

A HIGHLY SENSITIVE
METHOD FOR DETECTING
MINIMAL RESIDUAL
DISEASE IN B-CELL
CHRONIC LYMPHOCYtic
LEUKEMIA

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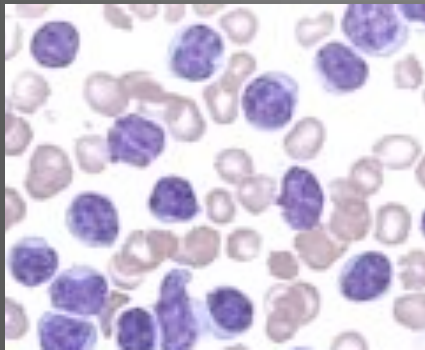
Virga Jesse Hospital, Hasselt, Belgium



B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

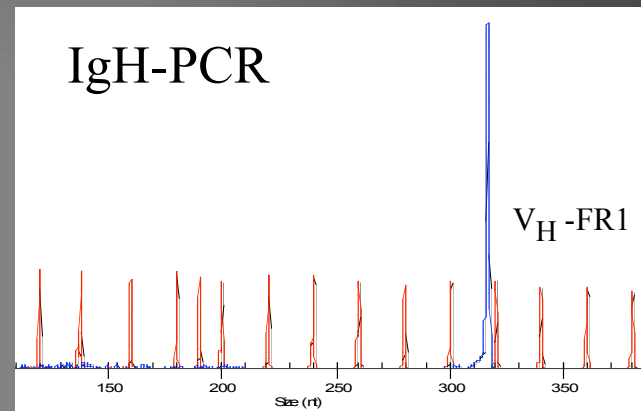
B-CLL is a **monoclonal** disorder characterized by a progressive **accumulation** of functionally **incompetent lymphocytes**.

Lymphocytosis > 5000 lymfo's/ μ l

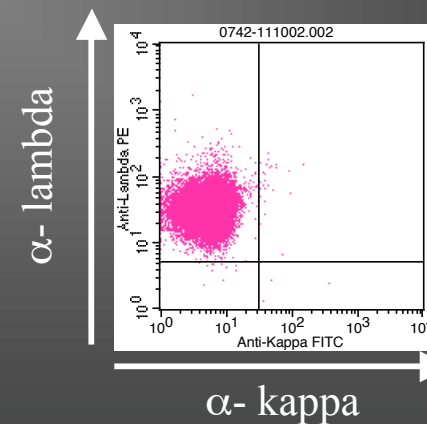
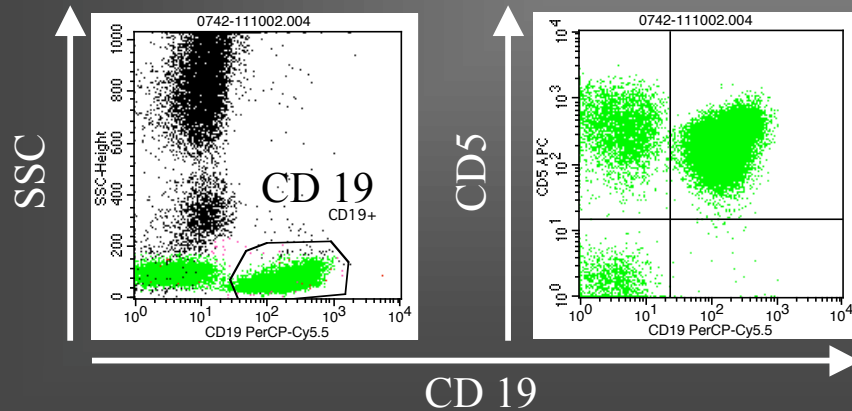


Clonality

IgH-PCR



FLOW: Aberrant expression of CD5 (T-cell)



DETECTION OF MRD IN B-CLL

- In the past: CLL considered to be a uniformly indolent disorder
various **prognostic parameters** predict outcome:
 - Chromosomal Aberrations
 - Ig-VH gene mutation status
 - CD38 overexpression
 - ZAP-70 overexpression
- **New therapies** (purine analogs (fludarabine) monoclonal antibodies (rituximab, alemtuzumab) can lead to the complete eradication of malignant B-cell clone → Complete Remission ↑↑↑
- **Eradication of MRD** is a prognostic parameter on itself

METHODS FOR DETECTION OF MRD IN B-CLL

- **Flowcytometry:**
 - CD19/CD5 sensitivity: 1×10^{-2}
 - CD19/CD5/kappa/lambda sensitivity: 1×10^{-3}
 - CD19/CD5/CD20/CD79b sensitivity: 1×10^{-5}
- Allele-specific oligonucleotide **(ASO)-PCR**
sensitivity: 1×10^{-6}
- New method: **iFISH on Flow-sorted CD19+CD5+ cells**

FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL

- 80% of CLL patients have **chromosomal aberrations** at diagnosis
- Patients with **del(17p)** have **bad prognosis** and are candidates for intensive therapy and monitoring of MRD
- **FISH** detection of chromosomal aberrations as a **marker of tumor load**: on pure CD19+CD5+ cell population to increase sensitivity

FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL

ADVANTAGES

- Increased sensitivity
- FISH analysis:
 - Pure cell population: microscopy is less time consuming
 - Small area on slide: less probe required
 - Less artefacts: easier interpretation

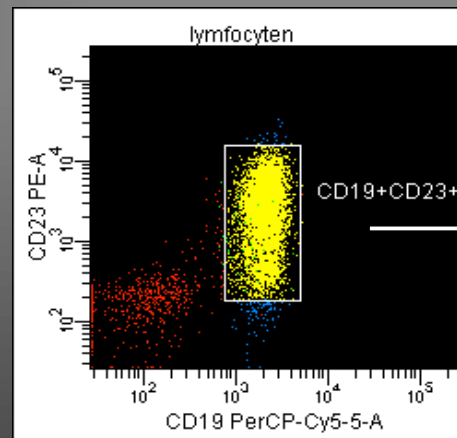
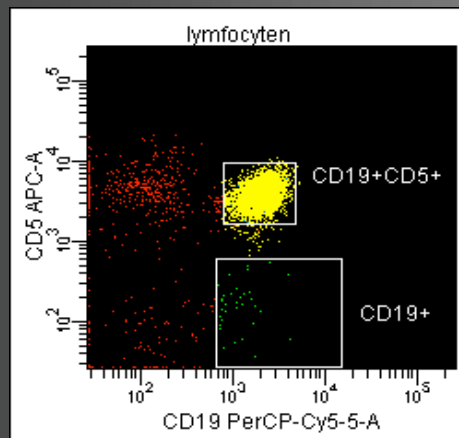
DISADVANTAGES

- Purchase of FACS sorter

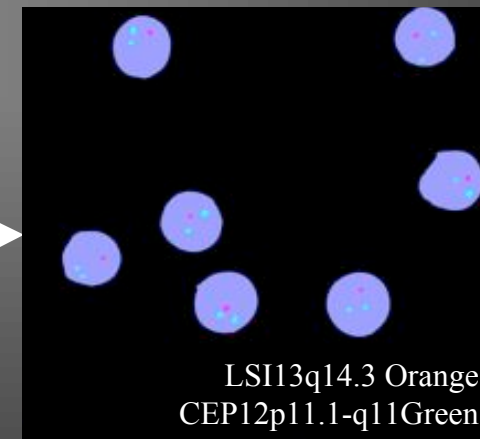
FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL

Experiment design:

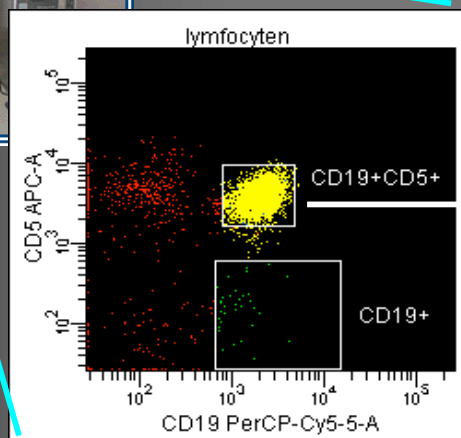
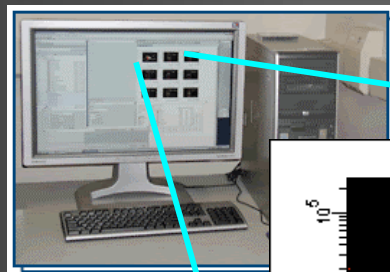
- Blood from newly diagnosed CLL patients
- Flowcytometry: cells labeled with CD5/CD19/CD23
- FISH: > 95% of CD19+/CD5+ have chromosomal aberrations



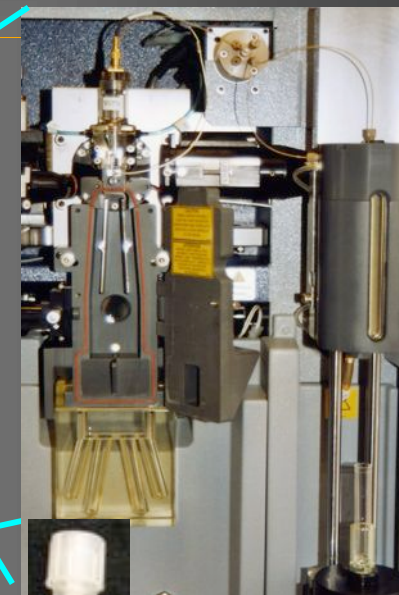
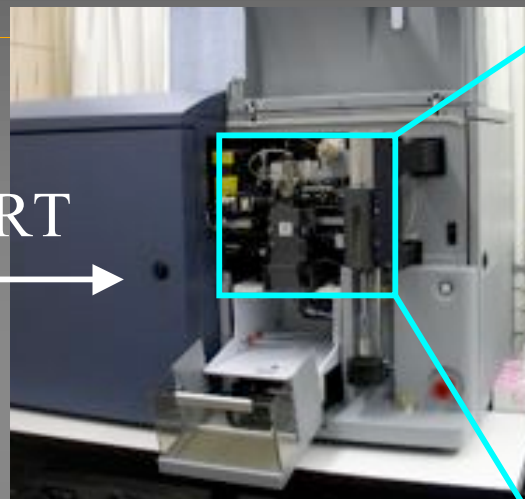
100 % del(13q)



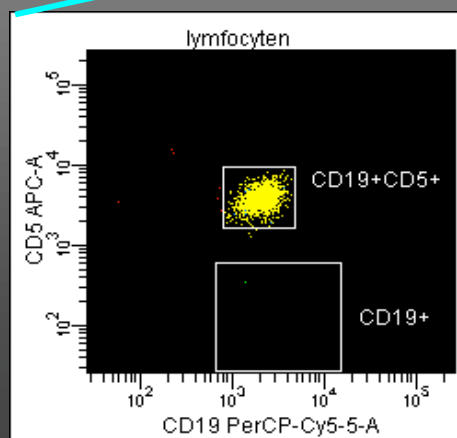
FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL



SORT

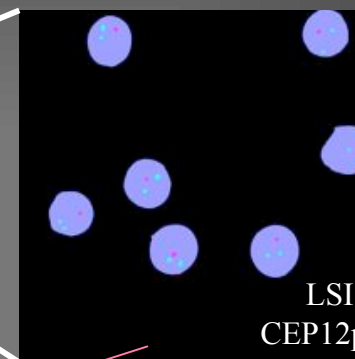
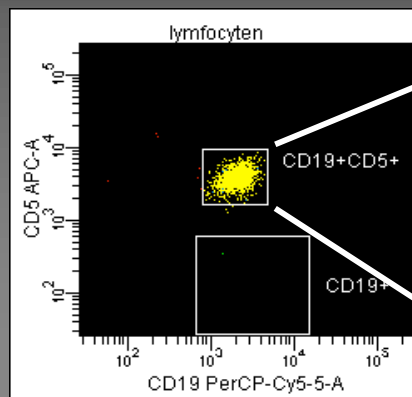


98% purity



FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL

SPIKING EXPERIMENT



B-CLL CELLS
CD19+CD5+CD23+

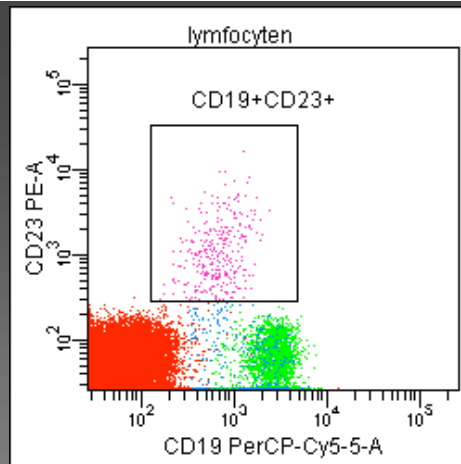
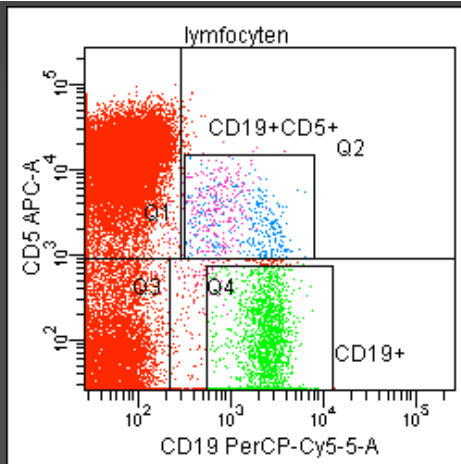
normal blood sample



NORMAL B-CELLS
CD19+CD5+

10⁻² 10⁻³ 10⁻⁴ 10⁻⁵ 10⁻⁶ to WBC

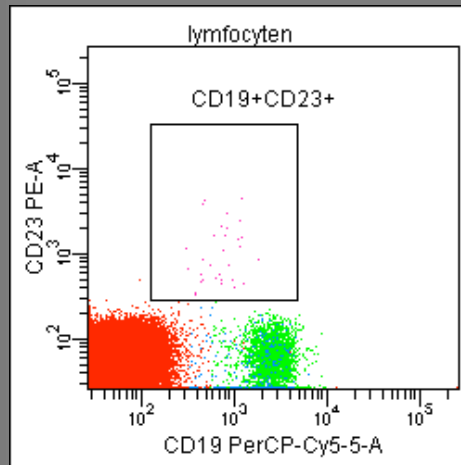
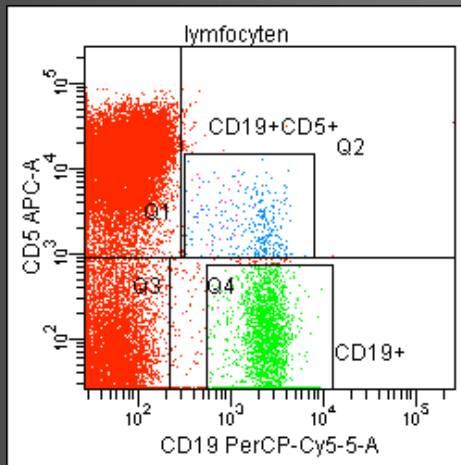
10⁻³



Tube: buis 3:1/1000

Population	#Events	%Parent	%Total
All Events	883,760		100.0
☒ totale populatie	392,977	44.5	44.5
lymfocyten	132,820	33.8	15.0
CD19+	6,398	4.8	0.7
CD19+CD5+	707	0.5	0.1
CD19+CD23+	329	0.2	0.0
☒ Q1	55,130	41.5	6.2
☒ Q2	4,469	3.4	0.5
☒ Q3	66,557	50.1	7.5
☒ Q4	6,664	5.0	0.8

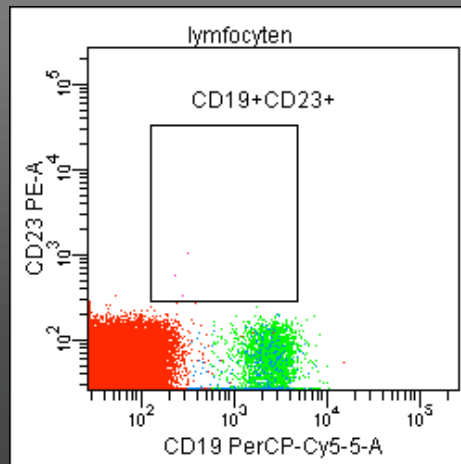
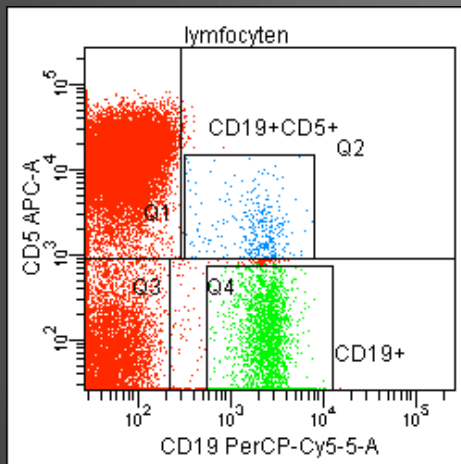
10⁻⁴



Tube: buis 4:1/10000

Population	#Events	%Parent	%Total
All Events	809,943		100.0
☒ totale populatie	377,119	46.6	46.6
lymfocyten	120,792	32.0	14.9
CD19+	6,222	5.2	0.8
CD19+CD5+	472	0.4	0.1
CD19+CD23+	33	0.0	0.0
☒ Q1	53,527	44.3	6.6
☒ Q2	4,045	3.3	0.5
☒ Q3	56,771	47.0	7.0
☒ Q4	6,449	5.3	0.8

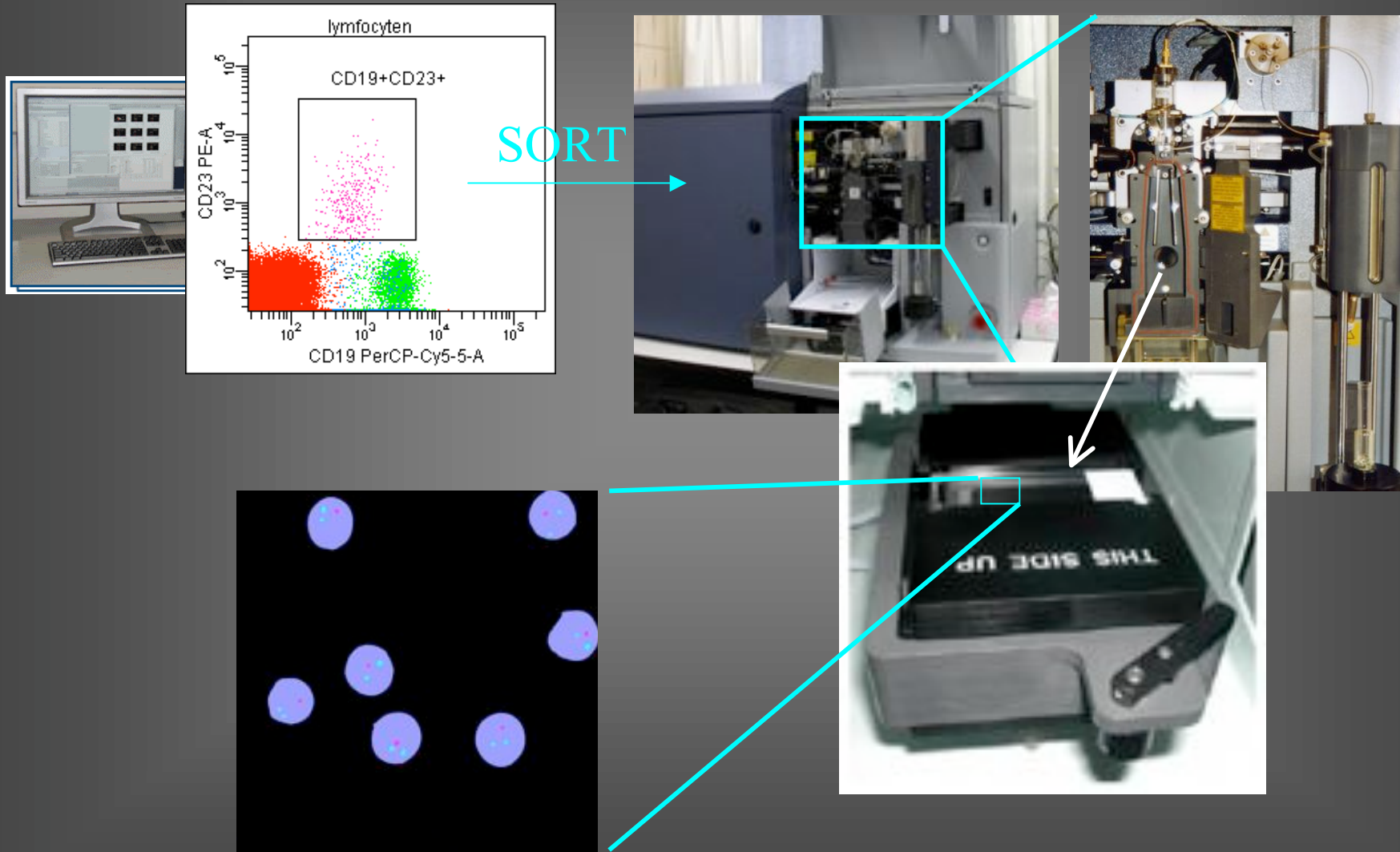
10⁻⁵



Tube: buis 5:1/100000

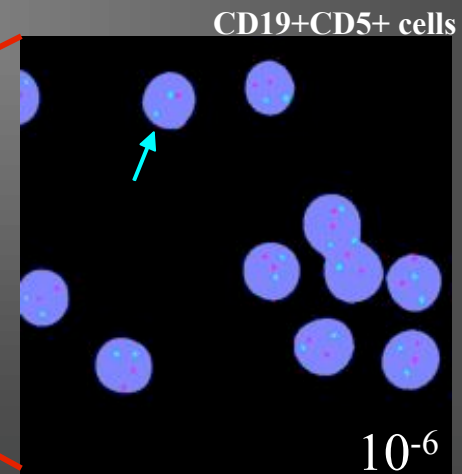
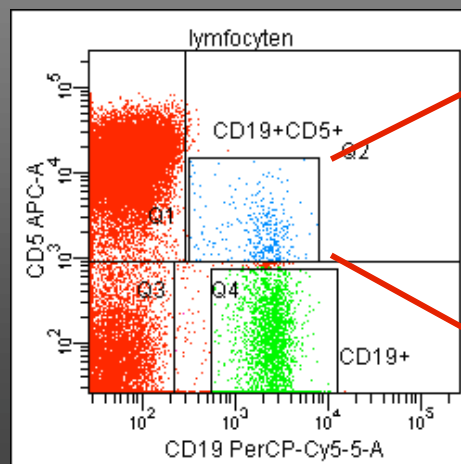
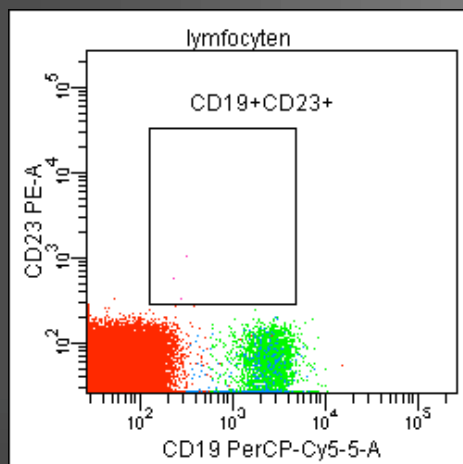
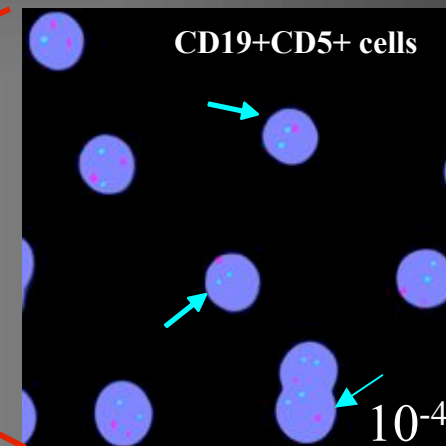
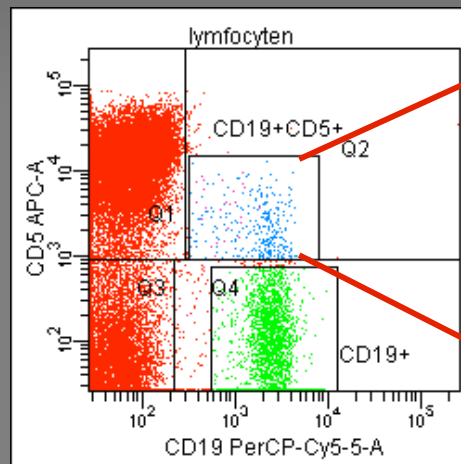
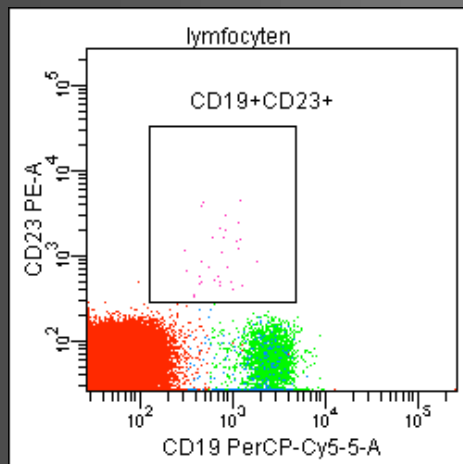
Population	#Events	%Parent	%Total
All Events	745,558		100.0
☒ totale populatie	362,520	48.6	48.6
lymfocyten	109,618	30.2	14.7
CD19+	6,113	5.6	0.8
CD19+CD5+	455	0.4	0.1
CD19+CD23+	3	0.0	0.0
☒ Q1	51,677	47.1	6.9
☒ Q2	3,969	3.6	0.5
☒ Q3	47,636	43.5	6.4
☒ Q4	6,336	5.8	0.8

FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL



FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL

DETERMINATION OF SENSITIVITY



FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL

VALIDATION OF SENSITIVITY

Dilution	TOTAL POPULATION		LYMPHOCYTES		CD23+/CD5+	FISH
	CD5+	CD23+	CD5+	CD23+		
NC	0,1631%	0%	0,7154%	0%	0%	2% (cut-off)
1 x 10⁻³	0,1799% (0,11%)	0,08% (0,12%)	0,5323% (0,30%)	0,2477% (0,32%)	46,53% (51,35%)	70%
1 x 10⁻⁴	0,1252%	0,0088%	0,3908%	0,0273%	6,99%	22%
1 x 10⁻⁵	0,1255%	0,0008%	0,4151%	0,0027%	0,65%	4%
1 x 10⁻⁶	0,1192%	0,0002%	0,409%	0,0007%	0,18%	0%

FISH ON FLOW-SORTED CD19+/CD5+ CELLS FOR DETECTION OF MRD IN B-CLL

CONCLUSION

- Sensitivity: 1×10^{-5} or even 1×10^{-6}
- **Purification** of CD19+/CD5+ for **sensitivity** in combination with **tumor specific** chromosomal **aberration** for **specificity**
(distinction from normal CD19+/CD5+ B-cells)
- Easy standardization:
 - only 2-color flowcytometry
 - commercial FISH probes
- Clinical validation necessary

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