

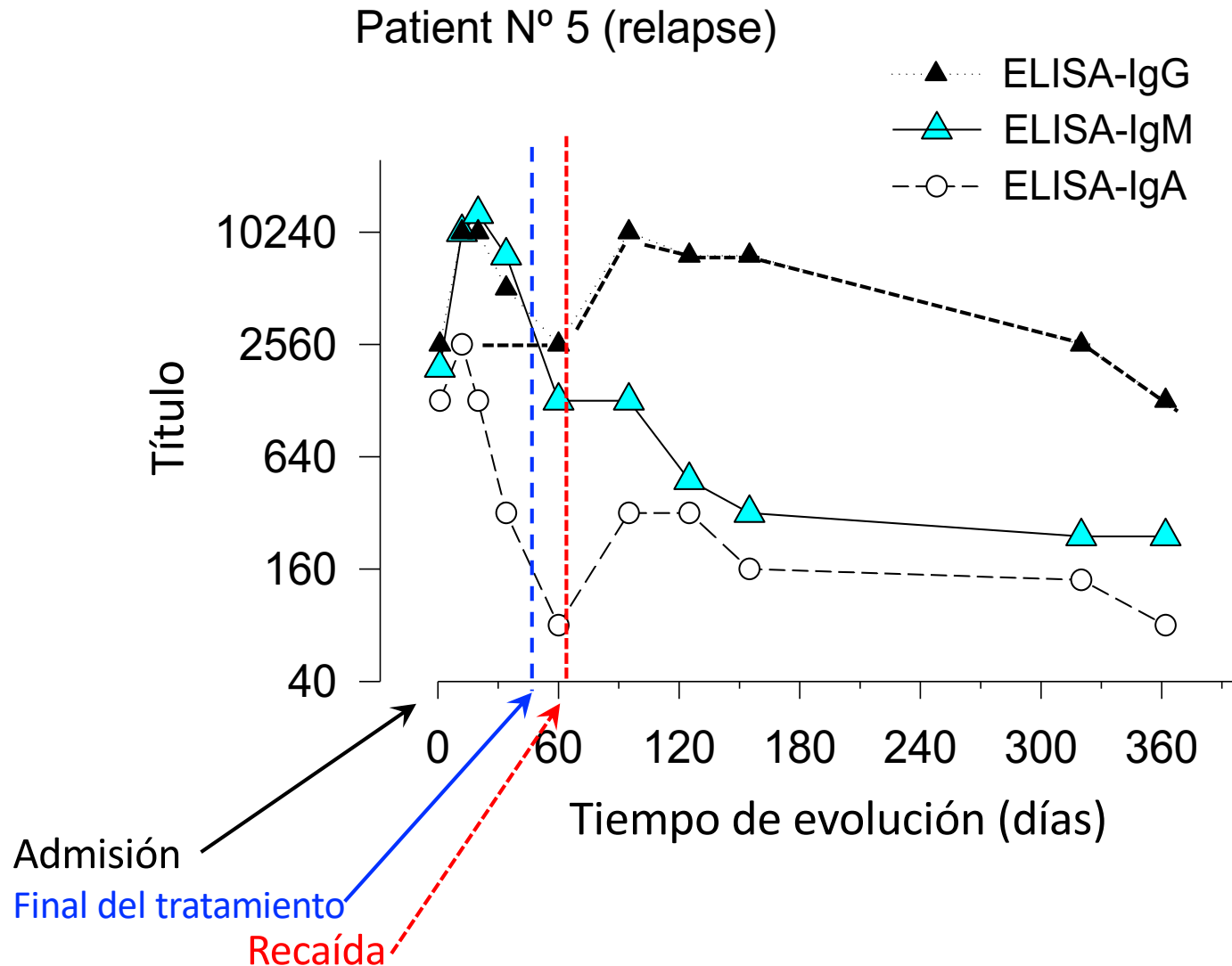


Pruebas que emplean bacterias completas como antígenos.

- Valor de las pruebas que detectan anticuerpos aglutinantes.
 - Antígeno febril.
 - Huddleson (o “standard plate agglutination test” [PAT]).
 - Seroaglutinación en tubo (o microplaca) sin o con mercaptanos (2-ME o DTT).
- La prueba de Coombs-*Brucella* para detectar anticuerpos no aglutinantes.
- Valor de las pruebas con bacterias completas que detectan anticuerpos aglutinantes y no aglutinantes.
 - Pruebas rápidas a pH ácido (Rosa de Bengala, “Card Test” y BPAT).
 - Brucellacapt.

¿Qué hay que detectar?

Perfil “ideal” de anticuerpos séricos: paciente con corto tiempo de incubación, diagnóstico temprano, recaída, seguimiento serológico continuo (i.e. una investigación).



Antígeno febril



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de Glanville, W. A., et al. 2017. Poor performance of the rapid test for human brucellosis in health facilities in Kenya. *PLoS Negl. Trop. Dis.* 11, 1-15. 10.1371/journal.pntd.0005508

Table 2. Comparison of FBAT, RBT, SAT Coombs IgG and LFA in the 825 sera.

FBAT	Nº positive sera				
	RBT	SAT	Coombs IgG ^a	LFA IgM	LFA IgG
Positive: 162	8	1 ^b	2	2 ^c	0 ^c
Negative: 663	0	0	0	n.d. ^d	n.d. ^d
Total: 825		1	3	5	0

a. A titre > 2 times the SAT titre was considered as positive.

b. This serum developed an atypical agglutination and only at a 1:80–1:160 titre.

c. Out of 148 positives tested.

d. n.d., not done.

Poor or no correlation was observed between FBAT results and most established risk factors for Brucella infection.

Huddleson



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Lucero, N. E., Bolpe, J. E. 1998. Buffered plate antigen test as a screening test for diagnosis of human brucellosis. *Journal of Clinical Microbiology*. 36, 1425-1427. 10.1128/JCM.36.5.1425-1427.1998.

Serum source (no.)	BPA (Buffered plate antigen)		PAT (Huddleson)			
	Negative	Positive	Negative	Positive at endpoint titer of:		
				1:25	1:50	≥1:100
Culture-positive patients (57) ^a	0	57	0	1	6	50
Suspected-brucellosis patients (142) ^b	0	142	0	1	20	121
Asymptomatic population (307) ^c	306	1	292	14	1	0

- Of the total of 57 positive isolates, 29 were *B. suis*, 15 were *B. abortus*, 6 were *B. melitensis*, and seven *Brucella* strains were not typed to the species level
- Patients with clinical evidence of brucellosis and positive by RB and CF.
- Asymptomatic population negative by RB and CF.

Lucero, N. E., Bolpe, J. E. 1998. Buffered plate antigen test as a screening test for diagnosis of human brucellosis. *Journal of Clinical Microbiology*. 36, 1425-1427. 10.1128/JCM.36.5.1425-1427.1998.

¡Protocolo!

1. 80, 40, 20, and 10 μ l of serum placed on 4-cm squares on a glass plate.
2. Then, 30 μ l of antigen was dropped onto each square and mixed and spread over 2 cm² or 3 cm² for the 80- μ l sample.
3. The plate was rotated to ensure mixing, allowed to stand for 4 min, rotated again and incubated 4 more minutes in a covered black box that has an oblique light onto the serum-antigen mixture.
4. The plate is tilted to allow the mixture to flow aside for the reading.

Otros comentarios en el artículo

*The TAT (i.e. SAT) has become the standard method, is the test recommended for collection of quantitative information on immune responses, and is **the most frequently used confirmatory serological test.***

Supplementary tests** such as the TAT and the CF **must be run on all BPA-positive samples to ensure diagnostic specificity.

(¡NO!)



Seroaglutinación en tubo (o microplaca) sin mercaptanos

(¡no 1960!)

Buchanan, T. M., Sulzer, C. R., Frix, M. K., Feldman, R. A. **1974**. *Brucellosis in the United States, 1960-1972. An abattoir-associated disease. Part II. Diagnostic aspects. Medicine (Baltimore)*. 53, 415-425

TABLE 1
*Results of Serologic Testing of Serum Specimens
from 46 Control Persons*

Titer	Number of serum specimens by test*				
	CA	Std	CF	2-ME	CARD (RBT)
>20	37	40	43	45	46 Negative
20	0	4	0	0	
40	0	1	1†	0	
80	0	1	0	1	
160	2	0	0	0	
320	0	0	0	0	
Total	45‡	46	44‡	46	

País no endémico
SAT (Std) \geq 1:160
2-ME \geq 1:160

* CA = Centrifugation agglutination; Std = Standard tube dilution agglutination; CF = Complement fixation; 2-ME = 2-mercaptoethanol agglutination, CARD = Card agglutination test.

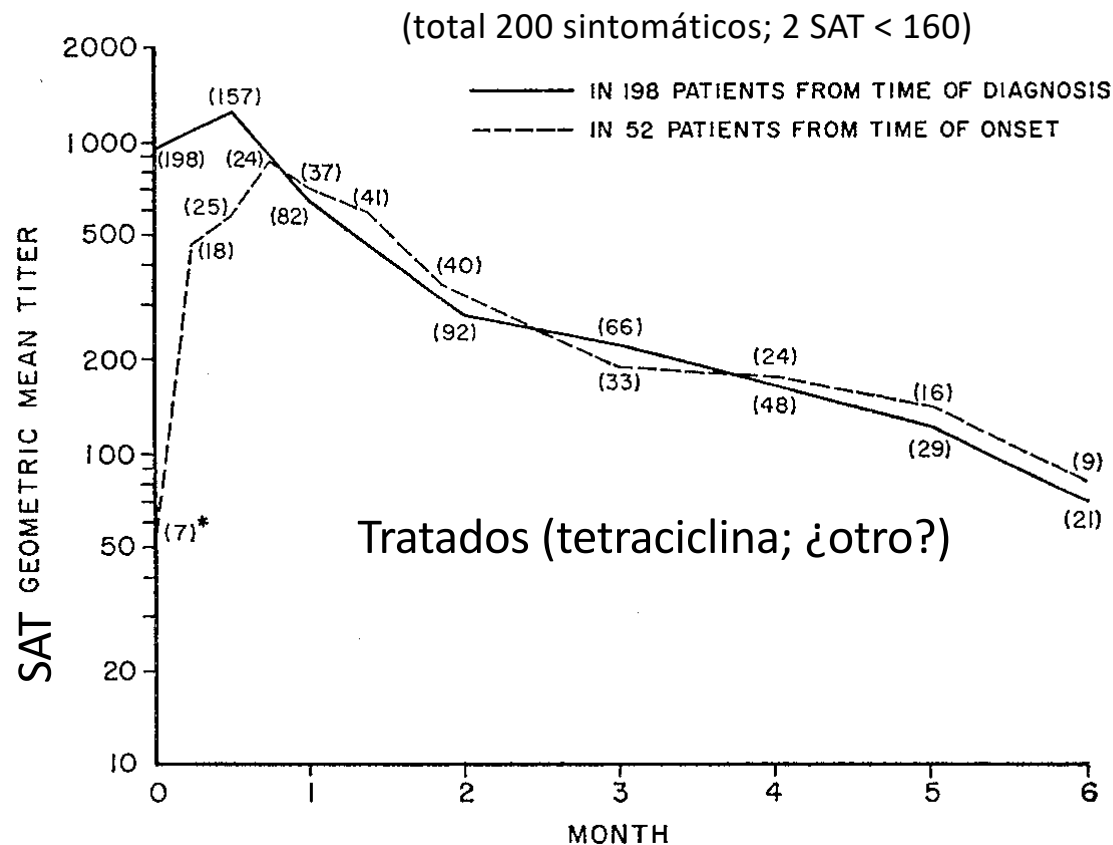
† Titer 32.

‡ Insufficient serum to complete one or more of the tests.



Seroaglutinación en tubo (o microplaca) sin mercaptanos

Buchanan, T. M., Sulzer, C. R., Frix, M. K., Feldman, R. A. 1974. Brucellosis in the United States, 1960-1972. An **abattoir-associated** disease. Part II. Diagnostic aspects. *Medicine (Baltimore)*. 53, 415-425



198 definidos como enfermos por SAT $\geq 1:160$ y seroconversión (incremento ≥ 2 o ± 4 títulos ¹)

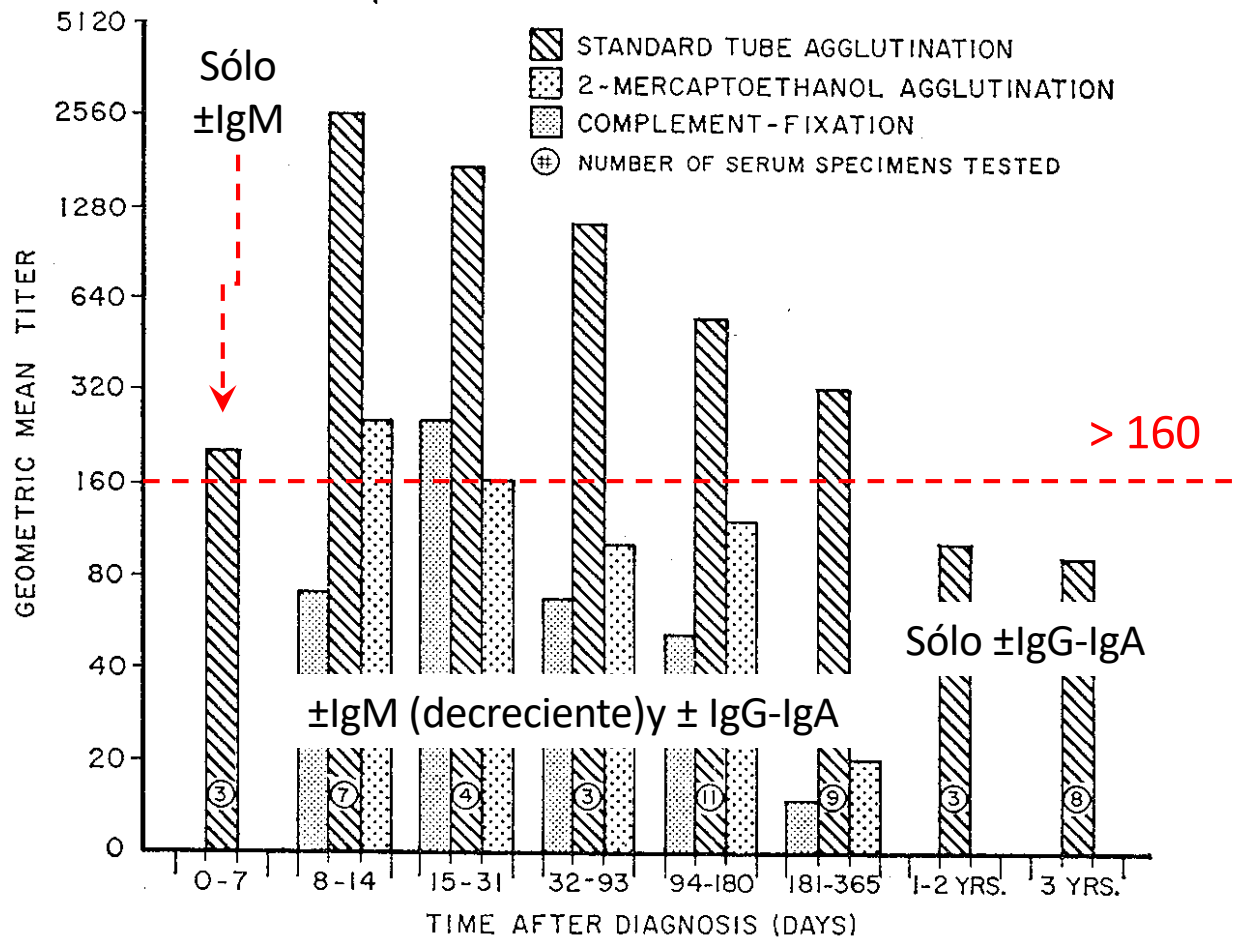
Son casos agudos con infección \pm reciente

1. Buchanan, T. M., Faber, L. C., Feldman, R. A. 1974. Brucellosis in the United States, 1960-1972. An abattoir-associated disease. Part I. Clinical features and therapy. *Medicine (Baltimore)*. 53, 403-413.



Seroaglutinación en tubo (o microplaca) con 2-ME

Buchanan, T. M., Sulzer, C. R., Frix, M. K., Feldman, R. A. 1974. *Brucellosis in the United States, 1960-1972. An abattoir-associated disease. Part II. Diagnostic aspects. Medicine (Baltimore). 53, 415-425*



* TESTS PERFORMED BY CDC LABORATORY

Fig. 2. *Brucella* antibody titers,* by interval after diagnosis.



Seroaglutinación en tubo (o microplaca) con 2-ME

Buchanan, T. M., Sulzer, C. R., Frix, M. K., Feldman, R. A. **1974**. *Brucellosis in the United States, 1960-1972. An abattoir-associated disease. Part II. Diagnostic aspects. Medicine (Baltimore). 53, 415-425*

Títulos de SAT (Std) y 2-ME en el suero de 91 trabajadores de un matadero expuestos a *Brucella* que eran asintomáticos

Titer		
	Std	2-ME
≤ 20	0	58
40	2	13
80	50	8
160	17	7
320	9	3
640	7	2
1,280	3	
2,560	1	
5,120	1	
> 10,240	1	

≥ 160

¿2-ME
confirmatorio
cuando la SAT
es positiva?



Seroaglutinación en tubo con 2-ME

Buchanan, T. M., Faber, L. C. 1980. 2-mercaptoethanol *Brucella* agglutination test: usefulness for *predicting recovery* from brucellosis. *J. Clin. Microbiol.* 11, 691-693.

92 casos de Buchanan et al. 1974 (tratados con tetraciclina; ¿otro?)

SAT

44 \geq 1:160 durante 1.5 años.

2ME

8 \geq 1:160 después de 1 año; (3 con tratamiento incompleto por intolerancia al antibiótico; 4 sintomáticos (“chronic brucellosis”)?);

4 \geq 1:160 después de 1.5 años; sintomáticos.

The results indicate that the 2ME test is superior to the SAT in evaluating the effectiveness of treatment and as a means to rule out a diagnosis of “chronic brucellosis”.

Pero la comparación SAT vs SAT-2ME sugiere que o la IgM (y las IgG-IgA) perduran más de 18 meses, o el 2-ME destruye también una proporción de IgG y/o IgA.



Seroaglutinación en tubo (o microplaca) sin mercaptanos

Foz, A., Arcalís, L. **1952**. Die *Komplementbindungs-Reaktion* in der Diagnose der menschlichen Brucellose. *Med. Microbiol. Immunol.* 136, 55-66

Títulos de seroaglutinación en tubo en 117 pacientes con brucelosis confirmada, todos positivos en la fijación de complemento (test IgG).

Tiempo de evolución (meses)	Nº	Nº (%) con un título:			
		≤ 1:40	<1:80	<1:160	≥ 1:160
Menos de 6	78	3 (3.8)	6 (7.6)	7 (8.9)	71 (91)
Más de 6	39	4 (10.2)	13 (33.3)	15 (38.4)	24 (61.6)
Total	117	7 (5.9)	10 (8.5)	22 (18.8)	85 (72.6)

Títulos diagnósticos que no solucionan la falta de sensibilidad de la SAT a 1:160 e incrementa inespecificidad (ver Tabla I de Buchanan et al. 1974)

37.2 % SAT negativos (→ 2ME negativos)

La prueba de Coombs en brucelosis

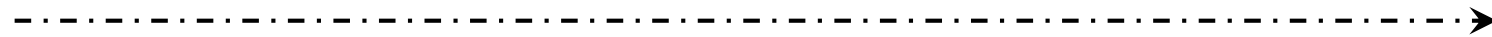
Seroaglutinación (SAT o MAT):¹ título diagnóstico $\geq 1: 160-320$

PERO:

corta evolución

estadios "intermedios"

larga evolución



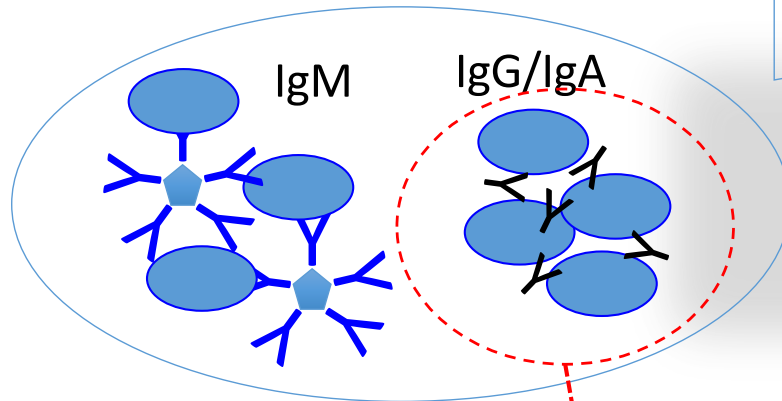
anticuerpos aglutinantes

anticuerpos no-aglutinantes

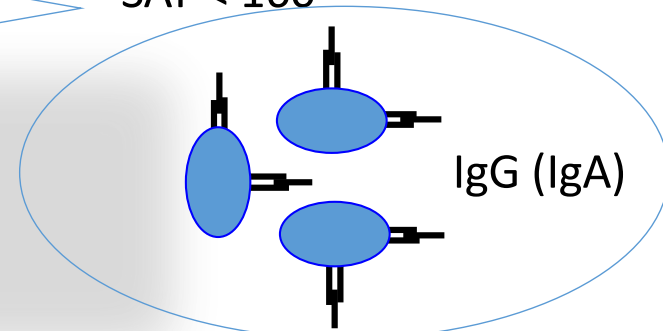
SAT "alta"

SAT decrece

SAT < 160



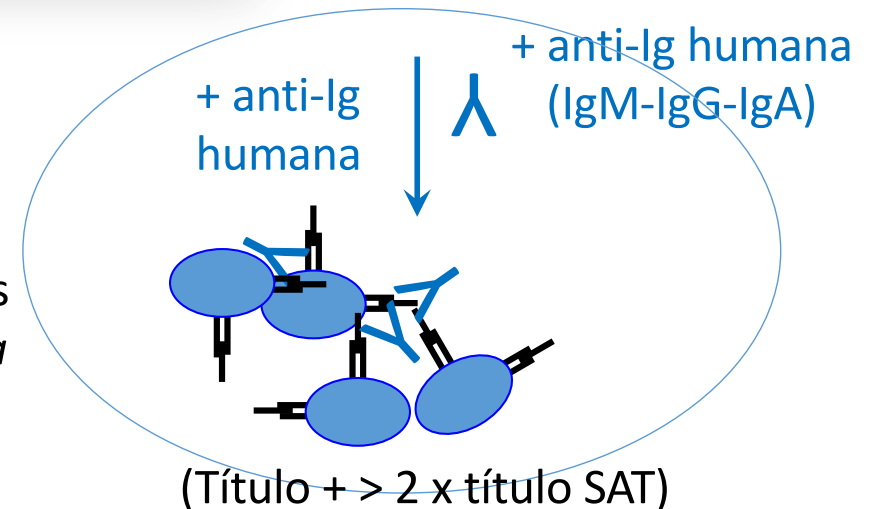
Aglutinantes y no-aglutinantes



¹ En tubo, SAT
en microplaca, MAT

β -ME

Coombs
Brucella





La prueba de Coombs en brucelosis

Kerr, W. R., Coghlan, J. D., Payne, D. J., Robertson, L. 1966. *The laboratory diagnosis of chronic brucellosis.* Lancet. 2, 1181-1183

Tabla. SAT y Coombs en 16 pacientes con brucelosis de más de 6 meses de evolución ¹

SAT		Nº / título en Coombs ‡					Nº positivos
Título	Nº	80	160	320	640	≥ 1280	
≤ 20	11	2		2	2	5	11
40	2			1		1	2
80	2			1			1 *
160	2					1	1

1. Brucelosis "crónica" en Kerr et al.; Spink la definía como de ≥ 6 meses de evolución

‡ "anti gamma globulin test (A.G.H.) en el artículo.

* no se hizo Coombs en un paciente de este grupo.

La prueba de Coombs en brucelosis



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Ratios of titers of Coombs and SAT and evolution of human brucellosis¹

Time of evolution (months)	Nº of patients studied	Coombs / SAT ratio
< 4	98	11.7
4 to 10	45	50.4
> 10	67	86.2

¹ Data from Foz, A. 1983. Brucelosis. In: E. J. Perea. Enfermedades infecciosas. Salvat Ed., Barcelona, Spain



La prueba de Coombs en brucelosis

Díaz, R., Maraví-Poma, E., Rivero, A. 1976. Comparison of counter immunoelectrophoresis with other serological tests in the diagnosis of human brucellosis. *Bull. World Hlth. Org.* 53, 417-424.

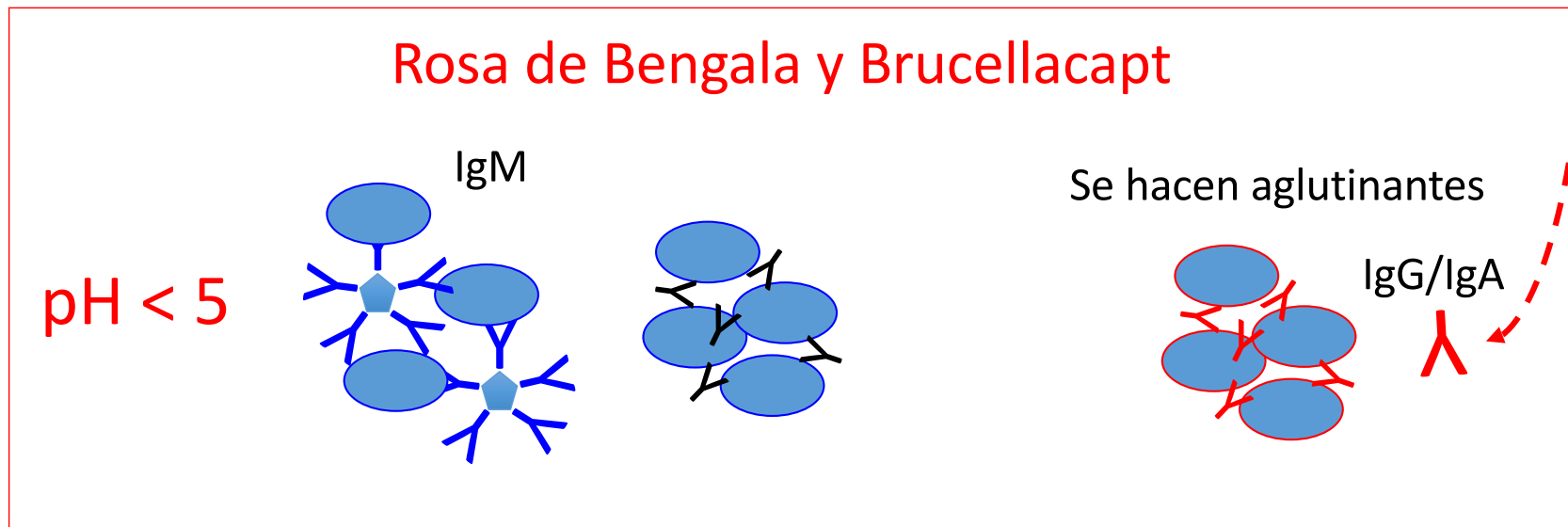
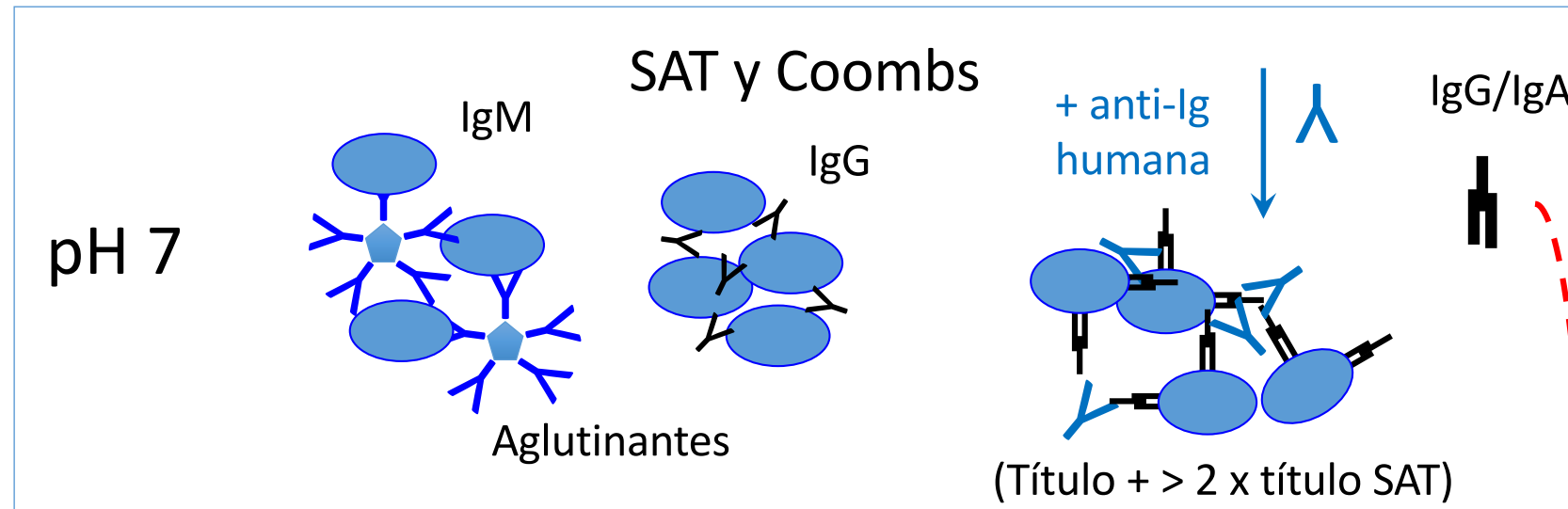
65 casos; clínica y epidemiología; 35 hemocultivos positivos; respuesta al tratamiento positiva

Table 2. Results of tests on sera from patients with clinical symptoms of brucellosis

Tube agglutination titre	Total No. of sera	No. of sera giving positive reactions in					
		rapid plate	Rose Bengal	Coombs	immunodiffusion		CIEP protein
					A & M	poly B	
20	1	0	1	1	1	0	1
40	2	1	1	1	0	0	2
80 ^a	6	2	6	6	4	0	6
160	11	9	11	9	11	1	10
320	4	2	4	4	4	1	4
640	20	19	20	16	19	5	20
1280	15	15	15	10	15	10	12
2560	4	4	4	2	4	3	4
5120	2	2	2	1	2	1	2
Total	65	52	64	50	60	21	61
% positive	86	80	98.5	76.9	92.3	32.3	93.8

^a Equivalent to 100 International Units.

La prueba de Coombs en brucelosis y el Brucellacapt



Rubio-Vallejo, M., Del Pozo, J. L., Hernández-Molina, J. M., Dorronsoro, I., Marrodan, T., Díaz, R. 2002. Diagnóstico de la brucelosis humana. Influencia del pH en la prueba de seroaglutinación y sobre la actividad aglutinante de los anticuerpos IgM, IgG e IgA. *Enferm. Infect. Microbiol. Clin.* 20, 144-149.



La prueba de Coombs en brucelosis y el Brucellacapt

Orduña-Domingo, A., Almaraz, A., Prado, A., Gutiérrez, M. P., García-Pascual, A., Dueñas, A., Cuervo, M., Abad, R., Hernández, B., Lorenzo, B., Bratos, M. A., Torres, A. R. 2000. Evaluation of an immunocapture-agglutination test (Brucellacapt) for serodiagnosis of human brucellosis. *J. Clin. Microbiol.* 38, 4000-4005.

Brucelosis de corta (“aguda”) y larga (“crónica”) evolución

TABLE 1. Distribution of results from sera of brucellosis patients and controls in serological tests

Titers ^a	No. of initial sera (n = 82)			No. of control sera (n = 412)		
	Brucellacapt	Coombs	SAT	Brucellacapt	Coombs	SAT
0			7	397	407	410
20	1		2			2
40		3	8		3	
80	3	4	11	11	1	
160	3	7	17	1	1	
320	2	8	20	2		
640	15	24	11			
1,280	9	24	5	1		
2,560	16	7	1			
5,120	13	5				
10,240	11					
20,480	2					
40,960	4					
81,920	3					

^a Inverse titer.



La prueba de Coombs en brucelosis y el Brucellacapt

Orduña-Domingo, A., Almaraz, A., Prado, A., Gutiérrez, M. P., García-Pascual, A., Dueñas, A., Cuervo, M., Abad, R., Hernández, B., Lorenzo, B., Bratos, M. A., Torres, A. R. 2000. Evaluation of an immunocapture-agglutination test (Brucellacapt) for serodiagnosis of human brucellosis. *J. Clin. Microbiol.* 38, 4000-4005.

TABLE 5. Relation between the titers in the Brucellacapt and Coombs anti-*Brucella* tests^a

Brucellacapt titer ^b	No. of samples with Coombs' anti- <i>Brucella</i> test titer ^b of:									
	0	40	80	160	320	640	1,280	2,560	5,120	Total
0	474	1	4							479
20	1	11	1							
40		10	3							
80	23	16	9	3						
160	5	14	24	3	3	1				
320		7	15	21	9	2				
640			5	17	19	10	2			
1,280			2	15	15	14	9			
2,560				8	7	19	15	1		
5,120					3	13	20	1		
10,240					1	4	10	3	1	
20,480								1	1	
40,960						1	1	1	2	
81,920								1	2	
Total	503									884

^a Correlation coefficient for all results was 0.956 ($P = 0.000$). Correlation coefficient for titers of $\geq 1/160$ (italic numbers) was 0.692 ($P = 0.000$).

^b Inverse titer.



La prueba de Coombs en brucelosis y el Brucellacapt

Brucelosis de corta (“aguda”) y larga (“crónica”) evolución

	Enfermos (n 82)	Sanos (n 412)
SAT \geq 1:160	54	0
Brucellacapt \geq 1:160	78	1

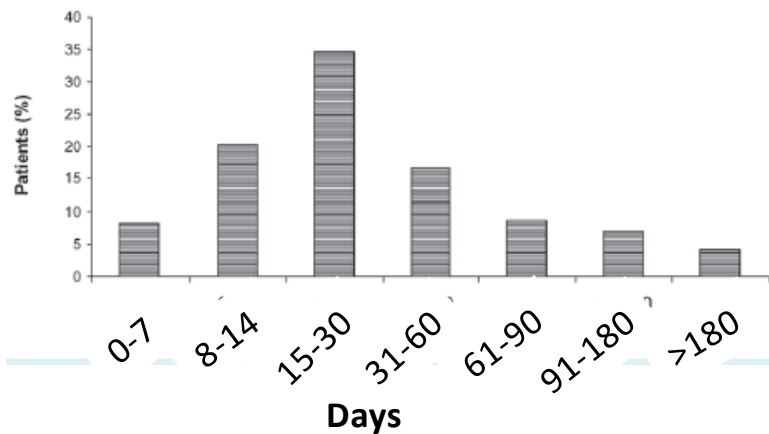
Adaptado de Orduña-Domingo A, Almaraz A, Prado A et al. Evaluation of an immunocapture-agglutination test (Brucellacapt) for serodiagnosis of human brucellosis. J Clin Microbiol. 2000;38:4000-4005.



La prueba de Coombs en brucelosis y el Brucellacapt

Bosilkovski, M., Krteva, L., Dimzova, M., Vidinic, I., Sopova, Z., Spasovska, K. 2010b. Human brucellosis in Macedonia - 10 years of clinical experience in endemic region. *Croatian Med. J.* 51, 327-336.

Figure 1.



Illness duration before diagnosis in 550 patients with brucellosis diagnosed and treated during 1998-2007 at the University Clinic for Infectious Diseases and Febrile Conditions in Skopje.

TABLE 3. Number (and percent) of patients showing the indicated anti-*Brucella* antibody titers as measured using the serum tube agglutination (STA) test, Coombs test, or Brucellacapt at the time of admission to hospital*

Titer	STA [†]	<i>Brucella</i> Coombs [†]	Brucellacapt ^{‡§}
≤1/80	24 (7)	0	0
1/160	34 (10)	28 (8)	4 (2)
1/320	51 (15)	43 (12)	8 (4)
1/640	82 (24)	67 (20)	20 (10)
1/1280	153 (44)	206 (60)	20 (10)
1/2560	ND	ND	29 (14)
1/5120	ND	ND	125 (61)

*Abbreviation: ND – not determined.

[†]Performed in 344 patients.

[‡]Performed in 206 patients.

[§]The numbers do not add up to 100% because of rounding.



La prueba de Coombs en brucelosis y el Brucellacapt

Gómez, M. C., Nieto, J. A., Rosa, C., Geijo, P., Escribano, M. A., Muñoz, A., López, C. 2008. Evaluation of seven tests for the diagnosis of human brucellosis in an endemic area. *Clin. Vaccine Immunol.* 15, 1031-1033

- 25 casos agudos (clínica, cultivo y SAT $\geq 1:160$).
- 90 donates de sangre sanos

TABLE 2. Accuracy indices of the tests^a

Test	Sensitivity	Specificity	PPV ^b	NPV ^c
RB ^d	1.00	0.97	0.89	1.00
MAT	0.92 ^e	1.00	1.00	0.98
Brucellacapt	1.00	1.00	1.00	1.00
IgG ELISA	0.84	1.00	1.00	0.96
IgM ELISA	0.60	1.00	1.00	0.90
IgA ELISA	0.96	0.98	0.92	0.99

^a Cutoff points for positive tests were as follows: RB, $\geq 1:1$; MAT and Brucellacapt, $\geq 1:160$.

^b PPV, positive predictive value.

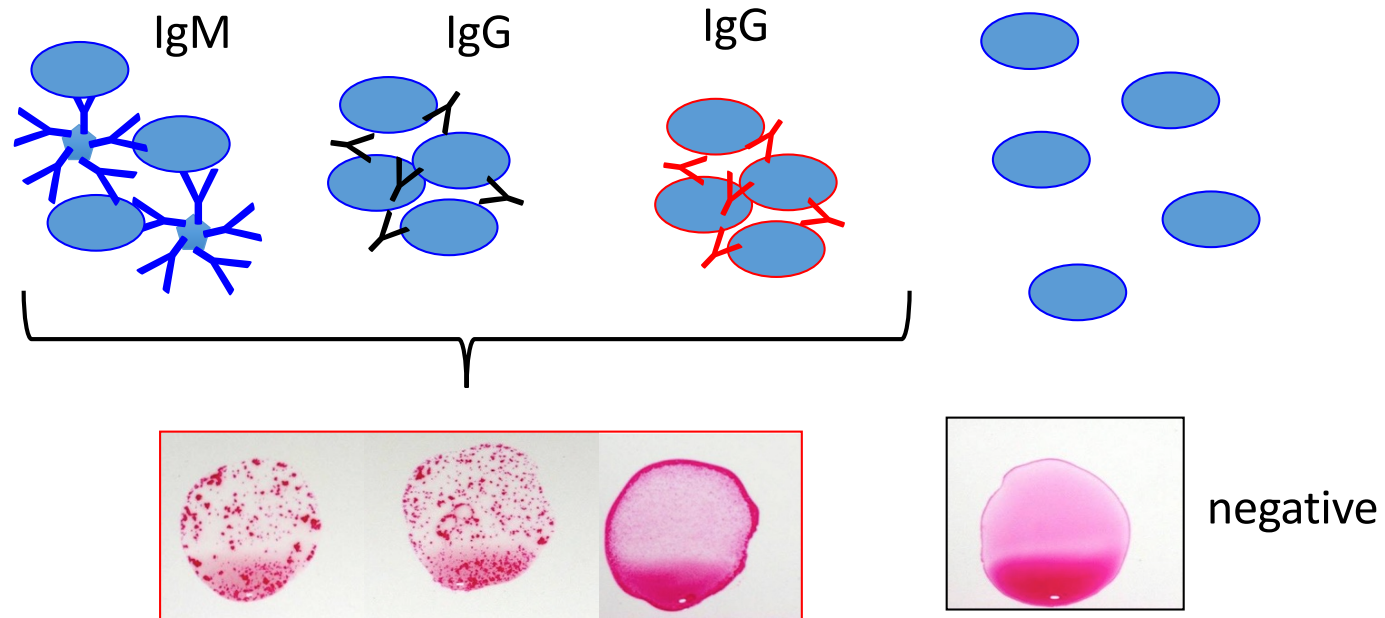
^c NPV, negative predictive value.

^d RB, Rose Bengal test.

^e Two patients had MAT titers of 1:80.

El Rosa de Bengala en brucelosis humana

pH 3.7



La "intensidad" (tamaño grumos) no guarda una relación unívoca con la cantidad de anticuerpos

Rosa de Bengala, “Card Test” y “Buffered Plate Agglutination Test” [BPAT]



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Lucero, N. E., Bolpe, J. E. 1998. Buffered plate antigen test as a screening test for diagnosis of human brucellosis. *Journal of Clinical Microbiology*. 36, 1425-1427. 10.1128/JCM.36.5.1425-1427.1998.

Serum source (no.)	(BPAT)		PAT (Huddleson)			
	BPA (Buffered plate antigen)		Positive at endpoint titer of:			
	Negative	Positive	Negative	1:25	1:50	≥1:100
Culture-positive patients (57) ^a	0	57	0	1	6	50
Suspected-brucellosis patients (142) ^b	0	142	0	1	20	121
Asymptomatic population (307) ^c	306	1	292	14	1	0

- Of the total of 57 positive isolates, 29 were *B. suis*, 15 were *B. abortus*, 6 were *B. melitensis*, and seven *Brucella* strains were not typed to the species level
- Patients with clinical evidence of brucellosis and positive by RB and CF.
- Asymptomatic population negative by RB and CF.

Rosa de Bengala, "Card Test" y "Buffered Plate Agglutination Test" [BPAT]



Díaz, R., Maraví-Poma, E., Rivero, A. 1976. Comparison of counter immunoelectrophoresis with other serological tests in the diagnosis of human brucellosis. *Bull. World Hlth. Org.* 53, 417-424.

65 casos; clínica y epidemiología; 35 hemocultivos positivos; respuesta al tratamiento positiva

Table 2. Results of tests on sera from patients with clinical symptoms of brucellosis

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					A & M	poly B	
20	1	0	1	1	1	0	1
40	2	1	1	1	0	0	2
80 ^a	6	2	6	6	4	0	6
160	11	9	11	9	11	1	10
320	4	2	4	4	4	1	4
640	20	19	20	16	19	5	20
1280	15	15	15	10	15	10	12
2560	4	4	4	2	4	3	4
5120	2	2	2	1	2	1	2
Total	65	52	64	50	60	21	61
% positive	86	80	98.5	76.9	92.3	32.3	93.8

^a Equivalent to 100 International Units. ¿100?

Rosa de Bengala, “Card Test” y “Buffered Plate Agglutination Test” [BPAT]



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Díaz, R., Casanova, A., Ariza, J., Moriyón, I. 2011. The rose bengal test in human brucellosis: a neglected test for the diagnosis of a neglected disease. *PLoS Negl. Trop. Dis.* 5, e950. 10.1371/journal.pntd.0000950.

Table 1. Results of SAT and RBT in 208 patients with brucellosis proved by bacteriological culture.¹

SAT		Nº positive in standard RBT protocol (%)	Nº RBT positive (%) at titers: ²	
Titer	Nº		≤ 4	≥ 8
≤ 1:20	6	6 (100)	2 (0,96)	4 (1,92)
≥ 1:40	202	202 (100)	26 (12,5)	176 (85,5)
≥ 1:80	201	201 (100)	25 (12,0)	176 (85,0)
≥ 1:60	185	185 (100)	13 (6,20)	172 (83,0)
≥ 1:320	160	160 (100)	2 (0,90)	158 (76,3)
≥ 1:640	136	136 (100)	0 (0,00)	136 (65,7)

1. Blood (n = 205) or abscess (n = 3) culture.

2. Titers correspond to plain serum (titer 1:2) or serum dilutions made in phosphate buffered saline and then tested with an equal volume of RBT reagent (1:4, etc.).

Puede haber prozonas (muy baja frecuencia)

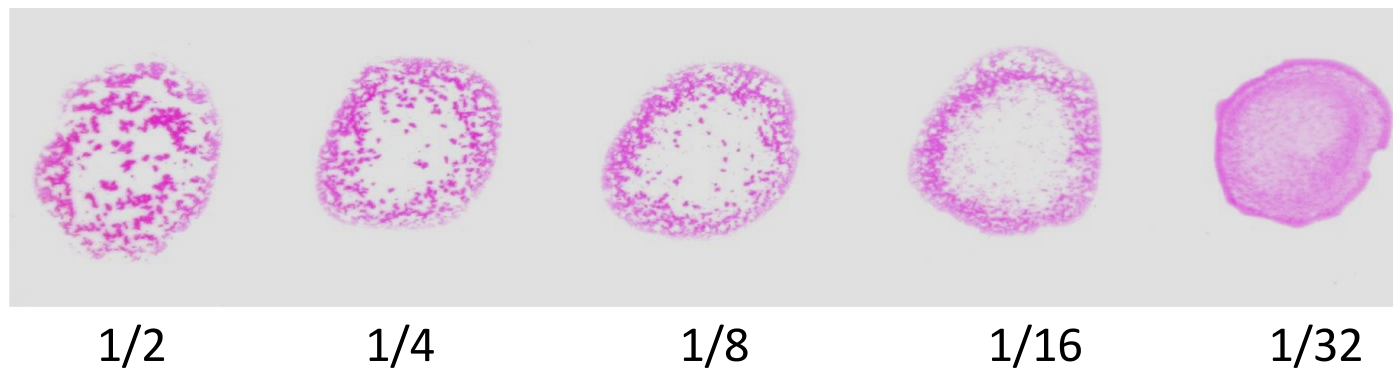
Elimina prozonas y efectos de bloqueo

Uso de diluciones de suero

1. Gotas (30 μL)
de suero salino

2. Suero: se hacen diluciones con las gotas

3. Cada dilución se testa con el reactivo



Rosa de Bengala, “Card Test” y “Buffered Plate Agglutination Test” [BPAT]



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Díaz, R., Casanova, A., Ariza, J., Moriyón, I. 2011. The rose bengal test in human brucellosis: a neglected test for the diagnosis of a neglected disease. *PLoS Negl. Trop. Dis.* 5, e950. 10.1371/journal.pntd.0000950.

Table 1. Serological tests in persons that had professional contact with *Brucella* but no symptoms

Group / Code ¹	Serum titers:					LFiC ³	
	RBT	SAT	Brucapt	Coombs IgG	cELISA % ²	IgM	IgG
Abattoir worker							
C-1 (0)	4	40	320	5120	65	0	3
C-1 (24)	4	40	320	5120	52	0	3
Meat processing plant worker							
C-5	2	160	80	640	25	0	2
Veterinarians accidentally injected with vaccine Rev1							
C-6 (0)	4	40	2560	5120	80	0	3
C-6 (18)	4	40	640	1280	83	0	2
Veterinarians that had contacts with infected animals							
C-11	2	80	160	1280	17	0	1
C-19	4	160	320	2560	24	0	1
C-20 (0)	0	<20	<20	20	3	ND ⁵	ND ⁵
C-20 (6)	8	640	ND ⁵	2560	ND ⁵	ND ⁵	ND ⁵

¹ For those persons that were sampled repeatedly, figures in parenthesis indicate the months at which the samples were taken

² % competitive index.

³ From 0 (negative) to 4 (strong positive).

⁴ Serum titers (number of precipitin lines).

⁵ ND, not done.

RBT results with sera samples from brucellosis patients and persons in contact with *Brucella* infected animals.¹

	Total N° of sera	N° (%) positive at dilution	
		≤ 1/4	≥ 1/8
Infected	210	209 (99,5)	195 (93,3)
Contact (asymptomatic)	105	21 (20,0)	1 (0,9)

¹ Medicine. 2002; 8(61):3289-3296 (Spanish edition)

Rosa de Bengala, “Card Test” y “Buffered Plate Agglutination Test” [BPAT]



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Araj, G. F., Lulu, A. R., Khateeb, M. I., Saadah, M. A., Shakir, R. A. 1988. *ELISA versus routine tests in the diagnosis of patients with systemic and neurobrucellosis. APMIS. 96, 171-176. 10.1111/j.1699-0463.1988.tb05286.x.*

Mizanbayeva, S., Smits, H. L., Zhalilova, K., Abdoel, T. H., Kozakov, S., Ospanov, K. S., Elzer, P. H., Douglas, J. T. 2009. *The evaluation of a user-friendly lateral flow assay for the serodiagnosis of human brucellosis in Kazakhstan. Diag. Microbiol. Infect. Dis. 65, 14-20.*

54-61 % sensibilidad

Díaz, R., Casanova, A., Ariza, J., Moriyón, I. 2011. *The rose bengal test in human brucellosis: a neglected test for the diagnosis of a neglected disease. PLoS Negl. Trop. Dis. 5, e950. 10.1371/journal.pntd.0000950.*

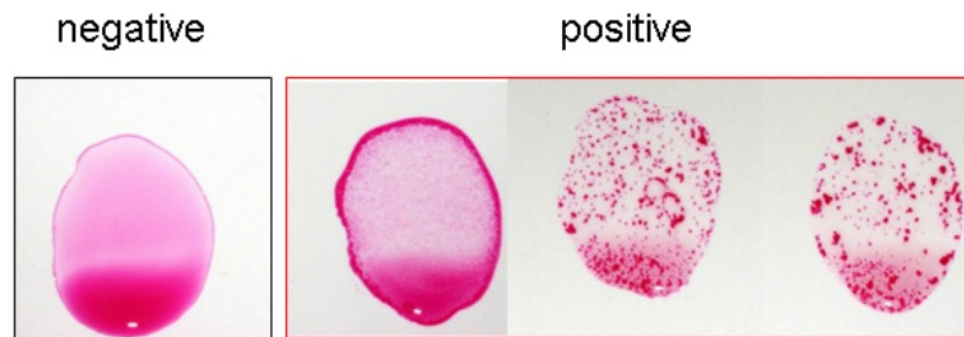


Figure S1. Different degrees of agglutination in RBT

Falsos “algoritmos”

Díaz Quiñónez, J. A. E.
(Editor), 2015, *Lineamientos para la vigilancia epidemiológica de brucelosis por laboratorio. VERSIÓN N°. 01. INDRE, 2015.*
Instituto de Diagnóstico y referencia Epidemiológicos “Dr. Manuel Martínez Báez”,
Secretaría de Salud,
Dirección General de Epidemiología, México.



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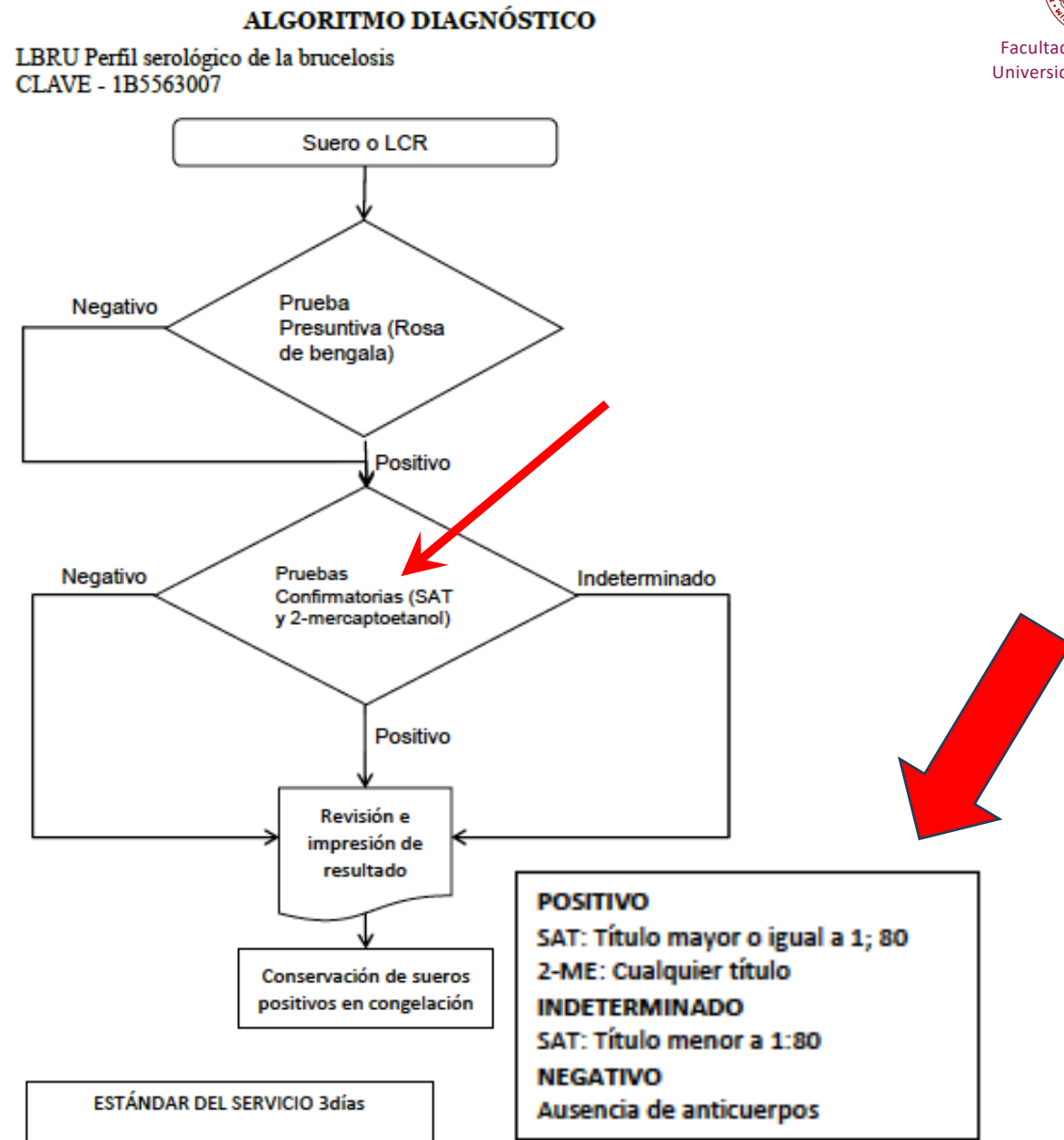


Figura. 3. Algoritmo para el diagnóstico serológico de Brucelosis

Falsos “algoritmos”



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Guzmán-Bracho, C., Salgado-Jiménez, B., Beltrán-Parra, L. G., Hernández-Monroy, I., Vargas-Pino, F., Rodríguez, D., López-Martínez, I., Pastén-Sánchez, S., González-Roldán, J. F., Membrillo-Hernández, J., Díaz-Quiñónez, J. A. 2020. Evaluation of serological diagnostic tests of human brucellosis for prevention and control in Mexico. *Eur. J. Clin. Microbiol. Infect. Dis.* 39, 575-581. 10.1007/s10096-019-03760-3.

We included individuals with clinical suspicion of brucellosis. Suggestive clinical data for identification of positive cases were fever, diaphoresis, headache, myalgia and arthralgia, and asthenia and adynamia, according to current regulations. Inclusion criteria were all patients with signs and symptoms suggestive of brucellosis and criteria for exclusion considered were all patients with a history of brucellosis and treatment as well as pregnant and hospitalized patients.

1. A conventional diagnostic algorithm (RB→SAT→SAT/SAT- 2ME) was applied to the 473 samples from endemic areas.
2. The RB test identified 165 samples as negatives (34.9%), which definitely rules out the presumptive diagnosis of brucellosis.
3. The remaining 308 were subjected to the SAT and SAT-2ME tests.
4. After this analysis, 222 were confirmed, indicating an efficiency in the screening of 72%.
5. Confirmed individuals represent 46.9% of the whole sample

ii86 / 308 (28%)
con diagnóstico
equivocado o no
diagnosticados!!

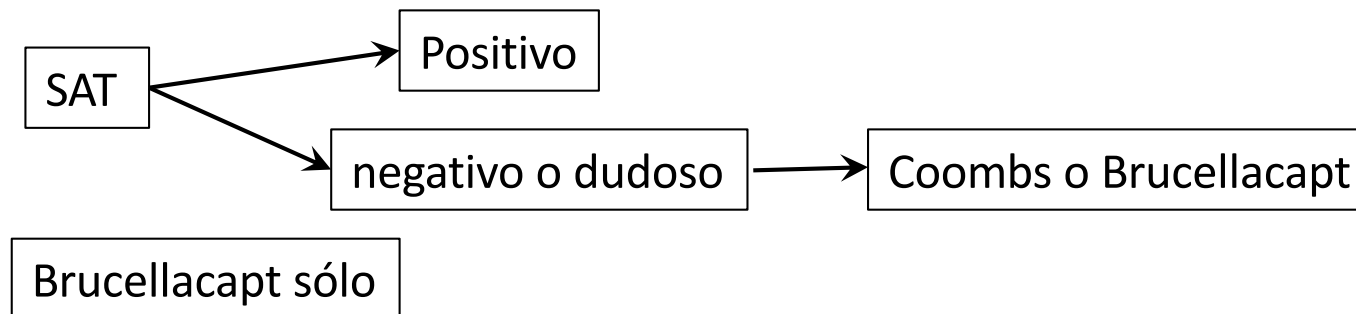
Conclusión y ejemplos



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Bosilkovski, M., Krteva, L., Dimzova, M., Vidinic, I., Sopova, Z., Spasovska, K. 2010b. Human brucellosis in Macedonia - 10 years of clinical experience in endemic region. *Croatian Med. J.* 51, 327-336.

- The serum agglutination test is an important diagnostic tool...
- The combination of the serum agglutination test and Coombs [...] may help to overcome the [...] false-negative results.
- [...] the Brucellacapt test has started to replace other serological tests.
- [...] at the time of admission [...] generally showed high anti-*Brucella* antibody titers, especially in the Coombs test and Brucellacapt.

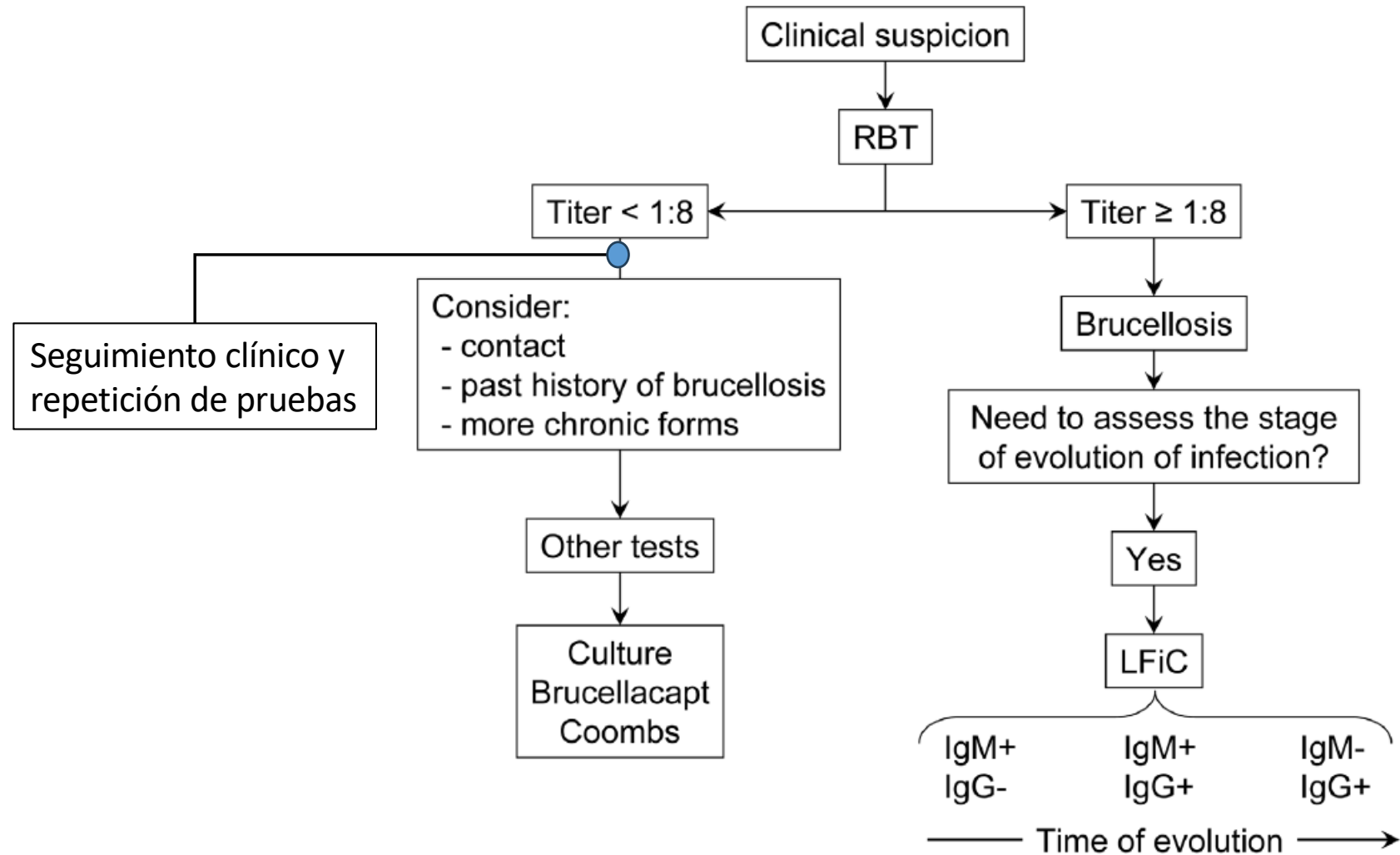


- In the case of inconclusive results but a high clinical suspicion of brucellosis, the patients were retested after 2-4 weeks to assess possible seroconversión.



Conclusión y ejemplos

Díaz, R., Casanova, A., Ariza, J., Moriyón, I. 2011. The rose bengal test in human brucellosis: a neglected test for the diagnosis of a neglected disease. *PLoS Negl. Trop. Dis.* 5, e950. 10.1371/journal.pntd.0000950.



Material complementario



Definición imperfecta de grupos

Serra, J., Viñas, M. 2004. Laboratory diagnosis of brucellosis in *a rural endemic area* in northeastern Spain. *Int. Microbiol.* 7, 53-58

62 patients diagnosed on the basis of clinical evidence and/or personal history.

Group 1 (n = 38) primary infections

Blood culture 0.92 sensitivity

RBT and agglutination $\geq 1/160$ was valid.

Group 2, (n = 24) previously exposed to the microorganism, i.e. re-infection of group 2 individuals occurred after long periods of time during which no active infection by *Brucella* had been detected.

Culture inappropriate (0.08 sensitivity).

SAT $< 1/160$ did not rule out brucellosis whereas.

RBT + Coombs' $\geq 1/320$ best (0.8 specificity; 1 sensitivity).

ELISA (for IgG, IgM, or both) did not improve diagnostic accuracy.