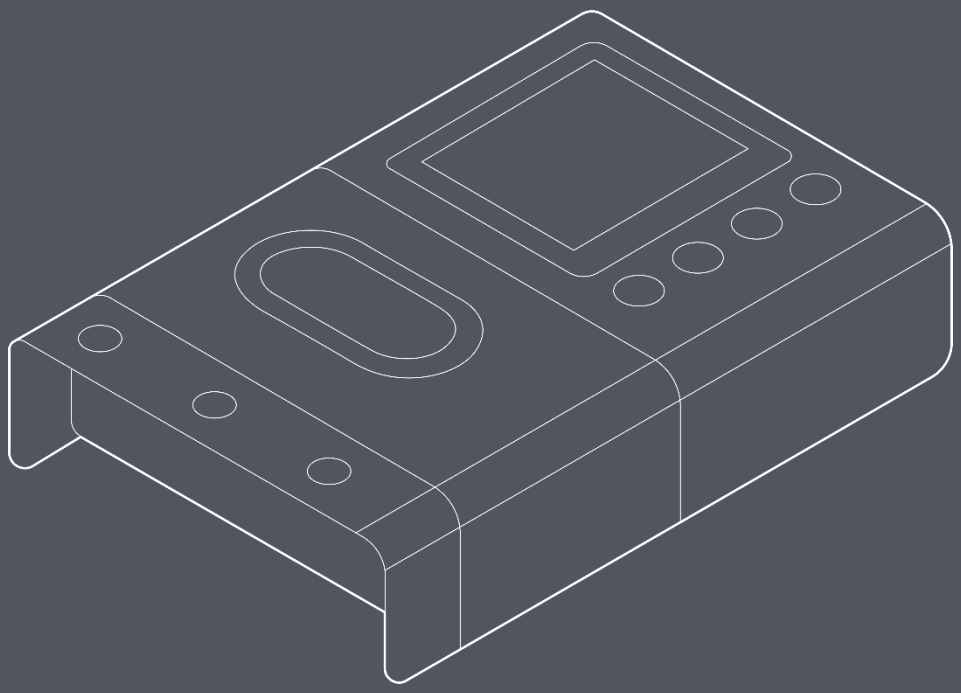
SBT

Application Note



BactoBox Linearity

Introduction

The linearity of an analytical procedure refers to its capability to yield test results that directly correlate with the concentration of a specific analyte in the sample. In this context, we present two examples of linearity response testing using the BactoBox: one with an early stationary stage culture of *Staphylococcus epidermidis* and the other with 1 µm silica microspheres, also known as QC beads.

Linearity response for a *Staphyloccus epidermidis* culture is excellent.

For the *S. epidimidis* example, a shake flask with tryptic soy broth (TSB) was inoculated with a fresh colony of *S. epidermidis* strain ATCC 12228 and incubated 12 hours at 37 °C. Disaggregation was done by vortexing with 3 mm glass beads for 3 min and the culture was placed in a cold bath to halt growth. The trend line below (Figure 1) reveals excellent linearity response (R^2 = 0.9975).

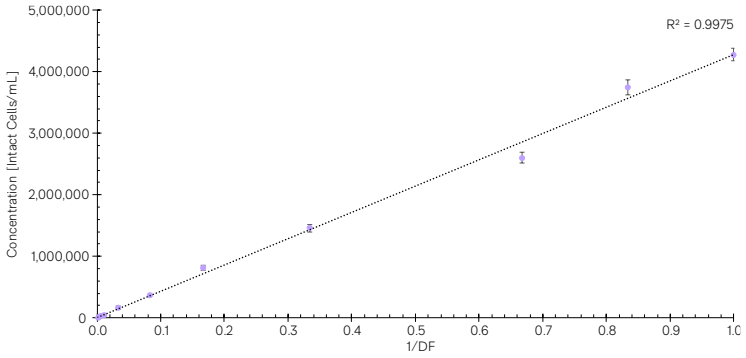


Figure 1: *S. epidermidis* linearity response. Concentration of intact cells as a function of 1/dilution factor. Triplicate measurements.

The above linearity response test operates under the presumption that there was no significant drift in bacterial concentration during the roughly 4-hour duration of the experiment. While efforts were made to maintain the bacterial concentration, fully stabilizing a bacterial culture is nearly unattainable. To compensate for potential drift, simultaneous plate counts were carried out using the same dilution series, as presented below (Figure 2). The improved R^2 value of 0.9982 indicates a near-perfect linear relationship. Additionally, a slope of 0.9947 suggests that the correlation between BactoBox's intact cell counts and Colony Forming Units (CFU) is almost an exact 1:1 match. A close examination of the error bars reveals that the BactoBox exhibits greater precision than plate counts, especially at elevated bacterial concentrations. The variability observed for the plate counts at higher concentrations can be attributed to the multiple dilutions needed to ensure 30-300 colonies per plate. For BactoBox, the minimal error bars observed at high concentrations on the log-log plot emphasize that BactoBox is especially precise at these higher concentrations.

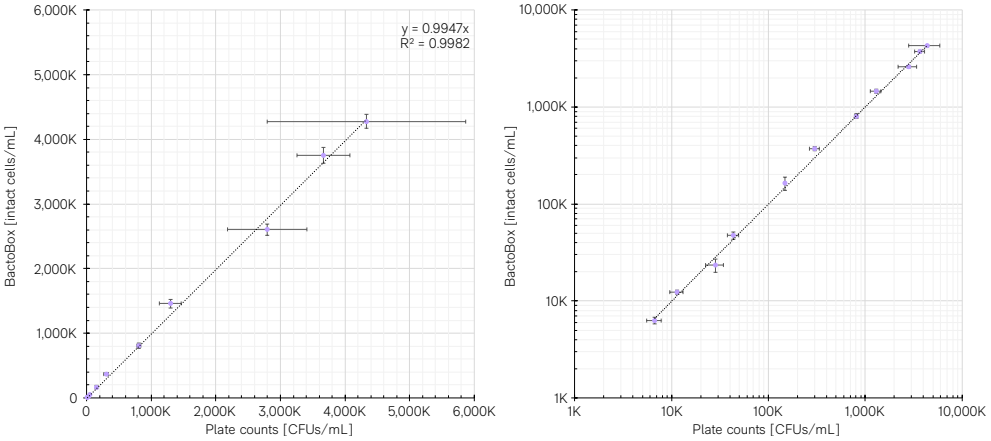


Figure 2: *S. epidermidis* linearity response test with head-to-head comparison to plate counts. Left: normal axes. Right: log-log chart. Average value of triplicate measurements for plate count (x-axis) and BactoBox intact cell concentration (y-axis). Y and X-axis error bars depict standard deviation for plate counts and BactoBox intact cell concentrations, respectively.

Linearity response for 1 µm silica beads is excellent at high concentrations.

As explained above, bacterial test suspensions can often drift in concentration within the duration of an experiment. Silica microspheres – on the other hand – do not replicate or die and therefore make a simpler analyte for investigation of linearity response. BactoBox precision for 1 µm silica beads is excellent for especially the three highest values (coefficient of variance of 1.9%, 1.6% and 2.9%).

The plot with normal axes below (Figure 3, left) show excellent linearity (R^2 = 0.9995) over the linear measurement range 50,000 to approx. 4.5 million total particles/mL. However, on the log-log plot (Figure 3, right), concentrations below 500,000 total particles/mL deviate from the ideal scenario, where target and actual concentration is identical (y=x line).

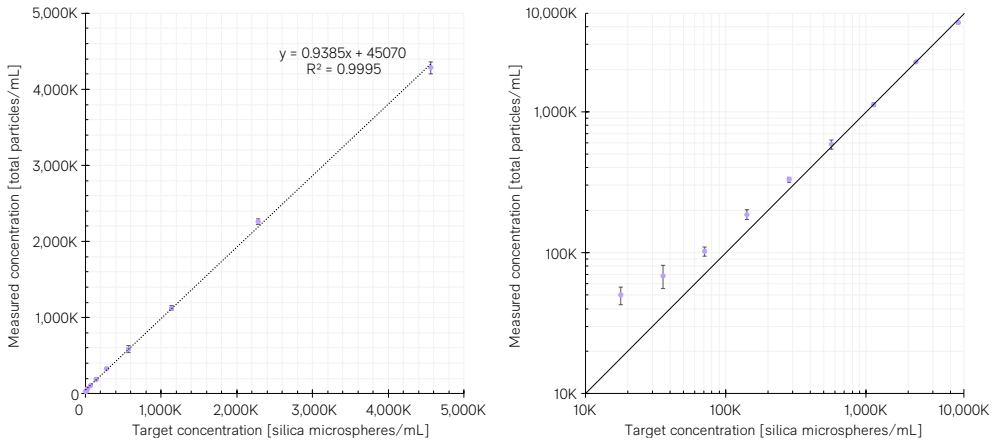


Figure 3: Silica microspheres linearity response. Measured BactoBox total particle concentration as a function of the target concentration determined from Multisizer 4e analysis. Data are depicted using normal axis on the left plot and log-log axes on the right plot. Triplicate measurements were done for each data point. Error bars represent standard deviation. The dotted line on the left plot is the trend line, while the fully drawn line on the right plot is an x=y plot to depict the ideal analytical performance.

The performance of BactoBox in measuring particles at lower concentrations reveals a loss of linearity that can be attributed to background noise from the instrument itself. Flow in BactoBox is driven by a peristaltic pump, where rollers force liquid forward. While such pumping principle is proven and robust, it leads to a spallation-induced release of small microplastic particles when the liquid is recirculated. Over extended measurement periods, this release averages approximately 10,000 to 100,000 particles/mL per sample. At concentrations exceeding 1 million total particles, this contribution is negligible, but at lower concentrations it is substantial. To understand this phenomenon better, we display the phase shift distribution of the particles; a metric that describe the electrical properties of the detected particles (Figure 4). It is seen that the distribution from a blank sample is practically identical to that of the lowest silica beads dilution, i.e. silica beads and spallation-induced microplastic particles have indistinguishable electrical properties.

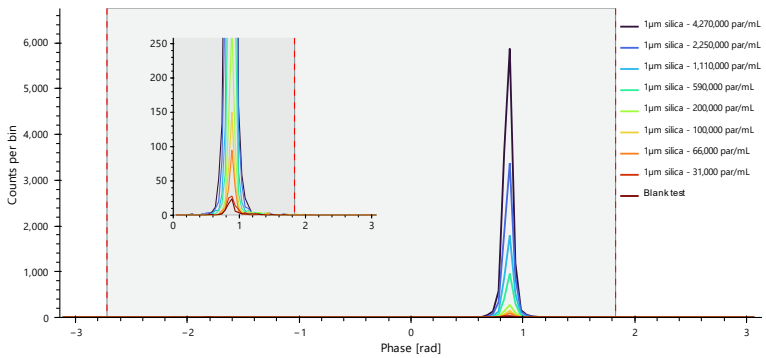


Figure 4: Phase shift histograms for 1 µm silica beads. Single-replicate representation of silica beads dilution series and a blank test. The inset provides a magnification of the non-conductive region. The numbers in the legend represent target concentration.

Contrarily, the *S. epidermidis* linearity test demonstrated impeccable linearity, even at lower concentrations (Figure 2). This can be attributed to the distinct electrical properties of intact bacterial cells (Figure 5), which differ markedly from those of spallation-induced microplastic objects. Below 160,000 intact cells/mL the spallation-induced microplastic objects (0.9 rad) are still observed in the phase shift distribution, but because this is nicely separated from the 1.8-2.2 rad population, microplastic particles do not interfere with classification of the intact cell populations (Figure 5, inset).

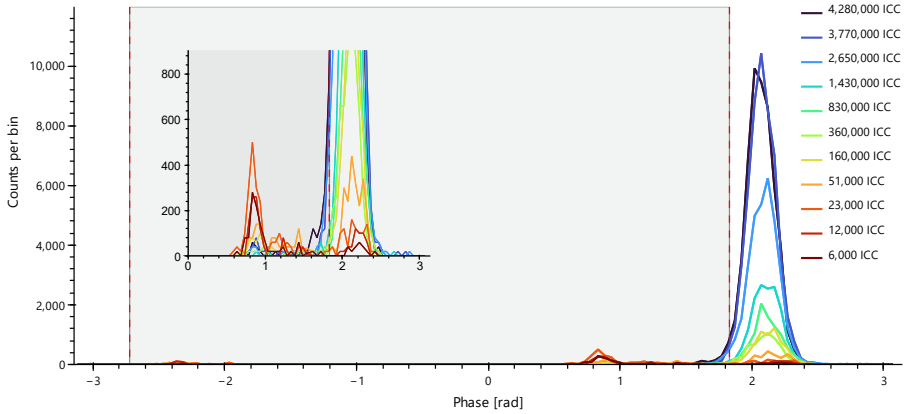


Figure 5: Phase shift histograms for *S. epidermidis*. Single-replicate representation of bacterial dilution series. The inset provides a magnification of the region close to the upper phase shift threshold, i.e. the dotted red line. The legend represents measured BactoBox intact cell concentrations.

Discussion

The linearity response test for *S. epidermidis* revealed that BactoBox exhibits excellent linearity across two orders of magnitude. Precision is typically highest for bacterial suspensions with concentrations up to approximately 5 million intact cells/mL. On the other hand, plate counts exhibit considerable imprecision at concentrations surpassing 1 million CFU/mL. The slope value of 0.9947 (Figure 2) indicates that, for the given S. epidermidis sample, BactoBox aligns almost perfectly with plate counts. *S. epidermidis* serves as an optimal example, given the ease in achieving a single-cell suspension with outstanding cultivability. However, bacterial samples that contain clumps or a significant number of viable-but-nonculturable (VBNC) bacteria might exhibit different slope values than the near-perfect 1 observed here.

The BactoBox's linearity response for 1 µm silica beads is outstanding for concentrations above 1 million total particles/mL (R^2 = 0.9995). However, at lower concentrations, the peristaltic pump tubing introduces a significant amount of microplastic objects. For applications requiring custom gating in the range of 0.8-1.2 rad, it is advisable to maintain total particle concentrations above 1 million particles/mL. Relevant applications include the enumeration of endospores, mycoplasma, smaller Gram-negative bacteria like *Pseudomonas*, and dead cells.

Conclusion

- Silica beads: BactoBox exhibits outstanding linearity for concentrations above 1 million total particles/mL (R^2 = 0.9995). At low total particle concentrations, deviations from the target concentrations occur due to the presence of spallation-induced microplastic objects.

- Bacteria: BactoBox demonstrates outstanding linearity across a working range of 10,000 to 5,000,000 intact cells/mL, with an R^2 value of 0.9982. Its correlation with plate counts is nearly a flawless 1:1 match. Beyond concentrations of 1 million intact cells/mL, BactoBox's precision distinctly surpasses that of plate counts.