



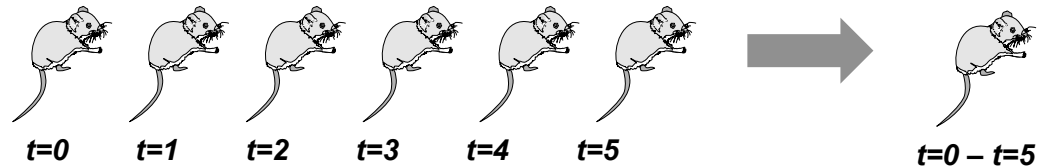
chimera biotec
ultra sensitive immunoassays

***Ultra-sensitive biomarker quantification
in 1 μ l to 5 μ l sample volume***

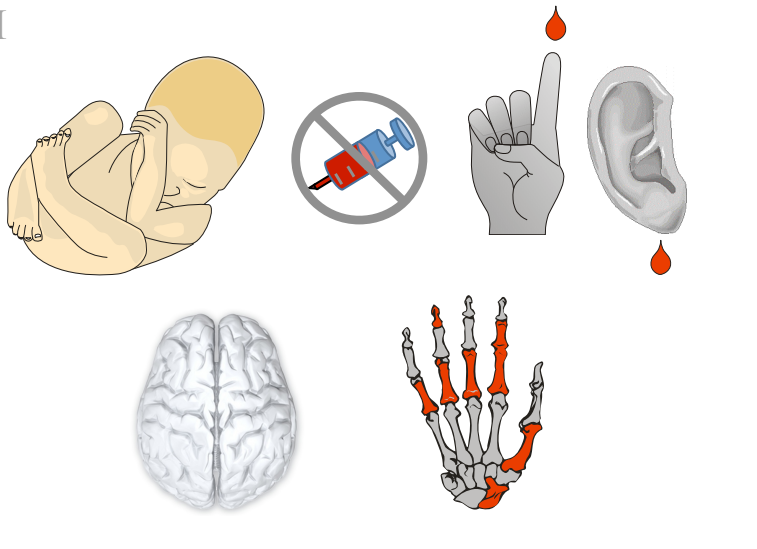
Dr. Beena Punnamoottil
Sen. Project Manager
Chimera Biotec GmbH
punnamoottil@chimera-biotec.com

Microsampling

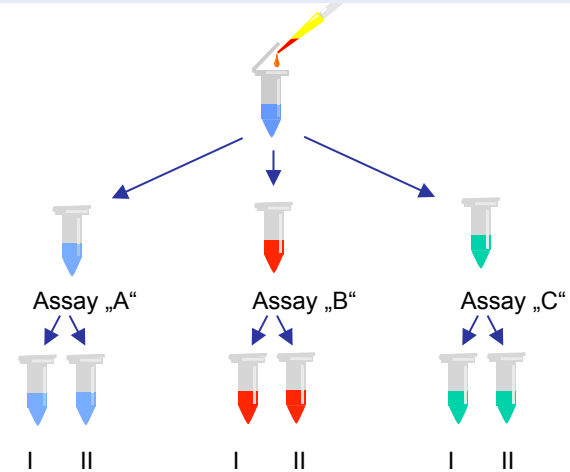
I



II

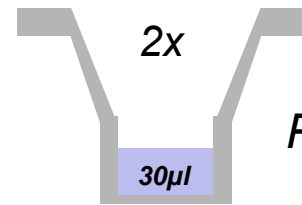
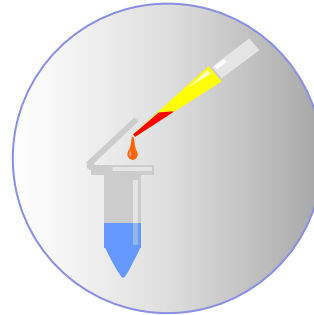
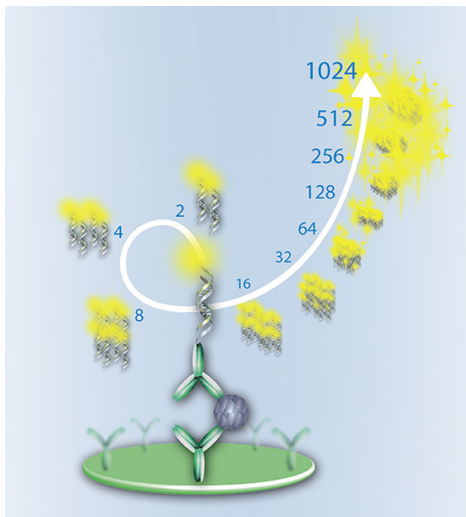


III



- I Pre-Clinical PK → *composite sampling vs. serial sampling from one animal*
- II Only small sample size available → *limited biofluids (e.g. liquor, synov. fluid...)*
Non-invasive sample drawing desirable → *prenatal, neonatal, pediatric*
- III One sample → *multiple biomarker analysis*

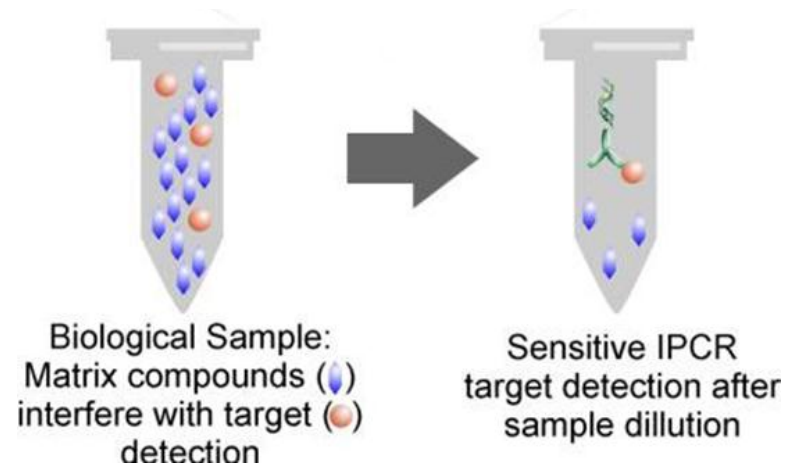
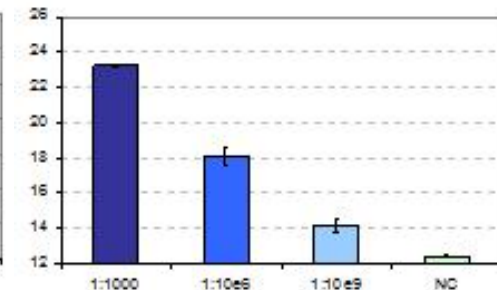
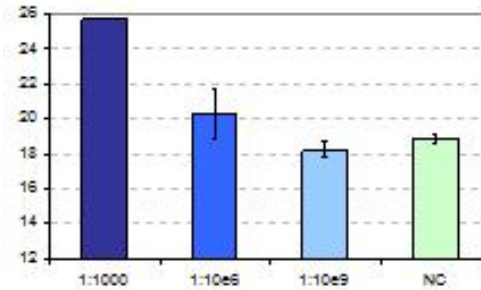
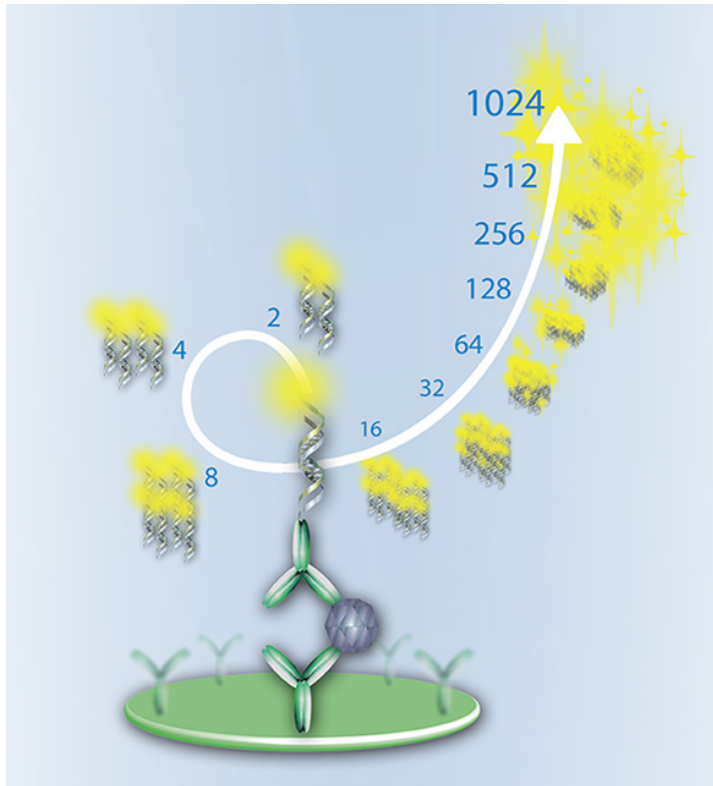
Low sample volume **vs.** High assay sensitivity ?



Pipetting volume = 70µl

The lower the neat sample volume, the higher the required sample dilution

Biomarker support feasible ?

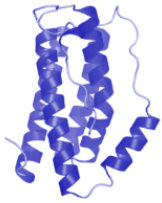


Imperacer®:

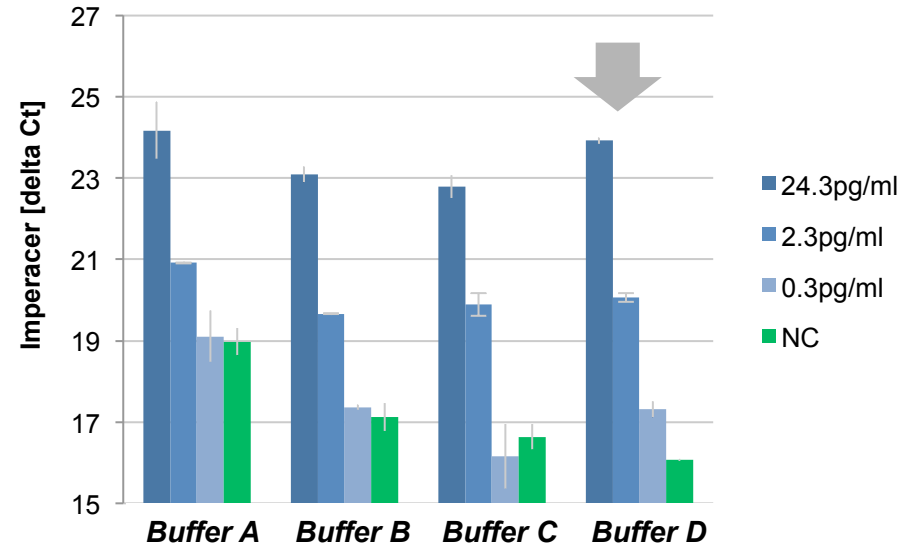
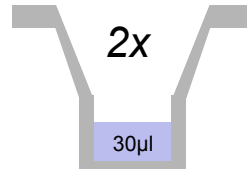
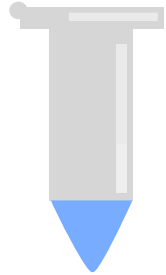
Exponential signal amplification

- ✧ *fine-tune sensitivity vs. sample consumption*
- ✧ *tolerate higher sample dilutions*
- ✧ *minimize neat sample volume*

Is biomarker quantification possible from less than 10µl neat sample?

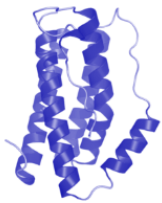


Interleukin 6

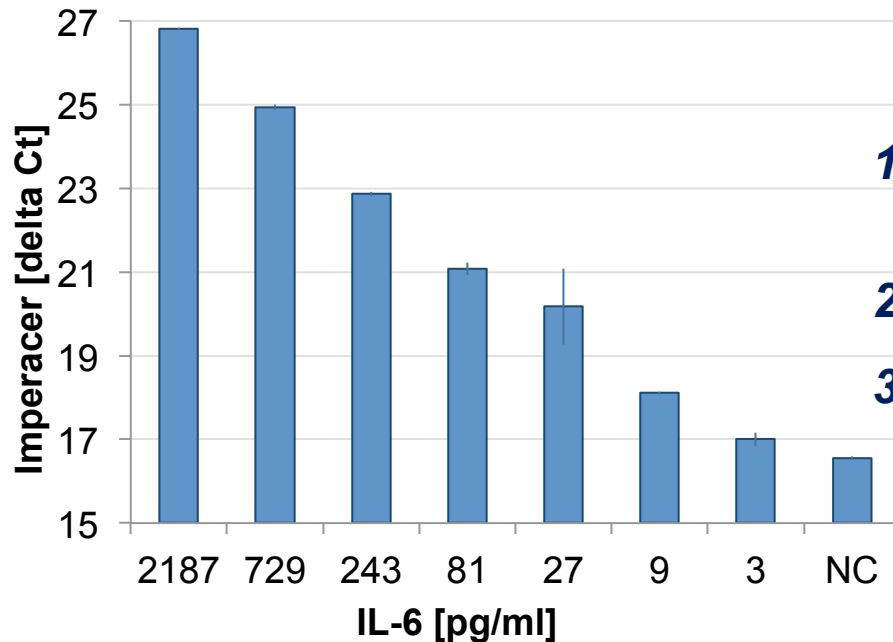
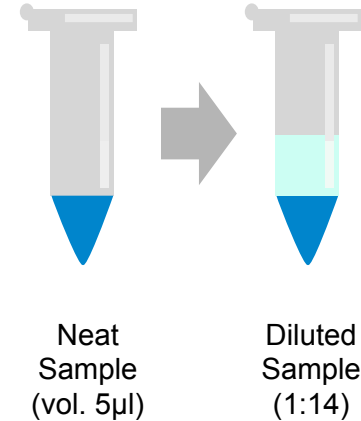
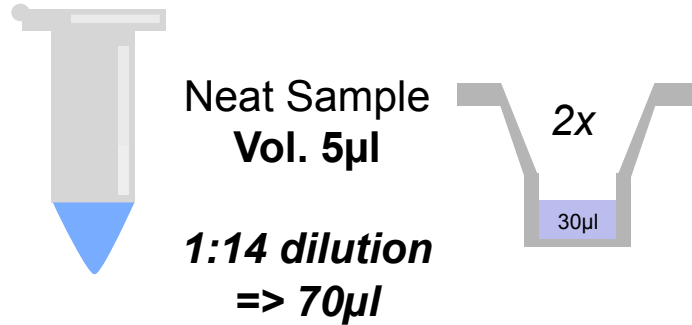


1. Fine-tuning of sample dilution in optimal buffer

Is biomarker quantification possible from less than 10µl neat sample?

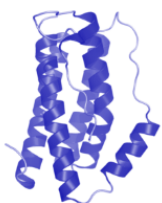


Interleukin 6

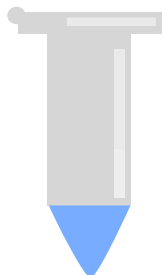


1. Fine-tuning of sample dilution in optimal buffer
2. Fine-tuning of quantitative assay range
3. Assay sensitivity: 3pg/ml - 2187pg/ml

Is biomarker quantification possible from less than 10µl neat sample?

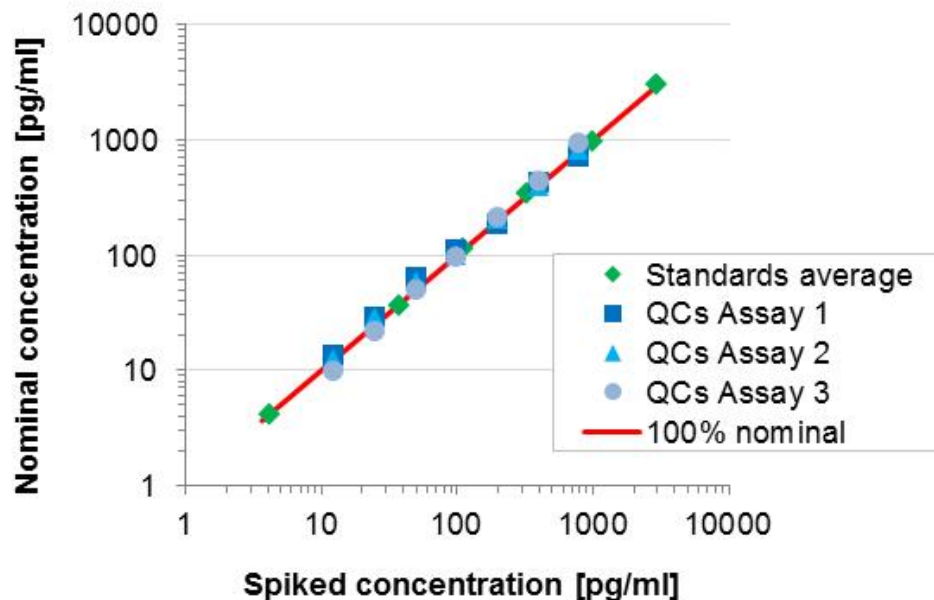


Interleukin 6



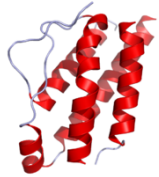
Neat Sample
Vol. 5µl

1:14 dilution
=> 70µl

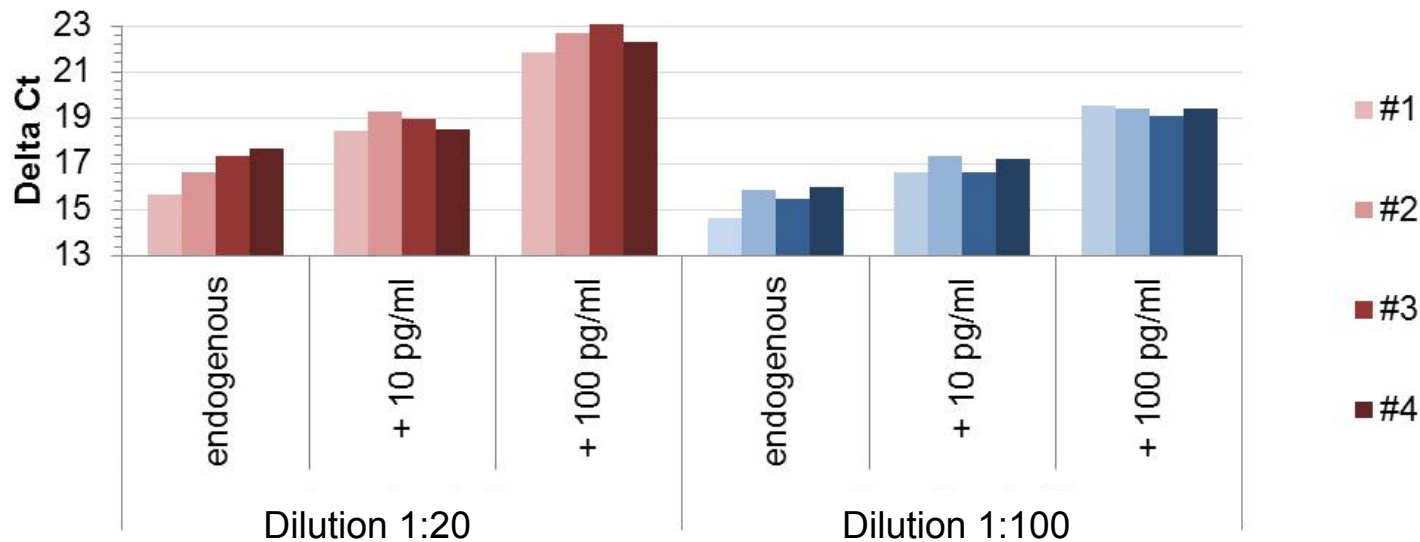
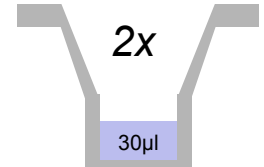


	nominal [pg/ml]	Calc. Conc. average	inter-assay		intra-assay	
			CV%	%RE	CV%	%RE
Standards	3000	3066.07	2.1	2.20	4.9 - 7.6	0.7 - 3.7
	1000	960.37	5.6	3.96	7.7 - 10.1	0.2 - 7.7
	333.33	342.18	8.1	2.66	8.8 - 9.2	3.2 - 8.5
	111.11	112.51	5.7	1.26	1.4 - 9.3	2.8 - 5.4
	37.037	36.12	2.1	2.49	7.2 - 13.7	1 - 4
	12.35	12.45	7.2	0.78	1.6 - 2.5	4.3 - 5.9
QCs	4.12	4.15	3.8	0.68	9.3 - 9.3	2 - 3.3
	800	809.41	14.5	1.18	0.3 - 15.3	0.1 - 16.5
	400	414.17	5.8	3.54	0.9 - 10.1	3.2 - 8.3
	200	198.92	5.9	0.54	0.7 - 9.7	2 - 7.3
	100	100.94	8.3	0.94	1.4 - 10.7	2.1 - 10.5
	50	56.87	11.8	13.75	1.3 - 13.7	1.5 - 23.6
	25	26.02	15.3	4.07	1.6 - 16.9	12.2 - 14.3
	13	11.80	16.1	5.61	9.3 - 13.2	1.4 - 22.4

How low can we go to trade off sample consumption vs. sensitivity?



Interleukin 2



How low can we go to trade off sample consumption vs. sensitivity?



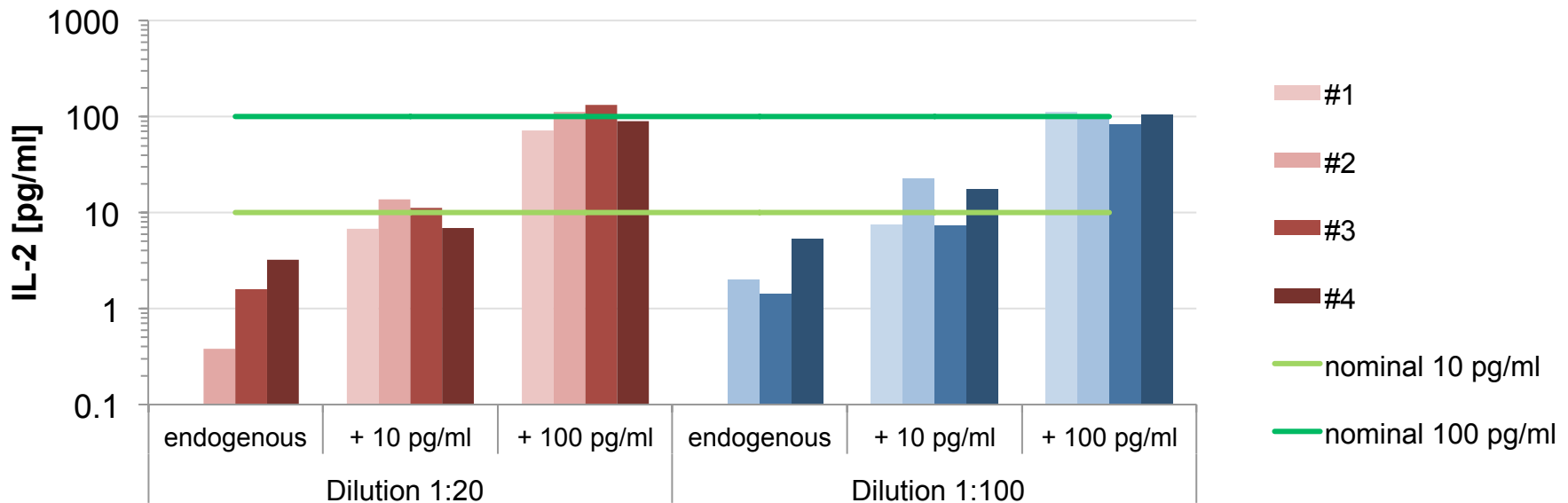
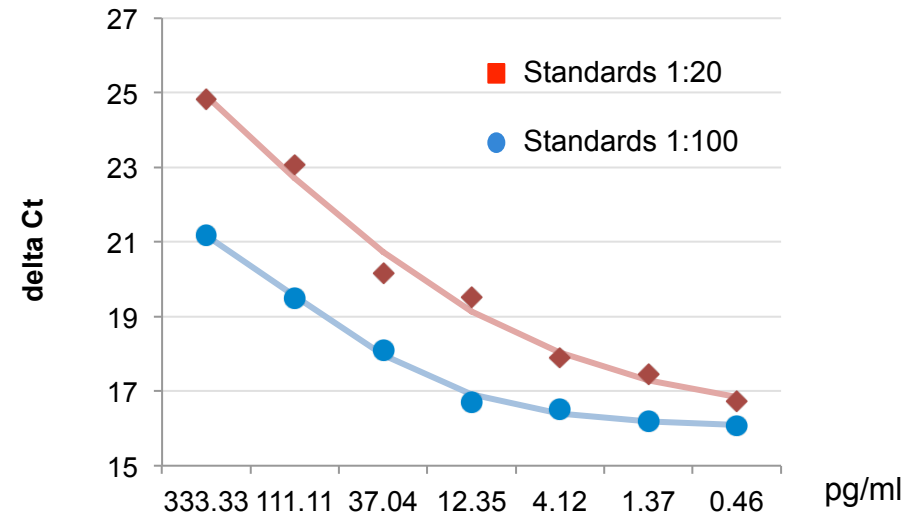
Interleukin 2



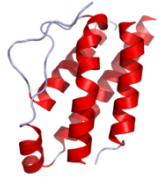
Neat Sample
Vol. 3.5µl



Neat Sample
Vol. 0.7µl



How low can we go to trade off sample consumption vs. sensitivity?



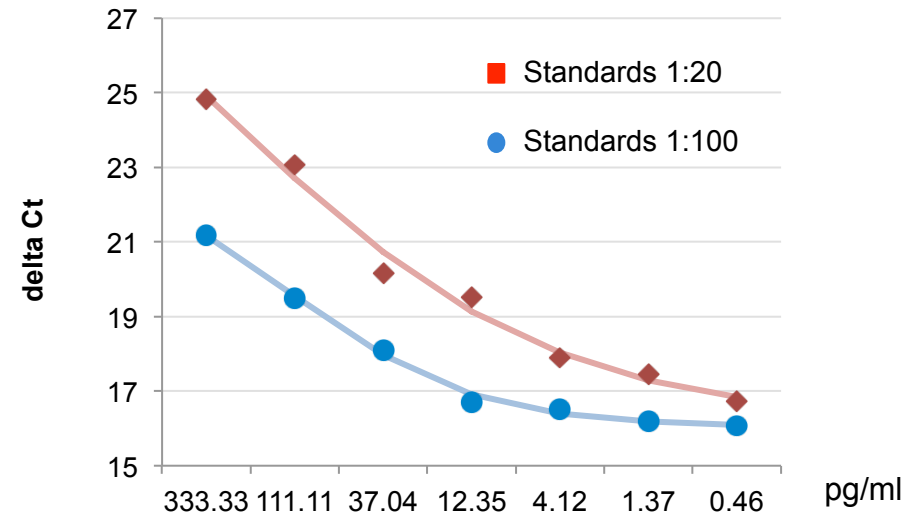
Interleukin 2



Neat Sample
Vol. 3.5µl

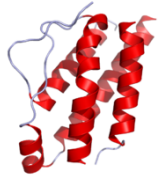


Neat Sample
Vol. 0.7µl



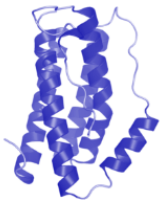
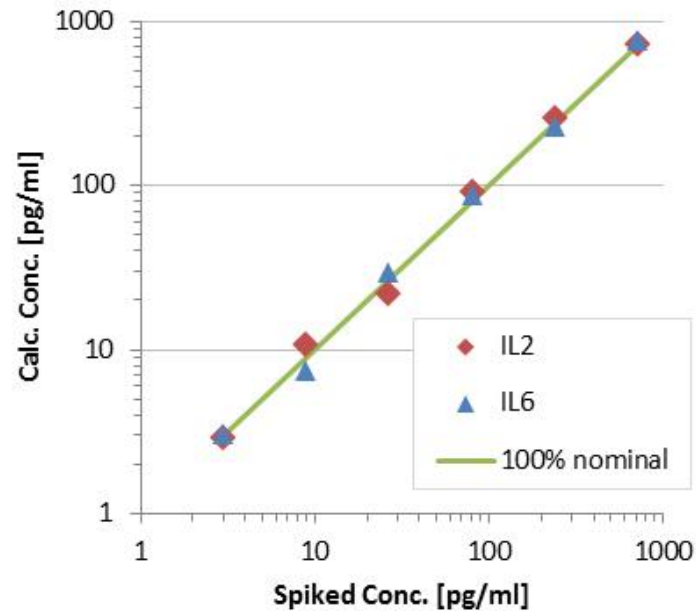
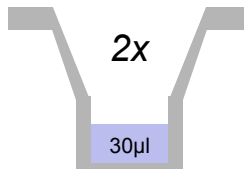
Biomarker quantification is possible with less than 5µl neat sample volume!

Multiple target quantification in less than 5µl neat sample?



Interleukin 2

Neat Sample
Vol. 2.5µl
1:28 dilution
=> 70µl

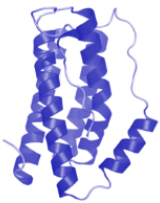


Interleukin 6

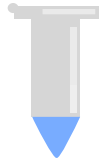
Neat Sample
Vol. 1µl
1:70 dilution
=> 70µl

nominal [pg/ml]	Calc. Conc [pg/ml]		Recovery [%RE]	
	IL2	IL6	IL2	IL6
729	716.8	763.7	1.7	4.8
243	253.8	224.7	4.4	7.5
81	91.6	87.0	13.1	7.4
27	21.6	29.4	19.9	9.0
9	10.6	7.4	17.5	17.3
3	2.9	3.0	4.4	1.1
NC	-	-	-	-

Multiple target quantification in less than 5µl neat sample?

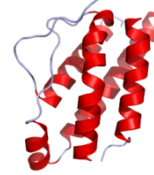


Interleukin 6

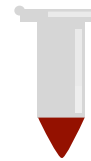


Neat Sample
Vol. 1µl

1:70 dilution
=> 70µl

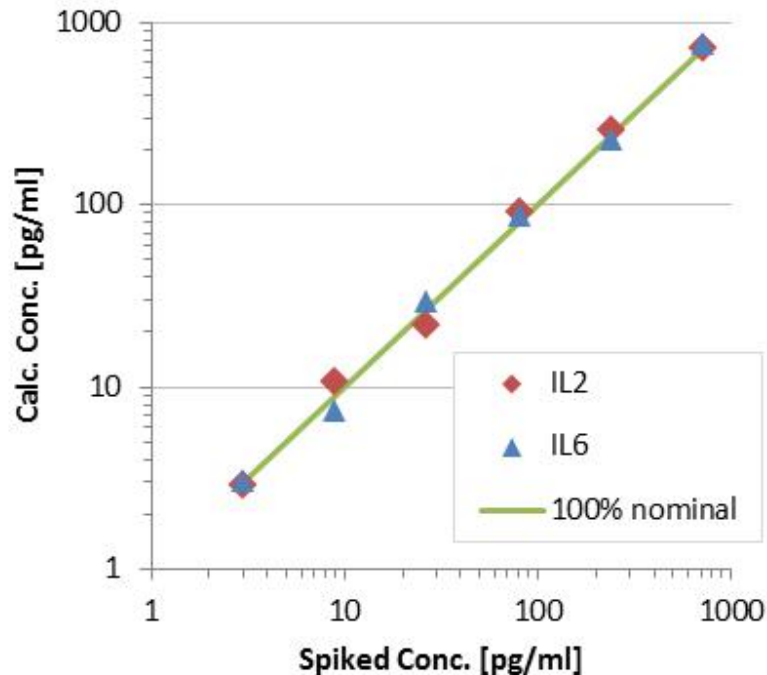


Interleukin 2



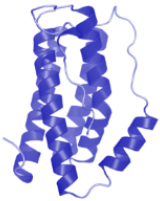
Neat Sample
Vol. 2.5µl

1:28 dilution
=> 70µl

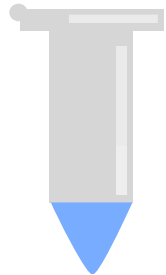


- Preliminary assay range from 3pg/ml to 729pg/ml for IL-2 and IL-6
- Required neat sample volume 2.5µl and 1µl respectively
- 5µl neat sample material is sufficient for multiple target quantification

Multiple target quantification in less than 5µl neat sample?

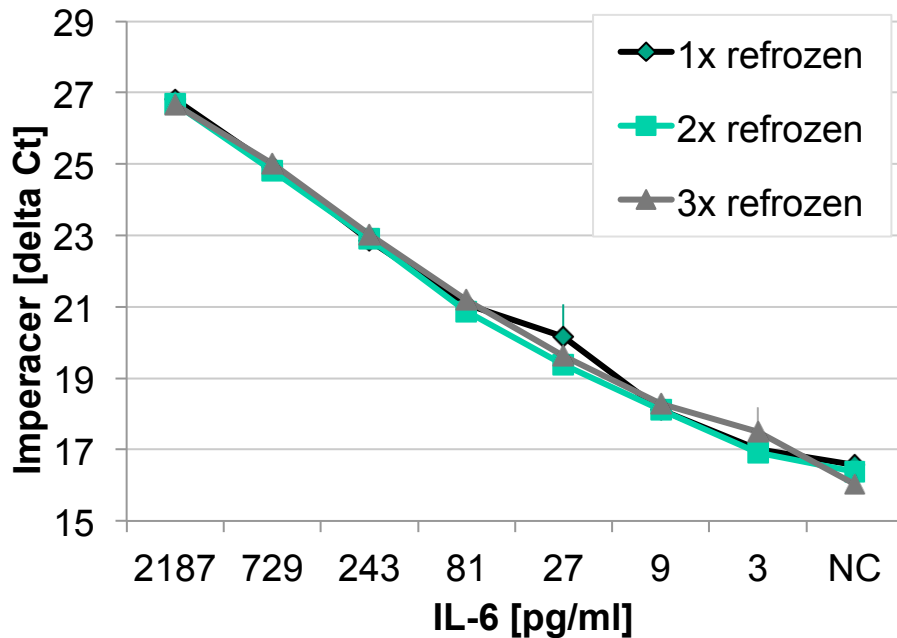
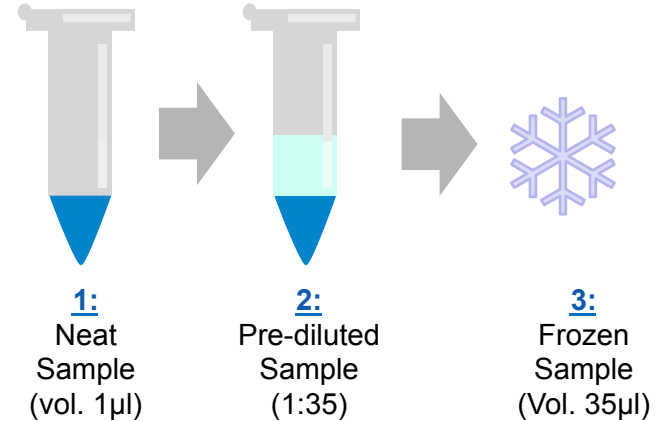


Interleukin 6



Neat Sample
Vol. 1µl

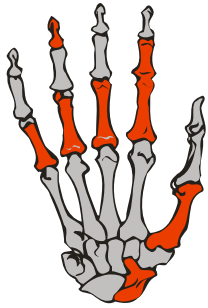
1:35 dilution
=> 35µl



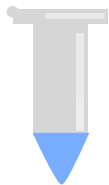
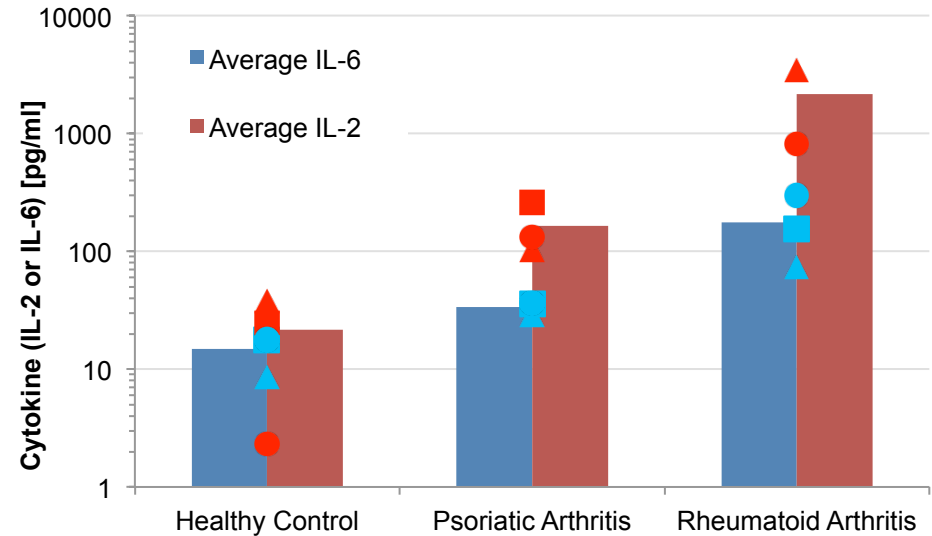
➤ **Pre-diluted sample stability confirmed**

➤ **Assay range: 3pg/ml - 2187pg/ml**

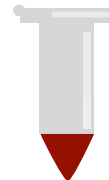
Multiple target quantification in diseased sample material?



- 3 healthy control sera
- 3 psoriatic arthritis sera
- 3 rheumatoid arthritis sera



IL-6:
Neat Sample Vol. 1 μ l
1:70 dilution

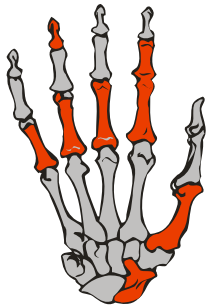


IL-2:
Neat Sample Vol. 2.5 μ l
1:28 dilution

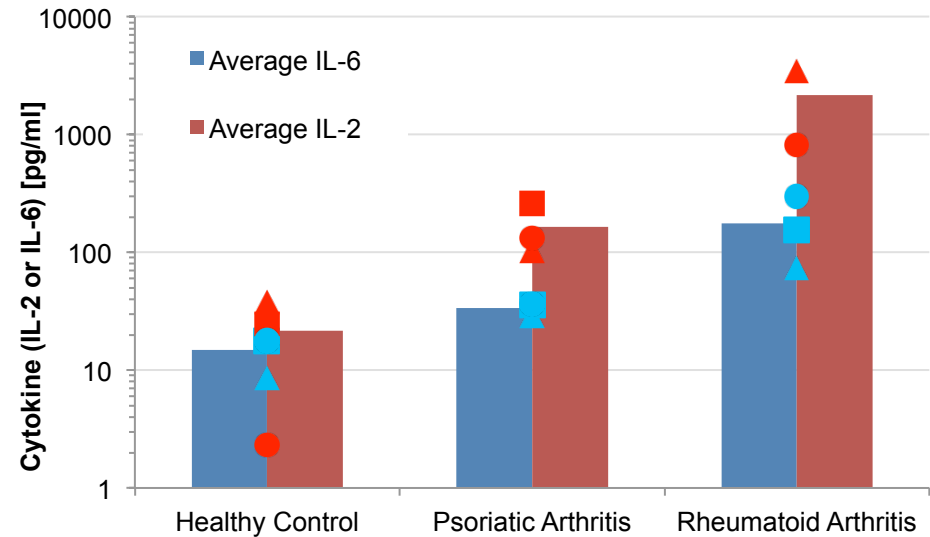
Each target quantified under best assay conditions in parallel (Imperacer[®] polyplex):

- Different assay strategies: Sequential (IL-6) & Combined incubation (IL-2)
- Imperacer[®] polyplex assay: Sample analysis in parallel
- Total sample: **Vol. \leq 5 μ l** for both targets in double determination

Multiple target quantification in diseased sample material?



- 3 healthy control sera
- 3 psoriatic arthritis sera
- 3 rheumatoid arthritis sera



Imperacer[®] polyplex assays for microsamples:

- ***Diseased individuals reveal significantly higher concentrations of tested cytokines***
- ***Individual disease status evaluation possible***
- ***Polyplex assays enable multiple target quantification with 5µl neat sample only***

Is biomarker quantification possible with less than 10 μ l neat sample?

- ✓ Yes, low level target quantification with **Vol. \leq 5 μ l** neat sample

How low can we go in regard to neat sample volume?

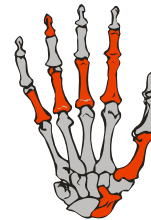
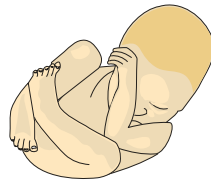
- ❖ Ultra-sensitive Imperacer[®] & fine tuning of dilution + assay strategy \rightarrow low level target quantification with Vol. \leq 1 μ l neat sample

What is the clinical/diagnostic value?

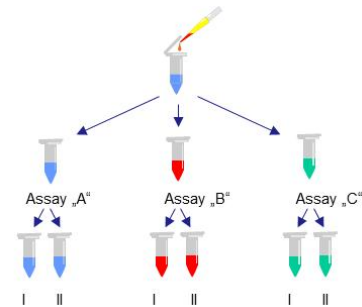
- Multiple target analysis is feasible in microsampling volume
- Biomarker quantification in microsamples can benefit pre-clinical and clinical applications in challenging matrices and low volume samples



Challenging Matrices



Low Volume Samples



Multiple Targets

Imperacer[®]

**Ultra-Sensitive Ligand-Binding Assays
and Method Development**

Thanks to:

Michael Adler

Hendrik Schröder

Mark Spengler

Christian Pieper

Chimera Lab-Team

*Dive deeper
into the proteome*



chimera biotec

ultra sensitive immunoassays

Contact:

Chimera Biotec GmbH

Dr. Beena Punnamoottil

email: punnamoottil@chimera-biotec.com

Phone: + 49 (0) 231 - 9742 840

Fax: + 49 (0) 231 - 9742 844

www.chimera-biotec.com

www.imperacer.com

Imperacer®

**Ultra-Sensitive Ligand-Binding Assays
and Method Development**

Thank you for your attention

*Dive deeper
into the proteome*



chimera biotec

ultra sensitive immunoassays

Contact:

Chimera Biotec GmbH

Dr. Beena Punnamoottil

email: punnamoottil@chimera-biotec.com

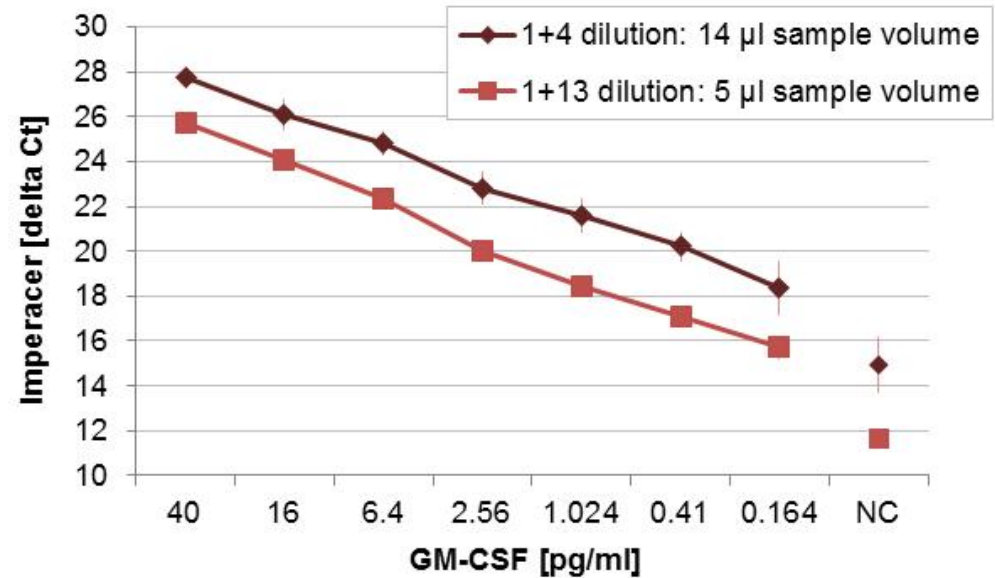
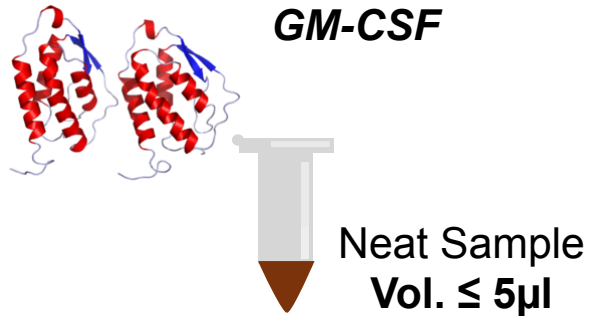
Phone: + 49 (0) 231 - 9742 840

Fax: + 49 (0) 231 - 9742 844

www.chimera-biotec.com

www.imperacer.com

Is biomarker quantification possible with less than 10µl neat sample?

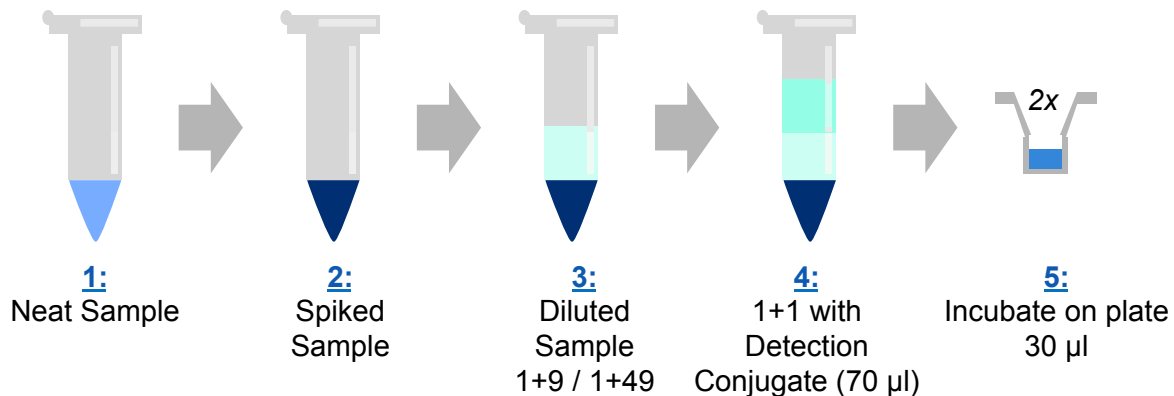


Imperacer[®] assays for microsamples:

- **Best sample dilution buffer for each target-matrix composition**
- **Best incubation strategy for each target-matrix composition**
- **Maintenance of assay sensitivity with 5µl neat sample only**

Assay Strategy

Combined incubation



Sequential incubation

