

NATIONAL PATHOLOGY ACCREDITATION ADVISORY COUNCIL

**REQUIREMENTS FOR CYTOGENETIC
TESTING**

(Third Edition 2013)

NPAAC Tier 4 Document

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The National Pathology Accreditation Advisory Council (NPAAC) was established in 1979 to consider and make recommendations to the Australian, state and territory governments on matters related to the accreditation of pathology laboratories and the introduction and maintenance of uniform standards of practice in pathology laboratories throughout Australia. A function of NPAAC is to formulate Standards and initiate and promote education programs about pathology tests.

Publications produced by NPAAC are issued as accreditation material to provide guidance to laboratories and accrediting agencies about minimum Standards considered acceptable for good laboratory practice.

Failure to meet these minimum Standards may pose a risk to public health and patient safety.

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Scope

The *Requirements for Cytogenetic Testing* is a Tier 4 NPAAC document and must be read in conjunction with the Tier 2 document *Requirements for Medical Pathology Services*. The latter is the overarching document broadly outlining Standards for good medical pathology practice where the primary consideration is patient welfare, and where the needs and expectations of patients, Laboratory staff and referrers (both for pathology requests and inter-Laboratory referrals) are safely and satisfactorily met in a timely manner.

Whilst there must be adherence to all the Requirements in the Tier 2 document, reference to specific Standards in that document are provided for assistance under the headings in this document.

This document is for use in Laboratories providing cytogenetic services.

Abbreviations

Ag	silver
AS	Australian Standard
DAPI	4', 6'-diamidino-2'-phenylindole
FISH	fluorescence in situ hybridisation
HGSA	Human Genetics Society of Australasia
ISCN	International System for Human Cytogenetic Nomenclature
ISO	International Organization for Standardization
NPAAC	National Pathology Accreditation Advisory Council
NOR	nucleolar organising region

Definitions

Ag-NOR	means a technique whereby a silver (Ag) salt is used to stain the nucleolar organising regions (NOR) of the five acrocentric chromosome pairs.
Analyse	means to evaluate each chromosome in a cell, either by comparing the homologues band for band through the microscope, on a high-resolution digital display, or by using photographs.
C-banding	means a technique that produces darkly stained bands specific only to those regions of the karyotype containing constitutive heterochromatin.
Count	means to enumerate the number of chromosomes in a cell. The chromosomes need not be banded. A count should include comment on any obvious structural aberrations.
Experienced cytogeneticist	means a person who has a minimum of two years of diagnostic human cytogenetics experience and who has been documented to be competent in cytogenetics according to the Laboratory's Quality System.
G-banding	means a technique that, following various pretreatments of chromosome preparations and staining with Giemsa (or similar) stain, produces characteristic alternating dark and pale bands along each chromosome. G-banding is the standard technique for chromosome identification in human cytogenetics.
Karyotype (noun)	means the array of a complete set of paired chromosomes displayed in standard arrangement.
Karyotype (verb)	means to arrange the banded chromosomes of a single cell in the standard arrangement (International System for Human Cytogenetic Nomenclature, ISCN). The arrangement may be achieved by physically cutting up a photograph or by using an image analysis system.
Q-banding	means a staining technique in which metaphase chromosomes are stained with quinacrine to produce temporary fluorescence on the chromosomes under ultraviolet illumination. Although the Q-bands are similar to G-bands, this method is useful for identifying the Y chromosome and certain DNA polymorphisms. However, Q-banding has been largely superseded in routine use by fluorescence in situ hybridisation (FISH) techniques.

R-banding (reverse banding)	means a chromosome banding technique that produces alternate dark and pale bands on chromosomes that are the reverse of the more commonly used G-banding (i.e. dark G-bands are pale in R-banding). Traditionally, R-banding has been more commonly used in European countries, especially France.
Requirements for Medical Pathology Services (RMPS)	<p>means the overarching document broadly outlining standards for good medical pathology practice where the primary consideration is patient welfare, and where the needs and expectations of patients, Laboratory staff and referrers (both for pathology requests and inter-Laboratory referrals) are safely and satisfactorily met in a timely manner.</p> <p>The standard headings are set out below –</p> <p>Standard 1 – Ethical Practice</p> <p>Standard 2 – Governance</p> <p>Standard 3 – Quality Management</p> <p>Standard 4 – Personnel</p> <p>Standard 5 – Facilities and Equipment</p> <p style="padding-left: 40px;">A – Premises</p> <p style="padding-left: 40px;">B – Equipment</p> <p>Standard 6 – Request-Test-Report Cycle</p> <p style="padding-left: 40px;">A – Pre-Analytical</p> <p style="padding-left: 40px;">B – Analytical</p> <p style="padding-left: 40px;">C – Post-Analytical</p> <p>Standard 7 – Quality Assurance</p>
Score (verb)	means to enumerate the presence or absence of a specific cytogenetic feature.

Introduction

This Tier 4 NPAAC document, together with the Tier 2 *Requirements for Medical Pathology Services*, is intended to be used in cytogenetics Laboratories to provide guidance on good practice in relation to cytogenetics and by assessors carrying out Laboratory accreditation assessments.

These Requirements are intended to serve as minimum Standards in the accreditation process and have been developed with reference to current and proposed Australian regulations and other standards from the International Organization for Standardization including:

AS ISO 15189 *Medical laboratories – Requirements for quality and competence*

These Requirements should be read within the national pathology accreditation framework including the current versions of the following NPAAC documents:

Tier 2 document

- *Requirements for Medical Pathology Services*

All Tier 3 Documents

Tier 4 Document

- *Requirements for Medical Testing of Human Nucleic Acids*

In addition to these Standards, Laboratories must comply with all relevant state and territory legislation (including any reporting requirements).

In each section of this document, points deemed important for practice are identified as either ‘Standards’ or ‘Commentaries’, as follows:

- A Standard is the minimum requirement for a procedure, method, staffing resource or facility that is required before a Laboratory can attain accreditation – Standards are printed in bold type and prefaced with an ‘S’ (e.g. **S2.2**). The use of the word ‘**must**’ in each Standard within this document indicates a mandatory requirement for pathology practice.
- A Commentary is provided to give clarification to the Standards as well as to provide examples and guidance on interpretation. Commentaries are prefaced with a ‘C’ (e.g. C1.2) and are placed where they add the most value. Commentaries may be normative or informative depending on both the content and the context of whether they are associated with a Standard or not. Note that when comments are expanding on a Standard or referring to other legislation, they assume the same status and importance as the Standards to which they are attached. Where a Commentary contains the word ‘**must**’ then that commentary is considered to be **normative**.

Please note that the Appendices attached to this document are **informative** and should be considered to be an integral part of this document.

Please note that all NPAAC documents can be accessed at
www.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-publication.htm

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1. Personnel

(Refer to Standard 4 in *Requirements for Medical Pathology Services*)

The number of Specimens processed by a person will depend on the experience of that person, their other duties, the degree of equipment automation and the complexity of the analyses.

The following ranges can be considered as a basis for calculating annual workloads:¹

- a) 250–350 lymphocyte cultures, or
- b) 250–350 prenatal cultures, or
- c) 250–350 solid tissues, or
- d) 150–250 haematological malignancy cultures, or
- e) 100–200 solid tumour cultures, or
- f) 400–500 metaphase/interphase fluorescence in situ hybridisation (FISH) tests, or
- g) 150–220 specialised FISH tests (e.g. multiple subtelomere).

2. Specimens and referral types

(Refer to Standard 6 in *Requirements for Medical Pathology Services*)

Prenatal (general)

S2.1 All unprocessed cultures must be kept for at least two days after a final result is issued/validated.

C2.1(i) The requirement that cultures are maintained for at least two days after the report is issued is to enable follow-up studies, if indicated, as well as the verification of Specimen identity.

If molecular genetic testing is also being performed on the prenatal Specimen, cultures may be required to be kept as a backup source for DNA extraction if insufficient DNA is extracted from the initial Specimen. The molecular genetics Laboratory performing the test should be consulted before culture disposal.

C2.1(ii) The possibility of maternal cell contamination, pseudomosaicism, true mosaicism and in vitro aberrations should be recognised, and the systems of culture and analysis used should be designed to detect and differentiate these.

C2.1(iii) Where adequate Specimen is available, duplicate or independently established cultures are recommended for all Specimen types.

S2.2 Each prenatal Specimen must be divided and cultured in two separate incubators, running on different electrical circuits if possible, and maintained with independent cell culture media and other reagents.

Chorionic villus

S2.3 Chorionic villus Specimens must be dissected free from decidua to reduce the chance of maternal cell contamination.

S2.4 All chorionic villus studies must include analysis of long-term cultures.

Fetal blood

S2.5 The Laboratory must ensure that fetal blood is tested to identify maternal blood contamination.

Bone marrow and tumour

In haematological and malignancy cultures, the likelihood of obtaining high-quality results should be optimised by using direct, short-term and synchronised cultures where practical.

When a B- or T-cell lymphoproliferative disorder is suspected, suitable mitogens should be used in additional cultures. Solid tumour cultures may require multiple and longer term cultures.

Chromosome instability syndromes

The rarity of chromosome instability syndromes requires that inexperienced Laboratories should refer cases to Laboratories with experience in diagnosing such disorders.

- a) Clastogen studies should only be undertaken using appropriate negative control Specimens (and positive control material if available).
- b) Control and test Specimens should be collected, processed, cultured and harvested in parallel.
- c) Where possible, controls should be appropriately matched and take into account sex, age, cigarette smoking and intercurrent illness.
- d) The Specimens should be analysed in a blinded fashion.
- e) Sufficient numbers of metaphases should be examined to verify the significance of detected chromosomal damage.

Environmental monitoring

Cytogenetics has been used as an adjunct to environmental monitoring by detecting chromosomal damage. This may be seen as increases in breakage, stable rearrangements or sister chromatid exchanges. Such testing is controversial and there is little scientific literature to justify its clinical use.

3. Chromosome analysis

(Refer to Standard 6 in *Requirements for Medical Pathology Services*)

General

- Hyper-, hypo- and pseudodiploid cells should be fully analysed unless mosaicism/clonality is established.

The total number of metaphases can be reduced to five in cases where a specific nonmosaic abnormality is being excluded or confirmed (e.g. a known familial translocation) or when confirming a previously detected trisomy. All these metaphases should be fully analysed.

- Coordinates of counted and analysed cells should be recorded to enable review of these cells as required.
- The quality of a chromosomal analysis depends on:
 - (a) the analysis of sufficient cells to adequately establish the true chromosome constitution
 - (b) the use of appropriate techniques to characterise an abnormality
 - (c) the production of cells with high-quality banding to allow resolution of bands appropriate to the likely type of abnormality
 - (d) the observational skill of the analyst.

Numbers of cells to be studied

- In general, routine cytogenetic analysis should consist of a minimum of:
 - (a) 5 banded cells analysed
 - (b) 10 cells counted [in addition to (a)].

Two of the fully analysed metaphases from (a) should be archived as images.

In cases where clinically relevant mosaicism is suspected, counting, analysis or scoring of at least 30 cells is recommended to exclude mosaicism of 10% at the 0.95 confidence level.²

For cancer cytogenetics, if no chromosomal abnormality is found in the first five cells analysed, then a further five cells are to be fully analysed and an additional 10 cells are to be counted.

FISH analysis may be the most appropriate method of confirming mosaicism or clonality if a suitable probe is available.

Prenatal studies

S3.1 Where analysis is performed on subcultured cells or suspension harvests, the analysis of prenatal cultures must be derived, where possible, from at least two independent cultures. As a minimum, five banded cells must be analysed.

S3.2 Where analysis is performed on primary colonies and sufficient colonies are available, no more than two cells must be counted and/or analysed from a single colony. If discrepant analysis is detected between two cells in a single colony, then further analysis of that colony is required.

C3.2(i) For in situ preparations, cells chosen should be selected from as many different colonies as are available, ideally representing at least two independent cultures.

C3.2(ii) If there are insufficient colonies or if only one culture is analysed, then a comment to this effect should be made in the report.³

C3.2(iii) A written procedure for delineating different types of mosaicism should be available within the Laboratory, noting that individual cases require careful assessment and discussion, and that the numbers of cells counted and analysed may need to be greater than the minimum guidelines.

C3.2(iv) The in situ colony method is recommended to facilitate the elucidation of mosaicism and in vitro abnormalities.

Acquired disorders (bone marrow, malignant tissue)

Where there is evidence of clonal evolution, sufficient cells should be examined to elucidate this. Since abnormal cells are often of poorer morphology than normal cells, this should be taken into account when selecting cells for analysis.

Banding methods

S3.3 Banding studies using G-bands must be routine in all karyotyping.

S3.4 Copies of the most recent International System for Human Cytogenetic Nomenclature (ISCN) must be readily available in the Laboratory.

C3.4(i) The ISCN defines levels of banding. These can be used as a guide for establishing the degree of resolution achieved in producing a result.

C3.4(ii) The ISCN level may be identified in the patient's report. An acceptable degree of resolution will vary with the abnormality expected.

³ See Gardner and Sutherland (2004). Mosaicism in prenatal diagnosis is dealt with extensively in Chapter 25.

- C3.4(iii) Additional banding/chromosome identification methods, such as R, Q, C, Ag-NOR, distamycin A and DAPI (4', 6'-diamidino-2'-phenylindole), should be available for appropriate cases.

Verification of chromosomal analysis

S3.5 A second experienced cytogeneticist must check all cases.

- C3.5 A second experienced cytogeneticist should check all cases by analysing every chromosome, band by band, in at least two complete cells, either by direct microscopy, photographs or digital images.

Karyotyping

S3.6 All cases must have at least two karyotypes/images prepared and archived as part of the patient's Laboratory record to allow retrieval of sufficient information to confirm the result.

Use of molecular techniques

S3.7 Validated molecular techniques must be used where there is proven benefit compared with conventional cytogenetics techniques.

S3.8 Fragile X testing must be carried out by molecular methods rather than cytogenetic methods.

4. Fluorescence in situ hybridisation

(Refer to Standard 4, Standard 5 and Standard 6 in *Requirements for Medical Pathology Services*)

Fluorescence in situ hybridisation (FISH) techniques

S4.1 Most established FISH methods would usually be within the routine repertoire of the diagnostic Laboratory, using commercially available probes. If not, then the Laboratory must have documented policies and procedures for referral of Specimens for FISH analysis.

C4.1(i) Routine FISH cytogenetics techniques include:

- chromosome painting
- identification of telomeric, subtelomeric and centromeric regions
- interphase analysis for aneuploidy
- locus-specific identification for microdeletion and other syndromes
- locus-specific hybridisation to demonstrate chromosomal rearrangements.

C4.1(ii) The Laboratory dealing with haematological referrals should be able to undertake:

- rearrangement analysis, using locus-specific probe combinations, and
- interphase analysis for the detection of low-level clones and graft/host chimaerism.

C4.1(iii) It is helpful to use hybridisation systems that include a control probe to tag the chromosome of interest. Such probes also afford a limited level of quality control by providing an internal control for the efficiency of the FISH procedure. For the detection of translocations in interphase nuclei, a probe set that gives an extra signal or double signals should be used, whenever possible.

S4.2 Where FISH is performed on any Specimen other than a standard cytogenetic Specimen (e.g. paraffin-embedded tissues, blastocyst/embryo biopsies), the Laboratory must ensure that it has the skills, expertise and collaborative and supervisory arrangements to perform and fully interpret the findings.

FISH analysis

S4.3 Sufficient numbers of metaphases, interphases or nuclei from cultured or uncultured cells must be analysed to ensure the statistical validity of the result. Signals must be scored by two independent analysts.

- C4.3(i) When used as the first line of analysis, the following minimum levels apply:
- For locus-specific probes, 10 cells should be scored to confirm or exclude an abnormality.
 - For prenatal interphase screening for aneuploidy, signals should be counted in a minimum of 50 cells for each probe.
 - For interphase screening for mosaicism or malignant clones, a minimum of 200 cells should be scored.
- C4.3(ii) While robust checking systems are still evolving, it is essential that all FISH analysis be independently checked. When used to confirm or extend the interpretation of abnormalities previously identified by other methods, it is necessary to score only three cells to confirm the abnormality and to have the interpretation checked.
- C4.3(iii) The number of nuclei examined will depend on the analytical sensitivity of the probe used and the confidence level required. It will also depend on the nature of the Specimen examined (i.e. constitutional or oncology). When determining cutoff values, reference can be made to Dewald et al (1998) and relevant documents relating to measurement of uncertainty.
- C4.3(iv) Scoring large numbers of metaphase or interphase cells for specific rearrangements increases the accuracy with which low-level clones can be identified and may permit the identification of cells that cannot be induced to divide in culture.
- C4.3(v) In the majority of malignancy investigations, in situ hybridisation will be undertaken in conjunction with karyotype analysis. The circumstances of each case and the results of any previous analysis must be taken into account when determining the appropriate techniques to be applied.

5. Laboratory performance

(Refer to Standard 2 and Standard 7 in *Requirements for Medical Pathology Services*)

Measures of performance

Based on success rates calculated from data submitted to the HGSA/Australasian Society of Cytogeneticists quality assessment scheme, the following figures provide a reasonable basis for estimating acceptable performance (expressed as the percentage of times when an answer is obtained):

- | | |
|-------------------------------|-----|
| a) amniotic fluid Specimens | 99% |
| b) chorionic villi Specimens | 98% |
| c) peripheral blood Specimens | 98% |
| d) bone marrow Specimens | 85% |

6. Reports

(Refer to Standard 6C in *Requirements for Medical Pathology Services*)

Reporting times

The decision to request recollection of a prenatal Specimen should be made in no more than 10 days.

A component of high-quality service involves producing results within a reasonable time. Adequately staffed Laboratories produce 90% of results within the times indicated:

Lymphocyte cultures	18 days
Bone marrow and tumour cultures	18 days
Amniotic fluid cultures	15 days
Chorion biopsies	15 days
Tissue cultures	28 days
Urgent lymphocyte, bone marrow or cord blood cultures	5 days

S6.1 Reports must include the following items:

- (a) the number of cells counted and metaphases analysed
- (b) the banding techniques applied (if the banding resolution is less than adequate for the referral and Specimen type, this must be stated; see Appendices A and B)
- (c) whether specific studies were undertaken (e.g. FISH studies)
- (d) the chromosome constitution using the most recent ISCN nomenclature.

C6.1(i) In general, reports of non-malignant tissues should only include true mosaicism.

C6.1(ii) A comment should be made if maternal cell contamination is detected in a prenatal culture.

C6.1(iii) Deciding what constitutes a non-clonal aberration is not straightforward (for guidance, see the current edition of ISCN, especially regarding determination of clonality in the cytogenetics of neoplasia.) To reach a decision on such matters, the application of general rules needs to be balanced by consideration of the specific clinical context.

- C6.1(iv) Similarly, in the reporting of prenatal studies, mosaicism is not relevant if the abnormal cells are regarded as having arisen during in vitro culture. Such origins indicate pseudomosaicism only. The alternative in vivo origin indicates a true mosaicism, which may involve either the foetus itself or the extra-embryonic membranes (or both). The distinction between such in vitro and in vivo origins of abnormality is problematic and requires very careful consideration of the cytogenetic data, the clinical context and the cell culture strategy used.
- C6.1(v) Normal chromosomal variants should not be reported as part of the ISCN description of the karyotype, but should be noted in the patient's Laboratory record.
- C6.1(vi) Normal variants that do not involve breakage and reunion of chromosomes are continuously variable (e.g. heterochromatin size, satellite size and fluorescent intensity), so assessment is, to a certain extent, subjective. 'Pericentric inversions' of heterochromatin are also variable.

7. Records

(Refer to Standard 6C in *Requirements for Medical Pathology Services*)

Records of images and worksheets

S7.1 Images must be duplicated and spatially separated for storage.

- C7.1 As a minimum, two banded metaphases **must** be kept, either as slides, as photographic negatives or as stored digital images where it can be demonstrated that the material will not degrade over the required period.

Records of FISH analysis

Records should identify the source and identification of the probe or primer, the number of cells scored and detailed hybridisation results.

Appendix A Assessment of banding quality of cytogenetics slide preparations (Informative)

Banding points	ISCN bands per haploid set	Examples
0		Unequivocal chromosome pairing is not possible
2	150	Can distinguish 8s from 9s Can distinguish 4s from 5s
4	400	Two distinct dark bands in 8p Two distinct dark bands in 9p Three distinct dark bands in 5q (5q14, 5q21, 5q23)
6	550	Four distinct dark bands in 18q 10q21, 10q23, 10q25 split 7q33 and 7q35 are clearly distinct 22q13.2 is visible
8	850	4p15.3 splits 5p15.32 is clearly visible 10q11.22 is clearly visible 11p14.1 should resolve from 11p14.3 20p12.1 and 20p12.3 are clearly visible

ISCN = International System for Human Cytogenetic Nomenclature

In practice, not all examples will appear simultaneously, and intermediate banding scores of 1, 3, 5 and 7 can be used to describe metaphases that do not fulfil all the criteria for even scores. This will be especially relevant for assessing metaphases between 550 and 850 bands.

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Appendix B Recommended minimum banding quality (Informative)

The recommended scores given below are defined as the lowest standard acceptable for a given reason for referral without issuing a qualified report.

Reason for referral	Banding points
Routine prenatal diagnosis (e.g. for age or biochemical pre-screens)	4
Aneuploidies and known large structural rearrangements	4
Expected small structural rearrangements, including their prenatal diagnosis	4
Possible small unknown structural anomalies (e.g. recurrent abortion, dysmorphic features, delayed development)	6
Microdeletion syndromes (FISH is the preferred method of analysis where available)	7

FISH = fluorescence in situ hybridisation

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Further information

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