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## **Detection of micro-organisms in compressed gases**

Validation of MAS-100 CG\*  
using compressed gases

Dr R. Ewald, Binningen, and R. Meier, Reitnau

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### Introduction

*Taking ISO standard 14698-1<sup>[1]</sup> into account, the test method using an MAS-100 CG and the membrane-filter method were compared in a given pressure range. Both methods were applied under identical test conditions. Compressed air was used as a pressure gas. Since ISO standard<sup>[1]</sup> Annex B (Guidance on validating air samplers) contains no instructions regarding the generation of aerosols in the pressure range concerned, this methodology had to be self-developed (the spinning-top or spinning-disc aerosol generator cannot be used in this pressure range). By using a self-developed ultrasonic nebulizing chamber, the spore suspension of *Bacillus subtilis* variation niger given in the ISO standard<sup>[1]</sup> could be nebulized and then the microbial count was determined by membrane filtration or by the MAS-100 CG.*

an ex-proof version and thus is suitable for use in explosion-proof zones. By default the MAS-100 CG is calibrated for air, nitrogen, argon and carbon dioxide, but it can also be calibrated for other gases.

### Material

MAS-100 CG Ex with sieve plate, 300 × 0.6mm holes, made of aluminum (CG)  
Membrane filter of mixed cellulose esters, 0.45µm pore size, sterilized (MF)  
Air compressor Atlas-Copco, prefilter (type DD32) and afterfilter ( type PD32), for separating oil, condensate and soil particles  
Ultrasonic nebulizing chamber, capacity approx. 500ml, made of clear polycarbonate  
Tryptic soy agar – Petri dishes, BioMérieux (TSA)  
Aqueous, concentrated suspension of *Bacillus atrophaeus* ATCC 9372 (former *Bac.subtilis v.niger*), Raven Biological Laboratories, Inc. Ethanol 80%

The bacterial suspension was prepared according to ISO standard 14698-1<sup>[1]</sup>; Annex B; B 2.2.1 ff., so as to obtain a concentration of 1.0–1.5 × 10<sup>4</sup>cfu/ml in 80% ethanol. To obtain this concentration, the required volume of bacterial stock suspension was diluted with 80% ethanol.

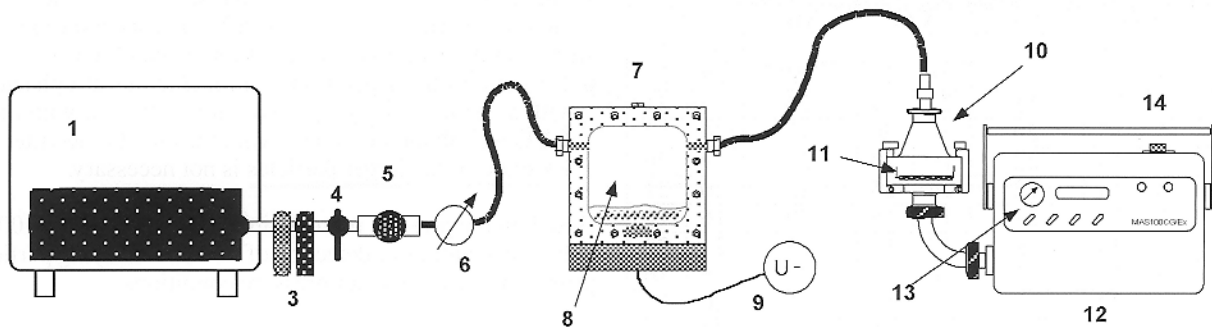
### Description of MAS-100® CG Ex

The MAS-100 CG Ex is an air-sampling system which is based on the well-known impaction principle of the MAS-100<sup>[3]</sup>. The micro-organisms are directly impacted on a 90-mm standard Petri dish filled with culture medium. This air sampler was specially developed for the microbiological monitoring of pressure gases. The micro-organisms are collected under working pressure, without risking a sublethal damage by the following decompression. The suction volume of 100 liters/minute is electronically controlled over a pressure range of 1.6 to 10 bar absolute pressure. Contrary to other systems, sampling is always performed in the defined pressure range. The instrument is delivered ex-works as

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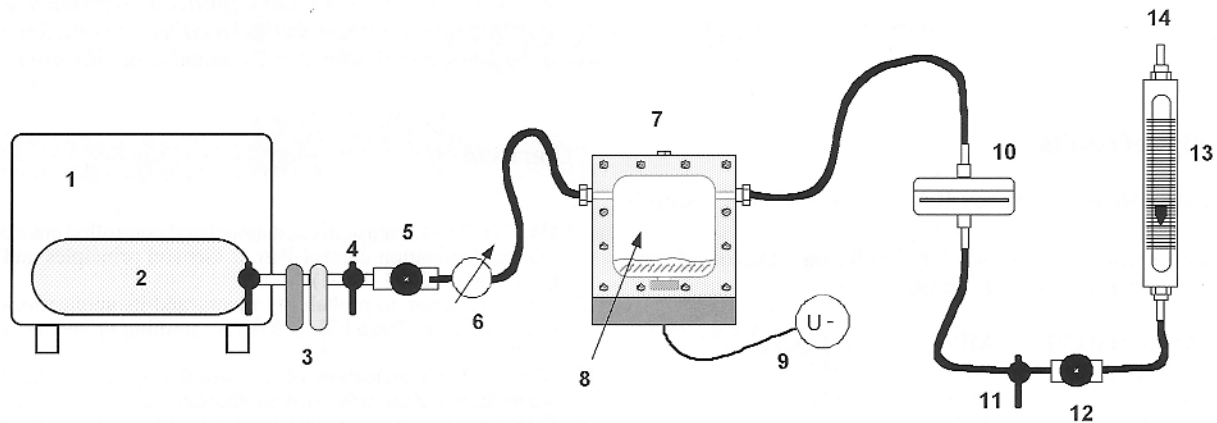
**Method**

Experimental setup for MAS-100 CG



1 compressor, 2 pressure tank, 3 prefilter and afterfilter, 4 stop valve, 5 pressure-control valve, 6 manometer, 7 nebulizer, 8 nebulizing chamber, 9 ultrasonics 10 pressurized sampling head, 11 Petri dish, 12 MAS-100 CG, 13 manometer, 14 air outlet

Experimental setup for membrane filtration



1 compressor, 2 pressure tank, 3 prefilter and afterfilter, 4 stop valve, 5 pressure-control valve, 6 manometer, 7 nebulizer, 8 nebulizing chamber, 9 ultrasonics, 10 filter holder, 11 stop valve, 12 control valve, 13 Rotameter, 14 air outlet

**Procedure**

100 ml of the bacterial suspension (106 spores) was filled into the nebulizing chamber and a (absolute) pressure of 2.6 bar was applied to the whole system. For a period of 30 seconds, a spore-containing aerosol was generated by means of ultrasonics. Subsequently this – visible – mist was either aspirated through a membrane filter, using a (dynamic) pressure of 1.6 bar, and placed on a Petri dish filled with TSA, or directly impacted onto a TSA Petri dish. (For technical reasons, the two test procedures could not be performed in parallel). The agar plates were then incubated at 30°C for 2 days.

With the filtration method, the aerosol was sucked off during one minute with an air flow rate of 10 liters/minute, which corresponds to the 20fold volume of the nebulizing chamber. In order to attain an approx. equally long rinsing period of the system, a test-gas volume of 250 liters was selected, which, under a dynamic pressure of 1.6 bar, corresponds to a collection period of approx. 1½ minutes.\*

With both procedures, a series of 10 individual samples was tested on each of three different days. The microbial recovery attained with the MAS-CG was expressed – according to the ISO standard – as a percentage of the membrane-filtration recovery. Additionally, the differences between the two procedures were checked statistically using the t test.

\* Boyle Mariotte Law: An empirical law which states that the product of pressure and volume is constant for an isothermal process;  $p \times V = \text{constant}$ . The MAS-100 CG is operated at a constant flow rate of 100 liters/minute, corresponding to a total collecting period of 2½ minutes for a sample volume of 250 liters and a pressure of 1 bar. At a pressure of 1.6 bar, the corresponding collecting period for a 250-litre sampling volume is 1 minute and 34 seconds.

**Results**

Colony count/TSA from 10 individual samples

Day 1			Day 2			Day 3		
MF	CG		MF	CG		MF	CG	
	r	Pr		r	Pr		r	Pr
12	18	19	15	21	22	22	24	25
18	20	21	11	23	24	24	20	21
21	15	15	20	9	9	11	26	27
20	10	10	22	19	20	31	19	20
22	13	13	22	24	25	23	20	21
23	20	21	27	15	15	29	16	16
29	29	30	19	17	17	23	21	22
28	18	19	12	20	21	21	16	16
19	19	20	22	17	17	25	20	21
16	15	15	35	25	26	27	24	25
<b>m= 20.8</b>	<b>m=18.3</b>		<b>m=20.5</b>	<b>m=19.6</b>		<b>m=23.6</b>	<b>m=21.4</b>	
<b>t=1.05</b>			<b>t=0.32</b>			<b>t=1.06</b>		

MF = Membrane filtration, CG = MAS-100 CG air sampler for pressure gases, r = number of colony-forming units (cfu) on the Petri dish, Pr = value with statistical correction according to Feller<sup>[2]</sup>, m = mean value

The barrier of significance for 2p 0.05 of the t test amounts to 2.10

**Interpretation of results**

(ISO standard 14698-1<sup>[1]</sup> ; Annex B.3 "Interpretation of results")

Collection efficiency of the MAS-100 CG for particles of approx. 1µm size (mean value from 10 samples)

	MAS-100 CG	MF	Efficiency
Day 1	m = 18.3	20.8	88%
Day 2	m = 19.6	20.5	96%
Day 3	m = 21.4	23.6	91%

As compared to the membrane-filter method, the MAS-100 CG has an average efficiency of 92%. According to the t test there is no significant difference between the microbial counts determined by the two procedures.

**Discussion**

By using ultrasonic nebulization, a spore-containing aerosol could be generated under pressure and the microbial count of this aerosol determined using the MAS-100 CG. Membrane filtration served as the method of reference. On three different days, a series of 10 individual samples was examined by each of the two methods and the results were statistically evaluated. All three test series yielded reproducible, corresponding results which could be statistically

proven by the t test. The average efficiency of the MAS-100 CG for approx. 1µm particles is 92%. Since the cut-off value calculated<sup>[4]</sup> amounts to 1.12µm, this means that practically all particles of larger diameter are separated by the MAS-100 CG.

As regards the determination of the separated particles, the ISO standard specifies the use of five different concentrations of potassium iodide solution, which should yield particle sizes of 0.8–15µm. As the separation rate for Bacillus atrophaeus spores – with a particle size of approx. 1µm – attained with the MAS-100 CG was about the same as with the membrane-filter method, a detection of the larger particles is not necessary.

Based on the present, reproducible results, the MAS-100 CG enables the quantitative detection of microbe-carrying particles (approx. 1µm in size) under pressure conditions.

**Summary**

*In a specially developed nebulizing chamber, a spore-containing aerosol was generated under pressure. The bacterial spore count of the aerosol was determined by the membrane filtration method and by means of an air sampler for pressure gases (MAS-100 CG). Based on three different test series, it could be proven that both test methods yielded statistically significant, reproducible results. By applying the impaction method a collection efficiency of 92% could be achieved as compared to membrane filtration.*

**Literature**

- [1] ISO 14698-1 Cleanrooms and associated controlled environments – Bio-contamination control-Part 1: General principles and methods (first edition 2003-09-01)
- [2] An introduction to probability theory and its applications, Volume One (W. Feller) Page 174–175 (d) A sampling problem / Feller et al. 1950
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- [4] Performance of Bio-aerosol Samplers: Collection Characteristics and Sampler Design Considerations A.Nevalainen et al., Atmospheric Environment Vol. 26A, No. 4, 531–540, 1992

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