

Comparative Evaluation of Ligand Binding Assay Platforms for Biomarker Quantification: Critical Considerations for Ensuring Data Quality and Project Success

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SELECTION OF AN APPROPRIATE LIGAND BINDING ASSAY (LBA) PLATFORM

Why?

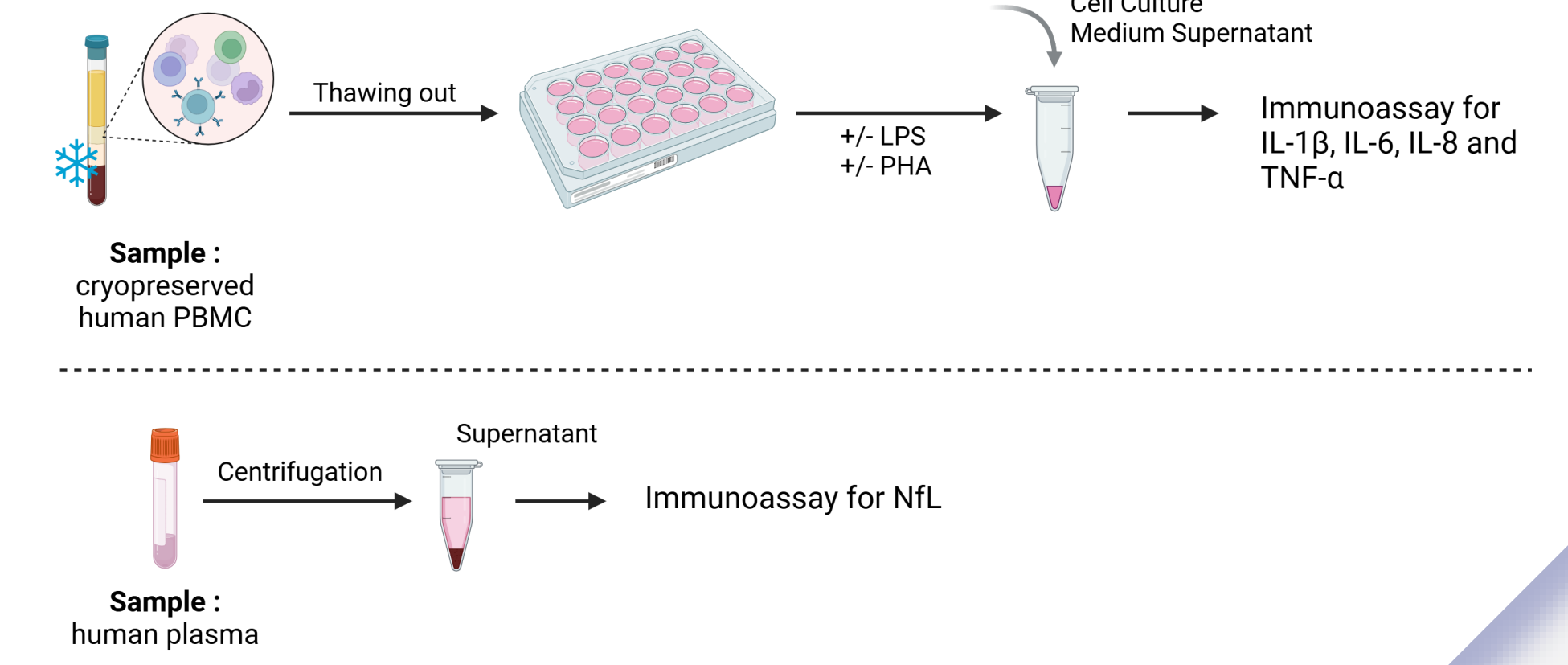
The Selection of an appropriate ligand binding assay (LBA) platform

- significantly influences the quality of data
- has a high impact on the overall success of the project

What?

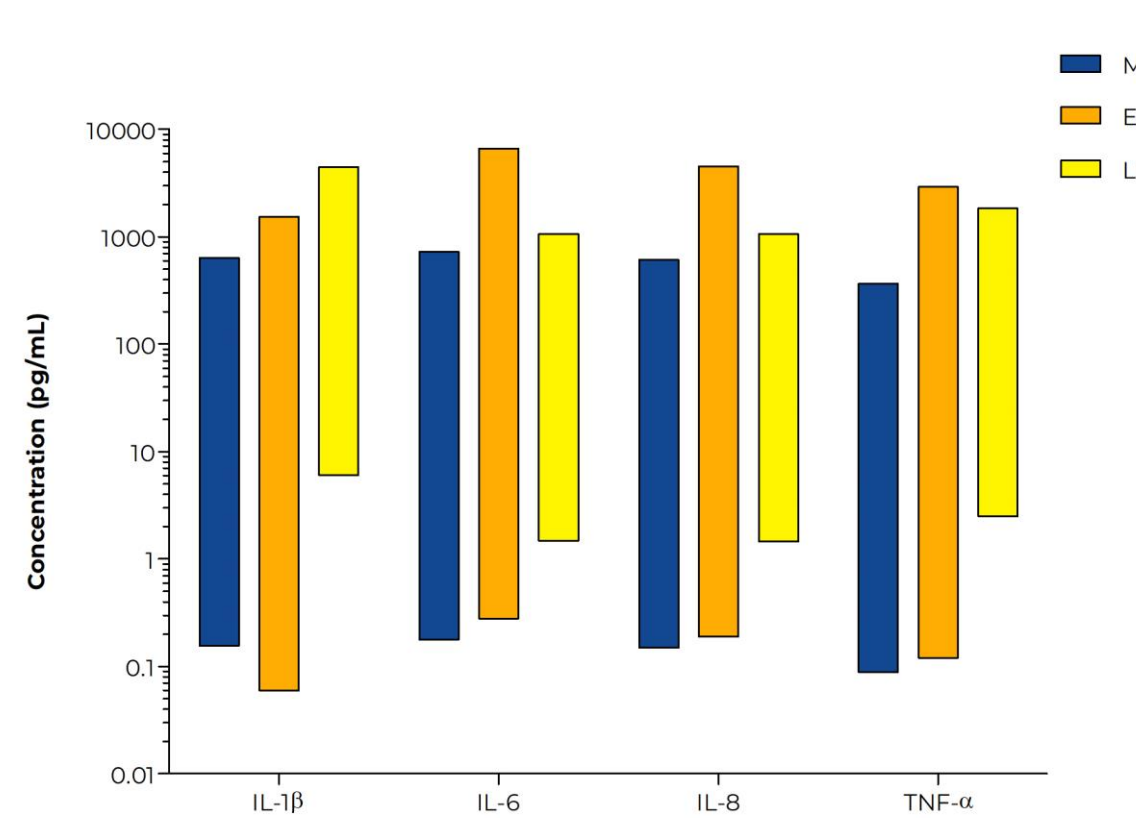
LBA platform	Biomarker	Parameter
<ul style="list-style-type: none"> MSD MESO QuickPlex SQ 120 (MSD) Ella™ Automated Immunoassay System (Ella) Luminex™ 200 Instrument System (Luminex) 	CRS* panel <ul style="list-style-type: none"> IL-1β IL-6 IL-8 TNF-α <small>* Cytokine release syndrome</small>	<ul style="list-style-type: none"> Sensitivity Performance Relative Performance Comparison to NIBSC standards
<ul style="list-style-type: none"> MSD MESO QuickPlex SQ 120 (MSD) Quanterix Simoa HD-X™ Automated Immunoassay Analyzer (Simoa HD-X) Fujirebio LUMIPULSE® G1200 Analyzer (Lumipulse) 	Neurotoxicity <ul style="list-style-type: none"> NfL 	<ul style="list-style-type: none"> Sensitivity Precision Relative Performance

How?



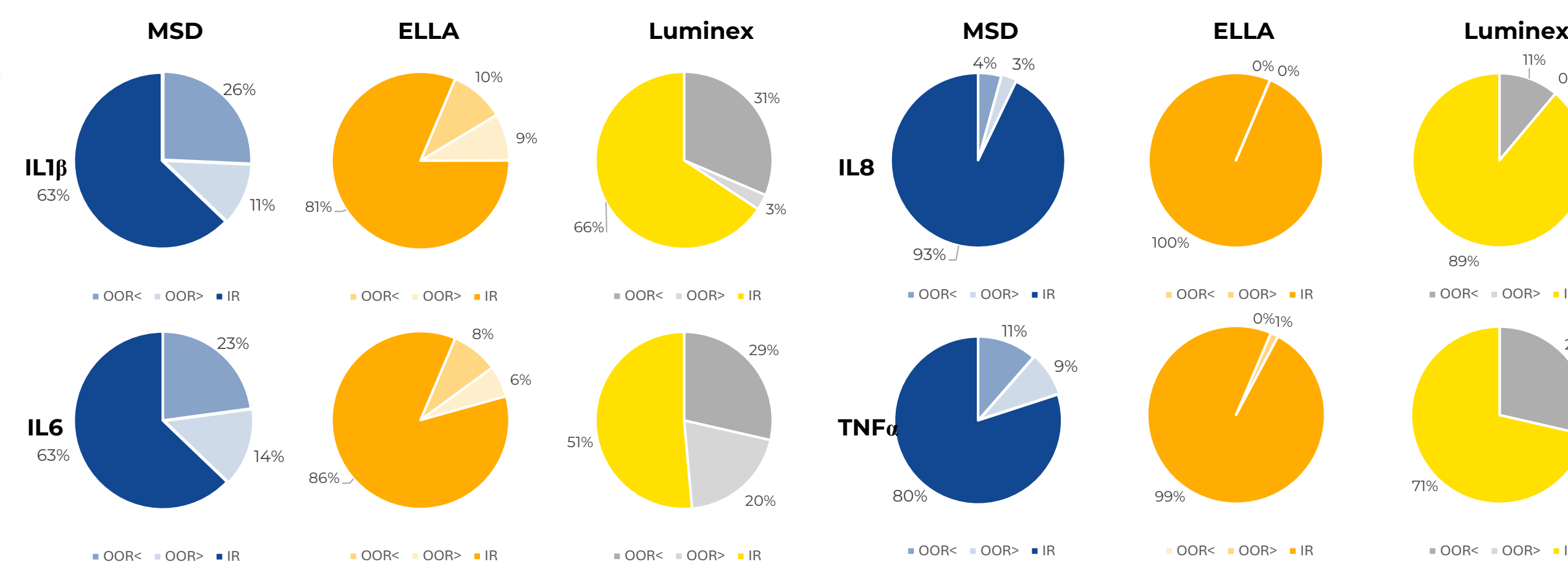
4 CYTOKINES, 3 LBA PLATFORMS

I. Dynamic range



- Large variation in cytokine levels → **ELLA & MSD** (dynamic range)
- Low cytokine levels → **ELLA & MSD** (highest sensitivity)
- High cytokine levels → **ELLA & Luminex** (highest ULOQ)

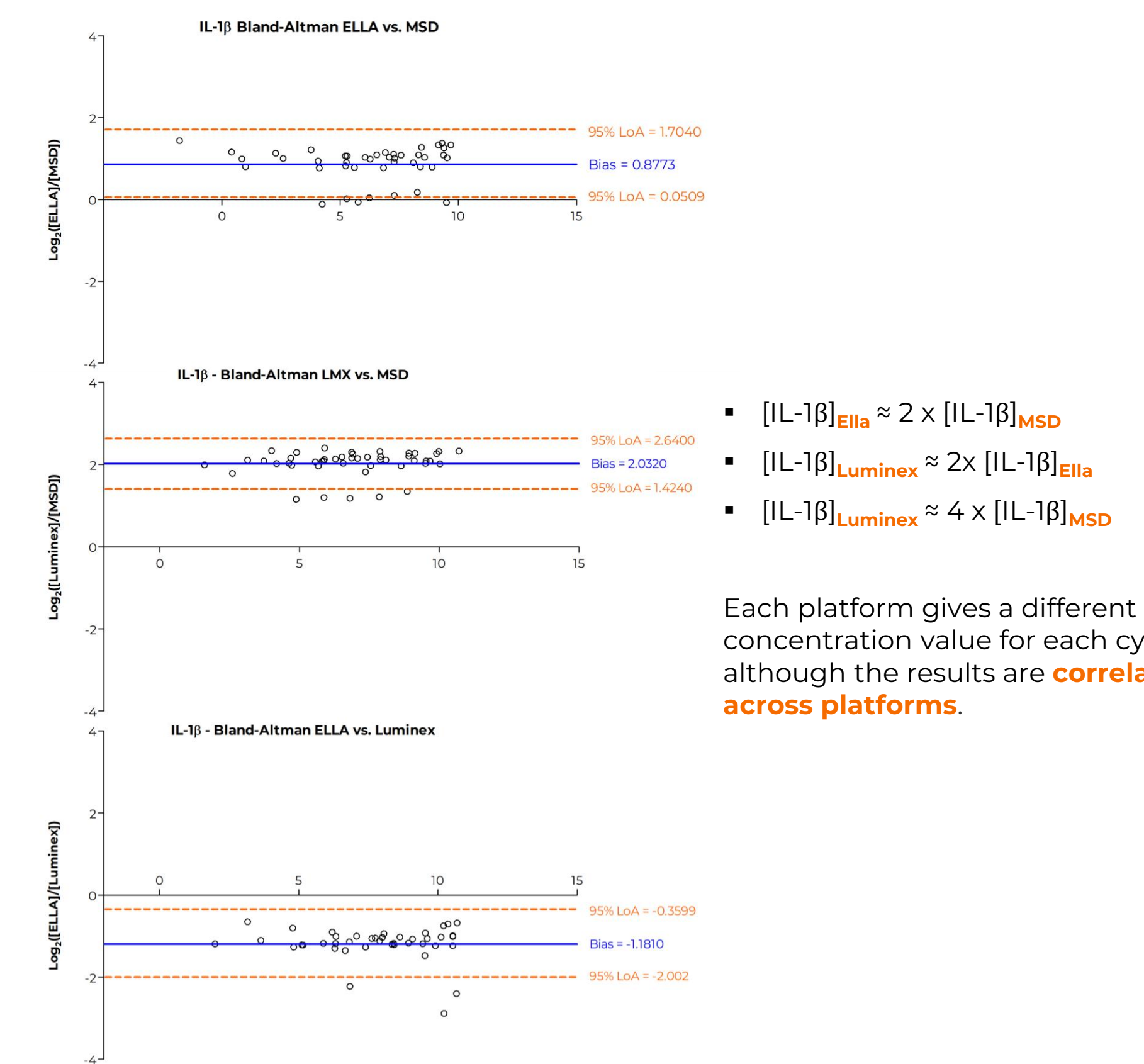
II. Proportion of samples within assay range



IR = In Range
OOR> = Out Of Range superior = above the ULOQ
OOR< = Out Of Range inferior = below the LLOQ

- OOR< → No result
- OOR> → Retest at a higher dilution

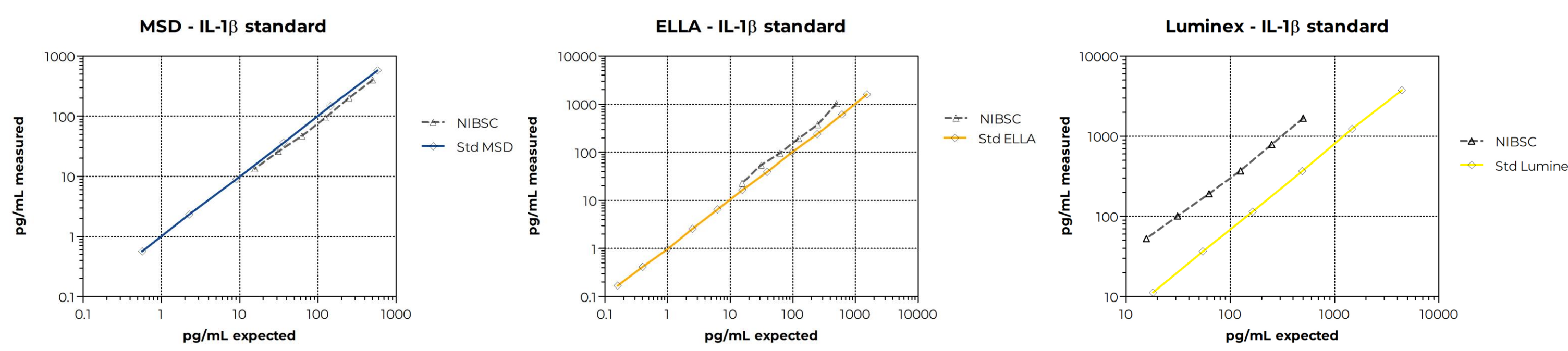
III. Agreement Analysis



- $[IL-1\beta]_{Ella} \approx 2 \times [IL-1\beta]_{MSD}$
- $[IL-1\beta]_{Luminex} \approx 2 \times [IL-1\beta]_{Ella}$
- $[IL-1\beta]_{Luminex} \approx 4 \times [IL-1\beta]_{MSD}$

Each platform gives a different concentration value for each cytokine, although the results are **correlated across platforms**.

IV. How kit calibrators compare to International Standards



NIBSC standards are spiked in plasma

- IL-1β levels measured with **MSD, ELLA & Luminex** correlate with NIBSC values
- The best correlation is observed for **MSD**. **Luminex** exhibits the highest bias (see Agreement Analysis in III.)
- This result is cytokine-dependent (see table in V.)

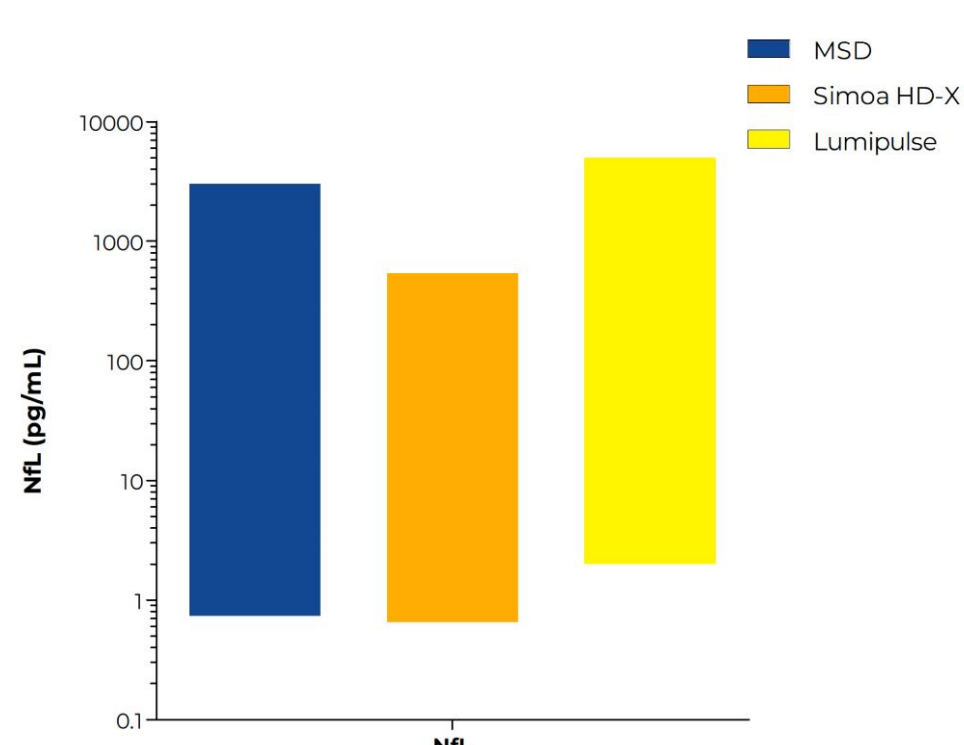
V. Overall comparisons for all four cytokines

		IL-1β	IL-6	IL-8	TNF-α
Mean bias between platforms	ELLA vs. LMX	0.44	0.89	0.83	1.21
	ELLA vs. MSD	1.84	1.99	0.89	2.80
	LMX vs. MSD	4.09	2.24	0.94	1.46
Mean % accuracy of international standards spiked in plasma	LMX	319.0%	71.0%	30.3%	101.2%
	MSD	79.5%	57.7%	53.2%	103.2%
	ELLA	163.50%	89.0%	24.7%	125.2%

- For all cytokines, a different value is provided by each platform
- The results are correlated across platforms
- The results correlate with NIBSC to a variable degree depending on the cytokine

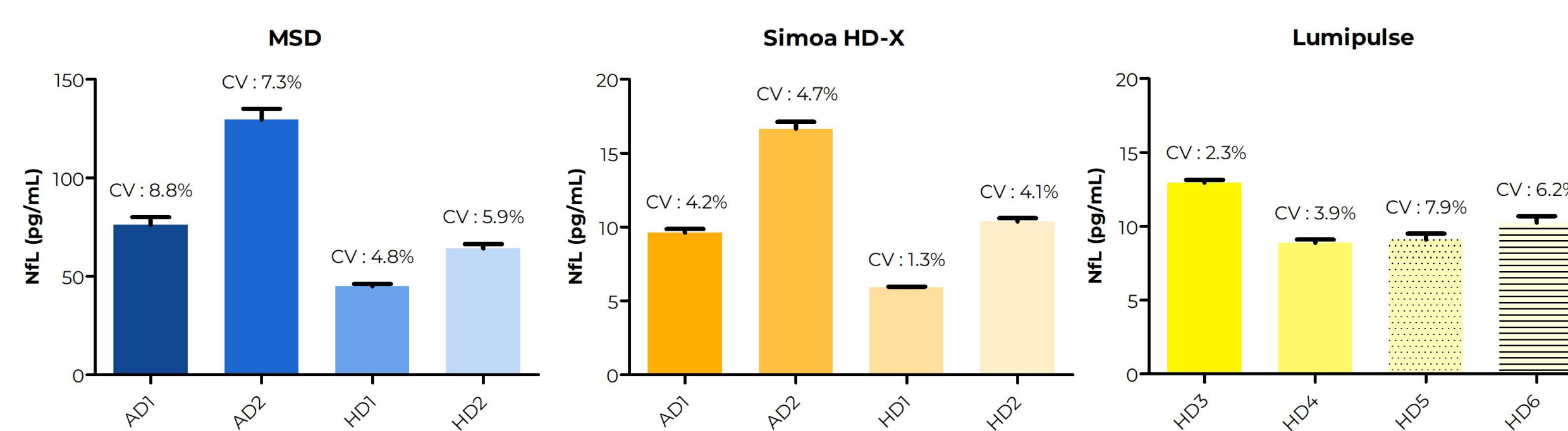
3 LBA PLATFORMS FOR NFL QUANTIFICATION

I. Dynamic range



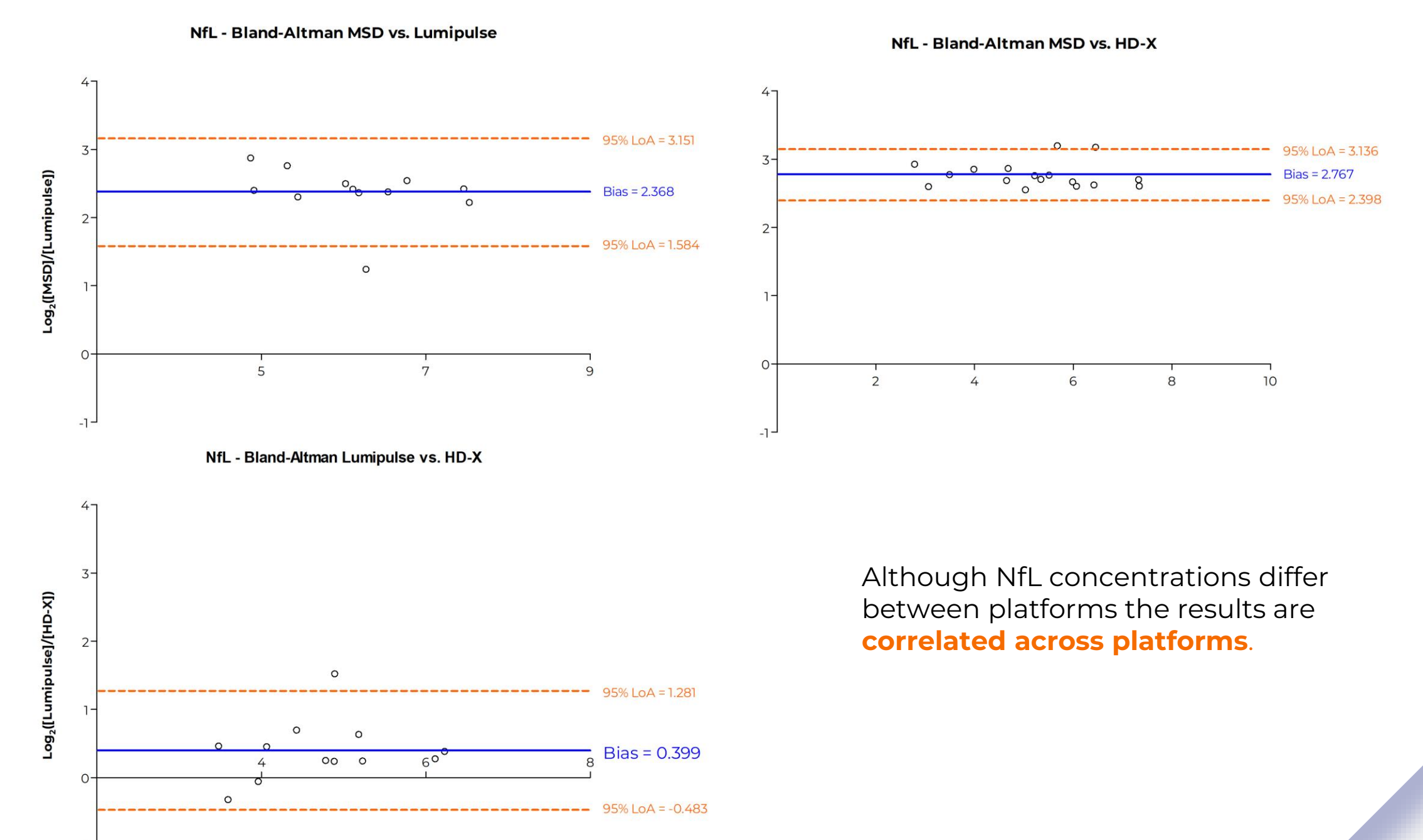
- Large variation in NFL levels → **MSD** (dynamic range)
- Low NFL levels → **Simoa HD-X** (highest sensitivity)
- High NFL levels → **Lumipulse** (highest ULOQ)

II. Intra-run precision



- Intra-run precision (n=3) was assessed using plasma from Healthy individuals (HD) or individuals with Alzheimer's disease (AD)
- CV < 10%, indicating **consistent results**

III. Agreement Analysis



Although NfL concentrations differ between platforms the results are **correlated across platforms**.

Conditions to set the project for success

- Platform-specific differences to be **carefully considered**.
- Selecting an LBA platform at the **project's outset** is crucial for the success of the project.
- Final choice to be based on the **Context of Use** of the biomarkers, i.e. on **multiplexing capacity**, expected **concentration range**, **sensitivity**, **throughput**, **sample volume**, and **scalability** of the assay.
- If switching platforms during clinical development, using **reference material** is essential for enabling cross-platform data comparison.

