Eur J Cancer Clin Oncol, Vol. 18, No. 8, pp. 713–721, 1982.
Printed in Great Britain.

0277-5379/82/080713-09$03.00/0

A Quality Control Study to Assess the Inter-laboratory Variability of Routine Estrogen and Progesterone Receptor Assays

DAVID T. ZAVA,* AENN WYLER-VON BALLMOOS,* ARON GOLDHIRSCH,* WERNER ROOS,† ATSURO TAKAHASHI,† URS EPPENBERGER,† STEFAN ARRENBRECHT,‡ GEORG MARTZ,‡§ GABRIELE LOSA,‖ FULGENCIO GOMEZ¶ and CHRISTIANE GUELPA**

**Ludwig Institute for Cancer Research (Bern Branch), Bern, Switzerland, †Department of Gynecology and Research, Kantonsspital, Basel, Switzerland, ‡Department of Internal Medicine, Universitätsspital, Zürich, Switzerland, ‖Ticino Institute of Pathology, Locarno, Switzerland, ¶Département de Medicine, Centre Hospitalier Universitaire Vaudois, Switzerland, **Center for Oncology-Hematology, Cantonal Hospital, Geneva, Switzerland and §Swiss Group for Clinical Cancer Research (SAKK)*

Abstract—*The steroid hormone receptor laboratories of Bern, Basel, Zürich, Locarno, Lausanne and Geneva have participated in a quality control study to assess potential inter-laboratory variability in results of tests for determination of steroid hormone receptor status in human breast tumor biopsies. Homogeneous breast tumor powders containing low, medium and high concentrations of estrogen receptors (ER) and progesterone receptors (PR) were prepared in Bern and dispatched on solid CO_2 to each laboratory within* 1 day *of preparation. Each laboratory was requested to assay each powder for ER and PR by their usual procedures. The results revealed that the quantitative discrepancies in ER and PR binding values among the participants could be attributed in part to variations in the methods used for measuring cytosol protein content and also to the differences in hormone receptor assay methods. Nevertheless, all of the laboratories were able to identify the samples containing low, medium and high concentrations of ER and PR.*

INTRODUCTION

IT IS now generally acknowledged that patients whose breast tumors contain estrogen receptors (ER) are more likely to respond to hormone therapy [1, 2] and have a significantly longer disease-free interval and survival [3]. The ER status of breast tumors has also been shown to be useful for selecting the patients with no tumor invasion of the lymph nodes who should be treated with more aggressive adjuvant chemotherapy [4]. Although the response rate to hormone therapy is high among patients with ER-positive (ER+) tumors, only about 60% of this group actually manifest an objective response. It is unclear as to why those in the remaining ER+ group do not respond; several theories have been formulated, however, including one that ER is defective [5] and another that a heterogeneous population of ER+ and ER-negative (ER−) tumor cells exists in the same tumor [5, 6].

Accepted 22 March 1982.

Progesterone receptor (PR) is an excellent marker for estrogen action since its concentration increases dramatically in both normal and neoplastic cells exposed to estrogen. PR in combination with ER has also been shown to be a better indicator of human breast tumor response to hormone therapy [2, 7]. Tumors which contain both ER and PR are more likely to be inhibited by hormonal manipulation than ER+, PR− tumors. For this reason, PR analysis has been included in the routine of most laboratories which assess human breast biopsies for steroid receptor.

ER and PR status in breast tumors is determined primarily for two purposes: first, to aid the clinician in choosing or rejecting hormone therapy as a palliative treatment for patients with systemic disease; and second, to contribute useful information to national and international study groups investigating the relevance of breast tumor hormone receptor status to other disease parameters such as menopausal status, histopathology, response to

therapy and epidemiology. For such retrospective comparisons of the various aforementioned disease parameters to be meaningful, it is important that the intra- and inter-laboratory variabilities of receptor data-gathering be reduced to a minimum. This requires that the procedures for tissue collection and the methods for receptor analysis be as uniform as possible.

Quality control studies are therefore necessary to identify potential differences in methodology and to test the feasibility of statistically comparing tumor receptor data with other disease parameters among the participants of large-scale trials.

A quality control study was organized among six laboratories which are members of the SAKK Steroid Receptor Study Group [8] to ascertain if the individual methods of the laboratories contributing ER and PR data to common breast cancer trials permit a legitimate statistical comparison of the receptor results. The study was designed to identify possible factors responsible for inter-laboratory variations in ER and PR assay results.

MATERIALS AND METHODS

Preparation and distribution of frozen tumor powders

Tissues for this study were derived from the residual portion of frozen (−70°C) human breast cancer biopsies (stored 1–12 months) which had been previously assayed for ER and PR. Frozen biopsies were pooled from three categories of ER and PR-binding profiles: those tumors containing less than 10 (Category A), 20–50 (Category B) and >50 (Category C) fmol ER and/or PR/mg cytosol protein. Approximately 15 g of frozen tumor specimens from each category listed above were combined and pulverized under liquid nitrogen to a fine powder. The powders were thoroughly mixed and pulverized twice more to assure tissue homogeneity.

Frozen powders of each category, coded A, B and C, were packed in small plastic vials (1.5 g/vial) and dispatched on solid CO_2 within 24 hr of preparation to each of the five participants. All samples arrived within 24 hr. There were no mishaps during transport which could account for erroneous ER or PR assay results.

Procedures for processing powders

Each participant was requested on receipt of the tumor powders to process the contents as follows:

1. Store the tissue at −70°C until assayed for ER and PR.
2. Process 1 g of each sample (A, B, C) by methods normally used in his particular laboratory.
3. Return 0.5 g of the original powder from each sample to the reference laboratory on solid CO_2.
4. Assay half of the cytosol prepared from 1 g of each powder (step 2) and snap-freeze (liquid nitrogen) the remaining half and return it frozen on solid CO_2 to the reference laboratory.
5. Describe specific details of the assay methods.
6. Record all results (e.g., counts per minute for each dose of radiolabeled estrogen or progestin, binding constants (K_d) according to the method of Scatchard [9], protein value of cytosol, fmol receptor/mg cytosol protein) on a questionnaire sent by the reference laboratory.
7. Assay tumor powders within two weeks.

Standard procedures used by the reference laboratory to measure ER and PR

One gram of frozen pulverized tissue was homogenized in 3 ml of Tris buffer (0.01 M Tris: HCl, pH 7.4 at 4°C, 0.0015 M EDTA, 10% glycerol, 0.001 M monothioglycerol) with a Polytron PT-ST homogenizer (Brinkman Instruments, Inc.) at a speed setting of 3.5 for 3 × 10-sec intervals. The homogenate was centrifuged at 100,000 *g* for 30 min at 0°C to sediment the crude cytoplasmic and nuclear debris. The resultant high speed supernatant (cytosol) was diluted to 10 ml with Tris buffer. Protein content of the diluted cytosols was determined by the method of Lowry *et al.* [10], with BSA as a standard protein.

The ER and PR contents of the diluted cytosols were determined by incubation of 100 μl cytosol (in duplicate) with 100 μl of five doses (0.4–6 nN) of [^{3}H]-2,4,6,7-estradiol (E, New England Nuclear) with and without a 100-fold excess of diethylstilbestrol (DES). PR was assayed with five doses (1–10 nM) of [^{3}H]-promegestone (R5020, New England Nuclear) with and without a 100-fold excess of non-labeled R5020.

The radioactive ligands and cytosol were incubated at 4°C for 3 hr. A 500-μl suspension of dextran-coated charcoal (DCC) (2.5 mg Norit A activated charcoal, 25 μg dextran in 1 ml Tris buffer, pH 8.0, 4°C) was added to each tube. The charcoal was resuspended every 5 min for 30 min and then sedimented by centrifugation at 4°C for 5 min at 2000 rpm.

The DCC-treated supernatant was combined with 3 ml of Beckman Ready-Solv scintillator

and counted in a Beckman Model LS8100 liquid scintillation counter with a counting efficiency of 28%. The ER and PR data were analysed by the method of Scatchard to determine the dissociation constants (K_d) and concentrations of ER and PR in each tumor specimen, expressed as fmol receptor per mg of cytosol protein.

Assay procedures used by participants

The distinguishing features of the methods used by each of the five participants (I–V), including the reference laboratory (REF LAB) for comparison, is illustrated in Table 1. All groups used the Tris buffer described above. Laboratories I and V measured the protein content of the tumor cytosols using spectrophotometric [11] and turbidimetry [12] methods respectively, as previously described. All laboratories assayed ER and PR with five concentrations of ligands, as indicated in the text, with the exception of laboratories IV and V, which used seven and six doses, respectively.

Inter-laboratory variations in data analysis

To check for inter-laboratory differences in methods of calculating the raw ER and PR binding data, Laboratory I sent to each of the institutions a questionnaire listing the unprocessed counts of a five-dose Scatchard analysis. Included was other pertinent information (assay volume, volume of DCC added, volume counted, protein concentration, efficiency of scintillation counter, specific activity of radiolabeled ligands used) necessary to make the appropriate calculations. Each laboratory was requested to calculate by their routine methods the following information: (a) the concentrations of ER and PR in fmol/mg cytosol protein, and (b) the binding constants (K_d) for each sample. Any comments concerning the binding plots (Scatchard analysis) were to be recorded on the questionnaire to help clarify discrepancies in results.

RESULTS

Intra- and inter-laboratory comparison of receptor results

The ER and PR binding values reported by each of the groups are represented in Table 2. The results are divided into four categories. The first category (Tumors Assayed in REF LAB) represents stored tumor powders which were assayed consecutively five times for ER and PR in the REF LAB during a one-month period. All participants were asked to assay the three different tumor samples during the same month. Category 2 (Powders Returned by Participants to REF LAB—Assayed in REF LAB) represents the 0.5 g of each frozen tumor sample which was returned to the REF LAB after having been sent to the participants. These results were used to assess loss of receptor content in the tumor powder during transport. Category 3 (Cytosols Prepared by Participants—Assayed in REF LAB) is essentially the same as Category 2 except that a portion of the cytosols prepared by the participants was snap-frozen after dilution and shipped back to the REF LAB. This enabled us to detect differences in methods for measuring cytosol protein content and also to identify differences in incubation conditions that would account for potential discrepancies in ER and PR binding sites, assuming that there was no loss of receptor from tumors frozen in buffer (Category 3) as compared with those frozen as powders (Categories 1 and 2). Category 4 (Powders Assayed by Participants) represents the ER and PR binding values for the three samples assayed by the participants.

The results of Table 2 reveal that the low, medium and high concentrations of tumor ER, but not PR, could be clearly distinguished, whether the tissue remained in the reference laboratory (Category 1), was returned by the participants in the same frozen powder originally delivered to them (Category 2) or was returned in the form of cytosol prepared from the frozen powder by the participants (Category 3). Only in three cases were the low binding (Sample A) ER values $\geq$10 fmol/mg protein. In each of these instances the binding constant (K_d) would be considered too high (>1 nM) for the estradiol-binding site to represent true ER, which characteristically falls within a K_d range of 0.1–0.5 nM. The results of the first three categories exclude any possibility that ER was destroyed in the frozen powders during transport or that differences in binding values were attributable to the methods used for preparing cytosols (i.e., buffer, homogenization, centrifugation techniques, etc.). Intra-laboratory variation in PR binding sites for the first three categories was much greater than for ER. In one case (Category 2—PR sample C), PR was almost three-fold higher than the other values of this category. Microheterogeneity of tumor powders may account for this quantitative discrepancy; however, the fact that the ER values from the same powder were quite uniform relative to the other powders of Category 2 makes this possibility unlikely. PR but not ER values (Category 2) were significantly lower than the other samples. Again, a clear explanation for this is not avail-

Table 1. Methods used by participants

Center	Homogenizer	Centrifuge speed (*g*)	(time in mins)	Ligands (dose* in nM)	Protein assay†	Cytosol vol. (ml)	Protein‡ (mg/ml)	Incubation vol.§ (ml)	Incubation time‖ (ER/PR)	DCC (ml)¶ (Time ER/PR)
REF LAB	Polytron	100,000	(30)	E + DES (0.2–3) R5020–R5020 (0.4–6)	Lowry	0.1	1.5–2	0.2	3 hr	0.5(30/30)
I	Ultra-Turrax	100,000	(60)	E ± DES (0.13–2) R5020– Pg (0.5–8)	Biorad	0.1	2	0.1	overnight	0.2(10/10)
II	Polytron	100,000	(60)	E ± DES (0.1–2) R5020- Pg (0.5–8)	Lowry	0.1	2	0.1	4 hr	0.2(15/15)
III	Heidolph	100,000	(30)	E ± DES (0.13–2)	Lowry	0.1	2	0.1	4 hr	0.2(30/30)
IV	Polytron	100,000	(60)	E ± DES (0.05–1) R5020–R5020 (0.6–5)	Lowry	0.1	2	0.2	22/4 hr	1(30/30)
V	Ultra-Turrax	100,000	(60)	E ± E (0.15–3)	Turbidimetry	0.1	2	0.2	overnight	0.25(30/30)

REF LAB = Ludwig Institute for Cancer Research, Inselspital, Bern; I = Dept. of Gynecology and Research, Basel; II = Dept. of Medicine, Zürich; III = Ticino Institute of Pathology, Locarno; IV = Dept. of Medicine, Lausanne; V = Center for Oncology-Hematology, Geneva.

*Final concentration of ligand.

†BSA was used as a standard protein in all laboratories.

‡Final diluted concentration of protein added to incubation.

§Total incubation volume.

‖Incubation time at 0–4°C.

¶Volume of DCC suspension (concentration as indicated in text) added to incubation tubes.

Table 2. ER and PR concentrations assayed in different institutions

CENTER	DATE	Estrogen receptor* Low A	Med B	High C	Progesterone receptor* Low A	Med B	High C
Tumors assayed in reference laboratory							
REF LAB	16.12.80	13	32	87	0	61	217
REF LAB	18.12.80	0	33	120	4	106	354
REF LAB	19.12.80	6†	63	139	4	140	167
REF LAB	2.01.81	3†	32	112	2	119†	268
REF LAB	16.01.81	6	34	115	0	42	259
Mean ± S.D.‡		6 ± 5	39 ± 12	115 ± 17	2 ± 2	93 ± 37	253 ± 62
Tumors returned by participants—assayed in REF LAB							
I –REF LAB	8.01.81	0	40	154	0	77	533
II –REF LAB	8.01.81	8	41	138	0	127	169
III–REF LAB	8.01.81	8†	37	75	7	65	145
IV–REF LAB	8.01.81	8†	32	74	5	97	145
V–REF LAB	16.01.81	8	28	109	0	22	33
Mean ± S.D.‡		6 ± 3	36 ± 5	110 ± 32	2 ± 3	78 ± 35	205 ± 170
Cytosols prepared by participants—assayed in REF LAB							
REF LAB–REF		10†§	29	97	0	47	145
I –REF LAB	8.01.81	2	—	115	0	—	41
II –REF LAB	8.01.81	10†	43	164	0	30	95†
III–REF LAB	8.01.81	2	25	50	0	25†	102†
IV–REF LAB	8.01.81	5†	41	(–)	0	83	87†
V –REF LAB	16.01.81	6	23	100	0	21	84
Mean ± S.D.‡		6 ± 3	32 ± 8	105 ± 37	0	41 ± 22	92 ± 31
Tumors assayed by participants							
I	22.12.80	4	115	281	41†§‖	134†	168
II	—	3	64	295	7	118	356
III	22.12.80	5	26	75	5	75	227
IV	17.12.80	5	25	105	0	76	109
V	—	26†§	85	326	0	61	100
Mean ± S.D.‡		9 ± 9	63 ± 35	216 ± 105	11 ± 15	93 ± 28	192 ± 93

*fmol ER or PR/mg cytosol protein.
†Binding constant (K_d) > 1 nM (ER) or >2 nM (PR).
‡Mean value of receptor content in each category ± standard deviation.
§Receptor values were difficult to evaluate based on Scatchard analysis.
‖Receptor content was based on the highest single dose (8 nM).

able since the ER data from this same powder was consistent with the other ER values within this category. It is apparent that the PR content of tumors was subject to greater diminution than that of ER during transport, especially in the form of cytosols (Category 3).

Although there was little intra-laboratory variation in the ER and PR content of tumor powders measured in the reference laboratory five consecutive times over a one-month period (Category 1), inter-laboratory concordance (Category 1 vs Category 4) was far less uniform. The quantitative values of ER and PR were either in accord with (Participants III and IV) or higher than (Participants I, II, V) those reported by the reference laboratory. Although there was not a consensus among the laboratories about the quantitative content of ER and PR in tumors, all groups (exceptions: Participant I, PR sample A, and Participant V, ER sample A, where the binding values either could not be evaluated based on Scatchard analysis or the binding constant (K_d) indicated a non-receptor estradiol binder) were generally able to qualify each tumor as containing low-, medium- or high-range ER and PR, even though some overlap in medium- and high-range binding did exist. Most important was that no laboratories reported either false-positive ER or PR values based on the Scatchard plot and a cutoff value of less than 10 fmol/mg cytosol protein considered as a negative receptor value. There were no significant group differences (exceptions mentioned above) in the binding constants (K_d's) reported, indicating that the ER and PR binding sites were of high affinity and limited capacity.

Standardization of ER and PR values according to the cytosol protein assayed in the reference laboratory

Since we had excluded the possibility that any quantitative differences in results were due

to receptor degradation during transport, at least for ER, or to the methods of preparing cytosols, it appeared that the inter-laboratory variations in ER and PR assay results (Category 1 vs Category 4) were caused by differences either in the assay methods themselves (Table 1) or in those for measuring cytosol protein. When the cytosols prepared by the participants and analysed for protein content were re-analysed in the REF LAB for protein content, there was an obvious discrepancy in the protein values of Laboratories I, II and V. These laboratories claimed to be using cytosols diluted to a final concentration of 2 mg/ml according to their methods of protein analysis. However, relative to the REF LAB, the protein content was actually much higher—about 3 mg/ml (Table 3). When the ER and PR contents of each tumor group were re-adjusted based on the REF LAB protein values (Table 4), the quantitative concordance in ER and PR values among the laboratories was somewhat improved.

Inter-laboratory variations in methods for calculating data

To assure that differences in final ER and PR concentrations were not due to differences in methods for calculating the binding data, each of the participating institutions was provided with precisely the same set of data and requested to calculate ER and PR binding sites and K_d's by their usual methods. As revealed in Table 5, all of the binding values are in close agreement, with the exception of ER, sample 3. It is noteworthy that differences in ER binding values for this sample are due to the difficulty in evaluating the Scatchard plot. Of further interest is that the high values reported by REF LAB and Laboratory V parallel the high binding constants reported for this sample. These results rule out the possibility that inter-laboratory differences in receptor content are due to methods of data calculation.

DISCUSSION

The primary goal of this study was to determine if laboratories which routinely assay steroid hormone receptors in breast tumor biopsies could accurately identify homogeneous frozen breast tumor powders containing low, medium and high concentrations of ER and PR and, if not, for what reasons.

It is clear that the differences in ER and PR values reported by the participants of this study are not the result of receptor decay during transport; nor could they be attributed to the methods of cytosol preparation or to differences in data processing. The results of this study do indicate, however, that inter-laboratory quantitative discrepancies in ER and PR binding sites arise because of differences in methods for quantitating cytosol protein and incubation conditions. When the ER and PR values were standardized according to the reference laboratory, inter-laboratory variation was significantly reduced but not eliminated entirely, revealing that the incubation conditions (Table 1) were also partly responsible for the quantitative differences seen. The only

Table 3. A comparison of cytosol protein values assayed by the participants and the reference laboratory

Center	Assay date	Method*	Protein (mg/ml) A	B	C
REF LAB	16.01.81	Lowry	1.5	1.6	1.3
REF LAB	22.01.81	Lowry	1.5	1.7	1.3
I	12.80	OD 230/260	2.1	1.9	2.1
REF LAB	08.01.81	Lowry	2.7	—	3.0
II		Lowry	2.0	2.0	2.0
REF LAB	08.01.81	Lowry	3.1	2.5	2.7
III	12.80	Lowry	2.0	2.0	2.0
REF LAB	08.01.81	Lowry	1.9	1.9	2.0
IV	12.80	Lowry	2.0	2.0	2.0
REF LAB	08.01.81	Lowry	1.7	1.6	1.6
V	12.80	Turbidimetry	2.2	1.9	1.8
REF LAB	08.01.81	Lowry	2.7	2.4	2.7

*Protein standard for all laboratories was BSA.

Table 4. Tumors assayed by participants but standardized according to protein values in the reference laboratory

		Estrogen receptor (fmol/mg protein)			Progesterone receptor (fmol/mg protein)		
		A	B	C	A	B	C
REF LAB*	(mean ± SD)	6 ± 5	39 ± 12	115 ± 17	2 ± 2	94 ± 37	253 ± 62
	I	4/2†	115/–	281/196	41/31	134/–	168/119
	II	3/2	64/51	295/218	7/5	118/94	365/263
	III	5/5	26/26	75/75	5/5	75/75	227/227
	IV	5/6	25/31	105/131	0/0	76/95	109/136
	V	26/21	85/67	326/218	0/0	61/48	100/67
Mean ± S.D.‡		9 ± 9/7 ± 7	63 ± 35/44 ± 16§	216 ± 105/167 ± 56	11 ± 15/8 ± 12	93 ± 28/78 ± 19§	192 ± 23/162 ± 72

*Mean values ± standard deviation for 5 assays (category 1, Table 2).

†Second value indicates that receptor content is readjusted according to cytosol prepared by participant, but assayed for protein by the reference laboratory.

‡Mean values ± standard deviation for participants excluding REF LAB (Category 4, Table 2).

§Participant I not included in calculations for mean.

Table 5. Inter-laboratory variance in methods for calculating data

	Estrogen receptor (a) sample			Progesterone receptor (a) sample		
	1	2	3	1	2	3
REF LAB	213	13	2655*	315	61	236
I	211	14	873	370	60	232
I	217	14	613	368	64	251
II	205	14	839	372	62	235
III	220	13	814	338	62	236
IV	230	14	1257*	375	60	236
V†	149	9	1154*	240	39	151

*Binding constant (K_d) was >1 nM (ER) and >2 nM (PR).
†Laboratory V failed to take a dilution factor (×1.5) into consideration when making calculations.

apparent discrepancies in methodology of the laboratories reporting the higher receptor values (I, II and V) were a longer incubation period (overnight for Participants I and V, as opposed to only three to four hours for other laboratories) and a shorter exposure time to DCC (10–15 min for Participants I and II, compared with 30 min for the other participants). Although the data are not shown, the reference laboratory re-assayed the same tumor samples using an overnight incubation but could not detect significant differences in the concentrations of ER or PR in comparison to three-hour incubations.

Other quality control studies carried out among national and international breast cancer trial groups [13–15] have shown that a large inter-laboratory variation in the concentrations of ER in frozen calf uterus does exist; however, these groups failed to identify the reasons. To our knowledge, this study is the first attempt to elucidate the reasons for the inter-laboratory variabilities in the measurement of ER and PR in frozen preparations of human breast tissue.

In view of the foregoing study and other quality control reports [13–15], it is apparent that certain quantitative differences in receptor values will persist among laboratories participating in common trials. It may not be realistic or even practical to expect these laboratories to quantitatively reproduce ER and PR binding data with strict inter-laboratory conformity. However, it should be possible to reduce significantly inter-laboratory variability in receptor results by standardizing the methods for protein analysis. This should allow groups participating in a common trial to qualify tumor receptor content more grossly into the categories of low-, medium- or high-binding. For the attending physician, this type of information should also be adequate to aid in the design of optimal endocrine therapy for the breast cancer patient.

We have focused primarily on the technical problems of the assay methods which can potentially cause quantitative variations in the ER and PR binding values reported by different laboratories. Our results have revealed that the incubation conditions account for the major discrepancies.

It should be recognized that other factors that influence the final tumor receptor content may overshadow the aforementioned assay problems experienced in hormone receptor laboratories. For instance, degradation of the thermolabile receptors in tumor specimens before the tumors reach the steroid hormone receptor laboratory may occur if proper cooling procedures are not practiced. In addition, assay of a portion of the tumor which is not representative of the tumor bulk may lead to erroneous receptor results. Both factors can significantly influence the final concentration of tumor receptor reported by the hormone receptor laboratory. Direct control of these problems is outside the realm of the steroid receptor laboratory.

A quality control study to detect significant inter-laboratory differences in post-surgical tumor collection methods or in the pathologists' methods for selecting a 'representative' section of the tumor bulk for hormone receptor assay is not possible, or would conceivably be very difficult. However, there are several ways of detecting such differences. First, if a participant in a multi-group trial consistently reports low ER and PR binding values relative to the group mean binding values, but has no problem in quantitating hormone receptors in standard preparations from a quality control study, then problems with tumor collection are strongly indicated. Second, histological

identification of tumor cells from a contiguous section of the biopsy specimen will verify that what is being assayed in the hormone receptor laboratory actually contains live tumor cells and not necrotic or fibrotic tissue.

In summary, the results of this quality control study reveal that the methods for measuring protein content of tumor cytosols and the variations in assay techniques account for the quantitative differences in ER and PR reported by different laboratories which assay ER and PR on a routine basis. Even so, all of the participating laboratories were able to classify tumor samples into low-, medium- and high-binding categories, although some overlap occurred with the medium- and high-receptor-binding categories. This should allow a comparison of ER and PR data with other disease-related parameters in the breast cancer trials in which these laboratories mutually participate.

Acknowledgements—Participants of this SAKK study would like to thank Brigitte Remy, Susanne Saurer, Madeleine Schmid, Giovanna Bosshard and others for their excellent technical assistance. DTZ wishes to express special thanks to Carol Wiedmer for invaluable critical manuscript review and preparation.

REFERENCES

1. ALLEGRA JC, LIPPMAN ME, THOMPSON EB *et al.* Estrogen receptor status: an important variable in predicting response to endocrine therapy in metastatic breast cancer. *Eur J Cancer* 1980, **16**, 323–331.
2. OSBORNE CK, YOCHMOWITZ MG, KNIGHT WA, MCGUIRE WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. *Cancer* 1980, **46**, 2884–2888.
3. BLAMEY RW, BISHOP HM, BLAKE JRS *et al.* Relationship between primary breast tumor receptor status and patient survival. *Cancer* 1980, **46**, 2765–2769.
4. VALAGUSSA P, DIFRANZO G, BIGNAMI P, BUZZANI R, BONADONNA G, VERONESI U. Prognostic importance of estrogen receptors (ER) to select node-negative (N−) patients for adjuvant chemotherapy (1981). Proceedings of the Third International Conference on the Adjuvant Therapy of Cancer, Tucson, Texas, March 1981, Abst. 42.
5. MAASS H, JONAT W, STOLBENBACK G, TRAMS G. The problem of non-responding estrogen receptor-positive patients with advanced breast disease. *Cancer* 1980, **46**, 2835–2837.
6. FISHER ER, REDMOND CK, LIU H *et al.* Correlation of estrogen receptor and pathologic characteristics of invasive breast cancer. *Cancer* 1980, **45**, 349–353.
7. BROOKS SC, SAUNDERS DE, SINGHAKOWINTA A, VAITKEVIVIUS VK. Relation of tumor content of estrogen and progesterone receptors with response of patient to endocrine therapy. *Cancer* 1980, **46**, 2775–2778.
8. EPPENBERGER U, ROOS W, HUBER P, CIKES M, STRASSER S, ROSSE G. Bestimmung von steroid hormonerezeptoren für eine endocrine therapie beim mammakarzinoma. *Schweiz Aerztezeitung* 1978, **33**, 1453–1454.
9. SCATCHARD G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci*, 1951, **51**, 660–672.
10. LOWRY DH, ROSEBROUGH WJ, FARR AL, RANDALL PJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1961, **193**, 265–275.
11. KALB UF, BESNLOHR RW. A new spectrophotometric assay for protein in cell extracts. *Biochemistry* 1977, **82**, 362–371.
12. LAYNE E. Spectrophotometric and turbidimetric methods for measuring proteins. *Methods Enzymol* 1957, **3**, 447–454.
13. ZAVA DT, JORDAN VC, GOLDHIRSCH A, WITTLIFF T. Steroid hormone receptor analysis: a quality control study. *Proc Am Assoc Cancer Res* 1981, **22**, 388.
14. RAAM S, GELMAN R, COHEN JL *et al.* Estrogen receptor assay: interlaboratory and intralaboratory variations in the measurement of receptors using dextran-coated charcoal technique: a study sponsored by E.C.O.G. *Eur J Cancer* 1981, **17**, 643–649.
15. KING RJB. Quality control of estradiol receptor analysis: the United Kingdom experience. *Cancer* 1980, **46**, 2822–2824.