LAPORAN PROJEK PENYELIDIKAN

GERAN INSENTIF KHAS UNTUK JURUTEKNOLOGIS MAKMAL PERUBATAN

PUSAT PENGAJIAN SAINS PERUBATAN

UNIVERSITI SAINS MALAYSIA

TAJUK : " Tumour Markers Assay Serum CA125 And Ca153. A Preliminary Report Of USM Experience."

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CONTENT	PAGE

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LIST OF FIGURES, TABLES AND DIAGRAMS

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Introduction :

Cancer is one of the most important medical problems in today's world and in Malaysia it is one of the leading causes of death.Although development of cancer takes a considerable amount of man's life span,it is however possible to detect its early pre-cancerous stage.Death due to cancer may be avoided if detected early and this is usually done by biochemical measurement of tumour markers.

Tumour Markers are substances which are related to the presence or progress of a tumour (1).
They include proteins such as enzymes and peptides which are secreted into body fluids by tumours and antigens which are expressed on cell surfaces.In contrast, tumour markers also play a critical role in the monitoring and treatment of patients with cancer . (2)

The method employed for this preliminary/pilot study is based on ELISA technique..The CA 125 enzyme immunoassay kit was purchased from TECO USA which uses a monoclonal antibody..It is specific for surface antigen derived from a papillary serous cyst adenocarcinoma. Elevated CA 125 serum levels are also found in patient with serious endometrium and fallopian tube.

The CA 15.3 enzyme immunoassay test kit was purchased from TECO USA which uses monoclonal antibodies to detect two antigenic sites associated with breast carcinoma cells.CA 15.3 is also useful for serial monitoring .

The presence of tumour cells in the blood can mean different things depending on the stage of the disease. .If tumour cells are found in the blood ,most likely it indicates a metastasis of the primary tumour. The presence of these cells in more advanced stages may indicate a more rapidly progressing disease.

Objective

The objective of this preliminary/pilot study is :

1.To find if serum CA 125 and 15.3 are specific and sensitive tumour markers for ovarian and breast cancer respectively during screening.

Usefulness of serum CA125

Currently the only tumour marker to have a well defined and validated role in the management of ovarian cancer is CA125.

Changes in the level of CA125 can be used as a reliable indication of response to progression according to various criteria but it does not yet have a clear place in diagnosis or prognosis.
CA125,a large glycoprotein of unknown functions is the most extensively researched tumour markers to be identified for epithelial ovarian cancer.

The serum concentration of CA125 is elevated by the vascular invasion,tissue destruction and inflammation associated with malignant disease and is elevated in over 90% of those women with advanced ovarian cancer(Tuxen et all1995) and 40% of all patients with advanced intra-abdominal malignancy .
Levels can also be elevated during menstruation or pregnancy and in other benign conditions such as endometriosis,peritonitis or cirrhosis,particularly with ascites.

Usefulness of serum CA15.3

There are a number of tumour markers that can help clinicians to identify and diagnose which breast cancer patients will have aggressive disease and which will have an indolent course.These markers include estrogen and progesterone receptors , DNA ploidy and percent-S phase profile ,epidermal growth factor receptor ,HER-2/neu oncogene, p53 tumour suppressor gene,cathepsin D, proliferation markers and CA15.3.

CA15.3 is most useful for monitoring patients post-operatively for recurrence, particularly metatastic diseases (4) . 96% of patients with local and systemic have elevated CA15.3 , which can be used to predict recurrence earlier than radiological and clinical criteria.

A 25% increase in the serum CA15.3 is associated with progression of carcinoma.(4). A 50% decrease in serum CA15.3 is associated with response to treatment.

METHODOLOGY

Patients

Since the establishment of the tumour marker unit at the Dept.of chemical Pathology in April 2000 about 91 blood specimen belong to patients from various wards such as Oncology ,O&G , Medical ,SOPD clinic and Radiotheraphy unit were received by the department. .Most request form were diagnosed as ovarian and breast cancer without other relevant information such as confirmatory benign or malignant stage(histology diagnosis report).

A study to establish normal value of CA125 and CA153 among local Kelantanese healthy subject were also conducted (N=17).(Table 7 and 8). Their blood sample were collected and allowed to clot, spinned,separated and the serum were stored at –20 C.

Total number of patients screened for serum CA125 is 56 while for serum CA153 is 35.

Measurement of Serum CA125 and CA 153

Both serum CA125 and CA15.3 were analysed using ELISA Kits purchased from TECO USA.(Attachment 1and 2)
The results were confirmed using Tumour Marker Control (Lyphochek) BIO RAD USA.(Attachment 3)

ENZYME LINKED IMMUNOABSORBENT ASSAY (ELISA)

Enzyme linked immunoabsorbent assay (ELISA) is a heterogeneous assay which is similar to the immunoradiometric assay except that an enzyme tag is attached to the antibody instead of a radioactive label. This assay has the advantage of avoiding radioactive materials and produces an end product that can be measured in a spectrophotometer. The antigen which is being studied is bound to the enzyme-labelled antibody and the excess antibody is removed.

Then the second antibody containing the enzyme is added followed by the substrate and cofactors necessary for visualization of enzyme activity.The amount of antigen present is directly related to the amount of enzymatic activity (substrate formed) during a detection incubation period. The sensitivity of the assay may be increased by increasing the incubation time for producing substrate.

In some cases, substrate formed may give an optical color change so that the detection of the tumour marker being measured can be determined by special reader that can identify the slight chances in color. This kind of special reader are called "microplate reader"(6).

CA125- Principle Of The Test ELISA Kit (5)
The Ca 125 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA125 molecule is used for solid phase immobilization (on the microtiter walls). A rabbit Anti –CA125 antibody conjugated to hoseradish peroxidase (HRPO) is in the antibody –enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies,resulting in the CA125 molecules being sandwiched between the solid phase and enzyme-linked antibodies.

After 3 –hour incubation at 37 C ,the wells are washed with water to remove unbound labelled antibodies. A solution of H202/TMB is added and incubated for 20 minutes, resulting in the development of a blue colour.The color development is stopped with the addition of 3 N HCL changing the color to yellow. The concentration of CA125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

CA153- Principle Of The Test ELISA Kit (4)

The Ca153 ELISA test kit is based on the principle of a solid phase enzyme-linked immunosorbent assay.The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA153 molecule used for solid phase immobilization (on the microtiter walls).A rabbit anti-CA153 antibody conjugated to horsedish peroxidase (HRPO) is in the antibody _enzyme conjugate solution.The test sample is allowed to react sequentially with the two antibodies ,resulting in the CA153 molecules being sandwiched between the solid phase and enzyme linked antibodies. After two separate 1 hr incubation steps at 37 C ,the wells are washed with water to remove unbound labeled antibodies. A solution of H202/TMB is added and incubated for 20 minutes ,resulting in the development of a blue color. The color development is stopped with the addition of 3N HCL changing the color to yellow. The concentration of CA153 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

RESULTS

Table 1 : Normal Serum CA125 value at Diagnosis.(<35IU/ml)

No	Patient	Diagnosis	CA125 At Diagnosis	Wad	Age	Race	Histopath Report.
1.	SZ	Ovarian Tumour	20.5	3s	33	M	c=confirmed Ca.
2.	SY	Rt.Ovarian Cyst	2.32	1s		M	
3.	SY	Lt.Ovarian Cyst	2.19	1s		M	c
4.	MRL	Granular Cyst	12.7	RT	30	M	c
5.	RA	Ca.Ovary	18.6	O/G		M	c
6.	CKCI	Ovarian Mass	14.5	2s	24	M	c
7.	MO	Ovarian Tumour	11.3	O/G	60	M	c
8.	SAY	Post R.Cophorectomy	1.9	Gynae	17	M	c
9.	NH	Ovarian Cyst	26.4	1s	18	M	c
10.	SRM	Ovarian Cyst	14.0	1s	47	M	c
11.	CKCI	Ovarian Tumour	15.5	2s	24	M	-
12.	RH	Molar Pregnancy	2.37	2s	36	M	-
13.	SRM	Ovaraian Cyst	14.0		47	M	-
14.	NH	Ovarian Cyst	5.9	5TB	12	M	-
15.	AMN	TRO Malignancy	10.6	O/G	28	M	-
16.	NHAK	Ovarian Mass	15.4	1s	18	M	-
17.	CH	RIF Pain	3.4	3u		M	-
18.	KELD	Ovarian Cyst	5.9	1s	64	M	-
19.	ZHM	Ovarian Ca	11.1	O&G	44	M	-
20.	MAIO*	Ovarian Tumour	8.4	O&G	61	M	-
21.	NUMY	Ovarian Tumour	10.5	O&G	31	M	-
22.	A970956	Ovarian Tumour	15.6	O&G	32	M	-
23.	NMS	Ovarian Tumour	8.2	O&G	50	M	-
24.	ZI	Ovarian Cyst	31.2	O&G		M	-
25.	KELD		5.9	O&G	64	M	-
26.	FC	Ovarian Ca	24.0	1U	-	C	-
27.	MSI	Dysplasia Polyp	2.5	3U	-	M	-
28.	HM	Ovarian Cyst	2.6	IU	-	M	-
29.	ZM	Ovarian Cyst	6.0	1U	-	M	-
30.	PUS	Endometrioses	2.4	O&G	40	M	-
31.	ROA	Ovarian Tumour	2.4	1U	-	M	-
32.	NOR	Ca Breast(Chemo)	26.8	RT	_	M	-
33.	NIS	Uterine Mass	25.2	1S	34	M	-
34.	SMH	Chondrosarcoma	7.9	4U	-	M	-

Statistic Report:

Total Screening : N=56

34x100/56 = 34 (60.7%) with various form of ovarian disease.

Corfirmed Cancer (Histopath Report)

N = 56

8x100/56 = 8 (14.3%) confirmed to have ovarian tumour/carcinoma.

- = Histopath report not available.

Table2 : Serum CA 125 Value Above Cut-Off Value (>35 IU/ml)

No.	Patient	Diagnosis	CA125 At Diagnosis	Wad	Age	Race	Histopath. Report.
1.	WZY	Ovarian Tumour	53.7	O/G	57	M	c
2.	MJA	Ca Ovary	>400.0	1s	76	M	c
3.	SM	Screening Ovary Ca	56.4	RT	28	M	c
4.	RZ_	Ovarian Ca	103	RT	32	M	c
5.	SBH	Cx.Ca	70.7	3s		M	-
6.	RAH	Ovarian Tumour	124.0	1s	17	M	-
7.	NII	Ca Ovary	124.9	O/G	30	M	-
8.	ZM	Ovarian Tumour	101.6	1s	23	M	c
9.	NR	Ca.Cervix	465.5	1s		M	-
10.	SS	Ovarian Cyst	85.4	RT	24	M	c
11.	NIA	Ovarian Cyst	94.1	O/G	45	M	-
12.	SHI	Ca Ovary	45.1	1s		M	-
13.*	ROI	Uterine Fibroid	100.0	1s	40	M	-
14.	MJA		467.8	1s	76	M	-
15.	ANS	Ca Colorectal	170	RT	26	M	-
16.	WZY	Ovarian Tumour	100.8	O&G	67	M	-
17.	NS	Ovarian Tumour	40.4	1s	53	M	-
18.	SI	Ca Ovary	62.2	1S	61	M	-
19.	CYCM	Malignancy Renal	>400.0	IU	-	M	-
20.	TR	Ovarian Cyst	83.7	1S	-	M	-
21.	CFCA	Ca Ovary	>400.0	ONCO	57	M	-
22.	RAS	Ovarian Ca	198.1	IU	25	M	-

Statistic Report:
N=56
22x100/56 = 22(39.2%)

Correlation with Histopath Report –Confirmed Ca
7x100/56 = 7(12.5%)

- It is interesring to note that patient with Ca Cervix has an elevated value of CA 125 ≅ 465.5 IU/ml
- - Histopath report not available (or not enough sample)

Table 3 : Normal Serum Ca15.3 value at Diagnosis.(<28 U/ml)

No	Patient	Diagnosis	CA15.3 At Diagnosis	Wad	Age	Race	Histopath Report.
1.	GCT	Ca.Breast	14.7	O/G	38	C	-
2.	FCL	Ca Breast	22.4	RT	52	C	-
3.	MMA	Ca(L)Breast	17.3	RT	67	M	-
4.	RZ	Adv.Breast Ca	28.2	3s	53	M	-
5.	NNM	Adv.Breast Ca	13.4	SOPD	63	C	-
6.	ZY	Ca.Breast	18.3	RT	62	M	-
7.	ZAR	Ca Breast	39.1	RT	41	M	-
8.	SED	Brain Lesion	30.5	7u	44	M	-
9.	SH	Fibroedenosis	8.9	SOPD	20	M	-
10.	ZS	Ca.Breast	21.1	RT	39	M	-
11.	CBCK	Ca Breast	13.8	Onco	79	M	-
12.	AE	Ca Breast	18.0	Onco	75	M	-
13.	HD	Ca Breast	11.3	RT	61	M	-
14.	ZI	Ca Breast	16.4	Onco	64	M	-
15.	CYY	Ca Breast	9.3	3s		C	-
16.	NJ	Ca Breast	16.4	SOPD	40	M	-
17.	RMN	Ca Breast	1.17	RT	43	M	-
18.	SD.	Ca Breast	13.4	RT	42	M	-
19.	YA	Ca Breast	8.5	Onco	77	M	-
20.	HAR	Ca Breast	43	RT	43	M	-
21.	MD	Ca Breast	53	RT	53	M	-

Statistic Report :

N=35
22x100/35 = 22(62.8%)

Confirmed Ca (Histopath Report) but low Ca153 : 3x100/35 = 3 (8.57%)
Comments : Patients on treatment (monitoring)

- Histopath report not available.

Table 4 :Serum CA 153 : Above Cut (>28U/ml)

No	Patient	Diagnosis	CA 15.3 At Diagnosis	Wad	Age	Race	Histopath Report
1.	SA	Ca.Breast	>120.0	3U	2s	M	-
2.	SA	Ca Breast	53.6	SOPD	2s	M	-
3.	RE	Ca Breast	77.8	RT	47	M	-
4.	CS	Ca Breast	94.3	3s	48	M	-
5.	LZH	Ceroblast	41.5	3U	40	M	-
6.	ZAH	Breast Abcess	>120.0	3U		M	-
7.	MES	Ca Breast	>240.0	3s		M	-
8.	RZ	Ad.Breast Ca	28.2	3s	53	M	-
9.	ZAR	Ca Breast	39.1	RT	41	M	-
10.	SED	Brain Lesion	30.5	7u	44	M	-
11.	NAM	Breast Ca	44.4	SOPD	-	M	-
12.	MNS**	MassL.Avilla	>240	4s	37	M	-
13.	HKL	Ca.Breast	>240	RT	49	C	-

Statistic Report
N=35
13x100/35 = 13(37.1%)

Confirmed Ca (Histopath Report)
3x100/35 = 3 (8.5%)

1 Male Patient Elevated Ca153 : MNS
1x100/35 = 1(2.8%)

- Histopath report not available.

Results :

Table 5 : CA125 Levels (N=56)

Cancer Patients –confirmed HPE report

CA 125 Below cut of value : 8 (14.3%)
CA 125 Above cut of value : 7 (12.5%)

All Patients

CA 125 Below cut of value : 34 (60.7%)
CA 125 Above cut of value :22 (39.2%)

Table 5a : CA125 level in patient diagnosed with ovarian cancer (N=15)

<35 U/ml = 8/15x100% = 53.3%
>35 U/ml = 7/15x100% = 46.7%

Table 6 : CA 15.3 Levels (N=35)

Cancer Patients -confirmed HPE report

CA15.3 Below cut of value : 5 (14.3%)
CA 15.3 Above cut of value : 3 (8.5%)

All Patients

CA15.3 Below cut of value : 22 (62.8%)
CA 15.3 Above cut of value: 13 (37.1%)

Table 6a : CA153 Level in patient diagnosed with breast cancer

<28 U/ml = 5/8x100% = 62.5 %
>28 U/ml = 3/8 x100% = 37.5 %

Healthy subject Normal Value (Table 7)

TEST	N	X	SD
CA153	17	5.25	4.55
CA125	17	9.28	3.94

Healthy subject Distribution Range (normal value) .

Additional information included :
Table 8 : Statistic of tumour markers done at Chemical Pathology Laboratory University Sains Malaysia Kubang Kerian Kelantan.

Table 6

1.	A117121	Left Breast: INFILTRATING DUCTAL CARCINOMA (GRADE 11, MODIFIED BLOOM AND RICHARDSON).
2.	B059775	Left ovary - Papillary Serous Cystadenocarcinoma with deposits in myometrium, parametrium and the omentum.
3.	031907	Gastric (antral) biopsy: WITHIN NORMAL
4.	B119648	Left Ovarian Cyst: IMMATURE TERATOMA (GRADE 11, NORRIS CLASSIFICATION)
5.	B122511	A: Right Ovary : MATURE CYSTIC TERATOMA;OMENTUM: METASTATIC ENDODERMAL SINUS TUMOUR
6.	A810975	A: Right Ovary: COMPATIBLE WITH A BENIGN HAEMORRHAGIC UNILOCULAR CYST B: Left Ovarian Mass: CLEAR CELL CARCINOMA, WITH CAPSULAR INVASION C; Omentum: NO MALIGNANCY SEEN
7.	A806853	Tumour From Right Lateral Aspect Of Vault: RECURRENT SEROUS PAPILLARY ADENOCARCINOMA.
8.	B113254	A: 'Mass': METASTATIC PAPILLARY SEROUS CYST ADENOCARCINOMA B: 'Right" Ovarian Mass: PAPILLARY SEROUS CYST ADENOCARCINOMA (WITH CONTRALATERAL OVARIAN TUMOUR METASTASES) Uterus: LEIOMYOMA
9.	B126154	Appendix: SUPPURATIVE APPENDICITIS
10.	A835360	Endometrial Sampling: INADEQUATE MATERIAL FOR INTERPRETATION.
11.	B111064	A: Cervix : CHRONIC CERVICITIS Uterus: SEROSITIS B: Peritoneal Biopsy: NON SPECIFIC CHRONIC INFLAMMATION C: Peritoneal Nodule: GRANULATION TISSUE D: Omentum: CHRONIC NON SPECIFIC INFLAMMATION E: Left Ovary: JUVENILE GRANULOSA CELL TUMOUR
12.	A040170	Left breast - Invasive Ductal Carcinoma (NOS - Grade II) (Bloom and Richardson). Endometrium: EARLY SECRETORY PHASE
13.	A834444	Left breast, mastectomy : INFILTRATING DUCTAL CARCINOMA (NOS/NST TYPE) (GRADE II / MOD. DIFF.)
14.	A091734	Breast, Right Lower Inner Quadrantectomy: DUCTAL CARCINOMA IN SITU, CRIBRIFORM TYPE, WITH MINIMAL INVASION. ESTROGEN RECEPTOR STATUS NEGATIVE.
15.	A052142	Right Breast Lump: INFILTRATING DUCTAL

		CARCINOMA (NOS) - POORLY DIFFERENTIATED WITH INVOLVEMENT OF THE MARGINS. (BLOOM AND RICHARDSONN GRADING) A: Right Mastectomy Specimen: REMNANTS OF MALIGNANCY SEEN (SURGICAL MARGINS ARE FREE OF TUMOUR)
16.	B115178	A: Left Ovarian Mass: SEROUS CYSTADENOMA AND SEROUS CYSTADENOFIBROMA OF LOW GRADE MALIGNANT POTENTIAL. B: Uterus, Cervix and right ovary: ENDOCERVICAL POLYP, ENDOMETRIAL POLYP AND A TINY SUBMUCOSAL LEIOMYOMA. NORMAL FALLOPIAN TUBES (R &L) (RIGHT) OVARY : A FOCUS OF HYPERPLASTIC STROMAL NODULE C: Omental Tissue: NO TUMOUR DEPOSIT
17.	A886276	Trucut Breast Biopsy: INFILTRATING CARCINOMA
18.	A077547	Right Breast: INFILTRATING DUCTAL CARCINOMA, HIGH GRADE (BLOOD AND RICHARDSON) WITH LYMPH NODE INVOLVEMENT
19.	B120320	A: MUCINOUS CYSTADENOMA - RIGHT OVARY B: NORMAL OVARIAN TISSUE - LEFT OVARY C; NORMAL FIBRO FATTY TISSUE = OMENTUM
20.	A214923	Endometrial Pipelle: INADEQUATE FOR HISTOLOGICAL DIAGNOSIS Right Ovarian Cyst: ENDOMETRIOTIC CYST
21.	A923523	Mastectomy Specimen: INFILTRATING DUCT CARCINOMA
22.	B150212	Left Ovarian Cyst: ENDOMETRIOTIC CYST
23.	B150828	Appendix: EARLY APPENDICITIS
24.	A150693	Pipelle: NON REPRESENTATIVE SAMPLE Right Ovarian Tumour: MUCINOUS CYST ADENOMA Uterus: ADENOMYOSIS Fibroid: ADENOMYOMA
25.	B150693	Gastric Biopsy: WITHIN NORMAL LIMITS
26.	B152122	A: Omentum: CONGESTION AND MILD ACUTE INFLAMMATION B: Right Ovarian Cyst: MUCINOUS CYSTADENOMA
27.		

TABLE 7

PRELIMINARY REPORT OF : HEALTHY SUBJECT DISTRIBUTION RANGE
TUMOUR MARKER TEST : CA15-3 (BREAST)
KIT (ELISA) : CA125 (OVARIAN)
SCREENING TEST FOR HEALTHY SUBJECT : CEA

NO.	LAB #	CA15-3 Ref. Value < 28 u/ml.	CA125 Ref.Value < 35 Iu/ml
1.	1	3.18 (-)	4.86 (-)
2.	3	7.30 (-)	15.7 (-)
3.	4	1.72 (-)	1.50 (-)
4.	5	1.33 (-)	4.17 (-)
5.	10	12.3 (-)	27.7 (1)
6.	8	3.9 (-)	2.04 (-)
7.	6	15.0 (-)	16.7 (-)
8.	4	7.48 (-)	5.25 (-)
9.	13	4.23 (-)	22.5 (-)
10.	15	9.76 (-)	5.08 (-)
11.	12	4.31 (-)	25.2 (-)
12.	17	0.86 (-)	2.10 (-)
13.	18	1.12 (-)	2.10 (-)
14.	19	1.30 (-)	6.57 (-)
15.	20	3.63 (-)	12.2 (-)
16.	21	0.22 (-)	2.70 (-)
17.	7	11.7 (-)	1.49 (-)
N		17	17
X		5.25	9.28
SD		4.55	8.94

TUMOUR MARKER ASSAY

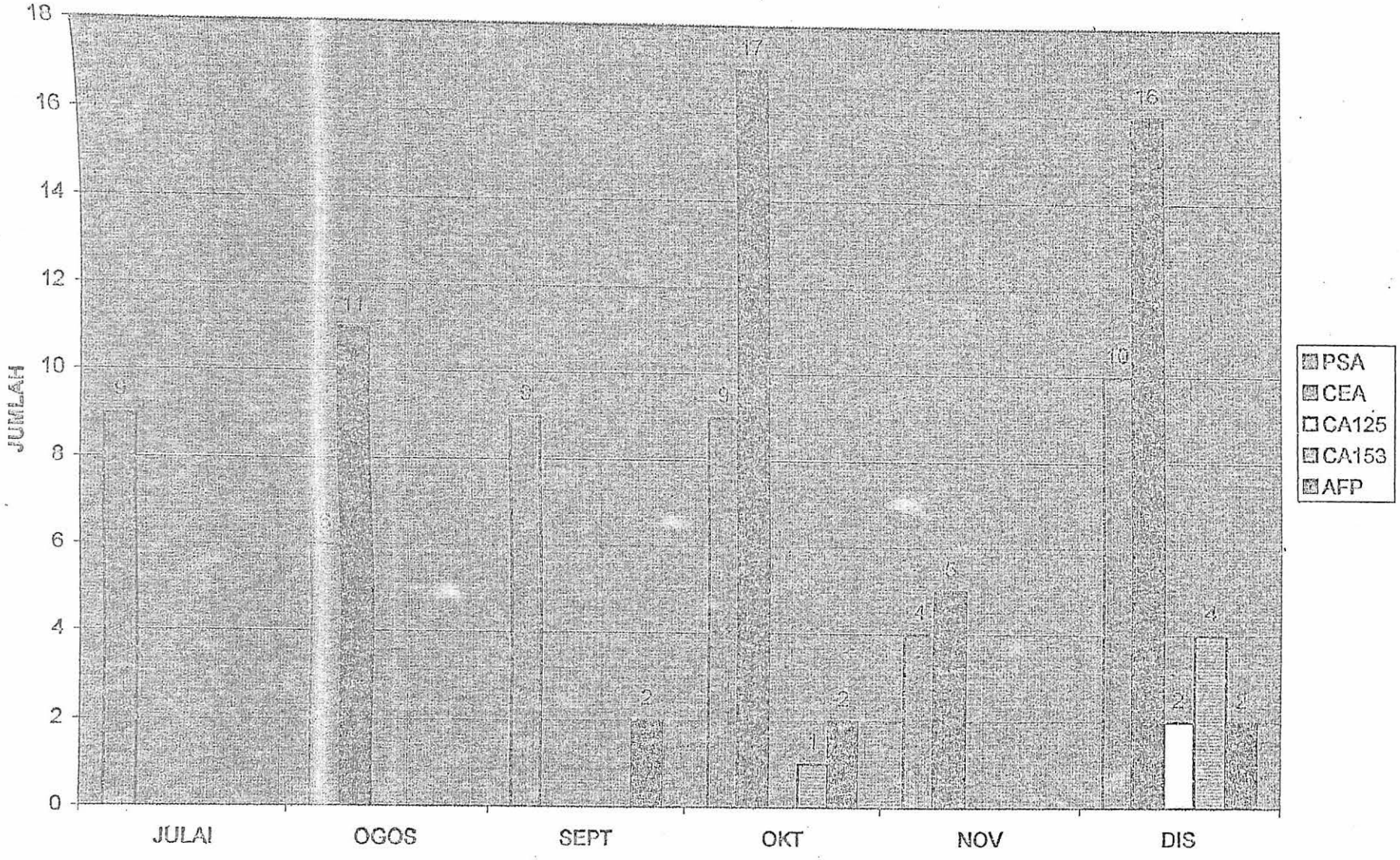
STATISTIC DATA REPORT

CHEMICAL PATHOLOGY LABORATORY

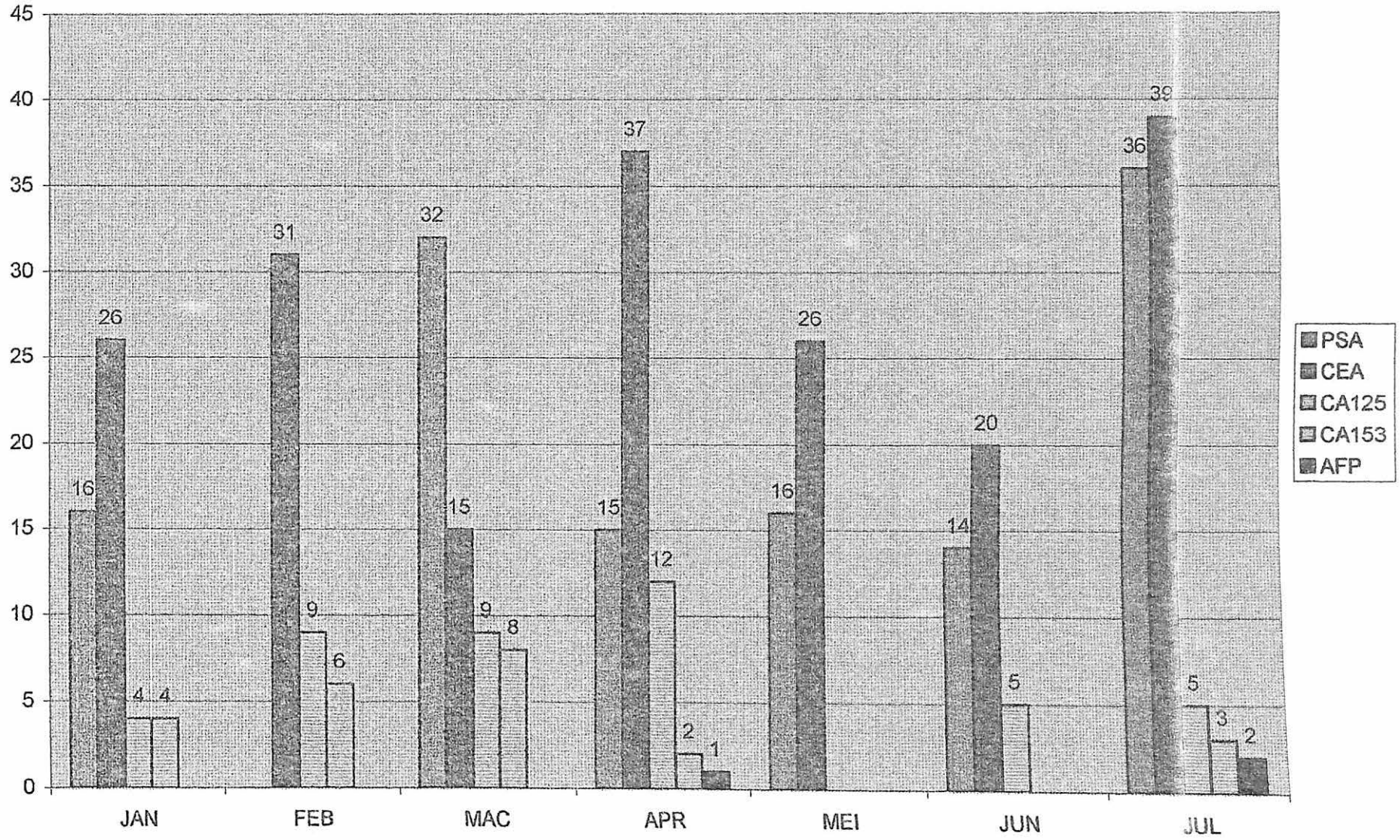
2000

Table 8

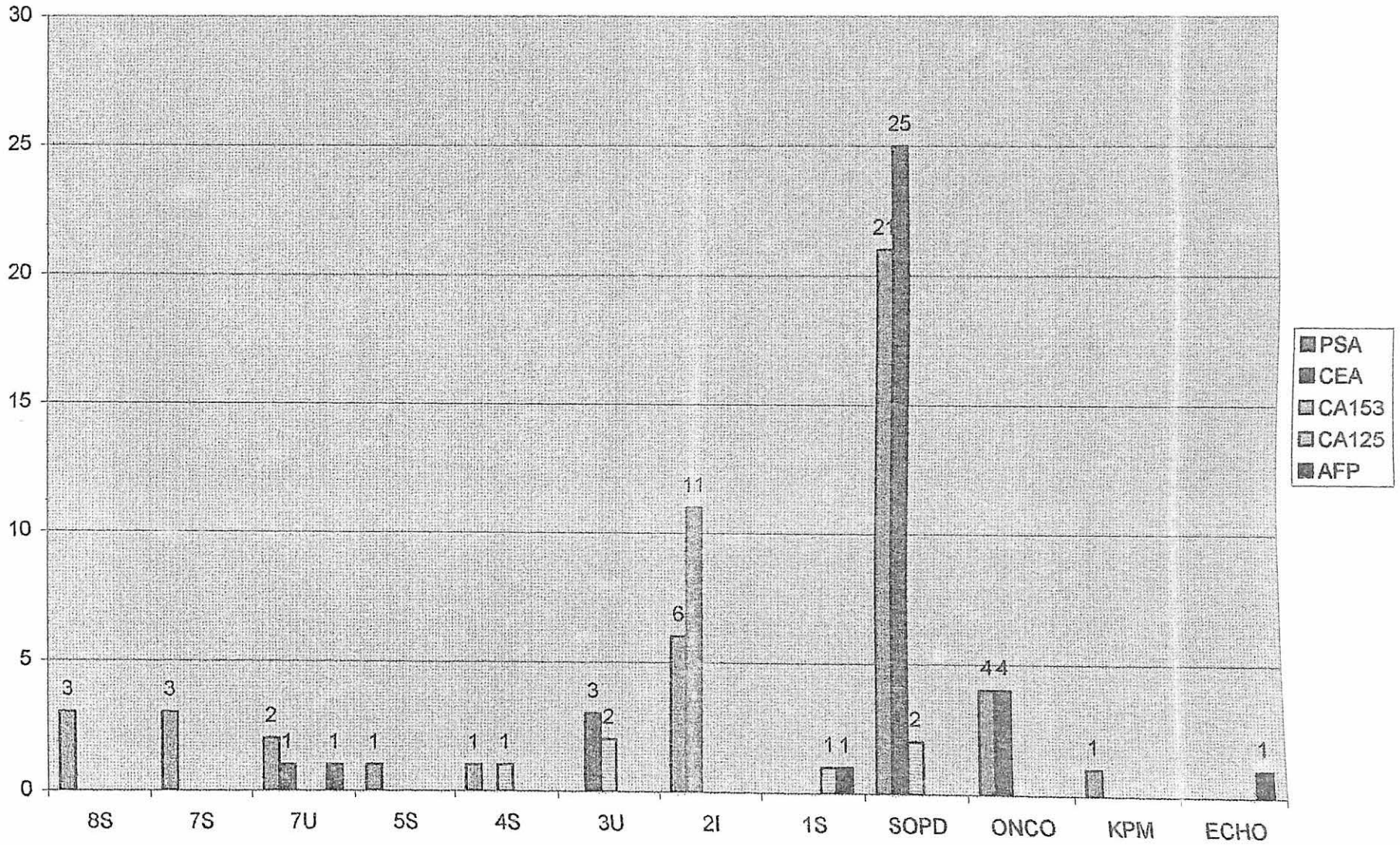
STATISTIK UJIAN PENANDA TUMOR MAKMAL PATOLOGI KIMIA 2000

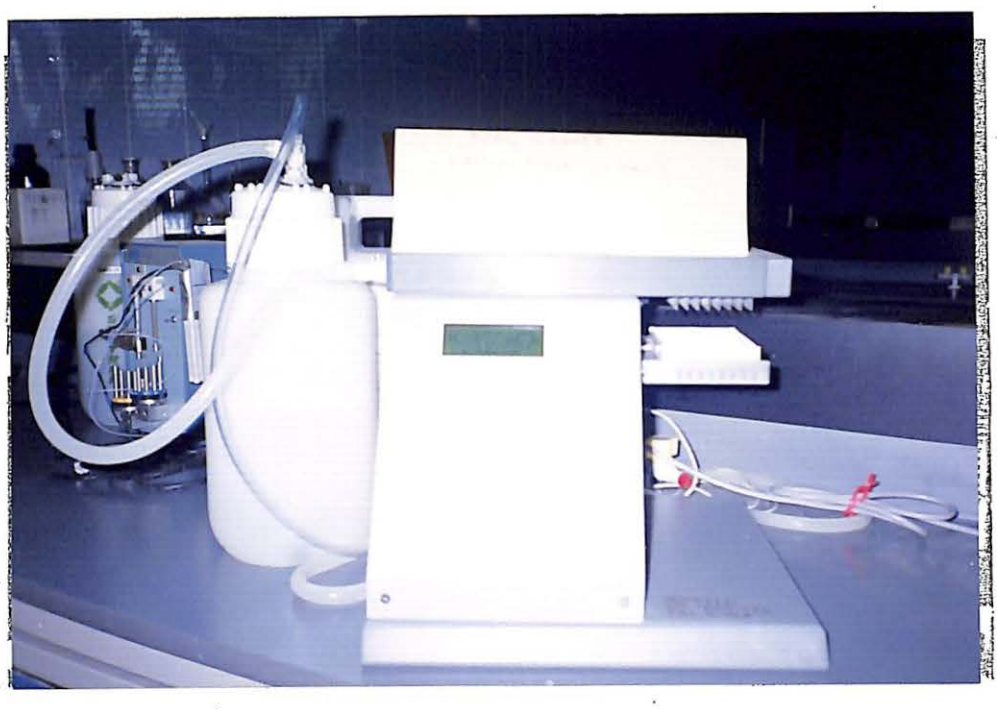


STATISTIK UJIAN PENANDA TUMUR MAKMAL PATOLOGI KIMIA JAN - JULAI 2001



PERMINTAAN UJIAN PENANDA TUMUR MENGIKUT WAD DAN KLINIK TAHUN 2000





3.1.1 Photograph 1 : The photograph shows the "*cell washer*" unit of (ELISA) method .

3.1.2 Photograph 2 : The photograph shows the "microplate reader" and the computer involved in (ELISA) method .

DISCUSSION :

Comments :(Summary Reports)

1.Cases of high valueof CA125 ;22(39.2 %)and CA15.3 , 13(37.1 %)belong to non cancer group would predict that patients may be at benign stage and not at malignant stage.

2. Significantly high values of CA125 ; 7 (12.5%)were found in ovarian canceas patients as confirmed by HPE report.

Significantly high values of CA15.3; 3 (8.5%) were found in breast cancer patients as confirmed by HPE report.

3. One case of elevated CA153 of male patient was found, 1(2.8%) during screening at diagnosis.This shows that even male patient is prone to have breast cancer.

4.Overall N=91 patients are predominantly Malays 86 (94.5%) and 5 (5.5%) are Chinese.This may reflect the dominant Malay population in Kelantan. Their age ranges from 18 – 61 yrs old. .

Conclusion

1. Elevated Ca 125,22 out of(39%) and Ca 153, 13 out of (37.1%) were considered imminent as our results show that it was possible to detect both cancers using ELISA technique .

2. Elevated level of serum CA125 above cut of value was seen in 39% of patient (22/56) diagnosed with ovarian cancer and
   Elevated level of serum CA 153 above cut of value was seen in 37.1% patients (13/35) diagnosed with breast cancer.

3. This study however was not able to get a full histological report of patients and thus correlation of the tumour markers with the stage of tumour was not able to be executed accurately.

   However an indication of its usefulness in aiding diagnosis of the respective cancers cannot be dismissed. We can see a high level of CA 125 in patients with ovarian tumour ε ovarian cyst (Table 2), ranging from 53.7 to 467.8 U/ml.

4. Our future study will include 1)a correlation study of serum CA153 ε CA 125 in both breast cancer ε ovarian cancer patients.
   1 i)a correlation study of serum CEA vesus serum CA15.3 Σ CA125 in both diseases :ie breast and ovarian cancer.

Suggestion And Review :

1.Since due to short period of time and limited funds this project would be versatile if more subjects being included and to study the clinical data of the patients with recurrent disease including the site of origin of the disease,histology,TNM.stage CA125 and CA15.3 level at the initial diagnosis after treatment and at recurrence.
And also the mean lead time between the elevation of the marker level and the clinical diagnosis of the recurrent disease .

2. Also other tumour markers assay such as correlation study between CA15.3 and CA27.29. Ca27.29 seems more sensitive than CA15.3 and confirm that CA27.29 may enter the diagnostic armentarium of tumour marker that are effective for breast cancer.(7)

3. To study in combination with CA125,CA15.3 wheather useful in early detection of relapse of ovarian camcer.

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Journal of Clinical Chemistry 45:10 , 1695-1909 (1999)

3.Yasmin Anum : Justification For setting Up Of Tumour Markers Laboratory/Centre At The Department Of Chemical Pathology. 22 Sept 1999 (Memo)

4. TECO Reagent Pamphlet : Breast Cancer Antigen Ca15.3 Enzyme Immunoassay Test Kit
Catalog Number : BC -1015

5. TECO Reagent Pamphlet : Cancer Antigen CA125 Enzyme Immunoassay Test Kit
Catalog Number : BC 1013

6.Perbandingan Keputusan Analisis "Tumour Markers " melalui Dua Kaedah iaitu ELISA Dan ECL Technologi _ Laporan Projek Penyelidikan DTMP 35/98

7. M.Gion , et all - Ca27.27 : a valuable marker for breast cancer management. A confirmatory multicentric study on 603 cases
European Journal Of Cancer 37 (2001) 355-363

8. T.Meyer and GJS Rustin – Role of tumour markers in monitoring epithelial ovarian cancer.
British Journal of Cancer (2000) 82 (9), 1535-1538.

9. TECO Reagent Pamphlet : TECO CEA microwell Test Kit Enzyme link immunosorbent assay (ELISA).

ATTACHMENTS

CANCER ANTIGEN CA125 ENZYME IMMUNOASSAY TEST KIT
Catalog Number: BC-1013

Enzyme Immunoassay for the Quantitative Determination of Ovarian Cancer Antigen CA125 in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

CA125 Enzyme Immunoassay

INTENDED USE

For the quantitative determination of the Cancer Antigen CA125 concentration in human serum.

INTRODUCTION

Cancer Antigen 125 (CA125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA125 is associated with a high molecular weight glycoprotein. Published studies have indicated that elevated serum CA125 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma.

The serum CA125 concentration is greater than 35 units per ml in 60% of women with ovarian cancer and >80% of patients with disseminated ovarian cancer. The serum CA125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serial determinations of serum CA125 as well as pelvic examination increase the test specificity. Serum CA125 concentration may be useful in monitoring treatment and distinguishing between good response to treatment and progressive malignant disease with poor therapeutic response. To date, CA125 is the most sensitive marker for residual epithelial ovarian cancer. CA125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

PRINCIPLE OF THE TEST

The CA125 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[12] The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA125 molecule is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA125 antibody conjugated to horseradish peroxidase (HRPO) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CA125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 3-hour incubation at 37°C, the wells are washed with water to remove unbound labeled antibodies. A solution of H_2O_2/TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 3N HCl changing the color to yellow. The concentration of CA125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Murine Monoclonal anti-CA125 coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml.
- CA125 reference standards containing; 0, 15, 50, 100, 200, and 400Unit/ml of CA125, 1 ml each, ready to use.
- Color Reagent A, 13ml.
- Color Reagent B, 13ml.
- Stop Solution (3N HCl), 10ml.

Materials required but not provided:

- Precision pipettes and tips, 0.1ml, 0.2ml, 1ml, and 5ml.
- Disposable pipette tips.
- Distilled water.
- Glass tubes or flasks to mix Color A Reagent and Color Reagent B solutions.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. To prepare H_2O_2/TMB solution, make an 1:1 mixing of Color Reagent A with Color Reagent B up to 1 hour before use. Mix gently to ensure complete mixing. The prepared H_2O_2/TMB reagent should be made at least 15 minutes before use and is stable at room temperature in the dark for up to 3 hours. Discard excess after use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 100µl of CA125 standards, specimens, and controls into the appropriate wells.
2. Dispense 100µl Enzyme Conjugate Reagent into each well.
3. Mix gently for 30 seconds. *It is very important to have complete mixing in this setup.*
4. Incubate at 37°C for 3 hours.
- *Prepare H_2O_2/TMB substrate 15 minutes before use.*
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Rinse and empty the microtiter plate 5 times with *distilled or deionized water. (Please do not use tap water.)*
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 200µl of H_2O_2/TMB solution into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.
9. Stop the reaction by adding 50µl of Stop Solution to each well.
10. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
11. Read the optical density at 450nm with a microtiter plate reader within 30 minutes.

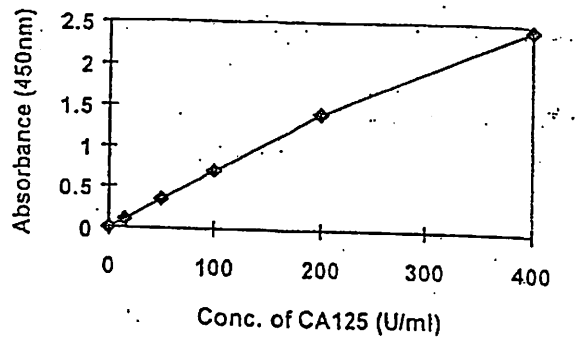
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CA125 in U/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against CA125 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

CA125 Values (U/ml)	Absorbance (450nm)
0	0.010
15	0.105
50	0.347
100	0.703
200	1.411
400	2.437



EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA125 assay values belo U/ml. The minimum detectable concentration of CA125 in assay is estimated to be 5 U/ml.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when assay procedure is carried out with a complete understan of the package insert instructions and with adherence to laboratory practice.
2. The wash procedure is critical. Insufficient washing will res poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be only as an adjunct to other diagnostic procedures information available to the physician.

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BREAST CANCER ANTIGEN CA15-3 ENZYME IMMUNOASSAY TEST KIT
Catalog Number: BC-1015

Enzyme Immunoassay for the Quantitative Determination of Breast Cancer Antigen CA15-3 in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

CA15-3 Enzyme Immunoassay

INTENDED USE

For the quantitative determination of the Cancer Antigen CA15-3 concentration in human serum.

INTRODUCTION

Breast cancer is the most common life-threatening malignant lesion in women of many developed countries today, with approximately 180,000 new cases diagnosed every year. Roughly half of these newly diagnosed patients are node-negative, however 30% of these cases progress to metastatic disease.

There are a number of tumor markers that can help clinicians to identify and diagnose which breast cancer patients will have aggressive disease and which will have an indolent course. These markers include estrogen and progesterone receptors, DNA ploidy and percent-S phase profile, epidermal growth factor receptor, HER-2/neu oncogene, p53 tumor suppressor gene, cathepsin D, proliferation markers and CA15-3. CA15-3 is most useful for monitoring patients post-operatively for recurrence, particularly metastatic diseases. 98% of patients with local and systemic recurrence have elevated CA15-3, which can be used to predict recurrence earlier than radiological and clinical criteria. A 25% increase in the serum CA15-3 is associated with progression of carcinoma. A 50% decrease in serum CA15-3 is associated with response to treatment. CA15-3 is more sensitive than CEA in early detection of breast cancer recurrence. In combination with CA125, CA15-3 has been shown to be useful in early detection of relapse of ovarian cancer. CA15-3 levels are also increased in colon, lung and hepatic tumors.

PRINCIPLE OF THE TEST

The CA15-3 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA15-3 molecule is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA15-3 antibody conjugated to horseradish peroxidase (HRPO) is in the antibody-enzyme conjugate solution. The test sample is allowed to react sequentially with the two antibodies, resulting in the CA15-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate 1-hour incubation steps at 37°C, the wells are washed with water to remove unbound labeled antibodies. A solution of H_2O_2/TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 3N HCl changing the color to yellow. The concentration of CA15-3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Muriine Monoclonal Anti-CA15-3 coated microtiter plate with 96 wells.
- Sample Diluent, 100 ml.
- Enzyme Conjugate Concentrate (22x), 1.0 ml.
- Enzyme Conjugate Diluent, 21 ml.
- CA15-3 reference standards, containing 0, 15, 30, 60, 120, and 240 Unit/ml. Liquid. 1 set. *These standards have been pre-diluted 51-fold. Please do not dilute them again.*
- Color Reagent A, 13 ml.
- Color Reagent B, 13 ml.
- Stop Solution (3N HCl), 10 ml.

Materials required but not provided:

- Precision pipettes and tips, 0.1 ml, 0.2 ml, 1 ml, and 5 ml.
- Distilled water.
- Disposable pipette tips.
- Glass tube or flask to mix Color Reagent A and Color Reagent B solutions.
- Vortex mixer.
- Absorbent paper or paper towel.
- A microtiter plate reader at 450nm wavelength, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater.
- Graph paper.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare working CA 15-3 Conjugate Reagent, add the entire 1.0 ml of Conjugate Concentrate (22x) to 21 ml of the Enzyme Conjugate Diluent (1:21 dilution) and mix well. The diluted Enzyme Conjugate Reagent is stable at 4° C for at least 4 months.
3. To prepare H_2O_2/TMB solution, make a 1:1 mixing of Color Reagent A with Color Reagent B up to 1 hour before use. Mix gently to ensure complete mixing. The prepared H_2O_2/TMB reagent should be made at least 15 minutes before use and is

stable at room temperature in the dark for up to 3 hours. Discard excess after use.

ASSAY PROCEDURE

1. *Patient serum and control serum should be diluted, 51 fold, before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20 µl serum with 1.0 ml Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS.*
2. Secure the desired number of coated wells in the holder. Dispense 200 µl of CA15-3 standards, diluted specimens, and diluted controls into the appropriate wells. Gently mix for 10 seconds.
3. Incubate at 37°C for 1 hour.
4. Remove the incubation mixture by emptying the plate content into a waste container.
5. Rinse and empty the microtiter plate 5 times with *distilled or deionized water. (Please do not use tap water.)*.
6. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Dispense 200µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds
8. Incubate at 37°C for 1 hour.
9. Remove the contents and wash the plate as described in steps 5-7 above. (5 – 6)
- *Prepare H_2O_2/TMB substrate 15 minutes before use.*
10. Dispense 200 µl H_2O_2/TMB substrate reagent into each well. Gently mix for 10 seconds.
11. Incubate at room temperature in the dark for 20 minutes.
12. Stop the reaction by adding 50µl of Stop Solution to each well.
13. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
14. Read the optical density at 450nm with a microtiter plate reader within 30 minutes.

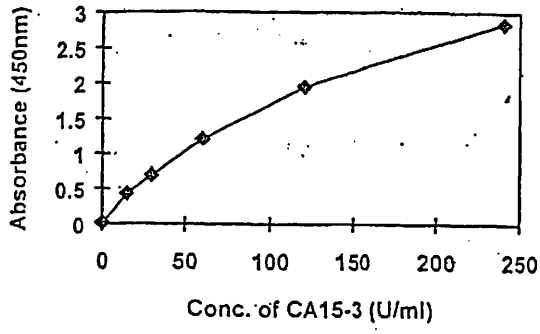
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CA15-3 in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y-axis against CA15-3 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

CA15-3 Values (U/ml)	Absorbance (450 nm)
0	0.021
15	0.425
30	0.693
60	1.214
120	1.956
240	2.845



EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA15-3 assay values below 35 U/ml. The minimum detectable concentration of CA15-3 in this assay is estimated to be 5 U/ml.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when t assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be us only as an adjunct to other diagnostic procedures a information available to the physician.

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