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# Comparative Study on Purification of IgG from Different Species by Protein "A" Affinity Chromatography

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## Abstract

Monoclonal antibodies (MAbs) developed in 1975 by Georges Kohler have been applied in many areas of research. Staphylococcal protein-A binds to most mammalian IgG and can be employed for detecting or purifying such antibodies. Affinity chromatography on staphylococcal Protein A column is extensively used for purifying Monoclonal and Polyclonal antibodies. IgG from serum samples of different species were purified by the method described in Protein "A" Agarose Affinity Chromatography kit supplied by Bangalore Genie Pvt Ltd, Bangalore India. IgG from serum samples of different species eluted as a single major peak. The descending order of affinity of Protein "A" for IgG from serum of different species are pig > human > rabbit > dog goat > sheep > buffalo > chicken respectively.

## Article Info

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## Keywords

Protein "A" Affinity Chromatography,
IgG purification,
Pig serum,
Human serum and
Rabbit serum

## Introduction

Immunity is the ability of an organism to resist a particular infection or toxin by the action of specific antibodies or sensitized white blood cells.

This defence mechanism is quite complex and involves many different cell types, molecules, and genes (collectively called immune system). The response of the immune system to invading foreign substances is called as the immune response.

Secreted antibodies (immunoglobulins) mediate a wide array of biological functions such as agglutination or aggregation, opsonisation, lysis and inflammation. (Kuby Immunology., 2003). Monoclonal antibodies (MAbs) developed in 1975 by Georges Kohler (Kohler, G and Milstein, C., 1975) have been applied in the fields of Immunology, Biotechnology, Biochemistry, Applied Biology and Pharmaceutical application.

## Protein "A"

Staphylococcal protein "A" is an IgG binding protein endured in secreted and membrane-associated form. It is a 42 Kilo Dalton protein (Sigma Product information). There are 4 Fc binding, highly homologous regions, each constituting of 58 to 62 amino acids. Staphylococcal protein-A binds to most mammalian IgG (Table I) and can be employed to recognize or purify such antibodies

Affinity chromatography on Staphylococcal Protein "A" column is extensively used for purifying Monoclonal and Polyclonal antibodies (Waliza Ansar and Shyamasree Ghosh., 2013) from biological fluids and cell culture media. Advantages of Protein-A column are (i) high

immunoglobulins binding capacity (ii) high flow rate with good resolution and (iii) highly stable column to all physical changes. The present study was aimed to evaluate the varying affinities of Staphylococcal Protein "A" towards IgG in serum samples from different species

## Materials and Methods

### *Serum Collection*

Human blood sample was obtained from a healthy donor after taking his consent and informing him about the purpose of our study. Blood samples from rabbit, pig, sheep, goat and chicken were collected from Chengicherla Abattoir located in Chengicherla village near Hyderabad, Telangana, India.

Dog blood sample was obtained from veterinary hospital, Sainikpuri, Secunderabad.

### Purification of IgG from Serum Samples of Different Species

Most commercially available affinity chromatography resins for purification of IgG are Protein "A" based (Hahn *et al.*, 2003; Hahn *et al.*, 2005 and Hahn *et al.*, 2006). IgG from serum samples of different species were purified by the method described in Protein "A" Agarose Affinity Chromatography kit supplied by Bangalore Genie Pvt Ltd, Bangalore India and Ramesh Kumar K (2014) (Ramesh Kumar K., *et al.*, 2014). All experiments were carried out in duplicates.1 ml fractions (total of 10 fractions) were collected and absorbance for the IgG present in each fraction was measured at 280 nm.

### Determination of IgG Concentration in Protein A Fractions Obtained from Serum Samples of Different Species

The protein concentration in the individual Protein "A" fractions were calculated according to the method published in Protein "A" Agarose Affinity Chromatography kit, Bangalore Genie Pvt Ltd, Bangalore India.

## Results and Discussion

### Purification of IgG from Serum Samples of Different Species

IgG from serum samples of different species were purified on Protein "A" Agarose affinity column and the absorbance values for each fraction is given in (table II). Elution profile for IgG purified from pig, human, rabbit, dog, goat, sheep, buffalo and chicken serum samples are shown in figures 1 to 8. As evident from the results presented in figures 1 to 8, IgG from serum samples of different species eluted as a single major peak and fraction number 2 in all the Protein "A" fractions manifested the highest absorbance values among the 10 fractions that were collected.

**Fig.1** Elution Profile of IgG Purified from Pig Serum Sample

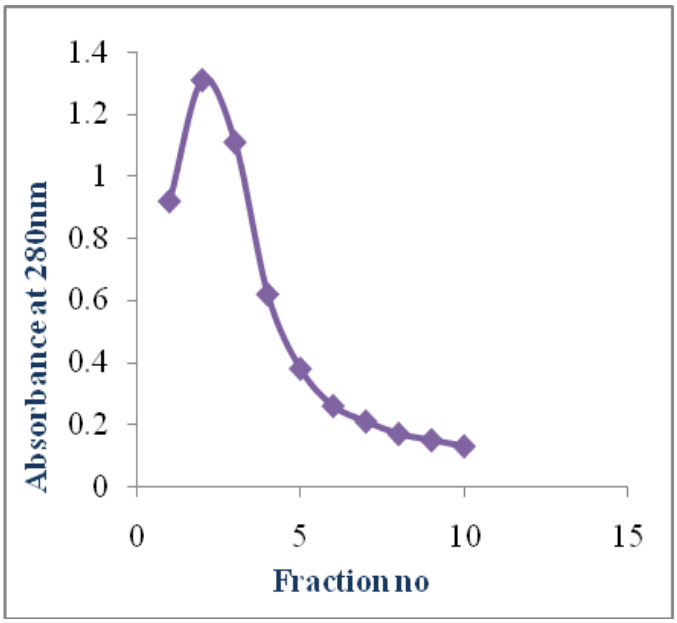


**Fig.2** Elution Profile of IgG Purified from Human Serum Sample

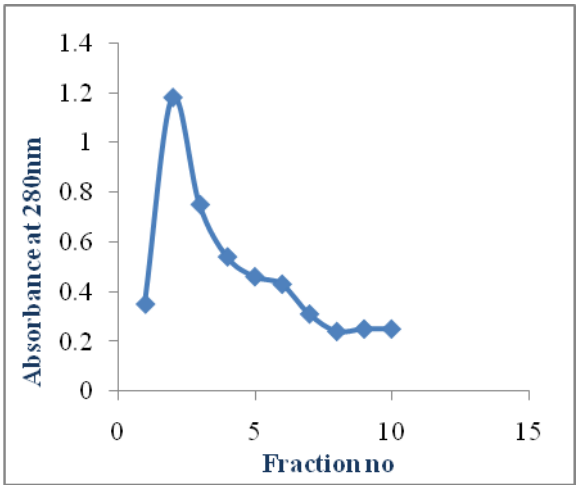



**Fig.3** Elution Profile of IgG Purified from Rabbit Serum Sample

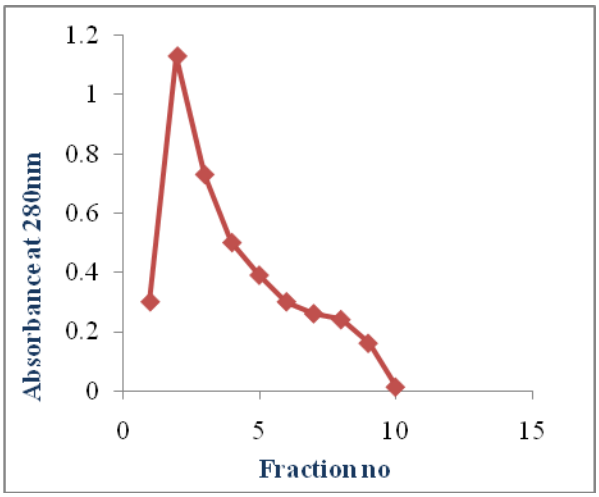



**Fig.4** Elution Profile of IgG Purified from Dog Serum Sample

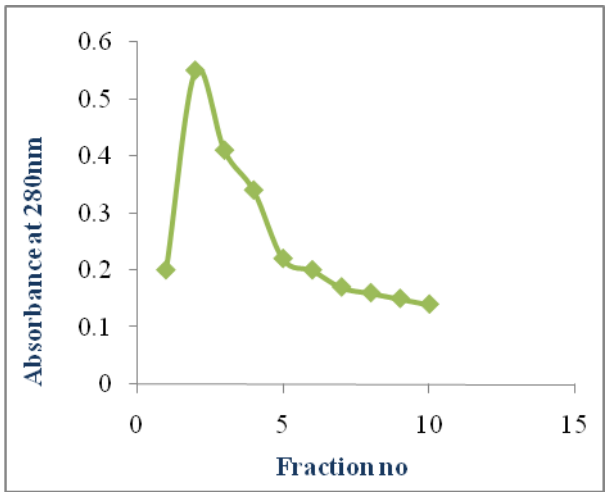


**Fig.5** Elution Profile of IgG Purified from Goat Serum Sample

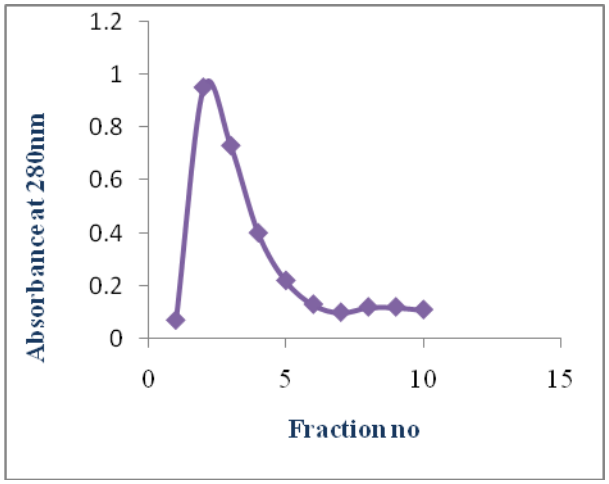



**Fig.6** Elution Profile of IgG Purified from Sheep Serum Sample

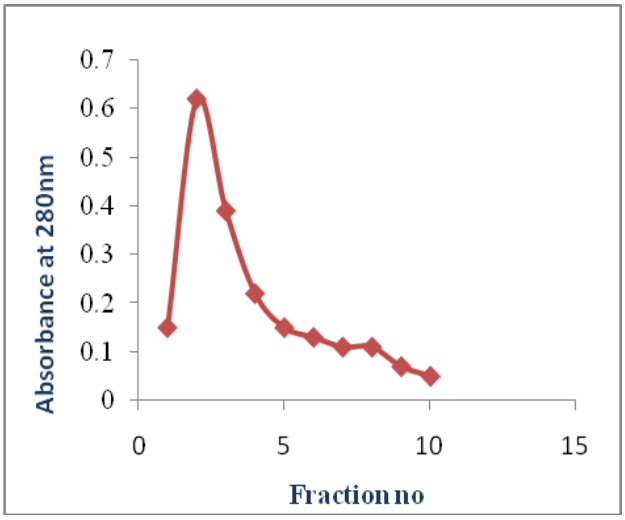



**Fig.7** Elution Profile of IgG Purified from Buffalo Serum Sample

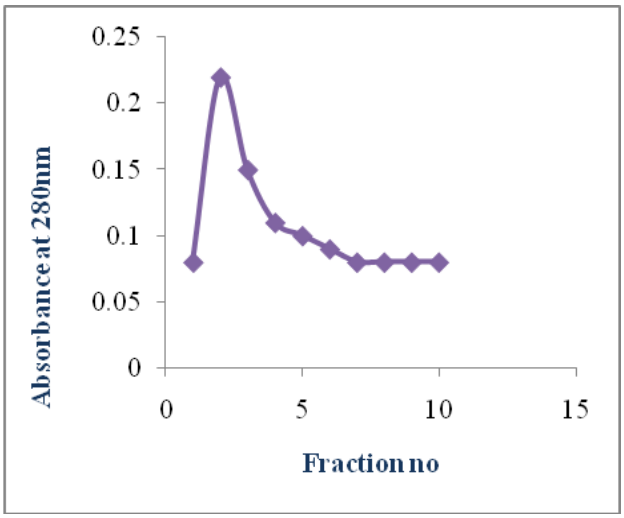
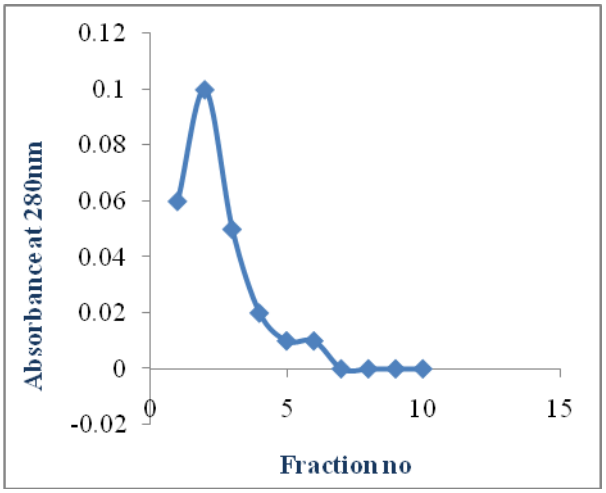


**Fig.8** Elution Profile of IgG Purified from Chicken Serum Sample




Serum sample (0.5 ml serum + 0.5 mL equilibration buffer) was loaded on protein A column that was previously equilibrated with equilibration buffer (PBS pH 7.4). Unbound materials were removed by washing and bound IgG was eluted with 0.1 M citrate buffer pH 4.6 as 1 ml fractions. A total of 10 fractions were collected. $A_{280}$ for each fraction was measured in a UV–Vis spectrophotometer and a graph was plotted with fraction number on X axis versus absorbance values on the Y axis.

**Fig.9** Concentration of IgG Purified from Serum Samples of Different Species (mg/mL)

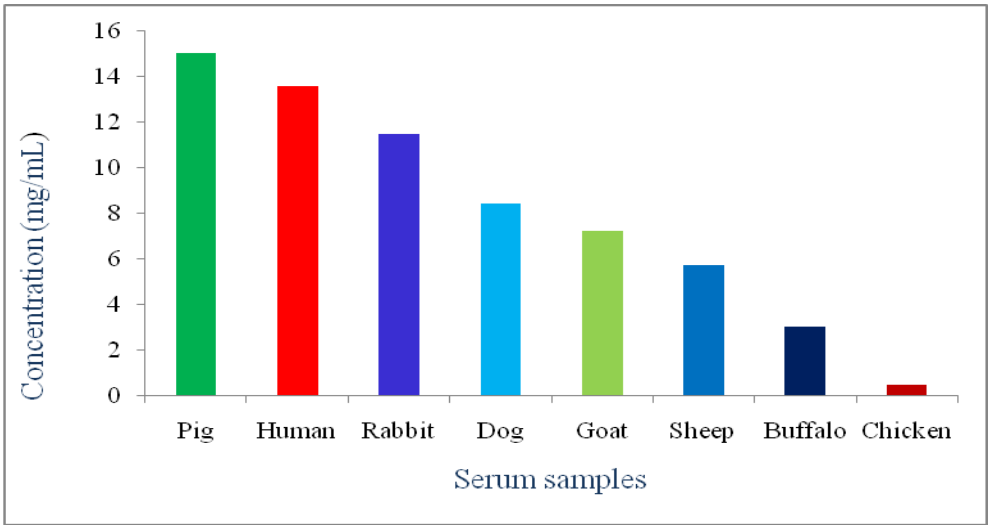



**Table.1** Relative binding affinity of IgG's From Different Species

| Species | Relative binding affinity IgG's (**Polyclonal)** |
|---|---|
| Human IgG | +++ |
| Rabbit | +++ |
| Pig | +++ |
| Dog | ++ |
| Buffalo | |
| Sheep | + |
| Goat | + |
| Chicken | - |

+++ Strong, ++ medium, + weak and - weak or no interaction

**Table.2** Absorbance Values for Protein Fractions Obtained for Different Species on Protein "A" Affinity Column

| Fraction. No | Pig serum $A_{280}$ | Human serum $A_{280}$ | Rabbit serum $A_{280}$ | Dog serum $A_{280}$ | Goat serum $A_{280}$ | Sheep serum $A_{280}$ | Buffalo serum $A_{280}$ | Chicken serum $A_{280}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.92 | 0.35 | 0.3 | 0.20 | 0.07 | 0.15 | 0.08 | 0.06 |
| 2 | 1.31 | 1.18 | 1.13 | 0.55 | 0.95 | 0.62 | 0.22 | 0.1 |
| 3 | 1.11 | 0.75 | 0.73 | 0.41 | 0.73 | 0.39 | 0.15 | 0.05 |
| 4 | 0.62 | 0.54 | 0.5 | 0.34 | 0.40 | 0.22 | 0.11 | 0.02 |
| 5 | 0.38 | 0.46 | 0.39 | 0.22 | 0.22 | 0.15 | 0.10 | 0.01 |
| 6 | 0.26 | 0.43 | 0.3 | 0.20 | 0.13 | 0.13 | 0.09 | 0.01 |
| 7 | 0.21 | 0.31 | 0.26 | 0.17 | 0.10 | 0.11 | 0.08 | 0 |
| 8 | 0.17 | 0.24 | 0.24 | 0.16 | 0.12 | 0.11 | 0.08 | 0 |
| 9 | 0.15 | 0.25 | 0.16 | 0.15 | 0.12 | 0.07 | 0.08 | 0 |
| 10 | 0.13 | 0.25 | 0.012 | 0.14 | 0.11 | 0.05 | 0.08 | 0 |

Serum sample (0.5 ml serum + 0.5 mL equilibration buffer) was loaded on protein "A" column that was previously equilibrated with equilibration buffer (PBS pH 7.4). Unbound materials were removed by washing and bound IgG was eluted with 0.1 M citrate buffer pH 4.6 as 1 ml fractions. A total of 10 fractions were collected. $A_{280}$ for each fraction was measured in a UV–Vis spectrophotometer.

**Table.3** Concentration of IgG Purified from Serum Samples of Different Species (mg/mL)

| Pig | Human | Rabbit | Dog | Goat | Sheep | Buffalo | Chicken |
|---|---|---|---|---|---|---|---|
| 15.04 | 13.6 | 11.48 | 8.44 | 7.24 | 5.72 | 3.04 | 0.48 |

## Determination of IgG Concentration in Protein "A" Fractions Obtained from Serum Samples of Different Species

Concentrations of IgG purified from serum samples of different species are presented in (table III) and figure 9. The highest concentration of IgG was observed in the case of pig serum sample and lowest IgG concentration was observed in the case of chicken serum sample. The descending order of IgG concentrations in the purified protein "A" fractions from serum samples of different species is pig > human > rabbit > dog > goat > sheep > buffalo > chicken respectively.

Our result and those reported in the literature clearly demonstrate the varying affinities of Protein "A" towards IgG in serum samples from different species. The descending order of affinity of Protein "A" for IgG from serum of different species are pig > human > rabbit > dog goat > sheep > buffalo > chicken. Our results are in accordance with those reported in the literature (Boyle, M. D. P. and K. J. Reis., 1987).

## Conflict of interest statement

Author declares that there is no conflict of interest.

## Acknowledgment

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