TECH TALK

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ISO Technical Report 14644-21 Published to Support ISO 14644 Parts 1 and 2

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ISO/TC 209 released its first Technical Report in 2023 to help clarify information regarding airborne particle counting provided in Parts 1 and 2 of ISO 14644. It also intends to dispel observed issues of misuse and misunderstanding of key sections of these standards with regard to airborne particle sampling.

Keywords
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Introduction

A new document joined the ISO 14644 series of cleanroom standards in August 2023. Number 21 in the series, this Technical Report (reference ISO TR 14644-21) is a different type of document from the cleanroom standards already published. Prepared by Working Group 15 (WG 15) of ISO Technical Committee 209 (ISO/TC 209) for cleanrooms, the Technical Report (TR) format was chosen to provide an easy and timely access to established consensus on airborne particle sampling techniques, in order to remedy perceived difficulties in understanding our current published standards.

It is important to stress that the TR format is by nature strictly descriptive and informative. A TR is not a standard, nor is it a manual, guide, or new scientific paper. ISO defines the role of a TR as follows:

- Contains information: e.g. data obtained from a survey, or an informative report, or information on the perceived "state of the art."
- Can provide a rationale for specific requirements in a related International Standard.

Further information on the types of ISO document, their nature and scope, can be found on https://www.iso.org/deliverables-all.html

Purpose and Intent of ISO TR 14644-21

In the present case, ISO TR 14644-21 supports the normative requirements and explains information regarding airborne particle counting provided in Parts 1 and 2 of ISO 14644. Aiming to promote better understanding of the intent and precise content of these standards, in the context of the scientific consensus on which they are founded, the document addresses observed issues of misuse and misunderstanding of key sections of ISO 14644 Part 1, the ISO

standard on cleanroom classification, and ISO 14644 Part 2, on cleanroom monitoring. These issues were apparent in some of the requirements for airborne particle sampling included in enquiry versions preparing the revision of EU and PIC/S GMP Annex 1, the regulation for production of Sterile Medicinal Products, and also more broadly, in requirements and observations of bodies involved in the accreditation of particle counting and measurement services. Clearly, they expressed concern over the large-particle losses inevitably encountered during airborne particle sampling, but in a confused manner, while apparently referring to sections of ISO 14644-1 that were not intended to be used in that way.

The initial ISO ad hoc group, and the subsequent Working Group 15 that produced the TR, brought together a broad range of skills and experience worldwide, with experts contributing from particle counter manufacturers, cleanroom testing and certification organisations, design and construction experts, and users involved in applications from sterile pharmacy to flat screen facility contractors.

The most urgent issue was to do with demands emerging in several fields, as mentioned above, that particle sampling tubes used together with light scattering airborne samplers (LSAPC) be limited to a maximum length of 1 metre. Tubes are used to connect a sampling probe located at a distance to an LSAPC. The demands were citing specifically Clause C.4.1.2 in *informative* annex C of ISO 14644-1:2015, out of the context intended by the ISO working group that drafted current Part 1. Beyond the misuse of an informative clause to found a requirement, such demands ignored the pure Macroparticle (equivalent optical diameter >5,0 µm) focus of informative Annex C, which is not in fact intended for classification purposes, but describes various means to quantify concentrations of large particles, outside the classification process. Within Annex C, only the mention of the M descriptor in Clause C.7 is linked to measuring and recording concentrations of large particles *alongside* true classification.

The rest of informative Annex C is devoted to measurement of large particles alone, by a variety of methods. And where Clause C.4.1.2 mentions use of an LSAPC, it is clearly stated that the instrument involved has a specific configuration destined solely for counting large particles: “Procedures for macroparticle measurement using an LSAPC are the same as those in Annex A for airborne particle count with one exception. The exception is that the LSAPC in this case does not require sensitivity for detection of particles less than 1 µm since data are required only for macroparticle counting.” We remind users that the method for classification as such is described in normative Annex A of ISO 14644-1:2015, and this only concerns the particle sizes and concentrations listed in Table 1, or Table E.1, of the 14644 Part 1 standard, by use of a suitable LSAPC.

Large particles can easily be lost in airborne sampling, and the loss can especially have significant impact where low concentrations are considered. The use by Clause C.7 of the M descriptor for large particle sizes, outside the sizes and concentrations defined in Table 1, allows expression of the uncertainty associated with sampling these large particles at the same time, and with the same instrument used for classification, where special requirements for such particles exist in use alongside classification. An example of this use is in sampling to classify

rooms or volumes to establish control of particle concentrations at both ≥0.5 µm and ≥5.0 µm sizes in life science and healthcare applications, such as EU GMP Annex 1, but many others exist.

It must be remembered that ISO/TC 209 work is not application-specific, and exists to cover many different uses of cleanrooms in varied fields of application. This does hold us to a certain concision in our standards. Informative annexes are used in standards to some extent to provide fuller information on application, but these do not constitute requirements. The TC considered that evidence of misuse of such information in field applications justifies fuller explanation of how to use ISO standard 14644 Parts 1 and 2, without altering the standards themselves.

Fundamentally, as mentioned above, ISO 14644-1 does not provide for classification of particle populations that are outside the specified lower threshold particle-size range, 0,1 µm to 5 µm, nor in concentrations deemed unreliable for classification. The basis of classification to comply with ISO 14644-1:2015 is in simple terms: classification has to do with the statistical prediction, based on sampling, that there is at least a 95 % level of confidence that at least 90 % of the cleanroom or clean zone locations possible will comply with the maximum particle concentration limit for the target class of air cleanliness. According to ISO 14644-1: 2015 Annex C, an M descriptor can be used to quantify populations of macroparticles (particles larger than 5 µm), but the associated measurement uncertainty, especially when low concentrations are considered, makes such quantification unsuitable for classification.

In monitoring, as indicated in ISO 14644-2, an LSAPC can also be used for airborne particle counting, but the counts obtained are often accompanied/supported or indeed replaced by other methods of particle counting and analysis, and the results are frequently associated with measurement of other parameters that attest the state of balance of the installation or device. The nature of monitoring is quite different from classification, having to do with measuring and analysing the change of state of conditions at specified (critical) locations over time, and really throughout the phases of a process or activity cycle, with regard to the validity of such conditions for the integrity of the activity considered, at the specified location. Following classification or quantification, there is no attempt to extrapolate results obtained at each location to a class prediction. Each location is considered separately and not in relation to a prediction of compliance (class) of a whole area.

The Introduction to the Technical Report outlines the basic concepts right away, presenting "the importance of understanding that

- For classification, the quality of the sample is the most important factor
- For monitoring, the quality of the data is the most important factor
- Direct sampling without tubing is preferred. However, sample tubing is sometimes necessary to get a representative sample at a significant or critical location
- To reduce sampling loss in tubing, this tubing is as short and straight as possible
- A sampling system is evaluated to assess the impact of any compromises in its set up."

And adds: "an evaluation of legacy systems can deem them suitable for continued use even if the installation is assessed as less than optimal." There may be no more precise configuration

that can be implemented, or the risk of losing continuity of monitoring data may outweigh the potential to obtain more precise particle counts.

Determination by the Ad-hoc Group

The ad hoc group of experts tasked to evaluate appropriate response identified that it would be advisable to broaden our subject beyond the immediate 'tube length' issue, to better address misapprehensions on the content of the standards and how they can be applied. It was deemed useful to remind users of the broader scientific consensus and practical experience regarding airborne particle sampling for classification and monitoring, on which the two central ISO 14644 standards have been built. This goes beyond the briefer content of ISO 14644 parts 1 and 2, and especially aims to provide users with sound information on how to evaluate their existing or intended sampling configurations and practices, and justify adoption of reasoned compromises, in situations where definition of an acceptable level of (larger) particle loss in the sampling system is of concern. Hence, the main burden of the TR is to inform users on how to make informed choices within the practical limits of measurement. The intent is primarily to help the field technician and his 'client' or manager appreciate and assess the challenges of an existing or planned airborne particle sampling system, step by step, enabling them to make informed choices that can be clearly justified with reference to sound knowledge and practice.

TR 21 addresses the issues involved in this process in the following logical and didactic sequence:

Chapter 4	Determination of airborne particle concentration: 1. General 2. Classification 3. Monitoring 4. Other LSAPC applications (i.e., that are not considered in this TR)
Chapter 5	Sampling airborne particles – things to consider 1. General 2. Instrument selection 2.1. Considered particle size selection 2.2. Required sample volume and flow rate 3. State of occupancy 4. Sample locations – points to consider 4.1. Sampling locations for classification 4.2. Sampling locations for monitoring 5. Instrument measurement issues 5.1. Sampling errors 5.2. Sample measurement errors 5.3. Sample tubing issues 6. Decision tree 7. Examples of use of the decision tree
Chapter 6	Verifying a system
Bibliography	

Chapter 4 sets out the fundamental concepts involved. In Clause 4.1, Figure 1 illustrates the relation between classification and monitoring, with regard to the Plan Do Check Act cycle of continuous improvement of quality.

We remind readers that: "All particle counting systems have the potential to delay or prevent a particle from reaching the counting mechanism of the LSAPC. The likelihood of this increases with the particle's size and the length and complexity of its pathway through the system. Good practice is applied to minimise particle loss and potential gain through shedding and false counts …" Note the wording is descriptive of good practice: the TR cannot issue guidance nor present new material.

Following the reminder of the fundamentals in Chapter 4, Chapter 5—things to consider—examines the practical challenges to be faced.

Clause 5.1 General begins: "Sampling airborne particle concentration at a specific location, to determine air cleanliness, cannot always be performed by placing the LSAPC directly at the location, due to limitations on access, instrument size and the need to avoid disturbance of a critical volume by the counter exhaust and heat gain. Consequently, a sample will often need to be drawn from the test location to the instrument for measurement." This is a practical necessity all users are familiar with, and one of the reasons why a flat limitation on tubing length is considered inappropriate by ISO/TC 209 and WG 15, in the context of counting particles simultaneously within and outside the tables defining sizes and concentrations suitable for classification.

In good practice, the approach taken to respond to this challenge ensures that:

- the sample is representative;
- a suitable volume is taken, relative to the considered particle size(s);[1]
- collecting the sample does not affect the operation of the process;
- the particles sampled do reach the device; and
- the location of the LSAPC is not influenced by other factors.

Clause 5.2 considers the range of instrument types available, and their suitability for different applications, summarised in a table. Selection of target particle sizes, sample flowrate, and sampling duration are discussed in this perspective. Most of sections 5.2 to 5.4 is a fuller reminder and explanation of what the standards say already.

In Clause 5.4.1 of the TR, we remind users that it can be necessary to position the sampling probe at a number of different locations, and potentially at different heights. Also, that as some particle loss always exists in airborne sampling, it can be prudent to use no tubing at all, so that

[1] Note that the need to perform either classification or monitoring will typically determine the type of instrument and the flow rate chosen, as high sample-flowrate instruments allow for a specific sample size to be taken in a shorter amount of time. Shorter sample duration time is beneficial for routine classification and facility monitoring in situations where individual samples are taken throughout an area and the instrument is moved between samples. Sample time is balanced against the statistical accuracy of the measurement, and the potential for monitoring a sample location's change of state over time – this implies a need to determine what constitutes a significant value, or variation of the value, obtained at a given location.

the sample is drawn directly from the sample probe into the LSAPC. However, there are many situations where this is not practical (cf 5.1). In such situations, it will be necessary to use a tube to connect the sampling probe positioned at the appropriate location to a counter placed at a distance. The TR here quotes Normative Clause A.5.1 of ISO 14644-1 and demonstrates that there is no specified requirement on limiting tubing length in the standard. The single appropriate recommendation in the standard, is to respect the manufacturer's specifications where possible. A number of considerations are mentioned, which are used reliably to mitigate loss and provide access with the least disturbance of the volume sampled, both for classification and monitoring.

The other factors influencing sampling with the LSAPC are discussed in the rest of this chapter, under Clause 5.5, instrument measurement issues. This section gives a broad overview of potential errors to be dealt with in particle counting. The various potential risks are presented under three headings:

a) sampling errors (5.5.2);

b) sample measurement errors (5.5.3); and

c) sample transportation errors (5.5.4).

Discussion of sample inlet design (usually isokinetic sample probe) and a reminder of the rules governing isokinetic sampling allows us to debunk another misconception. Most of us are familiar with the much repeated but rarely challenged airflow velocity value of 0,45m/s supposedly defining unidirectional airflow, and habitually associated with a tolerance of ±20%. This value has been apparently reinstated in the new EU Annex 1, whereas the previous version wisely acknowledged that inside confined volumes, there are sound reasons to select lower airflow velocities, precisely to maintain the desired flowline characteristics.

Besides the positioning and alignment issues, this clause reminds users that standard isokinetic sampling probes (ISP) supplied by counter manufacturers are designed to provide isokinetic sampling precisely at the default UDAF velocity reference value of 0,45m/s at the specific sampling rate of the counter. A number of experts in our working group expressed concern over the real-life issue of airflow velocity decay inside isolators, compounded by the variety of 'working heights' or critical locations that it may be necessary to sample because of process concerns. Some reassurance is available if one consults the well-established notion of 5% sampling error tolerance published from experiments by Belyaev and Levin in 1974. This tolerance value is robust across a range of airflow velocities and instrument flowrates, and allows an amount of leeway in use of standard probes and tubing section. This widely accepted tolerance, taken up in the previous US Federal Standard 209, implies for instance that a 28,3l/min flowrate counter using a standard probe and tube section would be within the range of acceptable sampling (±5% sampling error) for airflow velocities between 0,22m/s and 0,54 m/s, allowing adaptation to a number of frequently-encountered situations in low-velocity airflow installations and especially barrier technology applications.

Sample tubing issues such as tubing length, but also bends and material/surface considerations, are discussed in Clause 5.5.3. With the focus on larger particle loss that was

our initial brief, these issues are discussed in some length, with of course the reminder that sedimented particles may also shutter and provoke false positives. In general, the longer the tube, the greater the fallout, but the TR does also remind users that secondary forces such as electrostatic attraction, Brownian motion, diffusion and thermophoretic forces will act upon far smaller particles, that are also lost in transport.

Consideration of the effect of bends calls for closer assessment of commonly-touted values such as the 150 mm minimum radius mentioned in ASTM F50 for 28,3l/min counters, and a close look at the practicalities of routing tubing within a confined volume. Referenced studies do show that in these cases, the close succession of bends may be as impacting as the bend radius and section. The TR also reminds users that the careful selection of connectors and valves (e.g., in transit between inside and outside an isolator) is essential to avoid losing particles that the tube has conveyed adequately to that fitting.

These topics lead on to a demonstration of practical consideration of the key factors in a decision tree flowchart. A graphic overview, and illustration of the typical challenges to be assessed and corrected, if possible, are given as a quick reference, especially useful for the field technician called upon to assess the feasibility of obtaining an acceptable sample through an existing or projected configuration. Questions to be answered yes or no in the step-by-step critical path can include:

- Can the instrument inlet be positioned to face the airflow, at the correct sampling height, without tubing?
- If not, and tubing has to be used, are >5,0 µm particles of interest?

Various questions concern the length of tubing required, and the number of bends imposed by the sampling location. Other considerations of tube material, vibrations, earthing and fixing will also be assessed as required. In the easy path, no significant action is required, and the user can justify the robust nature of the configuration.

Mitigation and adaptation are suggested for some more-challenging cases. The worst-case situation resulting from the initial assessment involves tube length ≤2 m and <3 bends, the conclusion being that additional assessment will be required. Such assessment goes beyond the easy adjustments set out here.

Conclusion

Potential advanced requirements to mitigate problems identified may involve design of a less compromised installation, a hard look at existing data to assess its value despite identified losses, for example in monitoring by highlighting the system's ability to capture condition changes et critical locations. If the system requires radical change, then Clause 5.7.3.2 suggests starting points for redesign. An interesting point is the question of balancing theoretical calculation and prediction against actual measurement on a test bed simulation, to ensure no significant factors have been overlooked. We hope this approach will prove useful to prospective users, to debug and justify investment in better-performing, robust sampling systems. This should be of particular value for life sciences applications, and especially for

barrier technology equipment which concentrates such challenges, but where the GMP Annex 1 expects hard data and documentation, enshrined in the Contamination Control Strategy, to justify configurations which do not meet unrealistic guidance values featured in the Annex. Two published studies (Thaveau et al, Solmaz) are referenced in the TR Bibliography, to illustrate such approaches.

Some issues do remain in Annex 1, which l came into force in August 2023, but it will be possible to answer these in many cases by justification in the contamination control strategy documents that users will have to produce, based upon implementation of established and robust information conveyed in the Technical Report.

A prime use of the new ISO TR21 will be to inform users of the broader scientific consensus regarding airborne particle sampling for classification and monitoring, on which the ISO 14644 standards have been built. The document will also suggest avenues of investigation to enable users to test and evaluate the causes and extent of particle loss in sampling lines, and to decide appropriate technical responses.

For the future, it should be borne in mind that a Technical Report does not undergo the rigorous series of reviews, both within the technical committee and in the broader community, that a standard undergoes. For TR21, we anticipate some material will potentially require further refinement. Nonetheless, one of the drivers for choosing the Technical Report format was to publish material to coincide with the recent release of EU and PIC/S GMP Annex 1 and address emerging concerns in other fields.

It is felt within the technical committee that this document will either contribute material to the future revision of Parts 1 and 2 or, perhaps evolve to become a standard in its own right.

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IEST is the leading global nonprofit contamination control society and Secretariat for ISO Technical Committee 209 (ISO/TC 209), the committee developing the ISO 14644 Standards. IEST has served as the Secretariat for ISO/TC 209 for more than 30 years with an established international leadership role based on more than 50 years of expertise in cleanrooms and controlled environments.