Table 21-2B Air Classifications According to European Grade

	At rest		In operation	
Grade	**Particles ≥ 0.5 μm/m³**	**Particles ≥ 5 μm/m³**	**Particles ≥ 0.5 μm/m³**	**Particles ≥ 5 μm/m³**
A	3520	0	3500	0
B	35,200	0	350,000	2,000
C	352,000	2000	3,500,000	20,000
D	3,520,000	20,000	Not defined	Not defined

Table 21-2C Recommended Limits for Microbial Contamination According to European Grade Clean room Classification

	Recommended limits for microbial contamination			
Grade	**Air sample (CFU/m³)**	**Settle plates (CFU/4 hr)**	**Contact plates (CFU/Plate)**	**Glove print (CFU/Glove)**
A	<1	<1	<1	<1
B	10	3	5	5
C	100	50	25	–
D	200	100	50	–

source of smoke typically is a glycol-based[1] fog generator that clearly shows if there is any turbulence in the room(s). If there is any suspicion of a breach in the security of a clean room (e.g., an emergency door opened where the alarm did not work), application of a smoke test will determine if such a breach caused turbulence.

Air pressure differentials between rooms must be different by at least 12.5 Pascals (0.05 inches of water). At least 20 changes of air per hour are required for all clean rooms with no specific minimum given for Grade A/B or Class 100 rooms. Microbiological monitoring is performed with the expectation of zero growth of any plate at any time.

The area immediately adjacent to the aseptic processing line should meet a minimum of Grade C or Class 10,000 conditions under dynamic operations, although the current preference is Class 1000 or maintaining the entire aseptic filling room at Class 100.

All compressed gases used in aseptic processing must be free from demonstrable oil vapor, sterilized tanks must be held under continuous overpressure, and all gas filters must be periodically integrity tested.

HEPA filters are to be integrity tested twice a year using poly-alpha-olefin (PAO) aerosol. This aerosol is a polydisperse, nontoxic liquid that possesses a light scattering mean droplet diameter of 0.7 micrometers. Dioctyl phthalate (DOP) is another aerosol used, although PAO is more widely used. The starting liquid is heated to the point of vaporization and reconstituted into 0.3-μm particles to form a monodisperse aerosol. These single-size particles are diluted with air until a concentration of 100 μg/L is reached, and the aerosol-air mixture is passed through the filter. The sample rate should be at least 1 ft^3/min with the probe 1–2 inches from the face of the filter. Since the upstream aerosol concentration is known, and the photometer is linear, the downstream samples may be read out in percent of concentration. Typical readings at the filter face range from 0.004% to 0.008%. Any leak greater than 0.01% of the upstream concentration is considered a significant leak and the location of the leak needs to be repaired (patched). There should be no leaks around the filter seals. If 10% or more of the filter face fails this challenge test, the entire filter must be replaced.

The design of the building and facilities has been covered in Chapter 14. A summary of the requirements of facilities according to the FDA aseptic processing guidelines include proper ergonomics of all equipment used, minimization of entries and exits, proper design of airlocks,

[1] Water-based fog generators (e.g., carbon dioxide or liquid nitrogen) create an effluent that is heavier than air that may not accurately demonstrate actual air patterns.