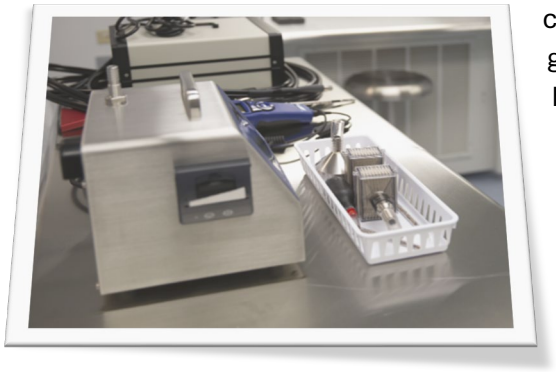
CriticalPoint
a trchealthcare brand

CriticalPoint Pearls of Knowledge — June 2025

The Blueprint to Certification: Total Particle Count Testing

Introduction

CriticalPoint continues its certification series with an in-depth look at individual cleanroom tests — what they are, how they're performed, and why they matter. In this month's issue, we focus on total particle count testing. According to USP <797>, engineering controls must function as designed to maintain airborne particles within acceptable limits during compounding.[1] However, USP chapters do not provide specific guidance on how particle count testing should be performed or how to determine acceptability — how can you be confident that your cleanroom and equipment meet these standards when reviewing test data?



We take a closer look at how engineering controls are designed to create low-particulate environments, why certifiers conduct particle count testing, and how to interpret the results when reviewing final reports as a pharmacy designated person (DP).

Particulate cleanliness is just a by-product

Engineering controls for cleanrooms and ISO Class 5 devices are designed to create low-particulate environments. This is achieved through carefully engineered airflow systems combined with HEPA filtration. HEPA filters, with their specific size, velocity, and filtration efficiency, allow forced airflow to pass through while capturing macro-particulates from the upstream, unfiltered "dirty" air. The air exiting the filter is clean and low in particulates.

The clean air is constantly circulated and mixed with return airflow inside the cleanroom or device. Over time, with enough air changes per hour, this process reduces the number of particles in the environment, creating a cleaner space. The environment becomes progressively cleaner, establishing the desired level of particulate control.

ISO Class	Particle Count/m^3
3	35.2
4	352
5	3520
6	35,200
7	352,000
8	3,520,000

Total particle count testing measures both the quantity and size of particles remaining in the environment. This testing evaluates the particulate cleanliness of the space and monitors the rate of particulate contamination introduced by people and activity during the test.

"True" dynamic conditions

Per USP <797>, total particle count testing must be conducted under dynamic operating conditions.[1] But what exactly does "dynamic conditions" mean, and why is it so important?

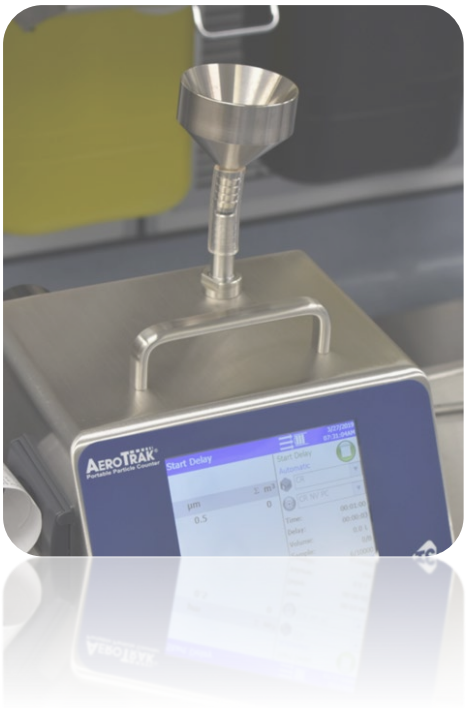
Dynamic conditions refer to a state where compounding operations are actively taking place and pharmacy staff are present in the cleanroom during the test. This activity can be either actual compounding or a simulated process, depending on the type of testing being performed.

For example, total particle count testing in a buffer room typically does not affect compounded sterile preparations within the ISO Class 5 environment. However, when testing occurs inside the ISO Class 5 environment itself, it's critical that simulated activity takes place, rather than mixing drugs for actual patients. The same principle applies to airflow smoke pattern testing within ISO Class 5 areas.

We want to avoid the scenario where, upon the certifier's arrival, pharmacy staff leave the cleanroom suite or step away from ISO Class 5 devices. This is not considered true dynamic testing. Why does this matter? Achieving acceptable particle counts under static conditions is relatively easy thanks to HEPA filtration and multiple air changes per hour maintaining ISO Class 5, 7, and 8 environments. The real challenge—and a true test of engineering controls—happens when material transfers, activity, and properly garbed personnel are present.

Testing under true dynamic conditions is essential because it challenges the engineering control design to maintain the assigned ISO classification. The results help identify any issues related to improper activities, behaviors, or garbing practices. Your typical dynamic conditions might also reveal if there are too many people present, exceeding the environment's capacity to maintain compliance.

The science of particle counters



Total particle count testing is one of the more complex and technical parts of the certification process. Fortunately, the math and science behind it are built into the particle counter's processor. Once the certifying technician determines the size of the space and how many samples are needed, the particle counter handles most of the work.

The particle counter uses a laser-based optical system to detect particles in the air. As air is drawn into the probe—typically, one cubic meter—it passes through tubing to the laser optics. When particles pass through the laser beam, they scatter light. This scattered light is detected by a photodetector, and the particle counter processes the data to report the number and size of particles based on its programmed settings.

Once a sample is complete, the device displays the results, showing the particle counts at various micron sizes for that specific sample location.

How many samples to take and where

Determining sampling locations is based on the area of the room or the interior square footage of the ISO Class 5 device. Certifiers measure the length and width of the space to calculate the total area in square meters. This value is then used to determine the minimum number of samples required in accordance with ISO 14644-1:2015,[2] a standard that defines air cleanliness classifications for cleanrooms and controlled environments. The larger the space, the more samples must be taken.

According to USP <797>, "sampling sites must be selected in all classified areas. Measurements of total airborne particles must be taken in each PEC at locations where there is greatest risk to the exposed CSPs … [and] should be taken at representative locations that reflect the quality of air in the

room(s).”[1] Applying a structured grid layout for sample locations ensures even distribution and uniformity of data across the space.

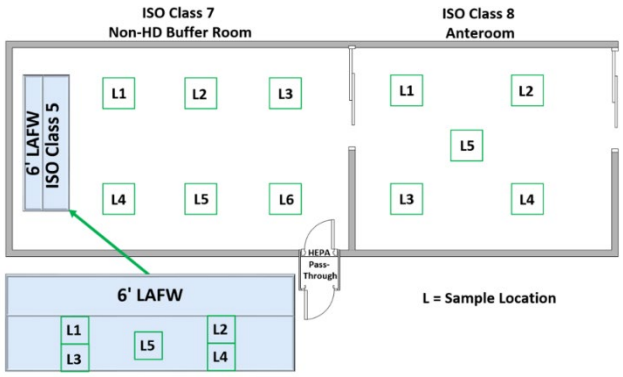
Table A.1 - Sampling locations related to cleanroom area	
Area of cleanroom (M^2) less than or equal to	Minimum number of sampling locations to be tested (N_L)
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10
56	11
64	12
68	13
72	14

From ISO 14644-1:2015, Table A.1

Sampling in ISO Class 5 environments is more nuanced. The particle counter’s probe must be oriented to detect downstream particles generated during simulated compounding activity. Proper placement is within six inches of the HEPA filter and upstream of the direct compounding area (DCA), where the greatest contamination risk exists.

Because ISO 5 devices are smaller, the total area may fall below the minimum sample threshold requiring only one sample point according to ISO 14644-1.[2] However, this may not provide an accurate representation of the environment’s cleanliness during active compounding. CAG-003 recommends three or more sample locations, especially when multiple compounding sites or connection points are present.[3]

USP also requires that all sampling sites and procedures be clearly described in the facility’s standard operating procedures (SOPs).[1] CriticalPoint recommends that pharmacies collaborate with their certifiers to determine the specific details and methodology for this specialized test. Documentation should include a general map of sampling locations along with a basic rationale for each site's selection. There’s no need to overcomplicate the pharmacy documentation—just focus on clearly recording the what, when, where, and why of the sampling process.



Sample procedure: total particle count testing

Particle count testing is typically performed first during certification to reduce the risk of inadvertent contamination or false negatives. Certification is not inherently a “clean” process—movement during testing (such as opening doors, taking airflow readings, or performing smoke studies) can generate a higher number of particles than normal.

To ensure accurate results, nonviable and viable particle testing should be completed before other disruptive certification tasks. This helps capture the most accurate particulate recovery rates under conditions that closely reflect actual use.

General testing procedure:

1. Determine the sampling plan based on organizational policy and the manufacturer’s instructions.
2. Perform zero-count calibration (zero filter check) to clear the channels and ensure accurate readings.
3. Sample according to the approved plan, ensuring even spacing and adequate area coverage as shown in the sampling diagram.
4. Orient the probe vertically.
5. Sample at “working height” (typical compounding height within the PEC or cleanroom).

6. Avoid sampling directly beneath HEPA filter diffusers, as this may give artificially low particle counts.

Post-sampling follow-up:

1. Verify that all results meet the design criteria for the room or device under dynamic conditions.
2. Retesting is acceptable if a sample location exceeds its assigned ISO classification. Consider retesting if:
   - There is excessive personnel movement or improper behavior.
   - Equipment generates unexpected levels of particulates.
   - The location is known to have high-activity or high-risk workflow zones.
3. Ensure all planned samples are taken and properly documented according to the sampling plan.

Reviewing certification documentation

The designated person is responsible for overseeing the performance and operation of the facility, particularly in areas where compounded sterile preparations (CSPs) are made. This includes ensuring that classified spaces meet the required air quality standards.

USP <797> specifically assigns the responsibility of reviewing all certification records to the designated person.[1] This review should go beyond simply confirming that areas "passed." Instead, it should involve understanding why an environment passed or failed by closely examining the data values in the certification documentation.

Environment Area (m^2)	Occupancy State	ISO Classification Limit (m^3)	Results
1.67	Dynamic/operational	3,520	PASS - ISO Class 5
Location 3	Location 4	Location 5	Location 6
12	7	63	N/A
11.16	Dynamic/operational	352,000	PASS - ISO Class 7
Location 3	Location 4	Location 5	Location 6
1,247	201	577	632
9.30	Dynamic/operational	3,520,000	PASS – ISO Class 8
Location 3	Location 4	Location 5	Location 6
1,567	5,841	4,471	N/A

Look for trends. Is the environment consistently meeting ISO classification standards? Are particle counts remaining stable or trending upward over time? Historical comparisons between certification reports can help identify whether performance is improving or declining.

While particle counts are a key indicator of cleanroom performance during certification, they are not the only measure. Ongoing viable environmental monitoring provides critical evidence that the room is maintaining a microbial state of control over time.

Some key documentation elements to look for during your review are outlined in CAG-003: Certification of Sterile Compounding Facilities for USP Compliance. The guidance recommends several comprehensive reporting and documentation components, including the following:[3]

- name and address of the testing organization and the date on which the test was performed
- current number and year of publication of the air cleanliness standard of ISO 14644-1:2015
- clear identification of the physical location of the cleanroom or clean zone
- testing (including reference to adjacent areas if necessary) and specific designations for coordinates of all sampling locations (a diagrammatic representation can be helpful)
- specified designation criteria for the cleanroom or clean zone, including the ISO class number, the relevant occupancy state(s), and the considered particle size(s)

- details of the test method used, with any special conditions relating to the test or departures from the test method, and identification of the test instrument and its current calibration certificate
- test results, including particle concentration data for all sampling locations
- report acceptance criteria
- provide a Pass or Fail statement based on the results of the test

The takeaway

Certification of facilities and equipment must be performed consistently, using methods that are both repeatable and clearly understood. Particle count testing is one such test. It provides a snapshot of the cleanroom's particulate levels and verifies that the environment meets the required classification at the time of testing under dynamic conditions.

However, particle count results reflect only that moment when testing was conducted and cannot guarantee air cleanliness between certification intervals. This is why ongoing viable air and surface monitoring, beyond the USP <797> minimum frequency, is essential. Routine environmental monitoring helps confirm that the cleanroom suite remains in a state of microbial control on a daily basis.

As part of the designated person's responsibilities, it is crucial to understand the fundamentals of both certification testing and environmental monitoring. A strong grasp of this data empowers you to recognize when a cleanroom may be trending out of control, identify potential root causes (e.g., increased personnel activity or equipment issues), and take corrective actions to reestablish engineering control and maintain a compliant environment under true dynamic conditions.

References

[1]United States Pharmacopeia USP <797> Pharmaceutical Compounding—Sterile Preparation. 2024

[2]ISO 14644-1:2015 Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness by particle concentration. Ed. 2, 2015.

[3]Controlled Environment Testing Association. CAG-003: Certification Guide for Sterile Compounding Facilities for USP Compliance. Revised 2022.