Pneumococcal Vaccine in Eosinophilic Asthma Study

RESEARCH PROTOCOL
Version 3 – 17 March 2016

A single-site trial to investigate the efficacy, safety and immunogenicity of conjugated pneumococcal vaccine (CJPV13, Prevenar) on airway inflammation in people with eosinophilic asthma

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SYNOPSIS

Title: A single-site trial to investigate the efficacy, safety and immunogenicity of conjugated pneumococcal vaccine (Prevenar 13) on airway inflammation in people with eosinophilic asthma

Primary Outcomes:

Induced sputum eosinophils, Treg cell numbers and function, Spn antibody analysis, fraction of exhaled nitric oxide (FENO)

Secondary Outcomes:

Asthma symptoms, lung function asthma related quality of life, asthma exacerbations, safety outcomes

Number of participants / centres:

100/1

Study Duration:

April 2016 – December 2018

Study Population:

Aged ≥ 18 years, male and female participants with eosinophilic asthma.

Never or ex-smokers

FEV_1 > 40% predicted

Confirmed variable airflow obstruction

No exacerbation for 4 weeks prior to study entry

Prescribed asthma therapy

Study Drug:

Prevenar13, 0.5ml intramuscular given in 2 doses at monthly intervals.

Study Design:

Randomised parallel group controlled trial

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LIST OF ABBREVIATIONS

AE	Adverse Event
AAD	Allergic airway disease
ACQ	Asthma Control Questionnaire
ADR	Adverse Drug Reaction
AHR	Airway hyperresponsiveness
AQLQ	Asthma Quality of Life Questionnaire
CD	Cluster of differentiation
CCQ	Common Cold Questionnaire
COPD	Chronic obstructive pulmonary disease
CTLA	Cytotoxic T-lymphocyte
ECG	Electrocardiogram
eNO	Exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second (in litres)
FoxP3	Forkhead box P3
FVC	Forced vital capacity (in litres)
GINA	Global initiative for asthma
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	Inhaled corticosteroids
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LABA	Long-acting beta agonist
MDI	Metered dose inhaler
MLN	Mediastinal lymph node
NHMRC	National Health and Medical Research Council
NHPA	National Health Priority Area
RCT	Radomised Controlled Trial
OVA	Ovalbumin
SAE	Serious Adverse Event
SOP	Standardised Operating Procedure
Spn	*Streptococcus pneumonia*
T_{reg}	Regulatory T cells
TGA	Therapeutic Goods Association
Th	T helper
VC	Vital Capacity
WAP	Written Asthma Action Plan

1 PURPOSE OF STUDY

Asthma is an immune mediated disease with characteristic patterns of airway inflammation that lead to increased airway responsiveness, variable airflow obstruction, and symptomatic episodes. There is an excessive prevalence of asthma in our community and people with current asthma need frequent, usually daily, medication to maintain control of their symptoms [1]. Modulation of the immune response holds the promise of early intervention in the asthmatic response resulting in long-lasting treatment effects. Various strategies have been proposed to modulate immune responses in asthma [2]. Based on recent epidemiological observations and new understanding of immune responses in asthma, vaccination using bacterial products is considered a promising approach [2,3].

In an extensive research programme, we have successfully achieved inhibition of the eosinophilic asthmatic response and consequent airway hyperresponsiveness and airway wall remodelling using vaccination with *Streptococcus pneumoniae* (Spn) in various forms. Specifically, we have shown that Spn infection [4], killed Spn [5], Spn components [6], and the Spn conjugate vaccine (Prevenar) [6] can all induce effective immunomodulation of asthmatic responses. Furthermore, we have established that the mechanism of these beneficial immunomodulatory effects involves the induction of protective T –regulatory cell responses. Crucially, we have now shown that vaccination with Prevenar can induce regulatory T cells that suppress the development and manifestations of allergic airways disease.

These observations provide a compelling case to investigate the therapeutic efficacy of Prevenar in asthma. This project will seek to translate our exciting experimental observations into clinical reality by establishing the beneficial effects of Prevenar on specific clinical outcomes in patients with asthma.

2 AIMS

2.1 Study objectives

To establish the efficacy, safety and immunogenicity of conjugate pneumococcal vaccine (Prevenar, CJPV) on airway inflammation and clinical outcomes in eosinophilic asthma.

The specific hypotheses that will be addressed are:

(a) Treatment with conjugated pneumococcal vaccine (Prevenar 13 CJPV13) will attenuate eosinophilic airway inflammation.

(b) Prevenar treatment will significantly improve health status and reduce asthma symptom scores, in patients with eosinophilic asthma.

In addition, a number of biological parameters will be assessed, and hypothesis-driven sub-studies will be conducted capitalising upon the expertise of the individual Principle Investigators and adding value to the data set.

This study will be conducted by leading Australian researchers who have extensive experience in asthma biology and clinical trials. The study results have a high likelihood of providing data that will lead to changes in clinical practice and directly address a major Australian healthcare priority.

3 BACKGROUND AND PRELIMINARY STUDIES

Asthma has now consistently been identified as a risk factor for invasive pneumococcal disease[7-11]. In the largest study to date [8], the odds ratio for invasive pneumococcal infection was 2.8 for low-risk asthma, and rose to 12.3 for high risk asthma [defined as >/=1 hospitalisation in the past 12 months. Asthma is also a risk factor for increased Spn colonisation [12] and neonates who are colonised with Spn may have a greater likelihood of developing asthmatic features at age 4 [13]. The mechanisms behind this observation are unclear. It is likely that there is impaired immunity to Spn, and that a common factor, for example deficient T_{Reg} cells, could be both a risk factor for Spn carriage and also for the development of asthma.

Limited observational studies suggest that pneumococcal vaccination may have beneficial effects on asthma outcomes. In a retrospective cohort study of elderly adults, Anasaldi *et al* found a reduced risk of hospitalisation for asthma in those who received pneumococcal vaccination [14]. A Cochrane review found no informative randomised controlled trials (RCTs) and called for a RCT to evaluate the efficacy of Spn vaccination in asthma[15]. One RCT [16] was identified that examined the effect of pneumococcal vaccination in combination with other interventions in children with asthma and recurrent (> 4 episodes in a year) otitis media. The study reported that the children treated with pneumococcal vaccination had a 30% reduction in asthma episodes over a 2-year period.

3.1 Spn and Asthma

Spn is a common human respiratory pathogen that is asymptomatically and commonly carried in the nasopharynx, with children being particularly susceptible to infection (60-90%)[17,18]. Few studies have investigated the relationship between Spn infection and asthma, but the limited evidence available suggests that:

(a) Asthmatics may be more susceptible to Spn infection [13] and invasive Spn disease [7-11],

(b) Susceptibility may result from reduced immune responses to Spn. There is good evidence for low Spn IgG responses in asthmatic children [19,20], and asthmatics have significantly lower serotype-specific pneumococcal antibody levels than non asthmatics[21], and most importantly

(c) Infection or immunisation with Spn may boost immune responses to Spn and moderate allergic responses, reducing the severity of asthma [14,16].

These observations suggest that there is an opportunity for the development of Spn-based immunomodulatory therapy for asthma. Our forerunner studies show that conjugate Spn vaccination can achieve this in model systems, and this trial will test, for the first time, the efficacy of this approach in human asthma.

3.2 Asthma and T regulatory cells

Asthma may develop because of a lack of *regulatory T cells (*T_{Reg}*)* and/or impaired function of T_{Reg} cells that normally suppress the inflammation that leads to asthma [4-6,22-23]. Forkhead box P3 $(FOXP3)^+CD4^+CD25^+$ regulatory T (T_{Reg}) cells control peripheral T-cell responses via cytokines such as IL-10 and/or TGF-β [24]. These cells promote inhalation tolerance, protect against aeroallergen sensitization, and limit the extent of allergic responses in tissues. For example, allergen induced airway hyperresponsiveness can be attenuated by T_{Reg} cells that are recruited into the airway mucosa [6,22]. In atopic people who are sensitized to aeroallergens, a failure of the limiting effect of T_{Reg} cells would potentially lead to repeated cycles of T_H2-cell-associated inflammation, stimulating the tissue repair responses that result in airway wall remodelling that is the hallmark of chronic human asthma. Conversely, and importantly, restoration of T_{Reg} responses by immunomodulatory therapy has the potential to limit allergic inflammation and the consequent tissue responses. The insufficiency of T_{Reg} cell function in allergic asthma can be reversed with allergen immunotherapy [23], resulting in an up-regulation of both the numbers and function of $CD4^+CD25^+FoxP3^+$ T_{Reg} cells. These data establish the principle of achieving beneficial immunomodulatory effects in asthma via the induction of T_{Reg} cells.

T_{Reg} cells are also induced by exposure to certain bacteria, including Spn, however no-one has yet attempted to use bacteria or their components to induce T_{Reg} cells to prevent and treat human asthma. We have shown

for the first time that Spn infection or exposure to killed Spn or Spn vaccines induces T_{Reg} cells and suppresses the development and progression of asthma in mice.

The induction of T_{Reg} cells and the T cell inhibitory and regulatory effects of infection on the expression of asthma can be translated into the treatment of human asthma by vaccination with specific bacterial components.

3.3 Immune responses induced by Spn

While anti-capsular antibodies offer some protection against Spn infection [27], innate immunity and T cells are also necessary. T cell development in response to Spn involves the generation of Th1 cells [26], as well as the production of IFN-gamma and IL-10 [26], which implicates the involvement of T_{Reg} cells. Furthermore, and most importantly, $CD4^+CD25^+$ T_{Reg} cells are crucial in mediating protection against Spn and are induced to substantially greater levels than by other Gram-positive bacteria [27].

3.4 Spn vaccines

Two Spn vaccines, Pneumovax 23 and Prevenar, are licenced for use in humans. Pneumovax 23 is composed of capsular polysaccharides from 23 Spn serotypes whereas Prevenar has 13 Spn polysaccharides conjugated to an immunogenic carrier protein. Pneumovax and Prevenar elicit different immune responses. Pneumovax generates a T-cell independent response, which is poorly immunogenic, resulting in a low antibody response of largely low-affinity IgM. The lack of conventional T-cell help results in failure to induced antibody affinity maturation, isotype switching or immunological memory. Polysaccharide protein conjugation enables Prevenar to elicit a T-cell dependent immune response and enhanced antibody production. Prevenar promotes affinity maturation, isotype switching and induction of immunological memory. Unlike Pneumovax, **Prevenar has been shown to induce T_{Reg} cells.**

3.5 Preliminary findings

Spn infection, killed Spn & Spn vaccine suppress Acute Allergic Airways Disease (AAD, experimental asthma) in mice (Figure 1)

Exposure to Spn infection, killed Spn or Prevenar, before, during or after sensitisation with ovalbumin (Ova) substantially reduces the hallmark features of AAD in murine models [28-30]. Exposure suppressed Ova-specific production of T_H2 cytokines (IL-5 and -13) by T cells isolated from both mediastinal lymph nodes (MLNs) and spleen. **This indicates that Spn has both local and systemic suppressive effects on T_H2 responses** in AAD. In addition, Spn suppressed the production of the T_H1 cytokine IFN-gamma These results are supported by the observation that Spn suppressed Ova-specific IgG1 and IgE (characteristic of T_H2 responses) as well as IgG2a (characteristic of T_H1 responses). The suppression of T_H2 responses correlates with suppressive effects on other hallmark features of acute AAD. Exposure also prevented eosinophil accumulation in the blood, bronchoalveolar lavage fluid, and lung tissue, reduced the numbers of mucus secreting cells in the airways and inhibited the development of airway hyperresponsiveness. The suppressive effects correlated with increases in $CD4^+CD25^+FoxP3^+$ T_{Reg} cells in the MLNs, lungs and spleens, which attenuated OVA-induced proliferation and cytokine release by T_H2 cells. Suppression of all features could be reversed by the depletion of T_{Reg} cells, demonstrating that the protective effects were mediated by Spn-induced T_{Reg} cells. The observation that killed Spn and Prevenar suppressed AAD demonstrates that exposure to Spn and its components are sufficient for suppression and that the infectious process is not required.

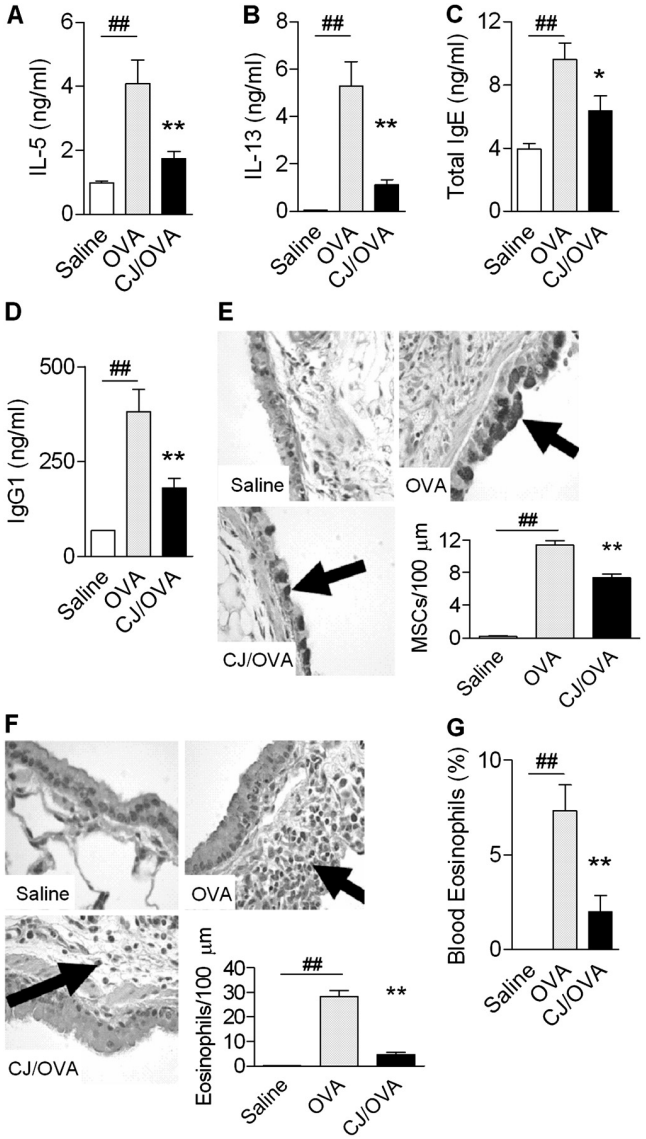

Figure 1: The inhibitory effect of conjugate vaccine (CJ) treatment on ovalbumin (OVA)-induced (A) interleukin 5 (IL-5) and (B) IL-13 release from splenocytes T cells, serum levels of (C) total immunoglobulin E (IgE) and (D) OVA-specific IgG1, numbers of (E) mucus-secreting cells (MSCs), numbers of (F) eosinophls in the airway tissue and (G) eosinophils in blood. Arrows indicate MSCs or eosinophils in airway tissue. Date represent mean±SEM from 6-8 mice. Significant differences between saline - sensitised (saline) and OVA-sensitised (OVA) controls are shown as ## $p<0.01$, significant differences between OVA-sensitised mice and CJ-treated OVA-sensitised mice are shown as *$p<0.05$, **$p<0.01$.

AntiCD25 treatment abrogates the protective response

To confirm the pivotal role of T_{Reg} cells in these responses, we used monoclonal anti-CD25 to specifically target the CD25 receptor on T_{Reg} cells. This leads to suppression of T_{Reg} cell responses, and inhibited the effects of conjugated pneumococcal vaccine on allergen induced airway hyperresponsiveness, eosinophilia and cytokine responses (Figure 2). CD25 also occurs on effector T cells, however, anti-CD25 treatment did not affect the development of any features of AAD in mice that were not treated with the conjugate vaccine. This demonstrates that the effect of anti-CD25 treatment is due to the suppression of vaccine-induced Tregs and not effector T cells.

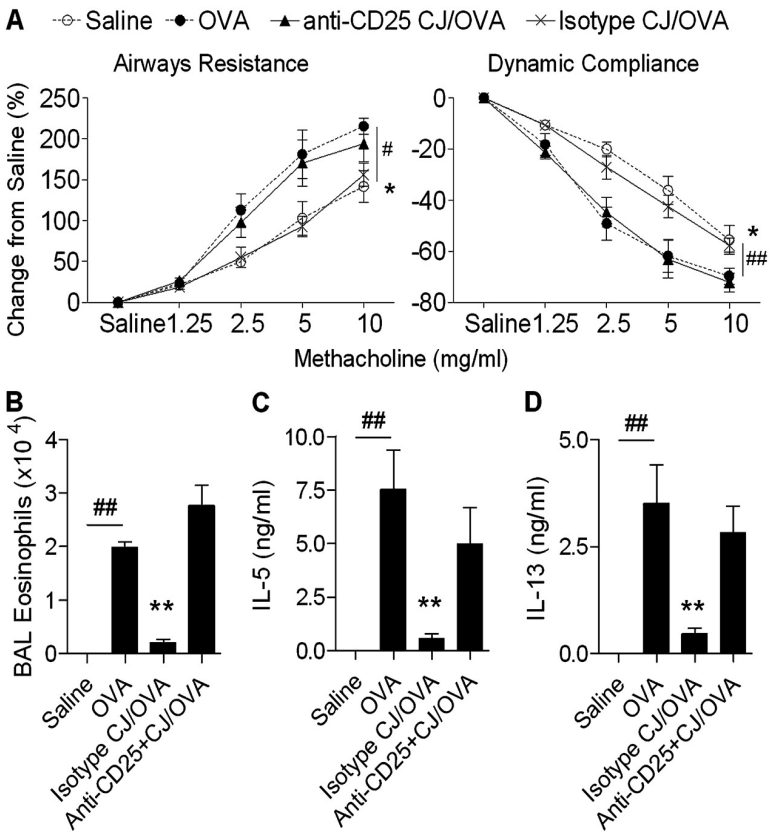

Figure 2 (left): The effect of anti-CD25 treatment on conjugate vaccine (CJ)-induced suppression of (A) airway hyper-responsiveness (AHR), (B) eosinophil numbers in bronchoalveolar lavage (BAL) and ovalbumin (OVA)-induced (C) interleukin 5 (IL-5) and (D) IL-13 release from mediastinal lymph node (MLN) T cells. Data are mean±SEM . Sig.differences between Saline and OVA are shown as #p<0.05, ##p<0.01. Sig. differences between OVA-sensitised mice and anti-CD25- and CJ-treated OVA-sensitised mice are shown as *p<0.05, **p<0.01. [P000759035]

Anti-CD25 targets the CD25 receptor on T regulatory cells. The beneficial effects of conjugated pneumocccal vaccine were inhibited by anti CD25, confirming a role for T_{Reg} *cells in this response.*

We also showed that conjugate pneumococcal vaccine-induced Tregs expressed increased levels of the functional markers CTLA4 and CD103, and suppressed the OVA-specific and non-specific (anti-CD3/CD28) proliferation and Th2 cytokine release of effector T cells in vitro, compared to OVA-induced Tregs.

C**ollectively, our results demonstrate that CJPV7 potently suppresses allergic airway disease through the induction of T_{Reg} cells. The next step is to assess its efficacy in human asthma.**

4 POPULATION AND STUDY DESIGN

A single-site trial will be conducted in participants with eosinophilic asthma, adherent to current asthma therapy. Participants will be recruited over 1 year and undertake a 2-week run-in before being randomly allocated to treatment with Prevenar13, 0.5ml intramuscular given in 2 doses at monthly intervals, or 1 dose Pneumovax 23 followed by 1 dose of sterile saline at a monthly interval. Participants will have the final study visit 12 months after the first vaccine dose. Participants will provide written informed consent. Approvals will be obtained from Human Research Ethics committees, the Therapeutic Goods Administration (CTN) and the trial registered (www.anzctr.org.au).

The study will recruit total of 100 participants. See discussion of sample size calculations below.

5 ASSESSMENTS

5.1 Schedule of visits and study procedures

5.1.1 Screening Visit (Visit 1)

Participants will be asked to withhold their asthma medications according to Table 1 and antihistamines according to Table 2.

The following assessments and procedures will be performed:

- Written informed consent will be obtained.
- Medical assessment (if required)
- Height and weight without shoes
- Skin prick tests with exposure to a range of common aeroallergens, including grasses, house dust mite, cat, dog and moulds (see table 2 for antihistamine withholding times). Note: allergens subject to availability.
- Medications: asthma and concomitant (Following screening, participants will be advised to consult study staff prior to taking ANY new medications – including over the counter medications).
- Asthma optimisation plan: eligible participants will be considered to be on optimal treatment in the run-in period if their current treatment matches treatment recommended by GINA guidelines.
- Smoking history
- Allergy history
- Spirometry (FEV_1 and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 1
- Juniper Asthma Quality of Life Questionnaire (AQLQ) (included in APPENDIX F)
- Juniper Asthma Control Questionnaire – 6 item (ACQ6) (included in APPENDIX F)
- History of variable airflow obstruction (within 10 years prior)
- Sputum induction using 4.5% hypertonic saline, to determine airway inflammatory cell type. If participant is able, a spontaneous sputum sample will be collected to determine airway inflammatory cell type in absence of an induced sputum sample.
- For participants who do not have a documented history of variable airflow obstruction within the preceding 10 years, bronchial responsiveness to 4.5% hypertonic saline will be performed in conjunction with sputum induction (for bronchial responsiveness, medication withholding times as outlined in tables 2 and 3). In the absence of bronchial responsiveness to 4.5% saline, salbutamol may be administered to assess reversibility (FEV_1 change >12%). If reversibility is not observed, a peak flow monitor may be provided and peak flow monitored over a 2 week period.

Table 1: Withholding times for asthma medications prior to bronchial hyperresponsiveness assessment and spirometry (ALL VISITS)

6 hours	12 hours	24 hours
Airomir	Austyn	Neulin SR
Asmol	Foradile	Slo-bid
Atrovent	Neulin	Theo-Dur
Atrovent Forte	Oxis	
Bricanyl	Seretide	
Combivent	Serevent	
Epaq	Singulair	
Intal	Spiriva	
Intal Forte	Symbicort	
Respolin		
Tilade		
Ventolin		

Table 2: Witholding times for antihistamines prior to skin prick test and bronchial hyperresponsiveness assessment (VISIT 1)

5 Days	5 Day (continued)	6 weeks
Aller G	Panquil	Hismanal
Andrumin	Periactin	
Avil	Phenergan	
Avil Retard	Polaramine	
Benadryl	Sinutab	
Claratyne	Sudagesic	
Demazin	Teldane	
Disolyn	Telfast	
Dramamin	Vallergan	
Panadol Sinus	Zadine	
	Zyrtec	

5.1.2 Run-in

Participants will then enter a run-in period of 2 weeks (up to a maximum of 8 weeks) to ensure disease stability and adherence to maintenance treatment.

5.1.3 Randomisation process

Randomisation will occur at visit 2 approximately 2 weeks after the screening visit. At the randomisation visit eligible subjects will be randomised to either Prevenar13 or Pneumovax23 . Random allocation will be achieved by computer-generated random number list and allocated by an independent data manager. Randomisation will be stratified according to asthma severity (GINA steps 1-3 or GINA steps 4-5) in order to prevent an imbalance between vaccine groups in asthma severity.

5.1.4 Randomisation (Visit 2- Treatment 1)

The following assessments and procedures will be performed:

- Medication use: asthma and concomitant
- Pre- and Post-bronchodilator spirometry (FEV_1 and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 1.
- AQLQ
- ACQ6
- Asthma medication adherence
- FENO
- Induced sputum eosinophils
- Venous blood for Treg cell numbers and function and Spn Antibody analysis
- Whole blood test to assess full blood count, & serum
- Exacerbations
- Adverse event assessment
- Urine pregnancy test to be performed on all female participants of child bearing potential, regardless of contraceptive methods. Not required on participants who are surgically sterile.
- Administer first dose of study drug. Participants who are clinically stable (no increase in symptom frequency or asthma medication use) and who meet the inclusion and exclusion criteria will be randomised to receive either prevenar at a total of 2 injections at 4 week intervals, or 1 dose of Pneumovax 23 followed by 1 dose of sterile saline at a 4 weekly interval.

5.1.5 Visit 3 (Treatment 2)

Participants will attend these visits at 4 week intervals (± 7 days).

The following assessments and procedures will be performed:

- Medication use: asthma and concomitant
- Pre- and Post-bronchodilator spirometry (FEV_1 and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2.
- AQLQ
- ACQ6
- Asthma medication adherence
- FENO
- Induced sputum eosinophils
- Venous blood for Treg cell numbers and function and Spn Antibody analysis
- Exacerbations
- Adverse event assessment
- Urine pregnancy test to be performed on all female participants of child bearing potential, regardless of contraceptive methods. Not required on participants who are surgically sterile.
- Administer second treatment dose (either Prevenar, or sterile saline if Pneumovax was administered at Visit 2)

5.1.6 End of treatment visit (Visit 4)

Participants will attend visit 4 weeks after Visit 3 (± 7 days).

The following assessments and procedures will be performed:

- Medication use: asthma and concomitant
- Pre- and Post-bronchodilator spirometry (FEV_1 and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 1
- AQLQ
- ACQ6
- Height and weight without shoes
- FENO
- Exacerbations
- Whole blood test to assess full blood count, & serum
- Asthma medication adherence
- Venous blood for Treg cell numbers and function and Spn Antibody analysis
- Sputum induction using 4.5% hypertonic saline to determine airway inflammatory cell type. Participants will be pretreated with 400µg salbutamol 10 min prior to sputum induction. If participant is able, a spontaneous sputum sample will be collected to determine airway inflammatory cell type in absence of an induced sputum sample.
- Adverse event assessment

5.1.7 End of study visit (Visit 5)

Participants will attend visit 12 months after Visit 2 (± 7 days).

The following assessments and procedures will be performed:

- Medication use: asthma and concomitant
- Pre- and Post-bronchodilator spirometry (FEV_1 and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2
- AQLQ
- ACQ6
- Height and weight without shoes
- FENO

- Exacerbations
- Asthma medication adherence
- Sputum induction using 4.5% hypertonic saline to determine airway inflammatory cell type. Participants will be pretreated with 400µg salbutamol 10 min prior to sputum induction. If participant is able, a spontaneous sputum sample will be collected to determine airway inflammatory cell type in absence of an induced sputum sample.
- Adverse event assessment

5.1.8 Study Withdrawal Visit

To be undertaken if participant withdraws from study during treatment or follow-up phase. The following assessments and procedures will be performed:

- Medications: Asthma and concomitant
- ACQ6
- AQLQ
- Exacerbations
- Adverse event assessment
- Pre- and Post-bronchodilator spirometry (FEV_1 and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2.
- Sputum induction using 4.5% hypertonic saline to determine airway inflammatory cell type (Visit 14). Participants will be pretreated with 400 µg salbutamol 10 min prior to sputum induction. If participant is able, a spontaneous sputum sample will be collected to determine airway inflammatory cell type in absence of an induced sputum sample.

6 SELECTION AND WITHDRAWAL OF PARTICIPANTS

6.1 Inclusion criteria

1. Able to provide informed written consent
2. Adults (≥18 years of age) with symptomatic asthma: ACQ6 $\geq$ 0.75 at screening
3. Confirmed variable airflow obstruction at screening visit or documented within the past 10 years:
   - Bronchodilator response >200ml OR > 12% (post-bronchodilator FEV_1 following administration of 400µg salbutamol, pMDI with spacer; after 10 minutes, or following administration of nebulised ventolin)

$$\% \text{ improvement} = \frac{FEV_1 \text{ post-bronchodilator} - FEV_1 \text{ baseline}}{FEV_1 \text{ baseline}} \times 100$$

   - Airway hyperresponsiveness (in response to any standard challenge agent)
   - Peak flow variability >12% when monitored over at least 1 week
   - FEV_1 variability > 12% (between two FEV_1 values measured within 2 months of each other)

   If not observed, then hypertonic saline challenge at visit 1 (pD_{15} < 15mL saline).
   - If pD_{15} < 15mL saline not observed, check bronchodilator response following saline challenge and provide peak flow meter for 2 weeks monitoring, if required.
4. Stable disease with no respiratory infection, asthma exacerbation, corticosteroid burst or use of antibiotics, or change in asthma therapy in the 4 weeks preceding screening
5. Maintenance asthma therapy. Asthma subtype will be determined using induced sputum - eosinophils ≥ 3% at screening for inclusion.
6. No active pulmonary (other than airway disease) or other system disease that would influence response to the vaccine or ability to assess asthma outcomes during the course of the study.
7. No contraindication to Prevenar and no prior pneumococcal vaccination with Prevenar ever.

8. Did not receive pneumococcal vaccination with Pneumovax in the past 8 weeks

10.1.1 Randomisation Inclusion

1. Asthma treatment optimised according to GINA guidelines (must be ≥80% adherence during run-in, repeat 2 week run in if adherence is <80%)
2. Stable asthma (ie. no increase in ACQ score >0.5 from visit 1, no exacerbation or respiratory infection during 2 week run in. Run in can be extended to up to 8 weeks from visit 1 to accommodate unstable asthma during run-in).
3. Confirmed variable airflow obstruction
4. Sputum eosinophils ≥3% at V1

6.2 Exclusion criteria

1. FEV1 < 40% predicted post-bronchodilator (excludes sputum induction based on safety grounds)
2. Current smoker
3. Ex-smokers who have quit within the last 6 months
4. Received pneumococcal vaccination with Pneumovax in the past 12 months
5. Treatment with oral corticosteroids 4 weeks prior to screening (unless a low dose is being taken on a long-term basis: ≤ 10mg for >3 months)
6. Pregnancy/breast feeding Respiratory disease other than asthma (e.g. active tuberculosis, bronchiectasis, emphysema, pulmonary fibrosis) that, at investigator's discretion, would adversely impact on study conduct.
7. Current lung cancer or other blood, lymphatic or solid organ malignancy
8. Inability to attend study visits
9. Unstable asthma: asthma exacerbation within 4 weeks of study entry
10. Cold or respiratory tract infection within 4 weeks of study entry
11. Participants who have participated in another investigative drug study parallel to, or within 4 weeks of study entry

6.3 Smoking History

Smoking may induce incomplete reversibility of airflow obstruction (COPD) that is associated with an intense inflammatory response in the small bronchioles that involve neutrophils and lymphocytes [31]. It is unclear whether Prevenar may or may not be effective in these processes. In this study it will be important to establish whether Prevenar is equally effective in asthmatics with a prior smoking history, where Prevenar may also target the airway inflammatory response induced by previous smoking. Ex-smokers will be included and randomisation stratified.

6.4 Withdrawal Criteria

In accordance with the Declaration of Helsinki, each participant is free to withdraw from the study at any time. The principle investigators also has the right to withdraw a participant from the study in the event of intercurrent illness, adverse event or other reasons concerning the health or well-being of the participant, or in the case of lack of cooperation.

Participants will be withdrawn if they are unable to tolerate the study medication, develop a severe adverse event that is potentially drug related, become pregnant during the study, or withdraw consent. Study treatment will be immediately withdrawn and where necessary medical assessment will be provided. Participants will attend a study withdrawal visit after cessation of the study medication and clinical care will be provided by study physicians or the participant's usual physician.

6.5 Adverse Events

Any serious adverse event will be reported in accordance with the institutional ethics committee guidelines

6.6 Recruitment

Eligible participants will be identified from the asthma clinic and research databases at John Hunter Hospital by clinical and research staff.

7 STUDY MEDICATION

7.1 Drug handling

Prevenar 13 is supplied as a suspension in 0.5 mL pre-filled syringes in packs of 1 and 10. It will be stored at the Insititute. Prevenar will be given at monthly intervals, totalling 2 doses. Pneumovax will be delivered as 1 dose, followed by sterile saline for the second dose. The vaccine will be delivered through intramuscular injections by medical or nursing research staff.

7.2 Concomitant asthma therapy

Participants will continue their usual maintenance and reliever asthma therapy according to their treatment requirements during the baseline observation period. Rescue medication in the form of a short-acting beta agonist will be used for symptom relief. Severe exacerbations will be treated using prednisolone according to current asthma therapy guidelines. Participants will be advised to consult study staff before taking ANY new medications (including over the counter medications).

7.3 Side effects of Prevenar

Prevenar and Pneumovax are clinically approved vaccines that are currently used worldwide. Potential minor side effects include local reaction around the injection site, chills, fever, headache, nausea, joint or muscle pain. Other side effects include allergic reactions, high temperature or rapid, shallow breathing. Any potential side effects will be monitored at the initial visit and periodically during treatment.

8 OUTCOMES

8.1 Efficacy

8.1.1 Sputum eosinophils

The primary study outcome is induced sputum eosinophils. It will be important to establish whether Prevenar will be able to reduce the number of eosinophils in sputum.

8.1.2 Health status and symptoms

We will evaluate health status using the Asthma Quality Of Life Questionnaire (AQLQ). This validated questionnaire assesses the effect of asthma on daily functioning, shortness of breath as well as night time asthma symptoms.

8.1.3 Others

Other study outcomes include FENO, spirometry, asthma exacerbations, AHR to 4.5% saline.

8.2 Immunogenecity

We will look into the effects of Prevenar on antipneumococcal antibody responses, Treg cell numbers and functional responses using CTLA4 and CD103 expression as markers of Treg functional status as well as the suppression of allergen-specific and non-specific effector T cell responses.

8.3 Safety

Analysis will constitute assessment of symptoms, vital signs, and respiratory specific safety assessment including measures of lung function before during and after administration.

8.4 Reporting procedures for adverse events and intercurrent illness

8.4.1 Screening visit (Visit 1)

Current medical illnesses and medications used to treat such conditions will be recorded at visit 1. Recurring symptoms associated with these pre-existing conditions will not be considered adverse events during the study unless they have a clinically significant increase in severity and/or frequency.

8.4.2 Treatment period (Visits 2-3)

Participants will be questioned at each visit about the occurrence of new or worsening symptoms.

8.5 Adverse Event (AE)

An adverse event (AE) is “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment” (ICH Good Clinical Practice).

Therefore, an AE can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of study product, whether or not related to Prevenar or Pneumovax.

8.6 Adverse Drug Reaction (ADR)

A response to Prevenar or Pneumovax which is noxious and unintended, and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases, or for modification of physiological function.

An Unexpected Adverse Drug Reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable Product Information for Prevenar or Pneumovax.

The appropriate clinical staff at each study centre will handle medical emergencies that involve study participants.

The Investigator will evaluate all adverse events as to:

- Maximum intensity
  - Mild (awareness of sign or symptoms, but easily tolerated)
  - Moderate (discomfort causes interference with usual activity)
  - Severe (incapacitating, or unable to do usual activities)
- Seriousness (see Serious Adverse Events)
- Duration: onset and stop dates
- Action taken
- Relationship to study drug

8.6.1 Recognition of adverse events

- Adverse events will be reported in the case report form in screened subjects during the run-in, where there is a protocol-specified intervention, including washout or discontinuation of usual therapy, or procedure.

- Exacerbations of asthma. An exacerbation of asthma will be defined as new onset or worsening of a complex of respiratory symptoms. Details on severity and characterisation of each exacerbation will be recorded in the participant's case report form.
- Illness during the study unrelated to asthma, if appropriate, will be managed by the referring doctor.
- Unexplained worsening in spirometry that requires intervention or further evaluation.

8.7 Serious Adverse Event (SAE)

8.7.1 Definition of SAE

An SAE is any medical occurrence that, at any dose:

- results in death;
- is immediately life-threatening (Note: the term 'life-threatening' in the definition of 'serious' refers to an even/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe);
- results in inpatient hospitalisation or prolongation of existing hospitalisation
- results in a persistent or significant disability/incapacity;
- is a congenital anomaly / birth defect; or
- is an important medical event or reaction.

Medical and scientific judgement should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalisation but might jeopardise the patient or might require intervention to prevent on of the other outcomes listed in the definition above. Examples of such events are intensive treatment in and emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisations, or development of drug dependency or drug abuse.

8.7.2 Follow-up and characterisation of SAE

- Any serious or significant AE, whether or not considered related to the study drug, and whether or not the study drug has been administered, must be reported immediately to: 1) Prof Peter Gibson by telephone / fax on telephone number (02) 4042 0143; and 2) Human Research Ethics Committee in accordance with their reporting guidelines.
- Any serious and Unexpected Adverse Drug Reactions must be reported immediately to) Prof Peter Gibson by telephone / fax on telephone number (02) 4042 0143; and 2) Human Research Ethics Committee in accordance with their reporting guidelines. In addition, ADRs are to be reported directly to the Therapeutic Goods Assocation (TGA) and Adverse Drug Reaction Unit (ADRU).
- An SAE, which persists or occurs within 30 days after cessation of the study treatment, will be followed up until the event or its sequelae resolve or stabilise at a level acceptable to the investigator.
- For all SAEs, the investigator is obliged to pursue and provide further information as requested by the Hunter Medical Research Institute. In general, this will include a description of the SAE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, including other medications and illnesses will be provided. The Principle Investigator's assessment of causality will also be provided. In the case of a subject's death, a summary of available autopsy findings must be submitted as soon as possible to the manufacturer or its designated representative.
- With respect to the reporting of AEs, it is important to distinguish between listed (expected) and unlisted (unexpected) AEs that relate to the study medication. A listed of AEs that relate to Prevenar can be found in the Product Information for Prevenar.

8.8 Outcome assessment

8.8.1 Induced sputum

Sputum induction will be performed as previously described [32] with selection and dispersion of muco-cellular clumps and performance of total and differential cell counts.

8.8.2 Treg cell analysis and Spn antibody analysis

Treg cell numbers and functional responses will be analysed using CTLA4 and CD103 expression as markers of Treg functional status as well as the suppression of allergen-specific and non-specific effector T cell responses.

8.8.3 Health status

Health status will be assessed using the AQLQ [33]. Asthma symptoms will also be assessed using the ACQ6.

8.8.4 Spirometry

Following baseline spirometry (CPFS/D™ USB Spirometer + BREEZESUITE software, Medgraphics), hypertonic (4.5%) saline will be nebulised using a De-Vilbiss ultrasonic nebuliser (Somerset, PA USA) and aerosol delivered via a one-way non-rebreathing Hans Rudolph valve box. Each participant will undergo a maximum of 16 minutes nebulisation time and nebulisation time within participants will be kept constant.

8.8.5 Asthma exacerbations

Asthma exacerbations will be defined as 'severe' or 'moderate' according to the ATS/ERS Asthma Outcomes Taskforce guidelines [35].

A 'severe' exacerbation will be defined as a participant who requires:

- Use of systemic corticosteroids, or an increase from a stable maintenance dose, for at least 3 days. (Courses of corticosteroids separated by 1 week or more will be treated as separate severe exacerbations);
- Hospitalisation or an emergency department visit requiring systemic corticosteroids.

A 'moderate' exacerbation will be defined as a participant who has/requires a:

- Deterioration in symptoms and increased rescue bronchodilator use, for at least 2 days;
- Deterioration in lung function and increased rescue bronchodilator use, for at least 2 days;
- Visit to the emergency department for asthma *not* requiring systemic corticosteroids

9 TIMELINE

- Recruitment: April 2016- February 2017
- Treatment: April 2016- April 2018
- Follow-up evaluation: June 2016- April 2018
- Analysis & Publication: April 2018- December 2018

10 STATISTICAL CONSIDERATION

10.1 Sample size

Alpha: 0.05; Power: 80%; Sample size required is 49 per group, as described below and allowing for dropouts.

ACQ: a clinically important difference is 0.5 units. In a previous study the response within each subject group was normally distributed with standard deviation 0.83. If the true difference in the experimental and control means is 0.5, we will need 44 experimental and 44 control subjects.

FEV1%predicted: In a prior study the response within each subject group was normally distributed with standard deviation 20.8. If the true difference in the experimental and control means is 15.5, we will need to study 29 experimental subjects and 29 control subjects.

Sputum eosinophils: In a previous study the response within each subject group was normally distributed with standard deviation of 16.28%. If the true difference in the experimental and control means is 10%, we will need to study 43 experimental subjects and 43 control subjects.

11 INNOVATION AND SIGNIFICANCE

Preliminary data indicate there is a strong likelihood of a successful efficacy outcome. This trial will establish whether Prevenar has a role in the treatment of eosinophilic asthma in adults.

This represents a new approach to asthma therapy that uses an approved vaccine that is accessible and can be implemented rapidly based on the results from this study. Spn vaccination for asthma represents a novel approach with a strong evidence base in experimental medicine. It has the potential to address many of the limitations of current therapy. There is now an opportunity to translate this into a viable commercial product that aims to effectively treat and prevent one of the world's most significant chronic diseases.

12 ETHICAL CONSIDERATION

Written informed consent will be obtained from each participant prior to the collection of any data or samples.

12.1 Study Drug

Prevenar and Pneumovax are registered and approved for long-term use in Australia. The vaccines have been used widely to protect individuals against disease caused by the bacteria *Streptococcus pneumonia* (pneumococcus). Overall, at the dose we propose to use, they are usually free of significant side effects. Potential side effects and toxicity concerns are described in detail above.

12.2 Sputum Induction

Sputum induction will be undertaken using 4.5% hypertonic saline. Lung function will be monitored using a spirometer before and after the administration of each saline dose to maximise safety. The test will be stopped at the participant's request or if lung function falls below a safe level. Sputum induction can cause cough and minor chest discomfort, and wheeze. This is brief and promptly responds to salbutamol, which will be provided for participants' comfort.

12.3 Allergy tests

Prick skin tests to a range of common allergens such as house dust mite, grass, animal hair and mould will be undertaken. This involves placing some liquid containing these substances on the forearm and lightly pricking the skin. The skin test may cause transient (20-30 min) itch if the participant is allergic to these substances. Participants will be offered the use of a cream to relieve the itch.

12.4 Blood tests (safety and substudies)

A small amount of blood (25 – 40 mL or approximately 1-2 tablespoons) will be collected. The collection of blood may cause slight localised bruising.

12.5 General considerations

All information obtained in this study will be available to the participant's general practitioner or specialist at their request. All participation is voluntary and participation will not affect the participant's current or future management. All information collected will be confidential and only accessible to the research team. Results of the study will be collated and communicated to the scientific community in a de-identified manner in which the identification of individual participants is not possible.

12.6 Serious Adverse Events

The Human Research Ethics Committees will be notified of any serious adverse event in accordance with local reporting guidelines.

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APPENDIX A INDIVIDUALISED WRITTEN ASTHMA ACTION PLAN

MRN:_____
Name:_____
Address:_____
DOB:_____

ASTHMA ACTION PLAN

WHEN WELL

Ventolin(Asmol) _____ Dose: 2 puffs when needed for asthma symptoms
Preventer _____ Dose: _____
_____ Dose: _____
Take Ventolin 2 puffs 10 minutes before exercise

WHEN NOT WELL

- If your peak flow reading does not reach 60 % of your best value, which is_____following your medication for a 24 hr period. OR
- If you are waking at night due to your asthma. OR
- If you require your Ventolin more frequently than usual and are not getting the same effect.

Then

- **Increase your** Ventolin: Take 2 extra puffs as needed up to 6 times per day
- Contact the study team to arrange a review (ph:_____)
- See your doctor if your symptoms are severe or not responding to Ventolin

FOR A SEVERE ATTACK

- If your peak flow does not reach 40 % of best value, which is_____ OR
- If you have a severe shortness of breath and can only speak in short sentences. OR
- If you are having a severe attack of asthma and are frightened. OR
- If you need to take your Ventolin more than 4 hourly and do not gain an effect.

Then

- **Take Ventolin 4 puffs : repeat if you do not improve**
- **Take mg of prednisone**
- **Seek medical attention immediately by calling an ambulance on 000**
- **Continue to use your Ventolin until help arrives**

Signature_____ Date_____

APPENDIX B PRICK SKIN TEST SOP

The allergy skin prick test is used to determine whether a patient/participant is atopic or not. A positive reaction to one or more allergens indicates atopy while no positive reaction to allergens implies the patient is not atopic. Mechanisms of action: the immediate reaction is mediated by degranulation of mast cells. Histamine is the main mediator responsible for the wheal and flare response.

The purpose of this method is to describe the method of carrying out a skin allergy test safely and ensuring that a reliable result is obtained.

1. Equipment:

1.1. Reagents (allergens stored in fridge)

1.1.1. Histamine: positive control 10mg/ml histamine hydrochloride
1.1.2. Negative control(Glycerin)
1.1.3. Allergens for testing

Important: Allergens should be at room temperature prior to testing. Remove from refrigerator 30 minutes prior to testing.

1.2. Materials

1.2.1. Biohazard bin for lancet disposal
1.2.2. Holder for allergens
1.2.3. Sigmacort Corticosteroid Cream
1.2.4. Alcohol wipes
1.2.5. Gloves
1.2.6. Flat surface such as a table
1.2.7. Skin Prick Test measuring slide, or ruler
1.2.8. Pillow armrest with 'Bluey' underpad placed under the arm
1.2.9. Skin Prick Lancets, Order from Bayer Diagnostics ph 1800 039 076, Code 2054254 box of 200)
1.2.10. Biro for marking subject's arm, marker pen for the skin
1.2.11. Tissues for removing allergen drops
1.2.12. Soap and water
1.2.13. Paper towel
1.2.14. Skin Prick Test Worksheet
1.2.15. Stopwatch/timer
1.2.16. Razor

2. Method

2.1. Pre Procedure

Request that the participant withholds antihistamine medication for **5** days (or 6 weeks for Hismanal) prior to the test. Supply them with the "Withholding Medication" list for Skin prick testing.

2.2. Procedure

The antigen is placed on the skin and introduced in the epidermis (prick test)

2.2.1. Allergens should be at room temperature prior to testing. Remove from refrigerator 30 minutes prior to testing.
2.2.2. Explain the test to the participant ie, place drops of solutions onto their forearm, lightly scratch them and see if they come up in a lump. Explain side effects that may be experienced: itchiness, redness and a lump like a mosquito bite and that any responses usually subside in about 20 minutes.
2.2.3. Consider contraindications to the test, question whether the participant has a known anaphylactic reaction to any allergen

2.2.4. As per worksheet question the participant as to whether they have had any antihistamines in the past **5** days. If yes, then reschedule skin prick test instructing the participant to withhold antihistamine use for at least **5** days before their visit.
2.2.5. Instruct the participant to lay their exposed arm (inner arm up) on top of a bluey underpad which has been positioned on a pillow on the table.
2.2.6. Gently wipe the arm with an alcohol swab to clean after putting on your gloves.
2.2.7. Using a biro, draw a grid (2 squares width by 6 squares length in the centre of the right forearm at least 5 cm above the wrist and 3 cm below the cubital fossa. The squares should be at least 2 cm apart to avoid false-positive reactions. Ensure there is minimal hair; shave if necessary.
2.2.8. Areas of thick hair, wounds, abrasions, scar tissue and veins should be avoided if possible.
2.2.9. Place a small drop of each allergen into a square. Allow a small amount of allergen to form at end of dropper. Touch liquid lightly to forearm. Surface tension will pull the allergen to the skin. Follow the order of the allergens on the results sheet. Do not change the order of allergens once determined.
Important: Do not allow dropper to touch skin as this will result in contamination of the allergen when the dropper is returned to the bottle.

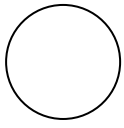
1.1.1.1 Right forearm

Antecubital fossa

Histamine (+)	Glycerin (-)
Grass Mix #7	D. Pter.
Cat Hair	Dog Hair
Mould Mix #10	Aspergillus fumigatus

Wrist

2.2.10. Apply one drop of each allergen next to the appropriate mark on the arm taking care to have the participant's arm flat to prevent allergen drop from running. Also take care not to touch the skin with the dropper to prevent potential contamination of the allergen bottle.
2.2.11. Release the lancet by twisting the plastic joint and pulling at the same time.
2.2.12. With the lancet exposed, prick the skin (using either the upward pressure or down pressure method) in the middle of the allergen drop and release skin gently. **DO NOT** flick the lancet across the skin as the skin opening should not be visible or draw blood.
2.2.13. Use a separate lancet for each allergen. Dispose of lancet in 'sharps' container.
2.2.14. Allow 1 minute and then blot excess allergen using tissues. Blot gently and ensure that allergens are not transferred to sites other than the position on the grid.
2.2.15. Ask the subject not to scratch the area and remind if necessary.
2.2.16. The histamine result (positive control) is measured at **10 minutes** (9 minutes after blotting) while the allergen results and negative control are read at **15 minutes** (14 minutes after blotting).
2.2.17. Record diameter of wheal (figure 2). For a wheal that is irregular in shape take the mean of the length of the longest diameter and the perpendicular length of the shortest diameter through the point of abrasion (figure 3). Add the two measurements together and divide by 2 to determine the mean. If the result of the wheal is circular, one measurement of the diameter (in mm) is sufficient. If you have difficulty determining the margin of the wheal, run a finger lightly over the area and observe the point at which the skin blanches. Where there is no response to a particular allergen a **zero** must be recorded on the Data Collection Sheet. Blank spaces and dashes may lead to confusion during data analysis. *Any weal that has a "tail" called a pseudopod can be regarded as a strong reaction. The length of the pseudopod is included in the measurement recorded.)* **IMPORTANT***:* Measure the WHEAL (lump) **NOT** area of erythema (redness).



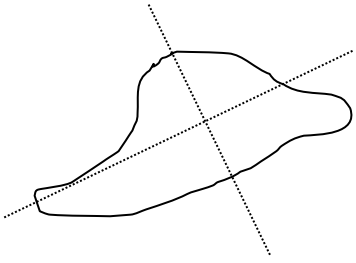


Figure 2 Figure 3

2.2.18. Confidence in the test result is determined by a positive reaction to the positive control (ie, histamine) and negative reaction to the negative control (ie, saline). If a negative reaction to positive control or a positive reaction to negative control is recorded the test will need repeating on the opposing arm.
2.2.19. When all measurements have been taken and on completion of recording results, remove biro marks with alcohol wipe.
2.2.20. Have the participant move over to the sink and wash their arm with the water and soap. Dry with paper toweling and apply corticosteroid cream to the area of itching and discomfort.
2.2.21. Explain to the participant that the reaction may last up to 24 hours after the test.
Important: Return allergens to refrigerator (4ºC) at completion of testing session.

3. Maintenance:

3.1. Ensure that the allergens are in date (this is recorded on the allergen bottle)
3.2. Ensure the exposed underarm is clean and free of wounds, etc (as detailed above)
3.3. The allergens are located in a particular order in the tray for the Pulmonary Function (PFT) Lab work, therefore ensure allergens are returned to tray in the order removed

4. Safety Precautions:

4.1. Return the allergens to the refrigerator.
4.2. Return the pillow, measuring slide and stopwatch to the correct locations
4.3. Document skin prick test worksheet into participant's file
4.4. Clean the work area with alcohol and paper toweling (alcohol is in spray bottle located on the sink bench)
4.5. As described above lancets is to placed into sharps container
4.6. Bluey and cup are to be placed into the contaminated bin.
4.7. Avoid any allergens where the patient knows they have a reaction to.
4.8. Explain to the participant that if they still have a reaction to any of the allergens after 24 hours to notify staff or seek medical attention

5. Troubleshooting:

5.1. Suspect or incorrect results require the test to be repeated.
5.2. Positive responses are known to decline after 50 years of age
5.3. Responses may be less with immunotherapy (record if the patient has received this treatment for any allergens to be tested).
5.4. Sensitive skin may induce false positives but should be screened by negative control

6. Allergen supply:

Link Pharmaceuticals:T: 1800 824 166 F: 1800 824 199

Allergen	REF #
Grass Mix #7	**HS 0850 TR**
D. Pteronyssinus	HS 6692 UP
Cat Hair	HS 4815 TR
Dog Hair	HS 4084 ED
Mould Mix #10	HS 5137 ED
Aspergillus fumigatus	HS 5021 ED
Glycerin: '-ve control'	HS 6806 ED

AUSPMAN MANUFACTURING FACILITY, PRINCESS MARGARET HOSPITAL, PERTH, WA

Histamine 10mg/ml	'+ve control'

ALLERGY SKIN PRICK TEST WORKSHEET

Has the participant taken Antihistamines within the past 5 days? (Hismanal 6 weeks) Yes ☐ No ☐

*If **yes** provide details do not continue test and reschedule appointment.*
How long since last antihistamine taken?

_____

_____

Start Time:_____

Test	Wheal Size (mm) x (mm)	Mean wheal size (mm)	Test	Wheal Size (mm) x (mm)	Mean wheal size (mm)
CONTROLS					
Histamine/ +ve Control ***(measure at 10 mins)***	____ X ____	____	Glycerin/ -ve Control (***measure at 15 mins)***	____ X ____	____
ALLERGENS (***measure at 15 mins)***					
Grass mix #7	____ X ____	____	D. Pteronyssinus	____ X ____	____
Cat Hair	____ X ____	____	Dog Hair	____ X ____	____
Mould Mix #10	____ X ____	____	Aspergillus fumigatus	____ X ____	____

** If the subject does not experience a reaction to an allergen, 0 should be entered for length and width.*

ATOPY positive if (any allergen wheal ≥ 3mm) Yes ☐ No ☐

Performed By: _____

APPENDIX C HYPERTONIC SALINE CHALLENGE SOP

Note: Sputum Induction can be conducted in combination with hypertonic saline challenge. A subject can be encouraged to provide a sputum sample throughout a challenge and after a $\beta_2$agonist has been given. The challenge ceases once $\beta_2$agonist has been given. Hypertonic saline inhalation after β_2 agonist is an induction (see SOP sputum induction).

1 Equipment:

- Refrigerator
- ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000 (JHH, PCH, RAH)/ NE-U12 Omron Ultrasonic nebuliser (SCGH)
- Hans Rudolph valve box, large 2 way No. 2700 (JHH, PCH, SCGH)
- Saliva trap (green)
- Vacumed 1001 mouthpiece
- Nebuliser cup and lid + tubing
- Spirometer + mouth pieces (CPFS/D™ USB Spirometer + BREEZESUITE software, Medgraphics)
- Volumatic/Spacer
- Stopwatch
- Calculator
- Nose clip
- Bench top Digital scales

2 Materials:

- 4.5% hypertonic saline – 200mL bottle
- Disposable mouthpiece for spirometer
- Salbutamol
- Saline Challenge Worksheet (Appendix C)
- Absorbent sheets (i.e. Blueys or underpads)
- Tissues

3 Set Up:

3.1 Calibrate spirometer

Assemble valve box

With clean hands, open sterile packet containing valve box (4 parts) and valves (2 valves wrapped in blue cloth, do not dispose of blue cloth). See Illustration 1 section 11.

Take the main body of the valve box (No.3 on Illustration 1, largest piece, contains the writing 2700 Series Large 2-Way NRBV and arrows) and 1 valve (No.4 on Illustration 1).

Ensure the valve is sitting firmly on frame (if not pull valve to completely cover frame).

Turn the main body of the valve box upright with the writing up or arrow ↑ and place the valve facing up or out of the main body (Illustration 2). Take the corresponding piece (No. 5 Illustration 1 clear – each piece only fits one spot) and screw (over the valve) to the main body.

Take the remaining valve (No.2 Illustration 1) and place downwards within the opposing end of the main body (follow flow arrows).

Attach the corresponding piece (No. 5 Illustration 1, white) to the main body of the valve box.

Attach the final piece (No. 6 Illustration 1 for mouthpiece) to the lower portion of the main body of the valve box (Illustration 3).

Take a Vacumed mouthpiece and attach to final piece attached to valve box. This can be hard to attach and may require stretching over connection at the base of valve box. Vacumed mouthpieces can be found on shelves opposite TB office or in the right hand draw of trolley within Pulmonary Function Lab.

Attach saliva trap (green attachment found on sink in drainer in clinical lab) to clear end of valve box or end displaying ↑ on main body of valve box.

Wrap blue cloth (original sterile wrapping cloth for valves) around Vacumed mouthpiece. Subjects will then recognize someone else has not recently used that mouthpiece.

Place assembled valve box on absorbent sheet in front of nebulizer.

3.2 Prepare nebuliser cup

Select a nebuliser cup and lid from drainer on sink in clinical lab.

Remove a 200mL bottle of 4.5% hypertonic saline solution from the refrigerator in the clinical lab. Check the expiry date. The hypertonic saline solution should be at room temperature when used.

Pour the hypertonic saline solution into the nebuliser cup ensuring the minimum level has been reached and the maximum level has not been exceeded. These levels are marked on the side of the nebuliser cup.

Place lid firmly on nebuliser cup, matching arrow on lid to arrow on cup.

Select a length of tubing (grey) hanging from rack above the sink in the clinical lab.

Attach one end of the tubing to the nebuliser cup lid (place grey rubber end of tubing over larger/higher outlet port on nebuliser cup lid).

Take nebuliser cup and attached tubing to digital scales and weigh to 1 decimal point. Record weight and cup selected on the Saline Challenge Worksheet in the 'Nebuliser cup pre test weight' section.

3.3 Prepare nebuliser

Place previously prepared nebuliser cup and tubing into the nebuliser, ensuring arrows on cup and lid are aligned with that on the nebuliser and elements on the base of the cup are aligned with those of the nebuliser

The nebuliser should be on the maximum output setting (dial on front of nebuliser turned fully to the right).

Attach white tubing on nebuliser to the nebuliser cup lid (smaller inlet port).

Attach the loose end of the tubing (grey) on the nebuliser cup to the free (opposite to saliva trap) end of the valve box. Secure firmly with micropore tape around tubing and valve box or use blue connector piece (in drainage tray).

Check the position of the valves and the tubing is connected correctly by reviewing the flow direction noted by the arrows on the valve box before commencing challenge.

4 Method:

Ensure that the subject has withheld their asthma medication for the required time (Table 3).

If a subject has not withheld their medications for the required length of time, the subject where possible should be rescheduled.

Document the type, amount and time of last asthma medication on the Saline Challenge Worksheet

Explain the purpose of the test, how it is to be conducted and possible side effects e.g. coughing, dry mouth, gagging, chest tightness, wheezing, dyspnoea, nausea and excess salivation

Measure the baseline spirometry FEV_1 and FVC as per ATS guidelines. Record highest FEV_1 and FVC on Saline Challenge Worksheet.

Calculate a 15% fall of the highest FEV_1 and record on Saline Challenge worksheet (FEV_1 x 0.85 = 15% fall).

**Ask the subject to apply the nose clip and insert the valve box mouthpiece into their mouth, in a similar manner to a snorkel.

Advise the subject to breathe normally (tidal breathing) and if they need to cough, to cough into the mouthpiece rather than removing the mouthpiece.

Also advise the subject, if they are uncomfortable at any time whilst on the nebuliser, they should raise their hand and the nebuliser will be stopped.

When the subject is comfortable, turn on the nebuliser (green switch on nebuliser press to left) and start the stopwatch when the first breath appears from the saliva trap end of the valve box.

Turn the nebuliser off (green switch press to right) after 30 seconds have elapsed.

Remove the mouthpiece from the subject's mouth and place the mouthpiece, opening down, on the absorbent sheet so any excess residue can be drained. This prevents a potential backwash of pooled secretions through the one-way valve.

Wait 1 minute after each nebulisation episode in order to record maximal bronchoconstriction.

Measure FEV_1 at the end of 1 minute and record on Saline Challenge Worksheet. Calculate the percentage of the FEV1 fall (Baseline FEV_1 – FEV_1) x 100 / Baseline FEV_1 and record on worksheet.

If the FEV_1 percentage fall is less than 15%, nebulisation should resume immediately the percentage of the FEV1 has been calculated. If the FEV_1 percentage fall is greater than 15% the challenge is complete and nebulisation ceased.

If the FEV_1 percentage fall is less than 15% repeat steps**, increasing the nebulisation time by doubling the time period: 1 minute, 2 minutes and 4 minutes. A 4-minute period is the maximum time for continuous nebulisation. Continue up to a cumulative time of 15.5 minutes.

Once a FEV_1 percentage fall of greater than 15% has been achieved, the nebulisation ceased, the subject should be given 400µg of salbutamol via a spacer. Wait 10 minutes and then measure FEV_1. Ensure the subject's FEV_1 has returned within 10% of the baseline measurement and that they are experiencing minimal discomfort before allowing them to leave a supervised area.

At the end of the procedure, remove the nebuliser cup and tubing (grey) without the valve box from the nebuliser and take to digital scales and again weigh to 1 decimal point. Record in 'Nebuliser cup post test weight' section on Saline Challenge Worksheet. The nebuliser output can be calculated by: (nebuliser cup pre test weight – nebuliser cup post test weight) / cumulative time (or time taken for subject FEV_1 percentage fall to be greater than 15%).

5 Maintenance:

Nebuliser output quality assurance should be completed monthly

Nebulisers to be serviced by Engineering yearly or as needed

Nebuliser cups may by taken to Engineering for repair or maintenance

6 Shutdown:

Following post test weighing, the nebuliser cup, lid and tubing can be cleaned. Remove the tubing and squirt Microshield 4 (pink) – chlorhexidine surgical handwash solution in tubing and rinse. Hang tubing up above sink to dry. Squirt Microshield 4 (pink) – chlorhexidine surgical handwash solution into nebuliser cup and fill with warm water and allow to soak for a minimum of 30 minutes, then empty, rinse and drain. The nebuliser cup lid should also be rinsed with Microshield 4 (pink) – chlorhexidine surgical handwash solution and drain.

Used spirometer mouthpiece to be placed in contaminated waste bin (next to sink)

Valve box, nose clip and spacer to be placed in white bucket in warm water + detergent. All to be placed in CSD red boxes daily for transfer to CSD for sterilization (red boxes in hall –opposite TB office).

The saliva trap should be removed from the valve box, rinsed with Microshield 4 (pink) – chlorhexidine surgical handwash solution and drain.

The laptop should be shutdown as appropriate

7 Safety Precautions:

All saline challenge procedures should be conducted in areas where in case of emergency resuscitation equipment is available. A doctor should also be available for assistance and to answer any queries during the procedure.

Saline challenge should not be performed on subjects with a FEV_1% predicted of < 40%

If the subject is clinically unstable or becomes symptomatic during the procedure, caution should be exercised when determining the length of each nebulisation. Monitor the FEV_1 at 1 to 2 minute intervals during each nebulisation if there is reason for concern.

Bench tops and chairs should be wiped with 70% ethanol (surface disinfectant) solution after each procedure

8 Troubleshooting:

If the subject wishes to cough during the procedure, they should be encouraged to cough into the nebuliser and not remove the mouthpiece

Remember the saline effect is cumulative, timing between nebulisations should be kept to 1 minute intervals according to subject comfort

Reassurance and encouragement is essential throughout the challenge

An absorbent sheet can be placed on the subject's lab to protect clothing (drip tray)

Where possible use a blue connection piece between tubing and valve box – micropore tape is difficult to remove for CSD

9 Spare Parts:

DeVilbus nebulisers can be purchased from Sunrise Medical Pty Limited, Castle Hill, Ph: 02 – 98993144.

Nebuliser cups can be purchased from Sunrise Medical Pty Limited, Castle Hill, Ph: 02 – 98993144.

Hans Rudolph valve boxes can be purchased from RJ & VK Bird, 54 Canterbury Road Middle Park VIC 3206, Ph: 03 – 96909898.

Vacumed mouthpieces can be purchased from RJ & VK Bird, 54 Canterbury Road Middle Park VIC 3206, Ph: 03 – 96909898.

Quotes should be acquired before ordering, Trevor may be able to assist.

SPUTUM INDUCTION (& HYPERTONIC SALINE CHALLENGE - IF APPLICABLE)

☐ **Induction Only** ☐ **Saline Challenge + Induction**
(if no documented variable airflow obstruction within last 10 years)
NB. **DO NOT** give bronchodilator (B_2) prior to saline challenge.

☞ *Please refer to sputum induction flowchart for safety guidelines.*

Mouth rinsed (x3)? ☐ Yes Nebuliser Make: _____

Asthma medications withheld? ☐ Yes 4.5% saline ☐ 0.9% saline ☐

IS THE BEST FEV_1, L *(POST B2 IF INDUCTION ONLY)* ≥ 40% PREDICTED? YES ☐ NO ☐	15% FALL FROM BASELINE FEV_1, L = 85% X BASELINE FEV_1, L _____
Nebuliser Cup Pre-weight (g): _____ Post-weight (g): _____	Pre-weight – Post-weight = Delivered dose (g): _____(mL)

Saline nebulised time *(adjust times if required)*	FEV_1, L effort			% fall from baseline FEV_1	Saline Induced Sputum (SIS) produced (✓)	B_2 required? Dose	Time paused	Recovery FEV_1, L (post B_2)
	1	2	3					
Baseline Best FEV_1, L (post B_2 – induction only)					Spontaneous Sputum (SS)?*			
30 sec								
1 min								
2 min								
4 min								
4 min								
4 min								

* NB. If spontaneous sputum (SS) is collected prior to induction, use new specimen jar for saline induced sputum (SIS).

Total cumulative induction time (mins) :_____ Time sputum sample collection completed : _____

HYPERTONIC 4.5% SALINE CHALLENGE ONLY:

Was a drop in FEV_1 of 15% observed? YES ☐ NO ☐ If NO → salbutamol + post-B2 spirometry ☐

If YES, was this within 15mL saline? YES ☐ NO ☐ If YES → participant ELIGIBLE for study.

💻 *Complete and attach a PD_{15} sheet to show volume of saline required for 15% drop in FEV_1.*

If NO to above Qs & FEV_1 reversibility <12%, issue peak flow meter for participant monitoring over 2 weeks

APPENDIX D SPUTUM INDUCTION SOP

Note: Sputum Induction can be conducted in combination with hypertonic saline challenge.

1 Equipment:

- Refrigerator
- ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000 (JHH, PCH, RAH)/ NE-U12 Omron Ultrasonic nebuliser (SCGH)
- Hans Rudolph valve box, large 2 way No. 2700 (JHH, PCH, SCGH)
- Saliva trap (green)
- Vacumed 1001 mouthpiece
- Nebuliser cup and lid + tubing
- Spirometer + mouth pieces (CPFS/D™ USB Spirometer + BREEZESUITE software, Medgraphics)
- Volumatic/Spacer
- Stopwatch
- Calculator
- Nose clip
- Bench top Digital scales

2 Materials:

- 4.5% hypertonic saline – 200mL bottle
- Disposable mouthpiece for spirometer
- Salbutamol
- Sputum Induction/Saline Challenge Worksheet
- Disposable cups x2, 1 with water
- Absorbent sheets (i.e. Blueys or underpads)
- Tissues
- Sputum collection jar and specimen bag

3 Set Up:

Calibrate spirometer.

Assemble valve box (Appendix C).

Prepare nebuliser cup (Appendix C).

Prepare nebuliser (Appendix C).

Check the position of the valves and the tubing is connected correctly by reviewing the flow direction noted by the arrows on the valve box before commencing induction.

4 Method:

Explain the purpose of the test, how it is to be conducted and possible side effects e.g. coughing, dry mouth, gagging, chest tightness, wheezing, dyspnoea, nausea and excess salivation.

Measure the baseline spirometry FEV_1 and FVC as per ATS guidelines.

Give the subject 4 puffs of salbutamol via a spacer. Wait 10 minutes and repeat spirometry.

Calculate a 15% fall of the highest FEV_1 post salbutamol and record on worksheet (FEV_1 post salbutamol x 0.85 = 15% fall).

Provide the subject with 1 cup of water and 1 empty cup. Ask the subject to rinse and gargle and spit in empty cup (x3). This will minimise squamous cell contamination of the specimen.

Dispose of cup, subject may retain a cup of water to drink during the procedure.

Instruct and demonstrate how to obtain sputum from the lungs by coughing and clearing the throat (deep cough and hack!).

**Ask the subject to apply the nose clip and insert the valve box mouthpiece into their mouth, in a similar manner to a snorkel.

Advise the subject to breathe normally (tidal breathing) and if they need to cough, to cough into the mouthpiece rather than removing the mouthpiece.

Also advise the subject, if they are uncomfortable at any time whilst on the nebuliser, they should raise their hand and the nebuliser will be stopped.

When the subject is comfortable, turn on the nebuliser (green switch on nebuliser press to left) and start the stopwatch when the first breath appears from the saliva trap end of the valve box.

Turn the nebuliser off (green switch press to right) after the required time as per protocol. This maybe 30 seconds if combined Saline Challenge/Sputum induction or up to 5 minutes if sputum induction alone.

Remove the mouthpiece from the subject's mouth and place the mouthpiece, opening down, on the absorbent sheet so any excess residue can be drained. This prevents a potential backwash of pooled secretions through the one-way valve.

Wait 1 minute after each nebulisation period.

Record FEV_1 at the end of 1 minute and record on worksheet.

During the 1-minute after nebulisation or after recording the FEV1 encourage the subject to cough in order to obtain a sputum sample. Ask them to cough, clear their throat and deposit all or any oral contents into the specimen container. Listen to the subject to ensure the sample is from the lungs and not post nasal secretions.

Calculate the percentage of the FEV_1 fall (Post B2 FEV_1 - FEV_1) x 100 /Post B2 FEV_1 and record on worksheet.

If the FEV_1 percentage fall is less than 15%, nebulisation should resume immediately the percentage of the FEV_1 has been calculated. Repeat steps ** either increasing or repeating nebuliser time according to the protocol. Continue up to a cumulative time of 15.5 minutes.

If the FEV_1 percentage fall is greater than 15% and there is not enough sputum produced, give the subject 4 puffs of salbutamol via a spacer. Wait 10 minutes and then measure FEV1. Ensure the subject's FEV1 has returned within 10% of the baseline measurement before recommencing nebulisation (maximum cumulative nebiliser time = 15.5 minutes).

Once an adequate specimen has been obtained, label the specimen jar as per protocol (including subject initials, subject number, SIS for saline induced sputum or SS for spontaneous sputum, date and time), place in specimen bag and refrigerate.

Contact lab staff for immediate pick up of specimen.

At the end of the procedure, remove the nebuliser cup and tubing (grey) without the valve box from the nebuliser and take to digital scales and again weigh to 1 decimal point. Record in "Nebuliser cup post test weight' section on worksheet. The nebuliser output can be calculated by: (nebuliser cup pre test weight – nebuliser cup post test weight) / cumulative time.

5 Maintenance:

Nebuliser output quality assurance should be completed monthly.

Nebulisers to be serviced by Biomedical Engineering yearly or as needed.

Nebuliser cups may be taken to Biomedical Engineering for repair.

6 Shutdown:

See Appendix C

Bench tops and chairs should be wiped with 70% ethanol (surface disinfectant) solution after each procedure.

7 Safety Precautions:

Stop the nebulisation if:

An adequate specimen has been produced and the FEV1 has dropped 15%

An adequate specimen has been produced and the cumulative nebulisation time is 15.5 minutes

The subject requests the nebulisation to stop

Ensure the subject's FEV1 has returned to within 10% of the baseline measurement and that they are experiencing minimal discomfort before allowing them to leave a supervised area.

All sputum induction procedures should be conducted in areas where in case of emergency resuscitation equipment is available. A doctor should also be available for assistance and to answer any queries during the procedure.

Normal saline (0.9%) may be used instead of hypertonic saline in acute subjects or for subjects with a FEV_1 < 40%.

If the subject is clinically unstable or becomes symptomatic during the procedure, caution should be exercised when determining the length of each nebulisation. Monitor the FEV_1 at 1 to 2 minute intervals during each nebulisation, if there is reason for concern.

Sputum induction procedures should be conducted in negatively ventilated rooms

8 Troubleshooting:

Many subjects will cough immediately the nebulisation finishes. This opportunity can be used to obtain sputum but exercise moderation so as not to tire the subject or cause a sore throat.

It usually takes a cumulative time of about 11 minutes to produce an adequate specimen. Listen for a moist cough as a sign the subject is ready to produce sputum.

Remember the saline effect is cumulative, timing between nebulisations should be kept to 1 minute intervals according to subject comfort.

Reassurance and encouragement is essential throughout the induction.

Communication with lab staff is essential to ensure consistent and adequate samples are being provided

Never dispose of a specimen (even if you think it is no good), this judgment should be made by the lab staff only.

If a subject provides a spontaneous sputum specimen (i.e. prior to induction), the specimen should be labeled as such and a new specimen jar used for the induced sputum specimen.

Subjects may prefer to provide a sputum specimen alone or standing.

9 Spare Parts:

See SOP Saline Challenge

Specimen jars and bags

10 Illustrations:

11 References:

ATS spirometry guidelines
Sputum Sample Collection video - Aventis
ERS Sputum Induction Guidelines

SPUTUM INDUCTION

☞ *Please refer to sputum induction flowchart for safety guidelines*.

Mouth rinsed (x3)? ☐ Yes Nebuliser Make: _____

Asthma medications withheld? ☐ Yes 4.5% saline ☐ 0.9% saline ☐

IS THE BEST FEV1 (L) *(POST B2)*	15% FALL FROM BEST BASELINE FEV_1, L
≥ 40% PREDICTED? YES ☐ NO ☐	= 85% X BEST BASELINE FEV_1, L _____
Nebuliser Cup	
Pre-weight (g): _____ Post-weight (g): _____	Pre-weight – Post-weight = Delivered dose (g): _____

Saline nebulised time *(adjust times if required)*	FEV_1, L effort			% fall from baseline FEV_1	Saline Induced Sputum (SIS) produced (✓)	B_2 required? Dose	Time paused	Recovery FEV_1, L (post B_2)
	1	2	3					
Baseline FEV_1, L (post B_2 administration)					Spontaneous Sputum (SS)?*			
30 sec								
1 min								
2 min								
4 min								
4 min								
4 min								

Cumulative induction time (mins) : _____ **Time sputum sample collection completed :** _____

* NB. If spontaneous sputum (SS) is collected prior to induction, use new specimen jar for saline induced sputum (SIS).

APPENDIX E EXHALED NITRIC OXIDE SOP (Ecomedics)

1 Purpose of the test

Nitric oxide (NO) is a small, highly reactive molecule, which can be produced by airway endothelial, epithelial and inflammatory cells.

NO triggers airway smooth muscle relaxation, helps to kill tumour cells, inhibit viral replication and other pathogens (bacteria, fungi). It is a marker of airway inflammation.

NO levels are elevated, compared with normal subjects in the following respiratory conditions:

- asthma
- eosinophilic bronchitis
- rhinitis
- bronchiectasis
- active pulmonary sarcoidosis
- active fibrosing alveolitis
- upper and lower respiratory infections.

Elevated eNO levels are correlated with airway hyperresponsiveness to methacholine

eNO levels are reduced, compared with normal subjects in the following conditions:

- current smoking
- severe COPD
- cystic fibrosis
- conditions where the motility of cilia is defective
- pulmonary hypertension
- α_1 antitrypsin deficiency.

The following additional factors may affect eNO levels:

- circadian rhythm. Measurements should be taken at a standardised time of day
- food and beverages. Ingestion of foods that contain nitrogen increase eNO levels, whilst alcohol has been shown to reduce eNO levels. Therefore, subjects should refrain from eating and drinking for at least one hour prior to the test
- Spirometry may transiently reduce eNO levels. Therefore spirometry should be performed after eNO measurement
- Strenuous exercise should be avoided one hour before the measurement
- Some medications affect eNO levels, therefore all medications administered, dose and time of last dose should be recorded.

important: Read the Ecomedics manuals prior to operation of the device.
Mobile telephones must be switched off in the testing area

Equipment:

2.1 Analyser CLD 88sp, NO Analyser (EcoMedics)

2.3 Flow head with DCR medium and spirette

3. Materials:

3.1Bacteria filter with mouthpiece(FP-MP/250 (Order Code) Air-Met Scientific(Supplier))

4. Set Up:

4.1 Prior to test, ensure daily calibration has been attended to (see Ecomedics manual)

4.2 Attach mouthpiece to flow head on handpiece of Ecomedics analyser

4.3 Double click on Spiroware 3 software icon on desktop computer.

4.4 Enter Username and password (lower case) and click onto ‘ Logon ‘ button.

4.5 If new participant, on right hand corner click onto ‘ Register new patient’

Enter participants details (first name – subjects initials, Last name – Study subject number) Ensure that ‘ Propose ID’ is clicked otherwise EcoMedics will give a warning message. Click ‘ Save’.

4.6 If previous participant, enter patients subject initials in top box to bring up patient details onto database list and double click on name. Click onto

‘ New Test’ on right hand side of screen.

4.7 Click onto ‘Single Breath Test’.

4.8 A blank grey screen will appear – no prompts are provided on screen to start test. Provide instruction to participant then the test can be commenced.

5. Method:

5.1 Patient should comfortably seated with good posture.

5.2 Instruct patient to place mouthpiece into mouth with a good seal around mouthpiece.

5.3 Participant is then instructed to take a full deep breath in with mouthpiece in mouth. The screen below will appear whilst breath is being taken in.

5.4 Once patient has taken full breath, instruct patient to exhale slowly and softly (enough to blow the red face into the upper grey section and turn the face green.) The patient needs to blow out the same steady pressure to keep the face icon green and in the grey sectio until the machine finishes reading the exhaled breathe. Once the manouvre is finished, the screen will switch to results page and participant can remove mouthpiece from mouth.

5.5 Once a valid measure is obtained, record on the CRF.

5.6ATS guidelines recommend a 30 sec rest window between manouvres.

5.6 The number of single-breath tests is not limited. ATS recommend 3 manouvres to be attended, all consistent with each other. There may be more than three tests carried out and unwanted tests may be deleted.

5.8 A test is successful if two measurements are within 5%, or three

manouvres within 10% deviation. For values below 10 ppb a deviation

of 1ppb for two measurements is acceptable. A successful test is displayed

in a window with indications of quality at bottom of screen.

6. Shutdown:

6.1 At end of procedure, close software, but leave the Ecomedics machine turned ON

7. Maintenance:

7.1 Mouthpiece should be disposed of immediately after each patient use.

7.2 Handpiece should be wiped down with alcohol wipes after each patient use.

7.3 Daily syringe calibration should be performed prior to first patient using

machine daily.

9. Troubleshooting:

9.1 See EcoMedics Manual CD88 (located in Resp CSU, in red folder next to

EcoMedics analyser).

9.2 Page Kelly Steel 6349 for other problems.

APPENDIX F QUESTIONNAIRES

ASTHMA CONTROL QUESTIONNAIRE

Circle the number of the response that best describes how you have been during the past week.

1 On average, during the past week, how often were you woken by your asthma during the night?

0 Never
1 Hardly ever
2 A few times
3 Several times
4 Many times
5 A great many times
6 Unable to sleep because of asthma

2 On average, during the past week, how bad were your asthma symptoms when you wake up in the morning?

0 No symptoms
1 Very mild symptoms
2 Mild symptoms
3 Moderate symptoms
4 Quite severe symptoms
5 Severe symptoms
6 Very severe symptoms

3 In general, during the past week, how limited were you in your activities because of your asthma?

0 Not limited at all
1 Very slightly limited
2 Slightly limited
3 Moderately limited
4 Very limited
5 Extremely limited
6 Totally limited

4 **In general, during the past week, how much shortness of breath did you experience because of your asthma?**

0 None
1 Very little
2 A little
3 A moderate amount
4 Quite a lot
5 A great deal
6 A very great deal

5 **In general, during the past week, how much of the time did you wheeze?**

0 Not at all
1 Hardly any of the time
2 A little of the time
3 A moderate amount of the time
4 A lot of the time
5 Most of the time
6 All the time

6 On average, during the past week, how many puffs of short-acting bronchodilator (eg Ventolin) Have you used each day?

0 None
1 1-2 puffs most days
2 3-4 puffs most days
3 5-8 puffs most days
4 9-12 puffs most days
5 13-16 puffs most days
6 More than 16 puffs most day

TOTAL SCORE (Questions 1-6) = _____
ACQ(6) = Total score/6 = _____

JUNIPER ASTHMA QUALITY OF LIFE QUESTIONNAIRE (AQLQ) (STANDARDISED)

1. Please indicate how much you have been limited by your asthma in strenuous activities (such as hurrying, exercising, running up stairs, sport) during the last 2 weeks. **Green card** ☐

2. Please indicate how much you have been limited by your asthma in moderate activities (such as walking, housework, gardening, shopping, climbing stairs) during the last 2 weeks. **Green card** ☐

3. Please indicate how much you have been limited by your asthma in social activities (such as talking, playing with pets/children, visiting friends/relatives) during the last 2 weeks. **Green card** ☐

4. Please indicate how much you have been limited by your asthma in work related activities (such as tasks that you have to do at work) during the last 2 weeks. **Green card** ☐

5. Please indicate how much you have been limited by your asthma in sleeping during the last 2 weeks. **Green card** ☐

6. How much discomfort or distress have you felt over the last 2 weeks as a result of chest tightness? **Red card** ☐

7. In general how often during the last 2 weeks have you felt concerned about having asthma? **Blue card** ☐

8. How often during the last 2 weeks did you feel short of breath as a result of your asthma? **Blue card** ☐

9. How often during the last 2 weeks did you experience asthma symptoms as a result of being exposed to cigarette smoke? **Blue card** ☐

10. How often during the last 2 weeks did you experience a wheeze in your chest? **Blue card** ☐

11. How often during the past 2 weeks did you feel you had to avoid a situation or an environment because of cigarette smoke? **Blue card** ☐

12. How much discomfort or distress have you felt over these 2 past weeks as a result of coughing? **Red card** ☐

13. How often during the past 2 weeks did you feel frustrated as a result of your asthma? **Blue card** ☐

14. How often during the past 2 weeks did you experience a feeling of chest heaviness? **Blue card** ☐

15. How often during the past 2 weeks did you feel concerned about the need to take medications for your asthma? **Blue card** ☐

16. How often during the past 2 weeks did you feel the need to clear your throat? **Blue card** ☐

17. How often during the past 2 weeks did you experience asthma symptoms as a result of being exposed to dust? **Blue card** ☐

Continued on next page.

18. How often during the past 2 weeks did you experience difficulty breathing out as a result your asthma? **Blue card** ☐

19. How often during the past 2 weeks did you feel you had to avoid a situation or an environment because of dust? **Blue card** ☐

20. How often during the past 2 weeks did you wake up in the morning with asthma symptoms? **Blue card** ☐

21. How often during the past 2 weeks did you feel afraid of not having your asthma medication available? **Blue card** ☐

22. How often during the past 2 weeks were you bothered by heavy breathing? **Blue card** ☐

23. How often during the past 2 weeks did you experience asthma symptoms as a result of the weather or air pollution outside? **Blue card** ☐

24. How often during the past 2 weeks have you been woken at night by your asthma? **Blue card** ☐

25. How often during the past 2 weeks have you had to avoid or limit going outside because of the weather or air pollution ? **Blue card** ☐

26. How often during the past 2 weeks did you experience asthma symptoms as a result of being exposed to strong smells or perfume? **Blue card** ☐

27. How often during the past 2 weeks did you feel afraid of getting out of breath? **Blue card** ☐

28. How often during the past 2 weeks did you feel you had to avoid a situation or environment because of strong smells or perfume? **Blue card** ☐

29. How often during the past 2 weeks has your asthma interfered with getting a good night sleep? **Blue card** ☐

30. How often during the past 2 weeks have you had the feeling of fighting for air? **Blue card** ☐

31. Think of the overall range of activities that you would have liked to have done during the past 2 weeks. How much has your range of activities been limited by your asthma? **Yellow card** ☐

32. Overall, among all the activities that you have done during the past 2 weeks, how limited have you been by your asthma? **Green card** ☐

(Response cards are included on next page)

Juniper AQLQ responses

RED CARD

1. A very great deal of discomfort or distress
2. A great deal of discomfort or distress
3. A good deal of discomfort or distress
4. A moderate amount of discomfort or distress
5. Some discomfort or distress
6. Very little discomfort or distress
7. No discomfort or distress

GREEN CARD

1. Totally limited, couldn't do activity at all
2. Extremely limited
3. Very limited
4. Moderate limitation
5. Some limitation
6. A little limitation
7. Not at all limited

YELLOW CARD

1. Severely limited – most activities not done
2. Very limited
3. Moderately limited – several activities not done
4. Slightly limited
5. Very slightly limited – very few activities not done
6. Hardly limited at all
7. Not limited at all – have done all activities that I wanted to do

BLUE CARD

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time