Immunohistochemical stainers
Overview
Pros and Cons

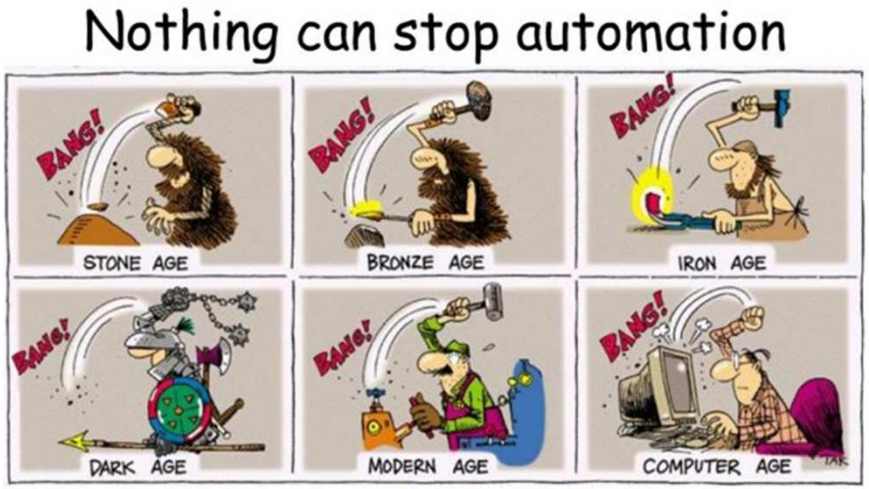
Søren Nielsen,
Director
NordiQC

IHC – Immunohistochemical stainers

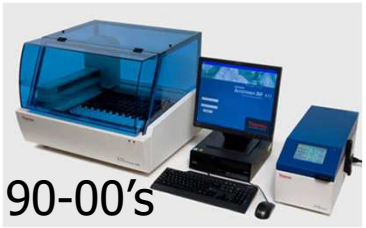
This lecture is meant to be a basis for an open discussion…
and not an attempt to promote any stainer / company ☺

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

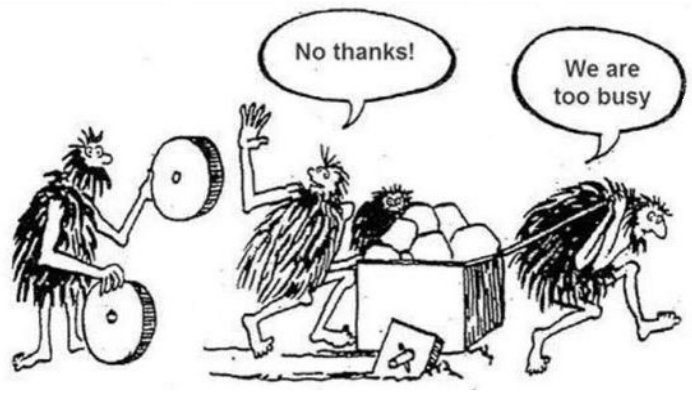
IHC – Immunohistochemical stainers



Manual



Semi-aut.



Fully-aut.

Fully-aut +

Multiplex (markers/assays)
Speed, etc

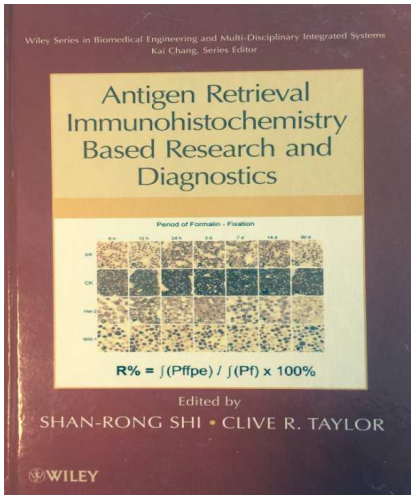
IHC – Immunohistochemical stainers

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

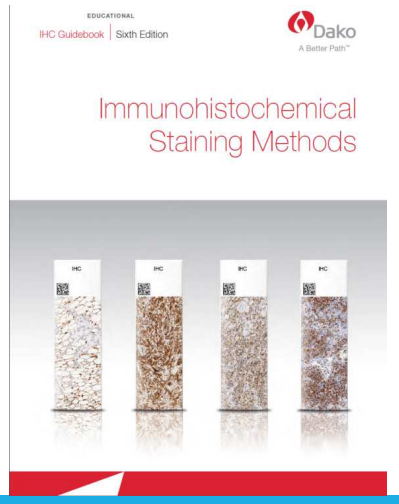
Dako
A Better Path™

Chapter 9

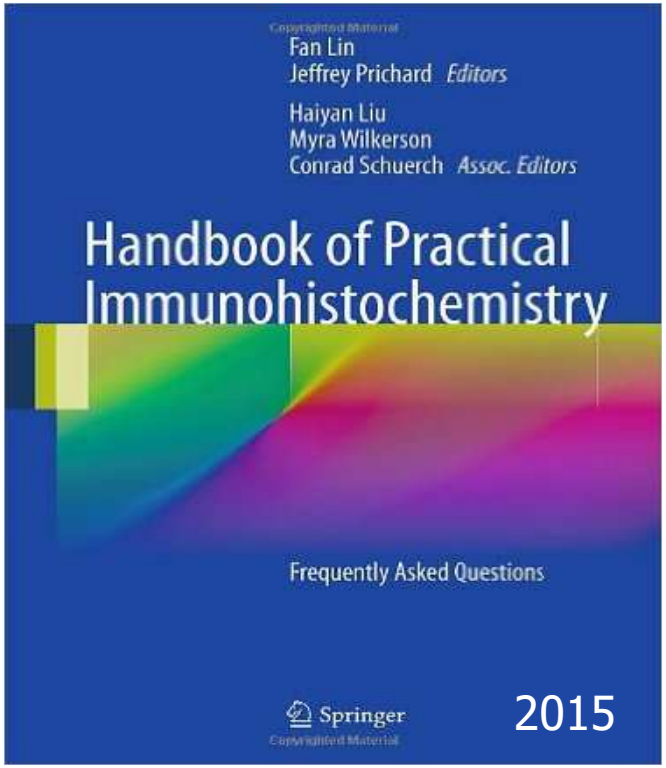
Automation in IHC

Ole Feldballe Rasmussen, PhD, MSc

2013/2021



IHC – Immunohistochemical stainers



Overview of Automated Immunohistochemistry

Jeffrey W. Prichard, DO

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

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

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

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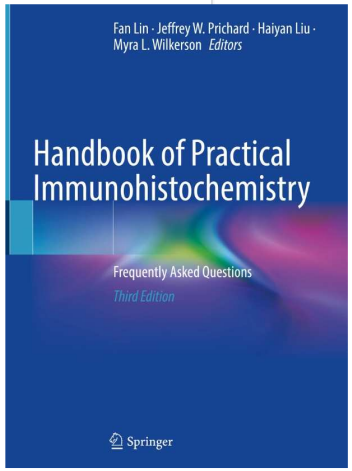
(*Arch Pathol Lab Med.* 2014;138:1578–1582; doi: 10.5858/arpa.2014-0083-RA)

Updated 2022 – 3’ version

IHC – Immunohistochemical stainers

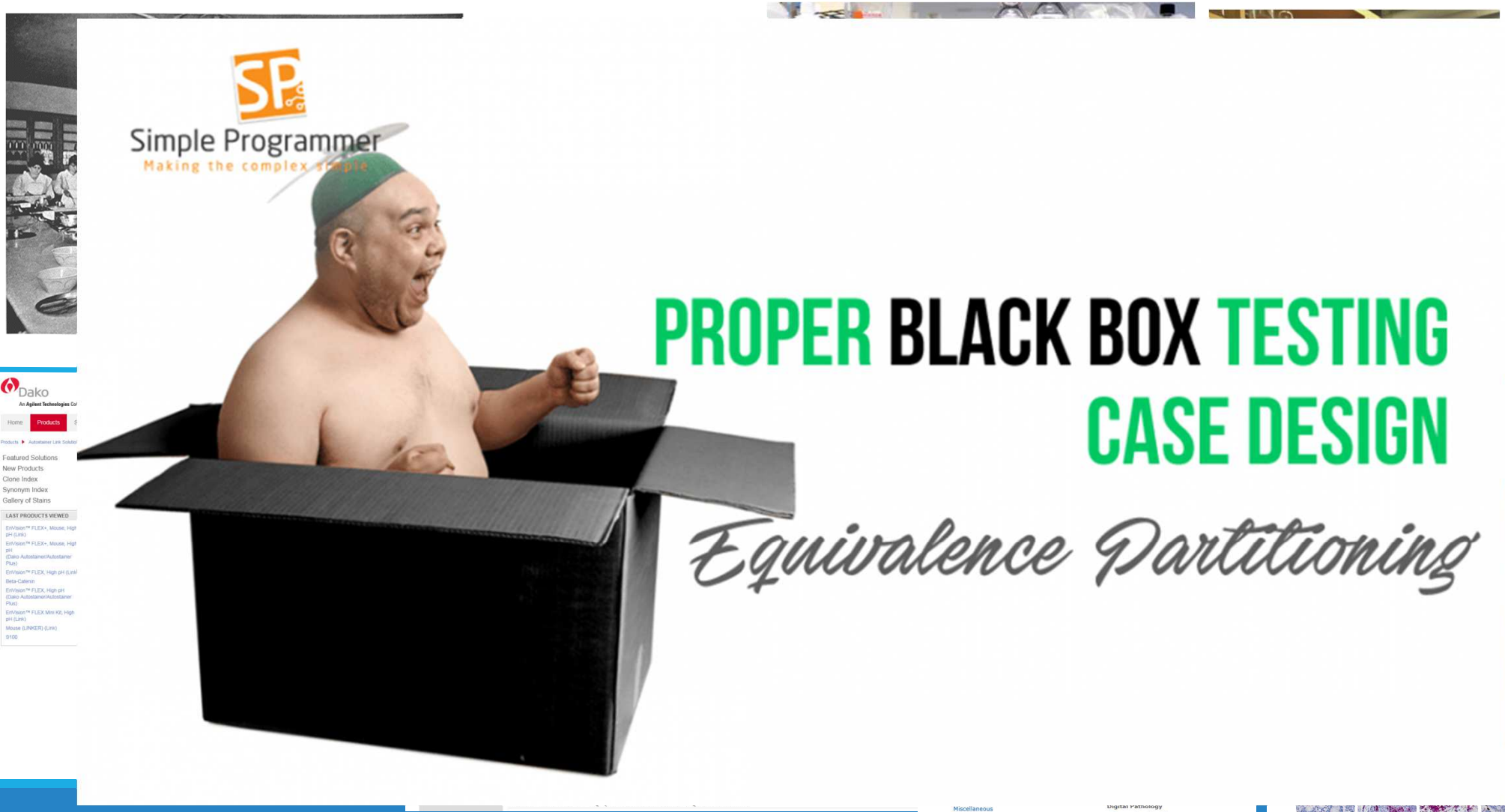


Abstract

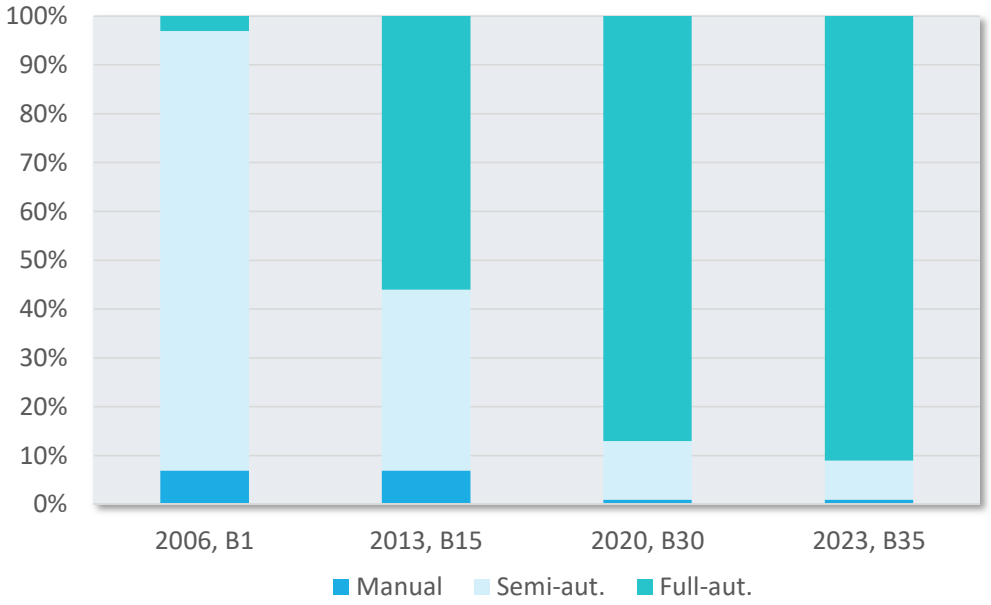
The past decade has produced major innovations and a great variety of features available in automated staining instruments. This chapter is a "buyer's guide" for automated IHC instruments. For those new to the topic of automated staining, it begins with discussions of the advantages and disadvantages 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.

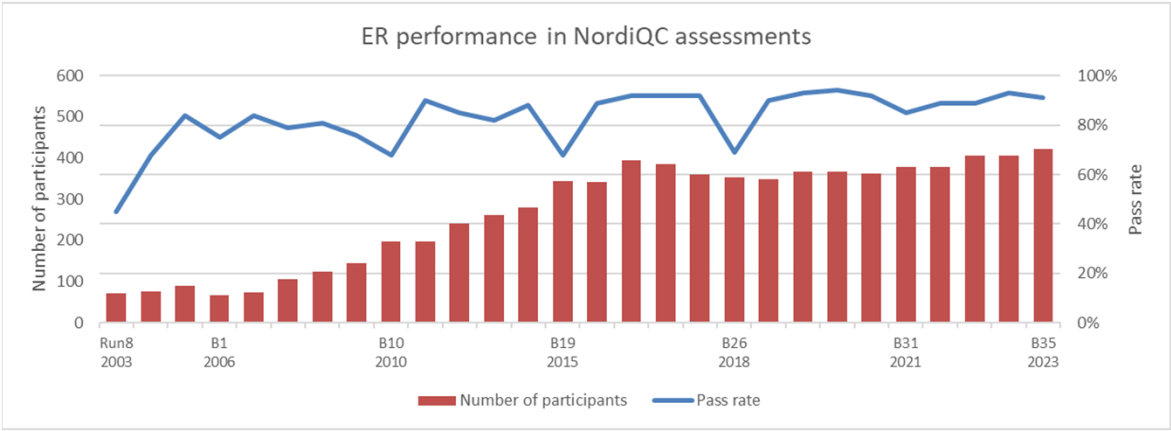
Updated 2022 – 3' version

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

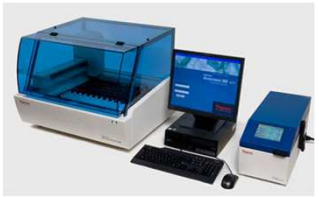


IHC – Immunohistochemical stainers





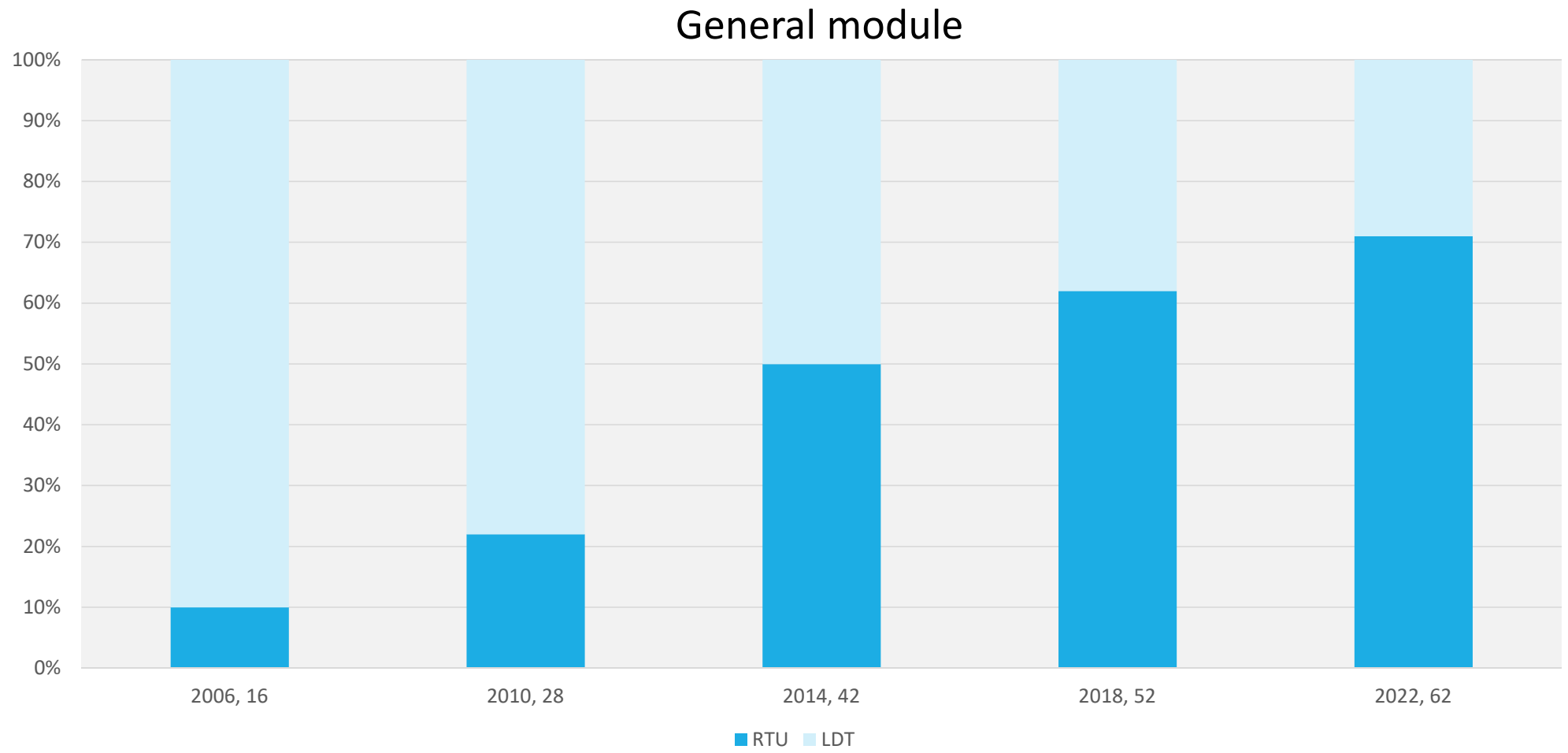
80-90's



90-00's

10-20's

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



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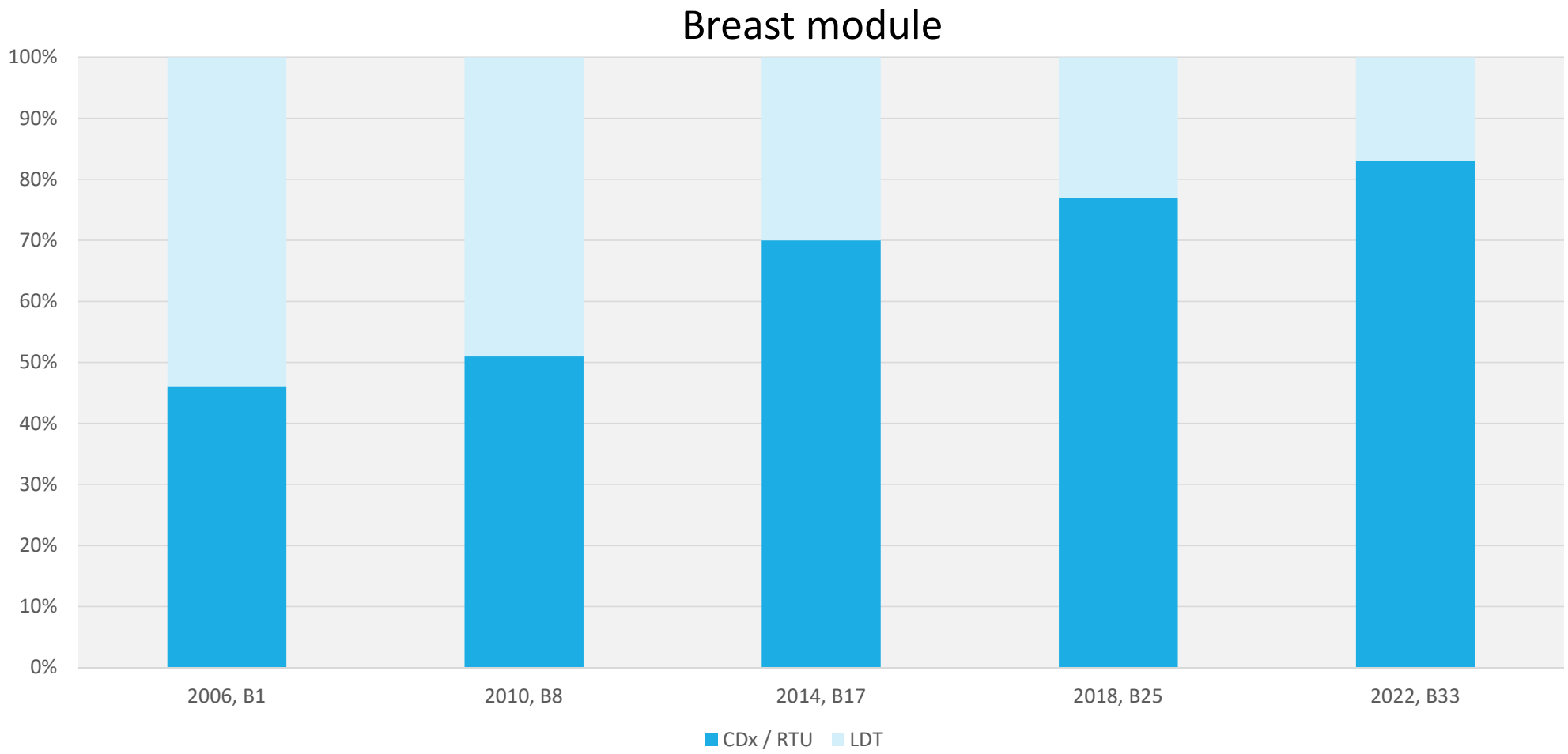
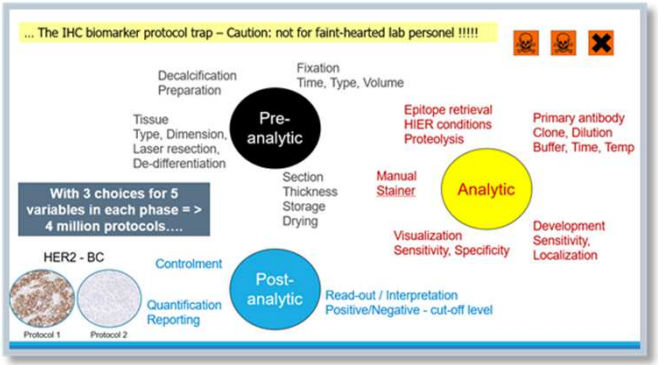


Harmonization of ”best practice” IHC methods

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

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:

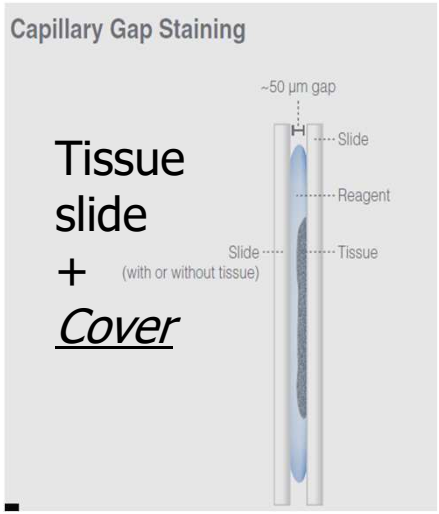
Leica:
Covertiles
Capillary

Dako:
Glass Lid
Dynamic gap

Sakura:
"*upside down*"
Capillary

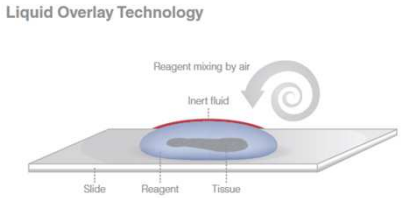
Technique;

To spread reagents and to avoid slides drying out

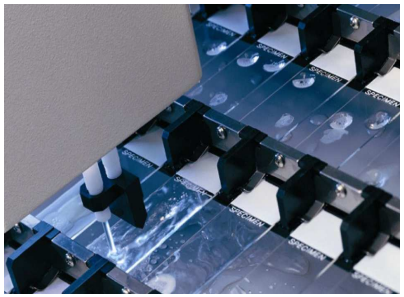


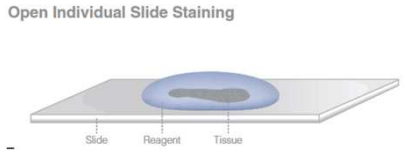
IHC – Immunohistochemical stainers

Flat labelling technology stainers:



Ventana:
+Mixing
+Overlay



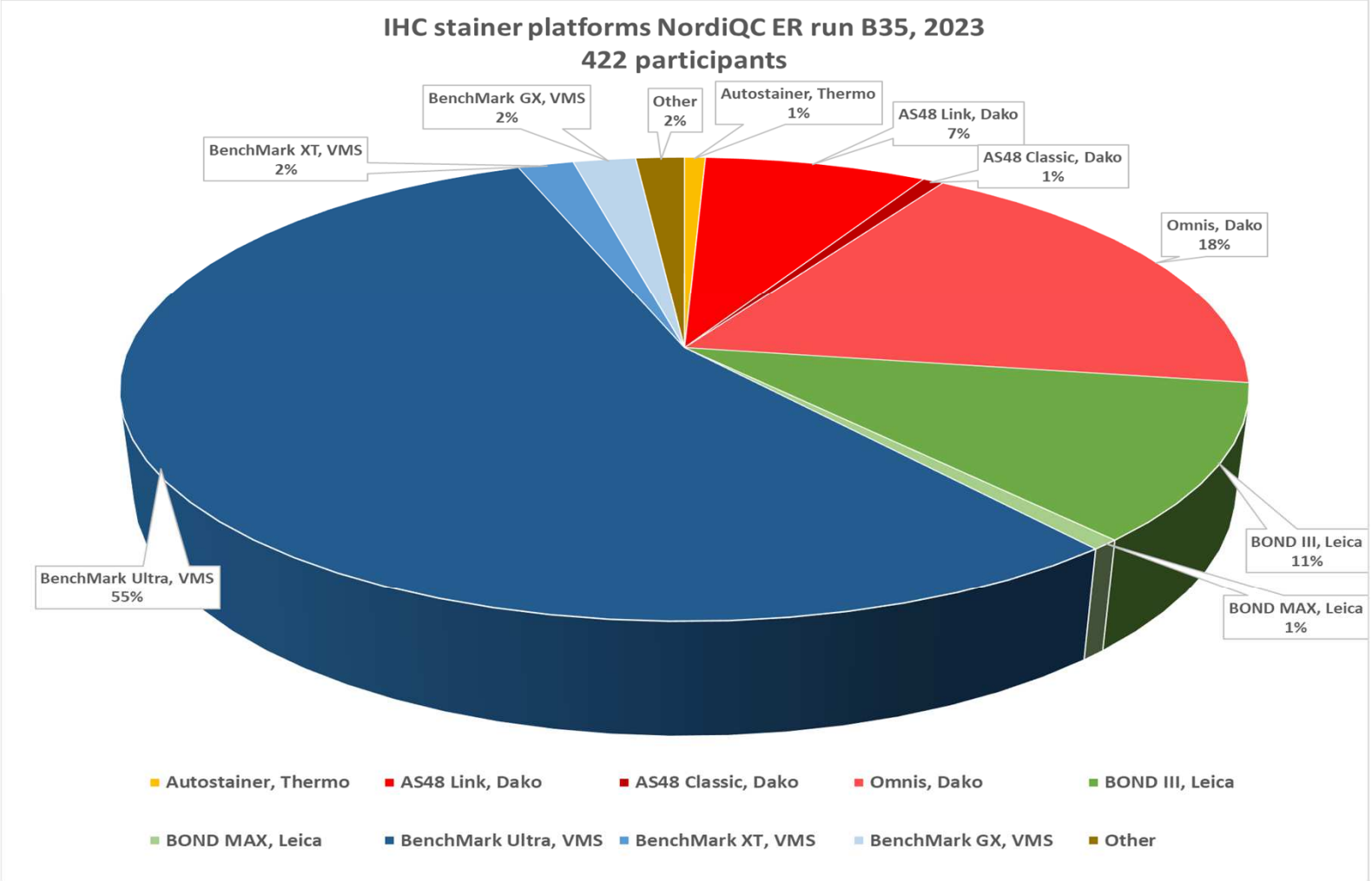


Dako:
-Mixing
-Overlay

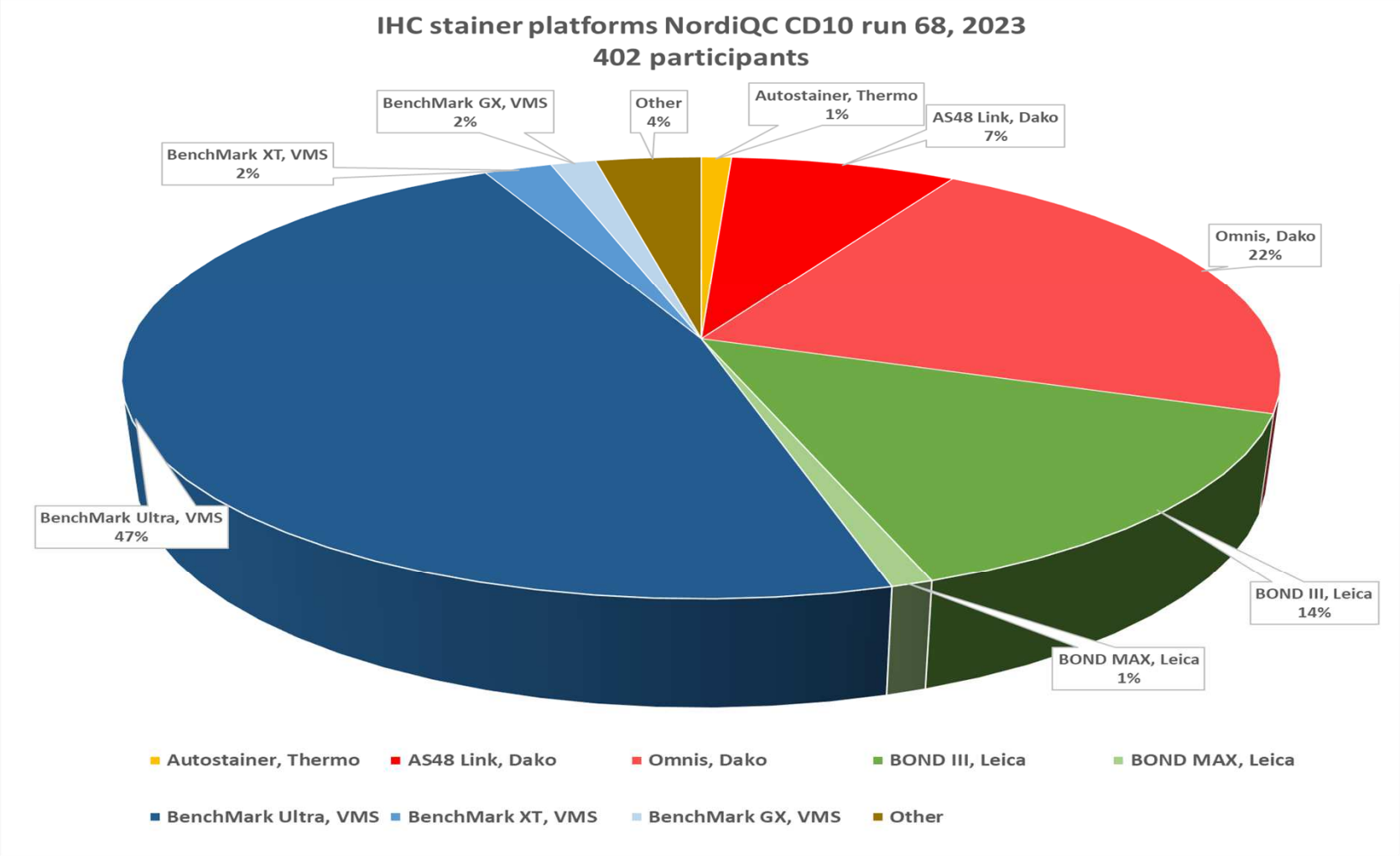
Technique;

Reagents are applied
+/- mixing
+/- overlay

IHC – Immunohistochemical stainers



IHC – Immunohistochemical stainers



IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

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Functionality – Workload – Workflow - Flexibility – Costs





IHC – Immunohistochemical stainers

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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.”

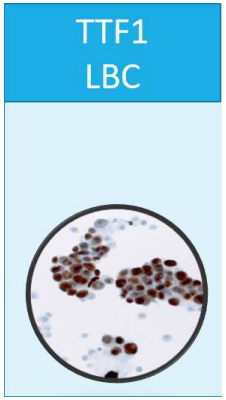
“When evaluating automated staining systems, the first thing to understand is that there is no, one “best system” on the market, for all purposes.”

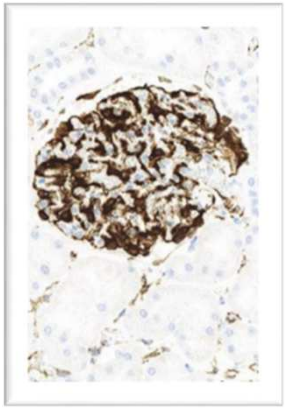
IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

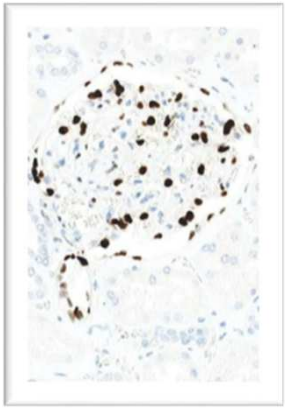
Functionality – Workload – Workflow - Flexibility – Costs

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





WT1 6F-H2
HIER



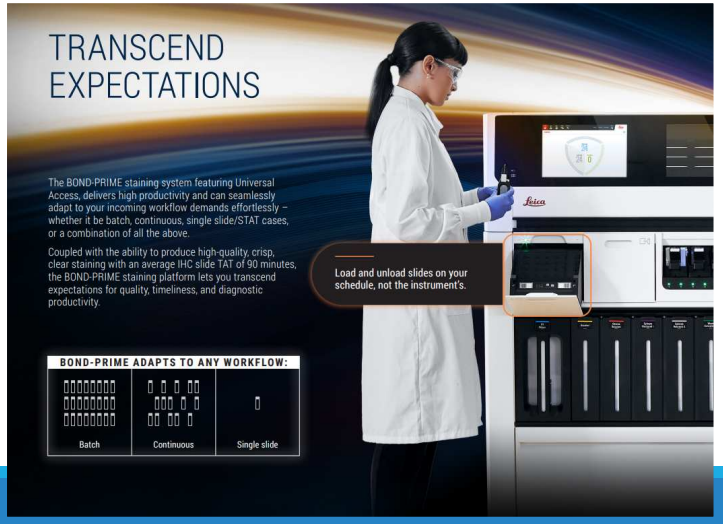
WT1 6F-H2
HIER + Prot.

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 ressources for maintenance
  - Frequency, extent, safety etc

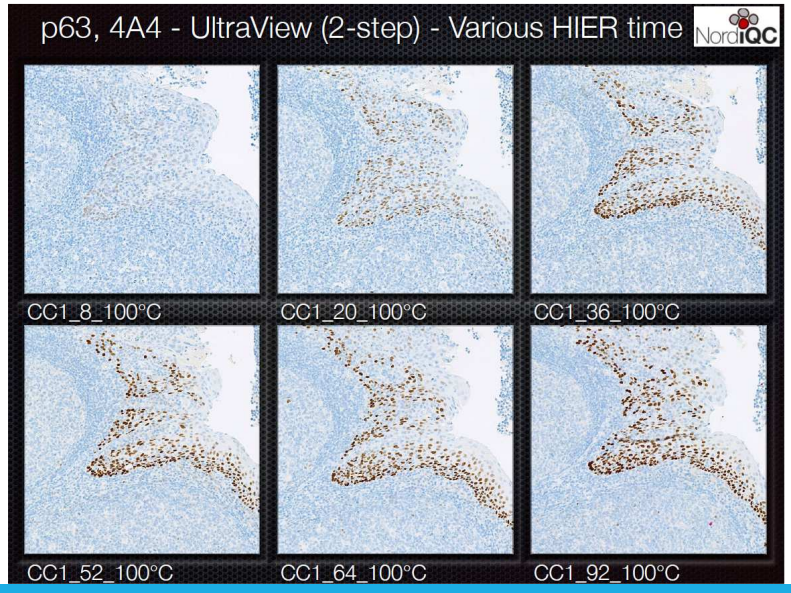


IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

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

- Software
  - Protocol set-up
    - HIER settings – time, temperature
    - Retrieval methods – single, combined
    - Adjustment of incubation times – Ab, detection, etc
    - Adjustment of incubation temp – Ab, proteolysis
    - Adjustment of protocol sequence – H_20_2 etc
    - Adjustment of reagent volume
    - Modification of protocol steps – addition/removal
    - Washing conditions – of low affinity Abs



IHC – Immunohistochemical stainers

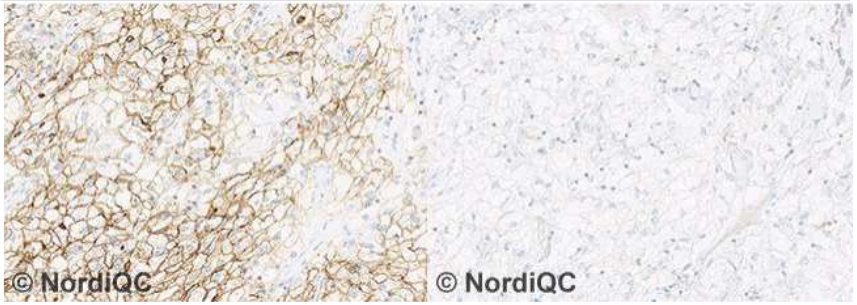
Automation of the IHC staining procedure:

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

- Reagents
  - HIER reagents
    - How many and which HIER 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

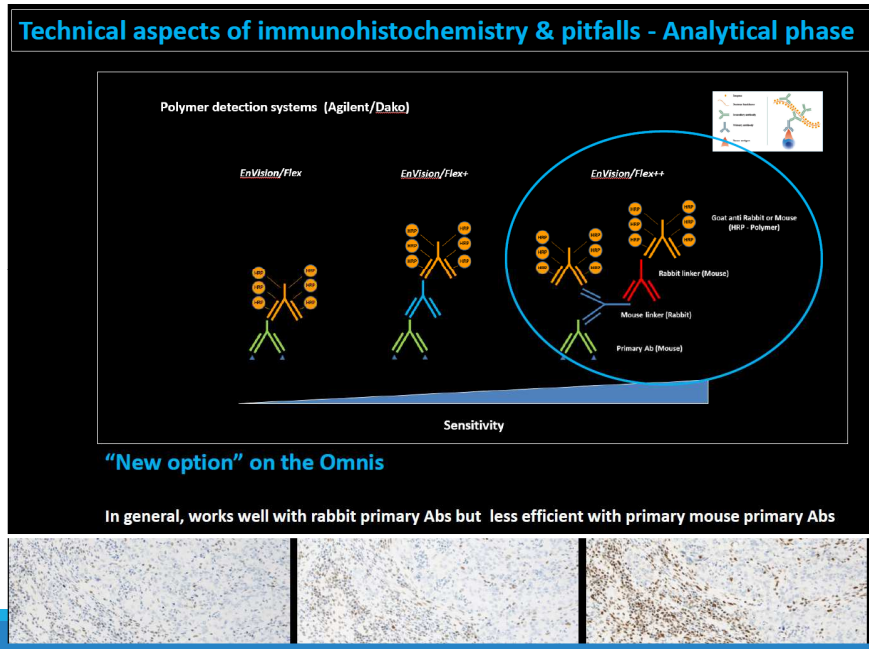


IHC – Immunohistochemical stainers

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 ?



IHC – Immunohistochemical stainers

Automation of the IHC staining procedure:

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

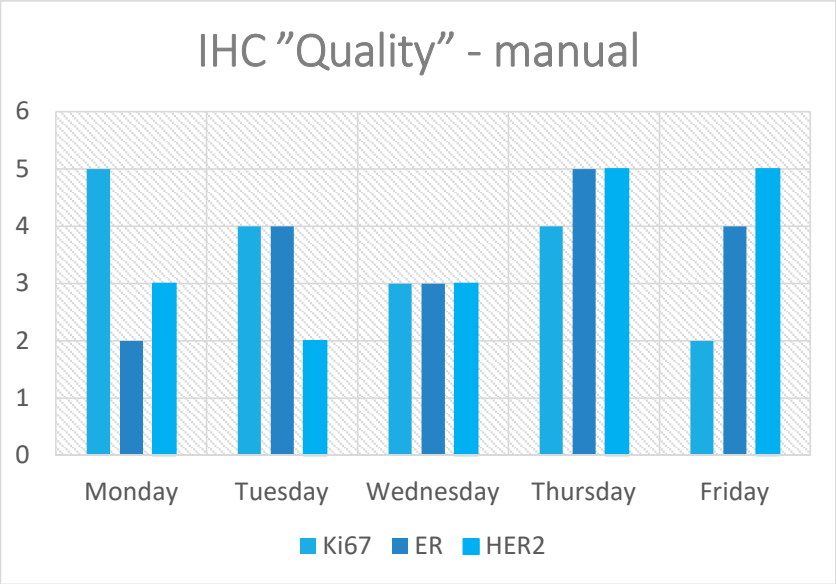
- Direct costs
  - Price pr instrument
  - Price pr slide
  - Preventive maintenance
- Indirect costs
  - Waste volumen
  - Daily maintenance (time used)
- ”Hidden costs”
  - Down-period – what is expected and accepted ?
  - Re-runs – what is expected and accepted ?
  - Assesscories needed/required
    - Empty vials for reagents, reagents, amp/linker, etc

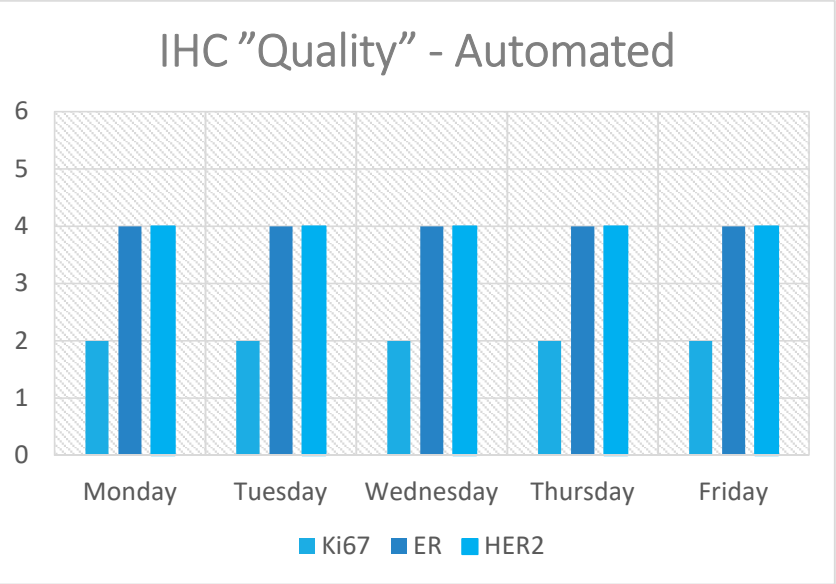
IHC – Immunohistochemical stainers

	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	√	(√)	√	√
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	√
…….					

Mitigation of RTU from one system to another can be challenging

IHC – Immunohistochemical stainers

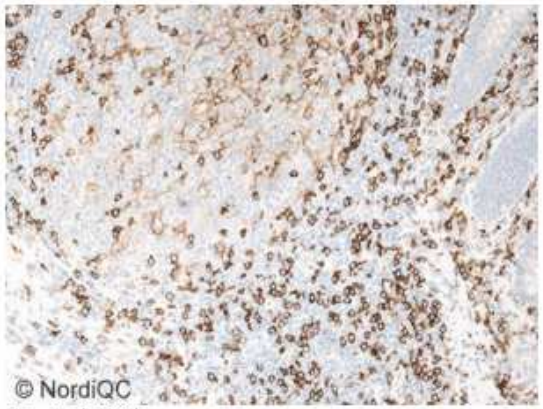


Fig. 1a (x200)
Optimal staining reaction for CD4 of the appendix applying the RTU assay IR649 (Autostainer, Dako/Agilent) based on the mAb clone 4B12, following vendor recommended protocol settings based on HIER in TRS (3-in-1) pH 9 and Envision FLEX+ as detection system.
All T-helper/inducer cells show 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.

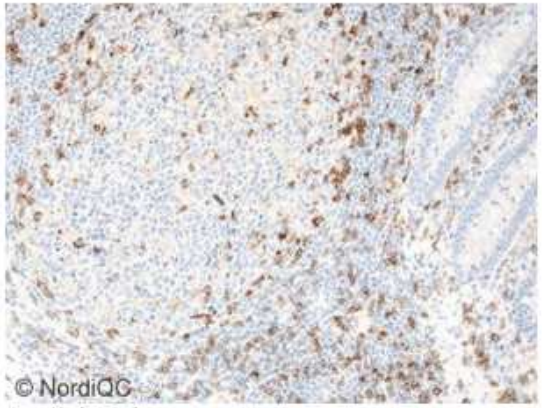
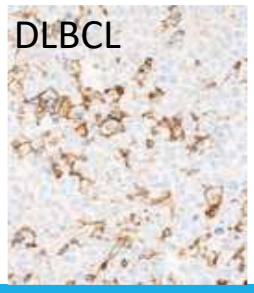
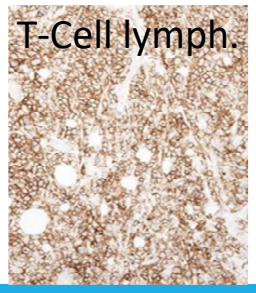
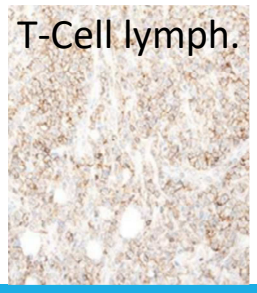


Fig. 1b (x200)
Insufficient staining reaction for CD4 of the appendix applying the same RTU system as in Fig. 1a, but used on the fully automated instrument Omnis (Dako/Agilent) with similar protocol settings as in Fig 1a - same protocol used in Figs. 2b - 4b.
The staining intensity is significantly 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.





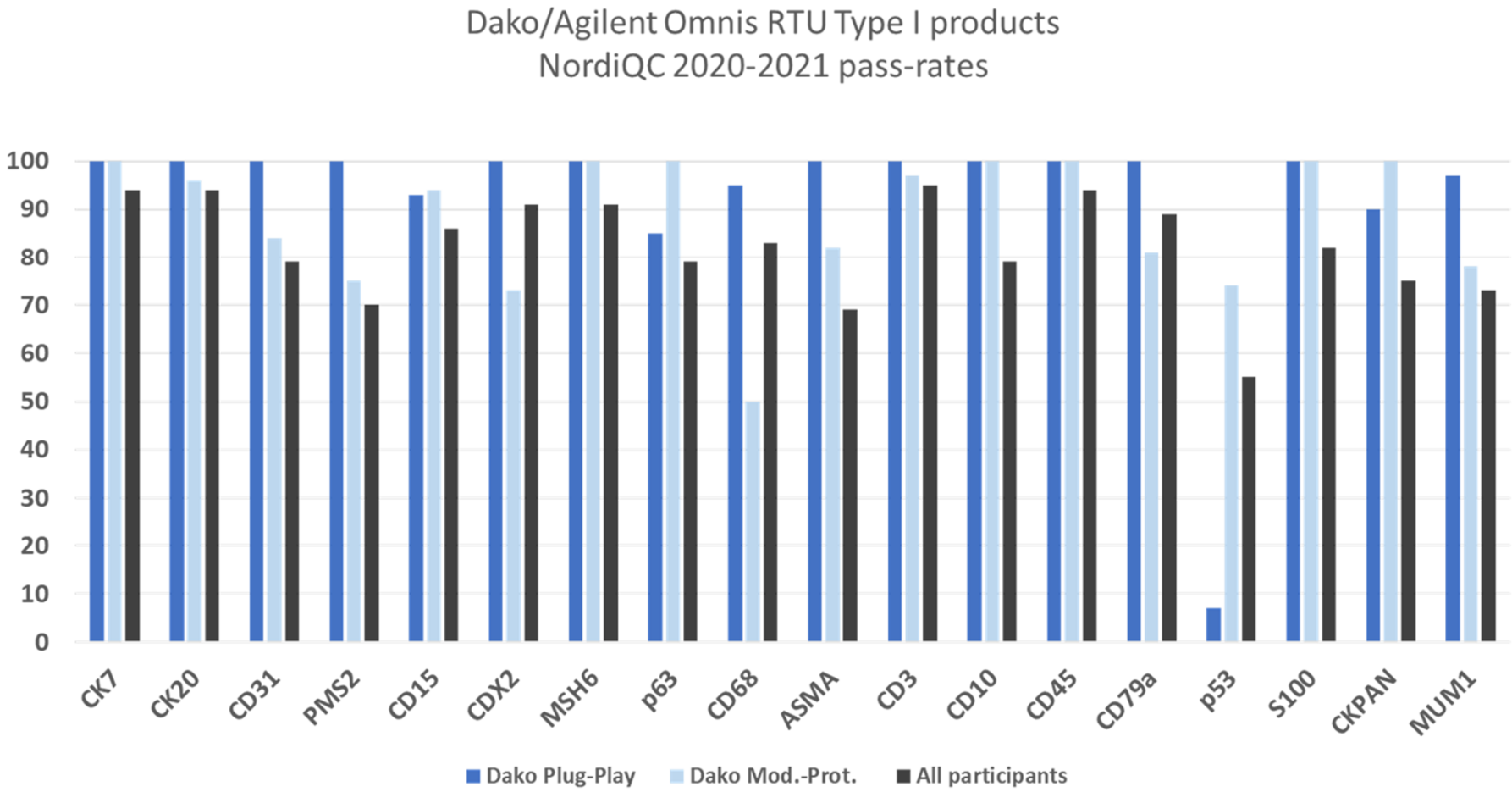


Pass rates for RTU's on "non-intended" platform;

	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



IHC – Immunohistochemical stainers

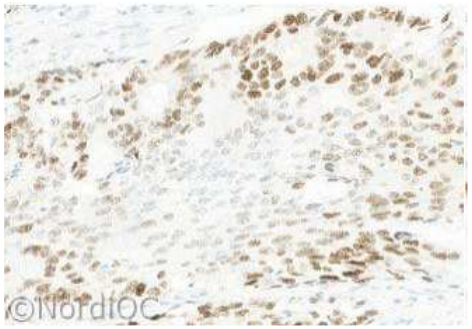
p53 run 38, 2013;

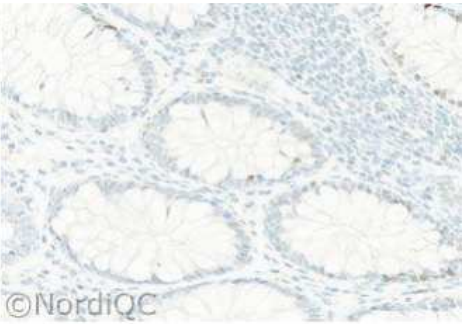
Purpose being demonstration of overexpression of p53 protein caused by TP53 mutation

p53 run 38, 2013;

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

Same level for other systems





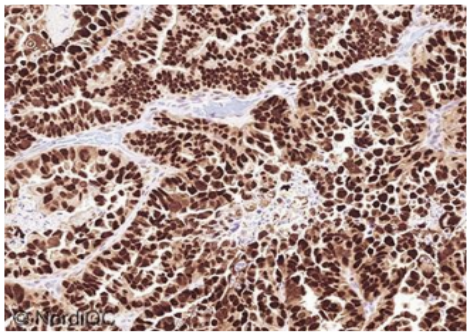
p53 run 63, 2021;

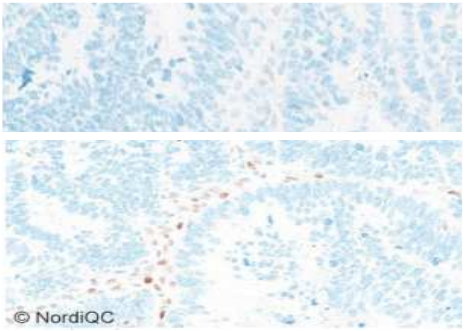
Purpose being demonstration of overexpression **and loss** of p53 protein caused by TP53 mutations

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)

IHC – Immunohistochemical stainers

Fully-automated systems: BenchMark Ultra, Ventana

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:

1. Place, start, walk
2. Flexible protocol set-up – ”30 stainers”
3. Wide range of sensitivity for detection systems
4. Wide range of RTU primary antibodies – Type I & II
5. IHC and ISH on same instrument / same slide

IHC – Immunohistochemical stainers

Fully-automated systems: BenchMark Ultra, Ventana

Functionality – Workload – Workflow - Flexibility – Costs

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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

IHC – Immunohistochemical stainers

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

IHC – Immunohistochemical stainers

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3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Good portofolio of RTU antibodies – plug-and-play

3 main Cons:

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

IHC – Immunohistochemical stainers

Fully-automated systems: Omnis, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:

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

IHC – Immunohistochemical stainers

Fully-automated systems: Omnis, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:

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

3 main Cons:

1. Limited portofolio of RTUs (I & II) & detection systems
2. Low affinity antibodies may show inferior performance
3. Less flexible protocol set-up

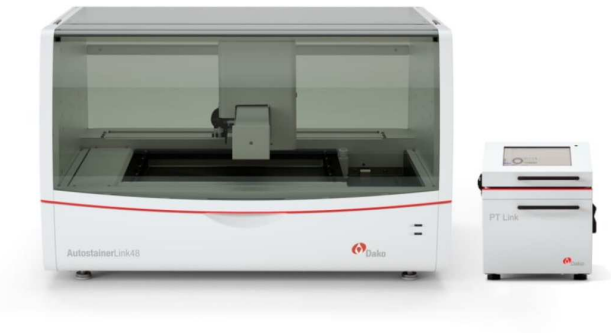
IHC – Immunohistochemical stainers

Semi-automated systems: AS-48, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:

1. Flexible protocol set-up – e.g. combined retr.
2. Flexible reagent choice – HIER buffer, detection system
3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Good portofolio of RTU's type I & II – plug-and-play



IHC – Immunohistochemical stainers

<u>Semi-automated</u> systems: AS-48, Dako

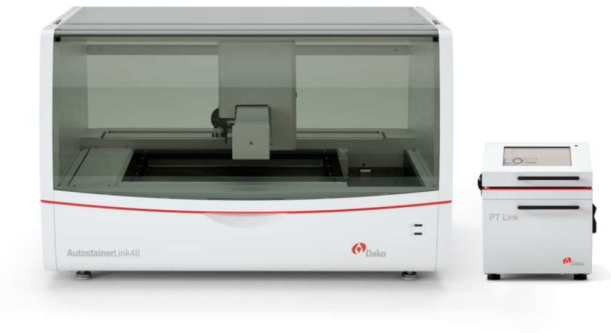
Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:

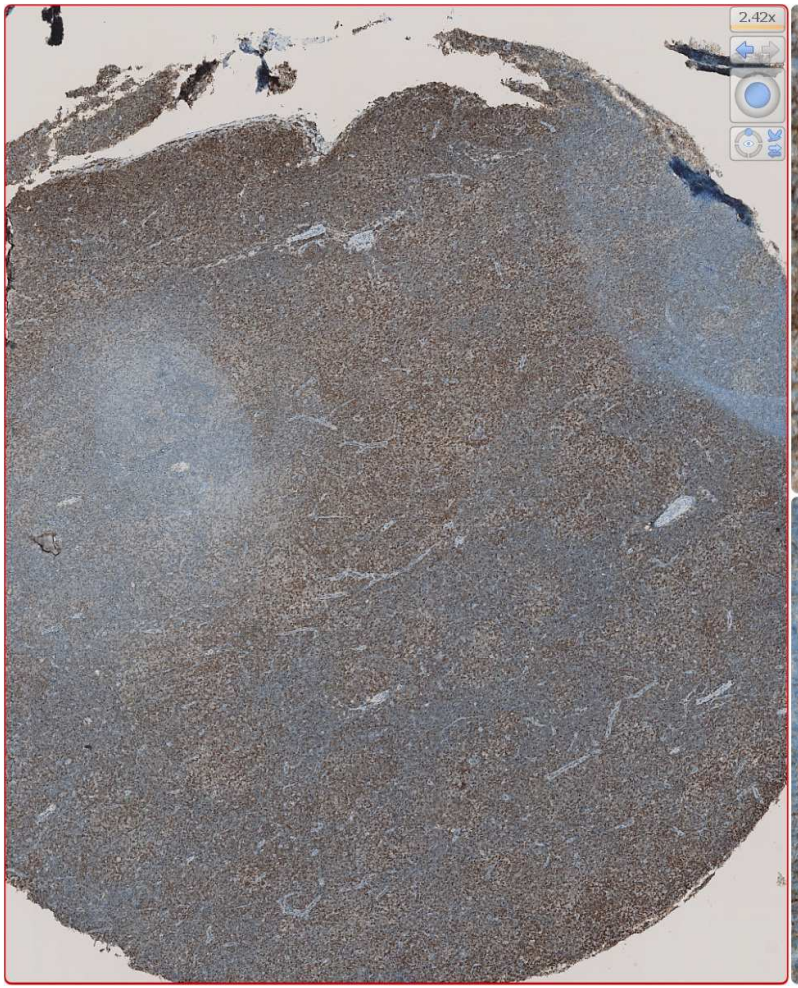
1. Flexible protocol set-up – e.g. combined retr.
2. Flexible reagent choice – HIER buffer, detection system
3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Good portofolio of RTU's type I & II – plug-and-play

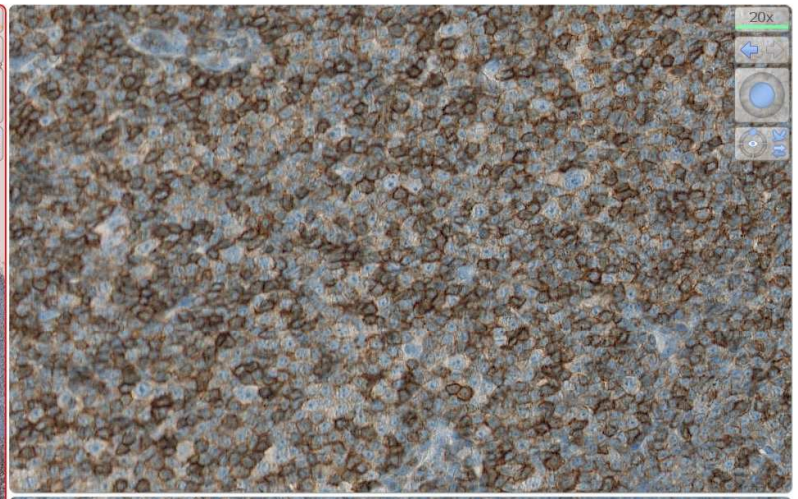
3 main Cons:

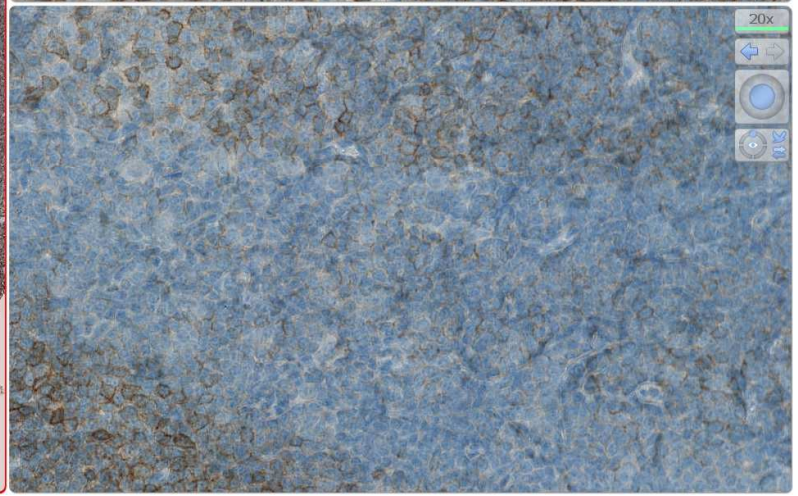
1. Increased manual interaction – 2 instruments needed
2. Primarily batch operation
3. High reagent volumen needed – 300 ul and >"dead-vol"



IHC – Immunohistochemical stainers



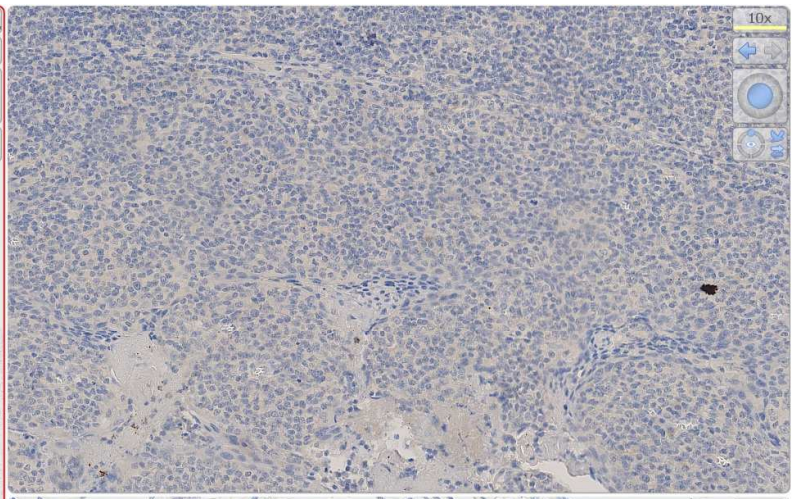


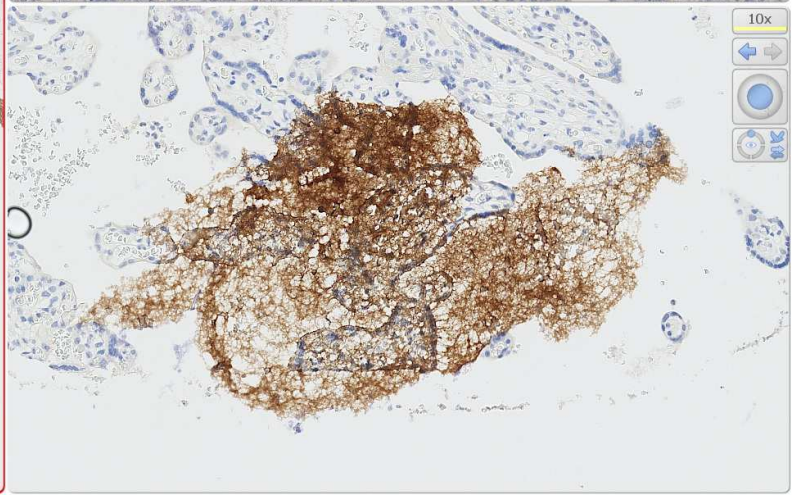


Staining issues
BenchMark, VMS;

Uneven weak and neg areas – air bubbles

IHC – Immunohistochemical stainers

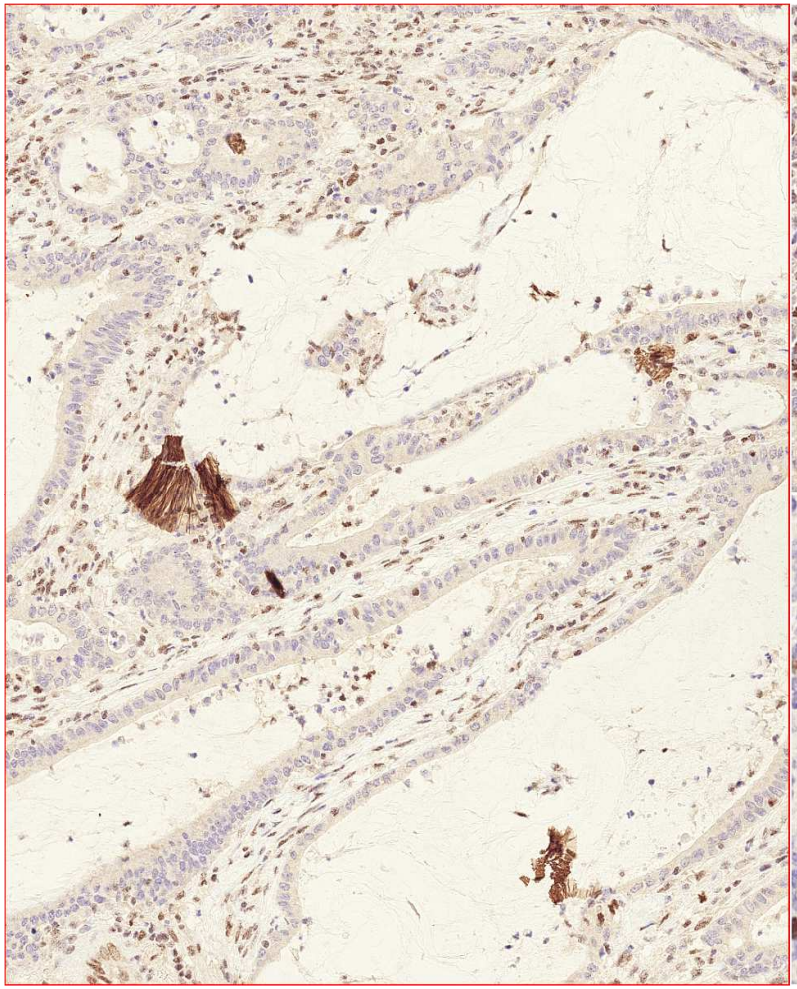


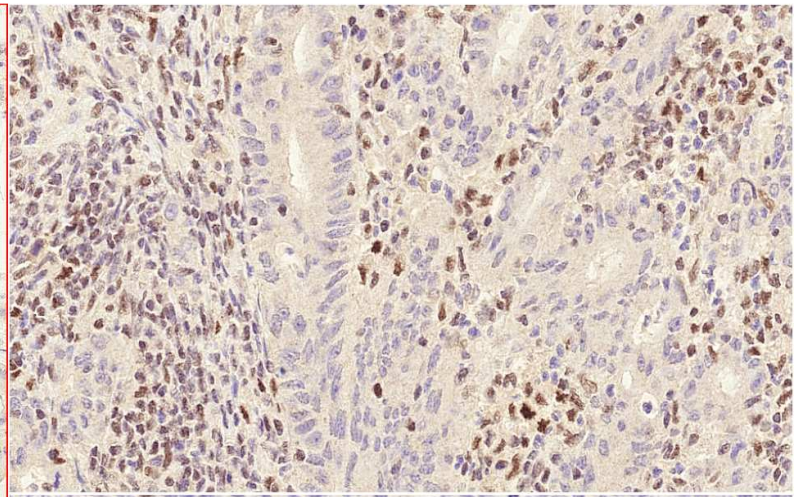


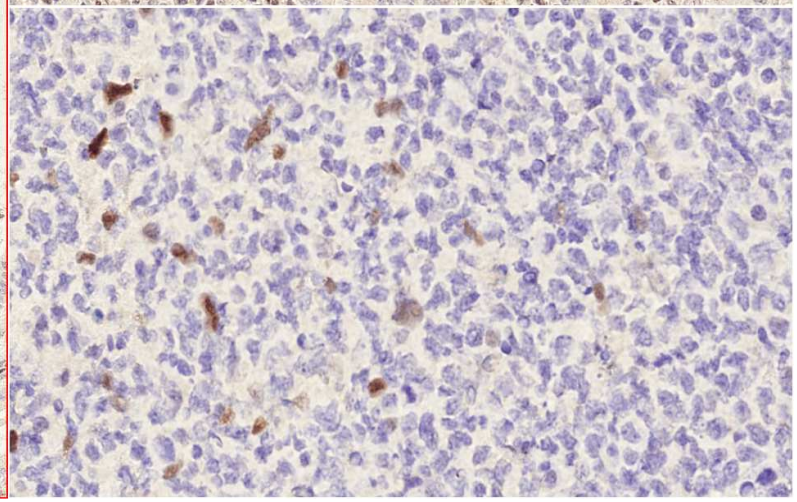
Staining issues
Bond, Leica;

Chromogen precipitates and general hue

IHC – Immunohistochemical stainers



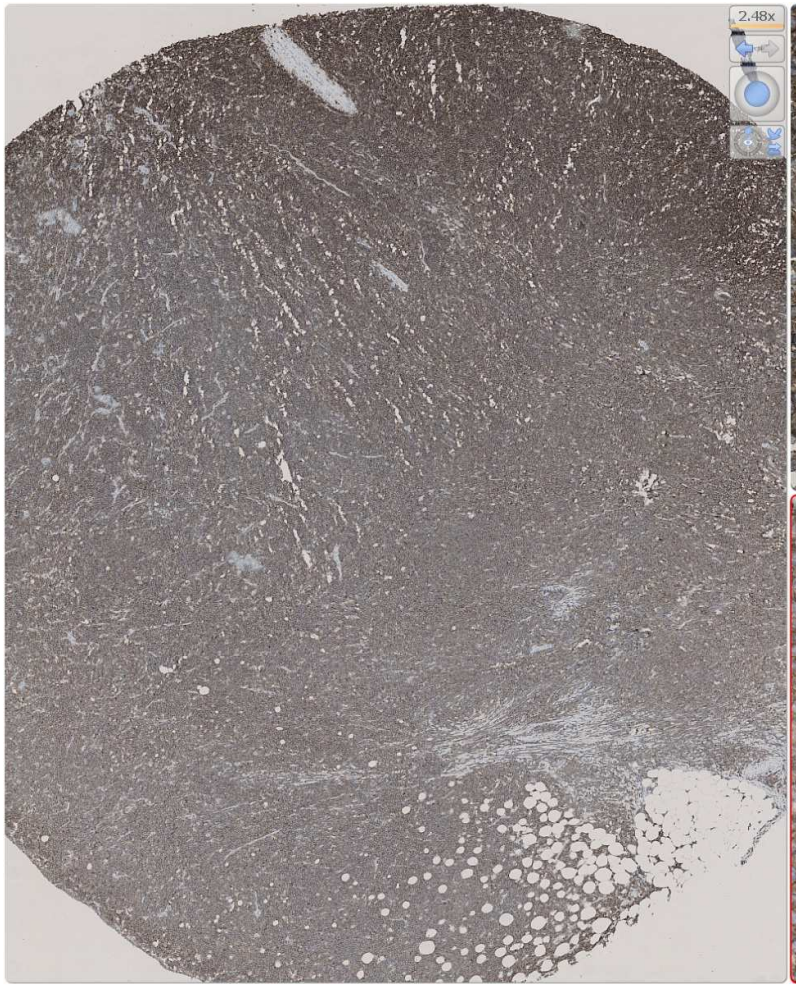


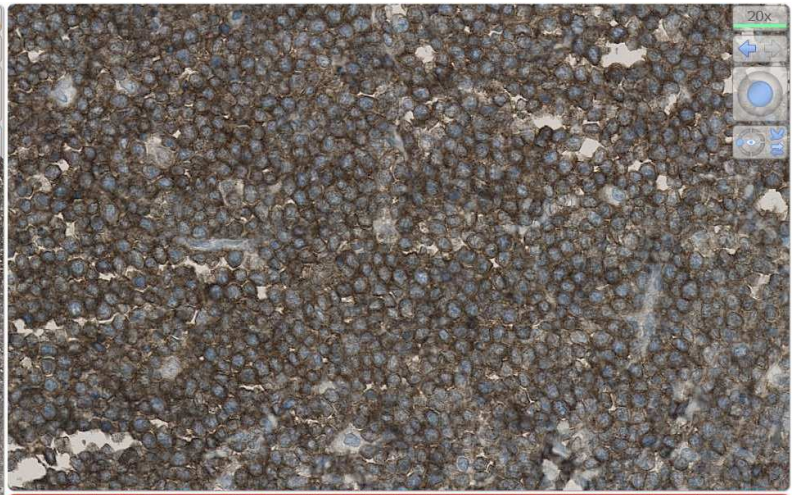


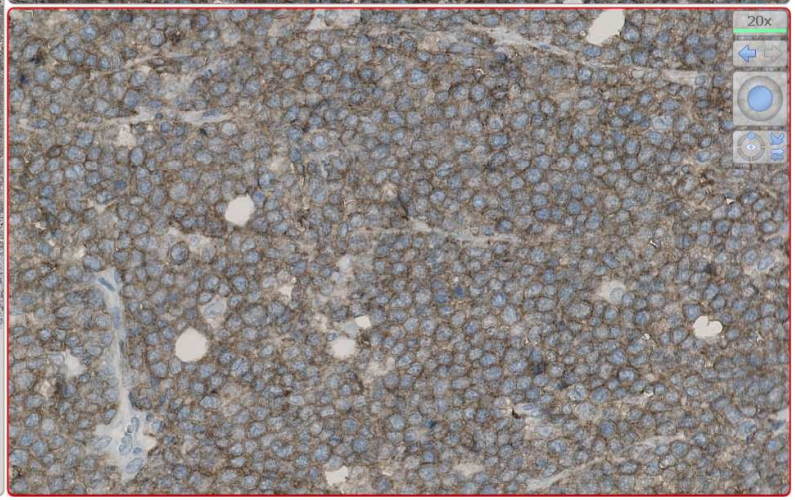
Staining issues
Omnis, Dako;

Chromogen precipitates, morphology and general hue

IHC – Immunohistochemical stainers







Staining issues
AS48, Dako;

Chromogen depletion, reagents not spread, drying out

Issue with rack levelling

IHC – Immunohistochemical stainers

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".

IHC – Immunohistochemical stainers

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†*

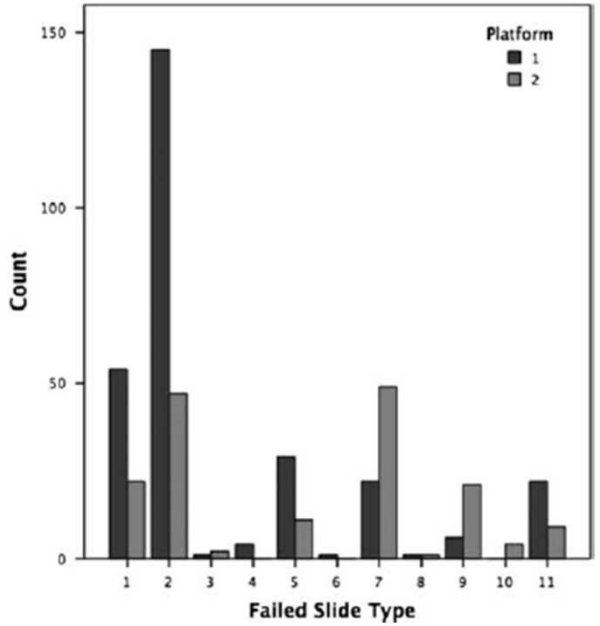


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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

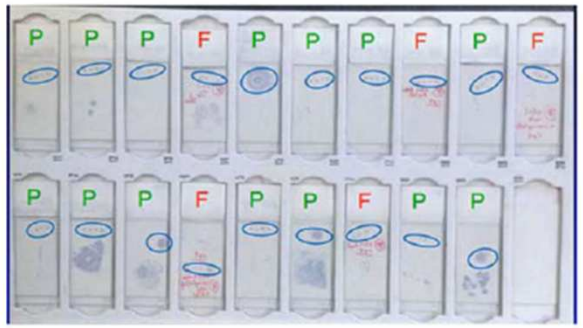
Lab related (22%)

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

Assay and/or Instrument (78%)

IHC – Immunohistochemical stainers

A



On-slide controls

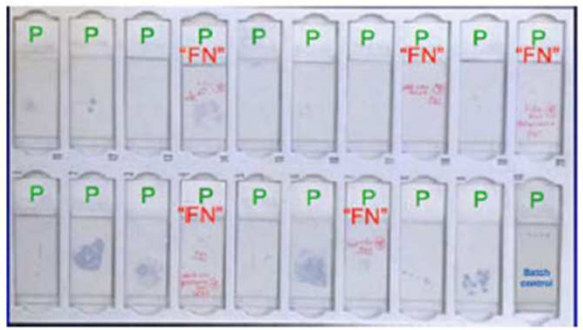
IHC slides stained for ALK (Class II),
same run, same instrument, same protocol
14/19 passed
5/19 failed

B

Batch-control - Theoretically:

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

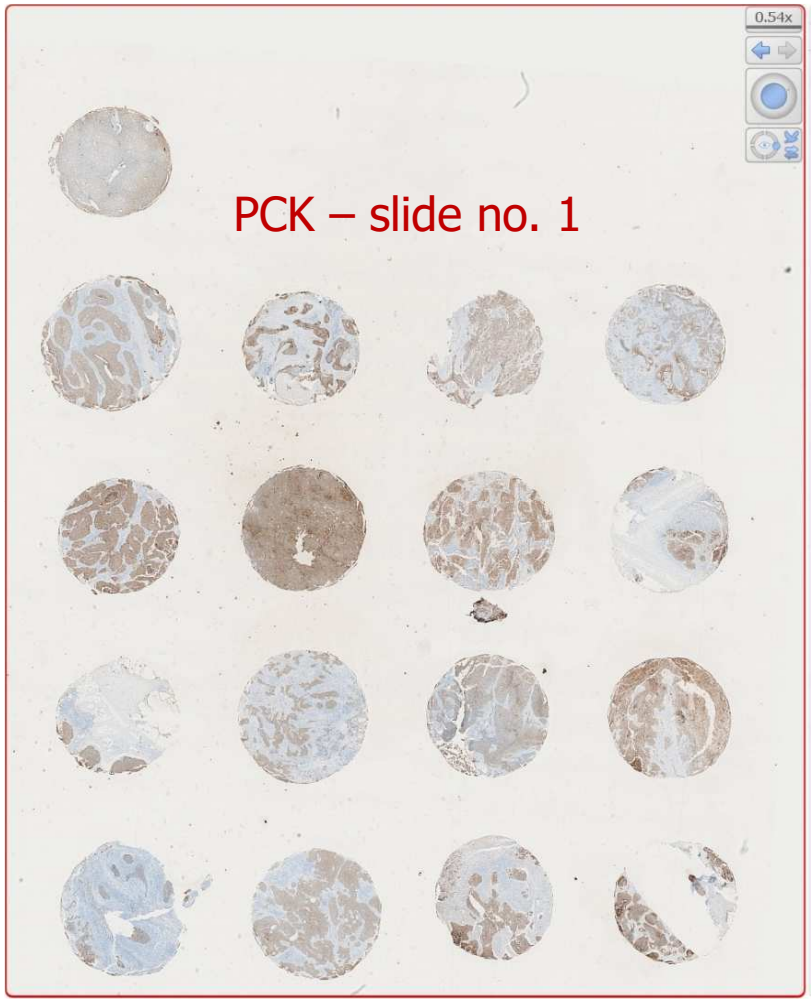
C



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

IHC – Immunohistochemical stainers

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

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

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

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

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

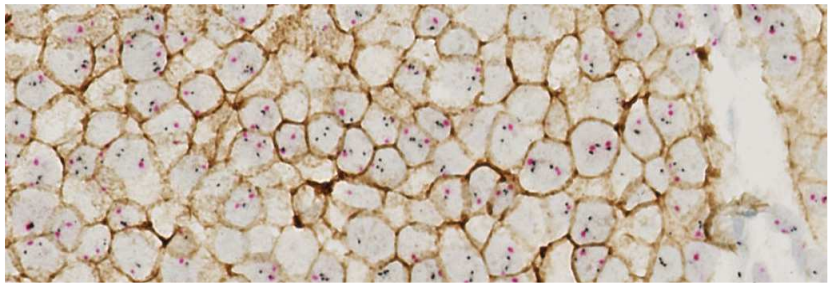
IHC – Immunohistochemical stainers

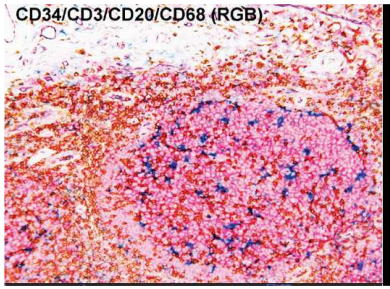
<u>Fully-automated</u> systems: Future …???

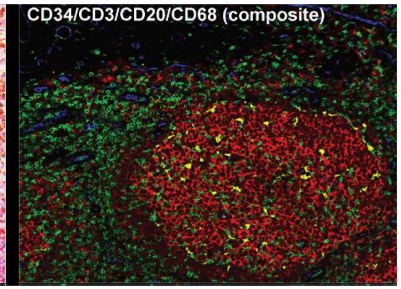
Functionality – Workload – Workflow - Flexibility – Costs

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. 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.

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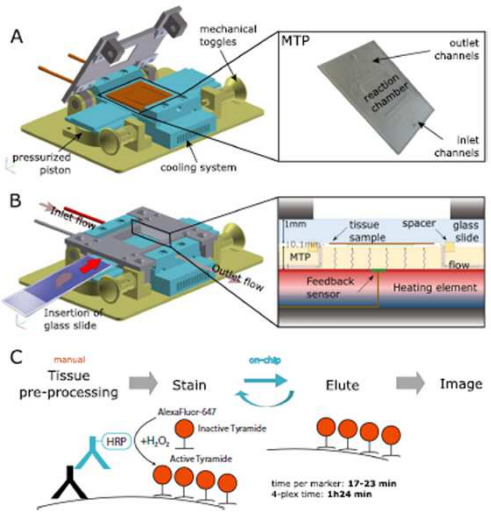
SCIENTIFIC REPORTS

OPEN

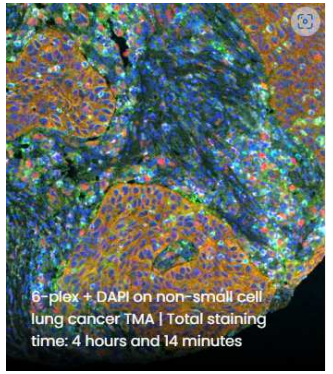
Ultra-fast and automated immunohistofluorescent multistaining using a microfluidic tissue processor

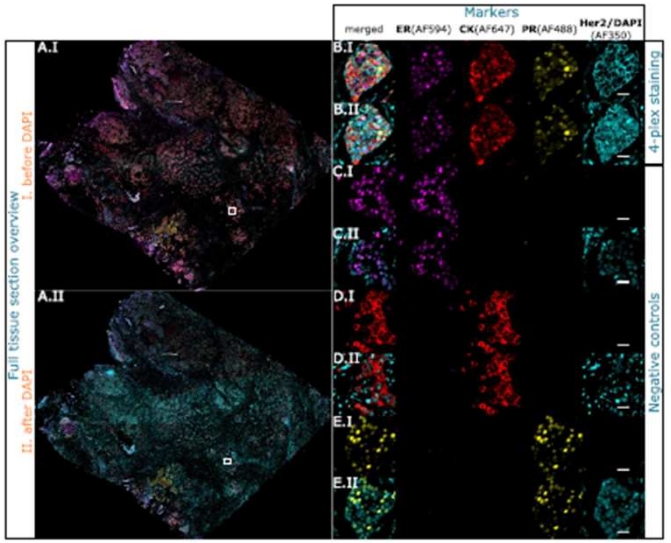
Received: 18 October 2017
Accepted: 28 February 2019
Published online: 14 March 2019

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



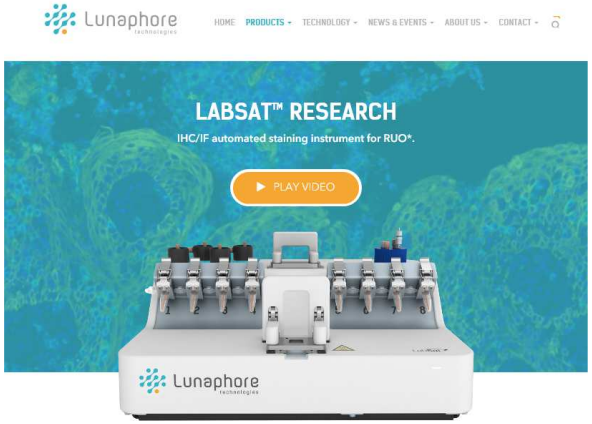
Step	Reagent	Incubation time min
1	anti-ER AbI	4
2	HRP-AbII	4
3	TSA-AF	2
4	Elution	6
5	anti-CK AbI	2
6	HRP-AbII	2
7	TSA-AF	2
8	Elution	4
9	anti-PR AbI	4
10	HRP-AbII	4
11	TSA-AF	2
12	Elution	6
13	anti-Her2 AbI	2
14	HRP-AbII	2
15	TSA-AF	2
	Total staining time	48 min
	Total staining time with washing steps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



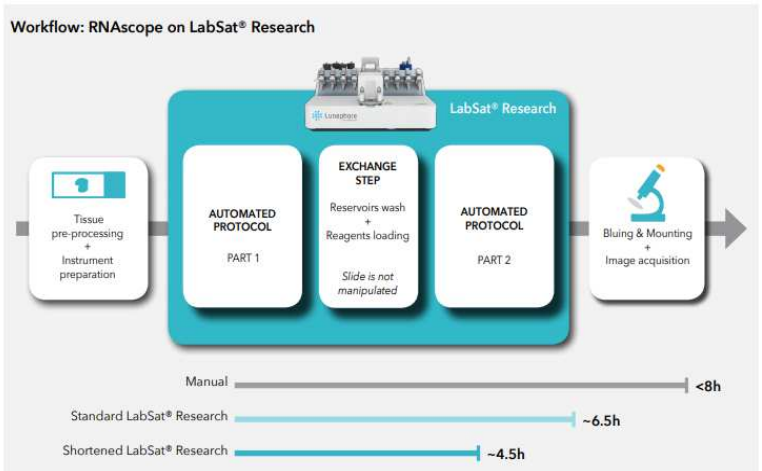


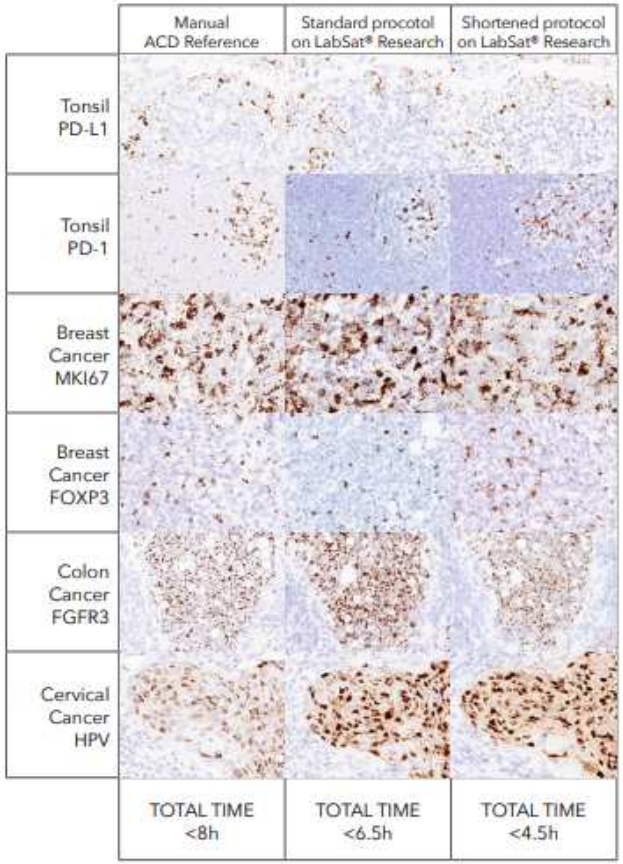
IHC – Immunohistochemical stainers

Fully-automated systems: Future …???



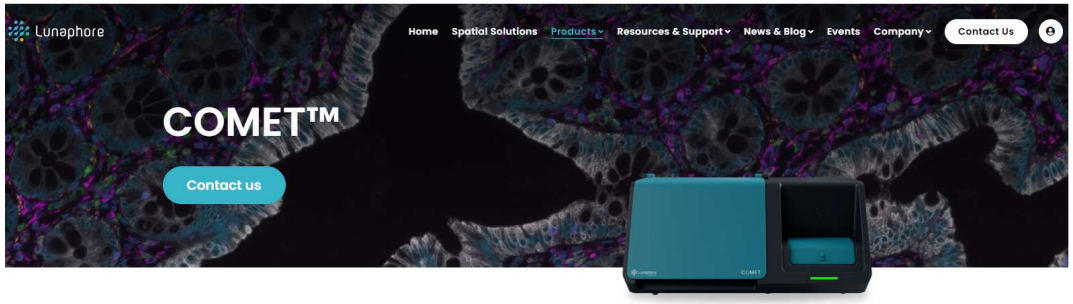
IHC
mRNA
lncRNA
miRNA
…



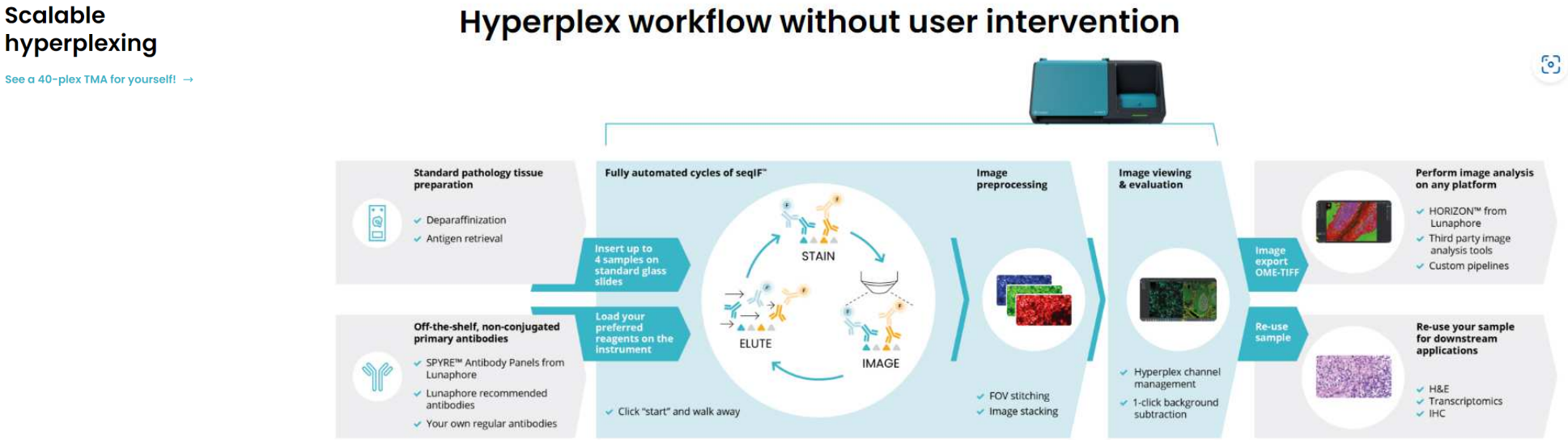


IHC – Immunohistochemical stainers

Fully-automated systems: Future …???

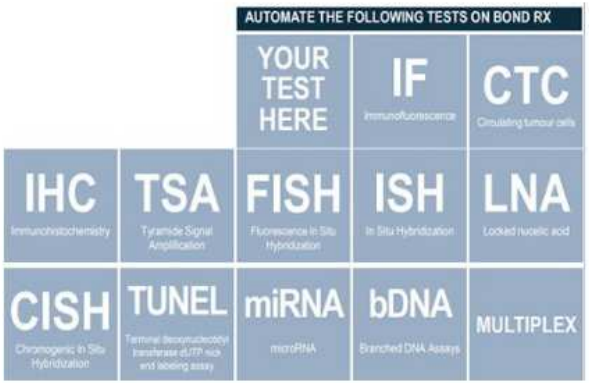


20 to 40-plex IHC system…..



IHC – Immunohistochemical stainers

Fully-automated systems: Future …???



Applications

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

IHC – Immunohistochemical stainers

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

Tissue-Tek Genie®
Advanced Staining System

Full automation,
true random access
for IHC and ISH

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

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

IHC – Immunohistochemical stainers

Conclusions:

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

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

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

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

IHC – Immunohistochemical stainers