WHITE PAPER

Improved Allele Specificity of Vitamin D BP ELISA Kits

INTRODUCTION

Vitamin D Binding Protein (Vitamin D BP), also known as DBP and Gc-globulin, is a 58 kDa glycoprotein that circulates at a high concentration in the serum and serves as a carrier protein for Vitamin D. In plasma, roughly 85–90% of circulating Vitamin D is bound to Vitamin D BP (1). Vitamin D BP transports Vitamin D and its metabolites and participates in many physiological functions; e.g., bone development. Alterations in Vitamin D BP activity is associated with many diseases (2).

Vitamin D BP is predominantly produced by hepatic parenchymal cells. Circulating levels of Vitamin D BP have been shown to decrease in liver failure and other liver diseases (3-4). Also, in view of the fact that Vitamin D BP is produced by the liver, its synthesis may additionally be reduced during physiological stress (5). In patients with active inflammation, there can be reduced hepatic production of Vitamin D BP (5). It has also been shown that circulating Vitamin D BP levels decrease in patients with cellular damage and tissue loss (6). Based on this data Vitamin D BP levels are expected to decrease in patients with Inflammatory Bowel Disease (IBD), such as Crohn's disease.

The human Vitamin D BP gene has numerous alleles and single nucleotide polymorphisms (SNPs) (2, 7). There are three dominant alleles of Vitamin D BP, which correlate with protein products (GC-1s, GC-1f and GC-2) distinguished by single amino acid substitutions (2). Compared to isoform GC-2, GC-1s has two-fold and GC-1f has four-fold higher binding affinity for Vitamin D (8).

The results from a publication noted that the original R&D Systems Vitamin D BP Quantikine® ELISA Kit (Catalog # DVDBP0) detected the GC-1f isoform with lower sensitivity than the GC-2 and GC-1s isoforms, resulting in underestimation of Vitamin D BP in African Americans who have primarily the GC-1f isoform (9). These results prompted us to investigate and then develop improved Vitamin D BP Quantikine and DuoSet® ELISA Kits, which now detect all three major isoforms similarly.

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RESULTS

With R&D Systems' continued effort to provide you with the best quality ELISAs on the market, an improved Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B) and Vitamin D BP DuoSet ELISA Kit (Catalog # DY3778B-05) have been developed that detect all three dominant Vitamin D BP isoforms (GC-1f, GC-1s and GC-2) similarly. The specificity differences between the original Vitamin D BP Quantikine and DuoSet ELISA Kits and the new versions of these assays are shown in Figure 1 and Figure 2, respectively. Both the new Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B) and Vitamin D BP DuoSet ELISA (Catalog # DY3778B-05) kits detect all three recombinant human isoforms expressed in HEK293 similarly.

Serum samples from healthy donors were evaluated with the original Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0) and the new Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B) as shown in Figure 3. The donors were either of African American descent (high prevalence of Vitamin D BP isoform GC-1f) or of Caucasian descent (high prevalence of Vitamin D BP form GC-1s). The original Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0) had higher sample values for the Caucasian donor population (mean 191,000 ± 69,300 ng/mL) than the African American donor population (mean 121,000 ± 42,600 ng/mL). The P value of 0.0389 suggests a significant difference between the two donor populations. These results are similar to what is reported in the literature (9). The new Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B) had similar sample values for the Caucasian donor population (mean 196,000 ± 29,700 ng/mL) and the African American donor population (mean 177,000 ± 20,000 ng/mL). The P value of 0.204 suggests there is no significant difference between the sample values of the donor groups.

Patient serum samples were evaluated with the new Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B). Circulating Vitamin D BP levels are expected to decrease in patients with liver disease (3, 4) and patients with active inflammation (5). The average serum value for apparently healthy donors was prominently higher than both Crohn's disease and liver disease patients (mean 245,000 ± 71,000 ng/mL, 205,000 ± 53,300 ng/mL, 125,000 ± 47,200 ng/mL respectively) as shown in Figure 4.

Figure 1

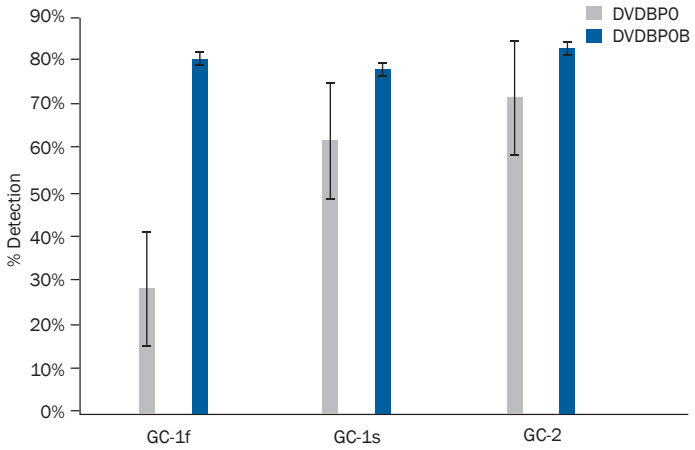

Figure 1. In Figure 1, the detection of recombinant human VDBP isoforms GC-1f, GC-1s and GC-2 were assessed using R&D Systems Vitamin D BP Quantikine ELISAs (Catalog #s DVDBP0, DVDBP0B). The new Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B) is a reliable means for detecting the main three isoforms.

Figure 2

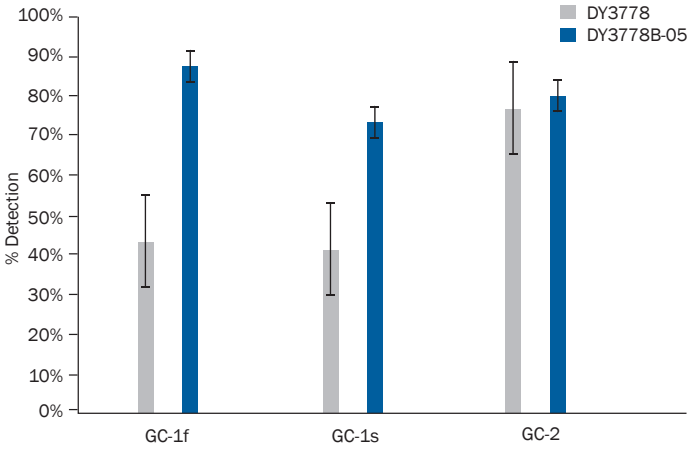

Figure 2. In Figure 2, the detection of VDBP isoforms GC-1f, GC-1s and GC-2 were assessed using R&D Systems Vitamin D BP DuoSet ELISAs (Catalog # DY3778, #DY3778B-05). The new Vitamin D BP DuoSet ELISA (Catalog # DY3778B-05) detects the main three isoforms similarly.

Figure 3

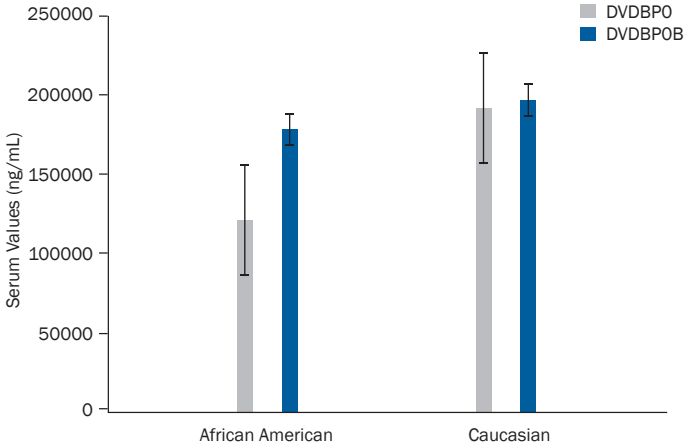

Figure 3. Serum values for healthy African American and Caucasian samples were obtained using R&D Systems Vitamin D BP Quantikine ELISA Kits (catalog # DVDBP0, DVDBP0B). n=10 for both African American and Caucasian donor samples. The original Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0) shows a significant difference between the average sample values from the donor groups, P value of 0.0389. The new Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B) detects samples from both donor pools similarly, P value of 0.204.

Figure 4

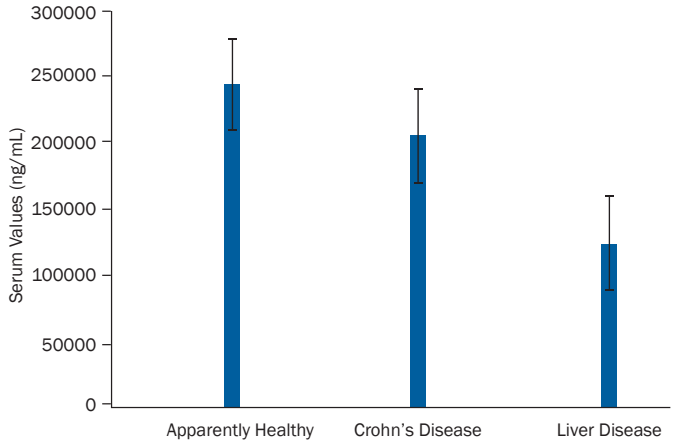

Figure 4. In Figure 4, Apparently healthy, Crohn's disease, and Liver disease patient serum sample values were assessed using R&D Systems Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B). n=50 for healthy donors, n=10 for Crohn's Disease patients and n=6 for liver disease patients.

DISCUSSION

ELISA kits remain the gold standard for quantitation of specific protein levels present in blood serum and plasma samples. R&D Systems is the top cited manufacturer of premium ELISA kits because we are committed to providing the highest quality products and customer service. We responded to our customer feedback on the Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0) to develop and release improved versions of both the Quantikine and DuoSet ELISA Kits. Both new kits utilize a different antibody that increases the specificity of GC-1f and now all 3 major alleles are detected similarly. The new Human Vitamin D BP Quantikine ELISA Kit (Catalog # DVDBP0B) now detects similar Vitamin D BP serum levels in donors from African American and Caucasian decent and shows the expected decrease in circulating levels of patients with IBD and liver disease.

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