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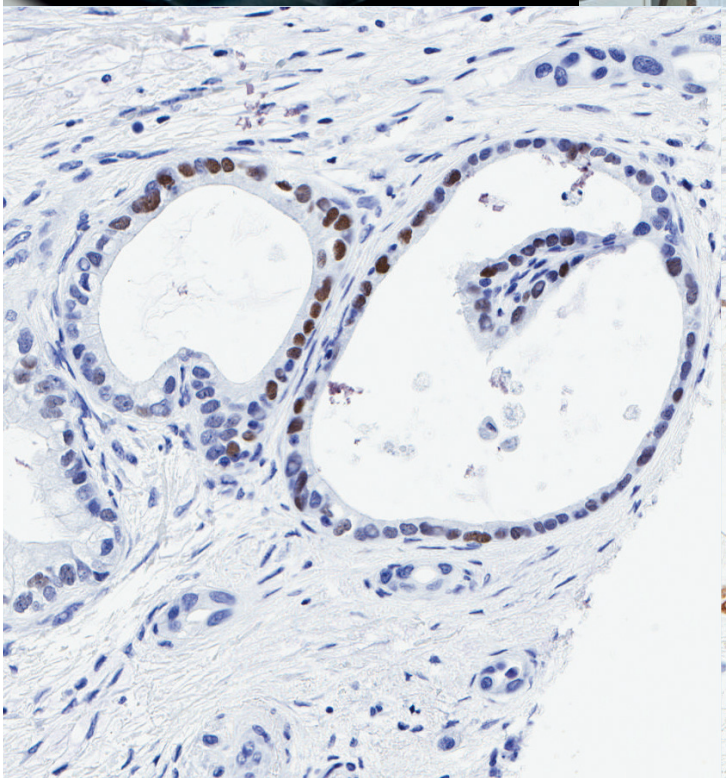
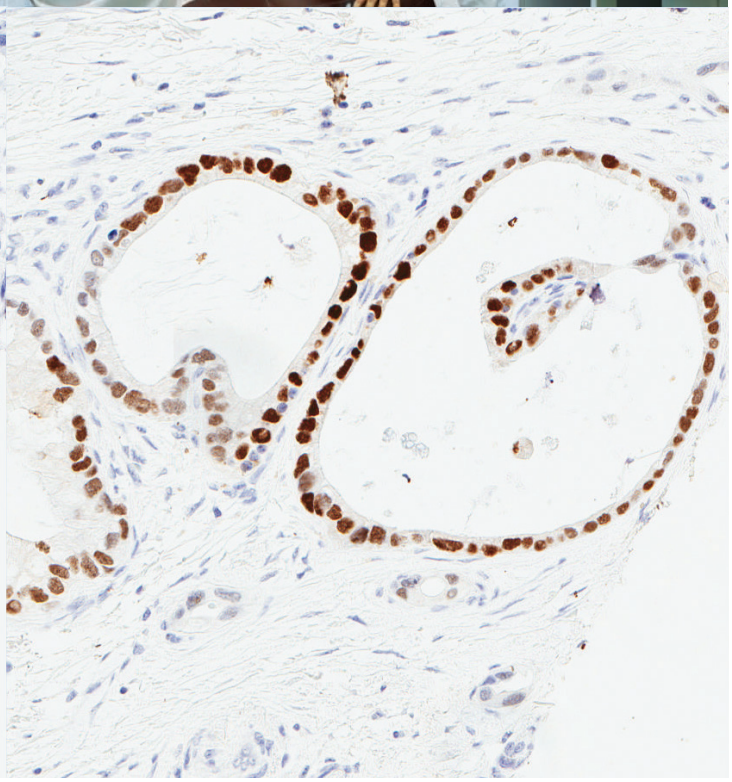
Ensure Accurate and Reliable IHC Results

Around 25% of IHC staining results are insufficient for diagnostic use, and 90% of those are false negatives[1, 2]





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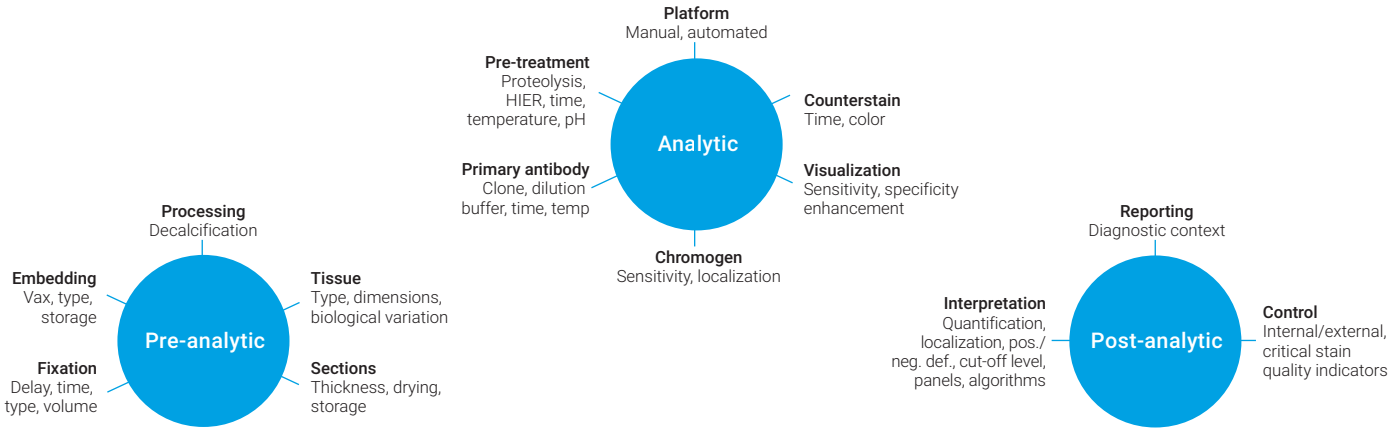
Is ~75% accuracy sufficient when there is a patient behind every stain?

IHC is a multi-layer technique and results are affected by the choices made to set up the test

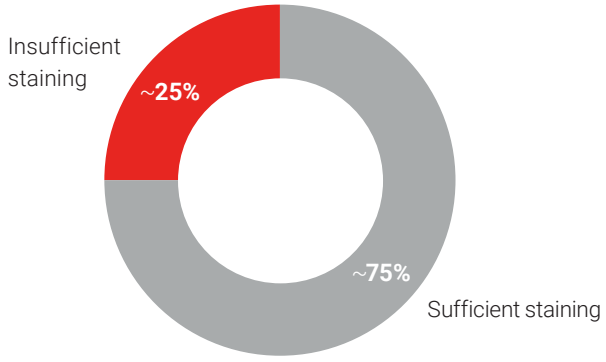
- IHC results are influenced by numerous factors from tissue sampling to interpretation
- With 14 steps in the entire process, and around three options at every step, there are 4.8 million possible combinations for each target
- Since all steps are interlinked, the right choices in each step of the process are vital to secure the right test outcomes



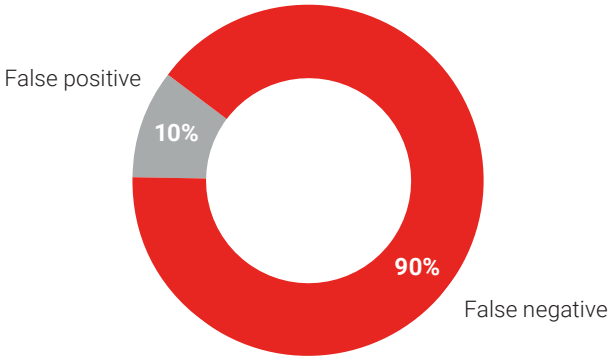
IHC is a powerful and well-established tool, but inaccurate results are a challenge

Data from the internationally recognized EQA* scheme, NordiQC**, shows that about 25% of IHC staining results are insufficient and can, in some cases, lead to inappropriate diagnosis. Of these, a startling 90% were characterized by either too weak or false negative results.[1, 2]

Analytical accuracy of laboratories' IHC tests[1]



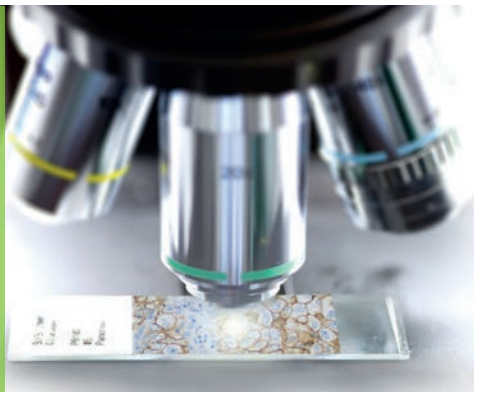
90% of insufficient results characterized by too weak or false negative results[1]



* *EQA: External Quality Assessment.*

** *NordiQC is a professional and scientific organization independent of economical or political interests. Pathology laboratories are invited to participate in the schemes and enrol by following the instructions at this website. See https://www.nordiqc.org/about.php for more details.*

Robust Assays Should Capture the Full Expression Range



High and low expressors are of equal importance in IHC staining[3]

Two tumors of the same type can have a variable expression level of a given protein. Visualizing the full range of tumor expressions is therefore a prerequisite for correct diagnosis in order not to miss the low expressing tumors.

As the image below shows, staining performance for high expression tissue structures may seem very strong or too intense, but that is needed to detect the low expressors.

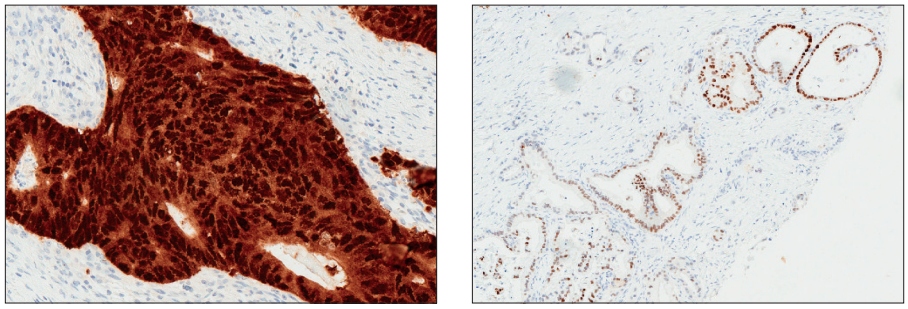
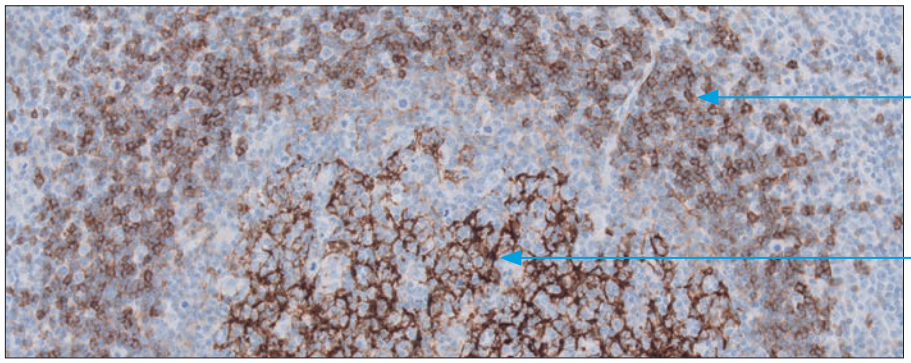


Figure 1. High expression staining image of colon adenocarcinoma (left), and low expression staining image of pancreas adenocarcinoma.

Tissue structures in non-neoplastic tissue can capture high and low protein expression levels:

- Tumors have heterogeneous protein expression, so identifying the optimal low expression tumor for assay calibration is difficult
- High and low expressing normal tissue has been identified for many different antibodies and the usage has been confirmed by EQA schemes and scientific publications



Low expressor: B cells in the mantle zone, weak to moderate staining reaction.

High expressor: Follicular dendritic reticulum cells in the germinal centers, moderate to strong staining reaction.

Figure 4. High and low expressing structures for CD23, in tonsil stained with GA781, FLEX Monoclonal Mouse Anti-Human CD23 Protein, Clone DAK-CD23, Ready-to-Use (Dako Omnis)

Low expressed tissue structures are sensitive to protocol modifications

- For optimal protocol calibration, sensitive tissue structures are needed to ensure the right analytical sensitivity and specificity
- This is important to secure that staining is detected in the full analytical range in the routine setting

High and low expression structures should be identified to minimize variation in tumor heterogeneity.

Reliable tissue controls are critical for monitoring IHC test reproducibility and accuracy

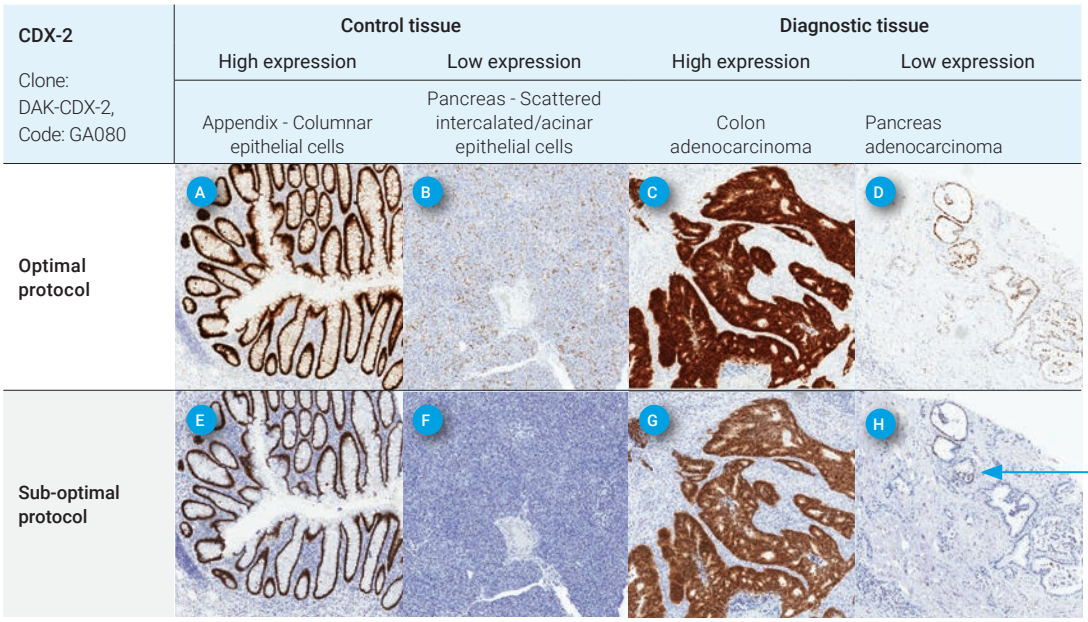
Mirroring the full expression range in the control tissue is important for reliable diagnostic results.

Appropriate tissue controls must therefore be used to monitor test correctness.

- Tissue controls with **high expression** levels should be used to confirm that the correct antibody was applied and that an adequate general technical quality was obtained
- Tissue controls with **low expression** levels can be used to confirm successful demonstration of the lower limit of detection and to minimize the risk of false negatives

Capturing both high and low expressions is necessary to minimize the risk of false negative results

Appropriate controls are needed both for protocol calibration and routine testing. Detecting the full protein expression range is critical to detect low expressors, even when staining performance may seem very strong or too intense for high expressors.



Staining signal in low expressing clinical tissue is almost lost when using sub-optimal protocol giving a is a higher risk of false negative.

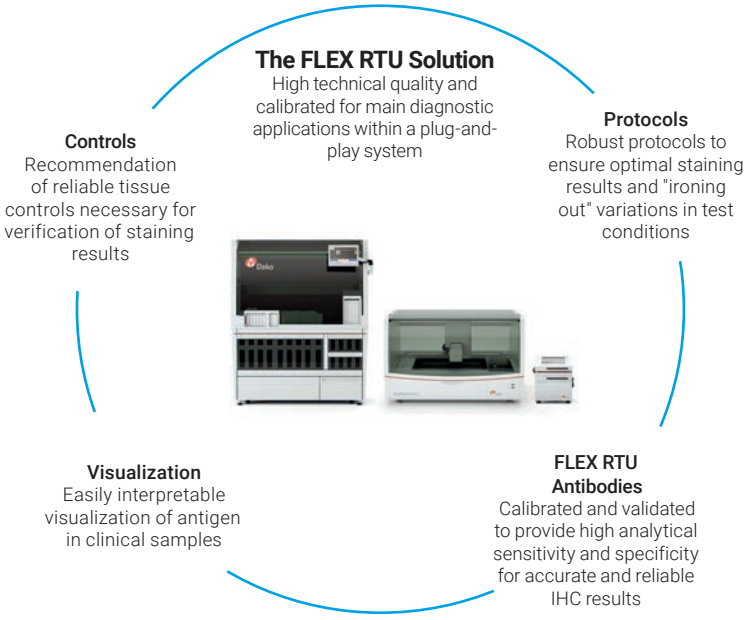
Figure 4. Examples of tissue stained with an optimal and sub-optimal protocol: (**A**) Control tissue with high expression show very strong staining using the optimal protocol. (**E**) Sub-optimal protocol decreases staining intensity. (**B**) Protocol parameters were optimized to include positive staining of low expressed tissue structures. (**F**) Decreasing the intensity resulted in a lack of staining for the low expression tissue with the sub-optimal protocols. (**C** and **G**) The sub-optimal protocol had no diagnostic implication for highly expressed antigens, but increased the risk of misdiagnosis on low expressed antigens (**F** and **H**).

Accurate and Reliable Staining Results Made Easy, Slide After Slide

The FLEX Ready-to-Use (RTU) Solution supports your lab in reducing the risk of false negative results

The FLEX RTU Solution provides your lab a simple and effective approach to the most difficult choices in the IHC staining process:

- Robust IHC tests based on carefully selected clones calibrated and validated for reliable diagnostic use, ensuring that the antigen is correctly demonstrated at both high and low expression levels in tissue
- The RTU antibodies are accompanied by appropriate plug-and-play protocols to provide reliable and reproducible diagnostic results
- The EnVision FLEX visualization system enables robust and clear signal amplification
- The FLEX RTU Atlas of Controls provides precise recommendations for appropriate tissue controls to verify staining results



Diagnostically important RTU IHC tests. Calibrated and validated for reliable diagnostic use. Provided as total plug-and-play solutions facilitating implementation for clinical use.

Delivering accurate staining results and improving time to diagnosis

The FLEX RTU Solution was developed in close collaboration with leading pathologists and lab managers.

The expert panel specified the required criteria and staining performance for each individual antibody.

Based on these guidelines, we developed a standard procedure and individual, but aligned, protocols for all FLEX RTU primary anitibodies that increase productivity without compromising the staining performance defined by the panel.

The Atlas of Controls

The Atlas of Controls provides recommendations for selection of tissue controls and examples of accurate reaction patterns required to confirm that a correct level of analytical sensitivity is obtained in each test.

The FLEX RTU delivers documented staining accuracy

The NordiQC assessment shows that the combination of robust IHC assays calibrated to capture the full protein expression range and with appropriate tissue controls delivers reliable diagnostic results.

Table 1. Four MMR markers evaluated by NordiQC

Run	# of labs	% of labs using vendor protocols (as is)	Performance with vendor protocols	% of labs modifying vendor protocols	Performance with modified vendor protocols
MLH1 run 56 2018	35	43% used Dako protocol	100% received sufficient or optimal	57% used a modified Dako protocol	90% received sufficient or optimal
MSH2 run 57 2019	66	21% used Dako protocol	100% received sufficient or optimal	79% used a modified Dako protocol	87% received sufficient or optimal
MSH6 run 52 2018	42	48% used Dako protocol	100% received sufficient or optimal	52% used a modified Dako protocol	91% received sufficient or optimal
PMS2 run 53 2018	41	49% used Dako protocol	100% received sufficient or optimal	51% used a modified Dako protocol	95% received sufficient or optimal

Less optimization needed when using FLEX RTUs

The Dako-recommended protocols generally performed better than the laboratory-modified protocols. There is therefore no need to optimize Dako protocols before validation if high and low expressors are identified in tissue processed according to the pre-analytical setup in the lab.

Of the labs assessed by NordicQC, 21% to 49% of the labs used the Dako-recommended protocol without modifications and achieved a sufficient/optimal pass rate of 100%. Labs which used a modified protocol achieved a pass rate of 87-95%.

Diagnostic Tech Support & Application Consultancy

Our local teams of highly skilled and experienced Application Consultants are ready to help you ensure optimal staining performance by supporting you in implementation, product training, and if needed, protocol optimization.

References

1. Nielsen S. External quality assessment for immunohistochemistry: experiences from NordiQC. Biotech Histochem 2015;90:331-40.
2. Mogens V, Nielsen S. Proficiency testing in immunohistochemistry—experiences from Nordic Immunohistochemical Quality Control (NordiQC). Virchows Arch (2016) 468:19–29.
3. Cheung CC, D'Arrigo C, Dietel M, et al. Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4: Tissue Tools for Quality Assurance in Immunohistochemistry. Appl Immunohistochem Mol Morphol 2017;25(4):227-230.

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