Early Detection Research Network

Trust, but Verify: EDRN Reference Laboratories

Daniel W. Chan, Ph.D., DABCC, FACB
Principal Investigator
Johns Hopkins University

EDRN Reference Laboratories

Early Detection Research Network

EDRN Reference Laboratories (BRL)

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EDRN Biomarker Reference Laboratories serve as the resource for clinical and laboratory validation of biomarkers, including technological development, standardization of assay methods and refinement.

All the reference laboratories are CLIA certified clinical laboratory.

Geographical Distribution of the EDRN Laboratories and Centers

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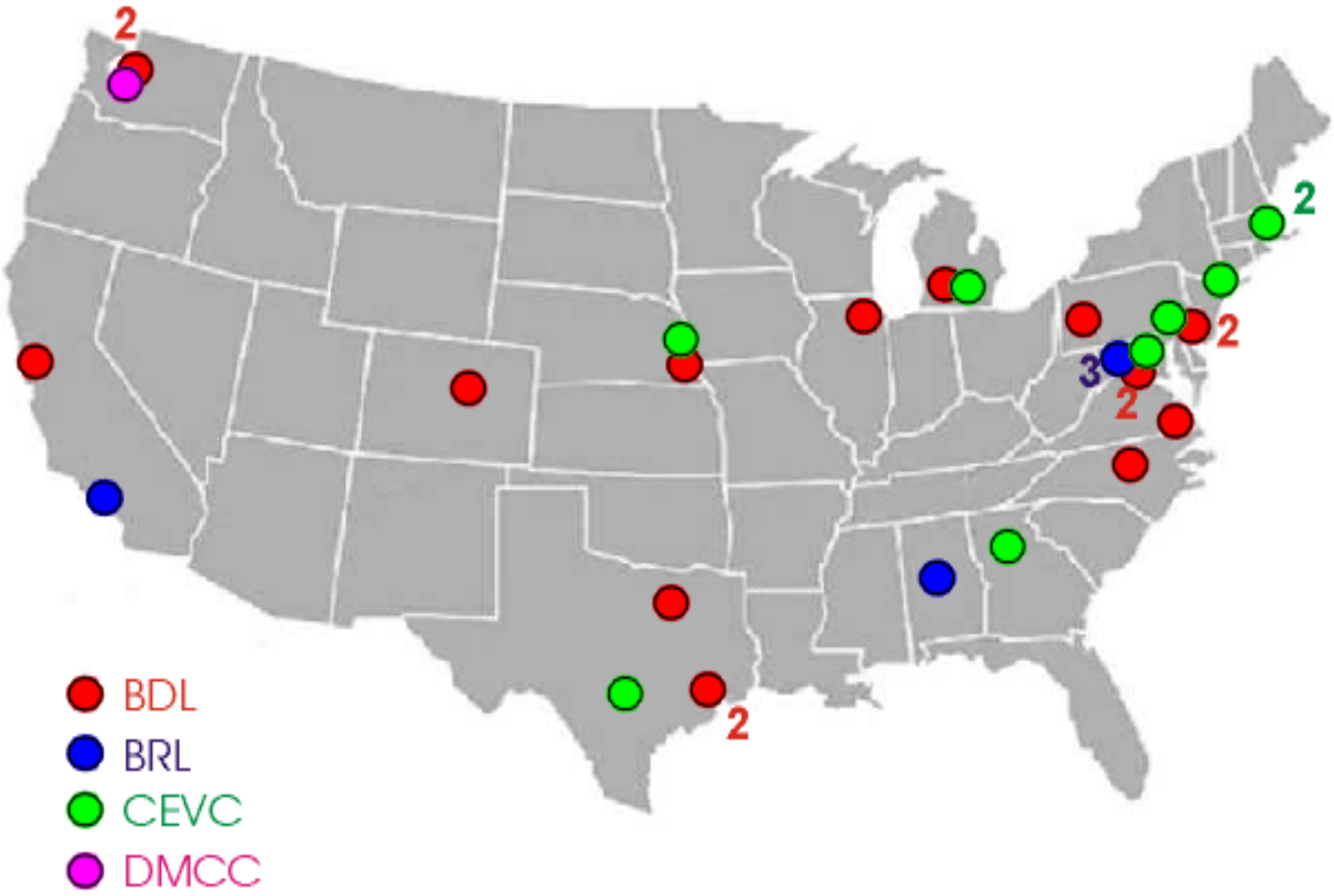

One of EDRN BRL's job is ……..

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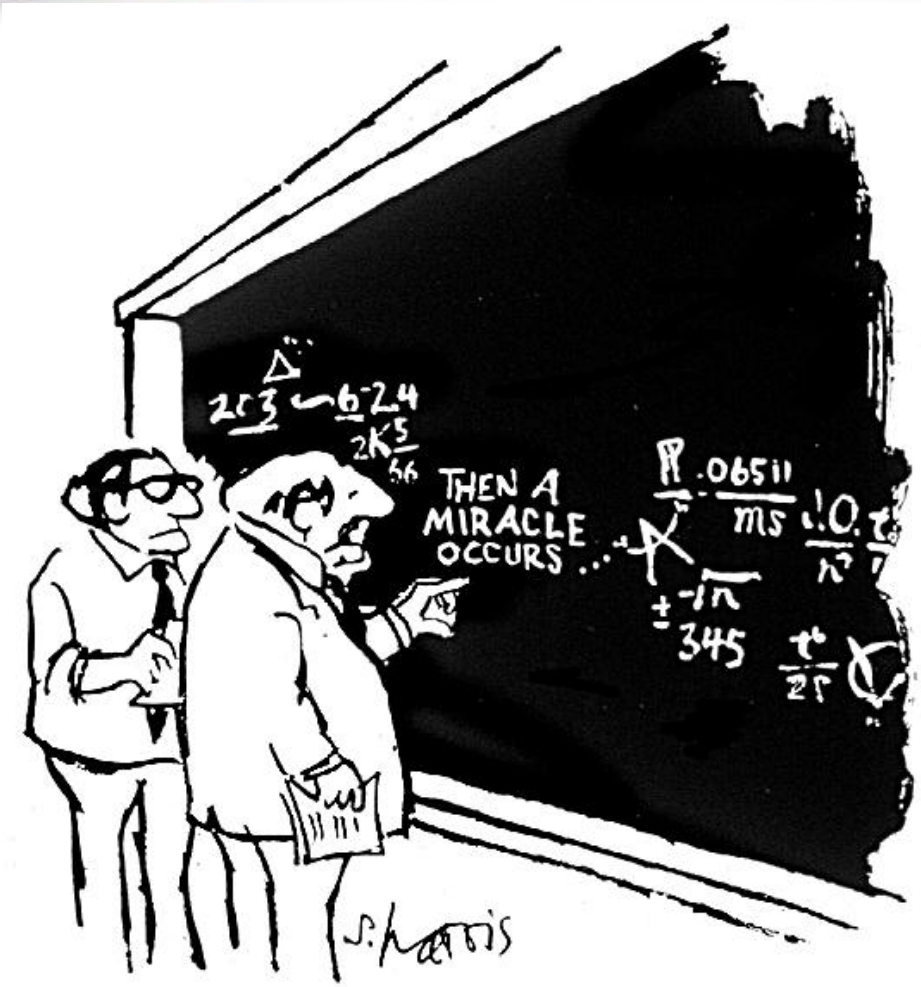

"I THINK YOU SHOULD BE MORE EXPLICIT HERE IN STEP TWO."

Who am I? What do I do?

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Daniel W. Chan, Ph.D., DABCC, FACB

Professor of Pathology, Oncology, Radiology and Urology

Director, Clinical Chemistry Division

Co-Director, Pathology Core Lab (CLIA and JCAHO certified clinical lab)

Director, Center for Biomarker Discovery

at The Johns Hopkins Medical Institutions in

Baltimore, Maryland

JHH Pathology Core Lab (Staff=250)

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JHH Cancer biomarkers clinical laboratory

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The Cancer Diagnostics Clinical Study Team

(Johns Hopkins Hospital)

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JHU Center for Biomarker Discovery (CBD)

Multi-disciplinary team (just like EDRN)

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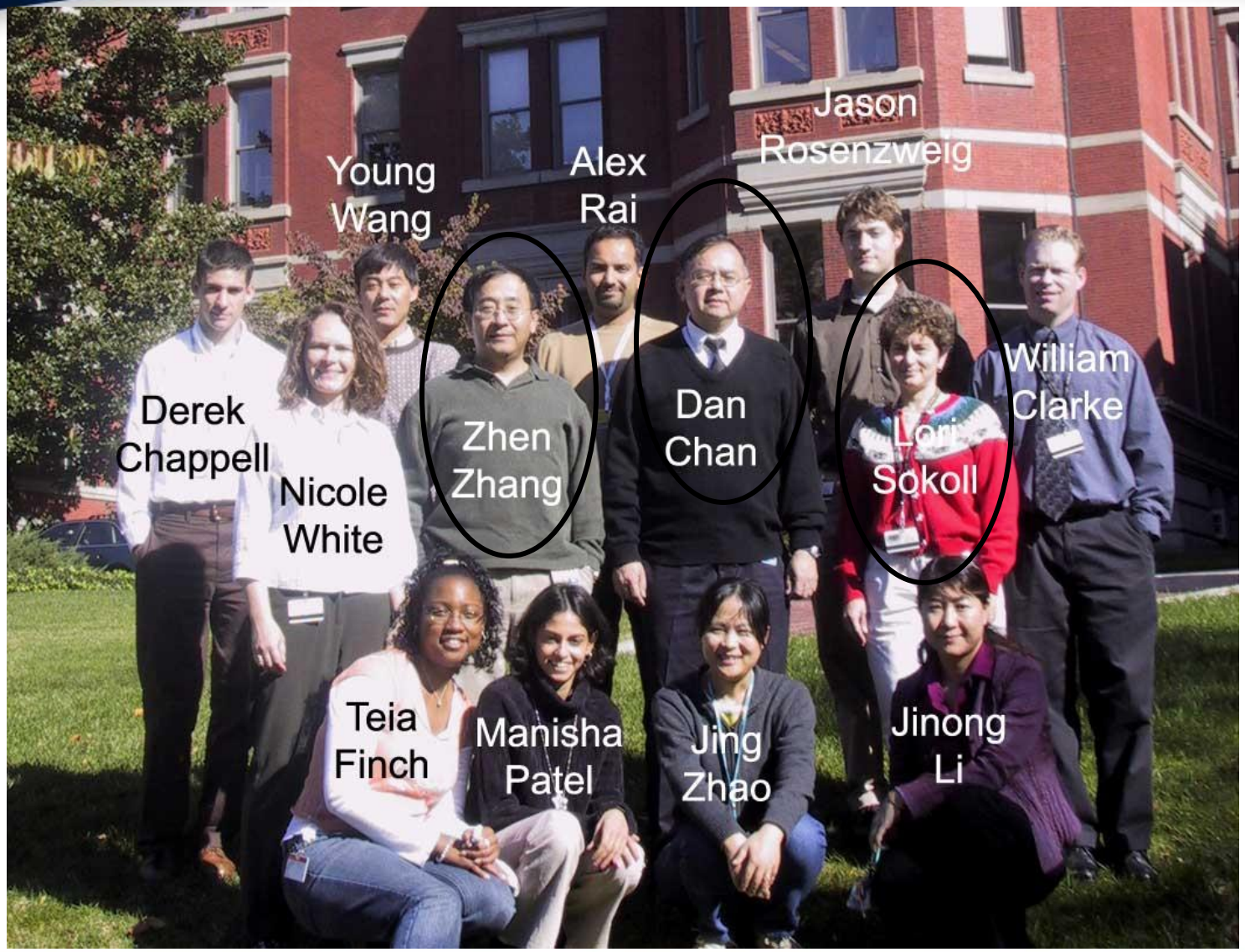

Our approaches to cancer biomarker discovery, validation and translation (CBD-JHU) *just like EDRN teams*

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- Select the right technologies: Protein array and/or mass spectrometry.
- Use well characterized clinical specimens – plasma, serum, urine, body fluid, tissue, cell: Pathology.
- Develop bioinformatics tools for data analysis and multiplexing of biomarkers: Engineering.
- Design multi-center case control study with extensive clinical validation to minimize the impact of possible confounding variables: Statistics.
- Discover and identify biomarkers (profile is not sufficient) with biological (clinical) significance: Cancer Biology.
- Translation of biomarker into multiplex clinical diagnostics: Clinical Chemistry.

Translation of Cancer Biomarker:

From discovery to clinical practice

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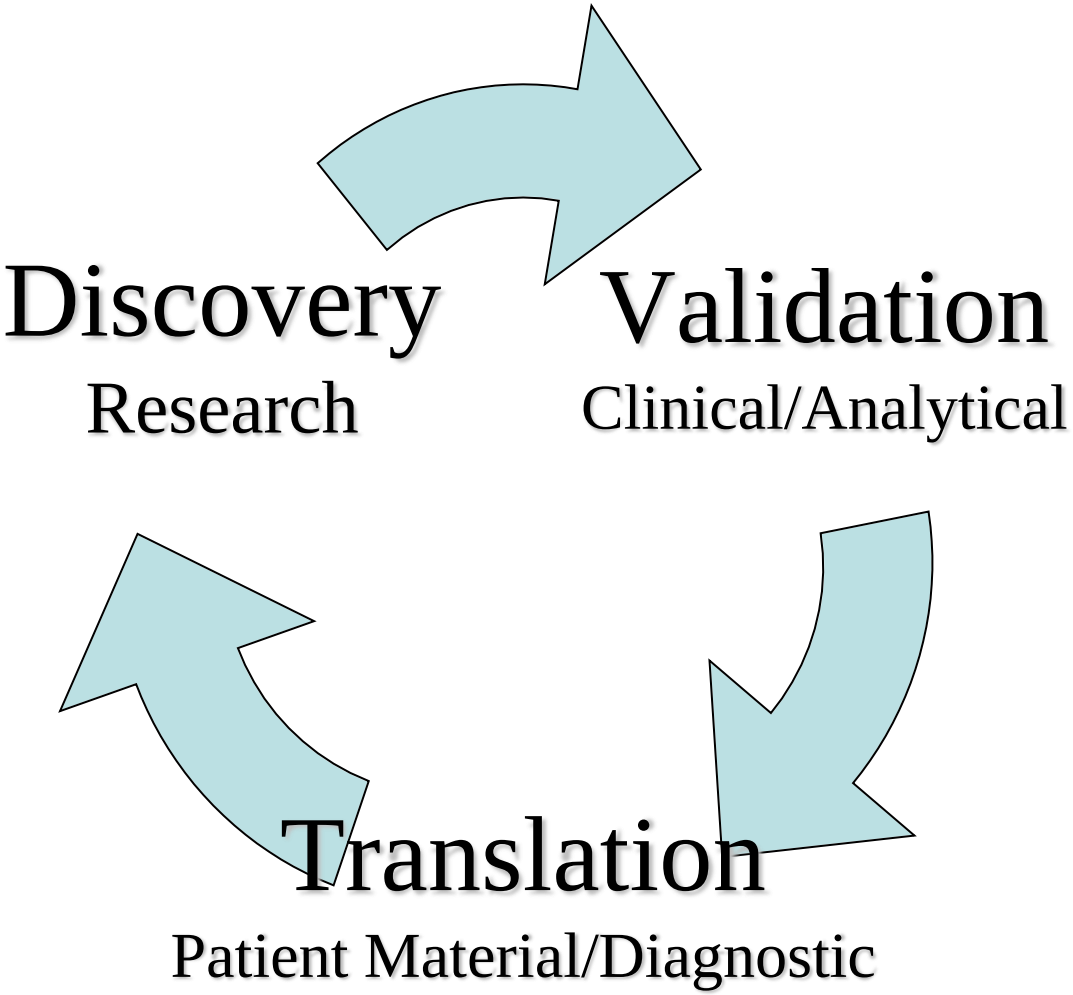

Why Pre-Validation?

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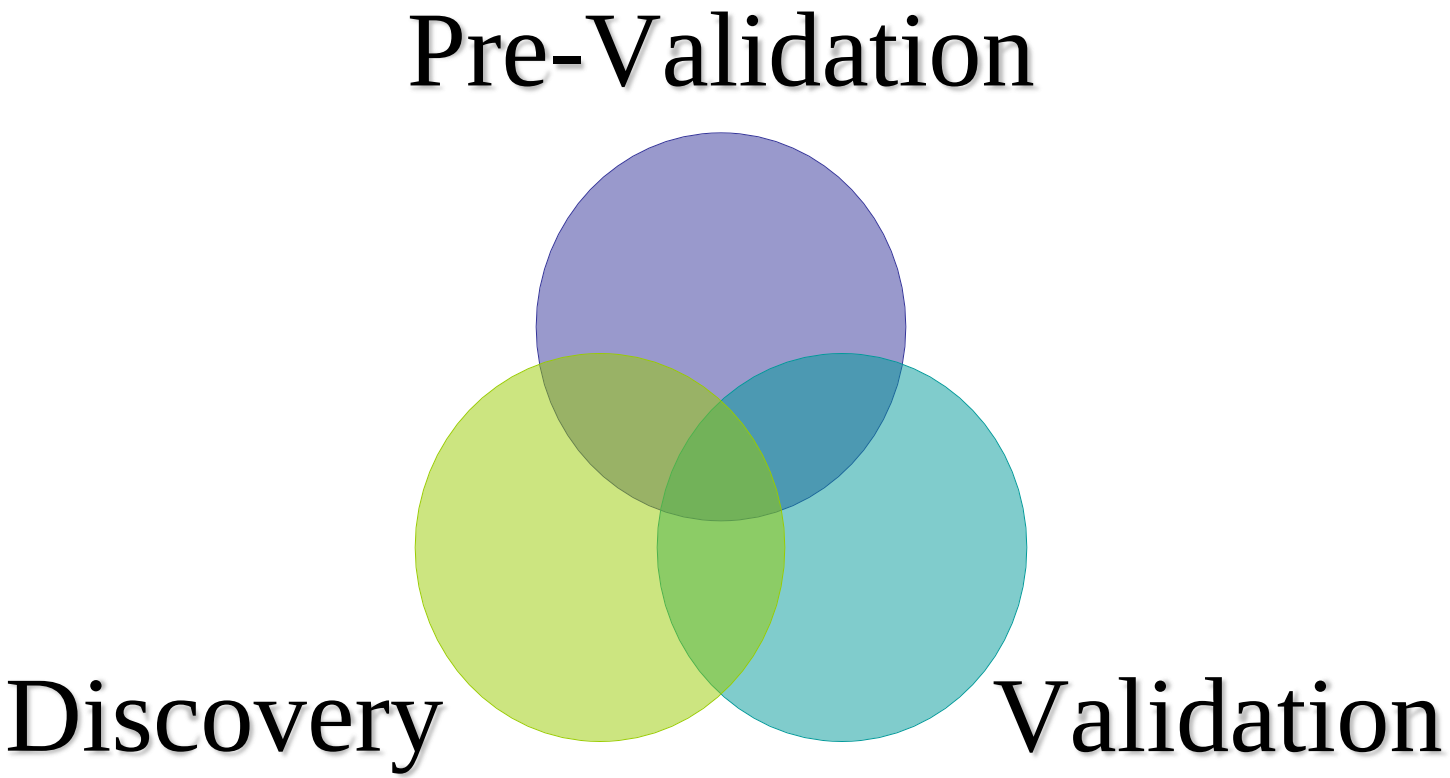

Why Clinical Specimen Reference Set?

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Purpose: To assess the analytical and clinical performances of one or more biomarkers (panel) in order to set priorities for further studies.

Performance assessment: To compare sensitivity, specificity, ROC analysis (AUC) of biomarkers using the same set of clinical specimen.

Outcome: Establish a cost effective (efficiency) process for pre-validation, validation and rapid translation of useful biomarkers into clinical practices.

Prostate Specimen Reference Set

Martin Sanda, M.D. (Chair)
Harvard Medical School

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- Collaboration between 3 Prostate CVEC sites (Hopkins, UT-SA, BIDMC).
- Case-control cohort of patients undergoing biopsy who have cancer or not (controls) N=120 per site.
- The resource: blinded sample set, standardized blood collection, standardized common data elements
- Specimen shipped to BRL (Dan Chan) for aliquoting, re-labeling, and shipping to four labs. Jacob Kagan did the blinding.

Early Detection Research

EDRN SC GU Group meeting 9/21/2005
I: Discovery II: Validation: Analytical and Clinical
III: Multi-Center Study

Recommendation	I	II	III	Speaker
Semmes: MS-Immunoassay	14			I
Wang: Immunomic Profiles	8	6		I-II
Sanda: Anti-AMACAR autoantibody	9	3	1	II
Liu: CD90, CD10, CA1	14			I
Sokoll: ProPSA	1	10	3	III
Veltri: PBOV-1	12	2		I
Zhang: Proteomic Markers	8	3		I
Smith: EPS DNA Methylation	12			I-II
Cairns: Methylation/Renal	8	7		I

Early Detection Research Network

EDRN SC GU Group meeting 9/21/2005 (Cont.)
I: Discovery II: Validation: Analytical and Clinical III: Multi-Center Study

Recommendation	I	II	III	Speaker
Diamandis: Human Kallikreins	4	9		II-III
Getzenberg: EPCA	6	7		II
Sen: Mytotic Kinases	11			I
Rittenhouse: PCA3	3	8	1	II

Outcomes - Recommendations

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- The top 5 biomarkers were selected for pre-validation using the prostate clinical specimen reference set (blinded): ProPSA, human Kallikreins, EPCA2, PCA3 and TSP1.
- Completed testing of all biomarkers from the 4 investigators and data sent to DMCC.
- Each investigator sent a report (2-3 pages summary) to the GU group.
- A committee reviewed the data and made recommendations for possible clinical validation.

Molecular Forms of Free PSA

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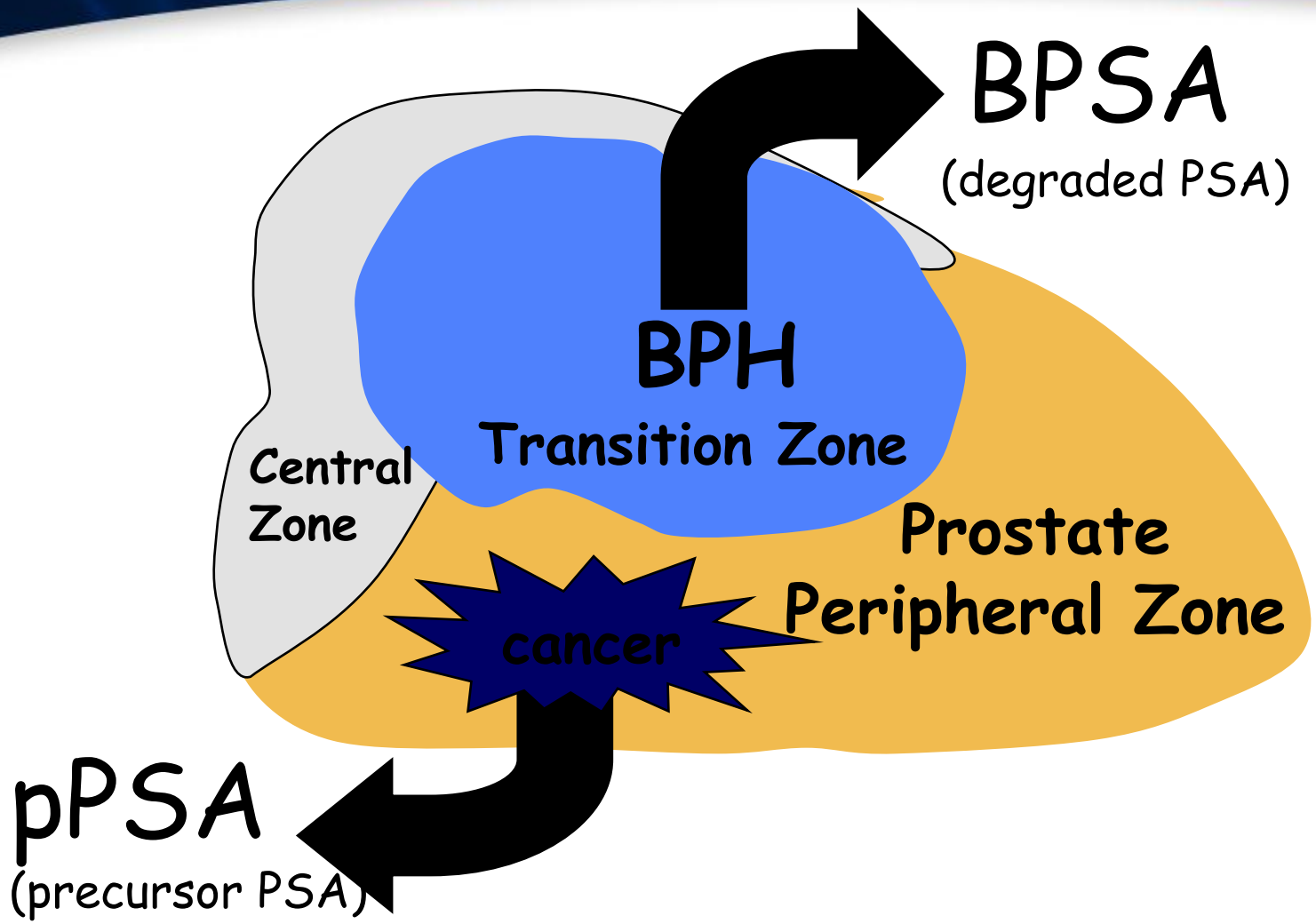
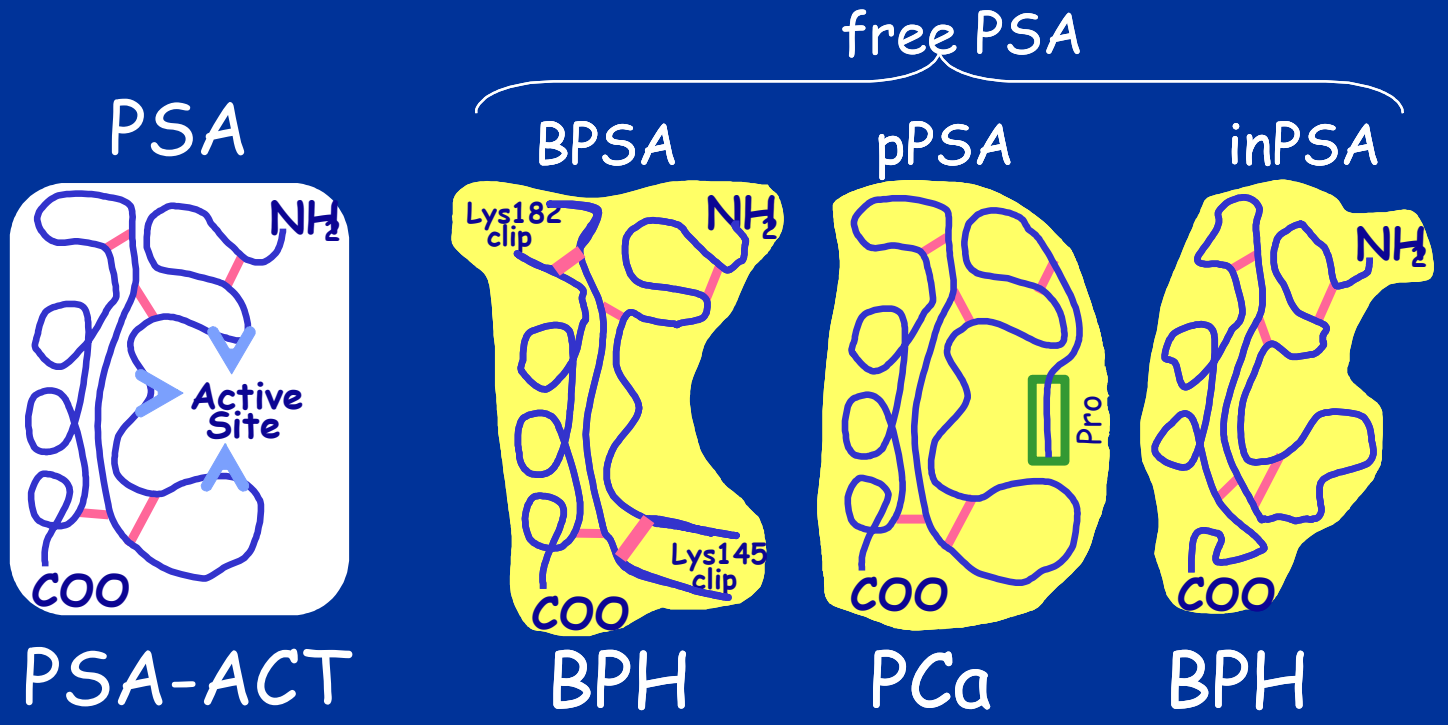
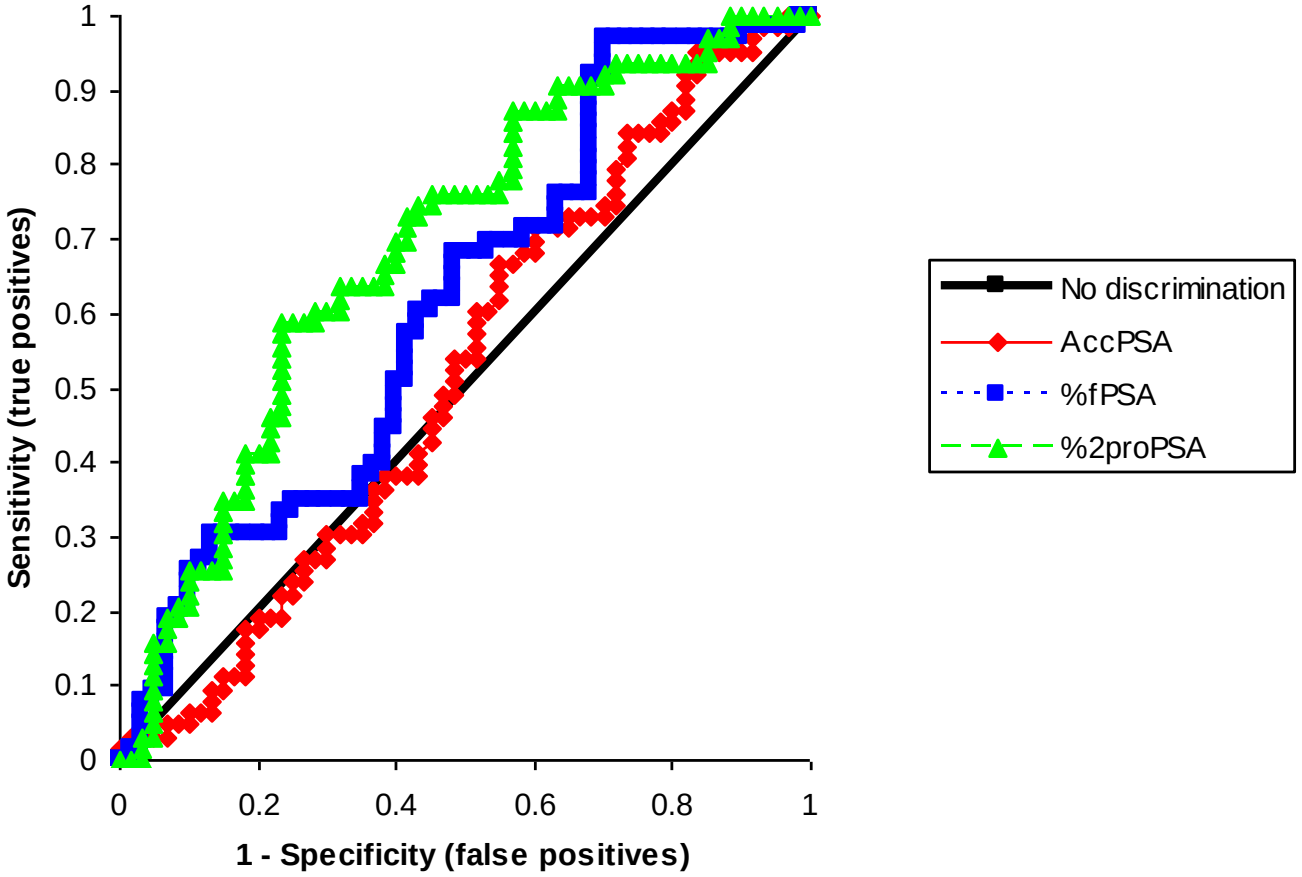


Molecular Forms of PSA in Serum

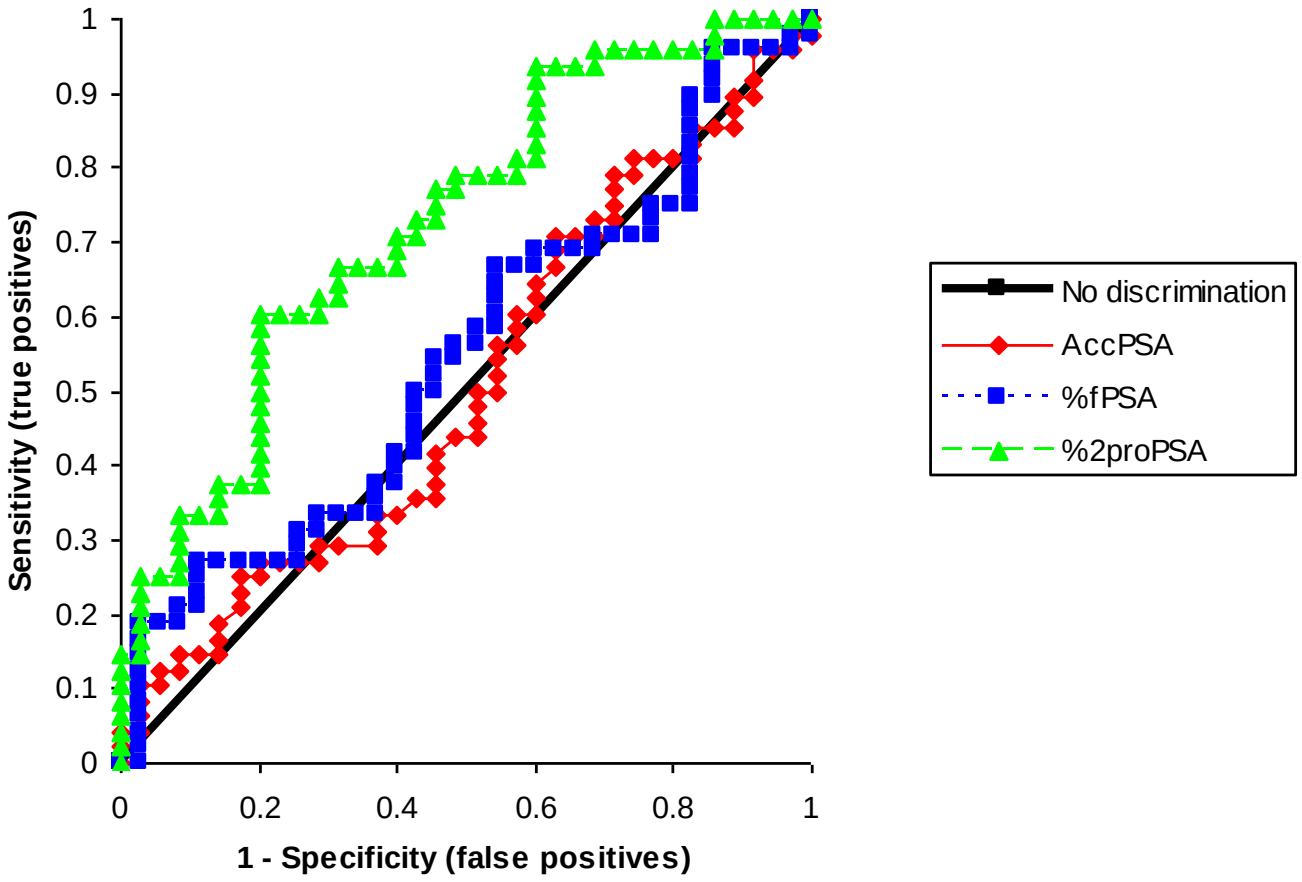


Mikolajczyk et al, Urology, **59**,797-802, 2002

ROC – All Data for Cancer Detection



ROC – PSA Range 2 to 10 ng/mL for Cancer Detection



PSA Isoform Study – Update as of today

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- Develped a public-private partnership between EDRN and Beckman-Coulter Company.
- Participated by the EDRN CEVC and reference lab.
- Design and conduct clinical trial of proPSA leading to FDA approval for clinical use.
- Multi-center clinical trial to start March 2008.
- Reagent and instrument will be provided by Beckman.
- Patient specimens and funding for the study will be provided by both Beckman and EDRN.

PCA3 Molecular Urine Test Study

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- Non-coding mRNA with low expression level in normal prostate cells and highly over-expressed in prostate cancer cells
- PCA3 presented at GU Collaborative Group Workshop (9/05) and selected as one of 4 markers to move towards validation
- At that time, appropriate samples were not available for further studies
- Prospectively collect samples to characterize the clinical utility of the PCA3 marker
- A public-private parternship bewteen EDRN and Genprobe Inc.

PCA3 Assay Procedure

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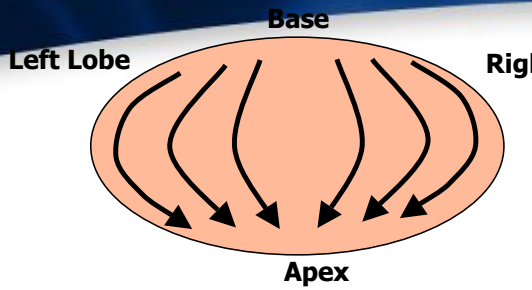
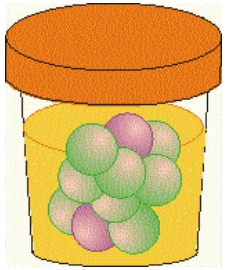


Digital Rectal Exam (3 strokes per lobe)



Urine Specimen

PCA3 and PSA mRNA concentrations measured in separate tubes

Quantitative ratio of PCA3/PSA mRNA = PCA3 Score

PCA3 Score < cutoff

Lower risk of positive biopsy

PCA3 Score ≥ cutoff

Higher risk of positive biopsy

Preliminary Data

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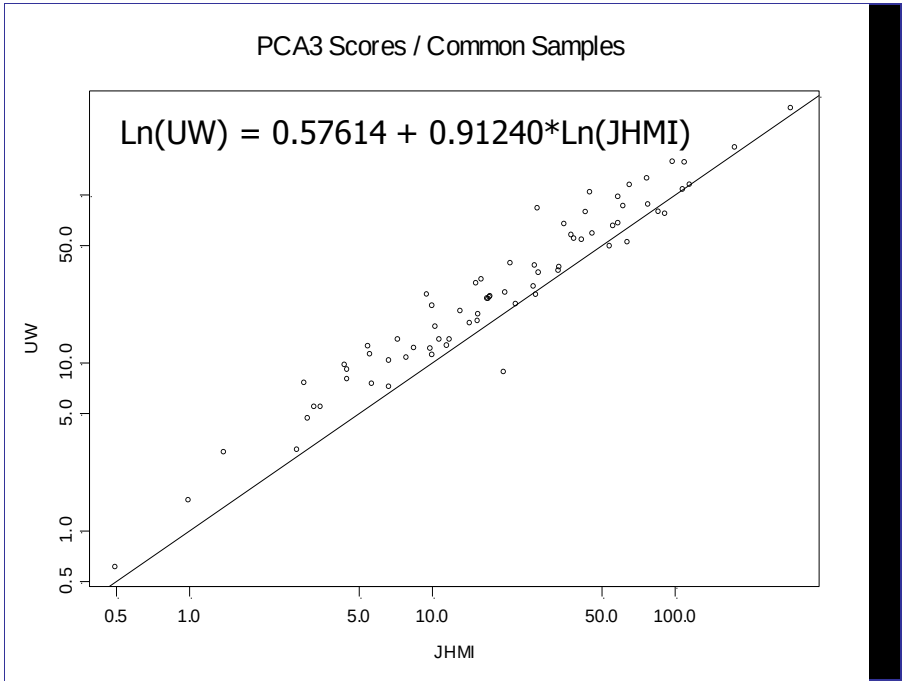

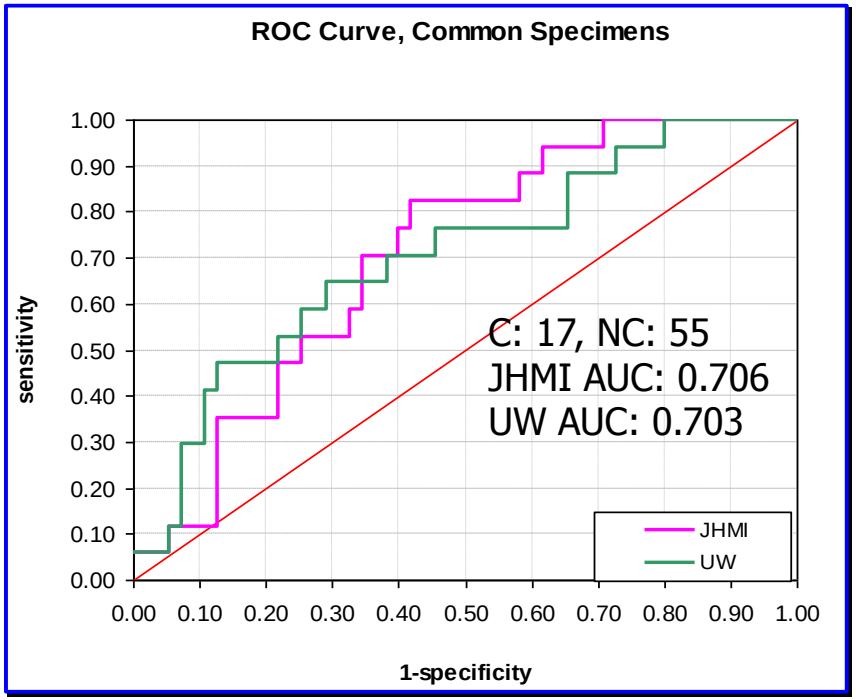

Study Proposal

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Global Hypothesis: Independent of serum PSA level, PCA3 score will define the risk of having cancer detected on prostate biopsy.

Specific Aims

A. Primary Specific Aims: To evaluate the PPV of PCA3 for initial biopsy population and NPV of PCA3 for repeat biopsy population in a multicenter prostate biopsy cohort of men without prior history of prostate cancer.

B. Secondary Aims:

1. To evaluate the sensitivity, specificity, PPV, NPV, and absolute risk prediction by PCA3 alone and multiplexed with other biomarkers and clinical variables in the detection of prostate cancer

2. To evaluate the correlation between PCA3 and prostate biopsy tumor grade

3. To evaluate the correlation between PCA3 and prostatectomy tumor grade and volume

4. To collect and bank urine/serum for pre-validation studies of gene fusion and other biomarkers

The analytical and clinical validation of DCP in Hepatocellular Carcinoma - UCLA BRL, David Chia, Ph.D.

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- In collaboration with Dr. Marrero (Univ. Michigan) to study biomarkers DCP (des-gamma carboxyprothrombin), AFP (α-fetoprotein), and AFP-L3% in early stage hepatocellular carcinoma.
- Validate the DCP assay from Sanko Junyaku Co. with DMCC.
- Validate the AFP, and AFP-L3% from Wako Diagnostics with DMCC.
- Perform DCP, AFP, and AFP-L3% on more than 800+ blinded samples of hepatocellular cancer cases and controls from the DCP study.
- Assay results were sent to DMCC for analysis.

The analytical validation study of prolactin in ovarian cancer - UCLA BRL

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Questions were raised on Dr. Gil Mor's study concerning prolactin level in ovarian cancer.

To validate the prolactin results, BRL tested 100 samples (ovarian cancers and controls) in a blinded fashion.

The results were analyzed by DMCC, and the results from BRL were highly correlated with Dr. Mor's result.

Why Proteomics Standards?

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To achieve high accuracy (measurement of protein concentration).

To obtain consistent results (both within and between labs).

To diagnose clinical conditions correctly (separation of disease from health).

All PSA assays give the same result?

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Total PSA in CAP Survey Material (1997 K-03)

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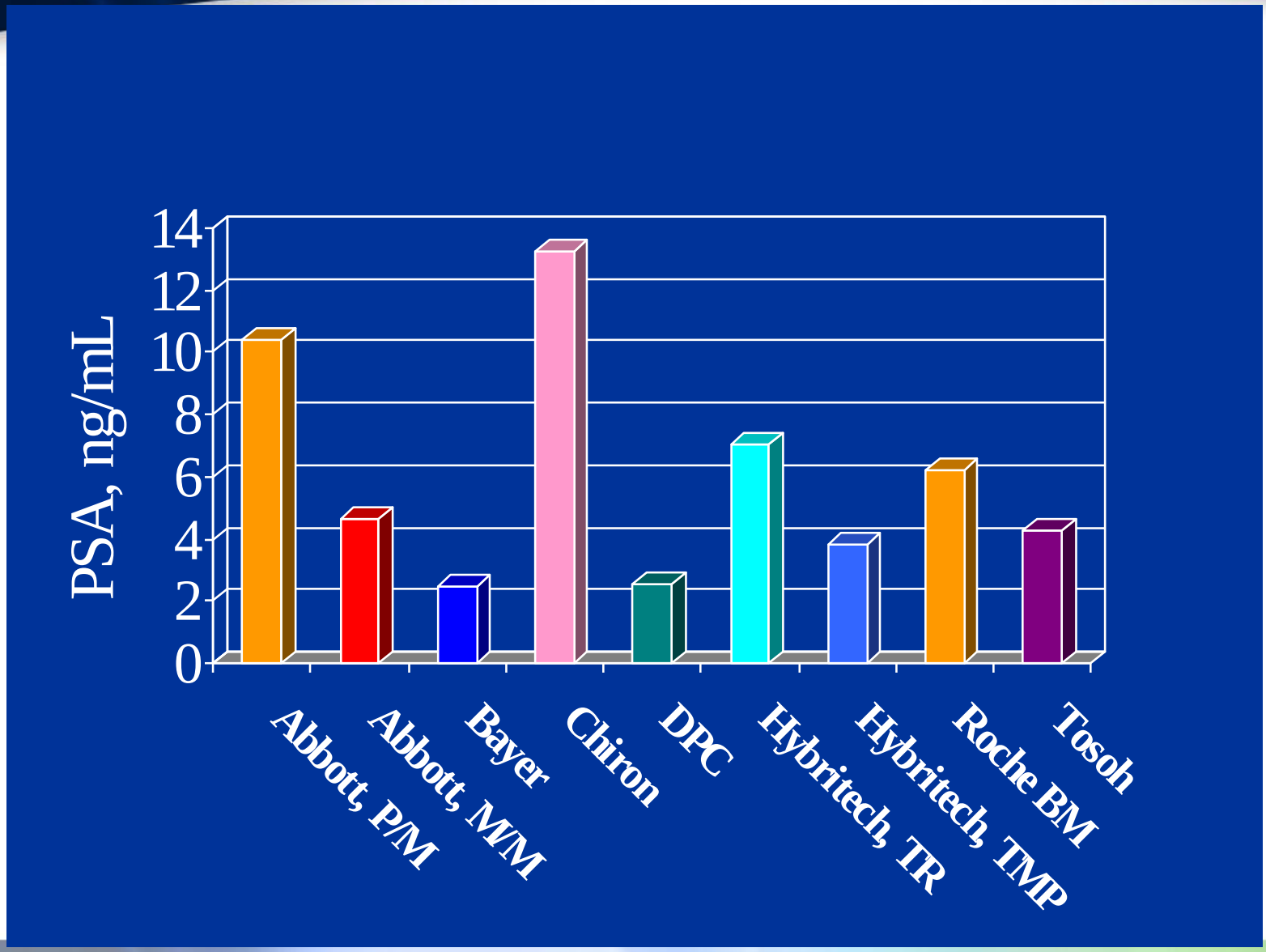
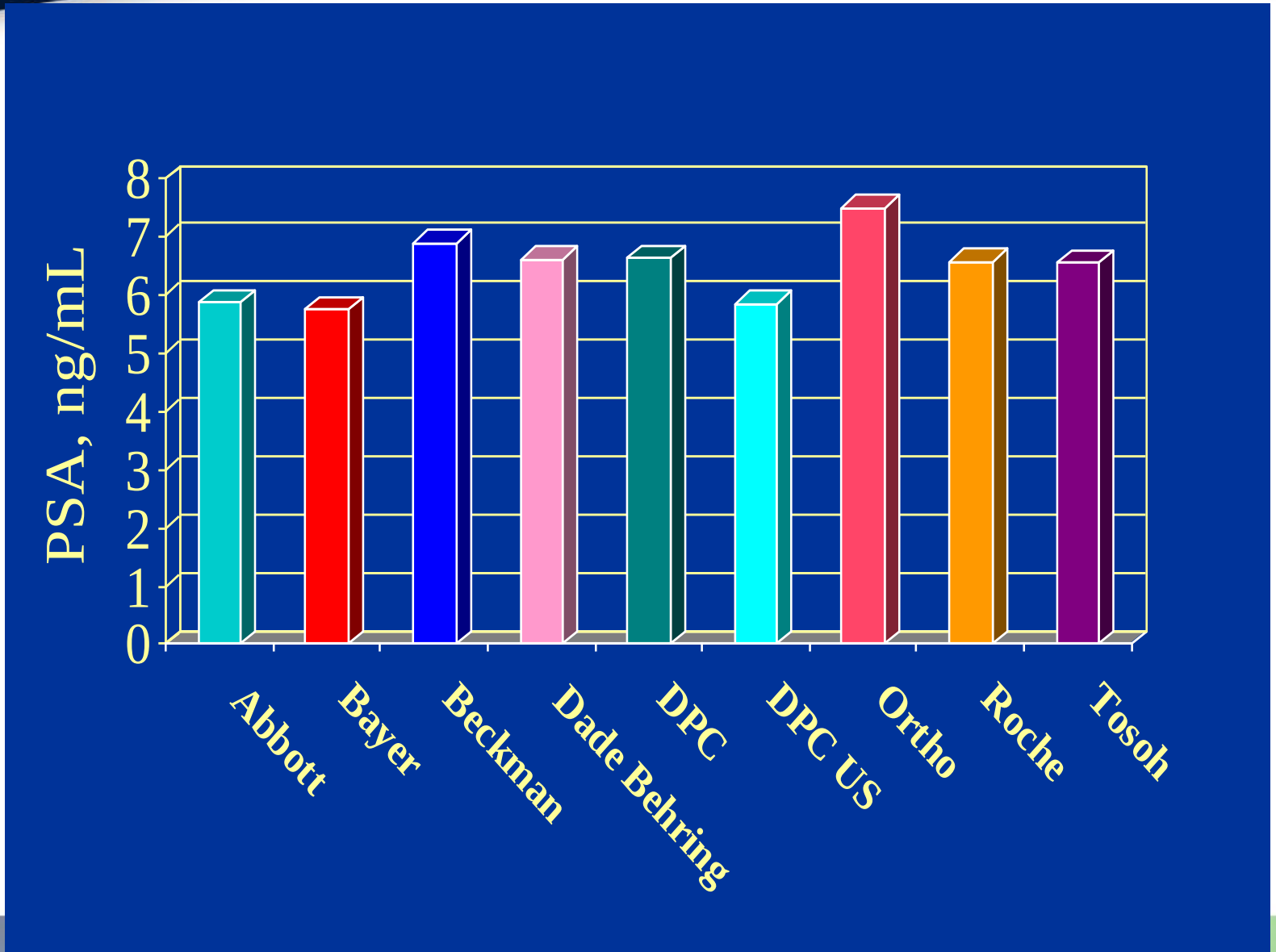


Total PSA in CAP Survey Material (2005 K-03)

Early Detection Research Network



PSA Standards and Assay Improvement

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PSA: WHO 1st IS 96/688 - 100% free, WHO 1st IS 96/700- 90% complexed and 10% free (1999).

Most PSA assays are more equimolar (2007) due to improvement in assay design: PSA epitopes, monoclonal antibodies, matrix effects and reaction kinetics.

NIST/NCI EDRN Workshop (2005): "Standards, Methods, Assays, Reagents, and Technologies (SMART) for Early Cancer Detection"

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Proposed Reference Standards for Cancer Proteomics Analysis:

- 1.Peptide mixtures
- 2. Single proteins
- 3. Mixtures of defined proteins (3-5)
- 4. Complex mixtures: Serum/plasma pools enriched with known cancer biomarkers

Barker PE, Wagner PD, Stein SE, Bunk DM, Srivastava S, Omen GS. Clin Chem 2006;52:1669

Cancer Proteomics Reference Materials (Complex mixtures) (EDRN – JHU & NIST BRLs)

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- JHU EDRN BRL and collaborators at NIST: Peter Barker, Ph.D. and David Bunk, Ph.D.
- The cancer proteomics reference materials consist of a normal serum pool from healthy individuals and a cancer pool prepared by spiking FDA approved cancer biomarkers into the normal base pool to simulate the cancer disease state.
- The reference materials are intended to be used for serum proteomics research for the early detection of cancer biomarkers, to aid in providing standardization across the proteomics research community, and for analytical instrumentation validation.

Measured Tumor Marker Results for Unspiked and Spiked Pools

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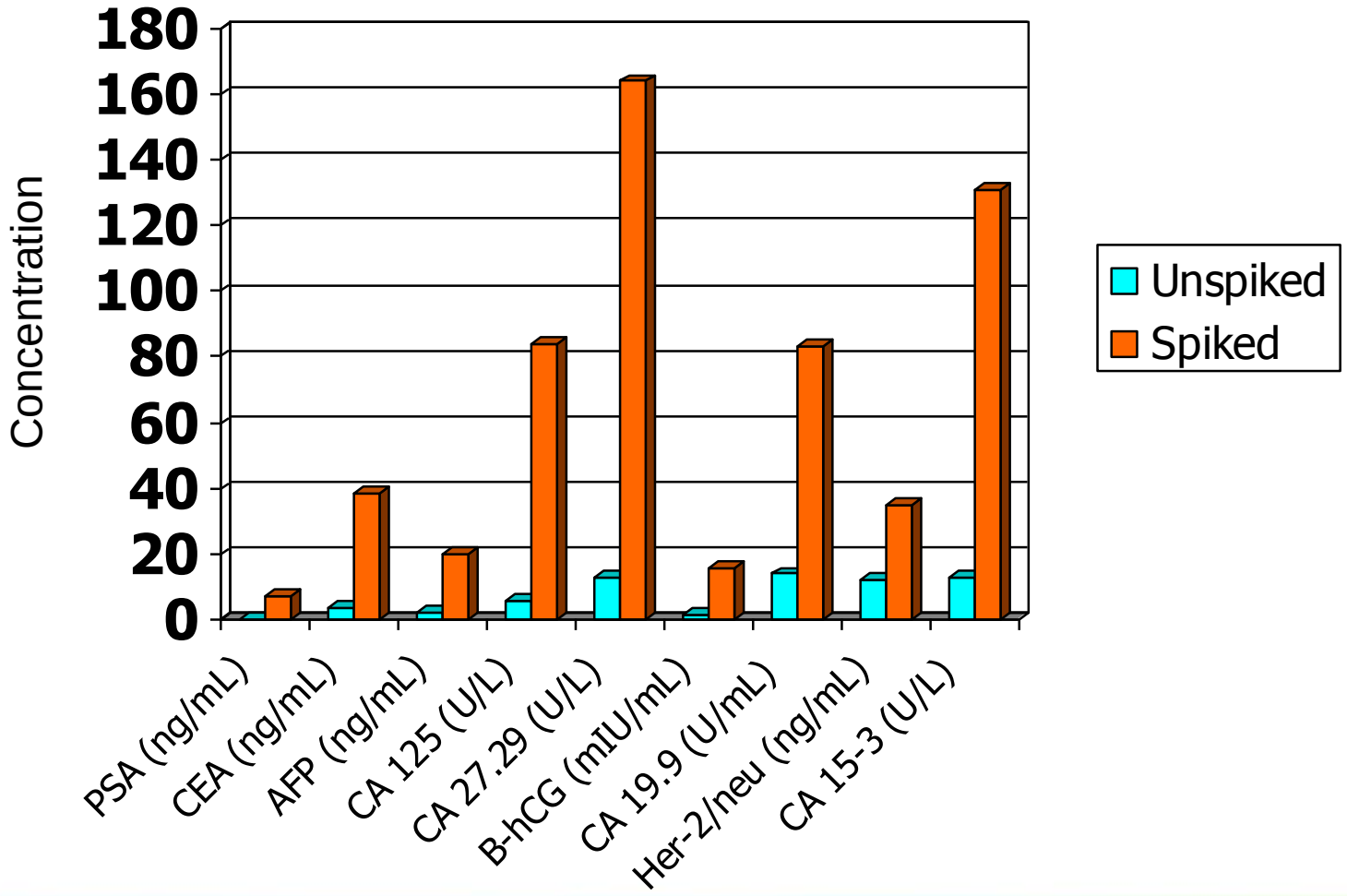

Other Proteins Measured in the Reference Material

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Total Protein	Alpha1-acid glycoprotein	SHBG	IGF-1
Albumin	Alpha2-macroglobulin	Beta2-microglobulin	LH, FSH
Transferrin	Apo A1, B	PAP	Growth Hormone
CRP	Ceruloplasmin	Free PSA	Insulin
IgA, IgM, IgG	Cystatin C	CK-MB	Prolactin
Rheumatoid factor	RPB	Troponin I	PTH
Haptoglobin	Soluble transferrin receptor	Myoglobin	proBNP
C3, C4	Calcitonin	Thyroglobulin	Osteocalcin
alpha1-antitrypsin	Gastrin	TSH	Ferritin

Multiplexing

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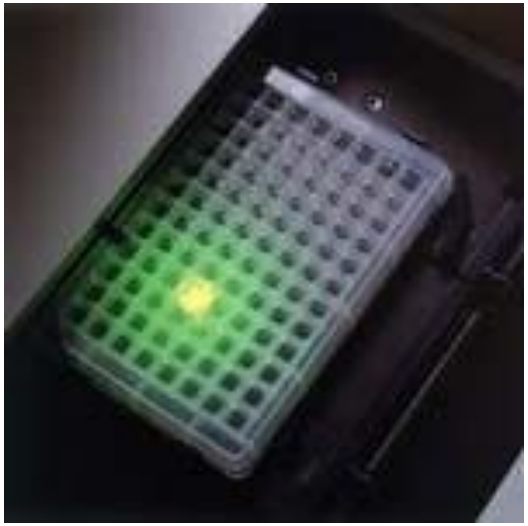
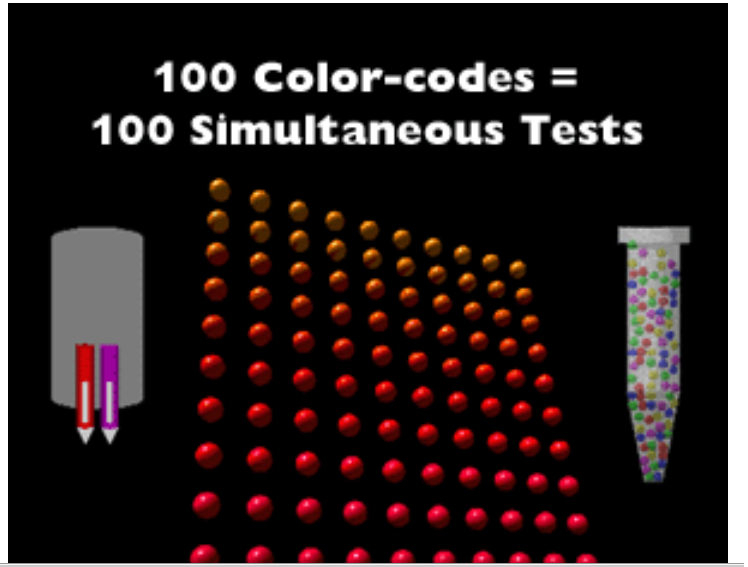
Cancer is heterogeneous





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12	M12	40.04	3.94	-4.62	0.467	0.776	0.359	0.853	0.089	0.321	0.442	0.472	0.173	0.364	1.021	0.32	0.828
26	M26	37.67	2.14	8.02	1.38	1.439	1.012	1.457	0.63	0.897	-0.649	0.85	0.396	0.698	1.564	0.219	1.527
45	M45	24.88	17.53	29.86	0.375	0.049	0.118	0.189	-0.504	-0.131	0.106	-0.571	-0.443	-0.535	-0.329	-0.744	-0.145
22	M22	-24.72	-15.4	14.66	0.399	0.542	0.41	0.637	1.299	-0.355	0.415	0.797	1.403	-0.248	0.547	-0.668	0.339
36	M36	24.13	-4.56	6.61	-0.183	0.163	-0.274	-0.356	-1.819	-1.028	1.199	0.298	-0.649	-0.879	-0.075	0.488	-0.625
14	M14	21.17	-19.14	-11.43	0.325	0.827	0.274	0.829	0.367	-0.687	0.25	0.308	0.69	0.348	0.879	0.117	0.743
31	M31	21.09	-21.91	3.26	0.511	0.611	0.553	1.201	0.733	-0.192	0.293	0.09	0.66	0.024	0.483	-0.484	-0.024
1	M1	-20.86	-21.18	-3.37	-0.057	0.271	-0.104	0.402	0.482	0.305	0.155	0.3	0.35	-0.542	-0.213	-0.32	0.214
52	M52	-18.76	-7.98	-10.21	-1.945	-1.829	-2.072	-1.859	-1.785	-2.078	-0.652	0.0	-1.639	-1.669	-2.146	-2.046	-2.074
9	M9	18.13	6.81	-17.73	0.232	0.647	0.0	0.464	0.149	-0.311	1.17	0.016	-0.667	-0.054	-0.457	-0.362	0.762
24	M24	-17.35	-23.53	17.23	0.749	0.739	0.385	0.936	1.197	0.569	-0.175	0.583	1.306	0.227	0.421	0.337	0.807
30	M30	17.26	-16.31	-3.4	0.543	0.263	0.069	0.381	0.295	-0.081	0.76	0.249	0.308	0.045	-0.049	-0.404	-0.035
29	M29	16.76	-18.91	3.74	0.744	0.894	0.267	0.938	0.339	-0.03	0.541	0.209	0.817	0.205	0.804	0.0	0.572
28	M28	16.16	-15.21	5.5	1.226	1.428	0.93	1.375	0.942	0.387	1.175	0.838	1.272	0.6	1.24	-0.501	1.087
35	M35	15.85	-9.34	6.82	0.332	0.5	0.096	0.243	-0.269	-0.31	1.653	0.611	0.106	-0.7	0.274	0.705	-0.057
41	M41	13.81	-11.68	-16.48	0.081	-0.152	-0.451	0.124	-0.404	-0.5	-0.347	-1.199	-0.351	-1.36	-0.62	-1.247	-0.522
8	M8	13.09	-9.19	-5.52	0.316	0.384	0.0	0.574	0.116	0.211	1.227	0.296	0.438	0.233	-0.313	0.517	0.398
25	M25	-12.7	-13.57	14.13	0.86	0.752	-0.028	0.804	0.611	0.605	-2.392	0.324	0.721	-0.102	0.632	-0.31	0.661
44	M44	-10.34	-1.66	6.0	-0.182	-0.352	-0.252	-0.019	-0.397	-0.344	-0.548	-0.437	-0.351	-1.117	-0.578	-0.864	-0.742
42	M42	9.56	-0.83	14.14	0.165	-0.093	-0.254	0.103	-0.408	-0.515	-0.082	-1.471	-0.45	-1.113	-0.622	-1.108	-0.536
43	M43	-9.49	2.54	4.72	-0.968	-1.006	-0.816	-1.313	-0.604	-0.909	0.298	-0.852	-0.701	-1.204	-1.134	-0.901	-0.959
10	M10	-9.44	-13.03	16.23	-0.277	0.075	0.484	0.294	0.363	-0.037	0.698	-0.089	0.681	0.205	0.0	-1.616	-0.077
6	M6	-8.77	1.01	10.54	-0.205	0.07	0.234	0.106	0.404	-0.487	0.813	-0.352	0.454	-0.162	-0.128	-0.031	0.159
7	M7	-8.14	-19.63	-3.26	0.482	1.215	0.497	1.107	0.915	-0.14	1.081	0.355	0.968	0.349	1.163	0.258	1.191
23	M23	-8.0	-13.77	8.71	-0.268	0.224	-0.035	0.492	0.58	-0.112	0.635	0.0090	0.836	-0.842	0.627	-0.17	0.521
53	M53	-7.98	3.32	-14.69	-2.066	-2.415	-2.13	-1.677	-1.756	-2.571	-0.889	-2.592	-1.777	-1.58	-2.558	-1.894	-2.267
48	M48	7.93	11.46	30.75	-1.428	-1.611	-1.265	-1.25	-1.699	-1.347	-0.466	-1.332	-1.455	-1.33	-1.449	-1.522	-2.077
17	M17	7.86	-33.59	-2.54	-0.062	-0.273	-0.366	0.744	0.272	0.145	0.593	-0.0090	0.539	0.0	0.113	-0.107	0.117
39	M39	-6.92	11.17	13.46	-0.332	-0.349	-0.655	-0.568	-0.665	-0.509	0.371	-0.514	-0.448	-0.789	-0.601	-0.758	-0.68

Multiplexing

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Rationale for multiple cancer biomarkers and multiplexing

- Improve sensitivity and specificity over individual markers
- Measure analytes simultaneously with small sample and reagent volumes

Caveat: these assays and assay systems must have the same characteristics of commercial ELISAs or immunoassay platforms with respect to

- Precision, Accuracy, Lower limit of detection, Interference characteristics and Reliability.

Types of technologies for multiplexing proteins

- Bead-based fluidics assays with antibodies conjugated to encoded beads and analysis by flow cytometry
- Spot-based microarrays with antibodies printed on the solid support and analyzed by imaging

Bio-Plex™ 2200

from Bio-Rad Laboratories

Roche IMPACT (Immunological Multi-Parametric Chip Technique) Multiplexing Platform

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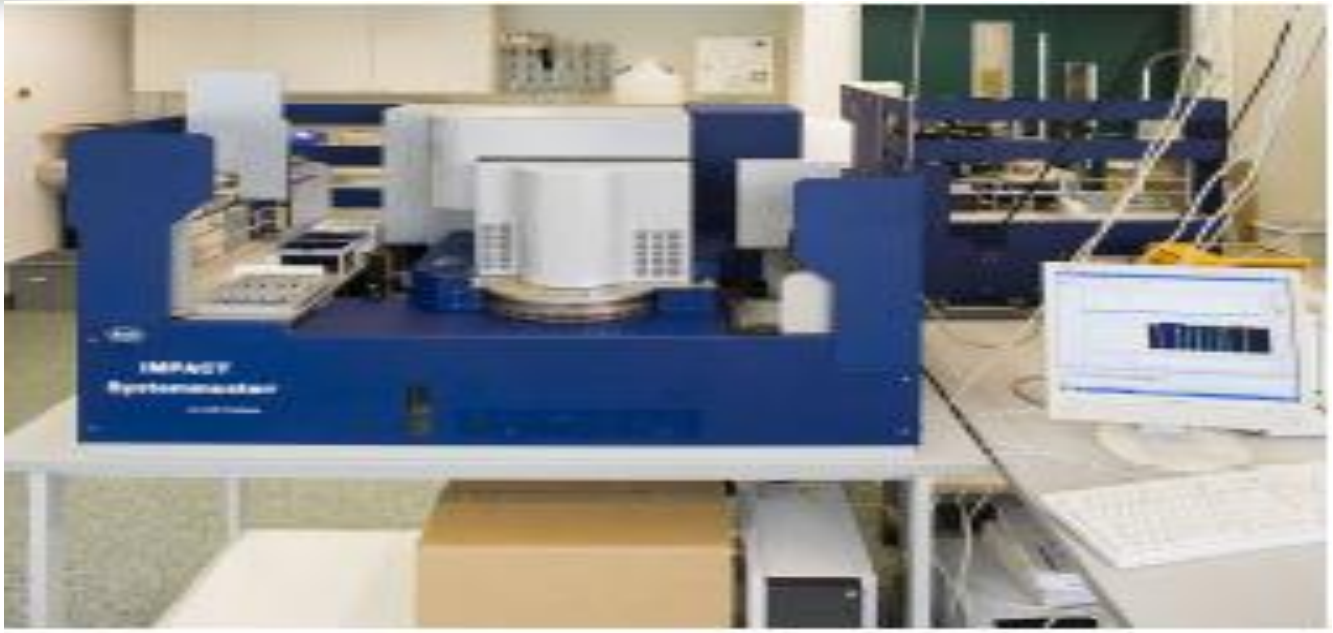
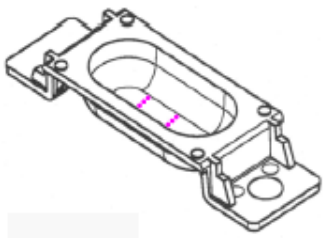
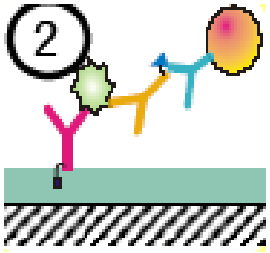


Chip Concept (*Up to 20 Analytes / Chip*)



Architecture	Polystyrene Chip
Capture	Antibodies/Antigen
Detection	Fluorescent Marker
Bioinformatics	Imaging Software



EDRN Reference Laboratories - conclusion

Early Detection Research Network

Cancer is a complex disease and requires a panel of multiple biomarkers.

Cancer diagnostics require high analytical and clinical accuracy.

High throughput multiplex nanotechnology should be developed for cost effective analysis.

Future diagnosis will be based on genomics, proteomics and imaging (PET, CT & MRI) to provide personalized medicine. *(As a leading imaging company, Siemens Medical acquired Bayer diagnostics, DPC and Dade Behring).*

EDRN BRL serves as the resource for clinical and laboratory validation of biomarkers, including technological development, standardization of assay methods and refinement.

EDRN BRL, working together with the BDL, CEVC and DMCC, is in a unique position to make significant impacts on biomarker discovery, validation and the rapid translation of cancer biomarkers into clinical practices.