Detection and diagnosis of fungal allergic sensitisation

Brett James Green

Central Clinical School, Discipline of Medicine
The University of Sydney
A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy
Declaration

I hereby declare that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any University. To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text. I am willing to make the thesis available for photocopy and loan if it is accepted for the award of the degree.

B.J. Green
Abstract

Airborne fungi are ubiquitous in the environment and human exposure is inevitable. Such fungi differ greatly in their taxonomic, physical, ecological and pathogenic characteristics. Currently, 69 000 species have been taxonomically classified and more than 80 of these are recognised to be aeroallergen sources. Many strategies have evolved to sample, identify and interpret fungal exposure to these species, however no strategy serves all purposes as exposure is a complex and dynamic process confounded by spatial, temporal and geographic variations in airborne counts, in addition to the inadequacies of the immunodiagnostic techniques available. To date, the interpretation of personal exposure and sensitisation to fungal allergens has been restricted to a few select species and the contribution of other genera, airborne hyphae and fragmented conidia to allergic disease are all poorly understood. The aim of the thesis was to utilize the Halogen Immunoassay (HIA) to diagnose fungal allergic sensitisation, to investigate the distribution and factors influencing allergens of fungi in the air and to understand what is actually inhaled in exposure settings. The novelty of the HIA derives from its unique ability to provide allergen sources that are actively secreted by the collected fungal spores and hyphae, which are bound to protein binding membranes (PBM) and then immunoprobbed.

In Chapter 2, the HIA was compared to the commercial in vitro Pharmacia UniCap assay (CAP) and the in vivo skin prick test (SPT), using 30 sera from subjects SPT positive to Aspergillus fumigatus and/or Alternaria alternata and 30 who were SPT negative to these fungi but sensitised to non-fungal allergens. Sera were analysed by CAP and the HIA against A. alternata, A. fumigatus, Cladosporium herbarum and Epicoccum purpurascens and compared statistically. Between 3% and 7% of SPT negative sera were identified to have specific IgE towards A. fumigatus and A.
alternata, respectively. For the SPT positive sera, significant associations were found between the HIA and CAP scores for all fungal species tested \((P<0.0001)\). Correlations between the HIA and SPT however, were weakly correlated for \(A.\ alternata\) \((r_s = 0.44, P<0.05)\) but not for \(A.\ fumigatus\).

In Chapter 3, personal exposure to indoor fungal aerosols was examined using the HIA to identify the fungal components that people were allergic to. Personal air sampling pumps (PASs) collected airborne fungal propagules onto PBMs for 2.5 hours indoors \((n=21)\). Collected fungi were incubated overnight in a humid chamber to promote the germination of conidia. The membranes were then immunostained with pooled human \textit{Alternaria} species-positive sera. All air samples contained fungal hyphae that expressed soluble allergens and were significantly higher in concentration than counts of conidia of individual well-characterised allergenic genera. Approximately 25\% of all hyphae expressed detectable allergen compared to non-stained hyphae \((P<0.05)\) and the resultant localisation of immunostaining was heterogeneous among hyphae. Fungal conidia of ten genera that were previously uncharacterised as allergen sources accounted for 8\% of the total conidia that demonstrated IgE binding.

In Chapter 4, the number and identity of fungi inhaled by 34 adults in an outdoor community setting was measured over 2 hour periods by people wearing Intra-nasal air samplers (INASs) and compared to fungal counts made with a Burkard spore trap and filter air samplers worn on the lapel. Using INAS, the most prevalent fungi inhaled belonged to soil borne spores of \textit{Alternaria, Arthrinium, Bipolaris, Cladosporium, Curvularia, Epicoccum, Exserohilum, Fusarium, Pithomyces, Spegazzinia, Tetraploa} and \textit{Xylariaceae} species, in addition to hyphal fragments. These results showed that inhaled exposure in most people varied in a 2-fold range with 10-fold outliers. In addition, the INAS and personal air filters agreed more with each other than with Burkard spore trap counts. The analysis was further confounded by different sampling efficiencies, locations of devices and ability to visualise and count fungal propagules.

In Chapter 5, a double immunostaining technique based on the HIA was developed and applied to the conidia, hyphae and fungal fragments of \(A.\ alternata, A.\ fumigatus\) and \textit{Penicillium chrysogenum} to discriminate between sources of allergens,
using IgE and to identify the fungi, using a fungal-specific antibody. The localisation of immunostaining was heterogeneous between both conidia and the state of germination with greater concentrations of double immunostaining detected following germination for each fungal species ($P < 0.0001$). Fragmented *A. alternata* hyphae and morphologically indiscernible fragments could be identified for the first time using this technique.

In Chapter 6, the factors affecting the release of allergen from the spores of eleven different species were studied. For nine of eleven species, between 5.7% and 92% of spores released allergen before germination. Ungerminated spores of *P. chrysogenum* and *Trichoderma viride* did not release detectable allergen. After germination, all spores that germinated eluted allergen from their hyphae. Upon germination there was a significant increase in the percentage of spores eluting detectable allergen ($P < 0.0001$) and the localisation of allergen along the hyphae varied between species. Increased elution of allergen post germination might be a common feature of many species of allergenic fungi following inhalation. Additionally, Chapter 6 explored the extent to which inhaled spores or hyphae germinate after deposition in the nasal cavity and thus cause exposure to allergens. Twenty subjects had their noses lavaged at three separate intervals, (1) at the beginning of the experiment, (2) after one hour indoors and (3) after one hour outdoors. The recovery of spores and hyphal fragments from the nasal cavity varied between individuals and was significantly greater after outdoor exposures. Germinated fungal spores were recovered often in high concentrations for *Aspergillus-Penicillium* species, however the proportion between ungerminated and germinated spores were much lower for other genera recovered.

**Conclusions:** Our analysis of cultured and wild-type fungi presents a new paradigm of natural fungal exposure, which in addition to commonly recognized species, implicates airborne hyphae, fragmented conidia and the conidia of a much more diverse range of genera as airborne allergens. Exposure is heterogeneous between individuals in the same geographic locality and the spectrum of fungal genera inhaled differs with the method of analysis. Many of the spores inhaled are likely to be allergenic, however upon germination there is an increased elution of allergen and this might be a common
feature of many fungal species following inhalation. This project also provides novel
techniques to diagnose fungal allergy by immunostaining wild-type fungi to which a
patient is exposed with the patient’s own serum. Such an immunoassay combines
environmental with serological monitoring on a patient specific basis and potentially
avoids many problems associated with extract variability, based on the performance of
current diagnostic techniques for fungal allergy.
Acknowledgements

Firstly, I need to thank Euan Tovey for finding the time to meet me in the spring of 2000 to discuss PhD projects in his laboratory. Not only is he a great role model to me of a brilliant and incredibly dedicated and competent scientist but he has also been a friend and taught me a great deal about life and the dedication that is required to achieve your goals. I am sincerely grateful for all of the hard work and support he has had to put in as my supervisor, including critiquing manuscripts, reviewing drafts of seminars and giving a helpful hand with methodological problems. I also appreciate the time and effort he has put into a number of project grant proposals that were to study different aspects of fungal allergic disease. I would also like to thank Euan for supporting me during the period around the death of my mother that occurred half way through my candidature, in addition to the financial support he provided, which enabled me to survive and live in Sydney. Thanks also for the financial support to travel to the American Academy of Asthma, Allergy and Immunology conferences in New York City (2002) and San Francisco (2004). I am indebted to you and I hope we can continue to have a fruitful collaborative relationship where I can repay you in some way for all you have done.

Next I would like to express enormous gratitude and pleasure of working side by side with Jason Sercombe. Jason has been a sincere, hard working and inspirational colleague, whom I think is a fantastic person and I have a lot of respect for. Although we did not work closely together at the beginning of my candidature, our paths eventually crossed during similar periods of our life and I think that our work for both of us during this time was a brief and provisional escape. I sincerely hope that he and Jenny have a happy life together and I am sure that the hard work that we have both put into the Halogen Immunoassay will eventually be recognized. Like Euan, I hope there is
scope to work together in the future, which would be spondiferous. Jason recruited the subjects and conducted the statistical analyses in Chapter 6, Part B.

I would also like to thank other members of the Woolcock Institute of Medical Research, in particular those belonging to the Woolcock Allergen Lab who helped out in some way throughout the duration of my candidature. Thank you Teresa Mitakakis for your advice with the fungal germination and the initial Halogen development for the other fungi, plus for taking me under your wings initially. Thanks to Tim O’Meara for providing valuable feedback on various manuscripts and seminars as well as for including me as the Palynologist responsible for counting the collected pollen and fungi for the pollen filter paper. Tim O’Meara conducted the ANOVA analysis in Chapter 4. Thank you to Wei Xuan for the statistical advice for each Chapter and Brett Toelle for providing information on subjects for the Materials and Methods section in Chapter 2. Thank you to Eija Yli-Panula and Marrku Viander for their friendly advice and assistance with data collection in Chapter 2. Thank you Daniel Crisafulli, Sandra O’Rouke, Leanne Poulos, Mary Garcia, Justine Lao, Kate Marriot, Sally Chris, Melanie Lean and Virginia Noronha for your valuable conversations and friendship particularly during lunch breaks and organized lab functions.

I would like to thank Murray Thomson and Warwick Britton for their support, co-supervision and morale boosting during the difficult periods of my candidature. Thanks to Andrew Williams from the Clinical Immunology Laboratory, Central Sydney Laboratory Service for serologically testing the human sera used in Chapter 2 and to the Cooperative Research Centre for Asthma for supporting this study. Thank you to Roger Shivas and Sophie O’Neal from the Queensland Department of Primary Industries for supplying all of the fungal isolates used in Chapters 2, 5 and 6. I would also like to thank the Department of Medicine, The University of Sydney and the Woolcock Institute of Medical Research for their support and providing a scholarship and wage.

Thanks also go to the academic and support staff of First Year Biology, The School of Biological Sciences, The University of Sydney for helping me find my other passion in life, which is teaching. In particular, I would like to acknowledge Peter McGee for providing valuable feedback regarding Mycological questions in relation to
my thesis and Charlotte Taylor for supporting my thoughts to integrate Medical Mycology into the first year syllabus for the subject, Concepts in Biology.

I have also been incredibly lucky to meet Detlef Schmechel and Don Beezhold from the Centers of Disease Control and Prevention who were willing enough to collaborate and share their molecular expertise, in addition to providing the monoclonal antibodies that were used in Chapter 5. I look forward to working with both of you more closely in the future. Thank you also to Shannon Rutherford and Rod Simpson for their continuing support and advice. I would also like to thank my mentor and friend Mary Dettmann, without you I would not be where I am today and I am grateful for all your advice and encouragement.

Out of the main area, I would like to thank all of my friends that made the transition from Brisbane to Sydney just that little easier and soon an unregrettable decision. Thanks to Fran and Larissa Marzcak for providing encouragement and supporting my move to Sydney, I will always remember your help.

I would like to dedicate this thesis for what it is worth, firstly to Mum. I know you wanted me to pursue my love of science and find my place in life, however there were a number of sacrifices that were required. I regret not being by your side when you needed me most when you found out that you had secondary cancer and during your first couple of doses of chemotherapy. You gave up so much for me throughout your life and I hope I was able to make you happy when it was really hard for you. I just wish you could be here so I could give you a big hug. I love and miss you so much and you will always be with me. I would also like to thank my Dad as well as the rest of my family in the United States for their continued support both financially and keeping me sane through the bad times and for being there whenever I needed a chat. I love you all very much.

Finally, I would like to also dedicate this thesis to my wife Katherine, who has supported me when I have needed it most. You have brought so much happiness to my life and I will always love you.


4. **Green BJ**, Schmechel D, Tovey ER. Detection of *Alternaria alternata* conidia and hyphae using a novel double immunostaining technique. *Clinical and Diagnostic Laboratory Immunology*, 12 (9): 1114-1116.


Conference Abstracts


Letters and Editorials


International Presentations


Published article abstracts

1. Green BJ, Mitakakis TZ, Tovey ER. Allergen detection from 11 fungal species before and after germination. Journal of Allergy & Clinical Immunology. 2003; 111: 285-289.

Abstract

Background: Allergens dispersed by airborne fungal spores play an important, but poorly understood role in the aetiology and exacerbation of asthma. Previous studies suggest that after germination, spores of Alternaria and Aspergillus release greater quantities of allergen than before germination. It is unknown if this is true of other allergenic fungi.

Objective: Our purpose was to investigate the release of allergen from a range of individual fungal spores before and after germination.

Methods: Allergen expression from spores of Alternaria alternata, Cladosporium herbarum, Aspergillus fumigatus, Botrytis cinerea, Epicoccum nigrum, Exserohilum rostratum, Penicillium chrysogenum, Stemphylium botryosum, Curvularia lunata, Trichoderma viride and Bipolaris spicifera was examined by Halogen immunoassays, using pooled serum IgE from fungal-allergic subjects. Spores were deposited onto protein binding membranes direct from culture. To germinate spores, samples were incubated in high humidity at room temperature for 48 hours. Ungerminated and germinated samples were then laminated with an adhesive film and immunostained by the Halogen assay. The samples were examined by light microscopy and positive counts (haloed particles) were expressed as percentages of total spores.

Results: For 9 of 11 species, between 5.7 - 92% of spores released allergen before germination. Spores of Penicillium and Trichoderma did not release detectable allergen.
After germination, all spores that germinated exhibited allergen elution from their hyphae. 8 of 11 species showed a significant increase (p<0.05) in the percentage of spores eluting detectable allergen. Localization of allergen along the hyphae varied with species, such that some eluted allergen mainly from hyphal tips and septal junctions, whereas others eluted allergen along the entire length.

**Conclusions:** Increased elution of allergen following germination may be a common feature of many species of allergenic fungi. Although allergens from both spores and hyphae were recognised by human IgE, the extent that human ‘exposure’ occurs to allergens eluted from inhaled spores or from hyphae that germinate following deposition in the respiratory tract, remains to be explored. The patterns of allergen expression may affect the clinical response to such exposure.

2. **Green BJ, Sercombe JK, Tovey ER.** Fungal fragments and undocumented conidia function as new aeroallergen sources. *Journal of Allergy & Clinical Immunology*. 2005;115 (5): 1043-1048.

**Abstract**

**Background:** More than 100 genera of fungal conidia are currently recognized as sources of allergens. The contribution of other fungal genera plus airborne fungal hyphae and fragmented conidia to allergic diseases is poorly understood.

**Objective:** We sought to investigate the expression of allergens from airborne wild-type fungi using the Halogen immunoassay, which uses allergic serum IgE to immunostain immobilized allergens extracted from individual fungal particles.

**Methods:** Airborne fungi were collected onto mixed cellulose ester protein–binding membranes for 2.5 hours with volumetric air pumps. Collected fungi were incubated overnight in a humid chamber to promote the germination of conidia. The membranes were laminated with an adhesive cover slip and immunostained with an *Alternaria* species–sensitive serum IgE pool. The samples were examined by means of light microscopy, and positively immunostained fungal particles were classified and counted.

**Results:** All air samples contained fungal hyphae that expressed soluble allergens and were significantly higher in concentration than counts of conidia of individual well-
characterized allergenic genera \((P<0.05)\). Resultant immunostaining of fungal hyphae was heterogeneous, and approximately 25% of all hyphae expressed detectable allergen compared with nonstained hyphae \((P<0.05)\). Fungal conidia of 10 genera that were previously uncharacterized as allergen sources were shown to demonstrate IgE binding to expressed antigens and accounted for 8% of the total airborne conidia count.

**Conclusions:** Our analysis of wild-type fungi collected indoors presents a new paradigm of natural fungal exposure, which, in addition to commonly recognized species, implicates airborne hyphae, fragmented conidia, and the conidia of a much more diverse range of genera as airborne allergens.


**Abstract**

Airborne fungi are ubiquitous in the environment and human exposure is inevitable. Such fungi differ greatly in their taxonomic, physical, ecological, behavioural, and pathogenic characteristics. Many strategies have evolved to sample, identify and interpret fungal exposure and their choice is determined by the hypotheses involved. While fungi can be sampled directly from surfaces, results do not generally reflect human exposure. For this reason, airborne spores are commonly sampled, by either filtration or impaction, using volumetric air samplers. Identification is commonly performed by either culture on nutrient medium or light microscopy using morphological criteria, although new techniques using DNA probes or characteristic antigens or toxins continue to be developed. Interpretation of such exposure data is both complex and contentious, but while there are numerous recommendations there is no consensus on exposure thresholds. A better understanding of the complex pathogenic roles of fungi and susceptibilities of their hosts will enable refinement of techniques for sampling and interpretation.
4. **Green BJ**, Schmechel D, Tovey ER. Detection of *Alternaria alternata* conidia and hyphae using a novel double immunostaining technique. *Clinical and Diagnostic Laboratory Immunology*, 12 (9): 1114-1116.

**Abstract**

A double immunostaining Halogen immunoassay was developed to identify aerosolized conidia, hyphae and fragments of *Alternaria alternata* using an anti-*Alternaria* polyclonal antiserum, while simultaneously, allergy to these components was concurrently determined using human IgE antibodies.
# Table of Contents

CHAPTER 1 .......................................................................................................................................................... 1

1. **INTRODUCTION........................................................................................................................................... 1**

1.1 **RESPIRATORY ALLERGY – A BRIEF HISTORY OF THE PROBLEM ............................................. 1**

1.2 **PREVALENCE OF ALLERGIC RESPIRATORY DISEASE ......................................................... 3**

1.3 **INNATE AND ADAPTIVE IMMUNITY ................................................................................................. 4**

1.4 **ALLERGENS AND SENSITISATION ............................................................................................................. 10**

1.5 **FUNGAL RESPIRATORY ALLERGY ........................................................................................................ 13**

1.5.1 **Fungal biology .......................................................................................................................... 15**

1.5.1.1 **Morphology.................................................................................................................................. 16**

1.5.1.2 **Growth and nutrition............................................................................................................... 18**

1.5.1.3 **Culture of fungi......................................................................................................................... 20**

1.5.1.4 **Reproduction............................................................................................................................. 22**

1.5.1.4 **Classification and taxonomy .................................................................................................... 24**

1.5.1.5 **Fungal aerospora and dispersal ............................................................................................... 26**

1.5.1.6 **Fungal spore deposition ............................................................................................................ 29**

1.5.1.7 **Medical disorders associated with fungi .................................................................................. 30**

1.5.1.7.1 **Cutaneous and subcutaneous infections................................................................................. 30**

1.5.1.7.2 **Systemic mycoses ................................................................................................................. 31**

1.5.1.7.3 **Fungal sinusitis ...................................................................................................................... 32**

1.5.1.7.4 **Invasive aspergillosis ............................................................................................................. 35**

1.5.1.7.5 **Mycotoxicosis ....................................................................................................................... 36**

1.5.2 **Airborne distribution............................................................................................................................ 38**

1.5.3 **Allergenic fungal extracts...................................................................................................................... 42**
CHAPTER 6 ................................................................................................................138

6. PART A. SPORE GERMINATION AND ALLERGEN RELEASE .........................138
  6.1 MATERIALS AND METHODS .................................................................139
    6.1.1 Fungal isolates .............................................................................139
    6.1.2 Cultured spore sampling ..............................................................139
    6.1.3 Immunostaining of cultured spore samples .................................139
    6.1.4 Statistical analysis ......................................................................140
  6.2 RESULTS .................................................................................................140
  6.3 DISCUSSION .........................................................................................141
  6.4 CONCLUSIONS .....................................................................................144

6. PART B. THE RELATIONSHIP BETWEEN INHALED FUNGI AND ..............155
  GERMINATION ............................................................................................155
  6.5 MATERIALS AND METHODS ...............................................................156
    6.5.1 Subjects .......................................................................................156
    6.5.2 Environmental exposure ..............................................................156
    6.5.3 Nasal lavage procedure .................................................................157
    6.5.4 PAS staining ................................................................................157
    6.5.5 Nasal lavage culture ....................................................................158
    6.5.6 IOM personal aerosol sampler counts .........................................158
    6.5.7 Statistical analysis .......................................................................158
  6.6 RESULTS .................................................................................................159
  6.7 DISCUSSION .........................................................................................160
  6.8 CONCLUSIONS .....................................................................................164

CHAPTER 7 ..........................................................................................................169
# List of Tables

**Table 1.1** Fungal genera frequently associated with IgE mediated (atopic) allergy…...56

**Table 1.2** Fungal nomenclature……………………………………………………………..57

**Table 1.3** Relevant characterised allergens from fungi and their function approved by the Allergen Nomenclature Committee………………………………………………...58

**Table 2.1** Inter-observer agreement between the HIA ordinal rank scores that were assigned by each independent observer…………………………………………………………..79

**Table 2.2** The number of serum samples identified by each immunodiagnostic technique to be either sensitive to fungi (+ve) or not sensitive to fungi (-ve)………………80

**Table 2.3** Spearman correlation results for the SPT fungal positive sera between the three-immunodiagnostic techniques………………………………………………………….81

**Table 4.1** Median, 25th and 75th percentile values of the total number of airborne fungi measured by three different air sampling methods in an outdoor environment………111

**Table 5.1** Summary of the proportion of ungerminated and germinated *A. fumigatus* and *P. chrysogenum* conidia treatments demonstrating double immunostaining with mAb and human IgE………………………………………………………………………………128

**Table 5.2** Summary of the proportion of ungerminated and germinated *A. alternata* conidia treatments demonstrating double immunostaining with pAb and human IgE…………………………………………………………………………………….136

**Table 6.1** Percentage of ungerminated spores and germinated spores with hyphae (± SE) eluting allergen, detected by the Halogen immunoassay…………………………146

**Table 6.2** Numbers of fungal colonies recovered by culture and the numbers of germinated and ungerminated fungal conidia recovered by PAS staining from nasal lavage samples………………………………………………………………………165
Table 6.3 Percentage of germinated *Aspergillus* and *Penicillium* conidia recovered from nasal lavage samples, grouped by experimental subject and environmental exposure.
List of Figures

Figure 1.1 Transverse sectional view of fungal hyphae, organelles and associated structures……………………………………………………………………………….60
Figure 1.2 Life cycle of Anamorphic fungi…………………………………………61
Figure 1.3 Morphological characteristics of asexual Anamorphic conidia and associated reproductive structures………………………………………………………….61
Figure 1.4 Number of fungal species represented in each fungal division…………62
Figure 1.5 Photomicrographs of fungal cultures in nutrient media and microscopic morphology of the spores. .................................................................63
Figure 1.6 Hirst-type volumetric spore trap………………………………………..64
Figure 1.7 Anderson sampler……………………………………………………64
Figure 1.8 IOM personal air sampler………………………………………………64
Figure 1.9 The Intra-nasal air sampler. The disassembled components for the nasal sampler – a soft silicon strap spans the septum of the nose and connects the two silicon frames and that house the collection cups……………………………………………65
Figure 1.10 The fully assembled Intra-nasal air sampler worn by a subject……65
Figure 1.11 Cross sectional view of a nose with the Intra nasal air sampler in place…65
Figure 1.12 Halogen Immunoassay system for fungi……………………………66
Figure 2.1 Germinated Alternaria alternata showing different intensities of immunostaining of conidia (arrow a) and hyphae (arrow b) using the Halogen immunoassay to detect the binding of human IgE (arrow c) to expressed allergens…..82
Figure 2.2 Germinated Aspergillus fumigatus showing different intensities of immunostaining of conidia (arrow a) and hyphae (arrow b) using the Halogen immunoassay to detect the binding of human IgE (arrow c) to expressed allergens…..83
Figure 2.3 Germinated *Epicoccum purpurascens* showing different intensities of immunostaining of conidia (*arrow a*) and hyphae (*arrow b*) using the Halogen immunoassay to detect the binding of human IgE (*arrow c*) to expressed allergens.84

Figure 3.1 Resultant immunostaining of airborne hyphal fragments confined to the hyphal tips (A-B, D, F; *arrow a*), regions of septation or cross wall linkages (C; *arrow b*) and around the entire fragment (E; *arrow c*). Fragmented conidia (G and H) expressed antigen localized at the site of fragmentation (*arrow d*).94

Figure 3.2 The proportion of airborne hyphal fragments not expressing detectable levels of fungal allergen was significantly higher than positively immunostained fragments (*P*<0.05).95

Figure 3.3 Total fungal counts demonstrated that airborne hyphal fragments were significantly higher in concentration compared to other fungal genera.96

Figure 3.4 Deuteromycete conidia (*arrow a*) and germinated conidia (*arrow b*) represented by *Alternaria* (A), *Aspergillus-Penicillium* (B), *Cladosporium* (C), *Curvularia* (D), *Exserohilum* (E) and *Pithomyces* species (F) demonstrated IgE binding to expressed antigens.97

Figure 3.5 Undocumented fungal genera demonstrated IgE binding to expressed antigens, including *Amphisphaeria* species (A), *Arthrinium* species (B), *Leptosphaeria* species (C), *Leptosphaerulina* species (D), *Myxomycete* spores (E), *Pleospora* species (F), *Spegazzinia* species (G), *Sporidesmium* species (H), Ascomycete cleistothecium of the Erysiphales (I), and germinated ascospores belonging to the Xylariaceae (J).98

Figure 4.1 The sampling site and surrounding vegetation with subjects participating in the study on SaD 1 with and inset of the map of Australia depicting the geographical position of Casino, northern New South Wales and the (□) sampling region.112

Figure 4.2 (A) The Intra-nasal air sampler. The disassembled components for the nasal sampler – a soft silicon strap spans the septum of the nose and connects the two silicon frames and that house the collection cups. (B) The fully assembled intra-nasal air sampler worn by a subject.113

Figure 4.3 The total number of fungal genera collected by each air sampling technique on each sampling day.114
Figure 4.4 The relative abundance of fungal spores belonging to the five most frequent genera inhaled by each subject. A. Sampling Day 1. B. Sampling Day 2…………….115

Figure 4.5 Photomicrographs of collected fungal conidia and hyphal fragments. A. Ascospores belonging to the Xylariaceae (arrow a) and hyphal fragments (arrow b) and B. fungal spores of Arthrinium (arrow c) and Curvularia (arrow d) species.............116

Figure 4.6 Comparison between the relative abundance of total fungal spores collected by left and right nostril INAS collection cups for the nine most frequent genera……..117

Figure 4.7 Correlation between IOM and INAS total fungal counts...............118

Figure 5.1 Dual immunostaining of culture derived A. fumigatus conidia (A-B) and germinated conidia (C-D) using mAb 18G2 (arrow a; red precipitate) and human serum IgE (arrow b; purple precipitate)……………………………………………………...129

Figure 5.2 Dual immunostaining of culture derived P. chrysogenum conidia (A-B) and germinated conidia (C-D) using mAb 18G2 (arrow a; red precipitate) and human serum IgE (arrow b; purple precipitate)……………………………………………………...130

Figure 5.3 Resultant double immunostaining of culture derived A. alternata conidia (A-C), A. alternata hyphal fragment (D) and germinated A. alternata conidia (E-F) using a pAb raised against a crude Alternaria extract (arrow a, red precipitate) and human serum IgE (arrow b, purple precipitate)……………………………………………….137

Figure 6.1 Individual (A) ungerminated and (B) germinated Exserohilum rostratum spores immunostained by the Halogen immunoassay with human serum IgE……….147

Figure 6.2 Individual (A) ungerminated and (B) germinated Bipolaris spicifera spores immunostained by the Halogen immunoassay with human serum IgE……………….148

Figure 6.3 Individual (A) ungerminated and (B) germinated Curvularia lunata spores immunostained by the Halogen immunoassay with human serum IgE…………………149

Figure 6.4 Individual (A) ungerminated and (B) germinated Epicoccum nigrum spores immunostained by the Halogen immunoassay with human serum IgE…………….150

Figure 6.5 Individual (A) ungerminated and (B) germinated Trichoderma viride spores immunostained by the Halogen immunoassay with human serum IgE…………….151

Figure 6.6 Individual (A) ungerminated and (B) germinated Botrytis cinerea spores immunostained by the Halogen immunoassay with human serum IgE…………….152

Figure 6.7 Individual (A) ungerminated and (B) germinated Aspergillus fumigatus spores immunostained by the Halogen immunoassay with human serum IgE……….153
Figure 6.8 Individual (A) ungerminated and (B) germinated *Cladosporium herbarum* spores immunostained by the Halogen immunoassay with human serum IgE........154

Figure 6.9 The nasal pool device used for nasal lavage........................................167

Figure 6.10 Photomicrographs of ungerminated and germinated fungal conidia and hyphae recovered from nasal lavage samples and stained with PAS......................168
List of Appendices

Appendix 1.1 Original Halogen immunoassay protocol.................................182
Appendix 1.2 Initial allergen detection protocol developed for spores of Alternaria
alternata developed by Teresa Mitakakis.............................................189
Appendix 2.1 Recipes for agars and buffers.............................................190
Appendix 2.2 Fungal germination Halogen immunoassay procedure..............191
Appendix 4.1 Coating Intra nasal air samplers with adhesive.......................192
Appendix 4.2 Calberla's stain for pollen grains......................................193
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>Immunoglobulin epsilon (ε)</td>
</tr>
<tr>
<td>TLRs</td>
<td>Toll-like receptors</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>APCs</td>
<td>Antigen-presenting cells</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex II</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodaltons</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>CFUs</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>AFS</td>
<td>Allergic fungal sinusitis</td>
</tr>
<tr>
<td>SPT</td>
<td>Skin prick test</td>
</tr>
<tr>
<td>INAS</td>
<td>Intra-nasal air samplers</td>
</tr>
<tr>
<td>HIA</td>
<td>Halogen immunoassay</td>
</tr>
<tr>
<td>CAP</td>
<td>Pharmacia UniCap</td>
</tr>
<tr>
<td>PBM</td>
<td>Protein binding membrane</td>
</tr>
<tr>
<td>SM</td>
<td>Skim milk</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PAS</td>
<td>Personal volumetric samplers</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Occupational Medicine</td>
</tr>
<tr>
<td>MPBM</td>
<td>Mixed cellulose ester protein binding membrane</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>SaD</td>
<td>Sampling day</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ICC</td>
<td>Intra class correlation co-efficient</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>pAb</td>
<td>Polyclonal antibody</td>
</tr>
<tr>
<td>PAS</td>
<td>Periodic acid Schiff</td>
</tr>
<tr>
<td>V8</td>
<td>Vegetable juice nutrient agar</td>
</tr>
<tr>
<td>RB</td>
<td>Rose-Bengal chloramphenicol nutrient agar</td>
</tr>
<tr>
<td>CFS</td>
<td>Chronic fungal sinusitis</td>
</tr>
</tbody>
</table>