

Application Note

Microbial Monitoring of Compressed Gas Lines — Meeting Regulatory Standards

Introduction

There is a need to monitor for particulates and microbial contaminants in pharmaceutical gas lines, whether they are used as a process gas, control gas with open vent into the clean room, or as an aspiration point within a process. The regulations state that all processes shall meet either defined limits as set in the ruling or guidance document or follow appropriate ISO standard.

Regulations

For cleanroom or clean air device monitoring of particulate and microbial contaminants the pharmaceutical industry is directed to the EU GMP Annex 1 2008, the FDA guidelines on aseptic manufacture and the USP/EP requirements for contamination limits. Where specific methods are not defined within the industry guidance it is important to look to ISO standards for methodologies for compliance.

Note:

This guidance does not lay down detailed methods for determining the microbiological and particulate cleanliness of air, surfaces etc. Reference should be made to other documents such as the EN/ISO Standards. (EU GMP Annex 1 2008)

The standard for review when ascertaining the test method for microbial monitoring of compressed gasses is therefore ISO8573-7 Compressed air —Part 7: Test method for viable microbiological contaminant content. This standard shall be reviewed within the context of this paper.

We also have direct industry input from regulatory bodies such as the FDA, 21CFR211 Current Good Manufacturing Practice for Finished Pharmaceuticals, requires that:

Equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing, or holding of a drug product. 21 CFR 211.46(b)

And in the FDA Guidance for Industry - Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practices it requires that the compressed gas line being used should be equivalent to, or better than the room air quality in which that air is being used.

A compressed gas should be of appropriate purity (e.g., free from oil) and its microbiological and particle quality after filtration should be equal to or better than that of the air in the environment into which the gas is introduced.

Therefore Air being used in an EU GMP Grade A or FDA Critical Environment should have the particulate and microbial burden not exceeding the environmental quality of that room.

FDA GUIDANCE TABLE 1- Air Classifications ^a

Clean Area Classification (0.5 μ m particles/ft ³)	ISO Designation ^b	$\geq 0.5 \mu$ m particles/m ³	Microbiological Active Air Action Levels ^c (cfu/m ³)	Microbiological Settling Plates Action Levels ^{c,d} (diam. 90mm; cfu/4 hours)
100	5	3,520	1 ^e	1 ^e
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

- a- All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.
- b- ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle concentration is equal to Class 100 and approximately equals EU Grade A.
- c- Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological action levels due to the nature of the operation or method of analysis.
- d- The additional use of settling plates is optional.
- e- Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

EU GMP ANNEX 1 - 2008

Clause 19. Recommended limits for microbiological monitoring of clean areas during operation:

Grade	Recommended limits for microbial contamination (a)			
	air sample cfu/m ³	settle plates (diameter 90 mm) cfu/4 hours (b)	contact plates (diameter 55 mm) cfu/plate	glove print 5 fingers cfu/glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Notes

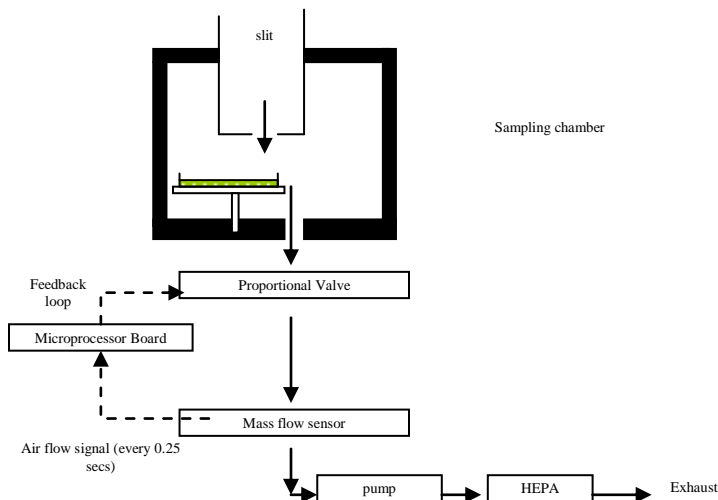
- (a) These are average values.
- (b) Individual settle plates may be exposed for less than 4 hours.

Method of Sampling

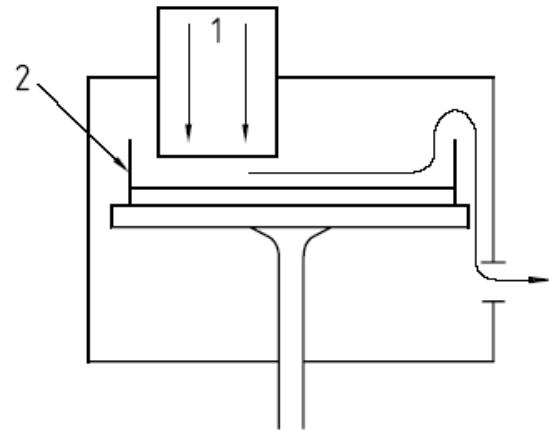
We have established that the routine testing and monitoring of compressed gasses is therefore required to meet current regulations for Pharmaceutical Manufacture, how is it done?

For this we should seek guidance from the ISO 8573-7. The scope of the ISO document covers a test method for distinguishing viable, colony-forming, microbiological organisms from other solid particles which may be present in compressed air. The standard provides a means of sampling, incubating and determining the number of microbiological particles. The test method is suitable for determining purity classes in accordance with ISO 8573-1, and is intended to be used in conjunction with ISO 8573-4 when there is need to identify solid particles that are also viable, colony-forming units.

The method is to expose a growth media, agar or similar, to the compressed gas air sample, use of a Slit-to-Agar impaction instrument is required along with depressurization of the gas. The flow rate of the gas is recorded and the measurement shall be taken within a period not exceeding 4 hours, however given the desiccation rate of most Agar media a 90 minute exposure time is required.



AirTrace Environmental Sampler Schematic



ISO8573-7 Preferred Sampler Schematic

The test method requires that:

- All equipment is sterilized prior to use
- Connect the sample inlet to the compressed gas line, including any electrical control connections to the instrument.
- Perform a blank of the gas in test, this allows a purge cycle of gas to be tested to clear out any remaining sterilant; this test is performed without agar.
- Inset a test 140mm agar plate onto the rotation platform.
- Remove the lid off the agar plate, close the sample lid of the Airtrace and store the agar plate lid in a sterile bag.
- The agar plate will automatically adjust to the slit optimal height on start-up of the Airtrace.
- Start the instrument and set the sampling parameters conducive to the sample requirements, 1m³ over a 90 minute period – or similar.
- At the end of sample remove the agar plate lid from the sterile bag, open the lid of the AirTrace and place the agar lid on the exposed plate. It should be noted that an indented quadrant of the 140mm agar will be indented due to impaction form the air flowing through the slit.
- Remove the agar plate, record the sample parameters, incubate the media at suitable temperature for the prerequisite period of time and record the cfu value.

An alternate technique can also be used to the slit-to-agar rotational plate and that is of the MiniCapt compressed gasses kit that fits to a standard MiniCapt environmental sampler. This uses a series of 20 static slits to impact the depressurized compressed gas onto a static plate. The technique is similar to above however there is no adjustment of media height to the slit, this is a fixed parameter.



Once the media has been incubated for the required period of time it can then be read for number of colonies, speciation as required and compared against the limits prescribed in EU GMP and FDA regulations for cleanroom control.

Testing should be repeated at a frequency that best reflects risk to finished product, this in turn is reflective of the cleanroom classification that the compressed gas is being used in.

Grade A and FDA critical areas should be monitored at least **Once per shift**; Grade B areas and FDA support areas **Once per day**; Grade C areas **Once per week** and Grade D areas **Once per month**.

For further information regarding the microbial sampling products identified in the paper please go to www.pmeasuring.com for technical information and other environmental applications.

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