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# Determination of carbon dioxide concentrations in air from livestock housing systems

Reference method using the lung method as applied by Wageningen Livestock Research

J. Mosquera, J.P.M. Ploegaert and G.C.C. Kupers

Report 1284



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Wageningen Livestock Research

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Wageningen, December 2020

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Samenvatting NL In dit document wordt de zogenaamde longmethode beschreven voor de bepaling van koolstofdioxideconcentraties (CO<sub>2</sub>) met gaschromatografie, zoals toegepast bij Wageningen Livestock Research. Deze methode wordt vaak gebruikt als de referentiemethode voor CO<sub>2</sub>-concentratiemetingen bij emissiemetingen uit verschillende landbouwbronnen (huisvesting, mestopslag). Het doel van dit document is om de prestatiekenmerken van deze methode te bepalen, en de procedure voor de implementatie van deze methode voor CO<sub>2</sub>-concentratiemetingen bij huisvestingssystemen in de praktijk uit te werken.

Summary UK This document provides a description of the lung method as applied by Wageningen Livestock Research (WLR lung method). This measurement method is commonly used as a reference method for carbon dioxide concentration measurements from agricultural activities (livestock housing, manure storage). The objective of this document is to specify the performance characteristics of the method, and the procedure for preparation and deployment of this method in practice to measure CO<sub>2</sub> concentrations from livestock housing systems.

This report can be downloaded for free at <https://doi.org/10.18174/536449> or at [www.wur.nl/livestock-research](http://www.wur.nl/livestock-research) (under Wageningen Livestock Research publications).



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Wageningen Livestock Research is ISO 9001:2015 certified.

All our research commissions are in line with the Terms and Conditions of the Animal Sciences Group. These are filed with the District Court of Zwolle.

Wageningen Livestock Research Report 1284

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# Foreword

The determination of the reduction potential of ammonia reduction measures requires accurate measurements of the ammonia emissions. For naturally ventilated livestock housing systems, the tracer gas ratio method using CO<sub>2</sub> (produced by animals and manure) as tracer is commonly used to estimate emissions from livestock housing systems. This approach requires accurate measurements of the CO<sub>2</sub> concentration inside and outside the livestock building.

This document was elaborated to give a description of the lung method as applied by Wageningen Livestock Research (WLR lung method) to measure CO<sub>2</sub> concentrations in air sampled inside and outside livestock buildings. This work was conducted to specify the performance characteristics of the method, and the procedure for preparation and deployment of this method in practice.

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# Summary

This document is elaborated to give a description of the lung method as applied by Wageningen Livestock Research (WLR lung method) to measure carbon dioxide (CO<sub>2</sub>) concentrations in the air entering and leaving livestock housing systems. Sampling in this method is based on the use of a 40 liter Nalophan sampling bag (type NA300 25 µm) inserted in a closed vessel. Air is sampled into the sampling bag after first being led through a dust filter at a constant flow rate (~20 ml/min) through Teflon or polyethylene sampling lines and critical orifices by using a pump that evacuates air out of the vessel. Air samples are then analysed in the laboratory by using a gas chromatograph with a Haysep Q column and a thermal conductivity detector. This method is commonly used as a reference method for carbon dioxide concentration measurements related to agricultural activities (livestock housing, manure storage). The objective of this document is to specify the performance characteristics of the method, and the procedure for preparation and deployment of this method in practice.

Laboratory calibration of the WLR lung method against gas standards (standard calibration gases; reference method) showed that the acceptance criteria for verification of trueness of measurements was fulfilled. The WLR lung method has been shown to have a repeatability (concentration difference between replicates) of less than 5%. A decay in concentration up to 1% per day has been observed (section 5.3), meaning that samples are advised to be analysed within 24 h to minimize this error. The combined (expanded) uncertainty of the WLR lung method is estimated to be 10%.



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# 1 Introduction and scope

Agriculture is the most important source of ammonia emissions in the Netherlands. Ammonia can be emitted at all stages of the manure management chain (grazing of animals on pasture, livestock housing, manures storage, manure treatment, manure application into the field). Emission from livestock housing systems is the main contributor to ammonia emissions from agriculture, followed by application of manure to land. Cattle produce the greatest amount of emissions in the livestock sector, followed by pigs and poultry.

Since the 1990s, a number of measures have been introduced in the Netherlands to reduce ammonia emissions from agricultural sources. Determination of the ammonia reduction potential of these measures relies, among others, on accurate measurements of the ammonia emissions. For naturally ventilated livestock housing systems, the current measurement protocol in the Netherlands specifies that emissions shall be determined by using the tracer gas ratio method. One of the options allowed in the protocol is to use CO<sub>2</sub> produced in the housing by animals and manure as a tracer gas. This approach requires accurate measurements of the CO<sub>2</sub> concentration inside and outside the livestock building. Gas chromatography is accepted as an accurate method to determine CO<sub>2</sub> concentrations in air.

This document is elaborated to give a description of the lung method as applied by Wageningen Livestock Research (WLR lung method) including: the sampling of air from agricultural sources, determination of the CO<sub>2</sub> concentration in the sampled air by gas chromatography, determination of its performance characteristics, and description of the procedure for preparation and deployment of this method in practice. Section 2 gives an overview of the normative references used in this document. The most important terms and definitions are presented in section 3. Section 4 gives a short description of the WLR lung method. In section 5, the performance characteristics of the WLR gas washing method are discussed. Finally, section 6 summarizes the main conclusions of the results presented in this document.

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## 2 Normative references

This document incorporates information from other normative references, including:

CEN/TS 14793:2017	Stationary source emission – Intralaboratory validation procedure for an alternative method compared to a reference method
NEN EN ISO 20988:2007	Air quality - Guidelines for estimating measurement uncertainty
NEN ISO 21748:2017	Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation
NEN-EN 12341:1998	Air quality – Determination of the PM 10 fraction of suspended particulate matter – Reference method and field test procedure to demonstrate reference equivalence of measurement methods (in Dutch)
ISO 5725-1:1994	Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

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## 3 Terms and definitions

**Accuracy (ISO 5725-1:1994):** closeness of agreement of a measurement compared to the true value. Combination of trueness (systematic errors) and repeatability (random errors).

**Air sample:** volume of air taken from the main air flow and directed to the measurement equipment to be analysed.

**Calibration:** procedure performed to determine the systematic difference of a value measured by a measurement method, and the true value provided by a reference method. Calibrations in the laboratory are usually performed by using standard calibration gases.

**Detection limit:** lowest value of the air quality characteristic which, with 95% probability, can be distinguished from a zero sample.

**Flow rate:** the rate at which a volume of air passes through a particular system.

**Limit of detection:** smallest concentration which can be detected, but not quantified, by using the selected measurement method.

**Measurement:** any physical dimension that is described by units.

**Precision:** see repeatability.

**Random errors:** unpredictable errors which average to zero.

**Repeatability (ISO 5725-1:1994):** the closeness of agreement between independent test results by measuring the concentration in duplicate (simultaneous measurements) with the same measurement method. Usually expressed in terms of standard deviation of test results. Repeatability depends only on random errors and has no relation with the true concentration value (see trueness).

**Systematic errors:** predictable errors leading to a constant or proportional deviation of the measurement result compared to the true value.

**Trueness (ISO 5725-1:1994):** closeness of agreement between the mean value obtained from a large series of test results and an accepted reference value. Usually expressed in terms of bias or systematic deviation (error).

## 4 Description of the method

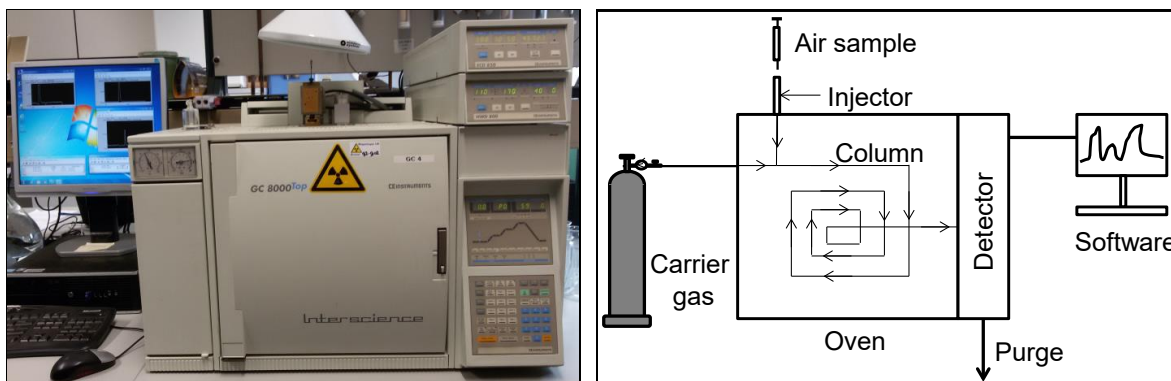
### 4.1 Measurement principle

The lung method is an active measurement method for point sampling, and gives an average CO<sub>2</sub> concentration over the whole sampling period. In this method, a 40 liter Nalophan sampling bag (type NA300 25 µm) is first inserted in a closed vessel and kept under vacuum conditions (Figure 4.1). An air sample is then taken from the sample location by using Teflon or polyethylene sampling lines, and sucking air from the container at a known flow rate (~20 ml/min) using a pump (Thomas Industries Inc., model 617CD32, Wabasha, Minnesota ,VS) and critical orifices (made of borosilicate glass and housed in a stainless steel container for protection; inside diameter: 8 mm; length: 80 mm). This creates an under pressure in the container, allowing the air sample to be drawn into the sampling bag, after first being led through a dust filter (type #1140, diameter: 50 mm, 5-6 µm, Savillex® Corp., Minnetonka, VS).



**Figure 4.1** Lung method. Closed vessels housing 40 liter Nalophan sampling bags (type NA300 25 µm) for air sampling. Air is sucked into the bags by using a pump (Thomas Industries Inc., model 617CD32, Wabasha, Minnesota ,VS), sampling lines (Teflon or polyethylene), and critical orifices (borosilicate glass (inside diameter: 8 mm; length: 80 mm), housed in a stainless steel container for protection; flow: ~20 ml/min).

Air samples are then analysed in the laboratory by using a gas chromatograph (Interscience/Carlo Erba Instruments Inc., Breda, the Netherlands, GC 8000 Top; column Haysep Q; detector: TCD (thermal conductivity detector); Figure 4.2). Gas chromatography relies on the individual separation characteristics (retention time) of different gases in an air sample between a mobile phase (an inert gas acting as carrier gas) and a stationary solid phase packed in a column. It involves the volatilization of the sample in a heated inlet port (injector), the separation of the components in a specially prepared column, the detection of each component by a detector, and the use of a carrier gas to transfer the sample from the injector through the column into the detector (Figure 4.2). The gas chromatograph is calibrated every year against a gas calibration standard (3500 ppm; see appendix 1). For the calibration line, ten dilution steps between 350 ppm and 3500 ppm (between 10% and 100% of the gas calibration standard) are applied, using the same dilution system (Signal 821) as described in section 5.1 and shown in Figure 5.2 (see also Appendix 2).



**Figure 4.2** Left: gas chromatograph (Interscience/Carlo Erba Instruments Inc., Breda, the Netherlands, GC 8000 Top; column Haysep Q; detector: TCD) used to determine the CO<sub>2</sub> concentration in the air sample. Right: schematic representation of gas chromatography.

## 4.2 Sampling procedure (field application)

For the application of the lung method in practice, the following steps must be followed:

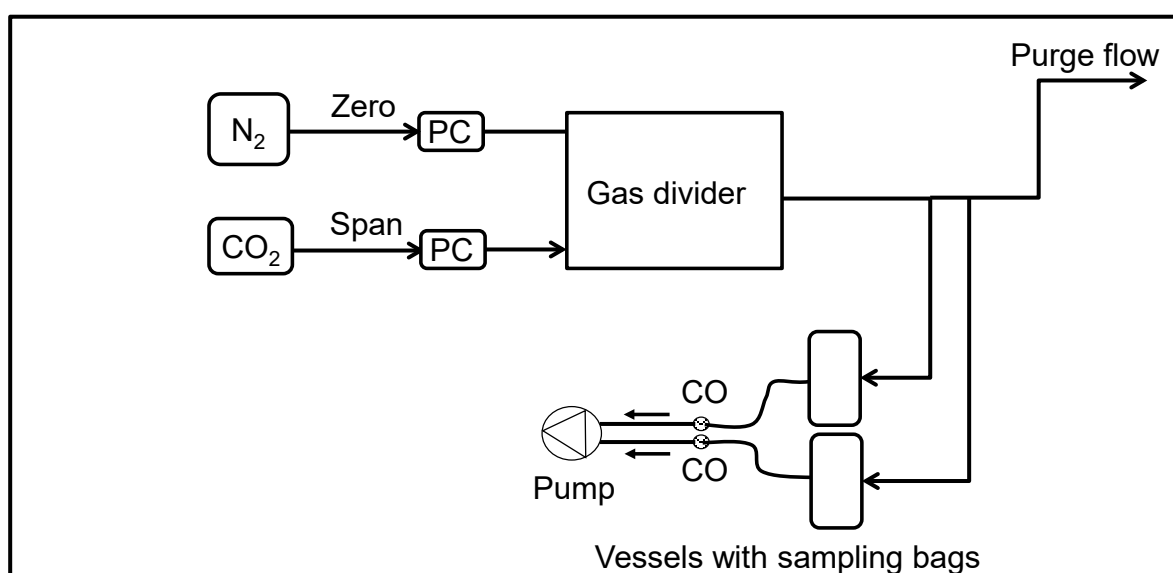
- Measurements performed in duplicate (two vessels), to get an accurate estimation of the concentration level; see Figure 4.1).
- At the measurement point, a filter (type #1140, diameter: 50 mm, 5-6 µm, Savillex® Corp., Minnetonka, VS) is placed to remove particulate matter from the air sample.
- The sampling line (Teflon or polyethylene) from the measurement point is connected to the inlet of the bag in the vessel. Heating and insulation of the sampling tubes is required when condensation problems in the sampling tubing may occur (large temperature differences along the sampling line).
- The outlet of the vessel is connected with the pump (Thomas Industries Inc., model 617CD32, Wabasha, Minnesota ,VS). In front of the pump, a critical orifice (borosilicate glass (inside diameter: 8 mm; length: 80 mm), housed in a stainless steel container for protection) is placed to provide a flow rate of approximately 20 ml/min.
- Start the measurements by switching on the pump, and write down the date and time at which the measurements have been started.
- Stop the measurements by switching off the pump. Write down the date and time at which the measurements have been stopped.
- The vessels with the sample bags are transported to the laboratory for analysis.

## 5 Performance characteristics

### 5.1 Verification of trueness of measurements

The lung method was calibrated in the laboratory against standard calibration gases (reference method) by following this procedure:

- A. First, bags were inserted into the vessels and cleaned by first filling them with N<sub>2</sub> and then sucking the air from the bag. This procedure was repeated twice.
- B. Then, overpressure (20 kPa) was created into the vessel to ensure the bags were completely empty before starting the experiment.
- C. The bags were filled with air from calibration CO<sub>2</sub> gas cylinders (see Appendix 1 for certificates of the standard gases used for the calibration) humidified before entering the bag to about 35% relative humidity and diluted with N<sub>2</sub> (gas cylinder; see Appendix 1) using different dilution steps (Table 2). The flow to the bags from the cylinders (N<sub>2</sub> and CO<sub>2</sub>) was regulated using a dilution system (gas divider type Signal 821; Figure 5.2 and Table 1; see Appendix 2 for the calibration certificate of this instrument), as shown in Figure 5.1.
- D. Finally, the air in the bags was directly (within 1 hour) analysed for CO<sub>2</sub> using gas chromatography (Interscience/Carlo Erba Instruments Inc., Breda, the Netherlands, GC 8000 Top; column Haysep Q; detector: HWD).



**Figure 5.1** Schema of the measurement set-up used for the calibration of the lung method. PC: Pressure controller; CO: critical orifice (flow restrictor).



**Figure 5.2** Dilution system (Signal 821) used in this study.

**Table 1** Dilution factors for different dilution steps for the Signal 821.

Settings	Dilution factor
10%	0.1012
20%	0.2008
30%	0.3002
40%	0.3990
50%	0.5006
60%	0.6004
70%	0.6993
80%	0.7992
90%	0.9000

**Table 2** Dilution steps for the calibration of the lung method.

CO <sub>2</sub> cylinder (ppm)	Setting (%) dilution system		Applied CO <sub>2</sub> (ppm)
	N <sub>2</sub>	CO <sub>2</sub>	
---	100	0	0
3500	90	10	352
3500	80	20	700
3500	70	30	1050
3500	60	40	1395
3500	50	50	1751
3500	40	60	2095
3500	30	70	2443
3500	20	80	2794
3500	10	90	3148
3500	0	100	3500

The null hypothesis to test the trueness of measurements when using the lung method is that the measured CO<sub>2</sub> concentrations with the lung method (and analysed using gas chromatography) and the standard calibration gases are similar. If the null hypothesis is fulfilled, a linear regression analysis ( $y=a*x+b$ ) of the lung method (x) against the standard calibration gases (y) results in a slope  $a=1$  and an intercept  $b=0$ . To test if this hypothesis was fulfilled, the following procedure was applied:

1. A linear regression analysis ( $y=a*x+b$ ) was performed based on the data set (CO<sub>2</sub> concentrations measured with the lung method (x) against standard calibration gases (y)). From this regression analysis, the following parameters were determined (Table 3):
  - a. Slope (a)
  - b. Intercept (b)
  - c. Coefficient of determination ( $R^2$ )
2. The two-sided acceptance intervals<sup>1</sup> were determined according to:
 
$$y = 0.9 * x \text{ ppm}$$

$$y = 1.1 * x \text{ ppm}$$

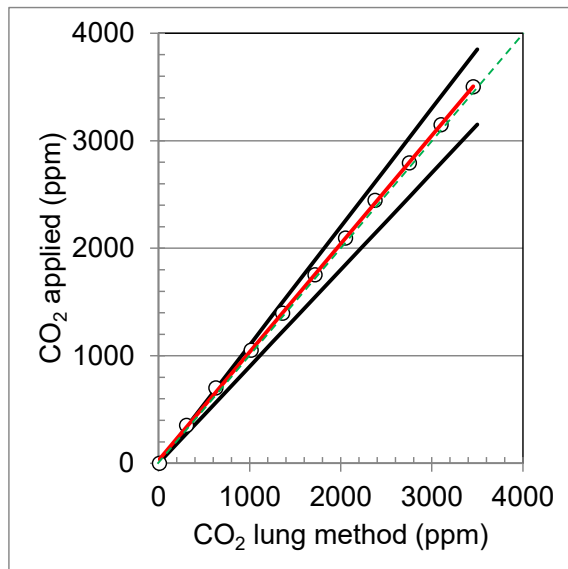
<sup>1</sup> An acceptance level of 10% is used, based on the expanded uncertainty of the WUR lung method as described in section 5.4

3. A graph was plotted showing the following information:
  - a. For all calibration steps ( $i=1\dots n$ ) applied in the calibration procedure, the value of the CO<sub>2</sub> concentration measured with the lung method ( $y_i$ ) and the applied CO<sub>2</sub> concentration according to the standard calibration gas ( $x_i$ ).
  - b. The line showing the null hypothesis ( $y = x$ ).
  - c. The regression line determined by the regression analysis (Table 3).
  - d. The two-sided acceptance intervals.
4. For acceptance, the following criteria shall be fulfilled:
  - a.  $R^2 \geq 0.95$
  - b. De regression line is bounded within the limits of the acceptance intervals.

The criteria for acceptance is fulfilled for the whole concentration range (Figure 5.3 and Table 3). The CO<sub>2</sub> concentration measured in the laboratory using the lung method was similar to the concentration supplied (by dilution) from the gas standards (gas cylinders) over the whole concentration range.

**Table 3** Linear regression (calibration) for the lung method.  $CO_{2\text{applied}} = a \cdot CO_{2\text{lung method}} + b$ .

Range	a	b	R <sup>2</sup>
0-3500 ppm	1.01	27.4	1.00



**Figure 5.3** Trueness of measurements (laboratory calibration) for the lung method. The red line represents the regression line of the measurements. The black lines represent the two-sided acceptance envelopes (10% acceptance level).

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## 5.2 Repeatability of concentration measurements (concentration differences between duplicates)

To test the precision (repeatability) of the concentration measurements (comparability of results between replicates), two vessels with sampling bags were prepared according to section 4.2 and exposed simultaneously to the same polluted air during measurements at different commercial farms. The dataset with the paired results was first analysed for outliers by using the box plot method with three times the interquartile range (IQR) as measure for extreme outliers:

$$IQR = Q3 - Q1$$

Q1: lower (first) quartile (25%)

Q2: median (second quartile)

Q3: upper (third) quartile (75%)

Outlier if: value > Q3+3\*IQR OR value < Q1-3\*IQR

Then, the selected dataset of parallel measurements (without outliers) was used to validate the repeatability of concentration measurements according to the following procedure:

1. Determine for all measurement periods ( $i=1\dots n$ ) the average concentration ( $Y_i$ ) of the two replicates ( $Y_{i1}, Y_{i2}$ ):

$$Y_i = \frac{(Y_{i1} + Y_{i2})}{2}$$

2. Determine the concentration difference ( $D_i$ ) between replicates 1 en 2:

$$D_i = (Y_{i1} - Y_{i2})$$

3. Determine the relative standard deviation ( $S_r$ ) of the measurements ( $n$ ) according to:

$$S_r = \sqrt{\frac{\sum_{i=1}^n (D_i / Y_i)^2}{2 * n}}$$

4. Determine the critical t-value ( $t_{f;95\%}$ ) for a two-tailed t-test with  $f=n-2$  degrees of freedom and a 95% confidence level.
5. Determine the two-tailed 95% relative confidence interval ( $CL_{95\%}^r$ ) of the measurements:

$$CL_{95\%}^r = S_r * t_{f;95\%}$$

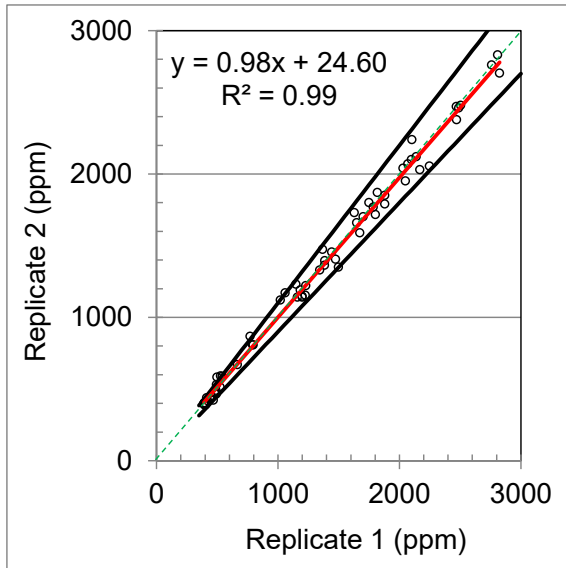
6. For acceptance<sup>2</sup>, the following criteria shall be fulfilled:

$$CL_{95\%}^r \leq 0.1 \text{ (10\%)}$$

Table 4 gives an overview of the measurement locations and number of measurements used in the analysis (outliers already excluded from this overview). Concentration differences between replicates are low, as shown in Figure 5.4. The criteria for acceptance of the method for repeatability of measurements is fulfilled ( $n=67$ ; relative average difference: 4%; median: 2%; standard deviation: 4%; 95%-confidence interval: 7%) for the whole concentration range (0-3500 ppm).

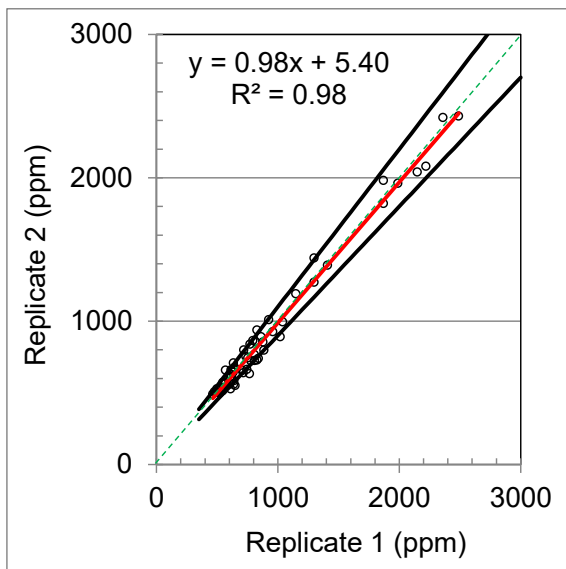
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<sup>2</sup> An acceptance level of 10% is used, based on the expanded uncertainty of the WUR lung method as described in section 5.4



**Figure 5.4** Concentration differences in samples taken in duplicate using the lung method. The red line represents the regression line of the measurements. The black lines represent the two-sided acceptance envelopes (10% acceptance levels).

When samples are taken simultaneously, but at different positions inside the animal house (see Table 5 for an overview of locations and number of measurements), a larger difference in concentration may be expected. This may in particular be the case in naturally ventilated dairy cattle buildings, where air is not always perfectly mixed and animals are not evenly distributed. Figure 5.5 shows that concentration differences between samples taken simultaneously but at different points in the building remains low ( $n = 68$ ; relative average difference: 6%; median: 5%; standard deviation: 5%; 95%-confidence interval: 11%).



**Figure 5.5** Concentration differences in samples taken simultaneously but not at the same point using the lung method. The red line represents the regression line of the measurements. The black lines represent the two-sided acceptance envelopes (10% acceptance levels).

**Table 4** Overview of measurements in duplicate using the lung method.

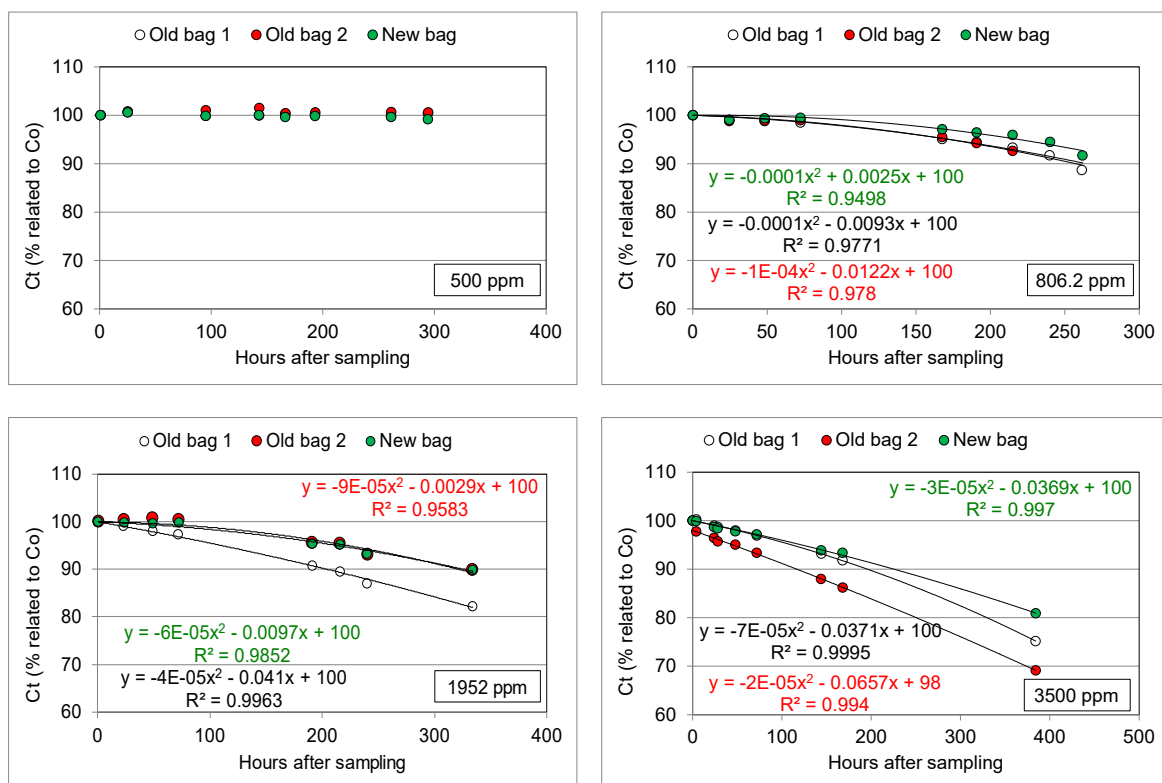
Concentration range	Animal category	Measurement locations	Number of measurements
0-3000 ppm	Cattle	---	---
	Pigs	4	16
	Poultry	9	51
TOTAL		13	67

**Table 5** Overview of measurements taken simultaneously but not at the same point using the lung method.

Concentration range	Animal category	Measurement locations	Number of measurements
0-3000 ppm	Cattle	11	46
	Goats	2	12
	Poultry	3	10
TOTAL		16	68

### 5.3 Stability of the sample in the sampling bag

Since the sample is not analysed directly, but transported to the laboratory, it is important to know how long the concentration in the bag remains stable (this is related to the permeability of the sampling bags being used). Figure 5.6 shows, for different concentration levels, the variation in concentration in time related to the time of sampling.

**Figure 5.6** Stability of the concentration in the bag expressed as the evolution in time of the relative concentration after sampling for different concentration levels in old and new sampling bags.

At low concentration levels, close to background levels, the concentration remains stable for a number of days. At higher concentration levels, a decay in concentration up to 1% per day has been observed (for a concentration level of 3500 ppm). Also, some differences were found between old and new bags. To minimize systematic errors, the sample shall be analysed within 24 hours after sampling.

## 5.4 Uncertainty analysis

The procedure used to evaluate the uncertainty associated with CO<sub>2</sub> concentration measurements using the lung method follows the manual for the expression of uncertainty in measurement (ERASER; JCGM, 2008). The uncertainty of the CO<sub>2</sub> concentration measurement using this method is evaluated with the help of the law of propagation of uncertainty. This requires the identification and quantification of all sources of uncertainty related to CO<sub>2</sub> concentration measurements carried out with the WLR lung method. These sources of uncertainty can be classified in the following categories:

- Repeatability of CO<sub>2</sub> concentration measurements ( $U_{dc}$ )
- Sampling time ( $U_t$ )
- Chemical analyses in the lab (reproducibility lab analyses;  $U_{lab}$ )
- Calibration ( $U_{cal}$ ).
- Interferences ( $U_{interf}$ ).
- Adsorption and desorption in piping and sampling system ( $U_{ads}$ ).

Addendum a). The repeatability of the measurements is defined as the absolute difference between the results of a duplicate measurement under similar conditions. At each measurement point, the measurements are performed in duplicate ( $i = 1, 2$ ) and the concentration at each measurement point is calculated as the average of the duplicate measurements:

$$C_{CO_2} = \frac{C_{CO_2}^1 + C_{CO_2}^2}{2}$$

According to section 5.2, a standard uncertainty of  **$U_{dc}=4\%$**  (normal distribution;  $k=1$ ) can be assigned for the repeatability of CO<sub>2</sub> concentration measurements.

Addendum b). The time is noted both at the beginning and at the end of the measurements. The expected uncertainty in these measurements is  **$U_t<0.5\%$**  (rectangular distribution; a 5-minute difference over a 24-hour period results in an uncertainty of 0.35%).

Addendum c). The concentration of CO<sub>2</sub> in the sample air collected in the sample bag using the lung method is determined in the laboratory of Wageningen Livestock Research using gas chromatography. The samples are normally analysed within 24 h, and during this time a decay in concentration up to 1% per day (see section 5.3) may be expected. According to these results, a standard uncertainty  **$U_{lab}=1\%$**  (normal distribution;  $k = 1$ ) can be assigned for the reproducibility of lab analyses.

Addendum d). The WLR lung method was calibrated in the laboratory against a primary standard (certified calibration gases, reference method). The procedure and the results are reported in section 5.1 and summarized in the table below.

**Table 6** Linear regression (calibration) of the WLR lung method.  $CO_{2applied} = a \cdot CO_{2lung\ method} + b$ .

Range	a	b	R <sup>2</sup>
0-3500 ppm	1.01	27.4	1.00

When the CO<sub>2</sub> concentration measurements are corrected by the results of the calibration procedure, the uncertainty in the calibration is equal to the uncertainty of the standard calibration gases used for

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the calibration. This source (calibration WLR lung method) is assigned a standard uncertainty  **$U_{cal}=2\%$**  (normal distribution;  $k = 2$ ).

Addendum e). By using the right column and detector, no interferences are expected when using gas chromatography to measure CO<sub>2</sub> concentrations in sampled air. This source of uncertainty is assigned a default uncertainty  **$U_{interf}=0\%$** .

Addendum f). Adsorption and desorption of CO<sub>2</sub> in the sampling lines is not expected to occur. This source of uncertainty is assigned a standard uncertainty  **$U_{ads}=0\%$**  (rectangular distribution).

The combined measurement Uncertainty ( $U_{comb}$ ) is determined according to:

$$U_{comb} = \sqrt{(U_{dc}^2 + U_t^2 + U_{lab}^2 + U_{cal}^2 + U_{interf}^2 + U_{ads}^2 + U_{flow}^2)} = \sqrt{(4^2 + 0,5^2 + 1^2 + 2^2 + 0^2 + 0^2)}$$

The expanded measurement uncertainty ( $U_{exp}$ ) is then determined according to:

$$U_{exp} = k * U_{comb}$$

For a 95% confidence interval, the cover factor ( $k$ ) is assigned a value of  $k = 2$ . The expanded measurement uncertainty with a 95% confidence interval is then  **$U_{exp}=10\%$** .

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## 6 Conclusions

In this document a description of the lung method as applied by Wageningen Livestock Research (WLR lung method) to measure carbon dioxide (CO<sub>2</sub>) concentrations in the air entering and leaving livestock housing systems has been presented. This means using a 40 liter Nalophan sampling bag (type NA300 25 µm) inserted in a closed vessel and kept under vacuum conditions. Air is sampled into the sampling bag after first being led through a dust filter at a constant flow rate (~20 ml/min) through Teflon or polyethylene sampling lines by using a pump and critical orifices. Air samples are then analysed in the laboratory by using a gas chromatograph with a Haysep Q column and a thermal conductivity detector.

Laboratory calibration of the WLR lung method against gas standards (standard calibration gases; reference method) showed that the acceptance criteria for verification of trueness of measurements (section 5.1) was fulfilled. Section 5.2 shows that the WLR lung method has a repeatability (concentration difference between replicates) of less than 5%. A decay in concentration up to 1% per day has been observed (section 5.3), meaning that samples are advised to be analysed within 24 h to minimize this error. The combined (expanded) uncertainty of the WLR lung method is estimated to be 10%.

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## 7 References

JCGM 2008. Evaluation of measurement data – Guide to the expression of uncertainty in measurement. Working group 1 of the Joint Committee for Guides in Metrology (JCGM/WG 1).

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## Appendix 1: Certificates of calibration standards (gas cylinders)

The calibration procedure required the use of a number of calibration standards (gas cylinders) providing different concentration levels. The following gas cylinders were used:

Gas	Concentration (ppm) according to certificate	Provider
CO <sub>2</sub>	3500 ± 2%	Scott specialty gases
N <sub>2</sub>	Pure (99.999%)	Scott specialty gases

# Certificate of 3500 ppm CO<sub>2</sub> gas cylinder



**Scott**  
specialty gases

**CERTIFIED MASTER CLASS**

*Single-Certified Calibration Standard*

Takkebijsters 48 4817 BL, Breda, The Netherlands Phone: +31(0)76-5711 828 Fax: +31(0)76-5713 267

## CERTIFICATE OF ACCURACY: Certified Master Class Calibration Standard

### Product Information

Project No.: 20-97567-003  
Item No.: 20020000560P50  
P.O. No.: WUR728325

Cylinder Number: 9907596  
Cylinder Size: 50  
Certification Date: 17Jun2014  
Expiration Date: 16Jun2017

### Customer

WAGENINGEN UR, ANIMAL SCIENCES GROUP  
T.A.V. JOHAN PLOEGAERT  
VEEHOUDERIJ BV  
VIJFDE POLDER 1  
6708 WC WAGENINGEN  
NEDERLAND

### CERTIFIED CONCENTRATION

#### Component Name

CARBON DIOXIDE  
NITROGEN

Concentration  
(Moles)

0,350 %  
BALANCE

Accuracy  
(+/-%)

2

### TRACEABILITY

#### Traceable To

VSL

APPROVED BY:

  
ING. C. V/D MOOSDIJK

SUPERVISOR:

  
C. DOOMEN

DATE:

17-6-14

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## SPECIFICATIONS

Component Name	Requested Concentration (Moles)	Certified Concentration (Moles)	Blend Tolerance Result (+/- %)	Certified Accuracy Result (+/- %)
CARBON DIOXIDE	0,35 %	0,350 %		2,00
NITROGEN	BAL	BAL		

## TRACEABILITY

Traceable To  
VSL

## PHYSICAL PROPERTIES

Cylinder Size: 50

Pressure: 150 BAR  
Expiration Date: 16Jun2017

Valve Connection: DIN-6

Min. Cyl. Pressure: 5 BAR

## SPECIAL HANDLING INSTRUCTIONS

Do not use or store cylinder at or below the stated dew point temperature. Possible condensation of heavier components could result. In the event the cylinder has been exposed to temperatures at or below the dew point, place cylinder in heated area for 24 hours and then roll cylinder for 15 minutes to re-mix.

Use of calibration standards at or below dew point temperature may result in calibration error.

## COMMENTS

# Certificate of N<sub>2</sub> gas cylinder



**Scott**  
specialty gases

European Headquarters

Takkebijsters 48 4817 BL, Breda, The Netherlands

Phone: +31(0)76-5711 828 Fax: +31(0)76-5713 267

## CERTIFICATE OF CONFORMITY

Project #: 20-04096-001  
Cylinder #: 9700339  
P.O.#: WUR883635  
Item #: 2001TSN2 10  
Date: 24Aug2015

WAGENINGEN UR, ANIMAL SCIENCES GROUP  
VEEHOUDERIJ BV, ZODIAC  
GEBOUW 122, DE ELST 1  
6708 WD WAGENINGEN  
NEDERLAND

Pure Material: TECHNISPEC NITROGEN

CAS# 7727-37-9

PURE MATERIAL: NITROGEN  
GRADE: 5.0  
PURITY: 99.999%

VALVE CONNECTION: DIN 10  
FILL PRESSURE: 200 BAR

CYLINDER SIZE: 10 LITER

Analyst:

  
R.J. HOFMEESTER

Approved By:

  
C. OOMEN

An Air Liquide Group Company

## Appendix 2: Calibration certificate of gas divider type Signal 821



SIGNAL GROUP LIMITED  
12 DOMAN ROAD, CAMBERLEY  
SURREY, ENGLAND GU15 3DF  
TELEPHONE: +44 (0)1276 682841 / 4 (4 LINES)  
FAX: +44 (0)1276 691302  
E-MAIL: INSTRUMENTS@SIGNAL-GROUP.COM  
SITE: WWW.SIGNAL-GROUP.COM

### CERTIFICATE OF CALIBRATION

CONSIGNEE Wageningen

ORDER NUMBER \_\_\_\_\_

W/O NUMBER \_\_\_\_\_

DATE OF CALIBRATION 01/12/2015

DESCRIPTION OF GOODS	SERIAL NO.	REMARKS
821S gas divider	4789	

### CALIBRATION RESULTS

DIVIDER SETTING	ACTUAL RESULT
100.00%	100.00
90.00%	90.00
80.00%	79.92
70.00%	69.93
60.00%	60.04
50.00%	50.06
40.00%	39.90
30.00%	30.02
20.00%	20.08
10.00%	10.12
0.00%	0.00

### CALIBRATION GAS DETAILS

CALIBRATION GAS TYPE	SUPPLIER	CERTIFICATE No.	TRACEABILITY
1000ppm C3H8/air	BOC	1433495	NPL

SIGNAL AUDIT PROCEDURE NUMBER TPB00101

UNCERTAINTY OF MEASUREMENT +/- 0.2% F.S.D.

Signal Group certifies that the materials and processes used in the calibration of the unit detailed above hereon unless otherwise stated conform in all respects to Signal Group Ltd procedures and specifications

SIGNED Roy Kinslow



To explore  
the potential  
of nature to  
improve the  
quality of life



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Wageningen Livestock Research creates science based solutions for a sustainable and profitable livestock sector. Together with our clients, we integrate scientific knowledge and practical experience to develop livestock concepts for future generations.

Wageningen Livestock Research is part of Wageningen University & Research. Together we work on the mission: 'To explore the potential of nature to improve the quality of life'. A staff of 6,500 and 10,000 students from over 100 countries are working worldwide in the domain of healthy food and living environment for governments and the business community-at-large. The strength of Wageningen University & Research lies in its ability to join the forces of specialised research institutes and the university. It also lies in the combined efforts of the various fields of natural and social sciences. This union of expertise leads to scientific breakthroughs that can quickly be put into practice and be incorporated into education. This is the Wageningen Approach.

