White Paper

RAININ
Pipetting 360°

Improving Qualitative ELISA Data

Some Benefits of Using Liquidator 96

ELISA (enzyme-linked immunosorbent assay), is a common technique in modern biological research. It is used to detect and quantify distinct proteins in complex mixtures of proteins.

ELISA can be subdivided into two mechanisms. The first, qualitative ELISA, allows a researcher to understand whether a certain protein is present or absent within a sample of biological fluid. The second, quantitative ELISA, allows not only allows for the determination of a protein's presence or absence, but also provides a concentration value for the protein within the sample. In order to carry out quantitative ELISA, an accurate standard curve must be generated to determine protein concentrations in experimental samples. If the standard curve in quantitative ELISA is inaccurate, then the calculated concentrations of protein within experimental protein samples will be incorrect and conclusions from the ELISA experiment might also be incorrect.

In the past, researchers using ELISA have relied on multichannel pipettes to carry out their assays. While multichannel pipettes are superior compared to single channel pipettes in accelerating ELISA protocols, limitations arise by using multichannel pipettes in quantitative ELISA. First, as quantitative ELISA is often carried out in 96-well plates, and multichannel pipettes are either 8- or 12-channels, it takes numerous repetitive pipetting steps to complete an ELISA assay. Second, the use of multichannel pipettes with 96-well plates can cause the skipping of rows, which can be severely detrimental for the conclusions of an experiment. Finally, the use of multichannel pipettes in quantitative ELISA can lead to erroneous standard curves, which lead to inaccurate data. The reason for obtaining imperfect data using multichannel pipettes arises from the reliance of ELISA data on a standard curve based on an enzymatic assay. During the last step of ELISA, an enzyme substrate is digested with the antibody-conjugated enzyme. If the duration of the enzymatic reaction is not consistent from row-to-row in the 96-well plate, like when a multichannel pipette is used for enzymatic reaction initiation, then the linearity of the generated standard curve will be affected.

In the most innocuous situation, the data points that deviate significantly from the standard curve can be excluded and the protein concentration in experimental samples can be calculated using an appended standard curve. The disadvantage of this is that data quality might suffer, and extra time may be needed to dilute samples to fall within the standard curve range. Though automated ELISA plate washers can be used to reduce the amount of repetitive pipetting steps, they are not precision liquid handling devices and cannot correct the problems of row skipping and incorrect enzymatic reaction initiation.

METTLER TOLEDO

Rainin Instrument has developed the Liquidator 96 for applications such as ELISA. Due to its ability to pipette 96 channels in unison, the Liquidator 96 improves quantitative ELISA in the following ways:

- Excess pipetting steps are eliminated, saving significant time for ELISA experiments.
- The possibility of skipping rows in 96-well plates is removed, which reduces the potential of having to repeat experiments.
- The range and linearity of standard curves are improved, which increases data quality and reduces the number of follow-up experiments.

96 aliquots are pipetted at the same time, removing the lag associated with sequential pipetting with a multi-channel pipette.After washing the resin to remove nonspecific contaminant proteins, the antibody-antigen protein complex is eluted and recovered.
In this paper we compared the performance of a multichannel pipette and the Liquidator 96 using the protocol below. (The protocol was adapted from Bio-RAD's Biotechnology Explorer-ELISA Immuno Explorer Kit; protein samples were obtained from this kit)

Using a 12-channel multichannel pipette, eight replicate standard samples of antigen protein were pipetted to the wells of a 96-well ELISA plate. Serial dilutions were carried out so that the concentration of antigen in the standard samples varied from 1 ng/µL to 63 ng/µL. After pipetting 50 µL of samples to wells, the plate was incubated for 5 minutes so that the antigen protein could adhere to the wells.

After standard sample preparation and the initial incubation, all subsequent steps were carried out with an 8-channel multichannel pipette, or the Liquidator 96. Excess antigen was removed by washing all 96 plate wells twice using PBST buffer (200 µL of buffer per wash step). Primary antibody was then added to the wells in 50 µL and the plate was incubated for 5 minutes at room temperature. The plate was then washed twice in the manner above. Horse radish peroxidase-conjugated secondary antibody was then added to the wells in 50 µL and the plate was incubated for 5 minutes at room temperature. Next, the wells were washed three times using PBST. Following this step, the colorimetric horseradish peroxidase substrate, TMB, was added in 50 µL and the plate was incubated at room temperature for 5 minutes. The reactions were then terminated by the addition of 20 µL of 0.18 M H_2SO_4. For quantification, plates were read in a Tecan multiwell plate reader at 450 nm. The absorbance values were averaged, and plotted against initial antigen concentrations.

Results and Discussion

The standard curve obtained by carrying out the ELISA experiment using a multichannel pipette was linear over low antigen concentration values, but deviated from linearity at high antigen concentrations. This is likely due to the inability to wash and incubate samples in each column of the 96-well plate for an equivalent amount of time. In terms of time needed to work through the ELISA protocol, the technique using the multichannel pipette required 1 hour and 25 minutes.

The standard curve obtained by using the Liquidator 96 in all ELISA steps is shown below. The standard curve is linear at both low and high antigen concentration values. The R^2 of this standard curve is greater than that obtained with the multichannel pipette. The higher quality

of the standard curve is probably due to the ability of the Liquidator 96 to deliver wash buffers and other liquids to the 96-well ELISA plate in parallel. Because of this feature of the Liquidator 96, each sample in the plate is treated equivalently. Regarding time, the Liquidator 96 was able to process this specific ELISA plate in 55 minutes.

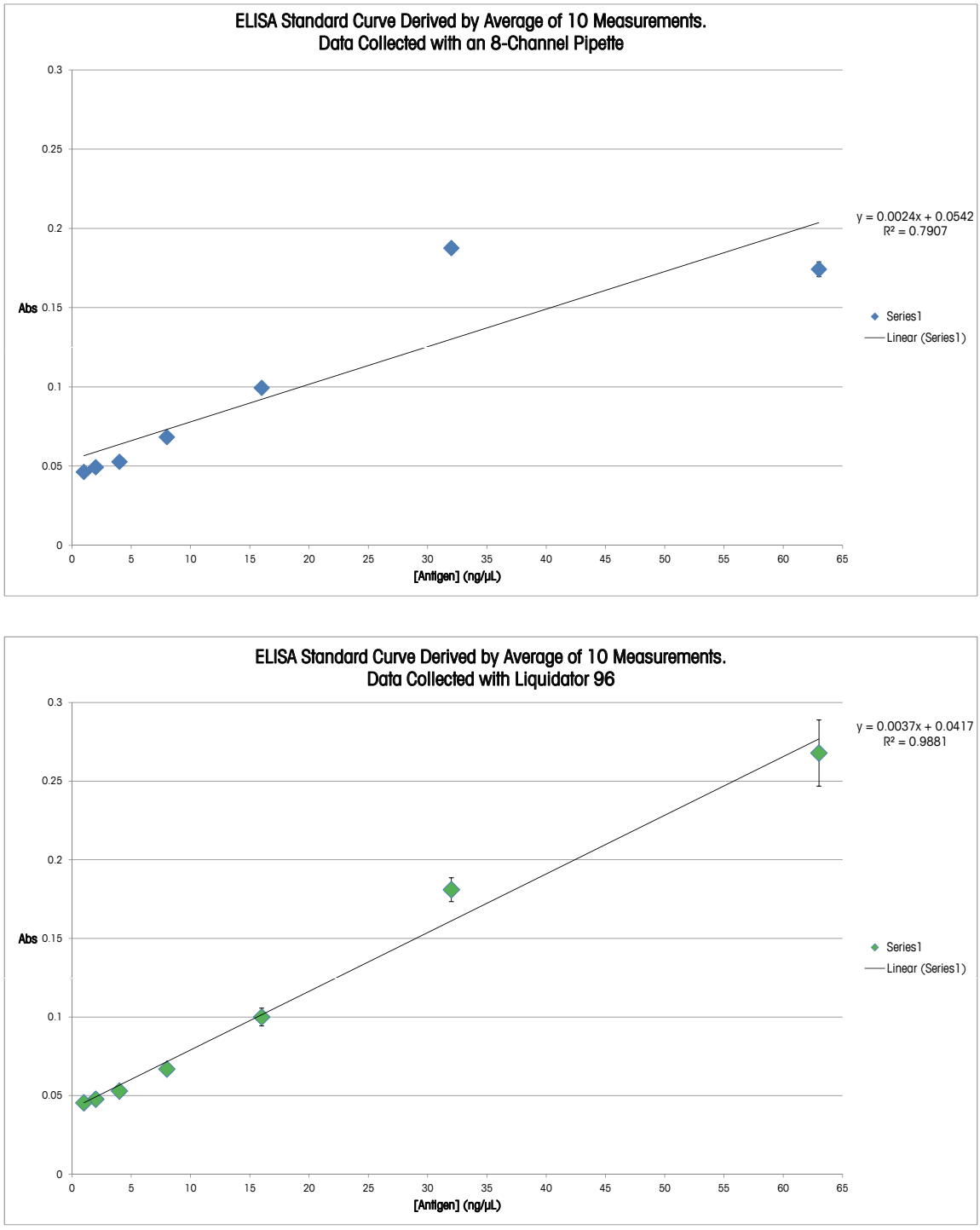

Figure 1. Standard Curve Data. (A) Averaged data of eight standard curves obtained using a multichannel pipette. Notice the deviation from linearity for the point beyond 32 ng/µL antigen. (B) Averaged data of eight standard curves obtained using the Liquidator 96. This line is more linear compared to when a multichannel pipette is used to generate standard curves. The R^2 of the standard curve generated with the Liquidator 96 is greater than the standard curve generated using a multichannel pipette.

Perspective

The Liquidator 96 is a manual pipetting system that is designed for 96- and 384-well plate assays. One such assay is ELISA which is used to detect antigen proteins within complex mixtures of thousands of proteins. ELISA can be carried out qualitatively or quantitatively, and in the latter case, the accuracy and precision by which an ELISA standard curve is generated can be critical. Multichannel pipettes cannot add buffer solutions and protein samples to all wells in 96-well plates simultaneously. For this reason, washing and incubation times might diverge for different samples in the same plate and the final standard curve might not be of high quality.

Key benefits that the Liquidator 96 offers over multichannel pipettes in ELISA are:

- The ability to add buffer and protein samples to all wells in 96-well plates simultaneously, ensuring equal treatment of all samples.
- Improved linearity and extended range of standard curves, which allows for the calculation of more accurate protein concentrations with the reduced need of diluting samples so that their absorbance values fall within standard values.
- The removal of the possibility of skipping wells in a 96-well plate, which could compromise the data from an otherwise sound experiment.
- The ability to process individual ELISA plates faster, which is exemplified by the Liquidator 96 completing the specific ELISA procedure described in this document in 55 minutes versus 1 hour 25 minutes using an 8-channel multichannel pipette.

Increased throughput and data quality are important for modern biological researchers. The faster samples can be processed and high quality data obtained, the more rapid valid conclusions can be reached and then published. Compared to multichannel pipettes, the Liquidator 96 can deliver both speed and high data quality to ELISA and other assays.

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