

Workshop in Diagnostic Immunohistochemistry Aalborg University Hospital, October 2nd-4th 2019

Immunohistochemical stainers Overview Pros and Cons

Søren Nielsen Director NordiQC



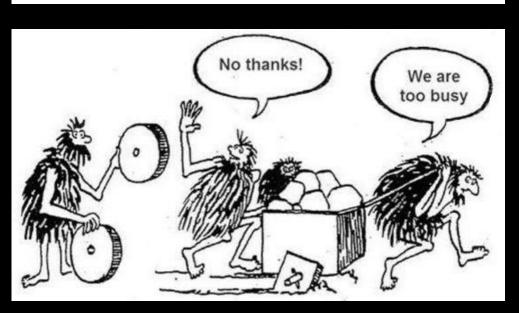
This lecture is meant to be a basis for an open discussion... and not an attempt to promote any stainer / company ©





Nothing can stop automation











Semi-aut.



Fully-aut.



Fully-aut +

Multiplex (markers/assays) Speed, etc



CHAPTER 9

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

DAVID G. HICKS and LORALEE MCMAHON

2010

Part II: The Potentials and Pitfalls

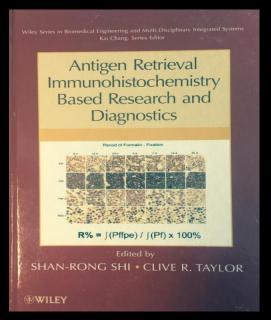


Chapter 9

Automation in IHC

Ole Feldballe Rasmussen, PhD, MSc

2013

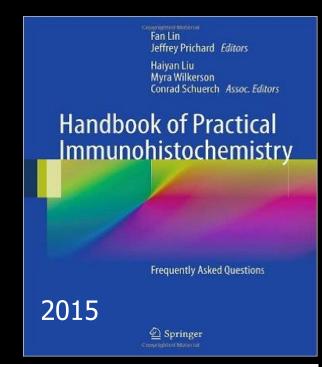








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Overview of Automated Immunohistochemistry

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)



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

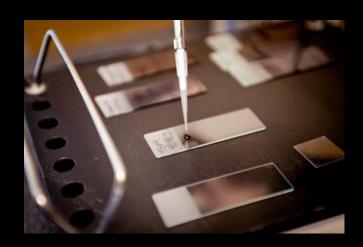
^{*} Haines DM, Chelack BJ. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin- fixed paraffin-embedded tissue for diagnostic pathology. J Vet Diagn Invest 1991; 3:101-12







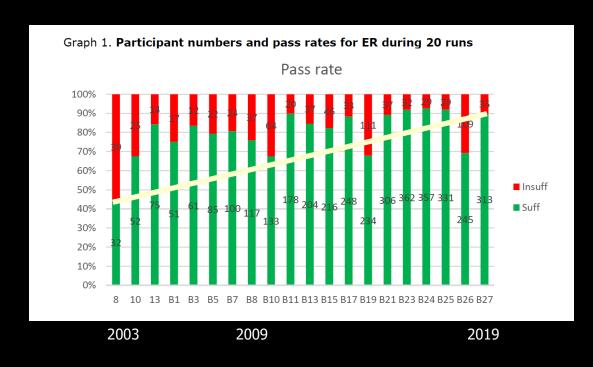




Challenge: Time, Standardisation, Traceability, Skills...



Estrogen receptor;



	2003 08 (n=71)	2009 B8 (n=154)	2019 B27 (n=349)
Manual	7%	5%	2%
Semi automated	90%	89%	14%
Fully automated	3%	6%	84%



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



Automation of the IHC staining procedure:

Initiated in late 80's

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

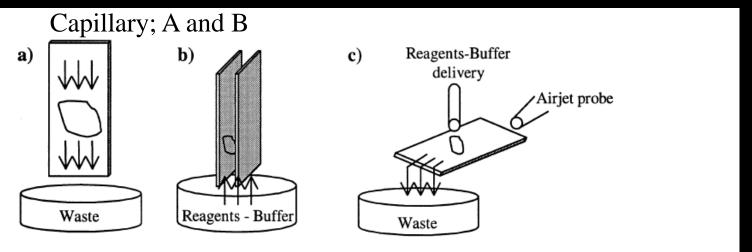


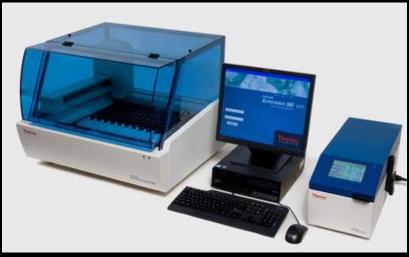
Fig. 1. Automation of IHC – Principles (a) top-down capillarity, (b) ascendant capillarity, (c) flat immunohistolabelling.

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



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



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)



Capillary gap technology stainers:

















Sakura: Glass Lid "upside down" Capillary

Technique;

To spread reagents and to avoid slides drying out

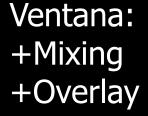




Flat labelling technology stainers:













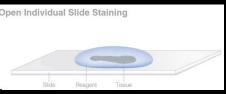




Dako:
-Mixing
-Overlay

Technique;

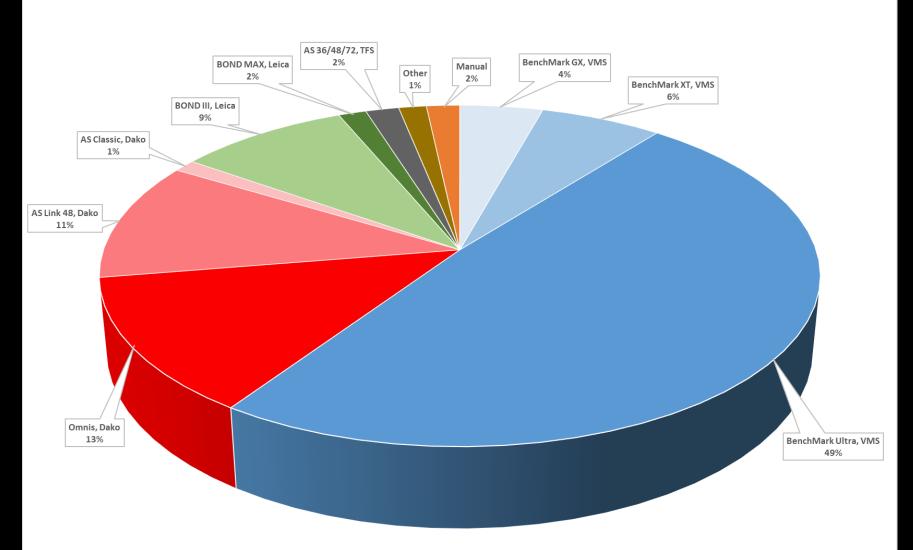
Reagents are applied +/- mixing +/- overlay



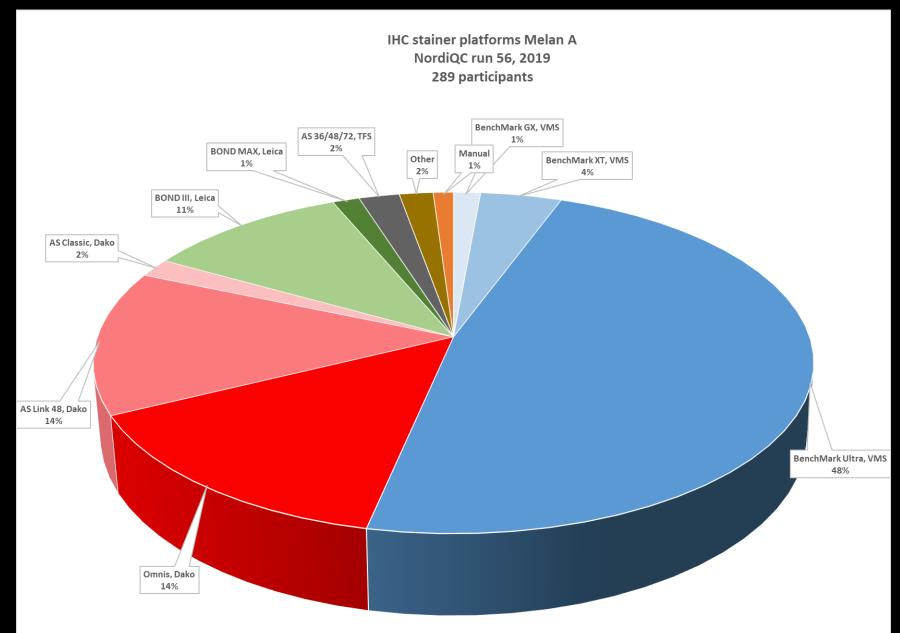














Automation of the IHC staining procedure:

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- 2. Reduce the technician workload used for IHC













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10.5858/arpa.2014-0083-RA)

"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."



Automation of the IHC staining procedure:

- 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



Automation of the IHC staining procedure:

- 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



Automation of the IHC staining procedure:

- 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



Automation of the IHC staining procedure:

- 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 ?



Automation of the IHC staining procedure:

- 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 ?



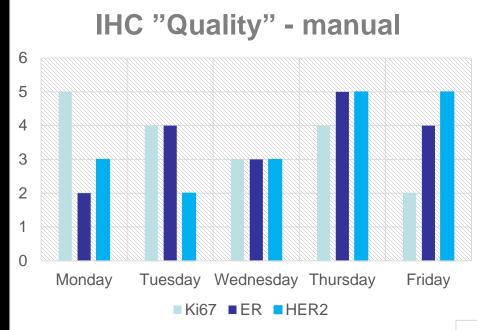
Automation of the IHC staining procedure:

- 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.



	Dako AS 48	Dako Omnis	VMS Ultra	Leica BOND III	Biocare Valent	Sakura Genie
Capacity	48	60	30	30	48	30
Reagents	64	60	35	36	44	39
Volume	200 ul	200 ul	100 ul	150 ul	300 ul	350 ul
Adjustable	Yes	No	No	Yes	Yes	No
Depar.	No	Yes	Yes	Yes	Yes	Yes
HIER	No	Yes	Yes	Yes	Yes	Yes
HIER buf. 3' party	- Yes	5 Yes	2 No	2 No	2 No	2 No
Comb. ret.	Yes	Yes – H+P	Yes	Yes – H+P	Yes	?
3' party reagents	Ab, enz, det, chr.	Ab, enz, det, chr.	Ab, enz	Ab, enz	Ab, enz, det, chr.	Ab
Protocol flexibility	High	Moderate	High	Moderate	High	Low
Any prot. / Any slide	Yes	No	Yes	No	Yes	Yes
Seq. DS	Yes	Yes	Yes	Yes	Yes	Yes
Sim. DS	Yes	Yes	No	No	Yes	No
ISH	No	Yes	Yes	Yes	No	No
RTU's no	116	76	283	155	86	128
CDx range	High	Low	High	Low	None	None





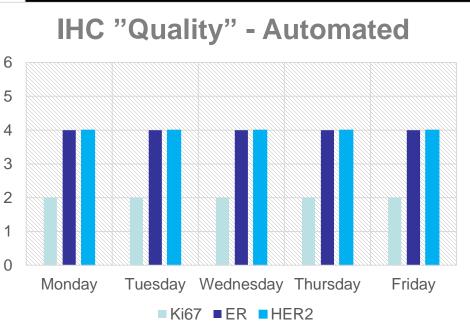
Manual processing induces lack of reproducibility

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





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



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3 main Cons:

- 1. Only CC1 applicable for HIER for IHC
- 2. Low affinity antibodies may show inferior performance
- 3. Maintenance time-consuming



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



Fully-automated systems: Bond-Max, Leica

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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



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



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



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



Semi-automated systems: AS-48, Dako

Functionality – Workload – Workflow - Flexibility – Costs

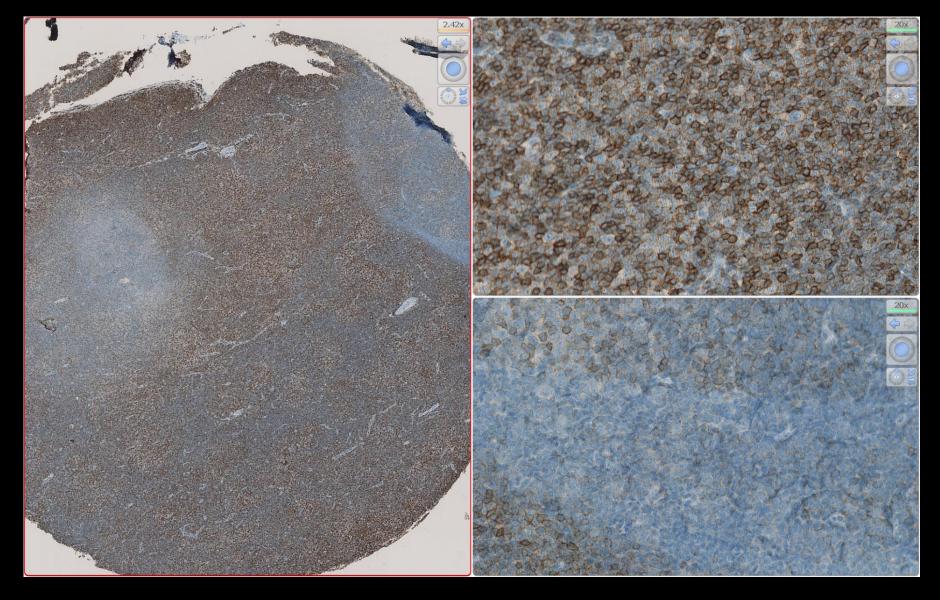
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3 main Cons:

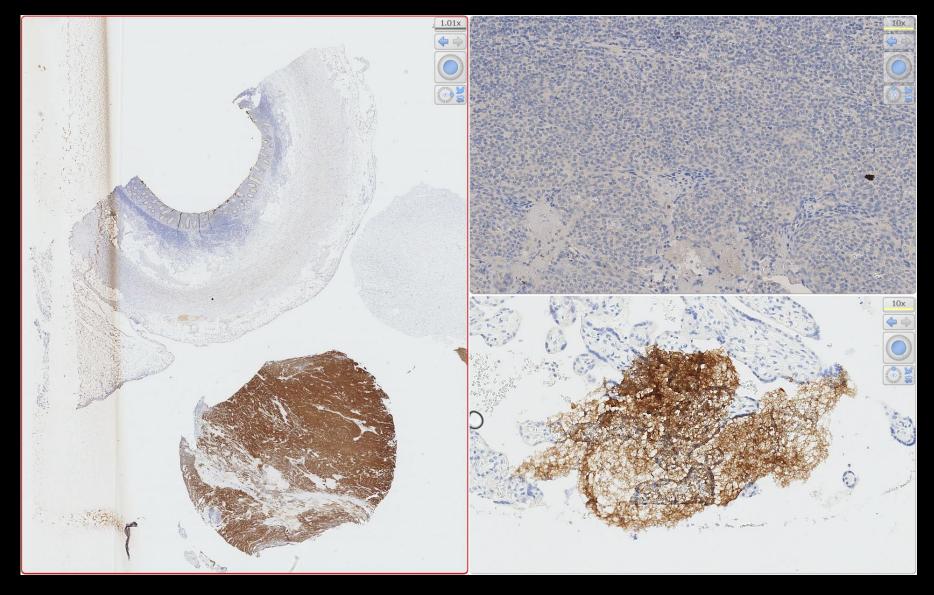
- 1. Increased manual interaction 2 instruments needed
- 2. Primarily batch operation
- 3. High reagent volumen needed 300 ul and >"dead-vol"





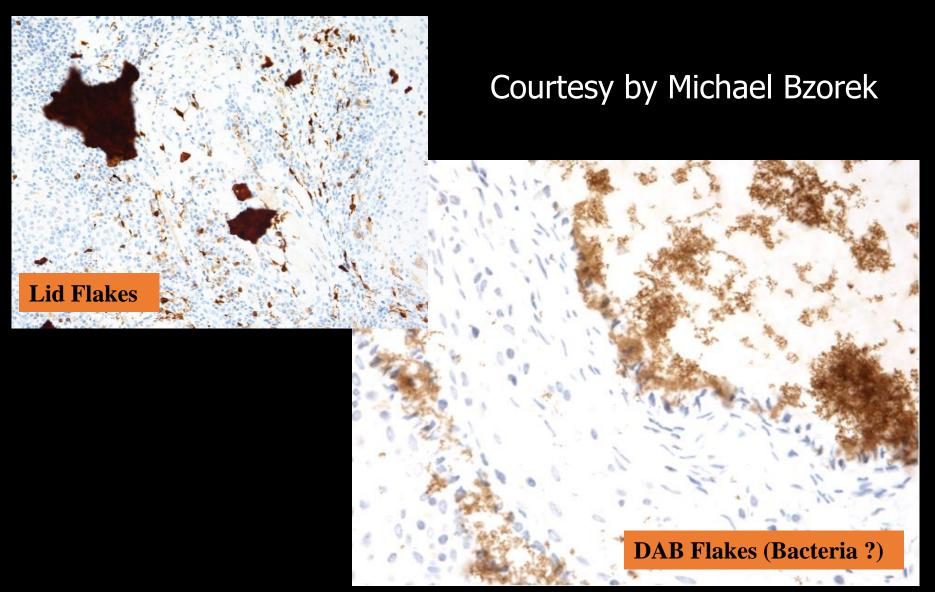
Staining issues; BenchMark, VMS – Uneven weak/neg areas – air bubbles





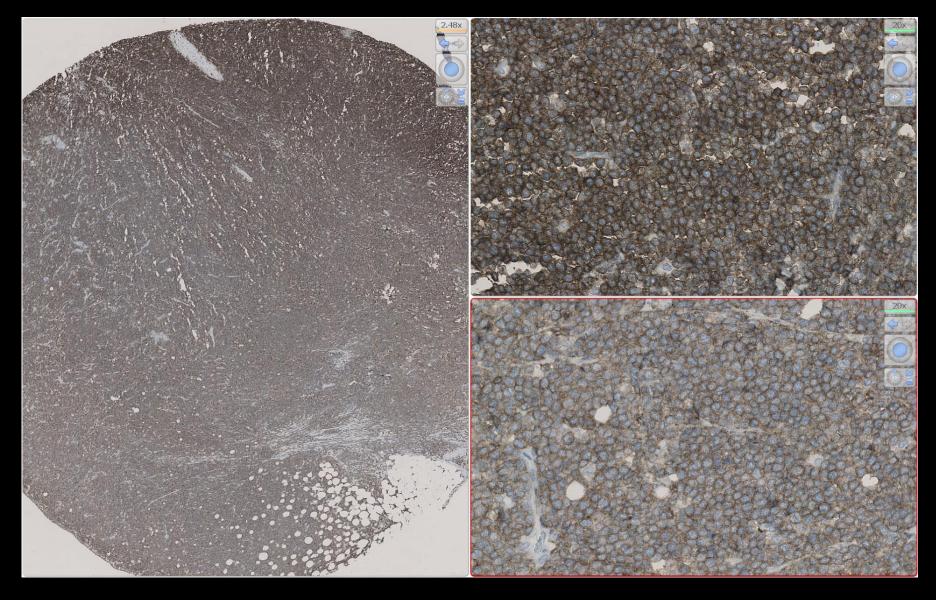
Staining issues; Bond, Leica – chromogen precipitates and general hue





Staining issues; Omnis, Dako – chromogen precipitates

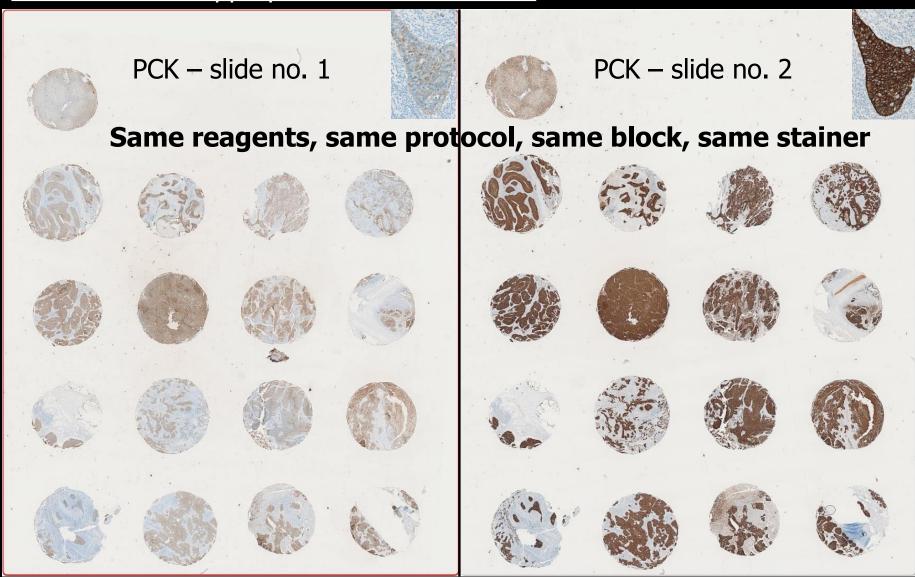




Staining issues; AS48, Dako – chromogen depletion or reagent not spread



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





REVIEW ARTICLE

(Appl Immunohistochem Mol Morphol 2015;23:1–18)

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

Emina E. Torlakovic, MD, PhD,*† Søren Nielsen, HT, CT,‡\$ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA), ||¶# John Garratt, RT,†** Blake Gilks, MD, FRCPC,†††

Jeffrey D. Goldsmith, MD,‡‡ Jason L. Hornick, MD, PhD,*\$\$ Elizabeth Hyjek, MD, PhD,*

Merdol Ibrahim, PhD,||| Keith Miller, FIBMS,||| Eugen Petcu, MD, PhD,||

Paul E. Swanson, MD,¶¶## Xiaoge Zhou, MD,***††† Clive R. Taylor, MD, PhD,‡‡‡

and Mogens Vyberg, MD‡\$

TABLE 3. (continued)

Special Considerations

Cut and submit "own on-slide control" if sending patients' unstained slides to another laboratory for IHC testing The positive controls should match patients' sample tissue processing so far as is possible

HC testing

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

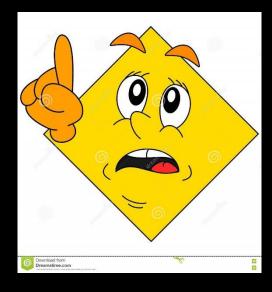
"Run" or "batch" positive controls are not recommended

Use on-slide positive controls

Date unstained slides with on-slide Without the date when the slides controls are prepared, it will be impossible.

Without the date when the slides are prepared, it will be impossible to determine if a unexpected weak result is due to variation in protocol or to an "expired" positive control

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



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



RESEARCH ARTICLE

(Appl Immunohistochem Mol Morphol 2017;25:308–312)

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. Torlakovic, MD, PhD†

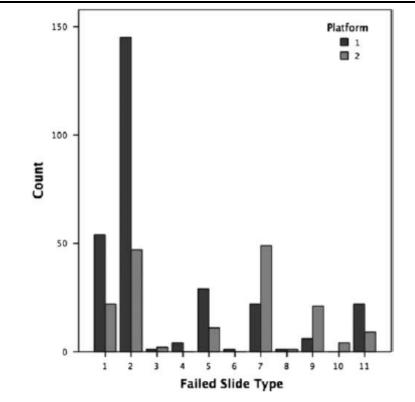


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

2% error rate (452/22.234 slides) Class I 0,8% - Class II 9,0%

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

Category 5,6,9,11

Lab related (22%)

Category 1,2,3,4,7,8,10

Assay and/or Instrument (78%)





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....)



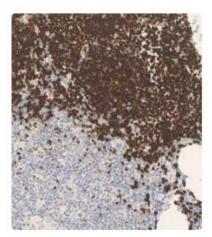
Automation in IHC reduces hands-on and improves consistency However the quality of the end result is less influenced by the function of the automated stainer compared to the impact of:

- 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

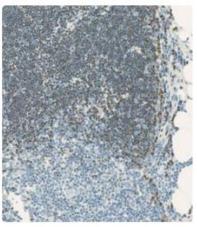


- Accuracy of the technical optimization and validation of the test
 Use of RTU formats not adequately calibrated etc
 - Terminal Deoxynucleotidyl Transferase (TdT)

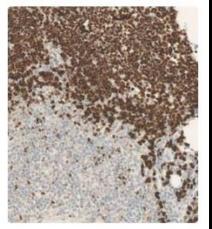
A comparison of Terminal Deoxynucleotidyl Transferase (TdT) Ready-to-Use antibodies from leading manufacturers on human thymus.



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.

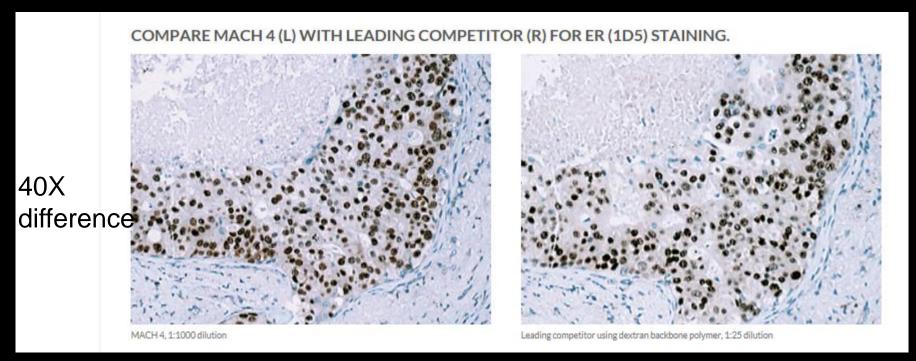
Difference less related to stainer performance compared to focus and precision of the companies protocol set-up.

^{*} independent analysis commissioned by Leica Microsystems and conducted by NordiQC according to the instructions for use and on the corresponding manufacturer's staining platform.



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 ?

....



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 UI.	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\$ 1\$ = 6.5 DKK	HIER ER2, pH 9 20m 20m primary 3-step pol. – refine 150 ul Ab 2.7\$ pr slide	HIER CC1,pH 8.5 48m 32m primary 3-step mul. – OptiV. 100 ul Ab 1.9\$ pr slide	HIER TRS,pH 9, 20m 20m primary 3-step pol. – Flex+ 300 ul Ab 3.5\$ pr slide



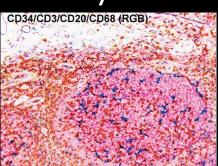
Fully-automated systems: Future ...???

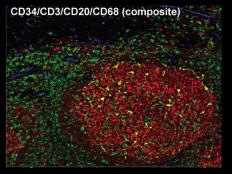
Functionality – Workload – Workflow - Flexibility – Costs

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

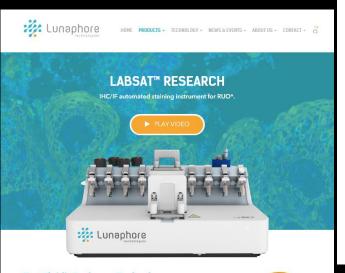


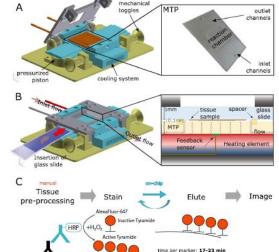






Fully-automated systems: Future ...???





Step	Reagent	Incubation time min
1	anti-ER AbI	4
2	HRP-AbII	4
3	TSA-AF	2
4	Elution	6
5	anti-CK AbI	2
6	HRP-AbII	2
7	TSA-AF	2
8	Elution	4
9	anti-PR AbI	4
10	HRP-AbII	4
11	TSA-AF	2
12	Elution	6
13	anti-Her2 AbI	2
14	HRP-AbII	2
15	TSA-AF	2
	Total staining time	48 min
	Total staining time with washing steps	1h24 min

Fast Fluidic Exchange Technology

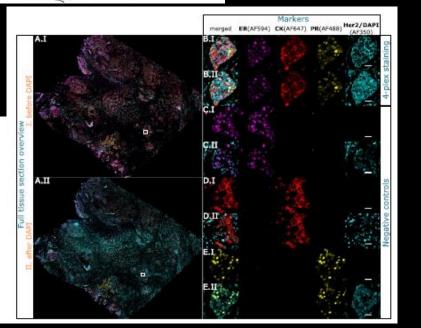
Lunaphore was born out of 9 years of R&D at the Swiss Federal Institute of Technology and develops laboratory automation solutions with potential for cancer research and tissue diagnostics* based on a unique microfluidic technology, called Fast Fluidic Exchange (FFeX).

Lunaphore's overall goal is to decrease assay time while maintaining the quality of its results

SCIENTIFIC REPORTS

OPEN Ultra-fast and automated immunohistofluorescent multistaining using a microfluidic tissue processor

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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 \otimes 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....



