

PDA SUGGESTED TEXT FOR ANNEX 1

4. Clean Zone Classification

Clean zones, cleanrooms and clean air devices, should be classified in accordance with EN ISO 14644-1. Classification should be clearly differentiated from operational process environmental monitoring. The maximum permitted airborne particle concentration for each grade is given in the following table.

Grade	At rest		In operation	
	0.5 microns	5.0 microns	0.5 microns	5.0 microns
A	3 500	20*	3 500	20*
B	3 500	20*	350 000	2000
C	350 000	2 000	3 500 000	20 000
D	3 500 000	20 000	Not defined	Not defined

*Whilst the maximum permitted number of particles at or greater than 5.0 microns is given as 20/m³, actual counts should routinely be lower than the maximum. If trend analysis shows a deviation from the norm an investigation/corrective action should occur. See clause 6.

For classification purposes, in Grade A zones, a minimum sample volume of 1m³ should be taken. Grade A and Grade B (at rest) is similar to EN ISO Class 5. For the purposes of clean zone classification studies EN ISO 14644-1 methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest considered particles size. Refer to Annex B in EN ISO 14644-1.

Particle counters with as short a length of tubing as possible should be used to minimise the loss of ≥ 5.0 micron particles, the transit tube length should not exceed the manufacturer's recommended length. Isokinetic sample heads should be used in unidirectional air flow systems.

"In operation" classification should be demonstrated during actual operations that incorporate routine and, as appropriate, worst case activities and conditions. EN ISO 14644-2 provides information on testing to demonstrate continued compliance with the assigned cleanliness classification.

5. Clean Zone Monitoring

Clean zones should be routinely monitored in operation with the monitoring locations based on a risk analysis study combined with the results obtained during the classification study of the cleanroom and/or device.

For Grade A zones a continuous or frequent sampling particle monitoring system should be used except where justified, e.g., the filling of live virus vaccines, powder filling operations. Grade B zones should be subject to scheduled routine monitoring. The particle monitoring systems may consist of independent particle counters or have one particle counter that is linked to a number of sampling ports that operate sequentially via a manifold system. The tube from the sample point to the sensor should be as short as possible ensuring the tube length and the radii of any tubing bends does not exceed the manufacturer's recommendations.

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The Grade A zone should be monitored for non-viable particles at a defined frequency such that interventions and other transient events would be captured and alarms triggered if excursions from normal operating values occurred.

Operational process environmental monitoring does not require the same sample volumes to be taken as for the classification studies of cleanrooms and clear air devices. The sample size taken for monitoring purposes will usually be a function of the sampling rate of the systems used.

6. In Grade A and B zones, the monitoring of the 5.0 micron particle concentration count takes on a particular significance as it is an important diagnostic tool for early detection of failure. The occasional indication of particle counts ≥ 5.0 microns may be false counts due to electronic noise, stray light, coincidence, etc. However consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated. Such events may indicate early failure of the HVAC system, filling equipment failure or may be a diagnosis of poor practices during machine set-up and routine operation.

7. It is accepted that it may not always be possible to demonstrate low levels of particles at the point of fill when filling is in progress due to the generation of particles or droplets from the product itself. The particle limits given in the table for “at rest” should be achieved after a short “clean up” period of 15-20 minutes (guidance value).

8. For Grade D areas in operation the requirements and limits will depend on the nature of the operations being carried out but the recommended “at rest” limits should be attained.

47. Validation of aseptic processing should include a process simulation test using a nutrient medium (media fill). In general, a microbiological growth medium such as soybean casein digest medium should be used. The process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all critical manufacturing steps subsequent to final product sterile filtration. The process simulation test should be performed as an initial validation with three consecutive satisfactory process simulation tests per processing line. Normally process simulation tests should be repeated twice per year for each processing line and after any significant modification to the HVAC system, equipment, process and number of shifts. Each person involved in aseptic processing should participate in at least one process simulation test per year.

The number of containers used for media fills should be sufficient to enable a valid evaluation. For small batches, the number of containers for media fills should at least equal the size of the product batch. The target should be zero growth and the following recommendations apply:

- i. When filling fewer than 5,000 units, no contaminated units should be detected.
- ii. When filling 5,000 to 10,000 units:
 - a. One (1) contaminated unit should result in an investigation, including the consideration of a repeat media fill.

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- b. Two (2) contaminated units are considered cause for revalidation, following an investigation.
- iii. When filling more than 10,000 units:
 - a. One (1) contaminated unit should result in an investigation.
 - b. Two (2) contaminated units are considered cause for revalidation, following an investigation.

For any run size, intermittent incidents of microbial contamination in media filled runs can be indicative of a persistent low-level contamination problem that should be investigated.

- 57. The bioburden should be monitored before sterilisation. There should be working limits on contamination immediately before sterilisation which are related to the efficiency of the method to be used. Bioburden assay should be performed on each batch for both aseptically filled product and terminally sterilised products. Where duplicate sterilising grade filters are used for aseptic processing or where overkill sterilisation parameters are set for terminally sterilised products the bioburden might be monitored only at suitable scheduled intervals. For parametric release systems, bioburden assay should be performed on each batch and considered as an in-process test. Where appropriate, the level of endotoxins should be monitored. All solutions should be passed through a micro-organism retaining filter sited, if possible, immediately before filling.
- 93. Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g., glass or plastic ampoules should be subject to 100% integrity testing. Samples of other containers should be tested and/or monitored for integrity according to appropriate procedures.

Vial filling operations should maintain the open or partially closed vial (as for lyophilisation vials) under Grade A conditions. As stoppered vials exit the Grade A zone, appropriate assurances should be in place to safeguard the product under local protection until completion of the crimping step. Using devices for on-line detection of improperly seated stoppers can provide additional assurance.