Handling & Pipetting Concentration Standards

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Particle count / concentration standards are routinely used as calibrators for automated cell (and particle) counters. They are suitable for instrument validation, routine QC calibration checks and corrections, and personnel training / proficiency programs. The use of external reference materials also aids in standardization between runs, instruments, laboratories, and over time.

Polymer microspheres are commonly utilized as surrogates for biologic particles such as cells, protein aggregates, etc. They offer high uniformity and consistency of results, ready availability, exceptional stability, and are not biohazardous. While non-biological surrogates have many advantages, it is important to note that there may be different handling considerations.

COUNT STANDARD SAMPLE PREPARATION

Proper mixing and sampling, and consistency in all aspects of the process will yield highest accuracy and reproducibility.

Mixing / Re-suspension

Proper attention must be given to mixing / re-suspension to ensure that the concentration of beads (and ratio if the suspension is a mixed population) is not altered. Inadequacies can have dual consequences: the prepared sample may not accurately represent the parent suspension, and the concentration of the parent suspension may migrate with repeated sampling, resulting in cumulative error.

Mixing to achieve a well-dispersed suspension is a simple process: roll or rotate the parent bottle (e.g. volumes of 5-20mL) for ~30 minutes, then immediately sample and run. In the case of our ViaCheck SingleShot™ packaging, vials are low volume, and several seconds of rigorous shaking or manual inversion is typically appropriate. Additional time may be required if suspensions are being used after extended storage (where they may have significantly or fully settled). High-energy mixing strategies (sonication, vortexing) may be appropriate in some circumstances, though they should be used judiciously as there have been reports of particle loss due to retention in caps, etc. Mixing and sampling procedures should be optimized and tested.

Pipetting

For concentration / count standards, volumetric error will translate into bead count error. Ensure that pipets are checked and calibrated regularly, e.g. weekly checks and annual calibration, or as appropriate for the needs of the program. Training and proficiency programs should be in place to ensure proper pipetting technique.

Timing

Many count standards are comprised of polymer spheres with diameters $> 2\mu m$ and typical bead densities of $\geq 1.05\ g/cm^3$. Aqueous suspensions will thus settle over time. Working quickly to pipet and run samples will aid in minimizing errors of this nature (i.e. where beads have settled and are not pipetted / aspirated, are not in correct analyzer focal plane, etc.).

INSTRUMENT CONSIDERATIONS

Instrument configuration, calibration status, and software version may impact results. Additional considerations may be outlined in the instrument user manual or determined during validation studies. In our experience, clean fluidics, filtered solutions, and low pre-run backgrounds (as low as possible) are universally important to accurate particle counting.

RELATED PRODUCTS

Cat. VC60N, VC70N, VC80N ViaCheck™ Concentration Controls (1e+6, 4e+6 or 8e+6 beads/mL; 20mL or SingleShot™ vials)

Cat. VC10B, VC25B, VC20B, VC30B, VC40B, VC50B ViaCheck™ Viability Controls (0%, 25%, 50%, 75%, 90%, 100% viable; 20mL or SingleShot™ vials)

Cat. CC03N, CC05N, CC10N, CC15N SureCount™ (1e+6 beads/mL in diameters of 3µm, 5µm, 10µm, or 15µm)

Cat. 580 Flow Cytometry Absolute Count Standard (1e+6 beads/mL, ~8µm full spectrum fluorescence)

See Product Data Sheets for additional use instructions.

FURTHER READING

1. Adams RB, Voelker WH, Gregg EC. (1967) *Electrical counting and sizing of mammalian cells in suspension: an experimental evaluation*. Phys Med Biol; 12(1):79-92.
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3. Barnard JG, Rhyner MN, Carpenter JF. (2012) *Critical evaluation and guidance for using the Coulter method for counting subvisible particles in protein solutions*. J Pharm Sci: 101(1):140-153.
4. Brando B, Göhde W Jr, Scarpati B, D'Avanzo G. (2001) *The "vanishing counting bead" phenomenon: effect on absolute CD34+ cell counting in phosphate-buffered saline-diluted leukapheresis samples*. Cytometry; 43:154-160.
5. Corvari V, Narhi LO, Spitznagel TM, Afonina N, Cao S, Cash P, et. al. (2015) *Subvisible (2-100µm) particle analysis during biotherapeutic drug product development: Part 2, experience with the application of subvisible particle analysis*. Biologicals; 43(6):457-73.
6. Kilbride K, Anglea R, Bavender A. (2017) *Optimization of Vi-CELL® XR settings for calibration checks using ViaCheck™ controls*. (Application Note 0708). Fishers, IN, Bangs Laboratories, Inc.
7. Kim J, Kim E-G, Bae S, Kwon S, Chun H. (2013) *Potentiometric multichannel cytometer microchip for high-throughput microdispersion analysis*. Anal Chem; 85(1):362-368.
8. Rainin Instrument, LLC. (2009) *Procedure for Evaluating Accuracy and Precision of RAININ Pipettes: Factory-Approved Method for Using Gravimetric Analysis*. (9920-335 Rev D). Oakland, CA. https://www.mt.com/dam/RAININ/PDFs/TechPapers/ab15.pdf