# ACS GUIDELINE FOR CLINICAL FLOW CYTOMETRY LABORATORY PRACTICE

## Fourth Edition 2025

Australasian Cytometry Society Guideline Document

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Second edition 2017 renamed and reformatted to be read '*ACS Guideline for Clinical Flow Cytometry Laboratory Practice*'.

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The Australasian Cytometry Society (ACS) was established in 1979 and incorporated in 1992 with the aim of promoting research, development and applications in, and to disseminate knowledge of flow cytometry.

A function of the ACS is to assist with development and application of clinical flow cytometry applications for hospitals and laboratories in the diagnosis and treatment of disease. This includes the preparation of guidelines and education programs.

Guidelines produced by the ACS are issued as reference material to provide laboratories and accrediting agencies with minimum requirements for testing considered acceptable for good laboratory practice.

Failure to follow these guidelines may pose a risk to public health and patient safety.

## SCOPE

The '*Guidelines for Clinical Flow Cytometry Laboratory Practice'* is an ACS document to be read in conjunction with the NPAAC Standards document *'Requirements for Medical Pathology Services*' or IANZ equivalent. 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 NPAAC Standards document, reference to specific Standards in that document are provided for assistance under the headings in this document.

The NPPAC Requirements encompass:
• documentation and accurate patient identification systems to minimise clerical errors and misidentification
• retention of records, data and documentation
• requirements necessary for the use of computers and computer software in clinical flow cytometry Laboratory practice
• quality assurance and quality control programmes for reagents, techniques and personnel

The '*Guidelines for Clinical Flow Cytometry Laboratory Practice*' document is a consensus recommendation by ACS members and associates for best medical laboratory practice for a procedure, method, staffing resource or facility. It encompasses any measures, procedures or considerations unique to operation of a clinical flow cytometry laboratory where they differ from a routine clinical pathology laboratory. It is a guideline not a standard.

The '*Guidelines for Clinical Flow Cytometry Laboratory Practice'* document is for use by clinical flow cytometry Laboratory personnel for the purpose of clinical flow cytometry testing and safe laboratory practice. In house departmental laboratory procedural and quality control documentation e.g. Haematology or Immunology Departments should be read in conjunction with this ACS document.

For details on procedural matters and methodologies readers are directed to the Reference List at the end of this document for ACS recommended published peer reviewed texts, articles and guidelines. Definitions given above are sourced from NPAAC.

## ABBREVIATIONS

| | |
|---|---|
| FCS | means flow cytometry standard, an electronic data file standard for the reading and writing of data from flow cytometry experiments |
| IVD | means in vitro diagnostic |
| IANZ | means International Accreditation New Zealand, accreditation body of the Testing Laboratory Registration Council of New Zealand |
| NPAAC | means National Pathology Accreditation Advisory Council |
| RMPS | means Requirements for Medical Pathology Services, Tier 2, NPAAC Standard |
| 7AAD | 7 Amino actinomycin D, a viability marker with DNA specificity |

## DEFINITIONS

| | |
|---|---|
| CD | Cluster of differentiation number used to classify cell surface molecular targets for immunophenotypic identification of cells e.g. CD3 for the pan T cell antigen |
| Cocktail | means an antibody reagent test mixture pre-prepared for use over the period of time validated. |
| Competent clinical flow cytometrist | means a person who has a minimum of two years clinical flow cytometry experience, and who has been documented to be competent in clinical flow cytometry according to the Laboratory's Quality System |
| Clinical flow cytometry supervisor | means a person who has a minimum of five years clinical flow cytometry experience, and who has been documented to be competent in clinical flow cytometry according to the Laboratory's Quality System |
| External Quality Assessment (EQA) | means a program in which multiple specimens are periodically sent to members of a group of Laboratories for analysis and/or identification, in which each laboratory's results are compared with those of other laboratories in the group and/or with an assigned value and reported to the participating Laboratory and others. Such a program may also compare an individual's results with their peer group. |
| Flow test | means a test carried out by flow cytometry |
| Guideline | means a consensus recommendation for best medical laboratory practice for a procedure, method, staffing resource or facility |

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| Guidelines for Clinical Flow Cytometry Laboratory Practice | means the overarching document broadly outlining standards for good clinical flow cytometry laboratory 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. |
| In vitro diagnostic medical device (IVD) | means a medical device test if it is a reagent, calibrator, control material, kit, specimen receptacle, software, instrument, apparatus, equipment or system, whether used alone or in combination with other diagnostic goods for in vitro use.<br>It must be intended by the manufacturer to be used in vitro for the examination of specimens derived from the human body, solely or principally for the purpose of giving information about a physiological or pathological state, a congenital abnormality or to determine safety and compatibility with a potential recipient, or to monitor therapeutic measures.<br>The definition of an IVD does not encompass products that are intended for general Laboratory use that are not manufactured, sold or presented for use specifically as an IVD. |
| In-house IVD | means an IVD that is developed de novo, or developed or modified from a published source, or developed or modified from any other source, or its intended purpose, within the confines or scope of a Laboratory or Laboratory network and is not supplied for use outside the Laboratory or Laboratory network.<br>Commercial IVDs being used clinically for a purpose other than that originally intended by the manufacturer are also classed as in-house IVDs and are subject to the requirements of this standard. |
| Marker | means an antibody directed to an antigen of interest in or on a cell used for diagnostic purposes |
| Quality Assessment | means a measurement and monitoring function of quality assurance for determining how well health care is delivered in comparison with applicable standards or acceptable bounds of care. |
| Quality Assurance | means part of quality management that involves a planned and systematic set of activities focused on providing confidence that quality requirements will be fulfilled and correct results are reported. |
| Quality control | means the study of those errors that are the responsibility of the Laboratory, and the procedures used to recognise and minimise them. This study includes all errors arising within the Laboratory between the receipt of the |

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| | Specimen and the dispatch of the report. On some occasions, the responsibility of the Laboratory may extend from the collection of the Specimen from the patient and the provision of a suitable container to the dispatch and delivery of the report.<br>Internal quality control:<br>means processes and activities that are used within the Laboratory to monitor the day-to-day operational and analytical performance of test procedures. These activities may include on-going instrument standardisation checks, instrument maintenance, analysis of control material, statistical or graphical assessment of results from control material. |
| Requirements for Medical Pathology Services (RMPS) Third Edition 2018 | 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.<br>The standard headings are set out below –<br>Standard 1 – The Role of Standards in the Australian Pathology Accreditation Framework<br>Standard 2 – Ethical Practice<br>Standard 3 – Risk Management<br>Standard 4 – Clinical Governance<br>Standard 5 – Quality Management<br>Standard 6 – Personnel<br>Standard 7 – Facilities and Equipment<br>A – Premises<br>B – Equipment<br>Standard 8 – Request-Test-Report Cycle<br>A – Pre-Analytical<br>B – Analytical<br>C – Post-Analytical<br>Standard 9 – Send away tests<br>Appendix A – Risk Assessment – Risk Points (Normative) |
| stain | means bind monoclonal antibodies to markers on cells of interest to |
| standard | means a minimum requirement for a procedure, method, staffing resource or laboratory facility that is required before a laboratory can attain accreditation |

# INTRODUCTION

This ACS document is intended to be used in clinical flow cytometry Laboratories to provide guidance on good practice in relation to flow cytometry and to assist assessors carrying out Laboratory accreditation assessments.

These Guidelines are intended to serve as consensus recommendations for best medical laboratory practice, have been developed by ACS members and associates with reference to other guidelines as published in peer reviewed journals. The ACS is the pre-eminent specialist society in clinical flow cytometry Laboratory practice in Australasia and New Zealand and has developed several guidelines relevant to this document.

These are Guidelines and not Standards. These Guidelines should be read in conjunction with the current version of the NPAAC Standards Tier 2 document *'Requirements for Medical Pathology Services*' or IANZ equivalent.

For clarification, Standards are described:

• A Standard is the minimum requirement for a procedure, method, staffing resource or laboratory facility that is required before a laboratory can attain accreditation. The use of the verb 'must' in standards indicates mandatory requirements for pathology practice.

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

• A Guideline is a consensus recommendation for best medical laboratory practice for a procedure, method, staffing resource or facility. Guidelines are prefaced with a 'G' (e.g. G2.2**)**. The use of the word 'should' in each Guideline within this document indicates a recommendation for good pathology practice.

• A Commentary may be provided to give clarification to the Guidelines 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.

Appendices if attached to this document are informative**,** that is explanatory in nature and may provide examples or information of a clinical nature and should be considered to be an integral part of this document.

Note: APC documents can be accessed at www.cytometry.org.au

# 1. PRE ANALYTICAL PHASE

Refer to NPAAC document *'Requirements for Medical Pathology Services'* for additional standards relating to minimum specimen labelling requirements, request forms, laboratory records, collection and transport conditions.

## 1.1 Specimen Collection and Storage

**For flow cytometry analysis appropriate anticoagulant and storage be used according to sample type and disease investigation[1,2,3]. Refer to assay specific ACS guidelines for further information.**

## 1.2 Specimen Transport

Refer to assay specific ACS test guidelines for transport, storage and rejection criteria conditions.

### G1.2 Specimen Viability

**Non-viable cells are a significant source of false positive staining. Viability testing is recommended for some tests on samples more than 24 hours after collection or if there is obvious deterioration of the sample[2,4].**

C1.2 Viability can be tested using 7AAD[2,5] or trypan blue[6]. Propidium iodide is not recommended due to instrument contamination and carryover.

## 1.3 Specimen Check

**G1.3.1 Primary collection samples must have the patients name and two identifiers (e.g. date of birth, medical record number, test/accession number), including collection date, sample type and/or collection site.**

C1.3.1 Secondary sample/assay tubes should must have patient name or part thereof and at least one identifier. Barcode alone is acceptable where this information can be extracted.

**G1.3.2 A protocol for return unlabeled/mismatch specimens according and criteria for rejection should be followed as described in the laboratory department manual.**

## 1.4 Antibody Reagents

**G1.4.1 New antibodies introduced into the laboratory should be correctly validated by clinical correlation[2,3].**

C1.4.1(i) Antibodies should be tested on a commercial reagent control or appropriate sample where available to determine whether they can be used to measure the level of antigen.

C1.4.1(ii) New Lot number and delivery of reagents should be tested where control reagents or suitable blood samples are available.

C1.4.1(iii) New Lot numbers, delivery date of reagents, expiry dates and in use dates should be recorded for each reagent.

**G1.4.2 Reagents should not be used beyond expiry dates.**

G1.4.2 (i) Laboratories must have a procedure in place to ensure validity if expired reagents are used

C1.4.2(ii) Reagent fluorescence intensity and marking may vary significantly beyond expiry.

**G1.4.3 Cocktail antibody preparations for flow tests should be correctly validated and each lot tested prior to use[1,2,3].**

**G1.4.4 Laboratories should adhere to manufacturer's instructions consistent with IVD guidelines.**

G1.4.4(i) Laboratories must have a procedure in place to ensure validity of a new reagent clone, manufacturer or catalogue number.

G1.4.4(ii) Laboratories must have a procedure in place to ensure validity of a deviation from manufacturer's instructions, including optimal reagent titration.

C1.4.4(iii) Excess antibody may lead to increased non-specific binding and quenching of fluorescence signals[4].

## 2. ANALYTICAL PHASE

Refer to NPAAC document '*Requirements for Medical Pathology Services'* for additional standards relating to sample analysis and performance measures.

### 2.1 Sample analysis

**G2.1.1 Sample preparation for any cellular based assay should seek to minimise manipulation of cells and maximise preservation of viability and antigen integrity.**

**G2.1.2 Appropriate software and gating should be used for individual flow tests[2,6,7].**

## 2.2 Performance Measures

**G2.2.1 Analysers should be set up, monitored, maintained and documented using appropriate material, and reviewed by a clinical flow cytometry supervisor[1,2,3].**

C2.2.1(i) Individual assay settings should be monitored and reanalyzed as needed e.g. daily QC beads for analyser performance, assay settings[2,3,4].

G2.2.1(ii) Variation from manufacturer's advice for use of instrumentation and reagents must follow current recommendations for IVD medical device and In-house IVDs.

**G2.2.2 A control should be prepared and run on a regular basis in parallel with patient samples where appropriate. A positive (and/or negative) reagent control should be run either daily or with each assay performed[3].**

C2.2.2 For some assays e.g. Lymphoma and Leukaemia screening, it is not practical or necessary to analyse a normal control sample on a daily or weekly basis if the laboratory is active and within-run positive and negative control results demonstrate appropriate reactivity.

**G2.2.3 Where absolute numbers (e.g. cells/uL) are reported, a control reagent should have specified ranges for the analytes measured, and reasons for deviations determined[3].**

**G2.2.4 A minimum number of target cellular events should be collected according to the flow test, reporting style suited (quantitative or qualitative) and criteria for rejection determined for individual flow tests[3].**

**G2.2.5 Sensitivities for each assay should be calculated, documented and reviewed when new assays are setup or when alterations to assay methods, technologies or reagents change[1,3].**

C2.2.5 Sensitivity data (eg Limits of Detection) should be included in reporting where appropriate[3].

**G2.2.6 Each laboratory should establish reference limits for antigens being tested where appropriate.**

## 3. POST ANALYTICAL PHASE

Refer to NPAAC document *'Requirements for Medical Pathology Services'* for additional standards relating to reports, record keeping, result validation.

### 3.1 Reports

**G3.1.1 Interpretation and report comments should be made and verified by a competent clinical flow cytometrist or suitably trained medical officer.**

**G3.1.2 Where quantitative (numerical) results are given, reference ranges should be provided where appropriate[2,3,8].**

**G3.1.3 Reports should be completed in a timely manner and in a time commensurate with clinical need. Depending on the test this should be less than a few hours e.g. CD34 pre harvest counts to no longer than 5 working days for haematology oncology reports.**

C3.1.3 Adequately trained staff and resources must be provided to meet these targets.

**G3.1.4 Measurement of uncertainty (MoU) should be calculated for flow cytometry assays according to the nature of the test performed.**

C3.1.4 Estimates of MoU should be made for all measurements commensurate with the quality requirements of the clinical application of the results. This does not apply to qualitative tests if they are not derived from a numerical value. MoU should be estimated for parts of procedures that generate numerical values eg where cut-offs are used. MoU should be estimated periodically eg annually. Individual ACS guidelines will make reference to estimation of MoU with examples given where appropriate [9,10].

## 3.2 Data Storage

**G3.2 Electronic data (FCS) should have backup copies and storage for the period required by NPAAC, IANZ.**

C3.2(i) FCS data should be traceable and include sample identifiers such as patient details, date and sample type[3,4].

C3.2 (ii) FCS files should be traceable, matching correctly to individual samples from a patient[2,3].

C3.2 (iii) Use of barcoding and reference to worklist data may help reduce error.

## 3.3 Laboratory Staff

**G3.3.1 Staff in a flow cytometry facility need a high level of skill to perform a unique variety of clinical tests using specialised equipment and software. There should be documented evidence staff are given sufficient time and resources to achieve and maintain their skills [(2)].**

C3.3.1(i) A competent flow cytometrist able to work unsupervised at all levels should have a minimum of two years full time equivalent clinical flow cytometry experience and have documented competency in clinical flow cytometry according to the Laboratory's Quality System[(3,4)].

C3.3.1 (ii) A sufficient number of cases studied over a given period is required to maintain proficiency [(1)]. This includes laboratory and medical staff.

C3.3.1 (iii) Staff reporting results should be annually tested for proficiency in use of gating software and interpretation of results[(3)].

**G3.3.1 Clinical flow cytometry laboratories should be supervised by a scientist with demonstrated experience in all aspects of flow cytometry testing, interpretation and quality management.**

C3.3.1 A clinical flow cytometry supervisor is able to oversee all aspects of the set up and running of a clinical flow cytometry laboratory including EQA, staff training, assay development and reporting.

They should have a minimum of 5 years full time equivalent clinical flow cytometry experience and have documented competency according to the laboratory's quality system.

## REFERENCES CITED


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### NPAAC Reference Documents

*Requirements for Medical Pathology Services,* Third Edition, 2018
Print ISBN: 978-1-76007-390-9, Online ISBN: 978-1-76007-389-3, Publications approval number: 12374

*Regulatory Requirements for In-house IVDs*, Version 2.2, September 2018, Australian Government, Department of Health, Therapeutic Goods Administration

*Requirements for the Development and Use of In-house In Vitro Diagnostic Devices (IVDs*,)
Fourth Edition 2018
ISBN: 978-1-76007-340-4; Online ISBN: 978-1-76007-339-8; Publications Approval Number: 11985

## PROCEDURAL REFERENCES

Further detail outside the scope of this document can be found in the references listed above to assist with further detailed descriptions of setting up and maintaining flow cytometry assays, instrumentation and quality systems.

**Review Committee**

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Further ACS clinical flow cytometry guidelines documents are available on the website: www.cytometry.org.au

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| Revision | Change Summary | Active Date | Review Committee Chair |
|---|---|---|---|
| First Edition | • First Edition | 2006 | Beth Rees |
| Second Edition | • Second Edition | 2017 | Neil McNamara |
| Third Edition | • Updated abbreviation. FCS<br>• Updated definition. Clinical Flow Cytometry Supervisor (adding C3.3.1)<br>• Minor miscellaneous corrections<br>• G1.4.2. Use of expired reagents<br>• G1.4.4. Manufacturer instructions for antibody reagents<br>• C3.1.1(iii) FC supervisor description<br>• Updated references and reviewers | 2020 | Neil Came |
| Fourth Edition | • G1.4.2 Use of expired reagents<br>• G1.4.4 Reagent titration<br>• C2.2.5 Reporting sensitivity data<br>• Measurement of Uncertainty | 2025 | Neil McNamara |