



Revista Brasileira de Ciência Avícola

ISSN: 1516-635X

revista@facta.org.br

Fundação APINCO de Ciência e Tecnologia
Avícolas
Brasil

de Freitas, ES; Soldá, A; Calegari, DLI; Munaretto, FC; Dietrich, GF; Back, A
Evaluation of Glycerin as Preserving Agent of Chicken Serum for Plate Agglutination Test
Revista Brasileira de Ciência Avícola, vol. 16, núm. 3, julio-septiembre, 2014, pp. 329-332
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

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■Keywords

Seroagglutination, glycerol, preservation, re-
frigeration, shelf life.

Evaluation of Glycerin as Preserving Agent of Chicken Serum for Plate Agglutination Test

ABSTRACT

Serum is widely used for the purpose of monitoring and diagnosis support for most of poultry diseases. In the case of the serum plate agglutination test (SPA), commonly used to detect antibodies for *Salmonella Pullorum* (SP), *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS), serum cannot be frozen because it may result in false positive. Without freezing, serum can last only for a few days. In this experiment, glycerin was evaluated as a serum preserving agent. About 50 samples for each disease and analyzed by SPA test previously were separated. Glycerin was added to serum from commercial chickens, with and without antibodies for SP, MG and MS, in the proportion of 1:1 (serum:glycerin) and kept at refrigerated conditions (2 to 8 °C). For four years they were tested by the SPA, initially weekly, afterward monthly and then annually. The results show that serum with glycerin give consistent and valid results according to the kind of antibodies present for the period tested. Sera that glycerin was not added to, the results were valid only for the first week. From the second week on, microbial growth affected the test results of the sera without glycerin. Our investigation shows that glycerin can be used to preserve chicken serum for SPA under refrigerated conditions. It is an easy, simple and cheap procedure that can extend serum shelf life, useful mainly for control sera.

INTRODUCTION

Serology is a laboratory method of analysis which aims to study the antigen-antibody reactions. Serum is routinely used to detect and/or evaluate its level of antibodies by an antigen. For its evaluation, both must be specific and homologous (Santos, 2009). Nowadays, this detection can be performed by various techniques, the most commonly used are ELISA (Enzyme-linked immunosorbent assay), HI (hemagglutination inhibition), AGP (agar gel precipitin), SN (seroneutralization), SAT (slow seroagglutination test) and SPA (serum plate agglutination test).

When using the SPA technique the antibody primarily detected is IgM, followed by medium-high titers of IgG (Back & Ishizuka, 2010). The SPA is a qualitative test with good sensitivity, but not with as good specificity. It is widely used for screening, monitoring and controlling typhoid *Salmonella* and Mycoplasmosis in poultry (Santos, 2009; Cardoso, 2009). However, for a definitive positive serologic diagnosis, it is necessary to perform a confirmatory test such as HI or ELISA (Back, 2010; I.N. MAPA 44/2001).

One of the limitations of the SPA is the sera preservation. It cannot be frozen because it may result in false positive due to a precipitation of some proteins and at refrigerated temperatures it remains viable only for a few days (Hay & Westwood, 2002). Glycerin has been used in



human and veterinary medicine for the preservation of different cell types (Alvarenga, 1992; Batista *et al.* 1996; Daleck *et al.* 1987; Mota *et al.* 2002; Nolasco *et al.* 2003; Pigossi, 1964; Pigossi, 1967; Reyes, 1993), microorganisms (Hubálek, 2003) and blood serum (Bier, 1982; Hay & Westwood, 2002). Its main advantages are low cost, antiseptic property, texture cell preservation, increase in strength without altering the degree of cell elasticity, facilitates handling and keeps its antigenicity properties (Alvarenga, 1992; Miller, 1972; Pigossi, 1964; Pigossi, 1967; Batista *et al.* 1996; Daleck *et al.*, 1987; Mota *et al.*, 2002; Nolasco *et al.* 2003; Reyes, 1993). Miller (1972) adopted the preservation of cells (bacteria) in glycerol at -20 °C for years. However, so far, there is no description of sera from birds preserved in glycerin at refrigerated conditions (2 - 8 °C) while maintaining its antigen-antibody reaction properties without undesired reactions.

This investigation aimed at evaluating the preservation of poultry sera used as control in SPA tests when kept refrigerated in glycerin at 2 - 8 °C.

MATERIALS AND METHODS

The present study was carried out at the serology Department of MercoLab Laboratory (Cascavel-PR, Brazil) from January 2010 to December 2013. The analyses were performed with poultry sera from commercial chickens (in nature) by the SPA test which is used to evaluate the presence of antibodies for *Mycoplasma synoviae* (MS), *Mycoplasma gallisepticum* (MG) and *Salmonella Pullorum* (SP).

About 50 positive and negative samples for each disease and analyzed by SPA test previously were separated and pooled. Each pool was divided into two fractions. One of 10 mL and the other of 5 mL. The fractions of 5 ml were added to 5 ml of neutral liquid glycerin, distilled twice (ratio of glycerin:serum was 1:1). No glycerin was added to the serum fraction of 10 ml. Each fraction, with and without glycerin, was subdivided into 20 aliquots of 0.5 ml each in eppendorf tubes and kept under refrigeration at 2-8 °C.

Sera were evaluated weekly for two weeks in January and monthly from February to December of 2010. In 2011 the samples were tested quarterly (Table 1). From then on, sera were evaluated annually. For all tests, commercial antigens were used for MS, MG e SP.

Serum and antigen were removed from the refrigerator and left at room temperature for one hour before being tested. Equal amounts (30 µl) of antigen and serum were pipetted and homogenized in a big

dropon in a glass plate, and rotated several times. The reading was performed after two minutes of the initial homogenization. The interpretation was based on the presence or absence of agglutination (small clots). Sera which agglutinated were considered positive and those with no agglutination were considered negative. The temperature of the test room was kept between 21 and 25 °C. All the test procedures described followed the manufacturer's specifications and general recommendations from Brazilian Ministry of Agriculture (MAPA) (BRASIL, 1995; BRASIL, 1994).

RESULTS AND DISCUSSION

Several methods and substances have been used to preserve cells, tissue and serum, such as low temperature (-20° C and -80 °C), lyophilization, mineral oil and cryopreservation. Preservation of microorganisms and tissues in glycerol has been used in laboratories worldwide, and meets most of the desirable characteristics of a good preservation method. In the case of poultry serum, antimicrobial agents such as sodium azide and thimerosal have been used for preservation, but the results have not always been satisfactory in our experience. Frequently, bacteria growth has been observed, invalidating the results.

The results of our investigation are shown in Table 1. In the first two trails, 1 and 7 days post refrigeration, the results of agglutination for MG, MS and SP showed no difference in serum with and without glycerin. All the positive serum presented results of agglutination and the negative control sera remained non-reactive, as expected. These results demonstrate that serum can be kept fresh at refrigerated temperatures (2-8 °C) for a week without affecting the results. It is known that this can vary depending on the degree of the bacterial contamination of the sera, which is related to the hygienic harvesting and handling of the samples (Personal communication). From day 14 on, in spite of the serum being kept at (2 - 8 °C), samples that did not have glycerin (*in nature*) added to it were inappropriate for analysis. They presented cloudy appearance (indicating microbial growth) and were discarded. The investigation continued only with the serum with glycerin, showing no evidence of microbial growth (transparent), demonstrating its property of preservation (antiseptic), as described by Alvarenga (1992).

According to MAPA (BRASIL, 1995; BRASIL, 1994) poultry sera for analysis must be refrigerated and processed until 3 days after the harvest. After this time,



Table 1 – Results of SPA test using sera stored under refrigeration with or without glycerin for a period of four years (2010 and 2013).

Period	Serum without glycerin				Serum with glycerin			
	MG (+)	MS (+)	SP (+)	C (-)	MG (+)	MS (+)	SP (+)	C (-)
January/2010 – day 1	+	+	+	-	+	+	+	-
January/2010 – day 7	+	+	+	-	+	+	+	-
January/2010 – day 14	*	*	*	*	+	+	+	-
February/2010	*	*	*	*	+	+	+	-
March/2010	*	*	*	*	+	+	+	-
April/2010	*	*	*	*	+	+	+	-
May /2010	*	*	*	*	+	+	+	-
June/2010	*	*	*	*	+	+	+	-
July/2010	*	*	*	*	+	+	+	-
August/2010	*	*	*	*	+	+	+	-
September/2010	*	*	*	*	+	+	+	-
October/2010	*	*	*	*	+	+	+	-
November/2010	*	*	*	*	+	+	+	-
December/2010	*	*	*	*	+	+	+	-
March/2011	*	*	*	*	+	+	+	-
June/2011	*	*	*	*	+	+	+	-
September/2011	*	*	*	*	+	+	+	-
December/2011	*	*	*	*	+	+	+	-
December/2012	*	*	*	*	+	+	+	-
December/2013	*	*	*	*	+	+	+	-

(MG +): *Mycoplasma gallisepticum* positive; (MS +): *Mycoplasma synoviae* positive; (SP +): *Salmonella Pullorum* positive; (C-): Negative control; (*): discarded.

sera should be frozen (-20 °C). Under manufacturer's specifications, no serum can be frozen for SPA testing, because it can lead to nonspecific reactions. This represents a limitation in the serum storage.

In the second year of evaluation (2011), the sera with glycerin were tested quarterly e in the third year on, annually, and the results were the same. These sera are still stored under refrigerated conditions (2 °C to 8 °C) for subsequent studies.

These results demonstrate that the use of glycerin in the serum, in the proportion of 1:1, is an alternative to preserve its SPA test properties for at least two years under refrigerated conditions. Therefore, this technique for its simplicity and low cost can be used routinely in laboratories to preserve serum. It becomes very important to preserve control sera for the routine testing. Under present refrigerated conditions, it does not last more than weeks without glycerin.

CONCLUSIONS

The results of our study showed that the serum with glycerin (1:1) under refrigerated conditions (2 °C to 8 °C) can keep its initial reacting properties for four

years for the SPA test, whereas serum without glycerin remain viable for a week.

This is an easy, low cost and simple procedure that can be widely used to preserve chicken serum for the SPA test. Its properties to inhibit bacterial growth can be used for any chicken serum that will be tested; however, it becomes especially important to extend shelf life of control serum that is needed in every testing. Good hygiene in the sera harvest can also added to longer preservation.

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