Selection of monoclonal antibodies using the Octet platform for diagnostic application: strengths and limitations

TURINI Marc

Pall ForteBio User Meeting
Lyon, 04th June 2015
Agenda

• Who we are?

• Use of Octet platform for diagnostic application at Beckman Coulter:
  - Selection of monoclonal antibodies with high sensitivity, low dissociation and no cross-reactivity
  - First screening of various monoclonal antibodies in order to perform a fast evaluation of critical raw materials

• Strengths and limitations
Agenda

• Who we are?

• Use of Octet platform for diagnostic application at Beckman Coulter:
  ➢ Selection of monoclonal antibodies with high sensitivity, low dissociation and no cross-reactivity
  ➢ First screening of various monoclonal antibodies in order to perform a fast evaluation of critical raw materials

• Strengths and limitations
Who we are?

Beckman Coulter, Inc.
Corporate History

1935
(Beckman) Founded by Dr. Arnold O. Beckman, National Technical Laboratories in Pasadena, CA – “Acidimeter”
Beckman Coulter, Inc. Corporate History

1948
(Coulter) Founded by Wallace and Joe Coulter – “Coulter Principle”
Who we are?

Beckman Coulter, Inc.
Corporate History

1950
Renamed Beckman Instruments, Inc.
1997
Beckman Instruments acquires Access product line from Sanofi Diagnostics Pasteur and in November the Coulter Corporation, the leading manufacturer of *in vitro* diagnostic systems for blood cell analysis.
1998
Beckman Instruments officially changes name to Beckman Coulter, Inc.
2001
BCI launched Access 2 instrument
2003
BCI launched DxI instrument
Who we are?

Beckman Coulter, Inc.
Corporate History

2011
BCI acquired by Danaher
Who we are?

PORTFOLIO SEGMENTATION

Environmental

2010 Revenues

$2.7B

Test & Measurement

$2.8B

Dental

$1.8B

Life Sciences & Diagnostics

$2.3B
Pro-forma
$6.0B*

Industrial Technologies

$2.8B

* Includes Beckman Coulter

HACH

LANGE

for water quality

TROJAN UV

for water and water treatment

ChemTreat, Inc.

Gilbarco Veeder-Root

Tektronix

Tektronix Communications

FLUKE

FLUKE Networks

Ormodo

Kerr

Leica Microsystems

RADIOMETER

Esco Artwork

AB SCIEX

Kollmorgen

Molecular Devices

Potter & Brum

Thomson

VIDEOJET

LINX

BECKMAN COULTER

We're better together.
Segmentation of the Worldwide IVD Market
$50.6 Billion in 2012

- Immunoassay 23%
- Clinical Chemistry 18%
- Hematology 5%
- Coagulation 4%
- Nucleic Acid/Microbiology 9%
- Blood Bank 7%
- Diabetes 25%
- Other 9%

Served Market: ≈ 50%

Source: BBC 2008 Market Book
BCI Market Research and IMB Update July 16, 2012
Who we are?

Total IA Market Share
InfoDynamics 2011

- Abbott: 22%
- Beckman: 8%
- Ortho: 4%
- Roche: 7%
- Siemens: 34%
- Other: 25%

Abbott
Beckman
Ortho
Roche
Siemens
Other
Who we are?

Immunoassay Platforms

Access (Base)

Access 2

DxC 600i (Access2+DxC600)

Dxl 800

Dxl 600

Lxl (Access2+Lx20)
Who we are?

Immuonoassay Platforms

Access (Base)

Access 2

DxC 600i (Access2+DxC600)

Dxl 800

Dxl 600

Lxl (Access2+Lx20)
Who we are?

Broad and Expanding Menu

Access Cardiac
Access Thyroid
Access Specialty
Access Tumor Markers
Access Reproductive
Access Metabolism
Access Blood Virus
Access Infectious Disease
Access Adrenal/Pituitary
Access Diabetes
What do we need to develop an efficient IA?

- The correct **choice of antibody** is usually the most important initial step in ensuring the correct **performance** in an assay.

Critical needs to develop the most performant IA:

- High sensitivity
- High specificity
- High stability
- Minimal matrix effect

Critical features of antibodies:

\[ \uparrow K_D, \uparrow K_{on}, \downarrow K_{off} \]

\[ \downarrow \text{cross-reactivity} \]

\[ \downarrow K_{off} \]

Consistent behaviors

- Ideally, a high number of antibodies have to be screened as quickly as possible to find the best pair.
Octet platform

Octet RED96 System
Agenda

• Who we are?

• Use of Octet platform for diagnostic application at Beckman Coulter:
  - Selection of monoclonal antibodies with high sensitivity, low dissociation and no cross-reactivity
  - First screening of various monoclonal antibodies in order to perform a fast evaluation of critical raw materials

• Strengths and limitations
1st example: Goal

Goal: select one antibody pair able to efficiently and stably bind antigen ABC but not B or C alone

- Initial raw materials evaluated → 11 different clones = 110 antibody pairs
1\textsuperscript{st} example: Determination of $K_D$

1\textsuperscript{st} step: Loading mabs on SA-Tips

→ Preparation range of dilution at 10-5-4-3-2-1 and 0µg/mL for all biotinylated mAb

Concentrations between 2 and 4µg/mL of biotinylated mAbs were chosen as optimal loading charge on SA-Tips
1<sup>st</sup> example: Determination of $K_D$

2<sup>nd</sup> step: Determination of $K_D$

→ Biotinylated mAbs loaded at 4µg/mL and dilution of ABC from 0 to 1µM

First evaluation of each antibody behavior
All antibodies were conserved for next step
1\textsuperscript{st} example: Antibody pair screening

3\textsuperscript{rd} step: Sequential binding for classical sandwich assay
1st example: Antibody pair screening

3rd step: Sequential binding for classical sandwich assay

Fast evaluation of all antibody pairs (≈ 14h for 110 combinations)
Save material (re-use ABC Ag and capture Aby)
1st example: Antibody pair screening

3rd step: Sequential binding for classical sandwich assay

27 antibody pairs were selected as the most sensitive as well as stable in the time
1st example: Antibody pair screening

4th step: Evaluation of antibody pair cross-reactivity

Remaining 27 antibody pairs were tested on B or C alone → no signal was expected
1\textsuperscript{st} example: Antibody pair screening

4\textsuperscript{th} step: Evaluation of antibody pair cross-reactivity

Over the 27 pairs tested only 1 show cross-reactivity
As B and C are small antigens, antibody pairs specific to one antigen were eliminated during the screening on ABC
Best antibody pairs on Octet give best results on Access platform
Agenda

• Who we are?

• **Use of Octet platform for diagnostic application at Beckman Coulter:**
  
  ➢ Selection of monoclonal antibodies with high sensitivity, low dissociation and no cross-reactivity

  ➢ **First screening of various monoclonal antibodies in order to perform a fast evaluation of critical raw materials**

• Strengths and limitations
2nd example: Goal

Goal: develop an immunoassay as sensitive as possible

Need to screen as quickly as possible a large number of antibodies

- Initial raw materials evaluated → 37 different clones
2nd example: Affinity determination

1st step: Optimisation of antibody loading on SA biosensors
2nd step: Determination of $K_D$

→ Biotinylated mAbs loaded at 10µg/mL and dilution of rec Ag from 0 to 30nM


2\textsuperscript{nd} example: Antibody pair selection

- 27 different clones = 702 antibody pairs to evaluate

Classical sandwich

Initial characterization

Affinity determination

Antibody pair selection on rec Ag using Octet
2\textsuperscript{nd} example: Antibody pair selection

Antibody #1 immobilized

Initial characterization

Affinity determination

Antibody pair selection on rec Ag using Octet
2nd example: Antibody pair selection

Antibody #1 immobilized

Initial characterization
Affinity determination
Antibody pair selection on rec Ag using Octet
2nd example: Antibody pair selection

Initial characterization

Affinity determination

Antibody pair selection on rec Ag using Octet
2\textsuperscript{nd} example: Antibody pair selection

| Antibodies | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 10 | 11 | 12 | 14 | 20 | 21 | 22 | 23 | 24 | 25 | 32 | 33 | 34 | 35 | 36 | 37 | 39 | 40 | 41 | 42 | 43 |
|------------|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1          |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2          |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 3          |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 4          |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5          |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 6          |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 7          |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 10         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 11         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 12         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 13         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 14         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 20         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 21         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 22         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 23         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 24         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 25         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 32         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 33         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 34         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 35         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 36         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 37         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 39         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 44         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 45         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 46         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 47         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 48         |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

Epitope binning matrix

Elimination of 160 antibody pairs
2nd example: Antibody pair selection

Initial characterization

Affinity determination

Antibody pair selection on rec Ag using Octet

Screening on native Ag
2nd example: Antibody pair selection

Concentration of native antigen was not sufficient for screening pairs using Octet platform

Antibody selection continued using ELISA assay

Initial characterization → Affinity determination → Antibody pair selection on rec Ag using Octet → Screening on native Ag
2nd example: Antibody pair selection

Initial characterization

Affinity determination

Antibody pair selection on rec Ag using ELISA

Screening on native Ag using ELISA

Evaluation on Access platform

Concentration of native antigen was not sufficient for screening pairs using Octet platform

Antibody selection continued using ELISA assay
Agenda

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- Use of Octet platform for diagnostic application at Beckman Coulter:
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- Strengths and limitations
Strengths and limitations of Octet platform

**Strengths**

- Time saving
  - Fast elimination of antibodies based on their kinetics
  - Fast elimination of antibody pairs binding the same epitope (epitope binning/cross-reactivity)
- Material saving
- Informations not available using ELISA screening ($k_{on}$, $k_{off}$)
  - Stable antibody pairs
- Format near to Access platform
**Strengths and limitations of Octet platform**

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Time saving</td>
<td>• Modification of mAbs</td>
</tr>
<tr>
<td>→ Fast elimination of antibodies based on their kinetics</td>
<td>→ Determination of affinity using anti-Fc tips did not give acceptable results</td>
</tr>
<tr>
<td>→ Fast elimination of antibody pairs binding the same epitope (epitope binning/cross-reactivity)</td>
<td>→ Epitope binning assays required SA-biotin loading system</td>
</tr>
<tr>
<td>• Material saving</td>
<td>• Binding of second Aby can be impaired by the binding of the first Aby → Aby pair missing?</td>
</tr>
<tr>
<td>• Informations not available using ELISA screening ($k_{on}$, $k_{off}$)</td>
<td>• Concentration of material not sufficient to test native antigen</td>
</tr>
<tr>
<td>→ Stable antibody pairs</td>
<td>• Buffer conditions impacting biosensors</td>
</tr>
<tr>
<td>• Format near to Access platform</td>
<td></td>
</tr>
</tbody>
</table>
Thanks

- Laurent Michaud
- Sandie Bonjean
- Christel Cusserne

- Charlène Le Bris
Move your lab forward with Beckman Coulter.