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ultra sensitive immunoassays

Imperacer (Immuno-PCR) method for ultra-sensitive quantification of Interleukin-6 to support clinical phase III trial

Authors: Mark Spengler, Nina Juergens and Michael Adler; Chimera Biotec; Emil-Figge Str. 76a; D-44227 Dortmund; Germany

Purpose

Pharmacodynamic (PD) markers have great impact on the development of Biotherapeutics for treatment of various diseases. While cytokines are highly important PD markers, patient levels are often below sensitivity limits of standard ligand-binding assay (LBA) technologies. More so, pre-clinical PD biomarker assessment, in addition to sensitivity, often demands for minimal sample volume requirement (<5-10 µl) of the LBA method.

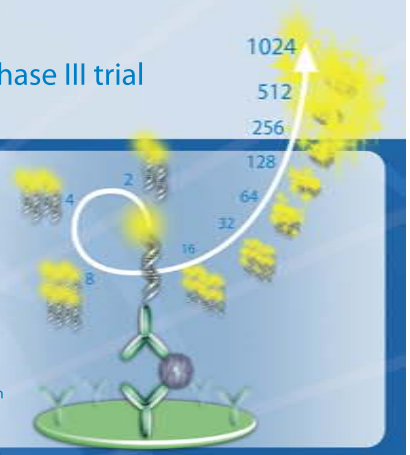
As one example, Interleukin-6 (IL-6) a multifunctional cytokine secreted by T cells and macrophages plays a major role in the regulation of the immune response, hematopoiesis, acute phase response, and inflammation. IL-6 therefore is an important PD marker in a variety of therapeutic areas, foremost autoimmune indications.

Here we describe Imperacer® assay development and bioanalytical method validation (BMV) in support of clinical phase-II and phase-III trials for development of autoimmune disorder therapies. In addition, adaptation for use of the assay in microsampling study support is demonstrated by limiting sample volume requirement to 1 µl per run in duplicate.

Method

Imperacer® sandwich assay in 96-well microplate format, using IL-6-specific capture reagent in combination with IL-6-specific, marker-DNA tagged detection conjugate for real-time PCR signal amplification.
Corresponding exploratory, ultra-sensitive chemiluminescence reference assay (Chimera Biotec) for discussion of sensitivity vs. sample volume requirement and appropriate assay modification.

Fig 1: Imperacer® Scheme



Sandwich assay set-up: IL-6-specific capture and IL-6 specific Imperacer® antibody-DNA detection conjugate. Subsequent amplification of immobilized DNA-tag permits exponential signal generation for highly sensitive quantification or extreme reduction of required sample volume.

Results

The full-validated Imperacer® method for clinical phase-II/III human serum or plasma sample testing support fulfills all relevant BMV guidelines for Biomarker LBA methods, with quantitative assay range of 0.3 – 5000 pg/ml. The assay allows bioanalysis in duplicate with sub-pg/ml sensitivity and sample requirement as low as 35 µl for an assay-run in duplicate. This sample requirement, including pipetting overhead, supports sample quantification from 100 µl study aliquots with backup volume for potential re-testing. Extreme sensitivity of the Imperacer® IL-6 method in combination with >4 log assay range leads to excellent performance in clinical trial phase II/III support of approx. 9000 samples with 1.7% BLQ and 0.1% ALQ results.
However, exponential signal amplification on the Imperacer® platform allows further reduction of sample volume requirement for microsampling studies. In contrast, an exploratory, ultra-sensitive chemiluminescence assay (Chimera Biotec), having an (non-validated) assay range of 0.5 – 1500 pg/ml, has an approx. 80% higher sample requirement of 200 µl for duplicate runs. By appropriate sample dilution in AnySource® buffers (Chimera Biotec) sample consumption can be drastically reduced. An exploratory IL-6 Imperacer® assay with sample requirement of 1µl per run in duplicate was developed (assay range: approx. 3 – 700 pg/ml). With this protocol, a parallel multi biomarker testing was demonstrated. The IL-6 microsampling assay was complemented by a separate IL-2 Imperacer® assay (3.6 µl sample requirement), enabling analysis of IL-2 and IL-6 with <5 µl sample volume for two separate runs in duplicate. This permits PD biomarker assessment in neonates, pediatric clinical trials or in pre-clinical setting even from single small rodents rather than from animal pools (e.g. earlap or heel prick blood sampling or serial blood sampling from the tail vein of a single mouse).

Fig 2: Accuracy and Precision of clinical IL-6 Imperacer® method

Fit-for-purpose validation of the Imperacer® human IL-6 method for use in phase-II/III clinical serum or plasma samples.
Sample dilution: 35 µl sample + 35 µl AnySource® sample dilution buffer (SDB 3100) (Chimera Biotec) for robust duplicate well analytical plate runs (30 µl diluted sample per well).
Standards s1- s8 prepared in target-free AnySource® SDB 2100 (Chimera Biotec);
QCs prepared in standardized human serum pool (human reference matrix (hRM); Chimera Biotec), to control quantification from serum or plasma. Endogenous level of IL-6 in hRM was found at approx. 1 pg/ml, QC levels were selected accordingly.
Note that LLOQ at 0.3 pg/ml is well separated from non-spiked negative control (NC): fit-for-purpose validation of the Imperacer® method was applied for best assay performance within the needed concentration range in clinical samples.

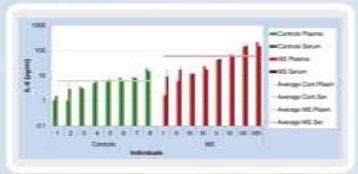


Fig 3: Application of clinical IL-6 Imperacer® method

Endogenous IL-6 levels as found in commercially available control samples from healthy volunteers and diseased (multiple sclerosis; MS) patients by analysis with the validated, clinical Imperacer® method. A broad endogenous concentration range from sub-pg/ml up to >100 pg/ml was observed. A different sample cohort revealed concentrations >1000 pg/ml (data not shown); MS patients typically revealed an approx. 10x higher concentration range compared to healthy volunteers.

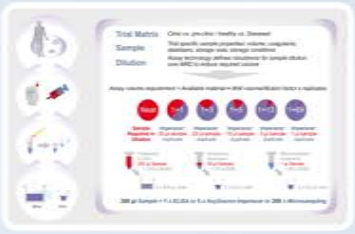


Fig 4: Assay sensitivity vs. sample volume requirement

In contrast to alternate, ultra sensitive IL-6 assays (e.g. chemiluminescence assay (Chimera Biotec)) exponential signal amplification on the Imperacer® platform allows fine-tuning of sample volume requirement vs. sensitivity by appropriate sample dilution in AnySource® buffers (Chimera Biotec) without total loss in assay signal.

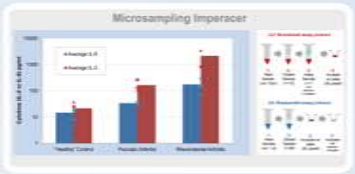


Fig 5: Separate-well multi Biomarker testing in microsampling support

Exploratory Imperacer® serum sample testing for IL-6 and IL-2 concentrations for microsampling study support. Shown here are results from healthy and diseased (Arthritis) patient samples purchased from commercial sample vendor. Sample requirement for duplicate-well assay runs: IL-6: 1 µl; IL-2: 3.6 µl.

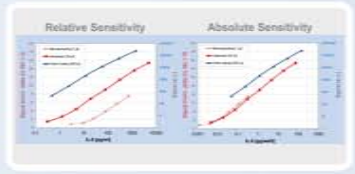


Fig 6: Sensitivity vs. sample volume requirement in IL-6 quantification

While the exploratory IL-6 chemiluminescence assay (Chem. Assay; Chimera Biotec) already demonstrated excellent sensitivity and assay range, the clinical support Imperacer® method allows for (fit-for-purpose) better sensitivity and even broader assay range for optimal assay performance. Of great advantage is the 80% lower sample consumption of the Imperacer® vs. Chem. assay.
A comparison of relative (pg/ml) vs. absolute (pg/well) sensitivity demonstrates the influence of sample volume on assay performance.

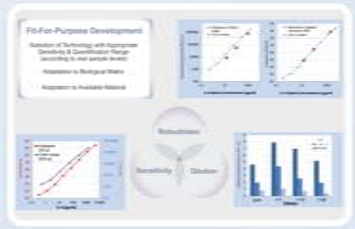


Fig 7: Fit-for-purpose development of assay characteristics

Trial specific fit-for-purpose assay development requires consideration of different parameters:

(I) Trial specific restrains: matrix, stabilizer, sample volume, storage conditions, etc.

(II) Pro's and con's of ligand-binding assay platforms

(III) Availability of appropriate reference matrix for standards and QCs and matching sample dilution buffers and protocols

Conclusion

An Imperacer® method for quantification of IL-6 in human serum or plasma was developed and validated according to BMV guidelines for Biomarker assays. Sensitivity (LLOQ: 0.3 pg/ml) and broad assay range (>4 log.; ULOQ: 5000 pg/ml) result in excellent method performance in clinical phase-II/III study support of approx. 9000 samples. Exploratory Imperacer® assays for IL-6 and IL-2 were developed to allow sample testing in duplicate from less than 5 µl total sample requirement at target levels compatible with tested individual samples (approx. 3 - 1000 pg/ml IL-2 or IL-6).