Epidem. Inf. (1987), **99**, 393–398
Printed in Great Britain

Serodiagnosis of leptospirosis in China by the one-point MCA method

By Y. ARIMITSU,* T. MATUHASI

Department of Applied Immunology, National Institute of Health, Tokyo, Japan

S. KOBAYASHI

Asaka Research Laboratories, Fuji Photo Film Co. Ltd Japan

T. SATO

Department of Research, Japan Lyophilization Laboratories, Tokyo, Japan

AND J. J. CUI

Chengdu Institute of Biological Products, China

(Accepted 27 March 1987)

SUMMARY

The one-point MCA method is very simple to perform and useful as a screening test in diagnosing leptospirosis in routine clinical laboratories.

The kit, sensitized with six serovars occurring in Japan, was also useful in detecting serum antibodies of patients with leptospirosis in China.

INTRODUCTION

The microcapsule agglutination (MCA) test has been developed to provide a simple method for the diagnosis of leptospirosis in clinical laboratories. This test utilizes chemically stable microcapsules (MC) on which are adsorbed leptospiral antigen replacing the use of sheep erythrocytes (Arimitsu *et al.* 1982).

We have developed the one-point MCA test further by using a single dilution of test serum. Because the technique is very simple and requires no special skill, the test can be easily used in diagnostic laboratories as a routine test for the diagnosis of leptospirosis.

Leptospirosis is prevalent in most parts of China; the disease has been observed commonly in rice-growing areas and frequently among rice farmers during the harvest season. In 1958, a large epidemic of leptospirosis involving 10000 people broke out at Sichuan Province in China. *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar *lai* was originally isolated from a patient.

At present, 13 serogroups and 59 serovars have been identified in China. The serogroup Icterohaemorrhagiae is the most common, and the serovar *lai* of this serogroup is the most prevalent infecting organism existing in the areas along the Yangtze River valley (Chen, 1985).

* Correspondence should be addressed to Dr Y. Arimitsu, Department of Applied Immunology, National Institute of Health, Kamiosaki 2-10-35, Shinagawaku, Tokyo 141, Japan.

In order to determine whether the one-point MCA method sensitized with multiple antigens may be used outside Japan, we applied this test for the detection of leptospiral antibodies in human serum samples from Sichuan Province in China. The MC antigens involved the following six serovars which have been commonly used in Japan: L. Icterohaemorrhagiae serovar *icterohaemorrhagiae*, L. Autumnalis serovar *autumnalis*, L. Hebdomadis serovar *hebdomadis*, L. Australis serovar *australis*, L. Canicola serovar *canicola* and L. Pyrogenes serovar *pyrogenes*.

MATERIALS AND METHODS

Leptospira serovars used for the microscopic agglutination (MA) test

The following six serovars were used in the MA test. L. Icterohaemorrhagiae serovar *lai*, L. Autumnalis serovar *autumnalis*, L. Hebdomadis serovar *hebdomadis*, L. Australis serovar *australis*, L. Canicola serovar *canicola*, and L. Pyrogenes serovar *pyrogenes*. All serovars except L. Australis serovar *australis* were obtained from Chengdu Institute of Biological Products. They were cultivated in Korthof medium containing 10% rabbit serum at 32 °C.

Sera

A total of 284 samples were obtained from Chengdu Institute of Biological Products in China. According to the results of tests carried out in Chengdu, 28 specimens were positive by the MA test and 11 doubtful, and 101 specimens were obtained from clinically unknown patients and 144 from healthy individuals. All sera were inactivated at 56 °C for 30 min.

One-point MCA test

Two reagents A and B were prepared by the method described previously (Seki *et al.* 1987).

Reagent A was sensitized with L. Icterohaemorrhagiae serovar *icterohaemorrhagiae*, L. Autumnalis serovar *autumnalis* and L. Hebdomadis serovar *hebdomadis*. Reagent B was sensitized with L. Australis serovar *australis*, L. Canicola serovar *canicola* and L. Pyrogenes serovar *pyrogenes*.

Test procedure

A 0·25 ml sample of diluent solution (1% bovine serum albumin-PBS, pH 7·2) was distributed into two disposable test tubes for each serum and added with 1 μl of serum specimen in each tube by using a loop and mixed well. Two drops (one drop 0·025 ml) of the A or B reagent were added into one of the two tubes respectively using a dropper and mixed well. The tubes were placed in a test rack with a horizontal mirror, avoiding any vibration. The agglutination patterns of the bottom were observed on the next day.

MA test

This was carried out by a modification of the Schüffner–Mochtar MA method (Galton *et al.* 1965). The titre was expressed as the highest dilution of the antiserum which showed 50% microscopic agglutination. Living antigens were used.

Table 1. *One-point MCA and MA tests on sera clinically and serologically diagnosed as leptospirosis*

Human sera no	One-point MCA		MA titres					
	A*	B†	L. *aust.*	L. *autum.*	L. *cani.*	L. *hebdo.*	L. *lai*	L. *pomona*
1	+	+	·	80	·	·	80	·
2	+ + +	+ + +	·	20	·	·	80	·
3	+ + +	+ + +	40	320	80	·	320	·
4	+ + +	+	·	80	160	·	160	·
5	+ + +	+ + +	·	40	160	·	320	·
6	+ + +	+ + +	·	160	40	·	160	·
7	+	+ + +	·	·	·	·	40	·
8	+ + +	+ + +	40	160	80	·	160	·
9	+ + +	+ + +	·	160	80	·	320	·
10	+ + +	+ + +	40	80	80	·	160	·
11	+ + +	+ + +	·	160	40	·	320	·
12	+ + +	+ + +	·	320	40	·	320	·
13	+ + +	+ + +	·	80	40	·	160	·
14	+ + +	+ + +	·	80	80	20	80	·
15	+ + +	+ + +	40	320	320	·	320	·
16	+	±	·	20	20	·	40	·

* A contains L. *icterohaemorrhagiae*, L. *autumnalis* and L. *hebdomadis*.
† B contains L. *australis*, L. *canicola* and L. *pyrogenes*.

Table 2. *One-point MCA and MA tests on sera from patients clinically diagnosed as leptospirosis*

Human sera no.	One-point MCA		MA titres					
	A*	B†	L. *aust.*	L. *autum.*	L. *cani.*	L. *hebdo.*	L. *lai*	L. *pomo.*
1	+ + +	+ + +	·	20	·	·	40	·
2	·	·	·	·	·	·	·	·
3	·	·	·	·	·	·	·	·
4	·	·	·	·	·	·	·	·
5	±	±	·	·	·	·	·	·
6	+ + +	+ + +	80	·	20	·	40	·
7	·	·	·	·	·	·	·	·
8	±	±	·	·	·	·	·	·
9	+	+	·	20	·	·	·	·
10	+ + +	+ + +	20	20	20	·	20	·
11	·	·	·	·	·	·	·	·

* A contains L. *icterohaemorrhagiae*, L. *autumnalis* and L. *hebdomadis*.
† B contains L. *australis*, L. *canicola* and L. *pyrogenes*.

Table 3. *Agreement of* 101 *diagnstic sera by one-point MCA and MA tests*

Positive by both tests	Negative by both tests	Positive by MA only	Positive by MCA only
34	50	13	4
33·7%	49·5%	12·9%	9%

https://doi.org/10.1017/S095026880006787X Published online by Cambridge University Press

Fraction of serum specimens by gel filtration

Serum (0·35 ml) was fractionated on a Sephacryl S-300 (Pharmacia Fine Chemicals, Sweden) column with a length of 90 cm and a diameter of 1·6 cm. Elution was performed with 0·15 mol/l phosphate-buffered saline (PBS). pH 7·2; 2·5 ml fractions were collected.

Optical absorption at 280 nm of eluted fractions showed three peaks corresponding to IgM, IgG and albumin respectively.

RESULTS

Sixteen of the 28 leptospiral positive sera which were indicated by one-point MCA tests carried out in Chengdu were examined by both one-point MCA and MA tests in the N.I.H. of Japan. All 16 sera were positive, as shown in Table 1. Thirteen out of 16 sera showed antibodies to L. Icterohaemorrhagiae serovar *lai*, L. Autumnalis serovar *autumnalis* and L. Canicola serovar *canicola*.

Eleven doubtful sera from the patients who were diagnosed only on their clinical symptoms were tested in the N.I.H. by one-point MCA and MA tests. The results of the two tests were in complete agreement, as shown in Table 2; four were positive and seven were negative.

Out of 101 sera from the patients lacking definite clinical information, 34 were positive by both tests, 50 were negative by both tests and 17 showed different results by the two methods; 4 were negative in the MA test but positive in the one-point MCA test, and 13 were positive in MA but negative in MCA. Level of agreement between the results of MA (positive at more than 10) and MCA was 83·2% (Table 3). Six of the 13 sera, which were positive by the MA test but negative by the one-point MCA test, were fractionated by gel filtration, and the MA test performed on IgM and IgG. However, the MA test failed to detect antibody in either fraction.

Sera from 144 healthy human beings were examined by both tests as the control. The results of the two tests showed a considerable disagreement; 65 sera were positive by the MA test, showing titres ranging from 20 to 80, but negative by the one-point MCA test. We later observed that sera from vaccinated people were included. The MA test could not detect antibodies in both fractions, even though the serum specimens showing high titre (80) were fractionated by gel filtration. On the other hand, the IgM fraction of a doubtful serum (no. 1) which showed MA titre of 40 to L. Icterohaemorrhagiae serovar *lai* was positive (4) by one-point MCA test but negative by MA test.

DISCUSSION

In 1958, a large explosive epidemic of leptospirosis involving 10000 people occurred at Sichuan Province in China. Since then, research on leptospirosis and vaccine has been undertaken. At present, human leptospirosis has been brought under control in most parts of China, except in a few provinces where systematic surveys have not been done.

Fieldmice (*Apodemus agrarius*) are heavily infected with leptospira, showing an

overall infection rate of 14·1% with L. Icterohaemorrhagiae serovar *icterohaemorrhagiae*. Leptospirosis is widely distributed, particularly in the southern parts of China; mass infection or sporadic cases frequently occur in rice farmers in the harvest season from July to October. Millions of farmers are immunized with vaccine every year in Sichuan Province (Chen, 1985). Because of this, it can be assumed that the antibody pattern of sera from Chinese people will be rather more complicated than in Japan. The sera from healthy volunteers used as controls contained many sera from vaccinated people who are often re-vaccinated at yearly intervals. This should be considered in the analysis of healthy sera used as controls as well as sera from some clinically unknown patients. It is reasonable that many healthy sera which were used as negative control showed MA titres of 40–80.

However, MA antibodies could not be detected in all the IgM fractions and most of the IgG fractions obtained from these sera by gel filtration, perhaps because the titre of the material was low. A low titre was detected in only a few IgG fractions. On the other hand, in a patient's serum which showed an MAT titre of 40, the MA test was negative on both IgM and IgG fractions but the MCA test was positive at a titre of 4 on the IgM fraction. Positive sera when tested by the one-point MCA test were usually obtained from the patients during the acute phase of the infection. It is presumed that in the patients showing positive MA results but negative in the one-point MCA, the pattern of antibody has shifted to IgG production over time, or that the patients had been vaccinated. Another problem which should be considered in such analysis is that there may be many cases with multiple leptospiral infections in China; this is rare in Japan.

At present, the one-point MCA reagents sensitized with six serovar antigens which are commonly used in Japan, have proved also to be quite useful to detect antibodies from leptospiral patients in China.

It is of interest to note that only four of 11 sera from patients who were diagnosed only on clinical grounds were true leptospirosis confirmed by laboratory tests and they were clearly recognized by the one-point MCA test. It is very difficult to confirm leptospirosis on clinical symptoms alone and laboratory tests are necessary.

The serological diagnosis of leptospirosis depends on the use of a live MA test. However, the method is tedious, live pathogenic leptospires are difficult to maintain and therefore it is not as useful a routine test in diagnostic laboratories. The one-point MCA test is very simple and easy to perform. It is also a relatively genus-specific test, and the kit sensitized with the six serovars occurring in Japan could be used to detect satisfactory antibodies of patient's sera with L. Icterohaemorrhagiae serovar *lai* in China. The results suggest that the kit could be used more routinely in other areas of the world.

The authors are deeply indebted to Dr R. Murata, honorary member of the National Institute of Health, for his useful advice in preparing the manuscript.

REFERENCES

ARIMITSU, Y., KOBAYASHI, S., AKAMA, K. & MATUHASI, T. (1982). Development of a simple serological method for diagnosing leptospirosis: a microcapsule agglutination test. *Journal of Clinical Microbiology* **15**, 835–841.

CHEN, T. (1985). Development and present status of leptospiral vaccine and technology of vaccine production in China. *Japanese Journal of Bacteriology* **40**, 755–762.

GALTON, M. M., SULZER, C. R., SANTA ROSA, C. A. & FIELDS, M. J. (1965). Application of a micro-technique to the agglutination test for leptospirosis antibodies. *Applied Microbiology* **13**, 81–85.

SEKI, M., SATO, T., ARIMITSU, Y., KOBAYASHI, S. & MATUHASI, T. (1987). One-point method for serological diagnosis of leptospirosis: a microcapsule agglutination test. *Epidemiology and Infection* **99**, 399–405.