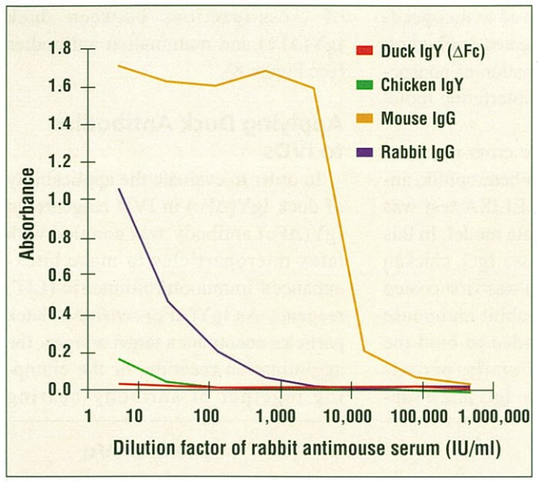

Figure 8. Binding of a rabbit antimouse antibody to different species' antibodies.

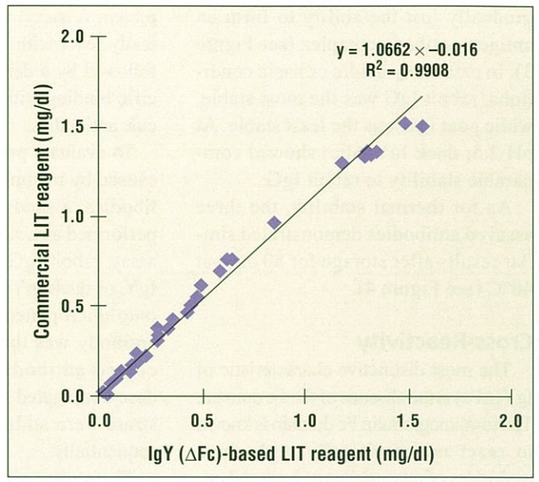

Figure 9. Correlation of IgY(ΔFc)-based CRP assay reagent and an FDA-approved commercial reagent.

particles in the presence of specific antigens, increases the turbidity of the reaction mixture, which can then be measured by automated clinical chemistry analyzers. Based on this principle, the concentration of an analyte in sample specimens can be determined automatically through an interpolation against calibrators.

The prepared high sensitivity C-reactive protein assay reagents have assay ranges of 0.01 to 2 mg/dl with less than a 5% coefficient of variation. Compared with an FDA-approved latex immunoturbidimetric assay, the IgY(ΔFc)-based LIT assay reagent showed good correlation, suggesting that IgY(ΔFc) is suitable for IVD applications (see Figure 9).

Conclusion

Searching for a suitable antibody is top priority for immunological IVD reagent manufacturers. An ideal antibody should possess the following characteristics:

- High specificity or affinity.
- No interference from molecules present in sample specimens.
- Stable assay or storage conditions.
- Easily obtained with continuing supply.
- Cost-effective.
- Minimal animal suffering.

Mammalian polyclonal antibodies, monoclonal antibodies, and avian antibodies each have their own unique advantages as well as limitations. Choosing the most suitable antibody depends on a thorough consideration of application fields, assay methods, and budget.

The overlooked IgY(ΔFc) could provide an option in both research and commercial fields, as well as in state-of-the-art biotechnology devices (e.g., antibody chips or biosensors that utilize antibodies as the main biological detection unit). The key biological features of the duck antibodies described above—namely the lack of Fc and the corresponding low interference and cross-reactivity with mammalian systems—make these antibodies alternative candidates for diagnostic applications in which such factors play a significant role.

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IVD Technology

Duck antibodies for IVD applications

Victor Chiou

A naturally existing analog to mammalian antibodies offers an alternative with broad applications.

In manufacturing immunological reagents for IVD applications, antibodies are the key components. For the most part, IVD companies employ mammalian antibodies to manufacture immunological reagents. Such antibodies include polyclonal antibodies, which are easy to produce, and monoclonal antibodies, which provide the analytical advantage of recognizing only one epitope on the target antigen.

However, in addition to the high cost of monoclonal antibodies, all mammalian antibodies have limitations. For example, the Fc domain of such antibodies is responsible for cross-reactions with interfering factors or Fc receptors, which constitute a family of cell-surface molecules that are expressed on almost every cell of the immune system.

In immunoassays, such mammalian antibodies can be nonspecifically bound by either human antianimal antibodies that arise as a result of exposure to a monoclonal antibody therapeutic or imaging agent (e.g., human antimouse antibodies), or rheumatoid factors that are present in the specimens of rheumatoid arthritis patients and some healthy individuals.[1,2] Such endogenous interfering antibodies usually exhibit broad reactivity and can result in false-positive or false-negative results in immunoassays. The consequences are that a patient may be required to undergo additional diagnostic testing or even unnecessary treatments. An antibody that can avoid such cross-reactions mediated by the Fc domain clearly has an advantage.

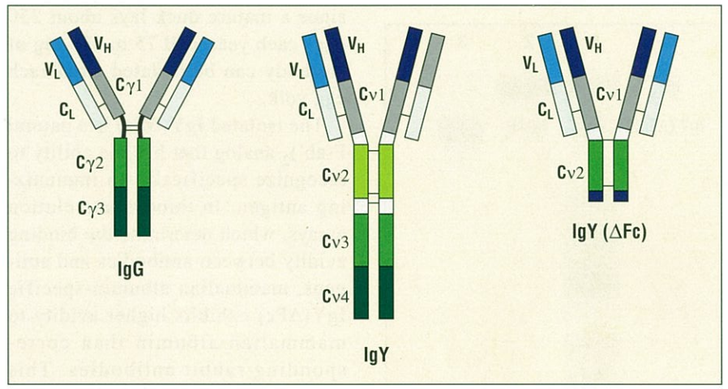

Figure 1. Molecular structures of mammalian IgG, chicken IgY, and duck IgY (ΔFc).

Avian Alternatives

With the purpose of finding a "better" antibody, research on avian antibodies has been going on since the 1960s. Avian antibodies refer to the antibodies that are produced in the serum or eggs of birds. Among the avian antibodies, immunoglobulin Y (IgY) has been the most extensively studied. IgY is the functional equivalent and evolutionary ancestor of mammalian immunoglobulin G (IgG) and is found in some birds, reptiles, and amphibians.[3] IgY contains molecules that possess two heavy and two light chains, with the heavy chains having one variable and four constant region domains (see Figure 1). It has a molecular weight of ~180 KDa and a sedimentation coefficient of 7.8 Svedberg units (S).

Chicken egg yolk IgY specifically has been advocated as an alternative to mammalian sources of antibodies because it offers a cheap, bloodless, and productive source.[4] IgY derived from chicken egg yolk also offers the advantage of not cross-reacting with mammalian antibodies, hence eliminating interferences in immunoassays.

A truncated version of IgY—IgY(ΔFc) or IgY(–Fc)—is found in ducks, some turtles, and lungfish. This truncated form of IgY, which may have evolved as a result of alternative mRNA splicing, lacks the two C-terminal domains in the heavy chains and is the structural equivalent of an $F(ab')_2$ fragment.[5] Due to the absence of the Fc domain, IgY(ΔFc) has a molecular weight of ~120 KDa and a sedimentation coefficient of 5.7 S. This unique ΔFc or –Fc

structure makes IgY(ΔFc) a potentially useful tool in producing reagents for use in immunoassays and other immunological applications.

Despite their potential, IgY antibodies are not extensively used in industry because of the difficulties involved in isolating them from chicken egg yolk. No chicken IgY-based IVD reagents are currently available on the market.[6] Similarly, the high lipid content in duck eggs makes duck egg yolk antibodies difficult to isolate, thereby hampering research into possible applications for IgY(ΔFc).

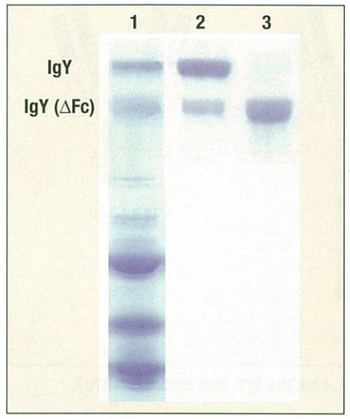

Figure 2. SDS-PAGE of duck egg yolk protein showing whole egg yolk protein (lane 1), egg yolk antibody (lane 2), and the IgY (ΔFc) antibody (lane 3).

Overcoming Isolation Problems

Recently, however, a procedure for isolating duck egg yolk antibodies has been developed. Through this procedure, which involves a short protocol of delipidization, salting out, desalting, and concentration, bulk-quantity duck IgY (ΔFc) can be isolated. The duck IgY(ΔFc) thus isolated has a purity of approximately 95% as shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (see Figure 2). Duck eggs serve as a cost-effective source of antibodies since a mature duck lays about 250 eggs each year, and 75 to 120 mg of antibody can be isolated from each egg yolk.

The isolated IgY(ΔFc) is a natural $F(ab')_2$ analog that has the ability to recognize specifically an immunizing antigen. In thiocyanate elution assays, which determine the binding avidity between antibodies and antigens, mammalian albumin-specific IgY(ΔFc) exhibits higher avidity to mammalian albumin than corresponding rabbit antibodies. This strong recognition and binding specificity of duck IgY(ΔFc) with mammalian antigens is perhaps due to phylogenetic differences between mammals and birds. For example, the differences between human and avian proteins are greater than those between human and chimpanzee proteins. This characteristic makes avian antibodies especially suitable for use in immunological assays involving mammalian specimens.

Stability Issues

Since the application of antibodies in biotechnology and medicine places a high premium on stability, experiments were conducted to compare the stabilities of rabbit, goat, and duck antibodies. In each case, antibodies to C-reactive protein (CRP) were selected for testing. These antibodies were stored at 40°C in an incubation chamber for up to 80 days, and subjected to low- and high-pH conditions for 4 hours, followed by a determination of remaining titers using single radial immunodiffusion.

In the pH experiments, all of the rabbit IgG, goat IgG, and duck IgY(ΔFc) antibodies remained stable during the 4-hour incubation period at pH 3.5 to 9. The antibody solution was adjusted from 3.5 to 9 by adding an HLL/NaOH glycine buffer, and then neutralized to pH 7. The capacity of the antibodies to form antigen-antibody complexes was then determined by single radial immunodiffusion. At pH levels of less than 3.5 or greater than 10 all of the antibodies gradually lost the ability to form an antigen-antibody complex (see Figure 3). In extremely acidic or basic conditions, rabbit IgG was the most stable, while goat IgG was the least stable. At pH 2.5, duck IgY(ΔFc) showed comparable stability to rabbit IgG.

As for thermal stability, the three assayed antibodies demonstrated similar results after storage for 80 days at 40°C (see Figure 4).

Cross-Reactivity

The most distinctive characteristic of IgY(ΔFc) is the absence of the Fc domain. The immunoglobulin Fc domain is known to react nonspecifically with some molecules of mammalian or bacterial origin.[7] Some substances, such as animal heterophile antibodies and bacterial proteins, are known to react with the immunological Fc domain. Because IgY(ΔFc) antibodies lack the equivalent Fc domain, an IgY(ΔFc) coating on microplates does not bind with molecules of rheumatoid factor, human complement C3, human complement C4, or staphylococcal protein A (see Figures 5–7).

By contrast, mammalian-antibody-coated plates exhibit high nonspecific binding to these molecules. In these experiments, normal human serum (for the complement C3 or C4 assay) or purified protein (for the rheumatoid factor or protein A assay) was used to nonspecifically react with the coated antibodies, followed by a determination of nonspecific binding with antiinterfering molecule antibodies.

To evaluate possible cross-reactions caused by mammalian heterophilic antibodies, a sandwich ELISA test was performed as a surrogate model. In this assay, rabbit IgG, mouse IgG, chicken IgY, or duck IgY(ΔFc) was first coated onto microplates. A rabbit antimouse antibody was then added to bind the coated antibodies. Finally, peroxidase-conjugated mouse IgG and a substrate were added sequentially.

The strong signal on the mouse IgG-coated microplates indicated specific capture of the peroxidase-conjugated mouse IgG by rabbit antimouse IgG. The signal on the rabbit-IgG-coated plates indicated nonspecific cross reactions with these antibodies. No signal was observed to indicate the presence of cross-reactions between duck IgY(ΔFc) and mammalian antibodies (see Figure 8).

Applying Duck Antibodies to IVDs

In order to evaluate the applicability of duck IgY(ΔFc) in IVD reagents, an IgY(ΔFc) antibody was coupled with latex microparticles to make latex-enhanced immunoturbidimetric (LIT) reagents. As IgY(ΔFc)-sensitized latex particles encounter a target antigen, the agglutination reaction, or the clumping together of antibody-bearing

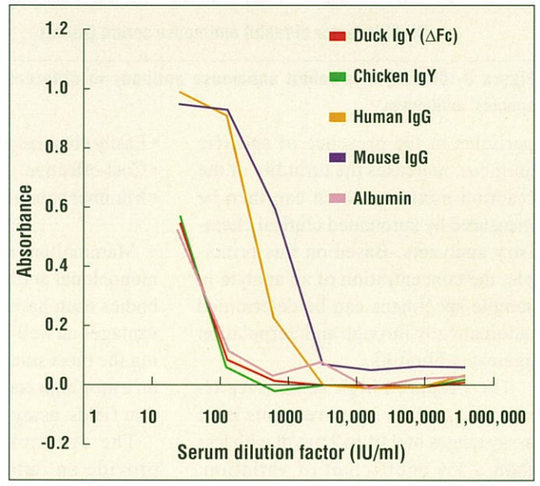

Figure 6. Nonspecific binding of antibodies to human complement C3.

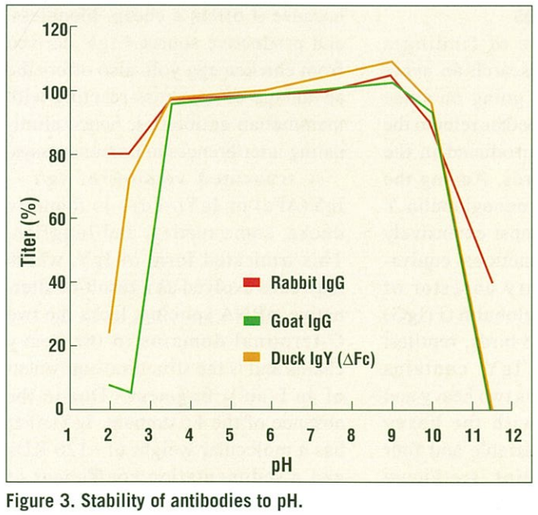

Figure 3. Stability of antibodies to pH.

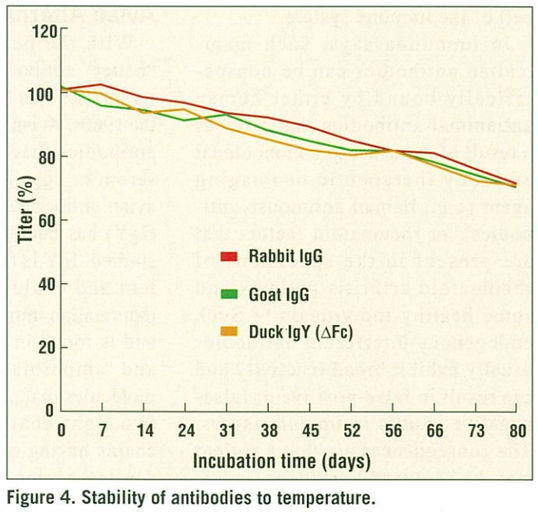

Figure 4. Stability of antibodies to temperature.

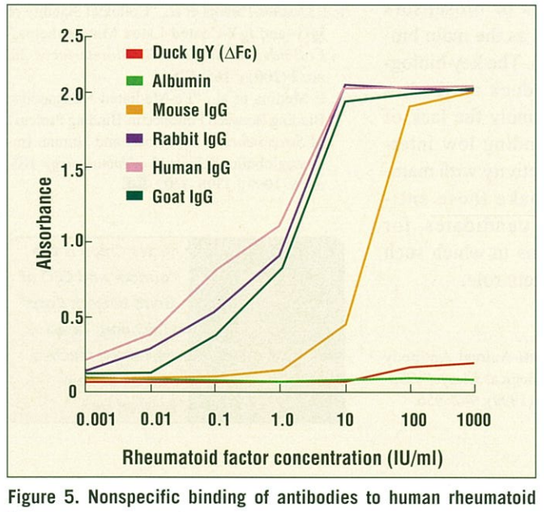

Figure 5. Nonspecific binding of antibodies to human rheumatoid factor.

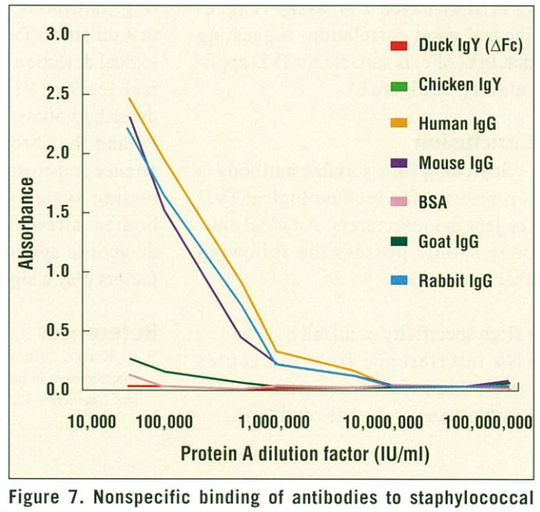

Figure 7. Nonspecific binding of antibodies to staphylococcal protein A.