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Identification of the Major Sources of Error in Estrogen Receptor Measurements for Individual Laboratories using both Tissue and Cytosol Samples

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Abstract—*The major sources of error between laboratories performing estrogen receptor measurements in tissue samples were identified for* 17 *participating laboratories in a trial conducted by the Australasian Quality Assurance programme. Both tissue and cytosol samples were provided, and the In-House assays were compared with the ER-EIA kit (Abbott Laboratories, U.S.A.) as a reference assay. For both the In-House and Abbott assays, tissue samples resulted in a between laboratory CV of about* 55% *and a within laboratory CV of about* 30%. *In contrast to tissue samples, the between laboratory CV for cytosol samples was reduced to* 41% *for the In-House assays and to* 33% *for the Abbott assay, whereas the within laboratory CV was reduced to* 10% *for both types of assay. The different methods of tissue homogenization by themselves were not found to be sources of error, and protein extraction efficiency from tissue was strongly correlated with protein measurement* ($P < 0.0005$). *The major sources of error due to protein measurement, cytosol preparation, In-House and Abbott assays were evaluated for individual laboratories. The results indicated absence of any major sources of error for four laboratories, while one, two and three or more sources were indicated for seven, three and three laboratories respectively. The conclusion that about half the participants need to improve their ER assays was confirmed by three independent reviews. Furthermore, the trial demonstrated that tissue samples are essential as quality assurance material for a realistic assessment of ER assays in biopsy specimens.*

INTRODUCTION

THE presence or absence of estrogen and/or progestin receptors (ER and PR) in breast cancer biopsies is widely used to predict response to endocrine therapy in breast cancer, and several national and international quality assurance programmes have been aimed at reducing between laboratory variation in receptor measurements [1–7]. The approach has generally been to reduce between laboratory variation by recommending standardized assays using cytosol samples as quality assurance material. Although cytosol samples are suitable for evaluating the receptor assays and the associated protein measurements, it has been recognized that cytosol preparation from tissue samples is itself a major source of error within and between laboratories [8–10]. Therefore, for quality assur-

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ance of receptor measurements in tissue samples (i.e. breast cancer biopsies), cytosol samples may have limited value as quality assurance material.

The aim of the current trial was to resolve the relative importance for individual laboratories of cytosol preparation, protein measurement and ER assay as major sources of between laboratory variation in ER measurements, using both tissue and cytosol samples as quality assurance material. The ER-EIA kit (Abbott Laboratories, U.S.A.) was used as a relative reference assay for comparison between the participants In-House assays. The detailed sources of error due to individual assay parameters as well as technical proficiency, were not evaluated since this approach was precluded by the large number of possible permutations.

MATERIALS AND METHODS

Tissue fragments and lyophilized cytosol samples were prepared from the same batch of calf uterus material in identical buffers (10 mM Tris, 1.5 mM EDTA, 5 mM molybdate and 1 mM monothioglycerol, pH 7.4) as previously described [10]. The reference laboratory allocated both tissue and cytosol samples at random to each of the participants. The participants did not know that each set of four tissue and four cytosol samples was identical or that they were prepared from the same material. By comparing the results from the two types of material, the quality of the cytosols prepared by the participants from tissue could be evaluated. The participants were asked to assay both sets of samples for ER with their In-House assays as well as with the ER-EIA kit (Abbott Laboratories, U.S.A., Cat. No. 1068). The Abbott kit served as an independent reference assay, since in contrast to the In-House assays, which were different between laboratories and which measure receptor binding (charcoal separation and Scatchard plots), the Abbott assay is an enzymetric monoclonal immunoassay. A proficiency test was conducted by Abbott Diagnostics, Australia, to ensure that all the participants were familiar with the kit prior to the trial.

In order to evaluate the protein assay as a between laboratory source of error, the participants were also asked to reconstitute the lyophilized cytosol samples in 10 ml of distilled water and to report the protein concentrations. Furthermore, in order to assess the protein extraction efficiency from tissue samples (% protein per weight of tissue), the participants were asked to measure weight of tissue prior to homogenization as well as volume and protein concentration in their cytosol preparations. Comparisons of protein measurements, extraction efficiencies and the results of two independent ER assays from both tissue and cytosol samples could then be made to evaluate the sources of error in ER measurements between laboratories.

The sources of error are inter-related in their effects on ER measurements, and linear regression analysis was used to compare related parameters. Average within laboratory CV was calculated as root mean square of the individual laboratories CV, between laboratory CV as the CV of the means for the laboratories, and overall CV as the CV of all the data.

RESULTS AND DISCUSSION

The results of ER measurements by 17 laboratories for both tissue and cytosol samples using In-House as well as the Abbott assays are shown in Table 1. It should be noted that, although three laboratories used the Abbott assay as their routine ER assay and one laboratory did not compare the Abbott assay with their In-House assay, precluding evaluation of the missing assay for these laboratories in subsequent figures and tables, sufficient data were returned to enable detailed comparisons between and within the laboratories. In addition, the results from protein measurements of the cytosol samples as well as protein extraction efficiencies of tissue samples are also shown in Table 1. It can be seen that a wide range of ER values were reported for both tissue and cytosol samples using both types of assays (from 29 to 234 fmol/mg protein). This range resulted in a between laboratory CV of about 65% for tissue samples and about 40% for cytosol samples. However, the participants obtained lower overall mean values for tissue compared to cytosol samples (about 110 and 150 fmol/mg protein, respectively). This appeared to be due to a relative under-estimation of ER in tissue samples compared to cytosol samples by some of the laboratories (see Table 1), and not due to degradation of ER in tissue samples during transport since no correlation between distance of transport and receptor measurements was observed. Furthermore, when two sets of tissue samples were sent to one participant with an interval of 3 weeks, the mean ER concentrations obtained in the two sets were not significantly different at 48 ± 12 (S.D.) and 46 ± 15 (S.D.) fmol/mg protein respectively.

When the results from the In-House assays are compared to the Abbott assay in Table 1, it can be seen that the mean ER results for the In-House assays were higher than for the Abbott assay (122 vs. 90 fmol/mg protein for tissue, and 161 vs. 140 fmol/mg protein for cytosol). The lower results with the Abbott kit were as expected since bovine ER, which was supplied as samples, has reduced cross-reactivity compared to human ER standard in the Abbott immunoassay [6, 11]. However, the reduced ER values obtained with the Abbott assay did not preclude the use of bovine material since a linear correlation was found between the two types of assay [11], and it is the relative differences

Table 1. Comparison of the mean ER measurements (fmol/mg *protein) between In-House and Abbott assays in tissue and cytosol samples for individual laboratories. The mean protein measurements* (mg/ml) *in the cytosol samples and the mean protein extraction efficiencies* (% *of tissue weight) are also shown)*

Lab I.D.	In-House		Abbott		Protein Concentration	Extraction Efficiency
	Tissue	Cytosol	Tissue	Cytosol	Cytosol	Tissue
1	46 (32)*	234 (14)	33 (33)	137 (14)	2.8 (15.6)	6.2 (5.6)
2	212 (28)	229 (4)	160 (38)	188 (8)	2.3 (2.3)	2.5 (3.0)
3	224 (27)	220 (8)	216 (18)	135 (8)	2.5 (5.3)	3.4 (9.0)
4†	—	—	15 (59)	92 (14)	3.3 (3.2)	4.1 (3.6)
5	203 (18)	231 (16)	105 (22)	153 (12)	1.9 (5.1)	2.5 (9.7)
6	75 (14)	71 (3)	97 (20)	96 (5)	1.5 (3.3)	1.9 (5.0)
7†	—	—	55 (15)	50 (5)	5.1 (2.3)	5.6 (4.1)
8	116 (34)	216 (2)	76 (47)	174 (1)	2.7 (0.2)	2.4 (1.6)
9	225 (39)	209 (9)	154 (52)	139 (13)	2.1 (6.0)	2.2 (16.7)
10	82 (13)	188 (11)	99 (24)	214 (14)	2.1 (9.3)	3.9 (20.0)
11	53 (76)	128 (12)	77 (61)	218 (6)	2.9 (3.8)	2.8 (7.6)
12†	—	—	40 (62)	125 (15)	2.4 (9.4)	2.1 (7.6)
13‡	129 (20)	137 (9)	—	—	2.8 (7.6)	3.1 (2.0)
14	76 (30)	112 (18)	72 (33)	160 (18)	1.4 (18.5)	1.1 (17.3)
15	106 (52)	106 (27)	89 (29)	158 (12)	1.2 (5.0)	1.4 (46.8)
16	129 (8)	138 (6)	65 (33)	86 (2)	3.9 (2.6)	4.6 (6.0)
17	29 (8)	38 (15)	96 (27)	115 (9)	3.6 (2.6)	3.0 (7.9)
Mean	122	161	90	140	2.6	3.1
Overall CV	62%	41%	65%	34%	37%	45%
Between CV	56%	41%	57%	33%	37%	45%
Within CV	27%	10%	33%	10%	5%	10%

*Within individual laboratory % CVs ($n = 4$).
†Laboratory only performed Abbott assay.
‡Laboratory only performed In-House assay.

between laboratories and assays that are used to unravel the sources of error.

It is important to note from Table 1 that the within laboratory CV was substantially lower than the between laboratory CV for all measurements, indicating that the sample homogeneity was adequate for evaluation of the sources of error between laboratories. In particular, the within laboratory CV was only about 30% for tissue and 10% for cytosol samples, whereas the between laboratory CVs were about 60% and 40% respectively. The increased within laboratory CV for tissue compared to cytosol samples is due to the additional error components of tissue heterogeneity and cytosol preparation from tissue. However, for these two sources of error, it has previously been shown that cytosol preparation is the major source of between assay error within a single laboratory [8]. Within laboratory CVs of about 35% for pulverized tissue and about 10% for cytosol samples were reported, which are comparable to the mean within laboratory CVs obtained in the present study using fragmented tissue samples and lyophilized cytosol samples.

It can be seen in Table 1 that the participants also obtained a wide range of protein concentrations from 1.2 to 5.1 mg/ml in the cytosol samples provided, and that the between laboratory CV for protein measurements of 37% was similar to the CV for ER measurements (about 40%). Likewise, the protein extraction efficiencies, calculated as % of tissue weight, varied greatly between the laboratories from 1.1 to 6.2%, and resulted in a CV of 45%, which was also comparable to that of the ER measurements.

The three main sources of between laboratory error in receptor measurements, identified here as variations in protein measurement, cytosol preparation and receptor assay (including data reduction), are interrelated in their effects on the obtained ER concentration. For example, the effect of protein under-estimation leading to over-estimation of receptor concentration may be cancelled by receptor degradation during cytosol preparation from tissue. Evaluation of the complex interactions between the sources of error was therefore approached by subdividing the wide ranges of results in Table 1 into classifications within subjective limits. The performance of individual laboratories could then be systematically evaluated using objective rules for the interactions between the major sources of error. Protein concentrations between 1.9 and 2.9 mg/ml in Table 1 were regarded as acceptable and classified as medium values, since a majority of laboratories were grouped

within these limits (see also Figs. 1 and 3). The expected effects of under- or over-estimation of protein concentration on the ER values could then be confirmed by the independent ER measurements in tissue and cytosol samples by the In-House and Abbott assays. Thus, excluding the three lowest and four highest protein values in Table 1, the between laboratory CV was reduced from 37% to a more acceptable value of 14%, giving an overall mean protein concentration in the cytosol samples of 2.45 mg/ml ($n = 10$). This classification of protein measurements is compared with the protein extraction efficiencies from tissue samples in Fig. 1. It can be seen that a highly significant correlation ($P < 0.0005$) was found between extraction efficiencies and protein measurements. The extraction efficiencies were therefore classified into low, medium and high (L, M and H) for maximum overlap with the protein ranges. The strong correlation between extraction efficiency and protein measurement suggest that, in this trial, differences in protein measurements between laboratories may be sufficient to account for the observed range of extraction efficiencies, irrespective of the homogenization method used.

The laboratories used three different methods of tissue homogenization; Microdismembrator, Polytron and Ultraturrax. Comparisons between homogenization methods and the protein extraction efficiencies as well as ER measurements in tissue samples are shown in Fig. 2. The differences in homogenization methods between laboratories had no significant effects on either extraction efficiencies or ER measurements, and it can be seen that similar ranges of values were obtained for any of the homogenization methods (see Fig. 2). Protein measurement therefore remained as a main source of error both for extraction efficiencies and ER assays between the laboratories.

Since ER values are expressed relative to protein concentration, over- or under-estimation of protein

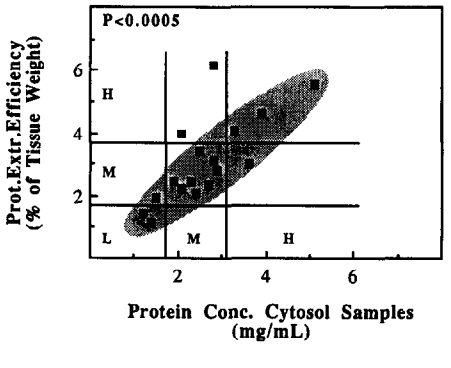

Fig. 1. Comparison of protein measurements in cytosol samples with extraction efficiencies in tissue samples. The shaded area indicates protein measurement as the major source of error for extraction efficiencies unrelated to homogenization method.

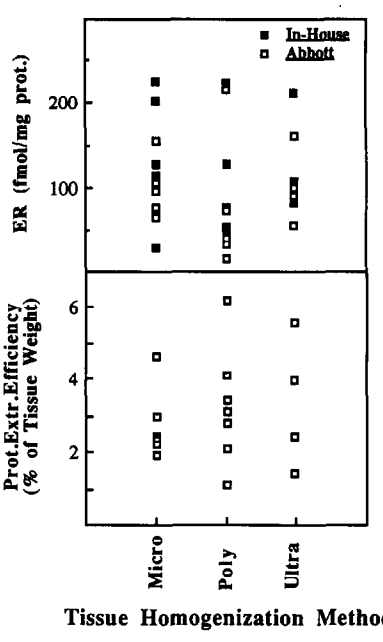

Fig. 2. The effect of different tissue homogenization methods (Microdismembrator, Polytron and Ultraturrax) on the ranges of ER concentration and protein extraction efficiency obtained by individual laboratories.

concentration would be expected to result in low or elevated ER values respectively. The protein concentrations measured in the standard cytosol samples were therefore compared with the ER values obtained by individual laboratories for the In-House and Abbott assays in Fig. 3. There was no significant correlation between protein and ER concentration as measured by the In-House assays, indicating that differences in protein measurements between laboratories were not sufficient to account for the variation in ER concentration. However, in contrast to the In-House assays, a significant correlation ($P < 0.025$) between ER and protein measurements was found for the Abbott assay in Fig. 3, indicating protein measurement as a major source of error between laboratories. The laboratories that obtained results consistent with the expected inverse relationship between protein and ER concentration have been highlighted by the shaded areas in Fig. 3. For these laboratories over-estimation of protein may account for under-estimation of ER, and the ER results were classified accordingly. However, additional sources of error are indicated for those laboratories that under-estimated both protein and ER. The data are therefore consistent with interpreting the classifications of medium protein and high ER concentrations by both the In-House and Abbott assays, as indicating absence of any sources of error. This interpretation may be confirmed by removing protein measurement as a source of error as described below, and by comparing the results from cytosol and tissue samples by both In-House and Abbott assays.

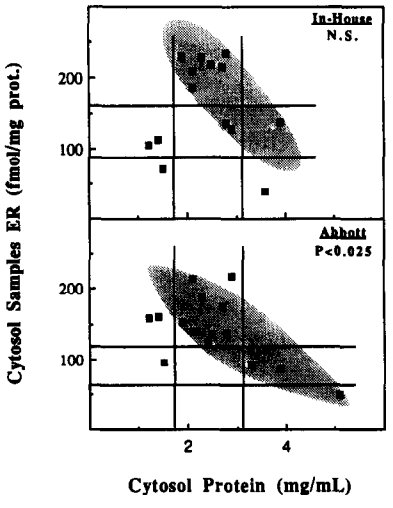

Fig. 3. Comparison of ER and protein measurements in cytosol samples with the In-House and Abbott assays. The expected inverse relationship between protein and ER concentrations is indicated by the shaded area.

For cytosol samples, variation in protein measurements between laboratories can be excluded from the ER results by expressing ER concentration relative to volume of cytosol (fmol/ml cytosol) instead of per weight of protein (fmol/mg protein). The comparison between ER per weight and volume is shown in Fig. 4. A significant correlation between ER expressed per weight and volume was found for both the Abbott and In-House assays ($P < 0.025$ and $P < 0.0005$ respectively, Fig. 4), confirming that differences in protein measurements between laboratories were not sufficient to account for the overall between laboratory variation in ER per weight obtained for either of the two assays. The shaded areas in Fig. 4 correspond to the laboratories within the shaded areas in Fig. 3. For these laboratories, over-estimation of protein was sufficient to account for under-estimation of ER concentration per weight since ER concentration calculated per volume was not different (see Fig. 4). However, receptor assays are indicated as sources of error for those laboratories that under-estimated ER concentration in cytosol samples as calculated per volume, since protein measurement and cytosol preparation from tissue can be excluded as sources of error.

The significance of cytosol preparation from solid tissue as a source of error between laboratories can be evaluated by comparing the ER ratios between cytosol and tissue samples since they were prepared from the same material (see Materials and Methods). The sources of error due to ER or protein measurements are then excluded, since they would be similar for both the cytosol and tissue samples within a laboratory, and therefore not affect the ER ratio between the two types of samples. The ER values for tissue samples are compared to those for cytosol samples for individual laboratories in Fig. 5, where the results grouped around lines of identity are indicated by the shaded areas. A significant correlation was found between tissue and cytosol values for the In-House assays ($P < 0.025$), while the correlation for the Abbott assay was not significant. This would suggest that for the In-House assays, cytosol preparation was not the only major source of error in ER measurements, whereas for the Abbott assay it was of major importance as compared to the sources of error due to ER and protein assays. It can also be seen in Fig. 5 that those laboratories that obtained high ER in tissue

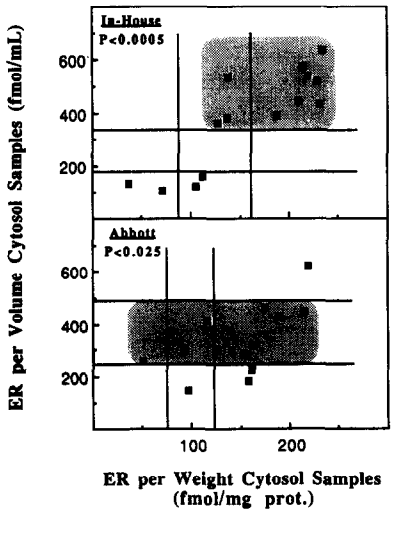

Fig. 4. Comparison of ER per weight and volume in cytosol samples with the In-House and Abbott assays. Under-estimation of protein concentration account for the results within the shaded area.

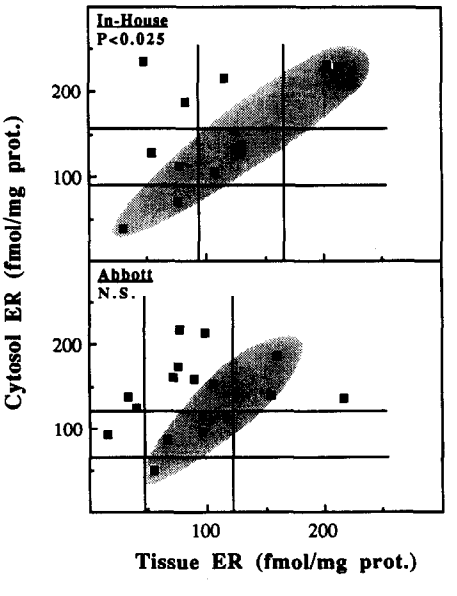

Fig. 5. Comparison of ER measurements in tissue vs. cytosol samples with the In-House and Abbott assays. The laboratories within the shaded areas obtained similar ER results in cytosol and tissue samples, thus excluding cytosol preparation as a source of error.

samples also obtained high ER in cytosol samples, which is consistent with absence of any sources of error for both types of samples. However, laboratories that obtained high ER in cytosol samples did not necessarily obtain high ER in tissue samples, indicating cytosol preparation from tissue as the main source of error (laboratories above the shaded areas). Particularly since an identical buffer to cytosol samples was used to homogenize tissue samples (see Materials and Methods), error in protein measurement can be excluded as a source of error for the relatively higher ER values (per mg protein) in the cytosols [12].

The importance of differences between the In-House assays as a source of error may be evaluated relative to the Abbott assay as shown in Fig. 6, since error in protein measurement would be expected to have a similar effect on ER concentration in both assays, and thus not affect the ER ratios between the two assays. A significant correlation was found for ER measurements between the In-House and Abbott assays for tissue samples ($P < 0.005$), but not for cytosol samples. The absence of correlation for cytosol samples indicates that differences between In-House assays is a source of error, since cytosol preparation and protein measurement are excluded, and higher between laboratory CV was obtained with the In-House assays compared to the Abbott assay (41% vs. 33%, Table 1). However, the correlation that shows up when tissue samples are used, despite the lack of correlation for cytosol samples, clearly identifies the cytosol preparation from tissue samples as a major source of error beyond differences in assays.

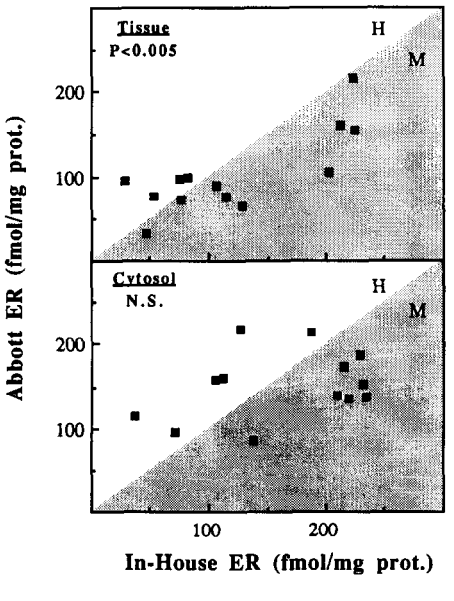

Fig. 6. Comparison of the In-House vs. Abbott assays for ER measurements in tissue and cytosol samples. The shaded areas indicate laboratories that obtained the expected relationship between the two assays due to the reduced cross-reaction of bovine ER in the Abbott assay.

Absence of errors due to either the In-House or Abbott assays are indicated for those laboratories below the diagonal lines in Fig. 6, since the ER ratio between the two independent types of assays is expected to be less than 1.0. Thus, it has previously been reported [6, 11] that bovine ER has reduced cross-reaction with human ER in the Abbott assay, whereas bovine and human ER are equivalent in the receptor assays (i.e. the In-House assays). Likewise, the reference laboratory obtained about 70% cross-reaction of bovine ER with human ER in the Abbott assay (unpublished results). Furthermore, the range of acceptable Abbott/In-House ratios from about 0.5 to 1.0 (see Table 3) is comparable to previously reported ratios of 0.38 [11] and 0.85 [13]. However, for the laboratories that underestimated ER by their In-House assays (above the diagonal lines) as well as for those without apparent assay errors, the wide ranges of ER results both below and above the diagonal lines indicates additional or other sources of error. However, the identity of all the major sources of error in ER measurements for individual laboratories can be evaluated by combining all the information outlined in Figs. 1–6.

INTERPRETATION AND CONCLUSION

The design of the trial allowed systematic evaluation of the likely sources of error both overall as well as for individual laboratories. In order to account for severity of error and facilitate evaluation, results were classified into high, medium and low (H, M and L) in concordance with the sources of error as discussed above. The classifications consistent with absence of any major sources of error were used to deduce the relationships between ER results in the presence of any single or combination of errors as shown in Table 2, according to the following four rules. *One*: error in protein measurement would result in over- or under-estimation in ER concentration per weight, but not per volume of cytosol. *Two*: receptor degradation during cytosol preparation from tissue samples would result in under-estimation of ER in the tissues, but not in the reference cytosol samples. *Three*: errors in the receptor assays would either under- or over-estimate ER in both tissue and cytosol samples, whereas the ER ratio in tissue over cytosol samples would be unaffected. *Four*: combinations of several sources of error can be identified by summation of the classifications consistent with each composite source of error.

The relative effects of the major sources of error due to cytosol preparation, protein measurement and In-House or Abbott receptor assays either as single sources or in combinations, could then be interpreted from the classifications for individual laboratories as shown in Table 3. Note that labora-

Table 2. Interpretation of the effect of single sources of error on classifications of results given in Figs. 1–6. Only classifications deviating from those expected in the absence of any sources of error are given. Several sources of error will give summations of the classifications resulting from each source of error

	Cytosol samples						Tissue samples			Tissue/cytosol	
Assay parameter unit	I-H protein (mg/ml)	I-H ER/weight (fmol/mg)	I-H ER/vol (fmol/ml)	Abbott ER/weight (fmol/mg)	Abbott ER/vol (fmol/ml)	A/I-H ER/weight ratio	I-H ER/weight (fmol/mg)	Abbott ER/weight (fmol/mg)	A/I-H ER/weight (ratio)	I-H ER/weight (ratio)	Abbott ER/weight (ratio)
Expected	M	H	H	H	M	M	H	H	M	M	M
High protein	H	L,M		L,M			L,M	L,M			
Low protein	L	vH*		vH*			vH*	vH*			
Cytosol preparation							L,M	L,M		L	L
Low In-House		L,M	L,M			H	L,M		H		
High In-House		vH*	vH*			L*	vH*		L*		
Low Abbott				L,M	L,M	L*		L,M	L*		
High Abbott				vH*	H	H		vH*	H		

*These classifications resulting from single sources of error were not encountered, but may have been encountered in the absence of additional sources of error.

Table 3. Sources of error for individual laboratories due to cytosol preparation (C), protein measurement (P), In-House (I) and/or Abbott (A) assay are evaluated from classifications given in Figs 1–6 (H, M and L), and interpretations given in Table 2. Only classifications deviating from those expected in the absence of errors are shown for individual laboratories

	Cytosol samples						Tissue samples			Tissue/cytosol		
Assay parameter unit	I-H prot (mg/ml)	I-H ER/weight (fmol/mg)	I-H ER/vol (fmol/ml)	Abbott ER/weight (fmol/mg)	Abbott ER/vol (fmol/ml)	A/I-H ER/weight ratio	I-H ER/weight (fmol/mg)	Abbott ER/weight (fmol/mg)	A/I-H ER/weight ratio	I-H ER/weight ratio	Abbott ER/weight ratio	Major source of error
Expected range	M 1.9–2.9	H 188–234	H 363–638	H 125–218	M 254–464	M 0.6–0.8	H 203–225	H 154–216	M 0.5–1.0	M 0.7–1.1	M 0.7–1.1	Nil
Lab 2												Nil
9												Nil
3											H	Nil
5								M				Nil
8							L	L		L	L	C
1							M	M		L	L	C
10						H	L	M	H	L	L	C
12*		—	—			—	—	L	—	—	L	C
16	H	M		M			M	M				P
7*	H	—	—	L		—	—	M	—	—		P
13†		M		—		—	M	—	—		—	I
4*	H	—	—	M		—	—	L	—	—	L	CP
11		M			H	H	L	M	H	L	L	CA
17	H	L	L	M		H	L	M	H			PI
6	L	L	L	M	L	H	L	M	H			PIA
15	L	M	L		L	H	L	M			L	PCIA
14	L	M	L		L	H	M	M			L	PCIA

*Laboratory only performed Abbott assay.
†Laboratory only performed In-House assay.

tory Nos. 4, 7, 12 and 13, as indicated in Table 3, only performed either the Abbott or the In-House assay, and therefore only were evaluated for the relevant assay. It can be seen that for four laboratories (returning complete sets of data), the results indicated absence of any major sources of error. One source of error only appeared to have affected the results from seven laboratories (cytosol preparation from tissue, protein measurement or In-House assay for four, two and one laboratories respectively). Two sources of error were indicated for three laboratories (cytosol preparation from tissue and protein measurement, cytosol preparation and Abbott assay, or protein measurement and In-House assay). Three or more sources of error were indicated for three laboratories.

Taking into account the severity of the sources of error, it is suggested that about half of the participants obtained acceptable results. Thus, eight laboratories obtained ER results in the tissue samples that were classified as medium or high in the absence or presence of only source of error. This analysis was of considerable interest to the participants, and was independently carried out by three

of the participants. All three reviews as well as the above evaluation nominated the same seven laboratories out of respectively seven, eight and 10 well-performing laboratories.

We conclude that tissue samples are essential as a realistic quality assurance material in assessing the reliability of ER assays on breast cancer biopsy specimens, since cytosol preparation from tissue was shown to be a major source of error. Identification of the technical details causing major errors for individual laboratories was outside the scope of the present study. However, it is recommended that particular attention should be focused on the method of cytosol preparation from tissue, since some laboratories under-estimated ER concentration in tissue, but not in cytosol samples. Furthermore, the large variation in protein measurements obtained between laboratories suggest that standardization of both the protein as well as the ER assays, would result in a reduction of between laboratory CV. The design of the trial allowed identification of the likely sources of error for individual laboratories, and should facilitate trouble-shooting and hence improve the reliability of ER assays as a diagnostic tool for prediction of response to endocrine therapy in breast cancer.

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