Immunohistochemical stainers
Overview
Pros and Cons

Søren Nielsen
Director
NordiQC
IHC – Immunohistochemical stainizers

This lecture is meant to be a basis for an open discussion... and not an attempt to promote any stainer / company 😊
IHC – Immunohistochemical stainers

Nothing can stop automation

Rang! Rang! Rang!
Stone Age, Bronze Age, Iron Age

Rang! Rang! Rang!
Dark Age, Modern Age, Computer Age

No thanks! We are too busy

Manual
80’s

Semi-aut.
90-00’s

Fully-aut.
00-10’s

Fully-aut +
Multiplex (markers/assays)
Speed, etc

10-20’s
CHAPTER 9

THE PROS AND CONS OF AUTOMATION FOR IMMUNOHISTOCHEMISTRY FROM THE PROSPECTIVE OF THE PATHOLOGY LABORATORY

DAVID G. HICKS and LORALEE MCMAHON

2010

Part II: The Potentials and Pitfalls

Chapter 9

Automation in IHC

Ole Feldballe Rasmussen, PhD, MSc

2013
Overview of Automated Immunohistochemistry

Jeffrey W. Prichard, DO

• Context.—The increasing demand for immunohistochemistry for clinical diagnostics, in combination with an ongoing shortage of staff in the histology laboratory, has brought about a need for automation in immunohistochemistry. The current automated staining platforms vary significantly in their design and capabilities.

Objective.—To review how technology has been applied to automating the process of immunohistochemical staining.

Data Sources.—Literature review, vendor interviews, and personal practice experience.

Conclusions.—Each of the commercially available, automated immunohistochemistry platforms has strategic design differences that produce advantages and disadvantages. Understanding those differences can help match the demands of testing volumes, turnaround time, standardization, and labor savings to the appropriate automated instrumentation.

IHC – Immunohistochemical stainers

Immunohistochemical staining procedure is a multiplex technique requiring a lot of hands-on when performed manually.

From deparaffination to counterstaining the IHC procedure at minimum requires 60-100 manual interactions and handling procedure on each slide to be stained. Capacity ?? (50-100 slides pr tech.*)

Preparation – sorting, deparaffination, epitope retrieval…. Application of reagents - pipetting
Secure even distribution – ”Pap-pen”
Avoid evaporation / secure moist – staining trays

IHC – Immunohistochemical stainers

Wash – Dry – Apply
Wash – Dry – Apply
Wash....... 

Challenge: Time, Standardisation, Traceability, Skills...
IHC – Immunohistochemical stainers

Estrogen receptor;

<table>
<thead>
<tr>
<th></th>
<th>2003 08 (n=71)</th>
<th>2009 B8 (n=154)</th>
<th>2019 B27 (n=349)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>7%</td>
<td>5%</td>
<td>2%</td>
</tr>
<tr>
<td>Semi automated</td>
<td>90%</td>
<td>89%</td>
<td>14%</td>
</tr>
<tr>
<td>Fully automated</td>
<td>3%</td>
<td>6%</td>
<td>84%</td>
</tr>
</tbody>
</table>
Automation of the IHC staining procedure:

1. To secure and improve consistency of the IHC assay compared to manual performance; intra- and inter-laboratory

2. Reduce the technician workload used for IHC

3. Improve IHC testing capacity in laboratory

4. Traceability / tracking of events

Key-driver: Automation = standardization
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

Initiated in late 80’s

Semi-automated systems - No depar or HIER (not invented 😊)

A: Cadenza, Shandon  
B: TechMate, Dako  
C: ES, Ventana

Fig. 1. Automation of IHC – Principles (a) top-down capillarity, (b) ascendant capillarity, (c) flat immunohistolabelling.
IHC – Immunohistochemical stainers

Most commonly used semi-automated stainners:

Autostainer Dako (Classic, Plus, 48Link)    Autostainer LabVision (36/48/72)

Parallel processing

1. Depar / dehydration / HIER – separately to IHC e.g. PT-module
2. IHC performed by stainer – blocking of enzyme to counterstaining
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

1. To secure and improve consistency of the IHC assay compared to manual performance; intra- and inter-laboratory

2. Reduce the technician workload used for IHC

2019: Fully automated with focus on 4 core elements

- Deparaffination
- Epitope retrieval (HIER and/or proteolysis)
- IHC protocol (1 or 2 markers)
- Counterstaining

Capillary; BOND Leica, Omnis Dako, Genie Sakura
Flat labelling; BenchMark Ventana, Valent Biocare, (AS48 Dako)
IHC – Immunohistochemical stainers

Capillary gap technology stainers:

Leica: Covertiles Capillary
Dako: Glass Lid Dynamic gap
Sakura: "upside down" Capillary

Technique:
To spread reagents and to avoid slides drying out

Tissue slide + Cover
IHC – Immunohistochemical stainers

Flat labelling technology stainers:

Ventana: +Mixing +Overlay
Biocare: -Mixing -Overlay
Dako: -Mixing -Overlay

Technique:
Reagents are applied +/- mixing +/- overlay
IHC – Immunohistochemical stainers

IHC stainer platforms Melan A
NordiQC run 56, 2019
289 participants
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

1. To secure and improve consistency of the IHC assay compared to manual performance; intra- and inter-laboratory

2. Reduce the technician workload used for IHC

Functionality – Workload – Workflow - Flexibility – Costs
"If you understand the needs of your laboratory and the capabilities of the various systems, you can find the best fit for your laboratory."

"If an automated IHC platform is chosen correctly to match the demands of testing, automation can provide necessary process improvement and cost savings needed in the modern practice of pathology."

"When evaluating automated staining systems, the first thing to understand is that there is no, one “best system” on the market, for all purposes."
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

**Functionality** – Workload – Workflow - Flexibility – Costs

- Sample type – FFPE / Cytology / Frozen sections
- Baking of slides
- Deparaffination
- Pre-treatment – HIER and proteolysis
- Combined retrieval – HIER+proteolysis / proteolysis+HIER
- Continuous loading
- Batch loading
- IHC / ISH?
- Coverslipping
- Temperature controlled – slides, reagents
- Waste handling – amount, separation
- Requirement of special utensiles – containers, slides, lids
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

Functionality – **Workload – Workflow** - Flexibility – Costs

- Capacity – pr run, .. day, .. week (no of units – back-up..)
- Place, start and walk
  - Interactions required – e.g. chromogen stability
- Sequential process
  - one instrument for all steps
- Parallel process
  - e.g. one instrument for HIER, one instrument for IHC
- Batch versus continuous load of slides
  - ”Whole” working process in dept must be incorporated
- Technician ressources for maintenance
  - Frequency, extent, safety etc
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

Functionality – Workload – Workflow - **Flexibility** – Costs

- **Software**
  - Protocol set-up
    - HIER settings – time, temperature
    - Retrieval methods – single, combined
    - Adjustment of incubation times – Ab, detection, etc
    - Adjustment of incubation temp – Ab, proteolysis
    - Adjustment of protocol sequence – H₂O₂ etc
    - Adjustment of reagent volume
    - Modification of protocol steps – addition/removal
    - Washing conditions – of low affinity Abs
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

Functionality – Workload – Workflow - **Flexibility** – Costs

- **Reagents**
  - HIER reagents
    - How many and which HIER buffers are offered?
    - Can 3’ party HIER buffers be applied?
  - Proteolysis
    - Which proteolytic enzymes are offered
    - Can 3’ party enzymes be applied
  - Primary antibody
    - 3’ party antibodies?
    - RTU antibodies available?
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

Functionality – Workload – Workflow - **Flexibility** – Costs

- Detection systems
  - Can 3’ party detection system be applied?
  - Reactivity – mouse-rabbit and other species?
    - Universal (MR), mono-specific?
  - Modularity – can sensitivity be adjusted?
    - Amplification step, Linker, different systems etc
- Dual staining capabilities
  - Are different chromogens offered from vendor
  - Can 3’ party chromogens be applied?
  - Simultaneously? (mono-specific system required)
  - Sequential?
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

Functionality – Workload – Workflow - Flexibility – Costs

- Direct costs
  - Price pr instrument
  - Price pr slide
  - Preventive maintenance
- Indirect costs
  - Waste volumen
  - Daily maintenance (time used)
- "Hidden costs"
  - Down-period – what is expected and accepted ?
  - Re-runs – what is expected and accepted ?
  - Assescories needed/required
    - Empty vials for reagents, reagents, amp/linker, etc
<table>
<thead>
<tr>
<th></th>
<th>Dako AS 48</th>
<th>Dako Omnis</th>
<th>VMS Ultra</th>
<th>Leica BOND III</th>
<th>Biocare Valent</th>
<th>Sakura Genie</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capacity</strong></td>
<td>48</td>
<td>60</td>
<td>30</td>
<td>30</td>
<td>48</td>
<td>30</td>
</tr>
<tr>
<td><strong>Reagents</strong></td>
<td>64</td>
<td>60</td>
<td>35</td>
<td>36</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
<td>200 ul</td>
<td>200 ul</td>
<td>100 ul</td>
<td>150 ul</td>
<td>300 ul</td>
<td>350 ul</td>
</tr>
<tr>
<td><strong>Adjustable</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Depar.</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>HIER</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>HIER buf. 3’ party</strong></td>
<td>-</td>
<td>Yes</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Comb. ret.</strong></td>
<td>Yes</td>
<td>Yes – H+P</td>
<td>Yes</td>
<td>Yes – H+P</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td><strong>3’ party reagents</strong></td>
<td>Ab, enz, det, chr.</td>
<td>Ab, enz, det, chr.</td>
<td>Ab, enz</td>
<td>Ab, enz</td>
<td>Ab, enz, det, chr.</td>
<td>Ab</td>
</tr>
<tr>
<td><strong>Protocol flexibility</strong></td>
<td>High</td>
<td>Moderate</td>
<td>High</td>
<td>Moderate</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Any prot. / Any slide</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Seq. DS</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Sim. DS</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>ISH</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>RTU’s no</strong></td>
<td>116</td>
<td>76</td>
<td>283</td>
<td>155</td>
<td>86</td>
<td>128</td>
</tr>
<tr>
<td><strong>CDx range</strong></td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

**Note:** Some values are abbreviated or require further context for full understanding.
IHC – Immunohistochemical stainers

Manual processing induces lack of reproducibility

Automation facilities reproducibility

Compromisation of protocol is needed to handle automated processing

Certain markers are severely affected

Flexibility of automation might compensate for the impact
IHC – Immunohistochemical stainers

**Fully-automated** systems: BenchMark Ultra, Ventana

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
1. Place, start, walk
2. Flexible protocol set-up – "30 stainers"
3. Wide range of sensitivity for detection systems
4. Wide range of RTU primary antibodies – class I & III
5. IHC and ISH on same instrument / same slide
IHC – Immunohistochemical stainers

**Fully-automated** systems: BenchMark Ultra, Ventana

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
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2. Flexible protocol set-up – “30 stainners”
3. Wide range of sensitivity for detection systems
4. Wide range of RTU primary antibodies – class I & III
5. IHC and ISH on same instrument / same slide

3 main Cons:
1. Only CC1 applicable for HIER for IHC
2. Low affinity antibodies may show inferior performance
3. Maintenance time-consuming
IHC – Immunohistochemical stainers

**Fully-automated** systems: Bond-Max, Leica

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
1. Place, start, walk
2. Flexible protocol set-up – e.g. combined retr.
3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Good portfolio of RTU antibodies – plug-and-play
IHC – Immunohistochemical stainers

**Fully-automated** systems: Bond-Max, Leica

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
1. Place, start, walk
2. Flexible protocol set-up – e.g. combined retr.
3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Good portofolio of RTU antibodies – plug-and-play

3 main Cons:
1. Covertile technique – precipitates and weak hue
2. Less flexible regarding continuous start – 3 x 10 slides
3. Limited portofolio of detection systems – DAB & RED
IHC – Immunohistochemical stainers

**Fully-automated** systems: Omnis, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
1. Flexible reagent choice – HIER buffers
2. Easy to use – loading, programming
3. High capacity and high daily throughput
4. IHC and ISH on same instrument
5. Temperature controlled reagents
IHC – Immunohistochemical stainers

**Fully-automated** systems: Omnis, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
1. Flexible reagent choice – HIER buffers
2. Easy to use – loading, programming, maintenance
3. High capacity and daily throughput
4. IHC and ISH on same instrument
5. Temperature controlled reagents

3 main Cons:
1. Limited portofolio of RTUs & detection systems
2. Low affinity antibodies may show inferior performance
3. Less flexible protocol set-up
IHC – Immunohistochemical stainers

**Semi-automated** systems: AS-48, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
1. Flexible protocol set-up – e.g. combined retr.
2. Flexible reagent choice – HIER buffer, detection system
3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Good portofolio of RTU antibodies – plug-and-play
IHC – Immunohistochemical stainers

**Semi-automated** systems: AS-48, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:
1. Flexible protocol set-up – e.g. combined retr.
2. Flexible reagent choice – HIER buffer, detection system
3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Wide portofolio of RTU antibodies – plug-and-play

3 main Cons:
1. Increased manual interaction – 2 instruments needed
2. Primarily batch operation
3. High reagent volumen needed – 300 ul and >”dead-vol”
Staining issues; BenchMark, VMS – Uneven weak/neg areas – air bubbles
Staining issues; Bond, Leica – chromogen precipitates and general hue
IHC – Immunohistochemical stainers

Staining issues; Omnis, Dako – chromogen precipitates

Courtesy by Michael Bzorek

Lid Flakes

DAB Flakes (Bacteria ?)
IHC – Immunohistochemical stainers

Staining issues; AS48, Dako – chromogen depletion or reagent not spread
Consider each slide position / chamber on the IHC stainer as an individual stainer and use appropriate on-slide controls.

**PCK – slide no. 1**

**PCK – slide no. 2**

**Same reagents, same protocol, same block, same stainer**
IHC – Immunohistochemical stainers

Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

“even for automated stainers, where it cannot be guaranteed that every slide in fact receives identical treatment”.

**TABLE 3. (continued)**

<table>
<thead>
<tr>
<th>Special Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cut and submit “own on-slide control” if sending patients’ unstained slides to another laboratory for IHC testing</strong></td>
</tr>
<tr>
<td>This is difficult if the sender does not know which IHC assays will be performed or if the sender does not have dIHC laboratory and has no positive controls</td>
</tr>
<tr>
<td><strong>Use on-slide positive controls</strong></td>
</tr>
<tr>
<td><strong>Date unstained slides with on-slide controls</strong></td>
</tr>
</tbody>
</table>

dIHC indicates diagnostic immunohistochemistry; iCAPCs, immunohistochemistry critical assay performance controls; SOP, standard operating procedure.
IHC – Immunohistochemical stainers

2% error rate (452/22,234 slides)
Class I 0.8% - Class II 9.0%

An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls

Carol C. Cheung, MD, PhD, JD,* † Clive R. Taylor, MD, DPhil,‡ and Emina E. Torklevic, MD, PhD†

**TABLE 1. Categories of Failed IHC Slides**

<table>
<thead>
<tr>
<th>Failed IHC Slide Category</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>On-slide control too weak, patient tissue negative</td>
<td>Correct primary Ab was applied, but test sensitivity is possibly too low</td>
</tr>
<tr>
<td>2</td>
<td>On-slide control negative, patient tissue negative</td>
<td>Total slide failure; the result of the test does not suggest possible cause of the failure</td>
</tr>
<tr>
<td>3</td>
<td>On-slide control too weak, patient tissue weakly positive but no internal control</td>
<td>May indicate decreased technical sensitivity</td>
</tr>
<tr>
<td>4</td>
<td>On-slide control negative, patient tissue weakly positive but no internal control</td>
<td>There is uncertainty whether the correct primary Ab was applied or if there was significantly decreased sensitivity</td>
</tr>
<tr>
<td>5</td>
<td>No on-slide control, patient tissue negative</td>
<td>Uncertain results; cannot distinguish if the staining was optimal, suboptimal, or total failure</td>
</tr>
<tr>
<td>6</td>
<td>No on-slide control, patient tissue positive</td>
<td>No internal control present; lesion positive; failed only if there is uncertainty over whether the proper primary Ab was applied</td>
</tr>
<tr>
<td>7</td>
<td>Failed signal-to-noise ratio</td>
<td>Usually too high background; potential false positive, involving both patient sample and on-slide external control</td>
</tr>
<tr>
<td>8</td>
<td>Counter staining problem</td>
<td>If severe, may render result uninterpretable</td>
</tr>
<tr>
<td>9</td>
<td>Wrong protocol</td>
<td>Wrong protocol selected when &gt;1 protocol for the given primary Ab exists in the system</td>
</tr>
<tr>
<td>10</td>
<td>Uneven staining</td>
<td>Large or critical areas of the patient tissue or controls were missed by uneven staining</td>
</tr>
<tr>
<td>11</td>
<td>Wrong control</td>
<td>Either wrong tissue control or areas relevant to the test were missing (detached during staining or paraffin block with control tissue cut through)</td>
</tr>
</tbody>
</table>

**FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.**

Category 5, 6, 9, 11
Lab related (22%)

Category 1, 2, 3, 4, 7, 8, 10
Assay and/or Instrument (78%)
IHC – Immunohistochemical stainers

On-slide controls
IHC slides stained for ALK (Class II), same run, same instrument, same protocol
14/19 passed
5/19 failed

Batch-control - Theoretically:
Batch control fail by same conditions as above
0/19 passed
19/19 failed (no consistent internal control...)

Batch-control - Theoretically:
Batch control pass by same conditions as above
19/19 passed
0/19 failed (the 5 failed slides not identified....)
Automation in IHC reduces hands-on and improves consistency. However, the quality of the end result is less influenced by the function of the automated stainer compared to the impact of:

- Quality of the tissue material (pre-analytics)
  - Automation will not compensate for delayed fixation etc

- Quality of the reagents used (sensitivity, specificity – analytics)
  - Use of detection system with low sensitivity etc

- Accuracy of the technical optimization and validation of the test
  - Use of RTU formats not adequately calibrated etc

- Interpretation of the test
  - Inadequate choice of control material etc
IHC – Immunohistochemical stainers

- Accuracy of the technical optimization and validation of the test
- Use of RTU formats not adequately calibrated etc

Terminal Deoxynucleotidyl Transferase (TdT)

A comparison of Terminal Deoxynucleotidyl Transferase (TdT) Ready-to-Use antibodies from leading manufacturers on human thymus.

Novocastra BOND Ready-to-Use TdT, PA0339, clone SEN28
Vendor 1 Ready-to-Use
Vendor 2 Ready-to-Use

Leica Biosystems BOND system using BOND Ready-to-Use TdT demonstrates high quality staining when compared directly to Ready-to-Use antibodies from other leading manufacturers on serially cut sections of human thymus. Images supplied by NordiQc.

* Independent analysis commissioned by Leica Microsystems and conducted by NordiQc according to the instructions for use and on the corresponding manufacturer's staining platform.

Difference less related to stainer performance compared to focus and precision of the companies protocol set-up.
IHC – Immunohistochemical stainers

Cautions to be taken when comparing the different solutions:

E.g. cost for primary Ab – Was same or similar test conditions applied

3-step polymer vs 2-step polymer?
Incubation times?
HIER settings – time, pH, temp etc?

…..
## Immunohistochemical Stainers

### Cautions to be taken when comparing the different solutions:

*E.g. cost for primary Ab – Was same or similar test conditions applied??*

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Bond-III</th>
<th>BenchMark Ul.</th>
<th>AS-48</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER, rmAb SP1</td>
<td>1:50</td>
<td>1:100</td>
<td>1:75</td>
</tr>
<tr>
<td>Ki67, mAb MiB1</td>
<td>1:100</td>
<td>1:200</td>
<td>1:200</td>
</tr>
<tr>
<td>Bcl2, mAb 124</td>
<td>1:100</td>
<td>1:25</td>
<td>1:100</td>
</tr>
<tr>
<td>CD10, mAb 56C6</td>
<td>1:20</td>
<td>1:40</td>
<td>1:40</td>
</tr>
<tr>
<td>CK-PAN, mAb AE1AE3</td>
<td>1:75</td>
<td>1:150</td>
<td>1:100</td>
</tr>
<tr>
<td>p504s, rmAb 13H4</td>
<td>1:100</td>
<td>1:100</td>
<td>1:150</td>
</tr>
<tr>
<td>Melan A, mAb A103</td>
<td>1:50</td>
<td>1:20</td>
<td>1:50</td>
</tr>
</tbody>
</table>

- **900$** pr ml Ab: 1 ul = **0.9$**
- **1$** = **6.5 DKK**

**HIER**
- **ER2, pH 9** 20m primary 3-step pol. – refine 150 ul Ab 2.7$ pr slide
- **CC1, pH 8.5** 48m primary 3-step mul. – OptiV. 100 ul Ab 1.9$ pr slide
- **TRS, pH 9, 20m** 20m primary 3-step pol. – Flex+ 300 ul Ab 3.5$ pr slide
IHC – Immunohistochemical stainers

**Fully-automated** systems: Future ...???

Functionality – Workload – Workflow - Flexibility – Costs

To come:

1. Multi-plexing
   1. IHC/ISH – information on both protein and gene level
   2. IHC trible/quadrable staining – less sample material
2. Reduced IHC staining time – shorter TAT required
3. Integration with image analysis for quantification
   1. Staining and scanning performed on same device
4. Increased demand for traceability of staining process
IHC – Immunohistochemical stainers

Fully-automated systems: Future ...???
Conclusions:

Automation in IHC is needed primarily to secure consistency of inter- and intralaboratory results and to reduce hands-on.

There is no perfect system 😞 all have pros and cons. Each laboratory has to select the system being most applicable and favourable for the needs and demands within the laboratory.

Use other laboratories to have a more objective view on the systems offered.

A combination of different systems might be the best solution, as the IHC tests can be performed on the system giving the best technical result and lowest price – drawback workflow....