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LYMPHOCYTE TRANSPORT BAG

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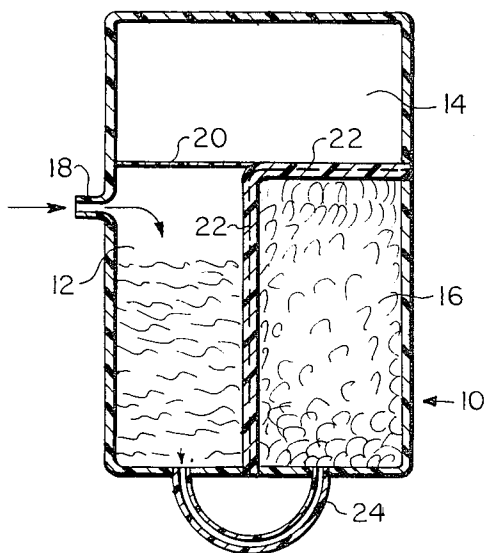


FIG. 1

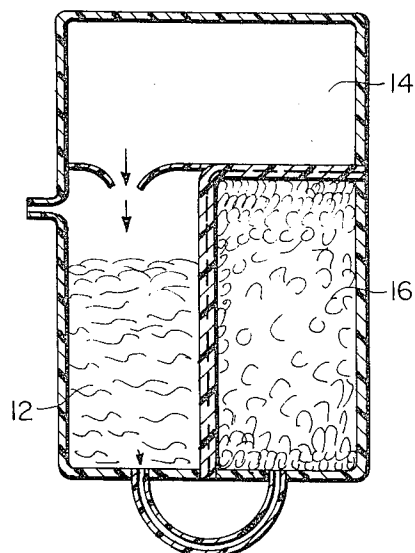


FIG. 2

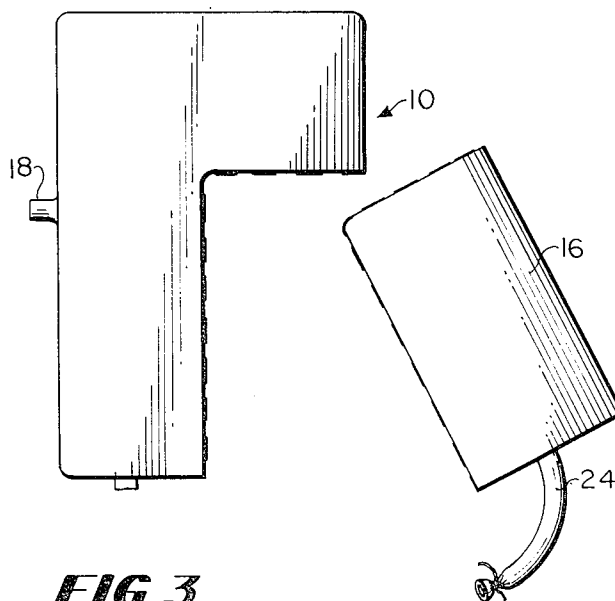


FIG. 3

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LYMPHOCYTE TRANSPORT BAG

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6 Claims

ABSTRACT OF THE DISCLOSURE

A compartmented mixing and shipping bag for blood. The bag, formed of plastic, is provided with three compartments; the first compartment, into which the blood is injected, contains nylon to which the undesirable granulocytes adhere. A second compartment contains a flushing ingredient and the seal between the first and second chambers is broken and the flushing medium is used to flush the lymphocytes from the first chamber into a third chamber. The third chamber is separated from the first two chambers and is used as a shipping container.

The present invention relates to a separating and shipping system and, more particularly, to the shipment of human blood lymphocytes for extended distances and without degradation.

Blood typing is an extremely important consideration in the transplantation of human organs in order to match donors and recipients for kidney, heart and other organs. The lymphocyte cytotoxicity method appears to be the most widely used for determination of human histocompatibility antigens. One of the advantages of the lymphocyte cytotoxicity test over the leukoagglutination test is the fact that lymphocytes can be kept in a testable condition longer than granulocytes can. This is an important consideration since the typing work is complex and the need exists to send samples to the limited number of qualified labs; lymphocytes will die within two days if blood is sent by currently available means. Accordingly, the need for better shipment methods is acute.

This need for the development of better means for transporting lymphocyte specimens is increased by recent evidence that HL-A antigens are important in man, with the attendant need to cross-check typing results in several typing laboratories. Collaborative efforts made possible by exchanges of sera have resulted in extremely rapid advances in the past few years and a system permitting more frequent exchanges of cells among leukocyte-typing laboratories can be expected to result in further advances.

It is, accordingly, an object of the present invention to provide for the improved shipping of unstable materials.

It is another object of the present invention to provide for the improved transportation of lymphocyte specimens.

It is another object of the present invention to provide an improved container for shipping lymphocyte specimens.

It is another object of the present invention to provide a simplified and inexpensive device and method for separating an undesirable or desirable blood component, retaining the desirable component and shipping the desirable component safely to a distant location.

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rating an undesirable or desirable blood component, retaining the desirable component and shipping the desirable component safely to a distant location.

These and other objects and the nature and advantages of the instant invention will be more apparent from the following detailed description of an embodiment, taken in conjunction with the drawing wherein:

FIG. 1 is a sectional view of an embodiment of a device in accordance with the present invention, showing one stage of its use;

FIG. 2 is a sectional view showing the device of FIG. 1 in a second stage of its use; and

FIG. 3 is a plan view of the device of FIGS. 1 and 2 shown in its third stage of its use.

A preferred embodiment of the present invention is illustrated in FIGS. 1-3 which shows a plastic bag divided into three sections or compartments. The basic principle of the bag is to remove the granulocytes from the blood in the first section of the bag, for it is the early death and subsequent release of enzymes from these dead cells which causes the death of lymphocytes. Thus, the bag 10, preferably formed of polyvinyl chloride, although it may be formed of any suitable plastic, is provided with a first compartment 12, a second compartment 14, and a third compartment 16. A blood inlet 18 is provided for the first compartment 12, and a frangible separating wall 20 is provided between the first and second compartments 12 and 14. A perforated separating wall 22 separates the third compartment 16 from the first and second compartments 12 and 14, and the interior of the first compartment 12 is connected to the interior of the third compartment 16 by means of a tube 24.

Very briefly, in usage, blood is injected into chamber 12, which contains a granulocyte adhering material such as nylon, through the port 18. After coating the nylon with blood, the seal 20 between the first chamber 12 and the second chamber 14 is broken, allowing the media in compartment 14 to flush the lymphocytes from the nylon in compartment 12 as illustrated in FIG. 2. Since granulocytes will be adherent to the nylon, they will not be flushed out into the third compartment 16 as will the lymphocytes. The third chamber 16, preferably containing an absorbent material such as cotton, serves to retain the lymphocytes, red cells and the media. The chamber 16 is separated along the perforation 22, the tubing 24 is cut close to chamber 12 and is then tied. The compartment 16 may then be mailed to any suitable lab with an attached label.

The flushing medium initially present in the second compartment 14 and in which the lymphocytes are transported ultimately in the third compartment 16 should be a tissue culture medium. One known tissue culture medium which has been tested and is highly effective is McCoy's medium; this medium includes L-glutamine, antibiotics and 30% fetal calf serum, and is known to be effective for propagating leukocytes, issues from biopsy specimens, and the most fastidious of primary and perpetually cultivated cell strains (McCoy, T. A. et al.; Proc. Soc. Exper. Biol. & Med., V. 100, pages 115-118, 1959). Other tissue culture media are also satisfactory, e.g. me-

dium 199 has been used successfully. A medium which is less complex than McCoy's medium may be used if desired.

The form of the nylon in compartment 12 is not critical and in one example nylon tricot has been used successfully. In other words, the nylon may be in woven fabric form, in fibrous form, in granular form. Although any type of nylon may be used, nylon 66 which is readily available in cloth form is preferred. Any material may be substituted for nylon which will serve the function of causing adherence of the granulocytes.

With regard to the adsorbent material in the shipping compartment 16, any absorbent pad may be used. Tampons produced by Tampex of Palmer, Mass. have been successfully used. The absorbent material in shipping container 16 serves to minimize leakage in case a tear develops in the container wall during shipping, and serves a supplementary purpose in trapping any granulocytes which may not have been removed in the first compartment 12.

If desired, a two chamber bag may be used in place of the illustrated three chamber bag 10. In this event, a chamber corresponding to the second chamber 14 of the three compartment bag 10 is eliminated and the tissue culture medium, used for flushing, is added from an outside source, such as through the same opening through which the blood has previously been injected.

In use (see "Transplantation," vol. 8, No. 3, published Sept. 26, 1969, pgs. 311-314), 15 milliliters of heparinized blood are injected into chamber 12 containing nylon tricot. The blood is kneaded well into the nylon and the granulocytes adhere to the fibers within 2-5 minutes. Then, 20 ml. of McCoy's medium without calf serum are added to flush out red cells and lymphocytes through the tube 24 and into the chamber 16. Chamber 12 must be squeezed tightly to transfer the blood-media mixture into chamber 16 containing an absorbent pad. Bag 16 is then torn along the perforated line and tube 24 is cut close to compartment 12; bag 16 is then tied and labeled and may, if desired, be sent through the mail by special delivery.

Upon receipt, the bag 16 is put in a 50-ml. syringe or a bag squeezing device, 10 ml. of saline are added and the bag is squeezed tightly with the plunger. The effluent is centrifuged, the buffy coat is removed, and the red cells are agglutinated in accordance with known procedures (e.g. Terasaki et al., "Ann. Rev. Med.," vol. 20, page 175).

Blood was received by ordinary air mail special delivery from England, Switzerland, Japan, Venezuela, Israel, and Canada with 80-90% viable cells. In all instances, the samples were received within 3½ days of bleeding. The yield has been in the range of 180,000 cells-1,000,000 purified lymphocytes. Considerable variability in yield was encountered, for many patients were uremic or cadaver donors. A trend toward loss in yield and in viability with time occurred. Blood from normal persons stored at room temperature for 7-8 days showed some loss in viability and yield but was suitable for typing. Over 180 tests could be set up readily by the microtest. At times, if the nylon had not been strongly squeezed by the sending center, it was possible to refush the nylon chamber if more lymphocytes were needed. Debris presumably contributed by lysed granulocytes, as well as by intact granulocytes, was almost completely absent in these preparations.

Whether lymphocytes stored for 7 days could be typed by the cytotoxicity test was then investigated. Storage could alter the cell membrane so that the cell might be more susceptible to lysis, or it could produce an apparent loss in antigenicity by loss in combining sites on the surface. The lymphocytes of 11 persons were tested accordingly on the day after bleeding and after 7 days of storage with 54-108 cytotoxic antisera representing 12 HL-A specificities are seen from the following table.

Effect of storage on HL-A typing lymphocytes *

Cell	HL-A specificities ^b	Antiserum reactions ^a				Discrepancy, percent
		F+S+	F-S-	F+S-	F-S+	
5 E.C.	1, 12	11	43	0	0	0
S.L.	3, 6, 7	12	39	2	1	5.5
H.U.	4, 5, 9, 11	13	32	6	3	16.6
R.C.	5	4	49	1	0	1.8
R.B.	4, 6, 9, 12	14	35	4	1	9.2
C.P.	5, 6	10	43	0	1	1.8
L.F.	2, 4, 6, 8, 10	16	34	2	2	7.4
B.M.	4, 5, 6	16	36	2	0	3.7
D.A.	1, 11	7	41	3	2	9.4
J.W.	1, 2, 6, 8, 10	38	58	1	9	9.3
M.Sch.	3, 4, 6, 7	33	67	2	4	5.6

* Lymphocytes were isolated from blood on the day of collection and following 7 days of storage. The cells were tested on a panel of 54-108 antisera representing 12 HL-A specificities.

^b 1, HL-A1; 2, HL-A2; 3, HL-A3; 4, Te4; 5, HL-A5; 6, Te7; 7, HL-A7; 8, HL-A8; 9, Te9; 10, Te10; 11, Te3; 12, Te12.

^a F, fresh cells; S, stored for 7 days.

Although some variations in reactions with different antisera were found, no changes in the HL-A classification of the cell were produced and no loss or gain of HL-A specificity occurred. With respect to each antiserum reaction, it can be noted that 84-100% of the reactions with each cell were reproducible after 7 days of storage, and the few discrepancies appeared to occur randomly.

It will be understood that various changes may be made without departing from the scope of the invention. For example, the flushing and storage fluid, i.e. the tissue culture medium, may vary in composition; further, changes may be made in the construction of the bag, i.e. for ease in mailing, another bag in which the nylon compartment can be separated from the second compartment may be provided, thus permitting the mailing of a simple small pad. Also, the selection of the material from which the bag is made variable.

The foregoing description of the specific embodiment will fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify such specific embodiment and/or adapt it for various applications without departing from the generic concept and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalence of the disclosed embodiments. It is to be understood that the phraseology and terminology employed herein is for the purpose of description and not of limitation.

What is claimed is:

1. A construction for the separation of blood components and the subsequent shipping of said components, said construction comprising:

means to receive and contain a blood sample, said means comprising a first compartment formed of flexible material, said compartment containing means to retain granulocytes from said blood sample, and said first compartment being further provided with means for the feeding thereto of a flushing tissue culture medium; and

a shipping container detachably connected to said first compartment and formed of a flexible material, and passageway means between said first compartment and said shipping container for the passage there-through of lymphocytes from said blood sample and said tissue culture medium.

2. A construction in accordance with claim 1 wherein said shipping container is filled with an absorbent material.

3. A device in accordance with claim 2 wherein said granulocyte retaining material in said first compartment is nylon and wherein said absorbent material in said shipping container is cotton.

4. A device in accordance with claim 1 of initially unitary construction and wherein said flexible material is plastic film, said first compartment being joined to said shipping container by a perforated connection, and said passageway means comprising a tube running between

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said first compartment and said shipping container independent of said perforation connection.

5. A device in accordance with claim 4 further comprising as part of said unitary structure means to initially contain said tissue culture medium, and wherein said means to feed said tissue culture medium to said first compartment comprises a frangible separation between said first compartment and said means to contain said tissue culture medium.

6. A device in accordance with claim 5 wherein said tissue culture medium is McCoy's medium.

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23—230 B, 253 R, 258.5, 292; 128—272; 195—1.8, 127;
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