

ISOBM Lunch symposium Munich, 2010



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Characteristics of Tumor Marker Multi-Constituent Controls

Purpose

- To monitor performance of laboratory testing methods
- Mimic patient sample

Demands

- Analyte levels at clinically relevant concentrations
- Matrix similar to patient sample
- Stability over long periods of time

Fujirebio Diagnostics goals

- Design a Tumor Marker specific control that:
 1. Contains most commonly used tumor marker analytes
 - Contains the novel ovarian cancer marker HE4
 2. Has clinically relevant levels of all analytes
 - Physiological relevant levels of screening marker PSA and its measurable forms
 3. Mimics true patient samples
 4. Long shelf life and lab usage

PRESENTING

Fujirebio Diagnostics Tumor Marker Control

Value Assignment Data

- Value assigned Multi-Constituent Control

- AFP, CEA, CA125, CA15-3, CA19-9, HE4			Roche Modular	Abbott ARCHITECT	Roche Modular	Abbott ARCHITECT
			Level 1	Level 1	Level 2	Level 2
	Unit	Reference range	Mean	Mean	Mean	Mean
- Clinically relevant analyte levels to assist decision points						
AFP	ng/mL	<10.0 ng/mL ⁴	20	21.5	268	282
CA 15-3	U/mL	<30 U/mL ⁴	18	15.5	124	136
CA 19-9	U/mL	<37 U/mL ⁴	17	48.5*	121	535*
CA 125	U/mL	<35 U/mL ⁴	19	25.2	287	365
CEA	ng/mL	Non-Smokers <3.0 ng/mL and Smokers <5.0 ng/mL ⁴	3.89	4.66	47.1	62.1
Ferritin	ng/mL	Male 20-250 ng/mL; Female 10-120 ng/mL ⁴	58.51	57.8	351	380
Free PSA	ng/mL	N/A	0.819	1.03	8.35	10.1
%Free PSA	%	≤25% ⁴	30%	37%	31%	36%
Total PSA	ng/mL	<4.0 ng/mL ⁴	2.74	2.79	26.6	27.9
HE4	pM	≤150 pM ⁵	N/A	83.1	N/A	841

⁴ Wu, Alan. Tietz Clinical Guide To Laboratory Tests. St. Louis: W. B. Saunders Company, 2006. Print.

⁵ Fujirebio Diagnostics AB Gothenburg, Sweden. HE4 EIA Assay Insert, 404-10US, 2008-06, F5908, r1

* Higher values as seen with Abbott ARCHITECT assay.

Multiple forms of PSA in serum

Two forms are detectable by immunoassay

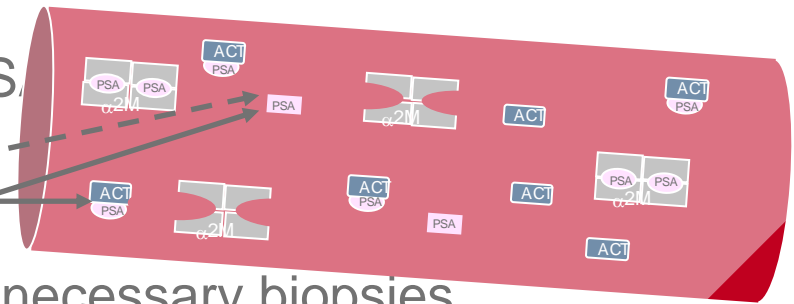
1) Major form, PSA complexed with alpha-1-antichymotrypsin (PSA-AC1) **Free PSA assay**

2) Minor form, uncomplexed or free PSA

- Cut off for % Free PSA at 25%

- $\% \text{Free PSA} = \text{Free PSA} / \text{Total PSA}$

Total PSA assay



- Detects 95% of cancers and avoid 20% of unnecessary biopsies

Healthy screening cohort involving 2323 participants (3)

- % Free PSA median was 26.8%

Conclusion

- Approx. 30% Free PSA is the most appropriate ratio for an assay control to monitor Free PSA and PSA assays

1. Lilja H, Ulmert D, and Vickers AJ. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. Nature Reviews Cancer 2008; 8: 268-278.

2. Catalona WJ, et al., Use of the Percentage of Free Prostate-Specific Antigen to Enhance Differentiation of Prostate Cancer From Benign Prostatic Disease. JAMA 1998; 279: 1542-1547.

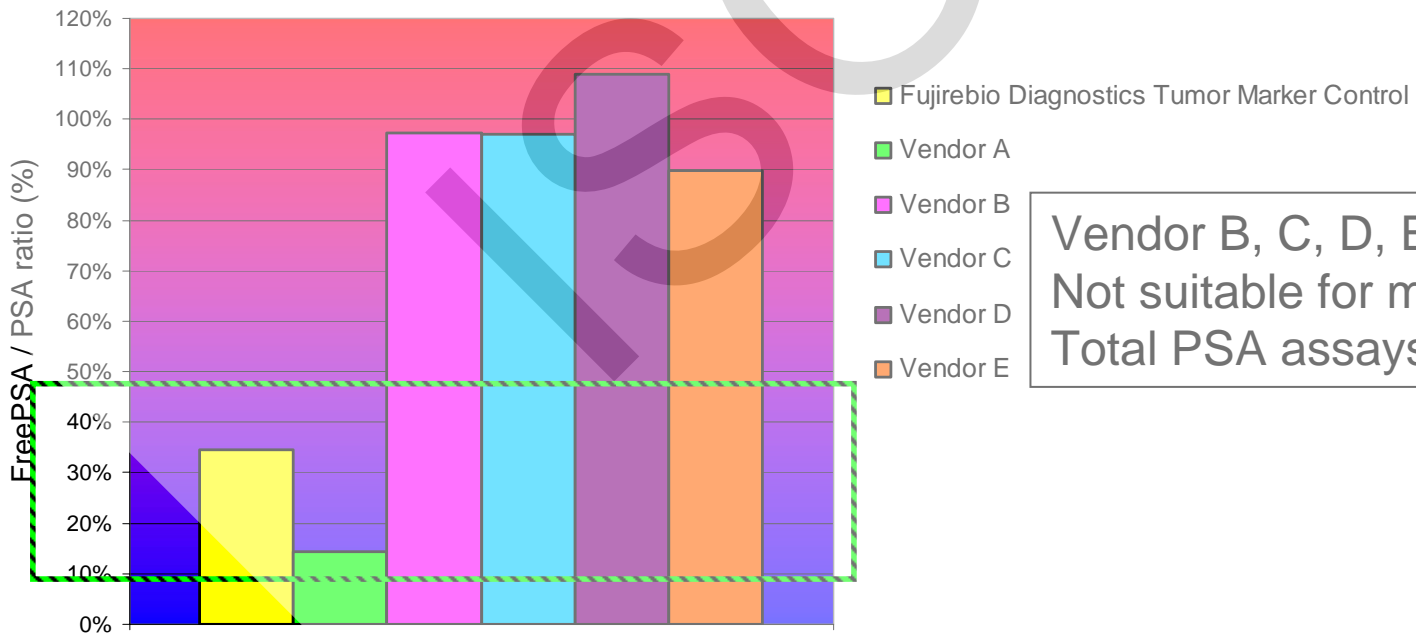
3. Chun FK.-H. Distribution of prostate specific antigen (PSA) and percentage free PSA in a contemporary screening cohort with no evidence of prostate cancer. BJU International 2007; 100: 37-41.

PHYSIOLOGICAL PSA LEVELS

Fujirebio Diagnostics Tumor Marker Control

- Physiological relevant PSA levels
 - Clinically relevant proportions of native PSA-ACT and Free PSA in a human serum based control
 - Suitable for monitoring of Free PSA and equimolar Total PSA assays

Free PSA / Total PSA ratio of Tumor Marker Controls
Abbott Architect values



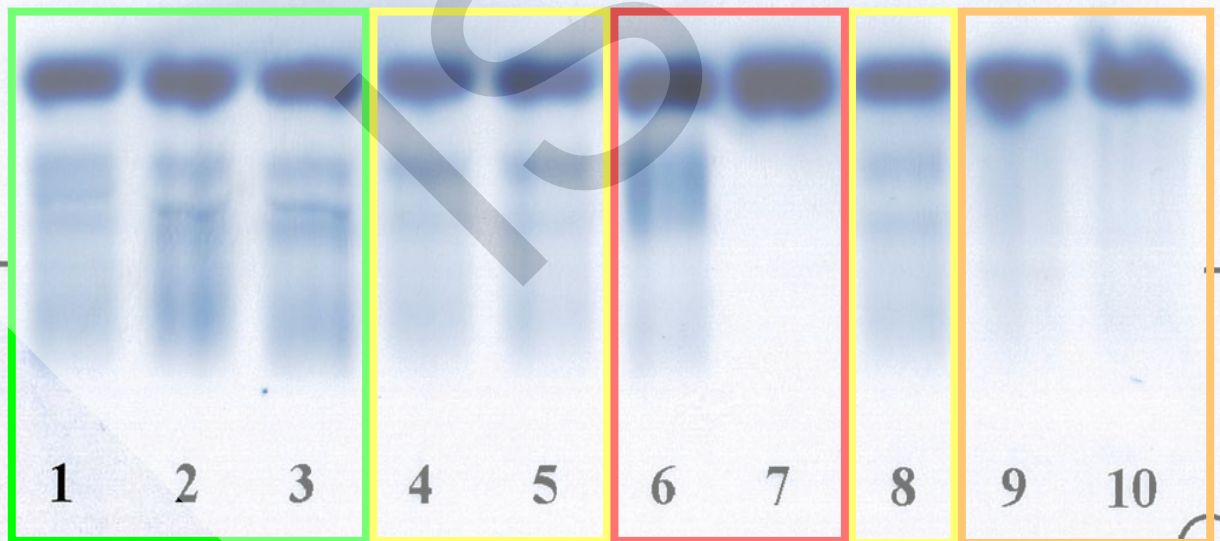
Vendor B, C, D, E not relevant %FreePSA.
Not suitable for monitoring of equimolar
Total PSA assays

CONTROL MATRIX

Fujirebio Diagnostics Tumor Marker Control

Serum Protein Electrophoresis

LANE	CONTROL	SPE PATTERN	MATRIX ACCORDING TO IFU
1-3	Normal Human Serum Control samples	SPE PATTERN AS EXPECTED	N/A
4, 5	Fujirebio Tumor Marker Control (108-20); 2 Lots	SPE PATTERN AS EXPECTED	Human serum, chemicals
6	Vendor A	NON SERUM BASED MATRIX	Human plasma, chemicals
7	Vendor B	NON SERUM BASED MATRIX	Human and animal constituents, chemicals
8	Vendor C	SPE PATTERN AS EXPECTED	Human serum, animal additives, chemicals
9	Vendor D	UNEXPECTED SPE PATTERN	Human serum, chemicals
10	Vendor E	UNEXPECTED SPE PATTERN	Human serum



GENERAL HUMAN SERUM BANDS

MIGRATION	BAND	MAIN PROTEINS
ANODE (+) +++++	Albumin	Albumin
↑	+++ Alpha-1 globulins	Alpha-1 lipoprotein
	++ Alpha-2 globulins	Alpha-2 macroglobulin, Haptoglobin
	+ Beta globulins	Transferrin, Beta lipoprotein, Complement protein 3, Fibrinogen B2
CATHODE (-) -	Gamma globulins	Immunoglobulins

CONTROL STABILITY

Fujirebio Diagnostics Tumor Marker Control

- Lyophilized
 - All analytes stable for >14 weeks at 37°C
- Reconstituted
 - All analytes are stable for 60 days when stored at $\leq -20^{\circ}\text{C}$
 - All analytes are stable for 14 days at 2-8°C
 - Free PSA is stable for 7 days at 2-8°C
- Freeze/thawing
 - All analytes stable for 9 freeze/thaw cycles at $\leq -20^{\circ}\text{C}$ to room temperature

SUMMARY

Fujirebio Diagnostics Tumor Marker Control

1. Contains common Tumor Markers including HE4
 - The only Multi-Constituent Control with both CA125 and HE4
2. Clinically relevant levels of all analytes
 - Bi-level control
 - Level 1: Around Cut-off
 - Level 2: Pathological range
 - % Free PSA of approx 30%
3. Mimics true patient samples
 - 100% Human serum matrix
 - Native antigen origin
4. Long shelf life and lab usage
 - Lyophilized product enables long shelf life
 - Reconstituted storage at $\leq -20^{\circ}\text{C}$ or $2-8^{\circ}\text{C}$ and freeze / thaw option

Kit configuration

Fujirebio Diagnostics Tumor Marker Control

- Kit Configuration
 - Bi-level Control
 - Level 1: 3 x 3 mL
 - Level 2: 3 x 3 mL
 - Lyophilized
 - Shipped at ambient temperature
 - Reconstituted with 3 mL distilled or deionized water
 - Available for IVD use in Europe (CE-marked)

