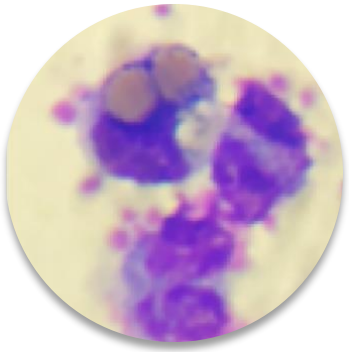
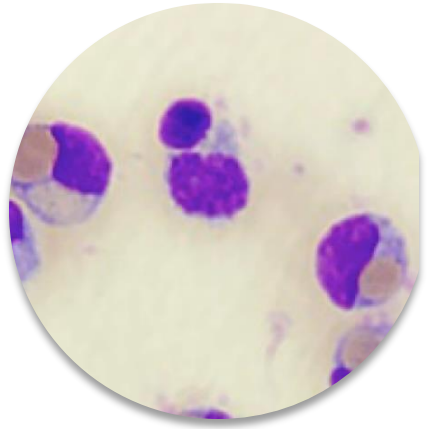
Técnicas para Valorar la capacidad lítica y fagocítica de anticuerpos anti-eritrocitarios





¨La técnica de MMA¨

Monocitos en monocapa

Grupo de trabajo de Hematíes y Donantes con fenotipos poco comúnes

GCIAMT

Grupo Cooperativo Iberoamericano de Medicina Transfusional

Dr. Diego Santoro
Dr. Sonia Gini Alvarez

Desarrollo

01
Introduction

02
Fundamento

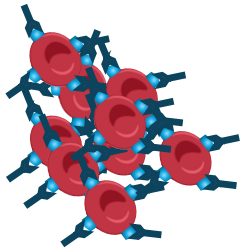
03
Tecnica MMA

04
¿Como y cuando utilizarla?

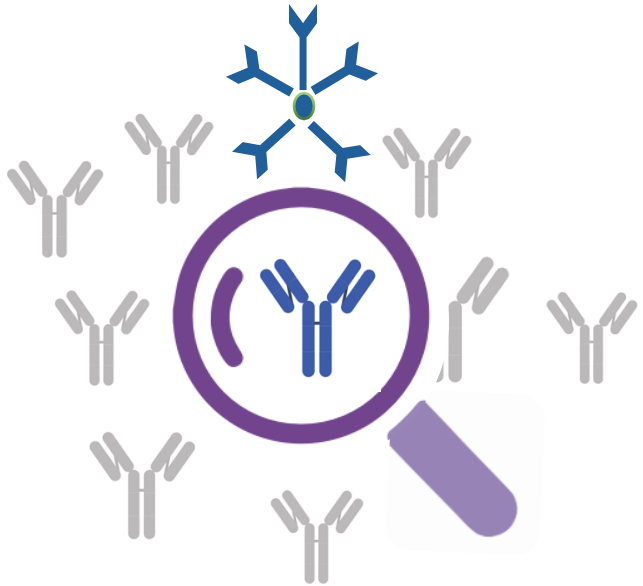
05
Conclusiones

06
Experiencia Paraguay

Valoración clínica de los anticuerpos



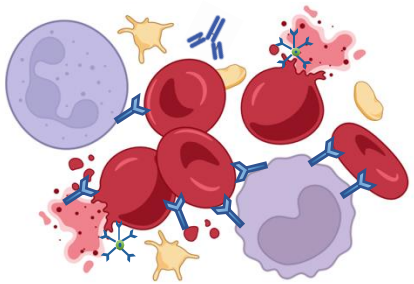
Técnicas serológicas



Características

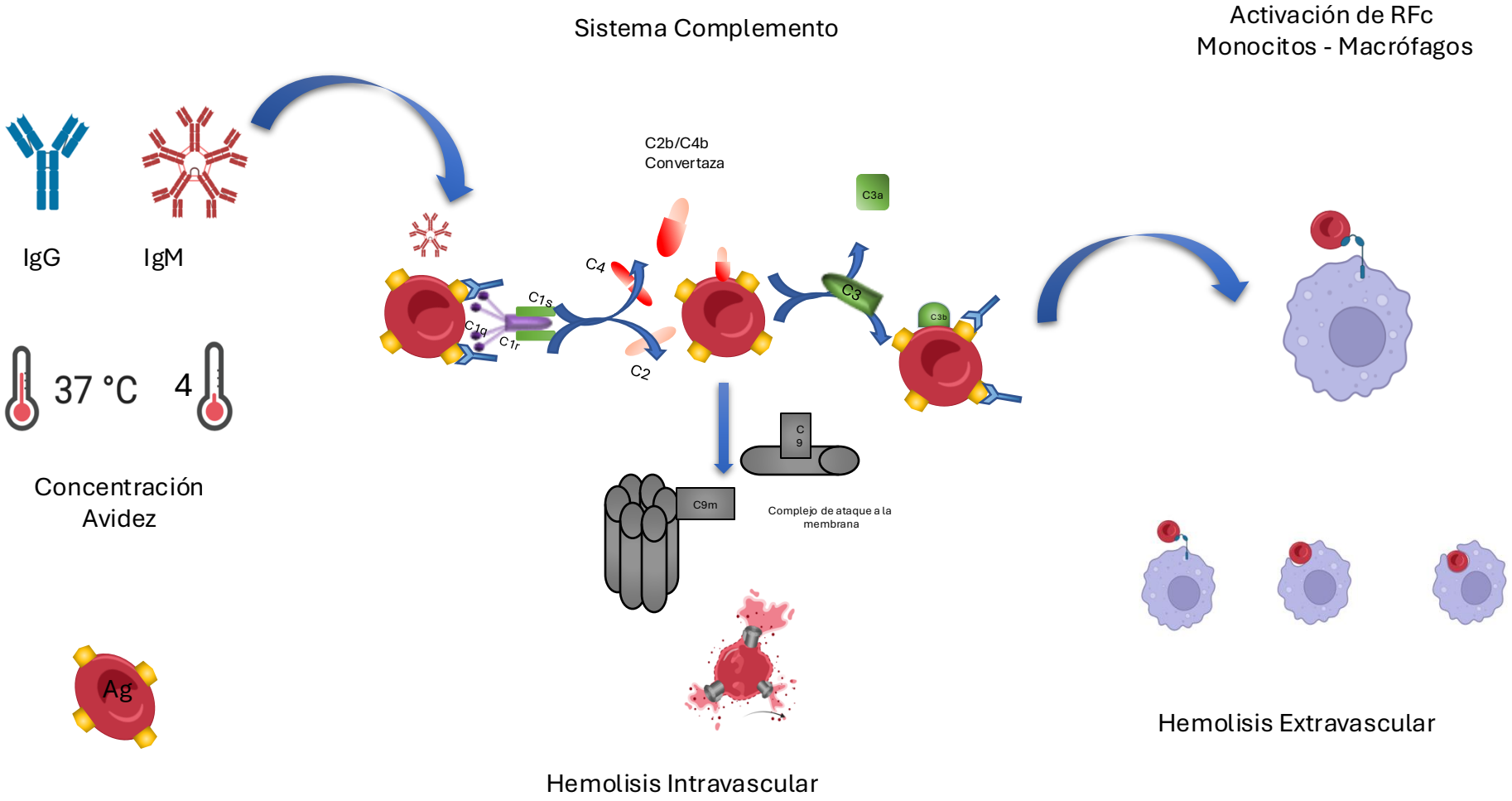
Anticuerpos clinicamente significativos

Capacidad de producir la destrucción acelerada de los glóbulos rojos

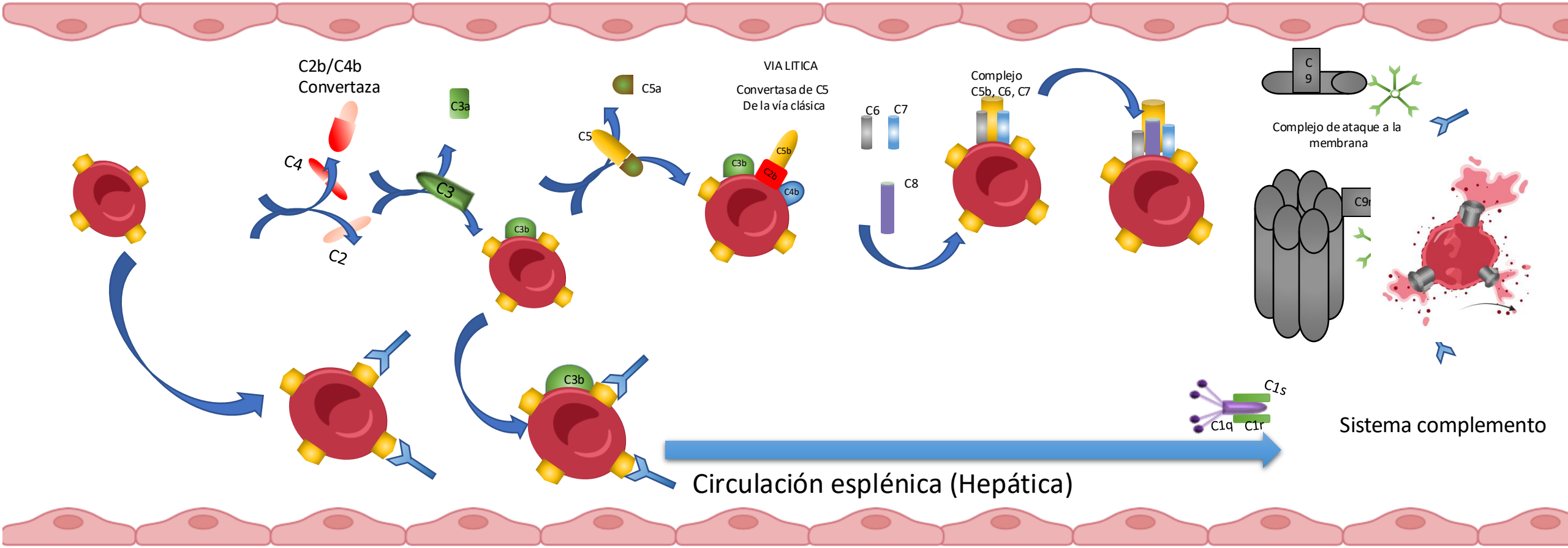


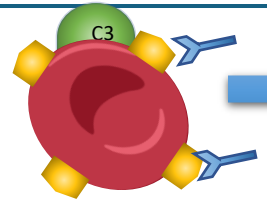
Seguridad transfusional – Materno fetal

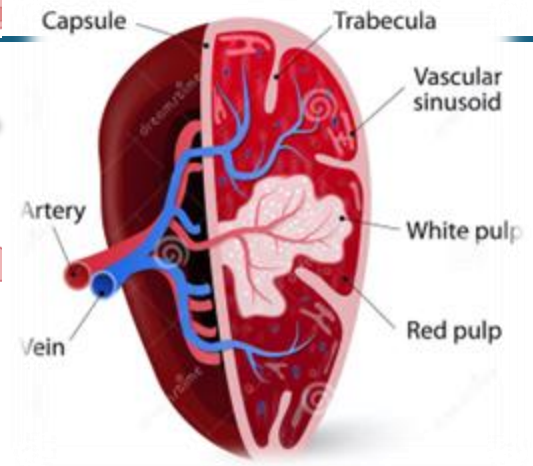
¿Cuáles son las características que debemos evaluar para prever si ese Ac va a tener repercusión clínica?

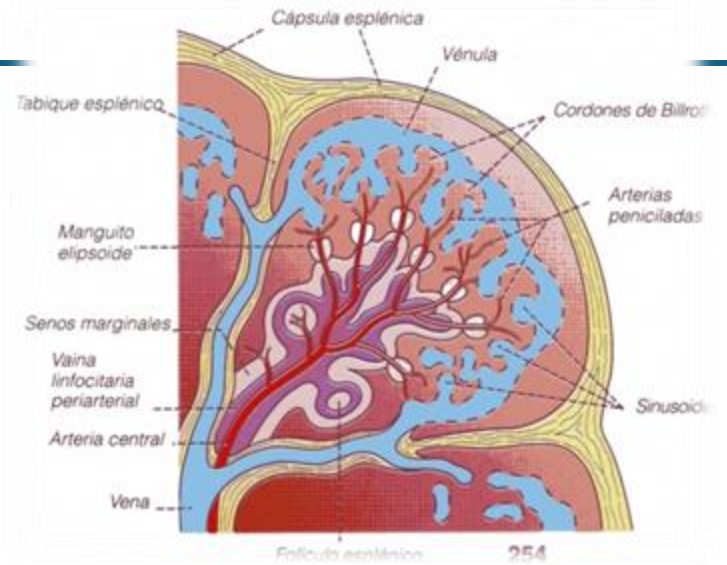


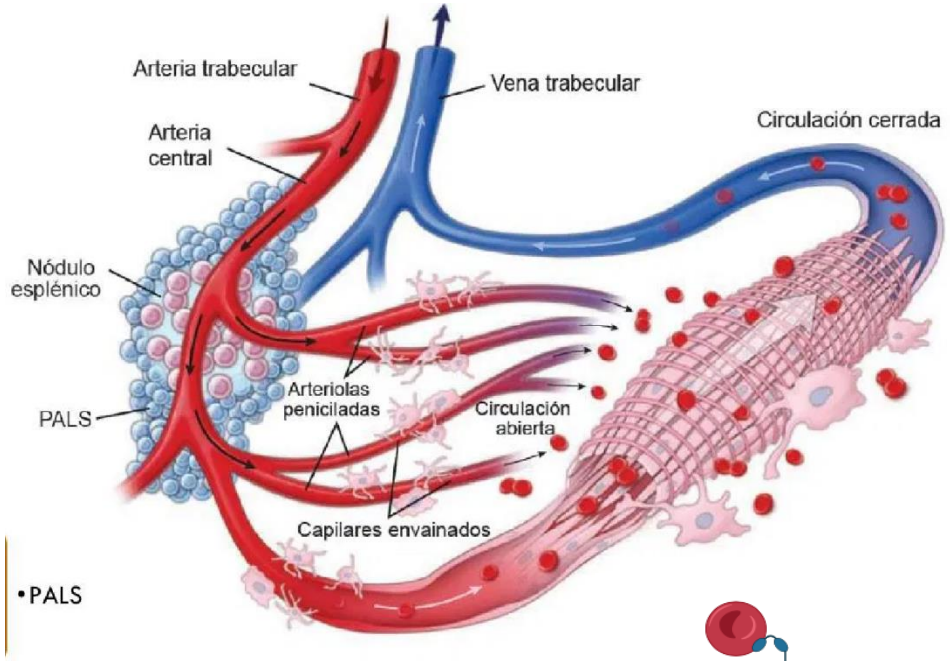
Hemolisis Intravascular











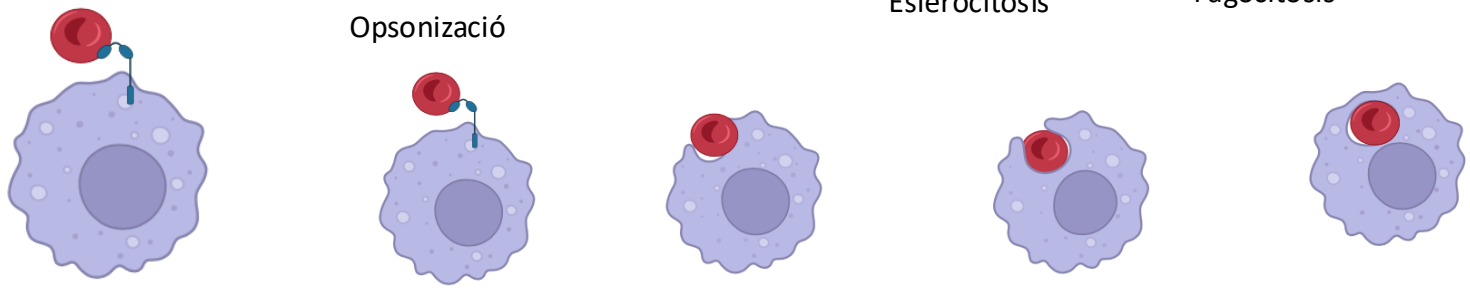
- PALS

Hemolisis Extravascular

Opsonizació

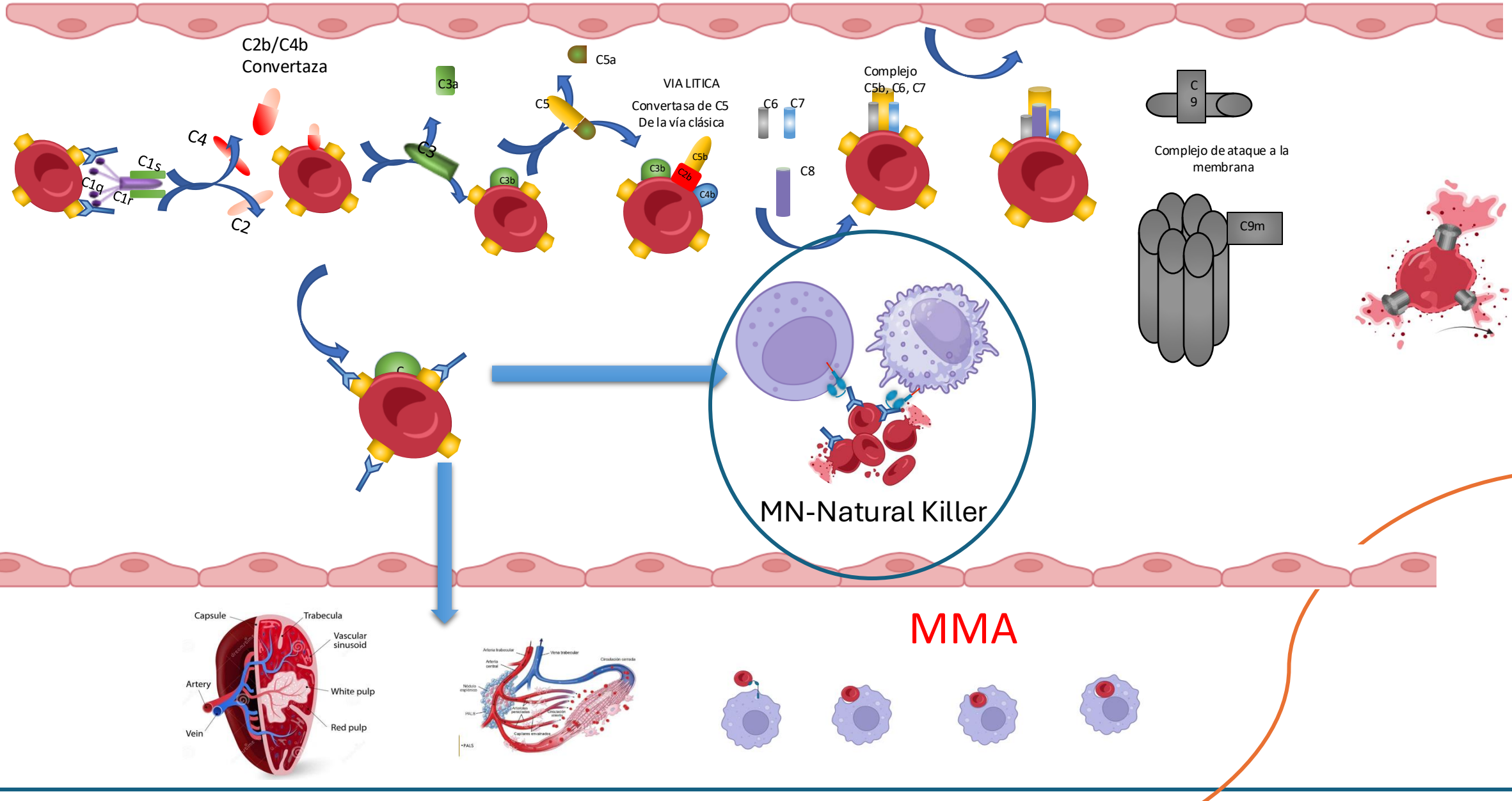
Fragmentación Esferocitosis

Fagocitosis



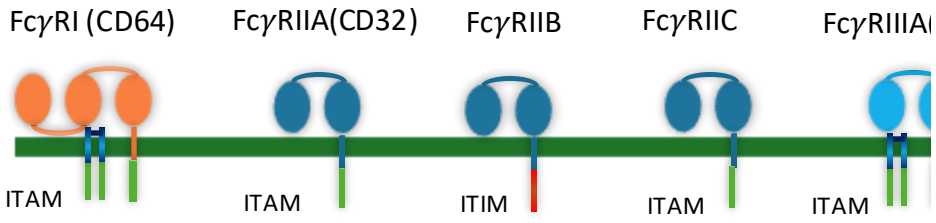
Receptores Fc y CR

Monocitos – Macrofagos

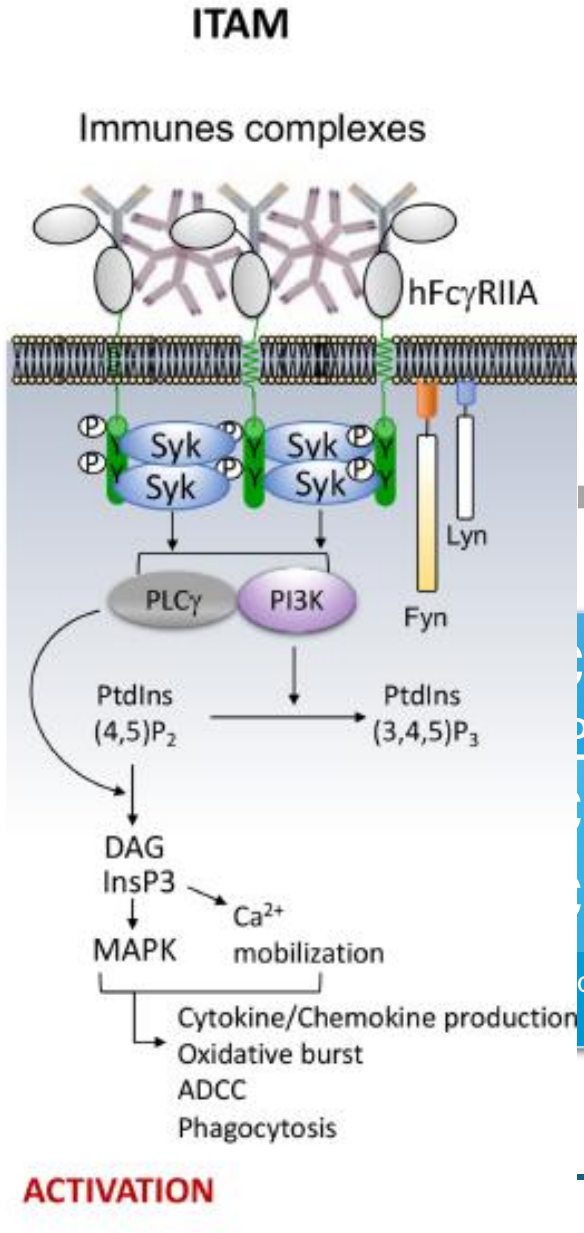

C2b/C4b
Convertaza
C1s
C1g
C1r
C4
C2
C3a
C3
C3b
C5a
C5
VIA LITICA
Convertasa de C5
De la vía clásica
C3b
C5b
C2b
C4b
C6
C7
C8
Complejo
C5b, C6, C7
C
9
Complejo de ataque a la membrana
C9m
MN-Natural Killer
Capsule
Trabecula
Vascular sinusoid
Artery
White pulp
Red pulp
Vein
MMA

Receptores Fc Monocitos-Macrofagicos

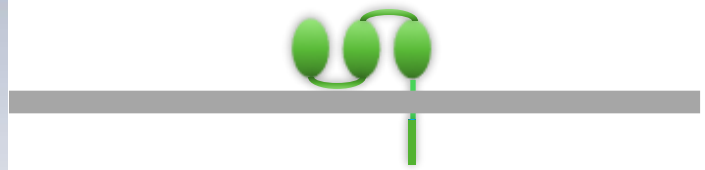
Receptores Fc de IgG macrofagicos



	Activacion	Activacion	Inhibicion	Activacion	Activa
Afinidad	Alta	Baja		Baja	Alta
IgG1	+++	+			++
IgG2	-	+		-	-
IgG3	+++	++			++
IgG4	++	-		-	-

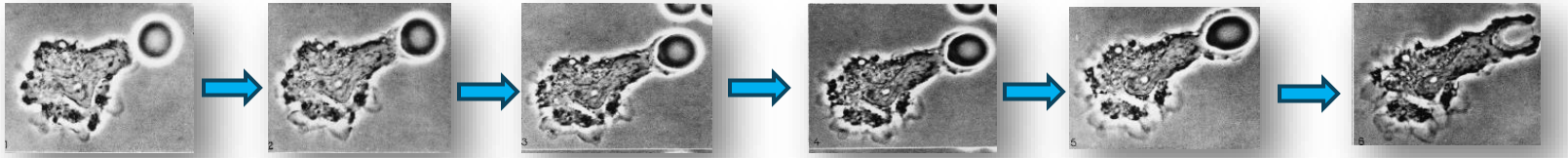


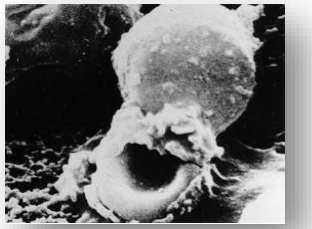
Receptores Fc de complemento



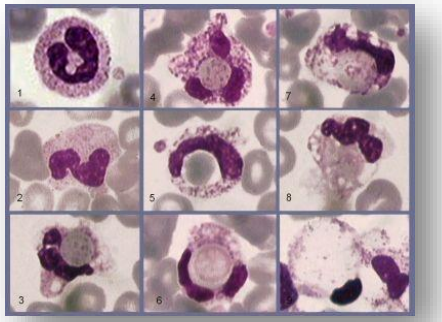
CR1 (D35)	CR2 (CD21)	CR3 (CD18)	CR4
C3b/ C4b	C3b	C3b	C3b
crofagos	Linfocitos B	Macrofagos	Macrofago s

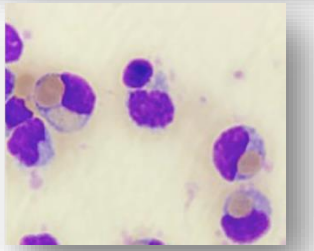
Proceso de fagocitosis

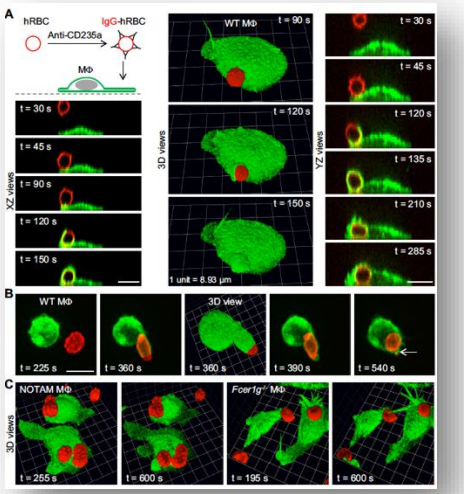


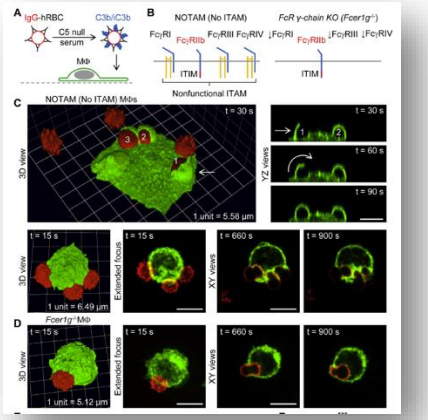


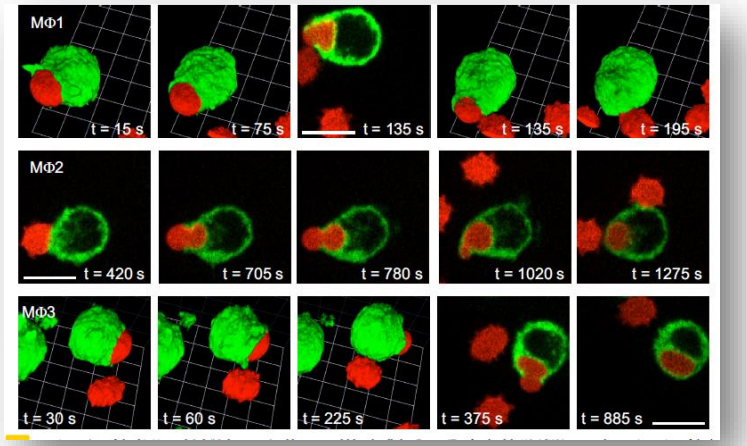
Petz, Lawrence D.
Immune hemolytic anemias /
Lawrence D. Petz,
George Garratty.

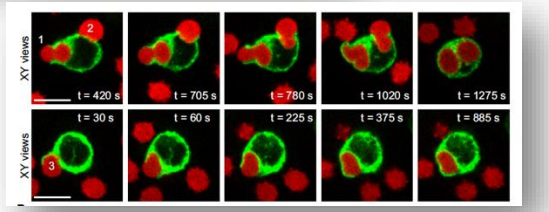




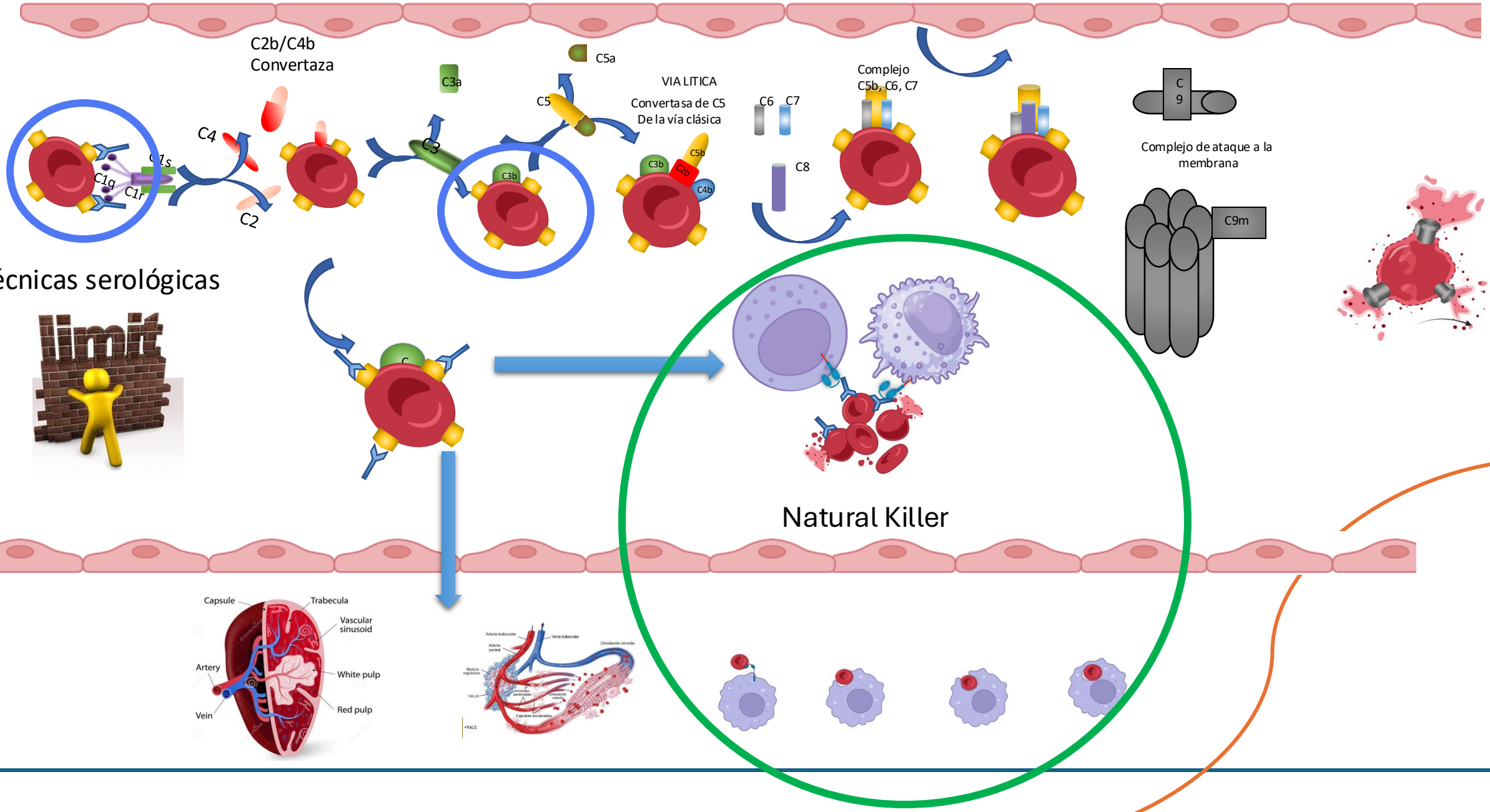




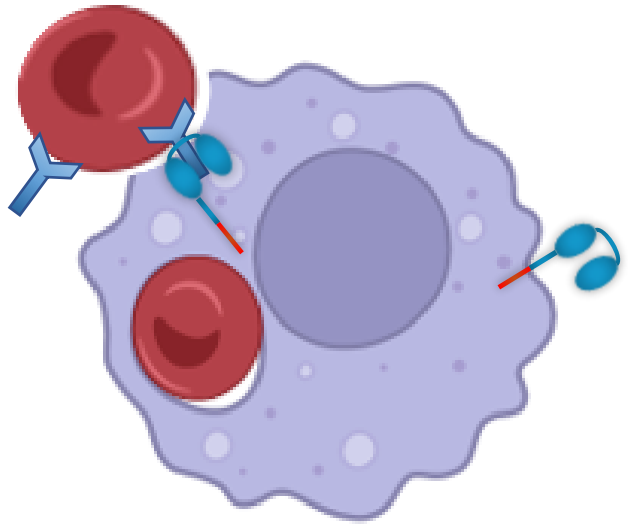




Walbaum S, Ambrosy B, Schütz P, Bachg AC, Horsthemke M, Leusen JHW, Mócsai A, Hanley PJ. Complement receptor 3 mediates both sinking phagocytosis and phagocytic cup formation via distinct mechanisms. J Biol Chem. 2021 Jan-Jun;296:100256. doi: 10.1016/j.jbc.2021.100256. Epub 2021 Jan 8. PMID: 33839682; PMCID: PMC7948798.


C2b/C4b
Convertaza
C1q
C1r
C1s
C4
C2
C3
C3a
C3b
C5
C5a
VIA LITICA
Convertasa de C5
De la vía clásica
C3b
C5b
C2b
C4b
C6
C7
C8
Complejo
C5b, C6, C7
C
9
Complejo de ataque a la membrana
C9m
Técnicas serológicas
Natural Killer
Capsule
Trabecula
Vascular sinusoid
Artery
White pulp
Red pulp
Vein

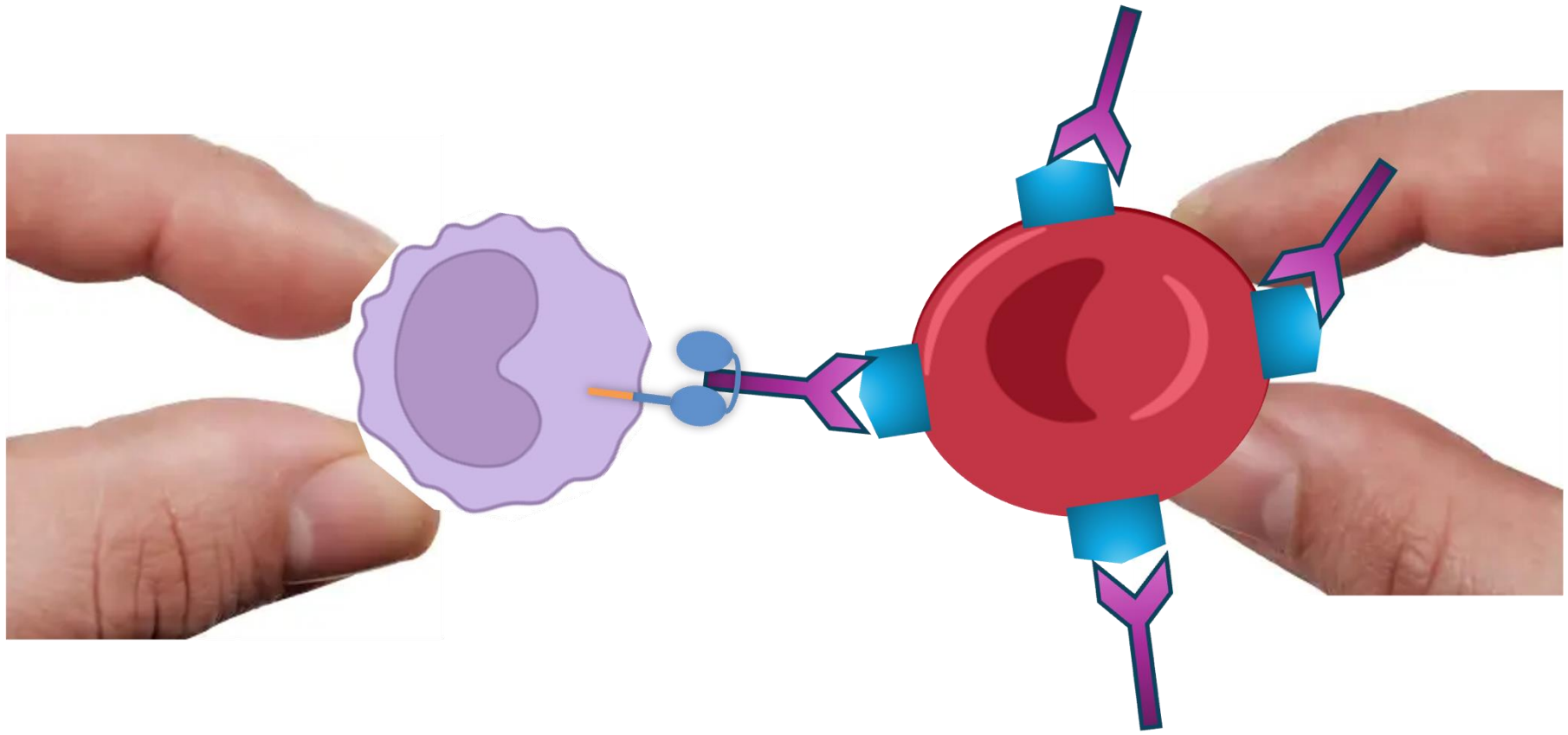
“p ntre
er e los
gló de las

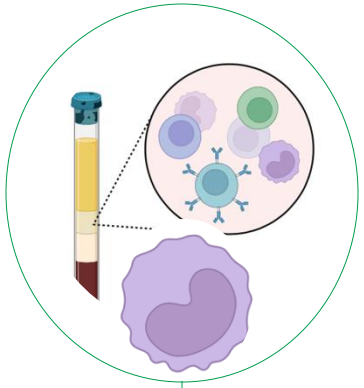


MMA

Adherencia y Fagocitosis

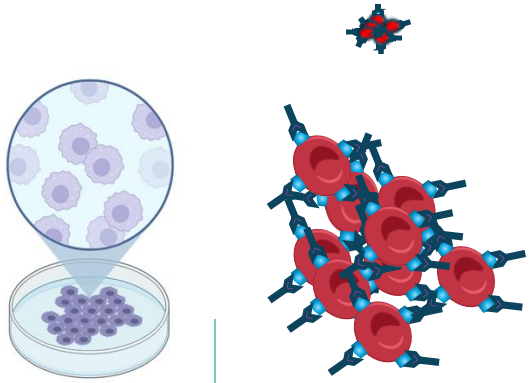
¿Cómo lo hacemos en el laboratorio?





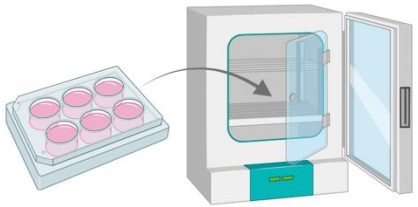
Separación de células mononucleares

Obtención y separación de las células mononucleares – Viabilidad – Prepar la suspensión de trabajo



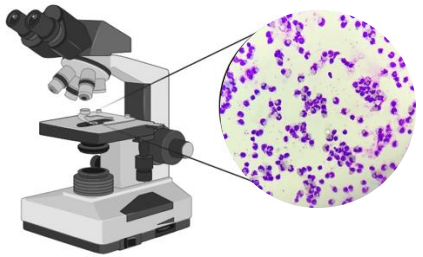
Formar la MMA Preparar los GR

Incubación y formación de la monocapa – Sensibilización de los eritrocitos con el Ac en estudio



Incubación

Incubación de los GR sensibilizados con la monocapa de monocitos Interacción con los receptores Fc

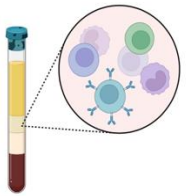


Visualización de la reacción

Acondicionamiento de la reaccion.Tinción y visualización e interpretación

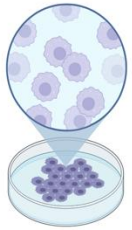
6-7hs

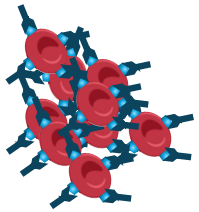
1



Tiempo 100min

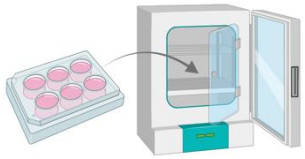
2





80min

3

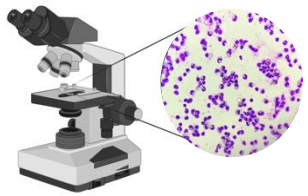


1h a 37ºC

100min

Lavados y Tinción

4



30min

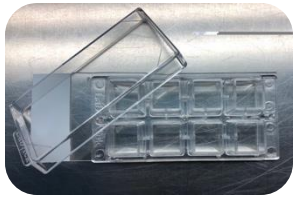
Placa de cultivo celular

Cabina de seguridad biológica

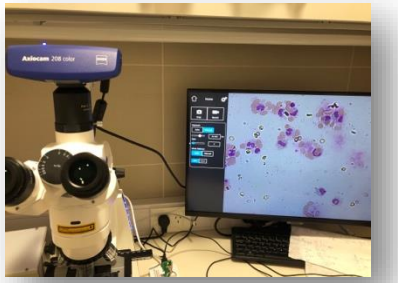
Incubadora CO2

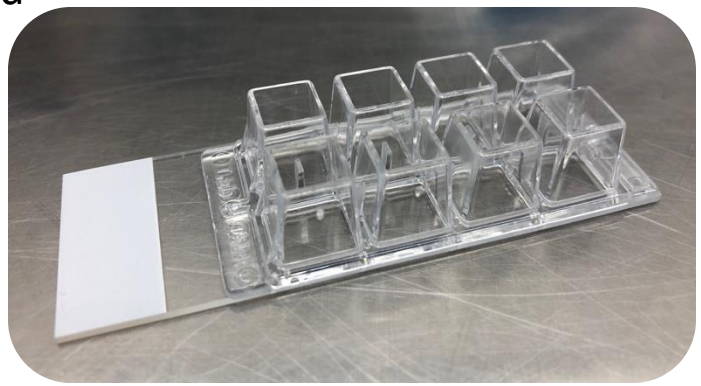
Medio cultivo celular

Gradiente de densidad



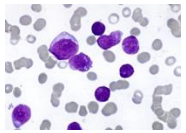
Microscopio





Suero fetal bovino

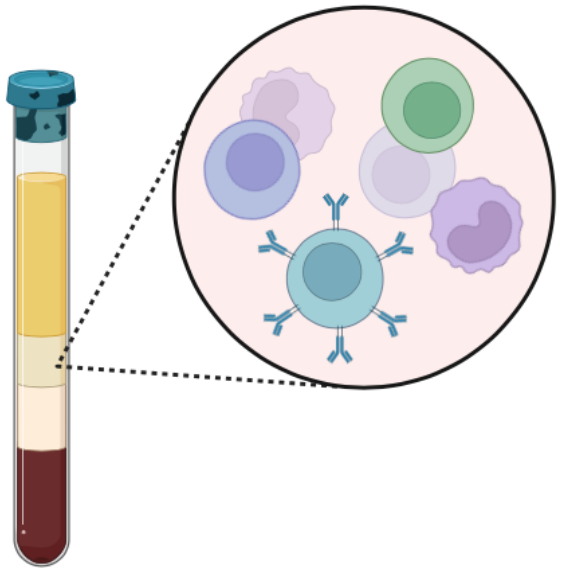
Tinción ***grunwald*** giemsa

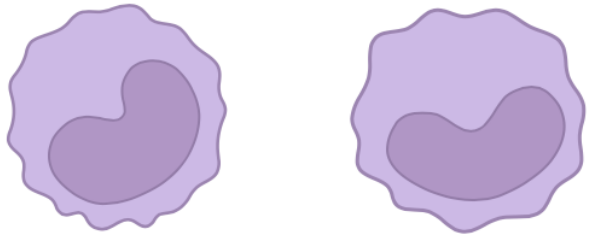


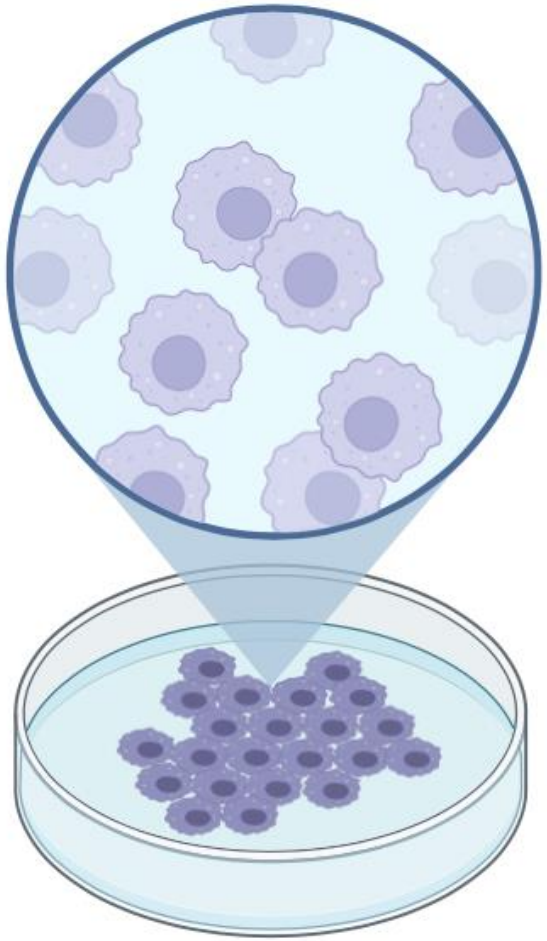
Pipeta automática

1

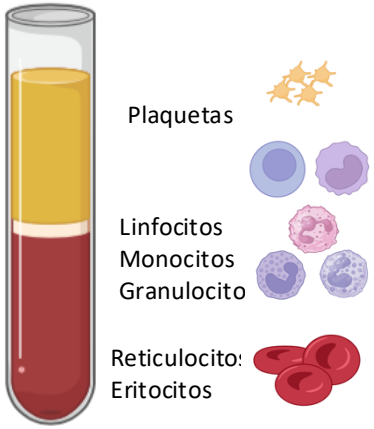
Obtención de células mononucleares

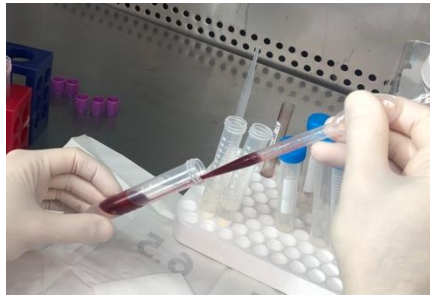


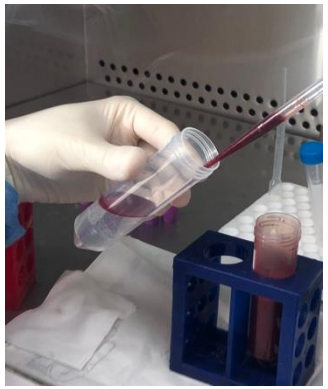


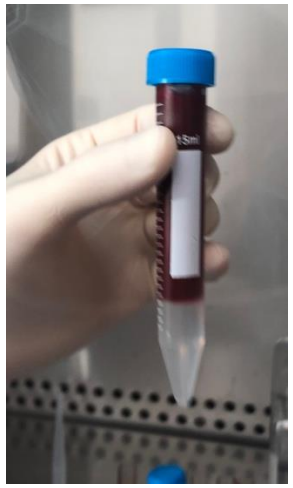


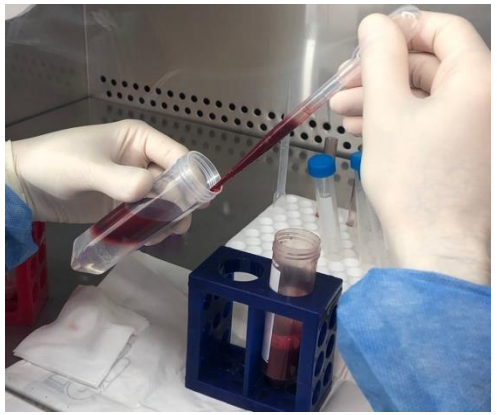
Separación de células mononucleares

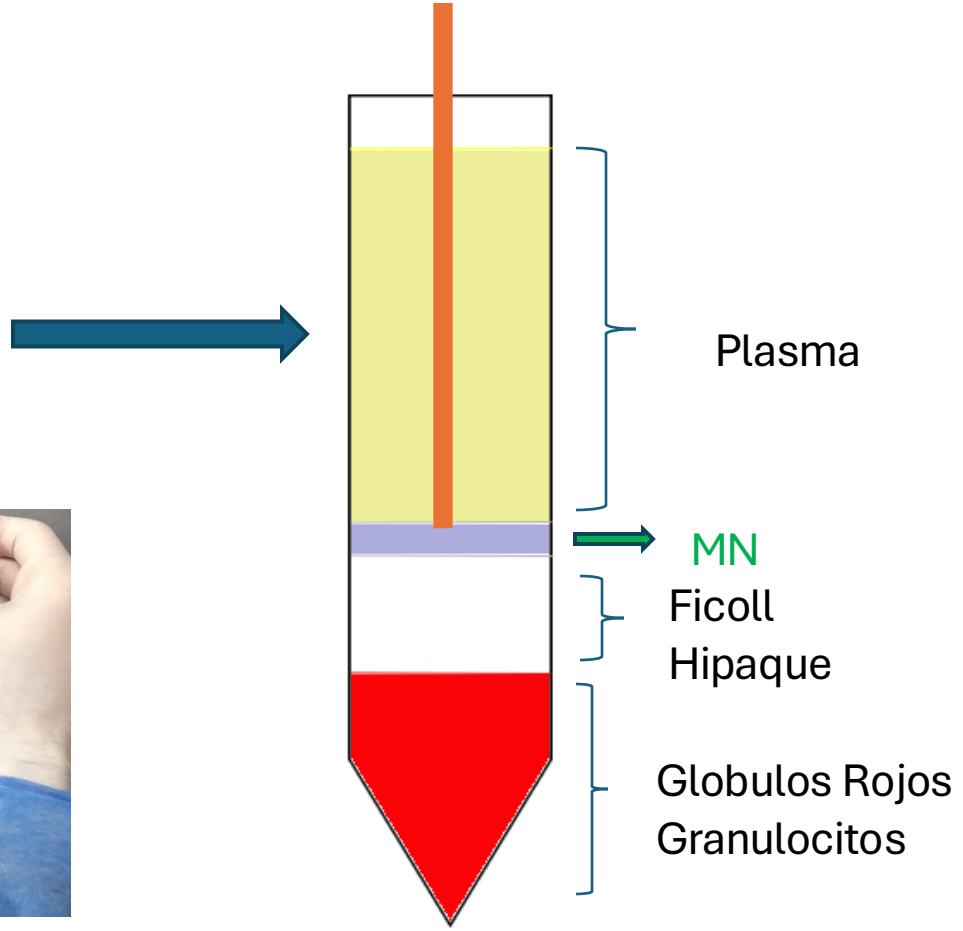






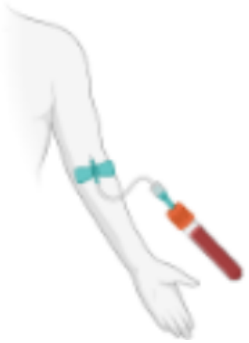






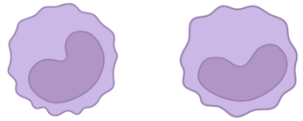
Fuente de Obtención Monocitos

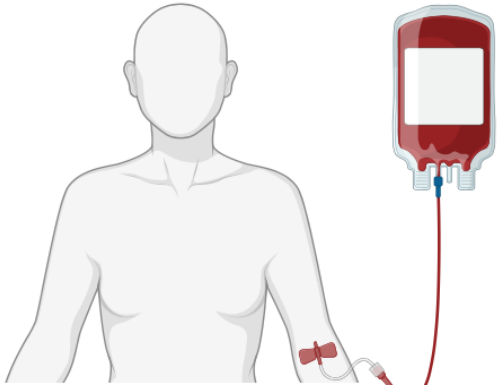
Blood sample collection (with, arm and vial)



Sangre entera (EDTA,ACD,heparina) 21ml

Autólogos o Alogénicos





Buffy coat

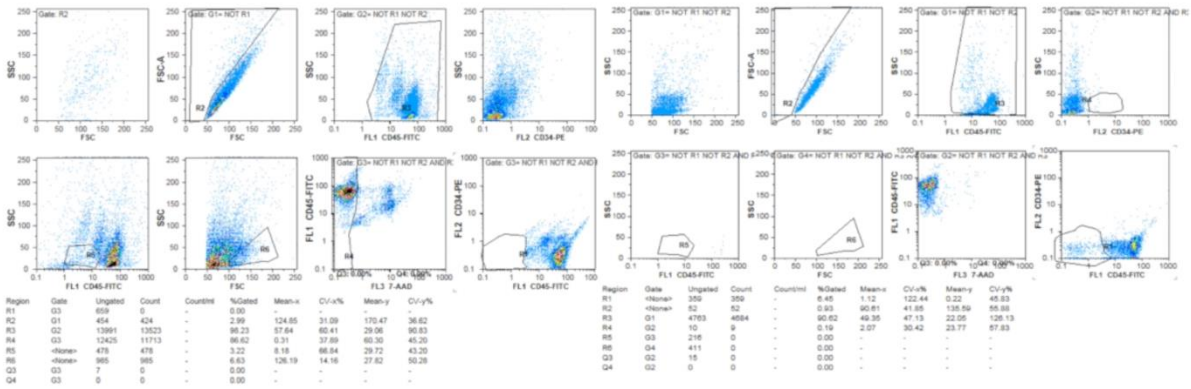
Optimal conditions for the performance of a monocyte monolayer assay
Tik Nga Tong,1,2 Emeralda Burke-Murphy,2 Darinka Sakac,2 Jacob Pendergrast,3 Christine Cserti-Gazdewich,3 Vincent Laroche,4 and Donald R. Branch1,2,3,5. TRANSFUSION Volume 56, November 2016

Criopreservación de células mononucleares

DMSO 20%

Monocitos-Criopreservados 86% viabilidad

Monocitos buffy 91% viabilidad

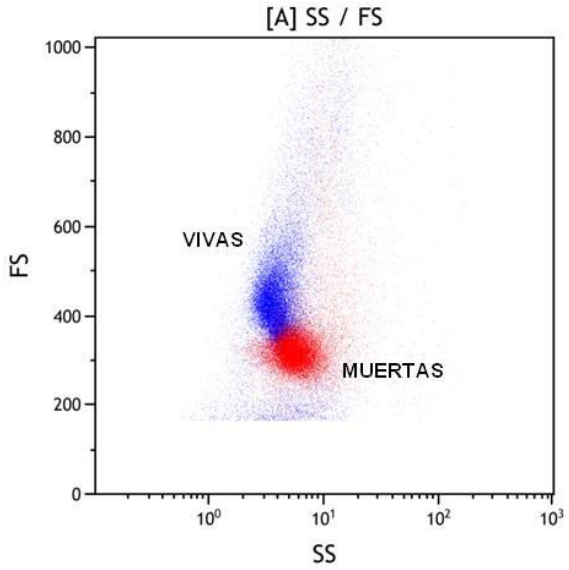


Control de viabilidad

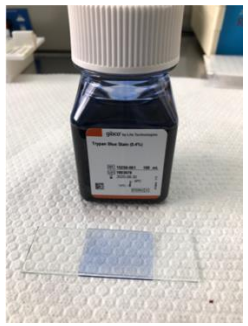
Citometria - tincion tripano

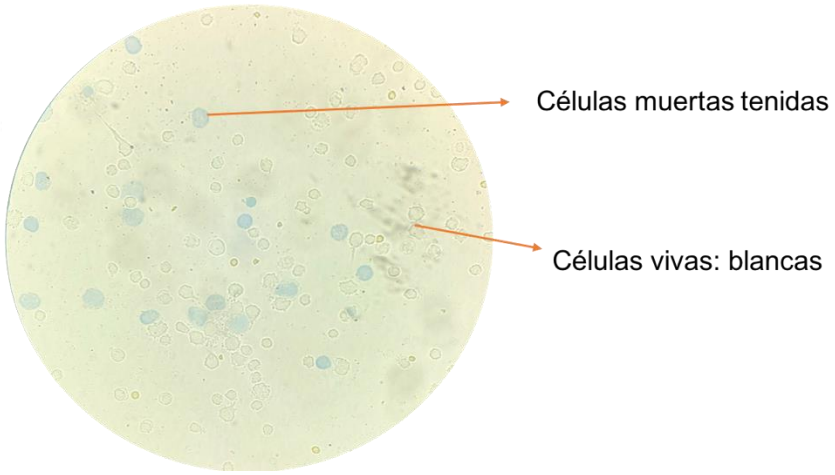
Control de viabilidad

Citometría de Flujo

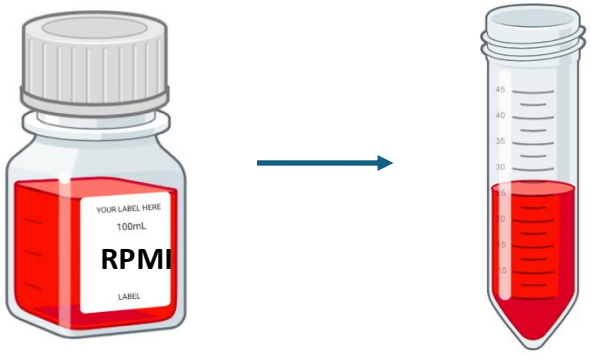


Azul de Tripano

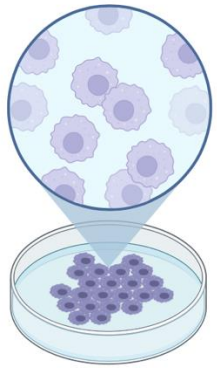


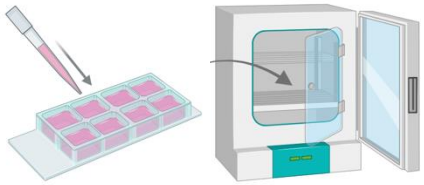


Preparación de la Monocapa de Monocitos



Suspensión de monocitos





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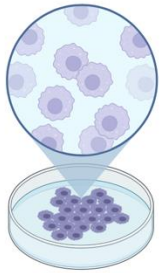
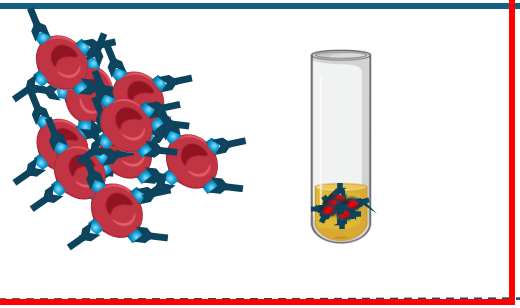


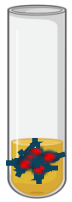


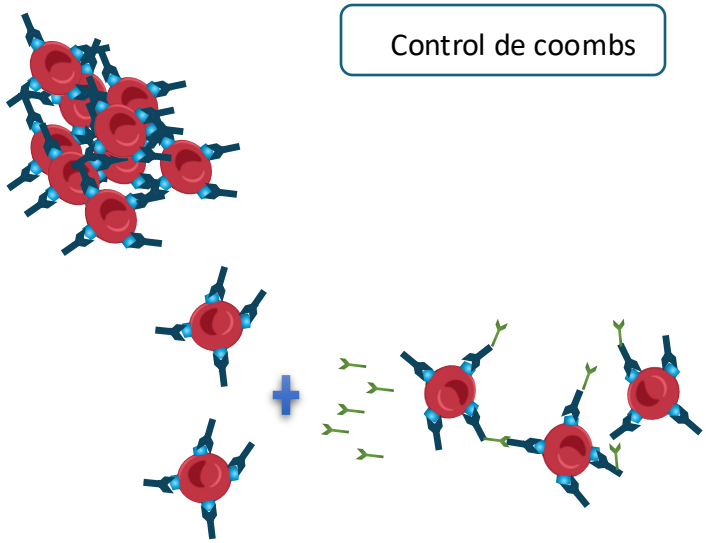
80min

Control de coombs

Suero con Ac a estudiar

Suspensión de GR
Con el Ag

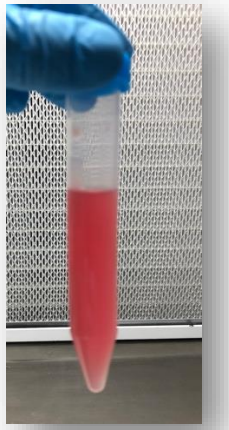


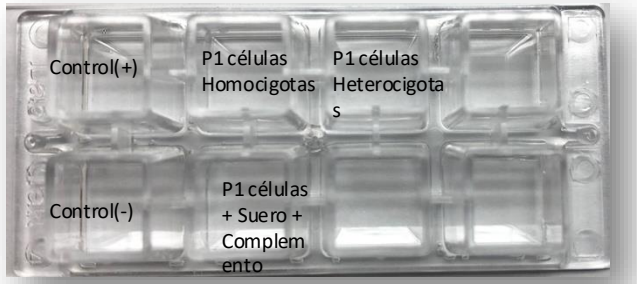


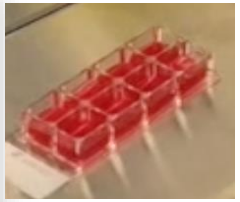
- Suero + Gr Ag positivo
- Suero + Gr Ag positivo + complemento
- Suero + Gr Ag negativo (control negativo)
- PBS + Gr Ag negativo (control negativo)
- Anti-D + Gr Ag positivo (control positivo)

Suspensión del paquete
GR con RPMI al 3-5%

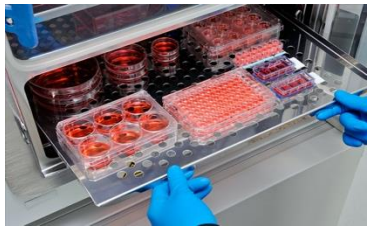
Sembrado placa







1 hora 37ºC



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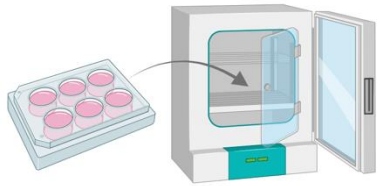


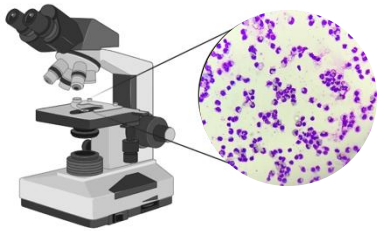
1h a 37ºC

100min

Lavados y Tinción

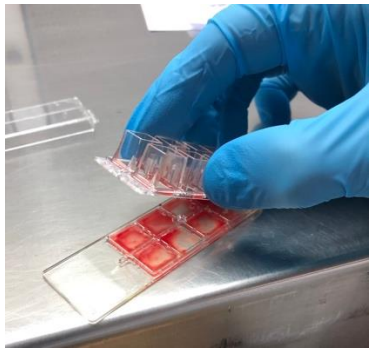
Lavados y Tinción

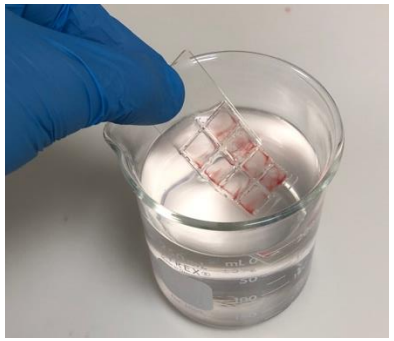
4



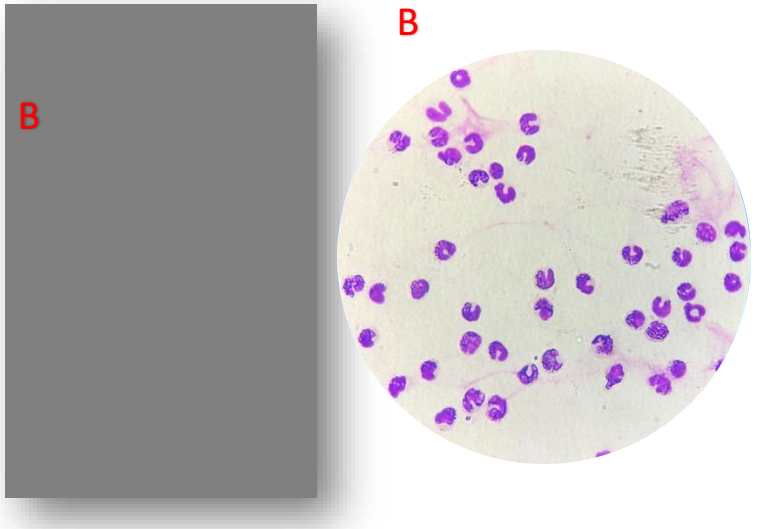
30min

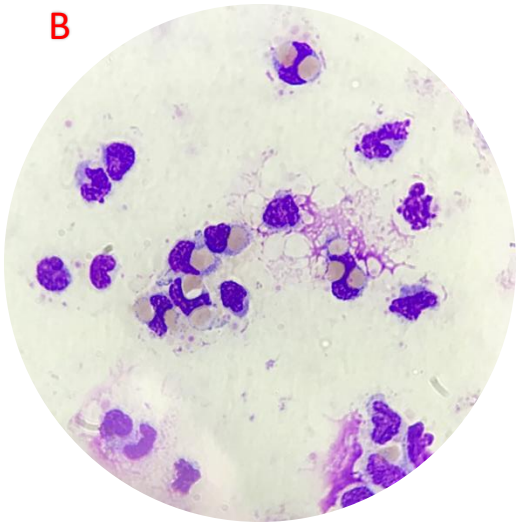
May Grünwald-Giemsa

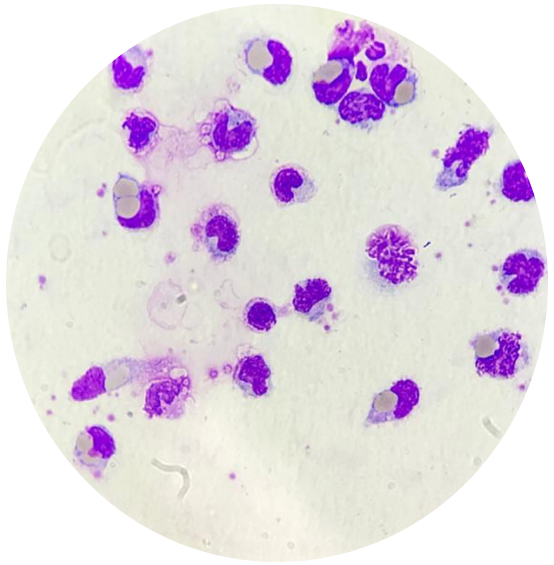




Monocitos

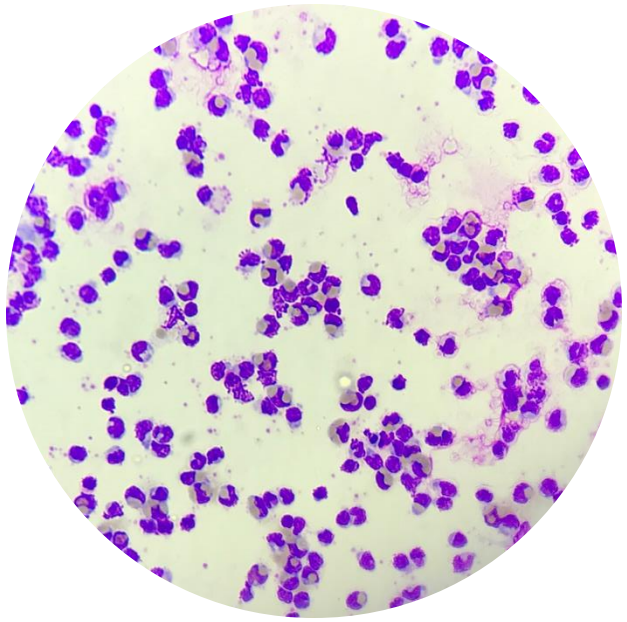






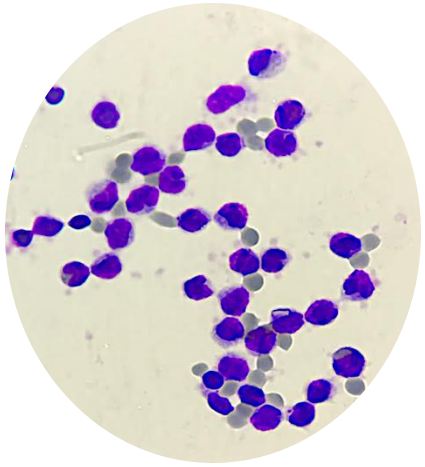
40x

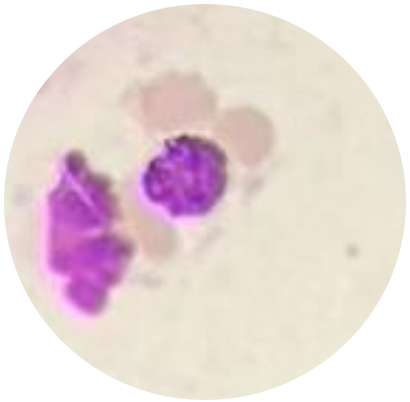
Fagocitosis



20x

Opsonización - Adherencia





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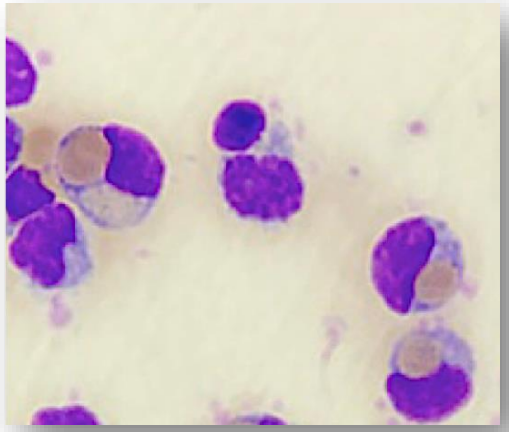

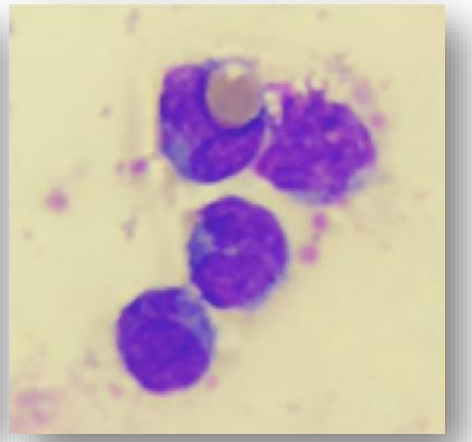

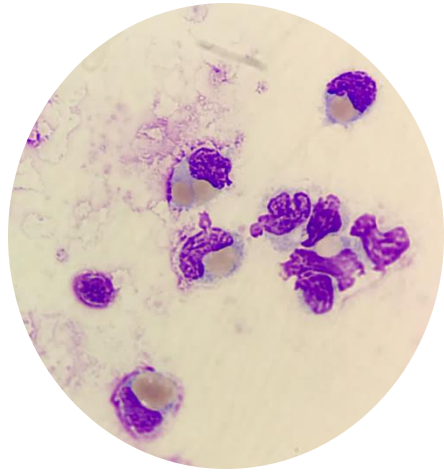

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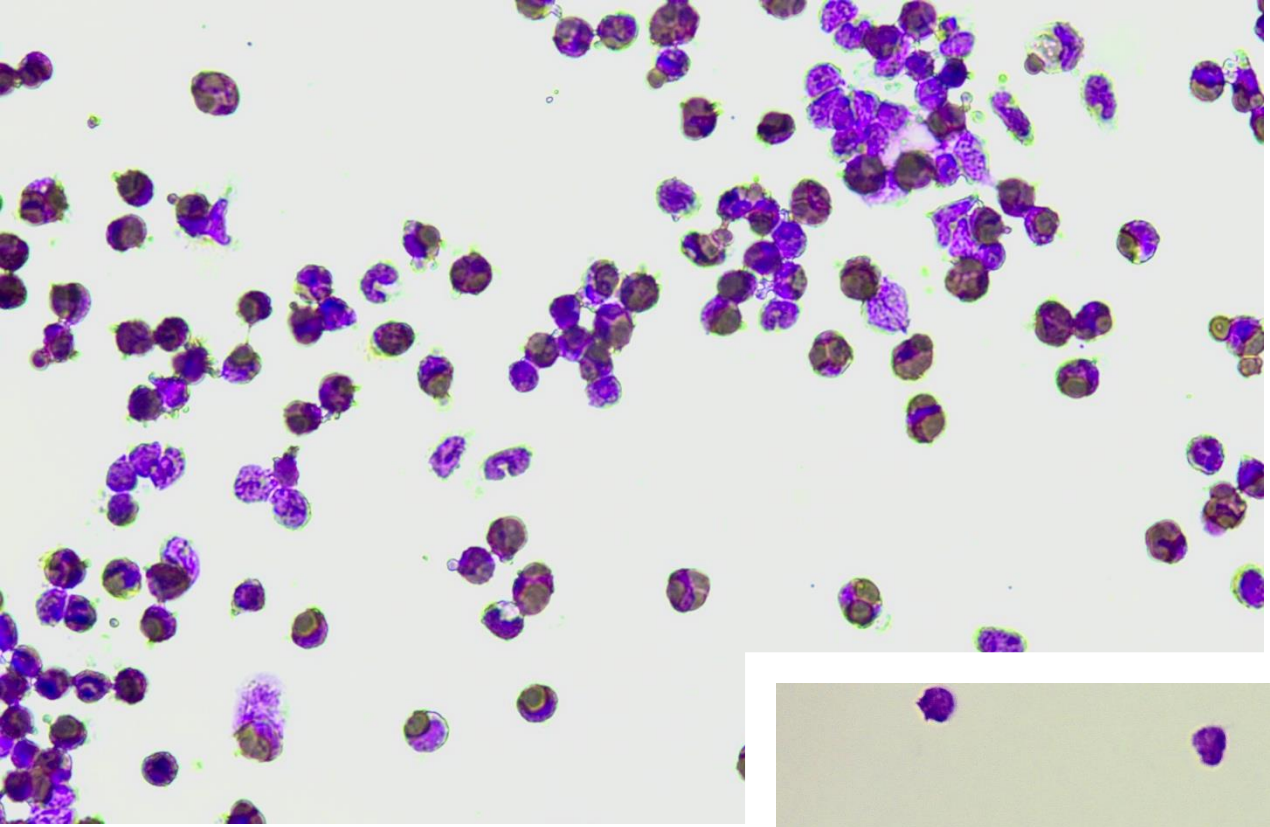
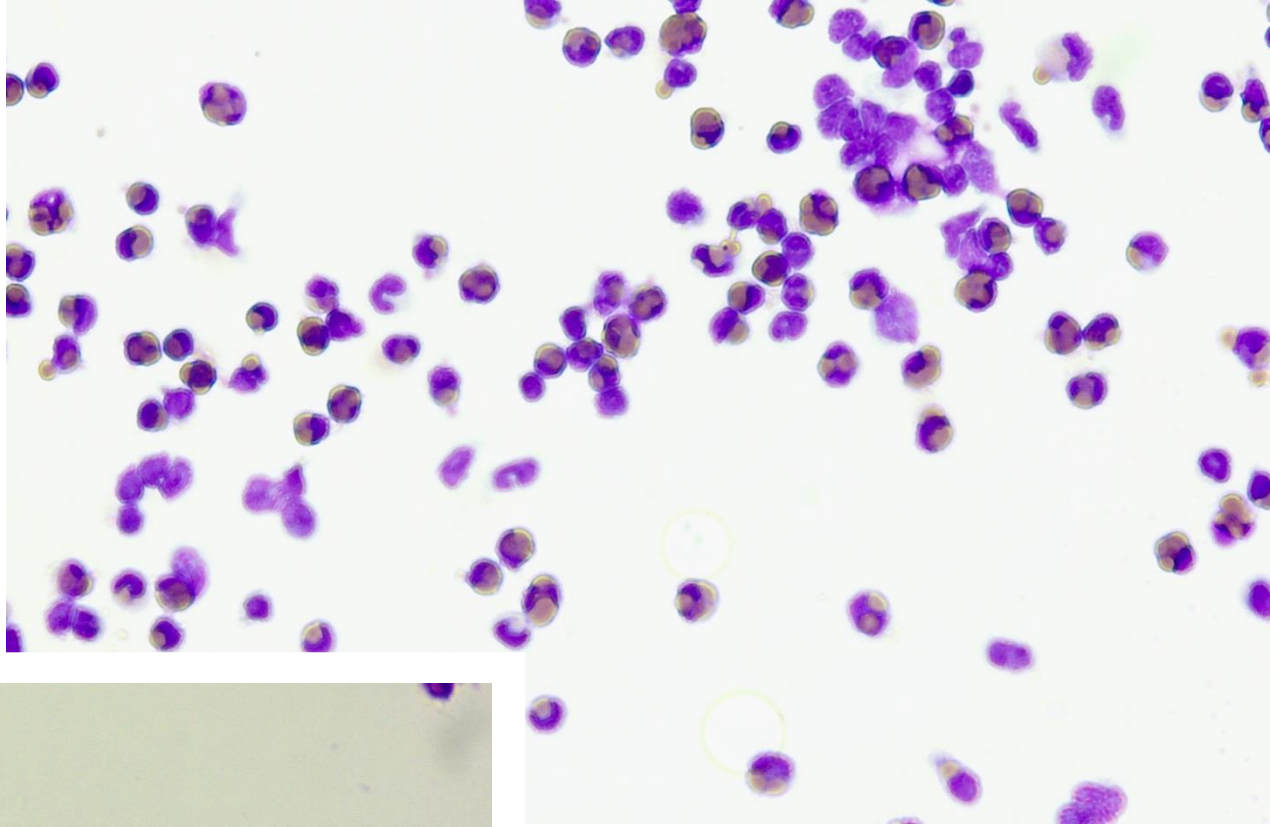
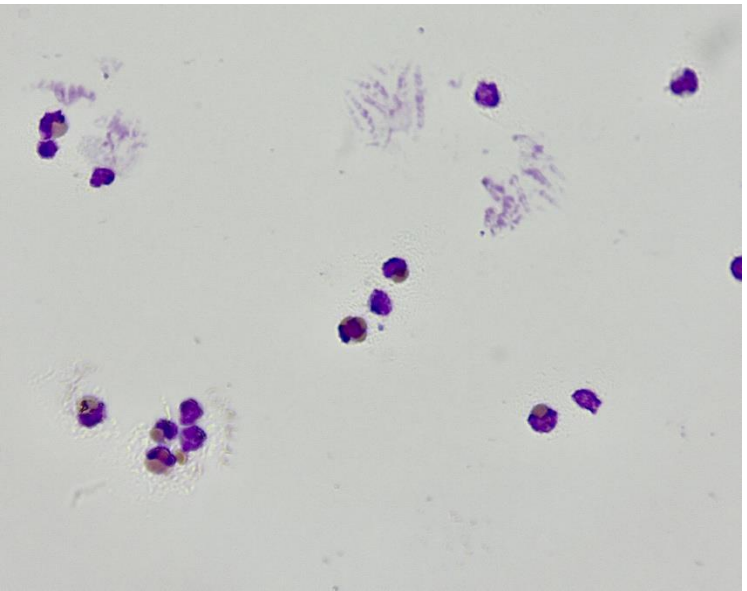
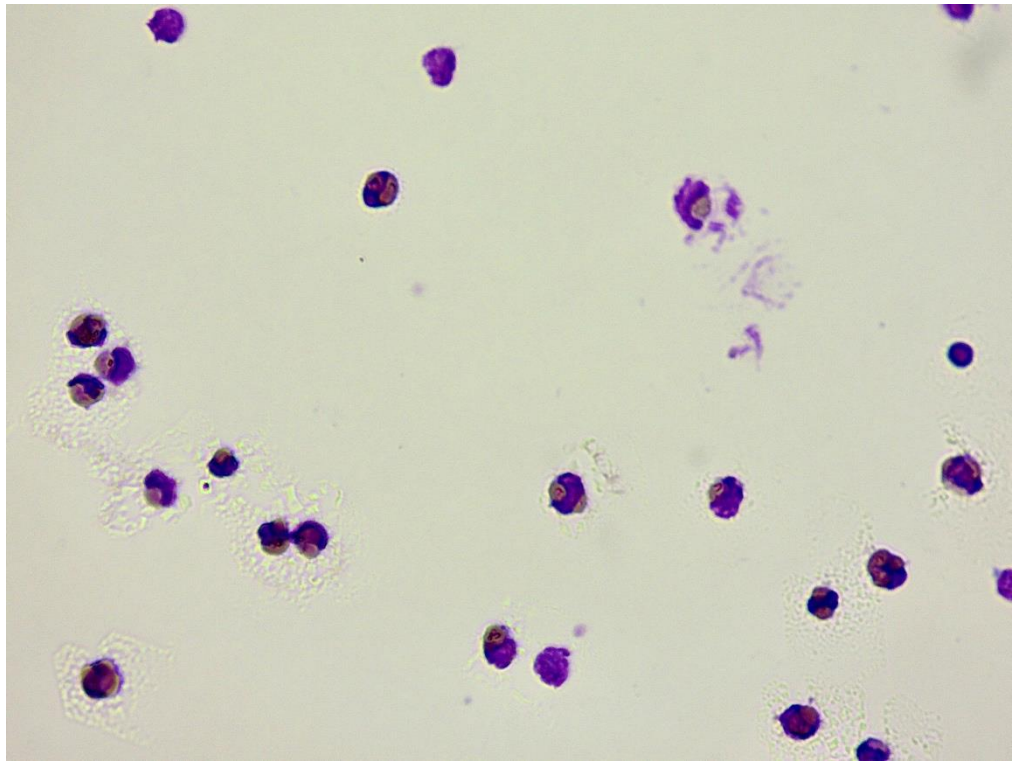
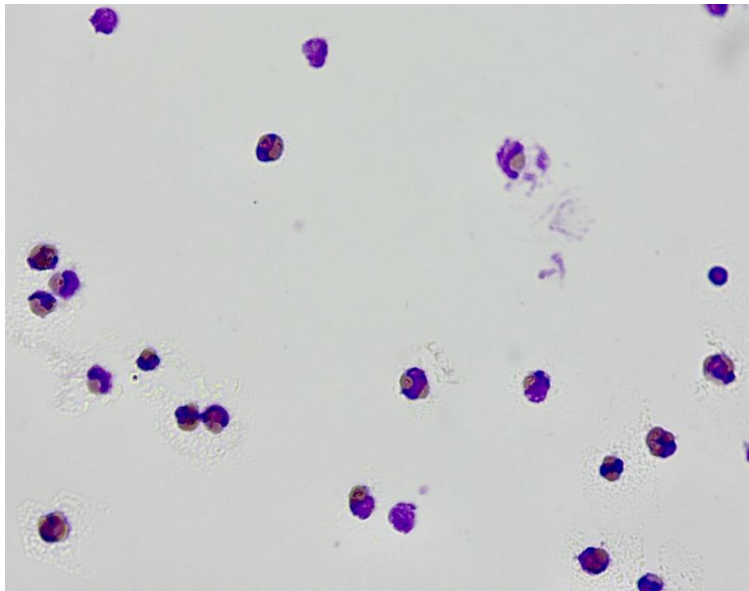
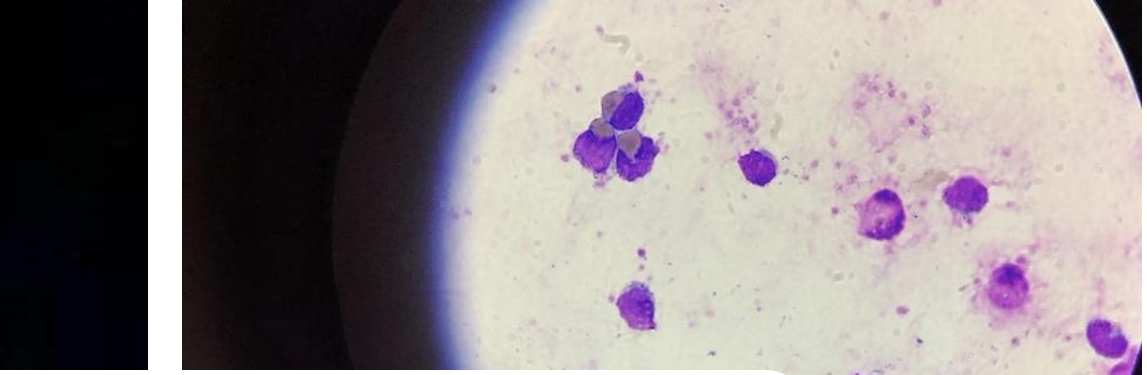
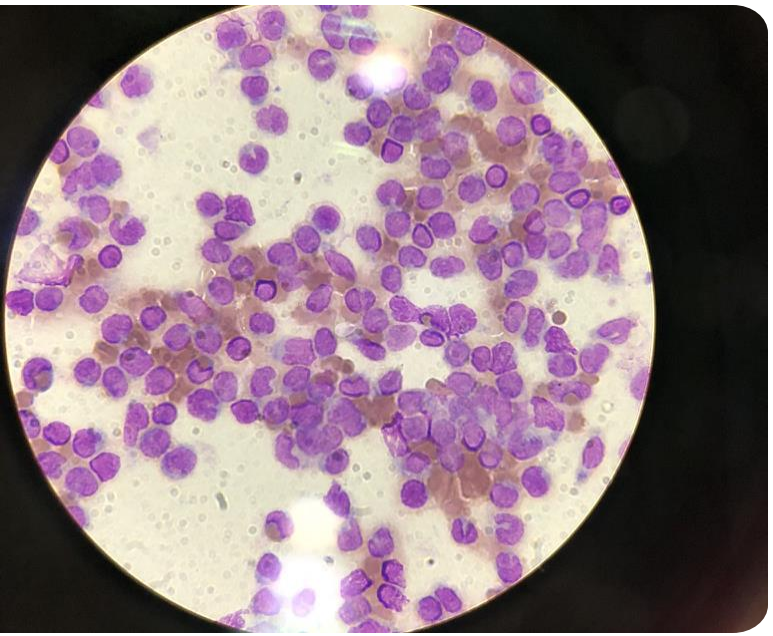
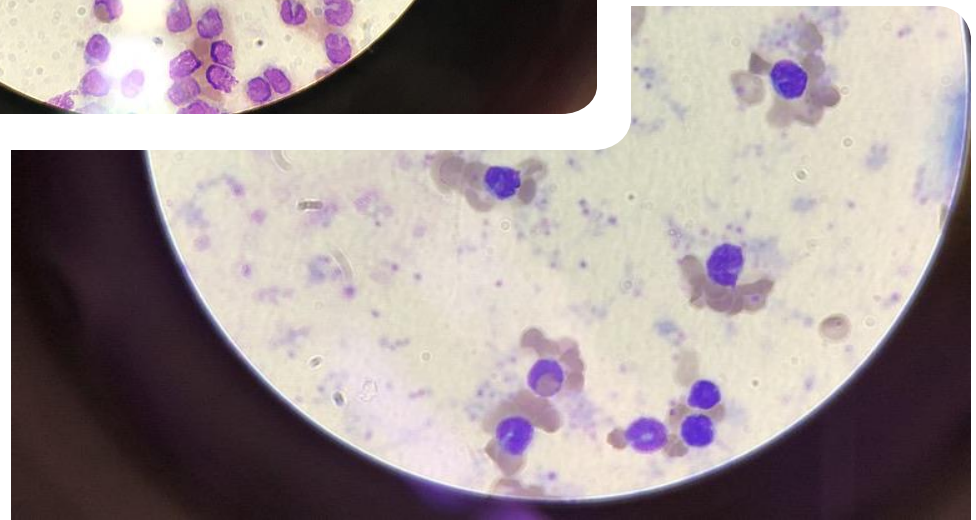
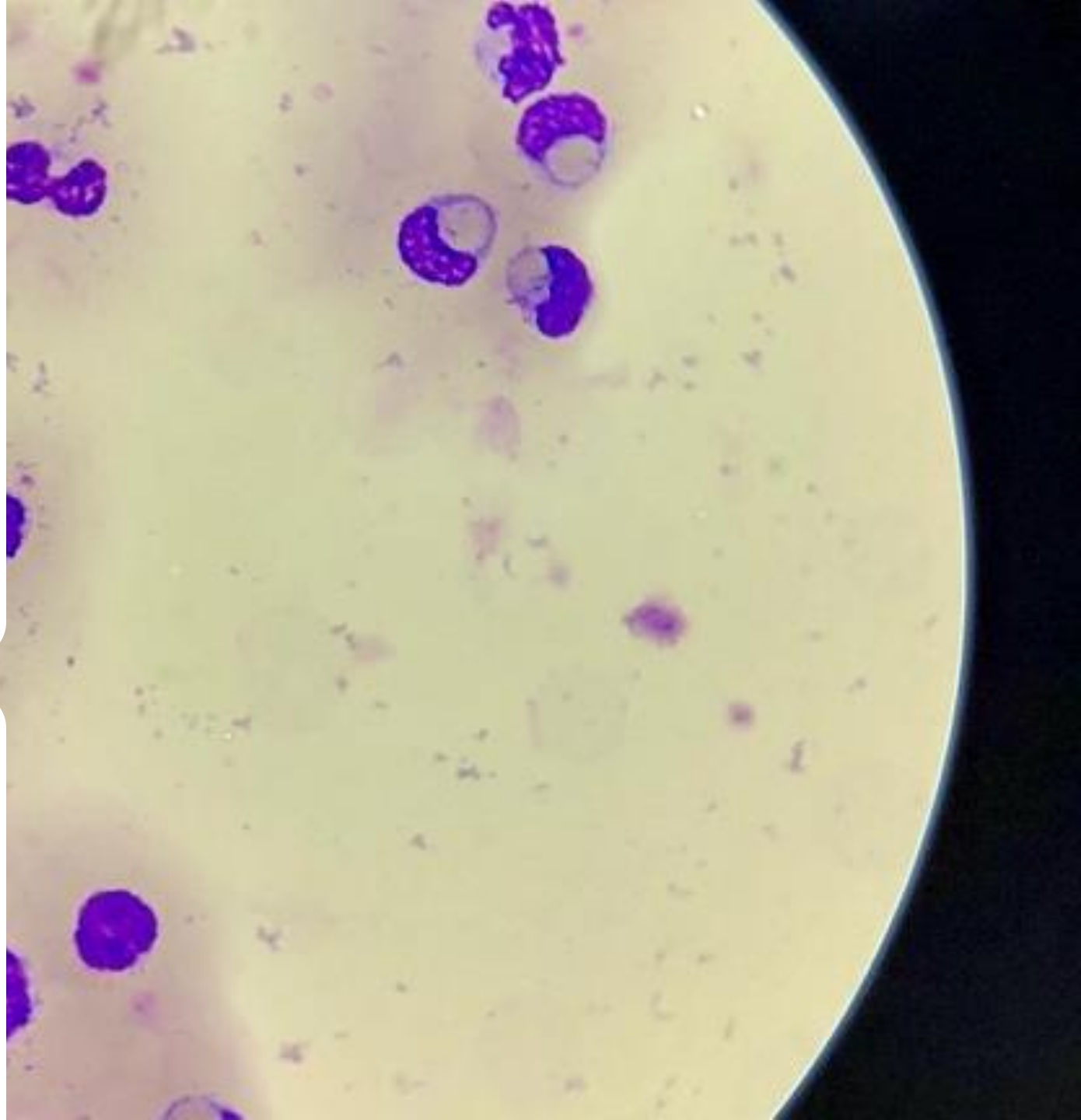
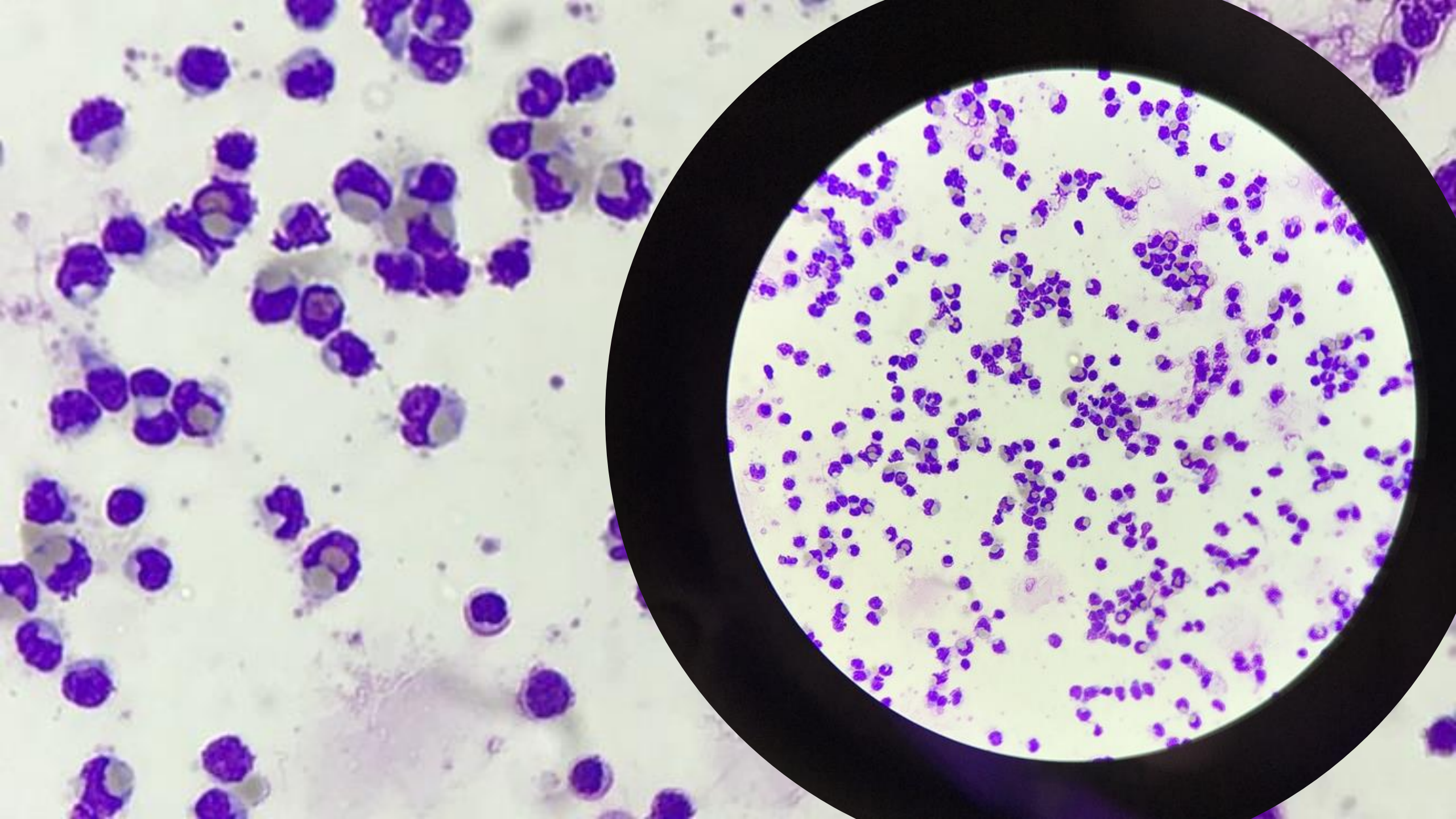










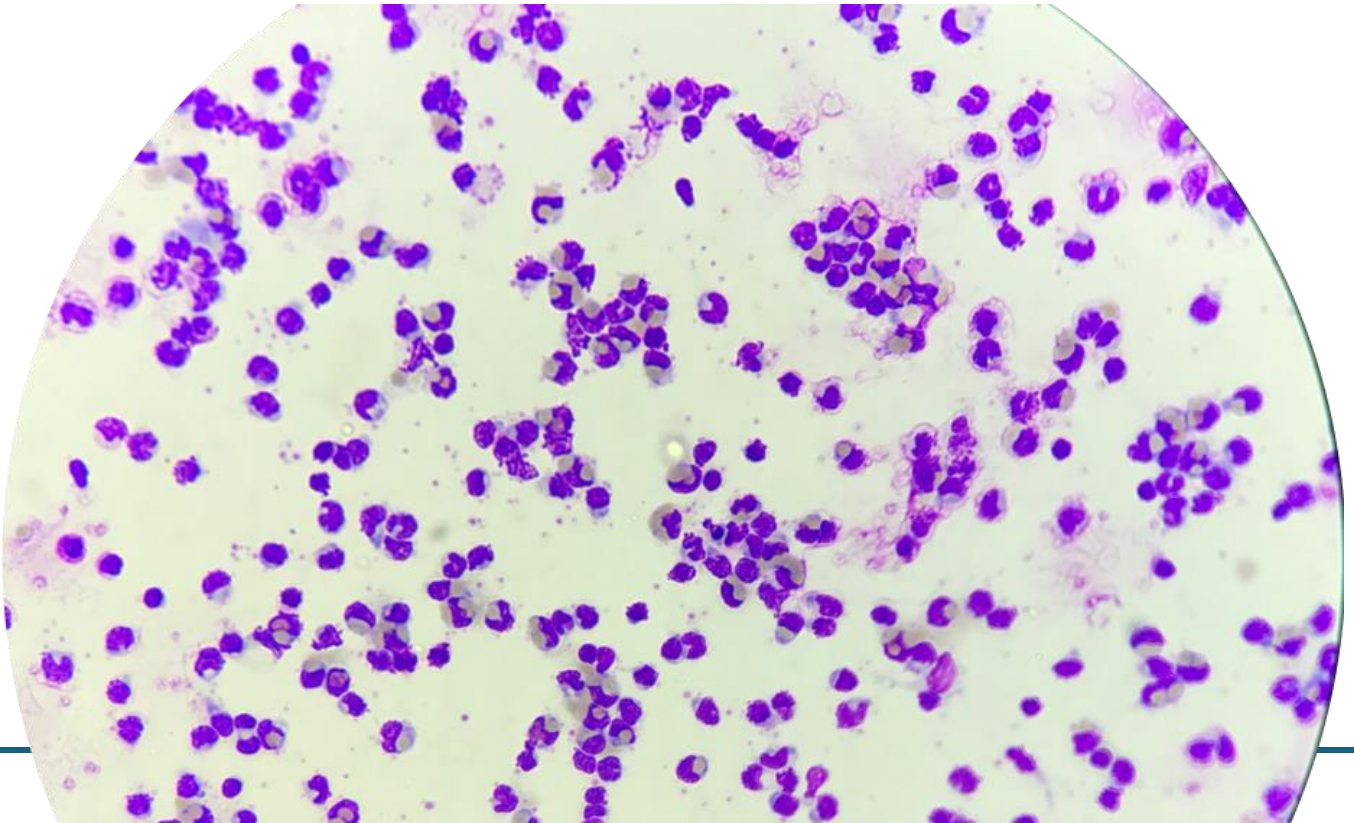


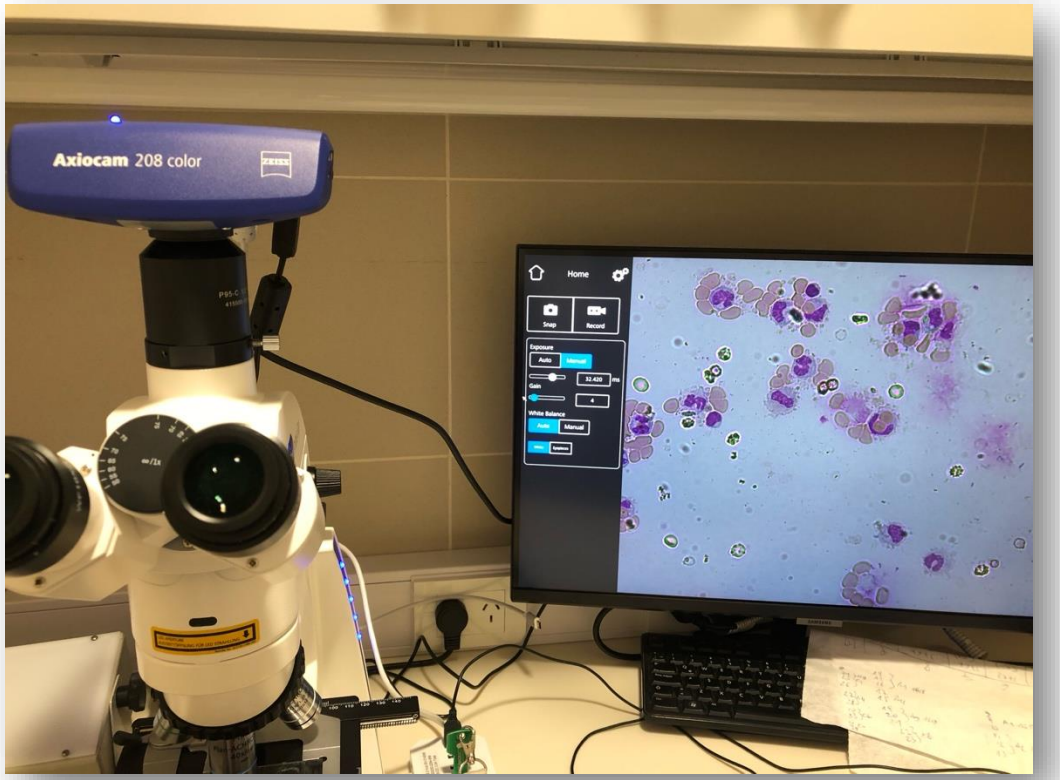




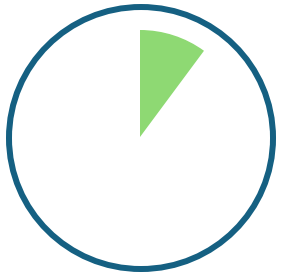
Calculo de adherencia y fagocitosis

- Lectura con Microscopía 40x o 100x
- Conteo de células (600 MN)
- Calcular porcentaje



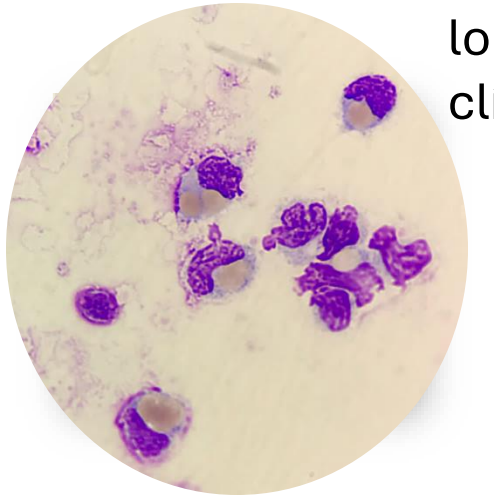


% adherencia y fagocitosis



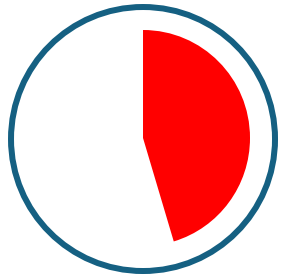
<5%

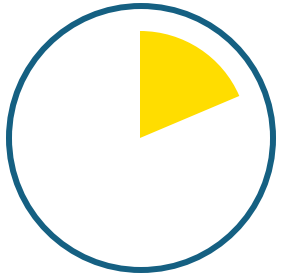
La destrucción de Gr es leve improbable.



>20%

Destrucción acelerada de los Gr con repercusión clínica.



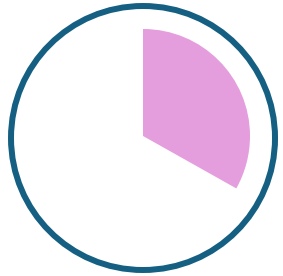


5-10%

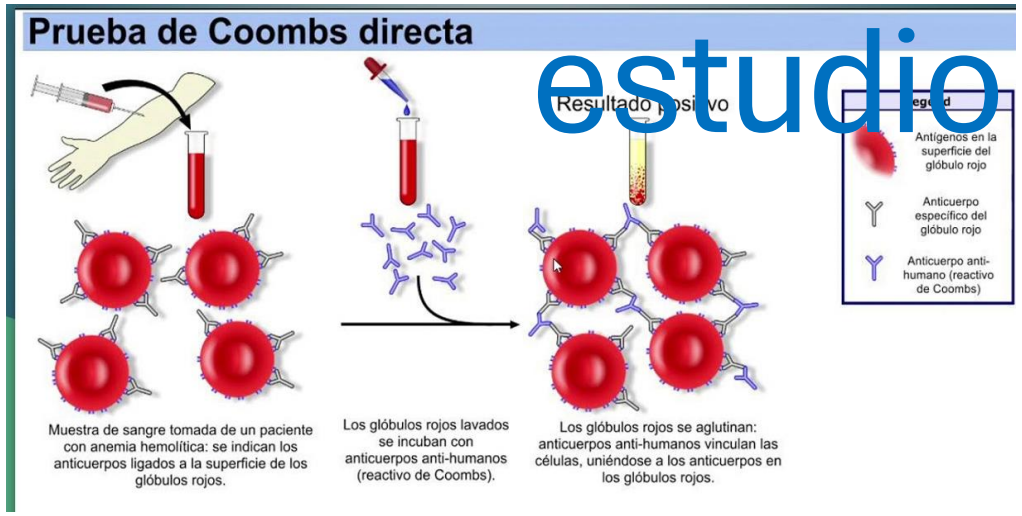
Probablemente haya destrucción acelerada de Gr pero con poca repercusión clínica

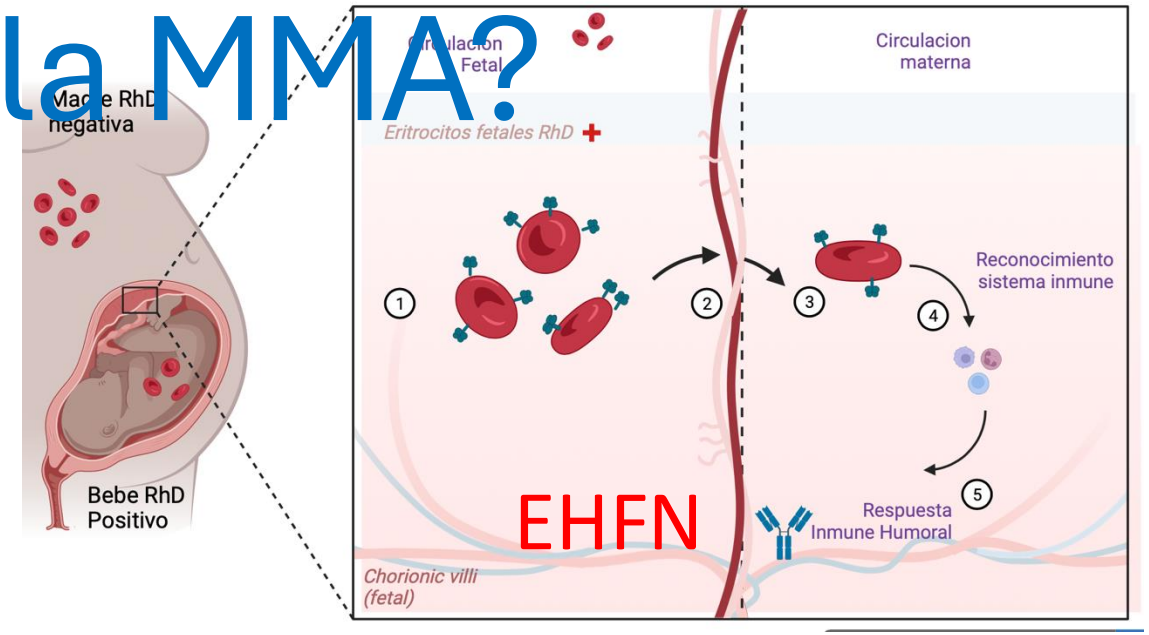
15-20%

????



¿Cuándo debemos recurrir al estudio de la MMA?





Autoanticuerpos – AHAI-PCD

A retrospective analysis of the value of monocyte monolayer assay results for predicting the clinical significance of blood group alloantibodies

Patricia A. Arndt and George Garratty

TABLE 2. MMA results of 251 unusual alloantibodies

System	Anti-	Number tested	Number (%) positive (>5% reactivity)	Number of positives with strength of reactivity 5.1-20%	>20%
Cartwright	Yt^a	73*	47 (64)	28	19
	Yt^b	2	2 (100)	1	1
Chido/Rodgers	Ch	4	4 (100)	2	2
Colton	Co^a	4*	2 (50)	0	2
	Co^b	1	1 (100)	1	0
Cromer	Cr^a	5	5 (100)	2	3
	Es^a	1	1 (100)	0	1
Diego	Di^a	1	1 (100)	0	1
	Di^b	4	4 (100)	1	3
	Wr^a	4*	2 (50)	2	0
Dombrock	Do^a	1	0 (0)	0	0
	Do^b	3	1 (33)	0	1
	Gy^a	6*	4 (67)	2	2
	Hy	8†	5 (63)	5	0
	Jo^a	5	4 (80)	3	1
Er	Er^a	1	0 (0)	0	0
Gerbich	Ge	27*	16 (59)	8	8
Globoside	P	1	1 (100)	0	1
	PP$_1$P^k	2	2 (100)	0	2
Hh	H	1	1 (100)	1	0
HLA	Bg^a (HLA-B7)	1	1 (100)	0	1
Indian	In^b	3	2 (67)	2	0
John Milton Hagen	JMH	4	2 (50)	1	1
Kell	Js^b	3	1 (33)	0	1
	Kp^b	4*	3 (75)	2	1
	K11	1	1 (100)	0	1
	Ku	1	1 (100)	0	1
Kidd	Jk3	1	1 (100)	0	1
Knops	Yk^a	3	1 (33)	1	0
	Kn^a	3*	2 (67)	2	0
	Kn/McC	5*	2 (40)	1	1
Landsteiner-Wiener	LW	1	1 (100)	0	1
Lutheran	Lu^a	2	2 (100)	0	2
	Lu^b	19	15 (79)	2	13
	Lu3	3	2 (67)	1	1
	Lu8	1	1 (100)	1	0
	Lu12	1	1 (100)	1	0
MNS	"MiIII"	1	1 (100)	0	1
	U	3	3 (100)	0	3
Rh	hr^B	1	1 (100)	0	1
	Rh29	1	1 (100)	0	1
	Go^a	1	1 (100)	1	0
Scianna	Sc1	1	1 (100)	0	1
Xg	Xg^a	3	3 (100)	1	2
Independent	AnWj	1	1 (100)	1	0
	At^a	3	3 (100)	0	3
	Jr^a	14*	5 (36)	3	2
	Lan	7	6 (86)	0	6
	Vel	5	5 (83)	0	5
Total		251	173 (69)	76	97

* One antibody of this specificity (two anti-Jr^a) was not tested in the presence of fresh normal serum (as a source of complement) and gave negative results by MMA.
† One antibody of this specificity was not tested without the addition of fresh normal serum (as a source of complement) and gave negative results by MMA.

TABLE 5. IgG subclassing results for 90 unusual alloantibodies

System	Anti-	Number tested	Number reactive with only one subclassing antiserum (number reactive with more than one subclassing antiserum) IgG1	IgG2	IgG3	IgG4
Cartwright	Yt^a	30	27 (0)	0 (0)	0 (0)	3 (0)
Colton	Co^a	1	1 (0)	0 (0)	0 (0)	0 (0)
	Co^b	1	1 (0)	0 (0)	0 (0)	0 (0)
Diego	Di^a	1	0 (0)	0 (0)	1 (0)	0 (0)
	Di^b	4	1 (0)	0 (0)	3 (0)	0 (0)
	Wr^a	1	1 (0)	0 (0)	0 (0)	0 (0)
Dombrock	Gy^a	5	2 (1)	1 (0)	0 (0)	1 (1)
	Hy	3	2 (0)	0 (0)	0 (0)	1 (0)
	Jo^a	1	1 (0)	0 (0)	0 (0)	0 (0)
Gerbich	Ge	14	11 (3)	0 (2)	0 (3)	0 (1)
Globoside	P	1	0 (1)	0 (0)	0 (1)	0 (0)
Indian	In^b	2	2 (0)	0 (0)	0 (0)	0 (0)
John Milton Hagen	JMH*	2	1 (0)	0 (0)	0 (0)	1 (0)
Kell	Js^b	1	1 (0)	0 (0)	0 (0)	0 (0)
	Kp^b	3	3 (0)	0 (0)	0 (0)	0 (0)
Knops	Yk^a	1	0 (0)	0 (0)	0 (0)	1 (0)
Lutheran	Lu^b	4	3 (1)	0 (1)	0 (1)	0 (0)
	Lu3	2	0 (2)	0 (1)	0 (1)	0 (1)
	Lu8	1	1 (0)	0 (0)	0 (0)	0 (0)
MNS	U	3	1 (2)	0 (1)	0 (2)	0 (2)
Rh	Rh29	1	1 (0)	0 (0)	0 (0)	0 (0)
	Go^a	1	1 (0)	0 (0)	0 (0)	0 (0)
Independent	At^a	2	1 (1)	0 (0)	0 (1)	0 (1)
	Lan	3	0 (2)	0 (0)	1 (2)	0 (0)
	Vel	2	1 (1)	0 (0)	0 (1)	0 (0)
Total		90	63 (14)	1 (5)	5 (12)	7 (6)

* Results on five additional anti-JMH (not tested by MMA): 1 = IgG1, 4 = IgG4.

TABLE 1. Examples of alloantibodies studied to determine the appropriate cutpoint for the MMA in Study I

Anti-	IAT	MMA result (% reactivity)	Response to transfusion of incompatible RBCs
Di^b	2$^1/_2$+	5.5	• Transfused three Di(b+) units; no clinical reaction but 6 days later Hb level dropped, bilirubin increased.[22]
Yt^a	1$^1/_2$+	10.8	• ^{51}Cr study = 100% @1 hr, 95% @24 hr; T_{50}Cr = 14 days (normal ≅ 28-32 days). • Transfused 15 least incompatible units; no signs of clinical reaction; bilirubin and lactate dehydrogenase values were unchanged.
Yt^a	1$^1/_2$+	0	• Transfused two Yt(a+) units two months after first MMA; no clinical reaction noted.[7]
	1+	16	• MMA repeated 5 months after transfusion of Yt(a+) RBCs; ^{51}Cr study = 80% @1 hr, <5% @24 hr; T_{50}Cr = 5 hr.[7]
Lu8	1$^1/_2$+	12-65	• Transfused three incompatible units → immediate transfusion reaction (temperature and blood pressure increased), Hb level dropped, bilirubin increased, urine bilirubin reported as "positive."[16]

Anti-	IAT result	MMA result
Jr^a	2+	2.1%-2.2%
Yt^a	2+	0.2%-0.7%
Lan	2+	1.5%-2.7%
Vel	Neg	7.2%-7.9%

J Maurer, S Nance, P Nickle. Relationship of Antibody Reactivity Strength to Reactivity in the Monocyte Monolayer Assay (MMA).*Transfusion*2018;58:187A

Antibody specificity	No. of alloantibodies evaluated	Range of antiglobulin test strength	No. showing significantly elevated phagocytic indices (PRBC)
Anti-Jk[a]	34	Micro‡–4+	11 (32%)
Anti-Jk[b]	3	Micro–2+	2 (67%)
Anti-Fy[a]	26	Micro–4+	16 (62%)
Anti-Kell	22	Micro–4+	16 (73%)
Anti-Rh$_0$(D)	16	1+–4+	12 (75%)
Anti-E	5	1+–4+	3 (60%)
Other Rh [C, c, e]	3	1+–4+	2 (67%)*
Anti-Vel	8	Micro–4+	2 (25%)
Anti-Yt[a] (Cartwright)	8	Micro–4+	2 (25%)
Anti-Ge (Gerbich)	9	Micro–2½+	2 (22%)
Others [M, S, s, Fy[b], Kp[b] Lu[a], Lu[b], Di[b], Co[a]]	14	Micro–4+	10 (71%)†
Totals	148	Micro–4+	78 (53%)

* Significantly elevated PRBC with 1/1 C, 1/1 c, 0/1 e.
† Significantly elevated PRBC with 1/2 M, 2/2 S, 1/1 s, 1/2 Fy[b], 1/1 Kp[b], 0/1 Lu[a], 1/2 Lu[b], 2/2 Di[b], 1/1 Co[a].
‡ Microscopically positive reactions.

Table 1
Monocyte index results for the 54 tested samples.

Blood system	Antibody specificity by gel cards	Number of patients	Monocyte index	
			≤5%	>5%
Usually clinically significant				
Rh	C	4	0	4
	E	6	1	5
	c	2	0	2
Kell	K	5	0	5
Duffy	Fy(a)	3	0	3
	Fy(b)	1	0	1
Kidd	JK(b)	3	0	3
Total		24	1	23
Usually clinically insignificant antibodies				
Luthern	Lu(a)	1	1	0
Lewis	Le(a)	1	1	0
Total		2	2	0
Sometimes clinically significant				
MNS	M	4	2	2
	S	1	1	0
	N	2	2	0
Autoantibodies		9	2	7
Multiple antibodies		4	1	3
Antibodies of undefined specificity		8	8	0
Total		28	16	12
Total		54	19	35

Table 2. NRLBGS MMA data 1995–2017 (used with permission)*

Anti-	TT	>3% C/NC	>3% C	>3% NC	≤3%	Anti-	TT	>3% C/NC	>3% C	>3% NC	≤3%
AnWj	2	1	0	0	1	Js[b]	1	1	0	0	0
At[a]	4	3	0	0	1	Kp[b]	6	2	0	0	4
Au[a]	1	0	0	0	1	Ku	1	1	0	0	0
Co[a]	2	2	0	0	0	Lan	11	7	0	0	4
Cr[a]	4	3	0	**1**	0	LU Sys	21	16	**2**	**1**	2
Di[b]	11	7	0	**1**	3	Lu[b]	14	12	0	0	2
Do[b]	5	0	0	**1**	4	LW	3	2	0	0	1
E	1	1	0	0	0	M	11	3	**1**	**1**	6
e	3	0	0	**2**	1	N	2	1	0	0	1
GE Sys	31	11	**1**	**4**	15	PP1P[k]	1	1	0	0	0
hr[B]	3	2	0	0	1	RH Sys	1	1	0	0	0
hr[S]	7	4	0	0	3	s	1	0	0	0	1
Hy	9	7	0	0	2	SC1	1	1	0	0	0
I	5	1	0	0	4	Tc[a]	2	1	0	0	1
JK3	1	0	0	0	1	U	4	2	0	0	2
Jo[a]	10	4	0	0	6	Vel	13	10	0	0	3
Jr[a]	15	7	**1**	**1**	6	Yt[a]	195	104	**5**	**10**	76

NRLBGS = National Reference Laboratory for Blood Group Serology; MMA = monocyte monolayer assay; TT = total antibodies per specificity tested; C = source of complement added to test (fresh inert serum); NC = no source of complement added to test; Sys = System.

*Normal range of MMA is 0–3% reactivity; values >3% are positive, ≤3% are negative.

Of note, the bolded blue numbers indicate where the test was only positive with the addition of a complement source (fresh inert serum) in the RBC-sensitization phase. Equally important to note is that some tests were only positive when no complement was added (also in bold font), thus confirming that separate red blood cell sensitization with and without fresh inert serum as a source of complement is important when performing the MMA.

Anti-	TT	POS	NEG
AnWj	2	1	1
At[a]	4	3	1
Au[a]	1	0	1
Co[a]	2	2	0
Cr[a]	4	4	0
Di[b]	11	8	3
Do[b]	5	1	4
E	1	1	0
e	3	2	1
GE Sys	31	16	15
hr[B]	3	2	1
hr[S]	7	4	3
Hy	9	7	2
I	5	1	4
Jk3	1	0	1
Jo[a]	10	4	6
Jr[a]	15	9	6

Anti-	TT	POS	NEG
Js[b]	1	1	0
Kp[b]	6	2	4
Ku	1	1	0
Lan	11	7	4
LU Sys	21	19	2
Lu[b]	14	12	2
Lw	3	2	1
M	11	5	6
N	2	1	1
PP1P[k]	1	1	0
RH Sys	1	1	0
s	1	0	1
Sc1	1	1	0
Tc[a]	2	1	1
U	4	2	2
Vel	13	10	3
Yt[a]	195	119	76

Nance SJ, Arndt P, Garratty G. Predicting the clinical significance of red cell alloantibodies using a monocyte monolayer assay. Transfusion. 1987 Nov-Dec;27(6):449-52. doi: 10.1046/j.1537-2995.1987.27688071692.x. PMID: 3686653.

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ISBT International Society of Blood Transfusion
ORIGINAL PAPER
Vox Sanguinis (2021)

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A fluorometric erythrophagocytosis assay using differentiated monocytic THP-1 cells to assess the clinical significance of antibodies to red blood cells

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www.ijbti.com
ORIGINAL ARTICLE PEER REVIEWED | OPEN ACCE

Evaluation of erythrocyte autoantibodies with flow cytometric phagocytosis assay

Shoichi Ito, Tomoko Hishinuma, Yoshiko Ogiyama, Tomomi Asano, Haruka Kagaya, Michiyo Irino, Hideya Hasegawa, Hiroshi Shimizu, Kenneth E. Nollet, Masayoshi Minegishi, Hitoshi Ohto

Ito et a CELLULAR IMMUNOLOGY 64, 359-370 (1981)

Comparison of Monocyte and Alveolar Macrophage Antibody-Dependent Cellular Cytotoxicity and Fc-Receptor Activity[1]

DAVID M. GARAGIOLA, THOMAS K. HUARD, AND ALBERT F. LOBUGLIO
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Premio "Dr. Luis Agote" - XVII Congreso Argentino de Medicina Transfusional
Aplicación de la prueba de la monocapa de monocitos (MMA) en el diagnóstico de la Enfermedad Hemolítica Feto Neonatal

Santoro DM*; Gamboa CV*; Grottola G*; Valiente VL*; Burgos Pratx LD*; Ielpi MR**; Camino PJ*; Scordo WE*; Salamone HJ*

ORIGINAL REPORT

A comparison of results from antihuman globulin-graded reactions with the monocyte monolayer assay

K. Bowman, L.A. Peña Marquez, L. Hawthorne, K. Billingsley, S. Kelham, S. Liang, and M. Kalvelage

In vitro cellular assays and other approaches used to predict the clinical significance of red cell alloantibodies: a review

R.M. LEGER

SEROLOGIC METHOD REVIEW

The monocyte monolayer assay, an *in vitro* method for prediction of *in vivo* survival of transfused incompatible red blood cells: a review

S.J.T. Nance

Monocyte monolayer assay in pre-transfusion testing: A magic key in transfusing patients with recurrent bad cross-match due to alloimmunization

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Video Article

Use of a Monocyte Monolayer Assay to Evaluate Fcγ Receptor-mediated Phagocytosis

Tik Nga Tong[1], Donald R. Branch[1,2]
[1]Department of Laboratory Medicine and Pathobiology, University of Toronto
[2]Centre for Innovation, Canadian Blood Services

Monocyte Monolayer Assay: An Efficient Noninvasive Technique for Predicting the Severity of Hemolytic Disease of the Newborn

SANDRA J. NANCE, M.S., MT(ASCP)SBB, JANICE M. NELSON, M.D., JANET HORENSTEIN, M.D., PATRICIA A. ARNDT, M.S., MT(ASCP)SBB, LAWRENCE D. PLATT, M.D., AND GEORGE GARRATTY, PH.D., F.I.M.L.S., M.R.C.PATH.

Vox Sang 1990;58:276–280

0042–9007/90/0584–0276 $2.75/0

Correlation of Monocyte-Monolayer Assay Results, Number of Erythrocyte-Bound IgG Molecules, and IgG Subclass Composition in the Study of Red Cell Alloantibodies Other Than D

B. Żupańska[a], *E. Brojer*[a], *J. McIntosh*[b], *H. Seyfried*[a], *P. Howell*[b]

[a]Institute of Haematology, Warsaw, Poland; [b]Regional Transfusion Centre, Manchester, UK

IMMUNOHEMATOLOGY

A retrospective analysis of the value of monocyte monolayer assay results for predicting the clinical significance of blood group alloantibodies

Patricia A. Arndt and George Garratty

Predicting the clinical significance of red cell alloantibodies using a monocyte monolayer assay

S. J. NANCE, P. ARNDT, AND G. GARRATTY

Predicting hemolytic disease of the newborn: a comparison of the monocyte monolayer assay and the chemiluminescence test

G.F. LUCAS, A.G. HADLEY, S.J. NANCE, AND G. GARRATTY

Case Report

Application of Monocyte Monolayer Assay technique to predict hyperhemolysis in patients with sickle cell disease

Dahra Teles Cruz, *Marina C.V. Conrado*, *Alfredo Mendrone*, *Carla L. Dinardo*

Fundação Pró-Sangue, São Paulo, SP, Brazil

REVIEW

PROCEEDINGS FROM THE INTERNATIONAL SOCIETY OF BLOOD TRANSFUSION WORKING PARTY ON IMMUNOHAEMATOLOGY WORKSHOP ON THE CLINICAL SIGNIFICANCE OF RED BLOOD CELL ALLOANTIBODIES, FRIDAY, SEPTEMBER 2, 2016, DUBAI

A review of in vitro methods to predict the clinical significance of red blood cell alloantibodies

S.J. Nance

IMMUNOHEMATOLOGY

Optimal conditions for the performance of a monocyte monolayer assay

Tik Nga Tong,[1,2] Emeralda Burke-Murphy,[2] Darinka Sakac,[2] Jacob Pendergrast,[3] Christine Cserti-Gazdewich,[3] Vincent Laroche,[4] and Donald R. Branch[1,2,3,5]

Landsteiner Award

Bioassays to determine the clinical significance of red cell alloantibodies based on Fc receptor-induced destruction of red cells sensitized by IgG

C.P. ENGELFRIET, M.A.M. OVERBEEKE, M.C. DOOREN, W.H. OUWEHAND, AND A.E.G.KR. VON DEM BORNE

CASE REPORT

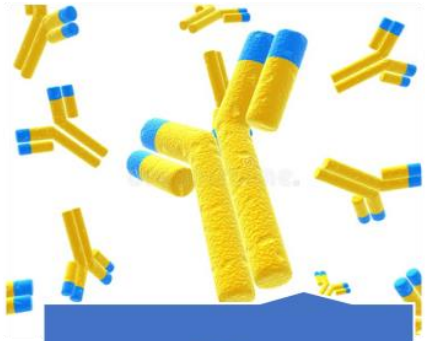
Management of pregnancy sensitized with anti-In^b with monocyte monolayer assay and maternal blood donation

R. Shree, K.K. Ma, L.S. Er and M. Delaney

Experiencia en el Hospital Italiano de Buenos Aires

anti-D	26
anti-E	11
anti-C	5
anti-M	3
anti-K	4
anti-S	3
anti-c	2
anti-Dia	1
anti-Fya	1
anti-Le(a)	1
anti-P	1
autoanticuerpo	2
Total	60

Test Monocapa



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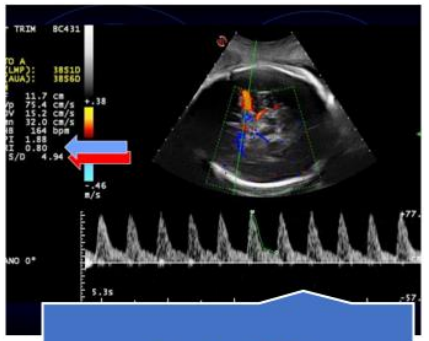
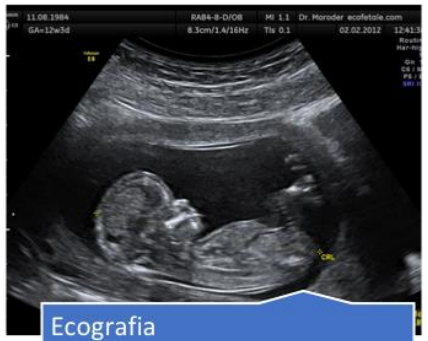


Doppler ACM



Ecografia

- Signos de Hidrops

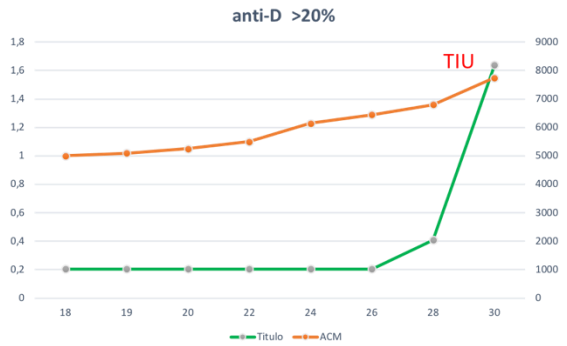
Post nacimiento

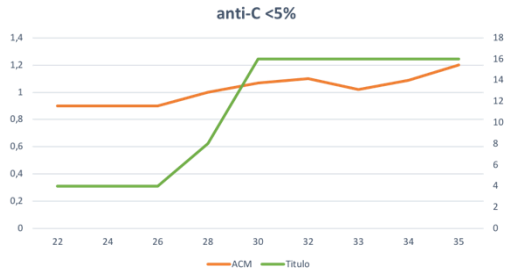
- Clinica

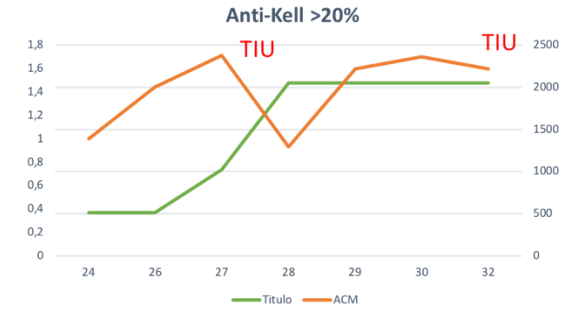
Premio “Dr. Luis Agote” - XVII Congreso Argentino de Medicina Transfusional.
Aplicación de la prueba de la monocapa de monocitos (MMA) en el diagnóstico de la Enfermedad Hemolítica Feto Neonatal.
Santoro DM; Gamboa CV; Grottola G; Valiente VL; Burgos Pratx LD; Ielpi MR;
Camino PJ; Scordo WE; Salamone HJ

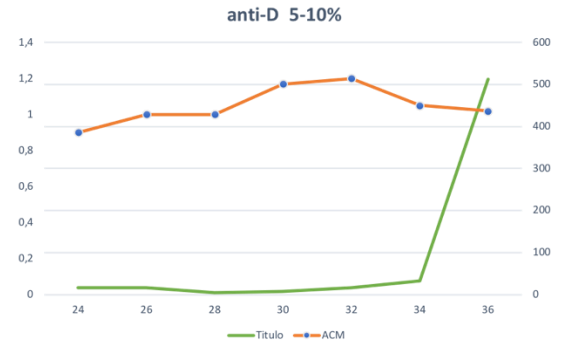
Paciente	MM%	Doppler	ECO	Título	TIU	Tx	BR	Lumn	RN
1	>20	>1,55	+	↑	SI	Si	Si	SI	V
2	>20	>1,55	+	↑	SI	Si	Si	Si	V
3	>20	>1,55	+	↑	Si	SI	SI	SI	V
4	>20	>1,55	+	↑	SI	-	-	-	MF
5	>20	>1,55	+	↑	SI	SI	SI	Si	V
6	>20	>1,55	+	↑	SI	SI	SI	SI	V
7	>20	>1,55	+	↑	SI	SI	Si	Si	V
8	5-10	<1,55	-	↑	No	No	Si	Si	V
9	5-10	<1,55	-	↑	No	No	No	No	V
10	5-10	<1,55	-	↑	No	No	No	No	V

anti-M	<5%	<1,5	0	-	-	No	No	No	S
anti-M	<5%	<1,5	0	-	-	No	No	No	s
anti-K	>20%	>1,5	+	↑	+	-	-	-	MF
anti-E	<5%	<1,5	+	0	-	No	No	No	S
anti-C	<5%	<1,5	+	0	-	No	SI	Si	S
anti-E	<5%	<1,5	+	↑	-	No	SI	si	S
anti-C	<5%	<1,5	0	0	-	No	SI	Si	S
anti-Dia	5-10%	<1,5	0	0	-	No	No	No	S
Anti-V	<5%	<1,5	0	O	-	No	No	No	S









Recomendación: Con un resultado de la prueba <10%, se recomienda repetir la prueba cada 2 semanas, cuando SG >32 Antes de las 32 semanas de gestación, es suficiente repetir la prueba cada 4 semanas.

Pacientes con diagnostico de AHAI

			Pre Tx					Post Tx				5 dias		10 dias		15dias	
Paciente	MM		**Hto**	**Hb**	**LDH**	**Bt**	**Hapto**	Hto	Hb	LDH	BT	Hto 5	Hb5	Hto 10	Hb 10	Hto 15	Hb 15
1	<5%	2 GR	20	7	482	6.43	<5.83	25.3	9.1	349	1.82	33.1/10.8	-	35		40	
2	5-10%	1GR	19.4	6.3	647	-	7.4	20,3	6,7	670	1,16	3 3/64	-	21	7	23	7,5
3	<5%	1GR	22,4	7,8	-	0,28	-	27,5	9,4	-	-	25	-				
4	<5%	1GR	16,2	5,9	193	2,41	<5.83	21,7	7,3	159	0,88	23	-	25		32	
5	<5%	1GR	20	6,8									-				
6	<5%	3GR	19,5	5,5	281	0,6	137	22,5	6,5	251	0,9	27,4	8,2	31,9	9,4	33,2	10,1
7	5-10%	2GR	19,9	7,2	486	1,22	<5,83	25,3	8,7	400	0,99	27,3	9,3	32,8	11	38,4	12,4
8	13%	5GR	18,5	5,9	1613	1,53	<5,83	20,6	6,9	462	1,07	20,5	6,6	27,2	9,8	33,9	11,2
9	>20%	6GR	12,8	4,5	-	1,64	<5,83	19,5	6,9	-	1,34	15,4	5,5	16,8	6	-	-
10	5-10%	2GR	10,1	3,9	750	9,66 1,26D	<5,83	15,3	6	760	9.16	17,4	6	20,1	7,1	20,2	7,3

Conclusiones

Es un estudio invitro que pondera la capacidad lítica de las Inmunoglobulinas.

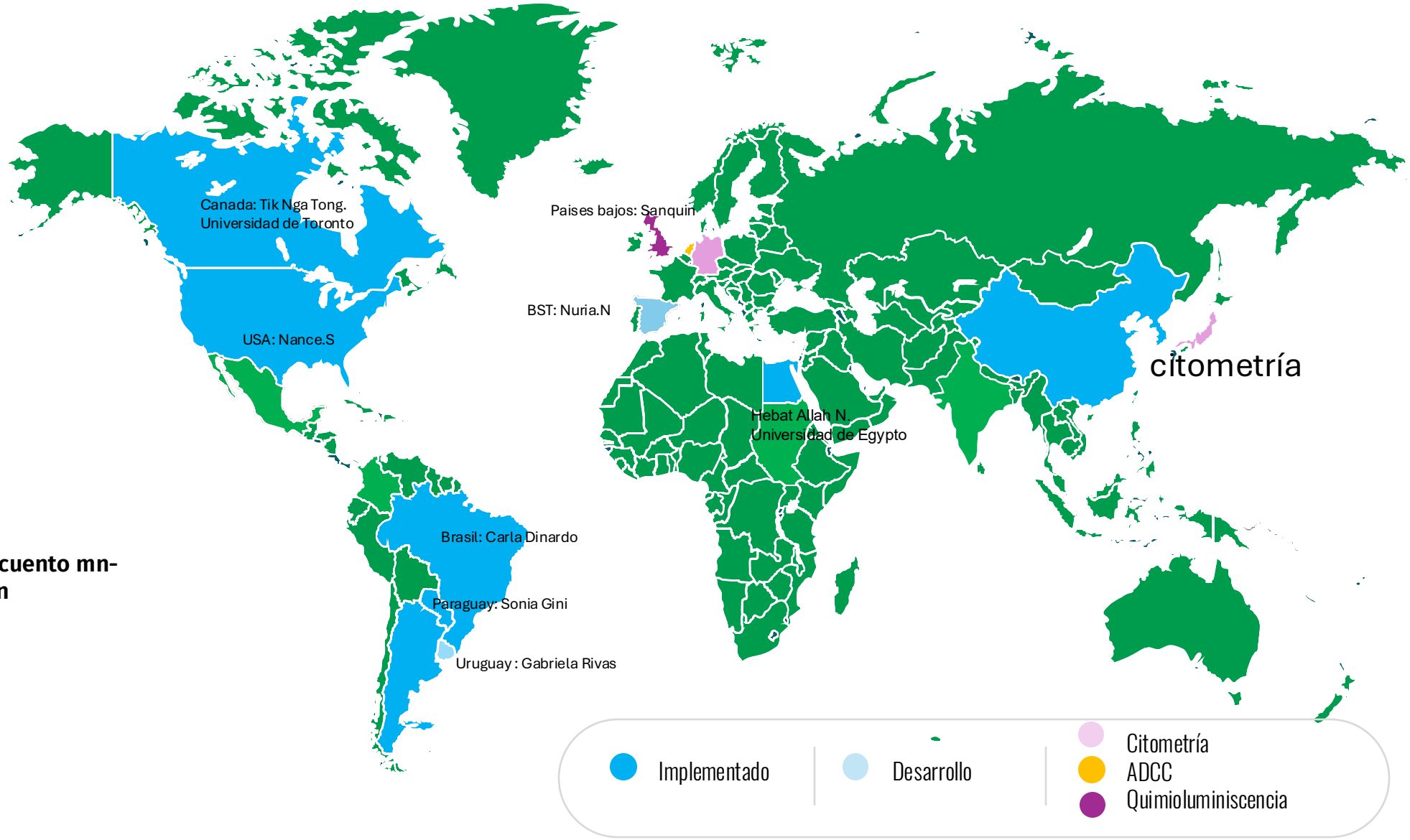
Los resultados presentan buena correlacionan con la evolución clínica del paciente.

Es una práctica operador dependiente, por los sucesivos pasos que demanda. Donde se requiere experiencia en el manejo celular.

Prueba utilizable en distintas situaciones clínicas donde no podamos tomar decisiones basadas en los métodos serológicos (Anticuerpos contra Antigenos de alta frecuencia, EFHN, AutoAc-AHAI)

No debe ser utilizada para suplantar las técnicas serológicas. Si no que deben complementar las mismas.

Actualidad de la utilización MMA



Fuente de células-recuento mn-
tiempo de incubación
Resultados
Validación
Control calidad

Hospital Italiano

Muchas Gracias

diego.santoro@hospitalitaliano.org.ar

Experiencia Paraguay

Dr. Sonia Gini Alvarez

GCIAMT

Grupo Cooperativo Iberoamericano de Medicina Transfusional

Estudio in vitro de la capacidad lítica de los Anticuerpos. La técnica de **MMA**

Dra. Sonia Gini Alvarez

Medicina Transfusional – Inmunohematología

Referente de Inmunohematología Pacientes
Area de Medicina Transfusional
Centro Productor de Sangre y Terapia celular
Hospital Central - IPS

Responsable del Área de Precursores Hematopoyéticos para TMO
Hospital de Clínicas Dpto de Hematooncología Pediátrica
FCM UNA

MMA IMPLEMENTACIÓN: 2022/2024

Primeros pasos:

- Gestión del equipamiento mínimo necesario, centrífugas adecuadas, campana de flujo, estufa de cultivo (incubador), microscopio. 2022
- Gestión y adquisición de los reactivos e insumos: Ficoll, Medio de cultivo celular RPMI, cámaras de cultivo y de contaje celular, colorantes para tinción, May Grumwald, Wright, Giemsa, Azul tripán. 2022
- **Desarrollo de las capacidades técnicas: 2 médicos, 7 bioquímicas, desarrollando los primeros protocolos. 2022**
- **Capacitación en la Fundación Pro Sangre, San Pablo** por 2 semanas de 2 bioquímicas. Ajuste del protocolo final. 2023
- Implementación del Ensayo: Estudio en gestantes y en los primeros casos clínicos. 2023 - 2024

CENTRO PRODUCTOR DE SANGRE Y TERAPIA CELULAR
INSTITUTO DE PREVISION SOCIAL - HOSPITAL CENTRAL
ASUNCION - PARAGUAY

GOBIERNO DEL PARAGUAY | PARAGUÁI REKUÁI

Implementación del ensayo en monocapa de monocitos (MMA) en el laboratorio de Inmunohematología

Objetivos

- Implementar el Ensayo en Monocapa de Monocitos como herramienta para predecir la capacidad hemolítica de los anticuerpos mediante el estudio de una población de gestantes sensibilizadas.
- Definir el procedimiento del ensayo que se adapta a las condiciones y necesidades de la institución

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Paciente	Edad Gestacional	Anticuerpo	Título	Anemia e hiperbilirrubinemia	Historial de transfusiones	Requerimiento de luminoterapia	IMR
DR	36 sem	Anti E	4	Sí	No	Sí	40%
MM	39 sem	Anti D	128	Sí	Sí + Exanguino	Sí	42%
NA	34 sem	Anti D	1024	Sí + 6 TIU	Sí	Sí	21%
NM	36 sem	Anti E	8	No	No	No	16.2%
PA	35 sem	Anti D	512	Sí	No	Sí	2.3%
PA	35 sem	Anti Fya	4	Sí	No	Sí	1.5%
PA	35 sem	Anti C	2	Sí	No	Sí	0.5%
RV	33 sem	Anti E	128	Sí	No	Sí	0.4%
RV	33 sem	Anti Jka	4	Sí	No	Sí	1.6%
SO	37 sem	Anti D	16	No	No	No	1.9%
VF	37 sem	Anti D	128	Sí	No	Sí	2.16%
VB	35 sem	Anti D	1024	Sí	Sí	Sí	11%
EA	35 sem	Anti D	1024	Sí	Sí	Sí	10%
PR	36 sem	Anti D	128	Sí	No	Sí	1.5%
AG	38 sem	Anti E	1024	Sí. Óbito del RN	Sí	Sí	42%

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Pacientes del Hospital Central de IPS	* Pesquisa de Anticuerpo Irregulares (PAI) de 3 células	TCD	Enzimático (Papaína)	4° C	DTT	* Panel de Identificación 11 células	* Autocontrol	Adsorciones Alogénicas con PEG	* Pruebas cruzadas	MMA	Observación
B. B. 73 años Diagnóstico: Insuficiencia Hepática A RHD: Negativo. Fenotipo RH: cDe Kell: Negativo. Fenotipo extendido: JK (a +, b -); Fy (a +, b -); S -; s +; M + N+	Panaglutinación: 2+	Negativo	Sensible	Negativo	Resistente	Panaglutinación: 2+ (Patrón homógeneo)	Negativo	PAI: Positivo luego de 3 Aloadsorciones, Anti D (en enzimas)	Incompatibles	**IMR ≤ 5%** transfusiones sin reacciones adversas.	Sospecha de Anticuerpos Anti Gerbich 2. Prueba cruzada GR de E.O. + Plasma de B.B.: Compatible
E. O. 72 años Diagnóstico: Estenosis aórtica severa O RHD: Positivo. Fenotipo RH: CDe Kell: Negativo. Fenotipo extendido: JK (a +, b -); Fy (a +, b +); S -; s +; M - N+; Dia -	Panaaglutinación 2+	Negativo	Sensible	Negativo	Resistente	Panaglutinación: 2+ (Patrón homógeneo)	Negativo	PAI: negativa pos 2 aloadsorciones	Incompatibles	**IMR ≥5%, IMR ≤ 5%** Se transfundieron 3 unidades de CGR con IMR≤ 5%	
J. G./ R.L. (O+) Diagnóstico: Puérpera B RHD: Positivo. Fenotipo RH: CDe Kell: Negativo. Fenotipo extendido: JK (a +, b -); Fy (a +, b -); S -; s +; M + N+	Panaglutinación: 2+	Negativo	**J.G.:Sensible** **R.L.: aumenta**	Negativo	Resistente	Panaglutinación: 2+ (Patrón homógeneo)	Negativo	PAI: Negativa luego de 1 Aloadsorción	Incompatibles COMPATIBLES con E.O y GR Ge -2-3,4 del GHNI. Confirmada: J.G. Ge -2-3,4	Pendiente	RN: TCD 1+/2+ PAI positivo. Sin afectación
M.E.A. /L.D. Diágnostico: anemia multifactorial/IRC Fenotipo extendido de M.E.A:JK (a +, b +); Fy (a -, b +); S -; s +; M +, N+;	Panaglutinación heterogénea	Negativo/DR	Negativo	Negativo	Resistente	Panaglutinación VARIABLE heterogénea	Negativo/DR	PAI: Se absorbe/No se absorbe	1 en 20 a 40	IMR ≤ 5%	Inhibición con plasma: negativa ABDE?
M.C.B.G. O RHD: Positivo. Fenotipo RH: cDEe Kell: Negativo. Fenotipo extendido: Jka+, Jkb-, M+, N+, S+, s+, Fya-, Fyb+, Dia-, Dib+ Diagnóstico: Tumor en Glomus Carotideo. Sin antecedentes de transfusión anterior	Panaglutinación . 2+	Negativo	Sensible: resultado negativo	Negativo	Resistente	Panaglutinación . Reacciones homogéneas de 2+	Negativo	No se adsorbe Pero no se inhibe con plasma	Incompatible con E.O y GR Ge -2-3,4 del GHNI.	<de 5% de IMR en PC con GR feno extendido compatible. Recibió 1 CGR alogénico compatible por MMA	anti Kanno??, se consideraron además anti Ge2 y otros acs con comportamiento ABDE

Pacientes de otras instituciones	* Pesquisa de Anticuerpo Irregulares (PAI) de 3 células	TCD	Enzimático (Papaína)	4° C	DTT	* Panel de Identificación 11 células	* Autocontrol	Adsorciones Alogénicas con PEG (Panel con fenotipo complementario)	* Pruebas cruzadas	MMA	Observación
M.D.E 26 años Diagnóstico: Pancreatitis Hipertrigliceridémica O RHD: Positivo. Fenotipo RH: CDe Kell: Negativo. Hospital Nacional de Itaugua	Panaglutinación	Negativo	Sensible	Negativo	Resistente	Panaglutinación	Negativo	PAI: Negativa luego de 1 Aloadsorción	Incompatibles	IMR ≥5%, IMR ≤ 5%	Serologicamente sugestivo de anti Ge2 Confirmado por BM
M.M. Diagnóstico: Prequirúrgico para cirugía de columna vertebral Hospital de Clínicas	Panaglutinación	Negativo	Disminuido	Negativo	Resistente	Panaglutinación: 2+ (Patrón homógeneo)	Negativo	PAI: Negativa luego de 1 Aloadsorción	Incompatibles	IMR ≥20%	Serologicamente sugestivo de anti Dib (CONFIRMADO Dib neg por BM)
G.D. 8 meses Dx: Esferocitosis hereditaria + Anemia Hemolítica Autoinmune Hospital de Clínicas	Panaglutinación	Positivo	Resistente			Panaglutinación: 2+ (Patrón homógeneo)	Positivo	Especificidad relativa auto anti D	Incompatibles	IMR ≥20% GR propios, Oneg y Opos: IMR ≤ 5%	Mejoría clínica significativa luego del tratamiento inmunosupresor
C.P. 50 años. Osteomielitis fémur. Hospital de Trauma CENSSA	Panaglutinación	DR	Sensible	Negativo	Resistente	Patrón heterogeneo	DR	No se adsorbe	1/30	I IMR ≤ 5%	Inhibición: No se inhibe. Transfusiones alogenicas sin EA Chido/Rogers?