



**Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, October 4-6 2023**

Immunohistochemical stainers

Overview

Pros and Cons

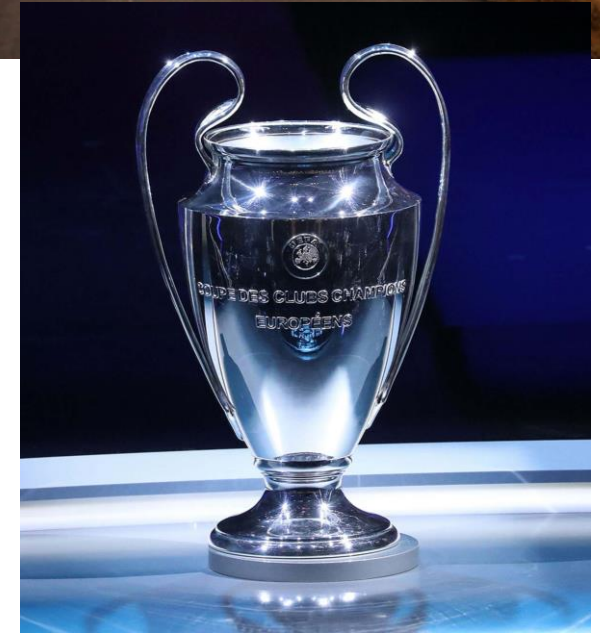
*Søren Nielsen,
Director
NordiQC*

IHC – Immunohistochemical stainers

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

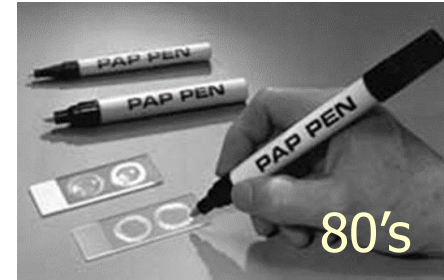


Photo by B.A. Rupert. Green Bay Press-Gazette

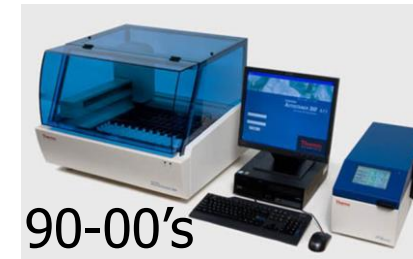


IHC – Immunohistochemical stainers

Nothing can stop automation



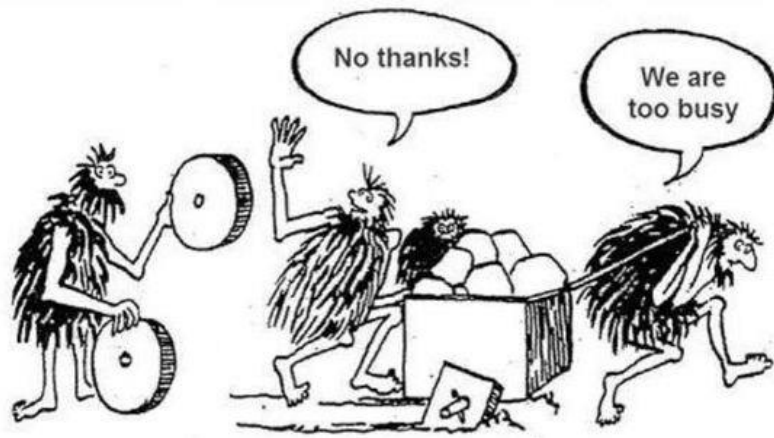
Manual



Semi-aut.



Fully-aut.



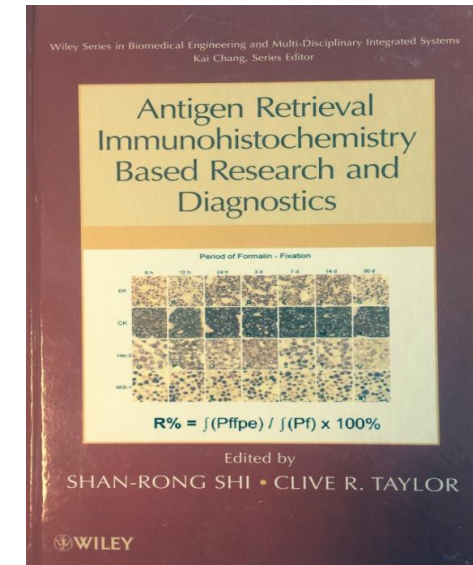
Fully-aut +
Multiplex (markers/assays)
Speed, etc

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

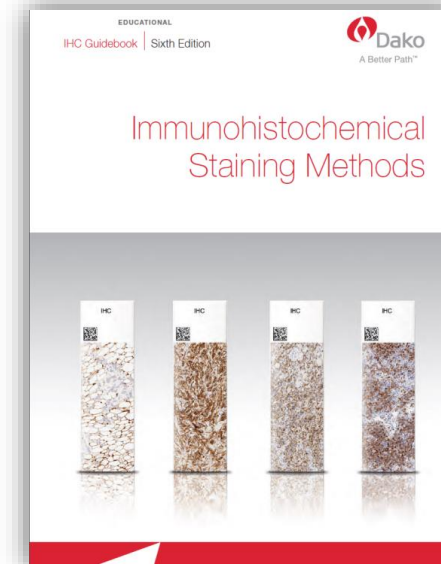


Chapter 9

Automation in IHC

Ole Feldballe Rasmussen, PhD, MSc

2013/2021



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Overview of Automated Immunohistochemistry

Jeffrey W. Prichard, DO

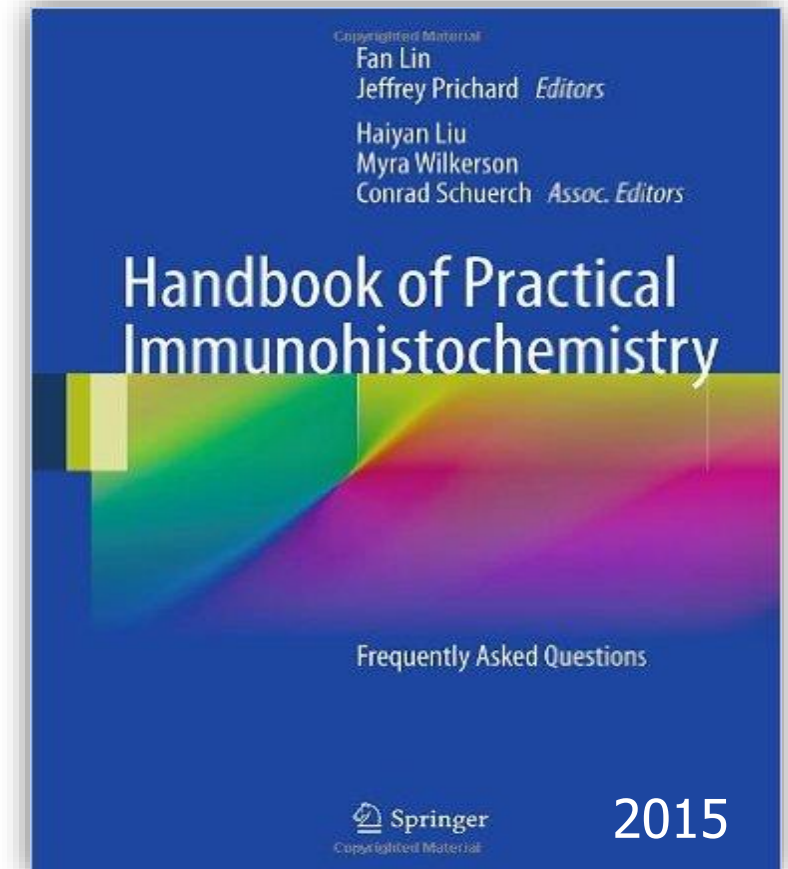
• **Context.**—The increasing demand for immunohistochemistry for clinical diagnostics, in combination with an ongoing shortage of staff in the histology laboratory, has brought about a need for automation in immunohistochemistry. The current automated staining platforms vary significantly in their design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



Updated 2022 – 3' version

[Automated Immunohistochemistry Overview](#)

Jeffrey W. Prichard, Angela K. Bitting
Pages 41-46

[Immunohistochemistry: An Agilent Perspective](#)

Ole F. Rasmussen, Lars Rudbeck
Pages 47-57

[The Leica Biosystems Perspective: From Excision to Imaging—Every Step Is Critical](#)

Douglas Coveney, Mandy Lindsay, Claire Kentler, Kellie Madigan
Pages 59-67

[Immunohistochemistry: Maixin Perspective](#)

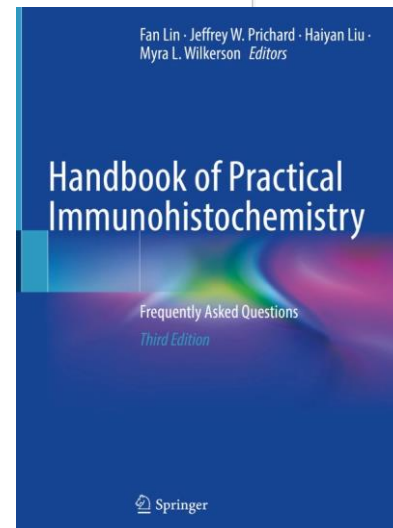
Xiaoya Wang, Qixin Lin, Yulin Xiong
Pages 69-75

[Immunohistochemistry: Roche Tissue Diagnostics Perspective](#)

Hiroaki Nitta, Mark D. Robida, Nate Polaske
Pages 77-85

Abstract

The past decade has produced major innovations and a great variety of features available in automated staining instruments. This chapter is a “buyer’s guide” for automated IHC instruments. For those new to the topic of automated staining, it begins with discussions of the advantages and disadvantages of automated versus manual staining techniques to help you decide if automation is the right choice for your laboratory. The basics of the general types of mechanics that differentiate the platforms are illustrated. Industry jargon about “open” and “closed” systems is better defined. To help with creating a thoughtful business plan to justify the budget expense of automation, the considerations that include the cost and potential savings of operating the equipment over and above the purchase price are presented. The different strategies for slide capacity and continuous processing that affect overall test throughput are described. A comprehensive feature comparison table is included to reveal how the current clinical instruments stack up side by side. With the information in this chapter, you will know how to evaluate whether an instrument is right for you and understand the value of technological advancements as they arrive in the future.



Updated 2022 – 3’ version

IHC being changed from “home-brew” to “Ready-To-Use”



SP
Simple Programmer
Making the complex simple



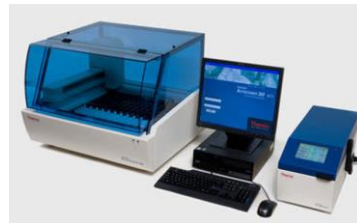
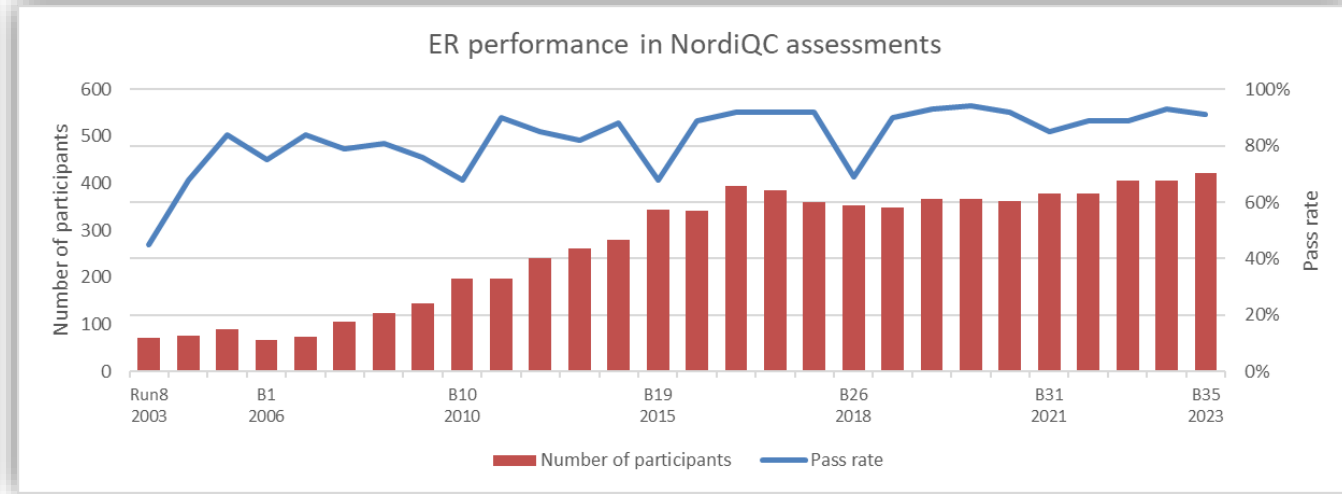
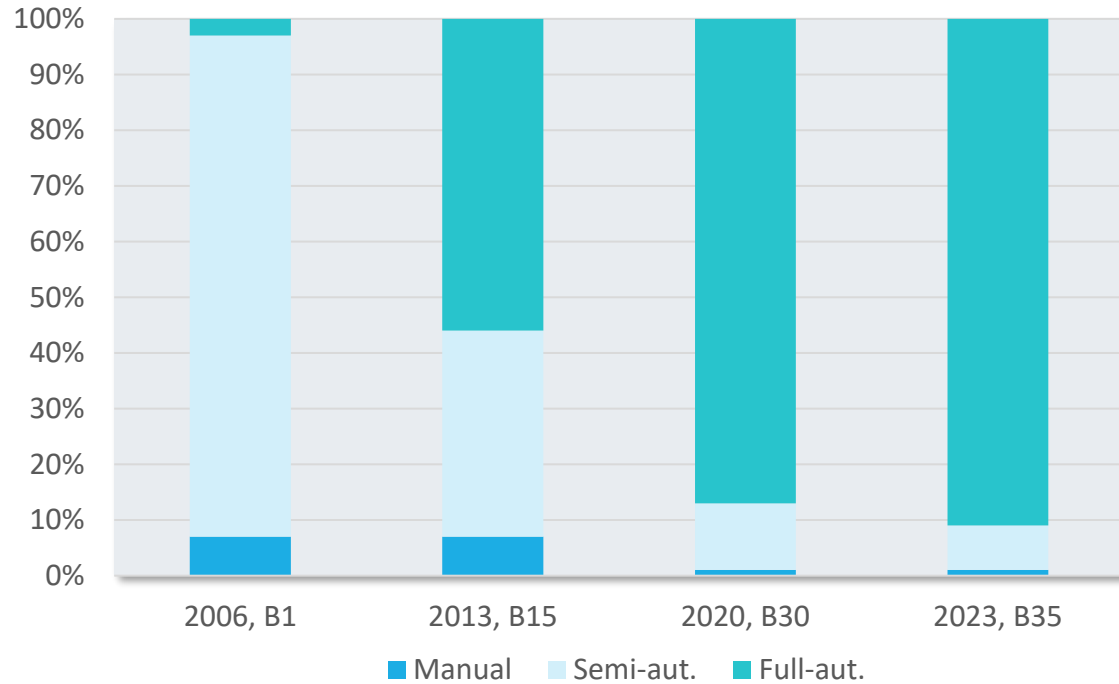
PROPER BLACK BOX TESTING CASE DESIGN

Equivalence Partitioning

Tek Genie®
Staining Catalog
september 2020



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

2023: Fully automated with focus on 4 core elements

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

Capillary; BOND and Prime 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;

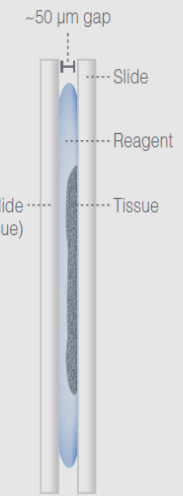
To spread reagents
and to avoid slides
drying out

Capillary Gap Staining

Tissue
slide

+ (with or without tissue)

Cover



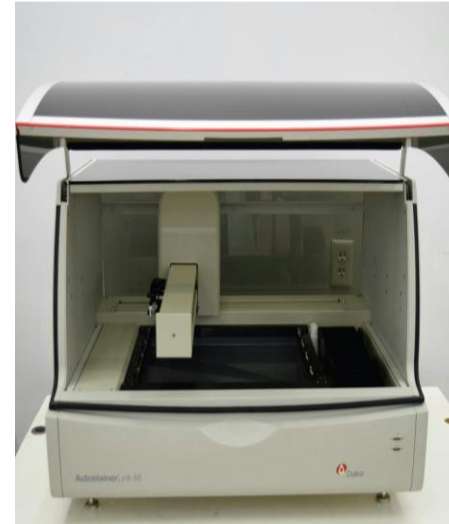
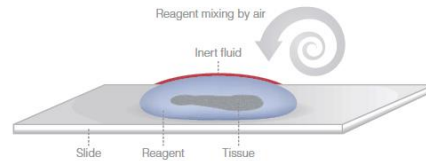
IHC – Immunohistochemical stainers

Flat labelling technology stainers:



Ventana:
+Mixing
+Overlay

Liquid Overlay Technology



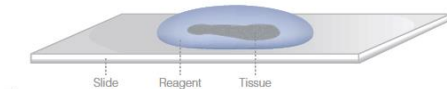
Dako:
-Mixing
-Overlay

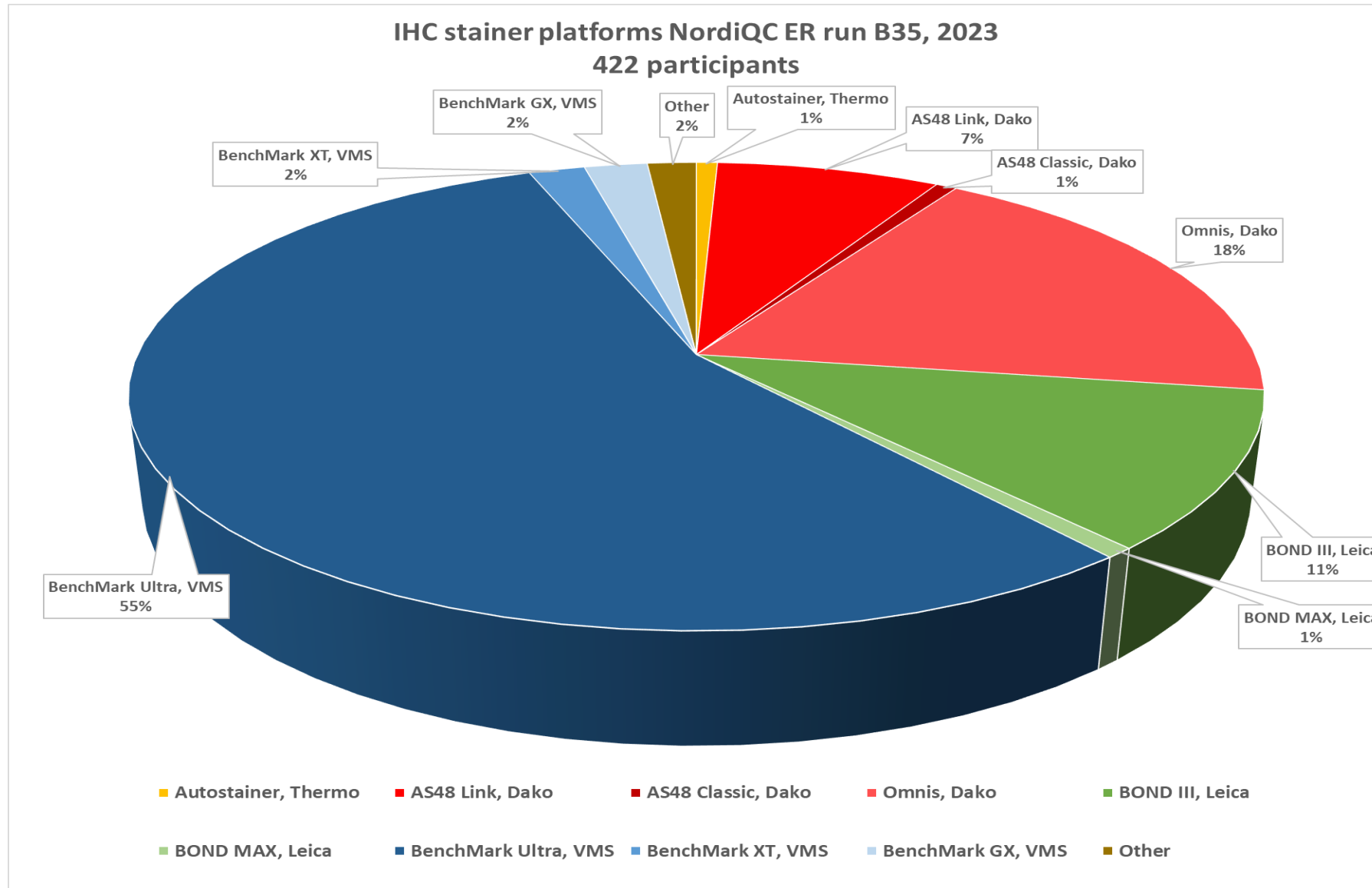


Technique;

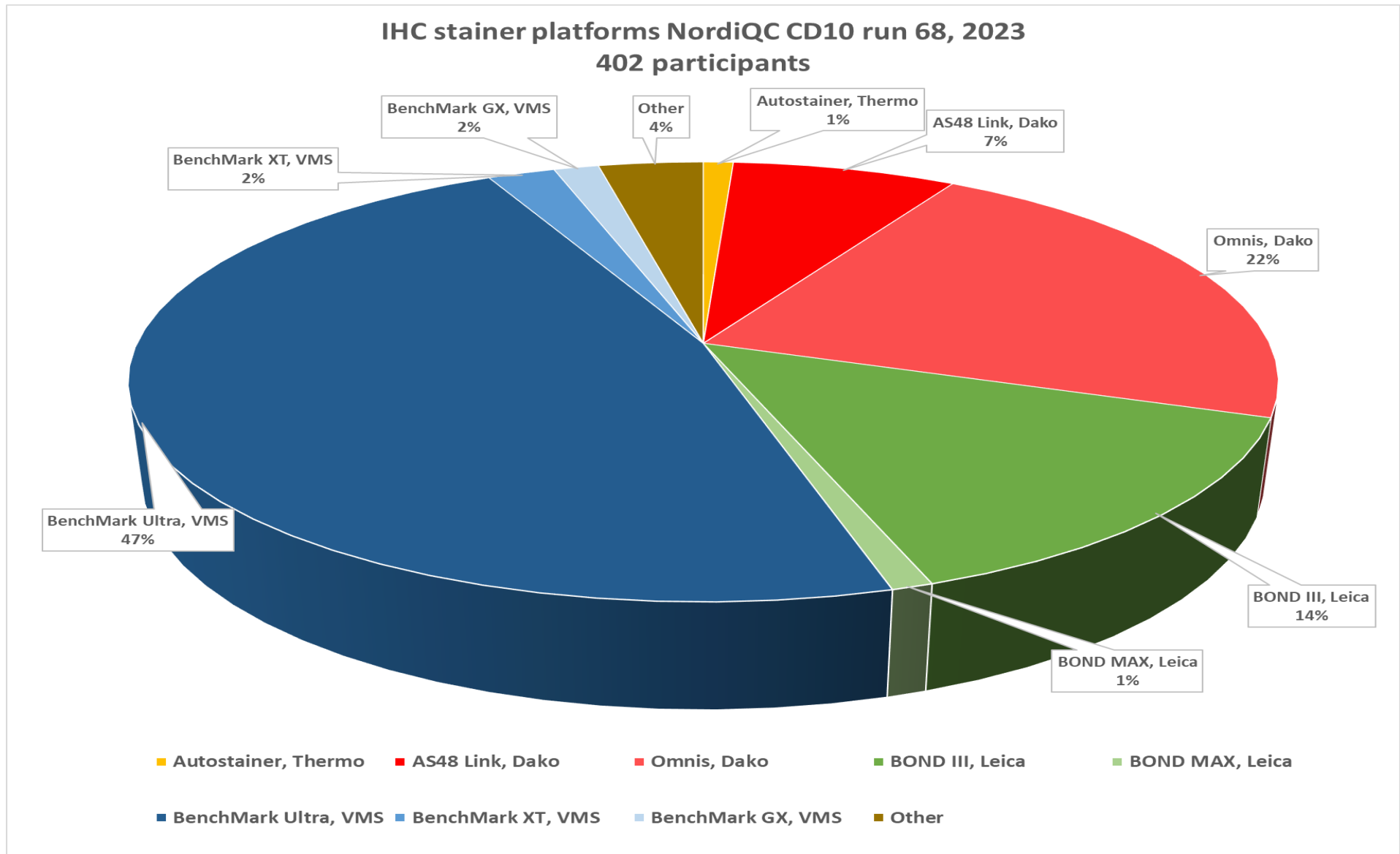
Reagents are applied
+/- mixing
+/- overlay

Open Individual Slide Staining





IHC – Immunohistochemical stainers

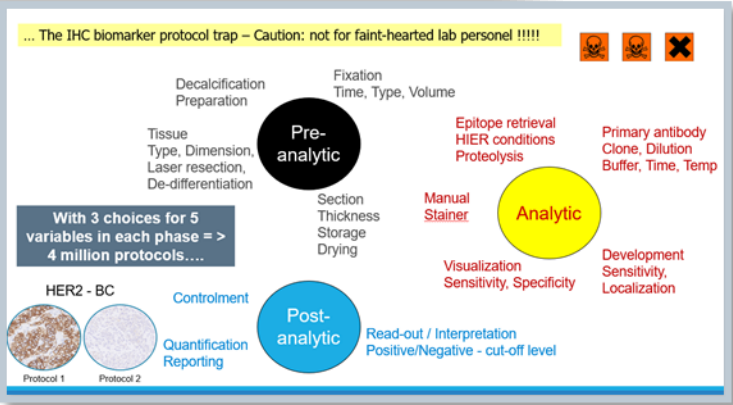


Harmonization of "best practice" IHC methods

TABLE 3. Harmonization of Protocols and Pass Rates for ER Among NordiQC Participants

Run No.	8	B1	B15	B32
Year	2003	2006	2013	2021
Ready-To-Use antibody (%)	17	20	66	88
Alkaline buffer for HIER (%)	75	85	94	96
Multimer/polymer detection system (%)	61	71	93	99
Fully automated IHC platform (%)	4	24	59	89
Pass rate (%)	50	75	77	89

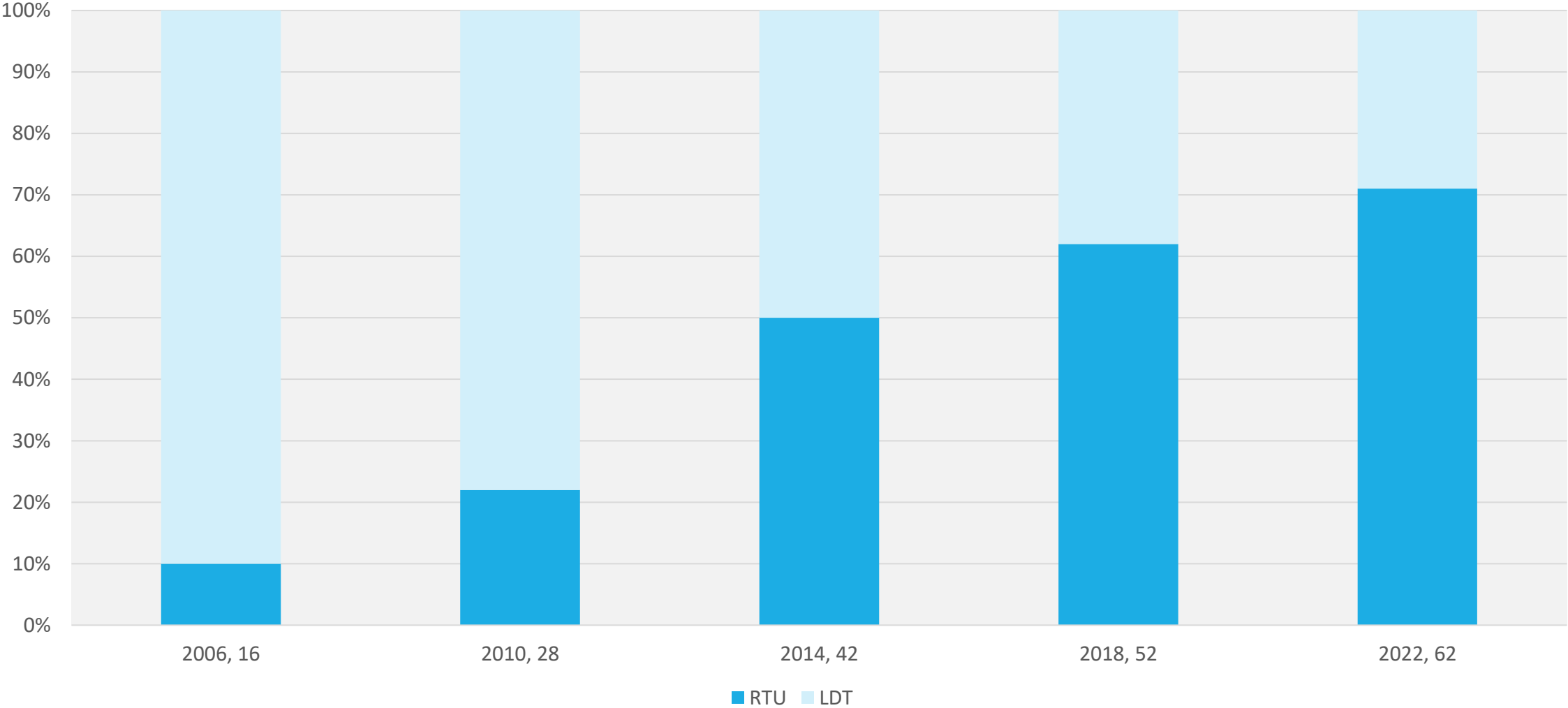
HIER indicates heat-induced epitope retrieval.



Nielsen, Søren, Bzorek, Michael, Vyberg, Mogens, Røge, Rasmus.
 Lessons Learned, Challenges Taken, and Actions Made for “Precision” Immunohistochemistry. Analysis and Perspectives From the NordiQC Proficiency Testing Program.
 Applied Immunohistochemistry & Molecular Morphology 31(7):p 452-458, August 2023. | DOI: 10.1097/PAI.0000000000001071

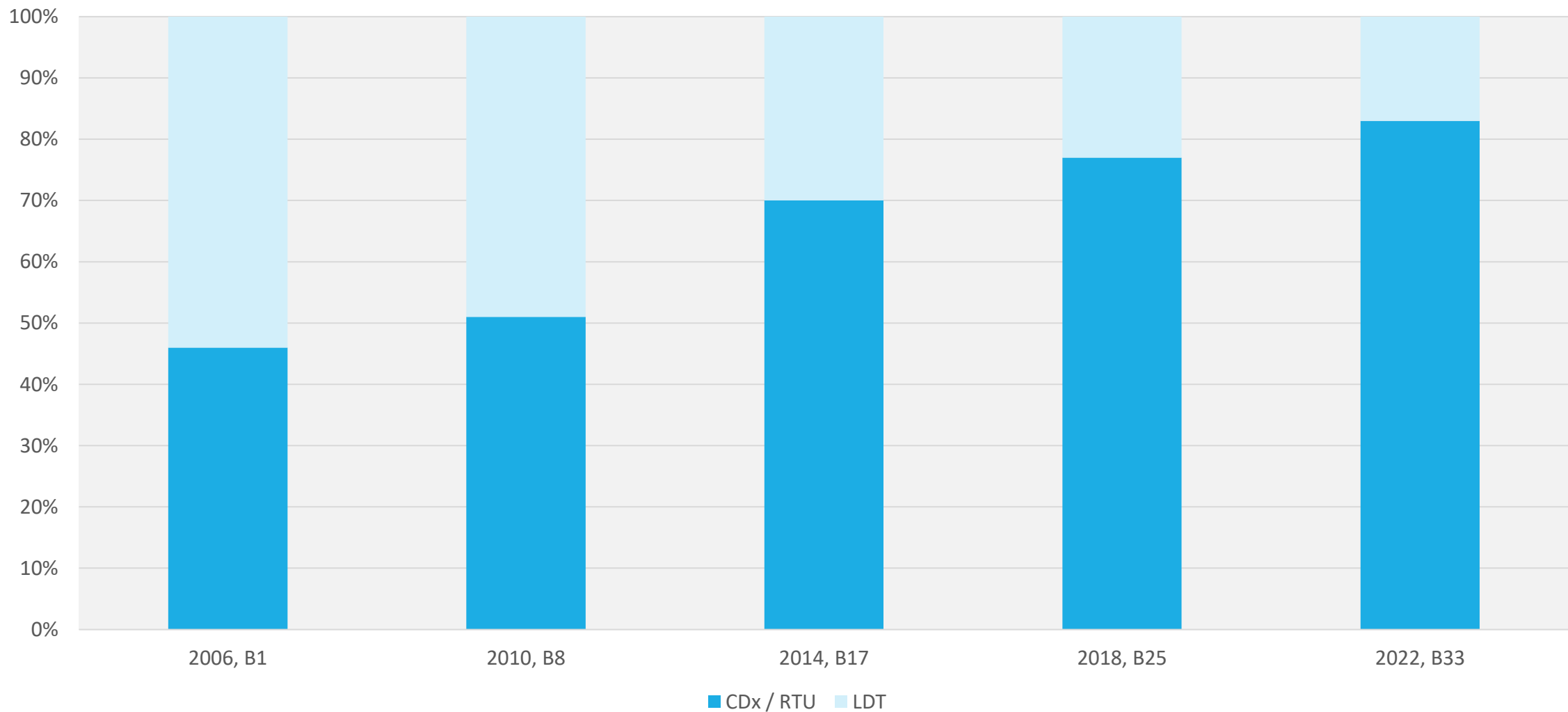
Proportion of protocols based on conc. and RTU formats in NordiQC

General module



Proportion of protocols based on conc. and RTU formats in NordiQC

Breast module



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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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)

“If you understand the needs of your laboratory and the capabilities of the various systems, you can find the best fit for your laboratory.”

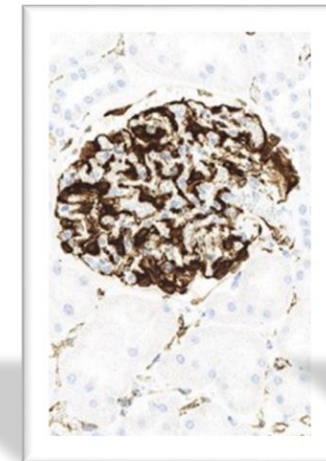
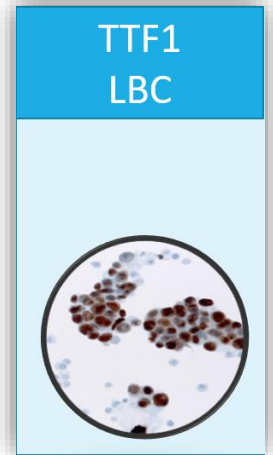
“If an automated IHC platform is chosen correctly to match the demands of testing, automation can provide necessary process improvement and cost savings needed in the modern practice of pathology.”

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

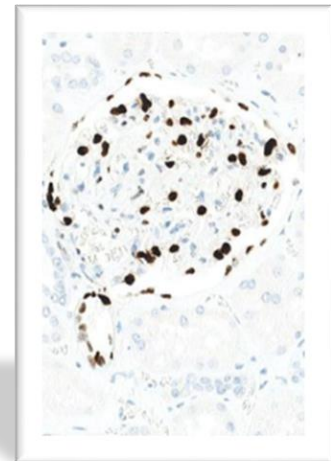
Automation of the IHC staining procedure:

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
-



WT1 6F-H2
HIER



WT1 6F-H2
HIER + Prot.

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 resources for maintenance
 - Frequency, extent, safety etc



Agilent Case Study: Dako Omnis Workflow

Complete Dako Omnis Installation Resulted in Improved Workflow

Agilent Dako

How one lab went from five stainers to two Dako Omnis platforms

It's not an easy decision to change an entire laboratory's way of working. Will the new solution deliver on the promises, or is it easier and more secure to just continue with the current setup? Providing evidence for improved workflow after the change is one way to be confident that Dako Omnis is the best choice.

An Agilent representative initially approached the laboratory from the Netherlands in 2017. After taking the time to learn about the laboratory setup and their challenges, the representative, working with Agilent's workflow specialists, was able to propose a new setup that could deliver:

- A well-organized workflow providing improved efficiency
- 1. More capacity
- 2. Faster time to case delivery
- Efficient merging of manual and automated processes
- 3. Less hands-on time
- 4. Continuous delivery of patient cases
- Quantitative information to support the proposal
- 5. Simulations and documented effects of improvements

To deliver on these commitments, the Agilent Workflow Team first proposed a plan to analyze the current setup in the lab and created a simulation of a future workflow. This analysis focused on the lab's instrument use patterns, including analysis of hands-on time and patient case delivery time for their current and proposed system.


Dako Omnis

Dako Omnis enables you to run your patient cases as they arrive to the lab in a fully automated and lean workflow. You can start staining immediately or overnight, as needed for your patient cases.

A 60-slide capacity, together with six independent staining units, enables optimized patient case management with continuous slide loading.

A capacity of 60 onboard, temperature-controlled reagents eliminates the need to constantly switch reagents saving hands-on time.

Ability to load and unload slides and reagents while the instrument is running frees-up time and supports an efficient laboratory workflow.



TRANSCEND EXPECTATIONS

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

Coupled with the ability to produce high-quality, crisp, clear staining with an average IHC slide TAT of 90 minutes, the BOND-PRIME staining platform lets you transcend expectations for quality, timeliness, and diagnostic productivity.

Load and unload slides on your schedule, not the instrument's.

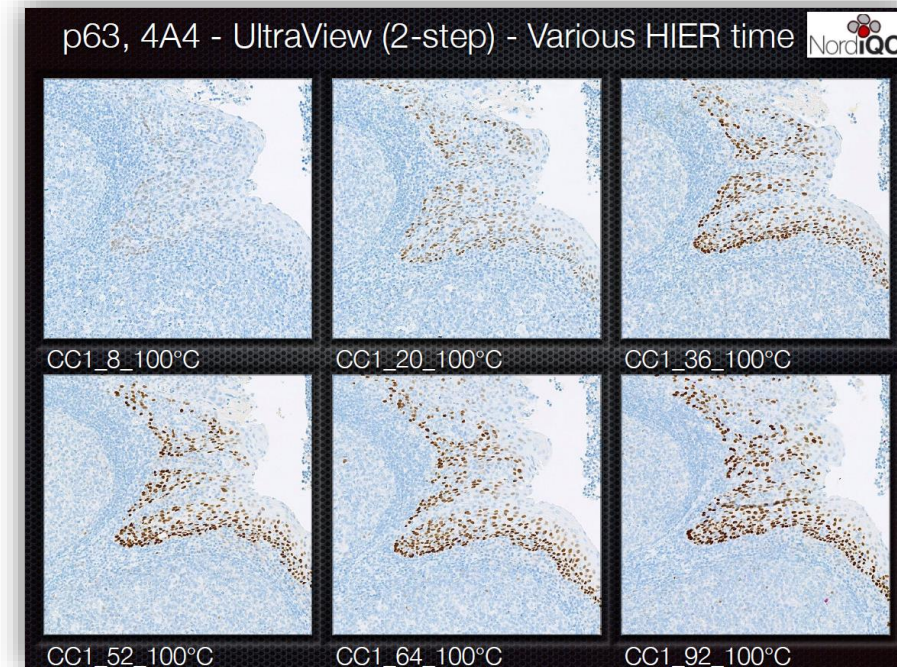
BOND-PRIME ADAPTS TO ANY WORKFLOW:

Batch	Continuous	Single slide
-------	------------	--------------

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

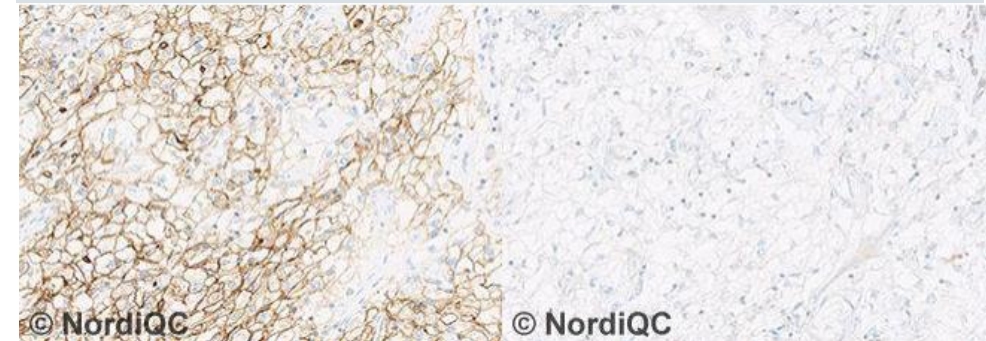
- Reagents
 - HIER reagents
 - How many and which HIER buffers are offered ?
 - Can 3' party HIER buffers be applied ?
 - Proteolysis
 - Which proteolytic enzymes are offered
 - Can 3' party enzymes be applied
- Primary antibody
 - 3' party antibodies ?
 - RTU antibodies available ?



EP-CAM in Renal Clear Cell Carcinoma
mAb clone Ber-EP4

HIER TRS low pH 6,1

HIER CC1 pH 8,5

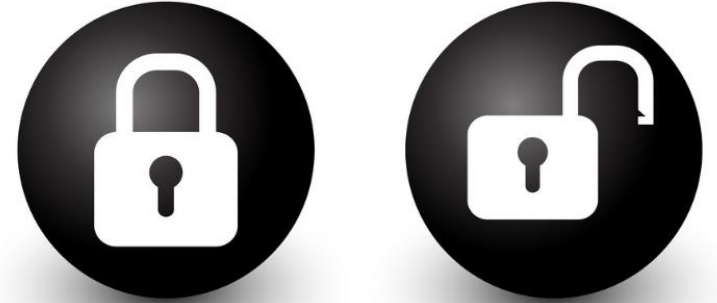


IHC – Immunohistochemical stainers

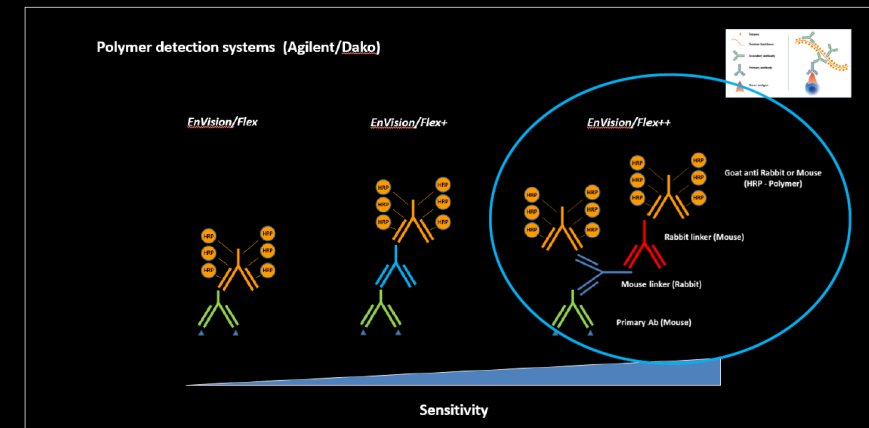
Automation of the IHC staining procedure:

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 ?

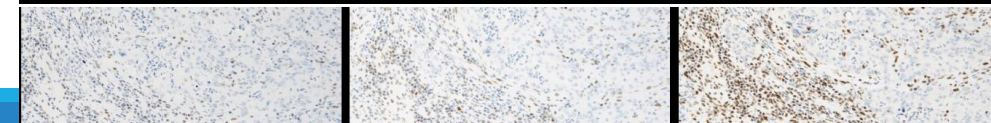


Technical aspects of immunohistochemistry & pitfalls - Analytical phase



“New option” on the Omnis

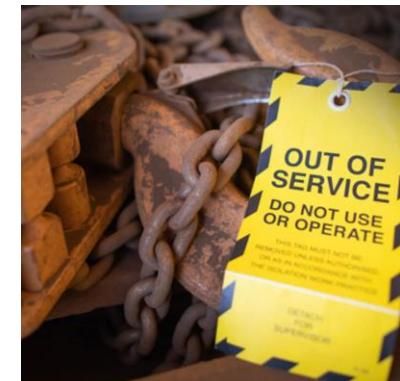
In general, works well with rabbit primary Abs but less efficient with primary mouse primary Abs



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

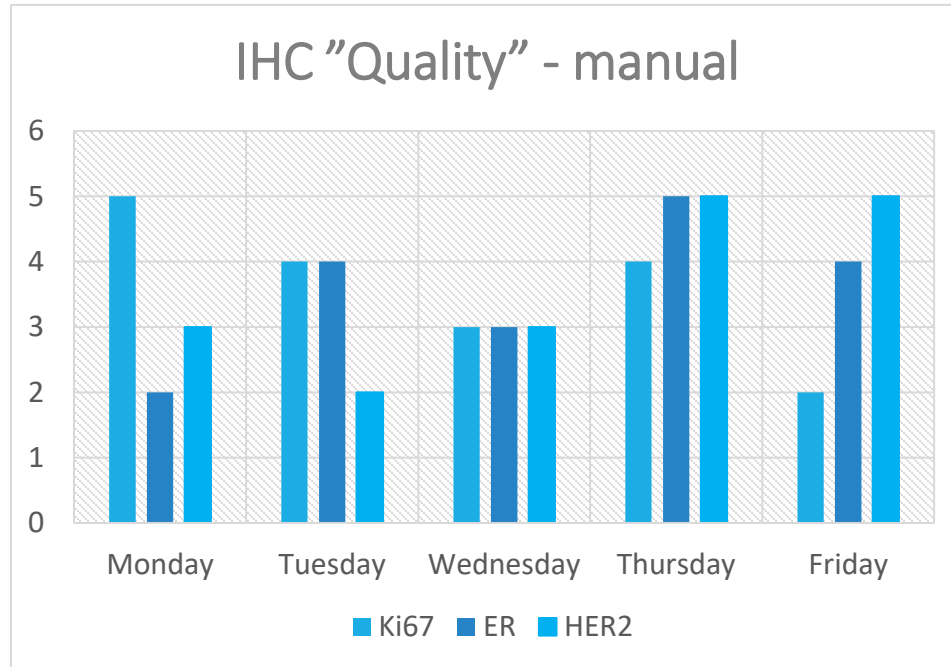


IHC – Immunohistochemical stainers

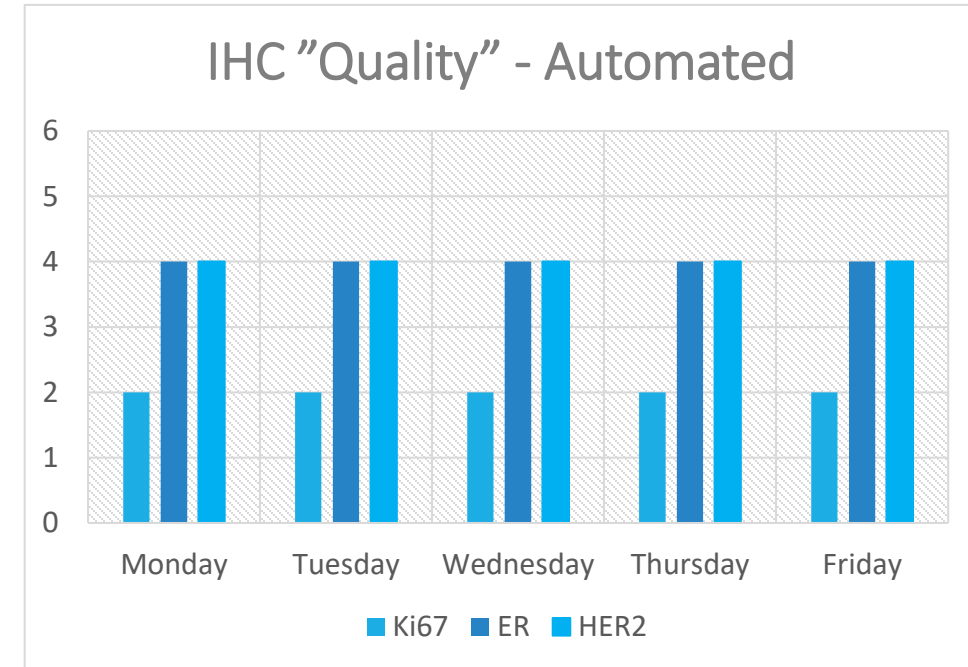
	Dako AS 48	Dako Omnis	VMS Ultra	Leica BOND III	Biocare ONCORE	Sakura Genie	Leica Prime
Capacity	48	60	30	30	36	30	24
Reagents	64	60	35	36	40	39	70
Volume	200 ul	200 ul	100 ul	150 ul	130 ul	350 ul	150 ul
Adjustable vol.	Yes	No	No	Yes	Yes	No	No
Depar.	No	Yes	Yes	Yes	Yes	Yes	Yes
HIER	No	Yes	Yes	Yes	Yes	Yes	Yes
HIER buf. 3' party	- Yes	5 Yes	2 No	2 No	2 No	2 No	2 No
Comb. ret.	Yes	Yes – H+P	Yes	Yes – H+P	?	Yes US/No EU	Yes
3' party reagents	Ab, enz, det, chr.	Ab, enz, det, chr.	Ab, enz	Ab, enz	Ab, enz	Ab	Ab
Protocol flexibility	High	Moderate	High	Moderate	High	Low	High
Any prot. / Any slide	Yes	No	Yes	No	Yes	Yes	Yes
Seq. DS	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sim. DS	Yes	Yes	No	No	Yes	No	Yes
ISH	No	Yes	Yes	Yes	Yes	Yes	Yes
RTU's no*	116	84	260	155	64	150	155
CDx range	High	Moderate	High	Low	None	None	None

* Estimate 09.2023 with uncertainties and changes due to IVDR and local regulations

IHC – Immunohistochemical stainers



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

Target	Clone	AS 48 Link	Omnis	BenchMark	Bond
ALK	D5F3	√	(√)	√	√
ASMA	1A4	√	(√)	FN,FP	√
Bcl6	PG-B6p	√	-	FN	(√)
BSAP	24	√	FN	FN	√
BRAF	VE1	(√)	FN	√	FN
Calretinin	Dak-Calret1	√	FN	FN	√
CD4	4B5	√	FN	FN	(√)
CD56	123C5	√	FN	FN	√
CDX2	DAK-CDX2	√	√	FN	√
CEA	II7	√	-	FN	√
CK-LMW	5D3	√	-	FN	√
Desmin	D33	√	FN	√	√
EPCAM	Ber-EP4	√	√	FN	FN
Hepatocyte	OCH1E5	√	-	√	FP
Melan A	A103	√	FN	FN	√
PAX8	MRQ-50, BC12	√	FN	FN	√
SATB2	EP281	√	(√)	√	(√)
SMAD4	B-8	√	FN	FN	√
.....					

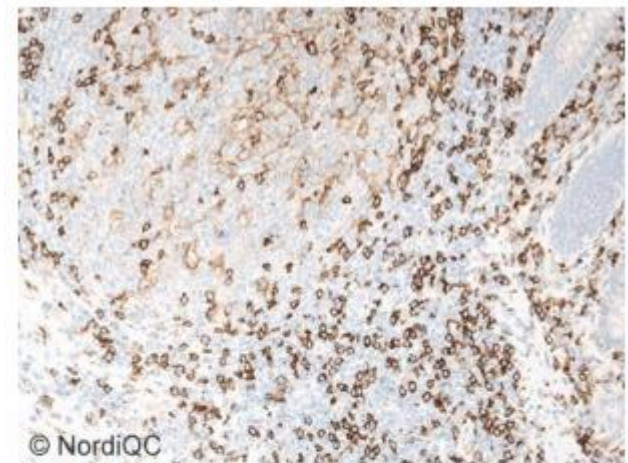
IHC performance challenges related to automation and clone choice

NordiQC data

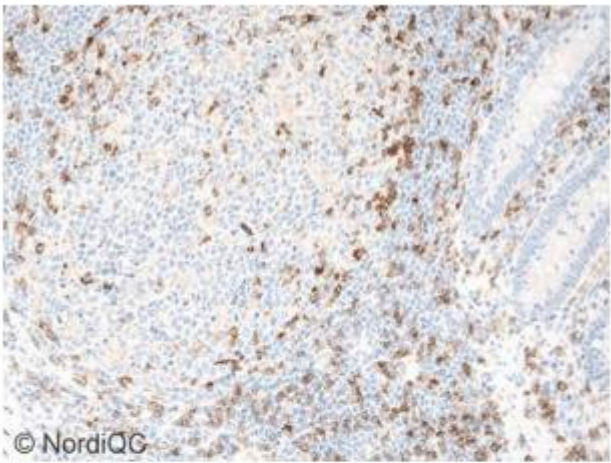
IHC – Immunohistochemical stainers

Target	Clone	AS 48 Link	Omnis	BenchMark	Bond
ALK	D5F3	√	(v)	√	√
ASMA	1A4	√	(v)	FN,FP	√
Bcl6	PG-B6p	√	-	FN	(v)
BSAP	24	√	FN	FN	√
BRAF	VE1	(v)	FN	√	FN
Calretinin	Dak-Calret1	√	FN	FN	√
CD4	4B5	√	FN	FN	(v)
CD56	123C5	√	FN	FN	√
CDX2	DAK-CDX2	√	√	FN	√
CEA	II7	√	-	FN	√
CK-LMW	5D3	√	-	FN	√
Desmin	D33	√	FN	√	√
EPCAM	Ber-EP4	√	√	FN	FN
Hepatocyte	OCH1E5	√	-	√	FP
Melan A	A103	√	FN	FN	√
PAX8	MRQ-50, BC12	√	FN	FN	√
SATB2	EP281	√	(v)	√	(v)
SMAD4	B-8	√	FN	FN	√
.....					

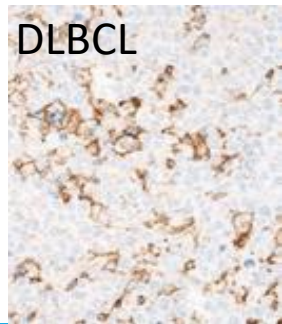
Mitigation of RTU from one system to another can be challenging



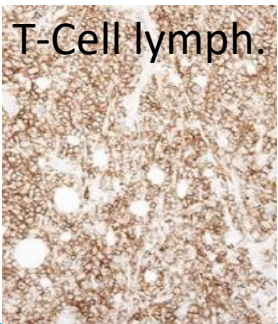
© NordiQC
Fig. 1a (x200)
Optimal staining reaction for CD4 of the appendix applying the RTU assay IR649 (Autostainer, Dako/Agilent) based on the mAb clone 4B12, following vendor recommended protocol settings based on HIER in TRS (3-in-1) pH 9 and Envision FLEX+ as detection system.
All T-helper/inducer cells show a strong and distinct membranous staining reaction. The germinal centre macrophages display a weak to moderate staining intensity. No staining reaction was observed in B-cells and epithelial cells of the appendix. Same protocol used in Figs. 2a - 4a.



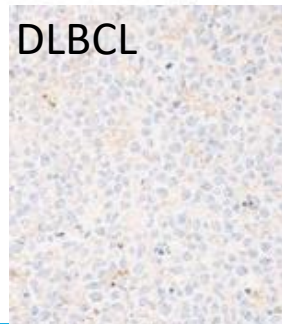
© NordiQC
Fig. 1b (x200)
Insufficient staining reaction for CD4 of the appendix applying the same RTU system as in Fig. 1a, but used on the fully automated instrument Omnis (Dako/Agilent) with similar protocol settings as in Fig 1a - same protocol used in Figs. 2b - 4b.
The staining intensity is significantly reduced in T-helper/inducer cells and germinal centre macrophages are false negative or only faintly demonstrated. This antibody clone provides too low analytical sensitivity on this particular platform (see description above) and should prompt laboratories to substitute to a robust primary Ab as e.g., the rmAb clones SP35 or EP204 - compare with Fig. 2a-4b.



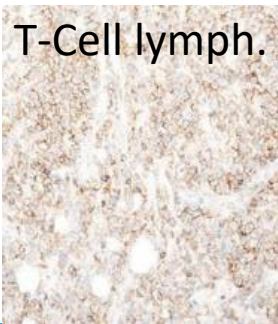
DLBCL



T-Cell lymph.



DLBCL



T-Cell lymph.

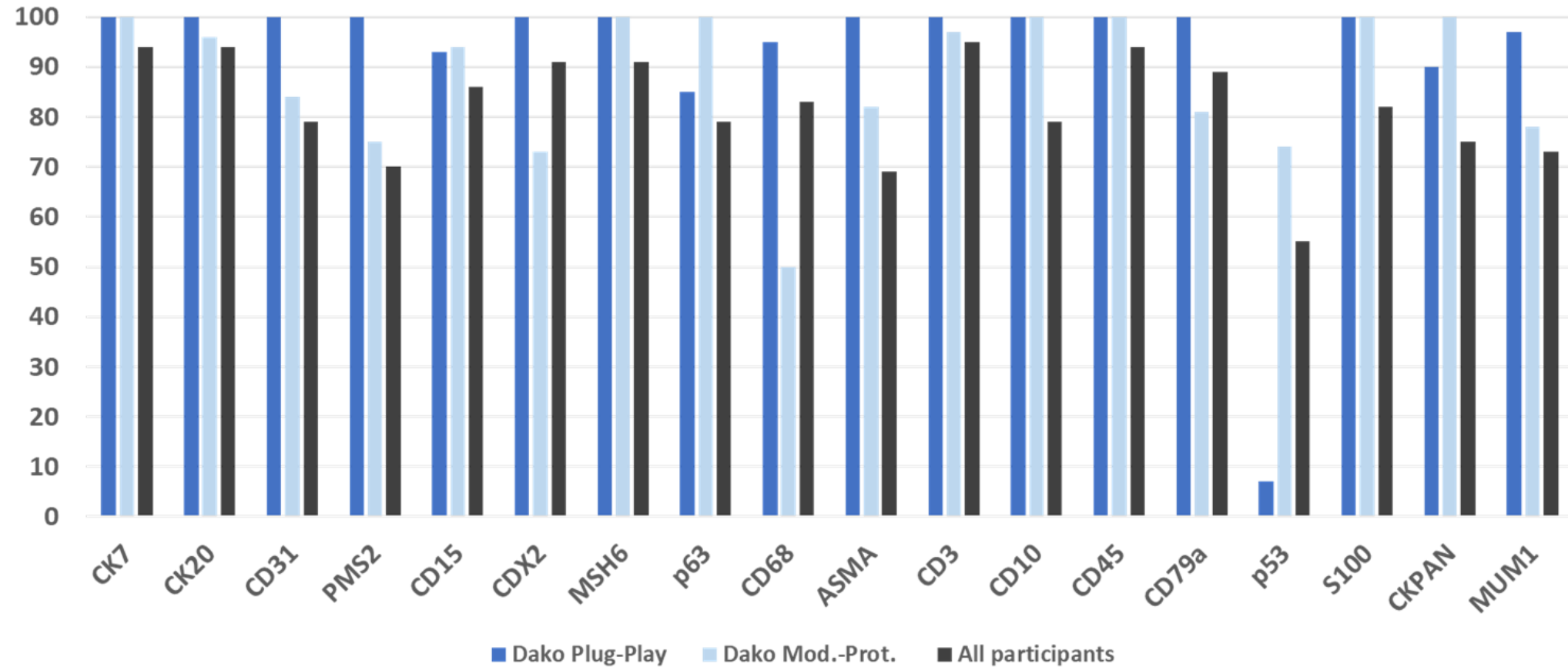
Pass rates for RTU's on "non-intended" platform;

	IR649 4B12 AS 48 Link	IR649 4B12 Omnis	Alternative clone* Omnis
CD4	93% (13/14)	0% (0/28)	100% (21/21)
	IR606 D33 AS 48 Link	IR606 D33 Omnis	Alternative clone** Omnis
Desmin	67% (18/27)	5% (2/37)	100% (7/7)

* SP35, ** BS21

IHC – Immunohistochemical stainers

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



IHC – Immunohistochemical stainers

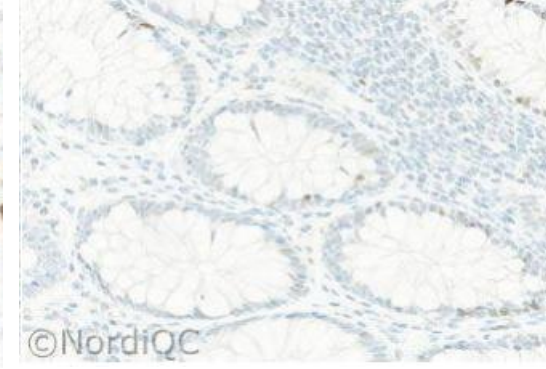
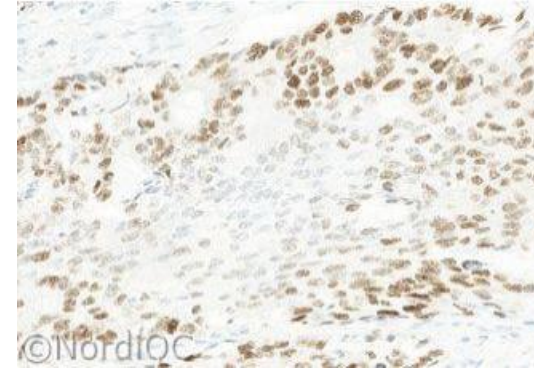
p53 run 38, 2013;

Purpose being demonstration of overexpression of p53 protein caused by TP53 mutation

p53 run 38, 2013;

Dako/Agilent RTU; 88% pass rate as "plug-and-play"

Same level for other systems



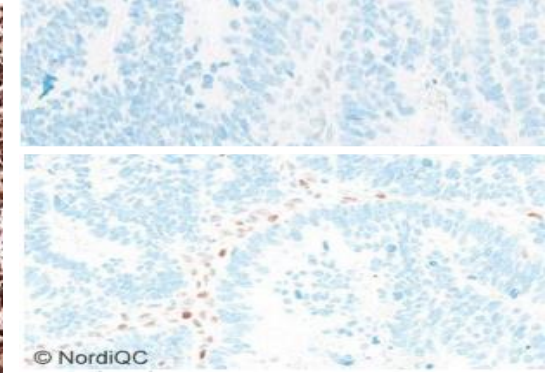
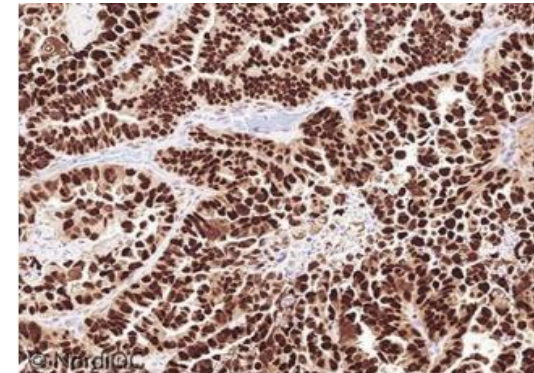
p53 run 63, 2021;

Purpose being demonstration of overexpression **and loss** of p53 protein caused by TP53 mutations

p53 run 63, 2021;

Dako/Agilent RTU; 3% pass rate as "plug-and-play"

Same level for other systems



IHC assays including RTU systems must always be developed and validated for its purpose(s)

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

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

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 portofolio of RTU antibodies – plug-and-play

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

3 main Cons:

1. Covertile technique – precipitates and weak hue
2. Less flexible regarding continuous start – 3 x 10 slides
3. Limited portfolio type II assays (CDx assays PD-L1 etc)

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
3. High capacity and high daily throughput
4. IHC and ISH on same instrument
5. Temperature controlled reagents

3 main Cons:

1. Limited portfolio of RTUs (I & II) & 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's type I & II – plug-and-play

Semi-automated systems: AS-48, Dako

Functionality – Workload – Workflow - Flexibility – Costs

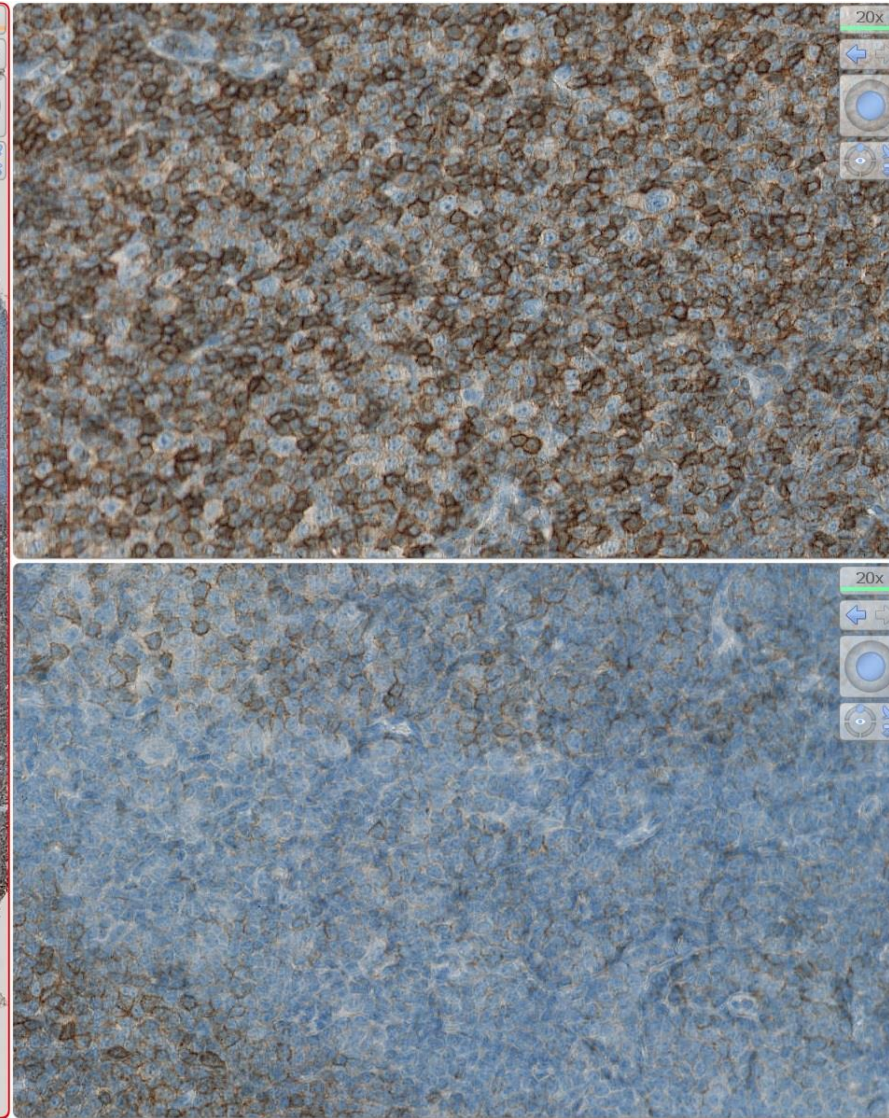
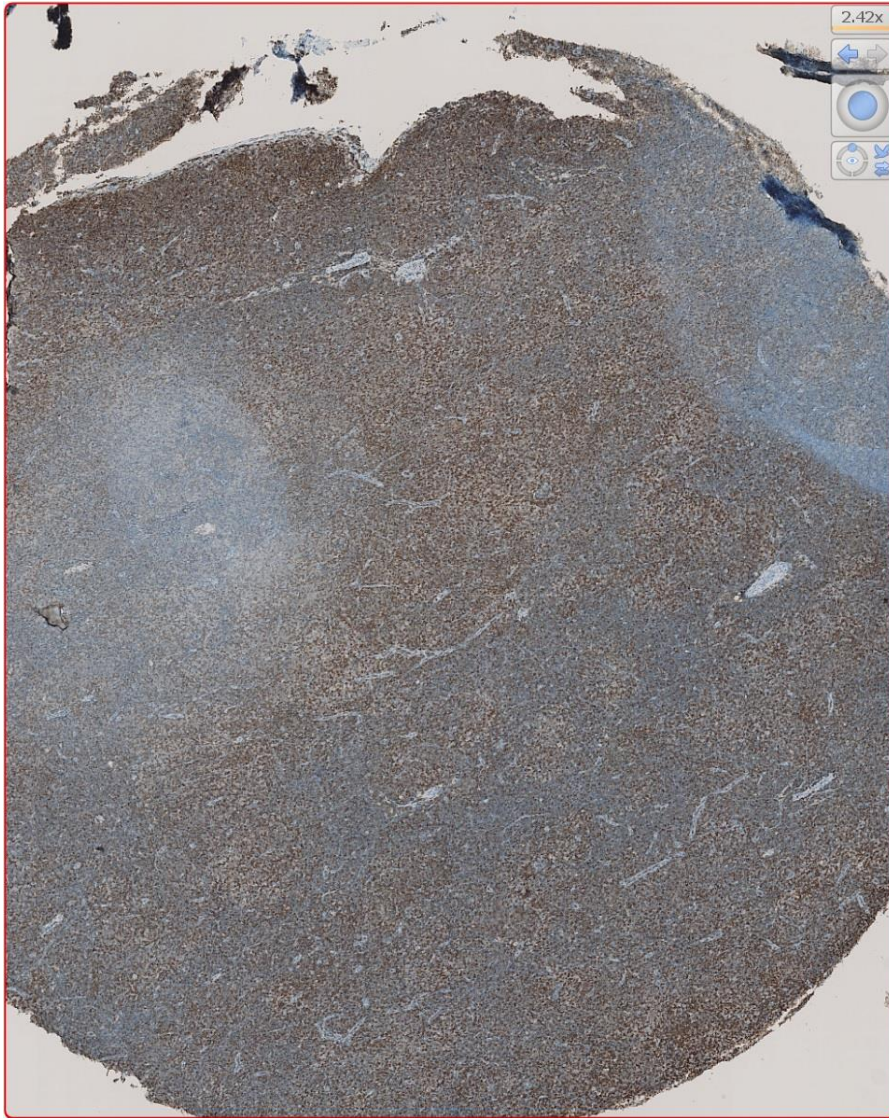
5 main Pros:

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. Good portofolio of RTU's type I & II – 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"

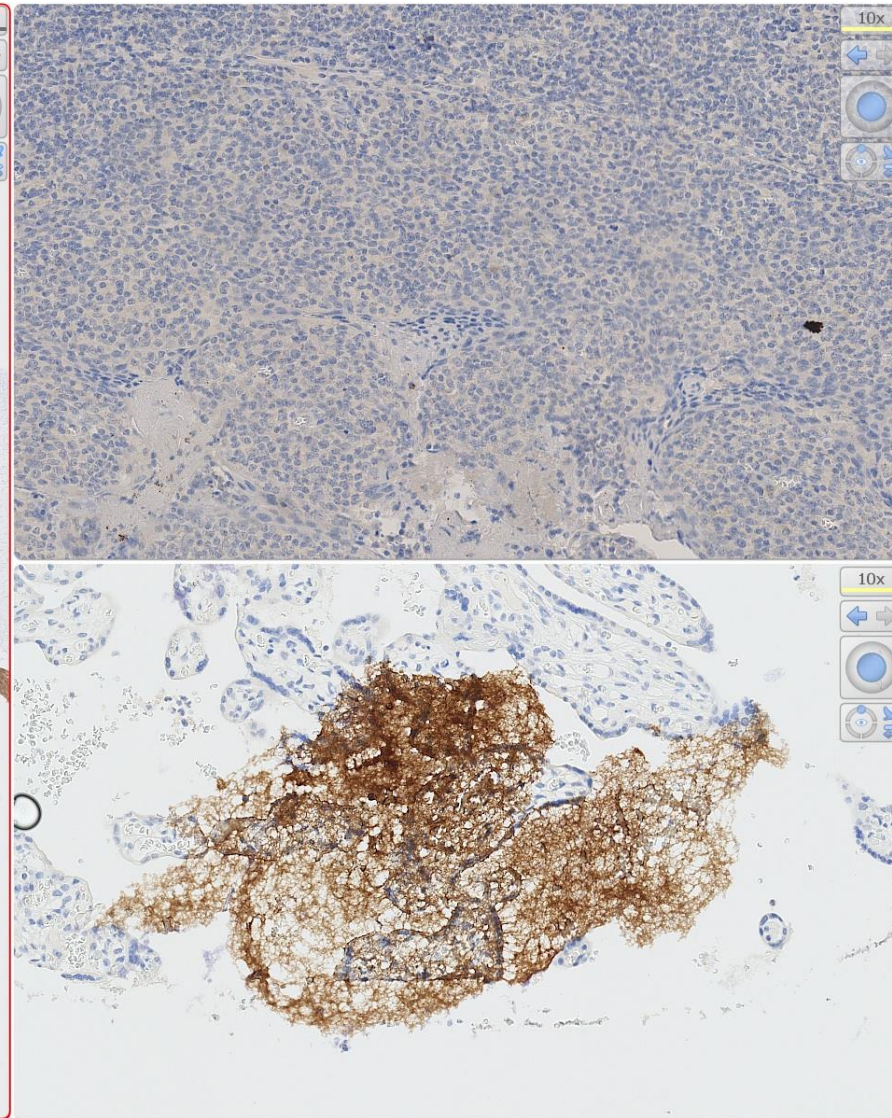
IHC – Immunohistochemical stainers



Staining issues
BenchMark, VMS;

Uneven weak and neg areas
– air bubbles

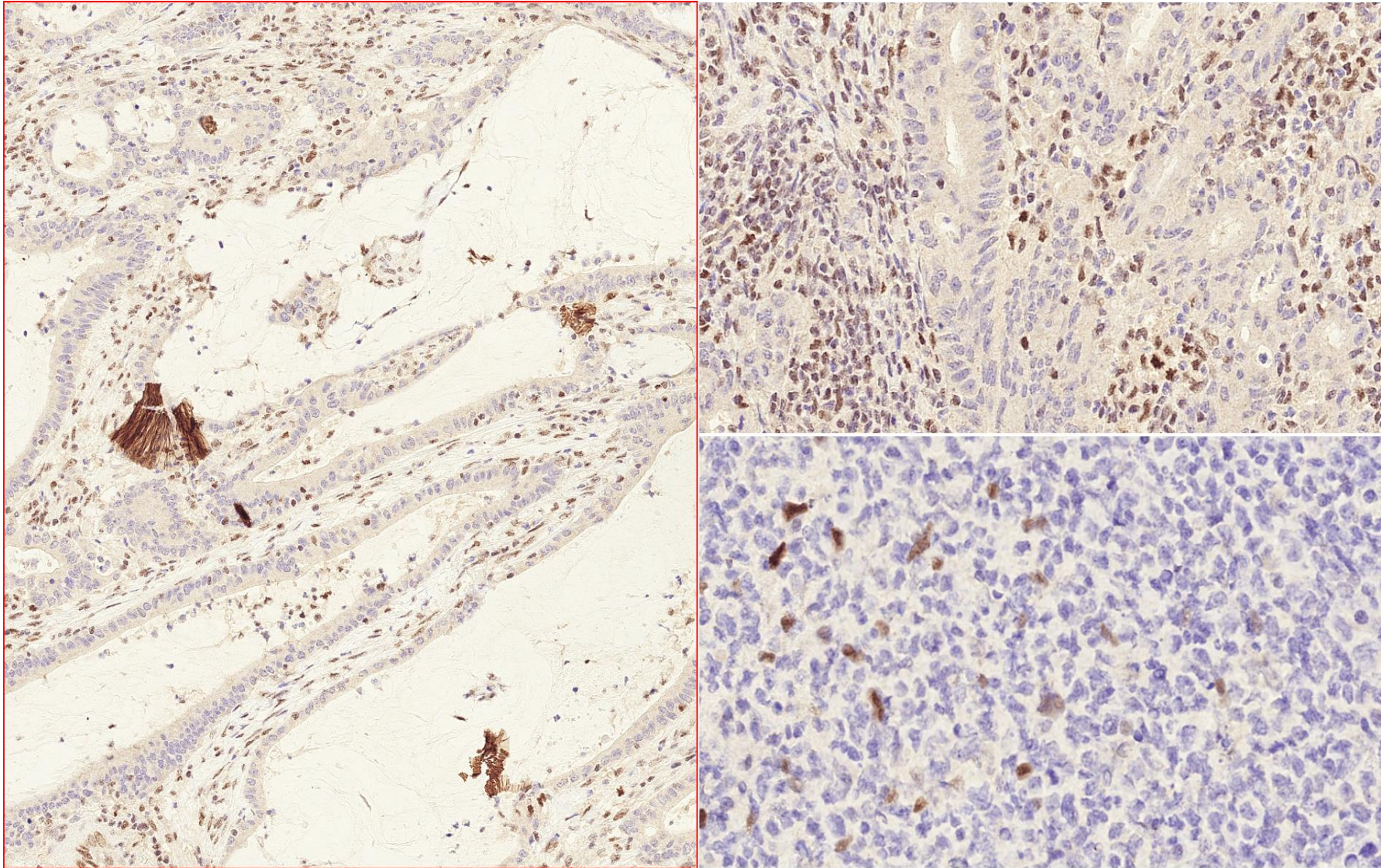
IHC – Immunohistochemical stainers



Staining issues
Bond, Leica;

Chromogen precipitates and
general hue

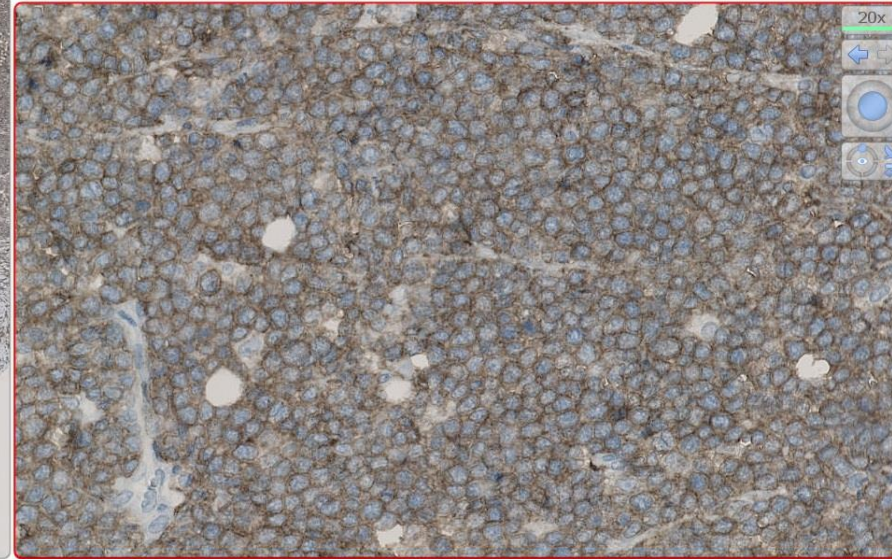
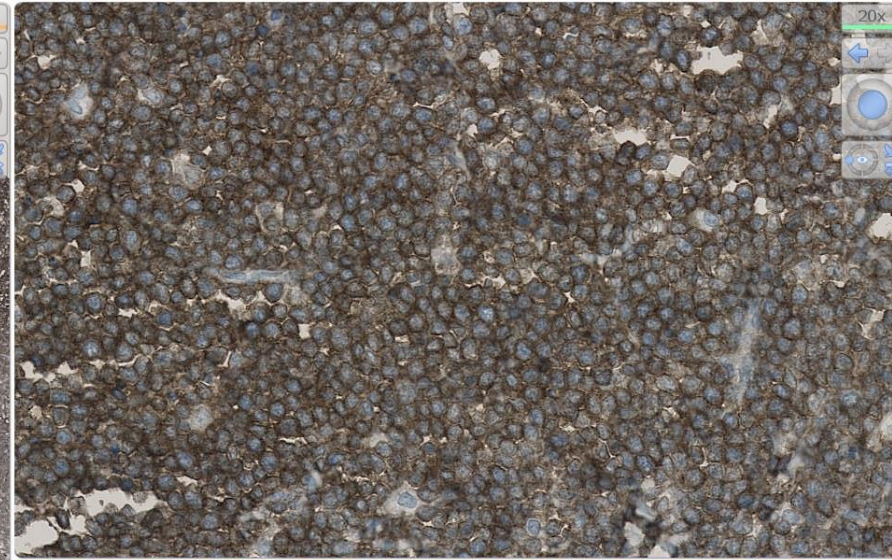
IHC – Immunohistochemical stainers



Staining issues
Omnis, Dako;

Chromogen precipitates,
morphology and general hue

IHC – Immunohistochemical stainers



Staining issues
AS48, Dako;

Chromogen depletion,
reagents not spread, drying
out

Issue with rack levelling

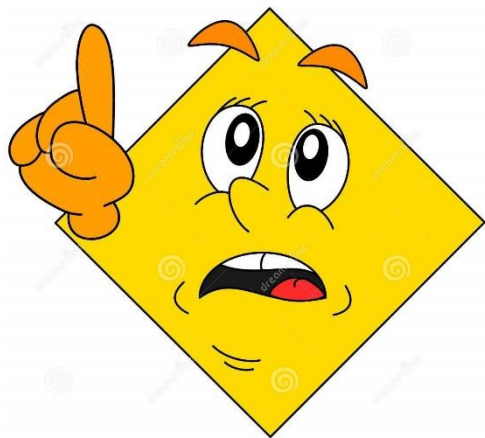


REVIEW ARTICLE

(*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,‡§



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Dreamstime.com

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†

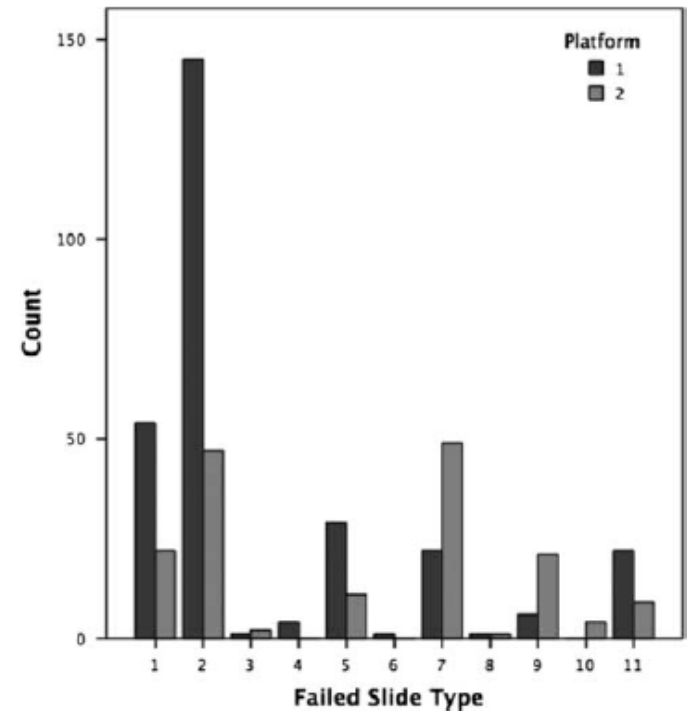


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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)

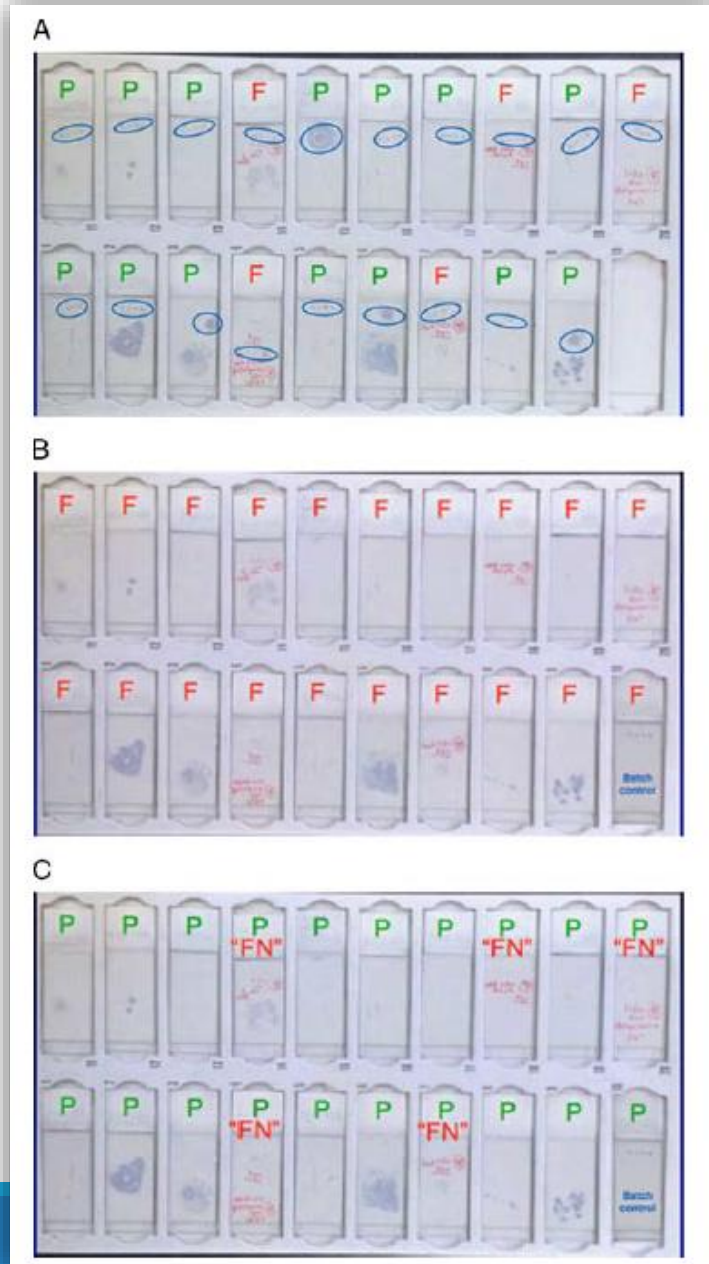
IHC indicates immunohistochemistry.

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:

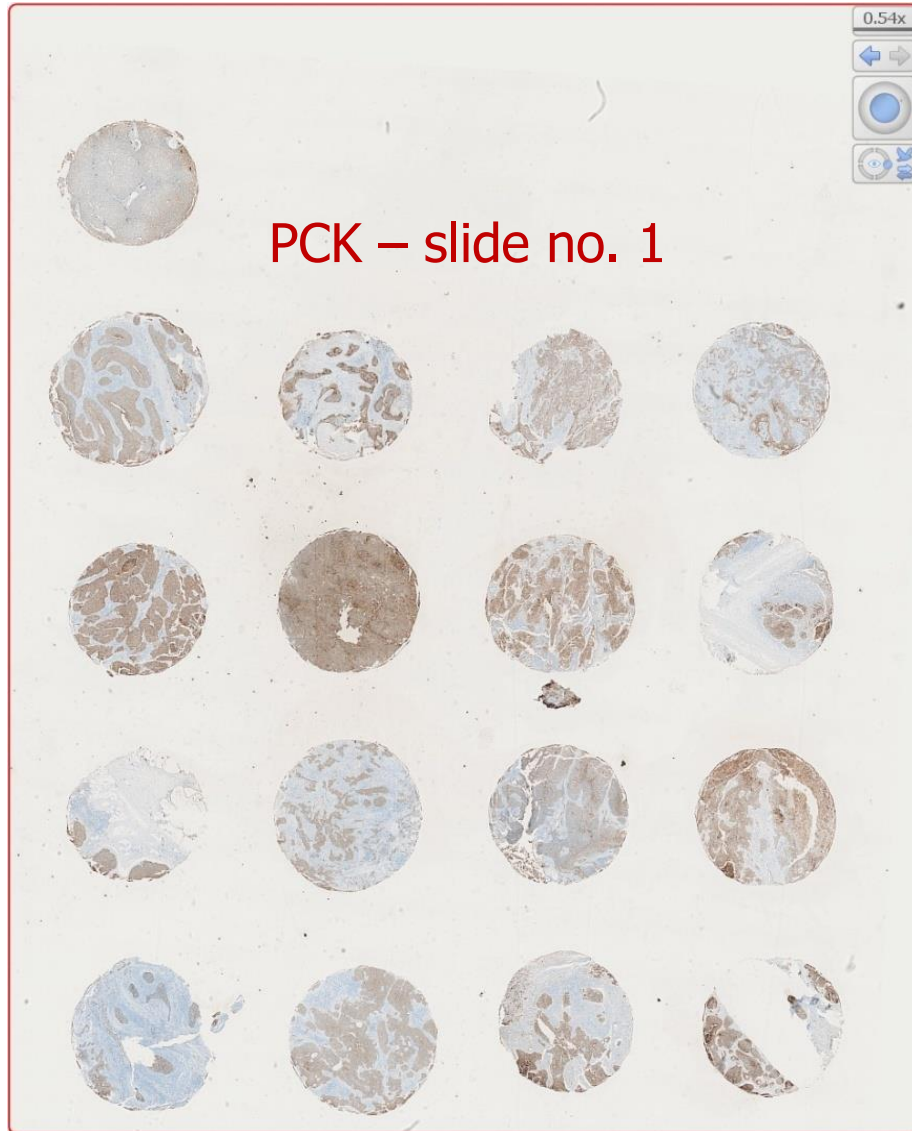
Batch control fail by same conditions as above
0/19 passed
19/19 failed (no consistent internal control...)

Batch-control - Theoretically:

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

IHC – Immunohistochemical stainers

PCK – slide no. 1



PCK – slide no. 2



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

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

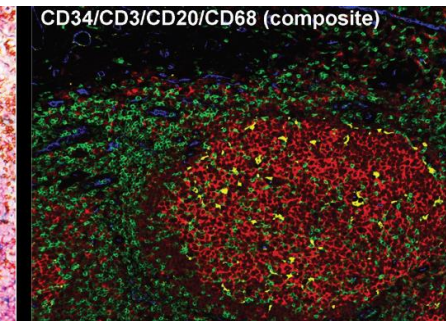
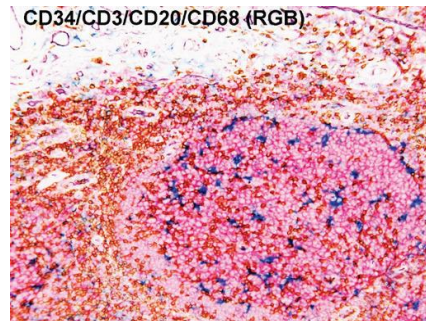
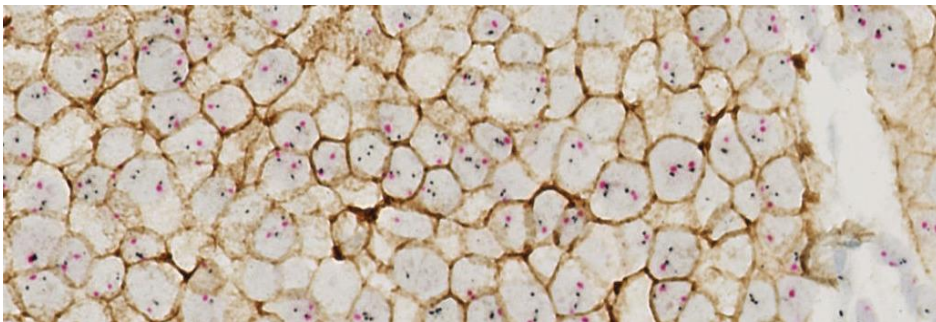
IHC – Immunohistochemical stainers

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. Multi-functionality – IHC, coverslipping, scanning in one device
6. New “players” on the market



IHC – Immunohistochemical stainers

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



Flexible automated system for researchers

LabSat™ Research is an ultra-rapid automated staining instrument based on an innovative microfluidic technology that is capable of carrying out IHC/IF staining cycles within a few minutes, in a highly precise and reproducible manner. This technology breakthrough together with a fully open system, bring a flexible solution at the reach of medium and small laboratories.

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

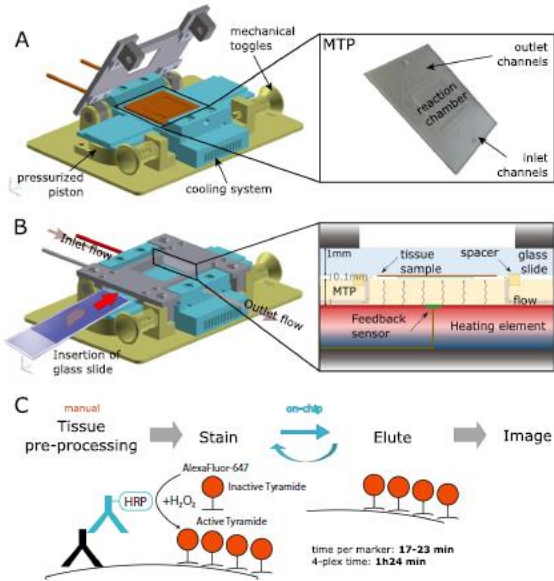
com/scientificreports

SCIENTIFIC REPORTS

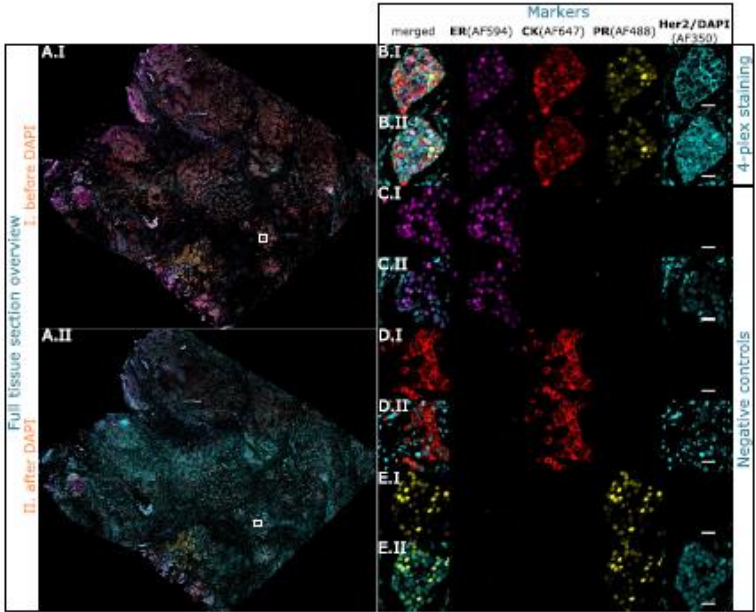
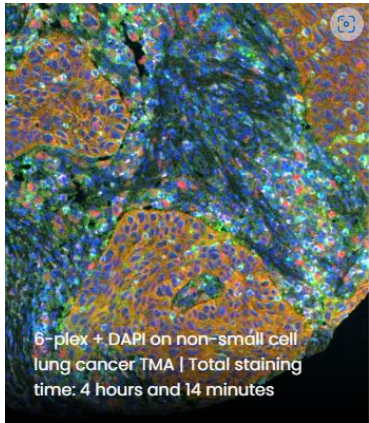
OPEN Ultra-fast and automated immunohistofluorescent multistaining using a microfluidic tissue processor

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

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



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

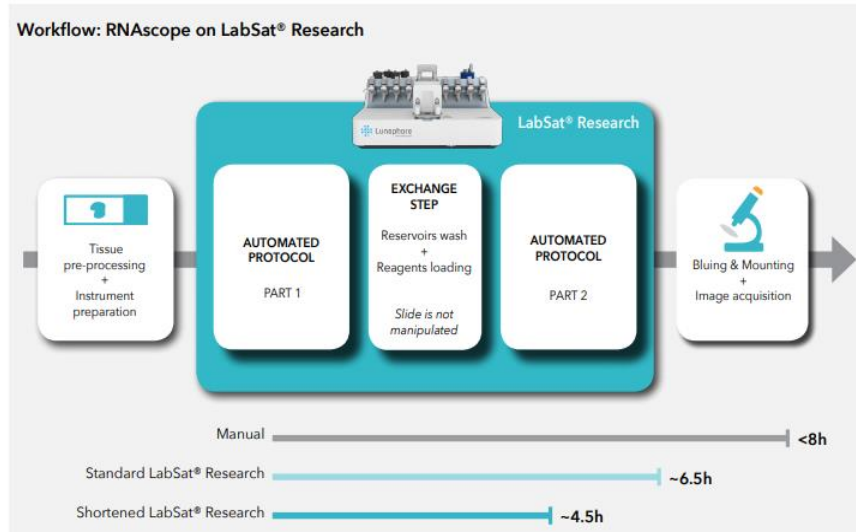


IHC – Immunohistochemical stainers

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



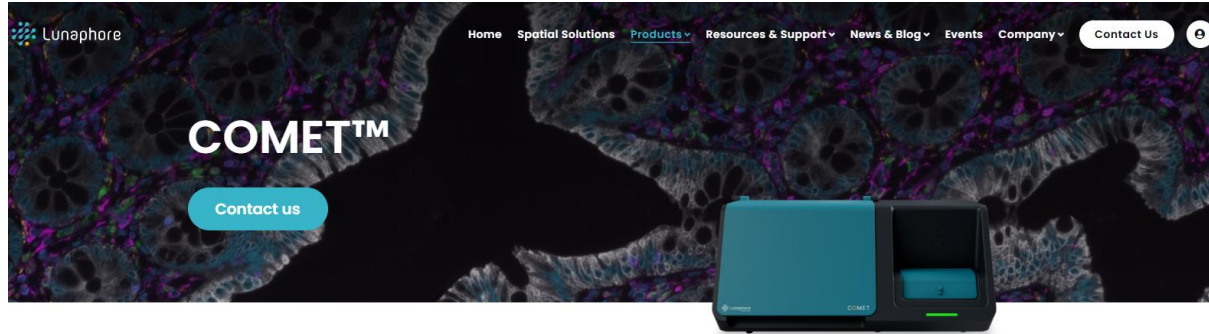
IHC
mRNA
lncRNA
miRNA
...



	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 ...???

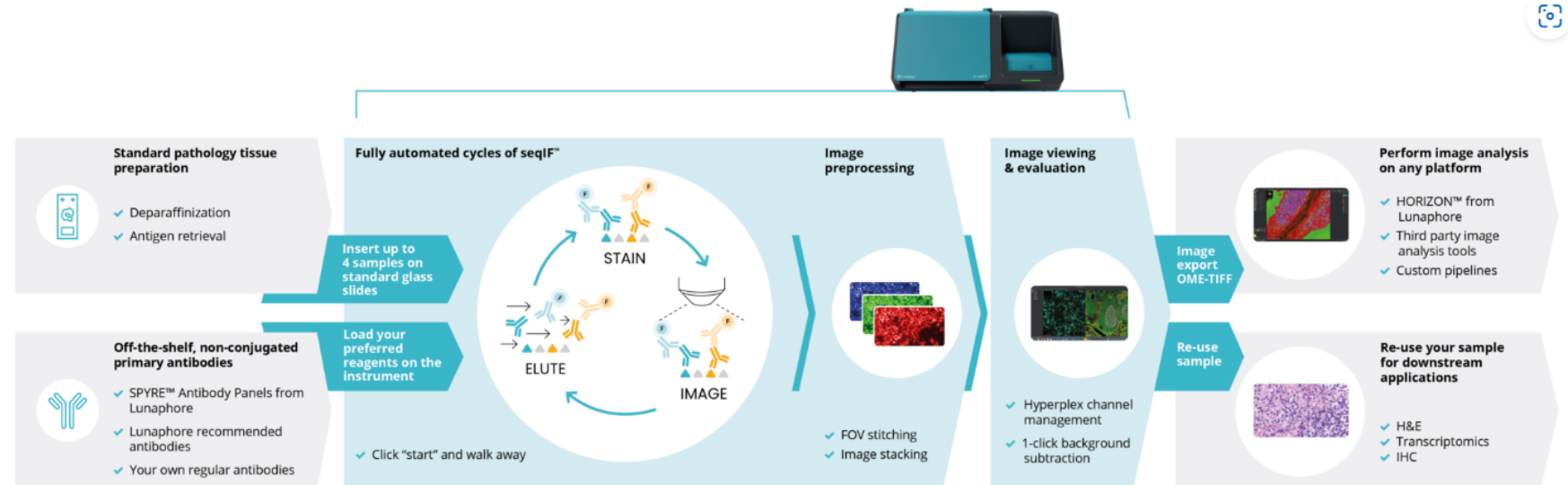


20 to 40-plex IHC system.....

Scalable hyperplexing

See a 40-plex TMA for yourself! →

Hyperplex workflow without user intervention



IHC – Immunohistochemical stainers

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



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



Full automation,
true random access
for IHC and ISH



Tissue-Tek Genie®
Advanced Staining System

IHC – Immunohistochemical stainers

Fully-automated systems: Future ...??? – New players



<https://www.celnovte-bio-tech.com/cnt360-m1/>

IHC – Immunohistochemical stainers

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



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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

