ABSTRACT

STEVEN PREISSLER. Aeromycologic Survey of a Specialized Patient Care Facility in a Hospital. (Under the direction of David A. Fraser)

A four-month, aeromycologic survey of a specialized patient care facility in a hospital was performed. The results of 446, two-stage impactor samples demonstrated the readiness of the facility for patient occupancy, and the effectiveness of the engineering controls. Furthermore, it was established, for the first time, that the likelihood of detecting fungal colony forming units in the patient room increased with the number of room occupants.

A single compartment model was used to rank the relative effectiveness of the engineering controls. The filters of the air handling unit were found to control fungal particles better than the recirculating room HEPA filter and the positive air pressure barrier. The limits of this agar impaction technique are discussed; also, a plan for further research, intended to contribute to better patient dose estimation and control, is presented.

ACKNOWLEDGEMENTS

To travel hopefully is a better thing than to arrive, and the true success is to labour. - R. L. Stevenson

I would first like to thank my academic and technical advisor, Dr. David Fraser; he has been a frequent and helpful listener on the occasions when a friendly ear was what I needed most. My thanks to my readers, Drs. Wayne Thomann and Jerry Tulis, for their time and thoughtful comments concerning this report. Dr. Thomann provided opportunity and guidance at the Medical Center for the experimental protion of this report. Dr. Tulis provided me with the foundations of Biohazard Science, which enabled me to complete this work. At the risk of stretching an analogy, Dr. Fraser provided an environment where the infectious principles of Dr. Tulis were applied to the host hospital via Dr. Thomann.

I am grateful for my five-month experience at the Medical Center, and to the individuals who contributed to my feeling "part of their team." I must acknowledge the help of the Environmental Safety Office Staff (especially Dr. Debra Hunt), the BMTU Staff (especially Dr. Bill Peters and Nurse Michael Plumer), the Clinical Laboratory Staff (especially the Mycology Dept.), the Design Department Staff (and their Contractor), and the Engineering and Maintenance Staff in the hospital.

I cannot mention everyone who has contributed to the process of my graduate studies which has culminated in the production of this report. In the Department, Dr. David Leith and Mr. C. L. Lassiter provided special technical and administrative assistance.

Finally, I would like to express my love and gratitude to my wife, Charu Krishnamoorthy, and my mother, Beatrice Preissler; they have been supportive and caring thoroughout the long course of my studies and I know that their pride in my accomplishments equals my own.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

0. INTRODUCTION

Imagine an apple tree in September. On one of the boughs is a big apple. A sudden gust of wind dislodges the apple, which falls and kills a small mouse which was running about in the grass.

If you investigate the cause of death of the mouse you run into difficulties.

-K.C. Winkler (112), from Jordan

Is the apple the cause of death? If it was the act of falling, the wind is the cause of the mouse's death. Maybe it was the lack of rainfall that summer which weakened the apple's attachment to the tree. But after all, fruit grows, gravity attracts, winds blow, and summer rainfall varies. If assigning cause (and Science) is not folly, then we must accept the notion that events are often the result of the combined forces of multiple causes. We should also note that the event may not be a unique result of combined forces. Had there been more rainfall that summer and the apple's attachment to the bough greater, a stronger wind may have dislodged the fruit with the same result.

Similar difficulties are encountered when assigning cause to deaths of hospital patients due to fungal infections. Outbreaks of nosocomial fungal infections have been associated with many different factors. An important factor is the concentration of fungal spores in the air. Frequently, occurrences of pulmonary mycoses have been associated with an absent, inadequate, or malfunctioning ventilation system.

In this report we propose a sampling strategy to identify factors associated with different levels of fungal spore concentration. Our method was applied to a specialized patient care unit in a hospital where fungal infections can be crucial.

This report is divided into six sections. The role of fungi in hospital infections is discussed in the first section. The specialized patients care area will be described in the second section with emphasis on the ventilation system. The goals of the survey are presented in this section. The sampling method will be explained in the third section. The fourth section contains summaries of the sampling results, assembled to identify factors associated with changes in contaminant concentration. General conclusions are presented in the fifth section with a discussion of the selected issues associated with the study results. The last section contains recommendations for further research. References and appendices follow the last section.

1. FUNGI & HOSPITAL INFECTIONS

Fungal organisms comprise a diverse kingdom of organisms important in completing the elemental cycle of carbon, by decomposing organic carbon to inorganic carbon (CO_2). This kingdom has demonstrated the ability to survive, grow, and reproduce throughout a wide range of environmental conditions, using an equally wide range of nutrient substrates. Fungi assimilate substrate nutrients and liberate airborne spores or conidia to reproduce. These qualities provide the basis for the wide occurrence of detrimental effects of fungi: biodeterioration of materials, products, and food; toxicoses; allergies; and mycotic infections.

1.1 Types of Infection: Mycotic infections may result from endogenous or exogenous fungi. Specialized, natural fungal flora of the skin or mucous membranes, like *Microsporum*, *Trichophyton*, *Epidermophyton*, *Geotrichum*, and *Candida*, cause diseases which are spread by direct or indirect contact between infected and non-infected individuals. These diseases are prevalent, mild, and seldom cause death.

On the other hand, exogenous fungi are unspecialized, free-living saprophytes. As natural inhabitants of the soil, they are rarely transmitted person to person. These fungi may be introduced into the host by trauma or injury. This results in subcutaneous infections or

mycotic mycetomas by a variety of dematiaceous fungi (chromoblastomycoses, and phaeohyphomycoses), *Rhinosporidium seeberi* (rhinosporodioses), *Loboa loboi* (lobomycoses), or *Sporothrix shenckii* (sporotrichoses) (88). Airborne spores or conidia of exogenous fungi may also enter the respiratory tract and cause pulmonary mycoses.

Airborne spores may infect normal or immunocompromised hosts. Diseases of the normal hosts include: histoplasmoses, blastomycoses, coccidiomycoses, and paracoccidioidomycoses. The etiologic agents associated with these diseases show important common characteristics: geographic restriction, dimorphism (as yeasts or spherioles), as well as infections resulting in benign granulomas. Diseases of normal hosts have a high prevalence and although usually a low fatality in endemic areas, secondary, progressive infections may be fatal.

1.2 Opportunistic Infection: Unlike agents infecting normal hosts, the airborne agents associated with opportunistic fungal infections are unspecialized, ubiquitous saprotrophs which may cause progressive disease. If the fungal organism can overcome the body's defense mechanisms and tolerate the temperature of the human body, they encounter abundant water, high carbohydrate levels, and available nitrogen sources. Air-transmitted exogenous fungi which have been associated with opportunistic infections are listed in Table 1.

An individual's intrinsic susceptibility to infection is influenced by factors such as age, sex, pregnancy, nutrition, and immune status. Congenital and acquired diseases may lower an individual's ability to resist fungal infection. Immunosuppression may also result as a side effect of medical treatments or the purposeful dimunition of the host's immuniologic response. Diseases and medical treatments which have been

TABLE 1. AIRBORNE EXOGENOUS FUNGI ASSOCIATED WITH OPPORTUNISTIC INFECTION (44)

Phylum Class, Order, Family Genus and Species
Zygomycota
Zygomycetes, Mucorales, Mucoraceae
Mucor
Rhizopus oryzae
R. rhizopodiformis
R. indicus
Rhizomucor pusillus
Absidia corymbifera
Mortierella
Basidiobolus haptosporus
Zygomycetes, Mucorales, Cunninghamellaceae
Cunninghamella elegans
Dikaryomycota
Ascomycetes, Aspergillales, Microascoceae
Petriellidium boydii[a]
Basidiomycetes
Cryptococcus neoformans
(Deuteromycota)
Deuteromycetes, Moniliales, Cryptococcaceae
Rhodotorula rubra
Trichosporon cutaneum
T. capitatum
Deuteromycetes
Aspergillus fumigatus
A. flavus
A. glaucus
Penicillium
Fusarium
Geotrichum candidum
Helminthosporium
Cephalosporium
Microsporum audouinii
Cladosporium trichoides
C. cladosporioides
Sporothrix schenckii

[a]formally called *Allescheria boydii*, perfect stage of *Monosporium apiospermum*

shown to contribute to air-transmitted opportunistic fungal infections are shown in Table 2.

TABLE 2. MEDICAL HOST FACTORS ASSOCIATED WITH AIR-TRANSMITTED FUNGAL INFECTION (98).

Fungi	Disease	Defect, Medicants, Procedures
Aspergillus	Chronic granulomatous disease	Polymorphonuclear leukocyte defect
	Rheumatoid lung	Corticosteriod therapy
	Leukemia during therapy	Antibiotic treatment
	Bronchiectasis	Cardiac surgery
Cryptococcus	Diabetes mellitus	Defect in cell-mediated immunity
	Hodgkin's disease	
	Sarcoidosis	Corticosteroid therapy
	Adrenal hyperplasia?	Immunosuppressive therapy
Mucor	Leukemia during therapy	
Zygomycetes	Diabetes mellitus	Polymorphonuclear leukocyte defect
	Burns	
	Leukemia during therapy	Parenteral drug use
	Chronic pulmonary disease	Severe malnutrition
	Renal acidosis	

1.3 Infection Transmission: Transfer of the fungal agent from its natural (or present) habitat to the compromised host has been documented in several episodes in which a sudden increase in infection has been detected. In 17 outbreaks shown in Table 3, an absent, inadequ- ate, or faulty ventilation system has been identified facilitating the agent transfer to the host. Ten outbreaks were traced to an inside source. An extramural source of the pathogenic fungus was identified in seven outbreaks. *Aspergillus fumigatus* is the agent usually associated with these mycotic infection epidemics.

1.4 Nosocomial Infections: Hospitals host a unique population requiring the need for prevention of opportunistic infections. In the mid-1970's, the rate of nosocomial infections in acute care, U.S. hospitals was estimated to be 5.7 per 100 admissions (45). The

TABLE 3. OUTBREAKS OF AIR-TRANSMITTED FUNGAL INFECTION WITH CONTRIBUTING FACTORS

Site of Mycotic Outbreak (Reference)	Cases	Patients' Predisposing Condition	Fungi[a]	Source of Fungi	Contributing Factor
Veterans' Administration Hospital of Buffalo, NY (39)	3	Open heart surgery	AFu	Pigeon excreta	Source near air intake Inadequate filtration
Baltimore City Hospital (24)	4	Renal transplant	AFu	Pigeon excreta	Source near air intake
Minneapolis Veterans' Hospital (61)	3	Renal transplant	AFu	Pigeon excreta	Malfunctioning exhaust
University of Maryland (2)	8	Leukemia	AFl	Fireproofing material	Hospital construction
Indianapolis Veterans' Hospital (11)	3	Renal transplant	AFl	Ceiling dust	Hospital construction
Texas Childrens' Hospital (67)	5	Leukemia	Asp	A/C coil & filter	Malfunctioning exhaust
Yale University Medical Center (42,76)	9	Oncology patients	AFu AFl	Construction dust?	Hospital construction
Milwaukee County Medical Center (62)	10	Renal transplant & Oncology patients	AFu AFl	Outside construction A/C filters	Inadequate filtration
North Carolina Memorial Hospital (94)	1[b]	Leukemia	AFl	Outside construction	Numerous ventilation defects
Hospital Clinico de San Carlos (50)	3	Leukemia	RPu	Refuse container	Source near air intake Inadequate filtration
Bellevue Hospital Center (59)	2	Premature infants	RIn	Ceiling dust	Hospital construction
Rosewell Park Memorial Institute (84,93)	10	Marrow transplant	AFu AFl	Road construction	Inadequate filtration
Fitzsimmons Army Medical Center (79)	11	Malignancies and steroids	Asp	Construction dust	Hospital construction
'Hospital A' (109)	5	Hematologic malignancies	Asp Zyg	Construction dust	Hospital construction
Childrens Hospital of Pittsburgh (107)	6	Immunocompromised children	AFu AFl	Utility room? A/C filters	Recirculating A/C Inadequate filtration
Westminster Childrens' Hospital (89)	6	Marrow transplant	AFu AFl	Construction dust	Hospital construction
Massachusettes General Hospital (51)	6	Immunocompromised adults	Asp	Construction dust?	Hospital construction

[a]Asp = Aspergillus spp.; AFl = A. flavus; AFu = A. fumigatus; RIn = Rhizopus indicus; RPu = Rhizomucor pusillus; Zyg = Zygomycetes;

[b]Eighteen additional patients exhibited colonization of the respiratory tract.

five-year average nosocomial infection rate in U.S. medical centers for the period 1980 through 1984 was 4.1 per 100 hospital discharges. As demonstrated by the data in Table 4, higher opportunistic infection rates are associated with the severity of the underlying illness and the prevalence of invasive medical procedures practiced by large teaching hospitals in this country. In these surveys (27-29), the causal agent is identified in approximately 85% of nosocomial infections. During the period 1980-1982, fungi represented 6% of the identified nosocomial pathogens. In 1983 and 1984, this proportion increased to 7% and 8%, respectively.

TABLE 4. UNITED STATES NOSOCOMIAL INFECTION RATES (27-29)

Number of Infections per 1000 Discharges

Year	National Average	In Large Teaching Hospitals
1980	32.5	39.5
1981	33.7	42.4
1982	33.2	42.1
1983	32.7	41.2
1984	33.5	41.4

A hospital setting also provides a high potential for control of the indoor environment. A highly trained staff, housekeeping service, food service, central ventilation, and plumbing utilities all contribute to this control. Additionally, prospective reimbursements paid according to patient diagnosis have added incentives to reduce nosocomial infections (110). These incentives remain despite efforts to reclassify patients to a higher paying diagnosis related group (46).

2. BONE MARROW TRANSPLANT UNIT (BMTU)

On May 31, 1988, the University Medical Center* opened a new bone marrow transplant unit on the top floor of the hospital. The surrounding medium-sized metropolitan area** lies in the eastern piedmont plateau at 406 feet above sea level, halfway between the Appalachian Mountains and the Atlantic coast, and experiences a humid subtropical climate (see Table 5). Approximately 125,000 people live within the 69.3 square miles of the city limits. July is the hottest month (mean temperature 78.3°F) and the wettest (mean rainfall of 5.19 inches) (41).

TABLE 5. REGIONAL CLIMATE (41)

Average annual temperature	59.9°F
Growing season	190-210 days
"Sunshine days"	230 days
Prevailing winds	southeast at 7.7 m.p.h.
Annual average rainfall	44.88 inches
Annual average snowfall	7.5 inches

The University Medical Center is a nationally known, not-for-profit, teaching and research hospital. It contains 990 beds, employs 5171 personnel, and receives nearly 34,000 admissions annually (6). Current nosocomial infection rates at the Medical Center and the

*Duke University

**Durham, North Carolina

associated proportion of identified pathogens are shown in Table 6. Efforts of the infection control department result in a high identification rate of bacterial and fungal infections.

TABLE 6. MEDICAL CENTER NOSOCOMIAL RATES (53)

Year	Rate per 100 Admissions	Percentage of Pathogens Identified	Fungal Proportion of Identified Pathogens
1986	5.2	93.0%	16.6%
1987	6.6	93.2%	19.9%
1988	6.7	91.3%	19.5%

2.1 Objectives of the Investigation: A four-month long survey was conducted at the newly opened bone marrow transplant unit to determine the efficacy of engineering controls and to assist with the clinical assessment of risk due to fungal spore exposure. Two additional questions were examined. The possibility of ranking components of the engineering design according to their importance in controlling fungal spores and identification of other environmental factors affecting fungal spore concentration in the patient rooms were investigated.

2.2 Risk of Fungal Infection: High doses of alkylating agents are used to treat advanced, often terminal, malignancies: carinomas, melanomas, sarcomas, and lymphomas. Alkylating agents crosslink double-stranded DNA, preventing the strands from separating for replication. These agents are non-specific, affecting not only the highly proliferative cancer tissue, but also the bone marrow, lymphoblasts, mucous membranes and skin, accounting for their immunosuppressive effects. Autologous (self-donated) bone marrow transplantation is a treatment for the effects of high-dose administration of alkylating agents.

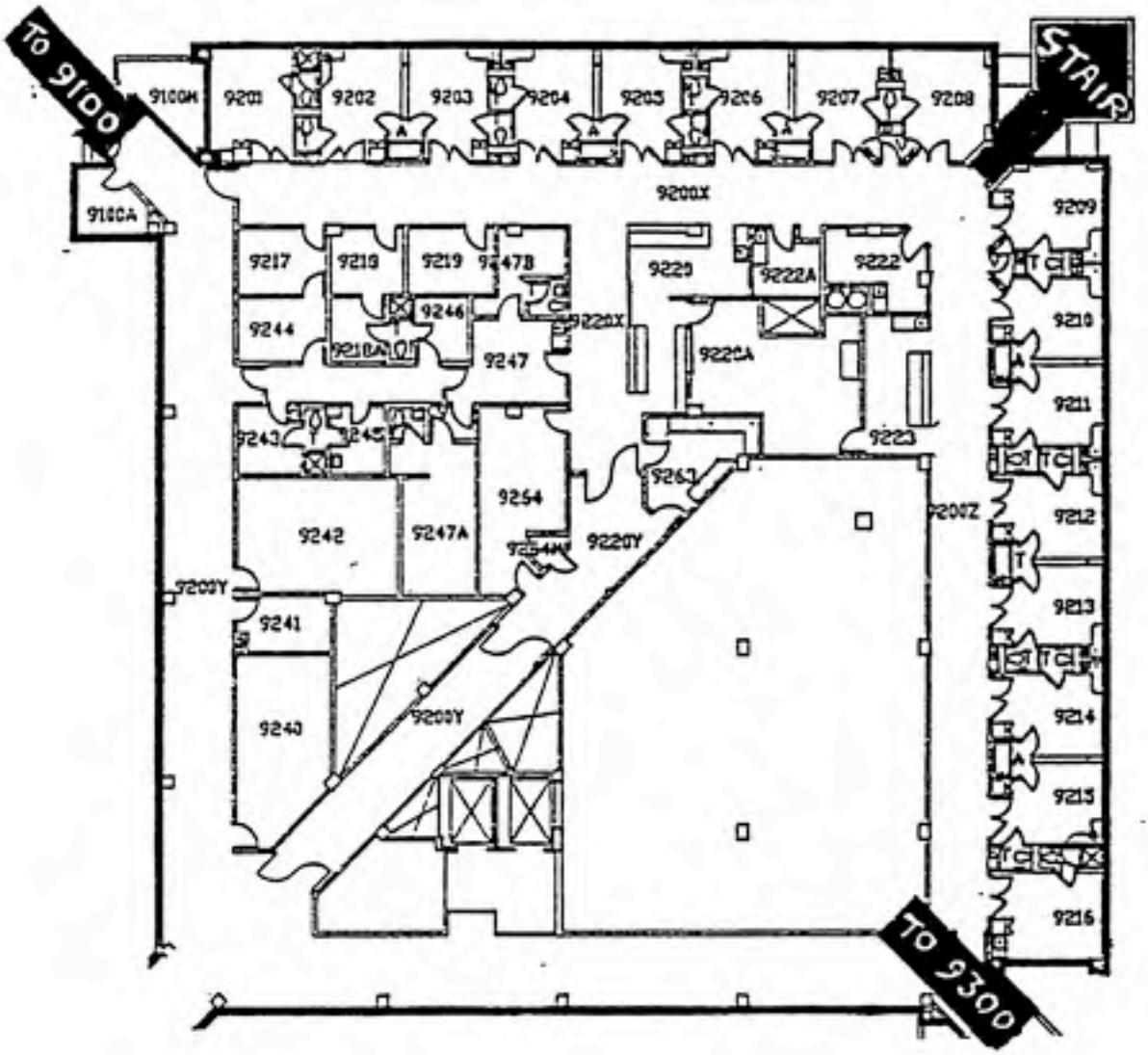
The average bone marrow transplant patient in this unit requires hospitalization for approximately one month. During this period,

alkylating agent chemotherapy is generally administered 11, 8, 7, and 4 days before bone marrow reinfusion (106). Neutropenia caused by chemotherapy predisposes these patients to viral, bacterial, and fungal infections. Of primary interest to us is the prevention of airborne fungal infection.

2.3 Infection Control Design: Control of infection at the bone marrow transplant unit is based on the broad principles of infection control: preservation and enhancement of the patient's protective defenses, identification and suppression of sources of the infective agent, protective isolation, and surveillence. These principles were considered in the design of the 3,700 square foot bone marrow transplant unit. A plan of the bone marrow transplant unit (BMTU) is shown in Figure 1.

A 24 foot-long airlock separates the unit from the remaining areas of the ninth floor. Staff and visitors don sterile gowns and disposable booties in the gowning area. The gowning area provides an entrance for equipment and food; the former stored, and the latter prepared in separate rooms. Equipment is disinfected in the equipment room before it is brought into the unit. Food is freshly prepared or heated, and known food sources of microorganisms, such as fresh raw vegetables, are prohibited. Visitors must wash hands with disinfectant, don gloves and surgical masks before entering patient rooms. Handwashing stations adjoin the 16 patient rooms. The ventilation system is designed to reduce patients' exposures to airborne fungal spores.

Nearly all the transplant patients' time is spent in their room. Clean air is supplied to the room and room air is continuously recleaned. Positive pressure prevents entry of presumably less clean



Patient Rooms	9201-9216
BMTU Hall	9200X, 9220X, 9200Z
Other BMTU Rooms	9217-9247A, except 9240-9242
Nurses Stations	9220 and 9223
Equipment Room	9264
Galley	9263
Gowning Area	9220Y
Airlock	9200Y

FIGURE 1. FLOOR PLAN OF THE BMTU

air from the bath, toilet, and unit corridor. A short description of the patient's room ventilation follows.

2.4 BMTU Ventilation: Two similar, 12½-ton, air handling units, Nos. AH-31 and AH-32, supply air to the bone marrow transplant unit. A schematic diagram of AH-31 is presented in Appendix A. Each air handling unit contains 120 square feet of pleated, glass-fiber prefilters (NU-CAP-G; Environmental Filter Corporation of Greensboro, NC) and post-filters (3XC-95; Cambridge Filter Corporation of Syracuse, NY). These filters have a combined efficiency of approximately 95%. These air handling units also contain steam humidifiers and coils for heating and cooling the filtered, humidified air. Approximately 72% of the air moved by AH-31 is recirculated from the hospital. The exact percentage of recirculated air is determined by the difference in temperature between the inside and the outside of the hospital. Cool and warm conditioned air from AH-31 is combined in a mixing box before it is supplied at a constant rate to the patient's room. The amount of air entering the patient's room when the toilet, bath, or hall door are opened is small compared to the amount of air supplied by the mixing box. A diagram of the patient room air supply and exhaust is presented in Figure 2. Note that AH-32 receives recirculated hospital air from areas it supplies, but these airflows are not shown in the figure.

A wall-mounted HEPA filter cleans recirculated air in the patient's room, providing approximately 39 room air changes per hour. Air is exhausted from the room through a ceiling mounted exhaust, bath exhaust, and toilet exhaust. The room remains at positive pressure with respect to the hallway. Air also leaves the room through the quarter to three-quarter inch corridor door undercut.

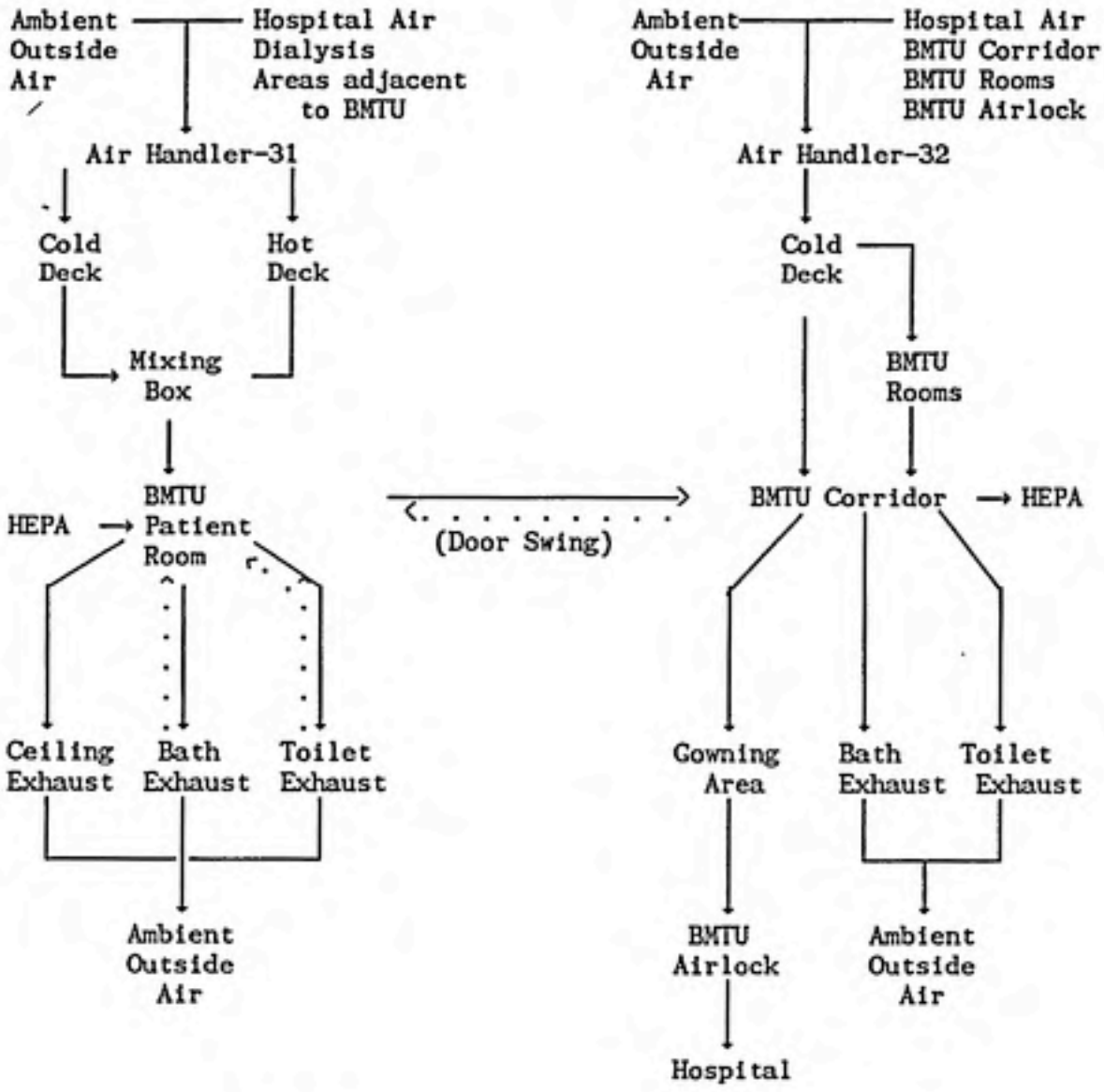


FIGURE 2. AIR FLOW IN THE BMTU

The bone marrow transplant unit corridor has a recirculating, roof-mounted, HEPA filter providing nine air changes per hour. The corridor remains at positive pressure to other unit rooms and offices (9217 - 9247A, except 9240 - 9242), galley (9263), and gowning area (9220Y). The gowning area is likewise positive to the unit's airlock, and the airlock is positive to the rest of the ninth floor.

3. SAMPLING METHOD

During the period April 25, through August 15, 1988, 446 samples of airborne spores were collected and analyzed from the bone marrow transplant unit and the hospital roof using a pair of two-stage sieve impactors (Andersen Samplers, Inc. of Atlanta, GA) and 100 mm petri dishes filled with Sabouroud agar (Table 7).

TABLE 7. IMPACTOR SAMPLE VARIABLES

Date and Time of Sampling
Location and Height of Sieve Impactor
Presence of HEPA Exhaust
Number of Room Occupants
Sample Duration
Date Petri Plates were Analyzed
Identification and Number of CFU on Top Plate
Identification and Number CFU on Bottom Plate
Impactor Operator and CFU Counter

The two-stage sampler collects particles on agar medium and is a simplified version of the six-stage Andersen impactor (8). The first and second stages of the aluminum sampler have two hundred, 1.5 mm diameter and 0.4 mm diameter holes, respectively. The design, with 45° countersunk holes are arranged in a radial pattern which incorporates the improvements of May (69). The design is reported to be efficient for collecting airborne particles in the size range 0.8 to 10.5 microns (105). Non-respirable microorganisms are collected on the first stage. The two-stage impactor has been recommended for dilute concentrations

(less than 1,000 particles per cubic meter) of large particles (greater than one micron diameter) (40). The samplers and vacuum pumps (Emerson Model 0522-V103-G18DX) were calibrated against a dry gas meter (Singer Model DTM-200) to pull one cubic foot of air per minute, as recommended by Andersen.

Impacted particles were collected on Sabouroud agar, one of the standard mediums used by medical mycologists. In the late 1950's this agar produced fungal colony counts similar to those from seven other media in an outdoor comparison, using a slit sampler (90). Twenty years later, in a similar comparison with seven media using a single stage sieve impactor, Sabouroud agar produced among the highest CFU recovery rates (23). It is a reproducible medium consisting of dextrose, peptone, pancreatic digest of casein, and peptic digest of animal tissue. Sabouroud agar plates used in this study were prepared in the Clinical Microbiology Laboratory of the University Medical Center, using standard procedures outlined in Appendix B. After sample collection, agar plates were incubated in the dark, at 30°C. Colonies of fungi were identified and counted after approximately three days. Unlike liquid impingement collection methods which reflect the total number of microbes suspended in the air, the agar impaction method of collection reflects the total number of airborne particles carrying fungi, spores, or conidia (113). The "positive hole" colony count adjustment method (63) was not used because it was not known whether a colony was observed in the jethole pattern.

Paired, 30-minute samples were collected simultaneously across control barriers: usually in the patients' rooms and the corridor outside the room. Longer sample periods dehydrate the agar. Shorter

sample periods were employed on the hospital roof to avoid overcrowding of fungal colonies. Samples were also collected in the galley, equipment room, gowning area, and airlock. Approximately 11% of the samples were collected outside the bone marrow transplant unit, as shown in Table 8 .

TABLE 8. SAMPLING SITE DISTRIBUTION BEFORE AND AFTER OCCUPANCY OF THE BMTU

Sampling Site	Before May 31, 1988	On or after May 31, 1988	Row Totals[a]
BMTU Areas			
Patient Room	29	145	174 (39 %)
BMTU Hall	29	136	165 (37 %)
Other Rooms	-	15	15 (3.4%)
Galley and Equipment Room	-	7	7 (1.6%)
Gowning Area	3	15	18 (4.0%)
Airlock	3	13	16 (3.6%)
Non-BMTU Areas			
Adjacent Areas	3	5	8 (1.8%)
Roof	-	43	43 (9.6%)
Total	67 (15%)	379 (85%)	446 (100%)

[a]Percentage of column total given in parentheses.

4. SAMPLE RESULTS

The results of sampling are presented in Appendix D, arranged in the order in which they are collected. In this section, summaries of the results and conclusions will be presented.

This section includes descriptions of the prevalence of fungal genera inside and outside the hospital. Statistics are presented for fungal concentrations collected by location and sample period. Abstracts of reports addressing each sample period accompany presentation of these statistics.

The effects of each engineering control measure on the concentration of fungal spores in the patient room are demonstrated in this section. The importance of each control measure were estimated and ranked based on experimental and hypothetical conditions. The effects of environmental factors on the concentration of fungal spores were also investigated. The results of these investigations are presented at the end of this section.

4.1 Types of Fungi: The genera of fungi identified in the hospital and on the roof are summarized in Table 9. High proportions of positive samples in the first column is a measure of consistent recovery of the corresponding organism. The second column shows the total number of colony forming units (CFU) detected in the samples.

Overall, 53% of the 3,369 CFU's collected in this study remained

TABLE 9. INVENTORY OF FUNGI FROM 446 IMPACTOR SAMPLES

	Indoor Results[a] for 330 m^3 of air		Outdoor Results[a] for 8 m^3 of air	
	Positive Samples	CFU	Positive Samples	CFU
Penicillium	30 (7.5)	≥ 1,262 (52.4)	28 (65.1)	132 (13.7)
Cladosporium	34 (8.5)	≥ 60 (2.5)	5 (11.6)	14 (1.5)
Aspergillus	28 (7.0)	49 (2.0)	19 (44.2)	27 (2.8)
Sterile Hyphae	9 (2.2)	≥ 25 (1.0)	0 (-)	0 (-)
Zygomycetes	6 (1.5)	8 (0.3)	6 (14.0)	6 (0.6)
Other Identified[b]	2 (0.5)	6 (0.2)	0 (-)	0 (-)
Fungi Identified	102 (25.4)	1,410 (58.5)	35 (81.4)	179 (18.6)
Fungi Unidentified	202 (50.2)	999 (41.5)	35 (81.4)	781 (81.4)
Overgrown	1 (0.2)	TMTC[c]	5 (11.6)	TMTC[c]
Total	402 (100.0)	> 2,409 (100.0)	43 (100.0)	> 960 (100.0)

[a]Parenthetical values are percentages of column totals.

[b]*Paecilomyces* and *Syncephalastrum*

[c]TMTC = Too Many To Count

unidentified. Inside the hospital, 41.5% of the spores were not identified. Outside the hospital, 81.4% were not identified. One agar plate collected adjacent to the bone marrow transplant unit and eight plates exposed to outside air became overgrown and the fungi could not be counted or identified.

Hospital results were collected over a four month period and roof samples were collected over four days. However, the results agree with previously established indoor/outdoor fungal air quality relationships. They are also consistent with previous surveys of the surrounding area and hospital air. *Cladosporium*, the most frequently recovered taxa outdoors in the United States (58), is likely to have been underrepresented among the identified outdoor fungi in this study, due to their relatively slower growth.

The overall hospital fungal spore concentration was 6% of the outdoor fungal spore concentration. Previously reported fungal concentrations using volumetric air sampling yielded "hospital to outdoor ratios" of 2% for mesophilic and 5% for thermotolerant fungi (66), 84% and 32% for *A.fumigatus* (100) , and 7% for thermophilic fungi (52). In this study *Penicillium*, *Cladosporium*, and *Aspergillus* were the most commonly collected taxa, indoors and out. Summaries of indoor air quality (74), and indoor/outdoor relationships (15) express a similar dominance of these genera. The types of fungi collected in this investigation agree well with the isolates from other hospital studies (77, 95), as well as those mentioned above (66, 100). Relative proportions of the taxa differ, probably due to geographic and climatic differences. The genera collected in the hospital are similar to those

obtained in a 1983 survey of molds in area homes using the gravity settling plate collection method (14).

4.2 Spore Concentrations: Summary statistics for the areas sampled are given in Tables 10 and 11. A log-probit plot of cumulative percent and CFU concentrations is provided in Appendix E. In the hospital, low fungal spore concentrations predominate the sample results. Forty-two percent of the hospital samples detected no CFU's. In contrast, outdoor samples were all positive and ranged from 44 to 316 CFU/m^3. While 30% of the roof samples contained half of the outdoor isolates, over half of all the indoor isolates collected on the bone marrow transplant unit and adjacent areas are contained in just 1.5% of the samples.

Second-stage CFUs, presumed to be the respirable portion, comprise 68% of the total indoor CFUs based on a sample-weighted average. The portion of outdoor respirable airborne spores is higher at 72%. This value is within 1.4 standard deviations of the indoor average proportion. When the overall proportion of respirable spores is calculated for all samples, inside and out, the sample-weighted result is 68.3% with a standard deviation of 3.3%. From these observations, we conclude that the respirable proportion of the CFUs is fairly constant. Therefore, isolates of both impactor stages were combined to report fungal spore concentrations.

Usually the results of each stage are separated to estimate potential harm based on lung penetration of the particles (105). Lung penetration is not an important factor in this study. Infectious syndromes of pathogenic fungi include rhinocerebral, rhinoorbital, and paranasal infection (13, 25, 32, 88). Invasive aspergillosis has also been associated with prior nasal colonization which may provide the

TABLE 10. NUMBER AND CONCENTRATIONS OF FUNGI BY SAMPLE LOCATION

	Number of Samples	CFU Detected	Percentage of Respirable CFU	Concentration Range (CFU/m^3)
BMTU Areas				
Patient Rooms	174	134	69%	0 - 36
BMTU Hall	165	1,212	67%	0 - 428
Other Rooms	7	13	85%	0 - 7.4
Galley and Equipment Room	15	407	63%	0 - 410
Gowning Area	18	426	67%	1.2 - 218
Airlock	16	148	64%	0 - 37
Non-BMTU Areas				
Adjacent Areas[a]	7	69	56%	4.9 - 22
Roof[b]	38	960	72%	44 - 316

[a]Area results do not include one sample with one overgrown plate.

[b]Area results do not include five samples with eight overgrown plates.

TABLE 11. SUMMARY STATISTICS OF FUNGAL CONCENTRATIONS BY SAMPLE LOCATION

	Percentage Sterile	Median Conc. (CFU/m^3)	Average Conc. (CFU/m^3)	Percentage of Samples Containing 1/2 CFUs
BMTU Areas				
Patient Rooms	77%	0	0.7	2.3%
BMTU Hall	19%	2.5	7.2	1.2%
Other Rooms	29%	2.5	2.3	29 %
Galley and Equipment Room	20%	3.7	33	6.6%
Gowning Area	0%	11.5	26	38 %
Airlock	6%	9.8	11	25 %
Non-BMTU Areas				
Adjacent Areas[a]	0%	11.5	9.7	38 %
Roof[b]	0%	184	144	30 %

[a]Area results do not include one sample with one overgrown plate.

[b]Area results do not include five samples with eight overgrown plates.

inoculum to the lung (1, 76). Further, the respirable portion of the CFUs is predictable.

4.3 BMTU Design Effectiveness: The ultimate success of the bone marrow transplant unit design can be judged by the absence of airborne fungal infection. A safe level of fungal spore concentration exposure has not been established for these susceptible patients. Other studies have found reductions in area spore concentrations to be associated with a decrease in the incidence of aspergillosis (91, 92, 94). The patients' rooms are the cleanest area sampled in terms of: the percent of sterile samples, the median spore concentration, the average spore concentration, or the peak spore concentration.

The highest concentration of spores was outside, on the hospital roof near the air handler inlets. Results are presented in Table 12. If these data are representative of the fungal spore challenge, the ventilation system reduced the contaminant concentration by an order of magnitude in the bone marrow transplant unit and two orders of magnitude in the patients' rooms.

TABLE 12. SUMMARY STATISTICS OF OUTDOOR FUNGAL CONCENTRATIONS

Location	Air Destination	Concentration (CFU/m^3) Median	Average	Peak
AH-31 Inlet	BMTU Patient Rooms	173	175	316
AH-32 Inlet[a]	All other BMTU Areas	158	150	287

[a]Area results do not include five samples with eight overgrown plates.

4.4 Filter Component Analysis: The effectiveness of the filter components of the ventilation system can be analyzed using the summary

in Table 12. Results from the bone marrow transplant areas have been tabulated by air handling unit and additional HEPA filtration. Concentration ratios were calculated in the last two columns to derive "contaminant control" factors: the median and average roof concentrations (from Table 12) were divided by the corresponding area spore concentration (e.g. Contaminant Control (C. C.) Factor of the AH-31 filter = AH-31 inlet average divided by average of patient room without a HEPA filter = 175/2.6 = 67). The higher the ratio, the more protection provided by the barrier.

The C. C. Factors in Table 13 are consistent with filter rating efficiencies and the rate of HEPA-filtered room air changes. The efficiency (7) of the pre- and post-filters in the air handlers are 35-40% (80) and 90-95% (26) , respectively. Their combined efficiency is expected to produce a dimensionless C. C. Factor of 15 to 33 under conditions similar to the ASHRAE test. The increase in the C. C. Factor to the room HEPA compared to the hall HEPA is likely due to the larger number of air changes per hour in the room.

TABLE 13. CONTAMINANT CONTROL FACTORS OF FILTER COMPONENTS IN THE BMTU

BMTU Area	Concentration (CFU/m^3) Median	Average	Barrier	C. C. Factor[a] Median	Average
Patient Room without HEPA	0	2.5	AH-31 filter only	NA	69
Patient Room with HEPA	0	0.5	AH-31 and HEPA filter	NA	350
Other BMTU Areas without HEPA	7.4	17.7	AH-32 filter only	22	8
Other BMTU Areas with HEPA	2.5	9.0	AH-32 and HEPA filter	65	17

[a]Concentration ratio of inlet spore concentration and area concentration.

The air handling units have identical filters, yet there is an eight-fold difference in the calculated C. C. Factors. The differences in spore challenge on the roof is less than 20%. The air handler inlets, due to their close proximity, are likely to encounter similar spore concentrations. This difference in C. C. Factors between filtered air supplied by the two air handlers cannot be explained by this analysis and suggests that the filters of AH-32 be examined for leaks.

4.5 Positive Pressure Barrier Analysis: Less air is exhausted from the patient rooms than is supplied creating a positive pressure air barrier. Positive pressure air barriers, throughout the unit, are shown in Figure 3.

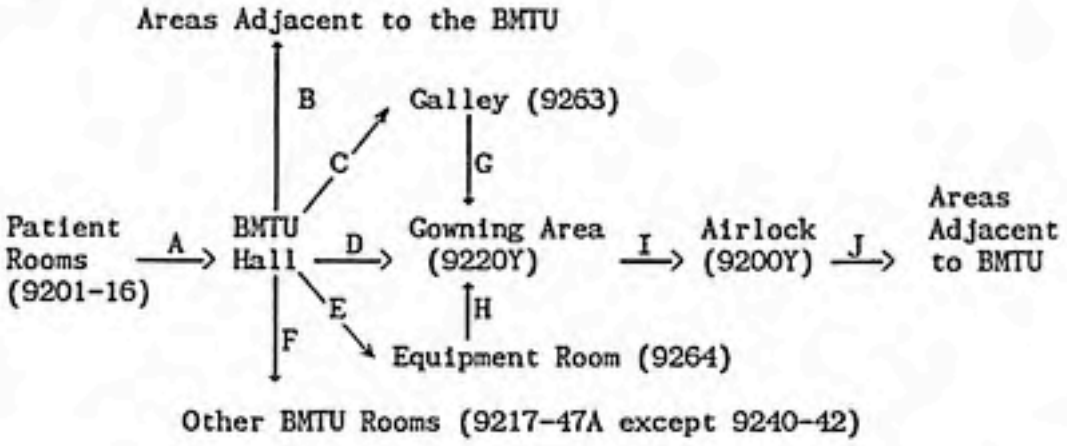


FIGURE 3. DESIGN OF AIR PRESSURE DIFFERENTIALS IN THE BMTU.

The arrows in Figure 3 identify the direction of airflow through the unit. Air flows from the patient room to the BMTU hallway (Label A). Air from the hallway leaves the BMTU (B), or enters other BMTU areas (Paths C, D, E, and F). The gowning area receives air from the hallway (D), galley (G), and equipment romm (H), before it leaves the BMTU (J), via the airlock (I). The positive pressure is designed to preserve upstream

areas of high air quality from contaminated, downstream areas. Simultaneous, paired samples on each side of the barrier test the effectiveness of the positive pressure design.

In Table 14, results of paired, simultaneous (collected within one hour) samples have been tabulated by airflow path. The proportion of samples in which the downstream spore concentration exceeded the upstream spore concentration is presented in the next to the last column of Table 14. If up- and downstream concentrations are equal or if no barrier exists, this proportion will be one-half. The p-value in the last column is the binomial probability that this proportion or a proportion of greater deviation would occur if no barrier existed (null hypothesis).

TABLE 14. PAIRED SAMPLE RESULTS FOR AIR FLOW ANALYSIS

Airflow Label	Upstream Side (US)/ Downstream Side (DS)	Number of Paired Samples Total	US<DS	US=DS	US<DS Fraction	P-value[a]
A	Patient Room / BMTU Hall	151	112	30	0.74	$< 10^{-6}$
B	BMTU Hall / Adjacent Areas	3	2	0	0.67	1.00
C	BMTU Hall / Galleys	7	1	1	0.14	0.22
D	BMTU Hall / Gowning Area	3	3	0	1.00	0.25
E	BMTU Hall / Equipment Room	6	1	0	0.17	0.22
F	BMTU Hall / Other BMTU Rooms	7	3	1	0.43	1.00
G	Galley / Gowning Area	4	2	0	0.50	1.00
H	Equipment Room / Gowning Area	4	4	0	1.00	0.13
I	Gowning Area / Airlock	15	3	1	0.20	0.06
J	Airlock / Adjacent Areas	5	2	0	0.40	1.00

[a]Probability that no barrier exists. See text for details.

Two airflow paths demonstrate an effective barrier at the 0.1 significance level, eight do not. A significant barrier exists between the patient room and the BMTU hallway. A significant barrier is also detected between the gowning area and the airlock, but air flows from an area of high spore concentration to an area less contaminated. At least five paired observations are necessary to demonstrate a significant barrier at the 90% confidence level. The ranking of the effectiveness of the positive pressure barriers below is based on the probabilities in Table 14. Underlined air flow labels indicate the barrier direction was reversed. That is, air flowed more often from a contaminated area to a cleaner one.

Significant barrier = A > <u>I</u> > H > <u>C</u>, <u>E</u> > D > G > B, <u>F</u>, <u>J</u> = no barrier detected

<u>4.6 Patient Risk Assessment</u>: Air sampling results from this study have been reported previously to assist in assessing patient risk to fungal spore exposure (33-35). Specifically, the following questions were addressed: Are fungal spore levels low enough in the newly constructed bone marrow transplant unit to transfer patients from the old unit? Have fungal spore levels remained at an acceptable level after patients and staff have occupied the unit? Are concentrations of fungal spores in the hallway of the unit low enough to allow patients to enter and remain in the hallway?

Portions of the data from the above mentioned reports are reproduced in Table 15. Results of air samples collected between July 19, and August 15, 1988, are also summarized in this table. Comments on each sampling period will follow. The new bone marrow transplant unit

TABLE 15. SUMMARY MEASURES FOR FOUR SAMPLING PERIODS

Location	Number of Samples	Number of Sterile Samples	Volume Sampled (m^3)	Number CFU Detected	Median Conc. (CFU/m^3)	Average Conc. (CFU/m^3)
April 25 to May 30, 1988						
Patient Rooms	29	21	29.7	62	0	2.09
BMTU Hall	31	10	31.7	82	2.45	2.58
All BMTU Locations	66	32	67.6	208	1.23	3.08
May 31 to June 17, 1988						
Patient Rooms	62	46	63.2	32	0	0.51
BMTU Hall	59	9	60.2	243	2.45	4.04
All BMTU Locations	129	38	131.6	340	1.23	2.58
June 20 to July 15, 1988						
Patient Rooms	36	28	36.9	19	0	0.52
BMTU Hall	31	9	31.8	90	2.45	2.83
All BMTU Locations	72	38	74.0	219	0	2.96
July 19 to August 15, 1988						
Patient Rooms	47	37	49.0	21	0	0.43
BMTU Hall	44	4	44.8	797	3.68	17.79
All BMTU Locations	127	44	130.4	1,573	1.23	12.07
April 25 to August 15, 1988						
Patient Rooms	174	132	178.8	134	0	0.75
BMTU Hall	165	32	168.5	1,212	2.45	7.19
All BMTU Locations	394	169	403.5	2,340	1.23	5.80

opened on May 31, 1988, which was the first day of the second sampling period.

4.7 Preoccupancy Results: Preoccupancy samples were collected where fungal spores were thought most likely to infiltrate from outside the unit and near the nurse's station in the center of the unit. Workmen made final adjustments to the ventilation system and electrical utilities during this period. Forty-eight percent of the samples collected on the unit showed no fungal growth. Of critical importance, only two *Aspergillus* spores, were detected, in the gowning area. No other pathogenic species were detected in the unit. The gowning area average spore concentration was five times the overall unit average during this period.

4.8 Early Occupancy Results: During the second sampling period of early unit occupancy, all rooms were surveyed. Of the samples collected during this period, 42% showed no fungal growth. As seen in Table 14 (page 26), room, hall, and overall unit median values remained unchanged. The average concentration for the room decreased and the hall average increased. The gowning area average remained at five times the overall unit average. The overall average spore concentration for the unit decreased but a significant increase in *Aspergillus* spores was observed.

Aspergillus spores were detected seven times more frequently during this early occupancy period compared to the preoccupancy period. Four *A. flavus* spores were collected between rooms 9213 and 9216 in the hall on four different days. One *A. niger* isolate was detected in room 9201, and twice in the hall: near room 9202 four days later, and near room 9207 six days after. Nineteen other *Aspergillus* spp. (not *flavus*,

fumigatus, or *niger*) were detected in the hall between the stairway door and the exit to 9300 on six different days.

4.9 Later Occupancy Results: The average found in the hall decreased during the third sampling period with little change in the other summary statistics. Fifty-two percent of the unit samples were sterile. *Aspergillus* recovery rates decreased to the preoccupancy level. Three species were recovered in the hall on three different days: *A. flavus* near 9210, *A. niger* near 9207, and one species (not *flavus*, *fumigatus* or *terrius*) was recovered just beyond the gowning area. The gowning area average doubled, increasing to more than ten times the unit average.

Patients were allowed to enter the hall for short periods of time (30 minutes, twice daily). The benefits of exercise and release from room confinement were regarded to exceed the fungal spore exposure risk. Based on results from the three sampling periods, the hall average is four times greater than the room average spore concentration. The resultant total spore exposure for a 24 hour period is expected to increase by one eighth:

$$\frac{23 \text{ hr. (room conc.)} + 1 \text{ hr. } (4 \times \text{room conc.})}{24 \text{ hr. (room conc.)}} = 1.125.$$

The proportionate increase in exposure to *Aspergillus* spp. is much greater.

4.10 New Occupancy Results: Hall and overall averages for the sampling period from July 19, to August 15, 1988 show a marked increase compared to the previous sampling period. These higher values result from incidences of brief, steep increases in *Penicillium* spore concentrations. The transitory increases occurred in the same area, near the galley on July 28, and July 29, 1988. Increases of this nature

have been documented during bedmaking (99), cleaning of overhead light fixture (86), and from rotting cabinet wood on other bone marrow transplant units (102). The values in the table can be recalculated without the results from these two incidences (Sample Nos. 329 to 346). The new hall and overall averages are 3.8 and 2.9 CFU/m^3, respectively. The hall median decreases to 2.45 CFU/m^3 and the overall median for the sample period remains 1.23 CFU/m^3.

The new hall average for the last sampling period remains elevated compared to the previous sampling period. Only 39% of the samples detected no fungal growth. The gowning area average concentration decreased from the previous sampling period. This average also decreased relative to the overall average. Similar to the second sampling period, the increase in hall average was accompanied by an increase in the detection rate of *Aspergillus* species.

A total of 15 CFUs of *Aspergillus spp.* were detected during the last sampling period. An *A. niger* species was detected in room 9212. Nine species were detected in the hall on six different days, including *A. terrius* on July 28, 1988. A total of five *Aspergillus* species were detected in the airlock, gowning and galley areas on three days, including *A. flavus* in the galley on August 1, 1988.

4.11 Baseline Concentrations: A summary of results for the complete survey divided by room, hall, and all locations are found on the bottom of Table 14. These values can be used as a basis of comparison for future air sampling results. The values listed in the table illustrate the amount of variation that might be expected from further sampling.

Concentration of spores in the patients' rooms remained low after unit occupancy. The average of all patient room samples after the unit

opened is 0.48 CFU/m^3 and only two *Aspergillus* spores were detected in 149 cubic meters of air sampled. It is important to note that no cases of fungal infection have been observed in the BMTU since it was opened. Therefore, it seems that the concentrations reported may approach an acceptable level.

The post-occupancy hall spore concentration is 8.3 CFU/m^3 and without the excursions of *Penicillium* is 3.7 CFU/m^3. The hall sampling results vary more than the patient room concentrations. The incidence of *Aspergillus* collection is also greater but remains infrequent. Similar concentrations of *Aspergillus* have been experienced in patient rooms on the Minnesota bone marrow transplant unit (86).

4.12 Ranking of Engineering Controls: Earlier in this chapter, effectiveness was demonstrated for each component of the engineering design : air handler filtration, recirculating HEPA filtration, and the positive pressure barrier between patient room and hallway. Two sources of difficulties arise when ranking the components of the unit engineering design by their importance in controlling fungal spore concentrations in the patient room. Conditions of spore challenge to the hospital unit change, sometimes very suddenly; roof and hall spore concentrations ranged one and two orders of magnitude in this study. The present set of conditions may rely on controlling a different source of spores than a future set of conditions. Also, in this study, no experimental controls existed to compare the lack of a positive pressure barrier and the absence of filtration on the air handling units.

Engineering controls can be ranked in their effectiveness if hypothetical conditions are considered. The positive effect of each component of the engineering design has been already demonstrated in an

earlier part of this section. We can rank the contributions of the parts of the engineering design by examining the expected effects of removing a single component or two components under conditions already found to exist during this study.

4.13 One Component Removal: A positive pressure barrier would not exist if the doors to the patient rooms were removed. Over time, the only difference in patient room and hall spore concentration would result from lack of air mixing. The ratio of hall to room concentration averages from bottom of Table 15 (page 28) is approximately ten. The combined volume of the patient rooms is twice the hall volume. Assuming perfect mixing, the patient room spore concentration would increase four-fold, based on a volume-weighted concentration:

$$\frac{2(\text{room conc.}) + 1(10 \times \text{room conc.})}{2 + 1} = 4 \text{ room conc.}$$

If the filters in AH-31 were removed, the room spore concentration would equal 28% of the roof spore concentration. This assumes that the behavior of the room spore concentration can be accurately described by steady state conditions of a single compartment model and that any losses in the other components of the ventilation system are negligible.

In 1946, Lidwell and Lovelock first proposed this single compartment model to describe the concentration of a tracer (2,4-pentanedione) in a room (65). More recently, Rhame (85) suggested such a model when considering the concentration of fungal spores in patient rooms on bone marrow transplant units.

After Ishizu (54), the amount of indoor pollution (fungal spores) can be derived from the following mass balance equation:

$$VdC = Gdt + C_i m\, Q_i dt - C\, m\, Q_i\, dt - C\, m\, Q_r \varepsilon dt$$

where V = volume of the room (m^3),

t = time (hour),

C = room spore concentration at any time (CFU/m^3),

C_i = inlet spore concentration supplied to the room (CFU/m^3),

G = spore generation rate inside the room (CFU/hr),

Q_i = inlet volume rate supplied to the room (m^3/hr),

Q_r = volume rate of recirculating filter (m^3/hr),

ε = efficiency of filter recirculating air (dimensionless),

and m = mixing factor (dimensionless).

Integrating with the boundary conditions $C = C_o$, the initial spore concentration (CFU/m^3) at t = 0, we obtain

$$C = C_o \exp\{-m(Q_i + \varepsilon Q_r)t/V\} + \frac{m C_i Q_i + G}{m(Q_i + \varepsilon Q_r)} [1-\exp\{-m(Q_i + \varepsilon Q_r)t/V\}].$$

When the generation rate, inlet spore concentration, filter efficiency, and volumetric flow rates remain constant, over a long period of time the exponential terms drop ($t \to \infty$, $\exp(-kt) \to 0$), and the steady state room concentration can be described,

$$C\ (CFU/m^3) = \frac{m C_i Q_i + G}{m(Q_i + \varepsilon Q_r)} \qquad \text{(Equation 1)}$$

Assuming air mixes perfectly in the room (m = 1), the HEPA filter removes all the fungal spores from air passing through the filter (ε = 1), and the roof spore concentration (C_i) remains constant:

$$C\ (CFU/m^3) = \frac{C_i Q_i + G}{Q_i + Q_r}. \qquad \text{(Equation 2)}$$

The room spore concentration would become 49 CFU/m^3, if typical values are substituted for these variables: C_i = 175 CFU/m^3; Q_i = 530 m^3/hr; Q_r = 1377 m^3/hr; and G = 0 CFU/hr for no source of spores inside the room. This calculated value for conditions without AH-31 filtration is 65 times higher than the measured room average of 0.75 CFU/m^3.

The effect of removing the recirculating HEPA filter from the patients' room can be calculated from the experimentally derived C. C. Factors in Table 13 (page 24). The ratio of the patient room C. C. Factor with HEPA filtration to that without HEPA filtration is approximately five. This experimental result agrees with that predicted from the above-mentioned, one-compartment model. Using the same variable values, the ratio of the room concentration without recirculating HEPA filtration to one with HEPA filtration is about four.

$$\frac{C_{\text{No HEPA}}}{C_{\text{HEPA}}} = \frac{(C_i Q_i + G)/ Q_i}{(C_i Q_i + G)/(Q_i + Q_r)} = \frac{Q_i + Q_r}{Q_i} = \frac{530 + 1377}{530} = 3.6.$$

Based on the expected effect of removing one of the engineering controls from the patient room, the following ranking can be formulated based on the expected increases in patient room concentration:

AH-31 filtration effect	>>	HEPA filtration effect	>	positive pressure barrier
(65X)		(5X)		(4X)

This analysis, of course, is based on specific hypothetical conditions. If this analysis is repeated using postoccupancy baseline averages for room and hall, the effect of removing the positive pressure barrier would exceed the effect of removal of HEPA filtration.

4.14 Two Component Removal: The positive pressure air barrier would rank as the least important among the engineering controls, if the roof inlet and patient room air remained unfiltered. The room air, at steady state, would approach concentrations on the roof. The presence of the positive pressure barrier would then prevent the "dirty" room air from being diluted with less contaminated hall air. Therefore, if two engineering control components are removed, the positive pressure barrier would provide a negative contribution to cleanliness in the patient room.

In hypothetical cases, when only one filter system is operating and no positive pressure barrier exists, the bone marrow transplant unit as a whole can be considered to be a single compartment. Single compartment model variables can be expressed as sums or weighted averages of airflow rates and concentrations supplied by the two air handlers. After substituting values for conditions found on the hospital unit in this study, the room concentration when AH-31 inlet supply is unfiltered exceeds the room concentration when it is filtered. Calculations are presented in Appendix F.

Based on the hypothetical conditions of removing two engineering controls from the patient room, the following ranking can be formulated:

AH-31 filtration effect (6X)	>	HEPA filtration effect (1X)	>>	positive pressure barrier (Negative)

This ranking agrees with the previous one-component rankings but the separation between the rankings differ.

4.15 Environmental Factor Analysis: Two directions were investigated to associate patient room concentrations with environmental conditions.

The first method consisted of ranking rooms by 'cleanliness' and identifying environmental factors associated with either a low or high level of cleanliness. The second method consisted of partitioning sample results by levels of environmental factors. The resultant contingency tables were examined to detect spore concentration differences associated with the levels of the environmental variables.

Two simplifications were made to assist in these analyses. First, the environmental factors were considered to be mutually independent. Second, the number of fungi collected per sample was used as the dependent variable, instead of the room spore concentration. Since the period over which airborne spores were collected in the patient room seldom deviated from 30 minutes, the volume of air sampled can be considered a constant. This constant can be multiplied by the room concentration to obtain the total number of fungi detected per sample.

4.16 Room Cleanliness Ranking: The average number of spores detected and the percentage of sterile samples were investigated as measures by which to rank room cleanliness. Since fungi were often undetected in patient rooms, results were hypothesized to fit a Poisson model. If the Poisson model fit, rooms could be judged more clean the lower the Poisson parameter. If a criteria for cleanliness can be established, environmental factors can be identified with levels of cleanliness.

The average number of spores detected were low and sample standard deviations were high. Most standard errors exceeded the half of the room average (see Table 16). No ranking could be justified by average number of spores detected because of the lack of separation between room averages and the large, standard errors.

TABLE 16. PATIENT ROOM RESULTS SUMMARY

Room No.	n	Fungi Detected[a] $\bar{X} \pm s/\sqrt{n}$	Percent Sterile[b] $P \pm \pi$	$\frac{(n-1)s^2}{\bar{X}}$	$\chi^2_{(n-1)}(0.05)$
1	19	0.53 ± 0.18	63 ± 11	20.4	9.4
2	12	0.08 ± 0.08	92 ± 8	11.0	4.6
3	12	0.83 ± 0.63	75 ± 13	63.4	4.6
4	12	1.00 ± 0.74	67 ± 14	72.0	4.6
5	17	0.29 ± 0.11	71 ± 11	12.0	8.0
6	12	0.50 ± 0.26	75 ± 13	17.5	4.6
7	11	0.55 ± 0.28	64 ± 15	16.0	3.9
8	4	0 ± 0	100 ± 0	-	-
9	11	0.18 ± 0.18	91 ± 9	2.0	3.9
10	9	0 ± 0	100 ± 0	-	-
11	5	6.40 ± 5.66	40 ± 22	100.2	0.7
12	13	0.62 ± 0.33	69 ± 13	27.8	4.6
13	10	0.10 ± 0.10	90 ± 9	9.0	5.2
14	9	0.56 ± 0.56	89 ± 11	40.0	2.7
15	4	0 ± 0	100 ± 0	-	-
16	14	2.57 ± 1.77	64 ± 13	221.5	5.2
All Rooms	174	0.787 ± 0.234	77.0 ± 3.2	2,160	210

[a] $\bar{X} \pm s/\sqrt{n}$ = Average Number of Fungi Detected ± Standard Error

[b] $P \pm \pi$ = Percentage of Sterile Samples ± Standard Error

A ranking on the basis of percent of sterile samples would fail for similar reasons. The percentage of sterile samples for the 16 rooms range from 40% to 100% (see Table 16). No fungi were found in three rooms (100% sterile samples). For the other thirteen rooms, the binomial model can be used to calculate the uncertainty in the percentage of sterile samples, P. This uncertainty is estimated by π, where $\pi = [(1-P)P/n]^{1/2}$. When the uncertainty of the percentage of sterile samples is considered, the data does not provide enough evidence to support ranking the rooms in terms percentage sterile.

Two methods were used to examine the goodness of fit of a Poisson model to the room results. In the first, expected Poisson frequencies

were calculated using each room average and compared to the study results. The goodness of fit test using the χ^2 failed to accept the model for all sixteen rooms. In the second method (68), ratios of room variances to room averages were compared to the suitable χ^2 critical value at 0.05 (See Table 16). The Poisson model was again rejected for all but the results from one room (room 9). It is concluded from both methods, that the patient room results, considered as replicates exhibit too much variability to admit the Poisson model.

4.17 Spore Prediction by BMTU Variable: Simple predictive relationships between the variables in Table 17 and patient room spore concentration (respirable concentration spore concentration or total spore concentration), number of spores detected, or spore generation rate were examined on scatterplots. The spore generation rates were calculated based on the one compartment model by rearranging Equation 2 (page 34):

$$G\ (CFU/hr) = C\ (Q_i + Q_r) - C_i\ Q_i.$$

Measured values for room concentration (C) and flowrates for the inlet supply and filter (Q_i and Q_r), along with postulated values for the inlet spore concentration (C_i) were used to calculate the hypothetical source (or sink, when negative) strengths for spores to reconcile the measured room concentration with the other room conditions.

No pattern was detected upon examination of the scatterplots, even when transformations of the BMTU variables were performed (73). Non-parametric analyses were performed to identify important variables before other predictive models were fit (multiple regression or ANOVA).

TABLE 17. INDEPENDENT BMTU VARIABLES

Date - d. from first sample day (4/2/88),
d. from BMTU opening (5/30/88);

Time - t. from earliest sample (7:42 am),
t. from noon (12:00 pm),
t. from 2 pm (2:00 pm);

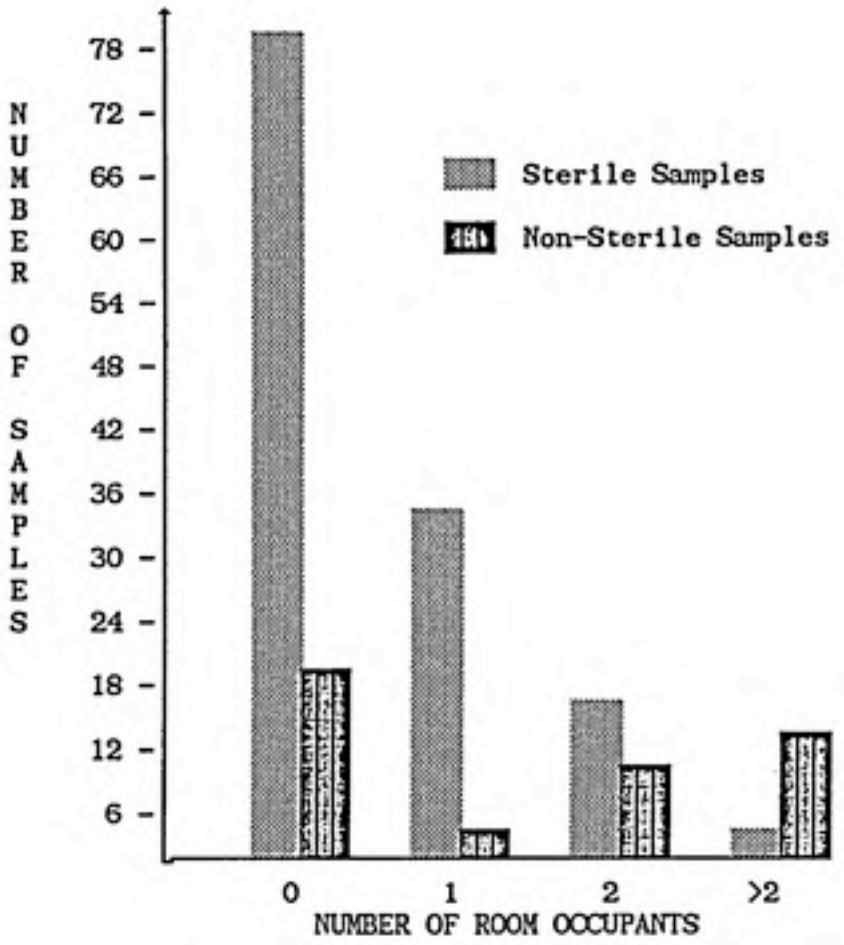
Occupancy - number of room occupants;

Location - l. of room from 9201,
l. from 9207;

Hall Concentration - respirable h.c.,
non-respirable h.c.,
total h.c.,
day's average h.c.,
day's vicinity average h.c.;

Contingency tables were assembled by counting sample results which showed the presence of airborne CFU's in different catagories of environmental variables. The chi-square statistic was computed and compared to published values to detect differences in the levels of the variable not likely to result by chance (49). Only one variable, the number of persons in the room, resulted in a significant ($p < 0.05$) chi-square statistic (see Figure 4).

Because only one variable was found to have a significant effect on the presence or absence of fungi in the patient rooms, no computer-intensive models were fit to the data.



$\chi^2_3 = 31.0$; P-value < 0.005

Occupants in Room	Sterile Samples[a]	Non-Sterile Samples[a]	Row Total
0	79 (81%)	19 (19%)	98
1	34 (92%)	3 (8%)	37
2	16 (64%)	9 (36%)	25
>2	3 (21%)	11 (79%)	14
Total	132 (76%)	42 (29%)	174

[a]Percentage of row total given in parentheses.

FIGURE 4. INFLUENCE OF THE NUMBER OF OCCUPANTS IN THE PATIENT ROOM ON THE DETECTION OF FUNGAL PARTICLES.

5. DISCUSSION AND CONCLUSIONS

The previous section demonstrated that, like the death of the field mouse, a number of factors can be associated with the presence of fungal spores in patient rooms. Chief among these factors are the engineering controls: air handler filters, recirculating HEPA filters, and positive pressure air barriers. The number of occupants was also associated with the detection of spores within the patient rooms. Infection control policies protecting immunocompromised patients were formulated based on these results.

The technique of paired, sieve samples used in this study was successful in obtaining the following objectives.

1) The efficacy of the engineering controls was established. Table 13 (page 24) demonstrates that the concentration of fungal particles is reduced by the filters in the air handling units, and by the recirculating HEPA filters in the patient rooms and the hallway. Positive pressure in the BMTU produced an effective barrier in only two of the ten airflows examined, as shown in Table 14 (page 26).

The relative effectiveness of the engineering controls is also seen in the frequency plots presented in Appendix E. There is a distinct separation between plots corresponding to the room amd the hall and the room and the outside. The success of the primary engineering objective of a clean patient room is demonstrated by these separations.

2) The method provided a basis for clinical risk assessment based on BMTU area cleanliness. Summaries are provided in sections 4.6 through 4.10 (pages 27-32). The patient room was found to be the most spore free and therefore the safest. Inside the hospital, the gowning area was found to be the area most contaminated with fungal particles. Furthermore, the appearance of potentially pathogenic spores was associated with higher spore concentrations in the unit.

Although the unit was not found to be free of pathogenic spores, the fungal particle concentrations were found to be low enough to allow patients to be moved into the unit. Also, BMTU hallway concentrations were found to be low enough to allow patients limited access to the hallway. Spore concentrations were expected to increase after patient occupancy because of the concurrent increase in unit activity and traffic from outside the unit. On the contrary, the period after occupancy showed a decrease in the patient room and overall BMTU unit averages (Table 15, page 28). This may have resulted from fewer disturbances due to BMTU construction and increased housekeeping during the post-occupancy period.

3) The engineering design components were ranked in importance in controlling fungal spores in the patient room. By using experimental data, hypothesizing engineering control failure, and applying a single compartment model, the filters in the air handling units were found to be the most important factor affecting spore removal. The recirculating HEPA filter in the room controlled fungal particles room better than the positive pressure air barrier. The positive pressure barrier was found to be the least important component in maintaining low patient exposures to fungal spores. (See pages 32-36)

Because of the lack of experimental controls, hypothetical conditions were postulated. The results agreed with theoretical expectations. Also, the removal of either one or two engineering controls resulted in the same engineering control ranking.

Although consistent, the present engineering control ranking has limited applicability. The discussion in sections 4.12 through 4.14 stressed the dependence of the ranking on temporary and somewhat subjective conditions. Likewise, because the rankings depend on data collected in this study, other locations with different climatic and ecologic conditions may experience different fungal challenges. These challenges will determine the relative importance of the engineering control components.

4) One environmental factor was successfully associated with the presence of fungal particles in the patient room. An increase in room occupants increased the likelihood of detecting fungal particles. As illustrated in Figure 4 (page 41), this result ($p < 0.005$) was obtained by applying non-parametric methods of analysis.

Further discussion in this section will be limited to three areas: BMTU air cleanliness and fungal infection (acceptability), limits of the impactor sampling method for risk assessment (accuracy), and recommendations for spore surveillance in the BMTU (evaluation). The validity and specific implications of each of the results presented in the previous section will not be directly addressed.

5.1 BMTU Air Acceptability: No incidence of air-transmitted fungal infections was detected during the study period. It may be concluded that the spore concentrations in the patient rooms were below harmful levels, preventing at-risk patients from being infected by pathogenic

spores. The veracity of this assertion relies on accurate disease rates and patient exposure estimates.

The detection of pulmonary fungal disease remains difficult (60, 88). This is borne out by the fact that treatment based soley on circumstantial evidence has been recommended by some investigators (1). In the past, a substantial proportion of cases of opportunistic fungal infection were detected post-mortem (12, 57).

Patient exposures may have been overestimated or underestimated in this study. Air sampling occurred during daytime and hours of high unit activity. Other investigators have demonstrated an association between high activity levels and high spore concentrations. Alternatively, patient exposures may have been underestimated if sampling did not occur during periodic episodes of high concentrations of fungal spores.

If future air sampling results are to be compared to the results of this survey, a few precautions must be considered. Estimates of patient exposure should be obtained by sampling techniques similar to the one used here. Sampling method, location, and time should be matched. Changes in patients' susceptibility (for example shorter or longer periods of neutropenia) should also be taken into account. A further difficulty arises from the fact that we do not yet have a suitable statistical model (Poisson, normal or lognormal) for this data. The Bioaerosols Committee of the ACGIH recognizes that the results of bioaerosol sampling rarely follow standard parametric distributions (16). Lack of a suitable model make comparisons a more complex task.

A review of the literature reveals that few studies have associated prospective volumetric sampling with rates of opportunistic fungal infection. Of the reports in Table 3 (page 7), only one, at the

University of Minnesota (86,87), shows that infection rates decreased with a reduction in average spore concentration (as a result of recirculating room HEPA filtration). When *Aspergillus* concentrations were reduced from 2.0 CFU/m^3 to 0.9 CFU/m^3, the cases of aspergillosis were reduced from 4 in 66 to 7 in 202 bone marrow transplant patients. Rhame and associates provide a good discussion of this risk factor and offer practical recommendations for the prevention of infection (86).

In the 1960's, medical applications of HEPA filtration began with local isolation for surgery (64), followed by patient isolation by a commercial device supplying HEPA filtered air, to an inflatable plastic tent (48). These applications were quickly applied to patients receiving chemotherapy for cancer (96). Concurrently, the need for skin, nose, throat, and gastrointestinal tract decontamination was recognized (20). Rooms with a wall or ceiling supplying unidirectional HEPA filtered air were developed (72) and evaluated with systemic or nonabsorbable antibiotics to complete patient decontamination (19).

These total protected (gnotobiotic) environments and antibiotic regimens have been shown to reduce (but not eliminate) the number of airborne or colonizing microorganisms and reduce infection rates. Demonstrated increases in remission rates and rates of patient survival have been difficult to reproduce. Various infection prevention strategies were investigated during the last two decades. Pizzo's schematic representation of the rising and falling levels of the use of infection prevention strategies is provided in Figure 5. As shown in the figure, gnotobiotic environments are experiencing renewed interest. Present applications include laminar airflow rooms for patients with solid tumors, similar to those cancers experienced by patients on this

BMTU. Pizzo and Levine present a good review of controlled environments (83).

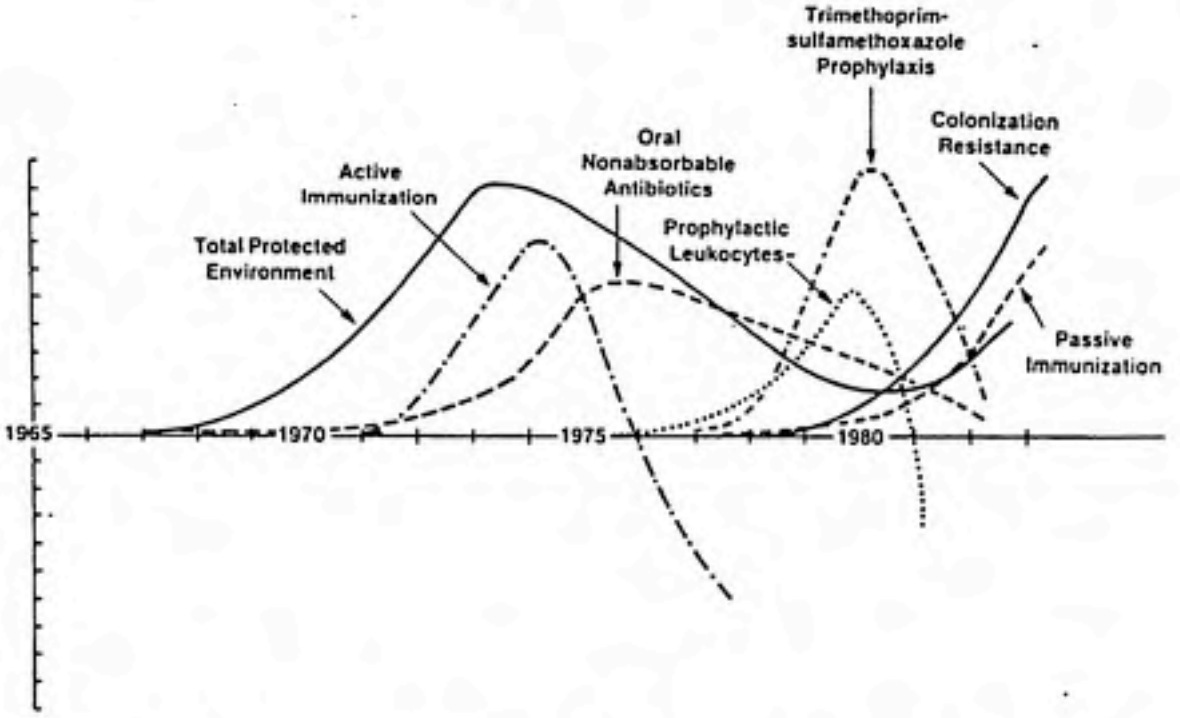


FIGURE 5. TWO DECADES OF INFECTION PRVENTION STRATEGIES. (82) The rising and falling of use reflect the ability to confirm (or fail to confirm) a reproducible benefit of a given regimen.

Criticism of patient isolation in gnotobiotic environments began in the early 1970's (55) and reemerged a decade later (10, 81). Currently, less expensive measures are being investigated and have been shown to be efficacious (75). The cost of providing unidirectional laminar air flow was a consideration in the design of this BMTU.

5.2 Exposure Assessment Accuracy: As previously mentioned, the two-stage sieve sampler employed in this survey collects the total number of airborne particles carrying fungi, spores, or conidia. For a variety of reasons this collection method underestimates the total

number of fungal microorganisms (22). The liquid impingement collection method, however, reflects the total number of fungi suspended in the air. Therefore, it is a more suitable method to estimate patient exposure, if patient infectivity is based on the total number of viable fungal organisms. One such liquid impinger system, the AGI 30 operated at 12.5 lpm, has been recommended as a standard method (21). The attachment of a pre-impinger to the AGI allows for added aerosol size discrimination (71).

If the size distribution of the CFUs (or the infection site, i.e., nares vs. lung) is a more important consideration then the total number of organisms, the six-stage Andersen sieve sampler is the recommended standard method (21). Other investigators have found that the two-stage sieve sampler produced lower values for airborne bacteria (30) and *Aspergillus fumigatus* (40) than the Andersen sampler. *A. fumigatus* collection efficiencies may be difficult to predict because of changes in median spore size (77). If the two-stage sieve sampler is used in future spore collections on the BMTU, it is recommended that a membrane filter be inserted behind the second stage and placed on agar after sampling, to detect spore breakthrough.

Additional comments concerning each sampling method follow. Sampling by either of these sieve samplers can be extended with the application of a thin wax film on the agar to prevent dehydration (70). A liquid impinger will require more careful handling, more time to manipulate the sample, and careful selection of the impingement liquid. The impinger liquid will require a surfactant to disperse hydrophobic spores. Whatever method is used to collect spores, field blanks should be collected routinely to monitor contamination.

Unlike the methods above, impaction devices like the Fort Detrich Slit Sampler, Fort Detrich Slit-Incubator Sampler, and the Casella Slit Sampler, give time-concentration relationships (113). These devices can detect transient increases in spore concentration. Besides providing exposure data, they can assist in the identification of factors contributing to these transient increases.

5.3 BMTU Surveillance: A complete program of infection control will include environmental surveillance. Surveillance goals for specialized patient care areas of a hospital are presented in the following hierarchy: detection of engineering control failure or building construction; BMTU air quality monitoring; and neutropenic patient exposure monitoring. Each of these objectives will be discussed separately.

Reported outbreaks of fungal diseases were listed in Table 3 (page 7). The causes contributing to these outbreaks provide a convincing argument for detecting engineering control failures and building construction. Checklists make surveillance relatively easy to implement. Corrective measures are unambiguous. This strategy of surveillance assumes that the BMTU is otherwise acceptable. It also assumes that the failure of engineering controls or building construction activity are the most important environmental determinants of risk of air-transmitted fungal disease.

Proper monitoring of BMTU air quality can not only detect the results of engineering control failure and building construction activity, but also identify other factors influencing BMTU air quality. Air quality monitoring may require more labor and instrumental analysis. An accurate historical basis for comparisons is necessary to identify

changes in air quality. Data may accumulate at a relatively slow rate and a detected change in air quality may be due to the interaction of many factors. This strategy of surveillance requires a long term commitment, recognizing that implementable recommendations to improve air quality will follow slow processes of data collection and analysis.

Patient exposure estimates extract the important information about BMTU air quality into measurements immediately affecting patient dose. Infection control efforts should be concentrated on limiting patients' dose until effective methods of antibiotic prophylaxis exist. Regrettably, patient exposure estimates are the most invasive, the most difficult to conduct, and the hardest to interpret. Again, multicausality makes it difficult to prescribe effective corrective measures to reduce patients' exposures.

The focus of environmental measurements should obviously support goals of infection control surveillance. A hierarchy of measurement objectives which could support a BMTU surveillance program, are presented in Table 18. The phenomena appearing down the list become more difficult to measure. They also become more applicable toward judgements of infective risk. Airborne measurement objectives in this table are nested. That is, any airborne objective listed, contains all contaminants lower in the table. Advantages and disadvantages for each of these environmental measurement objectives will be discussed below.

TABLE 18. POSSIBLE OBJECTS OF ENVIRONMENTAL MEASUREMENT

Settled or trapped fungal spores
Airborne particulates (total, nonspecific)
Airborne spores (viable and non-viable)
Viable airborne fungal organisms

Settled or trapped fungal spores are the easiest to collect. Included in these types of measurements are: air-cleaning filter sampling, wipe sampling, and gravity-settle plate collection methods. A reproducible method of recovery of spores from air cleaning filters must overcome difficulties of spore viability and efficiency of capture. Since roughing filters in the BMTU room HEPAs are replaced after each patient, analysis could provide qualitative information on the patient's most recent exposures. Wipe sampling and settle plate methods have poor efficiencies for smaller spores, like the conidia of *Aspergillus* species. These methods may be more applicable when fungal particles originate from attrition processes, like hospital construction. Despite the difficulties in interpretation, collection methods for settled or trapped spores are among the methods most often used.

Volumetric air measurements are more directly applicable to estimating patient exposure than the collection of non-airborne fungi. Recommendations for instrument and method selection of continuous, instantaneous, or time-integrative techniques, are available for airborne particulates (5), spores (78), and viable organisms (113). Ultimately, we are concerned with the number of viable pathogenic fungal organisms which may be inhaled by the neutropenic or otherwise, compromised patients. We could substitute measurement objectives and infer patient dose, if individual pathogens comprised a predictable portion of airborne fungal particles, spores (viable and non-viable), or total particulates. Presently, there is no evidence to suggest that this is so.

Currently, genus and species of many spores of the Deuteromycetes class cannot be identified except by culturing. Often the spores

themselves cannot be distinguished from inorganic particulates. In addition, spore viability cannot be determined except by culturing.

A successful program of infection control in the BMTU requires the involvement of the patient, medical staff, engineering and maintenance department, housekeeping personnel, and the infection control department. The various groups should participate in the selection of surveillance goals. Cooperation of all those concerned will greatly enhance environmental data collection and other activities focused toward environmental control.

6. FURTHER RESEARCH

In this study, fungal particles were measured in a specialized patient care area of a hospital. A number of factors were successfully shown to influence the presence of potentially infectious agents in the patient room. As the objectives of this study were met, the need for further investigation became apparent. Given the current limits of time, money and instrumentation, a strategy for further research is outlined below. The following plan emphasizes the accurate estimation of patient exposure to fungal organisms.

Additionally, this research would enable us to accomplish important goals. It would characterize the aerodynamics of the patient room, validating or invalidating the substitution of area sampling for breathing-zone sampling. It would identify and characterize variables to predict the room fungal spore concentration using a mass-balance model. And this strategy may identify the relationship between the concentration of airborne fungal organisms and other suspended particulates to simplify the analysis of air sampling.

Rooms like those in this BMTU have been shown to be less effective in controlling airborne microorganisms than unidirectional laminar air flow rooms (9). However, if patient exposures in rooms of this BMTU are found to be below infective levels, the expense of providing expensive unidirectional laminar airflow can be avoided.

6.1 Room Aerodynamics: The patient rooms have a relatively simple configuration. After careful measurements of room volume, room air supply, exhaust, etc., airflow can be characterized by velocity measurements at points in a three dimensional grid. A circuit of air is expected to pass through the HEPA filter unit to the patients' breathing zone and return to the HEPA filter. Measurements can be made to observe airflow pattern changes which result from different rates of HEPA filtration. If similar airflow patterns are achievable by replacing the HEPA filters with a simple diffuser, the effect of HEPA filtration can be quantified.

Using a suitable tracer gas and instrument which continuously records concentration of the gas, the mixing of the air in the room and decay rate of the tracer gas can be observed. Results should agree with concentrations based on a one-compartment model. Concentration measurements should be made in the patients' breathing zone and compared to other sampling areas in the room. To simulate possible contaminant sources, the tracer gas should be releases from areas near the air supply and all exhaust locations. The rate of HEPA recirculation should be varied in experimental trials. At this point, conclusions can be made as to whether area air sampling can be representative of breathing zone values for a gaseous contaminant.

After the behavior of the tracer gas is adequately characterized, the experiments should be repeated with a suitable aerosol tracer. Potassium iodide (36,37) and sodium chloride (38) particles have been successfully used as experimental tracers in a hospital setting. Previous studies have found aerosols transfer less effectively than gases (47). Observations of the aerosol decay rate and mixing behavior

of the room based on aerosol measurements are expected to be similar to gas values when a simple diffuser is substituted for the HEPA filter. Again, the aerosol measurements should stress the patient's breathing zone and the source of the aerosol generation should be near the air supply inlet, the ceiling exhaust, and undercuts of the bath, toilet and hall doors. When the HEPA filter is returned to the filter unit, measurements should agree with decay rates resulting from 100% filtration.

At this time, it can be determined whether area particulate sampling can be substituted for breathing zone sampling. If the substitution cannot be made, recommendations for locations of air sampling on the BMTU should reflect this fact. Otherwise, recommendations toward a single sampling location, producing results which represent the aerosol concentration in the room, can be made at this time.

6.2 Predictive Model: Results from air samples in the BMTU may be used to estimate patient breathing zone exposures. The AGI 30 or an equivalent method should be used. The AGI 30 method accurately measures the number of airborne fungal organisms.

If comparable results are obtained by using some other air quality measuring technique, it could be substituted for the AGI 30. The liquid impinger method is time-integrative. If the alternate method provides continuous monitoring, we may be able to detect instantaneous changes in air quality more accurately.

A thorough record of important variables influencing (or thought to influence) fungal spore concentration should be kept. Room and inlet spore concentrations should be measured. Spore concentrations outside

the room door should also be measured, as well as, frequency and speed of door opening. This can be accomplished by a rheostat connected to the door hinge. A strip chart recorder can be used to record the position of the door at any time.

One study (111) using a gaseous tracer, found that the amount of air transferred by opening a 3 ft × 7 ft door, was half a cubic meter. The result was shown to be independent of the temperature gradient between hall and room, for a six-second door opening. In another experiment, particularly well designed, Keimig and associates (56) showed that the velocity of door opening affected the transfer of airborne contaminants in a scale model. The same study showed that the present direction of door swing on the BMTU is more prone to transfering contaminants from the hall to the room.

Ultimately, the data collected should be assembled in the form of a mass balance equation (Equation 1, page 34), so that the validity of the model can be experimentally tested. Different factors influencing air quality may be identified by each method. When the methods agree, inferences may be made concerning the relationship between the different objects of measurement. Statistical methods of time series and autocorrelation may be used to analyze continuous data.

6.3 Surrogate Sampling: If a method of air quality monitoring produces comparable results to our standard method, it can be substituted to reduce analysis time, effort and expense. It should be clear, after enough data has been collected to test the one-compartment model, whether the alternate method selected is an adequate substitute for the AGI. If it is not clear, the comparison may resume. Otherwise, a different technique of air quality measurement may be selected and

compared to the AGI. Meanwhile, patient exposures are regularly estimated and, hopefully, additional factors influencing air quality recognized.

7. REFERENCES

1. Aisner, J., Murillo, J., Schimpff, S.C., *et al.*, "Invasive Aspergillosis in Acute Leukemia: Correlation with Nose Cultures and Antibiotic Use," Annals of Internal Medicine, Vol. 90, No. 1, pp. 4-9 (Jan. 1979).

2. Aisner, J., Schimpff, S.C., Bennett, J.E., *et al.*, "Aspergillus Infections in Cancer Patients: Association with Fireproofing Materials in a New Hospital," Journal of the American Medical Association, Vol. 235, No. 4, pp. 411-412. (Jan. 26, 1976).

3. Ajello, L., "Natural Habitats of the Fungi that Cause Pulmonary Mycoses," Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung I, Suppl. 8, pp. 31-42 (1980).

4. Alteras, I. and Lehrer, N., "Fungal Flora in the Air of a Large Hospital," Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung I, Suppl. 8, pp. 123 (1980).

5. American Conference of Governmental Industrial Hygienists, Air Sampling Instruments for Evaluation of Atmospheric Contaminants, Sixth edition, Cincinnati, Ohio, 1983.

6. American Hospital Association, A.H.A. Guide to the Health Care Field, Chicago, Illinois, 1988.

7. American Society of Heating, Refrigerating, and Air-Conditioning Engineers, Inc., "Method of testing air-cleaning devices used in general ventilation for removing particulate matter," ASHRAE Standard 52-76, New York, 1976.

8. Andersen, A.A., "New Sampler for the Collection, Sizing and Enumeration of Viable Airborne Particles," Journal of Bacteriology, Vol. 76, No. 5, pp. 471-484 (Nov. 1958).

9. Arlet, G., Gluckman, E., Gerber, F., *et al.*, "Measurement of Bacterial and Fungal Air Counts in Two Bone Marrow Transplant Units," Journal of Hospital Infection, Vol. 13, No. 1, pp. 63-69 (Jan. 1989).

10. Armstrong, D., "Protected Environments are Discomforting and Expensive and Do Not Offer Meaningful Protection," American Journal of Medicine, Vol. 76, No. 4, pp. 685-689 (April 1984).

11. Arnow, P.M., Anderson, R.L., Mainous, P.D., *et al.*, "Pulmonary Aspergillosis during Hospital Renovation," American Review of Respiratory Diseases, Vol. 118, No. 1, pp. 49-53 (July 1978).

12. Baker, R.D., "Leukopenia and Therapy in Leukemia as Factors Predisposing to Fatal Mycoses: Mucormycosis, Aspergillosis, Cryptococcus," American Journal of Clinical Pathology, Vol. 37, No. 4, pp. 358-373 (April 1962).

13. Baker, R.D., "Mucurmycosis (Opportunistic Phycomycosis)," pp. 832-918. In The Pathologic Anatomy of Mycosis: Human Infection with Fungi, Actinomycetes and Algae, R.D. Baker, ed., Springer-Verlag, New York, 1971.

14. Banerjee, U.C., Weber, P., Ruffin, J., *et al.*, "Airborne Fungi Survey of Some Residences in Durham, North Carolina, U.S.A.," Grana, Vol. 26, No. 2, pp. 151-157 (June 1987).

15. Benson, F.B., Henderson, J.J. and Caldwell, D.E., Indoor-Outdoor Air Pollution Relationships: A Literature Review, U.S. Environmental Protection Agency, National Environmental Research Center Publication No. AP-112, 1972.

16. Bioaerosols Committee, "Bioaerosols: Guidelines for Assessment and Sampling of Saphrophytic Bioaerosols in the Indoor Environment," Applied Industrial Hygiene, Vol. 2, No. 5, pp. R10-R16 (Sept. 1987).

17. Bodey, G.P., "Current Status of Prophylaxis of Infection with Protected Environments," American Journal of Medicine, Vol. 76, No. 4, pp. 678-684 (April 1984).

18. Bodey, G.P., Buckley, M., Sathe, Y.S., *et al.*, "Quantitative Relationships between Circulating Leukocytes and Infection in Patients with Acute Leukemia," Annals of Internal Medicine, Vol. 64, No. 2, pp. 328-340 (Feb. 1966).

19. Bodey, G.P., Freireich, E.J. and Frei, E., III, "Studies of Patients in a Laminar Air Flow Unit," Cancer, Vol. 24, No. 5, pp. 972-980 (Nov. 1969).

20. Bodey, G.P., Hart, J., Freireich, E.J., *et al.*, "Studies of a Patient Isolator Unit and Prophylactic Antibiotics in Cancer Chemotherapy - General Techniques and Preliminary Results," Cancer, Vol. 22, No. 5, pp. 1018-1026 (Nov. 1968).

21. Brachman, P.S., Ehrlich, R., Gabelli, V.J., *et al.* "Standard Sampler for Assay of Airborne Microorganisms," Science, Vol. 144, No. 3624, pp. 1295 (June 12, 1964).

22. Burge, H.A. and Solomon, W.R., "Sampling and Analysis of Biological Aerosols," Atmospheric Environment, Vol. 21, No. 2, pp. 451-456 (1987).

23. Burge, H.A., Solomon, W.R. and Boise, J.R., "Comparitive Merits of Eight Popular Media in Aerometric Studies of Fungi," Journal of Allergy and Clinical Immunology, Vol. 60, No. 3, pp. 199-209 (Sept. 1977).

24. Burton, J.R., Zachery, J.B., Bessin, R., et al., "Aspergillosis in Four Renal Transplant Recipients: Diagnosis and Effective Treatment with Amphotericin B," Annals of Internal Medicine, Vol. 77, No. 3, pp. 383-388 (Sept. 1972).

25. Byard, R.W., Bonin, R.A. and Haq, A.U., "Invasion of Paranasal Sinus by *Aspergillus oryzae*," Mycopathologia, Vol. 96, No. 1, pp. 41-43 (Oct. 1986).

26. Cambridge Filter Corporation, "Series 2 Hi-Flow Air Filters," Cambridge Bulletin 134, Syracuse, N.Y., 1978.

27. Centers for Disease Control, "Nosocomial Infection Surveillance, 1981-1982," CDC Surveillance Summaries, Vol. 32, No. 4SS, pp. 1SS-16SS (1983).

28. Centers for Disease Control, "Nosocomial Infection Surveillance, 1983," CDC Surveillance Summaries, Vol. 33, No. 2SS, pp. 9SS-21SS (1984).

29. Centers for Disease Control, "Nosocomial Infection Surveillance, 1984," CDC Surveillance Summaries, Vol. 35, No. 1SS, pp. 17SS-29SS (1986).

30. Curtis, S.E., Balsbaugh, R.K. and Drummond, J.G., "Comparison of Anderson Eight-Stage and Two-Stage Viable Air Samplers," Applied and Environmental Microbiology, Vol. 35, No. 1, pp. 208-209 (Jan. 1978).

31. Edmonds, R.L. (ed.), Aerobiology: The Ecological Systems Approach, Dowden, Hutchinson & Ross, Stroudsburg, Penn. 1979.

32. England, A.C., III, Weinstein, M., Ellner, J.J., et al., "Two Cases of Rhinocerebral Zygomycosis (Mucormycosis) with Common Epidemiologic and Environmental Features," American Review of Respiratory Disease, Vol. 124, No. 4, pp. 497-498 (Oct. 1981).

33. Environmental Safety Office, "Baseline Sampling Results," Duke University Medical Center, Durham, N.C., 1988.

34. Environmental Safety Office, "Occupancy Sampling Results," Duke University Medical Center, Durham, N.C., 1988.

35. Environmental Safety Office, "Preoccupancy Sampling Results," Duke University Medical Center, Durham, N.C., 1988.

36. Foord, N. and Lidwell, O.M., "Airborne Infection in a Fully Air-Conditioned Hospital. II. Transfer of Airborne Particles between Rooms Resulting from the Movement of Air from One Room to Another," Journal of Hygiene (Cambridge), Vol. 75, No.1, pp. 31-44 (Aug. 1975).

37. Foord, N. and Lidwell, O.M., "The Control by Ventilation of Airborne Bacterial Transfer Between Hospital Patients, and its Assessment by Means of a Particle Tracer. I. An Airborne-Particle Tracer for Cross-Infection Studies," Journal of Hygiene (Cambridge), Vol. 70, No. 2, pp. 279-286 (June, 1972).

38. Fraser, D.A., "An Innocuous Tracer Technique for Testing the Performance of Ventilation Systems," American Industrial Hygiene Association Journal, Vol. 26, No. 5, pp 490-497 (Sept.- Oct. 1965).

39. Gage, A.A., Dean, D.C., Schimert G., et al., "Aspergillus Infection after Cardiac Surgery," Archives of Surgery, Vol. 101, No. 3, pp. 384-387 (Sept. 1970).

40. Gillespie, V.L., Clark, C.S., Bjornson, H.S., et al. "A Comparison of Two Stage and Six Stage Anderson Impactors for Viable Aerosols," American Industrial Hygiene Association Journal, Vol. 42, No. 12, pp. 858-864 (Dec. 1981).

41. Greater Durham Chamber of Commerce, "Durham profile," pp. 3 and 4 of Community Services Pages. In Durham/Butner/Creedmoor/Raleigh Durham Airport/Research Triangle Park, General Telephone Co. of the South, Durham, N.C., 1988.

42. Greene, W.H. and Adriole, V.T., "An Outbreak of Nosocomial Invasive Aspergillosis," Abstract #546. In 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, 1980.

43. Gregory, P.H., The Microbiology of the Atmosphere, Second Edition, Leonard Hill, London, 1973.

44. Grieco, M.H., "Introduction to the abnormal Host and Complicating Infections," pp. 1-10. In Infections in the Abnormal Host, M.H. Grieco, ed., Yorke Medical Books, New York, 1980.

45. Haley, R.W., Culver, D.H., White, J.W., et al., "The Nationwide Nosocomial Infection Rate: A New Need for Vital Statistics," American Journal of Epidemiology, Vol. 121, No. 2, pp. 159-167 (Feb. 1985).

46. Haley, R.W., White J.W., Calver, D.H., et al., "The Financial Incentives for Hospitals to Prevent Nosocomial Infections under the Prospective Payment System: An Emperical Determination from a Nationally Representative Sample," Journal of the American Medical Association, Vol. 257, No. 12, pp. 1611-1614 (March 27, 1987).

47. Hambraeus, A. and Sanderson, H.F., "The Control by Ventilation of Airborne Bacterial Transfer Between Hospital Patients, and its Assessment by Means of a Particle Tracer. III. Studies with a Airborne-Particle Tracer in an Isolation Ward for Burned Patients," Journal of Hygiene (Cambridge), Vol. 70, No. 2, pp. 299-312 (June 1972).

48. Haynes, B.W., Jr. and Hench, M.E., "Hospital Isolation System for Preventing Cross Contamination by Staphyloccal and Psuedomonas Organisms in Burn Wounds," Annals of Surgery, Vol. 162, No. 4, pp. 641-649 (Oct. 1965).

49. Hays, W.L., Statistics, Holt, Rinehart and Winston, New York, 1963.

50. Hernanz, A.P., Fereres, J., Garraus, S.L., et al., "Nosocomial Infection by *Rhizomucor pusillus* in a Clinical Haematology Unit," Journal of Hospital Infection, Vol. 4, No. 1, pp. 45-49 (Mar. 1983).

51. Hopkins, C.C., Weber, D.J. and Rubin, R.H., "Invasive Aspergillosis Infection: Possible Non-ward Common Source within the Hospital Environment," Journal of Hospital Infection, Vol. 13, No. 1, pp. 19-25 (Jan. 1989).

52. Hughes, W.T. and Gosier, J.W., "Thermophilic Fungi in the Mycoflora of Man and Environmental Air," Mycopathologia et Mycologia Applicata, Vol. 49, No. 1, pp. 77-78 (Jan. 31, 1973).

53. Hunt, D.L., Personal Communication, 1989.

54. Ishizu, Y., "General Equation for the Estimation of Indoor Pollution," Environmental Science & Technology, Vol. 14, No. 10, pp. 1254-1257 (Oct. 1980).

55. Jameson, B., Gamble, D.R., Lynch, J., et al., "Five-year Analysis of Protective Isolation," Lancet I, No. 7708, pp. 1034-1040 (May 22, 1971).

56. Keimig, S.D., Galvin, M.L. and Sansone, E.B., "The Influence of the Direction of Door Swing and Door Velocity on the Transfer of Airborne Contaminants in a Scale Model," Applied Industrial Hygiene, Vol. 2, No. 3, pp. 103-107 (May 1987).

57. Keye, J.D., Jr. and Magee, W.E., "Fungal Diseases in a General Hospital: A Study of 88 Patients," American Journal of Clinical Pathology, Vol. 26, No. 11, pp. 1235-1253 (Nov. 1956).

58. Kramer, C.L., Pady, S.M., Rogerson, C.T. et al., "Kansas Aeromycology. II. Materials, Methods and General Results," Transactions of the Kansas Academy of Science, Vol. 62, No. 3, pp. 184-199 (Fall 1959).

59. Krasinski, K., Holzman R.S., Hanna, B., et al., "Nosocomial Fungal Infection During Hospital Renovation," Infection Control, Vol. 6, No. 7, pp. 278-282 (July 1985).

60. Krick, J.A. and Remington, J.S., "Opportunistic Invasive Fungal Infections in Patients with Leukemia and Lymphoma," Clinics in Haematology, Vol. 5, No. 2, pp. 249-310 (June 1976).

61. Kyriakides, G.K. Zimmerman, H.H., Hall, W.H., et al., "Immunologic Monitoring and Aspergillosis in Renal transplant Patients," American Journal of Surgery, Vol. 131, No. 2, pp. 246-252 (Feb. 1976).

62. Lentino, J.R., Rosenbranz, M.A., Micheals, J.A., et al., "Nosocomial Aspergillosis: A Retrospective Review of Airborne Disease Secondary to Road Construction and Contaminated Air Conditioners," American Journal of Epidemiology, Vol. 116, No. 3, pp. 430-437 (Sept. 1982).

63. Leopold, S.S., "'Positive Hole' Statistical Adjustment for a Two-Stage, 200-Hole-per-Stage Anderson Air Sampler," American Industrial Hygiene Association Journal, Vol. 49, No. 2, pp. A88-A90 (Feb. 1988).

64. Levenson, S.M., Trexler, P.C., Malm, O.J., et al., "A Disposable Plastic Isolator for Operating in a Sterile Environment," Surgical Forum, Vol. 11, pp. 306-308 (1960).

65. Lidwell, O.M. and Lovelock, J.E., "Some Methods of Measuring Ventilation," Journal of Hygiene (Cambridge), Vol. 44, No. 5, pp. 326-332 (May 1946).

66. Lidwell, O.M. and Noble, W.C., "Fungi and Clostridia in Hospital Air: the Effect of Air-Conditioning," Journal of Applied Bacteriology, Vol. 39, No. 3, pp. 251-261 (Dec. 1975).

67. Mahoney, D.H., Steuber, C.P., Starling, K.A., et al., "An Outbreak of Aspergillosis in Children with Acute Leukemia," Journal of Pediatrics, Vol. 95, No. 1, pp. 70-72 (July 1979).

68. Margolin, B.H., Resnick, M.A., Rimpo, J.Y. et al., "Statistical Analyses for *In Vitro* Cytogenetic Assays Using Chinese Hamster Ovary Cells," Environmental Mutagenesis, Vol. 8, No. 2, pp. 183-204 (1986).

69. May, K.R., "Calibration of a Modified Anderson Bacterial Aerosol Sampler," Applied Microbiology, Vol. 12, No. 1, pp. 37-43 (Jan. 1964).

70. May, K.R., "Prolongation of Microbiological Air Sampling by a Monolayer on Agar Gel," Applied Microbiology, Vol. 18, No. 3, pp. 513-514 (Sept. 1969).

71. May, K.R. and Druett, H.A., "The Pre-impinger, a Selective Aerosol Sampler," British Journal of Industrial Medicine, Vol. 10, No. 3, pp. 142-151 (July 1953).

72. Michaelsen, G.S., Vesley, D. and Halbert, M.W., "Laminar Flow Studied as Aid in Care of Low Resistance Patients," Hospitals, Vol. 41, No. 12, pp. 91-106 (June 16, 1967).

73. Montgomery, D.C. and Peck, E.A., Introduction to Linear Regression Analysis, John Wiley & Sons, New York, 1982.

74. National Research Council, Indoor Pollutants, National Academy Press, Washington, 1981.

75. Nauseef, .M. and Maki, D.G., "A Study of the Value of Simple Protective Isolation in Patients with Granulocytopenia," New England Journal of Medicine, Vol. 304, No. 8, pp. 448-453 (Feb. 19, 1981).

76. Newman, K.A., Schimpff, S.C. Young, V.M., et al., "Lessons learned for Surveillance Cultures in Patients with Acute Nonlymphatic Leukemia: Usefulness for Epidemiologic, Preventative, and Therapeutic Research," American Journal of Medicine, Vol. 70, No. 2, pp. 423-431 (Feb. 1981).

77. Noble, W.C. and Clayton, Y.M., "Fungi in the Air of Hospital Wards," Journal of General Microbiology, Vol. 32, No. 3, pp. 397-402 (Oct. 1963).

78. Ogden, E.C., Raynor, G.S., Hayet, J.V., et al., Manual for Sampling Airborne Pollen, Hafner Press, New York, 1974.

79. Opal, S.M., Asp. A.A., Cannady, P.B., Jr., et al. "Efficacy of Infection Control Measures during a Nosocomial Outbreak of Disseminated Aspergillosis Associated with Hospital Construction," Journal of Infectious Diseases, Vol. 153, No. 3, pp. 634-637 (March 1986).

80. Piccioli, J.E., Personal Communication, 1988.

81. Pizzo, P.A., "Do Results Justify the Expense of Protected Environments," In Controversies in Oncology, P.H. Wiernik (ed.), John Wiley & Sons, New York, 1982.

82. Pizzo, P.A., "Granulocytopenia and Cancer Therapy: Past Problems, Current Solutions, Future Challenges," Cancer, Vol. 54, Suppl. 11, pp. 2649-2661 (Dec. 1, 1984).

83. Pizzo, P.A. and Levine, A.S., "The Utility of Protected-Environment Regimens for the Compromised Host: A Critical Assessment," Progress in Hematology, Vol. 10, pp. 311-332 (1977).

84. Rhame, F.S., "Lessons from the Roswell Park Bone Marrow Transplant Aspergillosis Outbreak," Infection Control, Vol. 6, No. 9, pp. 345-346 (Sept. 1985).

85. Rhame, F.S., "Endemic Nosocomial Filamentous Fungal Disease: A Proposed Structure for Conceptualizing and Studying the Environmental Hazard," Infection Control, Vol. 7, Suppl. 2, pp. 124-125 (Feb. 1986).

86. Rhame, F.S., Streifel, A.J., Kersey, J.H. et al., "Extrinsic Risk Factors for Pneumonia in the Patient at High Risk of Infection," American Journal of Medicine, Vol. 76, Suppl. 5A, pp. 42-52 (May 15, 1984).

87. Rhame, F.S., Streifel, A.J., Stevens, P. et al., "Endemic Aspergillis Airborne Spore Levels are a Major Risk Factor for Aspergilloses in Bone Marrow Transplant (BMT) Patients," Abstract #147. In 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, 1985.

88. Rippon, J.W., Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes, Third Edition, W.B. Sanders, Philadelphia, 1988.

89. Rogers, T.R. and Barnes, R.A., "Prevention of Airborne Fungal Infection in Immunocompromised Patients," Journal of Hospital Infection, Vol. 11, Suppl. A, pp. 15-20 (Feb. 1988).

90. Rogerson, C.T., "Kansas Aeromycology. I. Comparison of Media," Transactions of the Kansas Academy of Science, Vol. 61, No. 2, pp. 155-162 (Summer 1958).

91. Rose, H.D., "Mechanical Control of Hospital Ventilation and Aspergillus Infections," American Review of Respiratory Disease, Vol. 105, No. 2, pp. 306-307 (Feb. 1972).

92. Rosen, P.P. and Sternberg, S.S., "Decreased Frequency of Aspergillosis and Mucormycosis," New England Journal of Medicine, Vol. 295, No. 23, pp. 1319-1320 (Dec. 2, 1976).

93. Rotstein, C., Cummings, M., Tidings, J., et al., "An Outbreak of Invasive Aspergillosis Among Allogeneic Bone Marrow Transplants: A Case-Control Study," Infection Control, Vol. 6, No. 9, pp. 347-355 (Sept. 1985).

94. Sarrubbi, F.A., Jr., Kopf, H.B., Wilson, M.B., et al., "Increased Recovery of *Aspergillus flavus* from Respiratory Specimens during Hospital Construction," American Review of Respiratory Disease, Vol. 125, No. 1, pp. 33-38 (Jan. 1982).

95. Sayer, W.J., Shean, D.B. and Chossieri, J., "Estimation of Airborne Fungal Flora by the Anderson Sampler versus the Gravity Settling Plate," Journal of Allergy, Vol. 44, No. 4, pp. 214-227 (Oct. 1969).

96. Schwartz, S.A. and Perry, S., "Patient protection in Cancer Chemotherapy," Journal of the American Medical Association, Vol. 197, No. 8, pp. 623-627 (Aug. 22, 1966).

97. Schimpff, S.C., "Protected Environments for the Treatment of High Risk Cancer Patients," In Controversies in Oncology, P.H. Wiernik (ed.), John Wiley & Sons, New York, 1982.

98. Sen, P. and Lauria, D.B., "Higher Bacterial and Fungal Infections," pp. 325-359. In Infections in the Abnormal Host, M.H. Grieco, ed., Yorke Medical Books, New York, 1980.

99. Solberg, C.O., Matsen, J.M., Vesley, D. et al., "Laminar Airflow Protection in Bone Marrow Transplantation," Applied Microbiology, Vol. 21, No. 2, pp. 209-216 (Feb. 1971).

100. Solomon, W.R., Burge, H.P. and Boise, J.R., "Airborne Aspergillus fumigatus Levels Outside and Within a Large Clinical Center," Journal of Allergy and Clinical Immunology, Vol. 62, No. 1, pp. 56-60 (July 1978).

101. Smolansky, S.J., "Aspergillosis of the Paranasal Sinuses," Ear Nose and Throat Journal, Vol. 57, No. 8, pp. 320-323 (Aug. 1978).

102. Streifel, A.J., Stevens, P.P. and Rhame, F.S., "In-Hospital Source of Airborne Penicillum Species Spores," Journal of Clinical Microbiology, Vol. 25, No. 1, pp. 1-4 (Jan. 1987).

103. Titche, L.L., "Aspergillosis of the Maxillary Sinus," Ear Nose and Throat Journal, Vol. 57, No. 9, pp. 398-401 (Sept. 1978).

104. Thirumalaikolundusubramanian, P., Uma, A., Chandramohan, M., et al., "Yeast and Mould Flora in Hospital Air and Hospital Patients in India," Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung I, Suppl. 8, pp. 115-122 (1980).

105. Turner, A.G. and Hill, N.F., "Calibration of the Anderson 2000 Disposable Air Sampler," American Industrial Hygiene Association Journal, Vol. 36, No. 6, pp. 447-451 (June 1975).

106. Vanacek, K. and Gilbert, C., "Autologous Bone Marrow Transplantation Research Protocol Overview," Duke University Marrow Program, Durham, N.C., 1988.

107. Wadowsky, R.M. and Benner, S.M., "Distribution of the Genus Aspergillus in Hospital Room Air Conditioners," Infection Control, Vol. 8, No. 12, pp. 516-518 (Dec. 1987).

108. Weems, J.J.,Jr., Andremont, A., Davis, B.J., et al. "Pseudoepidemic of Aspergillosis after Development of Pulmonary Infiltrates in a Group of Bone Marrow Transplant Patients," Journal of Clinical Microbiology, Vol. 25, No. 8, pp. 1459-1462 (Aug. 1987).

109. Weems, J.J.,Jr., Davis, B.J., Tablan, O.C., et al., "Construction Activity: An Independent Risk Factor for Invasive Aspergillosis and Zygomycosis in Patients with Hematologic Malignancy," Infection Control, Vol. 8, No. 2, pp. 77-75 (Feb. 1987).

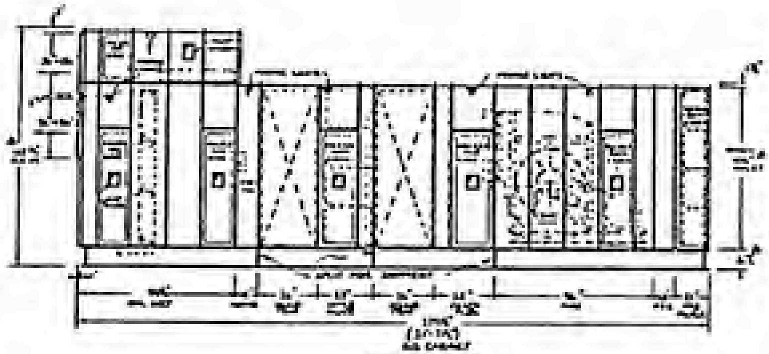
110. Wenzel, R.P., "Nosocomial Infections, Diagnosis-Related Groups, and Study on the Efficacy of Nosocomial Infection Control: Economic Implications for Hospitals under the Prospective Payment System," American Journal of Medicine, Vol. 78, Suppl. 613, pp. 3-7 (June 28, 1985).

111. Whyte, W. and Shaw, B.H., "Air Flow through Doorways." In Airborne Transmission and Airborne Infection, J.F. Ph. Hers and K.C. Winkler, eds., John Wiley & Sons, New York-Toronto, 1943.

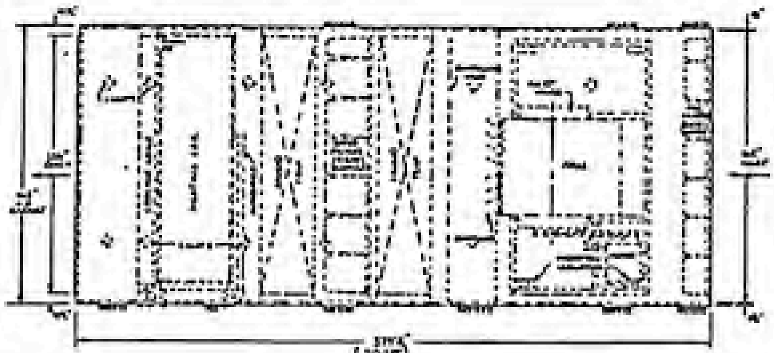
112. Winkler, K.C., "The Scope of Aerobiology," pp. 1-11. In Airborne Transmission and Airborne Infection, J.F. Ph. Hers and K.C. Winkler, eds., John Wiley & Sons, New York-Toronto, 1973.

113. Wolf, H.W., Skaliy, P., Hall, L.B., *et al.*, Sampling Microbiological Aerosols, U.S. Department of Health, Education, and Welfare, Public Health Service Monograph 60, 1964.

APPENDIX A



SIDE ELEVATION



PLAN VIEW

APPROX. UNIT WT.: 25,000#

77-30510-31

Sales Order No. 77-30510-31

Sales Engineer EH

Duke University Medical Center
Durham, North Carolina 27710

AH-31

Turner Construction Co.

Duke Hospital North,
Duke University, Durham, N.C.

Bellmuth, Obata & Kassabaum

Hayakawa Associates

48,000 CFM

5.8" T.S.P.

14 gauge

PACE GRAY ENAMEL

124W — 43¾" — 154" — 46.52

4 rows, 12 fins per inch, 5/8" OD, AL, 1448.1 MBH

571 — 21,540 — 53.3 — 103.3 — 96.6 GPM, 130° – 100° H₂O

.47 — 6.6 — 2.8 — 4 — 2½" — 2½" — ARI CERT.

106W — 3-30" — 154" — 96.25

6 rows, 10 fins per inch, 5/8" OD, AL, 1939.8 MBH — ARI CERT.

452 — 43,540 — 78.7 — 67 — 55.3 — 52.7 — 243.0 GPM, 42° – 58° H₂O

.52 — 5.7 — 3.0 — 4 — 2½" — 2½"

Cartridge (side load) 2-sets

Cartridge (rear load) 2-sets

MARATHON — 100 — 1750 — 3 — 460/3/60

O.D.P., HORIZ. — X

BY OTHERS

BROWNING (FIXED PITCH)

6R5V132 × 2 7/16 — 6.5SV150 × 2 15/16 — 3V × 150

NONE

"SARCO", 354 1⅛ SB STEAM, 12-PSIG, MODEL "MD-NHA", 12" VS (A-MANIFOLD)

2" spring defl. — flat X — MASON INDUSTRIES — X

"INDUSTRIAL ACOUSTICS CO", MODEL 3-S, 156" × 96" (2-REQ'D)

TVG — 7-7-77

77-30510-31

Sheet 2 of 2

DUKE HOSPITAL NORTH

DUKE UNIVERSITY MEDICAL CENTER

UNIT AH-31

CONSTRUCTION NOTES:

(1) CLASS II CONSTRUCTION. (WHEEL SHAFT & SCROLL.)
(2) BEARINGS WITH MINIMUM L-10 LIFE OF 200,000 HRS.
(3) DRAIN HOLE PROVIDED AT BOTTOM OF FAN HOUSING WITH THREADED PLUG.
(4) LUBRICATION LINES EXTENDED TO UNIT CASING WALLS. (1/4" O.D. NYLAFLOW PLASTIC TUBING & ALEMITE #1960-B PM ZERK FITTING.)
(5) FLEXIBLE CONNECTION ON FAN DISCHARGE (ELGEN DUCTLINER)
(6) MARINE LIGHTS PROVIDED IN ALL ACCESS SECTIONS. LIGHTS WIRED IN CONDUIT WITH SWITCH & DUPLEX RECEPTACLE.
(7) FAN SECTION PROVIDED WITH DOOR INTERLOCK SWITCH, TO SHUT FAN OFF WHEN DOOR IS OPENED. ("MICRO-SWITCH" CAT. #22 OR 23, AE SERIES.)
(8) WALK-IN ACCESS DOORS, WITH #260 VENTLOK & HANSEN #1 OSLN KEY LOCKING LATCHES. LATCHES OPERABLE FROM INSIDE & OUTSIDE CASING.

REQUIRED ON:

A) FAN SECTION
B) UPSTREAM COOLING COIL SECTION.

LATCH TYPE ACCESS DOORS, WITH #260 VENFLOK LATCHES. OPERABLE FROM INSIDE & OUTSIDE CASING.

REQUIRED ON:

A) HOT DECK
B) COLD DECK
C) AFTER FILTER, & SIDE LOAD PRE-FILTER SECTIONS
D) DISCHARGE PLENUM

(9) SECTIONS HAVING COOLING COIL OR HEATING & COOLING COILS SHALL BE OF DOUBLE WALL CONSTRUCTION, WITH 1 1/2" - 3 LB. DENSITY NEOPRENE COATED FIBERGLASS INSULATION. MIN. 24 GA. GALVANIZED STEEL LINER. THIS SHALL ALSO INCLUDE ALL DOWNSTREAM SECTIONS. ALL OTHER SECTIONS, INCLUDING PRE-HEAT COIL SECTION SHALL BE SINGLE WALL CONSTRUCTION. NO INSULATION OR INNER WALL LINER.
(10) DRAIN PAN DOUBLE CONSTRUCTED OF TYPE 304 STAINLESS STEEL. 1 1/4" CONDENSATE DRAIN. 1 1/2" - 3# DENSITY INSULATION.
(11) DIFFUSER PLATE IN FAN DISCHARGE SECTION. PERFORATIONS UNIFORMLY SPACED ON THE PLATE, PROVIDING OPENINGS HAVING A MINIMUM OF 60% FREE AREA OF THE PLATE.
(12) SQUAD TRAPS & FILTER ASSEMBLIES MOUNTED BY "PACE" AT FACTORY.
(13) HUMIDIFIER MANUFACTURED BY "SAMCO" - MOUNTED BY "PACE". CONTROLS BY CONTROL CONTRACTOR.
(14) VARIABLE VOLUME, MULTI-BLADED INLET VANES. VANE ASSEMBLIES EXTERNAL OF INLET CONE, INTERCONNECTED WITH SHAFT & SELF ALIGNING BEARINGS.
(15) COIL TUBE SHEETS SHALL BE MINIMUM 14 GAUGE GALVANIZED STEEL.

DUKE HOSPITAL NORTH			
DUKE UNIVERSITY, DURHAM, NORTH CAROLINA			
ENGINEER	HAYAKAWA ASSOCIATES		
ARCHITECT	HELLMUTH, OBATA & KASSABAUM	LOCATION	LEVEL - P AREA - B
CONTRACTOR	TURNER CONSTRUCTION CO	UNIT NO.	AH-31
SUB CONTRACTOR	INDUSTRIOTECH CONSTRUCTORS INC.	CONTRACT ORDER REFERENCE NO.	9-30

PACE

DRAWN BY	CHECKED BY	REVISIONS AND ISSUE RECORD (SHEET #'S AFFECTED)	BROD & McCLUNG-PACE CO.		DRAWING NO.
TVG			8800 S.E. McLOOD AVENUE PORTLAND, OREGON		77-30390-31
DATE 3/3/77	SCALE NTS				SHEET 1 OF 2

APPENDIX B

SAMPLING PROCEDURE

Materials

Pen and paper for recording sampling conditions
Vacuum pumps and 2-stage Andersen Impactors
Alcohol wipes and garbage bags
100 mm Sabouroud Agar Plates
Measuring tape and stopwatch
BMTU map and smoke tube with bulb
Ziplock Bags to secure exposed agar plates

Procedure

1. Record sampling conditions. Try to pair samples across control measures or barriers. i.e. across a closed door. Record DATE, TIME, SAMPLE DURATION, LOCATION (record on BMTU map), HEIGHT, SAMPLE TYPE (if not impactor, i.e. wipe), HEPA ON?, NUMBER OF ROOM OCCUPANTS, HALL DOOR OPEN?, VENTILATION ON?, COLLECTION OPERATOR, etc. Use NOC to specify Normal Operating Conditions.

2. Prepare 2-stage Andersen impactor by wiping above and below agar plate with an alcohol wipe prep pad. A finger inserted at the fold will allow two surfaces surrounding the sieve to be wiped with one sweep.

3. Assemble impactor with agar plates, verifying the plate is labeled correctly and is gently rocked into its correct position on the bottom of each stage. Place plate lids face down so no spores settle on lid while sampling.

4. Plug in vacuum pumps recording the time. Sample for 30 minutes. Record sample duration to the nearest one-half minute.

5. While sampling, smoke test rooms which communicate with the BMTU hallways, as well as the exits, gowning area, and workroom. Record results on BMTU map. If the smoke test indicates the air is flowing from the hall into the patient room, inform the Head Nurse, Michael Plumer.

6. After unplugging vacuum pumps, disassemble the impactor noting if the plates are correctly labeled.

7. Incubate plates at 30°C in the Clinical Microbiology Laboratory, agar side up, in the plastic Ziploc Bags.

8. After three days, record the number of colonies on each plate, identifying numbers of recognizable fungi. Confirm the presence of *Aspergillus* spp. or other potential pathogens with Medical Mycology.

APPENDIX C

AGAR PLATE PREPARATION.

Materials

Dextrose, 20g
Popypeptone, 10g
Agar, 20g
Distilled Water, 1000ml

Procedure

Mix thoroughly and heat with frequent agitation. Boil for approximately one minute until a solution occurs. Sterilize for 15 minutes at 121°C at 12-15 psi and allow to cool to 56°C. Dispense 18ml into 100 X 15 mm petri dishes using Pourmatic. When cool, label dish 'SAB' and heat seal in plastic sleeve. Refrigerate and store in walk-in cooler at 40°F

APPENDIX D

Sample No.	Date	Duration (min)	Occupancy Location	No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
1	880425	30	9211 Hall	NA	NA	NA	1Un	1.23
2	880425	30	9211 Room	0	no	NA	26Pe3Un	35.56
3	880425	30	9203 Hall	NA	NA	2Un	1Un	3.68
4	880425	30	9203 Room	0	yes	NA	NA	0.00
5	880506	30	9216 Room	0	no	NA	1Cl	1.23
6	880506	30	9216 Hall	NA	NA	NA	NA	0.00
7	880506	30	9213 Hall	NA	NA	NA	3Pe	3.68
8	880506	30	9213 Room	0	yes	NA	NA	0.00
9	880506	30	9204 Hall	NA	NA	NA	5Pe	6.13
10	880506	30	9204 Room	0	yes	NA	1Pe	1.23
11	880510	30	9201 Hall	NA	NA	NA	NA	0.00
12	880510	30	9201 Room	0	yes	NA	NA	0.00
13	880510	30	9220 Y	NA	NA	NA	1AF	1.23
14	880510	30	9200 Y	NA	NA	NA	NA	0.00
15	880511	30	9202 Hall	NA	NA	NA	NA	0.00
16	880511	30	9203 Room	0	yes	NA	NA	0.00
17	880511	30	9207 Hall	NA	NA	NA	NA	0.00
18	880511	30	9207 Room	0	yes	NA	NA	0.00
19	880512	30	9202 Hall	NA	NA	2Un	NA	2.45
20	880512	30	9202 Room	0	yes	NA	NA	0.00
21	880512	30	9206 Hall	NA	NA	NA	NA	0.00
22	880512	30	9206 Room	0	yes	NA	NA	0.00
23	880513	30	9205 Hall	NA	NA	2Un	1Un	3.68
24	880513	30	9205 Room	0	yes	1Un	NA	1.23
25	880513	30	9204 Hall	NA	NA	NA	2Un	2.45
26	880513	30	9204 Room	0	yes	NA	NA	0.00
27	880516	30	9213 Room	0	yes	NA	NA	0.00
28	880516	30	9213 Hall	NA	NA	NA	NA	0.00
29	880516	30	9216 Room	0	no	NA	NA	0.00
30	880516	30	9216 Hall	NA	NA	NA	NA	0.00
31	880516	34.5	9209 Room	0	yes	NA	NA	0.00
32	880516	34	9209 Hall	NA	NA	2HS	NA	2.16
33	880516	30	9205 Hall	NA	NA	NA	NA	0.00
34	880516	30	9205 Room	0	yes	1Cl	NA	1.23
35	880516	30	9201 Hall	NA	NA	2HS	NA	2.45
36	880516	30	9201 Room	0	yes	NA	NA	0.00
37	880520	30	9201 Room	0	yes	NA	NA	0.00
38	880520	30	9201 Hall	NA	NA	1Un	3Un	4.90
39	880520	30	9100 Y	NA	NA	5Un	7Un	14.71
40	880520	30	9205 Room	0	yes	NA	NA	0.00
41	880520	30	9205 Hall	NA	NA	1Un	NA	1.23
42	880520	31	9209 Room	0	yes	NA	NA	0.00
43	880520	30	9209 Hall	NA	NA	NA	2Un	2.45
44	880520	30	9213 Room	0	yes	NA	NA	0.00
45	880520	30	9213 Hall	NA	NA	NA	2Un	2.45
46	880520	30	9216 Room	0	no	1Un	24Un	30.66
47	880520	31	9216 Hall	NA	NA	4Un	11Un	17.80
48	880520	31	9300 Y	NA	NA	4Un	4Un	9.49
49	880520	30	9200 Entry	NA	NA	NA	4Un	4.90

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
50	880529	30	9200 Y	NA	NA	2Un	4Un	7.36
51	880529	30.5	9220 Y	NA	NA	1Un	NA	1.21
52	880525	30	9216 Room	0	no	NA	NA	0.00
53	880525	30	9216 Hall	NA	NA	1Un	2Cl	3.68
54	880525	30.5	9209 Room	0	yes	NA	2Cl	2.41
55	880525	30	9209 Hall	NA	NA	1Cl1HS	2Pe2HS	7.36
56	880525	30	9201 Room	0	yes	NA	1Cl1Pe	2.45
57	880525	30	9201 Hall	NA	NA	1HS	3Cl4Pe	9.81
58	880525	31	9205 Room	0	yes	NA	NA	0.00
59	880525	31	9205 Hall	NA	NA	NA	4Un	4.75
81	880530	30	9205 Hall	NA	NA	NA	NA	0.00
82	880530	30	9205 Room	0	yes	NA	NA	0.00
83	880530	30	9201 Hall	NA	NA	NA	NA	0.00
84	880530	30	9201 Room	0	yes	NA	NA	0.00
85	880530	30	9209 Room	0	yes	NA	NA	0.00
86	880530	30	9209 Hall	NA	NA	NA	1HS	1.23
87	880530	30	9200 Y	NA	NA	?Pe?HS	?Pe?Cl?HS	22.07
88	880530	30	9220 Y	NA	NA	?Pe?Un	?Pe1AT	46.60
89	880531	30	9216 Room	0	no	NA	NA	0.00
90	880531	30	9216 Hall	NA	NA	1Un	13Un1AF1AB	19.62
91	880531	30	9213 Room	0	yes	NA	NA	0.00
92	880531	30	9213 Hall	NA	NA	NA	4Pe	4.90
93	880531	30	9209 Room	0	yes	NA	NA	0.00
94	880531	30	9209 Hall	NA	NA	1Un	NA	1.23
95	880531	30	9201 Room	0	yes	NA	NA	0.00
96	880531	30	9201 Hall	NA	NA	3Un	1Un	4.90
97	880531	20	9205 Room	1	yes	NA	NA	0.00
98	880531	30	9205 Hall	NA	NA	NA	3Un	3.68
99	880601	30	9201 Room	1	yes	NA	NA	0.00
100	880601	30	9201 Hall	NA	NA	1Un	2Un	3.68
101	880601	31	9205 Room	2	yes	NA	NA	0.00
102	880601	31	9205 Hall	NA	NA	NA	2Un	2.37
103	880601	30	9209 Room	0	yes	NA	NA	0.00
104	880601	30	9209 Hall	NA	NA	2Un	6Un1AB	11.04
105	880601	30	9213 Room	0	yes	NA	1Un	1.23
106	880601	30	9213 Hall	NA	NA	2Un	14Un	19.62
107	880601	30	9216 Room	0	no	NA	NA	0.00
108	880601	30	9216 Hall	NA	NA	NA	1AB1AB22Un	29.43
111	880603	30	9216 Room	0	no	3Un	1Un	4.90
112	880603	30	9216 Hall	NA	NA	NA	2Un	2.45
113	880603	30	9201 Room	2	yes	1Un	NA	1.23
114	880603	30	9201 Hall	NA	NA	3Un	NA	3.68
115	880603	30	9213 Room	0	yes	NA	NA	0.00
116	880603	30	9213 Hall	NA	NA	1Un	1Un	2.45
117	880603	30	9209 Room	0	yes	NA	NA	0.00
118	880603	30	9209 Hall	NA	NA	1Un	2Un	3.68
119	880603	30	9205 Room	1	yes	NA	NA	0.00
120	880603	30	9205 Hall	NA	NA	NA	2Pe	2.45
121	880606	30	9201 Room	1	yes	NA	NA	0.00

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
122	880606	30	9201 Hall	NA	NA	5Un	5Un	12.26
123	880606	33	9205 Room	2	yes	NA	1Cl	1.11
124	880606	33	9205 Hall	NA	NA	NA	1Un	1.11
125	880606	29.5	9209 Room	0	yes	NA	NA	0.00
126	880606	30	9209 Hall	NA	NA	NA	6Un	7.36
127	880606	30	9213 Room	0	yes	NA	NA	0.00
128	880606	30	9213 Hall	NA	NA	1Un	8Un1AF1Pa	13.49
129	880606	30	9216 Room	0	no	1Un	3Un	4.90
130	880606	30	9216 Hall	NA	NA	1Cl	1AF1AB1AB14Un	22.07
131	880608	30	9201 Room	2	yes	1Un	NA	1.23
132	880608	30	9201 Hall	NA	NA	1Un	1Un	2.45
133	880608	30	9205 Room	1	yes	1Un	NA	1.23
134	880608	30	9205 Hall	NA	NA	NA	NA	0.00
135	880608	30.5	9209 Room	0	yes	NA	NA	0.00
136	880608	30	9209 Hall	NA	NA	1Un	2Un	3.68
137	880608	30	9213 Room	0	yes	NA	NA	0.00
138	880608	30	9213 Hall	NA	NA	1Un	1Ab6Ab3Ab17Un	34.33
139	880608	30	9216 Room	0	no	NA	NA	0.00
140	880608	30	9216 Hall	NA	NA	2Un	1Ab2Ab6Un	14.71
141	880609	30	9202 Room	1	yes	NA	NA	0.00
142	880609	30	9202 Hall	NA	NA	2Un	1Un	3.68
143	880609	30	9204 Room	2	yes	1Un	NA	1.23
144	880609	30	9204 Hall	NA	NA	3Un	2Un	5.13
145	880609	30	9206 Room	2	yes	1Un	NA	1.23
146	880609	30	9206 Hall	NA	NA	NA	NA	0.00
147	880609	30	9200 Y	NA	NA	1Un	NA	1.23
148	880609	30	9220 Y	NA	NA	1Un	3Un	4.90
149	880609	30	9208 Room	0	yes	NA	NA	0.00
150	880609	30	9208 Hall	NA	NA	NA	NA	0.00
151	880610	30	9201 Room	1	yes	1Un	1AN	2.45
152	880610	30	9201 Hall	NA	NA	NA	NA	0.00
153	880610	29.5	9203 Room	1	yes	NA	NA	0.00
154	880610	29.5	9203 Hall	NA	NA	NA	NA	0.00
155	880610	30	9207 Room	5	yes	3Un	NA	3.68
156	880610	30	9207 Hall	NA	NA	NA	1Un	1.23
157	880610	30.5	9210 Room	1	yes	NA	NA	0.00
158	880610	30.5	9210 Hall	NA	NA	NA	1Un	1.21
159	880610	30	9216 Room	0	no	NA	NA	0.00
160	880610	30	9216 Hall	NA	NA	NA	1Zy	1.23
161	880612	30	9200 Y	NA	NA	1Rh	1Rh	2.45
162	880612	30	9220 Y	NA	NA	7Un	1Rh2Un	12.26
163	880613	30	9201 Room	4	yes	NA	1Pe	1.23
164	880613	30	9201 Hall	NA	NA	1Un	NA	1.23
165	880613	30	9204 Room	2	yes	NA	NA	0.00
166	880613	30	9204 Hall	NA	NA	NA	NA	0.00
167	880613	30	9206 Room	1	yes	NA	NA	0.00
168	880613	30	9206 Hall	NA	NA	1HS	NA	1.23
169	880613	30	9208 Room	0	yes	NA	NA	0.00
170	880613	30	9208 Hall	NA	NA	2Un	1Cl	3.68

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
171	880613	30	9211 Room	0	yes	NA	1Pe	1.23
172	880613	30	9211 Hall	NA	NA	2Un	NA	2.45
175	880614	30	9214 Room	0	no	NA	NA	0.00
176	880614	30	9214 Hall	NA	NA	NA	2Un	2.45
177	880614	30	9207 Room	0	yes	NA	NA	0.00
178	880614	30	9207 Hall	NA	NA	2Un	1Un	3.68
179	880614	30	9210 Room	1	yes	NA	NA	0.00
180	880614	30	9210 Hall	NA	NA	2Un	1Un	3.68
181	880614	30.5	9212 Room	0	no	NA	NA	0.00
182	880614	30.5	9212 Hall	NA	NA	NA	1Un	1.21
183	880614	30	9202 Room	3	yes	NA	NA	0.00
184	880614	30	9202 Hall	NA	NA	1AN	NA	1.23
185	880614	30	9203 Room	1	yes	NA	NA	0.00
186	880614	30	9203 Hall	NA	NA	1Un	1Cl	2.45
187	880615	30.5	9202 Room	2	yes	NA	1Un	1.21
188	880615	30.5	9202 Hall	NA	NA	NA	3Cl	3.62
189	880615	30	9204 Room	2	yes	NA	1Cl	1.23
190	880615	30	9204 Hall	NA	NA	2Un	2Cl1Un	6.13
191	880615	30	9206 Room	2	yes	NA	NA	0.00
192	880615	30	9206 Hall	NA	NA	1Un	1Un	2.45
193	880615	30	9200 Y	NA	NA	4Un	3Un	8.58
194	880615	30	9220 Y	NA	NA	4Un1Cl	4Un	11.04
195	880615	30	9208 Room	0	yes	NA	NA	0.00
196	880615	30	9208 Hall	NA	NA	1Un	NA	1.23
197	880615	30	9215 Room	0	yes	NA	NA	0.00
198	880515	30	9215 Hall	NA	NA	1Un	1Un1Cl	3.68
199	880616	30	9202 Room	3	yes	4Un	4Cl	9.81
200	880516	30	9203 Hall	NA	NA	3Un	1Un	4.90
201	880616	30	9207 Room	1	yes	NA	NA	0.00
202	880616	30	9207 Hall	NA	NA	1Un	1AN	2.45
203	880615	30	9210 Room	1	yes	NA	NA	0.00
204	880615	30	9210 Hall	NA	NA	1Un	2Un1Cl	4.90
205	880616	30	9200 Y	NA	NA	1AF3Un	5Un	11.04
206	880616	30	9220 Y	NA	NA	12HS4Un1Cl	3Un3Cl	28.20
207	880616	30	9212 Room	0	no	NA	NA	0.00
208	880615	30	9212 Hall	NA	NA	1Un	NA	1.23
209	880516	30	9215 Room	0	yes	NA	NA	0.00
210	880616	30	9215 Hall	NA	NA	2Un	4Pe4Un	12.26
211	880617	30	9214 Room	0	no	NA	NA	0.00
212	880617	30	9214 Hall	NA	NA	NA	NA	0.00
213	880617	30	9202 Room	2	yes	NA	NA	0.00
214	880617	30	9202 Hall	NA	NA	1Un	NA	1.23
215	880617	30	9211 Room	0	yes	NA	NA	0.00
216	880617	30	9211 Hall	NA	NA	NA	NA	0.00
217	880617	30	9204 Room	2	yes	NA	NA	0.00
218	880617	30	9204 Hall	NA	NA	2Un	2Un	4.90
219	880617	30.5	9206 Room	0	yes	NA	NA	0.00
220	880617	30.5	9206 Hall	NA	NA	1Un	NA	1.21
221	880617	30	9208 Room	0	yes	NA	NA	0.00

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
222	880617	30	9208 Hall	NA	NA	NA	NA	0.00
223	880620	30	9202 Room	2	yes	NA	NA	0.00
224	880620	30	9202 Hall	NA	NA	2Un	2Un	4.90
225	880620	30	9204 Room	2	yes	7Un	1Un1Cl	11.04
226	880620	30	9204 Hall	NA	NA	NA	NA	0.00
227	880620	30	9206 Room	0	yes	NA	NA	0.00
228	880620	30	9206 Hall	NA	NA	3Un	1Un2Cl	7.36
229	880620	34	9200 Y	NA	NA	9Un	25Un	36.79
230	880620	34	9220 Y	NA	NA	12Un	21Un	35.70
231	880620	30	9210 Room	1	yes	NA	NA	0.00
232	880620	30	9210 Hall	NA	NA	1Un	1AF	2.45
233	880620	30	9216 Room	0	no	1Un	1Un	2.45
234	880620	30	9216 Hall	NA	NA	1Un	1Un	2.45
237	880622	30	9201 Room	1	yes	NA	NA	0.00
238	880622	30	9201 Hall	NA	NA	3Un2Cl	2Un1Cl	9.81
239	880622	30	9203 Room	0	yes	NA	NA	0.00
240	880622	30	9203 Hall	NA	NA	3Un3Cl	2Un	9.81
241	880622	30	9205 Room	1	yes	NA	NA	0.00
242	880622	30	9205 Hall	NA	NA	1Un	4Cl	6.13
243	880622	30.5	9207 Room	1	yes	NA	NA	0.00
244	880622	30.5	9207 Hall	NA	NA	2Un	1AN1Un1Cl	7.24
245	880623	30	9211 Room	0	yes	NA	2Un	2.45
246	880623	30	9211 Hall	NA	NA	2Un	2Cl2Un	7.36
247	880623	30	9215 Room	0	yes	NA	NA	0.00
248	880623	30	9215 Hall	NA	NA	1Un	NA	1.23
249	880627	30.5	9214 Room	0	no	NA	NA	0.00
250	880627	30.5	9214 Hall	NA	NA	NA	1Un	1.21
251	880627	30	9212 Room	0	no	NA	NA	0.00
252	880627	30	9212 Hall	NA	NA	NA	NA	0.00
253	880627	30	9222 Room	NA	NA	NA	NA	0.00
254	880627	30	9209 Room	0	yes	NA	NA	0.00
255	880627	30	9209 Hall	NA	NA	NA	NA	0.00
256	880627	30	9222 Stair	NA	NA	2Un	3Un1Cl	7.36
257	880704	30.5	9201 Room	1	yes	NA	NA	0.00
258	880704	30.5	9201 Hall	NA	NA	NA	NA	0.00
259	880704	30	9203 Room	3	yes	NA	NA	0.00
260	880704	30	9203 Hall	NA	NA	NA	NA	0.00
261	880704	30	9205 Room	3	yes	NA	1Cl	1.22
262	880704	30	9205 Hall	NA	NA	1Un	1Un	2.45
263	880704	30	9207 Room	2	yes	NA	NA	0.00
264	880704	30	9207 Hall	NA	NA	NA	NA	0.00
265	880704	30	9204 Room	1	yes	NA	NA	0.00
266	880704	30	9220 X	NA	NA	1Un	NA	1.23
267	880704	30	9200 Y	NA	NA	3Un	3Un	7.36
268	880704	30	9220 Y	NA	NA	32Un	5Un	45.37
269	880704	30	9202 Room	0	yes	NA	NA	0.00
270	880704	30	9206 Room	3	yes	2Un	NA	2.45
271	880708	30	9201 Room	1	yes	NA	NA	0.00
272	880708	30	9201 Hall	NA	NA	NA	1Un1Cl	2.45

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
273	880708	30	9203 Room	3	yes	NA	1Un	1.22
274	880708	30	9203 Hall	NA	NA	NA	NA	0.00
275	880708	30	9205 Room	2	yes	NA	NA	0.00
276	880708	30	9205 Hall	NA	NA	NA	NA	0.00
277	880708	32	9207 Room	5	yes	NA	1Cl	1.15
278	880708	32	9207 Hall	NA	NA	1Un	NA	1.15
279	880708	30	9210 Room	1	yes	NA	NA	0.00
280	880709	30	9210 Hall	NA	NA	NA	NA	0.00
281	880708	30	9204 Room	1	yes	NA	NA	0.00
282	880708	30	9220 X	NA	NA	1Un	NA	1.23
283	880708	30.5	9202 Room	2	yes	NA	NA	0.00
284	880708	30	9206 Room	1	yes	NA	NA	0.00
285	880715	30	9201 Room	0	yes	NA	NA	0.00
286	880715	30	9201 Hall	NA	NA	NA	1Un1Cl	2.45
287	880715	30	9203 Room	1	yes	NA	NA	0.00
288	880715	30	9203 Hall	NA	NA	2Cl	1Un	3.68
289	880715	30	9205 Room	2	yes	NA	NA	0.00
290	880715	30	9205 Hall	NA	NA	2Un	1Un	3.68
291	880715	32	9207 Room	3	yes	NA	1Un	1.15
292	880715	32	9207 Hall	NA	NA	7Un	11Un1HS	21.84
293	880715	32.5	9210 Room	1	yes	NA	NA	0.00
294	880715	32.5	9210 Hall	NA	NA	NA	1Un	1.13
295	880715	30	9202 Room	2	yes	NA	NA	0.00
296	880715	30	9206 Room	2	yes	NA	NA	0.00
297	880715	30	9204 Room	1	yes	NA	NA	0.00
298	880715	30	9220 X	NA	NA	1AB2Un	3Un	7.36
299	880719	30	9216 Room	0	no	NA	NA	0.00
300	880719	30	9216 Hall	NA	NA	NA	25Un1As	31.88
301	880719	30	9213 Room	0	yes	NA	NA	0.00
302	880719	30	9213 Hall	NA	NA	NA	1Un	1.23
303	880719	30	9209 Room	0	yes	NA	NA	0.00
304	880719	30	9209 Hall	NA	NA	NA	2Un	2.45
305	880719	30	9200 Y	NA	NA	10Un	3Un	15.94
306	880719	30	9220 Y	NA	NA	NA	12Un	14.71
307	880725	30	9207 Room	1	yes	NA	NA	0.00
308	880725	30	9207 Hall	NA	NA	2Un	NA	2.45
309	880725	31	9201 Room	0	yes	NA	NA	0.00
310	880725	31	9201 Hall	NA	NA	6Un	2As	9.49
311	880725	30	9203 Room	3	yes	NA	1Pe	1.23
312	880725	30	9203 Hall	NA	NA	NA	NA	0.00
313	880725	30	9205 Room	2	yes	NA	NA	0.00
314	880725	30	9205 Hall	NA	NA	NA	1AN	1.22
315	880726	30	9220 Y	NA	NA	5Un	3Un	9.81
316	880726	30	9200 Y	NA	NA	3Un	NA	3.68
317	880726	30	9210 Room	1	yes	NA	NA	0.00
318	880725	30	9210 Hall	NA	NA	1Un	1Un	2.45
319	880726	30	9206 Room	2	yes	NA	NA	0.00
320	880726	30	9206 Hall	NA	NA	1Un	NA	1.23
321	880726	31	9202 Room	2	yes	NA	NA	0.00

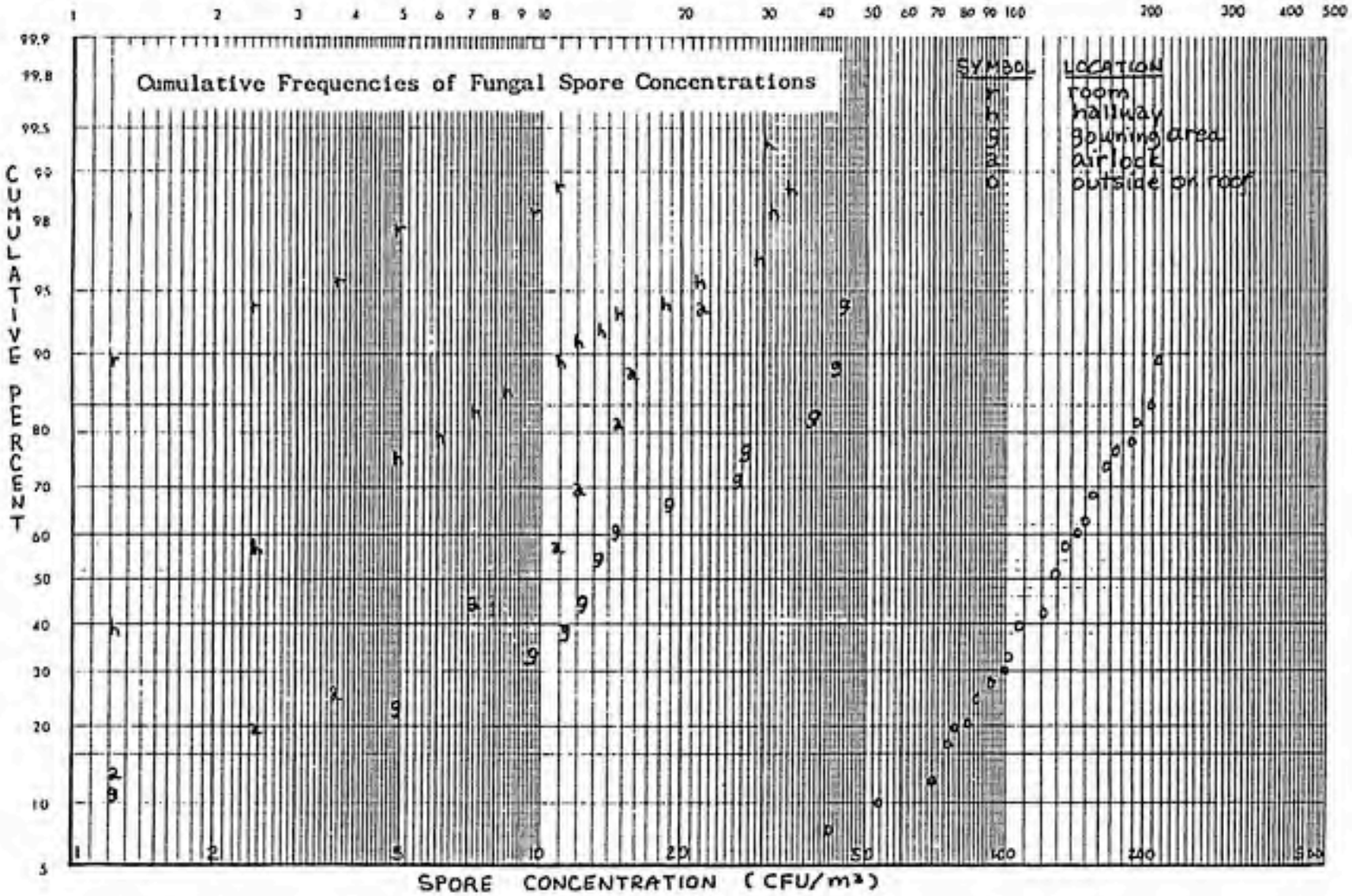
Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
322	880726	31	9202 Hall	NA	NA	NA	NA	0.00
323	880728	30	9215 Room	0	no	NA	NA	0.00
324	880728	30	9215 Hall	NA	NA	1Un	4Un	6.13
325	880728	30	9214 Room	0	no	NA	NA	0.00
326	880728	30	9214 Hall	NA	NA	1Un	6Un	8.58
327	880728	30	9212 Room	0	no	NA	NA	0.00
328	880728	30	9212 Hall	NA	NA	NA	3Un	3.68
329	880728	30	9220 X	NA	NA	2Un	NA	2.45
330	880728	30	9264 Room	0	NA	NA	1Un	1.23
331	880728	30	9264 Room	0	NA	NA	NA	0.00
332	880728	30	9220 Y	NA	NA	2Un	2Un	4.90
333	880728	30	9220 Y	NA	NA	1Un1Pe	2Un18Pe	26.98
334	880728	30	9263 Room	0	NA	2Un132Pe	200Pe	409.55
335	880728	30	9263 Room	1	NA	2Pe	32Pe	41.69
336	880728	30	9220 X	NA	NA	3Un144Pe	200Pe1Un1AT	427.94
337	880728	30	9200 Y	NA	NA	2Un	3Un7Pe	14.71
338	880728	30	9200 Entry	NA	NA	8Un	10Un	22.07
339	880729	30	9200 Entry	NA	NA	8Un	2Un1AN	13.49
340	880729	30	9200 Y	NA	NA	3Un	2Un1AN	7.36
341	880729	30	9220 Y	NA	NA	3Un23Pe	1Rh151Pe	218.26
342	880729	30	9220 X	NA	NA	89Pe	195Pe1Un	349.47
343	880729	30	9220 Hall	NA	NA	1Un	5Un2Pe	9.81
344	880729	30	9222 Hall	NA	NA	1Un	1Un	2.45
345	880729	30	9264 Room	0	NA	1Un	1Pe	2.45
346	880729	30	9263 Room	0	NA	2Un	1Un	3.68
347	880801	30	9200 Y	NA	NA	2Un	8Un	12.26
348	880801	30	9220 Y	NA	NA	9Un	1AN4Un2Cl	19.62
349	880801	30	9220 X	NA	NA	1Zy1Un	5Sy1AF2Un	12.26
350	880801	30	9263 Room	NA	NA	3Un	1As1Rh8Un	15.94
351	880801	30	9215 Room	0	yes	NA	NA	0.00
352	880801	30	9215 Hall	NA	NA	NA	6Un	7.36
353	880801	30	9213 Room	0	yes	NA	NA	0.00
354	880801	30	9213 Hall	NA	NA	NA	1Un	1.23
355	880801	30	9211 Room	0	yes	NA	NA	0.00
356	880801	30	9211 Hall	NA	NA	NA	NA	0.00
357	880802	30	9207 Room	2	yes	NA	1Pe	1.23
358	880802	30	9207 Hall	NA	NA	NA	1As2Un1Pe	4.90
359	880802	30	9206 Room	0	yes	NA	NA	0.00
360	880802	30	9206 Hall	NA	NA	NA	3Un	3.68
361	880802	30	9205 Room	0	yes	NA	NA	0.00
362	880802	30	9220 X	NA	NA	NA	2As1Pe9Un	14.71
363	880802	30	9204 Room	1	yes	NA	NA	0.00
364	880802	30	9204 Hall	NA	NA	1Un	2Un	3.68
365	880802	30	9203 Room	0	yes	NA	NA	0.00
366	880802	30	9202 Room	2	yes	NA	NA	0.00
367	880802	30	9201 Room	3	yes	1Un	NA	1.23
368	880802	30	9201 Hall	NA	NA	2Un	1Un	3.68
369	880805	30	9200 Entry	NA	NA	1Un	3Un	4.90

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
370	880805	30	9200 Y	NA	NA	4Un	4Un2C1	12.26
371	880805	30	9220 Y	NA	NA	6Un	5Un	13.49
372	880805	30	9220 X	NA	NA	2Un	5Un	8.58
373	880805	30	9263 Room	0	no	NA	1Un	1.23
374	880805	30	9264 Room	0	no	3Un	2Un	6.13
375	880805	30	9247 Room	0	no	1Un	NA	1.23
376	880805	30	9220 Hall	NA	NA	1Un	1Un	2.45
377	880805	30.5	9222 Room	NA	no	1Un	1Un	2.41
378	880805	30	9223 Hall	NA	NA	NA	1Un	1.23
379	880805	30	9210 Room	1	yes	NA	NA	0.00
380	880805	30	9210 Hall	NA	NA	1Un	1Un	2.45
381	880808	20	9216 Room	0	no	NA	NA	0.00
382	880808	30	9216 Hall	NA	NA	NA	NA	0.00
383	880808	30	9214 Room	0	no	NA	NA	0.00
384	880808	30	9214 Hall	NA	NA	5Un	2Un	8.58
385	880808	30	9212 Room	0	no	NA	NA	0.00
386	880808	20	9212 Hall	NA	NA	1Un	1Un	2.45
387	880808	30	9263 Room	0	no	1AB2Un	1Un1AN	6.13
388	880808	30	9264 Room	0	no	NA	3Un	3.68
389	880808	30	9220 Y	NA	NA	4Un	1Pe3Un	9.81
390	880808	30	9220 X	NA	NA	2Un	3Un1AB	7.36
391	880809	30	9201 Room	3	yes	1Un	1Un	2.45
392	880809	20	9201 Hall	NA	NA	NA	2Un	3.68
393	880809	30	9202 Room	1	yes	NA	NA	0.00
394	880809	30	9203 Room	3	yes	NA	NA	0.00
395	880809	30	9204 Room	1	yes	NA	NA	0.00
396	880809	30	9204 Hall	NA	NA	1Un	NA	1.23
397	880809	30	9205 Room	1	yes	NA	NA	0.00
398	880809	30	9205 Hall	NA	NA	1Un	NA	1.23
399	880809	30	9207 Room	1	yes	NA	NA	0.00
400	880809	30	9207 Hall	NA	NA	NA	1Un	1.23
401	880809	30	9210 Room	1	yes	NA	NA	0.00
402	880809	30	9210 Hall	NA	NA	1Un	NA	1.23
403	880811	30	9212 Room	0	no	NA	1Un	1.23
404	880811	10	AH32 Roof	NA	NA	O'grn	14Un	TMTC
405	880811	11.5	AH32 Roof	NA	NA	O'grn	O'grn	TMTC
406	880811	30	9212 Room	0	no	3Un	1AN	4.90
407	880811	11	AH32 Roof	NA	NA	O'grn	O'grn	TMTC
408	880811	21	AH32 Roof	NA	NA	O'grn	O'grn	TMTC
409	880811	55.5	9212 Room	0	no	NA	NA	0.00
410	880811	25	AH32 Roof	NA	NA	O'grn	2AC1AN48Un	TMTC
411	880811	11	AH32 Roof	NA	NA	8Un	30Un	127.08
412	880811	14	AH32 Roof	NA	NA	10Un	2AN26Un	99.85
413	880811	35	9212 Room	0	no	NA	2Un	2.10
414	880812	30	9200 Entry	NA	NA	6Un	O'grn	TMTC
415	880812	30	9200 Y	NA	NA	2Un	9Un	13.49
416	880812	30	9220 Y	NA	NA	8Un	2Un12y	13.49
417	880812	30	9220 X	NA	NA	NA	1Un	1.23
418	880812	30	9263 Room	0	no	NA	1Un	1.23

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
419	880812	30	9264 Room	0	no	NA	NA	0.00
420	880812	30	9247 Room	0	no	NA	6Un	7.36
421	880812	30	9220 Hall	NA	NA	NA	12Un	14.71
422	880812	30	9206 Room	1	yes	1Un	2Un	3.68
422	880812	30	9206 Hall	NA	NA	1Un	2Un	3.68
424	880812	30	9223 Hall	NA	NA	NA	1Un	1.23
425	880812	30	9222 Room	NA	NA	NA	2Un	2.45
426	880814	5	AH31 Roof	NA	NA	3Un	1AN8Pe10Un	161.86
427	880814	5	AH32 Roof	NA	NA	10Un	1AN9Pe1Un	154.50
428	880814	5	AH31 Roof	NA	NA	12y1Un	9Pe12Un1AN	176.57
429	880814	5	AH32 Roof	NA	NA	3Pe10Un	1Pe12y3Un	132.43
430	880814	5	AH31 Roof	NA	NA	8Un	14Un	161.86
431	880814	5	AH32 Roof	NA	NA	1Pe11Un2Cl	1AN5Pe4As7Un	235.43
432	880814	5	AH31 Roof	NA	NA	5Un	1Pe1Rh	51.50
433	880814	5	AH32 Roof	NA	NA	3Un	7Pe4Un2Cl	161.86
434	880814	30.5	9214 Room	0	no	5Un	NA	6.03
435	880814	30	9212 Room	0	no	NA	NA	0.00
436	880814	30	9212 Room	0	no	NA	NA	0.00
437	880814	30	9214 Room	0	no	NA	NA	0.00
438	880814	30	9246 Room	0	no	NA	2Pe	2.45
439	880814	30	9264 Room	0	no	NA	NA	0.00
440	880814	5	AH31 Roof	NA	NA	1Pe6Un	3Pe13Un	169.22
441	880814	5	AH32 Roof	NA	NA	1C15Un	5Pe1As12Un	183.93
442	880814	5	AH31 Roof	NA	NA	6Un	5Pe5Un	117.72
442	880814	5	AH32 Roof	NA	NA	2Un	6Pe1As9Un	132.43
447	880814	5	AH31 Roof	NA	NA	3Un	12Pe1Zy22Un1AN	286.93
448	880814	5	AH32 Roof	NA	NA	3Un	7Pe29Un	286.93
449	880814	5	AH31 Roof	NA	NA	1Pe4Un	7Pe1Rh14Un	198.64
450	880814	5	AH32 Roof	NA	NA	11Un	9Pe15Un1As	264.86
451	880815	5	AH31 Roof	NA	NA	NA	4Pe17Un1As	161.86
452	880815	5	AH32 Roof	NA	NA	3Un	10Un	95.64
453	880815	5	AH31 Roof	NA	NA	4Un	4Pe24Un1As	242.79
454	880815	5	AH32 Roof	NA	NA	2Pe7Un	1Pe7Un	125.07
455	880815	5	AH31 Roof	NA	NA	1Pe11Un	14Un	191.29
456	880815	5	AH32 Roof	NA	NA	2Un	3Un1As	44.14
457	880815	5	AH31 Roof	NA	NA	NA	1Pe27Un1AN	213.36
458	880815	5	AH32 Roof	NA	NA	1Pe5Un	6Un	88.29
459	880815	5	AH31 Roof	NA	NA	4Un1Cl	2Un	51.50
460	880815	5	AH32 Roof	NA	NA	1Pe6Un	2Pe9Un	132.43
461	880815	5	AH31 Roof	NA	NA	14Un	2Pe21Un	272.22
462	880815	5	AH32 Roof	NA	NA	3Un	1Pe19Un	169.22
463	880815	30	9214 Room	0	no	NA	NA	0.00
464	880815	30	9212 Room	0	no	NA	1Un	1.23
465	880815	30	9214 Room	0	no	NA	NA	0.00
466	880815	30	9212 Room	0	no	NA	NA	0.00
467	880815	30	9246 Room	0	no	NA	NA	0.00
468	880815	30.5	9264 Room	0	no	1Un	4Un	6.03

Sample No.	Date	Duration (min)	Location	Occupancy No.	HEPA	Analysis Results 1st Stage	2nd Stage	Fungus Conc. (cfu/m3)
469	880815	5	AH31 Roof	NA	NA	10Un	32Un1AT	316.36
470	880815	5	AH32 Roof	NA	NA	12Un	22Un2Pe1AT	272.22
471	880815	5	AH31 Roof	NA	NA	1Rh2Un	6Un	66.21
472	880815	5	AH32 Roof	NA	NA	9Un	14Un2Pe1AT	191.29
473	880815	5	AH31 Roof	NA	NA	10Un	12Un4Pe2AT	206.00
474	880815	5	AH32 Roof	NA	NA	7Un	7Un	103.00
475	880815	5	AH31 Roof	NA	NA	1Pe2Un	7Un1Pe1AT	95.64
476	880815	5	AH32 Roof	NA	NA	7Un1Cl	5Un1Pe1Cl	110.36

APPENDIX B


Cumulative Frequencies of Fungal Spore Concentrations
SYMBOL
LOCATION
room
hallway
Spuming area
airlock
outside on roof
CUMULATIVE PERCENT
SPORE CONCENTRATION (CFU/m³)
1
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
100
200
300
400
500
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
1
2
5
10
20
50
100
200
500

APPENDIX F

BMTU ANALYSIS BY A SINGLE COMPARTMENT MODEL

Let: Q_{31} = room inlet airflow rate, supplied by AH-31, (m^3/hr)

Q_{32} = airflow rate, supplied by AH-32, (m^3/hr) = 20 Q_{31}

Q_{rm} = room HEPA airflow rate, (m^3/hr) = 3 Q_{31}

Q_{hl} = hallway HEPA airflow rate, (m^3/hr) = 10 Q_{31}

C_{31} = AH-31 filtered, inlet spore conc., (CFU/m^3)

C_{32} = AH-32 filtered, inlet spore conc., (CFU/m^3) = 7 C_{31}

E = 1.00, (dimensionless)

G = 0, (CFU/hr)

Equivalent[a] one-compartment variables are:

Q_i = BMTU inlet airflow rate, supplied to 16 rooms and hallway

$= 16\ Q_{31} + Q_{32} = 16\ Q_{31} + 20\ Q_{31} = 46\ Q_{31}$

Conditions of AH-31 Supply	C_i(CFU/m^3)	Q_r(m^3/hr)
unfiltered	$\dfrac{69\ C_{31}\ 16\ Q_{31} + C_{32}\ Q_{32}}{16\ Q_{31} + Q_{32}}$	$13(3\ Q_{rm}) + Q_{hl}$
filtered	$\dfrac{C_{31}\ 16\ Q_{31} + C_{31}\ Q_{32}}{16\ Q_{31} + Q_{32}}$	Q_{hl}

By substitution of simplified equivalents into $C = C_i \dfrac{Q_i + G}{Q_i + E\ Q_r}$:

$$\frac{C_{unfilt}}{C_{filter}} = \frac{34.56\ C_{31}\left[\dfrac{46\ Q_{31}}{46\ Q_{31} + 49\ Q_{31}}\right]}{3.39\ C_{31}\left[\dfrac{46\ Q_{31}}{46\ Q_{31} + 10\ Q_{31}}\right]} = \frac{16.73\ C_{31}}{2.79\ C_{31}} = 6.0$$

[a]During this study, only 13 out of 16 patient rooms had HEPA filtration. Unfiltered room concentration equals 69 times C_{31} from Table 13 (page 24).