APPLICATION NOTE

Benchmark Analysis of Novel Portable Absorbance Microplate Reader

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INTRODUCTION

Since its introduction, Enzyme Linked Immunosorbent Assay (ELISA) has become a widespread and highly trusted tool to quantify a wide array of diverse analytes from small molecules to quaternary protein complexes. It has become a workhorse for a broad variety of research fields and is widely used for analytic and diagnostic purposes.

Laboratories and field-based research teams are hampered by the availability of affordable, portable, and low maintenance microplate reader instrumentation. This application note serves to analyze the performance of an ultra-compact microplate reader (**Figure 1**) with established benchtop instrumentation.

To compare both instrument types, a variety of experimental setups have been performed in parallel, using both sandwich and competitive ELISA (**Figure 2**) and the most common ELISA substrates for either type (TMB – tetramethylbenzidine and pNpp-p-Nitrophenylphosphate).

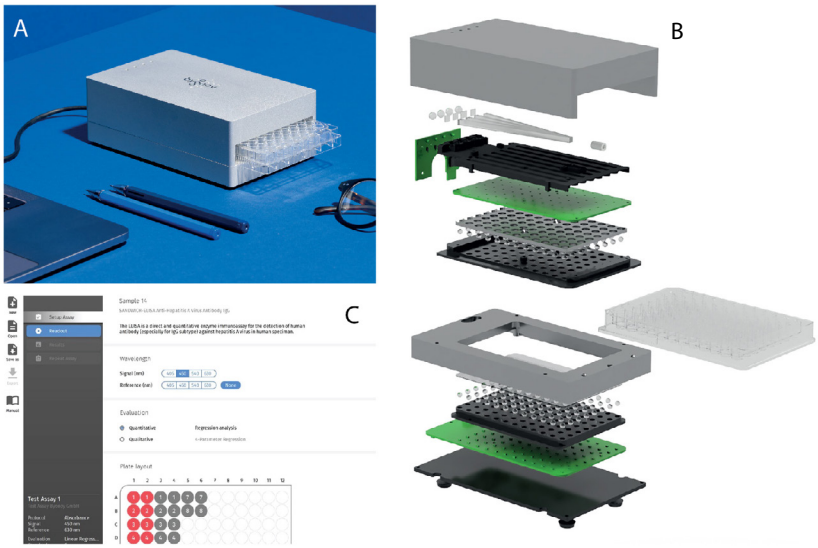

Figure 1: The Absorbance 96. The unique design relying on 96 individual detection units allows for a compact and maintenance-free, portable microplate reader. Open design enables plate loading. Plug-and-play functionality and power supply via USB result in maximal portability and flexibility. (A) *In-situ* appearance of the plate reader. (B) Explosion view. (C) Intuitive software interface.

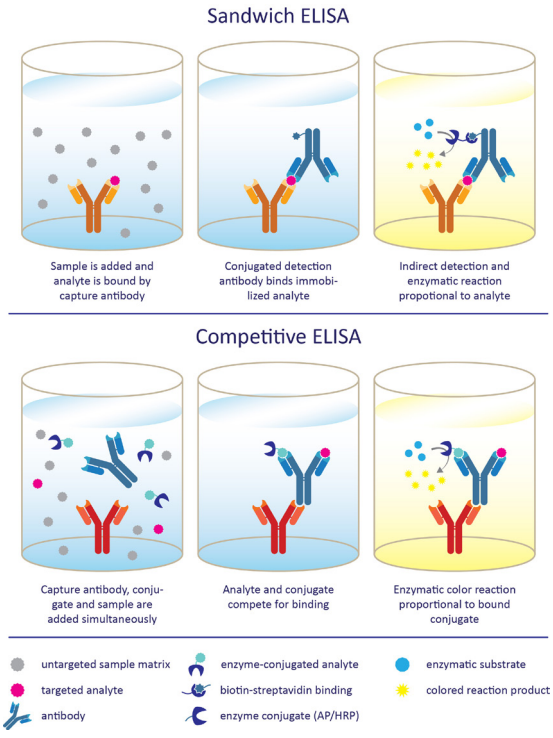

Figure 2: ELISA Principle (Sandwich and Competitive ELISA).

MATERIALS

- Absorbance 96 Microplate Reader, Enzo / Byonoy (ENZ-INS-A96)
- PowerWave™ XS2, Biotek® Instuments
- SpectraMax® 250, Molecular Devices
- IgG_{2b} (mouse) ELISA Kit, Enzo (ADI-900-110)
- TNF-α (mouse) ELISA Kit, Enzo (ADI-900-047)
- Cortisol ELISA Kit, Enzo (ADI-900-071)
- Aldosterone ELISA Kit, Enzo (ADI-900-173)

METHODS

Precision Testing

TNF-α (mouse) ELISA was run using a uniform pool of the analyte applied evenly into 80 wells of a mouse TNF-α 96-well plate. The remainder of the assay was carried out according to manufacturer's recommendations. The plate was measured with three devices: Absorbance 96, PowerWave XS2, and SpectraMax 250. Results were corrected against blank wells.

Intra- and Inter-Measurement Variation Testing

IgG_{2b} (mouse), TNF-α (mouse), and Cortisol ELISA were carried out according to manufacturer's recommendations. Plates were measured with two devices: Absorbance 96 and PowerWave XS2. Duplicate measurements were run with the same plate and obtained values were corrected against blank wells.

B/B0 Variation Testing

Aldosterone ELISA was carried out according to manufacturer's recommendations. Plate was measured with three devices: Absorbance 96, PowerWave XS2, and SpectraMax 250. Samples were run in duplicate and, the plate was read with all three devices. Afterwards, the plate was measured a second time with Absorbance 96 and PowerWave XS2. All values were corrected against non-specific binding control (NSB) wells.

RESULTS

For precision testing, well-to-well variation, and value variation of blank-corrected optical density (OD) values across devices were analyzed by measuring a homogenous, pre-developed TMP substrate (**Figure 3**). All three devices showed comparable data spread and no statistically significant differences. The Absorbance 96 showed negligibly higher inner quartile range (IQR) and extreme spread as compared to the other two devices, and variation of mean and median were comparable between all devices.

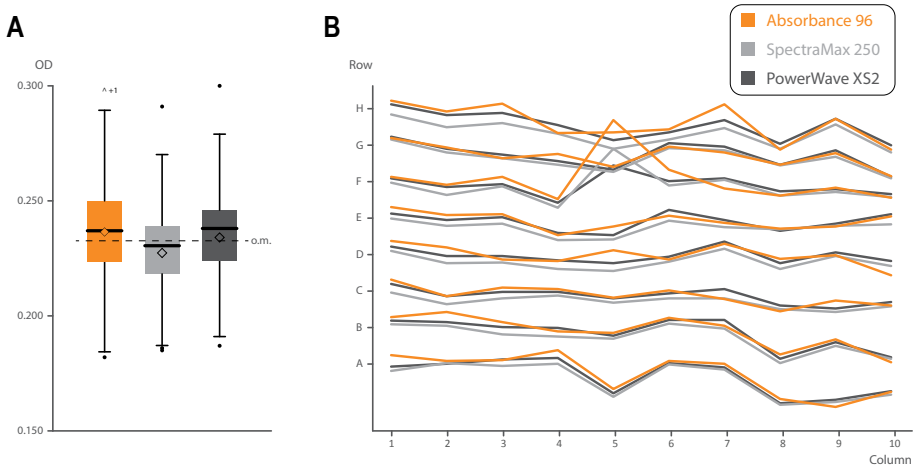

Figure 3: Precision Testing. TNF-α (mouse) ELISA was performed using a uniform solution of analyte across all analyzed wells. (A) Variation of wells within each device depicted as a box-and-whisker plot. (B) Relative OD value change across single rows of the plate for all three devices. Midline shows median, box indicates inner-quartile range (IQR) from first to third quartile, whiskers indicate +/- 1.5xIQR and dots show outliers. Rhombus shows the mean and dotted line shows the overall mean (o.m.) across all three devices.

For intra- and inter-measurement variation testing, result variations between duplicates within one measurement with a single device as well as variation of the mean of duplicates as determined with either device were compared and plotted graphically (**Figures 4-7**). Variations between two measurements or averages are both depicted as ΔOD as well as %CV. While ΔOD results in relatively high absolute values for normal changes of high optical densities, %CV tends to exaggerate minor changes in very low OD values.

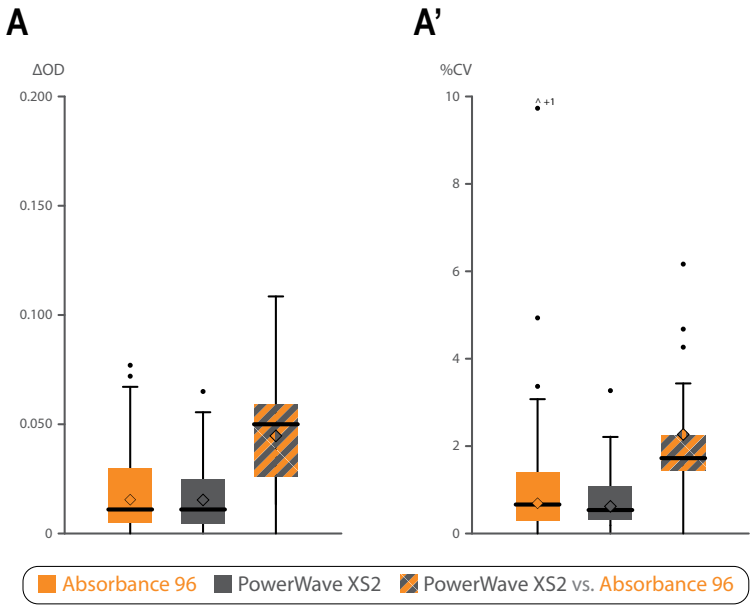

Figure 4: Intra- and Inter-Measurement Testing. IgG_{2b} (mouse) samples were pipetted in duplicates and variation between sample duplicates was determined with either device, as well as variation of the average between both devices. (A/A') Variation of sample duplicates per device and differences of sample mean between the devices are depicted as (A) OD as well as (A') %CV. Boxplot data depicted as box-and-whisker plot. Midline shows median, box indicates IQR, whiskers indicate +/- 1.5xIQR, and dots show outliers. Rhombus shows mean.

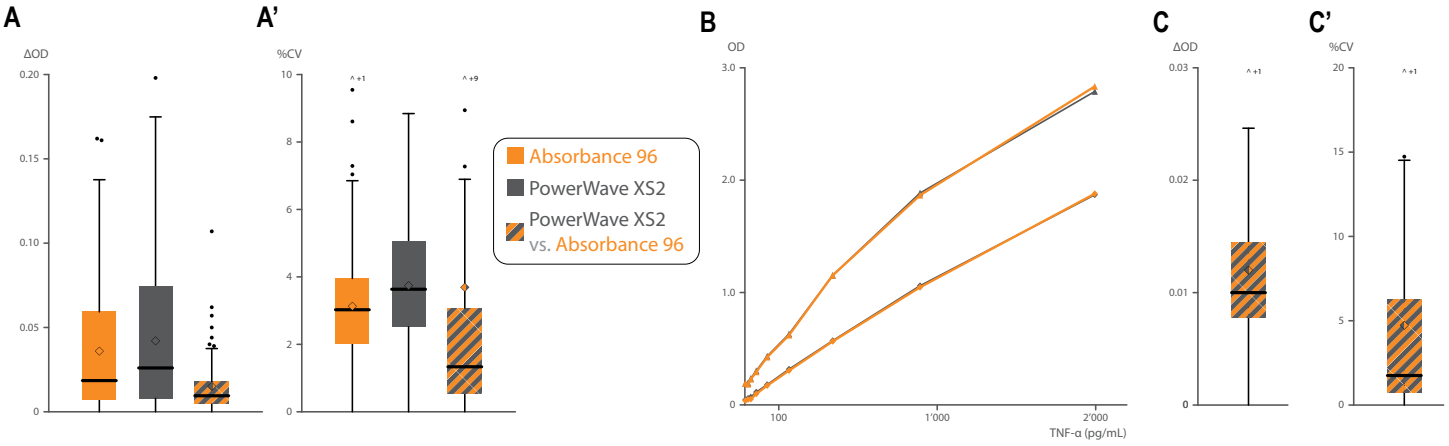

Figure 5: Intra- and Inter-Measurement Testing. TNF-α (mouse) ELISA was carried out and resulting plates were measured twice with each device. (A/A') Variation between repetitive reads within one device as well as the variation of the average of two measurements between both devices. Deviation of blank-corrected measurements depicted as (A) ΔOD or (A') %CV. (B) Two standard curves were generated and measured with each device. Measured OD values are plotted against known standard concentrations. (C/C') Variations of the obtained standard values between both devices depicted as (C) ΔOD or (C') %CV. Boxplot data depicted as a box-and-whisker plot. Midline shows median, box indicates inner-quartile range (IQR) from first to third quartile, whiskers indicate +/- 1.5xIQR and dots show outliers. Rhombus shows the mean.

Overall variability of measurements was low and at comparable levels between devices. Variation between reads of physical sample duplicates (**Figure 4**) as comparable between Absorbance 96 and PowerWave XS2 and is likely mainly rooted in variations of the separate reactions in each individual well. Variations between repetitive reads (**Figures 5-6**) were comparable for either device, independent of whether TMB or pNpp substrate-based ELISA kits were measured. Differences between the values as determined by either device (**Figures 4-6**), as seen by the difference of the mean of repetitive measurements with each device, were at the expected levels. All variations were well within the expected range with the 3rd quartile limit just at or below 5%CV.

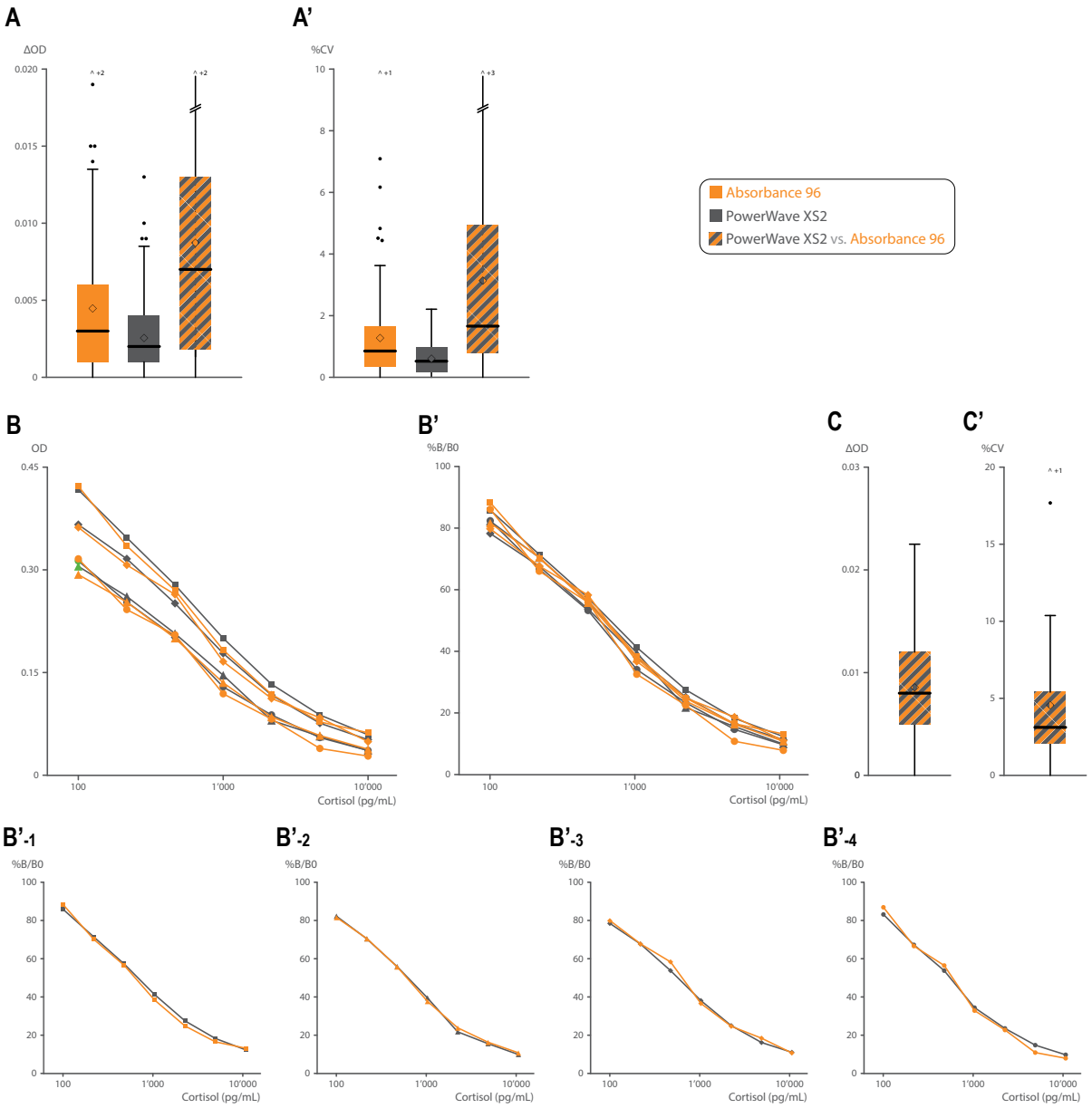

Figure 6: Intra- and Inter-Measurement Testing. Cortisol ELISA was carried out and resulting plates were measured twice with each device. (A/A') Variation between repetitive reads within one device as well as the variation of the average of two measurements between both devices. Deviation of blank-corrected measurements depicted as (A) ΔOD or (A') %CV. (B/B') Four standard curves were generated and measured with each device. (B) Measured OD or (B') B/B0 values are plotted against known standard concentrations. (B'-1 to B'-4) Each B/B0 standard curve as measured with each device shown separately for comparison. (C/C') Variations of the obtained standard values between each device depicted as (C) ΔOD or (C') %CV. Boxplot data depicted as a box-and-whisker plot. Midline shows median, box indicates inner-quartile range (IQR) from first to third quartile, whiskers indicate +/- 1.5xIQR and dots show outliers. Rhombus shows the mean.

Consistent and reliable standard curves are vital for consistent and meaningful ELISA results. Standard curve acquisition was comparable between Absorbance 96 and PowerWave XS2 (**Figures 5-6, B-C**).

B/B0 values determining the ratio of a sample to the maximum binding capacity of the assay is a very useful metric tool to analyze competitive ELISA assays. B/B0 variation testing was performed by measuring a single Aldosterone ELISA plate in either device (**Figure 7**), determining B/B0 values for each duplicate of samples and analyzing variance of obtained B/B0 values between reads, either repetitive reads in one device or reads across different devices. Variation between obtained B/B0 values for all samples on the plate was very low, and B/B0 values obtained with each device deviated in average only about 1%CV.

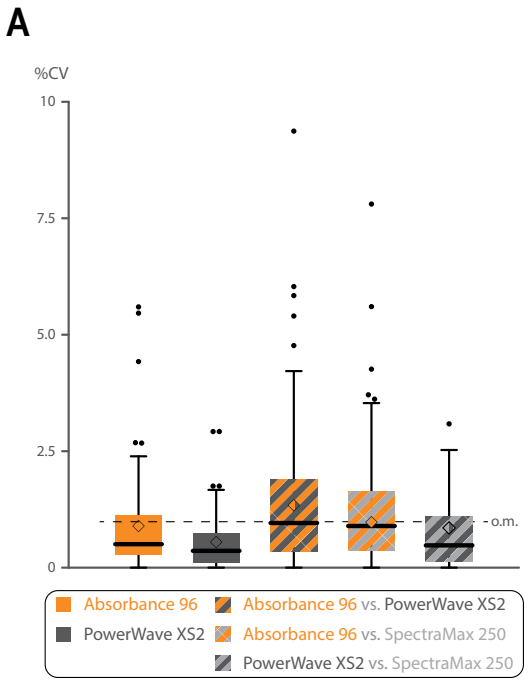

Figure 7: B/B0 Variation Testing. Aldosterone ELISA was performed and plate was measured using each plate reader. B/B0 values of sample duplicates were determined for each measurement run. Spread of %CVs of B/B0 are depicted as (A) %CV between two measurements. Boxplot data depicted as a box-and-whisker plot. Midline shows median, box indicates inner-quartile range (IQR) from first to third quartile, whiskers indicate +/- 1.5xIQR and dots show outliers. Rhombus shows the mean. Dotted Line shows the overall mean.

CONCLUSION

No significant performance differences were found between the portable, compact Absorbance 96 and standard benchtop microplate reader devices. This design allows the Absorbance 96 to fit into every lab, saving bench space and providing unprecedented flexibility. Versatile and maintenance-free, the Absorbance 96 works well with a broad range of Enzo's ELISA kits, as well as other absorbance-based cell and biochemical assays. User-friendly software allowing intuitive ELISA analysis is supplied with the device. Summarizing, the Absorbance 96 provides unmatched applicability to individual laboratories.

Visit www.enzolifesciences.com/Absorbance96 for more information including:

- References
- Cited Samples
- Other Application Notes

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