Influence of Sample Storage Conditions on Sweat Chloride Results

To the Editor:

Sweat chloride samples may be collected in areas of a hospital that are removed from the chemistry laboratory, and a delay in analysis may compromise the integrity of the sample. Clinical and Laboratory Standards Institute (formerly NCCLS) (CLSI) document C34-A3 (1) recommends that sweat chloride samples collected on filter paper or gauze be promptly weighed and then refrigerated in the event that the sample cannot be analyzed immediately. The citation supporting the CLSI requirements (2) did not examine other storage conditions. We investigated the stability of simulated sweat samples stored under various conditions for 3 to 5 days.

KCl (Aldrich Chemical) was dissolved in 100 mL of deionized water to achieve simulated sweat with concentrations of 17, 27, 41, 63, and 85 mmol/L. Samples were then prepared by adding filter paper (Grade H9262/H9263; Whatman) to snap cap vials (Fisher) that were preweighed with a Mettler AE240-S analytical balance. We pipetted 100 µL of each concentration onto the filter paper and immediately capped the vials. The day 0 vials were reweighed immediately. The vials for the other storage conditions were weighed just before chloride measurement. At the time of analysis, we added 5.5 mL of Chloridometer Acid Reagent (Labconco) to each vial, weighed the vial, and incubated it for 40 min at 21 °C–23 °C. The filter paper was then removed and the remaining volume was assessed gravimetrically to correct for any volume removed with the filter paper. Cou- lometric titration was performed as described in the CLSI guideline with a Labconco Digital Chloridometer (Model 4425000). Sweat chloride was calculated in millimoles of chloride per liter of sweat.

Sweat samples analyzed immediately (day 0) were used as controls in evaluating the influence of storage. The remaining samples were incubated at either room temperature (21 °C–23 °C) or refrigerated temperature (2 °C–8 °C) for 72, 96, and 120 h before analysis (Table 1). Increases in sweat chloride concentrations were highly variable, ranging from 20.9%–65.8% at room temperature and 2.9%–19.2% at refrigerated temperatures in vials stored for 3 to 5 days. These data support the conclusion that loss of water due to evaporation before weighing the vial plus the filter paper containing sweat caused unacceptable results. The chloride concentrations in the vials containing sweat plus acid diluent were nearly identical for each concentration, with a mean change of 0.6% (range, -1.6% to +7.1%) for all concentrations, regardless of storage condition. These data support the conclusion that no loss of chloride occurred during vial storage.

When the sweat weight from day 0 controls was used to recalculate the sweat chloride concentrations for day 5 storage conditions, the percentage change for room temperature and refrigerated samples ranged from +1.7% to +6.1% at both conditions, with low concentrations showing the greatest change in percentage (Table 1). This finding suggests that the weight of the sweat sample must be measured immediately but that the chloride can be measured up to 5 days later. Sweat chloride concentrations can be measured reliably because the sweat sample (typically 75–150 µL) is extracted into a relatively large volume of solution (5.5 mL) in the measurement vial. For example, we observed a worst-case loss of 32.7 mg from 101.1 mg of sweat, which represents a decrease in the measurement vial from 5601.1 mg to 5568.4 mg (0.58%). This small change would negligibly influence the calculated sweat chloride concentration as long as the original weight of the sweat was known.

Methods to prevent evaporation were investigated by storing simulated sweat samples under 6 conditions: at room temperature, refrigerated, at room temperature with a Parafilm seal, refrigerated with a Parafilm seal, at room temperature in a sealed plastic specimen bag (12 in × 15 in; VWR) and refrigerated in a sealed plastic specimen bag. Parafilm-sealed samples were prepared by wrapping a Parafilm strip (1 in × 12 in) tightly around the cap and vial. Samples were weighed at initial preparation, at 72 h, and at 120 h. Table 1 shows that refrigerated, Parafilm-sealed samples had the least evaporation (1.1% at 3 days and 2.4% at 5 days), whereas room temperature samples had the most evaporation (19.1% at 3 days and 32.3% at 5 days).

We conclude that sweat samples should be delivered immedi-
Table 1. Changes in sweat sample chloride concentration and weight when samples were stored for 3 to 5 days.

<table>
<thead>
<tr>
<th>Sample concentration and storage condition</th>
<th>Day 0 sweat Cl⁻, mmol/L</th>
<th>Day 3 sweat Cl⁻, mmol/L (% change)</th>
<th>Day 4 sweat Cl⁻, mmol/L (% change)</th>
<th>Day 5 sweat Cl⁻, mmol/L (% change)</th>
<th>Day 5 with the day 0 weight; sweat Cl⁻, mmol/L (% change)</th>
<th>Day 0 vial Cl⁻, mmol/L</th>
<th>Day 3 vial Cl⁻, mmol/L (% change)</th>
<th>Day 4 vial Cl⁻, mmol/L (% change)</th>
<th>Day 5 vial Cl⁻, mmol/L (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 mmol/L, room temperature</td>
<td>16.8</td>
<td>21.0 (+25.0%)</td>
<td>22.5 (+33.6%)</td>
<td>22.9 (+36.3%)</td>
<td>17.8 (+6.1%)</td>
<td>14.85</td>
<td>14.7 (-1.3%)</td>
<td>15.5 (+4.0%)</td>
<td>15.9 (+7.1%)</td>
</tr>
<tr>
<td>17 mmol/L, refrigerated</td>
<td>N/A*</td>
<td>18.1 (+7.6%)</td>
<td>17.3 (+2.9%)</td>
<td>20.0 (+19.2%)</td>
<td>17.8 (+6.1%)</td>
<td>N/A</td>
<td>15.3 (+2.9%)</td>
<td>14.8 (-0.1%)</td>
<td>15.9 (+6.9%)</td>
</tr>
<tr>
<td>27 mmol/L, room temperature</td>
<td>27.1</td>
<td>33.4 (+23.4%)</td>
<td>41.0 (+51.6%)</td>
<td>36.1 (+33.3%)</td>
<td>27.8 (+2.6%)</td>
<td>24.6</td>
<td>24.4 (-0.8%)</td>
<td>23.7 (-3.7%)</td>
<td>24.8 (+0.8%)</td>
</tr>
<tr>
<td>27 mmol/L, refrigerated</td>
<td>N/A*</td>
<td>28.7 (+6.1%)</td>
<td>28.9 (+6.8%)</td>
<td>30.8 (+13.7%)</td>
<td>28.3 (+4.4%)</td>
<td>N/A</td>
<td>24.3 (-1.2%)</td>
<td>24.0 (-2.3%)</td>
<td>25.2 (+2.4%)</td>
</tr>
<tr>
<td>41 mmol/L, room temperature</td>
<td>41.3</td>
<td>58.7 (+42.1%)</td>
<td>51.1 (+23.7%)</td>
<td>57.5 (+39.2%)</td>
<td>42.2 (+2.2%)</td>
<td>37.2</td>
<td>37.0 (-0.5%)</td>
<td>36.6 (-1.6%)</td>
<td>37.9 (+1.9%)</td>
</tr>
<tr>
<td>41 mmol/L, refrigerated</td>
<td>N/A*</td>
<td>43.2 (+4.6%)</td>
<td>44.4 (+7.5%)</td>
<td>46.1 (+11.7%)</td>
<td>42.8 (+3.7%)</td>
<td>N/A</td>
<td>37.1 (-0.3%)</td>
<td>37.3 (+0.3%)</td>
<td>38.2 (+2.7%)</td>
</tr>
<tr>
<td>63 mmol/L, room temperature</td>
<td>63.4</td>
<td>76.7 (+20.9%)</td>
<td>101.7 (+60.4%)</td>
<td>86.9 (+36.9%)</td>
<td>64.4 (+1.5%)</td>
<td>56.8</td>
<td>56.5 (-0.5%)</td>
<td>55.3 (-2.6%)</td>
<td>57.6 (+1.4%)</td>
</tr>
<tr>
<td>63 mmol/L, refrigerated</td>
<td>N/A*</td>
<td>67.5 (+6.4%)</td>
<td>67.2 (+5.9%)</td>
<td>71 (+11.9%)</td>
<td>64.2 (+1.2%)</td>
<td>N/A</td>
<td>56.6 (-0.4%)</td>
<td>56.2 (-1.1%)</td>
<td>57.6 (+1.4%)</td>
</tr>
<tr>
<td>85 mmol/L, room temperature</td>
<td>85.2</td>
<td>107.8 (+26.5%)</td>
<td>130.7 (+53.4%)</td>
<td>141.3 (+65.8%)</td>
<td>86.6 (+1.7%)</td>
<td>76.4</td>
<td>76.2 (-0.3%)</td>
<td>75.7 (-0.9%)</td>
<td>77.7 (+1.7%)</td>
</tr>
<tr>
<td>85 mmol/L, refrigerated</td>
<td>N/A*</td>
<td>91.7 (+7.6%)</td>
<td>88.9 (+4.4%)</td>
<td>96.8 (+13.6%)</td>
<td>86.6 (+1.7%)</td>
<td>N/A</td>
<td>76.9 (+0.7%)</td>
<td>76.4 (0%)</td>
<td>77.5 (+1.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evaporation</th>
<th>Day 0 weight, mg</th>
<th>Day 3 weight, mg (% change)</th>
<th>Day 5 weight, mg (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>101.1</td>
<td>81.8 (-19.1%)</td>
<td>68.4 (-32.3%)</td>
</tr>
<tr>
<td>Room temperature, in plastic bag</td>
<td>100.9</td>
<td>84.9 (-15.9%)</td>
<td>73.8 (-26.9%)</td>
</tr>
<tr>
<td>Room temperature, Parafilm seal</td>
<td>101.2</td>
<td>95.4 (-5.7%)</td>
<td>92.9 (-8.2%)</td>
</tr>
<tr>
<td>Refrigerated</td>
<td>100.9</td>
<td>97.4 (-3.5%)</td>
<td>93.1 (-7.7%)</td>
</tr>
<tr>
<td>Refrigerated, in plastic bag</td>
<td>100.9</td>
<td>96.9 (-3.9%)</td>
<td>92.6 (-8.2%)</td>
</tr>
<tr>
<td>Refrigerated, Parafilm seal</td>
<td>101.3</td>
<td>100.1 (-1.1%)</td>
<td>98.9 (-2.4%)</td>
</tr>
</tbody>
</table>

* All values are the mean of measurements of 2 vials.
* Percentage changes are from day 0.
* Sweat chloride concentrations at day 5 are recalculated with the day 0 control sweat weights (mean, 99.3 mg; range, 98.5–100.1 mg). Percentage changes are from day 0.
* Chloride concentration in the measurement vial (sweat plus diluent volume). Days 3 to 5 show percentage changes from day 0.
* N/A, not applicable.
* All values are the mean of measurements of 4 vials.
Letters to the Editor

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References


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Therapeutic Drug Monitoring of Busulfan

To the Editor:

We were excited to read the clinical case study recently published in Clinical Chemistry (1), because the dosing of busulfan is a topic of great interest; however, one of the authors’ Points to Remember was, “The therapeutic dose of intravenous busulfan is best predicted when the patient has achieved aCss” (1). We demonstrate that this statement is incorrect by addressing the question from both a theoretical and a practical point of view.

Busulfan pharmacokinetics are described by a 1-compartment model. Thus, with intravenous administration, the pharmacokinetics are described by the parameter’s clearance and volume of distribution. Because our aim is to achieve a constant area under the curve (AUC) for all patients, the clearance of busulfan is the most important parameter. The formula:

\[
\text{Clearance} = \frac{\text{Dose}}{\text{AUC}}
\]

demonstrates that if we know the clearance, we can adjust the dose to achieve a constant AUC. It does not matter if the patient is at steady state or not. Like other pharmacokinetic parameters, clearance displays some inter- and intraindividual variation, but other than a circadian rhythm, it does not change systematically with time over the 4 days of administration. Clearance may change when enzyme inducers or inhibitors are added to the medication, but the many published pharmacokinetic investigations of busulfan have not indicated nonlinear pharmacokinetics or autoinduction causing a change in clearance over time. Therefore, from a theoretical point of view, it does not matter when the blood sampling is done. From a practical point of view, it is appropriate to measure busulfan after the first dose to get the necessary information to be able to adjust the dose for subsequent administrations as quickly as possible. In previously published investigations on this 16-dose schedule, the therapeutic interval is always defined to the AUC from zero to infinity measured after the first busulfan dose (2).

We are not aware of how the mentioned therapeutic interval of 900–1350 µmol · L⁻¹ · min⁻¹ was defined. Fig. 2 of the case study by Johnson-Davis et al. suggests that the pharmacokinetic parameters do not change much over time. Rather, the authors appear to have calculated the AUC with noncompartmental methods and interpreted the AUC incorrectly by calculating the clearance from the AUC according to the above equation. However, this equation does not work correctly if the plasma busulfan concentration does not start at zero, i.e., if the patient had received previous busulfan doses. Therefore, the clearance values given in the authors’ Table 1 appear to be incorrect, except for the value after the first dose.

The 29.9% change in clearance from the first dose to the fifth dose is substantial (Table 1 in their case study), but it appears to be due to misinterpretation of the AUC. The values reported in the literature for intraindividual variation in clearance are not greater than

atately to the laboratory and that sweat sample weight be measured without delay. Once an accurate sweat sample weight is obtained, chloride can be measured within 5 days without affecting the reliability of the results, irrespective of storage conditions. A limitation of this study is that we examined vials from only 1 manufacturer. Other storage systems may have different sealing properties and may exhibit different effects due to sample evaporation.