reasonably well between euthyroid and hyperthyroid TSH concentrations. Even then, measurement of borderline TSH concentrations will give a considerable percentage of erroneous results (e.g., TSH of 1.5 milli-int. units/L, Table 1). In a typical clinical setting, the implications of poor interassay precision are even more serious than may be appreciated from these data for a normal population with gaussian distribution of TSH values because, in patients under treatment, TSH concentrations in serum tend to be more uniformly distributed over the normal and subnormal range, and the proportion of borderline cases is higher. This is not to say that diagnostic accuracy is necessarily highly correlated with interassay precision. Rather, in practice, the diagnostic accuracy of various TSH kits (or assays in general) will also depend on many other factors, such as (e.g.) the overall quality and characteristics of the kit, interfering substances, and the composition of the patient population to be tested, all of which influence and ultimately determine the clinical performance.

Apparently one and the same immunoassay TSH assay, in the hands of different individuals or laboratories, can be carried out with vastly different degrees of precision. The variations of interassay precision between laboratories are generally underestimated. For example, the fact that the interassay CV of 87% at the lower limit of the normal range, reported by Wilke and Utley for the Abbott assay, is greater by approximately a factor of 10 than the interassay CV of 7.7% (at the cutoff point) that our laboratory can currently achieve, with the same kit, in routine clinical use, underscores the magnitude of the problem.

In my judgement, extreme interassay imprecision should not be acceptable in scientific investigations or in clinical practice. There is little to be gained if a host of data are generated, before investigators learn how to perform these assays reasonably well. And clinical diagnosis and patient management could be compromised if physicians were to rely on test results with such high interassay variations. As a case in point, let us consider a patient under treatment for hyperthyroidism, on a certain regimen to maintain euthyroidism. His/her true serum TSH is steady at 2.0 milli-int. units/L. If this patient were followed up at certain intervals by TSH measurements with interassay CVs as high as those of Wilke and Utley, then there is statistically (Table 1) a high probability that TSH results would alternate between (a) normal and (b) abnormally low and that, consequently, proper treatment may be jeopardized. Adequate interassay precision is a prerequisite for high diagnostic accuracy and interassay variance also influences the reference intervals, intra-individual or interindividual variations, and results from method comparisons. Thus it would be in the interest of good science and good patient care to set specific criteria for what constitutes acceptable interassay precision for sensitive TSH assays—and to require investigators and clinical laboratories to meet these criteria if their data are to be published, or before they are used clinically.

Specific criteria that particularly address the interassay precision have been proposed recently (2). Briefly, it was suggested that interassay CVs at TSH concentrations below the lower limit of the reference interval do not exceed 15%. This criterion implies (automatically) that interassay and intra assay CVs across the entire working range of the assay are also <15%, because in immunometric assays, as a rule, the precision increases with the analyte concentration, and because intra-assay CVs are usually smaller than interassay CVs. Reports such as that by Wilke and Utley underscore the need for tighter standards and more general acceptance of appropriate performance criteria for interassay precision.

Table 1. Statistical Relationship between Precision and Analytical Accuracy

<table>
<thead>
<tr>
<th>TSH (μ)</th>
<th>CV, %</th>
<th>SD</th>
<th>z = (1.38 - x)/SD</th>
<th>% false results*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) CVs from ref. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>100</td>
<td>0.3</td>
<td>3.6</td>
<td>&lt; 1 (false neg)</td>
</tr>
<tr>
<td>0.5</td>
<td>90</td>
<td>0.45</td>
<td>1.95</td>
<td>2.6</td>
</tr>
<tr>
<td>1.0</td>
<td>70</td>
<td>0.7</td>
<td>0.54</td>
<td>29</td>
</tr>
<tr>
<td>1.5 (borderline)</td>
<td>55</td>
<td>0.83</td>
<td>-0.148</td>
<td>44 (false pos)</td>
</tr>
<tr>
<td>2.0</td>
<td>53</td>
<td>1.06</td>
<td>-0.589</td>
<td>28</td>
</tr>
<tr>
<td>2.48 (mean)</td>
<td>51</td>
<td>1.26</td>
<td>-0.073</td>
<td>19</td>
</tr>
<tr>
<td>2.66 (median)</td>
<td>50</td>
<td>1.33</td>
<td>-0.962</td>
<td>17</td>
</tr>
<tr>
<td>B) CVs ≤15%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>15</td>
<td>0.15</td>
<td>2.53</td>
<td>&lt; 1 (false neg)</td>
</tr>
<tr>
<td>1.5 (borderline)</td>
<td>15</td>
<td>0.23</td>
<td>-0.52</td>
<td>30 (false pos)</td>
</tr>
<tr>
<td>2.0</td>
<td>10</td>
<td>0.15</td>
<td>-0.80</td>
<td>21</td>
</tr>
<tr>
<td>2.48</td>
<td>15</td>
<td>0.30</td>
<td>-2.07</td>
<td>1.9</td>
</tr>
<tr>
<td>2.87</td>
<td>15</td>
<td>0.37</td>
<td>-2.97</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Calculations are based on Wilke and Utley's data for the Boets-Celltech kit (1): Lower limit of TSH reference interval = 1.38 milli-int. units/L, interassay CVs at various TSH concentrations are (A) taken from their Figure 1, or (B) set at 10-15%.

*% probability (derived for z from statistical tables) that results for various TSH concentrations are above (false negative) or below (false positive) the lower limit of the reference interval. This column indicates minimal percentages of false results that are expected on the basis of imprecision alone.

References


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What is Serum Ornithine Decarboxylase?

To the Editor:

Clinical chemists or veterinary clinical pathologists responsible for clinical chemistry in food, pharmaceutical, chemical, and contract toxicology research laboratories are occasionally asked to provide ornithine decarboxylase (ODC, EC 4.1.1.17) results for safety assessment studies. ODC appears on a list of suggested "blood" chemistry determinations with other common clinical laboratory tests in both FDA and EPA test guidelines (1, 2), so this would seem to be a logical request to many toxicologists and product regis
tration specialists. Unfortunately, their requests can rarely, if ever, be accommodated because measurement of ODC activity in "blood," plasma, or serum is not simple, and its numerical interpretation is unclear. Because "suggested tests" is often interpreted as "required tests" by those within both industry and government, some discomfort at the thought of not providing "everything" is bound to occur in those responsible for obtaining product approval from the government. (However, I am not aware of any instance where a regulatory agency has cited the failure to include ODC results in a safety assessment study as a deficiency.) For the benefit of all who must occasionally answer "the ODC question," I would like to report the following information.

ODC is the initial enzyme in the polyamine biosynthetic pathway and a key regulatory enzyme in the growth process (3). It is important for the control, proliferation, and differentiation of cell growth. Its activity is regulated by all known classes of hormones (4). Induction of ODC may be one of the common pathways whereby hormones with different mechanisms of action influence their target cells. ODC catalyzes the conversion of ornithine to putrecine, which is the rate-limiting step in the polyamine pathway (5). Polyamines are aliphatic polycarbons whose synthesis is markedly increased in fast-growing cells. Polyamine concentration and ODC activity have been used as markers of cell growth induced by hormones, growth factors, toxic stimuli, and tumor promoters (3, 5, 6). There is some speculation as to whether the gene for ODC is an oncogene, and there has been a great deal of research on the role of ODC in tumor promotion (3).

In the only published procedures for determining ODC activity, L[14C]ornithine is the substrate (4, 5, 7). Enzyme activity is measured by trapping 14CO2 evolved from the labeled ornithine. The amount of 14CO2 released by ODC is determined by liquid scintillation. The assay requires several hours to complete, and is usually run in duplicate or triplicate. It does not appear from published reports that the assay can be easily automated. The procedure most certainly cannot be considered a routine clinical laboratory test.

ODC activity has been measured in liver, prostate, skin, kidney, neuroplasmas, other tissues (3), and in cells in culture (7). There are also several reports on ODC activity in human erythrocytes from patients with malaria or uremia (4, 8). In a computerized search of the medical literature dating back to 1966, I was unable to locate a single report on serum or plasma ODC (9), although it is listed in a directory of rare analyses performed by commercial clinical laboratories in the U.S. (10). It seems unlikely that serum ODC would be a diagnostically useful enzyme anyway. Tissue ODC activity is difficult to measure accurately because of its very low intracellular concentration and very short half-life (10–15 min) (3). This would suggest that ODC activity in serum is extremely low. Little intracellular enzyme would be present to enter the blood, and any enzyme that did enter the blood would not accumulate.

The reason for the presence of ODC in a list of routine serum clinical chemistry tests in EPA and FDA regulations is unclear. Just how changes in serum or plasma ODC activity would be interpreted in safety-assessment studies is equally unclear. It does not appear that an assay for serum or plasma ODC has ever been validated or accepted widely enough to be cited in current medical literature. The presence of ODC in the government safety assessment regulations can cause confusion and concern among those responsible for product registration. If anyone has worked with serum ODC, perhaps he/she would consider this a good time to organize and publish the information. For the present, this information will hopefully assist those who must occasionally answer questions about ODC.

References

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CK-MB Assay Specificity

To the Editor:

In describing two interesting cases of macro-CK Type 1, Jay (1) states that "only [two] clinically available assays [are] specific enough for quantifying CK-MB in these patients." This statement is not accurate. For example, macro-CKs do not interfere in the (clinically available) immunoinhibition/immunoprecipitation assays of Wicks et al. (2), as shown in reference 2 and confirmed by others, including ourselves (3). Jay's caveats about immunoinhibition and electrophoretic assays are well taken.

References

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