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 turn-around-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.

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.

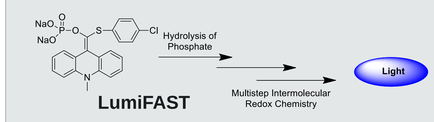


Figure 1 illustrates the active component and mechanism for chemiluminescence of Lumi-Phos 530 and LumiFAST substrates

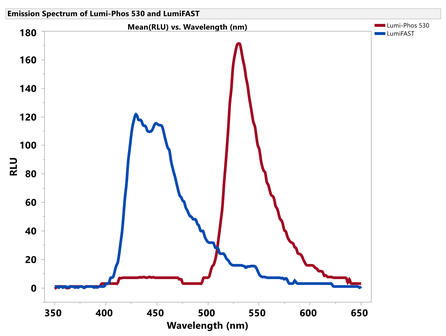


Figure 2 illustrates the emission spectrum of both substrates with an emission maxima around 430 nm for LumiFAST(blue) and 530 nm for Lumiphos-530(red)

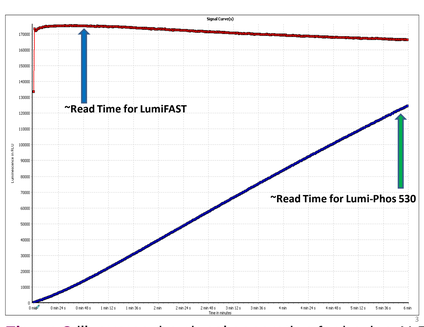


Figure 3 illustrates the signal generation for bovine ALP with both substrates. Peak intensity was seen with LumiFAST within 60 seconds after injection. Results obtained on a BMG Plate Reader over 6 minutes

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 ALP-based 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.

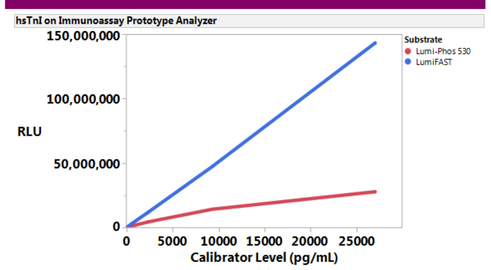
"Assay results shown were generated using immunoassay prototype systems and may not represent final product claims"

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



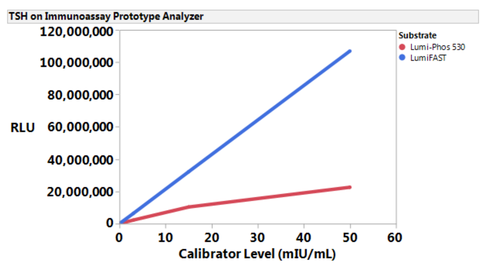
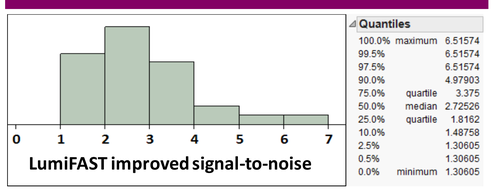


Figure 4 and 5 illustrates the signal generated with hsTnI and TSH calibration curves for both LumiFAST (blue) and Lumi-Phos 530 (red)

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



Quantiles		
100.0%	maximum	6.51574
99.5%		6.51574
97.5%		6.51574
90.0%		4.97903
75.0%	quartile	3.375
50.0%	median	2.72526
25.0%	quartile	1.8162
10.0%		1.48758
2.5%		1.30605
0.5%		1.30605
0.0%	minimum	1.30605

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

Improved Sensitivity with LumiFAST on prototype immunoassay analyzer

TSH Detection Capability (mIU/L)

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

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

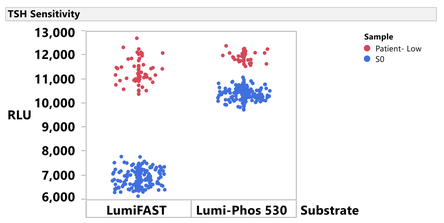
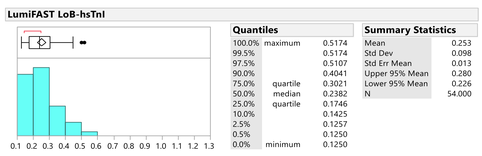


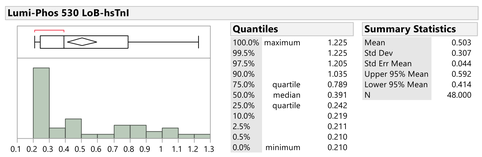
Figure 7 illustrates differences and distinction in RLU between low concentration TSH samples and zero calibrator with both substrates

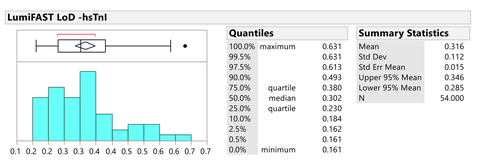
hsTnI Detection Capability (pg/ml)

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







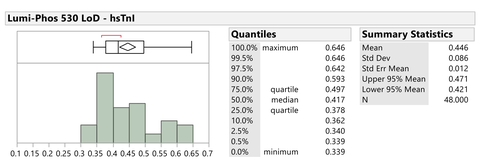
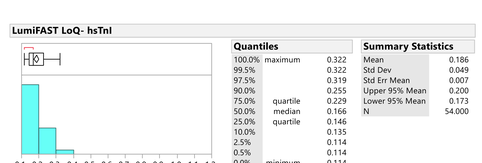


Figure 8A-D illustrates the Limit of Blank and Limit of Detection for hsTnI with both substrates on the immunoassay prototype analyzer. Better detection capability was seen with LumiFAST



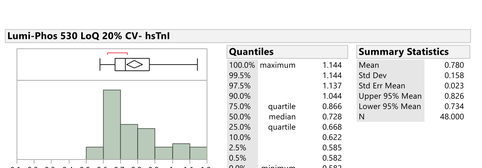


Figure 9A and 9B illustrates differences in LoQ (20% CV) for hsTnI (pg/ml) on immunoassay prototype analyzer

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
hsTnI	17	11

Enhanced Assay Specificity with LumiFAST on immunoassay 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

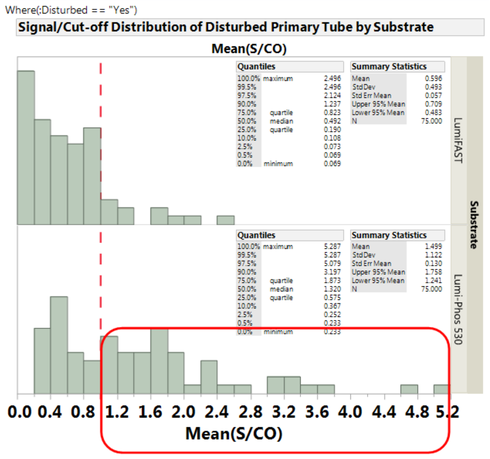


Figure 10A and 10B illustrates fewer reactive samples with LumiFAST Substrate compared to Lumi-Phos 530

Disturbed Tube Model- HBsAg

	LumiFast		Lumi-Phos 530	
Disturbed	Non-Reactive	Reactive	Non-Reactive	Reactive
No	74	1	68	7
Yes	65	10	29	46

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

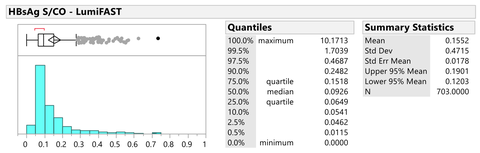
Reactive rate of HBsAg with high endogenous ALP Levels

	Non-Reactive	Reactive	Total
LumiFAST	441	30	471
Lumi-Phos 530	364	111	475

73% reduction in false reactives were seen with LumiFAST.

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



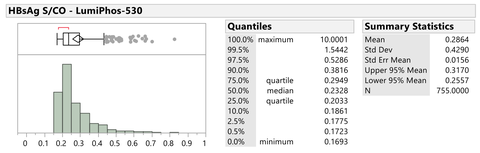
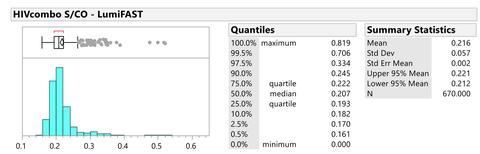


Figure 11A and 11B illustrates differences in signal to cut-off for HBsAg with LumiFAST and Lumi-Phos 530 for presumed normal samples on immunoassay prototype analyzer



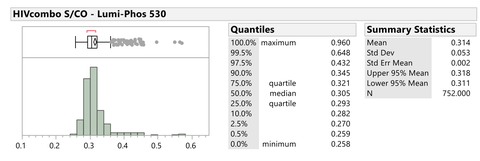
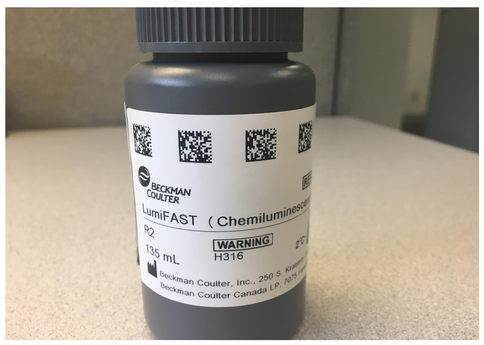


Figure 12A and 12B illustrates differences in signal to cut-off for HIV with LumiFAST and Lumi-Phos 530 for presumed normal samples on immunoassay prototype analyzer



CONCLUSION

The chemiluminescent substrate LumiFAST has been optimized to generate signal rapidly, improve signal-to-noise 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.

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

"This product is in development and is not available for sale. Pending achievement of CE compliance; not yet available for in vitro diagnostic use. Pending clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US. For Investigational Use Only."