MEDICAL GASES

MICROBIOLOGICAL QUALITY OF MEDICAL AND FOOD GASES REVIEW

SCIENTIFIC REPORT

Doc 232/20

EUROPEAN INDUSTRIAL GASES ASSOCIATION AISBL

AVENUE DES ARTS 3-5 • B – 1210 BRUSSELS
Tel: +32 2 217 70 98 • Fax: +32 2 219 85 14
E-mail: info@eiga.eu • Internet: www.eiga.eu

Doc 232/20

MICROBIOLOGICAL QUALITY OF MEDICAL AND FOOD GASES-REVIEW

Prepared by AHG-M.12
Authors: E. Pasinetti, L. Hogervorst Jensen, K. Herlem Allain, L, Frederiksen

Disclaimer

EUROPEAN INDUSTRIAL GASES ASSOCIATION AISBL
Avenue des Arts 3-5 B 1210 Brussels Tel +32 2 217 70 98 Fax +32 2 219 85 14
E-mail: info@eiga.eu Internet: www.eiga.eu

Table of Contents

1 Introduction

For the assessment of the microbiological quality of food and medical gases there are no specific normative references, either for parameters to detect and for the analytical aspects, or for limits to be respected. However, there is a reference in the European Pharmacopoeia with parameters and limits for non-sterile products for inhalation use, that fit well with the topic [1]. This reference is also reported in EIGA Technical Bulletin TB02, Microbiological Quality of Medical, Pharmaceutical and Food Grade Gases [2]. Therefore, the choice of parameters to be investigated is based on the acceptance criteria of the microbiological quality of non-sterile products for pharmaceutical use of the European Pharmacopoeia 10.0 in chapter 5.1.4, where the following maximum criteria are indicated for products for inhalation use [1]:

Total Aerobic Microbial Count: (10^2) 200 CFU/ml or CFU/g

Total Yeasts Microbial Count: (10^1) 20 CFU/ml or CFU/g

Staphylococcus aureus: absent in 1 g or 1 ml

Pseudomonas aeruginosa: absent in 1 g or 1 ml

Bile-tolerant gram-negative: absent in 1 g or 1 ml

It should be noted that the units of measurement, CFU/ml or CFU/g, are not representative of the matrix in study, which is a gas. For example, for oxygen breathing use, the dose is of hundreds of litres per hour.

Another useful reference is the criteria used for ambient air in cleanroom class D (clean areas for carrying out less critical stages in the manufacture of sterile products), where the microbiological quality specifies a maximum Total Bacterial Count (TBC) value of 200 CFU/m3 [3].

Gases are manufactured in completely closed systems, supplied under pressure, and with no contact with ambient air, humans or animals. Where gas is exposed to process water as part of the manufacturing process, the water quality is controlled (as defined in the EC Guide to Good Manufacturing Practice) using validated methods [4]. Either drinking water quality or specially treated water is used to assure microbiological contamination is controlled and minimised. For medical and food gases supplied in cylinders or cryogenic containers, the valve outlets are required to be covered after filling to ensure no contamination enters the valve. For cryogenic and liquefied gases supplied by tanker, the transfer hose is purged with gas prior to filling the tanker and prior to making the delivery to ensure that any contamination inside the hose is removed prior to the product being transferred.

In gas production processes there are no sterilisation treatments, however the conditions in which gases are produced, including temperature, pressure, and lack of humidity and nutrients, does not allow microorganism to proliferate [5]. Moreover, the decompression step when the gas is used significantly deteriorates the microbial cells, possibly by shear forces, and affect their ability to generate colony forming units (CFU) [6].
In particular, several researchers reported the microbial inactivation effects of compressed carbon dioxide (CO2) on different types of microorganisms in different experimental conditions of temperature, pressure and moisture. This inhibitory action has been increasingly applied to improve the hygiene of both liquid and solid foodstuffs by protecting them from bacterial spoilage [7]. However, there are some rare microorganisms able to thrive in extreme environments. These extremophiles include, for example, pressure-phile, anhydrobiotic and low pH-phile microbes [8].

It is clear that an experimental approach is important. In the bibliography there are some specific studies, article and technical papers reporting activities of sampling, analysis on different gases with related results. The present paper reports this data by references and supports the EIGA Technical Bulletin TB02 [2].

2 Scope and purpose

2.1 Scope

The scope of this scientific report specifically covers the microbiological quality requirements for gases used for medical, pharmaceutical and food applications. It covers compressed and liquefied gases supplied in high pressure cylinders, and cryogenic liquids supplied by tankers into bulk storage tanks or in portable cryogenic containers.

It covers the quality of the gas up to the point of delivery into the customer's storage tank or at the outlet valve in high pressure cylinders or portable cryogenic containers. It does not address the quality of the gas once it has been distributed to the usage point via the customer's pipeline system. It does not cover medicinal or food grade gases that are produced using either Pressure Swing Adsorption (PSA) or Air compressing plants on the customer's premises.

This scientific report is based on a bibliographic search on scientific articles on the matter. The bibliographic research strategy is given in chapter 7.

This publication relates only to the microbiological quality of the gas and does not cover the external condition of the container.

2.2 Purpose

This scientific report is intended as support to the EIGA Technical Bulletin 02 [2] and is for use by EIGA Members and national regulatory authorities involved in the regulation of manufacturing of medical, pharmaceutical and food gases.

3 Abstract of this Scientific Report

In recent years, there is a need to characterise potential impurities, including microbiological, present in gases. The drive, in addition to regulation, is linked to the requests from end users and authorities. As there is not a sterilisation step in gas production processes, gases are not defined as sterile, despite the extreme conditions existing in production in terms of temperature, pressure and lack of humidity and nutrients. In the present work, the microbiological aspects of gases for food and pharmaceutical use are presented with the related data from sampling to analytical activity. Different sampling methods have been tested and, to date, the best is the impaction method, validated with recovery tests with different American Type Culture Collection (ATCC) strains. For medical gases, the specific reference is the European Pharmacopoeia with parameters and limits for non-sterile products for inhalation use. The data collected, based on more than one thousand analyses, reveal an average microbial load of the order of approximately 10 CFU/Nm3 with maximum values of 100 CFU/Nm3 and 50 CFU/Nm3, respectively for bacteria and fungi, and an absence of pathogens and opportunistic microorganisms. The results refer both to liquid bulk and package gas production processes and considers the possible microbial contribution of the cylinder surfaces content. Data confirms that the microbiological quality of gases complies with the European Pharmacopeia acceptance criteria for inhalation use. A bibliographic search of scientific literature was performed and the most complete scientific approach is the one developed in Italy.

Key words: medical, food, technical and special gases; microbiological quality; gas sampling; gas analysis, recovery test, microbial counts.

4 Sampling, Analysis, Recovery test and Results

This chapter reports the main results relating to compressed gas sampling, microbiological analysis and recovery tests. The information is divided into paragraphs, each relating to a reference, as the reported experiences differ in the approach and operating methods.

In the technical paper Life in extreme environments, authors investigated if micro-organisms survive in compressed gas system, and if they survive, what appropriate methods are applied in order to collect and enumerate them [9].

Recovery tests have been done on compressed air in cylinders at pressures from 10 bar to 170 bar, on 13 microorganisms with impaction and filtration sampling.

The tested microorganisms were cultivated on Tryptone Soy Agar (TSA) and on Sabouraud Dextrose Agar (SDA). Results indicated that microorganisms can be collected and grow from compressed gas with recovery of 85% at 10 bar and 0% at 170 bar. Up to 10 bar, 11 of the 13 microorganisms survived and grew. Sampling methods tested were comparable. During the impaction sampling it is important to know, and to maintain constant, the impaction speed of the microorganisms on the agar surface, to have an uniform an reliable sampling performance.

In the scientific note Collaborative studies for the establishment of microorganisms in medicinal gases, microbiological data on air and nitrous oxide (N2O) medicinal gases are reported at hospital sites from four compressors, both lubricated with water and oil and oil free compressors, working at 8 to70 bar [10]. Two sampling methods based on impingement and filtration were tested. The two sampling methods were practical to use, however for both, the amount of detected viable microorganisms was at the detection limit. TBC (Total Bacterial Count) and Fungi plus API (Analytical Profile Index – enzymatic test) and endotoxins were analysed. The TBC was at the detection limit (2 CFU/m3 – 50 CFU/m3) with an absence of Fungi. API and endotoxin were below the detection limits. Positive plates resulted in 27% from filtration and 4% from impingement.

From the overall low level of gas contamination found in this study, authors concluded that there is no requirement for regular control and monitoring of microbial quality of the medicinal gas during production.

The paper Examination of microbiological according to the method of the European Pharmacopoeia while manufacturing medical gases reports tests performed on oxygen, nitrogen and argon from cylinders and air after molecular sieves in Air Separation Units (ASU) [11]. The survey also included the analysis of the inner surfaces of cylinders and of the ambient air in front of main air compressor. Membrane filtration method was used for gas sampling. Total aerobic and anaerobic bacterial count and fungi (yeasts and moulds) were analysed as analytical parameters on about 100 litres of samples, for n=15 cylinders and no microbial growth was detected after 7 days of incubation time.

The data showed that the initial microbiological contamination is clearly reduced by the production process, airborne microorganism was detected in air and after molecular sieves, and no microorganism was been detected from gases in cylinders.

The inner surfaces of the cylinders (15 cylinder) were slightly contaminated: aerobic count = 0-219 CFU/cylinder, anaerobic count = 0-48 CFU/cylinder and only on argon, yeast was absent and mould = 0-24 CFU/cylinder. By opening the cylinder valve during sampling, it is possible to have ambient air contamination.

The results show that there is no hygienic risk for patients treated with medical gases produced with the mentioned production process and that the process can be evaluated as valid from the microbiological point of view.

This validation is valid for two years, with no major changes to the production process. A periodically revalidation is recommended every two years by the author.

In the paper Impurities in gases and gas mixtures: metals and bacteria, sampling and analytical methods were tested over more than 10 years of activity in bulk and package gas production [12]. Hundreds of samples were taken with impaction method at the ASU plant, on gases in cylinders and on the inner surfaces, for air, oxygen, nitrogen, carbon dioxide, and nitrogen monoxide. The results made it possible to establish a specification for the microbiological quality of the produced gases, both for medical and for food gases, as <200 CFU/Nm3 and absence of pathogens (as acceptance criteria of the EU Pharmacopoeia). In this reference there are a series of research activities, divided by topic.

Figure 1-2-3: images of different sampling apparatus: bubbling, cyclone and impaction [12].

4.1 Gas Bubbling Sampling

The method is based on the bubbling principle of a known gas volume into a known volume of collection liquid, tested collection liquids include; Peptone, Tween 80, Antifoam A, Buffered peptone water, Tween 80, physiological solution and distilled water. Gas sampling took place directly from the cylinder, sampling about 1000 litres at a flow rate of about 120 litres/hour. Total Bacterial Count at 30°C and Fungi at 22°C analyses, with an incubations time of five days were performed, with blank tests as negative controls. Testing did not identify an operating mode that avoids sample contamination: 62% of the negative controls were positive, contamination occurs randomly using different operating modes. Similar results were obtained from the Fungi analyses. Therefore, the bubbling method is not suitable for assessing the microbiological quality of a compressed gas.

4.2 Gas Sampling with Cyclone

The sampling activity was performed on a carbon dioxide production line with a pressure reducer and a sterile collection lung connected to the collection system. The Coriolis®µ system (from Bertin Technologies, Montigny-le-Bretonneux, France) was tested. The microorganisms were captured by a sterile liquid solution (Airtest Solution Triton X 0.005%) then divided into two rates, one for molecular evaluation, while the remaining volume was used for TBC and fungi analysis. At the end of the incubation period the growth was verified, and colony isolation and identification were performed using the Vitek 2 system (Biomerieux, F).

For the molecular analysis, Universal primers 16S bacteria and Syber Green chemistry using Real Time PCR (Eco Real-Time PCR System) were used. For each sampling, 1000-3500 gas litres were collected.

The sampling (n=16) and analysis (n=47 samples) revealed the presence of bacterial and fungal contaminations at a level lower than 1 CFU/m3. For bacteria, in particular, it was also possible to identify the bacterial species isolated.

For analytical activities in molecular biology, four different methods of sample preparation and DNA extraction were tested using positive controls.

The best method selected for the subsequent steps was the centrifugation method accompanied by mineral oil lysis. To date, the lower limit of sensitivity achieved was about 300 CFU/m3 on a sampling volume of 3.25 m3.

4.3 Gas Impaction Sampling

Sampling is based on the tangential impact principle of a known gas volume on a plate containing agarised medium, according to ISO 8573-7, Compressed air. Part. 7: test method for viable microbiological contaminant content [13].

Gas sampling took place directly from the cylinder or a pressurised line using a pressure reducer with rubber holder. The sampler was connected with sterile tubes, sampling about 500-1000 litres at a flow rate less than 120 litres/minute.

The sampling was performed with a sampler for compressed gases (PBI International) with the detection of environmental conditions during time.

Microbiological gas results from Bulk Production

Samples were collected from an ASU production plant (downstream of the molecular sieves), from a carbon dioxide plant (downstream of the dust filter) and from an hydrogen plant (downstream of the filters, upstream of the pipeline) for the analysis of total aerobic and anaerobic bacterial count, fungi (moulds and yeasts), Bacillus cereus, Pseudomonas aeruginosa and Clostridium perfringens.

The results put in evidence show that the molecular sieves in an ASU production plant act as filtering elements for ambient air and that there was no correlation between microbial growth and gas humidity.

The average microbial growths within the units was a few tens of CFU/Nm3 with prolonged incubations time up to 15 days, and maximum values of Total Bacterial Count (TBC) at 30°C and Fungi at 25°C of 49 and 40 CFU/Nm3 respectively with an absence of specific organisms (opportunists and pathogens) with 30% of positive plates.

Table 1: microbiological results on gases from primary production – all the data (ASU, CO2, H2)

	TBC CFU/Nm^3	**Fungi** CFU/Nm^3	**Anaerobic** CFU/Nm^3	***B. cereus*** CFU/Nm^3	***C. perfringens*** CFU/Nm^3	***P. aeruginosa*** CFU/Nm^3
N° data	66	66	55	55	58	58
AVERAGE	**1.64**	**1.58**	**0**	**0**	**0**	**0**
MIN	0	0	0	0	0	0
MAX	49.0	40.4	0	0	0	0
N. Positive	18	21	0	0	0	0
N. Negative	48	45	55	55	58	58
% positive	**27.3**	**31.8**	**0.0**	**0.0**	**0.0**	**0.0**

Microbiological gas results from Secondary Production – Medical gases

A number of samples of oxygen, nitrogen, carbon dioxide, air and nitric oxide, have been analysed for a total of n=569 analyses in more than six years. The average microbial growths were within the units of CFU/Nm3 with prolonged incubations time with maximum values of TBC at 30°C and Fungi at 25°C of one hundred of CFU/Nm3, with an absence of specific organisms (opportunists and pathogens) with 30%-40% of positive plates. The maximum growth has been detected on oxygen and carbon dioxide with prolonged incubation times.

Table 2: microbiological results on packaged medical gases – all the data. Results in 5 days and at 15 days incubation. In the last row are reported the acceptance criteria for microbiological quality of non-sterile dosage forms [1]

	Standard incubation time					Prolonged incubation time				
	TBC/TAMC CFU/Nm^3	**Fungi** CFU/Nm^3	***S.aureus*** CFU/Nm^3	***P.aerug.*** CFU/Nm^3	***Entero*** CFU/Nm^3	**TBC/TAMC** CFU/Nm^3	**Fungi** CFU/Nm^3	***S.aureus*** CFU/Nm^3	***P.aerug.*** CFU/Nm^3	***Entero*** CFU/Nm^3
N° data	74	59	60	59	56	64	49	50	49	49
AVERAGE	**0.76**	**0.43**	**0.00**	**0.00**	**0.00**	**4.02**	**1.06**	**0.00**	**0.00**	**0.00**
MIN	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MAX	8.29	2.89	0,00	0,00	0.00	100	15.74	0.00	0.00	0.00
Positive N°	23	17	0	0	0	27	18	0	0	0
Negative N°	51	42	60	59	56	37	31	50	49	49
% positive	**31**	**29**	**0**	**0**	**0**	**42**	**37**	**0,0**	**0,0**	**0,0**
Ph.Eur. (CFU/ml)	200	20	absence	absence	absence	-	-	-	-	-

Microbiological gas results from Secondary Production (Package) – Food gases

Sample of oxygen, nitrogen, carbon dioxide, argon and mixture have been analysed for a total of n=324 analysis over six years. The average microbial growths are within the units of CFU/Nm3 also with prolonged incubations time with maximum values of TBC at 30°C and Fungi at 25°C of 74 CFU/Nm3 and 7 CFU/Nm3, with 17%-29% of positive plates. The maximum growth has been found on nitrogen, oxygen and carbon dioxide with prolonged incubation times. Some analysis on E.coli parameter have been always resulted negative. Prolonged incubation times allow a greater recovery, mainly for TBC.

Table 3: microbiological results on packaged food gases – all the data. Results in 5 days and at 15 days incubation

	standard incubation		prolonged incubation	
	TBC CFU/Nm^3	**Fungi** CFU/Nm^3	**TBC** CFU/Nm^3	**Fungi** CFU/Nm^3
N° data	92	49	114	69
AVERAGE	**0.16**	**0.30**	**1.99**	**0.66**
MIN	0,00	0,00	0.00	0.00
MAX	4.5	2.7	73.7	6.9
N. Positive	9	12	33	20
N. Negative	83	37	81	49
% positive	**9.8**	**24.5**	**28.9**	**29.0**

Inner cylinders surface - Washing water sampling and analysis

Washing waters sampled from cylinders of food gases (pure gas and mixture) and medical cylinders (oxygen, nitrogen, carbon dioxide, air) have been analysed for microbiological parameters, Total Bacterial Count, Fungi and E.coli.
The results put in evidence have an aerobic microbial count with range of 0 to 250 CFU/cylinder. The content, referred to the entire volume of the cylinders, is of the same order of magnitude of the data reported for gas matrix.

4.4 Recovery Tests from Cylinders

Tests were carried out on n=4 light-alloy cylinders of 20 litre water capacity each with brass valves and filled with nitrogen at about 150 bar, after a cleaning procedure with hot nitrogen.

The inoculum was performed using the Bacillus subtilis strain, ATCC 6633, and *Clostridium sporogenes* ATCC 19404, in the form of lenticules dissolved in 0.15 ml distilled water, inserted inside the cylinders before the filling. Three cylinders were used as positive controls while the fourth as a negative one. Sampling has been done with impaction method for the entire content of the gas and at the end the when cylinders were opened for sample recovery and related analysis on washing water (total aerobic microbial count and total anaerobic microbial count). The cylinders were sampled immediately after the filling, then after one and two months after the filling. The recovery tests with *B.subtilis* put in evidence show recovery efficiency of about 60% with widespread growth. The test was repeated with another bacterial strain, *C. sporogenes*, inoculating 58.7 CFU/cylinder with an average recovery equal to 83.5%: 86.9% in the first cylinder (51 CFU), 81.8% in the second (48 CFU) and 81.8% in the third (48 CFU) while the negative control remained negative.

The recovery values were obtained only from the liquid phase on cylinders' washing water, while in the gas phase the recovery was equal to zero. The results indicate that the microbial load remains adhered to the internal walls of the cylinder.

In the paper Microbiological Quality of Gases, a survey in a production sites was performed on food pure gases and mixtures in 40 L cylinders at 200 bar (nitrogen, carbon dioxide, oxygen and mixtures of nitrogen and carbon dioxide [70-80% and 30-20%]) and analyses performed using impaction and liquid impingement methods [14]. Average values of TBC = 0.96 CFU/Nm3 and Fungi = 0.60 CFU/Nm3 with maximums of 6 CFU/Nm3 and 3.8 CFU/Nm3 respectively. Among Fungi, only mould was detected. On 44 analyses, the positive plates were 41% for Total Bacterial Count and 30% for Fungi.

Some recognition by sequencing the genetic material has highlighted only the presence of organisms of environmental nature.

The results indicate that the impaction sampling was better than the impingement mrthod with low microbial count in food gases, less than 1 CFU/Nm3, both for TBC and Fungi, and with absence of pathogens.

Authors emphasised that as it is not possible to rule out a microbiological contamination, it is important to establish the quality of the gases.

In the paper Detection of micro-organisms in compressed gases, authors have studied a specially nebulising chamber, where a spore-containing aerosol was generated under pressure, to study microbial recovery (*Bacillus atrophaeus* ATCC 9372) with different sampling methods [15]. The

bacterial spore count of the aerosol was determined by the membrane filtration method and by means of an air sampler for pressure gases with impaction sampling method. Results indicated that both test methods yielded statistically significant, reproducible results. By applying the impaction method a collection efficiency of 92% could be achieved.

5 Discussion

This report has reviewed the scientific articles currently available concerning microbiological activity in food and medical gases. The report gathers the results of n=6 articles which involves thousands of analyses. The results cover the range of gases used in the medical and food sectors and, although they were obtained with different methodologies of sampling and analytical activity, they express a good case history. The gases have been sampled in production plants, both bulk and packaged productions, in Germany, in Italy, and in a Swedish hospital [11, 12, 14, 10]. All the authors conclude that the microbiological activity in food and medical gases is far below the acceptance criteria of the European Pharmacopeia with regard to microorganisms in non-sterile products for inhalation use (section 5.1.4).

Gases are produced under conditions, such as extreme temperatures, pressures, and lack of humidity and nutrients, that are not optimal for microbiological activity. The change of these conditions are also detrimental to the survival of microorganisms. Moreover, when using the gas from the cylinders, the gas decompression deteriorates, by shear forces, the microbial cells and inactivates them from generating colony-forming units (CFU). The review of these articles confirms the theory and experience, the microbiological activity in food and medical gases is very low.

Factors influencing the microbiological activity include the presence of humidity and nutrients. These factors are well controlled in the manufacturing and filling processes of gases by the use of well-established risk assessment and quality management systems. The water content in gases shall be very low (< 67 ppm for medicinal gases according to the European Pharmacopeia) and controlled by routine analysis. Cylinders are pressure-tested and inspected internally periodically in according with regulations (this is a direct control of presence of water). Microbiological contamination is also controlled by manufacturing the gases in entirely closed systems and supplied under pressure, ensuring no contact with ambient air.

The tested sampling systems (impaction, filtration and impingement) showed that they were comparable in terms of results obtained, whereas the bubbling method highlighted the presence of false positive results. In order to evaluate the most suitable sampling system, the results of the recovery tests (carried out only by some authors and only on Air [9] and Nitrogen [12]) should be evaluated. In the first case, the tests were performed with impaction and filtration sampling with comparable results on 13 microorganisms with recovery of 85% up to 10 bar and recovery of 0% at 170 bar due to the high pressure. In the second case, the gas was sampled by impaction on two bacterial strains with recoveries ranging from 60% to 86% in cylinders at 150 bar. This test also showed that the recovery, which was stable even after two months from the filling of the cylinders, was found only in the washing water of the inner walls of the cylinders and no recovery from the gas matrix. Moreover, in the tested conditions, some microorganisms prefer the inner cylinder walls as habitat and do not move into the gas. The results of the recovery tests performed with impaction and filtration sampling methods in a nebulising chamber with a spore-containing aerosol under pressure, confirm the validity of the systems tested with recovery yields of 92%. [15] To sum up, the impaction and filtration sampling methods, for which recovery tests are available, are comparable. Between the two techniques, the impaction method is to be preferred as it has a reference in an international standard for compressed air [13].

For the analytical activities, the results reported by all the authors, appear comparable as total microbial, bacterial and fungal count, with no growth or within few CFU/m3 units, with sporadic maximum values within 100 CFU/m3 [12].

It should be emphasised that even if sterile material is used, the sampling is mainly carried out at the production sites and thus environmental contamination cannot be excluded.

As for the microbiological indicator parameters reported in the Pharmacopoeia, for non-sterile products for pharmaceutical use (*S. aureus, P. aeruginosa* and Gram-negative bile tolerant), only one

author reports results on these specific indicators which are always negative [12]. In addition, these analyses were carried out with prolonged incubation times of the plates up to 15 days to confirm higher recovery yields. To complete the analytical investigations, this author reports research activities for the development of analytical methods in molecular biology, which have confirmed the same order of magnitude of the microbiological results obtained with traditional methods and the absence of pathogens. Overall, the microbiological investigation research carried out in Italy appear to apply the most complete scientific approach on all main types of gas productions with over a thousand of analyses, which allowed the establishment of microbiological quality of the gases at values of <200 CFU/m3 and the absence of pathogens.

Further investigations on the microbiological quality of the gases were conducted to evaluate the microbial contribution of the inner surfaces of the cylinders [11, 12]. The results showed that the microbial load (aerobic, anaerobic and fungal counts) was of the same order of magnitude as the one reported for the gas phase. Therefore, the microbiological contribution of the inner surfaces of the cylinders to the gas quality, is minor or negligible.

Each author carried out an evaluation on the microbiological quality of the gases and on the possible necessity of verification checks. All the studies confirmed that the analytical findings are within the acceptance limits of the Pharmacopoeia for non-sterile products for inhalation use, both for total microbial counts and on absence of pathogens (section 5.1.4). The findings over the years also showed that, although a microbiological contamination cannot be excluded, the production processes determined that gases are safe from a microbiological point of view with no hygienic risk to the end users [14]. The production processes are able to produce reproducible microbiological impeccable gases [11, 12].

There are different opinions concerning the need for periodical verification checks. Zingre et al suggested no need for regular control and monitoring of microbial quality of the medicinal gas during production [10]. Brill et al recommended that validation lasts for two years if no major changes occur to the production process, with a periodical revalidation every two years [11]. However, the author of this paper is a consultant to a testing lab and for this reason may have a different approach to the assessment on revalidation.

Revalidation is unnecessary where there is very limited detection of microbiological activity and the manufacturing processes do not favour conditions for growth.

Bissolotti et al performed multiple tests with scientific approach to validate the methods and deepen some themes, for example recovery tests on different gases, quality of the cylinder inner wall and new analytical approaches such as in molecular biology to obtain results in a shorter time (24 hours compared to 5 to 15 days) [12].

6 Conclusion

This report provides the results from sampling and analytical activities to evaluate the microbiological quality of food and medical gases, in production processes and at the outlet of cylinders. The gases investigated were, air, oxygen, nitrogen, argon, carbon dioxide, nitric oxide, hydrogen and mixtures.

Different gas sampling methods have been tested, some also with recovery test, including impaction, filtration, bubbling and impingement. Except for the bubbling method, the sampling methods are adequate to obtain significant results. Based on data, the impaction method provided most reliable data and this method is also reference in ISO 8573-7 on compressed air [13]. No correlations have been reported between the microbial loads and the operating parameters at the process productions.

Impaction and filtration sampling methods have been validated with recovery tests with different ATCC strains on air and nitrogen. On compressed air, the recovery has been of 85% at 10 bar and 0% at 170 bar. Up to 10 bar microorganisms do survive, while at 170 bar they do not. The recovery test on nitrogen, from 150 bar to ambient pressure, gave an average value equal to 84% in the liquid washing water of the cylinders (not in gas product phase). The results indicate that the microbial load remains adhered to the surface of the cylinder.

The microbiological analysis has been mainly done with the plate count method, with references in European Pharmacopoeia and ISO. Best growth rate has been documented with prolonged incubation time of two weeks. The parameters analysed are: Total Aerobic Microbial Count, Total Yeast microbial Count, Staphylococcus aureus, Pseudomonas aeruginosa and bile-tolerant gram-negative bacteria and also Total aerobic and anaerobic bacterial count, Fungi, Bacillus cereus, Clostridium perfringens and Escherichia coli.

All the data, more than a thousand of analyses, reveal an average microbial load of order of magnitude of few units of CFU/Nm3 with maximum values of 100 CFU/Nm3 and 50 CFU/Nm3 respectively for bacteria and fungi and absence of pathogens and opportunists. The results refer both to bulk and package production processes, with comparable data, also considering the possible microbial contribution of the cylinder surfaces content. Some results derived from analytical molecular techniques confirmed the same order of magnitude of the traditional approach.

In gases there is an extremely low level of microbial counts and absence of pathogens. Moreover, the manufacturing and filling processes conditions (temperature, pressure, low level of humidity and nutrients) are extreme and the growth conditions are unfavourable when storing gases in tanks and gas packages. Ambient cross contamination is not possible because gas is always produced, stored and transported in closed systems and packages. Finally, at the gas usage point, depressurisation during usage damages the microbial cells.

Based on the above discussion, analytical measurement data confirm that the microbiological quality of gases are well below the European Pharmacopeia acceptance criteria for non-sterile products for inhalation use.

Based on the review of the above documents it is not required to monitor the quality of gases compared to the acceptance limits of the European Pharmacopeia.

7 References

Bibliographic research strategy: the search was carried out on websites using the following keywords: microbiological quality of compressed gases, compressed gases microbiological assessment, microbiological analysis on compressed gases, medical and food gases microbial aspects, compressed gases sampling, microbial cell viability in compressed gases. Some of the following references were provided by the authors themselves.

[1] European Pharmacopoeia, 10th edition, section 5.1.4. Microbiological quality of non-sterile products for pharmaceutical use.
[2] EIGA TB 02 Microbiological Quality of Medical, Pharmaceutical and Food Grade Gases. www.eiga.eu
[3] GICR, 2018. Guideline: Convalide ambientali - Monitoraggi Particellari e Microbiologici (Environmental validation – Particle and biological monitoring).
[4] European Medicines Agency, 2018. Guideline on the quality of water for pharmaceutical use.
[5] Denoya C., 2015. Microbial survival in compressed gases under fast decompression to normal atmospheric conditions. Technical paper, Particle Measuring Systems.
[6] Sandle T., 2015. Microbiological assessment of compressed gases in pharmaceutical facilities. Journal of Validation Technology. Institute of Validation Technology.
[7] Debs-Louka E., Louka N., Abraham G., Chabot V., Allaf K., 1999. Effects of compressed carbon dioxide on microbial cell viability. Applied and Environmental Microbiology, 65(2): 626-631.
[8] Rothschild L.J., Mancinelli R.L., 2001. Life in extreme environments. Nature, Vol. 409.
[9] Zingre H., Meier R. Detection of micro-organisms in compressed gases. MBV AG.
[10] Hamilton L., Agerkvist I., Lofter A., Falt I.C., Wessen B., 2001. Scientific notes. Collaborative studies for the establishment of microorganisms in medicinal gases. Pharmeuropa, Vol.14, N.1.
[11] Brill F. et all., 2011. Examination of microbiological according to the method of the European Pharmacopoeia while manufacturing medical gases. Validation Report N. P11/013013. (MESSER document).
[12] Bissolotti G., Pasinetti E., 2019. Impurities in gases and gas mixtures: metals and bacteria. Article in review of Aqual, Accreditation and Quality Assurance Journal, Springer. Data presented at “Gas Analysis 2019” Symposium. (SIAD S.p.A. document).

[13] ISO 8573-7 Compressed air - Part 7. Test method for viable microbiological contamination content.
[14] Bissolotti G., Pasinetti E., 2013. Safety in Food Production: Microbiological Quality of Gases. Controlled & Modified Atmosphere Research Conference. (SIAD S.p.A. document).
[15] Ewald R., Meier R., 2004. Detection of micro-organisms in compressed gases. Swiss Pharma, 26, N.10a, 16-18.