US 20040209353A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0209353 A1**

Chien et al. (43) **Pub. Date: Oct. 21, 2004**

(54) **BIOLOGICAL SAMPLE STORAGE DEVICE AND METHOD FOR BIOLOGICAL SAMPLE CONTAMINATION TESTING**

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(21) Appl. No.: **10/733,766**

(22) Filed: **Dec. 12, 2003**

Related U.S. Application Data

(60) Provisional application No. 60/432,665, filed on Dec. 12, 2002. Provisional application No. 60/435,287, filed on Dec. 23, 2002.

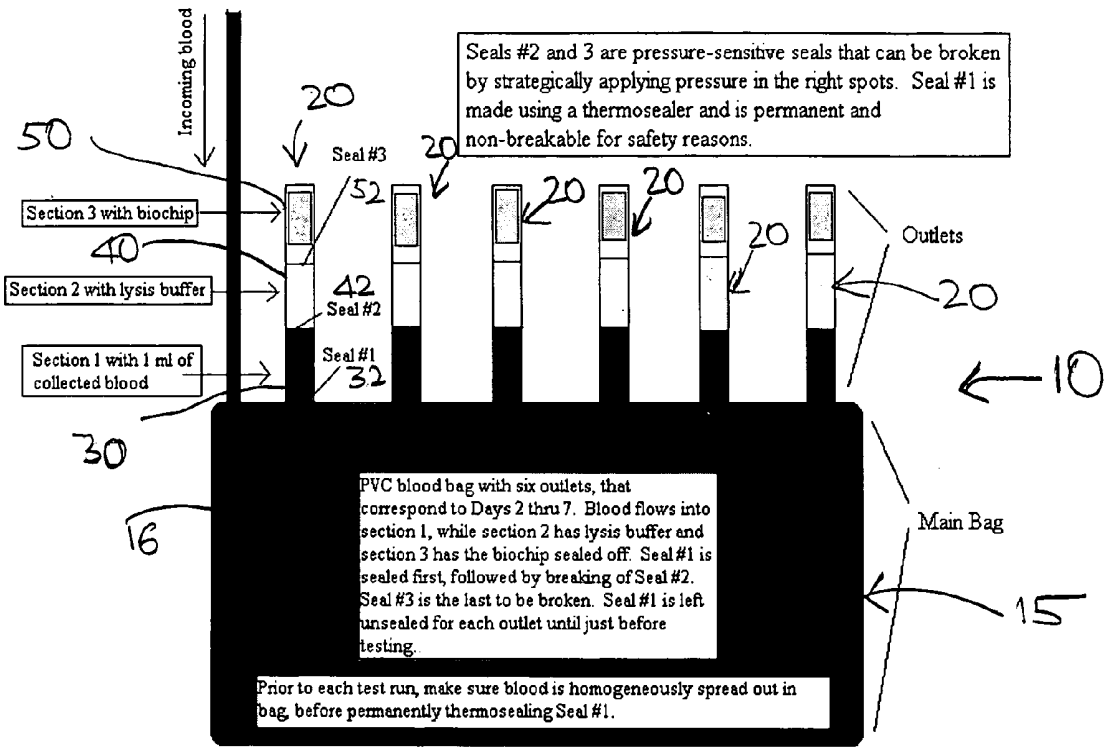
Publication Classification

(51) **Int. Cl.**[7] **C12Q 1/68**; A61M 5/32; C12M 1/34

(52) **U.S. Cl.** **435/287.1**; 604/162; 435/6

(57) **ABSTRACT**

The present invention is directed to a method for screening blood and blood products that utilizes a specialized blood and blood products storage device useful for storing and testing blood or blood products comprising a container having at least one compartment with one or more sections for storing and testing the blood or blood products for a target molecule. The method utilizes a homogeneous testing system that employs ribozymes, DNAzymes or catalytic antibodies.



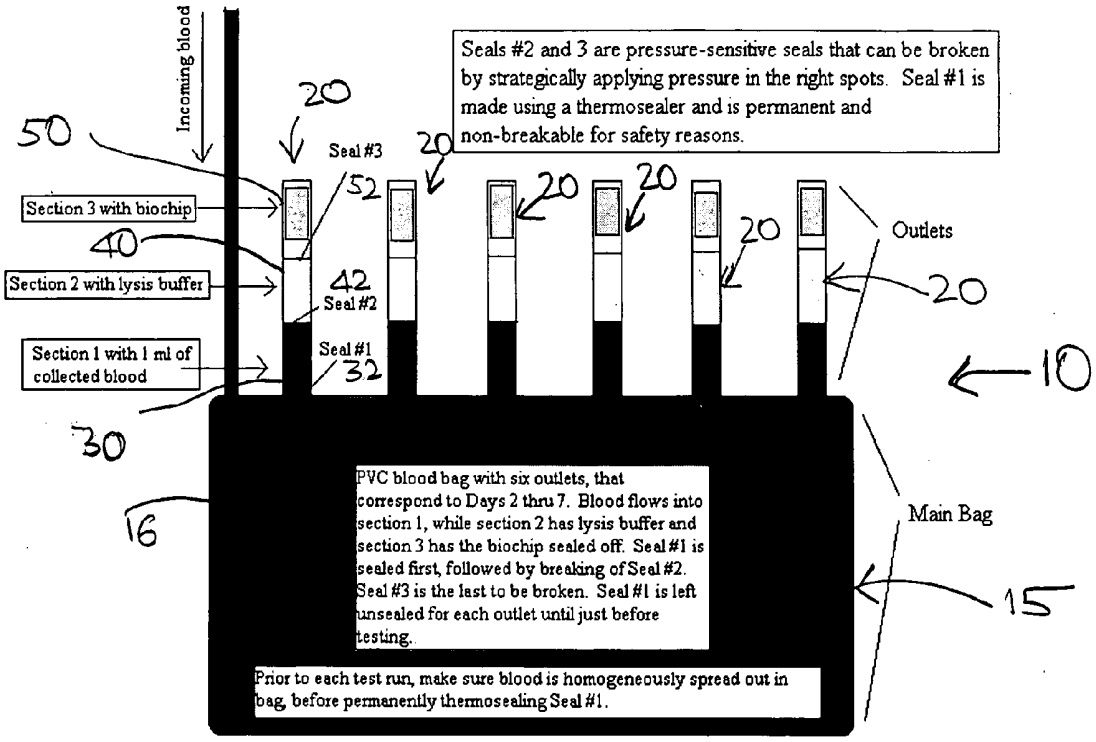

Incoming blood
Seals #2 and 3 are pressure-sensitive seals that can be broken by strategically applying pressure in the right spots. Seal #1 is made using a thermosealer and is permanent and non-breakable for safety reasons.
50
20
Seal #3
52
20
Section 3 with biochip
40
Section 2 with lysis buffer
42
Seal #2
Seal #1
32
Section 1 with 1 ml of collected blood
30
16
PVC blood bag with six outlets, that correspond to Days 2 thru 7. Blood flows into section 1, while section 2 has lysis buffer and section 3 has the biochip sealed off. Seal #1 is sealed first, followed by breaking of Seal #2. Seal #3 is the last to be broken. Seal #1 is left unsealed for each outlet until just before testing.
Prior to each test run, make sure blood is homogeneously spread out in bag, before permanently thermosealing Seal #1.
Outlets
Main Bag
10
15

FIGURE 1

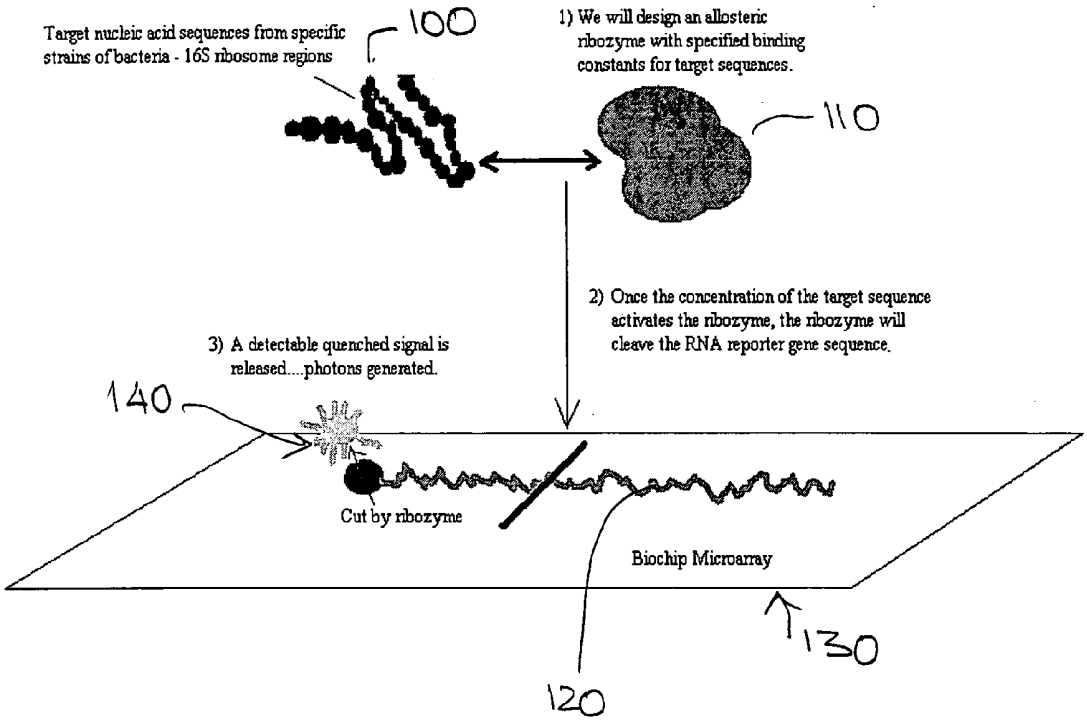

Target nucleic acid sequences from specific strains of bacteria - 16S ribosome regions
100
1) We will design an allosteric ribozyme with specified binding constants for target sequences.
110
2) Once the concentration of the target sequence activates the ribozyme, the ribozyme will cleave the RNA reporter gene sequence.
3) A detectable quenched signal is released....photons generated.
140
Cut by ribozyme
Biochip Microarray
130
120

FIGURE 2

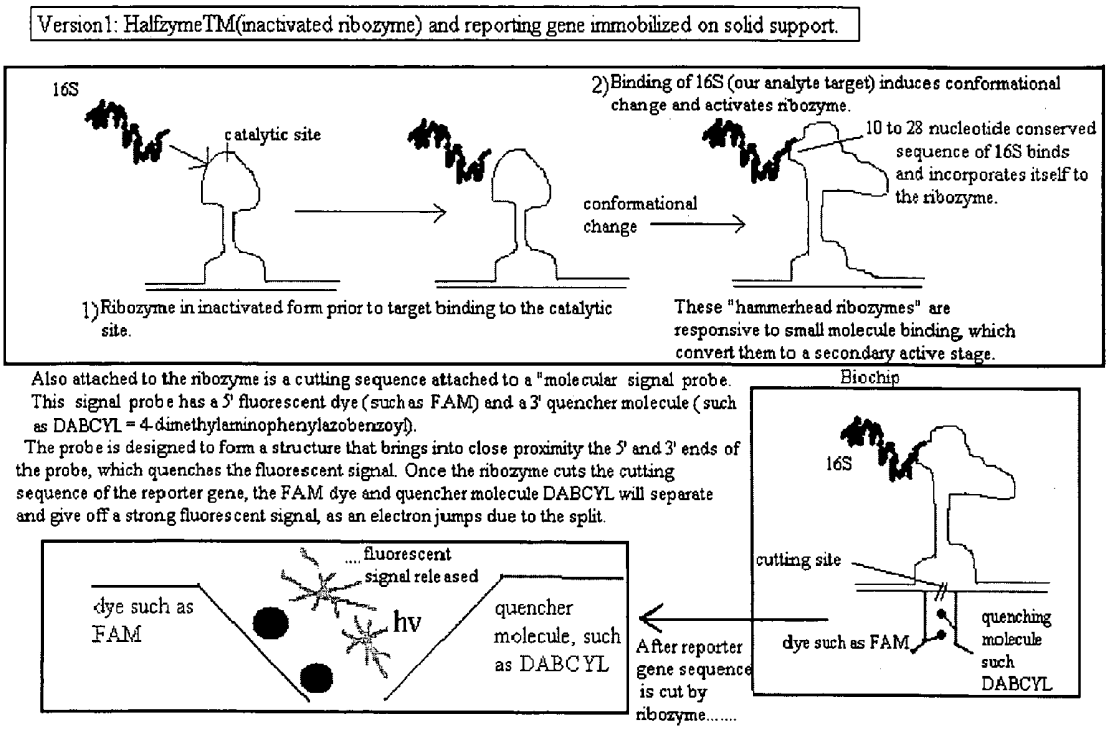

Version1: HalfzymeTM(inactivated ribozyme) and reporting gene immobilized on solid support.
16S
catalytic site
1)Ribozyme in inactivated form prior to target binding to the catalytic site.
2)Binding of 16S (our analyte target) induces conformational change and activates ribozyme.
conformational change
10 to 28 nucleotide conserved sequence of 16S binds and incorporates itself to the ribozyme.
These "hammerhead ribozymes" are responsive to small molecule binding, which convert them to a secondary active stage.
Also attached to the ribozyme is a cutting sequence attached to a "molecular signal probe. This signal probe has a 5' fluorescent dye (such as FAM) and a 3' quencher molecule (such as DABCYL = 4-dimethylaminophenylazobenzoyl).
The probe is designed to form a structure that brings into close proximity the 5' and 3' ends of the probe, which quenches the fluorescent signal. Once the ribozyme cuts the cutting sequence of the reporter gene, the FAM dye and quencher molecule DABCYL will separate and give off a strong fluorescent signal, as an electron jumps due to the split.
Biochip
16S
cutting site
dye such as FAM
quenching molecule such DABCYL
After reporter gene sequence is cut by ribozyme.....
fluorescent signal released
dye such as FAM
hv
quencher molecule, such as DABCYL

FIGURE 3

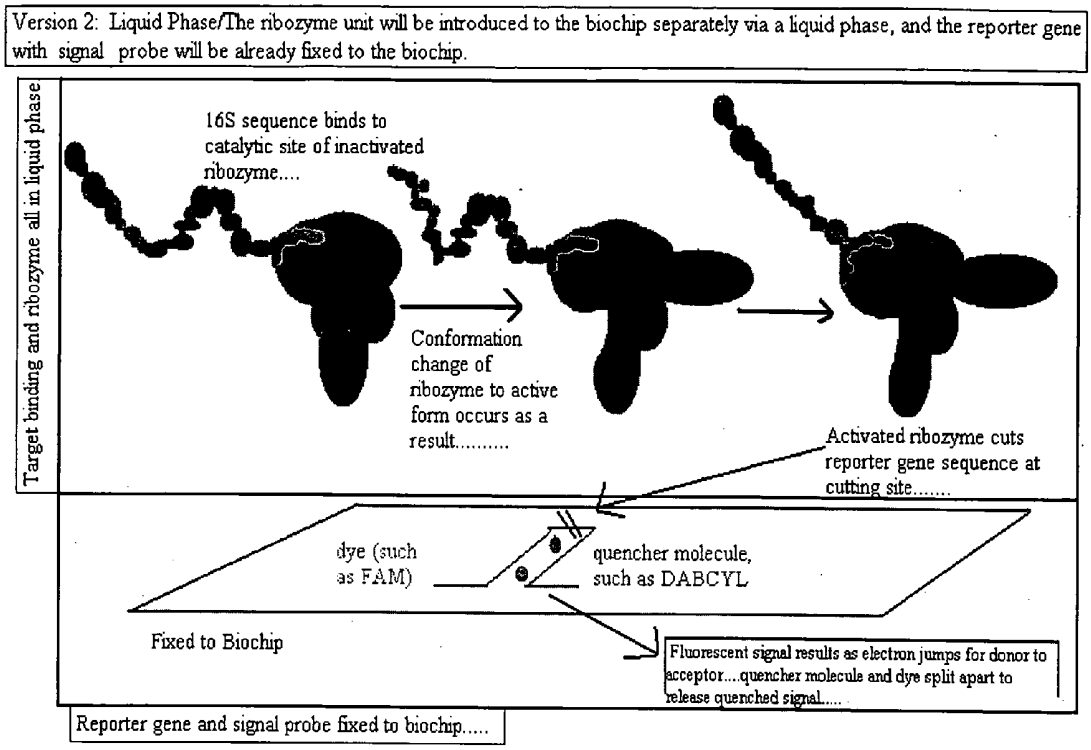

Version 2: Liquid Phase/The ribozyme unit will be introduced to the biochip separately via a liquid phase, and the reporter gene with signal probe will be already fixed to the biochip.
Target binding and ribozyme all in liquid phase
16S sequence binds to catalytic site of inactivated ribozyme....
Conformation change of ribozyme to active form occurs as a result.....
Activated ribozyme cuts reporter gene sequence at cutting site.....
dye (such as FAM)
quencher molecule, such as DABCYL
Fixed to Biochip
Fluorescent signal results as electron jumps for donor to acceptor....quencher molecule and dye split apart to release quenched signal....
Reporter gene and signal probe fixed to biochip.....

FIGURE 4

BIOLOGICAL SAMPLE STORAGE DEVICE AND METHOD FOR BIOLOGICAL SAMPLE CONTAMINATION TESTING

BACKGROUND OF THE INVENTION

[0001] This invention relates generally to the field of screening biological samples and specimens for contamination by a targeted molecule that is indicative of the presence of a pathogen, infectious agent or contaminating agent. More specifically, the invention is directed to a method of screening blood and blood products that utilizes a specialized blood and blood products storage device useful for both storing and testing blood and blood products. The storage device comprises a container having at least one compartment with one or more sections for storing and testing the blood or blood products for a target molecule that is indicative of the presence of a pathogen, such as a bacterium, virus, parasite, prion or other pathogenic agent.

[0002] Currently, blood banks use several individual assays to test for the presence of multiple agents or molecules associated with or indicative of a disease or condition. Exemplary agents or molecules include anti-HIV I/II antibodies, anti-HCV antibodies, HBV surface antigen, anti-HBC antibodies, liver enzyme (ALT), syphilis and HIV P24 antigen. Additionally, infectious agents such as pathogenic bacteria and fungi, prions and protozoa, including the causative agents of small pox, malaria, West Nile disease, Chagas disease, and variant Creutzfel Jacob disease (vCJD) may be detected. Additionally the TMA Procleix system is currently used to test for HCV and HIV-1 viral RNA molecules.

[0003] The current method involves collecting blood directly into a blood collection device via a collection needle and duct, and collecting a separate fraction of blood for testing/screening for various diseases. In addition to concerns about contamination of the blood by agents or molecules that indicate an infection in the blood donor, a concern also exists that the techniques used for blood collection may cause contamination of the collected blood. For example, when blood is collected via a needle, there may be bacterial contamination from skin or a hair follicle caught in the needle that could get into the collected blood, as well as bacterial contamination inadvertently introduced from other aspects of collecting and handling the blood and blood products. Contaminating bacteria replicate within blood and can cause adverse effects in recipients. For example, contaminating bacteria can produce high levels of endotoxins and other pathogenic components that cause severe health damage, including death. This problem is particularly keen for platelets, a blood product separated from the whole blood that must be stored at room temperature until use. Presently, platelets only can be stored up to 5 days, and must be discarded if not used during that time. If, however, the platelets could be tested quickly, simply and accurately for contamination immediately prior to use, the length of storage time could be increased.

[0004] Currently, there is an issue at blood banks and hospitals over the difficulty of detecting and evaluating bacterial and other pathogen levels in blood because the pathogens must replicate to reach threshold levels for detection. Detection at Day 0 of collection, therefore, is not a sufficient measure for determining that blood is free of bacteria and other pathogens. Also, some forms of bacteria take longer to replicate and to become detectable, particularly if the blood or blood products are stored at approximately 4° C.

[0005] Under current practices, blood banks must make a difficult decision as to what is an acceptable level of bacterial contamination for a blood product to be considered safe for release to a patient. Thus, the timing, simplicity and sensitivity of screening tests is an important consideration. Testing done at the time of collection, Day 0, or shortly thereafter needs to be extremely sensitive because it is likely that the number of bacteria present at Day 0 is very low. An extremely sensitive assay may result in a significant number of false positives, raising serious regulatory issues because the test should not result in every unit of blood product testing positive. A further consideration is that screening tests must be performed at the blood bank before sending the sample to a hospital, or alternately at the hospital, immediately prior to use. The screening tests therefore must be rapid and fairly simple, such that they may be performed by medical technicians.

SUMMARY OF THE INVENTION

[0006] The present invention provides a blood or blood products storage device for storing and testing blood or blood products that combines a unique configuration with a "real-time test" that includes state of the art diagnostic detection technology providing a visual signal detection of pathogen contamination, particularly bacterial, viral and parasitic contamination. More specifically, the claimed device comprises a container having at least one compartment with one or more sections for testing biological samples, particularly blood and blood products, for contamination by a pathogenic agent or a product of a pathogenic agent, such as a toxin. The invention also provides a method of using the device for testing samples for the presence of the pathogenic agent or product. The container design provides the opportunity for collecting, storing and testing, all in the claimed device. In one embodiment, the specialized multi-sectioned compartmentalized device is self-contained for the storage and testing of the blood or blood product without opening the container and exposing the sample to additional risk of contamination. Although the device and method preferably are designed to detect bacterial contamination in the blood, i.e., bacteria or a bacterial product such as toxins, the device and method can be used to detect the presence of any pathogen in the blood or blood product, such as viruses, viral components or products, parasites, parasitic components or products or other detectable components of the indicative of the presence of pathogenic agents.

[0007] The present device provides a self-contained storage and testing container that facilitates a fast and easy way to test blood or blood products throughout its shelf-life, while conserving collected blood and not exposing it to manipulations that may cause further contamination. The configuration of test reagents used in the device allows them to remain separated from the blood or blood product until the moment of testing.

[0008] An advantage of the invention is the concept of "real time testing," in which the presence of bacteria or any pathogen or their products of interest in the blood or blood product can be tested or monitored in real time, on an

ongoing basis up to the actual time of release of the blood or blood product to a patient. A further advantage of the invention is that the testing assay is a homogenous assay, meaning that there are no separate washing steps involved. The test reagents are mixed and the signals are generated and detected without a need for any further manipulations. Any known homogeneous signal detection assays that can be utilized as the signal-detection testing procedure with the disclosed device. Exemplary signal detection assays employ ribozyme, DNAzyme or catalytic antibody technology. Another employs B-cells engineered to express aeqorin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate presently preferred embodiments of the invention. Together with the general description given above and the detailed description given below, the drawings serve to explain principles of the invention.

[0010] **FIG. 1** shows one embodiment of a biological sample storage device of the present invention.

[0011] **FIG. 2** shows one embodiment of the testing reagents, utilizing as a solid support a biochip, and the relationship and interaction of the target molecule, the ribozyme and the RNA reporter gene sequences.

[0012] **FIG. 3** shows one embodiment of the interaction of the target molecule, the ribozyme and the RNA reporter gene sequences when the ribozyme designated as a "Halfzyme 9" and the reporter gene are immobilized on a solid support.

[0013] **FIG. 4** shows another embodiment of the interaction of the target molecule, the ribozyme and the RNA reporter gene sequences when the ribozyme is in a liquid phase and the reporter gene is immobilized on a solid support, such as a biochip or beads.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT(S)

[0014] The reference throughout this application alternately to blood or blood products is intended to encompass either of these biological samples. Further, the word "a" or "an" is intended to encompass one or more recited elements.

[0015] The present invention is directed to a biological sample storage device for storing and testing blood or blood products. The device comprises a container for receiving and storing blood or blood products, and at least one compartment for testing the blood or blood products. The compartment comprises at least a first section for holding a portion of the blood or blood products, and optionally testing the portion of the blood or blood products. The first section of the device is arranged contiguous to the container so that the blood products can flow from the container to the first section. The first section further is designed such that the first section can be sealed from the container, so that the blood products sealed into the first section can be used for testing. The device further comprises at least one additional section for testing the portion of the blood or blood products held in the first section, the additional section being arranged in sealed contact with another portion of the first section, different from a portion of the first section in contact with the container. The additional section comprises a pressure sensitive seal between the first section and the additional section that can broken by the application of pressure, such that breaking the seal in the additional section allows mixing of the contents of the first and additional sections. The container of the device comprises a plurality of compartments. In one embodiment, each of the compartments is arranged as a protruding element from the container.

[0016] In another embodiment, the device comprises a first section and at least one additional section comprising a second and a third section. The second section is arranged in sealed contact with another portion of the first section, different from a portion of the first section in contact with the container. The third section is arranged in sealed contact with another portion of the second section, different from a portion of the second section in sealed contact with the first section. Further, the second and third sections comprise pressure sensitive seals that can broken by the application of pressure such that breaking the seal in the second section allows mixing of the contents of the first and second sections and breaking of the seal in the third section allows transfer of the mixed contents of the first and second sections into the third sections. The second section contains a buffer, wherein the buffer is a lysis buffer or an isotonic buffer, and the third section contains test reagents for testing the transferred mixed contents from the first and second sections, wherein these test reagents are a catalytic molecule and a reporter sequence. In one embodiment, the catalytic molecule is an inactivated ribozyme, a DNAzyme or a catalytic antibody. In a further embodiment, the test reagents are an inactivated ribozyme and an RNA reporter sequence. At least one of the test reagents, the catalytic molecule or the reporter sequence is immobilized to a solid support or at least one of the test reagents, the catalytic molecule or report sequence is in a lyophilized form. In one embodiment, the blood products tested in the device comprise blood platelets.

[0017] Any number of solid support systems can be used for immobilizing test reagents. For example, the support may be a biochip, a bead, a gel, a microparticle, a membrane, a slide, a plate, a pellet, a disk, a capillary, a hollow fiber, a needle, a solid fiber or another form capable of providing a substrate for attachment of the test reagent(s). The support system may comprise a wide variety of compositions, including glass, plastic, silicon, alkanethiolate-derivatized gold, cellulose, low crosslinked and high crosslinked polystyrene, silica gel, polyamide, and the like.

[0018] In one embodiment, the blood or blood products storage device accommodates, in its compartment for testing the blood or blood products, biosensors capable of supporting a "self-performing" screening assay. For example, engineered B-cells with surface antibodies specific for target agents or molecules may be attached to a biochip. The engineered B-cells express cytosolic aequorin, a calcium-sensitive bioluminescent protein from *Aequoria Victoria* jellyfish. Once a target antigen binds to the B-cell, it will trigger an elevated intracellular calcium concentration, which will illuminate aequorin. See, e.g., Rider et al., Science, 301: 213-215 (2003). Such a "self-performing" screening assay is particularly advantageous because it also allows for continuous monitoring of a blood sample by visual inspection.

[0019] The invention is further directed to a method of testing blood or blood product for a target molecule indicative of contamination in the blood or blood product. The

method comprises (a) providing a sample of a blood product in a compartment of the storage device for storing and testing blood or blood products, which storage device comprises a container for receiving and storing blood or blood products and at least one compartment for testing the blood or blood products, wherein the compartment comprises at least a first section for holding a portion of the blood or blood products, and optionally testing the portion of the blood or blood products; (b) contacting the blood product in the compartment with a lysing buffer; (c) releasing the target molecule from the cells and protein in the blood product; and (d) detecting the presence of the target molecule. In one embodiment the target molecule is a 16S ribosomal RNA or a nucleic acid associated with a pathogen. The detecting step of the method may employ test reagents comprising a catalytic molecule and a reporter sequence, wherein the catalytic molecule is an inactivated ribozyme, a DNAzyme or a catalytic antibody. In a further embodiment, the test reagents are an inactivated ribozyme and an RNA reporter sequence. In this embodiment of the method, the inactivated ribozyme binds to the target molecule under sufficient conditions to activate the ribozyme that in turn cleaves the RNA reporter sequence and releases a detectable sequence.

[**0020**] In another embodiment of the method, the target molecule in the described method is a protein that is associated with or indicative of a pathogen. In the alternate method of testing a blood or blood product for these target proteins, which generally are cell surface proteins or secreted proteins of pathogens, such as bacteria, viruses, parasites and other pathogenic agents, the buffer is an isotonic buffer that dilutes the sample and does not lyse the cells or degrade the proteins. The method is performed in the same way as in the method of detecting the nucleic acid, but utilizes an isotonic buffer that does not lyse the cells or pathogen. The detecting step of the method employs test reagents comprising a catalytic molecule and a reporter sequence, wherein the catalytic molecule is an inactivated ribozyme, a DNAzyme or a catalytic antibody. In a further embodiment, the test reagents are an inactivated ribozyme and an RNA reporter sequence. In this embodiment of the method, the inactivated ribozyme binds to the target molecule under sufficient conditions to activate the ribozyme that in turn cleaves the RNA reporter sequence and releases a detectable sequence.

[**0021**] As shown in one aspect of the invention in **FIG. 1**, the present invention provides a storage device (**10**) for collection, storage and testing of blood or blood products comprising at least one compartment (**20**), preferably a plurality of compartments, each with at least two sections (for examples, the sections (**30**), (**40**) or (**50**)), with one section (**30**) for holding a portion of the blood product that will be tested and at least one additional section (for example, section (**50**)) for testing the portion of the blood or blood products present in the first section (**30**). Each of the compartments (**20**) is preferably arranged as a protruding element from the container (**15**) but a configuration of the compartment(s) within the device may be such that they are formed within the device and do not protrude outwardly from the device wall (**16**). Each of the compartments (**20**) of the device is arranged contiguously to the container (**15**) so that the blood products can flow from the container (**15**) to the first section (**30**) of the compartment (**20**). The first section (**30**) is designed such that it can be sealed from the container (**15**) so that the blood products sealed into the first section (**30**) can be used for testing but separated from the remaining blood product in the container to maintain the integrity of the product. Heat sealing is one method for sealing off the first section (**30**) from the container. Each of the compartments (**20**) further comprise at least one additional section (for e.g., section (**50**)) which contains the test reagents necessary for testing the blood product for contamination. Preferably this additional section comprises a second (**40**) and a third section (**50**), the second section (**40**) being arranged in sealed contact with another portion of the first section (**30**), different from a portion of the first section (**30**) in contact with the container (**15**), and wherein the third section being arranged in sealed contact with another portion of the second section (**40**), different from a portion of the second section (**40**) in sealed contact with the first section (**30**). Further, the second and third sections (**40**) and (**50**) include pressure sensitive seals that can be broken by the application of pressure, such that breaking the seal in the second section (**40**) allows mixing of the contents of the first and second sections (**30**) and (**40**), and breaking the seal in the third section (**50**) allows transfer of the mixed contents of the first and second sections (**30**) and (**40**) into the third section (**50**).

[**0022**] The blood products storage device (**10**) preferably is a bag composed of a flexible material, such as polyvinylchloride (PVC) or other similar type of material that will accommodate the design of the device and not adversely affect the blood product. For example, thermoplastic polyolefins, such as polyethylene, polypropylene, polybutalene and copolymers composed of such materials can be used. These can be made by Ziegler-Natta, metallocene or free-radical catalysts. Polyolefin blends also can be used in which polybutadiene and polyisoprene are mixed in, as well as ethylene co-polymers (EVA=ethylene vinyl acetate and EMA=ethylene methyl acrylate). Different nylon materials also may be used to prepare the device (**10**).

[**0023**] The container (**15**) includes at least one and preferably a plurality of compartments (**20**), each comprising at least two sections but more preferably three sections. In one embodiment, each of the compartments (**20**) of the container (**15**) comprises three sections (**30**), (**40**) and (**50**), however, the present invention may be performed using compartments with more or less sections than three if the selected testing method requires more or less test reagents and manipulative steps.

[**0024**] In all embodiments, the first section (**30**) of a compartment (**20**) is contiguous with the container so that it is in open connection to the container (**15**) and contains the same blood product as is in the container (**15**) prior to testing. Immediately prior to testing, the container (**15**) is agitated to ensure that the blood product in the first section (**30**) of a compartment (**20**) is representative of the blood product in the container (**15**). Then, this first section (**30**) of the compartment (**20**) is permanently sealed off from the container (**15**) by forming a seal between the container (**15**) and the first section (**30**) forming seal #1 (**32**) as depicted in **FIG. 1**. The second section (**40**) which is sealed off from the first section (**30**) and third section (**50**) via pressure sensitive seals (**42**) and (**52**), contains a buffer, in one embodiment a lysis buffer for lysing and degrading the cells, bacteria, any cellular or bacterial associated proteins and proteins present in the blood product. When the sample in the first section (**30**) of the compartment (**20**) is ready to test, the pressure

sensitive seal, Seal #2 (**42**), is broken with applied pressure, and the blood product sample held in the first section (**30**) mixes with the lysing buffer. After a sufficient period of time to achieve lysing of the blood product sample, seal #3 (**52**) is broken, allowing the contents of the first and second sections (**30**) and (**40**) to flow into the third section (**50**), which contains the test reagents required to perform the test to detect the presence of pathogen. In one embodiment, there are two test reagents in the third section (**50**), which may both be bound to a solid support, such as a biochip or bead, or lyophilized in the third section (**50**) so that they are maintained in a dry environment until use when they are solubilized by the sample/buffer mixture. When the reagents are bound to a solid support, they remain bound during the testing; however, if one or both of the test reagents are lyophilized in the third section (**50**) of the compartment (**20**), then these reagents will be in the liquid phase inside the compartment (**20**) when pressure sensitive seal #3 (**52**) between the second and third sections (**40**) and (**50**) is broken. The test reagents in the third section (**50**) now mix with the sample/buffer mixture and the level of contamination can then be detected visually after completion of signal activation in the compartment (**20**). This test generally takes about an hour and can be repeated for each of the compartments (**20**) on subsequent days depending upon the number of compartments (**20**) present in the container (**15**). Any number of compartments (**20**) can be present in the container (**15**) and the device can be designed to contain a suitable number of compartments (**20**) for the particular use of the device. However, it is most likely that six compartments (**20**) will be present in the container for testing from Days 2 to 7 as shown in **FIG. 1**. Thus, the device's contamination levels can be monitored everyday for a week or every few days to extend the testing period.

[**0025**] This test can be administered by hospital staff one to two hours before a transfusion to a patient, as the test requires only sealing seal #1 (**32**) and breaking seals #2 (**42**) and #3 (**52**) and reading the detection signals. Other advantages of this design are based on the optimization of the test itself.

[**0026**] Also the dry environment of the third compartment (**50**) assures stabilization of the test reagents involved in the detection method until the moment of testing. Any suitable test reagents can be employed as long as they provide the ability to detect the presence of the targeted molecule indicative of a contaminating pathogen. In one embodiment, the test reagents are allosteric enzymes and reporter gene sequences that can be cleaved by the allosteric enzymes and located in the third section (**50**) of each of the compartments.

[**0027**] An allosteric enzyme useful in the present invention is a catalytically inactive ribozyme that has a specific binding constant for a known target molecule that may be present in the blood product. The target molecule contains a sequence that activates the ribozyme which then cleaves a specific cleavage site in the RNA reporter sequence. Similarly, DNAzymes can be substituted for the ribozymes; however, in the discussions of the test reagents useful in this disclosure, ribozymes are used when DNAzymes could alternatively be used.

[**0028**] For detection of bacterial contamination, the preferred target molecule of the ribozyme is conserved bacteria-specific ribosomal RNA (rRNA), such as 16S rRNA, which detects the presence of bacteria in the blood product. In addition to targeting the specific sequences of 16S rRNA that indicates the presence of bacteria, specific ribozymes also can recognize sequences in the 16S rRNA to detect specific bacterial taxonomic family/genus/species to which the contaminating bacteria belong. For example, a first test is performed in one of the compartments in which the ribozyme targets the conserved 16S rRNA to detect the presence of the bacteria. Then, a plurality of compartments each containing a specific ribozyme that targets specific 16S rRNA is used to detect the genus and species, and possibly strain of a contaminating bacteria. See U.S. Pat. No. 5,679,520 for use of 16S rRNA to detect particular species of bacteria. This type of testing is useful because not all bacteria impose the same degree of health risk at a given level of contamination. Such a panel of further tests for bacterial species provide more information regarding whether the level of contamination with a particularly identified type of bacteria is acceptable or not in the blood product.

[**0029**] The bacteria that the device and test reagents are designed to test for are those that have contaminated the blood during collection and handling or bacteria that have infected the blood of the donor. The bacteria may be *Escherichia coli, Salmonella* species, *Campylobacter* species, *Listeria* species, *Enterococcus* species, *Neisseria gonorrhoeae, Enterobacter cloacae, Pseudomonas* species, *Proteus mirabilis, Legionella, Mycobacterium* species, *Chlamydia* species, other types of bacteria, protozoa and fungi.

[**0030**] The target molecule may also be nucleic acids, both RNA and DNA from viruses, such as HIV: long term repeated region (LTR), HBV: preS and S regions, HCV: 5' untranslated region (UTR), particularly regions of these viruses known to be indicative of the presence of these viruses. Additionally, the target molecule may be nucleic acids from parasites, such as *Plasmodium falciparum, Trypanosoma cruzi, Trypanosoma brucei, Babesia microti* and *Leishmania tropica.*

[**0031**] When nucleic acid is the target molecule, the lysis step ensures that the pathogenic-specific RNA sequences are properly exposed to allow for easy access for binding to and activation of the allosteric ribozyme. This is important because the allosteric ribozyme or DNAzyme generally can only recognize a range of 10-28 nucleotide sequences, disregarding secondary or tertiary structure as well. The need for an exposed linear epitope is satisfied by lysing the pathogen's cells. A ribozyme useful in the present invention is a ribozyme that has been designed to possess a specific binding affinity to a RNA target molecule to ensure binding and to possess sequences that cleave a designated RNA reporter sequence. Cruachem, Ltd. is a commercial source for these reporter molecules and provides services to design specific signal probes with the desired fluorescent dyes. This company is also a source of specifically designed ribozymes for specific assays. In one embodiment, the allosteric ribozyme is a catalytically inactive ribozyme, such as a "Halfzyme ϑ," a product of Ribozyme Pharmaceutical, Inc., Boulder Colo., ("RPI"), which is engineered so that the target molecule supplies the structural elements; i.e., the missing sequences required for ribozyme catalysis.

[**0032**] The test reagents, e.g., the allosteric ribozymes and the RNA reporter sequences, are sensitive to the pathogen

load in the blood product. In the case of bacterial detection, with the bacterial specific 16S rRNA sequence exposed in the lysate, the detection system utilizes sequence-specific binding and activation of enzymes, that cascade to a visible signal. Once the bacterial load reaches a certain level or concentration, a detectable signal is activated, which involves the capture of bacteria-specific sequences, through the use of technology of specifically designed allosteric enzymes. It is believed that a single bacterium present in the blood product is equivalent to 10^3-10^4 copies of 16S rRNA. A single bacterium is equivalent to one colony forming unit (CFU) of a specific bacteria, which is defined as a single bacterium that is able to clone itself into an entire colony of identical cells. Therefore, 10 CFUs would result in approximately 10^4-10^5 16S rRNA copies present in the lysis sample solution which provides sufficient analytes for a very sensitive assay. The 16S rRNA is the target of the assay.

[**0033**] Ribozymes can be designed to have specified binding constants with the desired target sequence. Depending on the concentration of the target molecule in the blood product, the allosteric enzyme/ribozyme is activated to recognize and cleave an RNA reporter sequence with a quenched signal attached to the solid surface or carrier or in solution in the lysed sample/buffer mixture. The ribozyme will not cleave the RNA reporter sequence if the concentration of the target 16S rRNA sequences is not high enough. Since the ribozyme is designed to have specified binding constants, the activation of the ribozyme is dependent on the concentration of the 16S rRNA target sequences present in the blood. The ribozymes that recognize target RNA can be prepared using known methods, as disclosed in RPI patents, such as U.S. Pat. Nos. 6,482,932; 5,496,698, and Vaish et al., *Nature Biotechnology,* Vol. 20, 810-815 (August 2002), all of which are incorporated by reference in their entirety.

[**0034**] The homogenous assay used in the present invention can utilize ribozymes, DNAzymes or catalytic antibodies as the signal-detection technology. The present invention is described in the context of utilizing ribozymes but any technology can be used with the disclosed blood storage device that provides a homogeneous assay and the generation of a detectable signal. The basic concept of the use of DNAzymes and catalytic antibodies are similar to the ribozymes described herein. Regulators or effectors bind to DNAzyme (instead of RNAzyme in the case of ribozyme) and activate the RNA-cleaving activity of the DNAzyme. DNAzymes may have better stability and easier to synthesize than ribozymes. Both DNAzymes and catalytic antibodies are known technologies that have been described in the scientific literature. The following publications describe this technology and the preparation and assays using these reagents are known in the prior art. For example, (1) A General Strategy for effectors-mediated Control of RNA-cleaving Ribozymes and DNA Enzymes, D. Y. Wang, B. H. Y. Lai and D. Sen, *J. Mol. Biol.* (2002), 318, 33-43; (2) A general approach for the use of oligonucleotide effectors to regulate the catalysis of RNA-cleaving ribozymes and DNAzymes. D. Y. Wang, B. H. Y. Lai, A. R. Feldman, and D. Sen, *Nuc. Acid Res.,* 2002, 30, 1735-42; (3) Cleaving DNA with DNA. N. Carmi, S. R. Balkhi, and R. R. Breaker. *Proc. Natl. Acad. Sci,* 1998, 95, 2233-2237; (4) A circular RNA-DNA enzyme obtained by in vitro selection. X D. Kong, S Z. Zhu, X J Guo, X P. Wang, H Y Zhang, and J. Zhang. *Biochem Biophys Res Commun.,* 2002, 292, 1111-5; (5) Catalytic DNA: a noval tool for gene suppression. M J Cairns, E G Saravolac and L Q Sun., *Curr Drug Targets* 2002, 3, 269-79; AND (6) Development of a Genetic Selection for Catalytic Antibodies. J. Gildersleeve, J. Janes, H. Ulrich, P. Yang, C. Barbas, and P. G. Schultz. *Bioorganic & Medicinal Chemistry Letters* 2002, 12 1691-94, which are all herein incorporated by reference.

[**0035**] Once the RNA reporter sequence is cleaved, the quenched signal is released, and detection is then complete. **FIG. 2** provides an example of the test reaction that occurs, showing that the target 16S rRNA sequence (**100**) from a specific strains of bacteria bind or hybridize to a specifically designed ribozyme (**110**) with a specified binding constant for sequences in the targeted 16S rRNA. When the concentration of the target 16S rRNA (**100**) sequence is sufficient to activate the ribozyme (**110**), the ribozyme (**110**) will cleave the RNA reporter sequence (**120**), that in this embodiment is attached to a solid support, such as biochip or beads, (**130**) as carrier. The biochip can be made from any suitable known materials, such as activated glass, plastic material, such as polystyrene or nylon, and ceramic materials. The beads likewise can be made from known materials, such as polystryrene. A detectable quenched signal (**140**) is released and photons are generated. This signal can be visually detected as a fluorescent signal using appropriate ultraviolet (U.V.) analytical equipment, such as a U.V. reading box, a fluorescence spectrophotometer (Beckman DU640 scanning U.V.-vis or Hitachi, San Jose, Calif.—Model F4500) or a fluorimeter, such as the Jenway Model 6200 Fluorimeter. A quantitative signal can be measured using a digital camera that scans U.V. light or fluorescence which are available from Kodak, BioRad or Alph Innotech, Inc. The compartment (**20**) can remain attached to the container (**15**) during the fluorescence signal determination or the compartment can be cleaved from the container (**15**) at the permanent Seal #1 (**32**) being careful not to damage the container (**15**) or the compartment (**20**).

[**0036**] In regard to the configuration shown in **FIG. 2**, it should be noted that only one terminal of the reporter gene, for example of a biotinylated molecule, need be attached to the solid support because the reporter gene and signal probe structure should have some mobility to allow the quencher molecule and reporter dye to split and diffuse away from each other. Thus, in a configuration of the test reagents where both the ribozyme and the reporter sequence are immobilized on the solid support at a portion of these molecules and these reagents should be immobilized so that the signal portion and the quencher portion of the reporter sequence are free to allow some mobility so that the signal can be released.

[**0037**] **FIGS. 3 and 4** provide more detailed disclosure of two configurations of the test reagents in the third section in the presence of the sample/buffer mixture used to detect specific bacterial 16S RNA.

[**0038**] In **FIG. 3**, the allosteric inactivated ribozyme and the reporter gene are immobilized on the solid support. Once the 16S rRNA target sequence binds to the ribozyme, it induces a conformational change and activates the ribozyme. More specifically, approximately a 10 to 28 nucleotide conserved sequence of 16S rRNA (conserved among all bacteria or conserved among a species of bacteria) binds and incorporates itself into the ribozyme. In theory, these ribozymes are responsive to small molecule binding,

which converts them to a secondary active stage. The ribozyme additionally contains a sequence that is capable of cleaving the RNA reporter sequence that also is bound to the biochip. When the ribozyme is activated by binding to the 16S rRNA, it then cleaves the RNA reporter sequence. The RNA reporter sequence is composed of a 5' fluorescent reporter dye, such as FAM (carboxyfluorescein) and a 3' quencher molecules, such as DABCYL (4-dimethylaminophenylazobenzoyl). Other reporter dyes that are available for use in the present invention include TET and JOE. The RNA reporter sequence is designed to form a structure that brings into close proximity the 5' and 3' ends of the probe, which quenches the fluorescent signal. Once the ribozyme cuts the cleavage site in the RNA reporter sequence, the FAM dye and the DABCYL separate and give off a strong fluorescent signal as an electron jumps due to the split of these two molecules.

[0039] **FIG. 4** provides an alternate configuration of test reagents in section **3**, in which the ribozyme is lyophilized and placed in the third section **(50)** of the compartment **(20)** or it is introduced through the wall of the third section in a liquid form via a syringe. The RNA reporter sequence is bound to the biochip. The 16S rRNA sequence binds to complementary sequences in the catalytic site of the ribozyme and activates it, forming a conformational change, which in turn cleaves the RNA reporter sequence at the cleavage site, which the ribozyme specifically recognizes. The signal is generated as described for **FIG. 3** above. The fluorescent signal results as an electron jumps from the donor to the acceptor and the quencher molecule (DABCYL) and dye split (FAM) apart to release the quenched signal.

[0040] A further test reagent configuration provides the ribozyme bound to the biochip and the RNA reporter gene in liquid form that is either lyophilized and placed inside the third section **(50)** of the compartment **(20)** or introduced via a syringe into the third section **(50)** in liquid form.

[0041] A further test reagent configuration provides both the ribozyme and the RNA reporter sequence in a liquid, either as lyophilized reagents in the third section or introduced via a syringe in solution into the third section **(50)**. The ribozyme and the RNA reporter sequence can be added simultaneously or sequentially with the simultaneous addition preferred. Alternatively, if these two test reagents were introduced in liquid form via a syringe, it would not be necessary to have a compartment with three sections. Only the first section **(30)** containing the blood sample and the second section **(40)** containing the buffer would be necessary. In this embodiment, the ribozyme and RNA reporter molecule could be introduced into the second section **(40)** via a syringe. If the test reagents are not immobilized, it is useful to include a solubilizing agent in the buffer at about 1-2%, such as DMSO (dimethylsulfoxide) or other comparable agents.

[0042] Alternatively, the allosteric ribozyme recognizes a protein rather than a nucleic acid. These ribozymes are known as "Allozymes," also produced by RPI, and are activated by the molecular recognition of a target protein. Vaish et al., *Nature Biotechnology,* Vol. 20, 810-815 (August 2002) describe allosteric ribozymes that recognize proteins and is incorporated in its entirety by reference. The target proteins that are recognized by the allozymes are cell surface proteins or secreted proteins of pathogens, such as bacteria, viruses, parasites and other pathogenic agents. If Allozymes are employed, the blood product is collected in the first section **(30)** of compartment **(20)** and sealed off at Seal #1 as discussed above. Pressure is placed on the pressure sensitive Seal #2 **(42)** and the blood product flows into the second section **(40)** which contains an isotonic buffer that dilutes the sample but does not lyse the cells or degrade the proteins present. After sufficient mixing in the dilution buffer, the pressure sensitive Seal #3 **(52)** is broken and the diluted blood sample flows into the third section in which the test reagents are immobilized or lyophilized and ready to go into solution. All of the various permutations of the combination, formulation and location of the test reagents applies to the detection of pathogenic proteins in the sample.

[0043] The container **(15)** can be configured to contain compartments in which some contain the lysing buffer and some contain the dilution buffer. Alternatively, the compartments can contain two sections, one for holding the blood sample to be tested and the other for holding the reagents. In this latter configuration, the buffer can be injected into the compartment. In another embodiment, the compartment can have one section for holding the blood sample. In this case, the appropriate buffer and test reagents can be injected into the compartment and read for signal release.

Experimental Section

EXAMPLE 1

[0044] The claimed device in the form of a PVC bag with six compartments for performing the contamination test is filled with blood or a blood product, such as the platelet fraction, as shown in **FIG. 1**. Each compartment corresponds to Days 2 through 7, respectively. Each compartment is of sufficient size to test 1 ml of the collected platelets, which means that section 1 of each compartment holds a volume of 1 ml. On Day 2, the bag is mixed and the platelet mixture flows into the first compartment. Seal #1 is permanently sealed. Pressure is then placed on section 1 to break the pressure sensitive Seal #2 allowing the platelets to flow into section 2 mixing with the lysis buffer contained in section 2. An effective lysis buffer is composed of a non-ionic detergent, such as Triton X-102 (1-2%), protease enzymes (such as protease K), Li chloride (1.0 M) and other protein denaturation reagents, such urea, guanidine HCl, EDTA (5 mM) or EGTA, and related inhibitors of nucleases; and related protein solubilizing reagents; Hepes (50 mM pH 7.5) and related buffering reagents. Any lysis buffer is intended to be useful in the present invention as long as it is capable of lysing cells, bacteria, and proteins and release the nucleic acids from cellular and bacterial protein components to provide targets for ribozyme activation without interference with the reporter gene sequence and functions. Other buffers and components of a lysis buffer that can lyse cells and degrade proteins to release 16S rRNA target sequences are known to persons skilled in the art. The buffer system for the ribozyme assay may include a combination of metal ions, such as Pb^{2+} and Nd^{3+} (neodymium), as well as divalent ions, such as Mg^{2+} and Mn^{2+}, for optimizing ribozyme activity, i.e., cleavage of the target sequence). It should be noted that only one terminal of the reporter gene, for example such as a biotinylated molecule, and that the reporter gene and signal probe structure must have some

mobility to allow the quencher molecule and reporter dye to split and diffuse away from each other. Thus, in a configuration of the test reagents where both the ribozyme and the reporter sequence are immobilized on the solid support, these reagents should not be immobilized at all points of the molecules, and in fact, the signal molecule and the quencher should be free to allow some mobility so that the signal can be released.

[0045] After lysing the platelet sample for a sufficient period of time, approximately 10 to 20 minutes to obtain a relatively clear mixture, to release the bacterial nucleic acid, the pressure sensitive seal #3 is broken to allow the mixed contents of the first and second sections **(30)** and **(40)** to flow into the third section **(50)**. If the numbers of bacteria have reached a certain concentration level, the number of copies of the 16S rRNA will be sufficient so that the allosteric enzyme is activated to cut the RNA reporter sequence and a detectable signal is released. The binding of the allosteric enzyme to the target sequence is determined by specified binding constants, and is dependent on the target sequence concentration.

[0046] Generally, the hybridization temperature at which the ribozyme binds to the target molecule depends mainly on two factors: (1) the length of the nucleic acid that is hybridized and (2) the salt concentration within the buffer system. A temperature range of about 45-60° C. is preferred. Known buffers that facilitate the hybridization without denaturing and changing the hybridization profile of the ribozymes is preferred and are known to persons skilled in the art. Ribozyme activation conditions are disclosed in several of the recited publications in this disclosure and can be utilized to perform the testing method of the present invention. Also applying a heat lamp or heat block to the compartment may be helpful to accelerate the assay as well as to increase its specificity.

[0047] The detectable sequence is fluorescent and can be visually read by an appropriate U.V. measuring device, such as a fluorescent spectrophotometer or U.V. light box. The total time for the assay is approximately 1 to 2 hours; however, the assay time can be shortened by heating the compartment once the test reagents are added, such as by using a U.V. light box that contains a heat lamp or a heating block.

[0048] The publications and patent documents cited herein are incorporated in their entirety by reference. Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification and the practice of the invention disclosed herein.

EXAMPLE 2

[0049] This example demonstrates the use of a biochip coated with antibody to capture corresponding antigen in blood and detection of the target antigen by mass spectroscopy.

[0050] A biochip (Ciphergen PS20 Protein chip) was covalently bound to a mouse monoclonal antibody against HIV P-24 core protein. The biochip then was placed in a tube where 12 ml of human blood was circulated a flow rate of about 30 ml/min at 37° C. HIV P-24 antigen was spiked in the blood at a concentration of 0-200 ng/ml. After 10 min of circulation, the chip was removed and the bound antigen was detected by mass spectroscopy (Ciphergen Protein Chip Reader).

[0051] The results showed that antigen can be detected at a concentration of about 8 ng/ml in a 12 ml blood sample. **FIG. 14** depicts a typical result.

EXAMPLE 3

[0052] This example demonstrates the use of a biochip coated with antigen to capture corresponding antibody in blood and detection of the target antibody by mass spectroscopy.

[0053] A biochip (Ciphergen PS20 Protein chip) was covalently bound to HIV P-24 core protein. The biochip then was placed in a tube where 12 ml of human blood was circulated at a flow rate of about 30 ml/min at 37° C. Mouse monoclonal antibody against HIV P-24 was spiked in blood at a concentration of 0-20 ug/ml. After 10 min. of circulation, the chip was removed and the bound antibody was detected by mass spectroscopy (SELDI-MS from Ciphergen).

[0054] The results showed that antibody can be detected at a concentration of about 1 ug/ml in a 12 ml blood sample.

We claim:

1. A biological sample storage device for storing and testing blood or blood products, comprising:

a container for receiving and storing blood or blood products; and

at least one compartment for testing the blood or blood products, wherein said compartment comprises:

at least a first section for holding a portion of the blood or blood products, and optionally for testing the portion of the blood or blood products.

2. The device according to claim 1, wherein the first section is arranged contiguous to the container so that the blood products can flow from the container to the first section.

3. The device according to claim 2, wherein the first section is designed such that the first section can be sealed from the container so that the blood products sealed into the first section can be used for testing.

4. The device according to claim 2, further comprising at least one additional section for testing the portion of the blood or blood products held in the first section, said additional section being arranged in sealed contact with another portion of the first section, different from a portion of the first section in contact with the container.

5. The device according to claim 4, wherein the additional section comprises a pressure sensitive seal between the first section and the additional section that can broken by the application of pressure, such that breaking the seal in the additional section allows mixing of the contents of the first and additional sections.

6. The device according to claim 1, wherein the container comprises a plurality of compartments.

7. The device according to claim 1, wherein each of the compartments is arranged as a protruding element from the container.

8. The device according to claim 7, wherein the first section is arranged contiguous to the container so that the blood products can flow from the container to the first section.

9. The device according to claim 8, wherein the first section is designed such that the first section can be sealed

from the container so that the blood products sealed into the first section can be used for testing.

10. The device according to claim 4, wherein the at least one additional section comprises a second and a third section, the second section being arranged in sealed contact with another portion of the first section, different from a portion of the first section in contact with the container, and wherein the third section being arranged in sealed contact with another portion of the second section, different from a portion of the second section in sealed contact with the first section.

11. The device according to claim 10, wherein the second and third sections comprise pressure sensitive seals that can broken by the application of pressure such that breaking the seal in the second section allows mixing of the contents of the first and second sections and breaking of the seal in the third section allows transfer of the mixed contents of the first and second sections into the third sections.

12. The device according to claim 11, wherein the second section contains a buffer, wherein said buffer is a lysis buffer or an isotonic buffer.

13. The device according to claim 11, wherein the third section contains test reagents for testing the transferred mixed contents from the first and second sections.

14. The device according to claim 13, wherein the test reagents are a catalytic molecule and a reporter sequence.

15. The device according to claim 14, wherein said catalytic molecule is an inactivated ribozyme, a DNAzyme or a catalytic antibody.

16. The device according to claim 14, wherein the test reagents are an inactivated ribozyme and an RNA reporter sequence.

17. The device according to claim 14, wherein at least one of the catalytic molecule and reporter sequence is immobilized to a solid support.

18. The device according to claim 14, wherein at least one of the catalytic molecule and reporter sequence is in a lyophilized form.

19. The device according to claim 1, wherein the blood products comprise blood platelets.

20. A method of testing a blood or blood product for a target molecule indicative of contamination in said blood or blood product, comprising

providing a sample of a blood product in a compartment of the storage device for storing and testing blood or blood products, comprising:

a container for receiving and storing blood or blood products; and

at least one compartment for testing the blood or blood products, wherein said compartment comprises:

at least a first section for holding a portion of the blood or blood products, and optionally testing the portion of the blood or blood products;

contacting the blood product in the compartment with a lysing buffer;

releasing the target molecule from the cells and protein in the blood product; and

detecting the presence of the target molecule.

21. The method according to claim 20, wherein the target molecule is a 16S ribosomal RNA or a nucleic acid associated with a pathogen.

22. The method according to claim 20 or **21**, wherein the detecting step employs test reagents comprising a catalytic molecule and a reporter sequence.

23. The method according to claim 22, wherein said catalytic molecule is an inactivated ribozyme, a DNAzyme or a catalytic antibody.

24. The method according to claim 22, wherein said test reagents are an inactivated ribozyme and an RNA reporter sequence.

25. The method according to claim 24, wherein the inactivated ribozyme binds to the target molecule, which activates the ribozyme that cleaves the RNA reporter sequence and releases a detectable sequence.

26. A method of testing a blood or blood product for a target molecule indicative of contamination in said blood or blood product, comprising

providing a sample of a blood product in a compartment of the storage device for storing and testing blood or blood products, comprising:

a container for receiving and storing blood or blood products; and

at least one compartment for testing the blood or blood products, wherein said compartment comprises:

at least a first section for holding a portion of the blood or blood products, and optionally testing the portion of the blood or blood products;

contacting the blood product in the compartment with a buffer to dilute the sample; and

detecting the presence of the target molecule.

27. The method according to claim 26, wherein the target molecule is a protein associated with a pathogen.

28. The method according to claim 26 or **27**, wherein the detecting step employs test reagents comprising a catalytic molecule and a reporter sequence.

29. The method according to claim 28, wherein said catalytic molecule is an inactivated ribozyme or catalytic antibody.

30. The method according to claim 28, wherein said test reagents are an inactivated ribozyme and an RNA reporter sequence.

31. The method according to claim 30, wherein the inactivated ribozyme binds to the target molecule which activates the ribozyme that cleaves the RNA reporter sequence and releases a detectable sequence.

* * * * *

专利名称(译)	生物样品储存装置和生物样品污染测试方法		
公开(公告)号	US20040209353A1	公开(公告)日	2004-10-21
申请号	US10/733766	申请日	2003-12-12
[标]申请(专利权)人(译)	希龙公司		
申请(专利权)人(译)	Chiron公司		
当前申请(专利权)人(译)	诺华疫苗与诊断，INC.		
[标]发明人	CHIEN DAVID PHELPS BRUCE H		
发明人	CHIEN, DAVID PHELPS, BRUCE H.		
IPC分类号	A61B5/00 A61M5/32 B01L3/00 B01L7/00 C07H19/00 C07H21/02 C07H21/04 C12M1/34 C12P19/34 C12Q C12Q1/68 G01N33/48 G01N33/53		
CPC分类号	A61B5/14557 B01L3/5027 B01L7/52 B01L2200/027 B01L2200/10 B01L2300/0636 B01L2300/0825 B01L2300/0877 B01L2300/0887 G01N33/5302		
优先权	60/435287 2002-12-23 US 60/432665 2002-12-12 US		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及一种用于筛选血液和血液制品的方法，该方法利用可用于存储和测试血液或血液制品的专用血液和血液制品存储装置，包括具有至少一个具有一个或多个部分的容器的容器，用于存储和测试目标分子的血液或血液制品。该方法利用均相测试系统，其使用核酶，DNA酶或催化抗体。