ACS GUIDELINE FOR

HLA-B27 IMMUNOPHENOTYPING

Second Edition 2017

Australasian Cytometry Society Guideline Document

First published 2009

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 The *Guideline for HLA B27 typing* is an ACS document for laboratories providing clinical flow cytometry services for HLA B27 typing. It should 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.

Reference 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

ACD	Acid citrate dextrose, anticoagulant
EDTA	Ethylendiamineteraacetic acid, anticoagulant
HLA	Human leukocytes antigen
PMT	Photomultiplier tube, voltage changes can affect gating of populations in scatterplots

DEFINITIONS

Competent flow cytometrist	means a person who has been documented to be competent in clinical flow cytometry for HLA B27 testing according to the Laboratory's Quality System
count	means to acquire events on a flow cytometer
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 antigen or protein on cells of interest used for diagnostic purposes
stain	means binding of antibodies to antigen or protein on cells of interest

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: ACSS 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 in addition to those shown below.

G1.1 Specimen Collection

G1.1.1 Sodium Heparin, EDTA or ACD anti-coagulated peripheral blood samples can be used.

G1.1.2 Samples should be tested as fresh as possible. The timeframe for testing should be validated by the testing laboratory.

C1.1.2(i) Samples may be suitable for testing for 5 days or more [(1)].
As samples age they may lose intensity of fluorescence staining [(2)].

C1.1.2(ii) When using commercial kits, it is recommended to follow the manufacturer's instructions for sample collection.

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 those shown below.

G2.1 Specimen Processing

G2.1.1 Whole blood lysis of red cells is recommended for routine analysis [(2; 3)] .

C2.1.1 A full blood count can be performed to determine if sufficient cells are available for processing, or whether dilution is needed to avoid possible false negative results.

G2.1.2 HLA-B27 analysis should employ a single direct fluorescence label for HLA-B27. The use of clones: ABC-m3, FD705 and GS145.2 are recommended [(2)].

C2.1.2(i) A second HLA-B27 marker (clone) is highly recommended to confirm positivity and assist with determining cross-reactivity [(3; 5)].

C2.1.2(ii) Labeling for HLA-B7 should be tested simultaneously or can be used as a blocking agent [(1).]

C2.1.2(iii) HLA-B7 is another member of the large HLA-B cross-reacting group antigens which shares common epitopes which are capable of cross staining with HLA-B27 [(2)] .

G2.2 Sample analysis

G2.2 Data acquisition: Forward Scatter and Side Scatter gating can be used for lymphoid population selection. HLA-B27 can be found on all lymphocytes.

C2.2(i) Addition of CD45 or CD3 (T cells) gating can help exclude non lymphoid populations and debris from the lymphocyte gate especially as samples age [(1).]

C2.2(ii) When using commercial kits, it is recommended to follow the manufacturer's instructions where given for gating analysis.

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G2.3 Performance Measures

G2.3.1 At least 2000 gated lymphocytes should be counted in each sample.

C2.3.1 This number assures with 95% confidence that the result is within 2% of the "true" value (binomial sampling); assuming that the variability of determining replicates is < 2%.

G2.3.2 Where possible control materials should be used each assay.

C2.3.2 Samples demonstrating HLA-B27 status from the previous day's run may also be used as control material to validate instrument and antibody performance on a regular period.

G2.3.3. Mean channel of fluorescence cut-offs should be determined at which HLA-B27 is excluded or demonstrated.

C2.3.3(i) Cut offs may be provided by use of reference beads, or determination by each laboratory. When using commercial kits, it is recommended to follow the manufacturer's instructions.

C2.3.3(ii) Where reference beads are not used, closer monitoring of analyser drift (eg PMT's) and testing of new reagent Lots is required by reference to positive and negative samples

G2.3.4 Where both HLA-B27 markers are indeterminate, samples should be forwarded to reference laboratories for testing [4; 6].

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 those shown below.

G3 Reports

G3 Results should be reported as: Positive, Negative or Indeterminate.

C3 Reports should comment indeterminate results are recommended for referral for HLA typing by molecular assay for HLA-B27 genotyping.

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2. Hulstaert F, Albrecht J, HaMet I, Lancaster P, Buchner L, Kunz J, Falkenrodt A, Tongio M, De Keyser F, Veys EM, Noens L, Mir N, Costello C, Becker R, Strauss K, An optimized method for routine HLA-B27 screening using flow cytometry. Cytometry (Commun Clin Cytometry) 18:21-29, 1994.
3. Levering, W. H., H. Wind, et al. (2003). Flow cytometric HLA-B27 screening: cross-reactivity patterns of commercially available anti-HLA-B27 monoclonal antibodies with other HLA-B antigens. Cytometry B Clin Cytom 54(1): 28-38
4. Lingenfelter, B., T. C. Fuller, et al. (1995). HLA-B27 screening by flow cytometry. Cytometry 22(2): 146-9.
5. Janssen WCM and Hoffman JJML (1997). HLA-B27 phenotyping with flow cytometry: further improvement by multiple monoclonal antibodies. Clin Chem. 43(10)1975-1981.
6. Steffens-Nakken HM, Zwart G, van der Bergh F. Validation of allele-specific polymerase chain reaction for DNA typing of HLA-B27. Clin Chem 1995;41 (5):687-692

PROCEDURAL REFERENCES

Recommended peer reviewed articles providing detailed methodologies for setting up assays, interpretation and reporting.

- Lingenfelter, B., T. C. Fuller, et al. (1995). HLA-B27 screening by flow cytometry. Cytometry 22(2): 146-9.

- Levering, W. H., H. Wind, et al. (2003). Flow cytometric HLA-B27 screening: cross-reactivity patterns of commercially available anti-HLA-B27 monoclonal antibodies with other HLA-B antigens. Cytometry B Clin Cytom 54(1): 28-38

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

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