NordiQC

Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, September 19th – 21st 2016

# Immunohistochemical stainers
# Overview
# Pros and Cons

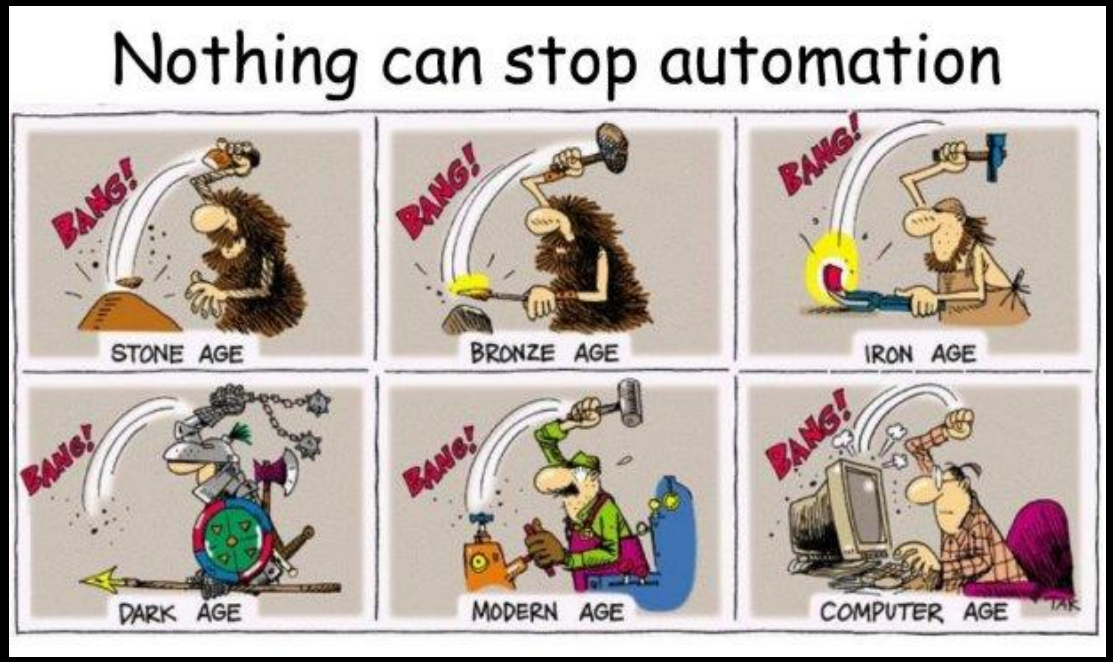
Søren Nielsen
Project coordinator & Scheme Manager
NordiQC
Aalborg University Hospital, Denmark

# IHC – Immunohistochemical stainers

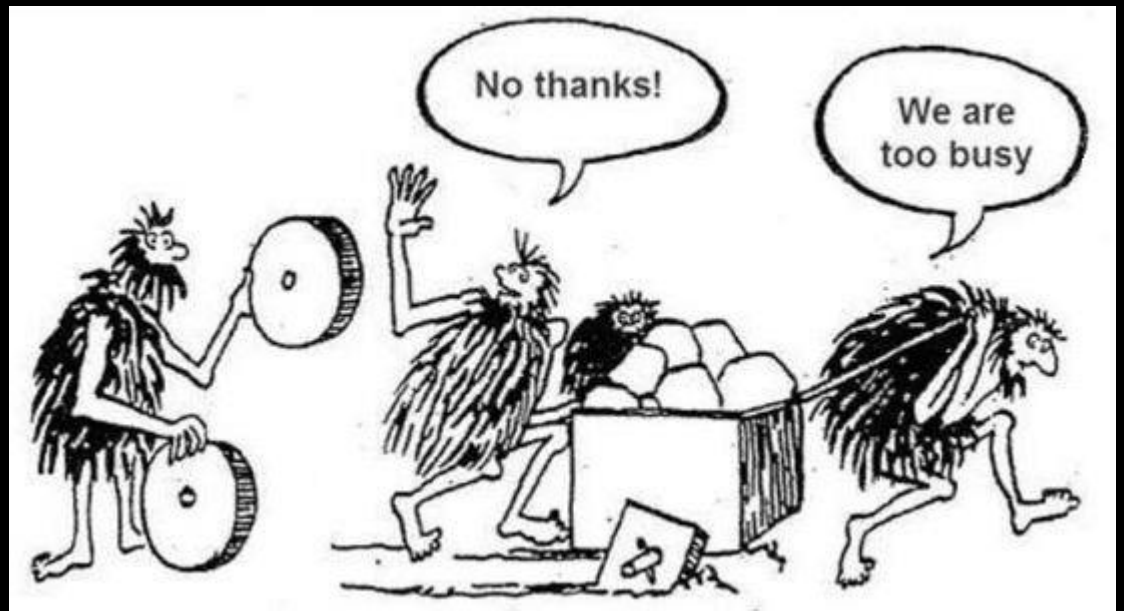
This lecture is meant to be a basis for an open discussion…
and not an attempt to promote any stainer / company ☺

Photo by B.A. Rupert. Green Bay Press-Gazette

# IHC – Immunohistochemical stainers







# IHC – Immunohistochemical stainers

ELSEVIER

Clinica Chimica Acta 278 (1998) 185–192

## Comparative evaluation of automated systems in immunohistochemistry

T. Le Neel[a], A. Moreau[b], C. Laboisse[b], A. Truchaud[a,*]

[a]*Engineering Laboratory, Institute of Biology, 9 Quai Moncousu, 44093 Nantes, France*
[b]*Laboratory of Pathology, Institute of Biology, 9 Quai Moncousu, 44093 Nantes, France*

**Product** focus

*www.mlo-online.com/articles/200801/0108product_focus.pdf*

## A review of automated slide stainers for IHC and ISH

By Joe Myers, MS, CT(ASCP)

2: http://www.mlo-online.com/articles/201207/automated-ihc-ish-slide-staining-systems.php

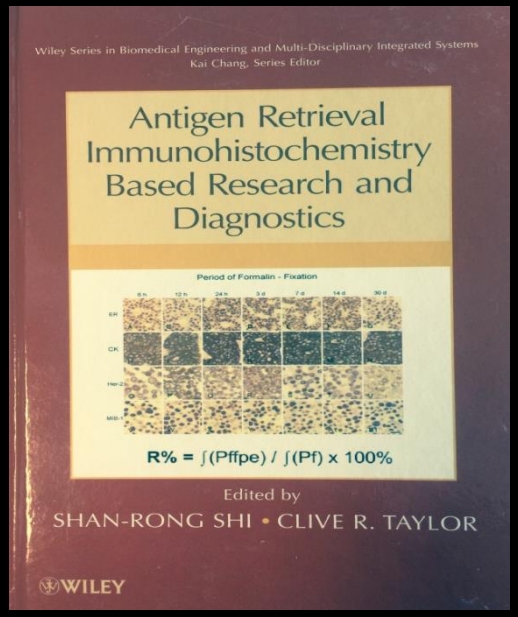
# IHC – Immunohistochemical stainers

CHAPTER 9

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

2010

DAVID G. HICKS and LORALEE MCMAHON




Part II: The Potentials and Pitfalls

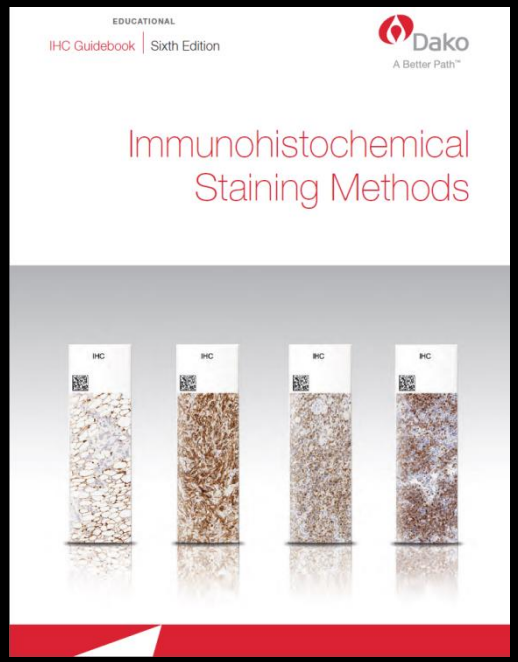
Dako
A Better Path™

Chapter 9

Automation in IHC

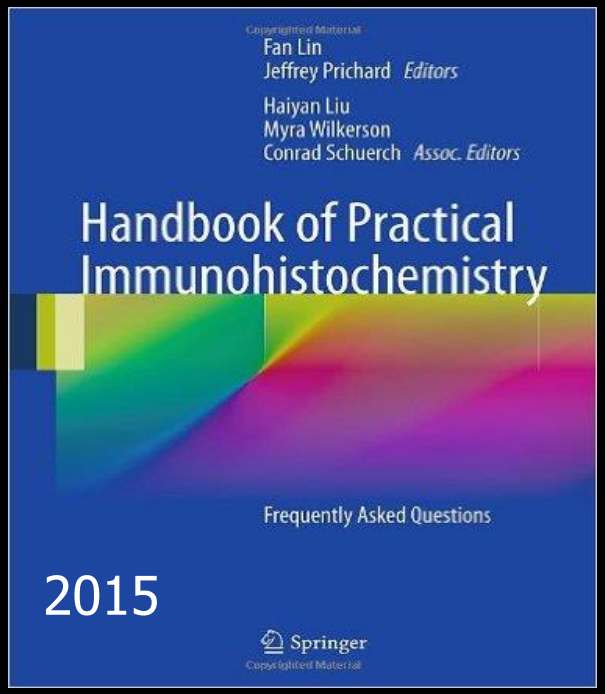
**Ole Feldballe Rasmussen**, PhD, MSc

2013



# IHC – Immunohistochemical stainers






## 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.



(*Arch Pathol Lab Med.* 2014;138:1578–1582; doi: 10.5858/arpa.2014-0083-RA)

# 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.**)

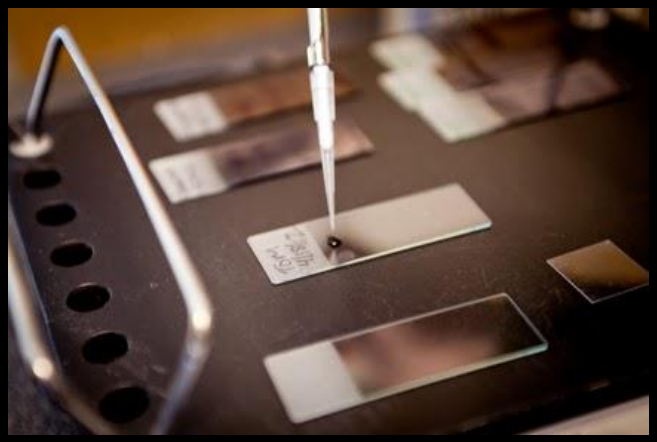
Preparation – sorting, deparaffination, epitope retrieval….
Application of reagents - pippetting

Secure even distribution – ”Pap-pen”

Avoid evaporation / secure moist – staining trays

* Haines DM, Chelack BJ. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin- fixed paraffin-embedded tissue for diagnostic pathology. J Vet Diagn Invest 1991; 3:101-12

# IHC – Immunohistochemical stainers

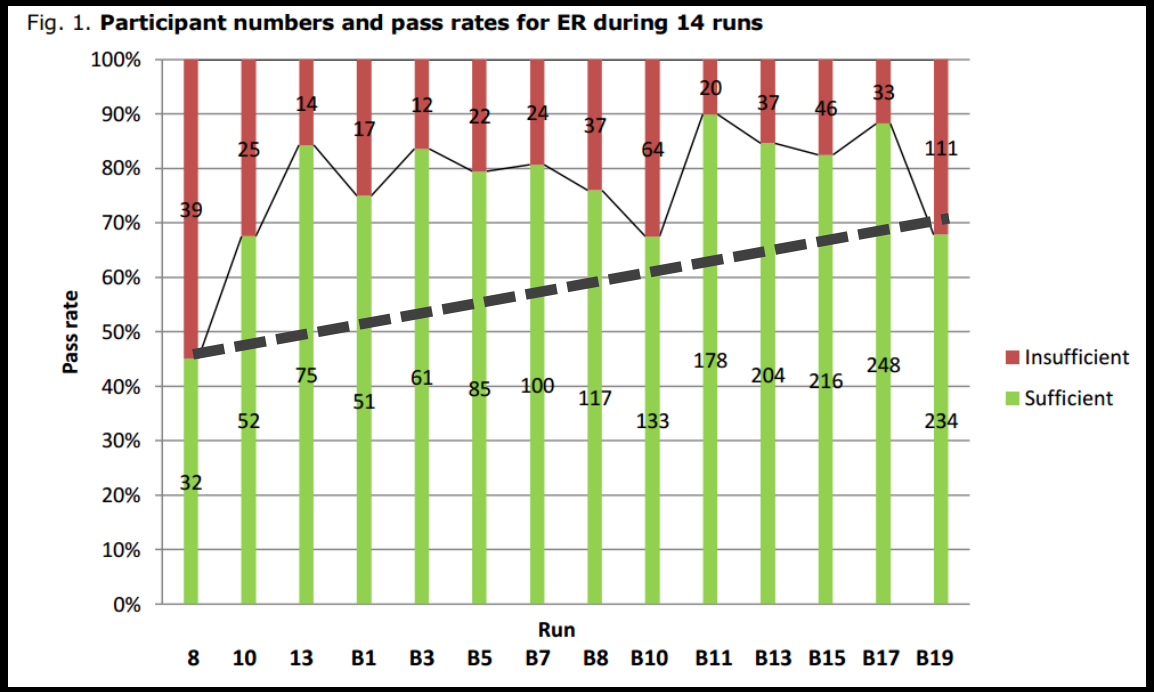



Wash – Dry – Apply
Wash – Dry – Apply
Wash…….

Challenge: Time, Standardisation, Traceability, Skills…

# IHC – Immunohistochemical stainers

## Estrogen receptor;




| | 2003<br>B8 (n=154) | 2015<br>B19 (n=345) |
|---|---|---|
| Manual performance | 5% | 4% |
| Semi automated system | 89% | 32% |
| Fully automated system | 6% | 64% |

# 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

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 (invented ☺)

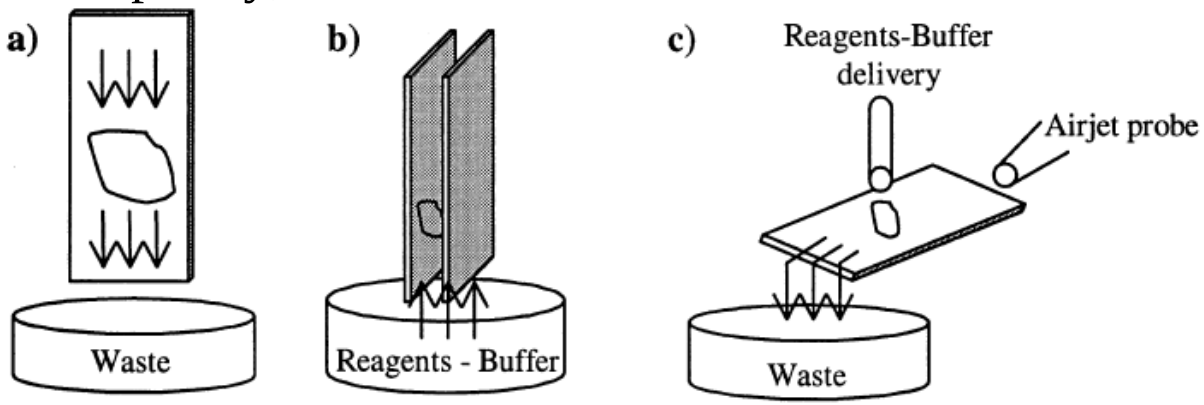
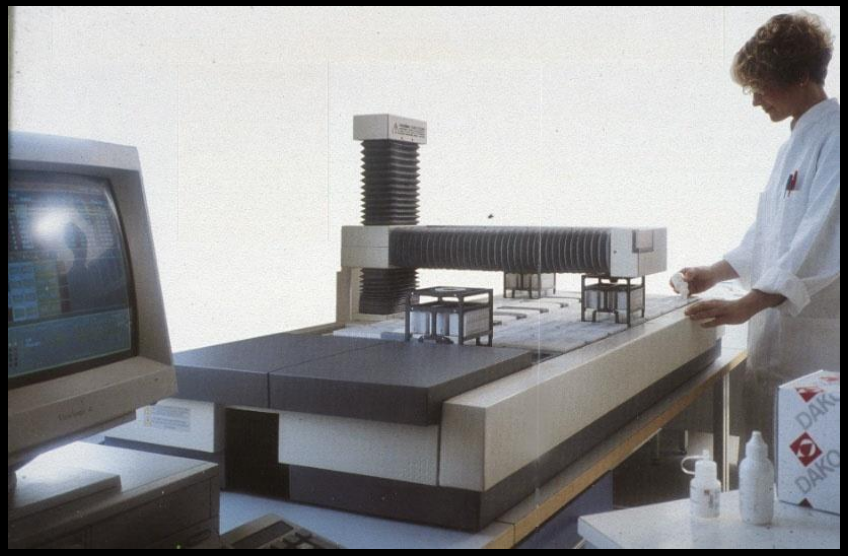



Fig. 1. Automation of IHC – Principles (a) top-down capillarity, (b) ascendant capillarity, (c) flat immunohistolabelling.

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

# IHC – Immunohistochemical stainers

Can be purchased on:
http://www.imebinc.com
App 2.000 $...



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

# IHC – Immunohistochemical stainers

Most commonly used semi-automated stainers:

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

2015: Fully automated with focus on 4 core elements

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

*Capillary; BOND Leica, OMNIS Dako*
*Flat labelling; BenchMark Ventana, Autostainer Dako / Thermo*

# 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



# IHC – Immunohistochemical stainers

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”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

- 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
- Etc

# 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 – $H_20_2$ 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 bufferes are offered ?
    - Can 3’ party HIER bufferes be applied ?
  - Proteolysis
    - Which proteolytic enzymes are offered
    - Can 3’ party enzymes be applied
  - Primary antibody
    - 3’ party antibodies ?
    - RTU antibodies available ?

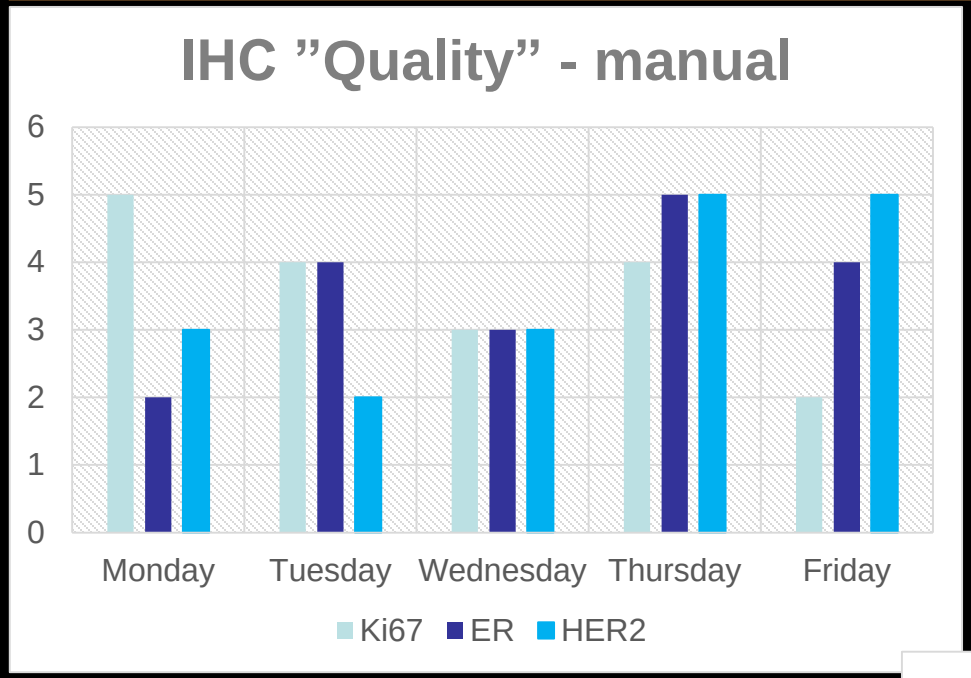
# IHC – Immunohistochemical stainers

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



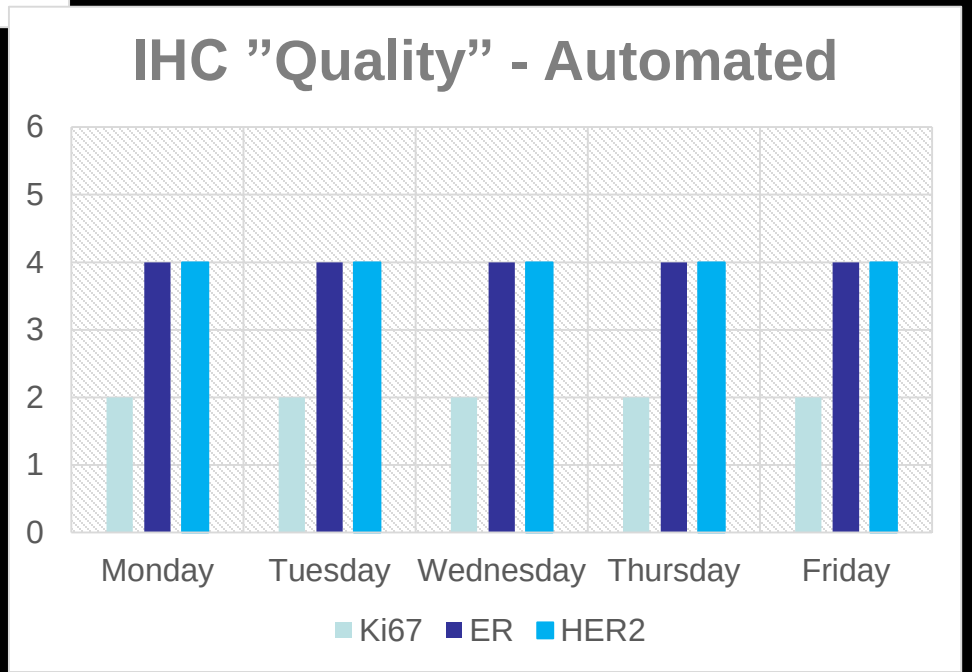

Manual processing induces lack of reproducibility

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

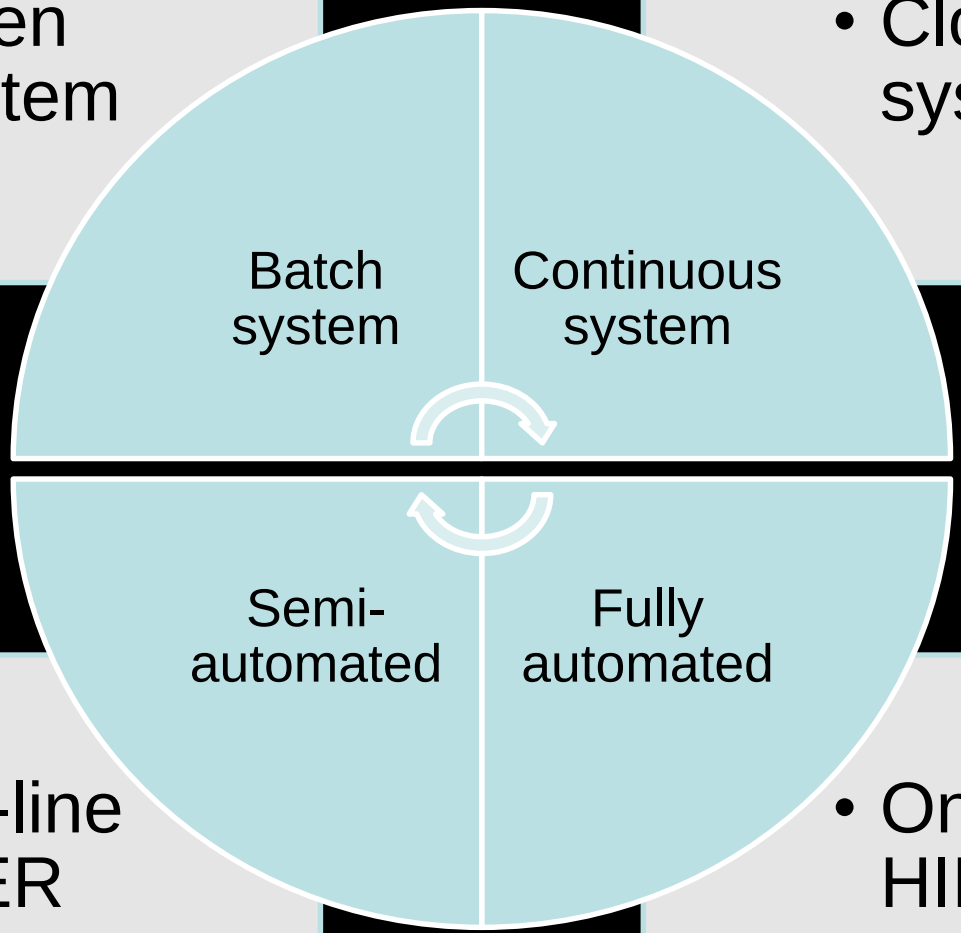
Automation of the IHC staining procedure:

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 ?
  - Assesscories needed/required
    - Empty vials for reagents, reagents, amp/linker, etc

# IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:




"Low costs"

"High costs"

High level of flexibility

High level of automation

# IHC – Immunohistochemical stainers

| | Autost. Dako/TFS | Intellip. Biocare | Oncore Biocare | Impath Pathc. | BOND III Leica | Bench. U VMS | Omnis Dako |
|---|---|---|---|---|---|---|---|
| Capacity | 48/36-72 | 50 | 36 | 36 | 30 | 30 | 60 |
| Reagents | 64 | 48 | 40 | 40 | 36 | 35 | 60 |
| Volume | 200 ul | 300 ul | 200 ul | 200 ul | 150 ul | 100 ul | 200 ul |
| Adjustab. | Yes | Yes | ? | ? | Yes | No | No |
| Depar. | No | No | Yes | Yes | Yes | Yes | Yes |
| HIER | No | No | Yes | Yes | Yes | Yes | Yes |
| HIER buf.<br>3’ part | -<br>Yes | -<br>Yes | 2<br>No | 2<br>No | 2<br>No | 2<br>No | 5<br>Yes |
| Comb ret | Yes | Yes | ? | ? | Yes – H+P | Yes | Yes - H+P |
| 3’part reagents | Ab, enz, det.,chr. | Ab, enz, det.,chr | Ab | No | Ab, enz | Ab, enz | Ab, enz, ,chr. |
| Any prot Any slide | Yes | Yes | Yes | Yes | No | Yes | No |
| Seq. DS | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Sim. DS | Yes | Yes | ? (Yes) | ? (Yes) | No | No | Yes |
| ISH | No | No | (Yes) | (Yes) | Yes | Yes | Yes |

# IHC – Immunohistochemical stainers

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

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:

1. Place, start, walk
2. Continuous and/or batch loading – ”30 stainers”
3. Flexible protocol set-up – e.g. combined retr.
4. Wide range of sensitivity for detection systems
5. IHC and ISH on same instrument / same slide..

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3 main Cons:

1. Only CC1 applicable for HIER for IHC
2. Low affinity antibodies mayl 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. Wide portofolio of RTU antibodies – plug-and-play

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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

**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
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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”

# 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 and protocols

# IHC – Immunohistochemical stainers

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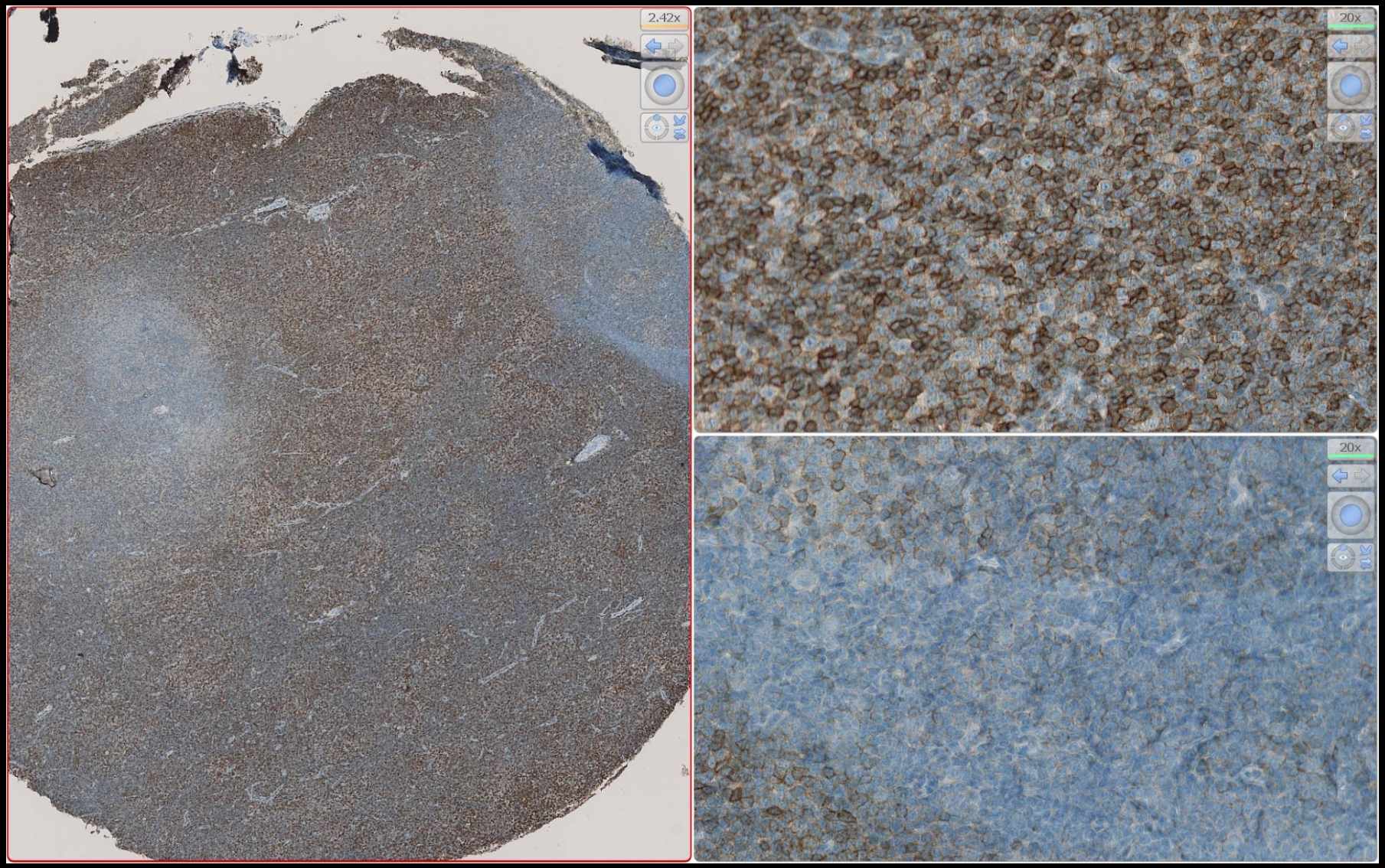
5 main Pros:

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 and protocols

3 main Cons:

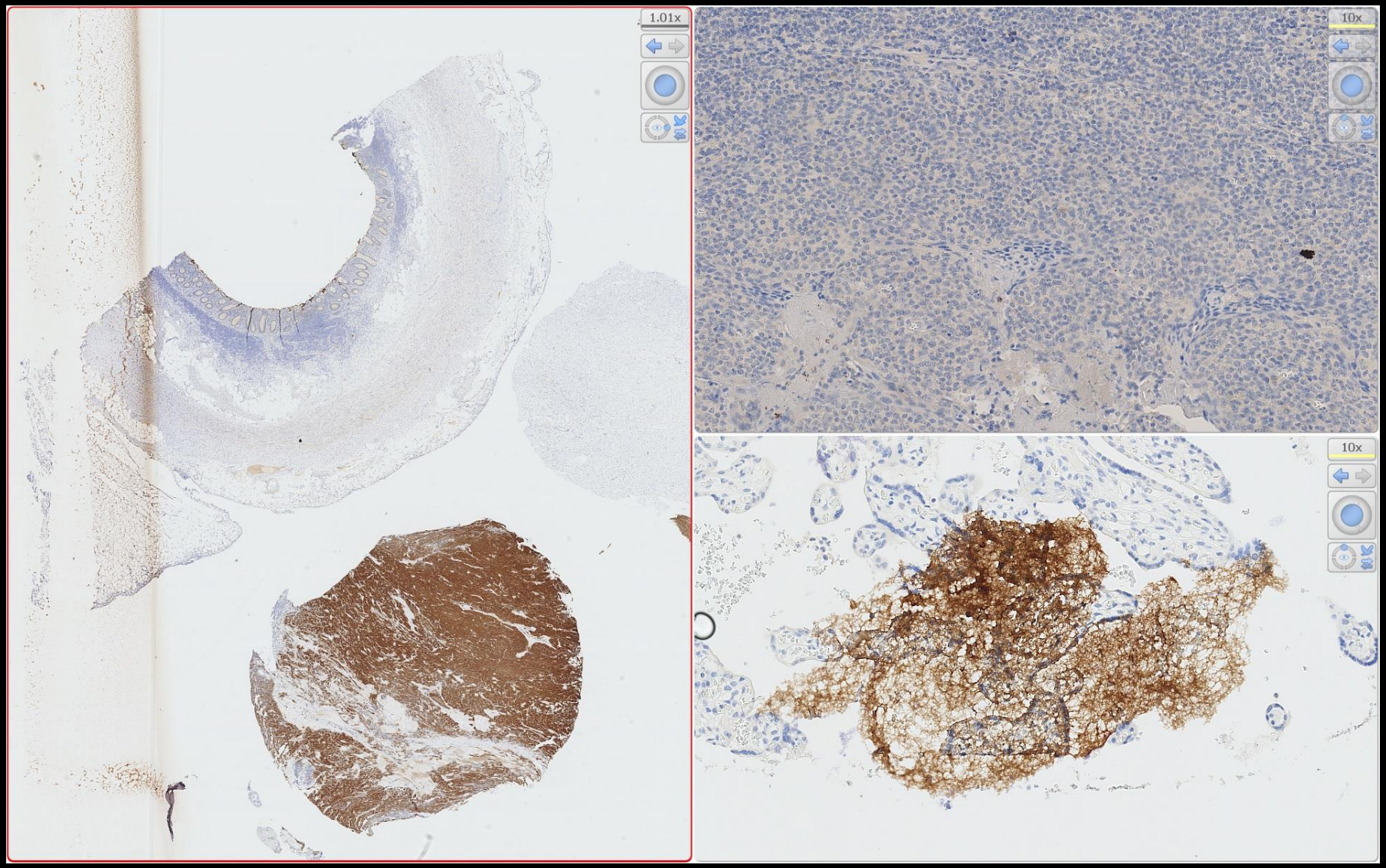
1. Limited portofolio of RTUs & detection systems
2. Limited experiences from the field – still new….
3. Less flexible protocol set-up

# IHC – Immunohistochemical stainers



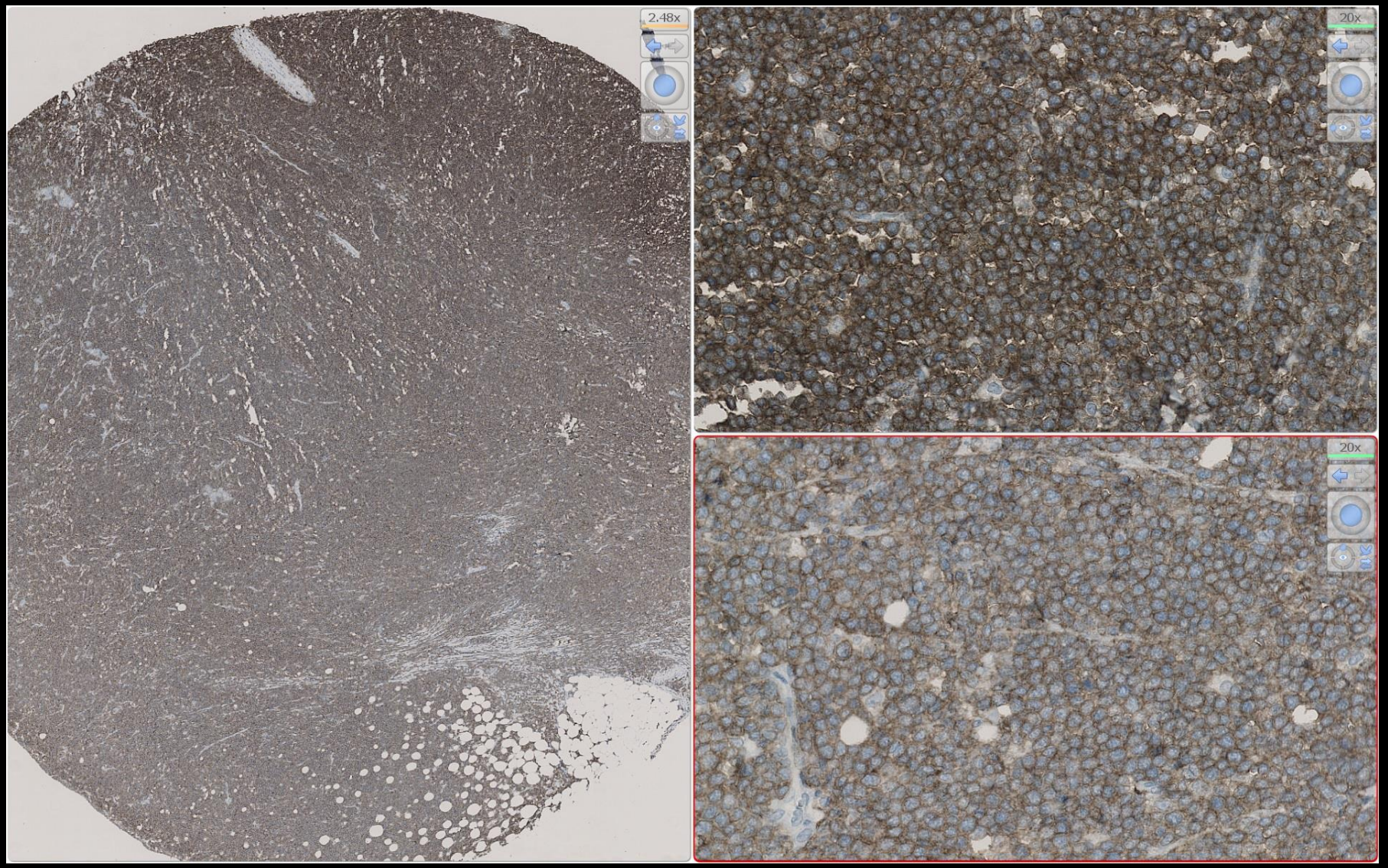

Staining issues; BenchMark, VMS – Uneven weak/neg areas – air bubbles

# IHC – Immunohistochemical stainers



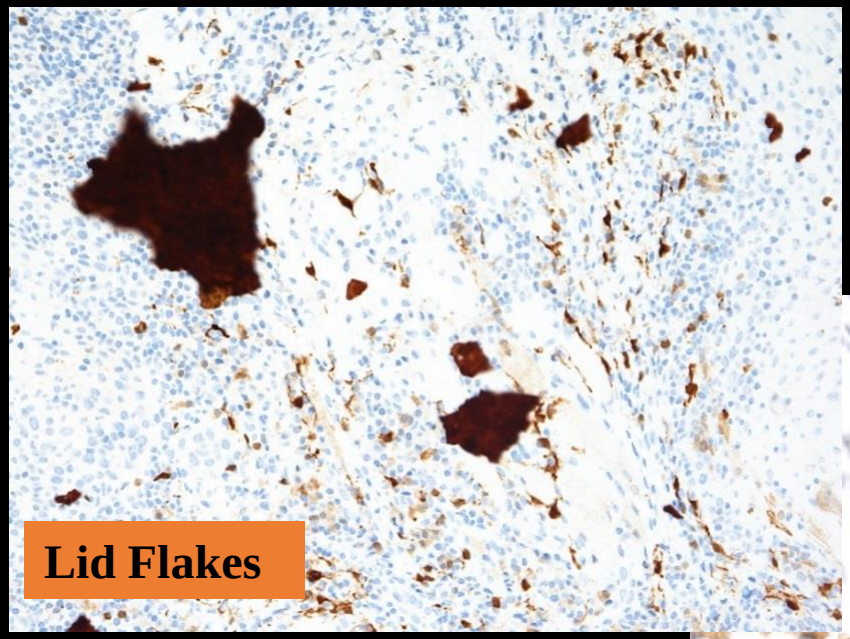

Staining issues; Bond, Leica – chromogen precipitates and general hue

# IHC – Immunohistochemical stainers

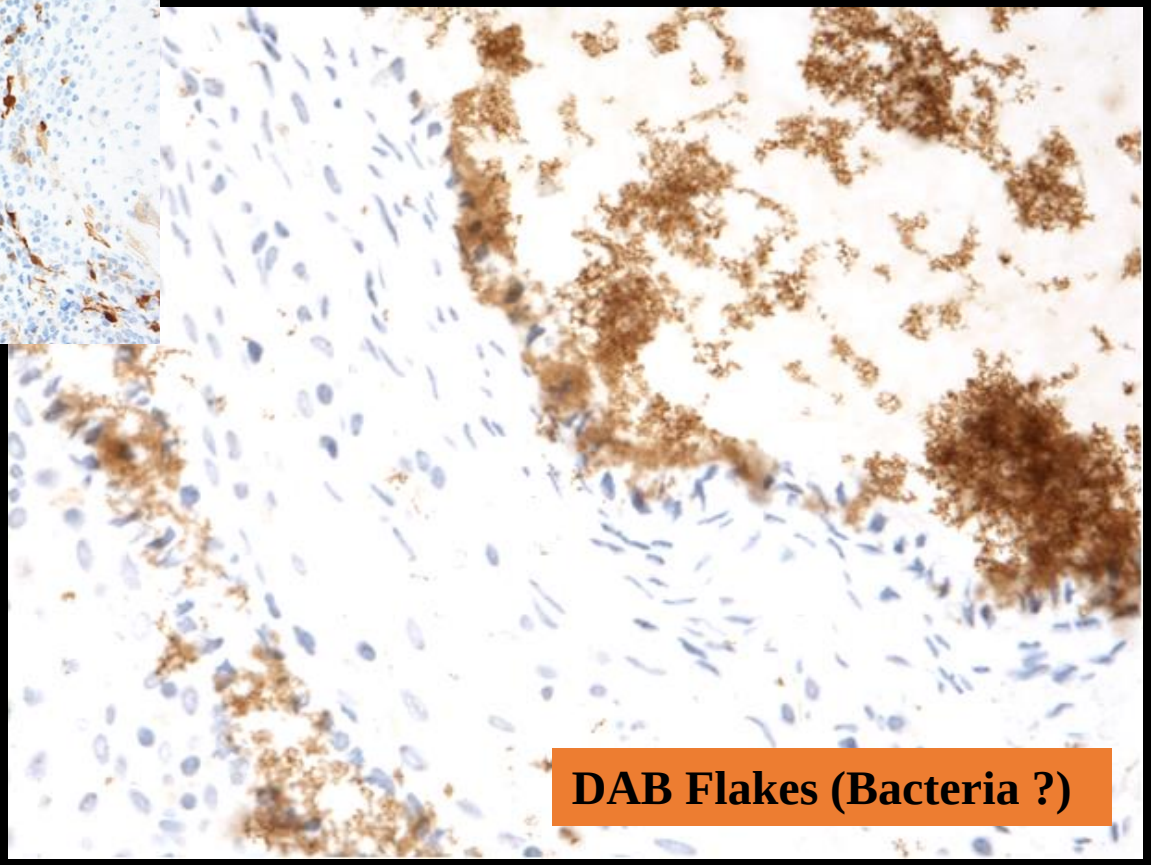



Staining issues; AS48, Dako – chromogen depletion or reagent not spread

# IHC – Immunohistochemical stainers



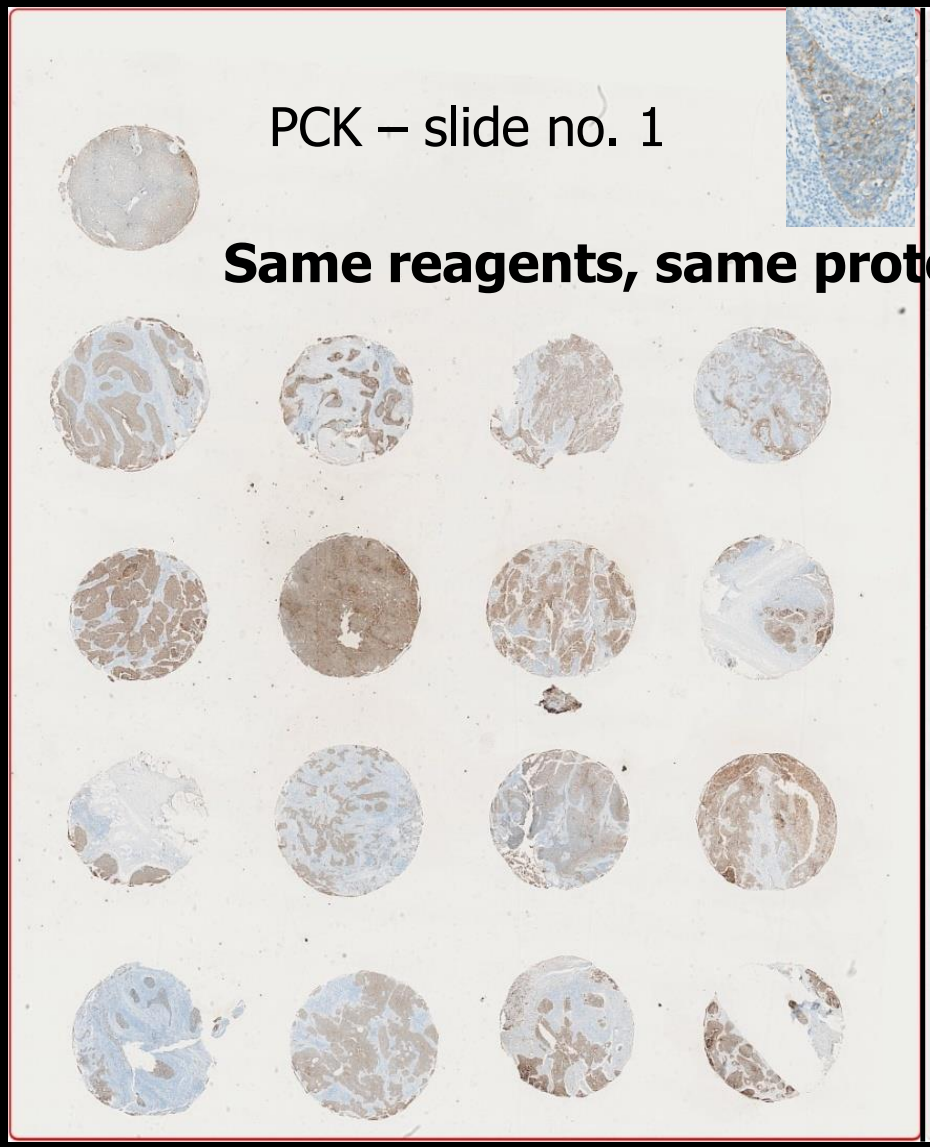

Courtesy by Michael Bzorek

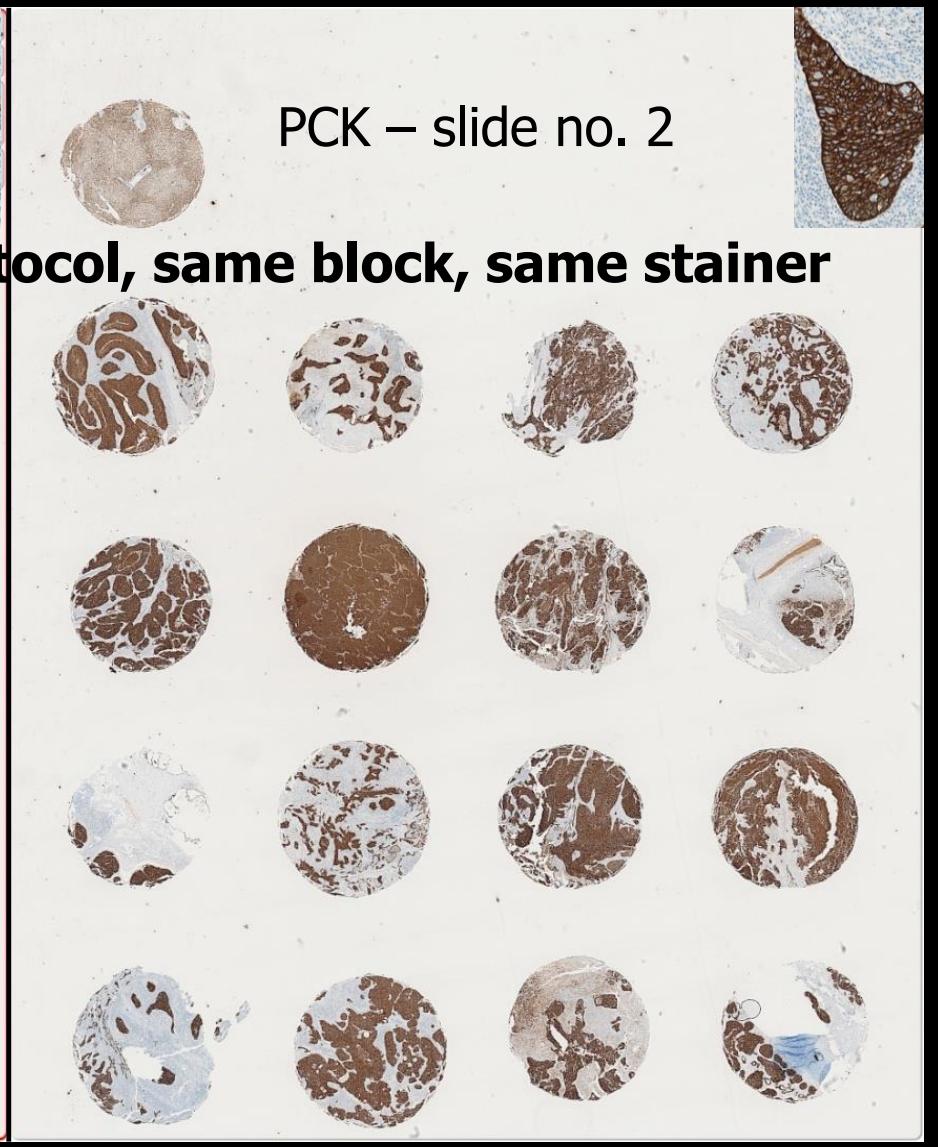



Staining issues; Omnis, Dako – chromogen precipitates

# IHC – Immunohistochemical stainers

Consider each slide position / chamber on the IHC stainer as an individual stainer and use appropriate on-slide controls




**Same reagents, same protocol, same block, same stainer**

# IHC – Immunohistochemical stainers

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

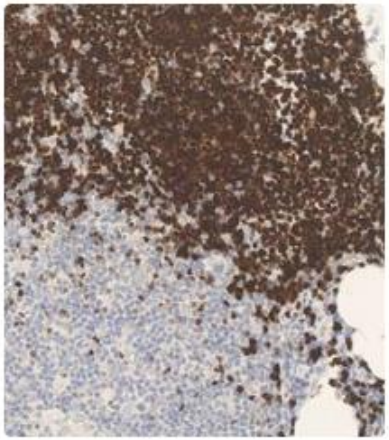
- Interpretation of the test
  - Inadequate choice of control material etc

# IHC – Immunohistochemical stainers

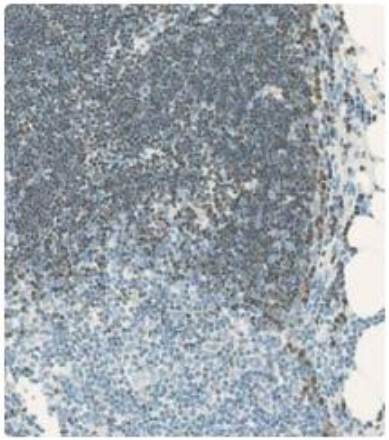
- Accuracy of the technical optimization and validation of the test
  - Use of RTU formats not adequately calibrated etc

## Terminal Deoxynucleotidyl Transferase (TdT)

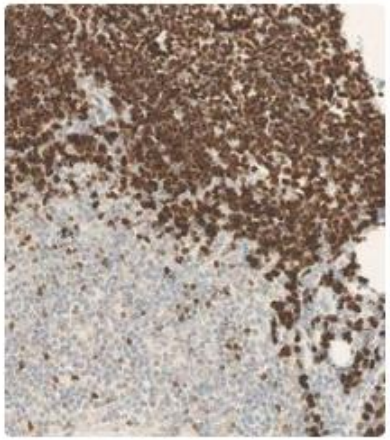
A comparison of Terminal Deoxynucleotidyl Transferase (TdT) Ready-to-Use antibodies from leading manufacturers on human thymus.



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.

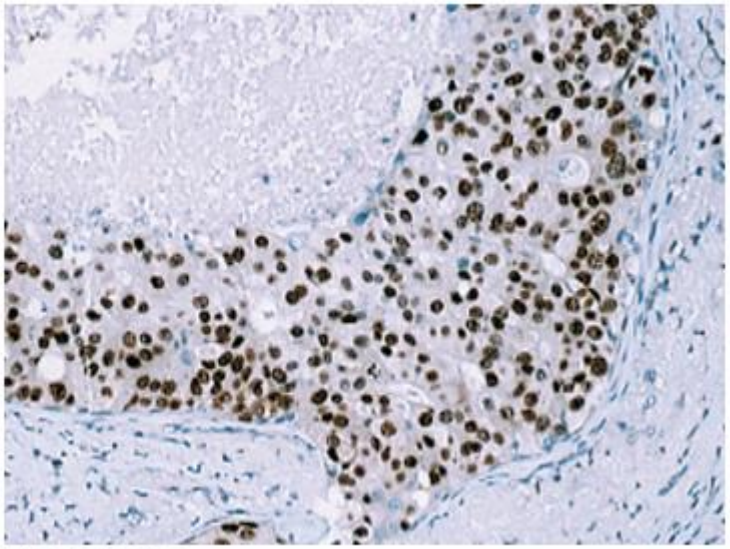
# IHC – Immunohistochemical stainers

Cautions to be taken when comparing the different solutions:

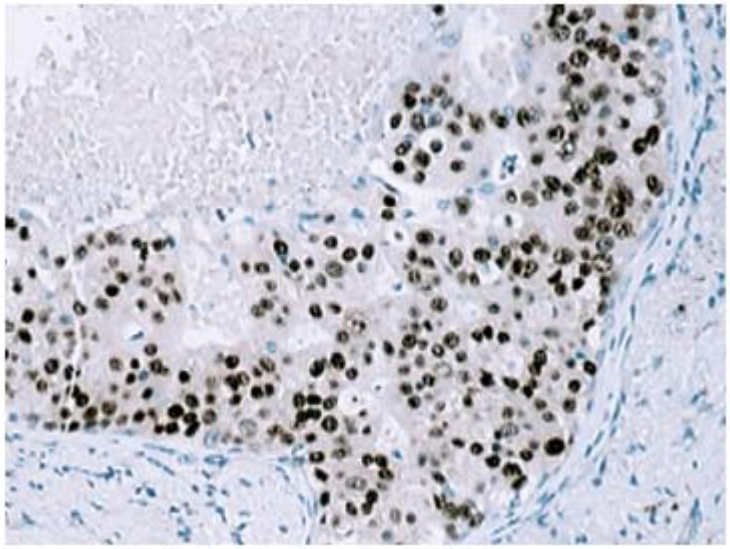
E.g. cost for primary Ab – Was same or similar test conditions applied

40X difference

COMPARE MACH 4 (L) WITH LEADING COMPETITOR (R) FOR ER (1D5) STAINING.



MACH 4, 1:1000 dilution



Leading competitor using dextran backbone polymer, 1:25 dilution

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

# 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 ??

| | Bond-III | BenchMark Ul. | AS-48 |
|---|---|---|---|
| ER, rmAb SP1 | 1:50 | 1:100 | 1:75 |
| Ki67, mAb MiB1 | 1:100 | 1:200 | 1:200 |
| Bcl2, mAb 124 | 1:100 | 1:25 | 1:100 |
| CD10, mAb 56C6 | 1:20 | 1:40 | 1:40 |
| CK-PAN, mAb AE1AE3 | 1:75 | 1:150 | 1:100 |
| p504s, rmAb 13H4 | 1:100 | 1:100 | 1:150 |
| Melan A, mAb A103 | 1:50 | 1:20 | 1:50 |
| 900$ pr ml Ab:1 ul = 0.9$<br>1$ = 6.5 DKK | HIER ER2, pH 9 20m<br>20m primary<br>3-step pol. – refine<br>150 ul Ab<br>2.7$ pr slide | HIER CC1,pH 8.5 48m<br>32m primary<br>3-step mul. – OptiV.<br>100 ul Ab<br>1.9$ pr slide | HIER TRS,pH 9, 20m<br>20m primary<br>3-step pol. – Flex+<br>300 ul Ab<br>3.5$ pr slide |

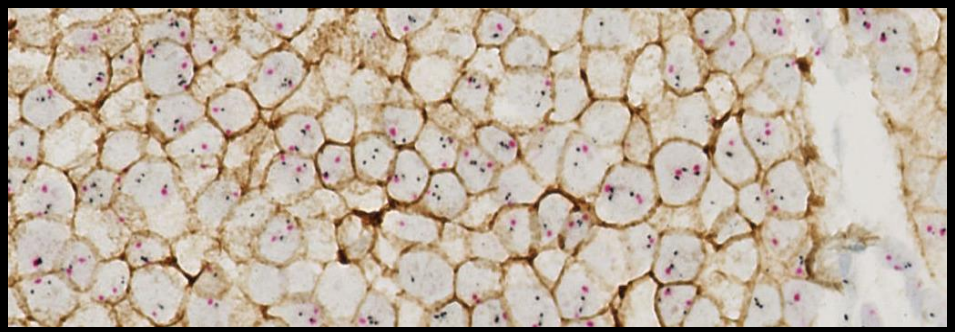
# IHC – Immunohistochemical stainers

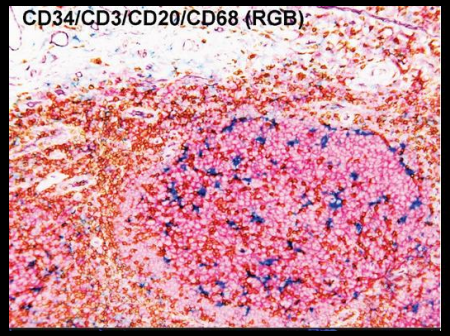
**Fully-automated** systems: Future …???

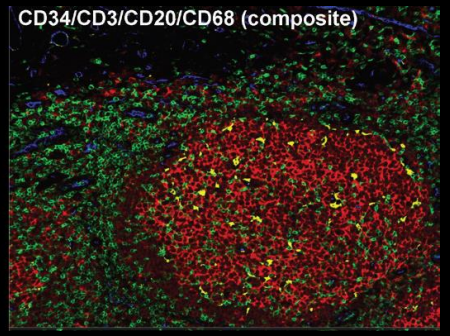
Functionality – Workload – Workflow - Flexibility – Costs

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

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….

# IHC – Immunohistochemical stainers