j.Egypt.net.med.Assoc 79, no 4. 997- 1005 (2019)

THE EFFICACY OF USING ANTIMICROBIAL PRESERVATIVES ON BRUCELLA ANTIGENS

By

Lamees A.El-Tantawy., Lubna, F.Farahat

VSVRI Department of Antigens and Serums, Abbasia, Cairo.

ABSTRACT

Eradication program of brucellosis is depending mainly on the detection of animal reactor by different brucella serodiagnosis tests. The usefulness of diagnostic tests depends on the accuracy of test result, which in turn depends on specificity and sensitivity of the test.

The standard serological tests such as Rose Bengal Plate Test (RBPT), Buffer Acidified Plate Test (BAPT),and Tube Agglutination Test (TAT) are routinely used for the accurate diagnosis of brucellosis.So in this study it was prepared proposed to establish the comparative analysis of the efficacy of adding preservatives such as phenol (0.35%), thimerosal (0.01%), and formaldehyde, (0.5%) on the morphological characters, reactivity performance and microbiological analysis of brucella antigen in order to turn out contamination to keep its efficacy along the shelf life time of them. After adding the preservatives to antigens, every antigen has been evaluated for the physical inspection, pH, auto agglutination, morphological characteristics, microbiological analysis and the sensitivity testes with positive and negative sera.

These parameters were equal in all antigens with no regard to the preservative used.

The best morphological profile with appropriate antigenic stability at 4°C has been noticed in formaldehyde preservative. So, it could be recommend using 0.5% formaldehyde as a preservative for antigens especially it is the most cost effective one for a long period of conservation.

Keywords:

Rose Bengal Plate Test, Buffer Acidified Plate Test, Tube Agglutination Test.

INTRODUCTION

Brucellosis is still a zoonotic disease of worldwide distribution and represents a public health hazard and economic importance. This disease has a direct impact on the economy of the developing countries.Proper diagnosis is the first step for its control and eradication.

The success of maintaining brucella free herds is determined by the efficacy of the serological test to detect infected animals tube agglutination test has been the principal conventionalserological test for diagnosis of brucellosis.(RBPT) is a simple spot agglutination test which is effective as rapid, screen,presumptive test for diagnosis of brucellosis. Among the rapid agglutination assay for brucellosis surveillance is BAPA**(OIE, 2012).**

(TAT), (RBPA) and (BAPA) antigens should be effectively preserved against microbial growth which might contaminant them during wrong use of bad handling storage error of these antigens in the field and depending affect the accuracy of the serological tests **(Gama *et al.*, 2013)** Preservatives are chemical substance whose role is to protect medical products as vaccines and antigens against harmful change caused by microorganisms as they inhibit the growth of microorganisms when added in proper concentration.The preservatives should be soluble, nontoxic, physiologically and chemically compatible**(Kallings (1966).**

The objective of this study was to evaluate the use of different concentration of different preservatives then detect the effect of addition of this preservative on the currently prepared antigens for examining serum samples.

MATERIAL AND METHODS

Antigens:

Three currently produced Brucella Antigens **(RBPA, BAPA, and TAA)** were prepared in sera and antigens department in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI) according to international rules regarding of pH and PCV **(Alton *et al.*, 1988 and OIE, 2016)**.

Preservatives:

Three types of Preservatives were used for each antigen as Phenol 0.35%, Thiomersal 0.01% v/v and Formaldehyde 0.5%.The three produced antigens were suspended in different preservatives solutions and stored at different temperatures (25 °C, 4°C,and-20°C) until use.

Quality control of the prepared antigens with different preservatives:

Antigen purity (Morphological analysis):

A smear of each antigen kept in each preservative on a glass slide and stained with Grams staining technique and examined microscopically to identify the best preservatives.

Sterility test:

Sterility of the examined antigens was done by culturing of the tested antigen (100µl of antigen) in each of 3 tubes of nutrient agar, Thioglycolate broth and Sabauraud's dextrose agar **Rybacki (1980)**. The tubes were macroscopically examined every day for forty days.

Antigen colour check:

The antigens suspensions were mixed thoroughly by gentle shaking and inversions, then the antigens colour were seen in the presence of indirect sunlight or white bright light.

The stained cell suspension should appear pink in (RBPA), blue in (BAPA) and white in (TAA) and when mixed with sera, the antigen produces a uniform coloration with no deposit.

Antigen sensitivity check:

The sensitivity of tested antigens was compared with a standards previous lot of antigens by carrying out the BCT in duplicate on a group of 10 bovine sera of varying degrees of reactions ranging from negative through 4+positive prepared by diluting a positive serum in phenol saline.

Antigen pH check:

The antigens suspensions were mixed thoroughly by gentle shaking and inversion, the antigens pH were measured the pH should be (3.65±0.05) in Rose Bengal Antigen and (3.70±0.03) in Buffer Acidified Plate.

RESULTS

Table (1): Sterility testing of antigens with (0.35%) phenol, (0.01%) Thiomersal and (0.5%) formaldehyde in different media.

Antigens	Months post preparation	Phenol (0.35%)			Thiomersal (0.01%)			Formaldehyde (0.5%)		
		N*	T*	S*	N*	T*	S*	N*	T*	S*
Rose Bengal Antigen	1st	All antigens with each preservative were sterile without any bacterial or fungus contamination when cultivated on different media.								
	2nd									
	3rd									
Buffer Acidified Plate Antigen	1st									
	2nd									
	3rd									
Tube Agglutination Antigen	1st									
	2nd									
	3rd									

N* =Nutrient agar.

T*=Thioglycolate broth.

S*=Sabauraud's dextrose agar.

Table (2): Physical inspection of different antigens with different preservatives.

Antigens	Preservatives		
	Phenol (0.35%)	Thiomersal (0.01%)	Formaldehyde (0.5%)
Rose Bengal Antigen	Normal physical inspection without any change in colour and no sediment		
Buffer Acidified Plate Antigen			
Tube Agglutination Antigen			

Table (3): The efficacy of different preservatives on auto- agglutination test of different types of antigens.

Antigens	Preservatives		
	Phenol (0.35%)	**Thiomersal (0.01%)**	**Formaldehyde (0.5%)**
Rose Bengal Antigen	**Negative**		
Buffer Acidified Plate Antigen			
Tube Agglutination Antigen			

Table (4): The sensitivity of antigens with different preservatives.

Numbers of Serum sample	Total numbers of tests	Antigens with different type of Preservatives								
		RBA Phenol (0.35%)	BAPA Phenol (0.35%)	TAA Phenol (0.35%)	RBA Thiomersal (0.01%)	BAPA Thiomersal (0.01%)	TAA Thiomersal (0.01%)	RBA Formaldehyde (0.5%)	BAPA Formaldehyde (0.5%)	TAA Formaldehyde (0.5%)
5 Negative serum samples	45 Tests for negative serum	The sensitivity of antigens in all tests not effected by different preservatives								
5 Positive serum samples	45 Tests for positive serum									
Total numbers of tests	90									

Table (5): The pH of antigens with different preservatives.

pH of				
Antigens before adding preservatives		Antigens after adding preservatives		
Rose Bengal Antigen	Buffer Acidified Plate Antigen	preservatives	Rose Bengal Antigen	Buffer Acidified Plate Antigen
3.65	3.70	Phenol (0.35%)	3.65	3.70
3.65	3.70	Thiomersal (0.01%)	3.65	3.70
3.65	3.70	Formaldehyde (0.5%)	3.65	3.70

Table (6): The stability of antigens kept at different temperatures.

Antigens	4°C	25°C	-20°C
Rose Bengal Antigen	Good reactivity	Slightly lower performance from those kept at 4°C	Worst morphological characteristics and the poorest reactivity that affect the sensitivity of antigens due to dissociation
Buffer Acidified Plate Antigen			
Tube Agglutination Antigen			

DISCUSSION

In the development of the diagnostic antigen, the most important item is its storage stability along its shelf life time. Preservative can play an important role in this point if it provides some essential properties such as broad spectrum activity against microorganisms, efficiency at low concentration, safety, stability at different temperatures and the most important point that it has no effect on sensitivity and specificity of the antigen **Karabit *et al.*, (1985).**

More recently, **Mayrink *et al.*, (2010)** proposed substituting thimerosal for phenol in the vaccine against American tegumentary leishmaniasis because it is considered to be less toxic. In this study we proposed to establish the best condition for preserving brucella antigens in

order to applicate serological tests with excellent performance. The main goal was evaluation of the morphological and microbiological characteristics as well as serological reactivity performance of antigens following usage of different preservatives at varied temperatures. All preservatives solutions were associated with no change in the morphological features this a gree with **Mayrink *et al.*, (2006)** who use phenol to preserve the immunogenic and biochemical properties of antigens was observed in the preservation of the Montenegro skin test in Brazil. Thimerosal has been used as an effective preservative in vaccines **(Fernandes *et al.*, 2008; Mayrink *et al.*, 1979)** but disagree with **Gama *et al.*, (2013).** Who observed that the morphological profile maintained in Thiomersal and Formaldehyde, but Phenol revealed it to be the worst preservative. Microbiological analysis revealed no contamination in the antigens among all preservatives and different storage temperatures (Table 1) these results were in agreement with **OIE (2000)**. It is important to highlight that a possible microorganism contamination of the antigens could lead to an unreliable evaluation of the serological reactivity as well as the morphological characteristics. Antigens shown no changes in physical inspections (colour) and without appearance of any sediment at different preservatives (Table 2), but **Gama *et al.*, (2013)** said the antigens observed in phenol did not show a good performance in the physical inspection. Under all preservatives conditions, the reactivity of the antigens kept at 4°C was the best amongst those maintained at 25°C and -20°C. Moreover, the maintenance of antigens at - 20°C was not indicated due to this temperature being associated with the worst morphological characteristics and the poorest reactivity that affect the sensitivity of antigens due to dissociation (Table 6) The diagnostic performance parameters obtained under different conditions of antigens evaluated by determine the sensitivity of antigens against positive and negative serum showed in (Table 4). All types of preservatives didn't effect on the pH of antigens in different storage temperature (Table 5). These results are matched with **Gama *et al.*, (2013).** Our findings demonstrate that preservation in formaldehyde and storage at 4°C provides the best conditions for preservation of antigens. We verified that these conditions ensure the best stability of morphological characteristics and excellent antigen reactivity as well as sterility of the antigen preparation. With the results obtained in this study it was possible to define the best conditions to be used for preserving the antigens.

CONCLUSION

The authors recommend using formaldehyde as a preservative in different types of Brucella antigens and store at 4°C provides good conditions for preservation of antigens.

They verified that those conditions ensure good stability of morphological characteristics and excellent antigen reactivity.

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