# ACS GUIDELINE FOR

# LYMPHOCYTE SUBSET IMMUNOPHENOTYPING

## Second Edition 2017

Australasian Cytometry Society Guideline Document

First published 2005

Second edition 2017 Reformatted to be read in conjunction with ACS '*Guidelines for Clinical Flow Cytometry Laboratory Practice*'.

## CONTENTS

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 '*Guideline for Lymphocyte Subset Testing'* 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.

References to specific guidelines in that document are provided for assistance under the headings in this document.

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

## ABBREVIATIONS

| | |
|---|---|
| EDTA | Ethylene-diaminetetraacetic acid |
| ACD | Acid Citrate Dextrose |
| WBC | White Blood Cells |
| SS | Side Scatter |

## DEFINITIONS

| | |
|---|---|
| count | means to acquire data on a flow cytometer |
| Dual platform (DP) | A method where two instruments are used for the determination of absolute numbers (e.g. Haematology and Flow Cytometry analysers) |
| 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. |
| markers | means antigens on cells of interest used for diagnostic purposes |
| platform | Instrumentation or analyser on which test assays are performed |
| Single platform (SP) | A method where a single instrument is used to determine absolute numbers of cells.<br>It uses only flow cytometry instrument and in traditional non volumetric method it is based on adding a known number of fluorescent microspheres to a known volume of sample enabling the determination of both absolute and percentage lymphocyte subsets in a single tube. |

# 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

# 1. PRE ANALYTICAL PHASE

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

## 1.1 Specimen Collection

**G1.1.1 Samples should be tested soon after collection. EDTA, ACD or Sodium Heparin anticoagulants may be used [(1)].**

C1.1.1(i) EDTA and ACD anticoagulated blood specimens are suitable if the specimen is to be processed within 24 hours of collection. After which the sample becomes depleted of granulocytes.

C1.1.1(ii) If the WCC and differential is obtained from the same sample used for flow cytometry then EDTA is recommended [(1)].

C1.1.1(iii) Sodium Heparin and ACD anticoagulated blood specimens are stable for 48 to 72 hours.

**G1.1.2 Dual platform methodologies require a total white cell count and differential should performed within the time frame specified by the manufacturer of the haematology instrument used.**

C1.1.2(i) For fresh samples (<48 hours) the WBC can be used in calculating T cell absolute numbers. For older samples (> 48 hours) lymphocyte numbers are preferred.

C1.1.2(ii) For distant laboratories and dispatch centers, a total white cell count and differential should accompany each specimen [(1)].

C1.1.2(iii) Single platform methods are preferred where a laboratory can demonstrate improved assay performance [(1)].

C1.1.2(iv) Where bead solutions are used they require a blood-to-bead ratio of approximately 1:1 (vol/vol) [(2)].

**G1.2 Specimen Transport**

**G1.2 Specimens should be maintained at 18-22 $^{o}$C in a leak proof container. Temperatures below 4 $^{o}$C and above 30 $^{o}$C must be avoided.**

## 2. ANALYTICAL PHASE

Refer to ACS *'Guidelines for Clinical Flow Cytometry Laboratory Practice'* for information regarding sample analysis and performance measures in addition to that shown following.

### G2.1 Sample analysis

**G2.1.1 Cell concentration needs to be considered where total white cells numbers are high.**

C2.1.1(i) Refer to manufacturers cell count ranges at which assays are validated. Otherwise cell numbers up to 1 x $10^6$ cells/tube may be acceptable.

C2.1.1(ii) Example: if WBC concentration is 10 x $10^9$/L (or 20 x $10^9$/L) and test volume is 100 µL (or 50 µL) the cell number per tube is 1x$10^6$.

**G2.1.2 All tubes need to include CD45. CD45 vs side scatter is recommended for gating lymphocytes, excluding monocytes and non lymphoid populations.**

**G2.1.3 A suitable panel for assessing lymphocyte subsets must include markers for T cells (CD3, CD4, CD8), B cells (CD19 or CD20), and NK cells (CD16 and/or CD56)[(1).]**

C2.1.3 CD20 may be blocked during the assay if it is the target of monoclonal immunotherapy.

**G2.1.4 To reduce test error sample manipulation should be minimised. Single platform methods must not have a wash step. Wash steps should be avoided for Dual platform methods [(1)].**

**G2.2 Performance Measures**

**G2.2.1 Count at least 5000 lymphocytes in each tube to ensure that enough cells have been counted when small lymphocyte subsets are analysed [(1)].**

**G2.2.2 Control material should be used and have validated ranges for the analytes measured.**

C2.2.2 A control should be run either daily or with each assay performed.

**G2.2.3 Analysis should include internal reliability checks of results.**

C2.2.3(i) The sum of T, B and NK cell percentages (the Lymphosum should be between 95 and 105% (minimally 90-110%) [(1)].

C2.2.3(ii) The sum of the CD4 and CD8 cell percentages should equal the total T-cell % and be within the range of ±5% to a maximum of ±10%

variability [1]. Values outside these ranges warrant further investigation. For example, co-expression of CD4 and CD8 or increase in gamma/delta T cells.

C2.2.3(iii) Replicate results within a panel (e.g. CD3+ %) for the same sample should be within 5% for CD45 v SS gating [1].

C2.2.3(iv) For SP methods the parameter 'Time' may be used to monitor instrument fluidic. Monitoring Bead Count Rate with time/fluorescence histograms can be used [2; 3; 4].

## 3. POST ANALYTICAL PHASE

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

**3. Reports**

**G3.1.1 For each of the lymphocyte populations and for the T cell subpopulation populations (CD3/4, CD3/8) absolute numbers should be reported.**

C3.1.1(i) Percentages may be reported in addition to absolute numbers.

C3.1.1(ii) Refer to clinician requirements for units of measure for reporting results. There are currently no consensus units of measure for lymphocyte subset enumeration.

**G3.1.2 Report data with corresponding reference limits of expected normal values.**

C3.1.2(i) Each laboratory should establish reference limits for antigens being tested where possible [1; 5].

C3.1.2(ii) Therapeutic ranges may be determined by clinicians for their own interpretation according to treatment.

## REFERENCES CITED


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2. Peeling RW, Sollis KA, Glover S, Crowe SM, Landay AL, Cheng B, Barnett D, Denny TN, Spira TJ, Stevens WS, Crowley S,Essajee S, Vitoria M, Ford N CD4 Enumeration Technologies: A Systematic Review of Test Performance for Determining Eligibility for Antiretroviral Therapy. PLOS ONE DOI:10.1371/journal.pone.0115019 March 19, 2015
3. Bergeron M, Lustyik G, Phaneuf S, Ding T, Nicholson JKA, Janossy G, Shapiro H, Barnett D, Mandy F. Stability of Currently Used Cytometers Facilitates the Identification of Pipetting Errors and Their Volumetric Operation: "Time" Can Tell All Cytometry Part B (Clinical Cytometry) 52B:37–40 (2003)
4. Lawrie D, Coetzee LM, Glencross DK. A model for continuous quality control incorporating sample-to-sample assessment of optical alignment, fluorescence sensitivity, and volumetric operation of flow cytometers. Cytometry Part B 2010; 78B: 201–210.
5. Whitby L, Whitby A, Fletcher M, Helbert M, Reilly JT, Barnett D. Comparison of methodological data measurement limits in CD4+ T lymphocyte flow cytometric enumeration and their clinical impact on HIV management. Cytometry Part B 2013; 84B: 248–254

## PROCEDURAL REFERENCES

The majority of laboratories performing Lymphocyte subsets use commercial kits which have widespread availability with detailed descriptions of methodology. For backgorund on the methods, interpretation and publications refer to:

WHO publication, Laboratory Guidelines for Enumerating CD4 T Lymphocytes in the context of HIV/AIDS, World Health Organisation Library Cataloging-in-Publication Data, ISBN 978-92-9022-298-9, June 2007

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ACS guideline documents are available on the website: www.cytometry.org.au