Comparison of Monoclonal Antibodies and Tritiated Ligands for Estrogen Receptor Assays in 241 Breast Cancer Cytosols

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Abstract

Estrogen receptor determinations have been performed on 241 cytosols from 160 breast cancer tumors using both radioactive ligands (3H)-estradiol, (3H)R2858) and monoclonal antibodies (Abbott ER-EIA Kit) in order to compare the two methods and to evaluate the clinical usefulness of the new immunological, simplified assay. Intra- and interassay reproducibility of the enzyme immunoassay (EIA) method was studied during a 6-month period on 35 standard curves with 4 different batches of monoclonal antibodies. Intraassay coefficients of variation studied on duplicates were smaller than 5% in most cases. Interassay reproducibility of the curves showed coefficients of variation lower than 10% except for standard 0 and 5 fmol/ml. Seven different control specimens provided by Abbott Laboratories were assayed with the EIA method, with interassay coefficients of variation from 1.7% [233.4 ± 4 (SD) fmol/ml] to 18.2% [18.5 ± 3.3 fmol/ml]. Pooled cytosols used as control for the dextran coated charcoal method had interassay variation coefficients between 3.8 and 11.4%. Reproductibility has been studied on clinical specimens assayed twice at two different periods with either EIA or dextran coated charcoal methods. Slopes obtained were 1.05 and 0.96, respectively. A good stability of EIA results was obtained with protein concentrations in the range 4–0.15 mg/ml cytosol. No significant effects of dithiothreitol or monothiolglycerol (1 mM) on EIA and dextran coated charcoal assay were observed. Eighty breast cancer cytosols were assayed with both EIA and Scatchard analysis. The slope of the regression curve obtained was 1.04 (r = 0.963). Cytosols were assayed by EIA and by a saturating concentration of tritiated ligand (5 nm). With 153 cytosols the EIA/5 nm slope was 1.34 (r = 0.978). This slope can be compared with the slope Scatchard/5 nm obtained with 90 cytosols: 1.29 (r = 0.985). Absence of cross-reactivity of monoclonal ER antibodies with progesterone receptor was observed.

Materials and Methods

Reagents. [2,4,6,7-3H]Estradiol (92 Ci/mmole) was obtained from Amersham International, Plc., Amersham, United Kingdom [11-methoxy-3H]Moxestrol (R2858) was obtained from N.E.N. Chemicals, Dreieich, West Germany. Other chemicals were analytical grade.

Tumors and Cytosols. Breast tumors used in this study were obtained through pathologists after confirmation of the neoplastic nature of the specimens. Tumors were stored in liquid nitrogen until used. Thirty tumors were obtained from the Faculté de Médecine Marseille (P. M. Martin) and 130 tumors were from the Centre François Baclesse, Caen (J. Goussard). Some of these tumors have been assayed twice for reproducibility studies, and finally 241 breast cancer cytosols were assayed by both EIA and DCC methods. For cytosol preparation, all procedures were carried out at 0–2°C. Tumors were homogenized using an Ultra-Turrax (five 5-s bursts) in 6 volumes of Tris homogenization buffer (100 mM-Tris-1.5 mM EDTA-10% glycerol-0.5 mM dithiothreitol-10 mM sodium molybdate, pH 7.4 at 2°C). Cytosol was obtained by centrifugation of the homogenate for 60 min at 40,000 x g; the first lipidic layer was discarded and the cytosol was collected in two cryobiological tubes and immediately stored in liquid nitrogen for further analyses of ER either by EIA or by DCC assay. Cytosol protein concentrations were determined by the method of Lowry et al. (3).

DCC Assay. Details concerning estradiol receptor assay have been described elsewhere (4, 5). For saturation technique and Scatchard analysis, 0.6,1.25, 2.5, and 5 nm concentrations of tritiated ligand were used. For cytosols with volumes that were too small for multipoint Scatchard analysis, a “near saturating” concentration of ligand was used (5 nm).

Abbott Enzyme Immunoassay. The Abbott Estrogen Receptor EIA system is a solid phase enzyme immunoassay based on the “sandwich” principle. Beads coated with rat (monoclonal) antibody to human ER are incubated with specimens (tissue cytosols), the appropriate standards and controls. During this incubation, ER present in the specimens, standards, and controls is bound to the solid phase. Unbound materials present in the specimens, standards, and controls are removed by aspiration of fluid and washing of beads. A second rat (monoclonal) anti-ER antibody conjugated with horseradish peroxidase is incubated with the beads and, if ER is present in the sample, the anti-ER:horseradish peroxidase conjugate is bound to the ER on the beads. Unbound conjugate is removed by aspiration and the beads are washed. The beads are next incubated with the enzyme substrate solution (hydrogen peroxide and o-phenylenediamine-2HCl) to develop a color which is a measure of the amount of bound anti-ER:horseradish peroxidase conjugate. The enzyme reaction is stopped by the addition of 1 N sulfuric acid and the intensity of color developed is read using a spectrophotometer at 492 nm. The intensity of the color formed by the enzyme reaction is proportional to the concentration of ER in the specimen within the working range of the assay. A standard curve is obtained by plotting the ER concentration of the standards versus the absorbance. The ER concentration of the specimens and controls run concurrently with the standards can be determined from the curve.

Introduction

The use of radioactive ligands for the determination of steroid receptors in biological tissues has been the main methodology during the past 15 years. These techniques present high precision and reproducibility but are not devoid of problems due both to tritiated ligands and to receptor molecules. Moreover, although exchange techniques exist, the presence of endogenous hormone is a limitation of the precise estimation of receptor content in the tumor. The recent preparation of specific monoclonal antibodies to estrogen receptor of human origin (1, 2) provides a new approach for the detection of receptor molecules in human breast cancer, whether or not they are occupied with hormone. As part of a multicenter study on Abbott ER-EIA in Europe, this paper reports the results obtained on ER determinations on breast cancer cytosols using both radioactive ligands and monoclonal antibodies in order to compare the two methods and to evaluate the clinical usefulness of this new immunological, simplified assay.

Results

ER-EIA Standard Curves

Five standards were prepared with the Abbott ER-EIA Kit, 0, 5, 25, 100, and 250 fmol/ml. Assays were performed on a 50% dilution of cytosols, allowing the kit to cover the range 0–500 fmol/ml. Over a 6-month period during the multicenter
trial, 35 curves were studied using 4 different batches of monoclonal antibodies. Absorbance results related to standard values are summarized in Table 1. Good reproducibility was obtained with coefficients of variation lower than 10% except for the lower standards, 0 and 5 fmol/ml. Intraassay repeatability was measured on duplicates with coefficients of variation lower than 5%.

Scatchard Analysis

ER determinations by Scatchard analysis were performed on 80 breast tumors, using either \[^{3}H\]moxestrol or \[^{3}H\]estradiol. All scatchard plots were easily linearized with correlation coefficients between 0.91 and 1. The mean apparent dissociation constant \((K_d)\) of receptor sites was 1.08 ± 0.65 nM with moxestrol (53 cytosols), and 0.16 ± 0.07 nM with estradiol (27 cytosols). Intraassay repeatability of the DCC method, measured on duplicates, had coefficients of variation lower than 2.5% for uninhibited cytosols (total binding) and lower than 6% for inhibited cytosols (nonspecific binding) (Table 2).

Control Specimen Analysis

During the multicenter study, control specimens were systematically assayed with each EIA experiment, in addition to a control provided in the ER-EIA Kit (Table 3). There was very good agreement between our results and the mean results of the European trial. Furthermore, three specimens (A, B, and C) were assayed daily on 5 consecutive days with the following results: \(A = 18.8 \pm 1\) fmol/ml (CV = 5%); \(B = 105.8 \pm 6.9\) fmol/ml (CV = 6.5%); and \(C = 233.4 \pm 4\) fmol/ml (CV = 1.7%). With the DCC method, pooled cytosols were used as controls. Five different pools were assayed during the study. Reproducibility of these controls had coefficients of variation between 3.8 and 11.4% (Table 4).

Clinical Specimen Reproducibility

The reproducibility of both EIA and DCC methods was studied on human breast cancer cytosols assayed twice, at two different periods during the clinical evaluation. Second assay results \((B)\) were compared to first assay results \((A)\) and the regression curves obtained were: \(B = 1.054 - 5.5 \times 10^{-5}\) fmol/mg protein for EIA (53 cases); and \(B = 0.964 + 9\) fmol/mg protein for DCC assay (42 cases) (Fig. 1).

Effect of Protein Concentration on EIA Results

Protein concentration of cytosols is one of the limitations inherent to labeled ligand technique, and DCC assays are often unstable for protein content lower than 1–2 mg/ml cytosol.

Table 1 ER-EIA: batch to batch evolution of standard curves with monoclonal antibodies

<table>
<thead>
<tr>
<th>Standards (fmol/ml)</th>
<th>Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>62761-HZ (N = 17)</td>
<td>Absorbance</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
</tr>
<tr>
<td>64830-HT (N = 9)</td>
<td>Absorbance</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
</tr>
<tr>
<td>68563-HZ (N = 6)</td>
<td>Absorbance</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
</tr>
<tr>
<td>67870-HT (N = 3)</td>
<td>Absorbance</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
</tr>
</tbody>
</table>

Table 2 Intraassay reproducibility of the dextran coated charcoal method

<table>
<thead>
<tr>
<th>Ligand concentration</th>
<th>0.62 nM</th>
<th>1.25 nM</th>
<th>2.5 nM</th>
<th>5 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.6–4.7)</td>
<td>(0.7–2)</td>
<td>(0.2–7.5)</td>
<td>(0.5–7.5)</td>
</tr>
<tr>
<td>Intraassay coefficient of variation on 30 duplicates (%)</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>(0–10.4)</td>
<td>(0–2.5)</td>
<td>(0–4.10.7)</td>
<td>(0–14.18)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>1.9</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>(0–28.1)</td>
<td>(0–12.3)</td>
<td>(0–13.2)</td>
<td>(0–14.8)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, extreme values of CV (%).

Table 3 ER-EIA controls

<table>
<thead>
<tr>
<th>Control</th>
<th>Control 1</th>
<th>Control 2</th>
<th>Control 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>kit</td>
<td>19</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>No. of assays</td>
<td>105.4</td>
<td>237.9</td>
<td>18.5</td>
</tr>
<tr>
<td>Mean (fmol/ml)</td>
<td>9.2</td>
<td>18.9</td>
<td>3.3</td>
</tr>
<tr>
<td>SD</td>
<td>8.8</td>
<td>7.9</td>
<td>18.2</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total binding</td>
<td>103</td>
<td>236</td>
<td>22</td>
</tr>
<tr>
<td>Mean of the European trial (8 laboratories)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 DCC method: intralaboratory quality control using pooled human breast cancer cytosols

With known ER and PR values and volume of each cytosol, 30 cytosols were mixed and a theoretical value was calculated. Comparison between theoretical values and results effectively obtained may be indicative of a high reproducibility of the method used. Interassay coefficients of variation as low as 3.8% can be obtained.

Table 4 DCC method: intralaboratory quality control using pooled human breast cancer cytosols

<table>
<thead>
<tr>
<th>Pool</th>
<th>Ligand</th>
<th>No. of assays</th>
<th>Result (mean ± SD)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>PHI</td>
<td>29</td>
<td>215 ± 24.3</td>
<td>11.4</td>
</tr>
<tr>
<td>MAR</td>
<td>20</td>
<td>230</td>
<td>288 ± 18.7</td>
<td>6.5</td>
</tr>
<tr>
<td>HYA</td>
<td>Estradiol</td>
<td>28</td>
<td>143.3 ± 11.5</td>
<td>8.0</td>
</tr>
<tr>
<td>LEO</td>
<td>Estradiol</td>
<td>15</td>
<td>142.3 ± 5.6</td>
<td>3.9</td>
</tr>
<tr>
<td>PAT</td>
<td>Estradiol</td>
<td>8</td>
<td>74 ± 3.2</td>
<td>3.8</td>
</tr>
<tr>
<td>PR</td>
<td>PHI</td>
<td>17</td>
<td>167.6 ± 20.6</td>
<td>12.2</td>
</tr>
<tr>
<td>PHI</td>
<td>ORG-2058</td>
<td>12</td>
<td>138.7 ± 7.7</td>
<td>5.5</td>
</tr>
<tr>
<td>MAR</td>
<td>19</td>
<td>429</td>
<td>426 ± 52</td>
<td>12.2</td>
</tr>
<tr>
<td>HYA</td>
<td>ORG-2058</td>
<td>28</td>
<td>140.9 ± 16.7</td>
<td>11.9</td>
</tr>
<tr>
<td>LEO</td>
<td>ORG-2058</td>
<td>13</td>
<td>138.8 ± 8.1</td>
<td>5.9</td>
</tr>
</tbody>
</table>

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MONOCLONAL ANTIBODIES FOR ER DETERMINATION

Table 5 Effects of monolthioglycerol and dithiothreitol on estrogen receptor results, assayed by EIA and Scatchard analysis

Presence of dithiothreitol or monolthioglycerol (1 mM) did not modify assay results. Statistics were performed on 3 consecutive assays. For differences between EIA and Scatchard results, see text.

<table>
<thead>
<tr>
<th></th>
<th>EIA</th>
<th>Scatchard</th>
<th>European result (fmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fmol/ml</td>
<td>fmol/mg protein</td>
<td>fmol/ml</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosol 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>12.5 ± 0.52 (a)</td>
<td>3.2 ± 0.04</td>
<td>69 ± 3.60 (b)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.2</td>
<td>1.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Cytosol 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>42.5 ± 7.07</td>
<td>10.8 ± 2.04</td>
<td>214 ± 14.2</td>
</tr>
<tr>
<td>CV (%)</td>
<td>16.6</td>
<td>18.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Cytosol 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>274 ± 19.82</td>
<td>319 ± 46.0</td>
<td>129 ± 24.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.24</td>
<td>14.4</td>
<td>19.0</td>
</tr>
<tr>
<td>Monothioglycerol</td>
<td>(N = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosol 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>12.3 ± 0.37</td>
<td>3.1 ± 0.08</td>
<td>67 ± 2.0</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.1</td>
<td>2.7</td>
<td>2.9</td>
</tr>
<tr>
<td>Cytosol 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>40.8 ± 5.8</td>
<td>10.5 ± 1.46</td>
<td>210 ± 16.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>14.4</td>
<td>13.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Cytosol 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>266 ± 10.07</td>
<td>310 ± 35.6</td>
<td>115 ± 15</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.8</td>
<td>11.5</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Fig. 1. Interassay reproducibility of DCC and EIA methods. Human breast cancer cytosols stored in liquid nitrogen were assayed twice in a period from 1 week to 6 months, with either the DCC or the EIA method. A, DCC method. [3H]R2858 and [3H]estradiol were used as ligands; regression curve obtained between first and second assay results was: (second assay) = 0.96 (first assay) + 9 fmol/mg protein. B, EIA method. The regression curve obtained between first and second assay results was: (second assay) = 1.05 (first assay) - 5.5 fmol/mg protein.

Fig. 2. Effect of protein concentration on ER-EIA. Assays were performed on cytosols diluted from 4 to 0.15 mg/ml protein. The mean result of each cytosol (fmol/mg protein) was taken as 100% and the percentage of deviation of the result of each protein dilution was measured and plotted in relation to protein concentration.

This factor was studied with ER-EIA by serial dilutions of cytosols, to obtain protein concentrations in the range 4–0.15 mg/ml cytosol. For each specimen, the result of each dilution assayed was compared to the mean result of all dilutions taken as 100% reference and plotted as a function of protein concentration. In this way 25 cytosols were diluted and assayed (Fig. 2) and very good stability was observed in the range of the protein concentration chosen, with sometimes an underestimation of ER content for highest cytosol protein dilutions (0.1–0.2 mg/ml).

Effect of Reducing Agents on ER-EIA

With classical DCC assays two different reducing thiols are used, dithiothreitol or monolthioglycerol, to protect the integrity of receptor sites. To study the effect of these agents on the ER-EIA and on the DCC assay, three control specimens were assayed three times with either dithiothreitol or monolthioglycerol (1 mM). There were no significant differences in results obtained with these reducing agents. Nevertheless, receptor contents varied with the method used (Table 5). Cytosol receptors from control 1 and 2 were calf uterus receptors, partially recognized by human monoclonal antibodies in the EIA, and the DCC assay was 5 times more effective in the detection of these receptor sites. Control 3 was extracted from MCF 7 cells, and the EIA method was twice as effective in detecting receptor molecules, in comparison with the DCC method, possibly due to the presence of endogenous estradiol preventing ligand binding.
Fig. 3. Stability of ER antigenic and binding sites after long-term storage. ER assays were performed twice on 63 human breast tumors stored in liquid nitrogen for more than 18 months. First assays were performed on fresh tissues with \(^{3}H\)R2858 as ligand. Second assays were performed 18 to 24 months later with EIA and at the same time with \(^{3}H\)R2858 and \(^{3}H\)estradiol as ligands. A, comparison between second assay by EIA and first assay by DCC method (\(^{3}H\)R2858; a); regression curve was: \(\text{EIA} = 0.90 (\text{DCC}) + 22.8 \text{ fmol/mg protein}, r = 0.968\). B, C, comparison between EIA and DCC performed at the same time; regression curve was: \(\text{EIA} = 1.36 (\text{DCC}) + 18 \text{ fmol/mg protein}, r = 0.951\) with \(^{3}H\)R2858; b as ligand and \(\text{EIA} = 2.05 (\text{DCC}) + 20 \text{ fmol/mg protein}, r = 0.940\) with \(^{3}H\)estradiol as ligand. D, second assay performed either by \(^{3}H\)R2858 or by \(^{3}H\)estradiol. Regression curve was: \(\text{EIA} = 1.47 \text{ (estradiol)} + 4 \text{ fmol/mg protein}; r = 0.920\).

Stability of ER Antigenic Sites and Binding Sites after a Long-Term Storage

ER assays were performed on 63 human mammary tumors stored in liquid nitrogen for more than 18 months, in order to study the stability of ER molecules, at the antigenic level with ER-EIA and at the hormone binding site level with the DCC method. DCC assays were first performed on fresh tumors (\(^{3}H\)R2858; Fig. 3, a) and a second assay was performed 18 to 24 months later using both EIA and DCC methods (\(^{3}H\)R2858; Fig. 3, b and \(^{3}H\)estradiol). Comparison between EIA and first DCC assay results (Fig. 3A) showed that antigenic sites were not modified by a long-term storage in liquid nitrogen; the regression curve obtained was EIA = 0.90 (DCC) + 22.8 fmol/mg protein (\(r = 0.968\)). Comparison between EIA and DCC results obtained at the same time yielded EIA = 1.36 (DCC) + 18 fmol/mg protein with \(^{3}H\)R2858 as ligand (Fig. 3B; \(r = 0.951\)), and EIA = 2.05 (DCC) + 20 fmol/mg protein with \(^{3}H\)estradiol as ligand (Fig. 3C; \(r = 0.940\)). The second assays performed both with tritiated R2858 and estradiol yielded the relation R2858 = 1.47 (estradiol) + 4 fmol/mg protein (Fig. 3D; \(r = 0.920\)). These results showed that receptor binding sites were lowered by long-term storage in liquid nitrogen.

Clinical Specimens

EIA-DCC Assay Comparison. Eighty human breast tumors were assayed with both EIA and Scatchard analysis. Among them, 20 were from premenopausal patients, 56 were from postmenopausal patients, and 4 were from patients whose hormonal status was unknown. The regression curve obtained between EIA (B) and Scatchard (A) was: \(B = 1.044 + 21 \text{ fmol/mg protein} (r = 0.963)\) (Fig. 4). When cytosol volume was too...
concerned. In terms of immunological structure, there may be some
endogenous estradiol, as for premenopausal women. Further
more, these patients were not on tamoxifen or steroid therapy
postmenopausal patients, values obtained by EIA were close to
levels obtained by EIA and DCC were significantly different,
found to be between 0 and 15 fmol/mg protein by EIA and 9
of 11 results were lower than 5 fmol/mg protein with both
methods. With tumors from premenopausal patients, mean ER
concentration of ligand (5 nM) was used. To correlate results
obtained by this technique with Scatchard analysis, 90 cytosols
were assayed by both methods and the relation between Scat-
chard (B) and single point analysis (A) was $B = 1.29A - 9$ fmol/
mg protein, $r = 0.985$. The single point method was
further compared with the ER-EIA on 153 cytosols from small
tumors and biopsies (30–100 mg, wet weight) and the relation
obtained between EIA (B) and DCC assay (A) was $B = 1.34A$
+ 5 fmol/mg protein, $r = 0.978$ (Fig. 5A). Eleven cytosols
assayed by DCC for which receptor results were between 0 and
10 fmol/mg protein were assayed by EIA. All results were to be
found to be between 0 and 15 fmol/mg protein by EIA and 9
of 11 results were lower than 5 fmol/mg protein with both
methods. With tumors from premenopausal patients, mean ER
levels obtained by EIA and DCC were significantly different,
92 and 68 fmol/mg protein, respectively ($P < 0.001$). For post-
menopausal patients, values obtained by EIA were close to
those obtained by DCC, 293 and 260 fmol/mg protein, respect-
ively, without statistical difference.

EIA-DCC Assay Discrepancy. Over 241 cytosols were assayed
by both methods. Two discrepancies were observed with two
70- and 72-year-old women, whose receptor site content was
low, 15 and 31 fmol/mg protein, respectively, and whose recep-
tor molecules, as determined by monoclonal antibodies, were
154 and 166 fmol/mg protein, respectively. These results were
confirmed twice and cannot be explained by high levels of
endogenous estradiol, as for premenopausal women. Further-
more, these patients were not on tamoxifen or steroid therapy
at the time of biopsy.

Absence of Cross-Reactivity with PR. ER assay with tritiated
ligand presents high specificity without any cross-reaction with
PR, as far as hormonal binding site of the molecule is con-
cerned. In terms of immunological structure, there may be some
likeness between ER and PR molecular structure leading to
some cross-reactivity in the EIA assay. In such a case, ER levels
determined with EIA should be artificially enhanced in the
presence of PR. In fact, cross-reactivity was never observed,
even for high levels of PR (Table 6).

Discussion

For 20 years, steroid receptor assays by tritiated ligands were
the sole methods used for estrogen and progesterone receptor
measurement in tumor tissues. Variations of results observed
in interlaboratory quality control programs emphasize the dif-
ficulties encountered with this method in which numerous
factors must be controlled to finally obtain high precision and
reproducibility. Our results show that such a precision and
reproducibility, for both intra- and interassay, can be obtained
with the DCC method and that this well controlled method can
be considered effective as a reference method to be used in
comparison with new technologies like ER-EIA (6–8). With
the DCC method, intraassay coefficients of variation as low as
0 to 0.2% can be eventually obtained on duplicates, never
exceeding 6%. The same intraassay reproducibility can be
obtained with ER-EIA. Interassay reproducibility studied with
both methods presents in most cases coefficients of variation
lower than 10%, for control specimens assayed by EIA as well
as for pooled cytosols assayed by DCC. Besides high precision
pipeting, criteria essential to reproducibility include good con-
trol of the DCC extraction step for the DCC method and a
time and temperature controlled step using o-phenylenediamine
with EIA. Because of the low level of protein concentration
needed, ER-EIA can be used for ER assays in small samples or
in drill biopsy samples with protein concentrations as low as
0.2 mg/ml, a condition that prohibits the use of the DCC
method which requires protein concentrations above 1 mg/ml
(9, 10). Long-term conservation studies on breast tumors stored
in liquid nitrogen show differences between antigenic site and
binding site stability. With monoclonal antibodies (11–13), it
has been shown that antigenic sites involved in ER-EIA are
different from binding sites, and our experiments show that
antigenic sites present a greater stability than binding sites,
when stored during 18 to 24 months. Furthermore, after stor-
age, a 50% decrease in ER results is obtained with the physio-
logical ligand estradiol compared to the synthetic ligand R2858,
showing some alteration of physiological binding sites. With
fresh tumors stored in liquid nitrogen for 1 or 2 weeks, there is
no difference between ER levels measured with tritiated estra-
MONOCLONAL ANTIBODIES FOR ER DETERMINATION

References


Comparison of Monoclonal Antibodies and Tritiated Ligands for Estrogen Receptor Assays in 241 Breast Cancer Cytosols


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