Serological Follow-up of Brucella Patients Using an Immunocapture-Agglutination test (Brucellacapt), Coombs anti-Brucella and LPS-ELISA tests.

Prado A, Gutierrez P, Duenas A, Miguel MA, Ruiz L, Mantecon MA, Bratos MA, Orduna A.
Department of Microbiology and Research Unit. University Hospital. Faculty of Medicine. Valladolid. Spain.

Introduction

We evaluated a new test for the diagnosis and follow-up of human brucellosis based on immunecapture-agglutination technique (Brucellacapt) compared with seroagglutination test (SAT), Coombs anti-Brucella test and IgG, IgA, and IgM ELISA against smooth lipopolysaccharide (S-LPS) from Brucella melitensis 16M.

Material and methods

We studied 82 initial sera from patients with a diagnosis of brucellosis and 277 follow-up sera from 53 patients of brucellosis. Four hundred and twelve sera from people living in rural areas with endemic brucellosis (Castilla y Leon, Spain) were included as a control group.
Brucellacapt (Vircell, Santa Fe, Granada, Spain), SAT, Coombs test (Linear Chemicals, Spain) and IgG, IgA and IgM ELISA against S-LPS from Brucella melitensis were performed for each serum sample. S-LPS was obtained by phenol-water extraction technique (Redfearn method). All sera from the same patient were studied simultaneously. The curve results were expressed as a percent of variation of the logarithm of the inverse of the titers of evolution sera in relation to the titers of corresponding initial sera.

Results

If a $\geq$ 1/160 diagnostic threshold titer was defined for Brucellacapt, SAT, Coombs tests, IgG ELISA, IgA ELISA and IgM ELISA, the sensitivities were 95.1, 65.8, 91.5, 92.6, 89.0 and 85.3% respectively. For the 412 control sera the specificities were 99.0, 100, 99.8, 97.8, 100 and 100% respectively.
The antibody titers of all serological test decrease after the beginning of the treatment. (Figure 1) One year later, the highest titers were obtained by IgG ELISA (90%). The lowest titers were obtained by SAT (66%) and ELISA IgM (70%). The curve of the follow-up titers of Brucellacapt was similar to the Coombs test and the titers persist high one year after treatment.

Conclusions

The study shows the highest sensitivity of Brucellacapt and a very good correlation between the Brucellacapt and the Coombs tests, with a high concordance between titers obtained in the two tests. The evolution of the titers after treatment is very similar in both tests.

TABLE 2: Sensitivity, specificity and likelihood ratio of the SAT, Coombs anti-*Brucella* and Brucellacapt® tests in the 82 diagnostic and 412 control sera.

Test**	Sensitivity (%)	Specificity (%)	Positive L.R* (CI 95%)	Negative L.R* (CI 95%)
Brucellacapt®				
20	100% (95.6-100)	96.4% (93.9-97.8)	27.4 (16.7-44.8)	0.000 (0.000 –0.057)
40	98.8% (92.5-99.9)	96.4% (93.4-97.8)	27.1 (16.6-44.5)	0.013 (0.002-0.068)
80	98.8% (92.5-99.9)	96.4% (93.4-97.8)	27.1 (16.6-44.5)	0.013 (0.002-0.068)
160	95.1% (87.3-98.4)	99.0% (97.3-99.6)	97.9 (38.4-251.8)	0.049 (0.019-0.120)
320	91.5% (82.7-96.1)	99.3% (97.7-99.8)	125.6 (43.0-369.5)	0.086 (0.042-0.167)
Coombs test				
20	100% (95.6-100)	98.8% (97.0-99.5)	82.4 (35.3-191.3)	0.000 (0.000-0.056)
40	97.6% (90.7-99.5)	98.8% (97.0-99.5)	80.3 (34.7-187.9)	0.024 (0.006-0.085)
80	96.3% (88.9-99.0)	99.5% (98.0-99.9)	198.4 (54.9-723.5)	0.036 (0.012-0.102)
160	91.5% (82.7-96.2)	99.8% (98.4-99.9)	376.8 (67.01-2135.7)	0.085 (0.042-0.166)
320	82.9% (70.7-89.1)	100% (99.1-100)	672.2 (70.7-6446.1)	0.1842 (0.113-0.286)
SAT				
20	91.4% (82.7-96.1)	99.5% (98.0-99.9)	188.4 (52.1-687.4)	0.085 (0.042-0.166)
40	89.0% (79.7-94.5)	100% (99.1-100)	733.5 (77.2-7030.2)	0.109 (0.058-0.195)
80	79.2% (68.6-87.1)	100% (99.1-100)	653.1 (68.7-6264.0)	0.207 (0.133-0.307)
160	65.8% (54.5-75.7)	100% (99.1-100)	542.6 (57.0-5210.5)	0.341 (0.248-0.449)
320	45.1% (34.2-56.4)	100% (99.1-100)	371.8 (38.9-3582.4)	0.548 (0.441-0.652)

*LR: Likelihood ratio
**Titer inverse

TABLE 1: Distribution of results from brucellosis patient sera and control sera in serologic tests.

	Initial sera (n=82)			*Follow-up sera (n=233)*			*Control sera (n=412)*		
*Titers**	Bruce llacapt	Coombs	SAT	Bruce llacapt	Coombs	SAT	Bruce llacapt	Coombs	SAT
0			7	8	20	48	397	407	410
20	1		2	0	0	22			2
40		3	8	0	8	30		3	
80	3	4	11	8	28	41	11	1	
160	3	7	17	21	53	53	1	1	
320	2	8	20	47	49	20	2		
640	15	24	11	37	40	14			
1280	9	24	5	45	33	4	1		
2560	16	7	1	34	1	1			
5120	13	5		24	1				
>10240	20			9					

Figure 1: Titer evolution of specific antibodies against Brucella melitensis

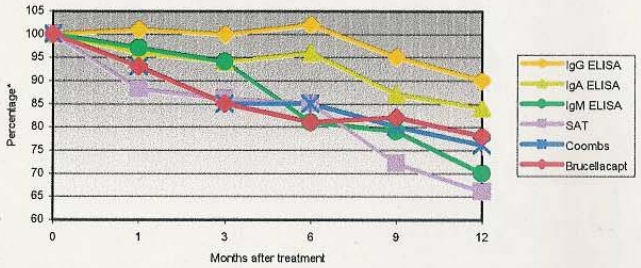
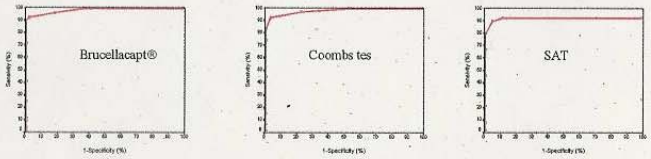


Figure 2. Diagnostic efficiency curves (Receiver Operating Characteristic Curve or ROC curve) for Brucellacapt®, Coombs test and SAT.



Area below the ROC curve: Brucellacapt®: 0.97852 (CI 95% 0.95109 - 0.99286); Coombs test 0.97611 (CI 95%: 0.94781 - 0.99146); SAT 0.91013 (CI.95%: 0.86649 - 0.94317)