SHORT REPORT

The effect of fixation and processing on the sensitivity of oestrogen receptor assay by immunohistochemistry in breast carcinoma

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Aims: To investigate the effect of different fixation and processing regimens on the assessment of oestrogen receptor (ER) by immunohistochemistry (IHC).
Methods: The ER results for 420 patients from seven different hospitals in which antigen retrieval and IHC were performed centrally were compared. The intensity of ER positivity was assessed semiquantitatively using the Quick score (range 0–7). The scoring profiles of cases from each different source (hospital) were compared to detect differences in the proportion of cases that were negative (Quick score = 0), moderately positive (Quick score = 1–5), and strongly positive (Quick score = 6–7).
Results: There were no significant differences (p = 0.3; χ^2 test) in the proportion of cases in each category.
Conclusions: None of the fixation or processing regimens had a significant adverse effect on the sensitivity of the ER assessment performed by automated immunohistochemistry.

T he assessment of the oestrogen receptor (ER) status of breast carcinomas is now routinely performed on formaldehyde fixed paraffin wax embedded sections by means of immunohistochemistry (IHC), and the results predict the tumour response to endocrine treatment as accurately as the dextran charcoal method.[1] Meta analysis has shown that women whose breast tumours are ER negative will gain no benefit from endocrine based treatments.[2]

It is possible that the initial handling of the specimen with variables such as rapid or delayed fixation, type of fixative, length of fixation, and processing schedule may affect the sensitivity of the subsequent immunohistochemical assay for ER. To investigate the effects of these parameters on the sensitivity of ER IHC, breast tumours from seven different hospitals were assayed using a standard antigen retrieval protocol and automated ER IHC with standard semiquantitative assessment of the results using the Quick score.

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MATERIALS AND METHODS

Case material

Formaldehyde fixed, paraffin wax embedded tissue from 447 patients from 11 different hospitals was submitted for ER assay by means of IHC. The local pathologist had selected a representative block containing tumour tissue. Sections (4 µm thick) were cut and were subjected to a standard antigen retrieval protocol (30 minutes of microwaving). Total numbers of cases from four of the hospitals were small (less than 10

cases) and these hospitals were excluded from the analysis. Analysis was performed on data from 420 cases from seven hospitals.

Participating histopathology departments were asked to submit details of their routine fixation times, fixative, and processing schedules for large breast specimens (not core biopsies).

ER assay

The ER IHC assay was performed using the 6F11 antibody and an automated immunostainer (Optimax plus; Menarini, Wokingham, UK). Bound antibody was detected using the Vector Elite link and label secondary detection system (Vector, Peterborough, UK). The laboratory is a participant in the UK National External Quality Assessment Scheme (NEQAS) for ER IHC and the assay has been validated against UK NEQAS standards.[3]

Assessment of positivity

The intensity of ER positivity was assessed semiquantitatively using the Quick score method, which takes into account intensity and distribution of positivity.[4]

Intensity

- Negative (no staining of any nuclei at high magnification) = 0.
- Weak (only visible at high magnification) = 1.
- Moderate (readily visible at low magnification) = 2.
- Strong (strikingly positive at low magnification) = 3.

Proportion of cells positive

The proportion of tumour nuclei showing positive staining was scored as follows: 0% = 0, 1–25% = 1, 26–50% = 2, 51–75% = 3, 76–100% = 4.

Quick scores

The score for intensity was added to the score for proportion to give a Quick score in the range of 0–7.

The scoring profiles of cases from each different source (hospital) were compared to detect differences in the proportion of cases that were negative (Quick score, 0), moderately positive (Quick score, 1–5), and strongly positive (Quick score, 6–7).

Statistical method

The data were analysed using a 3 × 7 contingency table and the χ^2 test. The null hypothesis is that the proportion of ER assay results in each category (negative, moderate, strong) is

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Table 1 Fixation and processing protocols for submitting laboratories

Hospital processing equipment	Fixative	Reagent	C	T	V/P	TD	Reagent	C	T	V/P	TD	Reagent	T	V/P	C	TD
1 Bayer VIP 2000	10% FS	Alcohol	7	37	+/+	370	Xylene	3	37	+/+	120	Wax	60	+/+	4	285
2 Leica TP 1050	10% NBFS	Industrial methylated spirit	5	A	+/+	300	Xylene	4	A	+/+	180	Wax	60	+/+	4	360
3 Shandon hypercenter XP	10% FS	Alcohol	6	A	–/–	390	IPA/chloroform 50/50	1	A	–/–	30	Wax	60	+/+	2	300
							Paraffin wx	2	A	+/+	120					
4 Leica TP1050	10% FS	Industrial methylated spirit	6	40	+/+	360	Xylene	3	40	+/+	180	Wax	56	+/+	3	240
5 Shandon Pathcentre	10% NBFS	IPA	6	A	+/+	420	Chloroform	2	A	+/+	180	Wax	56	+/+	4	360
							Chloroform/xylene (1/1)	1	A		90					
6 Shandon Pathcentre	10% NBFS	IPA	7	A	+/+	540	Chloroform	3	A	+/+	180	Wax	60	+/+	4	180
7 Shandon hypercenter XP	10% acetic FS	Alcohol	8	A	+/+	480	Xylene	2	37	+/+	180	Wax	60	+/+	2	240

Fixation and processing protocols for laboratories submitting paraffin embedded blocks for ER assay.
C, number of changes of reagent; T, temperature in degrees C; V/P, vacuum/pressure; TD, total duration of stage of processing (minutes); FS, formal saline; NBFS, neutral buffered formal saline; IPA, isopropyl alcohol.

Table 2 Results of statistical analysis

Hospital	Negative Quick score=0 Observed (expected)	Moderately positive Quick score=1–5 Observed (expected)	Strongly positive Quick score=6–7 Observed (expected)	Total
1	3 (6.15)	30 (33.68)	103 (96.17)	136
2	3 (1.18)	4 (6.44)	19 (18.39)	26
3	4 (226)	14 (12.38)	32 (35.36)	50
4	1 (1.09)	7 (5.94)	16 (16.97)	24
5	0 (1.36)	8 (7.43)	22 (21.21)	30
6	1 (2.99)	18 (16.34)	47 (46.67)	66
7	7 (3.98)	23 (21.79)	58 (62.23)	88
Total χ^2	19	104	297	420

Hospital	Negative Quick score=0	Moderately positive Quick score=1–5	Strongly positive Quick score=6–7
1	1.615+	0.401+	0.485
2	2.828+	0.923+	0.021
3	1.336+	0.212+	0.319+
4	0.007+	0.188+	0.056+
5	1.357+	0.044+	0.029+
6	1.321+	0.168+	0.002+
7	2.290+	0.067+	0.287+
Total χ^2=13.995; degrees of freedom, 12; p=0.304			

Numbers of observed cases in each of three levels of oestrogen receptor (ER) positivity on tumours processed in each of seven hospitals. Expected values are calculated based on the proportions of cases in all cases submitted (ER negative, 19; ER moderate, 104; ER strong, 297). The χ^2 values for each cell are shown, allowing identification of those results contributing most to the overall χ^2 value.

the same in material from the seven different laboratories. The overall frequency of ER negative assays is 19 of 420 (4.5238%). If the null hypothesis is true, then 4.5238% of the 136 assays from hospital 1 (6.15) should be ER negative. This is the expected value for ER negative tumours from hospital 1.

The χ^2 statistic is calculated ((observed − expected)^2/observed) for each cell in turn. The sum of these χ^2 values gives the overall χ^2 from which a probability that the null hypothesis is true is derived.

RESULTS
Table 1 provides the details of the routine fixation and processing practices in the seven participating departments. Formal saline without buffer is probably more acidic than the neutral buffered alternative. One laboratory uses acidified formal saline.

Table 2 shows the analysis of the ER assay results. The null hypothesis is that the proportion of ER assay results in each category (negative, moderate, strong) is the same in material from the seven different laboratories. The overall frequency of ER negative assays is 19 of 420 (4.5238%). If the null hypothesis is true, then 4.5238% of the 136 assays from hospital 1

(6.15) should be ER negative. This is the expected value for ER negative tumours from hospital 1.

The χ^2 test shows no significant differences in the proportion of cases in each category (p = 0.304). This indicates that none of the fixation or processing practices in the submitting hospitals had a significant effect on the sensitivity of the ER assessment performed by IHC centrally.

DISCUSSION
External quality assurance is recognised as an important activity for laboratories performing immunohistochemical assays.[5] The UK NEQAS IHC scheme offers a programme in which participating laboratories perform ER IHC on paraffin wax embedded material sent out from a central organising laboratory but collected from numerous different sources.[3] It is possible that differences in the time delay before tissue fixation, duration of fixation, and the type of fixative could have an effect on the sensitivity of ER IHC. All laboratories claimed rapid fixation for at least 24 hours, but in reality it is likely that tissues were fixed at different rates for varying lengths of time. A study of 25 tumours showed that a delay of up to 120 minutes in fixation resulted in a reduction of ER

Take home messages

- There were no significant differences between hospitals in the proportion of cases in each of the Quick score categories (negative, moderately positive, strongly positive)
- None of the fixation or processing regimens had a significant adverse effect on the sensitivity of the oestrogen receptor assay performed by automated immunohistochemistry.

immunopositivity, but the result was not significant.[6] Differences in processing times and reagents could also have an effect. Table 1 shows that although the general principles of fixation and processing to paraffin wax are common to all laboratories, there is a variation in the reagents used and times of exposure. This study was undertaken to search for evidence that these different practices in different departments have an effect on the results of the ER IHC assay. The analysis presented in table 2 shows that there is no significant difference in the proportion of negative, moderate, and strongly positive tumours from any of the seven submitting sources. This result suggests that when histological material from different sources is collected centrally and subjected to a common protocol of antigen retrieval, automated immunohistochemical analysis, and assessment the results are not compromised.

"It is possible that differences in the time delay before tissue fixation, duration of fixation, and the type of fixative could have an effect on the sensitivity of oestrogen receptor immunohistochemistry"

This type of analysis provides a useful quality control measure to determine that specimens derived from one particular source show different sensitivities on ER IHC.

CONCLUSION

This study provides no evidence that differences in handling, fixation, and processing of breast tumour tissue have an effect on ER assay sensitivity after antigen retrieval when the assay is performed on automated equipment.

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