# Do Plate Readers Agree?

# Understanding Performance Differences between Different Plate Reader Makes/Models

Tanya R. Knaide, John Thomas Bradshaw, Richard H. Curtis, Geary Ritter, Rachel Parshley


## Abstract

Microplate readers are used for numerous laboratory applications to measure results of chemical assays in microplates. Readers may be used to collect one or several types of measurements including absorbance, or optical density. However, like any type of laboratory equipment, not all plate readers are created equal and each lends different amounts of uncertainty to measurements collected, and therefore the overall results of the assays conducted. Characterization of plate reader performance enables users to harmonize groups of instruments, making results agree regardless of the plate reader employed, as well as to understand how the uncertainty of the plate reader measurements translates to their assay results. Herein, a specific procedure for the measurement of the variability between plate readers will be discussed.


## Introduction

Multiple plate readers are often used throughout a facility to measure the response in microplates for different assay types. However, QC of plate readers is often considered sufficient when PM service is performed or measurement of reader-specific calibration plates is completed for each individual reader. The performance of multiple plate readers, regardless of make or model, is often not directly compared even if those readers are performing the same task(s) for the same assay(s). Unknown performance differences between plate readers introduces an additional source of error to assays at perhaps the most critical step.

In this presentation, a method of measuring plate reader performance using Artel MVS dye solutions is described using six different plate reader types from different manufacturers. Using this method, groups of plate readers may be assessed and standardized, thereby minimizing the uncertainty contributed to assay results from plate reader measurements.

It should be noted that this procedure may be employed using different dyes at other wavelengths. In the example described, MVS Sample Solutions were used because they represent the wavelength of interest and are reliably manufactured. When repeating this procedure in an effort to standardize plate readers used for specific assays, appropriate dye solutions should be employed.

## Materials

- Single-channel Test Plate Readers A, B, D, E
- 8-channel Test Plate Reader C
- Single-channel Reference Plate Reader
- Interference filters for 520 nm (where appropriate)
- Calibrated syringes (or another transfer standard)
- Calibrated 1 mL or higher pipette with tips
- MVS Baseline Solution
- MVS Range A, B and E
- MVS Diluent
- 96-well Artel Verification Plates
- 384-well Artel Verification Plates
- MVS Calibrator Plate
- Multichannel pipette or automated liquid handler
- Amber bottles
- 5-place balance

## Method

**Discussion:**

A reference plate reader was established for this protocol (procedure not discussed). This plate reader complied with the following guidelines: the lowest number of individual measurement channels was considered to return measurements as close to the "true" value as possible.

This experiment compared average absorbance measurements at 520 nm between multiple plate readers. The absorbance measurements from each reader were compared to those from the reference reader to determine the off-set from the "true" absorbance at a specified absorbance range.

Various dilutions were made containing different mixtures of MVS Sample Solutions and Diluent. The dilutions were dispensed into 96-well and 384-well Artel Verification Plates as defined in Table 1. MVS Sample Solutions are manufactured with a high degree of reproducibility at six different concentrations that absorb light at 520 nm. Using these solutions provided a range of absorbance values at 520 nm which were measured by each plate reader under test.

All test readers and solutions were allowed to equilibrate in the testing environment for 1 hour prior to testing to minimize thermal influence on the measurements.

Plate reader settings including number of flashes and pause time between reads were tested and selected in a separate experiment. The settings used for the plate readers in this experiment are described in Table 2.

As with any QC procedure, materials and instruments used for this experiment were tested to ensure proper performance before beginning and technician skill level was adequate for the procedure.

**Procedure:**

- Using the calibrated syringe*, or a gravimetric large volume dilution procedure**, the dilutions described in Table 1 were prepared in amber bottles using the appropriate MVS Sample and Diluent Solutions. The appropriate volume of MVS Diluent was transferred to the bottle. Using the syringe, the appropriate Sample Solution volume was aspirated and dispensed into the Diluent-filled amber bottle. Each bottle was gently inverted at least 10 times to achieve complete mixing.
- Using a calibrated multichannel pipette, 200 µL of the prepared dilution was dispensed into each well of a 96-well Verification Plate, or 55 µL into a 384-well Verification Plate, respectively, as indicated in Table 1.
- Each sample plate was measured in the reader pool according to Table 3. To ensure that the baseline reading was not drifting due to thermal fluctuations of the readers, the two 96-well sample plates were read in close succession. To do this, both plates were run through the reader pool concurrently (e.g. plate one/reader one, plate one/reader two and plate two/reader one, etc.)

*Calibrated syringe dilution procedure:

a. Transfer desired volume of diluent to bottle using a reliable transfer method (gravimetric, pipette, etc.)
b. Using calibrated syringe and proper technique, transfer the required volume of sample solution to the diluent filled bottle.
c. Mix at least 10 times by inversion.

**Gravimetric large volume dilution procedure:

a. Place bottle with cap on 5-place balance and tare.
b. Remove the cap and dispense the appropriate volume of diluent into the bottle.
c. Replace the cap and record the weight.
d. Remove the cap again and dispense the appropriate volume of sample solution to the bottle.
e. Replace on the cap and record the weight.
f. Mix at least 10 times by inversion.

## Method (continued)

| Plate # | Target Abs. | Sample Solution | Sample Volume (µL) | Diluent Volume (µL) | Plate Type | Portion of plate |
|---|---|---|---|---|---|---|
| 1 | 0.15 | Range E | 20 | 60000 | 96 | Columns 1-4 |
| 1 | 0.25 | Range E | 30 | 60000 | 96 | Columns 5-8 |
| 1 | 0.475 | Range B | 3000 | 57000 | 96 | Columns 9-12 |
| 2 | 1.133 | Range B | 7500 | 52500 | 96 | Columns 1-4 |
| 2 | 2.265 | Range A | Neat | 0 | 96 | Columns 5-8 |
| 2 | 0 | Baseline | Neat | 0 | 96 | Columns 9-12 |
| | | | | | | |
| 1 | 0.15 | Range E | 20 | 60000 | 384 | Columns 1-4 |
| 1 | 0.25 | Range E | 30 | 60000 | 384 | Columns 5-8 |
| 1 | 0.475 | Range B | 3000 | 57000 | 384 | Columns 9-12 |
| 1 | 1.133 | Range B | 7500 | 52500 | 384 | Columns 13-16 |
| 1 | 2.265 | Range A | Neat | 0 | 384 | Columns 17-20 |
| 1 | 0 | Baseline | Neat | 0 | 384 | Columns 21-24 |

Table 1. Dilution proportions for experimental use

Plate Reader Settings

| Reader Type | Plate Type | # Flashes | Read Mode | Pause Time (ms) |
|---|---|---|---|---|
| Reader A | 96 | 10 | n/a | 20 |
| Reader B | 96 | 10 | n/a | 20 |
| Reader C | 96 | n/a | n/a | n/a |
| Reader D | 96 | n/a | 6 (10 ms) | 50 |
| | | | | |
| Reader A | 384 | 5 | n/a | 20 |
| Reader B | 384 | 5 | n/a | 20 |
| Reader C | 384 | n/a | n/a | n/a |
| Reader D | 384 | n/a | 7 (16.7 ms) | 50 |

Table 2. Plate reader settings employed

Reference Plate Reader: Single-channel, interference filter-based
Plate Reader A: Single-channel, monochromator-based
Plate Reader B: Single-channel, interference-filter based
Plate Reader C: 8-channel, interference filter-based
Plate Reader D: Single-channel, interference filter-based
Plate Reader E: Single-channel, monochromator-based
Plate Reader F: 8-channel, monochromator-based

Figure 1. Properties of plate readers assessed

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| •TR 1 | •TR 2 | •TR 3 | •TR 4 | •TR 5 | •RR | •RR | •TR 5 | •TR 4 | •TR 3 | •TR 2 | •TR 1 |

Table 3. Order of measurements collected. TR = Test Reader, RR = Reference Reader

## Results of Experiment

The plate readers described in Figure 1 were used to measure the absorbance of each well in the sample-filled microplates. Measurements collected during the experiment are summarized in the Figures 2-4. In Figure 2, all single-channel plate reader measurements are compared to the respective measurements from the reference reader at each absorbance level. Figures 3a and 4a compare the absorbance measured by each channel of 8-channel plate readers to the single channel of the reference plate reader. Figures 3b and 4 compare the absorbance measurement from each of the 8 channels to the median of all absorbance measurements collected by the 8-channel plate readers.

In this experiment, single-channel and multichannel plate readers similarly exhibited the largest deviation from the reference reader at absorbance levels below 0.5 OD, while the best agreement between channels and between readers occurred near 1.0 OD. Photometric noise represents a portion of the observed deviation from the reference at low absorbance levels and was measured for selected readers by collecting ten consecutive reads of the same artifact. The results are summarized in Table 4 and indicate that, while a greater amount of uncertainty is contributed by the noise to the total deviation from the reference at low absorbance levels, it only represents approximately 0.5% of the total deviation. Similar tests were completed in 384-well plates with comparable results.

| Reader | Nominal Absorbance | Standard Deviation | %CV |
|---|---|---|---|
| A | 0.15 | 0.00027 | 0.16% |
| A | 0.25 | 0.00027 | 0.11% |
| A | 0.5 | 0.00038 | 0.08% |
| A | 1.2 | 0.00067 | 0.06% |
| A | 2.4 | 0.0020 | 0.09% |
| B | 0.15 | 0.00072 | 0.46% |
| B | 0.25 | 0.00078 | 0.34% |
| B | 0.5 | 0.00094 | 0.21% |
| B | 1.2 | 0.0019 | 0.17% |
| B | 2.4 | 0.0059 | 0.26% |
| C | 0.15 | 0.00027 | 0.162% |
| C | 0.25 | 0.00027 | 0.112% |
| C | 0.5 | 0.00038 | 0.082% |
| C | 1.2 | 0.00067 | 0.057% |
| C | 2.4 | 0.0020 | 0.088% |

Table 4. Photometric noise measurement at key absorbance levels

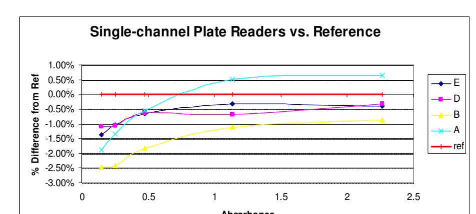


Figure 2.

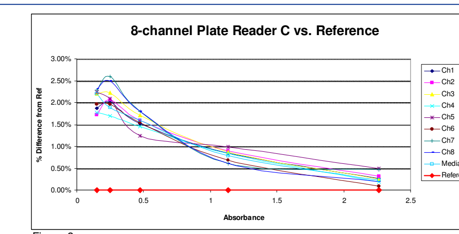


Figure 3a.

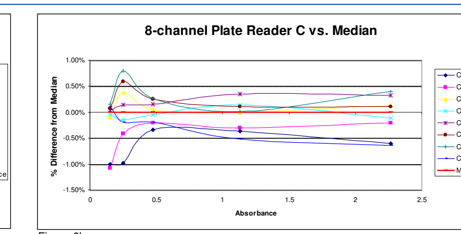


Figure 3b.

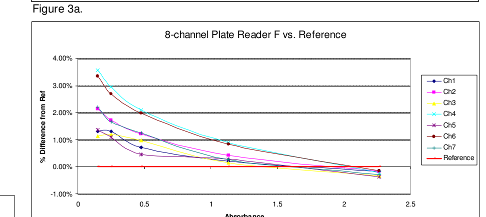


Figure 4a.

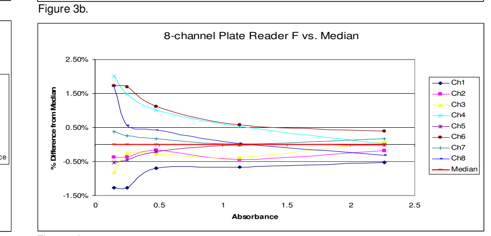


Figure 4b.

## Conclusions

Understanding the performance at critical wavelengths for a group of plate readers used to measure the response of plates from the same assay is critical to minimizing variability in assay results. While, in this experiment, the channels in each reader type trend in the same direction, it cannot be assumed that the observed behavior will repeat for other readers of the same type. Because more than one plate reader may be employed to measure multiple plates for the same assay step, it is equally as important to define the differences in performance from one reader to another as it is to control the performance of an individual plate reader to allow direct comparison of results. The measured variability from the plate readers included in this study alone may contribute up to 3.5% to the total uncertainty of the measurement unless corrections for the off-sets from reader to reader are introduced.

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