Hach Whitepaper

ISO 21501-4 Calibration of Air Particle Counters from a Metrology Perspective

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Abstract
The ISO 21501-4 calibration standard for Light Scattering Air Particle counters (LSAPC) is being increasingly adopted by pharmaceutical manufacturing companies. Calibration of APC instruments is a broad and complex subject of interest to a wide variety of stakeholders. For example production engineers, cleanroom validation technicians, micro-biologists and QA specialists. The informational needs of each of these groups vary considerably. Some will require to understand the 'regulatory and compliance' impact of the new standard whereas others have a more technical interest in the calibration itself. This paper is intended to serve the latter, essentially in a metrologists perspective.

Introduction

ISO 21501-4 is a relatively new calibration standard covering the performance and periodic calibration of light-scattering air particle counters (LSAPC or APC) throughout the instruments life. This international standard, which builds upon earlier regional standards and recommended practices characterized by a broader range of tests, deals comprehensively with statistical uncertainty and defines limits to a set of performance parameters which must be covered in routine periodic calibration. Conformance to ISO 21501-4 has ensured that APC instruments both size particles correctly to an acceptable uncertainty level as well as count particles accurately to a defined counting efficiency level.

Calibrating instruments to the new standard requires more sophisticated calibration systems in terms of equipment, methods and algorithms. This paper discusses the various elements of the standard, methods and required limits. A discussion on the combined uncertainty for the calibration is included in the appendix.

Historical Calibration Method and Standards

ISO 14644 is a cleanroom management standard, widely used for cleanroom classification and environmental monitoring. Despite the existence of ISO 14644, prior to 2007 there was no ISO standard addressing the calibration and performance of the particle counters (APC) used to classify cleanrooms to ISO 14644.

However, non-ISO standards and calibration methods guidelines did exist prior to ISO 21501-4 and were employed by most major manufacturers of optical particle counting instruments. The standards typically referred to at that time included:

ASTM F 328-98(2003) "Standard Practice for Calibration of an Airborne Particle Counter Using Monodisperse Spherical Particles" (withdrawn May 2007).

IEST-RP-CC014.1 "Calibration and Characterization of Optical Airborne Particle Counters" .

JIS B 9921:1997 - Japanese standard which comprehensively deals with APC design and performance, which is still widely regarded today.

While these standards were quite comprehensive in scope, they did not deal adequately with routine calibration and performance metrics throughout the life of the instrument. For example counting efficiency, a critical parameter, was not required to be re-assessed during periodic recalibration, particularly on-site calibrations.

The New ISO 21501-4 Calibration

To quote from the ISO 21501-4 standard:

"The purpose of this part of ISO 21501-4 is to provide a calibration procedure and verification method for particle counters, so as to minimize the inaccuracy in the measurement result by a counter, as well as the differences in the results measured by different instruments."

Simply put, the ISO 21501-4 standard ensures that APC instruments will size and count particles correctly, using a traceable reference instrument with the fundamental goal being that different APC models will closely correlate in terms of actual particle counts recorded.

Review of Particle Counting Technology

The particle detection technology employed within airborne particle counters used to classify cleanrooms is based on the phenomena of light scattering. When a light wave is incident on a particle it scatters radiation with the frequency of this radiation being the same as the frequency of the incident light. Fig. 1 depicts a typical optical particle sensor design comprising the primary elements. A sample flow path directs the sample air stream through the incident light source. An arrangement of lenses and/or mirrors collects the scattered light which is focused on to a detection device such as a photo-diode. The intersection of all 3 define the sensing zone. The scattered light intensity is a function of the size of the particle, the refractive index of the particle relative to the medium, the wavelength and intensity of incident light, and, the collection angle of the optics relative to the incident beam.

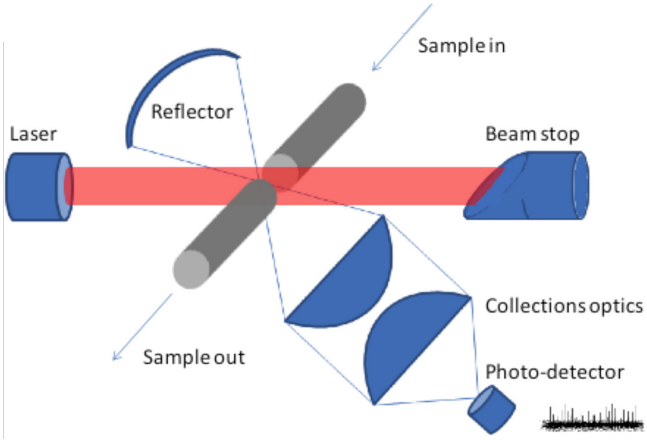

Fig. 1 Typical light scattering air particle sensor

The scattered light from each particle event on the photo-diode produces an electrical pulse. Fig. 2. Illustrates how these pulses are amplified and processed, typically using an analog to digital convertor (ADC) to determine pulse height.

Critical to APC counting and sizing is the relationship between pulse height and particle size and will be discussed later. Correctly detected and sized particles can be counted within predetermined size channels or bins. Totalized particle counts are typically presented to the user in terms of particle concentration to a pre-defined sample volume either via a local display or print-out, and/or alternatively transmitted over a network.

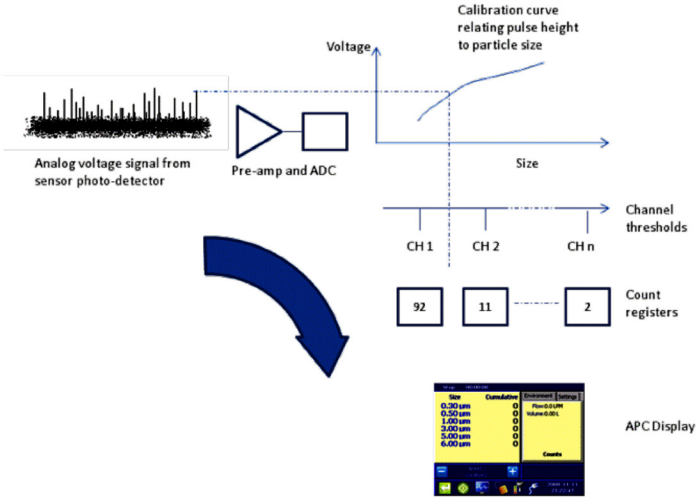

Fig. 2 Conversion of electrical pulses to particle counts.

Calibration

The calibration of APC instruments requires a number of specialized instruments and materials to conform to international standards. Most air particle counters used for cleanroom classification are calibrated using NIST traceable polymer microspheres or simply put; calibration spheres. Certain attributes of these calibration spheres are certified by the manufacturer, for example, the mean particle size of the spheres, the uncertainty of that mean as well as the standard size deviation, often expressed as c.v. (coefficient of variance), which is simply the standard deviation, expressed as a percentage of the mean, as shown in Fig. 3.

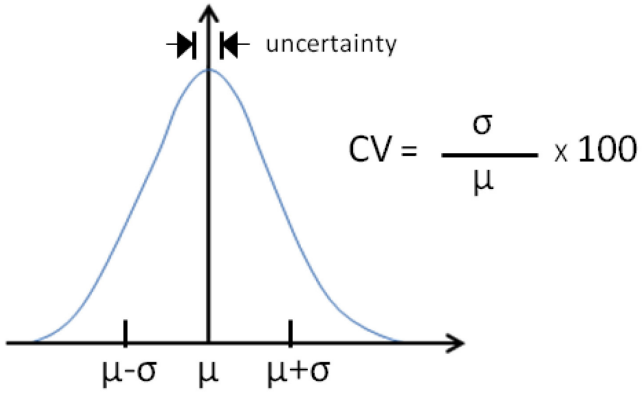

Fig. 3 Statistical parameters relevant to NIST standard particles

To calibrate an APC, a range of different standard calibration spheres are successively introduced at a controlled rate to the particle sensor, usually by means of an aerosol nebulizer whereby the standard particles (which are typically suspended in water) are atomized, dried and maintained as a controlled concentration in a clean air stream.

During each calibration sphere challenge, the distribution of pulse heights produced by the microspheres passing through the particle sensor are collected and displayed on a Pulse Height Analyzer (PHA). Fig. 4. depicts an example of a PHA output showing and APC sensors cumulated response to a mono-sized calibration sphere challenge with pulse height voltage on the horizontal scale and differential counts per bin on the vertical scale.

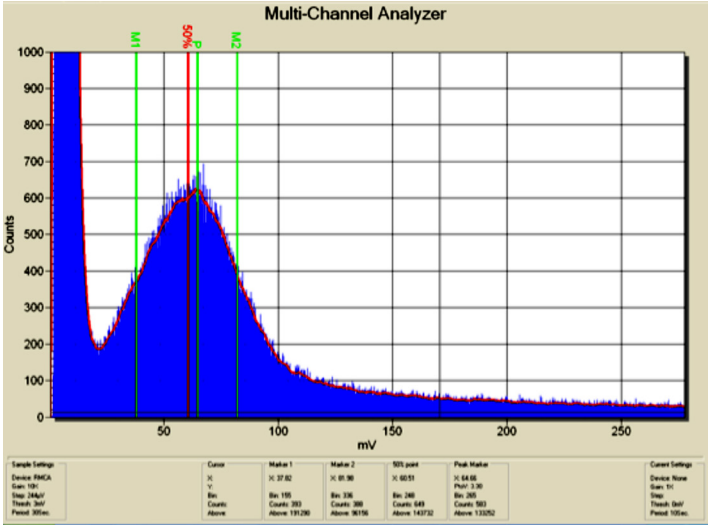

Fig. 4 Typical output form a PHA connected to an APC being challenged with calibration spheres

In an ideal world, all the pulses from particles of the same size would produce the same pulse height but in the real world they do not. It is an important point to note that the pulse heights produced by mono-sized calibration spheres are not all the same height for a number of reasons. In addition to the variance associated with the particles there are other variables involved, for example intensity variations across the illumination beam. This the reason the median of each calibration-sphere distribution is used to determine the calibration voltage points, which is essentially the voltage at which there are equal numbers of pulses above and below that voltage.

The ISO 21501-4 standard clearly defines how the median pulse height voltage of each distribution is established

By obtaining the relationship between pulse height voltage for each particle size within the dynamic range of the sensor, a calibration curve may be constructed. Fig. 5. depicts an example. A calibration curve is necessary as some minor interpolation is usually required, even though calibration spheres are generally available in sizes very close to typically measured particle size channels used to monitor or classify cleanrooms. Those familiar with the cleanroom management standard ISO 14644 will recognize the common particle sizes routinely measured. For example 0.3um, 0.5 µm, 1.0µm, 5.0 µm etc. If there is not an available size of calibration sphere for a specific size channel of interest, then a curve fit is used to interpolate the correct calibration voltage or ADC bin.

Individual manufacturers employ a variety of algorithms to fit the curve to the data points and to interpolate to desired channel size settings. Such interpolation does result in small but quantifiable errors in the channel size thresholds. ISO 21501-4 requires that the size setting error be calculated. This is an important parameter and is discussed further in the appendix dealing with calibration uncertainty.

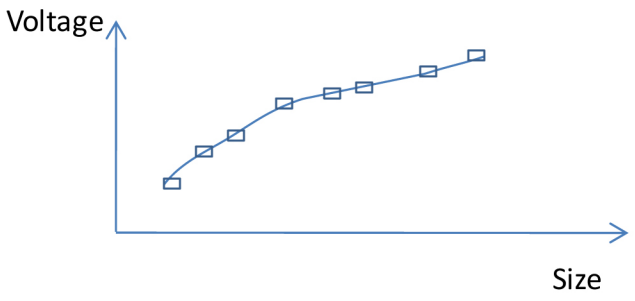

Fig. 5 Example APC calibration curve.

Particle Counting Instruments: Performance Metrics

ISO 21501-4 now mandates that in addition to the actual size calibration, certain performance parameters are measured and recorded on the calibration certificate. These include, counting efficiency, size resolution, false count rate and flow rate. These parameters are now discussed in more detail.

Counting Efficiency

We have described that when calibration spheres of a size equal to a particular APC size channel threshold are introduced to that APC, only 50% of them will be recorded as being greater than that size channel. It is here that the misconception often arises that the first channel of an APC only counts 50% of the "total" particles. It is quite erroneous to say that a particle sensor so calibrated,

when sampling real world distributions of particles, will only count 50% of the true value. In a real world sample, a particle passing through the sensor that produces a pulse exactly equal to the channel size threshold will have a 50% probability of being counted as being equal to or greater than that channel size as shown in Fig. 6 (a).

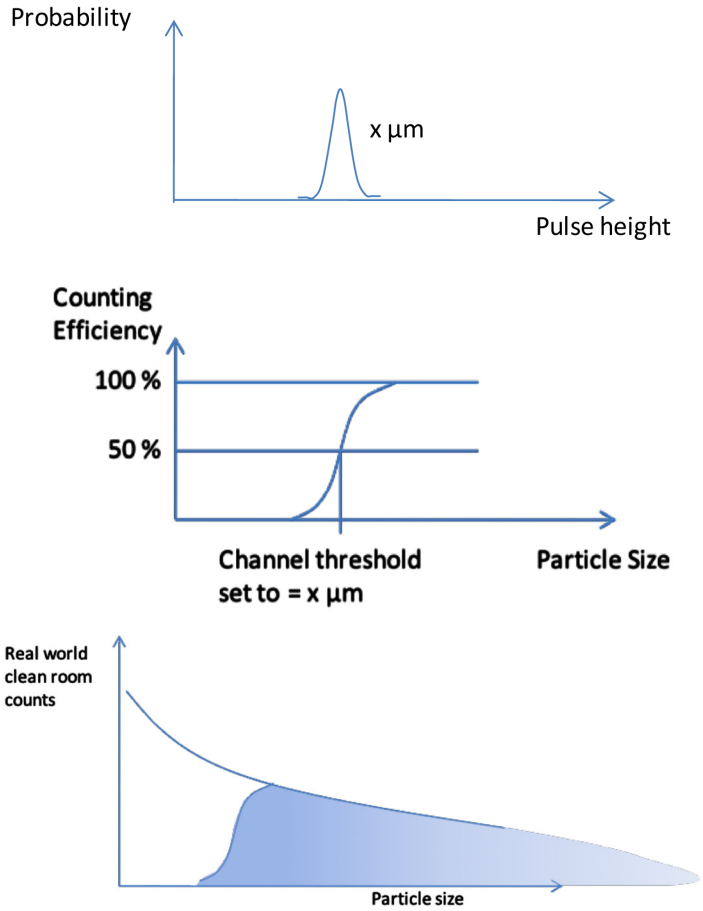

Fig. 6 Explanation of Counting Efficiency

(a) Particles exactly equal to any one size channel have a 50% probability of producing a pulse higher (or lower) than the channel size threshold.
(b) The same concept as (a) above presented in terms of "Counting Efficiency" versus "particle size".
(c) APC calibrated to the 50% counting efficiency point will count close to 100% of particles greater than the channel size threshold.

Referring to Fig. 6 (b), if an APC size channel is set to size x µm it can be seen that a particle of exactly x µm will have a 50% probability of being counted as being greater than x µm. It follows then that a particle slightly greater than x µm will have a greater than 50% probability of being counted. At some small increment beyond this there is a particle size that will have a 100% probability of being counted as being equal to or greater than x µm. Refer to Fig. 6 (c).

Returning to the ISO standard, in addition to the requirement that the first channel of an APC be calibrated at the 50% efficiency point, ISO 21501-4 also specifies at which point the first channel must reach 100% +/-10% counting efficiency. This is specified as being 1.5 to 2 times the channel 1 size.

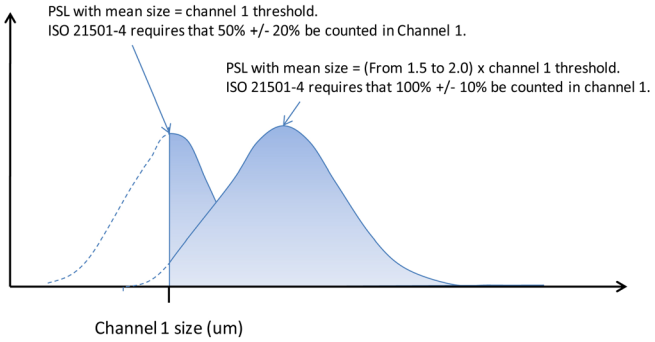

Fig.7 ISO 21501-4 specifications for channel 1 counting efficiency.

For example, consider an APC with channel 1 set to 0.5µm. To comply with ISO 21501-4, channel 1 must be 50% for 0.5µm particle and attain 100% +/-10% counting efficiency for particles with a size between 0.75µm and 1.0µm.

Verifying Counting Efficiency

In addition to ensuring that the APC size channels are calibrated to the 50% counting efficiency point, to comply with ISO 21501-4 it is necessary that after size calibration the APC is verified to count correctly by comparing it against a 100% counting efficiency reference APC. Refer to Figure 8.

Hach maintains high-sensitivity reference APC units with 100% counting efficiency where the size channel thresholds have been set to capture 100% of the CALIBRATION SPHERE challenge. These reference APC units are periodically verified as having 100% counting efficiency by comparison testing against a Condensation Nucleus Counter (CNC) with a sensitivity of about 0.01µm using particles generated from an Electrostatic Classifier. The theory and operation of these instruments is beyond the scope of this paper.

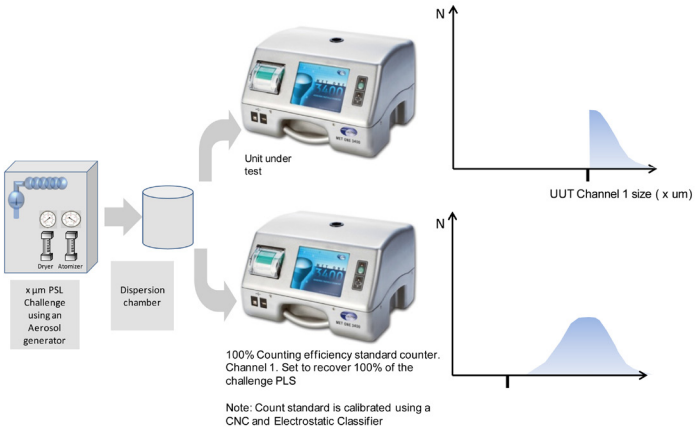

Fig. 8 Using a 100% counting standard to verify APC counting efficiency

Sizing Resolution

Sizing resolution refers to the ability of the optical particle sensor to differentiate between particles of similar size. Even if two identically sized particles were to pass through the sensing zone in close succession, they would be unlikely to produce electrical signal pulses of exactly the same height. Minor differences in particle velocity in addition non-uniformities in light intensity within the sensing zone and system noise will result in slightly different scattering energies and hence pulse height. Variance around the mean particle size is attributed not just to the variance of the CALIBRATION SPHERES but is also associated with the variance in pulse heights produced by the sensor in response to identically sized particles. To calculate the component of resolution contributed by the particle sensor, it is necessary to mathematically eliminate the contribution to total variance.

ISO 21501-4 requires that the size resolution of a particle counter be no greater than 15%

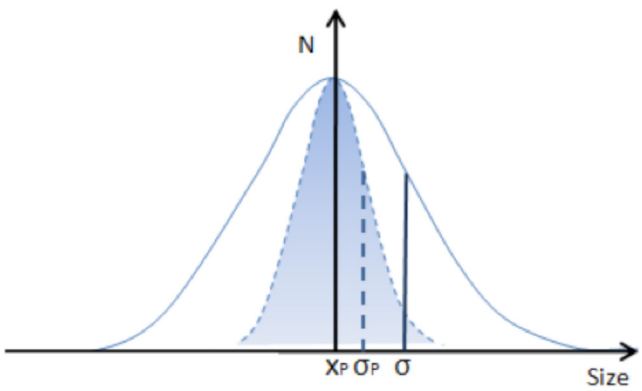

Fig. 9 APC sizing resolution

Size resolution is calculated as`

$$R\% = \frac{\sqrt{\sigma^2 - \sigma_p^2}}{\chi_\rho} \times 100\%$$

where

R is the sensor particle size resolution in %

σ is the observed standard deviation measure on the PHA

σP is the CALIBRATION SPHERE suppliers reported standard deviation

xP is the particle size of the calibration particles

False Count Rate

False count rate, sometimes referred to as zero-count rate, is the number of false counts recorded by the APC as a function of time when a known good filter is installed on the particle counter sample inlet. These “false” particle events are associated with a variety of sources intrinsic and extrinsic to the system, such as contamination, optoelectronic noise or cosmic radiation.

ISO 21501 does not specify an upper limit for APC false count rate but is does require that false count rate be measured and recorded on the periodic calibration certificate. The standard states that false count data should be statistically processed as a Poisson distribution using the 95% upper confidence limit. The false count rate is to be specified on the calibration certificate in terms of particles per cubic meter.

Sampling Flow Rate And Sample Time

Particle counts are typically reported by APC instruments as concentration per unit volume, for example particles per cubic foot or cubic meter. As a result, sample flow rate accuracy is important because the actual volume sampled for a fixed sample time will be impacted by flow rate errors. Sample time accuracy is also important for calculating the sampled volume at a give sample rate. Timing accuracy due to today's µ-processor based technology make it relatively easy to achieve +/- 1%, and accurate flow sensing technology can easily achieve flow rate accuracy to +/- 5%. The latter typically require that control systems built into the APC design. For example, the Hach MET ONE 3400 series APC employs altitude correction and volumetric measuring devices to provide real time control of sample flow rate to meet the ISO 21501-4 requirements.

Summary

ISO 21501-4 delivers an elevated level of confidence to the results obtained from APC instruments. Calibrating APC to this standard is particularly important when classifying cleanrooms to ISO 14644. Calibrating to comply with ISO 21501-4 is complex subject requiring more sophisticated equipment together with a rigorous understanding of the calibration process and associated parameters from a statistical perspective.

References

1. ISO 21501-2:2007(E), Determination of Particle Size Distribution – Single Particle Light Interaction Methods – Parts: Light Scattering Liquid-borne Particle Counter
2. ASTM F 328-98(2003) "Standard Practice for Calibration of an Airborne Particle Counter Using Monodisperse Spherical Particles" (withdrawn May 2007).
3. IEST-RP-CC014.1 "Calibration and Characterization of Optical Airborne Particle Counters" .
4. JIS B 9921:1997 - Japanese standard which comprehensively deals with APC design and performance, which is still widely regarded today.
5. ISO/IEC Guide 98-1:2009 Uncertainty of measurement.

Appendix

APC Calibration Uncertainty

Uncertainty, as defined in the ISO Guide to the Expression of Uncertainty in Measurement (GUM) is a..

"parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand."

For Airborne Particle Counters (APC), one of the more critical measurands, as defined in ISO 21501-4, is the particle counters' size setting accuracy, in other words the channel size threshold accuracy. The channel size accuracy will impact counting and sizing accuracy of APC.

Associated with the size setting accuracy is the size calibration uncertainty, which for APC instruments generally consists of several components. These components can be divided into two categories according to the methods used to evaluate them. These categories are known as Type A (random uncertainties) and Type B (systemic uncertainties) which are attributable to systematic effects, and can used to determine the size channel accuracy for a particular APC.

In analyzing Type B uncertainties associated with APC size calibration, we consider the measurement uncertainty associated with a variety of components such as the size of the polystyrene (PSL) particles, the pulse height analyzer (PHA), the voltmeter, the calibration curve and the flow meter. Example uncertainties values are provided in the Annex A of the ISO 21501-4 standard.

The following section examines the Type B uncertainties associated with the various components associated with APC calibration.

Calibration Spheres

An uncertainty of 2.5% is suggested by the ISO 21501-4 standard. Hach uses NIST traceable PSL particles, which are certified as having a size uncertainty of typically 1.2% or less for the size range between 0.1 micron and 10um, well within the scope of the ISO 21501-4 standard.

Pulse Height Analyzer (PHA)

The uncertainty of the PHA instruments used by Hach have been determined to have worst case accuracy of 2% with a standard uncertainty of 0.67% in the voltage domain. The offset voltage is ±2 mV, the corresponding standard uncertainty is ±0.67 mV. Converting to the particle size domain is specific to the APC in question. The Hach MET ONE Model 3423 APC for example translates this standard size uncertainty to 1.2%

Voltmeter

The uncertainty of voltmeters used by Hach is 0.1%. Again, applying the specific APC sensor calibration curve the standard size uncertainty attributable to the voltmeter as can be calculated. In the case of the Hach MET ONE Model 3423 this is 0.2%.

Flow Meter

Hach uses flow meters with a maximum 2.0% uncertainty. There is a relationship between particle induced signal pulse height and flow rate. For the Hach MET ONE 3400 series APC, we can establish the size standard uncertainty attributable to the flow meter as 1.2%.

Calibration Curve

Calibration curve uncertainty relates to the fit of the algorithm used to produce the calibration curve. Hach use a proprietary curve fitting algorithm for which the uncertainty is 1.4%.

Combining the above contributory factors, the combined uncertainty can be determined. All uncertainty components (standard deviations) are combined using the root-sum-squares to arrive at a ‘standard uncertainty which is the standard deviation of the reported value. For large degrees of freedom, it is recommended to use coverage factor k=2 to approximate 95% coverage (or called 95% confidence).

As shown in Table 1, the combined size uncertainty for APCs is 6.7% with a coverage factor of 2.

Size Uncertainty	Uncertainty
PSL Particles	**2.50%**
PHA	**1.20%**
Voltmeter	**0.20%**
Calibration Curve	**1.40%**
Flow Meter	**1.20%**
Standard Size Calibration Uncertainty (1 s.d.)	**3.30%**
Expanded (k=2) (2 s.d)	**6.70%**

Table 1 Combined Size Uncertainty of Hach MET ONE 3400 series <10% per ISO 21501-4

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