Imperacer® Immuno-PCR Technology

Assay Principle

**ELISA**
Antibody-enzyme conjugate

**Imperacer®**
Antibody-DNA conjugate
Readout by real-time qPCR
Signal amplification
Imperacer® Immuno-PCR Technology

Data readout:
- qPCR readout: normalized fluorescence
- Baseline calibration: set threshold
- Cycle time: Ct
- Concentration: nominal values of the calibrators
## Imperacer® Immuno-PCR Technology

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection range</td>
<td>Broad dynamic range</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Determination of endogenous levels of special interest analytes</td>
</tr>
<tr>
<td></td>
<td>Investigation of analytes at ultra-low concentration levels (fg/mL)</td>
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<tr>
<td>Dilution</td>
<td>Resolving matrix effects by sample dilution</td>
</tr>
<tr>
<td></td>
<td>Microsampling; low sample volume requirement</td>
</tr>
<tr>
<td></td>
<td>Improving drug tolerance in immunogenicity assays</td>
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</tbody>
</table>
### Imperacer® Immuno-PCR Technology

<table>
<thead>
<tr>
<th>Potential Challenges</th>
<th>Details</th>
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</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Highly specific reagents are needed, cross-reactivity will be amplified.</td>
</tr>
<tr>
<td>Robustness</td>
<td>Uniform handling procedures are essential</td>
</tr>
<tr>
<td></td>
<td>Dedicated lab operators are needed - training and routine is key</td>
</tr>
<tr>
<td>Reagents</td>
<td>Dependent on a single vendor: risk of vendor discontinuation</td>
</tr>
</tbody>
</table>
Combined services
Offered by Chimera Biotec and PRA Health Sciences

- Conjugate Synthesis and QC
- Maximizing assay sensitivity
- Method engineering
- Method qualification
- Method transfer
- Method validation
- Sample analysis

Chimera Biotec
ultra sensitive immunoassays

PRA Health Sciences
Equipment
Imperacer work station
• Reader (cycler)
• Washer, shaker, vortex
• Centrifuge

• Imperacer equipment coupled to PRA network
• Data analysis software Softmax PRO and Thermo Watson LIMS (21CFR part 11-compliant software)
• Validation of the work station
• Appropriate procedures are in place
Materials
Chimera: conjugated materials
Chimera: kit materials (buffers, consumables)
PRA: preparation of calibrators and QC samples

Lab Technician
PRA: technicians trained by Chimera
Chimera: on-site technical support if needed
Applications of Imperacer® Technology

PharmacoDynamics Exploratory
Anti-Drug Antibodies
PharmacoKinetics Toxicology

PharmacoDynamics BioMarker
Anti-Drug Antibodies
PharmacoKinetics Safety/Efficacy

Preclinical Phase Bioanalytical Support
Clinical Phase Bioanalytical Support
B. Biomarkers

The recommendations in this guidance pertain only to the validation of assays to measure in vivo biomarker concentrations in biological matrices such as blood or urine. Considerable effort also goes into defining the biological function of biomarkers, and confusion may arise regarding terminology. Information about defining the biological role of a biomarker is available on the FDA Drug Development Tools website.
Case-study of a PK study with Imperacer

Sponsor X:

“We want to determine PK concentrations in our clinical trial using an ultrasensitive assay performed on the Imperacer® platform. This assay should be validated according to FDA and EMA guidelines and according to all relevant white papers. Sample analysis should be performed with a full GLP claim.”
Case-study of a PK study with Imperacer

“Validated according to FDA and EMA guidelines and according to all relevant white papers”

Precision and Accuracy
- Mean bias within ± 20.0% (25.0% at LLOQ and ULOQ)
- Total precision (%CV) < 20.0% (25.0% at LLOQ and ULOQ)
- Total error < 30.0% (40.0% at LLOQ and ULOQ)

Dilution Linearity
Diluted samples should be within ± 20.0% from nominal value and precision should not exceed 20.0%

Optional: Integrity of Dilution

Selectivity (including diseased, lipemic and hemolyzed matrices)
80% of the matrices should be within ± 20.0% from nominal value (25.0% at LLOQ)

Stability (F/T, bench-top, frozen storage and stock stability)
Stability samples should be within ± 20.0% from nominal value and precision should not exceed 20.0%
Case-study of a PK study with Imperacer

“Sample analysis should be performed with a full GLP claim”

Quality System for the organization of lab studies which concerns Planning, Performing, Monitoring, Recording, Reporting and Archiving

Originally intended for non-clinical safety testing of pharmaceuticals, cosmetics, food additives, chemicals etc.

Adopted to support clinical studies as well

GLP does not assure good science
Applying this to the case-study:

“Ready-made” QC samples from an Imperacer kit can only be used if the preparation of these samples is documented within the validation study.

Alternative: preparation of QC samples within validation study.
Case-study of a PK study with Imperacer

“Method X”

Calibration range: 0.03 – 125 ng/mL
Aimed LLOQ: 0.09 ng/mL
LLOQ ELISA assay: 10.0 ng/mL: 100-fold increased sensitivity

Precision and Accuracy

<table>
<thead>
<tr>
<th></th>
<th>0.090 ng/mL</th>
<th>0.250 ng/mL</th>
<th>5.00 ng/mL</th>
<th>100 ng/mL</th>
<th>125 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>% CV</td>
<td>26.5</td>
<td>18.4</td>
<td>10.8</td>
<td>18.7</td>
<td>14.8</td>
</tr>
<tr>
<td>% bias</td>
<td>-8.8</td>
<td>-14.3</td>
<td>-15.7</td>
<td>5.1</td>
<td>-0.4</td>
</tr>
<tr>
<td>% Total Error</td>
<td>35.3</td>
<td>32.7</td>
<td>26.5</td>
<td>23.9</td>
<td>15.1</td>
</tr>
</tbody>
</table>
## Case-study of a PK study with Imperacer

### Selectivity

<table>
<thead>
<tr>
<th></th>
<th>0.135 ng/mL</th>
<th>Bias (%)</th>
<th>100 ng/mL</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix 1</td>
<td>0.167</td>
<td>23.7</td>
<td>110</td>
<td>10.3</td>
</tr>
<tr>
<td>Matrix 2</td>
<td>0.165</td>
<td>22.1</td>
<td>126</td>
<td>26.1</td>
</tr>
<tr>
<td>Matrix 3</td>
<td>0.175</td>
<td>29.8</td>
<td>133</td>
<td>33.4</td>
</tr>
<tr>
<td>Matrix 4</td>
<td>0.180</td>
<td>33.3</td>
<td>91.1</td>
<td>-8.9</td>
</tr>
<tr>
<td>Matrix 5</td>
<td>0.137</td>
<td>1.7</td>
<td>118</td>
<td>18.2</td>
</tr>
<tr>
<td>Matrix 6</td>
<td>0.152</td>
<td>12.8</td>
<td>112</td>
<td>12.0</td>
</tr>
<tr>
<td>Matrix 7</td>
<td>0.121</td>
<td>-10.1</td>
<td>83.4</td>
<td>-16.6</td>
</tr>
<tr>
<td>Matrix 8</td>
<td>0.139</td>
<td>3.1</td>
<td>93.0</td>
<td>-7.0</td>
</tr>
<tr>
<td>Matrix 9</td>
<td>0.101</td>
<td>-25.2</td>
<td>86.0</td>
<td>-14.0</td>
</tr>
<tr>
<td>Matrix 10</td>
<td>0.139</td>
<td>2.7</td>
<td>111</td>
<td>11.3</td>
</tr>
</tbody>
</table>
Assay criteria are dependent on multiple factors:

- Clinical phase (pre-clinical, early phase or late phase)
- Aim of the assay (PD, PK)

But does the assay platform also play a role in the determination of the assay criteria?

- How to interpret the guidelines for ‘newly emerging highly sensitive ligand binding methods’ which are not described in any white paper?
- Novel techniques might require an alternative approach
- Might the higher assay sensitivity require an adaptation of the common criteria?
Conclusions

- Development and validation of Imperacer® methods requires investment in time and dedication.

- With increased sensitivity and low sample volume requirement as compared to ELISA methods, Imperacer® can be a solution to specific problems.

- Would this assay be acceptable for use provided that the scientific justification is documented? More discussion is needed on this topic to reach a consensus.
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