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Microbiological and Particle Control of Compressed Gases

A review

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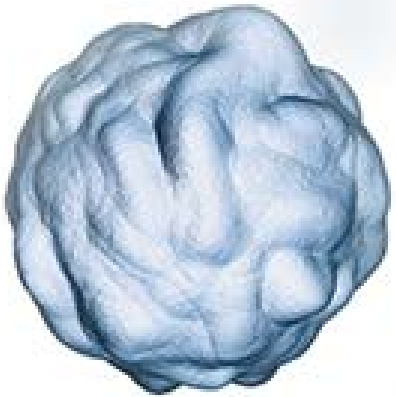
Agenda



- Compressed Gases as an Essential Part of an EM Program
- Application overview
- Microbial Survival in Compressed Gases Under Fast Decompression to Normal Atmospheric Conditions
- Instrument Selection Criteria and ROI
- Conclusion

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Compressed Gases as an Essential Part of an EM Program

Quality context and rationale

- Control of the environment in which pharmaceutical products are manufactured is a key element of Good Manufacturing Practices (GMP).
- Physical and microbial monitoring of manufacturing cleanrooms, Restricted-Access Barrier Systems (RABS), and isolators consists of clearly defined components.
- Absence of microbiological contamination is considered a critical quality attribute due to its potential to dramatically impact, directly or indirectly, the safety and/or the efficacy of the drug product.
- Compressed gas is considered a critical utility in the Pharma industry.
  - Direct product contact
  - Direct entry into clean environments

Physical monitoring of manufacturing cleanrooms

Components of an environmental monitoring program			
Type	Component	Characteristics	Purpose
Physical	• Total particulate of room or enclosure air + Compressed Gases	• Total inert and viable particles • Active sampling • Particle counter • Decompression adaptor	• Quality of environment • Root-cause analysis for source of contamination • Evaluation of compressed gas quality: Equal or better than air in the critical area
	• Differential pressures, positive pressure from a critical area to adjacent area	• 10 - 15 Pa difference • Sensor	• Prevents entry of contaminant particles from adjacent to critical clean area
	• Air changes • Unidirectional air flow	• Sensor	• Quality of environment
	• Temperature and relative humidity	• Thermometer, hygrometer sensors	• Product quality and personnel comfort

Microbial monitoring of manufacturing cleanrooms

Components of an environmental monitoring program

Type	Component	Characteristics	Purpose
Microbiological	• Viable counts of room or enclosure air & compressed gases	• Microbial counts by active (impactors and others) sampling • Passive (settle plate) air sampling • Sampling of gas by microbial gas sampling impactor with decompression adapter	• Quality of environment • Root-cause analysis for source of contamination • Evaluation of compressed gas quality: Equal or better than air in the critical area
	• Viable counts on personnel	• Effective personnel training and hygiene • Microbial counts by contact plates and swabs	• Contamination risk from operators • Evaluation of aseptic techniques and practices
	• Viable counts on surfaces	• Microbial counts by • contact plates & swabs	• Effectiveness of aseptic techniques and cleaning • Disinfection of area and equipment

Regulatory requirements

- There is a lack of specific guidelines/regulation for pharmaceutical applications.
- Key sources of information: FDA/GMP Guidelines
- Additional information may come from **ISO standard 8573**
- Key attributes for CG applications
  - Solid particles
  - Water
  - Oil
  - Microbiological burden

Regulatory requirements

*" A compressed gas should be of appropriate purity (e.g. from oil) and its microbiological and particle quality after filtration **should be equal or better than that of the air in the environment into which the gas is introduced**. Compressed gases such as air, nitrogen and carbon dioxide are often used in cleanrooms and are frequently employed in purging or overlaying."*

(FDA aseptic filling guide)

ISO – 8573

Part 1: Contaminants and purity classes

Part 2: Test methods for aerosol oil content

Part 3: Test methods for measurement of humidity

Part 4: Test methods for solid particle content

Part 5: Test methods for oil vapor and organic solvent content

Part 6: Test methods for gaseous contaminant content

Part 7: Test method for viable microbiological contaminant content

Part 8: Test methods for solid particle content by mass concentration

Part 9: Test methods for liquid water content

ISO 8573- 1

Solid particulate, water and oil concentration per ISO class							
ISO8573-1:2010 Class	SOLID PARTICULATE				WATER		OIL
	Maximum number of particles per m3			Mass Concentration mg/m³	Vapour Pressure Dewpoint °C	Liquid g/m³	Total Oil (aerosol liquid and vapour) mg/m³
	0.1 - 0.5 micron	0.5 - 1 micron	1 - 5 micron				
0	As specified by the equipment user or supplier and more stringent than Class 1						
1							01
2							.1
3							1
4							5
5							
6							
7							
8							
9							
X							10

- **NOTE: ISO 8573 classes are not identical to the ISO 14644 classes.**
- **If you choose to use the 8573 concentration limit table for your CG classification, make sure the appropriate particles sizes are considered, respective to your ISO 14644 class limits.**

Sampling point classification – Summary

- Typically, in an aseptic filling environment this table will not apply (equivalent to Class 0 in 8573-1).
- Manufacturers will need to define ther own specifications according EU/FDA GMP/ISO guidelines.
- In pharmaceutical production environments, the cleanroom user shall guarantee the compressed gas purity to meet or exceed the same cleanroom grade limits.
  - For example, a gas used in a Grade A/ISO 5 area should not exceed the Grade A/ISO 5 maximum permitted particle limit.

ISO 8573-7 test method for viable microbiological contaminants

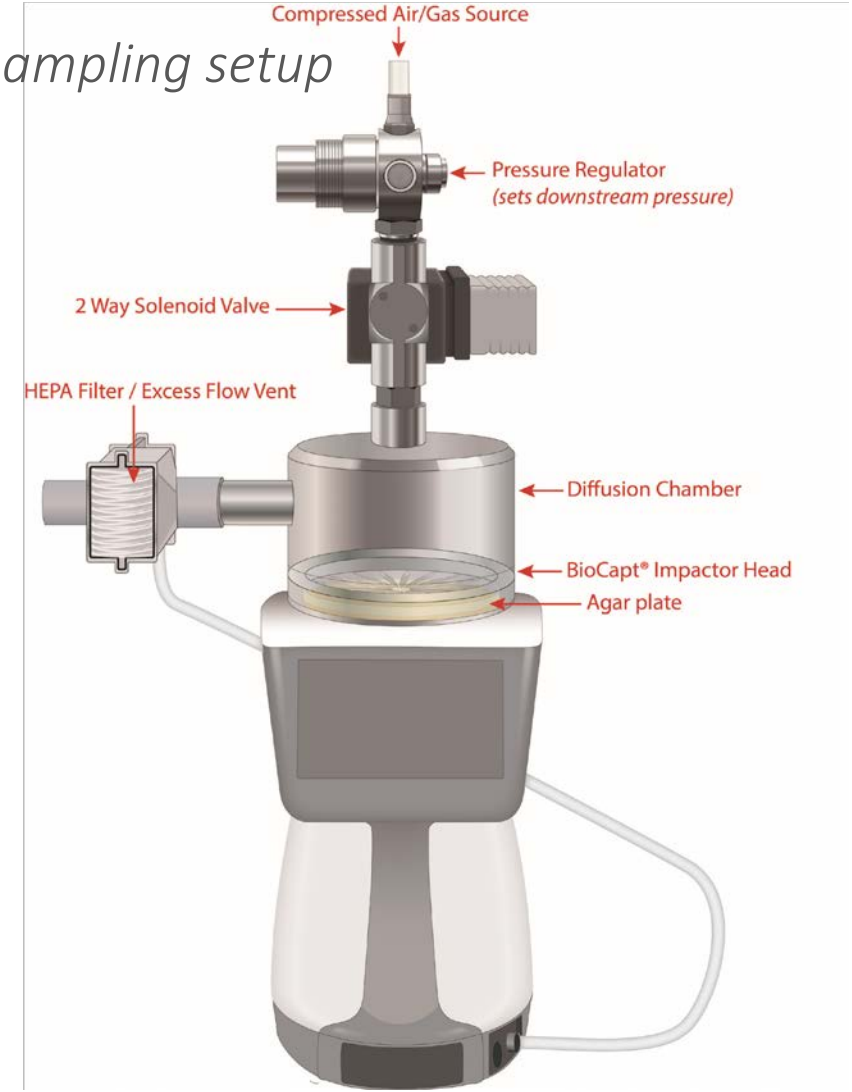
- ISO 8573 Part 7 provides a means of sampling, incubating and determining the number of microbiological particles.
  - Sampling method: Impaction (STA) together with the method given in ISO 8573-4
  - Isokinetic sampling of the air shall be carried out and reduced until it is within the range of the sampler as identified by the manufacturer.
  - Pressure reduction to atmospheric conditions and flow measurements shall be performed to establish compatibility with the manufacturer's recommendations or in accordance with ISO 8573-4.
  - Limits are not specified.

⇒Note: Investigations have shown that less than 50% of pharmaceutical plants WW use the impaction method (direct impaction or STA).

Example #1: Microbial CG sampling setup

MiniCapt Mobile CG Kit

- Special diffusion chamber mounted to sampler inlet
- Other end connected to the CG distribution lines
- Sampling at balanced flow
  - Compressed gas flow = sampling flow
- The impactor inlet pressure coincides with the pressure of the compressed gas kit diffusion chamber.
- Pressure regulator between the CG source & inlet
  - Safety
  - Regulate the flow rate of the gas into the diffusion chamber
- Solenoid valve connected downstream of the regulator allows synchronization of gas with the start of a sample.
- Available in 25, 50 or 100 LPM configurations
- Active monitoring of gas flow to detect changes



Example #2: Particle CG sampling setup

Lasair III HPD – High Pressure Diffuser

- Provides a plug & play solution for particle contamination control in CG.
- No pressure adjustment required
- **No additional calibration required**
- The Critical Orifice principle guarantees a stable flow in accordance with the instrument flowrate requirement.
- The HEPA filter guarantees any contaminated exceeding gas pressure will not contaminate the cleanroom.

- Available in 28.3, 50 or 100 LPM configurations
- Accept gas pressure from 25 to 100 psi
- Designed for portable or fixed installation

Sampling plan design – Microbial & particle CG monitoring

Frequency

- Initial qualification
  - 1 week daily
  - Monthly
- Routine monitoring
  - Quarterly
- Long term routine monitoring
  - Risk based approach
  - Annually to bi-annually

Location

- Initial qualification
  - Every sample location
- Routine monitoring
  - Worst case locations based on initial qualification
- Long term routine monitoring
  - Risk based approach based on historical data

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Applications

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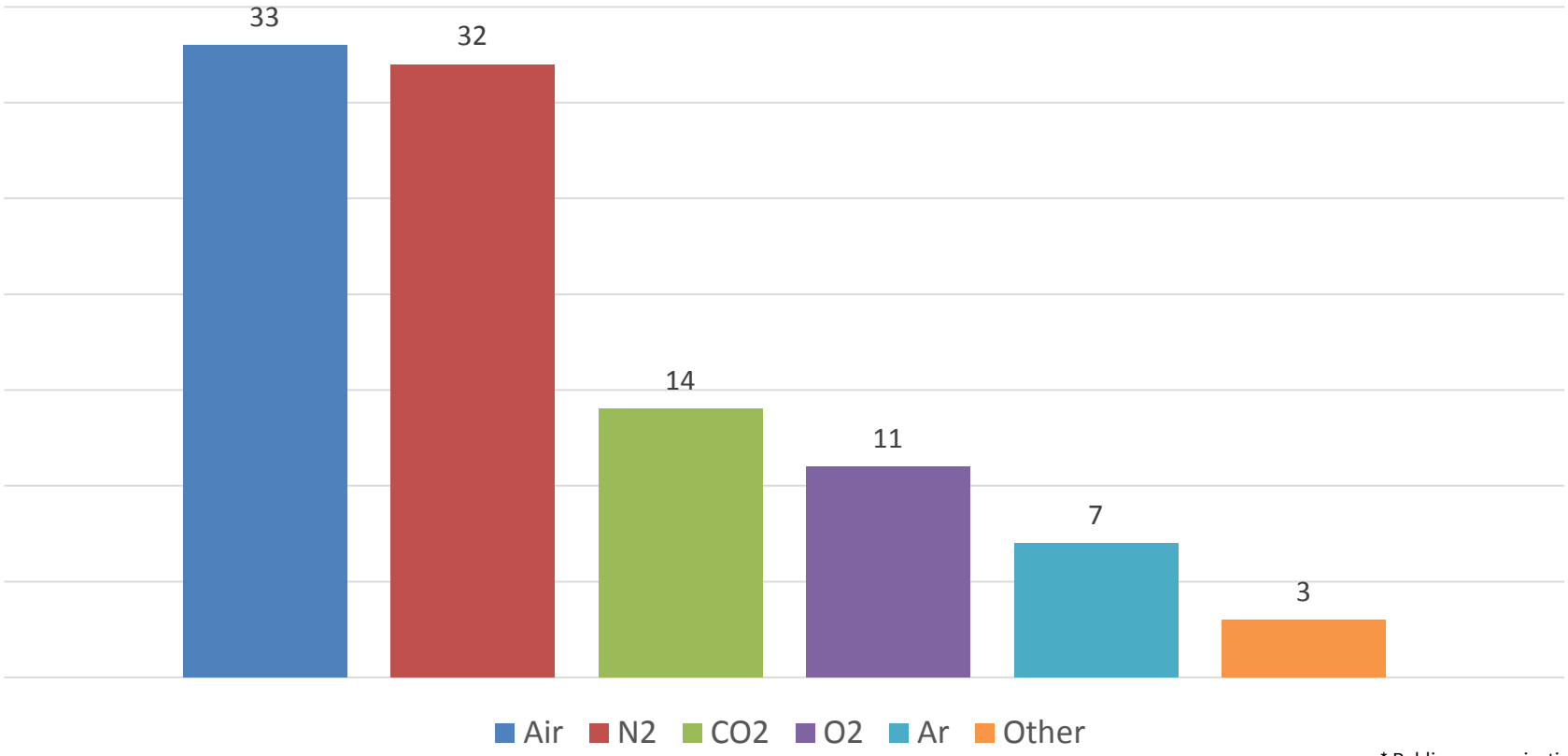
Gas applications in aseptic environments used in pharmaceuticals & biotechnology

Example of Gas Applications in Pharmaceutical Industry
Blanketing: Maintain an inert atmosphere above a liquid or powdered product inside a container. Prevention of product degradation/corrosion, control of volatile emissions.
Aseptic Packaging: Packaging of medical supplies Maintain sterility and cleanliness of the product by purging oxygen from the packaging
Pressure and Leak Testing: Inert gas to pressurize new, repaired, or modified tanks, pipelines, vessels, and process equipment in order to check their integrity and leak tightness.
Pressure Transfer: Transfer liquid or powder products to and from containers without requiring pumps
Filtration, Separation and Purification
Purging: The use of inert gas to displace air, flammable vapors, and contaminants from containers

*Use of compressed gases in Pharmaceutical Industry % **



* Public communication MerckSigma

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Microbial Survival in Compressed Gases Under Fast Decompression to Normal Atmospheric Conditions

Bacterial cells survive high pressures!

Bacterial cultures in food products require **2500- 3000 bar* to inactivate**[3]

Lessened microbial growth & viability on metabolic processes in vivo are seen **with pressures higher than 1000 bar***[5]

Cells treated at pressures of <1000 bar* showed **no significant loss** of viability[3]

Serratia and *Carnobacterium spp* **survive** conditions **similar to Mars** (low O2, <0°C, 7 mbar)[5]

***1 Bar = 0.1 Mpa = 14.5 PSI**

3 FDA. (2014). Kinetics of Microbial Inactivation for Alternative Food Processing Technologies - High Pressure Processing (HPP). A report of the Institute of Food Technologists for the Food and Drug Administration of the U.S. Department of Health and Human Services. Updated December 2014.

5 Nicholson, W. (2013). Growth of Carnobacterium spp. from permafrost under low pressure, temperature, and anoxic atmosphere has implications for Earth microbes on Mars. pnas 110(2):666- 671.

A comparison of bacterial recovery from compressed gases

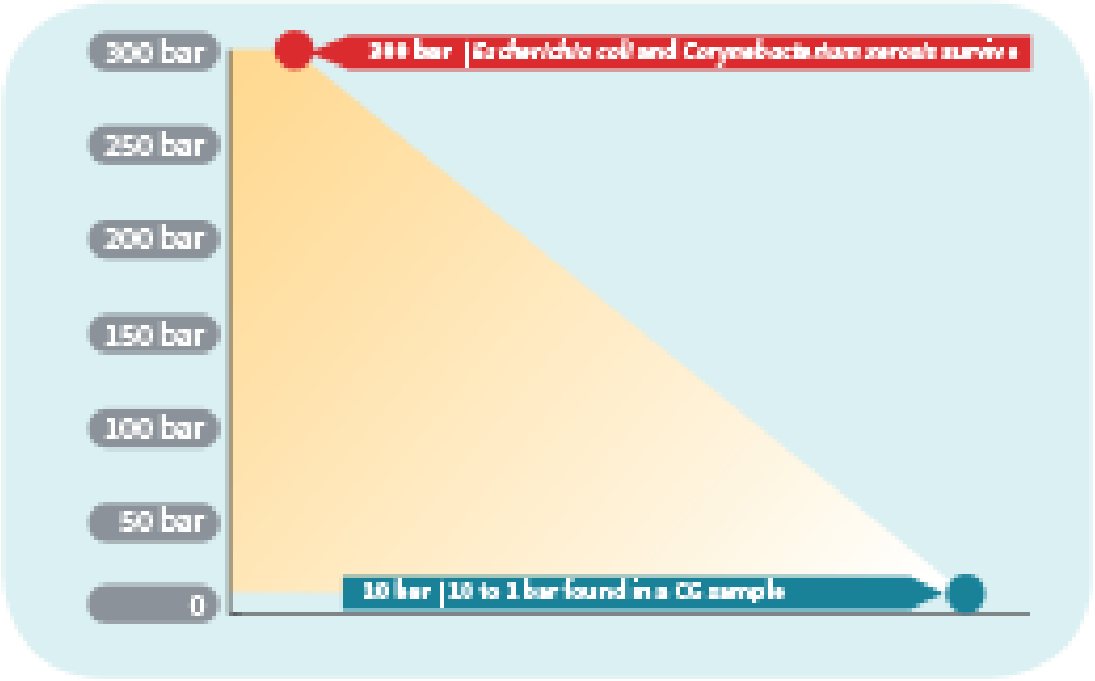
Results[2]

- 92% recovery using the MAS100 CG
- Bacterial cells maintained suitable growth, even up to 10 bar during the incubation.
- Compression cycle did not influence the viability of the tested microorganisms.

⇒Hypothesis from study: Sampling prior to decompression avoided potential microbial cell damage and lowered viability. This hypothesis is refuted by multiple studies.

2 Zingre, H. and Meier, R. (2013). Detection of Microorganisms in Compressed Gases. MBV AG Application Note. Stäfa, Switzerland

Microbial cells without gas vesicles easily survive fast decompression from 10 – 1 bar

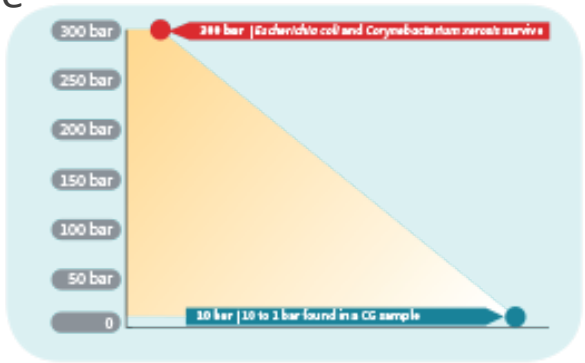


- Cells without gas vesicles were not harmed by decompression from up to 300 bar.[4]
- *Escherichia coli* and *Corynebacterium xerosis* survive rapid decompression from 300 bar (vs decompression from 10 to 1 bar found in a CG sampler]

4 Hemmingsen, B. B. & Hemmingsen, E. A. (1980). Rupture of the Cell Envelope by Induced Intracellular Gas Phase Expansion in Gas Vacuolate Bacteria. Journal of Bacteriology. 143 no. 2:841-846.

Decompression of sensitive bacteria

- Gram-negative, gas vacuolate bacteria (*M. aquaticus, P. pneumaticum, and M. glaucopis*) are known to be very susceptible to decompression.[4]
  - Populations of these cells were saturated with Ar, N_2 or He up to 100 bar. Gas phases of the vesicles remained intact.
  - Upon rapid decompression to atmospheric pressure, the vesicles expanded and ruptured the cells.
  - Viable counts indicated minimum pressures were between 25 - 50 bar.
  - Majority of the cell envelopes were ruptured at pressures between 50 - 100 bar



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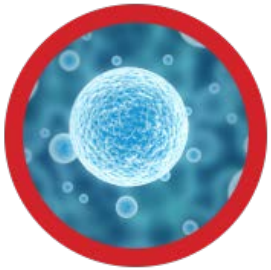
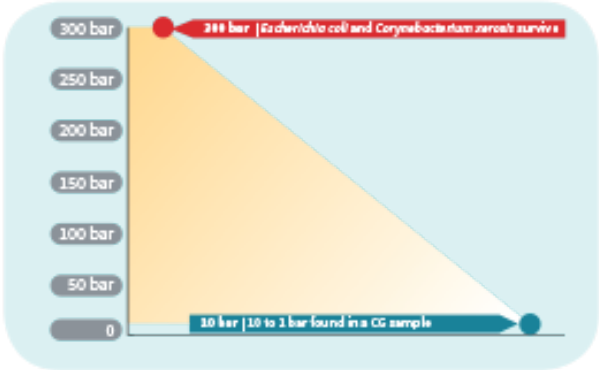
Fast decompression - conclusion

- The analogy of bacterial cells and human divers is wrong!



- Sensitive (vesicle containing) microorganisms are affected by rapid decompression.
- The viability is affected only when compression exceeds 25 bar to 1 bar.
- These conditions do not compare to realistic pharmaceutical settings.



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Instrument Selection Criteria and ROI

MICROBIAL Instruments or adaptors from multiple vendors are available

MAS CG EX®, (MerckSigma/MBV)

RCS ® CG Adaptor, (MerckSigma)

MiniCapt® Mobile, (PMS)

Instruments or adaptors from multiple vendors are available

Lighthouse High Pressure Diffuser

PMS - High Pressure Diffuser III

MET ONE High Pressure Diffuser

Climet High Pressure Diffuser

Challenges of CG monitoring

- Compressed gases need to be tested directly on-line
  - Especially in critical areas
- Reality: Monitoring points are not always established close to the point of use

⇒Size and weight of instrument mobile units

⇒Ease of use

⇒Software/data capabilities

⇒Cleanroom protection: HEPA filtration

Low accessibility of sampling points

Selection of Instruments - ROI

Challenge

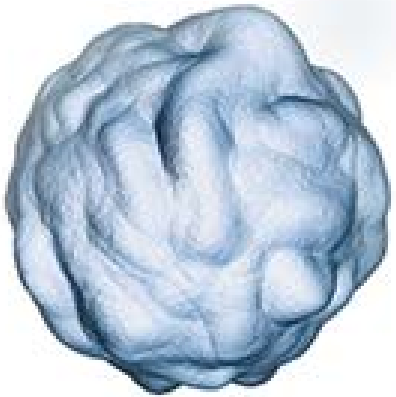
- Investment for rare measurement
- Full service program for a dedicated instrument (IQ/OQ + maintenance/calibration)

Solution

- External service provider
- Multi-functional instrument
  - Only cost of accessory
  - Full service program
  - Routine monitoring
  - Flexibility

Conclusions

Conclusions



- Compressed gas monitoring is an essential part of an EM Program.
- Microorganisms survive hyperbaric or hydrostatic pressures from 1 to 10 bar.
- Decompression from 10 bar to normal atmospheric pressure does not harm microorganisms.
- Instrument selection and ROI calculations follow specific criteria of size/weight and flexibility of functionality.

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Thank you

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