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Characterization of normal and pathological lymphoid populations: validation of a 10-colors flow cytometry protocol for routine diagnosis

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BACKGROUND - AIMS

Multiparametric flow cytometry (MFC) **10-colors** techniques are becoming routine, providing a great level of information., but remain complex techniques needing a thorough validation before it can be used as a routine-test for diagnosis in hematology or immunology.

We validated the technical performances of a new "2 tubes panel" compared to our previous 5-colors technique, for correct characterization of lymphoid abnormalities.

- A **"screening" tube containing 11 markers** in 9 colors.
- A **"Matutes tube"** performed when a monotypic population is detected in 1st tube.

Former 5-colors panel

FITC FL1	PE FL2	ECD FL3	PC5 FL4	PC7 FL5
Kappa	Lambda	CD19	CD5	CD20
CD45	CD4	CD8	CD3	CD7
CD45	CD16+CD56	CD19	CD3	CD2
FMC7	CD23	CD19	CD5	
CD22	CD10	CD19	CD5	CD38

10-colors panel

FITC	PE	ECD	PC5.5	PC7	APC	APC AA700	APC AA750	PacBlue	KrO
CD8 +Kappa	CD56 +Lambda	CD3	CD5	CD19	CD2	CD7		CD20 +CD4	CD45
	CD200	CD23	CD5	CD19	CD10	CD22	CD38	FMC7	CD45

METHODS

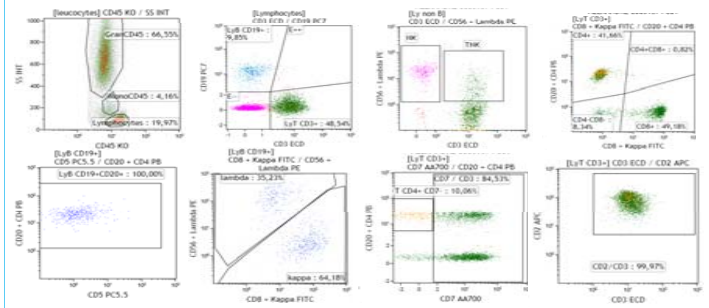
All antibodies provided by Beckman Coulter except kappa and lambda (Dako) and CD200 (BD Biosciences). Navios* (Beckman Coulter) and Kaluza* software (Beckman Coulter) were used. Usual MFI values were established for kappa, lambda, CD20, CD22 and CD200, in normal and CLL samples.

SAMPLES

Almost 100 samples were tested including

- normal cases,
- benign unbalanced lymphoid populations (*eg* HIV),
- various pathological contexts: CLL, other B-cell malignancies, T-cell malignancies (*eg* Sézary cells, prolymphocytic T-cell leukemia) and blastic infiltrations,
- quality assessment samples were also assayed.

Screening tube analysis

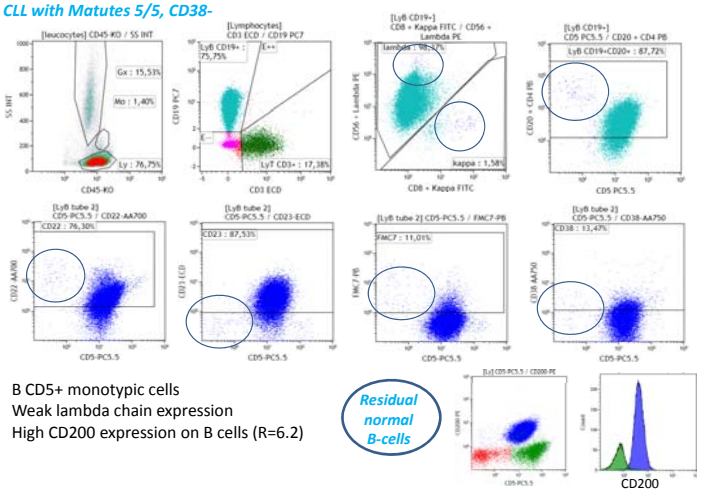


Tube « Matutes »: every marker is determined on the CD19+ b-lymphocytes gate.
Intensity of CD200 is determined with the B/T lymphocytes MFI-ratio.

RESULTS

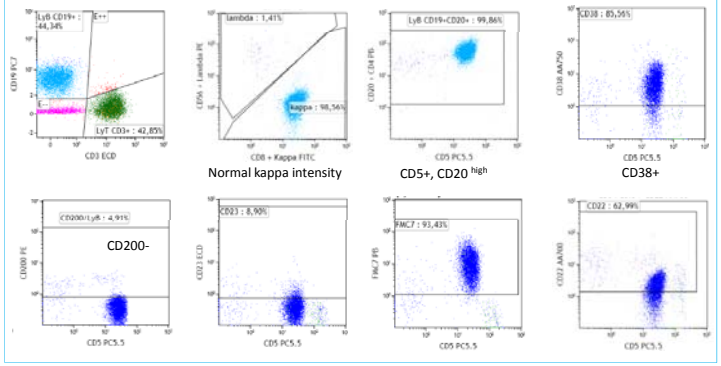
- No discordance with our previous technique.
- This new method provided a higher level of information, as more markers could be assessed together.
- The sensibility and specificity were thereby better with this technique than with our previous 5-colors protocol.

CLL with Matutes 5/5, CD38-

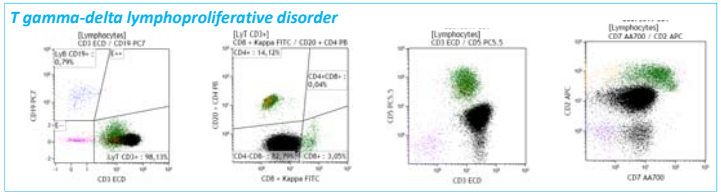


B CD5+ monotypic cells
Weak lambda chain expression
High CD200 expression on B cells (R=6.2)

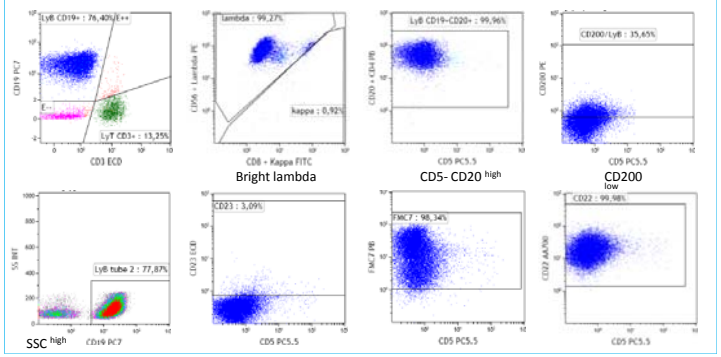
Mantle-cell lymphoma, Matutes 2/5



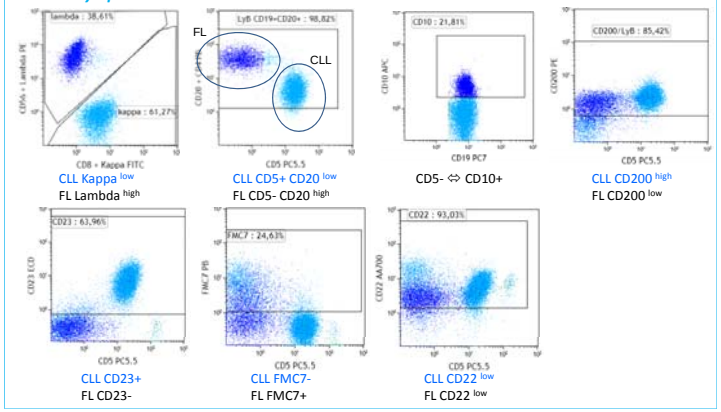
T gamma-delta lymphoproliferative disorder



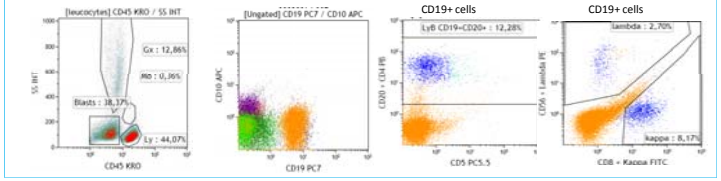
Marginal zone lymphoma (with villous lymphocytes)



Follicular lymphoma + CLL



B-ALL CD10- *Blasts CD45 low CD19+ CD20- CD10- kappa- lambda-*



CONCLUSION

This 10-colors technique with one 11-markers screening tube and one "Matutes"-tube for lymphocyte exploration provided **very satisfying results**. Hence it was adopted in our lab for routine-use and **submitted to accreditation**.
In addition to these technical issues, it is to note that this technique saves human resources (less manipulations), cytometer resources (faster acquisition) and reagents (no marker repeated in iterative tubes), **medico-economical cost** being also of importance nowadays.