Analytical and Clinical Performance of Improved Abbott IMx CA 125 Assay: Comparison with Abbott CA 125 RIA

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We compared the improved Abbott IMx cancer antigen (CA) 125 assay (cat. no. 7A89) with the Abbott CA 125 RIA. Serum specimens were from healthy perimenopausal women (n = 124) and from patients with benign gynecologic and nongynecologic diseases (n = 124), ovarian carcinoma (n = 104), or other malignancies (n = 193). The IMx assay detected as little as 0.193 kAU/L CA 125 (AU = arbitrary Abbott unit), demonstrated up to 29% overestimation upon serum dilution, low within-assay (2.7–5.6%) and between-assay (4.8–8.2%) CVs, and no high-dose hook effect ≤46 000 kAU/L nor interference from human anti-mouse antibodies in serum of women injected with OC 125 F(ab')2. Values by IMx were 20% lower than by RIA for healthy perimenopausal women (n = 100; IMx = 0.80 RIA - 2.5 kAU/L), and at least 50% higher for those with benign or malignant ovarian disorders at concentrations <100 kAU/L. Receiver-operating characteristic (ROC) curve analysis of ovarian neoplasms vs perimenopausal controls indicated a gain of specificity and sensitivity with the improved IMx assay over the RIA, but ROC performance was the same with either assay if patients with benign ovarian disorders were used as controls.

Indexing Terms: ovarian cancer/tumor markers/intermethod comparison/heterophile antibodies

A variety of monoclonal antibodies has been generated against antigens associated with a broad range of malignancies (1). The anti-cancer antigen (CA) 125 monoclonal antibody (OC 125) has been used to develop a serodiagnostic immunometric sandwich-type assay (IRMA) for ovarian carcinoma (2, 3).6 The specificity and the sensitivity of the CA 125 assay have been sufficient to support its evaluation in several clinical applications for use with ovarian cancer patients, including detection of disease prior to second-look surgery (4, 5), monitoring response to therapy (6), differential diagnosis (7), and even early detection of ovarian carcinoma (8). Thus, increased concentrations of CA 125 in serum are thought to indicate the presence of disease. Many of these measurements, including most of the clinical studies mentioned above, have been performed with the first commercially available one-step IRMA (3) developed by Centocor (Malvern, PA) in which 125I-labeled antibody to CA 125 is used as a tracer. Subsequently, three other assay test kits involving 125I-labeled OC 125 and five other immunoassay procedures involving enzyme-labeled OC 125 have been developed.

We have previously reported the analytical performance and comparability of five of the available CA 125 assay kits (9). Emphasis was given to method comparison analysis with orthogonal regression and to comparison of concordance and discordance of assay results of each kit on the basis of cutoff concentrations of 35 and 65 kAU/L (the arbitrary unit is related to an Abbott-maintained reference preparation; no internationally recognized standard is available at this time). The present study details the performance characteristics and clinical usefulness of the fully automated and improved IMx CA 125 procedure (Abbott Laboratories, North Chicago, IL). We have correlated CA 125 values obtained with the recalibrated and reformulated IMx assay and those obtained with the Abbott CA 125 RIA. Furthermore, this report attempts to discern (a) whether the recalibration provides good agreement of cutoff (35 and 65 kAU/L) with the Abbott RIA; (b) whether improved assay sensitivity and precision provides better distinction between patients with and without ovarian cancer; and (c) whether the combined application of polyclonal sheep anti-CA 125 antibody on microparticles and the OC 125 monoclonal antibody conjugated to alkaline phosphatase in the improved IMx assay further protects against falsely increased test results due to heterophile (human anti-mouse) antibodies (HAMA) in the serum specimens.

Materials and Methods

Description of immunoassays. The IMx CA 125 assay (cat. no. 7A89) is based on the microparticle enzyme immunoassay (MEIA) technology developed for the Abbott IMx automated immunoassay system. Currently, the IMx CA 125 assay is not available for sale in the US. The probe/electrode assembly delivers 150 µL of serum sample, specimen diluent (buffered sheep serum with protein stabilizers), and anti-CA 125 (sheep, polyclonal) antibody-coated microparticles in buffer to the incubation well of the reaction cell. After formation of antibody–antigen complex during incubation, an aliquot is transferred to the glass fiber matrix to which the microparticles are irreversibly bound, and the matrix is washed to remove unbound materials.

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6 Nonstandard abbreviations: CA 125, cancer antigen 125; HAMA, human anti-mouse antibodies; MEIA, microparticle enzyme immunoassay; ROC, receiver-operating characteristic; FIGO, International Federation of Gynecology & Obstetrics; and AUC, area under the curve.

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The OC 125 (mouse, monoclonal)—alkaline phosphatase conjugate in buffer (>0.1 mg/L) is dispensed onto the matrix to bind to the antibody—antigen complex. After another wash, 4-methylumbelliferyl phosphate substrate (1.2 mmol/L in buffer) is added to the matrix and the fluorescent product is measured by the MEIA optical assembly. Total procedure time is 42 min. Specimens with a CA 125 assay value >600 kAU/L are automatically flagged with the code “>600.” In this case, a manual dilution procedure of the specimen can be performed with the IMx CA 125 specimen diluent before pipetting the sample into the sample well. Six IMx CA 125 calibrators (0, 15, 50, 125, 375, and 600 kAU/L) are processed, and the point-to-point curve calculated by the built-in algorithm is stored. A Mode 1 calibrator of 50 kAU/L for system calibration verification and curve adjustment within the manufacturer’s specified preset limits is run with each series of 24 tests.

The IMx CA 125 assay has recently been improved by recalibration and reformulation. To more closely match the Centocor RIA and RIA II assays and allow for full market entry, Abbott has adjusted the CA 125 assay standards (~35%), increased the protein concentrations of accessory and specimen diluents, and improved the manufacturing process of the microparticles.

The Abbott CA 125 RIA Monoclonal is a double-determinant one-step sandwich-type IRMA identical to the Centocor CA 125 RIA procedure serving as the comparison method. This kit includes the monoclonal OC 125 antibody both as capture and tracer in a simultaneous sandwich format (3). According to the manufacturer’s instructions, 100-μL aliquots of five CA 125 calibrators (range 7–500 kAU/L) or 100 μL of serum specimen are pipetted into tubes and incubated for 20 h at room temperature, together with OC 125-coated beads and 125I-labeled OC 125. After the beads are washed to remove unbound materials, the radioactivity bound to the coated carriers, which is proportional to the concentration of CA 125 in the serum specimen, is counted in a gamma counter. Results of this assay were calculated with the 1224 MultiCalc Program (Wallac Oy, Turku, Finland). The minimum detectable concentration of this test is ~7.0 kAU/L.

Analytical evaluation. The performance of the IMx CA 125 MEIA was assessed by evaluating (a) the detection limit of the assay (defined as the lowest concentration of CA 125 that is >2 SD above the mean signal for 20 replicate determinations of the zero calibrator); (b) within-assay precision (three serum specimens with CA 125 concentrations 17–240 kAU/L in 20 replicates assayed three times) and between-assay precision (the same serum specimens run in duplicate in 73–79 consecutive assays run over >90 days), as well as construction of a precision profile (covering the entire dynamic range of both assay procedures and derived from the duplicate measurements of unknowns processed during the present study); and (c) accuracy testing, comprising checks of linearity and influence of supplied specimen diluent buffer (determined with three serially diluted serum specimens with high CA 125 concentrations); tests of the occurrence of a high-dose hook effect (defined as an analytical response below that of the highest calibrator of 600 kAU/L, in two undiluted specimens with a very high concentration of CA 125); and assessment of CA 125 measurements made with IMx and RIA in serum specimens from three ovarian cancer patients for possible interference from HAMA before and after OC125 F(ab’)2 injection (10).

Study material. The clinical evaluation material comprised serum samples from healthy perimenopausal women all with echoscopically normal ovaries (n = 124, median age 48 years, range 40–63) serving as controls, and serum specimens collected from pretreatment patients with benign gynecologic (n = 91, median age 41 years, range 14–77) and nongynecologic diseases (n = 33, median age 57 years, range 28–92); patients with ovarian carcinoma (n = 104, median age 54 years, range 17–81); and during follow-up of 8 patients with recurrent disease (n = 71), or other gynecologic (n = 81, median age 60 years, range 25–85) and nongynecologic (gastrointestinal) malignancies (n = 112, median age 65 years, range 32–86). The patients with ovarian neoplasms were staged according to recommendations of the International Federation of Gynaecology & Obstetrics (FIGO) (11) and those with gastrointestinal malignancies according to criteria of the International Union Against Cancer (12). All the serum samples were collected by venipuncture and kept frozen at −35°C until assay. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Statistics. From the CA 125 results for both tests we calculated frequency distributions of the healthy controls. For all the groups of clinical serum specimens examined we indicated the median, minimum, and maximum serum concentrations and calculated the percentages of CA 125 values that exceeded cutoff values of 35 and 65 kAU/L. The results obtained from healthy controls and those with benign ovarian disease and ovarian cancer were compared by linear regression analysis (13). To extend the comparison of methods, we also calculated the linear regressions of the two assays in eight follow-up cases of ovarian cancer patients. Diagnostic test performance was further evaluated by documenting the diagnostic accuracy of each test with receiver-operating characteristic (ROC) curves and calculation of the areas under these curves (AUC) (14).

Results

Analytical Evaluation

Sensitivity. The minimum detectable concentration of the IMx CA 125 assay was 0.193 kAU/L (mean ±2 SD), on the basis of 20 replicate measurements of the zero calibrator (mean 0.049, SD 0.072 kAU/L).

Linearity. The recoveries of three human serum specimens (mean concentrations 413, 451, and 399
Table 1. Dilution linearity of the Abbott IMx CA 125 assay.

| Serum sample | CA 125, kAU/L | Recovery, %
|--------------|---------------|-------------
|              | 1             | 2           | 3           |
| CA 125, kAU/L| 413           | 451         | 399         |
| No dilution  | 100           | 100         | 100         |
| Twofold dild.| 106           | 105         | 109         |
| Fourfold dild.| 115          | 110         | 119         |
| Eightfold dild.| 117         | 120         | 127         |
| 16-fold dild.| 123           | 129         | 121         |

* Recovery = (CA 125 measured/expected) × 100.

kAU/L) serially diluted 2-, 4-, 8-, and 16-fold increased progressively, ranging between 105% and 129% of the undiluted concentrations (Table 1). Unlike the CA 125 RIA, the IMx CA 125 assay did not show a high-dose hook effect up to 46 000 kAU/L, as demonstrated by two specimens.

**Precision.** Within-assay precision of the IMx assay was tested with three human serum specimens at CA 125 concentrations of ~19, 40, and 230 kAU/L. Replicates of 20 in each of three different assay runs revealed mean CVs ranging from 3.2% to 4.8%. To calculate the between-assay precision, we also assayed these serum specimens in 79 consecutive runs over >90 days. The resulting mean CVs ranged from 5.8% to 8.2%.

Figure 1 depicts the precision profiles of the IMx procedure and the RIA test. The first four ranges of CA 125 concentrations ≤35 kAU/L (0–3.5, 3.5–7.0, 7.0–15, and 15–35 kAU/L) showed CVs that decreased from 16% to 4.6% with the IMx, and, because of the detection limit of the RIA (>7.0 kAU/L), from 49% to 23% between 7.0 and 35 kAU/L if measured by RIA. The reproducibility of the IMx procedure for the remaining part of the dynamic range of the test (35–600 kAU/L) was always much lower (range 2.3–3.6%) than that obtained with the RIA procedure (range 6.1–9.8%).

**Method comparison of immunoassay procedures.** Fig. 2 shows the linear relations between the results obtained with the CA 125 IMx and RIA over the studied range of CA 125 concentrations (>7.0–23 000 kAU/L). Almost identical slopes were calculated for the entire group of healthy controls (0.80, Fig. 2A) and the patients with ovarian cancer (0.72, Fig. 2C) or benign ovarian diseases having CA 125 concentrations >100 kAU/L (0.88, Fig. 2B). Increased regression slopes were observed for the subsets with CA 125 values up to 100 kAU/L (5.00, Fig. 2A, 2B) for samples from women with ovarian cancer (1.67 and 2.45) or benign ovarian diseases (1.50 and 3.37).

**Interference of HAMA.** Prior to intravenous injection of radiolabeled OC 125 F(ab')2 fragments into three subjects, no relevant differences were observed between CA 125 measurements with the two assays.
Table 2. Comparison of CA 125 concentrations (kAU/L) determined by IMx and RIA in serum of three patients treated with OC 125 F(ab)\(_2\) 

<table>
<thead>
<tr>
<th>Patient</th>
<th>HAMA*</th>
<th>RIA</th>
<th>IMx</th>
<th>Week</th>
<th>HAMA</th>
<th>RIA</th>
<th>IMx</th>
</tr>
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<tr>
<td>1</td>
<td>&lt;28</td>
<td>300</td>
<td>200</td>
<td>2</td>
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<td>750</td>
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<td>25000</td>
<td>68000</td>
<td>71</td>
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<td>3</td>
<td>&lt;28</td>
<td>43</td>
<td>40</td>
<td>4</td>
<td>1500</td>
<td>2300</td>
<td>160</td>
</tr>
</tbody>
</table>

* HAMA were quantified with a homologous double-determinant OC 125 F(ab)\(_2\) assay expressing HAMA concentrations (kAU/L) in terms of CA 125 calibrator material.

Between 2 and 6 weeks postinjection, HAMA serum concentrations increased dramatically to 1100–27 000 kAU/L concomitantly with substantial increases of CA 125 concentrations with either assay procedure. RIA values were always substantially higher than IMx values.

Clinical Evaluation

Healthy controls. The CA 125 concentrations obtained with the IMx in the serum specimens from 124 perimenopausal women with echoscopically normal ovaries were invariably ≤35 kAU/L. With the CA 125 RIA procedure, only two sera exceeded this concentration (43 and 44 kAU/L, Table 3). Of all sera, 24 had CA 125 RIA values ≤7.0 kAU/L and were thus excluded from the method comparison depicted in Fig. 2. Of the 124 sera, 63 (51%) yielded CA 125 IMx concentrations below the detection limit of the RIA (≤7.0 kAU/L), and 16 of these sera had values ≤3.5 kAU/L (data not shown). The CA 125 values of 95% of all controls were ≤30 kAU/L by RIA, compared with 23 kAU/L by IMx.

Benign ovarian and other benign disease. The maximum CA 125 serum concentration observed in this group was 760 kAU/L as determined by IMx, and 810 kAU/L by RIA. The positivity rate of CA 125 (proportion of CA 125 values >35 kAU/L) yielded by the IMx procedure was 30%, and 27% in the case of RIA measurements. The sera from other benign diseases did not show differences between positivity rates with both assays (Table 3).

Ovarian carcinoma. In patients with malignant epithelial tumors of the ovary we used the CA 125 IMx to assay sera collected before primary treatment (Table 3) or during clinical follow-up (Table 4). Differences between the distributions of CA 125 IMx or RIA values of all FIGO stages were tested with two-sided Student's t-tests of the logarithmic CA 125 values and were in all FIGO stages highly significant (P <0.001). The CA 125 values of 95% of all controls were ≤30 kAU/L by IMx and ≤7.0 kAU/L by RIA, 63 (51%) yielded CA 125 IMx concentrations below the detection limit of the RIA (≤7.0 kAU/L), and 16 of these sera had values ≤3.5 kAU/L (data not shown). The CA 125 values of 95% of all controls were ≤30 kAU/L by RIA, compared with 23 kAU/L by IMx.

Other malignant diseases. No remarkable differences were observed between the respective positivity rates obtained for blood samples collected from the pretreatment patients with other gynecologic and non-gynecologic malignancies (Table 3).
CA-125 Assay Systems

IMx, AUC0.971
RIA, AUC=0.932

Sensitivity (true positive rate), %

1-Specificity (false positive rate), %

Discussion

The newly available Abbott IMx CA 125 assay procedure has been improved by restandardization of the calibrators. This has provided better agreement of values with other test methods such as Abbott and Centocor CA 125 RIA (15, 16). Comparison of the two assay procedures (Fig. 2) demonstrated a systematic overestimation of at least 20% by the RIA. The linear regression slope for CA 125 RIA values >7.0 kAU/L was 0.80 in the serum specimens from healthy perimenopausal women (n = 100) and 0.72 for the ovarian cancer patients with CA 125 values >100 kAU/L (n = 61) as well as those during follow-up of their recurrent diseases. The latter slope becomes substantially steeper, i.e., 1.67 and 2.45, if only CA 125 values ≤100 kAU/L are considered. This is in line with the calculated slopes of 1.50 and 3.37 for the patients with benign ovarian diseases whose CA 125 serum values were 7.0–35 and >35–100 kAU/L, respectively. Thus, we observe discrepancies between the slopes for CA 125 IMx vs RIA if either healthy women (slope <1.0) or those with ovarian disorders (with slopes >1.0 for both benign and malignant diseases) are evaluated. This means that CA 125 IMx values are higher than RIA in cases of ovarian disorders, whereas IMx values are lower than those measured by RIA in perimenopausal controls who have normal or diminished ovarian activity. The diversities in CA 125 measurements obtained with IMx and RIA confirm the observations on the calculated AUCs of the constructed ROC curves, which are similar for the respective ROC curves of benign vs malignant ovarian disease (Fig. 3B) and different (AUCIMx > AUCRI) for ROC curves of healthy perimenopausal controls vs patients with ovarian cancer (Fig. 3A).

The clinical evaluation of the IMx CA 125 assay demonstrated better specificity and sensitivity than the CA 125 RIA. At the internationally accepted CA 125 cutoff value of 35 kAU/L, FIGO stage I ovarian cancers were positive in 42% of cases with CA 125 IMx, but only 26% were positive by RIA——a gain of 16% in favor of the IMx assay. The positivity rates of both assays were similar (86%) for FIGO stage II ovarian cancers. The 99% CA 125 cutoff concentrations, i.e., the CA 125 value of the 122nd ranked sample, for the 124 healthy perimenopausal women tested were 29 kAU/L with the IMx and 32 kAU/L by RIA. Above these cutoffs, ovarian cancers (FIGO stage I) were positive in 58% of cases with the IMx assay, and 39% with the RIA. Compared with positivities measured at 35 kAU/L, the respective gains in positivity at these lower cutoff values were 16% in case of IMx and 13% with RIA. The lower serum cutoff figures of our control group indeed appear to have greater specificity and sensitivity than other potential control groups, as reflected by higher AUCs. Recently suggested guidelines for interpretation of AUC of ROC curves are: 0.5–0.7, low accuracy; 0.7–0.9, accuracies useful for some purposes; and >0.9, high accuracy (18, 19). Thus, as a quantitative measure of accuracy, the AUC of the IMx CA 125 assay of 0.971 means the IMx may be more useful than the RIA (AUC = 0.932) for further studies on screening of populations at risk for early ovarian cancer as recently reviewed by Bast (20) and others (21, 22). However, evaluations of the available screening studies on ovarian cancer have suggested that, if CA 125 is the only criterion, screening of a general (asymptomatic) population for occult ovarian cancer on a single occasion cannot be recommended, given considerations of costs and false-positive rates (20–22) because many factors other than cancer can lead to modest increases of CA 125 [e.g., the first trimester of pregnancy, benign gynecologic and pelvic inflammatory diseases, and some other disorders (20)]. Moreover, in the same group of asymptomatic women, CA
125 alone cannot discriminate between benign and malignant conditions of the ovary, although another study suggests that urinary gonadotropin fragment may provide the necessary additional information (23). Conversely, in women with symptoms of pelvic mass disorders, the much lower but equal AUCs for IMx and RIA (0.83, Fig. 3B) obtained with the group of benign ovarian diseases as controls suggest a similar performance of the two assay procedures, despite a report (20) that quantification of CA 125 is adequate for discriminating malignant from benign pelvic masses in postmenopausal women.

Another change made in the improved IMx CA 125 assay concerned the increased protein concentration of the specimen diluent buffer. This has been reported to improve dilution linearity (15), although we still observed nonlinear responses at higher dilutions (Table 1). Thus, the diluent buffer needs further improvement to overcome this matrix effect.

The improved IMx CA 125 test demonstrates an outstanding precision profile across the entire dynamic range and particularly in the very low concentration range. Consequently, if one decides to assay a serum specimen only once, the IMx assay is obviously the most suited candidate. The high precision obtained with the IMx MEIA system is probably due to the use of a closed system, because only reagents developed specifically for the instrument are applicable, with very strict test procedures. Another possibility for increased precision with the IMx kit may be the additional inclusion of a polyclonal anti-CA 125 serum as the capture antibody. Such an antibody may have an higher affinity for the CA 125 antigen than the monoclonal OC 125 used as signal antibody in the IMx assay and as both capture and signal antibody in the other CA 125 assay procedures.

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References