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| protocol  pipa |
| **P**redictors, **I**mmunopathogenesis and **P**rescribing in **A**ntibiotic allergy – A prospective multicenter cohort study |
| Protocol Number: HREC/15/Austin/75  Version: 6  Date: 2/10/2019 |
|  |
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| **STUDY SYNOPSIS** |  |

|  |  |
| --- | --- |
| Title: | **P**redictors, **I**mmunopathogensis and **P**rescribing in **A**ntibiotic allergy – A prospective multicenter cohort study |
| Short Title: | **PIPA** |
| Design: | Prospective multi-centre cohort study (including biobank) |
| Study Centres: | Austin Health  Peter MacCallum Cancer Centre (PMCC)  Alfred Health (Part 2/3 only) |
| Hospital: | Austin Hospital  Peter MacCallum Cancer Centre (PMCC)  Alfred Health (Part 2/3 only) |
| Study Question: | Can skin prick/intradermal testing (SPT/IDT) with appropriate oral challenge (OC) effectively remove an antibiotic allergy label?  Can SPT/IDT/OC improve antibiotic appropriateness and antimicrobial stewardship initiatives?  Does variation in HLA and/or immune receptors affect severe antibiotic allergies?  Are resident T-cells and their responses different within tissue than peripheral blood in those with delayed hypersensitivity.  What is the positive and negative predictive value of *ex vivo* T-cell enzyme linked immunospot (ELIspot) assays for delayed antibiotic allergies at various time points post reaction and in different populations (immunocompetent versus immunosuppressed)?  Are skin and peripheral blood T cell responses lost over time? |
| Study Objectives: | To understand the impact of clinical practice SPT/IDT/OC on antibiotic allergy labels, restricted antibiotic use and antibiotic appropriateness.  To understand the association between variation in HLA and/or immune receptor polymorphism and antibiotic allergy and characterize (immunophenotyped) T-cell immune responses in patients with delayed antibiotic allergy.  To understand the difference in T-cell populations in skin and peripheral blood during delayed hypersensitivity  To understand the longevity of T-cell responses in the skin and peripheral blood  To develop a biobank of specimens that can be used to explore the immunological mechanisms and genetic predictors of antibiotic allergy in the future |
| Primary Objectives: | Measure the effectiveness of SPT/IDT/OC in removing the antibiotic allergy label (Austin/PMCC only) |
| Secondary Objectives | Proportion of antibiotic usage (total courses) that is restricted (i.e. meropenem, ciprofloxacin, vancomycin, ceftriaxone) in patients with an antibiotic allergy label pre and post antibiotic allergy SPT/IDT clinical program (Austin/PMCC only)  Antibiotic appropriateness (median score) in patients an antibiotic allergy label pre and post antibiotic allergy SPT/IDT/OC clinical program (Austin/PMCC only)  Number of positive T-cell ELIspot assays in patients with delayed antibiotic allergy (delayed- IDT or oral challenge positive) (All sites)   * In those with positive responses the duration of positivity over time   Variation in HLA class I and II and immunoreceptors in patients with well phenotyped delayed hypersensitivity reactions to antibiotics (All sites) |
| Inclusion Criteria: | For SPT/IDT/OC performed as routine clinical practice:   * Age > 18 years * Ability to give informed consent * Identified by a specialist infectious diseases physician to have a clinical history of an adverse reaction to an antibiotic compatible with an immunologically mediated reaction that has resulted in chronic labeling and avoidance.   For T-cell ELIspot and HLA typing   * Ability to give informed consent * Identified by a specialist infectious diseases physician to have one of the following: * Clinical history of delayed antibiotic allergy * Positive delayed-IDT to an antibiotic(s) * Positive oral challenge to an antibiotic(s) |
| Exclusion Criteria: | Nil |
| Number of Planned Subjects: | 150 |
| Investigational product: | Not applicable |
| Safety considerations: | SPT/IDT will be performed as part of a new routine clinical practice at both sites. All antibiotics used for SPT/IDT are administered via a subcutaneous (SC) or intradermal (ID) route at a minute dose (significantly less than therapeutic) from well-established protocols used in successful clinical practice and international clinical guidelines. In the extremely rare event of an adverse reaction patients will be continuously supervised by a registered doctor were resuscitation facilities are immediately available.  Patients will be asked to contribute a large volume of blood (150 ml to facilitate the validation of ex vivo assays). This volume may be altered accordingly down to a minimum of 60 ml for patients acutely ill or not deemed suitable for a 150 ml draw. Patients may develop slight bruising over the venipuncture site. There are no other safety considerations for T-cell ELIspot and HLA/T-cell receptor sequencing. Some patients will be asked to contribute a small skin sample following biopsy. Patients may develop slight bleeding around the biopsy site. Some patients will be asked to contribute convalescent skin and blood samples post resolution of drug reaction.  Patient serum and peripheral blood mononuclear cells (PBMCs) used for initial T-cell ELIspot and HLA/T-cell receptor sequencing will be stored in a biobank as a DNA source following informed patient consent. |
| Statistical Methods: | Where the data distribution conforms to a Gaussian distribution the median will be compared using paired students t-tests. Where the data does not conform, the non-parametric Mann-Whitney U test will be used to assess differences. |
| Subgroups: | Immunocompromised host defined as:   * Steroids > 10mg daily for one month * Stem cell or solid organ transplant recipient * Autoimmune, inflammatory or connective tissue disorder requiring immunosuppressant therapy * Haematological malignancy or cancer |

## **Glossary of Abbreviations & Terms**

|  |  |
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| **Abbreviation** | **Description (using lay language)** |
| SPT | Skin prick test using a low dose of a drug or allergen to induce a localized (only) skin response in a patient with a reported allergy. |
| IDT | Intradermal prick test using a low dose of a drug or allergen to induce a localized (only) skin response in a patient with reported allergy. |
| OC | Oral challenge |
| Delayed-IDT | Delayed-Intradermal pick test using a low dose of a drug or allergen to induce a localized (only) skin response in a patient with reported allergy that occurs 24-72 hours post. |
| ADR | Adverse Drug Reaction |
| Type A ADR | A non-immune mediated ADR to a drug that is usually dose dependent and predictable (e.g. gastrointestinal upset) |
| Type B ADR | An immune mediated ADR to a drug that can be from preformed antibodies (immediate) or immune cell reaction (delayed) |
| Immunocompromised host | A patient with an impaired immune system secondary to condition such as cancer, transplantation or inflammatory /autoimmune disease requiring drugs that lower the immune response |
| DHR | Delayed Hypersensitivity Reaction – An allergy that is not immediate or anaphylaxis, generally after 72 hours post drug exposure. |
| HLA | Human Leucocyte Antigen –Genes that encode structures on cells that present proteins to the immune system. |
| TCR | T-cell receptor |
| T-cell ELIspot | T-cell Enzyme linked immunospot assay – An test tube investigation using patient white blood cells (T-cells) cultured with an antibiotic to measure the immune (cytokine) response. |
| Antibiotic Allergy De-labeling | The removal of a patients antibiotic allergy label following allergy assessment, skin prick testing/intradermal testing and/or oral challenge. |

## **Study Sites**

### Study Location/s

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Site** | **Address** | **Contact Person** | | **Phone** | **Email** |
| Austin Health | 145 Studley Road, Heidelberg VIC 3084 | Dr Jason Trubiano | | (03) 94966676 | Jason.trubiano@austin.org.au |
| Peter MacCallum Cancer Centre (PMCC) | 2 St Andrews Place, East Melbourne VIC 3002 | Dr Jason Trubiano | | (03) 96561111 | Jason.trubiano@petermac.org |
| Alfred Health | 55 Commerical Road, Alfred Health 3001 | | Dr Ar Kar Aung | (03) 90762000 | [a.aung@alfred.org.au](mailto:a.aung@alfred.org.au) |
| Vanderbilt Universitya | 1161 21st Avenue South  Nashville, TN 37232-2582 | Prof Elizabeth Phillips | | +1(615) 322-2035 | elizabeth.j.phillips@vanderbilt.edu |

aParticipants will not be recruited from this study site. This study site and its affiliated investigators will provide technical support for HLA typing and ELIspot testing.

## **Introduction/Background Information**

### Lay Summary

Doctors routinely treat patients who are ‘allergic’ to a drug or a range of drugs. An allergic reaction occurs when a person’s immune system reacts to a substance that is normally harmless. Allergic reactions to antibiotics are unpredictable, less dependent on the dose and potentially life threatening. The subsequent antibiotic allergy “label” attached to the patient has significant and potentially deleterious effects on antibiotic prescribing. Skin prick/intradermal testing and oral challenge (SPT/IDT/OC) using a range of antibiotics is not a new procedure, performed locally and internationally as a safe and effective method for correctly labeling patients as allergic or not for many years. However, the long-term effects of SPT/IDT on a patients allergy label and antibiotic usage is unknown. Genetic predictors and the immune response in patients with allergies to anti-infective drugs are still not well defined, however there are defined examples such as the association between a severe allergic reaction to abacavir (a HIV drug) and the gene HLA-B\*57:01 where avoidance of the drug in the at risk population is preventive.

In this multicenter prospective cohort study we plan to examine the downstream effects of antibiotic allergy SPT/IDT/OC program on long-term patient allergy labeling and antibiotic usage. As a follow on and audit of this new clinical practice we plan to perform three inter-related antibiotic allergy studies to examine the effects of a SPT/IDT testing on antibiotic usage and the role of advanced diagnostics. Part 1 of this project will only be undertaken at the Austin Health and Peter MacCallum Cancer Centre. Part 2 and 3 will recruit participants from all recruiting/participating sites.

Part 1) **The impact of skin prick testing on antibiotic allergy labels and antibiotic utilisation**

SPT/IDT/OC for the diagnosis of antibiotic allergy is widely described in clinical practice and in the literature, however the effects of an antibiotic allergy SPT/IDT/OC ‘de-labeling’ program on antibiotic usage, especially in the highest users of antibiotics (e.g. immunocompromised host) has not be undertaken. We plan to examine the effects of an antibiotic allergy SPT/IDT/OC clinical program performed as routine clinical practice on patient allergy labeling, restricted antibiotic usage and appropriateness. The SPT/IDT/OC testing will be performed as part of routine clinical practice. This part of study that requires prospectively collecting patient data requires no additional participant time. Data will be collected from (i) clinical notes/electronic medical record and (ii) SPT/IDT/OC results.

Part 2) **The relationship between genes and predisposition to antibiotic allergies**

Ten years ago the gene responsible for a severe potentially life threatening allergy to an anti-HIV drug called abacavir was discovered. It is now routine practice to test for the presence of this gene (HLA-B\*5701) prior to using abacaviir. In this project our aim is to identify variation in HLA and immune receptors that may predict how patients react to certain antibiotics.

Part 3) **The role of immune based blood tests to diagnosis antibiotic allergy**

There is little evidence to support the use of *in vitro/ex vivo* testing in the diagnosis of drug allergy and for many types of drug allergy such as immediate or suspected IgE allergy such as penicillin allergy SPT/IDT/OC is considered the gold standard. Currently problems exist both with false positive and false negative results with currently developed *ex vivo/in vitro* assays. We aim to determine if T-cell immune assays can confirm the presence of an antibiotic allergy, particularly in those with impaired immune function (e.g. bone marrow and solid organ transplant recipients). For cellular assays and HLA/TCR sequencing a single blood draw of 150mls that will take approximately 15 minutes to complete is required. This can be performed during the routine clinical visits. Patients will be given the option to consent to further blood draws and may be contacted in the future for future follow-up. Some patients with asked to contribute a skin sample and/or blister fluid (if applicable) for assessment of resident T-cell immune function.

The information obtained from these studies will aid the safe use of antibiotics in patients with reported antibiotic allergies. HLA/TCR sequencing and validation of *ex vivo/in vitro* assays will help in the immunophenotyping of drug reactions and will prove important to further understand the immunopathogenesis of these reactions and future ways to utilize preventative (screening) and diagnostic assays.

### Introduction

Antimicrobial allergies are commonly reported yet their validity and impacts are relatively unknown, The impact of a clinical practice antibiotic allergy SPT/IDT/OC on antibiotic usage and patient outcomes, particularly in the immunocompromised is unknown. The utility of *ex vivo* T-cell enzyme linked immunospot (ELIspot) assays to immunophenotyped reactions has been utilized but their more broad use as diagnostic assays and their durability over time has not been defined. Genetic variation in HLA and immune receptors (TCR) may identify the small subset of patients at risk of developing a severe immune mediated ADR however there are currently few examples where these discoveries have been made for anti-infectives (exception: flucloxacillin, amoxicillin-clavulanate, abacavir). The combined utility of these *in vivo*, *ex vivo* and *in vitro* studies in the prevention, diagnosis and management of antibiotic allergy is also unknown.

This research protocol will follow on from the routine clinical practice of antibiotic allergy SPT/IDT/OC (Figure 1). Clinical governance/Chief Medical Officer approval has been sought at both sites (attached). The SPT/IDT/OC protocols used will replicate those successfully published by co-investigator Prof Elizabeth Phillips. For the non-TGA approved Diater-DAP kit (penicillin kit) Austin HREC has successfully been sought to obtain authorized prescriber status. There are three-parts to this novel multicenter prospective cohort study including: (i) Prospective database of antibiotic allergy SPT/IDT and effects of allergy de-labeling and antimicrobial usage, (ii) the role of HLA/TCR sequencing in identifying those at risk for delayed antibiotic allergy and (iii) the utility of T cell ELIspot and flow cytometry as a tool for immunophenotyping and diagnosing delayed antibiotic allergy. From a single blood draw we aim to detect HLA associations with delayed antibiotic allergies and examine the utility of T-cell ELIspot. From skin samples of acute rashes we aim to compare resident dermal T-cell responses to those within peripheral blood. We focus our research on the *ex vivo* and *in vitro* testing for allergy assessment and diagnosis. This research program will enhance patient care via improving safe antibiotic prescribing and aid antimicrobial stewardship initiatives.

### Background information

Antimicrobial allergies are primarily reported for beta-lactam agents and although 10-15% of hospitalized patients are labeled ‘allergic’, 90% are negative on SPT/IDT.([1-3](#_ENREF_1)) In those with antibiotic allergies, especially the immunocompromised, the presence of an allergy label is associated with inappropriate antibiotic usage, higher readmission rates and restricted antibiotic use.([3-5](#_ENREF_3)) The impact of a negative SPT/IDT on antimicrobial usage and patient outcomes, particularly in the immunocompromised is unknown. The utility of *ex vivo* T-cell enzyme linked immunospot (ELIspot) assays and HLA typing to assign drug causality in delayed (T-cell) reactions is also ill defined.([6](#_ENREF_6), [7](#_ENREF_7)) The combined utility of these *in vivo*, *ex vivo* and *in vitro* studies in the diagnosis and management of antibiotic allergy is unknown. Pathways to prevent, diagnose and assign drug causality in antibiotic allergy are required.

Antibiotic allergy assessment with SPT/IDT/OC is safely performed as routine clinical practice in many local and international centres. Previous prospective studies and both international and local allergy society guidelines support the use of SPT/IDT/OC for antibiotic allergy assessment.([8-15](#_ENREF_8)) The dose of drug given for SPT/IDT/OC is minute, designed to cause the least amount of irritation as per published dosing guidelines. ([8](#_ENREF_8), [10](#_ENREF_10), [16-18](#_ENREF_16)) All the antibiotics used are TGA approved and have been safely administered via the intradermal route in published literature.([13](#_ENREF_13), [19-22](#_ENREF_19)) Such practices are performed at Royal Perth Hospital (WA, AUS) and Vanderbilt Medical Centre (TN, USA) under the guidance of Professor Elizabeth Phillips, a co-investigator on this project. . For immediate reactions OC is an absolutely essential step in the de-labeling of antibiotic allergy and SPT/IDT/OC is considered the gold standard with a 100% negative predictive value described for this combination for penicillin allergy. An antibiotic allergy SPT/IDT/OC clinical program using identical protocols to those presented here (Appendix 1) was performed in 405 patients (2008-2013) with beta-lactam antibiotic allergy without a serious adverse event reported. Re-labeling that consisted of either total removal of beta-lactam allergy label or allergy selective to only a single agent occurred in almost 90% patients. ([23](#_ENREF_23)) This study clearly validated the safety and efficacy of an antibiotic allergy SPT/IDT clinical program, however limited data still exists on the long-term impact on patient allergy labels and antimicrobial usage. We aim to examine the follow on effects of a routine clinical practice antibiotic allergy SPT/IDT program on long-term antibiotic allergy labeling, restricted antibiotic usage and appropriateness.

The role of genetic predictors such as HLA typing and immunodiagnostics in antibiotic allergy is ill defined. The variations in T cell responses within skin and in peripheral blood during a delayed hypersensitivity unknown. Links between delayed antibiotic allergy phenotypes and routinely used antibiotics are only reported for flucloxacillin and amoxicillin-clavulanic acid.([24](#_ENREF_24), [25](#_ENREF_25)) A genetic polymorphism (HLA-B\*57:01) was found to be responsible for a severe and potentially life threatening delayed allergy to the antiretroviral drug abacavir.([26](#_ENREF_26), [27](#_ENREF_27)) HLA-B\*57:01 testing is now routine clinical practice prior to prescribing abacavir.([28](#_ENREF_28)) The findings of this research have informed how adverse antimicrobial reactions, in particular delayed immune (T-cell) mediated allergies are triggered in some individuals but not others.([27](#_ENREF_27)) T-cell enzyme linked immunospot (ELIspot) assays measuring IFN-γ response to antibiotics have also been used to assist drug hypersensitivity causality investigations in patients with antimicrobial allergy.([6](#_ENREF_6), [7](#_ENREF_7), [29](#_ENREF_29)) T-cell ELIspot assays have mainly be described for nevirapine and abacavir.([30](#_ENREF_30), [31](#_ENREF_31)) The role of both HLA-typing and functional T-cell *ex vivo* assays to predict T-cell mediated reactions in antibiotic allergy is ill defined. Using these tools we aim to develop a specific immunophenotype of antibiotic reactions that can be in the future applied both preventively and diagnostically. Although this has been examined in some extent in HIV infected patients, another very poorly understood area is the impact on immunosuppression on *ex vivo/in vitro/in vivo* immune responses. We hope to better understand the impact of impaired immune function such as seen in transplant populations or patients with other underlying immunodeficiencies on T-cell ELISpot testing.

This research program will identify pathways to correctly label or de-label patients with antibiotic allergy and therefore approve antibiotic appropriateness and avoid potentially serious adverse drug reactions. At a community level the ability to use potentially more targeted and less restricted antimicrobial therapy can significantly aid antibiotic stewardship initiatives.

## **Study Objectives**

### Hypothesis

The hypotheses to be tested in this study are:

* That routine clinical practice antibiotic allergy SPT/IDT/OC testing provides long-lasting removal of antibiotic allergy labels and reduces restricted antimicrobial usage.
* That antibiotic allergy SPT/IDT/OC testing improves antibiotic appropriateness
* That antibiotic allergies are controlled by a group of genes (Class 1, Class II and Class III) located on an area known as the Major Histocompatibility Complex (MHC) also known as the Human Leucocyte Antigen or HLA.
* That variations exist in activated T-cell populations within skin and peripheral blood during a delayed (T-cell mediated) hypersensitivity
  + That these responses are lost over time
* That *ex vivo* T cell enzyme-linked immunospot (ELIspot) assays can identify delayed antibiotic allergies in the immunocompotent and immunocompromised host who have previous clinical histories consistent with such reactions.

### Study Aims

1. To assess if routine clinical practice SPT/IDT/OC can remove a patient antibiotic allergy label at long-term follow up.
2. To examine antibiotic usage, restricted antibiotic usage and antibiotic appropriateness pre and post the introduction of an antibiotic allergy SPT/IDT/OC testing program.
3. To identify variation within the HLA class I and/or II region and immune receptors (TCR) and severe antibiotic reactions
4. To identify differences in T-cell populations within skin and peripheral blood during delayed (T-cell mediated) hypersensitivity.
   1. To determine the longevity of T-cell responses
5. To determine the positive and negative predictive value of T-cell ELIspot tests in patients with delayed antibiotic allergy.
   1. Subgroup analysis: Immunocompromised hosts.

### Outcome Measures

*Primary outcome measures*

* Proportion of patients with an antibiotic allergy label post antibiotic allergy SPT/IDT testing clinical program (30days, 90days, 360days phone follow-up)

*Secondary outcome measures*

* Proportion of antibiotic usage (total courses) that is restricted (i.e. meropenem, ciprofloxacin, vancomycin, ceftriaxone) in patients with an antibiotic allergy label pre and post antibiotic allergy SPT/IDT clinical program
* Antibiotic appropriateness (median score) in patients with an antibiotic allergy label pre and post antibiotic allergy SPT/IDT clinical program
* Positive and negative predictive value T-cell ELIspot assays in patients with delayed antibiotic allergy (delayed- IDT or oral challenge positive)
* Associations of variation within class I and/or II HLA and immune receptors (TCR) in patients with positive (i) SPT/IDT/OC, (ii) T-cell ELIspot or (iii) clinical history of delayed antibiotic allergy as verified by a minimum of a “probable” score using a validated ADR causality assessment tool.

# **Study Design**

### Study Type & Design & Schedule

*Design:* Prospective multi-centre cohort study

*Population:* Adults aged > 18 years with reported antibiotic allergy

*Sites***:** Austin Health, PMCC, Vanderbilt University, Alfred Health

*Overview of study design & research program (Figure 1):*

*Part 1:* Prospective database of patients assessed as part of routine clinical practice antibiotic allergy SPT/IDT clinical program, including follow-up questionnaire(s). (Austin Health, Peter MacCallum Cancer Centre)

*Part 2 & 3:* *In vitro* and *ex vivo* testing of patients with a clinical history of delayed antibiotic allergy and/or positive *in vivo* antibiotic allergy testing (All sites\*)

*\**Baseline demographic and clinical information will be obtained from participants from sites partaking in ‘Part 2 or 3’ only. Convalescent samples will only be asked of patients reviewed at Austin Health and Peter MacCallum Cancer Centre clinical services

**Pre Study: Clinical Practice Antibiotic allergy SPT/IDT Program –** (April 2015 – ongoing**)**

* Established protocols for SPT/IDT and oral challenge as per Appendix 1
* Clinical governance approval by Chief Medical Officer obtained at both sites for this practice.
* For non-TGA approved penicillin kit (DAP-kit) Austin HREC ethics approval has been obtained.

**Part 1 – Impacts of antibiotic allergy SPT/IDT clinical program –** (April 2015 – ongoing)

* Prospective database of all patients referred to allergy clinic for routine clinical practice assessment
* Antibiotic appropriateness scores and restricted antibiotic usage (proportion of total antibiotic courses) pre and post antibiotic allergy assessment:
  + Data extracted from electronic medical record at both clinical sites
  + Comparison of antibiotic appropriateness (score) 60 days & 12-months pre and post antibiotic allergy SPT/IDT clinical program
    - Antibiotic appropriateness defined as per NAPS (National Antimicrobial Prescribing Survey)([32](#_ENREF_32))
  + Comparison of restricted antimicrobial usage as a proportion of total antibiotic courses 60 days & 12-months pre and post antibiotic allergy SPT/IDT clinical program
    - Restricted antibiotics: 3rd generation cephalosporins, carbapenems, fluoroquinolones and glycopeptides.
* Phone follow-up questionnaire (Appendix 2):
  + Patient: 30, 90, 360 days
  + Local medical practitioner: 30, 90, 360 days
  + Referring specialist (if applicable): 30, 90, 360 days

**Part 2 & 3 – HLA typing and T-cell ELISpot testing –** (April 2015 – July 2017)

* Negative patient controls: (patients with clinical history of immediate antibiotic allergy AND negative SPT/IDT/oral challenge)
* Test patients: History of delayed antibiotic allergy AND/OR positive SPT/IDT test
* Single blood draw at time convenient for patient after informed consent (100-150mls)
* DNA saved for HLA class I (ABC) and class I (DR,DQ,DP) typing and PBMCs cryopreserved for T-cell ELIspot, in vitro assays and for performing TCR sequencing on sorted T-cell populations.
* Some patients will be asked to provide a skin and/or blister fluid samples from an area of skin rash presumed secondary to a drug.
* Some patients will be asked to provide skin from an area of resolved rash AND/OR blood sample after complete resolution of drug reaction
* For patients not underdoing allergy clinical/testing assessment and only partaking in the single ‘blood draw’ of Part 2 and 3, baseline demographic and clinical information will be collected (as indicated in **methods** section)

**General considerations**

No home visits required, phone follow-up only.

No student involvement

All patient information will be re-identified and replaced with a unique study number.

* Processing of data only via study investigations only.
* Data kept within access database and destroyed following study completion. Data will be kept strictly confidential according to the National Statement on Ethical Conduct in Human Research 2007 and the Australian Code for Responsible Conduct of Research 2007. Data from the 1st of December 2019 will be transferred to Austin Health hosted version of REDcap and storage as outlined in **Section J.**

### Standard Care and Additional to Standard Care Procedures

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Standard Care Proceduresa** | | |  | **Additional To Standard Care** | | |
| **Procedure** | **Time/Visit** | **Dosage/Volume** |  | **Procedure** | **Time/Visit** | **Dosage/Volume** |
| Antibiotic Allergy SPT/IDT Clinical Program | Single or three visits | Clinical assessment  SPT/IDT  +/- oral challenge  Oral challenge alone |  | Blood draws | Single visit – 15 minutes with option to consent for further visits and blood draws | 100-150mls of blood |
|  | Skin sample | Taken at time of above visit for blood draw and/or subsequent clinical review | 3mm punch biopsies and/or blister fluid aspiration |
|  | Phone follow-up | 3 phone calls – 10 minutes each | 30days, 90days, 360days |

a Antibiotic allergy SPT/IDT and oral challenge will form routine clinical practice (Appendix 1) as approved by Chief Medical Officers at both clinical sites.

## Study methodology

1. **Pre Study: Clinical Practice Antibiotic Allergy SPT/IDT Program**

* Patients will be required to complete an informed consent to undergo antibiotic allergy SPT/IDT/OC (Appendix 3)

1. **Antibiotic Allergy SPT/IDT prospective database (Part 1)**

* Patient baseline demographic, allergy history and SPT/IDT/oral challenge results will be kept in a prospective database.
* **Please note**: Participants from sites undertaking in only the ‘blood draw’ component of the study (Part 2 and 3) will have baseline demographic and clinical information as indicated by - #.
* The data collected will be:
* Demographics (age, sex, ethnicity, country of birth)#
* Past medical history#
* Drug history (current) – Drug, dose, route of administration, frequency#
* Histopathology reports
* Family history of drug allergy or atopy# (Y/N). If Y list…
* Allergy(s) history# - Date of reaction, reaction description, hospitalisation (Y/N), prior referral to allergist/immunologist (Y/N), history of SPT/IDT (Y/N), history of oral re-challenge (Y/N).
  + If Y to SPT/IDT and/or oral re-challenge, List…
* Baseline questions:
  + Would you be happy to be re-challenged with the offending antibiotic if negative on SPT/IDT testing? (Y/N)
  + If the oral challenge allergy testing was negative would you be willing to take that antibiotic in the future? (Y/N)
* Inpatient admission(s) for infective diagnosis within last 12 months (Y/N)
  + If Yes:
    - Infective diagnosis(s)
    - Antibiotic allergy label (electronic medical record/drug chart)
    - Antibiotic(s) administered (agent, dose frequency)
    - Antibiotic(s) duration (days for each agent)
    - Admission duration (days)
    - Adverse events (Y/N), If Y List…
* Inpatient admission(s) for infective diagnosis 12 months post SPT/IDT (Y/N)
  + If Yes:
    - Infective diagnosis(s)
    - Antibiotic allergy labels (electronic medical record/drug chart)
    - Antibiotic(s) administered (agent, dose, frequency)
    - Admission duration (days)
    - Adverse events (Y/N), If Y List…
* Hospital outpatient antibiotic prescriptions (pharmacy records) in 12 months pre and post SPT/IDT (Y/N)
  + If Yes:
    - Infective diagnosis(s)
    - Antibiotic(s) administered (agent, dose, frequency)
    - Antibiotic duration (days)

1. **Phone follow up questionnaire (Part 1)**

* 30, 90, 360 days post allergy assessment. (Appendix 2)

1. **Blood draw (Part 2 & 3)**

* Informed patient consent required to collect blood for DNA and PMBC storage and baseline demographic and clinical information (if applicable) (Appendix 4):
* Positive IDT
* Positive oral challenge
* Negative controls
* Patients consenting to the HLA and T-cell ELIspot study will be asked to provide a one off blood sample of approximately 150mls. Peripheral blood mononuclear cells (PBMCS) will be isolated from this sample and stored at -80°C before use in the following experiments. If patients present with blisters associated with drug hypersensitivity blister fluid will be collected. The blister(s) will be punctured with a needle and the fluid collected in a syringe. PBMCS will be isolated from this sample and stored at -80°C before use in the following experiments.
* **Optional (skin biopsy)**
  + Patients consenting to the HLA and T-cell ELISpot study will be asked to provide (optional) skin and/or blister fluid sample. Mononuclear cells will be isolated from this sample(s) and stored and -80°C before use in the following experiments.
* Optional (convalescent samples)
  + Patients consenting to the HLA and T-cell ELISpot study will be asked to provide (optional) skin or blister fluid samples AND/OR repeat blood samples (100mls) after complete resolution of their drug reaction (minimum of 6 weeks post index reaction) - Austin Health and Peter MacCallum Cancer Centre patients only.

1. **T-cell ELIspot (Part 2 & 3)**

* We will test whether a range of antibiotics are able to elicit cytokine responses via T-cell ELISpot assay, from PBMCs of patients with a history of T-cell mediated antibiotic allergy (delayed allergy).
* *Methods:(*[*33*](#_ENREF_33)*)*
  + Cryopreserved PBMC are thawed and left to settle over night at 37°C. Viable lymphocytes are enumerated by trypan blue exclusion using a Neubauer haemacytometer. 200,000 cells resuspended in culture medium (10%FCS/RPMI-1640) are dispensed per well using a modified version of the previously described IFNγ ELISpot assay.76 Sterile 96 well nitrocellulose backed plates (MAIP S4510, Millipore, Bedford, USA) are coated with 2μg/mL IFNγ coating antibody (Mabtech, Naka Strand, Sweden) overnight 4°C in sterile Phosphate buffered Saline (PBS). Plates are washed with sterile PBS, blocked with culture medium (CM, 10% FCS/RPMI-1640 30minutes, room temperature [RT]), after which cells and stimulants (test drugs, dispensed in triplicate where possible at optimised dilution, with positive [anti-CD3 antibody Mabtech, Victoria, Australia] and negative [culture media alone] controls)- are dispensed for overnight incubation CO2, 37°C. Plates are then washed with sterile PBS. Biotinylated IFNγ (Mabtech, Victoria, Australia) added (2 hrs, RT), after which plates are washed and streptavidin horseradish peroxidise (Mabtech, Victoria, Australia) added (1 hr, RT). Plates are washed and developed with 100μL/well tetramethylbenzidine substrate (Mabtech, Victoria, Australia) for 10minutes at RT. Plates are washed extensively with MilliQ H20 and left to dry prior to analysis on the AID iSpot reader (AID, Strassburg, Germany) with AID software (5.0 B7337). Responses are determined by subtracting the mean of the negative control wells from the mean of triplicate stimulated wells. Results are presented as spot forming units/106cells (SFU). Responses are considered positive if they are > 50 SFU based on the distribution of the negative controls and test data. Utilizing an identical method, the cytokine capture/output may be changed from IFN-y to other commercial kits including: IL-10, IL-33, IL-2, IL-4. IL-5, IL-8, IL-22, IL-13, granulysin or granzyme B.

1. **HLA testing (Part 2 & 3)**

* *Methods:*
  + Limited genomic testing for HLA types. No additional genes sequences.
  + HLA-A,-B-,-C, -DR/DQ/DP 4 digit typing is performed on stored DNA using the 454 FLX platform.  Specific HLA Loci is PCR amplified using sample specific MID-tagged primers that amplify polymorphic exons from Class I (A, B, C Exons 2 and 3) and Class II (DQ, Exons 2 and 3; DRB and DPB1, Exon 1) MHC genes.  Amplified DNA products from unique MID tagged products (up to 48 MIDs) are then pooled in equimolar ratios and subjected to library preparation, quantitation and emulsion PCR suitable for entry into the 454 FLX sequencing pipeline.  Clonally enriched beads are used for 454 Titanium chemistry based sequencing on the 454 FLX+ sequencer. Sequences are then separated by MID tags and alleles called using an in house accredited HLA allele caller software pipeline using the latest IMGT HLA allele database as the allele reference library.

1. **Skin sampling (Part 2 & 3)**
   1. A 3mm punch biopsy will be performed on skin of an acute reaction, recovered skin and/or the skin of a positive intradermal or patch test. The desired site will be circled and the skin at each site removed via the above method. The specimen is then removed with sterile forceps and placed on sterile soaked gauze. Verbal wound care instructions will be given to the participant. Follow up will be given as appropriate. Skin biopsy samples are placed in a bottle of preservative free saline in a cooler for immediate transport to the lab. In patients with blisters evident, blister fluid will be aspirated with a 27-29guage needle attached to a 1ml syringe.
   2. Ten micrometer slices of skin will be processed for RNA extraction. Bulk RNA will then be subjected to gene expression immunophenotyping analysis using the NanoString nCounter assay. For this study, we will develop a custom panel of primers to evaluate a wide range of immune markers as well as transcripts for cytotoxic peptides known to be mechanistic in T-cell mediated drug hypersensitivity pathogenesis, utilizing flow cytometry (including CLA, CD3, CD8, CD4, CD45R0, CCR7, CD103 and CD69). These cells may also be stimulated utilizing the ELISpot platform in Section E.c.5.
   3. Using flow cytometry at The Peter Doherty Institute for Infection and Immunity, the presence, frequency and phenotype of subgroup T cells (MAIT – Mucosal associated invariant T cells) from previously collected patient PBMC and/or blister lfuid samples. An increased frequency of MAIT cells and an activated phenotype will indicate whether MAIT cells pay a role in antibiotic hypersensitivity. Using follow cytometry, MAIT cell activation following in vitro stimulation with culprit antibiotics will be determined. Briefly, MAIT cells in patient PBMCs/blister fluid versus health PMBCs, will be co-incubated with antibiotics and control MAIOT cell stimulus or no stimulus and 2 parameters of MAIT cell activation will be determine at several time points: cytokine production and MAIT cell proliferation. IN vitro, re-stimulation of MAIT cells by antibiotics will determine if MAIT cells in patient blood/blister fluids are activated by antibiotics. Flow cytometry data acquired will be analyzed with a flow cytometry specific software (FlowJo) for each chosen characteristic. Analysed data will then be plotted and analysed with statistics in respect to health donor control samples and negative control samples (no stimulus) using a graphing and analysis software.

## **Study Population**

### Recruitment Procedure

This research collaboration is a flow-on from new clinical practice, antibiotic allergy SPT/IDT clinical program at Austin Health (VIC, AUS) and Peter MaCallum Cancer Centre (VIC, AUS). This will be performed in collaboration with Professor Elizabeth Phillips (Vanderbilt University, TN, USA) who has performed antibiotic SPT/IDT testing successfully at multiple institutions over a 10-year period and published her experience. A prospective database of these patients and patient follow-up will form PART 1 of this study. Patients with a clinical history suggestive of non-immediate antibiotic allergy (presumed T-cell mediated), positive delayed-IDT test or positive oral challenge will be considered for enrollment in PART 2 & 3 of the study. Alfred Hospital patients will be identified by the Infectious Diseases Unit during admission.

### Inclusion Criteria

Age > 18 years of age

Ability to give informed consent

Identified by a specialist in the field of drug allergy as having a potentially immunologically mediated reaction (Type B) to an antibiotic.

### Exclusion Criteria

Inability to give informed consent

Age <18

Pregnancy

People with cognitive impairment, intellectual disability or mental illness

Identified by a specialist in the field of drug allergy as having a drug side effect or reaction that is not likely to be related to an immunologically mediated reaction associated with a drug or externally administered substance.

### Consent

Individual consent will be obtained via a structured template for each of the following (Appendix 3 & 4):

* Antibiotic allergy SPT/IDT clinical program and follow-up (clinical practice)
* T-cell ELIspot testing & HLA typing (single blood draw)
* Skin biopsies
* Blister fluid aspiration
* Biobank and future research (blood draws – as above)

# **Participant Safety and Withdrawal**

### Risk Management and Safety

Antibiotic allergy SPT/IDT/OC testing will be performed as routine practice. Patients will undergo antibiotic allergy SPT/IDT/OC as a clinical practice, separate from the outlined studies. **PART 1** of the study is the collecting of a prospective database of SPT/IDT patients therefore without clinical concern.

As a reference to SPT/IDT/OC safety:

* As demonstrated in the ‘introduction’ and ‘background’ sections it has been shown to have an excellent safety rating, local and international guidelines supporting it its clinical use.
* SPT/IDT/OC is performed in outpatient clinics within large tertiary hospital centres under complete specialist supervision with onsite medical emergency services available. This level of patient safety is beyond that normally provided in many drug allergy SPT/IDT/OC testing centres.
* Patients have a detailed allergy assessment prior to initial SPT testing. Concentrations of antibiotics will be given at the lowest published concentration via well-established clinical protocols.
* No long-term skin or systemic effects occur following testing and resolve generally within 24-48 hours. In rare occasions SPT/IDT/OC can cause more generalized rashes, urticaria or anaphylaxis. Patients will be monitored for safety throughout by trained personal. Following SPT/IDT/OC consultation is provided to ensure patients are adequately informed of the SPT/IDT/OC program results.
* Written information of the revised antibiotic allergy label is provided to the patients and respective clinicians to ensure the safe prescribing of antibiotics for that patient in the future. PART 1 of the study is collecting clinical and electronic medical data from this clinical practice in a prospective database, without safety concern.

For **PART 2 and 3** to minimize risk, extra blood and or blister fluid will be collected for this study at the same time as routine clinical visits (Figure 1). If applicable patients will be asked to provide 100-150mls of blood in order to test qualitative immune responses and HLA type. The risks associated with participating in this study relate to uncommon complications of venipuncture/skin biopsy and could include discomfort, bruising, infection, and/or superficial blood clot formation. Patients will be monitored for safety and comfort by trained clinical personnel.

### Handling of Withdrawals

Given the nature of this study and the fact that it is non-interventional apart from initial antibiotic allergy SPT/IDT clinical program, withdrawal of the patient from the doctor would be unlikely to happen.  Removal of the patient from the study would be at the patients’ request to withdraw and have their samples removed from storage. A consent form log is kept of all patients consenting for this study. Should a patient withdraw consent, that information will be entered into the consent log.

# **Statistical Methods**

### Sample Size Estimation & Justification

Pilot study data (Austin Health and PMCC) performed by this research group has enabled a more accurate estimate of the projected sample size. Combined with published data on the prevalence of antibiotic allergies (20-30%), number of SPT/IDT/OC possible in a 2 year period (405 in 6 years by Bourke et al., 2014) and rate de-labeling post SPT/IDT testing (80-90%) a conservative study size of n = 150 participants was established.([3](#_ENREF_3), [4](#_ENREF_4), [23](#_ENREF_23)) A more detailed explanation is described below:

**Austin Health pilot data**

A prospective 3-month study at Austin health in 2012 of 169 consecutive infectious diseases consults identified 35 (21%) patients with an antibiotic allergy label, 59% (22/37) of reported allergies were delayed antibiotic allergies. Beta-lactams were implicated frequently (26; 74%), with only 9 (25%) of patients with an antibiotic allergy label undergoing re-challenge. (*Trubiano, J et al. 2012 unpublished data*)

**Peter MacCallum Cancer Centre pilot data**

A retrospective review of cancer patients admitted with an infective diagnosis to PMCC (2012-2013) compared those with an antibiotic allergy label to those without, in regards to antibiotic usage and patient outcomes. From 199 patients, 47 (24%) had an antibiotic allergy label, 76% were presumed delayed antibiotic allergies. 40% of antibiotics courses restricted antibiotics in patients with antibiotic allergy versus 25% in those without. (*Trubiano, J et al. 2014 unpublished data*)

**Projected potential patients for antibiotic allergy SPT/IDT clinical program (Part 1)**

* N = 150 patients
* Projected proportion of patients that will have an admission pre and post is 30%, average antibiotic courses 3 per patient.
* Projected proportion of antibiotic courses that restricted pre SPT/IDT clinical program 0.40 compared with post 0.25.

**Projected potential participants for HLA assessment/T-cell ELIspot (Part 2 & 3)**

* 75-100 Patients
  + 50-70% of pilot patients with antibiotic allergy had history of severe and delayed antibiotic allergy
* Controls (1): 10 patients with negative SPT/IDT/OC and history of immediate antibiotic allergy
* Controls (2): 10 patients with negative SPT/IDT/OC and history of delayed antibiotic hypersensitivity

### Power Calculations

Power calculations are required for PART 1 as comparisons are made between pre and post antibiotic allergy SPT/IDT clinical program (i) antibiotic usage, (ii) restricted antibiotic use and (iii) antimicrobial appropriateness. The unit for consideration being antibiotic courses.

If assuming a before and after study design and a correlation coefficient of 0.2:

N = 121 (antibiotic courses) - 80% power and 5% significance.

N = 159 (antibiotic courses) For a 90% power and 5% significance.

### Statistical Methods To Be Undertaken

**General comments**

Basic statistical analysis via Stata will be performed via principle and secondary investigators. No additional support is expected to be required. Categorical variables will be summarized using frequency and percentage and compared using a chi-square test. Continuous variables will first be assessed for significant skew using a Shapiro-Wilk test. They will then be summarized using mean and standard deviation (SD) or median and inter-quartile range as appropriate and compared using a paired t-test or Wilcoxon signed-rank test as appropriate.

**PART 1: Antibiotic allergy SPT/IDT clinical program**

* Comparison of antibiotic appropriateness (mean/median scores) for cohort pre and post SPT/IDT clinical program (60 days and 12 months pre and post)
* Comparison of the proportions of antibiotic courses that are restricted antibiotics for cohort pre and post SPT/IDT clinical program (60 days and 12 months pre and post)
* This will be performed as a “before and after” analysis using a repeated measures regression model.
* The 60 day pre and post assessment will constitutes the primary analysis and then the 12 months pre and post will be the secondary analysis.
* Will also compare patients that had persistent allergy “labels” to those that had the allergy “label” removed via an exploratory post-hoc analysis.

**PART 2: HLA assessment and T-cell ELIspot testing**

* No statistical analysis required

# **Storage of Blood and Tissue Samples**

## Details of where samples will be stored, and the type of consent for future use of samples

For PART 2 & 3 of study the 100-150mls of blood draws from each patient will be separated and stored as PBMCs as per the methods. From the same blood sample limited genetic testing via HLA typing will be performed. The remaining whole blood (EDTA), serum and PBMCs will be stored in a created biobank at Austin Health for an indefinite period and may be used for future related non-genetic (cytokine profiling, flow cytometry, T-cell receptor profiling) and genetic studies (HLA typing) to investigate immune responses to antibiotic allergy and therefore participants will be asked for extended consent. The PBMCs will be used as a DNA source. The samples will be stored in -80 degree storage at study site(s).

# **Data Security & Handling**

### Details of where records will be securely kept & How long will they be stored

Along with the blood sample, we plan to collect clinical data in PART 1 (prospective database) extracted from the clinical electronic medical record and retained in a secure database. All data will be re-identified prior to any analyses and data sharing. Access to the original data will be restricted to study site(s) listed investigators. Furthermore, all laboratory personnel have been instructed about the proper notation of specimen requests on laboratory requisition forms. Consent documents will be stored in a separate location.

* Patient research data will only be accessed by the named investigators. Electronic records will be retained on password-protected computer(s) in databases requiring password access. This data will be stored separately from the master list of patient names.
* Any hard copies of data will be kept in locked facilities of the Austin Health (Department of Infection Control and Infectious Diseases)
* Any laptop computer will be password-protected and electronic records stored on it will be coded and in databases requiring password access. Only study investigations will have access to the data.
* Patient data will be only be transferred and analyzed in a coded form
* Individual patients will not be identifiable from the presented or published material
* Patient and research data will be stored on hard disk and CD-ROM for a period of at least 7 years. After 7 years these files may be destroyed by erasure and/or incineration (for CD-ROM) unless decided by the principle investigator.
* From 1st December 2019 data from existing database and any new prospective data will be stored in the Austin Health hosted version of REDcap. No change in data collection, storage terms or study numbers will be required, Processing of data only via investigators or appointed data managers. Data will be kept strictly confidential according to the National Statement on Ethical Conduct in Human Research 2007 and the Australian Code for Responsible Conduct of Research 2007.

**Figure 1: Austin Health and PMCC antibiotic allergy SPT/IDT clinical program**



**Appendix 1: Antibiotic Allergy Skin Prick Testing and Intradermal Testing protocols: (a) Short protocol, (b) long protocol.**

Macintosh HD:Users:jasontrubiano:Dropbox:antibiotic allergy:Antibiotic allergy ethics:Hypersensitivity/HLA ethics:Final ethics documents:PIPA Skin testing protocol (SHORT) - Appendix 1.pdf



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**Macintosh HD:Users:jasontrubiano:Dropbox:antibiotic allergy:Antibiotic allergy ethics:Hypersensitivity/HLA ethics:Final ethics documents:PIPA Skin testing protocol (LONG) - Appendix 1.pdf**

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**Appendix 2: Follow-up Questionnaire (phone)**

Name:­­­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Study Number: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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Time closest to SPT/IDT +/- oral challenge (circle): 30day, 90days, 360days post.

Date of SPTI/IDT +/- oral challenge: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Patient or clinician questionnaire (cicle)

**Questions:**

1. Would you consider yourself/patient allergic to any antibiotics? (Y/N). If Y… (list)

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1. Have you received/prescribed any antibiotics since SPT/IDT testing? (Y/N) if Y….(list agent, indication or reaction)
   1. Agent(1)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Indication(1)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Reaction(1)\_\_\_\_\_\_\_\_\_\_\_
   2. Agent(2)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Indication(2)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Reaction(2)\_\_\_\_\_\_\_\_\_\_\_
   3. Agent(3)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Indication(3)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Reaction(3)\_\_\_\_\_\_\_\_\_\_\_
   4. Agent(4)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Indication(4)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Reaction(4)\_\_\_\_\_\_\_\_\_\_\_
2. Would you be prepared to have/prescribe an antibiotic that you/the patient was previously labeled as allergic too? (Y/N/Not applicable)

**Appendix 3: Informed consent for SPT/IDT +/- oral challenge and phone follow-up (see attached)**

**Appendix 4: Informed consent for HLA and T-cell ELIspot testing from single blood draw (see attached)**

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