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How Many Particles, How Much Sample Volume?

How many particles are needed to demonstrate statistical confidence for cleanroom control and how much sample volume is required to see them?

There is increasing confusion over the requirements for compliance with EC GMP Annex 1 limits on 5.0 µm particles, especially when compared with the ISO 14644-1 calculations recommended for establishing conditions for such testing.

The problem arises when using the ISO 14644-1 calculations. The minimum sampling volume required by this formula for EC GMP compliance is huge.

$$Vs = \frac{20}{C_{n.m}} \times 1000$$

If we look at a Class A (ISO 5) environment and the particle size 0.5 µm, where $C_{n.m}$ is 3520 nm^{-3}, the equation becomes:

$$Vs = \frac{20}{3520} \times 1000 = 5.682 \text{ liters}$$

Figure 1. 1 CFM IsoAir Particle Counter

Using a 1 cubic foot per minute (CFM) instrument, this volume would take approximately 12 seconds to sample. A caveat in the rules requires that the sampling duration is a minimum of 1 minute, so that a full 28.3 liters would be drawn (28.3 l = 1 CFM).

However, if we look at the 5.0 µm requirement for the same Class A environment we are now faced with a limit of 1 nm^{-3} and the equation now becomes:

$$Vs = \frac{20}{20} \times 1000 = 1{,}000 \text{ liters}$$

This would now require a sample at each location of 1 m^3 and, if one were using a 1 CFM particle counter, the sample would take approximately 36 minutes. Prior to 2009, the value for the limit of 5.0 µm had been 1, as the cleanrooms should be essentially free from all large (macro) particles. This gave a 20,000 liter result that would have been over 11 hours for sample duration at each location. So a change was required and hence the new 2009 values.

The question is how to gain statistical confidence while maintaining a reasonable sample period.

History of Application

The history of this application dates back to the Federal Standard on cleanroom cleanliness, FS 209. When reviewing the original standard the minimum number of particles allowed in a sample was deemed to be 20. This number determined whether a sample was statistically significant for class limits of the 'U' descriptor. If a sample did not yield sufficient particles, a sequential sample technique could be employed. Sequential sampling is a technique that allows the total required volume to be divided into equal parts and essentially the proportion of required particles equally divided throughout each portion. Therefore, a large volume did not have to be sampled if the sub-sample showed sufficient evidence of cleanliness.

The statistical confidence of a population of these random particles was determined to have a minimum cutoff before special calculations were required to prove that lower numbers showed any statistical validity. When the revised ISO 14644-1 was released, it also contained this function of statistical confidence and it required a minimum of 20 counts per unit volume.

There is evidence that for certification purposes, either FS 209E or ISO 14644-1, a minimum number of particles is required. As particle counters have a fixed flow rate (1 CFM, 0.1 CFM, 50 LPM, or 100 LPM), so too is a fixed sample period determined by this calculation.

Figure 2. Lasair III Particle Counter available in 1 CFM, 50 LPM, or 100 LPM

Solutions for Certification

Two routes are available when looking at room certification. Either one can adopt the recommended minimum sample volume written in the EC GMP Annex 1, or one can use the sequential sampling technique identified in both the FS 209E and ISO 14644-1 standards.

1. Minimum Sample Volume from EC GMP Annex 1 September 2003 in the notes section immediately below the EC GMP classification table, Note '**a**', states:

   For routine testing the total sample volume should not be less than 1 m^3 for grade A and B areas and preferably also in grade C areas.

   Therefore, if "routine testing" (i.e., periodic room certification) is being performed, the sample volume needs to be 1 m^3 and not the calculated 20 m^3 if using ISO alone.

   The 2009 version clarified this by stating that the maximum permitted number of particles allowed is 20. So now the formula works to give a 1 m^3 sample volume and also by its inclusion of its opening for cleanroom classification, Clause **4**:

   4. Clean rooms and clean air devices should be classified in accordance with EN ISO 14644-1. Classification should be clearly differentiated from operational process environmental monitoring.

2. Sequential Sampling

   In sequential sampling the running total of the particles counted is compared with an expected count limit that is a function of the amount of sampling done. Sequential sampling typically requires less sampling than any single sampling plan having the same probability of false acceptance and false rejection.

   Figure 3 shows the boundaries of the sequential sampling plan that has been designed for use in this standard. The observed number of counts, *C*, is plotted against the expected number of counts, *E*, for air, which is precisely at the class limit. A full single sample corresponds to *E* = 20.

   Therefore, if the expected particle counts should be 10 and only 5 have been measured, the sampling can stop, the location declared a pass, and sampling can be started at the next location.

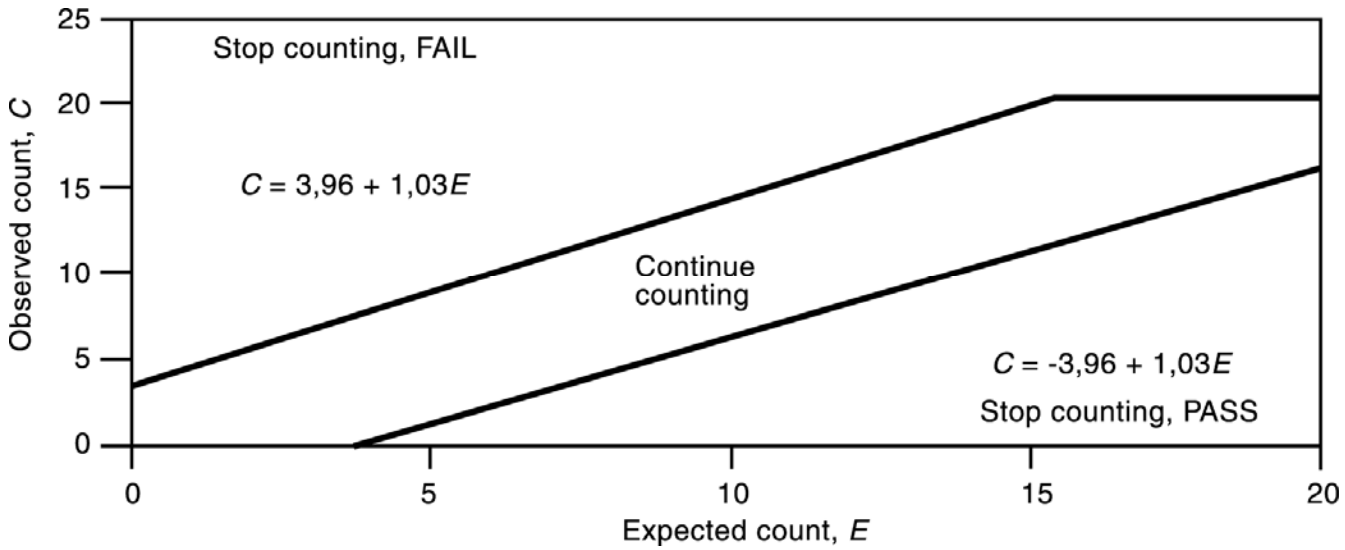

Figure 3. Sequential Sampling Boundaries

Solutions for Monitoring

In accordance with GMP regulations, clean rooms need to be routinely monitored in operation. The monitoring locations must be based on formal risk analysis obtained during the initial classification of the room. For Grade A zones a continuous or frequent sampling particle monitoring system should be used. The Grade A zone should be monitored at such a frequency that all interventions and other transient events are captured and alarms triggered if excursions from defined operating norms occur.

The sample volume at each monitoring location should also be based upon a risk assessment, as excessive data does not always tell you if a process is under control. Only suitable data will demonstrate that.

So what sample volume is required?

> *12. The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of clean rooms and clean air devices.*

Sample periods of 1 minute are normal and can be as short as 10 seconds for critical areas associated with high volume production. This will give much smaller sample volumes. However, statistical confidence over the process is now gained due to an increase in the number of individual samples taken.

In Grade A and B zones, the monitoring of the 5.0 µm particle concentration count is significant, as it is an important indicator of failure of sterility. An occasional indication of a single 5.0 µm particle count is typically a random count and has no foundation with an associated problem. However, consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated. So the limits attributable to 5.0 µm monitoring are based upon a frequency of event and not necessarily on a magnitude defined by an industrial standard.

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