Deciding the Optimum Interval between Specimen Collections: Theory and Nomograms

Callum G. Fraser and Margaret C. K. Browning

The time interval between collection of specimens from an individual patient is usually determined empirically. For analytes whose values decline according to first-order kinetics—for example, enzyme activities in serum after an acute myocardial infarction, tumor markers in serum after excision of the tumor, and drugs in serum after an overdose—the minimum time between collections (ΔT) depends on the elimination half-life (t) and the analytical precision (CV₀), according to the equation:

\[ \Delta T = (1/\log 2) \cdot t \cdot \log (2.33 \cdot CV₀/100 + 1) \]

Nomograms showing this relationship graphically have been generated.

Additional Keyphrases: sample collection · economics of laboratory operation · precision

The time interval between collection of specimens from the individual patient for assay of the same analyte depends on several factors. Empirical considerations—including local practice which has evolved over time, availability of the patient, and the frequency with which batches of samples are assayed in the laboratory—are undoubtedly important. In addition, more-objective criteria influence the choice of interval, such as the speed at which values for the analyte would be expected to change, and whether any changes, even if numerically significant, would lead to some change in treatment (T). Moreover, one should take into account the standard of analytical performance and the ramification of this on the magnitude of what is a statistically significant difference.

All analyte concentrations change with time. Some have diurnal, monthly, or seasonal rhythms. Most have what can be described as random fluctuation around an homeostatic set-point. Quantitative knowledge of the intra- and interindividual biological variation allows one to set desirable performance standards (2), decide which is the best test to use, assess the usefulness of conventional reference values (3), and ascertain the significance of numerical changes in serial results (4).

In certain clinical situations, analyte concentrations gradually decline, particularly if pre-analytical influences such as stress are minimal. Examples are (a) after an acute event, such as myocardial infarction or an episode of pancreatitis, (b) after surgical excision of a tumor that sheds a marker into the circulation, and (c) after an overdose of a drug or poison. In these and similar situations, one would like to know what time interval between specimen collections will best ensure that any observed change in results does in fact reflect removal of the analyte from serum. We have developed a theoretical model that allows such appropriate intervals to be defined.

Theory

Assuming that the removal of an analyte is a process that can be described by using first-order kinetics, the following relationship holds (5) (all logs are to the base 10):

\[ \log C_T = \log C_0 - \frac{KT}{2.303} \]

where Cₜ is the concentration or activity at time T, C₀ is the concentration or activity at zero time, and K is the first-order elimination rate constant.

It is also known that \( K = 0.693/t \), where \( t \) is the elimination half-life, and substitution in the above equation gives:

\[ \log C_T = \log C_0 - \left(0.693/t\right) \cdot T \cdot 2.303 \]

\[ = \log C_0 - 0.3 \cdot T/t \]

At any time, \( T_1 \), the concentration or activity, \( C_1 \), is given by

\[ \log C_1 = \log C_0 - 0.3 \cdot T_1/t, \]

and at a second time, \( T_2 \), the concentration or activity, \( C_2 \), by

\[ \log C_2 = \log C_0 - 0.3 \cdot T_2/t. \]
By subtraction,
\[
\log C_1 - \log C_2 = (0.3 \cdot T_2/t) - (0.3 \cdot T_1/t),
\]
\[
= 0.3 \cdot (T_2 - T_1)/t,
\]
\[
= 0.3 \cdot \Delta T/t,
\]
where \( \Delta T = T_2 - T_1 \).
This can be transformed to the following expression:
\[
C_1/C_2 = 2^{\Delta T/t},
\]
which is equivalent to
\[
C_1 = 2^{\Delta T/t} \cdot C_2.
\]
Subtracting \( C_2 \) from each side of the equation gives
\[
C_1 - C_2 = C_2 \cdot (2^{\Delta T/t} - 1).
\]
For two results to be analytically statistically different \( (P < 0.05) \), when only a decline is possible, the difference must be equal to, or exceed, 2.33 analytical standard deviations. Thus, in this model, \( C_1 - C_2 = 2.33 \cdot \text{SD}_A \), and substitution in the above equation gives the expression:
\[
C_2 \cdot (2^{\Delta T/t} - 1) = 2.33 \cdot \text{SD}_A.
\]
If the assumption is made that the analytical CV at \( C_1 \) and \( C_2 \) are not significantly different, then:
\[
\text{CV}_A = (\text{SD}_A/C_2) \cdot 100.
\]
This expression can be rearranged to
\[
C_2 = (\text{SD}_A/\text{CV}_A) \cdot 100
\]
and substitution in the above equation gives:
\[
(\text{SD}_A/\text{CV}_A) \cdot 100 \cdot (2^{\Delta T/t} - 1) = 2.33 \cdot \text{SD}_A,
\]
which, on rearrangement, leads to the expression:
\[
\text{CV}_A = (2^{\Delta T/t} - 1) \cdot 100/2.33.
\]
This expression may be simply rearranged to give:
\[
\Delta T = (1/\log 2) \cdot t \cdot \log (2.33 \text{ CV}_A/100 + 1).
\]

**Applications**

We considered using this model to derive **analytical goals for precision** in a manner analogous to the one we used previously to derive goals for therapeutic drug monitoring (6).

For example, concentrations of the ovarian-tumor-associated antigen CA-125 in serum decline after the tumor is completely removed. The half-life of CA-125 after complete tumor removal should correspond to the natural half-life of 4.8 days (7). If, after surgery, specimens were taken at successive 24-h intervals, the model suggests that the CV for the assay should be 6.7% . If this goal was achieved, then when two results differed by more than 15.5% a true decline would have occurred.

The model also demonstrates that less precision is needed the less frequently specimens are collected for analysis. For example, if specimens were taken every three days for CA-125 assays, an assay CV of 23.3% would suffice for correct identification of a significant decline.

While this approach to analytical goal-setting might be of utility if assay results were only used to monitor the decline in the concentration of the analyte in serum, we believe that it is not generally appropriate. Our rationale is as follows: Results from different specimens in the same analytical batch are used for many purposes in clinical medicine, including diagnosis, monitoring therapy, screening, and health maintenance. So as to make results as comparable as possible in all of these situations (and over time and locale), ideally a single method with total analytical error equal to or lower than the most stringent analytical goal should be used. Because it is widely suggested that this goal is the one based upon intra-individual biological variation, according to the postulate of Harris (8), that
\[
\text{CV}_{\text{analytical}} \leq \frac{1}{2} \cdot \text{CV}_{\text{intra-individual}},
\]
we believe that this particular strategy for analytical goal-setting currently is still the most appropriate.

In contrast, we believe the above-mentioned model is appropriate to determine objective goals for the time interval between specimen collections from an individual.

The equation shows that, for a given analytical CV, the relationship between the half-life and the time interval between collections is linear. Thus, a series of graphs can be constructed showing these relationships. The resulting nomograms are shown in Figure 1.

As examples, we considered assays of enzyme and isoenzyme activities in serum that can be, and are, used to monitor the patient who has had an acute myocardial infarction. Assays of aspartate aminotransferase activity (EC 2.6.1.1) in serum are widely used. This enzyme is present in both cytosol and mitochondria; cytosolic isoenzyme activity is the prominent one in serum, both in health and after uncomplicated infarction; its half-life is 11.8 h (9). As shown above, the frequency with which specimens can be logically collected depends upon the analytical precision. If the precision was such that the CV was 5, 10, 15, or 20%, then specimens should not be collected more frequently than at intervals of 1.9, 3.6, 5.1, or 6.5 h, respectively. Similarly, the half-life of the "cardiac" isoenzyme (LD1), of lactate dehydrogenase (EC 1.1.1.27) is 48 h (9), and therefore, if the CV is as above, 5, 10, 15, or 20%, then the time interval between specimen collection should not be less than 7.6, 14.5, 20.8, or 26.5 h, respectively.

We assessed the time intervals between specimen collection that would be allowable if aspartate aminotransferase activity assays met goals derived by using different strategies—for example, the fraction of the reference interval as proposed by Tonks (10), biological variation (11), the recently observed opinions of clinicians (12), and the state of the art achieved by the best 20% of laboratories participating in an international quality-assessment scheme (13). These are shown in Table 1, and significant differences are evident. We therefore believe that, when test results are used to follow changes over time, goals for the minimum time allowable between specimen collections should be calculated by using our model. For example, for aspartate aminotransferase, attainment of the widely quoted biological-variation-based goal means that specimens should not be collected within time intervals of less than 2.8 h. If for any compelling clinical reason more-frequent specimen collection was required, better precision would be mandatory; this could be achieved by various strategies, such as doing replicate analyses.

The model may have utility in considerations of the costs of health care. It is wasteful of resources to perform assays on specimens taken so frequently that significant changes cannot be identified because of the inherent lack of precision of the assay and the half-life of the analyte. Moreover, it is not necessary to incur considerable costs by keeping a patient in hospital for an extended period to collect additional specimens if two test results of adequate precision ob-
tained on an analyte with a short half-life can be quickly generated so as to assess whether or not true changes have occurred.

We have previously stated (2) that objective goals should be delineated for performance characteristics other than precision and accuracy. We believe that our model represents a contribution to this ideal. The model deals with those situations in which analyte concentrations decline with time. For certain of these, for example tumor markers, it is also important to be able to identify significant increases. Analytical plus intra-individual biological variation will then dictate the magnitude of change which is significant (4).

We thank Mrs. M. Cooper for her expert secretarial assistance.

References