References

1 World Health Organisation, Globocan 2002: Cancer Incidence, Mortality and Prevalence Worldwide

2 Practice Guidelines and Recommendations for use of Tumor Markers in the clinic (2002), *AACC Press*

3 Nussbaum, S., Roth, H.J., (2000). Human anti-mouse antibodies: pitfalls in tumor marker measurement and strategies for enhanced assay robustness; including results with Elecsys® CEA. *Anticancer Res. 2000 Nov-Dec; 20*(6D): 5249-52.

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Tumor marker testing

Going straight for the answer

Roche

cobas®

Life needs answers

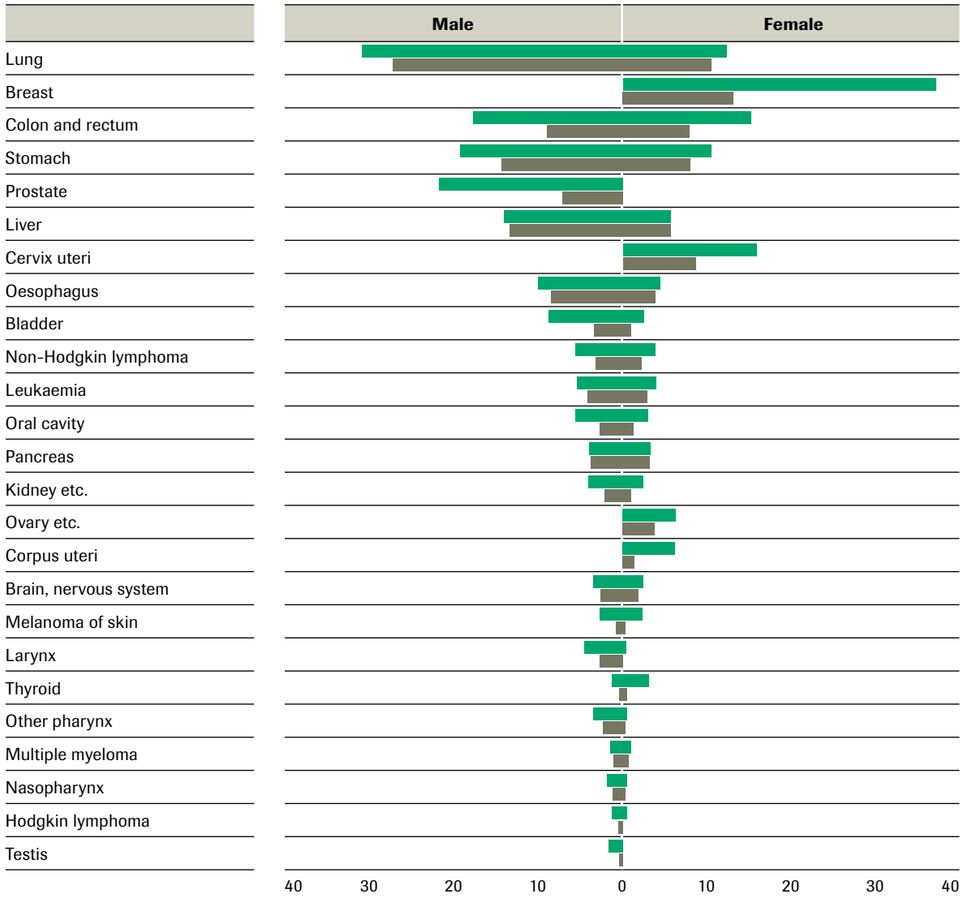
The indication

Every year there are more than 6 million deaths from cancer. 10 million new cases of cancer are diagnosed globally every year.[1] One third can be prevented and another third can be effectively treated, given early detection and treatment.[1]

The World Health Organization estimates that these figures will only worsen in the next 20 years, increasing to 10 million deaths and 15 million new cases annually. Cancer is responsible for 12% of all deaths in the world, and is the second most common cause of death in industrialized countries, second only to cardiovascular disease.[1]

Roche is firmly committed to leading the way in developing innovative cancer tests and drugs and remains dedicated to oncology research with major advances in early diagnosis.

World crude rate per 100,000 (all ages)



Legend:
Incidence
Mortality

Source:
GLOBOCAN 2002, IARC

Definition of tumor markers
Tumor marker recommendations

Definition of the term “specificity”
The specificity is the percentage of healthy individuals or individuals with benign disease in whom the test gives a correctly negative result.

A Tumor marker is a naturally occurring molecule that is measured in serum, plasma, or other body fluids or in tissue extracts or paraffin-embedded tissues (to identify the presence of cancer) to assess patient prognosis or to monitor a patient´s response to therapy with the goal of improving the clinical management of the patient. Tumor markers are found inside cells, both in the cytoplasm and nuclei, and they are associated with cell surface membranes. They also circulate in blood.

Definition of the term “sensitivity”
Sensitivity is the percentage of test results which are correctly positive in the presence of a tumor.

The ideal marker for the purpose of diagnosis would have two characteristics: it would be secreted into the blood in measurable concentration only after the cells that produce it had undergone malignant transformation, and detection of it would permit conclusions as to the site of the tumor from which it arose. Unfortunately markers with close to 100% specificity (undetectable in benign diseases and healthy individuals) and 100% sensitivity (always detectable even in the early stages of a tumor) do not exist.

The use of diagnostic tests in the clinical setting is highly controlled by regulatory bodies, but tumor markers have been particularly identified for special consideration. Regulatory bodies that control the use of tumor markers in the United States include the Food and Drug Administration (FDA), the Center for Medicare and Medicaid Services (CMS) through management of Medicare reimbursement. In Europe they are regulated under the In Vitro Diagnostics directive (IVD directive).

Although tumor markers have not proven useful as screening tests, they provide clinically useful information for the management of these patients, primarily as predictive indicators for selection of certain therapies as markers to monitor the clinical course of the disease.

Markers for breast cancer

Breast cancer remains one of the main causes of death for women in western countries, with a lifetime risk of developing this malignancy of 12.2% and a lifetime risk of death of 3.6%. Multiple factors are associated with an increase in breast cancer risk. These include genetic and familial factors, hormonal factors (early menarche, late menopause and late first pregnancy), diet, benign breast diseases (mainly associated with atypical hyperplasia) and environmental factors.

Presently, a large number of markers exist or breast cancer. These include MUC-1 (e.g. CA 15-3), CEA, oncoproteins, and cytokeratins (i.e. CYFRA 21-1). Of these, CEA and CA 15-3 are the most commonly used. Other members of the MUC-1-gene family such as MCA, CA 549, CA 27-29 and BRMA have a similar sensitivity and specificity to CA 15-3.

Recommendations for use of tumor markers in breast cancer, developed by the National Academy of Clinical Biochemistry (NACB, USA) and the European Group on Tumor Markers (EGTM) [2]

1. Estrogen and progestin receptor status should be used to identify those breast cancer patients most likely to respond to hormone therapy.
2. CA 15-3 or CA 27-29 determinations are useful for the early detection of breast cancer recurrences in patients previously treated for stage II and stage III carcinomas who are clinically free of disease. High CA 15-3 levels in a patient with breast cancer almost certainly indicate the presence of metastatic disease.
3. Decreasing concentrations of circulating CA 15-3 levels are indicative of successful therapeutic response. Persistent or increasing CA 15-3 levels are associated with disease progression and poor response to therapy. In addition, measurement of CEA levels is recommended for the early diagnosis of distant metastases.
4. Since elevated levels of CA 15-3 levels are observed in a number of other malignant and non-malignant diseases, its use is precluded in screening, diagnosis or staging of breast cancer.
5. The use of CA 27-29 in the clinical setting is restricted to the follow up of breast cancer patients with advanced disease.

Roche product base for the treatment of breast cancer:

- *Avastin®*
- *Bondronat®*
- *Herceptin®*
- *Xeloda®*

Markers for ovarian cancer

In the western world, gynecological cancers represent approximately 15% of all cancers in women and are responsible for approximately 10% of all cancer deaths. In terms of frequency, endometrial cancers are the most common, followed by cancers of the ovary and the uterine cervix. However, ovarian cancer has the highest mortality rate.

NACB and EGTM recommendations for use of tumor markers in ovarian cancer [2]

1. CA 125 serum levels should not be used for screening a general asymptomatic population to detect sporadic ovarian cancer.
2. CA 125 levels should be determined every six months with transvaginal sonography (TVS) performed annually as an aid in early detection of ovarian cancer in individuals with either a strong family history of breast or ovarian cancer, a demonstrated mutation in BRCA 1, BRCA 2 or a mismatch repair gene.
3. CA 125 levels should be determined in women presenting with pelvic masses to distinguish benign from malignant disease.
4. CA 125 levels should be determined during primary therapy to predict prognosis.
5. CA 125 levels may be used to document failure of salvage therapy.

Markers for prostate cancer

Prostate cancer is a frequent cause of death in men. Unfortunately, the majority of prostate cancers have spread beyond the gland when first diagnosed using the conventional detection method, digital rectal examination. Prognosis is poor and treatment options are limited to palliative therapy with late stage disease. With no curative therapy for advanced prostate cancer available currently the most promising alternative for improving the prognosis of patients with prostate cancer is enhance early detection.

NACB and EGTM recommendations for use of tumor markers in prostate cancer [2]

1. PSA must not be used alone, but should be evaluated in conjunction with digital rectal examination (DRE).
2. Given the controversy regarding the use of PSA for detecting very small tumors, a low cut-off (2 ng/mL) is not recommended.
3. The use of age specific reference ranges is recommended.
4. The use of percent free PSA is recommended as an aid in distinguishing prostate cancer from benign prostate hyperplasia (BPH) when the total PSA level in serum ranges from 4-10 ng/mL and digital rectal examination is negative. This recommendation is tempered by the need for proper validation of the medical decision limits for each combination of free and total PSA assays within each institution.
5. It is recommended that blood be drawn before any manipulation of the prostate and several weeks after resolution of prostatitis.
6. It is recommended that the following statements and information be appended to each report of results:
   - PSA is to be used in conjunction with digital rectal examination
   - The name of the assay
   - The analytical sensitivity of the assay
   - A valid reference range specifically generated for the assay used.

Markers for lung cancer

The poor prognosis of patients with lung cancer and the lack of effective therapy for recurrent disease limit the application of tumor marker determinations in lung cancer. Tumor markers can still be helpful in monitoring for treatment success or failure. The tumor markers most frequently used are: NSE (Neuron specific enolase), CEA (Carcinoembryonic antigen) and CYFRA 21-1 (Cytokeratin fragments).

EGTM recommendations for use of tumor markers in lung cancer [2]

1. CYFRA 21-1 (for non small cell lung cancers, NSCLC), CEA (for NSCLC), NSE and ProGRP (for small cell lung cancers, SCLC) should not be used for screening purposes because of lack of sensitivity and specificity.
2. CYFRA 21-1, CEA, NSE and ProGRP may be tested in lung cancer patients prior to first therapy. Where no biopsy can be obtained before surgery, measurement of all markers is necessary to identify the leading marker (the one present in highest concentration).
3. Where inoperable lung cancer is suspected but no biopsy is available, raised serum, ProGRP and NSE is suggestive of SCLC.
4. Follow up of asymptomatic patients after primary therapy of lung cancer is controversial. Serial determinations of the leading marker can help to assess the completeness of tumor removal and provide early indication of recurrence.
5. ProGRP and NSE can be measured during systemic treatment of SCLC to reflect response to therapy and to document progressive disease.
6. Careful attention to pre-analytical factors is essential. Specimens for NSE determination should be separated from the clot within 60 minutes of collection and hemolyzed samples should not be assayed. Vigorous mixing of serum samples after thawing should be avoided for cytokeratin measurements. Contamination of samples with skin or saliva must be avoided for SCC measurements. ProGRP must not be used in renal failure patients as this leads to falsely elevated results due to kidney impairment.

Markers for colorectal cancer

Colorectal cancer is a leading cause of cancer death and is second only to lung cancer. The five year survival is about 90 % when colorectal cancer is diagnosed at an early stage, but most cases are detected only after the cancer has spread and cure is not possible. Screening is key to controlling colorectal cancer since early detection and removal of adenomas that give rise to cancer have a major impact on survival. Tumor markers have not been useful in screening because of lack of specificity, but they are a useful adjunct in predicting recurrence and assessing efficacy of treatment.

NACB and EGTM recommendations for use of tumor markers in colorectal cancer [2]

1. CEA testing is not recommended for colorectal cancer screening.
2. CEA may be ordered prior to surgical intervention in patients with colorectal cancer to complement pathologic staging and treatment planning.
3. CEA should not be used in the immediate postoperative period because it may be released during the operation and high levels resulting from the operation may be misinterpreted as recurrent disease.
4. CEA testing may be performed postoperatively if resection of liver metastases would be clinically indicated.
5. CEA may be measured during treatment to monitor response to therapy and to document progressive disease.

Roche product base for the treatment of lung cancer:
- *Avastin®*

Roche product base for the treatment of colorectal cancer:
- *Xeloda®*

Consolidated workstations

Flexible platforms for Roche tumor marker tests
Elecsys® tumor markers are available on several Roche immunochemistry analyzers, meeting the workload requirements of a wide variety of clinical laboratories. These automated analyzers include:

- The Elecsys 2010 and the **cobas e** 411 analyzer for low to mid workloads
- The E170 and the **cobas e** 601 analyzer for mid to high workloads

In addition, the **cobas e** 411 and **cobas e** 601 analyzers have been designed to compliment a new generation of clinical chemistry analyzers for a complete Serum Work Area solution:

- The combination of the **cobas e** 411 and **cobas c** 311 analyzers completes the **cobas**® 4000 analyzer series for low to mid workload laboratories
- The combination of the **cobas e** 601 and **cobas c** 501 analyzers completes the **cobas** 6000 analyzer series for mid to high workload laboratories

To deliver streamlined testing processes and standardized patient results, Roche's line of immunochemistry analyzers shares common components, such as:

- Patented electrochemiluminesence (ECL) detection technology offering:
  - high sensitivity and specificity for unmatched analytical performance
  - broad measuring ranges for fewer test reruns due to out-of-rangeresults
  - 9, 18 and 27 minute measuring time for fast turnaround of results

- Identical reagent applications for standardized patient results across platforms
- Universal **cobas e** reagent packs for simplified logistics, convenient handling and long onboard stability
- Disposable pipette tips and sample cups for testing free from reagent and sample carryover
- Low sample volume requirements for convenience in drawing pediatric samples

The Roche tumor marker test menu
The analytical platforms

Roche tests for tumor markers per indication: Primary markers, secondary markers and tertiary markers
Adapted from: Sensible use of tumor markers, Fateh, Stieber, Hartmann Verlag, 1993

Indication	Tumor marker																		
	AFP	CA 125	CA 15-3/MCA	CA 19-9	CA 72-4	CEA	CYFRA 21-1	Ferritin	HE4	HCG	ß2 Microglobulin	NSE	ProGRP	Free PSA	Total PSA	SCC	S100	TG/<TG>
Biliary ducts				●														
Bladder							●											
Breast			●			●												
C-Cell						○												
Cervix						●										●		
Chorion										●								
Colon				○		●												
ENT (ear, nose, throat)						○										●		
Esophagus						●										●		
Germ cell	●									○								
Liver	●																	
Lung (SCLC)							○					●	●					
Lung (NSCLC)						○	●									●		
Lymphoma											●							
Malignant melanoma																	●	
Ovary		○			○			●										
Pancreas				●		○												
Prostate														○	●			
Stomach				●	●	○												
Thyroid						○												●
Tumor related anemias							●											

Legend:
- ● *Primary markers – gives significant guidance in the decision making process*
- ○ *Secondary markers – adds to decision making in combination with a primary method*
- ● *Tertiary markers – adds to decision making in combination with more than one other diagnostic procedure*

Roche reagent and application portfolio for consolidated tumor marker testing

Test	Cancer indications (primary and secondary)	Roche/ Hitachi systems	COBAS INTEGRA®	cobas e systems	MODULAR® *ANALYTICS* EVO	cobas c systems
AFP	Liver, testicles			●	●	
CA 125	Ovary			●	●	
HE4	Ovary			●	●	
CA 15-3	Breast			●	●	
CA 19-9	Pancreatic, colorectal			●	●	
CA 72-4	Gastric, colorectal			●	●	
CEA	Colorectal, lung			●	●	
CYFRA 21-1	Non small cell lung, bladder			●	●	
Ferritin	Tumor related anemia	●	●	●	●	●
HCG	Chorion			●	●	
ß2 Microglobulin	Multiple myeloma (non-Hodgkin)	●	●		●	●
NSE	Small cell lung			●	●	
ProGRP				●	●	
Free PSA	Prostate			●	●	
Total PSA	Prostate			●	●	
S100	Malignant melanoma			●	●	
SCC*	Lung (SCC), head&neck, cervix			●	●	
Thyroglobulin and antibody against thyroglobulin	Medullary, thyroid, carcinoma			●	●	

AFP = Alpha-fetoprotein, CA = Carcinoembrygenic antigen, CEA = Carcinoembryonic antigen, CYFRA 21-1 = Cytokeratin fragment 19, HCG = Chorionic gonadotropin, HE4 = Human epididymis protein 4, NSE = Neuronspecific enolase, PSA = Prostate-specific antigen, TG = Thyroglobulin, <TG> = Antibody against thyroglobulin, ProGRP = Progastrin-releasing peptide, SCC = Squamous cell cancer antigen

** in development*

Quality requirements of tumor marker assays – internal quality control

Topic	Recommendation for the laboratory [2]	How does Roche help to comply with recommendation
Assessment of reproducibility	Demonstration of intra-assay variability should be < 5% and inter-assay variability should be < 10%.	Fulfilled by our assays and provided in evaluation reports, see package inserts.
Need to establish criteria for assay performance	Selection of appropriate criteria for acceptability of internal quality control.	Help provided with evaluation data, clinical studies and peer reviewed publications, see package inserts.
Specimens closely resembling authentic patient sera	In general it is inadvisable to rely exclusively on QC materials supplied with the kit, and an authentic serum matrix control from an independent source should be included.	Kit controls do not always resemble authentic patient sera, but target values for controls are assigned in standardization using human serum samples to ensure lot to lot consistency, see Roche booklet Id-No: 03537595.
Utilize internal control specimens of concentrations appropriate to the appropriate to the clinical application	Negative and low positive controls should be included for all tumor markers, but there is also a need to cover the broader concentration range and to assess accuracy of dilution steps required for high concentration specimens.	Kit control 1 is adapted to the 95% percentile of healthy individuals. Kit control 2 is set to cover the pathological ranges frequently found in samples, see package inserts.
Assessment of assay interference	Occasionally checking for interference (heterophilic or other antibodies, clotting agents in blood clotting tubes) is desirable.	Tumor marker assays are made robust against interference using blocking proteins, fragmented antibodies or chimeric antibodies (Elecsys® CEA). [3]

Quality requirements – external quality assessment (EQA)

Topic	Recommendation for the laboratory [2]	How does Roche help to comply with recommendation
Utilize external quality assessment specimens of appropriate analyte concentration	Concentrations covering the range of assays are adequate. Analyte free serum is important to check baseline security for some analytes (AFP, HCG).	We are influencing the design of EQA schemes for appropriate design of samples (e.g. the German Society of Clinical Chemistry, DGKC and the National Quality Assessment UK, NEQUAS).
Assessment of assay stability	Can be accomplished by issuing repeat specimens of the same pool and comparing results over time	We make sure reagents are stable and lot to lot consistency is provided via an extensive standardization routine, see booklet Id-No: 3142850.
Demonstrating accuracy and stability of target values	These are usually consensus means as reference methods are not available, for the analytes. Validity of consensus means should be demonstrated by assessment of their stability (repeat distribution of same pool) as well as accuracy, by recovery experiments undertaken with spiked pools.	Where possible we standardize assays utilizing internationally accepted standards (Elecsys® CEA, AFP, free PSA, total PSA, HCG are standardized to international standard preparations). Where such a reference is not available, we provide traceability to a commonly accepted methodology, see package inserts.

Potential causes of erroneous tumor marker results

Topic	Recommendation for the laboratory [2]	How does Roche help to comply with recommendation
High dose hook effect	Tumor marker concentrations range over several orders of magnitude. Protocols permitting identification of high dose hooking are essential to avoid reporting misleadingly low results. Potential hook effects can be checked by assaying specimens at two dilutions.	Tumor marker assays are protected against hook effect by employing sufficient surplus of both tracer and catcher antibody, see package inserts.
Specimen carry over	Potentially a problem whenever very high concentration specimens are assayed. Should occasionally be checked.	Elecsys® 2010 and **MODULAR®** *ANALYTICS* <E> utilize exclusive tips to eliminate carry over.
Interference from heterophilic or human anti-mouse antibodies (HAMA).	Falsely high or low results may be obtained for patient specimens containing anti IgG antibodies capable of reacting with antibodies used in the assay. Presence of HAMA frequently induced in cancer patients who have undergone treatment with mouse monoclonal antibodies for imaging or therapeutic purposes, may also give erroneous results. The simplest check is re-assaying the specimen by a different method.	Tumor marker assays are protected against HAMA utilizing blocking proteins, fragmented catcher or tracer antibodies or chimeric antibodies (Elecsys CEA).[3]

Postanalytical requirements of particular importance to provision of a comprehensive tumor marker service

Topic	Recommendation for the laboratory [2]	How does Roche help to comply with recommendation
Clinical information from the requesting doctor	Encouraging clinicians to provide very brief clinical information (eg. “postoperative”, “post-chemotherapy”) is essential if any interpretation is to be provided, and may help to identify errors.	Provision of clear intended use statements in package inserts helps with the interpretation of results.
Availability of appropriate reference ranges	Usually derived from an appropriately matched healthy population, reference ranges for tumor markers are most relevant for patients before the initial treatment. Subsequently, the patient´s own baseline provides the most important reference point for interpretation of marker results. If this is well established, increases within the reference range are clinically significant.	Detailed information is given in the package inserts.
Knowledge of what constitutes a significant or clinically relevant change	The assessment should include contributions of both biological variation and analytical variation. A confirmed increase or decrease of 25% is frequently considered to be of clinical significance.	High precision assays reduce analytical error and allow to attribute changes in marker concentration to clinical outcome, see package inserts.
Defined protocol when changing methods	It may be helpful to the laboratory to indicate changes of method on tumor marker reports, but it is more helpful if the laboratory highlights whether any change is likely to have affected interpretation of the trend in marker result. Laboratories should always state the manufacturer’s name. Furthermore, when changing the assay manufacturer patient samples should be determined at least once in parallel to ensure proper comparison.	Provision of evaluation data to aid in method change, see various product information documents.
Knowledge about marker half lives	Defined as the time to 50% reduction of circulating tumor marker concentrations following complete removal of tumor tissue. Knowledge about marker half lives is of most relevance to interpretation of serum concentrations of certain markers, like AFP and hCG.	References provided upon request.

The benefits

Shaping tomorrow's laboratories today: workload, turn-around time, automation, integration and cost management are defining terms for the laboratory in the future.

Automated systems and consolidated tumor marker testing allows laboratories worldwide to generate information quickly, accurately, and cost effectively, providing the basis for improved decisions in healthcare.

Clinical requirements
All tumor marker test results within a short turnaround time. Reliable long term follow up through high lot to lot consistency of reagents. Direct feedback on the effectiveness of therapy.

Analytical requirements
Suitable for small to large workloads in central and satellite laboratories. Allowing consolidation of tumor marker testing with the advantages of a fully automated serum work area solution.

Reagents
- Quality proven worldwide
- Comprehensive portfolio
- Commitment to continuous extension of portfolio

Five year survival rate in %

Male		Female	
Lung	13.1%	Lung	17.6%
Stomach	23.1%	Stomach	27.3%
Colorectal	64.5%	Colorectal	64.3%
Bladder	81.2%	Corpus u teri	83.9%
Prostate	98.9%	Breast	88.7%

Source:
National Cancer Institute, SEER Cancer Statistics Review 1975-2005, USA