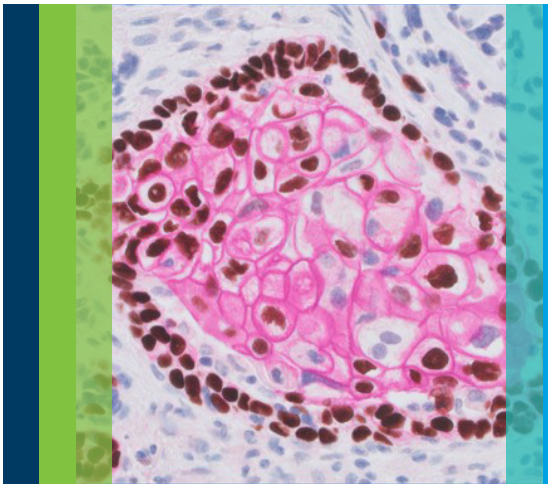

Agilent Case Study: Dako Omnis Solution

Double Staining IHC on Agilent Dako Omnis

Agilent Dako

More results with fewer resources, and positive impact on workflow.

Double immunohistochemistry (IHC) staining[1] on Dako Omnis utilizes two primary antibodies and two different color chromogens in a sequential double staining procedure. The two contrasting chromogens, DAB and HRP Magenta, delineate the presence of the different targets resulting in the combined visualization of the two target antigens on a single tissue section.

By producing double IHC stains on the same tissue slide, less tissue is used from the patient sample, which can be particularly relevant with small biopsy specimens or small areas of interest within a larger sample. This is especially useful when further testing will require use of the same sample, e.g. in molecular diagnostic studies. A reduction in the number of slides to be cut, stained, interpreted, and finally archived – while still providing the same amount of diagnostic information as multiple slides – supports workflow, throughput, and storage efficiencies for laboratories that adopt multiplex IHC staining.

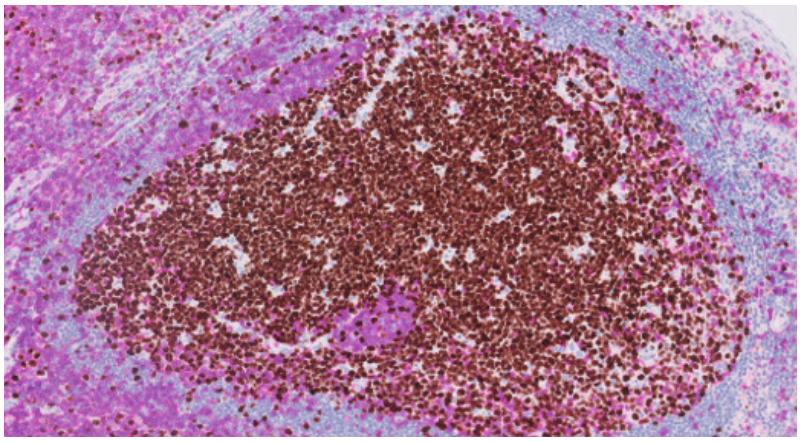

Figure 1. DAB: Ki-67 (Code GA626), nucleus, germinal center B cells of the dark zone, basal and parabasal squamous epithelial cells. Magenta: CD3 (Code GA503), cytoplasm and membrane, T cells in interfollicular areas and in germinal centers.

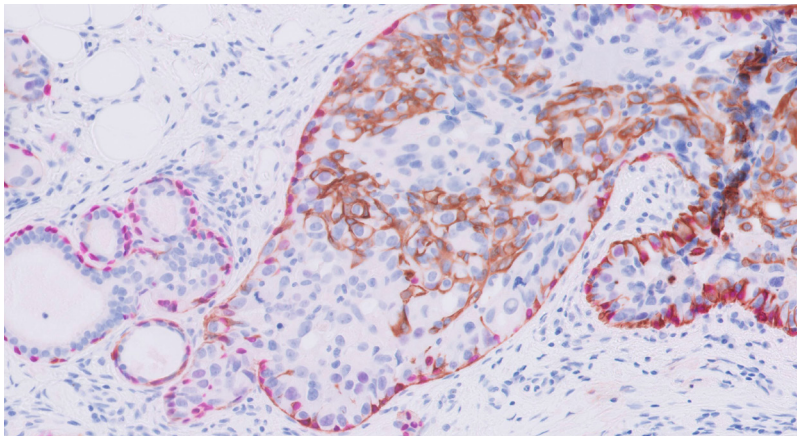

Figure 2. Magenta: p63 (Code GA662), nucleus, basal epithelial/myoepithelial cells of nonneoplastic ductal tissue and preserved basal epithelium of ducts with neoplastic growth. DAB: CK 5/6 (Code GA780), cytoplasm/membrane, focal expression in benign intraductal, proliferative cells surrounded by ductal carcinoma in situ (DCIS – negative for CK 5/6).

Agilent

Trusted Answers

Double staining on a Dako Omnis in Canadian pathology laboratories

A study was launched in Canada to evaluate the uptake of double IHC staining on Dako Omnis and its impact on workflow within the labs. At the time of the study, Dako Omnis instruments were installed in 23 laboratories.
Of these 23 labs, 15 labs were performing double staining using DAB and Magenta, in a sequential IHC protocol.

Table 1. Distribution of double stained slides from July 2020 to January 2021 in eight Canadian pathology labs.

Laboratory	Single IHC Slides	Double IHC Slides	% Double stain slides	Double stain test menu	Slides/ Dako Omnis
Lab 1	92005	3627	4%	3	16870
Lab 2	72522	2543	4%	5	15273
Lab 3	35604	699	2%	4	12595
Lab 4	32258	1702	5%	15	11532
Lab 5	30650	546	2%	5	16689
Lab 6	26502	2160	8%	5	10188
Lab 7	17190	949	6%	1	9374
Lab 8	10578	2588	24%	5	6598

Survey on perception of double staining on Dako Omnis

A Voice of the Customer survey was created, and eight labs were randomly selected from the 15 eligible labs. Individual interviews were conducted with the labs to determine how the quality and workflow impact of double staining on Dako Omnis is perceived and to compare this with the laboratory's metrics for workflow.

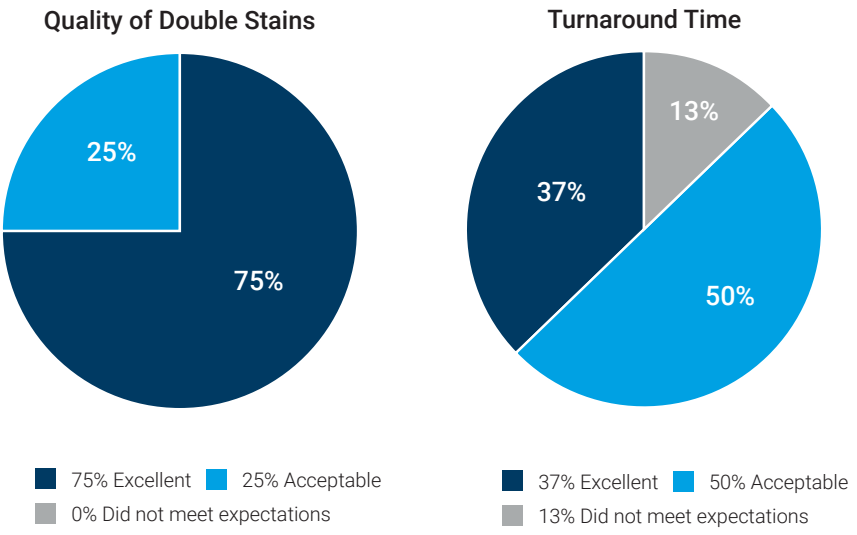

Figure 3. All labs reported the quality of the double stains produced using the combination of DAB and Magenta as good, with 75% assessing the results as excellent and 25% assessing the quality as acceptable. 87.5% of labs interviewed felt the slide turnaround time for double stains was good and met their expectations.

Interestingly, all labs stated that their pathologists would request more double stains to be added to the antibody menu if possible. This indicates that there is a demand for this type of testing by the pathologist staff.

Double Staining on Dako Omnis

Dako Omnis uses a sequential IHC staining method to produce double stains using a combination of the chromogens DAB and Magenta. Both DAB and Magenta are horseradish peroxidase (HRP)-catalyzed chromogens, meaning they function with the same HRP-based visualization system to give permanent staining results that do not require extra steps or special handling post staining[2]. Double IHC staining with Magenta on Dako Omnis requires the addition of only two extra vials when running EnVision FLEX visualization system for both DAB and Magenta, consuming very little of the instrument's 60 reagent positions.

Through customer interviews, these evaluation criteria were identified as critical for adoption of double staining IHC:

Critical requirements for implementation of double staining

- High-quality stain with clear color separation
- Turnaround time (TAT) that supports patient case management
- Workflow that minimizes impact on other stains
- Support and education around development and implementation

Analysis of double staining's impact on turnaround time

While all laboratories appreciated the quality of the stains produced with DAB and Magenta, one of the labs in the study believed there was a negative impact on their slide throughput due to the double stains. Another lab reported acceptable on the questionnaire yet indicated in the interview that they felt the double stains slowed down other single stains and extended their TAT without benefit. Interestingly, the labs with a negative perception also had the lowest volumes of double stains, while labs doing more double stains were also happier with double staining on Dako Omnis and the impact on workflow.

To dive deeper into why labs doing a higher volume of double stains were more positive about their experience, detailed slide information was pulled from the Dako Omnis database for each of the eight labs on four calendar days spanning from July of 2020 to January of 2021. Data was analyzed for each lab evaluating the TAT of patient cases, instrument capacity, slide rack utilization, slide availability, and rack loading pattern.

A simulator tool was developed to create charts where the daily workflow could be visualized easily.

Optimal use of double staining on Dako Omnis

It was found that mixing the double stain racks alongside the single stain racks throughout the day resulted in better workflow, with more available instrument capacity and an improved TAT compared with those labs only running the double stains overnight.

To further evaluate the hypothesis that more slides stained as double stains equaled better workflow, simulations were created for all eight labs showing the potential workflow impact if double staining was increased further. In the simulation, new double stains were 'created' for certain antibody combinations being frequently ordered together. This produced a customized recommendation of potential stain combinations built around each lab's unique ordering pattern. These customized combinations were then inserted back into the database and the corresponding single stains removed. Next, slide availability and loading times for the new double stains were matched to the original times as closely as possible. The resulting simulated daily slide flow of cutting, loading, staining, and unloading was then charted. The results were evaluated for impact on TAT and instrument capacity (see Table 2).

Table 2. Simulated effect of adding more double staining slides to existing workflow. Data based on the average of four selected days in the period July 2020 to January 2021.

Laboratory	Original workflow	New workflow	Avg. reduction in #racks	TAT change for affected racks
	Avg. Racks/Day	Avg. Racks/Day		
Lab 1	166.0	156.0	6%	Shorter TAT
Lab 2	132.8	125.0	7%	Most reduced to same day TAT
Lab 3	60.5	58.5	3%	Shorter TAT for most cases
Lab 4	50.0	46.0	9%	Shorter TAT for most cases
Lab 5	49.3	49.0	1%	Shorter TAT for some cases
Lab 6	62.3	56.8	12%	No change
Lab 7*	30.1	29.5	3%	No change
Lab 8	29.5	27.8	7%	No change

*This lab had a highly randomized ordering pattern. In order to identify enough possible combinations of potential double stains, an additional four days had to be added to the study.

The individual trial results ranged from no impact in labs that had a highly randomized ordering pattern resulting in few potential combinations to convert to double stains, and up to an 18% reduction in the number of racks needed on a single day in lab 4. The average over four days ranged from 1 to 12% fewer racks to be run if compatible single IHC stains were converted to double IHC stains. No negative effect on TAT could be identified in any of the labs.

Instrument capacity is a workflow metric used to evaluate the number of slides running on an instrument at any given time compared with the total number of slides the instrument is able to handle. How 'full' an instrument is, along with the number of available instruments, has a direct impact on the lab workflow by determining when slides can be loaded into available slide positions, which ultimately affects patient case TAT.

The simulation study showed that workflow efficiencies could be gained by increasing the number of double stain slides. Fewer slide racks need to be loaded due to the reduction in the number of single stain slides, which translates to less handling time. With fewer slide racks to be loaded, it would also open up additional capacity within the instruments, creating room for racks to be loaded earlier than had been done in the original day. Being able to load slides sooner would result in earlier staining completion times.

Double IHC staining offers the opportunity to improve efficiency through increased instrument capacity while still producing the same number of "test" results. In laboratories with constraints in human resources, operational space or funding for additional instrumentation, double staining is a way of handling the increased workload without additional resources.

The staining protocols for double IHC stains do take longer to complete compared with single IHC stains, but this amount of time was offset by the earlier available loading times for both the double and single IHC slides. This resulted in a reduction in the simulated turnaround time of the patient cases during the simulation.

Conclusions

The results of the study showed that double staining IHC on Dako Omnis can have a strong positive impact on a laboratory's workflow and help achieve more results with fewer resources.

Double staining on Dako Omnis

- Produces high-quality IHC results using two chromogens
- Increases slide capacity without additional instrumentation
- Has the potential to reduce patient case turnaround time
- Conserves patient tissue while maintaining the same diagnostic information
- Reduces hands-on time as fewer slides are handled
- Requires only two extra vials with limited impact on instrument reagent capacity

References

1. Silverstein, A.M. Contrasting fluorescent labels for two antibodies. J. Histochem. Cytochem. **1957**, 5 (1), 94-95. https://journals.sagepub.com/doi/pdf/10.1177/5.1.94-b.
2. Petersen, K. H.; Lohse, J.; Ramsgaard, L. Automated Sequential Chromogenic IHC Double Staining with Two HRP Substrates. PLoS One **2018**, 13 (11), e0207867. https://doi.org/10.1371/journal.pone.0207867

www.agilent.com

D71686_02

Published in the USA, April 12, 2023
29498 2023MAR23

Agilent
Trusted Answers