A FIELD COMPARISON OF FOUR BIOAEROSOL SAMPLERS FOR ENUMERATING AIRBORNE FUNGI

by

Kit Shan Lee

Hon. B.Sc., University of Toronto, 2000

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

School of Occupational and Environmental Hygiene

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 2002

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

School ~~Department~~ of Occupational & Environmental Hygiene

The University of British Columbia
Vancouver, Canada

Date Sept 3rd, 2002

Abstract

Introduction: No standard method exists for enumerating fungal aerosols, impeding the development of reliable exposure-response data. A field comparison of four bioaerosol samplers, the Reuter Centrifugal Sampler (RCS), the Andersen N6 Single Stage (N6), the Surface Air System Super 90, and the Air-o-Cell sampler (AOC), was conducted in a variety of public buildings for the measurement of fungal aerosols to compare sampling performance efficiencies and to collect baseline data for a pool of buildings

Methods: Sampling was conducted at 75 sites in public buildings from June-October 2001 in the greater Vancouver area, British Columbia. Four locations were sampled at each site (1 common area, 2 offices, and 1 outdoor sample). Each location was sampled in parallel, collecting approximately 150 litres of air for each sample. Malt extract agar was used for all growth media. Sequential duplicates were taken at each location. Fixed- and mixed-effects regression models were constructed to examine the relationships between each method pair and to develop between-sampler calibration equations. Samplers were also scored and ranked on a combination of performance and other sampler characteristics. A survey of a panel of academics and consultants that regularly used bioaerosol sampling equipment for fungal aerosols was conducted to guide the comparison.

Results: Data from approximately 592 samples (60 different buildings) were available for analysis from each instrument. Differences were found between samplers for overall yield, detection limits, and reproducibility. Fixed- and mixed-effect models indicated location of the sample to be a confounder in the relationship of all method pairs, and interaction was also found for all except the N6-RCS comparison. Six final models were suggested to serve as possible calibration curves to convert measurements made with one sampler to those made with another. Surveys from 10 professionals were available to weight the other sampler characteristics. The final ranking for this comparison had the N6 and AOC ranked highest and the SAS and RCS the lowest.

Conclusions: Concentration data is dependent on the sampling methodology utilized for assessment and should be considered before conducting investigations of bioaerosols in different environments. Exposure guidelines cannot be created until a standard methodology is available.

Table of Contents

List of Tables

List of Figures

Abbreviations and Symbols

ACGIH	American Conference of Governmental Industrial Hygienists
AND VI	Andersen Six Stage Sampler
AOC	Air-o-Cell Sampler
BCBC	British Columbia Building Corporation
CFU	Colony forming unit
CFU/m^3	Colony forming unit per metre cubed
d_{50}	Cut-off diameter
Geo Mean	Geometric mean
GSD	Geometric standard deviation
L	Litres
LOD	Lower limit of detection
Ln	Natural logarithmn
L/min	Litres per minute
N6	Andersen N6 Single Stage
RCS	Reuter Centrifugal Sampler
SAS	Surface Air System Super-90
SFHR	Simon Fraser Health Region
SD	Standard deviation
UBC	University of British Columbia
UDL	Upper detection limit
VAA	Vancouver Airport Authority

Acknowledgements

I would like to extend my sincerest thanks to the three extra-ordinary members of my thesis committee. Thank you to Mike Brauer, Kay Teschke, and to my supervisor, Karen Bartlett, for all your hard work, encouragement, and enthusiasm during all stages of this thesis. I feel very fortunate to have had the input from the three of you during the progress of my thesis.

Thank you to Julie Hsieh and Victor Leung for their assistance and hard work throughout the project. The completion of this project would not have been possible without the both of you. Also, a thank you to Don Strutt from the BCBC, Dave Bell from the UBC, Quinn Danyluk from the SFHR, and Dan Strand from the VAA for providing the sampling sites for this project, and to all the contacts at each site for their co-operation.

A personal thank you to the faculty and all my wonderful friends here at SOEH. My experience at UBC has definitely been fun and memorable, and would not have been the same without you all! My memories here will be remembered fondly.

Finally, a thank you to my family and good friends back home in Toronto, and in Rankin Inlet, Nunavut, for their prayers and support during my transition to the West. A special thank you to my father (baba), to whom I owe all my success and accomplishments. Thank you for your inspiration, love, and inexhaustible support.

Chapter 1. Background Information and Study Objectives

1.1. Indoor Air Quality and Bioaerosols

The indoor environment has typically been regarded as a place of refuge and safety from the dangers of the outdoor world. However, there is increasing concern for the influence the indoor environment has on health, especially after a study by Spengler & Sexton (1983) found that urban residents spend more than 90% of their day indoors. Table 1.1 shows a summary of some of the indoor pollutants of concern and their emissions sources.

Table 1.1. Indoor Air Pollutants and Major Sources (Spengler & Sexton, 1983)

Pollutant	Major Emission Source
	Origin: predominantly outdoors
Sulfur Oxides (gases, particles)	Fuel combustion, smelters
Ozone	Photochemical reactions
Pollens	Trees, grass, weeds, plants
Lead, Manganese	Automobiles
Calcium, chlorine, silicon, cadmium	Suspension of soils, industrial emissions
Organic Substrates	Petrochemical solvents, natural sources, vapourization of unburned fuels
	Origin: indoors or outdoors
Nitric Oxide, nitrogen dioxide	Fuel Burning
Carbon Monoxide	Metabolic Activity, combustion
Particles	Resuspension, condensation of vapours, combustion products
Water vapour	Biological Activity, combustion evapouration
Organic Substances	Volatilization, combustion, paint, metabolic action, pesticides
Spores	Fungi, moulds
Radon	Building construction materials (concrete, stone), water
Formaldehyde	Particleboard, insulation, furnishings, tobacco smoke
Asbestos, mineral, and synthetic fibres	Fire retardant materials, insulation
Organic substances	Adhesives, solvents, cooking, cosmetics
Ammonia	Metabolic activity, cleaning products
Polycyclic hydrocarbons, arsenic, nicotine, acrolein	Tobacco smoke
Mercury	Fungicides, paints, spills in dental-care facilities or labs, thermometer breakage
Aerosols	Consumer products
Microorganisms	People, animals, plants
Allergens	House dust, animal dander, insect parts

The focus of this thesis is on fungal aerosols in the indoor environment. Fungal aerosols (spores, fragments of conidia, hyphae) are classified as bioaerosols. Bioaerosols, defined by the American Conference of Governmental Industrial Hygienists (ACGIH, 1999), are airborne particles that are living or originate from living organisms. These include microorganisms (culturable, non-culturable, and dead microorganisms) and fragments, toxins, and particulate waste products from all varieties of living things. Bioaerosols are everywhere and can be modified by human activity. People are exposed to a wide variety of bioaerosols every day (ACGIH, 1999).

1.1.1. Indoor Mould in Residential and Occupational Settings

There is a growing concern over the link between microbial air pollution and human illness in both residential and occupational settings. A survey, conducted by Seitz (1989) from the U.S. Department of Labour, found that that 20-30% of all commercial buildings has significant air quality problems. The U.S. National Institute for Occupational Health and Safety (NIOSH) documented an increasing percentage of air quality complaints due to microbial disease, from 5% in the 1970-80 to 50% in the 1990's (Lewis, 1995). Illnesses such as Sick building syndrome (SBS) and Building Related Diseases (BRD) have been linked to indoor exposure to microbial air pollution (ACGIH, 1999).

Many residential studies have found consistent correlation between reported moisture, mould, and respiratory problems (Dales et al., 1990; Dales et al. 1991; Brunekreef et al., 1996; Pirhonen et al., 1996; Andriessen et al., 1998). There is an increasing concern and awareness among the public regarding mould in residences. In California, queries to the State IAQ program by phone have increased ten fold since 1997, and Internet traffic has tripled in the last three years (Waldman et al., 2002). News coverage has served as a medium to increase public awareness of the issues associated with indoor mould. Waldman et al., (2002) found a dramatic spike in phone calls to the state IAQ programs after the television show, *48 hours*, had a show focusing on indoor mould entitled, "Invisible Killers".

1.2. Health Effects from Fungi

Studies in both occupational (Reynolds et al., 1990; Eduard et al., 1993) and residential (Brunekreef et al., 1989; Reynolds et al., 1990; Dales et al., 1991; DeKoster & Thorne, 1995; Pirhonen et al., 1996; Andriessen et al., 1998; Koskinen et al., 1999) settings have found an association between adverse health effects and exposure to airborne mould. Exposure-assessment methods have been limited. Most of the large epidemiological studies conducted on health and mould have assessed exposure by questionnaire without objective measures for exposure. Dose-response data has been very difficult to obtain due to the lack of standardized methodology and is one of the main reasons why the American Conference of Governmental Industrial Hygienists (ACGIH) has resisted suggesting exposure limits.

Exposure to fungi occurs on a daily basis without any adverse health effects. However, exposure to specific fungi or fungal products can cause human disease. Fungi are most often associated with allergic disease, such as allergic rhinitis/conjunctivitis and allergic asthma,

and hypersensitivity pneumonitis (Pope et al. 1993). There are three main health effects associated with exposure to fungal aerosols (Shum, 2002; ACGIH, 1999):

1) Infection, such as infection of the skin, mucous membranes, hair, nails and respiratory passage,
2) Allergy, such as skin allergy, asthma, and hay fever from exposure to allergenic components of fungi, and
3) Toxic effects (from exposure to fungal metabolic products such as mycotoxins, and volatile organic compounds, and cell wall components, such as glucans.

Adverse respiratory effects, such as asthma and rhinitis, have been associated with fungal genera such as *Alternaria*, *Cladosporium*, and *Penicillium* (Lighthart and Mohr, 1994).

1.3. Fungal Aerosols and Exposure Assessment

In order to further elucidate the health effects caused by fungal aerosols by risk assessment, dose-response data (with baseline data) are needed. The development of dose-response data for fungal aerosols have been complicated by the lack of standardized methodology. In the study of health effects due to fungal aerosols, a variety of methods have been used to determine exposure. Without standardized methodology, it is difficult to develop dose-response data that reflects all the studies conducted.

1.3.1. Qualitative assessment by Questionnaire

Previous epidemiological studies (Brunekreef et al., 1989; Dales et al., 1990; Dales et al., 1991; Pirhonen et al., 1996; Andriessen et al., 1998; Koskinen et al., 1999) used questionnaires to assess exposure qualitatively. Participants were asked to note the presence of water stains or visible mould in their homes, and their responses were used as a measure of exposure. Another approach, utilized by Pirhonen et al., (1996), was to use a trained surveyor to assess the extent of water damage or mould in a home. A study testing the ability of questionnaires to assess exposure found questionnaires not to be a reliable assessment tool for exposure to mould (Ren et al., 2001). However, questionnaires are easy, and have the ability to collect some information without needing to spend money on other measures of exposures.

1.3.2. Air Sampling

Air sampling has also been conducted to determine exposure. There are also many methods to conduct air sampling for fungal aerosols. The measurement of total culturable fungi to reflect exposure to airborne mould has been used in residential (Reynolds et al., 1990; DeKoster & Thorne, 1995) and occupational settings (Rautiala et al., 1996; van Netten et al., 1997). Air sampling for total fungal spores have also been conducted (Neas et al., 1996; Rosas et al., 1998). Another approach is to determine exposure to total fungal matter by sampling for a marker (such as ergosterol, a cell wall component of fungi). It is not certain whether viable spores, total spores, or total fungal matter, are related to health effects, making it difficult to determine which methodology best reflects exposure.

1.4. Fungi – General Introduction

Fungi are eukaryotic organisms that rely on external food sources for nutrients. Fungi reproduce asexually and sexually and often produce spores, which may be allergenic to humans. Fungi have rigid cell walls made up of chitin (acetyl glucosamine polymer) and glucans. The membranes of fungi contain ergosterol, which has been used in many assays to assess overall fungal growth. Fungi can be multi-cellular (formed of microscopic branches called hyphae) or unicellular (yeasts). Fungal colonies are a visible mass of interwoven hyphae that form a mycelium (Otten & Burge, 1999). The term 'moulds' is derived from fungi that are typically found growing indoors (species of *Penicillium*, *Aspergillus*, and *Cladosporium*). The major groupings of fungi, with some examples that are commonly found in indoor air, can be found in Table 1.4.

Table 1.4. Classification of some common fungi found in indoor and outdoor air[1]

Phylum	Class	Genera
Zygomycota (conjugation fungi)	Zygomycetes	*Mucor*, *Rhizopus*
Ascomycota (sac fungi)	Ascomycetes	Yeast, *Alternaria, Aspergillus, Cladosporium*
Basidiomycota (club fungi)	Basidiomycetes	pink yeast (*Sporobolomyces*), *Cryptococcus*
Deuteromycota (imperfect fungi)	Blastomycetes	*Geotrichum, Candida albicans, Rhodotorula* spp.
	Hyphomycetes	*Trichoderma, Penicillium, Fusarium*
	Coelomycetes	*Phoma*

[1] adapted from Otten & Burge, 1999

The presence of fungi is universal in outdoor environments, comprising about 25% of all biomass on the planet (Miller, 1992). Fungi can grow on living and dead plants, animals, and other microorganisms. The main role of fungi is to break down organic matter. Some fungi, (*Aspergillus fumigatus*, *Aspergillus flavus*, and *Penicillium* spp.) have been found to favour low oxygen levels, dryness and heat, growing in places like composts and silos. Other fungi (*Basidiomycetes* class) live in the soil as parasites on plants. The most common outdoor fungi live on the surfaces of leaves (phylloplane fungi), and genera such as *Cladosporium*, *Alternaria*, *Epicoccum* and *Aureobasidium* comprise about 40-80% of propagules in outdoor air in surveys worldwide, while soil-borne fungi, such as *Aspergillus* and *Penicillium*, constitute a smaller percentage (Miller, 1992).

1.4.1. Environmental Factors Influencing Fungal Growth

The presence of airborne fungi indoors is mainly due to infiltration from the outdoor air. Spores can enter with ventilation air, or on the surfaces of people, animals or objects (Pope et al., 1993). Fungi can grow when the environmental conditions, such as moisture, temperature, light, and nutrients, are in the proper balance. Different types of fungi prefer different conditions. Table 1.4.1 provides a summary of the moisture, temperature, light, and nutritional requirements for each major group of fungi.

1.4.1.1.Moisture

Moisture is the most important factor that controls the growth of fungi. Mycologists use water activity (a_w) to measure the water availability within a substrate that an organism can use to support growth (Otten & Burge, 1999). Fungi can be grouped according to the water activity they require for growth. Most fungi are hydrophilic ('love' water, also known as xerotolerant), with an optimum a_w=0.90 (Otten & Burge, 1999). There is a range of water activities for each fungus with the optimum dependent on temperature and the amounts and types of nutrients available. In indoor environments, wet environments like basements, windowsills, shower stalls, and buildings that have water damage, tend to have mould growth.

1.4.1.2.Temperature

Temperature has an effect on biochemical reactions that occur within an organism, and can have an impact on fungal growth directly and indirectly (by the control of water activity). Typically there are minimum, maximum, and optimum ranges of growth. There are four classes of fungi according to temperature: mesophilic, psychrophilic, psychrotolerant, and thermotolerant fungi (Otten & Burge, 1999).

1.4.1.3.Light

Fungi require light mainly for spore production.

1.4.1.4.Nutrients

All fungi require an external source of carbon. In indoor environments, carbon sources include the starchy pastes from wallpaper, cellulose in paper and fabrics, the keratin of animal scales, and the lignin in wood. Fungi release enzymes that digest these materials into glucose. The glucose is then absorbed. Other nutritional requirements include nitrogen, phosphorus, sulfur, and manganese (Otten & Burge, 1999).

Table 1.4.1. Summary of Environmental Influences on Fungal Growth[1]

Environmental Factors	Groups	Characteristics	Examples
Water Activity	Hydrophilic	Min a_w>0.90	*Fusarium, Rhizopus, Stachybotrys* spp
	Mesophilic	Min a_w≥0.8≤0.9 Optimum a_w=0.9	*Alternaria, Epicoccum, Ulocladium, Cladosporium* spp, *Aspergillus versicolor*
	Xerotolerant	Min a_w<0.8 Optimum a_w>0.8	*Eurotium*, some *Penicillium* spp.
	Xerophilic	Min a_w<0.8	*Aspergillus restrictus*
Temperature	Mesophilic Psychrophilic Psychrotolerant Thermophilic	15-30°C <0-17°C <15-17°C 35-50°C	Most fungi *Acremonium psychrophilum* *Cladosporium herbarum* *Humicola lanuginosis* *(Thermomyces* spp.)
Light	Most Fungi	Some light to stimulate spore production	
	Dark spored fungi	Resistant to ultraviolet radiation	
Nutrients	Saprobes	Use non-living organic material, responsible for decay and nutrient recycling	Most indoor fungi (eg. *Aspergillus* spp. and *Penicillium* spp.)
	Parasites	Usually live as saprobes, but invade living tissues when a suitable host is available	Obligate parasites (plant rusts)
	Symbiotes	Live in association with another organism	Mycorrhizal fungi (including mushrooms), lichens

[1]adapted from Otten & Burge, 1999

1.4.2. Sources and Composition of Indoor Fungi

The composition of fungal aerosols indoors is dependent on the abundance and strength of sources, as well as mixing, dilution, and particle removal (Pope et al., 1993). Natural

aerosols are typically a mixed species, though in agricultural and indoor environments, aerosols from a single species may be found (Pope et al., 1993), and thus may increase the risk of exposure to species-specific toxins or allergens. A survey of 1,717 buildings across the United States, using the N6 sampler, found the most common culturable fungi indoors and outdoors to be *Cladosporium*, *Penicillium*, non-sporulating fungi, and *Aspergillus* for all seasons and regions (Shelton et al., 2002). The amount of fungi in the air also varies over seasons, and Shelton et al., (2002), found outdoor concentrations to be higher in summer and fall, and lower in winter and spring across all regions.

1.5. Guidelines for fungal aerosols – Sampling strategies and data analysis

In response to the growing concern for both workers and residents, guidelines have been outlined by a variety of organizations such as the American Conference of Governmental Industrial Hygienists (ACGIH, 1999), New York State Department of Health (New York City Department of Health & Mental Hygiene, 2002), and Health Canada (Nathanson, 1995). A more comprehensive review of the existing quantitative standards and guidelines is available by Rao et al., (1996).

No standards specifying acceptable concentrations of airborne fungi exists other than for certain occupational environments (manufacturing). No consensus health-based guidelines exist. The guidelines outlined in this thesis represent a variety of approaches in the assessment of fungal exposure. The development of an exposure guideline for fungal aerosols is hindered by the need for exposure-response and baseline data for fungal aerosols. The ACGIH states that until guidelines are developed for particular environments, it is important that investigators not invoke previously published numbers as exposure limits, most of which the original authors no longer support.

1.5.1. ACGIH Guidelines (ACGIH, 1999)

1.5.1.1. Guidelines and Recommendations

Currently, the ACGIH does not support any existing numerical guidelines for interpreting data on biological agents from source or air samples in non-manufacturing environments. They recommend gathering the best data possible, and the use of knowledge, experiences, expert opinion, logic and common sense to assist in the interpretation of results.

1.5.1.2. Exposure Assessment and Data Interpretation

The ACGIH suggest the comparison of indoor and outdoor concentrations (typically an indoor/outdoor ratio<1) and species composition to distinguish between 'problem' and 'non-problem' environments. The presence of an indicator species (i.e., fungi that indicate excessive moisture) or potentially pathogenic fungi (fungi that pose a specific health hazard) should be investigated (but compared to the presence outdoors first).

1.5.2. Health Canada (Nathanson, 1995)

1.5.2.1. Guidelines

Health Canada has published guidelines based on a 3-year survey of federal buildings. These guidelines state that the "normal" air mycoflora is qualitatively similar and quantitatively lower than outdoor air (federal government buildings, 3 year average 40 CFU/m^3), and thus recommend that investigations should focus on determining whether the indoor environment reflects the outdoor environment.

The Health Canada guideline states:

- The presence of significant numbers of pathogenic fungi (*Aspergillus fumigatus*, *Histoplasma*, and *Cryptococcus*), should be investigated
- Air intakes, ducts, and buildings should be kept free of bird or bat droppings
- The persistent presence of toxigenic fungi (*Stachybotrys atra*, toxigenic *Aspergillus*, *Penicillium*, and *Fusarium* spp) indicates further investigation may be needed
- Significant presence of one or more fungal species in indoor samples not represented by outdoor samples is evidence of fungal amplifier
- >50 CFU/m^3 of single species (except *Cladosporium* or *Alternaria*) may require further investigation
- <150 CFU/m^3 is acceptable in summer if there are a mixture of species and reflect the outdoor species composition; higher counts suggest dirty or low efficiency filters
- >500 CFU/m^3 acceptable in summer if species primarily *Cladosporium*, or other tree/leaf fungi, values higher indicate failure of filters or contamination in building
- Visible presence of fungi in humidifiers and on ducts, mouldy ceiling tiles and other surfaces require investigation and remedial action regardless of airborne spore load

1.5.2.2. Exposure Assessment and Data Interpretation

Air and surface sampling is recommended to determine contamination and quantitation. It is recommended that samples be taken while ventilation system is turned off, early on a Monday morning. Air intakes, air supply plenum, air outlets, at desk level, at several locations throughout area in question should be sampled. Health Canada recommends a rank order assessment of the data.

1.5.3. New York City Department of Health & Mental Hygiene (2002)

Most of this document focuses on remediation guidelines and procedures. This summary will only focus on the guidelines and exposure assessment section.

1.5.3.1.Guidelines

- The presence of mould, water damage, or musty odours, should be addressed immediately. The source(s) of water must be stopped and the extent of water damage should be determined

- Mould damaged materials should be remediated in accordance with the document

1.5.3.2.Environmental Assessment

The visual inspection is identified as the most important initial step in identifying a possible contamination problem. The extent of any water damage or mould growth should be visually assessed.

A visual inspection of the following should be done

- Ventilation systems (damp filters, overall cleanliness)
- Ceiling tiles, gypsum wallboard, cellulose materials
- A boroscope (view spaces in ductwork or behind walls) or a moisture meter may be helpful in identifying hidden sources of fungal growth and extent of damage

Other assessment methods

- Bulk, surface, and air monitoring not required to undertake remediation
- Bulk/Surface sampling conducted only when:
    - To identify specific fungal contaminants as part of medical evaluation
    - To identify presence/absence of mould if a visual inspection is equivocal
- Air Sampling conducted when
    - An individual has been diagnosed with a disease associated with fungal exposure
    - Evidence from visual inspection/bulk sampling that ventilation systems may be contaminated

Note: For air sampling, outdoor samples should be taken for comparison (at air intake preferably), bulk, surface, and air samples should be taken by trained individuals, and analysis should be conducted by an accredited laboratory.

Analysis of bulk and surface samples

- Presence of a few or trace amounts fungal spores should be considered background; amounts greater than this, or the presence of fungal fragments (hyphae, conidiophores) may suggest fungal colonization, growth, and/or accumulation at or near the sampled location

Analysis of air samples

- Indoor/outdoor comparison by concentrations and fungal type (genera and species)
- Levels and types of fungi found should be similar indoors compared to outdoors (non-problem buildings)

- Differences in levels or types of fungi may indicate moisture sources and resultant fungal growth may be problematic

1.5.4. Threshold Limit Values - ACGIH

An exposure standard by the ACGIH has five components (ACGIH, 1999):

1) Scientific basis for the standard
2) Sampling method
3) Analytical method
4) Sampling strategy
5) Limit value

Without a standardized protocol for evaluating the airborne concentration of fungal aerosols, further characterization of these health effects and the development of an exposure guideline are very difficult, and as stated by the ACGIH, no exposure standard can be defined. Standardized procedures provide a basis for comparing problem environments and are required to enable the assessment of workplace health for employees. Without scientifically valid standards and guidelines, arbitrary criteria may be adopted leading to inappropriate testing and test interpretation. Several review publications are available describing possible methodologies for assessing exposure, which include descriptions of available samplers (Griffiths et al., 1994; Dillon et al., 1996; Flannigan, 1997; NIOSH, 1998; Eduard & Heederik, 1998; Dillon et al., 1999; ACGIH, 1999; Macher, 2000).

1.6. Background to Sampling Methodology for Fungal Aerosols

1.6.1. Introduction to Air Sampling

To determine the air concentration for fungal aerosols, the methodology for most bioaerosol samplers involve three main steps:

1) Air Collection – air is collected at a known flow rate
2) Particle collection – particles are separated from the air stream and deposited onto a sampling medium
3) Analysis – particles on sampling medium are analyzed for fungal aerosols

At each of these steps, there are a variety of methods available to accomplish the task. The use of different methodologies to accomplish each of these steps results in different collection efficiencies between samplers.

1.6.2. Sampling Performance

The sampling efficiency is defined by the ACGIH, 1999 as a combination of three efficiencies:

1) Inlet sampling efficiency
This is defined as the efficiency of an inlet to entrain particles from the ambient environment without bias for particle size, shape or aerodynamic behaviour.

2) Particle Removal Efficiency
This is defined as the ability to remove particles from the sampled air stream and to deposit them onto a sampling medium.

3) Biological Recovery Efficiency
This is defined as the ability to deliver collected particles to an assay system without altering the viability, physical integrity, or other essential characteristics of the biological agents.

Factors that influence these efficiencies ultimately depend on the design of the instrument. The following summary will only cover the differences that are of importance to this study and is not a comprehensive evaluation of all methods available.

1.6.2.1. Inlet sampling efficiency – air collection

The efficiency with which bioaerosols are collected by a sampler is dependent on several factors: particle size, density, and shape; a sampler's face velocity, inlet design and orientation; and ambient wind velocity (ACGIH, 1999). It is best to sample air isoaxially (in the same direction as the air flow) and isokinetically (at the same velocity of the airflow). However, this is difficult in areas with airflow (such as mechanically ventilated rooms) that may have varying flow rates. In environments with moving air, the bioaerosol concentration can be overestimated through the capture of particles not in the true sample airstream or underestimated through failure to capture all particles in the airstream if air is not sampled isoaxially (Grinshpun et al., 1994).

Samplers must be able to draw in air at a known and constant rate for quantification to be accurate. Calibration of flow rate is important since the air concentration data is dependent on an accurate flow measurement. Flow rates are typically given by the manufacturer and are checked by a variety of calibration methods.

1.6.2.2.Particle Collection

The collection of particles from the sampled air is accomplished if the particle is successfully removed from the traveling air stream. Impaction, usually by inertia, is the primary method of accomplishing this task. Impaction depends on a particle's inertial properties (size, density, velocity) and also on the dimensions of the instrument (inlet nozzle, airflow pathway) (Hinds, 1999).

The methods evaluated in this study utilize two types of impaction:
1) Inertial
2) Centrifugal

1.6.2.3.Inertial Impaction

Inertial impaction is the most widely used method for particle collection. All inertial impactors operate on the same basic principles. In this study, three instruments, the N6, SAS and AOC, utilize inertial impaction onto a sampling medium to separate particles from the air stream. Aerosols are passed through a nozzle and the output stream (jet) directed against a flat plate (impaction plate) as demonstrated by Figure 1.6.2.3. This impaction plate forces the airflow to bend 90^0. Particles whose inertia exceeds a certain value cannot follow the air stream and impact onto the flat plate (Marple & Willeke, 1976).

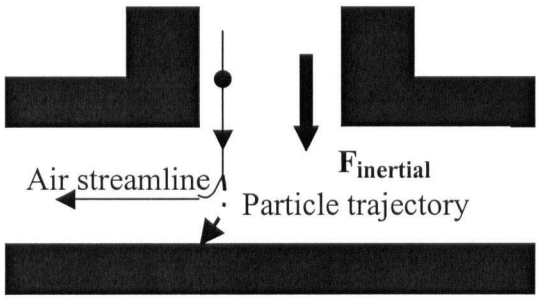


Figure 1.6.2.3. Inertial Impaction (adapted from ACGIH, 1999)

1.6.2.4.Centrifugal Impaction

Centrifugal impaction separates particles from an air stream by inertial impaction using centrifugal forces. Air is drawn in using a rotating impeller and the air stream is forced to make a 90^o turn when it hits the side of the sampling head (see Figure 1.6.2.4). The RCS uses centrifugal impaction to collect particles from the air stream. These particles are deposited onto an agar strip.

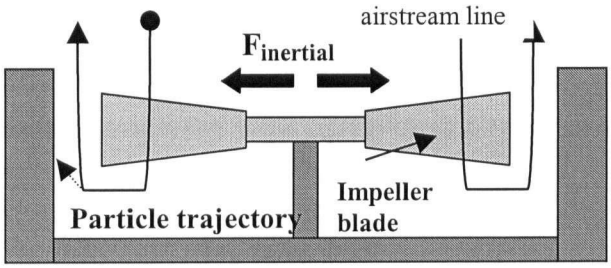


Figure 1.6.2.4. Centrifugal Impaction (adapted from ACGIH, 1999)

1.6.2.5.Particle collection efficiency (Cut-off diameter)

The efficiency at which different instruments collect particles is dependent on their specifications, such as flow rate and impaction principles. The cut-off diameter (d_{50}) designates the particle size at which half of the particles are removed and half pass through

the sampler, and therefore defines the particles sizes the instrument can collect (Hinds, 1999). Particles that are larger than this cut-off diameter are removed from the air stream at increasing efficiency and deposited on the sampling medium. The d_{50} is generally assumed to be the diameter above which all particles are removed, assuming that the instrument has a sharp cut-off curve. Fungal spores range from 0.5-20 μm (Hinds, 1999), but are typically larger than 2 μm. Instruments, such as the RCS, which have cut-off diameters greater than 2 μm will not be able to collect the smaller spores.

1.6.2.6. Biological Recovery Efficiency

This efficiency at which samplers deliver bioaerosols without destroying their biological activity or viability is not well characterized, but is dependent on many factors. For culture methods, the viability of the fungal spore is important. Exposure to heat, light, cold, dryness, toxic gases, shear and mechanical forces during sampling all affect the viability of bioaerosols. Stewart et al., (1995) suggest that impaction velocities used in different samplers may result in different metabolic and structural injuries to collected organisms. Continuous exposure to the sampling airstream can dry out microorganisms. It is very difficult to determine the biological recovery efficiency of sampling instruments, and this aspect has not been evaluated for most samplers.

1.6.2.7.Sampling Time and Collection Efficiency

Previous studies have shown varying collection efficiencies in sampling instruments due to differences in total sampling time. Folmsbee et al., (2000) found the highest average concentration (colony forming units/m^3) using a 2-minute sampling time with the Andersen Microbial Sampler compared to 1-, 3-, 4-, 5- or 6-minute samples. This may affect the results for sampling protocols that have varying sampling collection times. Sampling times are typically chosen based on the estimated prevailing bioaerosol concentration. This is to ensure that the samplers are operating within their detection limits, with shorter sampling periods for environments with higher bioaerosol concentrations, and longer sampling periods for environments with lower bioaerosol concentrations.

1.6.3. Analysis for fungal aerosols

Once the particles have been collected, analysis for fungal aerosols is performed. There is also a wide range of methods for analyses. Burge & Solomon (1993) stated that there are at least five major methods of analysis:

1) Culture
2) Direct microscopy
3) Bioassay
4) Biochemical assay
5) Immunoassay

Each of type of analysis has a different set of advantages and disadvantages. For this study, only the first two methods (culture and microscopy) were examined.

1.6.3.1. Viable versus Total Fungal Spores

Viable spores are those spores that are alive and have the capability of producing colonies. For analysis, a viable spore is a spore that is alive and remains alive during the sampling process. The measurement of viable spores as a representation of total fungal matter is an underestimate because: 1) not all spores are alive, and 2) not all 'live' spores that are sampled will remain viable after sampling. Whether viability is important in the development of illness has not been determined, and thus only measuring viable spores may not necessarily reveal the causative agent.

Total fungal spores represent all spores without any selection for viability. This can be determined using microscopy. It is also unknown whether the total fungal spore composition is important in the development of illness. Exposure to fungal aerosols occurs daily and can be at high magnitudes, especially in outdoor environments. Therefore, detecting the presence of fungal spores may not necessarily constitute a health hazard.

1.6.3.2. Culture (or viable) Methods

This method involves the deposition of the particles onto an agar medium that supports the growth of fungal aerosols into colonies. The ability to identify each colony to the species level makes this type of analysis method attractive. Quantitatively however, it underestimates airborne concentrations because of its dependence on viability. Only those spores that are alive in the aerosol and that remain alive during sampling, and that can grow under the provided conditions will survive (Burge, 1995). The colonies that do grow are enumerated following a set incubation period and an air concentration is defined as the total colony forming units (CFU) by the total air volume sampled. The viability of fungal spores on a sampling medium can be compromised by: impaction forces on spores, desiccation of the spores, nutrient conditions, temperature, and radiation environment. Also, some fungi grow more slowly, and may be out-competed on some growth media by faster growing fungi. For example, *Stachybortrys chartarum*, a fungal species that has thought to be the cause of toxic health effects, does not grow well on malt extract agar since it is usually out-competed by the faster growing species on the plate (ACGIH, 1999).

There are a wide variety of sampling media available, each with different nutrient compositions. For example, dichloran glycerol-18 is a sampling media that has a lower a_w, and also restricts the growth of colonies, preventing the overlap of colonies (Pitt & Hocking, 1997). Therefore, this medium is useful for the sampling of xerophilic fungi (fungi that colonize dry materials). The selection of medium is dependent on the hypotheses of the study. For this study, malt extract agar was used for all samplers employing growth media.

Malt Extract Agar (MEA)

MEA is used extensively as a medium for sampling the broad spectrum of fungi and is recommended by the ACGIH, Committee on Bioaerosols, for detection and enumeration of fungi in indoor environments (Burge et al., 1987). MEA does not support the growth of all

fungi. For high loads of fungal aerosols, colonies from faster growing fungi can easily overgrow the slower growing moulds, possibly masking colonies.

1.6.3.3.Microscopic Methods

This method, unlike culture methods, does not rely on the viability of fungal spores. Particles are impacted onto an adhesive slide. These slides are stained and then viewed under a microscope for enumeration. A variety of microscopic techniques, light, fluorescence and electron microscopy have been used to view fungal spores. For samplers using microscopy, total fungal spores or structures (including fragments) can be enumerated based on morphological characteristics. The disadvantage of the microscopic method is it can only speciate some fungal spores. For example, *Aspergillus* and *Penicillium* spores are indistinguishable by microscopy.

1.7. Study Overview

Adverse health effects, ranging from allergic to toxic, have been associated with exposure to indoor mould, but limitations in exposure assessment methods for fungal aerosols have made it difficult to obtain exposure-response information. No standardized methodology has been set for fungal aerosols, despite the wide variety of samplers that are commercially available. Standardized methods are needed to avoid inappropriate test interpretation.

The purpose of this study was to conduct a comparison of the following four widely used bioaerosol samplers:

1. Andersen N6 Single Stage Sampler (N6)
2. Reuter Centrifugal System Sampler (RCS)
3. Surface Air System Super-90 (SAS)
4. Air-o-Cell (AOC)

These samplers represent a variety of different methodologies for air and particle collection and different analyses for fungal aerosols. These samplers do not represent all the methods that are available, but are commonly used by professionals today for exposure assessment purposes.

1.7.1. Study Objectives

The objectives of the study were to compare the four samplers in the following manner:

1. To compare the field performances of each sampler by the following characteristics:
    - Proportion of samples below and above detection limits
    - Reproducibility (coefficient of variation)
    - Total Yield

2. To develop regression models for each method pair combination to examine the relationships between the four samplers.

3. To compare and rank the instruments on a combination of performance characteristics (reproducibility, proportion of samples beyond detection limits, overall yield, cut-off diameter) and other characteristics (cost, portability, ease of use, noise, sampling time, and historical and current use by industry).

1.7.2. Study Hypotheses (H_o)

Most bioaerosol samplers have typically been used interchangeably in the field. To reflect this general idea, the following study hypotheses were made:

Ho_1: No differences exist between the geometric means, detection limits, and reproducibility among the four samplers (RCS=N6=SAS=AOC)

Ho_2: Regression models between each method pair will show a linear and y=x relationship, with an intercept=0.

Ho_3: The four samplers will have the same score across all categories, resulting in an equal ranking for all four samplers

Chapter 2. Materials and Methods

2.1. Sampling Strategy for Field Comparison

2.1.1. Sampling Sites

The initial target number of sampling sites for field comparison was 75 public building sites. This was based on the definition of an individual sampling site as either:

1) A set of sampling locations in separate public buildings or
2) Two or more sets of sampling locations from the same public building that are on different floors, supplied by different air intakes or in different departments of the building.

To achieve this target, four organizations were contacted to provide a pool of public buildings as sites for the study:

2.1.1.1.The Building Corporation of British Columbia (BCBC)

The BCBC was established in 1977 to provide accommodation and real estate services to the provincial government. Since 1997, BCBC's mandate was expanded to enable the Corporation to provide its services to the broader public sector (Province of British Columbia, 2001). The BCBC was approached for a list of buildings.

2.1.1.2.The University of British Columbia (UBC)

The UBC is located at the western tip of the Point Grey peninsula near the city of Vancouver. The buildings on the campus are mostly new, though the history of UBC dates back to 1929 (The University of British Columbia, 2002). A general email was sent out to all the departments affiliated with UBC and interested parties were asked to reply directly.

2.1.1.3.The Simon Fraser Health Region (SFHR)

The SFHR provides a variety of health services to the residents from Burnaby, New Westminster, Coquitlam, Port Coquitlam, Port Moody, Anmore, Belcarra, Pitt Meadows, and Maple Ridge (SFHR 2002). Site locations and the sampling schedule were determined by consultation with the safety consultant for the SFHR.

2.1.1.4.The Vancouver Airport Authority (VAA)

The VAA is responsible for the management and operation of the Vancouver International Airport (YVR) (Vancouver International Airport Authority, 2001). Site locations and scheduling were determined by consultation with the occupational hygienist.

These four organizations provided a pool of buildings that represented a variety of building types in different areas of Vancouver (see Table. 2.3.1.).

Table 2.1.1. Locations and Buildings Types by Organization

	ORGANIZATION			
	BCBC	UBC	SFHR	VAA
Sampling Locations	Vancouver	Vancouver	Burnaby Port Moody	Richmond
Building Types	Government offices in large and small buildings, with either mechanical or natural ventilation	Administration offices, research laboratories, Performing arts theatre, Gymnasium, In large and small buildings, with either mechanical or natural ventilation	Hospitals, Long term care facilities, Administration Offices in large and small buildings with either mechanical or natural ventilation	Administrative offices in temporary mobile buildings that served as offices with either mechanical or natural ventilation

2.1.2. Site Selection and Sampling Location

A contact person, generally in the management level of the office, was provided for each site. From the pool provided, each site was contacted by telephone. 75 sampling sites from the greater Vancouver area, British Columbia, were chosen and scheduled for sampling based on the convenience and the availability of the occupants. Selection criteria for buildings consisted of only two parameters: 1) Buildings were public buildings (not residential) and 2) "Problem" buildings (buildings with publicized water damage or mould problems) were excluded.

At each sampling site, four locations were chosen, under consultation with the site contact, to be examined:

- One common room

A common room was defined to the contact as a place where people frequented but was not occupied by a specific person for a constant period of time. Examples were a reception area, kitchen, hallway, lounge or meeting room.

- Two individual rooms or offices

An individual room was defined as an area where specified occupants spent a constant period of time such as an individual or shared office.

- One outdoor location

The outdoor sample was taken close to the air intake if the building was mechanically ventilated. For locations where the air intake was not accessible or applicable, the samples were taken in a location where the security of the sampling instruments could be guaranteed (a place that was away from public traffic or that could be easily monitored by sampling technicians) and was chosen by consultation with the contact. Examples of outdoor locations suggested to the contact besides the air intake included the roof, the loading zone, balcony or outside a window.

2.2. Bioaerosol Samplers – General Introduction, Calibration, and Maintenance

The following sections describe the principles of each instrument based on the sampling protocol utilized by this study.

2.2.1. Reuter Centrifugal Air Sampler Standard (RCS)

The Reuter Centrifugal Sampler Standard (Biotest, Frankfurt, Germany) draws air into the instrument by an impeller (total sampling rate of 280 L/min, effective sampling flow rate of 40 L/min) from a distance of at least 40 cm (see Figure 2.1.1). Each strip contains 34 wells (34 x 1cm^2) filled with an agar medium (see Figure 2.1.1.1C.). The particles are collected by centrifugal impaction onto an agar strip (Biotest HYCON, Germany) at a d_{50}=4μm. The RCS is a self-contained, battery operated (4 alkaline 'D' batteries, electrical power=3 Watts) air sampler that can be set at 0.5-, 1.0-, 2.0-, 4.0- and 8.0- minute settings. The RCS can be used to enumerate fungi or bacteria.

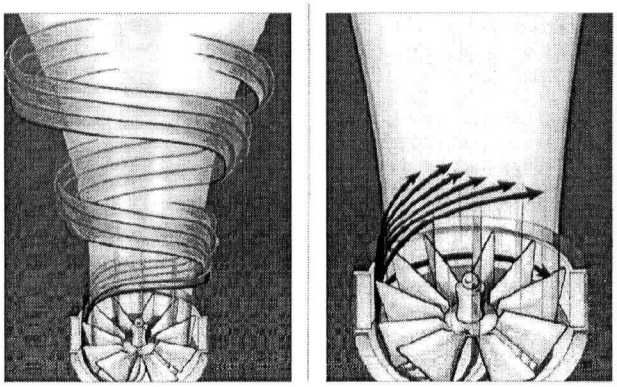


Figure 2.2.1. RCS Impeller Air Flow (Biotest, 1997)

2.2.1.1.Calibration

The RCS has a total sampling flow rate of 280 L/min. However, to calculate the volume of air sampled, 40 L/min is used (separation flow rate) based on an average particle diameter of 4 μm. The direct calibration by a primary method is not possible for this instrument because air enters and exits the instrument through the same opening. Instead, the manufacturer suggests two methods of verification that the total sampling rate of 280 L/min by:

1) **The impeller blade angle must be set to specification.**
A deviation in the blade angle of the impeller can alter the volume collected by the instrument (see Figure 2.2.1.1.B).

2) **The impeller rotation frequency must be set to 4,096±82 rpm (2% deviation).**
Deviations in the rotation frequency can be detected by timing the operation of the instrument on the 8-minute setting. The instrument generates 16 electronic pulses per revolution and the number of impulses for each running time is programmed into the unit.

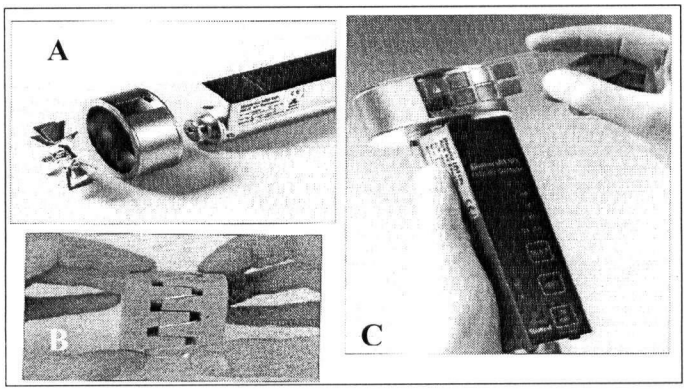


Figure 2.2.1.1. The RCS (Biotest, 1997)
A. RCS Sampler (Impeller/drum)
B. RCS Calibration Set
C. RCS Agar Strip

2.2.2. Andersen N6 Single Stage Impactor (N6)

The Andersen N6 Single Stage impactor (Graseby-Andersen, Atlanta, GA, USA) is a sieve type sampler that collects air through 400 holes (diameter of each hole=0.026 cm). The particles were collected at a d_{50}=0.65 μm by inertial impaction. The particles were deposited onto an agar medium (100 mm petri dish, Phoenix Biomedical, Canada). The N6 consists of an aluminum inlet cone, sampling stage, and a base plate held together by three spring clamps and sealed with O-ring gaskets. The N6 can be used to enumerate fungi or bacteria. The N6 is a modification of the Andersen Six Stage sampler. The Andersen Six-Stage sampler (Andersen, 1958) collects particles by drawing air through six successive stages. The N6 is equivalent to the sixth stage. A study by Jones et al. (1985) found that the N6 produces results in total CFU/m^3 that are comparable to the Andersen Six Stage sampler. However, the N6 does not have the size selection that the Andersen Six Stage sampler has and may be more prone to overloading. For 'clean' office environments, where lower counts are expected, the N6 serves as an attractive alternative to the Andersen Six Stage sampler because it is smaller and does not use as many sampling plates, reducing the cost in preparation and analysis.

2.2.2.1.Gilian AirCon-2 High Flow Pump and Calibration

A separate sampling pump is required to draw air through the impactor at 28.3 L/min. Air was drawn through the N6 using a Gilian® AirCon-2 High Flow pump (Sensidyne®, Clearwater, FL) set at 28.3 L/min. This pump was operated on its 4-hour rechargeable, 12V DC battery. The operating specifications for this pump are: airflow range of 2-30 L/min,

constant flow capabilities up to 1.5 psi pressure for the specified flow, operating temperature of -20°C to 45°C, and relative humidity of 0-95%. The flow rate is regulated internally by a critical orifice and was controlled by the flow adjust valve and rotameter. See Figure 2.2.2.1 for a schematic diagram of the pump.

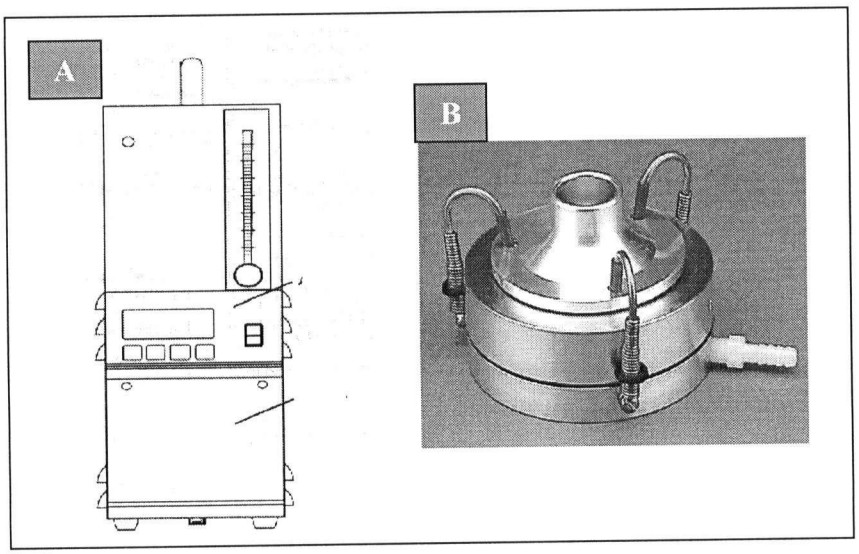


Figure 2.2.2.1. A. Gilian AirCon2 High Flow Pump (Sensidyne, 1990)
B. N6 Impactor (Thermo Andersen, 2002)

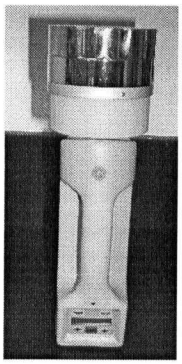
2.2.3. Surface Air System Super-90 (SAS)

The Surface Air System Super-90 (PBI International, Milan, Italy) is a self-contained, battery operated (rechargeable 8.4-Volt, 1.2 A/hr, nickel-cadmium battery), single stage, sieve type sampler (see Figure 2.1.3.). Air is drawn through a single impactor plate with 487-holes (diameter of each hole=0.1 cm). Particles are collected by inertial impaction and deposited onto an agar medium (84 mm maxi Replicate Organism Direct Agar Contact (RODAC) plate, Bioscience International, Rockville, MD) with a d_{50}=2-4μm. Air is drawn with an internal motor (6-Volt, 15 W) at 90 L/min and can be pre-set to collect 10, 20, 30, 50, 100, 200, 500 and 1,000 litres, with an option of 8 other volumes, and a max volume of 1,800 litres. The SAS can be used to enumerate fungi or bacteria. The SAS uses similar collection principles as the N6, but is a portable, self-contained air sampling system. The SAS has a very quiet motor (unlike the N6 pump) and is an attractive alternative for occupied spaces.

2.2.3.1.Calibration

Direct calibration of this instrument cannot be made because of the nature of the sampling head. Factory calibration of this instrument is recommended on an annual basis.

Figure 2.1.3. SAS-90 Sampler



2.2.4. Air-o-Cell Sampler (AOC)

The Air-o-Cell Sampler (Zefon International, St. Petersburg, FL) is a battery operated (rechargeable) glass slide impactor. An external sampling pump is required to draw air through the sampling cassette at 15 L/min. Particles are impacted inertially onto an adhesive coated slide (see Figure 2.1.4) with a stated d_{50}=2.6 μm. Analysis is done by microscopy. The Air-o-Cell sampler can be used to enumerate fungal spores, pollen, fibres, and other aerosols (cell fragments, combustion emissions, and insect parts).

2.2.4.1.Air-o-Cell Mini Pump and Calibration

Air was drawn through the cassette utilizing the Air-o-Cell Mini pump (Zefon International, St. Petersburg, FL, US). The mini pump operates on a rechargeable battery (lead acid) set at 15 L/min, and can sample at 1-, 5-, 10- and continuous minute timing intervals. The mini pump is calibrated by a rotameter that is provided with the pump.

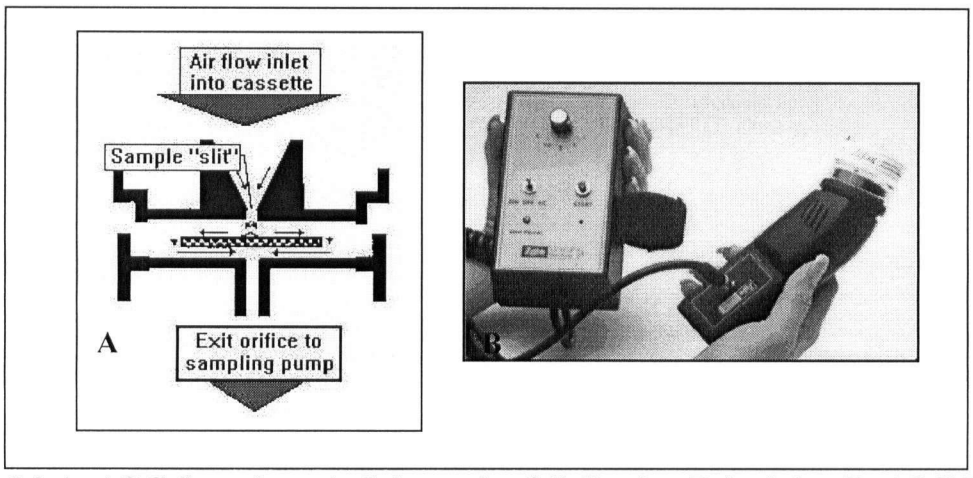


Figure 2.2.4. AOC Sampler – A. Schematic of Collection Principles, B. AOC Pump (SKC, Inc, 2002)

Table 2.2.5. Summary of Air Collection Characteristics

Sampler	Air Collection Methods	Flow Rate (litres/min)	Calibration
RCS	Air drawn in through one opening by impeller	40	No direct method, based on revolutions per minute of impeller blade
N6	Air drawn in through multiple holes (Sieve-type)	28.3	Primary standard or Factory
SAS	Air drawn in through multiple holes (Sieve-type)	90	Factory only
AOC	Air drawn in through one slit	15	Rotameter provided by manufacturer

Table 2.2.6. Summary of Particle Collection Methods and Efficiencies

Sampler	Impaction Method	d_{50} (µm)	Reference
RCS	Centrifugal	4.0	Macher and First (1983)
N6	Inertial	0.65	Andersen (1958)
SAS	Inertial	2-4.0	Lach (1985)
AOC	Inertial	2.3-2.4	Aizenburg et al. (2000)

2.3. Sampling Media

Figure 2.3 presents a picture of the N6, AOC, and RCS sampling mediums, respectively.

2.3.1. Culture Medium

The culture medium used in this study for the RCS, N6, and SAS, was malt extract agar (MEA). MEA consists of maltose, 12.75g/L, dextrin, 2.75g/L, glycerol, 2.35g/L, pancreatic digest of gelatin, 0.78g/L, and agar, 15.0g/L. It has a final pH of 4.6±0.2. A summary of the different sampling media used for each viable sampler is provided in Table 2.3.

2.3.2 Sticky glass slide

For the AOC, particles were collected by impaction onto a sticky glass slide contained in the Air-o-cell sampling cassettes. Cassettes were single use only and purchased directly from the manufacturer, therefore no preparation was required.

Figure 2.3. Sampling Medium – A. N6 plate, B. AOC slide, C. RCS Agar Strip

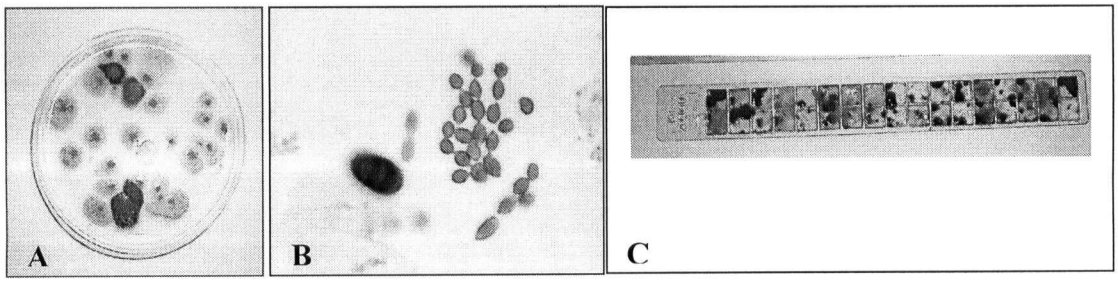


Table 2.3. Sampling Medium, Sampling Area, and Media Volume

Instrument	Sampling Medium	Sampling Area	Volume of Media
RCS	Agar Strips	34 cm^2	~9 mL
N6	Petri Dishes	78.5 cm^2	~40 mL
SAS	Maxi RODAC	55 cm^2	~20 mL
AOC	AOC Cassettes	16.5 mm^2	NA

2.4. Sampling Protocol

2.4.1. Daily Maintenance of Samplers

The RCS sampling head, the N6 impactor, and the SAS impactor plate were autoclaved at 121°C for 20 minutes before each sampling day. The AirCon-2 High Flow pump (115/230 V AC), SAS (220-240/110 Volts), and Air-o-Cell Mini Pump were charged overnight with their respective batteries.

2.4.2. Calibration Protocols for each Sampler

2.4.2.1. Calibration of the RCS

The impeller blade angle was checked with the provided calibration set prior to every sampling day. The impeller rotation frequency was verified to be within specifications after every battery change by timing the operation of the instrument set on the 8 minute setting with an external timer (Radioshack™ Dual Timer, Barrie, Canada). After every battery change, it was checked that, when set at the 8 minute setting, the instrument operated for 480±9 seconds (2% deviation). The 4 x 1.5 Volt 'D' alkaline batteries for the RCS were changed once every 2 weeks. The rotation frequency of the RCS impeller was also checked upon completion of the study by using a tachometer to determine the revolutions per minute and was found to be 4,096 rpm.

2.4.2.2. Calibration of the AirCon-2 High Flow Pump (for N6)

Direct calibration of the flow rate was not performed daily, but the flow rate of the pump was factory calibrated on October 27, 2000 by Sensidyne. The airflow of the pump was verified upon completion of the project using a Gillian Bubble Meter, an adapter that fit into the sampling inlet of the N6 impactor, and a petri dish with agar. Tubing was used to connect the adapter to the bubble meter. The pump was turned on and the flow rate was averaged over ten readings. The flow rate was found to be approximately 28 ±3 L/min. This methodology is similar to factory calibration (Thermo Andersen, personal communication).

2.4.2.3. Calibration of the SAS

Calibration of the flow rate for the SAS was performed at the end of the study, on October 28, 2001 (JBW & Associates, Inc, Frederick, MD). Factory calibration is conducted with a wind-tunnel calibration system, a thermoresistor anemometer (measures airflow), and a calibrated digital timer (Pratt, P., personal communication). The anemometer measures the

flow rate of the instrument in the wind tunnel. The instrument is also set to sample for 100-, 500-, and 1000 litres with a sampling plate in place, and timed with a calibrated timer. The final flow rate was found to be 97.5 L/min and all subsequent analyses were based on this flow rate.

2.4.2.4.Calibration of the Air-o-Cell Mini Pump (for AOC)

The sampling flow rate for the AOC was calibrated, prior to each sampling day, using the provided rotameter (Zefon International, St. Petersburg, FL). The rotameter was held upright in one hand and the pump was held in its inverted position in the other hand. Keeping the pump in its inverted position, the rotameter was attached to the mouth of the pump. The pump was then turned (still inverted). The pump was set at a flow rate of 15 L/min when the plastic float was within the marked black line of the rotameter.

2.4.3. Field Sampling Protocol

Sampling was conducted on weekdays from Monday to Thursday (June, July, August 2001) and Monday to Friday (September, October 2001) during normal work hours (8:30am-5pm). Within this time frame, four locations were sampled:

- One common room
- Two individual rooms or offices
- One outdoor location

The locations and times of sampling at each site were determined by consultation with the site contact after explaining the protocol and the definition for each location. The scheduling for sampling at each location was based on convenience and availability. Occupants were allowed to use the common areas and offices normally during sampling.

2.4.3.1.Instrument Set-up

For indoor locations, sampling was conducted as close to the center of the room as possible. A limit in the battery power of the AOC pump made access to electrical outlets necessary. For outdoor locations, sampling was conducted in an area that was out of the way from pedestrian or vehicle traffic. If possible, sampling was conducted under an overhang. During periods of rain when an overhang was not possible, an umbrella was used to prevent water from entering the instruments.

For every sampling run, each sampler was elevated on a tripod to approximately 1.5 metres to ensure that each sampler was drawing air from a similar air space (typically defined as 'the breathing zone'). Instruments were set up in a line in the same order, each with approximately 1 m^2 clearance between equipment. The AirCon2 high flow pump for the N6 was set up so that its exhaust was facing away from the samplers.

2.4.3.2.Instrument Specifications and Sample run time

Table 2.4.3.2 presents a summary of the flow rates, sample times and total volumes collected for each instrument. The goal was to sample approximately 150 L with each instrument. Instrument specifications did not allow for all four instruments to sample exactly 150 L. Therefore, the instruments were operated for a time period that approximated this volume as closely as possible. Due to the differences in sampling run times, instruments operated over slightly different time frames. The AOC was turned on first, followed by the N6, the RCS and lastly, the SAS to overlap air sampling. The RCS, AOC and SAS have internal timers and automatically shut off once sampling period was completed. For the N6 sampling pump, an external timer (Radioshack™ Dual Timer, Barrie, Canada) was set for 5 minutes. The air pump and timer were started simultaneously and both shut off immediately after the alarm went off.

Table 2.4.3.2. Target flow rate, sampling time, and sampling volume

Sampler	Flow Rate	Sample Time	Total Volume
RCS	40 L/min	4 min	160 L
N6	28.3 L/min	5 min	140 L
SAS	90 L/min	1min 20sec	150 L
AOC	15 L/min	10 min (indoors) 5 min (outdoor)	150 L (indoors) [1]75 L (outdoor)

[1]A lower volume was collected outdoors with the AOC to prevent overloading.

2.4.3.3.Air Sampling Protocol

Prior to each sampling run, the N6, SAS, and RCS sampling heads were thoroughly wiped with a 70% alcohol wipe. For the N6, a petri dish was placed onto the base of the sampling head. The lid of the petri dish was placed over the inlet of the N6 to prevent contamination. For the SAS, a RODAC plate was fitted onto the sampling head. The lid of the RODAC was removed immediately prior to sampling. For the RCS, the agar strip was removed from the plastic cover and inserted into the sampling drum. The RCS sampling head was capped with the provided plastic cover until sampling commenced. For the AOC, the sampling cassettes were unsealed and fitted onto the sampler head immediately prior to each sampling run. For the RCS, N6, and SAS, a sequential duplicate was taken in the exact same manner after the first run was complete for all instruments. For the AOC, a sequential duplicate was taken immediately after the first sample was completed. This protocol was repeated in the exact same manner in all four locations for each site. A unique 9-digit identification code was assigned to each sample taken. Sequential duplicates were given the designation 'R' immediately following the code.

Upon completion, samples were repackaged into an ice cooler and transported back to the laboratory at UBC. One field blank was taken for each instrument for each site and treated in a similar manner but not exposed to the air.

2.5. Laboratory and Sample Analysis Protocols

2.5.1. Sample Medium Preparation and Quality Control Protocols

25.2g of MEA media (BBL, Becton Dickinson and Company, Cockeysville, MD) was mixed with 750 mL of distilled water in a 1,000 mL wide mouth flask. The mixture was autoclaved at 121°C for 20 minutes on slow exhaust. The media was allowed to cool to 90°C and then put into a 60°C hot water bath until the flask was cool enough to touch with the inner arm. All media plates were prepared using sterile techniques. 40 mL of media was poured into 100 mm petri dishes (Phoenix Canada, Biomedical) for the N6. A 25 mL pipette was used to fill the 84 mm RODAC plates for the SAS with media. A 10 mL pipette was used to fill each well of the RCS agar strips with approximately 1 mL of media.

All poured media plates were left at room temperature for 72 hours prior to refrigeration to determine if contamination occurred during the pouring process. All field blanks were treated similarly to the samples to account for any transport contamination.

2.5.2. Incubation and Counting of Viable Samples (RCS, N6 and SAS)

Samples were taken back to the lab and incubated at room temperature (25°C±4) in the natural light and dark cycle (approximately 14 hrs light and 10 hrs of darkness for duration of study). RCS strips were incubated for 4 days, and the SAS and N6 samples were incubated for 5 days (a shorter incubation period was set for the RCS to prevent overgrowth). Total colony forming units (CFU) were determined utilizing a Scienceware® colony counter (Bel-Art Products, Pequannock, NJ) and a Scienceware® Mini Light Box (Bel-Art Products, Pequannock, NJ). Samples were placed on the mini light box bottom side up and a solid black dot was placed in the center of each colony using the colony counter. The total colony forming units were enumerated for each sample and recorded on data sheets. The samples were counted in a specific order with N6 samples first, then SAS and lastly RCS samples to facilitate data entry. Field blanks, followed by common room, room 1, room 2 and lastly outdoor samples were counted. Once counting was completed, samples were packaged and put into the fridge.

2.5.3. Slide preparation and examination of AOC Slides

2.5.3.1. Staining with Lactophenol Cotton Blue

There are several stains available, but for this study, lactophenol cotton blue was used. This stain targets chitin, a polymer of N-acetyl-D-glucosamine, which is a major component of the walls of fungal spores (Pitt and Hocking, 1997). Chitin is also found in the hard shells of crustaceans and insect exoskeletons.

2.5.3.2. Slide preparation

Cassettes were disassembled and the glass slide removed. Each AOC slide was stained with two drops of lactophenol cotton blue and then mounted onto a microscope glass slide. The

two drops of stain were placed in the center of a microscope glass slide. The AOC slide was slowly lowered onto the stain, with the sample side down, to facilitate even staining. The AOC slide was fixed onto the microscope glass slide by placing approximately 1 drop of Cytoseal™ XYL (Stephens Scientific, Kalamazoo, MI) in the center of each side of the AOC slide. The drops were spread along each side of the slide with a toothpick to form a permanent seal between the AOC slide and the microscope glass slide. Slides were dried for approximately 3 days in a fume hood and then stored in slide boxes until microscopic analysis was performed.

2.5.3.3.Enumeration of AOC Slides

Slides were counted using a modified version of the NIOSH Method #7400 (Fibres in air). Spores were counted using light microscopy (Jenamed2 Fluorescence microscope, Carl Zeiss Jena) set at 500x magnification. For each slide, one drop of Type B, formulae code 1248, non-drying immersion oil (R.P. Cargille Laboratories Inc, Cedar Grove, NJ) was used to facilitate viewing of the slide. The field diameter at 500x magnification was determined by using a Nikon (Japan) stage micrometer with 0.01 mm gradations (field diameter at 500x=360μm). Prior to counting, a survey of each slide was conducted to determine the general area of particle impaction. This survey was conducted to position the counting within the impaction area of the slide and not on the edge. Counting outside of the area of impaction could lead to underestimates of the actual spore counts. Counting proceeded systematically from the lower edge to top, from the left to right. Spores in the entire field of view were counted.

2.5.3.4.Counting Rules for Enumeration of Fungal Spores

Prior to counting slides, the microscopist underwent an initial training session with an experienced mycologist. Introductory concepts, such as the basic morphological features (size and shape) of fungal spores and the variety of stain colouration, were introduced in this session. Several texts (Malloch, 1981; Smith, 1990) were used as reference guides to the variety in size and shapes of fungal spores. The general counting rules for the AOC slides were:

1) Spores were defined as structures with:
    - Round or definite structure
    - Blue, brown, or yellow staining
    - A cell wall
    - A shape or size resembling the images in the reference texts (Malloch, 1981; Smith, 1990).

2) A maximum of 400 spores or 100 fields (whichever came first) were counted for each slide.

2.6. Data Analysis

2.6.1. Statistical Software

All data entry and analysis was conducted using SPSS 10.0 for Windows (©SPSS, Inc., 1989-1990), Microsoft® Excel 2000 (©Microsoft Corporation, 1985-1999) or S-Plus 2000 Professional Release 3 (©MathSoft Inc, 1988-2000).

2.6.2. Field Blanks

For viable methods, samples that had field blanks with colony formation were adjusted only if they contained the same type of fungal colony as the field blank. For microscopic methods, field blanks only provided means of determining if there was any significant factory contamination (eg. epithelial cells with fungal spores). If similar contamination was found on sample slides, the contaminants were not enumerated.

2.6.3. Values below the lower limit of detection (LOD) or above upper detection limit (UDL)

2.6.3.1.Detection limits

The detection limits of these samplers have not been defined by the manufacturer, but instead were defined in this study based on theoretical principles. Table 2.6.3 presents the theoretical limits (upper and lower detection limits) for each sampler.

2.6.3.2.Lower Limit of Detection (LOD)

The lower limit of detection (LOD) is defined as the air concentration of culturable fungi or spores that would result in the formation of one colony-forming unit on an agar medium or the impaction of one spore on a glass slide. Theoretically, the LOD can be adjusted by adjusting the volume collected (to decrease the LOD, the volume of air collected is increased).

2.6.3.3. Upper Detection Limit (UDL)

The upper detection limit has been defined as the air concentration that would result in a colony density on the sampling medium that would hinder the ability to distinguish between two colonies or two spores and can be adjusted by reducing the volume of air collected. For sieve type samplers (N6 and SAS), the UDL is suggested to be equal to the number of holes in the impactor plate (Dillon et al., 1996). For the SAS, the manufacturer suggests a UDL of 260 CFU (uncorrected) because numbers higher than this were believed to lead to inaccuracy (Pratt, P., personal communication). For the RCS, where the impaction of particles onto the medium is random, the UDL is defined as the maximum colony density of 5 CFU/cm^2 (for the RCS, 34cm^2 x 5 CFU/cm^2 $\approx$ 180 CFU) (Dillon et al., 1996).

Table 2.6.3. Lower and Upper Detection Limits

Sampler	Total Volume (l)	LOD	UDL
RCS	160	6 CFU/m^3	1,125CFU/m^3
N6	141.5	7 CFU/m^3	18,572 CFU/m^3
SAS	162.5	6 CFU/m^3	7,471 CFU/m^3
***AOC**			
Indoor	150 L	11 spores/m^3	[1]NA
Outdoor	75 L	22 spores/m^3	[1]NA

*Two LODs for AOC because different air volumes collected
[1]No UDL determined because no samples were overloaded to define a limit

2.6.3.4.Replacing values beyond detection limits

For samples outside of detection limits, values were replaced for statistical purposes and descriptive data summary. Samples below the LOD (zero values) were replaced with a value of 0.5 of the LOD. Overloaded samples were given a value equal to the UDL (Macher 1999).

2.6.3.5.Proportion of samples beyond detection limits

The total number of samples, below and above detection limits, was determined for each sampler and the respective proportions were calculated.

2.6.4. Positive Hole Correction for Multiple-hole Impactors

2.6.4.1. Introduction to the Positive Hole Correction

To avoid underestimating colony counts because of more than one particle impacting through the same hole, the application of a positive hole correction to counts has been used. Another option is using microscopy to enumerate impaction sites, but this is time consuming and labour intensive, and therefore the use of a correction table is more convenient. Several correction tables have been created, with one provided for the original Andersen Six-Stage Sampler by Andersen, 1958. The table created by Andersen (1958) was calculated by the formula shown by equation 1.

Equation 1:

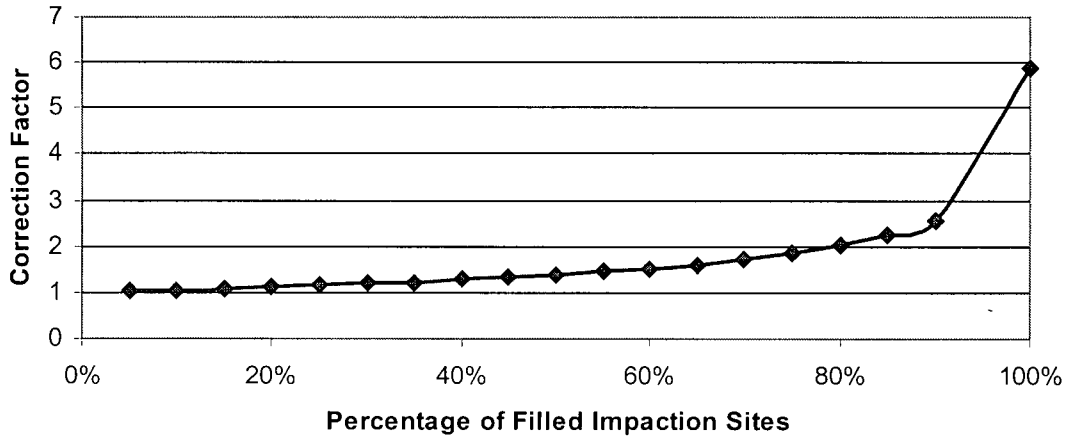
$$\Pr = N\left[\frac{1}{N}+\frac{1}{N-1}+\frac{1}{N-2}+\ldots+\frac{1}{N-r+1}\right]$$

Pr=expected number of viable particles to produce r positive holes
N = total number of holes per stage

Macher (1989) created a correction table with similar values as the one provided by Andersen (1958), but also provided standard deviations between observed and corrected counts. The standard deviation between observed and corrected does not exceed 10% until

the number of CFUs reaches about 75 for impactors with 400 holes. Therefore, the correction factor increases as the number of filled sites increased (Willeke and Macher, 1999, see Figure 2.6.4). Somerville and Rivers (1994), provide a simplified method to the one proposed by Macher (1989) and have similar results.

Figure 2.6.4. Correction Factors for Multiple Hole Impactors[1]



[1]based on Willeke and Macher (1999)

2.6.4.2. Application of the Positive Hole Correction

For the multiple-hole impactors (N6 and SAS), a positive-hole correction was applied to the count data prior to entry into database (Macher, 1989). The correction tables can be found in Appendix I.

2.6.5. Air Concentration Calculations

For all air concentrations, the actual volume sampled was used. Air concentrations (CFU/m^3) for viable samplers were determined using equation 1 (Macher, 1999). To calculate total spores/m^3 for the AOC, equation 2 was used. For all samplers, the mean air concentration between sequential duplicates was calculated for every sample pair.

Equation 1:

$$\frac{CFU}{m^3} = \frac{\text{Total CFU}}{\text{Volume of Air Sampled}(m^3)}$$

CFU/m^3 = Colony Forming Units per m^3 of air
Total CFU = Total Colony Forming Units

Equation 2:

$$\frac{Spores}{m^3} = \frac{N}{n} \times \frac{A}{a} \times \frac{1}{\text{Volume of Air Sampled}(m^3)}$$

N = total number of spores counted (spores)
n = total number of fields counted (fields)
A = total deposition area ($16.5mm^2$)
a = total area per field ($=\Pi r^2=0.10179mm^2$)

2.6.6. Method Comparisons

A total of six method pairs were compared:

i. N6 and RCS
ii. N6 and SAS
iii. SAS and RCS
iv. N6 and AOC
v. RCS and AOC
vi. SAS and AOC

Comparisons i to iii were between the viable samplers, and comparisons iv to vi were between the viable and microscopic methods. These method comparisons will be referred to in this section.

2.6.7. Reproducibility

To determine reproducibility, the coefficient of variation (see equation 3) was determined for each pair of sequential duplicates (Macher, 1999).

Equation 3:

$$CV(\%) = \frac{\text{Standard Deviation}(\text{Sample1}, \text{Sample2})}{\text{Mean}(\text{Sample1}, \text{Sample2})} \times 100\%$$

The arithmetic mean, median and standard deviations of the resulting coefficients of variation were determined for each instrument stratified into indoor and outdoor samples. The Kolmogorov-Smirnov test was applied to determine whether differences in CV existed between indoor and outdoor samples for each instrument. On stratified data for indoor and outdoor samples, the Wilcoxon Rank Signed test was performed on all six comparisons to determine whether differences in the CV existed between instruments.

2.6.8. Descriptive Statistics

Histograms of the fungal concentrations, overall and stratified into indoor and outdoor, were plotted for each instrument to visualize the distribution. The distributions were right skewed and values were transformed to the natural logarithm to accommodate parametric testing. Following transformation, the distributions were re-checked for normality.

Descriptive statistics (means, standard deviations, 95% confidence intervals, minimums and maximums) were determined for each instrument on transformed data stratified into indoor and outdoor samples. From these descriptive statistics, the geometric mean, geometric standard deviation, 95% confidence interval around the geometric mean, maximum and minimum values were calculated by exponentiating the respective values.

2.6.9. Total Yield Comparison

Differences between indoor and outdoor concentrations were determined by a two-sample t-test. Two-tailed paired t-tests between the methods for all six comparisons were conducted on the stratified transformed data to determine whether the geometric means significantly differed between instruments.

2.6.10. Linear Regressions

2.6.10.1. Introduction to Regression

For this study, regression modeling was used to examine the relationship of one sampler to another using the data collected from the field study, to examine the effects of location on the relationship between samplers, and finally to create a model for each method pair to serve as a calibration curve between methods.

2.6.10.2.Introduction to Confounding, Interaction, and Strength of Model

There were two main goals of the regression analysis conducted in this study:

1) To predict the mean concentration value from one sampler (dependent variable) by using the concentration information from another sampler and other important variables (independent variables).

2) To quantify this relationship between samplers by developing a calibration models using regression techniques.

To achieve the second goal, accurate estimates of the relationship between the samplers are important. Confounding and interaction are two possible influences on the accuracy of the estimates. Confounding defined by Kleinbaum et al., (1988) as 'the existence of meaningfully different interpretations of the relationship of interest when an extraneous variable is ignored or included in the data analysis'. Interaction, also defined by Kleinbaum et al., (1988), is 'the condition where the relationship of interest is different at different levels of the extraneous variable'. Interaction terms are included in regression models if the relationship between the dependent and independent variables (samplers) change at different levels of the independent (interaction) term. For the models in this study, location was investigated as a possible confounder and interaction term.

To determine the strength of the model to describe the relationship between the dependent and independent, the adjusted R^2 (the correlation of the model squared) is typically used.

The adjusted R^2 is the amount of variation in the data that is explained by the model, corrected for the number of variables in the model. The adjusted R^2 was used to compare the seven different models. Models with higher adjusted R^2 were considered 'better' at explaining the relationship.

2.6.10.3. Regression Methodology

Seven different linear regression models were constructed for each method comparison. A total of 42 models were created. Five regression models were constructed using fixed-effects linear regression by the Least Squares method. Two regression models were constructed using mixed-effects linear regression using the Restricted Maximum Likelihood method. The variables entered into the regressions are outlined in Table 2.6.10a and the characteristics of each regression method are outlined in Table 2.6.10b. Regression methods 1-3 were conducted to investigate confounding by location on the relationship between instruments. Regression method 4 includes the locator variable, and regression method 5 investigates the possibility of interaction between the instrument and location. Regression methods 6-7 are the mixed effects models.

The regression models were constructed in the following manner:

1) The N6 was always defined as the dependent variable. The N6 has been considered the reference standard, and has been suggested by the National Institute of Occupational Safety and Health to be the sampler to assess for bioaerosols (National Institute of Occupational Safety and Health, 1998).

2) The SAS was defined as the dependent variable for models with the RCS and AOC. This was because the SAS has similar operating principles to the N6.
3) The AOC was always the independent variable because it is the newest instrument on the market and utilized a different method of analysis.

Table 2.6.10a. Regression Variables

Fixed Effects Variables	Random Effects Grouping Variable (Mixed Effects Regression)
1. Instrument (β_1)	**1. Building**
a) Mean Ln RCS	
c) Mean Ln SAS	
d) Mean Ln AOC	
2. Locator (Indoor=0/Outdoor=1) (I/O) (β_2)	
3. Interaction Term (Instrument x Locator) (β_3)	

Table 2.6.10b. Regression Methods

Data Included	Model	Regression Type
1. Instrument only (all data)	$y=\beta_0+\beta_1 x$	Simple Linear
2. Instrument only (indoor data only)	$y=\beta_0+\beta_1 x$	Simple Linear
3. Instrument only (outdoor data only)	$y=\beta_0+\beta_1 x$	Simple Linear
4. Instrument + I/O variable (all data)	$y=\beta_0+\beta_1 x+\beta_2(I/O)$	Multiple Linear
5. Instrument + I/O variable + interaction term (all data)	$y=\beta_0+\beta_1 x+\beta_2(I/O)+\beta_3(x \cdot I/O)$	Multiple Linear
6. Instrument + I/O variable (all data) + Random effects for Building	$y=\beta_0+\beta_1 x+\beta_2(I/O)$ + Building	Mixed effects Linear
7. Instrument + I/O variable + interaction term (all data) + Random effects for Building	$y=\beta_0+\beta_1 x+\beta_2(I/O)+\beta_3(x \cdot I/O)$ + Building	Mixed effects Linear

β_0 = y-intercept
β_1 = slope for instrument independent variable
β_2 = coefficient for locator (indoor=0, outdoor=1)
β_3 = coefficient for interaction term (independent instrument variable x locator variable)

Fixed effect regressions were performed using SPSS for Windows. For these regression models, the mean and 95% confidence interval were reported for each coefficient. Residuals were checked for normality by plotting a Q-Q plot. To determine whether outliers had a strong effect on the model, the Cook's distance was determined for each data point. Finally, an adjusted R^2 was determined.

Mixed effects regressions were conducted using S-plus. The random effect was defined as the building identification number, set as a grouping variable. The mean and 95% confidence intervals were reported for each coefficient. Residuals were checked for normal distribution by plotting a Q-Q plot. To estimate the R^2 of these models, the predicted values were plotted against the dependent values. Simple linear regression was used to examine the agreement and determine an R^2 (for these models, the R^2 = adjusted R^2 since only one independent variable was put in the model).

2.7. Ranking of Instruments based on Performance and Sampler Characteristics

2.7.1. Rationale and Strategy for Comparison and Ranking

The purpose of this comparison was to:

1) Link the measured performance data determined by the field comparison with other sampler characteristics to see how each instrument would rank based on the scoring of these qualities,

2) To suggest a method by which users can select the most appropriate method for their needs.

The other sampler characteristics (cost, ease of use, portability, sampling time, and noise) were selected based on field experience with the instruments. Current and historical use in industry was also considered. For each characteristic, sub-categories were determined from field experience of the sampling technician (see Table 2.7). To determine weighting of each characteristic, a questionnaire was distributed to a panel of 14 professionals (see Table 2.7b).

Table 2.7a. Sampler Characteristics and Sub-categories

	Characteristic	Sub-Categories
Performance Characteristics	**d_{50}** (measure of particle collection efficiency	d_{50} found in literature
	Reproducibility	Coefficient of Variation between sequential duplicates for: Indoor Outdoor
	Total Yield	Overall mean for: Indoor Outdoor Proportion of Samples Below LOD Above UDL
Other Characteristics	**Cost**	Cost of Sampler+Sampling Pump Cost per sample (costs for media/sample, sampling plates or cassettes) Cost of analysis
	Portability	Weight of Sampler+Sampling Pump Size of Sampler+Sampling Pump Power Requirement Length of battery charge
	Ease of Use	On site calibration Speed of analysis (median time per sample) Incubation Pre-set sampling times Ease of sample preparation
	Noise	Average dB (A) emission from four points
	Sampling Time	Minimum volume sampled Maximum volume sampled Flow rate
	Industrial Use	Use of samplers in published literature Use by surveyed panel

Table 2.7b. Suggested panel for questionnaire

Name	Position	Company/Group
Evan Aldernaz	Industrial Hygiene Technologist	Pacific Environmental Vancouver, BC, CANADA
Karen Bartlett	Assistant Professor	School of Occupational and Environmental Hygiene, UBC Vancouver, BC, CANADA
Harriet Burge	Air pollution research specialist	Department of Environmental Health, Harvard School of Public Health Boston, MA, USA
Geoff Clark	Consultant	PE Services North Vancouver, BC, CANADA
Chris Collett	President	Chris Collett and Associates Vancouver, BC, CANADA
Mike Glascow	Operations Manager	Theodore Sterling and Associates Vancouver, BC, CANADA
John Holland	President	PHH Environmental Vancouver, BC, CANADA
Robert Lockhart	Occupational Hygienist	BC Research Vancouver, BC, CANADA
Janet Macher	Air Pollution Research Specialist	Environmental Health Laboratory California Department of Health Services, Berkeley, CA, USA
Bruce Miller	IAQ Specialist	Aerotech Laboratories Phoenix, AZ, USA
Ted Nathanson	IAQ Manager	Public Works and Government Services Canada Ottawa, ON, CANADA
Brad Prezant	President	Prezant and Associates Seattle, WA, USA
Mona Shum	Consultant	Exponent Incorporated, Menlo Park, CA, USA
Dr. Chin-Yang	President & Chief Microbiologist	P&K Microbiology Services, Inc Cherry Hill, NJ, USA

2.7.2. Methodology for Scoring and Ranking Samplers

Each sampler was evaluated and ranked using a scoring scheme based on:

1) Performance characteristics determined by this study (total yield, reproducibility) and described by previous literature (cut-off diameter)
2) Defined sampler characteristics weighted and ranked by a panel of professionals (cost, ease of use, portability, sampling time, and noise)
3) Any other characteristic deemed important by panel from questionnaire

The other sampler characteristics and their respective weighting in the ranking scheme were determined by conducting a small survey of professionals in the field. A list of fourteen

individuals was provided by one of the panelists. Each professional was contacted and interviewed using a questionnaire by telephone or email sampler (see Appendix II for a blank questionnaire). The questionnaire was structured into two sections. The first section asked these professionals to rank the importance of five characteristics (cost, portability, ease of use, noise and sampling time) in the design of a bioaerosol sampler: 1=not important, 2=somewhat important, and 3=very important. The second section of the questionnaire consisted of a series of questions that were more open ended to capture any other sampler characteristics that may be of interest and any opinions they may have on exposure assessment strategies for bioaerosol sampling.

2.7.3. Weighting of Characteristics

A maximum weighting of 5 was assigned for this comparison.

2.7.3.1.Performance Characteristics

The performance characteristics include reproducibility, cut-off diameter, total yield, and the proportion of samples beyond detection limits. These characteristics were each given the highest weight of 5 because performance was considered to be most important.

2.7.3.2.Other Sampler Characteristics

The weighting of the five other defined sampler characteristics were determined by the survey. The scores for each characteristic were summed over all the completed questionnaires. A maximum weighting of 5 was designated to the characteristic with the highest total score. The other characteristics were weighted by:

$$\text{Weighting} = \frac{\text{Total Score of Characteristic A}}{\text{Highest Score}} \times 5$$

The weighting of any other sampler characteristic (not specifically mentioned by survey) was based on the proportion of professionals that mentioned this characteristic (no scoring). The final weighting was equal to the proportion of professionals with responses including the factor multiplied by 5 (maximum weighting).

2.7.4. Evaluation and Scoring of sampler characteristics

For each characteristic, several factors (sub-categories) were used to define it. Within each sub-category, a score was assigned to each instrument in the following manner:

1) The 'best' instrument for each sub-category is given the score of '1' and the other instruments are assigned a score that is relative to the best instrument.
2) The total possible score is determined for each sub-category by summing all the scores for each instrument.
3) A final 'normalized' score for each sub-category was assigned to each instrument and is computed as:

Normalized score = Score assigned to instrument/Total score over all instruments

4) A final score was determined for each instrument for each characteristic as the total sum of all the normalized scores over all sub-categories.
5) The total score of each instrument is determined by adding the scores for each characteristic multiplied by their respective weighting.

2.7.5. Performance Characteristics – Sub-categories and Scoring Rationale

Particle collection efficiency (measured by the cut-off diameter), reproducibility, and overall yield were defined by several sub-categories and scored according to the rules outlined Table 2.7.5.

Table 2.7.5. Performance Characteristics

Factor	Sub-categories	Scoring Rules
Cut-off Diameter	d_{50} found in literature	Lower d_{50}, higher score
Reproducibility	Coefficient of Variation between sequential duplicates for:	
	Indoor	Lower CV, higher score
	Outdoor	Lower CV, higher score
Total Yield	Overall mean for:	
	Indoor	Higher mean, higher score
	Outdoor	Higher mean, higher score
	Proportion of Samples	
	Below LOD	Lower proportion, higher score
	Above UDL	Lower proportion, higher score

2.7.5.1.Rationale for Scoring of Cut-off Diameter

The cut-off diameter (also know as the d_{50}) defines the particle size at which 50% of them are collected. Generally, a lower d_{50} is desirable because it has the ability to collect the smaller spores. A higher score is assigned to samplers with smaller d_{50} to reflect this idea.

2.7.5.2.Rationale for Scoring Reproducibility

The reproducibility of each instrument was defined by two sub-categories: indoor CV and outdoor CV. Instruments with lower CV were given a higher score since this reflects a higher reproducibility between sequential samples.

2.7.5.3.Rationale for Scoring Total Yield

The total yield of the instrument was defined by 4 sub-categories: indoor geometric mean, outdoor geometric mean, proportion of samples below the LOD, and the proportion of samples above the UDL. Typically, a higher geometric mean sampled is considered more desirable since it is assumed that instrument has the ability to pick up more fungal spores

than others. Thus, samplers with higher geometric means were assigned a higher score. Samplers with lower proportions of samples less than the LOD or above the UDL were given higher scores. Instruments with a wider working range are typically considered 'better' instruments.

2.7.6. Other Sampler Characteristics – Sub-categories and Scoring Rationale

Cost, portability, ease of use, noise, and sampling time were defined by several sub-categories and then scored according to the rules shown in Table 2.7.6.

Table 2.7.6. Qualitative Factors and sub-categories

Factor	Sub-categories	Scoring Rules
Cost	Cost of sampler (including pump)	Lower cost, higher score
	Cost per sample (media+sampling plate)	Lower cost, higher score
	Cost of analysis*	Lower cost, higher score
Portability	Weight of sampler+pump	Lower weight, higher score
	Size of sampler+pump	Smaller size, higher score
	External Power Source (Y/N)	Yes=0, No=1
	Length of time battery charge lasts	Longer time, higher score
Ease of Use	On site calibration (Y/N)	Yes=1, No=0
	Speed of analysis	Shorter time, higher score
	Incubation	Yes=0, No=1
	Pre-set sampling times (Y/N)	Yes=1, No=0
Noise	Noise emission level (dBA)	Lower values, higher score
Sampling Time	Minimum volume it can sample	Lower values, higher score
	Maximum volume it can sample	Higher values, higher score
	Flow Rate	Higher values, higher score

*Note: determined by comparison to commercial lab (Aerotech Laboratories)

2.7.6.1.Information for Cost sub-categories and Scoring Rationale

All information for the cost of the sampler, cost of sampling pump and cost of factory calibration were collected from the manufacturer. American dollars were converted to Canadian dollars using the 1 US Dollar = 1.58 Canadian Dollars for all currency conversions.

The determine the cost per sample for each method, the following equation was used:

$$\text{Cost per sample} = \text{cost media/sample} + \text{cost/sampling plate}$$

The cost of the sampler was ranked separately from the cost per sample, and not integrated into this cost.

Cost of analysis for each sample was determined by contacting Aerotech Laboratories, Inc. Aerotech Laboratories offers analytical services to the indoor air quality industry including the analysis of culture and microscopic samples (Aerotech Laboratories, Inc. 2002).

Lower costs for each sub-category were assigned a higher score, because cheaper methods are more desirable than more expensive methods.

2.7.6.2.Information for Portability Sub-categories and Scoring Rationale

All information on the size, weight and battery charge lifetime were retrieved from the operation manuals for each of the instruments. The size of the sampler was determined as the total volume (length x width x height) of both the sampler and the pump. The power requirement was determined by field experience and not based on the information from the manufacturer.

A lighter and smaller sampler was defined as more portable, and therefore the scoring was higher for samplers smaller in size and lighter in weight. Samplers that do not require an electrical outlet does not restrict the user in sampling location, and therefore samplers that do not require an electrical outlet were assigned a higher score. A longer battery charge enables a user to take more samples in more locations, and thus samplers with a longer battery charge were assigned a higher score.

2.7.6.3.Information for Ease of Use sub-categories and Scoring Rationale

For all sub-categories, except for analysis speed, the scores were determined based upon experience with the samplers gathered during the field study.

Speed of Analysis – Method Protocol

To determine speed of analysis, a small subset of samples (see Table 2.7.6.3 for the number samples for each method) were timed using a timer (RadioShack™ Dual Timer, Barrie, Canada). The median time was determined for each method and used for comparison. This protocol was not considered at the start of the study, and therefore the numbers of samples that were evaluated are different.

Table 2.7.6.3. Sample Size for Speed of Analysis

Instrument	Sample Size
RCS	48
N6	49
SAS	39
AOC	6

On site calibration enables the user to verify flow rate directly, and therefore considered easier to use. Also, analyses that do not require incubation (thus user does not need to wait for a long period for results), and are faster to process (so more samples can be analyzed), were also considered easier methods, and assigned a higher score.

2.7.6.4.Noise Survey of Samplers and Scoring Rationale

Noise measurements were taken for each instrument using a modified method outlined by International Organization for Standardization (1993). Noise measurements were taken with a calibrated precision sound level meter with octave band analyzer (NA-29E Rion Company Ltd., Tokyo, Japan) in the acoustics laboratory at the School of Occupational and Environmental Hygiene. The acoustics laboratory resembles a typical office except that the walls and ceilings are very sound absorbent. A background noise value was first noted before any other measurements were made. Noise measurements for each instrument were taken one at a time. Each instrument was mounted onto their respective tripods to a height of approximately 1.5 metres. The sampler was turned on and four measurements were taken at right angles from one another approximately one metre away from the instrument at a height of approximately 1.55 metres. Each measurement was over a 15 second time period and the average of the value was noted. The instrument was not turned off between measurements. This procedure was repeated for every instrument. The final noise measurement was the average of the four measurements taken.

Samplers that had a lower noise measurement were assigned a higher score, because noise may prevent the user from taking samples.

2.7.6.5.Information for Sampling Time and Scoring Rationale

The information for sampling time was defined in the operating manuals for each of the instruments. The minimum volume is defined by the minimum pre-set time for each sampler (samplers without a pre-set time restriction, the minimum volume was equal to the volume collected for ten seconds) and their respective flow rates. The maximum volume was defined as the maximum volume that can be collected (if not limited to pre-set times, is equal to volume equivalent to sampling for duration of battery charge).

The scoring of this section was to give the highest score to the sampler that had the most flexible sampling volume range, so that a user could adapt it to a particular sampling protocol. Also, a higher flow rate was given a higher score because the time per sample would be smaller. This would enable the user to take more samples, or cut down on time required to conduct an investigation.

2.7.7. Previous and Current Use in Industry/Research

The respondents to the questionnaire also considered the use of the sampler in previous studies and by industry to be important factors. To estimate the frequency of use of each sampler, a restricted literature search was conducted in two journals, the American Hygiene Association Journal and Indoor air. The subject index in the December issue for each year from 1992-2002 was used to locate articles with the keywords listed in Table 2.7.7 and a final skim of all the articles over this time period was also conducted. The abstracts were read to determined whether the article was relevant and if so, which sampler was utilized in the study. For every study an instrument was used in, a score of 1 was assigned. For both

journals, the total tally was determined. To determine an overall score, the instrument with the most studies was given a score of 1.

Table 2.7.7. Keywords used in Literature Search

Keywords
Air sampling, airborne contaminants, bioaerosols, fungi, fungal aerosols, mold, mould, microbiological pollution, indoor air quality

The AIHAJ is published by the American Industrial Hygiene Association. The articles found in this journal cover research interests that span the science for occupational and environmental health (American Industrial Hygiene Association, 2002).

The Indoor Air journal is published by Blackwell Munksgaard. This journal publishes articles that describe research in the indoor environment of non-industrial buildings. Topics include health effects, thermal comfort, monitoring and modeling, source characterization, ventilation and other environmental control techniques (Blackwell Munksgaard, 2002).

These journals were recommended by a professional in the field based on the belief that they contained a majority of the articles that would be relevant for this study.

2.7.7.1.Measurement of Current Use

To determine the use of the instruments in industry/research, question #4 asked each respondent which sampling methodology they used. To determine the overall score for current use, the instrument used most by the professionals was given a score of 1.

2.7.7.2.Scoring Rationale

Samplers that were used as an exposure assessment measure in more of the selected published literature were scored higher to reflect that it was used more extensively than the others. Also, samplers that are used by more of the expert panel were also given higher scores since it indicates it may be used more widely than the others.

2.7.8. Final Ranking of Samplers

The final ranking of samplers was done by:

1) Calculating final scores for each sampler characteristic
2) Multiplying final scores by respective weighting
3) Summing over all the weighted scores for each instrument
4) Rank based on score – instruments with higher scores receive higher ranks

Final Score for each instrument was calculated with the following weighting scheme:

Final score=

Cost x 4 + Ease of Use x 5 + Portability x 4 + Sampling Time x 4 + Noise x 3 + Cut-off diameter x 5 + Reproducibility x 5 + Total Yield x 5 + Industrial Use x 4

Ranking was assigned to each instrument. Higher final scores resulted in higher ranks.

2.7.9. Scoring Analysis

A sensitivity analysis of the scores was conducted for each instrument by calculating the proportions of each score contributed by each sampler characteristic. The results of this analysis were used to elucidate what factors had a larger impact on the final ranking of the instrument and therefore may be more important to specific users or for specific uses.

Chapter 3. Results

3.1 Sampling Sites

A total of 75 sites from 61 different buildings sampled from June-October 2001. These buildings were provided by the BCBC, UBC, SFHR and VAA, and were all located within the greater Vancouver area, British Columbia. A public building was defined as a non-residential, and publicly administered building, where people were free to enter and exit. Data from one site were excluded because it did not fit this description of a public building, resulting in data from 74 sites (60 different buildings) for analysis. Table 3.1 shows the total number of sites and buildings from each organization.

Table 3.1. Building Pool Sampled

RESULTS	ORGANIZATION			
	BCBC	UBC	SFHR	VAA
Total # of sites (# of buildings)	25 (18)	35 (34)	11 (5)	3 (3)

3.2 Sample Size and Data Grouping

For each measurement method, a maximum of 592 samples (296 sequential duplicates) and 74 field blanks were possible. Table 3.2.1 reports the actual number of samples for each method. The SAS had to be sent away for repairs during the study, resulting in a total of 552 samples for this instrument.

Table 3.2.1. Number of Sample Means (total # of samples)

Sampler	Field Blanks	Overall Total	Indoors				Outdoor
			Common Room	Room 1	Room 2	Indoor Total	
RCS	74	**295** (590)	74 (148)	74 (148)	73 (146)	221 (442)	74 (148)
N6	74	**296** (592)	74 (148)	74 (148)	74 (148)	222 (444)	74 (148)
SAS	69	**276** (552)	69 (138)	69 (138)	69 (138)	207 (414)	69 (138)
AOC	74	**295** (590)	74 (148)	74 (148)	74 (148)	222 (444)	73 (146)

Table 3.2.2 reports the geometric mean concentrations (CFU/m^3 or spores/m^3) for each room type for each sampling instrument. No significant differences in concentrations between indoor locations were found when analyzed by one-way ANOVA and Bonferroni post-hoc adjustment for multiple comparisons. Therefore, the three indoor sites were grouped together for all subsequent analyses. A significant difference in concentration was found between all indoor locations compared to outdoors.

Table 3.2.2. Geometric Mean Concentrations by Location Type

Sampler	Room Type – Geo Mean[1] (GSD[2]) *Sample Size (N)*			
	Common Room	Room 1	Room 2	Outdoor
RCS (CFU/m^3)	108 (2.5) *74*	112 (2.7) *74*	126 (2.3) *73*	550 (1.8) *74*
N6 (CFU/m^3)	71 (4.3) *74*	64 (3.9) *74*	68 (4.0) *74*	691 (2.3) *74*
SAS (CFU/m^3)	17 (3.8) *69*	16 (3.0) *69*	17 (2.8) *69*	175 (2.7) *69*
AOC (Spores/m^3)	906 (3.6) *74*	998 (3.5) *74*	1,042 (3.3) *74*	10,577 (2.4) *73*

[1]Geometric mean
[2]Geometric standard deviation

3.3. Distribution of Measurements

Histograms of all the measurements (means of duplicate samples) for each instrument, stratified by indoor and outdoor sampling locations, are shown in Appendix III (Figure 3.3.1-3.3.8). The histograms all showed positively skewed distributions and transformation to the natural log were applied to the data. The histograms for the transformed data are also shown in the Appendix 3 resulting an approximately normal distribution. All parametric testing and regression analyses were conducted using the transformed data.

3.4. Descriptive Statistics

The geometric means, their 95% confidence intervals, standard deviations, arithmetic means and ranges for each method, are shown for indoor samples (Table 3.4a) and for outdoor samples (Table 3.4b) and illustrated by Figure 3.4.

Table 3.4a. Indoor Geometric Means with 95% CI, arithmetic means and ranges

Instrument	Geo Mean[1]	GSD[2]	95% CI[3]	Arith Mean[4] (SD)[5]	Range
RCS (CFU/m^3)	115	2.5	102-130	164 (142)	8-984
N6 (CFU/m^3)	68	4.1	56-82	168 (277)	3.5-2,484
SAS (CFU/m^3)	17	3.2	14-20	42 (145)	3-1,991
AOC (Spores/m^3)	980	2.4	832-1,155	2,118 (3,578)	21-29,555

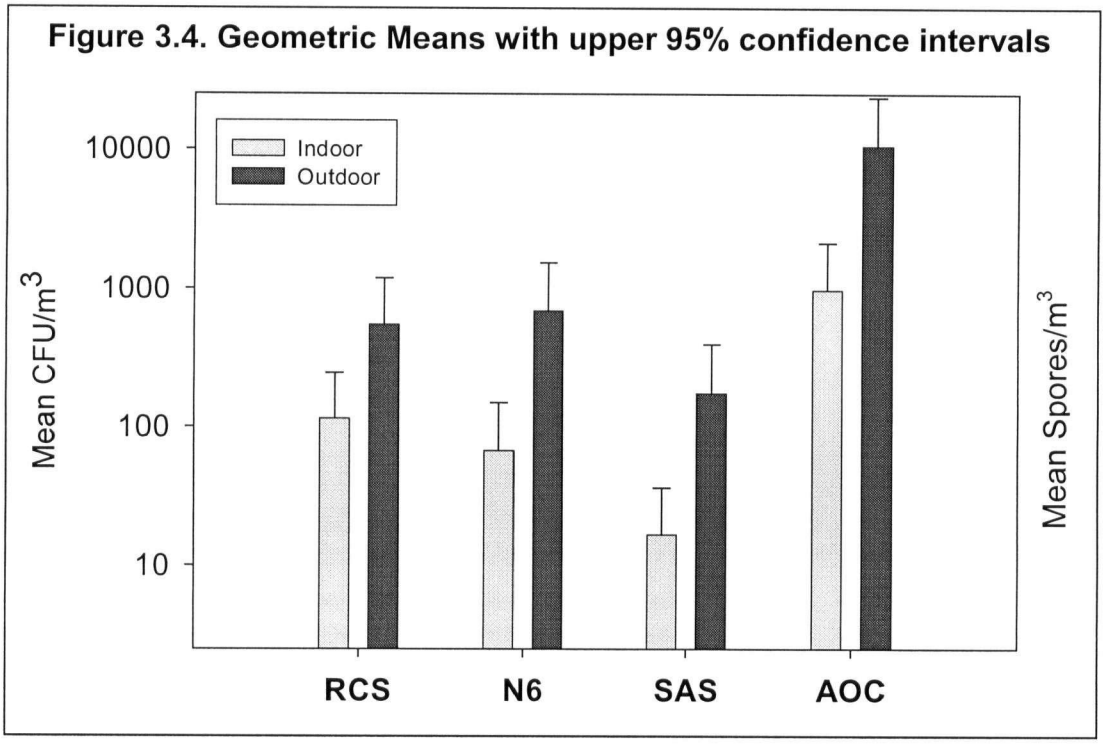
[1]Geometric Mean
[2]Geometric Standard Deviation
[3]95% Confidence Interval for the geometric mean
[4]Arithmetic Mean
[5]Standard Deviation

Table 3.4b. Outdoor Geometric Means with 95% CI, arithmetic means and ranges

Instrument	Geo Mean[1]	Geo Std Dev[2]	95% CI[3]	Arith Mean[4] (StdDev)[5]	Range
RCS (CFU/m^3)	550	1.8	478-634	651 (366.9)	141-1,130
N6 (CFU/m^3)	691	2.3	567-841	986 (1,015)	60-7,039
SAS (CFU/m^3)	175	2.7	138-223	308 (548)	18.5-4,394
AOC ($Spores/m^3$)	10,577	2.4	8,631-12,962	15,125 (13,759)	886-69,286

[1]Geometric Mean
[2]Geometric Standard Deviation
[3]95% Confidence Interval for the geometric mean
[4]Arithmetic Mean
[5]Standard Deviation for the arithmetic mean

Figure 3.4. Geometric Means with upper 95% confidence intervals



3.5 Limit of Detection

Table 3.5 shows the theoretical upper and lower detection limits and the proportion of samples that were outside detection limits (below the lower limit of detection and above the upper detection limit respectively) for each instrument.

Table 3.5. Proportion of samples beyond detection limits

Instrument	LOD	# of samples<LOD (%total samples)	UDL	# of samples>UDL (%total samples)
RCS	6 CFU/m^3	7 (1.2)	1,125 CFU/m^3	25 (8.4)
N6	7 CFU/m^3	24 (4.1)	18,572 CFU/m^3	0 (0)
SAS	6 CFU/m^3	84 (15.2)	7,471 CFU/m^3	0 (0)
AOC[1]				
·Indoor	11 spores/m^3	0 (0)	NA	0 (0)
·Outdoor	22 spores/m^3	0 (0)	NA	0 (0)

[1]Air volume sampled for indoor samples (150 L) different from outdoor samples (75 L)

3.6. Reproducibility of Sequential Duplicates

The arithmetic mean and median of the coefficients of variation for each sequential duplicate sample for each instrument, stratified into indoor and outdoor values, are presented in Table 3.6.1. The two sample Kolmogorov-Smirnoff test results are shown in Table 3.6.2. A significant difference between indoor and outdoor CV was found for all methods (with indoor>outdoor, $p<0.001$). The results of the Wilcoxon Signed Ranks test are presented in Table 3.6.3-3.6.4 and show that for CV, SAS>N6=RCS>AOC, for indoor and for outdoor, SAS>N6=RCS=AOC. Figure 3.6 shows a boxplot of the distributions of the coefficients of variation for each method.

Table 3.6.1. Reproducibility - Coefficient of Variation (%)

	Arithmetic Mean (*StdDev*)		Median		Range	
Instrument	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
RCS	30.9 (*26.1*)	17.7 (*19.3*)	25.7	10.2	0-138	0-92
N6	32.2 (*28.3*)	19.1 (*22.4*)	24.7	13	0-135	0-140
SAS	43.5 (*33.3*)	31.6 (*21.6*)	47.1	22.8	0-140	0-112
AOC	23.3 (*21.6*)	13.3 (*12.5*)	15.9	9.9	0-130	0.2-74

Table 3.6.2. Two-sample Kolmogorov-Smirnov between indoor/outdoor for CV (%)

	RCS	N6	SAS	AOC
Kolmogorov-Smirnov Z	2.203	2.248	2.155	1.988
Asymp. Sig. (2-tailed)	<0.001	<0.001	<0.001	0.001

Table 3.6.3. Wilcoxon Signed Ranks Test for Indoor CV (%)

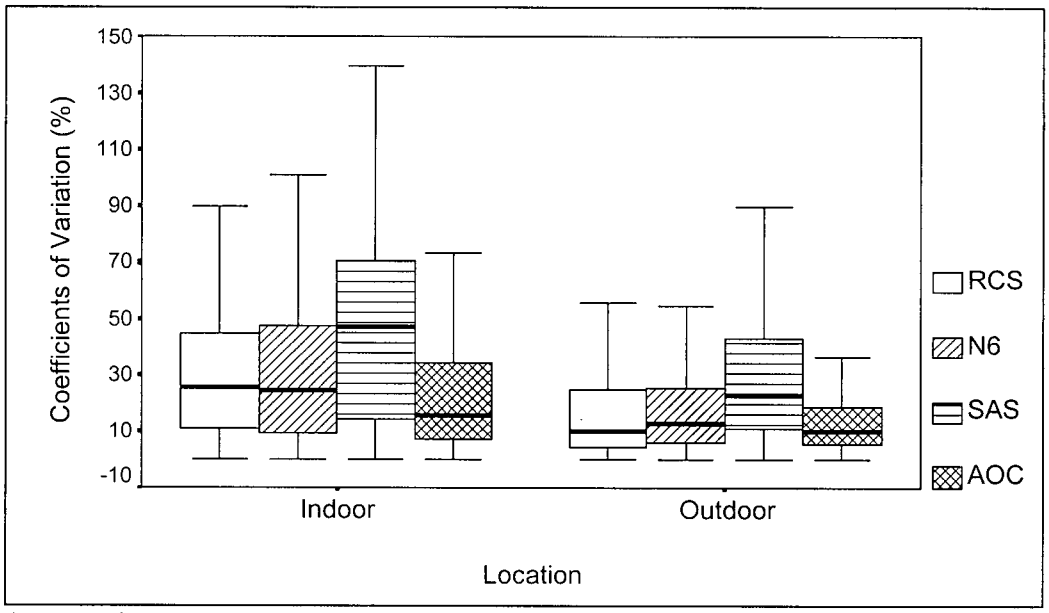
	N6-RCS	SAS-RCS	AOC-RCS	SAS-N6	AOC-N6	AOC-SAS
Z	-0.528[a]	-4.630[a]	-3.973[b]	-3.796[a]	-3.764[b]	-6.848[b]
Asymp Sig. (2 tailed)	0.597	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3.6.4. Wilcoxon Signed Ranks tests for Outdoor CV (%)

	N6-RCS	SAS-RCS	AOC-RCS	SAS-N6	AOC-N6	AOC-SAS
Z	-0.374[a]	-4.081[a]	-1.438[b]	-4.218[a]	-1.872[b]	-4.980[b]
Aymp Sig. (2 tailed)	0.708	<0.001	0.151	<0.001	0.061	<0.001

[a]based on negative ranks
[b]based on positive ranks

Figure 3.6. Boxplot* of Coefficients of Variation



*The solid bars represent the median, the top and lower edges of the box represent the 75th and 25th percentile, respectively, and the upper and lower whiskers represent the 95th and 5th percentile, respectively.

3.7 Inferential Comparisons of Geometric Means between Instruments

For all methods, outdoor concentrations were significantly greater than indoor concentrations (see Table 3.7.1 for two sample t-test). Significant differences between instruments were found with AOC>RCS>N6>SAS for indoor concentrations, and AOC>N6>RCS>SAS for outdoor concentrations (see Table 3.7.2 and 3.7.3 for paired t-test results).

Table 3.7.1. Two sample t-test of geometric means for Indoor/Outdoor

Instrument	Indoor	Outdoor	t	p
RCS (CFU/m^3)	115	550	-16.595	<0.001
N6 (CFU/m^3)	68	691	-17.017	<0.001
SAS (CFU/m^3)	17	175	-14.972	<0.001
AOC (Spores/m^3)	980	10,577	-18.049	<0.001

Table 3.7.2. Paired t-test results for indoor values on natural log transformed data

Pair	N	Paired Differences				t	p
		Mean	Std Dev	95% CI of difference			
				Lower	*Upper*		
Ln RCS - Ln N6	221	0.5070	1.1120	0.3596	0.6544	6.778	<0.001
Ln RCS - Ln SAS	206	1.9765	0.8959	1.8535	2.0996	31.666	<0.001
Ln RCS - Ln AOC	221	-0.1848	0.9748	-0.3140	-5.55E-02	-2.818	0.005
Ln N6 - Ln SAS	207	1.3851	0.9369	1.2567	1.5135	21.269	<0.001
Ln N6 - Ln AOC	222	-0.6987	1.1022	-0.8445	-0.5530	-9.446	<0.001
Ln SAS - Ln AOC	207	-2.1549	1.0981	-2.3054	-2.0044	-28.233	<0.001

Table 3.7.3. Paired t-test results for outdoor values on natural log transformed data

Pair	N	Paired Differences				t	p
		Mean	Std Dev	95% CI of difference			
				Lower	*Upper*		
Ln RCS – Ln N6	74	-0.2380	0.6550	-0.3897	-8.62E-02	-3.126	0.003
Ln RCS – Ln SAS	69	1.1829	0.6330	1.0308	1.3349	15.521	<0.001
Ln RCS – Ln AOC	73	-0.9990	0.8990	-1.2087	-0.7892	-9.494	<0.001
Ln N6 – Ln SAS	69	1.3834	0.5104	1.2607	1.5060	22.512	<0.001
Ln N6 – Ln AOC	73	-0.7567	1.1701	-1.0297	-0.4836	-5.525	<0.001
Ln SAS – Ln AOC	68	-2.2314	1.1651	-2.5135	-1.9494	-15.793	<0.001

3.8 Linear Regressions of Relationships between Instruments

A total of seven different regression models were created for each method comparison (total of 42 models). A scatterplot of the mean values, for both indoor and outdoor samples, and a table of the different regression models are presented for each method comparison.

For all fixed effects models, the Cook's distance for all data points were less than 0.3, and therefore, outliers were not removed. The residuals for all models (fixed and mixed) followed a normal distribution.

3.8.1 N6 versus RCS

Figure 3.8.1. Scatterplot of LN N6 versus LN RCS values
Overall Pearson Correlation = 0.759, p<0.001

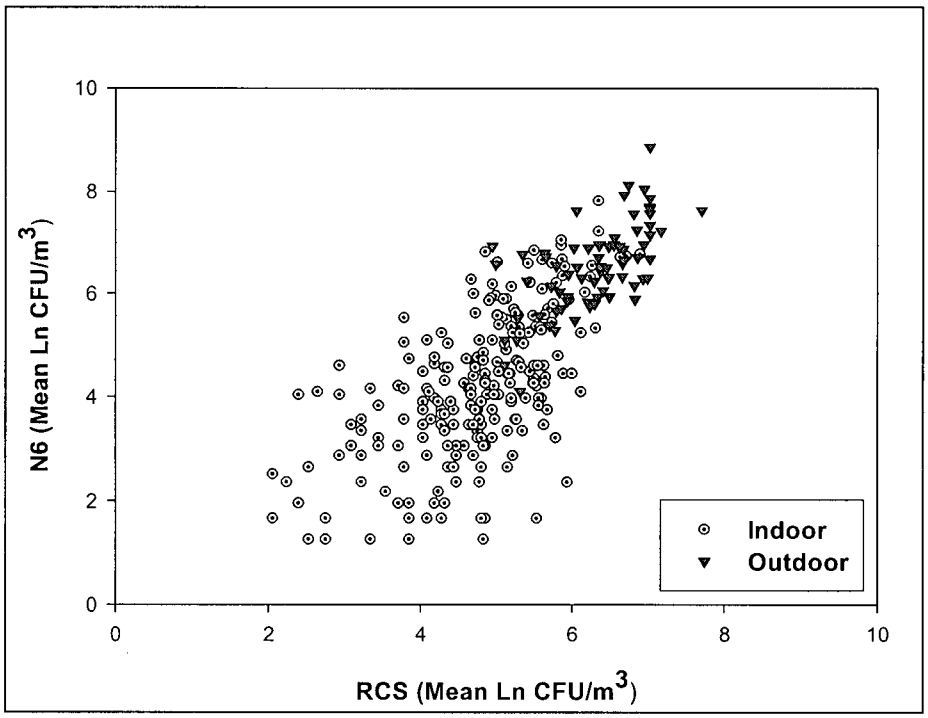


Table 3.8.1. Regression Models for Ln N6 (dependent) versus Ln RCS (independent)

Regression Model	Regression Coefficients 95% Confidence Intervals				adj R^2
	y-Intercept	Ln RCS	[1]Locator	[2]Interaction	
Overall Data	-0.997 *-1.582, -0.411*	1.129 *1.018, 1.241*	NA	NA	0.57
Indoor Data	-0.099 *-0.875, 0.677*	0.912 *0.751, 1.072*	NA	NA	0.36
Outdoor Data	0.901 *-0.690, 2.492*	0.893 *0.642, 1.144*	NA	NA	0.40
Overall + Locator	-0.088 *-0.751, 0.575*	0.909 *0.773, 1.046*	0.887 *0.544, 1.231*	NA	0.61
Overall + Locator + Interaction	-0.099 *-0.809, 0.610*	0.912 *0.765, 1.058*	1.000 *-1.538, 3.539*	-0.018 *-0.430, 0.393*	0.61
Mixed Effects + Locator	0.351 *-0.314, 1.016*	0.828 *0.693, 0.963*	1.016 *0.713, 1.319*	NA	0.78
Mixed Effects + Locator + Interaction	0.456 *-0.239, 1.151*	0.806 *0.664, 0.947*	-0.076 *-2.276, 2.125*	0.178 *-0.177, 0.534*	0.78

[1]Locator (indoor/outdoor) = 0 for indoor, 1 for outdoor, [2]Interaction = Ln RCS x Locator

3.8.2. N6 versus SAS

Figure 3.8.2. Scatterplot of LN N6 versus LN SAS values
Overall Pearson Correlation = 0.861, p<0.001

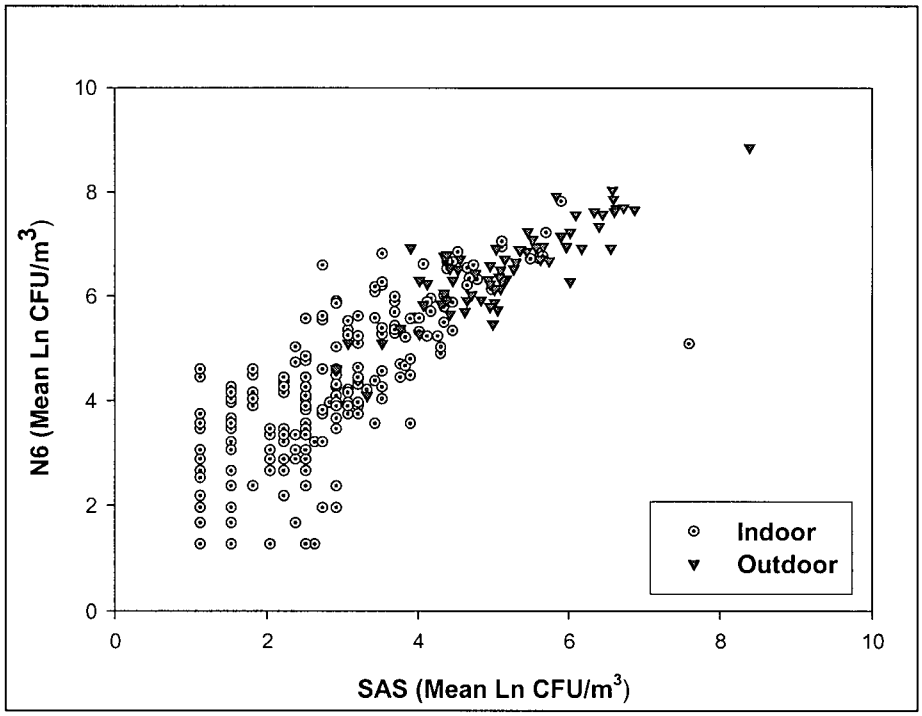


Table 3.8.2. Regression Models for Ln N6 (dependent) versus Ln SAS (independent)

Regression Model	Regression Coefficients *95% Confidence Intervals*				adj R^2
	y-Intercept	Ln SAS	Locator	Interaction*	
Overall Data	1.558 *1.311, 1.806*	0.946 *0.879, 1.012*	NA	NA	0.74
Indoor Data	1.528 *1.191, 1.866*	0.945 *0.835, 1.056*	NA	NA	0.58
Outdoor Data	2.803 *2.252, 3.353*	0.723 *0.618, 0.828*	NA	NA	0.74
Overall + Locator	1.651 *1.373, 1.928*	0.902 *0.813, 0.991*	0.229 *-0.084, 0.541*	NA	0.74
Overall + Locator + Interaction	1.528 *1.226, 1.831*	0.945 *0.846, 1.044*	1.275 *0.174, 2.375*	-0.222 *-0.446, 0.002*	0.74
Mixed Effects + Locator	1.947 *1.646, 2.247*	0.806 *0.717, 0.894*	0.454 *0.170, 0.738*	NA	0.84
Mixed Effects + Locator + Interaction	1.847 *1.524, 2.170*	0.840 *0.742, 0.938*	1.210 *0.214, 2.205*	-0.162 *-0.366, 0.042*	0.85

*Interaction = SAS x Locator

3.8.3. SAS versus RCS

Figure 3.8.3. Scatterplot of LN SAS versus LN RCS
Overall Pearson Correlation = 0.808, p<0.001

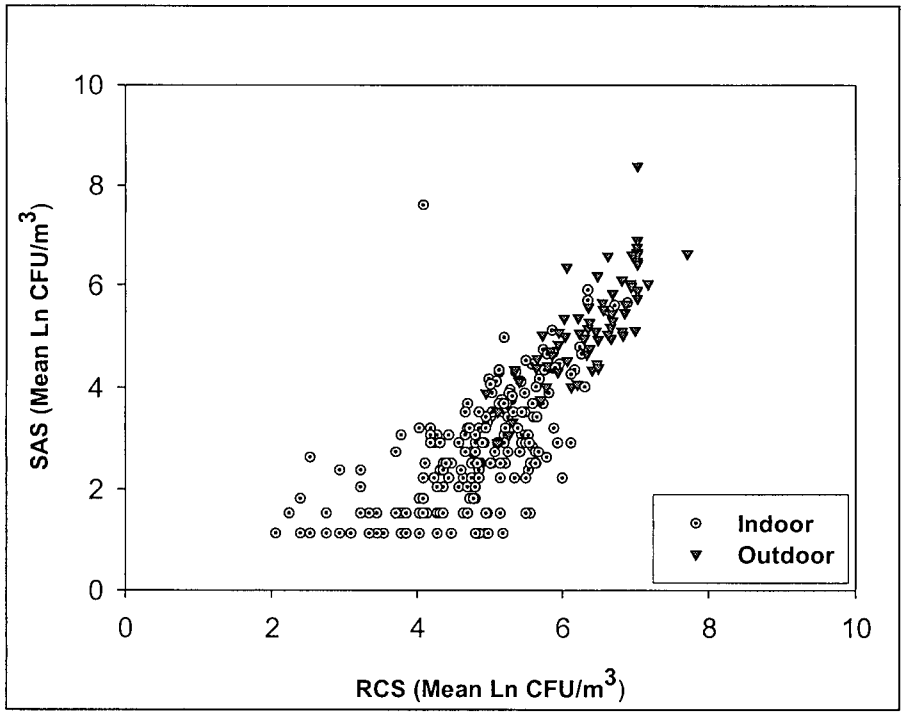


Table 3.8.3. Regression Models for Ln SAS (dependent) versus Ln RCS (independent)

Regression Model	Regression Coefficients *95% Confidence Intervals*				adj R^2
	y-Intercept	Ln RCS	Locator	Interaction*	
Overall Data	-2.498 *-3.022, -1.975*	1.139 *1.040, 1.238*	NA	NA	0.65
Indoor Data	-1.189 *-1.843, -0.535*	0.836 *0.702, 0.970*	NA	NA	0.42
Outdoor Data	-3.489 *-5.063, -1.914*	1.363 *1.116, 1.610*	NA	NA	0.64
Overall + Locator	-1.497 *-2.076, -0.918*	0.900 *0.782, 1.018*	0.948 *0.655, 1.241*	NA	0.70
Overall + Locator + Interaction	-1.189 *-1.797, -0.581*	0.836 *0.712, 0.960*	-2.300 *-4.517, -0.083*	0.527 *0.170, 0.884*	0.70
Mixed Effects + Locator	-1.396 *-1.999, -0.793*	0.885 *0.763, 1.008*	0.971 *0.688, 1.253*	NA	0.77
Mixed Effects + Locator + Interaction	-1.052 *-1.679, -0.425*	0.814 *0.686, 0.942*	-2.569 *-4.688, -0.451*	0.575 *0.234, 0.916*	0.79

*Interaction = Ln RCS x Locator

3.8.4 N6 versus AOC

Figure 3.8.3. Scatterplot of LN N6 versus LN AOC
Overall Pearson Correlation = 0.755, p<0.001

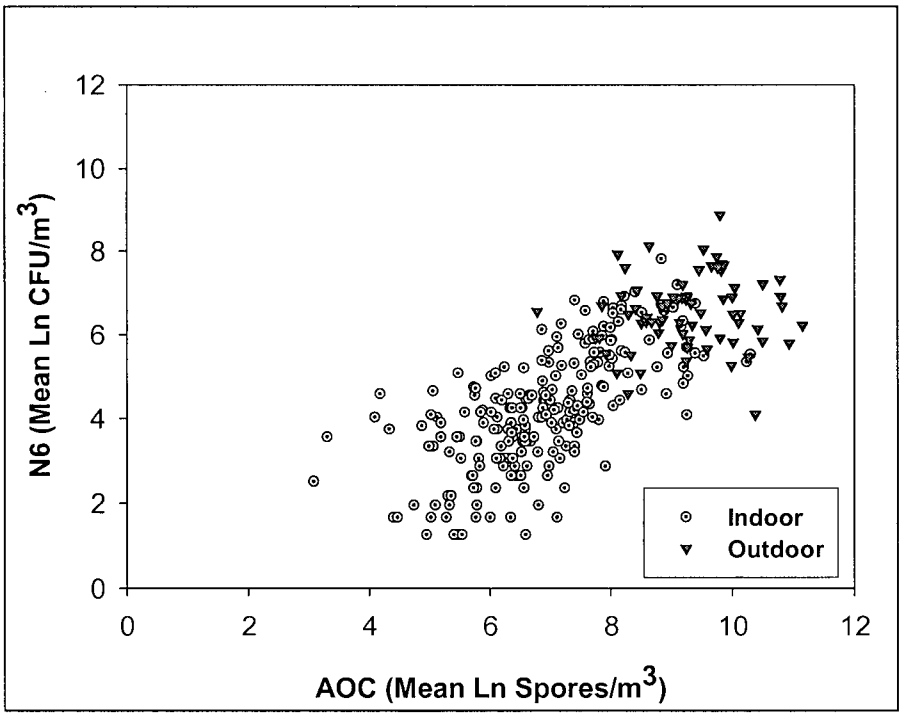


Table 3.8.4. Regression Models for Ln N6 (dependent) versus Ln AOC (independent)

Regression Model	Regression Coefficients *95% Confidence Intervals*				adj R^2
	y-Intercept	Ln AOC	Locator	Interaction*	
Overall Data	-1.148 *-1.754, -0.541*	0.794 *0.715, 0.874*	NA	NA	0.57
Indoor Data	-0.904 *-1.693, -0.114*	0.743 *0.631, 0.856*	NA	NA	0.43
Outdoor Data	5.790 *3.636, 7.944*	0.081 *-0.151, 0.312*	NA	NA	-0.01
Overall + Locator	-0.273 *-0.998, 0.452*	0.652 *0.548, 0.755*	0.771 *0.400, 1.142*	NA	0.59
Overall + Locator + Interaction	-0.904 *-1.658, -0.149*	0.743 *0.635, 0.851*	6.694 *4.075, 9.312*	-0.663 *-0.953, -0.372*	0.62
Mixed Effects + Locator	-0.439 *-1.219, 0.341*	0.681 *0.572, 0.791*	0.701 *0.364, 1.037*	NA	0.80
Mixed Effects + Locator + Interaction	-1.084 *-1.880, -0.288*	0.773 *0.662, 0.885*	5.911 *3.787, 8.035*	-0.586 *-0.822, -0.350*	0.82

*Interaction = Ln AOC x Locator

3.8.5. RCS versus AOC

Figure 3.8.5. Scatterplot of LN RCS versus LN AOC
Pearson Correlation = 0.756, p<0.001

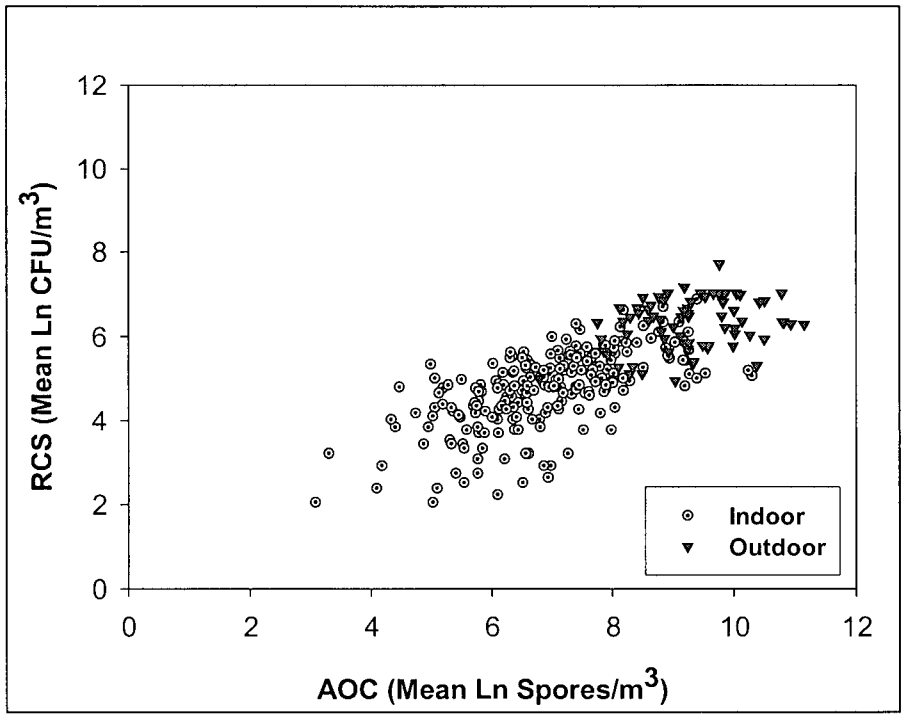


Table 3.8.5. Regression Models for Ln RCS (dependent) versus Ln AOC (independent)

Regression Model	Regression Coefficients *95% Confidence Intervals*				adj R^2
	y-Intercept	Ln AOC	Locator	Interaction*	
Overall Data	1.162 *0.758, 1.567*	0.531 *0.478, 0.584*	NA	NA	0.57
Indoor Data	1.528 *0.991, 2.065*	0.467 *0.390, 0.544*	NA	NA	0.39
Outdoor Data	4.310 *2.835, 5.784*	0.215 *0.057, 0.374*	NA	NA	0.08
Overall + Locator	1.769 *1.286, 2.252*	0.432 *0.363, 0.501*	0.533 *0.287, 0.779*	NA	0.59
Overall + Locator + Interaction	1.528 *1.013, 2.042*	0.467 *0.394, 0.540*	2.782 *1.003, 4.561*	-0.252 *-0.449, -0.054*	0.60
Mixed Effects + Locator	1.800 *1.263, 2.331*	0.424 *0.349, 0.500*	0.556 *0.312, 0.800*	NA	0.72
Mixed Effects + Locator + Interaction	1.445 *0.880, 2.009*	0.474 *0.394, 0.554*	3.576 *1.937, 5.215*	-0.338 *-0.520, -0.157*	0.74

*Interaction = Ln AOC x Locator

3.8.6. SAS versus AOC

Figure 3.8.6. Scatterplot of LN SAS versus LN AOC
Overall Pearson Correlation = 0.739, p<0.001

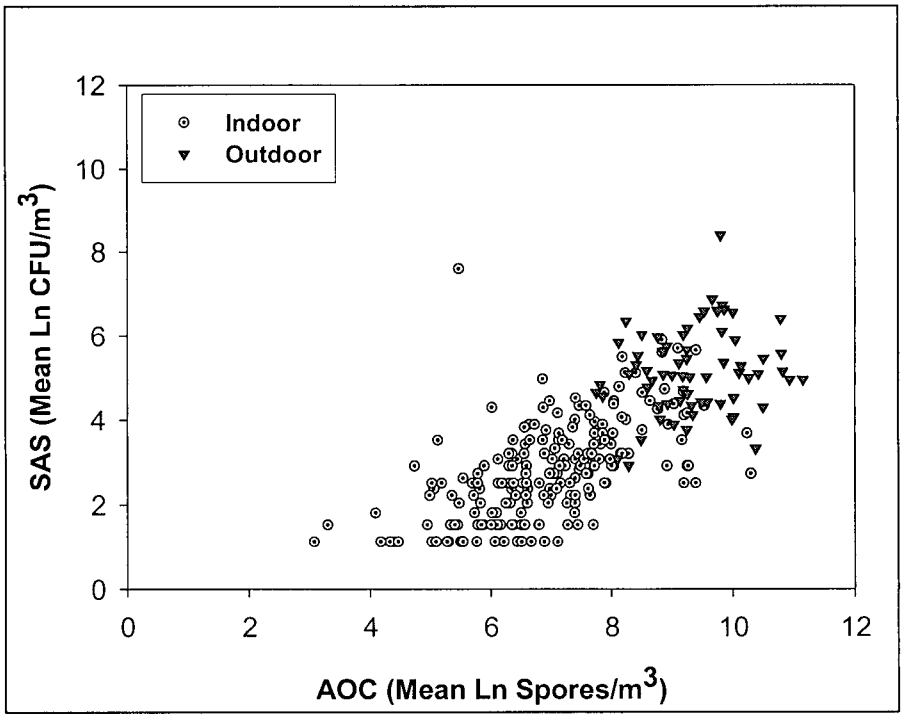


Table 3.8.6. Regression Models for Ln SAS (dependent) versus Ln AOC (independent)

Regression Model	Regression Coefficients *95% Confidence Intervals*				adj R^2
	y-Intercept	Ln AOC	Locator	Interaction*	
Overall Data	-1.997 *-2.596, -1.398*	0.716 *0.638, 0.794*	NA	NA	0.54
Indoor Data	-0.979 *-1.705, -0.253*	0.548 *0.445, 0.651*	NA	NA	0.35
Outdoor Data	2.964 *0.137, 5.790*	0.236 *-0.065, 0.536*	NA	NA	0.02
Overall + Locator	-0.723 *-1.419, -0.027*	0.511 *0.412, 0.609*	1.109 *0.753, 1.465*	NA	0.60
Overall + Locator + Interaction	-0.979 *-1.715, -0.244*	0.548 *0.444, 0.652*	3.943 *1.169, 6.716*	-0.312 *-0.615, -0.009*	0.60
Mixed Effects + Locator	-0.876 *-1.645, -0.106*	0.534 *0.426, 0.642*	1.050 *0.700, 1.400*	NA	0.74
Mixed Effects + Locator + Interaction	-1.164 *-1.969, -0.358*	0.575 *0.462, 0.688*	3.908 *1.353, 6.463*	-0.316 *-0.595, -0.036*	0.74

*Interaction = Ln AOC x Locator

3.9 Instrument Ranking based on Performance Sampler Characteristics

3.9.1. Questionnaires

Of the 14 people contacted by telephone or email to complete the questionnaire, 10 questionnaires were completed (response rate=71%).

Table 3.9.1 show the results of the questionnaires. A respondent code was applied to each questionnaire to keep answers confidential. A weighting was assigned to each factor by dividing the total score for each characteristic by the highest obtained score (of 25 for ease of use) and multiplying by 5.

Table 3.9.1. Questionnaire Results

Factor	Respondent Code										Total Score	Weighting
	A	B	C	D	E	F	G	H	I	J		
Cost	1	2	2	1	2	3	3	3	2	1	20	**4**[1]
Ease of Use	3	2	3	2	2	3	2	3	3	2	25	**5**
Portability	2	2	3	2	3	3	2	3	3	1	24	**5**
Noise	1	2	3	1	2	2	1	2	1	1	16	**3**
Sampling Time	2	2	2	2	2	2	2	3	3	1	21	**4**

[1]weighting (i.e., for cost, 20/25 x 5 = 4)

Other factors that were considered important by the panel were:

- Previous studies characterizing performance of instruments
- Use in industry

3.9.2. Format of Scoring Results

For all tables for both performance and other sampler characteristics, three values will be defined for each sub-category for each sampler. One represents the sampler characteristics, and the following two are scores that were calculated. The first score represents the ratio compared to the 'best' sampler. The second score, in parentheses is the normalized score and represents the fraction of the total score.

3.9.3. Performance Characteristics

3.9.3.1. Cut-off Diameter

Table 3.9.3.1 shows the scoring of each instrument based on their cut-off diameter that has been reported in published literature. The N6 scored highest (with the smallest cut-off diameter), and the RCS scored lowest (with the biggest cut-off diameter).

Table 3.9.3.1. Cut off diameter (μm)

Instrument	d_{50} (score)	Reference	Final Score
RCS	4.0 (*0.2*)[1]	Macher and First (1983)	**0.12**[2]
N6	0.65 (*1*)	Andersen (1958)	**0.59**
SAS	2-4 (*0.2*)	Lach (1985)	**0.12**
AOC	2.3 (*0.3*)	Aizenburg et al. (2000)	**0.17**
Total	*1.7*		1

[1]score assigned as a proportion of the best score (i.e., for the RCS, 4.0/0.65 = 0.2)
[2]score adjusted to 1 (i.e., for the RCS, 0.2/1.7 = 0.12)

3.9.3.2.Reproducibility

Table 3.9.3.2 shows the scoring based on the reproducibility found in this study. The AOC scored highest (best reproducibility) while the SAS scored lowest (worst reproducibility).

Table 3.9.3.2. Score for Reproducibility (Median CV)

Instrument	Indoor CV	Outdoor CV	Total Score	Final Score
RCS	25.7 0.6[1] (*0.24*)[2]	10.2 0.97 (*0.31*)	*0.55*[3]	**0.275**[4]
N6	24.7 0.6 (*0.24*)	13 0.76 (*0.24*)	*0.48*	**0.24**
SAS	47.1 0.3 (*0.12*)	22.8 0.43 (*0.14*)	*0.26*	**0.13**
AOC	15.9 1 (*0.4*)	9.9 1 (*0.31*)	*0.71*	**0.355**
Total	2.5 (*1.0*)	3.16 (*1.0*)	*2.0*	1

[1]score assigned relative to the 'best' score (i.e., for the RCS, 15.9/25.7=0.6)
[2]normalized score (i.e., for the RCS, 0.6/2.5=0.24)
[3]sum of normalized scores for all sub-categories (i.e., for the RCS, 0.24+0.31=0.55)
[4]total score adjusted to 1 for final score (i.e., for the RCS, 0.55/2.0=0.275)

3.9.3.3.Total Yield

Table 3.9.3.3 shows the scoring of each instrument based on their total yield from this study. The AOC scored highest for total yield, while the SAS scored lowest for total yield. Note that each sampler was weighted equally for the indoor and outdoor means to maintain the methodology between characteristics. It will be discussed later regarding the difference between the culture methods (RCS, N6, and SAS) and the microscopic methods (AOC) for overall yield.

Table 3.9.3.3. Scores for Total Yield

Sampler	Indoor Geo Mean	Outdoor Geo Mean	%<LOD	%>UDL	Total Score	Final Score
RCS	115 0.12[1] (*0.10*)[2]	550 0.37 (*0.19*)	1.2 0.83 (*0.39*)	8.4 0.23 (*0.07*)	*0.75*[3]	**0.19**[4]
N6	68 0.07 (*0.058*)	698 0.47 (*0.240*)	4.1 0.24 (*0.11*)	0 1 (*0.32*)	*0.728*	**0.18**
SAS	17 0.02 (*0.016*)	175 0.12 (*0.06*)	15.2 0.06 (*0.03*)	0.2 0.9 (*0.29*)	*0.396*	**0.10**
AOC	980 1 (*0.826*)	1,487 1 (*0.51*)	0 1 (*0.47*)	0 1 (*0.32*)	*2.126*	**0.53**
Total	1.21 (*1.0*)	1.96 (*1.0*)	2.13 (*1.0*)	3.13 (*1.0*)	*4.0*	1

[1]score assigned relative to the 'best' score (i.e., for the RCS, 115/980=0.12)
[2]normalized score (i.e., for the RCS, 0.12/1.21=0.24)
[3]sum of normalized scores for all sub-categories (i.e., for the RCS, 0.10 + 0.19 + 0.39 + 0.07 = 0.75)
[4]total score adjusted to 1 for final score (i.e., for the RCS, 0.75/4.0=0.19)

3.9.4. Other Sampler Characteristics

3.9.4.1. Cost

All costs were determined by costs incurred during the study. The cheaper sampler was assigned a higher score. Table 3.9.4.1a presents a summary of how the total cost per sample was determined for each sampler. Table 3.9.4.1b shows the results for each aspect of cost considered for each method. The N6 scored highest for this characteristic (cheapest overall), and the SAS scored lowest (most expensive overall).

Table 3.9.4.1a. Summary of Total Cost per Sample (Canadian Dollars)

Sampler	Media Cost/flask	#samples/flask	Media Cost/sample	Cost/sampling medium	Total Cost/sample
RCS	3.95	75	0.05	2.38	**2.43**
N6	3.95	17	0.23	0.27	**0.50**
SAS	3.95	37	0.11	1.13	**1.24**
AOC	NA	NA	NA	19.74[1]	**19.74**

[1]cost per AOC sampling cassette

Table 3.9.4.1b. Cost Summary in Canadian Dollars

Sampler	Instrument	Sample	Analysis	Score	Final
RCS	$3,436 0.62[1] (*0.28*)[2]	$2.43 0.20 (*0.12*)	$55 1 (*0.25*)	*0.65*[3]	**0.2167**[4]
N6	$5,294 0.40 (*0.18*)	$0.50 1 (*0.60*)	$55 1 (*0.25*)	*1.03*	**0.3433**
SAS	$9,095 0.23 (*0.1*)	$1.24 0.40 (*0.24*)	$55 1 (*0.25*)	*0.59*	**0.1967**
AOC	$2,126 1 (*0.44*)	$19.74 0.06 (*0.04*)	$55 1 (*0.25*)	*0.73*	**0.2433**
Total	2.25 (*1.0*)	1.66 (*1.0*)	4 (*1.0*)	*3.0*	1

[1]score based as a proportion of the 'best' score (i.e., for the RCS, $2,126/$3,436 = 0.62)
[2]normalized score, proportion of score to total score (i.e., for the RCS, 0.62/2.25 = 0.28)
[3]sum of all normalized scores (i.e., for the RCS, 0.28 + 0.12 + 0.25 = 0.65)
[4]score adjusted to '1' for final score (i.e., for the RCS, 0.65/3.0 = 0.2167)

3.9.4.2. Ease of Use

Ease of use was scored over 5 sub-categories. Results for the speed of analysis per sample are shown in Table 3.9.4.2a. Table 3.9.4.2b shows the scoring for ease of use. The sampler that had the ability to calibrate on site, a quicker sample analysis, did not require incubation of samples, had pre-set sampling times, and had sampling media that was easy to prepare, was assigned a higher score. The AOC scored highest for this characteristic (easiest to use), and the N6 scored lowest (most difficult to use).

Table 3.9.4.2a. Speed of Analysis Results (seconds)

Sample	N	Mean	Median	Range
RCS	48	41	24	6-130
N6	49	48	38	2-170
SAS	39	17	20	1-87
AOC	6	991	1,104	554-1,210

Table 3.9.4.2b. Scoring for Ease of Use

Sampler	On site calibration?	Speed of Analysis	Incubation[1]	Pre-set times[2]	Ease of Sample prep[3]	Score	Final
RCS	Yes 1[4] (*0.33*)[5]	24.5 sec[6] 0.82[7] (*0.35*)[8]	Yes 0 (*0*)	Yes 1 (*0.33*)	Low 0 (*0*)	*1.01*[9]	**0.203**[10]
N6	Yes 1 (*0.33*)	38 sec 0.53 (*0.22*)	Yes 0 (*0*)	No 0 (*0*)	Medium 0.5 (*0.25*)	*0.80*	**0.161**
SAS	No 0 (*0*)	20 sec 1 (*0.42*)	Yes 0 (*0*)	Yes 1 (*0.33*)	Medium 0.5 (*0.25*)	*1.0*	**0.200**
AOC	Yes 1 (*0.33*)	1,104 sec 0.02 (*0.01*)	No 1 (*1.0*)	Yes 1 (*0.33*)	High 1 (*0.5*)	*2.17*	**0.436**
Total	3 (*0.99*)	2.37 (*1.0*)	1.0 (*1.0*)	3 (*0.99*)	2 (*1.0*)	*4.98*	**1**

[1]Does the sample require incubation before analysis?
[2]Presence of pre-set sampling times on sampler
[3]Relative ease of preparing sampling medium for analysis
[4]'Yes' to direct calibration considered best, assigned a score of '1'
[5]normalized score by dividing score by total score (i.e., for the RCS, 1/3 = 0.33)
[6]sec=seconds
[7]score assigned as a proportion of the 'best' score (i.e., 24.5/20 = 0.82)
[8]normalized score (i.e., for the RCS, 0.82/2.37 = 0.35)
[9]sum of normalized scores (i.e., for the RCS, 0.33 + 0.35 + 0 + 0.33 + 0 = 1.01)
[10]score adjusted to 1 is final score (i.e., for the RCS, 1.01/4.98 = 0.203)

3.9.4.3.Portability

Portability was scored across four sub-categories and the results are shown in Table 3.9.4.3. The sampler that had a longer battery charge, did not require a power source, were smaller in size, and lighter weight was assigned a higher score. The RCS scored highest overall sub-categories (most portable), and the N6 scored lowest overall (least portable).

Table 3.9.4.3. Scoring for Portability

Instrument	Max Charge/battery	Separate source of power?	Size of Sampler cm^3	Weight kg	Total Score	Final Score
RCS	240 min 1[1] (*0.34*)[2]	No 1[3] (*0.33*)[4]	1,689 0.65 (*0.304*)	1.3 0.61 (*0.30*)	*1.274*[5]	**0.31**[6]
N6	240 min 1 (*0.34*)	No 1 (*0.33*)	5,938 0.18 (*0.084*)	11.17 0.07 (*0.03*)	*0.784*	**0.20**
SAS	150 min 0.62 (*0.21*)	No 1 (*0.33*)	3,572 0.31 (*0.145*)	2.185 0.36 (*0.18*)	*0.865*	**0.22**
AOC	80 min 0.33 (*0.11*)	Yes 0 (*0*)	1,095 1 (*0.467*)	0.794 1 (*0.49*)	*1.067*	**0.27**
Total	2.95 (*1.0*)	3 (*0.99*)	2.14 (1.0)	2.04 (*1.0*)	*3.99*	1

[1]score of '1' for highest battery charge (i.e., for the RCS, 240/240=1)
[2]normalized score (i.e., for the RCS, 1/2.95 = 0.34)
[3]'No' for a separate source of power is best, a score of '1' is assigned
[4]normalized score (i.e., for the RCS, 1/3 = 0.33)
[5]sum of normalized scores across all sub-categories (i.e., for the RCS, 0.34 + 0.33 + 0.304 + 0.30 = 1.274)
[6]total score adjusted to one to give final score (i.e., for the RCS, 1.274/3.99 = 0.31)4

3.9.4.4.Noise

Table 3.9.4.4 shows the results of the noise survey conducted for each instrument. The background noise level was 37.0 dB (A). The quieter sampler was assigned the higher score. The SAS scored highest (quietest sampler), while the N6 and AOC scored lowest (noisiest samplers).

Table 3.9.4.4. Noise Survey Results and score

Instrument	Measurement #				Arithmetic Mean (score)	Final Score
	1	2	3	4		
RCS	47.0	47.0	46.0	47.0	46.8 (*0.80*)[1]	**0.26**[2]
N6	59.0	58.5	59.5	59.5	59.1 (*0.63*)	**0.21**
SAS	37.5	37.5	37.5	37.5	37.5 (*1*)	**0.33**
AOC	60.0	60.5	61.0	59.5	60.2 (*0.62*)	**0.2**
Total					*3.05*	1

[1]score assigned as a proportion of the 'best' score (i.e., for the RCS, 37.5/46.8 = 0.8)
[2]normalized score (i.e., for the RCS, 0.80/3.05 = 0.26)

3.9.4.5.Sampling Time

Sampling time was scored over 4 sub-categories. Table 3.9.4.5 shows the scoring of each instrument for sampling time. The sampler that could collect a wider range of sampling volumes, and sampled at a higher flow rate was assigned a higher score. The N6 scored highest (best for sampling time), and the RCS and AOC scored (worst for sampling time).

Table 3.9.4.5. Scoring for Sampling Time

Instrument	Min Volume[1]	Max Volume[2]	Flow Rate	Total Score	Final Score
RCS	20 L 0.25[3] (*0.12*)[4]	320 L 0.05 (*0.032*)	40 0.4 (*0.211*)	*0.363*[5]	**0.12**[6]
N6	5 L 1 (*0.49*)	6,792 L 1 (*0.645*)	28.3 0.3 (*0.158*)	*1.293*	**0.43**
SAS	10 L 0.5 (*0.24*)	1,800 L 0.3 (*0.194*)	90 1 (*0.526*)	*0.96*	**0.32**
AOC	15 L 0.3 (*0.15*)	1,200L 0.2 (*0.129*)	15 0.2 (*0.105*)	*0.384*	**0.13**
Total	2.05 (*1.0*)	1.55 (*1.0*)	1.9 (*1.0*)	*3*	1

[1]Minimum volume sampled defined by a pre-set volume or a sampling time of 10 seconds (i.e., for the RCS, the minimum pre-set volume is 20 L)
[2]Maximum volume sampled defined by a pre-set volume or a sampling time = duration of battery charge (i.e., for the RCS, maximum pre-set volume is 320 L)
[3]score assigned as a proportion of the best score (i.e., for the RCS, 5/20 = 0.12)
[4]normalized score (i.e., for the RCS, 0.25/2.05 = 0.12)
[5]sum of normalized scores across sub-categories (i.e., for the RCS, 0.12 + 0.032 + 0.211 = 0.363)
[6]total score adjusted to 1 (i.e., for the RCS, 0.363/3 = 0.12)

3.9.5. Historical and Current use by Industry/Research

80% of the panel mentioned previous studies documenting efficiencies were important and therefore a weighting of '4' was given to the history category. Also, current use in industry as a standard was also important. Appendix IV presents the studies found for both journals in. Table 3.9.5 presents the results of the literature search conducted from 1992-May 2002 for AIHAJ and Indoor Air journals respectively. The sampler that was used in more studies, was assigned the higher score. To determine current use in industry, the results from the questionnaire were used. For every mention of each sampler (or a similar model), a score of '1' was assigned to that sampler. The N6 scored highest (used the most), while the RCS scored lowest (used the least).

Table 3.9.5. Scoring for Industrial (Historical and Current) Use

Instrument	Total Studies			Use by Panel	Total Score	Final Score
	AIHAJ	Indoor Air	Total			
RCS	2	2	**4 (*0.14*)**[1]	3 (*0.17*)	*0.31*[2]	**0.155**[3]
N6	15	2	**17 (*0.61*)**	7 (*0.39*)	*1.0*	**0.50**
SAS	3	3	**6 (*0.21*)**	2 (*0.11*)	*0.32*	**0.16**
AOC	1	0	**1 (*0.04*)**	6 (*0.33*)	*0.37*	**0.185**
Total	21	7	**28 (*1*)**	[4]18 (*1*)	*2.0*	1

[1]score assigned as a proportion of all studies (i.e., for the RCS, 4/28 = 0.14)
[2]total score across both sub-categories (i.e., for the RCS, 0.14 + 0.17 = 0.155)
[3]total score adjusted to 1 (i.e., for the RCS, 0.31/2=0.155)
[4]This number is greater than the number of people panel because some used more than one instrument.

3.9.6. Final results for comparison

Table 3.9.6 shows the total scores for each instrument section with the weighting incorporated as reflected by the following equation (overall scores shown in Table 3.9.6.1). The highest overall scores were assigned to the N6 and the AOC, while the SAS and the RCS were assigned lower scores.

Final Score =

Cost x 4 + Ease of Use x 5 + Portability x 5 + Noise x 3 + Sampling Time x 4 + Cut-off diameter (d_{50}) x 5 + Total yield x 5 + CV (Reproducibility) x 5 + Industrial* x 4

*Historical and Current use in Industry/Research

Table 3.9.6. Total Scores for each instrument with weighting

Sampler	d_{50}	CV	Total Yield	Cost	Ease of Use	Portability	Noise	Sampling Time	Industrial Use[1]
RCS	0.6	1.375	0.95	0.867	1.015	1.55	0.78	0.48	0.62
N6	2.95	1.20	0.90	1.373	0.805	1.00	0.63	1.72	2.00
SAS	0.6	0.65	0.50	0.787	1.00	1.10	0.99	1.28	0.64
AOC	0.85	1.775	2.65	0.973	2.18	1.35	0.6	0.52	0.74
Total	5	5	5	4	5	5	3	4	4

[1]Historical and Current use in Industry/Research

Table 3.9.6.1 Overall Scores

Instrument	Total Score	Rank
RCS	8.24	**2**
N6	12.58	**1**
SAS	7.55	**2**
AOC	11.63	**1**
Total	40	

3.9.7. Analysis of comparison

Figure 3.9.7 shows the distribution of scores of each method for the different characteristics they were scored on. Table 3.9.7 presents the highest scoring and lowest scoring categories for each instrument.

Figure 3.9.7. Total Normalized Scores for each Sampler

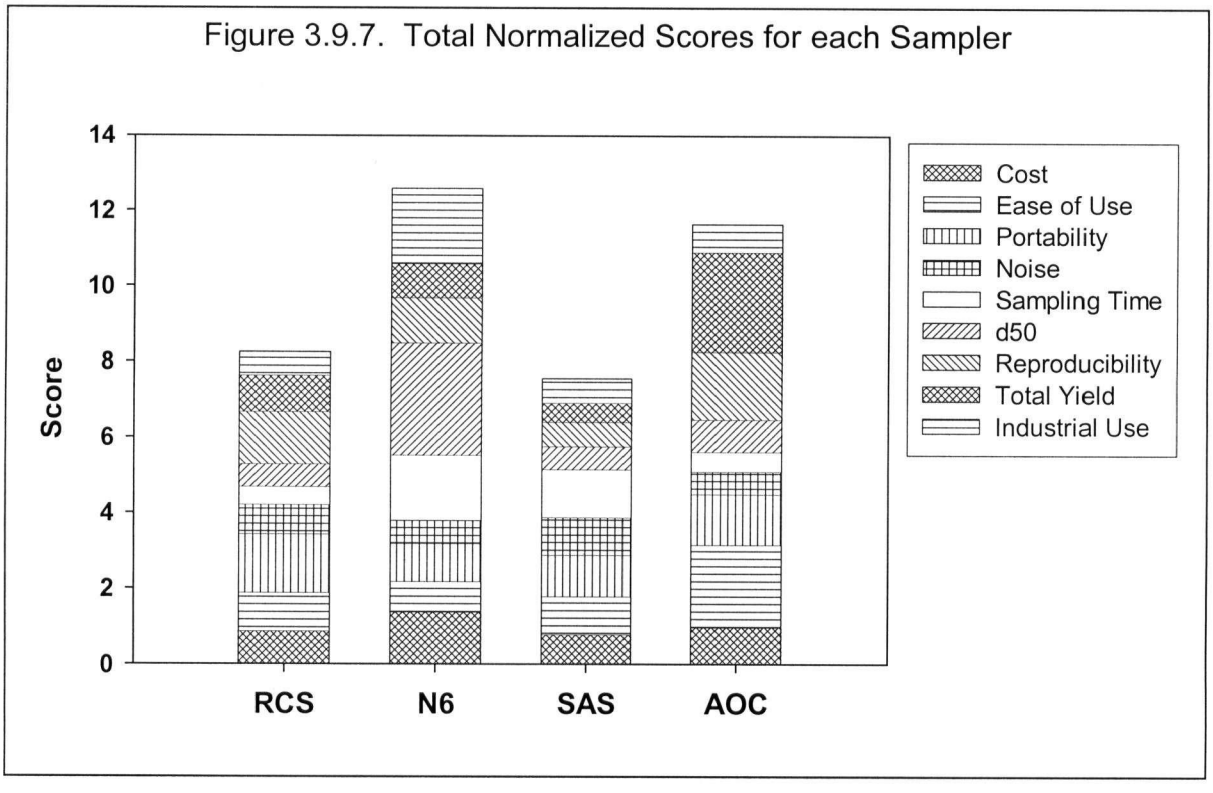


Table 3.9.7 shows the scoring characteristics for each sampler. The three highest scoring and the lowest scoring characteristics for each instrument are shown.

Table 3.9.7. Scoring Characteristics

Instrument	Highest Scoring	Lowest Scoring
RCS	Portability Reproducibility Ease of Use	Sampling Time d_{50}
N6	Cut-off diameter History and Use Cost	Ease of Use Portability
SAS	Noise Ease of Use	Total Yield Reproducibility Cost
AOC	Ease of Use Total Yield Reproducibility	Noise Sampling Time

Chapter 4. Discussion

4.1. Study Overview

The results of this study support the rejection of all original study hypotheses. The following lists the original study hypothesis, and the reasoning found from the study that rejects it:

Ho_1: No differences exist between the geometric means, detection limits, and reproducibility among the four samplers (RCS=N6=SAS=AOC).

A significant difference was found between samplers in the geometric means for indoor and outdoor samples (overall yield), detection limits, and reproducibility.

Ho_2: Regression models between each method pair will show a linear and y=x relationship and have a zero intercept.

All regression models had slopes that were not equal to 1 and most models had a significant intercept. Location was a confounder and for most models. Interaction was found as well for different levels of the location variable (i.e, the relationship found for indoor data was different from relationship found for outdoor data). 6 final models were presented to serve as calibration curves for each method comparison.

Ho_3: The four samplers will have the same score across all categories, resulting in an equal ranking for all four samplers

The samplers were ranked differently across each characteristic, with a final overall ranking of the N6 and AOC with the higher scores, and the RCS and SAS with the lower scores. Higher scoring characteristics differed between the N6 and AOC despite a similar score overall.

4.2. Proportion of Samples Beyond Detection Limits

Bioaerosol samplers typically do not have defined detection limits. Instead, the lower and upper detection limits were defined in this study based on theoretical principles. The results of this study indicate that theoretical limits do not reflect the actual detection limits and an attempt of defining the 'true' limits still needs to be made. These detection limits assume that the collection efficiencies of the samplers are perfect and do not account for any losses that may occur during sampling (do not take into account the biological recovery efficiency). Manufacturers should make an attempt to determine upper and lower detection limits for their sampler, or provide a guideline for protocols to be used in certain environments (clean versus dirty) and make the information available (Macher, 1997).

4.2.1. Lower Limit of Detection (LOD)

The LOD of concentrations in air is dependent on the volume of air collected (a higher volume of air gives a lower LOD). There were slight differences in the volumes collected by

each sampler: SAS (162.5 L)>RCS (160 L)>N6 (141.5 L) for the viable samplers, and AOC (150 L indoors and 75 L outdoors). For the viable samplers, the SAS and RCS theoretically have the lowest LOD followed by the N6. However, a higher proportion of the SAS samples (14.4%) were below the LOD compared to the N6 (4.4%) and RCS (0%). These results suggest that the LOD cannot be simply determined by the air volume alone and that another factor must be involved. For example, high flow rates (resulting in higher impact velocities onto the sampling medium) have been thought to result in a decrease in viability of bacterial spores (Stewart et al., 1995), and these results may be applicable to fungal spores. Also, higher flow rates have also been correlated with particle bounce off of the sampling medium (Hinds, 1999). The SAS has the highest flow rate of the three instruments and may have more samples below the LOD for all these reasons. This study however, did not directly study any of these aspects specifically and further study is needed to examine them.

The RCS had the fewest samples below the LOD. However, the actual flow rate of the RCS cannot be directly determined. The manufacturer recommends the use of 40 L/min as the effective sampling flow rate for data analysis and has been used by previous investigators to determine air concentration (Smid et al., 1989; Verhoeff et al., 1990). This flow rate is the effective sampling rate, where for particles with a d_{50}= 4μm, 100% collection efficiency occurs. A study (Macher & First, 1983) attempted to calibrate the RCS by creating an adapter that separated the inflow and the outflow and found a flow rate of 210 L. Previous studies have also used the total airflow rate of 280 L/min to derive concentration (Jensen et al., 1992). The total air volume collected can be at least 5 times higher than the volume specified by the manufacturer (Macher & First, 1983). Increasing the total volume collected reduces the LOD and may explain why the RCS had no samples below the LOD.

For the AOC, no samples were below the LOD, which was defined theoretically as the impaction of one spore on at least one of the fields of view. This is expected since it does not rely on the viability of spores and underscores the differences between methods. Whether this is the true LOD is still not certain, and the manufacturer should specify one in their technical manual.

4.2.2. Upper Detection Limit (UDL)

Overlapping of colonies can hinder the ability to distinguish between colonies if they reach a diameter beyond 10 mm (Dillon et al., 1996). Factors that affect the colony surface density include the bioaerosol concentration in sampled air, the sampler airflow rate, the sample collection time, the collection area, the nutrient concentration and the incubation conditions (Chang et al., 1995). For nutritious media like MEA, colony overlap is a problem, and a maximum colony density of 1 CFU/cm^2 instead of the 5 CFU/m^2 was recommended (Burge, 1987). This would change the UDL drastically for the RCS from 180 to 34 colonies. An UDL of 180 was used in this study, and it is possible that masking could have resulted in some incorrect counts, and perhaps a correction table (like the N6 and SAS) should be devised.

The RCS agar strips are the smallest in area (34cm^2), and therefore, are expected to be more prone to overloading compared to the other methods. Incubating the RCS for a shorter time

period compared to the N6 and SAS plates was an attempt to reduce overlapping. The RCS had the highest proportion of samples above the UDL (8.4%), while the N6 and SAS did not have any overloaded samples. Alternatively, this may be because of the larger air volume that may have been collected by the RCS. All the overloaded samples for the RCS were outdoor samples, and therefore, in situations with expected higher load, such as outdoors, a shorter sampling period (2 minutes) should be employed for the RCS. For the N6 and the SAS, the UDL were theoretically defined to be equal to the number of holes in the impactor, but for the SAS, 260 was used since the correction table provided by the manufacturer only went up to 260 colonies. Counts above this number, were believed by the manufacturer to be beyond the UDL (Peter Pratt, personal communication).

No UDL was defined for AOC because none of the samples were so overloaded that distinguishing between two spores was impossible. The counting rules of this method made it possible to read slides with a large load of fungal spores (maximum of 400 spores or 100 fields) without having to spend too much time on analysis. These slides are subject to interference by other particles. Slides that have a lot of material that are not spores (such as epithelial cells, insect, dirt and dust fragments) may make counting of spores nearly impossible if they crowd the slide. Also, the stain adheres to other particles besides fungal spores (chitin is also found in the exoskeleton of insects), and therefore may interfere with counting. No suggestion has been made by the manufacturer about how much interference is acceptable for the reading of the slide. This information should be provided in future manuals.

4.3. Reproducibility

Duplicate samples were taken sequentially. The airborne load of fungal aerosols is subject to change over time, and therefore the variation between sample 1 and sample 2 may not necessarily reflect the performance of the instrument, but instead the dynamic airborne environment. However, paired t-tests between sample 1 and sample 2 for each method showed significant differences only for the SAS sampler for indoor samples ($p=0.008$). Table 4.3 shows the results of the paired t-test between sample 1 and sample 2. The SAS sampled for the shortest period of time, and therefore, the time between samples were highest for the SAS. The AOC was the only sampler set to take samples one right after the other (no waiting in between except to remove old cassette and attach a new one). This may be why the AOC has the lowest CV between samples indoors. The AOC was close to significance for differences between samples for outdoor samples. This may be due to the change in sampling methodology for outdoor locations (more time between samples).

Parallel samples may be able to assess the true reproducibility of sampling methods, but since airborne loads also differ spatially, the spacing between two instruments may also affect the direct air concentration being measured by the sampler.

4.3.1. Indoor/Outdoor Differences

A difference in reproducibility was found between indoor and outdoor sampling locations for all samplers (indoor CV>outdoor CV). This suggests that either the fluctuations in the actual

collection efficiencies for each sampler occurred more frequently indoors, or that indoor environments tended to be more variable in airborne fungal load and are reflected in the sequential duplicate samples. From this data, fluctuations in airborne load as a reason for reduced reproducibility indoors can only be accounted for by the SAS, since a significant difference between sequential duplicate values was found only for that sampler. Another possibility may be simply due to the higher concentrations found outdoors. CV is calculated by using the standard deviation divided by the mean, and since a higher concentration is generally found outdoors, it may appear that the CV is lower. Table 4.3.1 presents a summary of the median standard deviations found between samples for indoor and outdoor for each sampler. Indoor standard deviations tend to be lower than those for outdoor samples, and therefore suggesting that outdoor environments tended to be more variable than indoor environments.

Table 4.3.1. Median Standard Deviation between Samples for Indoor and Outdoor

Sampler	Indoor Median Standard Deviation	Outdoor Median Standard Deviation
RCS	26	49
N6	20	67
SAS	9	30
AOC	153	993

4.3.2. Differences between samplers

A previous study found slightly different results in reproducibility between sequential duplicates (Verhoeff et al., 1990), however, their sample size was very small (n=10). They also found the SAS to have the lowest reproducibility with a CV of 23%, followed by the RCS with 21.4%, and then the N6, 14.0%. Despite the differences in CV between methods, the range of CV is wide for all methods (approximately 0-140% for indoor and outdoor), suggesting the sequential duplicates results in variability.

For the sieve samplers (SAS and N6), a previous study has shown that using the positive-hole correction emphasizes the differences between samples, therefore decreasing the reproducibility (Buttner & Stetzenbach, 1993). The actual value counted compared to the corrected value can be very different. As the number of counts increase, the difference between the actual count and the corrected count increases (Macher, 1989). Uncorrected counts were not investigated because it was beyond the scope of this study, but should be examined in further studies. The N6 has comparable reproducibility to the RCS despite this fact, but the SAS had the lowest reproducibility, which can be partly due to the correction. Fluctuating sampler characteristics have been found for the SAS (Buttner & Steztenbach, 1993), and this may also contribute to a higher CV between samples. This study did not research any of these reasons specifically and need to be studied further.

4.4. Total Yield

4.4.1. Indoor to Outdoor comparison

The indoor mean concentrations were found to be significantly lower than outdoor means for all methods. This suggests that sources for fungi were predominantly from the outdoors. Different loads and types of airborne fungi between indoor and outdoor can affect the performance of the instruments, and therefore, location must be considered when analyzing the performance of instruments. Table 4.4.1 compares the outdoor to indoor yield for each sampler. For the N6, SAS, and AOC, the outdoor geometric mean is approximately 10 times higher than the indoor geometric mean. For the RCS, only a 5-fold difference was found, and can be attributed to the lower UDL of the RCS. In outdoor samples, where the concentration is expected to be high, the RCS is more likely to overload compared to the other samplers.

Table 4.4.1. Comparison of Outdoor and Indoor Concentrations

Sampler	Outdoor Geo Mean[1]	Indoor Geo Mean	Ratio
RCS (CFU/m^3)	550	115	5x
N6 (CFU/m^3)	691	68	10x
SAS (CFU/m^3)	175	17	10x
AOC (Spores/m^3)	10,577	980	10x

[1]Geometric Mean

4.4.2. Viable versus Microscopic Methods

The AOC had the highest mean of all the methods. This was expected because of two main reasons:

1) The microscopic method does not rely on viability of fungal spores.

2) The microscopic method is more likely to distinguish between chains or clumps of spores, drastically increasing the final count. If a chain or clump of fungal spores impacted onto a culture medium (i.e., through one hole in the N6 or SAS impactor head), it would be more likely to only appear as one colony after incubation because the colonies would overlap over one another. A microscopic method, such as the AOC, where no cultivation is required, each spore in the chain can be counted.

A higher total yield may not necessarily make a particular sampling methodology 'better' than another. The ability to collect a wide variety of spore sizes and types should be considered as well. Some species of fungi are relatively benign, while others are responsible for a variety of health effects, and therefore, it is important that the sampling methodology can differentiate between the types of fungal spores. The sizes of fungal spores vary, and therefore, the efficiency at which these samplers can collect for certain fungal spores can be reflected by their cut-off diameter. Speciation of fungal spores is very difficult with the microscopic method, but can be accomplished relatively well using culture methods. Despite

the high numbers collected by the AOC, identification of the spores to the species level cannot be done for all spores, significantly limiting this method.

A previous study by Tsai et al., (1999), compared the AOC and N6, and also found the AOC to have a higher mean than the N6. However, that study counted total fungal structures and not only spores on the AOC (this study only counted spores) and may have increased the magnitude of difference between the two samplers.

This study did not compare the AOC to other microscopic methods, and therefore cannot determine whether the AOC has comparable performance. A laboratory study that compared the AOC with other microscopy methods (Aizenburg et al., 2000) found the AOC to have similar performance for enumerating total spores to be similar with the other methods. This was true for particles that were larger than its d_{50} (2.3μm) but does not hold for particles less than that size. Further research needs to be done on the AOC in comparison to other microscopic methods regarding its comparative collection efficiency and its ability to collect a wide range of spore types and sizes.

4.4.2.1.Comparison of Viable Samplers

For the viable samplers, the SAS had the lowest overall yield both indoors and outdoors. This is consistent with previous comparison studies utilizing other models of the SAS (Bellin & Schillinger, 2001; Mehta et al., 1996; Buttner & Stetzenbach, 1993; Verhoeff et al., 1990; Smid et al., 1989).

Between the culture methods, differences also exist on the ability to collect for a range of spore types and sizes. This is related to the cut-off diameter for each of these instruments. A smaller cut-off diameter allows the instrument to collect smaller spores more efficiently. Therefore, it is expected that instruments, like the N6, to be more efficient at collecting smaller fungal spores, and that instruments, like the RCS, be more efficient at collecting larger fungal spores. Bartlett et al., (2002), using the data collected from this study, found differences in collection efficiencies of each viable sampler for the recovery for different types of fungal genera. The N6 was found to detect more *Aspergillus* and *Penicillium* spores (spores typically 2-4 μm), while the RCS detected more yeast (spores typically 4-6 μm). These differences were beyond the scope of this study, but this is an important factor in evaluating the differences between total yield.

4.4.2.2.Microscopic Counting Method

The method used to enumerate fungal spores on the AOC slide is different from what was recommended by the manufacturer, but similar to methods used in previous studies (Aizenburg et al., 2000; Tsai et al., 1999). The manufacturer suggests counting at least 15% of the entire trace or 100 mould spores (whichever is first) at 600x magnification (specified for speciation). Air concentrations are determined by using the trace length of the AOC and the microscope field diameter. One field diameter is equivalent to one traverse. The manufacturer does not recommend use of the trace area for calculating the air concentration since it varies with flow rate and medium thickness. This method was not used because it

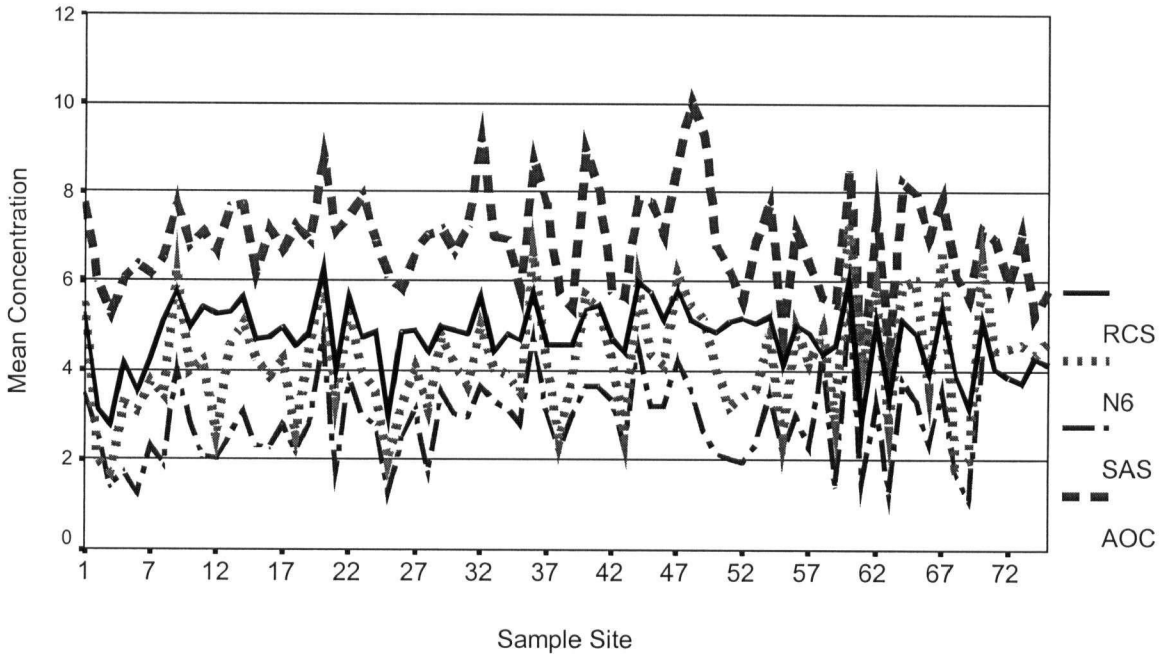
was unclear, but instead, a modified version of the NIOSH 7400 fibre counting method was used (National Institute of Occupational Safety and Health, 1994). It was assumed that media thickness did not vary significantly and the same flow rate was used throughout the study, so it was assumed that the specified trace area of 16.5 mm^2 to be accurate. However, no study has been conducted to determine whether this is a right assumption and may have affected the results. Also, no study has compared the different analysis methodologies to determine if they are different. The difference in methodology between this study and that of Aizenburg et al., (2000) and Tsai et al., (1999) was in the counting rules. Differences in the maximum number of fields and spores counted were evident, but no research has been done comparing the method recommended by the manufacturer and the method used for this study, so it is unknown whether these differences in enumeration had significant effects on the results.

4.4.3. Indoor Yields

Indoors, the RCS had a significantly greater mean than the other culture methods, which is also consistent with a previous study done by Verhoeff et al., 1990. However, as mentioned before, the flow rate recommended by the manufacturer of the RCS is not reliable and has been suggested to be as high as 210 L/min (Macher and First, 1983). This can decrease the indoor mean concentration of the RCS from 115 CFU/m^3 to about 23 CFU/m^3, which is similar to the geometric mean of the SAS (17 CFU/m^3). A second model of the RCS (the RCS plus) is similar to this model of the RCS (standard), but the flow rate can be calibrated directly to a flow rate of 50 L/min. Previous studies have shown the RCS Plus to have lower concentrations compared to the N6 but more similar to the SAS (Mehta et al, 1996; Buttner and Stetzenbach, 1993). The results from this study support this when using the total sampling flow for the RCS. Future investigations utilizing this model of the RCS should employ the total sampling flow rate to calculate air concentration.

Figure 4.4.3 shows the indoor mean concentrations detected by each instrument stratified by sample site. The AOC consistently gives higher means, while the SAS consistently gives the lowest. The RCS tends to have higher means than the N6, but is not found for all samples, possibly due to variations in environmental factors. If the total sampling flow rate for the RCS was used to determine concentration (280 L/min), the means for the RCS would be reduced by 7, making its geometric mean more comparable to the SAS. This would make the means from the N6 consistently higher.

Figure 4.4.3. Mean Indoor Concentrations by Sample Site

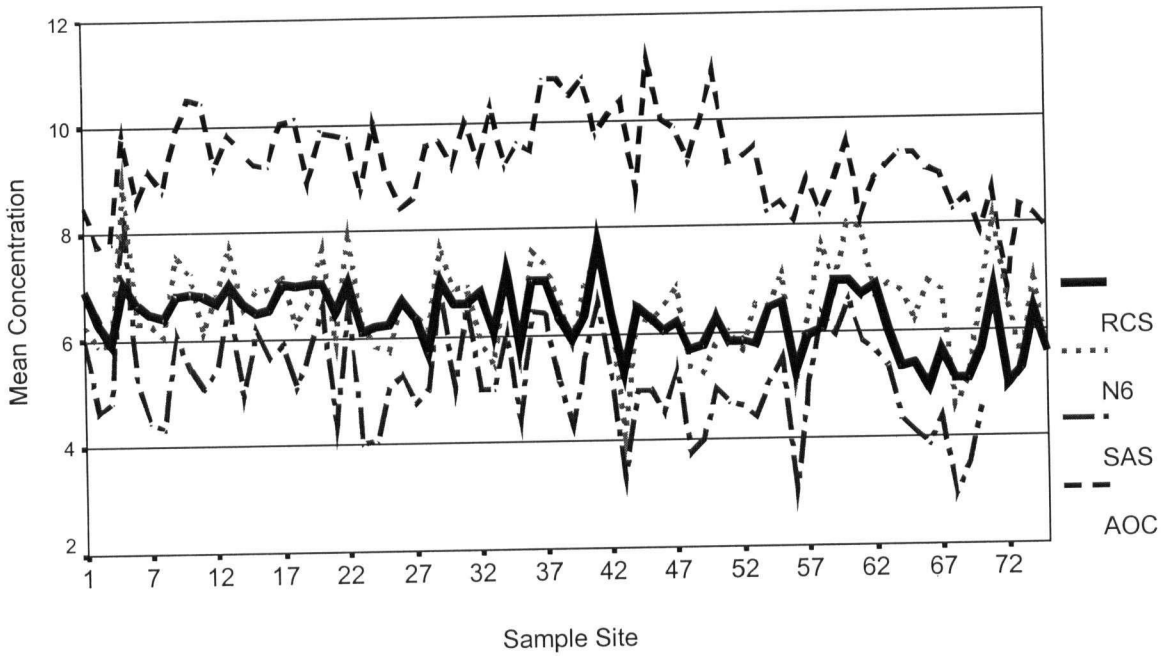


4.4.4. Outdoor Yields

Of the viable samplers, the N6 had the highest geometric mean (651 CFU/m^3). The change in the order from indoors to outdoors may reflect the lower upper detection limit of the RCS, or differences in the types of fungal spores outdoors (spores<4μm will not be collected by the RCS), or another factor that has not been determined. Also, the inability to calibrate the flow rate of the RCS is a problem, and can reduce the outdoor geometric mean from 550 CFU/m^3 to 110 CFU/m^3. Again, this is similar to the geometric mean found by the SAS (175 CFU/m^3). Therefore, in situations that are more likely to have higher counts (such as outdoors) the RCS would be easily overloaded so a lower volume should be sampled.

Figure 4.4.4 is a graph showing the outdoor mean concentrations for each sampler for each sample site and draws similar results as the indoor means. However, the N6 has the higher mean in outdoor samples, but again, the RCS and N6 cross lines for some samples.

Figure 4.4.4. Mean Outdoor Concentrations by Sample Sites



4.5. Regression Models

4.5.1. Advantages of Mixed Effects over Fixed Effects Modeling

Experiments are typically conducted such that measurements are made repeatedly over a period of time. For this study, repeat measurements were conducted within a particular sampling site over a course of four months. Therefore, correlation of measurements taken within one sampling day is expected. Fixed-effects linear regression ignores this aspect of the data, and treats each measurement as a separate measurement (Burton et al., 1998). Linear regression relies on the assumption that the data are independent of one another. This is not true if the measurements are repeated on the same day. Mixed effects regression can adjust for repeated measure, taking into account that the data comes from specific 'groups' (accounting for the dependence of the measurements on one another). Data were grouped according to the building, and thus for each building, a different model was created by changing the intercept only. This results in 60 parallel lines, and the model presented is an average of all the models. In general, all mixed-effects models had higher R^2.

4.5.2. General Trends in Fixed Effects Models

4.5.2.1. Confounding

For all method comparisons, location of the sample (indoor or outdoor) was found to be a confounding factor for the relationship between instruments (i.e. the slope of the relationship for overall data was different than the slopes predicted in stratified analyses when the locator

variable was not included). Therefore, location was an important variable when trying to assess the relationships between methods and should be included in any regression models.

4.5.2.2. Interaction

A change in the relationship (slope) between the two methods being compared was found when considering indoor data compared to outdoor locations data. This was found to be significant for all comparisons except for the N6-RCS, and borderline statistical significance for N6-SAS. Differences in upper detection limits, particle collection efficiencies, sampling environments, and data analyses may be the cause of this. Outdoor environments may have spores that have a wide range of sizes, and since these instruments collect particles at different efficiencies, this may affect the performance of these instruments. A negative interaction was found for all method comparisons except for the SAS-RCS, meaning that the outdoor slope was flatter than the slope for the indoor comparison. The UDL of the AOC has not been defined, but is not confined to the limitations of culture methods (such as overgrowth and overlapping). Also, for outdoor samples, the volume collected by the AOC was reduced, while the volumes were kept constant for the other methods. This may have affected the relationship outdoors since the UDL was increased significantly.

4.5.2.3. Variance explained by model (adjusted R^2)

The model including all the data had the highest R^2 than the stratification into indoor and outdoor models, perhaps partly due to the reduction in the number of data points. For the culture method comparisons (N6-RCS, N6-SAS, and SAS-RCS), the outdoor models had a higher R^2 (as high as overall models) compared to indoor models.

For comparisons with the AOC, stratification decreased the R^2 drastically, for both indoor and outdoor, which also may be partly due to the reduction in data points, and a greater variation in concentrations. The regression models comparing the viable samplers with the AOC, for only the outdoor samples, had a very low R^2 (R^2=-0.01 to 0.08). Indoor sampling time with the AOC was 10 minutes compared to 5 minutes for outdoor sampling. The reduction in total volume collected may have an influence on why the relationship seen indoors did not hold outdoors. Reducing the volume collected drastically increases the UDL.

Models including the dichotomous variable for location, with or without the interaction term, increased the R^2 to at least the value seen with the overall model. The R^2 was 0.60-0.62 for method comparisons with the AOC and ranged from 0.61-0.74 for culture comparisons. The N6-SAS comparison had the highest R^2=0.74. The collection principles of the N6 and SAS are very similar (single stage, sieve type impactor, culture method) with differences in flow rates and physical makeup and a high R^2 is not surprising. The lower R^2 is expected for comparisons of culture methods with the AOC since it uses a different analysis method (microscopy).

4.5.3. General Trends for Mixed Effects Models

The R^2 were higher for the mixed effects models, indicating that within-building correlation explained additional variance in the concentrations. Initially, both building and sampling date were considered as potential random effects, included since multiple measurements were taken over a four-month period, in four locations per day, and typically, a different building each day. Therefore, the correlation between sampling date and building was expected. To prevent problems from correlation between the two variables, only building was used as the random effect.

The models were first developed with only the dichotomous locator variable and the independent. For all models, the locator variable was significant. All mixed effects models were similar to the fixed effects model (coefficients within 95% confidence for both models) except for the N6-SAS combination. The mixed-effects model for N6-SAS put more emphasis on the y-intercept (constant) and the locator variable than the slope. This was also the case for all other comparisons with only the culture methods, though not significant. For comparisons with the AOC, more emphasis was put on the slope and less on the intercept and the locator variable. This suggests that for culture methods, the differences in environment (indoor compared to outdoor) have more impact on the relative performance of the instruments than the instruments themselves. For comparisons with the AOC, the instrument contributes more to the model.

4.5.4. Limitations of Regression Models

These regression models may serve as calibration equations. These models are based on data that were collected with specific volumes and instrumentation and may not necessarily be appropriate for data collected under different procedures. These models are also limited to the range of concentrations sampled by the instruments and should not be applied to data outside of this range.

For all models with the AOC, the outdoor data did not appear to have any slope. This suggests that culture methods do not correlate well with microscopy methods at high concentrations (as shown by the R^2 for the outdoor only model). It may be more appropriate to only use the models for indoor situations.
The detection limits differ between instruments and may have affected the regression models.

4.5.5. Final Models

The final models are presented in Table 4.5.4. The simplest model with significant coefficients was selected as the final model for each instrument pair. Only the N6-SAS model uses the mixed-effects model because there was a difference between the coefficients for the fixed effects regression compared to the mixed effects model.

Table 4.5.5. Final Models and Limits

Model	Range*
Ln N6 = 0.909 (Ln RCS) + 0.887 (I/O)[1]	N6: 7-8,000 RCS: 7-1,125
Ln N6 = 1.847 + 0.840 (Ln SAS)+ 1.210 (I/O) - 0.162 (Ln SAS*I/O)	N6: 7-8,000 SAS: 7-3,000
Ln SAS = -1.189 + 0.836 (Ln RCS) - 2.30 (I/O) + 0.527 (Ln RCS*I/O)	SAS: 7-3,000 RCS: 7-1,125
Ln N6 = -0.904 + 0.743 (Ln AOC) + 6.694 (I/O) - 0.663 (Ln AOC*I/O)	N6: 7-8,000 AOC: 20-59,874
Ln RCS = 1.528 + 0.467 (Ln AOC) + 2.782 (I/O) - 0.252 (Ln AOC*I/O)	RCS: 7-1,125 AOC: 20-59,874
Ln SAS = -0.979 + 0.548 (Ln AOC) + 3.943 (I/O) - 0.312 (Ln AOC*I/O)	SAS: 7-3,000 AOC: 20-59,874

[1]Indoor/Outdoor variable (=0 for indoor and =1 for outdoor)
*For RCS, N6, and SAS = CFU/m^3, for AOC = Spores/m^3

These models can serve as calibration curves for those who may have used other instruments to assess airborne mould, provided that it is the same instrument used in this study. Concentrations of one sampler can be related to another (such as a 'reference' sampler of their choice, typically N6). Regression modeling has never been used in previously published comparison studies to examine these relationships. This can be partially attributed to the fact that most studies conducted previously have very few samples. The models describing relationships of the N6, SAS, and RCS to the AOC is literally a comparison of viable and total spores. Therefore, for those who may be interested in getting a rough estimate of the total spores or total colony forming units if they have the value with another instrument, these equations may be used. However, these models say nothing about the speciation, which is an important result. This relationship may prove useful in determining the magnitude of difference between total and viable spores.

4.6. Ranking of Instruments by Performance and Other Sampler Characteristics

Air sampling for fungal aerosols is conducted during indoor air quality investigations and research studies, despite the lack of a standardized method. The sampler of choice varies by personal preferences and study hypotheses. The information obtained by this questionnaire provides a small sampling of what professionals use for exposure assessment and what their rationale behind their choice. Such information has not been provided previously. The questionnaires that were completed in this study may provide some information to explain what different researchers and consultants look for in choosing a bioaerosol sampler. The methodology used to rank these samplers may assist those who may need to choose a method based on particular traits. Table 4.6 summarizes the final normalized scores for each characteristic for each instrument without any weighting. With the information provided by this scoring scheme, a user can apply different weights to each characteristic to meet their sampling needs. For example, for a user who may be more interested in a sampler that is affordable, easy to use, and portable, the final scores obtained for those sections only can be summed for each sampler to determine which one may be best.

Table 4.6. Final Normalized Scores for each Characteristic by Sampler

Sampler	d_{50}	CV	Total Yield	Cost	Ease of Use	Portability	Noise	Sampling Time	Industrial Use
RCS	0.12	0.275	0.19	0.2167	0.203	0.31	0.26	0.12	0.155
N6	0.59	0.24	0.18	0.3433	0.161	0.20	0.21	0.43	0.500
SAS	0.12	0.13	0.10	0.1967	0.200	0.22	0.33	0.32	0.16
AOC	0.17	0.355	0.53	0.2433	0.436	0.27	0.20	0.13	0.185

4.6.1. Scoring Analysis for Performance Characteristics

4.6.1.1.Cut-off Diameter

The cut-off diameter was the only measure of particle collection efficiency available. Inclusion of speciation data would have influenced the scoring of each sampler, but this is dependent on what the study hypotheses are (sampler have different collection efficiencies for different fungal species). The N6 received the highest score because it has the lowest cut-off diameter. Typically, a lower cut-off diameter is more desirable because it has the ability to sample for smaller organisms. If the scores for this characteristic were removed, this would impact the final score for the N6 and it would have a score more comparable to the other samplers.

Another aspect of the N6 method that is not evaluated by this scoring is that it is derived from a method that can discriminate between particle sizes. None of the other samplers have this attribute. This was not included in this assessment, but should be noted as a favourable characteristic for the N6.

4.6.1.2.Reproducibility

The results for indoor and outdoor CV of the sequential duplicates for each sampler were used to assess reproducibility. Note that this is for sequential duplicates. For true duplicates (in which samples are taken concurrently) the scores may change, but no information was gathered from this study to address this. A high score was given to the AOC, and the lowest to the SAS.

4.6.1.3.Total Yield

No adjustment was made to account for differences between viable and microscopic methods for viability. This was because no rationale was available to score one type of method higher than the other. The best may be to not use the scores for overall yield to compare the AOC with the other samplers. Removing the overall yield score would result in the following ranking (total score): N6 (11.68)>AOC (8.98)>RCS (7.29)>SAS (7.05). Therefore, when total yield is removed, the N6 and AOC do not have similar scores. This does not change the relative scores between the culture methods (N6>RCS>SAS), because the N6 and RCS have similar scores for this characteristic, and the SAS has a very low score for total yield, and

therefore, the removal of the scores from this section does not affect its rank in the final score.

The scoring for this characteristic may not be representative of the actual performance of each instrument since speciation is also an important component of total yield. Speciation was not included because it was beyond the scope of this study. However, it can be inferred that the AOC would receive a low score for speciation. Microscopic methods cannot identify most fungal spores to the species level, and are unable to differentiate between certain fungal genera. It can also be inferred based on the information provided by Bartlett et al. (2002), that there are also differences between the collection efficiencies for particular fungal spores between instruments. However, scoring will also depend on which fungal spores the user is interested in. For example, if the user is interested in collecting *Aspergillus*, the N6 would receive higher scores, since it was found that the N6 had the highest collection efficiency for this type of spore (Bartlett et al., 2002).

4.6.2. Scoring Analysis for Other Sampler Characteristics

4.6.2.1.Cost

The N6 received the highest score for cost, followed by the AOC, and then the RCS and SAS. The cost per sample was highest for the AOC, but this was compensated by the lower cost of the instrument. The SAS was the most expensive instrument and the sampling plates were also of moderate cost, resulting in it being scored the lowest for this section. Depending on whether initial or continual operating costs are of concern, this score may need to be adjusted.

4.6.2.2.Ease of Use

The AOC received the highest score for this characteristic, despite a low score for the speed of analysis sub-category. This is mainly due to a high score assigned for incubation since no incubation time is required for this method. However, because the AOC and the other culture methods do not assess for the same type of fungal spores, it may not be relevant for some users regarding incubation, and therefore assigning a score for incubation may not be appropriate. Removing this score would result in the AOC having a similar final score to the SAS and the RCS.

The level of technical expertise required to enumerate samples was not assessed for this assessment, but should be considered. Counting of the AOC slides require more technical expertise than counting colonies.

The SAS and RCS have similar scores for this characteristic, and the N6 had the lowest. The SAS had a very high score for speed of analysis, and this may be due to the fact that there weren't as many colonies to count compared to the N6 and the RCS (reflected by low geometric mean). The N6 received a low score because it does not have pre-set times, and instead, a timer is required when sampling. The N6 could be improved in this aspect if a sampling pump was designed to have some pre-set times.

4.6.2.3.Portability

The RCS was assigned the highest score for this characteristic, and generally was one of the top scorers across each sub-category. This was expected since the RCS has been marketed based on its portable design. The lowest scoring was the N6, mostly due to the bulky sampling pump that is required to accompany the impactor. To improve the score for the N6, the design of the sampling pump will need to be improved upon, especially for those sampling protocols that require a lot of mobility (i.e., climbing up stairs). It was surprising that the SAS received a score similar to the N6 for portability, but is partly due to a lower score for a low battery charge lifetime.

4.6.2.4.Noise

Noise emissions by sampling instruments may be disruptive and annoying, especially in occupied spaces. However, since noise received the lowest weighting across all the characteristics, a lower score in this characteristic may not necessarily change the overall score. The SAS received the highest score for noise, making it an attractive choice for environments where noise can pose as a problem. The N6 and the AOC scored lowest for noise, and improvements in the design of both these instruments would be in the use of quieter sampling pumps.

4.6.2.5.Sampling Time

The N6 received the highest score for sampling time, mainly because of its flexibility in the range of sampling volumes. The presence of pre-set sampling times were ranked high in the ease of use section, but were ranked low for this section, and therefore the scores assigned for this section may have cancelled out the scores for the ease of use section.

4.6.2.6.Historical and Current Use in Research/Industry (Industrial Use)

The N6 received the highest score for both historical use in the published literature, and for current use by the expert panel. However, the AOC received a similar score to the N6 for current use by the panel, but scored the lowest for historical use. This suggests that the use of the AOC is widespread, despite the fact that it has not been studied or used extensively in the past.

4.6.3. Scoring Analysis - Overview

The highest rank went to the N6 and AOC, and the RCS and SAS were a second group. However, the N6 and AOC received a similar overall score for scoring high in different characteristics.

The N6 is still ranked high among the viable samplers, even though it was low scoring in the ease of use and portability sections, which were weighed highly by the panel. Its cost, collection efficiency, and historical and current use in the field overcame the lack of portability and ease of use.

The AOC, even though it hasn't been on the market for long, was also ranked high, mainly because of its high yield for spores, but also for its ease of use.

The RCS and SAS models evaluated in this study are older than the models that are currently on the market. The RCS Plus model has a higher flow rate of 50 L/min, though this would not change its score drastically. The SAS-90 with the large faceplate is not used often. P. Pratt stated that only approximately 5% of total customers have this type of faceplate (personal communication, August 1, 2002), and this is reflected in low score given for the history section of this comparison. A newer model of the SAS, the SAS-100, uses the smaller faceplate, has the option of choosing a faceplate with 0.75 mm holes, which would improve the cut-off diameter, and also samples at an even higher flow rate of 100 L/min. These improvements may improve the scoring of the SAS, but can only be fully assessed when its performance characteristics are researched. Therefore, the rankings given to these older models may reflect the scores that would be assigned to the newer models.

For the viable samplers, though the RCS and SAS are attractive alternatives, their performance as a methodology to enumerate airborne fungi is not comparable to the N6, as reflected in this comparison. The AOC, a microscopic method, is in widespread use, even though there are not many studies looking at its performance. The AOC is attractive because it is very easy to use, portable, and has the ability to detect higher concentrations of fungal spores than the N6 would. However, it cannot be said that the AOC and the N6 give the same information, and therefore the choice between these two samplers is defined by the objectives of the air sampling. Though the AOC may be able to detect more fungal spores than the N6, whether that is really an advantage or not has yet to be determined. Currently, the ability to detect more fungal spores is considered an advantage (Macher, 2000).

4.7. Study Overview of Strengths and Limitations

4.7.1. Strengths of Study

Previously published field studies of bioaerosol samplers have been small in terms of both the numbers of samples and sites (see Table 4.7), except for the one by Tsai et al., (1999). This study is unique because of its large sample size (74 sites x 4 locations/site x 2 samples/location = 592 samples/instrument), its wide variety of test environments (60 different buildings across greater Vancouver of different sizes and types), and its instrument comparison (no comparisons with these four instruments together have been done before). The variety in field conditions allows for these samplers to be challenged under many different environmental conditions.

Laboratory studies test samplers under controlled conditions. These conditions are rarely reproduced in the field, and thus, results from field studies, because of the varied particle size distributions, localized sources and low indoor air velocities, can provide additional information on sampler performance that may not agree with predictions based on laboratory experiments (Macher, 1997).

Table 4.7. Previous Relevant Field Studies

Reference	Environment	Agent	Instruments of interest	Sample Pairs
Bellin & Schillinger, 2001	4 buildings, University	Viable Fungi	N6 SAS-180	55
Tsai et al., 1999	Various buildings across US	Viable Fungi Total Fungal Matter	N6 AOC	1,431
Mehta et al., 1996	1 building (5 locations)	Viable Fungi	AND-II[1] SAS-90 RCS Plus	60
Verhoeff et al., 1990	11 houses in winter	Viable Fungi	N6 SAS-180 RCS	9
Smid et al., 1989	4 buildings (7 occupational environments)	Viable Fungi	N6 SAS-180 RCS	10
Mehta et al., 2000	1 building (5 locations)	Viable Bacteria	AND-II SAS-90 RCS Plus	60

[1]AND-II = Andersen Two-Stage Sampler

Previous field studies have not used regression techniques to evaluate the relationships between samplers. This could be due to the low numbers of samples that are typical in a field comparison of this nature. The calibration curves presented in this study may be used to estimate the general concentration between samplers, provided that all restrictions are met.

The comparison of the samplers based on a combination of performance and other sampler characteristics, using the opinions of an expert panel to guide the weighting, have not been published previously. The methodology of this ranking can serve as a template to future comparisons, or the results of this comparison can be used to score the samplers based on different weighting schemes to meet the needs of other users.

4.7.2. Limitations of study

Field studies are not able to control for environmental factors that may influence sampler performance. This makes it difficult to determine what influence they may have on the results of each sampler. Previous studies have shown environmental factors, such as relative humidity, to have an influence on the clumping of fungal spores (Reponen et al., 1996; Madelin & Johnson, 1992). Relative humidity and temperature measurements were made at each sampling location, but this data is not a part of this analysis and will be examined in the future. Other factors such as wind turbulence affect the inlet sampling efficiency for some samplers. Human activity, such as walking or vacuuming, has been found to increase the air concentration of fungi, which can affect how instruments perform (Buttner & Steztenbach, 1993). None of these factors were quantified and are only presented as possible sources of variation in sampler performance.

A sampling protocol typical for an office work environment was employed and the results of this study may not necessarily be applicable to other environments (such as agricultural sites) where characteristics, such as relative humidity, and temperature, may be drastically different from that of an office.

A randomized selection of buildings was not possible since the pool of buildings were not all available initially. Sampling dates were determined based on convenience of the occupants and compatibility with the schedule. Some offices were unoccupied at the time of sampling.

This study was conducted over one season (summer). A seasonal variation (Shelton et al., 2002; Lighthart & Mohr, 1994) and a diurnal variation (Lighthart & Mohr, 1994) in total fungal spores and viable fungal colonies have been documented, and may have added addition variation on the performances of the samplers depending on the time of day the sample was taken. The time of sampling varied between sites and scheduled based on convenience. This was not accounted for in the present analysis, and its effect on the instruments performance may need to be explored further.

The results from the field comparison show that there are many differences in performance characteristics between each sampler. These differences lead to varying results in exposure assessment, making direct comparisons virtually impossible. It is crucial that a standard methodology be defined prior to the definition of a guideline or exposure limit since the concentration is highly dependent on the methodology employed. This study was not designed to determine specifically what causes these differences in performance, but instead it is an attempt to determine the magnitudes of these differences and make some inferences about why these differences exist.

The questionnaire was distributed to a small panel and may not have captured the range of opinions among experts. No attempt at randomization was made during the process. 60% of the questionnaires were delivered over the phone and 40% were filled out directly by the respondent and differences in interpretation of the questions may have resulted. The characteristics evaluated were pre-determined by the author based on field experience. There may be missing elements that may be considered important by other experts. The sub-categories for each characteristic were also pre-determined by field experience, and may not have captured the opinions of the panel, who were only asked to rank each characteristic, and not what it was about each characteristic that was important. Also, sub-categories were kept small to simplify the scoring process and do not necessarily provide a comprehensive assessment of the characteristic.

The performance characteristics were ranked based on the results of this study. This study may not necessarily reflect all the possible settings in which these samplers would be used, and therefore the results may not be applicable to every situation. Only two journals were searched for published literature on the instruments.

Chapter 5. Conclusions and Recommendations

5.1. General Conclusions

The results of this study indicate that total fungal concentration data are dependent on the sampling methodology used for assessment. This should be taken into consideration because it introduces additional variability into exposure assessment studies. Therefore, data from one method cannot be directly compared to the results of another method. A decision on standardized methodology must be made in order to move onto the development of an exposure guideline. This guideline may need to include one for culturable and total fungal spores.

Standardized methodology must include the following:

- Sampler
- Sampling medium
- Sampling duration
- Sampler placement (elevation to a specified height)
- Sampling locations (for indoor and outdoor)
- Time of day for sampling
- Incubation period (culture methods)
- Staining (microscopic methods)
- Microscope magnification (microscopic methods only)
- Counting rules (especially for microscopic methods)

The choice of a standard method should reflect the study hypothesis.

The calibration curves developed in this study may be used, provided that the restrictions are met, to relate the information of a non-standard sampler to the standard (provided that the standard methodology is one of the samplers that were studied).

The methodology used to compare samplers based on a combination of performance and other sampler characteristics provides a framework for those who may be interested in comparing other samplers in a similar manner. This may also be useful to manufacturers who may be interested in seeing what aspects of their product stands out or needs improvement.

5.2. General Comments and Recommendations for Each Sampler

5.2.1. RCS

The RCS should not be used to make quantitative assessments because of the inability to calibrate its flow rate (affecting results for overall yield and detection limits). The instrument can be used to detect the presence of microorganisms, provided that they are within the size range that the RCS can detect. If used for quantitative assessments, for environments with expected high loads (such as outdoor samples), the 2-minute sampling period should be chosen to minimize overloaded samples. The use of 280 L/min instead

of 40 L/min may need to be used since calibration of the RCS does guarantee a total sampling flow rate of 280 L/min. Alternatively, one can use the RCS Plus model, in which calibration to a total sampling airflow of 50 L/min is possible.

5.2.2. N6

The N6, as found in the ranking, is still considered to be a 'better' method relative to the other three samplers in this study, because of its long history and performance characteristics. The most unattractive feature of the N6 is its lack of portability. Despite the emphasis placed on portability, the N6 was still the highest ranked instrument. Better performance characteristics and widespread use historically and currently helped balance out the lack of portability. Future work should investigate increasing the portability and reducing the noise emission of the N6 sampling pump. Though the N6 has been used as a replacement for the Andersen Six Stage, it does not have the ability to separate particles based on aerodynamic diameter (important in the study of health effects). However, in non-problem office environments, where the airborne fungal load is expected to be low, size-selection is not required, and the N6 can be used.

5.2.3. SAS

The SAS is more likely to report lower values compared to the other methods and this should be considered when using this sampler. A high proportion of the samples in this study were below the LOD, suggesting that for 'clean' environments such as mechanically ventilated offices, a higher volume of air (200 L) should be collected. Despite the lower yield, the SAS had the highest correlation with the N6 and use of the regression model for the N6-SAS (r^2=0.74) may be used to estimate concentrations that would have been produced with an N6 if it meets all the restrictions. The manufacturer of the SAS should try to improve on the collection efficiency of this instrument, especially in its yield.

5.2.4. AOC

The AOC was also ranked highly in this study due to its ease of use, portability, and high yield for fungal spores. However, its methodology is very different from the other samplers, and therefore the results of this study may not necessarily reflect its utility as a microscopic method. More research should be done on the performance characteristics of the AOC and its comparison to other microscopic methods. More work should also be done on standardizing a counting method for the AOC.

5.3. Final Conclusions and Recommendations

Each of the samplers assessed in this study have different advantages and disadvantages, and therefore, the definition of the 'best' sampler of the four is difficult. However, a comparison was conducted in this study using a combination of qualitative and performance characteristics. The results of the comparison indicate the N6 and the AOC to be higher ranked and the RCS and SAS to be ranked lower. Differentiation between

the N6 and AOC is not possible since these two samplers use different methodologies for analysis and are mutually complementary and exclusive because of this. However, with respect to culture-based methods, the N6 has still been found to be the better of the three methods evaluated in this study. The models of the RCS and SAS used in this study are old and will probably become obsolete as the improved models replace them. The use of the AOC as an enumeration method for fungi is promising, but further work needs to be done on characterizing its sampling and performance characteristics.

5.4. Future Research

Environmental measurements, such as temperature and humidity, should be examined to determine if they posed any effect on the efficiencies of these samplers. It has been shown that humidity may affect the agglomeration of spores.

The speciation data should be examined to determine whether it generally supports the published literature on cut-off diameters for these instruments. It would be expected that the N6 would be able to detect the smaller spores, while the RCS would be able to detect the larger spores better. Also, the effect of mechanical ventilation on overall yield may be examined.

Though portability and ease of use are important, the development of new methodologies addressing these issues should not result in compromising the performance characteristics of the sampler. The SAS and RCS are marketed as portable samplers, however, despite their ease of use and portability, they still ranked low in the comparison conducted in this comparison, mainly due to poor performance characteristics. Manufacturers should keep this in mind when improving upon or designing new bioaerosol samplers. The manufacturers of the SAS and the RCS should improve the particle collection efficiencies of their respective samplers. The manufacturers of the SAS may also want to reduce the sampling flow rate of this instrument because of the possibility that this may reduce the viability of collected fungal spores. The new model of the SAS has an even higher flow rate, and study is required to determine its collection efficiency.

Despite the lack of published literature, the AOC was ranked similarly to the N6, and is in widespread use. Very little is known on its collection efficiency and should be further researched.

References

Aerotech Laboratories, Inc. (2002). Aerotech Laboratories – Services. Retrieved August 2, 2002, from http://www.aerotechlabs.com/serv/serv.asp.

American Conference of Governmental Industrial Hygienists—Bioaerosols Committee. (1999). *Bioaerosols: Assessment and control.* Macher, JM (ed). Cincinnati, OH: ACGIH.

Biotest HYCON. (1997). *Standard RCS Air Sampler: Operating Manual.* Germany: Biotest.

International Organization for Standardization. (1993). *Acoustics – Noise emitted by machinery and equipment – Measurement of emission sound pressure levels at the workstation and at other specified positions – Method requiring environmental corrections (Draft).* International Organization for Standardization.

National Institute of Occupational Safety and Health. (1994). NIOSH Method 7400, Asbestos & Other Fibres using PCM, In Cassinelli, M.E. & O'Connor, P.F., Eds, *NIOSH Manual of Analytical Methods (NMAM®), 4th ed.*, (DHHS Publication 94-113).

National Institute of Occupational Safety and Health. (1998). NIOSH Method 0800, Bioaerosol sampling (Indoor air), In Cassinelli, M.E. & O'Connor, P.F., Eds, *NIOSH Manual of Analytical Methods (NMAM®), 4th ed.*, (DHHS Publication 94-113).

New York City Department of Health & Mental Hygiene. (2002). Guidelines on assessment and remediation of fungi in indoor environments. Retrieved August 2, 2002, from http://www.ci.nyc.ny.us/html/doh/html/epi/moldrpt1.html.

PBI International. (1998). *SAS Super 90 – Microbiological monitoring of the environment,* Milan, Italy: PBI International.

Province of British Columbia. (2001). The Building Corporation of British Columbia. Retrieved August 1, 2001, from http://www.bcbc.ca.

Sensidyne, Inc. (1998). Sensidyne Document No. F-PRO-3100 (REV D). *Gilian Aircon-2 High Volume Air Sampler: Operation & service manual.* Clearwater, Florida: Sensidyne, Inc.

Simon Fraser Health Region. (2002). Simon Fraser Health Region Website, Retrieved August 1, 2001, www.sfhr.com.

SKC, Inc. (2002). Air-o-cell sampling cassettes. Retrieved on August 1, 2002, from www.skcinc.com/prod/aoc.html.

Thermo Andersen. (2002). N6 Single Stage Impactor. Retrieved on July 29, 2002, from http://www.anderseninstruments.com/N6_Single_stage.htm.

University of British Columbia. (2002). The University of British Columbia Website. Retrieved August 1, 2001, from http://www.ubc.ca.

Vancouver International Airport Authority. (2001). *Vancouver Airport Authority – Who we are*. Retrieved August 1, 2001, from http://www.yvr.ca/authority/whoweare.index.asp.

Aizenberg, V., Reponen, Y., Grinshpun, S.A., & Willeke, K.. (2000). Performance of Air-o-Cell, Burkard, and Button samplers for total enumeration of airborne spores. *American Industrial Hygiene Association Journal*, Vol 61, p855-864.

Andersen, A.A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. *Journal of Bacteriology*, Vol 76, pp. 471-484.

Andriessen, J.W., Brunekreef, B., & Roemer, W. (1998). Home dampness and respiratory health status in European children. *Clinical and Experimental Allergy*, Vol 28, pp. 1191-1200.

Bartlett, K.H., Lee, K.S., Hsieh, J., Brauer, M., Black, W., Stephens, G., & Teschke, K. Sampling efficiencies of three bioaerosol samplers for culturable fungi under field conditions. *Epidemiology*, Vol 13, Supplement, pp. S207.

Bellin, P., & Schillinger, J.. (2001). Comparison of field performances of the Andersen N6 Single Stage and the SAS sampler for airborne fungal propagules. *Indoor Air*, Vol 11, pp. 65-68.

Blomquist, G., Palmgren, U., & Strom, G. (1984).Sampling og high concentrations of fungi. *Scandinavian Journal of Work, Environment, and Health*, Vol 10, pp. 109-113.

Blomquist, G., Palmgren, U., & Strom, G. (1984). Improved techniques for sampling airborne fungal particles in highly contaminated environments. *Scandinavian Journal of Work, Environment, and Health*, Vol 10, pp. 253-258.

Brachman, P.S., Ehrlich, R., Eichenwald, H.F., Gabellia, V.J., Kethley, T.W., Madin, S.H., Maltman, J.R., Middlebrook, G., Morton, J.D., Silver, I.H., & Wolfe, E.K. (1964). Standard sampler for assay of airborne microorganisms. *Science*, Vol 144, pp. 1295.

Brimblecombe, P., Blades, N., Camuffo, D., Sturaro, G., Valentino, A., Gysels, K., van Grieken, R., Busse, H.-J., Kim, O., Ulrych, U., & Wieser, M. The indoor environment of a modern museum building, the Sainsbury Centre for Visual Arts, UK. *Indoor Air*, Vol 9, pp. 146-164.

Brunekreef, B., Dockery, D.W., Speizer, F.E., Ware, J.H., Spengler, J.D., & Ferris, B.G. (1989). Home dampness and respiratory morbidity in children. *American Review of Respiratory Disease*, Vol 140, pp. 1363-1367.

Burge, H.A., & Solomon, WR. (1987). Sampling and analysis of biological aerosols. *Atmospheric Environment*, Vol 21 (2), pp. 451-456.

Burge, H.A. (1995). Aerobiology of the indoor environment. *Occupational Medicine: State of the Art Reviews—*, Vol 10, pp. 27-40.

Burge, H.A., & Otten, J.A. (1999). Fungi. In Macher, J.M. (Ed.), *Bioaerosols: Assessment and Control* (pp.19-1-19-13). Cincinnati, OH: ACGIH.

Burge, H.A., Macher, J.M., Milton, D.K., & Ammann, H.A. (1999). Data Evaluation. In Macher, J.M. (Ed.), *Bioaerosols: Assessment and control* (pp. 14-1-14-11). Cincinnati, OH: ACGIH.

Burge, H.A., Pierson, D.L., Groves, T.O., Strawn, K.F., & Mishra, S.K. (2000). Dynamics od airborne fungal populations in a large office building. *Current Microbiology*, Vol 40, pp. 10-16.

Burge, P., Boise, J.R., Rutherford, A., & Solomon, W.R. (1977). Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. *Mycopathologia*, Vol 61, pp. 27-33.

Burton, P., Gurrin, L., & Sly, P. (1998). Extending the simple linear regression model to account for correlated responses: An introduction to generalized estimating equations and multi-level mixed modeling. *Statistics in Medicine*, Vol 17, pp. 1261-1291.

Buttner, M.P., & Stetzenbach, L.D. (1993). Monitoring airborne fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling. *Applied and Environmental Microbiology*, Vol 59, pp. 219-226.

Chang, C.W., Hwang, Y.H., Grinshpun, S.A., Macher, J.M., & Willeke, K. (1994). Evaluation of counting error due to colony masking in bioaerosol sampling. *Applied and Environmental Microbiology*, Vol 60, pp. 3732-3738.

Chang, C.W., Grinshpun, S.A., Willeke, K., Macher, J.M., Donnelly, J., Clark, S., & Juozaitis, A. (1995). Factors affecting microbiological colony count accuracy for bioaerosol sampling and analysis. *American Industrial Hygiene Association Journal*, Vol 56, pp. 979-991.

Clark, S., Lach, V., & Lidwell, O.M. (1981). The performance of the biotest RCS centrifugal air sampler. *Journal of Hospital Infection*, Vol 2, pp. 181-186.

Crook, B. (1996). Review: Methods of monitoring for process micro-organisms in biotechnology. *Annals of Occupational Hygiene*, Vol 40, pp. 245-260.

Dales, R.E., Burnett, R., & Zwanenburg, H. (1990). Adverse health effects among adults exposed to home dampness and molds. *American Review of Respiratory Diseases*, Vol 143, pp. 505-509.

Dales, R.E., Zwanenburg, H., Burnett, R., & Franklin, C.A. (1991). Respiratory health effects of home dampness and molds among Canadian children. *American Journal of Epidemiology*, Vol 134, pp. 196-203.

Dawson, MW, Scott, JG, and Cox, LM. The medical and epidemiologic effects on workers of the levels of thermoactinomyces spp. Spores present in Australian raw sugar mills. *American Industrial Hygiene Association Journal*, Vol 57, pp. 1002-1012.

De Koster, J.A., & Thorne, P.S. (1995). Bioaerosol concentrations in noncompaint, complaint, and intervention homes in the Midwest. *American Industrial Hygiene Association Journal*, Vol 56, pp. 573-580.

Dillon, H.K., Heinsohn P.A., & Miller J.D., eds. (1996). *Field guide for the determination of biological contaminants in environmental samples*. Farifax, VA: American Industrial Hygiene Association.

Dillon, K.H., Miller, J.D., Sorenson, W.G., Douwes, J., & Jacobs, R. (1999). Review of methods applicable to the assessment of mold exposure to children. *Environmental Health Perspectives*, Vol 107, Supplement 3, pp. 473-480.

Duchaine, C., Mériaux, A., Brochu, G., and Cormier, Y. (1999). Airborne microflora in Quebec dairy farms: lack of effect of bacterial hay preservatives. *American Industrial Hygiene Association Journal*, Vol 60, pp. 89-95.

Duchaine, C., Mériaux, A., Thorne, P.S., & Cormier, Y. (2000). Assessment of particulates and bioaerosols in Eastern Canadian sawmills. *American Industrial Hygiene Association Journal*, Vol 61, pp. 727-732.

Eduard, W., Sandven, P., & Levy, F. (1993). Serum IgG antibodies to mold spores in two Norwegian sawmill populations: Relationship to respiratory and other work-related symptoms. *American Journal of Industrial Medicine*, Vol 24, pp. 207-222.

Eduard, W., & Heederik, D. (1998). Methods for quantitative assessment of airborne levels of noninfections microorganisms in highly contaminated work environments. *American Industrial Hygiene Association Journal*, Vol 59, pp. 113-127.

Fisk, W.J., Mendell, M.J., Daisey, J.M., Faulkner, D., Hodgson, A.T., Nematollahi, M., & Macher, J.M. (1993). Phase 1 of the California Healthy Building study: a summary, *Indoor Air*, Vol 3, pp. 246-254

Flannigan, B. (1997). Air sampling for fungi in indoor environments. *Journal of Aerosol Science*, Vol 28, pp. 381-392.

Folmsbee, M., Strevett, K., Stafford, K. & Evenson, C. (2000). The effect of sampling time on the total efficiency of the Andersen microbial sampler: a field study. *Journal of Aerosol Science*, Vol 31, pp. 263-271.

Griffiths, W.D., & DeCosemo, G.A.L. (1994). The assessment of bioaerosols: a critical review. *Journal of Aerosol Science*, Vol 25, pp. 1425-1458.

Griffiths, W.D., & Stewart, I.W. (1999). Performance of bioaerosol samplers used by the UK biotechnology industry. *Journal of Aerosol Science*, Vol 30, pp. 1029-1040.

Grinshpun, S.A., Chang, C.W., Nevalainen, A., & Willeke, K. (1994). Inlet characteristics of bioaerosol samplers. *Journal of Aerosol Science*, Vol 25, pp. 1503-1522.

Grinshpun, S.A., Willeke, K., Ulevicius, V., Donnelly, J., Lin, X. & Mainelis, G. (1996). Collection of airborne microorganisms: Advantages and disadvantages of different methods. *Journal of Aerosol Science*, Vol 27, Suppl 1, pp. S247-S248.

Henningson, E.W., & Ahlberg, S. (1994). Evaluation of microbiological aerosol samplers: a review. *Journal of Aerosol Science*, Vol 25, pp. 1459-192.

Hinds, W.C. (1999). *Aerosol Technology: Properties, behavior, and measurement of airborne particles*. New York: John Wiley & Sons, Inc.

Hocking, A.D., & Pitt, J.I. (1980). Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. *Applied and Environmental Microbiology*, Vol 39, pp. 488-492.

Hyvärinen, A., Reponen, T., Husman, T., Ruuskanen, J., & Nevalainen, A. (1993). Characterizing Mold Problem buildings – Concentrations and flora of viable fungi, *Indoor Air*, Vol 3, pp. 337-343.

Jensen, P.A., Todd, W.F., Davis, G.N., Scarpino, P.V. (1992). Evaluation of eight bioaerosol samplers challenged with aerosols of free bacteria. *American Industrial Hygiene Association Journal*, Vol 53, pp. 660-667.

Jensen, P.A. (1995). Evaluation of standard and modified sampling heads for the international PBI Surface Air System bioaerosol samplers. *American Industrial Hygiene Association Journal*, Vol 56, pp.272-279.

Jones, W., Morring, K., Morey, P., & Sorenson, W. (1985). Evaluation of the Andersen viable impactor for single stage sampling. *American Industrial Hygiene Association Journal*, Vol 46, pp.294-298.

Kleinbaum, D.G., Kupper, L.L., & Muller, K.E. (1988) *Applied regression analysis and other multivariable methods, second edition*. Boston, MA: PWS-KENT Publishing company.

Koskinen, O.M., Husman, T.M., Meklin, T.M., & Nevalainen, A.I. (1999). The relationship between moisture or mould observations in houses and the state of health of their occupants. *European Respiratory Journal*, Vol 14, pp. 1363-1367.

Krahmer, M., Fox, K., Fox, A., Saraf, A., & Larsson, L. Total and viable airborne bacterial load in two different agriculture environments using gas chromatography-tandem mass spectrometry and culture: a prototype study. *American Industrial Hygiene Association Journal*, Vol 59, pp. 524-531.

Lach, V. (1985). Performance of the surface air system air samplers. *Journal of Hospital Infection*, Vol 6, pp. 102-107.

Laitinen, S., Kangas, J., Liesivuori, J., Martikainen, P.J., Nevalainen, A., Sarantila, R., & Husman, K. Workers' exposure to airborne bacteria and endotoxins at industrial wastewater treatment plants. *American Industrial Hygiene Association Journal*, Vol 55, pp. 1055-1060.

Levy, J.I., Nishioka, Y., Gilbert, K., Cheng, C.H., & Burge, H.A. (1999). Variabilities in aerosolizing activities and airborne fungal concentrations in a bakery. *American Industrial Hygiene Association Journal*, Vol 60, pp. 317-325.

Lewis, F.A. (1995). Regulating Indoor Microbes: the OSHA proposed rules on indoor air quality. *International Conference on Fungi and bacteria in: Indoor Air Environments*, October 6, 1994, Saratoga Springs, N.Y. Eastern New York Occupational Health Programs, pp. 5-9.

Lighthart, B, & Mohr, A.J., eds. (1994). *Atmospheric microbial aerosols: Theory and applications*. New York: Chapman & Hall, Inc.

Luoma, M. & Batterman, S.A. (2000). Autocorrelation and variability of indoor air quality measurements. *American Industrial Hygiene Association Journal*, Vol 61, pp. 658-668.

Macher, J.M., & First, M.W. (1983). Reuter Centrifugal Air Sampler: measurement of effective air flow rate and collection efficiency. *Applied and Environmental Microbiology*, Vol 45, pp. 1960-1962.

Macher, J.M. (1989). Positive-hole correction of multiple-jet impactors for collecting viable microorganisms. *American Industrial Hygiene Association Journal*, Vol 50, p561-568.

Macher, J.M. (1997). Instrument performance criteria: Bioaerosol samplers. *Applied Occupational and Environmental Hygiene*, Vol 12, pp. 723-729.

Macher, J.M. (1997). Evaluation of bioaerosol sampler performance. *Applied Occupational and Environmental Hygiene*, Vol 12, pp. 730-736.

Macher, J.M. (1999). Data Analysis. In Macher, JM (ed), *Bioaerosols*, pp. 13-1-13-16. Cincinnati, OH: ACGIH

Malloch, D. (1998). *Moulds: Their isolation, cultivation, and identification*. Toronto: University of Toronto Press.

Marple, V.A., & Willeke, K. (1976). Impactor design. *Atmospheric Environment*, Vol 10, pp. 891-896.

McCullough, N.V., Brosseau, L.M., Vesley, D., & Vincent, J.H. (1998). Improved Methods for generating sampling and recovery of biological aerosols in filter challenge tests. *American Industrial Hygiene Association Journal*, Vol 59, pp. 234-241

Mehta, S.K., Mishra, S.K., & Pierson, D.L. (1996). Evaluation of three portable samplers for monitoring airborne fungi. *Applied and Environmental Microbiology*, Vol 62, pp. 1835-1838.

Mehta, S.K., Bell-Robinson, D.M., Groves, T.O., Steztenbach, L.D., & Pierson, D.L. (2000). Evaluation of portable air samplers for monitoring airborne culturable bacteria. *American Industrial Hygiene Association Journal*, Vol 61, pp. 850-854.

Miller, J.D. (1992). Fungi as contaminants in indoor air. *Atmospheric Environment*, Vol 26A, pp. 2163-2172.

Miller, J.D., Haisley, P.D., & Reinhardt, J.H. (2000). Air sampling results in relation to extent of fungal colonization of building materials in some water-damaged buildings. *Indoor Air*, Vol 10, pp. 146-151.

Morey, P.R. (1992). Microbiological events after a fire in a high-rise building. *Indoor Air*, Vol 3, No. 4, pp. 354-360

Nathanson, T. (1995). *Indoor air quality in office buildings: A technical guide*. Ottawa, Canada: Health Canada.

Neas, L.M., Dockery, D.W., Burge, H., Koutrakis, P., & Speizer, F.E. (1996). Fungus spores, air pollutants, and other determinants of peak respiratory flow rate in children. *American Journal of Epidemiology*, Vol 143, pp. 797-807.

Nevalainen, A., Pastuszka, J., Liebhaber, F., & Willeke, K. (1991). Performance of bioaerosol samplers: collection characteristics and sampler design considerations. *Atmospheric Environment*, Vol 26A, pp. 531-540.

Oxborrow, S., Fields, N.D., Puleo, J.R., & Herring, C.M. (1975). Quantitative relationship between airborne viable and total particles. *Health Laboratory Science*, Vol 12, pp. 47-51.

Parat, S., Fricker-Hidalgo, H., Perdrix, A., Bemer, D., Pelissier, N., & Grillot, R. (1996). Airborne fungal contamination in air-conditioning systems: Effect of filtering and humidifying devices. *American Industrial Hygiene Association Journal*, Vol 57, pp. 996-1001.

Pasanen, A.L. (2001). A review: Fungal exposure assessment in indoor environments. *Indoor Air*, Vol 11, pp. 87-98.

Peretz, C., Goren, A., Smid, T., and Kromhout, H. (2002). Application of mixed-effects models for exposure assessment. *Annals of Occupational Hygiene*, Vol 46, pp. 60-77.

Pirhonen, I., Nevalainen, A., Husman, T., & Pekkanen, J. (1996). Home dampness, molds, and their influence on respiratory infections and symptoms in adults in Finland. *European Respiratory Journal*, Vol 9, pp. 2618-2622.

Pitt, J.J, & Hocking, A.D. (1997). Methods for isolation, enumeration and identification. In *Fungi and food spoilage*, pp. 28-53. Cambridge: Blackie Academic and Professional.

Pope, A.M., Patterson, R., & Burge, H.A., eds. (1993). *Indoor allergens: Assessing and controlling adverse health effects*. Washington, D.C.: National Academy Press.

Rao, C.Y., Burge, H.A., & Chang, J.C.S. (1996). Review of quantitative standards and guidelines for fungi in indoor air. *Journal of Air and Waste Management Association*, Vol 46, pp. 899-908.

Rautiala, S., Reponen, T., Hyvarinen, A., Nevalainen, A., Husman, T., Vehvilainen, A., & Kalliokoski. (1996). Exposure to airborne microbes during the repair of moldy buildings. *American Industrial Hygiene Association Journal*, Vol 57, pp. 279-284.

Ren, P., Jankun, T.M., Belanger, K., Bracken, M.B., Leaderer, B.P. (2001). The relation between fungal propagules in indoor air and home characteristics. *Allergy*, Vol 56, pp. 419-424.

Reynolds, S.J., Streifel, A.J., & McJilton, C.E. (1990). Elevated airborne concentrations of fungi in residential and office environments. *American Industrial Hygiene Association Journal*, Vol 51, pp. 601-604.

Rosas, I., McCartney, H.A., Payne, R.W., Calderon, C., Lacey, J., Chapela, R., & Ruiz-Velazco, S. (1998). Analysis of the relationships between environmental factors (aeroallergens, air pollution, and weather) and asthma emergency admissions to a hospital in Mexico City. *Allergy*, Vol 53, pp. 394-401.

Schafer, M.P., Fernback, J.E., & Jensen, P.A. (1998). Sampling and analytical method development for qualitative assessment of airborne mycobacterial species of the Mycobacterium Tuberculosis complex. *American Industrial Hygiene Association Journal*, Vol 59, pp. 540-546.

Schillinger, J.E., Vu, T., & Bellin, P. (1999). Airborne fungi and bacteria: Background levels in office buildings. *Journal of Environmental Health*, Vol 62, pp. 9-15.

Seitz, T.A. (1989). NIOSH Indoor Air Quality Investigations: 1971-1988. *Proceedings of the Indoor Air Quality International Symposium*, Cincinnati, OH: National Institute of Occupational Health and Safety.

Shelton, B.G., Kirkland, K.H., Flanders, W.D., & Morris, G.K. (2002). Profiles of airborne fungi in buildings and outdoor environments in the United States. *Applied and Environmental Microbiology*, Vol 68, pp. 1743-1753.

Shum, M. (2002). An overview of the health effects due to mold exposure. *Proceedings: Indoor Air 2002*, July 3, 2002, Monterey, CA, pp. 17-22.

Sigler, L., Abbott, S.P., & Gauvreau, H. Assessment of worker exposure to airborne molds in honeybee overwintering facilities. *American Industrial Hygiene Association Journal*, Vol 57, pp. 484-490.

Smid, T., Schokkin, E., Boleij, J.S.M., & Heederick D. (1989). Enumeration of viable fungi in occupational environments: a comparison of samplers and media. *American Industrial Hygiene Association Journal*, Vol 50, pp. 235-239.

Smith, E.G. (2000). *Sampling and identifying allergenic pollens and molds*. San Antonio, TX: Blewstone Press.

Sorenson, W.G. (1999). Fungal spores: Hazardous to health? *Environmental Health Perspectives*. Vol 107, Supplement 3, pp. 469-471.

Spengler, J.D., & Sexton, K. (1983). Indoor air pollution: a public health perspective. *Science*, Vol 221, pp. 9-17.

Spicer, R.C., & Gagloff, H.J. (2000). Limitations in application of Spearman's rank correlation to bioaerosol sampling data. *American Industrial Hygiene Association Journal*, Vol 61, pp. 362-366.

Stewart, S.L., Grinshpun, S.A., Willeke, K., Terzieva, S., Ulevicius, V., & Donnelly, J. (1995). Effect of impact stress on microbial recovery on an agar surface. *Applied and Environmental Microbiology*, Vol 61, pp. 1232-1239.

Thompson, M.W., Donnelly, J., Grinshpun, S.A., Juozaitis, A., & Willeke, K. (1994). Method and test system for evaluation of bioaerool samplers. *Journal of Aerosol Science*, Vol 25, pp. 1579-1593.

Trunov, M., Trakumas, S., Willeke, K., Grinshpun, S.A., Reponen, T. (2001). Collection of bioaerosol particles by impaction: Effect of fungal spore agglomeration and bounce. *Aerosol Science and Technology*, Vol 35, pp. 617-624.

Tsai, S.M., Yang, C.S., Moffett, P., & Puccetti, A. (1999). A comparative study of collection efficiency of airborne fungal matter using Andersen Single Stage N6 impactor and the Air-o-Cell cassette. *Proceedings for Indoor Air '99*, Vol 2, pp. 776-781.

Van Netten, C., Brands, R., & Dill, B. (1997). Investigation and remediation of diesel converted trolley buses associated with extensive fungal growth and health complaints. *American Industrial Hygiene Association Journal*, Vol 58, pp. 726-731.

Waldman, J.M., Macher, J.M., McNeel, S., Wersinger, E., & Gilliss, D. (2002). How public agencies address indoor mold hazards in California. *Proceedings: Indoor Air 2002*, July 3, 2002, Monterey, CA, pp. 38-43.

Willeke, K., & Macher, J.M. (1999). Air Sampling in Macher, J.M. (Ed.), *Bioaerosols: Assessment and Control* (pp.11-1-11-25). Cincinnati, OH: ACGIH

Verhoeff, A.P., van Wijnen, J.H., Boleij, J.S.M., Brunekreef, B., van Reenen-Hoekstra, E.S., & Samson, R.A. (1990). Enumeration and identification of airborne viable mould propagules in houses. *Allergy*, Vol 45, pp. 275-284.

Appendix I:
Positive-hole Correction Tables for the N6 (Andersen, 1958) and SAS (PBI International, 1998)

Positive hole correction table: Positive hole counts (r) and corresponding corrected particle counts (P) (Andersen, 1958)

r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
1	1	46	49	91	103	136	166	181	241	226	333	271	453	316	624	361	931
2	2	47	50	92	105	137	168	182	243	227	335	272	456	317	629	362	942
3	3	48	51	93	106	138	169	183	245	228	338	273	459	318	634	363	952
4	4	49	52	94	107	139	171	184	246	229	340	274	462	319	639	364	963
5	5	50	53	95	108	140	172	185	248	230	342	275	465	320	644	365	974
6	6	51	55	96	110	141	174	186	250	231	345	276	468	321	649	366	986
7	7	52	56	97	111	142	175	187	252	232	347	277	472	322	654	367	998
8	8	53	57	98	112	143	177	188	254	233	349	278	475	323	659	368	1010
9	9	54	58	99	114	144	179	189	256	234	352	279	478	324	664	369	1023
10	10	55	59	100	115	145	180	190	258	235	354	280	482	325	670	370	1036
11	11	56	60	101	116	146	182	191	260	236	357	281	485	326	675	371	1050
12	12	57	61	102	118	147	183	192	262	237	359	282	488	327	680	372	1064
13	13	58	63	103	119	148	185	193	263	238	362	283	492	328	686	373	1078
14	14	59	64	104	120	149	186	194	265	239	364	284	495	329	692	374	1093
15	15	60	65	105	122	150	188	195	267	240	367	285	499	330	697	375	1109
16	16	61	66	106	123	151	190	196	269	241	369	286	502	331	703	376	1125
17	17	62	67	107	125	152	191	197	271	242	372	287	506	332	709	377	1142
18	18	63	69	108	126	153	193	198	273	243	374	288	508	333	715	378	1160
19	19	64	70	109	127	154	194	199	275	244	377	289	513	334	721	379	1179
20	21	65	71	110	129	155	196	200	277	245	379	290	516	335	727	380	1198
21	22	66	72	111	130	156	198	201	279	246	382	291	520	336	733	381	1219
22	23	67	73	112	131	157	199	202	281	247	384	292	524	337	739	382	1241
23	24	68	75	113	133	158	201	203	283	248	387	293	527	338	746	383	1263
24	25	69	76	114	134	159	203	204	285	249	390	294	531	339	752	384	1288
25	26	70	77	115	136	160	204	205	287	250	392	295	535	340	759	385	1314
26	27	71	78	116	137	161	206	206	289	251	395	296	539	341	766	386	1341
27	28	72	79	117	138	162	208	207	292	252	398	297	543	342	772	387	1371
28	29	73	81	118	140	163	209	208	295	253	400	298	547	343	779	388	1403
29	30	74	82	119	141	164	211	209	296	254	403	299	551	344	786	389	1438
30	31	75	83	120	143	165	213	210	298	255	406	300	555	345	793	390	1476
31	32	76	84	121	144	166	214	211	300	256	409	301	559	346	801	391	1518
32	33	77	86	122	146	167	216	212	302	257	411	302	563	347	808	392	1565
33	34	78	87	123	147	168	218	213	302	258	414	303	567	348	816	393	1619
34	36	79	88	124	148	169	220	214	306	259	417	304	571	349	824	394	1681
35	37	80	89	125	150	170	221	215	308	260	420	305	575	350	832	395	1754
36	38	81	91	126	151	171	223	216	311	261	423	306	579	351	840	396	1844
37	39	82	92	127	153	172	225	217	313	262	426	307	584	352	848	397	1961
38	40	83	93	128	154	173	227	218	315	263	429	308	588	353	857	398	2127
39	41	84	94	129	156	174	228	219	317	264	432	309	592	354	865	399	2427
40	42	85	96	130	157	175	230	220	319	265	434	310	597	355	874	400	*
41	43	86	97	131	159	176	232	221	322	266	437	311	601	356	883		
42	44	87	98	132	160	177	234	222	324	267	440	312	606	357	892		
43	45	88	99	133	162	178	236	223	326	268	443	313	610	358	902		
44	47	89	101	134	163	179	237	224	328	269	447	314	615	359	911		
45	48	90	102	135	165	180	239	225	331	270	450	315	620	360	921		

*Indicates quantitative limit of state (approximately 2628 particles) is exceeded

Correction Table to Adjust Colony Counts from a 487-hole Impactor using 84 mm Maxi-contact Plates (PBI International, 1998)

r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr
1	1	31	33	61	69	91	111	121	162	151	224	181	306	211	427	241	655
2	2	32	34	62	71	92	113	122	164	152	227	182	309	212	432	242	668
3	3	33	35	63	72	93	115	123	166	153	229	183	313	213	437	243	681
4	4	34	36	64	73	94	116	124	167	154	231	184	317	214	442	244	695
5	5	35	37	65	74	95	118	125	169	155	234	185	320	215	448	245	709
6	6	36	39	66	76	96	119	126	171	156	236	186	323	216	453	246	725
7	7	37	40	67	77	97	121	127	173	157	239	187	326	217	459	247	741
8	8	38	41	68	79	98	122	128	175	158	241	188	330	218	465	248	759
9	9	39	42	69	80	99	124	129	177	159	244	189	333	219	471	249	777
10	10	40	43	70	81	100	126	130	179	160	246	190	337	220	477	250	797
11	11	41	44	71	83	101	127	131	181	161	249	191	341	221	483	251	819
12	12	42	46	72	84	102	129	132	183	162	251	192	344	222	490	252	843
13	13	43	47	73	85	103	130	133	185	163	254	193	348	223	496	253	869
14	14	44	48	74	87	104	132	134	187	164	257	194	352	224	503	254	898
15	15	45	49	75	88	105	134	135	189	165	259	195	356	225	510	255	931
16	16	46	50	76	90	106	135	136	191	166	262	196	360	226	517	256	969
17	17	47	52	77	91	107	137	137	193	167	265	197	364	227	524	257	1012
18	19	48	53	78	92	108	139	138	195	168	268	198	368	228	532	258	1065
19	20	49	54	79	94	109	141	139	197	169	270	199	372	229	539	259	1130
20	21	50	55	80	95	110	142	140	200	170	273	200	376	230	542	260	1217
21	22	51	57	81	97	111	144	141	202	171	276	201	380	231	555		
22	23	52	58	82	98	112	146	142	204	172	279	202	384	232	564		
23	24	53	59	83	100	113	147	143	206	173	282	203	389	233	573		
24	25	54	60	84	101	114	149	144	208	174	285	204	393	234	582		
25	26	55	62	85	102	115	151	145	211	175	288	205	398	235	591		
26	27	56	63	86	104	116	153	146	213	176	291	206	402	236	601		
27	28	57	64	87	105	117	155	147	215	177	294	207	407	237	611		
28	30	58	65	88	107	118	156	148	217	178	297	208	412	238	621		
29	31	59	67	89	108	119	158	149	219	179	300	209	417	239	632		
30	32	60	68	90	110	120	160	150	220	180	303	210	422	240	643		

r = colony forming units counted

Pr = probable count

Appendix II:
Questionnaire for Sampler Comparison

Standardized Questionnaire – Qualitative Assessment of Bioaerosol Sampling

Telephone Interviews

People to Speak to:

- Bioaerosol Expert
- Equipment Manager
- Purchaser of sampling instruments (specifically for bioaerosol sampling)

Company Name:_____ Phone#:_____

Contact Info (name, position, #):_____

Questions:

1. How do the following factors affect your choice in a bioaerosol sampler? (1=not important, 2=somewhat important, and 3=very important)

a) Cost	1	2	3
b) Ease of Use	1	2	3
c) Portability (size/weight)	1	2	3
d) Noise	1	2	3
e) Sampling Time	1	2	3

2. Are there any other factors that you look for in a bioaerosol sampler?

_____

_____

_____

_____

3. What method do you currently employ for bioaerosol sampling?

_____

_____

_____

4. Why did you select this method?

_____

_____

_____

_____

5. Is your method viable/non-viable method? Why did you choose this type of method?

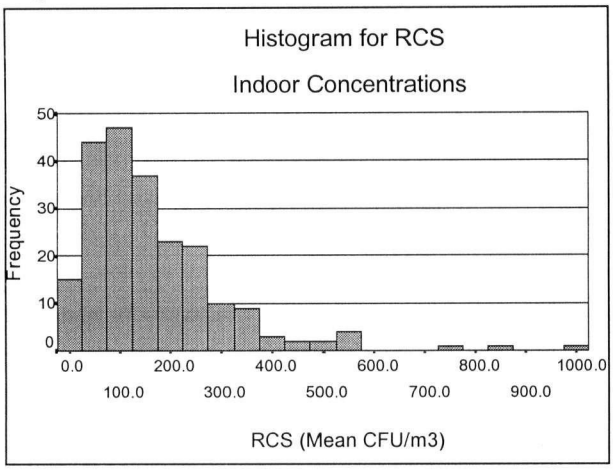
6. Do you have an analytical lab on site? If not, which lab do you employ for analysis?

7. Do you use any other methods for bioaerosol sampling (i.e. measure for surrogate markers such as beta-d-glucan or ergosterol, etc)?

Notes:

Appendix III:
Histograms of mean concentration data for untransformed and transformed data

Figure 3.3.1.



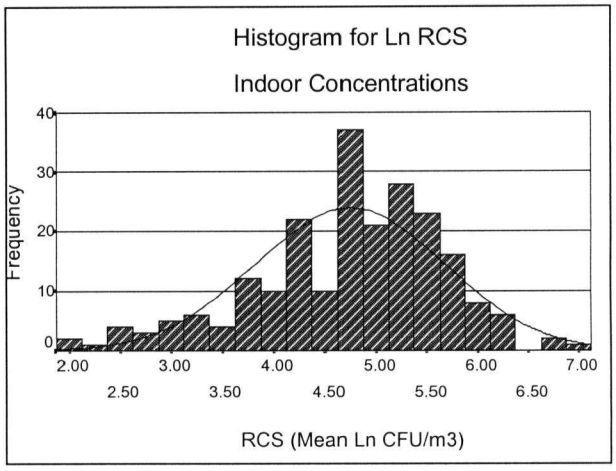
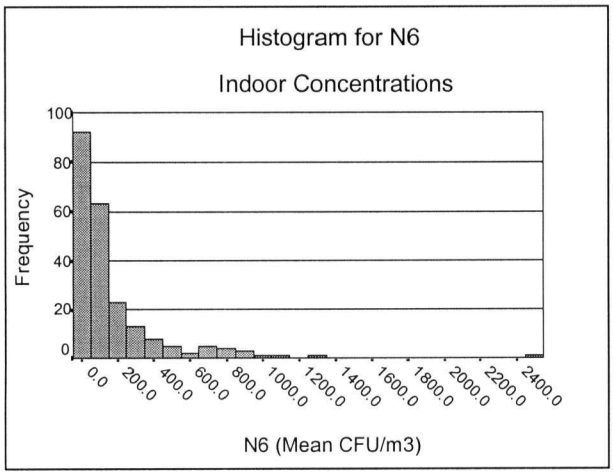


Figure 3.3.2.



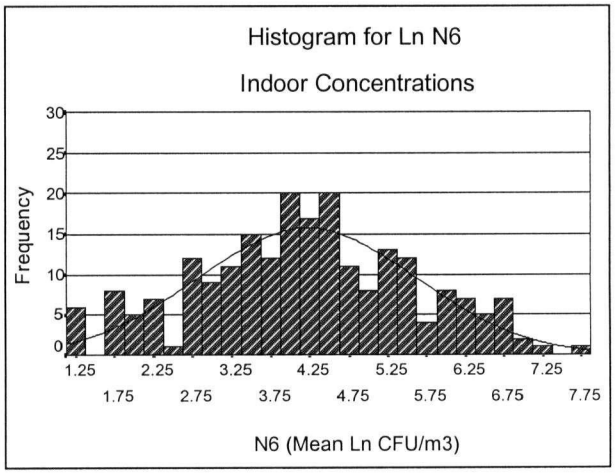
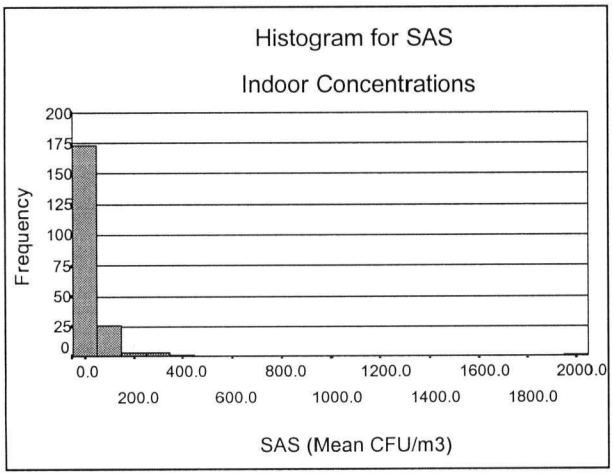


Figure 3.3.3.



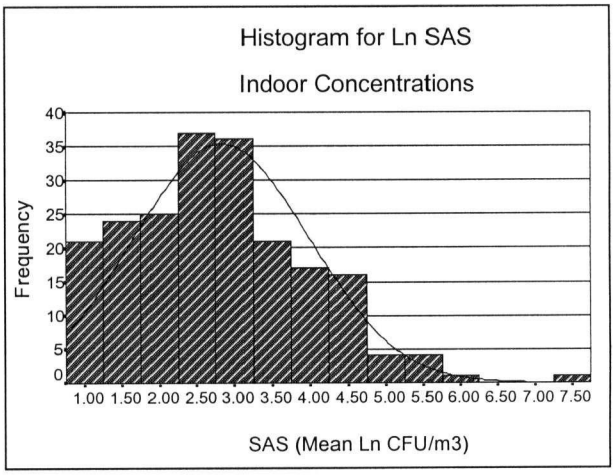
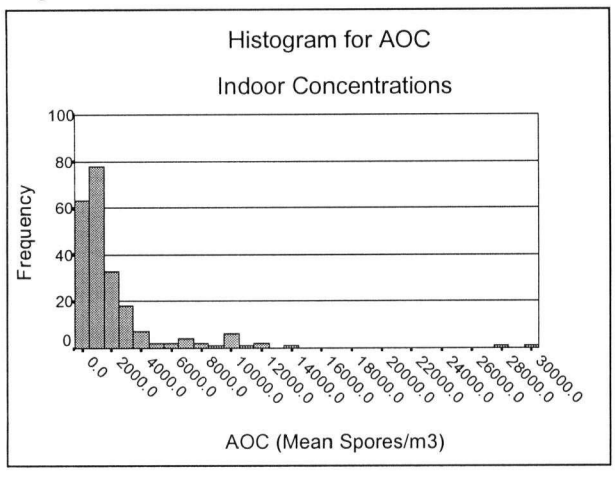


Figure 3.3.4.



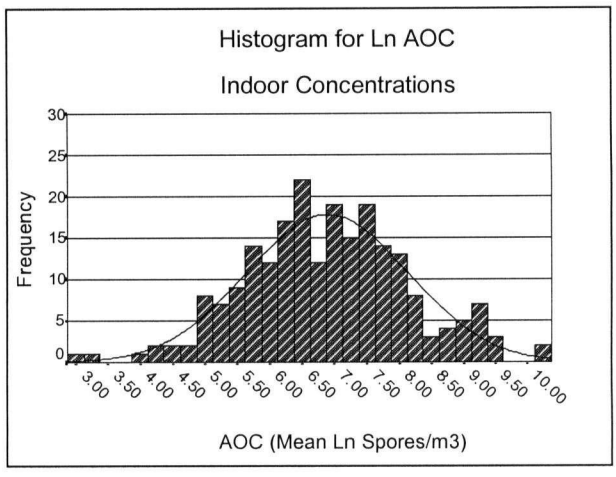
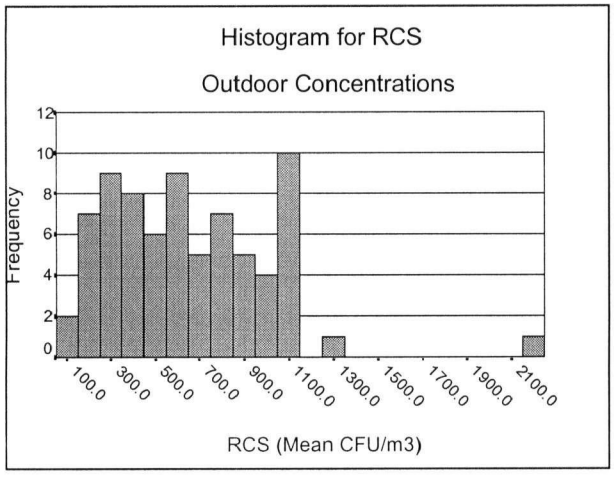


Figure 3.3.5.



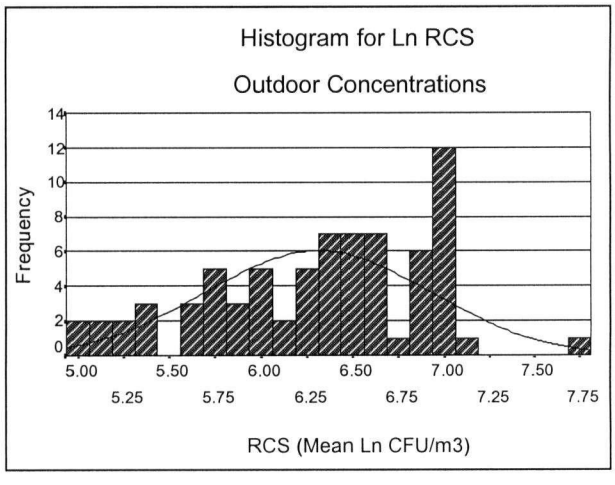
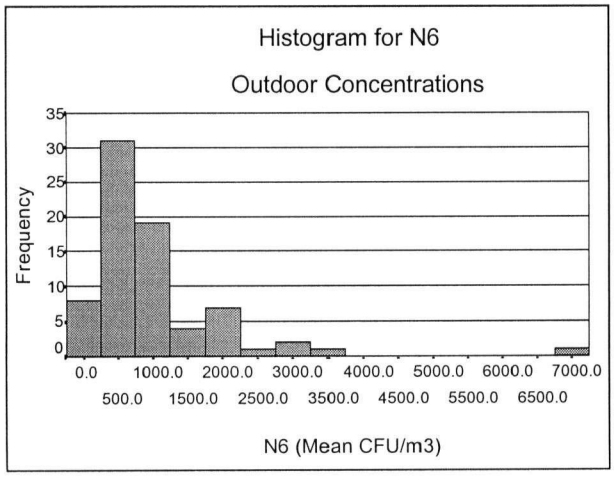


Figure 3.3.6.



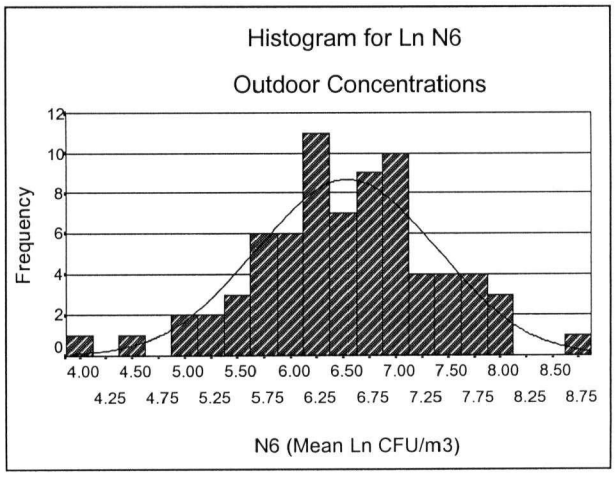


Figure 3.3.7.

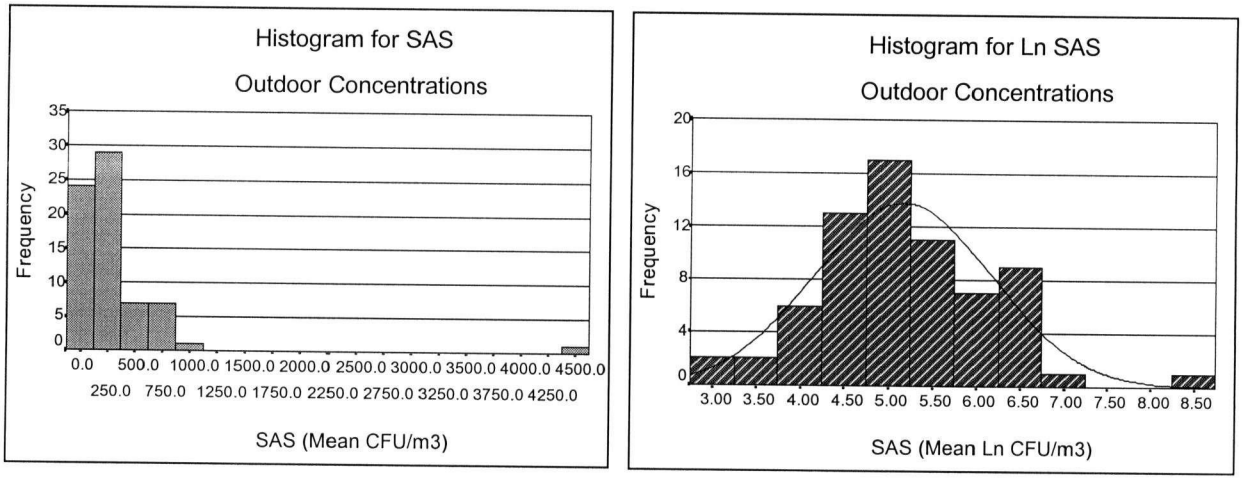
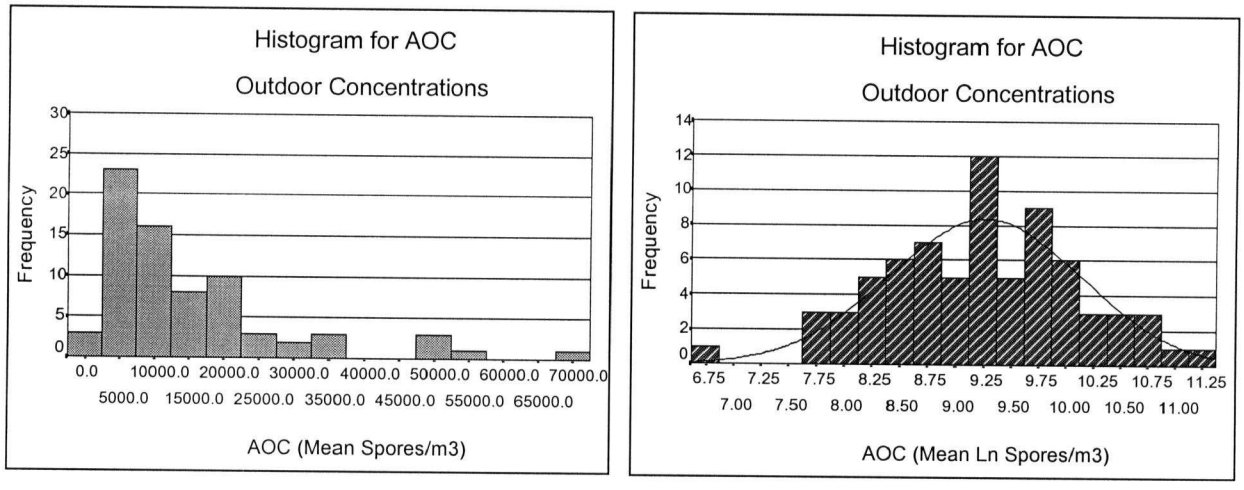


Figure 3.3.8.



Appendix IV:
Results for Literature Search in AIHAJ and Indoor Air for Sampler Comparison

Literature search results for the American Industrial Hygiene Association Journal

Year	#Reference	Instrument
1992	1. Jensen et al. (1992)	N6 RCS SAS
1993	0	
1994	1. Laitinen et al. (1994)	AND VI[1]
1995	1. Jensen (1995) 2. DeKoster et al. (1995)	SAS AND VI AMS[2]
1996	1. Rautiala et al. (1996) 2. Sigler et al. (1996) 3. Parat et al. (1996) 4. Dawson et al. (1996)	N6 RCS AND VI AND VI
1997	1. van Netten et al. (1997)	AND VI
1998	1. Krahmer 2. McCullough et al. (1998) 3. Schafer et al. (1998)	N6 AND VI AND VI
1999	1. Duchaine et al. (1999) 2. Levy et al. (1999)	AND VI N6
2000	1. Spicer et al. (2000) 2. Duchaine et al. (2000) 3. Mehta et al. (2000) 5. Aizenberg et al. (2000)	N6 N6 SAS-90, RCS+[3] AOC
2001	0	

[1]AND VI = Andersen Six-Stage model
[2]AMS = Andersen Microbial Sampler
[3]RCS+ = RCS Plus model

Literature search results for Indoor Air Journal

Year	Reference	Instrument
1992	0	
1993	1. Fisk et al. (1993) 2. Hyvärinen et al. (1993) 3. Morey, PR (1993)	SAS AND VI SAS
1994-1997	0	
1999	1. Brimblecombe et al. (1999)	RCS+
2000	1. Miller et al. (2000)	RCS
2001	1. Bellin & Schillinger (2001)	N6 SAS

Appendix V:
Conference Abstracts

American Industrial Hygiene Conference and Exposition, San Diego, CA, US
June 1-6th 2002
Platform Presentation: Tuesday, June 4th, 2002.

Lee, KS, Black, W, Brauer, M, Teschke, K, Stephens, G, Hsieh, J and Bartlett, K

A field comparison of methods for enumerating airborne fungal bioaerosols

Introduction: There is no standard method for enumerating airborne fungal bioaerosols in indoor air quality investigations. A variety of sampling instruments are available with limited knowledge of their comparative sampling efficiencies in field situations. A field comparison of three commonly used instruments was conducted in a variety of public buildings (office buildings, research institutions, hospitals, temporary mobile buildings) within southern British Columbia. The Andersen N-6 (N6), Surface Air System (SAS) Super 90 and Reuter Centrifugal Sampler (RCS), in combination with two types of media, malt extract agar (MEA) and dichloran glycerol-18 agar (DG18) were compared with respect to enumeration of culturable airborne fungal propagules.

Methods: Sampling was conducted from June-September at 50 different sites. At each site, four locations were sampled (1 common area, 2 offices and 1 outdoor sample). Each location was sampled in parallel with the three instruments, collecting approximately 150 litres for each sample. Sequential duplicates were taken for each media type. Samples were incubated at room temperature and the total colony forming units were determined for each. Data analysis was performed on log-transformed concentration data.

Results: A high correlation coefficient ($r>0.70$, $p<0.001$) with a significant difference ($p<0.001$) between the concentrations collected by each instrument for both media types resulted. Geometric mean concentrations (CFU/m^3) collected had the following order for MEA: RCS>N6>SAS (131.85>59.69>16.41 CFU/m^3 respectively) and DG-18, N6>RCS>SAS (58.57>38.36>16.03 CFU/m^3 respectively). A significant difference ($p<0.001$) was found between the MEA and the DG18 media for the RCS only. A significantly greater concentration ($p<0.001$) was found in naturally ventilated sites than in mechanically ventilated sites.

Conclusions: The differences in the field performance of these three instruments suggest that the results obtained for concentration of culturable fungal bioaerosols is dependent on the method employed for the assessment.

Indoor Air, 2002 in Monterey, CA, US.
June 30th-July 5th 2002
Poster Presentation, Monday July 1st, 2002.

A FIELD COMPARISON OF METHODS FOR ENUMERATING AIRBORNE FUNGAL BIOAEROSOLS

KS Lee[1*], W Black[2], M Brauer[1], G Stephens[2], K Teschke[1], J Hsieh[1] and K Bartlett[1]

[1]School of Occupational and Environmental Hygiene, University of British Columbia, Vancouver, BC, CANADA
[2]Dept of Pathology, University of British Columbia, Vancouver, BC, CANADA

ABSTRACT
A field comparison of three microbial samplers, the Andersen N6 single stage (N6), the Surface Air System 90 (SAS) and the Reuter Centrifugal Sampler (RCS), using malt extract agar (MEA) media, was conducted at 50 sites in public buildings in British Columbia, Canada. There were significant differences between sampling devices. Overall geometric mean concentrations were ranked in the following order for indoor: RCS>N6>SAS and for outdoor: N6=RCS>SAS. Naturally ventilated buildings also had higher concentrations of fungal aerosols compared to mechanically ventilated buildings. The results from this study indicate that concentration data are dependent on the methods used for assessment, and introduce additional variability in exposure assessment studies.

INDEX TERMS
Analytical methods, Fungi, Bioaerosols, Indoor Air Quality, Public Buildings

INTRODUCTION
Many studies have shown that exposure to indoor mould has been linked to adverse health effects. To further characterize these exposures, a reliable measurement method is needed. Currently, there is a wide variety of sampling instrumentation and analyses available but no standard method for enumerating fungal aerosols in indoor air quality investigations. Standardized methods are needed to avoid inappropriate test interpretation and comparisons between samples using different methods, however, there is no consensus among experts regarding which methodology should be used in fungal exposure assessments. For the commonly used sampling methods, little is known regarding their comparative sampling efficiencies in field settings. The purpose of this study was to evaluate the comparative field performances of three widely used instruments.

The Andersen N6 Single Stage (N6) (Graseby-Andersen, Atlantis, GA, USA), the Surface Air System 90 (SAS) (PBI International, Spiral System Instruments, Bethesda, MD, USA)

*Contact author email: kitshanl@interchange.ubc.ca

and the Reuter Centrifugal Sampler (RCS) (Biotest, Frankfurt, FRG) are three commonly used air samplers for enumerating viable airborne fungal propagules. These sampling devices all employ particle impaction onto culture media for analysis. A comparison of these three instruments using malt extract agar (MEA) media was conducted in a variety of public buildings (office buildings, research institutions, hospitals, temporary mobile buildings) within southern British Columbia.

METHODS

Sampling Devices

A general description of each sampling device is provided in Table 1.

Table 1. General Description of Sampling Devices

Instrument	*Particle Collection Method*	*Collection Plate*	*Flow Rate*	*Cut-off diameter d_{50} (μm)*
N6	400 hole Sieve impactor	100 mm Petri Dishes	28.3 L/min	0.65
SAS	487 hole Sieve impactor	84 mm Maxi Contact RODAC Plates	90 L/min	2.0-4.0
RCS	Centrifugal Impactor	34 well agar strips (Biotest)	40 L/min	4.0

Culture Media

MEA (BBL, Becton Dickinson and Company, Cockeysville, MD) is recommended by the ACGIH Bioaerosols Committee (Burge et al. 1987), and is a medium that supports a broad growth spectrum. It is composed of maltose, 12.75g/L, dextrin, 2.75 g/L, glycerol, 2.35g/L, pancreatic digest of gelatin, 0.78g/L and agar, 15.0g/L.

Sampling Protocol

Fifty sampling sites in public buildings were chosen from a pool of buildings administered by the Building Corporation of British Columbia, the University of British Columbia, and the Simon Fraser Health Region and scheduled based on convenience to the occupants.

At each site, 4 locations were chosen for sampling:

1. Common Area (Kitchen, Main Reception/Office space, Hallway)
2. Individual office or room
3. Individual office or room
4. Outdoors, as close as possible to the air intake

Air Sampling Protocol

The samplers were placed centrally within each room and were raised to a sampling height of approximately 1.5 metres. Table 2 shows the times and collected volumes for each sampler. Sequential duplicates were taken for each instrument for each media type. Between samples, each sampler head was thoroughly wiped with 70% ethanol. One field blank per day was included for each sample medium.

Table 2. Pre-set Sampling Time and Collected Volume

Instrument	*Sampling Time*	*Total Volume*
N6	5 min	141.5 L
SAS	1 min 40 sec	150 L
RCS	4 min	160 L

Laboratory Protocol

All samples and field blanks were incubated at room temperature in the natural light/dark cycle for the season. RCS strips were incubated for 4 days and SAS and N6 plates were incubated for 5 days. Total colony forming units were counted for each sample by using a magnified colony counter (Scienceware, Bel-Art Products, England).

Data Analysis

To account for the probability of more than one spore impacting through the same sieve hole, appropriate positive-hole correction factors for the count data were applied to the N6 (400 hole) and SAS (487 hole) colony counts. Samples below the limit of detection were given a value of 1 and samples above the upper detection limit were given a value equal to the upper detection limit for data analysis. Air concentrations were determined by dividing the total colony forming units (CFU) counted by the air volume sampled (CFU/m^3). Data analysis was performed using SPSS Version 10.0 statistical software package.

RESULTS

The 50 sites were sampled from June to Sept 2001. Concentration data were approximately log normally distributed. Table 3 provides a summary of the proportions of samples above and below detection limits for each method type, where LOD=limit of detection and UDL=upper detection limit.

Table 3. Proportion of samples above and below detection limits

Instrument	*N*	*%<LOD*	*%>UDL*
RCS	398	1.5%	14.6%
N6	400	6%	0.5%
SAS	400	26.5%	1%

The RCS had the fewest samples below the LOD but also had the highest number of overloaded samples. The N6 had the fewest overloaded plates (0.5%) while only 6% were below the LOD. The SAS had the most samples below the LOD.

Linear correlations between results for different samplers were used to examine agreement between the relative fungal concentrations measured and are presented in Table 4. Paired t-tests were used to determine whether the concentrations measured were the same between samplers (Figure 1).

Table 4. Overall linear correlations

	Pearson r	P
Device Comparison		
RCS-N6	0.833	<.001
RCS-SAS	0.843	<.001
N6-SAS	0.860	<.001

All correlations were significant ($p < 0.001$). Between samplers, correlations were high for all pairs, with the highest for the N6-SAS comparison.

Figure 1 presents mean indoor natural log (ln) concentrations for each method type. Differences between samplers were found for all combinations (paired t-tests, all $p<0.001$) with the following order, (geometric means are presented in Table 5), for indoors RCS>N6>SAS. For outdoors, the following order was found, N6=RCS>SAS. All indoor concentrations differed significantly from outdoor locations (see Table 5).

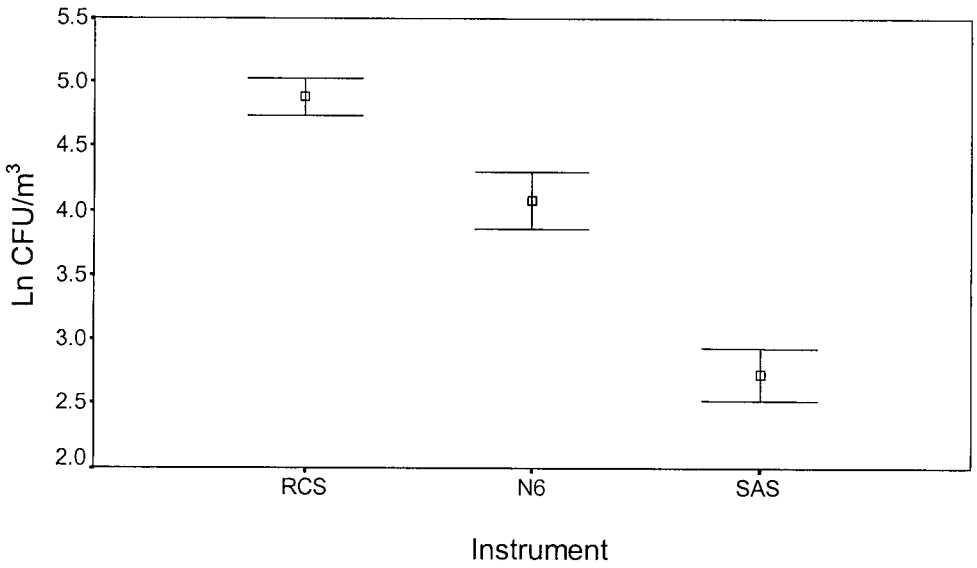


Figure 1. Instrument Comparison: Mean Indoor Ln CFU/m^3 with 95% Confidence Intervals

Table 5. Geometric Means for Indoor and Outdoor Data

Instrument	*Indoor Geo Mean CFU/m^3 (Geo Std Dev)*	*Outdoor Geo Mean CFU/m^3 (Geo Std Dev)*	*P*
RCS	131.1 (2.42)	679.4 (1.61)	<.001
N6	61.0 (3.92)	689.4 (2.27)	<.001
SAS	15.4 (3.50)	201.2 (2.60)	<.001

In the outdoor measurements, the results from the RCS (geometric mean=679.4 cfu/m^3) did not differ from the N6 (689.4 cfu/m^3) (paired t-test, $p=0.852$). The SAS still had the lowest mean (201.2 cfu/m^3).

The presence or absence of mechanical ventilation was found to have an influence on indoor concentrations. Of the 50 sampling sites, 11 were naturally ventilated buildings and 39 were mechanically ventilated. Indoor concentrations in naturally ventilated buildings were significantly greater (t-test, $p<0.001$) than indoor concentrations from mechanically ventilated buildings (see Table 6). Differences in airflow and relative humidity have been documented between buildings with mechanical versus natural ventilation (Parat *et al.*, 1997) and may contribute to the differences in the yields determined by the samplers.

Table 6. Indoor Concentrations based on Ventilation Type

Instrument	*Natural Ventilation Indoor Geo Mean CFU/m^3 (Geo Std Dev)*	*Mechanical Ventilation Indoor Geo Mean CFU/m^3 (Geo Std Dev)*	*P*
RCS	229.3 (1.89)	112.7 (2.42)	<.001
N6	244.1(2.37)	39.9 (3.36)	<.001
SAS	44.2 (2.38)	11.3 (3.26)	<.001

DISCUSSION

These data suggest that the measured concentration of fungal aerosol is highly dependent on the assessment method employed. Previous studies that compare the relative performance of instruments include laboratory and field studies. Laboratory studies provide important information regarding the instrument efficiency and performance, but are limited since settings in the field rarely reflect those in the lab. Field studies, though limited in their uncontrolled nature, provide some information in a more dynamic environment. Previous field studies (Bellin and Schillinger 2001, Mehta *et al.* 1996, Verhoeff *et al.* 1990, Smid *et al.* 1989) have been small in sample size and are limited in their diversity of sampling environments (mainly office buildings or residential homes from one area). This study is unique because it has a very large sample size (approximately 400 samples for each instrument) and a large variety of sampling sites (large and small offices, temporary mobile homes, hospitals, research institutes).

In terms of total yield, overall highest concentrations were found with the RCS, which is in agreement with a previous study by Verhoeff *et al.* (1990), however, this may be due to an underestimation of the sampling flow rate by the manufacturer. This model of RCS cannot be calibrated by measuring its flow. The only check given by the manufacturer is by determining the revolutions per minute of the impeller (it must be within 2% of 4,096 rpm to ensure a sampling flow rate of 40L/min). A previous study by Macher and First (1983) involved the development of an attachment to determine the flow rate of the RCS. Their results suggested that the quoted sampling flow rate might be an underestimate (may be five times higher than what the manufacturer stated).

The RCS had similar results in total yield to the N6 in naturally ventilated buildings and outdoors, suggesting that other factors, such as environmental conditions (concentration, relative humidity, temperature and ambient airflow), may have an impact on the performance of the methods. This needs to be further characterized in controlled conditions.

The RCS was the most sensitive method overall, but also had the highest number of overloaded samples that are probably due to the culture area (34x1cm^2 welled strips compared to 84mm plates for the SAS and 100mm plates for the N6) and larger volume sampled, therefore suggesting that for outdoor samples, a shorter sampling period (3 minutes) should be employed.

The SAS-90 consistently had the lowest mean concentrations of the three devices, despite differences in environmental settings (indoors, outdoors, mechanical ventilation, natural ventilation). This is in agreement with previous studies that have examined other models of the SAS (Bellin and Schillinger, 2001; Mehta *et al.* 1996; Verhoeff *et al.* 1990; Smid *et al.* 1989), suggesting that the SAS consistently underestimates airborne fungal concentrations. The collection efficiency of the SAS is lower than that of the N6 and may explain the difference in performance. It has also been suggested that high impact velocity onto a medium (from high sampling flow rates) result in more injury and loss of sample (Stewart *et al.*, 1995). The SAS-90 has the highest flow rate (150L/min) and this may account for the lower concentrations retrieved by this instrument.

CONCLUSIONS

The apparent concentration of airborne mould is highly dependent on the sampling and analytic method utilized by the investigator. Until methods can be standardized or fully characterized, the interpretation and comparison of results must be done with caution. Environmental conditions, such as airflow, relative humidity and temperature, may affect the performance of the different instruments and further study should be done to characterize these effects.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Workers Compensation Board of British Columbia for funding this project and all the contacts that provided the sampling sites.

REFERENCES

Bellin, P and Schillinger, J. 2001. Comparison of field performance of the Andersen N6 Single Stage and the SAS Sampler for airborne fungal propagules. *Indoor Air.* Vol 11, pp 65-68.

Burge, H, Chatigny, M, Feeley, J, *et al.* 1987. Bioaerosols: guidelines for assessment and sampling of saprophytic bioaerosols in the indoor environment. *Applied Industrial Hygiene.* Vol 9, pp 10-16.

Clark, S, Lach, V and Lidwell, OM. 1981. The performance of the Biotest RCS Centrifugal air sampler. *Journal of Hospital Infection.* Vol 2, pp 181-186.

Lach, V. 1985. Performance of the Surface Air System air samplers. *Journal of Hospital Infection.* Vol 6, pp 102-107.

Macher, JM. 1997. Evaluation of bioaerosol sampler performance. *Applied Occupational and Environmental Hygiene.* Vol 12 (11), pp 730-736.

Macher, JM and First, MW. 1983. Reuter Centrifugal air sampler: Measurement of effective airflow rate and collection efficiency. *Applied and Environmental Microbiology.* Vol 45, pp 1960-1962.

Mehta, SK, Mishra, SK and Pierson, DL. 1996. Evaluation of three portable samplers for monitoring airborne fungi. *Applied and Environmental Microbiology*. Vol 62, pp 1835-1838.

Parat, S, Perdrix, A, Fricker-Hidalgo, H, *et al.* 1997. Multivariate analysis comparing microbial air content of an air-conditioned building and a naturally ventilated building over one year. *Atmospheric Environment*. Vol 31, pp 441-449.

Pasanen, AL. 2001. A Review: Fungal exposure assessment in indoor environments. *Indoor Air*. Vol 11, pp 87-98.

Smid, T, Schokkin, E, Boleij, JSM, *et al.* 1989. Enumeration of viable fungi in occupational environments: A comparison of samplers and media. *AIHAJ*. Vol 50, pp 235-239.

Stewart SL, Grinshpun, SA, Willeke, K, *et al.* 1995. Effect of impact stress on microbial recovery on an agar surface. *Applied and Environmental Microbiology*. Vol 61, pp1232-1239.

Verhoeff, AP, van Wijnen, JH, Boleij, SM, *et al.* 1990. Enumeration and identification of airborne viable mould propagules in houses. *Allergy*. Vol 45, pp 275-284.