PRODUCT DEVELOPMENT OF HUMAN DIAGNOSTICS

From idea to product that can be sold

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November 27, 2014

From idea to product 1
Euro Diagnostica – past, present and future

**Unique Products**
- CCPlus
- Rapid test (CCPoint)
- Complement Screen
- ANCA Capture
- Tumour CgA, CgB, HE4 & CA125

**Dynex collaboration**
- EuroDiagnostica kits harmonized for automation

**Clinical competences**
- Wieslab laboratory service
- Patient sera bank (>80,000 sera)

**1992**
- Ferring Diagnostica SE
- BioCarb Diagnostics SE
- EuroDiagnostics NL
- Medscand Diagnostics-MILAB SE

**Innovative complete diagnostic, prognostic and treatment solution in autoimmunity**

**High profile collaborators**
- OEM partners Roche, Euroimmune, BioRad

27 November 2014
Developing cutting edge diagnostics

- Scientific, clinical & technical **competence** within Autoimmune, Complement and Oncology diagnostics
- **Innovative** developer of diagnostic kits
  - anti-CCP, ANCA, anti-GBM
- Excellence in **ELISA technology** and application science
- Strong academic research **collaborations**
- Proprietary, innovative development **partnerships**
- World leader in **RA testing**
State-of-the-art manufacturing

- In-house antigen production
- Controlled production environment
- Modern, cost effective manufacturing
- Knowledge, capacity and infrastructure for
  - up-scaling
  - multiplex-array manufacturing

Malmö, Lundavägen 151

High customer satisfaction; complaints <0.1% of orders processed
Wieslab Service Laboratory – an essential part of ED

- Analytical service to physicians in Sweden and the world
- Unique competence and clinical experience in Autoimmunity
- Daily diagnosing of patients using own products
- The latest assays
- In-house Clinical Immunologist
- iPhone and Android application to guide testing (Wieslab)
Introduction of new products to the world

- Regulatory experience
  - 87 products CE marked
  - 37 products FDA (510K) cleared
  - CE market PoC device / test
- Skilled and growing sales force
  - DACH, Netherlands, Nordic, UK
  - Distributors world wide
EuroDiagnostica distributor presence
Main product: Rheumatoid arthritis (RA) testing

- Incidence: 1-2%
- Anti-CCP
  - Best diagnostic marker
  - Superior specificity
  - Part of diagnostic guideline
  - Negative for 25% RA patients
- Anti-TNF therapy
  - Negative for 40% of patients
- Anti-CCP 10 M tests/yr
- RF 110 M tests/yr
Principle of the anti-CCP assay

RA patients recognize peptides / proteins which contain the amino acid “citrulline”

L-arginine residue (+ charged)

peptidylarginine deiminase (PAD)

Ca^{2+}

L-citrulline residue (neutral)
Development of the anti-CCP assay

**University of Nijmegen (Prof van Venrooij)**

*2000 First generation* anti-CCP assay (**CCP1**): -
based on the epidermal protein filaggrin

*2002 Second generation* anti-CCP assay (**CCP2**): 
- random peptide sequences not derived from the epidermal protein filaggrin
- improved sensitivity (up to 80%)

Commercialization by Euro-Diagnostica
DEVELOPMENT OF IN VITRO DIAGNOSTICS
From where do we get the product ideas?

- Improvement of own existing product
- Copy competitor product – “Me too”
- From other companies or inventors
- Continuous contacts with academic research
- Own brilliant ideas
A new test idea. How do you decide?

• Customer needs: what do the doctors want
• Marketable = can we sell and how much?
• ”Extremely interesting” = unique
• Time / money / resources
• Protection: Patentable?
What does it take for your biomarker to succeed commercially?

The unmet need:
• First in class vs. right timing (end user acceptance)
• The complete solution

The competitive edge:
• Protection: Patented invention vs. Freedom-to-operate

The financial upside:
• Investment vs. Risk vs. Future Profit
A good example of timing/patents/finance

The Anti-CCP story:

Scientists at Radboud Univ., Nijmegen:

0) 1996 Collaborate with Euro Diagnostica
   File patent: (Cyclic) citrullinated peptides: (C)CPs

1) 1998 Publish: RA is characterized by early generation of anti-citrullinated protein antibodies (ACPA)

2) 1998 License CCP to Euro Diagnostica

3) 2000 Launch of CCP-ACPA ELISA

4) 2001 File patent: Ideal CCP=“CCP2”

5) 2002 Launch of CCP2-ACPA ELISA

6) 2004 B2B sales: CCP2 for other ACPA assays

Euro Diagnostica’s income from sales of all CCP products is shared with Radboud Univ. through royalty payments.

The anti-CCP test is steadily substituting the classical test for RA: *Rheumatoid Factor*

Annual no. of CCP-tests >15 Mill (RF: 50-60 Mill).

A majority is based on CCP2 from Euro Diagnostica => Commercial success
Start with a **feasibility study**: *Can it work?*

- Does it measure what it should?
- Is it practical?
- Analytical sensitivity?
- Right patient samples available?!
- Disease controls available?
- Healthy control group?
- Stability >1 year?

A clear idea how the assay will be constructed
Design control process

A procedure that ensures:

- Controlled development process
- Documented product
- Safe product
- Can be registered in various countries
- Possible to sell
Design control— a regulated process; ISO

- Project initiation form
- Project leader/owner/group
- Set specifications
- Risk management
- Main plan and subplans
- Experimental work to meet specs
- Regular review meetings

Redo if changes
Design verification

- Design input meets output
- Freeze design

Design output and transfer

- Production instructions
- Transfer to production

Validation

- Determine that end product meets user requirements

Design History File

- Compilation of all significant data and events

Post market

- Complaint handling
- Vigilance
Project initiation/management:

Let’s start! Goals / Who should do what

Executive management decision to start
Design goals well defined
Financial Resources

Project leader (practical)
Project owner (commercial)

Project team, (minimum)
Technology / Production
Marketing
Independent reviewer = quality responsible
Project plan and subplans
Define what is needed to make a marketable product:

- Technical specifications: Sensitivity / Specificity etc....
- Other customer needs: Handling / stability ...
- Regulatory plan: CE labelling / cGMP / Risk management...
- Packaging / Labelling plan
- Production transfer plan: Training / Process validation
- Parts and raw material supply
- Legal plan: Patenting / agreements / royalties
- Environmental plan
- Verification / Validation plan
- Market plan: Customer segment / Launch plan ...
- Resources / Time
Design input = Specifications

Decide what must be achieved

- Performance
  - Assay basics – time / temp / reagents
  - Sensitivity (true positive)
  - Specificity (false positive)

- Physical design
  - Adapt to existing products
Risk management at every step

In a structured manner identify and mitigate:

- Severity + Frequency = Risk
  - Patient
  - Operator
- Risk versus benefit $\Rightarrow$ acceptable risk
- ISO standards
Do the work

- Develop prototype that meets specs
- Test, test, test, ....
- Design reviews
- If it does not meet specs then reevaluate specs + risk. Still acceptable?
An assay prototype: Neolisa CGA

Peptide calibrator checked against protein standard
Check the performance

Linearity

- Excellent linearity over the range of the assay interval
- Suitable for monitoring

![Scatter Plot CgA - sample 1](image)

\[ y = 1.0072x - 14.831 \]
\[ R^2 = 0.9994 \]

![Scatter Plot - CgA sample 2](image)

\[ y = 1.0051x + 0.4243 \]
\[ R^2 = 0.9992 \]

![Scatter Plot-CgA sample 3](image)

\[ y = 1.0108x - 20.337 \]
\[ R^2 = 0.987 \]
Check the performa: NEOLISA™ Chromogranin A

Interfering Substances

- Four samples were tested for each interferent
- Ratios for spiked samples / control samples calculated

<table>
<thead>
<tr>
<th>Substance</th>
<th>Final concentration</th>
<th>Mean ratio test sample / control sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin F (free/unconjugated)</td>
<td>20.8 mg/dL</td>
<td>0.98</td>
</tr>
<tr>
<td>Bilirubin C (conjugated)</td>
<td>20.0 mg/dL</td>
<td>0.99</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>4.84 g/L</td>
<td>1.02</td>
</tr>
<tr>
<td>Chyle (lipids)</td>
<td>1430 Formazine Turbidity Units</td>
<td>1.03</td>
</tr>
<tr>
<td>HAMA</td>
<td>54-1821 ng/mL</td>
<td>0.98</td>
</tr>
<tr>
<td>RF</td>
<td>1725-4773 IU/mL (Beckman)</td>
<td>1.00</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&gt; 1500 mg/mL</td>
<td>1.02</td>
</tr>
</tbody>
</table>
Check the performance: Neolisa CGA

No hook effect i.e. no false low value with very high samples
## How to separate disease from healthy

Set cut-off – Sensitivity vs Specificity

<table>
<thead>
<tr>
<th>CD=Celiac disease (gluten intolerance); TP= True positive (specificity)</th>
<th>TN= True negative (sensitivity); CI= confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>151 CD + 120 normaler (Positive test &gt; cutoff)</td>
<td>TP rate (Sensitivity)</td>
</tr>
<tr>
<td>2.8</td>
<td>0.881</td>
</tr>
<tr>
<td>2.8</td>
<td>0.874</td>
</tr>
<tr>
<td>2.8</td>
<td>0.868</td>
</tr>
<tr>
<td>2.9</td>
<td>0.868</td>
</tr>
<tr>
<td>3.2</td>
<td>0.861</td>
</tr>
<tr>
<td>3.2</td>
<td>0.854</td>
</tr>
<tr>
<td>3.7</td>
<td>0.848</td>
</tr>
</tbody>
</table>

In this case we chose 95% specificity which gives 87% sensitivity
Separate disease from healthy: NEOLISA™ Chromogranin A

Reference intervals

A ROC curve was created based on data from 107 samples from patients with NETs and 126 blood donor samples.

At a 97.5% reference interval the upper reference limit for the Neolisa CgA assay is 108 ng/mL.
Check the competition early: NEOLISA™ Chromogranin A
Clinical performance as compared to other ELISA CgA kits*

Summary of sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) according to each manufacturer’s expected reference ranges as stated in the IFU*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NEOLISA CgA</th>
<th>Chromo-A CgA (Cisbio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut off (IFU)</td>
<td>108 ng/mL or 3.0 nmol/L</td>
<td>94 ng/mL</td>
</tr>
<tr>
<td>Sensitivity (n=43)</td>
<td>63%</td>
<td>67%</td>
</tr>
<tr>
<td>Specificity (n=32)</td>
<td>100%</td>
<td>88%</td>
</tr>
<tr>
<td>PPV (n=75)</td>
<td>100%</td>
<td>88%</td>
</tr>
<tr>
<td>NPV (n=75)</td>
<td>67%</td>
<td>67%</td>
</tr>
</tbody>
</table>

**NEOLISA™ Chromogranin A**  
Clinical performance at fixed specificity*

<table>
<thead>
<tr>
<th></th>
<th>NEOLISA CgA</th>
<th>ChromoA CgA (Cisbio)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC</strong></td>
<td>0.94</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Specificity 90%</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>88%</td>
<td>67%</td>
</tr>
<tr>
<td>Cut off</td>
<td>1.91</td>
<td>94.0</td>
</tr>
<tr>
<td><strong>Specificity 97%</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>77%</td>
<td>63%</td>
</tr>
<tr>
<td>Cut off</td>
<td>2.21</td>
<td>103.3</td>
</tr>
</tbody>
</table>

Check the performance: NEOLISA™ Chromogranin A
Analytical Stability – Flexible handling of samples

Chromogranin A positive samples can be stored at 4-8°C for up to 7 days without loss of signal.

The stability claim includes serum, heparin plasma and EDTA plasma.

Please see separate report for details.*

*Doc no: Q12_2014-06-25 Specimen Stability
Common tech problems

- Analytical sensitivity not enough
- High background, false positives
- Immunological cross reactivity
- Incubation conditions not standard
- Shortage of raw materials or samples
- Poor stability <1 year at 4C
Verification

Check and document that assay meets specifications:
   Design Input = Design Output

Test with relevant patient material.

If OK – Freeze design
Production transfer

SOP:s + specifications

Process validation => Always same performance
  Machinery settings
  Processes, time/temp/stops

Training of personnel

Quality control procedures/samples
Production process validation:
Make sure it can be produced repeatedly. Take control of all steps.

Processes
• Test extremes – lowest/ highest temp. or longest/shortest time etc.
• Stoppage during production flow
• Different lots of critical raw materials
• Repeat process 3x

Equipment
Incubators – right temperature / even temp in cabinet
Bag sealers – tight and stable seal
Product validation

Does it meet customer/clinical demands? Is it safe to use?

Different ways:

- Theoretic evaluation
- Literature
- Clinical testing on representative material
- Simulated internal testing
- Field testing = “clinical trial”

Note! On end product or equal
Regulatory demands – *in vitro* diagnostics

**Europe – CE**
Self declaration if low risk / “Notified body” if higher risk
IVD Directive 98/79/EC

**USA – FDA certification**
510(k) – Simplest but not simple. Comparison to already approved product
PMA – (Very expensive)

**Japan – Ministry of Health & Welfare**
Approximately like FDA but very slow

**China**
Tell them everything – performance and how to manufacture
FDA Requirements

A **legal** procedure => legal evidence => documentation

Several ways:

510(k) compare w already certified product

Identify a **predicate** device (already approved) to compare with:

Show equal or better

PMA, do it all from the beginning. Expensive!

+ other variants

Many guidelines to follow
FDA Study Requirements..

B: Test population:

- 100+ patients with the disease(s)
- 50+ disease controls from other patients with similar diseases likely to be tested with assay
- 100+ other disease controls
- 50+ normal

Can be modified depending on disease and risk
FDA Study Requirements..

C: Assay performance studies

• **Cutoff study.**
  Analytical sensitivity. Reference range.

• **Cutoff validation.**
  Do not include samples used in method comparison or cutoff study.

• **Interference study.**
  Demonstrate minimal interference with potentially cross-reacting substances.

• **Cross-reactivity.**
  Analyze incidence of positive results in sera containing potentially cross-reacting analytes and associated disease populations.

• **Limit of Detection.**
  Follow CLSI EP-17A “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.”
C: Performance study, continued

- **Precision.**

- **Reproducibility.**
  Qualitative (if applicable) and Lot-to-lot. Reference CLSI EP12-A2 “User Protocol for Evaluation of Qualitative Test Performance.”

- **Linearity and Recovery.**

- **Stability.**
  Provide real time (if not available, accelerated) and open kit stability along with testing protocol.
FDA Study Requirements

D: Method comparison (a quick way):

• Test patients samples on device to be submitted and predicate (already approved) device.
• Test population should include >10% specimens near the assay cutoff
• Correlation analysis; Do the assays give same/similar results?
FDA Study Requirements

E: Clinical validation study – preferred way:

• Q: Does the assay give the clinical information needed?

• Include entire test population i.e. all kinds of patients that might be tested by the physician.
## PR3-ANCA: Clinical sensitivity and specificity

<table>
<thead>
<tr>
<th>Disease and control groups</th>
<th>Total number</th>
<th>Negative &lt;10 units</th>
<th>Equivocal 10-20 units</th>
<th>Positive &gt;20 units</th>
</tr>
</thead>
<tbody>
<tr>
<td>WG:</td>
<td>39</td>
<td>3</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>MP:</td>
<td>28</td>
<td>17</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Blood donors:</td>
<td>80</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SLE:</td>
<td>19</td>
<td>18</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Others:</td>
<td>32</td>
<td>32</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Anti-GBM:</td>
<td>18</td>
<td>17</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

WG = Wegener's granulomatosis,  
MP = microscopic polyangiitis  
SLE = systemic lupus erythematosus  
Others = Rheumatoid Arthritis, Ulcerative Colitis, etc.  
GBM = glomerular basement membrane

**Clinical sensitivity (shall be detected)**  
WG = 33/36 = 91.7 %; MP = 11/28 = 39.3 %

**Clinical specificity (control groups, not detected)**  
GBM = 17/18 = 94.4 %; SLE = 18/18 = 100 %; Others = 32/32 = 100 %; Donors = 80/80 = 100 %
Final things

Instructions for use – Languages!!

= Marketing and sales material
Design history file

Compile all documentation of the construction work in a fully traceable manner

Shall always be kept up to date!
Submit for registration

- CE for all Europe – Rapid process for low risk products, self declaration.
- FDA, USA – Complex increasing demands. Several months.
- Japan – Slow, 1-2 years
- China – All details including manufacturing

Regulatory demands are increasing!
SELL!

Always: Check that sales materials incl. web site etc. follows IFU *Intended use* section
Post market surveillance

Complaints
- Failed controls
- Too low/high signals
- Etc..

Vigilance reporting – lethal or serious harm.

Investigate, correct and report to respective medical agency
Design changes

Rarely everything is perfect. Changes are necessary.

Must follow design control process!
The reward: Your product helps patients!

Example: Vasculitis

Diffuse symptoms: weakness, slight fever

Progressively worse – eventually deterioration of kidney function leads to suspicion of rapidly progressing glomerulonephritis

Test for PR3-ANCA, MPO-ANCA and anti-GBM

If positive test results start aggressive treatment directly: Plasma exchange + immunosuppression to lower autoantibody levels

Save life/kidneys
Think ahead!