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Reproducibly Simulating Hemolysis, for Evaluating Its Interference with Chemical Methods

To the Editor:

Too often the effect of dissolved hemoglobin on results of determination of a plasma or serum constituent has been described without mention or adequate consideration of the system for introducing the hemolysis. Effects of this contaminant may differ from those reported because there is an uncritical use of hemoglobin experimentally added or failure to avoid excessive dilution of serum.

For example, five volumes of a 1 g/dl stock hemoglobin solution diluted with five volumes of serum produces a 500 mg/dl hemoglobin concentration in serum:water (1:1 by vol), whereas one volume of the stock diluted with nine volumes of serum produces a 100 mg/dl concentration in serum:water (9:1 by vol). Because the concentrations of serum constituents are so different, results obtained when one is studying (e.g.) the effect of hemolysis on direct-reacting bilirubin as measured by the Jendrassik-Grof procedure may be profoundly affected.

Hemoglobin should be added to serum with least possible dilution, so that it simulates the hemolysis created by erythrocyte destruction under the conditions of blood collection, or as it originates from blood clotting, processing, or treatment. A suggested procedure follows:

Wash the packed erythrocytes obtained from about 5 ml of heparinized whole blood three times with 10 ml of isotonic saline. Dilute the cells with an equal volume of water, and freeze overnight. After the cells are thawed and at room temperature, centrifuge to remove the stroma (simulating its removal when hemolyzed serum or plasma is obtained after centrifugation of whole or clotted blood). Determine the hemoglobin content of the supernatant fluid by an acceptable method (e.g., cyanmethemoglobin). Use the mean value of triplicates. One or two milliliters of the suspension is usually more than enough for any experiment. This “stock” hemoglobin should contain about 90-110 g/liter.

| Stock hemoglobin added, μl | Final volume, ml | Dilution factor, initial vol/final vol | Actual hemoglobin concn, mg/dl
|--------------------------|------------------|--------------------------------------|---------------------
| 0                        | 1.00             | —                                    | 0 (blank)           |
| 10                       | 1.01             | 100/101                              | 99                  |
| 20                       | 1.02             | 100/101                              | 196                 |
| 30 (20 + 10)^            | 1.03             | 100/103                              | 291                 |
| 40 (20 + 20)^            | 1.04             | 100/104                              | 385                 |
| 50                       | 1.05             | 100/105                              | 476                 |

^ a This must be calculated from the value obtained for the stock hemoglobin by using the dilution factor from the column to the left. For the values in this column, I assumed a hemoglobin concentration in the stock of 100 g/liter.
^ b Use “to contain” micropipets. While 30- and 40-μl pipets are not usually available, one may substitute the sizes shown.

To prepare an experimentally hemolytic serum or plasma, obtain “normal” serum or plasma free of suspected interferences, and add to 1.0-ml aliquots the volumes of the stock hemoglobin listed above.

In the above example, to add about 500 mg of hemoglobin per deciliter, the serum volume is increased by not more than 5%, resulting in a final value of 476 mg of hemoglobin. What volume of erythrocytes actually corresponds to this 500 mg of hemoglobin per deciliter? Is it a reasonable reproduction of inadvertent hemolysis?

Assume that a freshly drawn sample of whole blood contains 15 g of hemoglobin per deciliter. If the hematocrit is 45%, then the hemoglobin content of the erythrocytes is 33.3 g (or 33300 mg) per deciliter. What volume of erythrocytes would contain 500 mg of hemoglobin?

500 mg/(333 mg/ml) = 1.52 ml

Thus, in this example, a serum containing 500 mg of hemoglobin per deciliter has been diluted by the equivalent of 1.52 ml of erythrocytes —less than 2%. In the above-recommended procedure, the dilution would never exceed 5%, which is inconsequential. Even less dilution would be involved if a more concentrated solution were used.

Such considerations apply to other interferences similarly studied. Dilution of serum must be at a minimum.

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Screening for Cystic Fibrosis by Specific Ion Electrode

To the Editor:

In a paper entitled, “Direct Measurement of Chloride in Sweat with an Ion-Selective Electrode,” Szabo et al. (1) discuss a modified procedure for the Orion Skin Chloride Measuring System. There are several disturbing points raised in the paper that require clarification.

It is these authors’ contention that the system is used by many laboratories only as a screening device because readings are often irreproducible and falsely high. They claim that their modifications allow the system to be used for diagnosing cystic fibrosis. As the manufacturer of this system, I feel that the authors have misunderstood the purpose of this instrument. The Orion Skin Chloride Measuring System is not, and has never been represented as a diagnostic device. The system is a screening device, and is intended to allow rapid and convenient measurement of sweat chloride.

As with most screening tests, the possibility for borderline values and occasional false reading does exist. Several papers have been published that attest to the overall reliability of the system as well as the frequency of overlapping results (2–4). For example, Kopito and Shwachman show overlap in six of 122 cases (2). The necessity for having independent, confirmatory data prior to a final diagnosis is emphasized by these and other authoritative people in the field of cystic fibrosis.

Also, Szabo et al. suggest that an increase in pilocarpine from 64 to 500 mg/100 ml enhances the performance of the system, and alleviates the problem of insufficient sweat production. We agree that insufficient sweat production will give rise to high readings. However, we do not believe that the solution to this problem is to increase the concentration of pilocarpine. Very careful studies were conducted to determine the optimal current and pilocarpine levels necessary to produce adequate sweat for this determination. Excessive pilocarpine and/or high currents have been found to produce a painful skin irritation, especially in young children. The concentration of pilocarpine originally supplied will induce ample sweat for the measurement.

In our experience, most cases of inadequate sweat have been traced to the iontophoresis step. If, for any reason, the passage of current to the skin