
By definition, the probability that a single measured cardiac troponin result exceeds the estimated 99th percentile is equal to 0.01 (1%) for a person randomly selected from the general (reference) population. This is irrespective of the imprecision profile of the analytical method as well as the lack of standardization of cardiac troponin assays (1–7). However, the probability that a measured result exceeds the 99th percentile limit for a specific person from the reference population will vary depending on that person’s true concentration and on the imprecision profile of the specific analytical method. Furthermore, the overall probability that at least one of multiple measured values (the second or third measured cardiac troponin concentration in a timed series of cardiac troponin orders) exceeds the 99th percentile also will vary depending on the imprecision of the analytical method.

To investigate the influence of assay imprecision on the likelihood of misclassifying healthy individuals or patients without myocardial injury, we simulated the distribution of cardiac troponin I (cTnI) results in a general population and added random analytical error reflecting different assay imprecision profiles. One imprecision profile assumes a CV of 37.5% at a cTnI of 0.05 µg/L, decreasing to a CV of 25% at a cTnI of 0.07 µg/L, and a CV of 9.4% at cTnI of 0.14 µg/L. The second imprecision profile was obtained by multiplying the first imprecision profile by a factor of 0.40, which produces a CV of 10% at a cTnI of 0.07 µg/L. The distribution of “true” cTnI concentrations in the general population was simulated by generating 500 000 random values from an exponential distribution. The mean of the exponential distribution was selected so that the 99th percentile occurred at a cTnI concentration of 0.07 µg/L for the case where the imprecision profile had a CV of 25% at 0.07 µg/L. For each of the 500 000 true cTnI values, a gaussian-distributed random error was added that reflected the appropriate CV given the imprecision profile and true cTnI concentration.

The distribution of values for a cTnI assay, assuming an imprecision profile with a 25% CV at the 99th percentile limit of 0.07 µg/L, is shown in Fig. 1. The same cardiac troponin distribution, assuming that the imprecision profile of this assay is improved, now with a 10% CV at 0.07 µg/L, lowers the calculated 99th percentile from 0.07 µg/L to 0.063 µg/L (Fig. 1, top). This demonstrates that when the imprecision of an assay is improved at low concentrations, the 99th percentile limits will shift to lower values because the width of the distribution is reduced.

In a population of patients presenting with suspected acute coronary syndrome who are being ruled out for myocardial infarction, serial cardiac troponin orders at presentation (0 h), 6 h, and 12 h are recommended (1, 2). Also shown in Fig. 1 are the probabilities of at least 1 of 3 serially measured values exceeding the 99th percentile limits as a function of the cTnI concentration for the 2 different imprecision profiles. The vertical lines are the 99th percentile limits for the 2 imprecision profiles. The overall probability of a single measured result obtained at a patient’s presentation (t = 0 h) exceeding the 99th percentile limit is 0.01 for both imprecision values (Fig. 1, middle panel). The probability of a cardiac troponin result being falsely classified as positive (increased above the 99th percentile) during a second (t = 6 h) or third (t = 12 h) measurement for the 2 different imprecision profiles increases compared with the initial t = 0 h measurement. The overall probability that a result exceeds the 99th percentile is greater for the analytical method with a 25% CV than for the analytical methods with the 10% CV as follows: second measurement at t = 6 h, 0.016 vs 0.013; third measurement at t = 12 h, 0.020 vs 0.015 (Fig. 1, bottom panel). Thus, for 2 or 3 serial cardiac troponin measurements in clinical practice, an additional 3 or 5 of 1000 patients, respectively, are likely to be misclassified as false positives for the 25% CVtroponinimprecision assay. It should be noted, however, that for the first measurement (t = 0 h), 10 in 1000 patients will be misclassified as false positive regardless of assay precision. We believe that the clinical implications of this false-positive frequency are insiginificant.
On the basis of these findings we are of the opinion that irrespective of the total imprecision of an assay at the 99th percentile reference limit, only the 99th percentile cutoff value should be used for a respective cardiac troponin assay in clinical practice. This is even with the understanding that there is no standardization between cTnI assays because different assays may measure different forms of circulating cTnI. We recommend that all manufacturers continue to strive to consistently optimize their assays for the best imprecision at low concentrations, to as close to 10% as possible. Improved low-end sensitivity of cardiac troponin measurements has been shown to be of important prognostic value in patients with acute ischemic heart disease, with increases above the 99th percentile providing risk stratification (8–11). Therefore, the evidence we have shown does not support the concept that the lowest concentration to meet a 10% CV should replace the 99th percentile reference cutoff for the diagnosis of myocardial infarction (4, 5). The potential for a relatively few misclassifications during serial monitoring as a result of assay imprecision differences is unlikely to affect clinical decision practices. Furthermore, we support that both reference limit and imprecision studies conform to routine clinical and laboratory practices and be based on multiple lots of reagents, multiple runs, and multiple instruments. Additional studies will be needed to determine the impact of cardiac troponin assay imprecision and how improvements in immunoassay harmonization will effect clinical decision making for ruling in myocardial infarction. Finally, we are of the opinion that each cardiac troponin assay requires individual study to establish its performance for clinical decision making at its 99th percentile reference limit.

References
Gaucher disease is an inborn error of glycosphingolipid metabolism resulting from a deficiency of the lysosomal enzyme β-glucosidase (1, 2). Acid β-glucosidase is responsible for the cleavage of the β-glucosidic bond of its primary substrate glucosylceramide, an intermediate in the catabolism of globoside and gangliosides. Gaucher disease is the most prevalent lysosomal storage disorder of the bone marrow, and in some instances does not manifest until later adulthood. Type 2 is an acute neuronopathic form of the disease, characterized by early onset, severe central nervous system impairment, and death, usually by the second year of life. Type 3 is a subacute neuronopathic form, usually with a more chronic course and later onset than type 2.

More than 187 mutations in the β-glucosidase gene have been associated with Gaucher disease (4), although there are rare instances of mutations in the prosaposin gene producing a Gaucher phenotype (5). Defined correlations between genotype and phenotype are not otherwise apparent, and there is broad phenotypic expression among all genotypes. A notable exception is the association of homozygosity for the L444P mutation with neuronopathic disease; similarly, the presence of the N370S allele precludes neuronopathic disease (6, 7).

Patients with the nonneuronopathic form of the disease are treated with enzyme replacement therapy (8, 9), which may also slow the progression of neuronopathic disease (10). Early and accurate diagnosis for Gaucher disease, as well as the prediction of disease severity, is paramount for the efficacy of current and proposed treatment strategies. To address this need, we evaluated measurements of acid β-glucosidase activity and protein as markers for the diagnosis of Gaucher disease and for the prediction of neurologic involvement.

A monoclonal antibody (1D6.9.9) was produced (11) against recombinant human β-glucosidase (rhβ-gluc; Genzyme Corporation). An anti-rhβ-gluc polyclonal antibody was produced in sheep and affinity-purified as described for α-glucosidase (12). The purified polyclonal antibody was labeled with Eu³⁺ (13). Calibrators and quality-control material were prepared by diluting rhβ-gluc in working buffer [0.1 mol/L citric acid, 0.2 mol/L Na₂HPO₄ (pH 5.5), 10 g/L bovine serum albumin, 2.5 g/L taurocholate, and 2.5 g/L Triton-X-100] or DELFIA® assay buffer (Perkin-Elmer Life Sciences) to measure β-glucosidase activity and protein, respectively.

Beta-Glucosidase protein in cell extracts or dried filter-paper blood spots was determined by either 1- or 2-step immunoquantification assays, respectively. Briefly, microtiter plates were coated with monoclonal antibody 1D6.9.9 at 5 mg/L in 0.1 mol/L NaHCO₃ (100 μL/well; incubation for 16 h at 4°C). Wells were washed twice with DELFIA wash buffer (Wallac), and then samples or calibrators diluted in 100 μL of DELFIA assay buffer (Wallac) containing 200 μg/L Eu³⁺-labeled sheep anti-rhβ-gluc polyclonal antibody were added to each well. Plates were shaken (10 min), incubated (16 h at 4°C), and washed 6

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**Table 1. Performance of the assay for immunoquantification of β-glucosidase protein and activity.**

<table>
<thead>
<tr>
<th>Assay format</th>
<th>Detection limit</th>
<th>Intraassay CV, %</th>
<th>Interassay CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-step protein assay</td>
<td>2 pg/well</td>
<td>10 (50 pg/well)</td>
<td>16 (50 pg/well)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 (400 pg/well)</td>
<td>15 (400 pg/well)</td>
</tr>
<tr>
<td>One-step protein assay</td>
<td>2 pg/well</td>
<td>5 (50 pg/well)</td>
<td>17 (50 pg/well)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (400 pg/well)</td>
<td>17 (400 pg/well)</td>
</tr>
<tr>
<td>Activity assay</td>
<td>0.8 pmol·min⁻¹·well⁻¹</td>
<td>8 (148 pmol·min⁻¹·mL⁻¹)</td>
<td>22 (148 pmol·min⁻¹·mL⁻¹)</td>
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<tr>
<td></td>
<td></td>
<td>8 (1335 pmol·min⁻¹·mL⁻¹)</td>
<td>17 (335 pmol·min⁻¹·mL⁻¹)</td>
</tr>
</tbody>
</table>

* Concentration or activity at which the signal reached background + 3 SD.
* Values in parentheses are the concentrations at which the CVs were determined.
* Based on 30 measurements.
* Based on 16 measurements over 11 months.
* Based on 14 measurements, activity expressed per milliliter of reaction volume.
* Based on 33 measurements over 10 months; activity expressed per milliliter of reaction volume.