National Academy of Clinical Biochemistry Guidelines on Quality Requirements for the Use of Tumor Markers

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Abbreviations: EGTM, European Group on Tumour Markers; NACB, National Academy of Clinical Biochemistry; PSA, prostate-specific antigen

INTRODUCTION

Important clinical decisions are increasingly likely to be made on the basis of tumor marker results, whether for diagnosis (e.g. raised calcitonin in multiple endocrine neoplasia families), screening (e.g. raised PSA levels in asymptomatic men leading to prostate biopsy), prediction (e.g. HER2/neu positivity in breast cancer patients) or treatment monitoring (e.g. increasing CA125 levels in treated ovarian cancer patients). The primary aim of these NACB recommendations is to provide evidence-based information both about the optimal use as well as the limitations of these tests - which are often not "good news" tests – in a form that is widely available and that translates readily into clinical practice. Specific recommendations relevant to particular malignancies are presented in subsequent sections. Here, further developing earlier recommendations of the NACB and EGTM (1), we present quality requirements relevant to all tumor marker measurements under the following broad headings

- Pre-analytical requirements - choice of tumor marker, specimen type, specimen timing, sample handling.
- Analytical requirements – assay standardisation, internal and external quality control, interferences.
- Post-analytical requirements – reference intervals, interpretation and reporting of tumor marker results.

QUALITY REQUIREMENTS FOR TUMOR MARKER USE: NACB RECOMMENDATIONS

Table 1 presents a broad summary of the current recommendations of the NACB Sub-Committees, each of which have considered the use of tumor markers in a particular malignancy. The individual recommendations are further discussed [see web-linked sections], where possible ascribing levels of evidence using the tumor marker utility grading system (TMUGS) described by Hayes et al (2) [*See also Section 1*]. In this section we present broad recommendations relevant to optimal use of tumor markers (Tables 2-5), with particular reference to the markers listed in Table 1. The nature of some of the recommendations in this section precludes assigning them formal levels of evidence, as they essentially represent a common sense approach to the use of tumor marker tests, as previously described (3,4). However where possible literature evidence in support of the recommendations is cited.

Pre-analytical quality requirements

Reporting of erroneous tumor marker results is more likely to cause undue alarm to patients than is the case for many other laboratory tests. The laboratory must therefore exercise extra vigilance in ensuring that correct results are reported. Errors in the pre-analytical phase reportedly occur ten times as often as in the analytical phase [41% cf 4% in one study (23)]. As for other

analytes, the majority of pre-analytical errors for tumor markers will be simple specimen handling errors – e.g. inappropriate sampling handling, hemolyzed specimens, insufficient specimens, and incorrect specimens – and their occurrence should be minimized by adherence to good laboratory practice and assessment in an effective audit cycle. As outlined in Tables 2 and 4, there are a number of additional circumstances in which misleading results may be obtained, particularly for PSA and CA125. Implementing the NACB recommendations presented in Table 2 – in particular by discouraging inappropriate test requesting, ensuring appropriate specimen timing and requesting confirmatory specimens when required – should reduce the risk of causing patients unwarranted distress and the likelihood of unnecessary clinical investigations. With the advent of the electronic health record, every effort should also be made to link the ordering process with pre-analytical precautions available through a database of pre-analytical interferences.

Analytical quality requirements

The almost universal use of automated immunoassay analysers for many commonly requested tests means that responsibility for analytical quality now rests largely with the diagnostic industry, which must meet quality requirements defined by national or international regulatory authorities [e.g. US Food and Drug Administration (FDA) regulations, European Commission *In Vitro* Diagnostics Directive (IVDD)]. It is nevertheless crucial for satisfactory measurement of any analyte that laboratories independently monitor their own performance carefully, both to ensure that analyzers are being used appropriately and to confirm that individual methods are performing according to specification. This is best achieved by implementation of rigorous Internal Quality Control (IQC) and participation in well-designed Proficiency Testing [External Quality Assessment (EQA)] programs (1).

NACB recommendations for both IQC and PT are presented in Table 3. Most of these are common to all analytes, but several have particular relevance to tumor markers. Specimens for both IQC and PT should always resemble clinical sera as closely as feasible. Where clinical decision points are commonly employed, it is important to ensure stable and consistent performance, and inclusion of IQC specimens at concentrations close to such decision points is highly desirable. This is especially critical when screening asymptomatic individuals, e.g. for prostate cancer using PSA (24), or where chemotherapy may be instituted on the basis of a rising tumor marker level in the absence of other scan evidence, e.g. when monitoring testicular cancer patients with AFP or hCG (3). Similarly, by issuing specimens of the same low concentration pool repeatedly, PT schemes can provide valuable complementary information about the stability of results over time (13). Since cancer patients are often monitored using tumor markers over months or years, similar assessment of long-term assay stability is also desirable at other analyte concentrations.

Long-term monitoring presents major challenges as patients may change hospital and laboratories may change the tumor marker methods during the relevant time period. While ideally results obtained in different methods would be fully interchangeable, data from PT schemes confirm that this is not the case, with between-method coefficients of variation in excess of 20% still observed for some tumor markers (12). Major causes of observed between-method variation for these complex analytes include poor calibration, differences in the specificity of antibodies used, and differences in method design (25).

It should be possible to achieve reasonably standardized and accurate calibration, but only for those analytes for which a recognized International Standard (IS) or Reference Reagent (IRR) is both available (Table 6) and universally adopted by diagnostic manufacturers for primary calibration of their methods. Unfortunately, as yet there are no IS for any of the important CA series of tumor markers, a major gap that should be addressed urgently. Where relevant IS or IRR are available, recovery experiments undertaken by PT schemes (Table 3) provide (together with linearity and stability studies) the independent validation of consensus target values that is essential in a well-designed PT scheme. Conveniently, since PT schemes should be working towards improving between-method agreement, the same experiments also permit assessment of the correctness of calibration of individual methods and identification of those methods requiring improvement (e.g. methods over- or under-recovering the relevant IS by more than 10%). Long-term PT scheme data can also confirm the effect of successful introduction of a new IS. Data from the UK National External Quality Assessment Scheme (UK NEQAS) for PSA, for example, confirm that mean geometric coefficients of variation (which reflect scatter) decreased from 21.9% in 1995, before the 1st IRR was introduced, to 9.5% in 2004 (26).

The recently established IRR for isoforms of hCG (21) and PSA (22) provide additional tools for elucidating method-related differences associated with the second major cause of method-related differences, namely antibody specificity. Carefully designed experiments with the IRR for PSA and free PSA have allowed assessment of the calibration and equimolarity of assays for PSA, which are particularly critical in the context of prostate cancer screening. Similarly, experiments currently in progress with six recently established IRR for hCG isoforms should provide valuable information about what currently available methods for hCG really “measure” (21), an issue of major importance for oncology applications where recognition of a broad spectrum of hCG-related molecules is recommended (27). Complementary epitope-mapping projects such as those carried out under the auspices of the International Society for Oncodevelopmental Biology and Medicine (ISOBM) may enable broad recommendations to be made regarding the most clinically appropriate antibody specificities for some tumor markers, with some progress towards this objective already having been made for hCG (27).

The results of such studies may lead to better understanding of optimal method design for the complex tumor markers, thereby addressing the third major cause of method-related

differences. Differences in method design are likely to contribute both to the numerical differences in results observed, and to differences in method robustness to clinically relevant interferences, the most important of which are described in Table 4. Maintaining vigilant awareness of the potential for such interferences is very important. Ultimately, the most effective way of minimizing the risk of such interference leading to serious clinical errors is to promote regular dialogue between laboratory and clinical staff, thereby encouraging early discussion and investigation of any results that are not in accord with the clinical picture (3).

Post-analytical quality requirements

Provision of helpful reports following the NACB recommendations in Table 5 encourages good communication between laboratory and clinic, which is highly desirable to achieve best use of tumor marker tests.

CONCLUSION

Optimal use of tumor markers requires care and attention to detail in the pre-analytical, analytical and post-analytical phases of analysis, and should be achievable in laboratories fulfilling the quality requirements described here.

Table 1. Summary of current NACB recommendations for the use of tumor markers in specific malignancies. For explanatory details please see relevant sections of this document

	Screening / early detection	Diagnosis / case-finding	Staging / prognosis	Detecting recurrence	Monitoring therapy
Testicular tumors [Section 3A]	No tumor markers recommended	AFP, hCG, LDH	AFP, hCG, LDH	AFP, hCG, LDH	AFP, hCG, LDH
Prostate cancer [Section 3B]	PSA, cPSA, %fPSA [With DRE]	PSA, cPSA, %fPSA [With DRE]	PSA, cPSA [With DRE & biopsy Gleason Grade]	PSA, cPSA	PSA, cPSA
Colorectal cancer [Section 3C]	FOB [In subjects >50 years old; Genetic testing in high risk subjects]	No tumor markers recommended	CEA	CEA	CEA
Liver cancer [Section 3D]	AFP [In high risk subjects]	AFP	AFP	AFP	AFP
Ovarian cancer [Section 3E]	CA125 [Only in combination with TVUS for early detection in hereditary syndromes]	CA125 [Post-menopausal women only]	CA125	CA125	CA125
Breast cancer [Section 3F]	No tumor markers recommended	No tumor markers recommended	ER, PR, HER-2, uPA, PAI-1	No tumor markers recommended	CA 15-3, CEA [Monitoring advanced disease]
Gastric cancer [Section 3G]	No tumor markers recommended	No tumor markers recommended	No tumor markers recommended	No tumor markers recommended	No tumor markers recommended
Bladder cancer [Section 3H]	No tumor markers recommended	No tumor markers recommended	No tumor markers recommended	No tumor markers recommended	No tumor markers recommended
Pancreatic cancer [Section 3I]	No tumor markers recommended	CA19-9 [If used, **only** with CT or EUS and in an appropriate clinical context]	CA19-9	No tumor markers recommended	CA19-9 [During palliative therapy with imaging tests or after potentially curative surgery]
Cervical cancer [Section 3J]	No tumor markers recommended	SCC [Possibly in squamous cell cervical carcinoma]	SCC [Possibly in squamous cell cervical carcinoma]	SCC [Possibly in squamous cell cervical carcinoma]	SCC [Possibly in squamous cell cervical carcinoma]
Monoclonal gammopathies [Section 3K]	See manuscript [Table 2] for specific recommendations regarding the use of serum protein electrophoresis; serum immunofixation; free light chains, viscosity, β_2 microglobulin, urine protein electrophoresis, urine immunofixation and urine light chains				
Melanoma [Section 3L]	No tumor markers recommended	LDH; TA90-IC	MIA; S100; TA90-IC	MIA; TA90-IC	TA90-IC
Parathyroid gland tumors [Section 3M]	See manuscript [Table 2] for specific recommendations regarding the use of calcium and parathyroid hormone (PTH) in the management of patients with parathyroid gland tumors				
Neuroendocrine tumors [Section 3N]	See manuscript [Table 2] for specific recommendations regarding the use of tumor markers in the management of different neuroendocrine tumors				
Thyroid cancer [Section 3O]	No tumor markers recommended	No tumor markers recommended	No tumor markers recommended	Thyroglobulin; thyroglobulin antibodies	Thyroglobulin; thyroglobulin antibodies
Lung cancer [Section 3P]	No tumor markers recommended	See manuscript [Tables 2 and 3] for specific recommendations regarding the appropriate applications for tumor markers in small cell and non-small cell lung cancer if and when these tests are required			

CT, computed tomography; DRE, digital rectal examination; EUS, examination under ultrasound; FOB, fecal occult blood; LDH, lactate dehydrogenase; MIA, melanoma inhibiting activity

Table 2. NACB recommendations: Quality requirements in the pre-analytical phase

Requirements	Recommendations	Comments / specific examples	References
Analyte-related			
Type of specimen	Requirements should be checked in the product information supplied with the kit. It is the laboratory's responsibility to provide clear advice about the appropriate tube type for each test, thereby ensuring that manufacturers' instructions are always followed.	Serum or plasma are usually (but not always) equally appropriate. Gel tubes may not be suitable for some assays. Anti-coagulating agents such as ethylene- diamine tetraacetic acid (EDTA) may interfere in some detection methods.	3
	Standardised conditions of specimen collection and fixation are crucial for immunohistochemical analyses.	Immunohistochemical studies with tumor markers that do not define the type of specimen and fixative used definitely prejudice the value of the results.	4
Specimen stability	Tumor markers are generally stable, but serum or plasma should be separated from the clot and stored at 4°C (short-term) or -30°C (long-term) as soon as possible. For longer term storage specimens should be stored at -70°C.	The stability of total and free PSA under different storage conditions is especially critical in the context of a screening programme. [See (7) and **[Section 3B]**].	5
	Heat treatment (e.g. to deplete serum complement components or to inactivate HIV) should be avoided, particularly for hCG and PSA. At high ambient temperature the potential influence of transit time on analyte results should be considered.	HCG may dissociate at elevated temperature to form its free α- and β-subunits.	3,5
Patient-related			
Test selection	Ordering of tumor marker tests should be according to locally agreed protocols, based on established national and international guidelines.	NACB recommendations for use of tumor markers in routine clinical practice are summarized in **Table 1**, while those of other international groups have previously been reviewed elsewhere (8). Abbreviated versions of these recommendations, tailored for local practice, e.g. as by the Association of Clinical Biochemists in Ireland (9) or in user-friendly laboratory handbooks, are likely to be most effective.	6,7
	Although in certain circumstances tumor markers may aid in diagnosis, speculative measurement of panels of tumor markers ("fishing") should be discouraged.	PSA should never be measured routinely in females. CA125 should never be measured routinely in males. CA15.3 should only be measured routinely in males with an established diagnosis of breast cancer.	
	A pre-treatment specimen is always desirable.	Interpretation of subsequent results is aided by knowledge of the pre-treatment "baseline" level.	1
Specimen timing	No strong evidence of diurnal variation for most markers, so specimens can be taken at any time of day.		3
	Blood for PSA should be taken before any clinical manipulation of the prostate. Any measurements taken too soon should be repeated.	Prostatic biopsy, transurethral resection of the prostate, or traumatic catheterization may markedly elevate serum PSA and/or free PSA. [See (7) and **[Section 3B]** for details of recommended delay in sampling after these events.]	3,5
	Blood for CA125 should not be taken during menstruation, which may increase the serum concentration two to three-fold. A confirmatory specimen avoiding sampling during menses should be requested.		8

Clinical conditions	CA125 may be mildly elevated in endometriosis and the first two trimesters of pregnancy, and markedly raised in any patient with benign ascites. Careful interpretation of results for patients with these conditions is essential, and their implications should be noted on the clinical report.	Awareness of these *caveats* is essential for proper interpretation.	1,9
	Urinary tract infections and prostatitis may increase PSA markedly, and confirmatory specimens should be taken following successful antibiotic treatment.	Serum PSA usually falls relatively rapidly but results may take more than a month (up to 9 months in one study) to return to within normal limits.	10
	Renal failure is most likely to cause inappropriately elevated results for CEA and cytokeratins. For patients in this category this should be noted on the clinical report.	Awareness of these *caveats* is essential for proper interpretation.	3
	Cholestasis may markedly increase CA19.9 concentrations. For patients in this category this should be noted on the clinical report.	Awareness of this *caveat* is essential for proper interpretation.	3
Medication / other treatment	5α-reductase inhibitors [Finasteride (Proscar®; Propecia®), Dutasteride (Avodart®)] cause a median decrease in PSA concentration of ~50%. For patients in this category this should be noted on the clinical report.	Awareness of this *caveat* is essential for proper interpretation.	3
	Transient increases in tumor marker concentrations may occur following chemotherapy.	Awareness of this *caveat* is essential for proper interpretation.	3
	Cannabis may transiently increase hCG.	Awareness of this *caveat* is essential for proper interpretation.	11
	Smoking may slightly increase apparent CEA levels in some immunoassays.	Awareness of this *caveat* is essential for proper interpretation.	3
Specimen contamination	Salivary contamination can markedly increase apparent concentrations of CEA, CA19.9 and TPS. If contamination is suspected a repeat specimen should be requested.	Awareness of this *caveat* is essential for proper interpretation.	3

Table 3. NACB recommendations: Quality requirements for Assay Validation, Internal Quality Control and Proficiency Testing

Requirements	Recommendations	Comments / specific examples	References
Assay validation			
Well-characterized methods	Prior to their introduction in routine clinical practice, both immunoassay and immunohistochemical methods must be validated by defined and well-characterized protocol that meet regulatory guidelines (e.g. FDA approval in the United States, CE marking in Europe).	It is critically important that methods are properly validated prior to their introduction to avoid misleading reports both in routine clinical practice and in the scientific literature. Failure to have done so accounts for some of the past issues with diagnostic tests, particularly for immunohistochemistry and fluorescence *in situ* hybridization (FISH) testing.	4
	Methods for immunohistochemistry should be particularly carefully described if appropriate high quality reference materials are not available.	For example, tissue arrays with variable tumor marker amounts can be used.	4
Internal Quality Control			
Assessment of reproducibility	Intra-assay variability <5%; inter-assay variability <10%.	Manual and/or research assays may be less precise but PT data suggests these targets should be readily achievable for most analytes.	1,12
Established objective criteria for assay acceptance	Limits for assay acceptance should be pre-defined and preferably based on logical criteria such as those of Westgard.	IQC data should be recorded, inspected and acted upon (if necessary) prior to release of any clinical results for the run.	1,13
Appropriate number of IQC specimens.	The number of IQC specimens included per run should allow identification of an unacceptable run with a given probability acceptable for the clinical application.		1,13
Specimens closely resembling authentic patient sera	At least one authentic serum matrix control from an independent source should be included in addition to any QC materials provided by the method manufacturer.	Kit controls may provide an overly optimistic impression of performance, particularly if they are prepared by adding standard to an artificial matrix.	1,14
IQC specimens of concentrations appropriate to the clinical application.	Negative and low positive controls should be included for all tumor markers. The broad concentration range should also be covered and IQC specimens should ideally include a high control to assess the accuracy of dilution.	Where clinical decision points are commonly employed (e.g. PSA, 4 μg/L; AFP, 5-8 kU/L; hCG, 5 U/L), IQC specimens of these concentrations should be included.	1
Proficiency Testing			
PT specimens of appropriate analyte concentration	Concentrations should assess performance over the working range.	Distribution of occasional specimens of high concentration to check linearity on dilution and of specimens containing analyte-free serum to check baseline security for certain analytes (e.g. AFP, hCG) is desirable.	1
PT specimens closely resembling authentic patient sera	PT specimens should ideally be prepared from authentic patient sera, which for tumor markers may require dilution of high concentration patient sera into a normal serum base pool.	PT specimens prepared by spiking purified analyte into serum base pools provide an overly optimistic impression of between-method performance (e.g. for CEA, mean CVs of 14% cf 20% for pools containing patient sera)	1,14
PT specimens that are stable in transit	Evidence of the stability of PT specimens in transit should be available.	Stability in hot climates is particularly relevant for hCG and free PSA, but reliable data should be available for all tumor	1,12

		markers.	
Accurate and stable target values	The validity of the target values (usually consensus means) should be demonstrated by assessing their accuracy, stability and linearity on dilution.	Accuracy should be assessed by recovery experiments with the relevant International Standard [see Table 4], stability by repeat distribution of the same pool and linearity by issue of different dilutions of the same sera in the same serum base pool. Issue of PT specimens containing the IS can also for some analytes elucidate the extent to which different methods recognise different isoforms (e.g. hCG, PSA).	13
Assessment of assay interferences	Occasional specimens should ideally be issued to check for interference (e.g. from heterophilic and other antibodies, high dose hooking, etc).	The volume of sera required may preclude undertaking this for all participants, but by distributing such specimens to eg 5 users of a number of different methods valuable information about method robustness can be obtained and subsequently provided to all participants.	1
Evaluation of interpretation as well as technical results is required for PT of immunohistochemical tests	The interpretation of the pathologist as well as the technical aspects of the test must be evaluated.	Since immunohistochemistry reports routine include an interpretive comment, the accuracy of these should be rigorously and independently assessed.	4
Interpretative exercises and surveys	Occasional surveys are desirable to compare practice in difference laboratories.	PT schemes can make a powerful contribution to national audit by highlighting differences in reference intervals, reporting practice and interpretation of clinical results, particularly when the ethos of the schemes is educational rather than regulatory.	1,12
Provision of relevant educational updates to all participants	Incorporating regular updates to participants on new developments relevant to provision of a tumor marker service is desirable and can be conveniently done in Comments sections accompanying reports.	Surveys of recent literature can provide a helpful monthly addition to PT reports.	1,12

Table 4. NACB recommendations: Quality Requirements for minimizing risk of method-related errors in tumor marker results

Type of interference	Recommendations	Comment	Reference
Cross-reaction of closely related molecules	Manufacturers should provide clear information about the specificity of their methods. Users should be aware of the characteristics of the methods used.	Often helpful (eg when measuring PSA or hCG) but differences in recognition of the cross-reacting isoforms are likely to contribute to numerical differences in results.	3
High dose hook effect	Laboratories should have in place defined protocols for identifying specimens that have "hooked".	Tumor marker concentrations range over several orders of magnitude and may exceed the capacity of the solid-phase. Recognition of extremely high tumor marker concentrations is critically important where the malignancy is potentially fatal but curable, e.g. hCG in choriocarcinoma. .	3
Specimen carry-over	Laboratories should occasionally check the vulnerability of their tumor marker methods to carry-over from a preceding high concentration specimen.	Potentially a problem whenever very high concentration specimens are assayed.	3,15
Interference from heterophilic or human anti-mouse antibodies (HAMA)	Laboratories should be aware of the possibility of interference from heterophilic or human anti-mouse antibodies, particularly when results are not in accord with the clinical picture. Where interference is suspected this should be investigated, e.g. by re-assaying the specimen 1. After treatment with commercially available antibody blocking tubes 2. After addition of further immobilized normal, non-immune serum, Protein A or Protein G 3. After precipitation of immunoglobulins with polyethylene glycol (PEG) 4. In a different method, preferably using a different methodology (eg radioimmunoassay) 5. At several dilutions to assess linearity on dilution	Falsely high or low results may be obtained for patient specimens containing anti-IgG antibodies capable of reacting with antibodies used in the assay. Such antibodies may be of particularly high titre in patients who have undergone treatment with mouse monoclonal antibodies for imaging or therapeutic purposes. Serious clinical errors as a result of failure to recognise such interference have been most frequently reported for hCG. A high degree of suspicion is usually required for identification, which is facilitated by good communication between clinical and laboratory staff.	3,15

Quality Requirements [Section 2]

Table 5. NACB recommendations: Quality requirements in the post-analytical phase

Requirements	Recommendations	Comments / specific examples	References
Factual requirements			
Clinical information from the requesting doctor	Brief clinical information indicating the source of the suspected/diagnosed malignancy and the treatment stage (e.g. post-op) should accompany the specimen.	Such information is essential if any laboratory interpretation is to be made and may help to identify occasional laboratory errors (e.g. mis-sampling on an analyser).	1
Availability of appropriate reference intervals	Reference intervals should be appropriately derived using an appropriate healthy population.	Reference intervals are usually most relevant for cancer patients pre-treatment, after which the patient's own "baseline" provides the most important reference point for interpretation of marker results. If this is well-established, increases even within the reference interval may be significant.	1
	Reference intervals must be included when reporting results for immunohistochemical markers.	Reference intervals may include internal reference materials, e.g. normal ducts in breast tissue, and specify what staining they should have.	4
Interpretation criteria for immunohistochemical tests	Interpretation criteria must be well-documented and must be clearly stated in the clinical report.	For Her-2/neu testing by FISH, for example, it is essential to define clearly what the appropriate ratio of Her-2 signals/CEP 17 signals is for classification as unamplified (2.0 for Vysis probes), borderline amplified, etc.	4
Knowledge of what constitutes a significant change	The percentage increase or decrease that constitutes a significant change should be defined and should take account of both analytical and biological variation. Laboratories should be willing and able to advise on this issue.	A confirmed increase or decrease of ± 25% is frequently considered to be of clinical significance (16) but more work is required in this area, the importance of which has recently been illustrated for PSA (17).	1,16,17
Defined protocol when changing methods	Laboratories should have a defined protocol when changing tumor marker methods.	This may necessitate analyzing the previous specimen by the new method or requesting a further specimen to re-establish the baseline and/or confirm the trend in marker level.	1
Knowledge of tumor marker half-lives	Laboratories should be able to provide calculated tumor marker half-lives for the markers for which these are relevant (e.g. AFP, hCG).	Half-lives are defined as the time to 50% reduction of circulating tumor marker concentration following complete removal of tumor tissue.	1
Objective audit of tumor marker utility	Laboratories should be involved in on-going audit of the clinical utility of the results they provide.	This remains a priority and is being considered by a number of professional organizations.	1
Reporting requirements			
Cumulation of tumor marker results	Laboratories should provide fully cumulated tumor marker results. Graphical representations may also be helpful.	Helpful reports facilitate interpretation and communication between laboratory and clinic. It is helpful if the reports incorporate any brief clinical information available, particularly dates of operation etc.	1
Tumor marker method used	Laboratories should indicate the method used on the report form and highlight whether any change of method is likely to have affected interpretation of the trend in marker result.		1

Recommendations as to the appropriate frequency of tumor marker measurements	Laboratories should be willing to advise on the frequency of monitoring and the need for confirmatory specimens.	An apparent rise in marker concentration should always be confirmed by repeat measurement.	1
Communication between laboratory and clinical staff	Laboratories should always welcome and encourage good communication with clinical users of the service.	Good communication facilitates appropriate use of these (and other) tests.	1

Table 6. International Standards (IS) and Reference Reagents (RR) for major tumor markers

Tumor marker	Code	Year established	Description	Reference
AFP	IS 72/225	1972	Crude cord serum (50%)	18
CA125	-	-	-	-
CA15-3	-	-	-	-
CA19-9	-	-	-	-
CA72-4	-	-	-	-
CEA	IRP 73/601	1973	CEA purified from liver metastases to primary colorectal cancer	19
hCG	IS 75/537	1975	Purified urinary hCG, contaminated with hCGβ and hCGn	20
hCGα	IS 75/569	1975		20
hCGβ	IS 75/551	1975		20
hCG	IRR 99/688	2001	Highly purified urinary hCG, free from nicked forms and free subunits	21
HCGn	IRR 99/642	2001	Highly purified urinary hCG, partially degraded, missing peptide bonds in the hCGβ-40-50 region	21
hCGα	IRR 99/720	2001	Highly purified urinary hCGα, dissociated from hCG	21
hCGβ	IRR 99/650	2001	Highly purified dissociated urinary hCGβ, free from intact dimeric hCG, hCGα and hCGβn	21
hCGβn	IRR 99/692	2001	Partially degraded hCGβ, missing peptide bonds in the hCGβ-40-50 region	21
hCGβcf	IRR 99/708	2001	Residues hCGβn-6-40, joined by disulphide bonds to hCGβn-55-92	21
PSA	IRR 96/670	2000	90:10 ratio of bound : free PSA	22
fPSA	IRR 96/668	2000	Purified free PSA	22

International Standards and Reference Reagents are available from the National Institute for Biological Standards and Control, Potters Bar, Herts, UK [http://www.nibsc.ac.uk/catalog/standards/preps/sub_endo.html]

[hCG, human chorionic gonadotropin; IS, International Standard; hCGß, hCG ß-subunit; IRP, International Reference Preparation; hCGα, hCG β-subunit; hCGn, nicked hCG; hCGßn, nicked hCG ß-subunit; and hCGßcf, hCG ß-core fragment; PSA, prostate specific antigen; fPSA, free prostate specific antigen]

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