THERAPEUTIC ADVANCES in
Vaccines and Immunotherapy

Review

Monoclonal IgY antibodies: advancements and limitations for immunodiagnosis and immunotherapy applications

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Ther Adv Vaccines Immunother

2024, Vol. 12: 1–25

DOI: 10.1177/25151355241264520

***Abstract*:** Due to their high specificity and scalability, Monoclonal IgY antibodies have emerged as a valuable alternative to traditional polyclonal IgY antibodies. This abstract provides an overview of the production and purification methods of monoclonal IgY antibodies, highlights their advantages over polyclonal IgY antibodies, and discusses their recent applications. Monoclonal recombinant IgY antibodies, in contrast to polyclonal IgY antibodies, offer several benefits. such as derived from a single B-cell clone, monoclonal antibodies exhibit superior specificity, ensuring consistent and reliable results. Furthermore, it explores the suitability of monoclonal IgY antibodies for low- and middle-income countries, considering their cost-effectiveness and accessibility. We also discussed future directions and challenges in using polyclonal IgY and monoclonal IgY antibodies. In conclusion, monoclonal IgY antibodies offer substantial advantages over polyclonal IgY antibodies regarding specificity, scalability, and consistent performance. Their recent applications in diagnostics, therapeutics, and research highlight their versatility.

Plain language summary

Chicken egg yolk antibodies (IgY) and monoclonal antibodies: advancements and limitations for immunodiagnosis and immunotherapy applications

Chicken egg yolk antibodies (IgY antibodies) and monoclonal antibodies (mAbs) are two types of antibodies used in medical applications. IgY antibodies are cost-effective, stable, and specific, with the advantage of not triggering harmful immune responses. However, they may have limitations in identifying certain target areas and availability. On the other hand, mAbs are highly specific and can detect multiple target areas on antigens, but their production is expensive and may cause immune responses. Despite these drawbacks, both IgY antibodies and mAbs show promise in various applications such as infectious disease diagnosis, cancer treatment, and autoimmune disorders. Ongoing developments in antibody technology are likely to expand their applications in immunology. This review provides an overview of the strengths and limitations of IgY antibodies and mAbs in immunodiagnosis and immunotherapy, as well as their role in pandemic control.

Keywords: chicken egg yolk antibodies, hybridoma technology, IgY antibodies, immunodiagnosis, immunotherapy, monoclonal antibodies, pandemics

Received: 30 November 2023; revised manuscript accepted: 9 June 2024.

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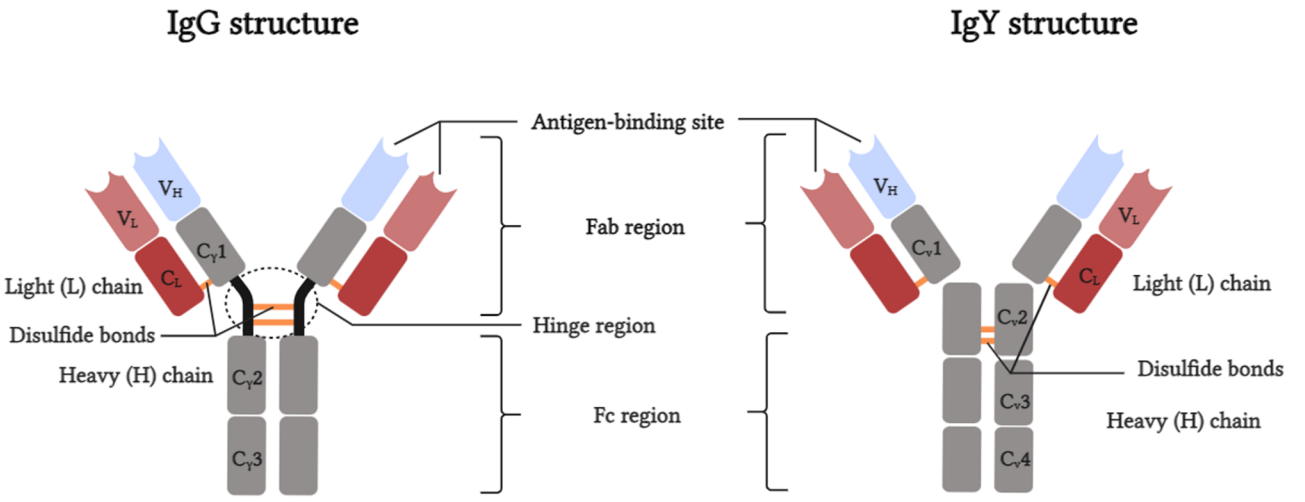
Overview of the IgY antibodies

IgY antibodies are immunoglobulin produced by birds, reptiles, lungfish, and amphibians. They are functionally similar to mammalian antibodies (IgG) but have some unique properties due to the phylogenetic distance between mammals and birds, making them useful in various research and diagnostic applications.[1,2] The molecular structure of IgY has some distinct properties in molecular mass, number of constant regions, presence or absence of hinge region which affects the flexibility of the antibody, as well as the isoelectric point which is greatly affected by the glycosylation or other side chains, and most importantly the potential binding of the antibody with the complement and mediating effector pathways and binding to rheumatoid factors (Figure 1). However, the Fc region of the IgY contains different carbohydrate chains than that of Fc of IgG, allowing the IgY to be transferred from the bloodstream to the egg while remaining highly intact.[3] Between the Fc (crystallizable fragment) and Fab (antigen-binding fragment) parts of mammalian IgG antibodies, is located the stretchy part of the polypeptide chain; the hinge region. It has disulfide bonds, which give the antibody flexibility and allow it to adopt multiple conformations. The hinge region is in charge of the antibody's flexibility and range of motion, allowing it to bind to antigens with varied spatial orientations and interact with immune cells and other effector molecules. IgY antibodies, on the other hand, lack the usual hinge region present in IgG antibodies limiting their flexibility.[4] They do, however, have a brief, stiff linker region between the Fab and Fc regions. This linker region comprises a few amino acids and lacks the disulfide linkages found in IgG antibodies' hinge region. Collectively these distinctive properties of IgY, as well as their greater stability, higher avidity, and lower Fc-mediated effector activities, are good for certain uses. Their stability and resistance to degradation, for example, make them appealing for use in oral immunotherapy and passive immunization techniques. Furthermore, their increased avidity can be useful in diagnostic applications.[5] IgY molecules contain two heavy and two light chains. The heavy chain consists of a variable domain



Molecular Mass	Number of Constant Domains	Hinge Region	pI	Concentration	Binding to Protein A/G
150 kDa	3 Heavy + 1 Light	Present	6.4-9.0	Serum=11 mg/ml	Yes

Thermal stability	Glycosylation	Flexibility	Complement Binding	Rheumatoid Factor Binding
Smaller	Smaller	High	Yes	Yes

Molecular Mass	Number of Constant Domains	Hinge Region	pI	Concentration	Binding to Protein A/G
180 kDa	4 Heavy + 1 Light	Absent	5.7–7.6	Serum=9 mg/ml Egg yolk=20mg/ml	None

Thermal stability	Glycosylation	Flexibility	Complement Binding	Rheumatoid Factor Binding
Higher	Higher	Reduced	No	No

Figure 1. Comparison between mammalian IgG and Avian IgY different biochemical structures and properties. IgY contains two glycosylation sites at the Cυ2 and Cυ3 while the IgG contains only one at the Cγ2.
Source. The antibodies diagrams were created using BioRender.com.

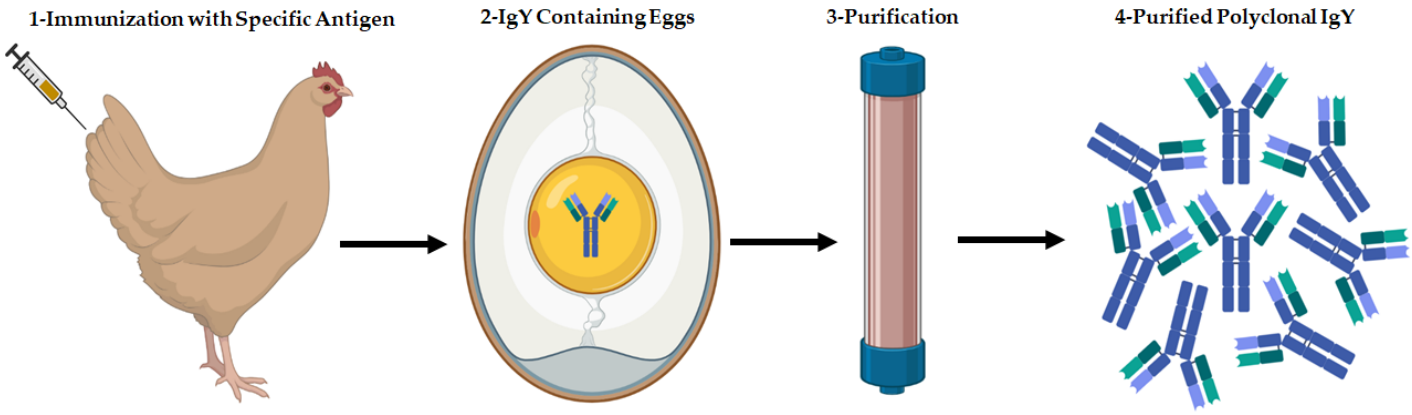


Figure 2. Diagrammatic procedures for the production of chicken egg polyclonal IgY.
Source. The figure is created using BioRender.com.

(VH) and four constant domains (CH1, CH2, CH3, and CH4)[5] (as shown in Figure 1). IgY immunotherapy has several attractive features, including lack of reactivity with the human complement system or binding to rheumatoid factor[6,7] the erythrocyte agglutinogens A and B,[8] and human Fc receptors, thereby preventing non-specific inflammation.[9]

Production and purification of polyclonal IgY antibodies

IgY antibodies are polyclonal, meaning they are produced by multiple B-cell clones and can recognize multiple epitopes on an antigen enhancing their usefulness and potential in various applications, in which the detection of multiple antigens is required.[10] They are mainly produced by immunizing birds, such as chickens, with an antigen of interest. The antibodies are then collected from the egg yolk, as they are concentrated in this part of the egg,[11] followed by purification (Figure 2). The process is relatively simple since its cost-effectiveness makes it a valuable tool in research, diagnostics, and potential therapeutics. Additionally, IgY antibodies can be produced against antigens that are difficult to obtain or purify, such as membrane proteins or small molecules.[3] In chickens, primary immune organs like bursa of Fabricius (B-cell maturation) and secondary immune organs such as spleen (antigen recognition), femur bone-marrow, Harderian glands, conjunctival-, bronchial- and gut-associated lymphoid tissue are all associated in presenting specific immune response within 4–6 weeks.[12,13] B cells rearrange their genes and change into mature antibody-making cells. IgY is taken into the egg yolk from the blood and transported across the yolk sac membranes during development.[14] IgY antibodies are known for their stability and resistance to degradation.

When stored properly, IgY antibodies can remain stable for an extended period. Under ideal conditions, IgY antibodies can maintain their activity for 5 years.[15] However, it's important to note that the stability of IgY antibodies can vary depending on several factors, including storage conditions, temperature, and handling procedures. To maximize the stability of IgY antibodies in chicken eggs, it is recommended to store them at low temperatures, typically between 2°C and 8°C. Storing eggs in this temperature range helps to slow down degradation processes and maintain antibody activity.[16,17] The resistance of IgY antibodies to fragmentation and proteolytic degradation was referred to as the high stiffness of the heavy chains due to the presence of proline and glycin amino acids at the CH1-CH2 and CH2-CH3 regions.[15]

IgY antibodies can be highly specific for their target antigen, with little or no cross-reactivity to other antigens.[18] This makes them useful in diagnostic applications where high specificity is required. They are stable molecules at a wide range of temperatures up to 70°C, pHs (3–11), salt concentration, and proteases.[19–22] Regarding protease susceptibility or resistance, IgYs were found to be moderately resistant to digestion by trypsin and chymotrypsin.[23] However, these

antibodies proved to be sensitive to pepsin and papain digestion when exposed to acidic pH levels below 5 and at 7, respectively.[23,24] To overcome this issue, IgYs were successfully coated with delayed-release capsules or specific protective formulas such as alginates, liposomes, gelatins, or other methods like attachment of antibodies to nanotubes or administration with egg proteins or milk.[25,26]

Generally, the process of *production* of IgY includes:

(A) *Immunization*: Poultry birds, mainly chickens, are injected with the antigen of interest which could be a protein, peptide, or other molecule that elicits an immune response in the bird. The antigen is commonly and preferably suspended in adjuvant Al_2O_3,[27] Freund's, $Al(OH)_3$,[28] before being injected, either intradermal, intramuscular, intraperitoneal and intravenous which is the most effective route in eliciting immune response compared to other routes.[29,30] Intramuscular injections are also common and carried out in multiple sites of the breast or pectoralis muscle.[31] This route has been shown to produce 10-fold higher titer than intradermal.[32] It is worth mentioning that the use of complete Freund's adjuvant resulted in high antibody titers but was accompanied by a decrease in egg production.[33] These authors attributed the low overall egg number to the localized inflammation after injections. Neck subcutaneous immunization has been reported.[3] Consequently, the purpose and characteristics of the intended polyclonal IgY may be evaluated before selecting an immunization protocol. Titer increase could be detected from the second week[34] or the fifth week[35] post injections. However, high antibody titers in egg yolk may be maintained through booster inoculations for more than 150 days.[36] The antigen administration protocols consider some critical factors such as hen's age, intervals between injections and specifically the interval between first and second injections. Karachaliou *et al.*[3] recommended using hens aged 3 months. Some protocols reviewed by Schade *et al.*[37] recommend antigen administration once/week for seven successive weeks, or the general protocol at days 0, 14, and 28, or weeks 0, 10, and 15, or immunization at 10-day intervals.

(B) *Egg collection*: After immunization, the animal begins to produce IgY antibodies that are normally concentrated in the egg yolk, rather than in the serum, as opposed to antibody distribution in mammals. Starting 1 week before immunization, eggs are collected weekly and the egg yolk is separated from the egg white and lyophilized.

(C) *Purification*: The IgY antibodies are purified from the egg yolk using a variety of methods, such as precipitation, ion exchange chromatography, or affinity chromatography. The choice of purification method depends on the specific properties of the IgY antibodies and the antigen of interest. This process is critical due to the nature of the contents of the egg yolk which affects the purity of the IgY. IgY purification can be easier compared to conventional IgG purification due to a few factors, such as the fact that IgY antibodies are naturally present in high concentrations in the egg yolk, making the starting material rich in antibodies. According to the literature, and depending on the chicken's age, Pauly *et al.*[38] showed total IgY content of 60–150 mg and 40–80 mg IgY per egg yolk[39] depending on the chicken's age.
Nevertheless, IgY antibodies have distinct physicochemical properties compared to IgG antibodies and may influence the purification steps. For example, IgY antibodies have a lower isoelectric point (pI) than IgG[17] more hydrophobic and stable for 15 min up to 70°C. IgY antibodies are highly active at pH between 4 and 11[9,40]and have a shorter half-life in the mammalian host than IgG because the Fc of the IgY cannot either bind the FcγRs of mammals) or protein G.[41] These differences between IgY and IgG enable the use of specific purification techniques optimized for IgY antibodies, reducing the complexity of the purification process.
IgY antibodies are highly specific to the antigen used for immunization. This

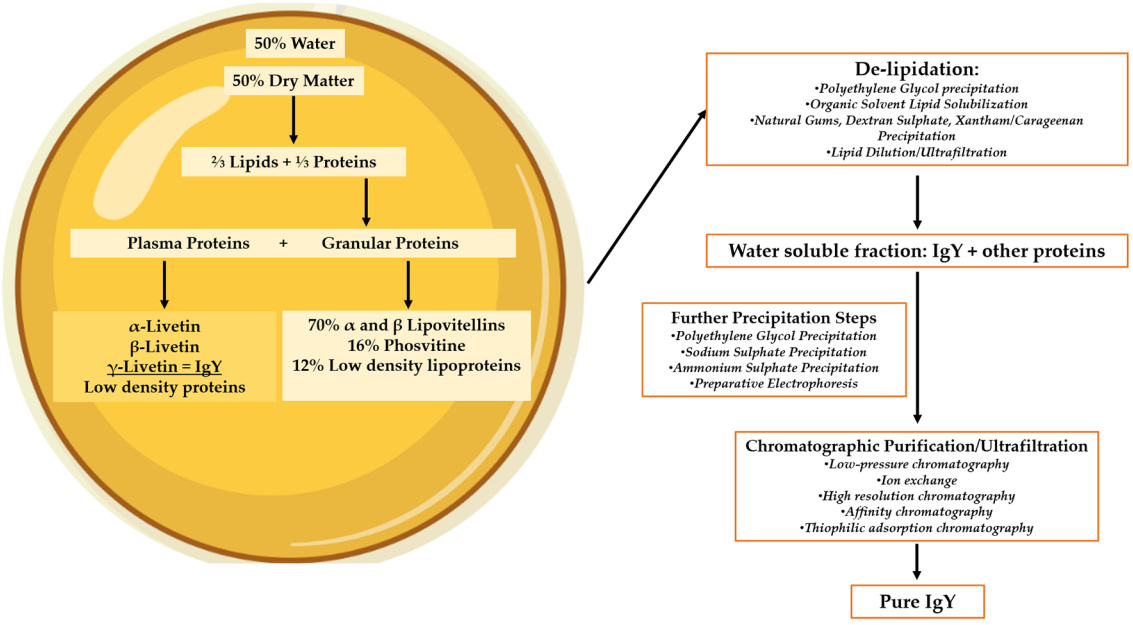


Figure 3. Protein and lipid content of egg yolk and main steps of IgY purification.

specificity can make the purification process easier by using antigen-specific matrices or affinity chromatography columns for affinity purification.[42] The contents of the egg yolk and the principles of purification are illustrated in Figure 3. The fundamental step is de-lipidation to remove the lipid portion using various precipitation possibilities to achieve a water-soluble fraction containing the IgY.[43] It is worth mentioning that IgY is a γ-livetin; a group of water-soluble proteins that represents a critical point during large-scale production of IgY to separate water-soluble contents from lipids.[2,44] De-lipidation involves the removal of lipid molecules, which may interfere with downstream applications and affect the stability and solubility of IgY. Various precipitation techniques can be used to achieve de-lipidation and obtain a water-soluble fraction containing IgY. These techniques may include methods such as water dilution method in an acid medium (pH 5), incubation for 4–6 h and the lipids being finally precipitated.[43] Other de-lipidation methods are carried out using solvent extraction (acetone, chloroform, isopropanol),[45] or by using polysaccharide solutions such as methylcellulose, dextran sulfate, carboxymethylcellulose, pectin, and λ-carrageenan. In brief, the specifically chosen method depends on the characteristics of the starting material and the desired purity of the IgY.[42,46] Following de-lipidation, the extraction of IgY is conducted through either precipitation, chromatographic methods, or filtration.[3] Several solutions like caprylic acid, polyethylene glycol, carrageenan, and saturated salts such as ammonium sulfate, sodium sulfate, and sodium chloride.[10,47–49] Huang *et al.*[50,51] showed that 20% ammonium sulfate precipitation is the best choice for IgY scale-up purification. Concerning chromatographic methods, ion exchange, low pressure, multi-column high-resolution systems, and affinity chromatography were used considering both protein A and G cannot bind IgY for affinity purification.[26,49,52] Nevertheless, chromatographic approaches are expensive and when compared to precipitation methods do not provide a substantial increase in the purity of IgY. Indeed, the

combination of methods can further increase the purity of IgY, for example, ammonium sulfate followed by ion exchange chromatography[49] or precipitation using Polyethylene glycol (PEG) and affinity purification.[39]

(D) *Quality control*: After purification, antibodies are tested for purity, specificity and activity. Various assays such as ELISA, Western blot, and immunohistochemistry can be used to test the antibodies. Critical Quality Attributes (CQAs) are the measurable characteristics of a product that are deemed critical to its quality, safety, and efficacy. Specific CQAs for IgY may vary depending on the intended use and manufacturing process, for example, purity, potency, specificity, stability, and consistency.[17] There are still several limitations to IgY antibody production.[53] The lack of standardization in the experimental animals (i.e. specific pathogen-free birds) for the production, extraction, and purification of IgY antibodies is one of the biggest problems that has slowed down product licensing and made it hard to agree on how IgY-based health products should be regulated and approved.[54] More safety studies are needed to evaluate their safety for use as human and veterinary therapeutics. Research is also needed to develop more industrial-scale standardized extraction and purification methods to fit the needs of clinical applications.[43,55]

(E) *Storage*: The purified IgY antibodies can be stored preferably at −20°C, even at room temperature, for long-term storage.[39,56] Alternatively, purified antibodies can be lyophilized for an even longer shelf life.[57] Unfortunately, freeze drying resulted in unfolding and structural changes due to dehydration stresses,[58] and consequently, IgY loses its binding activity to target antigen.[59] On the contrary, Karachaliou *et al.*[60] showed successful immunoreactivity of −30°C stored lyophilized IgY raised against prothymosin alpha in an ELISA assay.

The production and purification of IgY antibodies offer several advantages over mammalian antibodies, including lower cost, higher yield,[43] and lower risk of contamination by mammalian viruses or bacteria or other pathogens.[5] Annually, one hen can lay about 300–325 eggs which adds between 17 and 35 g of IgY per year per one hen.[39,61] However, the cost of IgY per g or mg produced is attributed to several factors, such as animal husbandry (housing, feeding, and veterinary care), antibody extraction and purification (chromatography, filtration, and reagents), research and development, and scale of production. Larger-scale production is generally more cost-effective due to the ability to spread fixed costs over a larger output.[17]

Production of monoclonal IgY antibodies (mIgY)

All well-known monoclonal antibodies are murine and a large number of biological targets are conserved between mammals. Immunologically, these antibodies could produce human anti-mouse antibody reactions if the murine antibodies are used to treat human diseases. Indeed, chickens were considered as alternative host, due to their phylogenic distance toward mammals, to produce antibodies against conserved molecules. Unfortunately, antibodies raised in chicken were found to be immunogenic in mammals[62] specifically the constant region,[63] leading to limited clinical applications. Therefore, different formats of chicken antibodies were applied using genetic engineering to overcome these challenges, such as recombinant, chimeric, and humanized antibodies. The newly humanized and chimeric formats reduced the immunogenicity and exhibited longer half-lives for pharmaceutical applications, diagnosis, and therapy[62,64,65] Nevertheless, the structural and functional studies of variable (V) and constant (C) regions of antibodies demonstrated that both domains influence each other. An allosteric (V to C) effect was attributed to antigen binding, resulting in conformational changes in the C-region.[66] The reverse (C to V) allosteric effect was generated due to the intrinsic sequences of the C-region.[67] Choi *et al.*[68] demonstrated that antibodies from avians and mammals share compatible V-C-region interfaces, which may facilitate the development of chimeric antibodies between mammals and Aves.

Different strategies were applied for developing monoclonal IgY (mIgY). Some protocols were based on switching the C- domain between Aves and mammals, using distinctive functional genes

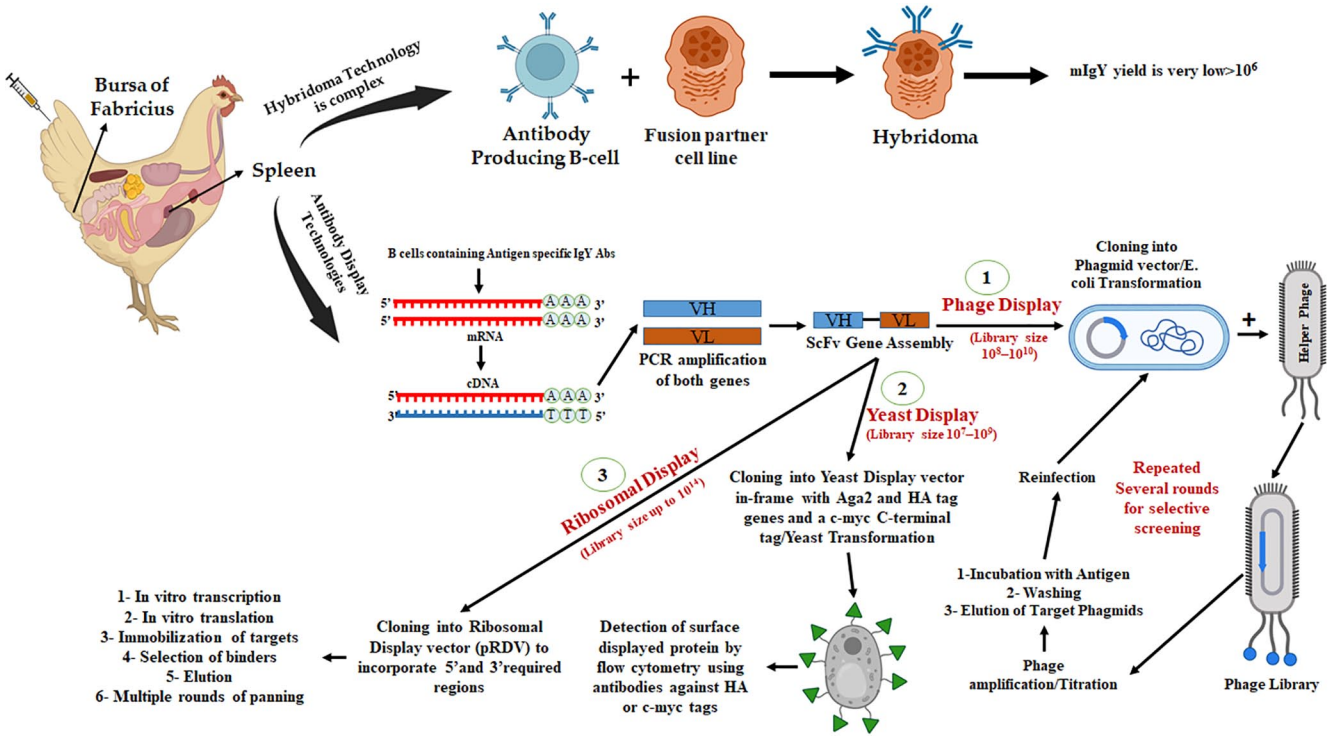


Figure 4. The most common approaches to generate monoclonal IgY (mIgY).
Source. The figure is created using BioRender.com.

of the variable region, hybridoma technology, antibody engineering using phage display or others.[69,70] The following Figures 4 and 5 show the most common techniques used to generate mIgY. In general, the combination of approaches such as monoclonal antibody engineering, IgY technology, generation of antibody library, and selection techniques has contributed significantly to expanding the applications and outcomes of mAbs derived from chicken.

Hybridoma approach

Chicken hybridomas have been generated using several fusion cell lines such as HU3R27, R27H4, and MuH1. Unfortunately, in several cases, the yield of clones that produce mAb was found to be less than 10^6 which is lower than the mouse system which produces approximately 10^7 mAb-producing clones.[71–73] The low yield was attributed to the fusion-partner cell lines which were derived from outbred chicken. This caused mismatching and mis-recombining between these fusion cells and the cells derived from the spleen of immunized chicken.[72] Moreover, Chicken hybridomas could not make ascites.[74] Another physiological factor affected the hybridoma approach where the fused cells are cultivated at 38.5°C rather than 37°C. Furthermore, mIgY cannot bind to protein A or G for one-step purification.[75] Alternatively, the chicken fast fast-growing lymphoma cell line; DT40 was used instead of hybridomas. These cells express a normal receptor for IgM on their surfaces and through the process of gene conversion, DT40 cells continue the diversification of the immunoglobulin loci.[76] DT40 cells produce IgM-type antibodies and undergo somatic mutations and gene conversion in the variable region of immunoglobulin during culture.[77,78] In brief, the gene conversion of the DT40 cells is activated by Trichostatin A (TSA, a histone deacetylase inhibitor).[79] This activation will generate an autonomously diversifying library (ADLib). Then, the DT40 clones producing antibodies are isolated from the ADLib using magnetic beads conjugated with the specific antigen (Figure 5).

However, several new antibody display approaches including the phage-, yeast surface-, and ribosomal-display approaches are available to generate mIgY. All these display techniques are based on the linkage between both genotype (RNA/DNA)

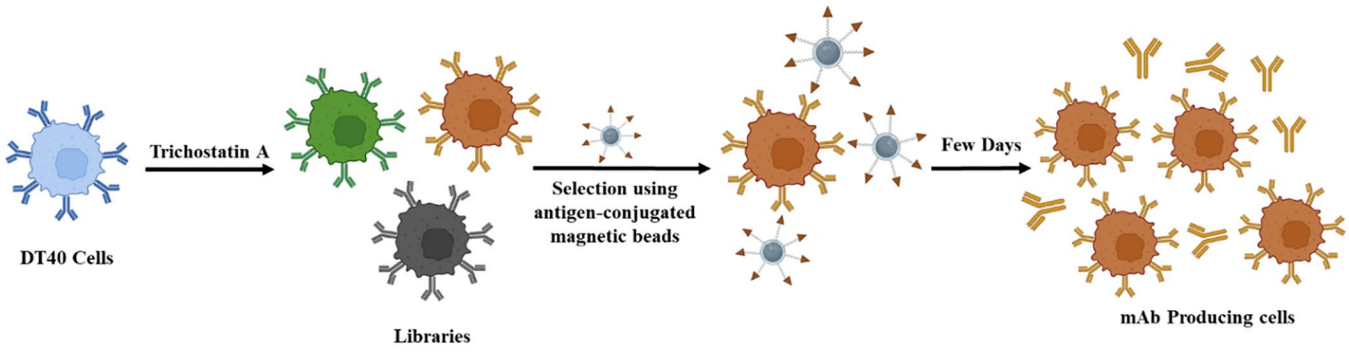


Figure 5. ADLib principle using DT0 cells.
Source. The figure is created using BioRender.com.

and phenotype for selection and affinity to mature a mIgY against a specific antigen.[65]

Phage display. RNA is purified from the isolated lymphocytes from the chicken spleen, reverse transcribed and PCR amplification of immunoglobulin's variable regions cDNA is carried out. The amplified DNA is ligated to suitable vectors which are used to transform *E. coli*. Next, *E. coli* are infected with helper phages. Bacteria, later, will release phages that now contain the vector DNA and the engineered antibody will be shown on its surface.[80] In contrast to the slowly growing hybridomas where 500–2000 clones would be expected, the resulting immortalized phage library has a titer of up to 10^9 *E. coli* cells.[81]

In recent decades, phage display was employed to generate the monoclonal IgY-single chain variable fragment (IgY-scFv) which has a high binding capacity to the targeted molecules from pathogens such as the protective antigen of *Bacillus anthracis*,[82] inactivated *Staphylococcus aureus*[83] or the carcinoembryonic antigen (CEA) epitope expressed on human colon carcinomas.[84] Other studies showed that generated IgY-scFv can be applied in immunoassays to detect either small molecules like gentamicin residue in edible food products of animal origin[85] or large molecules like the virus-like particles of canine parvovirus (CPV-VP2). The titer of amplified phage libraries for Gent-OVA-scFv, anti-CPV-scFv, and *S. aureus*-scFv was 1.65×10^9 pfu/ml, 8.2×10^{11} pfu/ml and 1.10×10^{10} pfu/ml, respectively. Recently, Ge *et al.*[86] showed efficient and sensitive binding of IgY-scFv to S1 protein of SARS-CoV-2 virus where the titer of the amplified phage scFv library was 2.8×10^{13} pfu/ml.

During the process of generating scFvs using phage display, both blood and splenic phage libraries can be used. Li *et al.*[85] revealed that the splenic library is superior as a chicken tissue for the preparation of phage library than blood tissue. They showed that the enrichment of phages after the third bio-panning was 5.8×10^6 pfu and 6.52×10^7 pfu for blood and spleen phages, respectively. In cases of immunization of chicken with whole pathogen or organism, several issues are still to be considered such as the particular position of the generated IgY-peptide, the anti-pathogen mechanism, and the pharmacokinetics and – dynamics of the peptide.

Yeast display. This approach is used to display secreted and cell surface proteins of mammals on the yeast cell wall. These proteins require specific posttranslational modifications such as proper folding and glycosylation, in the endoplasmic reticulum. The scFv antibody fragment is fused to the C-terminal region of the A-agglutinin-binding subunit (Aga2p) adhesion receptor of *Saccharomyces cerevisiae*.[87] The signal sequence of the Aga2p directs it to the surface where it becomes anchored to Aga1p through disulfide bonds.[65] The target scFv is cloned into yeast surface display vector with Aga2 and the hemagglutinin (HA) tag genes and c-myc C-terminal tag[88] (Figure 6).

Ribosomal display. It is based on the translation of a target mRNA (genotype) and the encoded protein (phenotype) remains physically attached

with its mRNA to the ribosome forming a complex.[89] To generate the protein-ribosome-mRNA (PRM) complex, the stop codon of the mRNA is deleted, the ribosome is stalled to the end of translation and finally, the budding protein is stretched by a spacer such as an immunoglobulin kappa to allow the release from the tunnel of the ribosome.[90] The general steps of the ribosomal display are listed in Figure 4. This approach can be performed using both prokaryotic and eukaryotic.[90]

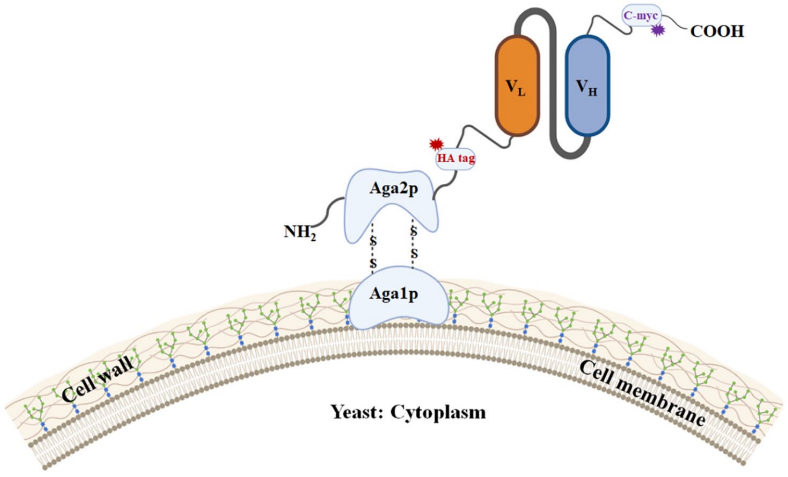


Figure 6. *Saccharomyces cerevisiae* surface display approach of scFv. *Source*. The figure is created using BioRender.com.

Diagnostic applications of IgY

Polyclonal IgYs have the potential to be used in research, diagnostic, and therapeutic applications due to their distinct antibody repertoire, including the broader epitope recognition ability of avian organisms compared to mammalian, the non-inductive response of the human complement, high yield production, and limited animal experimentation. IgY was used in ELISA, western blotting, immunofluorescence, and immunohistochemistry to detect, diagnose or neutralize pathogens. The performance of antibodies may vary between different immunoassays if there are conformational changes in the epitope structure. However, compared to mammalian IgG, IgY antibodies perform differently. In this respect, IgY was found to fail to bind to the rheumatoid factor (RF). RF can bind to the mammalian IgG during several immunoassays leading to false-positive ELISA results and this interference could be avoided by using IgY antibodies.[6]

IgY antibodies raised against spike protein were able to neutralize SARS-CoV and MERS-CoV viruses.[91,92] Furthermore, polyclonal IgY-based immunoswabs were used to detect SARS-CoV nucleocapsid protein in pig nasopharyngeal aspirates to 10 pg/ml.[93] IgY was used to detect the influenza-A matrix 2 proteins in ELISA and western blotting immunoassays, Dengue virus antigens in blood and serum, and reovirus and aquaculture antigens in infected birds and tissue samples.[54] ELISA tests used IgY antibodies to immunodiagnosis different antigens such as metacestodal antigens from *Taenia crassiceps* to diagnose human neurocysticercosis[94] antigens from *Ancylostoma ceylanicum* to detect infections of Hookworm[95] antigenic proteins of *Strongyloides venezuelensis* to diagnose human strongyloidiasis[96] In the poultry industry, chicken IgY raised against 67 kDa exoantigen released by *Fusarium verticillioides* as a secondary metabolite, was able to detect the target antigen in an indirect competitive ELISA (ic-ELISA) and other proteins in western blotting.[97] The ic-ELISA based on anti-67kDa IgY can be used to detect contamination of poultry feed by *Fusarium verticillioides*.

Immunohistochemistry along with ELISA and using of specific IgY against a 70 kDa Heat Shock recombinant protein (*Tg*HSP70) isolated from *Toxoplasma gondii* is an important tool to detect the Toxoplasma parasitic infection and quantify the circulating TgHSP70 in blood sera of mice.[98] Using flow cytometry as an immunoassay, polyclonal IgY against rabbit platelet factor 4 (PF4) was found to be sensitive and specific to detect PF4 in rabbit serum after activation of platelets by thrombin.[99] Other immunoassays like immunoprecipitation, electrophoretic mobility shift assay and immunofluorescence experiments using IgY antibodies against α subunit of the human hypoxia-inducible factor 1 (HIF-1a), showed exciting results whereas the application of mouse monoclonal anti-HIF1a was limited.[100] Taking into account the advantage that IgY does not induce Fc-dependent complement activation, IgY antibodies were used in immunofluorescence assays and conjugated to fluorescein isothiocyanate (FITC) to detect effectively *Campylobacter fetus* similar to the rabbit IgG conjugated to FITC. Interestingly, IgY showed less background as a result of the unspecific fluorescence compared to IgG.[101] In 2015, Bentes *et al.*[102] were

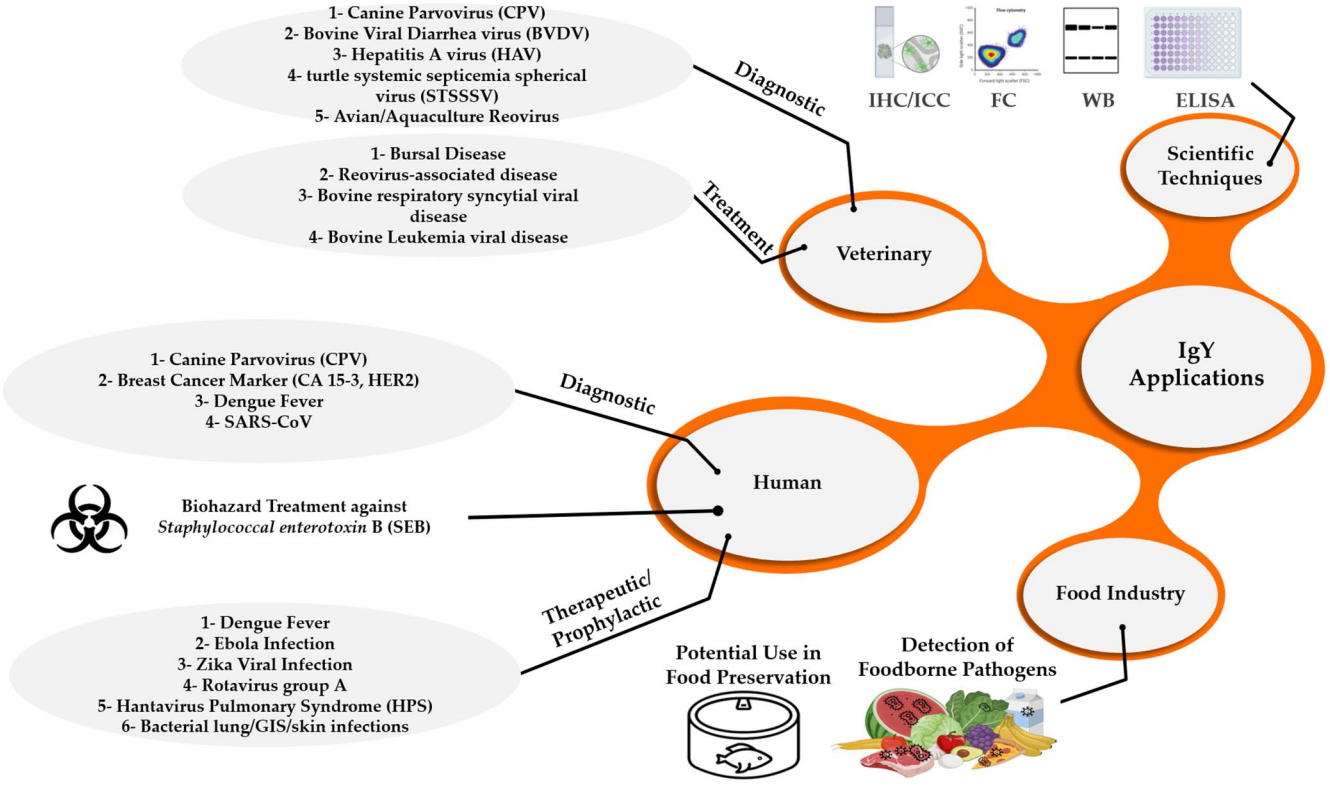


Figure 7. Potential applications of IgY technology.

able to detect the hepatitis A virus in frozen liver biopsies using anti-HAV-IgY through confocal microscopy.

Furthermore, IgY antibodies can be used to detect foodborne pathogens and allergens in food samples[103] providing a rapid and sensitive method for food safety testing Dou *et al.*[104] preventing or treating infectious diseases in animals as well as detecting specific pathogens in animal samples.[105] The following Figure 7 shows general applications of IgY in human and veterinary health with examples, in the food industry, and the scientific techniques that use IgY.

Therapeutic applications of IgY

Passive immunotherapy against human and animal infections was conducted and showed efficient therapeutic effects through mammalian-derived antibodies.[106] Unfortunately, the production costs and achieving necessary titers limit their applications for therapeutic purposes. Alternatively, pathogen-specific IgY emerged for passive immunization as a protective and effective approach.[40,107–109] Despite the major advantages of IgY such as nontoxicity, the limited number of animal-producing antibodies, and environmental safety, the use of specific pathogen-free birds and the efficacy of produced antibodies may elevate the production costs which will hinder its implementation in therapeutic purposes.[110] However, in poultry, these authors reported that using formalin-sterilized IgY may be cost-effective and alternative to attenuated vaccines since formalin did not interfere with the Fab antigen binding and did not activate Fc-complement.

Anti-inflammatory and immunomodulatory therapy

IgY antibodies can be used to target specific cytokines or immune cells implicated in inflammatory or autoimmune disorders. To determine the therapeutic possibility of allergic rhinitis, anti-IL-1β-IgY, and TNF-α-IgY were found to block IL-1β and TNF-α alleviating the inflammatory effects in the nasal mucosa and decrease the neutrophil, and lymphocyte infiltration, bronchoalveolar lavage fluid, and pathological inflammation

in lung tissues in guinea pigs.[111] The article by Leiva *et al.*[112] reviewed IgY-based biologicals for human medicine, including patent applications and clinical trials from 2010 to 2018. The authors discussed how IgY technology can lead to new ways of making biologicals to treat and prevent a wide range of infectious and non-communicable diseases.

In human medicine, IgY against gingipain from *Porphyromonas gingivalis* was able to decrease the hydrolytic activity of gingipain and consequently is effective against periodontitis in cultured human epithelial cells,[113] and adult dogs with periodontitis[114] IgY antibodies raised against the vacuolating toxin; one of the virulence extracellular proteins released by *Helicobacter pylori* and inducing apoptosis leading to epithelial cell damage was administered orally to mice and showed a reduction in eosinophilic infiltration of the stomach and protection of mice gastric tissues. El-Kafrawy *et al.*,[115] recently talked about how antimicrobial resistance is becoming more prevalent around the world. They also talked about how egg yolk IgY antibodies could be used to treat bacterial infections, especially those on the World Health Organization's priority list.

Antiviral therapy

By targeting particular viral proteins or blocking viral attachment to host cells, IgY antibodies can be utilized to prevent or cure viral infections. IgY antibodies specific to the influenza virus, for example, have been created as a possible treatment for influenza, Middle East Respiratory Syndrome (MERS) coronavirus infection and COVID-19.[38,48,49] In respect to the most recent pandemic; COVID-19, people were vaccinated to develop direct immunity against SARS-CoV-2. On the contrary, passive immunity was considered by using convalescent plasma from recovered persons. The use of preformed antibodies from humans requires donors, blood banks, analysis of the blood, and evaluation of the neutralization capacity of the plasma.[116] However, to achieve immediate and short-term immunity, large-scale production of specific antibodies, continuous supply of antibodies and highly purified antibodies, immunization of hens with the target antigen is a priority.[80]

The overall challenges confronting the monoclonal antibodies especially during pandemics, the mining of effective and economic approaches have been of great interest to use the IgY from egg yolk. Prophylactically, IgY can help humans during pandemics by lowering or blocking pathogen transmission or infection. IgY plays an important role in the control of pandemics such as COVID-19. Somasundaram *et al.*[80] provided overall importance to produce monoclonal IgY using the phage display method. They described the potential passive immunotherapeutic application of IgY for the treatment and prevention of SARS-CoV-2 infection, which is a simple, fast, and safe approach to treating patients efficiently. Phage display is currently utilized for creating a range of medical diagnoses and therapies, as well as to investigate antibody-antigen associations and SARS-CoV-2 epitope mapping. The IgY antibody has a higher binding affinity to the SARS-CoV-2 spike antigen than the routinely used IgG antibody for Lateral Flow Immunoassay (LFIA). However, the distinct pH stability, heat resistance, and storage capacity features of IgY, strongly suggest IgY is an excellent candidate for LFIA[117] as well as alternative diagnoses and treatments, preventing viral transmission and dissemination, and minimizing morbidity and mortality.[118,119] Chicken IgY is demonstrated to give prophylactic protection by coating the naso- and oropharynx with antibodies that inhibit viral entry into the respiratory system, from where it can travel throughout the body. These antibodies anti- H1N1-, H3N2-, and H5N1-IgY have been demonstrated to be exceedingly cross-reactive and protective where anti-H5N1-IgY cross-protected *in vivo* and *in vitro* against A/Puerto Rico/8/34 (H1N1).[118] Pandemics can be controlled and managed when used strategically in conjunction with vaccines and medications. Indeed, IgY offers the option of not having to rely on vaccines which are frequently selected for mutant strains leading to worse scenarios than what was originally present. Besides its cross-protective function, IgY can be manufactured and stored to be ready for viruses or other pathogens that are likely to trigger pandemics. As a result, control can be achieved rapidly before the situation spirals out of control, as we saw with COVID-19 at the commencement of the pandemic in 2019.

There are several studies about the beneficial neutralizing activities and prophylactic efficacy of IgY *in vivo* and *in vitro*[120–122] showed evidence of immunoglobulin Y antibodies' preventive

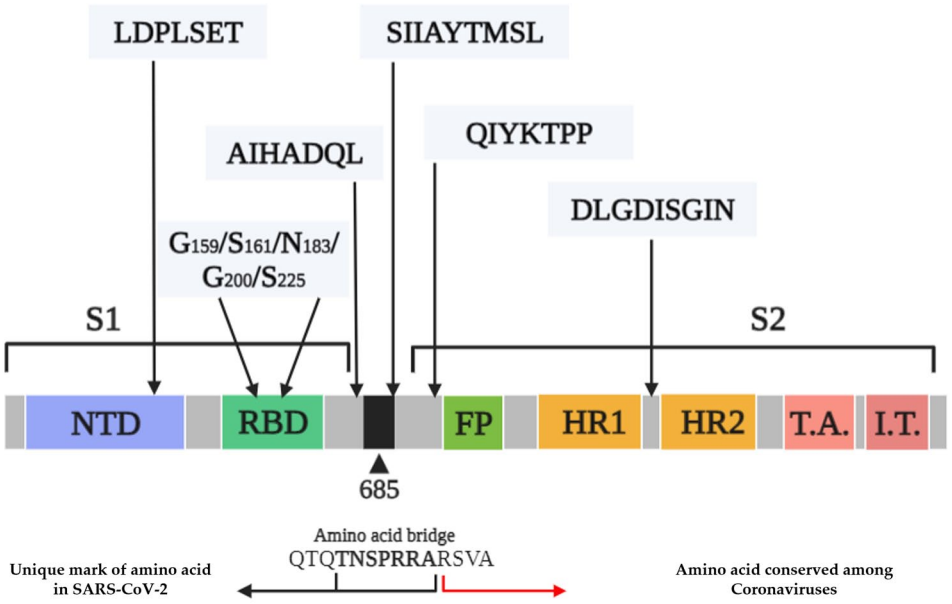


Figure 8. Different epitopes were identified on the S protein of SARS-CoV-2 and have significant binding to IgY.
Source. Figure is created using BioRender.com.
FP, fusion protein; HR1 and HR2, heptad repeat 1 and 2; I.T, the intracellular tail; NTD, the N-terminal domain; RBD, receptor binding domain; T.A: transmembrane anchor.

effectiveness against SARS-CoV-2 in mice. SARS-CoV-2 RBD protein was injected into Lohmann hens and IgY-Abs were collected from the eggs and analyzed using SDS-PAGE. Plaque reduction neutralization tests were used to assess antiviral activity. IgY-RBD effectiveness was also investigated in mice sensitized to SARS-CoV-2 infection through transduction with Ad5-hACE2 (moderate disease) or mouse-adapted virus (severe disease). In all cases, prophylactic intranasal injection of IgY-Abs reduced SARS-CoV-2 replication, morbidity, inflammatory cell infiltration, bleeding, and edema in the lungs and enhanced survival when compared to non-specific IgY-Ab control groups.[53] These findings suggest that more research into IgY-RBD antibodies in humans is necessary.

Consistent with the previously mentioned results, Ge *et al.*[86] developed IgY-single chain variable fragment (IgY-scFv) by using phage display and this IgY demonstrated significant binding capacity to the S1 fragment of the spike protein of SARS-CoV-2. The antibody formed double bonds with specific amino acid residues of the RBD (G159/S161/N183/G200/S225). Moreover, the connection between the antibody and antigen is facilitated through two distinct contacts or interactions occurring at certain amino acid positions on the antibody molecule. The various types of contacts that can occur include electrostatic interactions, hydrogen bonding, van der Waals forces, and hydrophobic interactions, among other possibilities. In aggregate, these interactions play a role in determining the binding affinity and overall stability of the antibody-antigen complex suggesting the potential of this IgY-scFv to be used in passive immunotherapy and immunoassays. Figure 8 shows five linear epitopes (two epitopes in the S1 subunit, two in the S2 subunit, and one epitope located at the S1/S2 cleavage region) on the S protein of SARS-CoV-2 virus which have been recognized by an IgY from egg yolk.[123] Proteome microarray showed high signals for epitopes LDPLSET, and SIIAYTMSL, relatively low signals for the three epitopes AIHADQL, QIYKTPP, and DLGDISGIN, and no signal in the RBD domain. Consequently, the S1/S2 epitope; SIIAYTMSL was suggested as a potential target for the IgY that would block the site of cleavage of S protein and consequently block the viral entry.

The IgY experimental and treatment trials proceed forward using models like mice, hamsters, young children, and Vero cells to study

the prospective role of IgY in controlling viral infections like Dengue (Mice),[124,125] Zika (Mice),[126] Hantavirus (Hamster),[127] rotavirus (Young children),[128] and SARS.[129] Goose IgYs were found to detect antigenic epitopes on the membrane, nonstructural membrane proteins, and envelop proteins of viruses like Zika, West Nile, and Dengue.[124]

Cancer therapy

IgY antibodies can be used to specifically target proteins in cancer cells, activating an immune response against them, and may induce apoptosis in cancerous cells paving the way to alternative therapeutics to minimize chemotherapeutic side effects. Two IgY antibodies specific to the human epidermal growth factor receptor 2 (HER2) with two domains HER2-A and HER2-B, for example, have been created as a possible treatment for breast cancer[130] showed that chicken IgY directed against 21 amino acid epitopes of the TNF-related apoptosis-inducing ligand (TRAIL-2R) receptor could induce apoptotic pathways in breast cancer cells. Successfully, a combined immunotoxin composed of chicken IgY, raised against the highly expressed CD133 receptor in glioblastoma, plus recombinant abrin A chain, was proposed as a novel immunotherapeutic of glioblastoma cancer.[131] Similar positive results were obtained upon using IgY anti-HER2 receptor coupled to single-walled carbon nanotubes to confer apoptotic signals in SK-BR-3 cells expressing the HER2 receptors led to cellular death.[132] The binding results of the complex to the receptor were confirmed using Raman spectroscopy.

Veterinary medicine

In different animals like calves, lambs, dogs, goats, cats, and poultry, the valuable applications of IgY antibodies have been described as comparable to studies in aquatic animals. Salmonella-specific IgY antibodies, for example, have been produced as a possible therapeutic for preventing salmonella infection in hens.[133] It was also suggested that IgY injections have a therapeutic effect in preventing shrimps from being infected by the White spot syndrome virus.[134] Although the application of IgY antibodies in therapies is currently in the beginning stages, interest in their potential uses is expanding. It is expected that further therapeutic uses for IgY antibodies will be identified. The following Table 1 shows examples of pathogens that IgY was generated against.

However, several veterinary diseases are detected or treated using the IgY technology with antibodies in the market or under discovery. Figure 9 shows the most recent veterinary therapeutics that use IgYs.[17] Several pathogens, such as bovine rotavirus, *Clostridium perfringens*, *Cryptosporidium parvum*, *Salmonella typhimurium*, *S. dublin*, *and E. coli* K99, are responsible for Calf diarrhea, porcine epidemic diarrhea virus, porcine rotavirus cause piglet diarrhea, Canine *distemper* virus causes canine disease, and *Salmonellosis* causes poultry diarrhea. The number of IgY products that are in the market or have been discovered for cattle diarrhea, piglet diarrhea, canine disease, and poultry diarrhea is 5, 5, 5, and 1, respectively.[17]

Early mortality syndrome in shrimps is caused by acute necrosis in the liver and pancreas.[17] Moreover, the production of IgY polyclonal antibodies is a straightforward and time-efficient technology that can be accomplished within a few months. It does not require advanced equipment or extensive expertise, making it accessible to a wide range of individuals. Additionally, IgY approach offers a high yield of antibodies and is cost-effective. Consequently, it holds promise for large-scale production aimed at controlling pandemics, particularly as an immunoprophylactic measure. This is especially relevant for low- and middle-income countries.

Recent applications of recombinant IgY monoclonal antibodies

It is crucial to bear in mind that these applications are examples of continued research and development in the field and with advancing technology and discoveries, there are increasing opportunities for using both polyclonal IgYs and recombinant IgY monoclonal antibodies. Some ongoing applications include Target therapeutics (IgY Monoclonal antibodies), R&D uses, & Diagnostic Applications. An interesting way of targeting cancer therapy is through the creation of recombinant IgY monoclonal antibodies. This comprises the use of antibody-drug conjugates and immune checkpoint inhibitors. These antibodies can attach to antigens associated with tumors selectivity, destroying tumor cells or activating the

Table 1. Examples of using IgY in veterinary applications.

Pathogen	Used molecule to produce IgY	Affected organism	Avian host	Proposed field of application	Reference
Vibrio anguillarum	*Whole pathogen*	Rainbow trout (*Oncorhynchus mykiss*)	Brown leghorn chicken (*Gallus domesticus*)	Passive immunization	Arasteh *et al.*[135]
WSSV	*Inactivated virus/a WSSV-DNA vaccine*	Shrimps and Crayfishes	Lohmann laying hens	Passive immunization	Lu *et al.*[136]
Edwardsiella tarda	formalin-killed bacteria	Japanese Eel, *Anguilla japonica*	Chicken	Protection/ treatment	Gutierrez *et al.*[137]
Yersinia ruckeri	formalin-killed whole cells	Rainbow trout (*Oncorhynchus mykiss*)	White Leghorn hens	Passive protection	Lee *et al.*[138]
WSSV	Truncated and fused WSSV enveloped protein, TrVP28:19	Shrimps	Chicken	Protection	Kim *et al.*[120]
Aeromonas hydrophila	Formalin-killed whole cells of *A. hydrophila*	*Carassius auratus gibelio*	White Leghorn hens	Protection	Li *et al.*[139]
WSSV	Binary ethylenimine (BEI)-inactivated virus and envelope protein VP28	*Chinese shrimp; Fenneropenaeus chinensis*	White Leghorn laying hens	Protection	Fu *et al.*[140]
Campylobacter jejuni	*C. jejuni* KC40 whole-cell lysate (EB1 + EB2) or Hydrophobic protein fraction only (EB2)	Ross broiler chickens	Brown Leghorn chickens	Passive immunization	Hermans *et al.*[141]
Hantavirus	DNA constructs	Rodents	Goose (*Anser domesticus*)	Protection	Haese *et al.*[127]

WSSV, White spot syndrome virus.

immune system.[131] For instance, diagnostic techniques used to detect biomarkers for diseases like cancer and cardiovascular infections have resorted to recombinant IgY monoclonal antibodies.[18,30,142,143] In the absence of a chicken myeloma fusion-partner cell line suitable for chicken hybridoma production, two ways can be chosen for selecting monoclonal IgY: either the antibody library approach involving methods such as phage display, ribosome display or yeast display or single B-cell cloning.[3,30,144,145] Recombinant mAbs require less animal usage than normal polyclonal IgY's. This is because more animals are needed only before getting an antibody library ready. Furthermore, stable CHO cell lines' developed antibodies exhibit a short manufacturing cycle with minimal batch-to-batch variation. Recombinant TK1 antibodies can now be purchased. However, none of these serum biomarkers have been proven useful in health screening or clinical oncology yet.[107] Wang *et al.*[107] conducted their investigation using a new automatic chemiluminescence sandwich-BSA platform and found out that the Human Thymidine Kinase 1 (hTK1)-IgY-rmAb assay is user-friendly and suitable for large-scale health screenings. Antibody mimetics might become an alternative to traditional antibodies in different biomedical applications where they are simply ineffective. Ge *et al.*[146] revealed that antibody mimetics may find diverse biomedical uses, particularly when conventional antibodies fail. To support this study, Ge *et al.*[146] created a smaller version of chicken IgY-mimetic peptide (IgY-peptide) using complementarity-determining regions from previously generated anti-CPV IgY-scFv made in chickens. The mimetic peptide did not cross-react with CDV or CCV; therefore,

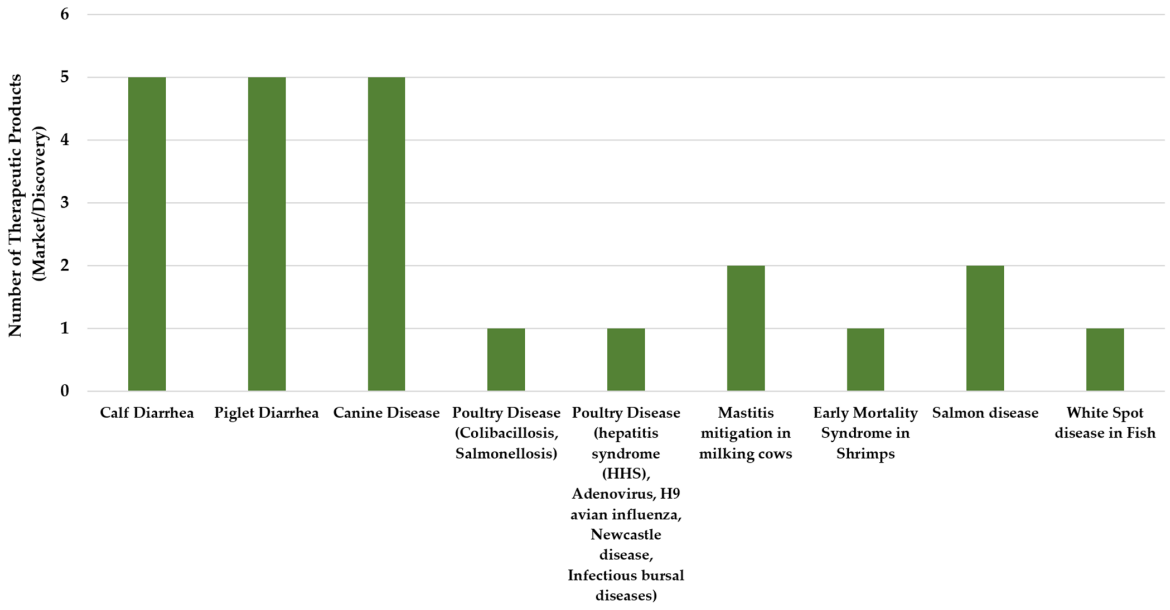


Figure 9. IgY-targeted veterinary diseases.

the specific protection exhibited by it toward CPV-infected Crandell-Rees Feline Kidney cells is exceptional. This paper presents the initial attempt at creating a synthetic antibody-like molecule as a functional mimic of IgY; herein, we present its simplicity and utility as applied to medicinal molecules.[146] Notably, in their study of the Eno1 protein of S. pneumoniae, Lee *et al.*[147] produced both polyclonal IgY and monoclonal scFv antibodies. It was discovered that recombinant spEno1 protein could specifically bind to polyclonal IgY antibodies from eggs that have been cleaned up. The findings reveal that these scFv antibodies can act as an immunotherapeutic drug to counter *S. pneumoniae* infections. According to a review article by Dou *et al.*[104] it has become the focus of how the serum IgY antibody of chicken egg yolk has gained recognition as a valuable resource in bioanalytics due to its versatility in productivity, affordable animal health care, comparable affinity, and high selected molecular specificity, among others. The problem with this is that there isn't any complete knowledge on how to do this efficiently using IgY-based assays for detecting chemicals and biological hazards in food samples, such as chemical contaminants or bacterial toxins. Dou *et al.*'s[104] paper gave a brief overview of the types, compositions, and synthesis methods for both polyclonal and monoclonal forms of IgY on the one hand while focusing more on the fundamental principles behind construction choice when designing an immunoassay, that is, risk-related issues. Lee *et al.*'s[148] research also suggest that anti-hEno1 protein-specific chicken-derived anti-human alpha-enolase (hEno1) IgY and scFv antibodies may prove very useful tools in diagnostics & therapeutics against lung cancer patients showing higher hEno1 protein levels.

Distinguishing advantages and limitations of IgY polyclonal antibodies and recombinant IgY monoclonal antibodies

Advantages of IgY polyclonal antibodies

Chickens produce antibodies, known as IgY polyclonal antibodies, when they come into contact with antigens. They are a diverse group of antibodies capable of binding many different epitopes on the target antigen.[30,149] These molecules display diverse specificities that make them useful in applications requiring the detection of multiple epitopes. In comparison to IgY monoclonal antibodies, the production of IgY polyclonal antibodies is typically more cost-effective. Large quantities of polyclonal antibodies can be generated from chicken eggs, making it possible to implement cheap production methods on a larger scale.[30] To generate them, chickens are immunized with

antigens relevant to the desired response, followed by collecting their eggs before isolating the yolk containing these specific immunoglobulins.[3] This process is less complicated and does not require advanced techniques such as hybridoma technology involved in producing monoclonal antibodies.[3] There are commercial vendors for IgY polyclonal antibodies available against various targets, including proteins, peptides or small compounds. Many trusted providers offer such kinds of reagents that have found a wide range of applications in research areas.[150]

Limitations of IgY polyclonal antibodies

However, there might be heterogeneity between batches of anti-IgY polyclonal sera obtained from different hens in terms of antibody composition and affinity. The lack of uniformity may lead to unreliable results, necessitating meticulous validation and characterization for every batch of these sera. Although some have broad specificity, other IgY polyclonal antibodies can only recognize a limited number of epitopes on target antigens. Some epitopes may be less immunogenic or not recognized by the chicken's immune system, leading to incomplete coverage of the target antigen.[107,115]

Advantages of recombinant IgY monoclonal antibodies

Recombinant IgY monoclonal antibodies are highly specific, targeting a particular epitope on an antigen using antibody-producing cells derived from a single clone.[65] This kind of specificity can be advantageous in situations where precise targeting is needed. Unlike polyclonal antibodies, recombinant monoclonal antibodies show consistent performance across different batches. They come from one source, ensuring that experimental variability remains low with highly reproducible results.[30] By characterizing them extensively, we can understand their binding affinities and kinetics more accurately, among other aspects. This is very useful to scientists investigating the effect of experimental conditions on their findings and how they can ensure that these findings are repeatable.[65] Researchers often screen and select monoclonal antibodies for specificity against the target antigen. Consequently, such reagents have a reduced ability to react with non-related molecules, thereby minimizing background signals.[151]

Limitations of IgY monoclonal antibodies

The unfavorable side of recombinant IgY monoclonal antibodies includes high costs, difficulty in identifying epitopes, and long research time.[65] While making recombinant monoclonal antibodies may be more expensive than polyclonal antibodies due to the need for more complicated methods such as hybridoma technology or recombinant expression systems,[65] it raises the issue of spending. The ability of recombinant monoclonal antibodies to recognize only one epitope on the target antigen could limit their applications in the detection of multiple epitopes. Some key steps involved in the generation of recombinant monoclonal antibodies are isolation and characterization of antibody-producing cells; production and screening for specificity, according to Leow *et al*.[65] This procedure takes longer compared to the faster making of polyclonal antibodies.[30,65]

Which is the best for low- and middle-income countries: poly or monoclonal IgY antibodies?

Various factors, such as cost, infrastructure, expertise, and accessibility, should be considered when choosing the right type of treatment for people living in poor and middle-income countries.[152,153] Polyclonal IgY synthesis is usually more economical than recombinant IgY monoclonal antibodies.[30] This can be done at a low cost by vaccinating chickens and collecting eggs.[17] Recombinant IgY monoclonal antibodies are produced through complex processes such as gene cloning, transfection, and purification of the antibody.[65] The process could be more costly and therefore require specialized equipment/expertise. The requirement for infrastructure and knowledge in making polyclonal IgY is somewhat less stringent. Routine laboratory settings will suffice while employing widely known methods, even in resource-constrained environments.[17] More advanced laboratory setups involving cell culture apparatuses and molecular biology know-how may be needed when developing recombinant IgY monoclonal antibodies[65] which may not be affordable or possible for some less-developed nations. Moreover, they recognize multiple epitopes on the target antigen, have a greater coverage area, and can detect it better than something else. We produce antibodies against diverse antigens, including intricate conserved sites, among others.[149] Single-clone-derived

monoclonal antibodies of recombinant Ig Y tend to be more specific because they come from a single clone of cells that make them.[65] Such entities can also be engineered to have specific attributes, such as modified effector functions or improved stability, thereby increasing the level of customization. LMICs can effectively execute the reasonably uncomplicated process of manufacturing polyclonal IgY with limited reliance on external supplies. Commonplace is the existence of hens for antibody production, and their availability is quite extensive.[65] On the other hand, getting specialized reagents, equipment, and knowledge could be a challenge for LMICs in producing monoclonal antibodies to recombinant IgY. This may handicap LMICs from accessing options that would enhance the development of recombinant IgY monoclonal antibodies because even technologies and precursors in this field are not always locally available.[17] The listed considerations therefore indicate that polyclonal IgY antibodies could well be a better choice for developing countries due to their cost-effectiveness, ease of production, and wider spread.[152,153] Nevertheless, proper assessment should always be carried out based on the specific requirements for each application as well as resource availability and accessibility. If there are certain applications where increased specificity and more tailored recombinant IgY monoclonal antibodies are important, it might be beneficial to engage with research establishments or groups having those tools through partnerships or collaborations.

Future directions and challenges in using polyclonal IgY and monoclonal IgY antibodies

One future direction is to develop engineered and modified polyclonal and monoclonal IgY antibodies to enhance their properties, such as by increasing their affinity or extending their half-lives.[154] This could further improve their efficacy in research, diagnostics, and therapeutics. In the future, it may be possible to combine polyclonal and monoclonal IgY antibodies with other types of treatment, such as small molecules or immune checkpoint inhibitors, to get better results from treatment.[80] We expect the use of polyclonal and monoclonal IgY antibodies as therapeutics to expand further, focusing on novel targets such as emerging infectious diseases, rare diseases, and personalized medicine approaches.[54,155,156] As technology improves, it may become possible to create quick and inexpensive diagnostic tests that use polyclonal or monoclonal IgY antibodies. This could improve healthcare access and patient outcomes, especially in resource-limited settings. It can be hard to make more polyclonal and monoclonal IgY antibodies because a lot of animals need to be immunized, and hybridoma technology is hard to understand.[65] Enhancing the efficiency and cost-effectiveness of production processes is essential to fulfilling the growing demand. The expenses associated with the manufacture, purification, and characterization of both polyclonal and monoclonal IgY antibodies can be a significant obstacle, especially in countries with lower and middle incomes.[17] It is crucial to tackling the cost concerns to ensure that they are affordable and accessible.[17] Both polyclonal and monoclonal IgY antibodies can display immunogenicity and cross-reactivity, which can cause problems in therapeutic applications. To address these problems, it is critical to improve the antibody engineering process and conduct thorough characterization.[30] Intellectual property problems may arise throughout the development and marketing of polyclonal and monoclonal IgY antibodies, particularly about patent protection and licensing agreements. Clear guidelines and limitations are essential to promoting innovation while ensuring fair and equal access to these vital resources.[157]

Chicken IgY presents an alternative and attractive approach to playing a role in pandemic control. The exponential growth in the number of registered manufacturing companies producing IgY products over the past 10 years, the number of issued patents, and the considerable volume of ongoing clinical trials are all signs of optimistic success in the IgY research field. Yakhkeshi *et al.*[17] recently published a review article that revealed a sharp increase in IgY patent applications after 2010, culminating in a peak of 77 patents in 2021. Furthermore, Yakhkeshi *et al.*[17] report that 73 industries actively market IgY products, with 46 in the diagnostic sector and 27 promoting biotherapeutics for use in human and veterinary medicine. IgY antibodies serve as primary and secondary antibodies, accounting for about 3729 and 846 products, respectively. Consumption of biotherapeutic products has significantly expanded as a food supplement and topical application in human and veterinary

medicine, which are in various stages of clinical research and have around 80 and 56 products available on the market, respectively.

Conclusion

In conclusion, IgY polyclonal antibodies possess a wide range of specificities, cost-effectiveness, and ease of manufacture. However, they may demonstrate variability between batches and have limited coverage of epitopes. Recombinant IgY monoclonal antibodies offer excellent specificity, uniformity, and clearly defined properties, but they can be more expensive and have restricted epitope recognition. The choice between polyclonal and monoclonal antibodies IgY is based on the application's specific needs, including the need for extensive or specific epitope identification, uniformity, and financial factors.

Declarations

Ethics approval and consent to participate

None.

Consent for publication

Not applicable.

Author contributions

Ashraf A. Tabll: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Supervision; Visualization; Writing (original draft); Writing (review & editing).

Yasser E. Shahein: Conceptualization; Investigation; Methodology; Supervision; Visualization; Writing (original draft); Writing (review & editing).

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Marija Glasnovic: Formal analysis; Investigation; Writing (review & editing).

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Acknowledgements

None.

Funding

The authors received no financial support for the research, authorship and/or publication of this article.

Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Not applicable.

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