ACS GUIDELINE FOR MINIMAL RESIDUAL DISEASE TESTING

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Australasian Cytometry Society Guideline Document

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

SCOPE

The ACS Clinical Guidelines taskforce have drafted an overarching Minimal (or measurable) Residual Disease (MRD) guideline document that, where relevant, refers to specific MRD guidelines for Acute Myeloid Leukaemia (AML), Acute Lymphoblastic Leukaemia (ALL), Chronic Lymphocytic Leukaemia (CLL) and Plasma Cell Myeloma (PCM). While other cell types may also need to be searched for at 'MRD levels', such as a PNH clone (see separate ACS guideline), aberrant mast cells, or Sezary cells, these are out of the scope of this document, and the reader should consult relevant literature, although similar underpinning principles apply.

MRD assessment refers to a sensitive assay designed to detect disease at a threshold of at least 1 malignant cell per 10,000 benign leucocytes remaining or re-emerging after therapy. MRD is proving a surrogate endpoint of survival for an increasing number of haematopoietic neoplasms. Despite advances in molecular techniques, flow cytometry remains advantageous due to broader applicability and accessibility.

There is a need for Australasian recommendations for MRD testing to facilitate assay implementation, uniformity across clinical flow cytometry laboratories, and quality assessment. A laboratory considering implementing these specialised assays requires access to experienced scientific and medical personnel, advanced computer software and storage capabilities, a sufficient clinical caseload and participation in relevant external quality assessment.

There are many international clinical guidelines published on MRD assessment by flow cytometry (FC), however the focus of the ACS Clinical Guidelines taskforce is to summate these resources into a consensus document for the use of ACS members to assist with setting up assays in a clinical flow cytometry laboratory, as well as serving as an audit curriculum for internal and external assessment.

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

ABBREVIATIONS

AML	Acute myeloid leukaemia
B/T-ALL	B- or T-acute lymphoblastic leukaemia
BM	Bone marrow
CLL	Chronic lymphocytic leukaemia
DFN	Different from normal
FC	Flow cytometry
HSCT	Haematopoietic stem cell transplant
LOD	Limit of detection
LLOQ	Lower limit of quantification
MM	Multiple myeloma
MFI	Median fluorescence intensity
MNC	Mononuclear cells
MRD	Minimal or measurable residual disease
PB	Peripheral blood
RT	Room temperature
WBC	White blood cells

DEFINITIONS

Stain	means binding of monoclonal antibodies to markers on cells of interest
MRD markers	means antigens on cells of interest used for MRD detection / enumeration
Experienced 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
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.
Sensitivity	means minimum level to which acquired data on a flow cytometer can detect cells of interest LOD & LLOQ can demonstrate achieved sensitivity in the test sample
Cell preparation	Includes procedures / actions to collect sufficient cells for test sensitivity
Analysis	set up with stain and flow cytometry data acquisition on cytometer
MRD panel	Selection of Antibodies required to detect cell markers in a given disease, able to phenotype residual disease for measurable detection level

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, and 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 Guideline for Clinical Flow Cytometry Laboratory Practice'* unless assay-specific requirements take precedence.

1.1 Sample Handling

G1.1.1	**Sample types:**	BM/PB (as required) must be in LiHep (non-gel) or EDTA
G1.1.2	**Collection:**	BM should be 'first pull' to avoid blood dilution
G1.1.3	**Transport:**	RT as per General FC Guidelines Sample should be kept at 18-22°C in leak-proof container; and must avoid temperatures below 4° C and above 30°C
G1.1.4	**Storage:**	RT unless validated for different temperature
G1.1.5	**Preparation:**	Must ensure minimal number of cells are tested to reach intended assay sensitivity
	C1.1.5 (i)	Pre-lysis (bulk RBC lysis) is recommended to maximise total WBC recovery and ensure the required cell concentration is achieved for intended test sensitivity
	C1.1.5 (ii)	Cell count should be checked prior to testing, and adjusted for the required cell concentration

1.2 Test Requests

G1.2.1	**Clinical Notes:**	Should state disease type, clinical time point (intention)
	C1.2.1 (i)	Verbal confirmation of these details maybe required and should be stated in the final report
	C1.2.1 (ii)	Clinical requirements may include: 'MRD', post induction/consolidation, pre/post HSCT, 'maintenance' or 'surveillance for re-emerging MRD'… I.e. While 'MRD' should ideally be explicitly stated in the referral, some special circumstances may require analysis at 'MRD levels' not immediately apparent unless the indication is discussed.

G1.2.2 **Collection details**: Date, time, and location should be clearly stated, to confirm time to analysis and any delay in transport

C1.2.2 Time to analysis should be within 24h of collection (12h for MM) unless validated for longer periods (e.g. 48 - 72h max).

2. ANALYTICAL PHASE

Refer to *'ACS Guideline for Clinical Flow Cytometry Laboratory Practice'* unless assay-specific requirements take precedence.

2.1 Recommended MRD markers

G2.1.1 Minimum markers that must be present in the MRD panel for gating:

These antigens are required for identification of all potential cells of interest within a particular lineage. However, an increasing challenge facing diagnostic laboratories undertaking MRD assessment is the use of therapy that is targeted toward and interferes with core antigens normally relied upon for initial gating such that this list will need to be adapted over time (e.g. see Pojero 2016 for MM and Cherian 2018 for B-ALL)

B-ALL: CD19, (occasionally CD22)
T-ALL: CD7
AML: HLA-DR, CD34, CD45, CD117
CLL: CD19
MM: CD19, CD38, CD45, CD138

G2.1.2 Minimum markers that should be included in a panel for MRD detection:

Informative MRD immunophenotypes are sufficiently different from those of normal cell populations by way of increased or decreased expression of antigens normally present, asynchronous expression of antigens for a particular maturational stage, or aberrant antigen expression. An MRD panel sufficient to distinguish abnormal from normal can be accommodated by a single 8-10 colour tube for B-ALL, CLL, and MM, although a two-tube panel is regarded as a reference standard for MM (Kumar 2016). The number of markers required for MRD assessment of T-ALL and AML are unlikely to be accommodated by a single tube.

B-ALL: **CD19**; CD20, CD22, CD10, CD34, CD38, CD45 CD58 (gating antigen/s in bold type).

C2.1.2 (i) Other markers in B-ALL MRD panel that may be used:
CD9, CD13/33, CD24 (gating), CD44, CD66b (exclusion of granulocytes), CD66c, NG2.

T-ALL: **CD7**; CD1a, CD2, smCD3, cytCD3, CD4, CD5, CD8, CD10, CD13, CD33, CD34, CD45, CD56 (NK contamination), CD99.

C2.1.2 (ii) Other markers in T-ALL MRD panel that maybe used:
CD117, CD123, TdT

AML: **HLA-DR, CD34, CD45, CD117**; (myelo-monocytic) CD4, CD11b, CD13, CD14, CD15, CD16, CD33, CD64; (stem cell) CD38, CD123; (cross-lineage), CD7, CD19, CD56

C2.1.2 (iii) Other markers in AML MRD panel that maybe used:
CD2, CD5, CD10, CD36, CD65, CD71, CD133, CD305.

CLL: **CD19**; CD5, CD20, CD22, CD43, CD79b, CD81

C2.1.2 (iv) Other markers in CLL MRD panel that maybe used:
CD3 (T-cell contamination), CD38, CD45, ROR-1

MM: **CD19, CD38, CD45, CD138**; CD27, CD56, CD81, CD117
cyt kappa, cyt lambda (EuroFlow reference standard).

C2.1.2 (v) Other markers in MM MRD panel that maybe used:
CD28, CD200, CD229, CD319, cytVS38c

G2.1.5 Intracellular analysis:

C2.1.3 (i) Recommended for MM in a second tube

C2.1.3 (ii) May be useful for T-ALL depending on MRD immunophenotype

2.2 Technical recommendations

G2.2.1 **Platform:** Must use a minimum 8 or 10-colour flow cytometry instrument in order to assess a sufficient number of key antigens (>6 colours) for modern MRD panels simultaneously. While previous published 4-colour panels for (example) CLL and B-ALL provided 10^{-4} sensitivity, demand for deeper response assessment has evolved in the context of 8-10 colour platforms (Rawstron 2013, 2016).

G2.2.2 **Instrument usage and maintenance:**

Must be in accordance with manufacturer recommendations

C2.2.2 Consider running bead-based controls to monitor assay voltages over time, and between instruments if applicable

G2.2.3 **Reagent recommendations:**

C2.2.3 Should use validated, stable cocktails for MRD panels

G2.2.4 **Computer:** Should be capable of analysis and storage of large data files

2.3 Performance Measures

Materials, parameters and data requirements for testing that must be achieved, to permit accurate and robust MRD reporting, i.e. for the MRD assay to be 'fit for purpose'.

G2.3.1 **Minimum number of target cells for an 'MRD positive' result (numerator):**

MRD detection (LOD), N = 20; MRD quantitation (LLOQ), N = 50* (Wood, Hedley, Selliah, Arroz)

C2.3.1 (i) *MRD quantitation, N=20-50 provided events form a well-defined cluster

C2.3.1 (ii) A cluster of 10-20 MRD events may be reported if the laboratory has sufficient experience and confidence, for example, if a previously known MRD phenotype is trackable and plausible across multiple plots/dimensions. (This will depend on disease type, with phenotypic shift more common in AML, and therapy-related down regulation of some antigens in B-ALL) (Wood)

G2.3.2 Minimum number of total cells to be analysed (denominator):

LOD/LLOQ at 10^{-4} requires 200 000/500 000 cells

LOD/LLOQ at 10^{-5} requires 2 x10^6/5 x10^6 cells (Hedley, Arroz)

C2.3.2 (i) #WBC (MNC for paediatric ALL C.O.G protocols). Some laboratories use total live cells following RBC lysis. There is no international consensus. Whatever denominator is used must be clearly stated in the report

C2.3.2 (ii) Aim for 10^6 WBC/tube for 10^{-4} and 10^7 WBC/tube for 10^{-5} prior to analysis (Hedley, Florez-Montero, Euroflow, Rawstron)

G2.3.3 QC materials / controls

C2.3.3 (i) Normal reference MFIs and normal reference phenotype of relevant haematological cell populations in PB/BM should be established for each disease type/MRD panel. Consider using normal population 'overlays/regions' if software allows.

C2.3.3 (ii) Dilution studies to at least required LLOQ for relevant disease should have been performed as part of the method validation

C2.3.3 (iii) Commercial controls +/or calibration particles may aid in procedural, antibody MFI and sensitivity level monitoring

C2.3.3 (iv) Internal sample controls. Residual normal cells of the *same* lineage as the target MRD cells may be used as an internal control for interpretation of antigen expression on MRD cells, or if absent, reliance on normal reference data.

C2.3.3 (v) Internal sample controls. Residual normal cells of *other* lineages, normally confined to BM serve as an important internal sample quality check post-acquisition when confirming a true MRD negative result.

C2.3.3 (vi) Correlation with other platforms testing MRD such as inter-laboratory sample exchange or participation in formal EQA represent important quality checks

G2.3.4 Recommended CV:

CV is dictated by Poisson statistics and the numerator a laboratory is prepared to accept as a positive result

Estimated (by Poisson): CV (%) = √numerator/numerator

= 10% for 100 events; 14% for 50 events; 22% for 20 events; 32% for 10 events

Derived (in-house): Min 5 replicates at low levels of sensitivity (performed as part of a dilution study)

G2.3.5 Establish Limit of Blank (LOB) & Limit of Detection (LOD)

Estimated: Can use estimated LOD, i.e. adopt commonly accepted numerator of 20 for LOD and 50 for LLOQ (as per 2.3.1), which is generally acceptable if LOB is <10 (usually)

OR

Derived (in-house): Min 5 replicates without measurand / or gating Ab

- ⇨ LOB = Mean of blank + 1.645 x SD of blank
- ⇨ LOD = Mean of blank + 3 x SD of blank

G2.3.6 Sensitivity limits:

Must be 10^{-5} for MM (IMWG) => need ≥2 x10^{6} WBC

Desirable 10^{-5} for CLL but unproven (European Medicine Agency)

Recommended 10^{-4} for CLL (ERIC, iwCLL); & ALL (COG, AIEOP/IBFM)

Recommended 10^{-3} for AML; uncertain b/w 10^{-3} & 10^{-4} (ELN)

G2.3.7 Measurement of uncertainty:

95% confidence limits of MRD of 20 cells [2xSD (√n)] = 11 - 29 (Hedley, Arroz)

G2.3.8 Interfering factors, limitations: Hypocellular patient and/or haemodilute sample collection; operator experience; monoclonal antibody therapy; down regulation of gating antigen/s, phenotypic shift (esp AML but also B/T-ALL), contaminating events.

C2.3.8 (i) Haemodilute samples should be avoided by dedicating 'first pull' to flow cytometry tube; may require liaison with proceduralist +/- education and training. 2 – 4 ml of BM sample is recommended (4ml for MM-MRD). Further aspiration from the same 'pull' is likely to become haemodilute.

C 2.3.8 (ii) Post-acquisition assessment for suboptimal, haemodilute or hypocellular (BM) samples should be performed and commented on in the final report; see C2.3.3 (v), G3.1.2 & G3.1.3. (Great importance is placed on this especially in the myeloma MRD literature; see Flores-Montero; Rawstron & deTute)

C2.3.8 (iii) Operator should be an experienced flow cytometrist trained in MRD testing procedures

C2.3.8 (iv) Interference by monoclonal antibody therapy (unexpected absence of expression of a usual gating antigen) should ideally be avoided by choice of appropriate MRD panel, or alternate gating strategy if possible, following communication between the clinical and laboratory teams prior to analysis

2.4 Quality Assessment:

C2.4.1 The MRD assay should be formally validated for use on clinical samples (minimum of 10 independent positive samples, depending on feasibility).

C2.4.2 **Intra-assay performance:** A measure of intra-assay performance may be assessed at the time of assay validation if sample type permits

C2.4.3 **Intra-laboratory performance:** May be assessed at the time of assay validation and when required, e.g. 6 – 12 monthly.

C2.4.4 **Inter-laboratory performance:** Consider comparison with a nominal reference sample, e.g. with an established assay at another department or reference centre at validation +/- periodically

C2.4.5 **External QAP:** Laboratory should participate in an external quality assessment program if available (Keeney)

C2.4.6 Laboratory should assess a reasonable number of normal control samples; approximately 10 – 20 per MRD panel type, depending on feasibility

C2.4.7 Laboratories should perform a substantial number of MRD assays per year; approximately 30 – 50 per disease type (Johansson).

C2.4.8 Some important considerations for Laboratories planning to implement MRD testing:

- Demand: Consider the number of MRD assays likely to be required per year
- Cost / turn-around-time of alternative arrangement (i.e. adopt MRD assay vs. sending -out)
- Commitment of clinical department / hospital
- Staff – medical and scientific; number and experience / need for up-skilling
- Equipment (cytometer/s, computer/s, data storage)
- Competing work flow; any alternate methodology (e.g. bulk lysis)
- Go-to laboratory/s: strongly recommend establishing a working relationship with experienced centre
- Ongoing maintenance / assessment of proficiency (i.e. No: of cases per year)

2.5 Gating Methods/Interpretation:

Refer to peer-reviewed journal reference material (Section 5), noting that there is no consensus method for AML MRD analysis.

2.5.1 General comments:

Should use pre-defined analysis algorithm / template in accordance with any relevant guidelines.

Fluidics and coincidence should be monitored during acquisition, with a time parameter vs. fluorescence plot and doublet exclusion plot included for post-analysis gating.

2.5.2 Reference or consensus gating methods

Validation	In general (see Selliah).
	CLSI (under development)
AML	No consensus
B-ALL	C.O.G or AIEOP-BFM (see Cherian; Dworzak)
T-ALL	No consensus (see DiGiuseppe)
CLL	ERIC (Rawstron)
	10-colour (Sartor)
MM	Euroflow (8 colour, 2 tube) (Flores-Montero)
	'MSK' (10 colour, single tube) (Royston)
	Detailed suggested method (see Soh KT).
	International (ICCS/ESCCA) consensus method is in development

3. POST ANALYTICAL PHASE

Refer to *'ACS Guideline for Clinical Flow Cytometry Laboratory Practice'* unless assay-specific requirements take precedence.

3.1 Clinical diagnostic reporting of MRD; minimum requirements

G3.1.1 Clinical notes / indication

Should include disease type and clinical time point (i.e. intention)

G3.1.2 **Body of report** should include

- Antibody panel
- Immunophenotype of the MRD clone(s) detected
- Level of sensitivity / Number of denominator events and denominator used
- Number of numerator events (and whether averaged per tube if applicable)

- Percent MRD (also for MM, % abnormal PC/total PC)
- Sample quality (stating reason if suboptimal, e.g. hypocellular sample, insufficient number of events, blood dilute, delayed processing).
- Report may also include: Instrument, number of tubes

G3.1.3 Conclusion:

C3.1.3 (i) Should state if MRD positive or negative and (if not already referenced), the amount of MRD, level of sensitivity, LOD & LLOQ

C3.1.3 (ii) State any disclaimers, e.g. diagnostic phenotype not known; suboptimal sample

C3.1.3 (iii) Include a caveat that results must be integrated with other clinicopathological information

3.2 Release and Storage of Data

3.2.1 Turnaround times:

3.2.1 (i) Should perform analysis within stipulated time frames, per C1.2.2

3.2.1 (ii) Report within local laboratory and clinical expectations (min 72h)

G3.2.2 Result Validation:

C3.2.2 Data should be analysed by fully trained experienced flow cytometry staff and validated by trained Pathologist

G3.2.3 Records, Data Storage and Recovery:

Refer to *'ACS Guideline for Clinical Flow Cytometry Laboratory Practice'*

C3.2.3 (i) Must use recommended National Pathology Accreditation Advisory Committee guidelines for data storage.

C3.2.3 (ii) Consider saving files with analysis strategy if software permits

C3.2.3 (iii) Data Types: Must use up to date flow cytometry standard file format (FCS files)

C3.2.3 (iv) Previous versions of FCS and/or analysis software should remain accessible with legacy software

G3.2.4 Confirmatory tests, referral to reference laboratories:

C3.2.4 Consider if required clinically; referral to another laboratory for parallel testing, or confirmation with molecular tests as applicable

4. APPENDIX

4.1 Summary Table

	B-ALL	T-ALL*	AML*	CLL	MM
Working Group	COG AIEOP/IBFM	COG AIEOP/IBFM	ELN	ERIC	EMN ESCCA/ICCS EuroFlow IMWG
Minimum panel *no universal consensus; may chose tube/s targeting relevant diagnostic markers	CD10, CD19 CD20, CD22 CD34, CD38 CD45, CD58	CD1a, CD2 CD3, cytCD3 CD4, CD5 CD7, CD8 CD10, CD13 CD33, CD45 CD56 CD34 CD99, TdT	DR, 2, 4, 5, 7, 11b, 13, 14, 15, 16, 19, 33, 34, 38, 45, 56, 64, 71, 117, 123	CD5, CD19 CD20, CD22 CD43,CD79b CD81	ESCCA/ICCS (2 tube, 8 colour) MSK (1 tube, 10 colour) CD19, 27, 38, 45, 56, 81, 117, 138, cytK, cytL
Gating	CD19, CD22, CD45	CD7	HLA-DR, CD34, CD45, CD117	CD19	CD19, CD38, CD45, CD138
MRD phenotype	10+/-, 19+, 20+/-, 22+/-, 34+/-, 38dim, 45-/dim, 58+/br	multiple	multiple	5+, 19+, 20-/dim, 22dim, 43+, 79b-/dim, 81-/dim	CD19-, 27-/dim, 38dim, 45-/dim, 56+, 81-/dim, 117+, 138br, K+/L+
Contamination	CD24/CD66b (neutrophils)	Back-gating	Back-gating	CD3+/CD19+ Some use	Sequentially gated out
Confounders	Anti CD19, CD20, CD22		CD34-, CD117- Minimal DFN Monocytic	Atypical cases; BTK inhibitors CD19dim	Anti CD38 Anti CD138
Solution	CD22 / CD24 gating		Software assisted gating; Difference from normal	Software assisted gating	CD38-multi-epitope CD229, CD319, cytVS38c
LOD required (ideal)	0.01% 200,000 cells (minimum)	0.01%	0.1%	0.001% 2x10^6 cells (minimum)	0.001%
QAP	UKNEQAS		UKNEQAS Trial program	UKNEQAS	UKNEQAS Trial program
When to test	Post induction Pre/post HSCT Maintenance	Post induction Pre/post HSCT Maintenance	Post induction Pre/post HSCT	Post induction Maintenance	Staging of MM, amyloid, plasmacytoma Post induction Pre/Post HSCT Maintenance Circulating PC at Dx

5. REFERENCES

General, LOD, LLOQ

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3. Johansson U, Bloxham D, Couzens S, et al. Guidelines on the use of multicolour flow cytometry in the diagnosis of haematological neoplasms. British Committee for Standards in Haematology. *Br J Haematol. 2014;165(4):455-488.*

4. Selliah N, Eck S, Green C, Oldaker T, Stewart J, Vitaliti A, & Litwin V. Flow cytometry method validation protocols. *Current Protocols in Cytometry 2019; 87:* e53. doi: 10.1002/cpcy.53

5. Keeney M, et al. A QA Program for MRD Testing Demonstrates That Systematic Education Can Reduce Discordance Among Experienced Interpreters

Pre-lysis

6. EuroFlow C. EuroFlow Standard Operating Procedure (SOP) for Bulk Lysis for MRD panels. 2018; https://euroflow.org/usr/pub/protocols.php
   (Regarded as the reference method for Myeloma MRD testing)

7. Muccio VE, Saraci E, Gilestro M, et al. Relevance of sample preparation for flow cytometry. *Int J Lab Hem 2018; 40: 152-158*
   (One of few published pre-lysis methods)

B-/T-Acute lymphoblastic leukaemia

8. Cherian S, Miller V, McCullouch V, et al. A Novel Flow Cytometric Assay for Detection of Residual Disease in Patients with B-Lymphoblastic Leukemia/Lymphoma Post Anti-CD19 Therapy. *Cytometry B Clinical Cytometry 2018; 94B: 112-120*

9. Cherian S, Stetler-Stevenson M. Flow Cytometric Monitoring for Residual Disease in B Lymphoblastic Leukemia Post T Cell Engaging Targeted Therapies. *Curr Protoc Cytom. 2018 Oct;86(1):e44.*
   (The primary application of this protocol is to identify residual B-LL in patients post anti CD19 therapy and/or anti CD22 therapy. Also if CD19 and/or CD22 expression is low or in the rare cases of B-LL which are CD19 negative)

10. Dworzak MN, Buldini B, Gaipa G, et al. AIEOP-BFM Consensus Guidelines 2016 for Flow Cytometric Immunophenotyping of Pediatric Acute Lymphoblastic Leukemia. *Cytometry B Clinical Cytometry 2018; 94B: 82-93*

(Includes a useful 'ready reckoner' for a more standardized application of Bethesda Guidelines for interpreting antigen expression)

11. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood. 2017;129(3):347-357.*

12. DiGiuseppe JA, Wood BL. Applications of Flow Cytometric Immunophenotyping in the Diagnosis and Posttreatment Monitoring of B and T Lymphoblastic Leukemia/Lymphoma. *Cytometry B Clinical Cytometry 2019; 96B: 256-265*
(Useful gating advice)

Acute myeloid leukaemia

13. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood. 2018;131(12):1275-1291.*
(The definitive document confirming the complementary value of 'leukaemia associated immunophenotype (LAIP) and 'difference from normal' (DFN) approach, threshold and time points; in particular, the lack of universal need for testing below 10^{-3})

Chronic lymphocytic leukaemia

14. Rawstron AC, Villamor N, Ritgen M, et al. International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. *Leukemia. 2007;21(5):956-964.*
(Three tube, four colour, showing Boolean gating algorithm)

15. Rawstron AC, Bottcher S, Letestu R, et al. Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL. *Leukemia. 2013;27(1):142-149.*
(Two tube, six colour)

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(Refer in particular to supplementary information; six - eight colour)

17. Sartor MM, Gottlieb DJ. A Single Tube 10-Color Flow Cytometry Assay Optimizes Detection of Minimal Residual Disease in Chronic Lymphocytic Leukemia. *Cytometry B Clinical Cytometry 2013; 84B: 96-103*

Multiple Myeloma

18. Rawstron AC, Orfao A, Beksac M, et al. Report of the European Myeloma Network on multiparametric flow Cytometry in multiple myeloma and related disorders. *Haematologica 2008; 93(3): 431-438.*
(Shows gating algorithm; see supplementary information)

19. Arroz M, Came N, Lin P, et al. Consensus guidelines on plasma cell myeloma minimal residual disease analysis and reporting. *Cytometry B Clinical Cytometry 2016; 90B: 31-39* **(Shows initial gating algorithm)**

20. Rawstron AC, de Tute RM, Haughton J, and Owen RG. Measuring Disease Levels in Myeloma Using Flow Cytometry in Combination with Other Laboratory Techniques: Lessons from the Past 20 Years at the Leeds Haematological Malignancy Diagnostic Service. *Cytometry B Clinical Cytometry 2016; 90B: 54-60*

    (Shows an example of post-acquisition sample quality check and rationale for triaging MRD samples at 10^{-4} before proceeding to 10^{-5} in a high throughput laboratory)

21. Pojero F, Flores-Montero J, Sanoja L, et al. Utility of CD54, CD229, and CD319 for the Identification of Plasma Cells in Patients with Clonal Plasma Cell Diseases. *Cytometry Part B Clinical Cytometry 2016; 90B: 91-100*

22. Soh KT, & Wallace PK. Monitoring of measurable residual disease in multiple myeloma by multiparametric flow cytometry. *Current Protocols in Cytometry*, 2019; *90:* e63. doi:10.1002/cpcy.63
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23. Soh KT, Tario JD, Wallace PK. Diagnosis of Plasma Cell Dyscrasias and Monitoring of Minimal Residual Disease by Multiparametric Flow Cytometry. *Clin Lab Med 2017;37:821–853*
    (Similar to ref 21 with suggested gating algorithm)

24. Roshal M, Flores-Montero JA, Gao Q, et al. MRD detection in multiple myeloma: comparison between MSKCC 10-color single-tube and EuroFlow 8-color 2-tube methods. *Blood Adv 2017; 1(12): 728-732*

25. Royston DJ, Gao Q, Nguyen N, et al. Single-Tube 10-Fluorochrome Analysis for Efficient Flow Cytometric Evaluation of Minimal Residual Disease in Plasma Cell Myeloma. *Am J Clin Pathol 2016; 146L 41-49*

26. Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia. 2017;31(10):2094-2103.*

    (Regarded as the reference MM MRD method by the International Myeloma Working Group; See Kumar 2016)

27. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol 2016;17: e328-e46.*

    (Introduces the response criterion; "Flow MRD-negative - Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher.")

PROCEDURAL REFERENCES

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

Editorial Committee

Neil Came, Gosia Gorniak, Aruna Kodituwakku, Vuong Nguyen, Mary Sartor

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

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