Nordic Immunohistochemical Quality Control

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Abstract   The use of immunohistochemistry by pathologists has been steadily, almost logarithmically increasing during the last decade. There is no scientific indication that this trend will reverse or slow down. At the same time, quality control issues have not followed this tempo. In particular, external quality control has been neglected. In 1999, nine Scandinavian laboratories established organization, Nordic Quality Control (NordiQC), to provide external quality control in immunohistochemistry for Denmark, Norway, Sweden, and Finland. This commentary briefly describes the work of this organization.

Immunohistochemistry (IHC) is widely used in routine diagnostic work and is a very common part of scientific reports in pathology and cytology, and its outcome is the basis of an expanding number of tumor diagnoses. Despite these facts, its standardization still lags behind (1,2). Since the IHC method is far from being standardized, staining quality can vary greatly among different laboratories depending on the technical expertise and protocols employed (3,4). Ultimately, the reported results and the diagnosis will not only rely upon the technical aspects of the staining, but also on the interpretation of the results. This is well illustrated by the study of Rhodes et al (5,6) and Balaton et al (7), who showed that the main problem in detection of estrogen and progesterone receptors is a technically suboptimal protocol, whereas one of the main problems in Her2/neu detection also originates in inappropriate interpretation of the results (8). Whereas internal quality control (IQC) procedures are essential for the reproducibility of the IHC performance in the indi-vidual laboratory, they will not necessarily identify a poorly calibrated IHC system giving insufficient staining (9). In contrast, external quality assessment (EQA) – a system which retrospectively and objectively compares staining results from many laboratories by means of an external agency, allows the identification of insufficient stains and inappropriate protocols, as well as identification of possible interpretation problems (8,9). In general, there are major benefits of external quality assurance which cannot be achieved by internal quality control programs alone. External quality assurance allows comparison of performance and results, serves as an early warning system for problems, identifies systematic kit problems, provides objective evidence of laboratory quality, serves as an indicator of where to direct improvement efforts, and identifies training needs. Therefore, just like for any other clinical laboratory testing, external quality assurance should be implemented in clinical immunohistochemistry laboratories.

Standardization vs Optimization

Since the results of immunohistochemical testing depend on preanalytical (fixation, tissue processing), analytical (staining methods), and postanalytical parameters (interpretation), the question is what needs to be standardized and what can be standardized at this time. The need for more stringent standardization of methods in immunohistochemistry of prostate lesions was recently stressed by Varma et al (10), even though no attempt was made to compare the results of immunostaining. In our opinion, it is the standardization of immunostaining results and standardization of the interpretation of the results that needs to be achieved. Any method that provides optimal results should be acceptable in clinical immunohistochemistry. At this point in time, fast development of new antibodies and detection systems does not allow standardization of methods. In general, it does not seem possible to standardize all the steps that have an influence on the results in clinical immunohistochemistry. Therefore, at this time, it may be more realistic to strive to optimize procedures, rather than standardize them.

By circulating serial sections from multi-tissue blocks to a large number of laboratories, only the analytical conditions, ie protocol steps, are involved, allowing a direct comparison between stains from many laboratories and hence identification of multiple parameters influencing the staining quality. Hence, external quality assurance may supply laboratories with guidelines on how to improve IHC staining if necessary.

NordiQC Organization

In the United Kingdom, the National External Quality Assessment Scheme for Immunocytochemistry (UK-NEQAS-ICC; *www.ukneqasicc. ucl.ac.uk*) has for several years carried out IHC quality assurance for about 450 laboratories in the United Kingdom, where it is compulsory, as well as in other countries. Some of their results were summarized in the publications mentioned above (5-8). In several Nordic and other countries, local networks for quality assurance have also been established. At the same time, the number of antibodies and methods has expanded tremendously, and due to limited capacity in many laboratories it may be increasingly difficult to keep up with quality demands. It is also very important to take into account that standards are not invariable items, but must be adjusted in parallel with the development in knowledge and technical possibilities. Protocol optimization based on updated standards accomplished by EQA is also of increasing importance because of the direct therapeutic consequences of detection of stand-alone markers, e.g., estrogen receptor, HER2/neu, and CD117. This has called for new EQA initiatives.

In 1999, nine pathology laboratories in Denmark, Finland, Norway, and Sweden met to find suitable means for optimizing methods and improving results of clinical IHC in the Nordic countries with initial support from DakoCytomation Norden. After a number of small test runs and two test runs open to all Nordic laboratories, Nordic immunohistochemical Quality Control (NordiQC) was established as an independent non-profit organization in January 2003 with Institute of Pathology, Aalborg University Hospital, as its domicile. From 2004, the capacity has been expanded to include a few laboratories outside the Nordic countries.

NordiQC is managed by a core group of four pathologists (one pathologist each from Denmark, Sweden, Norway, and Finland). Two histotechnicians have also been appointed.

The purpose of NordiQC is to arrange schemes for immunohistochemical stainings and provide examples of optimal stains and recommended protocols, as well as other information including descriptions of epitopes and technical solutions, primarily at the website *www.nordiqc.org*.

The NordiQC EQA consists of three annual runs, each catering for five markers selected among those commonly used for diagnostic purposes in pathology departments. Participants enroll by completing a web-based questionnaire detailing the technical variables. Multi-tissue blocks are made from several normal and tumor tissues selected to include cells with varying content of epitopes. For each marker to be demonstrated, two unstained slides are circulated to the participating laboratories, which are requested to perform stains using their standard protocols. The stains returned are assessed by a panel of four consultant pathologists and one technician, all experienced in assessing IHC slides. Each stain is by consensus marked as optimal, good, borderline, or poor, based on the staining intensity and localiza-

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tion in cells expected to stain, background staining, signs of cross-reactivity, and counter-staining.

The overall staining results are presented at the website *www.nordiqc.org*, together with an analysis of the protocols pointing out variables that are considered to be of importance for the staining quality. The origin of optimal stains and the associated protocols are published, encouraging technicians and pathologists to communicate directly. Individual scores are sent to all participating laboratories by e-mail. In the case of borderline or poor marks, specific suggestions for improvement are also included and the laboratories are offered reassessments.

As of November 2004, eight runs have been accomplished, comprising staining of 41 different epitopes which are frequently used by pathologists (for the list see *www.nordiqc.org*). The

number of participants has expanded from about 50 in the first runs to about 85 in the latest. The annual fee for participating in the EQA is DKK 5,500 (approximately €740).

A total of about 2,500 stained sections were assessed. The overall assessment results were: 35% – optimal, 33% – good, 21% – borderline, and 12% – poor. The overall results from runs 5 to 10 with 23 epitopes are summarized in Table 1.

Table 1. Examples of the staining results for testing runs with 23 epitopes

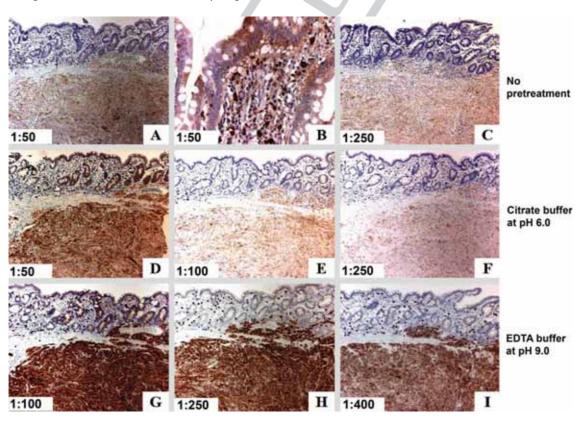| Testing run	Staining results (% laboratories)			
	poor	borderline	good	optimal
Run 5	5	20	40	35
Run 6	8	17	35	40
Run 7	10	20	30	40
Run 8	10	20	38	32
Run 9	20	20	30	30
Run 10	15	15	30	40

Figure 1. Testing of different conditions for CD117 (clone A4502) show that no pretreatment is necessary to achieve some staining of the GIST tumor of the small bowel and mast cells in the lamina propria (**A-C**). The staining of the tumor is rather weak, but further concentration of the primary dilution is not recommended because significant background is already present at the current dilution (**B**). To achieve results with no background, the primary antibody had to be further diluted. At the dilution with no background, no definite specific staining can be appreciated (**C**). However, by using heat-induced epitope retrieval in the microwave for 20 minutes in either citrate buffer at pH 6.0 (**D-F**) or EDTA buffer at pH 9.0 (**G-I**), much better signal to noise ratio is achieved and primary antibody can be further diluted resulting in better quality of the results with lower cost. The best results were achieved with primary dilution of 1:250 and pretreatment in EDTA buffer pH 9.0 (**H**).

The results of multiple testing runs show that, almost independent of epitope tested, about 1/3 of the laboratories will have optimal staining, 1/3 will have good staining, and 1/3 will have borderline and poor staining. Whereas some laboratories tend to produce more often than others optimal results, there was no laboratory that did not at least in some tests produce suboptimal results. Based on the analysis of the protocols submitted, the probable main causes of insufficient (ie, borderline or poor) stains were: inappropriate choice of antibody, antibody too diluted or too concentrated, insufficient or inappropriate epitope retrieval, and false positive staining due to endogenous biotin. Often, a combination of several of the above mentioned factors were identified. An example of how multiple factors may change the results of immunostaining is illustrated in Figure 1, which shows the outcomes of staining for CD117 based on different dilution of the primary antibody, as well different antigen retrieval methods.

The specific suggestions for improvement of protocols seem to be effective. For instance, when submitting stains for the second estrogen receptor (ER) run, 13 out of 25 laboratories which had insufficient stains in the first ER run changed their protocols according to the NordiQC recommendations (longer HIER time, alkaline HIER buffer, adjustment of antibody concentration). Of these, 10 (77%) improved their score from poor or borderline to good or optimal. Among the 12 laboratories that did not follow the recommendations, only 3 improved their score (25%).

Conclusion

External quality control is a very important part of quality control in immunohistochemistry laboratories. NordiQC experience indicates that a large number of laboratories would probably benefit greatly from participation in such programs. Ultimately, external quality control in immunohistochemistry has a potential to improve our diagnostic precision and patients' care.

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