High-Sensitivity Total IL-15 Quantification Using a Physiologically Relevant ELISA

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Background & Rationale

IL-15 regulates NK and CD8⁺ T-cell homeostasis and exists mainly as a heterodimeric IL-15/IL-15Rα complex. Most ELISAs quantify only free IL-15, missing the physiologic complex. PBL's assay uses a HEK293-expressed IL-15/IL-15Rα standard, capturing both free and receptor-bound IL-15, enabling accurate total IL-15 quantification in serum, plasma, and TCM.

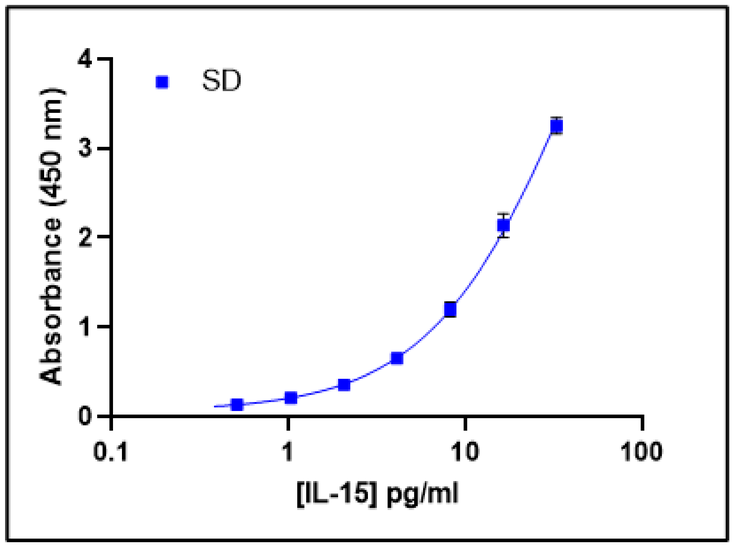

Fig 1. Representative standard curve demonstrating assay sensitivity (LLOQ = 0.5 pg/mL).

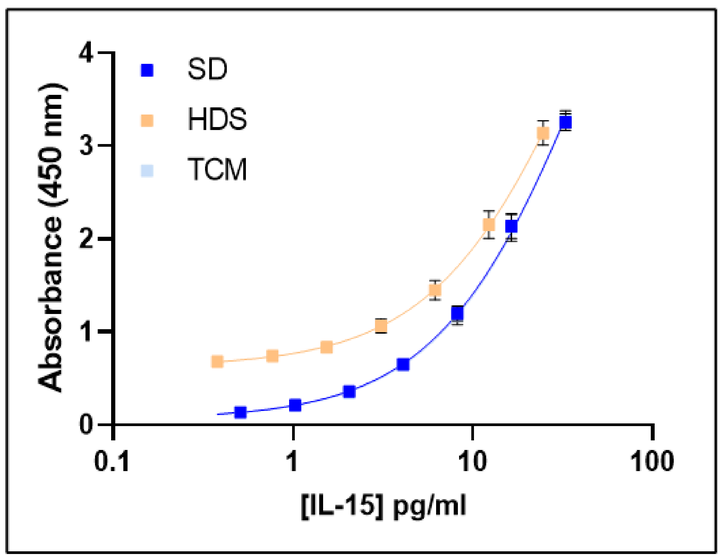

Fig 2: Representative standard curves in various matrices. (Note: SD and TCM curves overlap).

Physiological Relevance of Total IL-15 Measurement

Circulating IL-15 predominantly exists in complex with IL-15Rα, forming a stable heterodimer that extends cytokine half-life and governs trans-presentation to NK and CD8⁺ T cells. Quantifying total IL-15 (free + receptor-bound) provides a more biologically meaningful assessment of immune tone than measuring free cytokine alone. This physiologically relevant approach better reflects cytokine activity in translational and clinical studies targeting IL-15 pathways.

Specificity

No cross-reactivity detected with human IL-2, IL-4, IL-7, IL-9, IL-21, IFN-α, IFN-β1a, IFN-γ, IFN-ω; cynomolgus IFN-α2; cynomolgus/rhesus IFN-α; mouse IL-15, IFN-αA, IFN-β, IFN-γ; rat IL-15, IFN-α1, IFN-α14, IFN-β, IFN-γ; bovine IFN-γ; or pig IFN-α.

Precision

Intra-Assay Precision						
Sample	**1**	**2**	**3**	**4**	**5**	**6**
N	26	26	26	26	26	26
Mean (pg/ml)	4.17	4.42	4.20	2.81	3.85	3.87
Std. Dev.	0.18	0.09	0.18	0.10	0.13	0.13
CV (%)	4.4	1.9	4.3	3.6	3.3	3.3

Inter-Assay Precision							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Mean (pg/ml)	4.52	3.55	4.47	2.87	3.54	12.55	3.68
Std. Dev.	0.24	0.16	0.17	0.16	0.19	0.61	0.13
CV (%)	5.3	4.6	3.7	5.4	5.4	4.8	3.4

Inter-Batch Precision							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Lot 1 (pg/ml)	5.26	3.97	5.12	3.24	4.02	13.94	4.18
Lot 2 (pg/ml)	5.16	3.94	5.09	3.20	3.97	13.58	4.26
Lot 3 (pg/ml)	4.80	3.62	4.71	2.85	3.66	12.77	3.86
Mean (pg/ml)	5.07	3.84	4.97	3.10	3.88	13.43	4.10
Std. Dev.	0.24	0.19	0.22	0.21	0.20	0.60	0.21
CV (%)	4.7	5.0	4.5	6.8	5.1	4.5	5.1

Intermediate Precision							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
N	27	27	27	27	27	27	27
Mean (pg/ml)	5.07	3.84	4.97	3.10	3.88	13.43	4.05
Std. Dev.	0.63	0.49	0.57	0.38	0.45	1.30	0.40
CV (%)	12.5	12.9	11.5	12.4	11.6	9.7	9.9

Table 1. Precision was assessed under multiple conditions, including repeatability within runs, reproducibility across runs, operator variability, and reagent lot differences. Across all studies, %CV values remained ≤ 12 % across all precision assessment, supporting excellent repeatability and reliability of the total IL-15 ELISA.

Parallelism

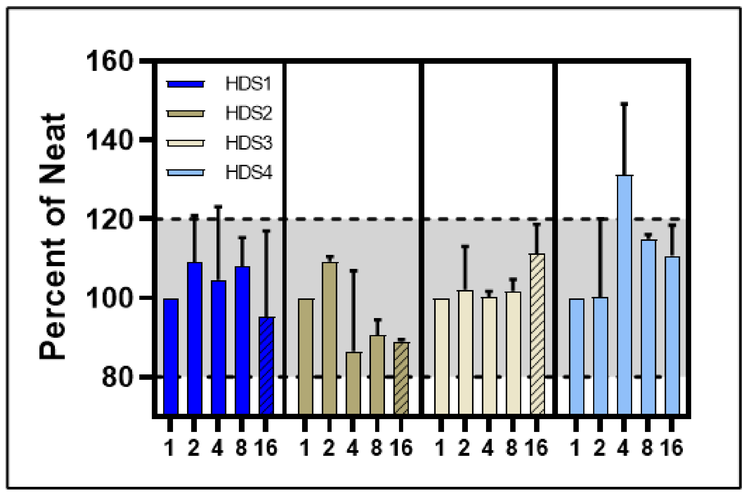

Fig 3. Parallelism was demonstrated for endogenous IL-15 in human serum samples, confirming accurate quantification of native IL-15 across dilution levels.

Performance Comparison

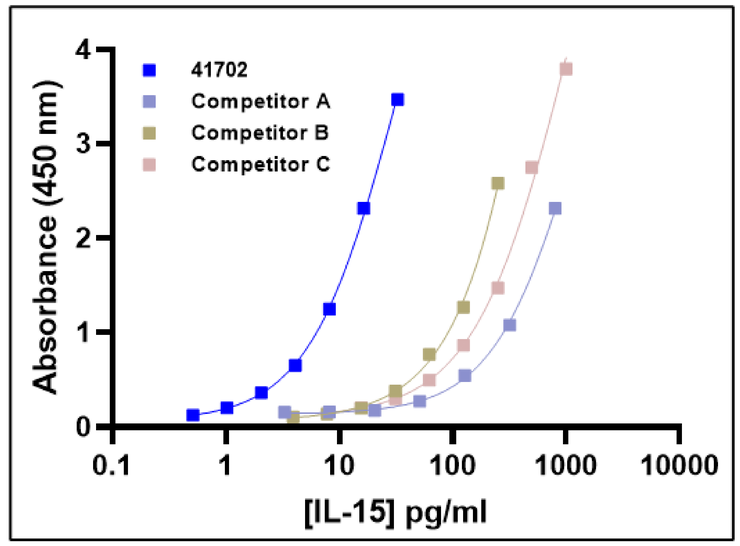

Fig 4. PBL's HEK-derived IL-15/IL-15Rα complex was assayed on three commercial IL-15 kits. Competitor ELISAs showed weak or absent response, indicating limited recognition of physiological IL-15 complex.

Conclusion

A physiologically relevant IL-15 standard enabled high assay sensitivity, strong precision, and reliable endogenous IL-15 measurement in serum and plasma. Parallelism confirmed accurate quantification across dilutions. Competing ELISAs showed limited detection of the IL-15/IL-15Rα complex, highlighting the advantage of a biologically relevant standard for translational studies.

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