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# Measurement protocol for emissions of bio-aerosols from animal houses

*Meetprotocol voor emissies van bio-aerosolen uit stallen*

A.J.A. Aarnink, Y. Zhao and N.W.M. Ogink



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Wageningen UR Livestock Research

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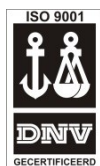
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# Foreword

Over the last decade the scale of especially pig and poultry production facilities in rural areas in the Netherlands has drastically increased. Residents living in rural areas with high animal production densities have been alarmed by possible effects of large scale livestock production on their health and their quality of life. Public health concern is mainly related to expected increasing concentrations of fine dust and dust components (e.g. endotoxins) in the environment and possible transmission of airborne diseases from animals to humans (zoonotic diseases). There is a strong need for information that quantifies emissions of these so-called bio-aerosols from livestock operations based on representative measured values. Accurate emission data are indispensable in assessing the exceedance of critical concentration and related risks of bio-aerosol components in residential areas surrounding livestock facilities. However a wide variety of methods for sampling and analysis for bio-aerosols are used in research, yielding data that cannot be compared with each other and that differ in quality with regard to determination accuracy. Establishing reliable emission data and comparable emission factors requires a standardized approach that prescribes sampling and measurement methods.

In this study a measurement protocol is developed for determining emissions of bio-aerosols from animal houses, making use of state of the art knowledge and methods for sampling and analysis. Where necessary the measuring protocol has been specified for certain types of bio-aerosols. This study was commissioned and financed by the Ministry of Economic Affairs.

Nico Ogink  
Programme coordinator  
Wageningen UR Livestock Research



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# Samenvatting

Mensen die in de omgeving wonen van nieuw te bouwen stallen of te maken krijgen met uitbreiding van stallen zijn bezorgd over mogelijke effecten hiervan op hun gezondheid en op de kwaliteit van hun leefomgeving. Deze bezorgdheid is vooral gerelateerd aan de verwachte toename van stofconcentraties in de omgeving en aan de mogelijke verspreiding van ziektekiemen (zoönosen) die via de stallucht worden uitgestoten. Dit is vooral actueel geworden na de Q-koorts crisis een aantal jaren geleden. Verder kunnen bepaalde componenten van kiemen, zoals endotoxinen, de longgezondheid aantasten. Meer kennis is nodig van de processen en factoren die de emissie van bio-aerosolen uit stallen beïnvloeden en van de mogelijkheden om deze emissies te reduceren. Bio-aerosolen zijn stofdeeltjes die geheel of gedeeltelijk een biologische oorsprong hebben. Deze deeltjes kunnen virussen, bacteriën, schimmels of componenten van deze micro-organismen bevatten. Om meer kennis op dit gebied te verkrijgen is het belangrijk om met een gestandaardiseerd meetprotocol te werken. Op deze manier kunnen resultaten van verschillende onderzoeken goed met elkaar worden vergeleken. De doelstelling van dit onderzoek was om een meetprotocol te ontwikkelen voor het bepalen van de emissie van bio-aerosolen uit stallen en voor het bepalen van de efficiëntie van potentiële reductiesystemen.

Bij onderzoek naar emissies van bio-aerosolen uit stallen kunnen verschillende typen micro-organismen en componenten van micro-organismen van belang zijn. Wat er precies gemeten moet worden hangt daarom af van het doel van het onderzoek. Wanneer het onderzoek is gericht op een bepaalde ziektekiem, dan wordt bij voorkeur de ziektekiem zelf gemeten. Echter, dit is waarschijnlijk niet in alle gevallen mogelijk. In dat geval en in het geval wanneer algemene data van emissies van micro-organismen worden gevraagd, dan is het belangrijk om bepaalde 'model' micro-organismen te selecteren. Deze 'model' micro-organismen moeten in detecteerbare aantallen aanwezig zijn. Voor bacteriën b.v. worden de volgende groepen vaak geanalyseerd in luchtmonsters van stallen:

- Totaal kiemgetal;
- Gram-positieve en Gram-negatieve bacteriën;
- Enterococci (Enterococcus), een belangrijk geslacht binnen de Gram-positieve bacteriën (b.v. E-faecalis);
- Enterobacteriën (Enterobacteriaceae), grote familie binnen de Gram-negatieve bacteriën (b.v. E-coli, Salmonella);
- Staphylococci (Staphylococcus), een geslacht binnen de Gram-positieve bacteriën. Deze groep bevat de Methicilline resistente Staphylococcus aureus (MRSA).

Veel virussen zijn alleen in detecteerbare aantallen aanwezig gedurende een besmetting met het virus. Sommige virussen komen echter regelmatig voor in stallen zoals somatische colifagen (alle diersoorten), hepatitis-E (bij varkens), IB virus (bij pluimvee) en influenza virus (bij varkens). Dergelijke veelvoorkomende virussen zouden als 'model' virus gebruikt kunnen worden. Microbiële componenten zoals endotoxine of beta-glucanen zijn meestal in detecteerbare hoeveelheden aanwezig in luchtmonsters. Ze kunnen ook in stofmonsters worden geanalyseerd, waardoor lange monsterperioden mogelijk zijn. PCR- (polymerase chain reaction) technieken kunnen worden gebruikt om DNA of RNA van micro-organismen in stofmonsters te bepalen. Bij deze analyse is echter geen onderscheid te maken tussen levende en dode micro-organismen.

Gebaseerd op literatuur, bestaande expertise en bestaande meetprotocollen voor ammoniak, geur en fijnstof wordt een advies gegeven voor de meetstrategie voor het bepalen van referentie emissieniveaus van micro-organismen en microbiële componenten. De volgende bemonsteringsstrategie wordt voorgesteld voor micro-organismen:

1. Monsternamen worden verricht op 4 verschillende bedrijven met een vergelijkbaar huisvestingssysteem om de variatie tussen verschillende meetlocaties, met verschillend management, te kunnen bepalen.
2. Monsternamen worden verricht gedurende 6 dagen per bedrijf. De monsterdagen worden verspreid over de verschillende temperatuurranges gedurende het jaar.

3. Tenminste één derde deel van de metingen (2 per bedrijf) wordt gedaan op dagen met een maximaal verwachte emissie, b.v. aan het eind van de groeiperiode of gedurende het reinigen van de stal. De rest van de metingen moet een representatief beeld geven van de gemiddelde emissie en van de variatie in de emissie.
4. Binnen een meetdag moeten de metingen vooral worden gedaan tijdens momenten met een verwacht hoog emissieniveau. In het algemeen is dat gedurende de dag bij een hoog activiteitsniveau in de stal. Aanvullende metingen moeten worden gedaan om inzicht te krijgen in de variaties binnen een dag.

De volgende meetstrategie wordt voorgesteld voor microbiële componenten:

1. De eerste twee punten zijn gelijk aan die voor micro-organismen.
2. Tussen dagen; monsters moeten zodanig over de dagen worden verspreid dat een representatief beeld wordt gekregen van de gemiddelde emissie. Dit betekent b.v. een evenredige spreiding over de groeiperiode van de dieren. Verder moet er goed inzicht worden verkregen in de normale spreiding van de emissies. Voor details van de verdeling van de meetdagen over de productiecycclus en over de normale management activiteiten zie Hoofdstuk 2 in het rapport van Ogink et al. (2011).
3. Binnen dagen; monsters moeten bij voorkeur worden genomen gedurende 24 uur of een meervoud hiervan. Wanneer de monsterperioden korter zijn dan moet een goed inzicht worden verkregen in de binnen-dag variatie, b.v. door het continu meten van de stof (PM10) concentratie gedurende 24 uur.

De emissie wordt berekend door de concentratie met het gelijktijdig gemeten ventilatieniveau te vermenigvuldigen. Het ventilatieniveau kan op de volgende drie manieren worden bepaald:

1. Met een meetwaaier;
2. Met behulp van de tracer gas ratio methode;
3. Met behulp van de CO<sub>2</sub> massa balans methode.

Wanneer bio-aerosolen worden bemonsterd dan moet de monsternamen efficiëntie bekend zijn. De monsternamen efficiëntie van een bio-sampler wordt bepaald door de fysische en de biologische efficiëntie. De fysische efficiëntie geeft aan hoe goed niet-levende deeltjes worden bemonsterd. Het aantal en de grootte van de bemonsterde deeltjes moeten representatief zijn voor het aantal en de grootte van de deeltjes in de lucht. De biologische efficiëntie geeft aan in welke mate micro-organismen de gevolgen van bemonsterings-stress overleven. De fysische efficiëntie ligt normaal gesproken in de range tussen 50 en 100%. De biologische efficiëntie kan echter veel sterker variëren, b.v. wanneer *Campylobacter jejuni* wordt bemonsterd met 'high volume sampler' OMNI-3000 of met de gelatine methode, kan de efficiëntie zakken tot 1 à 2% van de werkelijke concentratie. Gram-positieve bacteriën zijn in het algemeen meer resistent tegen bemonsteringsstress dan Gram-negatieve bacteriën en bacteriën zonder celwand. Wanneer in het onderzoek geen metingen nodig zijn van levende micro-organismen dan kunnen andere bemonsteringstechnieken worden gebruikt. Dan kunnen bemonsteringstechnieken worden overwogen die vergelijkbaar zijn met de bemonstering van stof. Een groot voordeel van de stofmonsternamenmethoden is dat de monsternameduur sterk kan worden verlengd, waardoor de detectielimiet sterk kan worden verlaagd.

Om de efficiëntie van reductiemethoden voor bio-aerosol emissies te bepalen moeten de volgende punten in acht worden genomen:

1. Het schaalniveau. In het algemeen wordt geadviseerd om systemen eerst te testen op lab-schaal, vervolgens op pilot-schaal en dan op volledige praktijkschaal.
2. De micro-organismen en/of de microbiële componenten die worden bemonsterd en geanalyseerd. Op pilot- en praktijkschaal is het in het algemeen moeilijk om de ziektekiemen waar het in feite om draait te bemonsteren, daarom worden in die situaties 'model' kiemen bemonsterd.
3. De opzet van het onderzoek. Om effecten van reductiesystemen te bepalen is het belangrijk om mogelijke variaties in tijd en ruimte zoveel mogelijk uit te sluiten. Daarom wordt geadviseerd om het effect van het reductiesysteem te bepalen in een directe vergelijking met een controlesysteem, op hetzelfde bedrijf en gedurende dezelfde periode. Wanneer de efficiëntie van een luchtwassysteem moet worden bepaald, dan worden concentraties gemeten aan de inlaatzijde van de wasser vergeleken met de concentraties aan de uitlaatzijde.

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De emissie van levende micro-organismen wordt weergegeven in aantal levende kiemen per tijdseenheid. De emissies worden berekend per dierplaats, waarbij wordt verondersteld dat bij het begin van de meetperiode de stal volledig bezet was. Om de reductie-efficiëntie te bepalen wordt eerst het geometrische gemiddelde (= mediaan) van de controle (= control) en van de proefbehandeling (= case) per locatie bepaald. Van deze medianen wordt de reductie-efficiëntie per locatie bepaald. Het overall gemiddelde wordt bepaald door het gemiddelde te nemen van de berekende efficiëntie per meetlocatie. De standaardfout van het gemiddelde wordt bepaald door de standaarddeviatie tussen locaties te delen door de wortel uit het aantal locaties (bedrijven).

In hoofdstuk 7 van dit rapport wordt een gedetailleerde beschrijving gegeven van de verschillende elementen die in het meetrapport van een emissieonderzoek van bio-aerosolen moeten worden opgenomen.

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# Summary

People living in the vicinity of newly build or enlarged livestock farms are concerned about possible effects on their health and their quality of life. This concern is mainly related to expected increasing concentrations of particulate matter (dust) in the environment and possible transmission of airborne diseases from animals to humans (zoonotic diseases). This became very actual after the Q-fever crisis some years ago. Furthermore, microbial components like endotoxin emitted from livestock farms could harm the respiratory system. More knowledge is needed on the processes and factors that are involved in the emission of these so-called bio-aerosols from animal houses and on the options to reduce these emissions. Bio-aerosols are a special type of airborne particles that originate or partly originate from biological sources. These particles may contain viruses, bacteria, moulds or components from these micro-organisms. To gain more knowledge in this area of research it is important to work with a more or less standardized measuring protocol, so results from different studies could be easily compared. The objective of this study was to develop a measuring protocol for determining emissions of bio-aerosols from animal houses and to determine the efficiency of potential reduction systems.

Within bio-aerosols different types of microorganisms and components of microorganisms could be of interest within emission research. What should be measured therefore depends on the purpose of the study. When the study is focussing on a certain pathogen, preferably the pathogen itself is measured. However, this might not always be possible. In that case and when general data on emissions of microorganisms are required, it is important to select some main 'model' microorganisms. These model microorganisms should be present in the animal house in detectable amounts. For bacteria, for instance, the following main groups are often analysed in (air) samples from animal houses:

- Total bacteria count;
- Gram-positive bacteria and Gram-negative bacteria;
- Enterococcus, important genus of the Gram-positive bacteria (e.g. E-faecalis);
- Enterobacteriaceae, large family of the Gram-negative bacteria (e.g. E-coli, Salmonella);
- Staphylococcus, also a genus of Gram-positive bacteria. This group includes the Methicillin-resistant Staphylococcus aureus (MRSA).

A lot of Viruses are only present in detectable amounts during an infection with the virus. Some viruses, however, are regularly present in livestock houses like somatic coliphages (all livestock), hepatitis-E (mainly in pigs), IB virus (in poultry) and influenza virus (in pigs). These widely present viruses could be used as 'model' virus in livestock houses. Microbial compounds like endotoxin or beta-glucans are generally present in detectable amounts in air samples. Especially while these compounds can be analysed within dust samples (long sampling periods are possible). Also PCR (polymerase chain reaction) techniques can be used to determine DNA or RNA from microorganisms within dust samples. However, no distinction then can be made between viable and non-viable microorganisms.

Based on literature, expert knowledge and based on the existing measuring protocols for ammonia, odour, and dust an advise is given for the measurement strategy for determining reference emission levels of microorganisms and microbial components from animal houses. Because of the differences in the mechanisms causing health effects, we make a distinction between the sampling strategy for microorganisms and the sampling strategy for microbial components. The following sampling strategy is proposed for microorganisms:

1. Samplings are done on 4 different farms with a similar housing system to be able to determine the variations between different locations and management of the farms.
2. Samplings are done during 6 days per farm. Sampling days are spread over the different temperature ranges of the year.
3. At least one third of the measurements (2 at each farm) are done on days with maximum expected emission rates, e.g. at the end of the growing period or during cleaning of the animal house. The rest of the samples should give an estimate of the average emission rate and its regular variations.

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4. Within a measuring day samplings should especially be done at moments of expected high emission levels, so during light hours with high activity levels within the animal house. Additional measurements should be done to get insight in the diurnal variations.

The following sampling strategy is proposed for microbial components:

1. The first two points are the same as for microorganisms.
2. Between days, the samplings should be divided in such a way that an accurate representative average emission rate can be obtained, e.g. equally spread over the growing period of the animals. Furthermore, a good insight in the regular variations should be obtained from the data, as well. For details on the division of the measurement days over the production cycle and over regular management activities see Chapter 2 in the report of Ogink et al. (2011).
3. Within days, samplings should preferably be done during 24 h periods or a multiple of this period. When sampling periods are shorter, a good insight in the diurnal variation should be obtained, e.g. by continuous measurement of dust (PM10) concentration during 24 h.

The emission is calculated from the concentration and the associated ventilation rate. The ventilation rate should be determined during the same period as the concentration measurements. The ventilation rate can be determined according three methods:

1. With a measuring fan;
2. By the tracer gas ratio method;
3. By the CO<sub>2</sub> mass balance method.

When sampling bio-aerosols the sampling efficiency should be known. The efficiency of a bio-sampler includes the physical and the biological efficiency. The physical efficiency describes how well non-viable airborne particles are sampled. The number and sizes of particles sampled should be representative for the number and sizes of particles in the air. The biological efficiency reflects how sampling stress affects viability of airborne microorganisms. The physical efficiencies are normally in the range of 50 to 100%. The biological efficiency, however, can vary a lot more, e.g. when sampling *Campylobacter jejuni* with the high volume sampler OMNI-3000 or with the gelatine method the sampling efficiency can be reduced to 1 or 2% of the real concentration. Gram-positive bacteria are generally more resistant to sampling stresses than Gram-negative bacteria and bacteria without cell wall. When a study does not require measurement of viable microorganisms other sampling techniques can be used. Then sampling techniques similar as used for sampling dust can be considered. A big advantage of the 'dust' methods is that the sampling duration can be largely prolonged, causing a large decrease of the detection limit.

To determine the efficiency of bio-aerosol reducing systems the following points should be considered:

1. The scale of the system. Generally it is advised to test systems first at lab-scale then at pilot-scale and then at full-scale.
2. The microorganisms and/or microbial components that are sampled and analysed. At pilot and full scale measurements generally the pathogens of interest are difficult to sample, therefore 'model' microorganisms should be sampled instead.
3. The experimental setup. To determine effects of reduction systems variations in space and time should be reduced as much as possible. Therefore it is advised to determine the efficiency of reduction systems by direct comparison with a control system, at the same location at the same time. When testing the efficiency of an air cleaning system, concentrations at the inlet side of the cleaning system are compared with the concentrations at the outlet side of the system.

The emission of viable microorganisms is given in number of viable microorganisms per time unit. The emissions are calculated per animal place, assuming that at the start of the measuring period the animal house was fully occupied. To calculate the reduction efficiency first the geometric means (= median) of the control and the case measurements are determined per location. From these medians the reduction efficiency per location is calculated. The mean of these calculated reduction efficiencies of the measured locations determines the overall mean reduction efficiency and the standard error of this overall mean is calculated by calculating the standard deviation in reduction efficiency between farms and divide this by the square root of the number of farms.

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In chapter 7 of this report a detailed description is given of the different elements that need to be included in the measurement report of a bio-aerosol emission study.

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# 1 Introduction

The increasing scale of especially pig and poultry production in the Netherlands is at odds with requirements on spatial planning, environment, and landscape. Public concern is mainly related to expected increasing concentrations of particulate matter (dust) in the environment and possible transmission of airborne diseases from animals to humans (zoonotic diseases). This became very actual after the outbreak of Q-fever some years ago. Furthermore, microbial components like endotoxin emitted from livestock farms could harm the respiratory system. The different types of aerosols originating or partly originating from biological sources are called bio-aerosols. In the rest of the report we therefore will talk about bio-aerosols as the general term for particles emitted from livestock houses that may contain viruses, bacteria, moulds or components from these micro-organisms.

Although different researchers mention airborne transmission as a possible route of spread of a number of infectious animal diseases and zoonosis, the exact (quantitative) contribution of airborne transmission is still unknown. The formation and emission of bio-aerosols is a multi-factorial process. Dust and micro-organisms can be carried with the airstream to the outside air. Emitted bio-aerosols are carried by the airstream over shorter or longer distances depending on their size and mass, the exhaust location, the ventilation rate, the exhaust airspeed, the meteorological and geographical circumstances. Depending on the half life time of a certain pathogen under the given circumstances, it can keep its infectivity during a shorter or longer period.

In the report of Aarnink et al. (2012) the following was recommended: 'more knowledge is needed on the processes in airborne transmission to determine the impact of this infection route in Dutch livestock production and to maximize the efficiency of reduction methods. It is recommended to study the number of micro-organisms and pathogens emitting from animal houses and the variations in emissions during the day and during the year. For these measurements a validated measuring protocol is needed.'

The objective of the study described in this report was to develop a measuring protocol for determining emissions of bio-aerosols from animal houses. When necessary the measuring protocol will be specified for certain types of bio-aerosols. The first part of a measuring protocol is the measuring strategy. For determining ammonia emissions (Ogink et al., 2005) and dust emissions (Hofschreuder et al., 2008) from animal houses such a measuring strategy has already been developed. That measuring strategy formed the basis for the measuring strategy of bio-aerosol emission. The proposed measuring strategy should also be suitable for determining the efficiency of technical systems to mitigate bio-aerosol emissions.

In chapter 2 it is reported what should or could be measured with respect to bio-aerosols. In chapter 3 the measurement strategy is discussed. In chapter 4 it is reported what kind of sampling equipment should be used. In chapter 5 a description is given how to determine the efficiency of bio-aerosol reducing systems. In chapters 6 the calculation method of bio-aerosol emissions and of the reduction efficiency is reported, while in chapter 7 the information that should be included in the measuring report is given.

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## 2 What to measure?

Within bio-aerosols different types of microorganisms and components of microorganisms could be of interest within emission research, therefore it is not easy to determine what should be measured. In fact it depends on the purpose of the study. Is the study focussing on a certain pathogen or is the focus more on giving general data on emissions of microorganisms. In the first case it is obvious that preferably the pathogen itself is measured. However, it might often be the case that this pathogen is not present at the moment of measurements or that the pathogen is so infectious that visiting such a farm is not desired. In such a case it might be preferred to measure a microorganism that is a good model for the pathogen that is wished to be studied. In the second case, general data on emissions of microorganisms are required, it is important to select some main 'model' microorganisms. These model microorganisms should be present in the animal house in detectable amounts. In such a case it is often desirable to measure groups of microorganisms. For bacteria, for instance, the following main groups are often analysed in (air) samples from animal houses:

- Total bacteria count;
- Gram-positive bacteria and Gram-negative bacteria;
- Enterococcus, important genus of the Gram-positive bacteria (e.g. E-faecalis);
- Enterobacteriaceae, large family of the Gram-negative bacteria (e.g. E-coli, Salmonella);
- Staphylococcus, also a genus of Gram-positive bacteria. This group includes the Methicillin-resistant Staphylococcus aureus (MRSA).

A lot of Viruses are only present in detectable amounts during an infection with the virus. Some viruses, however, are regularly present in livestock houses like somatic coliphages (all livestock) (Heijnen, 2011), hepatitis-E (mainly in pigs; (Wang et al., 2002)), IB virus (in poultry) and influenza virus (in pigs). These widely present viruses could be used as 'model' virus in livestock houses.

For human health, some microbial components are of importance, as well. The main biological components within the air emitted from animal houses are: lipopolysaccharide (LPS) or endotoxin, component from the outer membrane of Gram-negative bacteria; Lipoteichoic acid (LTA), a major constituent of the cell wall of Gram-positive bacteria; beta-glucans, present in certain fungi, bacteria, and in plants.

It can be concluded from this chapter that it cannot be prescribed what microorganisms should be measured. It fully depends on the kind of animal house that is going to be studied and on the purpose of the study. Often it is difficult to measure individual pathogens in detectable (cultivable) levels. Therefore often groups of similar micro-organisms are analysed. When using the polymerase chain reaction technique (PCR), microorganisms can be detected at much lower concentrations. However, this technique only proves the presence and with real time PCR (RT-PCR) also the quantity of a microorganism, but not whether these microorganisms are viable or dead.

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## 3 Measurement strategy

As was valid for the question what should be measured, the question of the measurement strategy that should be followed is also fully depending on the purpose of the study. When the purpose is to determine mean emission levels during the year, sampling should be done on representative days. This means that the measurement strategy should focus on achieving a representative sampling procedure that includes all relevant variation sources, e.g. measurements in the different seasons and measurements during the whole growing period of the animal. This kind of measurement strategy would be similar to the one developed by (Ogink et al., 2013b) for ammonia emission and (Hofschreuder et al., 2008) for dust emission. For microorganisms and microbial components this measuring strategy could be relevant for determining reference levels to characterize present housing systems. Because of high variations in these levels, this measuring strategy is of less relevance for determining efficiencies of reduction systems.

The measurement strategy reported by (Ogink et al., 2013b) and (Hofschreuder et al., 2008) is based on a variance component model in which three variance layers can be distinguished (Ogink & Klarenbeek, 1997). Based on this variance component model a sampling protocol was developed. This protocol prescribes inclusion of four farm locations in the sampling scheme and six measurements per location spread over one year, being randomly taken in subsequent two month periods. This procedure distributes seasonal variations that influence microorganisms concentrations and ventilation rates throughout a year equally and well balanced in the sampling scheme. For growing animals, like broilers or growing-finishing pigs, it is prescribed that measurements are spread over the growing period. It can be decided that for the sake of accuracy of the emission factor to be determined more measurements are done in e.g. the last part of the growing period, because this part largely contributes to total emission. When doing this, however, the emission factor cannot be determined by just taking the means of the measured values. In cases where regular management practices can be expected to affect emission levels, care should be taken that these practices are incorporated in the sampling scheme in such a way that samplings are well distributed over these management practices.

In former protocol it is also prescribed that sampling periods should last for 24 h to prevent systematic errors due to diurnal variations. When sampling viable (or more precise cultivable) microorganisms, however, sampling periods could not last for 24 h, while during such a long sampling period the viability of the microorganisms will be reduced. Due to lack of a standard, the sampling duration for airborne microorganisms in different studies varies, but is normally less than 1 hour. The duration is determined by taking account of the estimated concentrations of microorganisms and the characteristics of the sampler in use. The sampling duration can be set at a short period (a few minutes) when the total amount of microorganisms, normally abundantly present in livestock houses, is of interest. When the microorganism of interest is sporadically present, the period should be set long enough to collect enough microorganisms for further analysis. Some samplers have not been designed to be used for long sampling durations. For instance, severe evaporation of liquid medium in All Glass Impinger (AGI-30) occurs when the sampling period is long and this may affect its sampling efficiency. The recommended maximum duration of the sampling period for the AGI-30 is 30 minutes. The Andersen impactor may easily become overloaded when samples are taken in livestock houses (Thorne et al., 1992), therefore, the sampling duration is limited to minutes or even to seconds.

In studies where the viability of microorganisms is of less importance, PCR (polymerase chain reaction) analyses could be used. Within a PCR test a characteristic piece of DNA or RNA of a certain microorganism is replicated several times, making this test very sensible to detect microorganisms in low concentrations. The detection limit can be further reduced by long sampling periods. When using the PCR technique, 24 h sampling periods are not a problem. In this case 24 h dust samples could be used for detecting the aimed microorganisms.

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When studying viable microorganisms it is important to sample at representative moments during the day. As yet, little information is available from literature about diurnal variations in concentrations and emissions of microorganisms. To get an indication of these variations we performed a study in a house for growing-finishing pigs and in a house for broilers. Within this study total bacteria, *Enterococcus*, endotoxins, and PM10 (=particulate matter < 10µm) were measured at different moments during the day. The results of this study were reported in Aarnink et al. (2013). The main conclusions of this study were:

- Clear diurnal patterns were observed for concentrations and emissions of bacteria (total bacteria and *Enterococcus*), endotoxins, and PM10 in rooms for growing-finishing pigs and broilers.
- Bacteria and endotoxin concentrations were highest during the light periods and lowest during the dark periods inside the animal house. As was shown for dust, this seems to be related to the activity of the animals.
- Within this study, most variation in concentration and emission of total bacteria and endotoxin could be explained by the measuring moment during the day, while little variation was explained by the measuring day.
- For *Enterococcus* most variation could be explained by the measuring day, while little variation could be explained by the measuring moment during the day.
- Low correlations were found between bacteria concentrations (total bacteria and *Enterococcus*) and PM10 concentrations, possibly caused by a short survival time of bacteria in the air.
- Clear correlations in both animal houses were found between the logarithm of PM10 and the logarithm of endotoxin concentrations. In the house for growing-finishing pigs 53% of the variations in endotoxin concentration could be explained by variations in PM10 concentrations, while this was 69% in the broiler house.

Apart from diurnal and seasonal variations, large variations in microorganisms concentrations may occur caused by occurrences of infections inside an animal house. A clear example is the occurrence of Q-fever on goat farms. In normal situations *Coxiella burnetii*, the bacteria responsible for Q-fever infection, is not present in the goat house or at very low concentrations. However, during a period of an 'abortion storm' caused by *Coxiella burnetii*, high numbers of these bacteria are excreted by the goats (mainly in the placenta). These bacteria will partly become airborne and will be spread in the environment. Approximately one month after the lambing season most infections of people living in the vicinity of the infected goat farms were noticed.

Generally, pathogens can cause infections when a minimum infective dose is exceeded. Background concentrations are generally a lot lower than this minimum infective dose. The minimum infective dose is generally only exceeded in special circumstances, e.g. because of an infection on a farm. Therefore it is important to get good insight in the maximum emission levels of pathogens. For microbial components like endotoxins also average concentrations seem to be important, while endotoxins exhibit a more gradual effect on the respiratory system.

Former considerations makes it difficult to determine the optimum measurement strategy that could be used for all kinds of microorganisms and its components. The VDI guideline 4257/Part 1 states as general requirements on emission measurements of bio-aerosols and biological agents the following: 'Emission measurements shall be conducted in such manner that the results describe the plant emissions in a representative way and enable a comparison of plants and/or operating conditions. Representative emission measurements require knowledge of the correlation between the operating conditions and the emission relevant activities within the plant area and the functionality of all plant components (e.g. exhaust air treatment). As a rule emission measurements are conducted under typical operating conditions that can lead to characteristic emission spectra and/or the highest emissions.' Furthermore it states: 'a sufficient number of measurements, however at least six under operating conditions that, based on experience, can lead to the highest emissions.'

From our former considerations, from the existing measuring protocols for ammonia, odour, and dust, and from the stated requirements in VDI guideline 4257/Part 1 we come to an advise for a measurement strategy for determining emissions of microorganisms and microbial components from animal houses. Because of the differences in the mechanisms causing health effects, we make a

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distinction between the sampling strategy for microorganisms and the sampling strategy for microbial components.

The following sampling strategy is proposed for microorganisms:

1. Samplings are done on 4 different farms with a similar housing system to be able to determine the variations between different locations and management of the farms.
2. Samplings are done during 6 days per farm. Sampling days are spread over the different temperature ranges of the year.
3. At least one third of the measurements (2 at each farm) are done on days with maximum expected emission rates, e.g. at the end of the growing period or during cleaning of the animal house. The rest of the samples should give an estimate of the average emission rate and its regular variations.
4. Within a measuring day samplings should especially be done at moments of expected high emission levels, so during light hours with high activity levels within the animal house. Additional measurements should be done to get insight in the diurnal variations.

The following sampling strategy is proposed for microbial components:

1. The first two points are the same as for microorganisms.
2. Between days, the samplings should be divided in such a way that an accurate representative average emission rate can be obtained, e.g. equally spread over the growing period of the animals. Furthermore, a good insight in the regular variations should be obtained from the data, as well. For details on the division of the measurement days over the production cycle and over regular management activities see Chapter 2 in the report of Ogink et al. (2011).
3. Within days, samplings should preferably be done during 24 h periods or a multiple of this period. When sampling periods are shorter, a good insight in the diurnal variation should be obtained, e.g. by continuous measurement of the dust (PM10) concentration during 24 h.

The emission is calculated from the concentration and the associated ventilation rate. The ventilation rate should be determined during the same period as the concentration measurements. The ventilation rate can be determined according three methods:

1. Measuring fan
2. Tracer gas ratio method
3. CO<sub>2</sub> mass balance method

For more details on these methods we refer to Ogink et al. (2011) and the references included on this subject in that report.

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## 4 Choice of instrumentation

When sampling particles it is important that the sampler collects a representative number of particles, representative for the concentration in the air. It is a well-known fact, that small particles in air are easily collected in an inlet as they have that low inertia, that they follow the streamlines of the air surrounding them. For larger particles sampling is more complex. For sampling in a defined flow iso-axiality and iso-kinetic sampling are required (Hofschreuder et al., 2008). Such sampling can be achieved if the sampler inlet is in alignment with and facing the direction of air flow (iso-axiality) and if the air velocity within the sampler is the same as the ambient air velocity (iso-kinetic) (Zhang, 2004). However, true isokinetic sampling is very difficult in practice due to the variation in the surrounding air flow pattern (air direction and velocity) and/or the limited possibilities of some samplers (Liu & Pui, 1981).

When emissions of microorganisms and microbial components are of interest, the best sampling location is in or near the air outlet. Sampling inside the outlet (stack sampling), however, is generally not possible in animal houses, while the duct length is most of the time too short for representative measurements according to the stack sampling protocols. Therefore (Hofschreuder et al., 2008) advised to sample in the airstream towards the outlet of the animal house and not inside the outlet duct. When sampling at some distance from the outlet, the air velocity is low enough to avoid problems related to iso-kinetic sampling. For more details on iso-axiality and iso-kinetic sampling see (Hofschreuder et al., 2008).

Current samplers for airborne viable microorganisms are generally based on one of three main principles, i.e. impaction, impingement, or filtration (Table 1). These different sampling principles have their advantages and disadvantages. Samplers using the impaction principle, e.g. Andersen six stage impactor, can be used to distinguish the microorganisms according to their sizes. In addition, the bacteria impacted on agar plates may be directly incubated for viable counts. However, its susceptibility to overloading limits its sampling in livestock houses to short periods, which may result in non-representative samples. The problem of overloading can be overcome by using samplers with the impingement principle, because then the liquid samples may be decimally diluted and analysed. A disadvantage of this sampler, e.g. AGI-30, is that it may not be able to sample for a long time due to evaporation of the collection liquid. This could be overcome by automatic supplementation of the evaporated liquid, as is done e.g. in the OMNI-3000 (Evogen Inc., Kansas City, US). Filtration is a user-friendly method in a practical situation, but not suitable for sampling microorganisms that are vulnerable to dehydration stress.

**Table 1***Common samplers for airborne microorganisms (Zhao, 2011).*

Sampling principle	Collection medium	Example samplers
Impaction	Agar plate	Andersen One/Two/Six stage Impactor (Andersen Instruments Incorporated, Atlanta, USA); Casella Slit Sampler (Casella Ltd, Bedford, UK); Surface Air Systems (Cherwell Laboratories, Bicester, UK)
Impingement	Liquid medium	AGI-30 (Ace Glass, Vineland, USA) Multistage May Liquid Impinger (AW Dixon, Beckenham, Kent) OMNI-3000 (Evogen Inc., Kansas City, US)
Filtration	Filter	Dissolvable gelatine filter (Sartorius, Göttingen, Germany); Polytetrafluoroethylene (Sartorius, Göttingen, Germany)

When sampling bio-aerosols the sampling efficiency should be known to be able to get a reliable evaluation of the microbial concentration in the air. The efficiency of a bio-sampler includes physical and biological efficiency. The physical efficiency describes how well non-viable airborne particles are aspirated by the device's inlet and transported to the collection medium, referred to as inlet sampling efficiency, and how well the bio-sampler retains these particles in its medium, referred to as collection efficiency. The biological efficiency reflects the resistance of airborne microorganisms to sampling stresses. In some studies, the efficiency has been evaluated by comparing samplers side-by-side in an environment with unknown microbial concentration (Engelhart et al., 2007; Henningson et al., 1982; Thorne et al., 1992). This method easily ranks the performance of different samplers; however, it does not reveal whether the amount of microorganisms collected in the air samples accurately represents the microorganism content of the air, nor does it distinguish between the physical and biological efficiency. Other studies separately investigated the efficiency of bio-samplers in aerosol experiments, in which a known amount of microorganisms together with an indicator (either labelled microorganisms, or inert tracer compound) were nebulized in an isolator. The physical efficiency can be determined by comparing the amount of tracer collected by a bio-sampler with that collected by the reference sampler (a sampler that has a high physical efficiency); the biological efficiency is subsequently indicated by the change in the ratio of microorganisms/indicator. Using this method, Zhao et al. (2011; 2013) reported the efficiency of the Andersen Six Stage Impactor, AGI-30, OMNI-3000 and gelatine filter in sampling *Enterococcus faecalis*, *E. coli*, *Campylobacter jejuni*, *Mycoplasma synoviae* and Gumboro vaccine virus. Compared to the gelatine filter, which has a known high physical sampling efficiency, the AGI-30 had an average sampling efficiency of 74% and the OMNI-3000 of 49%. For the Andersen Six Stage Impactor the physical efficiency could not be determined because of a low recovery of the tracer uranine from the agar plates. The biological sampling efficiency of *Enterococcus faecalis*, *E. coli*, *Campylobacter jejuni*, *Mycoplasma synoviae* and Gumboro vaccine virus were not significantly different from 100% for AGI-30. This was also the case for sampling with the OMNI-3000, except for *Campylobacter jejuni* (sampling efficiency of  $1.0 \pm 1.0\%$ ), and for Gumboro vaccine virus ( $12 \pm 8\%$ ). For the gelatine filter the biological sampling efficiency was different from 100% for *E. coli* ( $38 \pm 10\%$ ) and *Campylobacter jejuni* ( $2.0 \pm 1.0\%$ ). The lower biological sampling efficiency of the OMNI-3000 seems to be related to the strong forces when mixing sampled air with the liquid, while the lower efficiency of the gelatine filter seems to be related to dehydration of bacteria on the filter.

It has been shown in previous studies that airborne Gram-positive bacteria are more resistant to sampling stresses than Gram-negative bacteria and bacteria without cell wall (Stewart et al. 1995; Landman et al. 2004). This was confirmed in the mentioned study of Zhao et al. (2011). The bacterial species without cell wall in the study of Zhao et al. (2011), *M. synoviae*, preserved its culturability comparably to those with cell wall, and sometimes even better than the two Gram-negative ones.

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When a study does not require measurement of viable microorganisms other sampling techniques can be used. Then sampling techniques similar as used for sampling dust can be considered. A big advantage of the 'dust' methods is that the sampling duration can be largely prolonged, causing a large decrease of the detection limit. Depending on the objective of the study dust within a certain fraction may be sampled, e.g. inhalable dust (particles which can be inhaled through the nose and mouth, generally smaller than 50  $\mu\text{m}$ ), PM<sub>10</sub> dust (particles smaller than 10  $\mu\text{m}$ ), respirable dust (particles smaller than 4  $\mu\text{m}$ ), PM<sub>2.5</sub> dust (particles smaller than 2.5  $\mu\text{m}$ ). For ambient air standards are based on PM<sub>10</sub> and PM<sub>2.5</sub>, while for occupational health standards are based on inhalable and respirable dust. In all these fractions non-viable microorganisms and microbial compounds can be determined. When endotoxins are the objective of the study binder-free glass fibre filters should be used while these filters usually give the highest recoveries (NEN-EN 14031, 2003). Other types of filters should therefore be validated toward filters of glass fibre.

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## 5 Determining efficiency of bio-aerosol reducing systems

To determine the efficiency of bio-aerosol reducing systems the following points should be considered:

1. The scale of the system, e.g. full-scale, pilot-scale or lab-scale.
2. The microorganisms and/or microbial components that are sampled and analysed.
3. The experimental setup.
4. Agricultural context.

### Ad 1. The scale of the system

Generally it is advised to test systems first at lab-scale then at pilot-scale and then at full-scale. The advantage of small scale, i.e. lab-scale, is that the study can be done under controlled circumstances. It also gives the opportunity to test and sample microorganisms that cannot be tested under pilot or full scale conditions, because it is required to do such tests under BSL (Bio-Safety Level) 1, 2 or 3 conditions.

### Ad 2. Microorganisms to be sampled and analysed

Only at lab-scale it is possible to test the effect of reduction systems on microorganisms that fall under BSL 1, 2, or 3. In some cases, when a farm is infected with a certain pathogen, it is possible to do full scale measurements. However, generally it is too difficult to fit everything together at full-scale. first a farm needs to have an infection with the pathogen under study, then the reduction system needs to be installed and then measurements needs to be performed. Therefore for pilot and full scale measurements it is better to work with model microorganisms, that are always, or almost always present on the farm. Possible model microorganisms are given in Chapter 2.

### Ad 3. Experimental setup

Concentrations of microorganisms and of microbial components are generally very variable. Differences in concentrations of 1 LOG<sub>10</sub> or 2 LOG<sub>10</sub> are easily found between different locations. Therefore, to determine effects of reduction systems variations in space and time should be reduced as much as possible. As stated under Ad. 1, reduction systems are first tested under lab-scale, then under pilot-scale and finally under full-scale. At all the different scales the reduction system should be tested against a control situation. This control system should be tested against the test system under the same conditions. Only the emission reducing system should be the difference, the rest should be as similar as possible. In practice this means that the control and the test system should be tested at the same location (= same lab or farm) and at the same moment (= same conditions).

From the considerations given above it can be concluded that efficiency of reduction systems should be determined by direct comparison with a control system, at the same location at the same time. This principle is used within the VERA-protocol (VERA, 2011). Therefore, when determining the efficiency of reduction systems at full-scale the VERA-protocol should be followed. The main principles of the VERA-protocol are (Ogink et al., 2013b):

- The case and the control situation should be measured at the same location (= same farm) and at the same moment.
- The case and control system should only differ in the reduction principle. The rest of possible influencing factors should be as similar as possible.
- The case and control are compared on at least two different locations.
- On each location measurements are done on at least 6 measuring days.
- The efficiency is determined as a reduction compared to the control situation.
- When testing the efficiency of an air cleaning system, concentrations at the inlet side of the cleaning system are compared with the concentrations at the outlet side of the system.

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When testing air cleaning systems the following points should be addressed:

- The samplers should be positioned in such a way in the inlet airstream and the outlet airstream that a representative sample of the airstreams are obtained. When there is a high spatial variation in concentration in one or both of the airstreams use of additional samplers should be considered. By measuring other components in the air, e.g. CO<sub>2</sub>, NH<sub>3</sub> and/or dust, a good impression might be obtained of the variation in concentrations in the airstream and about the optimal location of the sampling.
- The air velocity around the samplers should not be too high. When this is higher than 2.0 m/s the possible effect on the sampling should be discussed in the report. See for background information about this point the report of (Hofschreuder et al., 2008).
- Care should be taken that the outlet air is the same air as the inlet air. This is especially relevant when the air is directly exhausted to outside. It should be prevented that the sampled air is diluted with fresh outside air. See for possible solutions to prevent this dilution the report of (Hofschreuder et al., 2008).

The effect of reduction systems should preferably be tested at relative high concentrations / emissions of the bio-aerosols. It is important to get insight in the effect of the reduction system in the whole concentration / emission range, however more than 50% of the measurements should be done in the upper concentration / emission range.

#### Ad 4. Agricultural context

Aim of the measurements is to get a representative set of data on the efficiency of the bio-aerosol reducing system. This involves normal management procedures and no exceptional situation within the animal house. In this protocol the agricultural management conditions are prescribed as formulated in annex B of the most recent version of the ammonia measurement protocol for livestock housing (Ogink et al., 2013a). Management conditions are described for the main animal categories and are representative for animal production in the Netherlands. Factors included are:

- Description of the housing management
- Minimum use of the facility since construction.
- Climate management
- Feeding management
- Minimum production standards
- Health status and maximum mortality/culling rates
- Minimum size of the facility.
- Minimum occupancy rate in relation to the allowed number of animals.
- Management parameters that have to be recorded during a sampling period and reported

With respect to bio-aerosol emissions, the feed composition, the amount of feed and water consumed, and the amount of cleaning water seems less important and therefore do not necessarily need to be registered. On the other hand additional care has to be taken in case of bio-aerosol measurements that normal procedures with regard to cleaning, providing food and litter are followed. Care has to be taken that no extra measures are taken that eliminate bio-aerosol emissions and which are not representative for normal management.

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## 6 Calculation of bio-aerosol emissions and reduction efficiency

### Concentration

The concentration of viable microorganisms will generally be given in colony forming units per cubic meter of air (CFU/m<sup>3</sup>). CFU's are reported as 10<sup>x</sup> or as x LOG<sub>10</sub>. For non-viable microorganisms, determined with PCR-techniques, and for microbial compounds other units will be used, e.g. endotoxin concentration is given in ng/m<sup>3</sup> or in endotoxin units per m<sup>3</sup> air (EU/m<sup>3</sup>).

### Ventilation rate

The ventilation rate is determined in cubic meter per hour (m<sup>3</sup>/h).

### Emission

The emission of viable microorganisms is given in CFU per hour or in another time unit, e.g. sec, min or day. The emissions are calculated per animal place, assuming that at the start of the measuring period the animal house was fully occupied. During the production cycle some animals may be removed, because they get sick or because they die. No correction is made for this (small) reduction in number of animals. However, both, the number of animal places, as well as the average number of animals present, should be reported.

### Reduction efficiency

To calculate the reduction efficiency first the geometric means (= median) of the control and the case measurements are determined per location. From these medians the reduction efficiency per location is calculated. The mean of these calculated reduction efficiencies of the measured locations determines the overall mean reduction efficiency and the standard error of this overall mean is calculated by calculating the standard deviation in reduction efficiency between farms and divide this by the square root of the number of farms.

### Emission factor

When a yearly emission factor should be determined, e.g. for endotoxins, then the same procedure should be followed as the one for dust (Ogink et al., 2011), including the correction for the non-occupancy period (Ogink et al., 2011; Appendix C).

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# 7 Measuring report

The measuring report should contain at least the elements given below (Ogink et al., 2011). When additional information is required compared to the report of Ogink et al. (2011) it will be reported as 'Additional info:'.

## Introduction and objectives

This section contains the motives, the context, the stakeholders, and the objectives of the study.

## Material and methods

### *Housing system or emission reducing principle*

This section contains the description of the housing system(s) and, when relevant, the reduction system that are going to be studied.

### *Description of the measuring location*

- Description of the animal houses/compartments
- Orientation of the animal house(s) (North/South)
- Size of the animal house / compartment (l x w x h); preferably a schematic drawing is given
- Manure management
- Description of solid and slatted floors
- Number of animals, total and number per m<sup>2</sup> (conformity with animal welfare standards)
- Description of the ventilation system (inlet, outlet, type and size of ventilator(s)) and ventilation control (min and max ventilation, temperature control, heating system)
- Feeding system (hardware, ad libitum or restricted, feeding times, kind of feed)
- Drinking system (hardware, ad libitum or restricted)
- Bedding material (yes or no, kind of material, how often and how much added)
- Light regime (dark and light periods)
- Production data of the animals (could also be included in the Results section)

### *Additional info:*

- Regular activities during the measurement days inside or outside the animal house / compartment that might affect the results, e.g. health check on the animals. Irregular activities during the measurement days inside or outside the animal house / compartment that may affect the results are reported under the Results section.

### *Measurement periods*

Contains a scheme with the dates of the measurements and contains information about the conformity of this scheme with the measuring strategy.

### *Measuring method and measuring equipment*

- Description of the measuring method
- Measuring equipment
- Location of the measuring points and motivation for the choice of these points
- Calibration of measuring equipment
- Quality checks of the measurements, e.g. use of blank samples
- Additional measurements to check the functioning of the reduction system might be necessary, e.g. characteristics of the scrubbing water when using air scrubbers
- Data analyses and calculation methods

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## Results

This section contains the following:

- All relevant animal parameters, e.g. the health status of the animals, antibiotics or other use of medicines
- Inside and outside climate conditions during the measurements, e.g. temperature, relative humidity
- In a summary table or in a figure the measured ventilation rates, concentrations and emissions
- Mentioning of special circumstances / irregular activities that might have affected the results
- Information about the functioning of the reduction system in relation to the interpretation of the measured data
- Calculation of the mean emission data and of the mean emission reduction factor, including standard errors of these means (between farms).


## Discussion and conclusions

Results are discussed in relation to the working principle of the reduction system and in relation to known emission levels from literature of similar housing systems. In the discussion it should be mentioned whether the results are reliable, accurate and representative for the studied housing and reduction system. The report is finished with a conclusion section in which the main elements of the results and discussion section are summarized in a few concise and precise written sentences.

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