Simplifying Antibody Conjugation Process

Elimination of Column Separations Is One Benefit of Lightning-Link Technology

Nick Gee, Ph.D.

Antibodies are widely employed in the quantification of antigens in complex biological samples. Using techniques such as Western blotting, ELISA, and immunohistochemistry researchers are able to measure a single antigen, or perhaps a limited number of antigens, in each sample. In the post-genomics era, advances in multiplex immunoassay technologies now allow scores or even hundreds of antigens to be measured simultaneously.

While almost all antibody-based detection techniques require a label of some description, which confers measurability, the vast majority of commercially available antibodies are not labeled. Only antibodies with the greatest commercial value might be offered in conjugate form by suppliers, and then perhaps only with a few key labels.

In order to use unlabeled antibodies it is necessary to adopt an indirect detection method. In this approach, the primary antibody binds to its target antigen and is then detected with a secondary reagent, commonly another antibody that bears the required label. In multiplex assays it becomes increasingly difficult to create a panel of secondary reagents with the desired selectivity and lack of unwanted cross-reactions if there are more than two or three primary antibodies.

By covalently attaching the label directly to the primary antibody it is possible to overcome these difficulties and to reduce the complexity of immunoassays. Historically, antibody conjugation has been conducted by those with specialist knowledge of chemical-modification techniques. Most strategies for conjugating two molecules, A and B, involve chemical modification of each entity to introduce reactive groups.

The resulting derivatives of A and B are separated from excess chemical reagents by column chromatography and then mixed, with the aim of creating soluble AB conjugates. One difficulty is that the introduction of too many reactive groups can lead to the formation of polymers and insoluble aggregates. Today, because of significant advances in conjugation technology, the production of labeled antibodies is one of the simplest procedures performed in a research lab.

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Lightning-Link

Innova Biosciences (www.innovabiosciences.com) has developed a conjugation technology called Lightning-Link™ (*Figure 1)* that eliminates almost all of the steps employed in a traditional conjugation process. The elimination of column separations from Innova's process has probably had the greatest impact. Issues that have beset traditional conjugation procedures—losses of material, sample dilution, batch-to-batch variation and difficulties in scaling up—have now been removed.

The Lightning-Link process is summarized in *Figure 2*. The researcher pipettes the antibody to be labeled into a vial of lyophilized mixture containing the label of interest. Dissolution of the contents activates the chemicals that mediate the conjugation reaction. Despite its apparent simplicity, the Lightning-Link process is sophisticated and generates conjugates with performance characteristics identical with, or better than, those prepared with laborious multistep conjugation procedures.

Moreover, it is possible to use the resulting Lightning-Link conjugates without purification, as the byproducts of the reaction are completely benign. Because the reactive groups are created in situ in a controlled manner the risk of unwanted polymerization is reduced.

The approach is also tolerant of sodium azide, which is commonly employed as an antimicrobial agent in commercially available antibodies. In addition, BSA, another common additive, has only a modest impact on Lightning-Link conjugation reactions.

This simpler approach to conjugation is likely to shift the balance of indirect detection technologies toward those of direct detection. Researchers carrying out immunodetection procedures can eliminate the tedious secondary incubation and wash steps. Intuitively, one can also see how data quality is likely to be improved by a reduction in the number of assay variables.

In flow cytometry, it is quite common to combine three or more directly labeled primary antibodies, each with a different fluorescent label.

The benefits of simple conjugation technology and direct labeling are even greater in the case of multiplex immunoassay technologies, where large panels of antigen-specific reagents can be constructed from the best available antibody tools, without the usual limitations that apply to indirect detection methods.

Simple conjugation technologies also greatly facilitate conjugate optimization and scale-up.

It is now possible with just 100 micrograms of antibody to create 20 trial conjugates, allowing one to reach a level of performance that is limited only by the attributes of the antibody itself. A similar approach can also be used to identify the best antibody, or best antibody pair, from a library of monoclonal antibodies against a common antigen.

The best conjugate can be scaled easily as there are so few variables in the conjugation process. For example, in *Figure 3,* conjugates prepared at 50 microgram scale and 50 milligram scale show identical ELISA performance; both reactions required a hands-on time of 30 seconds.

We also expect simple state-of-the-art conjugation methods to be deployed for manufacturing of tomorrow's immunodiagnostics reagents, because of greater product consistency, higher yields, and reduced costs.

Available Labels

The labels presently available in the one-step Lightning-Link format include enzymes (horseradish peroxidase, glucose oxidase, and alkaline phosphatase), fluorescent proteins (phycoerythrin, allophycocyanin, PerCP), streptavidin, biotin, and fluorescent dyes that cover the entire visible spectrum. Recently, oligonucleotides have also been integrated into the Lightning-Link format allowing the development of reagents for use in ultrasensitive immuno-PCR diagnostics tests.

The availability of an increasing number of labels will facilitate the exploration of the human proteome. Researchers might also expect the percentage of commercially available antibody tools available in labeled form to increase, and for there to be a broader range of labels for each antibody.

Finally, in view of the simplification of bioconjugation technology one can imagine in the near future a vast library of exciting research tools, comprising millions of antibodies and several hundred labels, which can be combined in any way to create an almost infinite number of virtual conjugates, any of one which can be turned into reality on demand. GEN

Figure 2. Lightning-Link conjugation process: The conjugation reaction can be set up in seconds by adding the antibody to a special lyophilized mixture that contains the label of interest. The byproducts of the conjugation reaction are benign and the conjugate can be used directly from the pot without purification.

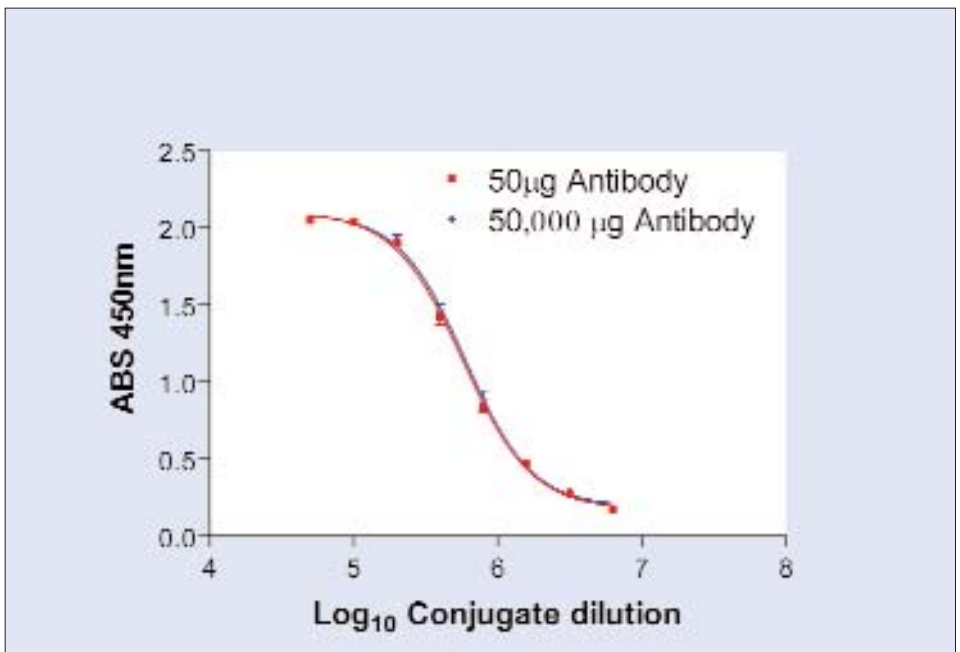

Figure 3. Comparison in ELISA of HRP conjugates prepared at two scales.

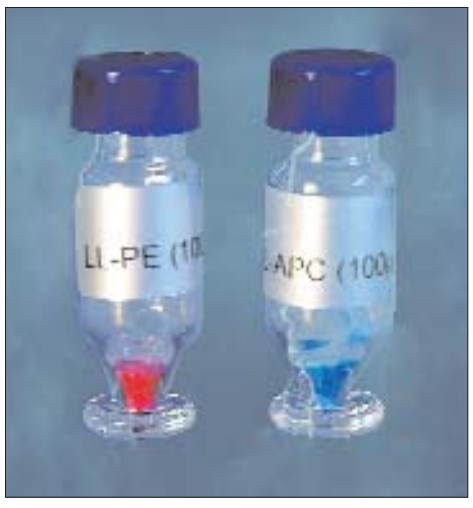

Figure 1. Lightning-Link eliminates almost all of the steps employed in a traditional conjugation process.