

IHC Stainer platforms

Overview, pros and cons

Bart De Wiest
Quality manager IHC
OLV Hospital, Aalst, Belgium

Donald Van Hecke
Lab & Quality manager
AZ St-Lucas, Brugge, Belgium

IHC - immunohistochemical stainers

Goal of this lecture:

to be a basis for an open discussion...

not to promote or disadvantage any stainer or company 😊

Disclaimer:

Information used is retrieved from literature, vendors, NordiQC database, daily IHC practice (Roche Benchmark Ultra / Leica Bond Max)

IHC - immunohistochemical stainers



ELSEVIER

Clinica Chimica Acta 278 (1998) 185–192

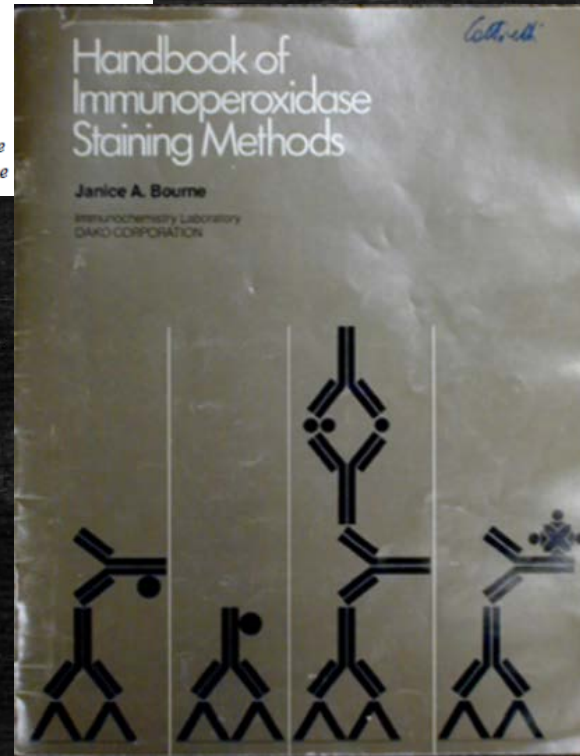


Comparative evaluation of automated systems in immunohistochemistry

T. Le Neel^a, A. Moreau^b, C. Labois^b, A. Truchaud^{a,*}

^aEngineering Laboratory, Institute of Biology, 9 Quai Moncousu, 44093 Nantes, France

^bLaboratory of Pathology, Institute of Biology, 9 Quai Moncousu, 44093 Nantes, France



Part II: The Potentials and Pitfalls



Chapter 9

Automation in IHC

Ole Feldballe Rasmussen, PhD, MSc



IHC - immunohistochemical stainers

- <https://www.thefreelibrary.com/A+review+of+automated+slide+stainers+for+IHC+and+ISH.-a0174196396> update 2008

Myers J. Automated slide stainers for special stains, immunohistochemistry, and in situ hybridization. Med Lab Obs.

Copyrighted Material
Fan Lin
Jeffrey Prichard Editors
Haiyan Liu
Myra Wilkerson
Conrad Schuerch Assoc. Editors

Handbook of Practical Immunohistochemistry

Frequently Asked Questions

Springer
Copyrighted Material

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)

1 Quality Management and Regulation	1
Jeffrey Prichard	
2 Technique and Troubleshooting of Antibody Testing	17
Fan Lin and Jianhui Shi	
3 Overview of Automated Immunohistochemistry	23
Jeffrey Prichard, Angela Bitting, and Joe Myers	
4 Automated Staining: Dako Perspective	31
Ole F. Rasmussen and Andreas Schønau	
5 Automated Staining: Ventana Perspective	37
Angela Sattler	
6 Tissue Microarray	45
Myra Wilkerson and Erin Powell	

IHC - immunohistochemical stainers

A review of automated slide stainers for IHC and ISH

By Joe Myers, MS, CT(ASCP)

This review of automated slide stainers for immunohistochemistry (IHC) and *in situ* hybridization (ISH) is intended to serve as an update to a previous paper,¹ published in this journal approximately four years ago. While the focus of the earlier version was simply to describe the basic mechanical differences between the automated IHC/ISH systems available at that time, this review is designed to provide a more detailed analysis of the capabilities and, where practical, dispel some of the myths associated with certain instruments and functions in the hope that future acquisition decisions will be based more on "good science" and cost effectiveness than on marketing "hype." An effort has been throughout this paper to provide interested parties with the information necessary to consider available systems with an open mind, being aware that the perceived advantages of a particular system may overshadow its otherwise unknown shortcomings.

The bottom line is that there is no perfect system — each has strengths and weaknesses that should be considered during the objective evaluation that usually precedes acquisition of capital equipment like IHC/ISH

apply reagents to slides. A summary of the most important features available on today's commercially available instruments is provided in Tables 1 and 2.

Applications and approaches

Although it may seem unnecessary to explain, IHC/ISH systems are, unlike instruments for hematological applications (e.g., Wright staining), specifically designed to perform more complex and time-consuming procedures. At this starting point, then, it is important to point out that there is actually very little difference between an instrument that is capable of performing IHC and one that is (also) capable of performing ISH, since both procedures involve, with one exception, the same fundamental mechanics. All IHC/ISH systems perform similar functions — that is: 1) provide an environment for reagents to react with specimen material during timed incubation periods, 2) apply unique reagents onto slides in a predetermined manner, and 3) apply rinse solutions onto slides at specified intervals (i.e., between incubation periods). The only additional function of instruments marketed specifically for ISH is the ability to

to use reagents them to create th does the system obtained (with tl bodies and prob the system vend (i.e., can you use or are you force sees fit?). Unfor classify and des degree of open operated, which tion will be pres examining som certain instrume other ways, cor is, therefore, en 1 and 2 for com systems.

Capacities and capabilities

For most people involved in the evaluation of IHC/ISH instruments, the primary criteria that are most often used for evaluation and/or acquisition decisions is the *quantity of slides* that a system can process in a single run and its *functional capabilities*, since these

Chapter 9

The Pros and Cons of Automation for Immunohistochemistry from the Prospective of the Pathology Laboratory

David G. Hicks, Lorelee McMahon

Book Editor(s): Shan-Rong Shi, Clive R. Taylor

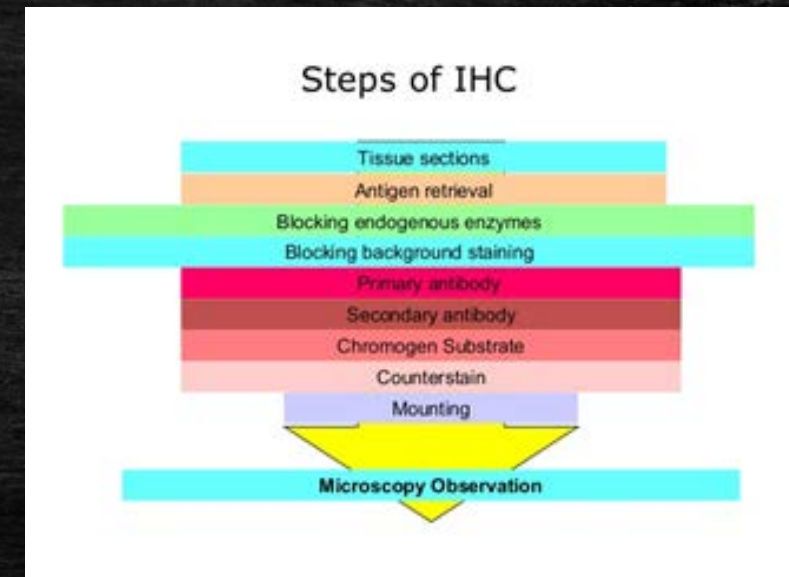
First published: 4 August 2010

<https://doi.org/10.1002/9780470875612.ch9>

bottom line of all these articles is that there is no perfect system -- each has strengths and weaknesses

IHC - immunohistochemical stainers

- Immunohistochemical staining is a multiplex technique requiring lots of hands-on time when performed manually.
- From deparaffination to counterstaining the manual IHC procedure requires at minimum 60-100 manual interactions on each slide to be stained. Capacity ?? (*50-100 slides per tech.**)
 - Preparation and sorting
 - Deparaffination, epitope retrieval (manually)...
 - Application of reagents – pipetting
 - Secure even distribution of reagents – “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

- Repetative: apply – wash – apply...
- Challenge: time, standardisation, skills (retrieval), traceability...

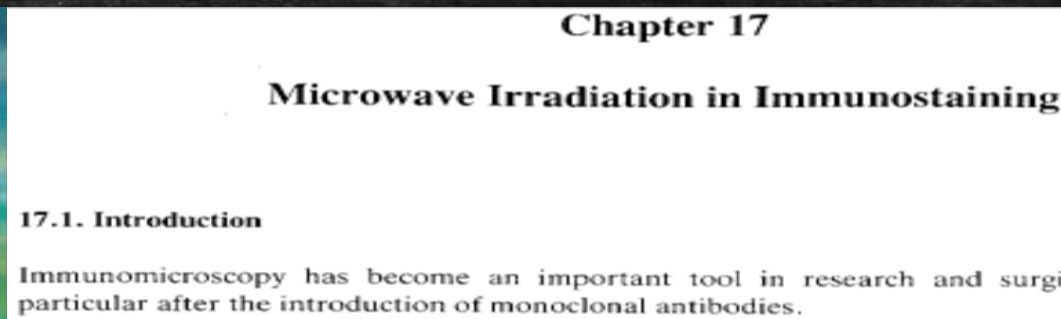
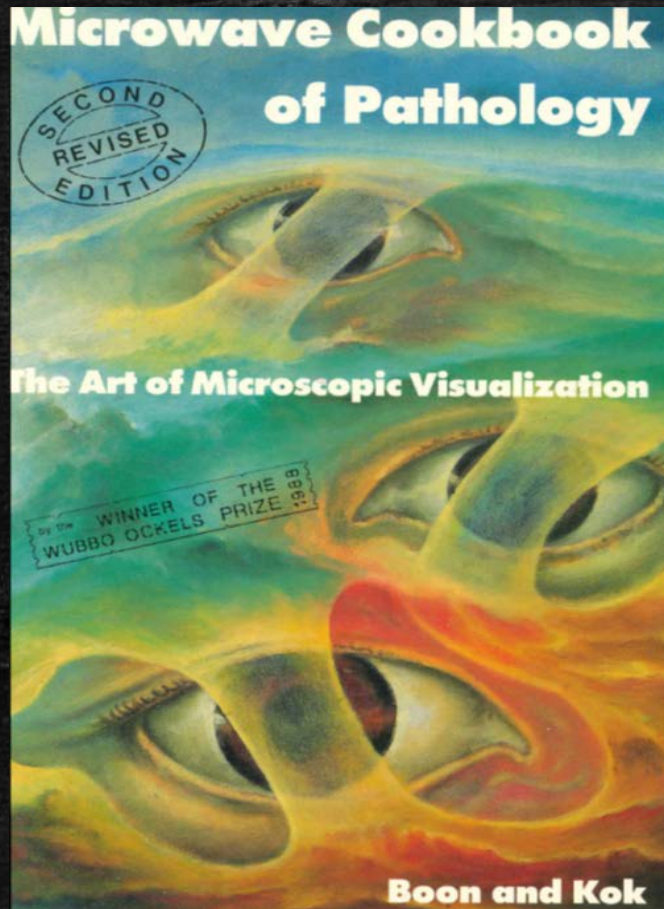


AR Pitfalls

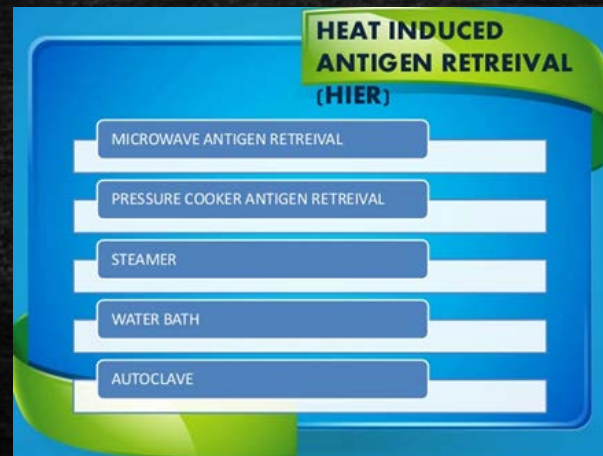
- No testing for pH stability in AR buffers
- Non testing of heating system for AR
- Not familiarizing yourself with the following prior to performing AR-IHC staining:
 1. The cellular localization of the antigen base
 2. Specificity of the primary antibody
 3. Previous IHC staining results from literature, especially from an experienced laboratory.
 4. Any adverse influence on the antigen from tissue fixation, processing, the necessity of any pretreatment procedures (heat-induced AR)
 5. Not reading the package insert! Information regarding reagents, antibody clone, detection systems, manufacturer, recommended concentration, etc.

IHC - immunohistochemical stainers

- Antigen Retrieval (AR): one of the most critical step.



Standardisation ?!



IHC - immunohistochemical stainers

Overview of Automated Immunohistochemistry

Jeffrey W. Prichard, DO

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

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

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

Conclusions.—Each of the commercially available, automated immunohistochemistry platforms has strategic design differences that produce advantages and disadvantages. Understanding those differences can help match the demands of testing volumes, turnaround time, standardization, and labor savings to the appropriate automated instrumentation.

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

“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

- Goals in automating the IHC staining procedure:
 - ✓ To secure and improve consistency of the IHC assay compared to manual performance ; intra-and inter-laboratory
 - ✓ Reduce the technician workload used for IHC
 - ✓ Improve IHC testing capacity
 - ✓ Traceability/ tracking of events (ISO 15189 standard)

Key-driver: automation = standardization

IHC - immunohistochemical stainers

- Automation of the IHC staining procedure:
 - ✓ Initiated in late 80's
 - ✓ Semi-automated systems : no on board deparaf. and HIER

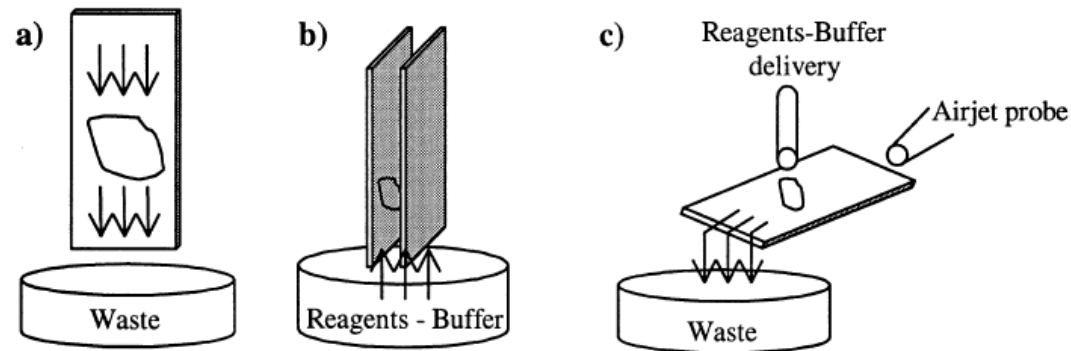


Fig. 1. Automation of IHC – Principles (a) top-down capillarity, (b) ascendant capillarity, (c) flat immunohistolabelling.

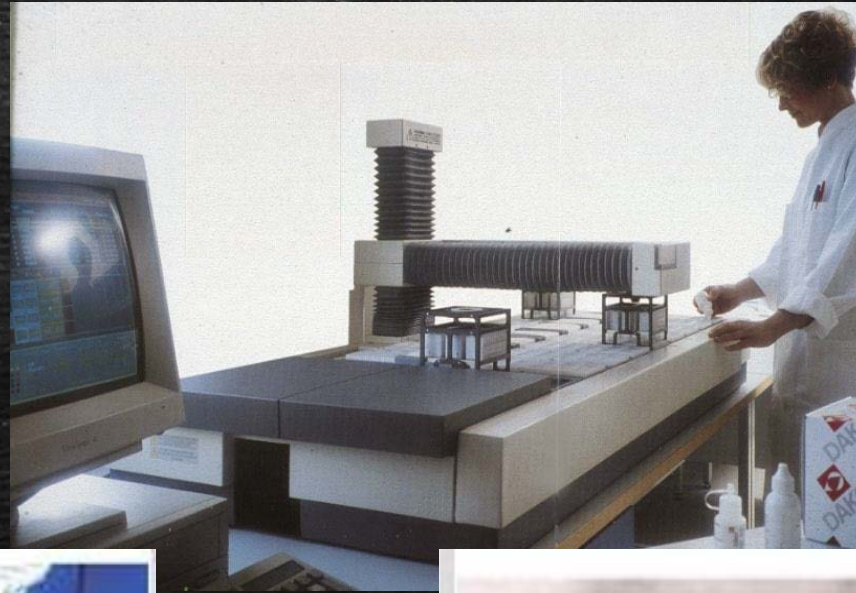
A: Cadenza, Shandon / B: TechMate, Dako / C: ES, Ventana

IHC - immunohistochemical stainers



The Midas e – an automated stainer

80's

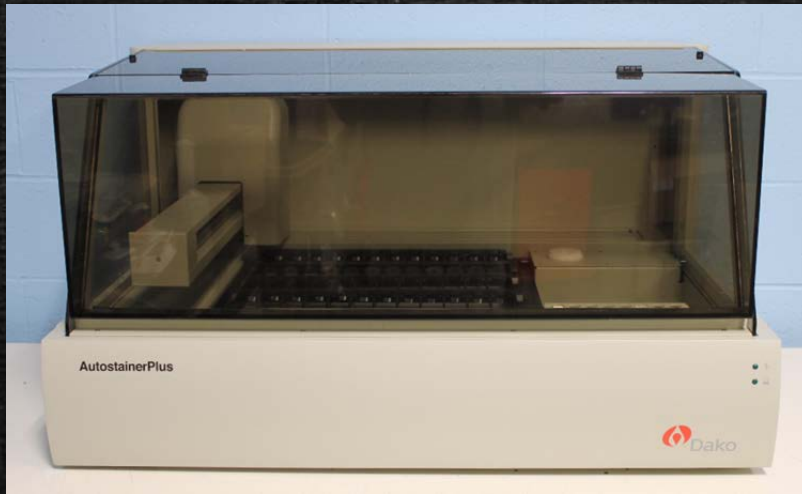


90-00's



IHC - immunohistochemical stainers

- 2000: most commonly used semi-automated stainers



Dako Autostainer (without PT module)



Ventana Nexes



Labvision Autostainer (36/48/72)

Staining procedure

1. Depar/ dehydration / HIER : separately to IHC (e.g. PT-module)
2. IHC performed by stainer : blocking of enzyme → counterstaining

IHC - immunohistochemical stainers

- 2017: Fully automated with focus on 4 core elements
 - ✓ Deparaffination
 - ✓ Epitope retrieval (HIER and / or proteolysis)
 - ✓ IHC protocol optimisation
 - ✓ Counterstaining

Capillary technique: BOND Leica, OMNIS Dako

Flatlabelling technique: BenchMark Ventana, Oncore/IntelliPATH Biocare

Functionality-Workload-Workflow-Flexibility-Costs



IHC - immunohistochemical stainers

Result: eg Estrogen receptor

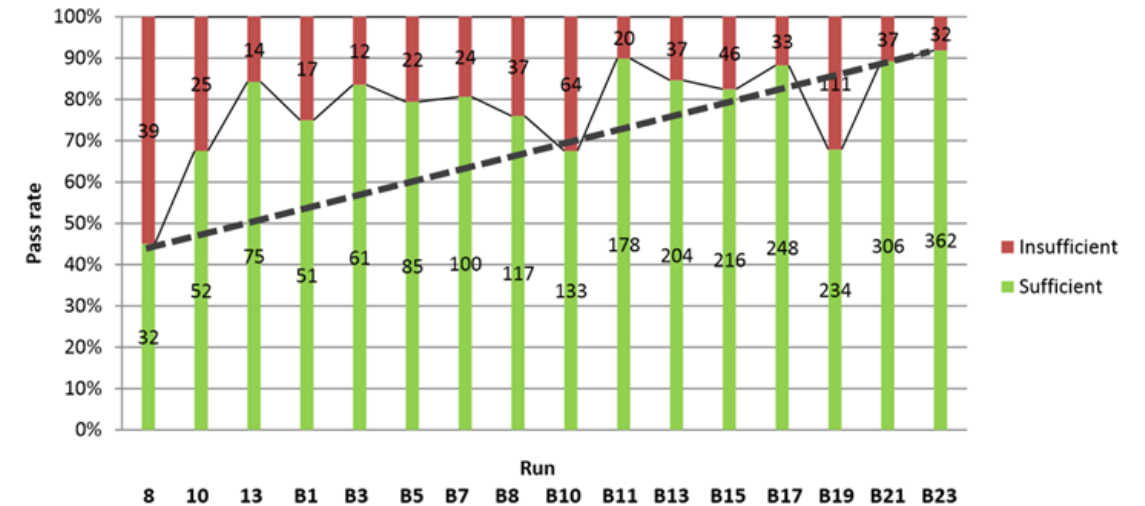
Manual <> Automated

Pass rate 75% → 92%

Performance history

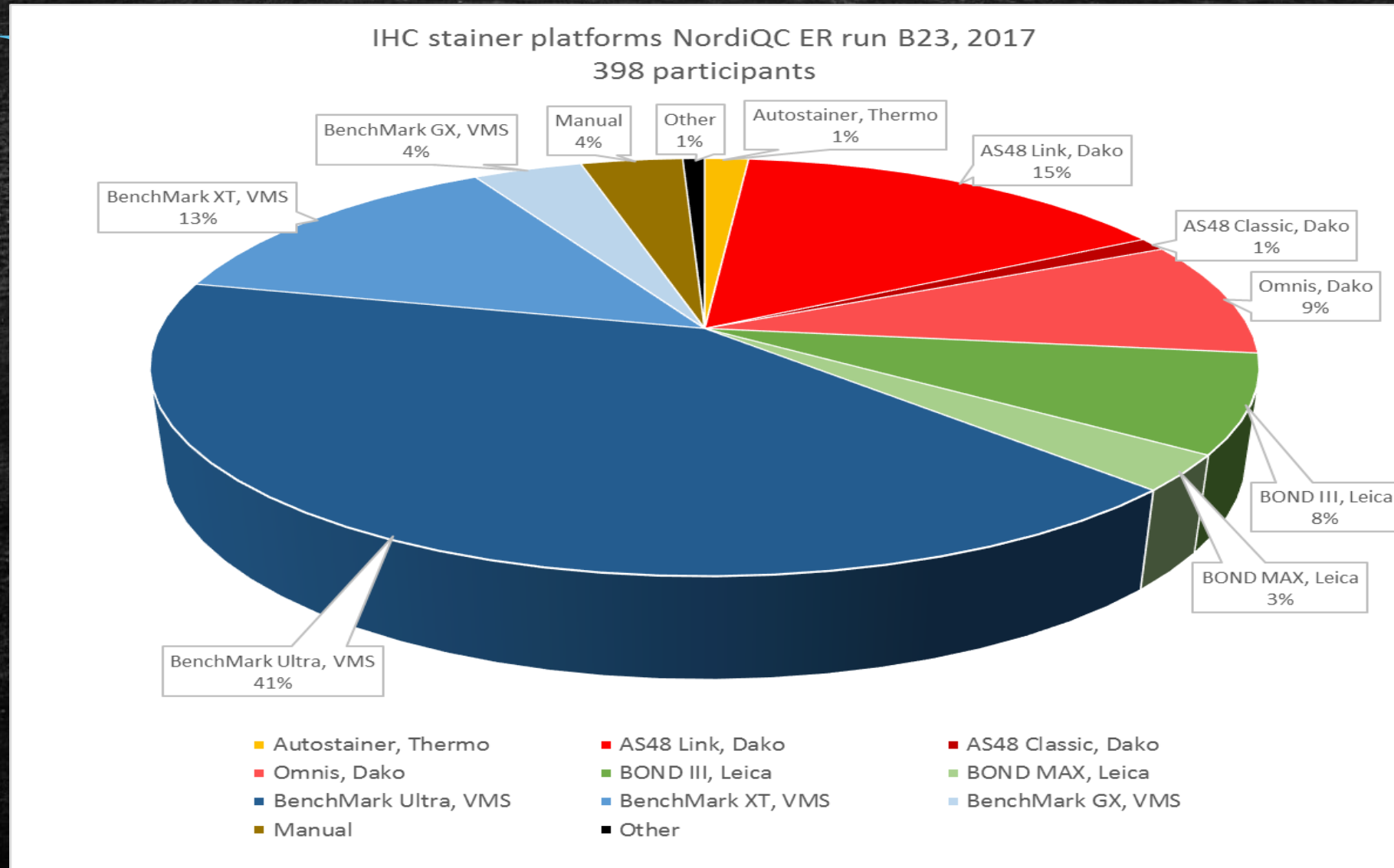
This was the sixteenth NordiQC assessment of ER. The proportion of sufficient results was similar compared to the latest run (see Figure 1).

Fig. 1. Participant numbers and pass rates for ER during 16 runs



	2009 B8 (n=154)	2017 B23 (n=398)
Manual performance	5%	4%
Semi automated system	89%	18%
Fully automated system	6%	78%

IHC - immunohistochemical stainers

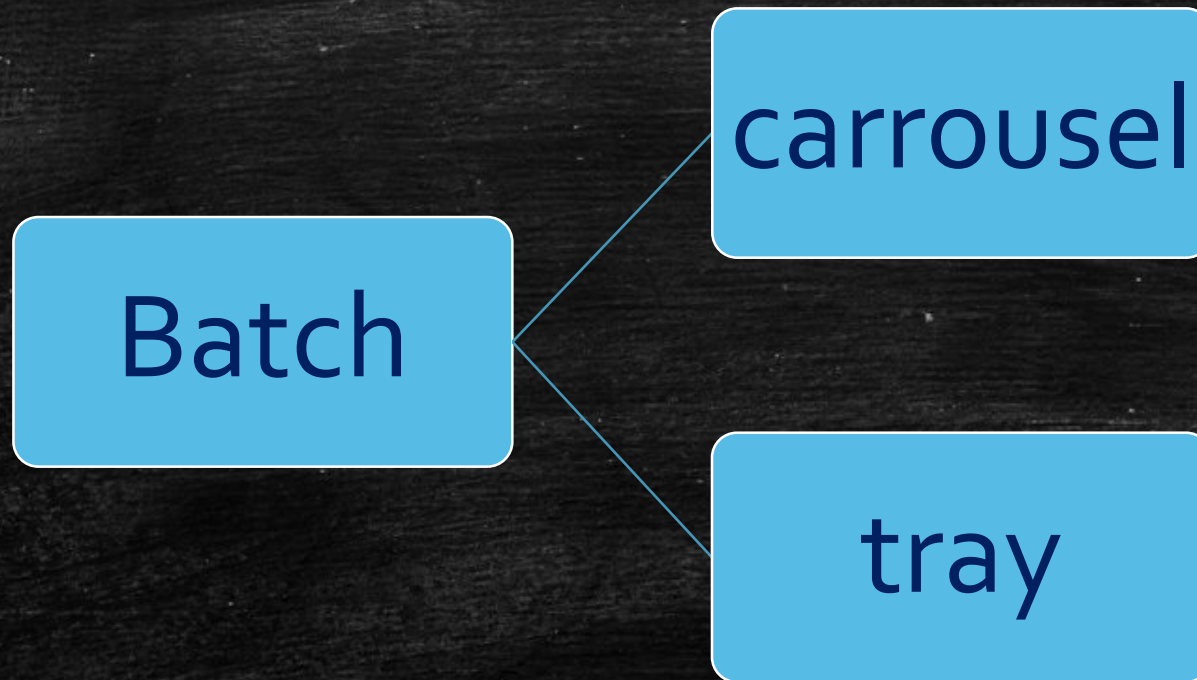


IHC stainers – Staining technologies

- IHC stainers inherent daily practice
 - Different vendors, platforms, etc...
 - Different staining technologies available
- ➡ How to choose the most optimal ?

IHC stainers – Staining technologies

- Slide processing method :



IHC stainers – Staining technologies

- Slide processing method :

Continuous loading

Tray 10 slides

3 x 10

5 x 10


Tray 5 slides

Single piece




IHC stainers – Staining technologies

- Open vs closed systems – reagent choice/usage :

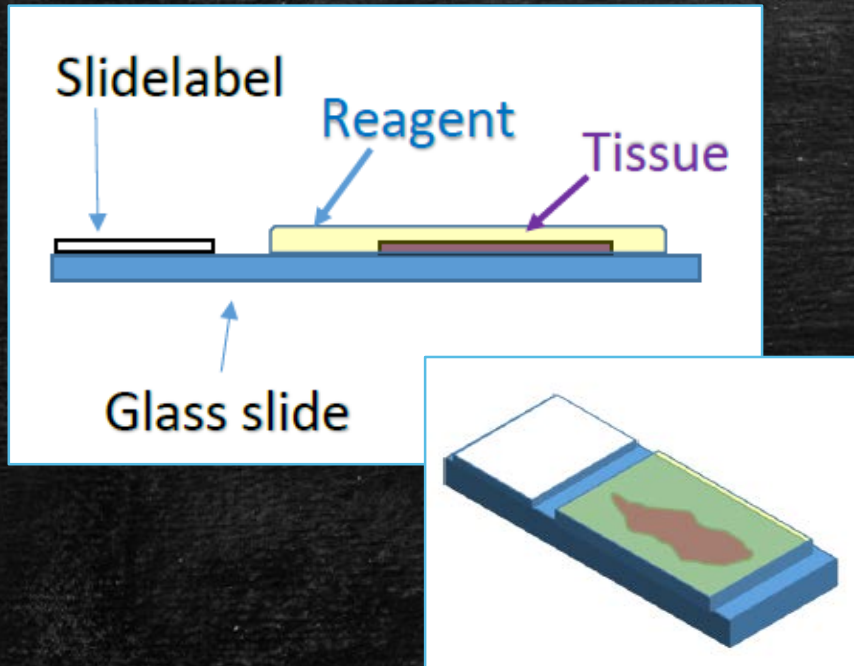


OPEN SYSTEM	CLOSED SYSTEM
Free choice visualization system	High degree of standardization (RTU)
Ab choice flexibility	High degree of consistency (RTU)
Research	Reduced hands on time



OPEN SYSTEM	CLOSED SYSTEM
More need for protocol optimization	Reduced staining protocol options
Increased risk manual error	Ab restricted choice vs optimal staining
Reduced staining consistency	Operational cost

IHC stainers – Staining technologies

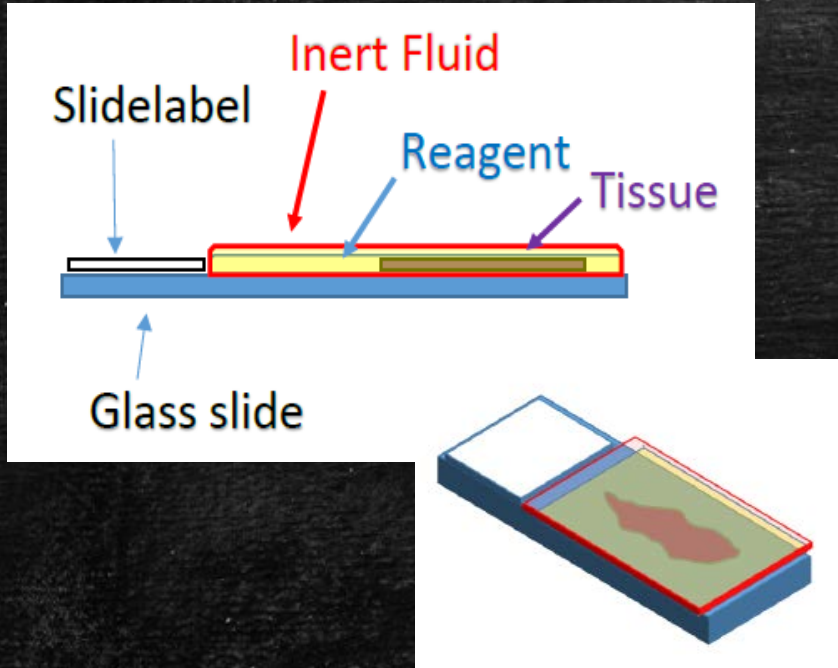


Open slide staining

- Volume - Drop zone
- Temperature – evaporation
- Leveling instrument & racks



IHC stainers – Staining technologies

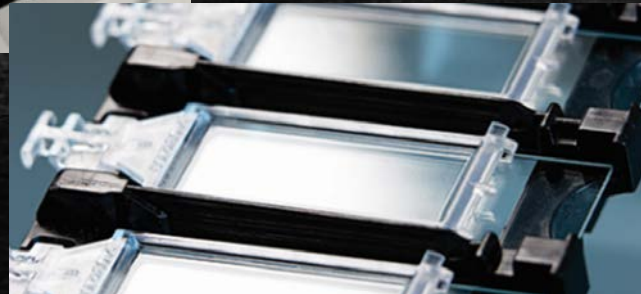
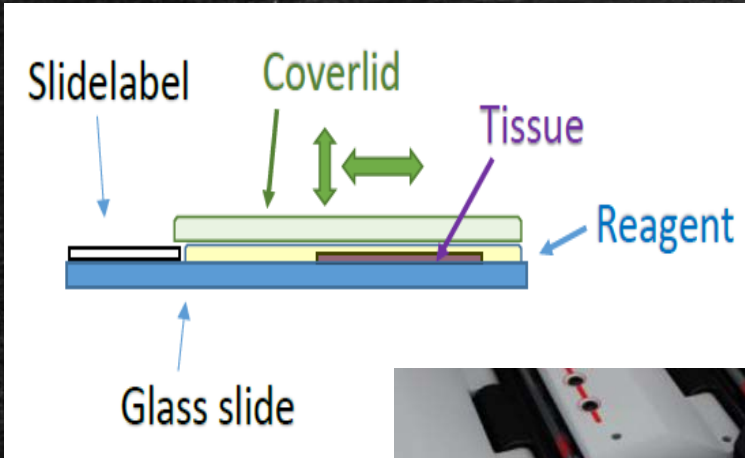


Liquid overlay staining

- Volume - Drop zone = slide
- Temperature – evaporation
- Leveling instrument & racks
- Removal inert fluid afterwards
- Slide choice + storage
FLUIDICS !



IHC stainers – Staining technologies



GAP staining

- GAP system vs free slide space
- Temperature – evaporation
- Homogeneous reactions
- Additional cleaning /waste

IHC stainers - Selection

- Budget (capital vs rental) , floor space, etc...
- How will the instrument be used ?
 - Type of samples (FFPE, CYTO)
 - Type of stains ? (IHC – double staining – ISH)
 - Workload : continuous – batch vs clinical service/added value
- Capacity needed ? Capacity vs loading system, protocol combinations
- Ease of use :
 - Daily usage, LIS connectivity, reagent management, slide management (e.g. LCS)
 - Software flexibility (protocol adaptations for optimization protocol)
 - Amount of maintenance
 - Waste

	Agilent - DAKO		BioCare Medical	Leica		Roche			Thermo Fisher Scientific	3D Histech
	Autostainer	Omnis	Intellipath	Bond Max	Bond III	Benchmark XT	Benchmark Ultra	Benchmark GX	Autostainer 480S-2D	iSACS
Automated staining steps										
Slide baking	NO	NO	NO	YES	YES	YES	YES	YES	NO	NO
Dewaxing	NO	YES	NO	YES	YES	YES	YES	YES	NO	NO
On board heating	NO	YES	NO	YES	YES	YES	YES	YES	NO	NO under development
On board in situ hybr	NO	DNA/RNA	RNA	DNA/RNA	DNA/RNA	DNA/RNA	DNA/RNA	DNA/RNA	RNA	
Counterstaining	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Dehydration & mounting	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES
Dimensions										
Type	Benchtop	Floor	Benchtop	Benchtop	Floor	Floor	Floor	Floor	Benchtop	Benchtop
Weight (kg) (Dry)	66,7	530	66	120	238	175	295	65	80	120 kg
Size (WxDxH)(cm)	89x66x68	150x80x177	102x64x61	76x77,5x70,3	79x80,6x137,8	89x66x153	112x84x159	51x55x124	128x67x58	112x66x78 cm
Slide management										
Barcode labelled slides	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Slide capacity	48	60	50	30	30	30	30	20	36-84	35
Batch size	4x12 slides	12x5 slides	5 x 10slides	3x10 slides	3x10 slides	1x30	30 x 1	1x20	3-7 x 12 slides	72
Processing capacity/24h IHC	144	165	150	90	90/120	90	90	60	240	70
Staining Protocols										
Standard template	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Custom template	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
IHC - ISH separate	NO	YES	NO	YES	YES	YES	YES	YES	NO	YES (NO FISH)
IHC - ISH combined	NO	YES (rack)	NO	YES	YES	YES	YES	NO	NO	YES (NO FISH)
Dual Staining	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO
Any protocol, Any position	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Protocol restrictions	NO	NO	NO	1/batch (tray)	1/batch (tray)	NO	NO	NO	NO	NO
Reagents										
Robotic movement	XYZ robotic arm	XYZ robotic arm	XYZ robotic arm	XYZ robotic arm	XYZ robotic arm	Rotary	Rotary	Rotary	XYZ robotic arm	XYZ robotic arm
Reagent positions	42	60	48	36	36	35	35	25	40	40
Reagent T° control	NO	YES	YES	NO	NO	NO	NO	NO	NO	YES
Barcode reagents	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Reagent application	Rinsed probe tip	Rinsed probe tip	Rinsed probe tip	Rinsed probe tip	Rinsed probe tip	Disp dispensers	Disp dispensers	Disp dispensers	Rinsed probe tip	Single use tips
Reagent incub method	Open slide	Dynamic gap	Open slide	Covertiles	Covertiles	LCS + air vortex	LCS + air vortex	LCS + air vortex	Open slide	Open slide
Staining Reagent incub T° (°C)	RT	32	RT	RT	RT	RT or 37°C	RT to 42°C	RT or 37°C	RT	RT
Adjustable dispensing vol	YES	YES	YES	YES (100/150µl)	YES (100/150µl)	NO	NO	NO	YES	YES - Specimen size
Open reagent system	YES	Prim Ab	YES	Prim Ab	Prim Ab	Prim Ab + Enz	Prim Ab + Enz	Prim Ab + Enz	YES	YES
Waste separation	YES	YES	YES	YES	YES	NO	NO	NO	YES	YES
Information technology										
LIS interface capability	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Units controlled/1 pc	5	8	4	5	5	4	8	4	4	1
Maintenance time (h)										
Cleaning procedure										
Daily	NO	NO	NO	YES	YES	YES	YES	YES	NO	NO
Weekly	NO	YES	NO	YES	YES	NO	NO	NO	NO	YES
Monthly	NO	YES	YES	YES	YES	YES	YES	YES	NO	NO
Quarterly (3m)	NO	NO	NO	NO	NO	YES	YES	YES	NO	YES
Partially automated	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Proprietary Issues										
Special accessories	PT Link	Reagent vials	Reagent vials	Covertiles	Covertiles	LCS	LCS	LCS	PT	
Unique features	Full open	Dynamic GAP technology DAB mixing vials slide movement reagentcheck FISH in 4h visual alarm	Simultaneous multiplex staining Reagent cooling chamber	DAB mixing vials low dead volume 30 ml RTU	Bulk dispensing robots DAB mixing vials visual alarm low dead volume 30 ml RTU		1 slide batch			Coverslipper Scanner volume reagent Scanner
Restrictions										No deparaffination.
Staining limitations	none		none	specimen vs covertile	specimen vs covertile	type glass slides vs fluidics	type glass slides vs fluidics	type glass slides vs fluidics	none	

- Important features :

 - ❖ On board heating
 - ❖ Slide capacity /batch size
 - ❖ Processing capacity/24h
 - ❖ Options protocols
 - ❖ Options reagents
 - ❖ Maintenance
 - ❖ Special requirements or features

IHC stainers – Considerations

- IHC stainer = AID to :

- Facilitate workload
- Reduce risk for errors
- Increase standardization & consistency
- Traceability

However ...

IHC stainers – Considerations

■ IHC stainer : optimal results

Instrument :

- ❖ Correct operationg (reagent application, incubation times, T°)
- ❖ Maintenance
- ❖ Scheduling/workflow

Clinical samples :

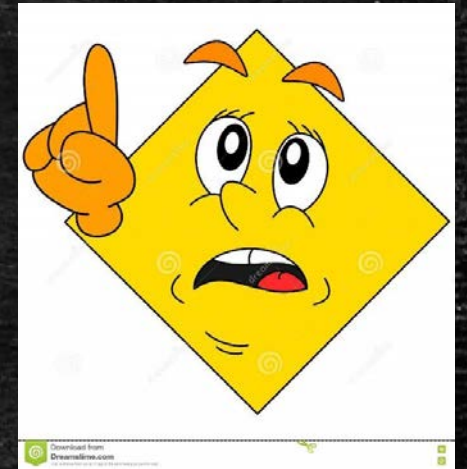
- ❖ Pre-analytical (time to fixation)
- ❖ Fixation (type, time)
- ❖ Sections
 - ❖ Thickness
 - ❖ Baking time & T°
 - ❖ Type of slide
- ❖ Storage

Reagents :

- ❖ Type Ab : conc. vs RTU
- ❖ Clones available
- ❖ Type detection system
- ❖ Optimized protocols
- ❖ Storage

IHC - immunohistochemical stainers

- Congrats, you switched to a stainer or you already have a stainer but ...



Do not trust your instrument
Staining issues will occur

IHC - immunohistochemical stainers

■ Staining issues

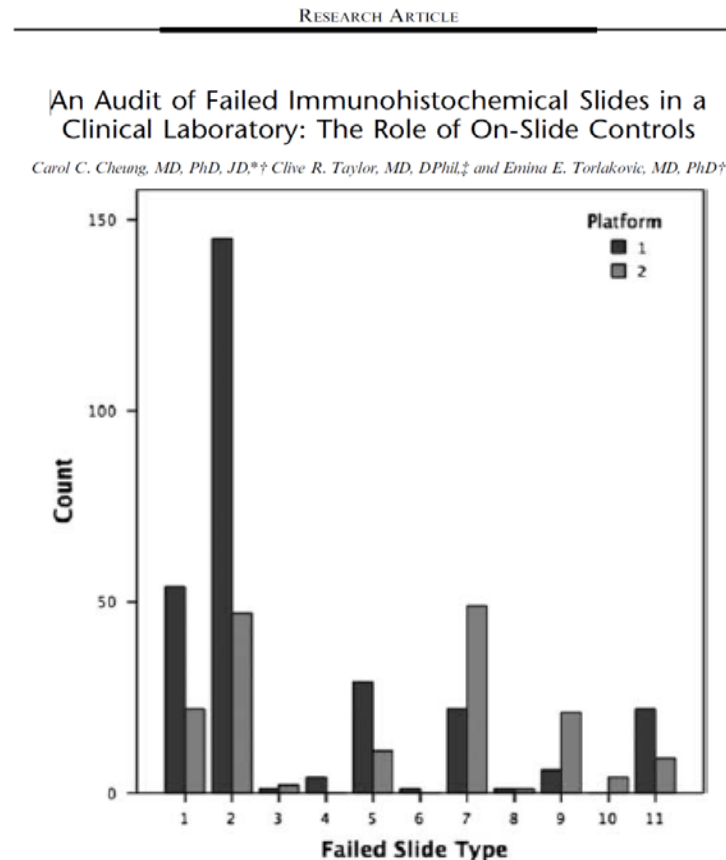


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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.

Lab related :category 5,6,9,11 (22%)

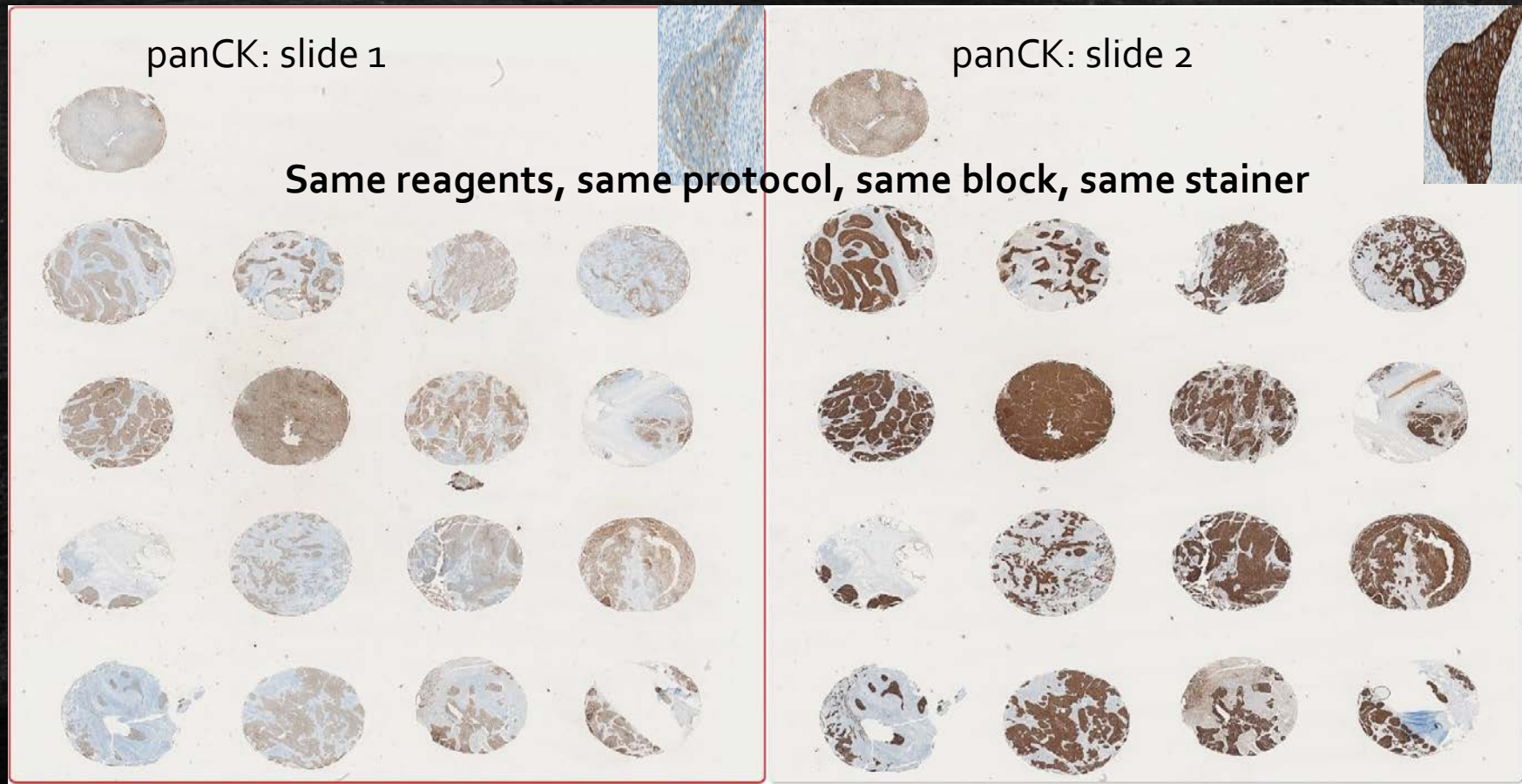
Assay and / or Instrument related: category 1,2,3,4,7,8,10 (78%)

Courtesy by S. Nielsen

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

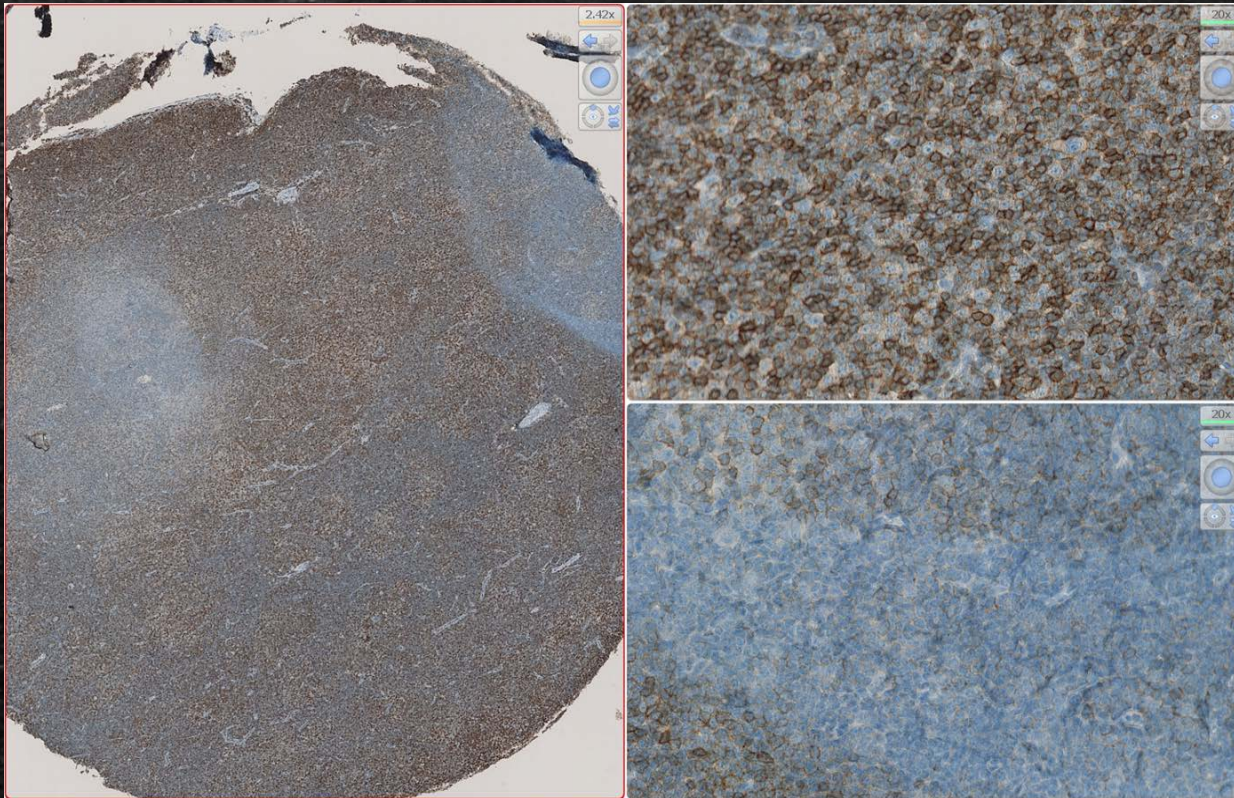
IHC - immunohistochemical stainers

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



IHC - immunohistochemical stainers

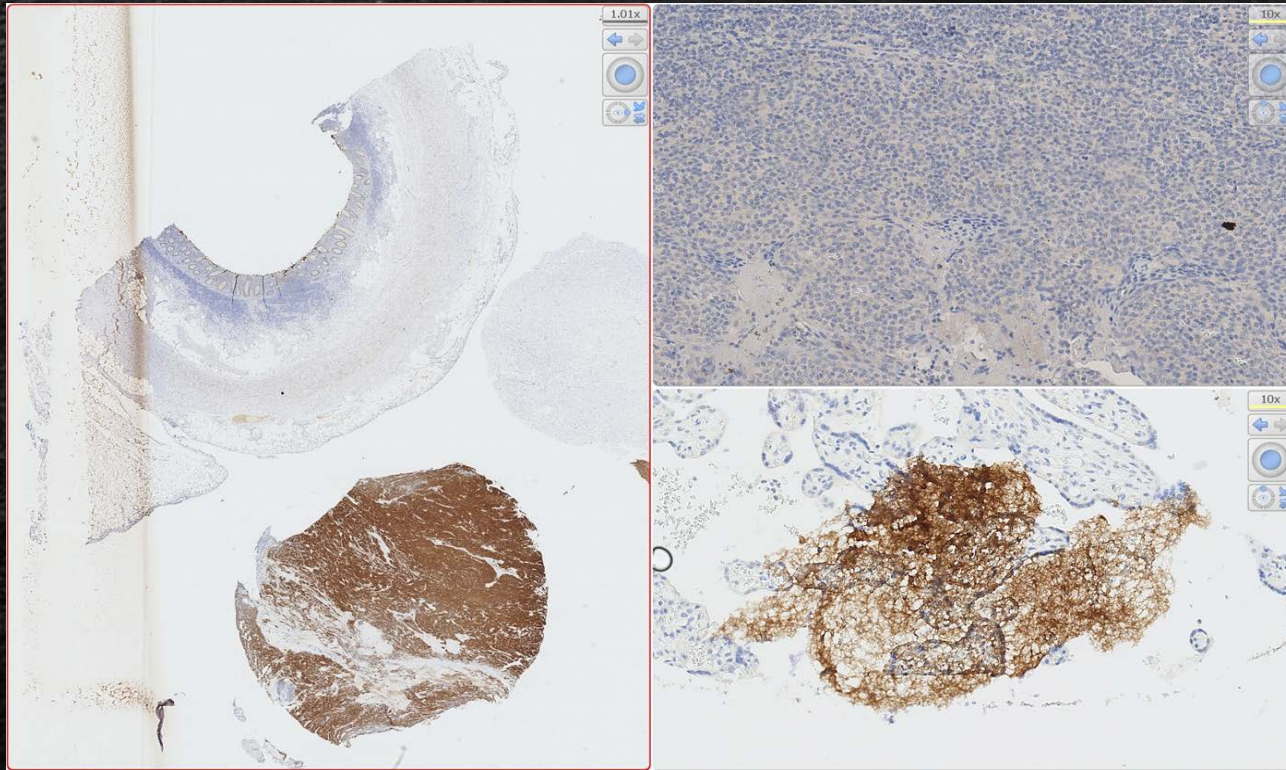
- Staining issues



Ventana BMK: uneven/weak areas: air bubbles

IHC - immunohistochemical stainers

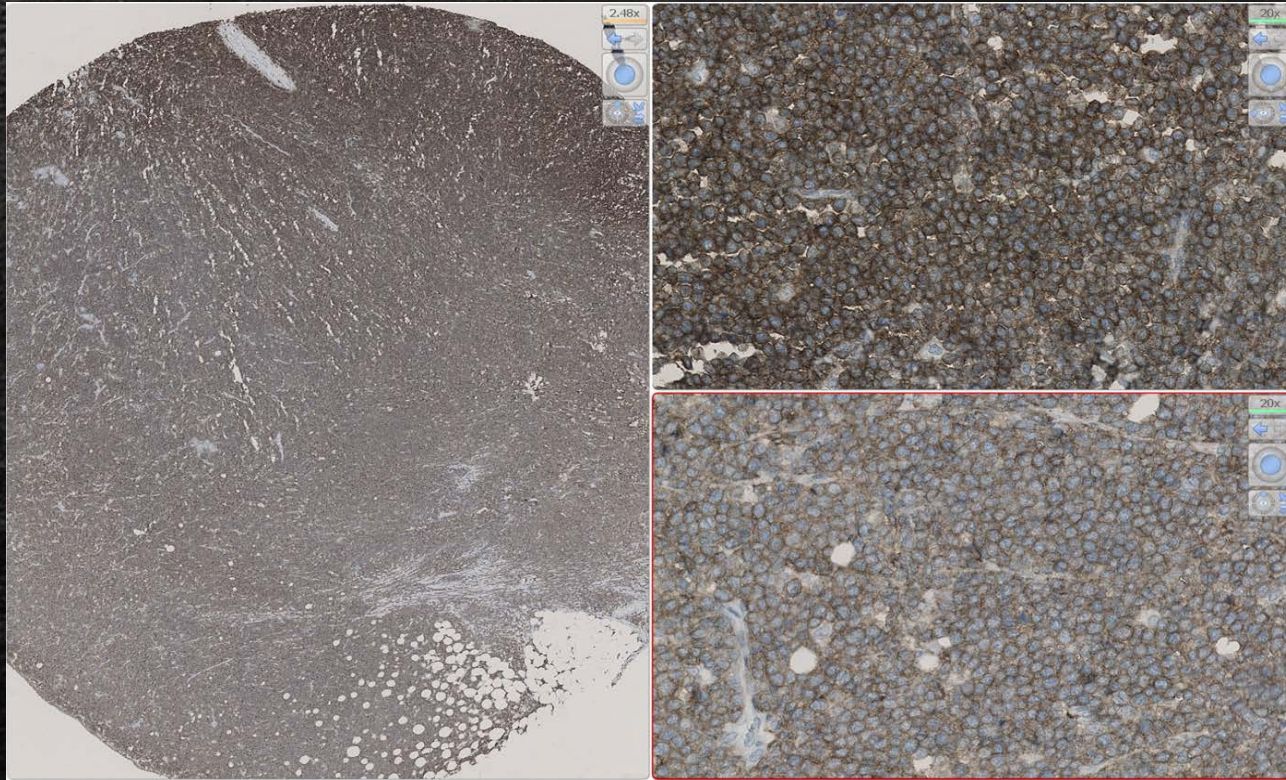
- Staining issues



Leica Bond: chromogen precipitates – general hue

IHC - immunohistochemical stainers

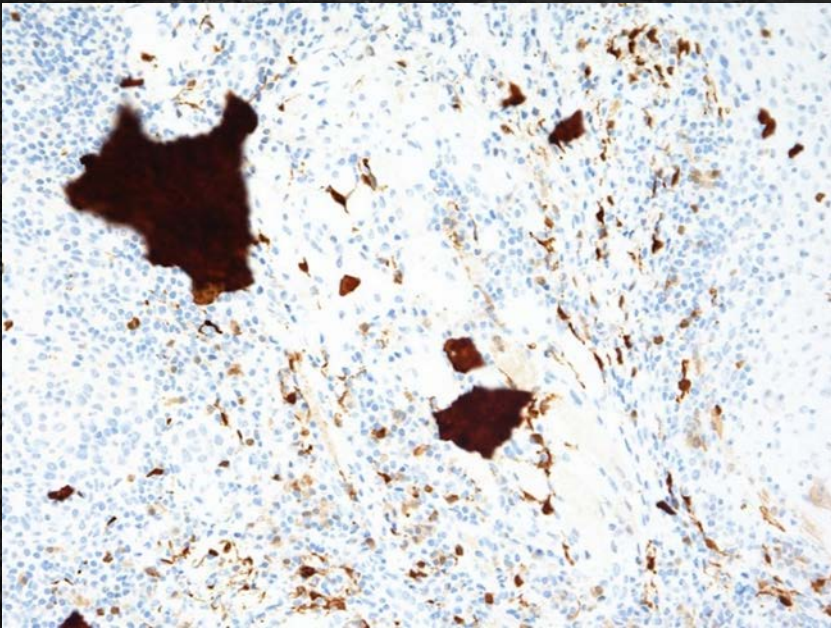
- Staining issues



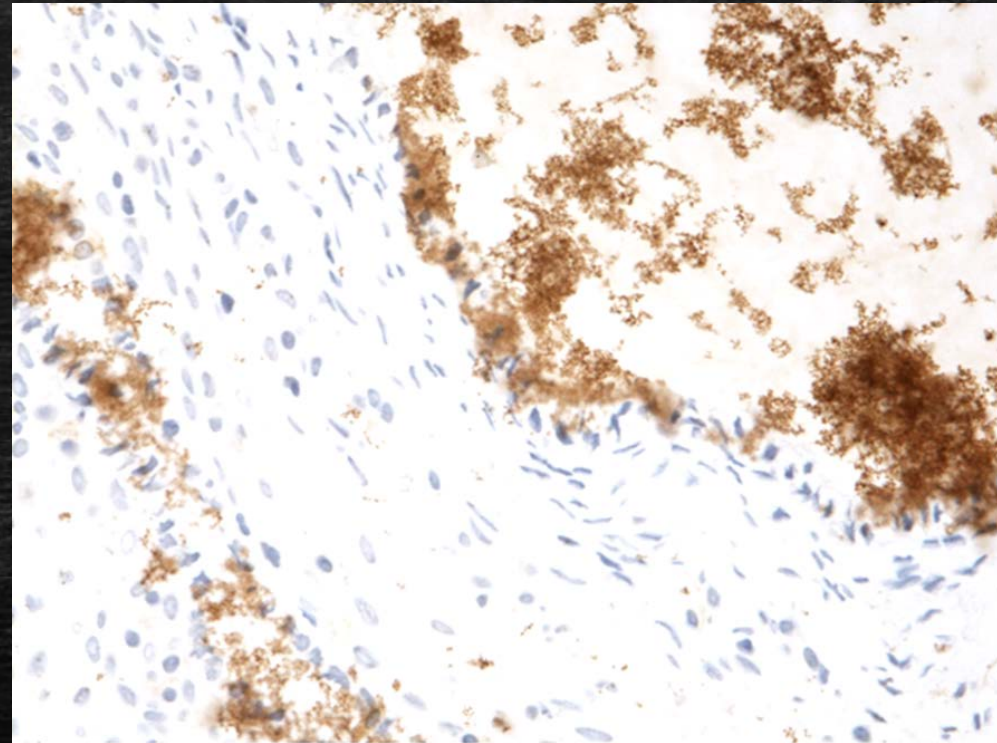
Dako Autostainer 48: chromogen depletion or reagent not spread

IHC - immunohistochemical stainers

- Staining issues



Lid Flakes



DAB Flakes (Bacteria ?)

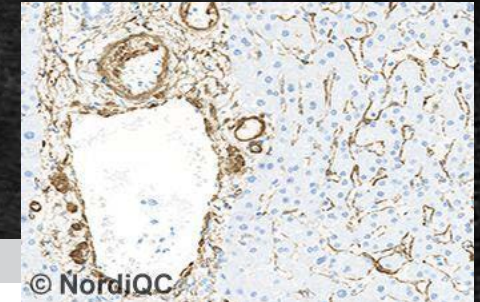
Dako Omnis: chromogen precipitates

IHC - immunohistochemical stainers

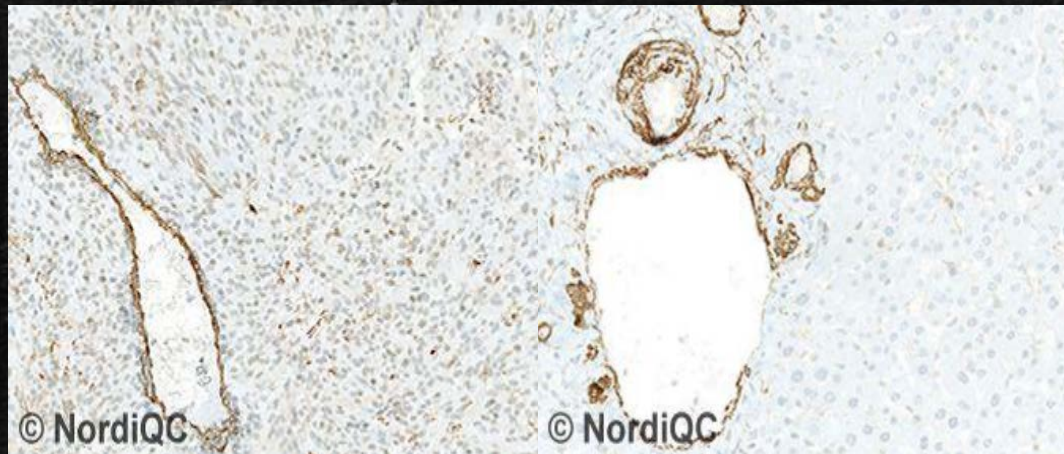
- Staining issues: less succesfull Abs on specific stainers

S. Muscle Actin: NordiQC run 44 (2015)

Ready-To-Use antibodies								
mAb clone 1A4 IR/IS611	44	Dako	23	13	7	1	82%	91%
mAb clone 1A4 760-2833	44	Ventana/Cell Marque	0	6	29	9	14%	-



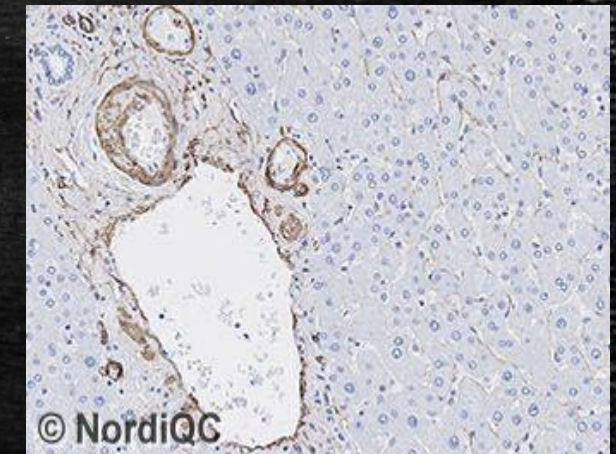
optimal



Ventana BMK XT: ultraView: too low sensitivity GIST (diffuse pos.) / liver perisinusoidal negative



Ventana BMK XT: optiView: increased sensitivity, but hepatocytes false positive

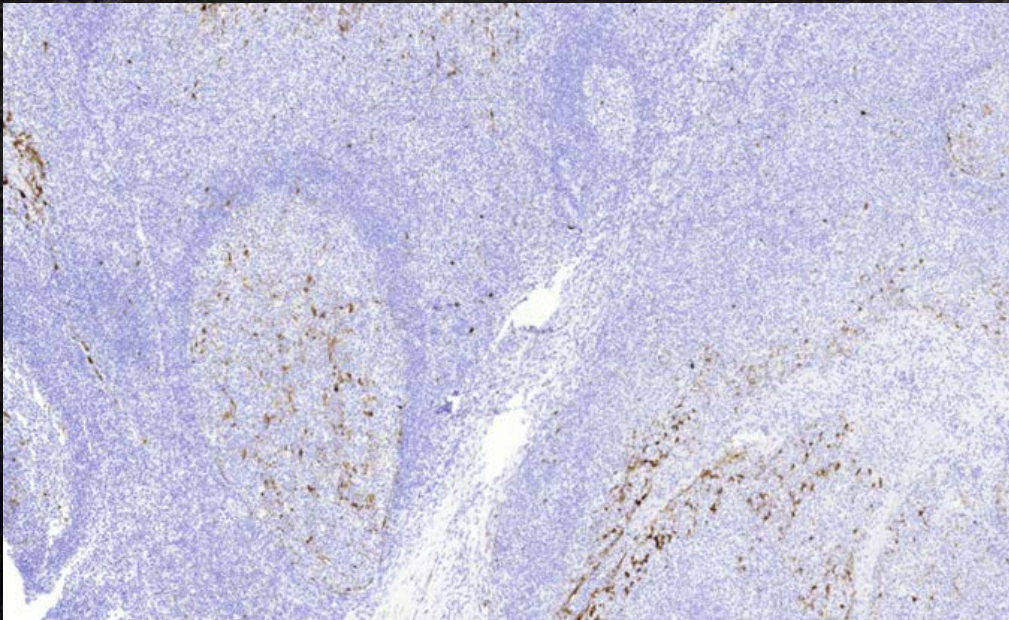


Ventana BMK XT: omitting HIER to reduce false positivity: too weak intensity/reduced staining

IHC - immunohistochemical stainers

- Staining issues: suboptimal use of RTU Abs -- diluted RTU

p16: Belgian EQA (2013)



Ventana BMK XT: clone E6H4: RTU: optimal result

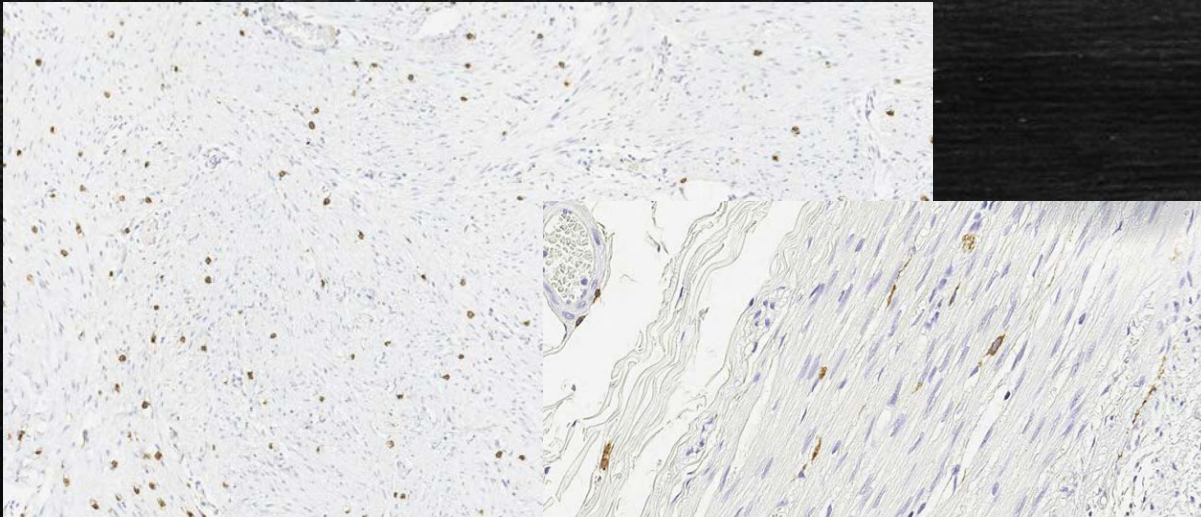


Ventana BMK XT: clone E6H4: RTU diluted: poor result (foll. dendr. cells too weak, background)

IHC - immunohistochemical stainers

- Staining issues: suboptimal use of conc. Abs -- too diluted

CD117: Belgian EQA (2013)



Dako Autostainer: polyclonal Ab: optimal diluted (1:100): optimal result: desmoid tumor mastcells and appendix endothelium/Cajal cells strong positive



Dako Autostainer: polyclonal Ab: too diluted (1:500) : borderline result: desmoid tumor mastcells and appendix endothelium/Cajal cells virtually negative

IHC - immunohistochemical stainers

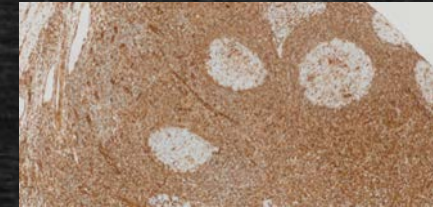
- Staining issues: wrong choice Abs

Vimentin: Belgian EQA (2016)

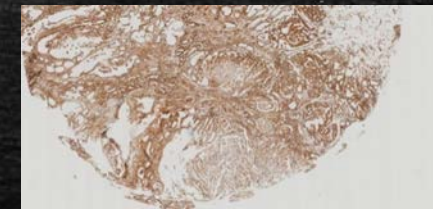


Leica Bond Max: SRL33 RTU: HIER pH6

Cf NordiQC Run 52 ,2018: borderline results



Ventana BMK Ultra: clone V9 RTU: optimal result



Renal Ca

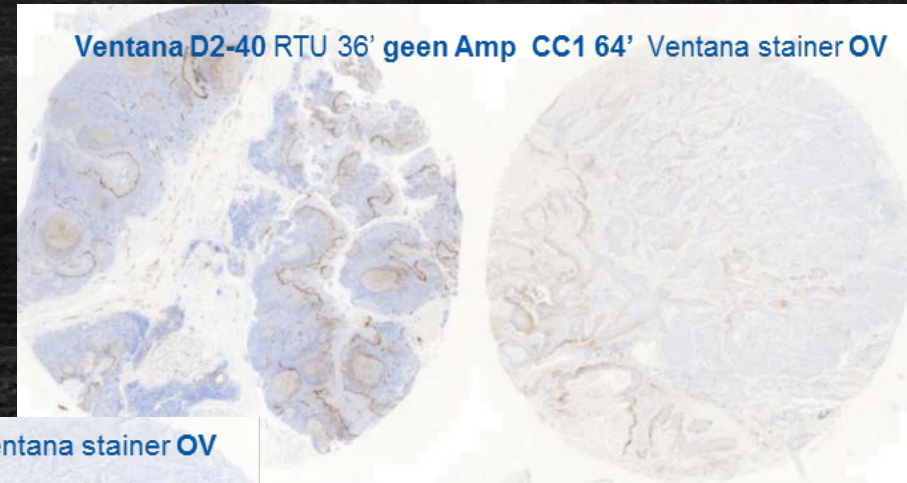
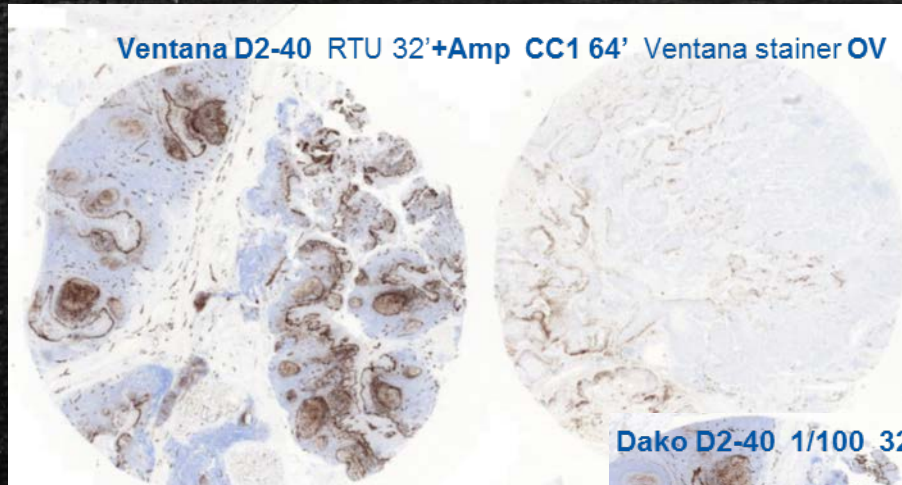
IHC - immunohistochemical stainers

- Staining issues: optimal calibration : same stainer/detection +- amplifier

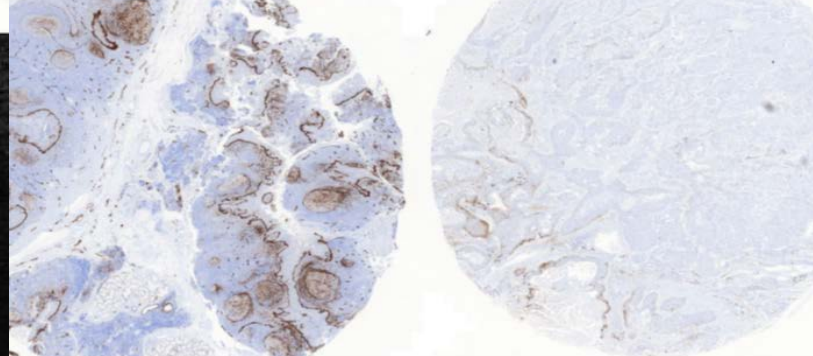
Optimal

Podoplanin: Belgian EQA (2015)

Borderline



Dako D2-40 1/100 32'+Amp CC1 32' Ventana stainer OV



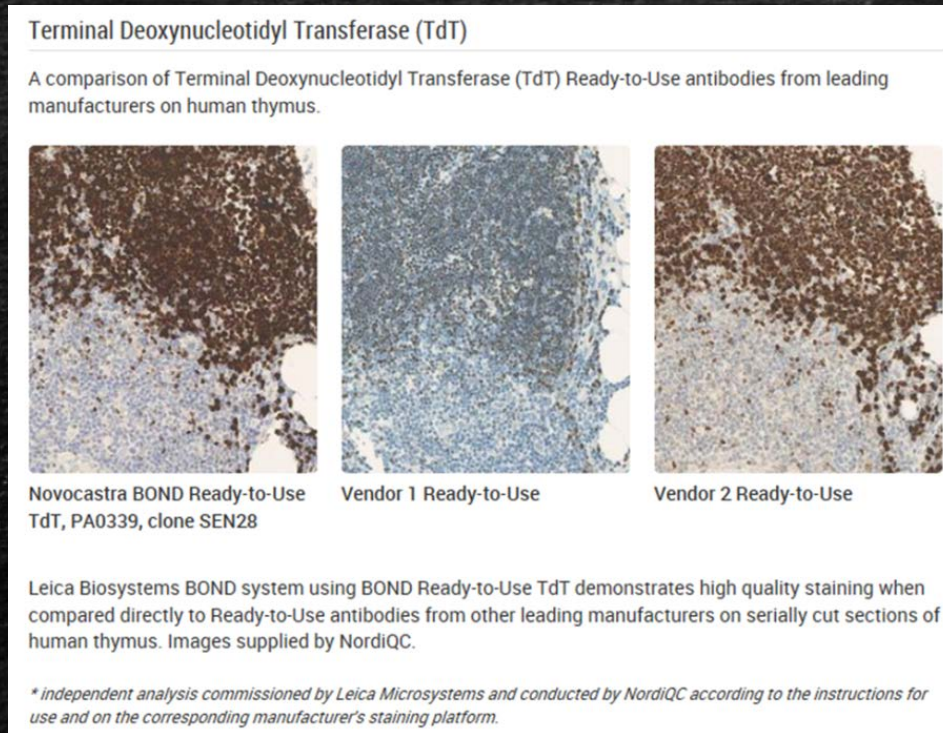
Good

Squamous cell Ca

IHC - immunohistochemical stainers

- Staining issues:

Accuracy of the IHC compromised by use of RTU formats not adequately calibrated etc



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

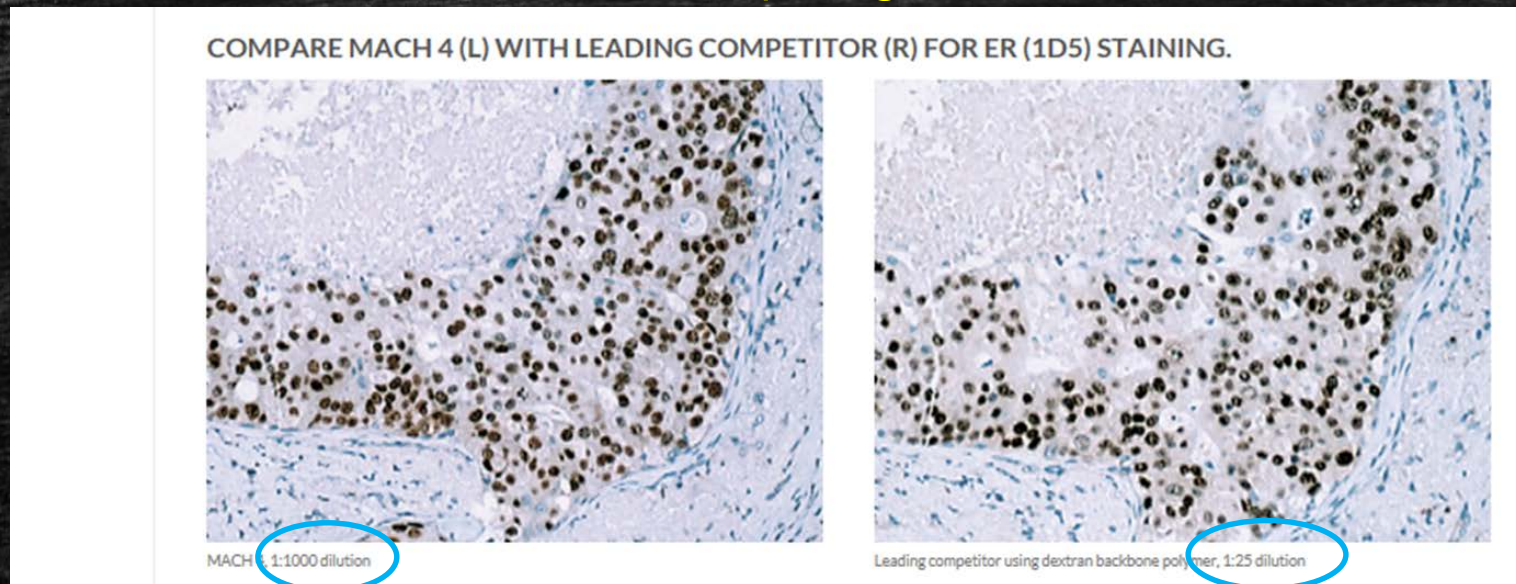


Use as a guideline

IHC - immunohistochemical stainers

- Staining issues:

Cautions to be taken when comparing the different solutions



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

Other: 3-step polymer vs 2-step polymer ? Incubation times ? HIER settings: time, pH, temp etc ?

IHC - immunohistochemical stainers

- Staining issues:

Cautions to be taken when comparing the different solutions

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

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

IHC - immunohistochemical stainers

- Conclusions

- Automation in IHC is needed primarily to secure consistency of inter-and intra laboratory results and to reduce hands-on time.
- There is no perfect system ☹ all systems have their pros and cons.
- Each lab has to select the system being most applicable and favourable for the needs and demands within the lab.
- Get in touch with other labs to have a more objective view on the systems offered.
- A combination of different systems might be a solution, as the IHC tests can be performed on the system giving the best technical result and lowest price – drawback: workflow, extra cost (purchase, expensive maintenance contracts)...
- Automation will not compensate for pre analytical errors (delayed fixation etc)

IHC - immunohistochemical stainers

Nothing can stop automation



