

# **Guidelines for the Use of Tumour Markers**

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by

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## INTRODUCTION

Because these assays are generally more expensive than most other routinely performed biochemical analyses, it is important that they are performed in a cost-effective manner. The purpose of this booklet is to give a brief background to enable the judicious use of the most widely performed cancer markers. We are aware that other cancer marker assays are performed in certain laboratories. Some of these may be included in a future edition of this publication.

While specific details relating to the most frequently used markers are listed below, we feel that it is important to make some general points about these tests.

1. No serum marker in current use is specific for malignancy.
2. Generally, serum marker levels are rarely elevated in patients with early malignancy. With a few exceptions, high levels are usually found only when patients have advanced disease.
3. No cancer marker has absolute organ specificity. PSA however, appears to be relatively specific for prostate tissue.
4. Apart from possibly HCG in choriocarcinoma, no marker is elevated in 100% of patients with a particular malignancy.
5. Requesting of multiple markers (such as CEA and the CA series of antigens) in an attempt to identify metastases of unknown primary origin is rarely of use.
6. Reference ranges for cancer markers are not well defined and are used only for guidance. Please note that a level below the reference range does not exclude malignancy while concentrations above the reference range does not necessary mean the presence of cancer. Changes in levels over time are likely to be more clinically useful than absolute levels at one point in time.
7. As many tumour markers lack agreed International Reference Preparations, different assay kits may give different results for the same sera.
8. Laboratories carrying out tumour marker tests should state the assay used on their report form.

### General References on Tumor Markers

Duffy MJ. Clinical use of tumor markers: a critical review. *Critical Review Clin Lab Sci* 2001;38:225-262.

Duffy MJ. Evidence for the clinical use of tumor markers. *Ann Clin Biochem* 2004;41:370377.

## ALPHA-FETOPROTEIN (AFP)

### Structure:

AFP is a 70 kDa glycoprotein homologous to albumin.

### Forms in Serum:

AFP exhibits microheterogeneity probably due to varying levels of glycosylation. AFP produced by malignancies appears to be more highly fucosylated than that formed by normal tissues.

### Physiological Function:

Appears to perform some of the functions of albumin in the foetal circulation.

### Malignancies With Elevated Levels:

Mainly confined to 3 malignancies, i.e.

- (a) Non-seminomatous germ cell tumours (NSGCT) of testis, ovary and other sites.
- (b) Hepatocellular carcinoma.
- (c) Hepatoblastoma (in children, extremely rare in adults).

AFP may be occasionally elevated in patients with other types of advanced adenocarcinoma. Proportion of patients with hepatocellular cancers having elevated levels is approx. 70 - 90% and proportion of patients with testicular germ cell tumours is approx. 40 - 60%. Please note however, that AFP levels are rarely elevated in patients with stage 1 testicular cancer.

### Benign Conditions Which May Have Elevated Levels:

Hepatitis, cirrhosis, biliary tract obstruction, alcoholic liver disease, ataxia telangiectasia and hereditary tyrosinaemia.

### Physiological Conditions With Elevated Levels:

Pregnancy and the first year of life. Infants have extremely high levels which fall to adult values between 6 months and 1 year of age. A slower than normal rate of fall may indicate the presence of a tumour.

### Main Clinical Applications:

- (a) In combination with HCG, for monitoring patients with NSGCT.
- (b) Independent prognostic marker for NSGCT (e.g. of the testis).
- (c) Diagnostic aid for hepatocellular carcinoma and hepatoblastoma.
- (d) Screening for hepatocellular carcinoma in high risk populations (e.g. China).
- (e) Screening for hepatocellular carcinoma in high-risk disease groups (e.g. haemochromatosis, hepatitis C).

### Malignancies in which Marker Should Not be Used:

AFP should not be used for tumours other than hepatocellular carcinoma, hepatoblastoma and NSGCT. In particular, AFP should not be used for metastasis to liver.

**Type of Sample for Assay:** Usually serum.

**Reference Range:** 0 - 10 KU/L or 0-12 µg/L

**T<sub>1/2</sub> in Serum:** Approx. 5 days.

### Comment on Assay:

Existing immunoassays appear to detect total AFP and do not discriminate between different glycosylated forms. Fucosylated forms can be identified by electrophoresis, isoelectric focusing and affinity chromatography.

### References:

1. Bosl GJ, Motzer RJ. Testicular germ cell cancer. N. Eng. Med. 1997; 337: 242.
2. International Germ Cell Cancer Collaborative Group. International germ cell consensus classification: a prognostic factor-based staging system for metastatic germ cell cancers. J Clin Oncol 1997;15:594-603.

## CA 125

### Structure:

CA 125 refers to the antigen originally detected by the OC-125 antibody. The protein detected by this antibody is Muc16.

### Forms in Serum:

The major forms in serum have molecular weights of 200 kDa to 400 kDa.

### Physiological Function:

None established.

### Malignancies with Elevated Levels:

- (a) Epithelial ovarian cancer; 80 - 85% of all cases; but only half of early (stage 1) cancer.
- (b) May be elevated in any adenocarcinoma with advanced disease.

### Benign Conditions Which May Have Elevated Levels:

Endometriosis, acute pancreatitis, cirrhosis, peritonitis, inflammatory pelvic disease. The presence of ascites (of non-malignant origin) can also give rise to elevated serum levels of CA 125. Peritoneal inflammation can also elevate CA 125 levels.

### Physiological Conditions with Elevated Levels:

Menstruation and pregnancy may be associated with moderately elevated serum CA 125 (usually not more than 100 kU/L)

### Main Clinical Applications:

- (a) While CA125 should not be used in screening asymptomatic women for sporadic ovarian cancer, its measurement in postmenopausal patients with pelvic masses may help differentiate malignant from benign lesions.
- (b) The rate of decline during initial therapy is a strong and independent prognostic indicator in ovarian carcinoma.
- (c) Monitoring treatment with chemotherapy.
- (d) Surveillance following initial treatment. This clinical value of this however, is unclear.

### Other Potential Uses:

Use of CA125 in screening for ovarian cancer is not recommended, except as part of prospective trials using CA125 in combination with ultrasound.

### Malignancies in which Marker Should Not be Used:

Non-ovarian cancers.

### Type of Sample for Assay:

Serum is recommended. CA 125 may be assayed on other fluid samples (e.g. ascitic fluid) but this cannot be recommended (outside of research projects), on analytical and interpretational grounds.

### Reference Range:

0 - 35 kU/L (most frequently used range) Please note however, that levels may be higher in premenopausal than postmenopausal women.

### T<sub>1/2</sub> in Serum:

Approx. 5-7 days.

### Comment on Assay:

Other assays such as ACS:OV appear to detect a similar antigen to CA 125.

### References:

1. Rustin GJS. The clinical value of tumour markers in the management of ovarian cancer. Ann. Clin. Biochem. 1996; 33: 284-9.
2. Duffy MJ, Bonfrer JM, Kulpa J, et al. CA 125 in ovarian cancer: European Group on Tumor Markers (EGTM) guidelines for clinical use. Int J Gynecol Oncol 2005; in press

**CA 15-3****Structure:**

CA 15-3 is a transmembrane glycoprotein encoded by the MUC1 gene. It is defined by reactivity with 2 monoclonal antibodies, i.e., DF3 and 115D8 in a sandwich immunoassay.

**Physiological Function:** May be involved in cell adhesion.

**Malignancies with Elevated Levels:**

Breast and other adenocarcinomas, especially with distant metastasis. Rarely elevated in patients with local breast cancer.

**Benign Diseases with Elevated Levels:**

Benign liver disease, possibly benign breast disease.

**Main Clinical Applications:**

- (a) For preclinically detecting recurrences in patients with diagnosed breast cancer. The clinical value of this practice is unknown
- (b) For monitoring the treatment of patients with advanced breast cancer, especially in patients with disease that cannot be evaluated using standard criteria.

**Other Potential Uses:** Assessing prognosis in breast cancer.

**Cancers in which Marker should not be used:**

Should not be used in cancers other than breast.

Reference range: 0 – 25 to 0-40 kU/L

**T<sub>1/2</sub> in serum:** Unknown

**Comment About Assay:**

Other assays such as BR 27.29 appear to measure the same antigen as CA 15-3

**References:**

1. Duffy M.J. CA 15-3 and related mucins as markers in breast cancer: a critical review. Ann. Clin. Biochem. 1999;36:579-586.
2. Anonymous. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1996; 14: 2843-2877.
3. Bast RC, Ravdin P, Hayes DF, et al. 2000 Update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 2001;19:1865-1878.

**CA 19-9**

**Type of Sample for Assay:** Serum.

**Reference Range:**

**Structure:**

A mucin which reacts with monoclonal antibody 111 6 NS 19-9.

**Physiological Function:**

May be involved in cell adhesion.

**Malignancies with Elevated Levels:**

Most pancreatic adenocarcinomas, approx. 50% of gastric carcinomas and approx. 30% of colorectal carcinomas.

**Benign Conditions which may have Elevated Levels:**

Acute and chronic pancreatitis, hepatocellular jaundice, cirrhosis, acute cholangitis and cystic fibrosis.

**Main Clinical Applications:**

- a) As a diagnostic aid for pancreatic carcinoma and
- b) Surveillance and monitoring therapy in patients with pancreatic adenocarcinoma.

**Other Potential Uses:**

Diagnostic aid in gastric and cholangio carcinomas. For colorectal cancer, CEA is generally more valuable than CA 19-9.

**Malignancies in which Marker should not be used:**

Non GIT malignancies.

**Type of Sample for Assay:** Serum

**Reference Range:** Very variable, from 0 - 37 U/L to 0 - 100 U/L.

**T<sub>1/2</sub> in Serum:** Approx. 1 day but can vary from less than 1 day to 3 days.

**Comment About Assay:**

Other assays such as the ACS:GI assay appears to detect a similar antigen to CA19-9.

**References:**

Duffy M.J. CA 19-9 as a marker for gastrointestinal cancers. Ann Clin Biochem 1998; 35:364.

## CEA

**Structure:**

A 200 kDa (approx.) glycoprotein.

**Physiological Function:**

Appears to play a role in cell adhesion and inhibition of apoptosis.

**Malignancies with Elevated Levels:**

Can be elevated in almost any advanced adenocarcinoma, i.e., where distant metastases are present. Almost never elevated in early malignancy.

**Benign Diseases Which May Have Elevated Levels:**

Hepatitis, cirrhosis, alcoholic liver disease, obstructive jaundice, ulcerative colitis, Crohn's disease, pancreatitis, bronchitis, emphysema and renal disease. Levels may also be elevated in apparently healthy individuals who smoke.

**Physiological Conditions with Elevated Levels:**

None to our knowledge.

**Main Clinical Applications:**

- a) In surveillance following curative resection of colorectal cancer.
- b) In monitoring therapy in advanced colorectal cancer. This is especially important when disease cannot be evaluated by standard criteria.

**Other Potential Uses:**

May also be useful in other gastrointestinal malignancies and as a "general purpose" marker for adenocarcinomas. Please note however, that CEA is rarely elevated in patients with any type of local cancer.

**Type of Sample for Assay:** Serum.

**Reference Range:** 0 - 3.5 µg/L to 0 - 5.0 µg/L

**T<sub>1/2</sub> in serum:** Approx. 3 days but can vary from 1 to 5 days.

**References:**

1. Duffy MJ. CEA as a marker for colorectal cancer: is it clinically useful. Clin Chem 2001; 47:624-630.
2. Anonymous. Clinical practice guidelines for use of tumor markers in breast and colorectal cancer. J Clin Oncol 1996;14:2843-2877.
3. Bast RC, Ravdin P, Hayes DF, et al. 2000 Update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 2001;19:1865-1878.
4. Duffy MJ, van Dalen A, Haglund C. Clinical utility of biochemical markers in colorectal cancer: European Group on Tumor Markers (EGTM) guidelines. Eur J Cancer 2003; 39:718-727.



## Human Chorionic Gonadotropin (hCG)

### Structure:

HCG is a heterodimer composed of 2 glycosylated sub-units (alpha and beta chains) non-covalently bonded. The alpha chain is almost identical to the alpha chain in TSH, FSH and LH. The beta chain is distinct from the corresponding chains in TSH and FSH but has a high degree of homology with LH over the first 75% of the amino sequence. HCG however, possesses a distinctive 24 amino acid carboxy-terminal extension.

### Forms in Serum:

HCG can exist in multiple forms including the intact 2-chain peptide, free alpha and beta chains, as well as various degradation products (e.g., beta core fragment).

### Physiological Function:

To maintain progesterone production by the corpus luteum during early pregnancy. hCG can be detected as early as one week after conception.

### Malignancies with elevated levels:

- (a) Virtually all patients with gestational trophoblastic disease (GTD) (i.e., complete and partial molar pregnancy, choriocarcinoma and placental site trophoblastic tumours).
- (b) Non-seminomatous germ cell tumours (NSGCT) (e.g., of testis and ovary).
- (c) Seminomatous germ cell tumours of testis (approx. 20%)
- (d) Can be produced by a small number of other malignancies.

### Benign Diseases With Elevated Levels:

Very few, e.g., ectopic pregnancy, pituitary adenoma.

**Physiological Conditions With Elevated Levels:** Pregnancy, after termination of pregnancy.

### Main Clinical Applications:

- (a) For monitoring patients with GTD.
- (b) In conjunction with AFP, for determining prognosis and monitoring patients with NSGCT of testis, ovary and other sites.

### Other Potential Uses:

- (a) Diagnostic aid for trophoblastic disease. Serum HCG levels do not usually differentiate between trophoblastic tumours and normal pregnancy. However, very high levels outside the range for twin pregnancies may lead to suspicion of a trophoblastic tumour. For diagnosing trophoblastic tumours, HCG assays are usually used in combination with ultrasound.
- (b) As a diagnostic aid for NSGCT. Please note however, that while elevated levels are found in 40-60% of patients with metastatic NSGCT of testis, levels are rarely increased in those with stage 1 disease.

### Diseases in Which Marker Should Not be Used:

HCG should not be used for malignancies other than GTD and NSGCT.

**Type of Sample for Assay:** Serum or urine

**Reference Range (serum):** 0 - 5 IU/L.

### T<sub>1/2</sub> in Serum:

Approx. 16 - 24 hours, decline may be biphasic with a second t<sub>1/2</sub> of 12.8 days.

### Comment About Assay:

When used as a tumour marker, assays for HCG should detect all the main forms, especially the intact molecule and beta-subunit.

Some hCG assays may give either false-positive or false-negative results. If false-positive results are suspected, then measure hCG on urine. Note: some methods for hCG cross-react with LH.

**References:**

1. Sturgeon, C. and McAllister, E.J., Analysis of HCG. Clinical applications and assay requirements. *Ann Clin Biochem* 1998;35:460.
2. Stenman U-H et al. Human chorionic gonadotropin in cancer. *Clinical Biochem* 2004;37:549-561

## Prostate Specific Antigen (total PSA)

### Structure:

A 28.4 kDa single chain chymotrypsin-like serine protease containing 237 amino acids and a member of the glandular kallikrein family.

### Forms in Serum:

Various molecular forms because of complex formation with protease inhibitors. Major immunoreactive form is PSA complexed with  $\alpha_1$ -antichymotrypsin (PSA-ACT). Other complexes occur such as PSA linked to  $\alpha_1$ -antitrypsin (trace quantity) and  $\alpha_2$ -macroglobulin (undetectable by current immunoassays). A non complexed free form (fPSA) represents 5 to 40% of the "total" PSA ( fPSA +  $\alpha_1$ -antichymotrypsin complex).

### Physiological Function:

Partially responsible for the liquefaction of semen to promote the release and motility of spermatozoa .

### Malignancy with Elevated Levels:

Present data suggests that prostate cancer is the only malignancy giving rise to elevated PSA levels in serum. However, PSA has been found in cells from various cancer types and different normal tissues. PSA is thus not completely prostate specific.

### Benign Conditions with Elevated Levels:

Benign prostatic hypertrophy (BPH), acute and chronic prostatitis, urinary retention. Transurethral resection of the prostate (TURP), prostate biopsy, prostate massage and ejaculation may give rise to transient elevated levels.

### Physiological Conditions with Elevated Levels:

None described.

### Main Clinical Applications:

- In combination with digital rectal examination PSA can aid the diagnosis of prostate cancer.
- Determining prognosis in patients with prostate cancer.
- Monitoring patients with diagnosed prostate cancer.

### Other Potential Uses:

The value of PSA in screening for prostate cancer is controversial and needs evaluation in randomized prospective trials. Two such trials are in progress with results expected in 2008.

### Malignancies in which Marker Should Not be Used:

Non-prostatic cancers.

**Type of Sample for Assay:** Serum or plasma.

### Reference Range:

0 - 4 ng/mL (most frequently used) but some advocate age-related reference ranges as follows:

<u>Age Range</u>	<u>PSA ng/mL</u>
40 - 49	0 - 2.5
50 - 59	0 - 3.5
60 - 69	0 - 4.5
70 - 79	0 - 6.5

**T<sub>1/2</sub> in Serum:** Approximately 2.5 days after radical prostatectomy. T<sub>1/2</sub> after radiotherapy may be many months.

### Effects of Urological Manipulations on PSA Levels.

DRE: May cause minor increases which are rarely of clinical significance  
 Prostate massage: May cause minor elevations in some patients.  
 Ejaculation: Results conflicting but may increase PSA levels.

TURP:	Increases PSA levels significantly. It is recommended to wait at least 6 weeks before drawing blood for PSA assay.
Needle Biopsy	As with TURP, increases PSA levels significantly. Wait at least 6 weeks before drawing blood for PSA assay.
Ultrasound:	Increases PSA levels in a minority of subjects.
Cystoscopy:	Flexible cystoscopy does not appear to increase PSA levels but rigid cystoscopy may increase levels.

#### **Effect of Drugs on PSA levels.**

Finasteride, a 5- reductase inhibitor used to treat PH, reduces PSA levels by approx. 50%.

#### **Comment about Assay:**

PSA assays should detect the free and complexed forms on an equimolar basis. Furthermore, the assay should be standardised against the First International Standard for PSA.

tPSA is stable for at least 6 hours at room temperature in uncentrifuged clotted blood. Five cycles of freezing and thawing caused no significant change.

#### **References:**

1. Duffy, M.J., PSA as a marker for prostate cancer. A critical review. Ann. Clin. Biochem. 1996; 33; 511.
2. Hernandez J, Thompson IM. Prostate-specific antigen: a review of the validation of the most commonly used cancer biomarker. Cancer 2004;101:894-904.
3. Han M et al. Prostate-specific antigen and screening for prostate cancer. Med Clin N Am 2004;88:245-265.
4. Price Cp, Allard J, Davies G, Dawnay A, Duffy MJ, France M, et al. Ann Clin Biochem 2001;38;188-216.

## PSA (free)

### Form in Serum:

As stated above, PSA exists in serum in both a bound and free form. The free form includes enzymically inactive prePSA, pro PSA, clipped PSA and the enzymatically active form of free PSA. The use of free/total PSA in men with PSA levels can reduce the number of unnecessary biopsies. The lower the %fPSA, the higher the probability of prostate cancer.

### Main Clinical Applications:

To enhance the specificity of total PSA in detecting prostate cancer, especially when total PSA values are between 4 and 10 ng/ml.

### Type of Sample for Assay:

Serum or plasma, assay should be carried out on same sample which had total PSA determined.

### Reference Range:

fPSA results can be used in 2 ways:

- Use of a single cut-off point. In a large prospective multi-centre study with a cut off point of <25% fPSA (ref 1), it was shown that unnecessary needle biopsies could be reduced by 20% while maintaining a 95% cancer detection rate with total PSA levels between 4 and 10 ng/ml.
- Probability of cancer: In the same multi-centre study, the following relationships were found between % fPSA and probability of prostate cancer.

% fPSA	% Cancer Probability
0-10	56
10-15	28
15-20	20
20-25	16
>25	8

### Comment about Assay

When using %fPSA, both the free and total PSA assay should be obtained from the same supplier. fPSA is less stable than total PSA, for medium and long-term storage, freezing at  $-70^{\circ}\text{C}$  is recommended. Assay of complex PSA, i.e. PSA bound to ACT, appears to give similar information to the free/total ratio in men with PSA levels between 2 and 10 ng/ml.

### Effect of Urological Manipulations on fPSA.

All the manipulations which increase total PSA levels also increase the free fraction as well as the % free.

### Effect of Drugs

While finasteride reduces both total and free PSA levels, it does not significantly change the % fPSA level.

### References

1. Chan D.W. et al. Analytical and clinical performance of Hybritech's Tandem-R free PSA assay during a large multi-center clinical trial to determine the clinical utility of percentage of free PSA. Clin Chem 1999; 45:1862.
2. Woodrum D.L. et al. Interpretation of free PSA clinical research studies for the detection of prostate cancer. J.Urol 1998; 159:5.
3. Roddam Duffy MJ, Hamdy FC, Mifford Ward A, Patnick J, Price C, et al. Use of PSA isoforms for the detection of prostate cancer in men with a PSA level of 2-10 ng/ml: systematic review and meta-analysis. Eur J Urol, in press

## Tissue Markers in Breast Cancer: Clinical Uses

### ESTROGEN AND PROGESTERONE RECEPTORS (ER AND PR):

#### Clinical Uses

- a) For predicting response to hormone therapy in patients with either early or advanced breast cancer.
- b) In combination with other factors, ER and PR may be used to determine prognosis.

Recommended Assay: Immunohistochemistry with a validated antibody.

#### References

1. Duffy MJ. Biochemical markers in breast cancer: which ones are clinically useful. Clin Biochem 2001;34:347-352.
2. Duffy MJ. Predictive markers in breast and other cancers. Clin Chem 2005;51:494-503

### HER-2 (c-ErbB-2)

#### Clinical Uses

- a) Mandatory uses: For the identification of patients who may be treated with trastuzumab (Herceptin) in the metastatic setting, and to identify patients that may be eligible for clinical trials of trastuzumab in the adjuvant setting.
- b) In combination with other factors, HER-2 may also be used to determine prognosis.
- c) Insufficient data is currently available to recommend HER-2 for predicting response to endocrine therapy or any type of chemotherapy.

#### Recommended Assay:

1. FISH or immunohistochemistry with validated antibodies and standardized methodology. For equivocal cases (i.e., those with scores of 2+), testing with FISH should be carried out.
2. The extracellular domain of HER-2 can be measured in serum. Serum HER-2 levels may be used for post-operative surveillance and monitoring therapy in patients with breast cancer. Based on evidence presently available, use of serum HER-2 has no advantage over CA 15-3. It may however, be of use if CA 15-3 is not elevated. Preliminary findings suggest that serum HER-2 may be of value in monitoring Herceptin therapy.

#### References

1. Duffy MJ. Biochemical markers in breast cancer: which ones are clinically useful. Clin Biochem 2001;34:347-352.
2. Duffy MJ. Predictive markers in breast and other cancers. Clin Chem 2005;51:494-503

## **UROKINASE PLASMINOGEN ACTIVATOR (uPA) AND PAI-1**

### **Clinical Uses**

Assay of uPA and PAI-1 can be carried out to identify lymph node-negative breast cancer patients that may not need or who are unlikely to benefit from adjuvant chemotherapy. The prognostic value of these factors for lymph node-negative breast cancer patients has been validated using both a randomised trial and a pooled analysis.

Recommended Assay: A validated ELISA

### **References**

1. Duffy MJ. Urokinase plasminogen activator and its inhibitor PAI-1, as prognostic markers in breast cancer: from pilot to level 1 evidence studies. Clin Chem 2002;48:1194-1197.
2. Janicke F, Prechtel A, Thomssen C, Harbeck N, Meisner C, Sweep F, et al. For the German Chemo N<sub>0</sub> Study Group. Randomized adjuvant chemotherapy trial in high-risk node-negative breast cancer patients identified by urokinase-type plasminogen activator and plasminogen activator inhibitor type 1. J Natl Cancer Instit 2001; 93:913-920
3. Look MP, van Putten WLJ, Duffy MJ, et al. Pooled analysis of prognostic impact of tumor biological factors uPA and PAI-1 in 8377 breast cancer patients. J Natl Cancer Instit, 2002; 94:116-128.