# Particle Count Standards

*Accusizer® SPOS system*

AccuSizer® customers often want to test system performance in order to verify that the system is working to specification. Entegris encourages these efforts and believes that a regular calibration/verification testing routine should be an integral component in owning these systems – either with testing by a certified representative or through internal testing. Particle size testing using a particle size latex (PSL) standard is an easy first step in tracking system performance. Checking the count accuracy is also a good idea, but slightly more complicated and we often spend a fair amount of time guiding customers through the suggested procedures presented in this technical note.

## INTRODUCTION

The AccuSizer is both a particle size analyzer and a particle counter, based on the principle of single particle optical sizing (SPOS). This principle requires sensor calibration using particle size standards. The calibration procedure relates pulse height generated as the particle passes through the sensor to particle size. A typical AccuSizer sensor is calibrated at the factory using 10 – 13 size standards across the dynamic range of the sensor. Once at a customer location the same complete size calibration can be performed, or one or more size standards may be used to test if the calibration is still accurate after some period.

We encourage customers to test the calibration using a size standard on a regular basis, the frequency being based on a risk assessment of how the data is used by the customer. A researcher using the system just as a quick check of approximate size, may test the calibration once a year, while a plant that runs their process based on the particle size result may test daily. Most customers check the sensor calibration every six months.

In addition to the particle size calibration, some customers also want to test the count accuracy of the system. The size and count accuracy are related since a shifted size calibration will also alter the count at a given size.

## PARTICLE COUNT STANDARDS

Far fewer particle count standards are available compared to the number of size standards. The three most validated count standards were created with the USP <1788> test[1] in mind.

- The USP Particle Count Set[2]
- The Micro Measurement Labs Particle Size/Count Standard[3]
- The Thermo Fisher Scientific Pharm-Trol Count Precision Size Standards[4]

These are all 15 µm nominal size standards where the concentration has also been tested and certified. The particle count/mL is typically between 3100 – 4400. The official particle count test in USP <1788> instructs the user to measure a blank, and then the count standard. The pass/fail criteria are based on the following equations:

- (Average count from the standard – average count from the blank)/sample volume
- Counts >10 µm/counts >15 µm

These calculated values must lie between the ranges stated on the count standard certificate. Systems passing this properly documented testing process can claim to be verified using the highest possible standard. When using the AccuSizer this count accuracy test should be performed using the SIS sampler with no dilution.

The official test described above is fairly expensive and some customers wish to verify count accuracy using a less formal procedure. Other customers wish to verify count accuracy at sizes other than 15 µm.

Entegris understands and supports these efforts, but cannot accept data from any random bottle of standards a customer picks up and decides to test with. Some customers take a particle size standard, measure the sample and then ask why the concentration does not match their expectations. This can lead to protracted discussions requiring considerable investigation into the sample used, measurement procedure, and result interpretation. Let's start with which samples are appropriate or inappropriate.

## INAPPROPRIATE STANDARDS

Most of the inappropriate data sent to Entegris was collected using particle size standards from Thermo Fisher Scientific (previously Duke Scientific Standards). These are perfectly fine particle size standards, and the concentration has been tested, but these samples were never meant to act as a count standard.

Samples such as the Thermo Fisher 3000 Series Nanospheres and 4000 Series Microspheres are particle size standards. They are appropriate for testing the AccuSizer sensor calibration to check if the expected size result is achieved when introduced to the system. These samples are not appropriate for testing the concentration value reported by the AccuSizer. The concentration in these samples are nominally around 1%, ranging from 0.2 – 4.8%. The concentration value is determined by calculating the known mass of material in a known volume and then reporting as approximate particles/mL. Although the standard manufacturer may be extremely experienced and operating under rigorous quality procedures, the concentration values reported (such as $1x10^7$ particles/mL) are approximate only and are not certified values.

## APPROPRIATE STANDARDS

Other standards are available from Thermo Fisher with values for both size and concentration that are appropriate for testing both size and count accuracy of the AccuSizer. These include the Pharm-Trol, Count-Cal, Validex, and Ezy-Cal samples. The size values for these standards are traceable to the standard meter through NIST. The concentration values for these standards are generated by measurement on certified systems and the values are appropriate for verifying the concentration results from the AccuSizer. No one can say a count value is NIST traceable to the meter – there is no way possible to achieve such a result — but these standards are acceptable options for testing the concentration values generated by the AccuSizer.

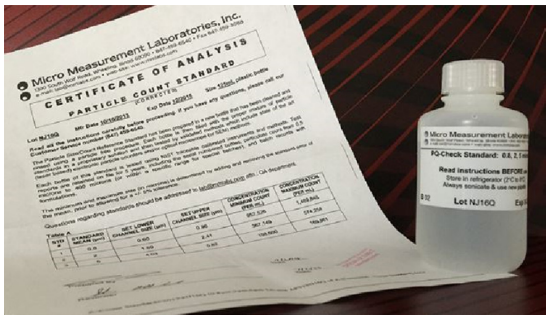


*Figure 1. Micro Measurement Labs trimix standard*

Micro Measurement Labs produces a count and size standard specifically for the AccuSizer. Entegris often refers to this sample as the "tri-mix" (see Figure 1) because three distinct size peaks are mixed into one container. Each bottle is filled with the proper mixture of particle standards in a proprietary solution and tested by validated methods including an SPOS particle counter and/or microscopic (optical or SEM) methods. The certificate of analysis (C of A) for this standard includes both the mean size peak and the expected count limits for each of the three sizes – typically 0.8, 2, and 5 µm.

## RESULT INTERPRETATION

Only the USP <1788> test includes official pass/fail criteria for particle count accuracy testing. Setting the expectations for less official testing is left up to the manufacturer and customer. It is not appropriate for customers to set their own internal specifications, measure as they please, and then call to question why these values were not met. The reasons why this is true should be obvious, but they include.

- Only officially published, ISO, ASTM, USP, etc. testing protocols have been reviewed and approved by both customers and instrument manufacturers
- Entegris would have to test a standard internally before we could state, with confidence, that the results lie within an acceptable specified range. We do not have the resources, or desire, to test all of the hundreds of available particle size standards.
- The calibration curve for the sensor would need to be current and accurate for a count test to also be accurate. While Entegris supports customers checking their own calibration curve, only factory tested, or field tested by an Entegris representative systems, are known to be in proper calibration.

But if a customer uses an appropriate standard on a recently calibrated system, what should the pass/fail criteria be? This answer depends both on the standard used and system being tested. The highest count accuracy is achieved when no sample dilution is involved - the AccuSizer SIS system. The AccuSizer APS and AD systems perform automatic dilution and generate accurate concentration data, but these concentration results are not as precise as SIS (no dilution) results. To understand why this is true, consider injecting 100 µL of the USP count standard into an AccuSizer AD system. To begin with, we are now sub-sampling from the original container rather than drawing the sample directly from the original into the sensor—error source #1. The AccuSizer then checks the concentration and decides if dilution is required. Once the concentration is within the desired range, the measurement begins. During this period, the sample is diluted below the originally intended value. Since the count rate is substantially lower, the result statistics are worse than the undiluted sample—error source #2. When testing on an AccuSizer APS the sample may go through two-stage dilution, increasing the error source #2. When testing the AccuSizer AD, we add ±10% to the expected result. When testing the AccuSizer APS we add ±13% to the expected result.

In addition to error sources #1 and 2, the uncertainty of the concentration value from the standard must also be added to the final pass/fail criteria. So if a customer is using a Thermo Fisher Count Cal standard on an AccuSizer AD system the pass/fail would be: 3000 particles/mL ±10%, plus ±10% when using the AccuSizer AD system = 3000 ±20%, or 2,400 – 3600 particles/mL

When testing the AccuSizer AD and APS systems we recommend using the Micro Measurement Labs count and size standard. The pass/fail criteria for this standard appears on the C of A. A typical range of results for this standard is shown in Figure 2.

When using this standard on either the AccuSizer AD or APS, the pass/fail criteria would be the exact values shown on the C of A. For example the reported concentration for the 5 µm peak would be between 108,600 and 169,861 particles/mL as seen in the last row of the C of A shown in Figure 2.

| Std # | Standard mean | Set lower channel size | Set upper channel size | Concentration minimum count (per mL) | Concentration maximum count (per mL) |
|---|---|---|---|---|---|
| 1 | 0.8 µm | 0.60 µm | 0.96 µm | 952,526 | 1,489,848 |
| 2 | 2 µm | 1.59 µm | 2.41 µm | 367,149 | 574,258 |
| 3 | 5 µm | 4.03 µm | 5.82 µm | 108,600 | 169,861 |

*Figure 2. C of A for the Micro Measurement Labs particle count and size standard*

## CONCLUSIONS

The AccuSizer SPOS system is a calibrated technique requiring regular testing to keep the calibration current and valid. Once the particle size calibration has been updated and verified, some customers like to also test the count accuracy. Official standards and procedures exist only when testing specific count standards on the AccuSizer SIS system with no dilution. Unofficial count accuracy testing is acceptable, but the procedures and results are outside of the control of Entegris unless performed by our own personnel on known samples. We remain happy to support all customer efforts, but hopefully this document explains why it is impossible to officially review and comment on count accuracy tests on inappropriate samples following uncertain procedures.

### References


[1] Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions Light Obscuration Particle Count Test/ Instrument Standardization tests/Particle Counting Accuracy - System Suitability, https://www.uspnf.com/notices/1788-determination-of-particulate-matter

[2] http://www.usp.org/reference-standards/find-reference-standard

[3] http://www.mmlabs.com/particle-countsize-standards.php

[4] http://www.thermoscientific.com/en/product/pharm-trol-count-precision-size-standards.html


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