Monobind Lab Tips

Fall 2010

Stored calibration curves and the use of 2PT adjusters

The calibration curve is very important because it shows the trend of the reacting analyte and is used to interpolate the control and/or sample values. If the calibration curve is not accurate the corresponding values calculated from the calibration curve will not be accurate. It is very important to run all the calibrators in duplicate for the first run of the assay. Once a 'master curve' has been established it is not required to run all calibrators for further runs of the assay within a 30-day period of the same reagent lot.

Once a 'master curve' has been established in an analyzer it is greatly suggested to run adjusters; Adjusters are calibrators that are near the two ends of the calibration curve that represent the variation that can occur between different runs of the assay. Monobind commonly refers to this as 2PT calibration, and its analyzers are programmed for this situation with necessary data analytics.

When running an assay with no adjusters the user assumes that there is no change in the assay, including the environment. This is not the best choice when a test is run in ambient conditions and/or on a laboratory bench that may be subject to drafts and environmental disruptions that may not be detrimental to the test but will affect the interpolation if adjusters were not run to compensate for these occurrences.

Whether a 'master curve' is being established or adjusters are being used in a run of an assay, it is best to run duplicates of each until experience with the assay shows significant positive reproduction through low CV% (e.g. <8% on Cal B is a good level). This will allow for sufficient data to determine if any bad duplicates, outliers, or environmental effects occurred. Once the assay's performance and the dispensing technique have been established, single wells of the calibrators/adjusters may be employed.

In a clinical setting there are three options given in an analyzer: New Curve, 2 PT, or Stored Curve. Table 1 shows the data that would be received from an analyzer when each method is used. The patient samples will be calculated based on the calibration curve option chosen. The New Curve and Stored Curve option use the values of the 'master curve' to calculate the sample

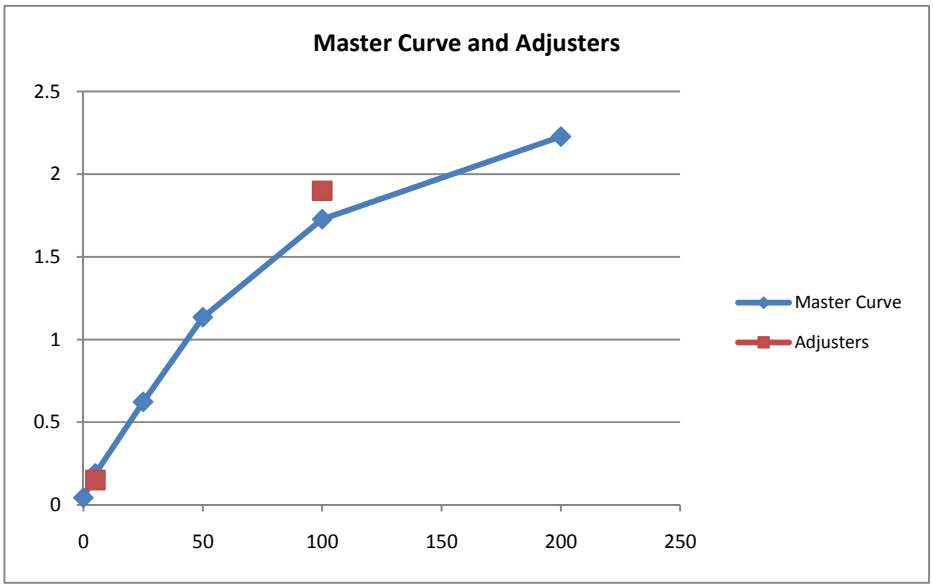
concentrations; The 2PT calibration option will use the values obtained by the adjusters to adjust the calculated sample values to reflect how the assay curve varied for that specific run of the test. The comparative values of each method can be seen more clearly in Graph 1 and Graph 2. Graph 1 depicts the Adjusters in relation to the Master Curve; the difference in the values does not seem very significant in value but when graphed it is more evident. Graph 2 shows the results of the interpolation of the sample values using each one of these methods. Again the absorbance values do not vary drastically from the ones received when the Master Curve was obtained but from the assay-to-assay variation, using strictly the stored curve gives drastically differing concentrations; however, when adjusters were used to calculate sample values the concentrations were those very similar to the 'master curve'.

At first thought, the user might believe using the stored curve will save time and resources but hopefully the data presented here explains otherwise. Every assay is different and as such some sample concentrations may not change between tests but adjusters will show when and where variation has occurred. The best option is to run a new calibration curve each time but results obtained from a stored curve with adjusters (2PT) can be used with confidence.

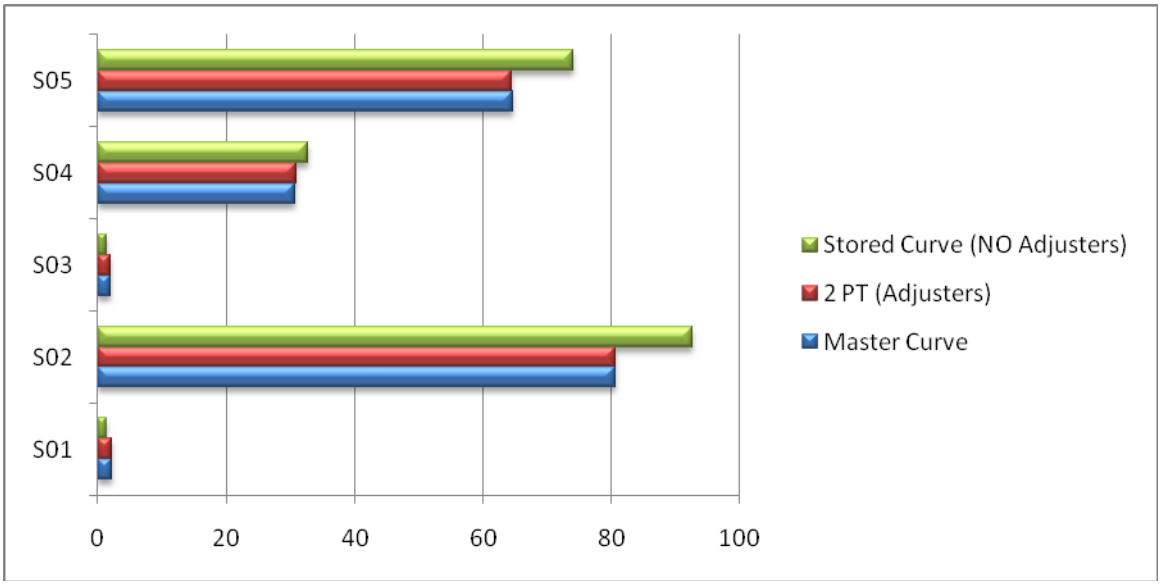
Table 1: Sample Assay Results received from ELISA Analyzer.

	CONC (ng/ml)	Master Curve	2 PT (Adjusters)	Stored Curve (NO Adjusters)
A	0	.0435		
B	5	.1900	.1500	
C	25	.6230		
D	50	1.1350		
E	100	1.7275	1.900	
F	200	2.2275		
S01		0.1095 ⇒ 2.25 ng/ml	0.086 ⇒ 2.23 ng/ml	0.86 ⇒ 1.45 ng/ml
S02		1.4975 ⇒ 80.59 ng/ml	1.64 ⇒ 80.72 ng/ml	1.64 ⇒ 92.62 ng/ml
S03		0.1045 ⇒ 2.08 ng/ml	0.082 ⇒ 2.06 ng/ml	0.082 ⇒ 1.31 ng/ml
S04		0.7405 ⇒ 30.74 ng/ml	0.780 ⇒ 30.88 ng/ml	0.78 ⇒ 32.67 ng/ml
S05		1.31 ⇒ 64.77 ng/ml	1.42 ⇒ 64.41 ng/ml	1.42 ⇒ 74.05 ng/ml

Graph 1: Master Curve and Adjusters



Graph 2: Sample Values Calculated using each calibration method



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