

## SUITABILITY OF THE MICROAGGLUTINATION TEST FOR DETECTION OF POST-INFECTION AND POST-VACCINATION Q FEVER ANTIBODIES IN HUMAN SERA

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*Summary.* — Serological examination by the microagglutination (MA) and complement-fixation (CF) tests of human sera collected before and after vaccination with Q fever chemovaccine revealed a higher sensitivity of the MA test for detecting both pre-vaccination antibodies reflecting a previous exposure to Q fever and post-vaccination antibodies reflecting vaccine immunogenicity. In persons serologically positive before vaccination the level of post-vaccination MA antibody response was indirectly proportional to the titres of prevaccination MA antibodies.

*Key words:* Q fever; man; infection; vaccination; serological tests

### Introduction

Of the battery of serological reactions available for diagnosis of rickettsial diseases (Newhouse *et al.*, 1978; Úrvölgyi, 1978), the MA and CF tests are the most commonly used in Q fever studies. The present paper compares the sensitivity of the MA and CF tests for detection of post-infection and post-vaccination Q fever antibodies in human sera collected during field vaccination against Q fever in Central Slovakia (Brezina *et al.*, 1981).

### Materials and Methods

Human sera were collected before and after vaccination with Q fever chemovaccine prepared as described (Brezina and Úrvölgyi, 1961). One vaccine dose represented an amount of soluble material extracted by trichloroacetic acid from 1 mg of dried highly purified phase I *Coxiella burnetii* organisms and dissolved in 1 ml of physiological saline (Brezina *et al.*, 1974). Some persons were given a second dose of the vaccine two weeks apart. Post-vaccination antibody response was determined three weeks after the second vaccine dose, i.e. 5 weeks after the first vaccine dose.

The sera were examined by the warm method of the CF test (Brezina *et al.*, 1970) and by the MA test according to Fiset *et al.* (1969) with artificial phase II *C. burnetii* antigen prepared by potassium periodate treatment of purified phase I *C. burnetii* suspensions (Schramek *et al.*, 1972). The sera were inactivated at 56 °C for 30 min before examined by the CF test; in the MA test non-inactivated sera were used. Sera with antibody titres of  $\geq 4$  in either test were considered positive.

### Results

#### *Comparison of MA and CF tests for detection of Q fever antibodies in human sera before vaccination*

Altogether 802 human sera collected before vaccination were examined to compare the sensitivity of the MA and CF tests for detection of pre-vaccination Q fever antibodies (Table 1). Of them, 160 sera (20.0 %) were positive in the MA test, but only 42 sera (5.2 %) reacted positively in the CF test. Among sera positive by the CF test, 13 (30.9 %) were negative by the MA test, but of those reacting positively in the MA test as many as 131 (81.9 %) were negative by the CF test. Conformity in positivity (3.6 %) and negativity (78.4 %) of both tests used was found in 82.0 % of all sera tested.

**Table 1. Comparison of the MA and CF tests for detection of Q fever antibodies in human sera before vaccination**

No. (%) of sera examined in the CF test	No. (%) of sera examined in the MA test		
	Positive	Negative	Total
Positive	29 (3.6%)	13 (30.9%)*	42 (5.2%)
Negative	131 (81.9%)**	629 (78.4%)	760
Total	160 (20.0%)	642	802 (100.0%)

\*Proportion related to the total number of sera positive in the CF test.

\*\*Proportion related to the total number of sera positive in the MA test.

The higher sensitivity of the MA than the CF test was evident also on comparing the levels of pre-vaccination antibodies (Table 2). Among sera positive by both tests, higher antibody titres were detected by the MA test in 20 sera, but by the CF test only in 2 sera. The same antibody titres by either test were found in 7 sera. The geometric mean titre of MA antibodies was 13, that of CF antibodies was 7.

**Table 2. The levels of pre-vaccination antibodies as detected by the MA and CF tests**

MA antibody titres	CF antibody titres							
	< 4	4	8	16	32	64	128	256
256	4	1		1			1	
128	1			1				
64	2				1			
32	9	3	1	2	2			
16	55	5	3	1				
8	29	1		1				
4	31	4	1					
< 4	629	8	4	1				

The figures refer to the number of sera with the given antibody titres.

*Serological conversion and antibody levels after vaccination of humans with one or two doses of Q fever chemovaccine as detected by the MA and CF tests*

When the MA and CF tests were compared for their sensitivity to detect post-vaccination antibodies to *C. burnetii*, the MA test revealed more positive sera (Table 3) with higher antibody titres (Tables 4 and 5) than the CF test. Of 105 sera collected after one vaccine dose, 74 (70.5 %) were positive by the MA test and 66 (62.8 %) by the CF test. Of 110 sera collected after two vaccine doses, serological conversion was demonstrated in 85 (77.3 %) by the MA test and in 72 (65.5 %) by the CF test. Though the proportion of negative sera was lower after two vaccine doses (16.4 %) than after one vaccine dose (24.8 %), vaccination with two doses of the vaccine did not substantially influence the proportion of sera positive by one test or another.

**Table 3. Serological conversion after vaccination of humans with one or two doses of Q fever chemovaccine as detected by the MA and CF tests**

No. of vaccine doses	No. of sera tested	No. (%) of sera positive by the MA test	No. (%) of sera positive by the CF test	No. (%) of negative sera
One	105	74 (70.5%)	66 (62.8%)	26 (26.8%)
Two	110	85 (77.3%)	72 (65.5%)	18 (16.4%)

A difference in the sensitivity of the MA and CF tests for detection of post-vaccination antibodies was more marked when antibody levels after one or two doses of the vaccine were compared. As shown in Table 4, after vaccination with one dose the MA test revealed higher antibody titres than the CF test in 68 sera, but the CF test only in 2 sera, and in 4 sera the same antibody titres were found. Besides that 13 sera positive by the MA test were negative in the CF test, but the reverse was true only for 5 sera. The geometric mean titre of MA antibodies was 69 and that of CF antibodies 20. A similar difference was observed after vaccination with two doses (Table 5),

**Table 4. Comparison of antibody titres determined by the MA and CF tests after vaccination with one vaccine dose**

MA antibody titres	CF antibody titres							
	<4	4	8	16	32	64	128	256
256			1	3	2	4		
128			1	3	4	2	1	
128			1	3	4	4	2	1
64	3	2		13	8		1	
32	6	3	2	3	1			
16	2	2		1				
8	2							
4								
<4	26	2		3				

The figures refer to the number of sera with the given antibody titres.

**Table 5. Comparison of antibody titres determined by the MA and CF tests after vaccination with two vaccine doses**

MA antibody titres	CF antibody titres							
	< 4	4	8	16	32	64	128	256
256			1	4	7	2		
128	6	1		2	4	3	1	
64	1	2	4	3	4	4	1	
32	4	3	3	4	2			
16	8	4	4	1				
8	1	1						
4								
< 4	18	3	3		1			

The figures refer to the number of sera with the given antibody titres.

i.e. higher antibody titres were detected by the MA test in 56 sera, by the CF test in one serum, and the same antibody titres by either test in 8 sera. Moreover, positivity by the MA test was demonstrated in 20 sera negative by the CF test, but only 5 sera positive by the CF test were negative in the MA test. The geometric mean titre was lower for both MA antibodies (56) and CF antibodies (18) than after vaccination with one dose.

*Relationship between the levels of pre-vaccination and post-vaccination agglutinating antibodies*

To determine whether a previous response to Q fever may influence the post-vaccination antibody response, 56 sera with titres of pre-vaccination agglutinating antibodies varying from 2 to 256 were examined by the MA test 5 weeks after vaccination with one dose of the vaccine. As follows from Table 6, the higher the level of pre-vaccination agglutinating antibodies, the lower an increase in MA antibody titres after vaccination.

**Table 6. Relationship between the levels of pre-vaccination and post-vaccination agglutinating antibodies**

Increase in titres after vaccination (-fold)	MA antibody titres before vaccination							
	2	4	8	16	32	64	128	256
128	1							
64	1	1						
32		5	2					
16	1	6	2	3				
8		2		2				
4		1	2	1	3			
2				5	2			
1				2	6	3	1	4

The figures refer to the number of sera with the given antibody titres.

*Discussion*

Serological diagnosis of Q fever is based mainly on the results of various modifications of agglutination and CF tests with natural or artificial phase II *C. burnetii* antigens (Úrvölgyi, 1978). To determine the suitability of one test or another, several aspects, namely the sensitivity of the test and the economy and ease of its performance must be borne in mind.

The agglutination tests are considered more suitable than the CF tests for early diagnosis of Q fever, but opinions concerning the persistence of agglutinating and CF antibodies are controversial. As to the MA test, its superiority to the CF test for detection of an early antibody response was proved for human and various animal sera (Fiset *et al.*, 1969; Kazár *et al.*, 1977; Řeháček *et al.*, 1978; Úrvölgyi and Brezina, 1978), but results differed when the levels of MA and CF antibodies in animal sera at later intervals post infection with *C. burnetii* were compared (Kazár *et al.*, 1977; Úrvölgyi and Brezina, 1978). In a field study in Central America (Peacock *et al.*, 1971), the MA test gave approximately similar results as the CF tests, but CF antibodies persisted at detectable levels longer than agglutinins. In other studies (Brezina *et al.*, unpublished data; Tokarevich *et al.*, 1979) agglutinins were detected along with CF antibodies in human sera as late as 20 years after *C. burnetii* infection, but the possibility of a repeated exposure to *C. burnetii* should be taken into account. The different results could be explained partly by differences in the antigenic preparations used and by inactivation of sera for the MA test in some studies.

In the present study, the MA test proved to be more sensitive than the CF test as evidenced by a higher proportion of positive sera and higher antibody levels detected. This was true not only for post-vaccination sera with a known interval of antigenic exposure, but also for sera collected before vaccination, in which an interval between exposure to infection and serological examination was unknown. The latter interval could have varied from months to years, because the natural focus of Q fever and first human cases in the area under study were recognized as early as in the 1960's (Řeháček *et al.*, 1970; Palanová *et al.*, 1978). No substantial difference in antibody response as measured by the CF and MA tests was observed, whether the vaccinees were given one or two vaccine doses. Post-vaccination antibody response was influenced, however, by the level of pre-vaccination antibodies.

One may object that the MA and CF tests do not measure the same type of antibodies. This theoretical objection is not very important from the practical point of view, namely in field studies, in which a rapid assessment of a previous exposure to Q fever and post-vaccination serological conversion is required. Advantages of the MA test (economy of time in performing and reading the test, and economy of reagents used) were already stressed by Fiset *et al.* (1969) who introduced this test. Since the MA test is easy to perform also in field conditions and because of its sensitivity for detecting both post-infection and post-vaccination antibodies, we recommend its use in field studies on Q fever.

## References

- Brezina, R., and Úrvölgyi, J. (1961): Extraction of *Coxiella burnetii* phase I antigen by means of trichloroacetic acid. *Acta virol.* **5**, 193.
- Brezina, R., Pospíšil, V., and Schramek, Š. (1970): Study of the antigenic structure of *Coxiella burnetii*. VII. Properties of phenol-extracted phase I antigenic component. *Acta virol.* **14**, 295–301.
- Brezina, R., Schramek, Š., Kazár, J., and Úrvölgyi, J. (1974): Q fever chemovaccine for human use. *Acta virol.* **18**, 269.
- Brezina, R., Kazár, J., Palanová, A., Tvrďá, B., and Schramek, Š. (1981): Očkovanie proti Q horúčke profesionálne exponovaných ľudí v okrese Veľký Krtíš. *Čs. Epidem.* **30**, 1–9.
- Fiset, P., Ormsbee, R. A., Silberman, R., Peacock, M., and Spielman, S. H. (1969): A microagglutination technique for detection and measurement of rickettsial antibodies. *Acta virol.* **13**, 60–66.
- Kazár, J., Schramek, Š., and Brezina, R. (1977): Analysis of antibody response and immunoglobulins in sera of rabbits and guinea pigs immunized with *Coxiella burnetii*. *Acta virol.* **21**, 246–255.
- Newhouse, V. F., Redus, M. A., Tzianabos, T., Shepard, C. C., and McDade, J. E. (1978): Toward more standard methods of serological diagnosis of rickettsial diseases, pp. 373–381. In J. Kazár, R. A. Ormsbee and I. N. Tarasevich (Eds): *Rickettsiae and Rickettsial Diseases*, Veda, Bratislava.
- Palanová, A., Řeháček, J., and Brezina, R. (1978): Epidemiology of Q fever in Slovakia, pp. 435–441. In J. Kazár, R. A. Ormsbee and I. N. Tarasevich (Eds): *Rickettsiae and Rickettsial Diseases*, Veda, Bratislava.
- Peacock, M. G., Ormsbee, R. A., and Johnson, K. M. (1971): Rickettsioses in Central America. *Am. J. trop. Med. Hyg.* **20**, 941–949.
- Řeháček, J., Áč, P., Brezina, R., and Majerská, M. (1970): Q fever investigation in Slovakia. I. Isolation of the agent from ticks and serological surveys in small mammals in the districts of Zvolen and Lučenec, Central Slovakia Region. *J. Hyg. Epidem. (Praha)* **14**, 230–239.
- Řeháček, J., Úrvölgyi, J., Brezina, R., Kazár, J., and Kováčová, E. (1978): Experimental infection of hare (*Lepus europaeus* L.) with *Coxiella burnetii* and *Rickettsia slovaca*. *Acta virol.* **22**, 417–425.
- Schramek, Š., Brezina, R., and Úrvölgyi, J. (1972): A new method of preparing diagnostic Q fever antigen. *Acta virol.* **16**, 487–492.
- Tokarevich, N. K., Druganova, L. P., Daiter, A. B., Amosenkova, N. I., Terentyev, V. F., and Charikova, A. A. (1979): Combined use of serological tests for diagnosis and study of Q fever (in Russian). *Zh. Mikrobiol. (Mosk.)* **1979** (12), 90–95.
- Úrvölgyi, J. (1978): New achievements in serological diagnosis of rickettsial diseases, pp. 361–371. In J. Kazár, R. A. Ormsbee and I. N. Tarasevich (Eds): *Rickettsiae and Rickettsial Diseases*, Veda, Bratislava.
- Úrvölgyi, J., and Brezina, R. (1978): Comparison of different antigenic preparations of *Coxiella burnetii* used for antibody detection in guinea pigs. *Acta virol.* **22**, 491–496.