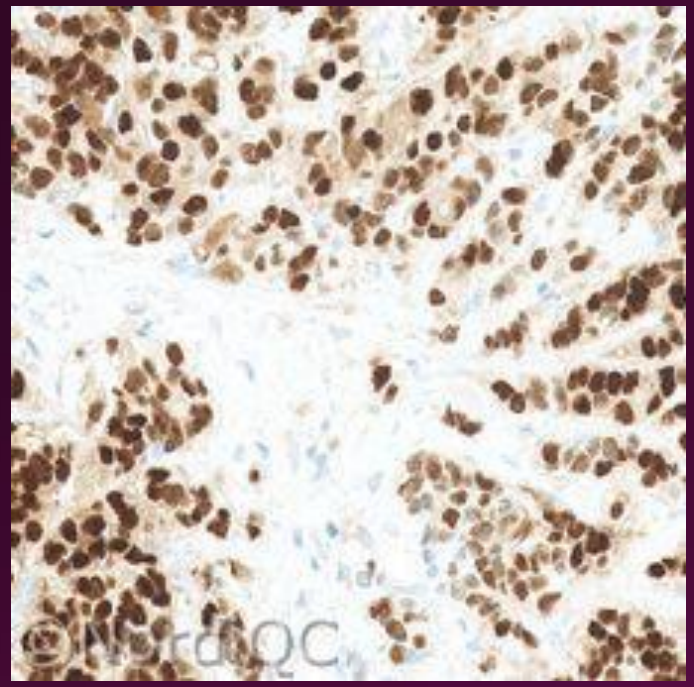
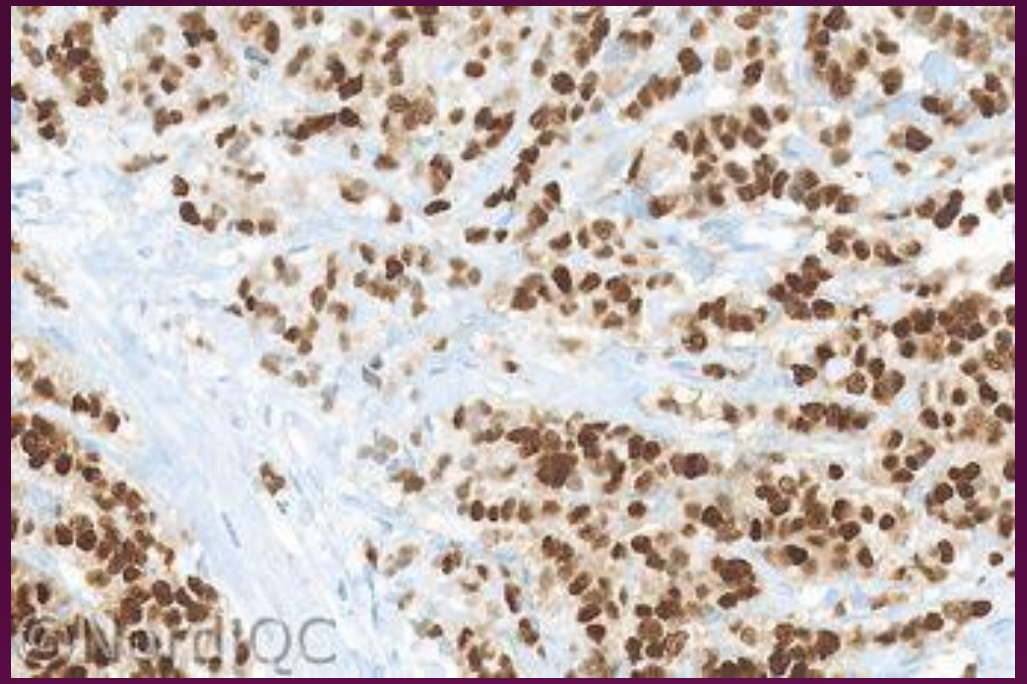
ER

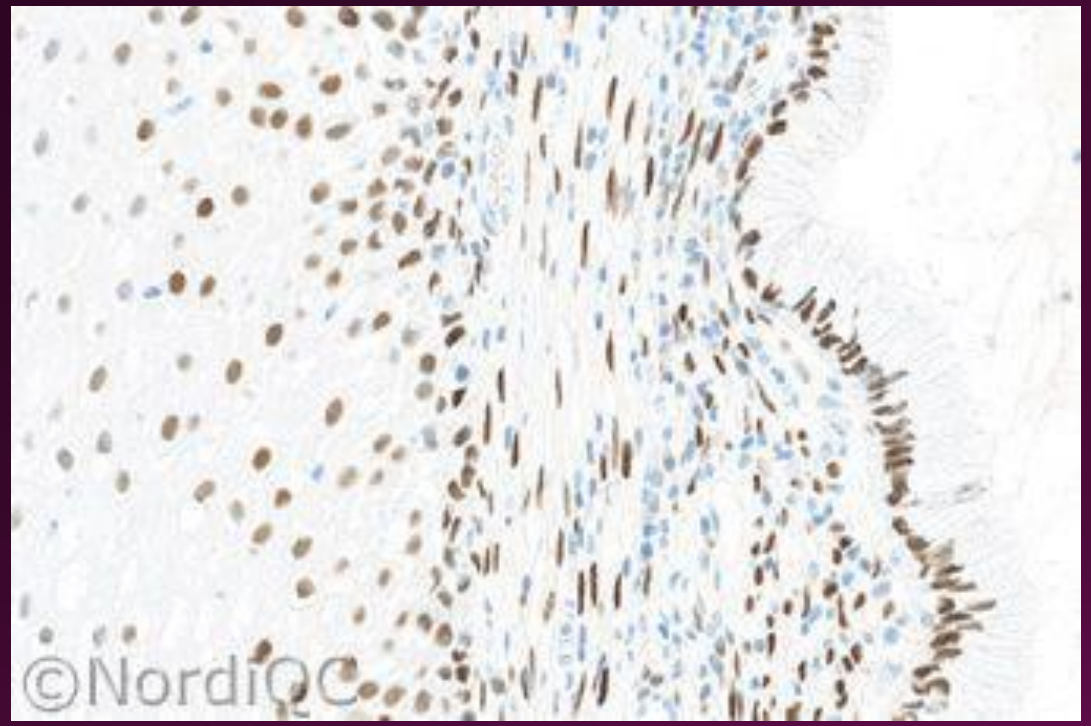


Optimal Protocol
Ductal carcinoma: Virtually all the nuclei of the neoplastic cells show a strong, distinct nuclear staining reaction with only a weak cytoplasmic staining reaction. No background staining is seen.

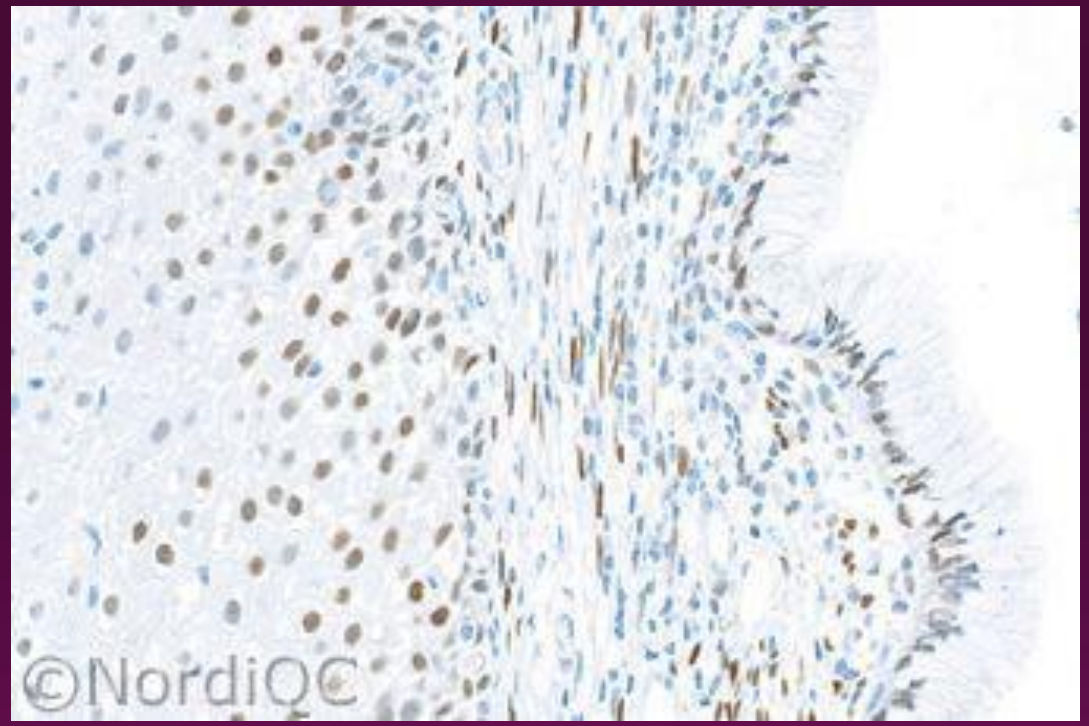


Insufficient Protocol
Ductal carcinoma: Virtually all the neoplastic cells are demonstrated but this protocol is insufficient as demonstrated by expression in normal cervix illustrating why tumor controls are not necessarily reliable for calibrating protocols

ER

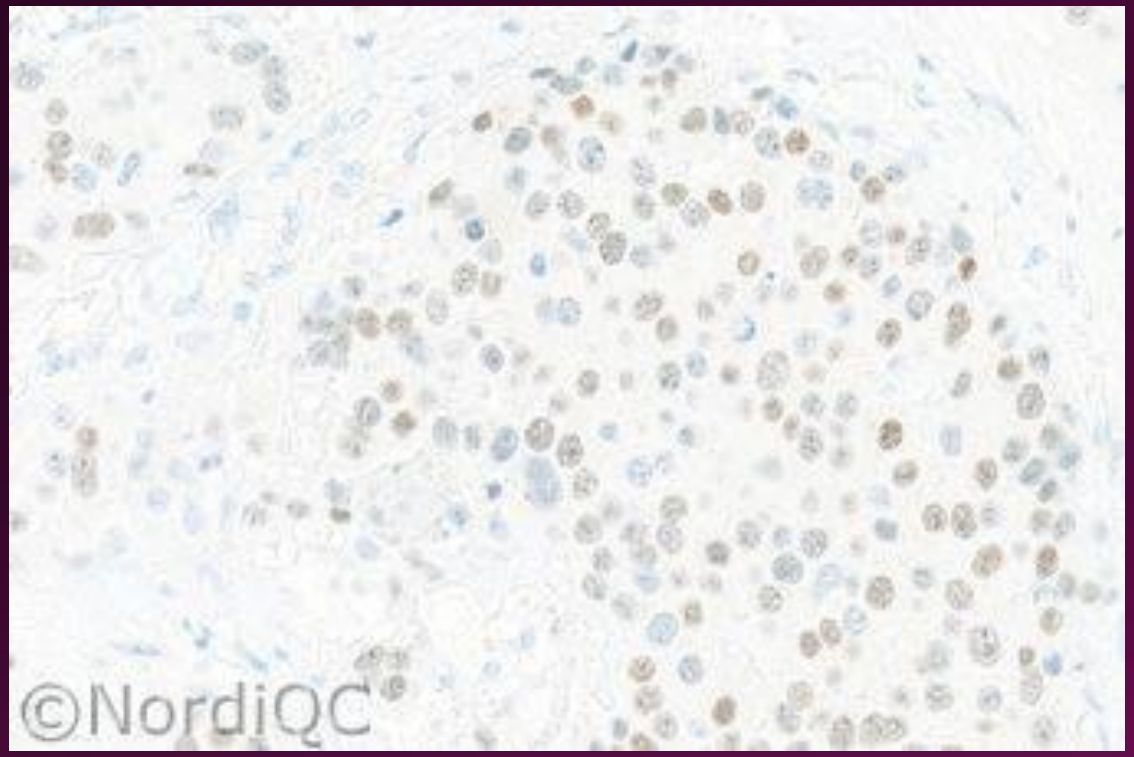


Optimal Protocol
Cervix: Virtually all the squamous and columnar epithelial cells show a moderate to strong, distinct nuclear staining reaction. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative.

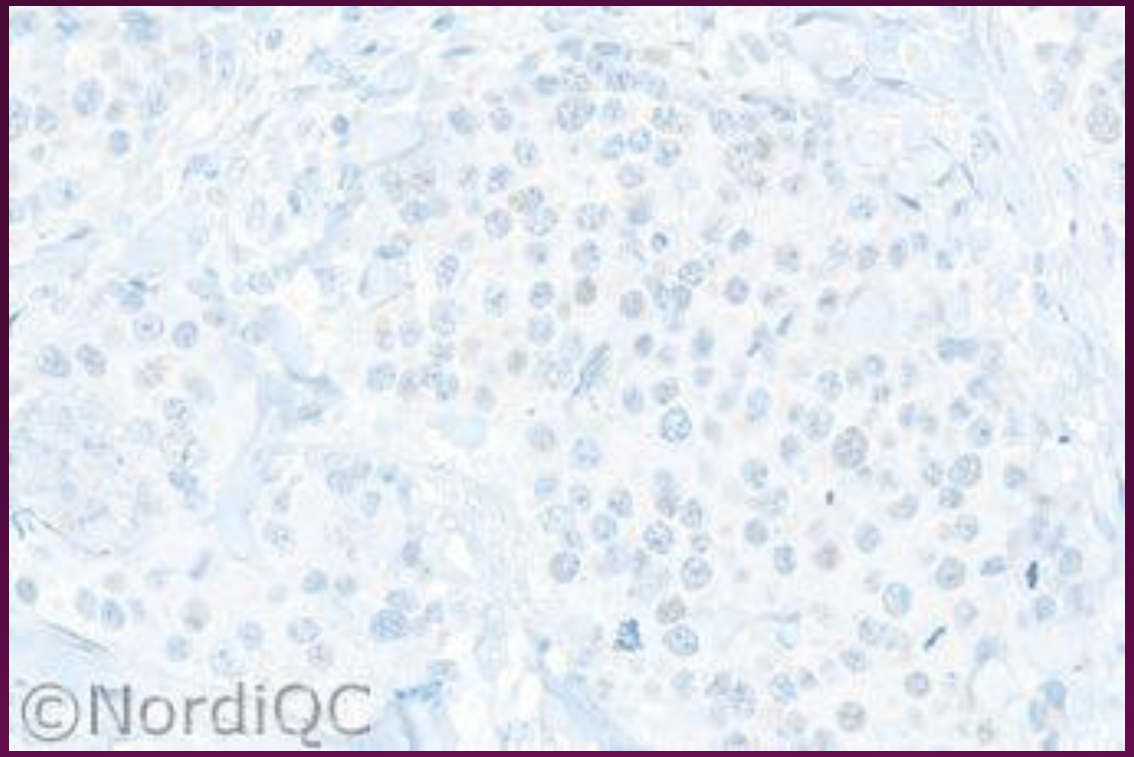


Insufficient Protocol
Cervix: The proportion and intensity of the staining reaction in the squamous and especially in columnar epithelial cells are reduced.

ER



Optimal Protocol
Ductal carcinoma: A weak but distinct nuclear staining reaction is seen in the appropriate proportion of the neoplastic cells and no background staining is seen.



Insufficient Protocol
Ductal carcinoma: A false negative staining result

Verification of Immunohistochemical Tests

- Assessing the performance of the immunohistochemical test on a case-by-case basis by verifying that the test is detecting the target antigen at the level of sensitivity for which it was calibrated to ensure that it will give the correct result on that case

- Positive control tissue
  - Internal
  - External
- Negative control tissue
  - Internal
  - External

Positive Controls

- Normal tissues with predictable antigen expression are highly recommended
- Tumor tissues have unpredictable and variable antigen expression and are not recommended as routine controls, although they may be needed in selected situations (MYF, OCT4, ALK)
- External control tissue must be present on the test slide for automated immunohistochemical instruments since each chamber represents an distinct reaction, requiring its own control tissue (batch controls are not valid)
- Internal control tissue (if present on the slide) should be evaluated

Evidence-Based Use of Positive Controls

- External positive controls are essential for
  - Verification that the IHC test is properly calibrated (quality indicators are appropriately expressed)
  - Ensuring that the antibody was dispensed and the appropriate IHC reaction occurred in the reaction chamber

- Internal positive controls are essential for
  - Determining that the antibody and reagents were appropriately delivered across the entire surface of the slide
  - Ensuring that the IHC protocol was able to adequately unmask tissue

Standardization of Negative Controls in Diagnostic Immunohistochemistry: Recommendations from the International Ad Hoc Expert Panel.

Torlakovic EE[1], Francis G, Garratt J, Gilks B, Hyjek E, Ibrahim M, Miller R, Nielsen S, Petcu EB, Swanson PE, Taylor CR, Vyberg M.

Appl Immunohistochem Mol Morphol. 2014 Apr;22(4):241-52.

Negative Controls

- Specific negative controls (negative tissue controls - NTC)
  - Internal or external control tissue that is known not to express the target antigen
  - Identifies unintended antibody cross-reactivity to cells or cellular components (false positive)

  - Reasons for false positive results in negative tissue controls
    - Too high concentration
    - Too long incubation with primary antibody
    - Too much HIER
    - Application of wrong antibody
    - Contamination of primary antibody with a secondary antibody
    - Cross reactivity of the primary antibody due to poor clone performance

Negative Controls

- Nonspecific negative controls (negative reagent controls - NRC)
  - Patient slide is exposed to IHC test in which various components of the test can be omitted
    - NRC-primAb:
      - Replaces primary antibody with nonspecific Ig
      - Not practical to run for every antibody
      - Can be performed if there is unexpected reaction in the on-slide negative control tissue
    - NRC-detSys:
      - Most commonly omits primary antibody and subsequently can omit each component of detection system
      - Required for avidin-biotin detection systems for identification of non-specific reaction due to endogenous biotin
      - Not required for polymer detection systems
  - Identifies unintended or non-specific reaction due to a component of the test (false positive)

External Quality Assurance Programs

Standard Terminology

- Quality Control
  - A set of procedures or rules intended to ensure that a test performs to a defined set of quality criteria.

- Quality Assurance
  - A program for the systematic monitoring and evaluation of the various aspects of a test to ensure that standards of quality are being met.
  - The means for periodically and systematically assessing whether the test is working properly (example: routine assessment of controls, calculating ER positive breast cancer rate and comparing to literature values, participation in external quality assurance programs)

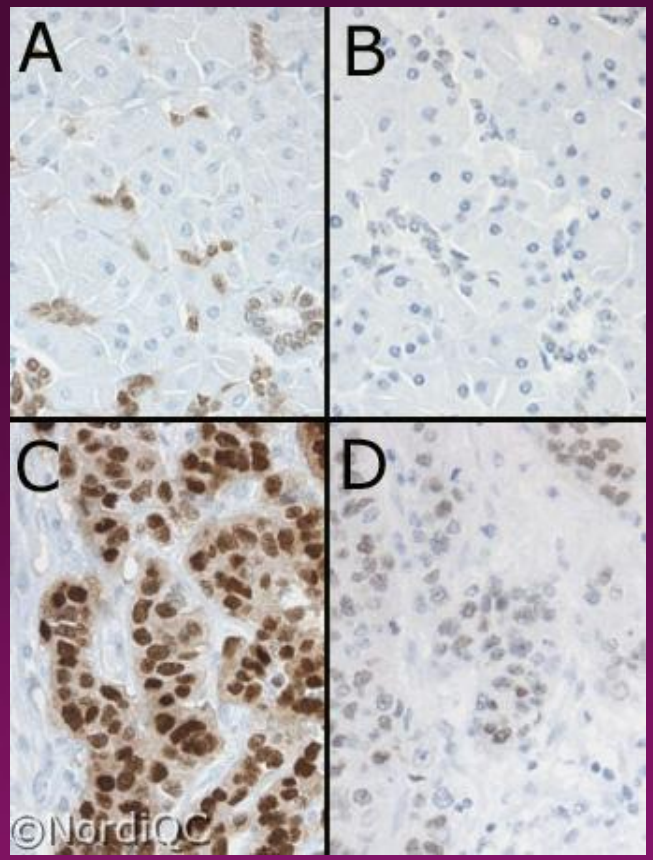
External Quality Assurance

- Nordic Immunohistochemical Quality Control (NordiQC)
  - Selected general antibodies
  - Breast Antibodies (ER, PR)
  - HER2/neu
- Canadian Immunohistochemistry Quality Control (CiQC)
  - Selected general antibodies
  - Breast Antibodies (ER, PR)
  - HER2/neu
  - HER2/neu FISH
- UK National External Scheme for Immunocytochemistry and in situ hybridization (UK NEQAS ICC & ISH)
- College of American Pathologists – External Quality Assurance / Proficiency Testing (CAP EQA / PT)
  - Selected general antibodies

NordiQC

Nordic immunohistochemical Quality Control

- External Quality Assurance Assessments in Immunohistochemistry
- General IHC module
  - Three times a year
- Breast cancer IHC module
  - Twice a year
    - ER, HER2, Ki67
    - PR, HER2, ECAD



NordiQC Assessment

- Optimal
  - Stain is perfect or close to perfect in all of the included tissues
- Good
  - Stain is fully acceptable in all of the included tissues.
  - Protocol may be optimized to ensure the best sensitivity or signal-to-noise ratio

Sufficient

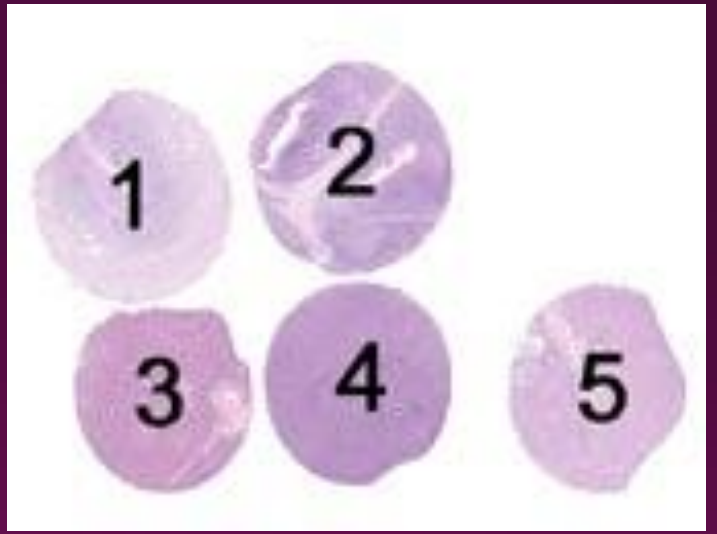
- Borderline
  - Stain is insufficient because of weak staining or false negative staining in one of the included tissues or a false positive staining reaction
  - The protocol should be optimized or the antibody should be changed
- Poor
  - Stain is considered very insufficient because of false negative staining of several of the included tissues or a marked false positive staining reaction
  - The protocol must be optimized urgently

Insufficient

NordiQC

The slide to be stained for CD79a :

1. Appendix
2. Tonsil
3. Precursor-B-acute lymphatic leukaemia (Pre-B-ALL)
4. B-chronic lymphatic leukaemia (B-CLL)
5. Plasmacytoma



NordiQC RUN 29 - 2010

The slide to be stained for CD79a :

1. Appendix
2. Tonsil
3. Precursor-B-acute lymphatic leukaemia (Pre-B-ALL)
4. B-chronic lymphatic leukaemia (B-CLL)
5. Plasmacytoma

BORDERLINE!

NordiQC RUN 29 - 2010

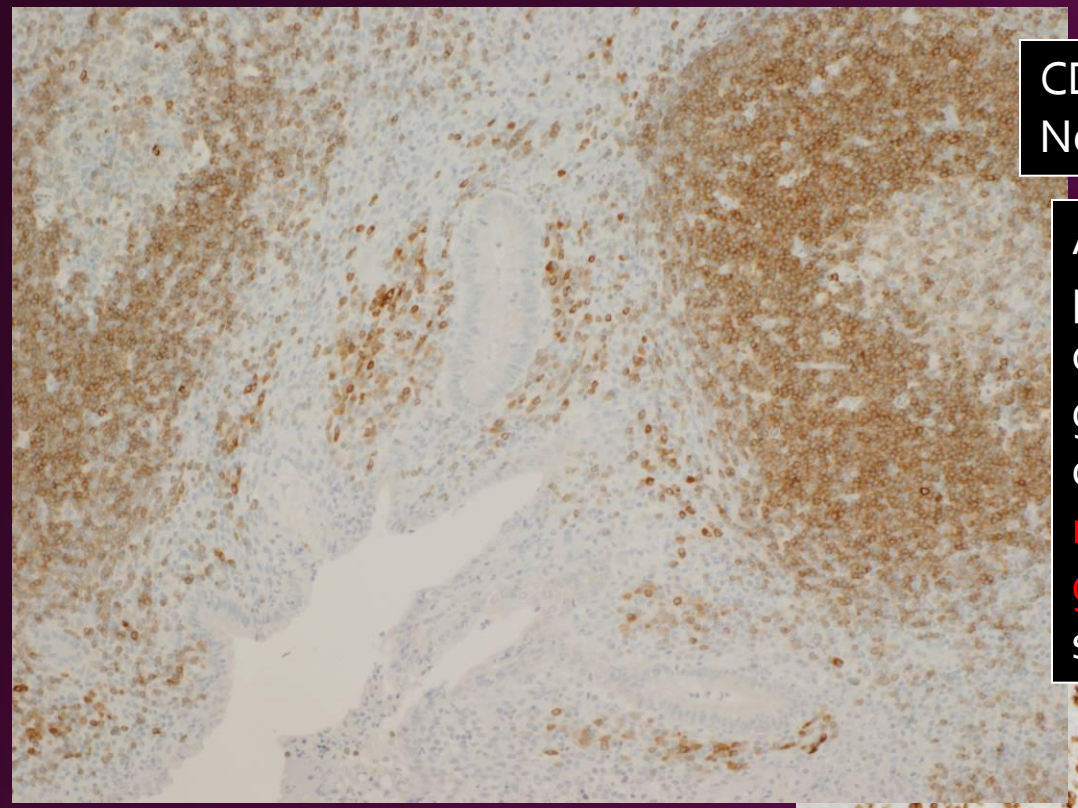
- CD79a
  - Borderline
  - Comments:
    - Weak
  - Suggestions:
    - Consider change of primary antibody and recalibrate
  - Change:
    - Switched antibody from Cell Marque to Ventana
    - Predilute (unchanged)
    - CC1 HIER 32 minutes → CC1 HIER 64 minutes
    - iView detection system → Ultraview detection system

NordiQC RUN 29 - 2010

- CD79a
  - Borderline
  - Comments:
    - Weak
  - Suggestions:
    - Consider change of primary antibody and recalibrate
  - Change:
    - Switched antibody from Cell Marque to Ventana
    - Predilute (unchanged)
    - CC1 HIER 32 minutes → CC1 HIER 64 minutes
    - iView detection system → Ultraview detection system

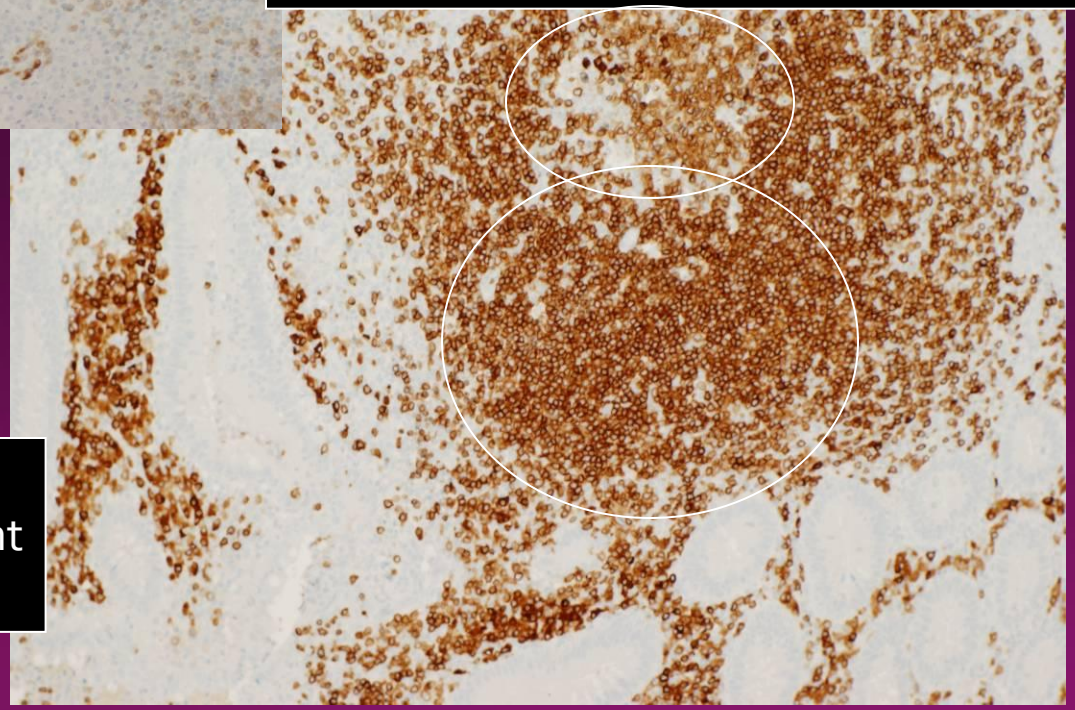
Reassessment: OPTIMAL !

NordiQC RUN 29 2010



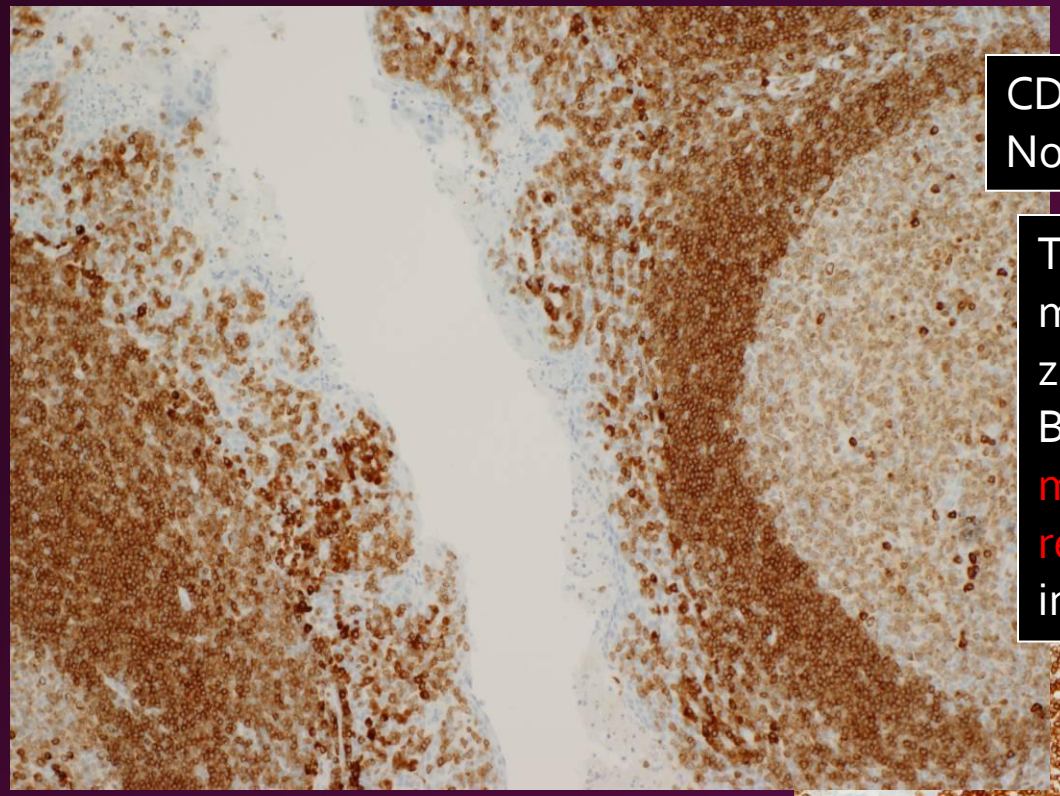
CD79a
NordiQC #1

Appendix – expect strong predominantly membranous staining of the mantle zone B-cells, late stage germinal centre B-cells, and plasma cells with at least moderate membranous staining reaction of the germinal centre B-cells in the secondary follicles



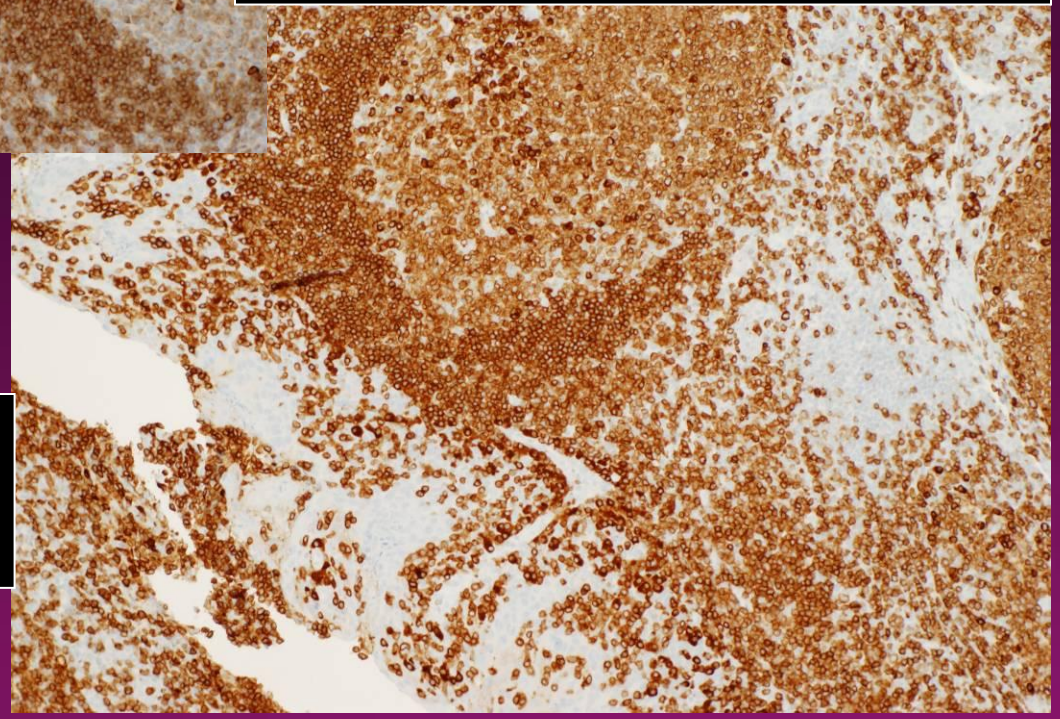
CD79a
Reassessment
NordiQC #1

NordiQC RUN 29 2010



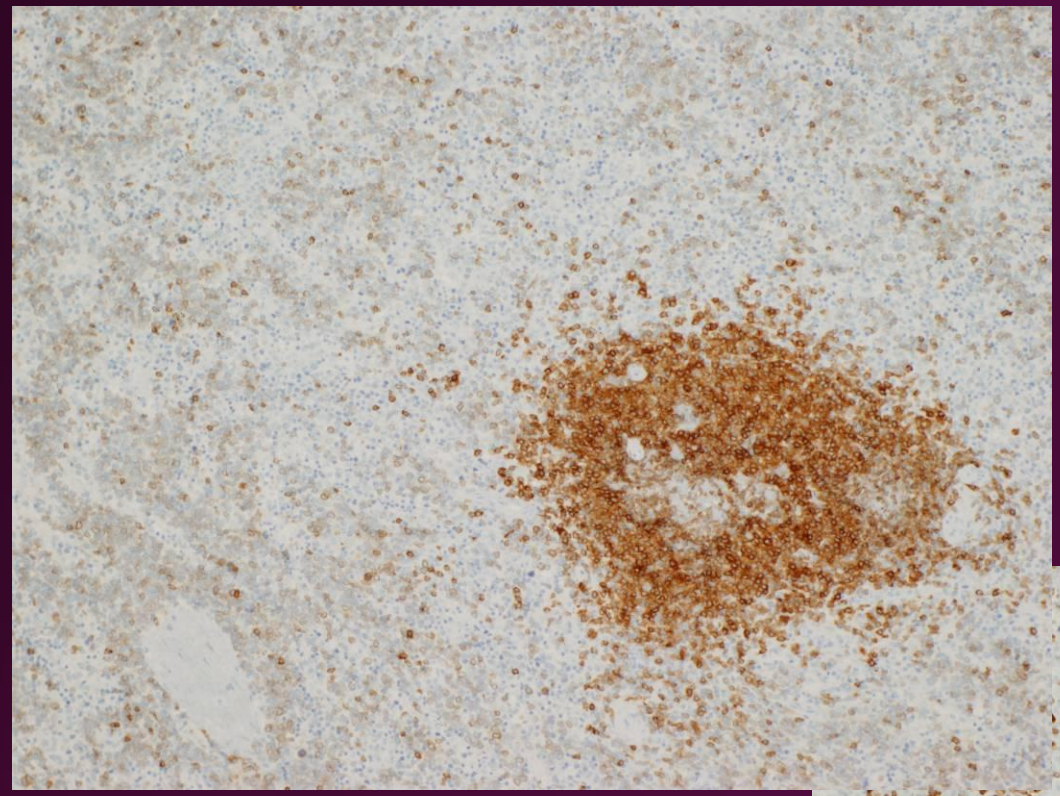
CD79a
NordiQC #2

Tonsil – expect strong predominantly membranous staining of the mantle zone B-cells, late stage germinal centre B-cells, and plasma cells with at least moderate membranous staining reaction of the germinal centre B-cells in the secondary follicles



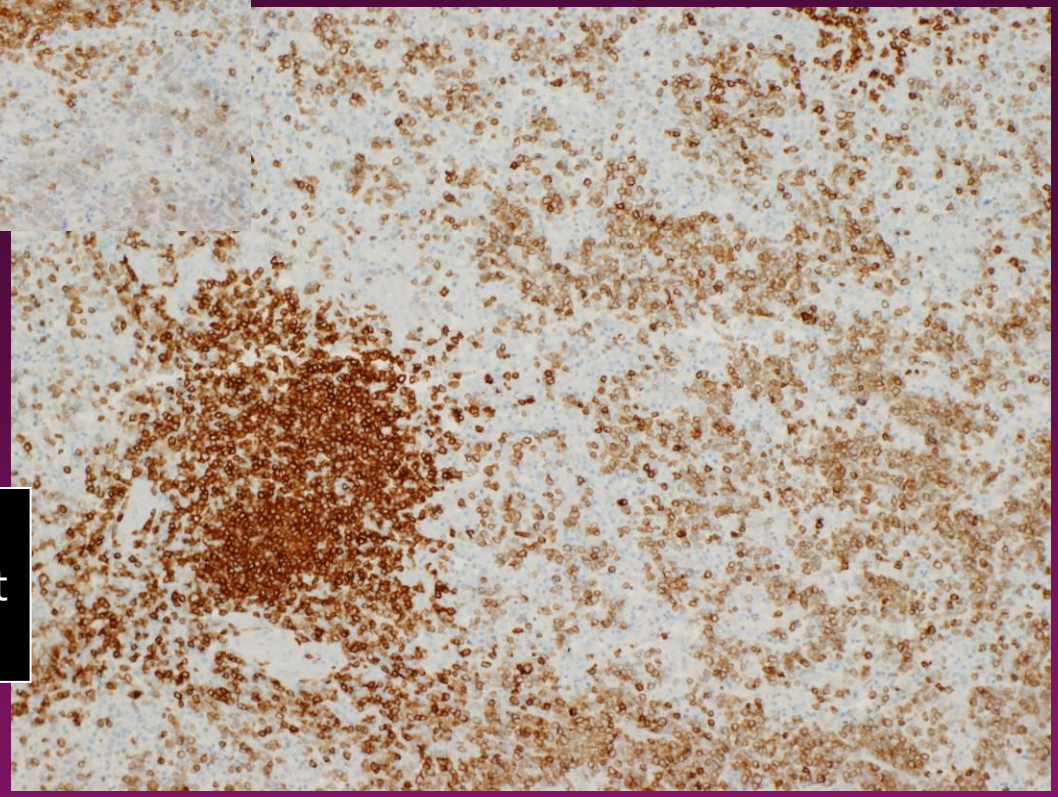
CD79a
Reassessment
NordiQC #2

NordiQC RUN 29 2010



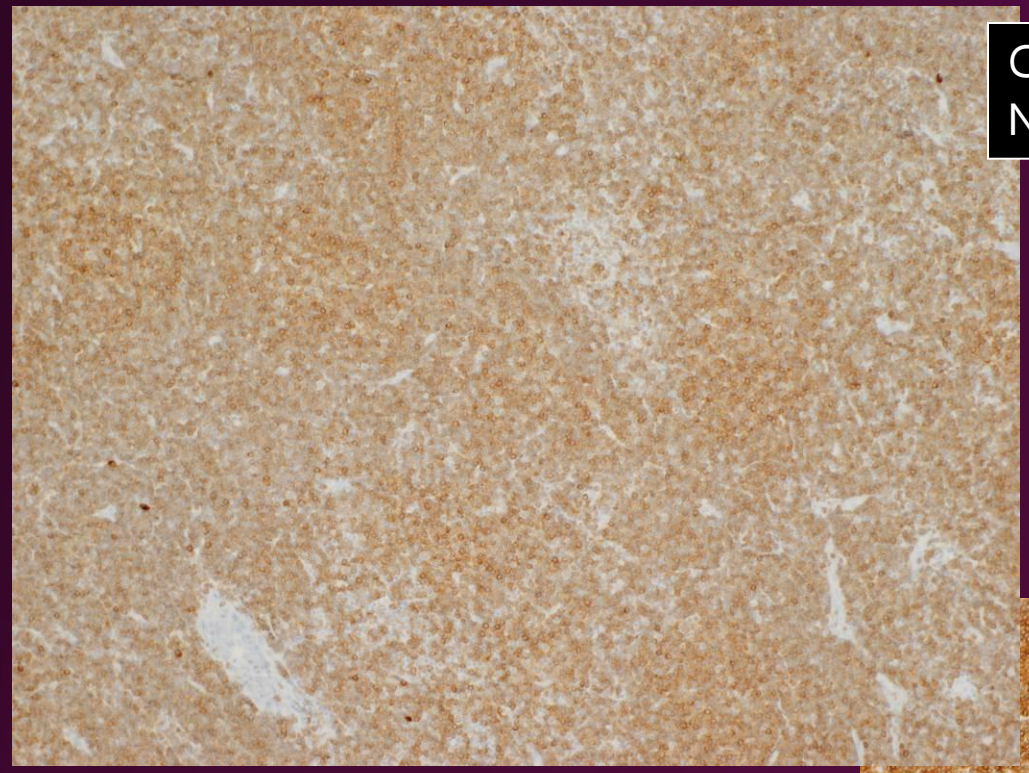
CD79a
NordiQC #3

Pre-B-ALL – expect at least weak to moderate predominantly membranous staining reaction in majority of neoplastic cells



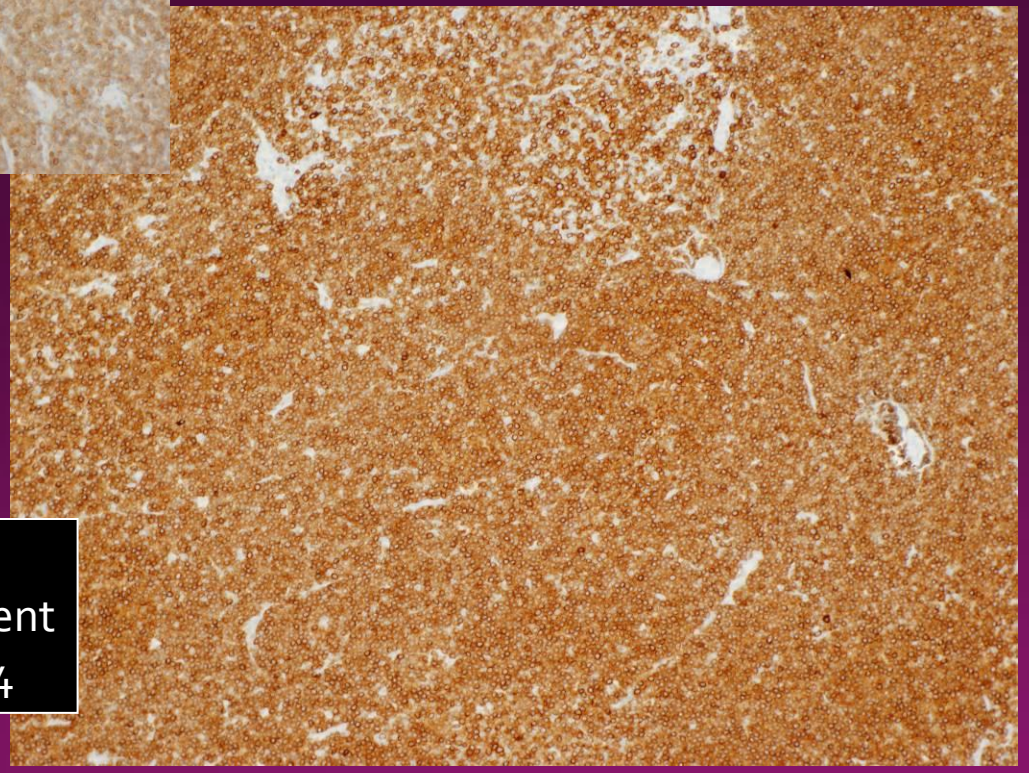
CD79a
Reassessment
NordiQC #3

NordiQC RUN 29 2010

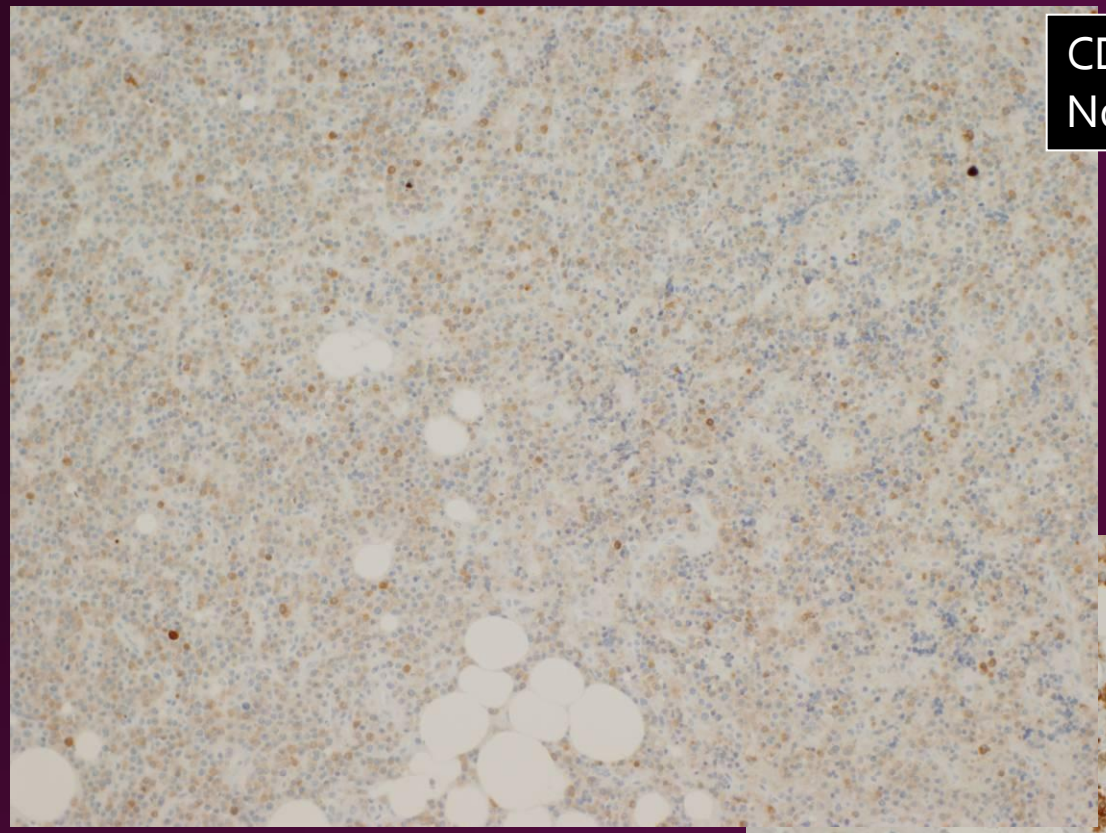


CD79a
NordiQC #4

B-CLL – expect moderate to strong membranous staining reaction in virtually all neoplastic cells

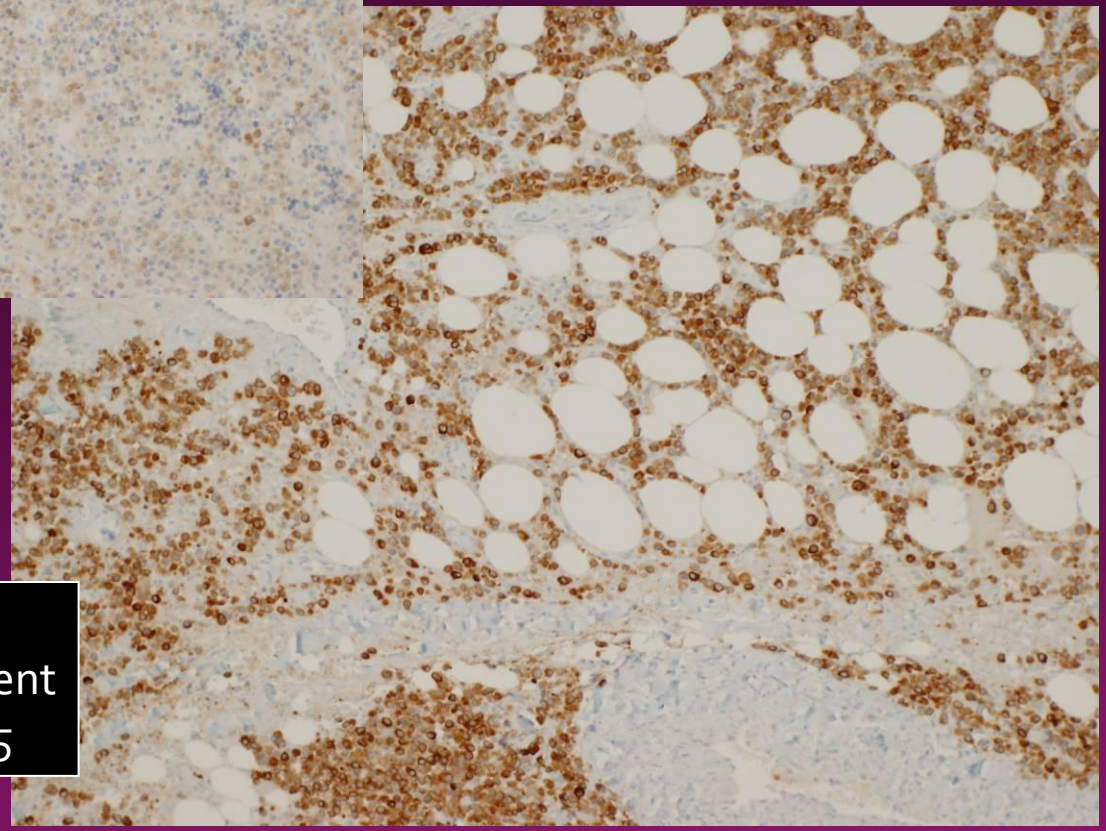


CD79a
Reassessment
NordiQC #4



CD79a
NordiQC #5

Plasmacytoma – expect at least weak to moderate cytoplasmic staining reaction in majority of neoplastic cells



CD79a
Reassessment
NordiQC #5

NordiQC RUN 29 2010

- Participation in external quality assurance programs is essential for ongoing evaluation of the immunohistochemical laboratory

- NordiQC assessments
  - Identify problems with immunohistochemical protocols
  - Provide suggestions for optimization of immunohistochemistry protocols

- NordiQC website
  - Explains expected patterns of epitope expression in normal tissues and neoplasms
  - Suggests optimal protocols for each antibody for a variety of platforms
  - Recommends appropriate control tissue for each antibody

- NordiQC should be used as a reference when
  - Selecting appropriate antibody clones and suppliers
  - Calibrating new immunohistochemical protocols
  - Selecting appropriate control tissue and verifying appropriate expression in these controls


Visits to
www.nordiqc.org
Greenland
Iceland
Canada
North Atlantic Ocean
México
Venezuela
Colombia
Brasil
Perú
Bolivia
Suomi
Россия
Russia
Kazakhstan
Монголия
Türkiye
Iraq
Afghanistan
Pakistan
China
India
Japan
Algeria
Libya
Egypt
Saudi Arabia
Mauritania
Mali
Niger
Chad
Sudan
Nigeria
Ethiopia
Kenya
DR Congo
Tanzania
Angola
Namibia
Botswana
Madagascar
Indonesia
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Afghanistan
Pakistan
Algeria
Libya
Egypt
Saudi Arabia
Mauritania
Mali
Niger
Chad
Sudan
Nigeria
Ethiopia
Kenya
DR Congo
Tanzania
Angola
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Japan
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Papua New Guinea
Indian Ocean

NordiQC Nordic immunohistochemical Quality Control

Home ■ Participation ■ Modules/tests ■ Assessments ■ Epitopes ■ Protocols ■ Techniques ■ Links

NordiQC is an independent scientific organization, promoting the quality of immunohistochemistry by arranging schemes for pathology laboratories, assessing tissue stains, giving recommendations for improvement and providing good protocols.

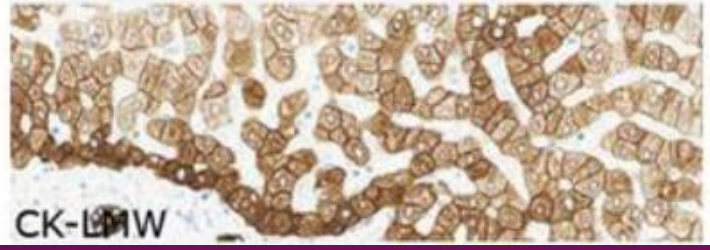
Update: 2013.10.16

Results from Run **38** and the NordiQC **Ki67** web module (Breast Cancer module run B15) are available, click on **Newsletter**

Protocol corrections for run **39, B16** and **H4,** go to **Participation**. Results are available by 7th December

See all 2013 tests on **Modules**.

See planned 2014 tests on **Future modules**



NordiQC

Nordic immunohistochemical Quality Control

Current protocols

Recommended protocols from NordiQC assessment schemes

Among protocols shown to give *optimal* staining results, one or more are selected to cover a spectrum of laboratories, antibodies, protocols and platforms.

Only the latest recommended protocols for each antibody/clone/epitope are listed here. Previously recommended protocols are not listed any longer but may be obtained from NordiQC on request.

Laboratories producing optimal stains are named in the protocols to encourage direct communication. If a laboratory wishes to remain anonymous, this must be specified when protocols are submitted.

mAb = mouse monoclonal antibody, rmAb = rabbit monoclonal antibody, pAb = polyclonal antibody

Protocols Run 38 (June 2013) and B15 (April 2013)

Epitope	Antibody	Platform			
		Dako	Leica	Ventana	Other
CD31	mAb JC70A	CD31-run38	CD31-run38	CD31-run38	CD31-run38
CDX2	rmAb EPR2764Y	CDX2-run38	CDX2-run38	CDX2-run38	CDX2-run38
	mAb DAK-CDX2	CDX2-run38	-	-	-
Cytokeratin, high molecular weight	mAb D5/16 B4	CK-HMW-run38	-	CK-HMW-run38	-
	mAb LL002	-	CK-HMW-run38	-	CK-HMW-run38
	mAb XM26	CK-HMW-run38	CK-HMW-run38	CK-HMW-run38	-
Cytokeratin, low molecular weight	mAb 5D3	CK-LMW-run38	CK-LMW-run38	-	-
	mAb C51	-	-	-	CK-LMW-run38
	mAb CAM5.2	-	-	CK-LMW-run38	-

Nordic Immunohistochemical Quality Control
Institute of Pathology, Aalborg University Hospital, Ladegaardsgade 3, P.O.Box 561, DK-9100 Aalborg, Denmark

Recommended protocol for CD31 (JC70A)

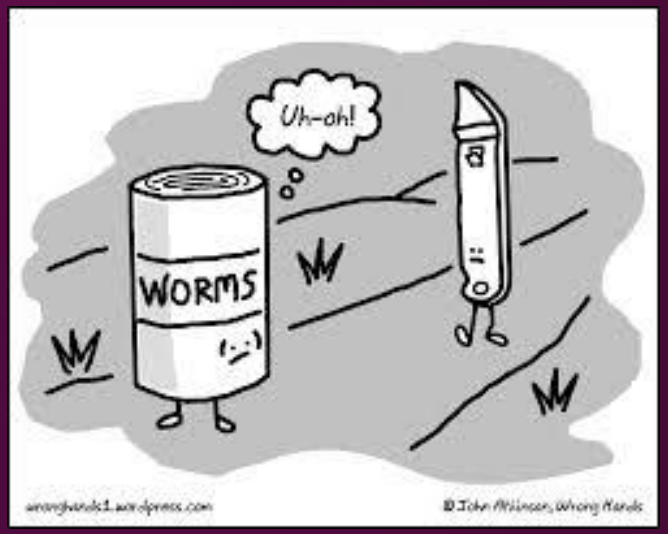
Obtained in General Module, run 38

Primary antibody	
Clone	JC70A
Producer	Dako
Product no. (Lot no.)	M0823 (00083027)
Dilution	1:50
Diluent buffer and additive(s)	Antibody diluent K8006, Dako
Incubation time / temperature	30 min./RT
Epitope retrieval, proteolysis	None
Proteolysis enzyme	None
Proteolysis time	None
Epitope retrieval, HIER	
Device	PT module
Buffer, pH	Target Retrieval Solution pH 6.1, Dako
Warm-up / heating max / resting time	15 min./20 min./15 min.
Maximum heating temperature	97°C
Visualization system	
Method	3-step polymer conjugate
Producer, product no.	Dako, K8002
Incubation time / temperature	15min. + 30min./RT
Chromogen	
Type	DAB
Producer, product no.	Dako, K8002
Incubation time / temperature	10 min./RT
Enhancement, type	CuSO4
Immunostainer	
Type	Autostainer Link 48, Dako

Main Reasons for Insufficient Protocols

- Use of less successful antibody
  - Clone
  - Supplier
  - Ready-to-use formation (predilute)
  - Platform dependent antibody
  - Lot-to-lot variation
  - MAG reaction (cdx2, synaptophysin, CK5/6)
- Inappropriate antibody dilutions
- Inappropriate epitope retrieval
- Inappropriate laboratory performance
  - Endogenous biotin reaction
  - Section drying-out after HIER
  - Technical platform error
  - Unexplained

Recommendations



- Review controls
  - On-slide controls are essential for automated immunostainers
  - Batch controls are not appropriate for automated immunostainers
  - Replace tumor control tissue with normal control tissue
  - Create normal control tissue arrays that incorporate multiple tissue types
    - Positive tissue control: strong expressor (for specificity) and low expressor (for sensitivity)
    - Negative tissue control: non-expressor (for specificity)
  - Negative reagent control required for Avidin-Biotin Detection Systems
  - Negative reagent control no required for Polymer Detection Systems
- Review protocols
  - Replace less successful clones with those recommended for your platform
  - Use HIER instead of proteolysis or no retrieval
  - Use high pH buffer systems rather than low pH buffer systems
  - Avoid Avidin-Biotin Detection Systems (endogenous biotin, low sensitivity)
  - Suggest Polymer Detection Systems (higher sensitivity)
- Participate in external quality assurance programs

- NordiQC evaluations show that up to 33% of participants are still insufficient and suggests problems with quality in the immunohistochemistry laboratory are widespread

- How many laboratories do not realize they have a problem because they do not participate in external quality assurance programs?

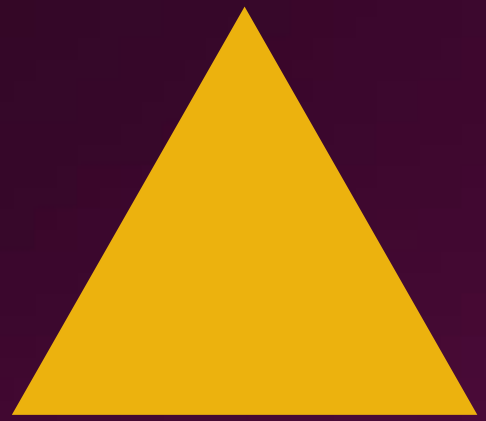
- How many false negative immunohistochemical tests are missed because there is no on-slide control tissue?

- How many companies continue to sell insufficient clones simply because they still have a market for them?

- How many scientific publications are performed with insufficient immunohistochemical protocols resulting in misleading results?

- What is the impact of inappropriate immunohistochemical test results for patient case?

Clinical Information



Morphology

Immunohistochemistry

The End