In summary, the present method allows the separation of several important steroids simultaneously and resolves cortisol, prednisolone, and methylprednisolone sufficiently to allow their accurate determination. The procedure is straightforward and robust, and therefore is suitable for any laboratory interested in the analysis of these steroids. Assays are usually run overnight for convenience, but they can be completed within 4 h if needed, which is quite sufficient for the clinical decision-making required for diagnosis of patients with congenital adrenal hyperplasia.

References

Quantitative Measurement of c-erbB-2 p185 and Mutant p53 Expression in Ovarian Neoplasms by Enzyme Immunooassay, Gamal M. Mabrouk,* Sabar A.A. Helal, Khaliil I. El-Lamie,* and Ali M. Khalifa (Oncology Diagnostic Unit, Dept. of Biochem. and 1Dept. of Gynecol. & Obstet., Ain Shams Faculty of Med., Cairo, Egypt; *author for correspondence: fax 20-2-285-9928)

Ovarian cancer is currently considered a leading cause of death among gynecological malignancies in the US [1, 2]. In Egypt, according to the National Cancer Institute records, ovarian cancer is the second most common malignancy in gynecological tumors (0.8%) after cervical cancer [3]. Prognostic markers c-erbB-2 p185 and p53 are overexpressed in ovarian malignancies, as shown by immunochemistry studies [4-8]. Most of these studies generally require tedious and time-consuming preparations of samples and must be interpreted by experienced personnel. Some other studies have quantified mutant p53 protein by immunofluorometry in sera, breast cancer tissue cytoplasmic extracts, malignant cell lines [9], and ovarian cancer [10]; however, results are expressed in arbitrary units without referral to the expression level of p53 in benign tumors. Other investigators have reported a high expression of c-erbB-2 in malignant breast tissue whole homogenate, as quantified by enzyme immunoassay (EIA) [11]. In this study we have tried to develop an easier and less time-consuming technique that would enable us to have cutoff values for p53 and c-erbB-2 assessed in their subcellular locations, i.e., nucleus and membrane, respectively. The use of such numerical values for laboratory diagnostic and prognostic assays would increase their sensitivities. Because EIA is considered an easy routine laboratory test, we thought that its use in detection of oncogene and suppressor gene products in subcellular fractions might be helpful in improving the accuracy of detection of these proteins [12].

All steps of sample preparation devised in our laboratory were carried out at 4 °C, and all reagents were purchased from Sigma Chemical Co. (St. Louis, MO). We used tissues excised from patients with benign tumors (n = 13)—serous cystadenoma (n = 3), benign ovarian cysts (n = 4), endometrial cysts (n = 2), and mucinous cystadenoma, sclerosing stromal tumor, cystic teratoma, and thecoma (n = 1 each)—and from patients with the most commonly seen malignant ovarian tumor serous cystadenocarcinoma (n = 18). Tissues were immediately washed in ice-cold saline and homogenized on ice in 10 mmol/L Tris buffer [pH 7.5; containing 100 mmol/L glycerol, 0.4 mol/L KCl, 10 mmol/L EDTA, 5 mmol/L benzamidine, 10 mmol/L 2-mercaptoethanol, 0.39 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 5 mg/L aprotinin] with an Ultraturrax T-25 homogenizer for three bursts of 55 s each separated by a pause for 1 min. The homogenate was filtered and then centrifuged at 4000g for 15 min with a Beckman Instruments (Brea, CA) CS-6R centrifuge to obtain the nuclear pellet. The pellet was dissolved in phosphate-buffered saline and sonicated for three bursts of 30 s each. After quantifying the protein concentration in the pellet by using Bradford's method [13] with bovine serum albumin as the calibrator, we applied the samples directly to ELSA plate wells for assay of p53 with an EIA kit from Oncogene Science (Uniondale, NY).

The supernatant fluid remaining after the above centrifugation was recentrifuged at 25 000g for 1 h with a Beckman L7 ultracentrifuge to obtain the cytosol; the membrane fraction was obtained by redissolving the pellet obtained in this second centrifugation in 10 mmol/L HEPES buffer (pH 7.5; containing 10 mmol/L EDTA, 5 mmol/L benzamidine, 10 mL/L Triton X-100, 10 mmol/L 2-mercaptoethanol, 0.39 mmol/L PMSF, and 5 mg/L aprotinin) and recentrifuging at the same speed for an additional 30 min. After measuring the protein concentration in the membrane fraction, we diluted the samples in sample diluent provided by the kit and analyzed them for c-erbB-2 p185, using another EIA kit from Oncogene Science.

We observed an overexpression of c-erbB-2 and mutant p53 proteins in malignant cases compared with benign ones (Fig. 1). The respective cutoff values for these analytes—0.5 fmol/µg (nmol/g) protein and 1.8 fmol/mg (µmol/g) protein—repre
We thank Abd El-Rahman M. A. Hammouda for his useful discussion.

Table 1. Sensitivity and specificity (%) and predictive values (%) at different cutoff values of c-erb B-2 for discriminating between benign and malignant ovarian tumors.

<table>
<thead>
<tr>
<th>Cutoff, fmol/μg protein</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Neg. predictive value</th>
<th>Pos. predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.5*</td>
<td>100</td>
<td>94</td>
<td>92</td>
<td>94</td>
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<td>&gt;0.3</td>
<td>83</td>
<td>94</td>
<td>77</td>
<td>89</td>
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<td>94</td>
<td>54</td>
<td>77</td>
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<tr>
<td>&gt;0.18</td>
<td>50</td>
<td>100</td>
<td>33</td>
<td>75</td>
</tr>
</tbody>
</table>

* 0.5 fmol/μg protein is the cutoff that maximizes the sum of sensitivity and specificity at which the highest predictive values are reached.

We recommend the routine use of this quantitative assay for both analytes in clinical laboratories to evaluate the prognosis of ovarian neoplasms and to adjust the disciplines of therapy after surgery.

We presented their greatest expression in benign tumors (Table 1). The cutoff value we observed for c-erbB-2 expression in serous cystadenocarcinoma cases between stage I with no lymph node deposits and stages II and III was 4.0 fmol/μg protein. We conclude that quantitative determination c-erbB-2 p185 and p53 helped us to have numerical values that discriminated between the expression of these markers in benign and malignant tissues. For c-erbB-2 only, the numerical assay results also helped to discriminate early from late stages of serous cystadenocarcinoma, whereas p53 values were not helpful for this.

References