HANDBOOK

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

1.1 Introduction

The Royal College of Pathologists of Australasia (the College) offers three certification modules in genetic/genomic immunopathology for those who have attained FRCPA.

These modules have been designed to satisfy the recent NPAAC Requirements, particularly as applicable to supervision of testing involving molecular diagnostics and genomics.

To competently 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 be integral to the work of immunopathology laboratories. These modules are designed to provide all immunopathologists with the opportunity to participate in accordance with NPAAC requirements.

1.2 General aims

The genetics/genomic certification modules build on discipline-specific Fellowship training. They are designed to build base knowledge, analytical and interpretive skills, quality assurance and communication capabilities relevant to genetics/genomic testing in Immunopathology.

This Handbook 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 immunopathology.

Completion of one or more modules will result in the 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.

Please note: Modules must be completed sequentially; ie. Module 1 is required for those who wish to complete Module 2, and Module 2 before commencing Module 3. There will be no opportunity to complete modules in any other order.

While candidates will gain understanding of the breadth of the field, they must remain aware of the limits of their own knowledge and appreciate when it is in the best interests of patients to refer to, or formally consult with, genetic pathologists or other appropriately credentialed colleagues.

1.3 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.

The three modules in this handbook 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).

1.4 Registration

Applicants must complete a registration form, obtainable from the RCPA website and submit this with the application fee. The form must clearly state the modules(s) being applied for. Applications for partial exemption from assessments (recognition of prior learning) must be submitted with supporting documentation at the time of registration.

1.5 Training structure and requirements

The modules are outcomes-based, with no fixed time requirements for completion. Candidates must participate in a range of laboratory-based experiences to achieve the listed outcomes. All specified assessment tasks must be completed satisfactorily to achieve certification.

1.6 Supervision

All training must be supervised by an approved Fellow of the RCPA with the required expertise. Supervisors are expected to monitor and provide regular feedback on the candidate’s developing

competence. Formal meetings with the candidate are expected to occur at least every three months.

The supervisor will complete a Supervisor Report at the end of the training period, along with a completed and signed portfolio summary sheet. If the training period exceeds twelve months, an additional annual Supervisor report will be required.

The supervisor will carry out workplace-based assessments or may delegate this responsibility to another suitably qualified pathologist or senior scientist.

1.7 Assessment

Assessment consists of a range of activities and workplace-based assessments to be documented in a portfolio, and formal examinations as prescribed for each module.

Portfolios may be maintained in paper-based and/or electronic format with back-up. Portfolios do not need to be submitted to the College. The supervisor will verify that all entries and assessment tasks have been completed on the portfolio summary sheet to be submitted with the final Supervisor Report for the module.

1.8 Continuing professional development

Activities carried out and documentation associated with the modules may be included, along with general immunopathology activities, in the relevant categories of the RCPA Continuing Professional Development Program (Professional Performance Framework). Following certification, specialists are expected to undertake ongoing professional development relevant to the modules for which they are certified.

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: HIV testing and detection of known variants in HLA alleles such as coeliac disease associated with HLA typing, HLA-B27, HLA-DR and HLA-DQ disease associated alleles) 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.

1.1 Learning outcomes

1.1.1 Theoretical and Technical Knowledge

- 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
- 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

1.1.2 Analytical and interpretive skills

- 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
- 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

1.1.3 Quality assurance

- Knowledge of technical performance, limitations and quality issues
- Recognition and troubleshooting of challenges
- Competence in monitoring data quality and result verification

1.1.4 Communication and Consultation

- 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
- Advice on appropriate follow-up genomic testing/other modalities as required (including testing of family members)

1.2 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.

1.3. Assessment

1.3.1 Portfolio requirements

Item	Requirements (number/type)	Documentation
Case Log	Summaries of all lab-based experiences, including analysis and reporting of 150 cases (may be simulated (ie. 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). (**note**: 60% of cases may be non-immunological if performed and fellow reporting supervised by a diagnostic genetic laboratory)	Log book to include test name; assay type; number of assays/ runs; test failures requiring review. (See Appendix 2)
Clinical Consultations	Fifteen (15) technically challenging or unusual cases/consultations involving lab data. (**note**: Examples of technical considerations/ challenges as listed in paragraph 1.2)	Clinical Consultations Sign-Off Form (Appendix 2)
Process-based Discussions (PdD)	• Write up (at least one (1) page description of case in addition to PbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of lab data. • At least one (1) PbD should be on further workup/management of findings.	PbD Assessment Form (Appendix 2)
Quality Assurance (QA) Activities Log	Eight (8) quality assurance activities. (**note**: You may include a significant/critical laboratory incident relevant to the module as a QA Audit)	Quality Assurance Activities Log Appendix 2)

QA Reflection Supervisor Report	Reflection on each of above One final report, plus additional annual report if module not completed in <12 months.	QA Reflection Form 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. (Supervisor Report, Appendix 2)
Portfolio Summary sheet	To accompany final Supervisor Report	Portfolio Summary Form

1.3.2 Formal examinations

See Appendix 1

1.3.3 Assessment matrix

See Appendix 3

Module 2: Targeted screening for undefined variants in genes associated with specific clinical phenotypes associated with immunological disease

This is complex and specialised training, which builds upon the sound genomic basics in Module 1.

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.

Methods 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

2.1 Learning outcomes

2.1.1 Theoretical and Technical Knowledge

- 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
- 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

- Knowledge of specific quality issues, validation and requirements of supervision of MPS (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 and understanding of nucleic acid preparation method(s), quantification/purity/intactness, storage/archiving
  - 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.

2.1.2 Analytical and interpretive skills

- 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

- 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
  - 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

2.1.3 Quality assurance

- Knowledge of technical performance, limitations and quality issues
- Recognition and troubleshooting of challenges
- Competence in monitoring data quality and result verification
- Knowledge of relevant ethical, clinical and regulatory structures/framework

2.1.4 Communication and Consultation

- Ability to provide clinically appropriate advice regarding contents of genomic reports including diagnostic, prognostic 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.
- Ability to recognise when complex test results mean that patient safety is best served by consultation with an expert colleague eg. a genetic pathologist

2.2 Examples of technical considerations/challenges

- 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.

2.3. Assessment

2.3.1 Portfolio requirements

Item	Requirements (number/type)	Documentation
Case Log	Summaries of all lab-based experiences, including analysis and reporting of at least 150 cases and 100 disease-associated variants. (**note**: 60% of cases may be non-immunological if performed and fellow reporting supervised by a diagnostic genetic laboratory).	Log book to include test name; assay type; number of assays/ runs; test failures requiring review. (See Appendix 2)
Clinical Consultations	Fifteen (15) technically challenging or unusual cases/consultations involving lab data. (**note**: Examples of technical considerations/ challenges as listed in paragraph 2.2). *NOTE: at least 1 case of evaluation of variants of uncertain significance MUST be included.*	Clinical Consultations Sign-Off Form (Appendix 2)
Process-based Discussions (PdD)	• Detailed discussion (at least one (1) page description of case in addition to PbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of lab data. • At least one (1) PbD should be on further workup/management of unusual such as an "additional" or "off target" finding.	PbD Assessment Form (Appendix 2)
MDT Participation	Five (5) MDT attendances	Multidisciplinary Meeting Sign-Off Form (Appendix 2)
Quality Assurance (QA) Activities Log	Eight (8) quality assurance activities (**note**: You may include a significant/critical laboratory incident relevant to the module as a QA Audit)	Quality Assurance Activities Log (Appendix 2)
QA Reflection	Reflection on each of above	QA Reflection Form
Supervisor Report	One final report, plus additional annual report if module not completed in <12 months.	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 methods, including internal and external QA; • working knowledge of method anomalies and associated trouble-shooting requirements. (Supervisor Report, Appendix 2)
Portfolio Summary sheet	To accompany final Supervisor Report	Portfolio Summary Form

2.3.2 Formal examinations

See Appendix 1

2.3.3 Assessment matrix

See Appendix 3

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

This is complex and specialised training and builds upon Modules 1 and 2.

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.

Methods covered

- Massively parallel sequencing using amplicon-based and hybridisation capture-based assays.
- Bioinformatics analysis of WGS/WES sequencing data (alignment, variant call and annotation).

3.1 Learning outcomes

3.1.1 Theoretical and Technical Knowledge

- 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
- 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
- Knowledge of specific quality issues, validation and requirements of supervision of MPS (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.
- 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 potential sources of error arising from massively parallel sequencing assays

- Wet lab considerations:
  - General practical skills and understanding of nucleic acid preparation method(s), quantification/purity/intactness, storage/archiving
  - 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.

3.1.2 Analytical and interpretive skills

- 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

- 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
  - 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
- Synthesis and clinical interpretation of laboratory data taking account of the clinical presentation, morphology, immunophenotype and other relevant non-genomic and genomic investigations
- 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
- 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

3.1.3 Quality assurance

- Knowledge of technical performance, limitations and quality issues
- Recognition and troubleshooting of challenges
- Competence in monitoring data quality and result verification
- Knowledge of relevant ethical, clinical and regulatory structures/framework

3.1.4 Communication and Consultation

- Close link with a clinical genomics service, particularly for whole genome /exome services.
- Ability to provide clinically appropriate advice regarding contents of genomic reports including diagnostic, prognostic 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, physicians, scientists and other referring specialists

3.2 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 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.

3.3. Assessment

3.3.1 Portfolio requirements

Item	Requirements (number/type)	Documentation
Case Log	Summaries of all laboratory-based experiences, including analysis and reporting of at least 150 cases with at least 100 unique variants. (**note**: 60% of cases may be non-immunological if performed and fellow reporting supervised by a diagnostic genetic laboratory)	Log book to include test name; assay type; number of assays/ runs; test failures requiring review (See Appendix 2)
Clinical Consultations	Fifteen (15) technically challenging or unusual cases/consultations involving laboratory data. (**note**: Examples of technical considerations/ challenges as listed in paragraph 3.2). *NOTE: at least 1 case of evaluation of variants of uncertain significance MUST be included.*	Clinical Consultations Sign-Off Form (Appendix 2)
Process-based Discussions (PbD)	• Detailed discussion (at least one (1) page description of case in addition to PbD cover sheet) on five (5) "challenging" cases that required use of multiple skills, including consideration of laboratory data. (**note**: discipline-specific discussion topics to be defined) • At least one (1) PbD should be on	PbD Assessment Form (Appendix 2)

	further workup/management of unusual such as “off target” findings.	
MDT Participation	Five (5) MDT attendances	Multidisciplinary Meeting Sign Off Form (Appendix 2)
Quality Assurance (QA) Activities Log	Eight (8) quality assurance activities (**note**: You may include a significant/critical laboratory incident relevant to the module as a QA Audit)	Quality Assurance Activities Log (Appendix 2)
QA Reflection	Reflection on each of above	QA Reflection Form (Appendix 2)
Supervisor Report	One final report, plus additional annual report if module not completed in <12 months.	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. Supervisor Report (Appendix 2)
Portfolio Summary sheet	To accompany final Supervisor Report	Portfolio Summary Form

3.3.2 Formal examinations

See Appendix 1

3.3.3 Assessment matrix

See Appendix 3

SECTION 3

Assessment

Assessment is by

- Formal examinations (see Appendix 1)
- A portfolio of evidence of having participated in a sufficient number and type of work activities (see Appendix 2)
- Satisfactory progress (supervisor reports) (see Appendix 2)

An assessment matrix is provided in Appendix 3.

Portfolio requirements

Portfolio activities are carried out in the workplace provides evidence that fellows have engaged in the appropriate number and type of work-based activities to build base knowledge, analytical and interpretive skills, quality assurance and communication capabilities relevant to genetics/genomic testing in Immunopathology. Requirements for each module are summarised in a table at the end of the module in Section 2.

Appendix 2 contains the forms and logbook pages for recording the portfolio activities. Please file the hard copy forms in a **portfolio folder** with separate sections as in the table provided in each module.

A soft copy **portfolio summary** (Excel spreadsheet) should also be compiled so that the fellow can keep track of what has been completed. The spreadsheet can be downloaded from the RCPA website. It is the fellow's responsibility to keep both hard and soft copy records up-to-date.

The portfolio summary spreadsheet should be appended to the annual supervisor reports and will be reviewed by the Registrar, Board of Education and Assessment and the Chief Examiner. Signatories and fellows may be contacted to confirm evidence of satisfactory completion.

Appendix 1: Examinations

There will be a multi-station examination consisting of two or three stations, each of twenty minutes, with two examiners for each. These will include stations focussing on practical cases; for example, practical cases for variant curation in Module 3.

Candidates will be allowed 1 hour pre-reading time, during which they may review data and reports, making notes where applicable, prior to discussion with the examiners.

The examination will focus on ability to:

- Analyse and interpret findings
- Explain principles of the test methods used, identifying any limitations
- Apply quality management principles and troubleshooting methods to explain and prevent possible sources of error
- Communicate clinically relevant conclusions and advice to referring doctors, including discussion of uncertainties
- Discuss broader health implications and/or ethical considerations relevant to the testing performed

Appendix 2: Forms and log pages for portfolio

Medical Genomics Immunopathology
Routine Case Log

How to use this form

From the beginning of training, Fellows should log all laboratory-based experiences, including analysis and reporting. Only runs that the Fellow has been directly involved with should be logged.

Modules 1: A minimum of 150 cases with 50 disease-associated variants should be recorded

Modules 2&3: A minimum of 150 cases with 100 disease-associated variants should be recorded

The log book should include:

- Test name
- Assay type
- Number of assays/ runs
- Test failures requiring review

At the end of each Module, the log should be sighted and signed off on the Supervisor Report.

Fellow Name			**Fellow ID**	**Module of Training (please tick)** ☐ **Module 1** ☐ **Module 2** ☐ **Module 3**	
	Date	***Test name***	***Assay type***	***Number of assays/ runs***	***Test failures requiring review***
1					
2					
3					
4					
5					
6					

7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
Final Outcome (please tick) ☐ Competent ☐ Not Competent					
Signature of Assessor			**Signature of Fellow**		
Name of Laboratory					

Medical Genomics Immunopathology
Clinical Consultations Sign-Off Form

How to use this form
From the beginning of training, Fellows should log consultations with clinical colleagues that involve significant, difficult or unusual cases.
A minimum of (fifteen) 15 consultations should be recorded during the Module.

Consultation type should be noted on the form as: ***ORAL****:Telephone Outpatient* ***(TOP) OR*** *Telephone Inpatient* ***(TIP)***
WRITTEN*: Outpatient* ***(OP) OR*** *Inpatient* ***(IP)***

At the end of each Module, this form and appended case lists should be sighted by the supervisor and signed off.

Fellow Name			**Fellow ID**	**Module of Training (please tick)** ☐ Module 1 ☐ Module 2 ☐ Module 3	
	Date	***Headline summary of case***	***Issue(s) raised by the case***	***Consult type***	***Fellow's role in the case***
	example	***eg. TPMT polymorphisms in azathioprine metabolism***	***Clinician seeking guidance on diagnostic possibilities and investigations***	***TOP / TIP OP / IP***	***Advice offered; review of results; follow-up discussion with referring clinician***
1					
2					
3					
4					
5					
6					
7					

8					
9					
10					
11					
12					
13					
14					
15					
Final Outcome (please tick) ☐ Competent ☐ Not Competent					
Signature of Assessor			**Signature of Fellow**		
Name of Laboratory					

Medical Genomics Immunopathology PbD Assessment Form

Process based Discussion

Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3
Assessor Name	**Assessor position** ☐ Pathologist ☐ Other (please specify)	

Techniques/methods (tick the box that applies).

1. ☐ Sanger sequencing
2. ☐ Multiplex-ligation primer amplification (MLPA)
3. ☐ Massively parallel sequencing (MPS) - targeted amplicon enrichment
4. ☐ Massively parallel sequencing (MPS) - hybridisation-based enrichment
5. ☐ Bioinformatics analysis of WGS/WES sequencing data

Please comment on whether these aspects of the Fellow's performance	**Yes**	**No**	**N/A**
Understands the principles of the method			
Has a working knowledge of instrument processes and maintenance requirements			
Has observed all phases of an assay successfully and been involved in the production of a valid result that can be reported			
Able to explain the Quality Controls procedures for this method, including internal and external Quality Assurance.			
Able to discuss anomalies and resolve uncertainties for the method			
Able to explain maintenance and trouble-shooting requirements for the method			

Please comment on other relevant aspects, especially on aspects for improvement (use the reverse side if insufficient room)

Final Outcome (please tick)

☐ Competent ☐ Not Competent

Signature of Assessor	**Signature of Fellow**

Name of Laboratory

Medical Genomics Immunopathology
Clinical/Multidisciplinary Meeting Sign Off Form

How to use this form
This form is to be used to record that the Fellow has fulfilled the following requirements:
Present cases at a minimum of five (5) clinical or laboratory meetings throughout the module.
The supervisor is asked to sign after each meeting to verify off the Fellow's participation.
At the end of the module, this form and appended case lists should be sighted by the supervisor and signed off.

Fellow Name:	**Fellow ID:**	**Module of Training** (please tick ☐ Module 1 ☐ Module 2 ☐ Module 3

	Meeting date	**Brief description of meeting; subject(s) of discussion**	**Did Fellow present cases? Y/N**
1			
2			
3			
4			
5			
6			
7			
8			

Final Outcome (please tick) ☐ Competent ☐ Not Competent

Signature of Assessor	**Signature of Fellow**

Name of Laboratory

Medical Genomics Immunopathology
Quality Assurance Activities Log

How to use this form
Eight (8) activities should be selected from the list. Items 9 and 10 are compulsory.

Use the Reflection Form in this handbook to write a brief reflection on what you learned from doing each activity (photocopy as many copies of the form as you need).
Keep the forms in your portfolio along with other specified documents if required
At the end of each rotation, the log should be sighted and signed off by the supervisor.

Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module **3**

	Quality activity	***Summary of Fellow's role in the activity or comment (where applicable)***	Date
1	Analyse the design and operating characteristics of a particular instrument or platform		
2	Work through the development of a new in vitro diagnostic test and the associated IQA processes		
3	Review relevant AS ISO standards (list documents reviewed)		
4	Review relevant NPAAC standards and guidelines (list documents reviewed)		
5	Review the laboratory's quality policy, including policy guiding response to unsatisfactory QAP results		
6	External QAP (particularly involvement with the HGSA/QAP, EMQN, ASoC programs)		
7	Active involvement in preparations for laboratory accreditation		

Medical Genomics Immunopathology
Quality Assurance Activities Log

How to use this form
Eight (8) activities should be selected from the list. Items 9 and 10 are compulsory.

Use the Reflection Form in this handbook to write a brief reflection on what you learned from doing each activity (photocopy as many copies of the form as you need).
Keep the forms in your portfolio along with other specified documents if required
At the end of each rotation, the log should be sighted and signed off by the supervisor.

Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3

	Quality activity	***Summary of Fellow's role in the activity or comment (where applicable)***	***Date***
8	Participate in a workflow check of effective/ efficient laboratory function		
9	**MANDATORY ACTIVITY** Review and update laboratory internal QC procedures. Include reports in portfolio.		
10	**MANDATORY ACTIVITY** Significant incident: Involvement in assessment, reporting and review, focussing particularly on the quality issues that were identified and addressed. **Minimum of one**. OR Quality audits: Conduct. Where possible, include comparison with relevant national/international guidelines. **Minimum of one**. Include documentation in portfolio. Use the reporting form in supplied in this Handbook		

Supervisor name……..... Signature…….…… Date…….....

Medical Genomics Immunopathology Quality Assurance Reflection Form

Fellow Name	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3
Nature of activity		
Your role in the activity		
Your reflection on what you learned from your involvement in this activity *Points to consider: -* - *Type of Activity* - *Actions undertaken* - *Findings* - *Resource considerations* - *Ethical considerations*		
Fellow signature		Date
Supervisor name (please print) and signature		Date
Name of Laboratory		

Medical Genomics Supervisor Report Immunopathology

Please review the Fellow's portfolio and logbook before completing this report

Fellow Name (please print)	**Fellow ID**	**Module of Training** (please tick) ☐ Module 1 ☐ Module 2 ☐ Module 3
Name of Organisation		
Training period: _____/_____/_____ to ___/_____/_____		
Name of supervisor (please print)	**Supervisor RCPA ID no.**	

Please inspect the forms in the Fellow's portfolio and use the Table below to record evidence of completion

Portfolio items for which there is a minimum requirement				
	Previous total (if applicable)	Number in current year/ module	Cumulative Total	Minimum for completion of module
Case Log				150
Clinical Consultations				15
Process-based discussion (PbD)				5
MDT Participation				5
QA Activities				8
QA Reflection				1 per each QA activity
Previous Supervisor's reports				1 report/ 12 months
Portfolio Summary				

Does the print-out of the portfolio summary spreadsheet accurately record the contents of the portfolio?

☐ Yes ☐ No

Please score the Fellow's performance using this scale

1 = Performance currently falls far short of expected standards for level of training.
There is a serious problem that may have implications for accreditation of the current training period. The problem must be stated clearly on the final page.

2 = Performance currently falls short of expected standards for level of training.
There is an area of lower than expected performance. The problem must be stated clearly on the final page.

3 = Performance is consistent with the expected level of training.
About 80% of fellows will merit this grade.

4 = Performance is better than expected for level of training.
About 10% of fellows will merit this grade.

5 = Performance is exceptional.
Very few fellows will merit this grade.

N/A = Not Applicable to this training period

	Score
The principles of all methods are understood	
Working knowledge of method anomalies and associated trouble-shooting requirements	
Working knowledge of instrument processes and maintenance requirements	
Successful generation of results from each method, at a quality level sufficient for reporting	
Working knowledge of method anomalies and associated trouble-shooting requirements	
Strong understanding of QC procedures for the methods, including internal and external QA	
Ability to provide clinically appropriate advice regarding contents of reports	
Ability to provide appropriate follow-up genomic testing/other modalities as required	
Ability to communicate in multidisciplinary meetings with pathologists, physicians, scientists and other referring specialists	

Overall evaluation

Have the outcomes of this module been satisfactorily achieved?

☐ Yes ☐ No

Is specific further professional development required? If yes, please outline process

☐ Yes ☐ No

Signatures

Fellow (please PRINT name and sign)	Date
Supervisor name (please PRINT name and sign)	Date
Other senior staff member/second supervisor (if applicable) (please PRINT name and sign)	Date
Head of Department (please PRINT name and sign)	Date

Comments by Fellow:

Please return this Supervisor's Report to:

The Royal College of Pathologists of Australasia
207 Albion Street
Surry Hills NSW 2010 AUSTRALIA

Faxed reports will not be accepted

THE ROYAL COLLEGE OF PATHOLOGISTS OF AUSTRALASIA USE ONLY

Signature

Registrar/Deputy Registrar, Board of Education and Assessment (please PRINT name and sign)	Date

Appendix 3: Assessment matrix

	Portfolio of workplace activities					Supervisor report	Oral exam
	Case Log	Consult -ations	PbD	MDT	QA Activity		
Theoretical & technical knowledge	Y	Y	Y	Y		Y	Y
Analytical and interpretive skills		Y	Y	Y	Y	Y	Y
Quality assurance			Y	Y	Y	Y	Y
Communication & consultation		Y	Y	Y	Y	Y	Y