

Performance Evaluation of an Automated Assay for the Measurement of LPL and HTGL Activity

Abstract

Background: Lipoprotein lipase (LPL) hydrolyzes triglycerides (TGs) into chylomicrons and VLDL particles during lipoprotein metabolism. Similarly, hepatic lipase (HTGL) is synthesized by hepatocytes and hydrolyzes TGs and phospholipids in chylomicron remnants, intermediate density lipoproteins and HDLs. LPL deficiency leads to hypertriglyceridemia with accumulation of chylomicrons. HTGL deficiency leads to hypercholesterolemia, hypertriglyceridemia and accumulation of β -VLDLs, chylomicron remnants, IDLs, TG-rich LDLs and HDLs. The conventional method for measuring LPL and HTGL activity uses ³H- or ¹⁴C-labeled trioleoyl glycerol and is not suitable for routine clinical measurement. A novel assay has been developed which is applicable to automated clinical analyzers. Here, we evaluated the performance of the new LPL and HTGL activity assay method in human post-heparin EDTA plasma (PHP) using the cobas c501 autoanalyzer.

Methods: LPL and HTGL activities were measured colorimetrically using two different channels on the cobas c501 autoanalyzer (Roche Diagnostics). The first channel contained apoCII, a cofactor required for LPL activity, and measured combined LPL and HTGL activities. The second channel lacked apoCII, measuring only HTGL activity. LPL activity was calculated from the difference between the two channels. The performance of the two channels as well as the calculated LPL activity were evaluated and validated using PHP through several experiments, including precision, linearity (2 samples), recovery (2 samples spiked to 3 different levels), sensitivity, reference interval (20 subjects) and stability (3 single donors). The within-run precision (WRP) and between-run precision (BRP) were evaluated using three in-house plasma controls with three different concentrations of LPL+HTGL and HTGL activities.

Results: For precision of both activity channels, coefficients of variation (CV) for all controls ranged from 0.9 – 4.5% for WRP, and 2.8 – 7.4% for BRP. For linearity, acceptable results ranged from 82.9 – 119.5% of targets with up to 16-fold dilution for both activity channels. For recovery, post-heparin plasma spiked into pre-heparin plasma demonstrated acceptable recovery of LPL and HTGL activities ranging from 100.0 – 117.1% of targets. The sensitivities for LPL+HTGL, HTGL and LPL activities in PHP were identified at 5, 17 and 11 U/L, respectively, with precision of \leq 20%CV. For the reference interval, LPL and HTGL activities were measured in 20 PHP samples from normal healthy volunteers. These results aligned with the range obtained by the manufacturer with values of 42 – 209 U/L and 198 – 859 U/L for LPL and HTGL activity, respectively. Accuracy was also verified using commercially available control samples and ranged from 90.0 – 108.6% of target. For short-term stability in post-heparin plasma, LPL+HTGL and HTGL activities were stable at 2 – 8 °C for up to 3 days, up to 1 day at room temperature, and stable for 3 additional freeze-thaw cycles.

Conclusion: The utility of the LPL/HTGL Activity was demonstrated with sufficient analytical performance. Overall, this assay on an automated platform is ideal for measuring LPL and HTGL activities in clinical trials.

Introduction

Identifying deficiencies in LPL and HTGL activities allows for a thorough understanding of lipid and lipoprotein metabolism in patients presenting with hypertriglyceridemia and/or hypercholesterolemia. The conventional method for diagnosing LPL or HTGL deficiency requires complicated, isotopic labeling not suitable for routine clinical measurement. A novel assay has been developed which is applicable to automated clinical analyzers. Here, we evaluated the performance of the new LPL and HTGL activity assay method in human post-heparin EDTA plasma (PHP) using the Roche Cobas c501 autoanalyzer.

Methods

The LPL/HTGL Activity Kit (IBL) was used to measure LPL and HTGL activities in post-heparin plasma (PHP) using two different channels on the Cobas c501 autoanalyzer (Roche Diagnostics). This assay is an automated kinetic colorimetric method using a natural long-chain fatty acid, 1,2-diglyceride in the presence and absence of apoCII, a cofactor required for LPL activity (see Reference 1). The first channel contained apoCII and measured combined LPL and HTGL activities. The second channel lacked apoCII, measuring only HTGL activity. LPL activity was calculated from the difference between the two channels.

The performance of the two channels were evaluated and validated using PHP through the following experiments:

Precision was evaluated using three in-house plasma controls with three different activities of LPL+HTGL and HTGL. The within-run precision (WRP) was evaluated by analyzing each assay control 10 times. Between-run precision (BRP) was evaluated by analyzing each assay control once in seven analytical runs performed over 6 days by 2 analysts.

Reference interval for LPL and HTGL activities was calculated from the mean \pm 2SD of 20 single-donor PHP samples. The manufacturer's recommended range was verified if $>$ 90% aligned.

Recovery was evaluated by spiking 2 pre-heparin pools (with no endogenous LPL activity) with post-heparin plasma to 3 different levels of activity. Recovery was considered acceptable if samples were within 80-120% of the expected values.

Accuracy was determined by analyzing commercially available high and low kit control samples 5 times in 1 run. Accuracy was acceptable if the %difference of the mean back-calculated values of the controls were within 80 – 120% of the target concentrations claimed by the manufacturer.

Sensitivity was assessed by serially diluting a PHP sample up to 16-fold and analyzed twice over three analytical runs performed over one day. Then confirmed using a second PHP sample serially diluted 16-fold and analyzed four times over 1 analytical run. The mean back-calculated values were plotted against the %CV at each level and a power-fit was used to calculate the LPL+HTGL activity with a CV = 20%. For HL activity, the activities were not low enough to reach CV \leq 20% and the lowest quantified dilution was assigned the LLOQ.

Linearity was evaluated using a single-donor PHP sample and control sample serially diluted 1:2 with saline up to 1:32 or 1:16 fold, respectively. Samples $<$ LLOQ or greater than the measurable range (GTR) were excluded from analysis. Linearity was acceptable if the %recovery of the back-calculated values for each dilution level were within 80-120% of the expected values.

Stability was evaluated using two single-donor PHP samples with high and low endogenous LPL and HTGL activity levels. Short-term stability was evaluated using the following conditions, 1) room temperature for 4 and 24 hours, 2) refrigerated (4°C) for 1 and 3 days, and 3) 1, 2 and 3 additional freeze/thaw cycles. A freshly thawed sample was included to represent the baseline for each level. For -70°C long-term stability, five single-donor PHP samples were assessed for a baseline, then analyzed at 1, 3 and 6 months). Samples were considered stable at a time point if the %difference of two of the three samples were \leq 20% of baseline, with no two consecutive time points \pm 30% from baseline for the LPL+HTGL and HTGL assays only. The long-term stability study is ongoing.

Results

Table 1: Precision

LPL+HTGL Within-Run Summary					LPL+HTGL Between-Run Summary				
Sample Name	Mean (U/L)	SD (U/L)	CV (%)	n	Sample Name	Mean (U/L)	SD (U/L)	CV (%)	n
QC - High	348	3	0.9	10	QC - High	321	19	5.8	8
QC - Med	273	6	2.1	10	QC - Med	267	7	2.8	7
QC - Low	129	2	1.6	10	QC - Low	123	8	6.5	7

HTGL Within-Run Precision					HTGL Between-Run Precision				
Sample Name	Mean (U/L)	SD (U/L)	CV (%)	n	Sample Name	Mean (U/L)	SD (U/L)	CV (%)	n
QC - High	226	10	4.5	10	QC - High	230	13	5.5	8
QC - Med	201	3	1.7	10	QC - Med	195	6	3.3	7
QC - Low	92	1	1.4	10	QC - Low	88	6	7.4	7

Figure 1: Reference Interval

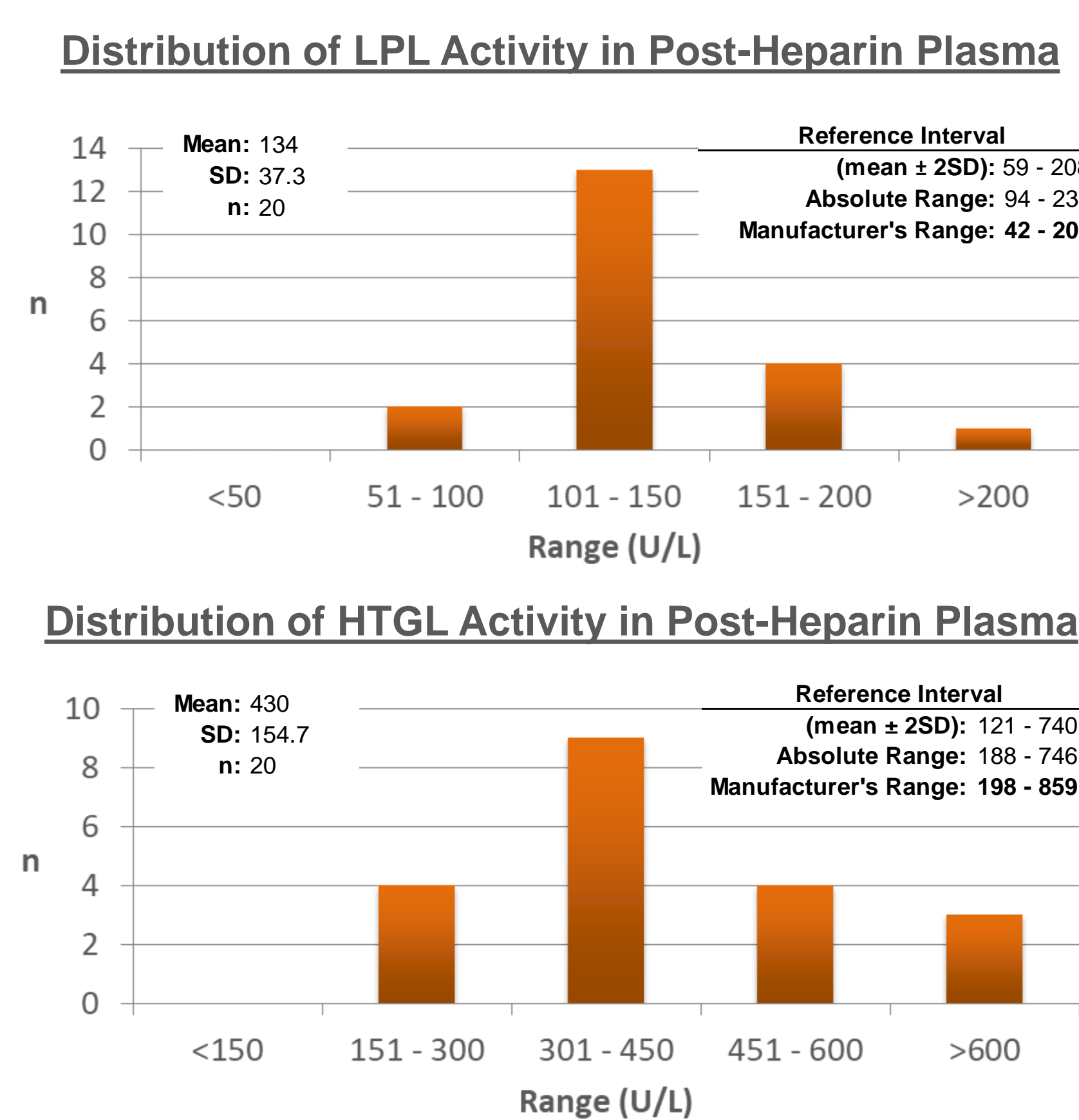


Table 2: Spike-in Recovery

Specimen	Theoretical Additive Amount (U/L)	Measured LPL Values (Diluent) (U/L)	Measured LPL Values (Plasma) (U/L)	Rec of LPL in Plasma from Diluent (%)
Sample 1	34	35	37	105.7
	17	17	17	100.0
	8	9	10	111.1
	Unspiked	0	0	NA
Sample 2	34	35	41	117.1
	17	17	19	111.8
	8	9	9	100.0
	Unspiked	0	0	NA

Specimen	Theoretical Additive Amount (U/L)	Measured HTGL Values (Diluent) (U/L)	Measured HTGL Values (Plasma) (U/L)	Rec of HTGL in Plasma from Diluent (%)
Sample 1	116	91	99	108.8
	58	42	46	109.5
	29	19	20	105.3
	Unspiked	0	0	NA
Sample 2	116	91	98	107.7
	58	42	47	111.9
	29	19	21	110.5
	Unspiked	0	0	NA

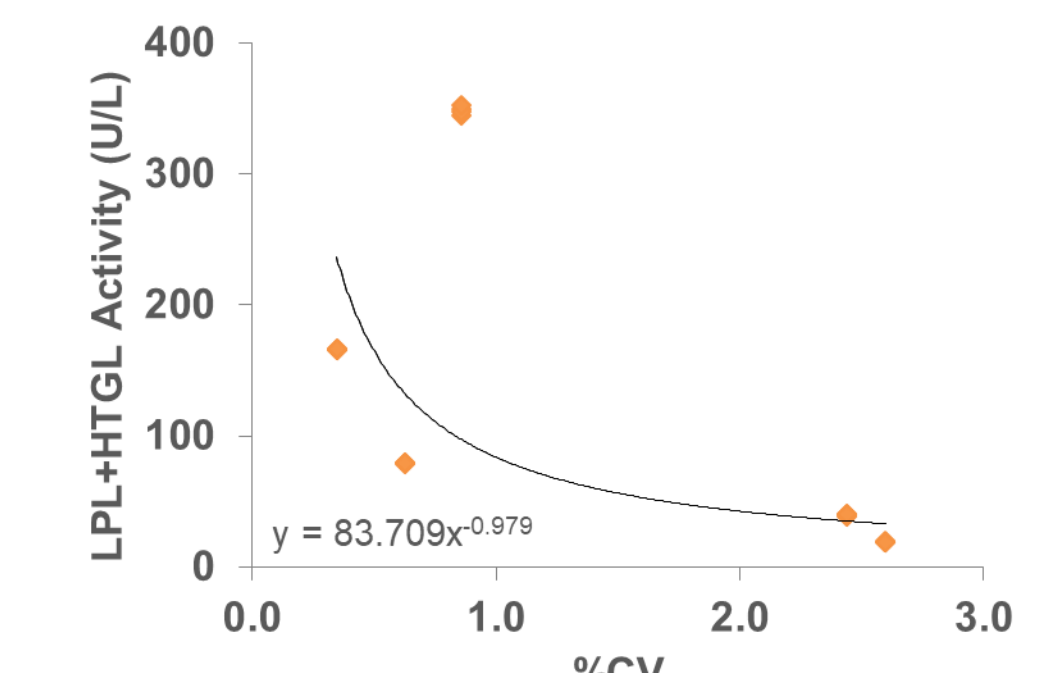
Table 3: Accuracy

Sample Name	LPL+HTGL Target (U/L)	Mean LPL+HTGL Measured (U/L)	SD (U/L)	CV (%)	n	Recovery (%)
Kit QC H	440	422	5	1.2	5	96.0
Kit QC L	212	201	3	1.3	5	94.6

Sample Name	HTGL Target (U/L)	Mean HTGL Measured (U/L)	SD (U/L)	CV (%)	n	Recovery (%)
Kit QC H	301	271	19	6.9	5	90.0
Kit QC L	141	132	6	4.3	5	93.6

Figure 2: Sensitivity

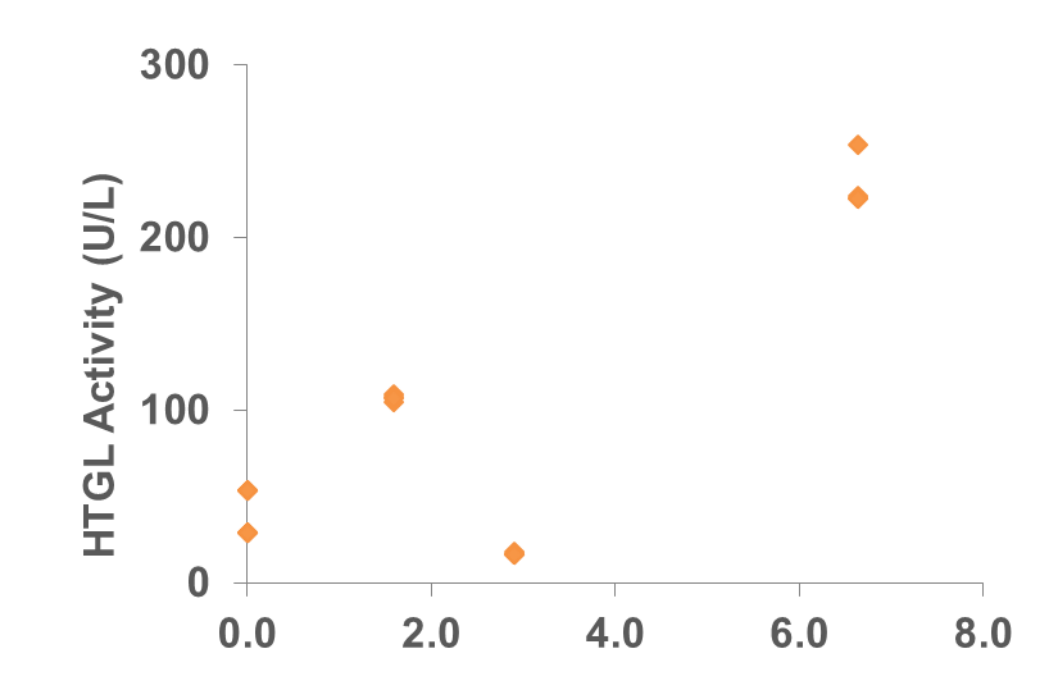
Sensitivity LPL+HTGL Activity in Post-Heparin Plasma



Sample	Rep 1	Rep 2	Rep 3	Rep 4	Mean	SD	%CV
LLOQ 1	345	350	348	352	349	3.0	0.9
LLOQ 2	166	167	166	167	167	0.6	0.3
LLOQ 3	80	80	79	80	80	0.5	0.6
LLOQ 4	38	40	39	40	39	1.0	2.4
LLOQ 5	19	19	20	19	19	0.5	2.6

Calculated LLOQ at %CV of 20 = 4 U/L from 20 values using the equation derived from a power fit.

Sensitivity HTGL Activity in Post-Heparin Plasma

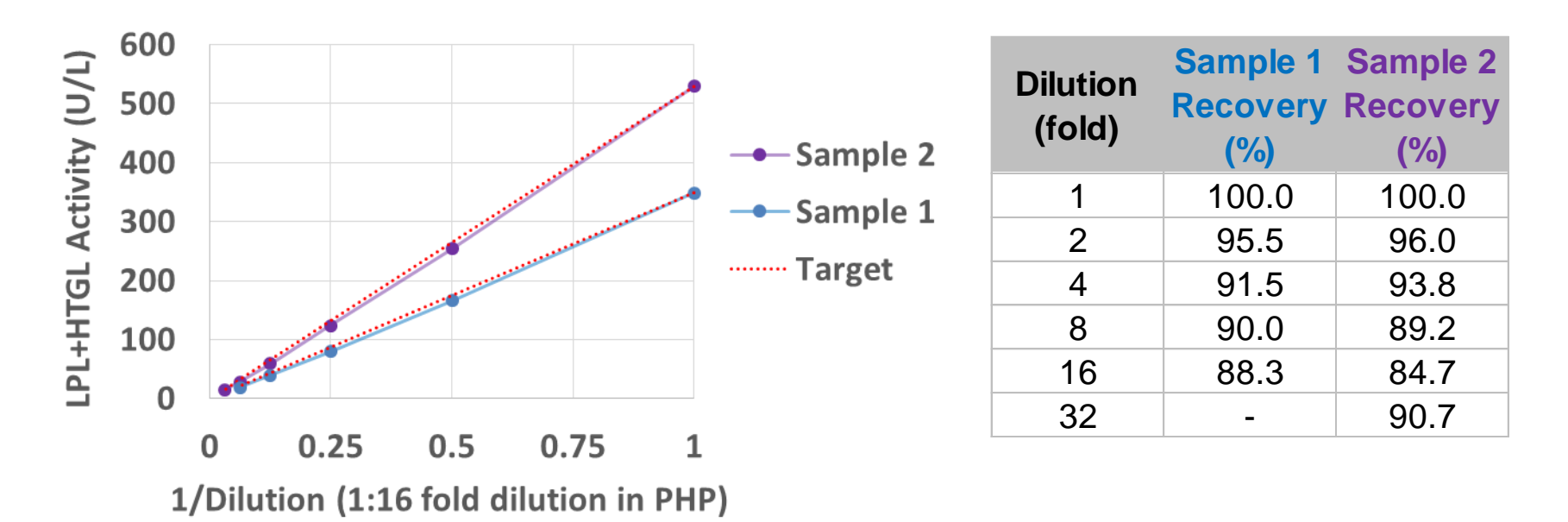


Sample	Rep 1	Rep 2	Rep 3	Rep 4	Mean	SD	%CV
LLOQ 1	254	224	223	223	231	15.3	6.6
LLOQ 2	105	108	109	107	107	1.7	1.6
LLOQ 3	54	54	54	54	54	0.0	0.0
LLOQ 4	29	29	29	29	29	0.0	0.0
LLOQ 5	18	17	17	17	17	0.5	2.9

Assigned LLOQ at %CV \leq 20 = 17 U/L

Figure 3: Linearity

Dilutional Linearity of LPL+HTGL Activity



Dilutional Linearity of HL Activity

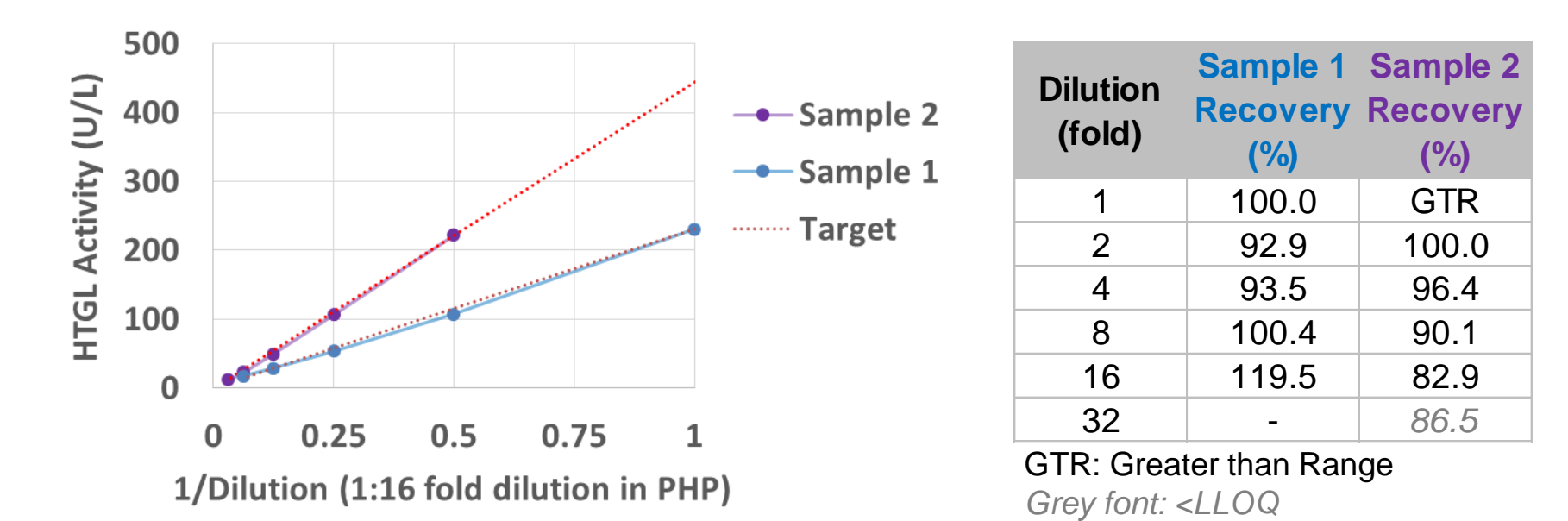


Table 4: Stability

	HTGL Activity	LPL+HTGL Activity
Room Temperature	24 hours	4 hours
4°C	3 days	3 days
Freeze/Thaw Cycles	3	3
-70°C (ongoing study)	up to 6 months	up to 6 months

Conclusion

The LPL/HTGL Activity assay manufactured by Immuno-Biological Laboratories provides a robust and cost-effective solution for activity assessment in post-heparin plasma. The utility of the LPL/HTGL Activity was demonstrated with sufficient analytical performance and met the acceptance criteria for precision, dilutional linearity down to 16-fold dilution, accuracy, spiked-in recovery, sensitivity, verification of the manufacturer's relative reference interval, short-term stability and long-term stability. Overall, this assay on an automated platform is ideal for measuring LPL and HTGL activities in clinical trials, especially for anti-apoCIII, anti-ANGPTL3 and anti-apo(a) drug targets. In addition, the LPL/HTGL Activity has great potential for future diagnostic use.

References

- Imamura, S., J. Kobayashi, S. Sakasegawa, A. Nohara, K. Nakajima, M. Kawashiri, A. Inazu, Yamagishi, J. Koizumi, and H. Mabuchi. 2007. A novel method for measuring human hepatic lipase activity in post-heparin plasma. J. Lipid Res. 48: 453-457.
- Imamura, S., J. Kobayashi, K. Nakajima, S. Sakasegawa, A. Nohara, T. Noguchi, M.A. Kawashiri, A. Inazu, S.S. Deeb, H. Mabuchi, and J.D. Brunzell. 2008. J. Lipid Res. 49: 1431-1437.