RCPA

The Royal College of Pathologists of Australasia

Extension of Scope of Practice in Molecular Genetics (NPAAC Supervision Certification Modules)

IMMUNOPATHOLOGY

TABLE OF CONTENTS

GLOSSARY

CPDP	RCPA Continuing Professional Development Program
(F)RCPA	(Fellow of the) Royal College of Pathologists of Australasia
IANZ	International Accreditation New Zealand
MDT	Multi-disciplinary team
NATA	National Association of Testing Authorities
NPAAC	National Pathology Accreditation Advisory Council
PPD	Personal Professional Development
RCPAQAP	RCPA Quality Assurance Programs Pty Ltd
SOP	Standard Operating Procedure
WHS	Workplace Health and Safety

SECTION 1

INTRODUCTION

The Royal College of Pathologists of Australasia (the College) offers a number of certification modules for Fellows of the Royal College of Pathologists of Australasia who have completed Fellowship in the discipline of Immunopathology.

The development of these certification modules has been required to satisfy the recent NPAAC requirements (http://health.gov.au/internet/main/publishing.nsf/Content/health-npaac-docs-supervision.htm), particularly as applicable to supervision of testing involving molecular diagnostics and genomics.

To adequately medically supervise a molecular/genomic service, immunopathologists need to have adequate and detailed knowledge of the wet and dry laboratory aspects of the technology and bioinformatics analysis. It is recognised that this knowledge may not be the same as the hands-on experience of the scientist in the genomics laboratory, however, supervising pathologists should be familiar with the limitations and strengths of the methodology, the ethical considerations of data use and reporting and the clinical relevance for assessing appropriate requesting and reporting.

It is anticipated that in the next few years, genomic analysis will increasingly become integral to the work of pathology laboratories. These modules are designed to provide all immunopathologist with the opportunity to participate in accordance with NPAAC requirements.

GENERAL AIMS OF THE TRAINING PROGAM

The genetics/ genomics certification modules build on discipline-specific Fellowship training. As well as gaining additional competencies in genetics/ genomics relevant to Immunopathology, candidates are expected to extend further their skills in management, research, scholarship, as well as the professional qualities they have been developing during their pre- and post-Fellowship years and will continue to develop during their professional life.

This Handbook outlines requirements for the Immunopathology Certification Modules. It is based on a common approach for Fellows in all pathology disciplines to develop/ demonstrate the minimum professional competencies required for safe clinical service provision of genetic/ genomic testing. As such, the certification modules and associated competency standards outlined within this handbook have shared features with the modules outlined in the equivalent handbooks for other disciplines.

The purpose of the modules outlined in this Handbook is to offer Fellows the opportunity to gain certification of expertise for a graduated range of genetic/ genomic testing categories specifically for clinical applications within their specific pathology discipline.

Completion of one or more modules would result in extension of scope of practice within Immunopathology to the limits defined for each module. The scope of practice would not extend into other discipline areas – for example, investigation of intellectual disability or prenatal diagnostic testing.

An essential part of sub-specialty genetics/ genomics training is for practitioners to gain sufficient understanding of the breadth of the field; are aware of the limits of their own knowledge and skills, and appreciate when it is in the best interests of patients to refer onto, or formally consult with genetic pathologists or other appropriately credentialed colleagues.

GENETIC AND GENOMIC TESTING

Immunology-related (allergy, immunodeficiency, auto-immunity, auto-inflammation, immune dysregulation) genetic and genomic testing includes *targeted* analysis for presence/ absence of predefined mosaic or clonal genomic variation; screening for undefined variants in a single gene; screening for undefined variants in a limited number of specified genes, and screening for undefined variants in a large number of specified genes.

Testing can also include *untargeted* screening of all chromosomes (karyotyping), higher resolution screening of all chromosomes (chromosomal microarray), whole exome screening, and whole genome screening.

Specialised genetic, genomic, epigenetic and gene expression tests are also emerging. They include targeted testing for uniparental disomy, chimerism, methylation anomalies and other epimutations, and gene expression profiling.

The goal of all genetic/ genomic investigations is to identify variants that are of direct relevance to the clinical phenotype in question. A growing number of tests may also simultaneously detect simultaneously additional disease-causing variants that are not directly relevant to the clinical indication for the specified test. Instead, these unanticipated findings may indicate a heightened risk of either having, or developing in the future, another unrelated illness. Additionally, tests that involve screening specified genomic sequences for undefined variants will inevitably identify variants that are of uncertain clinical significance.

To address adequately the wide range of professional competencies required to cover these activities, it is proposed that post-graduate training and assessment is delivered in three modules. The modules are a practical response to the gradient of technical and clinical complexity within genetic and genomic testing, as well the differing practical skill sets required across the spectrum of methods now available.

Completion of the preceding module(s) will be a prerequisite unless already accredited to supervise tests relevant to that module. However, even if exempt, the curriculum items listed in these modules will be expected core knowledge and assessable since they will be fundamental to each of the 'higher' level test methodologies.

Completion of these modules will not be required for Immunopathologists only undertaking supervision of coeliac disease associated HLA typing, HLA B27 or HIV testing (see module 1) which are covered in the immunopathogy training curriculum (Tier 1) or formal HLA testing for which there is an existing accreditation process via the American Society for Histocompatibility and Immunogenetics (ASHI).

SECTION 2

LEARNING OUTCOMES AND RECOMMENDED TRAINING ACTIVITIES

Module 1 – Targeted testing for presence/ absence of predefined genomic variation associated with immunological disease, by relevant molecular methods.

The purpose of testing in Module 1 is the identification or exclusion of individual variants with known clinical implications. A single assay may identify one or several variants, but the underlying principle is that the pathologist is not required to undertake analysis to determine whether the identified variant/s is/are clinically significant, as this is already established knowledge.

Examples of typical clinical applications include: –

- Diagnosis of diseases with limited allelic heterogeneity (e.g. MyD88 L265P polymorphism in Waldenstrom's macroglobulinaemia),
- Diagnosis within a family (following identification of the family-specific mutation),
- Pharmacogenetic variants (e.g.: HIV drug resistance testing, TPMT polymorphisms in azathioprine metabolism),
- Somatic "gain of function" variants (e.g. *FAS* or *STAT3* variants in ALPS phenotypes, following previous detection).

This module is available as a post-fellowship option for immunopathologists seeking to take responsibility for pathology tests in this category. Some examples of these clinical applications that are less complex with respect to interpretation, assay design and / or performance (eg: coeliac disease associated HLA typing, HLA B27, HIV testing) are incorporated in the trainee curriculum relevant to all immunopathology trainees. There is potential overlap with other subspecialties such as Haematology due to impacts on both systems from genetic variations in the same gene.

From the perspective of Immunopathology, the methodologies relevant to Module 1 are nucleic acid amplification-dependent assays (end point, quantitative and real-time), and Sanger Sequencing. It is anticipated that this module will be progressively incorporated into the Immunopathology fellowship curriculum. In time, the need for this module to be offered as a specific post-fellowship training module may gradually disappear. Meanwhile, there was general agreement that this module should be available as a post-fellowship option for Immunopathologists seeking to take responsibility for the broad range of pathology tests in this category.

Generic knowledge and practical skills

- design and maintenance of molecular suites
- appropriate specimen types and associated collection methods
- nucleic acid preparation method(s)
- nucleic acid quantity and quality indicators
- nucleic acid storage/archiving
- running and analysing nucleic acid amplification-dependent assays (end point)
- selection of appropriate control samples
- running and analysing nucleic acid amplification-dependent assays (quantitative, including associated practical numeracy skills)
- measurement of uncertainty (for quantitative assays)

- trouble-shooting failed quality indicators
- awareness of the unique characteristics of the specific nucleic acid amplification-dependent assay(s) being used.
- Principles of primer design, including probe design for real-time PCR based assays
- Principles of BLAST analysis

Additionally, the following crucial points:

- *ensuring that the assay is capable of detecting and, where required, measuring accurately the intended target*
- *ensuring that no amplification occurs in the absence of substrate*
- *confirming that the assay can distinguish "positive" from "normal"*

Post-analytic considerations

- Synthesis and clinical interpretation of laboratory data taking account of the clinical presentation, immunological phenotype and other relevant non-genomic and genomic investigations
- Ability to provide clinically appropriate advice regarding contents of reports including diagnostic, prognostic and therapeutic implications of the results.
- Ability to communicate results and provide interpretative discussion to referring specialists and in multidisciplinary meetings

Professional Competency

- **Log book** - summarising all lab-based experiences, which includes analysis and reporting of 200 cases (may be simulated (i.e. training sets) instead of actual from primary data, to the standards appropriate to a diagnostic as opposed to research setting) with at least 50 disease-associated variants, with no more than 10 of the same category. 60% of cases may be non-immunological if performed and trainee reporting supervised by a diagnostic genetic laboratory. Log book to include test name; assay type; number of assays/ runs; test failures requiring review.
- **Portfolio Requirements** - should also include:
    - *Fifteen (15) technically challenging or unusual cases/consultations involving lab. data. * See below for a list of examples of technical considerations/ challenges.*
    - *Five (5) MDT attendances,*
    - *Eight (8) quality assurance activities (including two (2) compulsory), and*
    - *One (1) significant/critical laboratory incident report relevant to the module*
- **Case-based Discussions** - *write up (2 page limit) of case in addition to CbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of lab. data. At least one (1) discipline-specific CbD should be on further workup/management of findings.*
- **Supervisor sign-off**
    - With sign-off indicating:
        - Successful review and interpretation of results, at a quality level sufficient for reporting
        - strong understanding of QC procedures for the methods, including internal and external QA
        - working knowledge of method anomalies and associated trouble-shooting requirements
- **Dry Practical** (practical cases)
- **Structured Oral**

*** Examples of technical considerations/challenges:**

- Evaluating primary specimen for suitability for molecular testing
- Normal cell contamination
- Investigations/actions when signal is detected in a no-template control
- Investigations/actions when positive control gives no result
- Investigations/actions when positive control gives an incorrect result
- Investigations/actions when DNA is of inadequate quantity for testing
- Investigations/actions when DNA is of inadequate quality/amplifiability for testing
- Investigations/ actions when sex discrepancy, discordant result, sample mix up identified
- Investigations/actions when amplification curve shows an unusual pattern/unusual Ct value (for real-time PCR based assays)
- Investigations/actions when control samples perform adequately but one or more sample does not pass QC metrics
- Investigations/actions when results are not consistent with standard targeted variant or with wildtype (i.e. may suggest a different variant at that locus)
- Investigations/actions when testing performed at another laboratory does not match with the result in your laboratory
- Validation process for a targeted assay
- Investigations/actions when an external QA program does not award full marks for genotyping
- Investigations/actions when an external QA program does not award full marks for result interpretation/reporting.

Module 2 – Targeted screening for undefined variants in genes associated with specified clinical phenotypes associated with immunological disease.

Examples of typical clinical applications for this module would include:

- Sanger sequencing of single candidate genes associated with particular disease entities (eg: *BTK* gene in boy with agammaglobulinaemia or *CYBB* gene in boy with CGD)
- Targeted gene panels for diagnostic confirmation of pre-defined inherited conditions (e.g. severe combined immunodeficiency, autoinflammatory conditions)

The unifying concept of this module is the detection of unknown genomic variation within a pre-defined list of genes for analysis (which is typically linked to a specific phenotype/clinical context), which allows for the practice and development of expertise within a defined phenotype.

The approach used to detect disease-linked variants will also yield the following outcomes:

- False positive results
- False negative results
- Detection of variants of uncertain clinical significance
- Detection of variants with partial penetrance or expressivity
- Findings that may allow reproductive choices

Typically, these assays are 5-30 gene amplicon panels, which are run for disease diagnosis or stratification. They may also include single gene- or single exon-sequencing for specific diseases.

Types of genomic variation covered

Targeted panels and associated bioinformatic pipelines may be designed to detect sequence variants, copy number changes and structural variants/fusions involving pre-defined genes/regions on the targeted panel, depending on the nature of the genomic variations contributing to the defined clinical phenotype.

Methodologies covered

- Sanger sequencing
- Multiplex-ligation primer amplification (MLPA)
- Massively parallel sequencing with a range of library preparation/ bio-informatic filtering
    - Targeted amplicon enrichment
    - Hybridisation-based enrichment

Knowledge and practical skills

General considerations

- This is complex and specialised training, which builds upon the sound genomic basics in Module 1.
- Knowledge of specific quality issues, validation and requirements of supervision of massively parallel sequencing (i.e *Requirements for human medical genome testing utilising massively parallel sequencing technologies, National Pathology Accreditation Advisory Council, 2017*)
- Knowledge of potential sources of error arising from massively parallel sequencing assays.

Wet lab considerations

- General practical skills understanding of nucleic acid preparation method(s), quantification/purity/intactness, storage/archiving (as per core module 1)
- Knowledge of technical performance, limitations and quality issues associated with different library preparation methodologies (amplicon, hybridisation based, use of unique molecular identifiers (UMI), etc)
- Knowledge of technical performance, limitations and quality issues associated with different sequencing technologies.

Dry lab considerations

- Knowledge of primary, secondary and tertiary analysis, variables and limitations
- Knowledge of relevant bioinformatics issues including performance and limitations of demultiplexing/alignment tools/variant callers, variant annotation strategies, bioinformatic methods of structural variant detection, reference generation strategies for copy number assessment, performance and validation of copy number calling algorithms
- Knowledge of data architecture, computing/processing/capacity issues and data security/privacy
- Knowledge of cloud based secondary and tertiary analysis systems.

Analysis considerations

- Detailed working knowledge of HGVS/ISCN nomenclature and its practical application
- Knowledge and application of pathogenicity classification systems for germline variants
- Management of incidental genomic findings more generally, and variant associated with significant clinical outcomes unrelated to the purpose of testing
- Knowledge of constitutional/germline variant curation strategy including understanding of variant annotation, advantages and limitations of cancer (e.g. COSMIC) and healthy population databases (e.g. gnomAD), locus specific databases/Clinvar, segregation analysis, modes of inheritance, curation of constitutional literature, mosaicism, appropriate germline samples, advantages and limitations of *in silico* prediction tools and splice prediction tools
- Integration of the genomic variations detected with accompanying immunophenotypic and clinical context of the patient in order to provide a clinically appropriate genomic report
- Knowledge of ethical, clinical and regulatory structures/framework around germline testing for inherited disease/predictive testing.

Post-Analytic Considerations

- Ability to provide clinically appropriate advice regarding contents of genomic reports including diagnostic implications, prognostic implications and therapeutic implications of detected genomic variations
- Advice on appropriate follow-up genomic testing/other modalities as required (including testing of family members)
- Involvement and ability to communicate in multidisciplinary meetings with pathologists, scientists and other referring specialists.

Professional Competency

- **Log book** - summarising all lab-based experiences, which includes analysis and reporting of at least 200 cases and 100 disease-associated variants. 60% of cases may be non-immunological if performed and trainee reporting supervised by a diagnostic genetic laboratory. *Log book to include test name; assay type; number of assays/ runs; test failures requiring review*
- **Portfolio Requirements** - should also include:
    - *Fifteen (15) technically challenging or unusual cases/consultations involving laboratory data. * See below for a list of examples of technical considerations/ challenges. NOTE: evaluation of variants of uncertain significance MUST be included.*
    - *Five (5) MDT attendances,*
    - *Eight (8) quality assurance activities (including two (2) compulsory), and*
    - *One (1) significant/critical laboratory incident report relevant to the module*
- **Case-based Discussions** - d*etailed discussion (at least one (1) page description of case in addition to CbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of laboratory data. At least one (1) CbD should be on further workup/management of unusual such as an "additional" or "off target" finding.*
- **Supervisor sign-off**
    - With sign-off indicating:
        - the principles of the method are understood
        - working knowledge of instrument processes and maintenance requirements
        - successful generation of results from each method, at a quality level sufficient for reporting
        - strong understanding of QC procedures for the method, including internal and external QA
        - working knowledge of method anomalies and associated trouble-shooting requirements
- **Dry Practical** (practical cases)
- **Structured Oral**

*** Examples of technical considerations:**

- Investigations/actions when DNA is of inadequate quantity for testing
- Sanger sequencing primer design
- Sequence with poor quality Phred score
- Trouble-shooting poor quality sequence
- Challenges associated with homopolymer runs
- Challenges with assay controls – positive and negative
- Trouble-shooting a massively parallel sequencing run with poor quality control metrics – wet lab and/or dry lab MLPA probe design
- Issues with sample depurination
- Trouble-shooting MLPA results – quantitation, standard deviation, abnormal marker patterns, low probe signals, apparent single exon deletion, etc.
- Investigations/actions when control samples perform adequately but one or more samples does not pass QC metrics
- Investigations/actions when testing performed at another laboratory does not match with the result in your laboratory
- Validation process for Sanger sequencing/ MLPA
- Validation process for massively parallel sequencing, including validation of bioinformatic pipeline

- Maintenance of bioinformatic pipeline, including version control, verification of new versions
- Assessment of variants of uncertain clinical significance (must be included)
- Consultation with colleagues regarding an incidental/secondary finding
- Investigations/actions when an external QA program does not award full marks for genotyping
- Investigations/actions when an external QA program does not award full marks for result interpretation/reporting.

Module 3 – Sequence-based screening for known and unknown variants in multiple genes, including genes potentially linked to clinical phenotypes that have not been previously diagnosed in the patient.

The unifying concept of this module is the application of massively parallel methodologies to screen for disease-causing and disease-associated genomic variants in large numbers of genes, including genes linked to immunological phenotypes that have not been previously diagnosed in the patient.

The purpose of genome-wide testing, when applied, would be to bioinformatically extract panels of genes relevant to immunological phenotypes, or for genome-wide analysis directed towards the diagnosis of immunological disorders.

The approach used to detect disease-linked variants will also yield the following outcomes:

- False positive results
- False negative results
- Detection of variants of uncertain clinical significance
- Detection of variants with partial penetrance or expressivity
- Findings that may allow reproductive choices

Wide scope sequencing may also yield other additional or "off-target" findings:

- Medically treatable disorders
- Serious, incurable conditions
- Variants conferring susceptibility to disease
- Variants with pharmacogenetic implications
- Mis-attributed paternity
- Close consanguinity

Types of genomic variation covered
Bioinformatic "pipelines" can be targeted to screen massively parallel sequencing output for nucleotide variants, copy number changes and structural variants/fusions across a large number of specified genes relevant to immunological phenotype.

Methodologies covered
Massively parallel sequencing using amplicon-based and hybridisation capture-based assays.

Bioinformatics analysis of WGS/WES sequencing data (alignment, variant call and annotation).

Knowledge and practical skills

General considerations

- This is complex and specialised training and builds upon Modules 1 and 2.
- Understanding of the nature of human genomic variation
- Differences between WES and WGS
- Sound working fluency in dealing with the range of expected challenging outcomes and additional, "off-target" findings.
- Knowledge of specific quality issues, validation and requirements of supervision of massive parallel sequencing (i.e *Requirements for human medical genome testing*

utilising massively parallel sequencing technologies, National Pathology Accreditation Advisory Council, 2017)

Wet lab considerations

- Refer to module 2.

Dry lab considerations

- Refer to module 2.

Additionally:

- Knowledge of read depth and coverage issues whilst using whole exome or genome techniques for rare phenotypes/inherited conditions

Analysis considerations

- Refer to module 2, in addition;
- Familiarity with methods for generating VCF files
- Principles of assembling diagnostic gene lists
- Segregation analysis including analysis of family trios for de novo and compound heterozygous mutations
- Principles of in silico analysis of germline variants
- Place of orthogonal testing
- VUS classification

Post-Analytic Considerations

- Refer to module 2, in addition;
- Awareness of the importance of data re-analysis
- Data storage
- Principles of data sharing
- Principles of variant reporting to ClinVar etc
- In principle understanding of the approach to functional analysis of VUS
- Writing a WES or WGS clinical report

Additionally:

- Close link with a clinical genomics service, particularly for whole genome /exome services.

Professional Competency

- **Log book** – summarising all lab-based experiences, which includes analysis and reporting of at least 200 cases with 100 unique variants. 60% of cases may be non-immunological if performed and trainee reporting supervised by a diagnostic genetic laboratory. *Log book to include test name; assay type; number of assays/ runs; test failures requiring review.*
- **Portfolio Requirements** - should also include:
    - *Fifteen (15) technically challenging or unusual cases/consultations involving lab. data. * See below for a list of examples of technical considerations/ challenges.*
    - *Five (5) MDT attendances,*
    - *Eight (8) quality assurance activities (including two (2) compulsory), and*
    - *One (1) significant/critical laboratory incident report relevant to the module*
- **Case-based Discussions** ***(note:** discipline-specific discussion topics to be defined)* - d*etailed discussion (at least one (1) page description of case in addition to CbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of lab. data. At least one (1) CbD should be on further workup/management of unusual such as an "additional" or "off target" finding.*
- **Supervisor sign-off**
    - With sign-off indicating:
        - the principles of the method are understood
        - working knowledge of instrument processes and maintenance requirements
        - successful generation of results from each method, at a quality level sufficient for reporting
        - strong understanding of QC procedures for the method, including internal and external QA
        - working knowledge of method anomalies and associated trouble-shooting requirements
- **Dry Practical** (variant curation, practical cases)
- **Structured Oral**

*** Examples of technical considerations:**

- Evaluating primary specimen for suitability for molecular testing
- Normal cell contamination
- Investigations/actions when signal is detected in a no-template control
- Investigations/actions when DNA is of inadequate quantity for testing
- Investigations/actions when DNA is of inadequate quality/amplifiability for testing
- Investigations/ actions when sex discrepancy, discordant result, sample mix up identified
- Investigations/actions when results may be confounded by repetitive DNA sequences/ pseudogenes
- Investigations/actions when testing performed at another laboratory does not match with the result in your laboratory
- Trouble-shooting a whole exome/genome sample/run with poor quality control metrics – wet lab and/or dry lab metrics
- Validation process for a targeted assay based on bioinformatic filtering of whole genome/exome sequencing data
- Panel selection from WES/WGS data, including technical and clinical considerations
- Approach to targeted genes which are not adequately covered for clinical reporting

- Investigations/actions when an external QA program does not award full marks for genotyping
- Investigations/actions when an external QA program does not award full marks for result interpretation/reporting
- Approach to assessment and reporting of variants of uncertain significance in the somatic and germline context
- Approach to assessment and reporting of 'susceptibility variants' or risk alleles in the somatic and germline context
- Consultation with expert colleagues regarding an incidental/secondary.

