

INFLUENCE OF TEMPERATURE, INCUBATION TIME, ANTIGEN DENSITY AND AGE OF THE CULTURE IN AGGLUTINABILITY OF LEPTOSPIRES

Núbia Seyffert*; Éverton Fagonde da Silva; Viviane Lorenzato Bermudez; Claudiomar Soares Brod

¹Centro de Controle de Zoonoses, Faculdade de Medicina Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brasil.

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ABSTRACT

In leptospirosis, the Microscopic Agglutination Test (MAT) is the basis of serological diagnosis and the most widely used. In our study, the MAT was applied to evaluate the influence of temperature, incubation time, antigen density and age of the culture in the agglutinability of Leptospire and the interference of these factors in diagnosis. Three serum samples with titers of 100, 800 and 25.600 to the serovar tande (local isolate) of canicola serogroup were used with different combinations among the four factors. There was a significant relation among all factors, specially between the antigen density and age of the culture.

Key words: *Leptospira*, microscopic agglutination, Leptospirosis, laboratorial diagnosis.

INTRODUCTION

Leptospirosis is an economically important zoonosis occurring worldwide in animals and humans (5). There is no sensitive, specific, low-cost, rapid and widely available diagnostic test for leptospirosis. Since 1926, a variety of tests has been developed for serodiagnosis of Leptospirosis (2,4,7). The MAT is the serological procedure most often used for the diagnosis of leptospirosis, but it is difficult to standardize (6). The MAT is the "gold standard" procedure for detecting leptospiral antibodies according to the Brazilian Ministry of Health.

MATERIALS AND METHODS

In our experiment, 3 serum samples with MAT titers of 100, 800 and 25600 against tande serovar were used. The antigen used had three different ages: 3, 10 and 28 days old and three different densities: 2×10^8 , 4×10^8 and 6×10^7 leptospiral organisms/mL, determined in a Petroff Hausser chamber. The sera were diluted in phosphate-buffered saline pH 7.2, starting at 1:12.5, and mixed with an equal volume of antigen (6). The

mixtures were incubated at 4°C, 30°C and 37°C and the agglutination was read after 15, 30, 60, 120, 180, 240, 300, 360 and 1440 minutes, by dark-field microscopy. The reported titer was the reciprocal of the highest dilution that agglutinated at least 50% of the leptospire, when compared to the buffer control (6).

RESULTS

After 594 readings the reactions with highest precocity followed in Figs. 1, 2 and 3.

All sera achieved 100% agglutination in 60 min. The sera with the highest titers required longer incubation time, regardless their age, density and temperature. At 37°C, there was desagglutination after 1440 minutes. At 4°C there was net agglutination since the first minutes of the reaction.

DISCUSSION

Many papers report comparative studies of factors that interfere in MAT. In 1926, Schuffner and Mochtar (3) described

*Corresponding author. Mailing address: Centro de Controle de Zoonoses, Faculdade de Medicina Veterinária, Universidade Federal de Pelotas. Campus Universitário, s/nº, Caixa Postal 354. 96010-900, Pelotas, RS, Brasil. E-mail: nbsey@bol.com.br

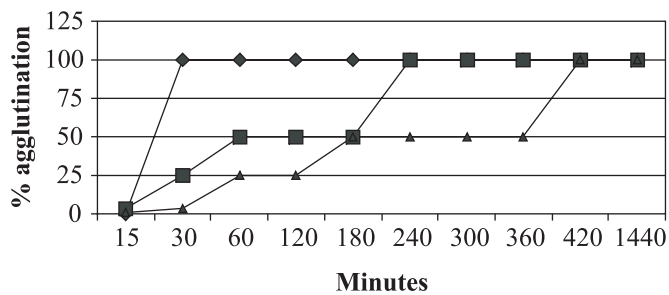


Figure 1. Percentage of agglutination of three sera (titers 100; 800 and 25600) incubated at 4°C, using three days old leptospirae and density of 6×10^7 leptospirae/mL.

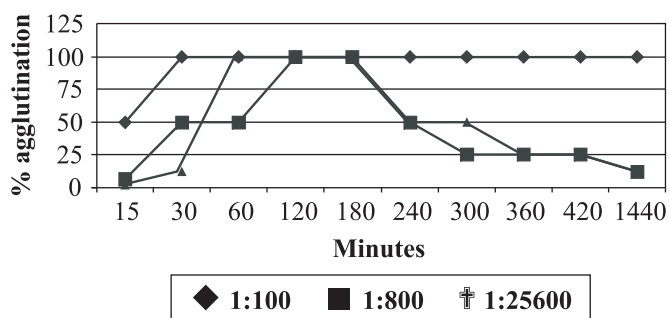


Figure 2. Percentage of agglutination of three sera (titers 100; 800 and 25600) incubated at 30°C, using three days old leptospirae and density of 2×10^8 leptospirae/mL.

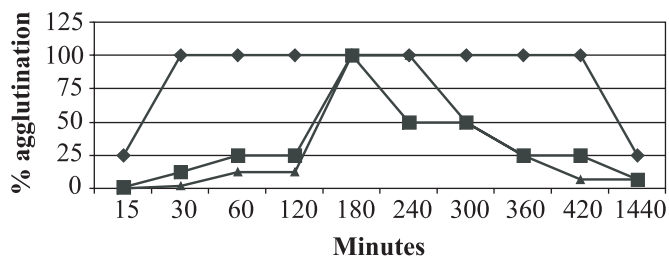


Figure 3. Percentage of agglutination of three sera (titers 100; 800 and 25600) incubated at 37°C, using ten days old leptospirae and density of 4×10^8 leptospirae/mL.

the phenomenon of agglutination in human and animal sera, when the reaction was read at 16-20 hours. Since then, the method was improved by other authors (1,3,9), who evaluated the influence of incubation time, incubation temperature, reading of the end-point titer and age of the culture. Four to 14 days old cultures, incubated at 30-32°C and containing $1-2 \times 10^8$ leptospiral organisms/mL are recommended for the test (6). In this study, it was observed that the serum with low titer (100) could be read from a 30 minutes of incubation, but the sera with

higher titers required longer than the recommended time to achieve their end-point titer. The 3 days old antigen achieved a better performance than the other antigens. The recommended density showed the best performance. Incubation at 4°C is important when the reading cannot be done within the recommended deadline. For a quick screening, in laboratories with intensive routine, the reading can be done with relative confidence from 60 minutes. The age and density of the antigen are important variables which may affect the results.

RESUMO

Influência da temperatura, tempo de incubação, densidade do antígeno e idade da cultura na aglutinabilidade das leptospiras

Na leptospirose, o teste de soroaglutinação microscópica (MAT) é a base do diagnóstico sorológico, sendo amplamente usado. Em nosso estudo, o MAT foi aplicado para avaliar a influência da temperatura, tempo de incubação, densidade do antígeno, a idade da cultura na aglutinabilidade das leptospiras e a interferência desses fatores na interpretação do diagnóstico. Três amostras de soro com títulos de 100, 800 e 25600 para o sorovar tande (isolado local), do sorogrupo canicola foram utilizadas com combinações diferentes entre os quatro fatores. Houve uma significativa relação entre todos os fatores principalmente a densidade do antígeno e a idade da cultura.

Palavras-chave: *Leptospira*, soroaglutinação microscópica, Leptospirose, diagnóstico laboratorial

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