

BEBPA'S 

HALLOWEEN HORRORS



**Assays that have scared
and scarred you!**

The Outlier Trap

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Assay Type: Binding Cell-Based Animal-Assay Other
(Please fill in)

Tell us what happened:

A statistically sound test for identifying outliers was misapplied to replicated dose-response measurements. On paper, the method was sound. But in practice, it was misused. The result? Distorted datasets and compromised data integrity, casting doubt on the reliability of the conclusions drawn. The story eventually reached resolution, but not without cost. Time, resources, and trust were spent reprocessing the results. And while lessons were learned, it's the kind of situation I wouldn't wish on anyone.



Statistical-sounding method for outlier removal → → Violation of data integrity

The validated method's SOP ABC-123, Data analysis section: "A data point may be removed with 95% confidence as a statistical outlier from three replicate measurements if its value falls outside the range of the mean $\pm 2 \times SD$ of the **remaining two replicates.**"

Assume $n=3$ readouts (Y-axis) for the same serial dilution (X-axis): A, B, and C, where C is farther from data point B, than data point A is from data point B.

↪ C is a suspect  , right? Let's interrogate it!

The mean and standard deviation are calculated for A and B. The deviation of A and B points from their mean is $A - \frac{A+B}{2}$ and $B - \frac{A+B}{2}$ OR $\frac{ab}{2}$. Hence, standard deviation s is

$$s = \sqrt{\frac{\left(\frac{ab}{2}\right)^2 + \left(\frac{ab}{2}\right)^2}{n-1}} = \sqrt{\frac{\left(\frac{ab^2}{4}\right) + \left(\frac{ab^2}{4}\right)}{2-1}} = \sqrt{\frac{\left(\frac{2ab^2}{4}\right)}{1}} = \sqrt{\frac{ab^2}{2}} = \frac{ab}{\sqrt{2}} = 0.71 \times ab, \text{ and}$$

$2s = 1.41 \times ab$, good, now... $\overline{mc} = \overline{bc} + \frac{ab}{2}$, but $\overline{bc} > ab$, which means

$\overline{mc} > ab + \frac{ab}{2} > \frac{2ab}{2} + \frac{ab}{2} > \frac{3ab}{2} > 1.5 \times ab$, therefore \overline{mc} will **ALWAYS** be greater

than "two-sigma" $2s = 1.41 \times ab$ and C will always be removed using this method.

This method is a statistical-sounding "scissors"  that a bioassayist can use to snip out the extreme "end" value from **any**  set of $n=3$ data points!

