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[54] **BLOOD REFERENCE STANDARD AND PROCESS FOR BLOOD GAS TEST**

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Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 397,347, Sept. 14, 1973, abandoned.

[52] **U.S. Cl.** ..... **23/230 B,** 252/408

[51] **Int. Cl.** **G01n 33/16**

[58] **Field of Search**..... 23/230 B; 252/408; 424/11

[56] **References Cited**

UNITED STATES PATENTS

3,466,249	9/1969	Anderson	23/230 B
3,629,142	12/1971	Marbach	252/408 X
3,681,255	8/1972	Wilfore	23/230 B

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[57] **ABSTRACT**

A stable blood reference standard and control for blood gas tests which includes the use of fluoride, citrate and iodoacetate.

11 Claims, No Drawings

BLOOD REFERENCE STANDARD AND PROCESS FOR BLOOD GAS TEST

This is a continuation-in-part of Copending application Ser. No. 397,347, filed Sept. 14, 1973, and now abandoned.

BACKGROUND OF THE DISCLOSURE

Blood gas tests are performed in most hospital laboratories on an emergency basis forthe purpose of diagnosing and treating abnormalities of pulmonary function and acid base balance. The three parameters are blood pH, $pCO_2$and pO_2. In performing the test, blood is drawn from the patient and introduced promptly into specialized equipment containing electrodes specific for each of the three blood-gas componetns. Since the blood sample deteriorates rapidly, it must be analyzed promptly, or within several hours if iced. As time progresses, however, the pH of the blood sample decreases, the pCO_2 increases, and the pO_2 decreases as a result of cellular metabolism. There is no recognized process in the prior art for preventing these changes from occurring in stored blood samples.

Present day blood gas equipment is standardized and controlled by cumbersome procedures using buffers for pH, and gases for pCO_2 and pO_2. Blood specimens of known pH, pCO_2 and pO_2 would be preferable for this purpose. However, the instability of these components in blood has made this approach impractical in the prior art.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a means of stabilizing the aforesaid components in blood, or a suspension of blood cells in an appropriate media, so as to facilitate the use of stored blood for standardizing and controlling the blood gas equipment. The process of the invention may also be used to stabilize, for example, a mixture of hemoglobin and buffer salts, which does not involve blood cells. The stabilizing agents are fluoride, citrate and iodoacetate. Each of these substances prevents a portion of the cellular metabolic processes which cause the changes in blood gases noted previously: fluoride by binding magnesium; citrate and iodoacetate by inhibiting different pathways in the metabolic processes.

It has been found that combinations of the above-mentioned inhibitors when added to blood or a suspension of blood cells, or a mixture of hemogloblin, stabilize the blood gases for periods of 30 days or longer when stored in closed containers under refrigeration.

The following example will further illustrate the present invention although the invention is not limited to this specific example which is provided by way of illustration and not limitation.

Fluoride	—	25 millimolar
Citrate	—	12 millimolar
Iodoacetate	—	5 millimolar (fluoroacetate may be substituted for iodoacetate)

It has been found that the concentrations listed above have been most efficient. However, it has also been found that the listed components may be varied considerably in concentration and in relation with each other without appreciably varying the blood gas stabilizing effect. For example, the following ranges of concentration have been found to be permissible, without significantly reducing the blood gas stabilizing effect.

Fluoride	10 to 100 millimolar
Citrate	3 to 50 millimolar
Iodoacetate	1 to 10 millimolar

In carrying out the process of the invention in accordance with one particular example, the following steps are presented:

Fresh blood is drawn and mixed with a solution containing concentrates of sodium fluoride, citrate and iodoacetic or fluoroacetic acid in sufficient quantity to yield the final blood concentrations mentioned above. For example, 25 milligrams of sodium fluoride and 93 milligrams of iodoacetic or fluoroacetic acid are dissolved in 10 ml of water and then mixed with 250 ml of blood. The citrate is also dissolved in water and mixed with the blood. These anions may be added in combination with any cation so long as the pH is adjusted to approximately 7.4 37°C. Following this the blood is stored in air tight containers ideally leaving little or no air space. After 2 weeks at refrigerated temperature the pH, pCO_2 and pO_2 are stabilized and remain unchanged for 30 days or longer if kept under refrigeration. In contrast, blood specimens without these preservatives show a progressive drop in pH and pO_2 and increase in pCO_2. Such changes are sufficient in magnitude to render untreated blood unsatisfactory as a blood gas standard or control.

As another example, fresh red cells are washed and suspended in an albumen glucose solution containing concentrates of sodium fluoride and citrate in sufficient quantity to yield the concentrations set forth above.

As another example, a mixture of hemoglobin (not cells) and buffer salts may be treated in the manner described above to stabilize the pH at about 7.40, the pCO_2 at about 40 mm, and the pO_2 at about 80 mm. Glycerol may be added to increase the viscosity of the mixture to about 3.5 centipoise, that of normal whole blood, and to act as a preservative. About a 50 percent solution at 37° C would have a viscosity of 3.5 centipoise, a 75 percent solution would have better preserving power, but the viscosity would be 9.4 centipoise.

It will be readily apparent to those skilled in the particular art under consideration that other examples of the invention described herein may be devised from an understanding of the foregoing specification without departing from the spirit and scope of the invention. It is intended in the following claims to cover all such modifications, variations and adaptations which are included within the scope of the invention.

What is claimed is:

1. A reference standard containing blood, blood cells, hemoglobin, or the like, reconstituted by the addition of a fluoride and an iodoacetate or a fluoroacetate.

2. A reference standard defined in claim **1**, in which the fluoride and iodoacetate or fluoroacetate are included with a concentration for the fluoride substantially in a range of **10–100** millimolar, and with a concentration for the iodoacetate or fluoroacetate substantially in a range of 1 to 10 millimolar.

3. The reference standard defined in claim **1**, and which also contains a citrate.

4. The reference standard defined in claim 2, and which further includes a citrate substantially in a range of 3 to 50 millimolar.

5. The reference standard defined in claim 1, in which the fluoride is sodium fluoride and the iodoacetate or fluoroacetate is iodoacetic or fluoroacetic acid.

6. The reference standard defined in claim 1, which comprises hemoglobin and glycerol in proportions to provide a viscosity of the order of 3.5 centipoise.

7. A process of providing a reference standard which consists of deriving a reference standard containing blood, blood cells, hemoglobin, or the like, dissolving sodium fluoride and iodoacetic or fluroacetic acid in water, and mixing the resulting solution with the reference standard.

8. The process defined in claim 7, and which further comprises the step of dissolving citrate in water and mixing the resulting solution with the reference standard.

9. The process defined in claim 7, in which 25 milligrams of sodium fluoride and 93 milligrams of iodoacetic acid are dissolved in 10 ml of water and then mixed with 25 ml of the reference standard or in multiples or sub-multiples thereof.

10. A process for providing a reference standard which consists of washing fresh red cells, dissolving sodium fluoride and citrate in an albumen glucose solution, and suspending the red cells in the solution.

11. The process defined in claim 7, which comprises deriving a unit of hemoglobin, and adding glycerol to the hemoglobin to increase the viscosity thereof to the order of 3.5 centipoise.

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