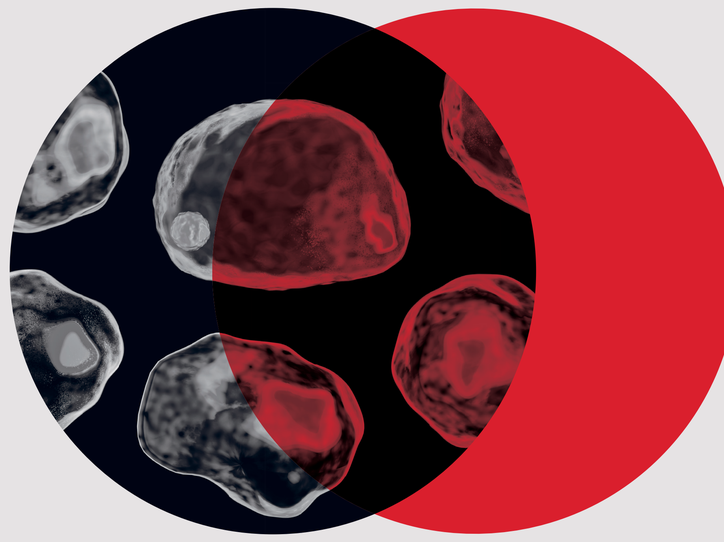
High variation in reporting of CD21^{lo} B cell population during a pilot external Quality Assurance Program



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Introduction

The diagnosis of Common Variable Immunodeficiency Disease (CVID) is complex and often delayed due to the multifaceted clinical phenotypes and manifestations. Immunophenotyping of B cells has become a valuable tool for the classification of immunodeficiency diseases, and particularly for CVID[1,2]. However, there is limited published data on the results of B cell subsets reported by Australasian Laboratories. As such, the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) undertook a 3 year pilot study to assess the variability in B cell analysis and reporting obtained from clinical immunology laboratories across Australia and New Zealand.

Method

Participating laboratories were provided with donor whole blood aliquots and performed flow cytometric analysis according to their standard methods. Samples from 2017 to 2018 were collected in lithium heparin tubes and dispatched in a cell culture medium or Streck™ cell preservative. Samples from 2019 onwards were collected in EDTA tubes and sent as neat aliquots.

Results for various B cell subset markers were returned to the RCPAQAP and analysed by examining the Coefficient of Variation (CV%) and a Bartlett's test to determine changes in variation across programs. A *p*-value of <0.05 for all analyses was considered significant.

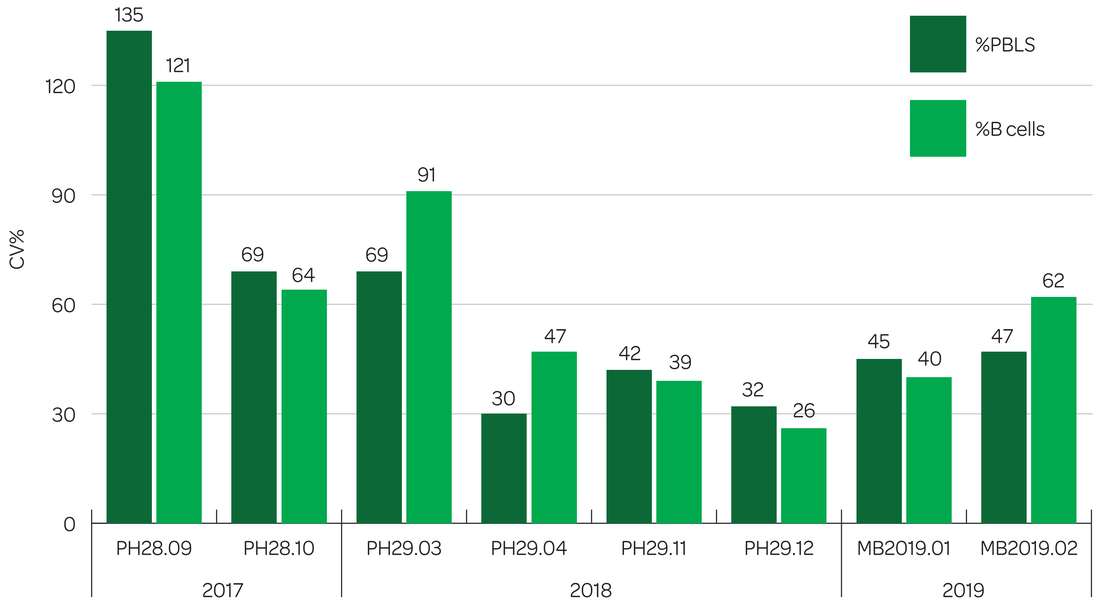


Figure 1. Coefficient of variation (CV%) for CD21^{lo} from 2017–2019. CV% is shown as a percentage of PBLs (dark green bars) and percentage of total B cells (light green bars).

The pilot program began in 2016, however results were so variable, that no meaningful analysis of the data could be performed. The CV% remained consistent for the various subset markers except the CD21^{lo} population, when reported as a percentage of both peripheral blood lymphocytes (PBL) and total B cells (Figure 1 and Table 1).

The CV% in the 2017 pilot decreased significantly from the first to the second survey, from 135% down to 69% of PBL and 121% down to 64% of total B cells. A further improvement was demonstrated in 2018 from 69% to 30% of PBL and from 91% down to 26% of total B cells. Encouragingly, the CD21^{lo} CV% for the first two surveys in 2019 has remained consistent at 45% and 47% of PBL and 40% and 62% of total B cells, across the participating laboratories. Over three years of pilot studies, variation in CD21^{lo} reported results has decreased for both the percentage of PBL (p<0.0001) and as a percentage of total B cells (p<0.0001) (Figure 2).

Table 1. Mean, SD and CV% of CD21^{lo} from 2017–2019.

Year	Program	Mean %PBL	No. Labs	SD	%CV	Mean %B cell	No. Labs	SD	%CV
2017	PH28.09	0.59	7	0.77	135	5.0	9	6	121
	PH28.10	0.56	7	0.42	69	8.4	9	5.1	64
2018	PH29.03	0.96	8	0.68	72	5.4	11	4.7	87
	PH29.04	1.1	8	0.34	30	8.6	11	4.6	47
	PH29.11	0.43	8	0.17	42	3.5	11	1.4	39
	PH29.12	0.74	8	0.24	32	7.4	11	1.9	26
2019	MB2019.01	1.1	10	0.49	45	10.4	13	4.2	40
	MB2019.02	0.28	9	0.13	47	2.5	12	1.5	62

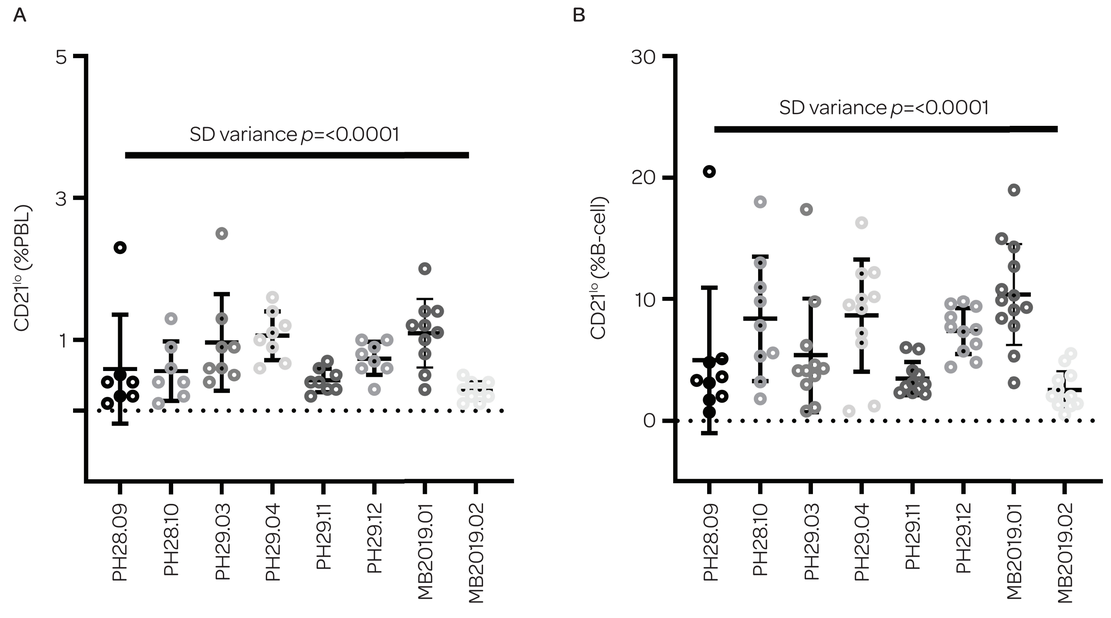
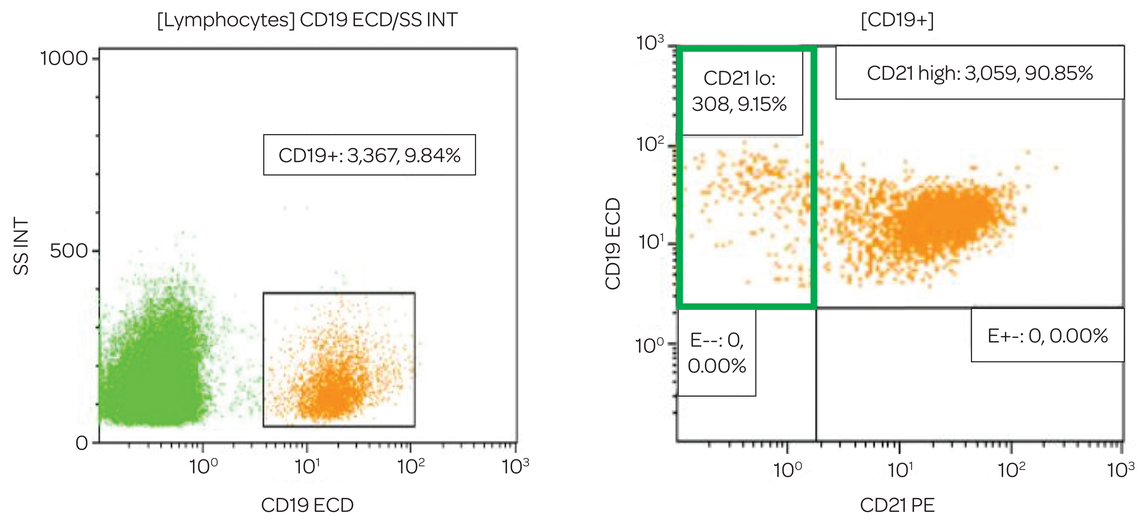


Figure 2. Standard deviation of CD21^{lo} from 2017–2019. Histograms represent data returned to RCPAQAP as a percentage of PBL (**A**) and as a percentage of total B cells (**B**).

Discussion

CD21^{lo} B Cells are IgM+/IgD+ polyclonal unmutated cells and expansion of this population has been reported in CVID patients with autoimmune cytopenia, particularly splenomegaly[3]. However, the enumeration of CD21^{lo} B Cells can be a challenging exercise as there is no clear differentiation of the population. Therefore gating of this marker is often based on subjective judgement or a static gate.



CD21 low cells (proportion of PBL)	0.90% of lymphocytes
CD21 low cells (proportion of B cells)	9.15% of B cells

Figure 3. Representative gating strategy for CD21^{lo} cell population. This demonstrates difficulty in gating CD21^{lo} population (green box) due to the inherent lack of cell surface marker which can give rise to variable subset reporting.

Plots courtesy of Immunology Department, John Hunter Hospital, NSW Health Pathology North.

The initial Memory B cell pilot in 2016 highlighted discrepancies in both the reporting algorithm (i.e. reporting of B cells subsets as % of PBL or B Cells) and the number and type of fluorochromes utilised. RCPAQAP Immunology has since encouraged the sharing of gating strategies and data plots between participating laboratories, facilitating a marked improvement in variation in subsequent years.

Conclusion

We have demonstrated a significant improvement in the variance of reported values for the CD21^{lo} B cell population in participating clinical immunology laboratories. These results indicate the value of external QAP to assess and maintain the quality of assay performance and interpretation thereby allowing clinicians to receive reliable and consistent pathology information for their patients.

References

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