ELSEVIER

Journal of Immunological Methods 225 (1999) 1–8

JOURNAL OF IMMUNOLOGICAL METHODS

Technical and clinical comparison of two fully automated methods for the immunoassay of CA 125 in serum

Guifeng Yan [a,b], Huangxian Ju [a,*], Zhichao Liang [b], Tingqing Zhang [b]

[a] *Department of Chemistry, Nanjing University, Nanjing 210093, China*
[b] *Department of Medical Examination, Cancer Hospital and Institute of Jiangsu Province, Nanjing 210009, China*

Received 14 August 1998; received in revised form 13 January 1999; accepted 20 January 1999

Abstract

The sensitivity and precision of two fully automated enzyme immunoassays, a chemiluminescent enzyme immunoassay (CLEIA) and an enzyme-linked immunosorbent assay (ELISA), for the determination of the ovarian carcinoma antigen CA 125 were evaluated by comparison with an immunoradiometric assay (IRMA). Sera were obtained from patients with ovarian carcinoma ($N = 28$ before treatment and $N = 24$ after treatment), digestive system cancer ($N = 21$ before treatment) and from healthy women ($N = 90$). The CLEIA showed a good agreement with the IRMA in terms of the positivity rate, accuracy and assay linearity, whereas the ELISA gave some false positive results. The mean value of CA 125 in the sera of healthy women was 14, 16 and 20 U/ml determined using the CLEIA, IRMA and ELISA procedures with standard deviations (SD) of 6.9, 7.3 and 8.8 U/ml, respectively. Both the reproducibility and precision of the CLEIA with coefficients of variation (CV) of 4.6% intra-assay and 7.6% inter-assay were better than those of the ELISA with CV of 6.2% intra-assay and 15.2% inter-assay ($N = 16$). We conclude that the CLEIA is the preferable method for CA 125 determinations and the diagnosis of ovarian carcinoma.

Keywords: CA 125; Chemiluminescent enzyme immunoassay; Enzyme-linked immunosorbent assay; Immunoradiometric assay; Ovarian carcinoma

1. Introduction

CA 125 is an antigen defined by the monoclonal antibody (MAb) OC 125 (Bast et al., 1980). It has been considered to be an especially valuable serum marker expressed by $> 80\%$ of patients with non-mucinous epithelial ovarian cancer (Endo et al., 1988). Thus, its measurement has been widely applied for the clinical diagnosis of ovarian cancer (Bast et al., 1981, 1983). Methods for detecting CA 125 in serum were firstly based on a radiometric technique (immunoradiometric assay; IRMA) using a one-step sandwich principle with ^{125}I-labeled OC 125 as the tracer antibody (Bast et al., 1983; Klug et al., 1984). Thereafter, this technique was further developed by changing the first MAb (Endo et al.,

Abbreviations: CLEIA, chemiluminescent enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; IRMA, immunoradiometric assay; EIA, enzyme immunoassay; CV, coefficient of variation; SD, standard deviation; MAb, monoclonal antibody

* Corresponding author. Tel.: +86-25-3592351; Fax: +86-25-3317761; E-mail: hxju@jlonline.com.

0022-1759/99/$ - see front matter
PII: S0022-1759(99)00023-X

1988; Kakizaki et al., 1993) or performing heterologous sandwich assays (Bonfrer et al., 1994). The specificity and the sensitivity of the CA 125 IRMA have led to its use in clinical applications for the diagnosis of ovarian cancer (Einhorn et al., 1986; Zurawski et al., 1987) and monitoring treatment (Lavin et al., 1987).

Subsequently, many highly sensitive non-radioactive methods have been developed. An enzyme-linked immunosorbent assay (ELISA) for CA 125 determinations was recommended by Abbott Laboratories (Chicago, IL). Kenemans et al. (1992) assessed the technical performance and clinical usefulness of a fully automated one-step ELISA, Enzymun-Test CA 125 in 10 European laboratories, in comparison with the results of the IRMA and EIA. In these assays, a MAb OC 125 and an anti-CA 125-horseradish peroxidase conjugate was used as the capture antibody and tracer, respectively. Deger (1993) developed a method for the ELISA determination of CA 125 using a streptavidin-coated polystyrene tube to immobilize biotinylated polyclonal anti-estradiol as the capture antibody for CA 125. In another study, Boerman et al. (1987) reported a two-step time-resolved immunofluorometric assay for the quantitative determination of CA 125 concentrations in serum. A rapid and sensitive chemiluminescent enzyme immunoassay (CLEIA) for tumor markers, such as CA 125, CA 19-9 etc., has also been described (Nishizono et al., 1991). More recently, Shima et al. (1992) evaluated the application of a fully automated CLEIA (Lumipulse 1200) in the determination of serum tumor markers. This technique also used the MAb OC 125 as capture antibody, but an alkaline phosphatase-labeled antibody and its substrate AMPPD as the tracer.

At present, many instruments for the assay of CA 125 in serum have been established by different companies (Zucchelli et al., 1994), and this has led to the development of alternative CA 125 assays. However, there have been many discrepancies among different assay methods even when the same MAb was used. Zucchelli et al. (1992) reported a methodological evaluation of immunoassays for four tumor markers on the automated ES 300 and ES 600 systems. In the present study, we have sought discrepancies in precision, positivity rate, assay linearity and reproducibility between commercially available CA 125 CLEIA and ELISA techniques in comparison with IRMA. Each of these assays was based on the same OC 125 MAb. The results should provide a scientific basis for selecting a non-radioactive CA 125 immunoassay for use in the diagnosis of ovarian carcinoma and for monitoring response to therapy.

2. Materials and methods

2.1. Description of the test

All three methods (CLEIA, ELISA and IRMA) are based on a solid phase sandwich immunoassay. One monoclonal anti-CA125 was coated on the surface of solid phase and another monoclonal or polyclonal anti-CA 125 was labeled with ^{125}I or enzyme as the tracer. CA 125 antigen present in the sample was 'sandwiched' between the antibodies. After incubation and washing, the enzyme activity bound to the solid phase was assayed by the chemiluminescent light emission with a luminometer in the CLEIA, or colorimetric reaction in the ELISA, or the tube was counted in a gamma counter in the IRMA. The intensity of signal was then converted using a calibration curve to give the amount of CA 125 present in the sample.

2.2. Materials and instruments

IRMA and CLEIA kits, including 500 assay tubes coated with murine monoclonal anti-CA 125, were purchased from Diagnostic Products (DPC, USA). The IRMA kit consisted of a ligand-labeled and an iodinated murine monoclonal anti-CA 125 in liquid form, a series of standard solutions of CA 125 with different concentrations from 0 to 1500 U/ml and a buffered wash solution. The CLEIA kit was composed of a buffer and an alkaline phosphatase conjugated rabbit polyclonal anti-CA125 in buffer and the solutions containing different concentrations of CA 125 in a non-human protein/buffer matrix. One vial containing CA 125-free non-human protein/buffer matrix, a substrate solution including a phosphate ester of adamantyl dioxetane in a 2-amino-2-methyl-

1-propanol (AMP) buffer and a probe wash module were obtained from Diagnostic Products (USA) for the CLEIA. The ELISA kits were obtained from Bioseed (USA). Each ELISA kit contained a monoclonal anti-CA 125 coated microtiter plate with 96 wells, CA 125 standard solutions, a solution of horseradish peroxidase conjugated monoclonal anti-CA 125, color reagent A and B and 2 N HCl.

The IRMA, CLEIA and ELISA procedures were performed with a FMJ-182 Immunoradiometric Gamma Counter (Shanghai, China), a Model H2686 Fully Automated Luminometer (DPC, USA) and a Model 550 Microplate Reader (Bio-Rad Pacific, Hong Kong), according to the instructions and assay procedures in the manufacturers' manuals. As a reference we used the standard solutions of CA 125 from the commercially available kits.

2.3. Sera

Serum specimens were obtained from 73 clinically diagnosed patients with ovarian carcinoma (28 untreated and 24 treated) and digestive system cancer (21 untreated) and from 90 healthy women. The sera were separated from the cells, without hemolysis, and were stored by frozen at −20°C for less than 2 months. Before assay, the samples were allowed to come to room temperature (ca. 20°C) and were mixed by gentle swirling.

2.4. Precision and reproducibility

Intra-assay precision was determined by assaying 16-fold replicates of one serum in the same run for both the CLEIA and ELISA procedures. Inter-assay precision was estimated by determining, in duplicate, CA 125 in one serum sample on 16 different occasions. The reproducibility tests of CLEIA and ELISA were carried out on 16 serum samples analyzed in duplicate.

3. Results and discussion

3.1. Clinical evaluation

We assayed, in duplicate, the CA 125 in serum samples from 28 untreated and 24 treated ovarian carcinoma patients, 21 digestive system cancer patients and 90 healthy women, by CLEIA, ELISA and IRMA. The statistical results and positivity rates are listed in Table 1.

3.1.1. Healthy control subjects

As shown in Table 1, the mean CA 125 value and the standard deviation (SD) for determinations by CLEIA on sera from 90 healthy women were similar to those obtained using IRMA. The two methods gave the same positivity rate and the results were

Table 1
Comparison of CA 125 determinations in sera using three methods

Sera	Results	CLEIA	ELISA	IRMA
Untreated ovarian carcinoma[a] ($N = 28$)	mean (U/ml)	86	102	98
	SD	111	85	123
	positivity rate (%)	82(23)[b]	96(27)[b], 89(25)[c]	82(23)[b]
Treated ovarian carcinoma patients ($N = 24$)	mean (U/ml)	30	38	44
	SD	38	44	50
	positivity rate (%)	25(6)[b]	33(8)[b], 29(7)[c]	25(6)[b]
Digestive system cancer ($N = 21$)	mean (U/ml)	18	32	23
	SD	13	20	15
	positivity rate (%)	14(3)[b]	24(5)[b], 14(3)[c]	14(3)[b]
Healthy women ($N = 90$)	mean (U/ml)	14	20	16
	SD	6.9	8.8	7.3
	positivity rate (%)	1.1(1)[b]	4.4(4)[b], 1.1(1)[c]	1.1(1)[b]

[a] Mean values and SD were calculated with 24 sera having less than 500 U/ml CA 125.
[b] CA 125 > 35 U/ml.
[c] CA 125 > 46 U/ml.

similar to those reported by Bast et al. (1983) and Kenemans et al. (1992), where the positivity rates were 1% and 1.9%, respectively. Whereas these values obtained from ELISA were rather high, the positivity rate was four times higher than for the other two techniques when using the same cut-off limit (35 U/ml). These discrepancies have also been noted in a study by Hashimoto et al. (1990) in which healthy women had highly increased CA 125 EIA values but normal CA 125 IRMA values. Thus, the ELISA apparently gives more false positive results (see also Section 3.2). However, if 46 U/ml (mean value + 3SD) were selected as the cut-off limit, the ELISA would give the same positivity rate.

3.1.2. Sensitivity for ovarian carcinoma

The concentration of CA 125 in ovarian carcinoma patients is dependent on therapy. In patients before primary treatment the value was very high (> 500 U/ml CA 125 in four cases of 28, which were not included in the calculations of mean and SD values listed in Table 1), and it markedly decreased after treatment (no evidence of disease). In both the CLEIA and the IRMA, the positivity rate was 82% before treatment, similar to that of Bast et al. (1983). This value differed significantly from the 25% positivity rate in cured patients (in clinical remission). These results are similar to those (85.7% and 21.7%) presented by Kenemans et al. (1992). However, the positivity rate using ELISA was up to 96%, indicating a higher sensitivity in the diagnosis of ovarian carcinoma. The positivity rates were 89% and 29% for untreated and cured patients when 46 U/ml was used as the cut-off limit of the ELISA, and these values are also higher than those obtained using the CLEIA and IRMA. The changes in CA 125

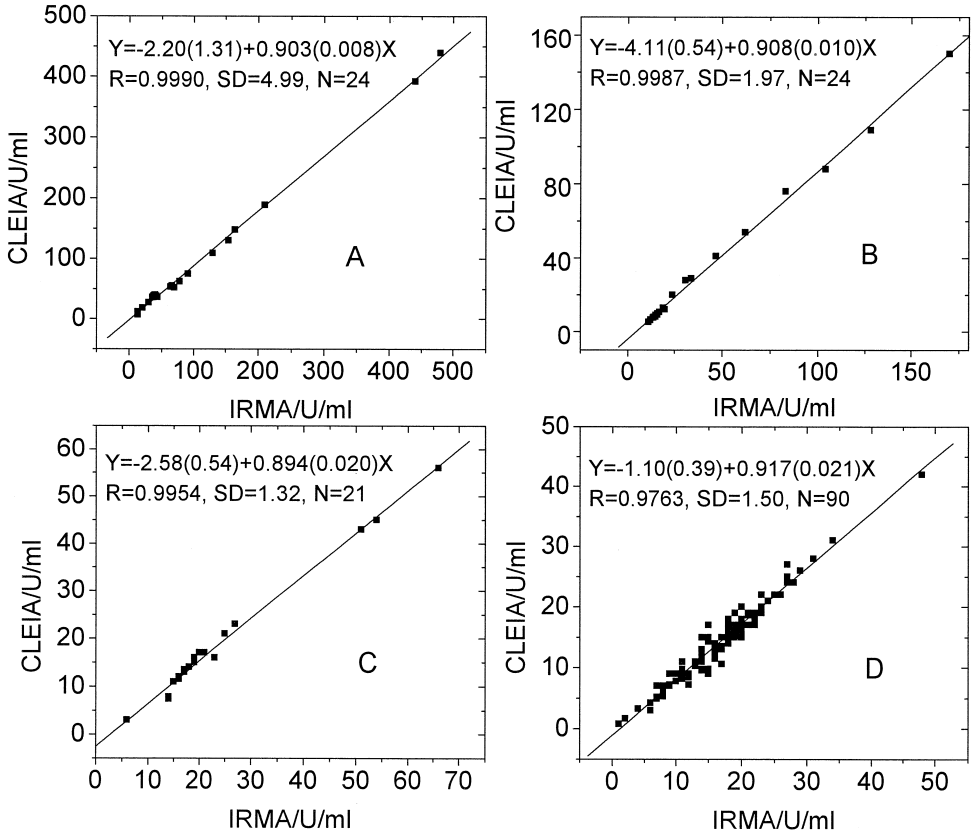

Fig. 1. Plots of CLEIA values vs. IRMA values of CA 125 in sera from untreated (A) and treated ovarian carcinoma patients (B), digestive system cancer patients (C) and healthy women (D) for comparison.

values and positivity rates indicate that CLEIA and ELISA can be used for monitoring the treatment of ovarian cancer patients with an accuracy comparable to IRMA, thereby providing alternative non-radioactive methods for CA 125 immunoassay.

3.1.3. Specificity

The mean concentration of CA 125 in sera from patients with different digestive system cancers is slightly higher than that from healthy women. No significant difference was observed in these values obtained using both CLEIA and IRMA ($P < 0.001$), although the CA 125 concentration range in patients was wider. The positivity rates of 14% in digestive system cancer patients and 1.1% in healthy blood are far lower than the values in ovarian carcinoma patients, indicating good specificity for the diagnosis of ovarian carcinoma. However, the mean CA 125 value in digestive system cancer patients using ELISA was 1.6 times that in healthy samples, furthermore, the ELISA gave a positivity rate of 24% using the same cut-off limit. Therefore, the specificity of the CLEIA was better than that of ELISA.

3.2. Technical evaluation

3.2.1. Correlation with immunoradiometric assay

As shown in Fig. 1, there is a linear relationship between the results of the CLEIA and the IRMA for sera from various groups of patients and healthy women, in assays performed in duplicate at the same time. Orthogonal regression analysis of these data ($P < 0.0001$) gave the linear equations and correlation coefficients shown in the figures (the data displayed in brackets are the errors). The slopes of plots of CA 125 concentrations for the CLEIA vs. the IRMA are almost identical for all cases, falling in the range of 0.894–0.917 with a mean value of 0.906. The higher CA 125 concentration, the better the

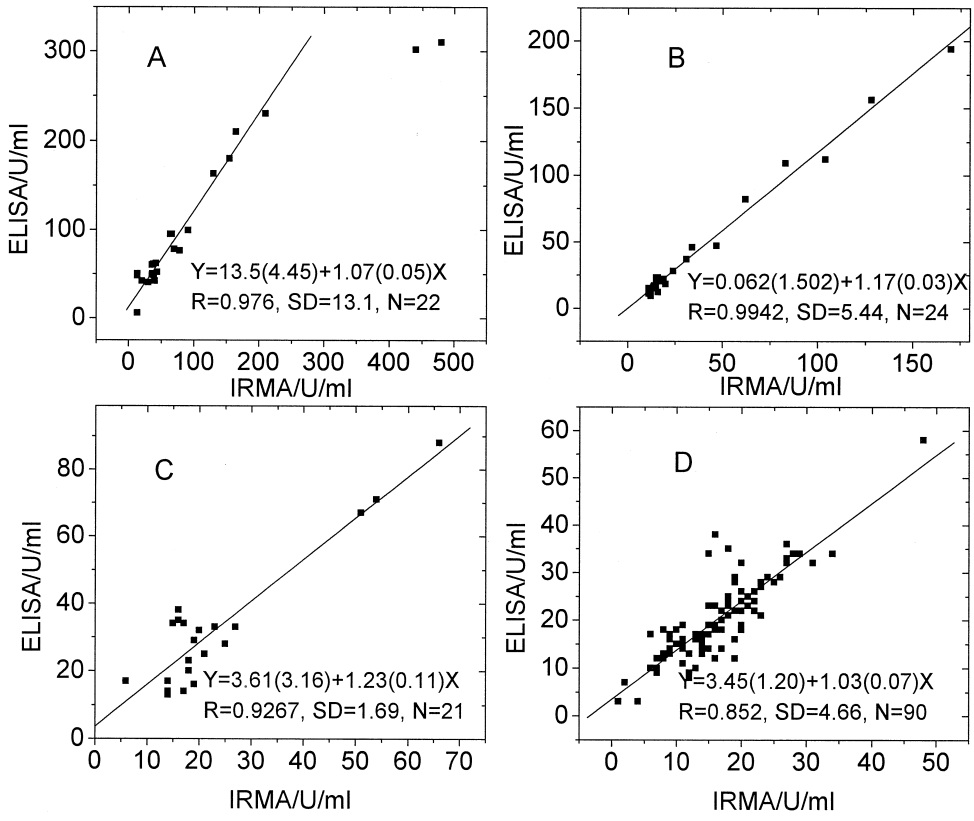

Fig. 2. Method comparison of the ELISA and the IRMA. The concentration of CA 125 was determined in sera from (A) untreated, (B) treated ovarian carcinoma patients, (C) digestive system cancer patients and (D) healthy women.

Table 2
Intra- and inter-assay coefficients of variation for CA 125 determined by the CLEIA and the ELISA

Methods	CLEIA	ELISA
Intra-assay ($N = 16$)	4.6% at 50 U/ml	6.2% at 53 U/ml
Inter-assay ($N = 16$)	7.6% at 37 U/ml	15.2% at 37 U/ml

correlation. The correlation coefficient was more than 0.998 for ovarian carcinoma patients, indicating a good correlation between the results obtained using the CLEIA and the IRMA.

Fig. 2 illustrates the results of orthogonal regression analysis of CA125 ELISA values vs. IRMA values compared under the same conditions as those in Fig. 1. The slopes of these plots are in the range of 1.03–1.23 with a mean value of 1.13. When the CA 125 concentration was more than 400 U/ml, the values on ELISA deviated from the line, indicating the linear range of the ELISA was narrower than those of the CLEIA and the IRMA. The correlation was worse and the values of the SD were rather large for all cases in comparison with those of CLEIA vs. IRMA values, particularly for samples containing low CA 125 concentrations. The larger errors possibly resulted from two factors, the influence of MAb coated wells and the immunoreaction between CA 125 and enzyme-labeled antibody and the interference of air or light with the colorimetric reaction. In

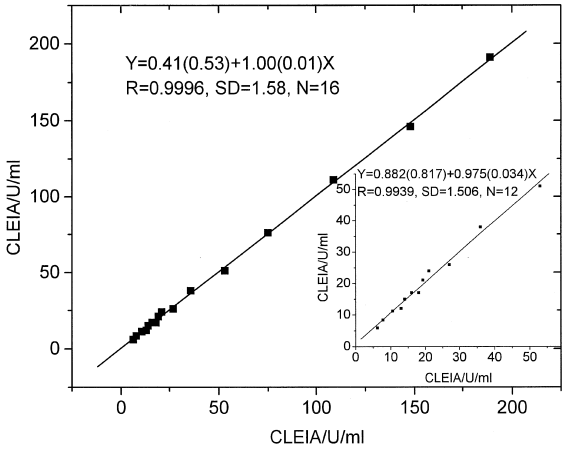

Fig. 3. Regression analysis of CA 125 concentrations determined in parallel procedures using the CLEIA. The inset shows the results obtained at low concentrations.

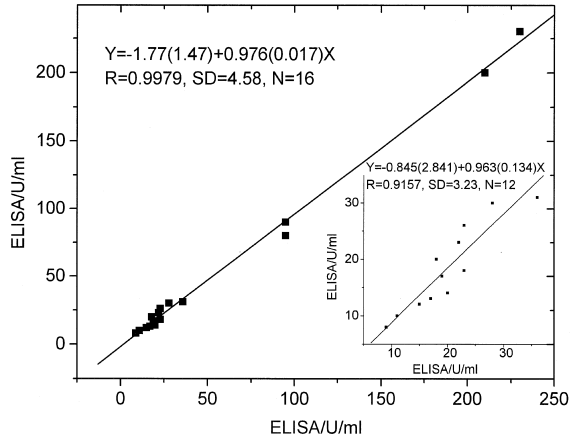

Fig. 4. Correlation between CA 125 concentrations determined in parallel ELISA procedures. The inset shows the results obtained at low concentrations.

the colorimetric reaction a reduced dye (e.g., 3,3′,5,5′-tetramethylbenzine (Madersbacher and Berger, 1991)) was used as the color reagent. The reduced dye is unstable in air and light and can react with oxygen by light catalysis (noted in the instructions of the ELISA test kits). Thus, the substrate reaction should be developed in the dark (Madersbacher and Berger, 1991). However, in practice the reaction is usually developed in air and light according to the instructions in the test kits.

3.2.2. Precision

We used four sera to perform precision tests of intra- and inter-assay variation with the CLEIA and ELISA procedures. Statistics were calculated for each from the results of 16 replicate measurements obtained in a single run or in 16 different runs. The results are shown in Table 2. It has been noted that all the coefficients of variation (CV) lie in the range recommended in the manufacturer's manuals and are similar to those reported by Nishizono et al. (1991). The intra-assay CV values exhibited less difference between two fully automated EIAs, while there was a larger discrepancy between the inter-assay CV values. The inter-assay precision test is based on a single well, and thus its error results from the determination of the products from hydrolytic (CLEIA) or colorimetric (ELISA) reactions. The smaller difference between the CV values of CLEIA and ELISA suggests that the effect of air or light on the colori-

metric reaction is secondary compared to the larger discrepancy between inter-assay CV values. The error in the tests of inter-assay variation results from the effects of MAb coated wells and immunoreactions between CA 125 and enzyme-labeled antibody as well as differences in procedure.

3.2.3. Reproducibility

In this work, the reproducibility of the CLEIA and the ELISA was evaluated by correlating parallel determinations of CA 125 in 16 sera over the concentration range of 0–250 U/ml. Fig. 3 shows the plot for results obtained with the CLEIA. The linear regression equation is $Y = 0.41 + 1.00X$ with a correlation coefficient of 0.9996 ($P < 0.0001$). When the concentrations of CA 125 are less than 50 U/ml, the correlation coefficient between twice parallel determinations is 0.9939, slightly worse than at high concentrations. The results of such regression analysis suggest that the reproducibility of the CLEIA is very good.

The linear regression equation of results for the ELISA is $Y = -1.77 + 0.976X$ with a correlation coefficient of 0.9979 ($P < 0.0001$) (Fig. 4), indicating a good correlation and good reproducibility. This regression analysis exhibits a larger SD, compared to the CLEIA. At CA 125 concentrations less than 50 U/ml the correlation is rather poor, and thus the error of the ELISA is larger at lower CA 125 concentrations. These results suggest that the experimental conditions influence the enzyme-catalyzed reaction in the ELISA more than that in the CLEIA.

Acknowledgements

Financial support for this work was provided by the Scientific Research Foundation for Returned Overseas Chinese Scholars, Ministry of Education of China and Cancer Hospital and Institute of Jiangsu Province.

References

Bast, R.C., Lazarus, H., Feeney, M., Knapp, R.C., 1980. Reactivity of a monoclonal antibody with human ovarian carcinoma. Am. Assoc. Cancer Res. 21, 207.

Bast, R.C., Feeney, M., Lazarus, H., Nadler, L.M., Colvin, R.B., Knapp, R.C., 1981. Reactivity of a monoclonal antibody with human ovarian carcinoma. J. Clin. Invest. 68, 1331.

Bast, R.C., Klug, T.L., St. John, E. et al., 1983. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. New Engl. J. Med. 309, 883.

Boerman, O.C., Thomas, C.M.G., Segers, M.F.G., Kenemans, P., Lovgren, T., Zurawski, V.R., Halsma, H.J., Poels, L.G., 1987. Time-resolved immunofluorometric assay for the ovarian carcinoma-associated antigenic determinant CA 125 in serum. Clin. Chem. 33, 2191.

Bonfrer, J.M.G., Baan, A.W., Jansen, E., Lentfer, D., Kenemans, P., 1994. Technical evaluation of three second generation CA 125 assays. Eur. J. Clin. Chem. Clin. Biochem. 32, 201.

Deger, A.U.W., 1993. Method for the determination of an immunologically bindable substance. Eur. Pat. Appl. EP 540, 037 (Cl. G01N33/543).

Einhorn, N., Bast, R.C., Knapp, R.C., Tjernberg, B., Zurawski, V.R., 1986. Preoperative evaluation of serum CA 125 levels in patients with primary epithelial ovarian cancer. Obstet. Gynecol. 67, 414.

Endo, K., Matsuoka, Y., Nakashima, T., Fujli, S., Kunimatsu, M. et al., 1988. Development of a new sensitive IRMA for CA 125: mixed use of two monoclonal antibodies reaction with separate epitopes. J. Tumor Marker Oncol. 3, 65.

Hashimoto, T., Ohba, N., Matsubara, F., 1990. Discordant CA 125 measurements in normal healthy women (Tech Brief). Clin. Chem. 36, 1377.

Kakizaki, J., Miyazaki, T., Maeda, T., 1993. Basic studies of ball ELISA CA 125-II kit for detection of serum cancer antigen 125. Kaku Igaku 30, 807.

Kenemans, P., Bon, G.G., Kessler, A.C., Verstraeten, A.A., van Kamp, G.J., 1992. Multicenter technical and clinical evaluation of a fully automated enzyme immunoassay for CA 125. Clin. Chem. 38, 1466.

Klug, T.L., Bast, R.C., Niloff, J.M., Knapp, R.C., Zurawski, V.R., 1984. Monoclonal antibody immunoradiometric assay for an antigenic determinant (CA 125) associated with human epithelial ovarian carcinomas. Cancer Res. 44, 1048.

Lavin, P., Knapp, R.C., Malkasian, G., Whitney, C.W., Berck, J.C., Bast, R.C., 1987. CA 125 for the monitoring of ovarian carcinoma during primary therapy. Obstet. Gynecol. 69, 223.

Madersbacher, S., Berger, P., 1991. Double wavelength measurement of 3,3′,5,5′-tetramethylbenzidine (YMB) provides a three-fold enhancement of the ELISA measuring range. J. Immunol. Methods 138, 121.

Nishizono, I., Iida, S., Suzuki, N., Kawada, H., Murakami, H., Ashihara, Y., Okada, M., 1991. Rapid and sensitive chemiluminescent enzyme immunoassay for measuring tumor markers. Clin. Chem. 37, 1639.

Shima, Y., Tsukada, T., Nakayarna, T., 1992. Evaluation of automated chemiluminescent enzyme immunoassay system, Lumipulse 1200: I. Characteristics of the instrument and properties of the reagents. Igaku to Yakugaku 28, 1247.

Zucchelli, G.C., Pilo, A., Jaworek, D., Masini, S., Chiesa, M.R., 1992. Immunoassay of CEA, CA19-9, CA 125 and CA 15-3 on the automated systems ES 300 and ES 600: methodological

evaluation from a multicenter collaborative study. Eur. J. Clin. Chem. Clin. Biochem. 30, 875.

Zucchelli, G.C., Pilo, A., Chiesa, M.R., Masini, S., Clerico, A., 1994. Analytical performance of CA19-9, CA 125 and CA15-3 assay as observed through an external quality assessment program. Int. J. Biol. Markers 9, 43.

Zurawski, V.R., Broderick, S.F., Pickens, P., Knapp, R.C., Bast, R.C., 1987. Serum CA 125 levels in a group of non-hospitalized women: relevance for the early detection of ovarian cancer. Obstet. Gynecol. 69, 606.