Understanding ISO 21501-4

ISO 21501 has the title *Determination of particle size distribution—Single particle light interaction methods*. Included with ISO 21501, there are four parts, of which, only Part 4 will be discussed in this paper:

- Part 1: Light scattering aerosol spectrometer (still undergoing preparation)
- Part 2: Light scattering liquid-borne particle counter
- Part 3: Light extinction liquid-borne particle counter
- Part 4: Light scattering airborne particle counter for clean spaces

The documentation and approval of ISO 21501-2, -3, and -4 subsequently replaces and cancels ISO 13323-1:2000. The ISO 21501 standard widens the scope of analysis to include methodology for airborne particle counting and liquid particle counting (both light scattering and extinction methods). Specifically, ISO 21501-4 provides a calibration procedure and verification method for airborne particle counters to minimize inaccurate measurements and reduce variations between different instruments. These new guidelines require pulse height analysis (PHA) for particle counter calibrations, which reduce inconsistencies.

Pulse Height Analyzer (PHA)

Particle counters employ solid-state photodetectors that convert detected light energy into electrical signals. The particle signals, known as pulses, leave the detector with proportional amplitudes of energy that represent the particles or photons witnessed by the detectors. If these pulses are sorted according to their height or magnitude, we could equate the pulses to energy received from the particle or photon. Electronic systems that analyze particle pulses and categorize their respective intensities are called *Pulse Height* Analyzers (PHAs).

Single channel PHAs count only pulses of certain amplitudes, which are equivalent to a particle or photon's "perceived" energy. Therefore, each pulse is placed into a counting bin, called a *channel*, and totaled with other similar amplitude pulses.

Adopting the New Standard

Cleanrooms that manufacture pharmaceutical products must follow aseptic manufacturing processes. Pharmaceutical manufacturers in the United States follow the guidelines set forth under current Good Manufacturing Process (cGMP), while pharmaceutical manufacturers within the European Union (EU), follow the EU's Good Manufacturing Practices (EU GMP) guideline.

The cGMP and EU GMP guidelines define limits for airborne particle counts, which are based upon specific cleanroom/clean space classification (e.g. Grade A, Grade B, Grade C, Grade D). However, the guidelines do not provide:

- The methods to determine the particle counts within a clean space
- The instrument to measure particle counts within a clean space
- The calibration methods for particle counters to assure data accuracy

Consequently, the EU GMP suggests pharmaceutical manufacturers follow ISO 14644-1 for determining particle counts and classifying cleanrooms (based upon airborne particle data) and ISO 14644-2 for instrumentation guidelines that demonstrate continued compliance. The new ISO 21501 provides calibration methods to assure data accuracy and meet the requirements for ISO 14644-1.

Introduction to ISO 21501-4

The ISO 21501-4 Introduction states:

> "*Monitoring particle contamination levels is required in various fields, e.g. in the electronic industry, in the pharmaceutical industry, in the manufacturing of precision machines and in medical operations. Particle counters are useful instruments for monitoring particle contamination in air. The purpose of this part of ISO 21501 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."*

The ISO 21501-4 Scope asserts:

> "*Instruments that conform to this part of ISO 21501 are used for the classification of air cleanliness in cleanrooms and associated controlled environments in accordance with ISO 14644-1*".

Consequently, cleanroom users should look to ISO 21501 as a method to meet cGMP, EU GMP, and other requirements.

Brief Overview of the ISO 21501-4 Test Method

- Calibration Particle: PSL particles traceable to an international standard with the standard uncertainty ≤ 2.5 %
- Sizing calibrated with a PHA @ median value for each size channel
- Pulse height distribution of PSL particle signals with noise must increase by 50 % from the minimum to the peak

Scope Defined Within ISO 21501-4

The ISO 21501-4 defines a particle size range from 0.1 – 10.0 µm. The particle counter is defined as an instrument used for classification of air cleanliness in cleanrooms and controlled environments in accordance with ISO 14644-1.

Specific parameters defined within ISO 21501-4:

- Size calibration:

    - When calibrating a light-scattering airborne particle counter (LSAPC) with calibration particles of a known size, the median voltage (or internal PHA channel) corresponds to the particle size. The median voltage (or internal PHA channel) should be determined by using a particle counter with variable voltage limit (or internal PHA channel) settings. The median voltage is the voltage (or internal PHA channel) that equally divides the total number of pulses counted. When a particle counter with variable voltage limit settings is not available, a PHA can be used in place of the counter.
    - Perhaps most important, if false counts or noise is present, the median voltage shall be determined by discarding the pulses due to "false particles". This is achieved by locating the peak due to real particles is more than double the density at the valley that separates if from the pulses due to "false particles".
- Verification of size setting:
    - The error in the particle sizes must be equal to, or less than, ± 10 %
- Counting efficiency:
    - 50 % (± 20 %) for particles close to the minimum detectable size
    - 100 % (± 10 %) for particles 1.5-2 times larger than the minimum detectable size
- Size resolution:
    - ≤ 15 % for calibration particles specified by the manufacturer
- False count rate:
    - When sampling clean air, the false count rate is a measure of particle concentration (counts per cubic meter) reported from the minimum size ranges.
- Maximum particle number concentration:
    - Specified by instrument manufacturer
    - Coincidence loss at this concentration must be ≤ 10 %
- Sampling flow rate:
    - The volumetric flow's uncertainty shall be ≤ 5 %
- Sampling time:
    - The sampling time duration's uncertainty must be ≤ 1 % of the preset values
    - This does not apply if the LSAPC does not have a sampling time control system
- Response rate:
    - The response rate must be ≤ 0.5 %
    - Determined by the test method in ISO 21501-4, section 4.9
- Calibration interval:
    - One year or less
- Test Report shall include:
    - Date of calibration
    - Particle sizes used for calibration
    - Flow rate
    - Size resolution (with the specific particle size used)

- Counting efficiency
- False count rate
- Voltage limit or channel of an internal PHA

21501-4 Objective and Specifications

The objective of ISO 21501-4 provides calibration and verification methods for airborne particle counters that minimizes inaccuracies in the particle counter's measurements and differences among other instruments.

- Sampling Flow Rate ≈ volumetric flow rate ± 5 %
- Counting Efficiency ≈ 50 % ± 20 %
  - @ particle size close to the 1st channel
- Counting Efficiency ≈ 100 % ± 10 %
  - @ particle size 1.5 to 2 times the 1st channel
- Particle Size Setting Error ≤ 10 %
- Instrument Resolution ≤ 15 %
  - @ the particle size specified by the counter manufacturer
- Zero count test ≈ ≤ 1 count / 5 minutes
- Maximum particle number concentration
  - specified by the manufacturer
- Coincidence loss ≤ 10 %
  - @ the maximum particle number concentration
- Sampling Time ≈ preset value (100 % ± 1 %)
- Response Rate ≤ 0.5 %
- Calibration Interval ≤ 1 year