High-Throughput Characterization of Monoclonal Antibodies Using the Octet HTX System

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Antibody Technology

- Antibody Core Facility at Novo Nordisk, providing poly- and monoclonal antibodies for multiple purposes:
  - Therapeutic monoclonal antibodies
    - Fully human
    - Humanized
    - Surrogate
  - Immunoassays
    - ELISA / AlphaLISA
    - Functional Cell Based assays
    - IHC
Diverse mAb repertoire is secured by access to complementary technologies

**In-vivo Technology**
- Immunized animal → B cells → mAbs

**Display Technology**
- Ab gene library → Display system → mAb
**In vivo** and display technologies are complementary in ability to deliver mAbs to different targets

<table>
<thead>
<tr>
<th></th>
<th>In vivo technology</th>
<th>Display technology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ab repertoire</strong></td>
<td>Natural</td>
<td>Designed</td>
</tr>
<tr>
<td><strong>Epitope</strong></td>
<td>Immune dominant epitopes</td>
<td>Unbiased</td>
</tr>
<tr>
<td><strong>Target limitations</strong></td>
<td>Challenging for self-antigens</td>
<td>Challenging for cellular targets</td>
</tr>
<tr>
<td><strong>Screening capabilities</strong></td>
<td>Screening on protein or cells using soluble IgG</td>
<td>Panning or FACS using recombinant protein</td>
</tr>
<tr>
<td><strong>Affinity</strong></td>
<td>Most times no need for affinity maturation</td>
<td>Affinity maturation process needed</td>
</tr>
<tr>
<td><strong>Timelines</strong></td>
<td>‘Slow’</td>
<td>Fast</td>
</tr>
</tbody>
</table>
In Vivo Technologies

Hybridoma Technology

- Conventional hybridoma technology
- Generation of murine and fully human mAbs
- ~250 Fusions/year
  - ~80 pl/fusion
  - >7500 samples/fusion

Rabbit mAbs

- Immunization of rabbits
- Ag-specific single B cell cloning from spleen
- Propagate B cells
- Recombinant cloning and expression
- ~6000 samples/sort
- ~300 recombinant mAbs
Display Technologies

Phage Display
- Conventional Phage display
- High diversity
- Panning / screening in ELISA
- Variable out-put

Yeast Display
- High diversity library
- Selection by FACS
- Labeled Ag
- Variable out-put
  - ~200-400 mAbs
mAb generation workflow

Hybridoma workflow

- Fusion
- 1st Screening (binding)
- Propagation
- 2nd Screening
- Small-Batch purification
- Characterization

Yeast-Display workflow

- Naïve selection
- Small-Batch purification
- Characterization
- Affinity maturation
- Small-Batch purification
- Characterization

Cell-line development
Screening and characterization of selected clones

- Analysis performed on Octet RED384 or Octet HTX systems

- Screenings primarily performed as single conc. kinetics – ie. one Ag Conc. Characterization primarily Binning.

- Performed on culture supernatants and/or purified mAbs

- Primary screening performed in 384 well format and 96 channels format allow fast screening of multiple plates

- Secondary screening performed on 300-500 samples

- Additional functional info. can be included
  - Competition
  - Domain specificity
  - Counter screen

<table>
<thead>
<tr>
<th></th>
<th>Octet RED384</th>
<th>Octet HTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro Plates</td>
<td>2 (96/384)</td>
<td>2 (96/384)</td>
</tr>
<tr>
<td>Affinity Range</td>
<td>1 mM - 10 pM</td>
<td>1 mM - 10 pM</td>
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<tr>
<td>MW</td>
<td>&gt;150 Da</td>
<td>&gt;150 Da</td>
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<tr>
<td>Min. Sample vol.</td>
<td>40 ul</td>
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<tr>
<td>Acquisition Rate</td>
<td>2, 5, 10 Hz</td>
<td>2, 5, 10 Hz</td>
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<tr>
<td>Spectrometers</td>
<td>16</td>
<td>16</td>
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<tr>
<td>Simultaneous Reads</td>
<td>16</td>
<td>96</td>
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</table>
HTX - Single conc. kinetics

Antigen

384 samples
# HTX - Single conc. kinetics

## Presentation Title

Date: 10

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### Table: Assay Data

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Sample</th>
<th>Plate</th>
<th>Step Name</th>
<th>Step Type</th>
<th>Sensor Type</th>
<th>Sensors</th>
<th>Room</th>
<th>Assay</th>
<th>Time</th>
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<tbody>
<tr>
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<td>A1</td>
<td>1</td>
<td>Elution</td>
<td>Elution</td>
<td>ACR (Active</td>
<td>C, F, C, F, C</td>
<td>98</td>
<td>channel</td>
<td>1:10</td>
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<tr>
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<td>98</td>
<td>channel</td>
<td>1:10</td>
</tr>
</tbody>
</table>

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### Diagram: Data Acquisition System

[Image of data acquisition system interface]

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### Notes

- In this step, the assay data will be recorded in the data acquisition system.
- Select a group of sensors and approve the currently selected step within the current area with a double click or right click for more options.

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*Image credit: novonordisk*
Reference subtraction
Reference subtraction

Buffer

Antigen

2x 192 Samples
Single conc. kinetics – Data evaluation
Single conc. kinetics – Data evaluation

- Primary screening of 864 samples (sup’s)
  - Capture on AMC

- Single point kinetics
  - One Ag conc.
  - Short dissociation time (10 min.)

- Instrument time ~7 hr.
  - Automated direct ELISA ~3 hr.
    - but only yes/no data

- Secondary screening on selected clones
  - Procedure identical to primary screening
Characterization – Epitope Binning

- An Epitope Bin can be described as the area on a given antigen covered by a mAb, ie. mAb footprint.
- Test for simultaneous binding of two mAbs to the same antigen
  - Competitive mAbs bind to the same bin
  - Non-competitive mAbs bind to different bins
Characterization - Binning

- **In Tandem**
  - Useful for multimeric or aggregated Ag
  - Require saturation

- **Classical Sandwich**
  - Useful for monomeric or nonaggregated Ag
  - If performed on AMC/AHC block excess sites using irrelevant IgG

- **Matrix binning**
  - “All vs. All”
  - Very laborious – but possible due to multiple channels on the HTX

- **Iterative binning**
  - Initial binning vs. subset of clones selected based on affinity/functionality. Based on these binning data, mAbs from new bins are binned vs. mAb population etc.

Matrix binning – 48x48 mAbs

- Assay performed “in Tandem”
  - Biotinylated Antigen on SA sensors
  - HTX in 48 channels mode
- 48 different mAbs as “mAb1” vs. one mAb as “mAb2”
  - 48 cycles
    - Max. 12 cycles/experiment
- Instrument time ~12 hr. – 48x48
  - ~3 hr. for 48x12
Matrix binning

- Build matrix based on response levels
  - Use “Process Epitope Binning” in the Analysis software
  - Difficult to assign common cut-off
  - Visual inspection needed
Iterative binning

- Assay performed as “Sandwich”
  - Capture on AMC/AHC sensors
  - Block with irrelevant IgG
  - Sensor binding check

- 48 mAbs vs. 2 mAbs
  - 2 cycles in one experiment
  - Instrument time ~1 hr.
Iterative Binning

- A panel of 48 mAbs from display platform and 48 mAbs from in vivo platform binned using the “Sandwich” set-up.

- Binned vs. 4 selected mAbs based on previous binning data, affinities and functional data.
  - 1st mAb: 2x48 panels / Antigen / 2nd mAb: selected mAbs

- Very good coverage. Tendency to different binning patterns for the two platforms.

- Larger set of mAbs:
  - Panel of 265 mAbs vs. 5 mAbs
Cell-line development

- Production cell-lines are established for selected lead mAb(s)
- Several rounds of clone selection
- Clones initially selected based on expression level.
Cell-line development

- Quantitation experiment with regeneration
  - 120 sec. @ 400 rpm
  - Post condition: 6x regeneration

- “Generic” Std. Curve used

- Measure 96 samples simultaneously
  - ~5 min/plate

- Re-use sensors and reagent plate
Summary

- The Octet HTX has been implemented in a high throughput mAb generation workflow.

- Analysis are performed at multiple stages in the mAb generation and production flow.
  - Screening and selection based on affinity
  - Epitope binning
  - Quantitation analysis

- Implementation of the Octet HTX has dramatically increased the throughput.