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The Effect of Temperature and Concentration on The Brucella Antigen Activity Used for Diagnosis of Brucellosis by Tube Agglutination Test

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تأثير درجة الحرارة والتركيز على فعالية مستضد البروسيلا في اختبار التلازن الأنبوبي

الخلاصة

تناولت هذه الدراسة تحضير مستضد التلازن الانبوبي من عترة قياسية لجرثومة البروسيلا (*Brucella Abortus S99*) حسب الطريقة الأوربية وبتراكيز مختلفة (1%، 2%، 2.5%، 3%، 3.5%، 4%، 4.5%، 5%) من خلال اضافة خلايا جرثومية من نفس العترة، وكذلك تم تعريض هذه التراكيز لدرجات حرارية مختلفة (60، 70، 80، 95، 110مْ) لغرض معرفة تأثير التركيز والحرارة على فعالية مستضد التلازن الانبوبي بالمقارنة مع المستضد المجهز من شركة بايومريو الفرنسية.

Abstract

The current study handled the preparation of a tube agglutination test antigen from a record culture *Brucella Abortus S99* according to the European method and at different concentrations (1%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5%), by adding additional microbial cells, these concentrations were compensated by different temperatures (60, 70, 80, 95 and 110^{0}C), to study the effect of the concentration and temperature on the activity of the tube agglutination test antigen in comparison with the antigen prepared by bio-Merieux.

Introduction

Tube agglutination test, used for diagnosis of brucellosis in animals, has been applied for the first time in 1907 by Crisuted and Alton in 1909 [9, 10, 15]. Also in 1988 Alton et. al., mentioned that, there are two types of antigens used in the tube agglutination test, and they are: the prepared antigen by the European method, by adding 20ml of phenol saline solution in each bottle and killing the microbial suspension at 60ْC, where the size of the PCV's are 4.5%, and diluted to 10:1 during test; and the other antigen is the one prepared using the American method in which the solution diluted to 100:1 when used [3]. Whereas, the French used an antigen prepared by adding (50-60) ml of phenol in each bottle

for one hour, where the size of the PCV's is 2%, and diluted to 10:1 when used[11].

As noticed, the characteristics of the tube agglutination test increases especially when using antigens that are of medial size (EDIT) is added and sodium salts to the final dilution to reduce the sensitivity of the antibodies [1, 6].

As clarified by Macmillan and Cockrem [8], animals affected by acute brucellosis show positive results to complement fixation and at high percentages, whereas lower percentages are show when the tube agglutination test is undertook [5].

Materials and Methods

1 - Microbial Culture:

Brucella Abortus S99 that was prepared by the WHO and it is a classical culture used in the preparation of antigens.

2 - Antigens:

The antigen specific to the tube agglutination test was used that was prepared by bio-Merieux and it was used for comparison with other antigens.

3 - Serum:

The national serum opposite to brucellosis and that is as efficient as the international standard sera (ISAB) 1000/international unit/ ml and that is prepared by injecting New Zealand laboratorial white rabbits at an average weight of 750 grams and of good health. The rabbits are injected with a microbial suspension of ($5x10^9$) of *Brucella Abortus S99*. Blood is taken from the fourth day after injecting the suspension, to calculate the level of the antibodies till the required level of antibodies is reached. Clinical cases of brucellosis sera were also used for different antibodies.

Preparation of Antigens:

The tube agglutination test antigen according to the European method [2, 4] and at different concentrations by adding different quantities of microbial cells to the same culture (1%, 2%, 2.5%, 3%, 3.5%, 4.5%, and 5%) and these concentrations were subjected to different temperatures (60, 70, 80, 95 and 110^0C) by putting them in a water bath for one hour to figure out the effect of concentration and temperature on the activity of the antigen in the tube agglutination test in comparison with the antigen provided by bio-Merieux[12], and to get different concentrations of the prepared antigens the following methods were used:

1 - Methods of weighing microbial cells:

Empty Conical tubes, specific to the centrifuge, of the same type and known weight, filled with 10ml of prepared antigen, and the tubes were put into the centrifuge at 3000 round/min for 75 minutes to obtain a complete residue of the microbial cells and to get rid of the floating liquid. The tubes were then weighed and the difference of the weight of the empty tubes and its weight with the microbial residue is the weight of the microbial cells and that represents (PCV) for the prepared antigen. Microbial cells or phenol saline solution is added according to the concentration desired.

2 - **Modified packed cells volume method**:

The method of calculating the size of PCV's was improvised to calculate the calibration of the brucellosis antigens, where capillary tubes of 7ml length and 1ml depth were used. These tubes were filled and the other end was sealed using fire [7], and these tubes were placed in the centrifuge at 1500 round/min for 10 minutes then the PCV of the tubes is calculated to figure out the percentage of the packed cells.

Using clinical cases:

To calculate the activity and sensitivity of the prepared antigens, sera of infected patients, cattle and sheep were used from different areas of Baghdad with different antibodies titrations.

Results

When comparing the results of the tube agglutination test antigens at different concentrations and temperatures with the antigens prepared by bio-Merieux, it shows sensitivity differences towards the antigens using concentrations (1% up to 2.5%) and at temperatures from (60–95^{0}C), whereas not much difference is noticed in the other concentrations and temperatures. A significant effect on the activity of the prepared antigens is shown at all concentrations at the high temperature 110^{0}C (Table 1).

When using serum of different titration from clinical cases infected with brucellosis (diagnosed by Rose Bengal and Wright tests), it shows that the antigen with lower concentration is less sensitive than the other antigens when using serum with lower titration (Table 2).

Discussion

The tube agglutination test antigen was prepared according to the European method [2, 4] and at different concentrations (1%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5%) by adding microbial cells of the same culture or the phenol saline solution to obtain the required concentration and then subjecting these concentrations to different temperatures by inserting them into a water bath for

one hour (60, 70, 80, 95 and 110^{0}C) to study the effect of temperature and concentration on the activity of the prepared antigen, it appeared that the low concentrations (1% up to 2.5%) gave sensitivity less than the higher concentrations (table 1), and that agrees with what was mentioned by Zelssing and Mansifeld [15], whereas doesn't agree with the antigen prepared by the French 2% (6), and also with the antigen prepared by the Americans that uses 1% [2, 13].

As of temperature it shows that there is a big effect when the temperature is high (110^{0}C) on the activity of the antigen (table 1), whereas there is not that much noticeable effect at the other temperatures (60, 70, 80, 95^{0}C) [2, 11].

As for weighing the microbial cells for the account of the concentrations of the prepared antigens goes back to the unavailability of hopken's vaccine tubes to calculate the PCV of the prepared antigen that are used by international laboratories, and sealing of the capillary tubes by heat for the account of the size of the packed cells is better than the clay sealing as it reduces the fault percentage [7, 14].

Concentration of prepared Ag	60 °C	70 °C	80 °C	95 °C	110 °C
1%	+ +	+ +	+ +	+ +	-
2%	+ +	+ +	+ +	+ +	-
2.5%	+ +	+ +	+ +	+ +	-
3%	+ + +	+ + +	+ + +	+ + +	+
3.5%	+ + +	+ + +	+ + +	+ + +	+
4%	+ + + +	+ + + +	+ + + +	+ + + +	+ +
4.5%	+ + + +	+ + + +	+ + + +	+ + + +	+ +
5%	+ + + +	+ + + +	+ + + +	+ + + +	+ +
Bio – Meriux Ag 4.5%	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

Table 1: The effect of temperature and concentration on the activity of prepared antigen by using prepared national serum.

Serum volume	1%	2%	2.5%	3%	3.5%	4%	4.5%	5%	Bio – Meriux

titration									Ag
0	-	-	-	-	-	-	-	-	-
1/10	-	-	-	+	+ +	+ +	+ +	+ +	+ +
1/20	-	-	+	+	+ +	+ +	+ + +	+ + +	+ + +
1/40	-	+	+ +	+ + +	+ + +	+ + + +	+ + +	+ + +	+ + + +
1/80	-	+	+ + +	+ + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
1/160	+	+ +	+ +	+ + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
1/320	+ +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
1/640	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

Table 2: The effect of concentration on the activity of prepared antigen using serum from clinical cases positive with Brucellosis

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