# High Pressure Diffusers Application Note

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ISO
9001

## High Pressure Diffuser


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## Contents

Compressed gas sampling for microorganisms and/or foreign particulate matter is an important part of contamination control assessment. This paper shall focus on contamination and monitoring of compressed gases in pharmaceutical production facilities.

## Monitoring High Pressure Gases

Compressed gases (CG) are introduced by means of a compressor into a special container (or tank) where they are stored until released for use. Any potential microbial contaminant present in the gas will be subjected to a compression level according to the standards of the compressed gas manufacturer.

Compressed gases are used at different stages of the pharmaceutical manufacturing process.

Compressed gases used in the production of pharmaceutical medicines that come into direct contact with the biopharmaceutical and pharmaceutical manufacturing products and other process components include: Nitrogen; Oxygen; Argon; Carbon Dioxide; and Air.

There are many applications in pharmaceutical manufacturing where CG are used, include blanketing, purging, overlaying, sterilization, packaging testing, product insulation from undesirable environmental effects such as oxidation, etc.

Among the different CG available, nitrogen gas has emerged as a leading source for pharmaceutical products produced for a broad range of applications.

High pressure gases are typically delivered in high-pressure cylinders or cryogenic vessels, constructed of aluminum, stainless steel, or other some other noncorrosive and nonreactive metal. These cylinders are filled from larger tanks. Most of the following gases have system pressures of about 50 to 55 psi to enhance breathability. Nitrogen pressure can reach 174 psi (~12 bar).

## TESTING AND STANDARDS

### MICROBIOLOGICAL CONTAMINATION

Microbial content itself does not influence the purity class assigned, although the standard recommends that microbial levels are assessed. Acceptable microbial numbers are subject to a separate assessment; with such an assessment is based on an

interpretation of GMP. For example, the 2004 FDA Aseptic Filling Guidance document states (4):

> "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."

With purity, many parts of the pharmaceutical industry will use class 1 compressed gas based on the maximum number of permitted particulates. The particle limits are:

| **ISO 8573 class** | **Particle size limits per $m^3$** | | |
|---|---|---|---|
| | **0.1 - 0.5 µm** | **0.5 - 1.0 µm** | **1.0 - 5.0 µm** |
| 1 | ≤ 20,000 | ≤ 400 | ≤ 10 |

Outside of the ISO 8573 standard, supporting information is contained within the ISPE Good Practice Guide - Process Gases (2011) (5). Table 7.1 (Ref. Appendix 1) of ISPE the guide indicates that particle counts (both viable and inert) should be:

> "Typically equal to the at rest condition of the area served."

That is, for EU GMP Grade A / ISO 14644 class 5 areas, the microbial count should be <1 CFU/$m^3$ and the particle levels conform to the area at rest ≤ 3,520 particles per $m^3$ @ > 0.5µm.

A separate standard exists for the production of compressed air (ISO 12500). ISO 12500 has no specific microbial testing requirements.

## MICROBIAL SURVIVAL

Based on the above, the maximum level of microorganisms will be applicable to the cleanroom class. This places a tight limit on ISO 14644 class 5 / EU GMP Grade A areas.

Although compressed gas and air systems are relatively harsh environments, they can aid microbial survival if there are available nutrients. The availability of nutrients is dependent upon the purity of the gas and airline. Nutrients suitable for metabolizing by microorganisms include water and oil droplets. Another factor that can affect survival is temperature, especially where temperatures are warmer. (Stewart, et al. 1995)

In addition to vegetative cells, bacterial spores are well equipped to survive the harsh environmental conditions. Spores are resistant to the types of temperature ranges and moisture levels found within compress gas lines. Another risk exists with biofilm, where microbial communities can potentially form and develop through attachment to air lines and tubing.

Although these risk factors exist, typically no microorganisms would be expected to be recovered from compressed gas lines. Research has shown that many microorganisms can survive and multiply in pressurized systems up to 10 bar, and approximately 85% are able to recover after being pressurized. (Zinge, 2013) Furthermore, it has been shown that *Escherichia coli* and *Corynebacterium xerosis* survive potentially explosive decompression from the extreme of 300 atm (= 300 bar), which is far from the decompression from 10 bar to 1 atm found in a CG sampler. (Zinge, 2013)

However, at 160 bar pressure (2,320.6 psi) and upwards, survival rates tend to be quite low. (Sandle, Aug 2015)

Although microbial contamination of compressed air or gas is a rare event, incidents can occur, and even low level counts require investigation. Sources of contamination include:

- Source of the gas. Intake air from surroundings which can contain oil, dirt/dust and moisture/ water vapor, and microorganisms.

- Piping distribution systems. Piping distribution and air storage tanks, more prevalent in older systems, will have contaminant in the form of rust, pipe scale, and mineral deposits in addition to bacteria.

- Bacterial retentive filter. The filter may become blocked, lose its integrity, or become wet.

- Compressor failure. The compressor itself can create a contaminated environment. For example; the compressor's pre-filters can become overloaded with dust and lint, causing the filter to cease functioning properly.

- Sample valve. The point-of-use sample valve may not be designed correctly or become faulty. Also, the type of valve may not have been properly selected – it should be a Teflon valve.

## NEED TO REDUCE PRESSURE

When sampling compressed gases, a regulated flow into the particle counter or microbial air sampler is requisite. Failure to regulate the flow rate can/will cause several problems:

- Particle Counter: This instrument counts and sizes particles based on an expected electrical response given the particle counters flow rate. The higher the electrical pulse, the greater the size of the particle. And conversely, the lower the electrical pulse, the smaller the particle. Subsequently, directly connecting a high pressure source at, for example, 50 psi to a 50 LPM particle counter will cause any particles in the gas to fly though the View-Volume (area inside the particle counter saturated by a laser beam) at a substantially accelerated rate, and will result in **gross under-sizing of the particle**.

- Microbial Air Sampler: Among other factors, the flow rate of the microbial sampler and the inlet geometry (i.e., the number and diameter of the holes in the sample head) are calculated to provide a given velocity for viable microorganisms impacting into agar. This flow rate and inlet geometry must be tested for **physical and biological efficiency** in accordance with ISO 14698. By directly connecting a high pressure source at, for example, 50 psi to a 100 LPM microbial sampler will increase in physical recovery; however, the accelerated impaction velocity will cause a significant decline in the percentage of microorganisms recovered. In more simple terms, it will result in a gross unfavorable impact on biological efficiency. (Stewart, et al., 1995) **Agar Desiccation** is another concern. Whatever growth medium is used, exposing it to high flow rates may cause the agar to desiccate, further reducing biological efficiencies.

- Possible Catastrophic Damage: Forcing high pressure gas into a particle counter or microbial sampler may cause a catastrophic failure of the exhaust filter. It may also cause damage to the pump or vacuum source, as well as other damage.

- Sample Volume Uncertainty: The particle counter or microbial sampler's sample time is based on the instrument's flow rate. For example, at 100 LPM, a sample time of 10 minutes is required for a cull cubic meter. Obviously, by directly

connecting high pressure gas to an instrument will likely result in gross over sampling.

Most pharmaceutical gases are typically stored at 10-11 bar (145 – 160 psi) or greater. The gas then passes through a regulator which reduces the pressure to somewhere generally between 4-7 bar (55 – 100 psi), and is next passed through a HEPA filter. Even at this reduced pressure, a further reduction in pressure is necessary to reduce the gas to atmospheric levels (~1.1 bar) allowing the particle counter or microbial sampler to draw the gas into the instrument normally.

## STERILIZATION

The head of the microbial sampler and any attachments must be sterile before use to avoid contamination and cross contamination. The culture medium used with the instrument should be sterile (normally by irradiation) and a representative item should have been tested for growth promotion. The sample head, tubing, and adapters should be autoclavable. Some users disinfect the tubes and hoses used to connect the sampler with a disinfectant such as 70% isopropyl alcohol. This is mentioned as an option in the ISO standard, although this is erroneously described as "sterilization." Where a disinfectant is used it is important to run the air through the sampler without any agar plate in place; this is necessary to evaporate the disinfectant and to remove any residues. The presence of disinfectant could potentially lead to a false negative.

## AGAR AND INCUBATION

Compressed air sampling should form part of an environmental monitoring program, along with cleanroom assessments. The program should take into account air points to be tested. This could be every point, points considered to be of greater risk such as product contact, or representative points along a loop. The frequency of testing must also be considered based on risk assessment.

The sampling time should be sufficient in order to sample one cubic meter of gas. After sampling, the agar plate is removed and incubated within a microbiology laboratory. At the end of incubation, the agar is examined for colony forming units. If colony forming units are recovered, these should be assessed against the appropriate limit. It is **good practice** to identify the contaminants recovered; the identification may provide important information to help determine the origin of the bacteria, and if is an objectionable species. [Sandle, 2015]

In terms of culture media, the Climet CI-9xA Series collects air samples onto either a 90mm petri dish or RODOC plate.

An appropriate agar must be selected. Per USP <1116>, a general microbiological growth medium such as soybean-casein digest medium (SCDM) is suitable for environmental monitoring in most cases because it supports the growth of a wide range of bacteria, yeast, and molds. This medium can be supplemented to minimize the effects of sanitizing agents or of antibiotics.(USP <1116>) Also, air samples taken for the purpose of measuring bacterial load utilize TSA (Tryptic Soy Agar) or soybean casein digest (SCDM) as growth media for collected organisms, while air samples taken to examine airborne fungal load are collected using MEA (Maltose Extract Agar) or Sabouraud Dextrose Agar. It is important to note that testing for fungi is only required for compounding categorized as high risk (USP 1116, *Revision Bulletin*, p. 25)

Most pharmaceuticals, however, use TSA, which is a generally nutritious medium designed to recover a range of bacteria and fungi. (Sandle, 2015)

The agar medium should be removed from the sampler as quickly as is practicable and transferred to the required incubator. This is to avoid the culture medium from drying out or deteriorating.

With the incubation conditions selected, the time and temperature should be suitable for the recovery of the a general range of microorganisms, particularly Gram-positive organisms given that such bacteria are better equipped to survive in dry environments (Moissl-Eichinger, 2012).

The typical requirement is to look for mesophilic bacteria and fungi (those that would grow across the temperature range 20-30$^{o}$C). Some users would elect to use one representative temperature whereas others would elect to use a two-step incubation regime, such as: (Sandle, 2014; and USP <1116>)

- 20-25$^{o}$C for 3-5 days, followed by
- 30-35$^{o}$C for 3-5 days.

The selected incubation time should be based on growth promotion studies. If certain microorganisms are considered a problem, alternate incubation times or culture media can be considered.

### SAMPLING VALVE

Whether performing viable or non-viable testing, the ON/OFF valve should be fully opened. We do not recommend users attempt to hand-regulate the pressure, but rather allow the high pressure diffuser to do its job. If too much gas is being exhausted, you may need a different diffuser.

### SAMPLING FREQUENCIES

The user will need to determine whether each compressed gas line requires testing and the frequency of testing. Certainly all product contact compressed gases should be assessed. A sampling plan should also consider and adapt to the following:

- Increased or reduced production schedules
- Seasonal changes
- Equipment changes and modifications
- Replacement of hardware or filters and dryers
- Inactivity of system.

### SAMPLE TEST REPORTS

Sample test reports are found in Annex A (non-viable particles) of ISO 8573-4 and Annex A (viable particles) of ISO 8573-7.

## Special Calibration of High Pressure Gases

Particle counters and microbial samplers are designed to operate in air.

Air (molecular density of 29) and Nitrogen (molecular density of 28) have approximately the same molecular weight. As a result, a particle counter or microbial sampler can sample either air or nitrogen without needing a special calibration.

Carbon Dioxide ($CO_2$) has a molecular density of 44.01, and requires a special calibration.

As a general rule, if it is not air or nitrogen, a special flow rate calibration is required, except for mixed gases where nitrogen is dominant.

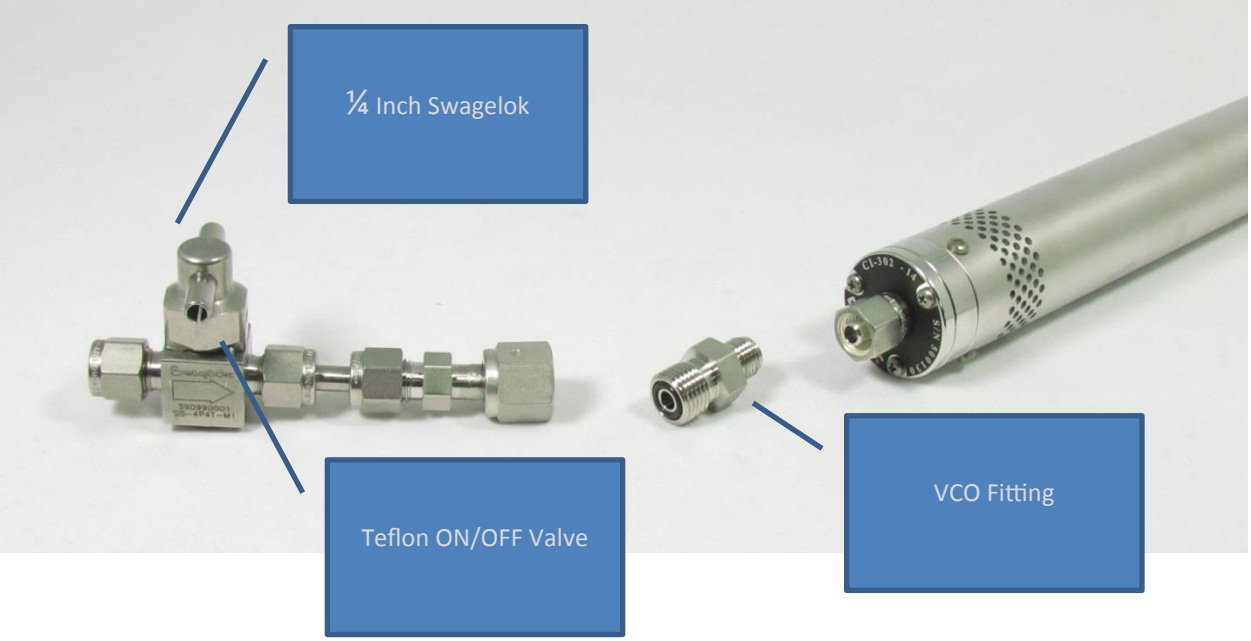
Some compressed gases, such has oxygen, may present hazardous conditions, such as explosive atmospheres. We do not recommend our particle counter or microbial sampler be used to test explosive or potentially explosive gases.

## Installing & Using a Climet CI-302

A High Pressure Diffuser (HPD) vents off un-needed gas pressure so that the particle counter or microbial sampler can draw air into the instrument normally.

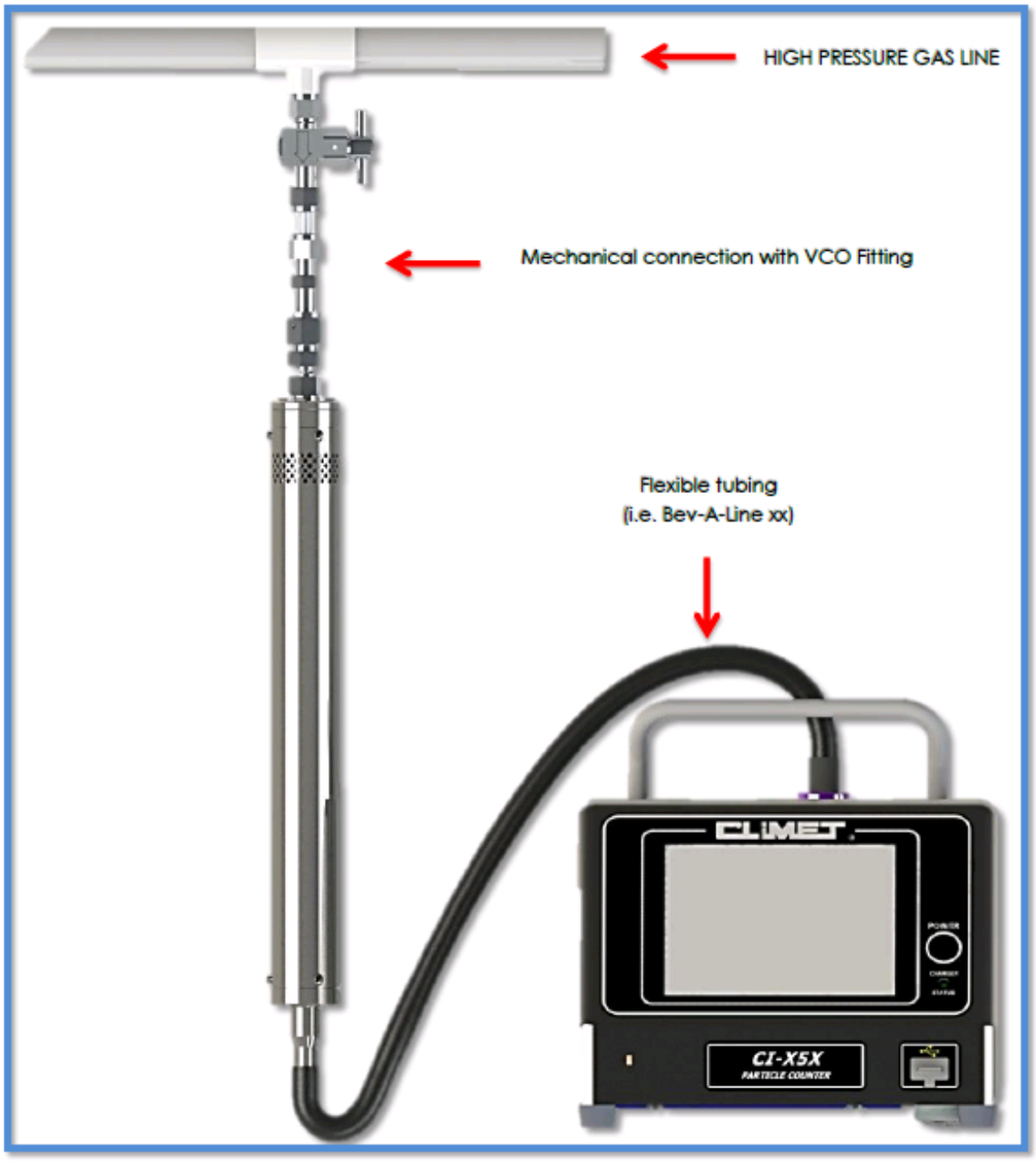
The Climet CI-302 high pressure diffuser has two ends:  a **high pressure end** and a **low pressure end**.

The standard connector on the **high pressure end** is a VCO fitting (by default), which can be directly connected to the high pressure gas line through additional hardware provided by Climet.

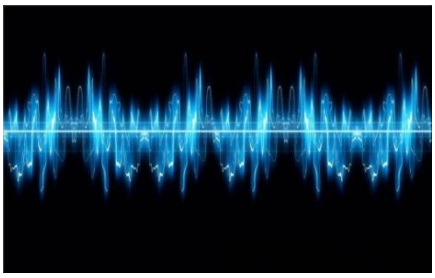



The **low pressure end** of the CI-302 has a tapered fitting (shown right), allowing Bev-A Line tubing to be fitted over it.

CI-302-XX High Pressure Diffuser




The location of the test point should be after the regulator and HEPA filter on the high pressure line. Tubing length should be 10 feet to mitigate high counts associated with sonic waves, and no sharp bends (Image above is for demonstration purposes only). Climet recommends use with a 1 CFM, 50 LPM, 75 LPM, or 100 LPM flow rate particle counter or microbial sampler.



As it relates to **particle counters**, one unfortunate byproduct of High Pressure Diffusers is the generation of sonic pressure waves. If the frequency is too low, an annoying audible high-pitch hum may become apparent; migrane headaches have been reported by some exposed for extended periods.

Climet has designed our CI-302 at the opposite end of the spectrum. However, when a paricle counter is used, we recommend 10 feet of Bev-A Line tubing be installed between the HPD and the particle counter to prevent sonic waves from causing false high counts.

As it relates to **microbial samplers**, a high pressure diffuser adapter head (shown right) will be required. The length of tubing is less critical as sonic waves will have no bearing on culturability of viable microorganisms.

Please, contact our Application Engineers for further questions or assistance.

# Appendix 1

Effective 23 October 2017:

<table>
<tr><th colspan="7">2011 ISPE Good Practice Guide - Process Gases, Table 7.1</th></tr>
<tr><th colspan="4">Particles</th><th colspan="2">Water</th><th>Oil</th></tr>
<tr><td colspan="3">By Particle Size<br>(maximum number of particles per $m^3$)</td><td>By Mass</td><td colspan="2">Aerosols or Vapor Pressure Dewpoint</td><td>Liquid</td><td>Hydrocarbons</td></tr>
<tr><td>0.10 - 0.5 microns</td><td>0.5 - 1.0 microns</td><td>1.0 - 5.0 microns</td><td>$mg/m^3$</td><td>°C</td><td>°F</td><td>$g/m^3$</td><td>$mg/m^3$</td></tr>
<tr><td colspan="3">Viable and Non-Viable Count Typically equal to the at rest condition of the area served</td><td></td><td>≤ -40</td><td>≤ -40</td><td>-</td><td>≤ 0.5</td></tr>
<tr><th colspan="4">Microbial Count</th><th colspan="3">Other Chemical Impurities</th></tr>
<tr><td colspan="2">Non-Sterile Applications</td><td colspan="2">Sterile Applications</td><td colspan="3">(Only as Applicable) Based on the generation technology and/or USP/EU Monograph</td></tr>
<tr><td colspan="2">Guideline limits to be established based on product bioburden limits. Typical level NMT 5 $cfu/m^3$</td><td colspan="2">As per viable particle requirements for Grade area where the product is exposed to the compressed gas (e.g., Grade A, Grade A/B, Grade B or Grade C)</td><td colspan="3">Gas Purity<br><br>Applicable for inert gases such as Nitrogen, as per USP/EU Monograph</td></tr>
<tr><td colspan="7">Air & Gas Specifications referenced above may be viewed and/or purchased from:<br>ISPE - International Society of Pharmaceutical Engineering</td></tr>
</table>

## Sources


EU GMP Annex 1. EU Guidelines to Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use. European Commission, EudraLex, Vol. 4, Brussels, 25 November 2008

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ISO 8573-4:2001 Test methods for sold particle content, International Standards Organization, Geneva, Switzerland

ISO 8573-7:2003 Test method for viable microbiological contaminant content, International Standards Organization, Geneva, Switzerland

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Moissl-Eichinger C, Rettberg P, Pukall R. (2012) The first collection of spacecraft-associated microorganisms: a public source for extremotolerant microorganisms from spacecraft assembly clean rooms, *Astrobiology* 12(11):1024-34

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