

DEVELOPMENT OF NOVEL ELISAs FOR TOTAL GDF-15 AND H-SPECIFIC GDF-15

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ABSTRACT

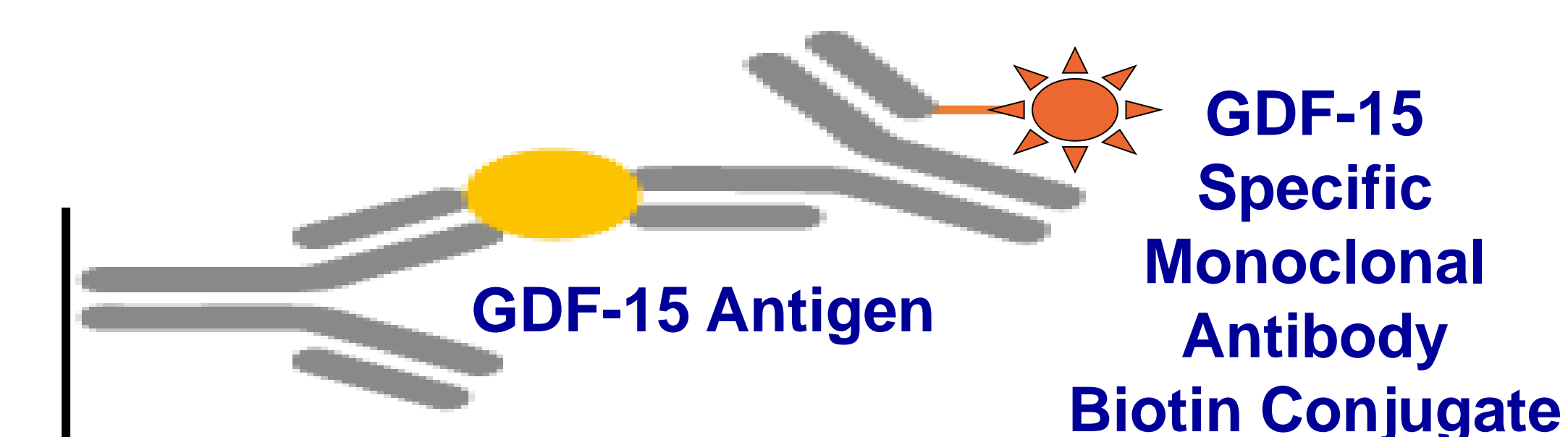
Background: To develop sensitive and specific ELISAs for the quantitative measurement of total human GDF-15 and H-specific (H202D mutant) GDF-15 in human serum, and other biological fluids. Growth/differentiation Factor 15 (GDF-15) is a divergent member of the TGF- β superfamily of growth factors. It is encoded in humans by a gene in chromosome 19. The human GDF-15 gene encodes for a protein of 308 amino acid residues which consists of a signal sequence (residues 1-29), pro-domain (30-194), and mature growth factor domain (195-308). The molecular weight of a mature GDF-15 dimer is 25 kDa. Approximately 25% of humans have a missense polymorphism in the GDF-15 gene resulting in mutation of histidine 202 to aspartate (histidine 6 in the mature domain), close to the N-terminus of the mature growth factor. This variant is associated with phenotypes in prostate cancer, hyperemesis gravidarum, (severe morning sickness in pregnancy), and rheumatoid arthritis.

Methods: Highly specific and reproducible total GDF-15 (AL-1014-r) and H-specific GDF-15 (AL-1018-r) ELISAs have been developed using specific monoclonal antibodies to help estimate the total and mutant (DD) concentration in serum in the respective assays. The ELISAs use 10 μ L sample volume and are calibrated to recombinant human GDF-15 from R & D Systems (Biotechne, USA). These ELISAs were validated for their specificity using HH, DD, HD recombinant preparations and their circulating levels in male, female, 1st, and 2nd-trimester serum samples.

Results: The Total GDF-15 ELISA assay detects total GDF-15 including histidine 202 to aspartate mutation (HH, HD & DD variant) in the mature domain in equimolar proportions. The H-specific GDF-15 assay is specific to histidine at 202aa in the GDF-15 sequence and does not detect histidine 202 to aspartate mutation (DD variant). Median Levels of total GDF-15 in healthy males (n=18), females (n=17), 1st trimester (n=20), and 2nd trimester (n=20) were 847.9 pg/mL, 1182.4 pg/mL, 12759.7 pg/mL, and 12951.2 pg/mL. Median Levels of H-specific GDF-15 in healthy males (n=18), females (n=17), 1st trimester (n=20), and 2nd trimester (n=20) were 723.2 pg/mL, 641.3 pg/mL, 8381.4 pg/mL and 4652.1 pg/mL. Method comparison between total GDF-15 and commercial GDF-15 assay using serum samples (HH, wild type) in the range of 400-2500 pg/mL yielded a slope of 0.98 (r=0.97). The HD and DD mutant samples in the total assay recovered at twice the concentrations of the commercial assay. The total and intact assays are highly reproducible with the total coefficient of variation less than 7%.

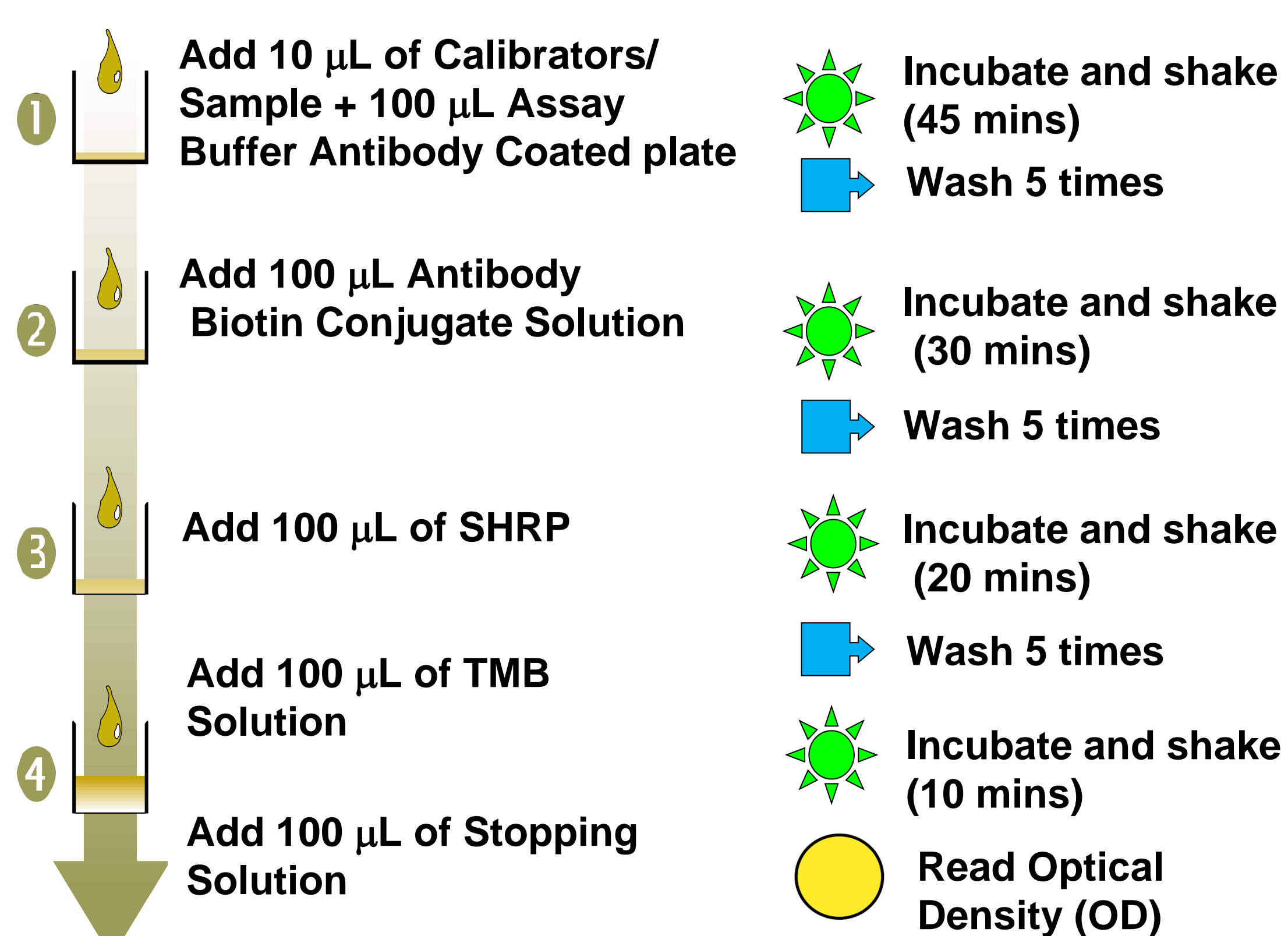
Conclusions: Use of total GDF-15 assay in combination with the GDF-15 H-specific assay (DD nondetectable) ELISA can accurately estimate the mutant (DD) concentration in serum. This will enhance the knowledge of the GDF-15 measurements in the literature as the commercial assays were compromised for its immunoreactivity to the HD DD genotypes which is highly prevalent.

GDF-15 ELISAs METHOD

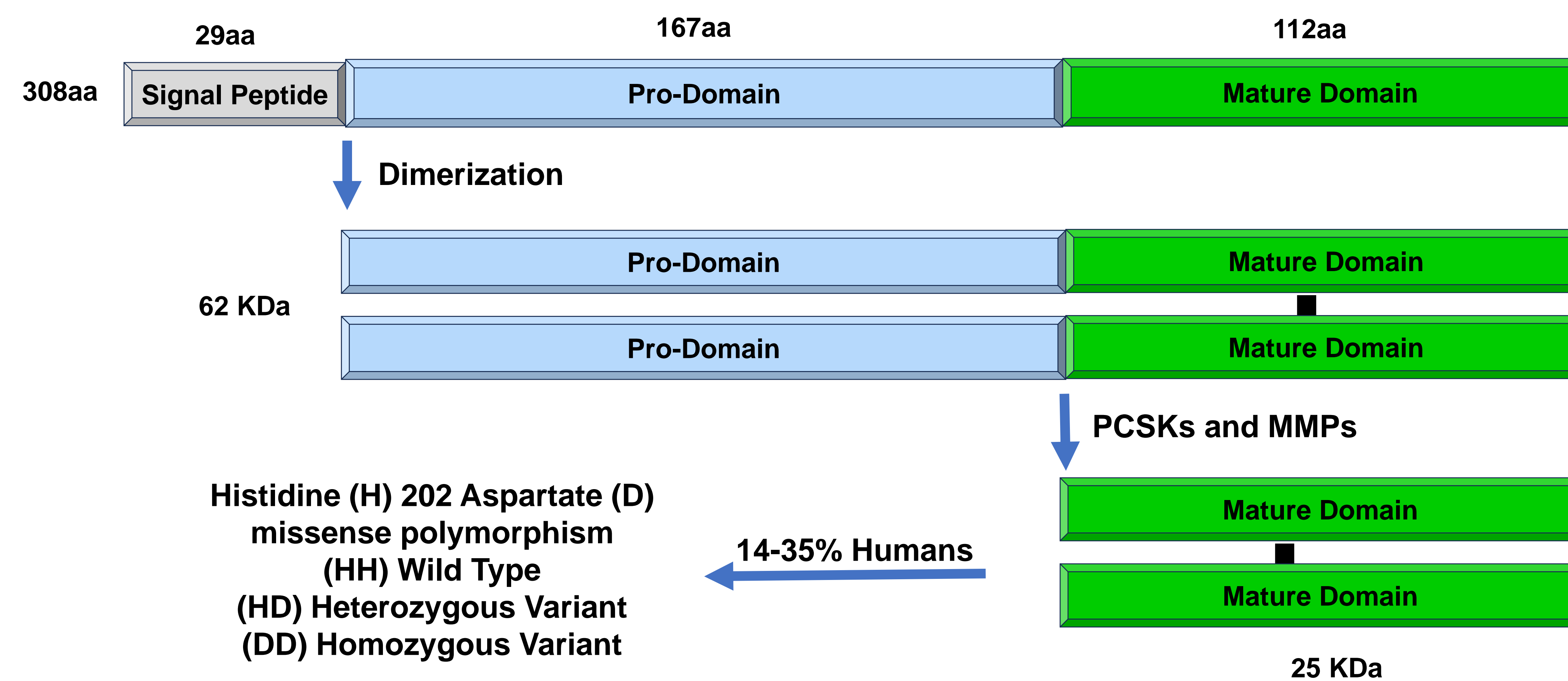


GDF-15 (Total and H-specific) Monoclonal Antibody Coated Plate

GDF-15 ELISAs Procedure

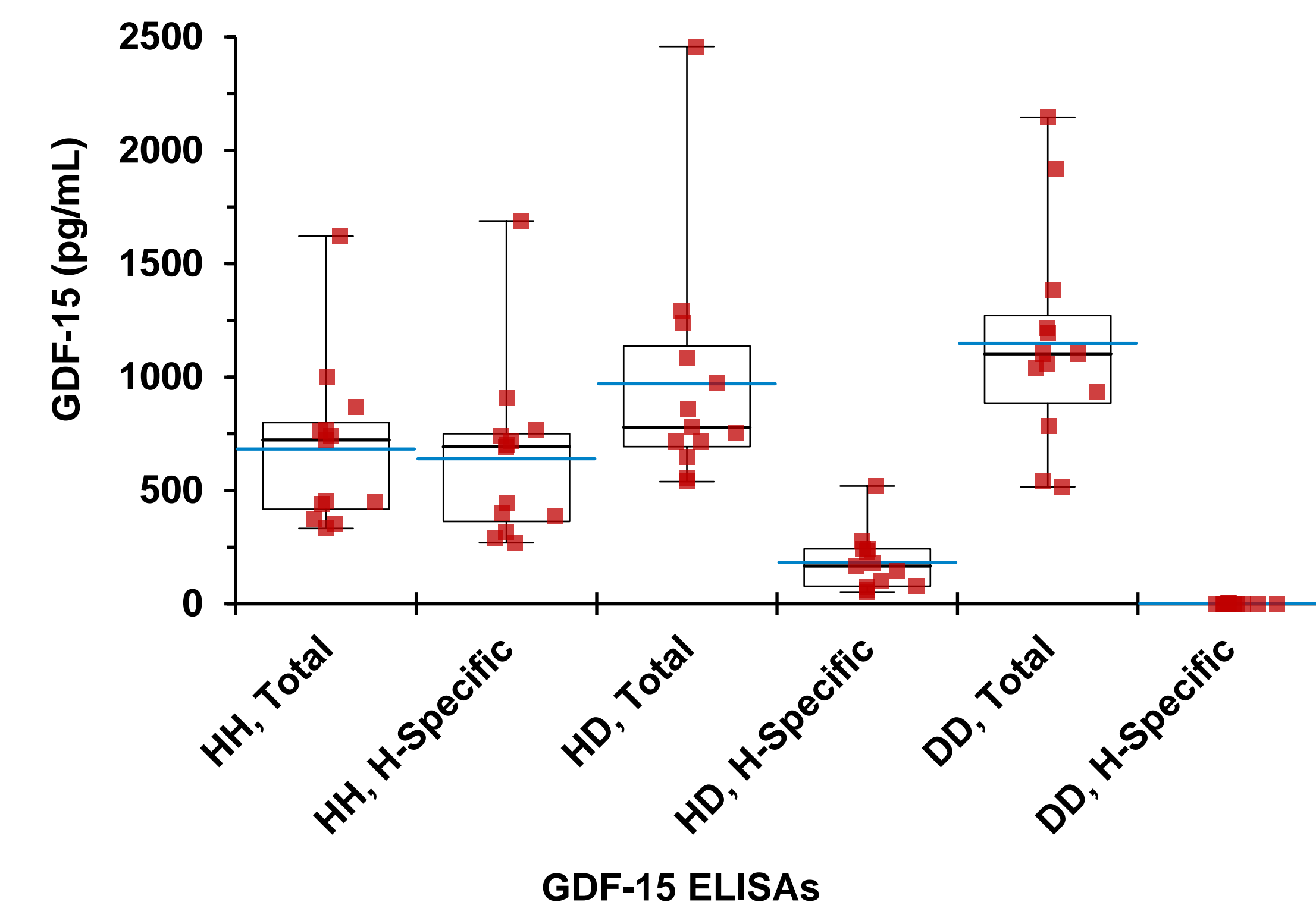


Prepro-Growth Differentiation Factor-15 Processing



Method Comparison

Total GDF-15 ELISA and H-Specific GDF-15 ELISA has been compared pairwise using 13 characterized pregnancy serum samples of human GDF-15 homozygotes for wild type (HH), heterozygous HD and the homozygous mutation (DD), in the range of 2.2-2500 pg/mL.



RESULTS: ANALYTICAL CHARACTERISTICS

ELISA Reagents	Antibody Pair Specificity GDF-15 H202D variant	Dynamic Range (pg/mL)	Assay Calibration Traceability	Limit of Detection (pg/mL)
Total GDF-15 AL-1014	HH, HD, and DD	15-3000	Recombinant Human Mature GDF-15	2.2
H-specific GDF-15 AL-1018	HH and HD	15-3000	Recombinant Human Mature GDF-15	2.4

Total and H-Specific GDF-15 Assays Imprecision

Reproducibility of the Total and H-Specific GDF-15 assays was determined using samples in the low, mid, and high concentration ranges. The study included a total of 6 assays, 3 replicates of each sample per assay (n=18) and the data were calculated based on EP10A-3 guidelines.

Total GDF-15 ELISA								H-Specific GDF-15 ELISA							
Sample	Mean Conc. (pg/mL)	Within run		Between run		Total		Sample	Mean Conc. (pg/mL)	Within run		Between run		Total	
		SD	CV	SD	CV	SD	CV			SD	CV	SD	CV		
Control I	184.0	4.3	2.3%	4.5	2.5%	6.2	3.4%	Control I	178.2	5.7	3.2%	4.9	2.7%	7.5	4.2%
Control II	482.7	18.8	3.9%	21.1	4.4%	28.2	5.8%	Control II	492.4	11.8	2.4%	0.0	0.0%	11.8	2.4%
Sample-1	1220.0	52.6	4.3%	19.8	1.6%	56.2	4.6%	Sample-1	1179.2	82.0	7.0%	0.0	0.0%	82.0	7.0%

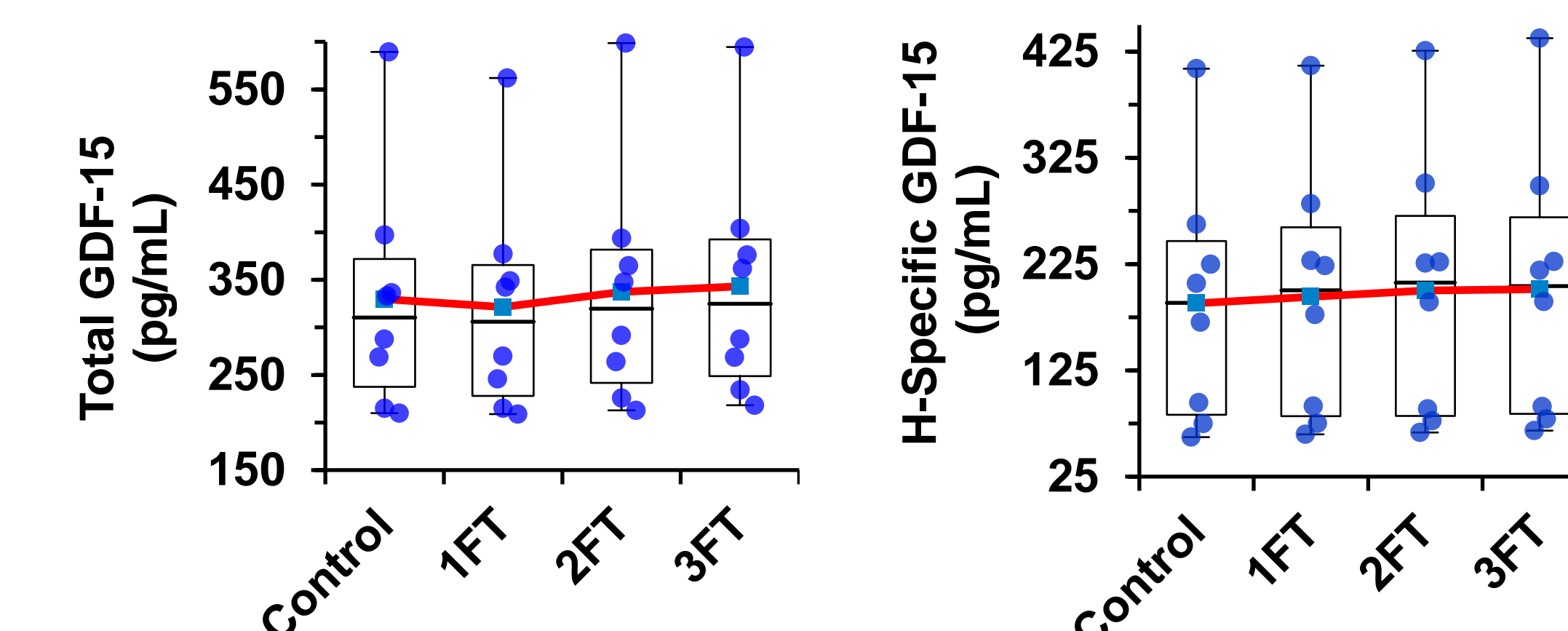
Total and H-Specific GDF-15 Assays Cross-Reactivity

The Total and H-Specific GDF-15 ELISA assay were tested on synthetic dimers of human GDF-15 homozygous for wild type (HH), heterozygous HD and the homozygous mutation (DD), Neurturin and GDNF (Glial Cell Line-derived Neurotrophic factor) when tested at higher physiological dose. The percent cross-reactivity results are tabulated below.

Total GDF-15 ELISA Assay				H-Specific GDF-15 ELISA Assay			
GDF-15 (202aa)	Expected GDF-15 Concentration (pg/mL)	Observed GDF-15 Concentration (pg/mL)	% Recovery	GDF-15 (202aa)	Expected GDF-15 Concentration (pg/mL)	Observed GDF-15 Concentration (pg/mL)	% Recovery
HH/Wild Type	500	529.9	106.0	HH/Wild Type	500	491.2	98.2
HD, DH, HH and DD/ Hetero-dimer	500	616.6	123.3	HD, DH, HH and DD/ Hetero dimer	500	154.3	30.9
DD/H202D homozygous variant	500	540.0	108.0	DD/H202D homozygous variant	500	0	Non Detectable
GDNF (Glial Cell Line-derived Neurotrophic factor)	50,000	0	Non Detectable	GDNF (Glial Cell Line-derived Neurotrophic factor)	50,000	0	Non Detectable
Neurturin	50,000	0	Non Detectable	Neurturin	50,000	0	Non Detectable

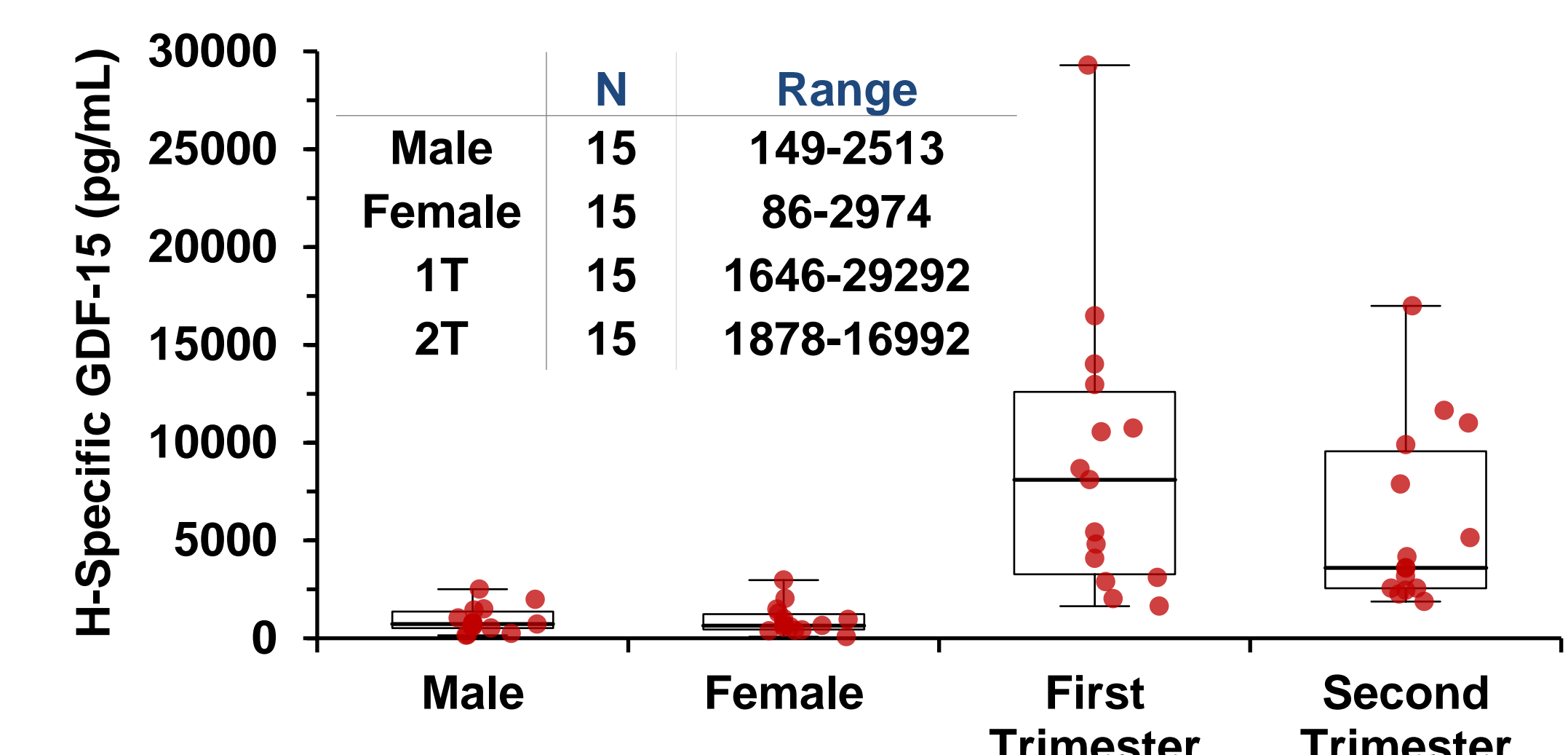
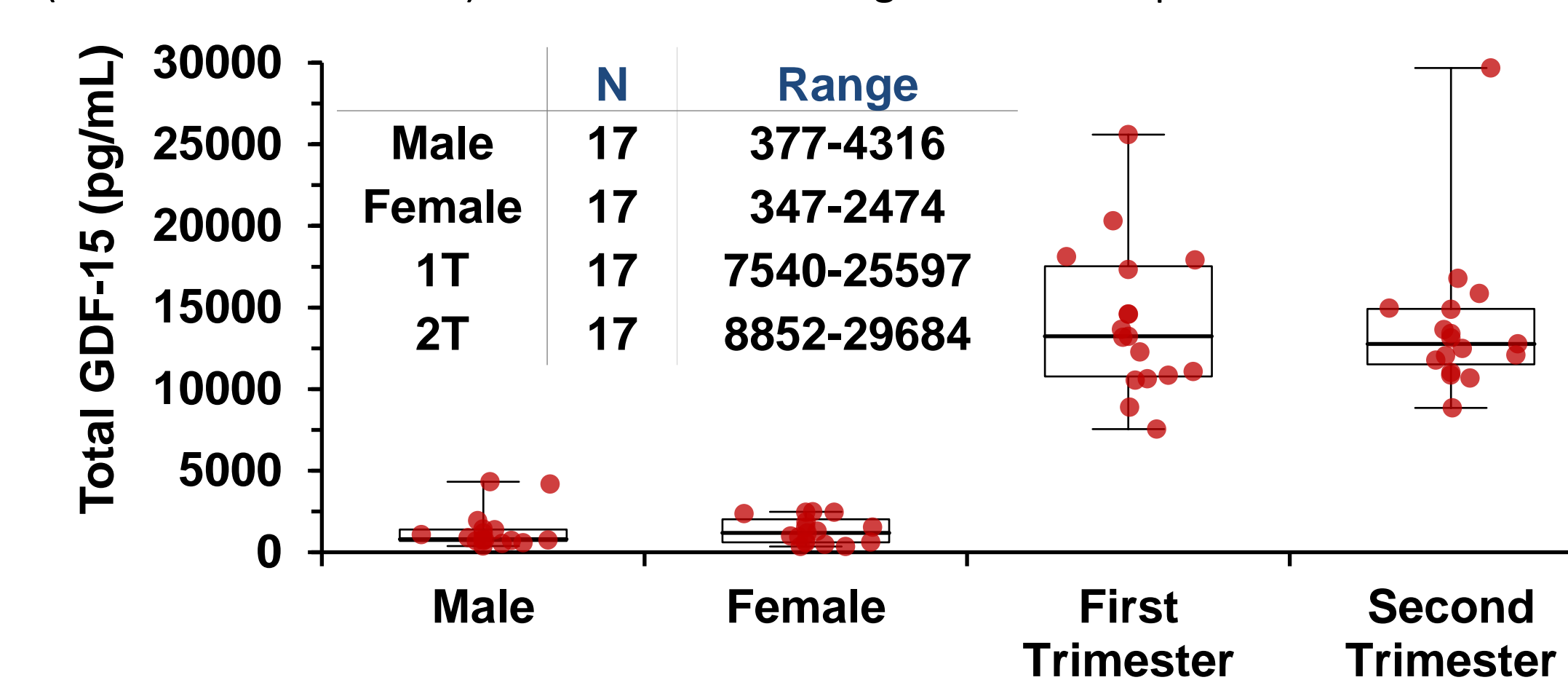
GDF-15 Sample Stability

Multiple K2EDTA plasma samples were tested for their stability upon freeze and thaw stress cycles in Total and H-Specific GDF-15 ELISA assays. The individual and mean concentrations have been plotted below.



Expected Ranges

GDF-15 concentration for ostensibly healthy males, females, and pregnant females (1st and 2nd trimester) were calculated using Total and H-Specific GDF-15 ELISA.



CONCLUSIONS

- Highly sensitive and specific H-Specific and Total GDF-15 ELISAs have been developed to reliably quantify GDF-15 in circulation.
- A combination of Total (AL-1014) and H-Specific GDF-15 (AL-1018) ELISA can be used to estimate the mutant (DD) concentrations in plasma.
- The stability of plasma specimen, low sample volume opens new avenue to re-analyze the residual samples from previous studies as many of these samples may be underestimated by commercial ELISAs.

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