

Experience exchange

Comparison of Coombs ' and immunocapture-agglutination tests in the diagnosis of brucellosis

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Brucellosis is an important zoonotic disease caused by bacteria of the genus *Brucella* encountered in animals such as cows , sheep , goats and pigs as well as in humans. It is one of the most widely seen infections and nearly half a million cases are declared annually. Endemic infections occur especially in the Mediterranean , Middle East , Latin America and Asia.¹ Seropositiveness ratios vary between 2% and 12% in Turkey.² The average annual number of cases declared to the Turkish Ministry of Health between 1991 and 2000 was 9000.³

Different clinical outcomes are observed in human brucellosis. An exact diagnosis is based on cultivation of the pathogen. Problems may occur during the diagnosis because of difficulties in the isolation of bacteria , and some laboratories may not have the capacity for *Brucella* culture. Yuong⁴ reported isolation rates of *Brucella* may differ between 15% to 70% and 50% to 90% in manual and automated systems , respectively. Thus , serology plays a major role in the diagnosis of brucellosis. Even so , there are some difficulties in the evaluation of test results.

The standard tube agglutination test (STAT) is used routinely in serological diagnosis , but false negatives may be observed due to blocking antibodies. However , the definitive test for *Brucella* is the *Brucella* Coombs ' test (CT).^{4 5} The aim of this study was to investigate the efficacy of the immunocapture-agglutination test (Brucellacapt , IcAT) in the diagnosis of *Brucella* infections by using the CT as a reference test.

METHODS

Samples

Ninety-two serum samples obtained from known

brucellosis patients and 40 from healthy individuals were included in the study. They were stored at -70°C until they were examined in the study.

Measurement

All the thawed sera were investigated for anti-*Brucella* antibodies by STAT , CT and IcAT , in that order. The antigen (Bacterial suspensions tube test , Biomedical Systems , Spain) used in STAT was studied after two-fold dilutions were performed from 1 : 20 to 1 : 5120 dilutions. The CT was performed in tubes to which STAT had been applied by adding Coombs serum (Lorne Laboratories Ltd. , UK) after they had been washed three times with 0.9 % NaCl.⁵ IcAT (Brucellacapt) test (Vircell SL , Spain) was performed according to the manufacturer 's instructions. The results were evaluated for the three tests using cut-off values of 1:160 and 1:320 titres , respectively.

Statistical analysis

Statistically significant differences between the performances of CT and IcAT , CT and STAT were tested by Fisher 's exact test. Relative sensitivities , specificities , positive predictive value (PPV) and negative predictive value (NPV) of IcAT and STAT were calculated by using CT as reference test. A *P* value less than 0.05 was considered statistically significant.

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RESULTS

In the trial group , when a cut-off titre of 1 : 160 was set , the positive rate was 80% by CT , 87% and 80% by IcAT and STAT , respectively. If a cut-off titre of 1 : 320 was set , these ratios were 76% , 86% and 70% , respectively (Table 1).

Table 1. Comparison of CT , IcAT and STAT

Cut-off values	CT (n)	IcAT (n)		STAT (n)		
		+	-	+	-	
1:160	+	(74)	72	2	72	2
	-	(18)	8	10	2	16
	Total	(92)	80	12	74	18
1:320	+	(70)	70	0	62	8
	-	(22)	9	13	2	20
	Total	(92)	79	13	64	28

CT : the Coombs ' test ; IcAT : the immunocapture-agglutination test ; and STAT : the standard tube agglutination test.

Only one sample from the control group was positive with 1 : 160 titre by CT , while one was 1 : 160 and one was 1 : 320 titres by the IcAT. All samples from the control group had titres of 1 : 80 by STAT.

The sensitivity , specificity , PPV and NPV of IcAT and STAT are summarized in Table 2. No significant differences (both $P > 0.05$ considering cut-off values of 1 : 320 and 1 : 160) were observed between the CT and IcAT in terms of positive ratios.

DISCUSSION

Coombs serum (anti-human serum globulin) is used in the detection of blocking antibodies observed in STAT.⁵ This situation broadens the diagnosis period and some cases are misevaluated when no request for CT is made by the clinician. An alternative test for detecting blocking antibodies will cut the number of misevaluated cases and shorten the diagnosis time. In this study , the efficacy of an easy , one-step immunocapture-agglutination method for detecting total anti-*Brucella* antibodies was examined.

Orduna et al¹ found 2- or 4-fold , 4- and 8-fold

higher titres with CT , IcAT and follow up sera , respectively , after the starting sera were found to have 1 : 160 titres with STAT in *Brucella*. Also , they reported STAT titres were frequently 1 : 160 in chronic cases and relapses , and , in contrast , CT and IcAT titres were 1 : 640 and 1 : 1280 , respectively. They also noted when low titred sera were tested , the titres found with CT and IcAT were correlated , but with titres of 1 : 160 , a smaller correlation was detected between these two tests. Gomez et al⁶ reported that CT and IcAT gave similar results with one or two dilution differences between 1 : 40 and 1 : 2560 titres. In this study , we observed higher titres in IcAT and CT than in STAT , as in the results of previous studies.

Orduna et al¹ found 1 : 20 – 1 : 640 titres in 54.8% with IcAT , 1 : 320 titres in 53.5% with the CT and 1 : 80 in 11.4% with STAT in a total of 157 sera obtained from *Brucella* suspected patients. When the cut-off titre value being 1 : 160 , they found that the sensitivities of these three tests were 95.1% , 91.5% and 65.8% , respectively. They determined the specificities of IcAT and CT to be 81.5% and 96.2% , respectively , in *Brucella* suspected cases. When the cut-off value was determined to be 1 : 320 , these ratios for the two tests were 97.4% and 100% , respectively.

In this study , when the results of CT were considered , and when a cut-off titre value of 1 : 160 was concerned , the sensitivity , specificity , PPV and NPV were 97.3% , 55.6% , 90.0% and 83.3% , respectively. These ratios were 97.3% , 88.9% , 97.3% and 88.9% , respectively , for STAT. When a cut-off value of 1 : 320 was concerned , the ratios of 100% , 59.1% , 88.6% and 100% and 88.6% , 90.1% , 96.9% and 71.4% were obtained by IcAT and STAT , respectively. The sensitivities of the IcAT for both cut-off titre values were correlated with the results found by Orduna et al,¹ while the specificities were somewhat lower. When the cut-off titre values of 1 : 160 and 1 : 320 were evaluated separately according to CT results , the sensitivity of the IcAT correlated well with CT results at both

Table 2. Sensitivity , specificity , PPV and NPV of IcAT and STAT (%)

Cut-off values	Sensitivity		Specificity		PPV		NPV	
	IcAT	STAT	IcAT	STAT	IcAT	STAT	IcAT	STAT
1:160	97.3	97.3	55.6	88.9	90.0	97.3	83.3	88.9
1:320	100	88.6	59.1	90.1	88.6	96.9	100	71.4

PPV : positive predictive value ; NPV : negative predictive value ; IcAT : the immunocapture-agglutination test ; and STAT : the standard tube agglutination test.

values. In this study , the positives were usually higher in CT and especially in IcAT than STAT. While these results were statistically significant between STAT and IcAT ($P < 0.01$) and between STAT and CT ($P < 0.01$), no significant difference was detected between IcAT and CT ($P > 0.05$). Serra et al⁷ also did not find statistical significance between IcAT and CT and reported that IcAT could be used instead of CT.

The cut-off value of STAT positivity was considered 1 : 160 and the results above this value were reported to be correlated with clinical outcome.^{2,3} Orduna et al¹ reported that a cut-off titre value of 1:320 was appropriate for the IcAT to correspond with the clinical condition of the patient.

In conclusion , it may be considered an advantage that the sensitivity of the IcAT is highly correlated with the CT while the lower sensitivity of the former is a disadvantage.

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