RCPAQAP

The Royal College of Pathologists of Australasia
Quality Assurance Programs

HA/2159

Haematology Immunophenotyping 2023

Survey Report

Survey 2, Closing Date 28 August 2023

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Haematology

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Survey: 2 **Open Date: 14 August 2023** **Closing Date: 28 August 2023** **Report Issue Date: 19 September 2023**

Summary of Performance

Target Source = Specific Target (Assessment is based on the z-score) **z-score = 2.0 – 3.0 (requires review)** **z-score = >3.0 (requires action)**

Performance Assessment

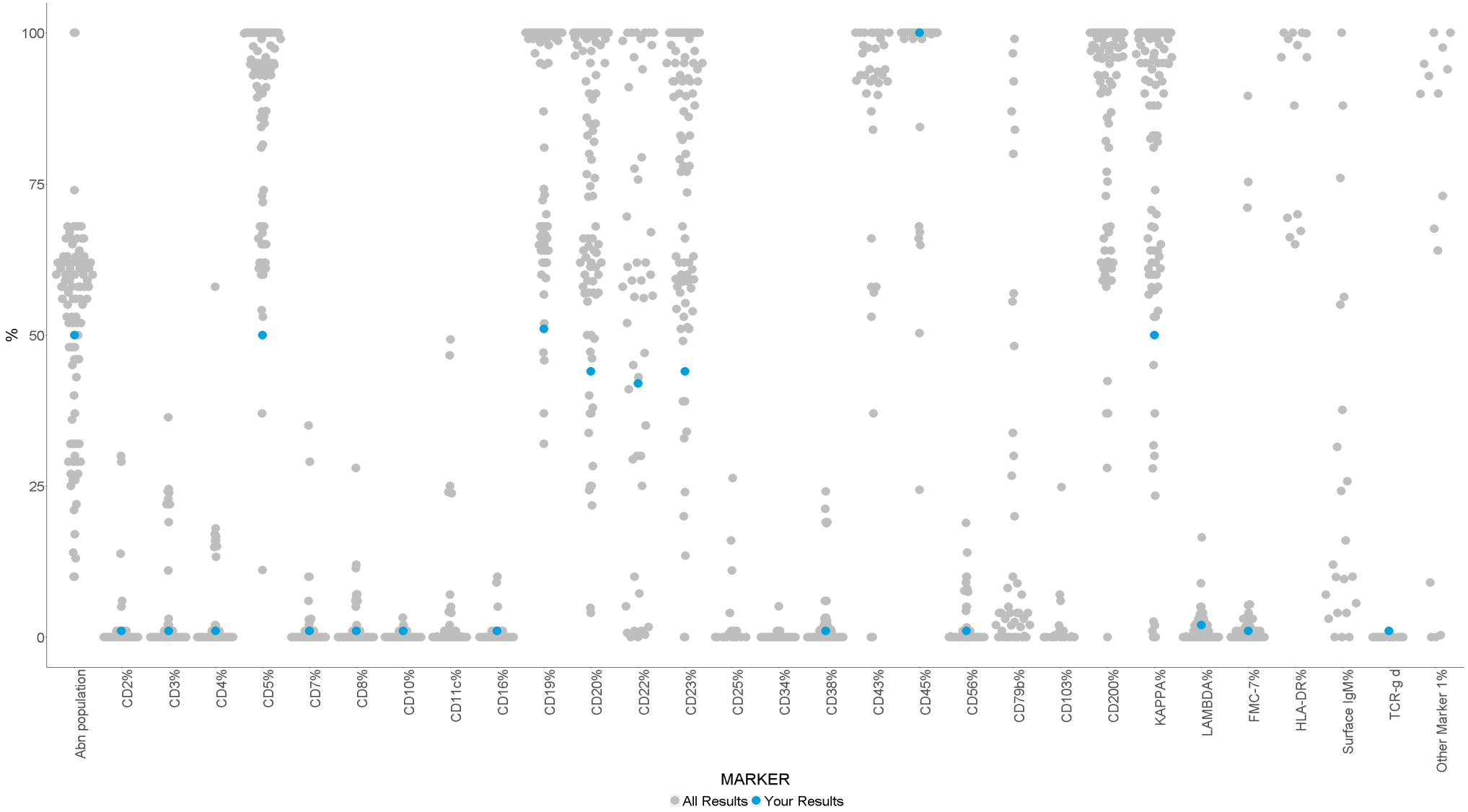
	Sample: HA-IP-23-02				
Test	**Your Result**	**Mean/Expected Result**	**Review**	**Z-score**	**nPart**
CD4%	1	3.7		-0.3	67
CD4 - Interpretation	Negative	Negative	Concordant		68
CD5%	50	86.8		-2.1	95
CD5 - Interpretation	Positive	Positive	Concordant		96
CD8%	1	1.7		-0.2	67
CD8 - Interpretation	Negative	Negative	Concordant		68
CD10%	1	0.3		1.3	89
CD10 - Interpretation	Negative	Negative	Concordant		91
CD19%	51	86.8		-1.9	95
CD19 - Interpretation	Positive	Positive	Concordant		96
CD20%	44	73.8		-1.2	97
CD20 Interpretation	Positive	Positive	Concordant		98
CD22%	42	53.8		-0.3	48
CD22 - Interpretation	Positive	Positive	Concordant		49
CD23%	44	74.6		-1.2	86
CD23 - Interpretation	Positive	Positive	Concordant		87
CD200%		84.2			81
CD200 - Interpretation		Positive	Not Assessed		82
KAPPA%	50	76.4		-0.9	96
KAPPA - Interpretation	Positive	Positive	Concordant		97
LAMBDA%	2	1.4		0.3	92
LAMBDA - Interpretation	Negative	Negative	Concordant		94
FMC-7%	1	5.7		-0.3	51
FMC-7 - Interpretation	Negative	Negative	Concordant		51
Diagnostic Interpretation	CLL/SLL-MBL	CLL/SLL-MBL	Concordant		99

Overall Performance

All results returned match target result.

Sample HA-IP-23-02 - Result review

Markers reported by greater than 10 participants



Sample HA-IP-23-02 - Result review

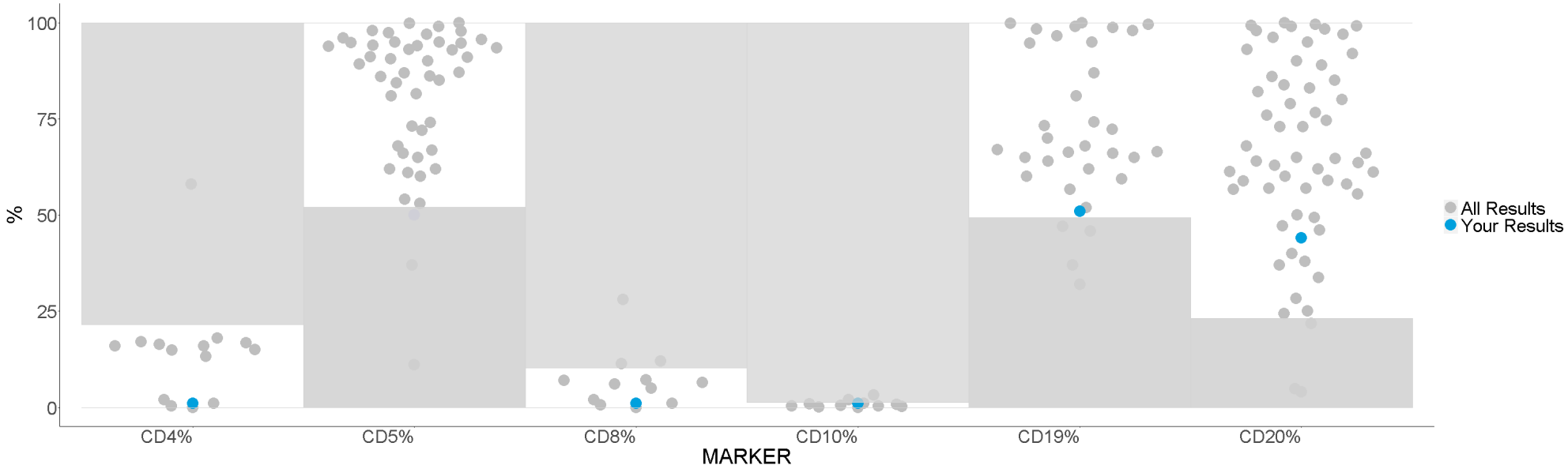
All Markers reported by Participants

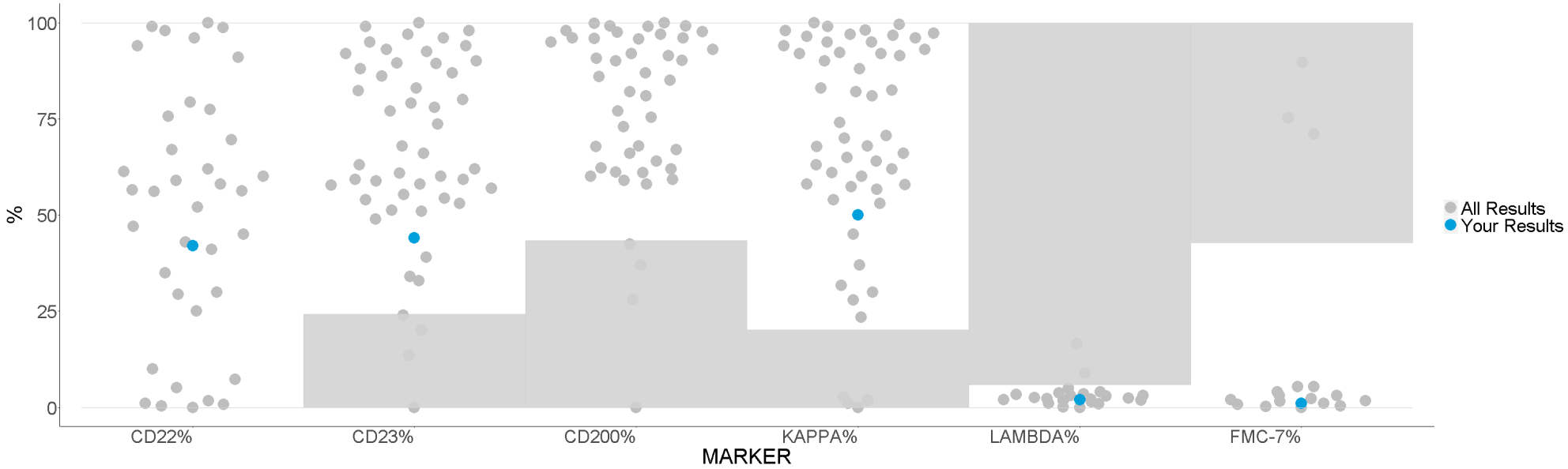
Marker	Median	Mean	Min	Max	nPart
Abnormal population	57.0	51.0	10.0	100.0	100
CD1a %	0.0	0.0	0.0	0.0	2
CD2%	0.0	2.6	0.0	30.0	35
CD3%	0.0	3.8	0.0	36.4	83
cytoplasmic-CD3%	1.0	9.6	1.0	26.7	3
CD4%	0.0	3.7	0.0	58.0	67
CD5%	94.7	86.8	11.1	100.0	95
CD7%	0.0	2.9	0.0	35.0	37
CD8%	0.0	1.7	0.0	28.0	67
CD10%	0.0	0.3	0.0	3.2	89
CD11c%	0.0	5.6	0.0	49.3	35
CD13%	0.0	0.0	0.0	0.0	1
CD14%	0.0	0.0	0.0	0.0	2
CD16%	0.0	1.1	0.0	10.0	30
CD19%	100.0	86.8	32.0	100.0	95
CD20%	76.6	73.8	4.0	100.0	97
CD22%	57.3	53.8	0.0	100.0	48
CD23%	82.7	74.6	0.0	100.0	86
CD25%	0.0	3.2	0.0	26.3	19
CD33%	0.0	0.0	0.0	0.0	1
CD34%	0.0	0.3	0.0	5.1	25
CD38%	0.4	2.3	0.0	24.1	66
CD43%	93.0	83.4	0.0	100.0	37
CD45%	100.0	95.3	24.4	100.0	59
CD52%	99.9	99.8	99.5	100.0	3
CD56%	0.0	2.2	0.0	18.9	57
CD57%	1.0	2.4	0.0	8.0	5
CD71%	35.1	35.1	35.1	35.1	1
cytoplasmic-CD79a%	100.0	100.0	100.0	100.0	2
CD79b%	4.0	20.7	0.0	99.0	43
CD103%	0.0	2.2	0.0	24.8	19
CD117%	0.0	0.0	0.0	0.0	1
CD123%	0.0	0.5	0.0	3.2	8
CD138%	0.5	0.5	0.0	1.0	2
CD200%	94.9	84.2	0.0	100.0	81

Marker	Median	Mean	Min	Max	nPart
KAPPA%	90.0	76.4	0.0	100.0	96
LAMBDA%	1.0	1.4	0.0	16.5	92
FMC-7%	1.0	5.7	0.0	89.6	51
HLA-DR%	96.0	86.8	65.0	100.0	14
IgG%	1.5	1.5	1.4	1.5	2
Surface IgM%	11.0	26.2	0.0	100.0	22
TdT%	0.0	0.0	0.0	0.0	1
TCR alpha beta%	0.0	0.0	0.0	0.0	3
TCR gamma delta%	0.0	0.2	0.0	1.0	15
Other Marker 1%	89.9	64.9	0.0	100.0	15
Other Marker 2%	0.8	5.5	0.0	29.0	6
Other Marker 3%	6.8	35.0	1.9	96.4	3

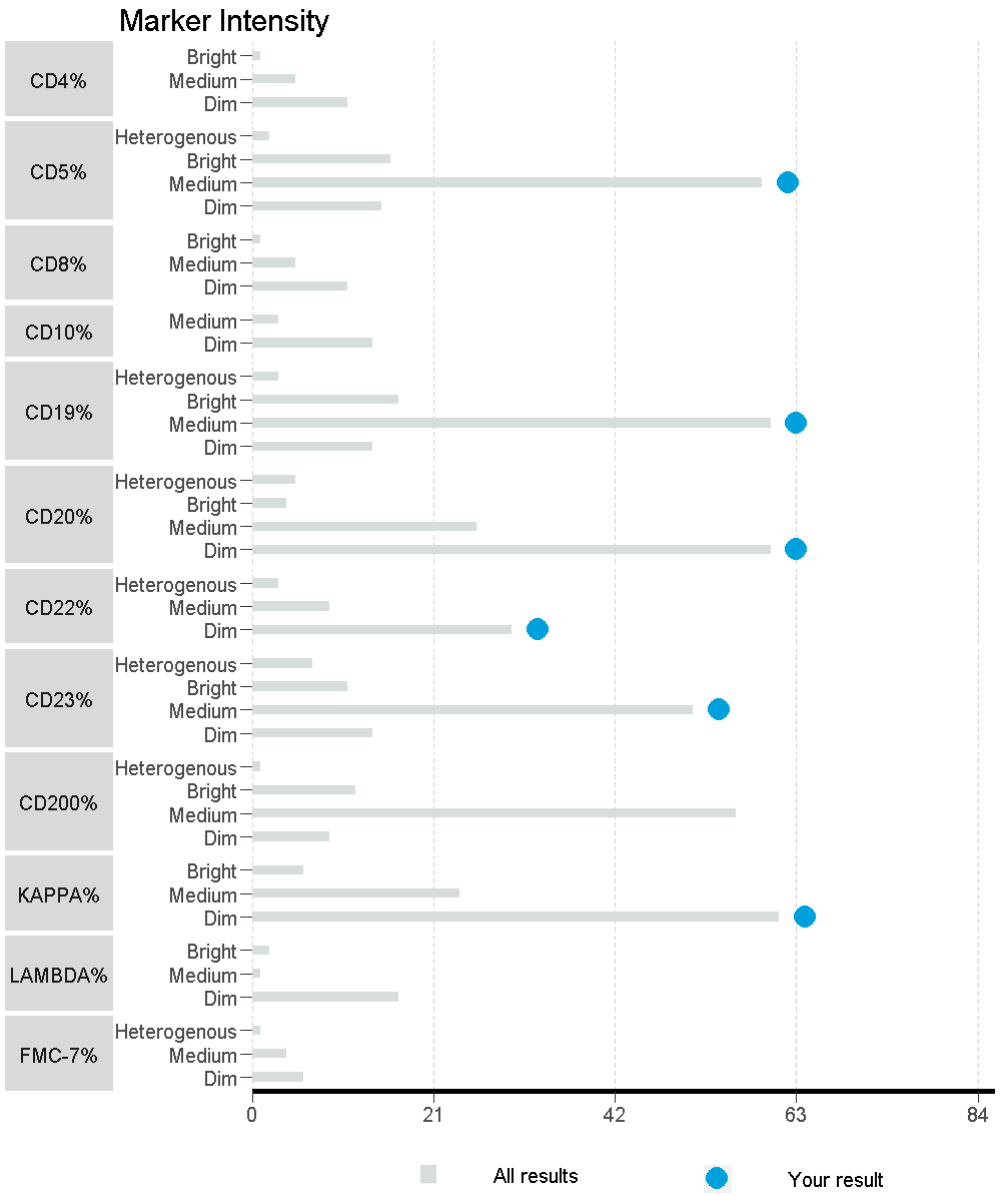
Sample HA-IP-23-02 - Result review

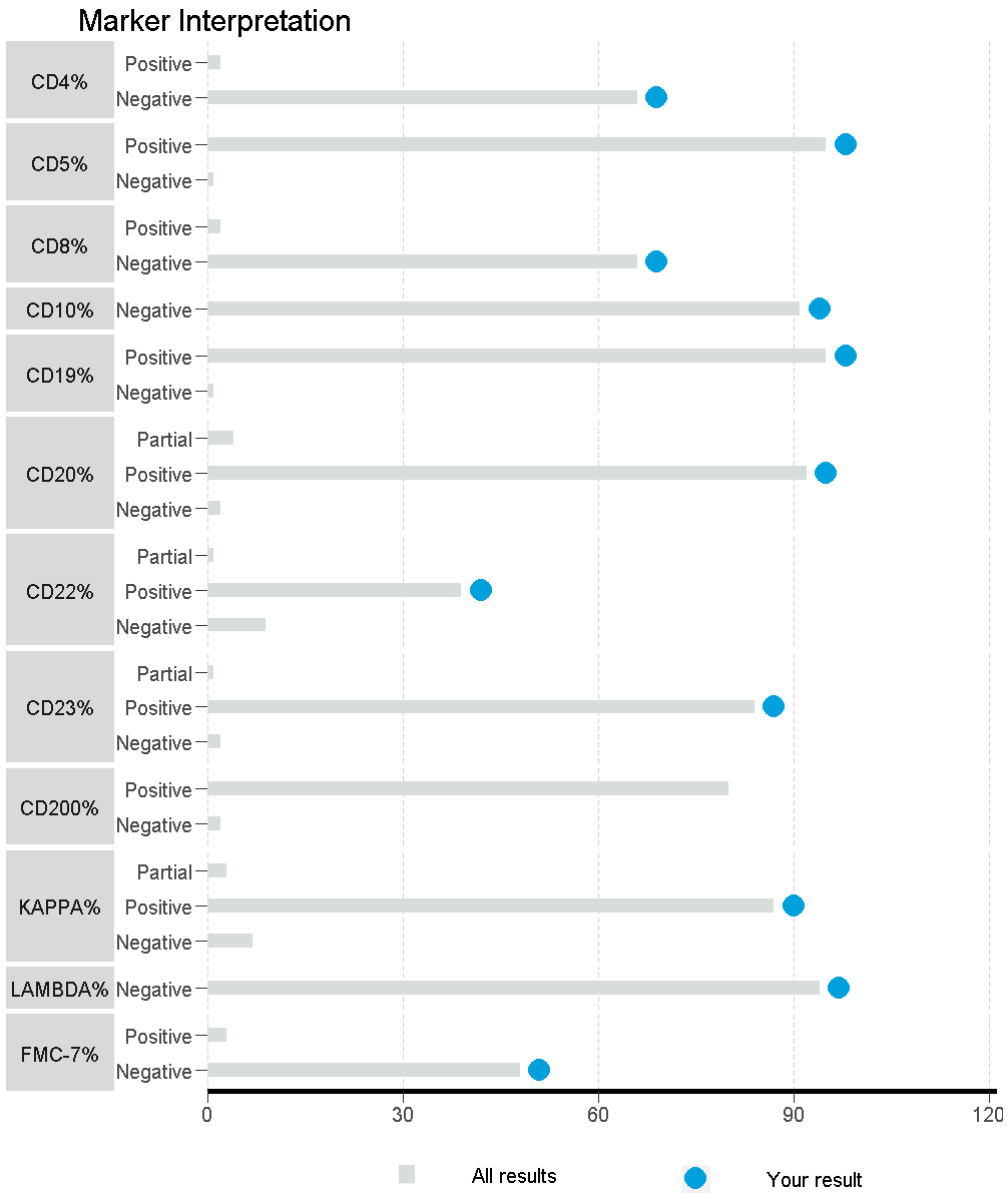
Markers analysed for assessment: Shaded area represent results outside the mean +/- 2SD





Sample HA-IP-23-02 - Result review

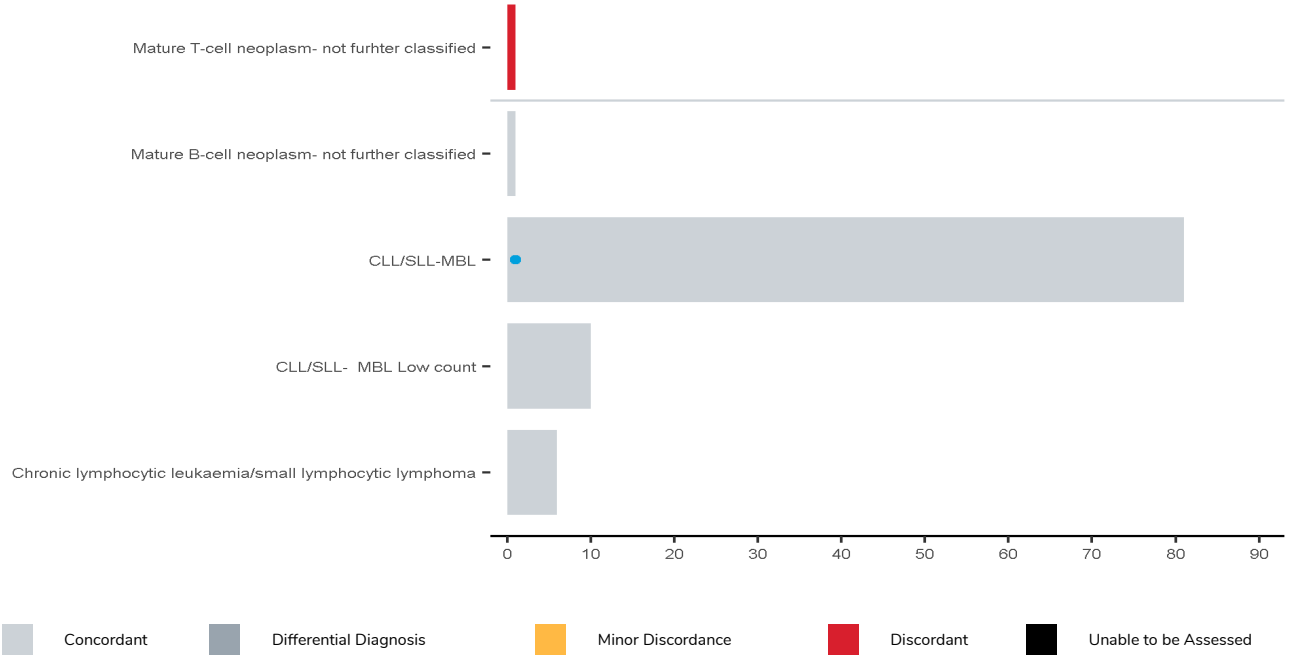




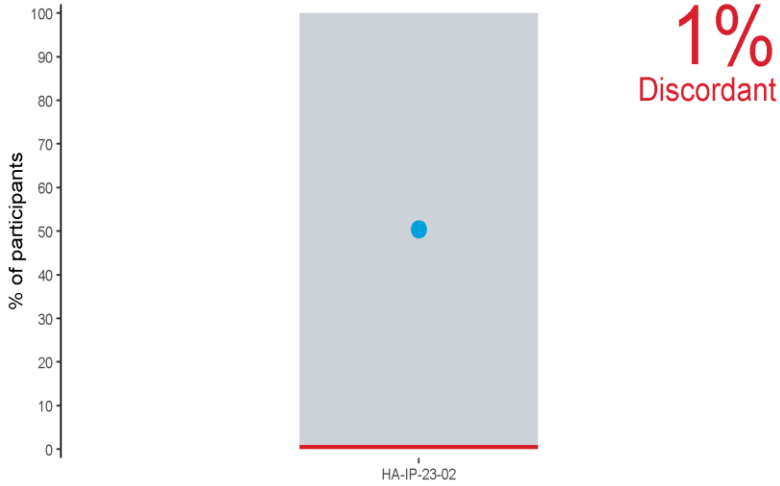
Interpretation

Clinical Notes	57 year old , male. On examination, abdomen was soft and non-tender and no palpable lymphadenopathy or organomegaly. Test LPD panel. WBC: 8.0 x10^9/L; RBC: 4.23 x10^12/L; HB:144 g/L; HCT: 0.42 L/L; MCV: 100 fL; MCHC: 342 g/L; PLT: 195 x10^9/L; Neutrophils: 3.3 x10^9/L ; Lymphocytes: 4.0 x 10^9/L; Monocytes: 0.5 x10^9/L; Eosinophils: 0.2 x10^9/L; Basophils: 0.1 x10^9/L.		
Target Diagnosis	CLL/SLL-MBL	**Your Result**	CLL/SLL-MBL
No. of participants	99	**Assessment**	Concordant

Participant Responses



Assessment Review



Discussion:

Source and preparation of samples

The survey sample was from a 57-year-old male. On examination, abdomen was soft and non-tender and no palpable lymphadenopathy or organomegaly. The full blood count results given were: WCC: $8.0x10^9$/L, RCC: $4.23x10^{12}$/L; Hb: 144 g/L, PLT: 174 x 10^9/L; HCT: 0.42 L/L; MCV: 100fL; MCHC: 342 g/L; PLT: 195 x10^9/L; Neutrophils:3.3 x 10^9/L; Lymphocytes: 4.0x 10^9/L; Monocytes: 0.5x 10^9/L; Eosinophils: 0.2x 10^9/L; Basophils: $0.1x10^9$/L. A digital image of the stained peripheral blood provided the images for the case study.

A peripheral blood sample was collected in lithium heparin, stabilised, aliquoted and dispatched on the same day. Participants were instructed to process the sample within 24 hours of arrival. The change in sample type (from cryopreserved to stabilised peripheral blood) is to provide a representative "real-time" sample. Also, the stabilised sample eliminates artefact induced by cryopreservation and subsequent thawing of samples[1].

Immunophenotyping of Case HA-IP-21-03

The peripheral blood film showed a population of small to intermediate-sized lymphoid cells with clumped chromatin and scant cytoplasm (Figure 1a and 1b). There were smudge cells noted on the blood film. The abnormal lymphoid population has the following immunophenotype (compared to normal B cell expression as recommended by Bethesda guidelines[2]): CD5+ CD19+ CD20+dim CD22+dim CD23+ CD43+ CD200+ and κ+. The abnormal population does not express CD3, CD4, CD7, CD8, CD10, CD38, CD56, FMC7, and λ.

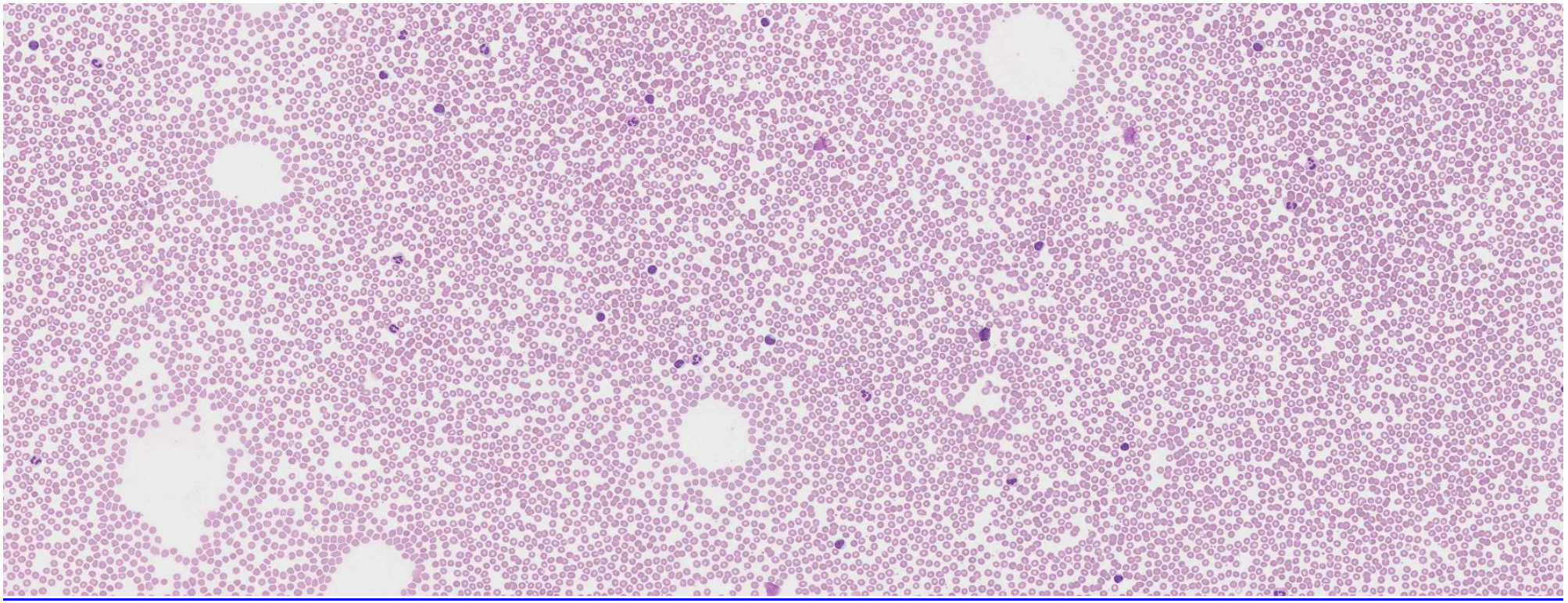


Figure 1a: HA-IP-23-02 Digital image low magnification (20x)

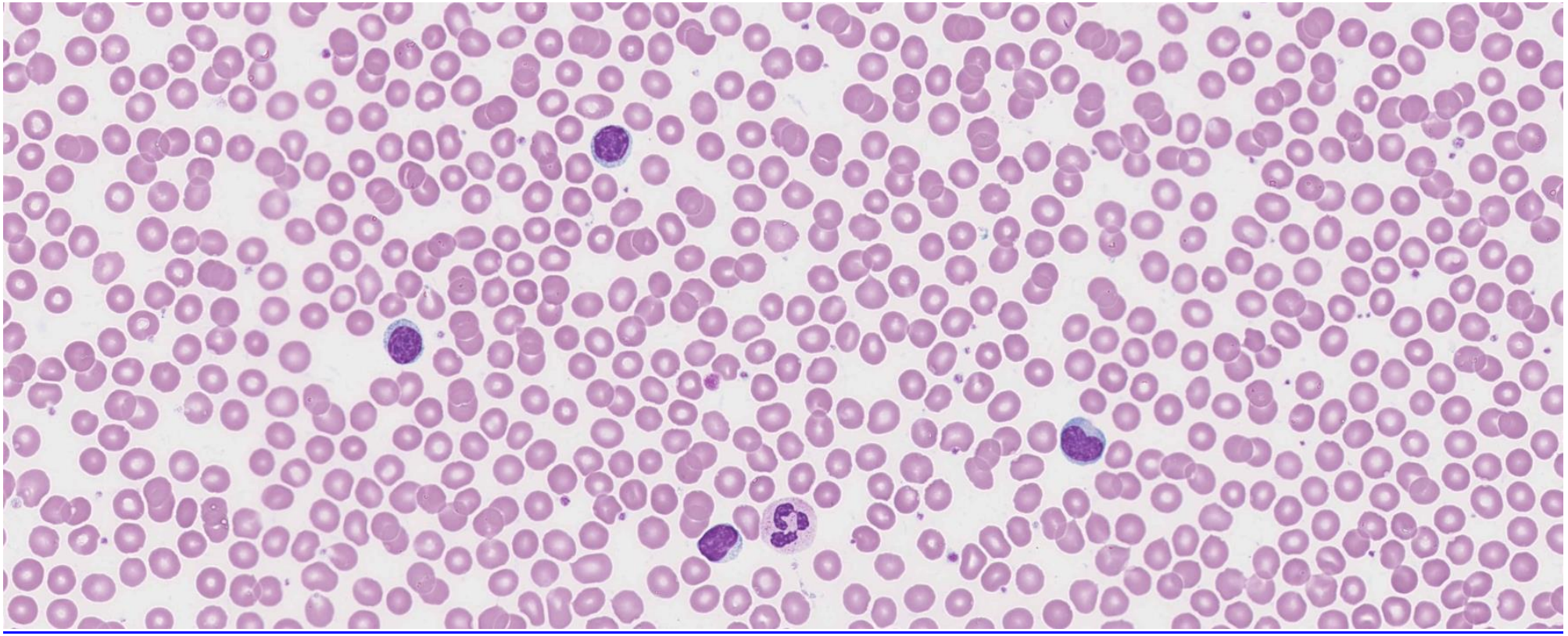


Figure 1b: HA-IP-23-02 Digital Image higher magnification (40x)

Interpretation of results

The immunophenotype of this case is consistent with a clonal B-lymphoproliferative disorder. The immunophenotype in conjunction with the WCC or lymphocytes count (~2.4 x 10^9/L clonal cells) was most consistent with Monoclonal B-cells lymphocytosis - chronic lymphocytic leukaemia type (high count)[3,4,5].

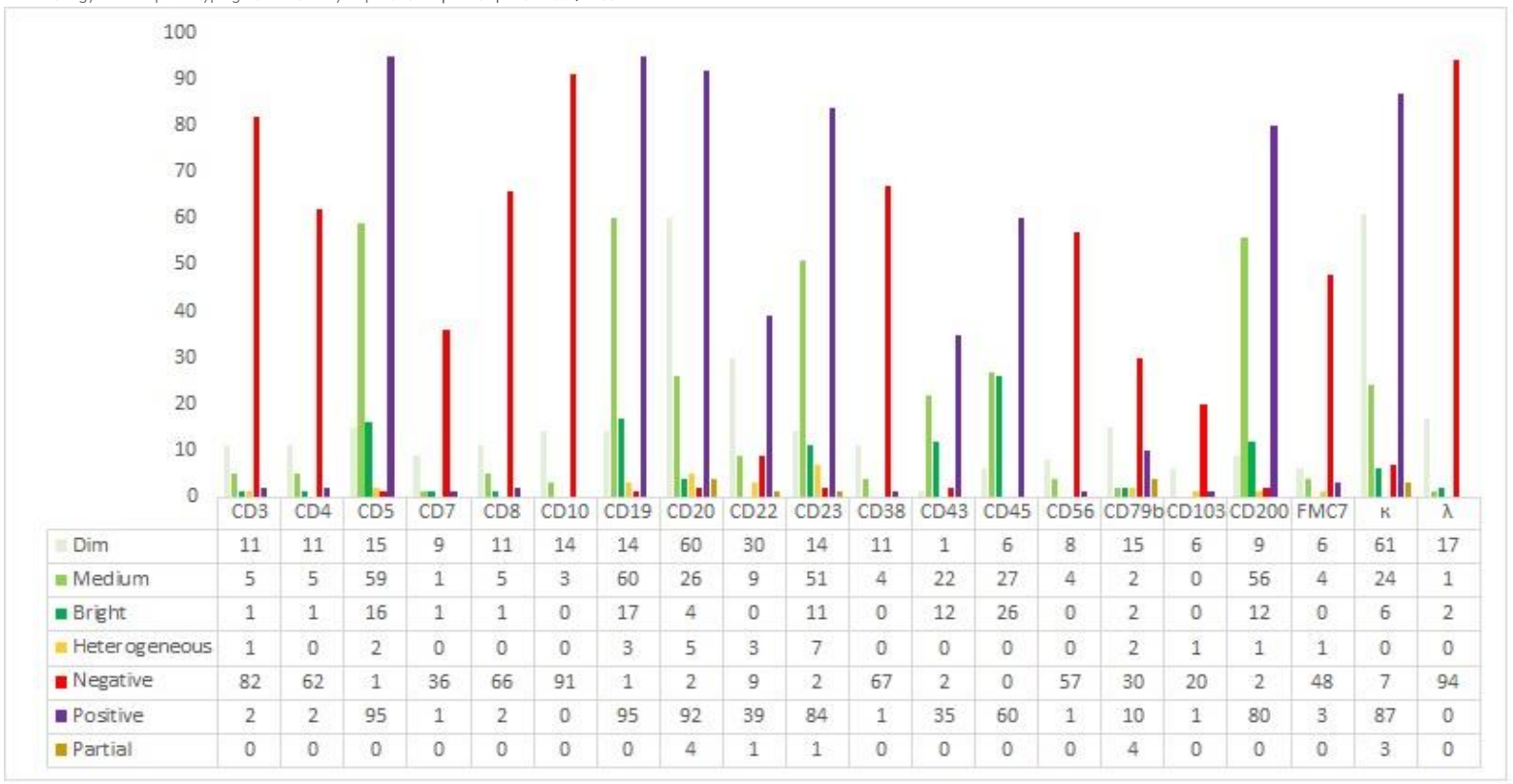
Comments on survey performance

The reported abnormal population accounted for a median of 57% of the total lymphocyte population, mean 51%, SD of 17.3 and a CV of 33.6%. The instruction to participants was to report the percentage positivity of the malignant (abnormal) population. (The denominator for the abnormal lymphoid population is total lymphocytes.)

Table 1 and the bar graph (figure 2) on page 11 of this report illustrate a summary of selected markers.

Table 1: Selected CD markers statistical analysis

CD Marker	Median	Mean	S.D	CV	No.
CD3	0.0	2.4	6.6	271.4	84
CD4	0.0	3.5	8.8	254.4	68
CD5	94.7	94.7	17.4	20.0	96
CD7	0.0	2.6	7.6	261.8	37
CD8	0.0	1.1	2.4	212.8	68
CD10	0.0	0.2	0.4	179.7	91
CD19	100.0	86.8	18.7	21.6	96
CD20	76.6	73.8	25.3	34.3	98
CD22	57.3	53.8	34.4	64.0	49
CD23	82.7	74.6	25.2	33.7	87
CD38	0.4	1.5	3.9	260.0	68
CD43	93.0	84.1	25.7	30.6	37
CD45	100.0	98.1	7.6	7.7	60
CD56	0.0	2.0	3.8	189.8	58
CD79b	4	20.7	31.8	153.6	44
CD103	0.0	1.0	2.1	211.4	21
CD200	94.9	84.2	20.4	24.2	82
FMC7	1.0	2.7	10.7	403.1	51
Kappa	90.0	76.4	28.1	36.8	97
Lambda	1.0	1.3	2.1	155.9	94



	CD3	CD4	CD5	CD7	CD8	CD10	CD19	CD20	CD22	CD23	CD38	CD43	CD45	CD56	CD79b	CD103	CD200	FMC7	κ	λ
Dim	11	11	15	9	11	14	14	60	30	14	11	1	6	8	15	6	9	6	61	17
Medium	5	5	59	1	5	3	60	26	9	51	4	22	27	4	2	0	56	4	24	1
Bright	1	1	16	1	1	0	17	4	0	11	0	12	26	0	2	0	12	0	6	2
Heterogeneous	1	0	2	0	0	0	3	5	3	7	0	0	0	0	2	1	1	1	0	0
Negative	82	62	1	36	66	91	1	2	9	2	67	2	0	57	30	20	2	48	7	94
Positive	2	2	95	1	2	0	95	92	39	84	1	35	60	1	10	1	80	3	87	0
Partial	0	0	0	0	0	0	0	4	1	1	0	0	0	0	4	0	0	0	3	0

Figure 2: HA-IP-23-02 Intensity and Interpretation for selected markers

There was a consensus in reporting a negative interpretation for CD3, CD4, CD8, CD10, FMC7 and lambda. Similarly, a consensus was achieved for the expression of CD5, CD19, CD20, CD22, CD23, CD43, CD200 and κ. The partial expression for CD79b was evident in the returned results where there was a 30 participant returned a negative and 10 participants returned a positive interpretation. It is pleasing to note the number of participants incorporating CD23 (87) and CD200 (82) on their panel to differentiate MCL from CLL/SLL/ CLL-type MBL.

Interpretation of comments on diagnosis

The patient had a history of CLL/SLL- MBL and attended the haematology clinic to investigate disease progression. In conjunction with the clinical notes, survey image and the positive interpretation of key markers (CD5, CD19, CD20, CD23, CD43, & CD200) and the absence of CD10 and FMC7 supports the diagnosis.

Eighty-two per cent (81/99) of participants submitted the target diagnosis. CLL/SLL-MBL low count, CLL/SLL and mature B-cell neoplasm, not further classified was considered a differential diagnosis. The returned results did not demonstrate the presence of an abnormal T cells. Therefore, a final interpretation of Mature T-cell neoplasm, not further classified was considered discordant.

References

1. Preijers F.W., et al., Fifteen years of external quality assessment in leukaemia/lymphoma immunophenotyping in the Netherlands and Belgium: a way forward. Cytometry Part B (Clinical Cytometry), 2016. 90B: 267-278.
2. 2006 Bethesda International Consensus Recommendation on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry: Optimal reagents and Reporting for the Flow Cytometric Diagnosis of Haematopoietic Neoplasia. Cytometry Part B (Clinical Cytometry) 72B: S14-S22 (2007).
3. Rawstron AC, et al. Reproducible diagnosis of Chronic Lymphocytic Leukemia by Flow Cytometry: An European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytometry Part B (Clinical Cytometry). 2018 Jan; 94:121-128
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5. Alaggio R, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022 Jul;36(7):1720-1748.

HA-IP-23-01: Correction.

The target interpretation for CD7 and CD25 should be Positive and Negative respectively.

Cumulative assessment

Survey 2	
Measurand	**Assessment**
CD4%	
CD4 - Interpretation	Concordant
CD5%	
CD5 - Interpretation	Concordant
CD8%	
CD8 - Interpretation	Concordant
CD10%	
CD10 - Interpretation	Concordant
CD19%	
CD19 - Interpretation	Concordant
CD20%	
CD20 Interpretation	Concordant
CD22%	
CD22 - Interpretation	Concordant
CD23%	
CD23 - Interpretation	Concordant
CD200%	
CD200 - Interpretation	Not Assessed
KAPPA%	
KAPPA - Interpretation	Concordant
LAMBDA%	
LAMBDA - Interpretation	Concordant
FMC-7%	
FMC-7 - Interpretation	Concordant
Diagnostic Interpretation	Concordant

Survey 1	
Measurand	**Assessment**
CD2%	
CD2 - Interpretation	Not Assessed
CD3%	
CD3 - Interpretation	Not Assessed
CD4%	
CD4 - Interpretation	Not Assessed
CD5%	
CD5 - Interpretation	Not Assessed
CD7%	
CD7 - Interpretation	Not Assessed
CD8%	
CD8 - Interpretation	Not Assessed
CD10%	
CD10 - Interpretation	Not Assessed
CD16%	
CD16 - Interpretation	Not Assessed
CD23%	
CD23 - Interpretation	Not Assessed
CD25%	
CD25 - Interpretation	Not Assessed
CD45%	
CD45 - Interpretation	Not Assessed
CD56%	
CD56 - Interpretation	Not Assessed
Diagnostic Interpretation	Not Assessed