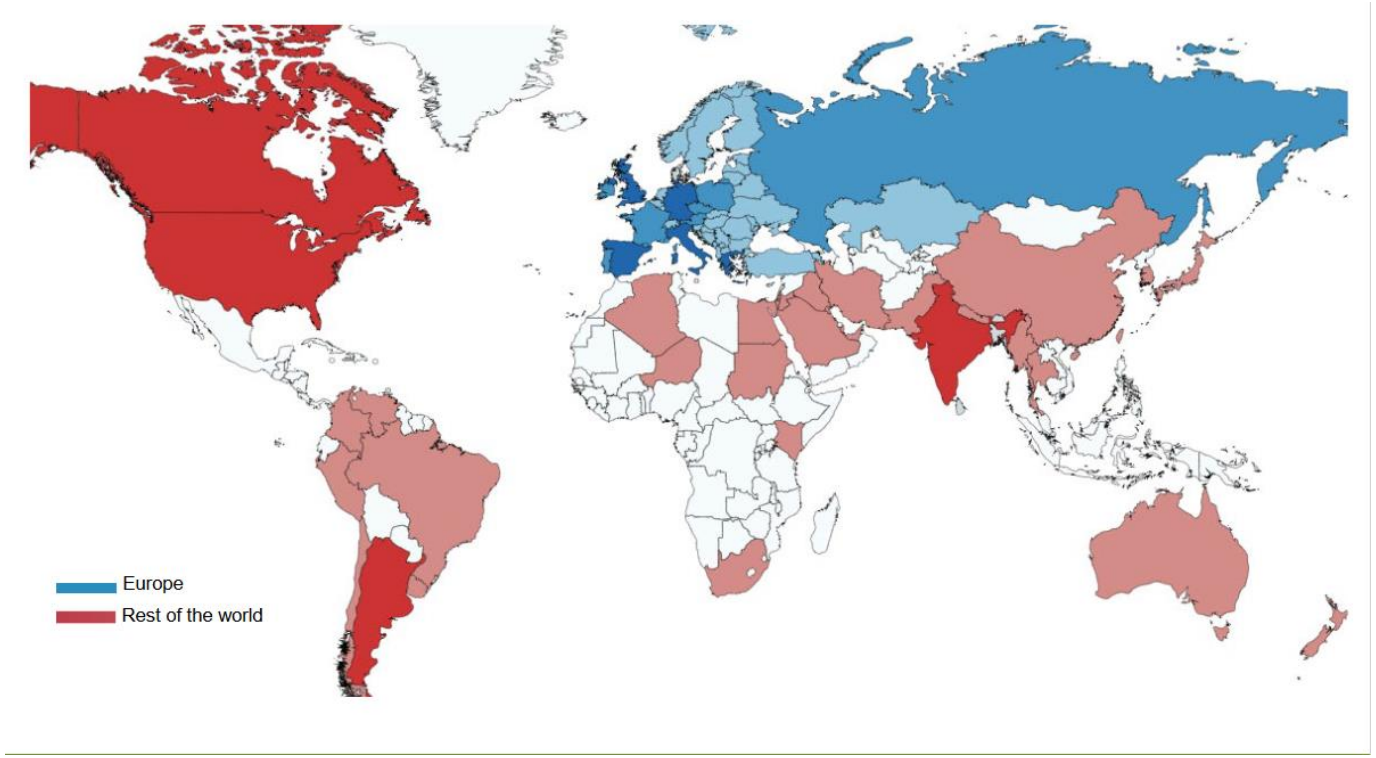
ERIC guidelines for MRD assessment in CLL 2022

https://barcelo.eventsair.com/eric-mrdc-certification/mrd-guidelines-2022/Survey/Landing

- Cellular technical approach
- Operational considerations
- Reporting MRD results



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Development of the ERIC CLL cellular MRD approach

Tested 35 markers reported to be differentially expressed in CLL vs. normal B-cells in 50 configurations

Identified the 3 combinations with the lowest false-positive rate and highest reproducibility

Consensus 5-tube 4-marker panel

FITC	PE	PerCPCy5.5	APC	Aim
kappa	lambda	CD19	CD5	Clonal assessment
CD45	CD14	CD19	CD3	Limit of detection
CD20	CD38	CD19	CD5	CLL quantification
CD81	CD22	CD19	CD5	CLL quantification
CD79b	CD43	CD19	CD5	CLL quantification

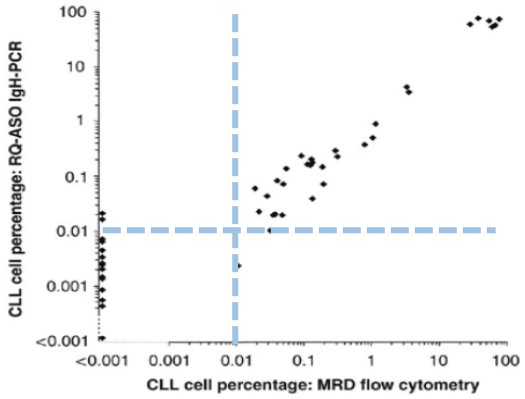
International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia

AC Rawstron[1], N Villamor[2,3], M Ritgen[4], S Böttcher[4], P Ghia[5], JL Zehnder[6], G Lozanski[7], D Colomer[2,3], C Moreno[2,3], M Geuna[8], PAS Evans[1], Y Natkunam[6], SE Coutre[6], ED Avery[9], LZ Rassenti[9], TJ Kipps[9], F Caligaris-Cappio[5], M Kneba[4], JC Byrd[7], MJ Hallek[10], E Montserrat[2,3] and P Hillmen[1]

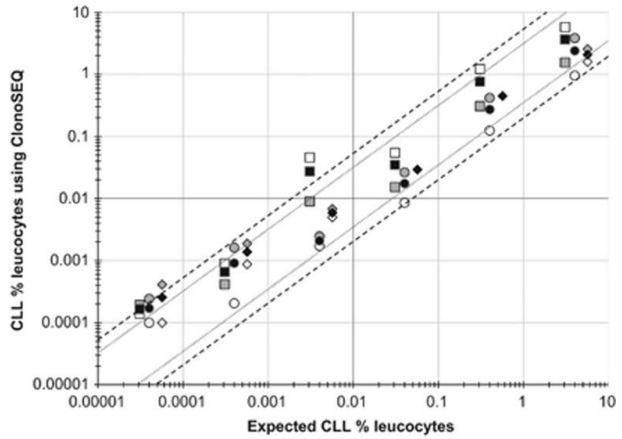
Markers	Tubes	Detection limit	Cells required for 0.01% – LoD
4	4	0.005%	4–20 million
6	2	0.001%	2–10 million
≥6	1	0.001%	1–5 million

Rawstron AC, *et al. Leukemia* 2016; 30:929-936;
Rawstron AC, *et al. Leukemia* 2013; 27:142–149;

Concordance and linearity with IGHV qPCR and high throughput sequencing



Leukemia 2007; 21:956–64;



Leukemia 2013; 27:142–149;

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ERIC standard for Flow Cytometry MRD Detection: can be adapted with additional markers

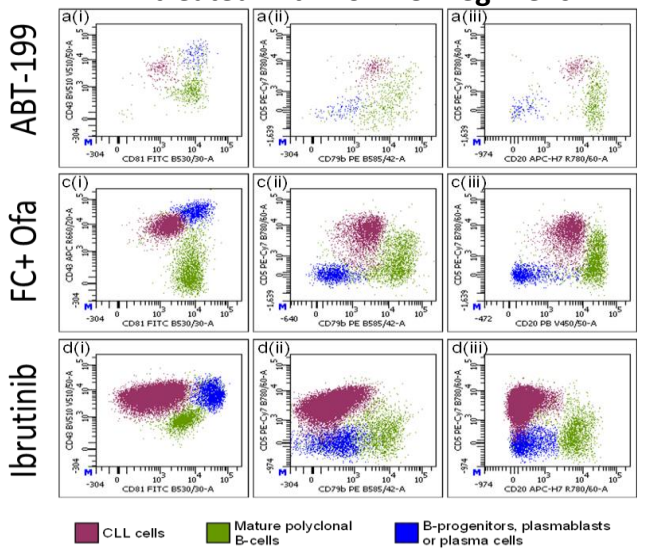
Requires ≥6 markers to achieve 0.01% – available to most labs Can achieve 0.001%

The core panel must meet these 6 specifications, but is flexible thereafter

Backwards-compatible and applicable to current treatments

Antigen	Typical expression (% positive vs control)	Control population in normal peripheral blood		Minimum relative fluorescence intensity (preferred)
		Positive	Negative	
CD5	Positive (>20%)	CD3+ T-cells	CD19+ B-cells	>30 (>65)
CD20	Weak	CD19+ B-cells	CD3+ T-cells	>10 (>20)
CD43	Positive (>20%)	CD3+ T-cells	CD20+ B-cells	>15 (>40)
CD79b	Weak	CD20+ B-cells	CD3+ T-cells	>15 (>30)
CD81	Weak	CD3+ T-cells	Granulo-cytes	>12 (>20)

Examples of MRD analysis in patients treated with non-FCR regimens



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Cellular analysis: technical questions

	Yes	No	Not sure
The core marker panel should be updated (slides 2-5)	39%	22%	38%

Additional markers which should be considered for the core panel	Required	Recommended	Not informative	Not sure
ROR1	33%	44%	0%	22%
CD3	18%	35%	24%	24%
CD27	29%	25%	24%	35%
CD200	29%	12%	24%	35%

CD20 vs. CD22	CD20 is more informative	CD22 is more informative	Both CD22 & CD20 are required	Either CD22 or CD20 is suitable	Not sure
CD20 vs. CD22	41%	12%	22%	5%	12%

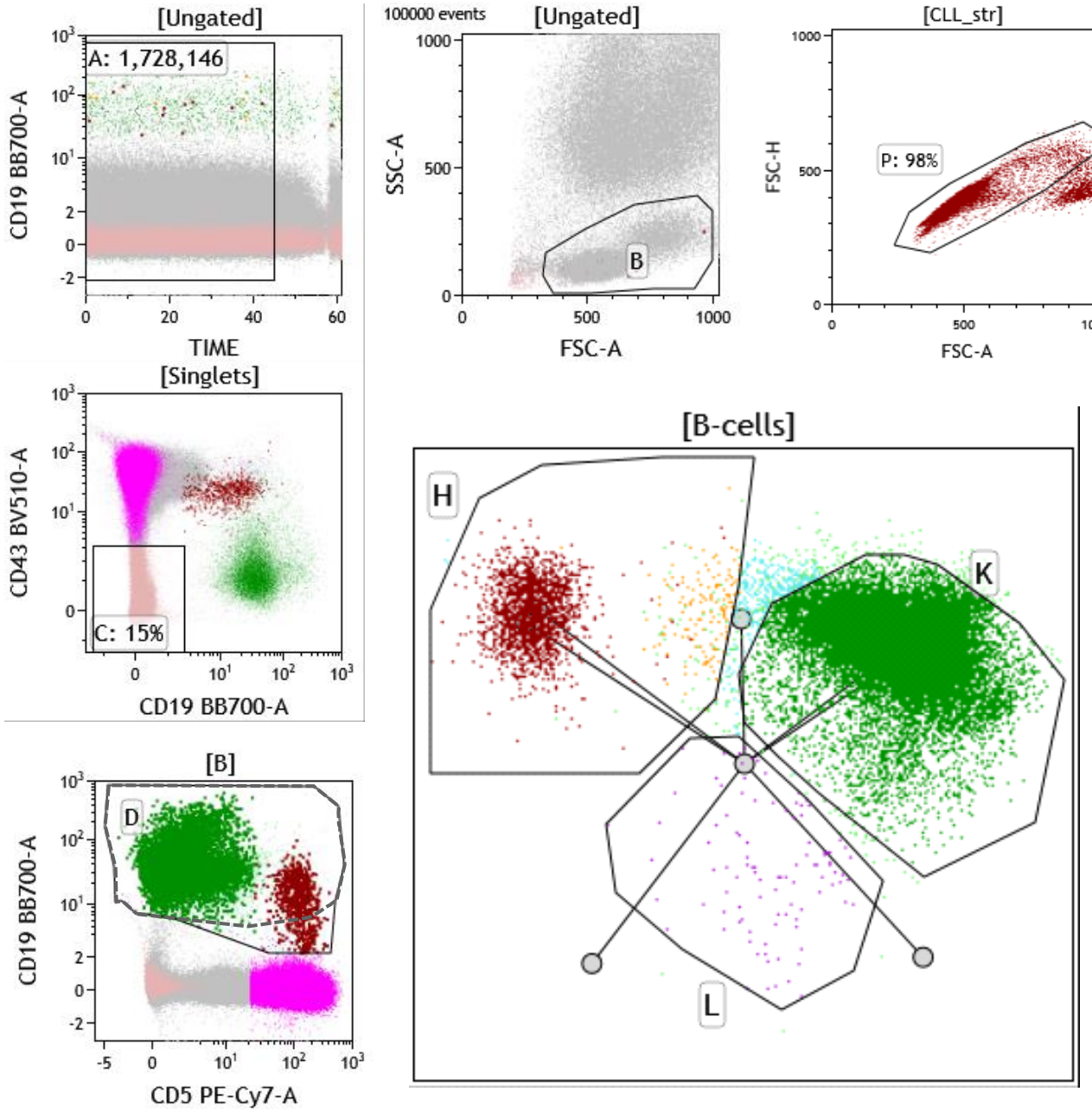
	Required	Recommended	Not informative	Not sure
CD19/CD5 clonality assessment in addition to any MRD panel	29%	41%	12%	18%
Pre-treatment immunophenotyping	41%	53%	0	6%
Early evaluation during novel treatment to check for phenotype shift	24%	41%	18%	18%

→ Spread of results: put these questions (reframed depending on ELN survey) to all ERIC members

Until automated analysis is available… CLL MRD analysis “requirements” and recommendation as an alternative to “fixed” gating strategy

- Time gate to exclude artefacts caused by unstable flow rates, cell clumps, fluidics blockage or air bubbles
- Light scatter gates to (i) include mononuclear cells and exclude debris and (ii) cell doublets
- Gates to identify (i) total leucocytes and (ii) B-lineage cells
- Separating CLL cells from normal B-cells, progenitors/plasma cells and contaminating non-B cells.
- **Tested at an ERIC educational workshop14/15 participants not performing CLL MRD flow**

→ Appendix 1 of MRD guidelines



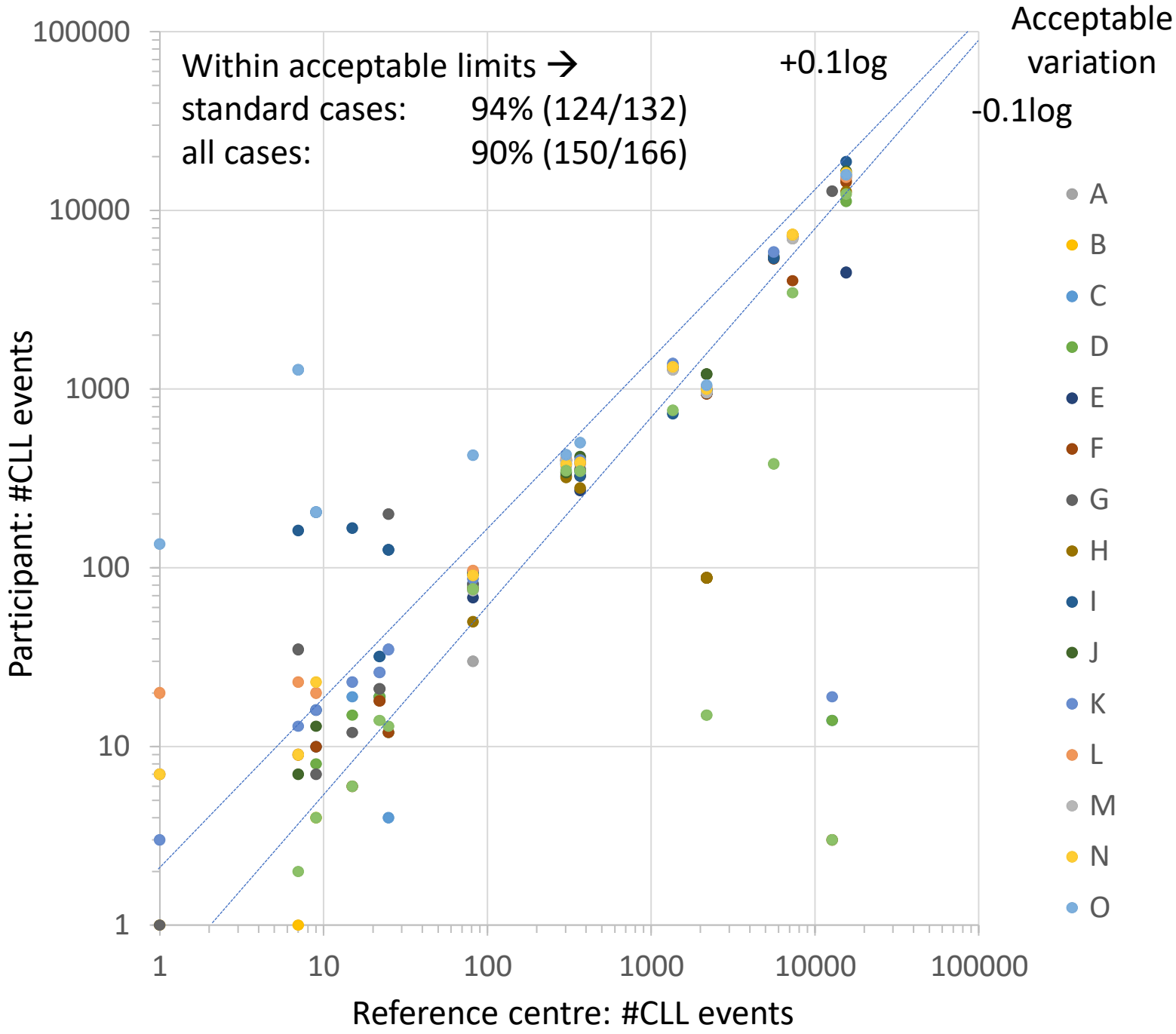
Workshop results

- Concordance at IWCLL 0.01% threshold →
- standard cases: 96.2% (127/132)

		Reference	
		<0.01%	≥0.01%
Participant	<0.01%	31	1
	≥0.01%	4	96

- all cases: 92.2% (153/166)

		Reference	
		<0.01%	≥0.01%
Participant	<0.01%	49	6
	≥0.01%	7	104



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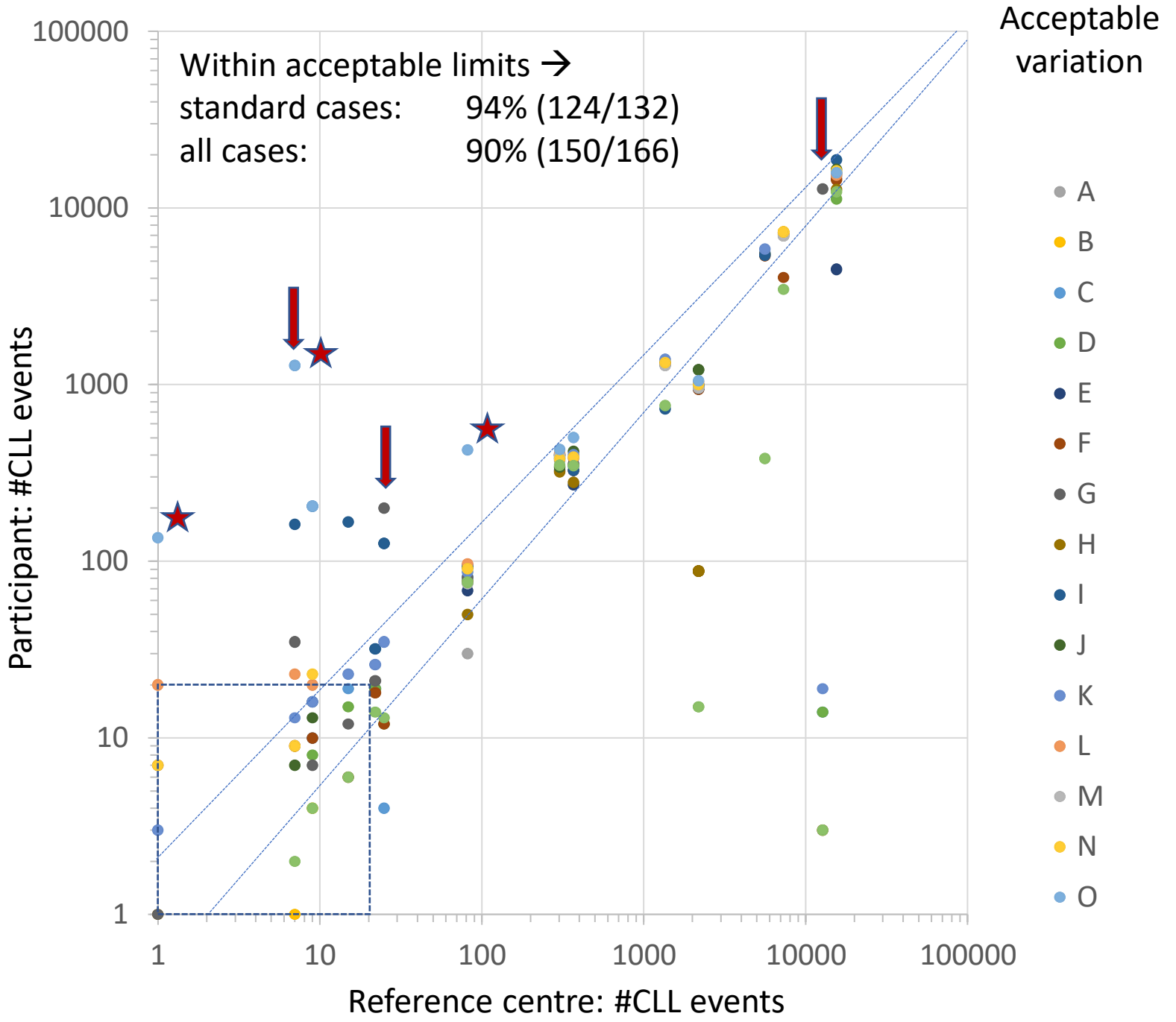
	≥0.01%	4	96

Difficult case → general guidance

Individual issue → education



MRD analysis CERTIFICATION

- Certify an individual for data analysis using a standard set of FCS files
- Register → download files → analyse using your own software/strategy
- Upload results

Please provide the following information:

CaseID *

Number of CLL events *

Number of total leukocyte events *

MRD status at the iwCLL Threshold

- ○ **CLL cells >= 0.01% of leukocytes**
- ○ **CLL cells < 0.01% of leucocytes**
- ○ **Unsuitable for MRD analysis**
- ○ **Other**

Optional: 2022 classification MRD level

Please select — MRD3 / MRD4 / MRD5

Optional: 2022 classification MRD status

Please select — Detectable or undetectable

Optional: Data quality

Please select

ERIC should seek evidence and/or consensus on the following topics to include in any update on updated general MRD guidance:

The updated guidance should include:	Yes	No	Not sure
Guidance on when to use PB vs. BM	100%	0%	0%
Guidance on MRD timepoints should be included	94%	0	6%

→ Follow-up survey to identify key timepoints of interest

Criteria for reporting individual samples and summarising MRD status independent of assay type (? also independent of disease type)

	Yes	No	Not sure
The proposed criteria for reporting individual samples are acceptable (slide 22)	77%	0%	23%
The proposed criteria for reporting categorical MRD status are acceptable (slide 23-26)	77%	0%	23%

Reporting individual MRD results: point estimate (CLL % of total cells), #total cells (DNA equivalent), limit of detection, limit of quantitation

- Limit of detection = 20 / total cells, limit of quantitation = 50 / total cells

- CLL cells = 0.02% of leucocytes
  - Total leucocytes = 498072, limit of detection = 0.0040%, limit of quantitation = 0.010%.
- CLL cells not detected (<0.0040%).
  - Total leucocytes = 498072, limit of detection = 0.0040%, limit of quantitation = 0.010%.
- CLL cells detected below the quantitative range (~0.007%, range 0.004-0.01% of leucocytes).
  - Total leucocytes = 498072, limit of detection = 0.0040%, limit of quantitation = 0.010%).
- Suspicious of residual disease
  - Below limit of detection
  - Below quantitative range
  - Different / atypical / non-CLL phenotype

Reporting individual MRD results: point estimate (CLL % of total cells), #total cells (DNA equivalent), limit of detection, limit of quantitation

Summarising MRD results: appropriate for any validated quantitative method and potentially applicable to many quasi-quantitative assays

MRD classification	Neoplastic cells / total normal cells	Neoplastic cells % of total cells	Scientific notation	Cell required for flow cytometry	Cells (DNA) required for molecular analysis
MRD3	<1/ thousand	<0.1%	10E-3 (10^{-3})	>20 thousand	>3 thousand (0.02µg DNA)
MRD4	<1/ 10 thousand	<0.01%	10E-4 (10^{-4})	>200 thousand	>30 thousand (0.2µg DNA)
MRD5	<1/ 100 thousand	<0.001%	10E-5 (10^{-5})	>2 million	>300 thousand (2µg DNA)
MRD6	<1/ million	<0.0001%	10E-6 (10^{-6})	>20 million	>3 million (20µg DNA)
MRD7	<1/ 10 million	<0.00001%	10E-7 (10^{-7})	>200 million	>30 million (120µg DNA)

Measurable residual disease in chronic lymphocytic leukemia: expert review and consensus recommendations

Leukemia (2021) 35:3059–3072

William G. Wierda[1] · Andrew Rawstron[2] · Florence Cymbalista[3] · Xavier Badoux[4] · Davide Rossi[5] · Jennifer R. Brown[6] · Alexander Egle[7] · Virginia Abello[8] · Eduardo Cervera Ceballos[9] · Yair Herishanu[10] · Stephen P. Mulligan[11] · Carsten U. Niemann[12] · Colin P. Diong[13] · Teoman Soysal[14] · Ritsuro Suzuki[15] · Hoa T. T. Tran[16] · Shang-Ju Wu[17] · Carolyn Owen[18] · Stephan Stilgenbauer[19] · Paolo Ghia[20] · Peter Hillmen[21]

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ERIC MRD guidelines 2022: detectable vs undetectable

	Detectable		Undetectable		
MRD classification	**MRD range**	**assay/sample limit of detection**	**upper limit of MRD**	**lower limit of MRD**	**assay/sample limit of detection**
MRD2	10E-3 to <10E-2 0.1% to <0.99%	≤10E-3	<10E-2	Not known	≥10E-3
MRD3	10E-4 to <10E-3 0.01% to <0.099%	≤10E-4	<10E-3	Not known	≥10E-4
MRD4	10E-5 to <10E-4 0.001% to <0.0099%	≤10E-5	<10E-4	Not known	≥10E-5
MRD5	10E-6 to <10E-5 0.0001% to <0.00099%	≤10E-6	<10E-5	Not known	≥10E-6
MRD6	10E-7 to <10E-6 0.00001% to <0.000099%	≤10E-7	<10E-6	Not known	≥10E-7
MRD7	10E-8 to <10E-7 0.000001% to <0.0000099%	≤10E-8	<10E-7	Not known	≥10E-8

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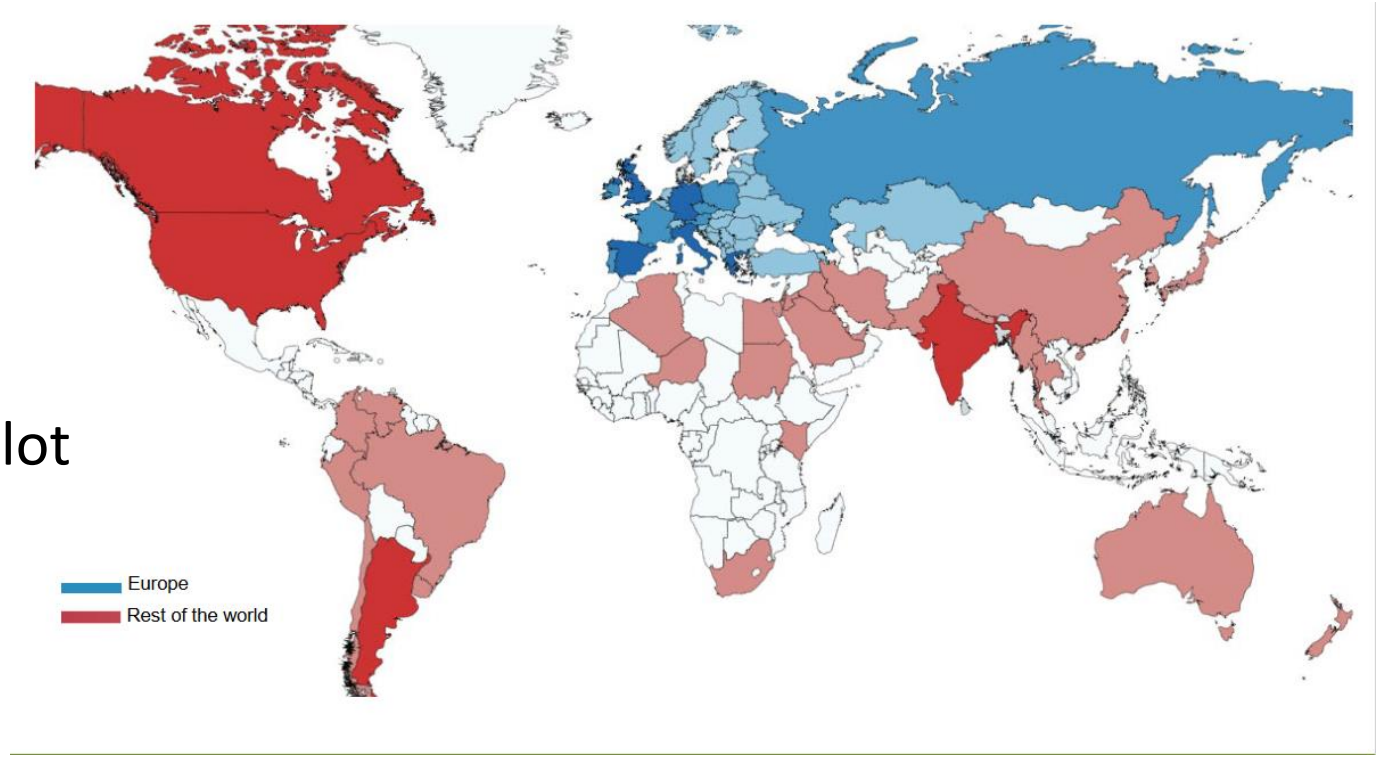
- Cellular technical approach
  - Data set to test inclusion of ROR1 ready to distribute
  - Cellular analysis requirements developed and tested
  - Analysis certification program in pilot
- Operational considerations
  - Further polling for key timepoints
- Reporting MRD results



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