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CLIPR™
Chemiluminescent Plate Reader

Application Note

Performance Evaluation of Various Imagers for UltraHTS

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INTRODUCTION

We have recently developed a standard protocol to evaluate detector performance[1] and we have applied it to all our readers in order to facilitate the transition between assay development and HTS. In addition, we are evaluating different platforms for ultraHTS (uHTS; 1536-well microplates and beyond) in order to make the right investments in the next generation of readers.

Luminescence, SPA and fluorescence are the detection modes best suited to work in miniaturized formats. In the past year, several companies have launched different imagers that are able to read high density plates fast and accurately in one or more of the above modes. For a couple of months, we had the luxury of having four imagers installed in our facilities, one acquired (LEADseeker™™), and three kindly offered for evaluation (NorthStar™, CLIPR and Viewlux™). Therefore, we have been able to compare the performance of these imagers using our standard protocol to evaluate detectors. Additionally, we have analyzed how they work in comparison with traditional photo-multiplier tube-based (PMT) readers.

Most of the work has been focused on luminescence, the only detection mode in common for all imagers analyzed. SPA detection has also been analyzed extensively when possible (LEADseeker™ and CLIPR). Our LEADseeker™ was only recently upgraded to work on fluorescence, and the Viewlux was available just for a short time, therefore no systematic data is presented for this mode.

Although our target was to evaluate these readers with 384- and 1536-plates, the lack of a suitable liquid handler for 1536-plates at the time this evaluation was performed forced us to focus on 384-plates.

Molecular Devices

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IMAGERS EVALUATED

	Vendor	Detection Modes	Format
Imagers			
LEADseeker™	Amersham PB	Lum, SPA, FI	96, 384, 1536
CLIPR	Molecular Devices	Lum, SPA	96, 384, 1536
NorthStar™	Applied Biosystems	Lum	96, 384, 1536
Viewlux™	PerkinElmer Wallac	Lum, FI, TRF, Abs*, FP*	96, 384, 1536
PMT Readers			
TopCount	Packard	Lum, SPA	96, 384
Trilux	PerkinElmer Wallac	Lum, SPA	96, 384

*Not available in the instrument tested

Table 1: Imagers evaluated.

LUMINESCENCE

Plate Evaluation and Choice

The following 384-plates were tested on each imager in the luminescence mode:

- Packard Optiplate 384 (white)
- Falcon # 35-3906 (white)
- Labsystems Cliniplates (white and black)
- Costar # 3705 (white)

In each case, the plate type that performed best was chosen for further tests. Signal-to-background (S/B) and cross-talk were taken into account as main parameters for this selection. For example, for CLIPR, the data collected (Table 1) showed that despite a lower light output, resulting in a poorer S/B, the lack of cross-talk made the white Cliniplate our plate of choice. All imagers showed a significant reduction in light output and background with this plate, resulting in lower S/B to those observed with other white plates. However, there is virtually no cross-talk with this plate. It was chosen for all imagers but NorthStar™, for which the Costar was the best option.

	% Cross-Talk at 12.5 pM Luciferase	Signal-to-Background at 400 fM Luciferase
Cliniplate	0.01	54
Falcon	6.13	73
Costar	0.99	89
Packard	1.51	81

Table 2: Performance of different white 384-well plates in CLIPR (Steady-Glo reagent, 30 second readings). Cross-talk calculated as defined in the sensitivity template below.

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Luciferase Substrate Evaluation and Choice

Three luciferase substrates from Promega and one from Molecular Devices were compared.

Luciferase Substrate Reagent	Half-Life
Promega Luciferase Assay System (LAS)	5 minutes
Promega Brite-Glo Luciferase Assay System	30 minutes
Promega Steady-Glo Luciferase Assay System	5 hours
CLIPR Luciferase Assay Kit	5 hours

Table 3: Luciferase substrates and their half-lives

The Steady-Glo system was chosen for this evaluation because of its enhanced stability, which allowed it to read the same plates in different readers without any significant change in signal. More data on reagents comparison is provided in another communication to this meeting.[2]

- Test buffer: 20 mM Tris pH 7.5, 100mM NaCl, 0.05% Tween 20
- Firefly luciferase was obtained from Sigma

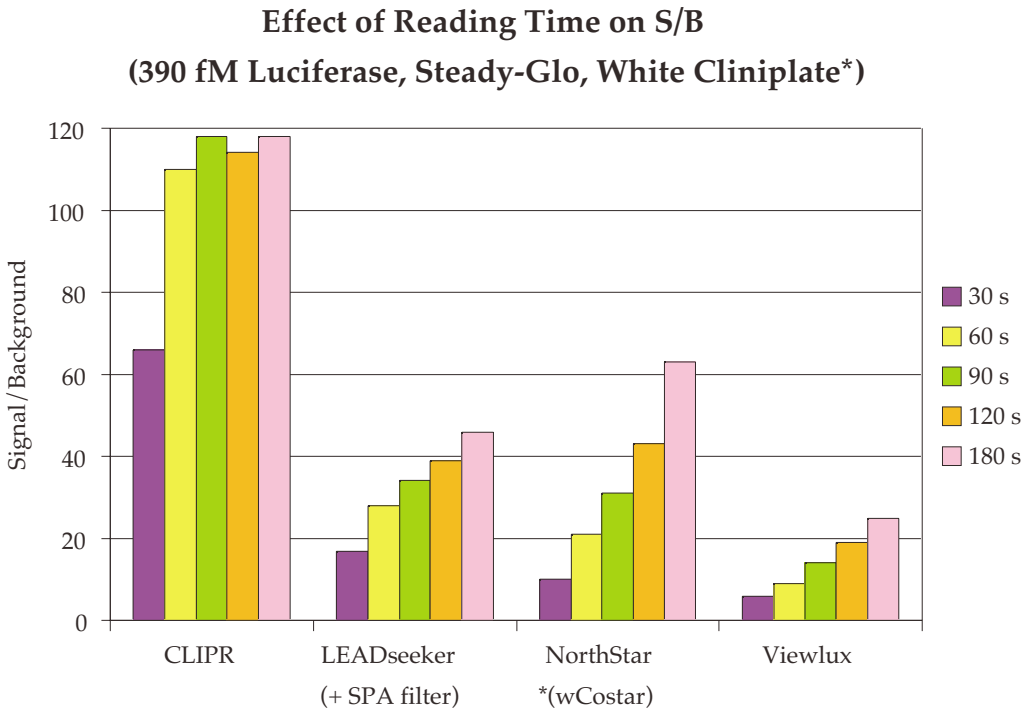


Figure 1: Effect of reading time on S/B. Except for CLIPR, all other imagers show an improved S/B at longer exposure time. This is another factor to consider when optimizing a luminescence test.

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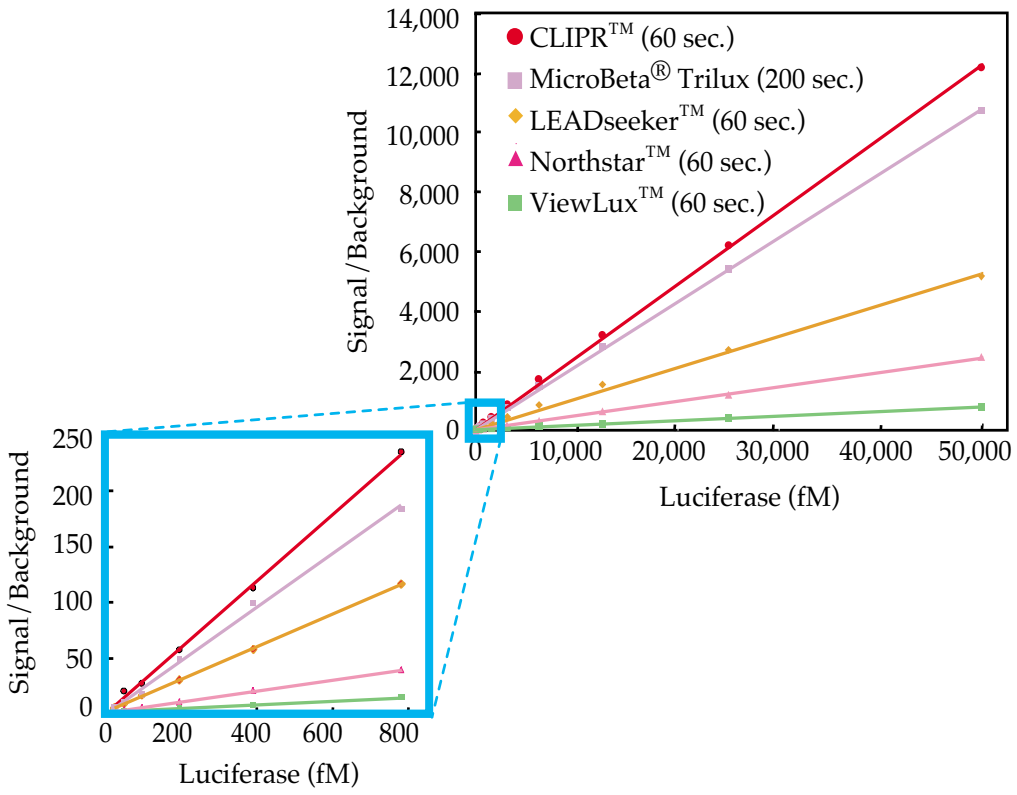


Figure 2: The linear response of all imagers throughout the whole range of light input is outstanding. The variability of readings is also extremely low at all levels of light (see Table 4 and Figure 3).

Instrument	S/B at 50 pM Luciferase: Read Time[1]	Dark Adapted	Normal Lighting	% Cross-Talk[2] at 50 pM Luciferase (Normal Lighting)
Imagers				
CLIPR	80	12,200	8,900	0.2
LEADseeker™	100	5,150	2,750	0.1
NorthStar™	72	2,500	550	0.4
Viewlux™	150	950	825	0.1
PMT-Based				
Trilux	215	10,700	ND	0.1
TopCount	600	12,300	ND	1.3

ND = Not Determined

[1] Including plate transport time. Similar times are expected for 1536-well plates using imagers.

[2] % cross talk = [(empty adjacent well counts - background counts)/ sample counts - background counts]*100

Table 4: Summary of different readers' performance as luminometers (data collected with Steady-Glo and a white Cliniplate).

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Variability Test

- 384-well white Cliniplates plates homogeneously filled with a Multidrop dispenser
- Three concentrations of luciferase were tested
- Duplicate plates of each concentration were made
- Same plates were read in all imagers
- One of the plates was read three times to determine inter-reading variability

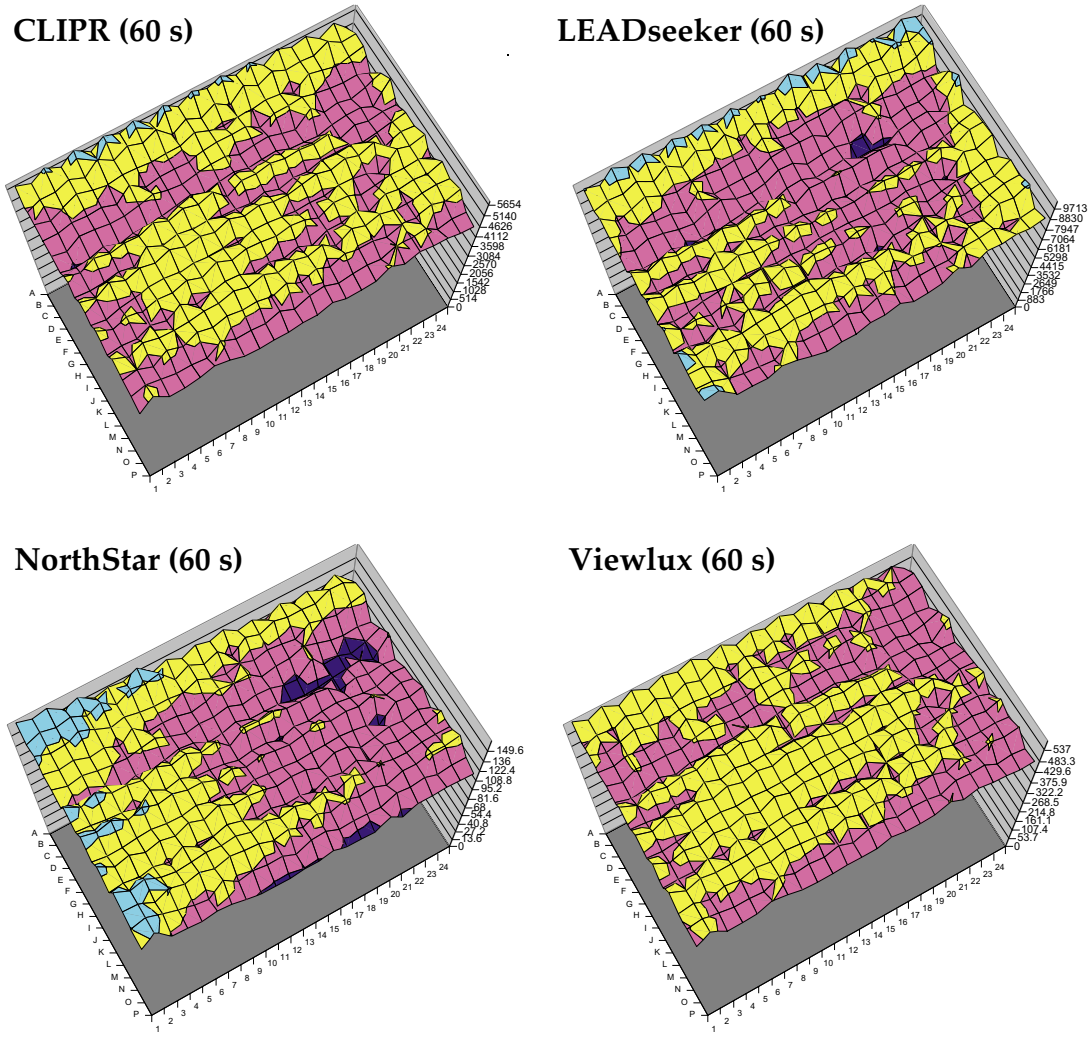


Figure 3: Example of the variability observed with each of the imagers reading the same uniformly filled plate. A relatively low concentration of luciferase (10 pM) and the low bright Steady-Glo reagent were used in this experiment. Each color corresponds to 10% of the average. The variability of dispensing, although expected to be low, is also contributing to the overall variability of the reading. Because the data were collected using the same plate in every instrument, any liquid handling error should have affected every readout equally.

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fM Luciferase	Plate#	%CV CLIPR	%CV LEADseeker™	%CV Northstar™	%CV ViewLux™	%CV TriLux
100	1	2.9	6.5	n/a	n/a	8.5
100	2	2.8	6.0	n/a	n/a	8.3
1,000	1	5.1	6.5	9.2	4.6	6.0
1,000	2	5.0	6.6	9.3	4.8	6.0
10,000	1	4.7	5.9	7.0	3.7	5.8
10,000	2	5.6	6.3	6.6	4.4	6.7

Table 5: Summary of results of the variability tests.

EXAMPLE OF REPORTER-GENE ASSAY READ IN CLIPR

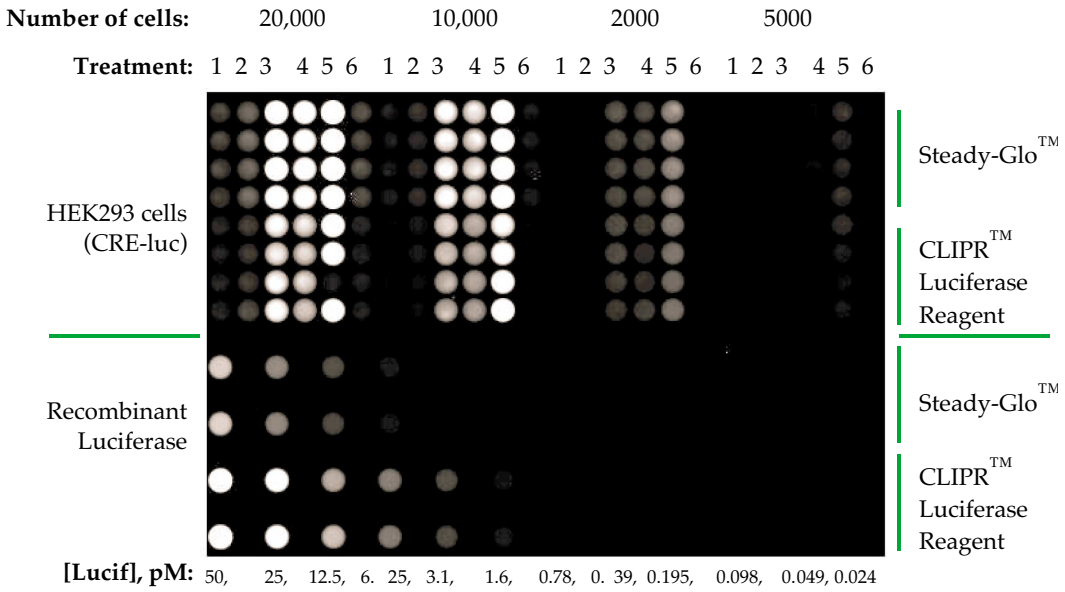


Figure 4: HEK293 cells stably transfected with a known Gs-coupled receptor were dispensed at different cell densities into a 384-well plate. Several treatments were tested and after four hours incubation, luciferase reagents were added.

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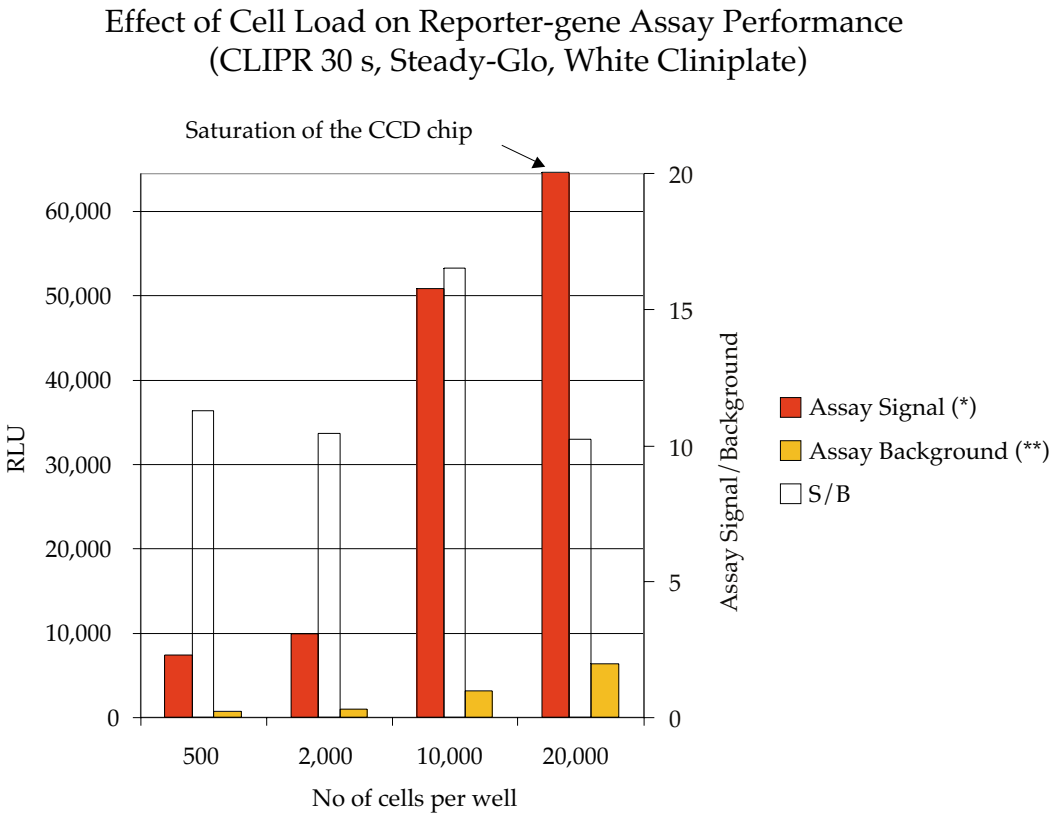


Figure 5: Increasing the quantity of cells per well does not always improve the performance of the assay. Much shorter reading times (<1 sec.) have been proven to give satisfactory results when dealing with these assays. If brighter reagents are used (e.g. Bright-Glo), it becomes very easy to saturate the reader.

Conclusions: Luminescence

- The choice of plate type should be carefully studied for each instrument and application.
- The choice of reagent and reading time provides a wide range of possibilities when planning luminescent uHTS assays.
- All imagers tested provide excellent sensitivity and low variability as luminometers at an outstanding throughput (approximately 50–250 1536-well plates per hour).
- Thus, they are suitable for uHTS of a great range of luminescent assays.
- CLIPR and LEADseeker are the most sensitive of the four imagers tested (LEADseeker was tested with an SPA filter not designed for luminescence. A more appropriate filter for luminescence is being developed by APB).
- The CCDs of the CLIPR and LEADseeker imagers are cooled to below –100 °C reducing the dark noise greatly in comparison to that of NorthStar™ and ViewLux™, which are cooled to –40 °C. This higher sensitivity is required to read SPA assays but it is not really a need for reporter-gene assays.

SCINTILLATION PROXIMITY ASSAY (SPA)

Assay Model for Tritium

- 3H Biotin mimics a product or a bound ligand
- 3H Leu mimics a substrate or an unbound ligand (there is no binding of Leu to the Streptavidin-coated beads, so that is a best case scenario without non-specific binding)

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- Total "substrate" /"ligand" amount is set at 0.1 μCi/well, typically 0.2 μM in a 384-well, 1 μM in a 1536-well plate
- 0.2 mg/well of Streptavidin-coated beads (all bead types tested)
- In a simulation of an assay in which we observe 10% depletion of substrate or bound ligand, 0.01 μCi 3H-Biotin and 0.09 μCi 3H-Leu are added per well to mimic a positive control (0% inhibition), and 0.1 μCi3H-Leu to mimic the negative control (100% inhibition of an enzyme or 100% displacement of a ligand)

	LEADseeker (rlu)				CLIPR (rlu)				Topcount (cpm)			
	YS[1]	YO	PVT[1]	PS	YS	YO	PVT	PS	YS	YO[1]	PVT	PS[1]
Signal				328	306	525	39	244	7425		1131	
Bckg				45	23	5.5	2.1	61	93		36.2	
CV Signal		7.2		4.3	2.4	5.9	8.3	4.1	4.8		6.4	
CV Bckg		14		6.4	8.6	16	19	0.8	26.2		22	
S/B [2]		40		7.3	13	95	18.0	4	80		31	
Z' [2]		0.77		0.82	0.9	0.81	0.71	0.83	0.85		0.78	

[1] Not readable
[2] S/B below 3 and/or Z'<0.4 are considered unacceptable (Z' as defined by Zhang et al.[3])

Figure 6: Simulation of an assay with 10% substrate depletion of ligand bound with 0.1 μCi of 3H substrate or ligand added. Data shown are from 16 replicates of each point with beads and reagents dispensed by hand pipetting.

	LEADseeker (rlu)				CLIPR (rlu)				Topcount (cpm)			
	YS[1]	YO	PVT[1]	PS	YS	YO	PVT	PS	YS	YO[1]	PVT	PS[1]
Signal		102		76	33	66	3.4	65	800		104	
Bckg		15		50	2.4	5	3.9	62	85		30.8	
CV Signal		7.8		2.9	11	2.3	17	1.7	6.6		16	
CV Bckg		16		1.3	17	12	126	2	19.7		25	
S/B [2]		6.6		1.5	14	13	0.9	1	9.4		3.4	
Z' [2]		0.64		-0.7	0.61	0.9	-32	-1.5	0.71		0	

[1] Not readable
[2] S/B below 3 and/or Z'<0.4 are considered unacceptable

Figure 7: Simulation of an assay with 1% substrate depletion of ligand bound with 0.1 μCi of 3H substrate or ligand added. Data shown are from 16 replicates of each point with beads and reagents dispensed by hand pipetting.

Conclusions (SPA)

- The performance of the new YO beads in either LEADseeker™ or CLIPR is similar to the performance of the traditional YSi beads in TopCount.
- Due to its current radiometric filter (590–630 nm), LEADseeker™ cannot read "blue" beads, while CLIPR does a good job with YSi beads. The emission of light by PVT beads is less efficient and so they are more difficult to read.

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FLUORESCENCE

ViewLux™ could only be evaluated for a very limited time. Our LEADseeker has only recently been upgraded. From our limited experience, both instruments perform similarly to traditional fluorescence readers such as Victor2, with lower variability. ViewLux™ is also capable of reading time-resolved fluorescence. Typical reading times for these modes are in the 10-30 sec. range.

ACKNOWLEDGEMENTS

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