Osmolality Gaps: Diagnostic Accuracy and Long-Term Variability, John Krahn and Annu Khajuria (Department of Clinical Biochemistry, St. Boniface General Hospital, and University of Manitoba Medical School, Winnipeg, Manitoba, Canada; * address correspondence to this author at: St. Boniface General Hospital, Winnipeg, Manitoba, Canada R2H 2A6; fax 204-231-2656, e-mail jkrahn@sbgh.mb.ca)

Background: The osmolal gap (OG) is a screening test for the detection of toxic volatiles such as methanol and ethylene glycol. We used mean values of patient data to assess the diagnostic accuracy and long-term stability of OG measurements.

Methods: In a prospective study period in 2003, all requests for volatiles had OGs calculated and quality-control samples were analyzed for OG. ROC curves were constructed to determine whether OG could predict the presence of toxic volatiles in serum. This was also done in a retrospective study for data from 1996 to 2004. Our laboratory database was searched for all emergency room patients for the period of 1996 to 2004 who had tests ordered that allowed us to calculate OGs.

Results: For the prospective study period in 2003, the ROC areas indicated that we could accurately predict the presence of toxic volatiles but at markedly different decision cutpoints depending on the formula used. These cutpoints ranged from +10 to +33 mosmol/kg. In the retrospective study, the mean OGs in the patient population for each of the 3 formulas increased by 12 mosmol/kg from 1996 to 2004. For this reason, the diagnostic accuracy was poor when all data were analyzed together.

Conclusions: Under properly controlled conditions, the OG has high sensitivity and specificity for detection of poisoning with some volatiles. Over the long term, however, use of the reference interval of −10 to +10 mosmol/kg yields poor diagnostic accuracy because mean OGs are not constant over time. Bedside calculation is not advisable.

Calculation of serum osmolality from the serum concentrations of sodium, glucose, and urea is a long-standing practice (1–3). In earlier times, calculation of the osmolality gap (OG), defined as the difference between the measured and calculated osmolality, allowed clinicians to estimate the concentration of ethanol in a patient’s blood and provided evidence of suspected toxins, such as ethylene glycol and methanol, in the blood (4–7). Because glucose and ethanol contribute more to osmolality than is inferred from their molar concentrations (8–12), the OG can be misleadingly high in the presence of increased glucose and will be higher than expected for the concentration of ethanol.

The performance of the OG in a diagnostic sense is unknown. There is no evidence that simple bedside calculations can be used to either rule in or rule out a poisoning. Formulas 1, 2 (12), and 3 (1) below accurately predict the measured osmolality (OSMm).

\[
\text{OSM}_{\text{CLSA}} = 1.86 \text{ (sodium + potassium)} + \text{urea} + 1.15 \text{ (glucose)} + 1.2 \text{ (ethanol)} + 14
\]

\[
\text{OSM}_{\text{C2}} = 2 \text{ (sodium)} + \text{urea} + 1.15 \text{ (glucose)} + 1.2 \text{ (ethanol)}
\]

\[
\text{OSM}_{\text{DORWART}} = 1.86 \text{ (sodium)} + \text{urea} + \text{glucose} + \text{ethanol} + 9
\]

We applied these formulas for calculated osmolality (OSMc) to data generated in our laboratory on control specimens and on patient samples from the emergency room, and calculated the OG for each OSMc. Osmolality was measured by freezing-point depression with a Fiske 2400 osmometer (Fiske Associates). Electrolytes, glucose, urea, and ethanol were determined on high-volume analyzers [Hitachi 717 (1996–2002) and Roche Modular System (2003–2004); Roche Diagnostics]. Collection tubes were Becton Dickinson SST and PST Vacutainers from 1996 until 2002 and Corvac PST thereafter. The calculations were all done in SI units and expressed in mosmol/kg or mmol/L as appropriate. The respective OG values were calculated as \( \text{OG} = \text{OSM}_{\text{m}} - \text{OSM}_{\text{c}} \). The prospective period of the study lasted from April to October of 2003. During this period, all requests for toxic volatiles also had all analytes measured to allow calculation of OG. We used 2 quality-control specimens (Bio-Rad MultiQual; Bio-Rad Laboratories) during that period and calculated OG on the control material.

We also carried out a separate retrospective search for all requests for toxic volatiles that also had the other laboratory tests performed to allow calculation of the OG. The practice in our region is that all requests for toxic volatiles are screened by an on-call clinical chemist. The data were carefully examined, and only diagnostic data were retained (without patient identifiers).

We performed ROC analysis on both the prospective and retrospective data and determined diagnostic specificity and sensitivity and areas under the curves (AUC). To determine whether the OG remained constant over long time intervals, we calculated mean OGs on data in 6-month intervals where sufficient tests had been ordered on emergency room patients to allow this calculation. This was done on data from 1996 to 2004. The statistical analysis was performed with the Microsoft Excel® add-in Analyze-It®. For the ROC analysis, any methanol or ethylene glycol >1 mmol/L was considered positive. To determine sources of variability in OG values, we assessed the data from quality-control specimens. The biggest contributions to the OSMc came from sodium and OSMm. It follows that imprecision in the OG results should be related to imprecision in sodium and OSMm.
For both of these analytes as well as for OG, we calculated the difference from the mean value of that control specimen for each data point. This allowed us to do linear regression analysis of the differences in the OG vs the differences in OSM\textsubscript{m} and measured sodium.

During the prospective study, the quality-control samples that were run daily for the entire period demonstrated the stability of the analytical processes over that entire period. This study also revealed that the variability in the OG was largely attributable to the variability of OSM\textsubscript{m}, with little imprecision attributable to sodium (see the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol52/issue4). We do not have this type of data for the retrospective period.

Shown in Table 1 are the decision cutoffs at which 100% sensitivity was achieved, i.e., the OG value at which all toxic poisoning would be detected. ROC curves during the prospective study had high AUCs. Formulas 1–3 were able to correctly predict whether a toxic volatile was present; the decision cutoffs for formulas 1–3 were /H10/H11001/H11001/H14/H11001/H13 mosmol/kg, respectively, in agreement with their mean OGS of 0, 2, and 20 mosmol/kg in the study period. For the retrospective period, the AUCs varied between 0.79 and 0.80. The decision cutoffs to achieve 100% sensitivity were /H10/H12/H19, /H10/H14, and /H10/H17 mosmol/kg, respectively, for the 3 formulas.

The mean OG values for each 6-month period from 1996 to 2004 are shown in Fig. 1. These statistics include all specimens (even those that contained ethanol). There is a difference of 20 mosmol/kg in the OG between the Dorwart formula and formulas 1 and 2. The Dorwart formula gave a mean OG of 8 mosmol/kg in 1996, which increased to 15–20 mosmol/kg in 2003–2004. Formulas 1 and 2 gave mean OG values of /H10/H12/H19 to /H10/H12/H17 in 1996, but had increased to /H12/H10/H19 to /H10/H12/H22 mosmol/kg in 2003–2004. It is apparent that all formulas show a large shift in the mean OG over time. We have not been able to identify the reason for the change of the mean OGS. When we subjected these data to ROC analysis for each 6-month period, the decision cutoffs required to achieve 100% sensitivity showed the same trend as the mean OG values. This meant that the assumption of a reference interval of /H10/H12/H10 to /H10/H12/H20 mosmol/kg was wrong and therefore was not reliable. Throughout the period during which the mean OG values changed, the laboratory’s proficiency testing programs showed acceptable performance of the constituent analytes.

This study shows that OSM\textsubscript{c} and OG formulas can be a highly effective screening method, over a relatively short time period of a few years when careful quality-control procedures are followed, for the identification of patients poisoned with toxic volatiles. However, the drift in mean OG values over a longer time reduces the effectiveness and may account for the proliferation of formulas. This finding limits bedside calculations based on a reference interval of /H10/H12/H19 to /H10/H12/H20 mosmol/kg. It is evident that these calculations are effective only if they are validated on appropriate reference populations and if strict quality procedures are subsequently followed to ensure that the calculations continue to function in the intended manner.

### References


### Table 1. ROC statistics for the study period and data for the period from 1996 to 2004.

<table>
<thead>
<tr>
<th>Formula*</th>
<th>AUC</th>
<th>mosmol/kg at 100% sensitivity</th>
<th>Specificity, %</th>
<th>TP, n</th>
<th>TN, n</th>
<th>FP, n</th>
<th>FN, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospective study (n = 49)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.95 (0.89–1.0)</td>
<td>10</td>
<td>86</td>
<td>6</td>
<td>37</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.96 (0.89–1.0)</td>
<td>14</td>
<td>88</td>
<td>6</td>
<td>38</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.91 (0.80–1.0)</td>
<td>33</td>
<td>74</td>
<td>6</td>
<td>32</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Retrospective study (n = 136)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.80 (0.68–0.90)</td>
<td>0</td>
<td>21</td>
<td>24</td>
<td>23</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.80 (0.68–0.90)</td>
<td>4</td>
<td>25</td>
<td>24</td>
<td>28</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.79 (0.68–0.90)</td>
<td>7</td>
<td>11</td>
<td>24</td>
<td>12</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* The formulas are described in the text.

b TP, true positive; TN, true negative; FP, false positive; FN, false negative.

Fig. 1. Mean OG values from 1996 to 2004.

Data points represent mean OG values (in mosmol/kg) for patient data in 6-month intervals. The means include all data on all specimens that were submitted for ethanol measurements. □ OSM\textsubscript{C1.86}; △ OSM\textsubscript{C2}; • OSM\textsubscript{C-DORWART} (formulas 1–3 in the text).
Clinical Chemistry 52, No. 4, 2006 739

Mulitplex Tetra-Primer Amplification Refractory Mutation System PCR to Detect 6 Common Germline Mutations of the MUTYH Gene Associated with Polyposis and Colorectal Cancer, Patrizia Piccoli,1,7 Martina Serra,1,14 Viviana Gismondi,2 Simona Pedemonte,1 Fabrizio Loiacono,1 Sonia Lastraioli,1 Lucio Bertario,2 Maria De Angioletti,4 Lili-ana Varesco,2 and Rosario Notaro1* (1 Laboratory of Human Genetics, Medical Oncology C, and 2 Hereditary Tumors Unit, IST, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy; 3 Preventive-Predictive Medicine Unit, Istituto Nazionale Tumori, Milan, Italy; 4 Istituto di Genetica e Biofisica “Adriano Buzzati Traverso”, Consiglio Nazionale delle Ricerche, Naples, Italy; † these authors contributed equally to this work; * address correspondence to this author at: Laboratory of Human Genetics, Medical Oncology C, IST, Istituto Nazionale per la Ricerca sul Cancro, Largo R. Benzi, 10, 16132 Genova, Italy; fax 39-010-560-0066, e-mail rosario.notaro@istge.it)

Background: We describe a simple tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) for detecting MUTYH mutations, which are associated with colorectal adenomas and colorectal cancer.

Methods: We designed specific T-ARMS-PCR assays for 6 mutations (Y165C, G382D, 1395_7delGGA, Y90X, 1103delC, and R231H) selected on the basis of the frequency of their occurrence. We also designed a set of 3 multiplex T-ARMS PCR assays, each for detection of 2 mutations. We tested DNA samples from patients with attenuated or classic adenomatous polyposis coli and no detectable APC germline mutations.

Results: All mutations were easily detected with both the specific and multiplex T-ARMS-PCR assays. Results were confirmed by DNA HPLC analysis in all 54 patients, and each mutation was confirmed by direct DNA sequencing.

Conclusions: T-ARMS-PCR does not require any special equipment, and it provides rapid, reproducible, and cost-effective detection of common MUTYH mutations. Multiplex T-ARMS-PCR allows the detection of 6 common MUTYH mutations with use of as few as 3 single tube PCR reactions. It could be useful to carry out large population-based epidemiologic studies.

© 2006 American Association for Clinical Chemistry

MUTYH-associated polyposis (MAP) is an autosomal recessive syndrome associated with biallelic germline mutations in the base excision repair gene MUTYH (OMIM #608456) (1). MUTYH biallelic germline mutations have been found in 4%–33% (2, 3) of patients with multiple colorectal adenomas and in 7.5%–29% of patients who have attenuated or classic adenomatous polyposis coli and no detectable APC germline mutations (2, 4–6). Population-based studies suggest that biallelic MUTYH germline mutations might be also responsible for ~0.5% of unselected colorectal cancers (7, 8).

At least 23 different putative pathogenic mutations have been identified as widespread in the MUTYH gene (9). Two of these mutations (Y165C in exon 7 and G382D in exon 13) account for at least 70% of the mutant MUTYH alleles (2, 6), and at least 1 of them is found in more than 80% of Caucasian MAP patients (2, 4–6, 10, 11). In addition, these 2 mutations have been found in the general Caucasian population with a frequency of ~0.5% (1, 2, 8, 12, 13). Other mutations may be frequent in patients from some populations; e.g., the homozygous E466X (exon 14) mutation has been found in 3 patients from unrelated Indian families (3). Recently, we found that in Italian patients a 3-bp deletion in exon 14 (1395_7delGGA) is relatively frequent (5) and that each of the mutations Y90X (exon 3), 1103delC (exon 12), and R231H (exon 9) represents more than 6% of mutant MUTYH alleles (14).

The identification of germline mutations in both MUTYH alleles in patients with multiple colorectal adenomas or colorectal cancer has clinical relevance because their siblings may also have a very high risk of cancer. Thus, genetic testing for MUTYH mutations should be offered, after appropriate counseling, to individuals with multiple colorectal adenomas and to members of their families; it may also be offered to individuals with early-onset colorectal cancer (9, 15). In addition, because some of the pathogenic MUTYH mutations have relatively high frequencies in the general population and heterozygotes may also have an increased risk of colorectal cancer (8, 12), more widespread genetic testing for MUTYH mutations, perhaps in any individual with colorectal cancer, may be advisable.

Many methods, such as single-strand conformation polymorphism analysis and DNA HPLC (dHPLC), are suitable for MUTYH mutation detection. These methods, however, require specialized equipment and, most impor-