28 & 29
SEPTIEMBRE
2023

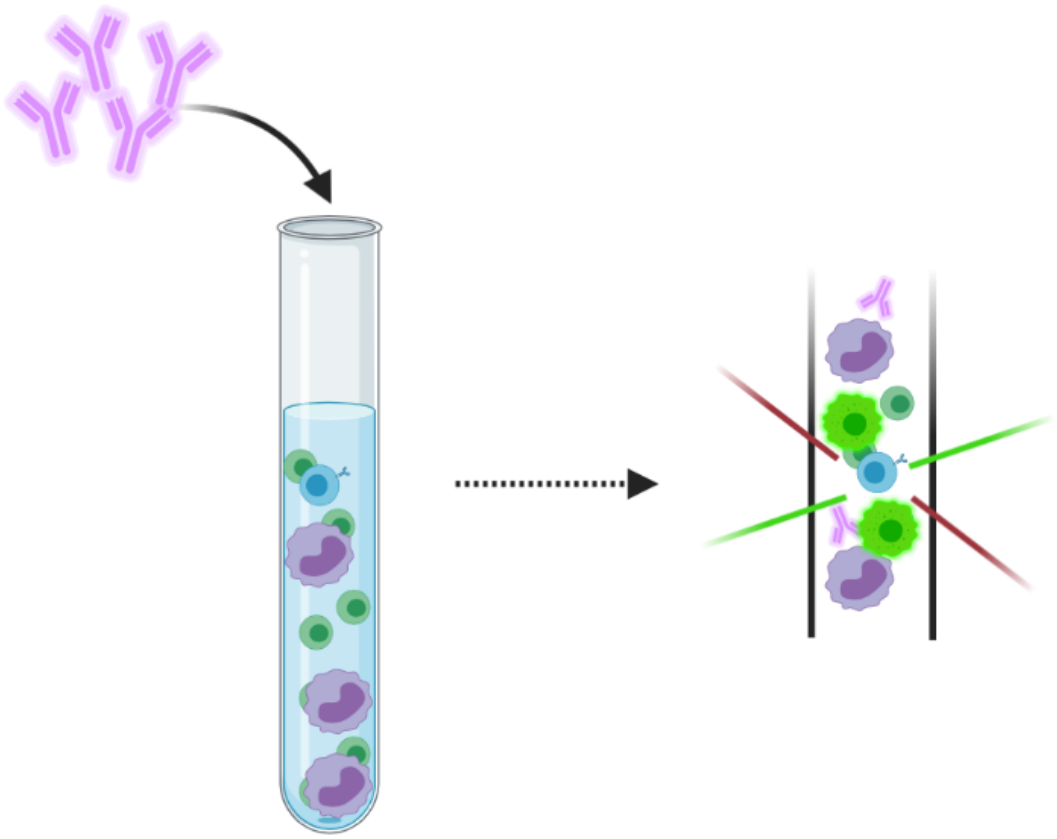
6º CURSO PRÁCTICO
CITOMETRÍA DE FLUJO

Análisis de líquido cefalorraquídeo mediante citometría de flujo

Lourdes Martín
Centro de Investigación del Cáncer, Universidad de Salamanca

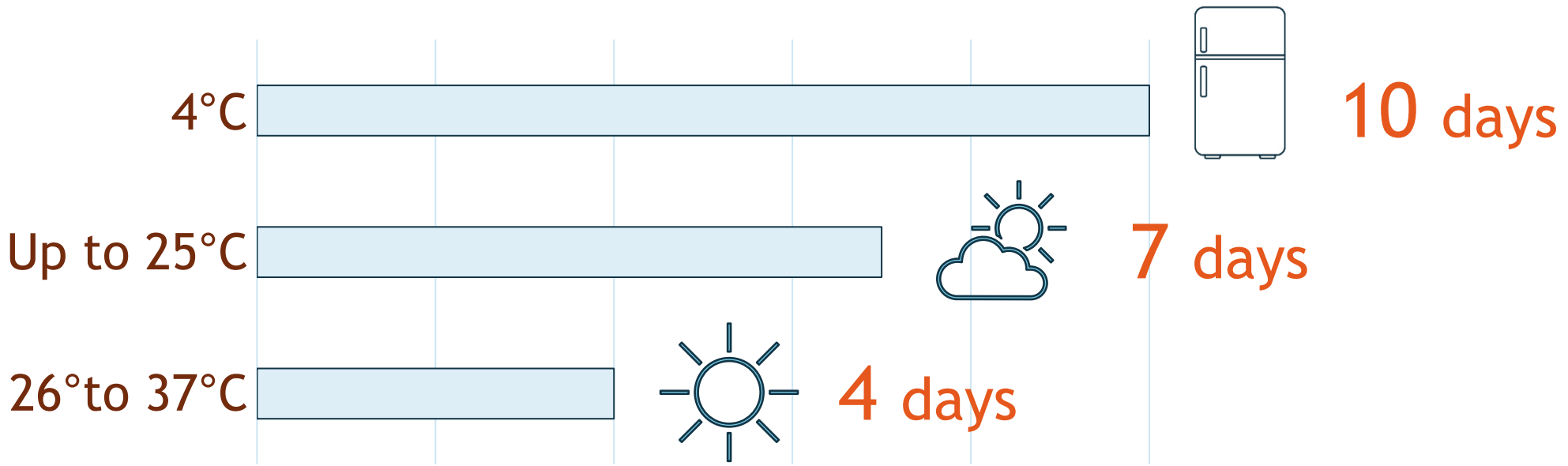
CIC Centro de Investigación del Cáncer
VNiVERSiDAD D SALAMANCA
EuroFlow
IBSALUD Instituto de Investigación Biomédica de Salamanca

Aspects to be considered in CSF processing by NGF



- ✓ Low number of cells
- ✓ Low cell concentration
- ✓ Low volume of sample
- ✗ **Short cell viability**

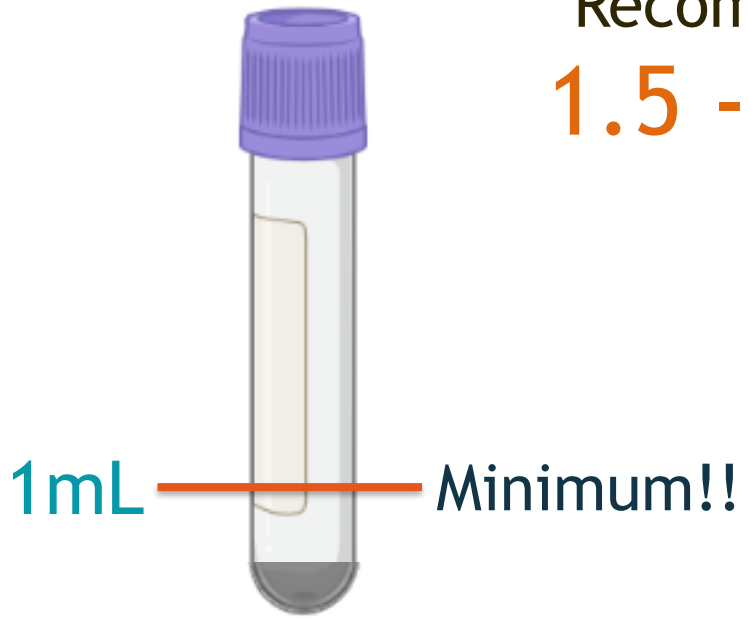
Collecting CSF sample in a stabilizing agent tube (i.e. TransFix) delays notably viability decay



Compatible with NGF, FISH and molecular biology studies

Keep at room temperature until use

Collecting CSF sample in a stabilizing agent tube (i.e. TransFix) **delays notably viability decay**



Recommended sample volume

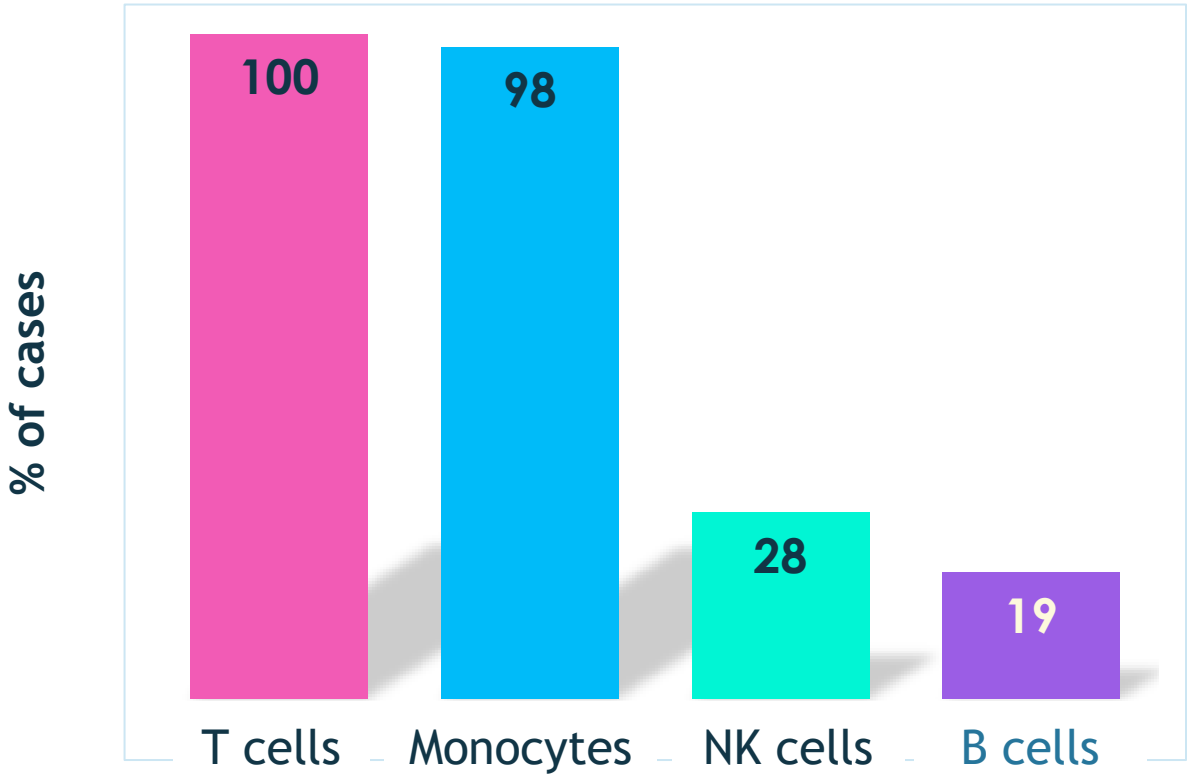
1.5 - 2mL (35 - 40 drops)

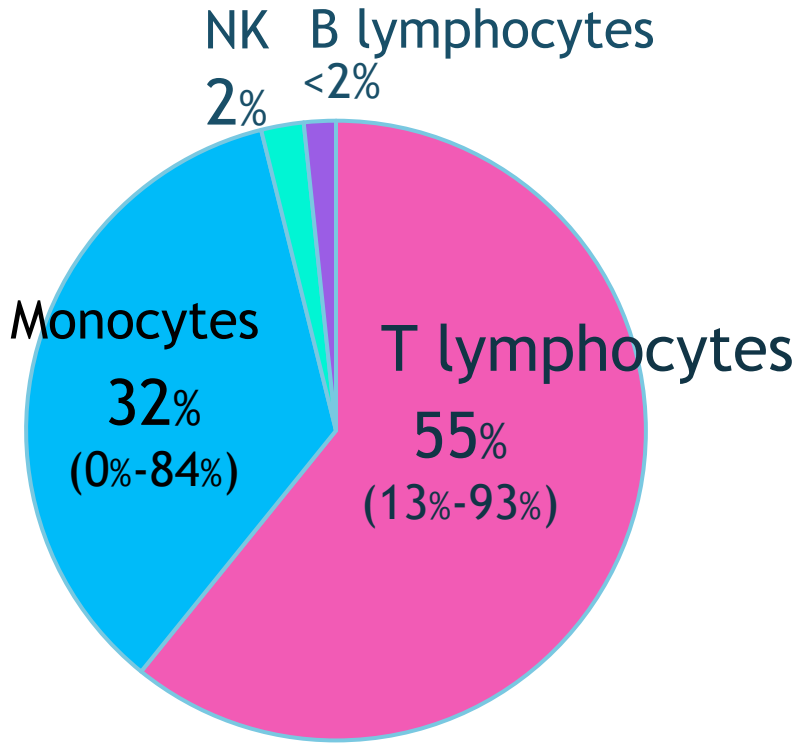
Add immediately!!

Factors to consider:

Intracellular antigens detection only possible in the short term

Cellular composition of non-pathological CSF samples

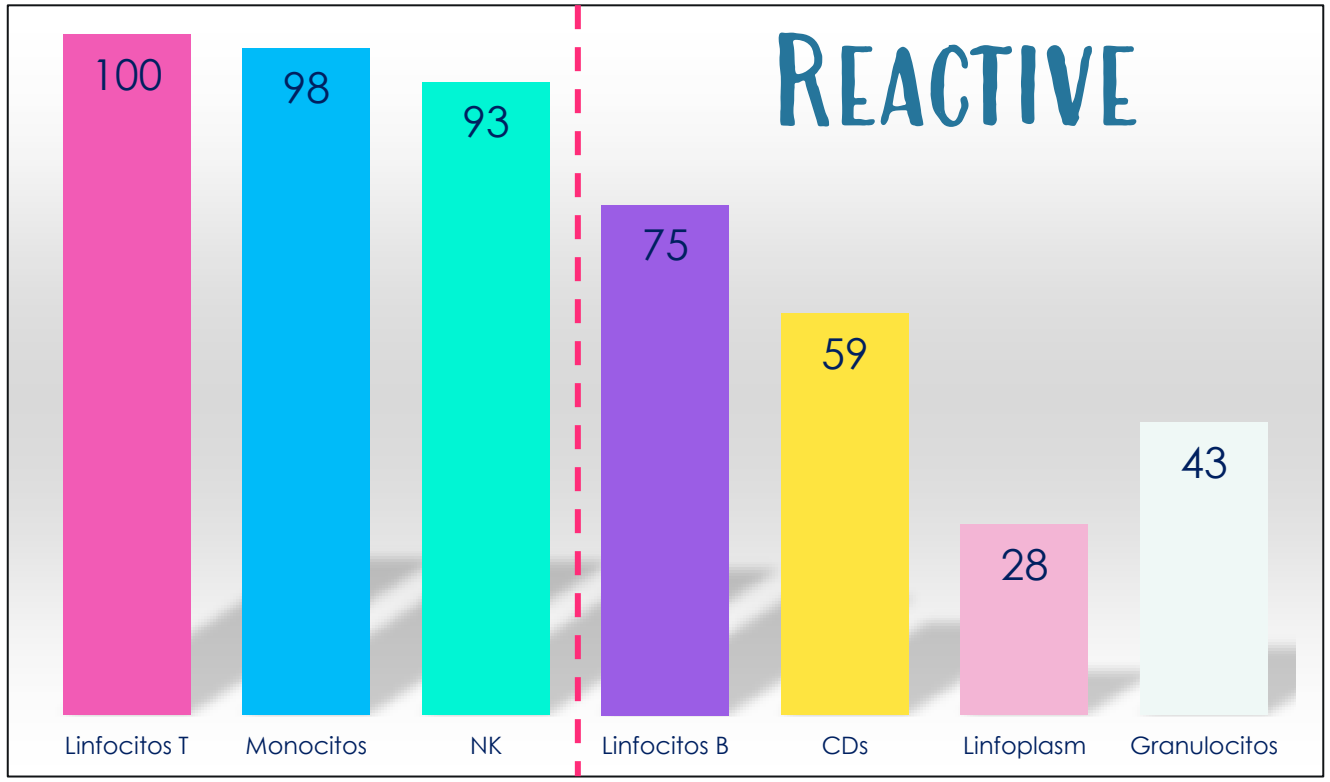


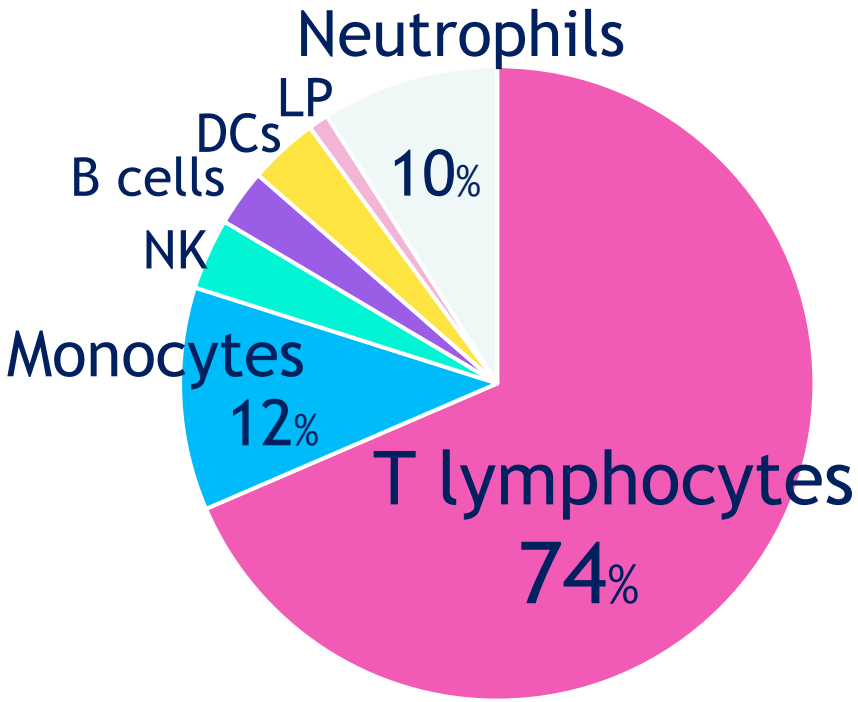


CELL COUNT ≈ 1 $cell/\mu L$

Martín-Martín et al, unpublished data & de Graaf et al. 2008

Populations in reactive CSF





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Increased cellularity

≥5 cells/µl

Martín-Martín et al, unpublished data & de Graaf et al. 2008

CSF screening tube - Euroflow Small Sample Tube (SST) -

PacB	PO	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
CD20	CD45	CD8 + sIgλ	CD56 + sIgκ	CD4	CD19	CD3 + CD14	CD38

SST kit

Pacific Blue™	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CD20	CD45	CD8 + SmIgLambda	CD56 + SmIgKappa	CD4	CD19	SmCD3 + CD14	CD38

EuroFlow

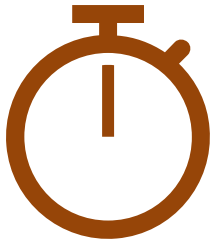
A preliminary result can be given in less than two hours

Screening tube analysis
(1/3 of sample)

CD20/CD45/CD8+sIgLambda/CD56+sIgKappa/CD4/CD19/CD3+CD14/CD38

negative

POSITIVE



<2 hours

EuroFlow protocol 2nd step

negative

Increase sensitivity and specificity with remaining sample (2/3)

negative

doubtful

POSITIVE

DOUBTFUL

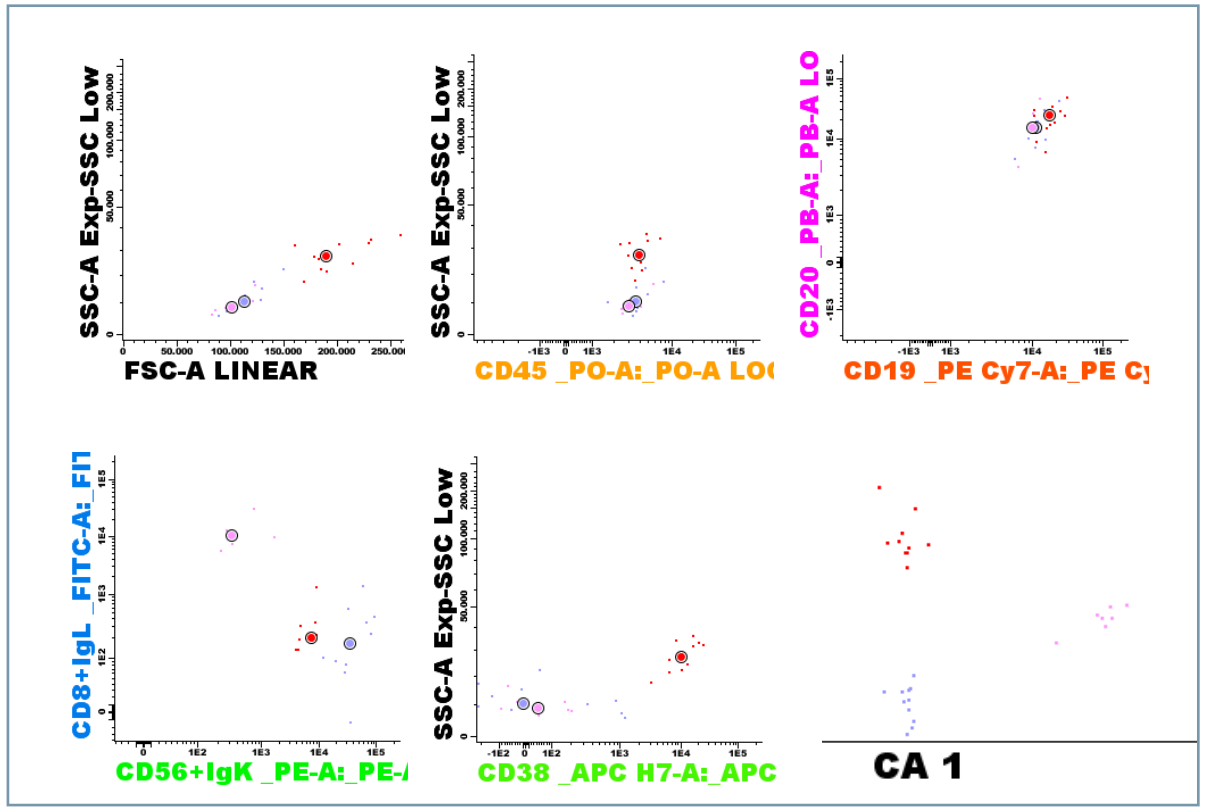
CLEARLY POSITIVE

Distribute remaining sample (2/3) for complete characterization

POSITIVE

SST AG&I example of low-level involvement and coexistence of normal B cells

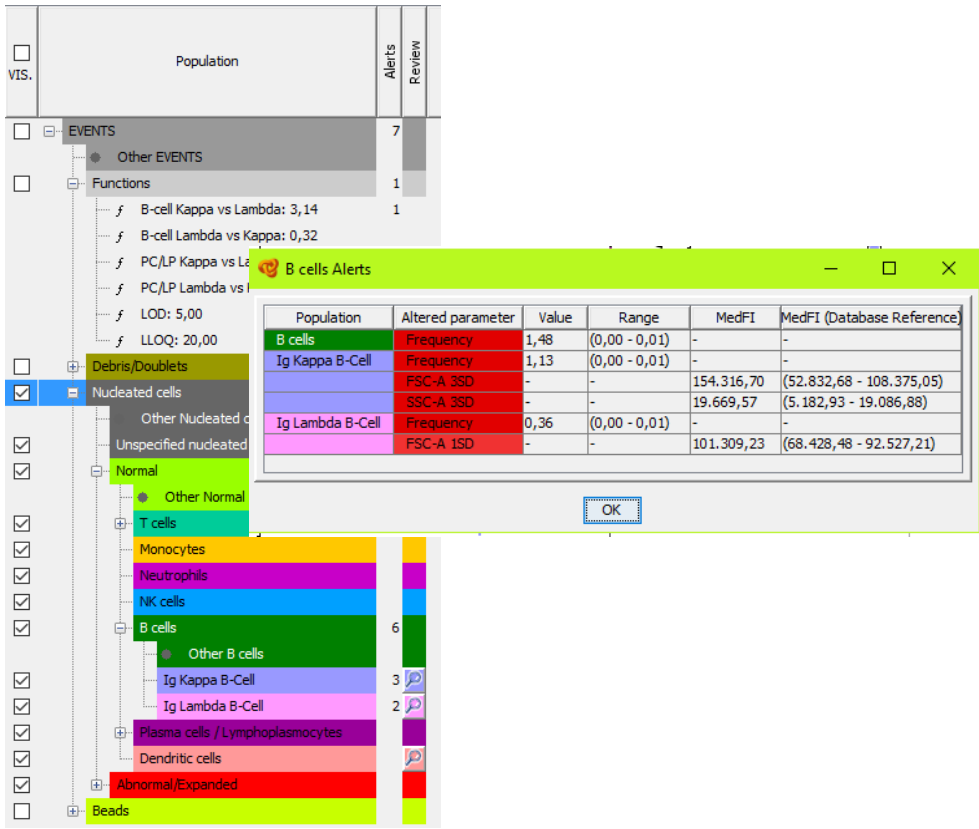
Example of DLBCL



- Ig Kappa⁺ B cells
- Ig Lambda⁺ B cells
- Tumor B cells

Alerts

If user classifies those checks into normal Ig Kappa B cells:



SST AG&I report

CELLULARITY (estimated based on total nucleated cells analyzed)

Population	Frequency	Reference (%)	Events/µl	Reference (events/µl)*
Nucleated cells	100	-	**12.9**	(0.00 - 5.00)
Normal	79.1	-	**10.2**	(0.40 - 3.17)
T cells	49.1	-	**6.3**	(0.15 - 1.83)
CD4+CD8-	25	-	**3.2**	(0.08 - 1.43)
CD4-CD8+	18.4	-	**2.4**	(0.04 - 0.40)
CD4+CD8+	0.00	-	0.00	-
CD4-CD8-/dim0	5.8	-	0.7	-
Monocytes	12.4	-	**1.6**	(0.08 - 1.11)
Neutrophils	0.8	-	0.1	(0.02 - 0.43)
NK cells	7.4	-	**1**	(0.00 - 0.05)
B cells	0.00	-	0.00	(0.00 - 0.03)
Ig Kappa B-cell	0.00	0.01	0.00	-
Ig Lambda B-cell	0.00	0.01	0.00	-
Plasma cells / Lymphoplasmocytes	0.00	0.01	0.00	-
Ig Kappa PC/LP	0.00	0.01	0.00	-
Ig Lambda PC/LP	0.00	0.01	0.00	-
Dendritic cells	9.4	-	**1.2**	(0.01 - 0.18)
Abnormal mature SIg Kappa+ B-cells	**20.9**	-	**2.70**	-

IMMUNOPHENOTYPIC DESCRIPTION OF ABNORMAL/EXPANDED CELLS

CD8-sIgLambda^{-} FSC-A^{normal} CD19^{+} SSC-A^{normal} CD38 _APC H7-A^{lo} CD20 _P8-A^{lo} CD56+IgK _PE-A^{+} CD45 _PO-A^{+}

COMMENT

NEGATIVE RESULT

Based on the results of this analysis, no pathological cells were detected in the cerebrospinal fluid sample tested. These findings suggest that the sample appears to be free from the specific types of abnormal cells analyzed.

POSITIVE RESULT below LOD

No pathological cells were detected in the cerebrospinal fluid specimen tested with a sensitivity of 5 cells per sample (4 pathological cells are identified below the LOD). Patient follow-up is recommended according to clinical criteria.

POSITIVE RESULT below LOQ

16 abnormal cells were detected in the cerebrospinal fluid sample, however, the concentration is below the limit of quantification for our assay. Therefore, it is not possible to accurately determine the exact concentration of cells in the sample, however, the presence of pathological cells can be confirmed.

POSITIVE RESULT

Our analysis of the received cerebrospinal fluid sample detected the presence of Abnormal/Expanded mature SIg Kappa+ population at a frequency of 0.09 cells/µL, which corresponds to 0.10% of the total cell count. These findings are consistent with infiltration of the central nervous system by pathological cells.

REACTIVE CSF

It is worth noting the elevated -5.01 cells/µL- and unusual populations observed in the sample, which may suggest a reactive process such as infection, inflammation, or autoimmunity.

Based on the results of this analysis, no pathological cells were detected in the cerebrospinal fluid sample tested. These findings suggest that the sample appears to be free from the specific types of abnormal cells analyzed.

**de Graaf et al, Cytometry, 2011*

PRÁCTICA