

**ACS GUIDELINE FOR**

**HAEMATOLOGY ONCOLOGY**

**IMMUNOPHENOTYPING**

**Second Edition 2017**

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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 ACS '*Guideline for Leukaemia Lymphoma Immunophenotyping*' is an ACS document to be read in conjunction with the ACS document '*Guidelines for Clinical Flow Cytometry Laboratory Practice*'. The latter overarching document broadly outlines guidelines 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.

This document is for use in laboratories providing clinical flow cytometry services.

## ABBREVIATIONS

ACD	Acid Citrate Dextrose
CSF	Cerebrospinal fluid
EDTA	Ethylene-diaminetetraacetic acid
WBC	White Blood Cells
RPMI	Roswell park Memorial institute medium, a sample preservative
Hanks	Hank's balanced Salt Solution, a sample preservative
FNAB	Fine Needle Aspirate Biopsy

## DEFINITIONS

CD	Cluster definition number used to identify individual markers eg CD3 for the pan T cell antigen
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
count	means to acquire data on a flow cytometer
Guideline	means a consensus recommendation for best medical laboratory practice for a procedure, method, staffing resource or facility
Guidelines for Clinical Flow Cytometry Laboratory Practice (GCFCLP)	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.
marker	means an antibody directed to an antigen of interest in or on a cell used for diagnostic purposes

## INTRODUCTION

This ACS document, together with '*Guidelines for Clinical Flow Cytometry Laboratory Practice*', 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.

These are Guidelines and not Standards. These Guidelines should be read in conjunction with the current version of the ACS '*Guidelines for Clinical Flow Cytometry Laboratory Practice*'. For clarification Standards are described as:

- 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: ACS documents can be accessed at: [www.cytometry.org.au](http://www.cytometry.org.au)

## **1. PRE ANALYTICAL PHASE**

Refer to ACS 'Guidelines for Clinical Flow Cytometry Laboratory Practice' for additional information regarding minimum specimen labelling requirements, request forms, collection and transport conditions.

### **G1.1 Specimen Collection and Storage**

For flow cytometry analysis appropriate anticoagulant and storage be used according to sample type and disease investigation.

#### **G1.1.1 For peripheral blood, bone marrow aspirates EDTA, lithium/sodium heparin or ACD solution may be used according to test procedures <sup>(1; 2)</sup>.**

C1.1.1(i) EDTA samples are better tested up to 48 hours, lithium heparin 48 to 72 hours <sup>(1; 2; 3)</sup>, ACD up to 72 hours <sup>(1)</sup>.

C1.1.1(ii) Lithium/sodium Heparin is preferred as sample integrity is preserved for a longer period. EDTA is useful as it allows morphologic assessment and FBC differential testing. ACD is not recommended for bone marrow aspirates where incorrect sample to anticoagulant ratio can alter pH and reduce cell viability <sup>(1)</sup>.

#### **G1.1.2 Peripheral blood, bone marrow aspirates can be stored at 18-25°C <sup>(1; 2)</sup>.**

C1.1.2 For B-cell clonality and myeloid studies storage at +4°C is recommended <sup>(2)</sup>.

#### **G1.1.3 Tissue biopsies in isotonic medium (such as phosphate buffered saline, Hanks, or RPMI) usually do not require anticoagulant. Specimens should be immersed in appropriate medium immediately on collection <sup>(1)</sup>.**

#### **G1.1.4 CSF should be processed as soon as possible, ideally not more than eight hours after collection. CSF usually does not require anticoagulant and may**

**benefit from stabilizing reagents<sup>(1; 3)</sup>.**

**G1.1.5 Other fluids (e.g. pleural, FNAB): Anticoagulants are not necessary. EDTA, Lithium/sodium heparin may be used. ACD is not recommended<sup>(3)</sup>. Fluids should be tested within 24 h and up to 72 hours if stored at 4°C.**

**G1.1.6 The sample type and site and time of collection should be provided on the sample tube/container along with patient identifiers. This information should be included in the final report.**

**G1.1.7 A total white cell count and differential on all peripheral blood samples should be performed at the laboratory initiating the request. For distant laboratories and dispatch centers a white cell count, differential and unstained blood film should accompany each specimen.**

## **G1.2 Specimen Transport**

**Samples must be delivered to the laboratory as soon as possible to minimise loss of cell viability.**

C1.2(i) Tissue samples should be transported in tissue culture media for transport at 18-22°C. Where transport media is not used, store in saline or similar at 4°C and analyse within 24 hrs.

C1.2(ii) All other specimens should be maintained at 18 to 22°C in a leak proof container. Temperatures below 4°C and above 30°C must be avoided<sup>(1)</sup>.

C1.2(iii) Viability testing is recommended for some tests on samples more than 24 hours after collection or if there is obvious deterioration of the sample. Non-viable cells are significant source of false positive staining.

C1.2.(iv) Delay in sample testing should be indicated in the final report.

### **G1.3 Test Requests**

**Requests for leukaemia/lymphoma testing should include any relevant clinical observations and history to assist with appropriate screens to be undertaken.**

C1.3(i) It is critical the correct screen is performed particularly where rare or unusual diagnosis are to be considered.

C1.3(ii) Previous abnormal immunophenotypes should be noted either on the test request or by search of laboratory records. This is to ensure relevant markers are investigated for residual disease/relapse.

### **G1.4 Antibody Reagents**

**Fluorescent marker antibodies used in panels should be validated by clinical correlation following IVD standards.**

C1.4(i) Leukaemia/lymphoma *screens* should include CD45 <sup>(4)</sup>.

C1.4(ii) Lymphoma *screens* should include as a minimum markers for

- B cells: CD19, CD20, CD10, CD5, kappa/lambda light chains
- T/NK cells: CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD56 <sup>(4)</sup>.

C1.4(iii) Leukaemia *screens* should include as a minimum markers for:

- Maturation: CD45, CD34, CD10, CD117.
- Myeloid lineage: CD13, CD14, CD33, CD64, HLADR
- Lymphoid lineage: CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD19, CD20, CD56, kappa/lambda light chains <sup>(4)</sup>

C1.4(iv) Intracytoplasmic markers: MPO, CD79a, CD3 may assist with lineage definition for leukaemia screens.

C1.4(iv) Additional markers should be tested to assist diagnosis following the current WHO Leukaemia/Lymphoma Classification <sup>(5)</sup>.

## **G1.5 Sample Preparation**

**Samples should be prepared with total white cell count  $\leq 20 \times 10^9/L$  <sup>(6)</sup>. If higher consider diluting the sample to a cell concentration to this range in isotonic medium.**

C2.1.1(i) This is assuming antibodies for lymphocytes were titred to saturate target antigens usually at  $1 \times 10^6$  cells in 100 uL of sample <sup>(6)</sup>. Refer to manufacturers cell count ranges at which assays are validated.

C2.1.1(ii) For paucicellular samples, buffy coat preparations may be useful.

## **2. ANALYTICAL PHASE**

Refer to ACS 'Guidelines for Clinical Flow Cytometry Laboratory Practice' for additional information regarding sample analysis and performance measures.

### **G2.1 Sample analysis**

**G2.1.1 A minimum of 5,000 cellular events in the target gate (e.g. lymphocytes, blasts) should be acquired where possible.**

C2.1.1 Acquisition of a minimum of 5,000 events of any cell type is required to achieve a sensitivity of 2% (for 100 target events). To achieve an intra-assay CV of 10% a minimum of 100 target events would be required. Laboratories need to assess the desired sensitivity of assays performed <sup>(2)</sup>.

**G2.1.2 Secondary sample/assay tubes should have patient name or part thereof and at least one identifier. Barcode alone is not acceptable.**

**G2.1.3 Analysis should be performed using CD45, FSC and SSC parameters.**

C2.1.3(i) Side scatter linear or logarithmic can be used. Logarithmic may be more useful for leukaemia analysis.

C2.1.3(ii) It is recommended event count versus time be recorded to monitor fluidics, and FSC Area versus Height gated to exclude doublets <sup>(2)</sup>.

**C2.1.4 Analysis should be performed by a competent flow cytometrist with documented competency in clinical flow cytometry analysis of leukaemia and lymphoma.**

C2.1.4(i) A sufficient number of leukaemia and lymphoma cases should be studied by laboratory and medical staff over a given period to maintain proficiency <sup>(1)</sup>.

C2.1.4(ii) All leukaemia, lymphoma reports should be validated by two competent staff members and include examination of scatterplots by both.

**G2.2 Performance Measures**

**G2.2.1 For 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.**

C2.2.1 Where absolute numbers (eg cells/uL for blood samples) are reported, a control reagent should be periodically tested have specified ranges for the analytes measured, and reasons for deviations determined.

## **G2.2.2 Account should be made for all populations tested.**

C2.2.2(i) Lymphoma screens: A lymphosum of B, T and NK cell populations can be calculated for blood and bone marrow samples. Lymphocyte gated populations unaccounted for >10% for bloods and >20% for bone marrow may require further investigation.

C2.2.2(ii) Leukaemia screens: All CD45 negative populations need to be examined. The absence of CD34, CD117, TdT does not preclude malignancy from blast gated populations.

C2.2.2(iii) Tissues and body fluids: Screens should include markers for all major lineages to optimise identification of majority populations.

## **3. POST ANALYTICAL PHASE**

Refer to ACS '*Guidelines for Clinical Flow Cytometry Laboratory Practice*' for additional information regarding reports, record keeping, result validation, follow up tests.

### **3.1 Reports**

#### **3.1.1 Reports should identify any abnormal population gated, its size, markers tested, relevant staining reactivities, and a diagnosis where possible <sup>(2)</sup>.**

C3.1.1(i) CD number listing should be in numeric then alphabetic listing order

C3.1.1(ii) CD numbers may be accompanied by descriptive and grouped by cell types/function.

C3.1.1(iii) Staining intensity should be reported e.g. dim, bright. It is not necessary to report percent positivity for individual markers <sup>(2)</sup>.

**G3.1.2 Reports should be completed in a timely manner, no longer than 5 working days for leukaemia and lymphoma immunophenotyping.**

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## **PROCEDURAL REFERENCES**

For background on the methods, interpretation and publications refer to the references cited in this guideline.

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Further ACS clinical flow cytometry guidelines documents are available on the website:

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