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Spike-and-recovery and linearity-of-dilution assessment

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Introduction

Spike-and-recovery and linearity-of-dilution experiments are important methods for validating and assessing the accuracy of ELISA and other analytical techniques for particular sample types. Spike-and-recovery is used to determine whether analyte detection is affected by a difference between the diluent used to prepare the standard curve and the biological sample matrix. [*Sample matrix* is either a neat (i.e., undiluted) *biological sample* or a mixture of the biological sample with *sample diluent*.] Linearity of dilution refers to the predictability of spike or natural sample recovery for known dilution factors in the desired assay range. The two kinds of information are related, and experiments can be designed to test both spike recovery and dilutional linearity simultaneously. The following discussion considers each one separately.

Spike and Recovery

A. The Meaning and Purpose of Spike-and-Recovery Assessment

In spike-and-recovery, a known amount of analyte is added (spiked) into the natural test sample matrix and its response is measured (recovered) in the assay by comparison to an identical spike in the standard diluent. ELISA methods involve comparison of test samples to a standard curve prepared using known concentrations of the analyte (e.g., purified recombinant protein). The goal in assay development is to maximize signal-to-noise ratio while achieving identical responses for a given amount of analyte in *standard diluent* (the standard curve) and *sample matrix* (biological sample + sample diluent). The sample matrix may contain components that affect assay response to the analyte differently than the standard diluent. A spike-and-recovery experiment is designed to assess this difference in assay response.

B. Performing a Spike-and-Recovery Experiment

To perform a spike-and-recovery experiment, a known amount of analyte is added to the sample matrix and standard diluent, and the two sets of responses are compared based on values calculated from a standard curve. If the recovery observed for the spike is identical to the recovery obtained for the analyte prepared in standard diluent, the sample matrix is considered valid for the assay procedure. If the recovery differs, then components in the sample matrix are causing the difference, and adjustments must be made to the method to minimize the discrepancy.

C. Correcting for Poor Spike-and-Recovery Results

Two kinds of adjustments can be made to re-optimize an ELISA method when a spike-and-recovery experiment detects a discrepancy between standard diluent and sample matrix. One strategy is to use a standard diluent whose composition more closely matches the final sample matrix. For example, culture medium could be used as the standard diluent if the samples will be culture supernatants. If the standard diluent had previously been optimized for signal-to-noise performance, changing the diluent to match the sample matrix performance will result in decreased assay range, sensitivity or signal-to-noise ratio. In some cases, a compromise may be necessary.

The second strategy for correcting discrepancy between standard diluent and sample matrix is to alter the sample matrix. If neat biological sample had been used, retest it upon dilution in standard diluent or other logical “sample diluent”. For example, if an undiluted serum sample produces poor spike and recovery, perhaps one that is diluted 1:1 in standard diluent will work better. If the level of analyte in the diluted sample is sufficient to be detected by the assay, this method will correct many recovery problems. Better results for a sample matrix may be obtained by altering its pH (e.g., to match the optimized standard diluent) or by adding BSA or other purified protein as a carrier/stabilizer. Be aware that the best *sample* diluent will not necessarily be the same as the best *standard* diluent; for example, serum samples contain considerable background protein (e.g., albumin and immunoglobulins) while the purified recombinant protein used as a standard may not contain any carrier protein. In this case, the best *standard* diluent may be phosphate-buffered saline (PBS) that contains 1% BSA, while the best *sample* diluent for the serum may be PBS without any additional protein.

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Linearity of Dilution

A. The Meaning and Purpose of Linearity-of-Dilution Assessment

A linearity-of-dilution experiment provides information about the precision of assay results for samples tested at different levels of dilution in the chosen sample diluent. Linearity is defined relative to the calculated amount of analyte based on the standard curve, not relative to the raw absorbance measurements (the best fit standard curve usually is not linear). If the linearity is good over a wide range of dilution, then the assay method provides flexibility to assay samples with different levels of analyte (e.g., a sample with high levels of analyte can be diluted several-fold to ensure that its values fall within the standard curve range and compared to a low-level sample that is assayed without dilution).

B. Performing a Linearity-of-Dilution Experiment

There are two ways to perform a linearity-of-dilution experiment. The usual method involves using a low-level sample containing a known spike of analyte (or just a high-level sample without spike), and then testing several different dilutions of that sample in the chosen sample diluent. An alternative method involves first preparing several different dilutions of a low-level sample and then spiking the same known amount of analyte into each one before testing. Assay recovery is assessed by comparing observed vs. expected values based on non-spiked and/or neat (undiluted) samples.

C. Interpreting Linearity-of-Dilution Results

Poor linearity of dilution indicates that the natural sample matrix, the sample diluent and/or standard diluent affect analyte detectability differently. This difference may be caused by dilution of components in one solution that inhibits or enhances detection in the assay method compared to the other solutions. The causes of poor linearity of dilution are, therefore, related to the same causes of poor spike-and-recovery. The goal in either case is to bring parity between standard curve (standard diluent) and sample matrix (neat sample fluid and sample diluent). If desired, a single experiment can be performed using a checkerboard matrix of spike levels, sample types, sample diluents and dilution factors to simultaneously assess both spike recovery and linearity of dilution.

Example Data

Table 1. ELISA spike and recovery of recombinant human IL-1ß in nine human urine samples. Samples were assayed by adding 50 µl of sample and 10 µl of spike stock solution calculated to yield the intended 0, 15, 40 or 80 pg/ml spike concentration. Values reported for spiked samples reflect subtraction of the endogenous (no-spike) value. Recoveries for spiked test samples were calculated by comparison to the measured recovery of spiked diluent control. Diluent for the diluent control and preparation of spike stock solutions was the same as the standard diluent. All values represent the average of three replicates.

Sample	No Spike (0 pg/ml)	Low Spike (15 pg/ml)	Medium Spike (40 pg/ml)	High Spike (80 pg/ml)
Diluent Control	0.0	17.0	44.1	81.6
Donor 1	0.7	14.6	39.6	69.6
Donor 2	0.0	17.8	41.6	74.8
Donor 3	0.6	15.0	37.6	68.9
Donor 4	0.0	15.1	36.9	67.8
Donor 5	0.5	12.5	33.5	63.6
Donor 6	0.0	14.0	33.5	68.7
Donor 7	0.0	14.4	38.5	69.6
Donor 8	7.1	16.3	41.4	69.5
Donor 9	0.7	12.4	37.6	68.2
Mean Recovery (+/- S.D.)	N.A.	86.3% +/- 9.9%	85.8% +/- 6.7%	84.6% +/- 3.5%

Table 2. Typical presentation for summarizing spike and recovery results. (Data from Table 1.)

Sample (n)	Spike Level	Expected	Observed	Recovery %
Urine (9)	Low (15 pg/ml)	17.0	14.7	86.3
	Med (40 pg/ml)	44.1	37.8	85.8
	High (80 pg/ml)	81.6	69.0	84.6

Table 3. ELISA linearity-of-dilution results for three human IL-1ß samples. Dilutions were made in a previously chosen sample diluent. Observed values were assessed relative to the assay standard curve produced by an IL-1ß standard prepared in a previously chosen standard diluent.

Sample	Dilution Factor (DF)	Observed (pg/ml) × DF	Expected pg/ml (neat value)	Recovery %
ConA-stimulated cell culture supernatant	Neat	131.5	131.5	100
	1:2	149.9		114
	1:4	162.2		123
	1:8	165.4		126
High-level serum sample	Neat	128.7	128.7	100
	1:2	142.6		111
	1:4	139.2		108
	1:8	171.5		133
Low-level serum sample spiked with recombinant IL-1ß	Neat	39.3	39.3	100
	1:2	47.9		122
	1:4	50.5		128
	1:8	54.6		139

Note: Although the colon symbol is more generally used to denote a ratio, sample dilutions in ELISA use the colon to denote a fraction (e.g., 1:2 means 1 part diluted to a final total volume of 2 parts).

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