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FINAL REPORT TITLE PAGE

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List of Terms and Abbreviations

ACH	Air changes per hour, a way to quantify ventilation relative to room volume.
AIR facility	Airborne Infections Research facility. Located in Mpumalanga Province. Site of controlled experimental studies on TB transmission and control interventions.
CADR	Clean air delivery rate – used to characterize room air cleaning devices.
CDC	Centers for Disease Control and Prevention (US).
CSIR	Council of Scientific and Industrial Research. A South African quasi governmental organization involved with research on the built environment.
Eq ACH	Equivalent ACH,
FAST campaign:	**F**ind cases **A**ctively**, S**eparate and **T**reat**,** campaign based on outcome of these experiments, that effective treatment rapidly stops transmission of MDR**.**
HEPA Filtration:	High Efficiency Particulate Air filters – used in room air cleaners tested in this project
MDR-TB	*Mycobacterium tuberculosis* resistant to at least INH and rifampin.
Mtb	*Mycobacterium tuberculosis*
MRC	Medical Research Council of South Africa, the lead partner in this project.
PPD	Purified Protein Derivative, the substance in the tuberculin skin test, or TST
SARS	Severe Acute Respiratory Syndrome, cause by a virus of the same name.
TB	Tuberculosis, disease caused by infection with *Mycobacterium tuberculosis*
TST	Tuberculin Skin Test
Upper room UVGI:	The use of 354 nm ultraviolet irradiation in the upper room to disinfect room air, ideally used with a paddle fan for good air mixing, as in this study.
UVGI	Ultraviolet germicidal irradiation, 254 nm UV used to disinfect air
XDR-TB	Xtensively Drug Resistant *Mycobacterium tuberculosis,* resistant to INH, rifampin, a fluoroquinolone, and an injectable beyond streptomycin.

Abstract

Background: This project aimed to test interventions to reduce the risk of airborne infection to workers. We used patients with multidrug resistant tuberculosis (MDR-TB) for our study because airborne person to person transmission is substantially the same for all infections with airborne potential, including SARS and influenza.

Methods: Our study site was the already established Airborne Infections Research (AIR) Facility, adjacent to a regional MDR-TB referral center, Mpumalanga, South Africa, to test 3 conventional interventions of interest: 1) upper room ultraviolet germicidal irradiation (UVGI), 2) room filtration air cleaners; and 3) surgical masks on patients. In this unique facility, guinea pigs breathing exhaust air from the clinical ward serve as quantitative air samplers for transmission from TB patients. The experimental plan was similar for all 3 interventions tested. For each of 5 experiments of 2-3 month total exposure, a series of 15-27 consenting, newly admitted infectious patients with MDR-TB served for periods of 2 to 4 weeks each to expose180 healthy guinea pigs. The guinea pigs were divided into two identical exposure chambers, each receiving equal exhaust air from the AIR facility on alternating days. The intervention being tested on the ward was also applied on alternate days, with exhaust air being delivered to one guinea pig chamber (intervention chamber) on the days the intervention was on in the patient rooms; and to the control exposure chamber on the days when the intervention was off. The difference in infection rate between the two chambers directly measured the effectiveness of the intervention for the entire patient cohort.

Results: 1) UVGI was found to be 80% effective with a ceiling fan providing good air mixing; 2) Room air filtration machines delivering (clean air delivery rate, CADR) of approximately 15 room air changes per hour (ACH) reduced the risk of infection by an estimated 20%, but this was not statistically significant; and 3) Surgical masks on patients were 53% effective in reducing infection of the guinea pigs. Another highly important but unanticipated observation made during these studies was that in the non intervention arm, transmission varied greatly from study to study depending on the presence or absence of patients among the cohort with undiagnosed extensively drug resistant TB (XDR-TB) who were inadequately treated with the standard South African treatment for MDR-TB.

Conclusions: This study: 1) confirmed that UVGI with ceiling fans can be highly effective under real world conditions, 2) that room air filtration machines were surprisingly ineffective under the same conditions, and 3) for the first time, that surgical face masks on patients were 53% effective in preventing transmission. Finally, and perhaps most importantly for global TB control efforts, these studies demonstrated that effective MDR treatment rapidly and markedly inhibited transmission from MDR patients, but not from unsuspected XDR patients. The latter observation will have important implications for the safety of MDR treatment in hospitals, clinics, and in the community. All of these results will impact on the protection of workers in the US and around the world for years to come.

Section 1

Significant (Key) Findings: This research project aimed to quantitatively test 3 conventional control strategies intended to protect workers from airborne infections. Specifically we wanted to see if: 1) surgical masks on individuals with airborne infections would reduce transmission by at least 50%; 2) whether *portable room air disinfection units would reduce transmission by at least 75%; and 3) whether* upper room germicidal air disinfection would reduce transmission by at least 75%.

The following constitute our key findings:

a. Simple **surgical masks** on infectious patients with MDR-TB were approximately 53% effective in preventing transmission to hundreds of sentinel guinea pigs in our South African Airborne Infections Research (AIR) Facility.
b. **Portable air (filtration) cleaners** that provided 15 equivalent room air changes per hour were estimated to be only 20% effective and did not reach statistical significance.
c. **Upper room germicidal ultraviolet** air disinfection fixtures with ceiling mixing fans were approximately 80% effective.
d. **Impact of treatment.** Another key finding of this research, not part of the planned experiments, may have the greatest impact on global TB control, and also has importance for workers caring for patients with MDR TB in the United States and other low-burden settings. In the control (non-intervention) arms of the 5 experiments conducted we observed wide variation in the rates of guinea pigs infected. Although patients were selected by uniform criteria believed to be associated with infectiousness (presence of cough, lung cavities on chest x-ray, TB organisms in sputum, absence of significant therapy), rates of guinea pig infection ranged from 1 to 77%. In our 3rd study of UV air disinfection, for example, 27 patients selected with the above criteria infected just 1 of 90 guinea pigs over 3 months (1%), whereas in the mask study, 17 apparently similar patients infected 77% of 90 guinea pigs over 3 months. These unexpected observations were puzzling at first, until we analyzed the results of bacteriology tests from the patients and from some guinea pigs that showed that there were no patients with extensively drug resistant (XDR) TB in the 3rd UV study, whereas there were XDR patients in the other 4 experiments, and in a pilot study where transmitted strains proved to be XDR. As had been shown for drug susceptible TB in similar studies 60 years ago, *it appears that effective treatment (that is, MDR treatment for MDR TB) suppresses infectiousness almost completely and almost immediately*, whereas infective treatment (MDR treatment for XDR TB) fails to suppress transmission.

Translation of Findings:

These 4 key findings indicate that:

a) **Surgical masks on patients** were intermediately effective in reducing cough-generated infectious particles. Moreover, these results were obtained under study conditions where nurses constantly reminded patients to wear their masks all day, except for eating or washing. It is likely that without as much supervision, surgical masks on patients would be less effective. On the other hand, surgical masks are probably best used short term, for example, during transport of infectious patients for tests, where good adherence may be easier on patients and easier to supervise. From that perspective, our results may underestimate effectiveness. Considering both perspectives, these results may provide a reasonably good estimate of the efficacy of surgical masks in preventing airborne transmission to workers and other patients.
b) **Portable filtration air cleaners** were not effective as used. We chose to test very well-made air cleaning units made in Switzerland, and ran them at flow rates that produced the equivalent of approximately 15 room air changes per hour (clean air delivery rate, CADR). To make these results comparable to the UV study, we kept the ceiling paddle fans on. This CADR was in addition to the 6 actual room air changes per hour produced by the air handling system in the AIR facility. We were quite surprised that the impact of these machines on guinea pig infection rates was minimal. It is possible that repeated evaluations with other configurations would show greater efficacy, but it is unlikely that efficacy could reach much more than the 53% produced by masks on patients, or the 80% efficacy found for upper room germicidal air disinfection with mixing fans. Moreover, many air cleaning machines that we see used in hospitals for airborne infection control have much lower CADRs and would be expected to be even less effective.
c) **Upper room UVGI fixtures with ceiling mixing fans** were highly effective in preventing airborne infection as used. This was not surprising as Escombe had shown about 73% efficacy using similar methods in Lima, Peru.

d) The findings on the **impact of effective treatment** suggest that if patients can be quickly identified and place on effective treatment (that is, tailored to their drug resistance pattern), transmission will stop almost immediately. In our study, patients were usually started on therapy the same day that they entered the AIR facility, not after days or weeks of therapy. The reduction in transmission occurred long before cough resolved or infectious organisms disappeared from their sputum. The rapid impact of treatment on TB transmission of drug susceptible TB was observed in epidemiological and similar experiments with guinea pigs in the past, but those important results have been overlooked in recent years, resulting in prolonged hospitalization around the world, often for many months until sputum tests show the absence of infectious organisms.

Outcomes/ Impact:

a) Surgical masks on patients

i. Potential impact: For those who are overly reliance on surgical masks on patients to prevent airborne infection, these data show that they are only about 50% effective, and could reduce false confidence. However, 50% effective is in theory equivalent to doubling ventilation, which is often difficult to do, especially in poor settings. For those dismissive of the use of surgical mask use, we have provided the first data on efficacy under real life conditions, and could lead to increased use with realistic expectations.

ii. Intermediate impact: These data have only recently been published, but have gotten significant attention, including a story for the popular press by Reuters. These data are likely to be used soon in cost-effectiveness studies in comparing surgical masks to various other interventions for use for airborne infections such as TB, and those with airborne potential like influenza and SARS.

iii. Final impact: There has not been time to evaluate the final impact of these data. It will be difficult to measure the impact of these data on infections among workers, which is why our study was done.

b) Room air filtration machines

i. Potential impact: These data, especially when replicated, will discourage the use of air cleaning devices for airborne infection control. Although they have the potential to be effective, as we see them used in hospitals around the world, they are unlikely to be highly effective.

ii. Intermediate impact: This was our last experiment and the data has only recently been analyzed, but will be published in the near future. We are already incorporating these findings in airborne infection control trainings around the world for engineers and architects.

iii. Final impact: There has not been time to assess the final impact of these data and it is extremely difficult to measure the impact of airborne infection control interventions in the workplace.

c) Upper room UV air disinfection

i. Potential impact: These data provide strong support for the broader application of upper room UVGI fixtures with ceiling mixing fans for airborne infection control. It further validates previous data suggesting that this technology is robust under hospital conditions as different as Lima, Peru and Mpumalanga, South Africa. It is particularly well suited to

ii. Intermediate impact: Upper room UVGI with mixing fans is currently in use, but has been controversial. These data support the immediate development and dissemination of improved application guidelines, the encouragement of improved and affordable fixtures, and the inclusion of UVGI in engineering and infection control training.

iii. Final impact: There has been insufficient time to assess the full impact of these data. Experience has shown that it will be difficult to measure the impact of UVGI on health care worker safety. This was an important rationale for the current study.

d) Impact of effective treatment on transmission

i. Potential impact: Combined active cough surveillance, rapid molecular diagnostics (Xpert TB), and effective treatment guided by molecular drug susceptibility testing could revolutionize TB infection control globally.

ii. Intermediate impact: These data are being used now as part of a new USAID/TB CARE "core" TB IC campaign called, "F-A-S-T" standing for Find cases Actively (by molecular diagnostics), Separate, and Treat (based on molecular drug susceptibility testing).

iii. Final impact: It is too soon to say how much impact this data will have on TB infection control.

Testing Interventions to Prevent Human-Generated Occupational Airborne Infections

Specific Aims *(from the original proposal)***:** Faced with the emerging threats of avian influenza, SARS, multidrug-resistant *M. tuberculosis* (MDR-Mtb), and bioterrorism, there is an urgent need not only to test conventional approaches to protecting workers from airborne infections, but to develop new, potentially more effective technologies and to test them in a standardized way as they become available.

Our specific aims are to test three types of infection control interventions for efficacy in reducing airborne transmission under similar conditions of human infectious source strength, host susceptibility, and physical environment. The first intervention is perhaps the most basic form of source control - a surgical mask on the infectious individual intended to reduce the numbers of large droplets and droplet nuclei released. The remaining interventions are forms of air disinfection in common use that have never been subjected to rigorous controlled testing against human-generated infectious aerosols under real life conditions. Of these three interventions, two (masks and portable air disinfection units) can be deployed quickly as an emergency response, and one (upper room UV) would be part of a pre-planned prevention strategy. All three interventions are applicable to a wide variety of airborne infectious agents. Our three principal hypotheses and their corresponding specific aims are:

A.1. Rapid response interventions

1. **Hypothesis:** Surgical masks on individuals with airborne infections will reduce transmission by at least 50%.
    a. **Specific Aim:** To determine if surgical masks on individuals with airborne infections are at least 50% effective in reducing transmission.
2. **Hypothesis:** Portable room air disinfection units will reduce transmission by at least 75%.
    a. **Specific Aim:** To establish a relationship between portable air disinfection unit clean air delivery rate (CADR) and protection from human-source airborne infection in a well-mixed room.
        i. **Sub-hypothesis:** Clean air delivery rate (CADR) is the critical determinant for portable air disinfection units, regardless of the method of disinfection
        ii. **Sub-hypothesis:** A commonly used office-type portable air disinfection unit with limited CADR will be less than 75% effective in preventing transmission.

A.2. Pre-planned preventive intervention

1. **Hypothesis:** Upper room germicidal irradiation will reduce transmission by at least 75%.
    a. **Specific Aim:** To determine if upper room UVGI reduces transmission from human-generated infectious aerosol by at least 75% in a well-mixed room at low humidity.
        i. **Sub-hypothesis:** Mechanical air mixing is an essential component of upper room germicidal air disinfection.
        ii. **Sub-hypothesis:** High humidity reduces the efficacy of upper room germicidal air disinfection against human-generated airborne infection.

B. BACKGROUND AND SIGNIFICANCE

Some respiratory infections, such as measles and tuberculosis, are known to be spread almost exclusively by the airborne route. Other infections, such as influenza and SARS, have been clearly shown to have airbornepotential[1-3], but the relative importance of the airborne route compared to large droplet spread remains unclear. Still other infections, such as anthrax and smallpox, while not normally significant airborne threats in nature, are potential biological weapons and could have devastating consequences in the workplace. Apart from quarantine of known infectious sources, the conventional defenses against airborne spread to workers include

building ventilation and other means of air disinfection, personal respiratory protection, and source control, i.e.,administrative controls, and masks on potentially infectious sources. However, these interventions have never been rigorously tested against human-source contagion under controlled conditions. One limitation to

testing such interventions is the inability to quantitatively culture human-generated airborne viruses and bacteria of interest from the air under real life conditions because of low concentrations and competing environmental organisms[4]. Molecular detection methods are under investigation, but currently available methods are qualitative and unable to distinguish infectious from inactivated infectious agents[5]. Microorganisms grown in culture have been artificially aerosolized in various media (Table 2) at high concentrations in small or large chambers and successfully collected with mechanical air sampling over relatively short periods[6]. We and others have used this approach to test upper room various air disinfection technologies, but is unclear whether the results accurately predict the protection possible against aerosol generated by humans episodically coughing and sneezing from respiratory infections under real life conditions[7,8]. Surrogate test organisms such as *Escherichia coli* and *Serratia marcescens* have long been used in when the organism of interest is unavailable, hazardous, or difficult to grow[9,10]. As a prototype airborne infectious pathogen, *Mycobacterium tuberculosis* (*Mtb*) can be used to study interventions aimed at a variety of other agents that are completely or partially airborne. Unlike influenza and other respiratory infections that are often transient and seasonal, human-generated Mtb aerosols can be studied at any time due to the chronicity of the disease and its unfortunate high prevalence in many parts of the world. Interventions proven to be effective against human-generated *Mtb* are likely to be as or more effective against other airborne microorganisms, since *Mtb* is particularly resistant to decontamination and killing due to its protective cell wall.

Multidrug-resistant (MDR) *Mtb* is an especially valuable airborne test agent because patients may remain sputum smear and culture positive for months despite the best available therapy[11,12]. *Mtb* also remains a significant airborne threat to workers in the United States, accounting for many millions of dollars of worker testing, prophylactic treatment, and infection control interventions annually. Newly revised and expanded CDC guidelines to protect health care workers from occupational TB attest to its ongoing importance in the workplace[13]. Globally, *Mtb* transmission, especially MDR-*Mtb* transmission, poses a serious threat in all institutional settings, from hospitals to prisons, especially among HIV-infected persons.

Using the well-established method of guinea pig air sampling described below, human-generated *Mtb* airborne transmission can be studied quantitatively, and the effectiveness of various interventions measured during 3-month exposure experiments. We are unaware of an airborne infection other than *Mtb* where human sources of infectious aerosol are continuously available and where proven quantitative air-sampling methods exist to rigorously test control interventions under real life conditions.

The **Airborne Infections Research (AIR) facility**, located in Witbank, South Africa, has been established through the collaboration of the Medical Research Council (MRC) of South Africa (SA), the US Centers for Disease Control and Prevention (CDC), the SA Council for Scientific and Industrial Research (CSIR) and Harvard University, with the cooperation of the provincial Department of Health. Opened in January 2005, the AIR facility benefited from substantial initial funding by USAID (through CDC), the SA MRC, and several other sources, with contributions by CSIR, Harvard University, and the Brigham and Women's Hospital, Boston. This is the first request for major outside funding to test infection control interventions, one of the main purposes for which the facility was designed. Based on the historic Baltimore Veteran's Administration Hospital experiments on airborne transmission by Richard L. Riley and colleagues[14-16], this updated, state-of-the-art apparatus efficiently delivers infectious aerosols generated by TB patients on a six-bed experimental ward to two large exposure chambers containing a total of up to 360 pathogen-free guinea pigs..

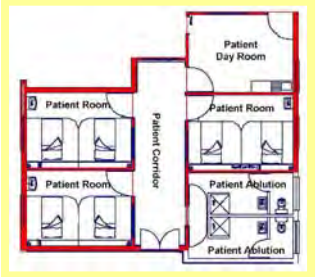


Figure 1, above: a) outside view of AIR facility; b) floor plan of clnical unit, showing 3 patient rooms with two beds each, day room, and lavatories; 4) corridor in patient unit showing entrance to one 2-bed room; and 4) guinea pig cages in one of two identical exposure chambers.

Guinea pigs are highly susceptible to as little as a single inhaled droplet nucleus of human Mtb, but are unaffected by background airborne contaminants that make culture impossible[17]. Knowing their average minute ventilation, guinea pigs have served as quantitative air samplers for airborne infectious droplet nuclei generated by patients[18]. Variable host susceptibility as well as variable environmental conditions are minimized in this experimental design. Variable infectious source strength of different patients is controlled by exposing guinea pigs in two identical exposure chambers to air from the same patient rooms on alternate days corresponding to the application on alternate days of control and experimental conditions in those rooms (Figure 1). The operative assumption is that, on average, the 6 patients are about as infectious one day to the next. Thus, different infection control interventions will be tested under conditions of similar host susceptibility (guinea pigs), physical environment (the AIR facility), and human infectious source strength (the 6 patients on the ward). Moreover, compared to most clinical trials, the high rates of infection observed in initial experiments
indicate that multiple control interventions can be tested sequentially over relatively short time periods.

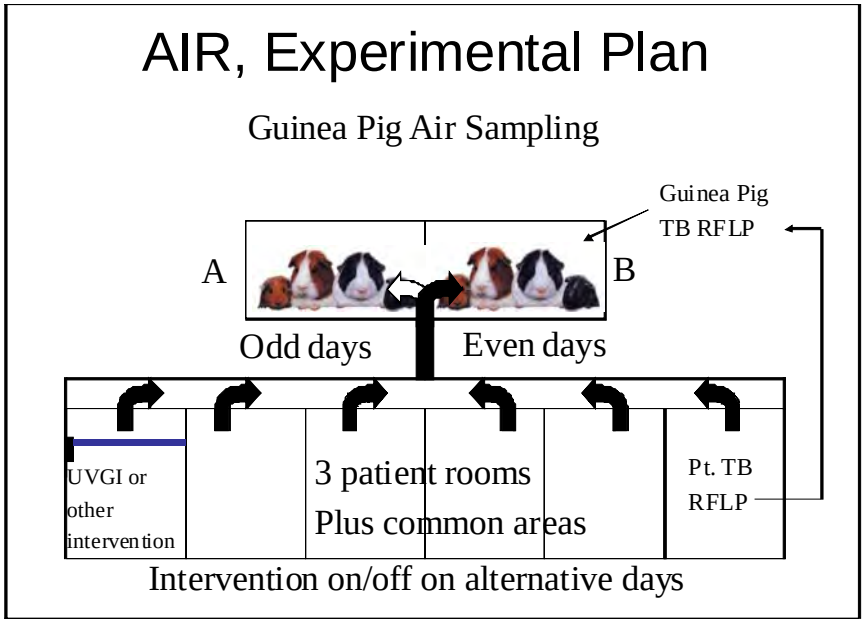


Figure 2: General experimental plan for testing all 3 hypotheses, assuming that patients are equally infectious on alternating days when the intervention (surgical mask, air filtration, UVGI air disinfection) is in use.

Surgical face masks worn by potentially infectious persons to reduce the generation of infectious aerosols is an officially recommended method of source control that can be applied quickly in response to an outbreak situation to reduce person-to-person transmission[13]. However, the utility of this simple intervention has never been rigorously tested against human-generated aerosols due to the lack of appropriate testing methodology. There is no substitute for testing persons with airborne infection while wearing masks under real life conditions. This intervention can be readily tested in a controlled experiment in the AIR facility. The results will have important implications for protecting workers and for resource allocation. Many millions of dollars are spent annually on surgical masks for infectious persons around the world in an effort to protect workers and others. This simple intervention may be effective, or it may give workers false confidence. It is vital to know exactly how well this simple intervention works.

Portable air disinfection units can also be applied emergently. The use of portable air cleaners in homes and offices has steadily grown over the last decade, and it is now estimated that 1 in 10 American households own some form of device for air cleaning[19]. They are also commonly used in the workplace. They are sold for a range of purposes, from removing smoke to reducing allergens and respiratory infections. Larger commercial devices are used in congregate settings such as hospitals, clinics, nursing homes, and homeless shelters. However, the performance of portable air cleaners varies greatly depending on their design and application**.** An American National Standards Institute (ANSI) testing procedure is designed to evaluate these devices by comparing the decay curves of small, medium, and large particulate test contaminants, with and without the

device in use[20]. The result is an assigned clean air delivery rate (CADR) for each of 3 test aerosols – smoke, dust, and pollen. It is much more difficult to test portable air cleaners against infectious aerosols and these devices have never been evaluated against human-generated infectious aerosols in a real world setting. The AIR facility was specifically designed for this kind of testing. Portable air disinfection units utilize a variety of technologies, including HEPA filtration, ultraviolet germicidal irradiation, and electrostatic air disinfection. An important metric is the single-pass retention or inactivation rate regardless of the method used21. At Harvard we have recently used a variety of aerosolized microorganisms to test a novel multistage electrostatic/filtration air disinfection technology, which had been developed to function for long periods with low maintenance in the Soyuz space capsule. The device demonstrated single-pass inactivation rates, approaching 100% for all species except fungal spores. In this proposal we plan to test a version of this technology against human-generated aerosol at three different flow rates in well mixed rooms so that a curve of CADR vs. air disinfection efficacy can be drawn. In contrast, because they are inexpensive and already widely available in office settings, we also propose to test an office-type portable HEPA filtration room unit to determine its efficacy as a function of its CADR.

Air disinfection by **upper room ultraviolet germicidal irradiation (UVGI)** requires pre-installation as a preventive strategy. One advantage over forced air moving systems is that large volumes of upper room air can be treated at once[22]. Normal room convection currents or mixing fans deliver contaminated air from the breathing space. One early field trial suggested good efficacy against measles transmission in schools[23], and another less rigorous study suggested efficacy against influenza transmission in a hospital24, but these studies have never been replicated under more controlled conditions against any other human-generated infectious aerosols. Brickner and the Harvard members of this research team conducted a multi-site, placebo controlled trial of UV air disinfection to reduce TB transmission in homeless shelters, but low infection rates under placebo conditions, and poor retention of homeless subjects led to inconclusive results[25]. Several groups have studied aerosolized test organisms in both small and room-scale exposure chambers and have confirmed high rates of air disinfection (Table 2)[8,26-30]. Consistently, room air mixing has been found to improve UV air disinfection while high humidity has been found to diminish it [8,27,28,31-33]. We plan to test the efficacy of upper room UVGI against human-generated aerosol and to measure the effects of room air mixing and high humidity under real world conditions.

The interventions to be tested in this proposal are largely conventional, but unproven under real world conditions. However, we are collaborating in the development of several **novel source control and air disinfection strategies**. New dry powder aerosol formulations and delivery systems for inhaled anti-infective agents are likely to rapidly reduce transmission in addition to any therapeutic benefits derived from treating the infection [34,35]. An inhaled non-specific (and non-therapeutic) rheological-active agent to reduce the production of respiratory droplets is also under development[36]. Another novel environmental intervention under development is aerosolized germicidal ionized water[37]. All of these novel approaches can be evaluated in the AIR facility as they become available for clinical testing.

C.1. General design and plan

The same general research design applies to testing each of the interventions (Figure 1). Following the general plan, an outline for each experiment focuses on the specific intervention being tested. Detailed methods conclude this section in the original proposal, but are not repeated here.

C1.1. Infectious sources

Six patients will be recruited from among recent admissions to the main MDR-TB hospital based on criteria associated with infectivity (sputum smear positive for Mtb, lung cavitation, cough, early in treatment). Patients will be replaced on the ward after 7 to 21 days. A total of 28 different patients occupied the ward during the 4-month pilot study, although some patients stayed longer than 21 days, and 3 patients returned to the ward

while still infectious. Recruitment and retention of patients in the AIR facility has not been a problem because it is less crowded (3 beds to a room elsewhere) and the amenities are better (air conditioning and television). **Rationale:** For the air disinfection experiments, the patients participate primarily as sources of cough generated infectious aerosol. They reside on the ward, as they would in the main MDR hospital ward, except that they agree to remain indoors 20 out of 24 hours as they receive the standard South African treatment regimen for MDR-TB. They are replaced when it is estimated that they are becoming less infectious or at any time before that if they request to return to the main TB hospital. Only in the facemask study (detailed below) are they asked to actively participate in the intervention under study.

C.1.2. Plan and rationale for controlled intervention studies

Air is exhausted from each of the 3 2-bed rooms, the corridor and the common room at the level of the breathing zone and delivered to one or the other of the two guinea pig exposure chambers; each containing 90 pathogen-free guinea pigs (see power calculation re. numbers of guinea pigs). On alternated days when an infection control interventions is being tested, all of the air goes to exposure chamber A (**Figure 1**); and when the intervention is off (control conditions), all exhaust air goes to exposure chamber B. At the end of the planned 3 month exposure, guinea pigs in Exposure chamber A will have been exposed for a total of 6 weeks only on days when the intervention is on, and those in chamber B, for 6 weeks, only on days when the intervention is off.

Rationale: The alternate day strategy assures that the infectious source strength is approximately equal during control and intervention days. This would less likely to be true if each intervention or control exposure period lasted several days or weeks.

C.1.3. Tuberculin skin testing scheme for guinea pigs and interpretation

Each pathogen-free guinea pig has a baseline PPD (TST-0) and after 30, 60, and 90 days of exposure (TST1-3). A final PPD will be done at 120 days, 30 days after exposure ends (TST-4). Based on the results of the preliminary study, in consultation with McMurray, any skin test induration (2 mm or more) will be considered indicative of infection17,58. Infected animals are removed and replaced by PPD-negative unexposed animals after each testing so that the majority of animals remain fully susceptible and testable for new infection (see detailed methods that follow and the Appendicles for more information on skin testing).

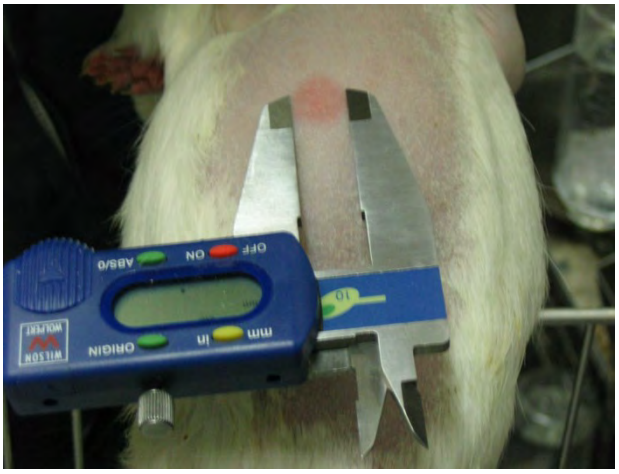


Figure 3. Tuberculin skin test result on a depilitated guinea pig back, and measured with a electronic digital caliper.

Figure 4: Time line for tuberculin testing guinea pigs

	Exposure starts			**Exposure ends**	**Experiment ends**
Time:	0	30	60	90	120 days
Basline	TST-0	TST -1	TST-2	TST-3	TST-4

C.1.4. Data expected, analysis, and interpretation
A rate-ratio test, assuming Poisson counts, will be used to determine significant differences n the rates of guinea pig infections between chambers A (intervention) and B (control).

C.1.5. Power calculations
A detailed power analysis based on the infection rates observed in the pilot study appears in the Appendix of the original proposal. Based on the monthly infection rate of approximately 30% observed in the pilot study, and exposure duration of 3 months, a protective effect of 50% or more would be detected with a power of 0.8 if 51 guinea pigs are used in each exposure chamber. For interventions such as upper room UV, protection in the range of 75% is possible based on experimental room studies. However, these experiments are powered to detect a smaller effect size of 50% in order to allow for the possibility of infection rates lower than previously observed. For that reason, 90 animals per chamber has proven to be highly effective in these studies.

These experiments are being conducted under very similar conditions allowing comparisons between arms. Although each of our experiments have been powered for the comparison of the intervention and control arms, there will also be sufficient power to detect effects of moderate to large size (e.g., rate ratios > 4) across the different intervention arms. For example, we will compare the intervention arms of Experiments 2 and 4 to determine the effect of low versus high humidity (controlling for mechanical air mixing). Comparisons will be made using a rate-ratio test, assuming Poisson counts.

C.1.6. Anticipated problems and solutions
Because these experiments all follow the same design template, the first experiment will guide subsequent studies in terms of infection rates, magnitude of effect, duration, and numbers of guinea pigs required. Subsequent experiments can be adjusted based on those studies.

Any patient who at any time and for any reason chooses to withdraw form the study will be returned to the general MDR-TB hospital and replaced by a new consenting subject. The excluded patient will continue to receive exactly the same treatment and care in the main MDR-TB hospital as in the AIR facility.

C.1.7 Infection control on the experimental ward: In the process of publishing our first intervention paper resulting from these studies (on the efficacy of surgical masks), the question of how much infection control on the AIR facility (i.e., ventilation, UVGI, respiratory use) was required was raised by one reviewer, resulting in a delay in publication and an in-depth discussion among our team members, the chair of the Partners IRB, ethicists, and our in-country partners, especially those at the CSIR who are charged with TB IC building guidelines. For all 3 of the experiments reported here, as outlined in the original proposal, 6 ACH were used in the AIR facility. The following is the **summary** of a long response to the editor in question, resulting in publication of the paper, and a clearer stance going forward with other publications and future experiments.

1. The MDR/XDR-TB situation in South Africa is dire and worsening – much worse than that in Peru and other MDR hot spots, in part because of the prevalence of HIV. Current hospitalization conditions are desperate throughout the country and unlikely to improve without solid evidence to support policy change. Interventions like full respiratory isolation, negative pressure, 12 ACH, and the use of upper room UVGI are generally not available. The country is (wisely) moving toward community-based treatment, not the building of isolation rooms. The exact course of MDR management will be guided by the quantitative research that the AIR facility can uniquely provide.
2. The CDC recommended 12 ACH applies to newly constructed airborne infection isolation rooms in the US. 6 ACH is acceptable for existing isolation facilities in the US. These are not international standards, and do not apply to our non-isolation TB wards in South Africa. Most South African hospitals are naturally ventilated, which means highly variably ventilation - with especially low

ventilation at night and in cold months when windows are closed. **If additional ventilation is deemed necessary for MDR-TB, the South African standard is 6 ACH.**

3. MDR patients in South Africa are usually not isolated from each other, but **drug susceptible, MDR, and XDR patients are separated.** As in the main MDR-TB hospital, patients in the AIR facility freely interact with each other, and **subjects released from the AIR facility return to the main MDR wards after 2 weeks regardless of smear status, per routine local practice.**
4. In the expert opinion of our consortium, including the CSIR, an assured **6 ACH by mechanical ventilation on the less crowded, better supervised 6-bed AIR facility represents a substantial improvement of TB IC conditions compared to estimated average ventilation in the rest of the MDR facility**, where windows are often closed and night and in cool weather. *We strongly believe that subjects and HCWs in the AIR facility are at reduced risk of TB transmission compared to those on in the main MDR hospital.*
5. Upper room **UVGI is not routinely used on MDR wards in South Africa, and there is an official moratorium on its used at this time – pending our evidence of efficacy and national guidelines.**
6. **Ethically conducted air disinfection research at the unique AIR facility is a critical source of controlled trial data to support TB IC policy in South Africa, but <u>it cannot be done if extraordinary means are imposed to essentially stop all transmission to the guinea pigs.</u>**
7. When, as a result of TB IC implementation policies based on rigorous research, air disinfection practices in South Africa approach those in the US and other low-burden settings, transmission will have been reduced and our guinea pig air sampling research methodology may no longer be feasible, but at that point it will also be much less important. **At present, however, we believe we are both protecting both research subjects and staff compared to current standards, and providing the evidence essential for more effective implementation policies in South Africa and globally.**

D. Results:

D.1 Accomplishments and limitations. With the assistance of a supplemental grant from NIOSH and a no cost extension, all 3 original specific aims have been accomplished over the course of this project. We have tested surgical masks on patients, filtration machines, and upper room UVGI. The actual order that the experiments were conducted, however, was not as presented in the proposal, based on rapid deployment (masks and portable filtration) or pre-planning (UVGI), but on global priorities as we perceived them, i.e., use of UVGI, masks on patients, and finally portable air filtration. However, we will present the results as originally proposed, for consistency with the Specific Aims, above.

For a variety of reasons, fewer experiments than originally projected were possible. The experiments became much more expensive than originally projected by the cost of our pilot study. A major factor was the need to provide **24/7 nursing coverage** for the 6 patients on the AIR facility when occupied. Originally this was supposed to be covered by the hospital, but the hospital itself had changed hands several times over the course of this project, the nursing shortages in South Africa had worsened, and the hospital said it simply could not hire enough nurses to cover our experiments. That same nursing shortage made hiring agency nurses very expensive. We then turned to hiring mostly retired TB nurses to staff the unit during 3-4 month experiments, but even this was/is a much higher cost that had been anticipated. There is a long list of other unanticipated costs, but another major barrier to our experiments has been ongoing administrative changes at the Medical Research Council, aimed to stem corruption and discrimination, that have made (and continue to make) it virtually impossible to purchase necessary supplies and services. Under new rules, supplies can only be published through the main MRC headquarters in Cape Town and only by description, not by product number from a specific source. Likewise, it has not been allowed to re-hire the same qualified consultants over and over again. Job opportunities are supposed to be more broadly distributed, apparently regardless of qualifications. For example, we were unable to re-hire the local Witbank retired engineer who keeps the AIR

facility functional despite the absence of any conceivable skilled alternative. This resulted in the premature ending of our last experiment (air filtration) when the local project supervisor did not feel he could ask the engineer or the animal support staff to continue working another month with no chance of compensation. Fortunately, that experiment is still interpretable, but it illustrates the logistical and fiscal problems that we have had to overcome to complete this study. For an upcoming CFAR (NIH) funded clinical trial, we have moved the responsibilities for purchasing supplies and hiring staff out of the MRC and to the University of Pretoria, a long-time partner, much less affected by government regulations. Finally, a new president of the MRC has been hired and there is hope that these onerous rules will be overturned shortly. In any case, we now have an alternative strategy for working around the MRC if needed.

D.2 Results of the 1st intervention study, testing the efficacy of surgical masks on patients (*Hypothesis 1 in our Specific Aims: Surgical masks on individuals with airborne infections will reduce transmission by at least 50%)*

The surgical masks that were selected for testing were readily available, inexpensive, over-the-ear surgical masks depicted below, in Fig. 5. Further details are available in the publication, currently available on line (*Published ahead of print on February 9, 2012, doi: 10.1164/rccm.201107-1190OC Am. J. Respir. Crit. Care Med. February 9, 2012 rccm.201107-1190OC).* The patients were asked, and reminded by the nurses and by simple ward signage, to wear the masks as much as possible, 7 AM to 7 PM, every other day, during which time the ward exhaust air was directed to the appropriate guinea pig chamber. On alternate days, during the same time period, exhaust air went to the control guinea pig exposure chamber. Patients were not expected to wear the masks while eating lunch, while sleeping (during which time air was not being sampled), or while outside for as long as 4 hours per day. Nurses were instructed in their proper use and a system of spot checks was devised to monitor mask usage by patients.

Figure 5 Simple surgical mask tested

The results of monthly TST testing of guinea pigs in the intervention chamber (breathing exhaust air 7 AM-7 PM on days when surgical masks were being worn) compared to guinea pigs in the control chamber (breathing exhaust air 7 AM – 7 PM on alternate days when surgical masks were not being worn) are shown in Table 1, below.

Guinea Pig Group	TST 0	TST 1	TST 2	TST 3	TST 4	Total
Intervention (90)	**0**	**1**	**10**	**20**	**5**	**36**
Control (90)	**0**	**4**	**15**	**39**	**11**	**69**

Table 1 Surgical mask and control TST results

The statistical analysis by Kaplan-Meier plot (Figure 6, below) takes into account both the decreasing number of vulnerable guinea pigs as infections progress and the added power of temporal trends. It indicates that wearing surgical masks, as described, under the conditions extant in the AIR facility ward was 53% effective in reducing transmission to sentinel guinea pigs. This is fortuitously close to the 50% protection proposed in the hypothesis.

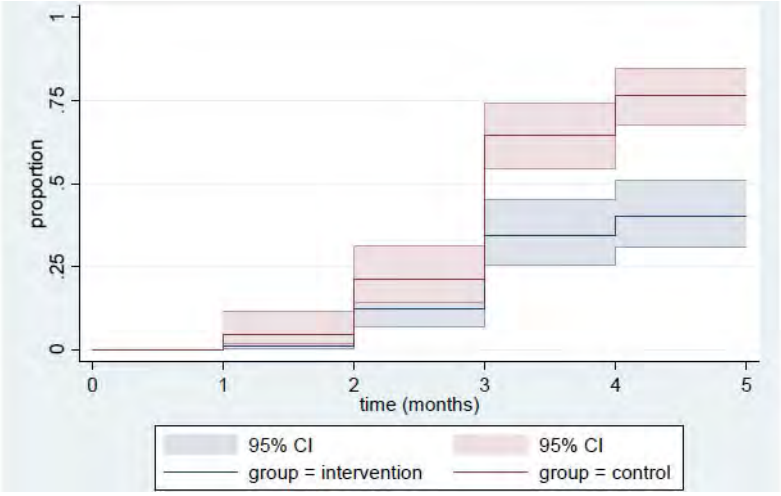


Figure 6 Surgical masks. Kaplan Meier plot of hazard of becoming infected over time under control or intervention conditions indicating an estimated 53% protection.

We never planned additional experiments for this intervention, for example, different style surgical masks, nor were particulate respirators ever considered for this application, as discussed further, below.

D.3 Results of the second intervention study, testing the efficacy of portable (filtration) room air cleaners. (Hypothesis 2 in our Specific Aims: Portable room air disinfection units will reduce transmission by at least 75%)

This was the final intervention study tested, with the help of both supplemental funding and a no cost extension. Because we knew that we could only test one device, the choice of the portable air cleaner was the subject of much discussion in the research consortium. The high tech Russian device originally proposed was dismissed as too costly to be practical. At the other extreme, there was support for testing the small cylindrical Honeywell air cleaners that are ubiquitous in many hospitals. Those too were dismissed as unlikely to move enough room air (CADR) to be effective, and because of their great potential to re-entrap the same processed air because of their design. We settled on testing a very high-quality, tall, high-capacity Swiss-made (IQ Air CleanZone, SL) room air cleaner, capable of producing adequate CADR and able, by design, to avoid excessive air re-entrapment.. A sample device was shipped to Harvard School of Public Health for testing.

IQAir Filter Data 12/10/10					
	Outlet				
Fan Speed	Average Velocity (ft/min)	Area (ft^2)	Volume (ft^3/min)	Volume Ratio (Inlet:Outlet)	Penetrance (%)
1	237	1.0	220	0.81	0.10
2	344	1.0	330	0.66	0.11
3	503	1.0	480	0.76	0.11
4	731	1.0	690	0.83	0.10
5	1010	1.0	960	0.79	0.10

Table 2 Room air cleaner, average velocity and filter penetrance with test inert polystyrene particles.

Table 2, above, shows the results of internal performance testing. Table 3, below, shows the room volume of the AIR facility clinical suite, patient rooms, corridor, and common room, each of which were equipped with an identical room air cleaner. As can be seen, at the 3rd setting, at which the machines were whisper quiet, but still generating enough airflow to produce the equivalent of a nominal 18.0 to 22.5 ACH in the various spaces, not considering re-entrapment of air (short circuiting), which was not estimated.

Room	Dimensions, meters	Volume, meters	Volume, cf	ACH at 480 cfm
Patient room C1	3.0 x 4.7 x 2.6	36.7	1295	22.0
Patient room C2	3.2 x 4.8 x 2.6	39.9	1410	20.4
Patient room C3	2.9 x 4.8 x 2.6	36.2	1278	22.5
Corridor	2.0 x 8.7 x 2.6	45.2	1596	18.0
Patient day room	3.3 x 4.8 x 2.6	41.2	1455	19.8

Table 3 Volume of clinical areas and estimated ACH (CADR) delivered by air cleaners set at 480 cfm speed.

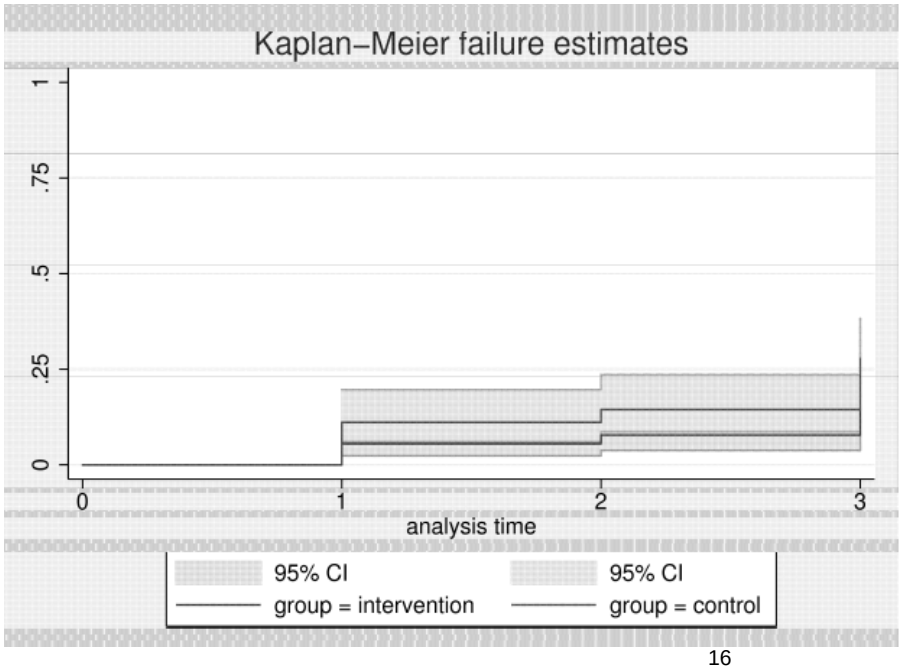
Study logistics: The room air filtration machine is shown here at the foot of the bed, opposite the discharge and exhaust vents above the heads of the beds. The discharge from the room air cleaner is at the top, and the intake from the bottom. Concern was raised over possible interference between ventilation discharge into the room and discharge from the room air cleaner in the opposite direction. For the actual tests, the device was placed between the beds, on the same side as the ventilation system discharge and exhaust grills. The machine were automatically turned on and off every other day at 7 AM, with exhaust air simultaneously directed to the appropriate guinea pig chambers. Ceiling fans were left on during both control and intervention periods to assure complete air mixing and allow more direct comparison with the upper room UV studies (see below).

Figure 7 IQAir CleanZone SL Air Cleaner in position in one of 3 AIR Facility patient rooms.

The results of monthly skin testing of control and intervention chamber guinea pigs is shown below in Table

Guinea Pig Group	TST 0	TST 1	TST 2	TST 3	Total	Censored
Intervention (90)	**0**	**5**	**2**	**13**	**20**	**70**
Control (90)	**0**	**10**	**3**	**12**	**25**	**65**

Table 4 Room air cleaner results of skin testing intervention and control guinea pigs.



As noted at the beginning of the results section, this study was terminated for logistical reasons before a planned 4th and final TST could be obtained. We will never know if TST 4 would have led to a significant difference between the conversion rate between the control and intervention guinea pig chambers, but it is clear that one more data point would not likely have resulted in a meaningfully higher estimate of efficacy.

The hazard ratio: 1.31 with a p-value of 0.4 This represents an estimated 20% efficacy, which was not statistically significant.

This intervention data was the most recently collected (the study ended in December, 2011), and the manuscript is being prepared by our South African colleagues. The testing of several room air cleaners was planned, but cost and time considerations were prohibitive. The possible reasons for these disappointing results are considered in the Discussion section.

D.4 Results of the third intervention study, testing the efficacy of upper room germicidal ultraviolet air disinfection (UVGI). (Hypothesis 3 in our Specific Aims: Upper room germicidal irradiation will reduce transmission by at least 75%)

D.4.1 **Selecting the UVGI fixtures to be tested.** This was actually the first of the three intervention tested and the research consortium was concerned that the fixtures tested not only represent the best available, but also that this not be a test of any one manufacturer's equipment, the results of which could then be used as marketing. Rather, we decided to develop technical specifications for fixtures in order to solicit samples of UV fixtures that would undergo in-house testing for compliance with the specifications. The following specifications were developed and circulated to the 3 major manufactures in the US and to known manufacturers in South Africa. In addition, the opportunity to donate fixtures meeting the following specifications for this experiment was widely advertised in South Africa using standard methods for the equitable solicitation of bids.

Fixture specifications:

UV fixtures will be selected according to the following specifications:

1. *Physical and functional characteristics*
   a. A fixture with an open port for UVGI of approximately 400 cm^2 ($\pm$ 20%) irradiating the room when mounted on a wall and containing commercially available low-pressure mercury vapor lamp(s) that emit UV radiation predominantly at 254-nm (these must be low-ozone emitting lamps).
   b. If applicable, a cut-off switch that turns off the lamps whenever the fixture is opened for servicing.
   c. Devices may be placed in the open port (such as vanes or louvers) to confine the emitted UV radiation to a wide, shallow horizontal path.
   d. Emission port beam-focusing devices and all interior devices, including lamps, must be easily accessible with simple tools (e.g., screwdriver or adjustable wrench for cleaning and servicing.
   e. Installation of standard electronic ballast and standard electrical parts throughout shall meet the latest revision of relevant SABS, ISO, CEN, BSS, DIN or equivalent American standard, listed in order of preference.
   f. The unit shall be capable of operating at 220 VAC ($\pm$20 VAC) and 50 Hz.
   g. The fixture shall have the maximum length flexible cord allowed by the standards listed above and have no plug.
   h. A warning notice in words and cartoons on the exposed face of the fixture citing the danger of looking at the bare lamps without eye and skin protection.

2. *UVGI fixture performance criteria:*
   a. A reading of not less than 250 $\mu W/cm^2$, one meter out from the face/opening of the fixture, on the horizontal centerline of the UVGI beam.
   b. Not less than 5% of input wattage emitted as 254-nm radiation.
   c. 254-nm direct (not reflected) irradiation readings not greater than 0.4 $\mu W/cm^2$ at 2.0m (6.5 ft) above the floor. The ceiling height is 2.6m (8.5 ft).
   d. If the wall- or ceiling-mounted fixtures have not been previously characterized by Harvard University, School of Public Health, they will be tested for compliance with stated criteria prior to acceptance.

Fixture selection results: Prescreening of proposed fixture specifications sent in my manufacturers in South Africa and 3 domestic manufactures resulted in only 2 fixtures, both made in the US, that came close to meeting criteria. One fixture (Atlantic Hygieaire) required no modifications and a second (Lumalier) built a custom, higher output model to approach the specified output when tested at Harvard. Both fixtures were tested and deemed acceptable for use in the study. Each manufacturer donated 6 wall fixtures for the study, one from each for each of the spaces listed in Table 3, and one to be kept in reserve if a fixture failed. Spare lamps for each fixture were also on hand.

One fixture of each type was installed in each room, one on the (door) side, and the other on the widow side of the room. The fixtures were not positioned exactly opposite each other, but staggered to give a more optimal distribution of UV flux in the room. Figure 9 shows one of the wall UV fixtures in place, the arrangement of ventilation discharge and exhaust grills in a patient room, and the position of a ceiling paddle fan intended to assure good air mixing.

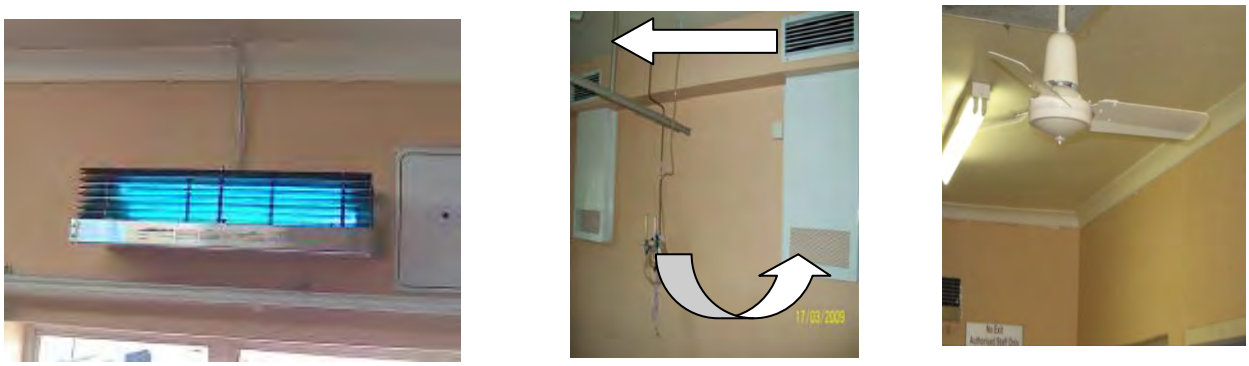
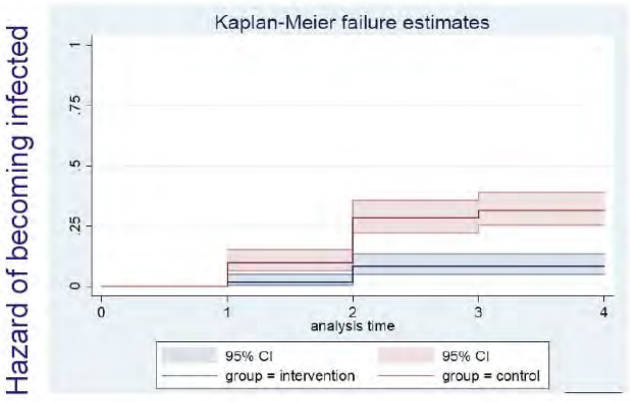


Figure 9 a) UVGI wall fixture, b) room ventilation arrangement, and c) ceiling paddle fan in use during experiments.

D 4.2 Logistics of the studies and results. This first intervention study was begun in September and expected to be completed before the South African summer and Christmas holidays, during which time patients often go home for a time, even when on MDR-TB treatment. As seen in Figure 10, however, there were relatively few infections noted during the months preceding the holidays when the experiment (UV1) had to be terminated. In order to achieve enough control infections to satisfy the power calculations, a second, nearly identical experiment (UV2) was conducted after the New Year. During this experiment we apparently had many more infectious patients, allowing a more robust testing of the intervention. The experiments were so similar that their analysis was combined, as seen in Figure 10. The resulting hazard ratio is consistent with an efficacy estimate of approximately 80%.

	Intervention	Control
UV1		
TST-1	0	1
TST-2	0	3
TST-3	0	5
TST-4	0	0
TOTAL	0	9
UV2		
TST-1	3	17
TST-2	12	30
TST-3	0	1
TOTAL*	15	48

*p<0.0005



Combined hazard ratio 4.9 (CI.95: 2.8, 8.6)

The only change made between UV1 and UV2 was to relocate the exhaust ports to positions indicated in Figure 9b. Originally, both the ventilation discharge vent and the exhaust vent were located close to the ceiling, the former over the later. Concern was raised during UV1 that the exhaust might be selectively drawing air from the irradiated upper room zone rather than from the breathing zone where health care workers sampled the air. All 3 engineers on the project felt that the ceiling mixing fans obviated those concerns since the room should approach perfect mixing. However, to be sure that this was not happening, the exhaust ventilation duct was lowered between UV1 and UV2 as shown in Fig 9b. UV1 contributed only 9 of the combined 57 (16%) control infections, not greatly affecting the highly significant results if there was, in fact, a sampling bias in the original configuration. This later configuration (9b) has been used in all subsequent experiments (surgical mask and room filtration) studies.

A third UV study was attempted, to look at the impact of **high humidity** on the efficacy of UVGI, as proposed in the application. However, as in UV1, UV3 produced only 1 infection in the control guinea pig chamber despite subject selection criteria that had not changed from UV1 and UV2. We considered this experiment a failure and went back to reconsider our subject selection criteria. Many experiments later we would understand that this variation in infectivity was directly the result of effective treatment, as explained below in section D 5. We also had technical difficulty maintaining humidity in the AIR facility at or near the 80% level planned. Our intention was to complete the other intervention studies and return to the high humidity experiment, but time, funding, and the other limitations listed above conspired not to make this happen. It is among the experiments that we plan to do if our renewal application is successful.

D 5 Other results - the impact of chemotherapy.

Another key finding of this research, not part of the planned experiments, may have the greatest impact on global TB control, and also has importance for workers caring for patients with MDR TB in the United States and other low-burden settings. In the **control (non-intervention) arms of the 5 experiments** conducted (including a pilot study prior to NIOSH funding) we observed wide variation in the rates of guinea pigs infected. Although patients were selected by uniform criteria believed to be associated with infectiousness (presence of cough, lung cavities on chest x-ray, TB organisms in sputum, absence of significant therapy), rates of guinea pig infection ranged from 1 to 77%. In our 3rd study of UV air disinfection, for example, 27 patients selected with the above criteria infected just 1 of 90 guinea pigs over 3 months (1%), whereas in the mask study, 17 apparently similar patients infected 77% of 90 guinea pigs over 3 months. These unexpected observations were puzzling at first, until we analyzed the results of bacteriology tests from the patients and from some guinea pigs that showed that there were no patients with extensively drug resistant (XDR) TB in the 3rd UV study, whereas there were XDR patients in the other 4 experiments, and in a pilot study where transmitted strains proved to be XDR. As had been shown for drug susceptible TB in similar studies 60 years ago, *it appears that effective treatment (that is, MDR treatment for MDR TB) suppresses infectiousness almost completely and almost immediately*, whereas infective treatment (MDR treatment for XDR TB) fails to suppress transmission.

These results are summarized below, and are being prepared for publication as preliminary observations, since these experiments were not planned or funded to collect and perform exhaustive mycobacteriology. A RO1 has been submitted by our South African colleagues to definitively study the impact of treatment on MDR-TB transmission. However, these observations have already had a profound impact on how TB IC is being considered in high burden settings. A new appreciation for the role of rapid diagnosis, rapid drug susceptibility testing, and rapid, effective treatment has emerged as a direct result of these experiments. A new campaign, sponsored by USAID, called **FAST**, is calling for **F**inding cases **A**ctively (through cough surveillance), **S**eparation until results are known, and the rapid implementation of effective **T**reatment based on rapid DSTs.

	# Patients/ Exp. Duration	% guinea pigs infected (# exposed)	Patients # XDR (MGIT)
Pilot	**26* / 4 mos**	**74% (360)**	**3/11**
Exp 1	**24 / 3 mos**	**10% (90)**	**5/10**
Exp 2	**15 / 2 mos**	**53% (90)**	**2/11**
Exp 3	**27 / 3 mos**	**1% (90)**	**0/21** ***0/27 (LPA)***
Exp 4	**17/ 3 mos**	**77% (90)**	**2/10**

Table 5 Impact of treatment on transmission - preliminary observations from the control arms (no interventions) of the 4 NIOSH-funded experiments reported here (Experiments 1-4).

E. Future studies: In addition to the important results reported here, this project allowed the AIR facility to becoe fully operational as an important test facility for conventional and novel airborne infection control interventions. The following grants for future studies have been granted or are under review:

1. Fogarty UV air disinfection research. The PI received a Fogarty Stimulus Innovation grant to develop better, more effective and more efficient UVGI fixtures for the developing world. Three post-doctoral students were hired (2 US, I South African) for a year of intensive research on UV performance and fixture design. A continuation grant proposes to test two novel UV strategies resulting from that grant at the AIR facility in South Africa.
2. US-India collaboration on UV fixture design. Related to the Fogarty, the PI is collaborating with the India National Institute of Design to develop UV technology for use in India where UVGI is needed, but almost unknown. NIH funds are being sought, and resulting products will be tested at the AIR facility.
3. Our NIOSH renewal application proposes to test 3 novel airborne transmission strategies at the AIR facility: 1) novel UVGI fixtures, 2) inhaled saline to reduce droplet nuclei generation, and 3) triethylene glycol vapor as an environmental control intervention for a variety of airborne and droplet borne infections, including TB.
4. Our immediate next study at the AIR facility, funded by NIH, is a clinical trial of a novel inhaled, dry powder antibiotic (colistin) to prevent the spread of TB. This will be the first trial of an inhaled antibiotic for TB, the first use of colistin for TB, and a novel use of the AIR facility. The antibiotic is widely used by aerosol for cystic fibrosis patients.

F. Discussion:

The results of testing the surgical mask are straightforward, but a few additional comments are warranted. Surgical masks are optimally used short-term, in waiting rooms, and for transport to and from procedures, for persons with known or suspected airborne infections before effective treatment is initiated, in the case of TB. Longer term, there are many comfort and logistical issues around eating, talking, and sleeping. In the AIR facility our methodology required that we test surgical masks during prolonged use, even though shorter term use is preferable. For that reason, our results could underestimate their effectiveness under more realistic shorter term use where adherence could be higher. On the other hand, our results reflect study conditions, with nurses reminding patients to use their surgical mask, and there may be less enforcement under real world conditions. In sum, we believe that this study may be a reasonable estimate of the efficacy of surgical masks short term, where compliance can be expected, under real world conditions. Surgical masks function as barriers for relatively large respiratory droplets, not filters, and we do not believe that surgical mask of a different shape would perform substantially better. No surgical mask or particulate respirator is designed to contain the force of a cough. We did not consider testing respirators in this study because they are expensive and designed to protect the wearer, not the environment.

The results of the air filtration study are troubling. With 18-22.5 equivalent ACH we expected air disinfection results similar to those obtained by UVGI. Doubling equivalent ventilation from 6 to 12 ACH should reduce risk by about 50%, and doubling it again to 24 ACH should further reduce risk to about 25% of the original risk, or about as much as UVGI reduced risk. Our best explanation at this point is that our arrangement of the machines and beds relative to the HVAC system discharge and exhaust vents was such that infectious droplet nuclei were entrained by the ventilation system before they had an opportunity to be captured by the room air cleaner. This was our last experiment and the filtration machines are still in place, even though the clinical unit is now unoccupied. We plan to use smoke tests and particle counting to further explore the reasons for these unexpectedly poor results despite ample CADR, at least in theory. Finally, if our research funding is renewed, we plan to make every effort to repeat the filtration study with greater attention to the air flow dynamics in the room, possibly using CFD.

The results of the studies of upper room UVGI are also clear. As reported by Escombe[38] in a similar study, we have also demonstrated highly significant efficacy of upper room UVGI with ceiling fans under real hospital conditions. The remaining question is efficacy under high humidity conditions. Escombe monitored humidity and reported that his results reflected fluctuations in humidity from 50-95% during his nearly 2-year study, but recorded an overall efficacy of 73% non-the-less. Our attempt at creating high humidity conditions in a 3rd UV study were stymied by technical difficulty achieving continuous high humidity, and by only 1 guinea pig infection over a 3 month exposure to 27 patients believed to be highly infectious with MDR-TB. We now know, in retrospect, that the cohort of 27 patients, unlike all of the other cohorts studied, happened not to contain a single patient with XDR TB. Therefore, all patients were on effective therapy and essentially were non-infectious from the first day in the AIR facility. If we our renewal application is funded we hope to repeat a high humidity experiment using better humidification methods and over a longer period in expectations of enrolling some infectious patients, as we have done in all other experiments.

Finally, these observations on the impact of treatment on MDR-TB transmission have great potential to change how MDR-TB is treated globally, especially in light of new, rapid, molecular diagnostics and drug susceptibility testing. Xpert TB diagnostic platform is being rolled out throughout sub-Saharan Africa by a variety of projects, for the first time allowing the diagnosis of MDR-TB within hours of a patient presenting with cough. Combined with active case finding based on cough surveillance, and the rapid implementation of effective treatment based on rapid drug susceptibility testing, the global approach to TB infection control could change markedly, in part as a result of the fortuitous observations we have made in the control (non-intervention) arms of these NIOSH-funded experiments. Further prospective studies are pending funding to further support these findings.

G. Summary: As predicted, UVGI was found to be approximately 80% effective, with a ceiling fan providing good air mixing. However, to our surprise, room air filtration machines delivering approximately 18-22.5 ACH reduced the risk of infection only by an estimated 20%, and this was not statistically significant. That study needs be repeated. As predicted, surgical masks on patients were about 53% effective in reducing transmission to guinea pigs. Another highly important but unanticipated observation made during these studies was in the non-intervention arms, where transmission varied greatly from study to study depending on the presence or absence of patients among the cohort with undiagnosed extensively drug resistant TB (XDR-TB) who were inadequately treated with the standard South African treatment for MDR-TB. These preliminary observations point to a rapid and previously unrecognized impact of effective treatment on transmission.
In conclusion**,** this project has: 1) confirmed that UVGI with ceiling fans can be highly effective under real world conditions, 2) shown that room air filtration machines were surprisingly ineffective under the same conditions, and 3) shown for the first time, that surgical face masks on patients were 53% effective in preventing transmission. Finally, and perhaps most importantly for global TB control efforts, these studies demonstrated that effective MDR treatment rapidly and markedly inhibited transmission from MDR patients, but not from unsuspected XDR patients. The latter observation will have important implications for the safety of MDR treatment in hospitals, clinics, and in the community. All of these results will impact on the protection of workers in the US and around the world for years to come.

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Notes: Label: 21185519

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Publications:

Dharmadhikari AS, Mphahlele M, Stoltz A, Venter K, Mathebula R, Masotla T, Lubbe W, Pagano M, First M, Jensen PA, van der Walt M, Nardell EA. Surgical Face Masks Worn By Multidrug-Resistant Tuberculosis Patients: Impact on Infectivity of Air on a Hospital Ward. Am J Respir Crit Care Med. 2012 Feb 9. [Epub ahead of print]

This is the first of 3 planned intervention publications resulting directly from this NIOSH grant, and a 4th paper on the impact of treatment derives from observations on infection rates in the control arms of the 4 NIOSH-funded experiments. This paper on surgical masks represents the first demonstration of the effectiveness of masks on patients for any infectious agent. The results should be applicable to influenza and other infections with airborne potential.

Program Director/Principal Investigator (Last, First, Middle): Nardell, Edward

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Testing Interventions to Human-Generated Occupational Airborne Infections

Total Enrollment: 132 **Protocol Number:**

Grant Number: R01OH009050

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	0	0	0	0 **
Not Hispanic or Latino	60	71	1	132
Unknown (individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total of All Subjects*	60	71	1	132 *
Racial Categories				
American Indian/Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	60	71	1	132
White	0	0	0	0
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	**0**	0
Racial Categories: Total of All Subjects*	60	71	1	132 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	**0**	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	**0**
Black or African American	0	0	0	0
White	0	0	0	0
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics or Latinos**	0	0	0	0 **

* These totals must agree.
** These totals must agree.

FINANCIAL STATUS REPORT

(LongForm)

(Follow instructions on the back)

1. Federal Agency and Organizational Element to Which Report is Submitted	2. Federal Grant or Other Identifying Number Assigned by Federal Agency	OMB Approval No.	Page 1 of pages	
NIH - National Institute for Occupational Safety and Health	**5R01OH009050-05**			

3. Recipient Organization (Name and complete address, including ZIP code)
Brigham & Women's Hospital
75 Francis Street
Boston MA 02115

4. Employer Identification Number	5. Recipient Account Number or Identifying Number	6. Final Report	7. Basis
04-2312909	**101478**	[X] YES [ ] NO	[x] CASH [ ] ACCRUAL

8. Funding/Grant Period (See instructions) From: (Month, Day, Year)	To: (Month, Day, Year)	9. Period Covered By This Report From: (Month, Day, Year)	To: (Month, Day, Year)
8/1/2006	**12/31/2011**	**8/1/2010**	**12/31/2011**

10. Transactions:	I Previously Reported	II This Period	III Cumulative
a. Total outlays	**$1,054,800.00**	**$320,491.06**	$1,375,291.06
b. Refunds, rebates, etc	$0.00	$0.00	$0.00
c. Program income used in accordance with deduction alternative	$0.00	$0.00	$0.00
d. Net outlays (*Line a less the sum lines b and c)*	$1,054,800.00	$320,491.06	$1,375,291.06
Recipient's share of net outlays, consisting of:			
e. Third party (in-kind) contributions	$0.00	$0.00	$0.00
f. Other Federal awards authorized to be used to match this award	$0.00	$0.00	$0.00
g. Program Income used in accordance with the matching or cost share alternative	$0.00	$0.00	$0.00
h. All other recipient outlays not shown on lines e, f, or g	$0.00	$0.00	$0.00
i. Total recipient share of net outlays (*Sum of lines e, f, g and h)*	$0.00	$0.00	$0.00
j. Federal share of net outlays (*line d less line i)*	$1,054,800.00	$320,491.06	$1,375,291.06
k. Total unliquidated obligations			$0.00
l. Recipient share of unliquidated obligations			$0.00
m. Federal share of unliquidated obligations			$0.00
n. Total Federal share (*sum of lines j and m*)			$1,375,291.06
o. Total Federal funds authorized for this funding period			**$1,379,062.00**
p. Unobligated balance of Federal funds (*Line o minus line n*)			$3,770.94
Program income, consisting of:			
q. Disbursed program income shown on lines c and/or g above			$0.00
r. Disbursed program income using the addition alternative			$0.00
s. Undisbursed program income			$0.00
t. Total program income realized (*Sum of lines q, r and s)*			$0.00

11. Indirect Expense	a. Type of rate (Place "X" in appropriate box)			
	[ ] Provisional	[x] Predetermined	[ ] Final	[ ] Fixed
	b. Rate	c. Base	d. Total Amount	e. Federal Share
	26.00%	**85,061.93**	$22,116.10	**$22,116.13**
Use if 2 rates	b. Rate	c. Base	d. Total Amount	e. Federal Share

12. Remarks: (Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.)

There are direct costs expenses in the amount of $213,313.03 which have been excluded from the indirect costs expense base.

Completed by Adam Tuleja, Research Finance Specialist, 617-954-9776

13. Certification: **I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.**

Typed or Printed Name and Title	Telephone (Area code, number, and extension)
Steve Quinn, Research Finance Manager	617-954-9849
Signature of Authorized Certifying Official: Steven T. Quinn	Date Report Submitted: 3/16/12

Form Approved Through 06/30/2012
OMB No. 0925-0001

Department of Health and Human Services

Final Invention Statement and Certification

(For Grant or Award)

DHHS Grant or Award No.
5R01OH009050-05

A. We hereby certify that, to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period

08/01/2006 *original effective date* through 12/31/2011 *date of termination*.

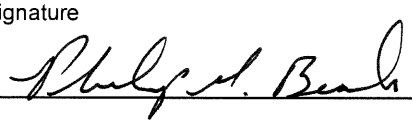
B. Inventions (Note: If no inventions have been made under the grant or award, insert the word *"NONE"* under Title below.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
	NONE	
(Use continuation sheet if necessary)		

C. Signature — This block ***must*** be signed by an official authorized to sign on behalf of the institution.

Title
Senior Grants Administrator, Signing Official

Typed Name
Philip M. Beals

Signature


Date
03/15/2012

Name and Mailing Address of Institution
The Brigham and Women's Hospital, Inc.
Research Administration
75 Francis Street
Boston MA 02115

HHS 568 (Rev. 06/09)

Report Date:	April 3, 2012	Grant Number:	R01OH009050
Project Title:	Testing Interventions to Human-Generated Occupational Airborne Infections	Project Period:	8/1/2006-12/31/2011
Grantee Name:	Edward Nardell, MD	Project Officer:	Joan Karr, PhD
Grants Management Officer:	Larry Guess	Grants Specialist:	Mary Pat Shanahan

Description of Item: i.e. pH Meter	Mfr.[1] i.e. Fischer	Serial Number	Quantity	Condition[2]	Location[3]	Purchase Cost	Date Received [mm/dd/yyyy]
NONE							

[1]Mfr. (Manufacturer)
[2]Condition: (Excellent) (Good) (Fair) (Poor) (Inoperable)
[3]Location: complete physical address

For Government Use Only, not to be completed by the Grantee
Property Administrator & PO Disposition Recommendation and Instructions:

Description of Item	Disposition[1]	Address[2]
[Copy from above]	_____Transfer Title _____Retain and Compensate Awarding Agency _____ Return to Program Office _____Other (explain)	Attn: [Project Officer] CDC / NIOSH 1600 Clifton Road, NE MS E-74 Atlanta, GA 30329-4018
[Copy from above]	_____Transfer Title _____Retain and Compensate Awarding Agency _____ Return to Program Office _____Other (explain)	

[1]Check the appropriate disposition
[2]CDC Warehouse is the central receiving point for delivery of all non-hazardous and non-perishable supplies and equipment, CDC –AM–2004-03, update 2010