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Purification of Antihuman Immunoglobulins by Polyethylene Glycol Precipitation

SHAHID MAHBOOB†, MUHAMMAD HASSAN, AZHAR MAHMOOD AND ROBINA YAQUB‡
Post-Graduate Department of Zoology, Government College, Faisalabad–Pakistan
†Corresponding author: Email: rsmahboob@yahoo.com
‡*Department of Zoology, Azad Jammu and Kashmir University, Muzzafarabad–Azad Kashmir*

ABSTRACT

This study was conducted to produce antihuman immunoglobulins (Igs) in rabbits and purify them from the hyperimmune sera by polyethylene glycol (PEG) precipitation technique. Human serum was inoculated as twin and multiple shot strategies keeping one group of rabbits as control (non-inoculated). Results revealed that difference ($P< 0.001$) of total protein between twin and multiple shot rabbits; twin shot and control rabbits, and between multiple shot and control rabbits. Mean values of AGPT titres of Igs were significantly higher in multiple shot than those in twin shot rabbits. Similarly, mean values of IHA titres of Igs were significantly higher in multiple shot than those in twin shot rabbits. Differences of antihuman Igs between before and after isolation by PEG were highly significant. The comparative estimation of antihuman Igs revealed that mean values of IHA titre were significantly higher than those of AGPT titre, both in twin and multiple shot rabbits.

Key Words: Antihuman immunoglobulins; Polyethylene glycol; Serum; AGPT; IHA

INTRODUCTION

There are various techniques employed for the purification of immunoglobulins (Igs) from serum. Most of the methods require organic solvents, sophisticated equipment and are time consuming (Wallmann *et al.,* 1990). Polyethylene glycol (PEG) precipitation technique is simple, inexpensive, quick and gives high purity of Igs (Polson, 1990). This technique was first developed by Stevens and Bridts (1981) for rapid determination of IgG containing circulating immune complexes using PEG and radioactively labeled protein A. Shaoqin *et al.* (1987) isolated human IgG by PEG. The resulting IgG had greater specific activity and recovery as compared to $(NH_4)_2SO_4$ precipitation. The best results were revealed by using 16% PEG and 0.025 M phosphate-buffered saline at pH 7.8. Shah *et al.* (1993) reported that serum samples from sheep, goats and buffaloes were treated with different concentrations of PEG 6000 ranging from 1-10% for different periods (i.e. 5-30 min). The protein content was enhanced by increasing concentration of PEG and time.

This paper describes the production of antihuman Igs in rabbits and their purification from the hyperimmune sera by PEG precipitation technique.

MATERIALS AND METHODS

A total of 27 adult male rabbits (*Oryctolagus cuniculus*, strain chinchilla) were selected for the production of antibodies. These animals were acclimatized for 15 days. The rabbits were fed with green fodder, pulse and tap water during experimental period. During winter, temperature was maintained by electric heater. After providing standard management conditions, all the rabbits were randomly divided into three groups with three replicates designated as Group A, B, and C. Each group comprised of total nine animals tagged with different code numbers and were housed in separate iron cages. Hair from the ventral side of the rabbits, were removed with the help of Samsol hair removing cream in order to expose the skin for injection.

Collection of human serum. 100 mL of human B+ve blood was obtained from the peripheral vein of healthy donor with sterilized disposable plastic syringe. The blood was preserved without anticoagulant in sterilized test tubes and let it stand in vertical position until clot was formed. The test tubes were placed at refrigeration temperature (40ºC) for 6 h; serum oozed out was taken with the help of sterilized Pasteur pipette, divided into aliquots of 1.5 mL in eppendeorfs and stored in a deep freezer at –20ºC till analyzed.

Inoculation of rabbits with human serum. Human serum was used as antigen to produce hyperimmune sera in rabbits. The rabbits of group A and B were subjected to twin and multiple shot modules, respectively, while group C rabbits were kept as control. Group A rabbits were inoculated intracutaneously with human sera (as antigen) by twin shot schedule. The twin shot schedule comprised two heavy doses of the serum: first dose of 2.0 mL and second dose of 4.2 mL after 12 days. Group B rabbits were inoculated with the same serum through multiple shot schedules. The multiple shots schedule consisted of five doses of the serum: 0.2, 0.4, 0.8, 1.6 and 3.2 mL, increasing gradually after each three-day interval between two consecutive doses. Group C rabbits were kept as control (non-inoculated) for comparison.

Collection of hyperimmune serum from rabbits. Blood

from rabbits of all the three groups (A, B and C) was collected first by vein puncturing and then by slaughtering the animals 28 days after first inoculation. The blood was collected in beakers and allowed to clot in slanting position for one hour. After clotting, the blood was kept in refrigerator (4ºC) over night. Next day, the oozed sera were collected by Pasteur pipette and preserved at -20ºC in aliquots of 1.5 mL in eppenderofs till further use.

Estimation of total immunoglobulins protein in rabbit serum. Total immunoglobulin proteins in the sera from inoculated and controlled rabbits were determined by using Biuret method (Gornall *et al.,* 1949).

Isolation of rabbit antihuman Igs by PEG precipitation method. Hyperimmune sera obtained from the rabbits were processed for isolation of Igs through PEG precipitation technique as follows. PEG was adjusted to the working concentration by mixing 6 mL of 20% PEG with 3 mL 0.2 M ethylene diamine tetra acetic acid (EDTA) and 1 mL vernal buffered saline (VBS). Then, 30 µL PEG working solution was added to 150 µL of the rabbit serum, mixed thoroughly and left over night at 4ºC temperature. The mixture was centrifuged at 4000 rpm for 20 min at 4ºC. Then, the tubes containing the mixture were placed in ice and removed the supernatants. The precipitate was re-suspended with 2 mL of 20% PEG in 0.01M EDTA in VBS. The suspended precipitate was centrifuged at 3000 rpm for 20 min at 4ºC. The supernatant was removed and re-dissolved the precipitate complex.

Titration of antihuman antibodies. The titre of antihuman antibodies was determined by agar-gel precipitation test (AGPT) as described by Hudson and Hay (1980).

Titration of purified rabbit antihuman Igs against human serum (as antigen). Igs against human serum (as antigen) were identified and titrated through indirect haemagglutinaion (IHA) test as followed by Aliev *et al.* (1989).

RESULTS AND DISCUSSION

Total immunoglobulin proteins were measured as 6.297±0.006, 6.416±0.015 and 6.194±0.000 g/100 mL in twin, multiple and controlled rabbits, respectively (Table I).

Table I. Total immunoglobulin proteins in sera of inoculated and controlled rabbits

Treatments	Total Igs (g/100 mL) Mean ± SE
Twin shot rabbits	6.297 ± 0.006
Multiple shot rabbits	6.416 ± 0.0015
Controlled rabbits	6.194 ± 0.000

Mean values of AGPT and IHA titre of rabbit antihuman Igs measured before isolation were found to be 12.6 and 25.4, and 25.4 and 64.0 in twin and multiple shot strategies, respectively (Table II). Mean values of AGPT and IHA titres of antihuman Igs, determined after isolation by PEG precipitation technique, were 64.0 and 101.6, and 101.6 and 203.2 in twin shot and multiple shot rabbits, respectively (Table III).

Table II. Rabbit antihuman Igs titre measured by AGPT and IHA before isolation through PEG

Treatments	AGPT titre Mean ± SE	IHA titre Mean ± SE
TS rabbits	12.6 ± 0.12	25.4± 0.10
MS rabbits	25.4 ± 0.14	64.0 ± 0.14

TS= Twin shot; MS=Multiple shot

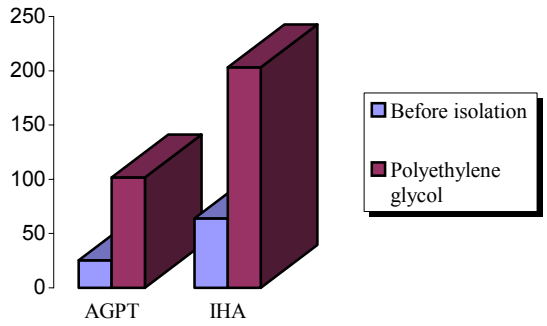
Table III. Rabbit antihuman Igs titre measured by AGPT and IHA after isolation through PEG

Treatments	AGPT titre Mean ± SE	IHA titre Mean ± SE
TS rabbits	64.0 ± 0.07	101.6 ± 0.10
MS rabbits	101.6 ± 0.13	203.2 ± 0.14

TS= Twin shot; MS=Multiple shot

The comparative estimation of antihuman Igs showed that mean values of IHA titre were significantly higher than those of AGPT titre in multiple shot rabbits (Fig. 1).

Fig. 1. Comparative estimation of antihuman Igs titre in multiple shot rabbits



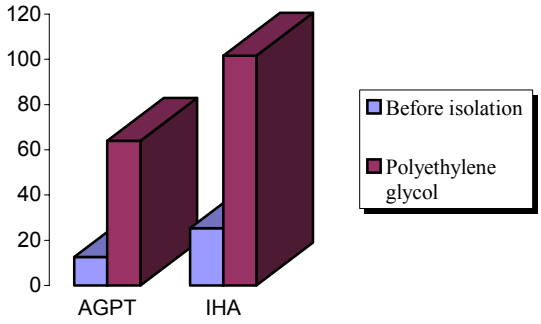
Similarly, mean values of IHA titre were found to be higher than the AGPT titre (Fig. 2).

In the current study, PEG precipitation technique was found to be simple, easy and economical (Polson, 1990) for purification of Igs. PEG has multiple hydroxyl groups, which become highly reactive in aqueous phase. In the aqueous phase, PEG acts as a nucleophile that is anion and can attract positively charged substances, thereby it produces electrostatic changes in water. Therefore, in a PEG medium the hydrophobic interaction of antibody molecules are considerably enhanced which separate them from the

other proteins of the suspensions (Shafique, 1996).

The results revealed higher mean values of antihuman Igs titres in multiple shot rabbits. The difference of antibody titre may probably be due to dose schedule, as multiple shot rabbits were injected with human serum (as antigen) five times by equal intervals of days, while the twin shot rabbits were inoculated with the same amount of the serum, but only two times. These findings are in agreement with those reported by Ahmad (1996). Higher values of IHA titre than those of AGPT titre indicated that IHA test was more sensitive than AGPT. These results are in conformity with those by Anwar (1993).

Fig. 2. Comparative estimation of antihuman Igs titre in twin shot rabbits



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