USP Compendial Particle Measurements with the Aura System

Introduction

Subvisible particulate matter present in biopharmaceutical injections and parenteral infusions has been strongly linked with immunogenicity[1]. USP <788> is the compendial chapter referenced by the FDA that establishes biopharmaceutical lot release guidelines and limits for subvisible particles[2]. The two methods described in USP <788> are light obscuration (USP <788> Method 1) and membrane microscopy (USP <788> Method 2). Until recently, light obscuration had virtually replaced membrane microscopy due to its lack of automation along with its error prone and arduous manual analysis. However, the USP <788> chapter and its informational chapter <1788> recommends membrane microscopy over light obscuration when handling complex, high-viscosity and low-volume samples, which is where a large part of biopharmaceutical development is now focused. Most significantly, USP membrane microscopy methods allow for automated image analysis, as established in the support chapter <1788> in 2020[3,4]. This change established Backgrounded Membrane Imaging (BMI) technology, which powers the Aura™ system, as a fully compendial method.

This application note describes how to generate accurate, USP <788> compliant subvisible particle data in biopharmaceutical samples using volumes ranging from 10 µL to as much as 10 mL. Recommendations on how to adhere to USP Method 2 recommended workflows are also shared and analyzed in detail.

Recommended USP Workflow

A subvisible particle counting protocol using the Aura system has been developed following the FDA's USP <788> Method 2 guidelines, including the recommended two membrane rinsing steps (Figure 1). The recommended protocol involves five sequential steps, including pre-washing, backgrounding the membrane, filtering the sample, post-filtration washing, and measuring the particles. This method does not substantially increase the time required to run an assay on the Aura and can be completed using either manual pipetting or an automated, robotic liquid handler.

To perform a USP <788> compliant method on the Aura system:

- Step 1: Rinse (pre-wash) the membrane with ultra-pure water to remove any undesired dust or soluble material on the membrane.
- Step 2: Background image the membrane.
- Step 3: Load the sample.
- Step 4: Rinse (post-wash) the membrane with ultra-pure water to remove any soluble material from the filtered samples. This post-wash step can be repeated as many times as necessary to exclude soluble material from the count data, as recommended by the USP.
- Step 5: Reinsert the membrane plate into the Aura instrument for particle measurement.

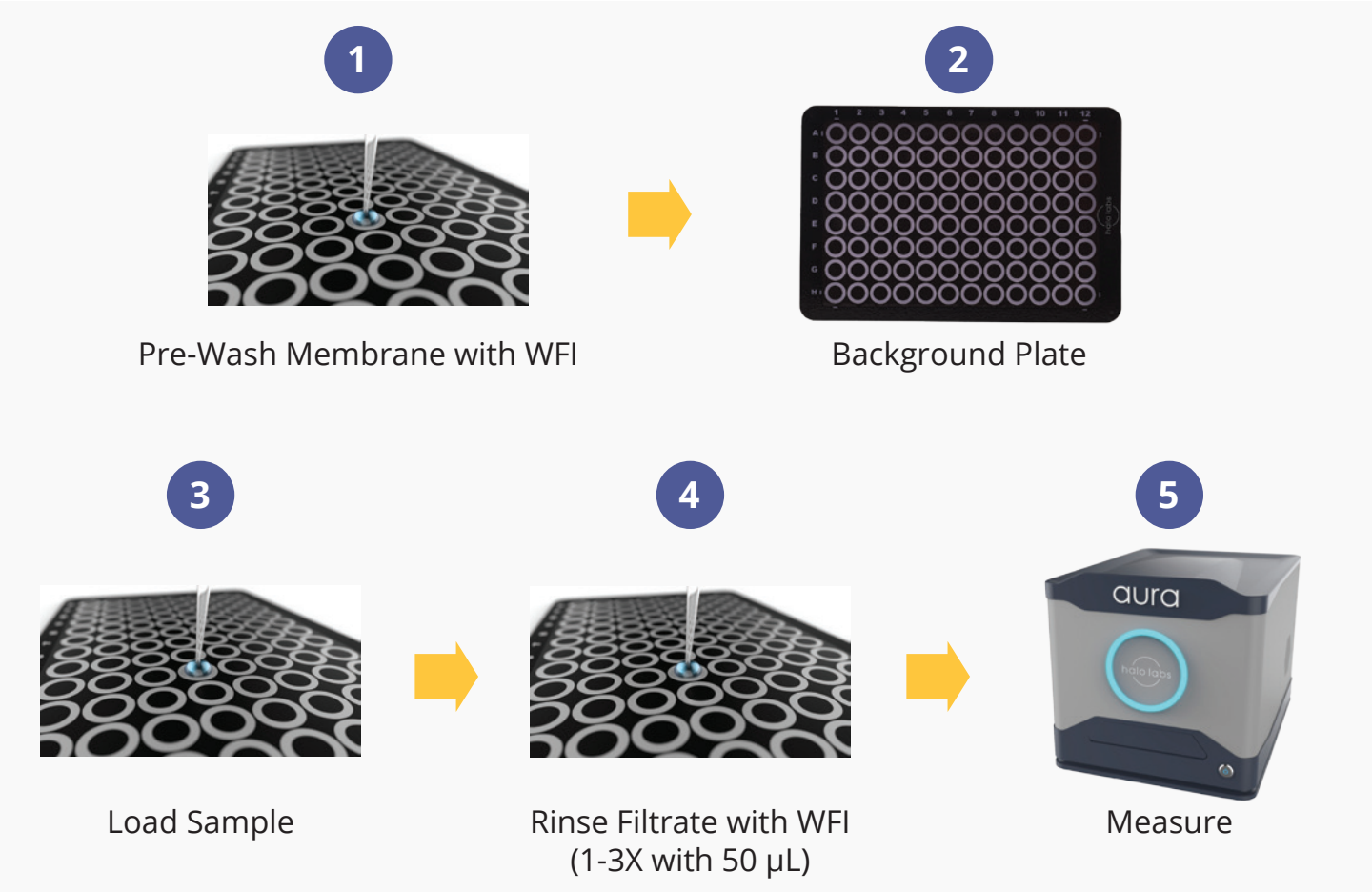

Figure 1: Aura system workflow using USP <788> recommendations. Step 1: Pre-wash. Step 2: Background the membrane plate. Step 3: Load the sample. Step 4: Post-wash. Step 5: Measure particles.

The impact of pre-washing on counts is demonstrated in Figure 2. The wells that were pre-washed exhibited, on average, a reduction of 1–2 particles in the >10 µm range, and no change for the cumulative bin range >25 µm. The impact on the size range of 2 µm–10 µm (a size range that is not considered in USP <788>) was more significant, with an average reduction of 17 particles. While potentially useful if adhering to the USP <788> large volume guidelines, pre-washing the membrane has little impact on formulation ranking, but is potentially important when single particle precision is required.

The impact of post-washing on filtered hIgG aggregates with ultra-pure water is demonstrated in Figure 3. Post-

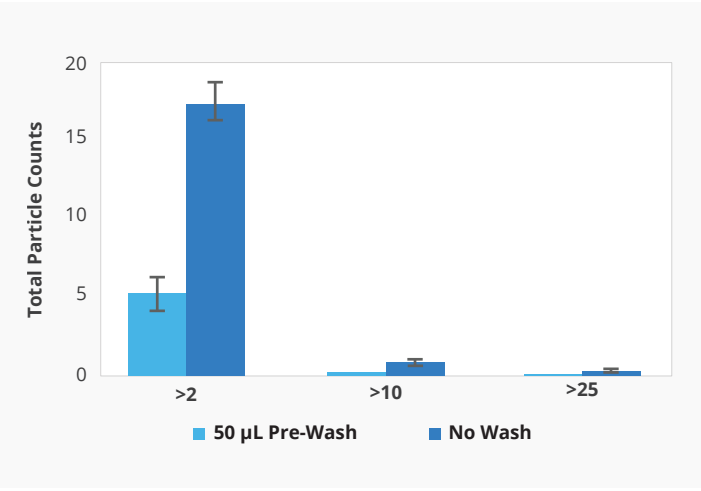

Figure 2: Impact of pre-washing the membrane on particle counts.

washing removes all soluble material including soluble proteins, and any salt crystals in the buffer that are not meant to be counted according to the USP <788> guidelines. As shown here, soluble material accounted for roughly 20% of overall total counts (>2 µm) for this sample but negligible amounts in the >10 µm and >25 µm USP bins. This soluble vs. non-soluble material experiment is very informative and simple to run using the clone plate features function, as explained in detail in Application Note 3, *Washing for More Accurate Particle Counts*. Repeated measurements on the same sample make assay validation very straightforward and robust. It allows sample variability to be treated separately from instrument variability – a feature available with the Aura system but not with light obscuration or flow imaging methods.

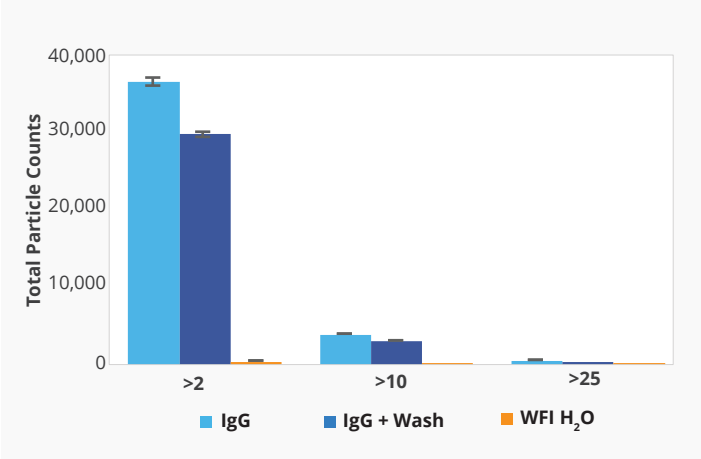

Figure 3: Impact of post-washing the membrane on particle counts.

Particle Vue software version 3.1 and beyond is powered with a powerful feature for subtracting background counts. Like traditional plate readers, a well, row, or column can be used to account for background counts. If desired, buffers, water, and environmental contamination can all be treated as background and referenced out of the total counts. A well can even be referenced against itself to account for any background material present before samples were filtered (Figure 4).

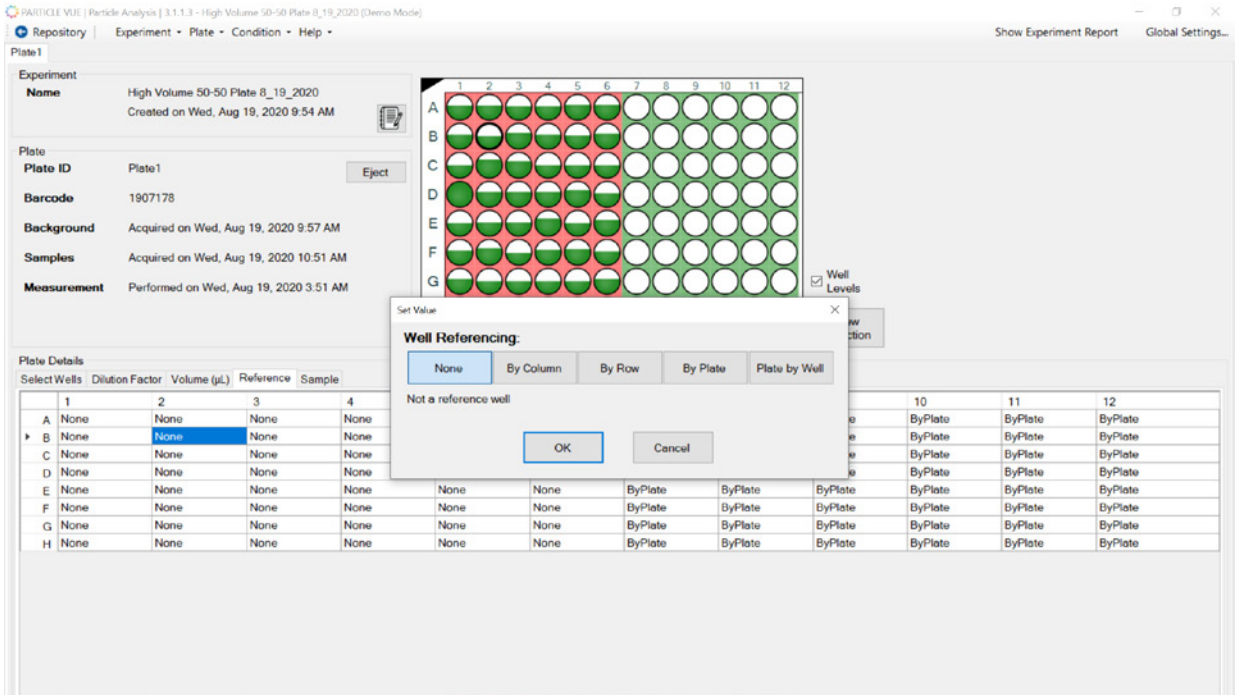

Figure 4: Well referencing using Particle Vue software version 3.1 and beyond.

Particle Size Using the Aura System

The Aura instrument's digital resolution is first established by calibrating the optics to an ASTM glass calibration slide (Figure 5a). The sizing is verified for particle detection using bead size standards (Figures 5b and 5c). Bead standards between 5 µm and 20 µm are measured within ±0.5 µm of their mean while beads 30 µm or larger are measured within ±1 µm.

Particle Counting Using the Aura System

Counting hIgG Subvisible Particles in Low and High Sample Volumes

BMI's high refractive index contrast methodology enables high sensitivity and reliable protein aggregate detection and quantification at volumes as low as 10 µL or as large as 10 mLs. The count data from Figure 6 was obtained using the USP-inspired workflow shown in Figure 1.

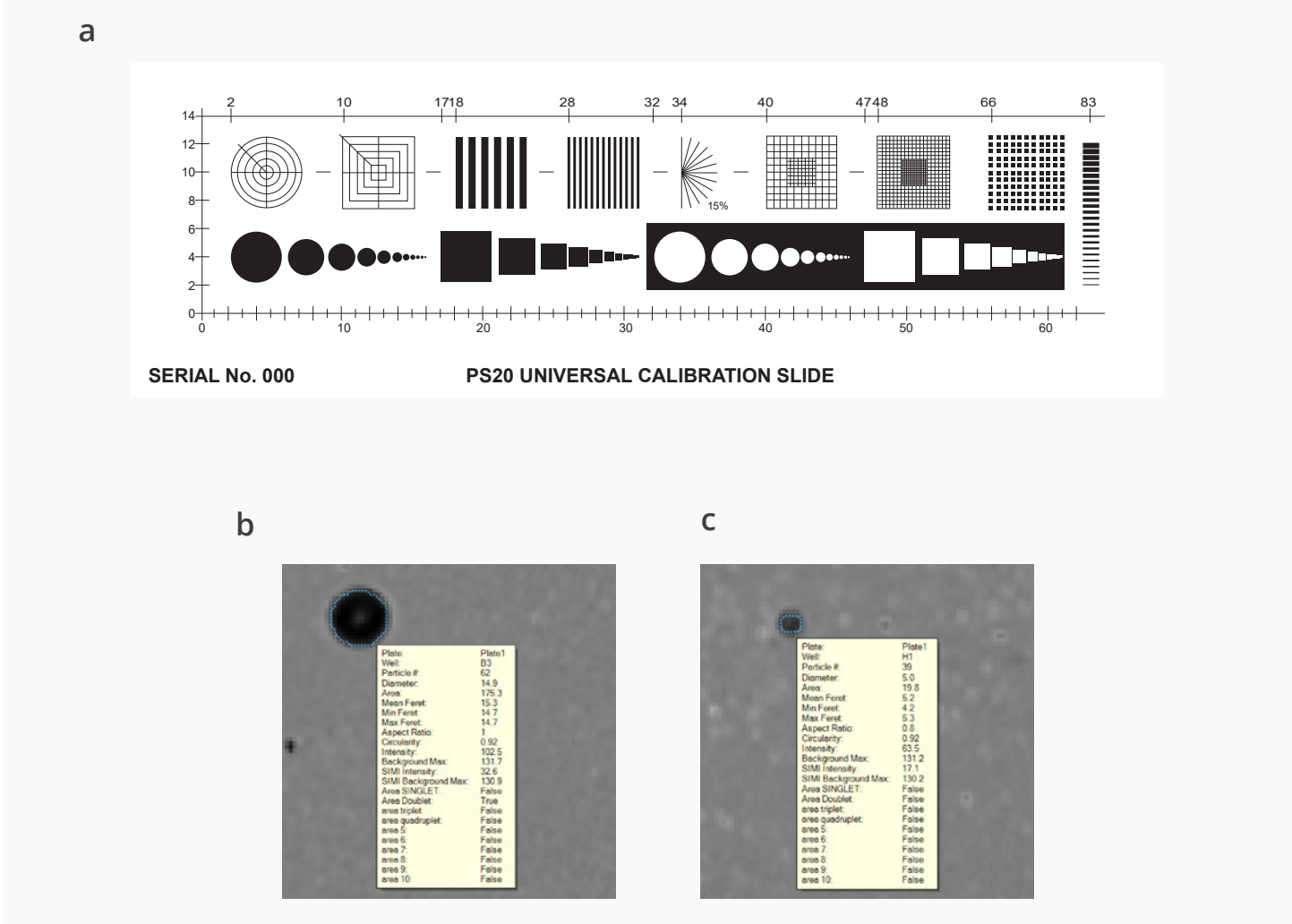

Figure 5: Sizing with the Aura system. (A) Calibration with ASTM Microscope Slide. Particle size verification with (B) 15 µm and (C) 5 µm polystyrene bead standards as shown in the captured images from the Aura Particle Vue software tooltip.

The average counts (≥2 µm) vs. cumulative well volume for an entire 96-well plate was demonstrated in Figure 6, where each well contains 50 µL of hIgG aggregates generated by rotation. 96 wells were pre-washed with water for injection (WFI), as recommended by the USP <788> Method 2 guidelines, and the entire plate was referenced against itself as shown in Figure 4 to exclude any background material. In this experiment, the average counts (≥2 µm) remained close to 60 k/mL and fluctuated by a max of 5% from the mean. The starting point of N=3 wells (150 µL of cumulative volume) yielded virtually the same results as 4.8 mL of sample (all 96 wells) and the cumulative volumes were consistently below 15%, representing the variability in a heterogeneous sample. This demonstrates that low sample volumes can predict the outcomes of large sample volumes.

Counting Bead Standards from Low to High Volumes

Counting with BMI is accurate and verifiable with USP bead count standards. In Technical Note 1, *USP Particle Count Standards with Aura and Particle Vue 3.1*, we described how Particle Vue software's expression engine can correctly measure USP bead count standards, and further corroborated those counts with manual verification. Those results were subjected to a four-instrument and three-user study summarized in Figure 7. Each user processed eight wells, each containing 50 µL of 10 µm bead count standards measuring 3k/mL ±10%, and another eight wells, each containing 50 µL of 25 µm bead count standards measuring 3k/mL ±10%. In both cases, automated and manual counting resulted in the USP bead count standards being measured within the stated specifications.

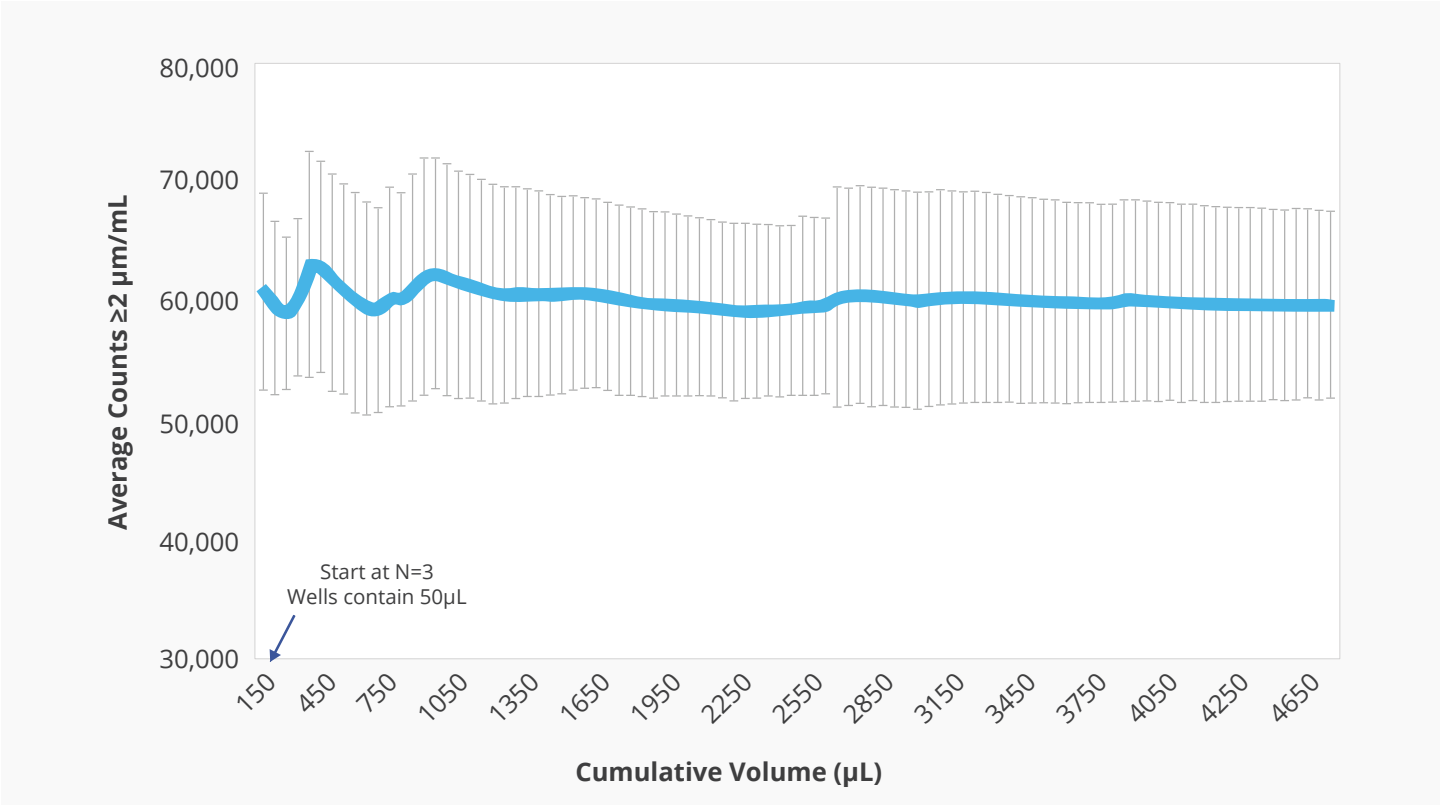

Figure 6: Counting hIgG protein aggregates volumetrically.

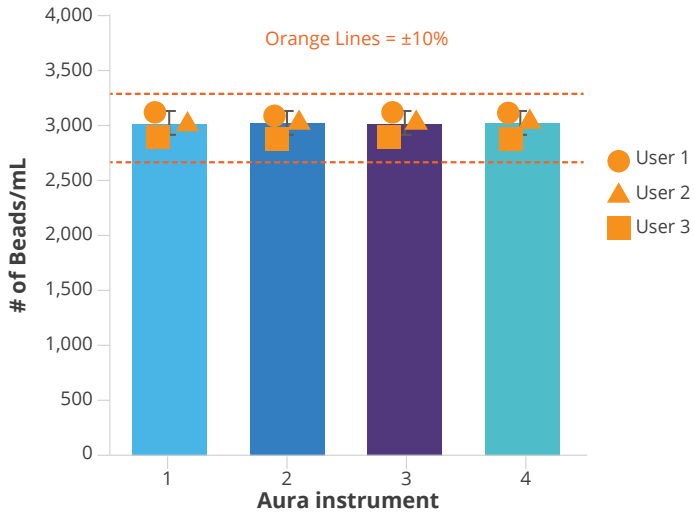

Figure 7: Inter-instrument, inter-user experiment counting standard bead count samples with the Aura system and and manual counts

Furthermore, manual counting validated the automated counting algorithm employed in the Aura platform.

As shown in Figure 8, the Aura system accurately counts samples from low volumes (150 µL in triplicate) to multi-plate experiments close to 10 mLs in volume. The cumulative volumes obtained in this multi-plate experiment matched exactly the 10% specified by the USP beads vendor.

In addition to handling large volumes, the Aura platform accurately detects subvisible particles from volumes as little as 5 µL of sample. Figure 9 demonstrates the counting linearity for serial dilutions of rotated hIgG aggregates from wells containing 5 µL and 10 µL of sample, with n=3 well replicates for each dilution condition. All results far exceeded an R^2 linearity of 0.99, indicating low-

Figure 8: 10 µm bead count standards vs. cumulative volume

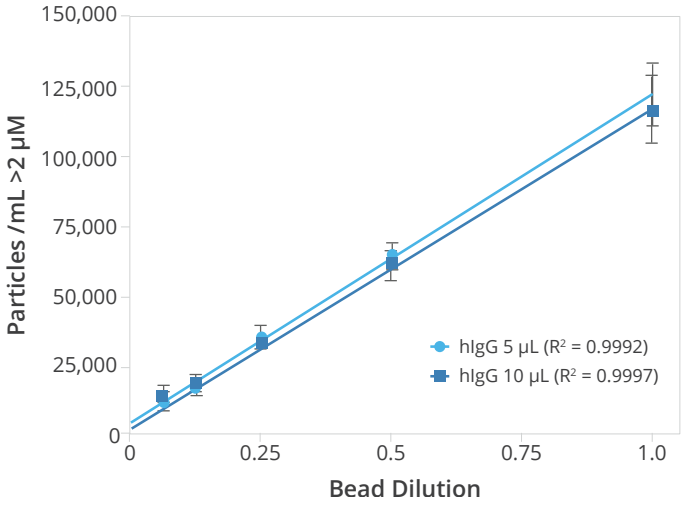

Figure 9: hIgG counts linearity for ultra-low well volumes of 5 µL and 10 µL

volume measurements are highly reproducible and that the Aura is appropriate for low-volume USP methods.

Discussion

The Aura platform, powered by BMI, provides a USP <788> compendial method that is rapid and robust. Unlike other subvisible particle analysis methods, the Aura can handle a broad range of sample types and volumes, enabling continuity of method from candidate selection to lot release. The Aura system's high refractive index contrast, 100% sampling efficiency, ability to re-analyze the same sample, and ability to detect particles in one field of focus enables subvisible particle detection accuracy that is unmatched by any other method. In addition, the Aura offers a high-throughput 96-well plate format that can be fully automated by using standard laboratory liquid handlers.

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