

A NEW AND IMPROVED CHEMILUMINESCENT SUBSTRATE

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BACKGROUND

Beckman Coulter is developing a new immunoassay system that will run current Access immunoassays as well as additional new menu. Goals for this new system include improved turnaround-times for all assays, thereby meeting STAT test requirements while improving overall platform throughput. A key component of the new system is a new chemiluminescent substrate employed to generate the light signal response. This new substrate is composed of a buffered surfactant enhancer system supporting an alkaline phosphatase-sensitive acridan. When the acridan is triggered in-situ, it forms a dioxetanone which immediately decomposes and emits light.

METHODS

LumiFAST formulation was optimized to work with Access immunoassays. Luminometer read time was assessed by determining the change in relative light unit (RLU) signal over 9 to 72 seconds using an ALPbased enzyme test method and several commercialized Access immunoassays. Improved signal-to-noise performance was demonstrated by comparing calibration curves from several immunoassays generated using Lumi-Phos 530 and the new chemiluminescent substrate LumiFAST. The impact of non-specific signal from endogenous ALP was determined by assessing a panel of patient samples previously identified to contain these interferents, using assays tested with both substrates.

Improved Sensitivity with LumiFAST on prototype immunoassay analyzer

TSH Detection Capability (mIU/L)

LumiFast	LoB	LoD	LoQ 10% CV	LoQ 20% (
Mean	0.00035	0.00074	0.0034	0.00130
Std Dev	0.00008	0.00018	0.0009	0.00031

Mean 0.00073 0.00157 0.0079 0.0028 Std Dev 0.00017 0.00038 0.0029 0.0008	Lumi-Phos 530	LoB	LoD	LoQ 10% CV	LoQ 20% CV
Std Dev 0.00017 0.00038 0.0029 0.0008	Mean	0.00073	0.00157	0.0079	0.0028
	Std Dev	0.00017	0.00038	0.0029	0.0008

TSH Sensitivity		
13,000	•	Sample
	•	Patier

Improved Time to first result with LumiFAST on immunoassay prototype analyzer

Time to first result in minutes					
Assay Name	Lumi-Phos 530	LumiFAST			
Intact PTH	14	8			
Total βHCG	17	11			
hsTnl	17	11			

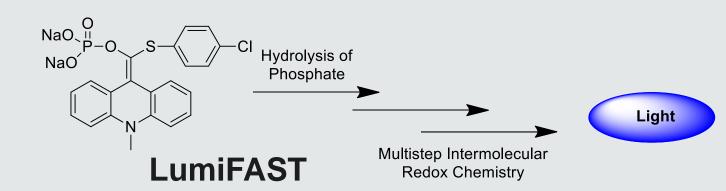
Better Discrimination of Non-Reactive Samples from Cut-off with LumiFAST on immunoassay prototype analyzer

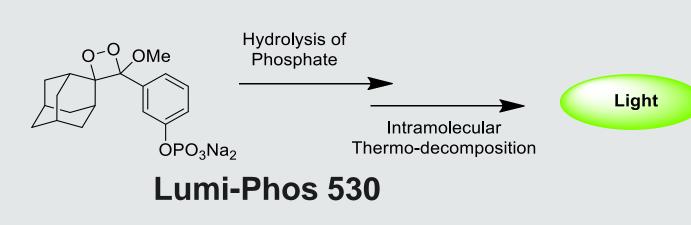
Approximately 500 presumed non-reactive samples were tested using the HIV and HBsAg assays with both Lumi-Phos 530 and LumiFAST chemiluminescent substrates

	Quant	tiles	Summary Statistics		
	100.0%	maximum	10.1713	Mean	0.1552
	99.5%		1.7039	Std Dev	0.4715
	97.5%		0.4687	Std Err Mean	0.0178
	90.0%		0.2482	Upper 95% Mean	0.1901
	75.0%	quartile	0.1518	Lower 95% Mean	0.1203
	50.0%	median	0.0926	Ν	703.0000
_	25.0%	quartile	0.0649		
	10.0%		0.0541		
	2.5%		0.0462		
	0.5%		0.0115		
0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9	9 1 0.0%	minimum	0.0000		

Lumi-Phos 530 (also known as LP-530) has desirable sensitivity, background luminescence and open bottle stability, but needs 6.3 minutes for signal generation on automated immunoassay systems. The new substrate formulation was optimized for immunoassay specificity, compatibility, sensitivity and is suitable for use with all forms of ALP employed by Access assays with a much shorter time to signal generation.

Comparison of the Lumi-Phos 530 substrate to the new chemiluminescent substrate LumiFAST was done on a immunoassay prototype analyzer to understand the performance characteristics.





"Assay results shown were generated using immunoassay prototype systems and may not represent final product claims"

RESULTS

Luminometer read time is approximately 5 minutes shorter for the new substrate than for Lumi-Phos 530. Three- to six-fold increases in signal-to-noise performance were demonstrated across the Access immunoassays. Samples with known high endogenous ALP activity displayed greater than 50% reduction in spurious elevations (fliers) when using the new chemiluminescent substrate as compared to the values observed with the same samples using Lumi-Phos 530.

Lumi-Phos 530 on Access 2	LumiFAST on Immunoassay Prototype analyzer
6.3 Minutes signal generation	1 Minute signal generation
Minimum 18 hour room temperature equilibration before use	No room temperature equilibration before use

Calibration Curve Signals

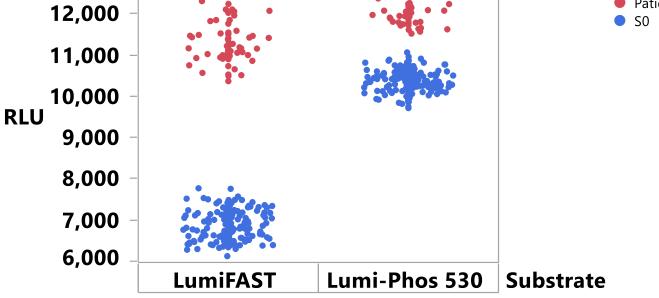


Figure 7 illustrates differences and distinction in RLU between low concentration TSH samples and zero calibrator with both substrates

hsTnI Detection Capability (pg/ml)						
	LumiFAST	LoB	LoD	LoQ 10% CV	LoQ 20% CV	
	Mean	0.25	0.32	0.43	0.19	
	Std Dev	0.10	0.11	0.12	0.05	

Lumi-Phos 530	LoB	LoD	LoQ 10% CV	LoQ 20% CV
Mean	0.50	0.51	1.91	0.78
Std Dev	0.31	0.23	0.44	0.16

	Quant	iles		Summary Statis	stics
	100.0%	maximum	0.5174	Mean	0.253
	99.5%		0.5174	Std Dev	0.098
	97.5%		0.5107	Std Err Mean	0.013
	90.0%		0.4041	Upper 95% Mean	0.280
	75.0%	quartile	0.3021	Lower 95% Mean	0.226
	50.0%	median	0.2382	Ν	54.000
	25.0%	quartile	0.1746		
	10.0%		0.1425		
	2.5%		0.1257		
	0.5%		0.1250		
0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.	3 0.0%	minimum	0.1250		

Lumi-Phos 530 LoB-hsTnl				
	Quantiles		Summary Sta	tistics
	100.0% maximum	1.225	Mean	0.503
	00 50/	4 225	CL I D	0 207

1.205

0.391

0.210

0.210

0.631

0.631

0.613

0.493

0.380

0.302

0.230

0.184

0.162 0.16

0.161

0.646

0.642

0.593

0.497

0.417

0.378

0.362

0.340

0.339

0.339

0.007

0.173

97.5%

90.0%

75.0%

50.0%

25.0%

Ouantile

99.5%

97.5%

90.0%

75.0%

50.0%

25.0%

0.0%

100.0% maximum

0.0% minimum

100.0% maximum

median

quartile

minimum

90.0%

75.0%

50.0%

25.0%

0.0%

immunoassay prototype analyzer. Better detection capability

median

quartile

0.0% minimum

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1 1.1 1.2 1.3

0.1 0.2 0.2 0.3 0.3 0.4 0.4 0.5 0.5 0.6 0.6 0.7 0.7

0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7

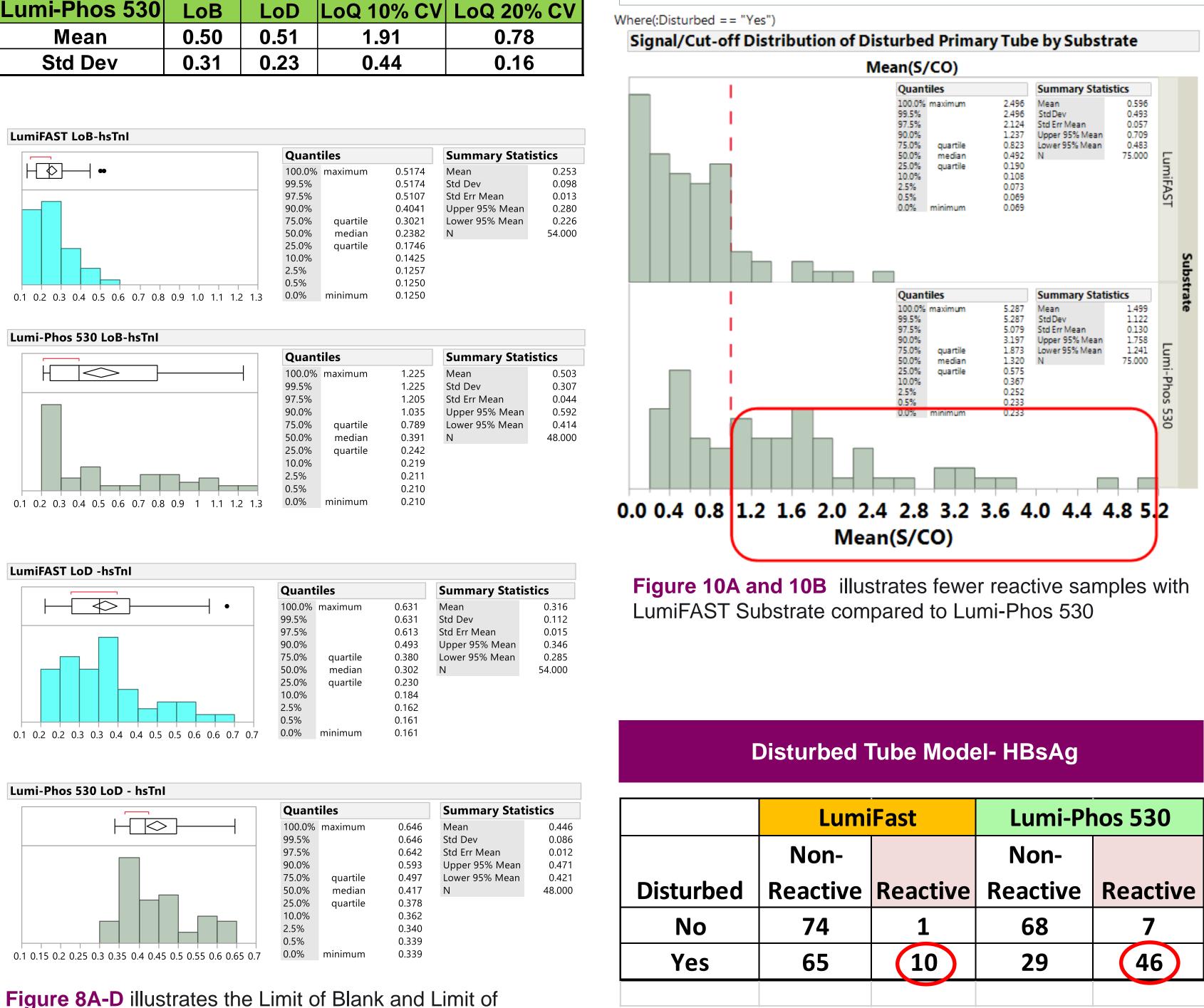
LumiFAST LoD -hsTnl

Lumi-Phos 530 LoD - hsTnl

nhanced Assay Specificity with LumiFAST on
nmunoassay prototype analyzer

An in-house model of incorrect primary tube handling, which leads to neutrophil contamination of plasma samples, is referred to here as the "disturbed tube" model". Neutrophil ALP generates non-specific signal in some immunoassays.

False positive results due to this endogenous ALP was evaluated by testing samples subjected to the "disturbed tube model" using normal plasma. HBsAg reactivity (S/CO) with both LumiFAST and Lumi-Phos 530 is shown below



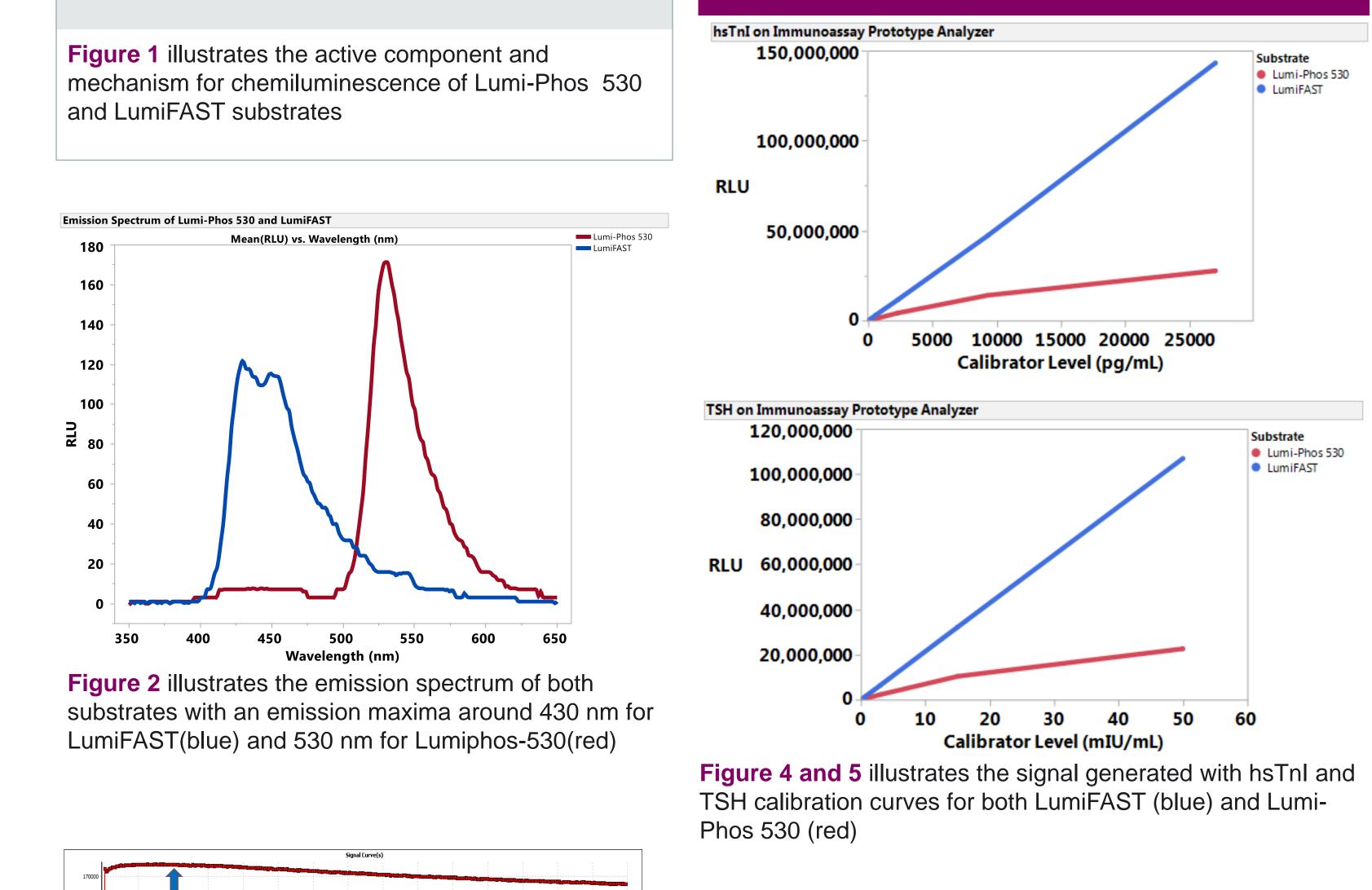
	Quantiles			Summary Statistics	
	100.0%	maximum	10.0001	Mean	0.2864
	99.5%		1.5442	Std Dev	0.4290
	97.5%		0.5286	Std Err Mean	0.0156
	90.0%		0.3816	Upper 95% Mean	0.3170
	75.0%	quartile	0.2949	Lower 95% Mean	0.2557
	50.0%	median	0.2328	Ν	755.0000
	25.0%	quartile	0.2033		
	10.0%		0.1861		
	2.5%		0.1775		
	0.5%		0.1723		
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1	0.0%	minimum	0.1693		

Figure 11A and 11B illustrates differences in signal to cutoff for HBsAg with LumiFAST and Lumi-Phos 530 for presumed normal samples on immunoassay prototype analyzer

	Quant	iles		Summary Stati	istics
	100.0%	maximum	0.819	Mean	0.21
	99.5%		0.706	Std Dev	0.05
	97.5%		0.334	Std Err Mean	0.00
	90.0%		0.245	Upper 95% Mean	0.22
	75.0%	quartile	0.222	Lower 95% Mean	0.21
	50.0%	median	0.207	Ν	670.00
	25.0%	quartile	0.193		
	10.0%		0.182		
	2.5%		0.170		
<mark>↓ ╿ ╿ ╿ ┝╤╒╔╤╤╼╤</mark> ┍╾┯╼╤	0.5%		0.161		
0.2 0.3 0.4 0.5 0.6	0.0%	minimum	0.000		

	Quant	iles	Summary Statistics		
	100.0%	maximum	0.960	Mean	0.314
	99.5%		0.648	Std Dev	0.053
	97.5%		0.432	Std Err Mean	0.002
	90.0%		0.345	Upper 95% Mean	0.318
	75.0%	quartile	0.321	Lower 95% Mean	0.311
	50.0%	median	0.305	Ν	752.000
	25.0%	quartile	0.293		
	10.0%		0.282		
	2.5%		0.270		
	0.5%		0.259		
0.2 0.3 0.4 0.5 0.6	0.0%	minimum	0.258		

Figure 12A and 12B illustrates differences in signal to cut-

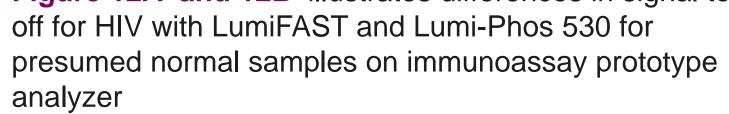


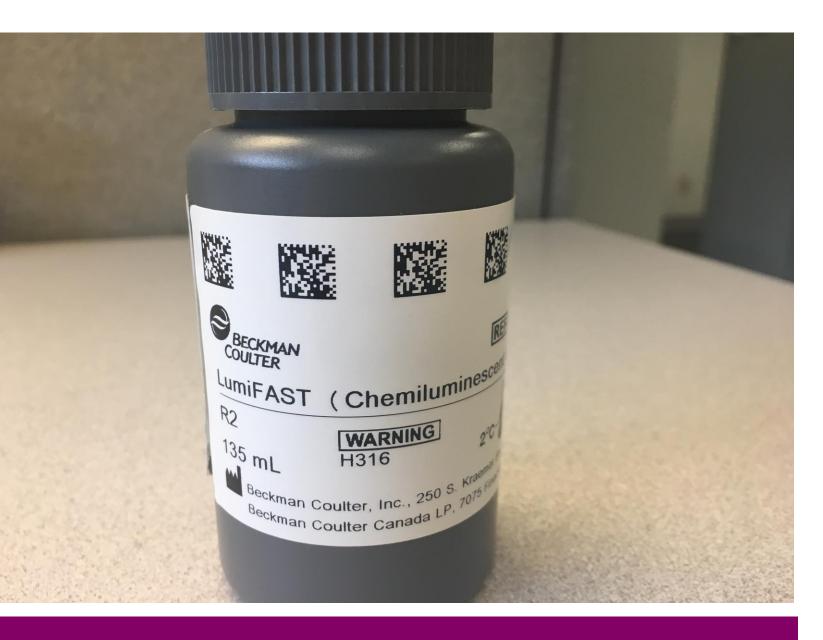
was seen with LumiFAST LumiFAST LoQ- hsTnl Quantile Summary Statistics HØ-----100.0% maximum 0.322 99.5% 0.322 Std Dev Std Err Mean 97.5% 0.319 90.0% Upper 95% Mean 75.0% 0.229 Lower 95% Mean 50.0% 0.166 N median

Detection for hsTnI with both substrates on the

78% Reduction in false reactives with LumiFAST compared to Lumi-Phos 530

Patient samples screened for high levels of endogenous ALP were used and were tested for HBsAg reactivity using both Lumi-Phos 530 and LumiFAST substrates





CONCLUSION

The chemiluminescent substrate LumiFAST has been optimized to generate signal rapidly, improve signal-tonoise performance, and reduce non-specific background from endogenous alkaline phosphatase (ALP) in comparison to Lumi-Phos 530. This new substrate presents the opportunity to significantly shorten the time to first result while simultaneously improving assay sensitivity.

Improvement in assay sensitivity (signal-to-noise) with LumiFAST compared to Lumi-Phos 530

⊿ Quantiles

99.5%

97.5%

90.0%

75.0%

50.0%

25.0%

10.0%

2.5%

0.5%

100.0% maximum 6.51574

0.0% minimum 1.30605

6.51574

6.51574

4.97903

1.48758

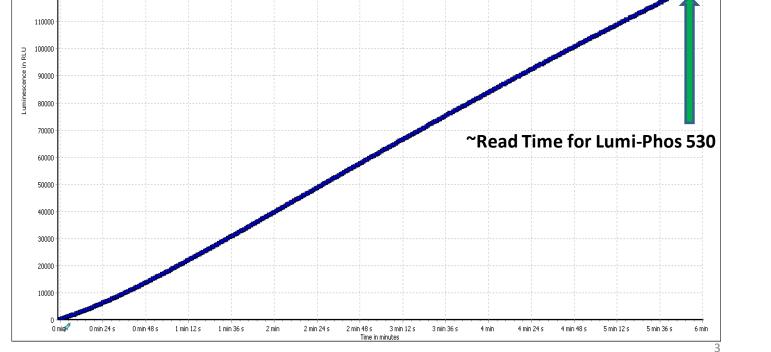
1.30605

1.30605

quartile 3.375

median 2.72526

quartile 1.8162



~Read Time for LumiFAST

Figure 3 illustrates the signal generation for bovine ALP with both substrates. Peak intensity was seen with LumiFAST within 60 seconds after injection. Results obtained on a BMG Plate Reader over 6 minutes

Figure 6 illustrates the signal-to-noise improvement across the current immunoassay menu. A 2.5 fold median increase in signal-to-noise was seen with LumiFAST compared to Lumi-Phos 530

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LumiFAST improved signal-to-noise

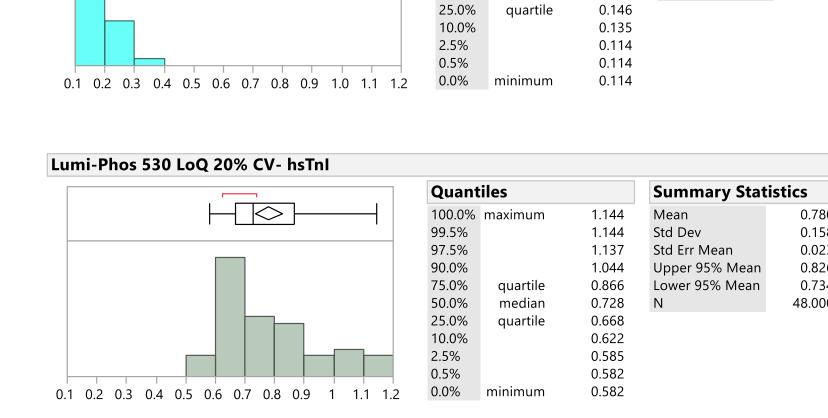


Figure 9A and 9B illustrates differences in LoQ (20% CV) for hsTnI (pg/mI) on immunoassay prototype analyzer

Reactive rate of F Levels	BsAg with I	high endoge	enous A
	Non-		
	Reactive	Reactive	Tota
LumiFAST	441	30	471
Lumi-Phos 530	364	111	475

73% reduction in false reactives were seen with LumiFAST.

Benefits:

Shortened Time to first result by ~5 minutes

- Improved assay sensitivity by reducing signal to noise
- Improved specificity for assay ALP Reduced magnitude of falsely elevated signals due to endogenous alkaline phosphatase
- Improved discrimination of non-reactive and reactive results

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