Hierarchical Modeling of the ELISA Process

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Abstract:

Enzyme-linked immunosorbent assays (ELISAs) are used to measure protein concentrations in biological samples, such as plasma or serum. Most assays are conducted using 96-well plates that include known samples for calculating a calibration curve as well as control samples for estimating variation within and between plates. While the calibration curve reduces systematic error introduced during the assay, the control samples indicate remaining systematic and random error. This remaining error is expressed in terms of the coefficient of variation: the ratio of the sample standard deviation to its mean.

Insulin-like growth factor-I is a mitogenic and anti-apoptotic protein associated with many types of cancer (e.g., breast (4) and prostate (2)). Between-plate (inter-assay) coefficients of variation of more than 10 percent are not uncommon for published IGF-I ELISA results (e.g., (3), (1)). Taken solely as an assay reliability indicator, this variation is ignored during subsequent analyses.

If samples are randomly assigned to wells and plates in an IGF-I ELISA experiment, the measurements should be log-normally distributed, given appropriate cofactors such as age. However, plate-specific variations not accounted for by the standard curve, such as may occur when unknown samples are thawed, may cause departures from the expected distribution. Possible departures include shifted means as well as differing variances of the distributions from plate to plate.

Using hierarchical regression models, inter-assay variation among ELISA plates can be considered. This approach more closely models the true assay process than do traditional models. The statistical software programs R and WinBUGS are used to construct the hierarchical models.

References