A new era of recombinant proteins has arrived. Scripps Laboratories is leading the way in developing novel recombinants to meet the needs of the diagnostic industry.

The last few years have brought monumental changes to the clinical diagnostic industry. The Covid-19 pandemic and the war in Ukraine have exposed vulnerabilities in global supply chains and underscored the value of risk management and disaster preparedness. Biological reagents, like purified human proteins, are critical to the diagnostic industry and these recent events have disrupted the steady supply of these essential raw materials. This jeopardizes the diagnostic industry's ability to keep test kit manufacturing on-schedule and maintain timely delivery of the life-saving assays needed in hospitals, reference laboratories, clinics, and doctor's offices around the world. Fortunately, there is a solution to this and Scripps Laboratories is leading the effort by developing sustainable, top-performing recombinant proteins that bypass the risks associated with the outdated supply chain structures in place today.

Shortcomings of Native Proteins

Traditionally, clinical diagnostic manufacturers have had two choices when considering proteins for their assay systems: native sourced and recombinant. Native proteins are those purified from human and other mammalian glands, organs, tissues, and fluids. Depending on the tissue source and the amount of protein needed, industrial-scale production and purification for the diagnostic industry can require dozens, hundreds, or even thousands, of individual donors.

Supply Chain Vulnerability / Unpredictable Supply

Relying on the availability of hundreds or thousands of donors is a high-risk venture and is ill-suited for the diagnostic industry. We have witnessed supply chain interruptions worldwide and the availability of human donor materials has become scarce and, in several instances, donor materials are not available at all. This vulnerability leads to unpredictable availability of these crucial donor materials. As a result, dozens of critically important native proteins, which were once abundantly available, are now in scarce supply.

Risk of Infectious Disease

Native proteins possess other characteristics that make them problematic in a regulated industry like clinical diagnostics. In addition to being reliant on individual donors, they run the risk of carrying and transmitting infectious disease. It is not possible to predict what unknown agent of infectious disease may be present in human tissues and fluids. Nor do we know where an outbreak may strike and what the global impact may be. We have seen this recently with Covid-19 and historically with outbreaks of Zika, Ebola, the H1N1 swine flu, HIV, and more. Biohazard risk is intrinsic to native proteins, as it is not possible to test for infectious agents that are not known to exist.

Protein Contaminants / Long Production Lead-Times

Native proteins are prone to contamination with proteins that are present in the starting material. Often, these contaminants are similar to the target protein being purified, making them difficult to remove. This results in long production cycles and decreased yields of the final product. Contaminating proteins

may also interfere with the function of a diagnostic assay, even if present in trace amounts.

Variable Production Yields

Over the last twenty years, we have seen the quality of native raw materials available to reagent manufacturers decline considerably. Many of the glands and organs donated for protein purification are damaged or diseased and contain only small amounts of the target protein. This has a pronounced, negative impact on production, as the yield of final product varies widely from one purification batch to the next, based on the quality of the starting material. Further, the purification of native proteins often employs multiple chromatographic steps, each of which results in additional loss of the target protein. The unpredictability of protein yield requires native proteins to carry a higher price to account for low-yield production cycles.

Benefits of Recombinants

In stark contrast to native proteins, recombinants offer several characteristics that make them ideal for the diagnostic industry. Notably, recombinant proteins are not dependent on glands, organs, and tissues provided by individual human donors. Native raw material supply is notoriously inconsistent, often resulting in production delays. Conversely, recombinant proteins are not bound by the limitations of outdated raw material supply chains. They are purified from cell lines that can be grown and harvested on-demand and, therefore, recombinants are not susceptible to the disruptions that are now common in traditional supply chains.

No Risk of Infectious Disease

The presence of an infectious disease agent is a serious, inherent danger when working with proteins purified from native sources. As we have seen with Covid-19, it is not possible to test for biohazardous or infectious materials that are not known to exist. This risk is carried through to the purified protein and, as such, native proteins are provided with a warning to handle as though the product is potentially infectious. This can present regulatory challenges for diagnostic test manufacturers as they import or export their test kits and components to locations around the world.

Recombinant proteins do not undergo such regulatory scrutiny, as they are purified from cell culture or fermentation systems. These protein production methods are not human sourced and are not considered biohazardous. As a result, recombinant proteins are not subjected to the regulatory restrictions found with native proteins.

No Protein Contaminants / Short Production Lead-Times

We discussed above the issues created by the protein contaminants encountered in native protein production. Recombinant protein purification is not encumbered by this as the cell culture or fermentation supernatant does not contain the biological detritus present during native tissue extractions. This facilitates the production of a highly purified final product that is highly unlikely to contain even trace amounts of native protein contaminants.

Readily available starting material and the absence of native protein contaminants result in short production lead-times for recombinant proteins. In some cases, native proteins can take as long as 5-6 months to purify, as contaminants are often very difficult to remove. In contrast, recombinants can be produced in weeks, rather than months. In addition, recombinant cell culture and fermentation systems are easily scalable, which enables on-demand production of large batches of protein.

Predictable Production Costs / Stable Pricing

The costs associated with recombinant protein production are predictable. Cell culture and fermentation reagents are easy to obtain and their prices are stable. Furthermore, the shorter production cycle of recombinant proteins reduces the production cost of the final product.

When considered collectively, the factors presented here demonstrate how recombinant proteins can be produced safely, consistently and more economically than native proteins. The results are highly purified, consistent biological reagents that can be produced in sustainable and renewable systems.

Developing Recombinants for Diagnostic Applications

Recombinant proteins offer many advantages over native proteins, but those features are irrelevant if the recombinant does not perform in a diagnostic assay system. Recombinant proteins have been available for biomedical research and pharmacological use since the early 1980s, but their use in clinical diagnostics has been limited. Native proteins performed better and were in ample supply, so there was no incentive for diagnostic companies to switch to recombinant proteins. Conditions today are quite different, however, and recombinant proteins will be essential in keeping the diagnostic industry moving forward.

The most challenging recombinant proteins to produce are those with complex, multi-subunit structures and those that are glycosylated or undergo post-translational modification. Two divisions of diagnostic medicine that involve proteins with complex structures include metabolic function and reproductive endocrinology. Both areas rejected the recombinant proteins that were available over the last thirty years, due to poor performance of the recombinants and ample supply of native proteins. Today, a new line of recombinant proteins from Scripps Laboratories, developed for diagnostic applications, meets and exceeds the performance challenges presented by structural complexity and post-translational modification.

One of the most intricate and complex structures in clinical diagnostics is found in the protein ferritin, an iron-storage protein and biomarker of anemia. Ferritin is composed of 24

subunits, with a varied mixture of heavy-chain and light-chain subunits, produced in the liver and spleen. Recombinant ferritin has been available since the early 1990s, but these early forms were highly unstable, making them unsuitable for use in a diagnostic assay.

Scripps Laboratories has produced a recombinant, multi-subunit form of ferritin that is stable and performs comparably to native ferritin in a variety of antibody-based assay systems. Currently, it is in advanced stages of evaluation for use in clinical immunoassays around the world. It can be produced in great quantities with relative ease, thus, meeting the most stringent requirements of the diagnostic industry: reliable performance and continuous supply.

In reproductive endocrinology, human chorionic gonadotropin (hCG), follicle stimulating hormone (FSH), and luteinizing hormone (LH) are essential for monitoring and detecting pregnancy, fertility, and ovulation. Structurally, these three hormones are heterodimer glycoproteins, making them difficult to produce as recombinants. Recombinant forms of hCG, FSH, and LH have been available for several decades, purified from mammalian cell culture systems like CHO and HEK293, which possess the cell mechanisms required for glycosylation. The presence of glycosylation alone, however, is not sufficient to assure the production of a quality recombinant, as poor performance of the previously available recombinants prevented their acceptance in the diagnostic industry.

With nearly forty years of native protein purification expertise, Scripps Laboratories solved the recombinant performance issues with careful project planning, meticulous screening, and thorough testing in multiple antibody-based assay systems. Additionally, we use purification processes that maintain the structural integrity and antibody-binding capability of the final product. The result of our system of recombinant protein development is clear: Today, all of our recombinant hormones have been approved, or are undergoing evaluation, for use worldwide in clinical diagnostic immunoassays.

Conclusion

The work being done at Scripps Laboratories is dispelling a significant misconception about the performance of recombinant proteins in antibody-based assay systems. The long-held opinion that recombinants don't function as well as native proteins in immunoassays is simply no longer true. Performance, reliability, and availability can now be used to describe recombinant proteins for use in the clinical diagnostic industry. A new era of reliable recombinant proteins has dawned and Scripps Laboratories is leading the way.

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