United States Patent [19]

Eppes

[11] **3,836,335**

[45] **Sept. 17, 1974**

[54] **REAGENT STORAGE AND DISPENSING SYSTEM**

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[73] Assignee: **Corning Glass Works,** Corning, N.Y.

[22] Filed: **June 1, 1973**

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[52] **U.S. Cl.** ..... **23/259,** 23/253 R, 222/386.5

[51] **Int. Cl.** **G01n 1/10**

[58] **Field of Search** ..... 23/253, 230, 259, 292; 222/395, 386, 386.5; 141/130; 73/425.4 R

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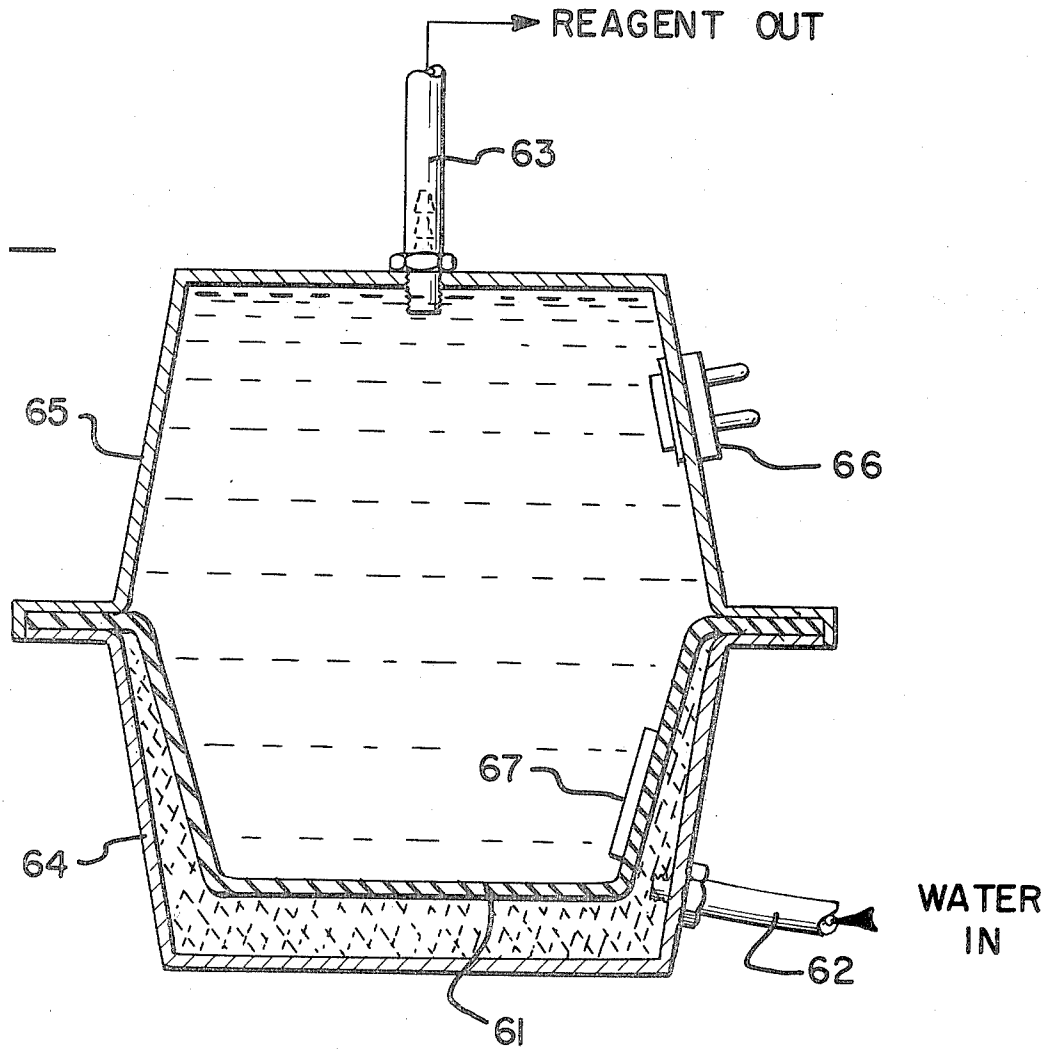
Primary Examiner—R. E. Serwin

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[57] **ABSTRACT**

Pressure vessels each have a movable separator which divides the vessel into two chambers. During filling, reagent is supplied to one chamber. During a dispensing operation, water pressure is applied to the other side of the separator. Solenoid valves are selectively operated to dispense accurate volumes of reagent from the vessels under pressure supplied by the water on the other side of the separator.

10 Claims, 8 Drawing Figures



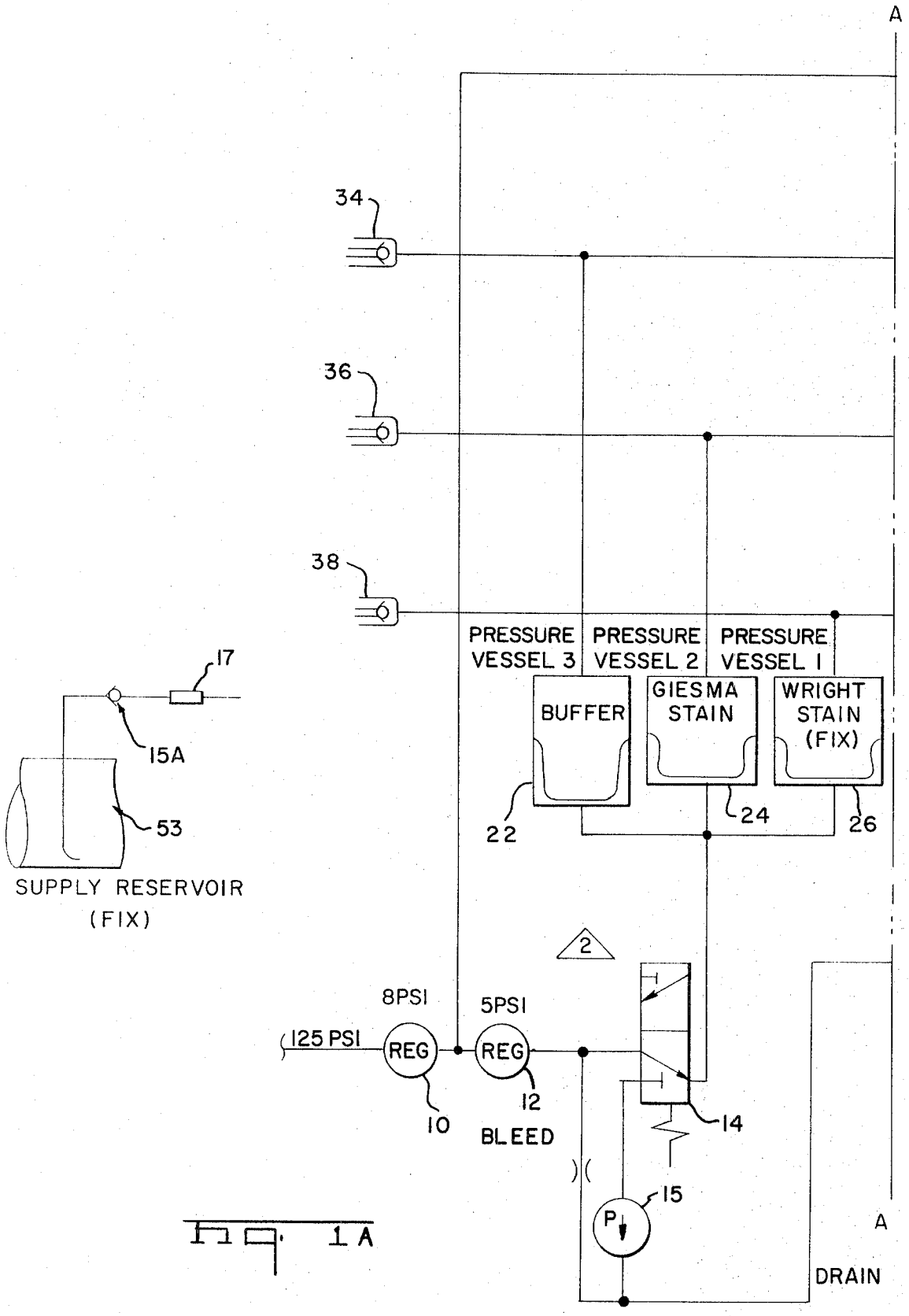

A
34
36
38
PRESSURE VESSEL 3
PRESSURE VESSEL 2
PRESSURE VESSEL 1
BUFFER
GIESMA STAIN
WRIGHT STAIN (FIX)
22
24
26
17
15A
53
SUPPLY RESERVOIR (FIX)
2
8PSI
5PSI
125 PSI
REG
REG
10
12
BLEED
14
15
P
A
DRAIN

Fig. 1A

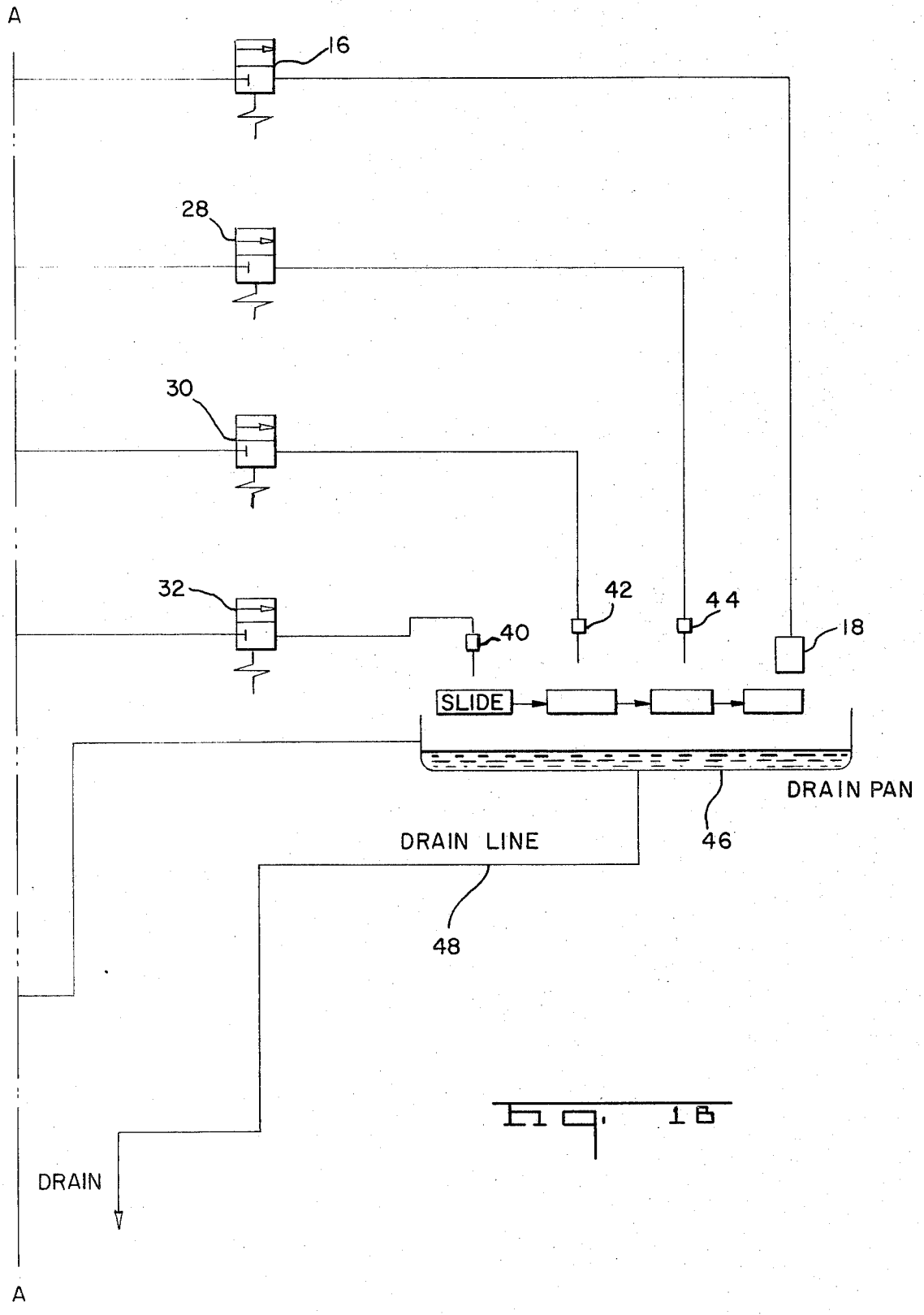


Fig. 1B

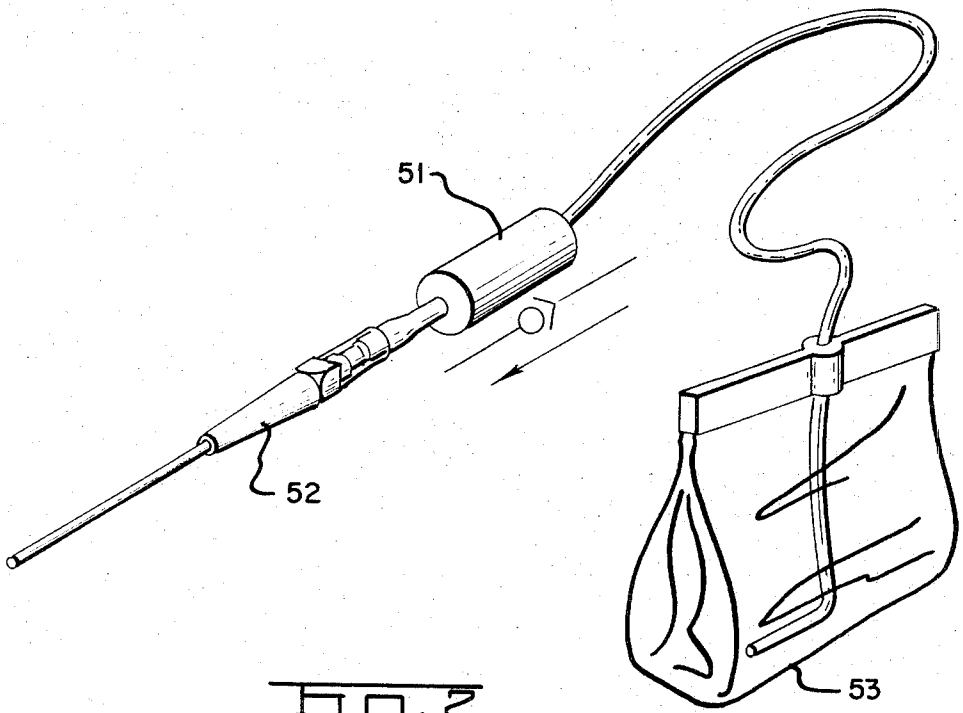


Fig. 2

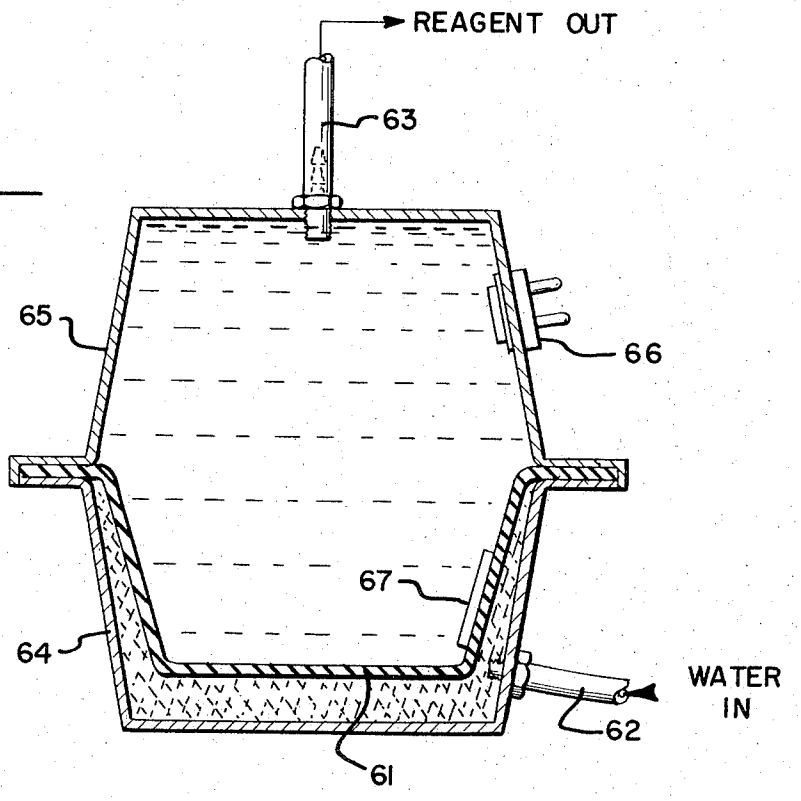


Fig. 3

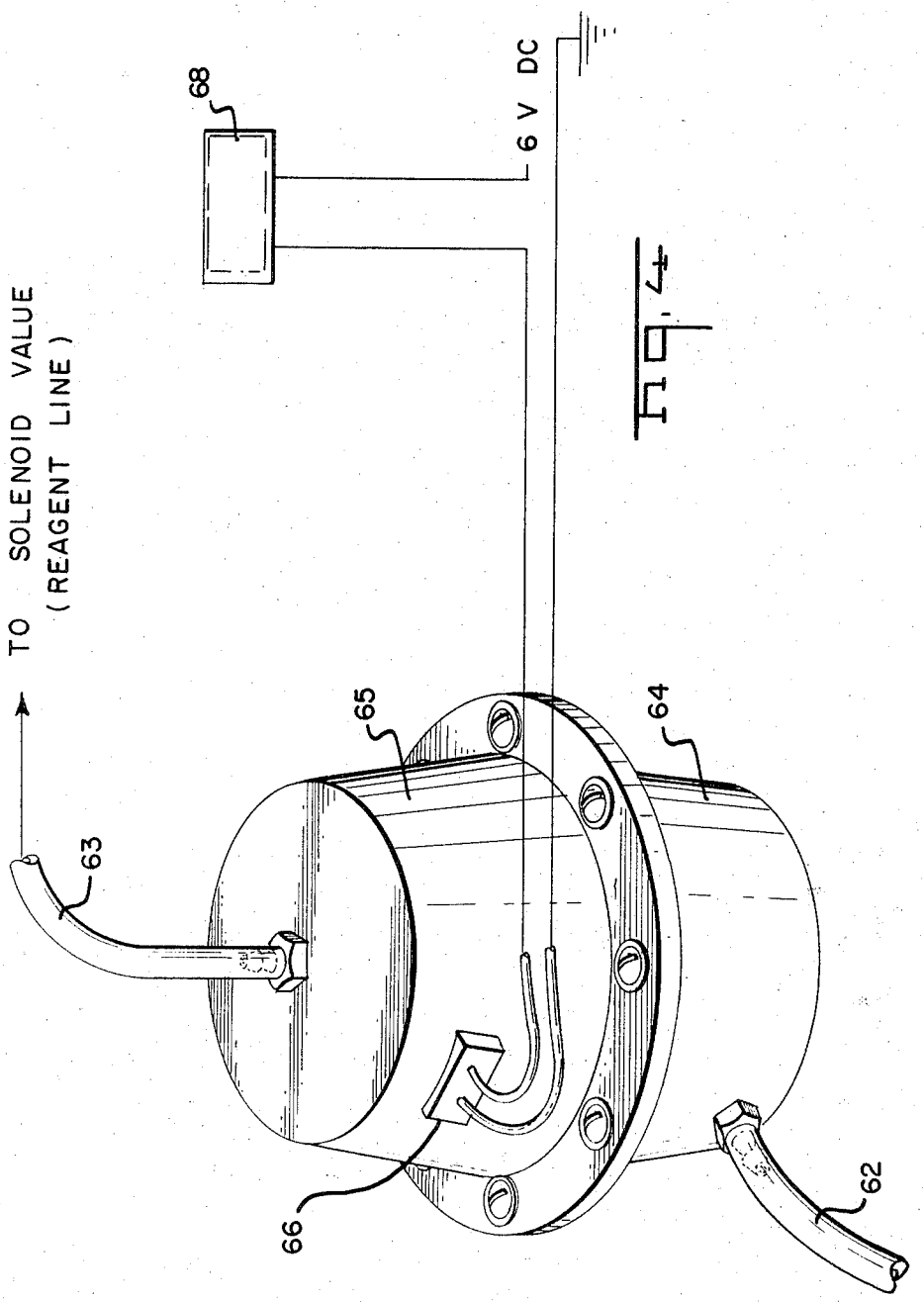


Fig. 4

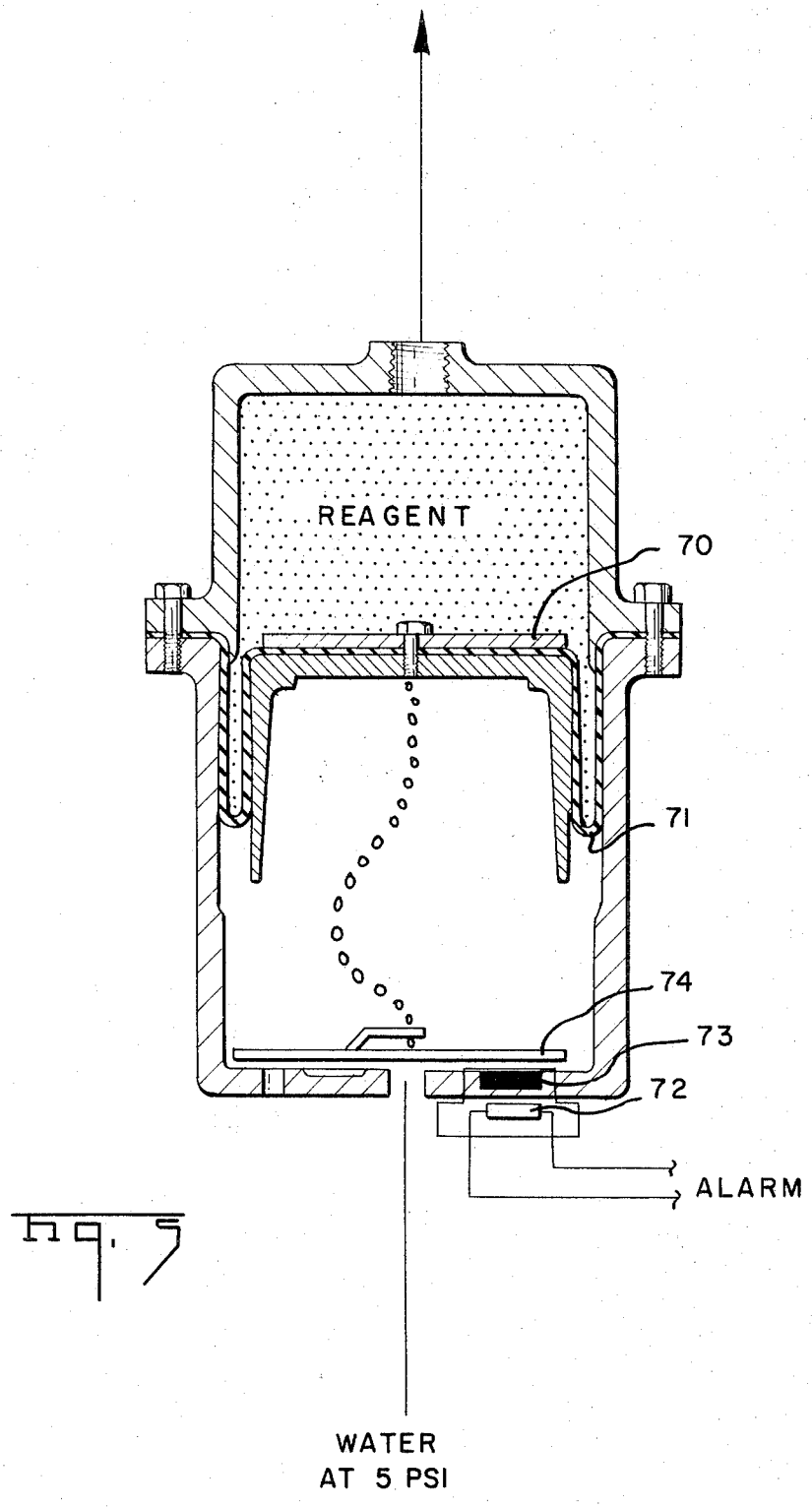


Fig. 5

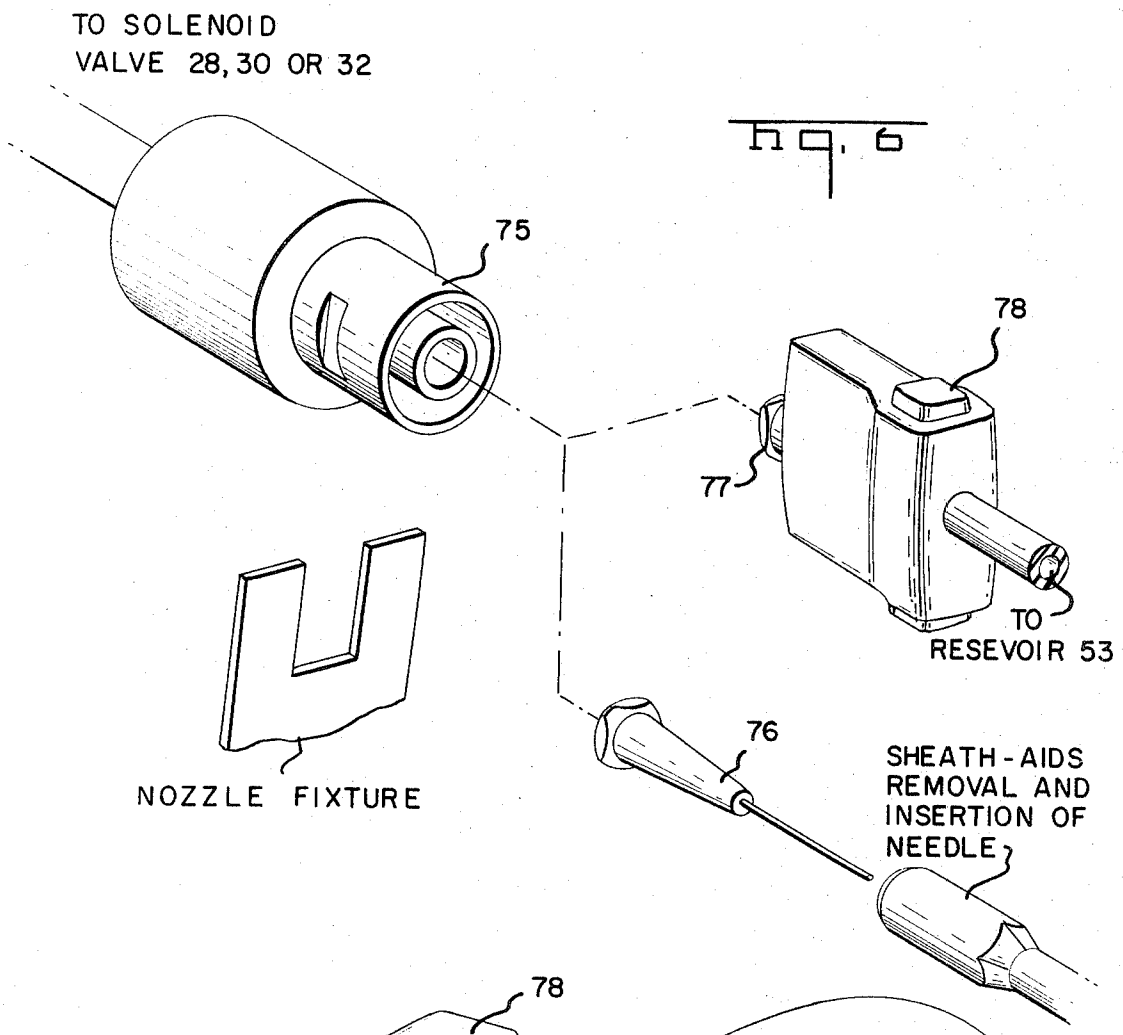

TO SOLENOID
VALVE 28, 30 OR 32
75
78
77
TO
RESEVOIR 53
NOZZLE FIXTURE
76
SHEATH - AIDS
REMOVAL AND
INSERTION OF
NEEDLE

Fig. 6

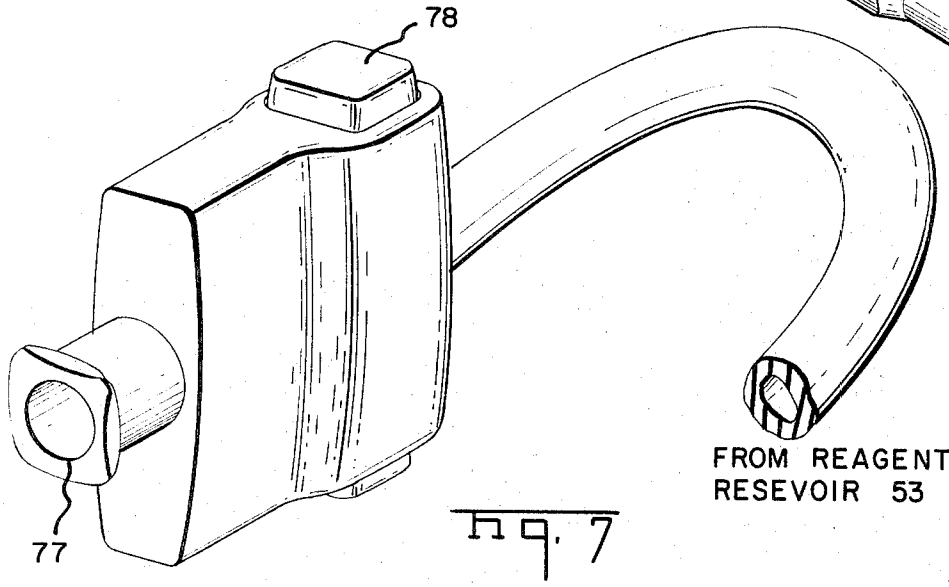

78
77
FROM REAGENT
RESEVOIR 53

Fig. 7

REAGENT STORAGE AND DISPENSING SYSTEM

BACKGROUND OF THE INVENTION

This invention relates to reagent storing and dispensing systems and more particularly to a system for storing and dispensing fix, stain and buffer reagents for staining a film of blood cells on a laboratory slide.

In the analysis of blood samples, the blood is smeared on a laboratory slide and the smear is stained. By counting the leukocytes on the stained smear, laboratory technicians perform what is referred to as a white blood cell differential. Automation of this differential has significant economic impact because the differential is performed so frequently at every hospital. A thesis by J. W. Bacus, "An Automated Classification of the Peripheral Blood Leukocytes by Means of Digital Image Processing," University of Illinois, Chicago, 1971, describes one automated system.

Copending application Ser. No. 353,004, filed Apr. 20, 1973, Douglas A. Cotter, "Image Scanning Converter for Automated Slide Analysis," describes a system developed by my co-workers for automatically scanning and digitizing the count of the leukocytes on the stained smear.

SUMMARY OF THE INVENTION

In accordance with this invention a reagent storing and dispensing system includes a plurality of pressure vessels each having a movable separator which divides the vessel into two chambers. Reagent is supplied to and stored in one of the chambers. A regulated water pressure is applied to the other chamber. solenoid operated valves are selectively actuated for intervals of time which dispense accurate volumes of reagent from the vessels under pressure supplied by the water pressure against the separator. In this manner, accurate volumes of reagent are supplied and the time at which they are delivered is easily adjustable. Furthermore, the pressure vessel of this invention effectively isolates the reagent from moisture which can be absorbed from the air or from any other material with which the reagent may react.

Further in accordance with this invention the pressure vessels are filled from flexible supply reservoirs. In one embodiment of the invention the connection between the resevoir and the pressure vessel includes a needle fitting connected to the solenoid valve and a mating fitting connected to the reservoir. After the pressure vessel is filled, a needle-like dispenser is placed on the needle fitting. The needle-like dispenser supplies accurate volumes of reagent passed by the associated solenoid valve.

The foregoing and other objects, features and advantages of the invention will be better understood from the following more detailed description and appended claims.

DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B together show the reagent storage and supply system;

FIG. 2 shows the supply reservoir;

FIG. 3 is a cross-section of the pressure vessel;

FIG. 4 is a view of the outside of the pressure vessel;

FIG. 5 shows a modification of the pressure vessel;

FIG. 6 shows a modified connection of the flexible supply reservoir; and

FIG. 7 shows the mating fitting of FIG. 6.

DESCRIPTION OF THE PREFERRED EMBODIMENT

In FIGS. 1A and 1B the system is shown in the pressurized stand-by condition. Water at line pressure passes through the pressure regulator **10** where pressure is reduced to approximately 8 to 10 lbs. per square inch. (PSIG. Part of the available water is applied to solenoid valve **16.** (The solenoid valve **16** is depicted in its closed condition. In the notation used two states of the valve are depicted by two blocks. The lower block depicts the closed condition whereas the upper block depicts the open condition of the valve.) When the solenoid valve **16** is actuated, available water passes through the valve **16** to the rinse discharge tube **18.**

Water at 8 PSI is also applied to the regulator **12** which reduces the pressure to approximately 5 PSIG. Water at this pressure passes through the solenoid valve **14** into the water containing chambers of the pressure vessels **22, 24** and **26.** Each of the pressure vessels are divided into two chambers by a separator as will be described more fully with respect to FIGS. **3** and **5.**

In the staining of blood smeared laboratory slides, pressure vessel **26** will normally contain a fix, such as Wright stain, in the reagent containing chamber. Pressure vessel **24** contains a stain such as Giesma stain and pressure vessel **22** contains a buffer in the reagent chamber. Lines on the reagent side of each pressure chamber are connected to solenoid operated valves **28, 30** and **32** which dispense reagent from the pressure chambers **22, 24** and **26** respectively.

These valves are connected to dispensers **40, 42** and **44** which sequentially apply the reagents to the slide. The dispensers **40, 42** and **44** are of an appropriate diameter which typically is 0.023 inches. They are individually removable for ease of cleaning or for replacing with dispensers of a different diameter to achieve different flow rates.

The solenoid valves **28, 30** and **32** control when and how much reagent is dispensed. The time in the process cycle at which the valve is energized determines when the liquid is dispensed. The length of time that the valve is energized determines the volume which is dispensed.

Liquid from the rinse process is collected in the drain pan **46** and carried away by the drain line **48.**

When it is desired to refill the pressure vessels **22, 24** and **26** with reagent, the solenoid valve **14** is actuated to provide a connection between the bottom chambers of the pressure vessels and a pump **15.** The pump **15** is wired in parallel with the solenoid valve **14.** When a fill push button, not shown, is depressed both the solenoid valve **14** and the pump **15** are energized. Water is pumped from the bottom chambers of the vessels **22, 24** and **26** and into the drain pan **46.** In one embodiment of the invention new reagent is supplied to each of the vessels during a fill operation through bladder valves **34, 36** and **38.** Bladder valves **34, 36** and **38** are respectively connected to the pressure vessels **22, 24** and **26.** These bladder valves are part of a connection between a supply reservoir **53** containing the appropriate reagent and the pressure vessel in which this reagent is to be stored. In FIG. **1A** the connection between the supply reservoir and the pressure vessel is shown as

including a valve 15A and a mating fitting 17 which, in the first embodiment to be described is a probe.

The supply reservoir of this first embodiment is shown in more detail in FIG. 2. It includes a flexible collapsible container 53. Typically this is constructed of three mil polyethylene. A line from the supply reservoir leads to a check valve 51 and thence to a dispensing probe 52. When the probe 52 is inserted into a bladder valve 34, 36 or 38 reagent from the supply reservoir flows into the pressure vessel.

A first embodiment of the pressure vessel is shown in FIGS. 3 and 4. It includes the flexible membrane 61. One type of suitable material for this impermeable membrane is butal rubber on a dacron matrix. The membrane is attached around the periphery of the approximate middle of the vessel. The membrane is sufficiently flexible to extend almost completely to one side of the vessel. As shown in FIG. 3, the membrane extends to the bottom of the vessel when it is filled with reagent. As the reagent is emptied from the vessel the membrane 61 follows the reagent and isolates it from air or other material with which the reagent might react. In particular, those reagents containing methyl alcohol may otherwise absorb moisture from the air. The membrane 61 is sufficiently flexible to extend completely to the top of the vessel when emptied of reagent.

Water enters the pressure vessel through the inlet 62 and reagent leaves the vessel through the outlet 63. The pressure vessel includes a bottom portion 64 and a top portion 65 which are secured together with the diaphragm between them. The lower portion 64 and the upper portion 65 of the pressure vessel are constructed of a nonmagnetic material such as acetal copolymer.

A warning system informs the operator when the reagent supply is near depletion. A magnet 67 is attached to the membrane. As the reagent is depleted the membrane is forced upward until the magnet 67 approaches the reed switch 66 which is attached to the vessel wall. When the magnet is sufficiently close, the switch closes completing the circuit to an indicator light 68 (FIG. 4) which is mounted in a suitably visible location on the machine. The operator is advised of a nearly depleted reagent.

The operation of the system is described with reference to FIGS. 1A and 1B. The procedure for filling a dry system is as follows. The line leading to the regulators 10 and 12 is pressurized. The valve 14 is in the position shown. Water pressure of approximately 8 PSI is applied to solenoid valve 16 and water pressure of approximately 5 PSIG is applied to the water containing chambers of pressure vessels 22, 24 and 26. The flexible membrane in each pressure vessel is deformed and either compresses the air on the opposite side of the membrane to 5 PSIG or fills the available space in the chamber, whichever occurs first. The solenoid valves 28, 30 and 32 are momentarily opened by a manual override. Any trapped air is bled out insuring that all membranes in the pressure vessels are forced completely to the reagent side of the pressure vessel.

The procedure from this point on is the procedure used to fill a previously charged system. Valve 14 and pump 15 are energized. The fitting 17 on the supply reservoir 53 is fitted to the proper bladder valve.

Because water is being drawn from the water chamber of the pressure vessel, flow of reagent is induced from the supply reservoir 53, through fitting 17 and a bladder valve (34, 36 and 38) into the reagent chamber of the pressure vessel. Flow will continue until the pressure vessel is completely filled with reagent or the valve 14 and pump 15 are de-energized.

It would seem possible to fill the pressure vessels by applying a positive pressure directly to the flexible supply reservoir by squeezing it. However, in this case it is possible to rupture the supply reservoir by applying too great a pressure in this manner. The arrangement by which reagent is drawn out of the supply reservoir is preferred.

When the system has been filled the probe or probes are disconnected from their respective bladder valves. Solenoid valves 28, 30 and 32 are momentarily opened to bleed any air that may be trapped in the reagent side of the system. The system is now in the dispensing mode and is ready for use. Since the pressure vessels 22, 24 and 26 are all subjected to 5 PSIG, energization of solenoid valves 28, 30 or 32 will allow the associated reagent to flow. The pressure in the system, the viscosity of the reagent and the time the solenoid valve is energized are all closely controlled. Therefore, the volume of reagent dispensed is repeatable within 10 percent.

A modification of the pressure vessel is shown in FIG. 5. The pressure vessel has a separator which includes a piston 70 and a flexible boot 71. This allows the piston 70 to travel all the way to the bottom of the pressure vessel when it is filled with reagent or all the way to the top of the vessel when the reagent is depleted. The warning system for warning the operator when the reagent supply is depleted includes a reed switch 72, a magnet 73 and a shunt 74. When there is reagent in the vessel, the magnet 73 is shunted by the shunt 74. However, when the reagent supply is depleted the shunt 74 is lifted off the magnet 73. The magnet 73 energizes the reed switch 72 thereby activating the alarm. When the vessel is refilled the shunt 74, which is nickel plated mild steel, contacts the magnet 73 thereby diverting its field away from the reed switch. The reed switch opens and turns off the "low reagent" alarm.

A modification of the connection between the reagent reservoir and the pressure vessel is shown in FIGS. 6 and 7. In this modification the bladder valves 34, 36 and 38 and their connections to the pressure vessels are eliminated. The dispensers 40, 42 and 44 of FIG. 1B are modified so that they are used both for dispensing reagent and for refilling the the pressure vessel. As shown in FIG. 6, each dispenser includes a male Luer needle fitting 75. During dispensing, a needle 76 is attached to the needle fitting 75. The needle 76 may be a standard medical needle which provides the desired flow rate of staining reagent to the slide. Use of such a needle has the advantage that it can be periodically replaced; otherwise dried reagent tends to change the size of the opening in the dispenser changing the flow rate.

When it is desired to refill a pressure vessel, a mating female Luer fitting 77 is placed on the needle fitting 75. This fitting may include a simple check valve. Or, as shown in FIG. 7 an alternate action valve actuator 78 may be provided to actuate a valve. The valve actuator is pushed one way to open the valve connection between the reservoir and the fitting. It is pushed the other way to close the valve and prevent air from entering the reservoir.

The operation of the modified system is as follows.

The needle 76 is removed from the needle fitting 75 connected to the pressure vessel to be refilled. This assures a maximum flow and hence a minimum filling time. The female Luer fitting 77 is connected to the needle fitting 75. The valve actuator 78 is operated to set the valve in the open position. The "fill" push button is depressed. This energizes valve 14 and pump 15 (FIG. 1A). When the pressure vessel is filled, the valve actuator 78 is depressed to set the valve in a closed position. The fitting 77 is disconnected from the fitting 75. The needle 76 is reattached to the fitting 75 and the system is again ready for dispensing.

While a particular embodiment of the invention has been shown and described, various modifications are within the true spirit and scope of the invention. The appended claims are intended to cover any such modifications.

What is claimed is:

1. A system for storing and dispensing reagents which stain a film of blood cells deposited on a laboratory slide comprising:
   - a plurality of pressure vessels each having a movable separator inside said vessel to divide each vessel into two chambers,
   - means for supplying reagents to one chamber of each pressure vessel during filling thereof,
   - means for applying a regulated fluid pressure to the chamber on the other side of the movable separator in each pressure vessel, and
   - a plurality of solenoid operated valves, one for each pressure vessel, said solenoid valves being selectively actuated for intervals of time which dispense accurate volumes of reagent from said vessels under pressure supplied by the fluid on the other side of said movable separator.

2. The system recited in claim 1 wherein said separator is a piston.

3. The system recited in claim 1 wherein said separator is a flexible, impermeable membrane attached to the inside of said vessel.

4. The system recited in claim 3 wherein said membrane is attached around the periphery of the approximate middle of each vessel, and wherein said membrane is sufficiently flexible to extend almost completely to one side of said vessel when said vessel is completely filled with reagent and to the other side of said vessel when emptied of reagent whereby said reagents are isolated from materials with which they may react.

5. The system recited in claim 4 further comprising:
   - a magnet attached to said flexible membrane, and
   - a reed switch positioned in the chamber containing reagent so that it is actuated by said magnet when the reagent supply is near depletion.

6. The system recited in claim 1 wherein there are three pressure vessels containing a fix, a stain and a buffer, the solenoid operated valves connected to said vessels being sequentially operated to apply fix, stain and buffer to said slide.

7. The system recited in claim 1 wherein said means for supplying reagents comprises:
   - a flexible supply reservoir containing a reagent, and connections between said supply reservoir and one of said pressure vessels.

8. The system recited in claim 7 wherein said connections include a bladder valve connected to said pressure vessel and a probe on said supply reservoir, said probe being inserted into said bladder valve during filling of said pressure vessel.

9. The system recited in claim 7 wherein said connections include:
   - a plurality of needle fittings, one connected to each of said solenoid valves,
   - a plurality of needle-like dispensers connectable to said fitting for dispensing said accurate volumes of reagent, and
   - a plurality of mating fittings, one connected to each flexible supply reservoir and each being connectable to one of said needle fittings for supplying reagent from said supply reservoir to a pressure vessel.

10. The system recited in claim 9 wherein each of said mating fittings includes a valve which permits flow of reagent from said supply reservoir and which blocks flow of air into said reservoir.

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