Comparison of various platforms for Large Molecule analysis

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Introduction of the Imperacer® technology

EBF

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Scientific Director

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- Introduction of PRA and Chimera
- Immuno-PCR technology
- Comparison of assay platforms
- Applications for the Imperacer® technology
- Cytokine assay development and validation
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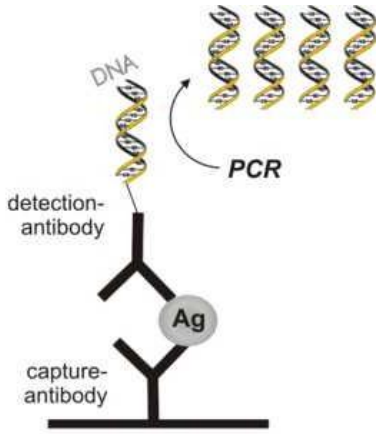
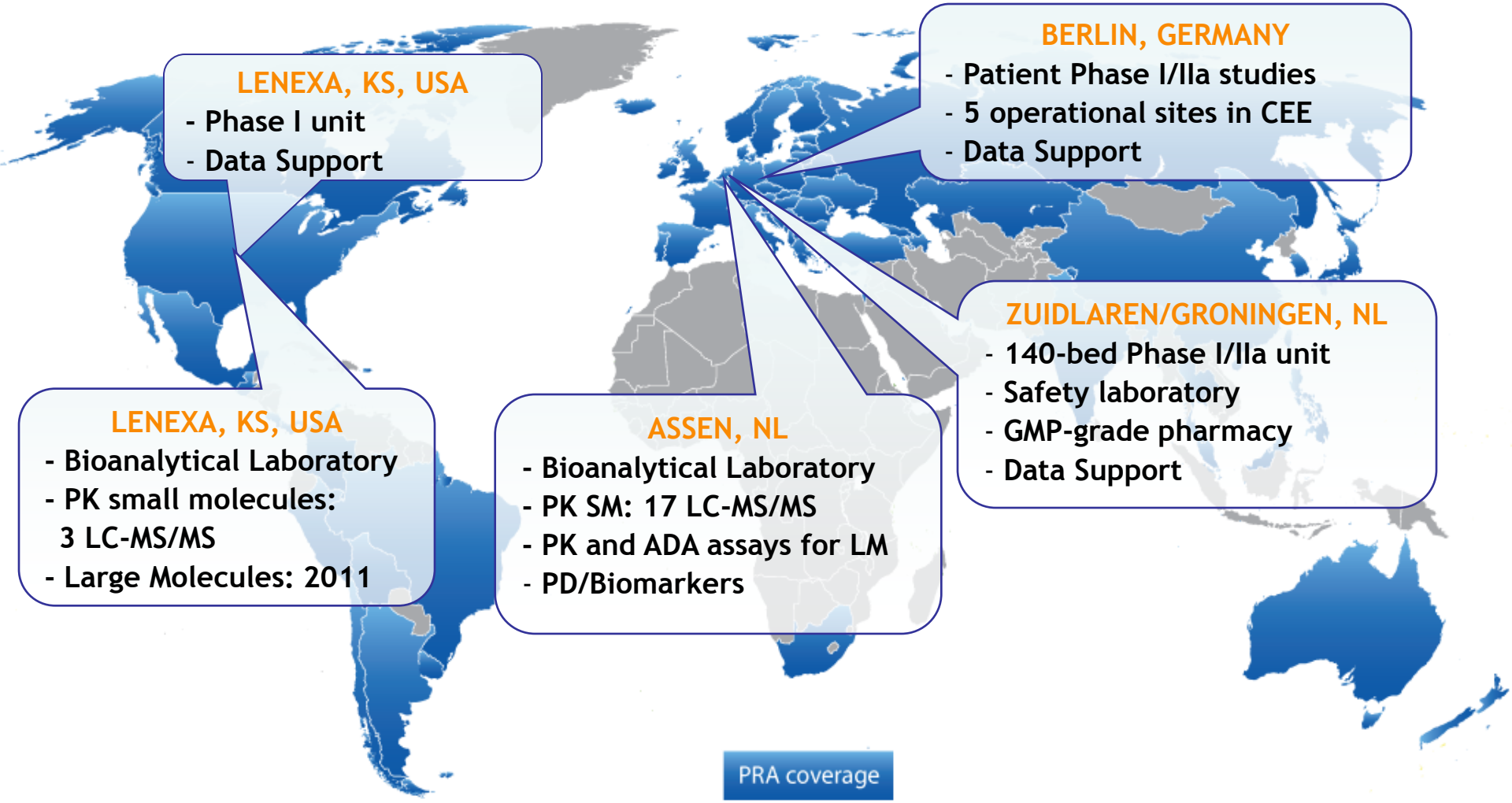


PRA Early Development Services



PRA Clinics and Labs in NL

1. Hospital based Phase-I/IIa unit
   - Microbiology Unit
2. Phase I unit with:
   - Safety Laboratory (fully automated)
   - Microbiology Unit
   - Full GMP pharmacy
   - Dedicated ^{14}C clinic
3. Recruitment and Screening Center
4. Bioanalytical Laboratory (PK, PD, ADA-assays; FACS; Biohazard and Isotope labs)
5. CLL - Central Laboratory services

Supporting Drug Development

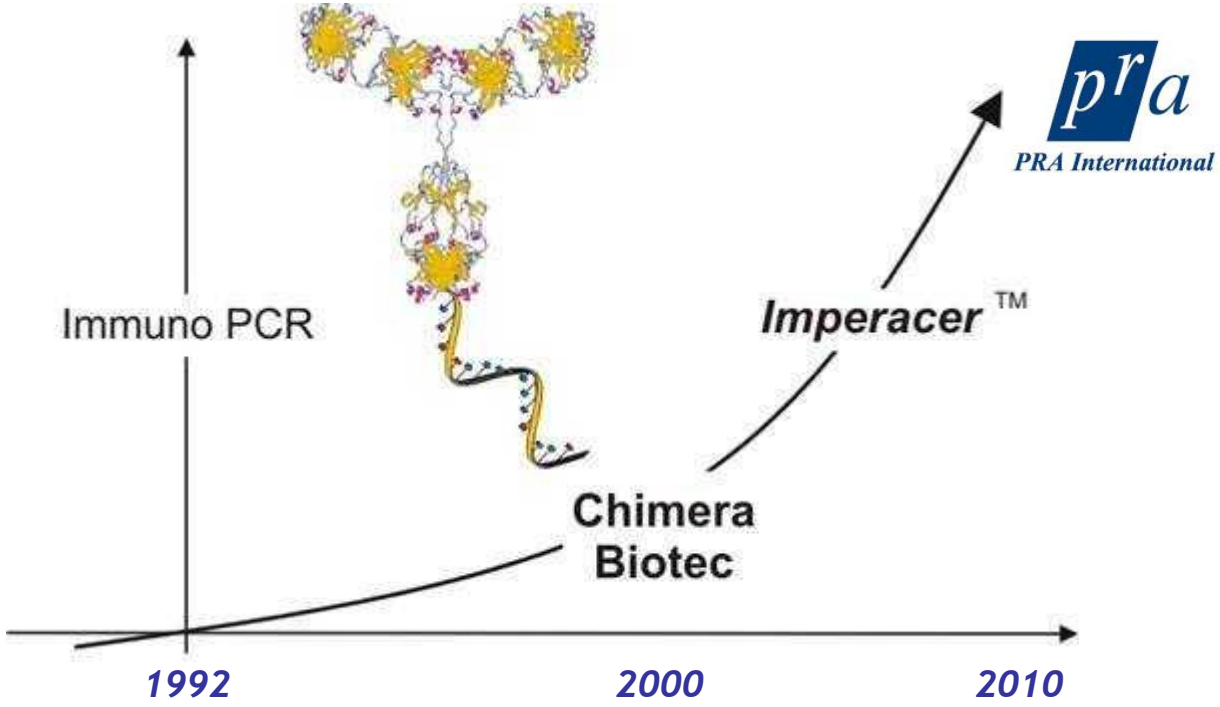
Bioanalytical Laboratory Services Across All Phases

Chimera Biotec

chimera biotec
ultra sensitive protein detection

- Manufacturer of the Imperacer® platform
- Focus on ultra-sensitive immunoassays
- Laboratory in Dortmund, Germany
- Founded in 2000
- Marketing Imperacer® since 2004
- Staff of 20 specialists
- Collaboration with **PRA International** for GLP studies

Immuno-PCR Development



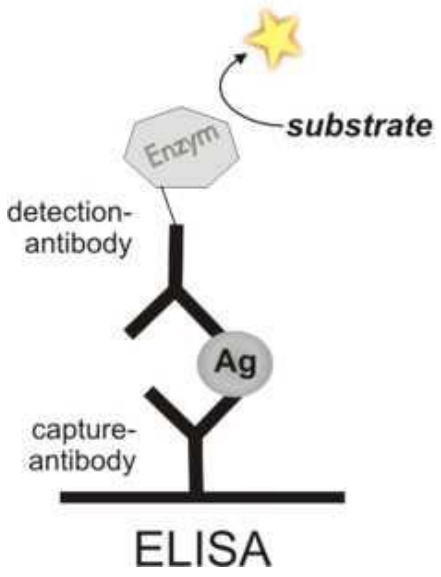
1992 - Start of Immuno-PCR technology

2000 - Foundation of Chimera Biotec

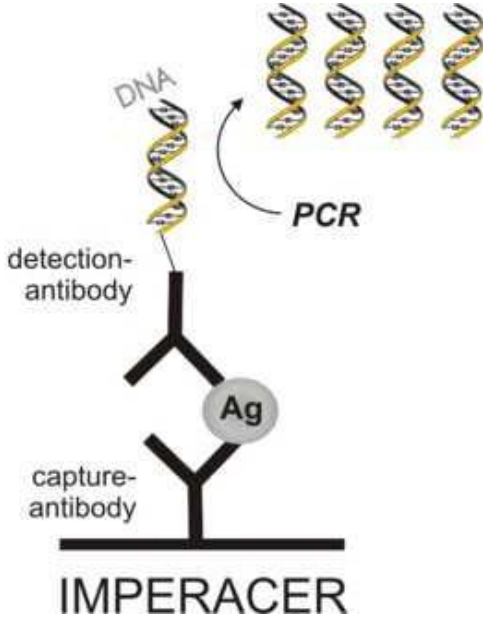
- → 210 Projects realized since 2004
- → 10 out top 25 Pharma/Biotech use Imperacer®

2010 - Collaboration with **PRA International** for GLP studies

Immuno-PCR Technology



Improved performance

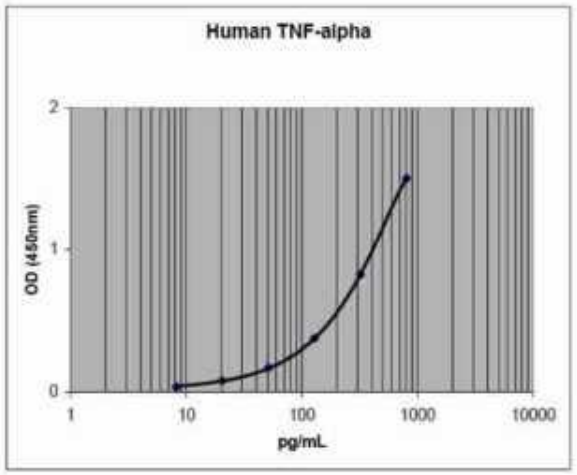


- Antibody-Enzyme Conjugate
- Conventional UV/Vis

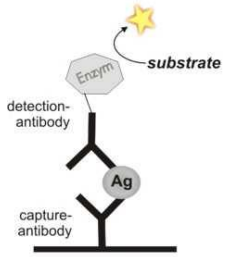
- Antibody-DNA Conjugate
- Real-time PCR

ELISA compared to Imperacer®

TNFa ELISA kit (Biosource)

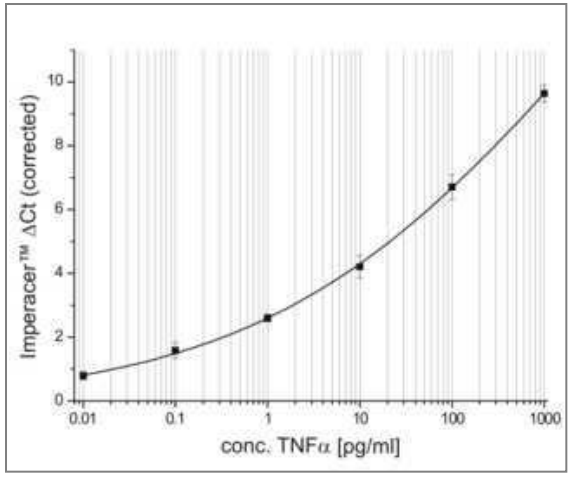


LOD: 10 pg/ml

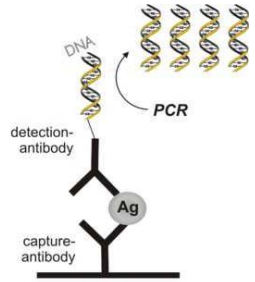


Improved performance

TNFa Imperacer® kit

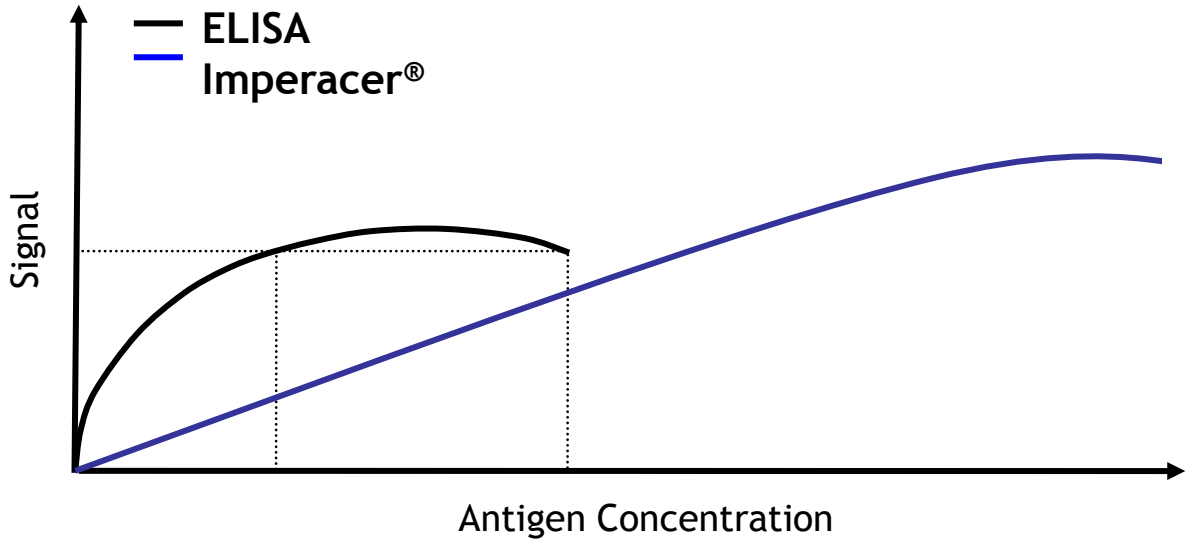


LOD: 0.01 pg/ml



Same Antibody pair

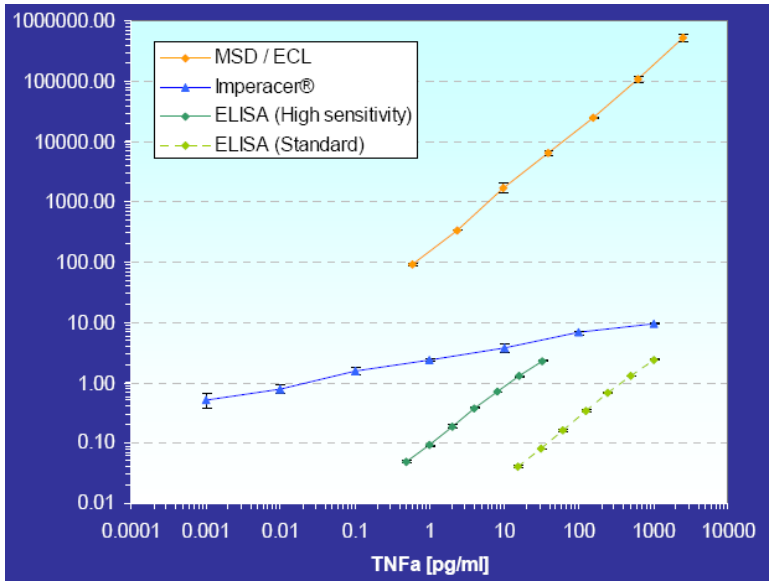
ELISA compared to Imperacer®



Advantages of the Imperacer® platform:

- Higher sensitivity
- A wider dynamic range (3-6 orders of magnitude)
- Imperacer® allows shifting the hook-effect through dilution

MSD compared to Imperacer®



LOD in human serum	Imperacer (pg/ml)	MSD (pg/ml)	Increase in sensitivity by factor
IL-2	0.10	1.3	13
IL-6	0.10	0.7	7
IL-11	0.80	n/a	-
IL-12	0.05	n/a	-
IL-17	0.02	0.4	20
IL-23	0.20	n/a	-
TNFα	0.01	1.1	110
TNFα (buffer)	0.001	0.7	700

Advantages of the Imperacer® platform:

- Increase in sensitivity by a factor 7-700
- A wider dynamic range (6 *vs.* 4 orders of magnitude)

  - *MSD: LOD is defined as the NC value plus 2.5-times standard deviation*
  - *Imperacer: LOD is defined as the NC value plus 3-times standard deviation*

Comparison of Assay Platforms

Assay platforms for anti-drug antibody (ADA) analysis

Assay format	Acid treatment requied	Typical sensitivity in ADA	Typical drug tolerance	Pros	Cons
Standard ELISA	Yes	~10 ng/mL	>1:100	• Generic reagents and instrumentation	• Multiple steps • Low drug tolerance
ELISA Drug Depletion		~10 ng/mL	~1:300	• Good drug tolerance • Generic reagents and instrumentation	• Laborious • Requires large sample volume • Increased variability of results
Solution ELISA		~10 ng/mL	~1:400	• Good drug tolerance • Generic reagents and instrumentation	• Multiple sequential assay steps • Requires high quality STV plates
Gyros		~10 ng/mL	>1:100	• Automation • Assay Time <2hrs	• Single provider of technology • Currently not available at CROs • Specialized instrument and consumables
AlphaLISA		~20 ng/mL	~1:100	• Homogeneous assay without washing steps	• Single provider of technology • Specialized instrument and consumables • Narrow dynamic range and hook effect
MSD		~10 ng/mL	~1:100	• Fewer steps than ELISA • Wider dynamic range	• Single provider of technology
Imperacer	No	~0.5 pg/ml	~1:1000	• Good drug tolerance • Good assay sensitivity • No pre treatment required • Wide dynamic range	• Single provider of technology • Limited availibility at CROs

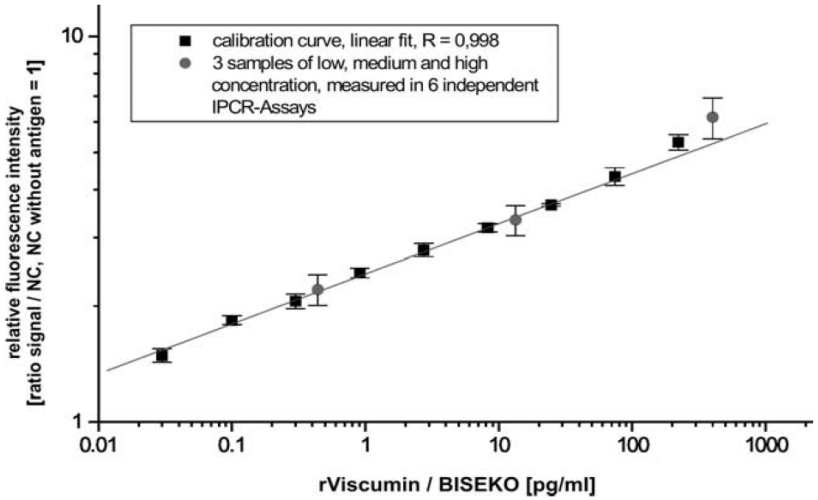
Data: Amaravadi et al., AAPS, November 2008; Spengler et al., BBRC, 2009.

Imperacer® Applications

PK analysis: drug assay in a Phase I study

- Phase I clinical study
- Solid tumors; Intravenous Aviscumine
- 41 patients

Aviscumine



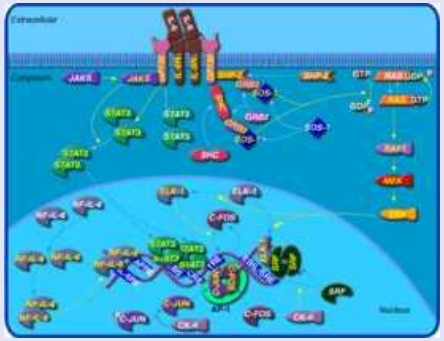
Validated Imperacer® Assay

- Correlation coefficient: >0.99
- Quantification range: 0.1 - 1000 pg/mL
- LOD: 0.004 pg/mL
- Average recovery (citrate plasma): 86%

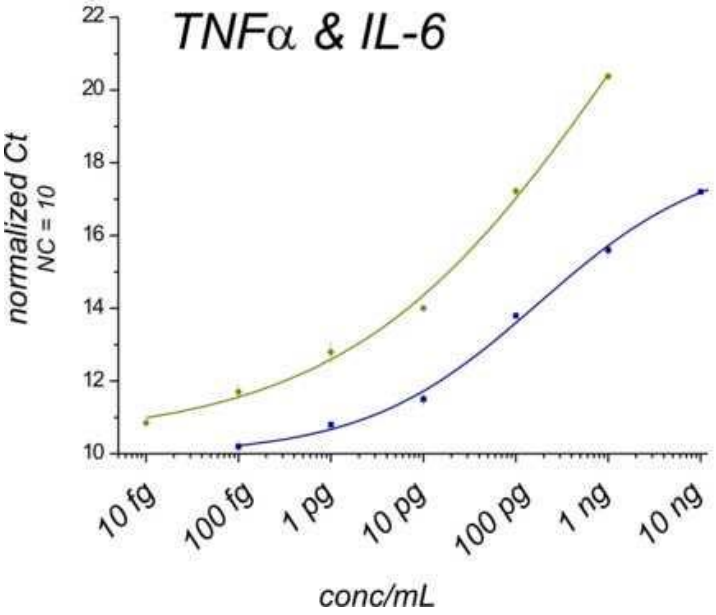
Schöffski, P. et al., Annals of Oncology 15 (2004), 671

Imperacer® Applications

PD analysis: Cytokines in inflammation



Imperacer® Cytokine assays (e.g. IL-6, IL-8 and TNFa)

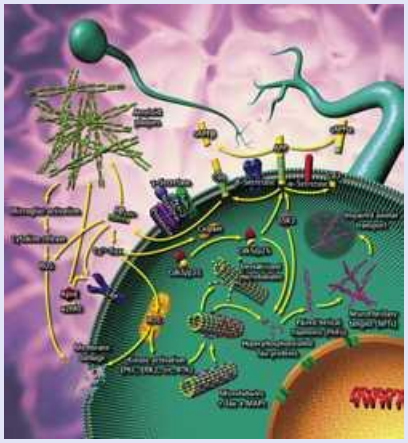


- Sensitivity: Variations in endogenous levels
- Dynamic range: Elevated levels in inflammation
- Various assay formats: Free *vs.* bound Cytokines

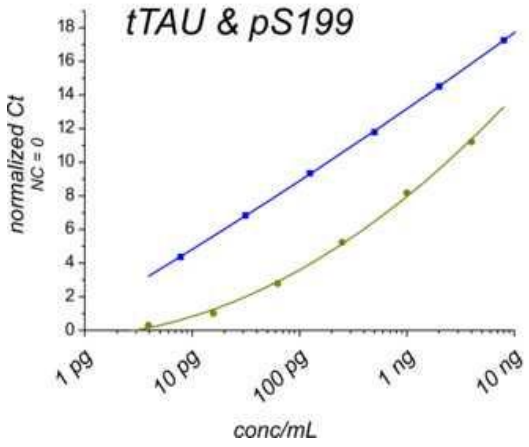
Imperacer® Applications

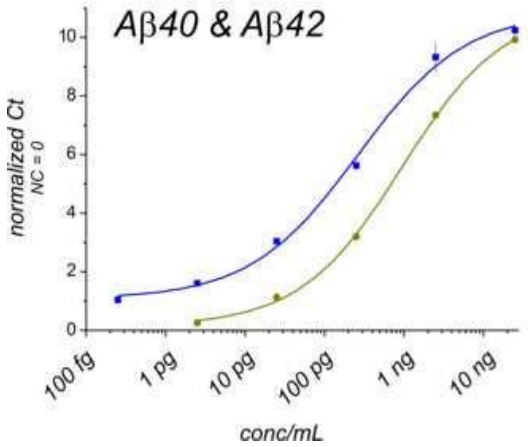
Diagnostics: Alzheimer's Disease

www.calbiochem.com



Alzheimer´s Disease pathway





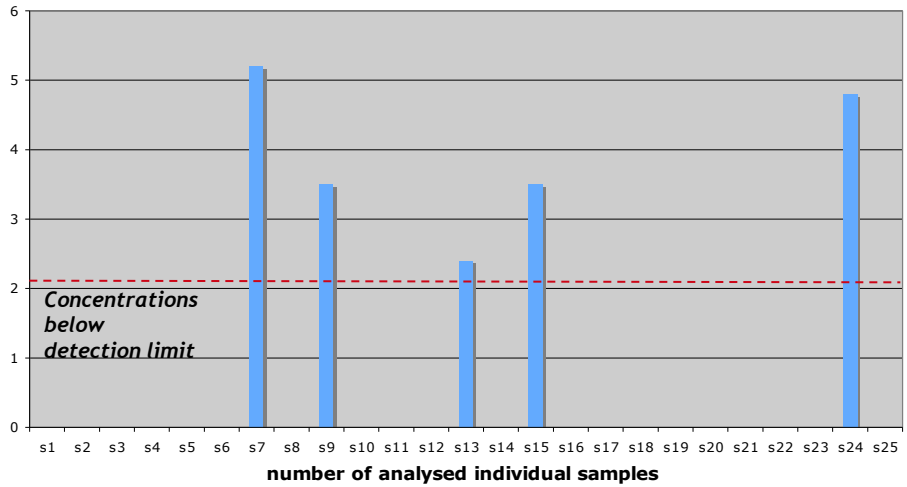
Imperacer® enables the detection of:

- Less than 10 pg/mL phosphorylated TAU in only 2 µl CSF sample
- Less than 5 pg/mL Amyloid beta 40 and 42 in CSF sample

Imperacer® Applications

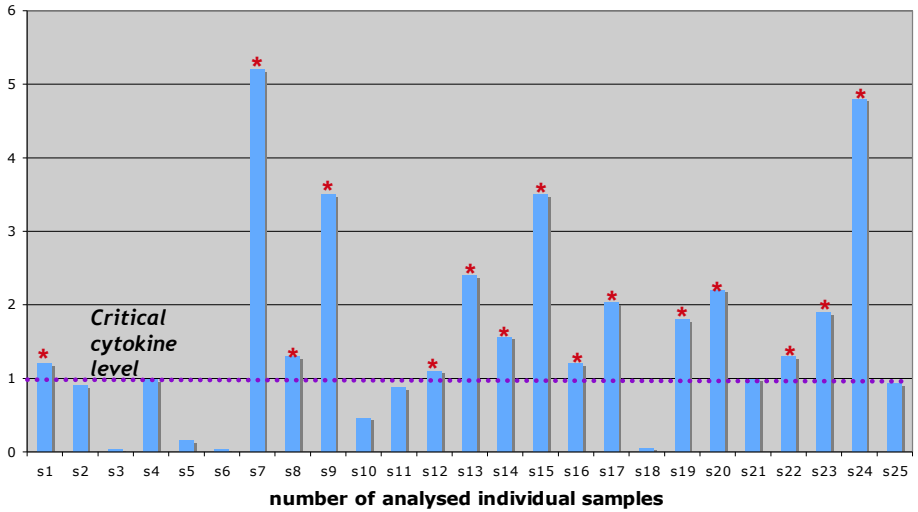
Clinical trial screening: study inclusion (e.g. anti-cytokine drug)

Cytokine detection common technology



- Cytokine screening with common technology (e.g. ELISA)
- Most patients with elevated levels are not detected as concentrations are <LOD

Cytokine detection Imperacer® technology



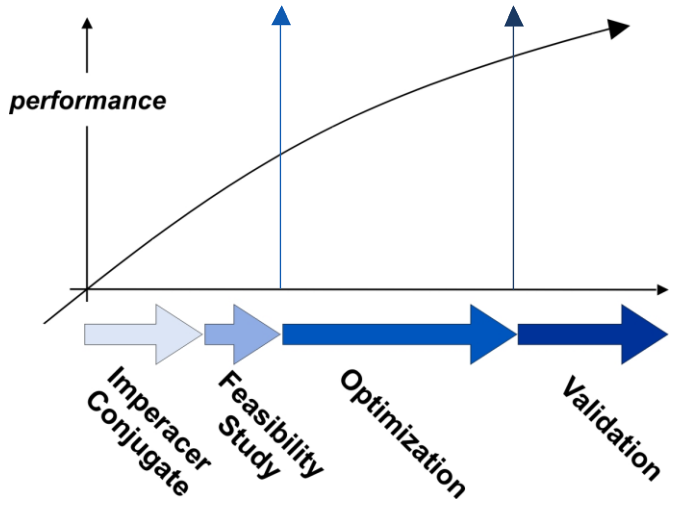
- Cytokine screening with Imperacer® technology
- Detection of low level concentrations
- Identification (and follow-up) of patients with critical cytokine levels

Ultra-sensitive patient screening enables dramatic increase in responder rate.
Current FDA guidelines support patient testing prior to study enrollment.

Development of a Cytokine Assay

Development of an Imperacer® assay

1. Conjugate synthesis
2. Feasibility study
3. Assay optimization
4. Manufacturer assay validation (**Chimera**)
5. Fit-for-purpose (GLP) validation (**PRA**)

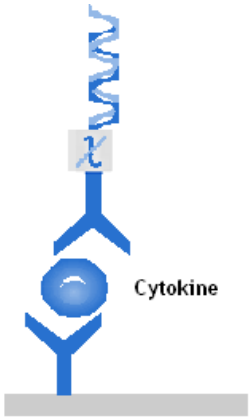


Key facts

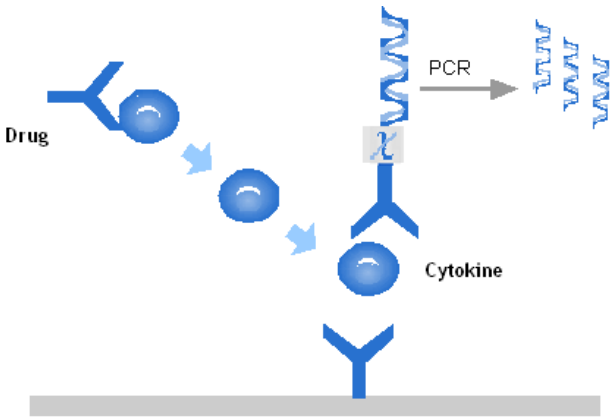
Antibodies:	Customer/Commercial - Choice of right Antibody-pair is key
Sensitivity:	Maximally achievable
Deliverables:	Manufacturer validated kits and detailed report
Duration:	12 to 16 weeks
Validation:	4 to 8 weeks (GLP or GLP-like, depending on purpose)

Cytokine Assay Development

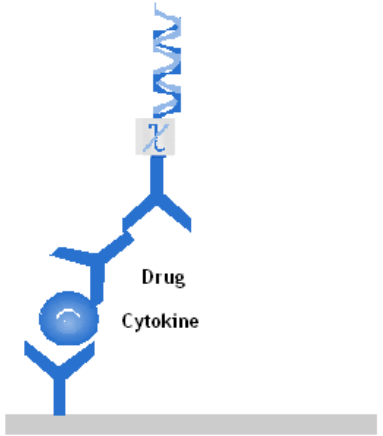
Imperacer® assays for a specific Cytokine: Free, Total and Bound



Free fraction



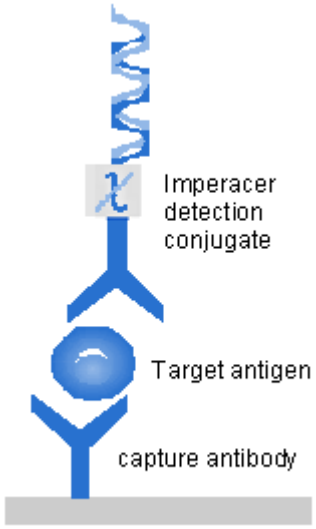
Total cytokine assay



Drug-bound fraction

Cytokine Assay Development

Imperacer® assays for a specific Cytokine: Free assay

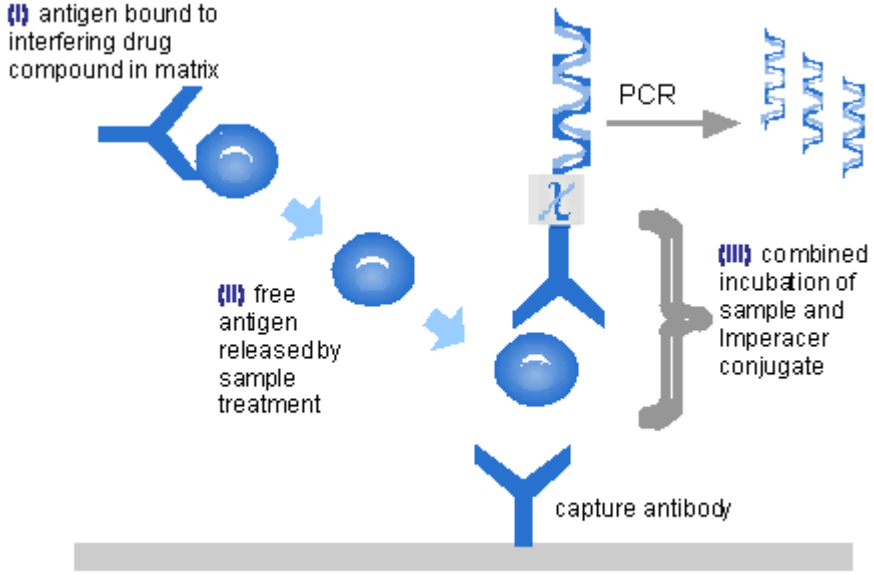


Free Assay

Sandwich Imperacer® assay to detect the free Cytokine with highest sensitivity.
The detection conjugate will not bind to drug-bound cytokine.

Cytokine Assay Development

Imperacer® assays for a specific Cytokine: Total assay

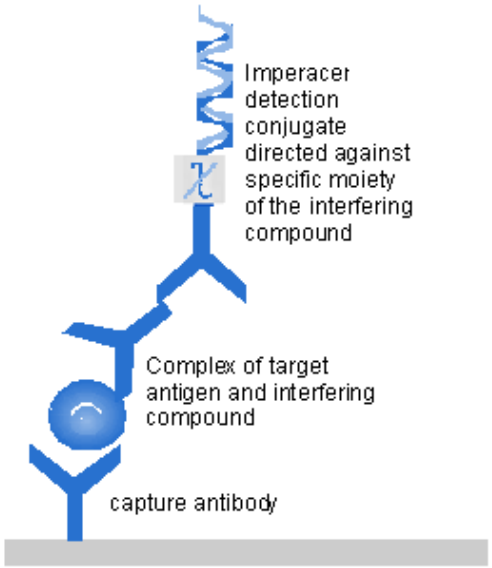


Dissociation Assay

1. Release Cytokine from drug (pH or organic solvent).
2. Dilute sample in buffer including the detection conjugate to neutralize the dissociation procedure.
3. Incubate the conjugate containing sample on the capture plate.

Cytokine Assay Development

Imperacer® assays for a specific Cytokine: Drug-bound assay



Complexed Cytokine Assay

1. Capture by Cytokine-specific capture antibody.
2. Detection by drug-specific Imperacer® detection conjugate.

Free Cytokine Assay Validation

Validation of the specific Free Cytokine Imperacer® assay

Calibration

- Regression model: 4PL regression
- Validated range: 0.059 - 6000 pg/ml
- Back-calculated results: $r^2 > 0.99$

LLOQ: 0.059 pg/ml (in human serum)

Spike recovery: 104 - 109% (at 0.100, 10.0 and 100 pg/ml)

Precision

- Signal: 0.4 - 1.1% (intra CV%); 0.4 - 1.8% (inter CV%)
- Concentration: 4.0 - 20.0% (inter-assay CV%)

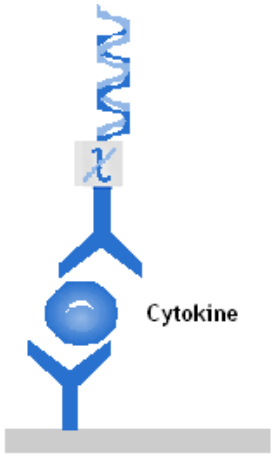
Specificity (matrix variability)

- Spike recovery: 79 - 128% (10.0 pg/ml in healthy subjects and patients)
- Endogenous levels: ND - 0.745 pg/ml

Freeze/thaw stability: 1.0 - 9.9% (CV% of QCs at 3 levels; 3 F/T cycles)

Robustness (QCs at 3 levels)

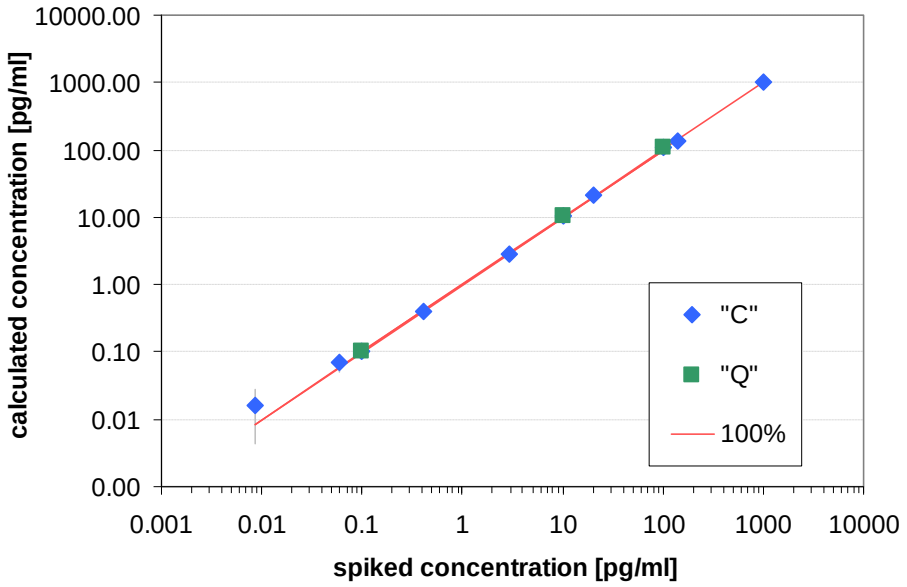
- Buffer *vs.* matrix: 0.5 - 1.9% (inter-matrix CV%)
- Instrument variation: 3.0 - 24.0% (CV%; 3 non-normalized instruments)
- Lot-to-lot variation: 3.0 - 19.0% (CV%; 2 batches of conjugate)



Cytokine Assay Validation

Free Cytokine assay - Summary

- Calibration curve in analyte-free proxy-matrix
- QCs spiked in human serum
- LLOQ: 0.059 pg/ml (59 fg/ml) in human serum
- Good spike-recovery, precision and stability
- GLP validation and analysis at PRA International



Conclusions

The **Imperacer®** low fg/ml sensitivity assays enable:

- Determination of endogenous levels of special interest analytes
- Investigation of analyte variations at ultra-low concentration levels
- Resolving matrix effects by dilution
- Immunogenicity assays with excellent drug tolerance
- Specific assays for total, bound and free analytes

PRA International offers in collaboration with **Chimera Biotec**:

- Development and optimization of custom tailored Imperacer® assays
- Validation and bioanalysis under GLP conditions

Presence at EBF 2010

Presence at EBF 2010 - Third Open Symposium:

- PRA International - exhibition booth
- Martin Nemansky, PRA International - Current presentation
- Mark Spengler, Chimera Biotec - Presentation on Dec. 3, 12:20 hours
- Michael Adler et al., Chimera Biotec - Poster presentation

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