TRF assay performance of the EnVision Nexus multimode plate reader.

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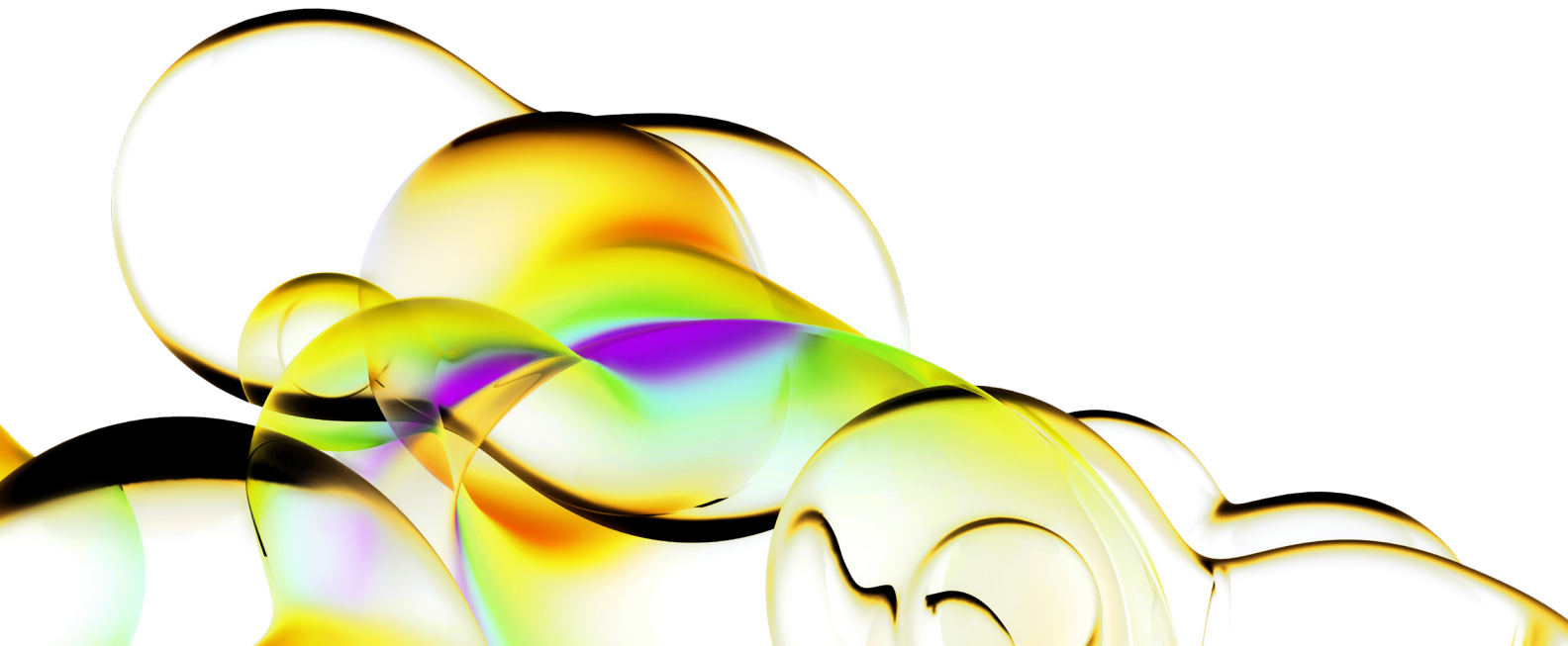
Highlights

- Remarkable HTRF performance
- High speed
- Excellent reproducibility
- Ease-of-use

Introduction

Immunoassays are an integral part of research and drug discovery. There are various formats available offering different specifications and performance parameters. HTRF™ (Homogeneous Time Resolved Fluorescence) and LANCE™ (Lanthanide Chelate Excite) are no-wash technologies, that combine standard FRET (Fluorescence Resonance Energy Transfer) technology with time-resolved fluorescence (TRF) measurements.

TRF measurements offer unparalleled sensitivity and specificity by an almost complete elimination of background fluorescence. This technology utilizes fluorophores like lanthanides because of their extended fluorescence lifetimes, enhancing the detection of low-concentration analytes.



Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) emerges as a specialized application within TRF technology, facilitating precise quantification of biomolecular interactions in drug discovery and molecular biology. Exploiting energy transfer between fluorophores, TR-FRET probes proximity-based interactions accelerating drug development efforts.

The EnVision Nexus™ is a high-throughput multimode plate reader offering remarkable speed and sensitivity for TRF technology using simultaneous dual detection and laser excitation. Combining the sensitivity of our no-wash HTRF and LANCE assays with the speed and ease-of-use of the EnVision Nexus system, this solution offers fast, easy, and reliable performance. In this technical note, we established the EnVision Nexus' TR-FRET capability with the HTRF reader control kit and illustrated its performance for four popular HTRF assays. Additionally, we compared the performance to its predecessor, the EnVision™ 2105 multimode plate reader for both lamp- and laser-based excitation of the respective TR-FRET based donors.

Materials and Methods

Plate readers

- EnVision Nexus multimode plate reader
- EnVision 2105 multimode plate reader

Microplates

- ProxiPlate™-384 Plus (White), part number: 6007290
- OptiPlate™-1536 (White), part number: 6004290

Assay kits

- HTRF Insulin Mouse Serum Detection Kit, part number: 62IN3PEF
- HTRF IP-One Gq Detection Kit, part number: 62IPAPEB
- HTRF Human and Mouse Advanced phospho-ERK (Thr202/Tyr204) Detection Kit, part number: 64AERPEH
- HTRF Human and Mouse Advanced phospho-ERK (Thr202/Tyr204) Detection Kit, Control Lysate, part number: 64AERTDA
- LANCE Ultra cAMP Detection Kit, part number: TRF0262
- HTRF Reader Control Kit, part number: 62RCLPEA

Sample preparation

Samples were prepared in triplicates and according to the kit instructions. Total sample volumes per well were 20 µL for the HTRF reader control kit in 96 well plates and 15 µL in 384 well plates and 8 µL in 1536 well plates for all other kits. For evaluating the plate reader performance standard, dose response curves were measured. Standards were prepared according to the kit instructions where available. For the phospho-Erk kit a 1:2 serial dilution of control lysate in lysis buffer was used. The cAMP standard curve was prepared in PBS. All samples were incubated in reaction tubes and dispensed into multiwell plates. HTRF reader control kit samples were dispensed into the included low volume HTRF 96 well plate (20 µl). For the other kits, samples were dispensed into ProxiPlate-384 Plus (White) and OptiPlate-1536 (White).

The HTRF reader control kit was incubated for 3 hours, the LANCE cAMP and IP-One kits were incubated for 1 hour, and the phospho-ERK and Insulin Mouse Serum kits were incubated overnight (~18 hours). Assay performance was evaluated by comparing Z' values and Delta F (assay window):

Delta F calculation:

$$100 * \frac{HTRF\ Ratio\ (Sample) - HTRF\ Ratio\ (negative\ control)}{HTRF\ Ratio\ (negative\ control)}$$

Z´ calculation:

$$1 - \frac{3\sigma(positive\ control) + 3\sigma(negative\ control)}{\mu(positive\ control) - \mu(negative\ control)}$$

Secondary analysis and data visualization: Revvity Signals™.

Measurements

All samples were measured both in the well-by-well standard mode (Std) and in the faster on-the-fly mode (OTF). The latter uses only one flash per measurement to avoid the need to stop motions of the scanning stage for each well. Donor and acceptor channels were detected simultaneously using dual detector mode on both instruments.

Table 1: Plate Reader settings. EnVision Nexus measurement times have been set to match the flash number used for EnVision 2105.

Instrument settings						
Plate reader	**Measurement mode**	**Light source**	**Measurement time (ms)**	**Flashes**	**Delay**	**Window**
EnVision Nexus	Std	Lamp	200 (100)§	50	60 (50)*	400 (200)*
		Laser	50 (100)§	12	60 (50)*	400 (100)*
	OTF	Lamp		1	60 (50)*	400 (200)*
		Laser		1	60 (50)*	400 (100)*
EnVision	Std	Lamp	200	100	60 (50)*	400 (200)*
		Laser	50	3	60 (50)*	400 (100)*
	OTF	Lamp		1	60 (50)*	400 (200)*
		Laser		1	60 (50)*	400 (100)*

* Settings for LANCE cAMP kit measurements.

§ Settings for HTRF reader control kit measurements.

Results

The performance of immunoassays is always limited by several factors including the underlying biology, the affinity of the antibodies, the assay technology, sample media and carriers, and the detection instrument. Here we are comparing a range of assays with different biologies: the HTRF IP-One, LANCE Ultra cAMP, HTRF Insulin Mouse Serum, and HTRF Phospho ERK1/2 kits to demonstrate the improved performance of the EnVision Nexus. We look at crucial parameters for assay performance such as dynamic range and Z'-score to assess the system's robustness.

The superior dynamic range of the EnVision Nexus for all assays is shown in figure 1. The differences in performance when comparing the EnVision Nexus with the EnVision, and comparing lamp- and laser-based measurements can vary between assays. The LANCE Ultra cAMP and Insulin Mouse Serum assays show a superior performance with the laser-based technology in comparison to the lamp-based equivalents. Furthermore, EnVision Nexus' lamp-based technology shows comparable or better dynamic ranges than the laser-based technology of the EnVision 2105.

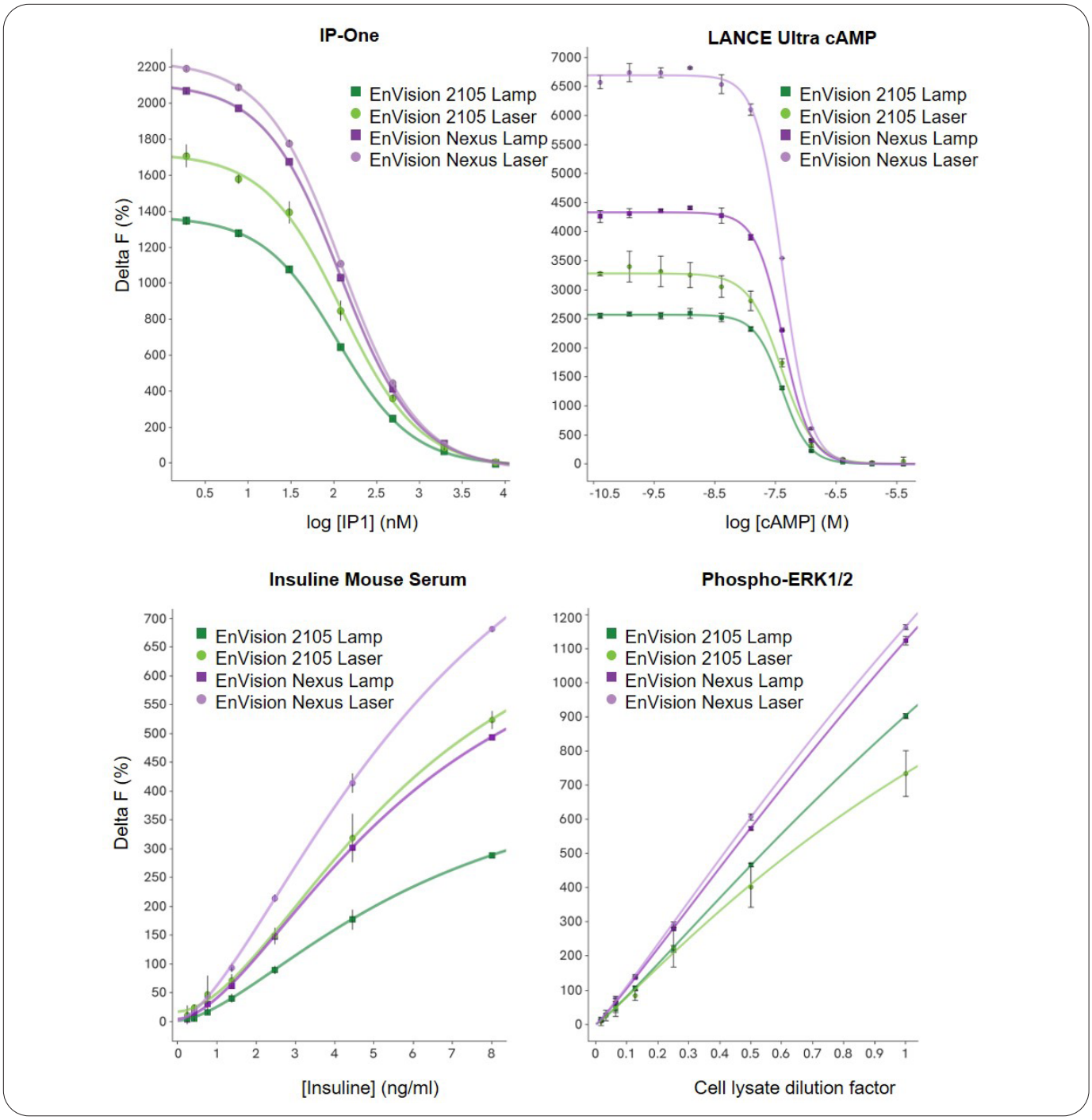


Figure 1: Delta F performance of the EnVision Nexus versus the EnVision 2105 comparing both detection modes lamp and laser.

Here we show that the EnVision Nexus delivers the highest assay performance regardless of assay, plate format, or excitation source. For all assays, we see excellent Z′ values using the standard measurement protocols for both lamp and laser excitation. Achieving similar or better Z′ values with the laser when compared to the lamp with a 4x shorter measurement time highlights the significant advantage of the laser-based technology for TR-FRET measurements on the EnVision Nexus for high-throughput screening applications (Figure 2).

Even under challenging conditions like 1536 well plates, we can show the superior performance of the OTF mode in conjunction with the laser-based technology. Using only 1 flash we can reduce the acquisition time of a 1536 well plate from 2 minutes and 30 seconds to approximately 30 seconds while maintaining excellent Z′ scores (Figure 2).

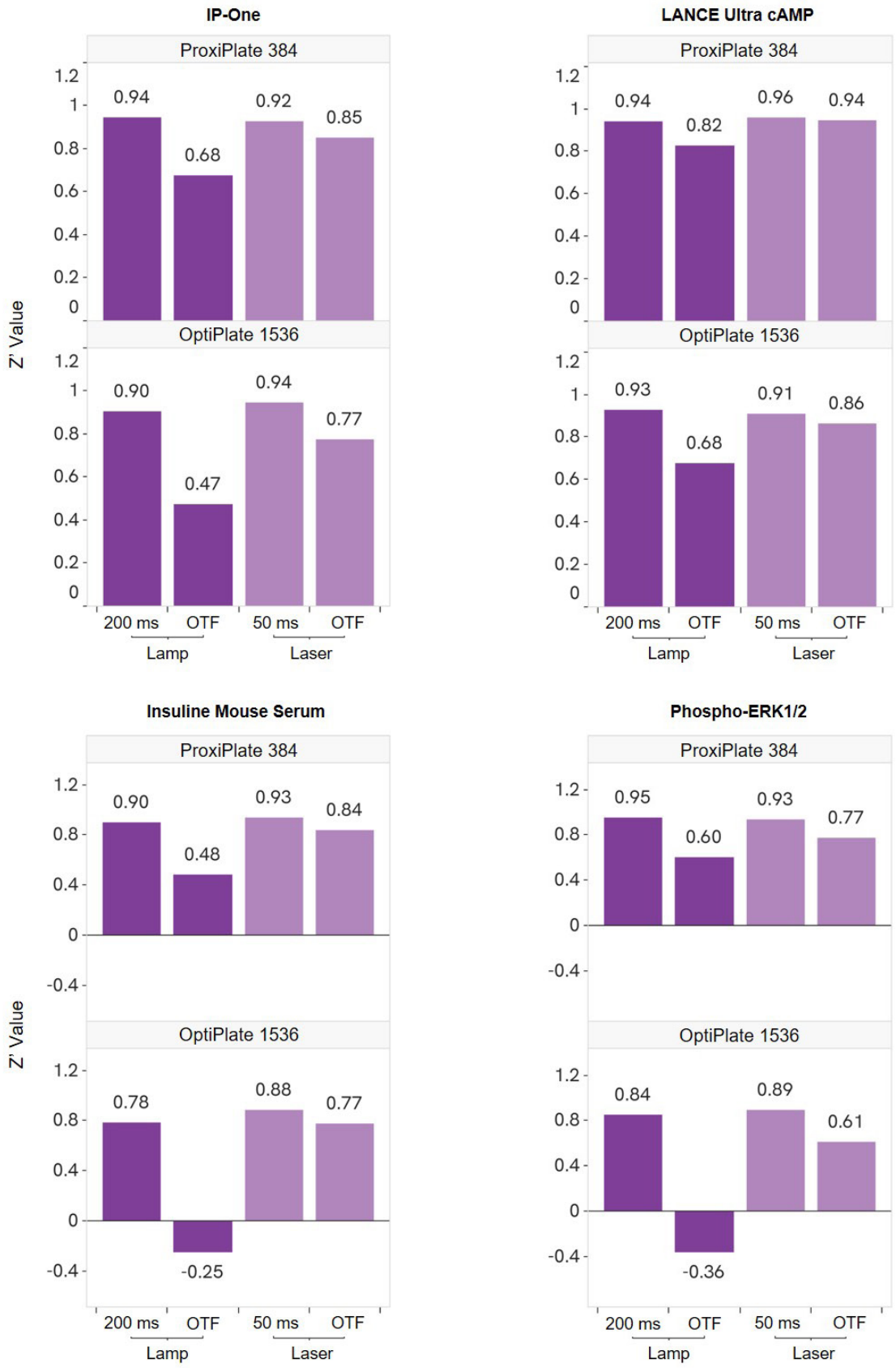


Figure 2: EnVision Nexus Z′ performance compared between assays, plate formats, excitation technologies and reading speeds.

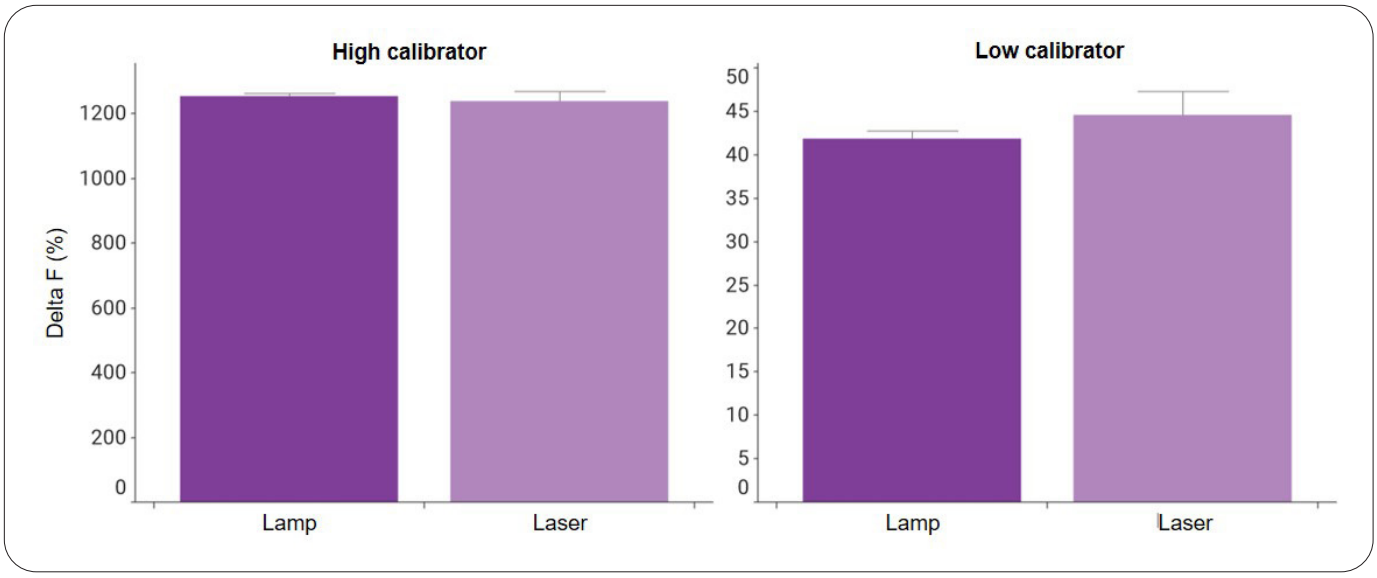


Figure 3: Delta F% reproducibility between different EnVision Nexus readers using the HTRF reader control kit high and low calibrators for lamp (n=5) and laser (n=4).

To demonstrate reproducibility between five different EnVision Nexus systems, we compared the calibrator probes of the HTRF reader control kit with each other. With a CV < 2.5 % and < 10 % for the high and low calibrator probes, respectively, we can show an excellent reproducibility between different EnVision Nexus systems.

Conclusions

In conclusion, the EnVision Nexus multimode plate reader shows excellent assay performance (Z', Delta F). While the EnVision Nexus offers similar measurement speeds, it exceeds the EnVision 2105 at key performance parameters qualifying the EnVision Nexus to be our next-generation gold standard system.

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