Immunohistochemical stainers

Overview
Pros and Cons

Ole Nielsen,
Dept. of Pathology
Odense University Hospital

With compliments to
Søren Nielsen,
NQC Scheme manager, 2003-2016

IHC – Immunohistochemical stainers

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

IHC – Immunohistochemical stainers

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. Laboisse[b], A. Truchaud[a,*]

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[b]*Laboratory of Pathology, Institute of Biology, 9 Quai Moncousu, 44093 Nantes, France*

Product focus

www.mlo-online.com/articles/200801/0108product_focus.pdf

A review of automated slide stainers for IHC and ISH

By Joe Myers, MS, CT(ASCP)

2: http://www.mlo-online.com/articles/201207/automated-ihc-ish-slide-staining-systems.php

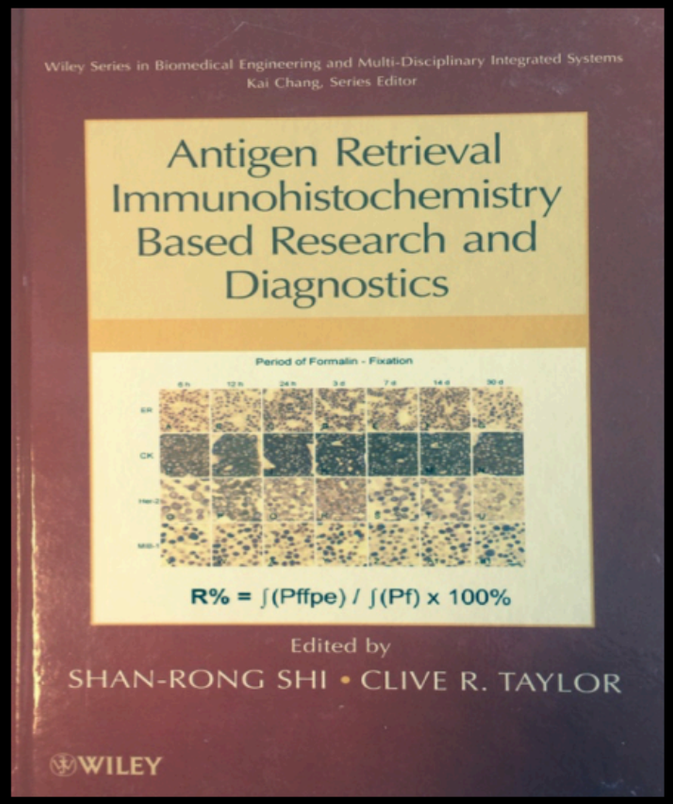
IHC – Immunohistochemical stainers

CHAPTER 9

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

2010

DAVID G. HICKS and LORALEE MCMAHON



Part II: The Potentials and Pitfalls

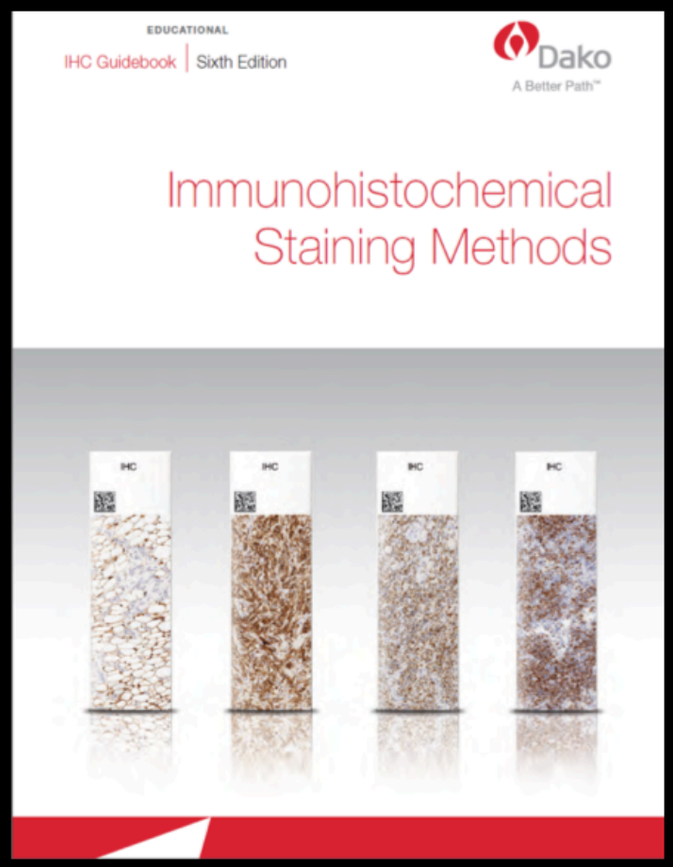
Dako
A Better Path™

Chapter 9

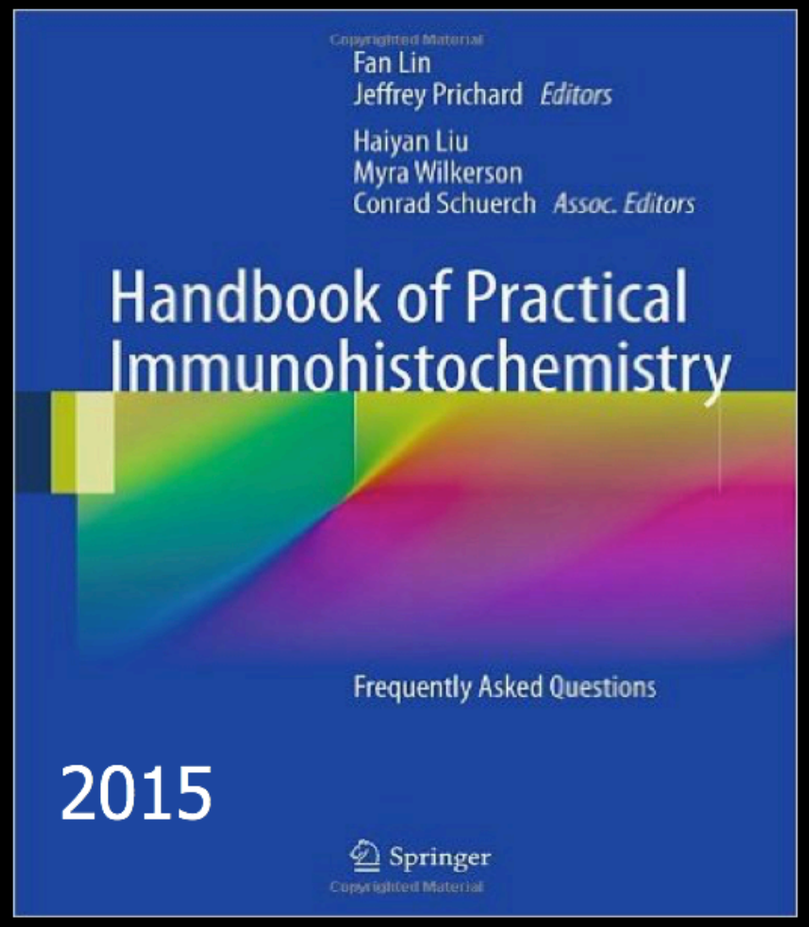
Automation in IHC

Ole Feldballe Rasmussen, PhD, MSc

2013



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)

IHC – Immunohistochemical stainers

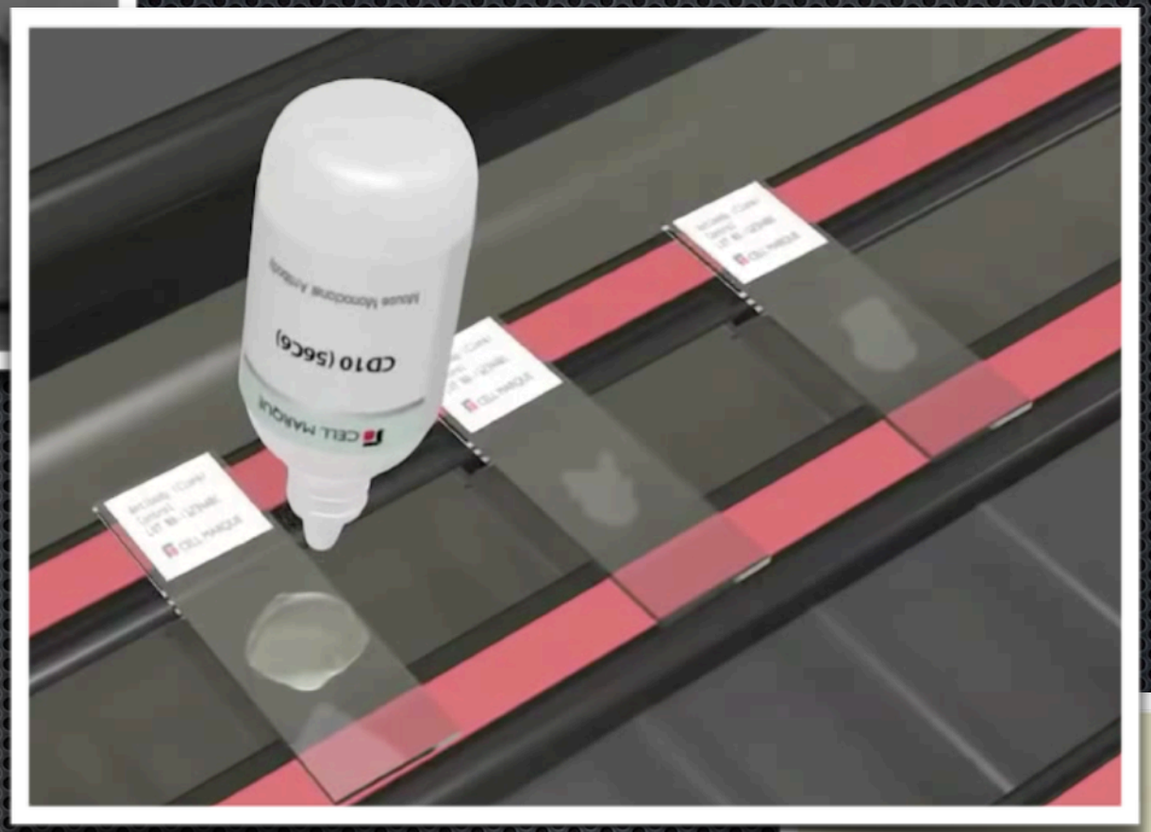
Immunohistochemical staining procedure is a multiplex technique requiring a lot of hands-on when performed manually.

From deparaffination to counterstaining the IHC procedure at minimum requires 60-100 manual interactions and handling procedure on each slide to be stained. Capacity ?? (50-100 slides pr tech.*)

Preparation – sorting, deparaffination, epitope retrieval….
Application of reagents - pippettingSecure even distribution – ”Pap-pen”Avoid evaporation / secure moist – staining trays

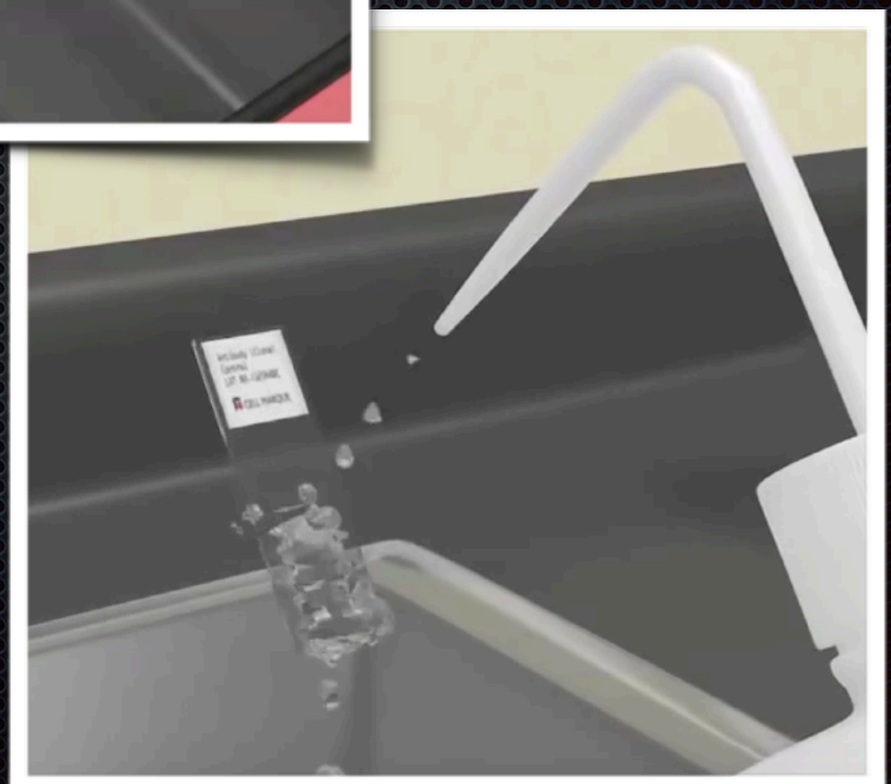
* Haines DM, Chelack BJ. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin-fixed paraffin-embedded tissue for diagnostic pathology. J Vet Diagn Invest 1991; 3:101-12

IHC – Immunohistochemical stainers



Manual staining:
Wash – Dry – Apply
Wash – Dry – Apply
Wash…….

Challenge: Time, Standardisation, Traceability, Skills…



IHC – Immunohistochemical stainers

ER assessments	2003 B8 (n=154)	2017 B23 (n=398)
Manual performance	5%	4%
Semi automated system	89%	18%
Fully automated system	6%	78%

IHC – Immunohistochemical stainers

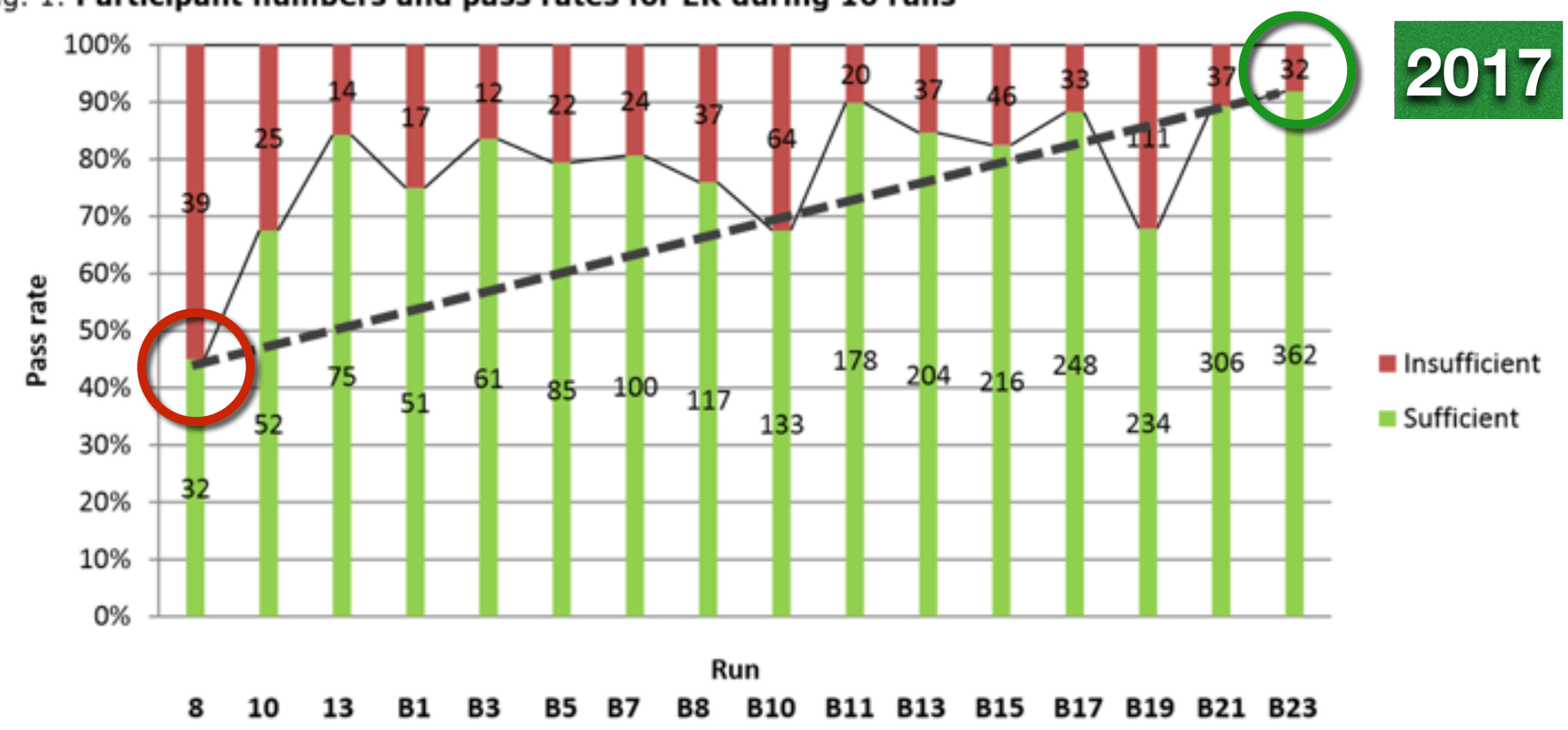
ER assessments	2003 B8 (n=154)	2017 B23 (n=398)
Manual performance	5%	4%
Semi automated system	89%	18%
Fully automated system	6%	78%

Caution: Introduction of new rmAb's and detection-systems in the same period

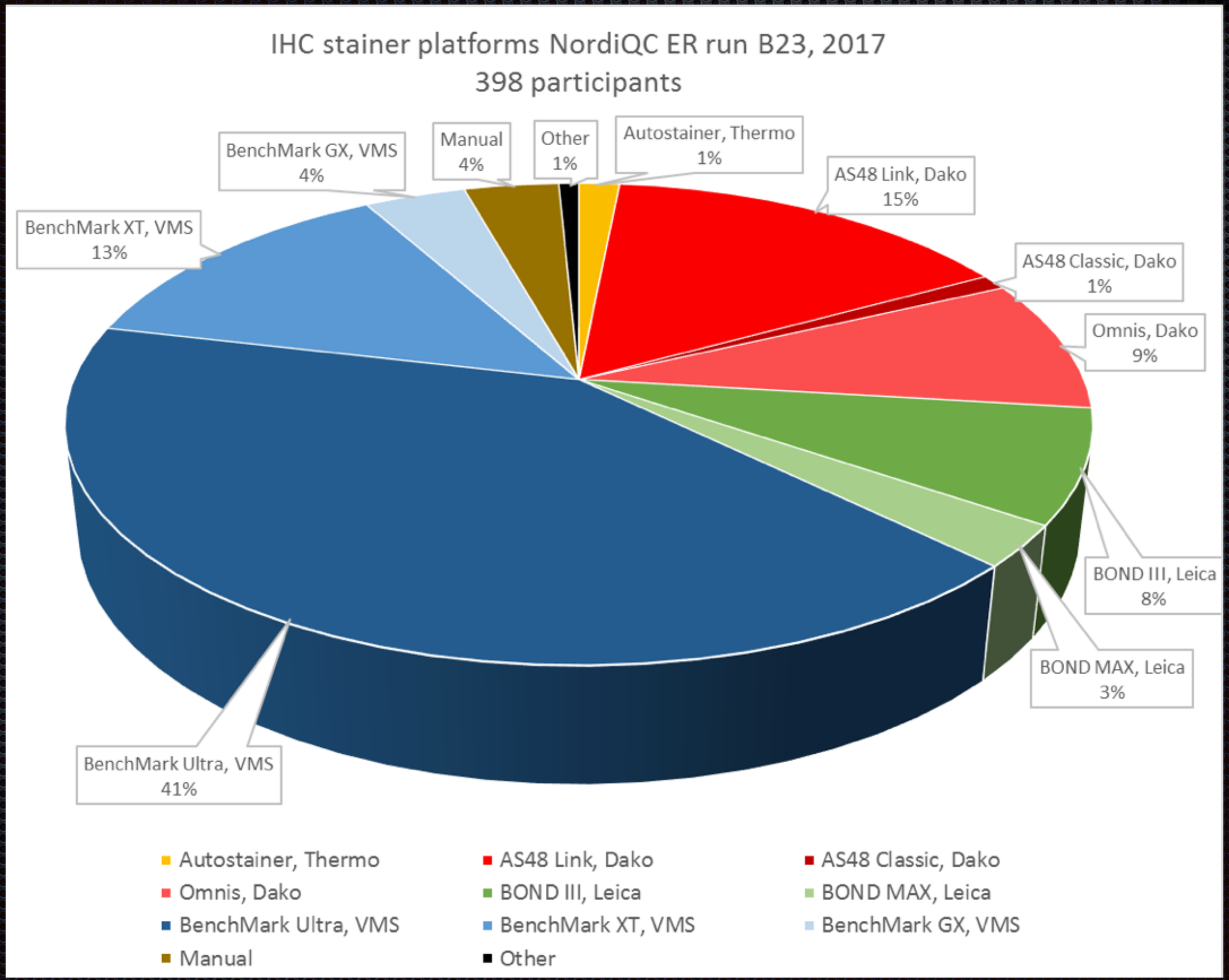
2003

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**



IHC – Immunohistochemical stainers



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
3. Improve IHC testing capacity
4. Traceability / tracking of events

Key-driver: Automation = standardization

IHC – Immunohistochemical stainers

History of IHC automation:

Started in the late 80's

Various semiautomated systems (No depar or HIER)

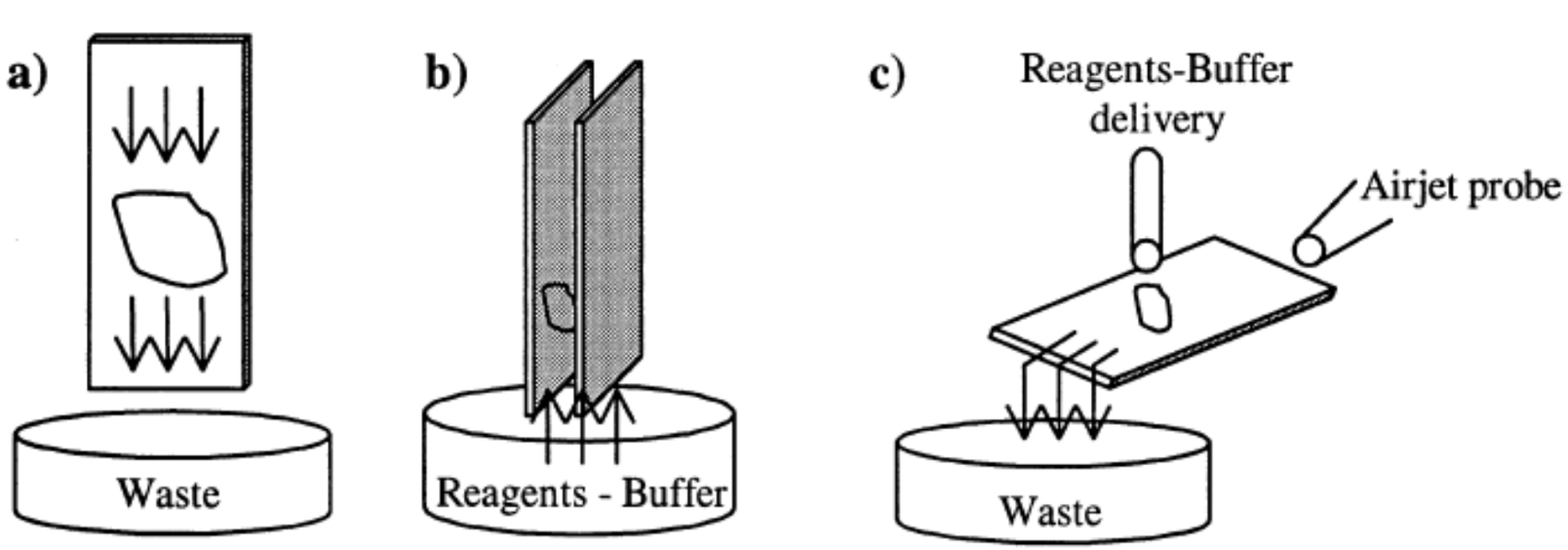


Fig. 1. Automation of IHC – Principles (a) top-down capillarity, (b) ascendant capillarity, (c) flat immunohistolabelling.

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

IHC – Immunohistochemical stainers

The first generation stainers - late 80's

TechMate, Dako

ES, Ventana

Cadenza, Shandon

IHC – Immunohistochemical stainers

Most commonly used semi-automated stainers

Autostainer, Dako (Plus, 48Link)

Autostainer, LabVision (36/48/72)

Parallel processing

1. Depar / dehydration / HIER – separately to IHC e.g. PT-module

2. IHC performed by stainer – blocking of enzyme to counterstaining

IHC – Immunohistochemical stainers

Fully automated stainers
Performs:

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

IHC – Immunohistochemical stainers

Fully automated stainers

Stainer	Company	Principle	Capacity
BenchMark Ultra	Ventana/Roche	Flat labelling	30 slides
Bond III/Max	Leica	Capillary	30 slides
OMNIS	Dako/Agilent	Dynamic capillary	60 slides
Oncore	Biocare	Kinetic chamber	36 slides
Tissue-Tek Genie	Sakura	Capillary	30 slides
Xmatrx ELITE	BioGenex	Flat labelling / Micro-chamber	40 slides

IHC – Immunohistochemical stainers

Which instrument should I choose?

- Functionality
- Workload
- Workflow
- Flexibility
- Cost

IHC – Immunohistochemical stainers

Overview of Automated Immunohistochemistry

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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 staining procedure: Functionality

- 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
- Others

IHC – Immunohistochemical stainers

Automation of the staining procedure: Workload/workflow

- 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 staining procedure: Flexibility

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 – H202 etc
  - Adjustment of reagent volume
  - Modification of protocol steps – addition/removal
  - Washing conditions – of low affinity Abs

IHC – Immunohistochemical stainers

Automation of the staining procedure: Flexibility

Reagents (I)

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

IHC – Immunohistochemical stainers

Automation of the staining procedure: Flexibility

Reagents (II)

- 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 staining procedure: 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

	Autost. Dako/TFS	**Intellip. Biocare**	**Oncore Biocare**	**Impath Pathc.**	**BOND III Leica**	**Bench. U VMS**	**Omnis Dako**
Capacity	48/36-72	50	36	36	30	30	60
Reagents	64	48	40	40	36	35	60
Volume	200 ul	300 ul	200 ul	200 ul	150 ul	100 ul	200 ul
Adjustab.	Yes	Yes	Yes	Yes	Yes	No	No
Depar.	No	No	Yes	Yes	Yes	Yes	Yes
HIER	No	No	Yes	Yes	Yes	Yes	Yes
HIER buf. 3’ part	- Yes	- Yes	2 No	2 No	2 No	2 No	5 Yes
Comb ret	Yes	Yes	?	?	Yes – H+P	Yes	Yes - H+P
3’part reagents	Ab, enz, det.,chr.	Ab, enz, det.,chr	Ab	No	Ab, enz	Ab, enz	Ab, enz, ,chr.
Any prot Any slide	Yes	Yes	Yes	Yes	No	Yes	No
Seq. DS	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sim. DS	Yes	Yes	? (Yes)	? (Yes)	No	No	Yes
ISH	No	No	(Yes)	(Yes)	Yes	Yes	Yes

IHC – Immunohistochemical stainers

Fully-automated systems: BenchMark Ultra, Ventana

5 main Pros:

1. Place, start, walk
2. Continuous and/or batch loading – ”30 stainers”
3. Flexible protocol set-up – e.g. combined retr.
4. Wide range of sensitivity for detection systems
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, Leica

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. Wide 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 of detection systems – DAB & RED

IHC – Immunohistochemical stainers

Fully-automated systems: OMNIS, Dako

5 main Pros:

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

3 main Cons:

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

IHC – Immunohistochemical stainers

Fully-automated systems: Autostainer-LINK48, Dako

5 main Pros:

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

3 main Cons:

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

IHC – Immunohistochemical stainers

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

Cautions to be taken when comparing the different solutions:

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

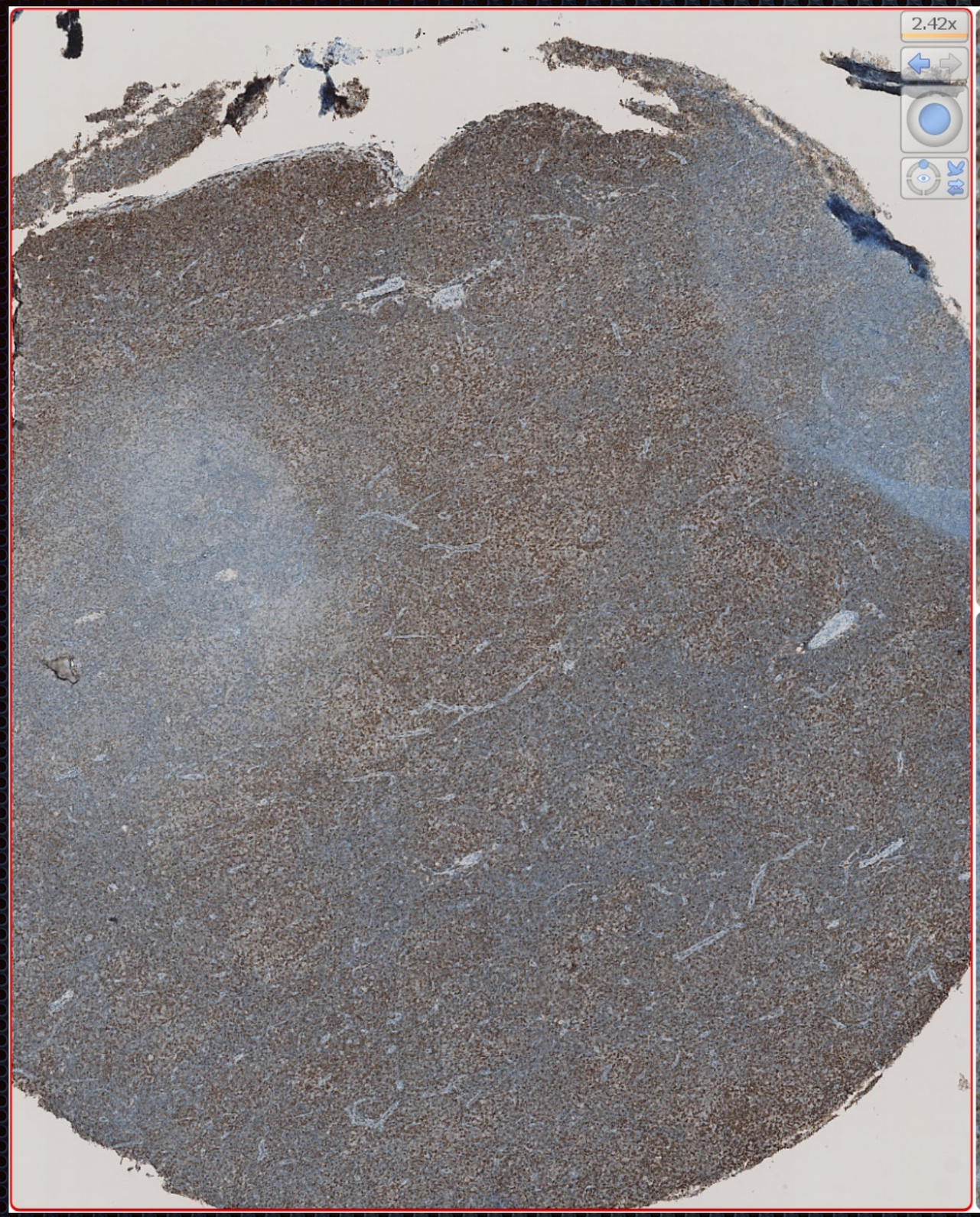
	Bond-III	**BenchMark Ultra**	**AS-48**
ER, rmAb SP1	1:50	1:100	1:75
Ki67, mAb MiB1	1:100	1:200	1:200
Bcl2, mAb 124	1:100	1:25	1:100
CD10, mAb 56C6	1:20	1:40	1:40
CK-PAN, mAb AE1AE3	1:75	1:150	1:100
p504s, rmAb 13H4	1:100	1:100	1:150
Melan A, mAb A103	1:50	1:20	1:50
900$ pr ml Ab:1 ul = 0.9$ 1$ = 6.5 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

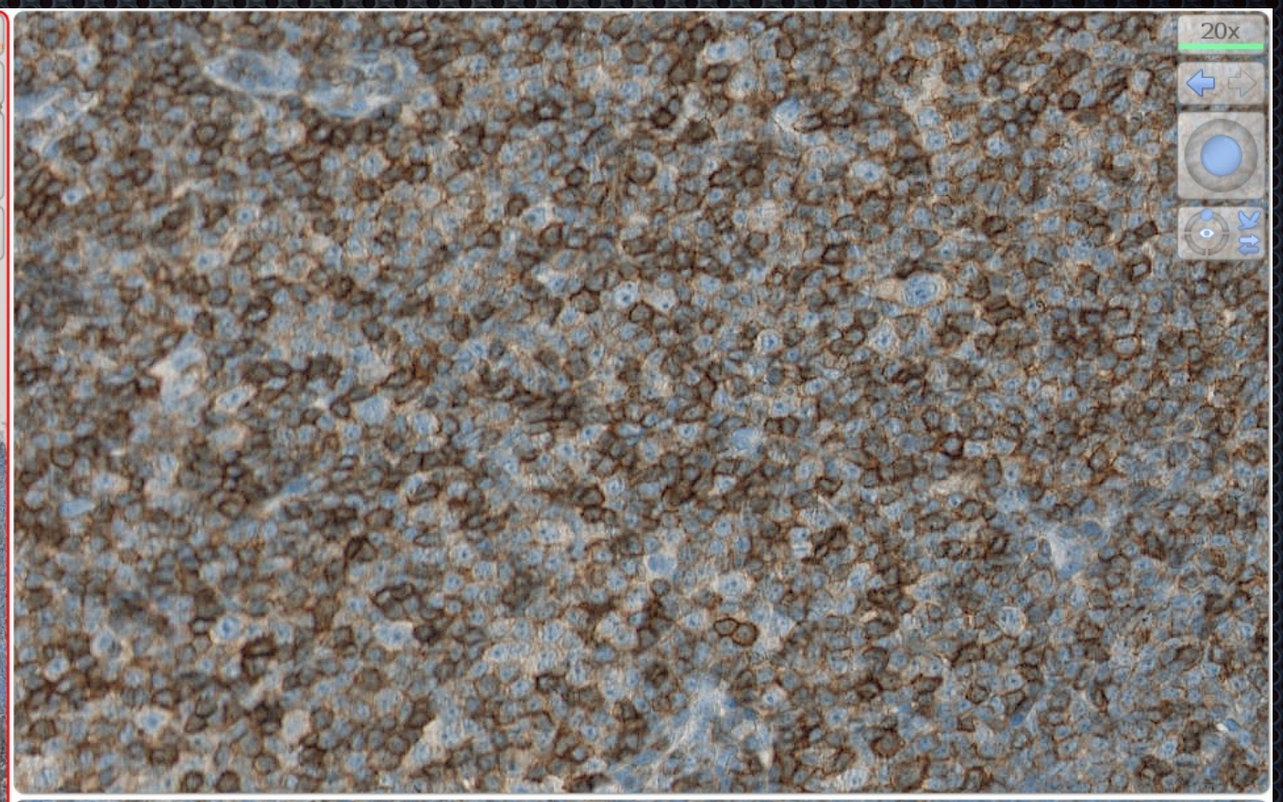
Data from Dept. of Pathology, Aalborg University Hospital

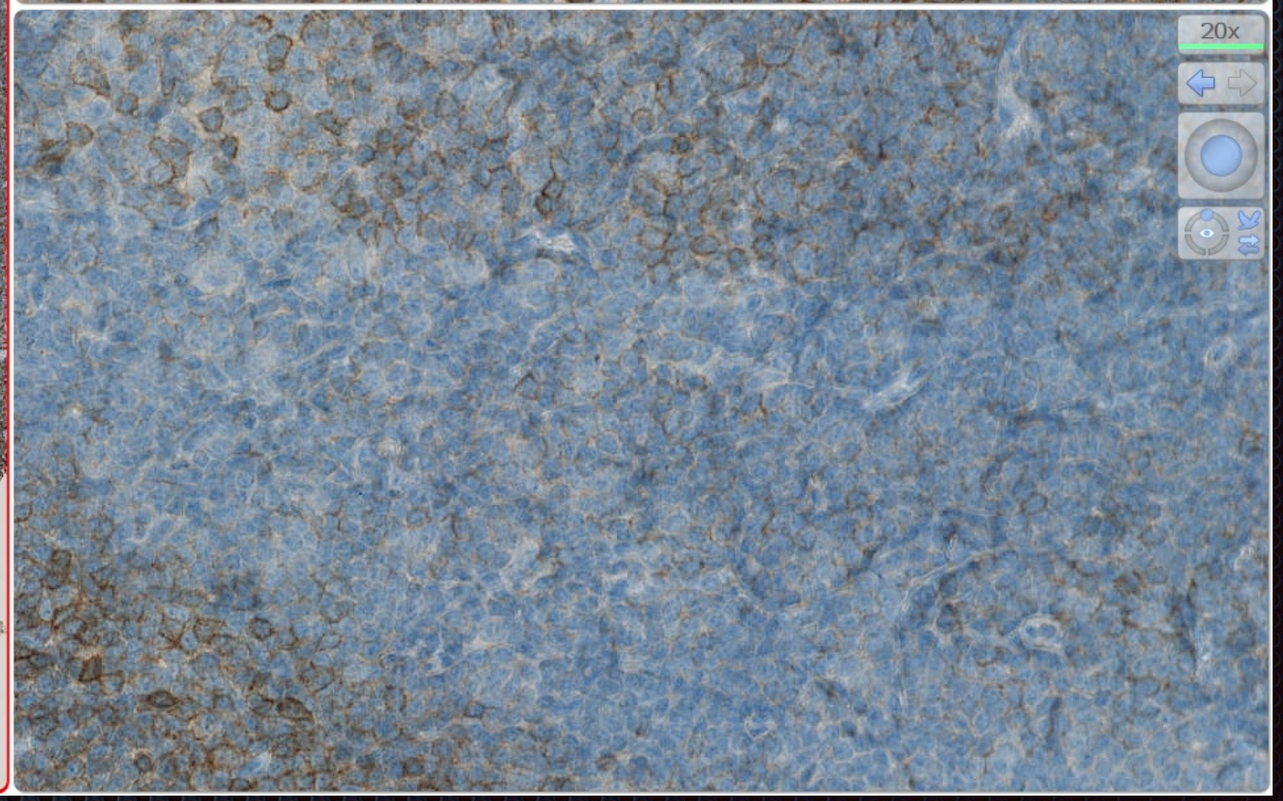
IHC – Immunohistochemical stainers

Staining issues; TechMate – Staining gradient, imprint pattern – air bubbles

IHC – Immunohistochemical stainers

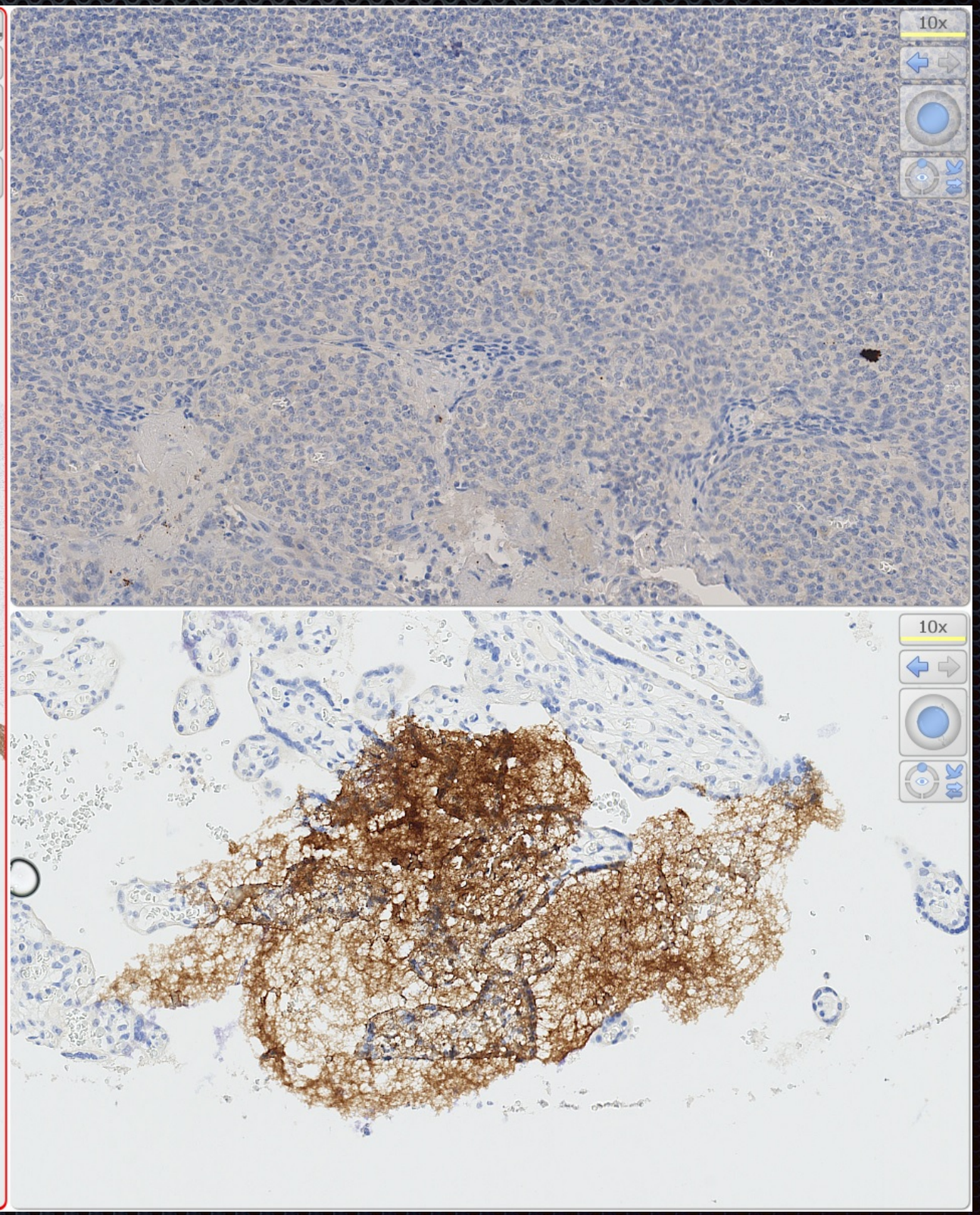






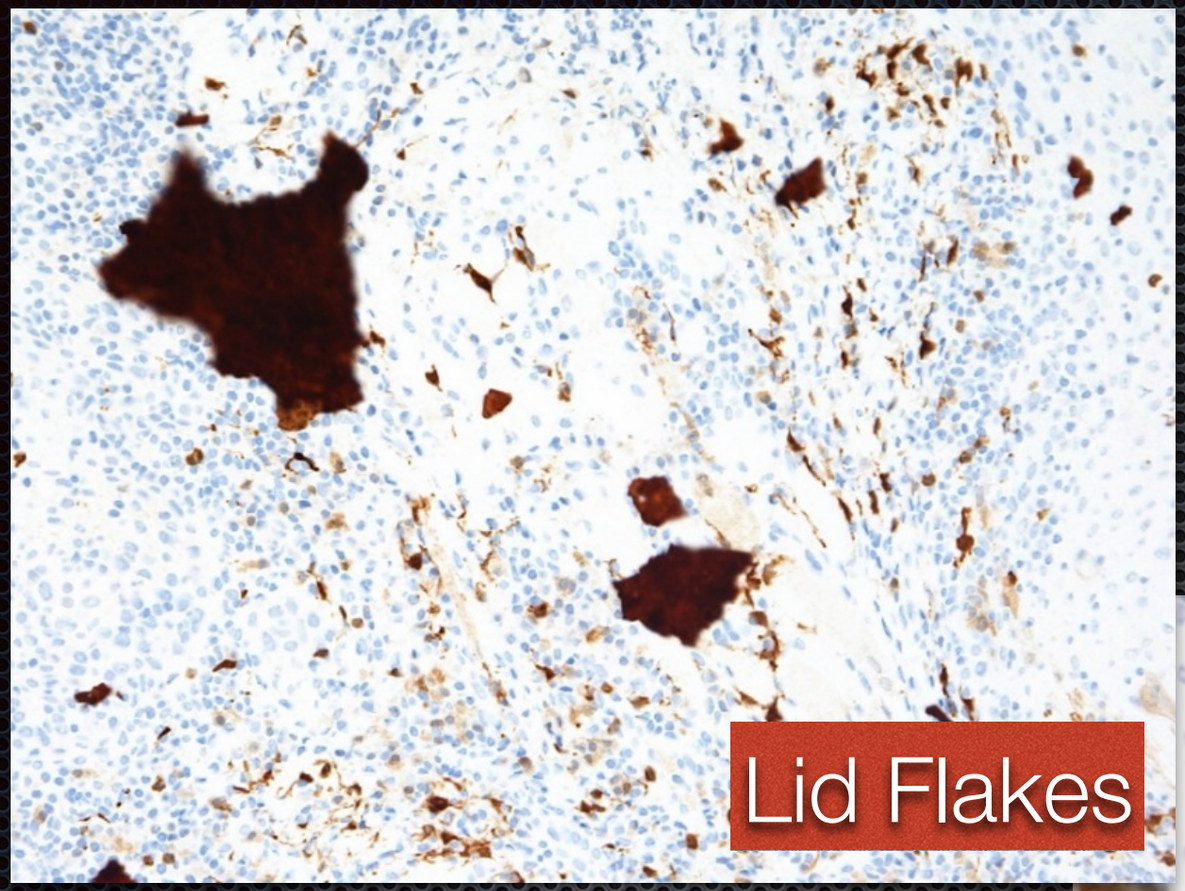
Staining issues; BenchMark, VMS – Uneven weak/neg areas – air bubbles

IHC – Immunohistochemical stainers

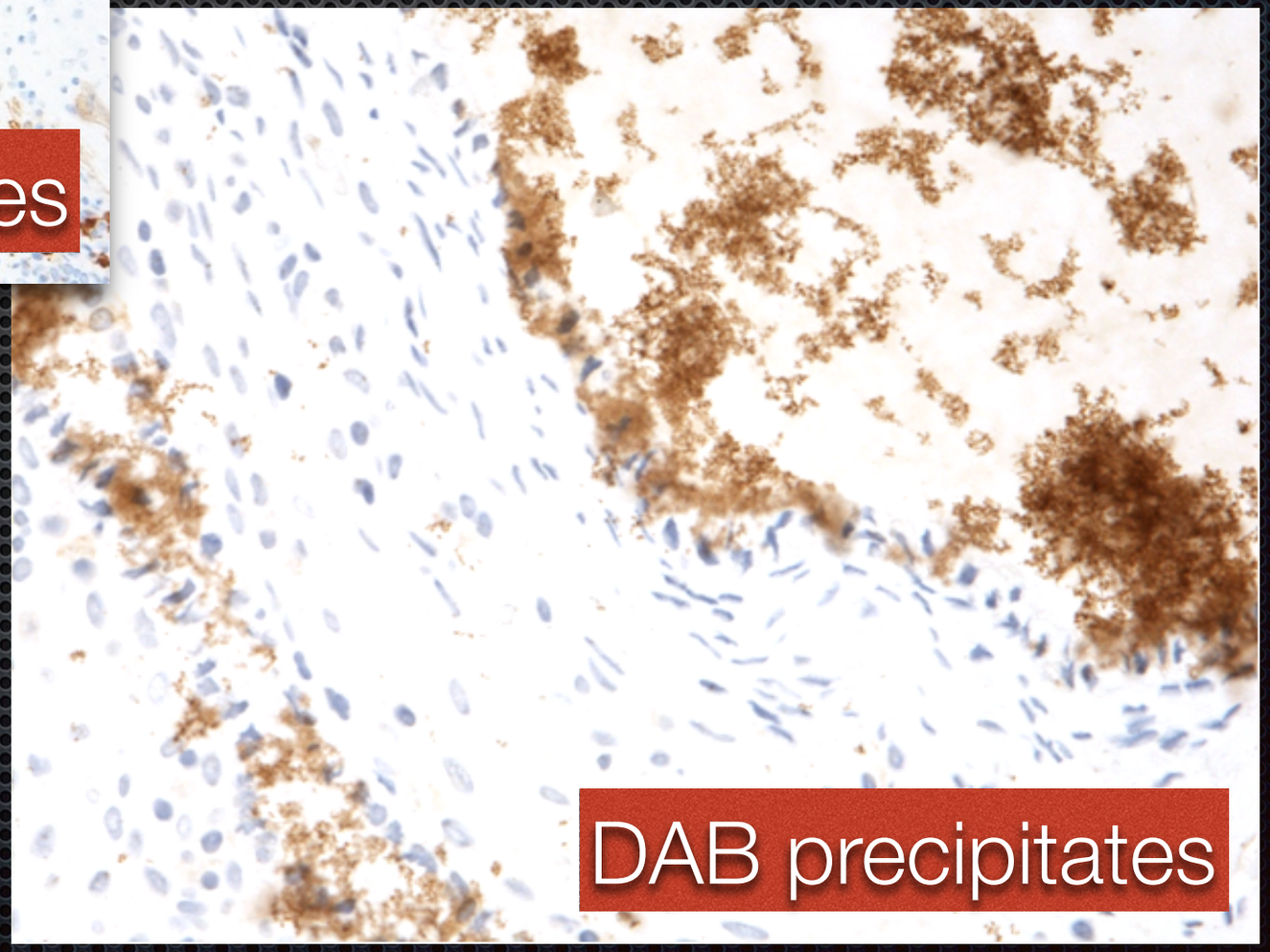


Staining issues; Bond, Leica – chromogen precipitates and general hue

IHC – Immunohistochemical stainers

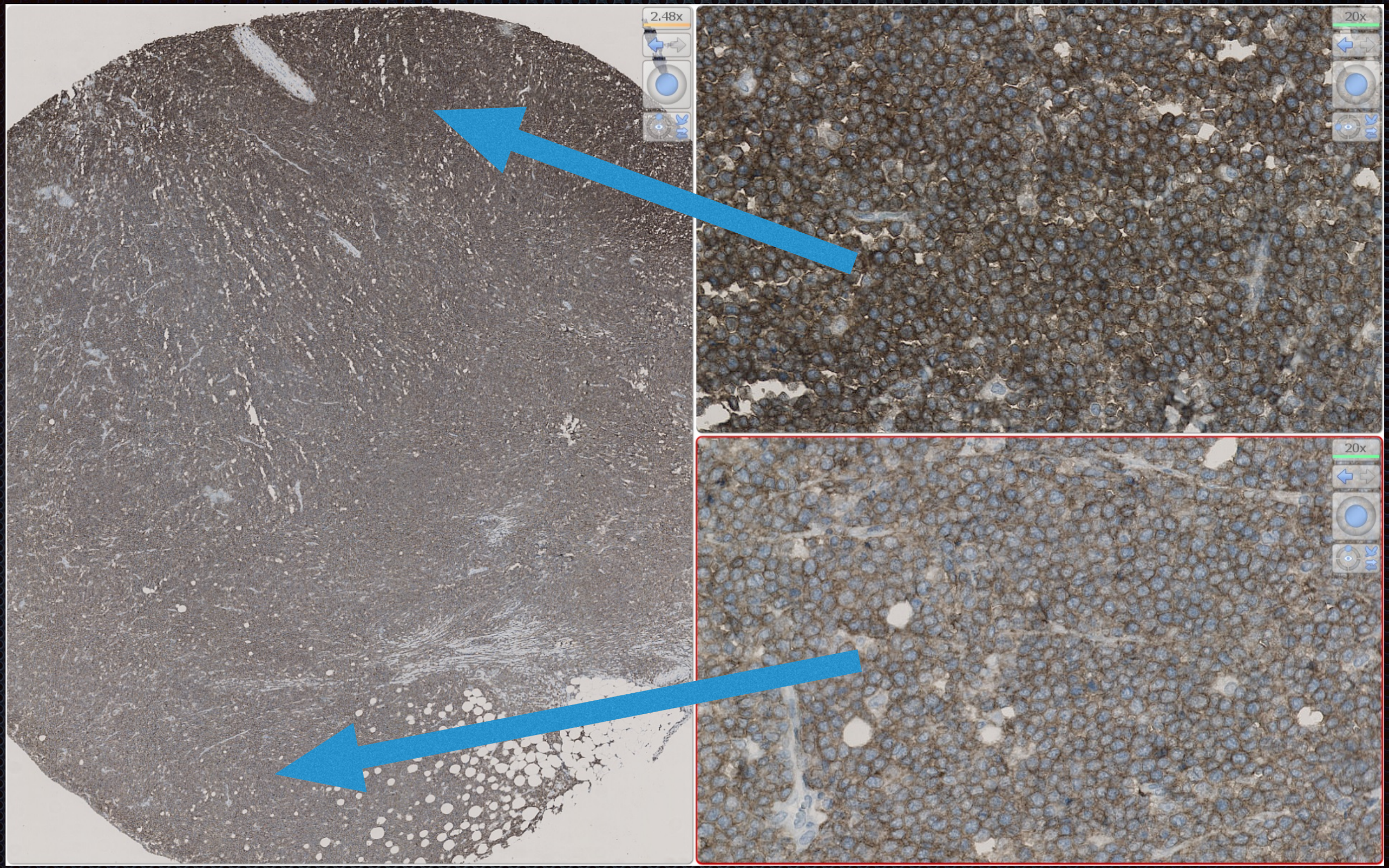


Courtesy by Michael Bzorek



Staining issues; Omnis, Dako – chromogen precipitates

IHC – Immunohistochemical stainers



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

IHC – Immunohistochemical stainers

PCK – slide no. 1

PCK – slide no. 2

Same reagents, same protocol, same block, same stainer

On-slide positive controls

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

IHC – Immunohistochemical stainers

REVIEW ARTICLE

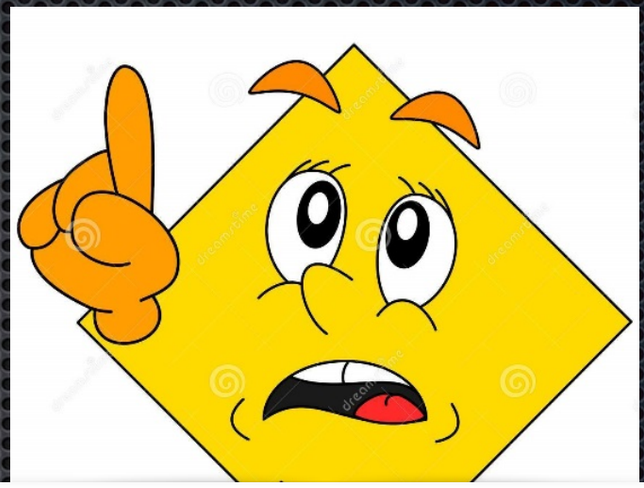
Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Emina E. Torlakovic, MD, PhD,† Søren Nielsen, HT, CT,‡§ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA),‖¶# John Garratt, RT,†** Blake Gilks, MD, FRCPC,† †† Jeffrey D. Goldsmith, MD,‡‡ Jason L. Hornick, MD, PhD,*§§ Elizabeth Hyjek, MD, PhD,* Merdol Ibrahim, PhD,‖‖ Keith Miller, FIBMS,‖‖ Eugen Petcu, MD, PhD,‖ Paul E. Swanson, MD,¶¶## Xiaoge Zhou, MD,***††† Clive R. Taylor, MD, PhD,‡‡‡ and Mogens Vyberg, MD‡§*

TABLE 3. (*continued*)

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



On-slide positive controls

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

IHC – Immunohistochemical stainers

RESEARCH ARTICLE

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†*

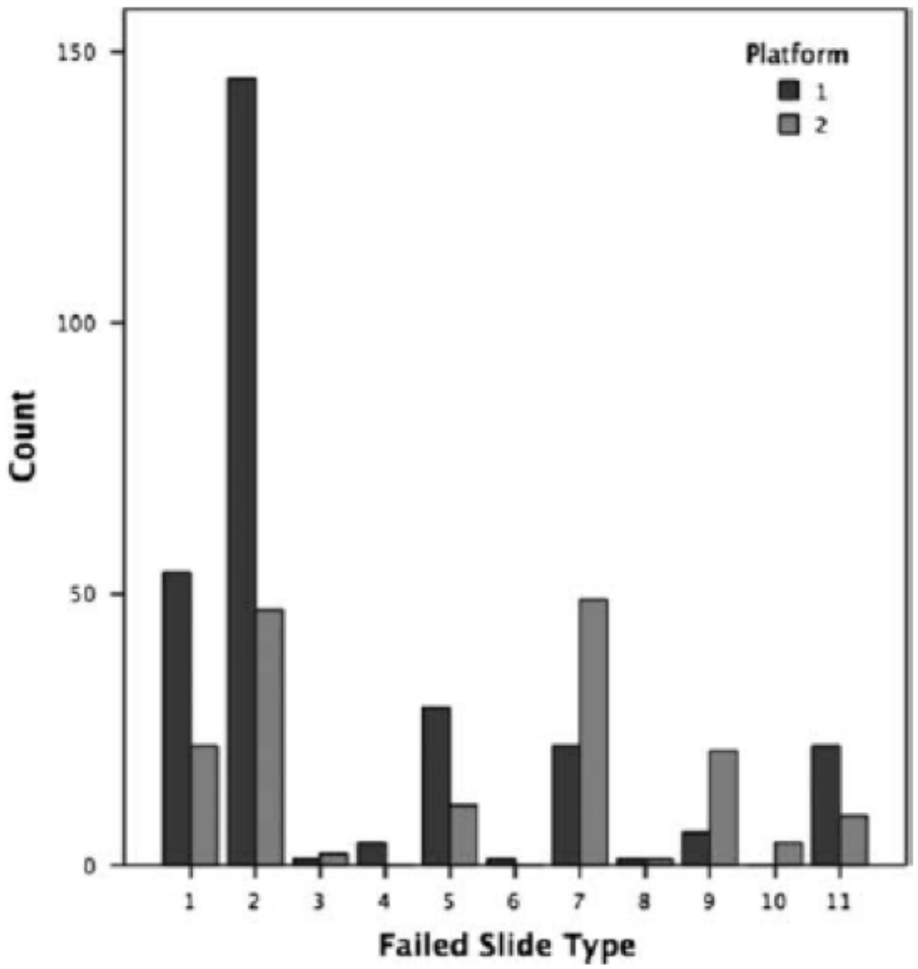


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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%)

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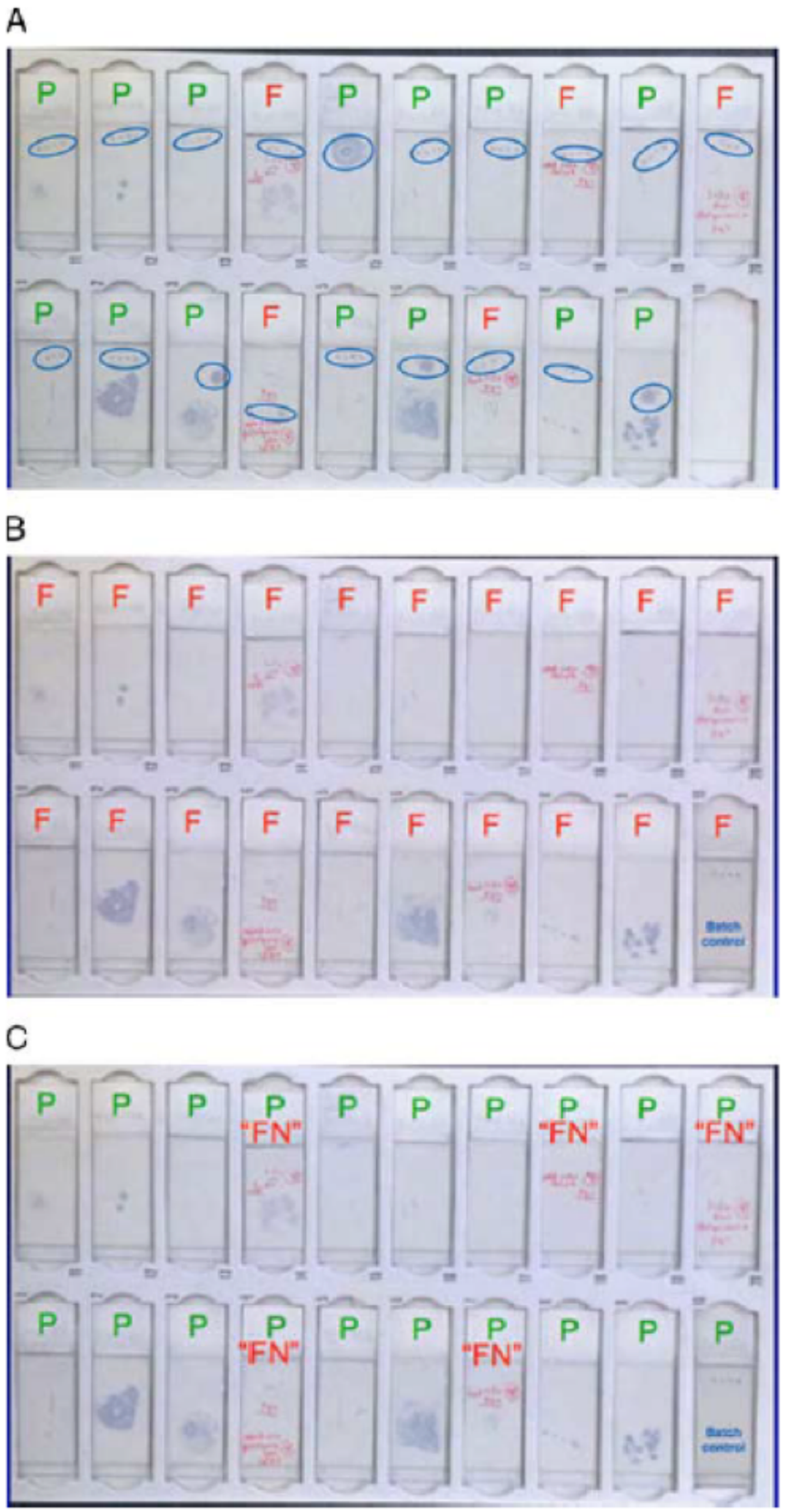


On-slide controls
IHC slides stained for ALK (Class II),
same run, same instrument, same protocol
14/19 passed
5/19 failed

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

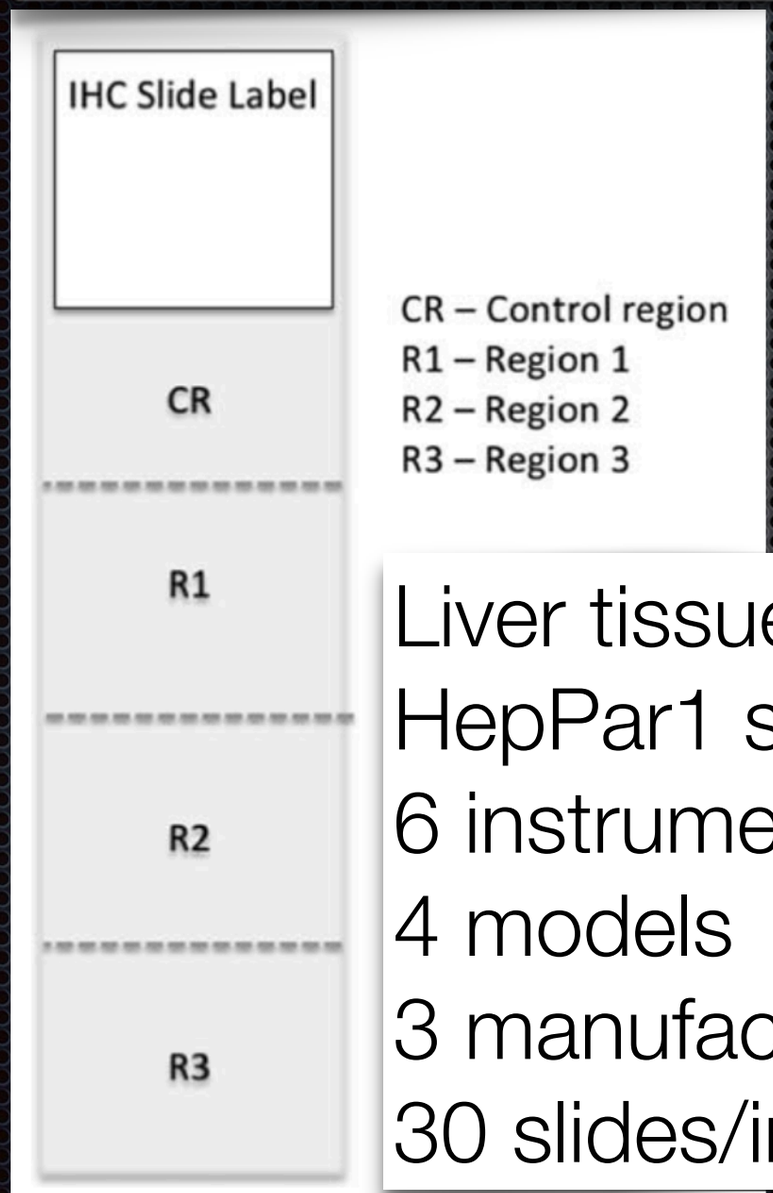
Batch-control - Theoretically:
Batch control pass by same conditions as above
19/19 passed
0/19 failed (the 5 failed slides not identified….)

IHC – Immunohistochemical stainers

Uneven Staining in Automated Immunohistochemistry: Cold and Hot Zones and Implications for Immunohistochemical Analysis of Biopsy Specimens

Carol C. Cheung, MD, PhD, JD,† Paul E. Swanson, MD,‡ Søren Nielsen, BMS,§ Mogens Vyberg, MD,§ and Emina E. Torlakovic,*

(*Appl Immunohistochem Mol Morphol* 2018;26:299–304)



Liver tissue
HepPar1 stain
6 instruments
4 models
3 manufacturers
30 slides/instrument

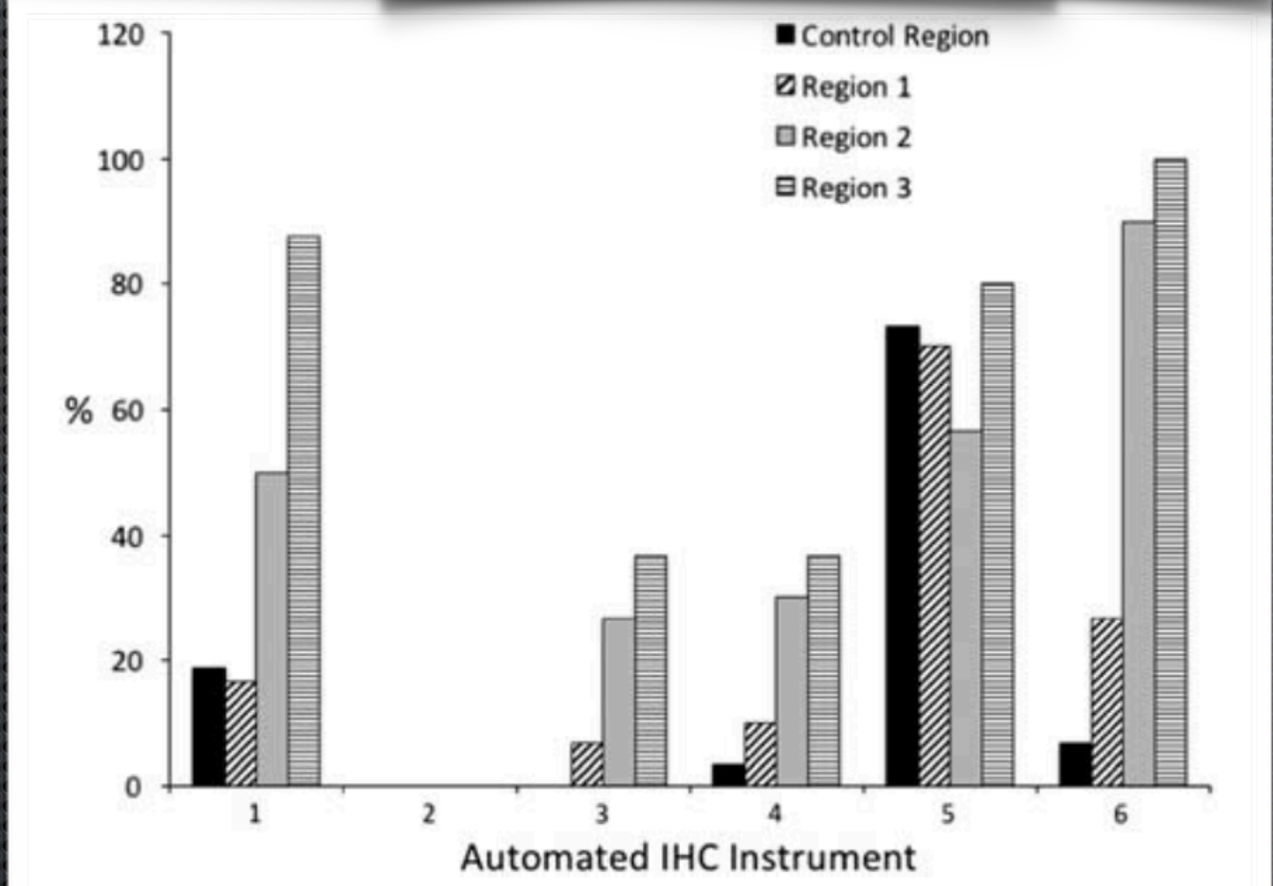


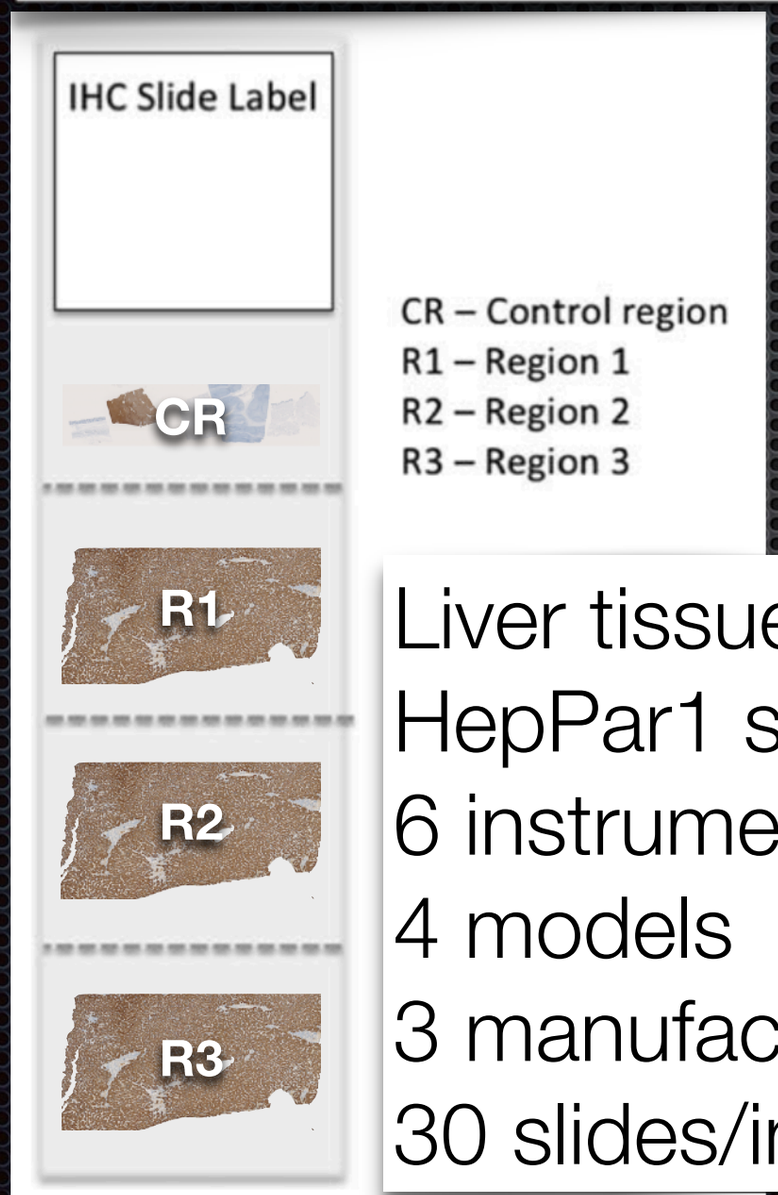
FIGURE 2. The percentage of slides with areas of decreased staining (cold zones) differed between regions of the same slide and between automated immunohistochemistry instruments. Differences were observed even between instruments of the same model/manufacturer (instruments 3, 4, and 5).

IHC – Immunohistochemical stainers

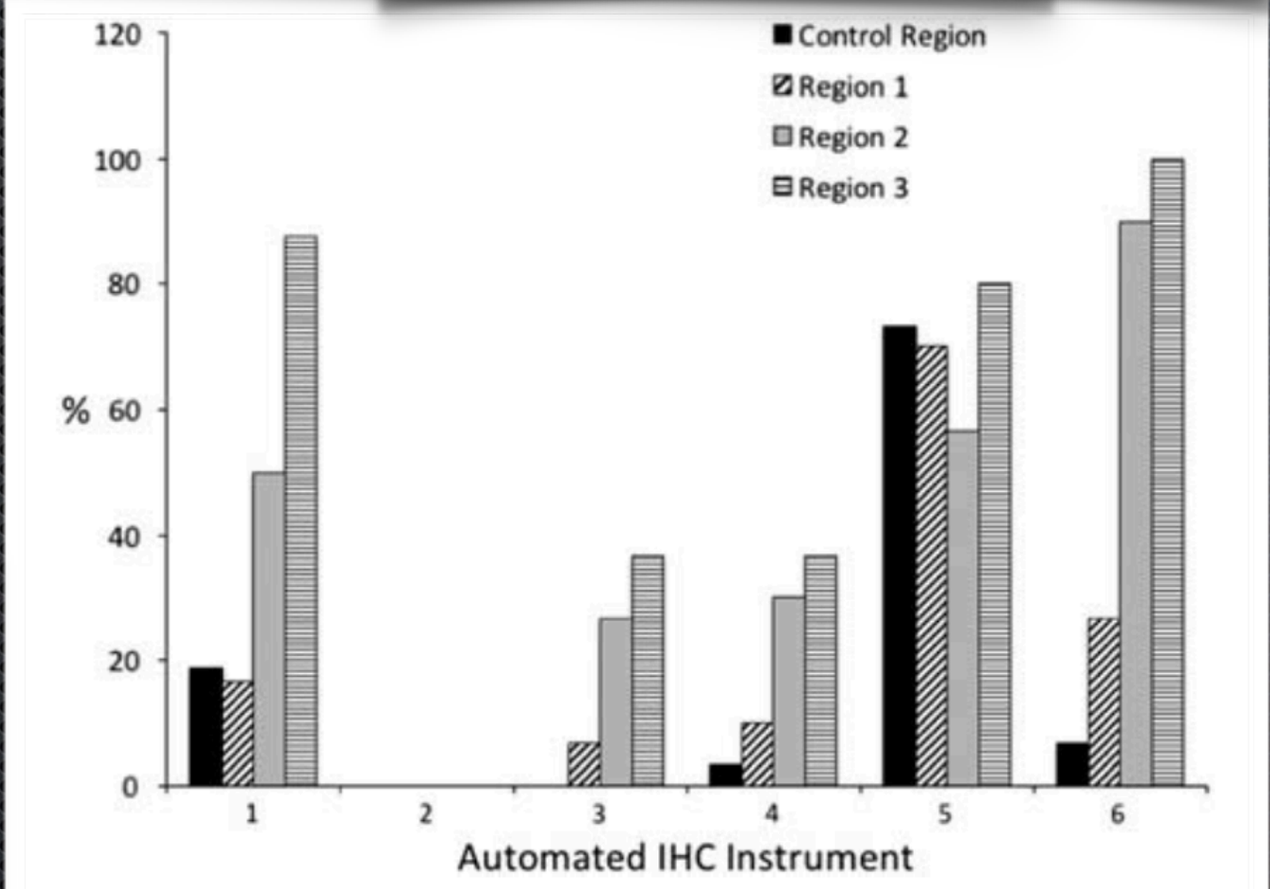
Uneven Staining in Automated Immunohistochemistry: Cold and Hot Zones and Implications for Immunohistochemical Analysis of Biopsy Specimens

Carol C. Cheung, MD, PhD, JD,† Paul E. Swanson, MD,‡ Søren Nielsen, BMS,§ Mogens Vyberg, MD,§ and Emina E. Torlakovic,*

(*Appl Immunohistochem Mol Morphol* 2018;26:299–304)



Liver tissue
HepPar1 stain
6 instruments
4 models
3 manufacturers
30 slides/instrument



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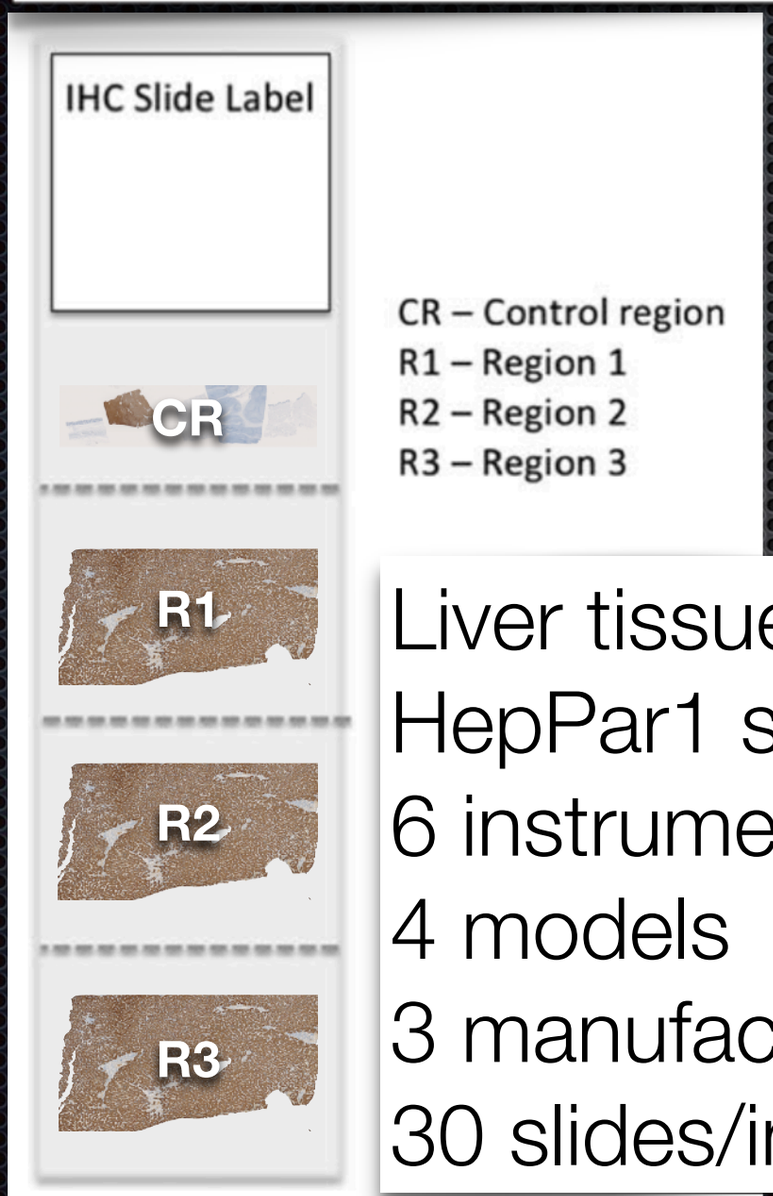

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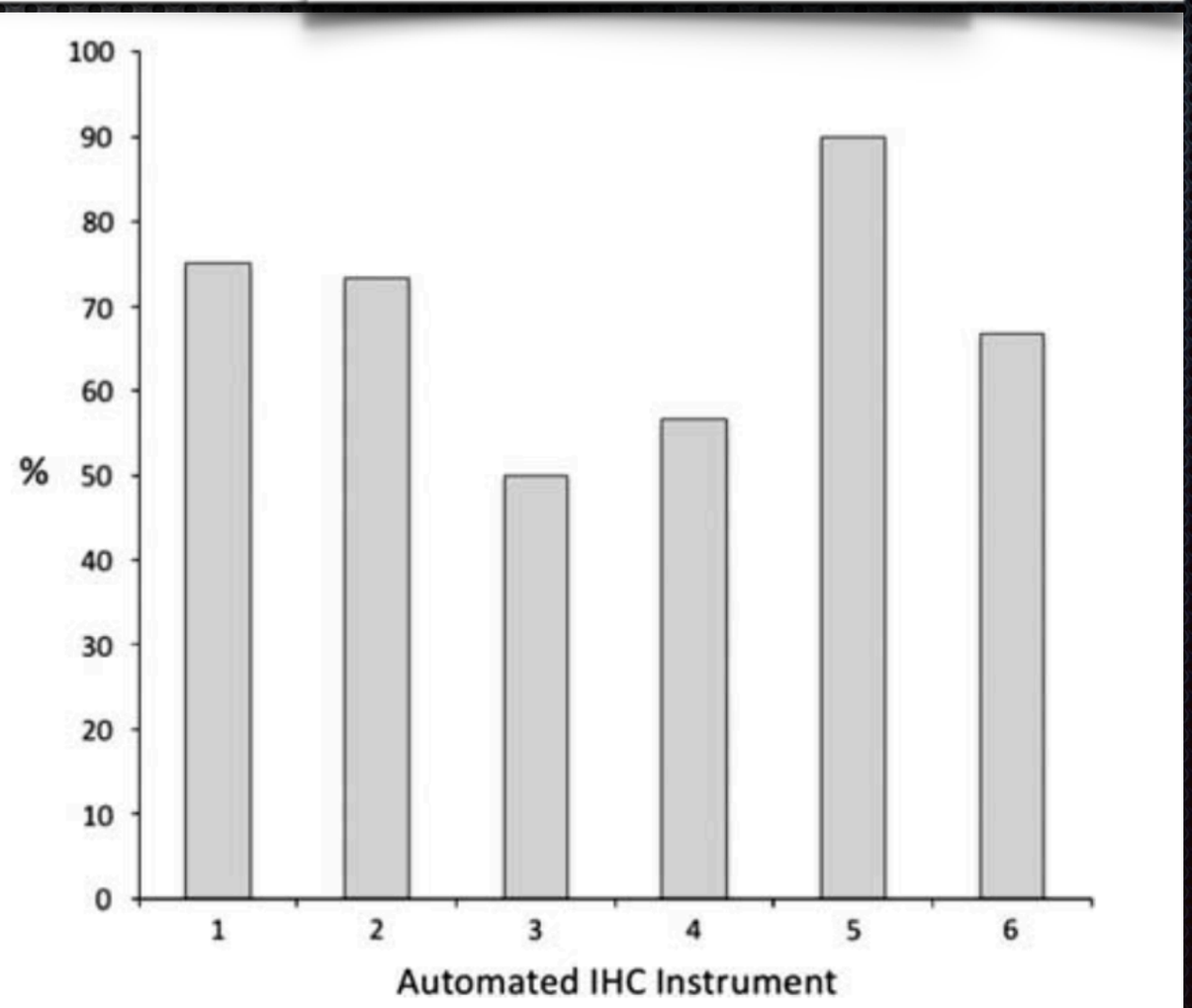

FIGURE 3. Patches/spots of increased staining (hot zones) were frequently observed on slides from all automated immunohistochemistry instruments.

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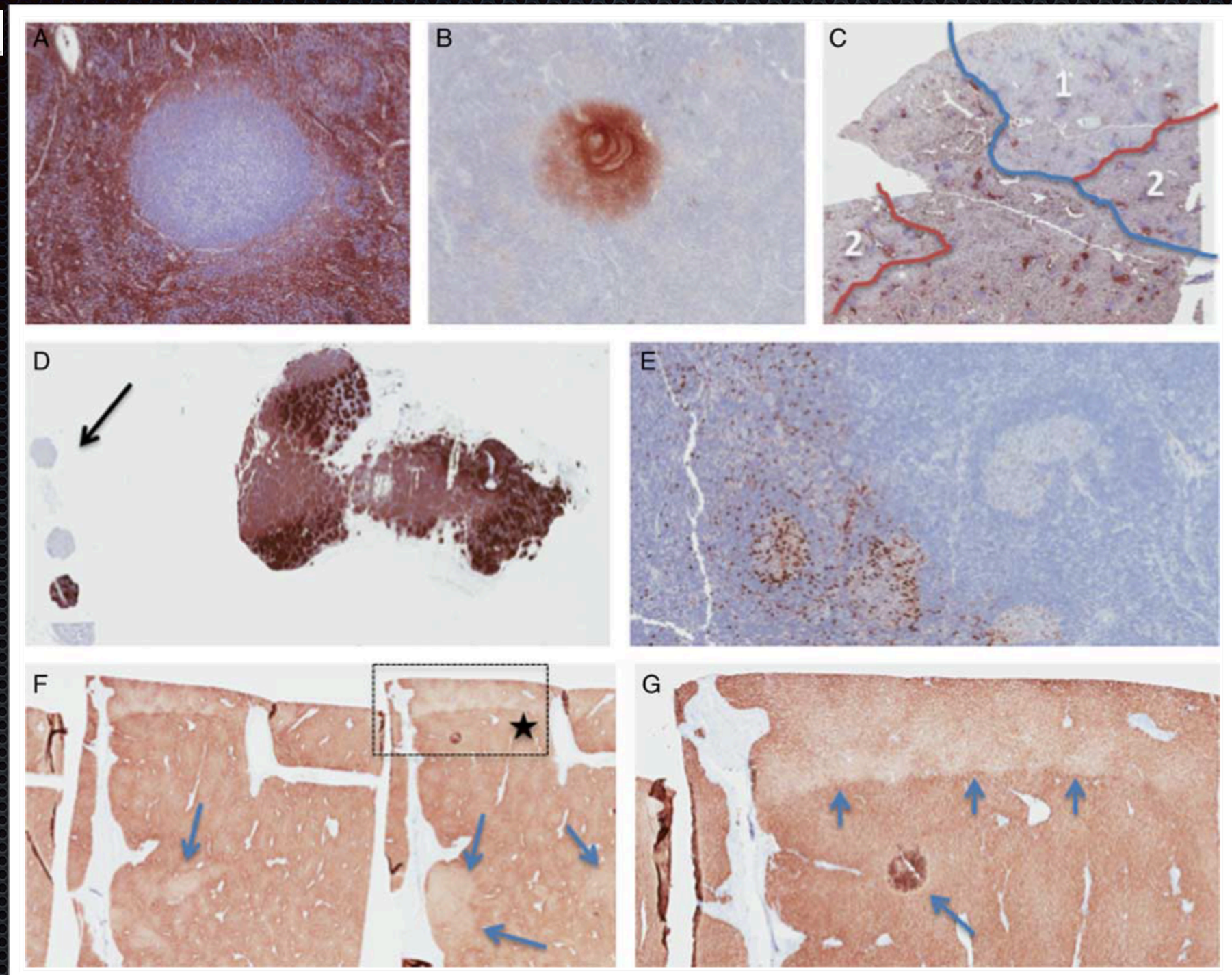

FIGURE 4. A, Small and medium sized cold zones are frequently circular. B, Hot zones are typically small and circular. C, Geographic areas with variable levels of decreased staining (1, moderate to severe; 2, mild). D, Despite the on-slide control (arrow) appearing to be "perfect," the entire patient test sample is affected by overstaining. E, Even when staining is significantly decreased, some evidence of a "positive" internal control can be detected, which may be misleading. F, Slide from the HepPar1 assay showing various patterns of uneven staining (arrows) including decreased lateral staining, small hot zones, as well as "vortex-like" decreased staining; areas that are central to the slide are most often most severely affected by "vortex-like" pattern (authors' observation; this parameter was not scored). G, Magnification of section ★ from image (F) showing that both increased and decreased staining can be present in the same slide (arrows).

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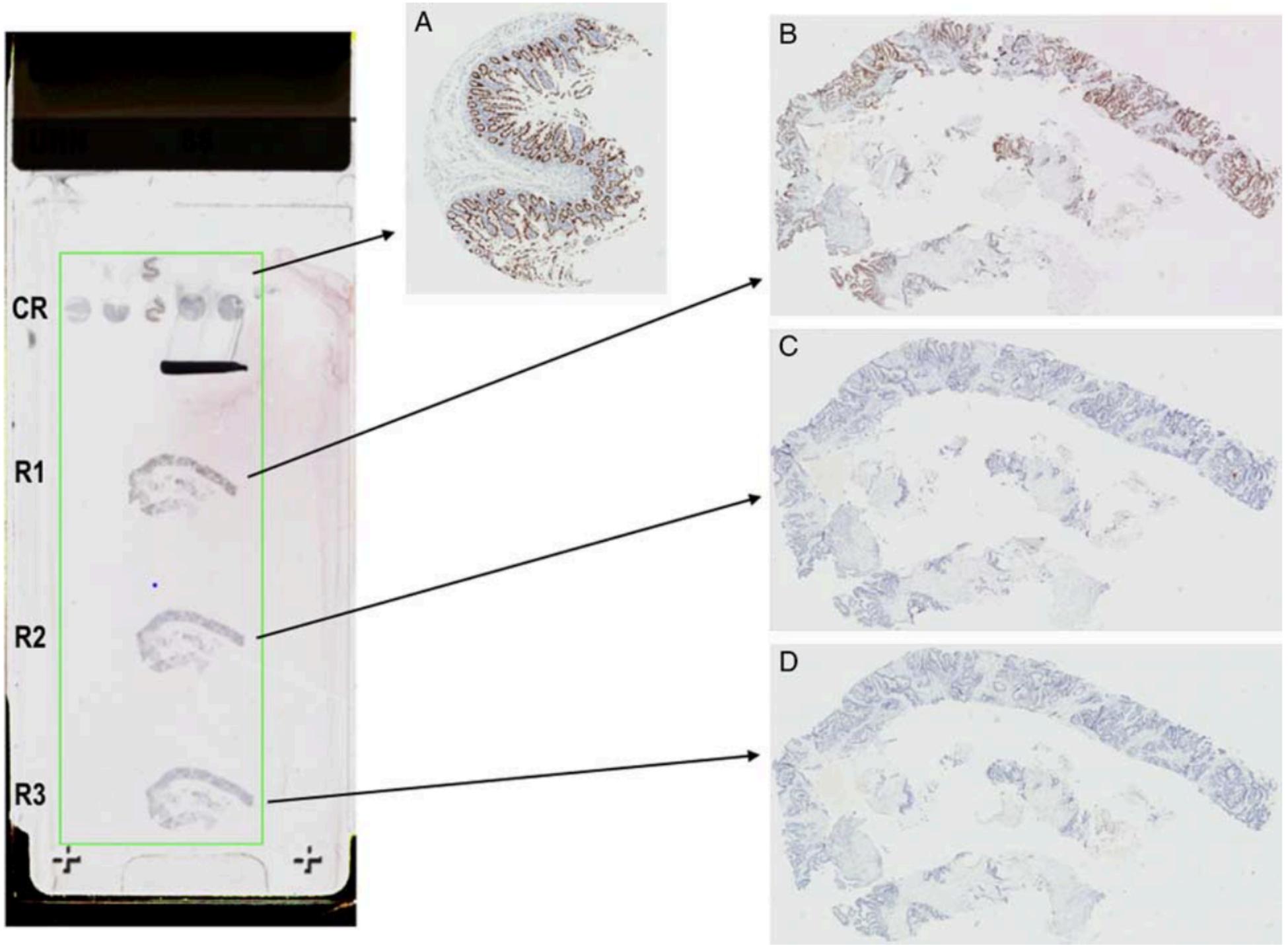

FIGURE 5. A slide stained for CDX2 with an on-slide control (OSC) in the control region (CR) and three serial sections of a needle core biopsy in region 1 (R1), region 2 (R2), region 3 (R3). A, Representative (high expressor) core from the OSC showing nuclear signal for CDX2 in colonic epithelium. B, Section of tumor in R1 showing nuclear positivity for CDX2. C, Serial section of tumor in R2 showing no signal for CDX2. C, Serial section of tumor in R3 showing no signal for CDX2.

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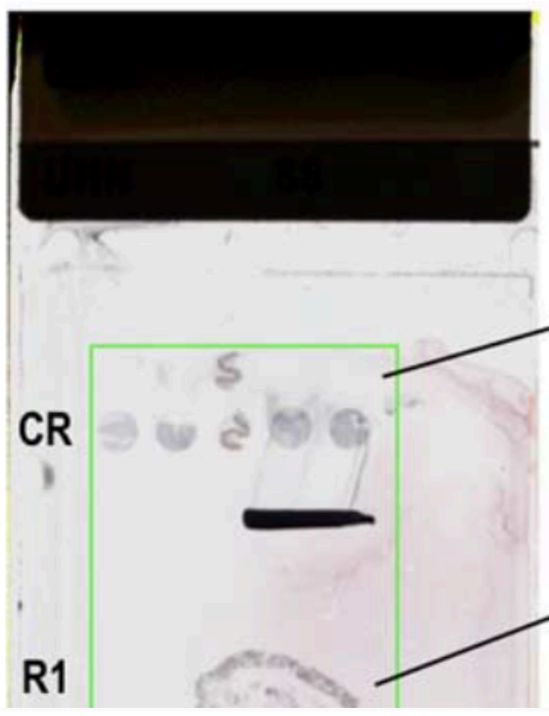

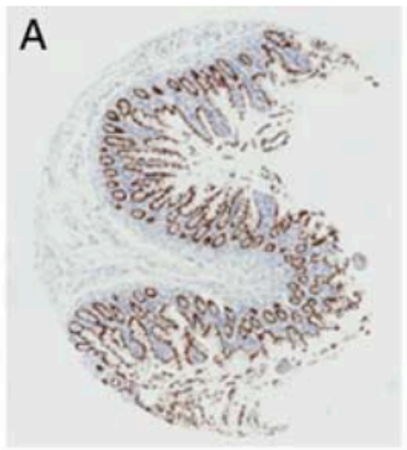

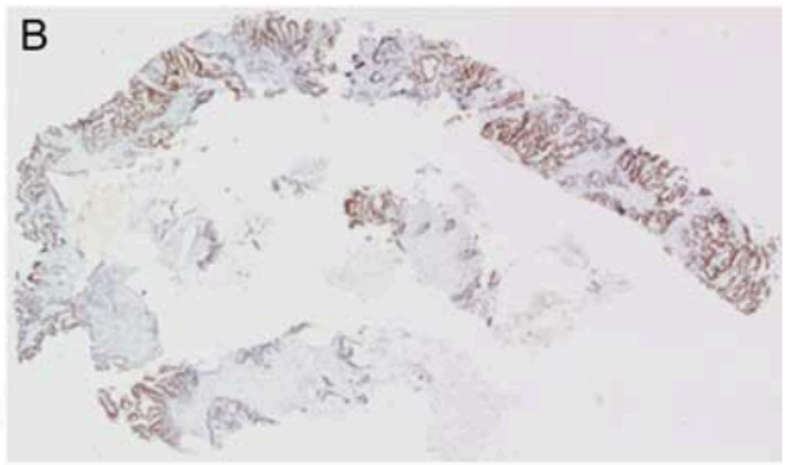

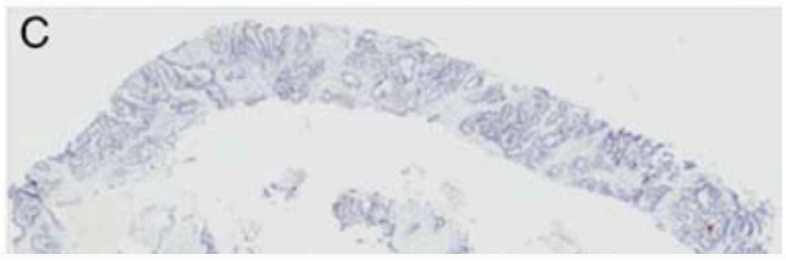

*"**Results:** Only 8% of slides showed completely uniform staining. Uneven staining (UES), including areas of both increased and decreased staining, occurred with all instruments. Decreased staining was often zonal, involving large regions of the slide. Decreased staining mostly localized in an instrument-dependent manner. Increased staining tended to occur in small foci with a random distribution. "*

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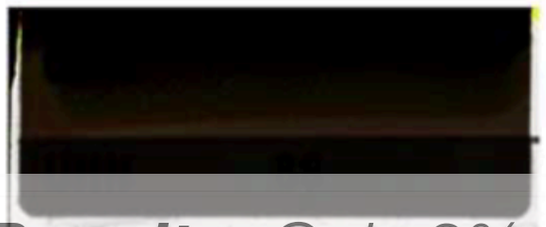

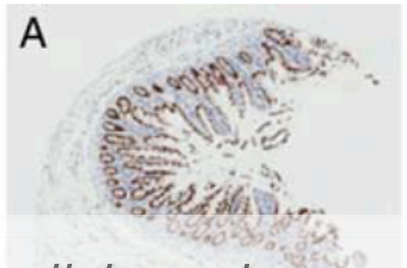

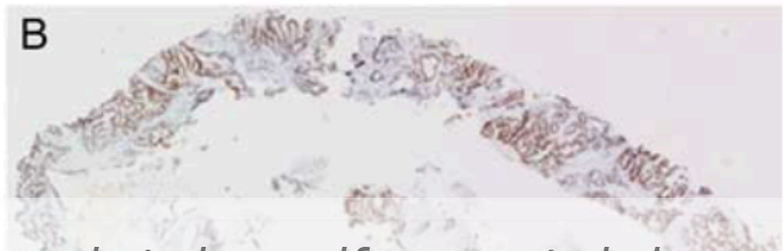

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*"**Conclusions:** The common occurrence of UES (particularly decreased staining) has important implications for the reliable read-out of IHC assays on biopsy samples. **Baseline and periodic quality assurance testing for UES is recommended for all automated IHC instruments."***

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Conclusions:

- Automation in IHC is needed primarily to secure consistency of inter- and intralaboratory results and to reduce hands-on.
- There is no perfect system ☹ 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.
- Test for uneven staining* before choosing system.
- 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….

**Appl Immunohistochem Mol Morphol 2018;26:299–304)*