Response of Red Blood Cell Control Materials to Altered Testing Conditions

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ABSTRACT

While many features are regularly considered in selecting control reagents, the responsiveness of these products to methodologic errors is frequently overlooked. To address this issue with regard to commercially prepared whole blood cell controls, mean cell volume (MCV) determinations were performed on several preserved red blood cell controls and fresh blood in the presence of various artifacts. The effects contaminating the isotonic diluent with water, saline, or bleach were compared. Although our results indicate that preserved and fresh RBCs generally behave similarly, large differences were observed in the responses to hypotonic stress among controls and between controls and fresh specimens. As this may have clinical relevance, our data highlight the need for testing the responsiveness of controls to commonly encountered methodologic variables as part of the routine evaluation of these products.

Introduction

Use of commercially prepared whole blood cell controls to monitor performance of cell counters in the clinical hematology laboratory is an accepted practice. Ideally, these reagents would behave identically to fresh human blood when tested, yet possess indefinite stability, thus allowing repeat testing and long-term instrument monitoring. Use of controls that are less responsive to altered testing conditions than fresh patient specimens might allow significant sources of error to go undetected. Alternatively, processed control cells which show greater responsiveness to changes in test variables than fresh blood cells could draw attention to consequential methodologic variation. Accordingly, experiments were carried out on six commercial cell control preparations.
by comparing their responses to controlled artifacts with those of fresh human blood.

Materials

Fresh Whole Blood Specimens

Patient samples collected in ethylenediamine tetraacetic acid (EDTA) were selected daily from among the specimens submitted to the clinical hematology laboratory in a large hospital for routine complete blood cell counts. These samples were chosen to represent a spectrum of patient diagnoses and a wide range of mean cell volumes (MCVs). Five to 11 samples from different patients were evaluated each day of the study. A fresh specimen from a normal volunteer was also included each day.

Commercial Cell Controls

Preparations of the following preserved commercial cell controls were tested: *4C,* Diff—Trol,** Counter-Check,† CBC—3D,$ Para 8|| and Interscience Multi-Parameter Hematology Control with Platelets.¶ Control preparations were stored and used in accordance with the manufacturers’ recommendations in the package inserts.

Methods

Sample Preparation

The effects of hypotonicity, hypertonicity, and bleach on the mean cell volume were examined. These conditions were achieved by adding contaminating solutions to conventionally prediluted specimens. Fresh whole blood and commercial preparations were treated identically. Twenty ml aliquots of each specimen were prepared in duplicate with isotonic buffered saline‡‡ to have a final dilution of 1:50,000. All such duplicates were randomly processed in each run. Exactly one minute prior to testing, the specific solution used to induce the artifact was introduced and the mixture gently swirled. Pilot studies were used to determine the concentration of solution required to produce clinically important artifactual changes in the MCV of fresh RBC’s. Osmolality was determined by freezing point depression. Each experiment was performed on a separate day with a separate set of fresh specimens.

Instrumentation

The Coulter ZBI equipped with an MCV/Hct accessory‡‡ was used for all MCV determinations and RBC counts. It was operated in accordance with the manufacturer’s instructions1. The MCV was calibrated daily using fresh whole blood samples. After testing each sample, the orifice was flushed until background counts fell below 50 per μL.

MCV Experiments

The effect of hypotonic media on the MCV was assessed by pipeting 1.0, 2.5, or 4.0 ml of deionized water into each 20 ml sample prediluted in isotonic diluent. The final osmolalities of the isotonic diluent similarly diluted were 325, 300, and 280 mOsm as compared with 337 mOsm for the unaltered diluent.

The effects of hypertonicity were evaluated by pipeting 2.0 ml of hypertonic saline (20 gm NaCl in 0.5 L deionized water) solution into each sample. The final osmolality was 398 mOsm.

* Coulter Diagnostics, Hialeah, FL.
† Dade, Miami, FL.
‡ Diagnostic Technology, Hauppauge, NY.
§ R & D Systems, Minneapolis, MN.
|| Streck, Omaha, NE.
¶ Interscience Diagnostics, Fairview, OR.
†† American Scientific Products, McGaw Park, IL.
‡‡ Coulter Electronics, Hialeah, FL.
The effects of two concentrations of bleach on the MCV of fresh blood and control samples were studied: (1) 100 μL of pure bleach in each 20 ml specimen and (2) 2.5 ml of a 1:1000 dilution of pure bleach in the 20 ml specimens. The final osmolalities of the samples were 358 mOsm and 306 mOsm, respectively.

**Osmotic Fragility Studies**

Osmotic fragility testing was performed according to standard methods\(^2\). Samples of five preserved controls were incubated with seven solutions of sodium chloride ranging in concentration from 0 to 70 percent. A fresh sample from a normal volunteer was included as a control.

**Results**

The change in MCV observed following the introduction of each artifact is expressed as the mean percent change above or below baseline measurements. Results of testing duplicate samples agreed within two μm\(^3\)/(fL).

**Hypotonic Stress**

The changes in MCV of samples in response to a hypotonic environment are shown in figure 1. The label, "fresh," indicates the pooled results for the six fresh whole blood specimens in diluent having concentrations of 325, 300, and 280 mOsm. The average percentage increase in MCV above baseline for fresh specimens was 1.9 percent, 5.8 percent, and 10.4 percent, respectively. The Dade reagent cells responded most like fresh human blood, showing MCV increases of 1.2 percent, 3.5 percent, and 8.2 percent, respectively. Nearly all fresh samples expanded more than those of the preserved controls at each dilution of isotonic saline. The cause of shrinkage of the 4C cells at 325 mOsm is not known but may be related to the osmolality of its preservative diluent.

**Hypertonic Stress**

In table I are depicted the changes in MCV following incubation of controls and patients in hypertonic saline. The decrease in MCV of five of the six controls tested appeared comparable to fresh blood. Interscience cells shrunk, however, over 1.5 times more than any other sample tested.

**Bleach Effects**

The effects of concentrated and dilute bleach on erythrocyte MCV are summarized in table II. All samples contracted markedly in pure bleach. Dade cells changed the most in volume and Interscience the least.

**Osmotic Fragility Studies**

Results of osmotic fragility testing of three control preparations resembled those obtained with the fresh control (table III). Slight differences were noted between the preserved cells and fresh blood.
TABLE I

Incubation with Hypertonic Saline

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Percent Change MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Para 8*</td>
<td>-11.4</td>
</tr>
<tr>
<td>Interscience</td>
<td>-17.8</td>
</tr>
<tr>
<td>Diff-Trol*</td>
<td>-7.8</td>
</tr>
<tr>
<td>4C*</td>
<td>-6.3</td>
</tr>
<tr>
<td>Countercheck*</td>
<td>-9.6</td>
</tr>
<tr>
<td>CBC-3Df</td>
<td>-10.0</td>
</tr>
<tr>
<td>Fresh blood§</td>
<td>(-8.1 to -11.4)</td>
</tr>
</tbody>
</table>

*High, normal range controls tested  
†Normal range control tested  
§Eight samples tested

Comment

Preparation of cells for use as quality control products requires modifications which permit long-term storage and repeat analysis. Unfortunately, these modifications may alter normal membrane properties and thus render control cells unlike native human blood cells. The effects of osmotic matrix on MCV determinations on both patient Hematology Survey samples is well known. Our observations show that control materials vary in their sensitivity to altered testing conditions and in their similarity to fresh blood. Our data confirm that control cells are less distensible than fresh human erythrocytes and vary in their response to hypotonic stress. Decreasing the osmolality from 337 mOsm to 300 mOsm caused the MCV of patients to increase 5.8 percent on average, whereas the five control preparations showed an average increase of only 2.6 percent. Further reduction to 280 mOsm caused the MCV of fresh RBCs to increase over 10 percent, yet the increase in the MCV of commercial controls varied from 2.4 percent to 8.2 percent. Therefore, it is possible that under certain circumstances some commercial controls might falsely signal that the test system is "in control" in the face of a large systematic error. Dade control cells appeared most like human blood in this experiment; Coulter 4C cells, the least. The Dade cells appeared to attain a progressively higher percent increase in MCV as the osmolality decreased, whereas the other products demonstrated a progressive decrease. The relevance of this is unclear, but it suggests some products more closely resemble the human cells than others (figure 1). Controls and patient cells responded similarly in hypertonic media. Interscience reagent, which had the highest baseline MCV, shrank 17.8 percent, which was significantly more than the other products tested.

Bleach is commonly used to remove debris from the orifice of automated cell counters. Our data suggest that concentrated bleach causes both patient and control RBCs to crenate markedly without producing cell lysis. Dilute bleach causes fresh and control RBC's to swell to a variable degree (table II). The effects of dilute bleach on the MCV are out of proportion to the hypotonicity.

Osmotic fragility testing demonstrated slight differences between some pre-

TABLE II

Effect of Bleach on Mean Cell Volume

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent Change in MCV After Exposure to Concentrated Bleach</th>
<th>Percent Change in MCV After Exposure to Dilute Bleach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Para 8*</td>
<td>-34.8*</td>
<td>+27.2†</td>
</tr>
<tr>
<td>Interscience</td>
<td>-28.2</td>
<td>+8.4</td>
</tr>
<tr>
<td>Diff-Trol*</td>
<td>-35.4*</td>
<td>+12.0†</td>
</tr>
<tr>
<td>4C*</td>
<td>-33.2*</td>
<td>+21.4†</td>
</tr>
<tr>
<td>Counter-Check</td>
<td>-33.0*</td>
<td>+8.7†</td>
</tr>
<tr>
<td>3D</td>
<td>-21.6*</td>
<td>+21.6</td>
</tr>
<tr>
<td>Fresh Blood§</td>
<td>-36.9</td>
<td>+15.7</td>
</tr>
</tbody>
</table>

(-34.0 to -38.2)  (+8.7 to +19.0)

*High, normal, low range controls tested  
†High, normal range controls tested  
§Twelve samples tested in concentrated bleach; eight samples tested in dilute bleach.
Osmotic Fragility Testing of Commercial Cell Controls
Percent Lysis at Various Saline Concentrations

<table>
<thead>
<tr>
<th>Percent Saline</th>
<th>Control 4C</th>
<th>Para 8</th>
<th>Diff-Trol</th>
<th>CBC-3D</th>
<th>Countercheck</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>60</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>50</td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>40</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>30</td>
<td>0.3</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>20</td>
<td>26.0</td>
<td>20.0</td>
<td>6.0</td>
<td>34.0</td>
<td>60.0</td>
</tr>
</tbody>
</table>

Preserved and fresh erythrocytes. Two preparations, Countercheck and CBC-3D, appeared to lyse slightly more readily than fresh blood, possibly reflecting relatively more cellular rigidity.

Our results demonstrate that fresh and preserved RBCs frequently respond differently to artifacts such as alteration of osmolality or exposure to bleach. Most importantly, some of these differences in responses may be of sufficient magnitude to influence medical decisions and could adversely affect patient care. The large artifacts required to reproduce our findings seem unlikely to occur during routine testing of patient specimens. Therefore, it is anticipated that all of the commercial control materials evaluated would perform acceptably in most situations. However, it is not possible to predict all the possible artifacts which may be encountered during laboratory testing. Thus, it would seem prudent to select control preparations which respond most similarly to native human blood. Presumably, by studying the responses of control preparations and fresh blood to experimentally induced artifacts, it is possible to obtain data which are required to make this comparison.

Generally, a control material is selected on the basis of cost considerations, safety and convenient packaging, vial-to-vial variability, long term stability, and independent assayability. However, this product, although judged favorably, may, in fact, be undesirable in that it does not fulfill its cardinal purpose—detection of methodologic errors which yield spurious patient results. Therefore, the evaluation of quality control material should include testing its responsiveness to a series of variables or interferences likely to occur in the laboratory environment.

Acknowledgments
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References