



Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, October 5-7th 2022

Immunohistochemical stainers

Overview

Pros and Cons

Søren Nielsen
Director
NordiQC

IHC – Immunohistochemical stainers

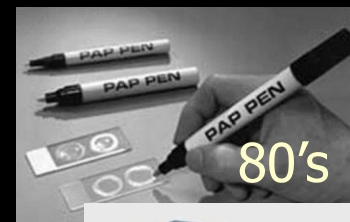
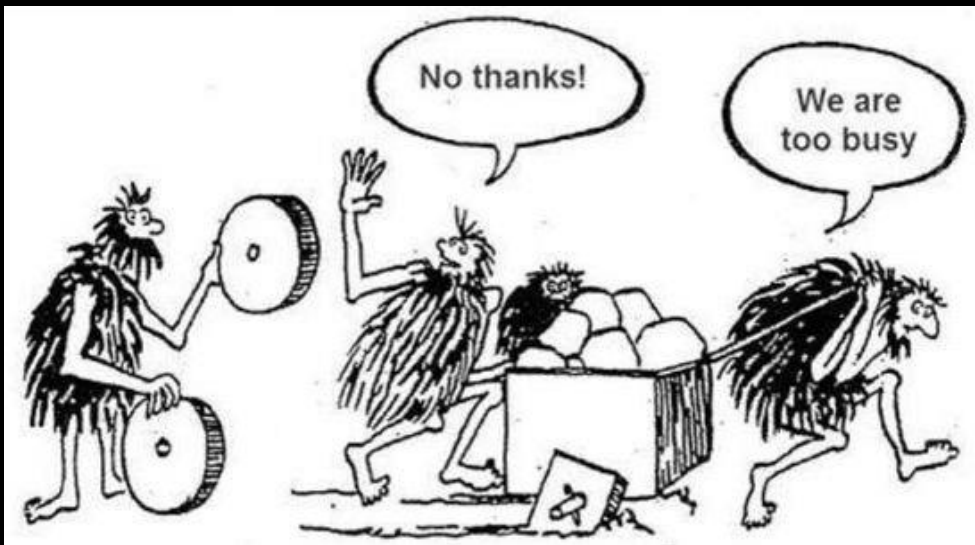
This lecture is meant to be a basis for an open discussion...
and not an attempt to promote any stainer / company 😊



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

IHC – Immunohistochemical stainers

Nothing can stop automation



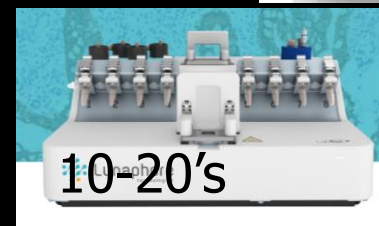
Manual



Semi-aut.



Fully-aut.



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

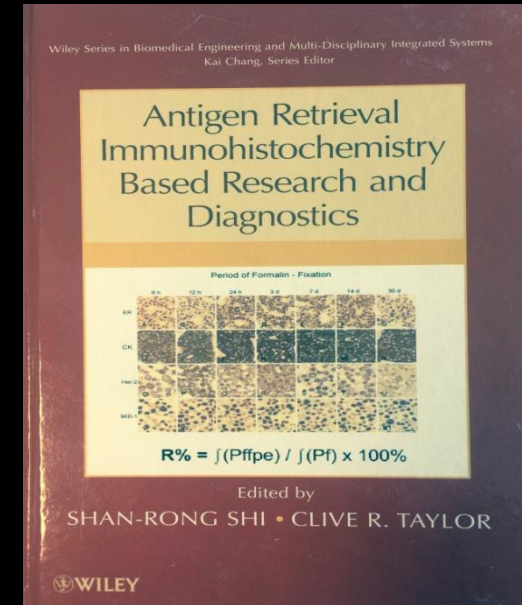
IHC – Immunohistochemical stainers

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

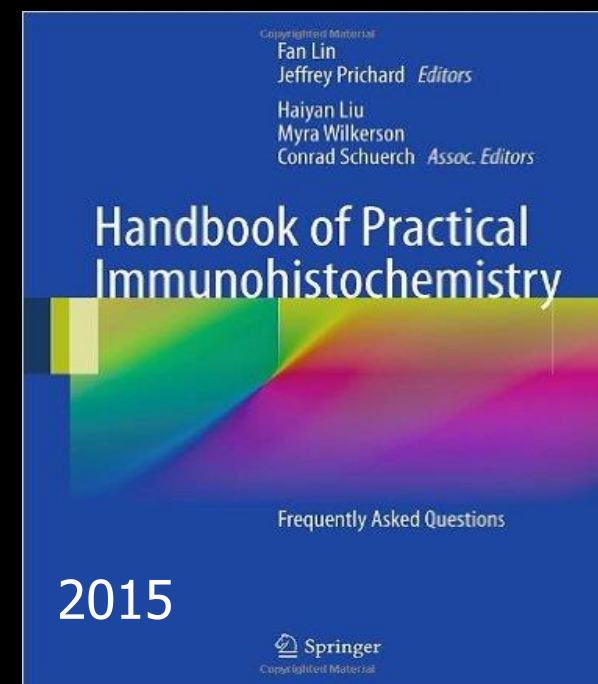
Automation in IHC

Ole Feldballe Rasmussen, PhD, MSc

2013



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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 - pipetting

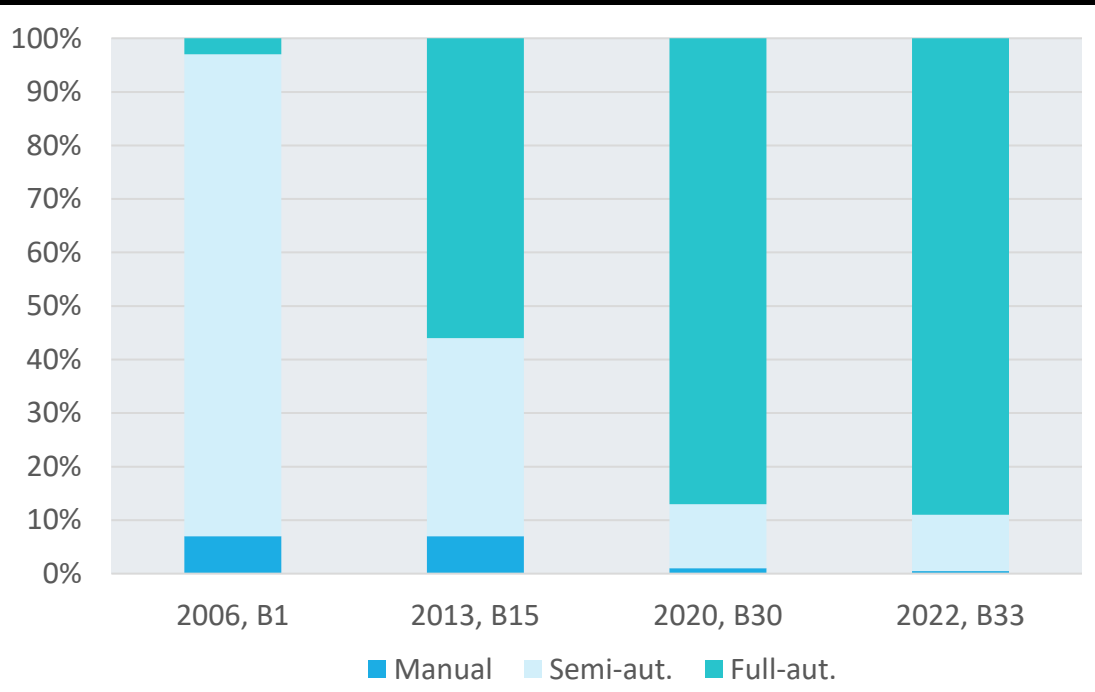
Secure even distribution – "Pap-pen"

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 – NQC ER

Change from semi-automated IHC platforms to fully-automated platforms



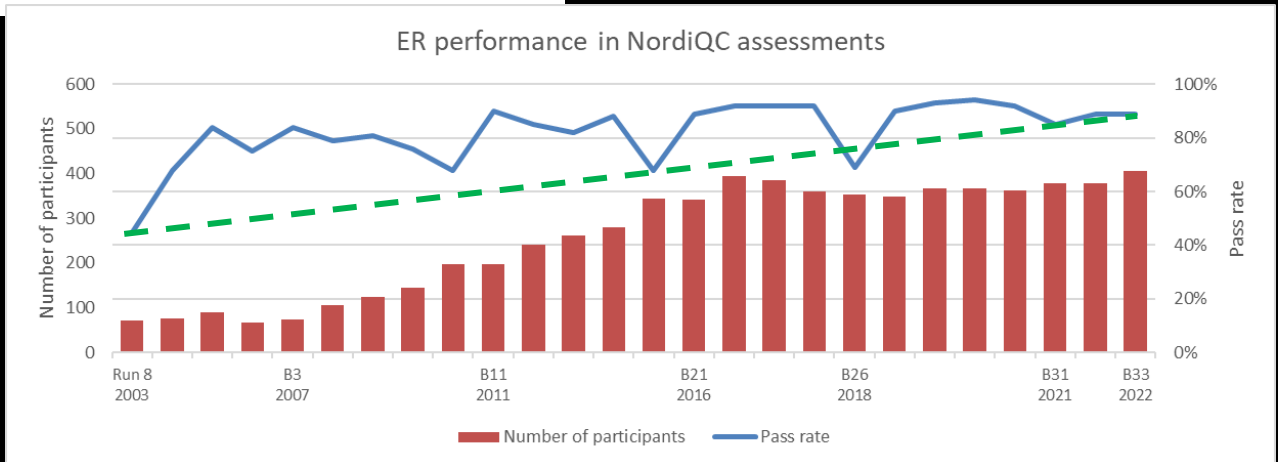
80's



90-00's



10-20's



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

2021: 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, Oncore Biocare, (AS48 Dako)

IHC – Immunohistochemical stainers

Capillary gap technology stainers:



Leica:
Covertiles
Capillary



Dako:
Glass Lid
Dynamic gap



Sakura:
"upside down"
Capillary

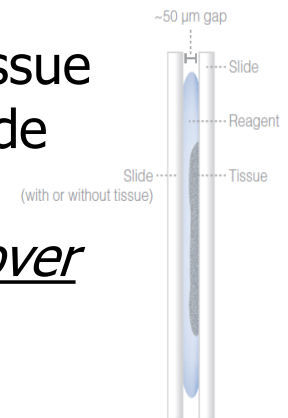
Technique;
To spread
reagents and
to avoid slides
drying out

Capillary Gap Staining

Tissue
slide

+

Cover

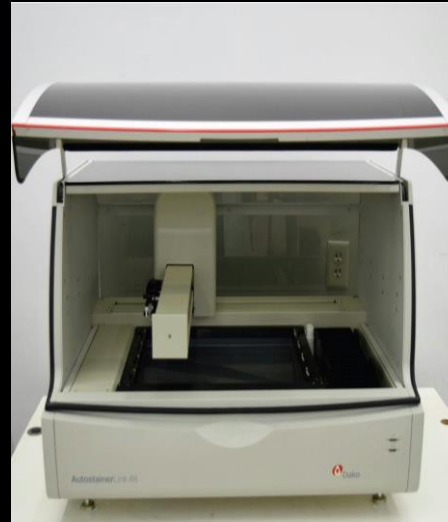


IHC – Immunohistochemical stainers

Flat labelling technology stainers:



Ventana:
+Mixing
+Overlay

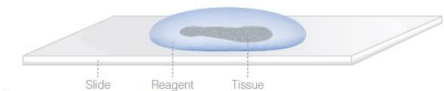


Dako:
-Mixing
-Overlay

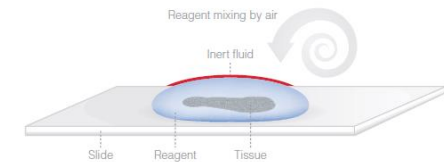
Technique;

Reagents are applied
+/- mixing
+/- overlay

Open Individual Slide Staining

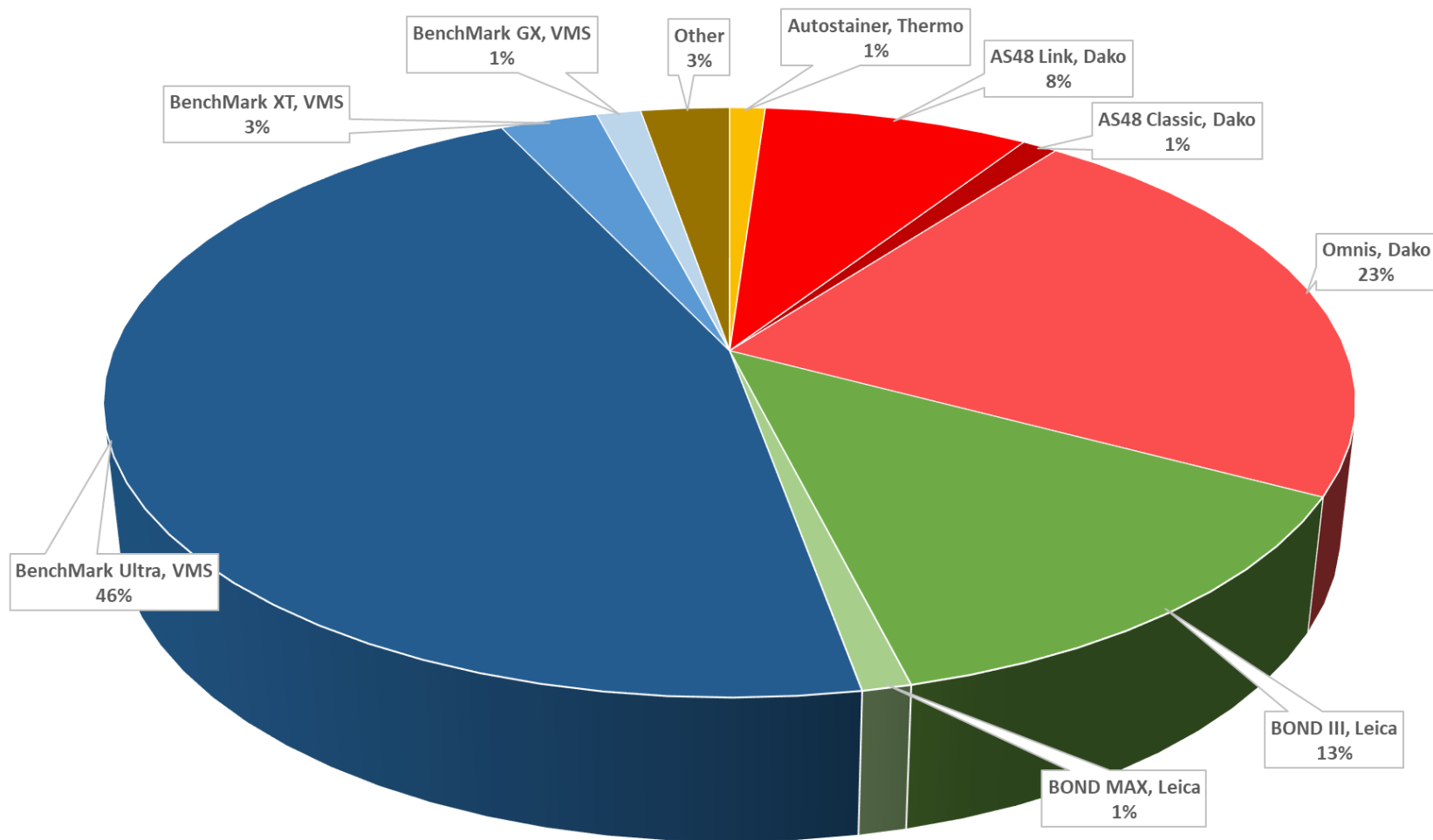


Liquid Overlay Technology



IHC – Immunohistochemical stainers

IHC stainer platforms NordiQC CD30 run 65, 2022
366 participants

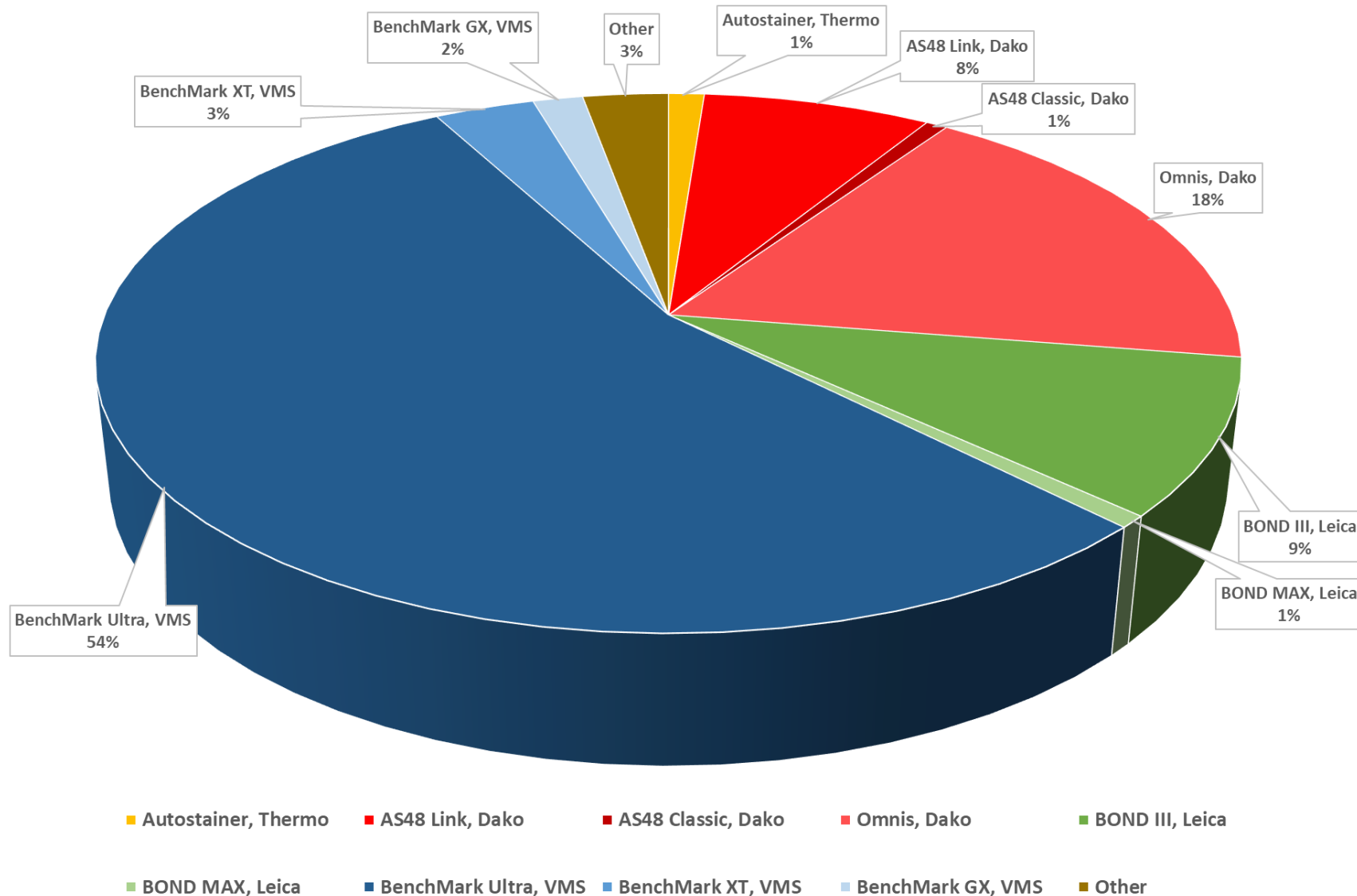


Autostainer, Thermo AS48 Link, Dako AS48 Classic, Dako Omnis, Dako BOND III, Leica
BOND MAX, Leica BenchMark Ultra, VMS BenchMark XT, VMS BenchMark GX, VMS Other

IHC – Immunohistochemical stainers

IHC stainer platforms NordiQC ER run B33, 2022

407 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



Overview of Automated Immunohistochemistry

Jeffrey W. Prichard, DO

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(*Arch Pathol Lab Med.* 2014;138:1578–1582; doi: 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:

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
-

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 resources for maintenance
 - Frequency, extent, safety etc

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

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 ?

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 ?

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

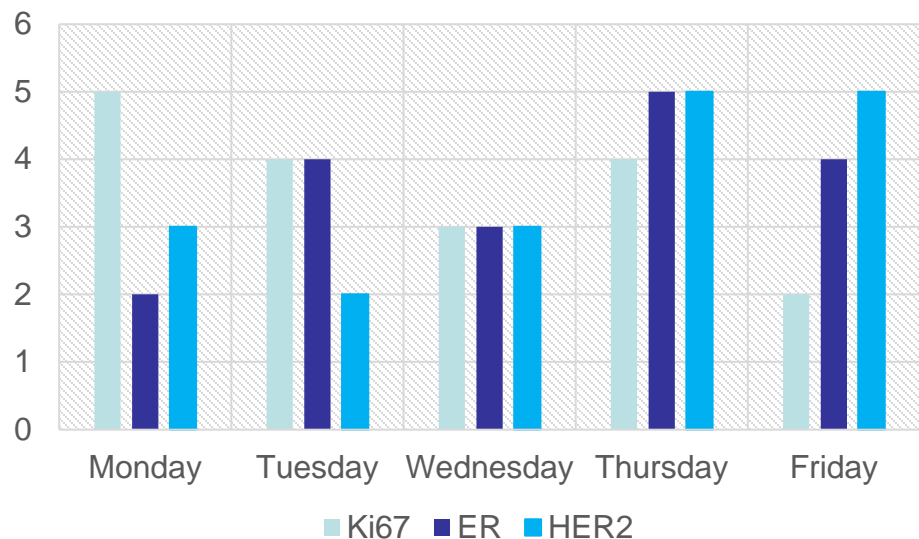
IHC – Immunohistochemical stainers

	Dako AS 48	Dako Omnis	VMS Ultra	Leica BOND III	Biocare ONCORE	Sakura Genie
Capacity	48	60	30	30	36	30
Reagents	64	60	35	36	40	39
Volume	200 ul	200 ul	100 ul	150 ul	130 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 US/No EU
3' party reagents	Ab, enz, det, chr.	Ab, enz, det, chr.	Ab, enz	Ab, enz	Ab, enz	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	Yes	Yes
RTU's no*	116	84	280	155	64	150
CDx range	High	Moderate	High	Low	None	None

* Estimate 09.2022 with uncertainties and changes due to IVDR and local regulations

IHC – Immunohistochemical stainers

IHC "Quality" - manual



Manual processing induces lack of reproducibility

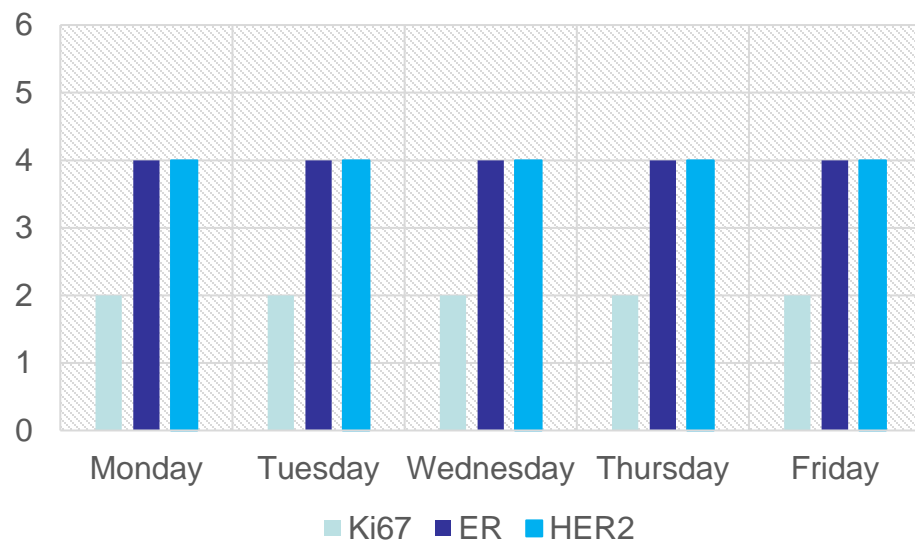
Automation facilities reproducibility

Compromisation of protocol is needed to handle automated processing

Certain markers are severely affected

Flexibility of automation might compensate for the impact

IHC "Quality" - Automated

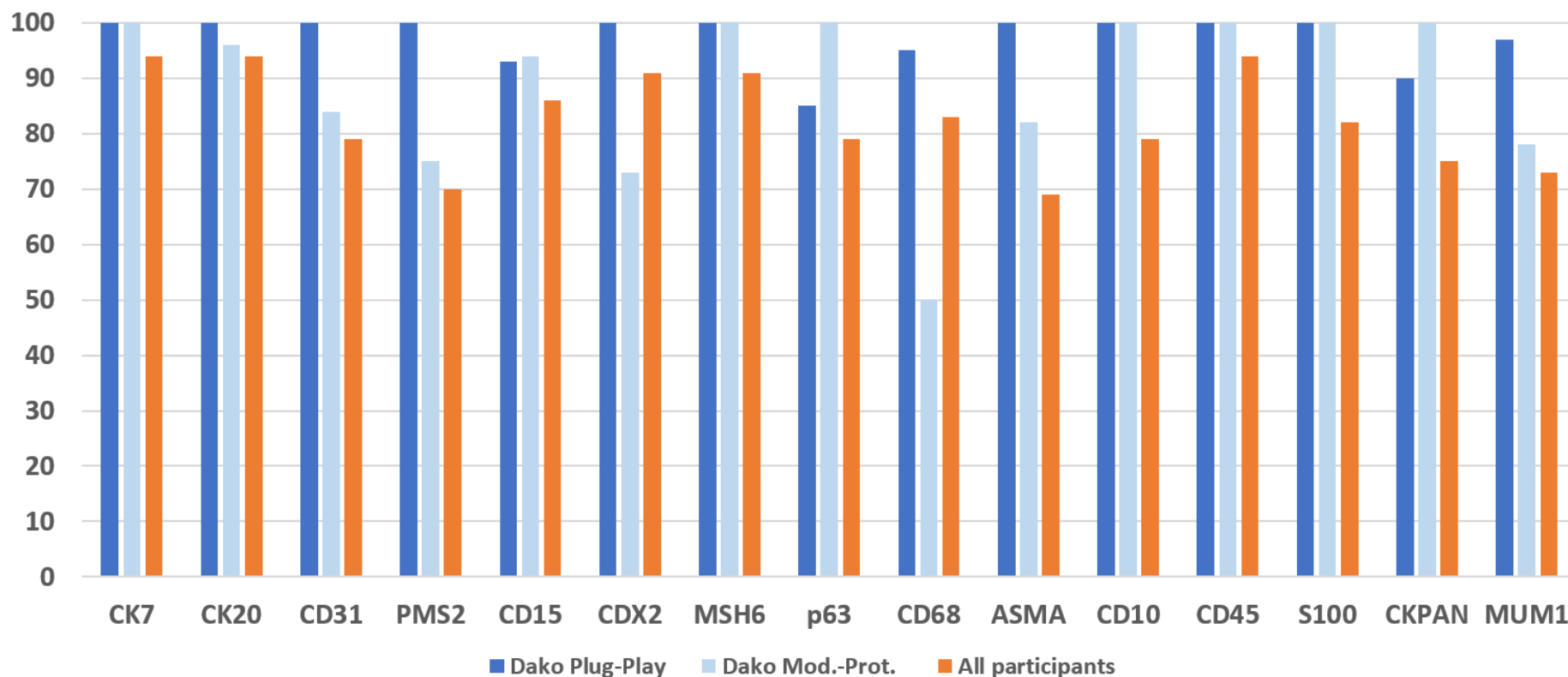


IHC performance challenges related to Automation NordiQC data

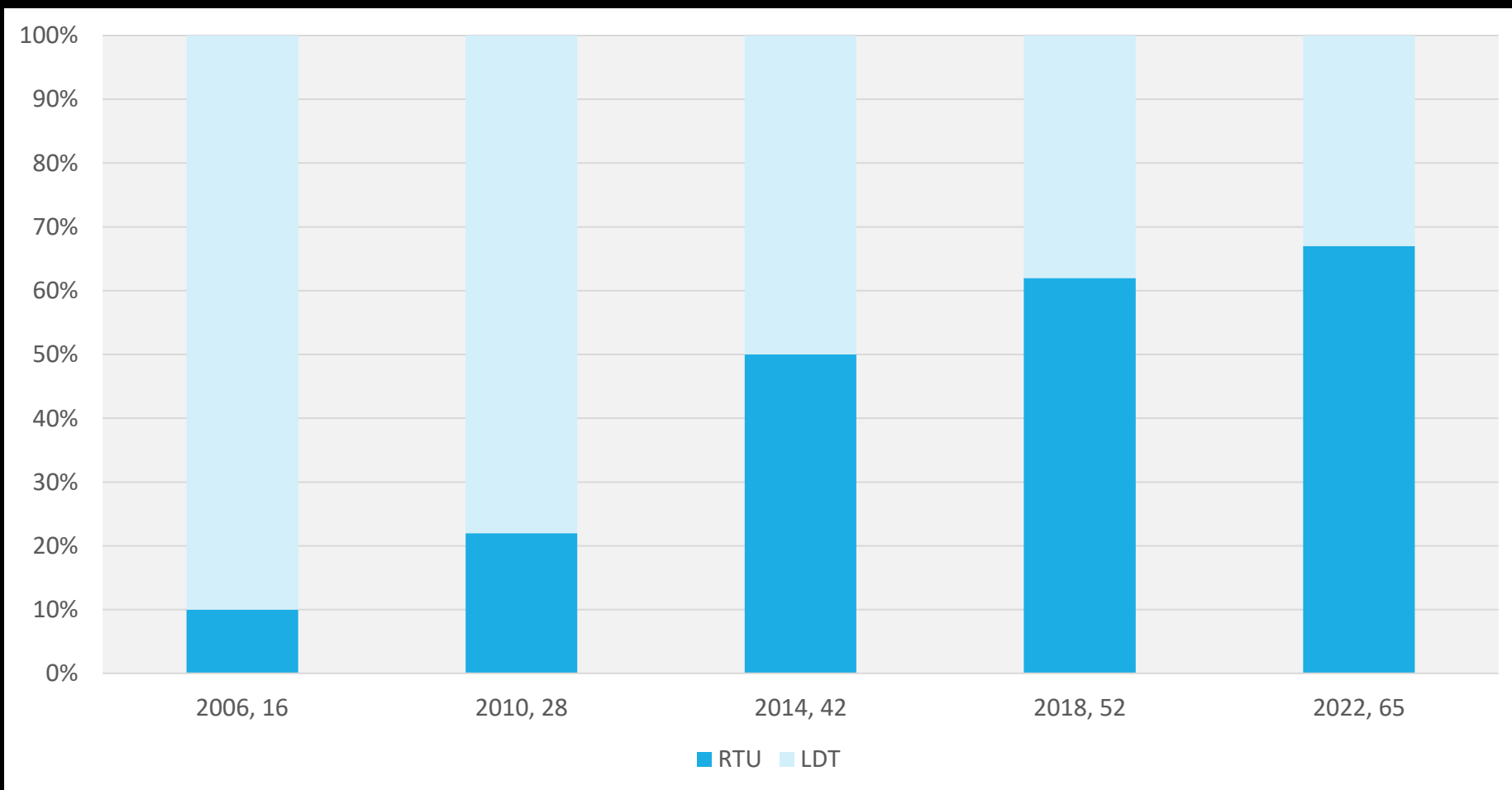
Target	Clone	Semi-automated	BenchMark	Omnis	Bond
ALK	D5F3	√	√	(√)	√
ASMA	1A4	√	FN, FP	√	√
Calretinin	DAK-Calret1	√	FN	FN	√
CD4	4B5	√	FN	-	(√)
CD56	123C5	√	FN	FN	√
CDX2	DAK-CDX2	√	FN	√	√
CEA	II-7	√	FN	-	√
CK-LMW	5D3	√	FN	-	√
Desmin	D33	√	√	FN	√
EPCAM	Ber-EP4	√	FN	√	FN
HEPATOCYTE	OCH1E5	√	√	-	FP
Mel. A (sexcord)	A103	√	FN	FN	√
PAX8	MRQ-50, BC12	√	FN	FN	√
SATB2	EP281	(√)	√	(√)	(√)
SMAD4	B-8	√	FN	FN	√

IHC – Immunohistochemical stainers

General module; Dako/Agilent Omnis RTU Type I products
NordiQC 2020-2021 pass-rates



General module; RTU & LDT IHC assays



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 – Type I & II
5. IHC and ISH on same instrument / same slide

Fully-automated systems: BenchMark Ultra, Ventana

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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

Fully-automated systems: BOND III, 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

Fully-automated systems: Bond III, Leica

Functionality – Workload – Workflow - Flexibility – Costs

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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 portfolio 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 portfolio of RTUs & detection systems
2. Low affinity antibodies mayl 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

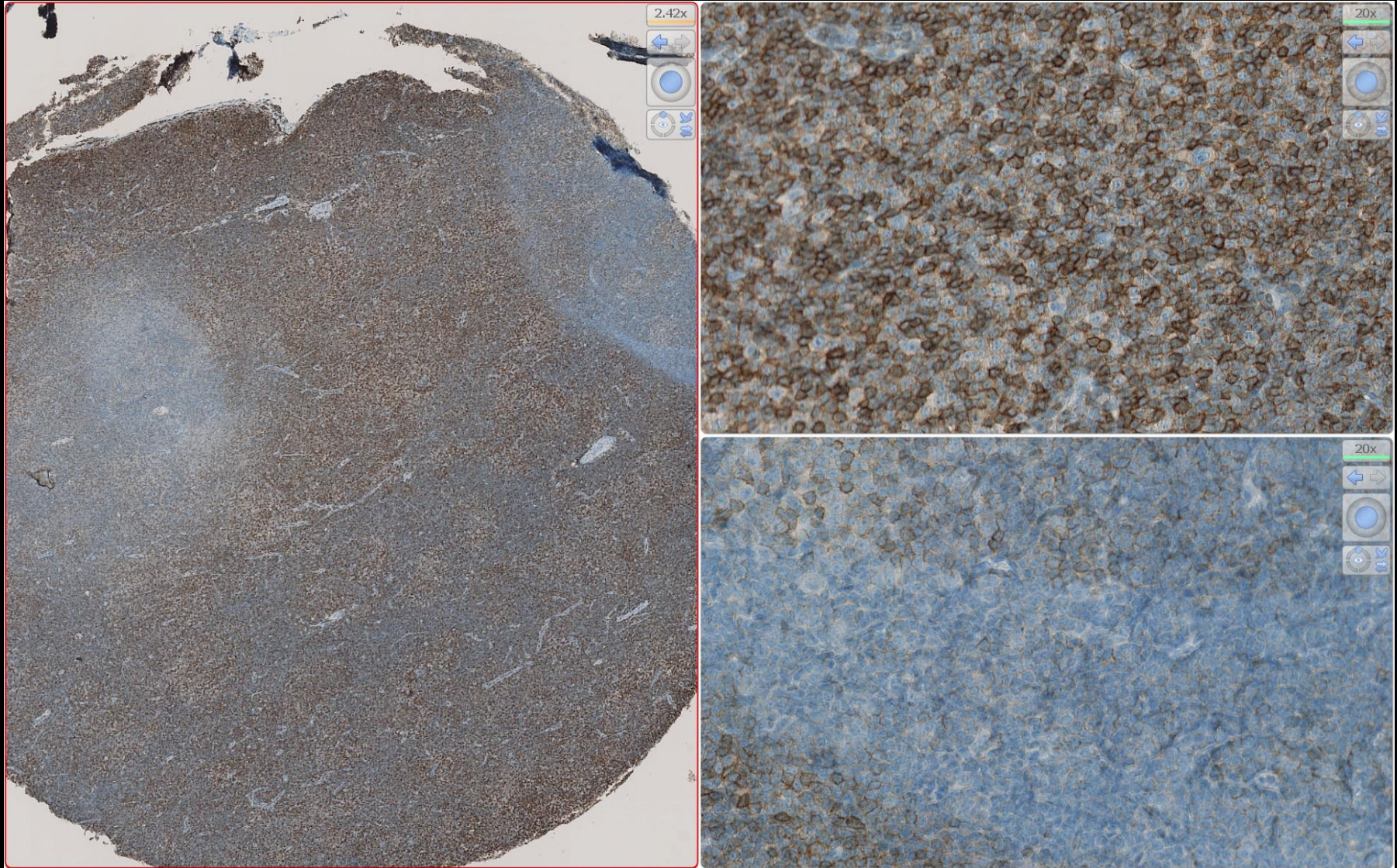
5 main Pros:

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:

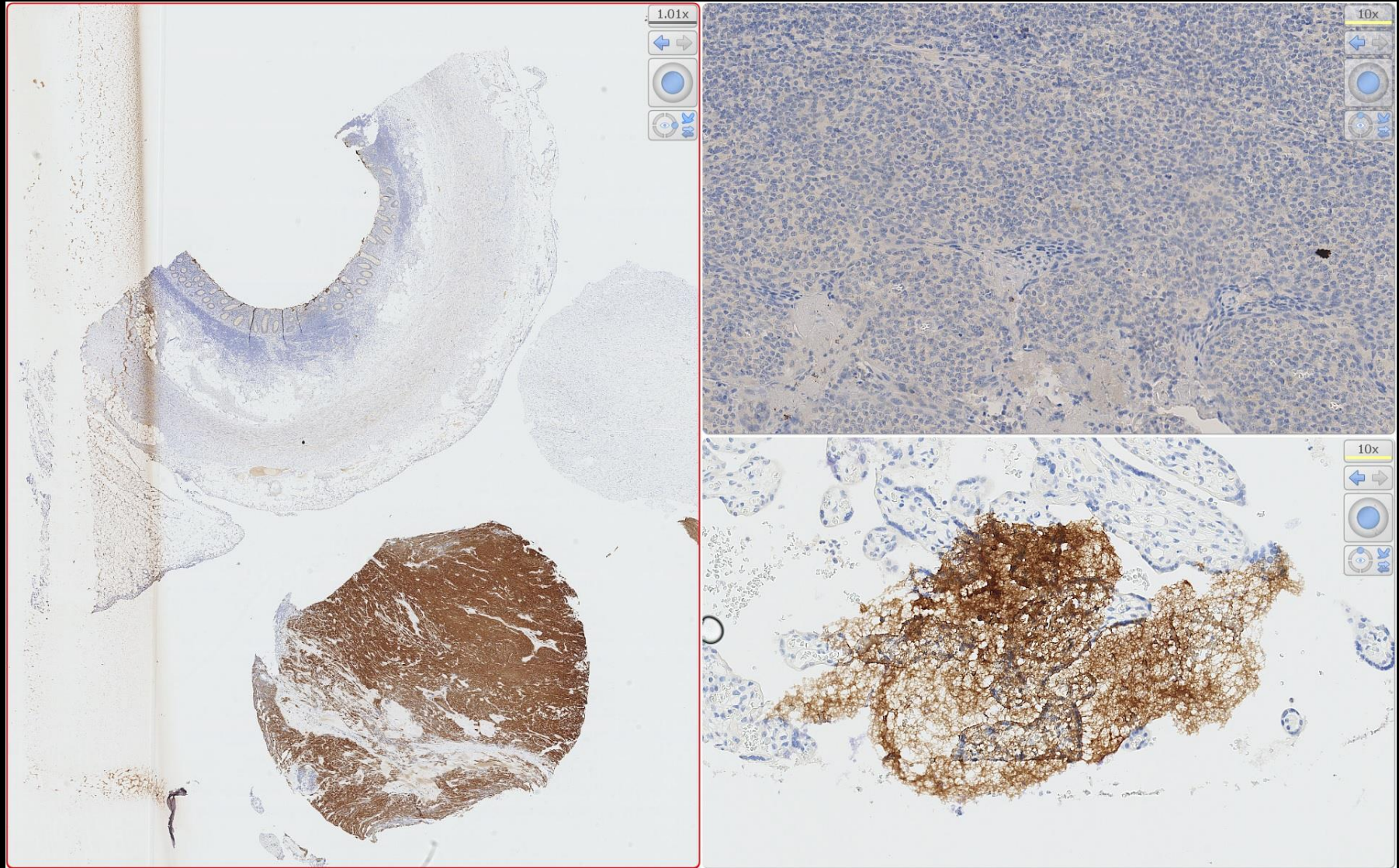
1. Increased manual interaction – 2 instruments needed
2. Primarily batch operation
3. High reagent volumen needed – 300 ul and >"dead-vol"

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

Courtesy by Michael Bzorek



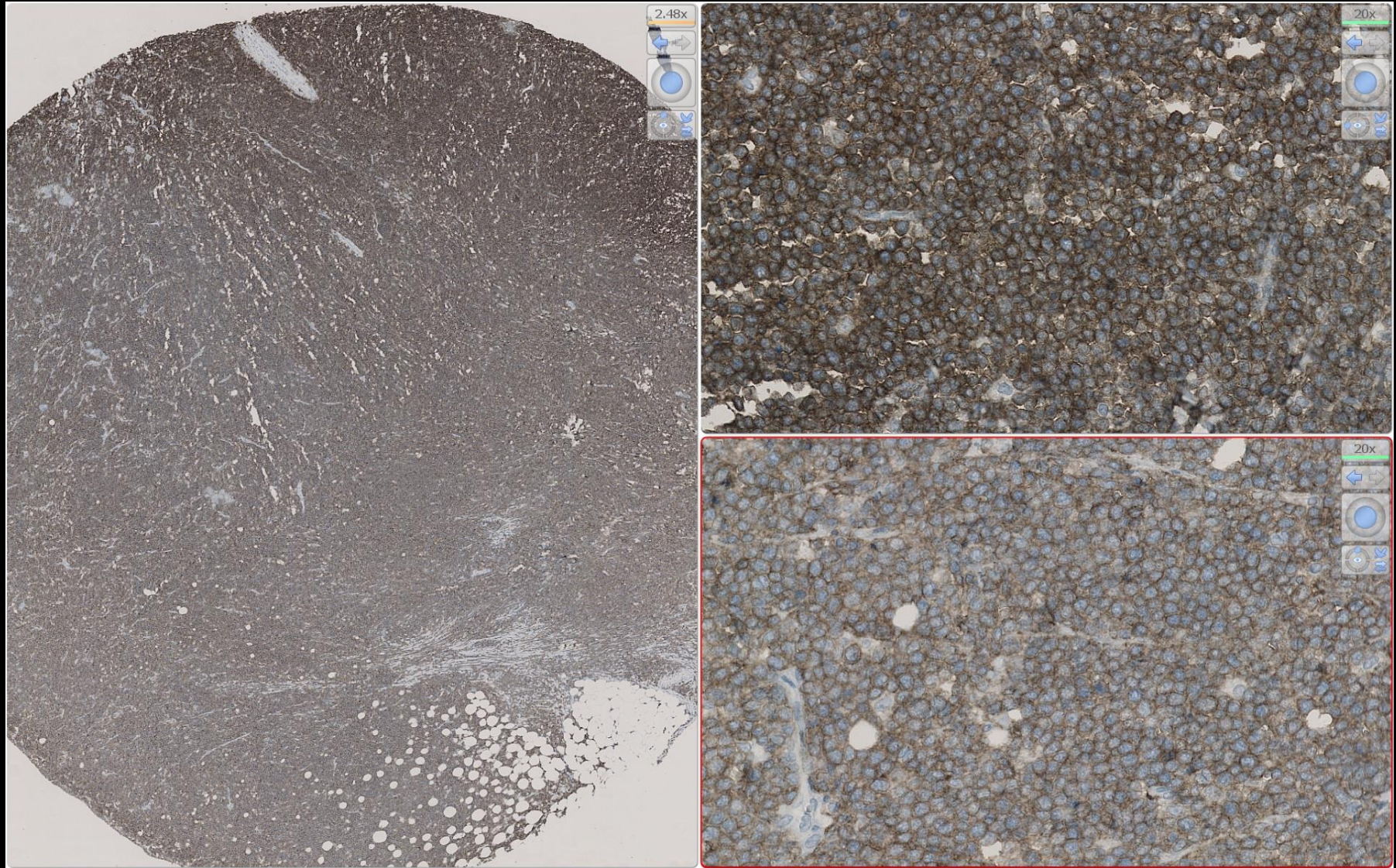
Lid Flakes



DAB Flakes (Bacteria ?)

Staining issues; Omnis, Dako – chromogen precipitates

IHC – Immunohistochemical stainers



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

REVIEW ARTICLE

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

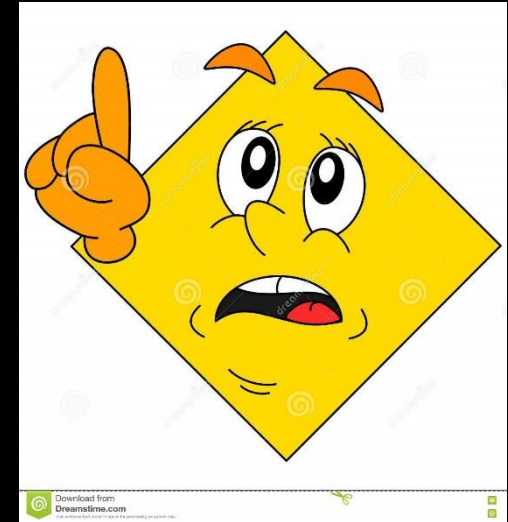
Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Emina E. Torlakovic, MD, PhD,*† Soren 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 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
Use on-slide positive controls	“Run” or “batch” positive controls are not recommended
Date unstained slides with on-slide controls	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†

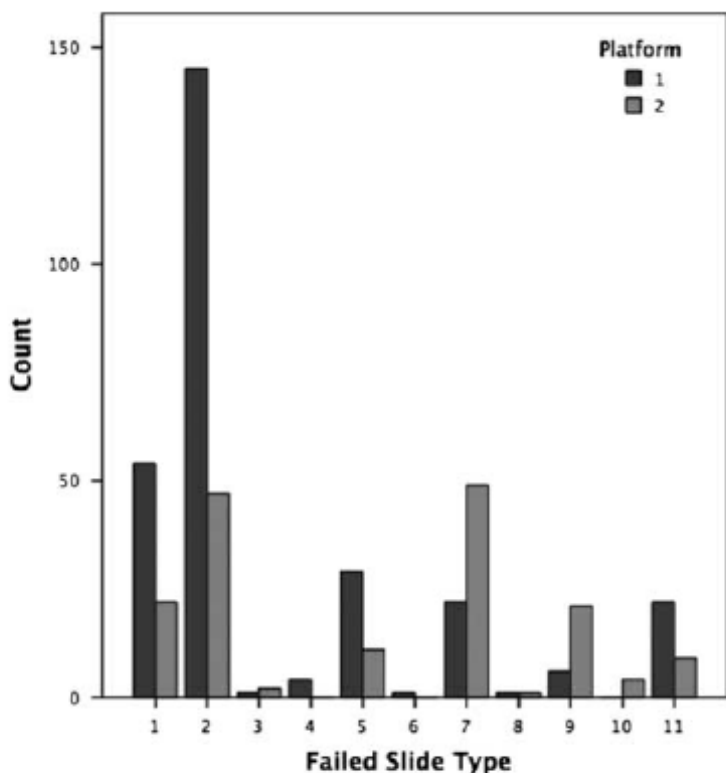


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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%)

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

IHC – Immunohistochemical stainers

A



On-slide controls

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

B



Batch-control - Theoretically:

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

C



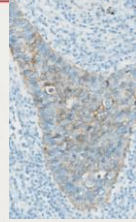
Batch-control - Theoretically:

Batch control pass by same conditions as above
19/19 passed
0/19 failed (the 5 failed slides not identified....)

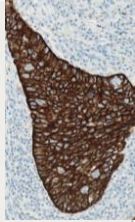
IHC – Immunohistochemical stainers

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

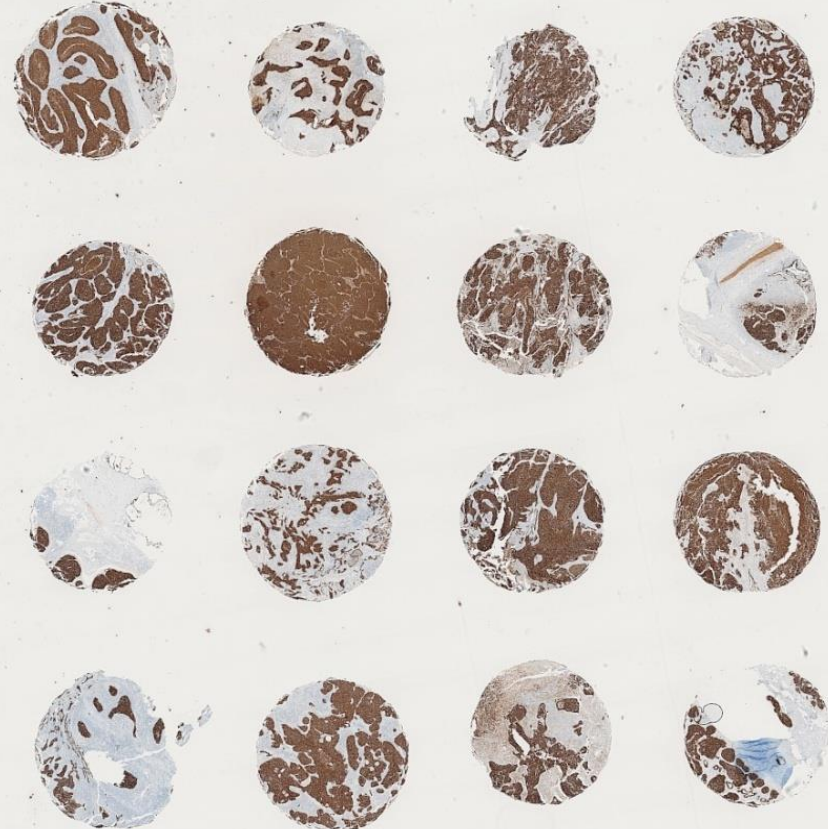
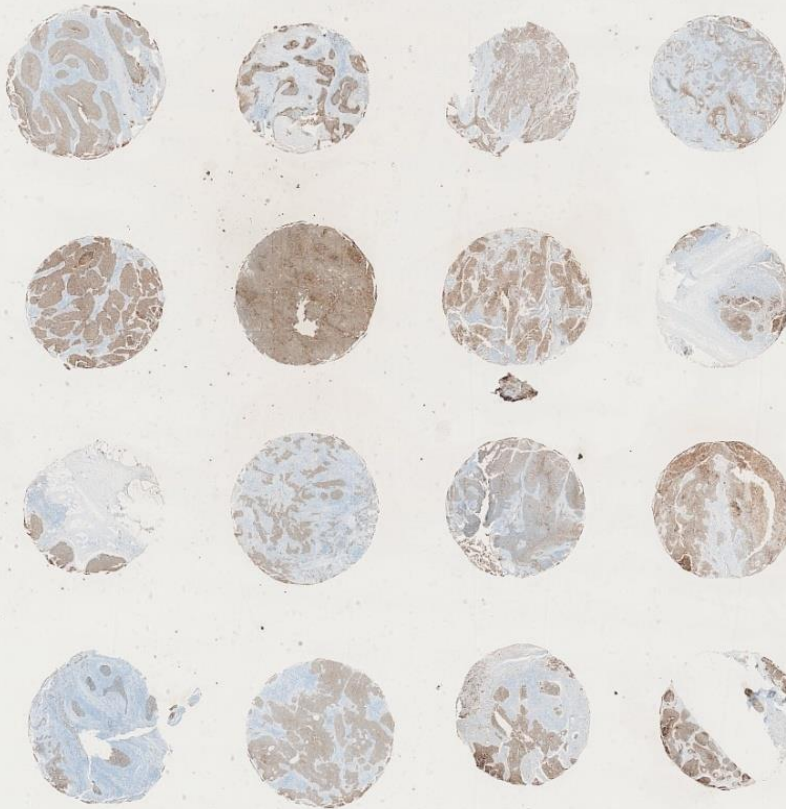
PCK – slide no. 1



PCK – slide no. 2



Same reagents, same protocol, same block, same stainer



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:

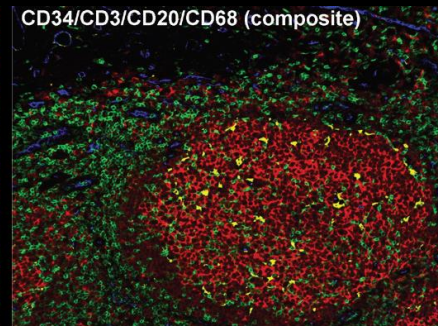
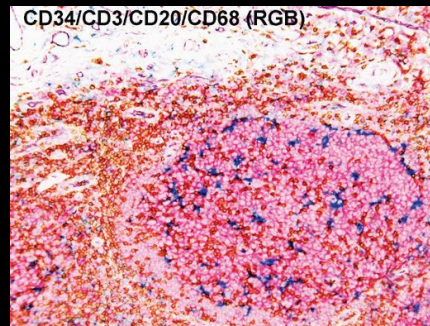
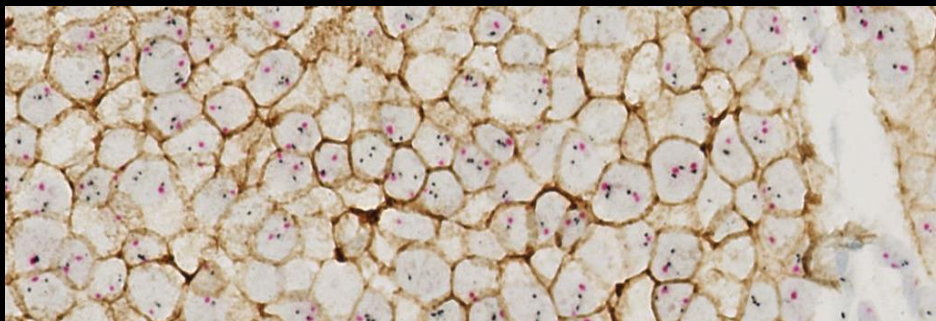
- Quality of the tissue material (pre-analytics)
 - Automation will not compensate for delayed fixation etc
- Quality of the reagents used (sensitivity, specificity – analytics)
 - Use of detection system with low sensitivity etc
- Accuracy of the technical optimization and validation of the test
 - Use of RTU formats not adequately calibrated etc
- Interpretation of the test
 - Inadequate choice of control material etc

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 triple/quadruple staining – less sample material
2. Reduced IHC staining time – shorter TAT required
3. Ability to perform ISH for miRNA and similar gene targets
4. Increased demand for traceability of IHC process (ISO)
5. New “players” on the market

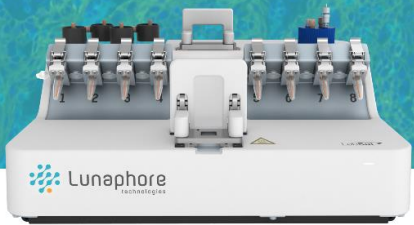


IHC – Immunohistochemical stainers

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

Lunaphore
LABSAT™ RESEARCH
IHC/IF automated staining instrument for RUO*.

▶ PLAY VIDEO



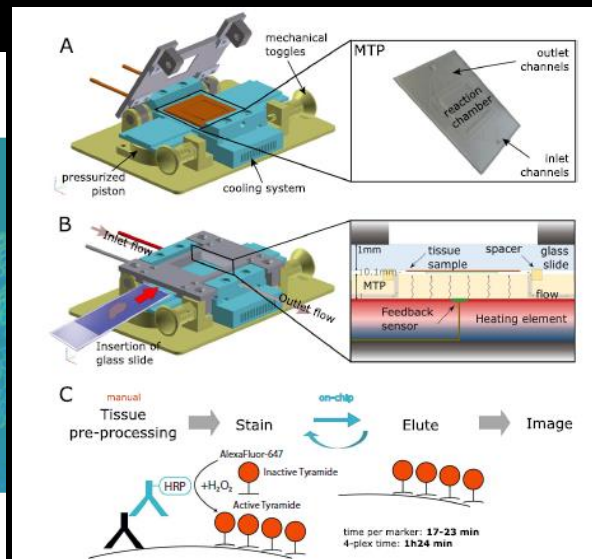
Fast Fluidic Exchange Technology

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.

* For Research Use Only. Not for use in diagnostic procedures.

FFeX



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

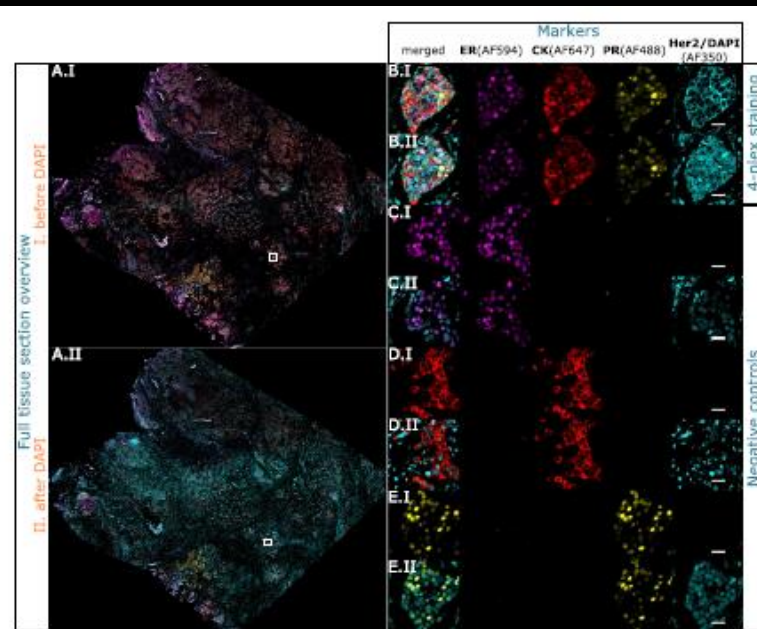
SCIENTIFIC REPORTS

OPEN

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

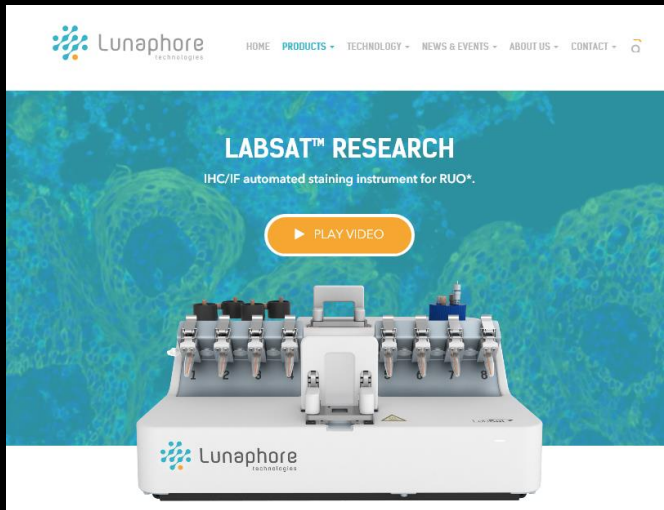
Giulia Cappi, Diego Gabriel Dupouy, Marta Aurelia Comino & Ata Tuna Ciftlik

Received: 18 October 2017
Accepted: 28 February 2019
Published online: 14 March 2019



IHC – Immunohistochemical stainers

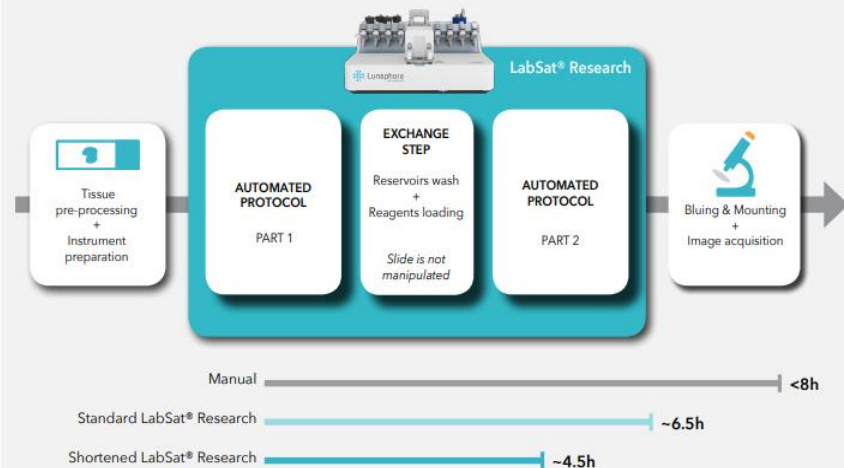
Fully-automated systems: Future ...???



mRNA
lncRNA
miRNA

...

Workflow: RNAScope on LabSat® Research



	Manual ACD Reference	Standard protocol on LabSat® Research	Shortened protocol on LabSat® Research
Tonsil PD-L1			
Tonsil PD-1			
Breast Cancer MKI67			
Breast Cancer FOXP3			
Colon Cancer FGFR3			
Cervical Cancer HPV			
	TOTAL TIME <8h	TOTAL TIME <6.5h	TOTAL TIME <4.5h

IHC – Immunohistochemical stainers

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

Leica BOND RX



AUTOMATE THE FOLLOWING TESTS ON BOND RX

AUTOMATE THE FOLLOWING TESTS ON BOND RX				
YOUR TEST HERE		IF	CTC	
IHC	TSA	FISH	ISH	LNA
Immunohistochemistry	Tyramide Signal Amplification	Fluorescence In Situ Hybridization	In Situ Hybridization	Locked nucleic acid
CISH	TUNEL	miRNA	bDNA	MULTIPLEX
Chromogenic In Situ Hybridization	Terminal deoxynucleotidyl transferase dUTP nick end labeling assay	microRNA	Branched DNA Assays	

Applications

IHC & multiplex IHC
Gene & protein IHC/ISH
mRNA ISH
miRNA ISH
DNA ISH

IHC – Immunohistochemical stainers

Fully-automated systems: Future ... New players



AS300/330 Fully Automatic IHC Stainer



Fully-automated systems: Future ... New versions and updates



Home · IHC & ISH · BOND IHC-ISH Instruments & Solutions · BOND-PRIME Discovery Hub

BOND-PRIME Discovery Hub

Transcend Expectations

The BOND-PRIME IHC & ISH staining platform delivers high productivity, featuring Universal Access, and can seamlessly adapt to your incoming workflow demands – whether it be batch, continuous, single slide or STAT cases, or a combination of all the above.

[GET A QUOTE FOR THE BOND-PRIME >](#)



The BOND-PRIME IHC & ISH Stainer Unveiled

Designed and manufactured in Australia, watch the global unveiling of the BOND-PRIME IHC and ISH stainer as it was revealed to a live Australian audience at the 7th International DIHC Conference.

[WATCH THE UNVEILING HERE](#)

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

