

Evaluation of the Effect of Changing Concentration and pH on The Activity of Rose Bengal Antigen

Muthanna, I. Al-Ezzi

University of Al-Mustansiriyah, College of Pharmacy

Department of Pharmaco-therapeutics

الخلاصة:

شملت هذه الدراسة تحضير مستضد الروزبنكال باستخدام صبغة الروزبنكال 1% وصبغة Brilliant green 0,5% وبتراكيز مختلفة من المستضد وعلى هيئة حجم خلايا مضغوطة أو مرصوصة (Packed cell volume) (4%, 5%, 6%, 7%, 8%, 9%, 10%) ودارى تخفيف الأَس المختلفة الهيدروجيني (pH) (3.0، 3.5، 3.65، 4.0، 4.5، 5.0) لدراسة تأثير الأَس الهيدروجيني والتراكيز المختلفة للمستضد على اختبار الروزبنكال. أظهرت النتائج أن فعالية المستضد تزداد عندما يكون التركيز أكثر من 7% والأَس الهيدروجيني (PH) من 3.65 ولغاية 4.0.

Abstract

The present study was conducted to prepare Rose Bengal antigen by using Rose Bengal stain 1% and brilliant green 0.5%. These antigens were prepared using different concentrations (4%, 5%, 6%, 7%, 8%, 9% and 10%) and different buffer diluents with different pH (3.0, 3.5, 3.65, 4, 4.5, 5.0) to study the effect of different antigen concentrations and pH on the Rose Bengal test. It was observed that the best results were obtained when the concentration of the antigen was more than 7% and the pH range of 3.65 to 4.0.

Introduction

The Rose Bengal test is one of the simple and rapid tests used to diagnose brucellosis in humans and animals and the antigen used is a bacterial culture consisting of denaturalized bacteria stained with Rose Bengal 1% [5, 17].

One of the properties of the Rose Bengal test is that it detects the infections in its early phases [4]. Behind this ability is that the immunoglobulin type M (IgM) is the precedent type after infection [7]. It becomes more active than immunoglobulin type G (IgG) with both its subtypes IgG1 and IgG2 [1,3]. Therefore, IgM is produced as a response to S19 vaccine [6, 18].

It has also been noticed that the pH for Rose Bengal antigen increases the activity of the bodies specific to brucellosis and reduces the sensitivity of the

characteristic antibodies ^[8, 11]. Rose Bengal antigen is used to diagnose *Brucella canis* using a microbial culture from *Brucella ovis* at a temperature where the concentration of the prepared antigen is 6% and Phosphate buffer solution (PBS) of concentration 0.1 with pH 7.0 is also added and it is then stained using Rose Bengal 1% ^[13].

The purpose of this study was to detect changes on the Rose Bengal antigen affinity after changing antigen concentration and pH.

Materials and Methods

1 - Microbial culture:

*Brucella abortus*_S99 provided by the W.H.O. and was considered a standard culture used in the preparation of antigens ^[20].

2 - Solutions:

- **Phenol Saline Solution:** This solution was used to prepare the antigens and was prepared according to Alton et al, ^[2].
- **Buffered diluents:** This solution was used to dilute the antigens and it was prepared according to Alton et al, ^[2] and OIE ^[15]. Different concentrations were prepared according to the pH required by changing the amount of lactic acid (UBP 86%) or NaOH (3.0, 3.5, 3.65, 4, 4.5, 5.0).

3 - Stains:

- **Rose Bengal:** Rose Bengal 1% solution was prepared (Rose Bengal provided by BDH) ^[2, 10, 16].
- **Brilliant green:** Brilliant green 0.5% solution was prepared (Brilliant green provided by BDH) to be used as a replacement to Rose Bengal when preparing the antigen.

4 - Antigens:

The antigen was prepared at different concentrations (4%, 5%, 6%, 7%, 8%, 9% and 10%) by using Rose Bengal 1% and brilliant green 0.5% according to Alton et al, ^[2] and OIE ^[15], by using a dilute buffer (PBS) of reduced pH (3.0, 3.5, 3.65, 0.3, 4.0, 4.5, and 5.0) to study the effect of concentration and pH on the efficiency of the prepared antigen.

5 - Serum:

Standard serum that was prepared by injecting rabbits with the same microbial culture (*Brucella abortus* S99) which was used to obtain standard sizes of the antibodies (1/640) ^[20]. Additionally, serum from clinical cases, infected with *Brucella*, with different titrations (1/10, 1/20, 1/40, 1/80, 1/160, 1/320) was also used to study the sensitivity of the antigen.

Results

The results of the present study showed that there is no effect on the sensitivity of the prepared antigen when using brilliant green as a replacement to Rose Bengal when used at a concentration of 0.5%, but it showed a clear effect on the pH and concentration on the efficiency of the prepared antigen where it was noticed that the concentration of the antigen should not be lower than 7% and that the best pH for the antigen is (3.65 – 4.0) whereas the rest of the concentrations and pH are less sensitive (Table 1).

The sensitivity of the antigen was reduced when the pH was less than 3.65 and more than 4.0 and concentration less than 7% to detect the lower titration of antibodies (Table 2).

Discussion

The sensitivity of the Rose Bengal test depends on many factors which include, dilution buffer, pH and the concentration of the microbial culture of the prepared antigen ^[13, 19], and brilliant green stain 0.5% was used as a substitute to Rose Bengal 1% and that was because this stain doesn't have an effect on the sensitivity of the antigen, and due to its availability in laboratories and using this concentration goes back to the strength of the stain where it was noticed that when used at 1% it left a mark on the slide specific to the test even after filtration.

It was also noticed that the prepared antigen when the pH of the buffer is (3.65 up to 4.0) is of high sensitivity to the antibodies ^[8, 9, 10, 12, 14, 16], whereas it was noticed that when the concentration of the antigen was less than 7% the sensitivity reduces and that conflicts with what Lisle and Carmichael ^[13] mentioned that was prepared at a concentration of 6%.

Types of Ag according to dyes and pH		Ag concentration						
		4%	5%	6%	7%	8%	9%	10%
3.0	R.B.	-	-	-	+	+	+	+
	B.G.	-	-	-	+	+	+	+
3.5	R.B.	-	-	+	++	+++	+++	+++
	B.G.	-	-	+	++	+++	+++	+++
3.65	R.B.	-	+	+++	++++	++++	++++	++++
	B.G.	-	+	+++	++++	++++	++++	++++
4.0	R.B.	-	+	+++	++++	++++	++++	++++
	B.G.	-	+	+++	++++	++++	++++	++++
4.5	R.B.	-	-	+	++	++	++	++
	B.G.	-	-	+	++	++	++	++
5.0	R.B.	-	-	-	+	+	+	+
	B.G.	-	-	-	+	+	+	+

R.B. = Rose Bengal antigen
 B.G. = Brilliant green antigen
 - = Negative (No agglutination)
 ± = Suspected
 + = 25% agglutination
 ++ = 50% agglutination
 +++ = 75% agglutination
 ++++ = 100% agglutination

Table 1: The effect of pH & different concentrations of antigen on the Rose Bengal test by standard serum.

Types of Ag according to dyes and pH		Ab volume titration	Ag concentration						
			4%	5%	6%	7%	8%	9%	10%
3.0	R.B.	1/10	-	-	-	-	-	-	-
	B.G.		-	-	-	-	-	-	-
3.5	R.B.	1/20	-	-	+	+	+++	+++	+
	B.G.		-	-	+	+	+++	+++	+++
3.65	R.B.	1/40	-	+	++	+++	++++	++++	++++
	B.G.		-	+	++	+++	++++	++++	++++
4.0	R.B.	1/80	-	+	++	+++	++++	++++	++++
	B.G.		-	+	++	+++	++++	++++	++++
4.5	R.B.	1/160	-	-	-	+	++	++	++
	B.G.		-	-	-	+	++	++	++
5.0	R.B.	1/320	-	-	-	-	+	+	+
	B.G.		-	-	-	-	+	+	+

◉R.B. = Rose Bengal antigen
 B.G. = Brilliant green antigen
 - = Negative (No agglutination)
 ± = Suspected
 + = 25% agglutination
 ++ = 50% agglutination
 +++ = 75% agglutination
 ++++ = 100% agglutination

Table 2: The effect of pH & different concentrations on the Rose Bengal test by using clinical cases serum.

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