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Short Communication

Non-linearity within the primary measurement range of a lipase assay as the cause of a gap in the interpatient lipase results distribution

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ABSTRACT

Objectives: Interpatient distribution data for lipase (Roche Cobas® assay) showed an unexpected data gap, where no results were reported. This gap occurred beginning at a point just above the assay's primary measurement range (i.e., above the cutoff (300 U/L) for automated repeat-on-dilution). Calculation or other errors within the automated dilution process were ruled out. Linearity of assay results was investigated.

Design and methods: Linearity of experimental sample dilution series data was assessed by correlation coefficient, intercept, and constancy of slope.

Results: Dilution experiment data demonstrated a discontinuity of results between 300 and 400 U/L consistent with the observed gap in patient data. Although data within the presumed linear range of the assay had a high linear correlation coefficient ($r2 > 0.99$), a non-zero intercept and progressively variable slope were inconsistent with linearity. Although the assay was assessed as linear by the College of American Pathology linearity survey, survey data also demonstrated non-linearity for this assay when analyzed for slopes and intercept.

Conclusions: Non-linearity in the presumed linear range of an assay can produce gaps in patient data above a repeat-on-dilution cutoff. As in this instance, CAP linearity surveys may not identify certain forms of non-linearity.

In a recent review of the distribution of patient results for lipase (reference interval: 13–60 U/L) using the Roche Cobas® c501 assay at Jefferson University Hospitals (JUH), we discovered that the laboratory had produced no results in the range of 300–393 U/L during a five-month interval (Fig. 1). To corroborate this finding, an additional review of data from a second 5-month interval also showed a similar gap (between 300 and 380 U/L) in patient results. Across institutions, an inquiry showed that a smaller results gap (between 300 and 344 U/L) was also present in data from a 6-month interval at The Johns Hopkins Medical Institutions (JHMI) obtained using the Roche Cobas® c701 instrument. These gaps were for values directly above the upper limit (300 U/L) of the primary measurement range (presumed linear range) for the Roche Cobas® lipase assays in use at both institutions.

A systematic error in the automated dilution process was initially evaluated as the cause of the observed gap in lipase results. Review of instrument and middleware programming ruled out a calculations error. Further, manually diluted samples (m) provided results comparable to those generated through automated on-board dilution (a): $a/m = 1.03 \pm 0.02$ (2.1%) ($n = 4$, lipase ranging from 360 U/L to 1800 U/L).

We then examined results of serial dilutions of samples from above and across the cut-off of the upper limit of the primary measurement range. Samples having measured lipase >300 U/L were used as basis samples to produce parallel dilution samples, using saline as a diluent, with dilution factors (d) varying between 1 (undiluted) and 0 (diluent only). These samples were analyzed using the Roche Cobas® lipase colorimetric assay using the Cobas® c501 instrument. The assay uses an artificial substrate in the presence of colipase. A product of lipase activity on this substrate is chromogenic via decomposition. A rate measurement of color development is converted to a returned value for lipase activity in the sample based on a two-point calibration curve. Final returned values for samples having an initial response above the primary measurement range (>300 U/L) are obtained via an automated repeat-on-dilution process performed by the instrument. According to the package insert, samples are diluted ten-fold with saline and re-analyzed via the rerun function. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10. In the dilution experiments performed, linearity of the returned data subset within the manufacturer's primary measurement range (<300 U/L) was assessed by linear regression to obtain the correlation coefficient and intercept. Constancy of slope across the dilution series was also assessed by

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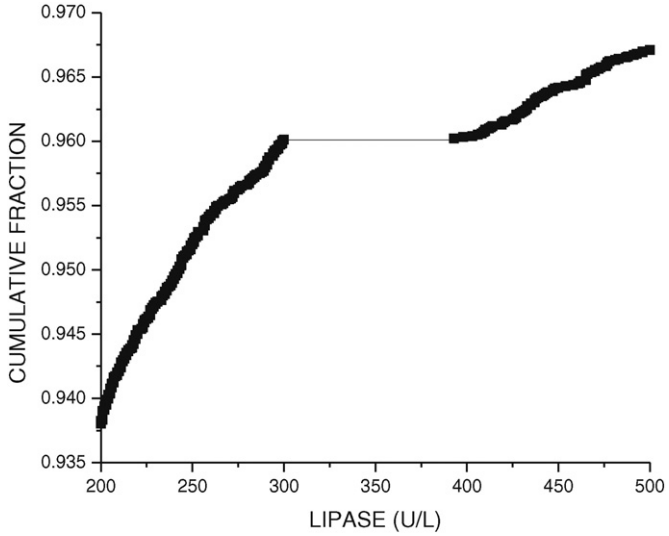

Fig. 1. Cumulative patient results distribution data for lipase (detail) across a 5 month interval (July–November, 2014) at JUH using the Roche Cobas® c501 lipase assay. Results are of data obtained across multiple instruments and across two separate hospital laboratories (total number of results = 10,329). In this dataset, no results were reported between 300 U/L and 393 U/L.

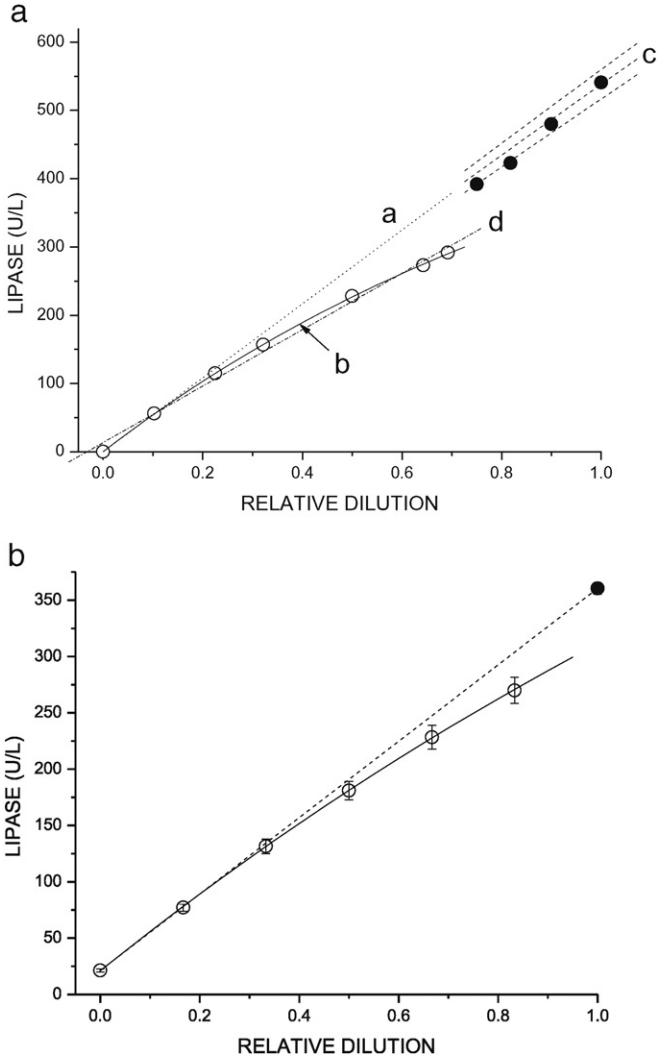

Fig. 2. A. Results of a sample dilution experiment. Parallel dilutions (relative values, x-axis) were produced from a single patient sample using normal saline as a diluent. Diluent measurement (<3 U/L) was assumed for simplicity to be zero. Open circles: returned results (y) <300 U/L (i.e., results within the primary measurement range); closed circles: returned results >300 U/L (i.e., results involving automated repeat-on-dilution performed by the instrument). Line *a*: calculated results curve based on sample #1 and assumption of perfect linearity. Line *b*: fitted curve for primary measurement range data (empirical; arbitrarily modeled as a simple exponential function y(x): $y = A(1\text{-}exp.(-x/B))$; A = 625, B = 1.11). Line *c*: projected curve for results >300 U/L after repeat-on-dilution with imprecision (average ± 2 SD, for CV = 2%), based on the empirical fitted curve for the primary measurement range data. Line *d*: linear regression curve for points within the primary measurement range (<300 U/L): $y = 413\,x + 13.4$; $r^2 = 0.993$. The results shown in this figure are representative of multiple experiments that demonstrated a disjunction of the results curve at y = 300 U/L. Additional numerical data for this figure are given in Table 1A. B. CAP linearity survey results (group mean ± 1SD; n = 16) for the Roche Cobas® c700 lipase assay (June 2014 [1]). Open circles: results (y) <300 U/L (i.e., results within the primary measurement range); closed circle: result >300 U/L (i.e., a result involving automated repeat-on-dilution performed by the instrument). Note that the zero relative dilution sample in the survey had non-zero lipase activity. Dashed line: calculated results based on sample #1 and assumption of perfect linearity when using the zero relative dilution sample (#7) as the diluent. Additional numerical data for this figure are given in Table 1B.

calculation of slopes from a fitted function characterizing returned data within the primary measurement range.

Results of a representative sample dilution experiment are shown in Fig. 2A and Table 1A. Results were disjointed between samples which underwent on-board dilution (solid circles) and those which did not (open circles). It is visually apparent that the data for samples within the primary measurement range (<300 U/L) were non-linear, exhibiting a progressive decrease in slope as a function of the dilution, x. Using an empirical function fitted to these data (Fig. 2A), there was a 46% decrease in slope of the response curve between the lowest and highest points for results reported within the primary measurement range. Despite this regular curvature of the response curve within the primary measurement range of the assay (<300 U/L), the linear correlation coefficient of the data in this range was nonetheless high ($r^2 = 0.993$). However, linear regression of data within the primary measurement range (<300 U/L) also produced a distinctly non-zero intercept (13.4 U/L) incompatible with the diluent measurement.

Whereas the on-board dilution procedure of the assay uses saline as a diluent, use of serum as diluent in a parallel dilution experiment as described above also produced a discontinuity in results at the repeat-on-dilution cutoff as observed in Fig. 2A. Thus, a matrix effect differentiating between serum and saline dilutions was unlikely to be operative in the production of this discontinuity.

The discontinuity in the data shown in Fig. 2A is the result of the non-linearity of the data within the primary measurement range. A forward projection of the regular curve formed by points below the repeat-on-dilution range intersects a result response of 300 U/L at a relative dilution of x = 0.726. According to this regular curve, a ten-fold dilution of this relative concentration (x = 0.0726) corresponds to a result response of 39.6 U/L. Correspondingly, a 10× projection of results from a dilution of 300 U/L is a result of 396 U/L. Thus, in this experiment, any result requiring dilution for a specimen initially reading greater than 300 U/L was projected to be a value of at least 396 U/L. The net effect is that there was a swath of results between 300 U/L and 396 U/L in this experiment that were unattainable. The magnitude of the data gap in this example is in close agreement with the magnitude of the discontinuity observed in Fig. 2A (the difference between the lowest repeat-on-dilution sample and the highest non-repeat-on-dilution sample) and also to the magnitude of the data gap that was observed in the patient distribution at JUH over a period of months (Fig. 1).

Non-linearity of data within the primary measurement range for the Roche Cobas® lipase assays is also evident in CAP linearity survey results. In such surveys, a dilution series of samples as prepared by CAP are measured by participants. CAP then assesses linearity of results using the known relative dilution factors for each sample. As an example, group mean survey results for participants using the Roche Cobas® c701 instruments (n = 16) are shown in Fig. 2B and Table 1B