bodily fluid such as blood, amniotic fluid, or cerebrospinal fluid, or that it may be transported in such fluids.

[0033] A variety of conventional solid supports are suitable for the present invention, including beads, fibers, gels, hollow fibers, and membranes. The supports for binding means may also be other materials such as proteins, soluble polymers, and the like that are not necessarily solid. In some embodiments, the support may be a cellulose-based material, while in others it is a synthetic polymer. Frequently, the solid support is removable from the container so that it can be readily disposed of by incineration, for example, after use, and in some embodiments the entire container is similarly combustible for easy disposal. In some embodiments, however, a surface of the tubing or adsorption container itself serves as the support for at least a portion of a binding means. Typically, one should use a support capable of holding a large load of binding means, preferably enough to remove at least one mg of bacteria. Where a binding means is associated with a solid support that is disposed inside an adsorption container, preferably the solid support has a surface area to volume ratio of at least about 4 to 1 in order to minimize the size of the adsorption container required.

[0034] The device or system of the invention may comprise more than one adsorption container, and where multiple containers are employed, the containers may be the same or different. One container may be sized to treat an average patient by determining an amount of the binding means that would ordinarily be used for a particular situation, and sizing the container to hold enough of the solid support to display enough of the binding means to treat one patient. For convenience, one can

size the container so as to contain enough of the binding means to provide sufficient binding capacity to remove a predetermined amount of a pathogen from a patient, for example; the treating physician can then estimate the number of containers necessary to treat an assayed level of infection, or the number of times the binding means must be either replaced or regenerated to achieve the desired effect.

[0035] For convenience, the adsorption container may be adapted to permit the binding means to be replaced easily. Thus the adsorption container may include a port or aperture through which the binding means may be inserted and removed, regardless of whether the binding means is associated with a solid support or with another type of carrier.

[0036] The binding means can be any conventional means for binding to a bacterium, fungus, virus, toxin, abnormal cell, or endogenous substance or other detrimental entity to be removed. Typically, the binding means is immobilized or bonded to a carrier or solid support in an amount sufficient to remove at least 1 mg of infecting pathogen or the associated toxins; however, the capacity of the binding means to be used depends on the substance or pathogen or cell type to be removed, and a suitable amount of the binding means may be larger or smaller than this. In the case of extremely potent toxins such as diphtheria, tetanus, and certain natural toxins, the removal of microgram quantities may be adequate to rescue a patient.

[0037] Suitable binding means include Con A, lectins, and immunoadsorbents such as immunoglobulins, monoclonal antibodies, and polyclonal antibodies as well as antibody fragments and polymerized antibodies or polymerized antibody fragments. Other binding means such as chelating agents that are well-known in the art are also included. As used herein, unless otherwise indicated, the term 'antibody' or 'antibodies' encompasses both monoclonal antibodies and polyclonal antibodies. In some embodiments of the invention it is preferred to use monoclonal antibodies, and in others it is preferred to use polyclonal antibodies.

[0038] In some embodiments, an immunoglobulin is used as the binding means. For example, Protein A of *Staphylococcus* species is a protein expressed on the external cell wall and is known to bind to immunoglobulins such as rabbit IgG or human IgG, even though the organism from which the IgG was obtained had not been exposed to or immunized against Protein A. Thus IgG from a human or a rabbit could be used as the binding means in an embodiment of the invention to remove a

Staphylococcus pathogen such as methicillin-resistant *Staphylococcus aureus* (MRSA) from the blood of an infected subject. For this purpose, the IgG would be associated with a support such as a membrane and disposed and confined within an adsorption container, through which the blood of an infected subject would be circulated to effect removal of at least a portion of the infecting microorganism. The use of human IgG is preferred in some embodiments, which include embodiments of the devices and methods that are adapted to remove MRSA from the blood of an infected subject. Methods for preparation of each of these types of binding means, and of associating them with a support, are well known in the art.

[0039] The binding means can be associated with its carrier or solid support by any suitable means. Methods for attaching immunoglobulins, antibodies and chelating agents to solid supports are well known. Examples include non-covalent adhesion to certain surfaces such as polyvinylidene fluoride (PVDF, or Immobilon P®), as well as nitrocellulose, nylon and modified versions of these materials, and beads of hydrophilic materials such as agarose or other carbohydrates, which may allow for some covalent bonding as well.

[0040] Covalent attachment is also well known for immobilization of certain binding means such as proteins and antibodies; methods for this form of attachment of the binding means include linking them to the support through bifunctional reagents such as glutaraldehyde, EDAC or suberimidate, where one reactive functional group of the reagent attaches the reagent to the support and the other functional group of the reagent attaches the reagent to the binding means to be affixed, or through reactions of a binding means component with functionalized polymer surfaces containing, e.g. isocyanate, hydroxyl, carboxyl, sulfhydryl, amino, or imidate groups, or an activated form of one or more of such groups.

[0041] The binding means may also be linked to a support through chemical or immunochemical means or through well-known combinations of high-affinity reagent pairs such as avidin or streptavidin plus biotin. By way of example only, streptavidin can be attached to the support, and biotin to the binding means, and when brought into contact, the two will become strongly associated by the affinity of biotin for streptavidin. Where this approach is used, it is often desirable to employ a linker between the support or binding means and the high-affinity reagent used to associate the binding means with the support. Methods for using these approaches are

summarized, e.g., in *Immunoassay*, E.P. Diamandis and T.K. Christopoulos, ed., Academic Press, San Diego, CA, pp. 216-222 (1996), which is incorporated by reference.

[0042] In some embodiments, the device is adapted to remove bacteria. In particular, it may be configured to remove drug resistant pathogenic bacteria, typically from the blood or spinal fluid. According to some estimates, up to 40% of *Streptococcus pneumoniae* infections in the U.S. are now resistant to both penicillin and erythromycin, two broad-spectrum antibiotics of very different structures. T.M. Powledge, *New Antibiotics—Resistance is Futile*, PLoS Biology, 2(2), 151-154 (2004). Such antibiotic resistance and multi-drug resistance is increasingly common and problematic, and as the resistant pathogens lead physicians to increasingly rely on the newest antibiotics, resistance to them quickly arises and spreads, even in hospital settings. The methods and devices of the invention are especially useful for the treatment of pathogens that are resistant to at least one of the antiviral and/or antibiotic treatments currently available. In particular, the methods and devices are useful in the treatment of infections that show multi-drug resistance, including *Staphylococci* such as MRSA, vancomycin-resistant *Enterococci*, and multidrug resistant *Streptococcus pneumoniae*.

[0043] In some embodiments of the invention, the pathogen is a bacterium such as a species of *Bacillus*, *Meningococcus*, *Streptococcus*, *Clostridium*, *Staphylococcus* including MRSA (methicillin-resistant *S. aureus*), or *Paratuberculosis*. Bacteria and viruses that are resistant to the available antibiotics and antivirals are especially appropriate targets for removal by the methods and devices of the invention, as are bacteria and viruses that have been modified to function as bioweapons that may resist known drugs. Also, bacteria or viruses that produce or induce production of a toxin are of special interest, because the device and methods provide ways to remove the toxin as well as the pathogen. The devices and methods are especially suitable for infection or illness caused by these pathogens and their by-products, including bacteria that cause tetanus, botulism, pneumonia, tuberculosis, anthrax, pertussis, cholera, shigella, toxic shock syndrome, diphtheria and the like.

[0044] In other embodiments, the pathogen is a virus, excluding virus from the family *Retroviridae*. Thus the methods and devices of the invention are useful to treat a bodily fluid that is infected with viruses from at least the following families:

Bunyaviridae, Filoviridae, Hepadnaviridae, Herpesviridae, Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Poxviridae, Reoviridae, Rhabdoviridae, Arboviruses, and *Togaviridae*. These families include, respectively, the Hanta virus; Marburg and Ebola viruses; hepatitis B virus; herpes viruses and mononucleosis; influenza virus; measles and mumps viruses; polio virus; smallpox virus; tick fever virus; rabies virus; West Nile virus; and dengue fever, rubella and yellow fever viruses. The methods may be suitable for use with other viruses as well, as long as the virus is present at least partly in a bodily fluid.

[0045] The methods permit removal of fungi and certain parasites, too, so long as at least one part of the life cycle of the entity occurs in a treatable bodily fluid. Parasite infections that may be treated with the present methods and devices include various forms of trypanosomiasis such as African sleeping sickness and Chagas disease, schistosomiasis, babesiosis, filariasis, and malaria (plasmodium infections).

[0046] Fungal infections treatable by the methods and devices are primarily those classified as systemic fungal diseases. These most often occur in individuals having compromised immune function, but can occur in otherwise healthy individuals. Examples of these include histoplasmosis, coccidioidomycosis, blastomycosis, paracoccidioidomycosis, sporotrichosis, cryptococcosis, systemic candidiasis, aspergillosis, mucormycosis, mycetoma, chromomycosis, and phaeohyphomycosis. As with bacteria, there is increasing occurrence of resistance to the limited variety of available antifungals, and resistant systemic fungal diseases are especially appropriate for treatment with the devices and methods of the invention.

[0047] Especially where the pathogen is a bacterium, fungus, or parasite, it may produce one or more toxins, and in some embodiments the invention provides adsorption containers and methods to remove one or more toxins produced by pathogens instead of or in addition to removing the infecting pathogen. The removal of other materials produced by a bacterium or virus or by a parasite is also within the scope of the invention: such methods can be used to disrupt the life cycle or reproduction of pathogens or parasites by removing a material essential to the pathogen, in order to assist the patient's recovery or facilitate the effectiveness of conventional treatments. In some embodiments, the devices of the invention are configured to remove a pathogen or parasite as well as a detrimental substance caused by or produced by that pathogen or parasite.

[0048] Similarly, the device and methods of the invention can be used to remove from a bodily fluid of a patient any other detrimental substance that can be selectively bound and removed. The detrimental substances may be toxic compounds, either natural or synthetic, and they may include endogenous substances that are present in undesirably high concentrations, where an improved physiological condition would be achieved by reduction of the concentration of the substance. The methods and devices are especially suitable for the removal of prions; toxins produced by the pathogens described above; poison substances such as ciguatoxins, aflatoxins, tetrodotoxin, ergot and other alkaloids, pesticides and the like that can be ingested and enter the bodily fluids, either by accidental poisoning, or as impurities in foods, or by deliberate delivery such as when employed as a weapon; and hormones, leptins, cytokines, enzymes and the like that are overproduced in the body under certain circumstances, or that may be present in undesirably high amounts on an ongoing or periodic basis.

[0049] Toxins that may be ingested from food sources such as marine fish and algae include paralytic shellfish toxins such as the saxitoxins; neurotoxic shellfish toxins such as brevetoxins; ciguatera toxins such as ciguatoxins, maitotoxins, ostreocin, and palytoxins; diarrhetic shellfish toxins such as okadaic acid, and dinophysistoxins; amnesic shellfish poisons such as domoic acids; and cyanobacterial toxins such as saxitoxins, anatoxins, microcystins, and nodularins. These are suitably removed by the devices and methods of the invention, and binding means such as antibodies for many of these toxic compounds are known in the art.

[0050] Endogenous substances that are suitable for removal by the methods and devices of the invention include, but are not limited to, enzymes, hormones or cytokines present in excessive amounts, including materials such as TNF-α, leptin, apolipoprotien E, angiotensinogen, adipsin, plasminogen activator inhibitor (PAI-1), C-reactive protein, tissue factor, and TGF-β, which are associated with or upregulated in obesity; autoantibodies and related overactive immune disorders that may cause rheumatoid arthritis, lupus, connective tissue diseases, SLE, renal disease, Sjogren's syndrome, scleroderma, interstitial lung disease, polymyositis, CREST syndrome, Wegener's granulomatosis, glomerulonephritis, ALS / Lou Gehrig's disease, IBD, Graves disease, Crohn's disease, other forms of arthritis, and related disorders; and

excess ferritin, prolactin, or erythropoietin (EPO). Again, binding means such as antibodies for many of these substances are known.

[0051] In another aspect, the invention provides methods of using the devices described herein to treat patients in need of at least partial removal of a pathogen or other detrimental entity from a bodily fluid. Typically the methods are used to remove a targeted pathogen, substance, or cell type from blood, plasma, or cerebrospinal fluid, and the treated fluid is subsequently returned to the treated subject's body by conventional means. The methods of the invention may be used once to treat an acute condition such as accidental ingestion of a toxin, or to reduce the load of a pathogenic bacterium such as to permit antibiotic treatment of the infected individual. They may also be used periodically, as once per day, or once per week, or twice a week, or from once to about ten times per month to treat chronic or recurring conditions. They may also be used continuously for a period or hours or more, in certain situations, such as in preparation for or during recovery from surgery, where it is desirable to control constituents such as cytokines or proteins that promote inflammation or undesired immune reactions, for example, or where cancer cells in the blood can be intercepted to deter metastasis while treatments are delivered.

[0052] When the treated fluid is blood or plasma, it may be removed from the subject with a needle by conventional means, and returned via the same needle or a separate needle. Needles having parallel or concentric pathways for removal and return of blood are known in the art and may be used to minimize the subject's discomfort during the procedure. Where the treated fluid is cerebrospinal fluid, it may be accessed by known methods using a needle as is typically done for a spinal tap. Like blood, it can be returned to the subject with the same needle used to extract it via a parallel or concentric pathway. However, because the cerebrospinal fluid is not circulated within the body in the efficient manner that blood is, it is sometimes preferable to employ a second needle to return cerebrospinal fluid at a location that is fluidly connected to but remote from the location where the fluid is extracted. Thus the device or system may comprise two separate needles, so the cerebrospinal fluid can be extracted at a point in the lower spine and returned at a point in the upper spine or in the skull, or vice versa. Also, where the fluid to be treated is cerebrospinal fluid, it may be advantageous to minimize the volume of the adsorption

container and the fluid handling system, and the system and adsorption container should be sized accordingly. The amount of binding means is also typically less in this application, and treatment with an anticoagulant is unnecessary.

[0053] The methods and devices can be used in combination with other medical treatments such as before, during or after a surgical procedure, e.g. to remove angiogenesis factors that promote cancer growth; in connection with an organ or tissue transplant procedure where it may be sued to reduce rejection disorders or to reduce inflammation due to cytokine production; during dialysis to remove a specific impurity or to remove two or more materials, e.g. as a replacement for or supplement to kidney dialysis; or in connection with diagnostic procedures where a foreign substance needs to be removed from a bodily fluid after it has served its purpose as an imaging agent, for example. They can also be used in conjunction with radiotherapeutic, chemotherapeutic and antibody treatments, to remove a therapeutic agent that has served its purpose, where prolonged exposure to the therapeutic agent(s) may cause increasing injury after the initial benefits have been achieved.

[0054] Often the methods of the invention are used one or two or three times, such as when they are used to rescue a victim of an acute infection or poisoning episode. Alternatively, these methods may be used intermittently and repeatedly to modify a patient's body chemistry in a desired way by removing a substance, such as to increase the likelihood that a woman will be able to conceive by removing hormones that prevent ovulation, or increase a man's sperm count by removing antibodies or other substances that are harmful to his sperm. Similarly, the methods and devices may be used to remove excessive immunochemical substances as a treatment for rheumatoid arthritis, type I diabetes, lupus or similar conditions where the patient's immune system forms detrimental self-directed substances, or to remove excessive or defective proteins such as those forming plaques associated with Alzheimer's disease, or prions such as those associated with Creutzfeldt-Jacob disease.

[0055] In some embodiments, the treatments may be administered at an appropriate time to enhance opportunities for conception, where either a male or a female may be treated to increase the subject's fertility because of the presence of detrimental substances such as antibodies to a man's sperm, or prolactin inhibiting the woman's ovulation. Methods to select the appropriate time based on the ovulation cycle of the female are known. In other embodiments, the methods provide

periodic treatments of chronic conditions such as lupus, arthritis, Alzheimer's disease, obesity, fibromyalgia, chronic fatigue syndrome, inflammation, and the like. Such treatments may be administered at a frequency that can be individually tailored to the particular subject's needs by a treating physician.

[0056] In some embodiments, the devices and methods may be utilized in the home without the need for a physician, which is particularly helpful in the treatment of chronic conditions such as arthritis, cancer, Alzheimer's, and the like, and for assisting conception. Means for withdrawing and returning blood to a patient in a home setting are known in the art, and are suitable for use with the methods and devices of the invention.

[0057] In another aspect, the fluid to be treated to remove a detrimental entity is admixed with a soluble binding means prior to passage through the adsorption container, where the soluble binding means comprises a capture means. These embodiments operate on a principle similar to that of a sandwich assay used in certain analytical systems. The soluble binding means is specific for binding to the pathogen or detrimental substance to be removed and is linked to a capture means. The soluble binding means binds to the pathogen or substance to be removed when it is admixed with the fluid to be treated. The detrimental entity is thereby linked to the capture means portion of the soluble binding means. The binding means in the device of the invention in these embodiments comprises a binding means that is specific for the capture means: by binding to the capture means, the device affixes and removes the pathogen or detrimental entity from the treated fluid. This aspect of the invention is suitable for use with any of the embodiments of the devices described above, as well as with devices known in the art for removal of a substance from blood.

[0058] The soluble binding means for these embodiments may be administered to the patient to be treated, prior to application of the methods described herein. Alternatively, the soluble binding means may be admixed with the fluid to be treated after removal of the fluid from the patient, to minimize exposure of the subject to the soluble binding means. The soluble binding means is then permitted to associate with or bind to the detrimental entity in the treated fluid. The bodily fluid is then circulated through an adsorption container which comprises a binding means that will specifically bind to the capture means portion of the soluble binding means. In these

embodiments, the binding means in the adsorption container will substantially remove the soluble binding means from the treated fluid before the fluid is returned to the patient. This minimizes exposure of the patient to the soluble binding means, and it also permits a single adsorption container to be used for removing a variety of substances or pathogens, provided that a soluble binding means is employed for each such substance or pathogen to be removed, and that each such soluble binding means comprises a capture means that the adsorption container's binding means can affix. It may also permit the use of a binding means with especially high binding capacity, which is of value to ensure that large pathogens and parasites, for example, are bound tightly enough that they remain affixed to the binding means confined in the adsorption container. For example, the binding means in the adsorption container may be a relatively small molecule such as a polypeptide, so that the carrier or support on which the binding means is located may carry an especially large load of the binding means, relative to the loading that would typically be achieved with a binding means such as an antibody. These embodiments can employ various combinations of capture means and binding means specific for the capture means, including an antibody – peptide combination, many of which are known in the art. In some preferred embodiments one of these two entities comprises biotin and the other comprises streptavidin or avidin.

[0059] In still other embodiments, the methods and devices can be used to remove abnormal cells from the blood, such as to treat leukemia, myeloma or sickle cell disease. As long as the cell to be removed has a unique surface component that can be exploited to selectively bind that type of cell, the methods and devices are useful for removal of the cell.

[0060] In certain embodiments, one can provide at least two different binding means together in one adsorption container, such as a binding means for each of two or more separate bacterial strains, or for a bacterium and at least one toxin produced by that bacterium, or for at least two different epitopes on the pathogen or toxin to be captured. In some embodiments, the binding means for a particular toxin or pathogen includes many different antibodies that recognize different epitopes on the target organism or molecule, reducing the likelihood that mutations will escape recognition.

[0061] In embodiments of the invention that include more than one binding means in a single adsorption container, the binding means in such embodiments may be alike, e.g. each one may be an antibody; or they may be of different types, such as a polymerized antibody in combination with an antibody fragment and/or an immunoglobulin. In some such combinations where more than one binding means is present each binding means is specific for binding to a different entity, e.g. a different microorganism or virus, or a different toxin. In other such combinations where more than one binding means is present, at least two of the binding means are specific for parts of the same entity; thus two or more antibodies having specific affinity for two different surface proteins of a single infecting pathogen may be used together, or two or more antibodies having specific affinity for two different epitopes on a single toxin produced by an infecting pathogen may be used together.

[0062] Similarly, one can provide a series of containers to be used concurrently, each containing a separate binding means in order to remove two or more different materials or bacterial strains. In that vein, one can provide for containers that can attach to each other in a serial fashion. For example, each end of a container of the device can be provided with a threaded inlet or outlet port so that an adsorption container containing a binding means for a bacterium can be threaded onto, or linked by conventional tubing to, a container for the associated bacterial toxins, if desired. This enables the device to include multiple containers with the same or different binding means, and provides the flexibility for the user to determine how much binding capacity to use for a particular subject and what combination of detrimental entities are to be removed by the treatment.

[0063] In some embodiments, which may employ more than one binding means, the invention provides for removal of some or all of the major metabolic by-products that are typically removed by kidney dialysis. This accomplishes at least partial replacement of a dialysis treatment, and can thus increase the time intervals between dialysis treatments; it also reduces some of the adverse effects of dialysis, whereby certain desirable substances such as nutrients and vitamins are lost. Thus the device may be adapted to remove urea, creatinine, uric acid, and the like to reduce dependence on typical kidney dialysis treatments.

[0064] Where two or more different binding means are included, they may both be disposed on a single support or substrate, or they may be on separate supports so the user can adjust the amount of each selective binding means used. Thus in certain embodiments, a first binding means selective for a species of bacterium may be employed in combination with a second binding means specific for a toxin produced by that species of bacterium. In such embodiments, the first and second binding means may be on a single support such as a single membrane or fiber. Alternatively, the first and second binding means may be adhered to supports separately, such as to beads, for example; this permits the user to control both the amount and the ratio of the binding means used for a particular subject according to the level of bacteria and of toxin that the user anticipates will need to be removed for the particular patient being treated, by simply selecting an appropriate amount of each type of bead. Similarly, two or more binding means on supports, which may be similar or different, can be deployed in a single container or they may be deployed within separate containers.

[0065] Also, where two or more binding means are to be used concurrently, they may be physically separated within a single adsorption container of the device so that they effectively occupy different compartments. For example, at least one such binding means may be partitioned by a semi-permeable membrane so that it is physically separated from the compartment containing the cells of blood to be circulated through the adsorption container. Thus the blood cells may flow through an adsorption container of the device in hollow fibers, for example, while at least one binding means is outside the hollow fibers, and preferably is associated with a separate support that can be removed and optionally replaced, such as a membrane, fiber, or bead. The detrimental entity to be removed must be able to exit through the porous material comprising the hollow fibers in this configuration, so it can reach the binding means. The pores of the semi-permeable membrane are thus sized so that the pathogen or toxin that is to be removed can leave the blood cells by passing through the pores, which are also sized so that blood cells cannot pass through. Blood cells then are confined to one compartment within the adsorption container, such as the interior of the hollow fibers, while a binding means may be in another compartment, such as outside the hollow fibers. This arrangement is possible because most pathogenic bacteria are small in comparison to a red blood cell, and

other blood cells are typically larger than the red blood cells. Suitable pore sizes for these configurations are typically 0.01 to 10 μm, since red blood cells are about 10 μm in diameter; in some embodiments where such semi-permeable membranes are employed, the pore size is preferably less than about 9 μm.

[0066] Where more than one binding means is employed, one may likewise be placed within a compartment of the adsorption container that is accessible to the blood cells, while another may be in a separate compartment defined by a semi-permeable membrane. This physical separation from the blood cells permits a binding means that is separated by the membrane from the blood cells to be treated with methods or reagents that might harm the blood cells; thus it permits the separated binding means to be regenerated or reactivated during operation, or it may permit the bacterium or toxin that becomes affixed to the binding means to be irradiated, for example, to inactivate the pathogen or toxin while the blood cells remain separate and protected.

[0067] In embodiments where a binding means is separated from the blood to be treated by a semi-permeable membrane, the membrane may have a pore size that permits the material or bacterium which is to be removed from the subject's blood to pass through, while preventing blood cells from escaping. This minimizes the loss of blood cells, but may permit some plasma components to leave the compartment containing the blood cells, thus configurations that minimize the volume of the compartment that the blood cells do not reach are sometimes preferred. In some embodiments, the semi-permeable membrane will thus have a pore size that is less than the size of a red blood cell, which is approximately 10 microns in diameter; such pores will prevent loss of red blood cells and of larger cells such as lymphocytes. They will also permit most bacteria, as well as viruses and toxins, which are typically smaller than the smallest bacteria, to escape from the compartment where the blood cells are circulating. These entities (bacteria, virus, toxins) can then contact binding means that cause them to be retained in the adsorption container.

[0068] Because most pathogenic bacteria are smaller than a red blood cell, it is possible to select a membrane that permits the infecting bacterium to pass out of the compartment containing blood cells while it retains the blood cells. See, e.g. Textbook of Biochemistry with Clinical Correlations, 5th ed., T. Devlin, ed., pg. 4: most prokaryotes have a volume between 1/1000 and 1/10,000 of the volume of

eukaryotic cells. While bacteria vary in size and shape, most have at least one dimension that is less than 1-2 microns; by comparison, a red blood cell is roughly 10 microns across. See, e.g., the following web site, developed by the New York State Department of Health, which provides photomicrographs showing bacteria in the presence of red blood cells and other blood cells, most of which are larger than red blood cells: <http://www.wadsworth.org/chemheme/heme/microscope/celllist.htm>. Toxins are of course much smaller than cells: even large proteinaceous toxins such as the toxins produced by *C. difficile* discussed above, are much smaller than even a small bacterium. Thus toxins can diffuse through a semi-permeable membrane having pores that are far smaller than a red blood cell, which allows them to contact a binding means that is physically separated by such a semi-permeable membrane from the cells of the treated blood or plasma. Selection of a membrane with suitable pore size for a given separation is within the skill of an ordinary practitioner, and can be guided, for example, by the Membrane Filtration Handbook, 2d ed., Nov. 2001, which was written by J. Wagner, and published by Osmonics, Inc., a division of GE, and is incorporated herein by reference.

[0069] Where a binding means is physically separated from the blood cells by a semi-permeable membrane, the binding means is not necessarily attached to a solid support. Thus, for example, this binding means may be a polymerized antibody or a polymerized antibody fragment, which may remain dissolved or suspended in a liquid medium, or may be attached to or associated with a non-solid support or contained within a liposome, for example, that is too large to pass through the pores of the membrane to enter the compartment occupied by the blood cells. As long as the pore size of the membrane is small enough to prevent the red blood cells from escaping from their compartment, which channels them through the container, and to prevent the binding means from passing through the membrane to enter the compartment containing the blood cells, the binding means need not be adhered to a solid support. In this way, the entity to be retained (pathogen, bacterium, virus, toxin) can contact the binding means, while blood cells being circulated through the adsorption container cannot contact the binding means. This permits the use of a broader range of binding means, since it prevents the binding means from becoming saturated by blood cells in the event the blood cells have some affinity for the binding means, and it prevents the binding means from causing a cellular immune response in the

subject's blood. Thus a polymerized antibody or polymerized antibody fragment may be kept separate from the blood cells by a semi-permeable membrane having a suitable pore size, while toxins or small pathogens such as mycobacteria may pass through the pores to be affixed by the binding means. Similarly, a binding means may be adhered to a gel or soluble polymer materials of suitable size to prevent passage of the polymer+binding means conjugate through the semi-permeable membrane pores, permitting the use of binding means without a solid support.

[0070] Where the pathogen to be removed is comparable in size to a blood cell, treatment of blood by the methods of the invention requires the blood to directly contact the binding means. This permits removal from blood of aberrant cells that are similar in size to a blood cell or larger, and it permits the removal of parasites and fungi that may be too large to be separated from blood cells based on size alone. In some preferred embodiments of the invention, the device and methods are used to treat blood, and at least one binding means of the invention is disposed so that it directly contacts the blood during operation.

[0071] In some embodiments, the device further includes means for regeneration of the binding means, either during operation or between uses. The binding means may become partially saturated with the bound cells or toxins during operation, and it may be advantageous in some cases to re-use the binding means, necessitating its regeneration. The binding means can typically be regenerated between uses by conditions known to reduce its affinity for the bound material, such as, for example, exposure to a rinsing solution, which may include higher or lower pH or high salt conditions, for example, or it may include, e.g., a solution of an amino acid such as glycine or a hypertonic solution of glucose or salts. Therefore, the device optionally includes means for such regeneration of the binding means while it remains inside the adsorption container, typically comprising a three-way valve attached to the inlet means or attached to a tube leading to the inlet means and one attached to the outlet means of the container, plus a pump means to circulate the appropriate rinsing solution through the adsorption container.

[0072] Typically, rinsing for regeneration is conducted by stopping flow of the treated fluid through the adsorption container; and passing the appropriate rinsing solution through the adsorption container for a suitable amount of time. In preferred embodiments, regeneration of the device is achieved by completely stopping the

circulation of a bodily fluid through the device, and optionally flushing the bodily fluid from the adsorption container to prevent it from being either contaminated by or damaged by the regeneration treatment or reagent. Flushing of the fluid from the device may be achieved with an isotonic aqueous solution, or it may be accomplished with a low-pressure gas flow. Where a gas flow is used to blow the fluid out of the adsorption container, it is often desirable to employ a gas trapping system whereby the fluid can be returned to the treated subject without injecting gas into the subject. Thus it is typically desirable to employ a trapping device to capture the gas and permit only the treated fluid to be delivered to the patient.

[0073] Alternatively, the binding means may be removable from the adsorption container, and regeneration of the device may be achieved by removing the used binding means and replacing it with fresh binding means. This is often particularly easy when the binding means is not on a solid support, as the binding means may then quickly be purged and replaced using fluid handling systems. The used binding means that is removed can, of course, optionally be regenerated off-line.

[0074] In embodiments where one or more binding means is separated from the compartment within the container that blood cells can occupy, the regeneration means may include a second inlet and a second outlet on the container that provide fluid access to the portion of the container where the binding means is confined and a pumping means to circulate the rinsing solution through that portion of the container, or to pump a solution or suspension of the binding means out of the adsorption container and replace it with a fresh supply. In such embodiments, it may be possible to regenerate the binding means during operation, since the binding means can be accessed without losing the precious blood cells. It may also be possible to replace the binding means in this way, especially in embodiments wherein the binding means is not associated with a solid support and can be flushed out quickly without applying chemical agents to displace bound cells or substances from the binding means. In either case, the regeneration means also may include a reservoir of a rinsing solution, means to circulate the rinsing solution through the adsorption container, and a receptacle to collect the rinsate that exits the container during the regeneration operation. Typically, though, the bodily fluid to be treated, which is usually blood in these embodiments, would not be flowing through the adsorption container while the regeneration solution is present in the container.

[0075] One embodiment of the invention is a device, referred to as an emergency bacterium and/or toxin removal device (EBTR), which has at least a solid support disposed and confined within the adsorption container and a binding means associated with the solid support that is specific for affixing an infecting pathogen such as a bacterium that is causing a severe infection and/or for affixing a toxin from such a pathogen. It operates by selectively removing the pathogens and/or toxins from a subject's blood; thus the blood of an infected subject is temporarily taken from the subject and passed through the extracorporeal adsorption container. Preferably, the blood passed through the container directly contacts the binding means disposed within the container. By passing the infected blood through the container, at least a portion of the infecting bacterium and/or bacterial toxins is removed by becoming affixed to the binding means disposed and confined in the container. The treated blood is returned to the patient, whether it is a human or an animal.

[0076] For the purposes of the present invention, "severe peripheral bacterial infection" includes situations where the patient has a level of either a bacterium or a mycobacterium in the peripheral system that is sufficiently high so that the use of an antibiotic at that stage of infection would put the patient at a significant risk of induced bacteremia or septicemia from the killing of the infecting bacterial load and/or the peripheral levels of associated bacterial toxins, and also includes the patient having a level of bacterium that is antibiotic resistant, either from environmental exposure or genetic manipulation of the bacterium or mycobacterium. The term also refers to such infections wherein the level of toxins released from the infecting microbe has reached a stage where the patient is at risk from the effects of the toxin on the body, including hemorraghic or edemic destruction of cells. Examples of severe peripheral bacterial infections include an infecting microbe (bacterium or mycobacterium) from the *Bacillus, Meningococcus, Streptococcus, Clostridium, Staphylococcus, or Paratuberculosis* species. The detection of bacterial infections can be determined by a number of conventional diagnostic means.

[0077] Another aspect of the invention provides adsorption containers containing the binding means needed for the devices and methods described herein. Thus in some embodiments, the invention provides an adsorption container such as a column that contains suitable binding means for use in the devices and methods described

herein; for example, the binding means may be an antibody, antibody fragment, or polymerized antibody or antibody fragment that is specific for binding to a pathogen such as one of the bacteria, virus, or fungus species listed above, particularly a resistant bacterium such as MRSA, or is selective for binding a toxin produced by a resistant species such as MRSA, or both. The adsorption container may be of glass or polymer materials, or it may be metal. Frequently it is made primarily of glass for easy sterilization in embodiments where it is to be re-used, or it is made of plastic for low cost and easy incineration in embodiments where it is to be disposed of after a single use or after use for treating a single patient. The binding means may be attached to or associated with any suitable support as described herein, or it may be separated from the first compartment where blood cells circulate by a semi-permeable membrane as described herein, and disposed in a compartment of the adsorption container where the blood cells cannot contact it.

[0078] Another aspect of the present invention provides methods for treating the blood or other bodily fluid of a patient by removing at least a portion of a detrimental entity. The first step is to identify a detrimental entity to be removed from a subject's bodily fluids, and to determine which fluid(s) require treatment. A suitable binding means is then identified and prepared, such as by production of antibodies specific for the particular pathogen or toxin, and is placed in the container of the device, in association with a support that will retain the binding means within the container while it is being used to treat the contaminated bodily fluid. Alternatively, the binding means may not be associated with a solid support, if it is retained in the adsorption container by a semi-permeable membrane that prevents it from contacting the blood cells in a fluid that circulates through the container. Typically, the subject to be treated is then connected to the extracorporeal adsorption container by conventional means to permit the subject's blood or cerebrospinal fluid to be circulated through the container, much as a kidney patient would be connected to a dialysis machine. For example, the subject may be connected to the device via flexible tubing: a needle on one end of the tubing is used to pierce the subject's skin and enter a vein or artery, while the other end of the tubing connects to a pump or adsorption container of the device described above. The blood is circulated through at least one container that has been charged with a suitable binding means, thereby cleansing the blood by removing at least a portion of the infecting pathogen and/or

toxins. The blood that exits the adsorption container is then conducted by another tube to means for returning the blood to the subject being treated, such as a second needle or to a fitting on the first needle, if the first needle is adapted to return blood to the subject.

[0079] In typical embodiments, the device described herein is part of a system that extracts blood or other bodily fluid from a patient, treats the fluid to remove a pathogen or other detrimental entity, and returns the blood or other fluid to the patient. This system may include means for regeneration of the binding means as described above. It is important to maintain control of the operating temperature and pressure of the fluid as it circulates through the device and returns to the patient. Optionally, the system therefore includes a pressure measurement means and/or a pressure regulating means, each of which is known in the art. It also may include temperature regulating means, which may surround or regulate the temperature of the adsorption container to protect the treated bodily fluid and to maintain stable binding conditions. In order to regulate the temperature at which the device operates and the subject's fluid is maintained, the system may include means for warming and/or cooling the subject's blood. The system may further include access points that provide means to sample and/or monitor the fluid being treated, and for adjusting its chemistry, such as through the addition of pharmaceuticals, nutrients, gases, electrolytes, and the like. It may also include means for replacing a cartridge in a column, or for exchanging one column (e.g., an adsorption container) with another column, which may be a new or regenerated column with the same binding means as the first column, or may comprise a different binding means complementary to that in the first column. Means for monitoring the temperature, pressure, and chemistry of blood in such circulation devices are well known in the art, e.g., from kidney dialysis systems and methods, as are means for exchanging either a cartridge or an entire adsorption container / column.

[0080] Typically, a pumping means such as a peristaltic pump is used to move the treated bodily fluid through the device, and typically if the fluid is blood, it is treated with an anticoagulant such as heparin to prevent it from clotting during the process. Thus the system often includes a pumping means, such as those typically used in dialysis systems. The treated blood or fluid is returned to the patient after treatment, though it may be circulated through an adsorption container or several for an

extended period of time as needed to effect sufficient removal of a pathogen or toxin or other detrimental entity. Typically, the fluid is treated until a pathogen load has been reduced to a level such that the use of an antibiotic does not put the patient at a significant risk of induced bacteremia, fungemia, or septicemia, or until the immediate threat from a toxin has been reduced, or until a suitable reduction in the level of an endogenous substance such as a hormone or cytokine has been achieved.

[0081] To speed up the patient's recovery and reduce the risk of bacterial overload, one can treat a patient's bodily fluid with the extracorporeal container to remove bacteria and/or toxins before treating the patient with antibiotics, so that the bacterial load is reduced enough to lower the likelihood that antibiotic treatment will induce bacteremia. Alternatively, the treatment may be done concurrently with an antibiotic treatment, especially if the extracorporeal device is configured to remove a toxin produced by the pathogen and the antibiotic treatment is likely to induce release of the toxin; or it may be employed after an antibiotic or antiviral treatment has been administered as a means to further reduce the bacterial or viral load.

[0082] In some applications, it is preferred to monitor the treated fluid for either the reduction in the level of pathogen or the associated toxins after a set treatment period or during operation. Also, any antibiotic treatment of the patient may be curtailed until an infecting pathogen load has been lowered to an acceptable risk level if the antibiotic treatment is likely to unleash a load of toxin. One can thus avoid inducing a toxin overload that could result from suddenly killing pathogens while they are extremely numerous, which could release a harmful or fatal flood of toxins, i.e., to avoid a Herxheimer effect.

Brief Description of the Drawings

[0083] FIGURE 1 is a sectional view of an extracorporeal container 12 of the present invention, showing inlet means 14; outlet means 16; solid support 20; and a specific binding means 22; and means 18 for retaining the solid support inside the container.

[0084] FIGURE 2 illustrates the Anthrax Infection and associated load of toxic components.

[0085] FIGURE 3 illustrates the Antibiotic Strategy and some of its advantages and disadvantages.

[0086] FIGURE 4 illustrates an EBTR Emergency Bacteria and Toxin Removal and certain methods to provide information about the stage of an infection and to follow the progress when an EBTR is used, using antibodies to each of several detrimental entities involved in a bacterial infection such as anthrax.

[0087] FIGURE 5 illustrates an EBTR Unit in greater detail (pump means omitted for clarity), illustrating a variety of different antibodies that may serve as binding means, any of which may be included to treat various aspects of an infection by removing different detrimental entities associated with the infection.

[0088] FIGURE 6 illustrates the Typical Appearance of EBTR Units.

[0089] FIGURE 7 illustrates a Use of EBTR for a patient having secondary phase anthrax infection.

[0090] FIGURE 8 illustrates a process for Patient Selection for EBTR, which includes determination of a secondary phase of infection, and rapid quantitation of detrimental factors associated with the infection.

[0091] FIGURE 9 illustrates an EBTR Treatment.

[0092] FIGURE 10 illustrates the EBTR Treatment Monitoring, and optional treatment with an antibiotic accompanying the EBTR treatment.

[0093] FIGURE 11 illustrates the Functional Capacity of One Gram of Affinity-Purified Antibody for Lethal Toxin, and compares it to a lethal dose of anthrax toxin.

[0094] FIGURE 12 illustrates the Functional Capacity of One Gram of Affinity-Purified Antibody for Bacillus Anthracis and a possible EBTR composition for removing Lethal Factor, Edema Factor, Protective Antigen, and the anthrax bacterium itself.

[0095] FIGURE 13 illustrates an EBTR Antibody Leaching Control for production of an EBTR.

[0096] FIGURE 14 illustrates the EBTR Major Advantages over Antibiotics for an EBTR.

[0097] FIGURE 15 illustrates the Application of EBTR to Septicemia as an example of its value, especially for treating multi-drug resistant infections.

EXAMPLES

[0098] The following examples describe particular embodiments of the invention, which illustrate the principles of construction and operation of the methods, devices and systems described herein. The invention is illustrated by examples describing a device and method for removing bacteria from blood; the same operating principles apply to removal of other detrimental entities from blood, and to the treatment of other bodily fluids. Those skilled in the art will recognize that many variations and combinations of the features exemplified and those described elsewhere herein are also feasible, and such variations and combinations are included within the scope of the invention.

Removal of MRSA from a Patient's Blood

[0099] Immunoglobulin G (IgG) is harvested from rabbits by methods known in the art. About 5 mg of IgG is applied to a sheet of nitrocellulose or polyvinylidene fluoride, which is then dried. The treated sheet is blocked with a solution of BSA in buffer, and is rinsed and again dried. It is then rolled up tightly and placed in a commercially available glass column with end fittings for circulating fluid through the column. The column is sized so that the rolled up sheet fills the column volume enough to ensure that the fluid pumped through the column will contact the IgG-treated surfaces substantially. The entire apparatus is sterilized by suitable means not detrimental to the bound IgG.

[0100] A patient afflicted with methicillin-resistant *Staphylococcus aureus* (MRSA) is treated with a suitable dose of heparin, taking into account the subject's size and health, and is connected to the device by a standard blood collection device, using a needle in a vein in the subject's arm to extract blood that is fed into the inlet of the column. A peristaltic pump is used to pump the blood at a suitable rate of about 30-300 mL / minute into the device; a slow rate is used initially, then the rate is

increased while maintaining a pressure that is suitable for the column and ensures the patient's comfort and safety. The outlet of the column is connected to a separate tube that directs the treated blood back to the treated subject: it is returned to the subject by a second needle which may be located in a vein in the subject's other arm. The treating physician then monitors the patient during the treatment, which continues until approximately two times the total volume of the patient's blood has been passed through the column. Thus at a flow rate of 100 mL/min, the treatment would require about two hours for a patient having about 6 liters of total blood volume. The patient is then disconnected from the unit, and is promptly treated with a combination of antibiotics selected for their potency against MRSA, which kills the remaining MRSA without unleashing a lethal dose of bacterial toxins due to the reduced level of MRSA in the patient's blood after treatment with the device and method of the invention.

Description of An EBTR Unit

[0101] An embodiment of the extracorporeal adsorption container (10) used in the present invention is shown in the FIGURE. A disposable glass or polypropylene column (12) has a conventional inlet fitting (14) at the proximal end and a conventional outlet fitting (16) at the distal end. Medical grade silicon tubing can be connected to each end. The inlet end can have affixed to it a shutoff valve and a first 14 gauge hypodermic needle. The end of the outlet silicone tubing can have connected to it a blood administration set and a shutoff valve and a second 14 gauge needle.

[0102] Inside of the column is a bacterium and/or toxin binding means and the associated solid support. At the inlet and outlet ends are 80 micron nylon nets (18) for retaining the solid support within the container while allowing blood cells to pass through safely. The solid support comprises agarose particles (20), such as CN-Br activated Sepharose 6B available from Amersham Biosciences (Piscataway, New Jersey). Antibacterial antibodies and anti-bacterial toxin antibodies (22) are affixed to the agarose support by conventional means according to instructions from the manufacturer using sterile solution and glassware that has been previously sterilized. For example, in the case of an EBTR unit for severe anthrax infection, once can use affinity-purified goat anti *bacillus anthracis* antibodies and goat anti *bacillus*

anthracis toxin antibodies available from Scantibodies Laboratory, Inc. (Santee, California).

Production of *Bacillus Anthracis* Antibodies

[0103] To create affinity purified anti *bacillus anthracis* polyclonal antibodies, one first uses inactivated *bacillus anthracis* available from the Centers for Disease Control (Atlanta, Georgia) or other qualified institutions as the immunogen for injection into the animal (typically a goat). The inactivated organism is suspended in a solution of 0.85 M sodium chloride to become the aqueous immunogen for injection. The aqueous immunogen for injection is mixed with an equal volume of Freund's complete adjuvant (a mixture of light mineral oil and mannide monooleate and inactivated *mycobacterium tuberculosis bacilli*). The resulting mixture is homogenized to produce an aqueous/oil emulsion for injection into the animal for the primary immunization. The immunogen dose is approximately 100-500 micrograms of *bacillus anthracis*. The goats are injected monthly with the same dose of immunogen complex except no *mycobacterium tuberculosis bacilli* is used in these subsequent injections. The goats are bled monthly under sterile conditions, starting approximately three months after the primary immunization. The serum (or antiserum) is derived from each bleeding by separating under sterile conditions the red blood cells from the blood by centrifugation and removing the antiserum, rich in antibodies against the *bacillus anthracis*.

[0104] To purify the antiserum for the desired antibody against *bacillus anthracis*, one packs a chromatography separation column with heat inactivated *bacillus anthracis* bound to cross linked agarose beads (such as CN-Br activated Sepharose 4B from Amersham Bioscience, Piscataway, New Jersey) according to the instructions from the manufacturer using the sterile solutions and glassware that has been previously sterilized. The column (which also has been previously sterilized) is packed with the *bacillus anthracis* bound to agarose and the column is washed and equilibrated with sterile 0.01 M phosphate buffered saline (PBS). The antiserum is 0.22 micron filtered and loaded onto the column and washed with sterile 0.01 M PBS in order to remove other serum components and the antibodies that are not directed against *bacillus anthracis*. The bound specific goat anti *bacillus anthracis* polyclonal antibody is eluted from the immobilized *bacillus anthracis* in the column by passing

an elution solution of sterile 0.1 M glycine hydrochloride buffer, pH 2.3-2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of sterile 1 M phosphate buffer, pH 7.5 or by buffer exchange with sterile 0.01 M PBS under sterile conditions, as is know to those of skill in the art. This affinity-purified goat anti *bacillus anthracis* polyclonal antibody is further 0.22 micron filtered and stored at 2-8 degrees centigrade.

[0105] One can repeat the above procedure so as to make affinity-purified goat antibodies against the associated *bacillus anthracis* toxins, including any or all of the *bacillus anthracis* protective antigen, edema factor, lethal factor, edema toxin and lethal toxin.

[0106] The affinity purified goat anti *bacillus anthracis* antibodies are bound to cross linked agarose beads (CN-Br activated Sepharose 6B which is available from Amersham Bioscience, Piscataway, New Jersey) according to instructions from the manufacturer using sterile solutions and glassware that has been previously sterilized.

Production of an EBTR Unit

[0107] One can produce an EBTR unit suitable for use to treat a human patient or domesticated animal in the following manner. A 200 ml glass chromatography column with inlet and outlet connectors and 80 micron nets at both inlet and outlet ports is sterilized. Sterile medical grade silicone tubing is attached to both the inlet and outlet of the column. A sterile shutoff valve is attached to the inlet tubing and a blood administration set with shutoff valve is attached to the outlet tubing. Needles (14 gauge) are attached to the ends of the inlet and outlet tubing. One gram of the affinity-purified goat anti *bacillus anthracis* bound to 200 ml of Sepharose 6B agarose beads is packed into the column. A peristaltic pump is attached onto the inlet tubing and the column is washed with sterile saline. With the sterile saline in place in the inlet and outlet tubing and the column, the shutoff valves are closed and the sterile unit is sealed under sterile conditions. An EBTR unit to remove products of the *bacillus anthracis* (i.e. toxins) is made by filling the column with goat antibodies to *bacillus anthracis* toxins (PA, EF, of LF) that are bound to agarose beads (produced in a manner analogous to the goat anti *bacillus anthracis* antibodies described above). Typically, a 200 ml EBTR unit is capable of removing about one milligram of an infecting bacterium.

Patient Selection for the Use of an EBTR Unit

[0108] Often a patient having a peripheral bacterial infection is not clinically diagnosed until the infection progresses into a severe peripheral bacterial infection. While it is possible to use an EBTR unit soon after a bacterial infection occurs, practically, in most cases the infection will not be identified until it is severe. For example, a *bacillus anthracis* infected patient typically will have passed into the secondary phase of infection by the time of diagnosis, and as such, is a candidate for the present invention. Candidacy can also be determined by employing a rapid quantitative assay of the blood level for a particular infecting agent, such as *bacillus anthracis* and/or quantitative rapid tests for the toxic byproducts of the infecting agent, for *bacillus anthracis*, namely protective antigen, edema factor, lethal factor, edema toxin and lethal toxin, as illustrated in the Drawings. These rapid assays have the advantage of providing objective quantitation to the process of selecting patients for treatment. The selection process using the quantitative rapid test for *bacillus anthracis* can be based on a fairly low level of infecting bacteria. The administration of bacteriocidal antibiotics can bring about the accelerated release of life threatening toxins, i.e., the patient can die from toxin loads, even if the bacteria has been substantially reduced or effectively eliminated. The selection process using the quantitative rapid test for the products of *bacillus anthracis* is based on the critical life threatening threshold level of toxins already in the patient's blood. As in the case of establishing LD50 (lethal dose at which 50% of a population would die) one can determine separate threshold cutoff's for differing infections.

Treating a Patient with an EBTR Unit

[0109] Before using the EBTR unit such as described above, a patient is injected with about 100 units per kilogram of patient body weight of sodium heparin, available from Wyeth-Ayerst (Philadelphia, Pennsylvania). To use the EBTR unit, the container is removed from its sterile sealed packaging and the inlet silicone tubing is connected to a blood peristaltic pump capable of delivering 100-300 ml per minute of blood, available from Baxter Healthcare (Deerfield, Illinois). The patient is placed in a supine position and both of the points of entry for the arm brachial veins are wiped with appropriate sterilant. The bottom inlet needle is inserted into one of the brachial veins of the patient and the pump speed is increased to 50 ml/min to allow blood to

fill from the bottom of the EBTR unit. The EBTR unit is rotated to assure that no air is trapped in the unit. When blood has filled the EBTR unit and the blood administration set and with no air in the outlet line, the 14 gauge outlet line needle is inserted into the patient's other brachial vein. The pump speed is increased to 300 ml/min. During the EBTR treatment the patient's levels of *bacillus anthracis* and the levels of the products of *bacillus anthracis* are quantitatively assessed by quantitative rapid tests.

[0110] During the EBTR treatment, rapid quantitative tests can be used to assay blood levels of *bacillus anthracis* and levels of *bacillus anthracis* toxins. Typically, one would not treat the patient with antibiotics while using the EBTR unit, so as to avoid the release of further toxins into the peripheral system; however, in some embodiments, the treatment with the unit is concurrent with administration of an antibiotic so that the unit can remove one or more of the bacterial toxins that are released as the bacterium is killed. The use of EBTR treatment can be halted when the infection and toxin levels have been reduced to a point where the use of antibiotics will not set the patient at risk from subsequent release of *bacillus anthracis* toxins. One should note that one does not have to remove substantially all infecting bacterium or bacterial toxins from the patient, though this is preferable. Alternatively, one can discontinue EBTR treatment when the levels are low enough that typically the patient's immune system is able to overcome immunologically residual *bacillus anthracis* levels and clear the associated toxins naturally. Of course, such a decision should be made by the attending physician and is specific for each patient, depending upon numerous known factors. Due to the potential infectious nature of anthrax, the used EBTR unit is incinerated.

Admixing the Treated Fluid with a Soluble Binding Means

[0111] A patient having severe bacterial spinal meningitis is identified, and the infecting bacterium is identified. Antibodies are raised against the lipopolysaccharide coat of the bacterium by conventional methods, and are linked to streptavidin using known methods employing a dicarboxylic acid linker. This provides a soluble binding means, that is a conjugate comprising an antibody specific for the infecting pathogen linked to streptavidin, which serves as the capture means. An adsorption column is prepared by filling a sterile column with polystyrene beads that have been

functionalized by known methods to display an amine functional group, which was then linked to biotin using standard amide formation chemistry. The column is sized according to the size of the patient to be treated.

[0112] The patient is anesthetized and a spinal tap needle is inserted into the patient's lower spine to draw out infected cerebrospinal fluid. The needle is connected by thin tubing to a low-volume pump, which is connected to the adsorption column described above. The outlet of the adsorption column is connected to a second thin tube that will return the treated cerebrospinal fluid to the subject at a point in the spine near the base of the subject' skull.

[0113] Once the flow of spinal fluid has begun, a solution of the antibody-streptavidin conjugate described above is injected by a syringe pump into the spinal fluid just before the fluid enters the peristaltic pump, so that the antibody-streptavidin conjugate mixes efficiently with the fluid before it enters the adsorption container, which permits the conjugate to bind to the infecting pathogen before the fluid reaches the adsorption container (the column). Inside the column, the biotin binding means affixes the infecting pathogen to the beads by its interaction with the streptavidin portion of the antibody-streptavidin conjugate. The subject is treated for a time determined by the treating physician to be sufficient to decrease the bacterial load enough to assist the patient's recovery using conventional antibiotic treatments. Optionally, the antibody-streptavidin conjugate may also be used in an analytical test to determine when the bacterial load has been substantially reduced, using a conventional sandwich-type assay and a suitable visualization agent.

[0114] The ordinarily skilled artisan can appreciate that the present invention can incorporate any number and combination of the preferred features described above.

[0115] All publications or unpublished patent applications mentioned herein are hereby incorporated by reference thereto.

[0116] Other embodiments of the present invention are not presented here which are obvious to those of ordinary skill in the art, now or during the term of any patent issuing from this patent specification, and thus, are within the spirit and scope of the present invention.

I Claim:

1. A device for removing a detrimental entity from a bodily fluid, wherein the entity to be removed is not a virus of the *Retroviridae* family, said device comprising:

an adsorption container having inlet means and outlet means;

pump means for circulating blood or plasma through the adsorption container; and

a first binding means which has a specific affinity for the entity to be removed,

wherein the first binding means is confined within the adsorption container, so that a bodily fluid circulated through the container directly contacts the first binding means,

whereby circulating a bodily fluid through the container removes at least a portion of the detrimental entity from the treated fluid.

2. The device of claim 1, wherein the detrimental entity is a xenobiotic substance or a pathogen.

3. The device of claim 1, wherein the detrimental entity is an endogenous substance, a prion, or an aberrant cell type.

4. The device of claim 2, wherein the pathogen is a virus, parasite or fungus.

5. The device of claim 2, wherein the pathogen is a multi-drug resistant bacterium.

6. The device of any of claims 1-5, wherein the first binding means comprises at least one immunoadsorbent.

7. The device of claim 6, further comprising a second binding means.

8. The device of claim 7, wherein the second binding means comprises at least one antibody, antibody fragment, or a polymerized antibody or antibody fragment, or an immunoglobulin.

9. The device of claim 2, wherein the pathogen is a virus selected from the following families: *Bunyaviridae, Filoviridae, Hepadnaviridae, Herpesviridae, Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Poxviridae, Reoviridae, Rhabdoviridae,* and *Togaviridae.*

10. The device of any of claims 7-9, wherein the bodily fluid to be treated comprises blood or plasma.

11. The device of any of claims 7-9, wherein the bodily fluid to be treated comprises cerebrospinal fluid or amniotic fluid.

12. A device for removing a pathogenic bacterium or fungus from blood, said device comprising:

an adsorption container having inlet means and outlet means;

pump means for circulating blood through the adsorption container; and

a first binding means,

wherein the first binding means is confined within the adsorption container where the detrimental entity in blood circulated through the container can contact the first binding means while blood cells circulated through the container cannot contact the first binding means,

and the first binding means has a specific affinity for the detrimental entity to be removed,

whereby circulating blood through the container removes at least a portion of the detrimental entity from the treated blood.

13. The device of claim 12, wherein the first binding means is partitioned by a semi-permeable membrane from the blood cells circulated through the container.

14. The device of claim 13, wherein the semi-permeable membrane has an effective pore size between about 0.01 microns and 10 microns.

15. The device of claim 13, wherein the semi-permeable membrane has an effective pore size less than 9 microns.

16. The device of any of claims 12-15, wherein the first binding means comprises at least one immunoadsorbent.

17. The device of claim 16, wherein the pathogenic bacterium to be removed is selected from the group consisting of *Bacillus*, *Meningococcus*, *Streptococcus*, *Clostridium*, *Staphylococcus* including MRSA (methicillin-resistant *S. aureus*), and *Paratuberculosis*.

18. The device of claim 17, wherein the detrimental entity to be removed comprises methicillin-resistant *Staphylococcus aureus*.

19. The device of any of claims 17-18, further comprising a second binding means, wherein the second binding means is specific for at least one detrimental entity.

20. The device of claim 19, further comprising means for at least partially regenerating the adsorbent capacity of at least one binding means.

21. A method for treating a bodily fluid to remove a detrimental entity, the method comprising:

circulating the bodily fluid through an adsorption container, wherein:

the adsorption container has an inlet means and an outlet means;

a first binding means is confined within the adsorption container so that a bodily fluid circulated through the container directly contacts the first binding means;

and the first binding means is specific for the detrimental entity to be removed;

whereby circulating the bodily fluid through the adsorption container removes at least a portion of the detrimental entity.

22. The method of claim 21, wherein the detrimental entity is a pathogen,

wherein the method further comprises circulating the bodily fluid through an adsorption container comprising a second binding means,

wherein said second binding means is specific for removal of at least one toxin produced by the pathogen.

23. The method of claim 22, wherein the second binding means is separated by a semi-permeable membrane having a pore size between about 0.01 and 10 microns from blood cells present in any bodily fluid that is circulated through the container.

24. The method of any of claims 21-23, wherein the first binding means is specific for *Bacillus*, *Meningococcus*, *Streptococcus*, *Clostridium*, *Staphylococcus* including MRSA (methicillin-resistant *S. aureus*), or *Paratuberculosis*.

25. The method of claim 24, wherein the first binding means is specific for a multi-drug resistant bacterium.

26. The method of claim 25, wherein the detrimental entity is a multi-drug resistant bacterium.

27. The method of any of claims 25-26, wherein the detrimental entity is methicillin-resistant *Staphylococcus aureus*.

28. The method of claim 27, wherein the first binding means comprises an immunoabsorbent.

29. The method of claim 28, wherein the first binding means comprises an antibody or an immunoglobulin.

30. The method of any of claims 28-29, wherein the bodily fluid comprises blood or plasma.

31. The method of claim 30, wherein the bodily fluid comprises cerebrospinal fluid or amniotic fluid.

32. The method of claim 21 or claim 31, wherein the first binding means is specific for a prion, virus, parasite or a fungal pathogen.

33. The method of claim 21 or claim 31, wherein the detrimental entity comprises an endogenous substance or a xenobiotic substance.

34. The method of claim 33, wherein the first binding means is not associated with a semi-permeable membrane.

35. A method of removing a detrimental entity from a bodily fluid, said method comprising:

admixing the bodily fluid with a soluble binding means,

wherein the soluble binding means comprises a capture means and wherein the soluble binding means is specific for binding to the detrimental entity to be removed,

allowing the soluble binding means to associate with the detrimental entity to be removed, and

circulating the bodily fluid through an adsorption container,

wherein a first binding means is confined within the adsorption container, which first binding means is specific for binding to the capture means of the soluble binding means,

whereby circulating the bodily fluid admixed with the soluble binding means through the adsorption container removes at least a portion of the detrimental entity.

36. The method of claim 35, wherein the detrimental entity is an aberrant cell, pathogen, or parasite.

37. The method of claim 35, wherein the detrimental entity comprises a prion, a xenobiotic substance or an endogenous substance.

38. The method of any of claims 35-37, wherein the soluble binding means is admixed with the bodily fluid to be treated prior to removal of the bodily fluid from the patient.

39. The method of claim 38, wherein the soluble binding means is admixed with the bodily fluid to be treated after removal of the bodily fluid from the patient.

40. The method of claim 39, further comprising circulating the bodily fluid through an adsorption container in which a second binding means is confined,

wherein the second binding means is specific for a toxin produced by the detrimental entity,

whereby at least a portion of the toxin produced by the detrimental entity is removed.

41. The method of claim 40, wherein the bodily fluid is blood or plasma.

42. The method of claim 40, wherein the bodily fluid is cerebrospinal fluid or amniotic fluid.

43. The method of any of claims 39-42, wherein the soluble binding means comprises an immunoadsorbent.

44. The method of claim 43, wherein the capture means or the first binding means comprises biotin.

45. The method of claim 44, wherein the capture means or the first binding means comprises streptavidin or avidin.

FIGURE 1

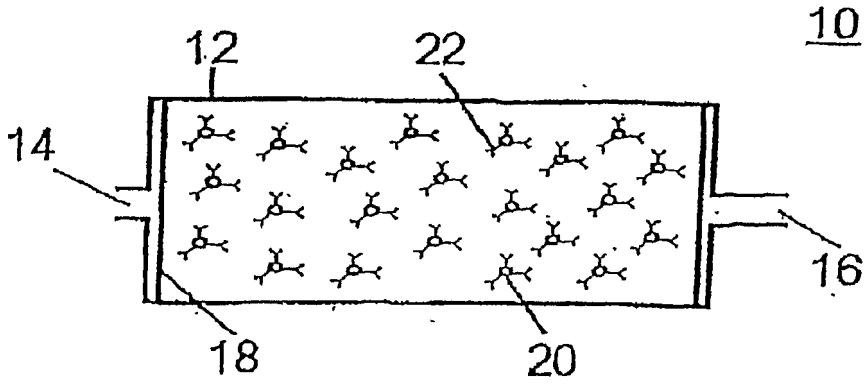


FIGURE 2

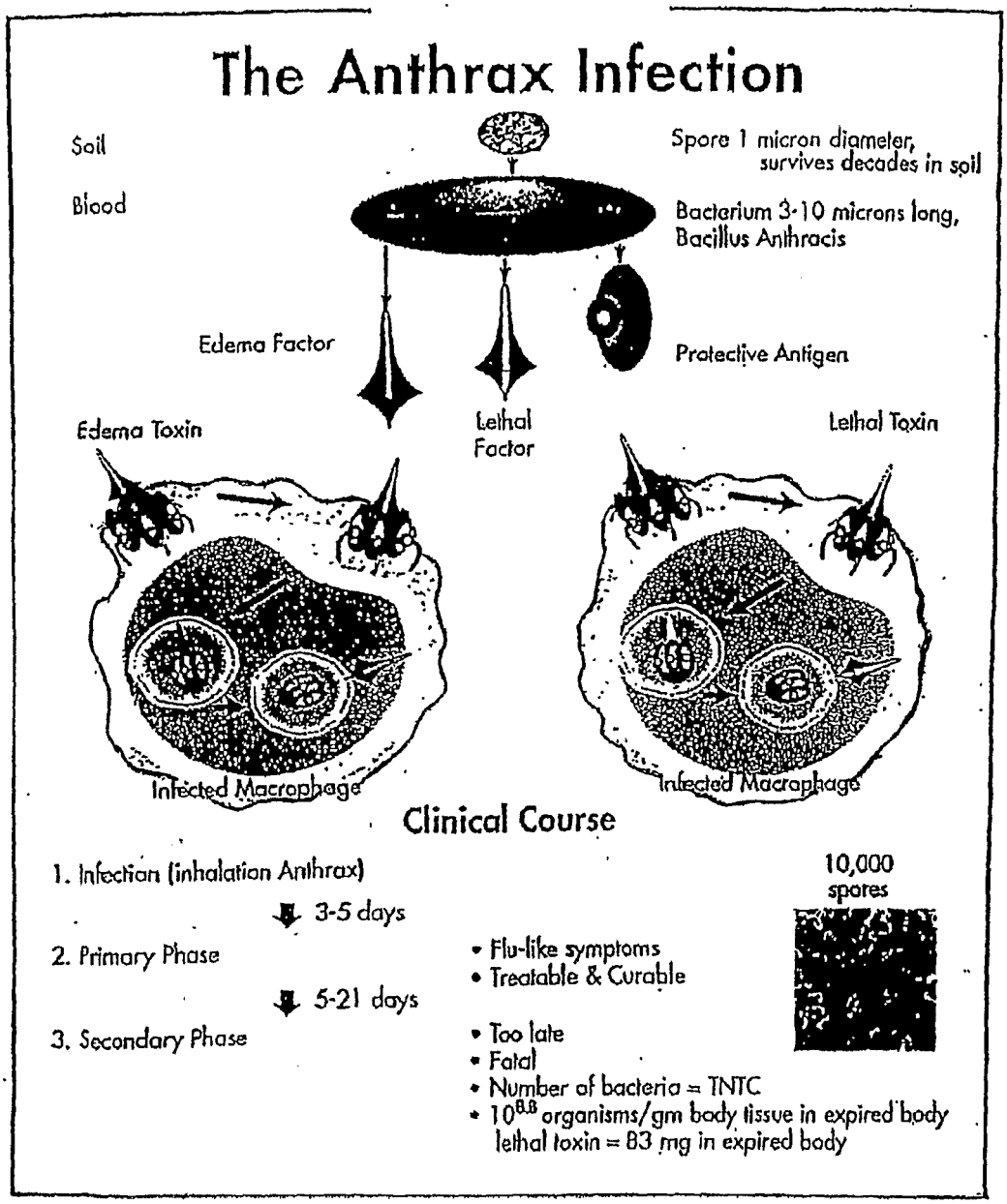
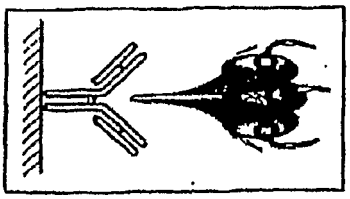

The Anthrax Infection
Soil
Blood
Spore 1 micron diameter, survives decades in soil
Bacterium 3-10 microns long, Bacillus Anthracis
Edema Factor
Protective Antigen
Lethal Factor
Edema Toxin
Lethal Toxin
Infected Macrophage
Infected Macrophage
Clinical Course
1. Infection (inhalation Anthrax)
3-5 days
2. Primary Phase
• Flu-like symptoms
• Treatable & Curable
5-21 days
3. Secondary Phase
• Too late
• Fatal
• Number of bacteria = TNTC
• $10^{8.8}$ organisms/gm body tissue in expired body
lethal toxin = 83 mg in expired body
10,000 spores

FIGURE 3

The Antibiotic Strategy

Advantages

1. Anthrax is easy to kill. (Cipro, Doxycycline, etc.)
2. Curable if antibiotics used in primary phase.



Disadvantages

Minor

1. Some side effects.
2. Incomplete course of treatment may result in selection of a resistant strain.

Major

1. Ineffective against Toxins*, the actual cause of death. "Patients die with sterile blood."
2. Ineffective against genetically modified strains*.
3. Dangerous to use in secondary phase because they accelerate death by causing rapid toxin release.
4. Ineffective against resistant strains*.

FIGURE 4

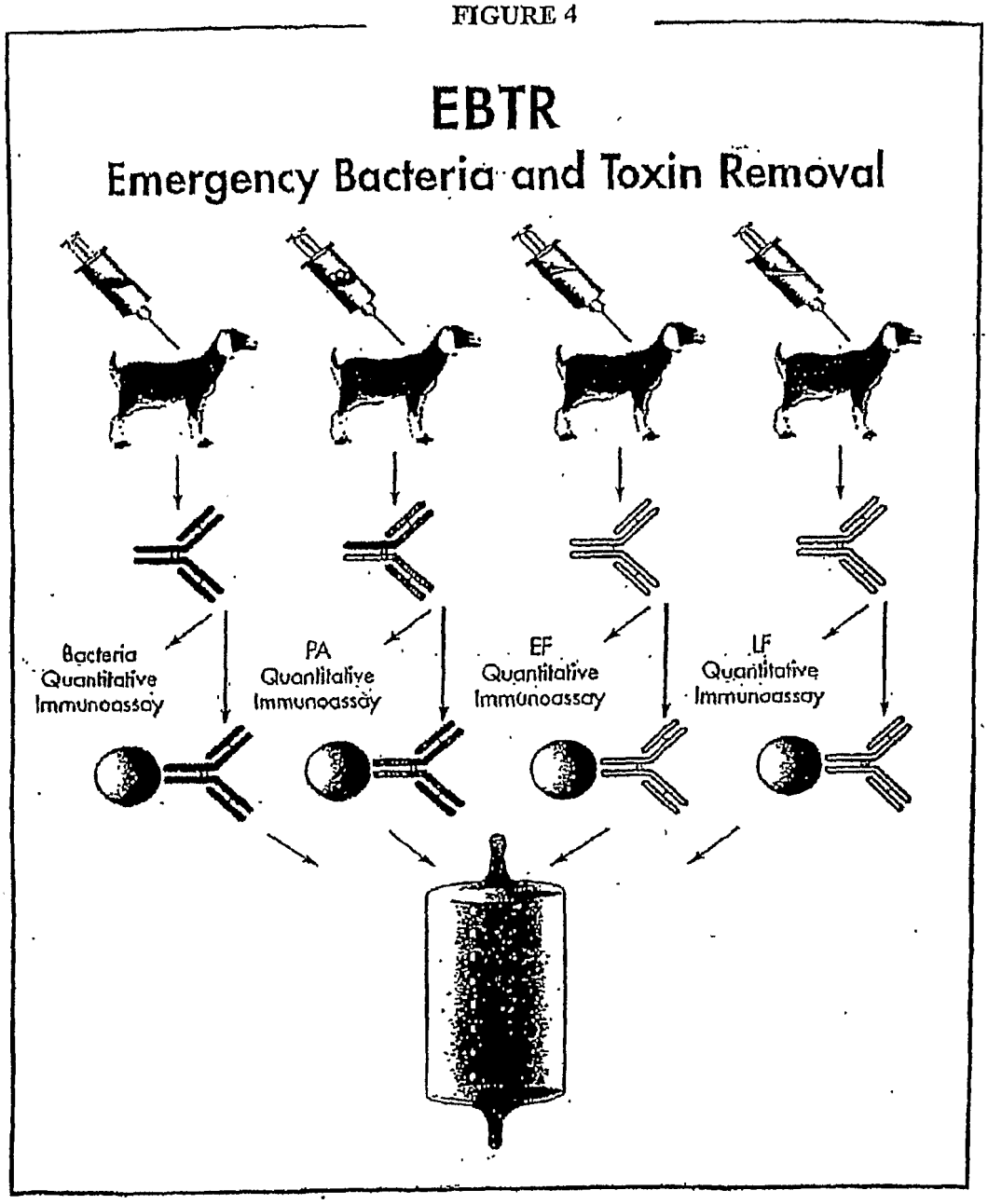

EBTR
Emergency Bacteria and Toxin Removal
Bacteria Quantitative Immunoassay
PA Quantitative Immunoassay
EF Quantitative Immunoassay
LF Quantitative Immunoassay

FIGURE 5

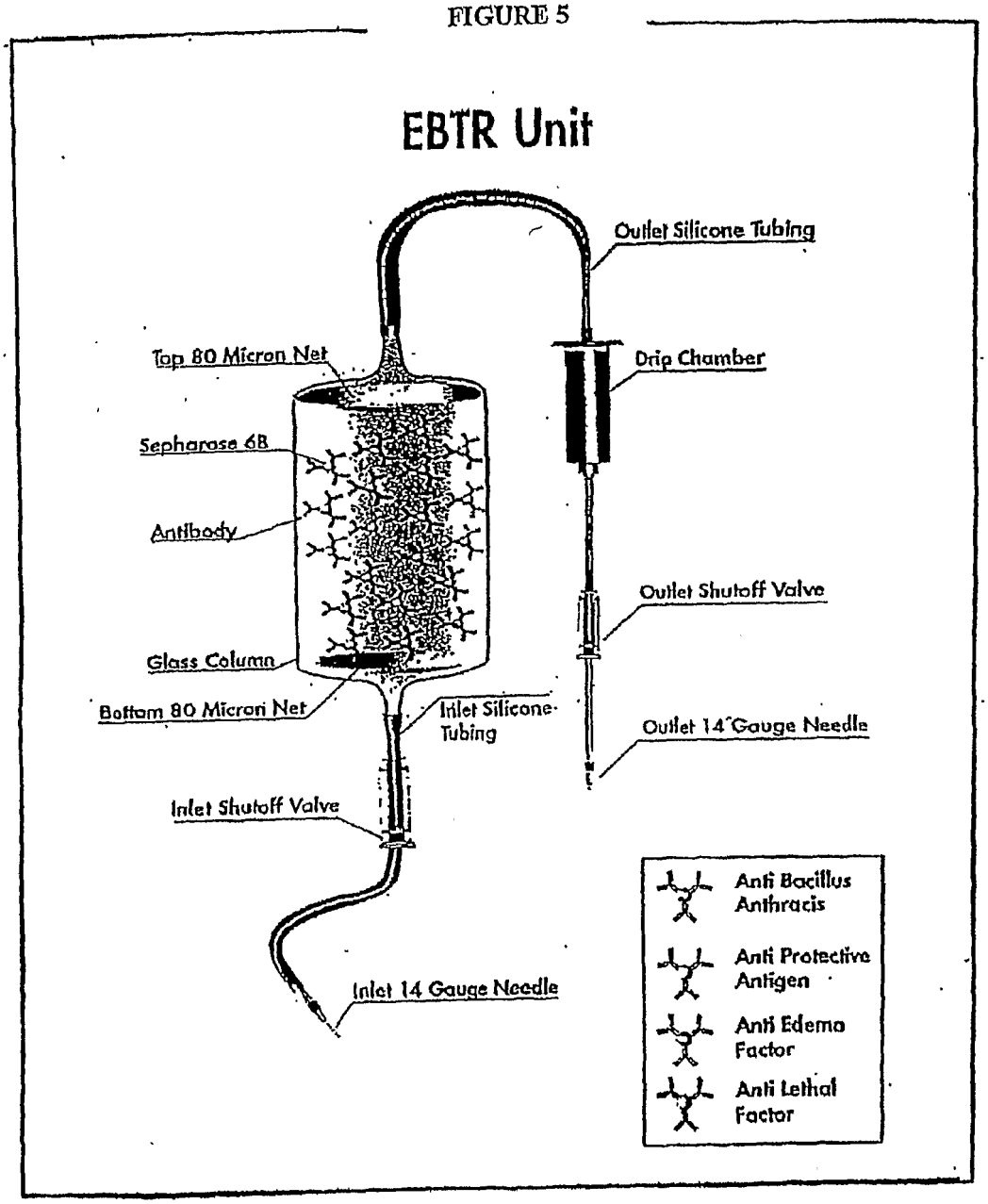
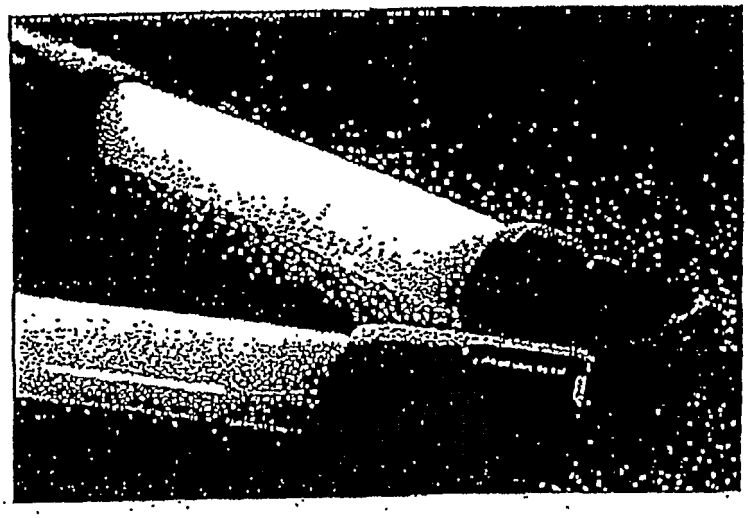

EBTR Unit
Outlet Silicone Tubing
Top 80 Micron Net
Drip Chamber
Sepharose 6B
Antibody
Outlet Shutoff Valve
Glass Column
Bottom 80 Micron Net
Inlet Silicone Tubing
Outlet 14 Gauge Needle
Inlet Shutoff Valve
Inlet 14 Gauge Needle
Anti Bacillus Anthracis
Anti Protective Antigen
Anti Edema Factor
Anti Lethal Factor

FIGURE 6

Typical Appearance of EBTR Units



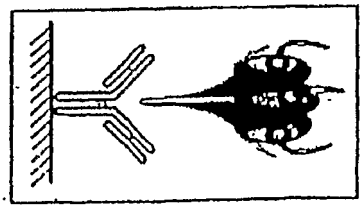


FIGURE 7

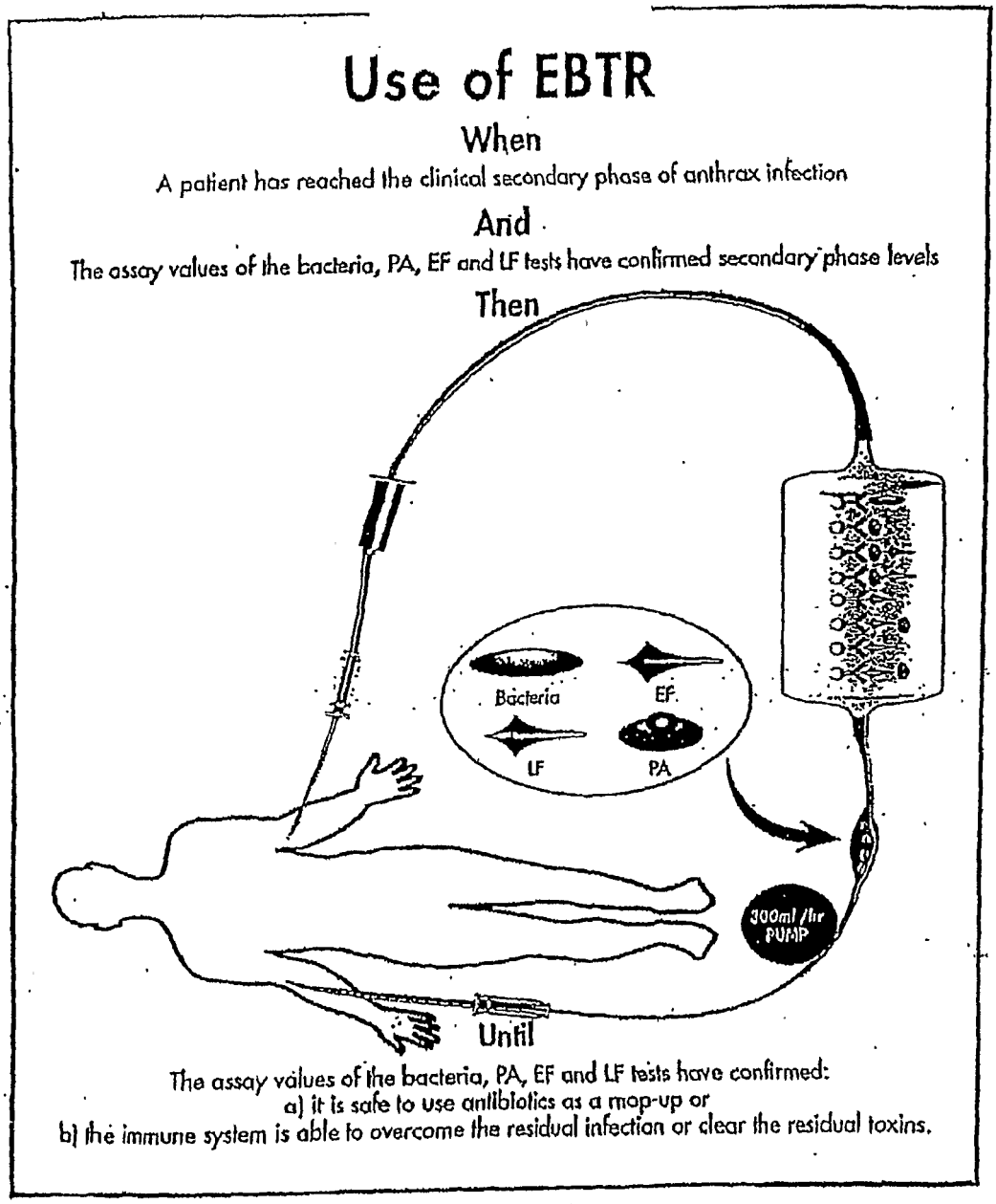

Use of EBTR
When
A patient has reached the clinical secondary phase of anthrax infection
And
The assay values of the bacteria, PA, EF and LF tests have confirmed secondary phase levels
Then
Bacteria
EF
LF
PA
300ml /hr
PUMP
Until
The assay values of the bacteria, PA, EF and LF tests have confirmed:
a) it is safe to use antibiotics as a mop-up or
b) the immune system is able to overcome the residual infection or clear the residual toxins.

FIGURE 8

Patient Selection for EBTR

Patient selection would be based on:

a) Clinicol symptoms indicating secondary phase of infection alternatively or in conjunction with

b) Rapid quantitative blood assays (objective evaluation) for bacillus and/or toxins reaching thresholds (i.e. approaching LD50 levels) based on the level of bacteria at which the use of antibiotics puts the patient at risk through release of a flood of toxins

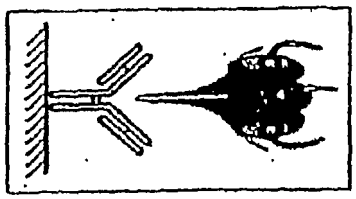


FIGURE 9

EBTR Treatment

1. Patient is injected I.V. with 100 units heparin/kg body weight.
2. EBTR unit is removed from its sterile, sealed package.
3. Inlet silicone tubing (bottom of column) is placed in a 100-300 ml peristaltic blood pump (battery or AC powered unit for field deployable use).
4. 14 gauge needle is inserted into the brachial vein and shutoff valves are opened.
5. Pump speed is increased to 100 ml/minute.
6. EBTR unit is filled with blood and rotated to expel any trapped air.
7. When blood has filled the unit, an outlet line 14 gauge outlet needle is inserted into the patient's other brachial vein.
8. Pump speed is increased to 300 ml/minute.

FIGURE 10

EBTR Treatment Monitoring

During the EBTR treatment, blood levels of the bacillus and its toxins are assayed to determine the point when

either

(a) antibiotics may be used safely as a mopping up procedure without risk of toxin overload,

or

(b) the immune system may be able to clear residual bacteria and toxins.

Disposition of EBTR Unit

After use, the EBTR unit is incinerated

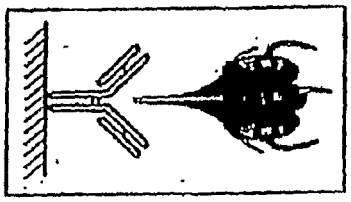


FIGURE 11

Functional Capacity of One Gram of Affinity-Purified Antibody for Lethal Toxin

1. Activity of Lethal Toxin

   235 units per mg protein

   Vick, et.al. 1968. "Neurological and Physiological Responses of the Primate to Anthrax Toxin." *J Infect Diseases 118:85-96.*

2. Lethal Dose

   1.85 units per gm body weight for primates

   Vick, et.al. 1968. "Neurological and Physiological Responses of the Primate to Anthrax Toxin." *J Infect Diseases 118:85-96.*

   150 lb. human = 68,182 gms x 1.85 = 126,263 units / 235 units / mg = 537 mgs

3. Molecular weight of Lethal Toxin = 93,000 (LF) + 90,000 (PA) = 183,000

   therefore, 537 mgs = 1.77×10^{18} molecules

4. 1 gm of anti-Lethal Factor antibody (m.w. = 155,000) = 3.89×10^{18} antibody molecules

5. Therefore, there are 2.2 times excess antibodies

FIGURE 12

Functional Capacity of One Gram of Affinity-Purified Antibody for Bacillus Anthracis

1. Expired guinea pig had 10^{88} organisms per gm of whole body tissue (Figure 10^{9})
2. 150 lb. human weighs 68,181 gms - when expired, corresponds to 6.82×10^{13} organisms
3. 1 gm of anti Bacillus Anthracis antibody (m.w. = 155,000) = 3.89×10^{18} antibody molecules
4. Therefore, there are 57,000 antibody molecules for each Bacillus Anthracis organism

Preliminary Composition of EBTR

1. 0.4 gm anti Lethal Factor Antibody
2. 0.2 gm anti Edema Factor Antibody
3. 0.2 gm anti Protective Antigen Antibody
4. 0.2 gm anti Bacillus Anthracis Antibody

FIGURE 13

EBTR

Antibody Leaching Control

1. Following production, each EBTR unit is thoroughly washed with alternating high and low pH solutions and finally rinsed and stored in isotonic saline solution.

2. The final isotonic saline solution rinse is assayed for goat IgG with an immunoassay sensitive to 10 picograms/mL.

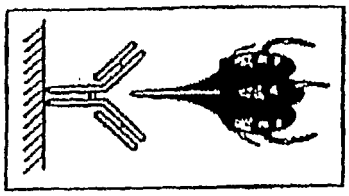


FIGURE 14

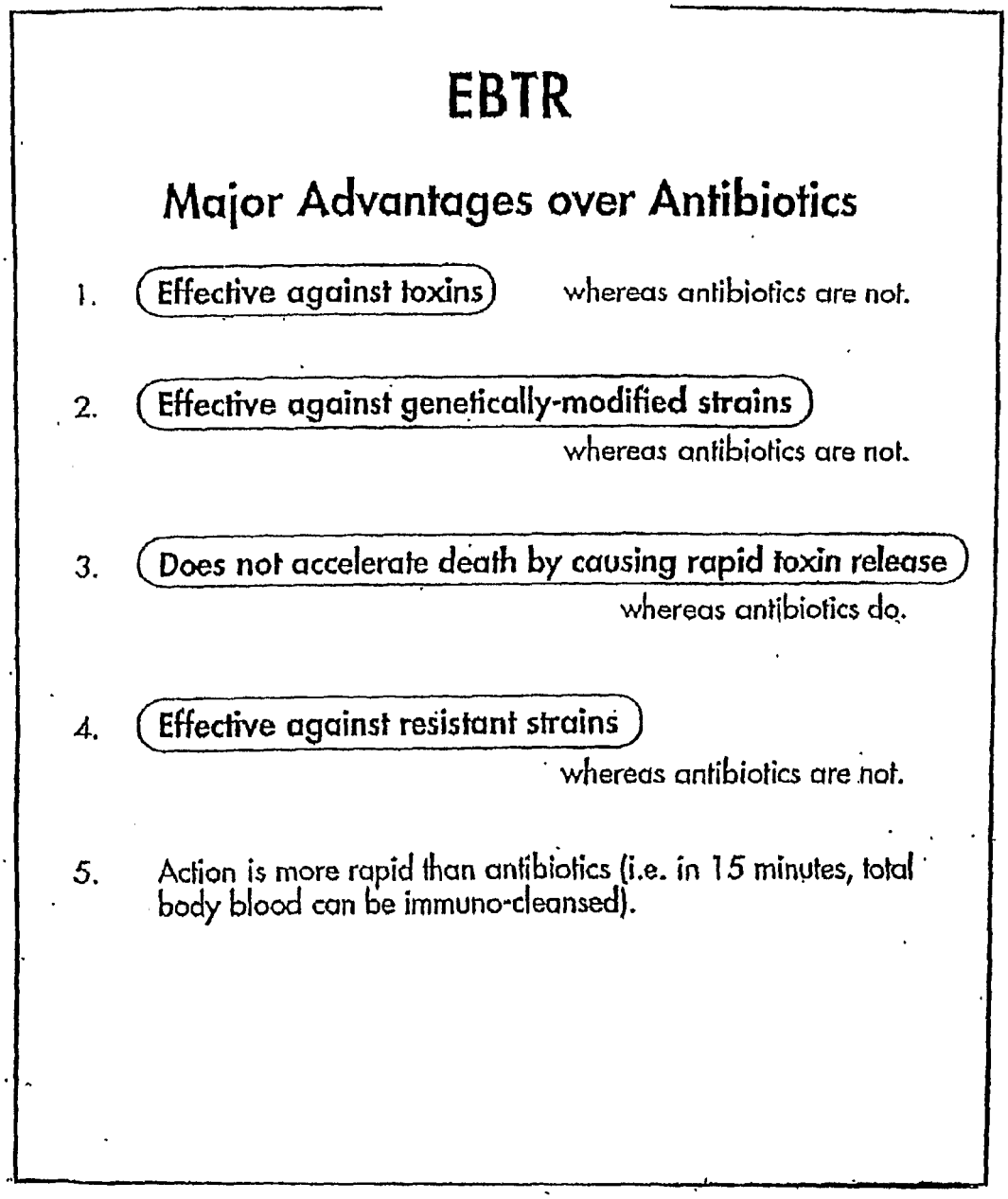
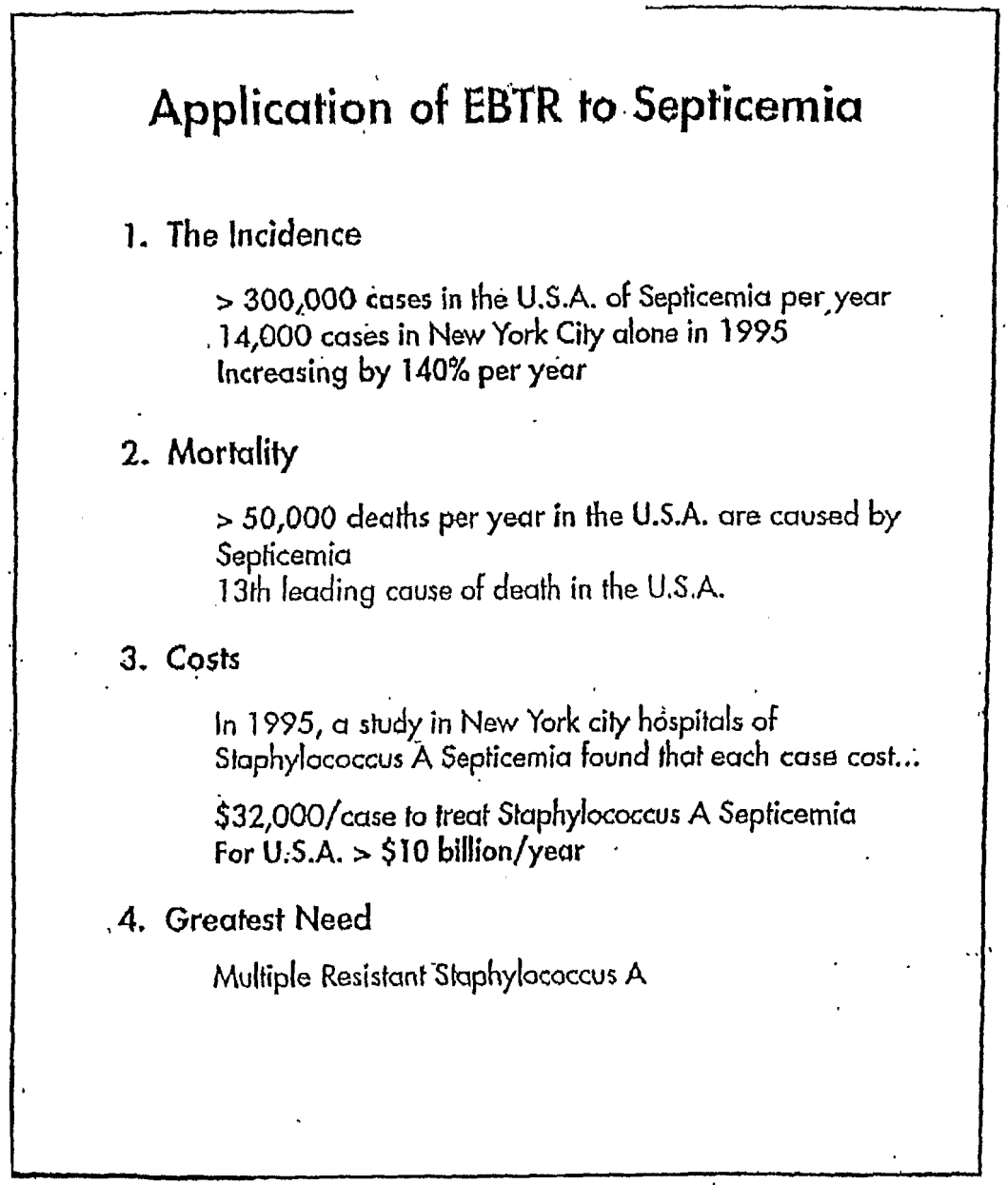

EBTR
Major Advantages over Antibiotics
1. Effective against toxins whereas antibiotics are not.
2. Effective against genetically-modified strains whereas antibiotics are not.
3. Does not accelerate death by causing rapid toxin release whereas antibiotics do.
4. Effective against resistant strains whereas antibiotics are not.
5. Action is more rapid than antibiotics (i.e. in 15 minutes, total body blood can be immuno-cleansed).

FIGURE 15


Application of EBTR to Septicemia
1. The Incidence
> 300,000 cases in the U.S.A. of Septicemia per year
14,000 cases in New York City alone in 1995
Increasing by 140% per year
2. Mortality
> 50,000 deaths per year in the U.S.A. are caused by Septicemia
13th leading cause of death in the U.S.A.
3. Costs
In 1995, a study in New York city hospitals of Staphylococcus A Septicemia found that each case cost...
$32,000/case to treat Staphylococcus A Septicemia
For U.S.A. > $10 billion/year
4. Greatest Need
Multiple Resistant Staphylococcus A

专利名称(译)	用于从体液中去除病原体和有害物质的方法和装置		
公开(公告)号	EP1909833A2	公开(公告)日	2008-04-16
申请号	EP2006785015	申请日	2006-06-16
[标]申请(专利权)人(译)	SCANTIBODIES LAB		
申请(专利权)人(译)	SCANTIBODIES实验室，INC.		
当前申请(专利权)人(译)	SCANTIBODIES实验室，INC.		
[标]发明人	CANTOR THOMAS L		
发明人	CANTOR, THOMAS, L.		
IPC分类号	A61K39/395 C12M1/34 B01L3/02 C12M1/36 C12M3/00 C12Q1/00 C12Q1/68 G01N15/06 G01N33/53 G01N33/543 G01N33/566		
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摘要(译)

本发明涉及通过使流体通过体外吸附容器从体液中除去有害实体的新方法和装置。该装置具有限制在容器内的结合装置，并且结合装置特定用于固定待除去的实体，其可以是以不希望的高水平存在的病原体，毒性物质，朊病毒，异常细胞或内源性物质。通过使体液通过容器，除去至少一部分有害物质。然后将处理过的液体返回患者体内。