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Production of Antibodies in Chickens

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Summary

Chickens, as a source of desired antibodies, represent an alternate animal system that offers some advantages with respect to animal care, high productivity and special suitability of avian antibodies for certain diagnostic purposes. Despite being an excellent counterpart to mammal IgG chicken IgY antibodies still represent an underused resource. This may be due to the lack of information concerning the possibility of production and application of IgY or their use is being hampered by problems with keeping the chickens and with IgY isolation. As a suggestion how to overcome IgY isolation problems a new immunoaffinity isolation method is presented here. The main purpose of the present work is to provide information on developments and possibilities in the production of chicken IgY. Polyclonal, monoclonal and recombinant forms of IgY, successfully produced so far, as well as their applications are summarised. This article should be a contribution to the efforts of the European Centre for the Validation of Alternative Methods (ECVAM), whose main goal is to promote the scientific and regulatory acceptance of alternative methods, which are of importance to the bioscience and which reduce, refine or replace the use of laboratory animals.

Key words: antibodies, chicken egg yolk, chicken IgY, monoclonal antibodies, recombinant antibodies, phage display, antibody library, transgenic chickens, immunoglobulin transport

Introduction

Antibodies presently available for research, diagnostic and therapies are mostly mammalian monoclonal or polyclonal antibodies. Antibody production requires the use of laboratory animals, at least in the first step. Traditionally, bigger animals such as horses, sheep, pigs and also rabbits and guinea pigs, were used for the production of polyclonal antibodies, while mice and rats were used as a source of spleen for the production of monoclonal antibodies. Nowadays, most frequently chosen mammals for polyclonal and monoclonal antibody production are rabbits and mice, respectively. Both technologies have their advantages but also disadvantages. The major problem of monoclonal antibody production is that some antigens are weakly or not at all immunogenic for mice. In polyclonal antibody production purification of antibodies from mammalian blood has been found low yielding and laborious in many cases. Both technologies also involve some steps each of which causes distress to the animals involved: *i*) the immunisation itself, *ii*) collecting of blood samples and *iii*) bleeding (or sacrifying for spleen removal), which is a prerequisite for antibody preparation. Disadvantages of the available techniques and concern for animal rights enhance the interest in developing alternative methods for the production of antibodies.

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Although the fact that immunised hens transfer immunoglobulins from the serum to the egg yolk has been known for over a hundred years, this alternative possibility of producing antibodies has attracted attention only in the last decade (*1–4*). Techniques for the production of chicken polyclonal, monoclonal and recombinant antibodies as well as techniques for their isolation from different sources, manipulation and use are completely comparable to the production of mammal antibodies. Even more, some new techniques, limited strictly to the chicken antibodies, were developed and will be discussed in this paper. Although IgY technology, as it was named by Schade *et al*. (*2*), has some disadvantages, it is worthy of attention since it has some major advantages, great applications and promising future.

In the sense of animal protection the use of chickens for antibody production represents a refinement in that the painful collecting of blood samples and final sacrificing are replaced by collecting eggs with the synthesised antibodies in egg yolk. Since chickens produce even larger amounts of antibodies than laboratory rodents, this technology also means the reduction in the number of animals used in particular experiment.

Structure and Characteristics of Avian IgY Versus Mammal IgG

Initially, avian serum immunoglobulins were classified as IgG-like immunoglobulins that are transferred to the egg yolk (*5*). In 1969 Leslie and Clem (*6*) showed experimental data proving profound differences in their structure and proposed the name IgY. Now IgY is recognised as a typical low-molecular-weight (*i.e.* non-IgM) serum antibody of birds, reptiles, amphibia and lungfish, and as an evolutionary ancestor of IgG and IgE antibodies that are unique to mammals only (*6,7*). Among all birds, chicken IgY is most frequently studied, best described and characterised. General structure of IgY molecule is the same as of IgG with 2 heavy (Hν) chains with a molecular mass of 67–70 kDa each and two light (L) chains with the molecular mass of 25 kDa each (Fig. 1). The major difference is the number of constant regions (C) in H chains: IgG has 3 C regions (Cγ1 – Cγ3), while IgY has 4 C regions (Cν1 – Cν4). One additional C region with two corresponding carbohydrate chains has a logical consequence in a greater molecular mass of IgY compared to IgG *i.e.* 180 and 150 kDa, respectively. IgY is much less flexible than IgG due to the absence of the hinge between Cγ1 and Cγ2, which is a unique mammalian feature. There are some regions in IgY (near the boundaries of Cν1–Cν2 and Cν2–Cν3) containing proline and glycine residues enabling only limited flexibility (*7*). IgY has isoelectric point 5.7–7.6 and is more hydrophobic than IgG (*8*). Regarding the relatively high core body temperature of chickens, which is 41 °C, it is not surprising, that half-life time of IgY is in months and that they retain their activity after 6 months at room temperature or for one month at 37 °C (*4*).

Structure differences are reflected in different molecular and biochemical interactions. Most biological effector functions of immunoglobulins are activated by the Fc region (*9*), where the major structure difference between IgG and IgY is located. That is why typical IgG-Fc dependent functions are essentially different in IgY molecule. First, IgY do not activate the complement (*10*), second, IgY do not bind to protein-A and G (*11,12*), third, IgY do not bind to mammalian antibodies *i.e.* to rheumatoid factors (RF, an autoantibody reacting with the Fc portion of IgG) (*13,4*) or to HAMA (human anti-murine antibodies) (*4*), and fourth, they do not bind to cell surface Fc receptor (*14*). These differences in molecular interactions bring great advantages to the application of IgY antibodies that were successfully applied in a variety of methods in different areas of research, diagnostics, medical application and biotechnology (*15*).

Chicken Polyclonal Antibody Production – IgY Technology

Using chickens as the immunisation hosts brings a number of advantages (*16,2*). Avian maternal serum antibodies are transferred to egg yolk (*5*). Thus, simple collection of eggs instead of invasive blood sampling and bleeding has an advantage concerning the welfare of the immunised animals. From the evolutionary point of view chickens as an antibody producing animals also have a great advantage (*4*). Namely, greater phylogenetic difference between the immunised animal and the animal that was the source of antigen increases the immune response. That is especially important when dealing with highly conserved mammalian proteins (*17*) such as hormones (*18*) or prions (*19*). Chicken immune system will recognise epitopes on the mammalian protein more readily and will often detect epitopes that differ from the epitopes detected by mammals, such as mice or rabbits. Keeping chickens as laboratory animals is inexpensive and requires almost the same procedures as keeping other laboratory animals. Housing conditions dictated by some species-specific behaviour must be assured as it is required by the state or international veterinary administrations. On principle, chickens bred for egg production or SPF (bred for free of specific pathogens) can be used as well as outbred chickens. When immunising chickens, two facts have to be considered: *i*) 10–15 % of outbred chickens are non-responders or low-responders to certain antigens (such as *Salmonella pullorum* bacterium, human serum albumin) (*3*); *ii*) several chicken viral diseases may cause immunomodulatory effects which can interfere with antibody production. Therefore, the use of SPF chickens is necessary at least for the production of therapeutic antibodies (*3*). Different immunisation protocols, using different adjuvants, antigen dose and volume, route of injection, vaccination frequency and interval were described (*3*). Basically different immunising protocols for each antigen and for each animal species have to be tested to find out which method induces the highest serum or/and egg yolk antibody titer. Usually 10–100 μg (*15,20,21*) of protein antigen in final volume of 1 mL is applied intramuscularly in the breast muscle at two or three injection sites of 7 to 8-week-old chicken. To avoid an eventual local tissue reaction the Freund's incomplete adjuvant could be efficiently used even for the first immunisation (instead of Freund's complete adjuvant, which is usually

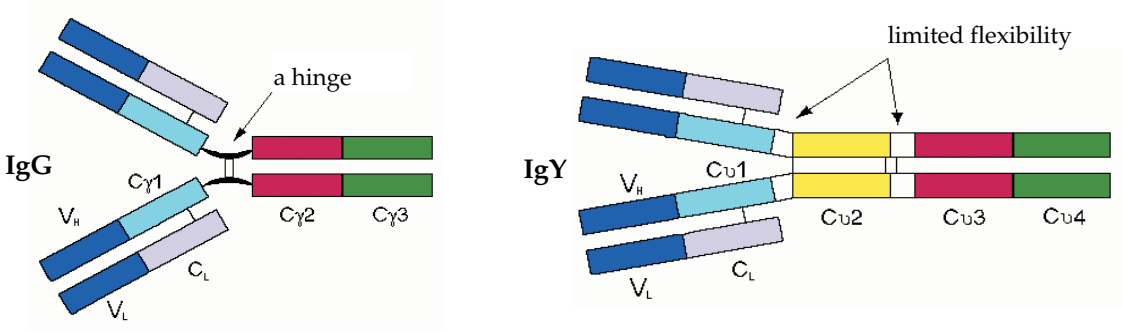

Fig. 1. Structure of IgG and IgY

used at that point when immunising mammals). Vaccination frequency and interval depend on the immunogenic potential of antigen itself and on adjuvant used (*22*). Often, reported interval is two to four weeks (*15, 20,21*). The presence of yolk antibodies should be checked two weeks after the second immunisation. When the antibody titer decreases booster immunisations can be given during the whole laying period. A laying hen produces five to six eggs per week. Average volume of egg yolk (15 mL) contains 50–100 mg of IgY, of which 2 to 10 % are specific antibodies (*3*). Compared to the production of polyclonal antibodies in mammals, this is significantly greater yield that only large mammals, such as a horse, can reach. Comparison between the rabbit and chicken polyclonal antibody production is presented in Table 1.

Egg yolk contains large amount of lipids. To avoid problems in immunoassay isolation of IgY from the egg yolk should be carried out as a final step of IgY technology. Though they do not bind to protein A and G, which is a popular method for isolation of IgG, there are many other methods enabling a successful purification (*23–25*). Frequently cited methods are affinity purification (*15,20,26*), ammonium sulphate or PEG precipitation (*27,28*) and many other methods have been compared and discussed as well (*4,25,27,29–33*). We recommend a new method, an immunoaffinity purification of IgY that was developed in our laboratory and will be discussed at the end of the article. IgY antibodies can be efficiently conjugated by different molecules that are used for conjugation of mammalian antibodies such as biotin (*23*), fluorescein (*34*) and peroxidase (*35*). They are sensitive to papain and pepsin digestion, therefore Fab and Fab' fragments can be produced (*36*).

IgY Application

Chicken polyclonal antibodies were produced against a number of antigens (summarised in Table 2) and were applied in many different methods for various purposes (as a research, diagnostic, therapeutic reagents, as a tool for purification or detection of antigens and as a protective agent in passive immunization; summarised in Table 2), where they provide an excellent alternative to or substitution for their mammalian counterparts. IgY was demonstrated to work in practically all tested immunological methods that were traditionally developed for mammalian IgG *i.e.* immunofluorescence, immunoenzyme techniques, immunoelectrophoresis and Western blotting, immunohistochemistry and many others (*15*).

In diagnostics, when components of human serum are to be examined, the human complement system, RF and HAMA are major sources of interference and false positive results in many immunoassays when mammalian IgG antibodies are used as diagnostic reagents. Since IgY do not react with mammalian IgG or complement system they offer a special advantage in these assays, which is reflected in great reduction of background and false results. In the last 5 years IgY were reported to be used as successful agents for passive and protective immunisation against gastrointestinal pathogens in humans and animals, as immunotherapeutic agents against pathogens that are difficult to treat with traditional antibiotics, as useful tool in cancer research, diagnostics and therapy (summarised in Table 2).

Table 1. Comparison of rabbit and chicken polyclonal antibody yield during a two-week period following the second immunisation

	Rabbit	Chicken
Number of animals	1	1
Method of sampling	Bleeding (20 mL/week)	daily collecting of eggs
Sample volume (in 2 weeks)	40 mL of blood	14 eggs = 210 mL of egg yolk*
Amount of total antibodies	200 mg	1120 mg**
Amount of specific antibodies	5 % (10 mg)	2–10 % (22.4–112 mg)
Rabbits/chicken – total***	5–6	1
Rabbits/chicken – specific****	2–11	1
Presence of other Ig	IgM, IgA, IgE	None

*average volume of egg yolk is 15 mL; **average amount of IgY is 80 mg per one egg yolk; ***No. of rabbits that produce an equal amount of total antibodies as one chicken in a two-week period; ****No. of rabbits that produce an equal amount of specific antibodies as one chicken in a two-week period

Table 2. Antigens used to produce specific polyclonal IgY and their applications

Antigen	Application	Reference
Human IL-6	RE	*37*
Human manose 6-phosphate/insulin-like growth factor-II receptor	RE	*38*
Human transferin	RE	*39*
Canine distemper virus	RE	*40*
α-subunit of hypoxia-inducible factor 1 (HIF-1)	RE	*15, 20*
Cathepsin D	RE	*41*
Helyobacter pylori	RE	*42*
Cholera toxin B	RE	*43*
Rabies virus	RE	*44*
Sendai virus	RE	*45*
Rabbit muscle actin	RE	*46*
Human rotavirus	RE, DE	*47*
Bovine growth hormone and prolactin	RE, DE	*14*
Lactoferin	RE, DE	*48*
Activin A	RE, DE	*49*
Parathyroid hormone-related protein	RE, DE	*50*
Mucin-like glycoprotein A	RE, DE	*51*
B-casokinin 10	RE, DE	*52*
α-subunit of insulin receptor	DE	*53*
Toxoplasma gondii	DE	*54*
Rat liver cytosolic casein kinase II	DE	*55*
Chlamidiae	DI	*56*
Newcastle disease virus	DI	*3*
African horse sickness virus	DI	*57*
Campylobacter fetus	DI	*58*
Cartilage gp-39	DI	*59*
Hepatitis B surface antigen	DI	*60*
Potato virus	DI	*60,61*
Human blood antigens	DI,RE	*62,63*
Influenza virus	DI,RE	*64*
AsialoGM1 (T-antigen in human colorectal adenocarcinom)	DI, RE	*65*
Calf thymus RNA polymerase II	DI,RE,TE	*66*
Plasmid (naked) DNA	DI, TE	*67*
E7 oncogenic protein of human papillomavirus type 16	DI, TE	*68*
P110 protein (antigen of human stomach cancer)	DI, TE	*69*
Ig and blood components of several mammalian species	DI, PU	*3,70,71*
Human α-2 plasmin (its carboxy-terminal peptide)	PU	*72*
Porcine endothelial cells	TE	*73*
E. coli	PI	*24,61,74*
Animal venoms (snake, viper, rattlesnake, scorpion)	PI	*29,75,76*
Streptococcus mutans glucan binding protein B	PI	*77,78*
Porcine epidemic diarrhea virus	PI	*79*
Yersinia ruckeri	PI	*80*

RE, research reagent; **PU**, purification of antigen; **DE**, detection of antigen; **DI**, diagnostic reagent; **TE**, therapeutic reagent; **PI**, used for passive immunization

Chicken Monoclonal Antibodies

In 1975 Kohler and Milstein (*81*) described the method for the production of monoclonal antibodies (mAb). By fusion of antibody producing mouse cells with their malignant counterparts (neoplastic B cell line) they developed such a powerful technique that they were awarded the Nobel Prize in Physiology and Medicine in 1984. Although the continuous use of the technique for almost over 30 years has enormously improved each step in this technology it still has some limitations and disadvantages. Some originate from the fact that the majority of mAbs are of mouse origin (some are of rat and very rare of human origin). First, mammalian mAb are not the most suitable for diagnostics when mammalian serum components are involved; second, evolutionary conserved antigens can be weak or not at all immunogens for mammals. At least these two limitations can be overcome by using chickens as an immunised donor of spleen cells. The technique originally developed for the production of mammalian mAb needed some modifications but the general idea and protocol remained the same. The major problem was the establishment of corresponding fusion partner to chicken B cell that would enable a successful fusion and HAT selection.

First chicken monoclonal antibodies (chmAb) were reported in 1991 by Nishinaka *et al.* (*82*). They used four TK^- chicken myeloma B cell lines (R27H1-4) and produced chmAb against keyhole limpet haemocyanin. In the process of HAT selection some HAT insensitive cells

Table 3. Chicken monoclonal antibodies produced so far

Antigen	Fusion partner	Application	Reference
Keyhole limpet haemocyanin	R27H1-4	RE	*82*
Hanganutziu-Deicher (HD) antigen	R27H4	DI	*84, 85*
Eimeria acervulina	R27H4	DE, RE	*21, 86, 87*
Human IgG	MuH1, MuH4	RE	*83*
Bovine prion protein peptide B204	MuH1	RE, DE, DI	*19*
Human prion protein peptide H25	MuH1	RE, DE, DI	*21*

RE, research reagent; **DE**, detection of antigen; **DI**, diagnostic reagent

Table 4. Chicken recombinant antibodies generated by phage display or Ig library

Source of RNA/ Ag*	Fragment	Application	Reference
Bursal lymphocytes/ 0	scFv	ME, RE	*92*
Spleen cells/murine serum albumin	scFv	ME, RE	*93*
Hybridoma cells/N-terminal PrP	scFv	RE, DE	*94*
Hybridoma cells/*Eimeria acerulina*	scFv	ME, RE, DT	*95-97*
Spleen cells + bone marrow/ hapten fluorescein	scFv, diabody, chicken/ human chimeric Fab	ME	*98*

*cells that were used for RNA extraction / antigen that was used for immunisation or hybridoma cells that are specific for; **ME**, introduction of methodology; **RE**, research; **DE**, protein (antigen) detection; **DI**, diagnostic reagent

appeared and inhibited the initial growth of hybrids and decreased the chances of selection of desired clones. So the additional improvements of chicken myeloma B cells were necessary. This was achieved by introduction of ouabain resistance into the TK^- myeloma cells. By establishing two chicken B myeloma cell lines MuH1 and MuH4, successors of R27H1 and R27H4 respectively, Nishinaka *et al*. (*83*) greatly increased the fusion efficiency. In the last five years some useful ch mAbs were produced (summarised in Table 3) with the greatest achievement in 1998 and 1999 when the team of Prof. Haruo Matsuda produced chmAbs against synthetic bovine prion protein peptide (*19*) and against the N-terminal part of human prion protein (*13*). They produced chmAbs that are suitable tool for immunological and diagnostic analyses of prion diseases in humans and other mammals and demonstrated how the problem of high protein homology in mammals can be overcome by using phylogenetic distant chickens in antibody production.

Recombinant Chicken Antibodies from Phage Display and Library

Limitations of mAbs, particularly when used for human therapy, where human mAbs are most desirable, dictated a new approach in the production of antibodies. The polymerase chain reaction (PCR) enables rapid cloning of particular protein genes, in this case Ig genes, mostly those encoding variable (V) domains. The use of surface-display vectors for displaying polypeptides (Ig molecules or their fragments) on the surface of bacteria and filamentous phage (now termed as phage display technology), combined with *in vitro* selection technologies, enabled the synthesis and selection of tailor-made antibodies. In fact, antibodies were the first proteins to be displayed successfully on the surface of phage in 1990 (*88*). A direct application of phage technology is cloning the Ig genes and creating an antibody library that gives rapid access to the expressed genes. Repertoires may be created from Ig genes of hybridomas (from mAbs production), or from B cells from spleen of native or immunised donor. Compared to hybridoma technology, many more Abs may be accessed from the material of a single donor. The selected Abs have higher affinity than mAb and can be rapidly produced in higher amounts. Nowadays phage displayed active antibody fragments (Fab, variable fragment Fv, single chain variable fragment scFv and construct, which use two different polypeptide chains, V_{H1}–V_{H2} and V_{L1}–V_{L2}, known as diabody fragments) of mouse and human origin are routinely produced and used as well as Ig libraries from a variety of species (*89*). Both technologies, phage display and construction of antibody library were also used for the production of chicken antibodies or their fragments (Table 4). Chickens offer two advantages: first, they respond to evolutionary conserved mammalian proteins used as antigens; second, formation of immunoglobulin repertoire in chickens is different from mammals, which enables slightly easier manipulation. Molecular diversification of Ig in mammals occurs through the somatic recombination of a diverse set of Ig variable (V), diversity (D) and joining (J) gene segments for heavy and light chains (*90*). In chickens, single functional Ig V and J gene segments of heavy and light chain (each) undergo V(D)J rearrangement and further diversity is achieved by gene conversions *i.e.* by transplanting blocks of sequences from upstream pseudogenes in both heavy and light chain V regions (*91*). This greatly simplifies the representative amplification of V region genes since only two pairs of primers are needed. Daves *et al*. (*92*) first demonstrated feasibility of generating chicken phage antibodies. They constructed the scFv library using total RNA from bursal lymphocytes of unimmunised chicken and selected by display technique phage with specific scFv for different antigens. Yamanaka *et al*. (*93*) constructed the library from RNA extracted from spleen cells isolated from the chicken that was immu-

nised with murine serum albumin and selected antigen--specific scFv by panning. Many authors reported that hybridoma cells produce too low levels of antibodies (*94–97*). In order to produce larger amount of mAbs Nakamura *et al*. (*94*) generated scFv using phage display. The source of RNA were chicken hybridoma cells secreting mAbs to the N-terminal part of mammalian prion protein (PrP). Low yield in hybridoma produced mAbs was also the main reason why Kim *et al*. (*95*), Min *et al*. (*96*) and Song *et al*. (*97*) produced scFv antibodies from VH and VL genes of hybridomas producing mAbs specific for different proteins of *Eimeria acervulina*. Perhaps the major contribution to the development of chicken recombinant antibodies in last few years was made by Andis-Windhoph *et al*. (*98*), who demonstrated the selection of antigen-specific clones from three libraries constructed from the same animal. They combined the spleen cells and bone marrow, extracted RNA and constructed two scFv libraries, one for monomeric scFv and the other for diabody formation. The third library was chicken-human chimeric Fab antibody library, where they also demonstrated the improvement of technique by using a new variant of the pComb3 phage display vector system. They developed the methodology that would produce specific chicken antibodies for clinically relevant antigens.

Expression of Genetically Engineered Antibodies in Chicken Eggs

Although the recombinant antibodies revolutionised the production of therapeutic antibodies, the need for inexpensive and efficient production still persists. Chicken eggs have long been recognised as a potential source of pharmaceutical product and represent a low cost, high-yield bioreactor system. Egg white and egg yolk are sterile, their proteins can be fractionated with different technologies and combined with the egg industry's capacity to produce thousands of eggs per day. Thus, large quantities of proteins can be obtained. Numerous proteins are secreted into the egg white or egg yolk and different strategies have been used to develop transgenic chickens which would produce eggs containing new drugs to treat serious diseases including cancer. Recombinant human antibodies are one of these promising drugs that were demonstrated to be successfully produced through chickens. As the chicken oocyte develops, receptors on its membrane sequester large amounts of maternal serum IgY into the egg yolk. Sherie *et al*. (*99*) described the sequences in IgY that are important for such receptor-mediated transport and demonstrated why only some Ig can be transported into the egg yolk. Namely, human IgG and IgA that were transported to egg yolk possess the corresponding sequences (or their homologues), while chicken IgA, IgM and the truncated form of duck IgY that was not transported through the yolk sac membrane lack such sequences. Their results offer the possibility to direct the deposition of other therapeutically useful proteins into the egg yolk and are also in agreement with the results described four years earlier by Mohammed *et al*. (*100*). They injected intravenously human IgG and human IgA into the hens and both molecules were detected in egg yolk. As with chicken IgA, human IgA was deposited into egg white. A second approach that Mohammed *et al*. (*100*) presented is the establishment of transgenic chickens using stably transfected DT40 cell lines that secrete recombinant human IgG (rhIgG) and IgA (rhIgA). Intravenously injected cells colonised the chicken host, and the secreted rhIgG and rhIgA were deposited into the egg yolk. The major disadvantage of this approach, which is not tolerable, is that rhIgG and rhIgA elicited a chicken anti-human immune response, therefore a majority of egg yolk antibodies would be anti-rhIgG/A IgY. In conclusion they suggest that by suppressing the expression of endogenous chicken Ig, it would be possible to generate transgenic hens that deposit mg amounts of a single defined rhAb.

Immunoafinity IgY Isolation

Avian technology has many advantages over the genetic modification of mammals, who were developed to produce drugs in their milk, and certainly has a great future. Chicken eggs can be produced fast, with low cost and high yield and long term production can be achieved. That is why several avian transgenic companies entered the scene. Though the oral administration of specific antibodies by eating eggs is an attractive approach to establish protective immunity against gastrointestinal pathogens and is becoming more and more interesting as increasing number of antibiotic resistant bacteria develops, the deficiency of this approach is in the great reduction of IgY antibody activity in gastrointestinal tract under acidic conditions. To overcome this problem the liposomal encapsulation of IgY was proposed by Shimizu *et al*. (*101*). For this purpose and for all technologies described above, IgY isolation as a final step is required. To optimise the isolation of IgY from all sources, either egg yolk, chicken serum, hybridoma culture supernatant or bacterial cell lysat, immunoaffinity isolation method was developed in our laboratory. Mouse anti chicken IgY mAbsM2, specific for Hν chain, were produced (*102*) and coupled with CNBr activated Sepharose 4B according to the producer's manuals (Sigma). Chicken serum IgY, egg yolk IgY (after lipid elimination) and IgY from hybridoma culture supernatant were applied to the column and after washing with PBS (pH=8), pH conditions were gradually decreased (pH=4; 3.5; 3; 2.5; 2). As it was demonstrated by dot immunobinding assay (DIBA) (*103*) IgY were eluted at pH=3 and 2.5 (Fig. 2).

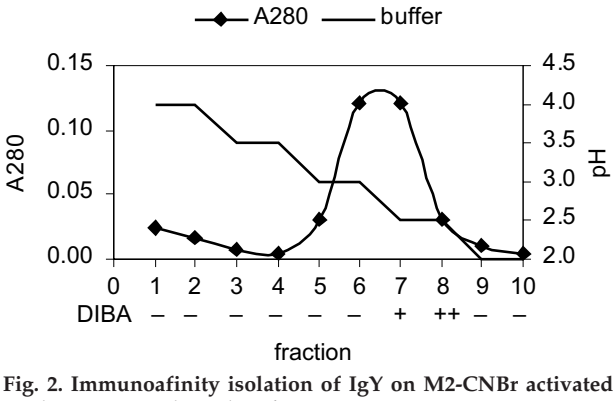

Fig. 2. Immunoafinity isolation of IgY on M2-CNBr activated Sepharose 4B and results of DIBA

Clone 1F5/3G2 that produces mAbM2 is stable, so mAbs can be produced at any time. M2 themselves, soluble or bound to CNBr activated Sepharose, are highly stable as well, so after regeneration the column can be used many times. Except for egg yolk, where the first step is lipid removal, this is very fast and standardised, one-step method that could substitute many different less specific biochemical methods for IgY isolation, which still remains a necessary step for the successful application.

In conclusion, it should be pointed out that scientists working with antibodies, either producing or using them, could take into consideration alternative methods instead of the production of antibodies in mammals. Beside that, producing IgY in chickens could sometimes be even more successful than producing mammalian IgG, this alternative method is in agreement with international effort to reduce, refine and if possible, to replace animals in experimentation (*104*).

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Proizvodnja antitijela u pilića

Sažetak

Pilići kao izvor potrebnih antitijela imaju neke prednosti, u usporedbi s drugim životinjama koje se koriste za njihovu proizvodnju, kao što su velika produktivnost i osobita prikladnost u njima proizvedenih antitijela za određene dijagnostičke svrhe. Usprkos tome što je izvrstan pandan IgG-u sisavaca, IgY antitijela pilića još je uvijek nedovoljno iskorišteni resurs. Možda je tome uzrok nedostatak informacija o mogućnostima proizvodnje i primjene IgY ili je njegovo korištenje ometano problemima čuvanja pilića i izolacijom IgY. U radu je prikazan novi imunoafinitetni postupak izolacije kako bi se prevladali problemi vezani za izolaciju IgY. Glavna je svrha ovoga rada informacija o razvoju i mogućnostima tijekom proizvodnje IgY pilića. Sažeto su prikazani poliklonalni, monoklonalni i rekombinantni oblici IgY, do danas uspješno proizvedeni, a i njihova primjena. Rad je doprinos nastojanjima Europskog centra za pravovaljanost alternativnih postupaka (ECVAM), čija je glavna svrha promocija i prihvaćanje znanstvenih i regulacijskih alternativnih metoda, važnih za bioznanost, čime se smanjuje, profinjuje i nadomješta korištenje laboratorijskih životinja.