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# Production and potential applications of Egg Yolk antibodies (Igy) as anti-bacterial prophylactic uses for infectious diseases

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**Abstract**
Immunoglobulin Y (IgY) is the major antibody produced by chickens (*Gallus gallus domesticus*). It is continually synthesized at a large scale, secreted into the blood and transferred to the egg yolk, where it accumulates. To obtain specific IgY antibodies against an antigen of interest, egg-laying hens are immunized with the antigen; following this, antibodies are purified from their egg yolks. IgY purification is an easy, fast and low-cost isolation process that produces a high quantity of specific antibody. IgY can be used in various immunological assays and may offer better results than traditionally used mammalian antibodies The yolks of eggs laid by immunized chicken have been recognized as an excellent source of polyclonal antibodies for over a decade. This simple noninvasive approach presents an appealing alternative to conventional polyclonal antibody production methods. This review offers summarized information about egg yolks from immunized hens may be a reliable source for antibody production, which can be employed for immunological studies.

**Keywords:** Immunoglobulin Y; Antibody; Chicken eggs; Immunization

## Introduction

Chickens are potent antibody producers that can serve as a successfül substitution of marnrnals. Chickens offer several advantages in terms of economical and practical aspects. Nowadays, most frequently chosen mammals for polyclonal and monoclonal antibody production are rabbits and mice respectively. Both technologies have their advantages but also disadvantages. Major problem of monoclonal antibody production is that some antigens are weakly or not at all immunogenic for mice. In polyclonal antibody production purification of antibodies from mammalian blood has been found to be low yielding and laborious in many cases. Both technologies also involve some steps each of which causes distress to the animals involved i) the immunization itself, ii) collecting of blood samples and iii) bleeding, which are a prerequisite for antibody preparation (Mojca Narat, 2003) [16].

Mamrnals such as rabbits, mice, sheep and goats are traditionally the rnost cornmon sources of antibody production that require an adequate amount of antibodies with high specificity and high avidity (Warr, 1982) [25]. Chickens are recognized as a potential alternative for antibody production since they rneet the above requirements and moreover have advantages over marnmals. During the past 20 years, the use of chickens instead of mammals for antibody production has increased. A major advantage of using birds is that the antibodies can be harvested from the egg yolk instead of serum, thus making blood sampling obsolete. In addition, the antibody productivity of an egg-laying hen is much greater than that of a similar sized mammal (Hau & Hendriksen, 2005) [10]. Purification of immunoglobulin from mammalian blood is time-consuming and expensive. Today, hens are recognized as a convenient and inexpensive source of antibodies.

## Antibodies from eggs

Immunoglobulins (antibodies) can be readily produced in eggs by immunising hens against specific antigens, serum antibodies of hyperimmunised hens are efficiently transferred and accumulated in the egg yolk. These Immunoglobulins can have broad applications from developing immunoassays to treating disease. Researchers have used egg antibodies in passive immunotherapy to treat a range of other diseases from bovine rotavirus in cattle to Mastitis in dairy cattle (Coleman, 1998). Antibody production in eggs is particularly advantageous because hens can be effectively immunised, antibodies are readily deposited in the yolk, and eggs are a convenient and inexpensive food source. IgY is successfully used in medical immune testing, diagnosis, heterografts and therapy. The use of chicken IgY in a double antibody sandwich ELISA for detecting African horse sickness virus by Du-Plessis *et al.* (1999) [4].

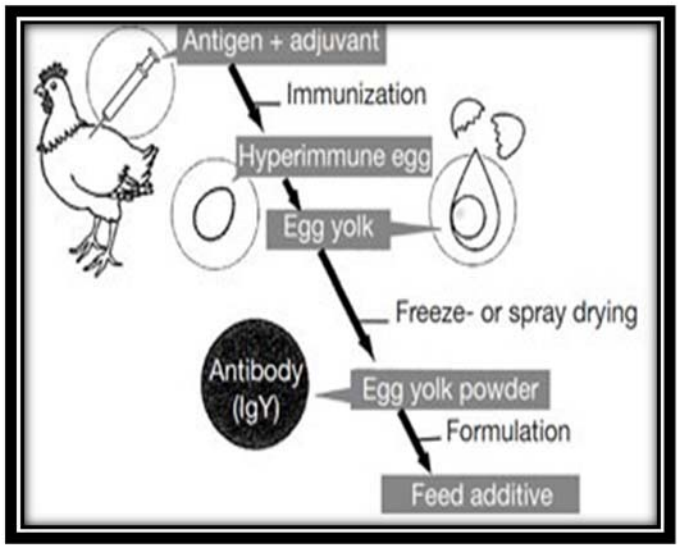
New vaccine technology has led to vaccines containing highly purified antigens with improved tolerability and safety profiles, but the immune response they induce is suboptimal without the help of adjuvants. Gottstein and Hemmeler, 1985 reported Chickens store high contents of IgY in the yolk and are considered to be efficient antibody producers.

## Immunoglobulins

The discovery and use of antibiotics and vaccination in animal agriculture have evolved from the management of small poultry flocks in the era prior to 1890s (Wehman, 1892) [26] to the large consolidated units of today. The antibodies present in egg yolk have been termed IgY (Hatta *et al.*, 1990) [9]. Thus, it is possible to obtain pathogen-specific IgY antibody from eggs laid by hens immunized against antigen (Shimizu *et al.*, 1988) [21]. Since poultry farming is carried out on a large scale globally, eggs may be a suitable source of antibody for passive immunization, which requires large amounts of antibodies.

Over the past few years, we have successfully used the chicken egg yolk system to produce polyclonal antibodies to enamel proteins and other calcified tissue matrix proteins (Nanci *et al.*, 1996) [17]. Furthermore, the amount of antibodies produced from an egg is equivalent to that from 200 to 300 ml of mammalian blood, and the costs for animal care per unit production of antibodies are much lower in chicken than in mammals. However, the practical use of IgY in research and diagnostics is limited due to complex and time-consuming purification steps associated with the further purification of IgY (Akita and Nakai, 1992) [1].

**IgY production**




**Isolation and purification methods for IgY**

Several methods were described in the 1950s for purifying IgY based on the strategy of separation of proteins (levitins) from lipoproteins (lipovitellins) and the rest of the yolk lipids using extraction with organic solvents with rather low yields of antibody. However, purification methods based on organic solvents like chloroform remain in use. Other methods are based on affinity chromatography or on dilution of the yolk followed by a freezing-thawing process after which the process consists of ion exchange chromatography and salt precipitations often combining a number of salts like for e.g. polyethylene glycol (PEG), dextran sulfate, dextran blue, sodium sulfate, ammonium sulfate, caprylic acid and sodium citrate. Hatta (1990) [9] reported that the IgY remaining in this supernatant was isolated by DEAE-Sephacel column followed by salting-out with sodium sulfate resulting in almost pure IgY (98%) and the yield was 70-100 mg per egg. Water dilution method found to be superior in terms of ease of use and large scale production of IgY. This is simple rapid and efficient means of purifying IgY with high activity (Akita & Nakai, 1993) [2].

**Properties of IgY**

Laying hens transfer large amounts of immunoglobulin from serum to egg yolk of their eggs, where it serves as a means of passively protecting the developing chicks (Kariyawasam *et al*., 2004) [12]. An average egg may contain 100~150 mg of yolk immunoglobulins (IgY), and substantial amounts of specific antibodies may be collected and purified from the eggs of immunized hens (Akita and Nakai, 1992) [1].

**Antibody application**

The existence of antibody in humans and anirnals is one of the marvelous phenornena in the biological world. The function of antibody as a defence mechanisrn is so complicated that incessant studies have been long carried out to unveil antibody related facts. On the basis of knowledge about the reaction of antibody with antigen, antibody has been used for a wide range of applications. In other words, the binding activity of antibody against antigen, which is the first step to process antigen, has made it possible to develop antibody technology in many areas such as biological and medical science. Various antibody sources, including colostrum of cows, miIk and blood of animals, thus, have been explored to obtain antibody suitable for different applications.

**Applications of IgY in Human and Veterinary Medicine**

Chicken egg yolk is also a valuable source of antibody, possessing advantageous characteristics for broad application. Food science and product technology is a potential area in which chicken egg yolk antibody (IgY) may be practically applied due to increasing interest in value-added foods and the applicability of food-based IgY. Therefore, the imrnunological property of IgY, which can serve as 'value' in foods, needs to be investigated to provide more evidence for IgY application.

IgY Abs are used successfully in immunohistochemistry for detection of antigens of viral, bacterial, plant and animal origin, and also to assess the incidence of intestinal parasites in domestic animals Schniering, (1995) [20] and the contamination of foods with toxins or drugs Pichler *et al.*, 1998. [18] During the past decade, IgY Abs have increasingly been used in therapy or prophylaxis of disease and also in the new context of socalled “functional food”.

The availability of large amounts of relatively inexpensive IgY from egg yolks makes it feasible to use these antibodies for passive immunization by oral administration or injection (Carlander *et al*., 2000) [3]. The efficacy of this approach has been shown in human and veterinary medicine for rotavirus diarrhea in humans. In aquatic species, IgY against *Edwardsiella tarda* was administered orally to passively immunize Japanese eels. These studies demonstrated that IgY could serve as an effective means against bacterial and viral infections (Van Nguyen *et al*., 2006) [24].

**Applications of IgY in Biomedical Research**

Powdered whole eggs or yolks have been used as an inexpensive alternative for the IgY treatment of enteric diseases in veterinary medicine. The most famous example of a successful therapeutic/prophylactic use of IgY is the treatment of calves and piglets with specific Abs against *Escherichia coli*, rotaviruses and coronavirus Ebina, (1996) [5]. Studies using both animal models and trials in field herds have been carried out. These studies confirmed that treatment of diarrhoea in calves and piglets with specific egg yolk Abs has achieved significant prophylactic and therapeutic benefits. Sunwoo *et al*. 2002 were able to demonstrate *in vitro* a marked growth inhibiting effect of specific IgY on *E. coli* 0157:H7, and showed that the growth inhibition was actually caused by the binding of specific IgY to the bacterial surface antigens, which caused significant changes in the bacterial surface structure.

Another effect of IgY binding to bacterial surface antigens is a marked impairment of bacterial attachment to the intestinal mucosa Lee *et al*., 2002. Thus, therapeutic IgY administration could reduce the clinical use of antibiotics, and so could lower the risk that bacteria will develop antibiotic resistance.

**Applications of IgY in Bacterial growth inhibition**

Monoclonal antibodies which are raised against one particular epitope of antigen have been utilized for a wide range of studies. Their characteristic of recognizing only one target epitope can be advantageous to studies on characterizing specific rnolecules of an antigen in cornparison to polyclonal antibodies. There have been some reports on bacteriostatic or bacteticidal effects of monoclonal antibodies against particular molecules possibly associated with bacterial growth (Yamaguchi *et al*., 1997; Lin *et al*., 1998) [27, 14]. In other words, the reaction of antibodies with bacteria could result in the inhibition of bacterial growth *in vitro* without complement or phagocytosis.

**Future appIications**

Today, there is no doubt that chicken Abs can be produced and used, with minor modifications, in similar ways to the use of mammalian Abs. It is to be expected that studies on the therapeutic or prophylactic use of IgY Abs will be intensified in future. In particular, because of the increasing resistance of microorganisms to antibiotics, research on all aspects related to the development of specific IgY against pathogenic microorganisms will have to be intensified. IgYs can be used both in veterinary medicineand in human medicine.

Advantageous properties as well as existing applications of IgY have spurred extensive studies to explore the potential of IgY for possible applications. In a recent study, the inhibitory effect of IgY on pig to-human xenograft rejection was demonstrated, showing the possibility of IgY application in pig-to-hurnan xenotransplantation (Fryer *et al.*, 1999) [6]. The success of monoclonal antibody-based immunotherapy in colorectal cancer as reported by Holz *et al*. (1996) [11] rnay suggest an additional future application of IgY. Accordingly, the range of areas in which IgY can be applied may be remarkably extended as studies in progress provide more results.

- Economical and practical irnmunization of antibody in donor animal;
- Determination of most immunogenic antigen to raise most effective antibody;
- Large-scale production of antibodies in large quantities;
- Sterile, safe, and stable preparation without loss of antibody activity;
- Available measurement of effective amount of specific antibody;
- Synergistic effect of antibody;
- Adverse effect of antibody;
- Long-term and stable storage.

**Conclusion**

IgY technology more popular and to convince the scientific community of its significant advantages. Chickens have the potential to be used to complete the spectrum of animals used for Ab production. The significant potential of avian antibodies for further use in immunodiagnostics and identification of disease markers, immunotherapy and the treatment and prevention of disease is expected. Since lot of benefits of IgY technology and its universal application in both research and medicine, it is expected that IgY will play an increasing role in research.

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