APPARATUS FOR INACTIVATING INFECTIOUS AGENTS IN AND RESISTING COAGULATION OF BIOLOGICAL FLUIDS.

References cited:
PATENT ABSTRACTS OF JAPAN, vol. 12, no. 230 (C-508), 29 January 1988
DIALOG INFORMATIONAL SERVICE, file 154: Medline 83-90, acc. no. 04860858; W.A. ANDRES, acc. no. 83093858
PATENT ABSTRACTS OF JAPAN, vol. 5, no. 194 (C-82), 11 September 1981

1061 Lindendale Drive
Mt. Lebanon, PA 15243 (US)

1061 Lindendale Drive
Mt. Lebanon, PA 15243 (US)

Representative: Howden, Christopher Andrew et al.
FRITZ FORRESTER & BOEHMERT
Franz-Joseph-Strasse 38
D-80801 München (DE)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).
Description

This invention relates to apparatus for containing biological fluid. In one aspect, the invention relates to such apparatus incorporating means for inactivating infectious agents in biological fluids, whilst in another aspect, the invention relates to apparatus for containing blood and which is adapted to resist coagulation of the blood.

In the evaluation and care of patients, it is often necessary to obtain blood samples. The routine procedure for drawing blood from a patient requires a needle holder, a disposable needle, and multiple evacuated tubes. A disposable needle is attached to the holder, the needle is inserted into the patient's vein and the evacuated tube is inserted through the opposite end of the needle into the holder. The evacuated tube is then allowed to receive the derived quantity of blood. The tube is then removed and another may be employed, if desired.

During the above process, the health care worker may be exposed to blood which drips from the end of the needle or may be injured by sticking the needle into his or her skin. In addition, the cap may accidentally come off of the tube or the tube may break, splashing the health care worker with blood. Laboratory workers, also, are exposed to the blood in the process of handling the blood filled tube and in disposing of the needle.

If a patient's blood contains infectious agents, health care workers may be exposed to these infectious agents and thus are at risk of acquiring infection. The Center for Disease Control has estimated that 500 to 600 health care workers are hospitalised annually due to occupationally acquired Hepatitis B Virus ("HBV"), U. S. Dept. of Labor and U. S. Dept. of Health and Human Services, Joint Advisory Notice on Protection Against Occupational Exposure to HBV and HIV, pages 1-13 October 30, 1987. Of these, over 200 deaths resulted. Other infectious agents, such as Human Immunodeficiency Virus ("HIV"), Human T Lymphotrophic Virus I ("HTLV I"), and Cytomegalovirus ("CMV") cause infections less often, but still pose a significant threat to the health care worker.

It is known to provide a blood collection tube and method that attempts to disinfect infectious viral contaminants instantaneously when the blood is taken. United States Patent No. 4,675,159 discloses using a disinfectant material, such as aldehyde (with glutaraldehyde being preferred), in connection with a blood collection tube. The amount of aldehyde based disinfectant positioned in the container is adjusted to provide an ultimate concentration of aldehyde in the blood specimen of about 1 to 2.5 percent by weight and is buffered to a pH of about 7.2 to 8.5. The aldehyde based substances disinfect blood by crosslinking and/or polymerizing amino groups on the surface of the infectious agent.

The problem with using aldehyde based substances is that the above-described polymerization distorts and destroys the structure and function of proteins. When used in biologic situations, a glutaraldehyde, for example, does not distinguish between the amino groups of infectious agents and the amino groups of the patient's blood and serum proteins. Ultimately, this cross-linking leads to coagulation which renders the blood sample unsuitable for routine processing. Also, the patient's altered proteins are unsuitable for analysis. Lowering of glutaraldehyde concentrations is not a solution because of the loss of ability to inactivate the infectious agents.

Other disadvantages of aldehyde based disinfectants are that they are unstable and that they cannot be used with heparin.

United States Patent No. 4308232 discloses an anticoagulant stopper coating which resists adherence of cells to the stopper. The coating consists of a blood anticoagulant, a binding agent layer, and an outer layer of silicone oil.

United States Patent No. 3890955 discloses a vacuum indicator. A standard blood collection tube having a partial vacuum pressure and a needle for removing blood from a patient is provided. A material, which is coated on the tube, changes colour to indicate when vacuum pressure is lost in the tube.

United States Patent No. 3901219 discloses a blood collecting container and method consisting of a tube having a stopper and a piston barrier which holds a chemical which can be added to the collected blood. When blood is introduced into the tube, the piston barrier descends (Figure 2) until reaching constriction. Figure 3 illustrates the apparatus with collected blood after it has been centrifuged.

EP-A-0116793 discloses a method of detecting pathogenic organisms in biological samples such as blood. Added to the sample which is suspected of being contaminated with a particular pathogenic organism are particles of magnetic gel to which are adhered antibodies specific for an antigen of the organism. The gelled particles are withdrawn from the medium by applying a magnetic field and are seeded on an appropriate culture medium. Wells are formed in the culture medium near to the particles and are filled with serum against the pathogenic organism to be detected and the presence of the pathogen is detected by the effect of the serum on the germ colonies grown from those originally adhering to the magnetic gel particles.

WO-A-8204264 discloses a method by which, inter alia, the quantity of a given micro-organism in a fluid sample can be determined. The fluid sample is exposed to a gel carrier to which are bound antibodies specific to the micro-organism concerned. The carrier, with the captured micro-organisms, is then exposed to a nutrient medium and the number of micro-organisms assessed by detecting the quantity of a
metabolic product produced in a given time, for example by detecting the pH change in a given time. US-A-4553553 discloses a device for use in detecting micro-organisms in blood circulated extracorporeally, the device comprising a housing packed with a particulate adsorbent capable of binding bacteria, fungi and viruses, the housing being closed at one end by a piston having a hollow piston rod extending from the housing and being closed at its other end by a closure providing a flow passage therethrough. In use, the housing is connected in a conduit carrying blood, the free end of the piston rod and the flow passage through the closure being connected with the conduit so that the blood flows through the housing and through the packing of the particulate adsorbent. After a time, the housing is disconnected from the blood supply, a rinsing agent is passed through the housing, then a nutrient broth. After incubation, the adsorbent medium is expelled into a Petri dish by operation of the piston rod.

Biotecnology. November 1988, I-FU TSAO et al: "The removal of adventitious viruses and virus-infected cells using cellular adsorbent: A feasibility study", pages 1330-1333 discloses a method of removing viruses from blood products by contacting the blood products with beads or membranes to which are bound cells having a known membrane receptor specificity for the viral contaminants to be removed from the blood products.

TROMB. RES., September 15th, 1982, 27(6) pages 703-712 W.A. Andes "IgM anticoagulant with acquired abnormalities in factor VIII" describes a study of the effect of IgM Lupus anticoagulant on coagulation tests and on factor VIII related antigen in a patient with lymphocytic lymphoma and suggests that corresponding antibodies may serve as disease markers.

Despite these known apparatus and methods there still remains a need for an effective way to prevent accidental infection of health care workers.

It is also desired, in evaluating blood constituents such as blood gases and potassium, to obtain an anticoagulated specimen. It is known to add EDTA (or equivalent chemical agents such as oxalate and citrate) or heparin to the drawn blood to obtain an anticoagulated specimen. The EDTA chelates calcium ions. These calcium ions are necessary for the functioning of many of the blood clotting factors (factors II, V, VII, VIII, IX and X). By removing the calcium, the effective functioning of the blood clotting factors is inhibited and the blood does not clot. Heparin functions through interaction with Antithrombin III and binding to factors XII, XI, IX, X and II. The binding inhibits the functioning of the factors and resists coagulation.

The problem with using chelating agents such as EDTA is that these cause water to leave cells and enter the plasma which causes mild dilution of the plasma. In addition, chelating agents cannot be used for blood gas determinations and when ions, such as calcium, are desired to be measured.

Heparin, which is a heterogeneous mixture of linear and ionic polyelectrolytes, is not uniform in its effects. Heparin preparations have ranging solubilities and anticoagulative properties, and so must be used in excess to achieve reproducible coagulation. Heparin also precipitates fibronectin, a blood protein, which clogs the small orifices of blood gas instruments. Heparin binds calcium and therefore is not suitable for ionized calcium determinations. Likewise, heparin may bind certain antibiotics such as aminoglycosides.

Thus, there remains a need for a method and apparatus for inactivating infectious agents in and resisting coagulation of biological fluids. This method should not only effectively disinfect infectious agents, but also not adversely affect the other constituents of the particular biological fluid to be analyzed. There also remains a need for a method of resisting coagulation of biological fluids that can allow effective analysis of certain blood constituents such as blood gases and potassium.

It is an object of the invention to provide an apparatus which meets these needs.

According to one aspect of the invention, there is provided an apparatus for containing biological fluid from a patient comprising a biological fluid container means having disposed therein a solution containing an antibody for inactivating infectious agents in said biological fluid, said apparatus including a needle and holder means to hold said needle, said holder means associated with said biological fluid container means, whereby said needle draws said biological fluid from said patient and deposits the same into said biological fluid container means.

In use of the apparatus, the antibody reacts rapidly with the biological fluid to inactivate the infectious agent therein as the biological fluid enters the tube. After the biological fluid is introduced in the tube, the infectious agents will bind to the antibodies, thus reducing the chance that the health care worker will be exposed to the infectious agent.

According to another aspect of the invention, there is provided an apparatus for containing blood from a patient comprising: a blood container means having disposed therein antibody blood factor anticoagulant for resisting coagulation of said blood; a needle; and holder means to hold said needle, said holder means being associated with said blood container means, whereby said needle draws said blood from said patient and deposits the same into said blood container means such that blood coagulant factors in said blood will be inhibited so that said blood sample will be uncoagulated.

The antibody or antibodies having an anticoagulative effect on the blood allow evaluation of certain blood constituents such as blood gases and potassium.
These and other objects will become apparent from the description of the invention with reference to the drawings appended to this application.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows a blood collection tube having an amount of an antibody disposed therein.

Figure 2 shows a blood collection tube having an amount of an antibody and an amount of silicone gel disposed therein.

Figure 3 shows a blood collection tube in which the antibody and an anticoagulant are disposed.

Figure 4 shows a blood collection tube having contained therein an amount of anticoagulative antibody/antibodies.

**DESCRIPTION OF THE PREFERRED EMBODIMENT**

As used herein, "patient" means members of the animal kingdom including humans, regardless of whether the person or animal is ill.

Referring to Figure 1, there is shown a standard blood collection tube 10 that is used in connection with drawing blood from a patient. This tube is equipped with a stopper 12 which consists of a body portion 14 made of rubber and a needle entry portion 16. The blood collection tube may, for example, hold about 10.00 ml of fluid. As is well known, this blood collection tube 10 is used with a needle holder and a disposable needle (both not shown). This disposable needle is attached to the holder, the needle is inserted into the patient's vein and the needle entry portion of the stopper 12 is inserted through the opposite end of the needle into the holder. The blood collection tube 10 is then allowed to receive blood and when the desired amount of blood has been collected the blood collection tube is removed and another may be used. In this fashion, multiple tubes of blood may be obtained from a single needle stick.

The blood collection tube 10 of Figure 1 contains a desired amount of antibody 22. The antibody is in a liquid form, such as an electrolyte solution composed predominantly of NaCl in physiologic concentrations. Because of the high specificity and collection of antibodies, in general, only minimal volumes of antibody will be needed. The volume of antibody 22 disposed in the tube 10 is about .01 ml to 1.00 ml of antibody, with .10 ml to .20 ml being preferred. This will provide a dilutional effect of approximately .01 to 2.0 ml, with .10 to .20 ml being preferred. The antibody will be about .10% to 20% of the volume of the blood with about .50% to 1.00% being preferred.

The antibody can either be a polyclonal or a monoclonal antibody, used either alone or in combination.

Upon the introduction of blood, the swirling action of the blood entering the evacuated tube will mix the antibodies and blood. Further mixing may be achieved by inverting the tube, as is commonly done following the collection of blood. Antibodies have high affinity constants, in the range of 10⁻⁹ to 10⁻¹² L/M, and react rapidly and completely with their preferred antigen (e.g., infectious agents or coagulation proteins) to form a complex, as is illustrated by the following:

\[
\text{Antibody (Ab) + Infectious Agent (IA) \rightarrow Antibody - Infectious Agent Complex (IA - Ab)}
\]

Following formation of the complex, certain blood proteins called complement proteins bind to the antigen-antibody complex. The binding of complement may lead to the direct inactivation of infectious agents or to the phagocytosis of the complex and agent by the white blood cells present in blood.

\[
\text{Ab - IA + Complement (C) \rightarrow Ab - IA - C (destruction or inactivation of infectious agent)}
\]

\[
\text{Ab - IA - C + White blood cells \rightarrow phagocytosis (inactivation of infectious agent)}
\]

These reactions take place quickly and are completed in seconds to minutes. The binding of antibodies to infectious agents in a liquid phase is fast and complete, usually occurring in less than 1 second. Solid phase reactions (reactions when the antibody is bound to beads or coated to the tube) take longer, on the order of several minutes.

These antibodies have been demonstrated to be effective in inactivating HIV and HBV. Centers for Disease Control: Recommendations For Prevention Of HIV Transmission In Health Care Settings. Morbidity and Mortality Weekly Report Supplement, 36 (25) 15-165, August 21, 1987.

Referring now to Figure 2, a blood collection tube 30, having a similar stopper 12 as was described in Figure 1, is shown having an amount of antibody 22 and a silicone gel 32. The amount of silicone gel used is about .50 ml to 2.00 ml with about .50 ml to 1.00 ml being preferred. As the silicone gel 32 is inert, it does not dilute the blood collected in tube 30. As is known to those skilled in the art, the silicon gel 32 is used to facilitate the separation of the serum from the cellular elements of a patient's blood. Silicon gel 32 is preferred for those assays requiring serum. The cellular constituents will be separated from the serum and intracellular viruses will therefore no longer be available for infecting the laboratory worker.

Figure 3 shows a blood collection tube 50 and stopper 12 having an antibody 22 along with an anticoagulant 52. The anticoagulant 52 (indicated by squigly lines) can be selected from the group consisting of heparin, EDTA and citrate. The amount of anticoagulant 52 is about .10 to 2.00 ml, with about .50 to 1.00 ml being preferred, or about 10% to 20% by volume to the amount of blood collected in the tube 50.

It will be appreciated that only certain examples of combinations of antibody, anticoagulant, and sili-
cone gel were discussed hereinafter. The invention, however, is not limited to these combinations.

It will be appreciated that the invention cannot be used to analyze the presence of antibody-specific virus or antibody to a specific virus. That is, if antibody HBV and HIV is added to a tube, the blood sample obtained in that tube cannot be used to evaluate the patient’s antibodies to HIV and HBV or analyzed for the presence of HIV or Hepatitis B Surface Antigen.

The invention provides a blood collection apparatus that not only inactivates viruses and other infectious agents present in a patient’s blood, but also does not have a measurable effect on other constituents of the blood, therefore, allowing the blood to be used for the analyses for which the blood was drawn.

As it was mentioned hereinafore, it is sometimes desirable to obtain an anticoagulated blood sample. The invention provides for using antibodies to clotting factors in the blood and to resist coagulation in blood samples obtained for laboratory analysis.

Figure 4 shows a biological fluid containing tube 100 (with stopper 12) in which is contained an amount of antibody 22 and a blood factor antibody 102 (indicated by triangles). The antibody 102 binds to one or several of the blood coagulation factors including factors I, II, V, VII, VIII, IX, X, XI, XII and resists coagulation of the blood. The antibodies 102 differ in specificity (the compound to which the antibody binds) from anti-infectious agent antibodies. Coagulation factor antibodies bind to the specific active portions of coagulation proteins, thereby inhibiting their biological function. The binding of the antibody to the specific coagulation protein inhibits the coagulation process. The uncoagulated blood may then be assayed or the formed elements (red blood cells while blood cells) may be separated and the plasma used for analysis.

The coagulant antibodies can be produced in goats, rabbits and horses or may be monoclonal antibodies which are produced through immunization of rats or mice. These antibodies are easy to produce, inexpensive and do not pose a chemical or biologic hazard to health care workers.

Antibody anticoagulation has the benefits of heparin (minimal chelating properties, negligible water shift and low ionic concentration) and lacks heparin’s disadvantages (uniform preparation, noninteraction with fibrinogen and calcium). Antibody anticoagulated blood may be used to assay blood gases, ionized calcium, potassium, aminoglycoside antibiotics, and cell numbers.

Claims

1. An apparatus for containing biological fluid from a patient comprising a biological fluid container means (10) having disposed therein a solution containing an antibody (22) for inactivating infectious agents in said biological fluid, said apparatus including a needle and holder means to hold said needle, said holder means associated with said biological fluid container means, whereby said needle draws said biological fluid from said patient and deposits the same into said biological fluid container means.

2. The apparatus of claim 1, including silicone gel (32) disposed in said biological fluid container means (30).

3. The apparatus of claim 1 or claim 2, including an anticoagulant (52) disposed in said biological fluid container means (50).

4. The apparatus of claim 1, wherein said antibody is selected from the group consisting of monoclonal antibodies and polyclonal antibodies.

5. An apparatus for containing blood from a patient comprising: a blood container (50) means having disposed therein antibody blood factor anticoagulant (52) for resisting coagulation of said blood; a needle; and holder means to hold said needle, said holder means being associated with said blood container means, whereby said needle draws said blood from said patient and deposits the same into said blood container means such that blood coagulant factors in said blood will be inhibited so that said blood sample will be uncoagulated.

6. The apparatus of claim 5, wherein said antibody blood factor anticoagulant is produced in an animal.

7. The apparatus of claim 5, wherein said antibody blood factor anticoagulant is a monoclonal antibody produced by immunizing rats or mice.

Patentansprüche

1. Eine Vorrichtung zur Aufnahme von biologischer Flüssigkeit von einem Patienten, die eine Behältereinheit für biologische Flüssigkeit (10) umfaßt, die darin angeordnet eine Lösung aufweist, die einen Antikörper (22) zur Inaktivierung von infektiösen Erregern in besagter biologischer Flüssigkeit enthält, wobei besagte Vorrichtung eine Kanüle und Haltemittel zum Halten besagter Kanüle einschließlich, wobei besagte Haltemittel mit besagter Behältereinheit für biologische Flüssigkeit verbunden sind, wodurch besagte Kanüle besagte biologische Flüssigkeit von besagtem Patienten abnimmt und dieselbe in besagte Behältereinheit für biologische Flüssigkeit abgibt.
2. Die Vorrichtung nach Anspruch 1, die Silicongel (32) einschließt, das in besagter Behältereinheit für biologische Flüssigkeit (30) angeordnet ist.

3. Die Vorrichtung nach Anspruch 1 oder Anspruch 2, die ein Antikoagulan (52) einschließt, das in besagter Behältereinheit für biologische Flüssigkeit (50) angeordnet ist.

4. Die Vorrichtung nach Anspruch 1, wobei besagter Antikörper aus der Gruppe ausgewählt ist, die aus monoklonalen Antikörpern und polyklonalen Antikörpern besteht.

5. Eine Vorrichtung zur Aufnahme von Blut von einem Patienten, die umfaßt: eine Behältereinheit für Blut (50), die darin angeordneten Antikörper-Blutfaktor-Antikoagulans (52) zur Verhinderung der Koagulierung von besagtem Blut aufweist; eine Kanüle; und Haltemittel zum Halten besagter Kanüle, wobei besagte Haltemittel mit besagter Behältereinheit für Blut verbunden sind, wodurch besagte Kanüle Blut von besagtem Patienten abnimmt und desselbe in besagte Behältereinheit für Blut abgibt, so daß Blutgerinnungsfaktoren (52) in besagtem Blut inhibiert werden, so daß besagte Blutprobe nichtkoaguliert sein wird.


Revidications

1. Appareil pour contenir un fluide biologique de patient comprenant un moyen servant de conteneur pour le fluide biologique (10) dans lequel se trouve une solution contenant un anticorps (22) pour l'inactivation d'agents infectieux dans ledit fluide biologique, ledit appareil incluant une aiguille et un moyen servant de support pour supporter ladite aiguille, ledit moyen servant de support étant associé audit moyen servant de conteneur pour le fluide biologique, par lequel ladite aiguille prélève ledit fluide biologique dudit patient et dépose celui-ci dans ledit moyen servant de conteneur pour le fluide biologique.

2. Appareil de la revendication 1, contenant du gel de silicone (32) qui se trouve dans ledit moyen servant de conteneur pour le fluide biologique (30).

3. Appareil de la revendication 1 ou de la revendication 2, contenant un anticoagulant (52) qui se trouve dans ledit moyen servant de conteneur pour le fluide biologique (50).

4. Appareil de la revendication 1, dans lequel ledit anticorps est choisi dans le groupe formé par des anticorps monoclonaux et des anticorps polyclonaux.

5. Appareil pour contenir le sang d'un patient comprenant : un moyen servant de conteneur pour le sang (50) dans lequel se trouve un anticorps anticoagulant dirigé contre les facteurs du sang (52) pour s'opposer à la coagulation dudit sang ; une aiguille ; et un moyen servant de support pour supporter ladite aiguille, ledit moyen servant de support étant associé audit moyen servant de conteneur pour le sang, par lequel ladite aiguille prélève ledit sang dudit patient et dépose celui-ci dans ledit moyen servant de conteneur pour le sang de telle façon que les facteurs coagulants du sang (52) dans ledit sang soient inhibés et que ledit échantillon de sang ne coagule pas.

6. Appareil de la revendication 5, dans lequel ledit anticorps anticoagulant dirigé contre les facteurs du sang est produit par un animal.

7. Appareil de la revendication 5, dans lequel ledit anticorps anticoagulant dirigé contre les facteurs du sang est un anticorps monoclonal produit par l'immunisation de rats ou souris.