



APLICACIÓN DE LA CITOMETRIA CUANTITATIVA EN LA PRÁCTICA ASISTENCIAL:

SEGUIMIENTO DEL TRATAMIENTO CON ANTI-CD49D (NATALIZUMAB)

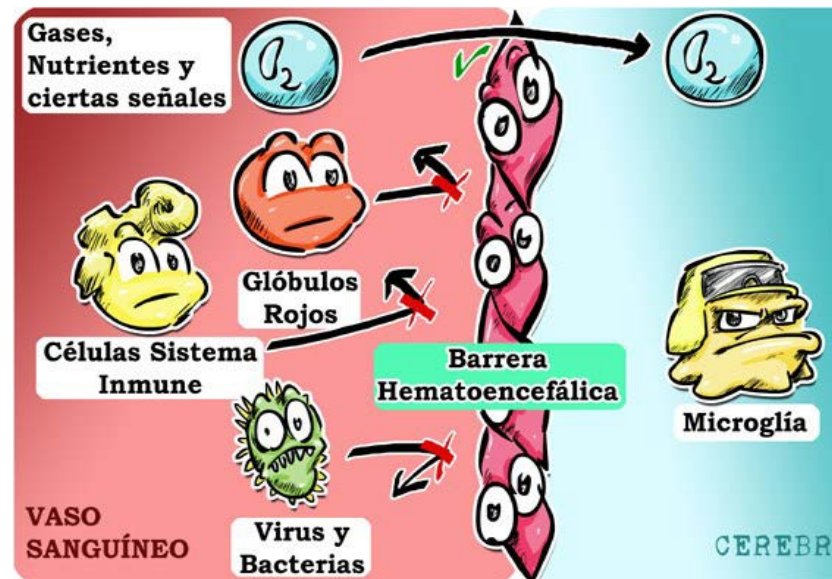
**ARMANDO RUIZ BARZOLA
(TEL)**

¿QUÉ ES EL NATALIZUMAB?

Anticuerpo monoclonal humanizado, inhibidor de la migración leucocitaria a través de la barrera hematoencefálica.

¿Qué es la Barrera Hematoencefálica?

Es un sistema de protección que sirve para controlar y restringir el paso de sustancias tóxicas entre la circulación sanguínea y el fluido cerebral.





VIRUS JOHN CUNNINGHAM

- También llamado virus JC.
- Es un virus común del sistema nervioso central.
- Se encuentra de manera inactiva.
- Se reactiva en pacientes inmunodeprimidos.
- **Leucoencefalopatía multifocal progresiva (LMP).**



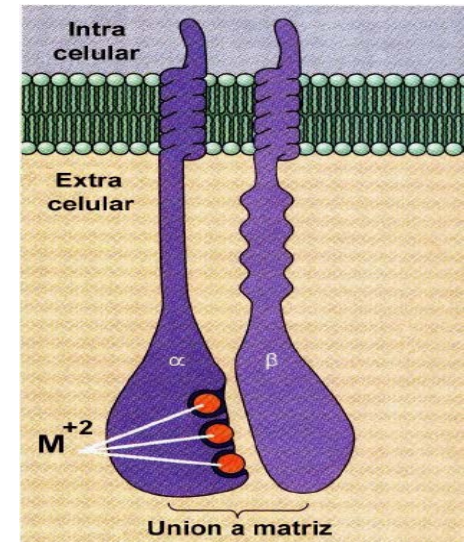
FUNCIÓN DEL NATALIZUMAB

Inhibir selectivamente las moléculas de adhesión y unirse a la subunidad **alfa-4** de las integrinas humanas, expresadas en la superficie de todos los leucocitos excepto de los neutrófilos.

¿Qué es una Integrina?

Son receptores que median la adhesión celular y regulan la formación de complejos de señalización.

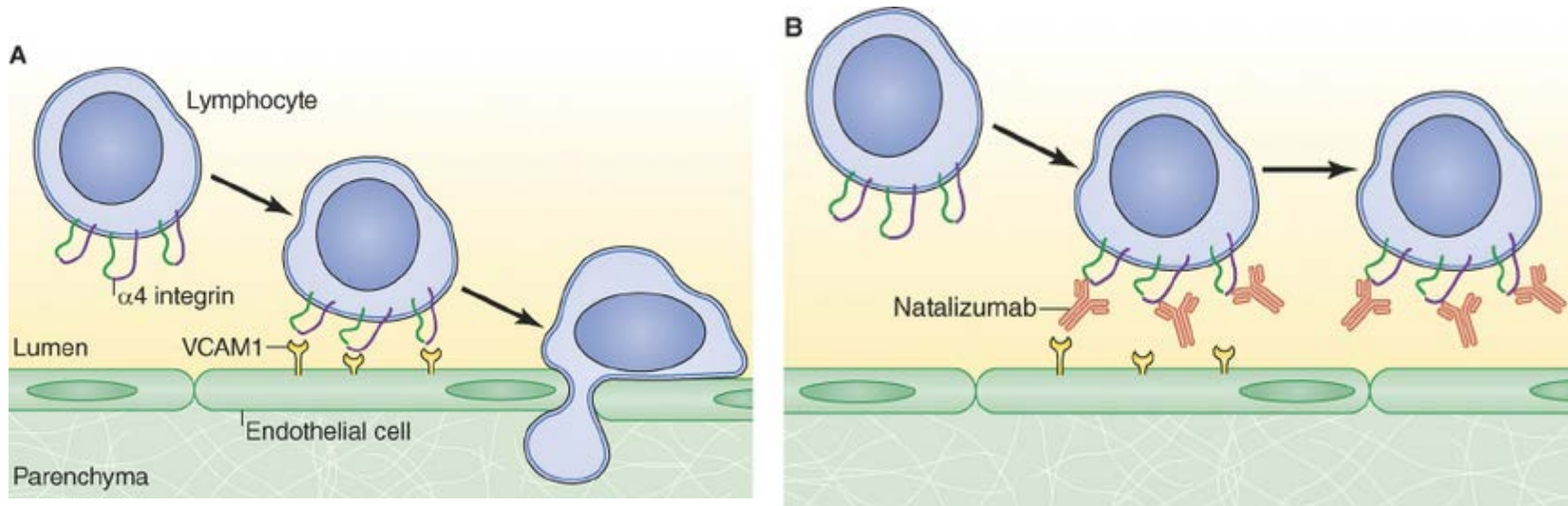
La subunidad **Alfa-4** es conocida por el marcador **CD49d**



MIGRACIÓN LEUCOCITARIA



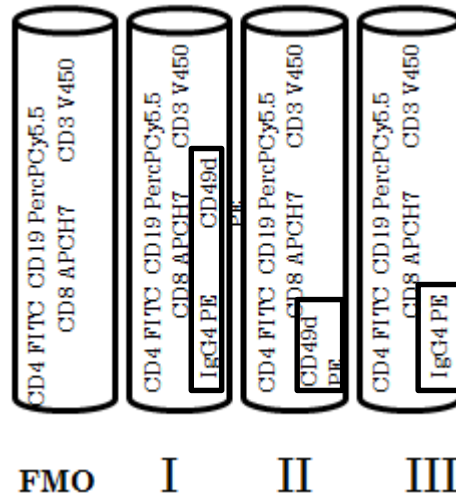
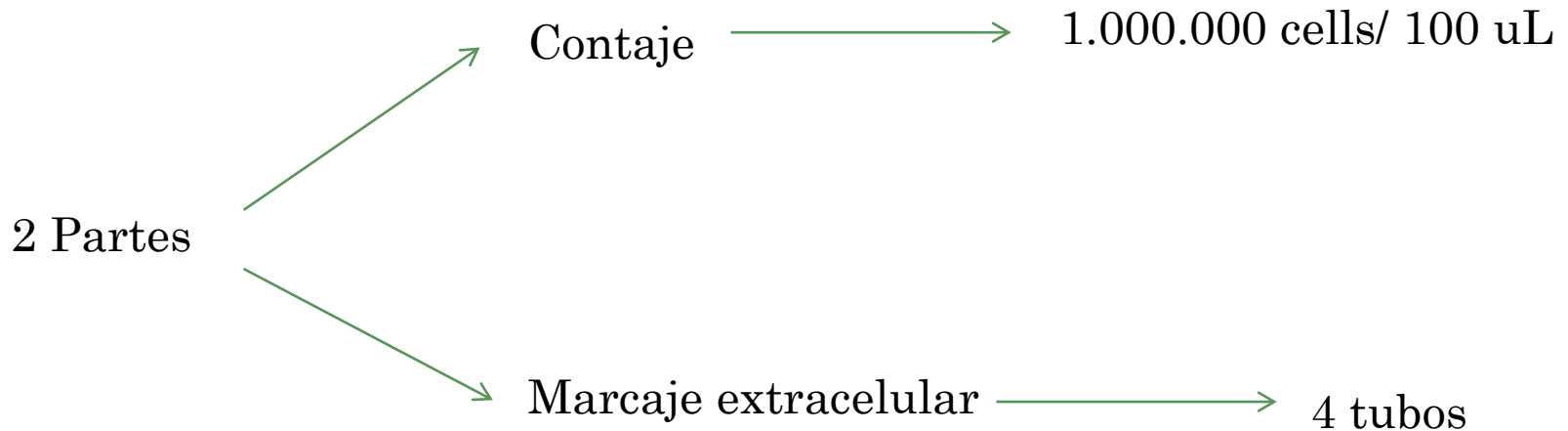
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¿Qué pretendemos obtener con la técnica?



PROCEDIMIENTO DE LA TÉCNICA



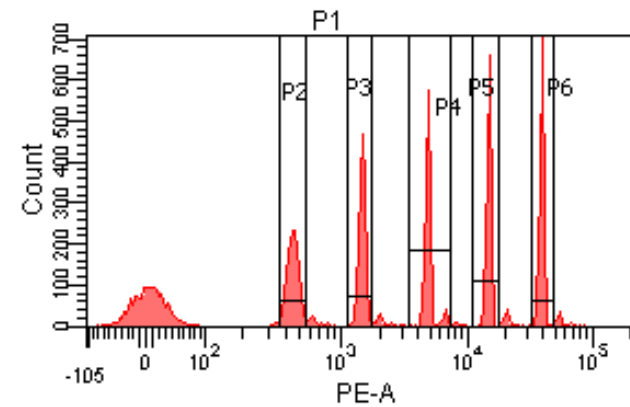
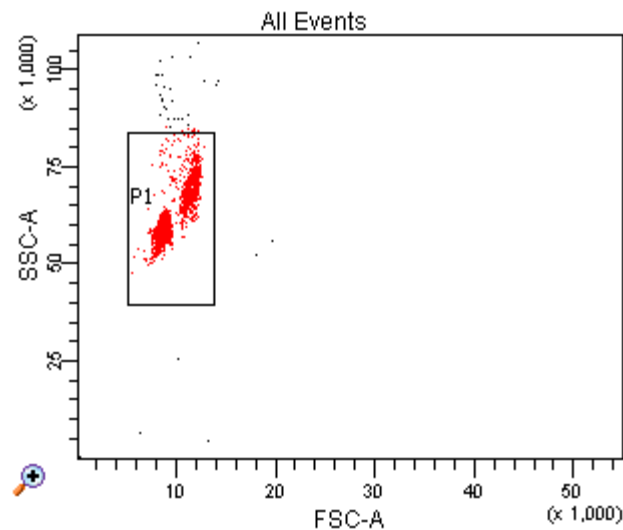
CD4 FITC
CD8 APCH7
CD19 PercPCy5.5
CD3 V450



RAINBOW PE



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Population	Parent N...	PE-A Geo Mean	PE-A Median	PE-A SD
<input checked="" type="checkbox"/> P2	P1	445	445	39
<input checked="" type="checkbox"/> P3	P1	1,449	1,455	79
<input checked="" type="checkbox"/> P4	P1	4,780	4,735	471
<input checked="" type="checkbox"/> P5	P1	14,267	14,386	596
<input checked="" type="checkbox"/> P6	P1	37,873	38,058	2,041

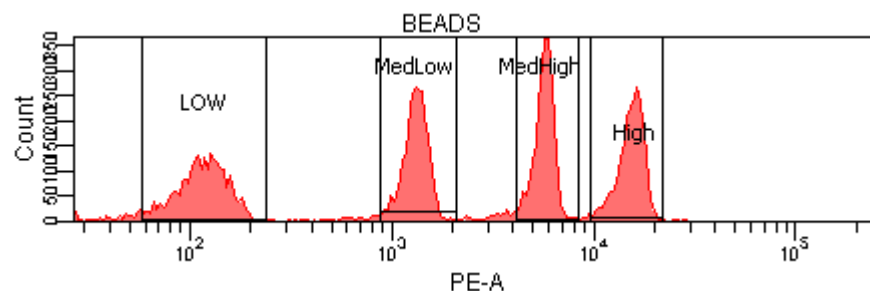
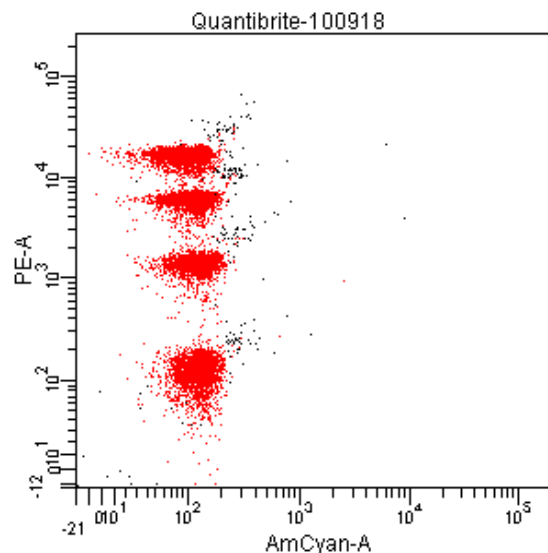
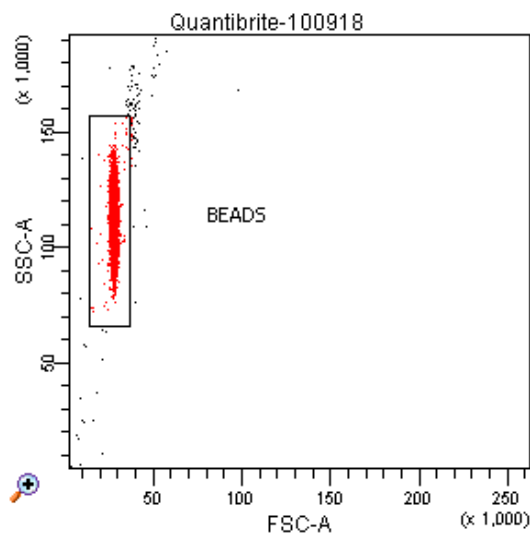
(Peak 4): 4.800 (4.650 - 4.950)



Quantibrite PE



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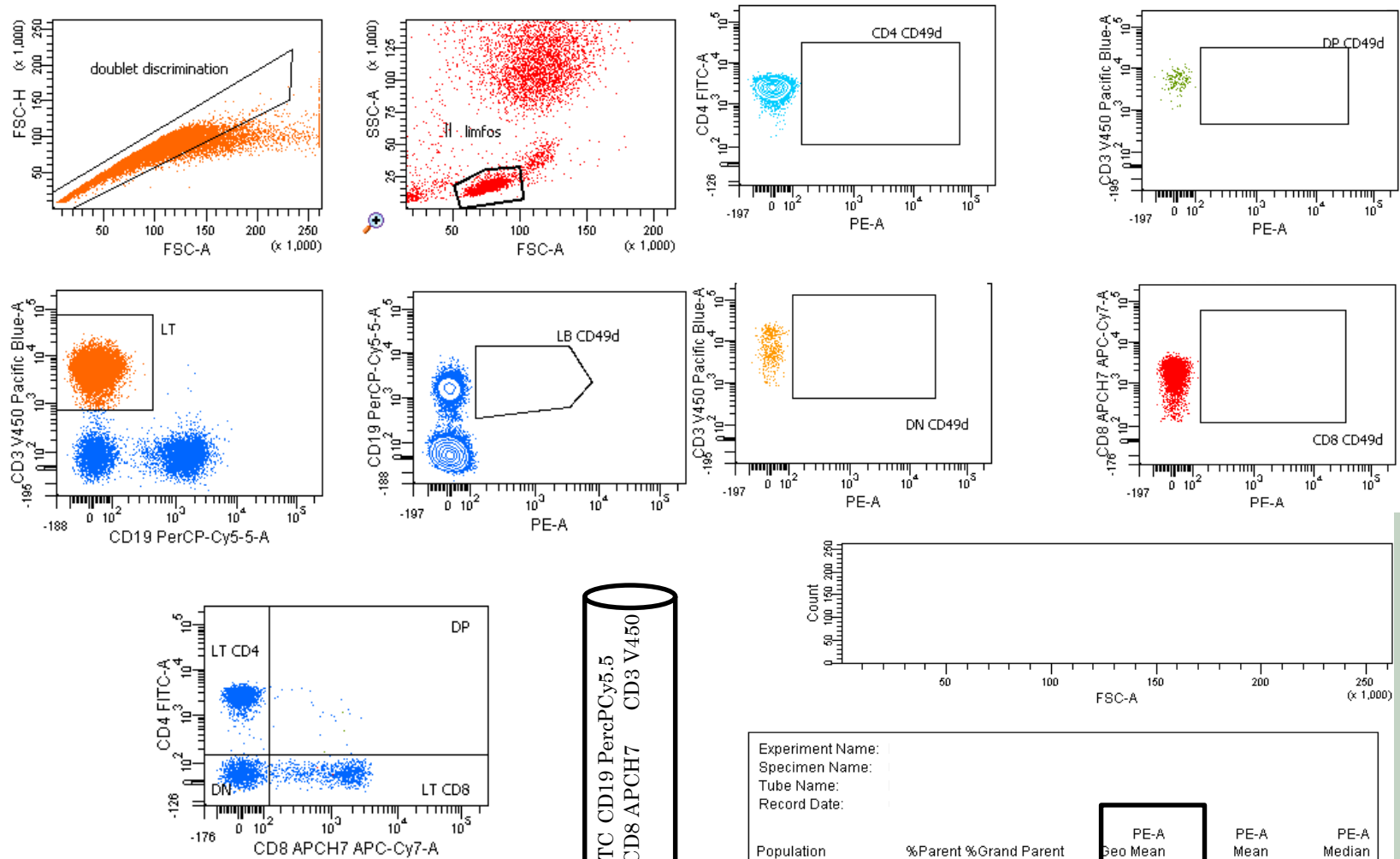


Population	#Events	PE-A Geo Mean
<input checked="" type="checkbox"/> LOW	2,345	116
<input checked="" type="checkbox"/> MedLow	2,421	1,317
<input checked="" type="checkbox"/> MedHigh	2,439	5,709
<input checked="" type="checkbox"/> High	2,328	15,397





ANÁLISIS



CD4 FITC CD19 PercPCy5.5
 CD8 APCH7 CD3 V450

FMO

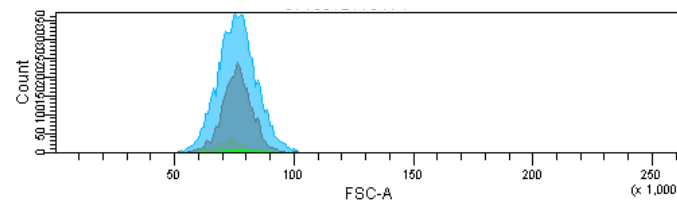
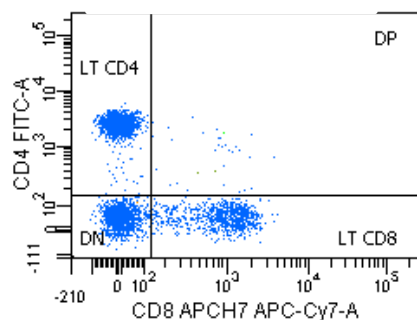
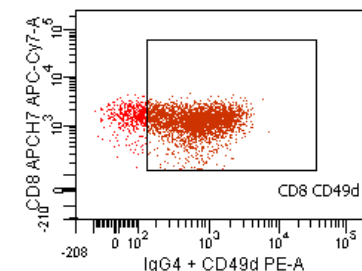
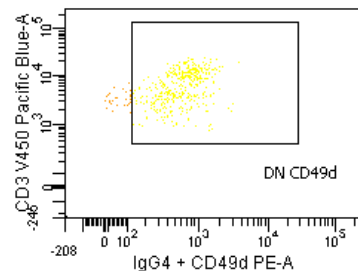
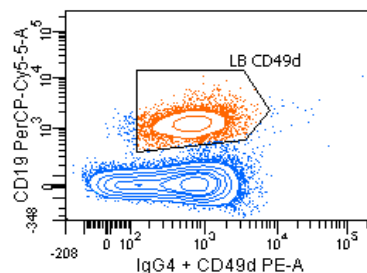
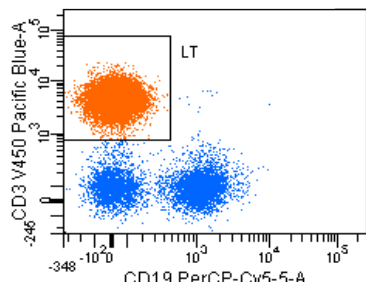
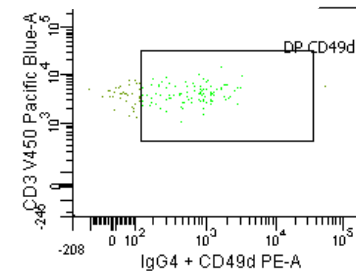
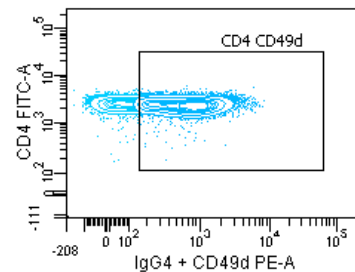
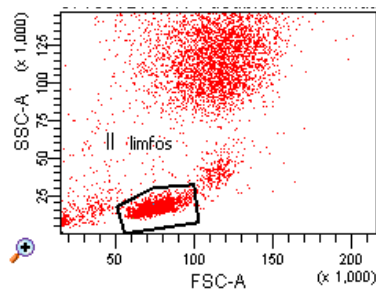
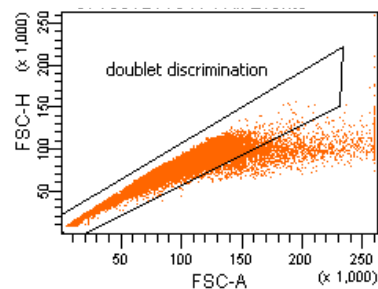
Experiment Name:
Specimen Name:
Tube Name:
Record Date:

Population	%Parent	%Grand Parent	PE-A Geo Mean	PE-A Mean	PE-A Median
CD4 CD49d	0.0	0.0	####	####	####
DP CD49d	0.0	0.0	####	####	####
DN CD49d	0.0	0.0	####	####	####
CD8 CD49d	0.0	0.0	####	####	####
LB CD49d	0.0	0.0	####	####	####

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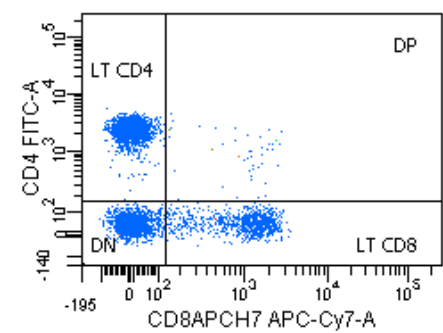
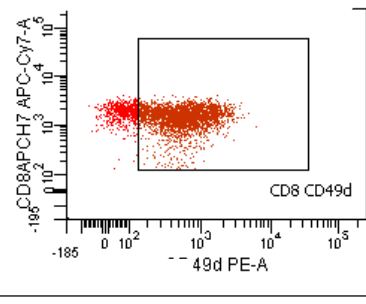
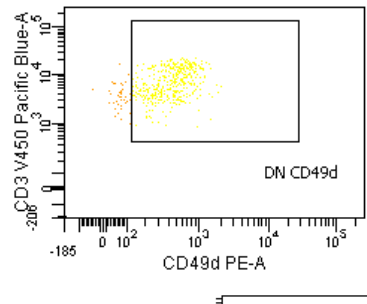
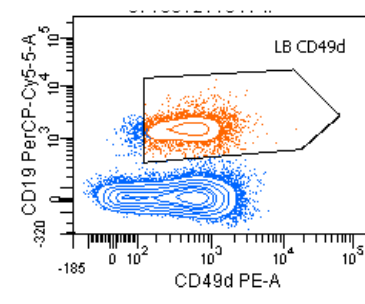
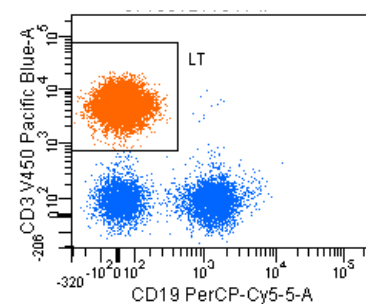
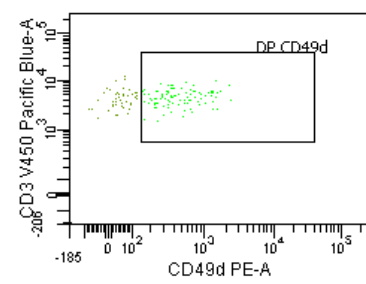
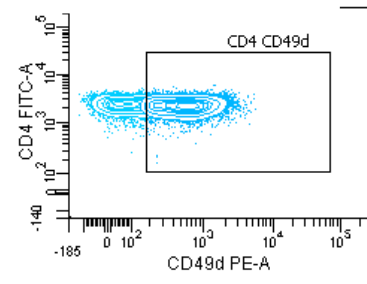
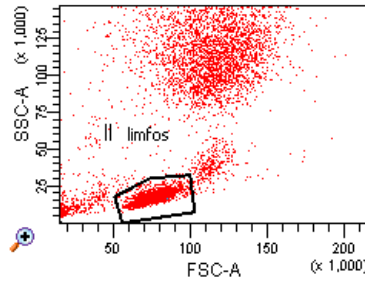
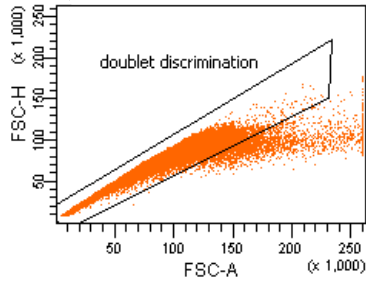
Experiment Name:
Specimen Name:
Tube Name:
Record Date:

Population	%Parent	%Grand Parent	IgG4 + CD49... Geo Mean	IgG4 + CD49... Mean	IgG4 + CD49... Median
CD4 CD49d	70.6	49.6	673	919	691
DP CD49d	72.4	0.7	683	913	829
DN CD49d	93.5	2.9	602	724	625
CD8 CD49d	87.1	22.4	700	930	741
LB CD49d	17.8	5.1	715	920	730

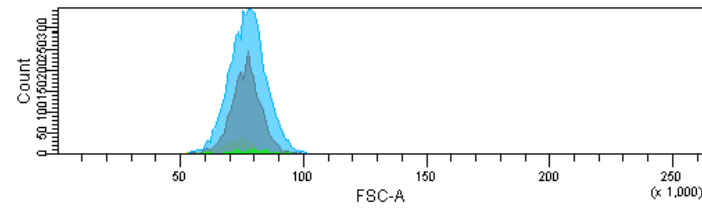
ANÁLISIS



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CD4 FITC CD19 PercPCy5.5
 CD8 APCH7 CD3 V450
 CD49d
 PE

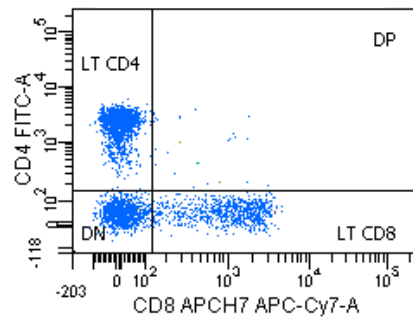
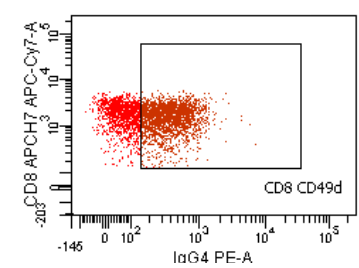
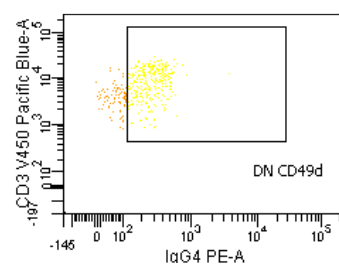
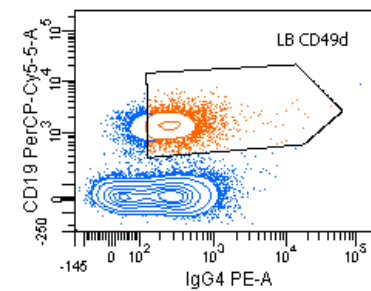
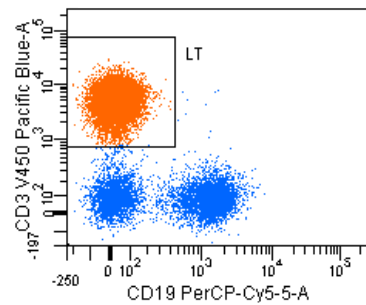
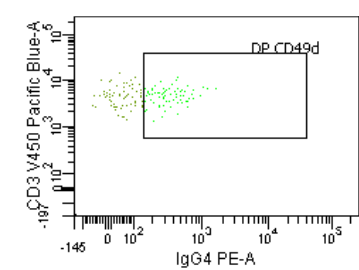
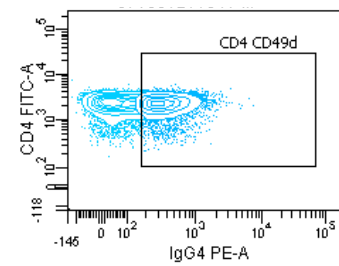
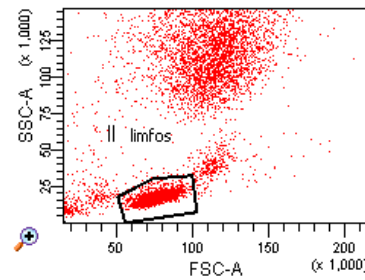
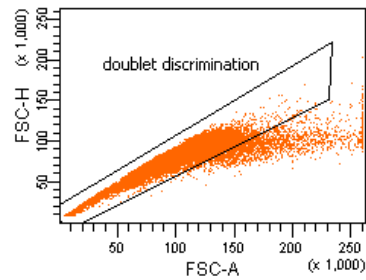


Experiment Name:
Specimen Name:
Tube Name:
Record Date:

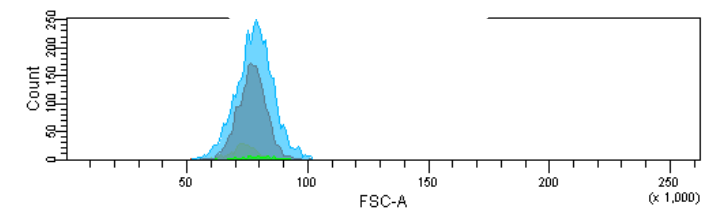
Population	%Parent	%Grand Parent	CD49d PE-A Geo Mean	CD49d PE-A Mean	CD49d PE-A Median
CD4 CD49d	62.5	43.7	595	755	591
DP CD49d	67.4	0.7	480	612	398
DN CD49d	91.2	3.1	443	520	475
CD8 CD49d	81.9	21.0	588	752	592
LB CD49d	17.4	4.8	547	702	559



ANÁLISIS



CD4 FITC CD19 PerCPcy5.5
 CD8 APCH7 CD3 V 450
 IgG4 PE



Experiment Name:
Specimen Name:
Tube Name:
Record Date:

Population	%Parent	%Grand Parent	IgG4 PE-A Geo Mean	IgG4 PE-A Mean	IgG4 PE-A Median
CD4 CD49d	43.5	30.4	403	480	375
DP CD49d	51.8	0.5	343	398	307
DN CD49d	76.2	2.3	271	304	266
CD8 CD49d	83.2	18.5	373	447	355
LB CD49d	13.3	3.8	339	496	309

RESULTADOS



Quantibrite

Population	#Events	PE-A Geo Mean
<input checked="" type="checkbox"/> LOW	2,345	116
<input checked="" type="checkbox"/> MedLow	2,421	1,317
<input checked="" type="checkbox"/> MedHigh	2,439	5,709
<input checked="" type="checkbox"/> High	2,328	15,397

	GeoMean	Log10 GM	PE/B	Log10 PE/B
L	116	2,064457989	474	2,675778342
ML	1317	3,119585775	5359	3,729083757
MH	5709	3,756560043	23843	4,377360899
H	15397	4,18743611	62336	4,794738931

$$y = 1,0005x + 0,6107$$

$$R^2 = 1$$

CD49d				
PE GeoMean	Log PE GM	Log PE/cell	PE mol/cell ABC	
595	2,774516966	3,383525203	2418,383678	LT CD4 CD49d
480	2,681241237	3,290296089	1951,1744	LT DP CD49d
443	2,646405726	3,255475988	1800,843565	LT DN CD49d
588	2,769377326	3,378388132	2389,94624	LT CD8 CD49d
547	2,737987326	3,347013819	2223,380638	LB CD49d

hulgG4				
PE GeoMean	Log PE GM	Log PE/cell	PE mol/cell ABC	
403	2,605305046	3,214397847	1638,316659	LT CD4 CD49d
343	2,53529412	3,144421909	1394,510887	LT DP CD49d
271	2,432969291	3,042148217	1101,915309	LT DN CD49d
373	2,571708832	3,180818423	1516,416225	LT CD8 CD49d
339	2,530199698	3,139330033	1378,256449	LB CD49d

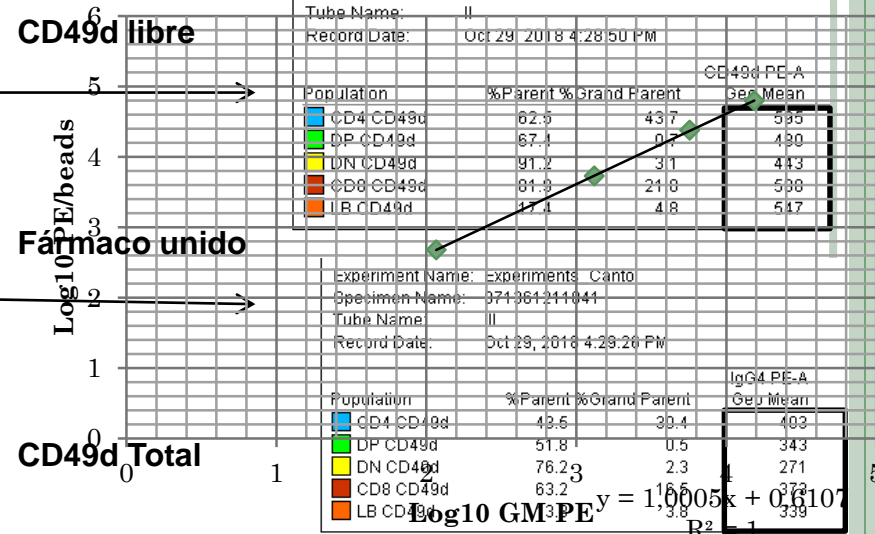
hulgG4+CD49d				
PE GeoMean	Log PE GM	Log PE/cell	PE mol/cell ABC	
709	2,850646235	3,459616427	2881,485424	LT CD4 CD49d
732	2,864511081	3,473474344	2974,91351	LT DP CD49d
602	2,79596491	3,38860219	2446,820949	LT DN CD49d
700	2,84509804	3,454071665	2844,926198	LT CD8 CD49d
736	2,866877814	3,475839894	2991,161721	LB CD49d

OCUPACIÓ (%)	
67,74428199	LT CD4 CD49d
71,47033533	LT DP CD49d
61,1888412	LT DN CD49d
63,44980483	LT CD8 CD49d
61,989226	LB CD49d

CD49d libre

Fàrmaco unido

CD49d Total



* Sat. (%) = $\frac{\# \text{ bound NTZ molecules per cell surface}}{\# \text{ CD49d molecules per cell surface}} \times 100$

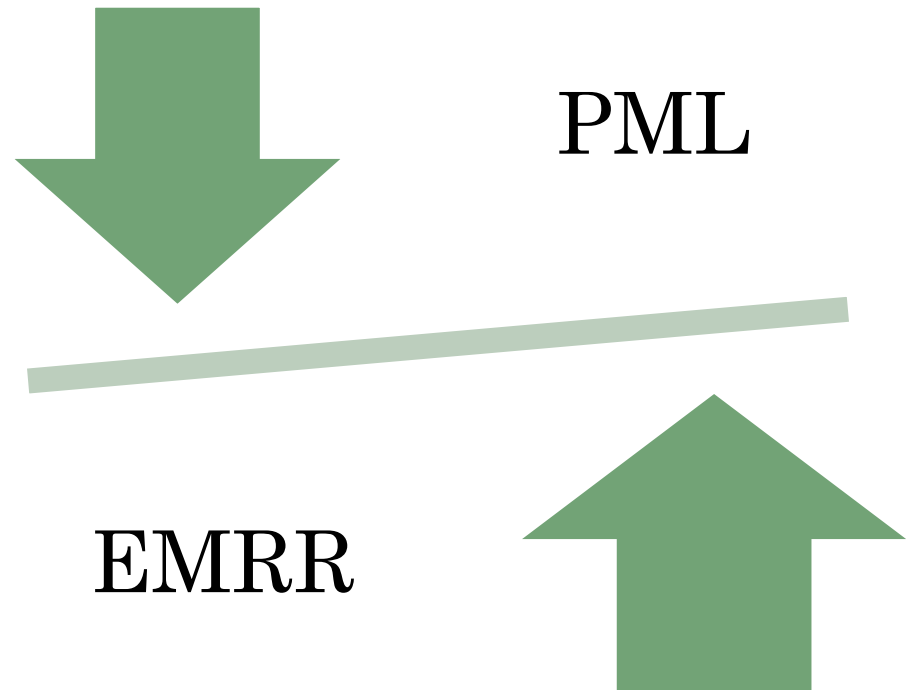
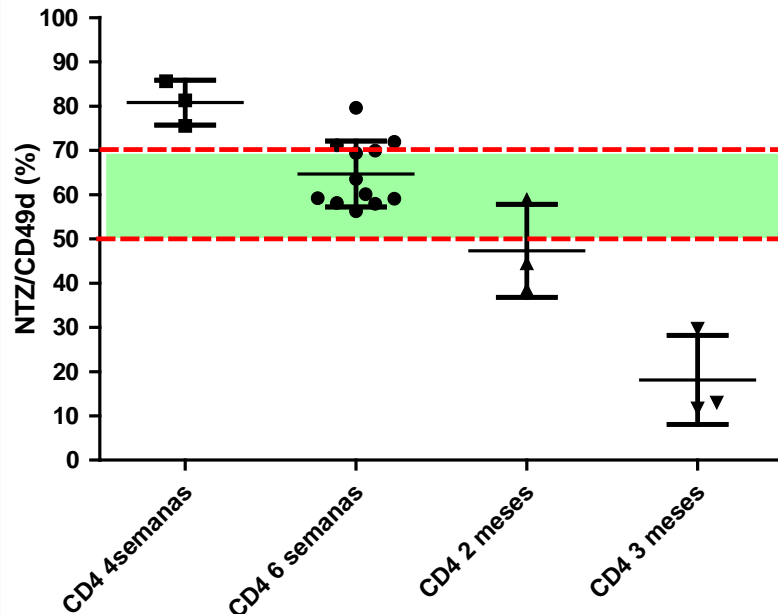
** Free CD49d = $\# \text{ CD49d molecules per cell surface} - \# \text{ bound NTZ molecules per cell surface}$

Experiment Name: Experiments_Canto			
Specimen Name: 071361211841			
Tube Name: I			
Record Date: Oct 29, 2018 4:28:15 PM			
Population	%Parent	%Grand Parent	IgG4 + CD49... I Geo Mean
CD4 CD49d	68.1	47.8	709
DP CD49d	69.4	0.1	732
DN CD49d	93.5	2.9	602
CD8 CD49d	87.1	22.4	700
LB CD49d	18.2	5.2	736

Monitoring CD49d Receptor Occupancy: A Method to Optimize and Personalize Natalizumab Therapy in Multiple Sclerosis Patients.

Puñet-Ortiz J1,2, Hervás-García JV3, Teniente-Serra A1,2, Cano-Orgaz A4, Mansilla MJ1,2, Quirant-Sánchez B1,2, Navarro-Barriuso J1,2, Fernández-Sanmartín MA5, Presas-Rodríguez S3, Ramo-Tello C3, Martínez-Cáceres EM1,2.
Cytometry B Clin Cytom. 2018 Mar;94(2):327-333. doi: 10.1002/cyto.b.21527. Epub 2017 Apr 17.

CD4 Saturación





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¡Muchas Gracias!

