Testing for Creatine Kinase and Creatine Kinase-2 in Ontario: Reference Ranges and Assay Types
A. R. Henderson,1 M. J. McQueen, R. L. Patten, S. Krishnan, D. E. Wood,2 and S. Webb

In 1991, 246 and 136 Ontario laboratories performed total creatine kinase (CK; EC 2.7.3.2) and creatine kinase-2 (CK-2) assays, respectively. A questionnaire mailed to these laboratories requested information about the types of assay used, the origin of their reference ranges, and the source of their instruments and reagents. All laboratories used current test formulations for CK, although seven laboratories did not assay at 37 °C. For CK, 69% of all laboratories reported different upper reference limits for men and women (5th–95th percentiles: 160–250 and 115–215 U/L, respectively); 31% reported similar ranges for both sexes. Fifty-six percent derived their own ranges; the remainder used either kit inserts or literature references, and nearly 60% of this latter group claimed to have validated these suggested ranges before use. For 6% of all laboratories, their pediatric ranges were similar to their adult ranges. For CK-2, only 32% used their own reference range; the remainder used kit inserts or literature references, but only 49% of this group validated these ranges before use. Reference limits (5th–95th percentiles) for CK-2 were as follows: activity 6–24 U/L; fraction of CK, 0.022–0.06; and, for mass assays, 5–10 μg/L and relative index 0.015–0.04.

Additional Keyphrases: isoenzymes • proficiency testing

Mandatory external quality assurance for Ontario laboratories comprises licensing and inspection by the Ontario Ministry of Health and, since 1974, mandatory proficiency testing by the Ontario Medical Association.

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Materials and Methods

Laboratories. The 246 and 136 Ontario laboratories licensed, respectively, to determine total CK and CK-2 were surveyed by an LPTP postal inquiry to each laboratory director in April 1991.

CK questionnaire. The LPTP Committee asked for the
assay temperature, thiol activator(s), adenylate kinase inhibitor(s) used in the assay, and the manufacturer's method insert. All likely thiol activators and adenylate kinase inhibitors were listed on the questionnaire, so the respondent merely had to check the appropriate boxes. In addition, we requested the current reference ranges for male, female, and pediatric populations, and details of how and when these ranges were obtained (population, number of subjects, preanalytical conditions, etc.). Alternatively, if the reference range was not derived by the laboratory, we asked for its source—manufacturer's kit insert or a literature reference. We also asked whether the laboratory had validated such a reference range in any way. Finally, comments were requested on any difficulties encountered while obtaining such ranges. Those 196 (79.7%) laboratories that provided unsatisfactory or incomplete data received a second request for further data.

**CK-2 questionnaire.** The content of this questionnaire was similar to that for total CK, described above. In addition, we asked whether the laboratory used the fraction of CK activity due to CK-2, or the relative index in the case of mass (immunological) assays (relative index = CK-2 (µg/L)/total CK (U/L)), or CK-2 activity alone. If this last mode of reporting was used, the respondent was asked to justify this procedure with a literature reference. Here, 114 (83.8%) laboratories provided unsatisfactory or incomplete data and received a second request for further data.

**Analysis of questionnaire responses.** There was an eventual 100% response to the questionnaires. All responses were entered into a MUMPS database program, written in-house in the Department of Clinical Biochemistry, University Hospital, London, Ontario, which was used for all subsequent analyses. Each laboratory was coded by its three-digit LPTP number and by the three-character method code assigned to the assay of total CK (i.e., thiol activator, analyzer, reagent source) and CK-2 (i.e., measurement principle, analytical device, reagent source).

**LPTP total CK survey results.** The results of an LPTP survey, conducted in January 1991, for two specimens with total CK activities near the upper reference limit for men (all-method means of 183 and 229 U/L), were categorized by analyzer type.

**Results**

**Total CK Assay**

Of the 246 laboratories, 97% used an assay temperature of 37 °C; the remaining 7 laboratories used 30 °C. The characteristics of the total CK assays are listed in Table 1.

The major analyzer types, used at 37 °C, are described in Table 2. Eight analyzer types accounted for 79% of the analyzers used in the Province for total CK analyses. Figure 1 shows the upper reference limit for men by analyzer type. We also classified upper limits for men by some locations (Figure 2). Data obtained from Hamilton (Figure 2C), where there is a citywide laboratory system, should be contrasted with data from London (Figure 2D), where there is not. A group (n = 11) of adjacent community hospitals has partially completed the process of generating a group reference range for their entire district (Figure 2F).

**Upper reference limits (37 °C).** Of the 239 laboratories, 74 (31%) had the same reference ranges for both men and women (a further 6% did not report any reference value for women at all): the 5th–95th percentiles were 130–253 U/L (median 206 U/L). Of the 239 laboratories, 41 (17.2%) used their own reference range, 36 used the manufacturer's kit insert range, and 4 used a range from a literature source; 7 of these laboratories used more than one source for their reference ranges.

Of the remaining 165 laboratories (69%), 93 (38.9%) used their own reference range, 72 used the manufacturer's kit insert range, and 8 used a range from a literature source. Twelve laboratories used more than one source for these ranges. Of the 68 laboratories using a manufacturer's or literature reference range, 48 (70.6% of 68 laboratories) claimed to have validated the ranges they were using. When the ranges for each sex were dissimilar, the following 5th–95th percentiles (and median values) were reported for the total CK assays performed at 37 °C: men (n = 237), 160–250 (205); women (n = 142), 115–215 (155); male–female difference (n = 142), 17–90 (60); laboratories reporting their own range (n = 93), 110–243 (205); and, laboratories

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**Table 1.** Characteristics of CK Assays (n = 246)

<table>
<thead>
<tr>
<th>Thiol activator</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetyl-L-cysteine (NAC)</td>
<td>219</td>
<td>89.0</td>
</tr>
<tr>
<td>Dithioerythritol (DTE)</td>
<td>11</td>
<td>4.5</td>
</tr>
<tr>
<td>Monothioglycerol</td>
<td>12</td>
<td>4.9</td>
</tr>
<tr>
<td>NAC and DTE/dithiothreitol</td>
<td>4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**Table 2.** Analyzers (n = 246) Used to Assay Total Creatine Kinase Activity

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kodak Ektachem and DT60</td>
<td>43</td>
<td>17.5</td>
</tr>
<tr>
<td>Roche Cobas MIRA</td>
<td>39</td>
<td>15.9</td>
</tr>
<tr>
<td>Hitachi systems</td>
<td>36</td>
<td>14.6</td>
</tr>
<tr>
<td>Technicon RA-1000</td>
<td>20</td>
<td>8.1</td>
</tr>
<tr>
<td>Abbott Spectrum</td>
<td>17</td>
<td>6.9</td>
</tr>
<tr>
<td>Coulter DACOS</td>
<td>14</td>
<td>5.7</td>
</tr>
<tr>
<td>Abbott bichromat systems</td>
<td>14</td>
<td>5.7</td>
</tr>
<tr>
<td>Baxter Paramax</td>
<td>11</td>
<td>4.5</td>
</tr>
<tr>
<td>IL Dimension</td>
<td>10</td>
<td>4.1</td>
</tr>
<tr>
<td>IL Monarch</td>
<td>10</td>
<td>4.1</td>
</tr>
<tr>
<td>Other analyzers*</td>
<td>32</td>
<td>13.0</td>
</tr>
</tbody>
</table>

* Beckman systems, Roche Cobas FARA, Ciba-Comin EXPRESS, Du Pont acc, Olympus systems, Technicon AXON, IL Genesis, American Monitor KDA, Gilford systems, and ENI systems.
The distribution of total CK upper reference limits for men for all assays performed at 37°C, by geographical location is shown in Figure 2. A, Greater Toronto; B, Ottawa; C, Hamilton; D, London; E, Windsor; F, district hospital group.

Table 3. Characteristics of CK-2 Assays (n = 136)

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoinhibition</td>
<td>74</td>
<td>54.4</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>29</td>
<td>21.3</td>
</tr>
<tr>
<td>Microparticle enzyme immunoassay (Abbott)</td>
<td>14</td>
<td>10.3</td>
</tr>
<tr>
<td>Du Pont column</td>
<td>8</td>
<td>5.9</td>
</tr>
<tr>
<td>Fluorometric enzyme immunoassay (Dade Stratus)</td>
<td>6</td>
<td>4.4</td>
</tr>
<tr>
<td>Immunoenzymetric (Hybritech Inc.)</td>
<td>5</td>
<td>3.7</td>
</tr>
</tbody>
</table>

reported upper reference limit for men and the survey results (data not shown).

CK-2 Assay

The types of assay used by the 136 laboratories to detect CK-2 are shown in Table 3. Most of the immunoinhibition assays were from Roche (37.8%), Kodak (23%), Boehringer Mannheim (17.6%), and Sigma (10.8%); the remainder were from various other suppliers (10.8%). These data indicate a shift from immunoinhibition methods (63% of the 123 laboratories reported in reference 2) to the newer immunoassay (mass assay) techniques—a trend that seems likely to continue, given the ease of use of the newer methods.

Ten laboratories (7.4% of 136 laboratories) reported a
screen and confirmation strategy for performing CK-2 assays. Seven used an immunoinhibition assay for screening; if the result was positive, six laboratories confirmed by electrophoresis, and one by using another type of immunoinhibition assay. The remaining three laboratories screened with a mass assay and confirmed by electrophoresis.

Upper reference limits. Of the 136 laboratories performing the CK-2 assay, 43 used their own values, 94 used the manufacturer's kit insert range (and 47% of these users validated these ranges), and 13 used a range from a literature source, of whom 62% validated the selected ranges. Fourteen laboratories used more than one source for these ranges. Of the 43 (31.6% of 136 laboratories) using their own range, only 2 had different ranges for men and women. Of the 94 (69.1% of 136 laboratories) using a kit insert range, only 4 had sex-specific ranges. None of the laboratories using a literature range had sex-specific ranges.

Although 98 laboratories reported upper reference ranges for CK-2 activity in men, only 89 laboratories also provided values for women (six of which differed from the range for men). For all assays based on the measurement of activity, the 5th–95th percentiles for men and women were both 6–24 U/L (fraction: 0.022–0.06), whereas for all mass assays (n = 24) the corresponding figures are 5–10 μg/L, with a relative index range of 0.015–0.04.

Reference ranges differed within each methodology (Figure 3). Ranges are shown for laboratories that used electrophoresis (Figure 3, A and B) or non-Kodak immunoinhibition methods (Figure 3, C and D). Upper reference ranges for men for Kodak users (n = 17 not shown) were 16 (n = 11), 15 (n = 3), and 10 U/L (n = 3), with a fraction of 0.04 (one each reported 0.05 and 0.06). Responses were sufficient to display mass assay ranges for only the Abbott IMx (Figure 3E).

The methods of reporting CK-2 results are listed in Table 4. Most laboratories used both activity (or mass) and CK-2 fraction of total CK activity (or relative index).

Discussion
Understanding of the Principles of the Assay

The responses to the questionnaires gave considerable insight into the extent of the understanding of the principles of the CK assay and of the factors that affect the assay. Respondents were senior laboratory staff (sometimes the laboratory director); nonetheless, a surprising lack of comprehension of the features of an adequate CK assay was often evident. For example, some respondents were content to indicate that the assay did not contain any adenylate kinase inhibitor at all, even though all assays used in the Province actually contain such compounds (Table 1). This finding indicates a serious deficiency in the knowledge of senior clinical chemistry staff about this assay. Indeed, 36% and 29% of the respondents omitted to mention the existence of one or more of these inhibitors in the total CK assay and CK-2 activity assays, respectively. Fewer than 3% of all respondents omitted mention of the presence of thiol agents in both assays. That these lacunae in understanding were not unique was underscored by the previously mentioned failure to recognize that male, female, and pediatric reference ranges differ and that a single, sex-neutral range is unacceptable.

Total CK

Nearly all laboratories used the currently recommended formulation for the total CK assay (7), or
modified formulations that correlate well with the recommended formulation, and include adenylate kinase inhibitors (8). We therefore expected to obtain a fairly uniform reference range throughout the Province. That is not what we found.

The 95th percentile obtained for the upper reference limit for men at 37°C is very close to those reported by Miller et al. (9) in a study of 550 men and 580 women and Lott et al. (10) from a population of ~8000 men and women. Although there can be no “gold standard” for such values, the essential reliability of the majority of the Ontario data appears to be confirmed by the agreement with these very extensive US data. However, the validity of values >250 and <160 U/L reported by 20 laboratories for the upper reference limit for men, and >215 and <115 U/L reported by 7 laboratories for the upper reference limit for women, when using an optimized assay at 37°C, must be questioned. Likewise, despite the significant difference between total CK values in men and women (9–14), 37% of Ontario laboratories licensed to perform total CK assays did not recognize this difference. Finally, we seriously question the validity of the very wide scatter of the reported differences between the values for men and women, which suggests that the original values may not truly represent each sex group. No laboratory described the effect of age on the reference range for men, although both Miller et al. (9) and Lott et al. (10) clearly demonstrated this effect.

Considerable racial heterogeneity exists in populations, and these racial differences profoundly affect total CK values (12–13) — to the extent that different reference race limits have been suggested (12). In general, such heterogeneity should broaden the reported ranges, but in the 56% of Ontario laboratories that actually derived their own reference ranges, such an explanation seems unlikely, because several of these laboratories reported rather low values.

Almost all of the plots in Figure 1 show, for the majority of users, a central plateau or a series of plateaus with a mid-point near 200 U/L. This is also the median value of the data obtained for men, which agrees with the extensive US data mentioned above. In other words, the central tendency for each instrument and reagent combination is consistent from instrument to instrument. However, as already mentioned, some ranges for men extend to extreme values. How did these arise? Some values obtained by the laboratory were stated to have been deliberately set low, e.g., in the range 80–130 U/L, to rule out myocardial infarction (this point will be discussed later). Other values, in the same range, were claimed to have been taken from the kit insert, but the accompanying insert did not support these claims and no effort had been made to validate such values locally. Of the 11 values >250 U/L, two were borderline (233 U/L) and the remainder were claimed to be based on kit insert values, only some of which could be verified by the LIPTP Committee from the attached inserts. The majority of the “rogue” values seen in the plots by location (Figure 2) derive from those already mentioned; we assume that future efforts to standardize reference ranges within a community will remove these anomalies. The differences between different laboratories in the same location are of considerable concern. For example, in one community, a total CK activity of 220 U/L is abnormal in three laboratories but normal in another three. (A similar set of circumstances was found for the seven laboratories reporting anomalous ranges for women—these will therefore not be discussed further.)

We also asked for details of the process by which laboratories obtained their own reference ranges. Several reported that their records were missing, or staff had changed, so that details could not be provided other than the actual ranges themselves. Record keeping of such data obviously requires much more attention from hospital authorities and regulatory or accreditation organizations. Of the 134 laboratories who obtained their own reference ranges (including the 41 laboratories that had similar reference ranges for men and women), 83 (61.9%) either provided histograms of their data showing their selected cutoff values or explicitly stated their method of deriving their reference range; the majority took a non-parametric approach and selected the central 95th percentile range. Most laboratories observed a positively skewed distribution of values. Many of the laboratories stated the numbers of subjects used—ranging from 15 in a small community hospital to 1490 in bigger commercial and hospital laboratories, where data from several hospitals had been pooled. The population used usually included outpatients, staff, some inpatients, or groups of subjects undergoing insurance examinations. Many laboratories attempted to have equal numbers of male and female subjects, but others did not state their sex ratio. One community hospital mentioned that, because many of their subjects performed heavy manual labor, they had to adjust the resulting reference range downwards to be more representative of the general population; this was one of the few laboratories to appreciate the effect of exercise on the CK values (15). One laboratory enclosed a copy of their preanalytical protocol for deriving a reference range: volunteers were asked about their state of health; the time of last meal and fluid intakes; any medication use, including aspirin, sedatives, tranquilizers, or birth control drugs; and the performance of vigorous exercise within the last 48 h. A small laboratory, with a correspondingly small operating budget, commented on the expense of deriving a reference range (because they were using an expensive immunoassay). Several respondents mentioned that obtaining a reference range was beyond their capabilities: they were already understaffed and simply did not have the time. Many laboratories, both hospital-based and commercial, pooled data and resources to obtain a more useful estimation of CK ranges in a locale (see, e.g., Figure 2C). The most commonly cited literature reference used by laboratories as a source for their reference range was Tietz (16).

Many of the responses to the questionnaire indicated that a major use of the total CK reference limit is to rule

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in, or out, myocardial infarction—although this use demonstrates a naive understanding of the entire process. Such a process actually requires the careful prior selection of subjects (17), a process that is not usually performed and one that produces decision thresholds that may differ widely from the conventionally derived upper reference limit. For example, whereas a conventionally derived upper reference limit for men (based on results for >200 healthy hospital staff) was 174 U/L, the decision thresholds for ruling in myocardial infarction were 250, 400, and 800 U/L for test specificities of 90%, 95%, and 99%, respectively, when an appropriately matched nondiseased population was used (18). By contrast, the decision thresholds for ruling out myocardial infarction, for test sensitivities of 90%, 95%, and 99%, were 40, 30, and 20 U/L, respectively, 6 h after the onset of chest pain (18).

CK-2

Table 3 shows the present distribution of CK-2 methods in the Province, 82% of which are activity-based assays. The data in Table 3 should be compared with recent US evidence, i.e., a survey of the College of American Pathologists (19) in which 62% of the participants used mass assays, whereas immunoinhibition, electrophoresis, and ion-exchange (Du Pont) assays each accounted for <15% of participants.

The CK-2 upper reference limits are shown in Figure 3 for selected methods: there appear to be grounds for a review of some of these upper reference limits or for considering a change in methodology. When the 5th–95th percentiles are taken into account (see Results section), the mass assays clearly demonstrate a much tighter distribution. These Ontario data are reflected in pilot studies conducted by this LPTP Committee and also in the results of the survey already referred to (19).

About half of those laboratories that used kit insert or literature CK-2 reference values validated them by examining diagnostic records in consultation with clinical staff. However, such a process could introduce a selection bias, which might explain some of the extremely high activities in Figure 3 (A and C).

To our knowledge, this is the first survey of the reference ranges in use in a single health-care administrative unit, involving nearly 10 million people. Our findings are not reassuring. As a result of this survey, the LPTP Enzyme and Lipids Committee has formulated, and has proceeded to implement, the following objectives:

* derivation of acceptable CK reference ranges by any laboratory that (a) has ranges falling outside the 5th–95th percentiles (men: 160–250 U/L; women: 115–215 U/L) established by this survey or (b) does not have appropriate differences between men, women, and pediatric reference ranges (as a corollary, we wish to encourage those laboratories that derived their ranges from kit inserts or literature references to validate these ranges within a local setting).

* standardization of CK reference ranges within a community.

* exploration of educational initiatives for improving knowledge of the essential elements of a satisfactory CK assay.

References


