

Challenges and Clinical Impact of Immunoassay Standardization

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The “Ideal” Tumor Marker

- **Quantitative level of TM reflects tumor burden with high diagnostic sensitivity (few FNs) and specificity (few FPs).**
- **Measured easily, reliably and cost-effectively using an assay with high analytical sensitivity and specificity.**
- **Test results influence patient care and especially outcome**

Tumor Markers Routinely Used in Monitoring Cancer

<u>Cancer</u>	<u>Tumor Marker</u>
Breast	CA 15-3, BR 27.29
Colorectal	CEA , CA 19-9
Gastric	CA 72.4, CA 19-9, CEA
Lung	NSE, CYFRA 21.1
Prostate	PSA , PAP
Ovarian	CA 125
Thyroid	Calcitonin, Thyroglobulin
Trophoblastic	HCG
Pancreatic	CA 19-9, CEA
Hepatocellular	AFP , CA 19-9
Bone	BAP, Osteocalcin, NTx

STANDARDIZING TUMOR MARKERS

- THE MAJORITY OF TUMOR MARKERS ARE **PROTEINS OR GLYCOPROTEINS**.
- STANDARDIZING TM IS MORE COMPLEX THAN STANDARDIZING A GLUCOSE OR CREATININE METHOD.
- THERE ARE INTERNATIONAL STDS FOR PROTEINS AND GLYCOPROTEIN BUT NOT ALWAYS HELPFUL.
- TUMOR MARKER STANDARDIZATION IS BASED ON **ANTIBODY** RECOGNITION OF A **SPECIFIC** EPITOPE ON A PROTEIN OR GLYCOPROTEIN.

Standardization of Tumor Markers

- Methods of standardization
 - WHO or National Standards
 - Purified materials
 - HARMONIZATION -Calibrators adjusted to match reference standard

WHO Tumor Marker Standards

- Examples

AFP 72/225

Calcitonin 89/620

CEA 73/601

hCG 99/688

hCG, β subunit 99/650

PSA (90:10) 96/670

Free PSA 96/668

<http://www.nibsc.ac.uk/products/directorylist.asp>

WHO or International Standards

- **Advantages**
 - **Consistent** source of primary reference material.
 - Antigen well **defined** and **characterized**.
 - Usually **broad consensus** across manufactures.
 - **Available to most manufactures.**
 - **Consistency** with new generation of product.

WHO or International Standards

- Disadvantages
 - **Matrix effect**- Assays may identify standards prepared in buffers/proteins differently than **human serum**.
 - **Standards may not dilute linearly.**
 - Long term **stability** of primary standards may be an issue.
 - **Epitope variability**- variances due to cleavage, binding proteins, steric hindrance and post synthesis processing
 - **Secondary standards** made against the primaries may use different source of material.
 - **Antigens elaborated by tumors may have a different molecular construct compared to standards.**

Antibody Defined Tumor Markers

- Examples

CA 125

CA 19-9

CA 50

CA 549

CA 15-3

CA 72-4

CA 27.29

- ❖ Standardization depends on (a) biochemical properties of the antigen, (b) epitope specificity of antibody and (c) assay format.
- ❖ Tissue culture mucin core proteins are similar to proteins elaborated by tumors except for tandem repeats. But, GLYCOSYLATION is highly variable between benign and tumor antigens.

Antibody Defined Tumor Markers

- **Advantages**

- Antibody specificity should be the same across manufactures
 - Ideally when the same antibodies are used, assays should detect the same component
 - In practice specificity often varies greatly due to the complexities of the antigens and antibodies
- Same material can be used for primary and secondary standards
- Standardization would be concordant between manufactures

Antibody Defined Tumor Markers

- **Disadvantages**

- **No industry standardization**
- There are rarely true **reference assays** to standardize against!
- Setting and maintaining primary standards is extremely complicated
 - Reference assay comparison (if there is one) is a **snap shot in time**
 - Difficult to prevent assay **drift and shifts.**
 - Epitopes often present on very **different backbones**
- Antigenes can be obtained from very different sources
 - Many **cell lines** express epitopes on different molecular backbones leading to large differences in antibody recognition and apparent concentration
 - Antibodies to **conformational epitopes** may be especially sensitive
 - **Tumors** produce widely varying antigens which may, and do, **differ from native antigens**

SUMMARY OF TUMOR MARKER STANDARDIZATION

TUMOR MARKER	DISCRIPTION	CLINICAL USE	STANDARDIZATION
AFP	GLYCOPROTEIN	HCC, GERM CELL	WHO
B ₂ -MACROGLOBULIN	LC OF MHC CLASS I ANTIGEN	LYMPHOPROLIF. DISEASE; MYELOMA	WHO
CALCITONIN	POLYPEPTIDE, 3.6 KD	MEDULLARY THYROID CARCINOMA	WHO
CA ₁₅₋₃	GLYOPROTEIN; MC _{115D8} & DF ₃	BREAST CA.	Ab
CA ₁₉₋₉	GLYCOLIPID; DEF. MC _{1116NS}	PANCREATIC CA	Ab
CA ₁₂₅	GLYCOPROTEIN; DEF. MC _{OC125} & M ₁₁	OVARIAN CA	Ab
CEA	GLYCOPROTEIN	CA OF GI TRACT	WHO
hCG	DIMERIC GLYCOPROTEIN	GERM CELL TUMORS	WHO
PSA	GLYCOPROTEIN, SERINE PROTEASE	PROSTATIC CA	WHO
THYROGLOBULIN	GLYCOSYLATED IODOPROTEIN	PAP & FOLL THYROID CA	WHO
TPA	CIRC. COMPLEX CYTOKERATIN	CA BREAST, LUNG, GI TRACT	PURIFIED

CAP PROFICIENCY TEST FOR CA125 1/09

VENDOR	TM-01	TM-02	TM-03
Abb, AR	167	57	20
B-C	82	26	9
Roche	125	41	15
Siemens, c'taur	114	37	15
Siemens, Imlte	71	24	8
Tosoh	176	58	18
Ortho, Vitros	113	34	10

CAP PROFICIENCY TEST FOR CA 15-3 1/09

VENDOR	TM-01	TM-02	TM-03
Abb,AR	12	67	33
B-C	8	36	18
Roche	11	56	31
Siemens	12	69	37
Tosoh	NA	NA	NA
Ortho ,Vitros	12	62	31

NYS-DOH PROFICIENCY TEST FOR CA125 1/09
120 LABS PARTICIPATED USING 10 METHODS

Vendor	1	2	3	4	5
Abb, AR	29	66	55	39	48
B-C	20	51	41	28	36
Roche	20	47	39	28	34
Siemens, c'taur	23	56	46	33	40
Siemens, Imlte	18	49	39	27	33
Tosoh	33	85	68	47	60
Ortho,Vitros	21	54	44	31	37

NYS-DOH PROFICIENCY TEST FOR CA15-3 1/09

105 Labs participated using 6 methods

Vendor	1	2	3	4	5
Abb,AR	51	27	40	37	40
B-C	44	22	37	32	25
Roche	36	18	28	25	33
Siemens, c'taur	52	26	42	37	46
Siemens, Imlte	95	46	74	67	47
Ortho,Vitros	45	22	34	31	35

PROFICIENCY TEST ANALYSIS FOR PSA

Compilation of PT for PSA and Documentation of Calibration

	Calibration	API	CAP	AAFP	AAB	ACP-MLE	NYS	WSLH	TOTAL
Abbott: IMx,Axsym, Axsym Plus, Arch	WHO	350	245	27	114	82	21	35	874
Biomerieux: VIDAS	WHO	301		29	45	27		6	408
Roche: COBAS, Elecsys	WHO	128	323		40	17	35	30	573
Siemens: Dimension, ADVIA Centaur,	WHO	717	874	19	220	51	111	96	2088
Siemens: Immulite 3rd Generation	Hybritech	31	12				7	5	55
Beckman Coulter: Access, Unicel	WHO		14				4		18
Beckman Coulter: Access, Unicel	Unspecified	382	306	8	108			51	855
Beckman Coulter: Access, Unicel	Hybritech		408			28	53		489
Ortho Clinical Diagnostics: VITROS	Hybritech	97	191		28		19	11	346
Qualigen Fastpack	Hybritech	245		7		10		4	266
TOSOH: AIA	Hybritech	323	39	20	71	53	13	20	539

TUMOR MARKERS PERFORMED

MSKCC CLINICAL CHEMISTRY SERVICE

<u>Year</u>	<u>CEA</u>	<u>PSA</u>	<u>FPSA</u>	<u>CA125</u>	<u>AFP</u>	<u>CA19-9</u>	<u>HCG</u>	<u>CA15-3</u>
1987	10925	569	---	1246	3313	---	3297	0
1990	11822	5152	---	2449	3411	---	3555	3135
1995	16834	11931	---	3857	3423	---	3439	10353
2000	24668	19432	2095	7654	3835	649	3566	15506
2002	26717	20595	1423	10709	4273	1453	3998	16951
2004	31838	20414	1189	15357	4823	2706	4227	19753
2006	35928	22709	1587	19394	5331	3552	4849	22387
2007	39994	23549	1738	19874	5546	4238	5158	23484
2008	42250	24304	842	19778	5661	5251	5218	24680
2009	44390	24175	563	18845	6510	5935	6118	26260
2010	45957	24845	1121	18529	6768	6789	6430	26940

Two Main Problems with Early PSA Assays

- Non-equimolar assays – free and complexed forms of PSA not measured equally in some assays
- Different reference standards – for the gravimetrically measurable amount of PSA in samples

Development of PSA as a Tumor Marker

First quantified in serum by immunoassay at Roswell Park by Wang, et al



1980

Development of PSA as a Tumor Marker

1st Stanford Conference
on International Standardization
of PSA Immunoassays



1992

A horizontal black arrow pointing to the right, representing a timeline. A diagonal line connects the arrow to a yellow box above it. The year '1992' is written below the arrow at the point of connection.

Development of PSA as a Tumor Marker

Hybritech assay approved for use
in the detection of PCa
(with DRE, men over 50)



1994

Development of PSA as a Tumor Marker

WHO International Reference Preparation
96/670 (total)
96/668 (free)



1999

Development of PSA as a Tumor Marker

Clinical implications of differences in PSA results related to re-standardization are recognized and reported



Development of PSA as a Tumor Marker

First manufacturers
re-standardize their
assays to WHO



1999

Concordance at 4.0 ng/mL cut-off

WHO Calibration	Hybritech Calibration		
	≤ 4.0 ng/mL	> 4.0 ng/mL	Total
≤ 4.0 ng/mL	47	38	85
> 4.0 ng/mL	0	170	170
Total	47	208	255

18% (38/208) of cases may not receive follow-up at a 4.0 ng/mL WHO calibration cutoff

BIOMARKER or TUMOR MARKER DEVELOPMENT

ALL ANSWERS MUST BE **YES!**

■ ANALYTICAL

- Can the Tumor Marker be measured accurately and reproducibly?
- Can the Tumor Marker distinguish normal from cancer?
- Is the Tumor Marker assay standardized?

■ CLINICAL

- Does the Tumor Marker provide additional clinical information not otherwise available for patient management?
- Will the Tumor Marker improve clinical outcome?

CONCLUSIONS

- **Standardization of TM** should be encouraged because it makes inter-laboratory testing **clinically useful, clinical collaboration feasible** and clinicians will more readily accept the data.
- Standardization of many currently used TM may not be practical since labs **object to changes** in cut point and it is difficult to define antigen epitopes. Harmonization of TM assays is a practical solution that would allow clinical comparison of different assays.
- All future development of TM and Biomarkers should be **standardized from the inception of the assay. IVD buy-in is encouraged.**