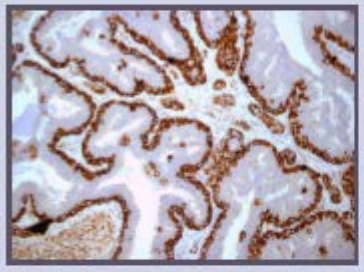
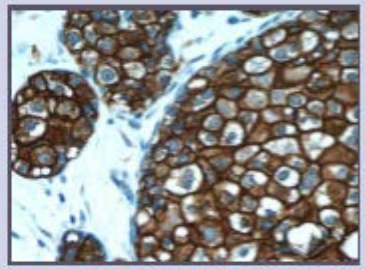




NordiQC

TATA MEDICAL CENTER

ISIMm
International Society for IHC and Molecular Morphology

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

Immunohistochemical principles
The technical test approach

Analytical parametres II

Søren Nielsen
Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

IHC – The Technical Test Approach

CME - DAY 1 Thursday - 04.01.2018	
09:00 – 13:00	***Morning session – the IHC and molecular laboratory. Moderator: CliveTaylor***
09:00 – 09:30	Søren Nielsen: Pre-analytical IHC parameters
09:35 – 10:10	Søren Nielsen: Analytical IHC parameters I (clone selection, protocol optimization, automation)
10:15 – 10:45	*Coffee break*
10:45 – 11:30	Mogens Vyberg: The impact of proficiency testing on lab immunoassays
11:35 – 12:10	Søren Nielsen: Analytical IHC parameters II (control selection)
12:15 – 12:55	T. S. Sridhar : Molecular studies on FFPE tissue
13:00 – 14.30	*Lunch break*
14:30 – 17:00	***Afternoon interactive Parallel IHC Session***
	IHC session, technicians | *IHC session, pathologists*
14:45 – 15:45	Søren Nielsen: Technical pitfalls, trouble shooting, internal quality control - **for technicians** Taylor, Bhargava, Vyberg: Diagnostic pitfalls, trouble shooting - **for pathologists**
15:45 – 16:15	*Coffee break*
16:15– 17:15	Søren Nielsen: Technical pitfalls, trouble shooting, internal quality control (cont'd) - **for technicians** Taylor, Bhargava, Vyberg:Diagnostic pitfalls, trouble shooting (cont'd) - **for pathologists**

Daily QC and IHC stainers

IHC – The Technical Test Approach

Issues to be adressed for IHC assay implementation:

1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
2. ”Evaluation of the robustness of the IHC assay – impact on pre-analytics
3. Evaluation of the analytical sensitivity/specificity
4. Identification of most robust controls providing information that the established level of detection is obtained in each test performed in daily practice.

<u>Tissue controls are key element</u>

IHC – The Technical Test Approach

External tissue control tool-box:

Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical assay performance controls	Maps Ab reaction pattern	Fixation time Fixative(s) Decalcification	Range of relevant expression levels	Range of relevant expression levels	Reproducibility
					Method of transfer proof
High expression Low expression No expression	With expression No expression		With expression No expression	High expression Low expression No expression	
			20/40 of each Type I/II IHC	+ relevant cut-off	

IHC – The Technical Test Approach

- The NordiQC focus areas
  - Central protocol elements for an optimal staining
    - Antibody selected
    - Antibody dilution range / Ready-To-Use
    - Epitope retrieval
    - IHC detection system & stainer platforms
  - Recommendable control and identification of critical quality stain indicators / iCAPCs
    (Which tissue ? Which cells ?, How must they look ?)

IHC – The Technical Test Approach

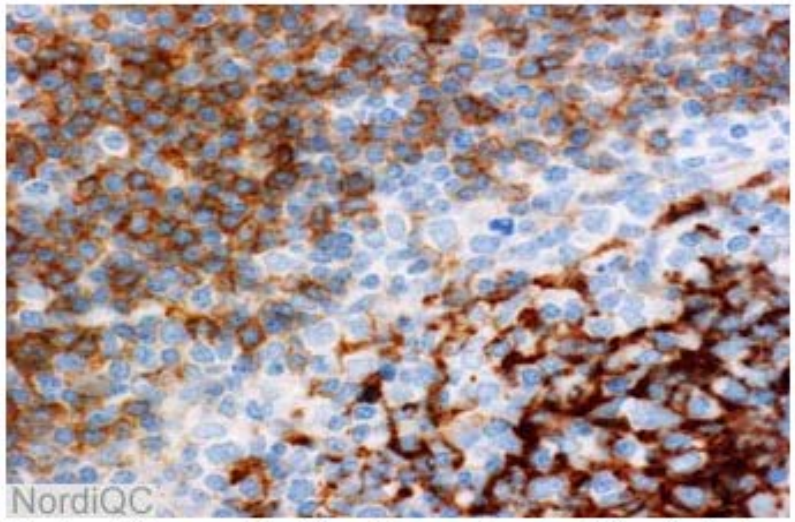


Fig. 2a. High magnification of the optimal staining in Fig 1a of the secondary follicle in the tonsil. The activated B-cells show a distinct continuous membranous reaction.

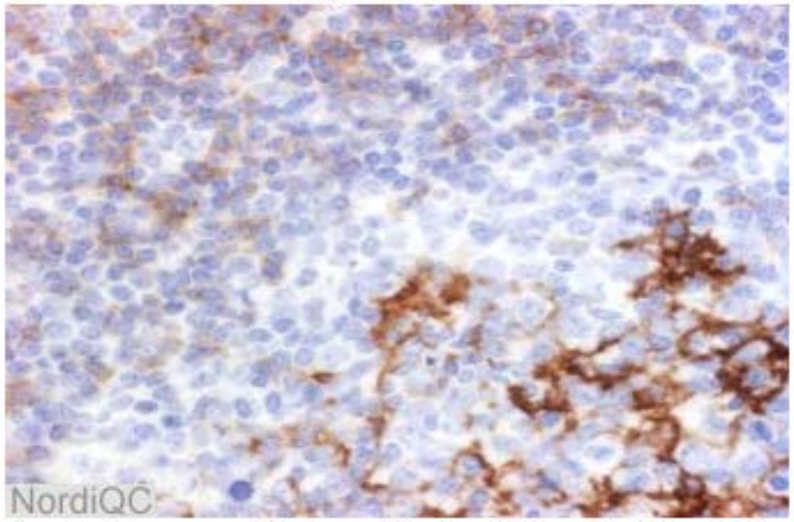


Fig. 2b. High magnification of the insufficient staining in Fig 1b of the secondary follicle in the tonsil (same field as in Fig 2a). The activated B-cells only show a weak imprecise reaction.

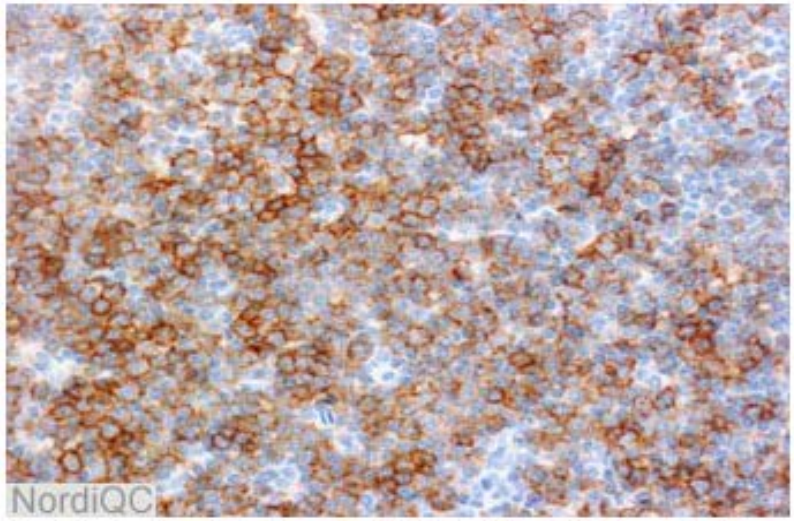


Fig. 3a. Optimal staining for CD23 of the B-CLL no 4 using same protocol as in Fig 1b and 2 b. The majority of the neoplastic cells show a strong and distinct membranous staining.

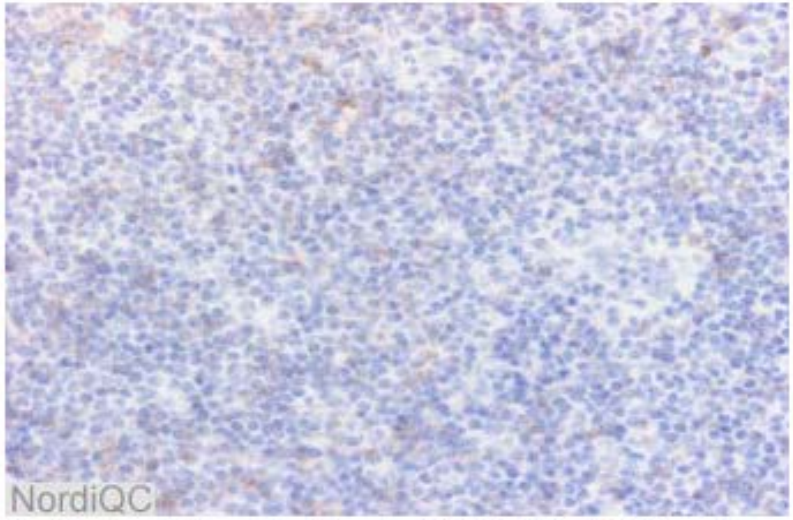


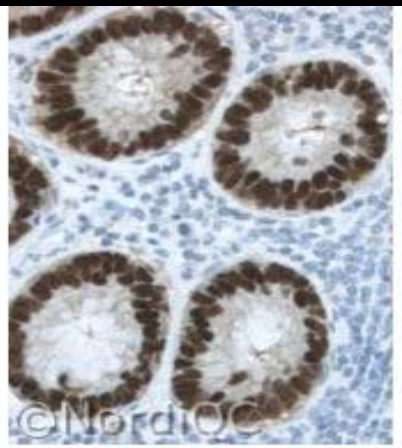
Fig. 3b. Insufficient staining for CD23 of the B-CLL no 4 using same protocol as in Fig 1b and 2 b. The neoplastic cells are virtually negative.

CD23

iCAPCs:

Activated B-cells in mantle z.

IHC – The Technical Test Approach



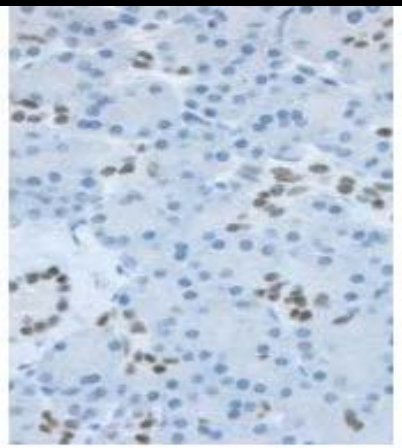


Fig. 1a. Optimal staining for CDX2 using the mAb clone CDX2-88.
Left, colon: A strong nuclear staining is seen in all the enterocytes with a minimal cytoplasmic reaction.
Right, pancreas: A weak to moderate staining is seen in the majority of the ductal epithelial cells.

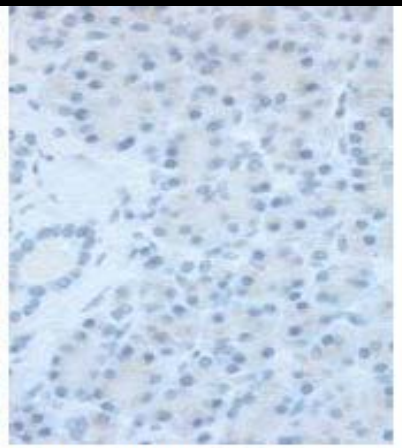
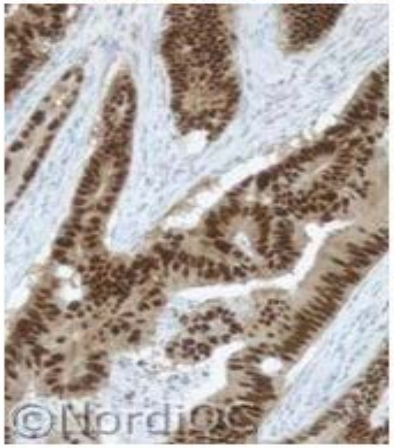


Fig. 1b. Staining for CDX2 using the mAb clone CDX2-88 with an insufficient protocol.
Left, colon: A moderate to strong nuclear staining is seen in all the enterocytes.
Right, pancreas: No nuclear staining is seen in the ductal epithelial cells. Also compare with Fig 2b – same protocol.



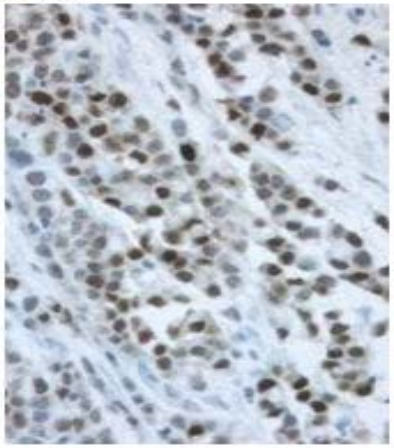
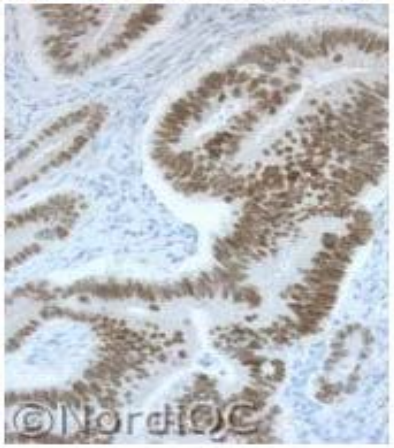


Fig. 2a. Optimal staining for CDX2 using same protocol as in Fig. 1a.
Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show an intense staining while the cytoplasmic compartment is weakly stained.
Right: Colon adenocarcinoma with low expression of CDX2: The majority of the neoplastic cells show a moderate to strong nuclear reaction.



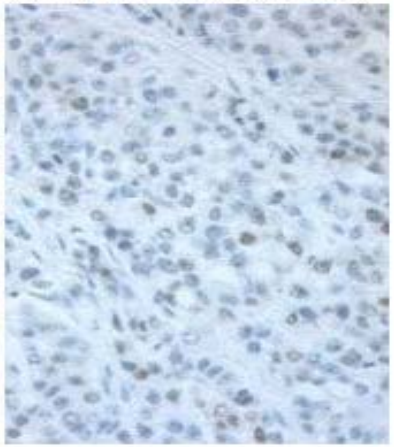


Fig. 2b. Insufficient staining for CDX-2 using same protocol as in Fig. 1b.
Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show a moderate staining, while the cytoplasmic compartment is almost negative.
Right: Colon adenocarcinoma with low expression of CDX2: Only scattered neoplastic cells show a weak nuclear reaction.

CDX2

iCAPCs:

Pancreatic duct ep. cells

IHC – The Technical Test Approach

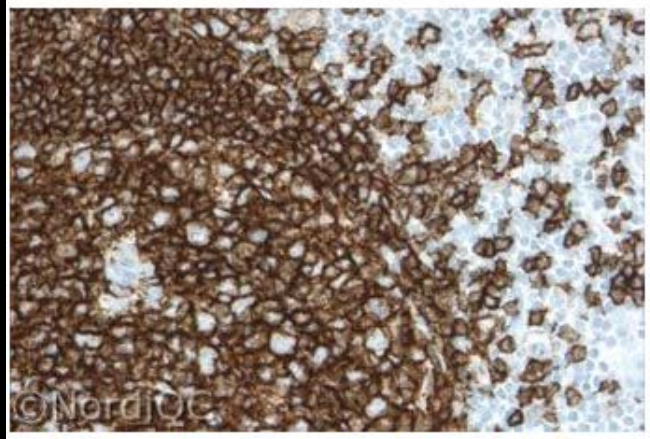


Fig. 1a. Lymphatic tissue in the appendix showing an optimal staining reaction for CD20 using the mAb clone L26 in a RTU format on the BenchMark platform. HIER was performed using Cell Conditioning 1. A very strong membranous staining reaction is seen in virtually all the B-cells.

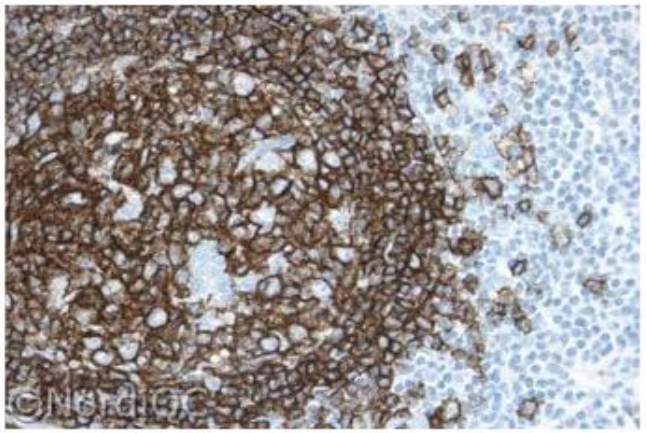


Fig. 1b. Lymphatic tissue in the appendix. Same field as in Fig. 1a. Insufficient staining for CD20 using the mAb clone L26 in a RTU format at the BenchMark platform. No HIER was performed. A moderate to strong staining reaction is seen in virtually all the B-cells. The normal B-cells are high expressors of CD20, hence the relatively strong reaction. Even so, the staining intensity should be improved in order to detect low expressors of CD20 (e.g. B-CLL in Fig. 2a and 2b).

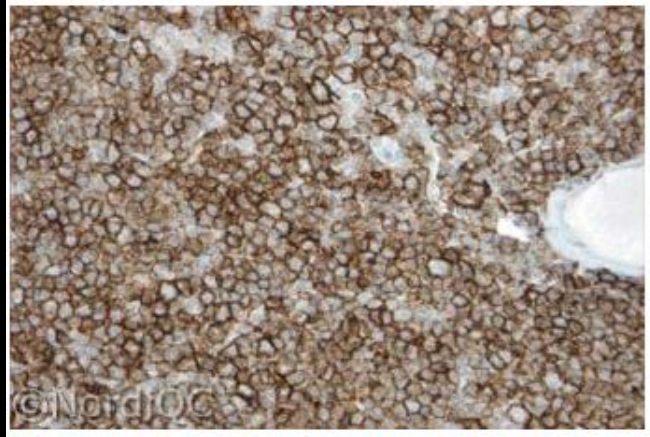


Fig. 2a. B-CLL. Optimal staining reaction for CD20. Same protocol as in Fig. 1a. A moderate to strong membranous staining is seen in virtually all the neoplastic cells.

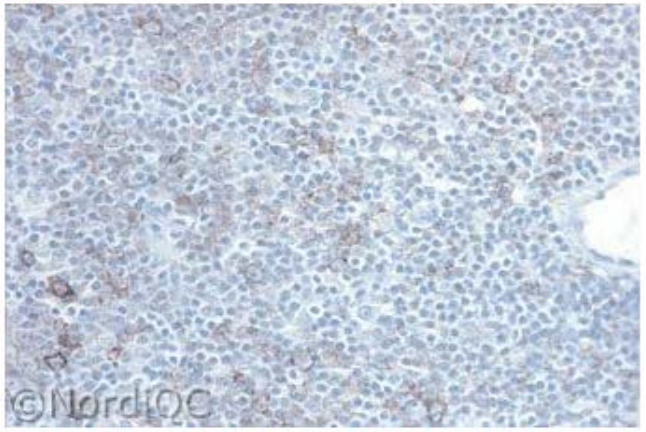


Fig. 2b. B-CLL. Insufficient staining for CD20 using the same protocol as in Fig. 1b. Omitting HIER, only scattered cells are positive. The majority of the neoplastic cells are negative. Compare with the optimal result in Fig. 2a, same field.

CD20:

iCAPCs:

????

ASAP….

As strong as possible…

IHC – The Technical Test Approach

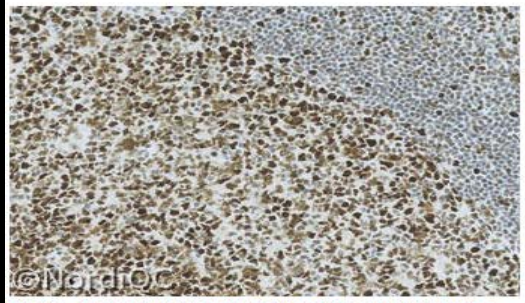


Fig. 1a. Optimal staining for MSH6 of the tonsil using the rmAb clone EP49 optimally calibrated, HIER in an alkaline buffer and a 3-step polymer based detection system.
Virtually all the mantle zone B-cells show a distinct, moderate to strong nuclear staining, while the germinal centre B-cells show a strong nuclear staining.

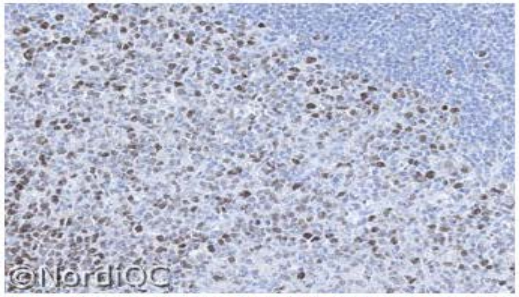


Fig. 1b. Insufficient staining for MSH6 of the tonsil using the mAb clone 44. by a protocol with a too low sensitivity (2-step polymer and too low. conc. of the primary Ab), same field as in Fig. 1a.
Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing limited MSH6 are virtually unstained.
Also compare with Figs. 2b. & 3b., same protocol.

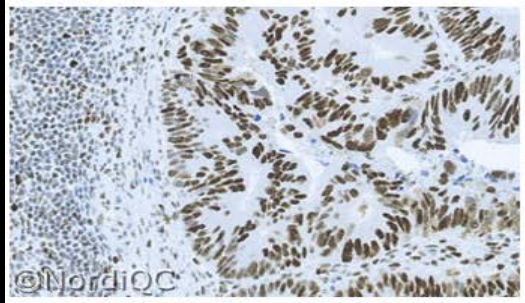


Fig. 2a. Optimal staining for MSH6 of the colon adenocarcinoma no. 3 with intact MSH6 protein using same protocol as in Fig. 1a.
The majority of the epithelial and the stromal cells show a moderate to strong nuclear staining. No background staining is seen.

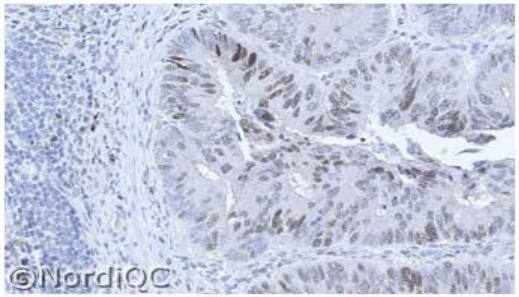


Fig. 2b. Insufficient staining for MSH6 of the colon adenocarcinoma no. 3 with intact MSH6 protein using same protocol as in Fig. 1b., same field as in Fig. 2a.
The proportion of positive cells and the intensity of the staining reaction is significantly reduced compared to the result in Fig. 2a.
Also compare with Fig. 3b., same protocol.

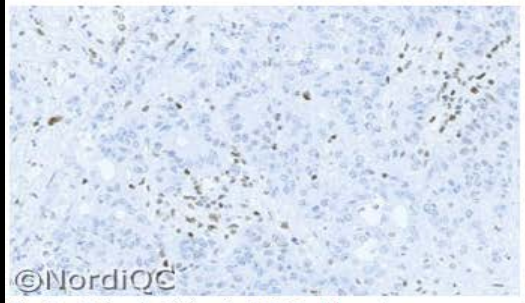


Fig. 3a. Optimal staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Figs. 1a. & 2a.
The neoplastic cells are negative, while the remnants of entrapped lymphocytes and stromal cells show a distinct nuclear staining, serving as internal positive control.

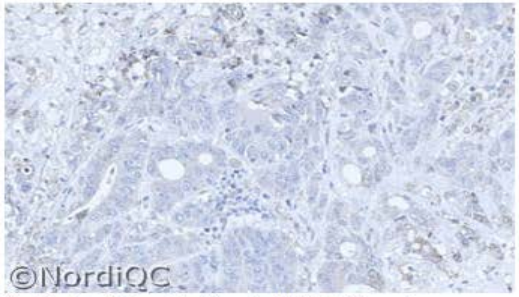


Fig. 3b. Insufficient staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Figs. 1b. & 2b., same field as in Fig. 3a.
No nuclear staining reaction is seen in the neoplastic cells, but as virtually no nuclear staining reaction is seen in the normal cells as stromal cells, the staining pattern can not reliably be interpreted. Also note the weak cytoplasmic staining complicating the interpretation.

MMR:

iCAPCs:

Mantle zone B-cells in tonsil

++++++++

(internal control)

Stromal cells!!

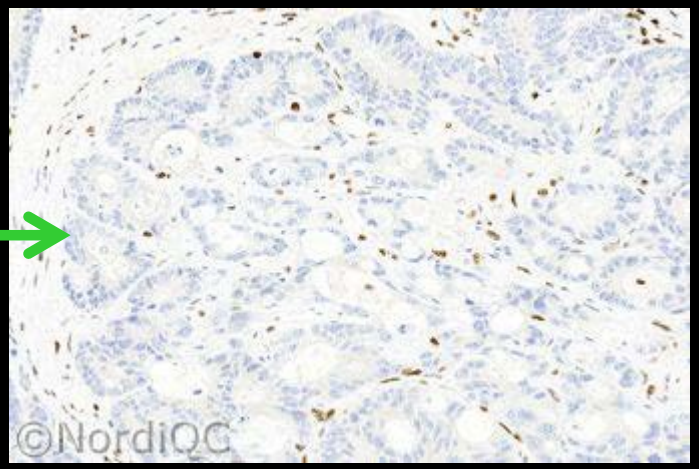
IHC – The Technical Test Approach

TABLE 2. Examples of IHC Assays Where Preferential Use of Internal Positive Controls Recommended

IHC Assay	Use	Comments
Cytokeratin 5	Demonstration of basal cells in glandular structures of prostate to differentiate between benign (positive) and malignant (negative) glands	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control Tested sample may be completely negative if no normal tissue is present
Mismatch repair proteins (MLH1, MSH2, PMS2, MSH6)	Absence of expression in the cells of colon or endometrial adenocarcinoma is abnormal; patients referred for molecular testing to rule out Lynch Syndrome	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control
SMAD4/Dpc4	Ubiquitously expressed tumor suppressor Ag that is inactivated in about 55% of pancreatic adenocarcinomas	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control
PTEN	Ubiquitously expressed; loss of expression is associated with carcinogenesis, cancer progression, and drug resistance	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control

Internal postive tissue controls;

Principally ideal as processed identically to patient relevant material evaluated



If internal positive control is neg or dubious – test is repeated

IHC – The Technical Test Approach

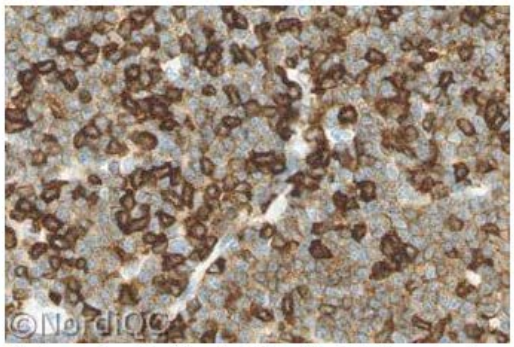


Fig. 4a. Optimal staining for CD5 of the B-CLL no. 5 using same protocol as in Figs. 1a - 4a.
The majority of the neoplastic cells show a moderate and distinct staining reaction, while the infiltrating normal T-cells show a strong staining reaction.

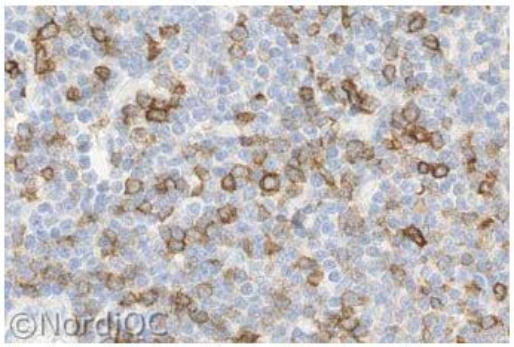


Fig. 4b. Insufficient staining for CD5 of the B-CLL using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The neoplastic cells are virtually negative and only the normal T-cells are clearly demonstrated.

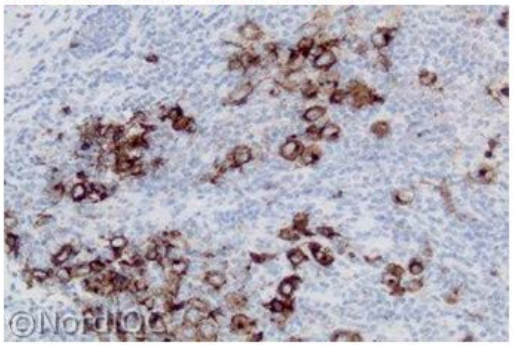


Fig. 2a. Optimal CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

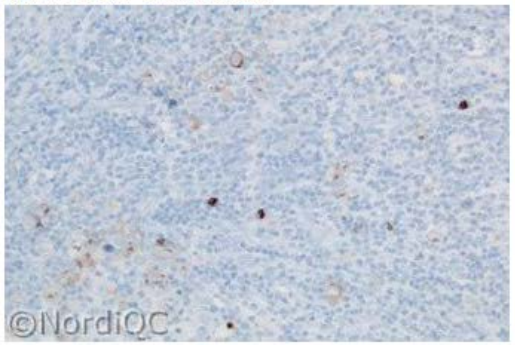


Fig. 2b. CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak staining - same field as in Fig. 2a.

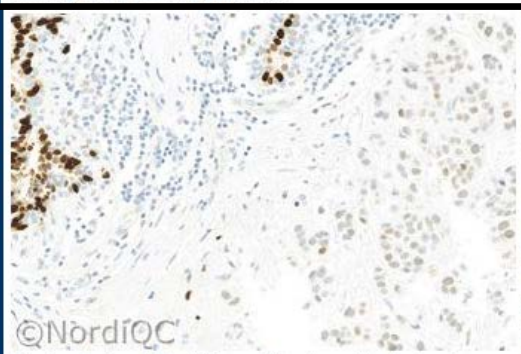


Fig. 3a. Optimal ER staining of the breast ductal carcinoma no. 3 with 60 – 80 % cells positive. A weak but distinct nuclear staining is seen in the appropriate proportion of the

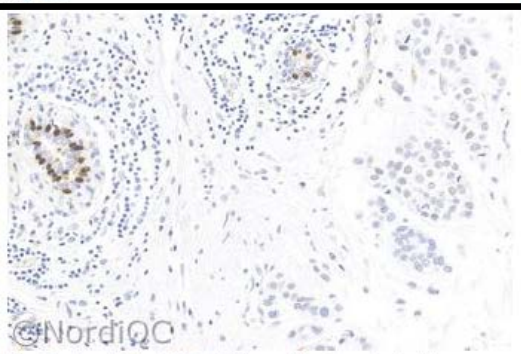
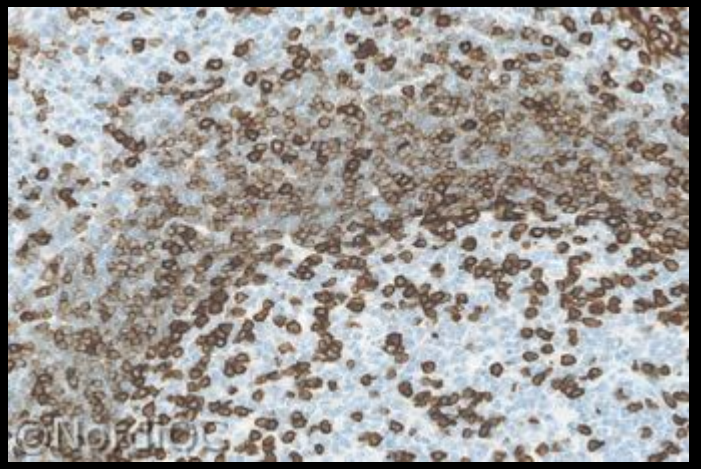


Fig. 3b. Insufficient ER staining of the breast ductal carcinoma no. 3 with 60 – 80 % cells positive using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a.

Internal positive tissue controls;

In general not applicable as positive controls due to levels of expression may not be relevant for level of test calibration

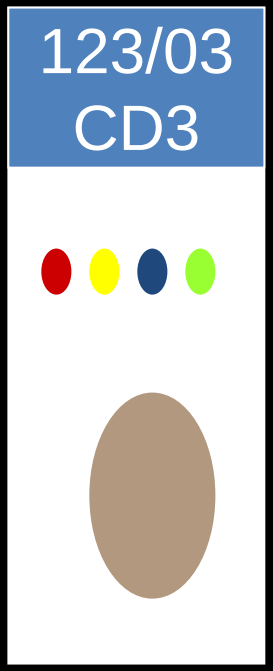
e.g. CD5, CD15, CD34, CD45, CD56, S100, ER, PR etc

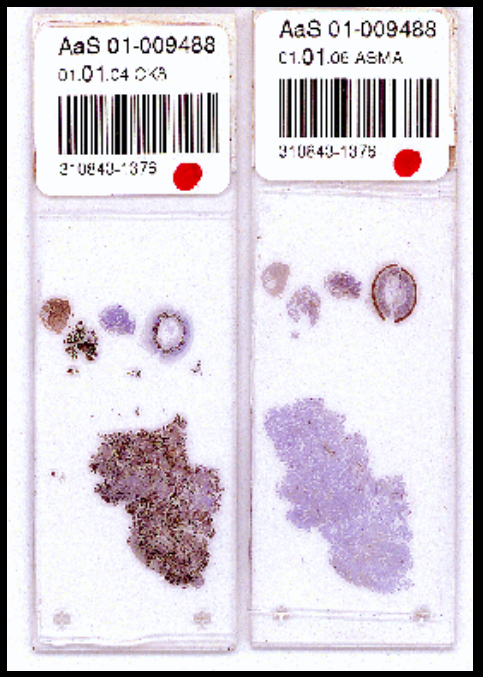


IHC – The Technical Test Approach

"Ideal" daily <u>on-slide</u> control for the majority of routine markers:

Appendix
Liver
Pancreas
Tonsil





Each slide stained and evaluated has essential information of the obtained sensitivity and specificity

In contrast only using 1 external tissue run control, no information is available for the single slide evaluated

IHC – The Technical Test Approach

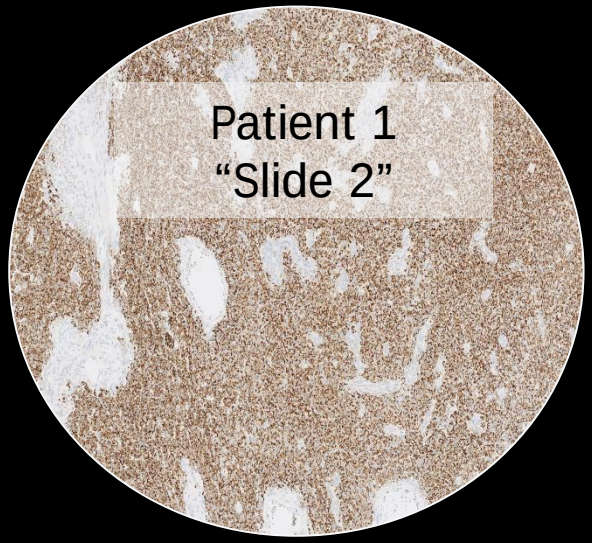
	TMA on-slide control	One batch control	Remarks
Missing reagent FN in patient test	Yes	No – only control slide	Potential internal pos. control only indicator of protocol performed
Wrong antibody FP in patient test	Yes	No – only control slide	
Inappropriate protocol performance - Drying out etc FN / FP in patient test	Yes	No – only control slide	Potential internal pos. control only indicator of protocol performed
	Errors seen for all IHC automated and semi-automated IHC platforms		

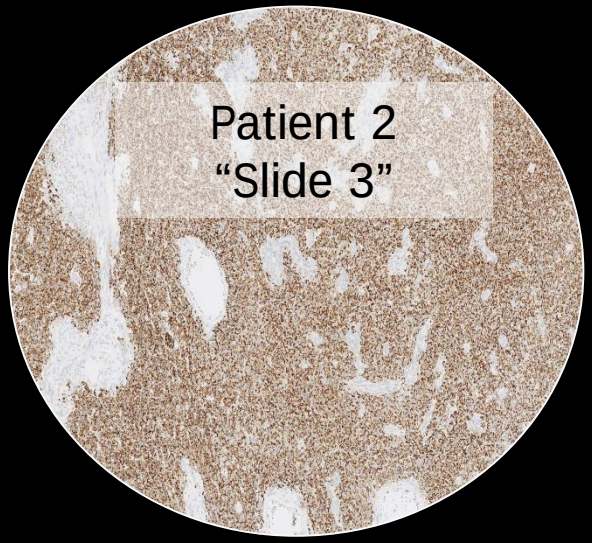
IHC – The Technical Test Approach

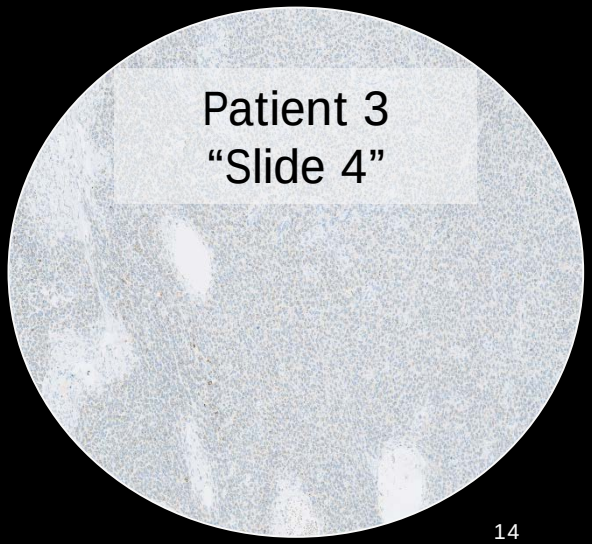
“Patient” 3 IHC assay level could be related to:

1. Biology
2. Tissue processing
3. Missing reagent or other technical issue

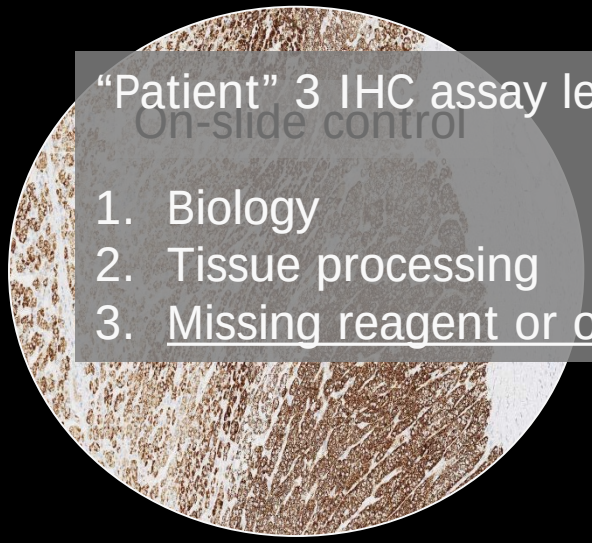
Melan-A in sex cord tumours





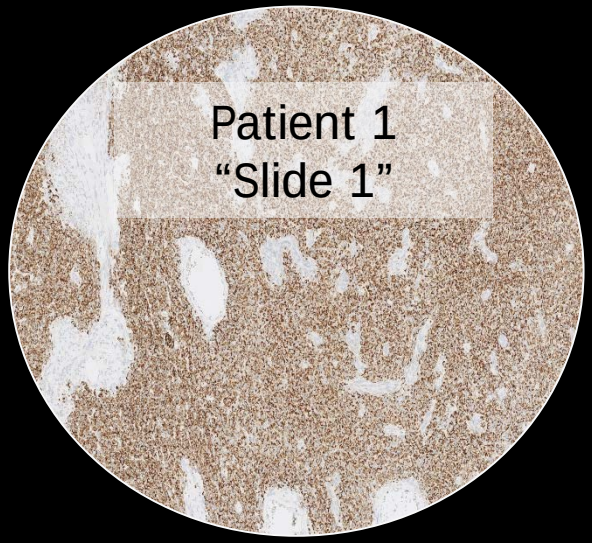


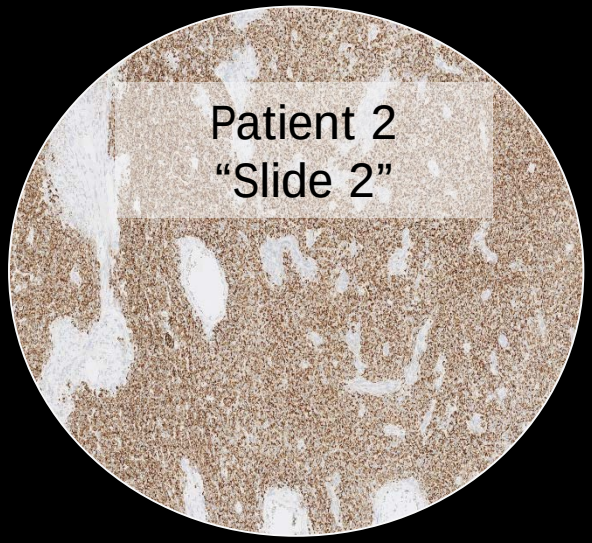
IHC – The Technical Test Approach

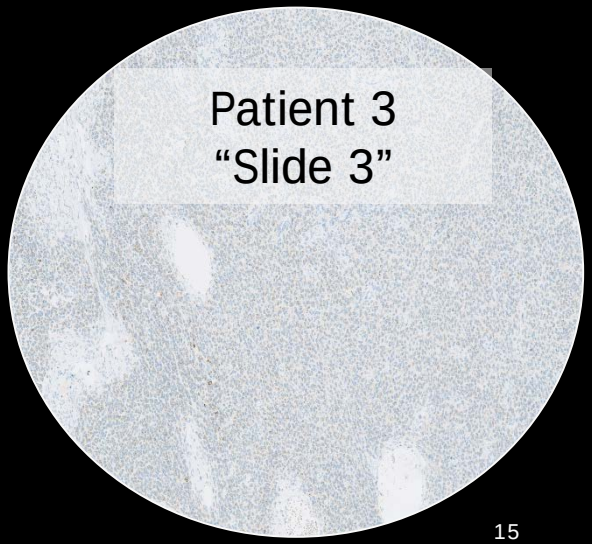


"Patient" 3 IHC assay level could be related to:

1. Biology
2. Tissue processing
3. Missing reagent or other technical issue

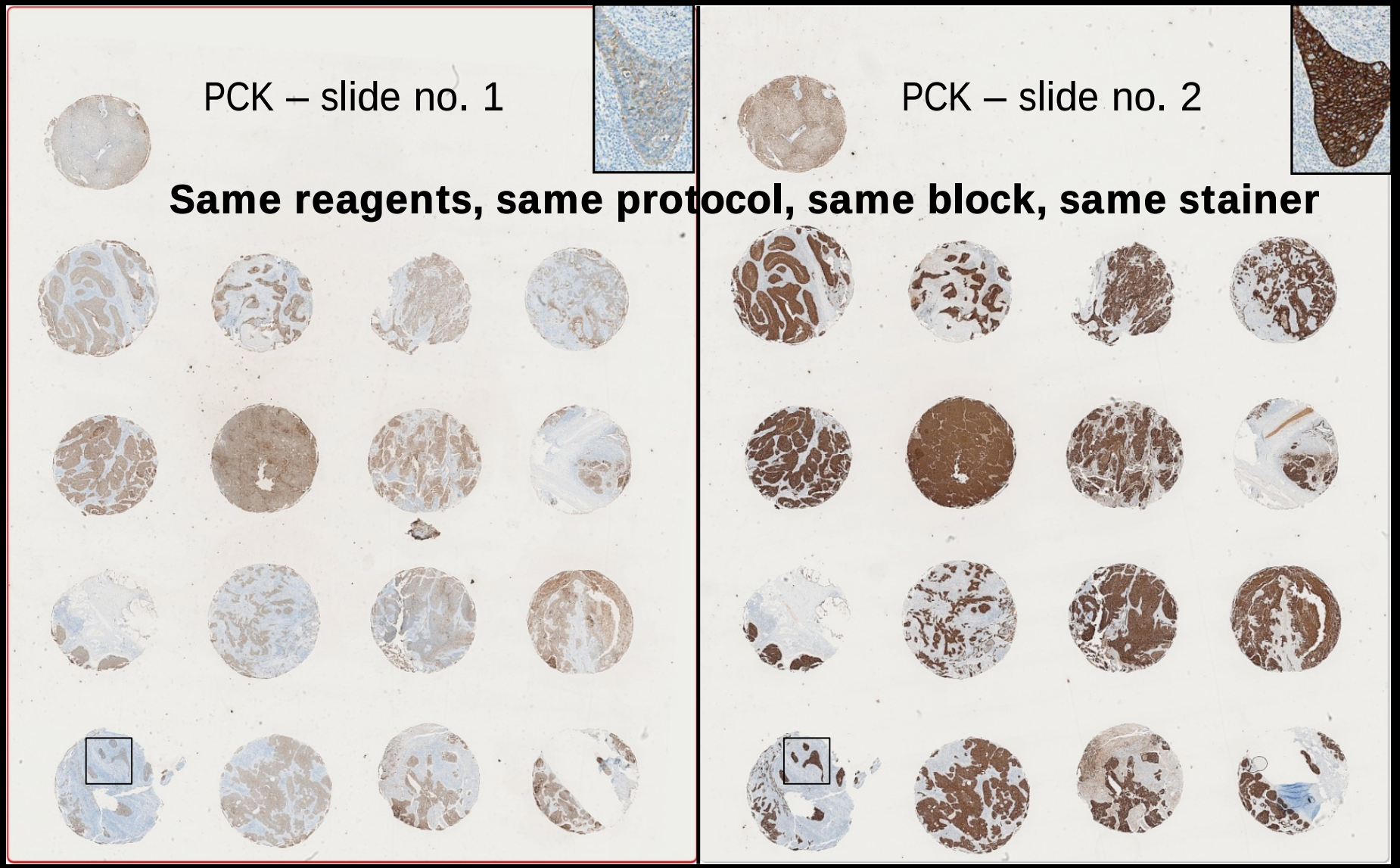






IHC – The Technical Test Approach

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



IHC – The Technical Test Approach

REVIEW ARTICLE

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

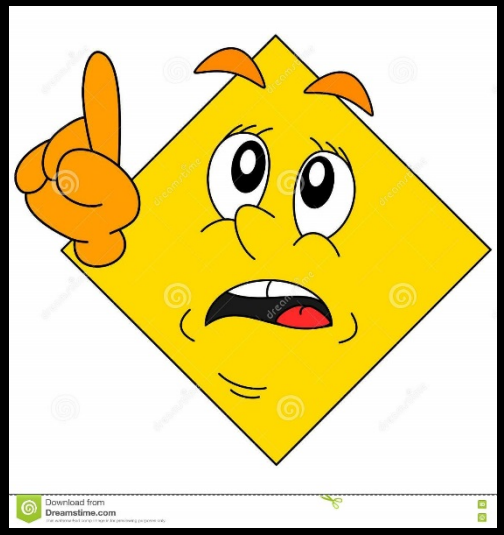
Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Emina E. Torlakovic, MD, PhD,† 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.



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

IHC – The Technical Test Approach

RESEARCH ARTICLE

(*Appl Immunohistochem Mol Morphol* 2017;25:308–312)

An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls

Carol C. Cheung, MD, PhD, JD,† Clive R. Taylor, MD, DPhil,‡ and Emina E. Torlakovic, MD, PhD†*

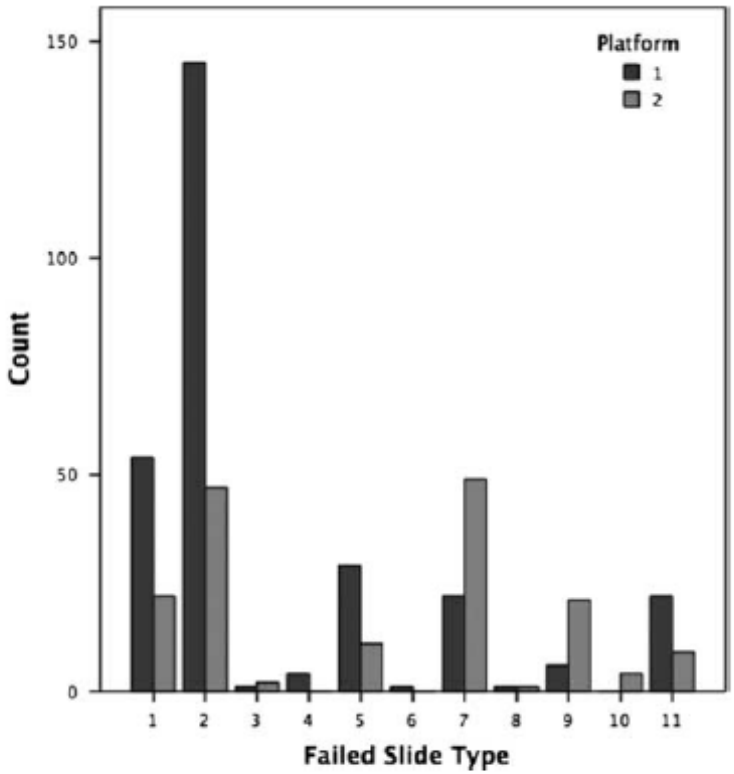


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

TABLE 1. Categories of Failed IHC Slides

Failed IHC Slide Category	Description	Comments
1	On-slide control too weak, patient tissue negative	Correct primary Ab was applied, but test sensitivity is possibly too low
2	On-slide control negative, patient tissue negative	Total slide failure; the result of the test does not suggest possible cause of the failure
3	On-slide control too weak, patient tissue weakly positive but no internal control	May indicate decreased technical sensitivity
4	On-slide control negative, patient tissue weakly positive but no internal control	There is uncertainty whether the correct primary Ab was applied or if there was significantly decreased sensitivity
5	No on-slide control, patient tissue negative	Uncertain results; cannot distinguish if the staining was optimal, suboptimal, or total failure
6	No on-slide control, patient tissue positive	No internal control present; lesion positive; failed only if there is uncertainty over whether the proper primary Ab was applied
7	Failed signal-to-noise ratio	Usually too high background; potential false positive, involving both patient sample and on-slide external control
8	Counter staining problem	If severe, may render result uninterpretable
9	Wrong protocol	Wrong protocol selected when >1 protocol for the given primary Ab exists in the system
10	Uneven staining	Large or critical areas of the patient tissue or controls were missed by uneven staining
11	Wrong control	Either wrong tissue control or areas relevant to the test were missing (detached during staining or paraffin block with control tissue cut through)

IHC indicates immunohistochemistry.

Category 5,6,9,11

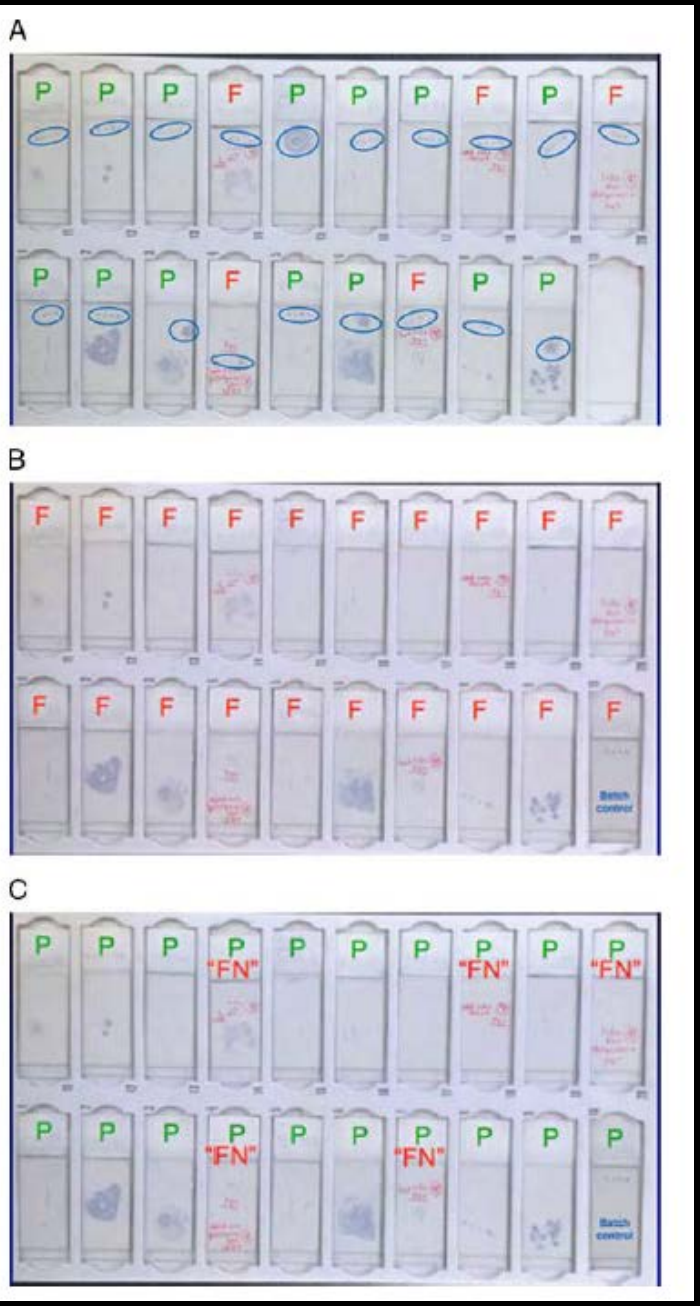
Lab related (22%)

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

Assay and/or Instrument (78%)

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

IHC – The Technical Test Approach



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

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

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

IHC – The Technical Test Approach

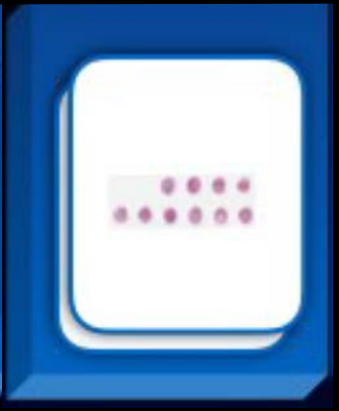
Conclusions:

Controls are essential to evaluate IHC results:

- Tissue controls used to calibrate IHC assay
- Tissue controls processed by variables applied in the laboratory is needed to evaluate on robustness
- Tissue controls to evaluate analytical potential
- Tissue controls to monitor consistency of IHC assay

IHC – The Technical Test Approach

External tissue control tools:

Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical assay performance controls (iCAPCs)	Maps Ab reaction pattern	Fixation time Fixative(s) Decalcification	Range of relevant expression levels	Range of relevant expression levels	Reproducibility Method of transfer proof
High expression Low expression No expression	With expression No expression	High expression Low expression No expression	With expression No expression 20/40 of each Type I/II IHC	High expression Low expression No expression + relevant cut-off	

IHC – The Technical Test Approach

Conclusions:

Focus on external tissue controls is central to standardize and optimize IHC:

- On-slide TMA controls are preferable to 1 bacth control
- Internal tissue controls are of limited value
- Negative reagent controls are only essential for biotin-based detection systems
- Negative reagent controls can be valueable for non-biotin based systems e.g. if pigment, frozen sections..

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

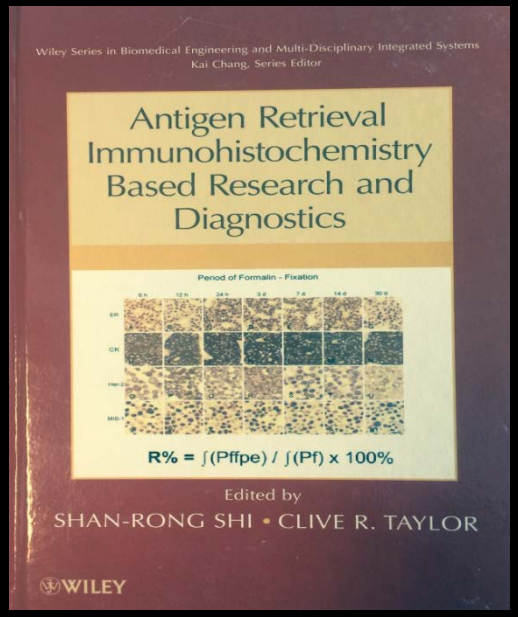
IHC – Immunohistochemical stainers

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

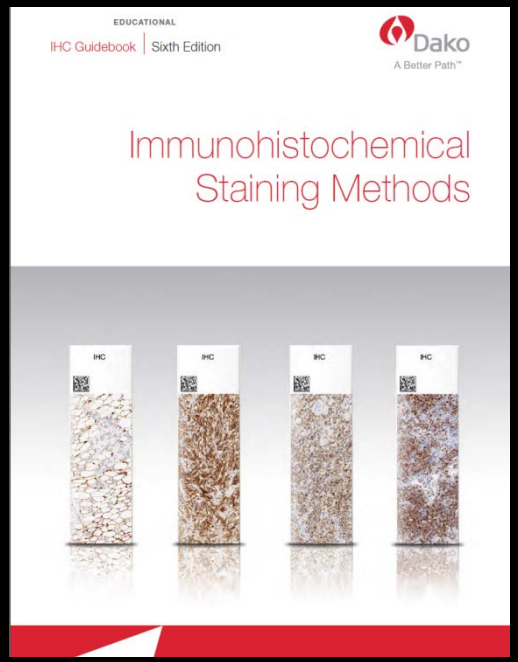
Dako
A Better Path™

Chapter 9

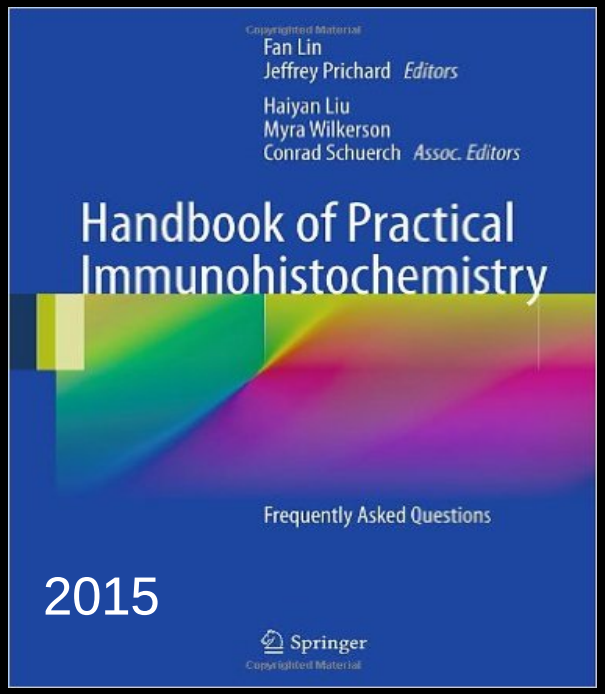
Automation in IHC

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.**)

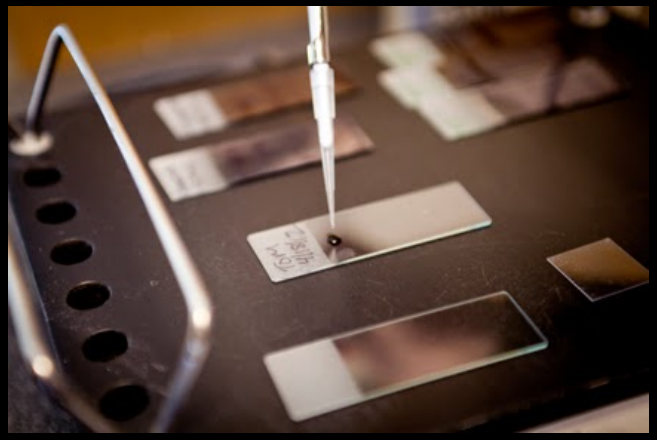
Preparation – sorting, deparaffination, epitope retrieval….
Application of reagents - pippetting

Secure even distribution – ”Pap-pen”

Avoid evaporation / secure moist – staining trays

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

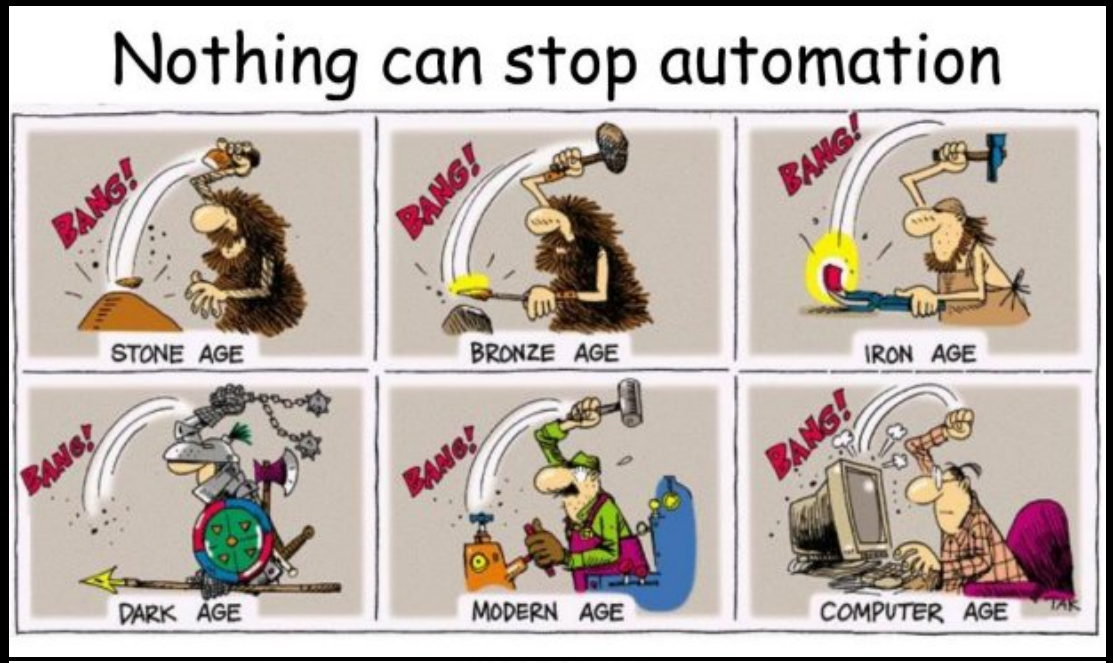
IHC – Immunohistochemical stainers

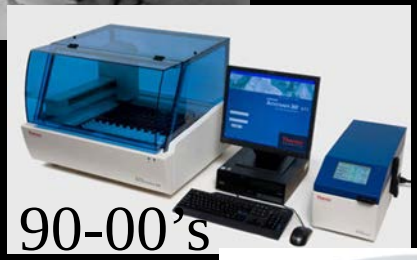


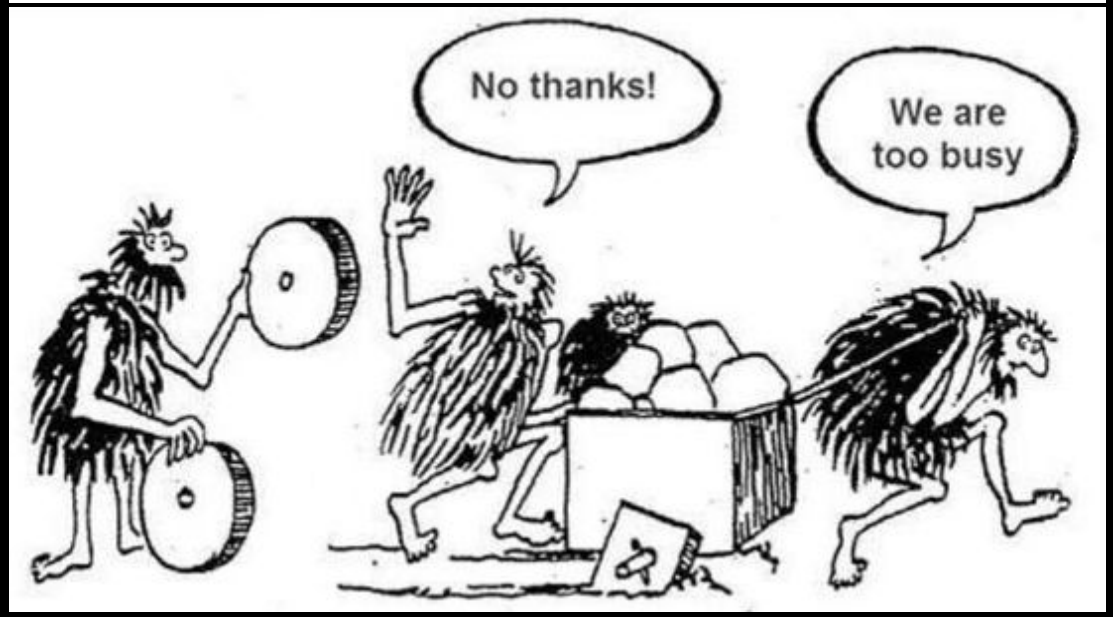
Wash – Dry – Apply
Wash – Dry – Apply
Wash.....

Challenge: Time, Standardisation, Traceability, Skills...

IHC – Immunohistochemical stainers





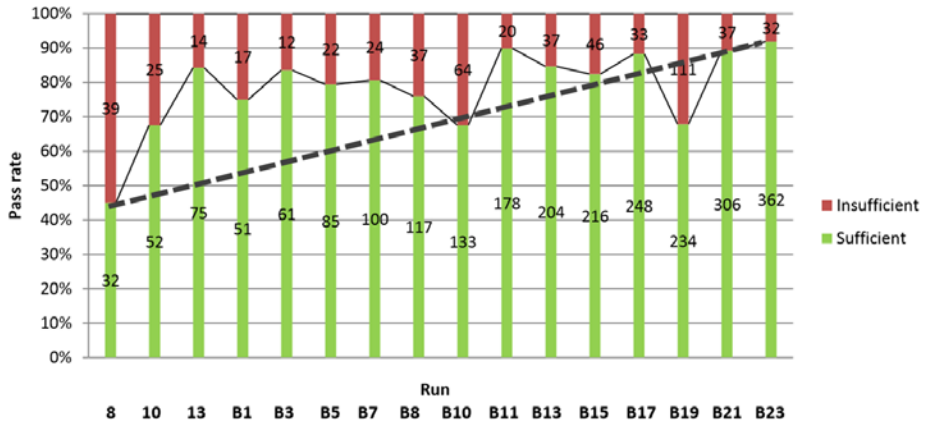


IHC – Immunohistochemical stainers

Estrogen receptor;

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).

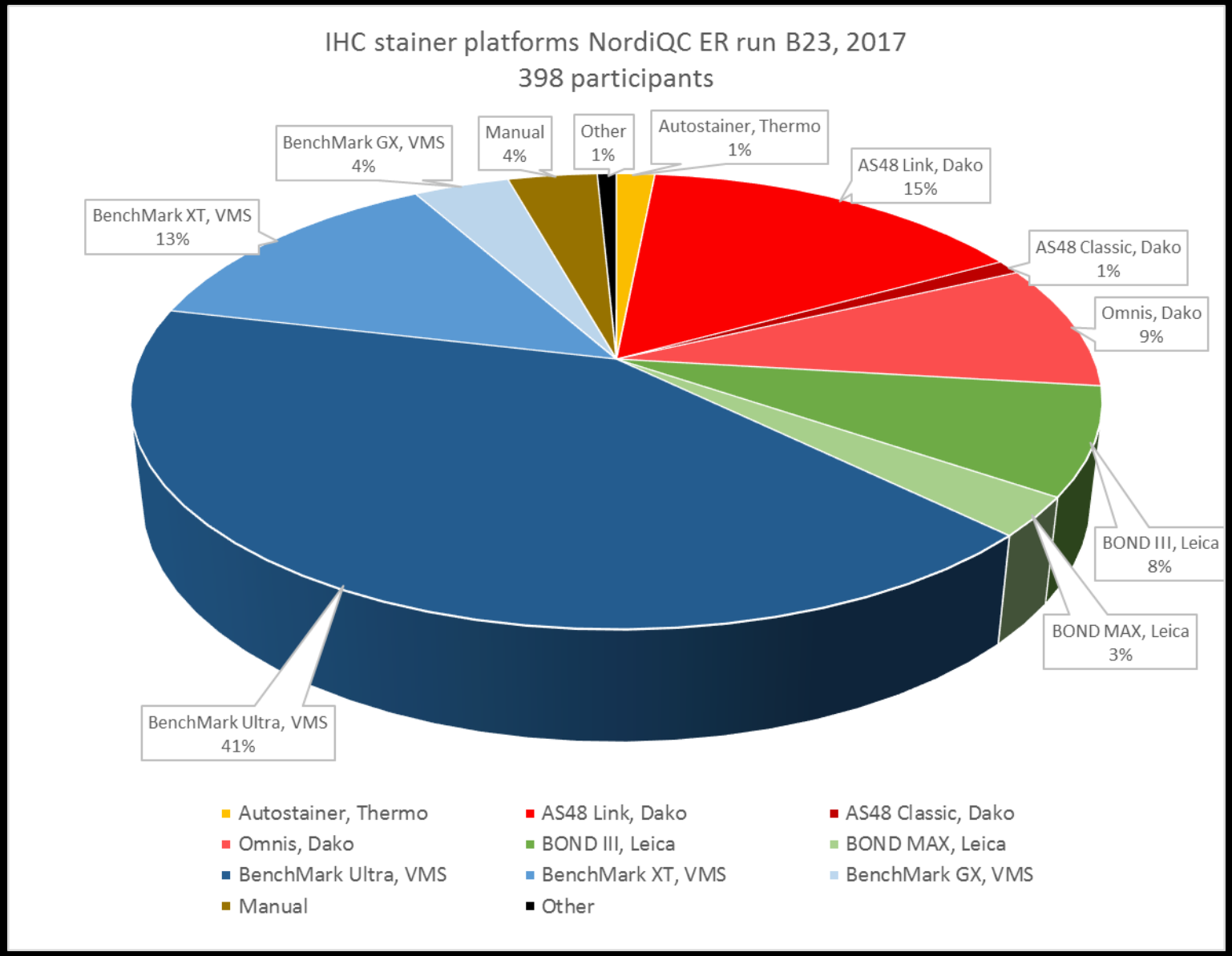
Fig. 1. **Participant numbers and pass rates for ER during 16 runs**



2003 2017

	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



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

Automation of the IHC staining procedure:

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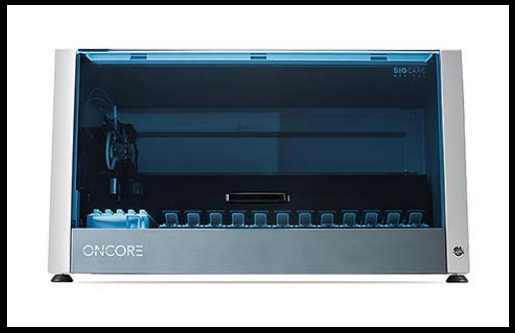
2. Reduce the technician workload used for IHC

Functionality – Workload – Workflow - Flexibility – Costs



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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

	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

Functionality – Workload – Workflow - Flexibility – Costs

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..

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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-Max, 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. Wide portofolio of RTU antibodies – plug-and-play

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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

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 and protocols

IHC – Immunohistochemical stainers

Fully-automated systems: Omnis, Dako

Functionality – Workload – Workflow - Flexibility – Costs

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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

Semi-automated systems: AS-48, Dako

Functionality – Workload – Workflow - Flexibility – Costs

5 main Pros:

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

IHC – Immunohistochemical stainers

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Functionality – Workload – Workflow - Flexibility – Costs

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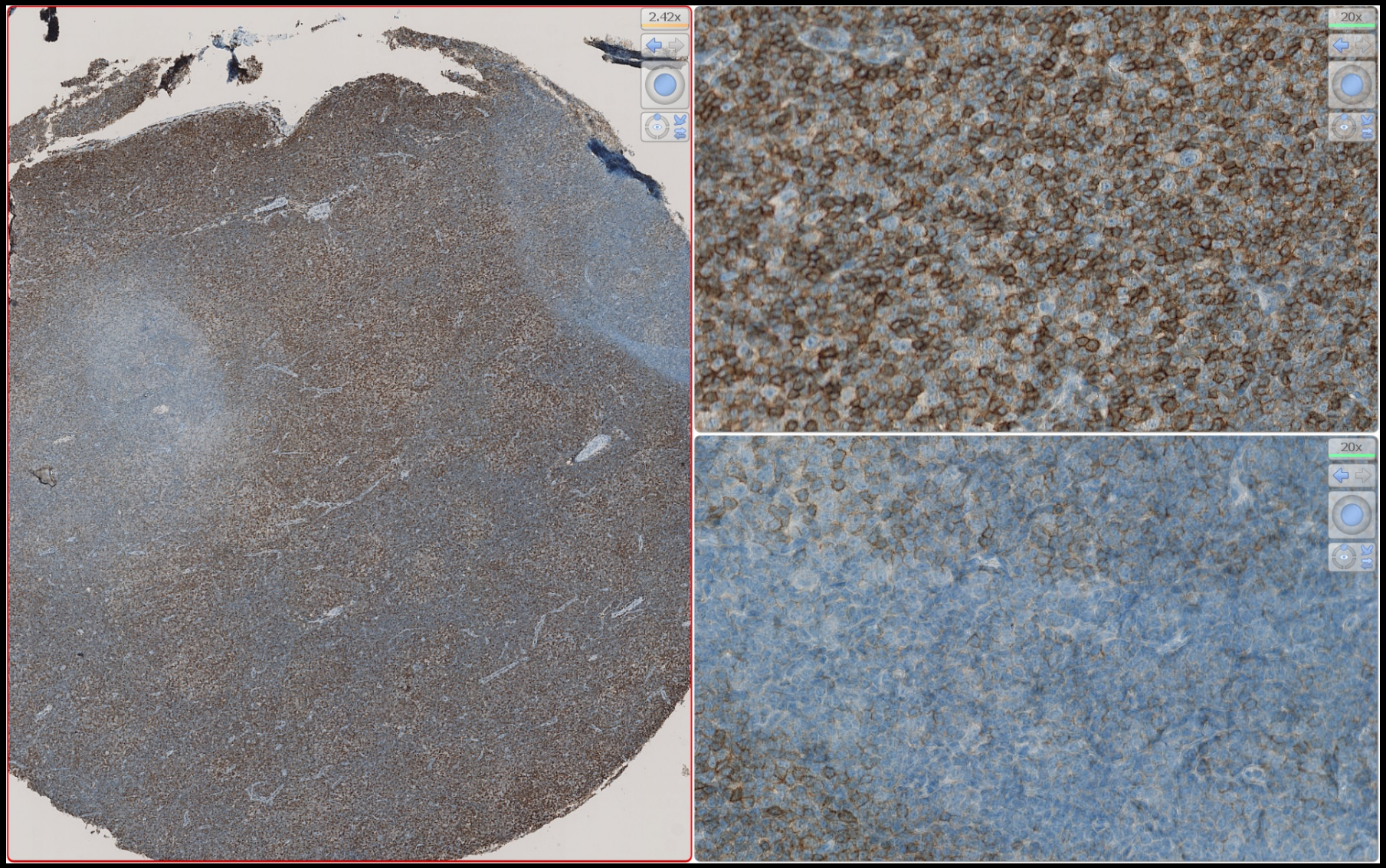
2. Flexible reagent choice – HIER buffer, detection system
3. Both low and high affinity primary antibodies work
4. Easy to use – loading, programming, maintenance
5. Wide portofolio of RTU antibodies – plug-and-play

3 main Cons:

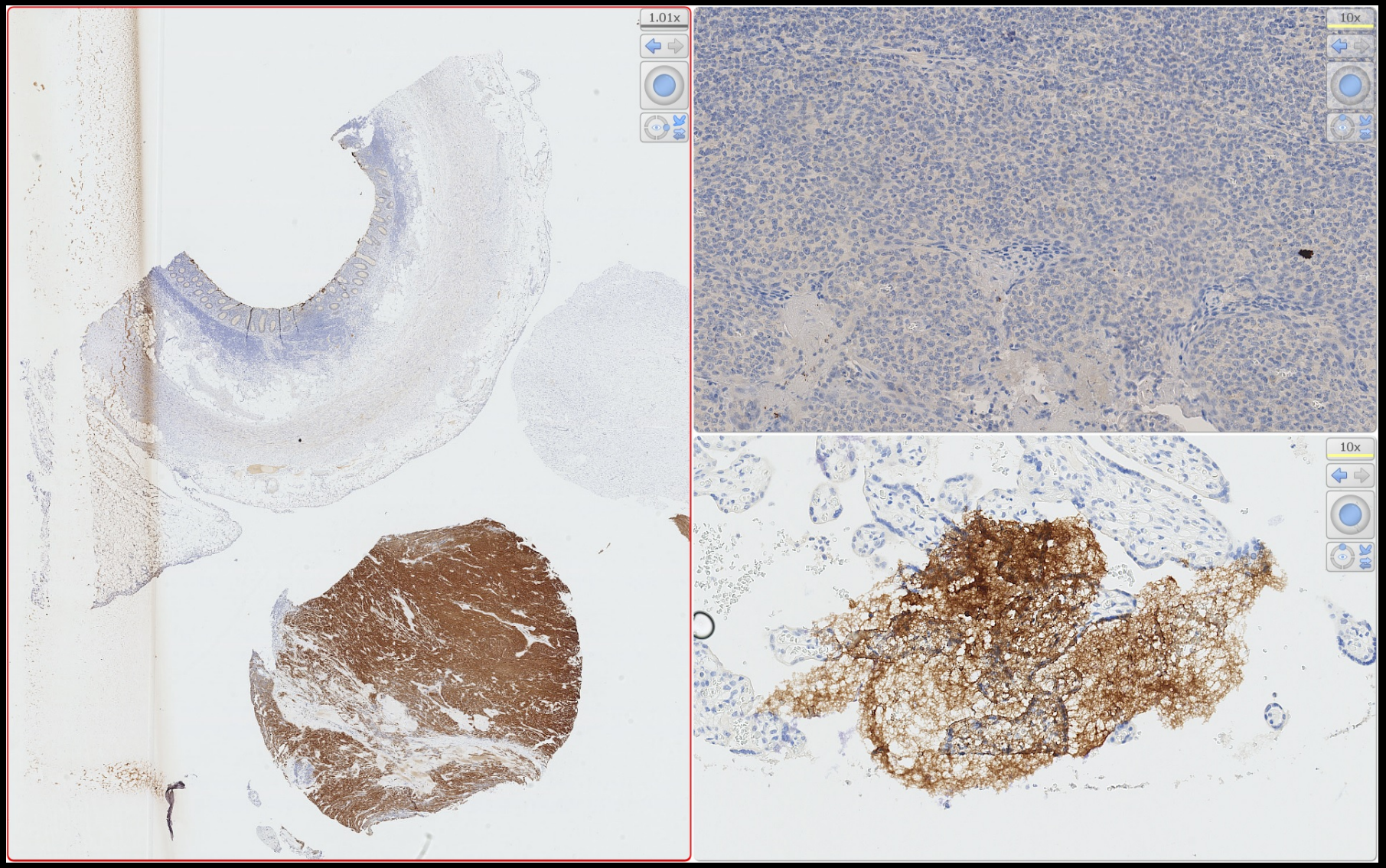
1. Increased manual interaction – 2 instruments needed
2. Primarily batch operation
3. High reagent volumen needed – 300 ul and >”dead-vol”

IHC – Immunohistochemical stainers



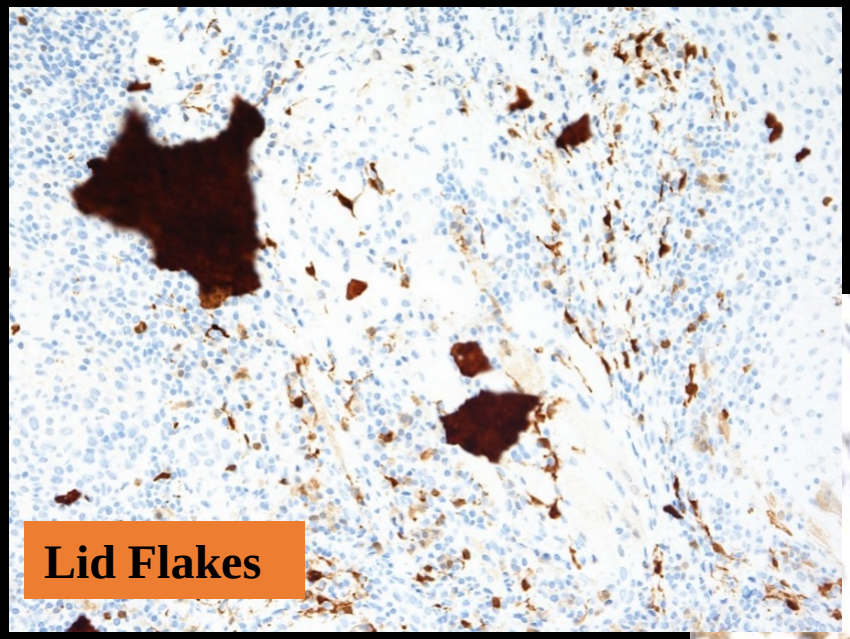
Staining issues; BenchMark, VMS – Uneven weak/neg areas – air bubbles

IHC – Immunohistochemical stainers

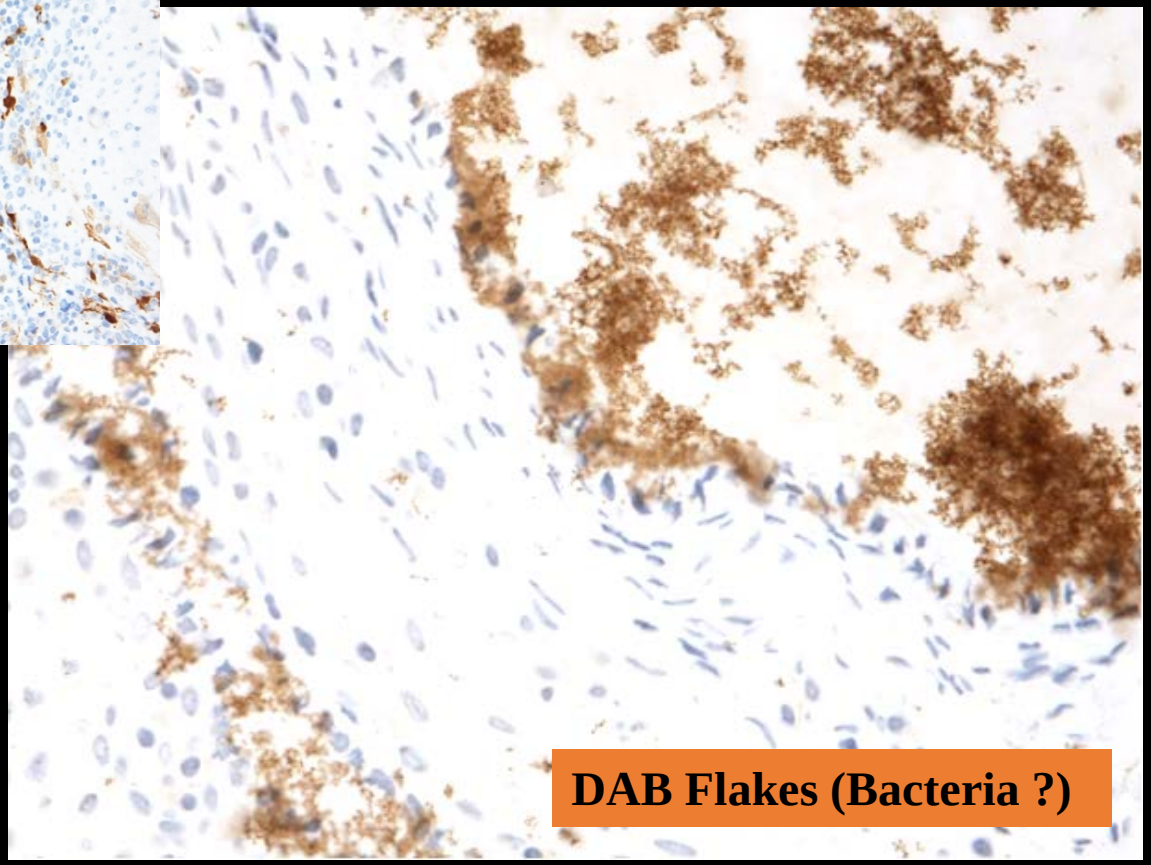


Staining issues; Bond, Leica – chromogen precipitates and general hue

IHC – Immunohistochemical stainers

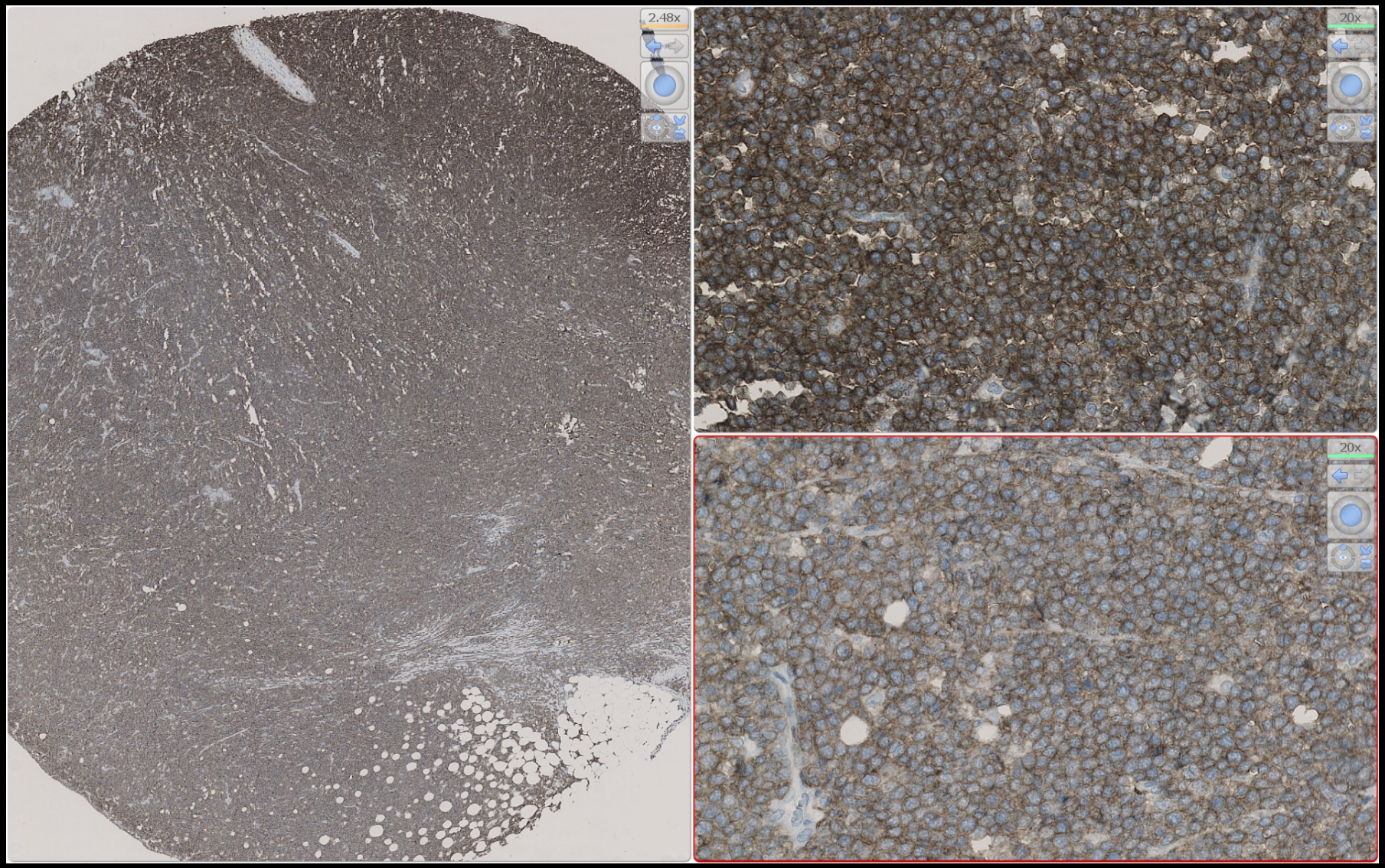


Courtesy by Michael Bzorek



Staining issues; Omnis, Dako – chromogen precipitates

IHC – Immunohistochemical stainers



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

IHC – Immunohistochemical stainers

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

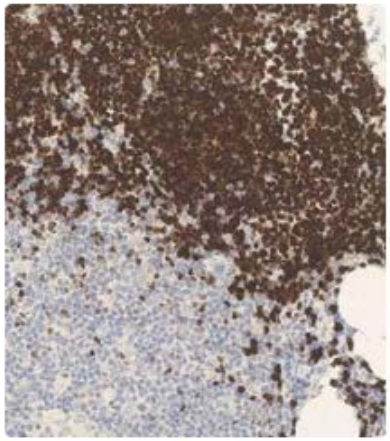
- Interpretation of the test
  - Inadequate choice of control material etc

IHC – Immunohistochemical stainers

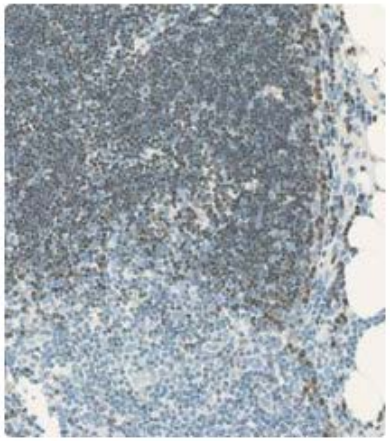
- Accuracy of the technical optimization and validation of the test
  - Use of RTU formats not adequately calibrated etc

Terminal Deoxynucleotidyl Transferase (TdT)

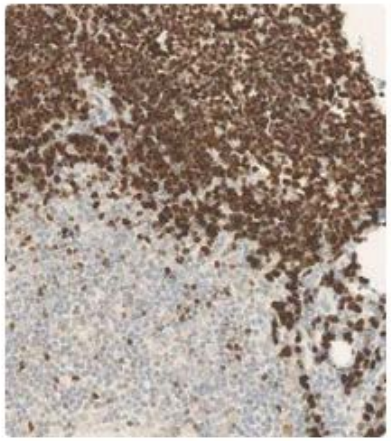
A comparison of Terminal Deoxynucleotidyl Transferase (TdT) Ready-to-Use antibodies from leading manufacturers on human thymus.



Novocastra BOND Ready-to-Use TdT, PA0339, clone SEN28



Vendor 1 Ready-to-Use



Vendor 2 Ready-to-Use

Leica Biosystems BOND system using BOND Ready-to-Use TdT demonstrates high quality staining when compared directly to Ready-to-Use antibodies from other leading manufacturers on serially cut sections of human thymus. Images supplied by NordiQC.

** independent analysis commissioned by Leica Microsystems and conducted by NordiQC according to the instructions for use and on the corresponding manufacturer's staining platform.*

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

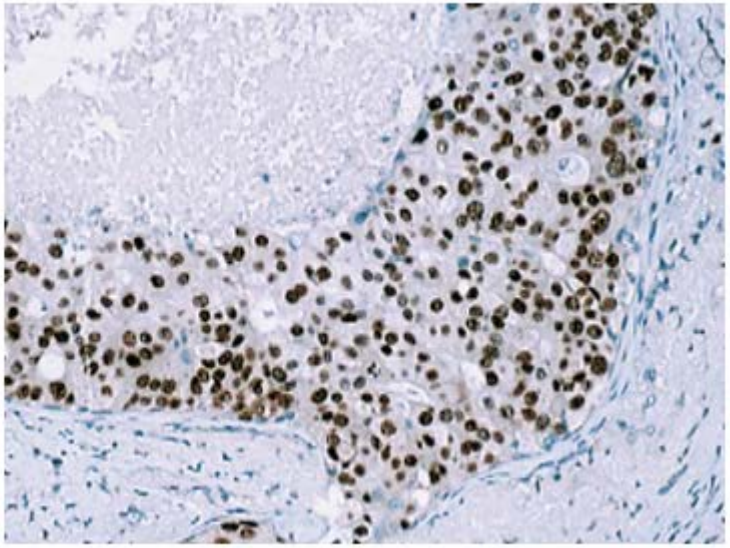
IHC – Immunohistochemical stainers

Cautions to be taken when comparing the different solutions:

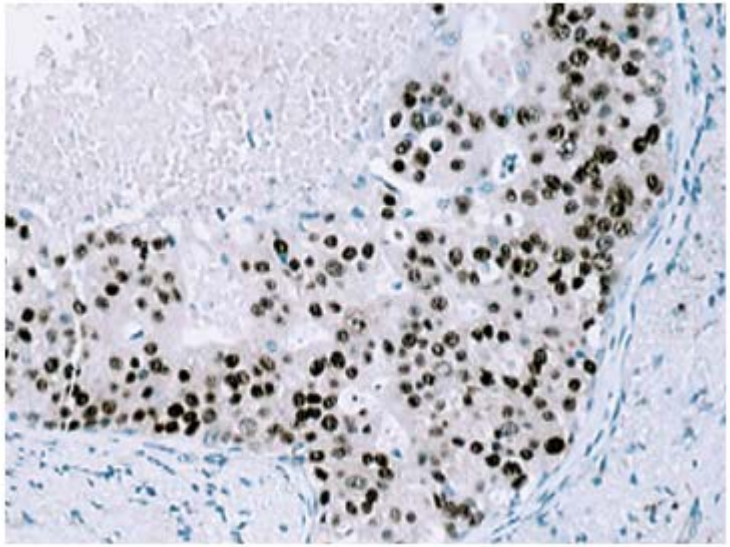
E.g. cost for primary Ab – Was same or similar test conditions applied

40X difference

COMPARE MACH 4 (L) WITH LEADING COMPETITOR (R) FOR ER (1D5) STAINING.



MACH 4, 1:1000 dilution



Leading competitor using dextran backbone polymer, 1:25 dilution

3-step polymer vs 2-step polymer ?
Incubation times ?
HIER settings – time, pH, temp 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 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

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