

## Short reports

## Audit and internal quality control in immunohistochemistry

P Maxwell, W G McCluggage

## Abstract

**Aims**—Although positive and negative controls are performed and checked in surgical pathology cases undergoing immunohistochemistry, internal quality control procedures for immunohistochemistry are not well described. This study, comprising a retrospective audit, aims to describe a method of internal quality control for immunohistochemistry. A scoring system that allows comparison between cases is described.

**Methods**—Two positive tissue controls for each month over a three year period (1996–1998) of the 10 antibodies used most frequently were evaluated. All test cases undergoing immunohistochemistry in the months of April in this three year period were also studied. When the test case was completely negative for a given antibody, the corresponding positive tissue control from that day was examined. A marking system was devised whereby each immunohistochemical slide was assessed out of a possible score of 8 to take account of staining intensity, uniformity, specificity, background, and counterstaining. Using this scoring system, cases were classified as showing optimal (7–8), borderline (5–6), or unacceptable (0–4) staining.

**Results**—Most positive tissue controls showed either optimal or borderline staining with the exception of neuron specific enolase (NSE), where most slides were unacceptable or borderline as a result of a combination of low intensity, poor specificity, and excessive background staining. All test cases showed either optimal or borderline staining with the exception of a

single case stained for NSE, which was unacceptable.

**Conclusions**—This retrospective audit shows that immunohistochemically stained slides can be assessed using this scoring system. With most antibodies, acceptable staining was achieved in most cases. However, there were problems with staining for NSE, which needs to be reviewed. Laboratories should use a system such as this to evaluate which antibodies regularly result in poor staining so that they can be excluded from panels. Routine evaluation of immunohistochemical staining should become part of everyday internal quality control procedures.

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Keywords: immunohistochemistry; audit; internal quality control

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Table 1 Details of the 10 most commonly used antibodies

Antigen/antibody	Clone	Supplier	Dilution	Pretreatment
CD45	2B11+ PD7/26	Dako	1/50	Microwave
CD20	L26	Dako	1/50	Microwave
CD3	Pab	Dako	1/50	Microwave
CAM5.2	CAM5.2	Becton Dickinson	1/10	Trypsin
AE1/3	AE1/3	Dako	1/50	Trypsin
CEA	11-7	Dako	1/50	Trypsin
NSE	Pab	Incastar	1/2	None
Chromogranin A	Dak-A3	Dako	1/50	None
S100	Pab	Signet	1/20	Trypsin
Desmin	D33	Dako	1:100	None

Pab, polyclonal antibody.

In recent years, increasing attention has focused on pathology laboratories with regard to many aspects of the quality of routine work. Internal quality control procedures should be in place in all laboratories whereby a variety of criteria, including the standard of staining, are checked routinely on a daily basis. These procedures, as well as being part of internal quality control, are assessed by bodies such as Clinical Pathology Accreditation (CPA), UK. Histopathology laboratories should also routinely audit part of their own work and this is carried out in many institutions. For example, laboratories may audit a proportion of randomly selected biopsies. During this audit, many factors pertaining to the biopsy might be evaluated including accuracy of clerical details, turnaround time, quality of staining, and pathological content and accuracy.<sup>1–3</sup> To date, there has been little focus on the quality of immunohistochemical staining and, apart from the routine performing and checking of positive and negative controls, there are few recommendations for internal quality control of immunohistochemistry. The aim of our study was to perform a retrospective audit to assess the quality of immunohistochemical staining in our institution. To this end, we devised a scoring system that allows comparison of immunohistochemical staining between cases and antibodies over a period of time.

Table 2 Scoring system used in our study

Staining criteria	Score and criteria for scoring			
Staining intensity	0 (no staining)	1 (weak staining)	2 (moderate staining)	3 (strong staining)
Uniformity of staining	0 (not uniform throughout)	1 (uniform throughout)	NA	NA
Specificity of staining	0 (non-specific staining present)	1 (only specific staining present)	NA	NA
Absence of background staining	0 (excessive background staining that interferes with interpretation)	1 (background staining present but does not interfere with interpretation)	2 (no background staining present)	NA
Counterstaining	0 (inadequate)	1 (adequate)	NA	NA

NA, not applicable.

Table 3 Scores of positive tissue controls in immunohistochemistry 1996–8

Antigen/antibody	Unacceptable (%) Score 0–4	Borderline (%) Score 5–6	Optimal (%) Score 7–8
CD45 (n = 72)	1.4	33.3	65.3
CD20 (n = 70)	0	10	90
CD3 (n = 69)	1.5	17.4	81.1
CAM5.2 (n = 71)	2.8	36.6	60.6
AE1/3 (n = 69)	7.3	30.4	62.3
*CEA (n = 71)	7.0	23.9	69.1
NSE (n = 58)	36.1	60.4	3.5
*Chromogranin A (n = 67)	10.5	26.8	62.7
S100 (n = 72)	0	25.0	75.0
Desmin (n = 64)	1.6	12.5	85.9

\*Includes two slides where inappropriate tissue was used as control (scored as 0).

## Materials and methods

### SPECIMENS

Cases were retrieved from the files of the department of pathology, Royal Group of Hospitals Trust, Belfast. Two positive tissue controls (where available) from each month over a three year period (1996–8) for the CAM5.2 and AE1/3 antibodies and antibodies directed against CD45, CD20, CD3, carcinoembryonic antigen (CEA), neurone specific enolase (NSE), chromogranin A, S100, and desmin were retrieved from file (table 1). These ten antibodies were chosen because they were the most commonly used antibodies during the study period. All routine test cases undergoing immunohistochemistry within the months of April were also retrieved. Where a test case was negative with an antibody and where there was no internal positive control, the positive tissue control for that antibody performed on that day was used. Negative controls, where the primary antibody was replaced with buffer (Tris buffered saline), for all cases were also reviewed.

### IMMUNOHISTOCHEMICAL STAINING

All slides were stained manually using a standard methodology of peroxidase streptavidin-biotin (Duet StABC; Dako, Ely, Cambridgeshire, UK) with diaminobenzidine as the chromogen. Counterstaining was with Harris's haematoxylin. All antibody incubations were conducted at room temperature for 30 minutes. Some cases, as well as undergoing manual staining, were also stained using an automated immunostainer (Ventana NEXES; Ventana, Strasbourg, France), which was being evaluated in our department during part of the study period. Automated protocols followed the manufacturer's recommended procedures with antibody incubations at 37°C for 30 minutes using the Ventana detection and counterstain systems. Pretreatment by microwaving was conducted using a Matsui domestic oven delivering 850 W for 20 minutes in 0.01 M citrate buffer (pH 6.0). Trypsin digestion (ICN, Aurora, Ohio, USA) was performed using a 0.1% solution in 0.1% calcium chloride at

37°C (pH 7.8) for 10 minutes. Protein digestion on the Ventana NEXES was performed at 37°C using the manufacturer's digestion kit.

### SLIDE ASSESSMENT

Each slide was assessed out of a possible score of 8. Parameters measured (table 2) were staining intensity (0, 1, 2, 3), uniformity (0, 1), specificity (0, 1), absence of background staining (0, 1, 2), and counterstaining (0, 1). A score of 0–4 was considered to be unacceptable, 5–6 borderline, and 7–8 optimal. In cases where the intensity of staining was 0 (negative), the staining was considered to be unacceptable and all other parameters were also considered to be 0. If the degree of background staining was judged to interfere with interpretation (a score of 0), the stain was also considered unacceptable and given a score of 0. In those cases where both manual and automated immunostaining were performed, the final numerical scores were compared. The two authors assessed each slide over a double headed microscope.

## Results

There was no staining of negative controls. Table 3 shows the numbers of positive controls examined and the proportions of these showing unacceptable, borderline, and optimal staining. Four slides (two staining for CEA and two for chromogranin A) were completely negative as a result of the selection of an inappropriate positive control. These were scored as 0. Most positive tissue controls showed optimal staining and in most cases staining was either borderline or optimal. The exception was staining for NSE where there were consistent problems: staining was typically weak and lacking in specificity, with excessive background. Using the same control material, chromogranin A staining was superior with only 10% of cases showing unacceptable staining.

There were 44, 42, and 46 test cases for review in 1996, 1997, and 1998, respectively. Of these, six of 44, five of 42, and six of 46, respectively, were not on file at the time of review. Within the test cases, 55 different antibodies were used, ranging in frequency from 1 to 39 requests. All test cases audited (including those that were negative and where the positive tissue control for that day was used), showed either borderline or optimal staining except for a single case of staining for NSE, which was unacceptable owing to non-specific staining and excessive background staining. Table 4 shows the percentage scores for each of the criteria for those slides stained manually. As can be seen, over 90% of cases gained maximum marks for staining intensity, uniformity, specific-

Table 4 Scores in percentages of all test slides reviewed in our study, including positive tissue controls where the test case slide was negative

Staining criteria	Scores for each year											
	0			1			2			3		
	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997	1998
Staining intensity	0.0	0.0	0.0	0.8	1.1	0.0	0.0	8.9	8.3	99.2	90.0	91.7
Uniformity of staining	5.0	4.4	0.0	95.0	95.6	100.0	NA	NA	NA	NA	NA	NA
Specificity of staining	0.8	6.7	2.8	99.2	93.3	97.2	NA	NA	NA	NA	NA	NA
Absence of background staining	0.3	1.1	0.0	36.1	17.8	38.9	63.6	81.1	61.1	NA	NA	NA
Counterstaining	0.0	1.1	0.0	100.0	98.9	100.0	NA	NA	NA	NA	NA	NA

Total slides reviewed by year (manual methodology only): 1996, 119; 1997, 90; 1998, 72.  
NA, not applicable.

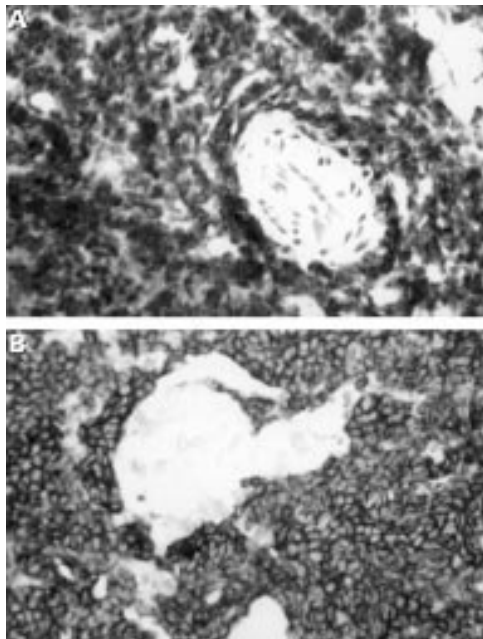


Figure 1 Comparison of staining for CD20 between manual (A) and automated (B) methods. Automated immunostaining resulted in more intense, crisp membrane staining.

city, and adequacy of counterstaining. Background staining was more of a problem, with only 61–81% of cases achieving the maximum score.

Twenty five different antibodies were used for automated immunostaining in our study. Overall, automated staining resulted in higher scores than manual staining. The overall mean score for manually stained slides was 7.6, whereas the mean score for automated stained slides was 7.9. Slides stained on the Ventana NEXES generally showed more intense staining of serial sections than those stained manually. In addition, background staining with the Ventana NEXES was eliminated without affecting the intensity of staining (fig 1). One exception to this was *bcl2* staining, which required an amplification protocol supplied by the manufacturer. There was generally no difference in the uniformity or specificity of staining or the adequacy of counterstaining between the manual and automated methods.

### Discussion

The aim of our study was to evaluate the standard of immunohistochemical staining in our department, which comprises a busy teaching hospital. External quality assurance

programmes such as that managed by UK National External Quality Assurance (UKNEQAS) for Immunocytochemistry (London) and a laboratory's own internal quality control systems are two means of assessing performance in immunohistochemistry. The UKNEQAS organisation holds regular regional workshops and updates participants through official publications. Internal quality control systems, however, have been more difficult to formalise and, although positive and negative control material are checked on a daily basis, more formal assessment is probably not carried out in most laboratories. Organisations such as the National Committee for Clinical Laboratory Standards (NCCLS) (USA) have published guidelines on best practice, and recent publications have shown that there is an interest in setting out goals and objectives for quality control procedures in immunohistochemistry.<sup>4,5</sup> Other groups have attempted to identify good practice and have made recommendations regarding quality standards in immunohistochemistry.<sup>6</sup> These quality issues are likely to assume increasing importance with the advent of clinical governance. Data from UKNEQAS for Immunocytochemistry show that increasing numbers of laboratories sometimes struggle to maintain standards.<sup>7</sup>

The internal quality control procedures carried out in our laboratory, namely positive tissue controls containing the antigen under test and a negative control section from each test block, appear to meet the minimum required criteria of those that are reasonably expected to be conducted by a routine diagnostic immunohistochemistry laboratory. Although it is common practice to review at the end of each day both sets of controls along with the test material, we have conducted a three year review of a proportion of the positive control material of the most commonly used antibodies in our laboratory, together with a proportion of test cases. Our study assesses only the standard of immunohistochemistry and makes no attempt to determine whether reporting pathologists have used an appropriate or adequate panel of antibodies, or whether they have interpreted the results correctly.

The scoring system we devised was an attempt to assess the elements looked for when examining an immunohistochemical slide. We did not consider the problem of interobserver and intra-observer variation of this scoring system but, rather, the two authors examined the slides together using a double headed micro-

scope. The intensity of staining was judged on a four point scale from negative (0) to intense (3). The specificity and uniformity of staining and the adequacy of counterstaining were scored as either 0 or 1. The degree of background staining was judged on a reversed three point scale from 0 (background stain interferes with interpretation) to 2 (no background). Other scoring systems such as that used in the UKNEQAS for Immunocytochemistry scheme score each slide out of a total of 20, this being a composite score from four independent assessors each scoring out of 5. In this scoring scheme, however, criteria vary and are dependent on the antibody under examination.<sup>8</sup> Using our system, we found that evaluating different antibodies on a common scale was possible, allowing for comparison between antibodies over a period of time.

The review of positive tissue controls showed that with most antibodies there was optimal or borderline staining, with only a small proportion showing unacceptable staining. Most test cases also showed optimal or borderline staining. The exception to this was staining for NSE, where only a small proportion of control cases showed optimal staining and over one third were unacceptable. The main problems were low intensity of staining, non-specific staining, and excessive background. Staining for NSE has a reputation for poor specificity and this was confirmed in the study, suggesting that NSE positivity is not conclusive evidence of neuroendocrine differentiation. Many pathologists still use antibodies to NSE as part of a panel to confirm neuroendocrine differentiation and we suggest that alternative antibodies such as those to chromogranin A and PGP 9.5 might be more suitable for this purpose. However, it might be that other laboratories may achieve better results with NSE staining using the same or a different antibody. Other laboratories may find consistent problems with other antibodies and might wish to exclude these from their immunohistochemical panels.

Automated immunohistochemistry using the Ventana NEXES system marginally improved the overall scoring, usually by producing a very clean background without loss of intensity. The exception to this was bcl2 staining, which required an amplification protocol provided by the manufacturer. This shows that each antibody must be evaluated individually

when introducing automated immunohistochemical staining into a laboratory. Although the overall mean score for automated immunostaining (7.9) was only marginally greater than that for manual staining (7.6), there was a trend towards greater intensity with a cleaner background. The cost of such automated systems and the effect automation may have on staffing levels are beyond the scope of this paper.

The number of cases not retrievable from the files is perhaps excessive, but is indicative of the diverse system of reporting and the ongoing research interests of a large teaching hospital. Implementing a system of review, such as we are suggesting, and reporting the incidence of missing slides to pathologists and laboratory staff may result in an improved awareness of the need to return slides for filing and to file the slides correctly.

In summary, this retrospective audit describes a method for improving the daily internal quality control of immunohistochemical staining. Laboratories might wish to carry out similar procedures on a regular basis to ensure that their immunohistochemistry is of a high standard. In this way, antibodies that consistently result in substandard staining can be identified. Steps can be taken to correct this, either by using different protocols or by excluding these antibodies from routine use. We would recommend that laboratories devise a system such as ours to assess their standard of immunohistochemical staining. This assessment should be performed regularly as part of internal quality control.

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