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Use of immunochemistry in Britain: EQA Forum antibody usage questionnaire

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SUMMARY A questionnaire was prepared under the auspices of the Department of Health with the aim of defining the extent and nature of immunocytochemistry use within pathology departments. The questionnaire was circulated to 320 pathology laboratories within the United Kingdom, and a total of 178 replies were received, representing a response rate of 56%. One hundred and thirty eight (78%) of the respondents used immunocytochemical techniques: 64 used immunocytochemical kits, including 35 district general hospital and 29 teaching hospital laboratories. An extensive range of antibodies was being used on a variety of tissues, epithelial and lymphoid markers far exceeding all other antibodies. Several differences in the numbers of cases and the types of tissues studied were identified among laboratories. The techniques used, the problems encountered, and the procedures followed with unsatisfactory reagents were also analysed. Finally, an assessment of the resources allocated to immunocytochemistry, both in terms of staff and reagent costs was made. Taking into account the response rate of 56% and the uncertainty that all pathology departments in the United Kingdom had been circulated, the estimated annual total costs for immunocytochemistry for all pathology laboratories in the United Kingdom was £5·4 million.

The use of immunocytochemical techniques has become widespread in the United Kingdom, but few data are available on exactly how and to what extent these techniques are used. A questionnaire circulated to district general hospital laboratories in 1986 indicated that 70·7% of these laboratories were using immunocytochemistry in routine diagnostic practice,[1] but no comparable data are available for teaching hospital laboratories.

A questionnaire was prepared by two of the authors (EH, IL) with the aim of defining the extent of use of immunocytochemistry within pathology departments in the United Kingdom, the type and range of tests being performed, the techniques being used, the problems associated with immunocytochemistry and the extent of kit use were analysed. Differences between methods of use in laboratories in district general hospitals and teaching hospitals were also defined. An assessment of the associated staff and reagent costs was used to provide an estimated total cost for all pathology departments in the United Kingdom.

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Methods

The questionnaire (copy available on request) was circulated to a total of 320 pathology laboratories throughout the United Kingdom using a list of histopathologists and immunopathologists provided by the Royal College of Pathologists. Information sought included types of antibodies used, types of tissues studied, and numbers of cases analysed. The extent of kit use, the commercial suppliers used, and technical details involved in immunocytochemistry were also requested. The results were collated, entered into a computerised database, and analysed statistically.

Table 1 *No (%) of laboratories and hospitals replying to questionnaire*

	District general hospital	*Teaching hospital*	*Total*
Laboratory type	126 (71)	52 (29)	178 (100)
Hospital type	118 (74)	41 (26)	159 (100)
No performing immunocytochemistry*	91 (72)	47 (90)	138 (78)
No using kits†	35 (28)	29 (56)	64 (36)
Percentage using kits‡	38	62	46

*Percentage calculated from number of laboratories/total number of respondents (n = 178).
†Percentage calculated from number of laboratories using kits/total number of respondents (n = 178).
‡Percentage calculated from number of laboratories using kits/total number of immunocytochemistry using laboratories (n = 138).

Results

Of the 320 laboratories, 178 (56%) replied to the questionnaire, about 70% of which were in district general hospitals and the rest in teaching hospitals (table 1). The actual proportion of hospital types was slightly different because some hospitals had up to three laboratories actively using immunocytochemical methods as part of the diagnostic service. The proportion of teaching hospital respondents (65%) was higher than the proportion of district general hospital respondents (53%). Table 1 shows the number of both hospital laboratory types performing immunocytochemistry, together with the number using immunocytochemical kits. Forty (22%) laboratories reported no use of immunocytochemical techniques, five in teaching hospitals and 35 in district general hospitals.

Table 2 shows the types of tissues which had been subjected to immunocytochemical analysis, the number of cases studied, and the mean number of antibodies used to study each case during a single month. Although there were almost twice as many district general hospital laboratories as teaching hospital laboratories using immunocytochemistry, the number of cases was much higher in teaching hospitals for all tissue types studied. Analysis of lymphoid and epithelial samples far exceeded that of other tissues in both hospital laboratory types. Lymph node analysis was performed more frequently than analysis of other tissues in teaching hospital laboratories, but in district general hospital laboratories analysis of epithelial tissues predominated. The mean number of antibodies for each case was consistently higher in teaching hospital laboratories than in district general hospital laboratories.

Table 3 *Suppliers*

Supplier	*No (%) of laboratories*	*Supplier*	*No (%) of laboratories*
Dako	135 (98)	BDH	29 (21)
Becton Dickinson	76 (55)	Ortho	22 (16)
Unipak	55 (40)	Biotest	18 (13)
Sigma	53 (38)	Coulter	15 (11)
Miles	48 (35)	Nordic	14 (10)
Bionuclear	47 (34)	RIA	8 (6)
Amersham	41 (30)	Cambridge	4 (3)
Janssen	35 (25)	Travenol	3 (2)
Seralab	35 (25)		

Percentages calculated from number of laboratories/total number of laboratories using immunocytochemistry (n = 138).

The companies supplying antibodies to the 138 laboratories using immunocytochemistry are listed in table 3. Dako supplied almost all the laboratories using immunocytochemistry; among the other suppliers only Becton Dickinson exceeded 50%. Ortho supplied only 16% of laboratories with primary antibodies, whereas they supplied 46 (32%) of laboratories using kits (table 4). When asked to recommend a particular manufacturer, 93 (67%) of the immunocytochemistry laboratories suggested Dako, with much smaller numbers recommending Becton Dickinson, Eurodiagnostics, or other companies.

Table 5 lists the primary antibodies used by the laboratories in order of greatest use, and illustrates the very wide range of antibodies being used. Not surprisingly, in view of the fact that lymph node analysis was frequently performed by both hospital types,

Table 2 *Tissue tests carried out during March 1987*

	No of cases			*Mean No of antibodies/case*		
Tissue type	*Total*	*District general hospital*	*Teaching hospital*	*Total*	*District general hospital*	*Teaching hospital*
Renal	332	79	253	7·0	5·4	8·7
Skin	379	128	251	4·8	4·6	5·2
Lymph node	714	236	478	6·2	5·1	8·3
Epithelial	772	396	376	2·8	2·6	3·3
Endocrine	179	51	128	2·8	2·3	3·1
Germ cell	144	25	119	2·5	2·1	2·9
Soft tissue	205	64	141	3·8	3·5	4·1
Others	356	196	160	2·7	2·3	3·2
Total	3081	1175	1906	4·1	3·5	4·9

Table 4 *Kit suppliers*

Supplier	No of laboratories: Teaching hospital	No of laboratories: District general hospital	Total (%)
Dako	24	33	57 (40)
Ortho	20	26	46 (32)
Vector	5	3	8 (6)
ICN	2	4	6 (4)
CMD	0	5	5 (4)
Amersham	4	0	4 (3)
BSC	4	0	4 (3)
Others	6	6	12 (8)
Total			142 (100)

leucocyte common antigen and immunoglobulins were used most often.

The immunocytochemical techniques used and the number of problems associated with these techniques are shown in table 6. Laboratories frequently used more than one technique routinely, and often used other techniques occasionally. Pronounced differences between district general hospital laboratories and teaching hospital laboratories were not noted. The techniques most frequently used routinely were peroxidase antiperoxidase (PAP) 73 (53%) and the indirect immunoperoxidase methods 62 (45%). Occasional problems with all the techniques used were reported by up to half of the users, most problems being encountered with the PAP technique and least with immunofluorescence. Avidin-biotin (30, 22%) and alkaline phosphatase anti-alkaline phosphatase (18, 13%) were less frequently used, and the number of problems met with using these techniques was slightly lower than those encountered with PAP.

The questionnaire requested details of further aspects of the techniques used, including chromogens, buffers, and fixation for both paraffin wax embedded and frozen material. Diaminobenzidine (DAB) was the commonest chromogen in use, but other frequently used chromogens were Fast red and AEC (3-amino-9-ethylcarbazole). Both TRIS and phosphate buffered saline (PBS) buffers were used extensively. Neutral buffered formalin was the fixative favoured by most laboratories for both large and small specimens 78 (62%), with smaller numbers of laboratories 31 (26%) using formol-saline. Other fixatives such as unbuffered formalin, formol sublimate, and Bouin's fluid were less frequently used (9%). Seventy (59%) laboratories preferred acetone as the fixative for frozen sections.

Use of kits was reported by 64 laboratories, 35 in district general hospitals and 29 in teaching hospital laboratories (table 1). Of the total number of laboratories in district general hospitals using immunocytochemistry, 38% used kits, in contrast to teaching hospital laboratories, where 62% used kits.

Table 5 *Primary antibodies used*

Antibody	No (%) of laboratories	Antibody	No (%) of laboratories
Leucocyte common antigen	123	Glial fibrillary acidic protein	61
Immunoglobulins	118	Prostatic acid phosphatase	59
Keratin	116	Fibrinogen	57
Epithelial membrane antigen	114	Thyroglobulin	57
Carcinoembryonic antigen	100	Anti-Leu M1	46
Alpha-1-antitrypsin	100	Hepatitis B antigen	43
S100	99	Calcitonin	40
Lysozyme	98	Papillomavirus	33
Alpha-feto-protein	89	Somatostatin	31
T cell	88	Insulin	29
B cell	86	Glucagon	29
Chorionic gonadotrophin	84	Pre-albumin	28
Prostate specific antigen	83	Pituitary hormones	24
Vimentin	80	Herpes simplex virus	22
Factor VIII related antigen	77	Myoglobin	21
Desmin	70	Human placental lactogen	19
Complement	63	Alpha-1-anti-chymotrypsin	15
Neuron specific enolase	61	Neurofilaments	13

The number of different kits used for each laboratory varied between one and nine, with most laboratories using just one or two types of kit. Table 4 shows that Dako supplied 57 (40%) and Ortho 46 (32%) of the kits used. Between them, Dako and Ortho supplied 45 (70%) of the laboratories with at least one kit.

One hundred and forty two different types of kits were used, and these could be divided into two broad groups. The first and largest group (n = 100) comprised those kits which include one or more primary antibodies such as prostatic acid phosphatase and hepatitis B surface antigen. The second group (n = 42) were kits for one technique only which did not include a primary antibody—for example, ABComplex HRP (Dako k355), Universal PAP kit. Table 7 shows the range of kits used by the laboratories in the first group. A wide range of antibodies was being used in kit form,

Table 6 *Techniques used and problems encountered*

Technique	No (%) of users Usual/occasional*	No (%) of users with problems†
PAP	73 (53)/31 (22)	53 (51)
Indirect	62 (45)/34 (25)	45 (47)
Immunofluorescence	40 (29)/23 (17)	23 (37)
AB	30 (22)/28 (20)	23 (40)
APAAP	18 (13)/35 (25)	25 (47)
Direct	12 (9)/20 (14)	13 (41)

*Percentages calculated from number of laboratories using technique/total number of laboratories using immunocytochemistry (138).
†Percentages calculated from number of laboratories reporting problems/number of laboratories using technique.

Table 7 *Use of kits with a primary antibody*

	Total	District general hospital/Teaching hospital
Immunoglobulins/lymphoma markers:		
Anaplastic tumour kit (LCA and EMA)	7	7/0
IgA, IgG, IgM	2	2/0
Kappa, lambda, lysozyme	2	2/0
Leucocyte common antigen	2	2/0
Kappa	1	1/0
Lambda	1	1/0
J-chain	1	0/1
Lymphoma identification kit	1	1/0
Hormones:		
Pancreatic and related hormones	17	2/15
Pituitary hormones	4	1/3
Others	6	1/5
Enzymes:		
Prostatic acid phosphatase	7	5/2
Lysozyme	1	1/0
Alpha-1-antitrypsin	1	1/0
Alpha-1-antichymotrypsin	1	1/0
Tissue specific markers/other cellular antigens:		
Keratin	5	5/0
Carcinoembryonic antigen	5	4/1
Factor VIII related antigen	3	3/0
Prostate specific antigen	3	2/1
S100	3	2/1
Neuron specific enolase	2	1/1
Myoglobin	2	0/2
Alpha-fetoprotein	2	1/1
CA 125	2	0/2
Oestrogen receptors	2	1/1
TDT	2	1/1
Epithelial membrane antigen	1	1/0
Glial fibrillary acidic protein	1	1/0
Infective agents:		
Hepatitis B surface antigen	7	5/2
Others	6	3/3
Total	100	58/42

LCA = leucocyte common antigen, EMA = epithelial membrane antigen.

and some differences among the kits used by laboratories in district general hospitals and teaching hospitals were noted.

A breakdown of the techniques used in all 142 different kits is provided in table 8, and the techniques provided in kit form are also shown. The most frequently used technique was the PAP technique, but kits providing PAP as a technique only, without a primary antibody, were rarely purchased, and ABC kits were preferred.

FINANCIAL CONSIDERATIONS

Tables 9 and 10 deal with the resources allocated to immunocytochemistry within the 138 laboratories. A total of 240·6 staff (expressed in whole time equivalents) was used by the laboratories in performing immunocytochemical techniques. The total number of whole time equivalents at the Scientific (PTA) grade was very low (5·5), and most work was being performed by MLSO (PTB) grades.

Table 8 *Techniques used in kits*

	All kits		'Technique kits'	
Technique	Total	District general hospital/Teaching hospital	Total	District general hospital/Teaching hospital
Peroxidase-anti-peroxidase	89	51/38	3	3/10
Avidin-biotin-peroxidase	25	11/14	22	10/12
Indirect immunoperoxidase	9	8/1	0	0/0
Streptavidin-biotin-peroxidase	6	4/2	4	2/2
Dinitrophenol localisation system	4	0/4	4	0/4
Immunogold-silver	4	2/2	4	2/2
Avidin-biotin-alkaline phosphatase	2	2/0	2	2/0
Immunofluorescence	2	0/2	0	0/0
Avidin-biotin-glucose oxidase	1	0/1	1	0/1
Total	142	78/64	42	19/23

Table 10 shows that both reagent and staff costs were considerably higher in teaching hospital than in district general hospital laboratories, and the total cost of immunocytochemistry in teaching hospital laboratories was about twice that in district general hospital laboratories. A small number of the laboratories were unable or unwilling to supply detailed figures for reagent costs, and an estimate has been made for those missing values using the mean values derived from those laboratories (n = 131) who did provide detailed figures. The final estimate of likely total expenditure in the United Kingdom was again based on the mean values for staff and reagent costs to provide an estimate of expenditure for a total of 320 laboratories (240 district general hospital and 80 teaching hospital laboratories).

Table 9 *Resources – staff numbers*

	No of staff (whole time equivalents)		
	District general hospital	Teaching hospital	Total
MLSO PTB:			
Senior chief	6·0	4·9	10·9
Chief	22·7	17·6	40·3
Senior	37·9	30·3	68·2
Basic grade	44·2	52·9	97·1
Junior	9·7	8·9	18·6
Total	120·5 (1·3)*	114·6 (2·4)*	235·1 (1·7)*
Scientific PTA:			
Principal	0·0	0·0	0·0
Senior	1·5	1·0	2·5
Basic grade	0·0	3·0	3·0
Total	1·5	4·0	5·5
Total staff	122·0 (1·3)*	118·6 (2·5)*	240·6 (1·7)*

*Means calculated from number of staff/total number of laboratories using immunocytochemistry.

Table 10 *Resources – reagent and staff costs*

	Costs (£) (mean value below)		
	District general hospital	*Teaching hospital*	*Total*
Reagents*	146 924 (1 670)	185 576 (4 316)	332 499 (2 538)
Staff	1 099 781 (12 086)	1 043 400 (22 200)	2 143 181 (15 530)
Total	1 246 705 (13 700)	1 228 976 (26 148)	2 475 680 (17 940)
Estimate for missing reagent costs	5 010	17 264	22 274
Estimated total	1 251 715 (13 755)	1 246 240 (26 516)	2 497 955 (18 101)
Estimate for all pathology laboratories in United Kingdom†	3 301 200	2 121 280	5 422 480

*Based on 131 replies (88 District general hospitals, 43 Teaching hospitals).
†Based on 320 laboratories (240 District general hospitals, 80 Teaching hospitals).

Assuming that the average laboratory buys one kit of each type a year, the percentage of the total amount spent on immunocytochemical reagents a year can be estimated. Laboratories in teaching hospitals used an average of 8% of the total amount spent on immunocytochemical reagents to buy kits; the corresponding figure for laboratories in district general hospitals was 28%. In district general hospital laboratories 75% of the total amount spent on kits was used to buy complete kits—that is, those in which a primary antibody is included; in teaching hospital laboratories this proportion was lower (60%).

Discussion

Responses from the 178 laboratories indicated that about 70% of district general hospital laboratories were using immunocytochemistry, while for teaching hospital laboratories this figure was 90%. The small number of teaching hospital laboratories not performing immunocytochemistry can be explained by the inclusion of some specialised departments such as forensic and ophthalmology laboratories. All of the teaching hospital laboratories that were not highly specialised were using immunocytochemical techniques routinely. Among those district general hospital laboratories not performing immunocytochemistry at the time the questionnaire was circulated, many indicated that they would either be setting up these techniques in the near future, or that they would like to see immunocytochemistry techniques established within their laboratories, provided that adequate resources could be obtained.

Teaching hospital laboratories performed immunocytochemical tests on a much higher number of cases than district general hospital laboratories, and the mean number of antibodies used to analyse each case was consistently higher in teaching hospital laboratories than in district general hospital laboratories. In teaching hospital laboratories lymph node analysis exceeded that of other tissues by far, while although lymph node analysis was frequently performed in district general hospital laboratories, epithelial tissues were more commonly studied. The larger number of antibodies used for each case in teaching hospital laboratories implies that these laboratories more frequently analysed tissues using panels of antisera.

The reasons for these variations between hospital laboratory types are probably complex, and may include factors such as availability of resources and a differing emphasis on research and developmental aspects of immunocytochemistry. Although the questionnaire asked laboratories to estimate the relative proportions of diagnostic and research work performed, some laboratories found this difficult to answer, because they could not clearly separate the diagnostic from the research and developmental applications of their work. From the 126 laboratories able to provide an answer, it was calculated that 92% of immunocytochemistry in district general hospital laboratories and 62% in teaching hospital laboratories is diagnostic. This difference may in part account for some of the differences between hospital laboratory types.

A larger proportion of laboratories in teaching hospitals (64%) use kits when compared with laboratories in district general hospitals (38%). Because kits supply reagents pre-diluted and ready to use, it was anticipated that their use would appeal more to smaller laboratories in district general hospitals with relatively less experienced staff. A detailed breakdown of the costs involved in kit usage, however, shows that district general hospital laboratories use a much larger proportion of the money they spend on immunocytochemical reagents to buy kits (28%) than teaching hospital laboratories (8%). Differences in the types of kits purchased by the hospital laboratory types were also noted. For example, the Anaplastic Tumour Kit, marketed by Dako, and intended to allow anaplastic tumours to be divided into the two broad groups of carcinoma or lymphoma, was used exclusively by district general hospital laboratories. This may partly reflect the much higher percentage of diagnostic immunocytochemistry done in district general hospital laboratories (92%) in contrast to teaching hospital laboratories (62%). Almost all of the kits detecting hormones were bought by laboratories in teaching hospitals, which again may reflect a higher interest in this field in teaching hospital laboratories. Although lymph node analysis was more frequently

performed in teaching hospital than in district general hospital laboratories, most of the kits for identification and characterisation of lymphomas were bought by laboratories in district general hospitals.

Occasional problems with detection techniques were encountered in up to half the laboratories with all the techniques used. The number of problems met with was slightly lower with the avidin-biotin and APAAP techniques than with the PAP technique, but this may reflect the lower incidence of usage of these techniques by the responding users. The avidin-biotin technique is more frequently used in kit form than other detection techniques (table 8), although the majority of "complete" kits—those detailed in table 7—used the PAP technique.

The respondents were asked what procedures were followed when unsatisfactory reagents were discovered. The general low level of response (61 laboratories) to this question could indicate either a general satisfaction with the currently available reagents, or that without some system of quality control many of the laboratories were unaware that the particular antibodies in use were unsatisfactory. The two most common responses were either to exchange the reagent for another sample of the same antibody, or to change the supplier. Serious defects should be reported to the Department of Health NHS Procurement Directorate.

The estimated total annual cost of immunocytochemistry in the United Kingdom may well be an underestimate as the precise number of laboratories is uncertain. The information provided by the Royal College of Pathologists was in the form of a set of addresses of known immunopathologists and histopathologists in the United Kingdom. About a quarter of the addresses were home addresses rather than those of the individual departments, and the precise number of laboratories still has to be established. Until a more accurate list is compiled the overall usage must be regarded only as an estimate, and any detailed analysis of costs should take this factor into consideration.

Considerable sums of money are involved, and it seems likely that much duplication of testing of new reagents is being performed. The Department of Health has already commissioned a number of studies of readily available commercial primary antibodies and detection techniques.[2] Several of the respondents to the questionnaire previously circulated by Hall *et al*[1] indicated that the establishment of a national database supplying data of this kind would be welcomed. At a time when there is considerable emphasis on cost reduction, many find excessive the costs of investigating new sources of reagents or redoing tests performed with antibodies or kits which turn out to be unsatisfactory.

We thank all those respondents who took the time and the trouble to reply to this questionnaire. We also thank Dr Howard Pringle for his invaluable help in helping to set up the database, the secretarial staff of the University of Leicester department of pathology for entering the data, and the Royal College of Pathologists for supplying the list of pathologists.

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