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Life Sciences

A high recovery and high purity data analysis strategy for rare abnormal CD5+ ROR-1+ B cells using the DuraClone RE CLB Tube

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IN THIS PAPER YOU WILL

Follow a mixed boolean / multivariate gating strategy to identify and characterize abnormal mature $CD5^+$ $ROR\text{-}1^+$ B cells with high recovery and high purity using Kaluza 1.5a data analysis software

See an analysis example for a small mature abnormal $CD5^+$ $ROR\text{-}1^+$ B cell population exposing abnormal features compared to the physiological phenotype

See analysis examples of small mature abnormal $ROR\text{-}1^+$ B cell population with dim expression of CD19, CD43 and CD5 and lacking detection of CD20

Introduction

Small populations of abnormal mature B cells can be efficiently detected due to their abnormal expression characteristics of CD5, CD20, CD43, CD79b, CD81 and ROR-1 (Table 1). To assure discrimination from the overlapping phenotype of greatly abundant normal B cell populations, a boolean gating strategy is necessary that cumulates differential expression profiles from each single marker.

ANTIGEN	NORMAL MATURE	ABNORMAL MATURE	NORMAL IMMATURE
CD5	Neg, partially +	+ / ++	Neg, partially +
CD19	++	+ / ++	+ / ++
CD20	++	Low / +	Low / +
CD22	++	Low / +	Low / +
CD43	Neg, partially low / +	+ / ++	Low / + (pre-B)
CD45	++	+ / ++	+
CD79b	+ / ++	Low / +	Low / +
CD81	+ / ++	Low / +	++
ROR-1	Neg	+ / ++	NEG / + (Pre-B)

Table 1. Phenotypic features of mature normal, mature abnormal and immature normal B cells[1,3]. Of note, depending on the donor the B cell expression patterns may deviate from those shown in the table.

The DuraClone RE CLB Tube uses CD45 and CD19 as gating markers with CD45 also enabling quantification of the B cell compartment relative to the leukocyte event count. The antibody doses accommodate staining of 300 µL of whole blood.

DuraClone RE CLB tube

Detection of CD45^{+}CD19^{+}ROR-1^{+}CD5^{++}CD43^{++}CD81^{dim}CD20^{dim}CD79b^{dim} B cells											
DuraClone RE CLB Tube	PB	Kr0	FITC	PE	ECD	PC5.5	PC7	APC	APC-AF700	APC-AF750	Quality Standard
B80393 (25 tests RU0)	CD20	CD45	CD81	ROR-1	-	CD79b	CD19	CD5	-	CD43	ISO 9001-2008

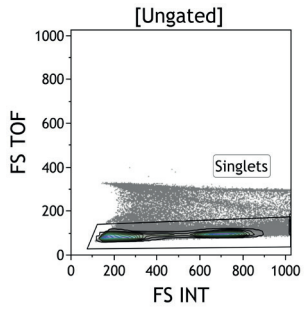
Materials

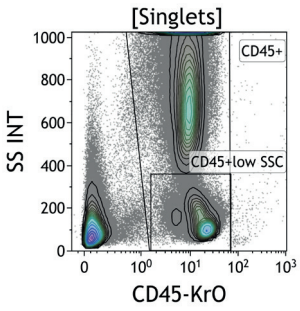
- Kaluza Analysis Software 1.5a or higher.
- Display with 1024×768 resolution minimum (1920×1200 or higher resolution recommended).
- Windows 7 SP1, Windows 8.1 or Window 10 operating system.
- 3Ghz CPU or higher / Quadcore recommended.
- Datasets obtained with the DuraClone RE CLB tube (B80393, please see instructions for use for sample preparation and flow cytometry data acquisition).

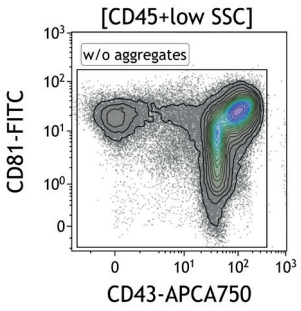
Tips for success

- The better your computer performance is (e.g. ≥3GHz Quadcore CPU), the shorter data recalculation times upon gating adjustments will be when handling large rare event data sets.
- Start with “oversized” gates for high recovery, then increase specificity by narrowing your gates.
- Always double-check plausibility by reviewing the features of identified target cells as colored events in plots that are early in the gating strategy (e.g. scatter gate), in the radar plot.

Gating example for a small mature abnormal CD5$^+$ ROR-1$^+$ B cell population exposing abnormal features compared to the physiological phenotype







1. Removal of doublets

Plot: contour with density

Gate: ungated

Approach: enrichment of singlets by selection of events with lowest Time of Flight (TOF) signals

2. Removal of debris

Plot: contour with density

Gate: singlets

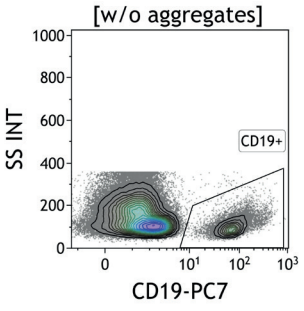
Approach: selection of events with med or higher CD45 and sideward scatter smaller than granulocytes

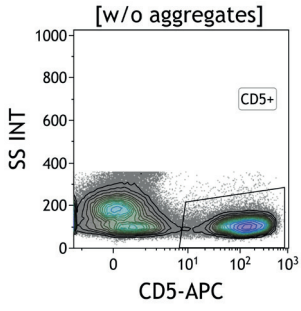
3. Removal of dye aggregates

Plot: contour with density

Gate: CD45$^+$lowSSC

Approach: discrimination of dye aggregates with high APC-AF750 fluorescence and events with high green autofluorescence





The CD5 gate does not have to capture all CD5+ events. This gate is merely to define a T cell-containing reference population for adjusting gates on CD81, ROR-1, CD79b, CD20, CD5 and CD43, please see the following plots.

4. Identification of B cells

Plot: contour with density

Gate: w/o aggregates

Approach: gating of CD19$^+$ events

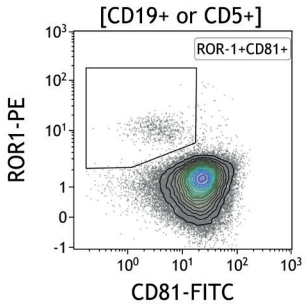
5. Identification of CD5$^+$, including T cells (partial)

Plot: contour with density

Gate: w/o aggregates

Approach: gating of CD5$^+$ events

Boolean



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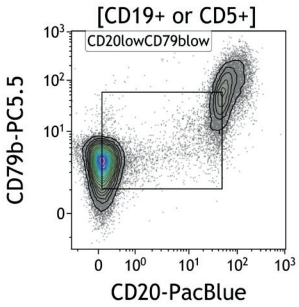

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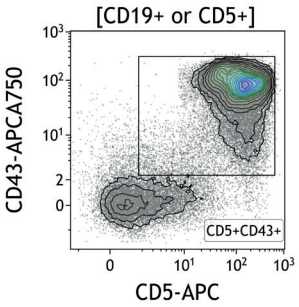


6. Selection of events with abnormal expression pattern for CD81 and ROR-1

Plot: contour with density

Gate: $CD19^{+}$ or $CD5^{+}$

Approach: adjustment of gate encompassing $CD81^{low}$ $ROR\text{-}1^{+}$ events using the $CD81^{++}$ $ROR\text{-}1^{neg}$ population (e.g. T cells) as internal reference

7. Selection of events with abnormal expression pattern for CD20 and CD79b

Plot: contour with density

Gate: $CD19^{+}$ or $CD5^{+}$

Approach: adjustment of gate encompassing $CD20^{low}CD79b^{+/low}$ events using the $CD79b^{++}$ $CD20^{++}$ and the $CD20^{neg}$ $CD79b^{low/neg}$ population (e.g. T cells) as internal reference

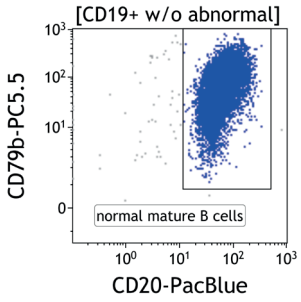
8. Selection of events with abnormal expression pattern for CD5 and CD43

Plot: contour with density

Gate: $CD19^{+}$ or $CD5^{+}$

Approach: adjustment of gate encompassing $CD5^{+/++}$ $CD43^{+/++}$ events using the $CD5^{++}$ $CD43^{++}$ population (e.g. T cells) and the $CD5^{low}/CD43^{neg}$ population (e.g. normal B cells) as internal reference

Boolean



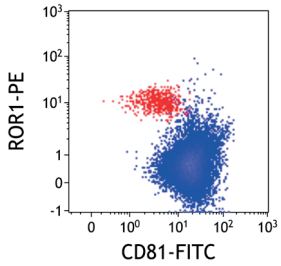
9. Purification of the normal mature B cell population

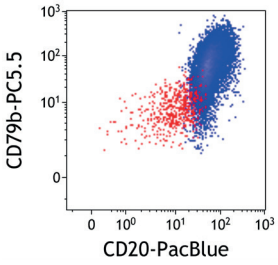
Plot: gate coloring

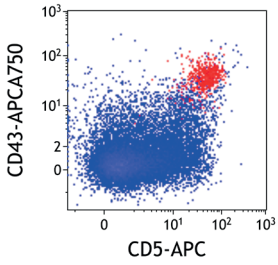
Gate: $CD19^{+}$ w/o abnormal cells

Approach: gating of a homogeneous cluster of $CD20^{++}$ $CD79b^{+/++}$ events

Boolean





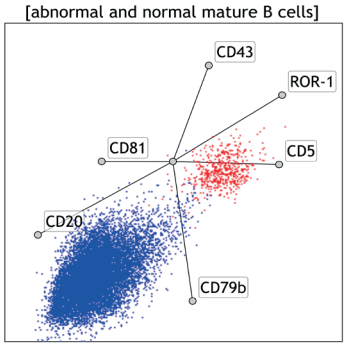


10. Verification of normal and abnormal B cells expression patterns

Plot: density overlay

Gate: a) normal mature B cells, blue
b) abnormal mature B cells, red

Approach: confirmation of differential cluster classification of normal and abnormal B cells



11. Verification of normal and abnormal B cell expression patterns - multivariate view

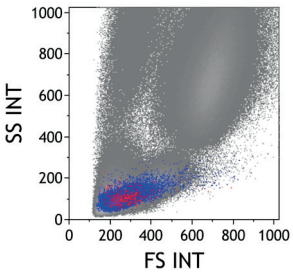
Plot: radar

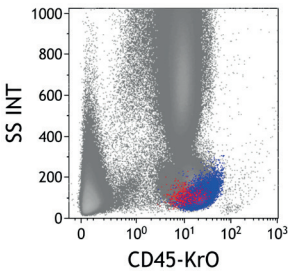
Gate: abnormal and normal mature B cells

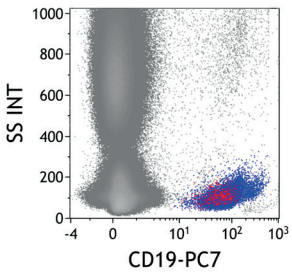
Approach: confirm cluster separation in a 2D projection of CD5, CD20, CD43, CD79b, CD81 and ROR-1.

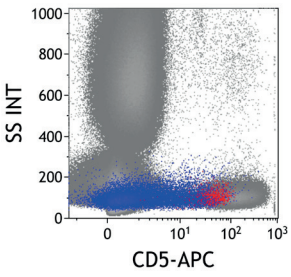
There are many possible radar plot configurations that allow for the shown discrimination. An easy way to set up a functional radar plot is as follows:

1. Positive (ROR-1, CD5, CD43) and negative (CD20, CD79b, CD81) deviations from normal expression should be grouped and these groups should point into opposite directions.
2. Strong deviations in expression should be assigned longer axes than moderate differences in expression. Please note, the cluster of normal B cells will reside in a constant position while the position of the abnormal cluster may vary.









12. Verification of typical scatter and CD5/CD19/CD45 characteristics

Plot: density overlay

Gate: a) w/o aggregates, grey
b) normal mature B cells, blue
c) abnormal mature B cells, red

Approach: confirm typical mature B cell positioning with lymphocyte-like scatter and high CD45 expression, the latter being slightly reduced for mature abnormal B cell events. Furthermore review of CD19-positivity and differential CD5 expression.

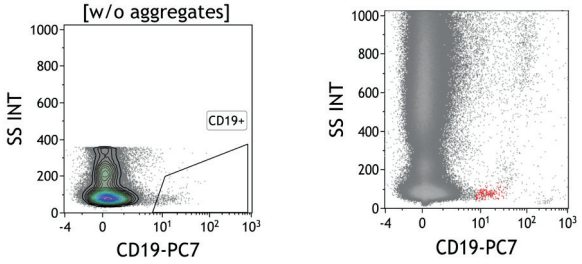
GATE NAME	GATE LOGIC
Abnormal and normal mature B cells	"Abnormal mature B cells" OR "normal mature B cells"
Abnormal mature B cells	$CD19^+$ and ROR-1^+ $CD81^+$ AND $CD20^{low}$ $CD79b^{low}$ AND $CD5^+$ $CD43^+$
$CD19^+$ OR $CD5^+$	$CD19^+$ OR $CD5^+$
$CD19^+$ w/o abnormal	$CD19^+$ and (not "abnormal mature B cells")

GATE	NUMBER
Abnormal mature B cells	584
Normal mature B cells	16,913
$CD45^+$	854,745

The results can be displayed in an Information plot: in the shown example, abnormal B cells have been detected at a frequency of 0.068% $CD45^+$

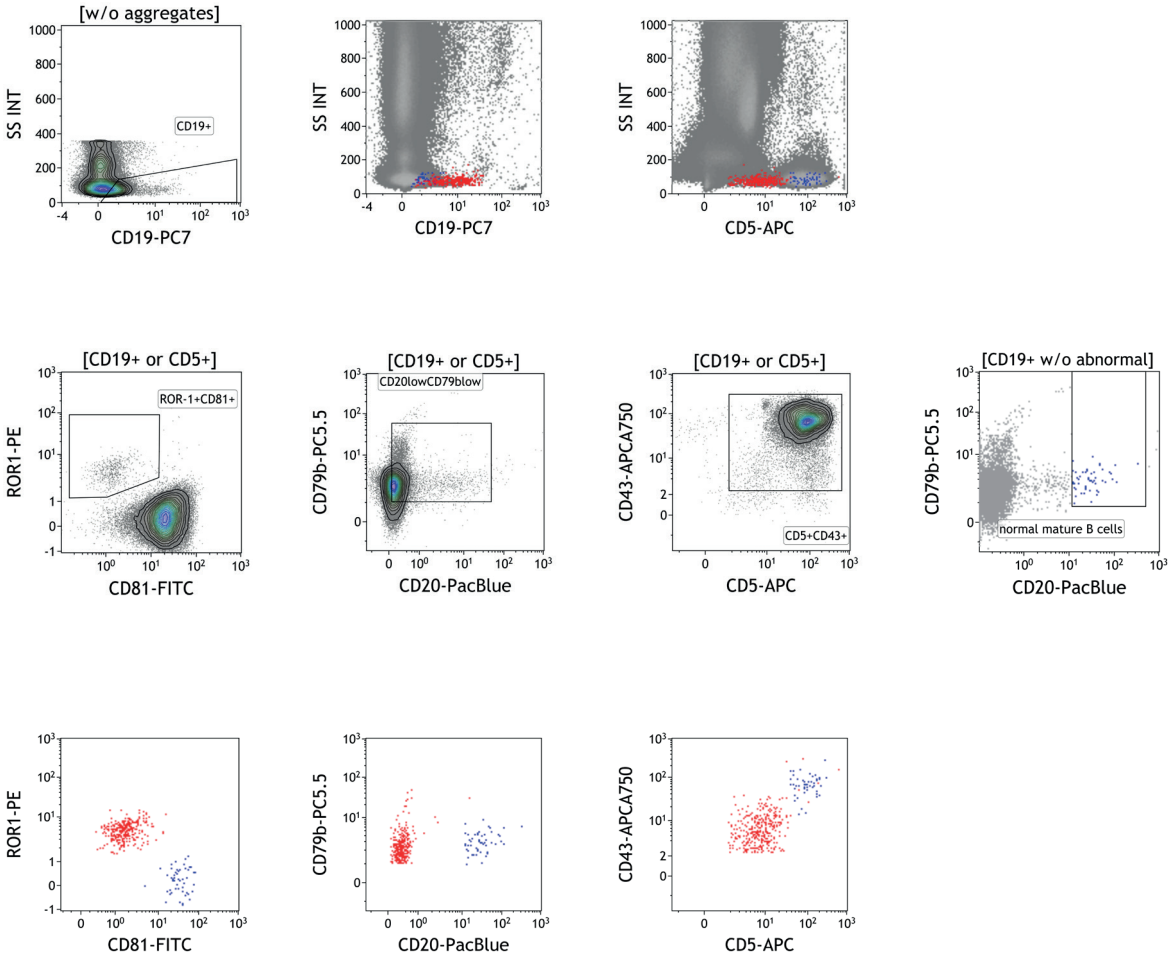
Gating example for a small mature abnormal $CD5^+$ ROR-1^+ B cell population with DIM expression of CD19, CD43 and CD5 and lacking detection of CD20

CD19 positivity is a very early step in the gating sequence to identify mature B cells. As in interventional clinical research studies CD19 expression may be down-regulated, CD19-clustering should be verified in a backgating control display. Also, CD20 detection may be impaired or lost in context of anti-CD20 targeting in clinical research studies.



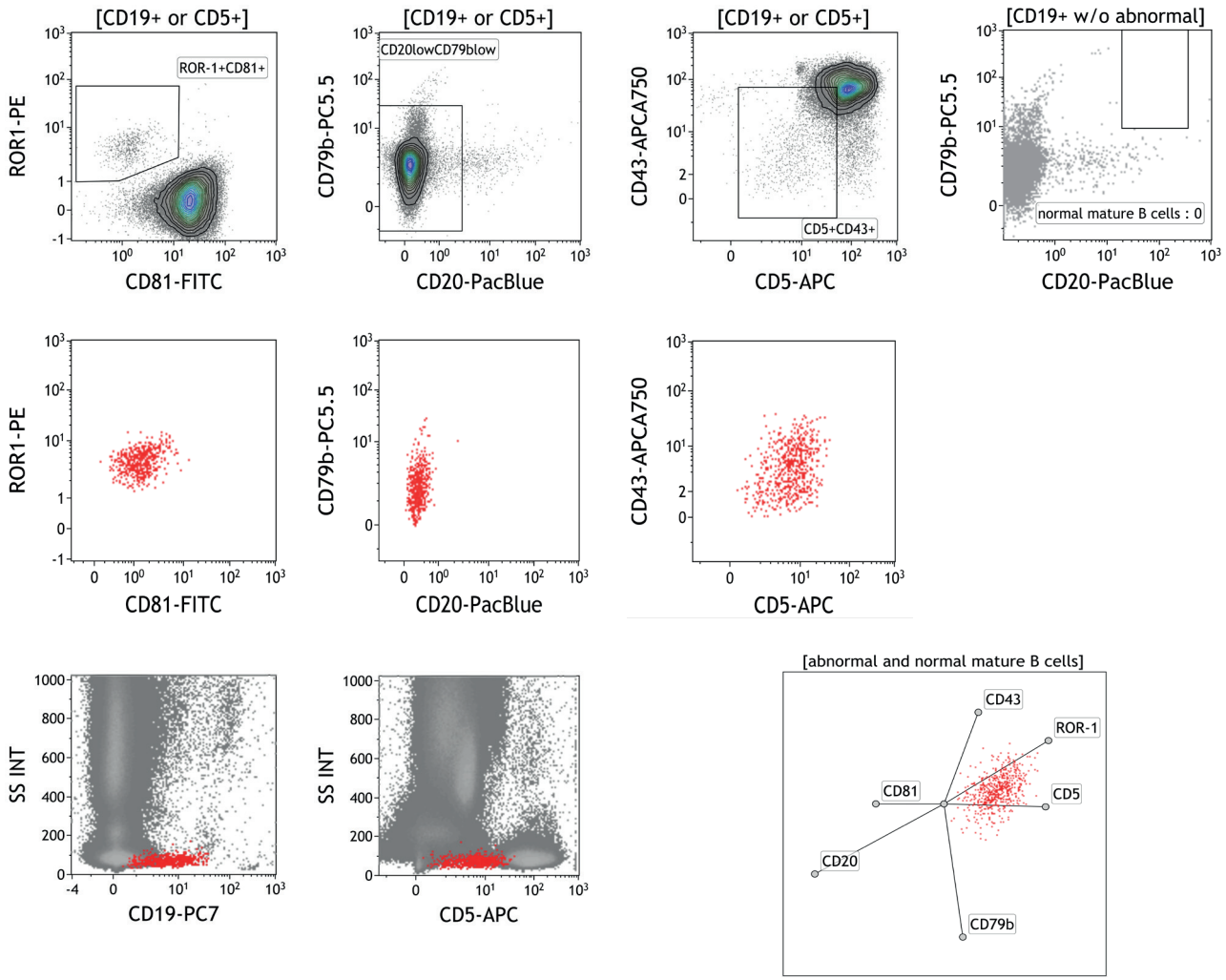
Typical gate positioning delivers incomplete capturing of CD19 population

CD19 positivity may be very dim, overlapping with other lymphocyte populations. The backgating control in a CD19 vs side scatter density overlay plot indicates the truncation of the CD19 cluster by the typical gate positioning (best seen at low graphical plot resolution).



Extension ("oversizing") of the CD19 gate improves capturing of the CD19$^+$ population but also encompasses contaminating events

The backgating control in a CD19 vs side scatter density overlay plot indicates a complete CD19 cluster and scattered contaminating events outside of the cluster, also seen as blue events in backgating/control plots. Assurance of complete CD19$^+$ capturing and neutralization of gate contamination requires correct identification of normal and abnormal B cell events, hence adjustment of the classifying gates across CD81vs ROR-1, CD20vs CD79b, CD5vs CD43 (please see following plots).



Adjusting gates for identification of normal and abnormal B cells assure complete B cell capturing and removes contamination

The following steps are guided by coniuous visual inspection of all color-coded backgating/control plots. (1) Positioning of the $CD20^{low}$ $CD79b^{low}$ gate to encompass completely the CD20-negative cluster and (2) positioning of the $CD5^{+}$ $CD43^{+}$ to exclude $CD5^{++}$ $CD43^{++}$ events and to comprise $CD5^{med}$ $CD43^{med}$ events assure complete capturing of abnormal B cells. Exclusion of $CD79b^{dim/neg}$ events from the normal B cell gate removes events contaminating the $CD19^{+}$ gate (blue events in preceding section). The CD19 and CD5 backgating plots as well as the radar plots now show a homogeneous cluster of red abnormal events.

GATE	NUMBER
Abnormal mature B cells	589
Normal mature B cells	0
$CD45^{+}$	451,291

The results can be displayed in an Information plot: in the shown example, abnormal B cells have been detected at a frequency of 0.131% $CD45^{+}$.

Conclusions

The specific antigen expression profile for CD5, CD20, CD43, CD79b and CD81 as well as for the highly discriminative ROR-1 antigen can be used very efficiently for the cytometric detection of abnormal cells in research studies of mature B cell disorders.

Notes

The results shown here represent data generated on the Beckman Coulter Navios Flow Cytometer and were kindly provided by Dr. Michaela Patz and Prof. Karl-Anton Kreuzer from the Laboratory of Molecular Hematology and Oncology at the University of Cologne. The described Kaluza analysis protocol is available for download:

https://www.beckmancoulter.com/coulter-flow-cytometry/duraclone-rare-event-solutions

References

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FLOW-2523APP-03.17