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Assessment of Brucellosis Card test in screening patients for brucellosis

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SUMMARY

The Brucellosis Card test (Brewers' Diagnostic Kits, Hynson, Westcott and Dunning, Inc., Baltimore, Md.) was evaluated in relation to the Brucelloslide test (bioMérieux, France), the microagglutination test (MAT) and the demonstration of brucella-specific IgG, IgM and IgA in an enzyme-linked immunosorbent assay (ELISA). A total of 573 serum specimens was tested. These included sera from patients with acute brucellosis (159), chronic brucellosis (23) and patients who had been diagnosed previously as having had brucella infection (155). Control groups consisted of patients with diseases other than brucellosis (52), others with non-infectious diseases (20), and healthy individuals (164). The Card test detected 100% of the patients with acute and 61% of the patients with chronic brucellosis. The sera from the control groups were all negative. Similar results were obtained with the Brucelloslide test and the MAT. The ELISA test detected brucella-specific Ig of all classes in the serum of patients with acute brucellosis, and IgG and IgA in the serum of patients with chronic brucellosis. In the latter group, IgM was also detected in 32% of the sera. Twenty-three per cent of sera with titres of 20 by the MAT were positive on the Card test and had ELISA titres for IgM, IgG and IgA of 400. Characterization of the antibodies involved in the Card test showed that sera with IgM ELISA titres of 1600, or an IgM titres of 800 together with IgG and IgA titres $\geqslant 200$ were Card test positive. Higher IgG ($\geqslant 1600$)) plus IgA ($\geqslant 400$) titres were required to produce a positive Card test in the absence of IgM or when the IgM titre was $\leqslant 200$. The Card test has a potential value as a rapid screening test for humans with acute brucellosis and shows similar results to Brucelloslide and MAT tests. ELISA, however, remains the most reliable test for diagnosis of brucellosis especially in patients with chronic and complicated stages of the disease.

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INTRODUCTION

Agglutination tests on slides, cards, or plates have been used as simple and rapid techniques for screening human and animal sera and human cerebrospinal fluid (CSF) for the presumptive diagnosis of brucellosis (Davies, 1971; Diaz, Maravi-Poma & Rivero, 1976; Diaz *et al.* 1978; Russell, Patton & Kaufmann, 1978; Araj *et al.* 1986*a*, *b*). Several such tests are commercially available. The most commonly used in Europe and the Middle East include the Brucelloslide test (bioMérieux, France), which uses *Brucella abortus* cells stained with Rose Bengal (RB) as antigen, and the slide test with *B. melitensis* and *B. abortus* stained antigen from Wellcome Diagnostics (Temple Hill, Dartford, England), Oxoid (Basingstoke, Hampshire, England) and Baltimore Biological Laboratory (BBL, Cockeysville, Maryland, USA). The efficacy of these tests vary as reported in limited studies (FAO/WHO, 1971; Diaz, Maravi-Poma & Rivero, 1976; Diaz *et al.* 1978). In a study comparing counter-immunoelectrophoresis (CIE), Brucelloslide, plate agglutination and standard agglutination in tubes (SAT), the Brucelloslide test correlated very well with the SAT and appeared to be one of the most promising methods for supporting the diagnosis of clinical brucellosis (Diaz, Maravi-Poma & Rivero, 1976). Similar findings for the RB were also observed in a recent study that evaluated several tests including SAT, brucella-stained antigen (Wellcome), microagglutination test (MAT) and the enzyme-linked immunosorbent assay (ELISA) for the diagnosis of both acute and chronic brucellosis in humans (Araj *et al.* 1986*a*). Although ELISA was found to be the most reliable technique, the Brucelloslide showed comparable results to the MAT and significantly more reliable results compared to the other slide agglutination tests. In addition, no significant differences were observed among slide agglutination tests using brucella stained antigen from Wellcome, BBL, or Oxoid (G. F. Araj, unpublished observation).

The antigen for the Brucellosis Card (Card) test (Brewers' Diagnostic Kits) was originally developed by the United States Department of Agriculture, National Veterinary Services Laboratory, Ames, Iowa, for rapid screening of animal sera. The test is based on the agglutination reaction using a suspension of whole *B. abortus* cells (strain 1119–3) stained with Rose Bengal dye and buffered at pH 3·65 to inhibit non-specific agglutinins (Rose & Roepke, 1957; Corbel, 1972). In field trials, this Card test was found to be an accurate indicator of brucellosis in animals (Nicoletti, 1967; O'Reilly & Cunningham, 1971; Davies, 1972). However, only a few studies have been reported about its use in the diagnosis of human disease (Nicoletti & Fadai-Ghotbi, 1971; Buchnan *et al.* 1974; Russell, Patton & Kaufmann, 1978). The Card test was found to be satisfactory in the diagnosis of patients with acute brucellosis but there clearly remained a need for further investigation to assess its use for the diagnosis of chronic brucellosis.

Characterization of the class(es) of immunoglobulins (Ig) involved in the agglutination reaction of the Card test has not been completely determined. A study in bovine sera reported that the antigen in the Card test reacted only with IgG (Corbel, 1972). Other studies reported reactions with IgG, IgM and IgA both in sera from bovines (Lambert & Amerault, 1962; Levieux, 1974; Patterson Deyoe & Stone, 1976) and from humans (Heremans, Vaerman & Vaerman, 1963;

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Wilkinson, 1966; Diaz, Maravi-Poma & Rivero, 1976; Russell, Patton & Kaufmann, 1978).

This study compares the use of the Card test with the Brucelloslide, MAT and ELISA tests in detecting brucella specific antibodies in sera from patients with acute and chronic brucellosis. It also reports on the class(es) and titres of immunoglobulin(s) involved in the Card reaction.

MATERIALS AND METHODS

Patients' sera. The study was carried out on 573 serum specimens from separate individuals living in Kuwait. The sera were collected at the Medical Department of Jahra Hospital from patients diagnosed as having or having had brucellosis and from controls. All sera were stored at −20 °C until tested. The patients and controls were placed in groups on the basis of comprehensive clinical assessment, epidemiological information and laboratory findings as previously reported (Araj *et al.* 1986*a*; Lulu *et al.* 1988). Patients were considered to have acute brucellosis when they showed appropriate signs and symptoms and had a history of illness of < 2 months duration. All sera from these patients showed either an MAT titre $\geqslant 160$, a fourfold rise, or, in the ELISA test elevated brucella specific IgM alone ($\geqslant 800$), or IgG ($\geqslant 1600$) together with IgM ($\geqslant 400$) and IgA ($\geqslant 200$) as previously reported (Araj *et al.* 1986*a*). Patients were considered to have chronic brucellosis when signs and symptoms persisted for more than a year with either a MAT titre of $\geqslant 80$, or an ELISA IgG titre of $\geqslant 1600$ alone, or together with IgA titre of $\geqslant 200$ (Araj *et al.* 1986*a*) and whose symptoms subsided within 3 months of a course of anti-brucella treatment. The patients and controls were grouped as:

Group 1: 159 patients with acute brucellosis of whom 83 had positive blood cultures for *B. melitensis*.

Group 2: 23 patients diagnosed as having chronic brucellosis.

Group 3: 52 patients with infectious diseases other than brucellosis from whom the following organisms had been isolated:- *Salmonella* sp. (10), *Escherichia coli* (10), *Haemophilus influenzae* (6) *Pseudomonas* sp. (6), *Klebsiella* sp. (6) *Streptococcus pneumoniae* (5), *Staphylococcus aureus* (5), *Listeria* sp. (4).

Group 4: 20 patients with non-infectious diseases which included diabetes (9 patients), rheumatoid arthritis (5), systemic lupus erythematosis (4) and leukaemia (2).

Group 5: 164 healthy adults who had been living in Kuwait for 3 years or more and who had no history of brucellosis or of the ingestion of raw milk or its products. Their MAT titres were all < 10.

Group 6: 155 stored serum specimens from follow-up patients who had had brucellosis, had been treated and were in remission. The sera were selected according to their MAT titres as follows: 75 had titres of < 10, 26 of 10, 22 of 20, 18 of 40 and 14 of 80. They were selected to determine the minimal titres in MAT and ELISA tests in relation to Card test positive reactions.

Brucella microagglutination test. The MAT was carried out on doubling dilutions of sera from 1:10 to 1:1280 as described earlier (Bettelheim, Maskill & Pearce, 1983) using *B. melitensis* tube agglutination antigen (courtesy of National Veterinary Services Laboratories, Ames, Iowa).

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Table 1. *Serological findings in patients with brucellosis and controls*

Study groups	No. of specimens tested	No. (%) positive in brucella test					
					ELISA†		
		Brucello-slide	Card	MAT*	IgG	IgM	IgA
Acute brucellosis							
Culture positive	83	83(100)	83(100)	83(100)	83(100)	83(100)	83(100)
Culture negative	76	76(100)	76(100)	76(100)	76(100)	76(100)	76(100)
Chronic brucellosis	23	14(61)	14(61)	14(61)	23(100)	7(32)	23(100)
Other infections	52	0	0	0	0	0	0
Non-infectious diseases	20	0	0	0	0	0	0
Healthy individuals	164	0	0	0	0	0	0

* MAT titres $\geqslant 80$.

† ELISA significant titres considered were IgG $\geqslant 1600$; IgM $\geqslant 400$; IgA $\geqslant 200$ (see ref. 1).

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Table 2. *Distribution of the 155 specimens from follow-up brucella patients according to MAT titres in relation to Brucelloslide and Card test results*

MAT titres	Total no. of specimens	No. (%) of specimens giving positive reactions in: Brucelloslide	Card
< 10	75	0	0
10	26	0	0
20	22	5 (23)	5 (23)
40	18	12*(67)	12 (67)
80	14	13 (93)	13 (93)

* One specimen was positive by Brucelloslide and negative by Card. One specimen was positive by Card and negative by Brucelloslide.

Brucelloslide test. The Brucelloslide test (lot no. 03408, bioMérieux) was carried out according to the instructions of the manufacturer.

Brucellosis card (*Card*) *test*. The Card test (Brewers' Diagnostic Kits) was provided by courtesy of the National Veterinary Services Laboratories, Ames, Iowa. The test was carried out according to the instructions of the manufacturer.

Culture and identification of microorganisms. Culture and identification of microorganisms from blood specimens were performed according to standard clinical microbiological procedures (Lennette *et al*. 1985). All patients had at least two pairs of blood samples cultured for brucella or other microorganisms. The brucella agar medium was supplemented with human plasma negative for brucella antibodies. The brucella cultures were reported as negative after 6 weeks of incubation.

Brucell ELISA test. The ELISA antibody titres of brucella specific IgG, IgM, and IgA in serum specimens were determined as previously described (Araj *et al*. 1986*a*). Briefly, immunoplates (Nunc, Denmark) were coated with heat-killed *B. melitensis* antigen followed by addition of twofold dilutions of serum specimens from 1:100 to 1:12800. Alkaline phosphatase-conjugated anti-human IgG, IgM or IgA (Sigma Chemical Co. St Louis, MO, USA) were added to the appropriate wells and the reaction indicated by addition of *P*-nitrophenyl phosphate (Sigma). The plates were read at 405 nm using a Titertek multiscan spectrophotometer (Flow laboratories, Scotland). The titres of each immunoglobulin class in each specimen was calculated using cut-off optical densities of two SD above the mean reading of control specimens.

RESULTS

A summary of the serologic results is presented in Table 1. All sera from patients with acute brucellosis were positive in all tests. In specimens from patients with chronic brucellosis only 14 out of 23 (61%) were positive in the Brucelloslide, Card, and MAT tests. The ELISA tests were positive in all sera from acute and chronic patients for IgG and IgA and in 7 of 23 (32%) for IgM. Sera from patients in the control groups were uniformly negative in all tests.

A comparison of the results for the Brucelloslide and Card tests in the 155

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Table 3. *Comparison of results of 155 serum samples in the Card and Brucelloslide tests in relation to the MAT test and ELISA tests for brucella-specific IgG, IgM and IgA titres*

MAT titres	Card or Brucello-slide results	No. of specimens	ELISA Ig	No. of specimens with ELISA titres						
				≤ 100	100	200	400	800	1600	≥ 3200
< 10	−	101	G	2	8	15	20	26	20	10
			M	19	20	30	23	8	1	—
			A	32	20	22	19	6	1	1
20	−	17	G	—	1	3	2	3	4	4
			M	1	—	6	5	5	—	—
			A	3	4	3	3	3	1	—
	+	5	G	—	—	—	1	2	2	—
			M	3	1	—	—	1	—	—
			A	—	—	—	1	1	—	3
40	−	6	G	—	—	—	1	4	—	1
			M	—	1	4	1	—	—	—
			A	1	1	2	1	1	—	—
	+*	10	G	—	—	—	1	3	1	5
			M	—	2	1	5	2	—	—
			A	—	—	4	4	1	—	1
80	−	1	G	—	—	—	—	—	1	—
			M	1	—	—	—	—	—	—
			A	—	—	—	—	1	—	—
	+	13	G	—	—	—	1	1	2	9
			M	—	2	2	4	5	—	—
			A	—	1	—	1	4	2	5

* Two specimens were excluded since one was positive by Brucelloslide and negative by the Card test while the other specimen was negative by the Brucelloslide and positive by the Card test. Both specimens were positive in ELISA IgG, IgM and IgA.

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Table 4. *Data on positive and negative Card tests in relation to brucella ELISA and MAT titres*

	Positive Card results with minimum ELISA titres for			Negative Card results with maximum ELISA titres for		
MAT	IgG	IgM	IgA	IgG	IgM	IgA
80	6400	400	200	—	—	—
	3200	200	400	—	—	—
	1600	400	200	—	—	—
	800	400	400	—	—	—
	400	1600	200	—	—	—
	400	400	400	—	—	—
	200	800	200	—	—	—
40	3200	400	200	1600	400	200
	3200	200	400	800	400	200
	1600	200	400	400	200	200
	800	400	400	200	400	100
	100	1600	100	100	800	< 100
20	400	400	400	1600	400	200
	—	—	—	800	200	< 100
	—	—	—	400	400	< 100
	—	—	—	400	200	200
10	—	—	—	3200	100	100
	—	—	—	1600	100	100
	—	—	—	800	200	400
	—	—	—	400	200	200

samples in relation to the MAT titres is presented in Table 2. The Brucelloslide and Card tests show similar results at all MAT titres except at the MAT titre of 40 where one specimen was positive by Brucelloslide and negative by Card test and another specimen was positive by Card and negative by Brucelloslide. All specimens with MAT titres $\leqslant 10$ were negative by both the Brucelloslide and Card tests. The number of samples positive on the Brucelloslide and Card test increased with increasing MAT titre. The distribution of ELISA titres for brucella-specific IgG, IgM and IgA in these specimens is presented in Table 3. All three antibody classes were present in the great majority of the specimens irrespective of the MAT, Card or Brucelloslide results. The ELISA titres tended to increase as the MAT titres increased.

Data comparing minimum ELISA titres of IgG, IgM and IgA with MAT titres and positive Card tests is presented in Table 4. Twenty-three per cent of the samples with MAT titres of 20 were Card test-positive and had ELISA titres of 400 each for brucella-specific IgG, IgM and IgA. Other samples that also had MAT titres of 20 but were Card test-negative had other combinations of ELISA Ig titres, generally higher IgG, the same or lower IgM and lower IgA titres.

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DISCUSSION

This study indicates that the Brucelloslide, MAT and the Card tests show comparable results when used for the detection of brucella-specific antibodies in sera from patients with acute or chronic brucellosis. The sensitivity in acute and chronic cases of brucellosis was 100 and 61% respectively. In addition, all tests in this study showed high specificity since none of them gave positive results with control specimens. Similar results have been reported for the Brucelloslide (Diaz, Maravi-Poma & Rivero, 1976; Diaz *et al.* 1978) and the Card tests (Russell, Patton & Kaufmann, 1978). Buchanan *et al.* (1974) found that 92% of 38 patients tested within 6 months after onset of clinical disease to be Card test-positive. Nicoletti & Fadai-Ghotbi (1971) tested serum from 236 humans suspected of having brucella infections and found that 55 of 56 (98·2%) standard tube agglutination-positive sera were also positive by the Card test and recommended wider utilization of the Card test in the diagnosis of acute brucella infection. However, they felt that further investigation of the Card test was necessary before being used in the diagnosis of patients with chronic brucellosis. Russell, Patton & Kaufmann (1978) reported a sensitivity of 95·3% and a specificity of 84·1% for the Card test as compared to SAT when testing a total of 1701 serum specimens from persons with various degrees of potential of exposure to brucella organisms or cross-reactive antigens. They also found the Card test to be negative when testing serum specimens from patients with tularaemia or who have been vaccinated for cholera.

The ELISA shows high sensitivity (100%) for detection of brucella-specific IgG, IgM and IgA antibodies in sera of patients with acute brucellosis and equally sensitive (100%) in detecting brucella-specific IgG and IgA in sera of patients with chronic brucellosis. These results confirm the previous reports in the evaluation of ELISA, slide, tube and MAT assays in the diagnosis of patients with acute and chronic brucellosis as well as those with CNS brucellosis (Araj *et al.* 1986*a*, *b*).

Possible false-positive and -negative results have been reported in the serological tests of brucellosis (Kerr *et al.* 1968; Araj *et al.* 1986*a*). The Card test, as with the other serologic tests, remains positive for a variable period of time after patients have been treated and cured of brucellosis. Thus, caution is warranted in interpreting these tests in relation to the patient's disease history since the long-term persistence of antibody could lead to over-diagnosis. False-negative results have also been encountered in agglutination tests especially at the early stages of the disease where antibodies have not been produced in sufficient amount for the agglutination reaction to become positive. Moreover, blocking antibodies or a prozone effect resulting from the use of undiluted serum may contribute to the false-negative findings and could compromise the sensitivity and value of these agglutination tests especially when used as screening tests (Kambal *et al.* 1983). These false-negative results have been overcome by the use of ELISA (Sippel, El-Masry & Farid, 1982; Araj *et al.* 1986*a*, *b*) or the immune radiometric assay technique (Parratt *et al.* 1977).

The variation in results obtained with different tests may be attributed to the involvement either of different classes of Ig or of different components of the brucella antigen or both (Diaz, Maravi-Poma & Rivero, 1976). These factors,

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together with tne class and level of Ig, were found to be important in the diagnosis of brucellosis (Araj *et al.* 1986*a*). From the present study it appears that a brucella-specific IgM ELISA titre of at least 1600 or an IgM titre of 800 in combination with an IgG and IgA titre of $\geqslant 200$ is necessary before the Card test becomes positive. If the IgM titre is $\leqslant 200$ or absent, a higher IgG titre ($\geqslant 1600$) in combination with a higher IgA titre ($\geqslant 400$) is necessary. These findings are consistent with those which reported that the Card test detected the three Ig classes; IgG, IgM and IgA and were thus similar to those in agglutination tests (Hermans, Vaerman & Vaerman, 1963; Wilkinson, 1966). In addition, IgM alone at appropriate concentration could mediate the agglutination reaction in the Card and Brucelloslide tests as reported earlier (Diaz, Maravi-Poma & Rivero, 1976). Moreover, as shown in the present study IgG and IgA in sufficient concentration could mediate the agglutination reaction which is contrary to the findings of Corbel (1972) that the Card test antigen reacts only with IgG antibodies.

In conclusion, the Card test is as sensitive as the Brucelloslide and MAT tests in detecting brucella-specific antibodies in the sera of patients with acute and chronic brucellosis. This test has a potential value as a rapid technique for screening human sera for the investigation of patients suspected of having acute brucellosis. However, the more sensitive ELISA test should be the method of choice for the diagnosis of brucellosis especially in chronic and complicated stages of the disease.

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