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 Multiple Myeloma (MM). 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 10^4 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 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

AIEOP-BFM	Associazone Italiana Ematologia Oncologia Pediatica – Berlin-Frankfurt-Munster group
AML	Acute myeloid leukaemia
B/T-ALL	B- or T-acute lymphoblastic leukaemia
BM	Bone marrow
CD	Cluster of differentiation number used to classify cell surface molecular targets for immunophenotypic identification of cells
CLL	Chronic lymphocytic leukaemia
COG	Children's Oncology Group
Cyt	Cytoplasmic (marker)
DFN	Different from normal
EDTA	Ethelene-diamine-tetraacetic acid
ERIC	European Research Initiative on CLL
FC	Flow cytometry
HSCT	Haematopoietic stem cell transplant
iwCLL	International Workshop on CLL
LOD	Limit of detection
LLOQ	Lower limit of quantification
Marker	means an antibody directed to an antigen of interest in or on a cell used for diagnostic purposes
MM / PC	Multiple myeloma / Plasma cells

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 (for unsupervised MRD analysis)	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 MRD analysis 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 / Cocktail	Selection of Antibodies required to detect cell markers in a given disease, able to phenotype residual disease for measurable detection level / which has been pre-prepared and validated as fit for purpose for a defined period of time.

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.

G1.1 Specimen Collection, Transport and Storage

G1.1.1 Sample types

BM/PB (as required) must be in heparin or EDTA.

G1.1.2 Collection

BM should be collected as 'first pull' to avoid blood dilution.

G1.1.3 Transport

Samples should be transported at 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

Samples should be stored at RT unless validated for a different temperature.

G1.1.5 Preparation

BM/PB (as required) must be in heparin or EDTA

A minimal number of cells must be tested to reach intended assay sensitivity.

C1.1.5 (i) Pre-lysis (bulk erythrocyte 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.

G1.2 Test Requests

G1.2.1 Clinical Notes

Disease type, clinical time point (intention) should be stated.

C1.2.1 (i) Verbal confirmation of these details may be required and should be stated in the final report.

C1.2.1 (ii) Clinical requirements may include: 'MRD', post induction/consolidation, pre/st HSCT, 'maintenance' or 'surveillance for re-emerging MRD' That is 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.

G2.1 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. Pojero 2016 for MM; 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. This list is not exhaustive.

There may be markers expressed by the tumour cells at diagnosis which are not in the list. It may be advantageous to include the assessment of antigens known to be expressed by the neoplastic population, representing a 'personalisation' of the MRD assessment, although work towards standardization of international consensus panels may ultimately limit this.

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, CD73, CD81, CD123, CD304, NG2.

T-ALL: **CD7,** CD2, surface membrane CD3, cyt CD3, CD4, CD5, CD8, CD10, CD13, CD33, CD34, CD45, CD56 +/- CD16 (to exclude NK cell contamination).

C2.1.2 (ii) Other markers in T-ALL MRD panel that may be used:

CD1a, (CD16), CD99, 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 may be 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 may be used:

CD3 (T-cell contamination), CD38, CD45, CD200, 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 may be used:

CD28, CD200, CD229, CD319, cytVS38c

G2.1.3 Intracellular analysis

Intracellular analysis should be used where recommended.

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

C2.1.3 (ii) Recommended for T-ALL.

G2.2 Technical recommendations

G2.2.1 Platform: Must use a minimum 8 or 10-colour TGA registered clinical flow cytometry instrument in order to assess a sufficient number of key antigens (>6 colours) for modern MRD panels simultaneously, more efficiently, faster, with less tubes.

C2.2.1 While previous published 4-colour panels for (example) CLL and B-ALL provided 10-4 sensitivity, demand for deeper response assessment has also evolved in the context of 8-10 colour platforms (Rawstron 2013, 2016).

G2.2.2 Instrument usage and maintenance

Instrument use 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

Reagents should be validated, stable cocktails for MRD panels.

G2.2.4 Computer

Computers should be capable of analysis and storage of large data files.

G2.3 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 The minimum number of target cells for an 'MRD positive' result (numerator) MRD detection (LOD) is N = 20; MRD quantitation (LLOQ), N = 50* (Wood 2016, 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 including physical characteristics. (This will depend on disease type, with phenotypic shift more common in AML, and therapy-related down regulation of some antigens in B-/or T-ALL) (Wood 2016, Roshal 2010).

G2.3.2 A minimum number of total cells need to be analysed (denominator)

LOD/LLOQ at 10^{-4} (0.01%) requires 2 x10^5/5 x10^5 cells

LOD/LLOQ at 10^{-5} (0.001%) 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 erythrocyte lysis. There is no international consensus. Whatever denominator is used must be clearly stated in the report. However, total WBC is the default denominator in adult MRD analysis.

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

C2.3.2 (iii) While a minimum numbers of total cells have been recommended, it is preferable to acquire more total events where possible in order to either make the demonstration of MRD more convincing or improve the probability of true MRD negativity.

G2.3.3 QC materials and controls

QC materials and controls must be used in assay validation and monitoring.

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 can be used for checking antigen expression. 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) QC and analysis strategies as described in peer reviewed publications are recommended.

AML: No specific consensus gating strategy. Bethesda Guidelines on reporting of antigen fluorescence intensity and distribution (Wood 2007) provide general guidance on the interpretation of "Negative, Positive, or Partially expressed" and "Dim, Bright, and Heterogeneous". Applying these principles to small numbers of MRD events is however difficult.

B/T-ALL: The AIEOP-BFM (Dworzak) provide a useful pictorial description of a 'Bethesda-style refinement of the tripartite system' which specifies how to interpret antigen expression by applying the Bethesda Guidelines using a more standardised algorithm.

CLL: ERIC (Rawstron 2007, 2013, 2016) provide a specific sequential Boolean gating strategy that separates CLL MRD from normal B-cells.

MM: Applying the 'Bethesda-style refinement of the tripartite system' (Dworzak) to plasma cells was troublesome even for an international group of experts (Scott) despite referring to consensus recommendations (Arroz). I.e. erythroblasts were recommended as an appropriate negative control. Internal positive controls were listed as B-cells for CD19 and CD81, NK cells for CD56, and myeloid progenitors and mast cells for CD117.

Nevertheless, as the discrimination of a neoplastic PC population can be very difficult at low level MRD, reliance on comparison with normal reference immunophenotypic data for interpretation of antigen expression intensity was considered just as important. Euroflow concluded that cytoplasmic light chain expression was ultimately required for definitive evidence of MRD at very low levels of sensitivity (Flores-Montero).

C2.3.3 (v) Internal sample controls for specimen quality. 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 (this has been specifically addressed for MM in (Arroz, Rawstron, Flores-Montero).

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

G2.3.4 Recommended Coefficient of Variation (CV) (Wood, Hedley, Selliah, Soh)

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) (Selliah, Soh)

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 $\geq 2 \times 10^{6}$ 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; (10^{-4} ideally, but significance is uncertain b/w 10^{-3} & 10^{-4} (ELN)

G2.3.7 Measurement of uncertainty

95% confidence limits of MRD of 20 cells [2xSD ($\sqrt{n}$)] = 11 - 29 (Hedley, Arroz)

G2.3.8 Interfering factors, limitations

MRD testing should consider effects of hypocellular marrows or haemodilute sample collection; operator experience; monoclonal antibody therapy; down regulation of gating antigen/s, phenotypic evolution during treatment (especially B/T-ALL) and at relapse (especially AML), contaminating events. (See also review by Hulspas et al).

C2.3.8 (i) Haemodilute samples must be avoided by dedicating 'first pull' to flow cytometry tube if flow cytometry is the most important bone marrow test contributing to the clinical decision. May require liaison with proceduralist +/- education and training. The first 0.5 – 1ml is ideal. 2 – 4 ml of BM sample is sometimes recommended but further aspiration from the same 'pull' is likely to be 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) Antigen-targeted therapeutic agents such as chimeric antigen T-cell receptor, bi-specific T-cell engager or monoclonal antibody therapies are likely to result in the (sometimes unexpected) absence of expression of a usual gating antigen. This should ideally be avoided by choice of an appropriate MRD panel beforehand following communication between the clinical and laboratory teams prior to analysis, and an alternate gating strategy may be required (e.g. Cherian).

G2.4 Quality Assessment

C2.4.1 The MRD assay must 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 (proficiency testing)**

should 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**

MRD testing laboratory should participate in an external quality assessment program if available (Keeney).

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

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

C2.4.8 Laboratories planning to implement MRD testing should make important considerations:

- Demand: Consider the number of MRD assays likely to be required per year
- Choice of panel in the absence of consensus: I.e. bespoke vs. published vs. commercially prepared
- 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)

G2.5 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

C2.5.1(i) A pre-defined analysis algorithm / template should be used in accordance with any relevant guidelines.

C2.5.1(ii) The use of sequential Boolean gating strategies are particularly useful at separating a cell population of interest from overlapping normal populations or contaminating events (Hulspas).

C2.5.1(iii) Fluidics and coincidence should be monitored during acquisition, with a time parameter vs. fluorescence plot and doublet exclusion plot included for post-analysis gating (Johansson).

2.5.2 Reference or consensus gating methods

While some principles of gating are provided, the recommendation is to seek an experienced MRD centre against which to validate the assay and gating set-up, and provide ongoing support. File swapping is recommended.

2.5.2(i) **Validation**

In general, refer to NPAAC document, *Requirements for the Development and Use of In-house In Vitro Diagnostic Devices* (See also Selliah).

Clinical and Laboratory Standards Institute (CLSI, USA) (under development)

2.5.2(ii) Gating and analysis strategies as described in peer reviewed publications are recommended.

AML

No consensus algorithm.

Examine a relevant immunophenotypic ‘space’ for abnormal myeloid progenitors in AML cases out of all WBC using an ‘integrated leukaemia associated immunophenotype (LAIP) based difference from normal (DFN) approach’ (Shuurhuis).

‘Spaces’ to examine depend, to some extent, on the diagnostic immunophenotype: E.g. CD34+, CD117+, CD34+/CD38- (‘leukaemic stem cell’), CD45/SSC progenitor region, or more targeted region if appropriate. E.g. CD34+/DR-. CD34-/monocytic AML is particularly challenging and ‘operational sensitivity’ should be reported with caution in these instances.

B-ALL

C.O.G (requires C.O.G. website log in). An outline of C.O.G. plots is provided in DiGiuseppe. See also Borowitz.

AIEOP-BFM (Dworzak).

For B-ALL MRD analysis following anti-CD19 therapy, see Cherian. Gate on CD19+ viable cells and then assess difference from normal.

T-ALL

No consensus (see DiGiuseppe). Gate CD7+ viable cells (unless CD45+, then use WBC). Focus on cytCD3+/CD3- (or CD3+dim) cells, and exclude NK-cells, which express cytCD3.

CLL

ERIC (Rawstron). WBC based on CD43 and light scatter properties. Gate on CD19+ mononuclear cells with light scatter characteristics consistent with lymphocytes and then assess difference from normal.

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) (Arroz). Refined consensus method is in development. Use EMN recommended Boolean gating of CD38+, CD138+, and CD45 out of 'all WBC plus plasma cells', and then assess difference from normal.

3. POST ANALYTICAL PHASE

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

G3.1 Clinical diagnostic reporting of MRD; minimum requirements

G3.1.1 Clinical notes / indication

Clinical notes should include disease type and clinical time point (i.e. intention).

G3.1.2 Body of Report

The body of the report should include:

- Antibody panel
- Immunophenotype of the diagnostic clone/s
- 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 of Report

The conclusion of the report should include:

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.

G3.2 Release and Storage of Data

3.2.1 Turnaround times

C3.2.1 (i) Analysis should be performed within stipulated time frames, as per C1.2.2.

C3.2.1 (ii) Reports should be completed within local laboratory and clinical expectations (min 72h).

G3.2.2 Result Validation

Data must be analysed by fully trained experienced flow cytometry staff and release of the clinical report must be authorised by a suitably trained Haemato- or Immuno- Pathologist credentialed by the employing institution.

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 Confirmatory tests should be considered if required clinically; referral to another laboratory for parallel testing, or confirmation with molecular tests as applicable.

APPENDIX

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 CD9	CD2 smCD3, cytCD3 CD4, CD5 CD7, CD8, CD10, CD13 CD33, CD45 CD56 CD34	HLA-DR, CD2, 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	CD10+/-, 19+, 20+/-, 22+/, 34+/-, 38+/-, 45-/dim, 58+/or br	multiple	multiple	CD5+, 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% 2x10^5 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 Pre/post immunotherapy 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

Abbreviations for Table:

COG, Children's Oncology Group

AIEOP-BFM, Associazone Italiana Ematologia Oncologia Pediatica – Berlin-Frankfurt-Munster group

ELN, European Leukaemia Net

ERIC, European Research Initiative in CLL

EMN, European Myeloma Net

ESCCA, European Society for Clinical Cell Analysis

ICCS, International Clinically Cytometry Society

IMWG, International MyelomaWorking Group

Sm, Surface membrane

Br, Bright

DFN, Difference from normal

BTK, Bruton's tyrosine kinase

QAP, Quality assessment program

UKNEQAS, United Kingdom National External Quality Assessment Service

HSCT, Haematopoietic stem cell transplant

REFERENCES

General, LOD, LLOQ

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PROCEDURAL REFERENCES

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

Editorial 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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