Review Article

International Journal of Clinical and Medical Case Reports

An Overview of The Production, Extraction And Applications of Egg Yolk Antibody (IgY)

Hadis Gandomkar[1], Mir Mohammad Reza Hosseini[2] and Ahmad Zavaran Hosseini[1*]

[1]Department of Immunology, Factually of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[2]Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Corresponding Author:**
Ahmad Zavaran Hosseini,
Department of Immunology, Factually of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: zavarana@modares.ac.ir

Received Date: 29 April 2024
Accepted Date: 06 May 2024
Published Date: 13 May 2024

Citation:
Ahmad Zavaran Hosseini. An Overview of The Production, Extraction And Applications of Egg Yolk Antibody (IgY). International Journal of Clinical and Medical Case Reports 2024.

1. Abstract

Hens produce a class of antibodies called IgY whose natural role is to protect the chicken and the growing chick inside the egg. The overall structure of the IgY molecule is similar to mammalian immunoglobulin. Egg yolk antibody (IgY) can be a suitable alternative to IgG due to its unique characteristics, cost-effectiveness, and compliance with the ethical principles of working with animals. The process of immunization of chickens, production of specific antibodies against antigens, and purification of IgY is called IgY technology. In this review article, we will investigate how to produce IgY antibodies, immunization in chickens, various methods of antigen administration (muscular, subcutaneous, intravenous, etc.), IgY extraction from egg yolk, and its purification. Finally, some diagnostic and therapeutic applications of this antibody have been investigated.

2. keywords:
Egg yolk antibody, IgY technology, Diagnostic, Therapeutic applications

3. Introduction

Antibodies are an important tool in biomedical research and diagnostic methods, but unfortunately, the production of these proteins is expensive and many countries cannot manufacture them on a large scale. Historically, polyclonal antibodies have been produced in rabbits and other rodents such as mice and guinea pigs, as well as farm animals such as horses, sheep, and goats; Their production process includes aggressive methods of immunization process, frequent injections, and blood sampling, which in some cases is accompanied by bleeding and on the other hand, it causes an immune response in humans. In recent years, by creating humanized mouse antibodies or using hybridoma lines, many immunogenic issues have been solved, but it has added additional cost to the production of these proteins. This is inefficient for producing antibodies against high-volume proteins. IgY antibody is present in high volume in bird eggs and the cost of its extraction and production is cheap (1-4). Hens produce a class of antibodies called IgY whose natural role is to protect the chicken and the growing chick inside the egg. Although chicken IgY is the usual molecular model for IgY antibody demonstration; This antibody is also present as the dominant serum immunoglobulin in other species of birds, amphibians, and reptiles (4-7).

In 1893, German physician Felix Klemper designed an experiment that showed that acquired immunity against tetanus toxin was transferred from chickens to egg yolks. For the first time in 1969, by Klemm et al., based on the physico-chemical and antigenic characteristics and insufficient similarity with other human immunoglobulins, the name IgY was given to the egg yolk antibody (8, 9). The term “IgY technology” was first used in 1995. The process of immunization of chickens, production of specific antibodies against antigens, and purification of IgY is called IgY technology. This technology has made great progress in recent years and has good potential in the field of medical use; It is also recommended by the European Center for the Validation of Alternative Methods (ECVAM) as an internationally standardized technology to replace mammalian IgG antibody for animal welfare purposes (10-12). The overall structure of the IgY molecule, similar to mammalian immunoglobulin, has two light chains and two similar heavy chains. The heavy chain is represented by the letter υ or Y and consists of one variable domain and four constant domains (IgG has three constant domains and is represented by the letter γ) (13-15).

3.1. IgY antibody production

Chickens have 3 classes of immunoglobulins (IgM, IgY, IgA). IgY is present in blood and eggs. Birds have a unique two-step transport system to transfer IgY from the mother’s circulation to the egg. Eggs are used instead of blood due to the ease of extracting antibodies from eggs compared to blood, as well as the observance of ethical principles and animal welfare (17, 20). The production process of IgY antibodies from chickens includes various steps such as immunization of chickens,

collection and separation of egg yolks, and extraction and purification of antibodies (17).

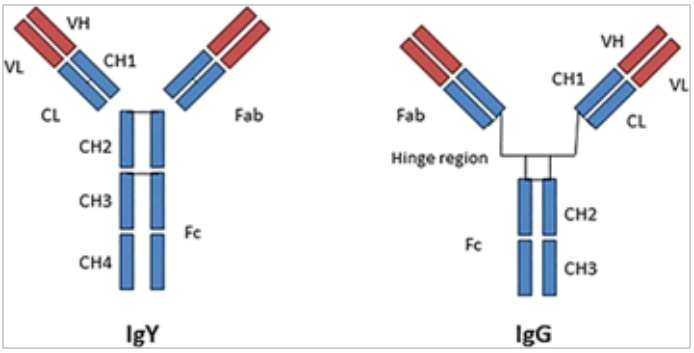

Figure 1: Structural comparison of IgY and IgG antibodies (16).

Some of the features and advantages of IgY antibody, based on which this technology can be used as an alternative in research, diagnostic, and therapeutic approaches, are :

1. Antibody extraction from egg yolk is a non-invasive method to preserve the issue of animal welfare and ethics (17).
2. IgY antibody naturally accumulates in high amounts in egg yolk, and each hen lays an average of one egg per day; As a result, antibody production in this way is cost-effective (17).
3. IgY antibody, compared to its mammalian counterpart (IgG), has unique features such as not binding to the Fc receptor and rheumatoid factor and mammalian complement proteins (17).
4. Birds separated from mammals more than 300 million years ago, while mammals share a common ancestor of 98 million years; These results indicate less homology and greater immunization between the two groups, and the possibility of obtaining antibodies against highly conserved mammalian proteins as well as against proteins that normally escape the mammalian immune system (17).

3.2. Immunization in chickens:

Immunization in chickens is a critical step in the process of producing biologically active antibodies with high specificity in large quantities. Factors such as the breed of chicken and its rearing conditions, type and source of antigen, methods of antigen inactivation, amount of antigen used, type of adjuvant, choice of route of antigen administration, and intervals between immunizations need to be carefully considered during the design of a study (17). Antibody titers should be carefully monitored during the immunization period and the immunization schedule adjusted according to the observed antibody response (21, 22).

3.3. Antigen sources:

Proper design of high-quality antigens is a prerequisite to obtaining high titer-specific IgY antibodies. Immunogenicity (ability to induce humoral and cellular immune responses) and antigenicity (specific recognition by antibodies produced in the immune response) are two desirable antigenic properties for the development of high-performance antibodies. Things like complete bacteria or viruses, virus-like particles, haptens, recombinant proteins, etc. can be used as antigen sources (22-31).

Table 1: Comparison of some characteristics of IgY (chicken), IgG (rabbit) and IgG (human) antibodies

Reference	IgG (Human)	IgG (Rabbit)	IgY (chicken)	Specificity
-17	Blood, milk	Blood	Egg	Antibody source
(18, 19)	IgG1: 9 mg ml-1 from serum	5 mg ml-1 of blood	50-100 mg per egg yolk	Average antibody
	IgG2: 3 mgml-1			
	IgG3 and IgG4: 0.5 and 1mg ml-1			
	Total plasma concentration: 13.5mg ml-1			
-17	----------	200 mg per rabbit	1000-2800 mg per chicken	Antibody extracted monthly
-17	150	150	180	Molecular weight (kilodaltons)
(17, 19)	Yes	Yes	NO	Binding to mammalian complement or Fc acceptor
(17, 19)	Yes	Yes	NO	Cross reaction with rheumatoid factor
(17, 19)	Yes	Yes	NO	Cross-reactivity with human anti-mouse antibody
-17	Yes	Yes	NO	Reaction with hetero-agglutinins

4. Antigen administration methods

Due to the biological, anatomical, and physiological features of bird species, there are different ways of immunization in chickens, such as intramuscular injection (IM), subcutaneous injection (.S.C), intravenous (.I.V), nose and eye drops, oral and aerosol drops. The best route of administration should be chosen according to the characteristics of the antigen and the nature of the adjuvant. The most common method of immunization in chickens is injection in several points. Although intramuscular injection is the most widely used method of antigen administration to chickens; Other methods such as shotgun immunization and group immunization are also increasingly being used (32-36). During a study in 2005, specific IgY antibody titers were compared after antigen

injection through S.C., I.M., and I.V.; And it was shown that a higher titer of antibody is produced in subcutaneous injection than in intramuscular injection. Intravenous route should be used without adjuvant. The combination of intramuscular injection and finally intravenous injection often significantly increases the IgY titer. To avoid an anaphylactic reaction, the intravenous injection (without adjuvant) should be done very slowly (approximately 500 microliters in 15 minutes) (32).

Group immunization generally includes oral immunization, immunization with nasal drops, immunization with drinking water, and immunization with aerosol drops. Immunization by nasal drops is usually used to stimulate the body to produce local and systemic antibodies (through mucosal absorption). This method is mostly applicable to live attenuated vaccines (32, 35). Immunization by gene gun: the basis of this method is high-speed and high-pressure injection of foreign DNA coated on gold and tungsten particles. The skin of the breast and thighs of chickens is suitable for this purpose. The feathers of the target area should be removed and then the superficial stratum corneum should be scraped with a razor and the area should be disinfected with alcohol. The whole process must be done in a dry and clean environment. The advantages of gene gun technology include its simplicity, the ability to quickly and easily transfer genes, and the need for a small number of therapeutic genes. The antibody level in chicken blood after immunization with a gene gun is 1.5 times higher than this level in intramuscular injection; While the amount of DNA used in intramuscular injection is only 3% of this amount (36-38).

4.1. Extraction and purification of IgY antibody:

Obtaining IgY antibody from high-purity egg yolk is a multi-step process. The first step is to remove lipids from the yolk, the second step is to extract IgY from the remaining mixture, and the final step is to use chromatographic methods (17). The egg yolk is surrounded by a two-layer vitelline membrane. Yolk content is approximately 48% water, 33% lipid, and 17% protein. After dilution and low-speed centrifugation, the yolk is divided into two main parts, the larger part of which is plasma and the smaller part is formed by granules. IgY antibody is located in the plasma part. Some features of IgY antibody that are important in extraction are molecular weight, isoelectric pH, solubility, stability against pepsin, and stability at specific temperature and pH (39-41).

4.2. Separation of yolk from egg white:

The first step in extracting IgY from the yolk is to separate the yolk from the egg white (which mainly contains protein). First, the shell is carefully broken and the yolk is separated using an egg separator (40, 42-44).

4.3. Delipidation of egg yolk:

One of the main challenges in extracting IgY from egg yolk is the removal of lipids. The general strategy for extracting IgY from the yolk involves a preliminary step in which the granules are removed by one of several methods, leaving the IgY in the plasma fraction (17, 41). Available methods for delipidation include dilution with water, use of polyethylene glycol (PEG), precipitation with anionic polysaccharides, extraction with organic solvent, and use of special chemicals (17, 45-51).

4.4. IgY extraction after delipidation:

After delipidation, what remains contains IgY antibodies, other water-soluble proteins, and some small lipids or lipoproteins. It is possible to remove the remaining lipoproteins by adding 0.1% charcoal at pH=4. The main protein components of plasma include IgY, livetin-α (chicken serum albumin), and livetin-β (alpha 2 glycoprotein). The available methods for the purification of IgY after delipidation include salt precipitation, polyethylene glycol precipitation, filtration, two-phase aqueous systems, and chromatography (hydrophobic, gel filtration, affinity, and thiophilic) (52-62).

5. Diagnostic and therapeutic applications of IgY:

5.1. ELISA method design:

Immune System AB is a small Swedish biotech company working with the production of avian antibodies. Started in 1983, the company initially focused on the production of avian antibodies for diagnostic purposes, and for the past 20 years has focused on the use of avian antibodies for therapeutic applications. Immunological determination of protein A in solution containing IgG presents significant challenges, especially when using mammalian detection antibodies because they compete with antibodies in solution for binding to protein A. One of the few antibodies that does not bind to protein A is IgY. This company has developed an ELISA test to determine protein A based on chicken antibody affinity. In this sandwich ELISA, a chicken anti-protein A antibody acts as capture, and another chicken anti-protein A antibody acts as detector. This method can detect less than 1 ng/ml of protein A both in IgG-free solutions and in solutions containing milligram amounts of IgG. The production of this kit has recently been transferred to IgY Lab Systems AB (63, 64).

5.2- Helicobacter pylori:

Helicobacter pylori is a gram-negative human pathogen that infects more than 50% of the population worldwide, and the level of infection reaches more than 70% in developing countries (Bravo et al. 2018). This bacterium can cause gastrointestinal cancers, gastrointestinal ulcers, and gastritis. One of its pathogenic mechanisms is the production of urease enzymes and the neutralization of stomach acidity. VacA, CagA, OipA, NapA, HpaA, and FlaA, B are some of the genes involved in its pathogenicity. Antibiotic treatment can effectively eradicate Helicobacter pylori infections, but in 10-20% of cases of multi-drug resistance, it causes the infection to persist, and as a result, alternative methods are needed. One such alternative is passive immunization by oral administration of IgY developed against Helicobacter pylori (IgY-Hp). In vivo studies, the outstanding effects of antibacterial IgY antibodies have been widely evaluated in human volunteers and rodent models, and also by in vitro studies, the effect of IgY on host tissues and cells or bacterial growth and virulence factor activities has been evaluated. It has been shown that anti-Helicobacter pylori IgY effectively inhibits its growth and increases bacterial agglutination in vitro. In addition, it strongly inhibits the urease

activity associated with Helicobacter pylori and ammonia production, as well as the cytopathic effect of this bacterium on cultured cells. The development of foods and drinks that are combined with anti-urease IgY has become relatively popular. For example, in a study in mouse models, it was found that the synergistic effect of administering Lactobacillus and Helicobacter pylori anti-urease IgY antibodies was more effective. A clinical study conducted with 42 Helicobacter pylori-positive volunteers showed that regular consumption of 150 ml of yogurt, 3 times a day, for 4 weeks, significantly reduced urea breath test values and also showed that anti-urease IgY It is stable up to 65°C and degraded to only 85% during 3 weeks of storage, so both properties support food industry standardization methods. It has also been shown that oral administration of anti-VacA IgY, in infected female C57BL/6 mice, is associated with a protective effect against Helicobacter pylori colonization and histological changes in gastric tissue (65-71).

5.3- Streptococcus mutans and tooth decay:

Oral diseases affect about 3.5 billion people worldwide, including dental caries, periodontal diseases, oral and pharyngeal cancers, oral lesions of HIV/AIDS origin, oral and dental trauma, cleft lip and palate, noma (severe oral gangrene) and acute necrotizing ulcerative gingivitis (ANUG). Oral diseases lead to pain, discomfort, disfigurement, and even death. Some of them are largely preventable and treatable in early stages, however, treatments are usually expensive. Therefore, low-cost and accessible solutions for the prevention and treatment of oral and dental diseases are essential. Tooth decay is the main oral health disease in all age groups from children to the elderly. Streptococcus mutans is an anaerobic and gram-positive coccus. Its cariogenic potential is mainly due to three features: 1-Extracellular synthesis of glycan polymers from sucrose, which supports local matrix formation and thus microbial colonization. 2- Acidification 3- High resistance to low pH (acidity) which allows it to grow in such conditions. In addition, the unique environmental conditions created help other species to grow and increase activity. Measures such as the widespread use of fluoride in food products or those such as chlorhexidine (CHX) or triclosan are commonly prescribed to prevent or reduce colonization of Streptococcus mutans. Passive immunization against Streptococcus mutans using IgY antibodies has been widely reported. A study by Carlander et al. was one of the first to reveal the persistence of IgY. In this study, IgY antibodies against Pseudomonas aeruginosa were used and it was shown that after washing the mouth with IgY solution in the evening, the next morning, 18 out of 19 people still had active antibodies in their saliva, although the antibody could not be detected 24 hours later of prescription in many people. The effect of mouthwashing with anti-streptococcus-mutans IgY in human volunteers shows a decrease in the ratio of S.mutans to its total in the plaque of treated individuals. In addition, anti-Streptococcus mutans IgY in toothpaste has been effective in reducing primary dental caries in human volunteers, and the use of tablets containing IgY against Streptococcus mutans cell-associated glucosyltransferase (CA-GTF) significantly decreased their numbers in human test groups (72-75).

5.4- Coronavirus or severe acute respiratory syndrome (SARS-CoV2):

Coronaviruses are involved in causing respiratory infections in humans with a range from mild symptoms to fatal pneumonia. To date, three highly pathogenic strains have been the cause of major outbreaks and pandemics, the most recent of which is SARS-CoV-2. In late 2019, SARS-CoV-2 emerged as a new type of coronavirus in Wuhan, China, becoming a global health emergency and socioeconomic crisis requiring the development and implementation of effective diagnostic, preventive, and therapeutic strategies to fight it. The main structural proteins of SARS CoV-2 include spike (S), membrane (M), envelope (E), and capsid (N). SARS-CoV-2 S protein binds to the receptor of angiotensin-converting enzyme2 (ACE2) on human alveolar epithelial cells. Considering the structure and role of protein S in SARS-CoV-2 infection, it is considered the main objective of antibody-mediated neutralization. The potential of chicken IgY to treat COVID-19 has been investigated by several laboratories. Lu et al. used the entire extracellular domain of protein S as an immunogen (amino acids 1-1213). He et al. used the second S1 in a baculovirus-insect cell (BAC) expression system and reported the production of IgY antibodies capable of binding to both SARS-CoV-2 and SARS-CoV. IgY neutralization assay was performed on pseudovirus derived from lentivirus that has S protein on the cover and luciferase reporter gene. Quantification of luciferase reporter gene expression levels in ACE2-expressing Hela cells after pseudovirus infection showed that anti-SARS-CoV-2 S1 IgY antibody effectively neutralized the pseudovirus (76-81).

5.5- Influenza:

Influenza viruses are a group of enveloped viruses containing single-stranded RNA. There are three main types of influenza virus: A, B, and C. Influenza A virus strains infect both humans and animals, rapidly giving rise to potentially lethal variants responsible for widespread outbreaks and pandemics. Influenza vaccines are not 100% effective, but in the past decades, they have played an important role in controlling certain seasonal epidemics and reducing mortality, and various strategies have been used over the years to optimize the vaccine, however, alternative prevention and treatment approaches are needed to combat influenza infection. IgY antibodies have been developed against both influenza A and B. Studies on avian influenza A (A/H5N1) have been performed in human species. Adachi and his colleagues immunized ostriches with two sets of antigens (recombinant H5 proteins from A/H5N1 birds and a mixture of HA antigens from human influenza vaccine strains). Anti-H5 IgY showed potent inhibitory activities against H5N1 and reduced cytopathic effects in MDCK cells and prevented the death of embryonic chicks after viral inoculation. Several studies show consistent and successful results in targeting IgY antibodies against human influenza A/H1N1 as well as A/H3N2 strains (82-85).

5.6- Antitoxin and immunotoxin:

mAb production is one of the most advanced technologies in the pharmaceutical industry. Monoclonal antibodies are now widely used to treat various diseases such as immunodeficiency and cancer. In one study,

phage display technology was used to obtain specific anti-CD19 Nbs from an immune Nb library derived from dromedary camel. This is the first report on the production of nanoparticles against human CD19. Following in vivo analysis, the produced Nbs can be used as research and diagnostic tools as well as in drug delivery systems against B-cell malignancies and autoimmune diseases (86-88). In another study to produce a monoclonal antibody (mAb) against the human epidermal growth factor receptor (EGFR), five 6- to 8-week-old female Balb/c mice were first vaccinated against A431 tumor cells that express more EGFR in their membranes. Fusion of mouse spleen immune cells with SP2/0 cells (myeloma cells) was performed in the presence of polyethylene glycol (PEG) and monoclonal antibody was produced on a large scale in vitro. The results of this experiment showed that such monoclonal antibodies against EGFR can be used in the diagnosis and treatment of tumors with membrane EGFR (86). Immunotoxins (ITs) for cancer treatment are composed of antibodies related to toxins, and as we know, vascular endothelial growth factor (VEGF) plays a key role in tumor angiogenesis, and blocking VEGF-2 receptor (VEGFR2) inhibits angiogenesis and tumor growth. The aim of the study was the production of anti-VEGFR2/rPE 38 IT (Pseudomonas exotoxin) to test its cytotoxic activity and its mechanism of action, the results of which show a decrease in cell viability and an increase in apoptosis, increasing the possibility of using it for cancer treatment (89). Poisons are harmful substances of different chemical nature that are produced in living organisms and cells. Several microbial strains have produced toxins that cause disease in humans, and antitoxins are used to counteract their effects. Currently, antitoxins are prescribed by doctors in clinics that provide treatment for diphtheria, tetanus, botulism, and shock-septic syndrome. Adverse reactions such as serum sickness, anaphylaxis, and the risk of transmission of blood-borne pathogens from mammals to humans pose limitations to antitoxin therapy. Chickens have shown different resistance and responses to toxin immunization, and IgY-based antitoxin can be efficiently produced and overcome some of the limitations of mammalian-derived antitoxins (90).

Enterohemorrhagic Escherichia coli (EHEC) is an important bacterial pathogen that causes several human diseases, including hemorrhagic colitis, diarrhea, renal failure, and hemolytic uremic syndrome (HUS). It has been shown that EHEC produces two types of Shiga pseudotoxins (Stxs) as the main virulence factors associated with pathogenesis (Stx-1 and Stx-2). Normally, Stxs enter the intestinal lumen and then enter the systemic circulation and bind to specific receptors on cells; where they cause cell death. Stxs belongs to the AB5 family of toxins (a catalytic A subunit and a pentameric B subunit). IgY antibody was raised against each of these two toxins (Stx1,2) and was initially shown to block cytotoxicity in Vero or HeLa cells. Further studies showed that IgY protects mice against various levels of toxins. These findings support the idea that anti-Stx IgY antibodies can be used as prophylactic agents for Stx-related diseases in EHEC infections (91-94). Enterotoxigenic Escherichia coli (ETEC) is the main cause of diarrhea in children under 5 years of age in developing countries. Heat-sensitive (LT) and heat-stable (ST) enterotoxins are the main virulence factors of ETEC. In the evaluation of lactating mice, it was shown that IgY antibodies can neutralize the natural toxicity of STa and STb (95, 96).

5.7- Snake venom:

Effective treatment for poisoning is immunotherapy, which is based on the immunization of animals and purification of immunoglobulins, mainly from serum. In 2009, WHO listed snakebite as a neglected disease. The WHO has estimated that 10 million vials of antidote are needed globally each year. As reported by Davey et al., anti-venom antibodies are present in egg yolk up to 100 days after immunization. Against Brazilian snake venom, an IgY antibody was produced that recognizes its toxic and lethal components and inhibits the hemolytic activity dependent on phospholipase A2 (17, 97, 98).

5.8- Cancer:

Biomarkers play an essential role in early detection of cancer. Accurate quantification of specific biomarkers is critical for making sound treatment decisions. In practice, immunohistochemistry (IHC) is the most important diagnostic method to evaluate the expression of protein biomarkers in tissue biopsy. However, IHC is largely qualitative. The low specificity of mammalian IgG antibodies used to capture analytes and the fluorescence instability of organic dyes used as diagnostic agents are among the factors that have hindered the development of quantitative IHC. Avian IgY antibodies have very attractive biochemical, immunological, and manufacturing advantages over IgG and are therefore better alternatives in diagnostic applications. The use of IgY in immunoassays can potentially eliminate false positives and often produces little background and interference (99). Helicobacter pylori is an important risk factor for stomach cancer. Its eradication prevents the recurrence of stomach cancer. Monoclonal antibodies that bind to extracellular epitopes of the domain of growth factor receptors can prevent receptor-ligand interaction and receptor dimerization. It can be assumed that oral IgY does not cause any allergic reactions and can be used as a preventive treatment for gastric cancer caused by Helicobacter pylori or as a general prevention against inflammation caused by this bacterium. In addition, due to its high stability, it can be used as a food supplement, which increases the availability of this therapeutic tool worldwide (14, 100, 101). Glioblastoma is the most common malignant tumor of the central nervous system. Despite research into treatment, the prognosis is poor. Malignant glioma stem cells (MGSCs) are the main cause of treatment failure and increased tumor recurrence. In general, cancer stem cells (CSCs) express prominin-1 (CD133), which is considered as a potential therapeutic target. The design, production, purification, and use of IgY anti-CD133 immunotoxin is the main goal of the research and the results have shown that IgY-abrin immunotoxin has cytotoxic activity against CD133 + MGSCs, which provides a new approach for glioblastoma immunotherapy (102).

6. Conclusion:

The newest findings using IgY have demonstrated the adaptability of this technology. Obtaining IgY from Hens presents several technical

and economic advantages over mammalian IgG, and as described in this review, IgY technology has a wide range of applications in human and veterinary health. Among the advantages of this technology, the replacement of invasive antibody collection by its extraction from eggs is one of the most interesting, considering the animal welfare benefits. Using this technology, large amounts of antibodies can be obtained with lower production costs and less damage to animal health. Due to its structural differences and phylogenetic distance, IgY is more specific for diagnostic use and displays greater avidity for mammalian conserved proteins than IgG, being, therefore, a significant alternative in the search for more effective diagnostics and therapies. In addition, given its proven capacity to neutralize microorganisms, IgY signifies an important therapeutic source in times of increasing resistance to antibiotics and the appearance of viral diseases for which there is no treatment.

References:

1. Porstmann T, Kiessig S. Enzyme immunoassay techniques an overview. Journal of immunological methods. 1992;150(1-2):5-21.
2. Gosling JP. A decade of development in immunoassay methodology. Clinical chemistry. 1990;36(8):1408-27.
3. Carlander D. Avian IgY antibody: in vitro and in vivo: Acta Universitatis Upsaliensis; 2002.
4. LARSSON A, MELLSTEDT H. Chicken antibodies: a tool to avoid interference by human anti-mouse antibodies in ELISA after in vivo treatment with murine monoclonal antibodies. Hybridoma. 1992;11(1):33-9.
5. Leiva CL, Gallardo MJ, Casanova N, Terzolo H, Chacana P. IgY-technology (egg yolk antibodies) in human medicine: A review of patents and clinical trials. International immunopharmacology. 2020;81:106269.
6. Schade R, Schniering A, Hlinak A. [Polyclonal avian antibodies extracted from egg yolk as an alternative to the production of antibodies in mammals-a review][Article in German]. ALTEX-Alternatives to animal experimentation. 1992;9(2):43-56.
7. Tini M, Jewell U, Camenisch G, Chilov D, Gassmann M. Generation and application of chicken egg-yolk antibodies. Comparative biochemistry and physiology part A: molecular & integrative physiology. 2002;131(3):569-74.
8. Klemperer F. ber naturliche immunitat und ihre verwerthung fur die immunisierungstherapie. Archiv fur Experimentelle Pathologie und Pharmakologie. 1893;31:356-82.
9. Leslie GA, Clem L. Phylogeny of immunoglobulin structure and function: III. Immunoglobulins of the chicken. The Journal of experimental medicine. 1969;130(6):1337-52.
10. Russell WMS, Burch RL. The principles of humane experimental technique: Methuen; 1959.
11. Schade R, Staak C, Hendriksen C, Erhard M, Hugl H, Koch G, et al. The Production of Avian (Egg Yolk) Antibodies: IgY: The Report and Recommendations of ECVAM Workshop 211, 2. Alternatives to laboratory animals. 1996;24(6):925-34.
12. Huopalahti R, Anton M, López-Fandiño R, Schade R. Bioactive egg compounds: Springer; 2007.
13. Amro WA, Al-Qaisi W, Al-Razem F. Production and purification of IgY antibodies from chicken egg yolk. Journal of Genetic Engineering and Biotechnology. 2018;16(1):99-103.
14. Müller S, Schubert A, Zajac J, Dyck T, Oelkrug C. IgY antibodies in human nutrition for disease prevention. Nutrition journal. 2015;14(1):1-7.
15. Shimizu M, Nagashima H, Sano K, Hashimoto K, Ozeki M, Tsuda K, et al. Molecular stability of chicken and rabbit immunoglobulin G. Bioscience, biotechnology, and biochemistry. 1992;56(2):270-4.
16. Pereira E, Van Tilburg M, Florean E, Guedes M. Egg yolk antibodies (IgY) and their applications in human and veterinary health: A review. International immunopharmacology. 2019;73:293-303.
17. Zhang X-Y, Vieira-Pires RS, Morgan PM, Schade R. IgY-Technology: Production and Application of Egg Yolk Antibodies: Basic Knowledge for a Successful Practice: Springer; 2021.
18. Murphy K, Weaver C. Janeway's immunobiology: Garland science; 2016.
19. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology, -South Asia Edition-E-Book: Elsevier Health Sciences; 2021.
20. West Jr AP, Herr AB, Bjorkman PJ. The chicken yolk sac IgY receptor, a functional equivalent of the mammalian MHC-related Fc receptor, is a phospholipase A2 receptor homolog. Immunity. 2004;20(5):601-10.
21. Woolley JA, Landon J. Comparison of antibody production to human interleukin-6 (IL-6) by sheep and chickens. Journal of Immunological Methods. 1995;178(2):253-65.
22. He J-X, Thirumalai D, Schade R, Zhang X-Y. Chronobiological studies of chicken IgY: monitoring of infradian, circadian and ultradian rhythms of IgY in blood and yolk of chickens. Veterinary Immunology and Immunopathology. 2014;160(3-4):266-72.
23. Kassim N, Mtenga AB, Shim W-B, Chung D-H. The in vitro and in vivo efficacy of hen IgY against Vibrio parahaemolyticus and Vibrio vulnificus. 2012.
24. Dong D, Liu H, Xiao Q, Li R. Affinity purification of egg yolk immunoglobulins (IgY) with a stable synthetic ligand. Journal of Chromatography B. 2008;870(1):51-4.
25. Nasiri K, Zibaee S, Nassiri M, Tahmoorespur M, Haghparast A. Production of specific IgY antibody to the recombinant FanC protein produced in Escherichia coli. Iranian Journal of Basic Medical Sciences. 2016;19(8):883.
26. Jahandar MH, Nassiri M, Nasiri K, Haghparast A. Production and purification of specific igy against invg protein of salmonella typhimurium. International Journal of Infection. 2019;6(2).
27. Liu XS, Yang WJ, He JX, Zhao JZ, Justo GC, Zhang XY. Preparation of artificial antigen and egg yolk-derived immunoglobulin (IgY) of florfenicol amine for ELISA assay. Journal of Environmental Science and Health, Part B. 2014;49(2):109-15.
28. Zhu Y, Ma Y, Lu M, Zhang Y, Li A, Liang X, et al. Efficient production

of human norovirus-specific IgY in egg yolks by vaccination of hens with a recombinant vesicular stomatitis virus expressing VP1 protein. Viruses. 2019;11(5):444.

29. Dong W, Zhang H, Huang H, Zhou J, Hu L, Lian A, et al. Chicken IgY Fc linked to Bordetella avium ompA and Taishan Pinus massoniana pollen polysaccharide adjuvant enhances macrophage function and specific immune responses. Frontiers in Microbiology. 2016;7:1708.
30. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. Nature reviews Drug discovery. 2018;17(4):261-79.
31. Dai Y-C, Zhang X-F, Tan M, Huang P, Lei W, Fang H, et al. A dual chicken IgY against rotavirus and norovirus. Antiviral research. 2013;97(3):293-300.
32. Schade R, Calzado EG, Sarmiento R, Chacana PA, Porankiewicz-Asplund J, Terzolo HR. Chicken egg yolk antibodies (IgY-technology): a review of progress in production and use in research and human and veterinary medicine. Alternatives to laboratory animals. 2005;33(2):129-54.
33. Cai Y, Chen J, Guo J, Chen S, Tong X, Tian L. Dynamic observation of chicken egg yolk antibodies against soluble egg antigen of Schistosoma japonicum obtained by two immunization routes. Zhongguo ji Sheng Chong xue yu ji Sheng Chong Bing za zhi= Chinese Journal of Parasitology & Parasitic Diseases. 2010;28(2):121-4.
34. Tokarzewski S. Influence of enrofloxacin and chloramphenicol on the level of IgY in serum and egg yolk after immunostimulation of hens with Salmonella enteritidis antigens. Polish Journal of Veterinary Sciences. 2002;5(3):151-8.
35. Noormohammadi AH, Whithear KG. Comparison of the short-term and long-term efficacies of the Mycoplasma gallisepticum vaccines ts-11 and 6/85. Avian Pathology. 2019;48(3):238-44.
36. Gary EN, Weiner DB. DNA vaccines: prime time is now. Current Opinion in Immunology. 2020;65:21-7.
37. Sanford JC, Klein TM, Wolf ED, Allen N. Delivery of substances into cells and tissues using a particle bombardment process. Particulate Science and Technology. 1987;5(1):27-37.
38. Witkowski PT, Bourquain DR, Hohn O, Schade R, Nitsche A. Gene gun-supported DNA immunisation of chicken for straightforward production of poxvirus-specific IgY antibodies. Journal of immunological methods. 2009;341(1-2):146-53.
39. Schjeide O, Wilkens M, McCandless RG, Munn R, Peterson M, Carlsen E. Liver synthesis, plasma transport, and structural alterations accompanying passage of yolk proteins. American Zoologist. 1963:167-84.
40. Sunwoo HH, Gujral N. Chemical composition of eggs and egg products. Handbook of food chemistry: Springer; 2015. p. 331-63.
41. Anton M. Egg yolk: structures, functionalities and processes. Journal of the Science of Food and Agriculture. 2013;93(12):2871-80.
42. Ji S, Ahn DU, Zhao Y, Li K, Li S, Huang X. An easy and rapid separation method for five major proteins from egg white: Successive extraction and MALDI-TOF-MS identification. Food Chemistry. 2020;315:126207.
43. Fishman JB, Berg EA. Isolation of IgY from chicken eggs. Cold Spring Harbor Protocols. 2018;2018(6):pdb. prot099150.
44. Staak C, Schwarzkopf C, Behn I, Hommel U, Hlinak A, Schade R, et al. Isolation of IgY from yolk. Chicken egg yolk antibodies, production and application: IgY-technology. 2001:65-107.
45. Hansen P, Scoble JA, Hanson B, Hoogenraad NJ. Isolation and purification of immunoglobulins from chicken eggs using thiophilic interaction chromatography. Journal of immunological methods. 1998;215(1-2):1-7.
46. Akita E, Nakai S. Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with an enterotoxigenic E. coli strain. Journal of immunological methods. 1993;160(2):207-14.
47. Ntakarutimana V, Demedts P, Van Sande M, Scharpe S. A simple and economical strategy for downstream processing of specific antibodies to human transferrin from egg yolk. Journal of immunological methods. 1992;153(1-2):133-40.
48. Son AP. Isolation of IgY from the yolks of eggs by a chloroform polyethylene glycol procedure. Immunological investigations. 1990;19(3):253-8.
49. Chang H-M, Lu T-C, Chen C-C, Tu Y-Y, Hwang J-Y. Isolation of immunoglobulin from egg yolk by anionic polysaccharides. Journal of Agricultural and Food Chemistry. 2000;48(4):995-9.
50. Jensenius JC, Andersen I, Hau J, Crone M, Koch C. Eggs: conveniently packaged antibodies. Methods for purification of yolk IgG. Journal of immunological methods. 1981;46(1):63-8.
51. Polson A, Coetzer T, Kruger J, Von Maltzahn E, Van der Merwe K. Improvements in the isolation of IgY from the yolks of eggs laid by immunized hens. Immunological investigations. 1985;14(4):323-7.
52. Ko K, Ahn D. Preparation of immunoglobulin Y from egg yolk using ammonium sulfate precipitation and ion exchange chromatography. Poultry Science. 2007;86(2):400-7.
53. Chalamaiah M, Esparza Y, Temelli F, Wu J. Physicochemical and functional properties of livetins fraction from hen egg yolk. Food bioscience. 2017;18:38-45.
54. Pauly D, Chacana PA, Calzado EG, Brembs B, Schade R. IgY technology: extraction of chicken antibodies from egg yolk by polyethylene glycol (PEG) precipitation. JoVE (Journal of Visualized Experiments). 2011(51):e3084.
55. Saxena A, Tripathi BP, Kumar M, Shahi VK. Membrane-based techniques for the separation and purification of proteins: An overview. Advances in colloid and interface science. 2009;145(1-2):1-22.
56. Hernandez-Campos FJ, Brito-De la Fuente E, Torrestiana-Sanchez B. Purification of egg yolk immunoglobulin (IgY) by ultrafiltration: effect of pH, ionic strength, and membrane properties. Journal of agricultural and food chemistry. 2010;58(1):187-93.
57. Freire MG, Claudio AFM, Araujo JM, Coutinho JA, Marrucho IM, Lopes JNC, et al. Aqueous biphasic systems: a boost brought about by using ionic liquids. Chemical Society Reviews. 2012;41(14):4966-95.

58. Freire MG, Neves CM, Marrucho IM, Lopes JNC, Rebelo LPN, Coutinho JA. High-performance extraction of alkaloids using aqueous two-phase systems with ionic liquids. Green Chemistry. 2010;12(10):1715-8.
59. Pereira MM, Cruz RA, Almeida MR, Lima ÁS, Coutinho JA, Freire MG. Single-step purification of ovalbumin from egg white using aqueous biphasic systems. Process Biochemistry. 2016;51(6):781-91.
60. Jadaun GPS, Dixit S, Saklani V, Mendiratta S, Jain R, Singh S. HPLC for Peptides and Proteins: Principles, Methods and Applications. Pharmaceutical Methods. 2017;8(1).
61. Xia H-F, Lin D-Q, Chen Z-M, Yao S-J. Purification of immunoglobulin of egg yolk with hydrophobic charge induction chromatography: Comparison of operation modes with packed bed and expanded bed. Separation Science and Technology. 2012;47(16):2366-72.
62. Xia W, Lu H, Li Y, Cao J, Zhou X, Zhang X, et al. Purification of chicken IgY by binding capture using elastin-like polypeptide-tagged immunoglobulin-binding domain of streptococcal protein G. Veterinary immunology and immunopathology. 2017;192:13-9.
63. Lindmark R, Thorén-Tolling K, Sjöquist J. Binding of immunoglobulins to protein A and immunoglobulin levels in mammalian sera. Journal of immunological methods. 1983;62(1):1-13.
64. LARSSON A, WEJÅKER P-E, SJÖQUIST J. Chicken anti-protein A for the detection and capturing of protein A from Staphylococcus aureus in the presence or absence of mammalian IgG. Hybridoma. 1992;11(2):239-43.
65. Bravo D, Hoare A, Soto C, Valenzuela MA, Quest AF. Helicobacter pylori in human health and disease: Mechanisms for local gastric and systemic effects. World journal of gastroenterology. 2018;24(28):3071.
66. de Brito BB, da Silva FAF, Soares AS, Pereira VA, Santos MLC, Sampaio MM, et al. Pathogenesis and clinical management of Helicobacter pylori gastric infection. World journal of gastroenterology. 2019;25(37):5578.
67. Solhi R, Alebouyeh M, Khafri A, Rezaeifard M, Aminian M. Inávitro evaluation of cross-strain inhibitory effects of IgY polyclonal antibody against H. ápylori. Microbial pathogenesis. 2017;110:682-7.
68. Shimamoto C, Tokioka S, Hirata I, Tani H, Ohishi H, Katsu K-i. Inhibition of Helicobacter pylori infection by orally administered yolk-derived anti-Helicobacter pylori antibody. Hepato-gastroenterology. 2002;49(45):709-14.
69. 70. 70. Wang B, Yang J, Cao S, Wang H, Pan X, Zhu J, et al. Preparation of specific anti-Helicobacter pylori yolk antibodies and their antibacterial effects. International Journal of Clinical and Experimental Pathology. 2014;7(10):6430.
70. Aiba Y, Umeda K, Rahman S, Nguyen SV, Komatsu Y. Synergistic effect of anti-Helicobacter pylori urease immunoglobulin Y from egg yolk of immunized hens and Lactobacillus johnsonii No. 1088 to inhibit the growth of Helicobacter pylori in vitro and in vivo. Vaccine. 2019;37(23):3106-12.
71. Hong KS, Ki M-R, Ullah HA, Lee E-J, Kim YD, Chung M-J, et al. Preventive effect of anti-VacA egg yolk immunoglobulin (IgY) on Helicobacter pylori-infected mice. Vaccine. 2018;36(3):371-80.
72. Dye BA. The global burden of oral disease: research and public health significance. Journal of dental research. 2017;96(4):361-3.
73. Alejandra BM, Daniel OM. Virulence factors of Streptococcus mutans related to dental caries. Staphylococcus and Streptococcus. 2020;11:9.
74. Nguyen SV, Icatlo Jr FC, Nakano T, Isogai E, Hirose K, Mizugai H, et al. Anti–cell-associated glucosyltransferase immunoglobulin Y suppression of salivary mutans streptococci in healthy young adults. The Journal of the American Dental Association. 2011;142(8):943-9.
75. Carlander D, Kollberg H, Larsson A. Retention of Specific Yolk IgY in the Human: Oral Cavity. BioDrugs. 2002;16:433-7.
76. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. New England journal of medicine. 2020.
77. Kotta S, Aldawsari HM, Badr-Eldin SM, Alhakamy NA, Md S, Nair AB, et al. Combating the pandemic COVID-19: clinical trials, therapies and perspectives. Frontiers in molecular biosciences. 2020;7:606393.
78. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet. 2020;395(10223):497-506.
79. Zhou G, Zhao Q. Perspectives on therapeutic neutralizing antibodies against the Novel Coronavirus SARS-CoV-2. International Journal of Biological Sciences. 2020;16(10):1718-23.
80. Lu Y, Wang Y, Zhang Z, Huang J, Yao M, Huang G, et al. Generation of chicken IgY against SARS-COV-2 spike protein and epitope mapping. Journal of Immunology Research. 2020;2020.
81. Wei S, Duan S, Liu X, Wang H, Ding S, Chen Y, et al. Chicken Egg Yolk Antibodies (IgYs) block the binding of multiple SARS-CoV-2 spike protein variants to human ACE2. International immunopharmacology. 2021;90:107172.
82. Jester BJ, Uyeki TM, Jernigan DB. Fifty years of influenza A (H3N2) following the pandemic of 1968. American journal of public health. 2020;110(5):669-76.
83. Sautto GA, Kirchenbaum GA, Ross TM. Towards a universal influenza vaccine: different approaches for one goal. Virology journal. 2018;15(1):1-12.
84. Adachi K, Handharyani E, Sari DK, Takama K, Fukuda K, Endo I, et al. Development of neutralization antibodies against highly pathogenic H5N1 avian influenza virus using ostrich (Struthio camelus) yolk. Molecular Medicine Reports. 2008;1(2):203-9.
85. Adachi K, Takama K, Tsukamoto M, Inai M, Handharyani E, Hiroi S, et al. Ostrich produce cross-reactive neutralization antibodies against pandemic influenza virus A/H1N1 following immunization with a seasonal influenza vaccine. Experimental and Therapeutic Medicine. 2011;2(1):41-5.
86. Baradaran B, Hosseini AZ, Majidi J, Farajnia S, Barar J, Saraf ZH,

et al. Development and characterization of monoclonal antibodies against human epidermal growth factor receptor in Balb/c mice. Human antibodies. 2009;18(1-2):11-6.

87. Banihashemi SR, Hosseini AZ, Rahbarizadeh F, Ahmadvand D. Development of specific nanobodies (VHH) for CD19 immuno-targeting of human B-lymphocytes. Iranian journal of basic medical sciences. 2018;21(5):455.
88. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nature reviews drug discovery. 2010;9(10):767-74.
89. Safari E, ZAVARAN HA, Hassan Z, Khajeh K, SHAFIEE AM, Baradaran B. Cytotoxic effect of immunotoxin containing the truncated form of Pseudomonas exotoxin A and anti-VEGFR2 on HUVEC and MCF-7 cell lines. 2014.
90. Schmitt CK, Meysick KC, O'Brien AD. Bacterial toxins: friends or foes? Emerging infectious diseases. 1999;5(2):224.
91. 92. 92. Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. Clinical microbiology reviews. 1998;11(3):450-79.
92. Saxena SK, O'brien A, Ackerman E. Shiga toxin, Shiga-like toxin II variant, and ricin are all single-site RNA N-glycosidases of 28 S RNA when microinjected into Xenopus oocytes. Journal of Biological Chemistry. 1989;264(1):596-601.
93. Neri P, Tokoro S, Kobayashi R, Sugiyama T, Umeda K, Shimizu T, et al. Specific egg yolk immunoglobulin as a new preventive approach for Shiga-toxin-mediated diseases. PloS one. 2011;6(10):e26526.
94. Parma YR, Chacana PA, Rogé A, Kahl A, Cangelosi A, Geoghegan P, et al. Antibodies anti-Shiga toxin 2 B subunit from chicken egg yolk: isolation, purification and neutralization efficacy. Toxicon. 2011;58(4):380-8.
95. Field M. Modes of action of enterotoxins from Vibrio cholerae and Escherichia coli. Reviews of infectious diseases. 1979;1(6):918-26.
96. You J, Xu Y, Li H, Wang L, Wu F, Xu F, et al. Chicken egg yolk immunoglobulin (IgY) developed against fusion protein LTB–STa–STb neutralizes the toxicity of Escherichia coli heat-stable enterotoxins. Journal of applied microbiology. 2014;117(2):320-8.
97. Devi CM, Bai MV, Krishnan L. Development of viper-venom antibodies in chicken egg yolk and assay of their antigen binding capacity. Toxicon. 2002;40(7):857-61.
98. Almeida C, Kanashiro M, Rangel Filho F, Mata M, Kipnis T, Dias da Silva W. Development of snake antivenom antibodies in chickens and their purification from yolk. Veterinary Record. 1998;143(21):579-84.
99. Xiao Y, Gao X. Use of IgY antibodies and semiconductor nanocrystal detection in cancer biomarker quantitation. Biomarkers in medicine. 2010;4(2):227-39.
100. Kasper S, Schuler M. Targeted therapies in gastroesophageal cancer. European Journal of Cancer. 2014;50(7):1247-58.
101. Rahman S, Van Nguyen S, Icatlo Jr FC, Umeda K, Kodama Y. Oral passive IgY-based immunotherapeutics: a novel solution for prevention and treatment of alimentary tract diseases. Human vaccines & immunotherapeutics. 2013;9(5):1039-48.
102. Chavez-Cortez E-G, Vargas Felix G, Rangel López E, Sotelo J, Martínez-Canseco C, Pérez-de la Cruz V, et al. Production and evaluation of an avian IgY immunotoxin against CD133+ for treatment of carcinogenic stem cells in malignant glioma: IgY immunotoxin for the treatment of glioblastoma. Journal of Oncology. 2019;2019.