Why Recombinants Are Essential
for Assay Development and Production

By David A. George

Scripps Laboratories says that if native proteins are unavailable, research organizations and diagnostic companies should look to recombinant replacements

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The COVID-19 pandemic and geopolitical conflict in Eastern Europe have done irreparable damage to the underpinnings of the clinical diagnostic industry. These events significantly impacted the supply of the starting materials needed to purify proteins that are essential to diagnostic test kit development and production. Today, proteins purified from glands and organs (native proteins) are in dire short supply. Fortunately, Scripps Laboratories is developing a new era of recombinant proteins for research and diagnostic assay development. Functional recombinant products are now available to the industry, and they offer several advantages over native proteins.

In a previous article in this publication, we stated that structural complexities and post-translational modifications constitute challenges that can limit the functionality of recombinant proteins available to the diagnostic industry (George, DA. Recombinant Proteins Benefit the Clinical Diagnostic Industry. *GEN* 2022; 42(9): 20–22). In the same article, we demonstrated that those challenges can be met by employing strategic project planning, protein-specific purification techniques, and extensive product testing. Since the time of that publication, native starting material shortages have become more severe, creating an urgent need for functional recombinant proteins that can be integrated into a myriad of clinical diagnostic assay systems.

Outlined here are examples of new recombinant proteins in the areas of anemia, cardiology, and endocrinology. These new recombinants are shown to be comparable to native proteins, establishing their suitability for immediate use in research and clinical diagnostics.

Anemia—Liver function and vitamin B12 absorption

Assessment of liver function is a vital measure of one's health. It is also an area in which tissue shortages have negatively impacted the supply of a key liver biomarker, ferritin. Ferritin is the body's primary iron storage protein, and its measurement is used to assist with the diagnosis of iron-deficiency anemia, hemochromatosis (excess iron absorption), liver disease, and adult Still's disease (a rare type of arthritis).

Ferritin is produced by several tissues in the body, but it is most abundant in liver and spleen. The quality and accessibility of the livers and spleens needed to purify native ferritin began to diminish approximately a decade ago. Currently, spleens are scarce, and livers available for purification are diseased, resected, or otherwise damaged. This negatively impacts the amount and quality of ferritin available from donor tissues, resulting in a shortfall of the native protein. The shortage of ferritin from human tissues is now extreme, exacerbated by recent global events.

Ferritin is a complicated molecule, composed of varying combinations of heavy-chain and light-chain subunits. In the past, this complex structure made recombinant ferritin difficult to produce. Scripps Laboratories, however, has developed a recombinant form of ferritin that resembles the native protein in physical and performance characteristics.

The recombinant product is apoferritin, which is the ferritin molecule devoid of iron, but its physical characteristics are indistinguishable from native ferritin. Upon SDS-PAGE, the recombinant and native forms run at nearly identical molecular weights. In addition, western blot analysis confirms the pres-

ence of heavy-chain and light-chain ferritin in both products. (See scrippslabs.com/recombinant-apoferritin for data.)

Perhaps the most important attribute of a recombinant protein is its performance on commercial analyzers. Consistent, reproducible results are ideal when considering a recombinant as a replacement for a native protein. As can be seen in the *Table*, the correlation of recombinant apoferritin on two different clinical analyzers is exceptional and matches that of native ferritin. The correlation (ratio) between recombinant apoferritin on the two instruments ranged between 1.01 and 1.02. Similarly, the correlation measured for native ferritin ranged between 1.01 and 1.07.

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In addition to its exemplary physical and performance characteristics, recombinant apoferritin can be produced in *Escherichia coli*, enabling gram-scale production. Given the supply-chain issues associated with native ferritin, recombinant apoferritin is a highly suitable replacement in research and diagnostic applications.

The challenges associated with native protein purification are not limited to human-derived proteins. Animal-sourced proteins are also impacted by inconsistent starting material supply. This is evident in the shortage of quality porcine intrinsic factor, a key component of vitamin B12 assays.

The diagnostic industry has used intrinsic factor purified from porcine stomach for several decades. The supply of this native protein has not been an issue. However, changes in extraction procedures at abattoirs worldwide adversely affected the quality and availability of porcine intrinsic factor. The native material available today performs poorly and is expensive to produce. In response to these problems, using the human gene sequence, Scripps Laboratories developed a recombinant form of intrinsic factor. that performs well in vitamin B12 assay development.

In work performed by Calbiotech (El Cajon, CA), recombinant human intrinsic factor from Scripps Laboratories was conjugated to biotin and used as a capture reagent in a vitamin B12 ELISA. Data generated from 36 samples were compared to results obtained from the kit-supplied capture reagent, made with native porcine intrinsic factor.

Figure 1 shows a scatter plot of the vitamin B12 sample recoveries for both reagents. Linear regression analysis demonstrates a strong positive correlation between the data sets obtained with native and recombinant intrinsic factor, recording an R-squared (R^2) value of 0.9979.

The poor performance and rising cost of native porcine intrinsic factor make recombinant human intrinsic factor an

Recombinant proteins are meeting the challenges presented by worldwide native tissue shortages. Today's recombinants match the performance standards set by native proteins and are a sustainable resource for the research and diagnostic communities.

Table. Concentrations of recombinant apoferritin and native ferritin determined with different analyzers

	Concentration (ng/mL)		
Sample Description	*Siemens Centaur CP*	*Beckman UniCell Dxl 800*	*Ratio (CP/Dxl 800)*
Recombinant apoferritin	*155.2*	*153.7*	*1.01*
Recombinant apoferritin	*164.9*	*161.3*	*1.02*
Native ferritin (Bio-Rad Lyphochek, Level 1)	*52.3*	*48.2*	*1.07*
Native ferritin (Bio-Rad Lyphochek, Level 2)	*138.7*	*137.8*	*1.01*
Native ferritin (Bio-Rad Lyphochek, Level 3)	*372.5*	*350.9*	*1.06*

excellent and sustainable alternative. It is physically and functionally similar to the native protein. In addition, recombinant intrinsic factor can be produced in large scale in *E. coli*, providing an economic benefit to researchers and diagnostic manufacturers.

Cardiology—Acute myocardial infarction

Early diagnosis of acute myocardial infarction (AMI) saves lives, making rapid tests for AMI essential tools in cardiac care. A typical rapid test for AMI looks for the presence of protein biomarkers shed from damaged heart muscle. Common biomarkers include creatine kinase MB (CK-MB), myoglobin, and subunits of the protein troponin. Troponin subunits I (TnI) and T (TnT) are viable cardiac biomarkers, but we will focus on TnT here.

The shortage of human organs has impacted the purification of these native cardiac biomarkers. Human hearts are available for purification purposes, but supply is erratic, tissue quality is poor, and costs are volatile. Fortunately, recombinant forms of CK-MB and TnT are available that rival the native proteins in purity and antibody reactivity.

Recombinant CK-MB from Scripps Laboratories matches native CK-MB in physical and performance characteristics. Upon SDS-PAGE, recombinant CK-MB runs at the expected molecular weight for native CK-MB. In addition, creatine kinase enzymatic assays demonstrate the absence of CK-BB and CK-MM enzymatic activity. (See scrippslabs.com/recombinant-ck-mb for data.)

Recombinant proteins are timely replacements for native proteins across a spectrum of clinical diagnostic assays.

When assayed on clinical immunoanalyzers, recombinant CK-MB exhibits specific activity comparable to that for native CK-MB. (Enzymatic activity was measured kinetically by the CK-NAC assay at 37°C. CK-MB mass was measured on a Siemens Centaur CP immunoanalyzer.) For several lots of recombinant CK-MB, the specific activity was 505–631 units/mg; for several lots of native CK-MB, it was 531–645units/mg.

As with CK-MB, recombinant TnT is available, but data are not presented here. (See scrippslabs.com/recombinant-troponin-t.)

Additional advantages of recombinant cardiac markers are their means of purification. Starting materials for the recombinants are readily available and can be produced in nonmammalian expression systems, such as *E. coli*. This allows rapid, large-scale purifications, which assure steady supplies and provide sustainable, affordable alternatives.

Native cardiac markers have served the diagnostic industry well for many years, but it is time to move beyond them. Recombinant biomarkers are available that are economical to produce and that match native proteins in purity, antibody reactivity, and in the case of CK-MB, enzymatic activity.

Endocrinology—Thyroid function and reproductive biology

Human hormones are perhaps the type of native protein affected most severely by the raw material crisis. Many of these hormones derive from the pituitary, which is a very small gland, approximately the size of a pea. This means several thousand glands, from several thousand donors, are needed for a single purification batch of a native hormone. Such large-scale consumption of a native raw material is unsustainable, and the diagnostic industry is in dire need of recombinant replacements.

Recombinant hormones have been available since the late 1980s, but they were not adopted by the diagnostic industry due to poor performance in antibody-based assay systems. Recent advancements in recombinant expression and purification methods, however,

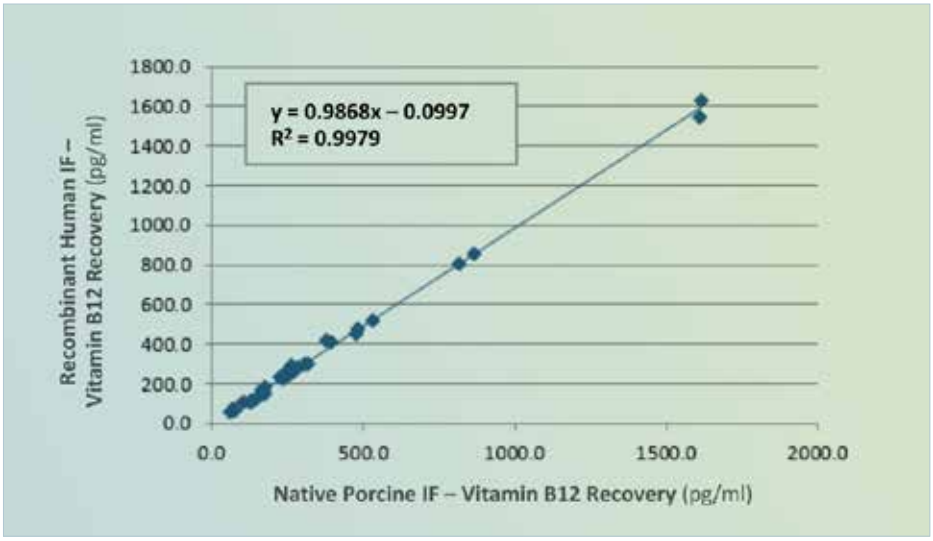

Figure 1. Linear regression analysis of vitamin B12 sample recovery using recombinant human intrinsic factor–biotin and native porcine intrinsic factor–biotin conjugates in a vitamin B12 ELISA. Data generated by Calbiotech (El Cajon, CA).

enabled Scripps Laboratories to produce recombinants that rival the performance of native hormones.

Thyroid function and reproductive biology are two areas of clinical diagnostics in which immunoreactive recombinant proteins are now available. Diagnostic testing in both areas has increased dramatically in recent years, and native raw material supply chains cannot keep up with the industry's demand for purified hormones.

Native hormones in short supply include thyroid stimulating hormone (hTSH), which regulates thyroid function, and the reproductive hormones human chorionic gonadotropin (hCG), follicle-stimulating hormone (hFSH), luteinizing hormone (hLH), and prolactin (hPRL). The global demand for these hormones is extraordinarily high, and recombinant alternatives are essential to sustain the diagnostic testing industry. Newly developed recombinant forms of hCG, hFSH, hLH, hPRL, and hTSH from Scripps Laboratories have all been tested and approved for use in clinical diagnostic assays worldwide.

Physically, each of the recombinant hormones displays SDS-PAGE, HPLC, and western blot data consistent with the expected values for each protein. (See scrippslabs.com/recombinant-hormones for data.) Regarding performance on clinical immunoassay systems, for brevity, we present data here for hCG only. Recombinant hCG and native hCG were analyzed on two different clinical immunoanalyzers: a Roche cobas 8000 and a Siemens Centaur CP. *Figure 2* demonstrates excellent correlation for both recombinant hCG and native hCG between the two instruments. Linear regression analysis shows a positive correlation of 0.9998 for recombinant hCG and 0.9997 for native hCG on both analyzers. This demonstrates the suitability of the recombinant as a replacement for the native hormone.

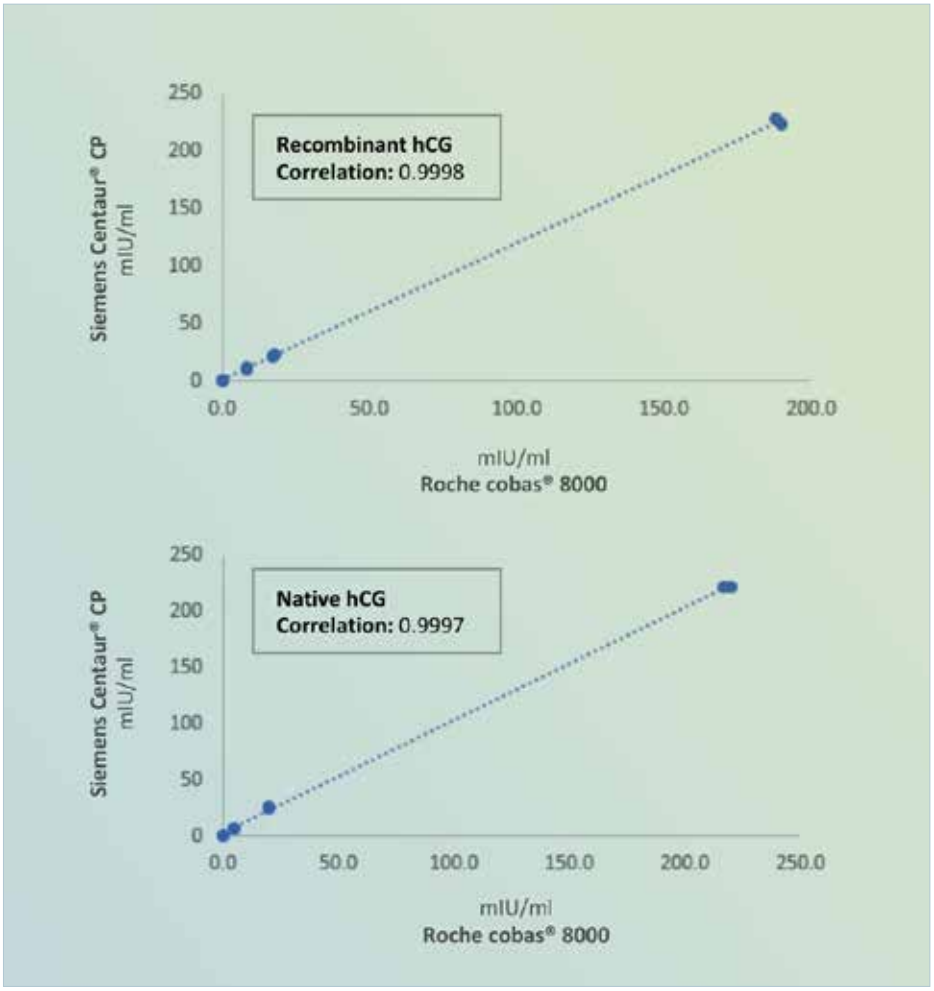

Figure 2. Linear regression analysis of recombinant human chorionic gonadotropin and native human chorionic gonadotropin on the Siemens Centaur CP and Roche cobas 8000 clinical immunoanalyzers.

Conclusion

Recombinant proteins are now being approved for use at an increasingly swift pace. Novel recombinant forms of apoferritin and intrinsic factor are being adopted for use in anemia and metabolism, replacing the hard-to-find native proteins. Tissue shortages and cost increases impacting the field of cardiac care are being remedied with recombinant forms of CK-MB and TnT. Similarly, antibody-reactive, recombinant forms of hCG, hFSH, hLH, hPRL, and hTSH are replacing native hormones in tests for thyroid function and reproductive biology.

Recombinant proteins are proving to be suitable and timely replacements for native proteins across a spectrum of clinical diagnostic assays. Recombinants can be produced more economically than their native counterparts, and they are similar to natives in performance and physical appearance.

Given the current and future state of native starting material supplies, Scripps Laboratories is utilizing emergent technological methods to develop recombinant proteins that meet the needs of a changing industry. The data presented here demonstrate that the recombinants of today are well suited to serve the research and diagnostic communities now and into the future. GEN

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