Validation of Carcinoembryonic Antigen and Carbohydrate Antigen 19-9 Measurements in Pancreatic Cyst Fluid with a Serum-Based Immunoassay

To the Editor:

Imaging techniques, cytology, and biochemical analysis (including carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) concentrations) of pancreatic cyst fluid obtained by endoscopic ultrasound-guided fine-needle aspiration are used to differentiate pancreatic cyst lesions, particularly those with malignant potential (1, 2). American College of Gastroenterology guidelines have outlined the published data relating to the diagnostic performance of pancreatic cyst fluid CEA and CA 19-9 (2) and concluded that cyst fluid CEA is the single most important factor in determining pancreatic cyst etiology. It is important, however, to appreciate that diagnostic cutoff values may be assay dependent. Many published reports do not describe the tumor marker assays used for analyzing pancreatic cyst fluid. In addition, the analytical validity of applying methods for serum tumor markers to the analysis of pancreatic cyst fluid does not appear to have been investigated in these studies. We investigated the analytical validity of the use of Roche E170 immunoassays for the measurement of CEA and CA 19-9 in pancreatic cyst fluid.

We investigated pancreatic cyst fluid samples (n = 21) obtained by ultrasound-guided fine-needle aspiration during routine investigation. Samples were stored at −20 °C and were thawed and vortex-mixed before analysis. CEA and CA 19-9 were measured with a Roche Modular Analytics E170 instrument. CEA and CA 19-9 were measured in a series of dilutions of each sample, which were performed manually with Elecsys Diluent Universal (Roche Diagnostics). The limits of quantification are 1–1000 µg/L for the CEA assay and 2–1000 kU/L for the CA 19-9 assay. The in-house CV data (from serum analysis) are 4.4% at 3.21 µg/L CEA, 3.0% at 37.1 µg/L CEA, 7.5% at 12 kU/L CA 19-9, 2.8% at 20 kU/L CA 19-9, and 2.7% at 89 kU/L CA 19-9.

In the case of CEA, 7 samples exhibited nonlinearity upon dilution (Table 1), with a progressive increase in the apparent CEA concentration as the dilution factor increased (3-fold in the most extreme case). In the case of CA 19-9, 7 samples exhibited nonlinearity upon dilution (Table 1), with an increase in the apparent CA 19-9 concentration as the dilution factor increased (60-fold in the most extreme case). In another sample (sample Q, Table 1), the CA 19-9 concentration was 452 kU/L when it was measured undiluted, but upon dilution the concentration increased to above the upper limit of detection (1000 kU/L). At a dilution of 1 volume in 100 000, the apparent CA 19-9 concentration was 1 160 000 kU/L.

Of the 21 samples of pancreatic cyst fluid examined, 9 exhibited marked nonlinearity in the measurement of CEA and/or CA 19-9 upon dilution. There are a number of possible explanations for this finding. First, there may be differences in the antigen and immunoreactivity within cyst fluid compared with those of calibrators or serum. Although the CA 19-9 antigen in serum is associated with a mucin (3), it was originally discovered in gastrointestinal tumor cells as a ganglioside (4). The form present in cyst fluid is not known. CEA is known to display considerable heterogeneity, particularly in the extent and type of glycosylation (5). Second, matrix effects associated with cyst fluid, such as viscosity and the presence of nonvisible cellular debris, may contribute to the nonlinearity observed. None of the examined samples appeared viscous during manual dilutions, however, and there was no relationship between nonlinearity and the presence/absence of mucin in cytoclogic examinations. Third, the high-dose hook effect can lead to inaccurate results. The anomalies in CA 19-9 concentration noted upon dilution of sample Q are clearly due to a hook effect.

In summary, this investigation has raised concerns about the performance of the Roche Elecsys assays for CEA and CA 19-9 in samples of pancreatic cyst fluid. Deviations from linearity on dilution are particularly marked for CA 19-9 measurement in certain samples. Other immunoassays for CEA and CA 19-9 may also be affected. These findings could have implications for the use of tumor marker analysis of cyst fluid in the investigation of pancreatic cysts. Caution should be exercised when measuring CEA and CA 19-9 concentrations in pancreatic cyst fluid with assays designed for the analysis of serum or plasma. Nonserum samples tested with such assays should always be subjected to additional quality-assessment measures, such as serial dilutions and spike recovery experiments. Further analytical validation is needed for assays used to measure CEA and CA 19-9 in pancreatic cyst fluid. The type of assay used and any investigation into its validity should be stated in future published studies that involve the measurement of these analytes in pancreatic cyst fluid.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the...
Table 1. Concentrations of CEA and CA 19-9 in undiluted and diluted samples of pancreatic cyst fluid.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Sample</th>
<th>CEA, (\mu g/L)</th>
<th>CA 19-9, kU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undiluted</td>
<td>Dilution factor</td>
</tr>
<tr>
<td></td>
<td>(\times 5)</td>
<td>(\times 10)</td>
</tr>
<tr>
<td>A</td>
<td>&lt;1</td>
<td>U\textsuperscript{c}</td>
</tr>
<tr>
<td>B</td>
<td>&lt;1</td>
<td>U</td>
</tr>
<tr>
<td>C</td>
<td>&lt;1</td>
<td>U</td>
</tr>
<tr>
<td>D</td>
<td>&lt;1</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>1.3</td>
<td>U</td>
</tr>
<tr>
<td>F</td>
<td>2.4</td>
<td>U</td>
</tr>
<tr>
<td>G</td>
<td>3.7</td>
<td>U</td>
</tr>
<tr>
<td>H</td>
<td>19.6</td>
<td>—</td>
</tr>
<tr>
<td>I</td>
<td>39.4</td>
<td>—</td>
</tr>
<tr>
<td>J</td>
<td>41.1</td>
<td>13.5 (67.5)</td>
</tr>
<tr>
<td>K</td>
<td>47.8</td>
<td>—</td>
</tr>
<tr>
<td>L</td>
<td>50.8</td>
<td>12.5 (62.5)</td>
</tr>
<tr>
<td>M</td>
<td>83.5</td>
<td>—</td>
</tr>
<tr>
<td>N</td>
<td>90.9</td>
<td>18.4 (92)</td>
</tr>
<tr>
<td>O</td>
<td>114.3</td>
<td>27.5 (137.5)</td>
</tr>
<tr>
<td>P</td>
<td>161.4</td>
<td>—</td>
</tr>
<tr>
<td>Q</td>
<td>504.1</td>
<td>145.7 (728.5)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Results are not shown when both CEA was \(<10 \mu g/L\) and CA 19-9 was \(<10 kU/L\), when analyzed undiluted and diluted (\(n = 4\)). Numbers in parentheses are results back-calculated to give values equivalent to undiluted samples. Boldface results demonstrate nonlinearity upon dilution.

\textsuperscript{b} Detection of mucin upon cytologic examination.

\textsuperscript{c} U, under the detection limit.

\textsuperscript{d} This result for sample Q was subject to a hook effect.

References


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The Osmolar Gap: What Has Changed?

To the Editor:

The osmolar gap (OG)\(^1\) is calculated in the emergency department (ED) when ingestion of ethylene glycol, methanol, isopropyl alcohol, acetone, or other osmotically active substances are suspected. Serum osmolality is measured and also calculated with the variables serum glucose, sodium, and blood urea nitrogen (BUN) concentrations. The difference between the measured and calculated osmolality is defined as the OG. Boyle et al. (1) recently cautioned against using the OG as a screening tool for toxic-alcohol poisoning. One reason relates to the debate about what constitutes a normal OG and the variations in the range of osmolar gaps (2). To address what constitutes a normal OG, we determined the OG range for healthy subjects and examined data from hospitalized patients by use of several published equations for calculating serum osmolality.

After obtaining informed consent, we collected blood from 126 self-reported healthy subjects. The serum osmolality glucose, potassium, sodium, and BUN were within respective reference intervals (Tables 1 and 2). The OG log-transformed parametric reference intervals (central\(_{95\%}\)) were 8 to 11 mOsm/kg and 3 to 22 mOsm/kg by use of Eqs. 1 and 2, respectively.

General equation (2,4)

\[
\text{Osm} = 2(\text{Na}^+) + \frac{\text{Glucose}}{18} + \frac{\text{BUN}}{2.8} + \frac{\text{EtOH}}{4.6}
\]

Rasouli and Kalantari (5)

\[
\text{Osm} = 1.897(\text{Na}^+) + \frac{\text{Glucose}}{18} + \frac{\text{BUN}}{2.8} + \frac{\text{EtOH}}{4.6} + 13.5
\]

Krahn and Khajuria (4)

\[
\text{Osm} = 1.86(\text{Na}^+) + 1.15\left(\frac{\text{Glucose}}{18}\right) + \frac{\text{BUN}}{2.8} + 1.2\left(\frac{\text{EtOH}}{4.6}\right) + 14
\]

Glaser (3) and Krahn and Khajuria ([4]; see Dorwart’s equation)

\[
\text{Osm} = 1.86(\text{Na}^+) + \frac{\text{Glucose}}{18} + \frac{\text{BUN}}{2.8} + \frac{\text{EtOH}}{4.6} + 9
\]

Medians of OGs have been observed in renal failure (2). In 2007–09, ethanol caused a 54% increase in the median OG despite a correction factor being used for its presence. However, the 95% CIs of the OG upper reference limits overlap. A small number of measured osmolalities were followed up with volatile screens, including ethanol; the clinical sensitivities and specificities of the OG (Eq. 1) for identifying ethanol were (16%, 93%) and (78%, 47%), respectively for ED records identified 157 patients in 1998 and 117 patients in 2007–2009 for whom a serum osmolality measurement was ordered. Cases were eligible if serum glucose, potassium, sodium, BUN, ethanol, and osmolality were measured simultaneously. Cases were excluded when ethylene glycol, isopropyl alcohol, or methanol was detected. In 1998, there were 45 positive volatile screens (43 ethanol, 1 ethylene glycol, 1 acetone). In 2007–09, 54 positive screens were confirmed (32 ethanol, 6 ethylene glycol, 2 methanol, 5 isopropyl alcohol, 9 acetone). Serum osmolality was calculated by use of several equations (2–5), and a general correction for ethanol was included. Because large OGs have been observed in renal failure (2), we assessed the renal function of each subject by use of the Modification of Diet in Renal Disease equation to estimate the glomerular filtration rate (eGFR).

Renal insufficiency (eGFR < 60 mL/min/1.73 m\(^2\)) was present in 14% and 35% of the 1998 and 2007–09 ED patients, respectively. Median OGs, calculated by use of Eq. 1, were 0 mOsm/kg (central\(_{95\%}\) = 11 to 19 mOsm/kg) and 16 mOsm/kg (central\(_{95\%}\) = 2–36 mOsm/kg) for 1998 and 2007–09 patients with renal insufficiency. When eGFR was > 60 mL/min/1.73 m\(^2\), the median OGs were –2 mOsm/kg (central\(_{95\%}\) = –12 to 22 mOsm/kg) and 11 mOsm/kg (central\(_{95\%}\) = –3 to 39 mOsm/kg) for 1998 and 2007–09, respectively. Renal insufficiency did not appear to affect the OG.

Using Eq. 1, in 1998 the median OGs were –2 mOsm/kg (central\(_{95\%}\) = –12 to 20 mOsm/kg) and –2 mOsm/kg (central\(_{95\%}\) = –14 to 17 mOsm/kg) for positive and negative ethanol screens, respectively. Using Eq. 1, in 2007–09 the median OGs were 17 mOsm/kg (central\(_{95\%}\) = 1–44 mOsm/kg; 90% CI of the 97.5th percentile upper reference limit 36–54 mOsm/kg) and 11 mOsm/kg (central\(_{95\%}\) = –3 to 36 mOsm/kg; 90% CI of the upper reference limit 31–41 mOsm/kg) for positive and negative ethanol screens, respectively. In 2007–09, ethanol caused a 54% increase in the median OG despite a correction factor being used for its presence. However, the 95% CIs of the OG upper reference limits overlap.

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1 Nonstandard abbreviations: OG, osmolar gap; ED, emergency department; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.
Affect the OG. Therefore, acetone did not negatively affect acetone results, respectively. In 1998, hyperglycemia increased the median OG and the 97.5th percentile upper reference limit of 38–100 mOsm/kg, which is consistent with published mean OGs of 8–15 mOsm/kg (4). Physicians must have the correct OG reference interval for the equation they use.

### Table 1. Reference intervals of measured analytes.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Central 95%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>87 (16.8)</td>
<td>85</td>
<td>56–137</td>
<td>52–137</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.1 (0.3)</td>
<td>4.2</td>
<td>3.5–4.8</td>
<td>3.2–4.9</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>140.8 (1.7)</td>
<td>141</td>
<td>137–144</td>
<td>135–146</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>13.4 (3.4)</td>
<td>13.8</td>
<td>7.6–20.7</td>
<td>5.9–23.2</td>
</tr>
<tr>
<td>Osmolality, mOsm/kg</td>
<td>292.6 (4.8)</td>
<td>292</td>
<td>283–303</td>
<td>282–303</td>
</tr>
</tbody>
</table>

### Table 2. Osmal gaps and osmolality for healthy and ED patients in 1998 and 2007–2009.

<table>
<thead>
<tr>
<th>Osmal gaps*</th>
<th>n</th>
<th>General (Eq. 1)</th>
<th>Eq. 2</th>
<th>Eq. 3</th>
<th>Eq. 4</th>
<th>Eq. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>126</td>
<td>−8 to 11</td>
<td>3 to 22</td>
<td>−7 to 12</td>
<td>−10 to 9</td>
<td>−9 to 11</td>
</tr>
<tr>
<td>ED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>156</td>
<td>−11 to 19</td>
<td>1 to 32</td>
<td>−11 to 21</td>
<td>−20 to 14</td>
<td>−18 to 16</td>
</tr>
<tr>
<td>2007–09</td>
<td>104</td>
<td>−1 to 36</td>
<td>9 to 45</td>
<td>−1 to 37</td>
<td>−6 to 25</td>
<td>−5 to 27</td>
</tr>
</tbody>
</table>

* Central 95% osmolar gaps, log-transformed parametric.

1998 (n = 43) and 2007–09 (n = 28). The clinical sensitivities of the OGs, calculated by use of Eqs. 1–5, with respect to detecting volatile substances, were 92%, 100%, 92%, 92%, and 85%, respectively. Eq. 5 showed less clinical sensitivity to the presence of volatiles. Acetone, a metabolite of isopropyl alcohol and a metabolic product that is increased in patients with ketoacidosis, did not affect OG. In 1998, there was 1 positive acetone. Using Eq. 1, in 2007–09 the median OGs were 16 mOsm/kg (central95% = 1–3 mOsm/kg; 90% CI of the upper reference limit of 38–100 mOsm/kg) and 11 mOsm/kg (central95% = 1–3 mOsm/kg; 90% CI of the upper reference limit of 31–40 mOsm/kg) for samples with positive and negative acetone results, respectively. Therefore, acetone did not affect the OG.

Hyperglycemia (>200 mg/dL) affected 9% and 17% of the 1998 and 2007–09 ED patients, respectively. In 1998, hyperglycemia had a minimal affect on the OG (median 11 mOsm/kg, central95% = 14 to 21 mOsm/kg), but in 2007–09 hyperglycemia increased the median OG and the 97.5th percentile upper reference interval of 17 mOsm/kg, central95% = 1–48 mOsm/kg).

Although it has been suggested that the OG has increased over time (4), we found that the reference interval of −8 to 11 mOsm/kg calculated by use of Eq. 1 is consistent with the general rule of −10 to 10 mOsm/kg for healthy subjects (1). We concluded that renal insufficiency, ethanol, and acetone have minimal influence on the observed OG ranges in our ED. The apparent increase observed in ED patients may reflect more selective ordering, as evidenced by the 67% reduction in orders from 1998–2008, and possibly an increase in the number of patients with diabetic ketoacidosis.

Recognizing the limitations of using the OG is important. If a patient’s normal baseline OG is −8 mOsm/kg and the patient presents in the ED with an OG of 10 mOsm/kg, although within the reference interval, this change in OG (18 mOsm/kg) may result from toxic ingestion. Finally, the equation used to calculate serum osmolality must be considered when evaluating the OG. Note that Eq. 2 requires a higher OG reference interval of 3 to 22 mOsm/kg (mean OG 12 mOsm/kg), which is consistent with published mean OGs of 8–15 mOsm/kg (4).

### References


Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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First- and Second-Trimester ADAM12s in Down Syndrome Screening

To the Editor:

ADAM12s \(^1\) (a disintegrin and metalloprotease 12s), a placenta-derived glycoprotein involved in tissue growth and differentiation, has been shown to be an early screening marker for trisomy 21 before the 10th week of gestation \((1)\) and a potential second-trimester marker. A recent report suggested that measurements of a serum marker in both the first and second trimesters (repeated measures) could provide better performance than single measurements in either trimester \((2)\).

We sought to demonstrate the potential value of such repeated measures of ADAM12s for the screening of Down syndrome.

A cohort of 7194 women underwent integrated biochemical screening before amniocentesis at the Department of Obstetrics and Gynecology, University of Messina, between December 1, 2006, and March 31, 2009. After collection, the maternal serum samples were stored at \(-80^\circ\text{C}\). We selected paired first- and second-trimester samples from 19 Down syndrome pregnancies (cases) from frozen storage for ADAM12s measurement. Of the Down syndrome cases, 14 were identified in the first trimester and 5 in the second trimester.

ADAM12s was measured in frozen samples from 562 unaffected pregnancies of the same gestational age \((\text{GA})\) by means of 2 regression equations derived from the first- or second-trimester control groups. QC samples derived from pooled serum stored at \(-80^\circ\text{C}\) had ADAM12s concentrations of 86.5, 415.8, and 917.5 ng/L and were measured in duplicate at the beginning and at the end of each run. The mean CVs were 4.9%, 2.9%, and 3.5%, respectively.

The weighted log-linear regression model described by Royston and Thompson \((4)\) with the model parameters \((\text{mean and SD})\) for the first and the second trimesters and taking into account the correlation coefficients between trimesters for ADAM12s in Down syndrome and unaffected pregnancies.

The weighted log-linear regression equations for the first and second trimesters in the unaffected group were, respectively:

\[
\text{ADAM12s}_{\text{first}} = 10^{0.0269 \cdot \text{GA} + 0.613}
\]

\[
\text{ADAM12s}_{\text{second}} = 10^{0.0108 \cdot \text{GA} + 1.72}
\]

where \(\text{GA}\) is expressed in days. The ADAM12s MoM values were re-
Letters to the Editor

Maternal serum ADAM12s values in 17 singleton pregnancies with Down syndrome and in 562 unaffected pregnancies.

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Marker</th>
<th>Mean (SD)</th>
<th>DR (95% CI), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected</td>
<td>A12_1T</td>
<td>0.0135 (0.1919)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A12_2T</td>
<td>−0.0041 (0.1473)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A12 ratio</td>
<td>−0.0176 (0.1843)</td>
<td></td>
</tr>
<tr>
<td>Down syndrome</td>
<td>A12_1T</td>
<td>−0.1562 (0.2517)</td>
<td>41.2 (18.4–67.1)</td>
</tr>
<tr>
<td></td>
<td>A12_2T</td>
<td>0.1269 (0.2154)</td>
<td>35.3 (14.2–61.7)</td>
</tr>
<tr>
<td></td>
<td>A12 ratio</td>
<td>0.2831 (0.1451)</td>
<td>52.9 (27.8–77.0)</td>
</tr>
</tbody>
</table>

* ADAM12s results are expressed as log MoM values in the first trimester (A12_1T) and the second trimester (A12_2T) and as the ratio of MoM ADAM12s in the second trimester to MoM ADAM12s in the first trimester (A12 ratio). DR values are given at a 5% FPR.

where W is body weight in kilograms. The log MoM values are displayed in Table 1. The geometric mean for ADAM12s MoM in the first-trimester cases was 0.654 at a median GA of 9.1 days, significantly lower than in the unaffected group (MoM, 1.003; P = 0.014). The geometric mean for the second-trimester ADAM12s MoM for the cases was 1.361, significantly higher than in the unaffected group (MoM, 1.000; P = 0.024). The ratio of the second-trimester MoM to the first-trimester MoM for the cases was 1.883, significantly higher than for the unaffected group (MoM, 0.985; P < 0.0001).

The between-trimester ADAM12s correlation coefficient was 0.430 for the unaffected pregnancies and 0.591 for the cases. At an FPR of 5.0%, the DR was 42.1% in the first trimester and 35.3% in the second trimester; the DR for the ratio of the second trimester value to the first trimester value was 52.9%. In the same cases, a biochemical integrated test (pregnancy-associated plasma protein A in the first trimester; α-fetoprotein, unconjugated estriol, and human chorionic gonadotropin in the second trimester) gave a DR of 70.6% at a 5.0% FPR. These DRs were lower than the expected rate of 90% reported in the literature (5), likely owing to the small number of cases considered and the number of positive pregnancies with spontaneous miscarriage before second-trimester sampling.

Adding the ADAM12s ratio to the serum integrated test increased the DR by about 6%. This performance was obtained with samples at very early GAs (7–10 weeks), whereas at 11–13 weeks, the more commonly tested first-trimester period, the use of ADAM12s was questionable (6).

The present data confirm that the ADAM12s concentration is relatively low in Down syndrome cases before 10 weeks of gestation and increases appreciably in the second trimester. This pattern is similar to that for pregnancy-associated plasma protein A in Down syndrome, and it constitutes the basis for the use of ADAM12s across trimesters in prenatal screening.

Despite the small number of cases, our data suggest that use of the ratio of second-trimester to first-trimester MoM values for ADAM12s could provide better performance than single MoM measurements in either trimester alone. Furthermore, ADAM12s may be useful in combination with other markers in the current integrated biochemical test. Further studies are needed to confirm these first limited results.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References
The Effect of Sample Hemolysis on Cardiac Troponin I and T Assays

To the Editor:

Cardiac troponin I and T (cTnI and cTnT) assays are used for the diagnosis of acute myocardial infarction (AMI) (1). Recent improvements in these assays have lowered the imprecision and detection limit so that the assays meet guidelines for using the 99th percentile cutoffs for the respective assays, namely 34 ng/L for cTnI (24, 36, 49 ng/L) and 13 ng/L for cTnT (6, 12, 23 ng/L). Indices were measured as recommended by the manufacturers. The Vitros 5600 measures the indices using the residual sample left in the sample tip. The Modular measures indices on the chemistry module by taking an aliquot of the patient specimen and diluting it in 0.9% NaCl. For both instruments, algorithms convert the absorbance measured at wavelength pairs into qualitative values that correlate with estimated concentrations of the sample interferent.

According to the recommendations of the National Academy of Clinical Biochemistry, a 20% change in cardiac troponin value is suggestive of an acute myocardial infarction that is either evolving (cardiac troponin increasing) or resolving (cardiac troponin decreasing) (3). For both assays, a hemolysis index of around 150 caused a >20% change in cTn (Fig. 1), which equates to a hemoglobin concentration of 1.9 g/L. It has been suggested that at baseline concentrations of cTn, δ changes of >20% are needed for improved clinical specificity and, thus, laboratories must consider carefully what constitutes a clinically significant change in cardiac troponin (4).

One important aspect of these experiments is that they were carried out at cardiac troponin concentrations close to the 99th percentile for each assay. When the same experiments were done at higher cardiac troponin concentrations, a clinically significant effect (for example, ±20%) was not observed. This is understandable because a change of 10 ng/L at a concentration of 10 ng/L represents a 100% change, whereas at 100 ng/L it represents a 10% change, demonstrating the need to do these types of experiments at critical concentrations for any analyte. Many reagent package inserts contain limited information on interferences and often only on what concentration of interfering material interferes with the assay, with no information on what concentrations of analyte were tested. If the analyte concentration was relatively high, the effect of the interfering material may not be observed. The hs cTnT brochure states that samples are unaffected by Hb <0.1 g/dL, and samples showing visible signs of hemolysis may be interfered. There is no indication of what concentrations of cTnT were tested. In the cTnI brochure, there is a table indicating the effect of increasing sample Hb, but this was tested at 0.006 μg/L, which is half the stated limit of detection for the assay.

Hemolysis has been reported to be as high as 8.8% for samples collected in an emergency department (5). In my own hospital, the number of cTnT requests from the emergency department rejected because of hemolysis interference...
is 3.9%. Studies indicate that the contemporary cTnl and high-sensitivity cTnT assays I tested are sufficiently affected at relatively low degrees of hemolysis to indicate that interference must be monitored for every specimen. With the advent of integrated analyzers that incorporate both chemistry and immunoassays, I advocate that laboratories investigate how interferences such as hemolysis affect key assays, and that indices be measured on all samples for which cardiac troponin has been requested. If this is not possible, at least a visual examination of the sample quality should be done before analysis.

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Assessing the Performance of Point-of-Care Hemoglobin A1c Systems

To the Editor:

Lenters-Westra and Slingerland (1) recently reported results of a comparative performance study of several point-of-care (POC)1 tests for hemoglobin A1c (Hb A1c), and we commend them for providing useful new information on instrument performance. We also believe that the results should be interpreted strictly in terms of the facts, including consideration of discrepancies between the study design and the instructions provided by the manufacturers.

Bayer’s A1CNow+® Multi-Test A1C System was included in the initial phase of this study, but testing was not continued for the main phase of the study after the local distributor of that product concluded that the preliminary CLSI EP-10 results did not warrant further testing. The authors reported that these results were probably due to EDTA interference. As a POC test, the A1CNow+ test is primarily intended for capillary blood samples, and the instructions for use specify use of heparin-containing collection tubes when using venous blood.

The authors’ title, “Six of Eight Hemoglobin A1c Point-of-Care Instruments Do Not Meet the General Accepted Analytical Performance Criteria,” and its conclusion are misleading when 7 instruments at most were used according to the manufacturers’ operating instructions; thus, performance conclusions are warranted only for these 7 instruments. No conclusion should be drawn regarding the performance of the A1CNow+ test because it was not used according to manufacturer’s labeling in the preliminary stage and thus was not included in the final study. In the Results section of the Abstract, the authors state that 2 of 8 manufacturers decided not to continue the study because of the disappointing EP-10 results, a simplification that omits the relevant fact that inappropriately obtained blood samples were used for the A1CNow+ test.

In the same issue of this journal, Bruns and Boyd (2) contributed an editorial offering further interpretation and an opinion of the Lenters-Westra and Slingerland report. They paraphrase the same misinterpretation by stating, “Two of the 8 manufacturers withdrew from the study after initial unpromising results with their POC methods.” This statement again misrepresents the reason for the withdrawal in the case of the A1CNow+ test and further supports our belief that there will be misunderstandings because of the conclusions and overall impression provided by the report.

Furthermore, the Results section and the Acknowledgments at the end of the Lenters-Westra and Slingerland report indicate that the study authors communicated directly with a local unaffiliated distributor for the Bayer A1CNow+ device in lieu of direct communication with the manufacturer (Bayer). We point out that Bayer was not asked to comment on the study protocol before its execution and thus did not have the opportunity to comment on the resulting negative bias when the test was used with EDTA-containing blood.

A challenge to future researchers (and their reviewers) examining the performance of POC Hb A1c devices would be to include an analysis of all the relevant information to provide a broader context for interpretation. In Lenters-Westra and Slingerland’s report, it is apparent that there was variation among the laboratory reference methods, although they were all controlled and calibrated in the authors’ laboratory. Reference 17 in their report is cited as a source of concern regarding the accuracy of

1 Nonstandard abbreviations: POC, point-of-care; Hb A1c, hemoglobin A1c.
POC instruments, yet this reference describes an accuracy drift over time that was as large in the central laboratory instrument as it was in the POC device (3). Survey results from the College of American Pathologists (4) indicate that in the field, variation within and between laboratory-based methods can be comparable to or greater than some of the POC results reported by Lenters-Westra and Slingerland, and an analysis of these trends was given in the report by Holmes et al. cited by these authors. Including such considerations would shed light on realistic performance expectations with the current state of laboratory methods for Hb A1c. Although performance should be of primary concern, additional clinical considerations, such as patient access, cost, portability, convenience, and the impact of immediately available Hb A1c results, would also bring added value to the context of this discussion.

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In Reply

We thank Drs. Irvin, Knaebel, and Simmons for their questions, and appreciate the opportunity to reply.

The evaluation protocol was designed by the authors and sent for comment either to the manufacturers, or to their local distributors. We expected local distributors to communicate with their manufacturer regarding the protocol. We reject the notion that we approached an unaffiliated distributor, because the distributor we contacted is registered on the Bayer HealthCare website (1). This local distributor had no comments on the study proposal. Moreover, the local distributor gave us the results of 9 A1CNow® InView™ field evaluation studies, all of which used whole-blood samples anticoagulated with EDTA. Although the professional-use product insert (2) states that heparin tubes are preferred for venous blood collection, it does not give specific warnings regarding the disadvantages of EDTA blood. If these disadvantages are important, their omission in the Warnings and Precautions section is a major oversight, because EDTA tubes are commonly used for the determination of hemoglobin A1c in the clinical laboratory. Such information should have been provided to the local distributors and further detailed in the product insert.

The EP-10 results were sent to the local distributor and discussed in person. The possible effect of EDTA was discussed and we offered the option of repeating the study with capillary blood at the diabetes care center, but the local distributor was not interested.

After publication of our article (3) we offered the manufacturer an option of repeating the study with heparin blood and sent them a draft proposal. Our only requirement was that regardless of outcome, the study results could be published. The manufacturer declined our initial offer, but suggested that we apply for a Bayer Diabetes Care Independent Research Grant. We understand that the manufacturer has very specific regulatory and compliance measures to meet to satisfy legal requirements, but in this case we felt their response was somewhat overcautious. In addition, to maintain our investigative independence, up to that point we had accepted only reagents from the various manufacturers, and no other support or grants from involved industries. Therefore, we respectfully declined the invitation to apply for further research grants. However, in our opinion, the manufacturer, by declining our offer to repeat the study with heparin blood, missed an opportunity to further extend the knowledge regarding the analytical performance of the A1CNow+ Multi-Test A1C System.
We are in agreement with the authors’ opinion that certain hemoglobin A1c laboratory methods also have problems concerning analytical performance and stated this in the podcast accompanying our article (4). Because College of American Pathologists survey results represent the aggregate results of many different laboratories, not just the performance in one laboratory, it is difficult to make any comparison of College of American Pathologists results with those presented in our report.

At this moment we are preparing a report on our comparison of the analytical performances of individual laboratories. We would not be surprised if the performance of some point-of-care methods turns out to be better than some laboratory-based methods. It is important that the limitations of both current point-of-care instruments and laboratory methods be understood by healthcare professionals, because these may have important clinical implications. This was pointed out in the editorial by Bruns and Boyd (5).

This study will be repeated at our own expense with heparinized whole blood.

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