GI04
Date
Time

Paul Lampe, Ph.D., Fred Hutchinson Cancer Research Center

Validation of early detection biomarkers for colorectal cancer

Paul D. Lampe, PhD

Professor and Head, Translational Research Program
Fred Hutchinson Cancer Research Center

Professor, Department of Global Health
University of Washington

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Colon CVC EDRN Project:
Phase 3 validation of early detection biomarkers for advanced adenoma and colon cancer

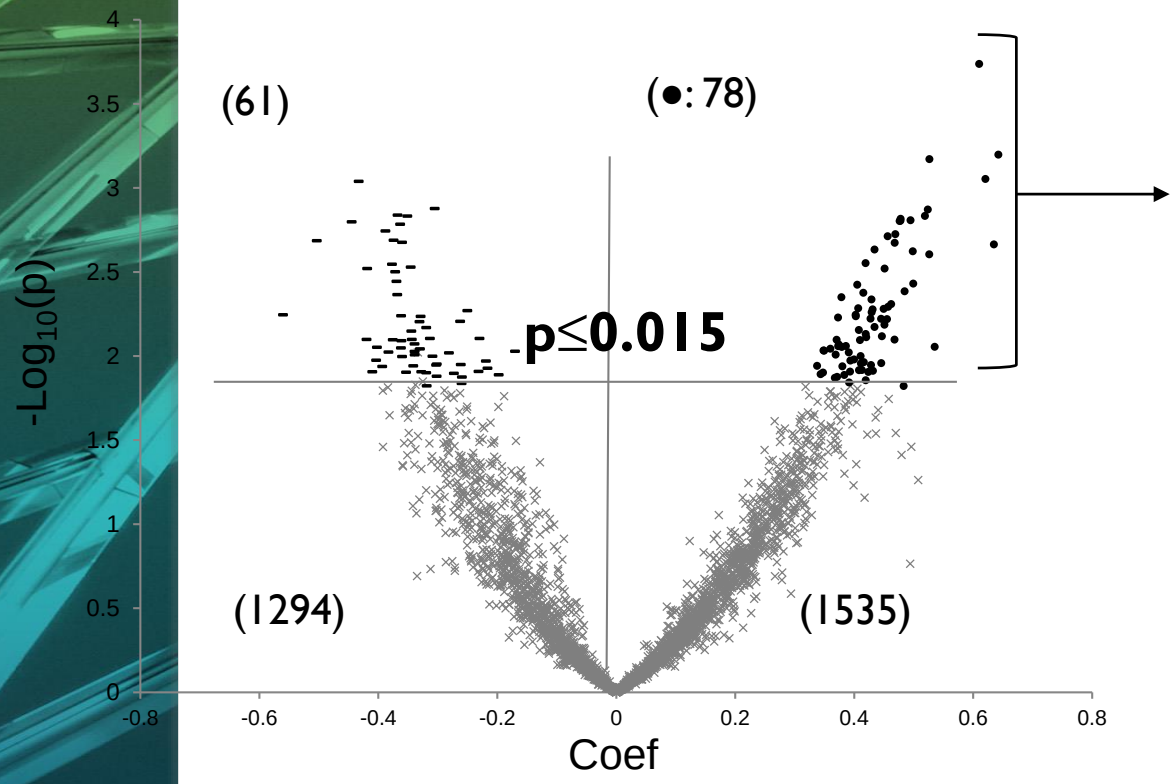
Background:

1. Colonoscopy and FIT reduce mortality.
2. Low acceptance rate (65% at best).
   - inconvenience
   - cost
   - insurance
3. Proteins in blood might be an alternative

Intended clinical applications:

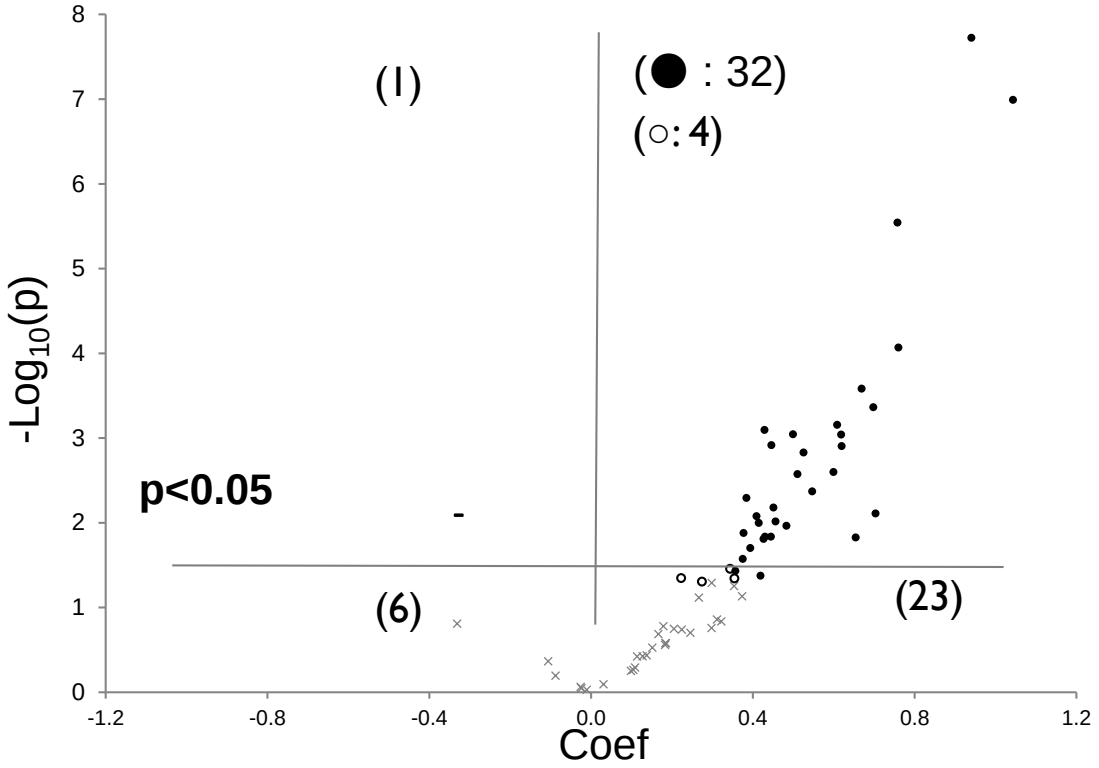
1. Identify people unwilling to undergo or with no access to colonoscopy who should be prioritized for colonoscopy (improve/replace stool tests).
2. Among symptomatic patients identify those who have a very low risk of cancer and can avoid colonoscopy.

Discovery - CHS Prediagnostic
(79 cases vs 79 controls)



Expression in cases:
- ● Increased (p≤0.015, AUC>0.60)
- - Decreased (p≤ 0.015, AUC>0.60)
- x Insignificant (p ≤ 0.015)

Confirmation - EDRN Diagnostic
(120 cases vs 60 controls)



Expression in cases:
- ● Increased (p<0.05, AUC≥0.60)
- ○ Increased (p<0.05, AUC<0.60)
- - Decreased (p<0.05)
- x Insignificant (p>0.05)

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ROC analysis of biomarker combination calculated for CHS and EDRN samples

Panel	Samples	Case category	Case	Ctrl		BAG4+IL6ST+VWF+EGFR*				Case	Ctrl		BAG4+IL6ST+VWF+CD44*			
			n	n		AUC	95%CI	Sens	95%CI	n	n		AUC	95%CI	Sens	95%CI
4-protein	CHS	All cases	66	66		0.81	0.74-0.88	40.9%	0.23-0.65	66	66		0.79	0.72-0.87	42.4%	0.15-0.64
		0-1 year	31	66		0.86	0.78-0.93	51.6%	0.23-0.77	31	66		0.84	0.76-0.92	50.0%	0.16-0.78
		1-3 years	35	66		0.77	0.68-0.86	31.4%	0.14-0.54	35	66		0.75	0.65-0.85	34.3%	0.09-0.54
	EDRN	All adenomas	20	23		0.83	0.68-0.95	55.0%	0.20-0.90	20	23		0.85	0.66-0.95	52.6%	0.21-0.95
		AA+Stage I+II	21	23		0.88	0.76-0.97	61.9%	0.29-0.95	21	23		0.82	0.75-0.98	60.0%	0.25-0.95
		All cancers	23	23		0.90	0.80-0.98	65.2%	0.35-0.96	23	23		0.88	0.78-0.98	54.5%	0.27-0.95
		All cases	43	23		0.87	0.76-0.95	55.8%	0.35-0.91	41	21		0.85	0.74-0.95	51.2%	0.32-0.90
4-protein + glycomic*	CHS	Prediagnostic	56	54		0.84	0.77-0.91	46.4%	0.27-0.73	32	33		0.84	0.75-0.94	50.0%	0.13-0.78
		0-1 year	25	54		0.87	0.79-0.94	56.0%	0.28-0.80	13	33		0.84	0.73-0.96	46.2%	0.00-0.85
		1-3 years	31	54		0.82	0.73-0.91	38.7%	0.23-0.71	19	33		0.84	0.73-0.95	52.6%	0.21-0.84
	EDRN	All adenomas	21	20		0.88	0.77-0.94	72.2%	0.28-0.94	17	21		0.80	0.65-0.94	52.9%	0.24-0.88
		AA+Stage I+II	19	20		0.91	0.83-1.00	78.9%	0.37-1.00	18	21		0.86	0.75-0.97	61.1%	0.33-0.94
		All cancers	21	20		0.93	0.85-1.00	85.7%	0.43-1.00	20	21		0.89	0.79-0.98	65.0%	0.35-0.95
		All cases	39	20		0.90	0.82-0.98	79.5%	0.41-0.95	37	21		0.85	0.75-0.95	59.5%	0.35-0.86

*EGFR or CD44's sialyl Lewis A and X information was added to the combinations. Coefficients were calculated for CHS All cases. AUC: area under the curve, CI: confidence interval, Sens: sensitivity at 90% specificity

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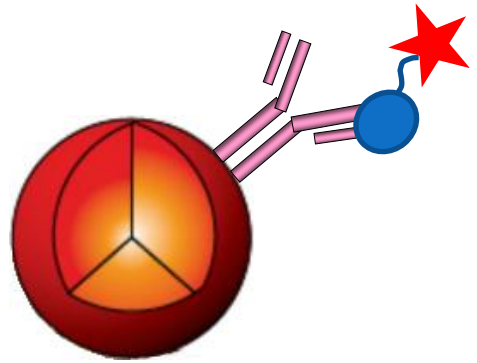
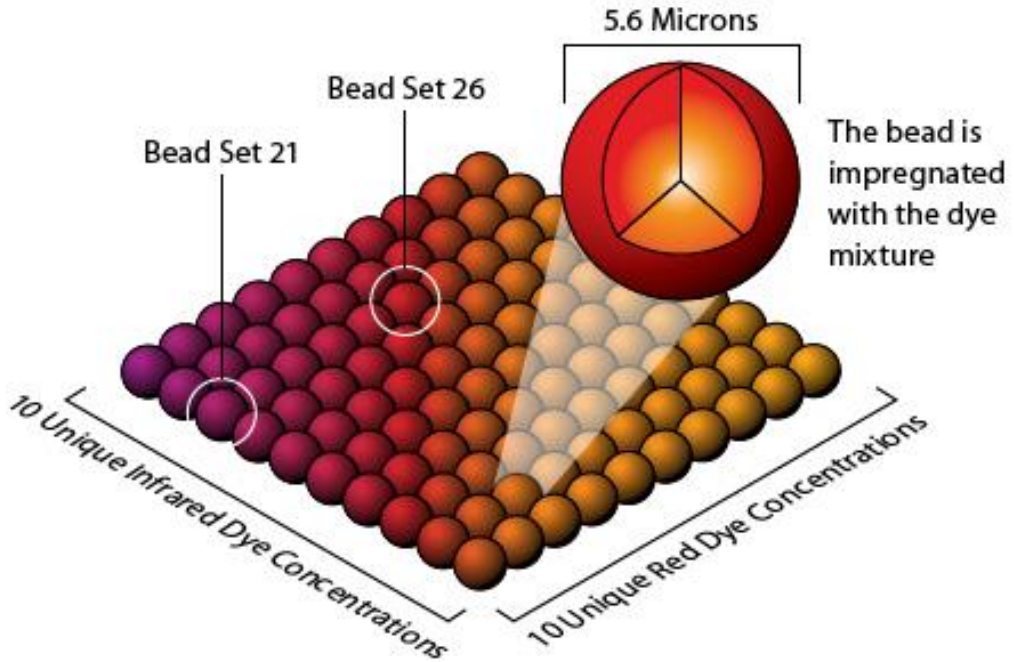
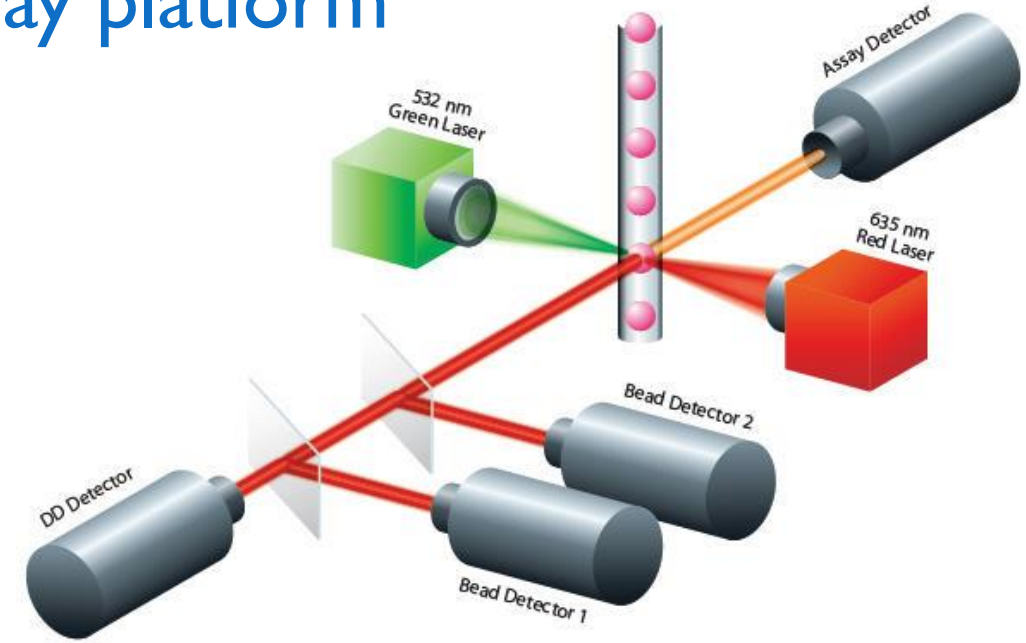
Transfer to quantitative, high-throughput multiplex technique: modified Luminex

- Bead-based technology
- Suitable for multiplexing
- Antibody-based assay
  - Directly translate from microarray platform







ROC analysis of biomarker combination in Japanese cohort

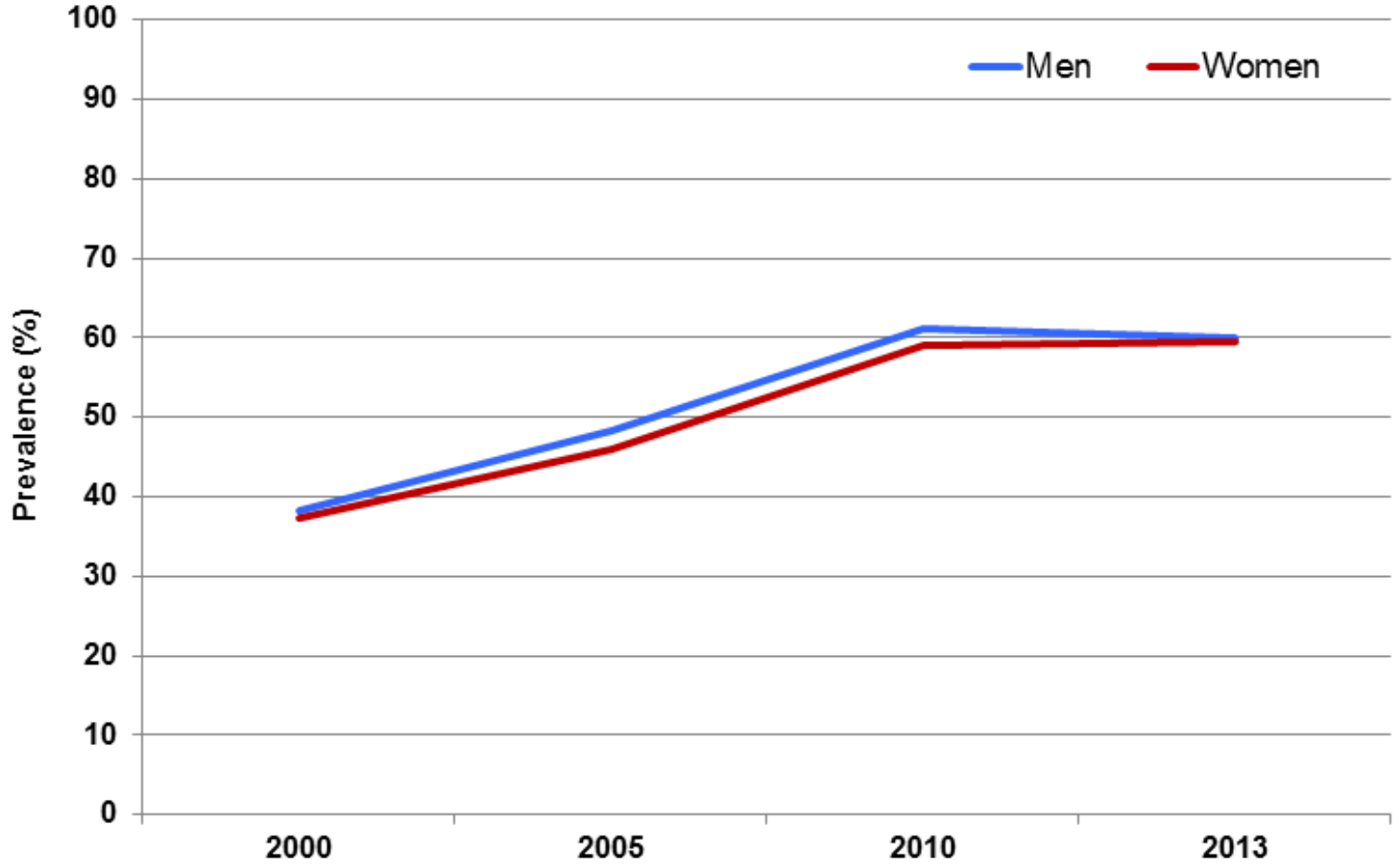
Case	n	Ctrl	n	BAG4+IL6ST+VWF+CD44*+EGFR*			
				AUC	95%CI	Sens	95%CI
I	114	All ctrls	386	0.79	0.74-0.85	62.3%	0.54-0.71
II	155	All ctrls	386	0.85	0.81-0.89	71.6%	0.63-0.79
III	147	All ctrls	386	0.88	0.85-0.92	77.6%	0.68-0.85
IV	98	All ctrls	386	0.90	0.86-0.95	81.6%	0.73-0.89
All cancer	514	All ctrls	386	0.86	0.83-0.88	73.0%	0.68-0.78
All cancer	514	Normal	168	0.84	0.81-0.87	70.0%	0.62-0.75
All cancer	514	Polyps	159	0.87	0.85-0.90	76.5%	0.72-0.81
All cancer	514	Colitis	59	0.87	0.84-0.90	73.3%	0.66-0.80

Sera: 514 cases, 386 controls (168 normal, 159 low risk polyps, 59 UC)

Intended clinical applications

1. Develop a test that improves on existing fecal tests, such as FIT and Cologuard for use in people unwilling to undergo or with no access to colonoscopy who should be prioritized for colonoscopy (i.e., improve/replace existing FIT and Cologuard tests)
2. Among symptomatic patients identify those who have a very low risk of cancer and can avoid colonoscopy.

Trends in Colorectal Cancer Screening, Adults 50 years and older



*Either a fecal occult blood test within the past year or sigmoidoscopy within the past 5 years or colonoscopy within the past 10 years. Note: Estimates are age adjusted to the 2000 US standard population.

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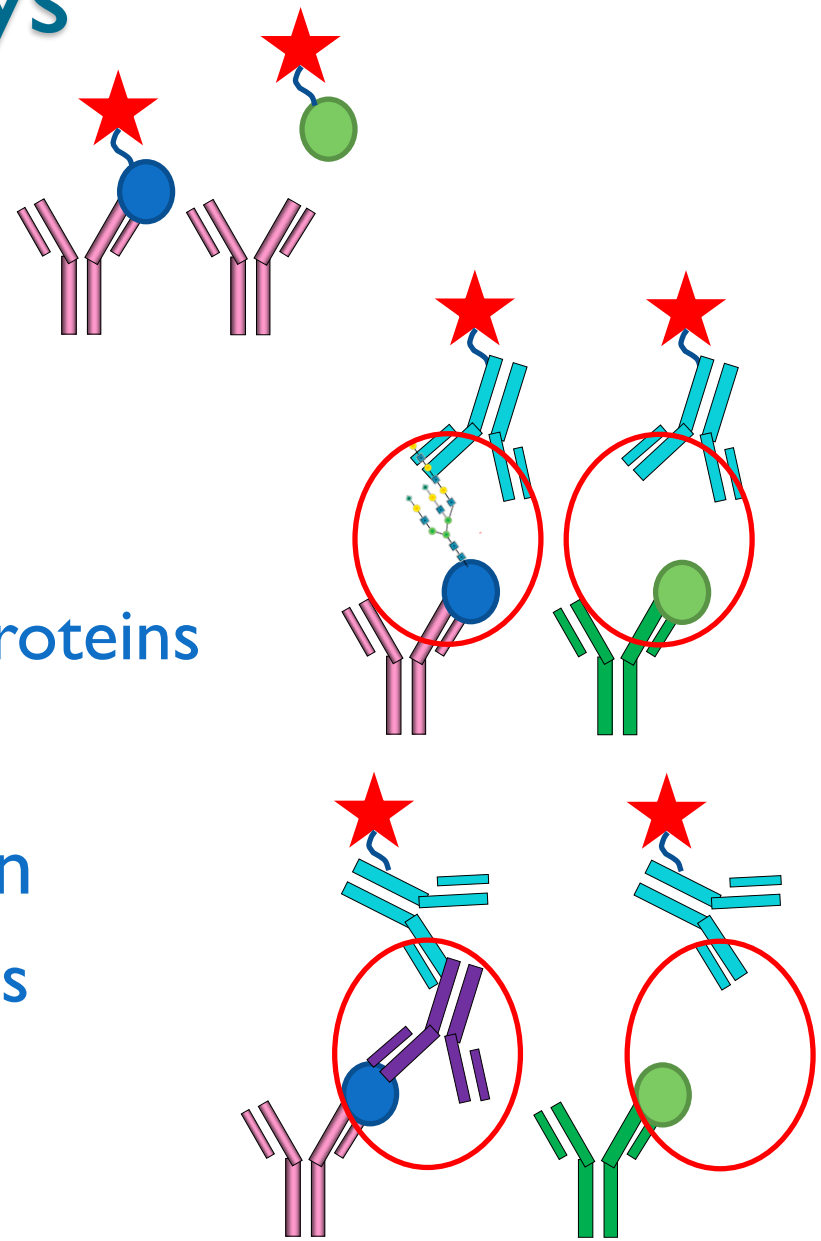
Source: American Cancer Society

Versatility of Antibody Arrays

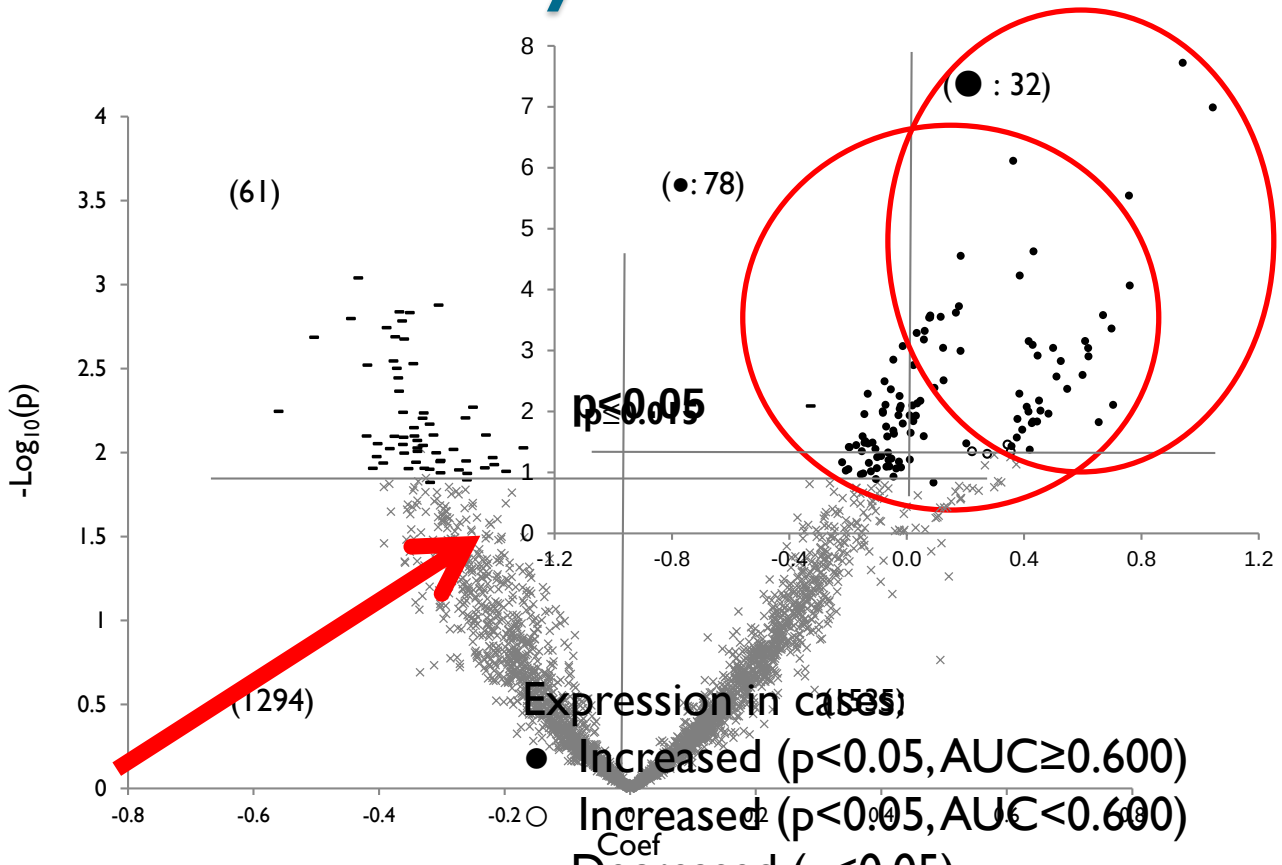
- Proteomic analysis
  - Abundance of individual proteins
- Glycomic analysis
  - Sialyl Lewis A/X features on specific proteins
- Autoantibody complex with antigen
  - Immune response to aberrant proteins



Completed Colon cancer studies

- Cardiovascular Health Study (CHS)
  - Prediagnostic samples (≤ 3 years prior to diagnosis)
  - 79 cancers, 79 matched controls
- EDRN samples (GLNE 7)
  - Diagnostic (cancers and adenomas)
  - 60 cancers, 60 adenomas (30 advanced), 60 controls
- Ogaki Hospital samples from WAKO Diagnostics
  - Diagnostic, PRoBE-compliant
  - 514 cancers, 168 healthy controls, 218 low-risk controls

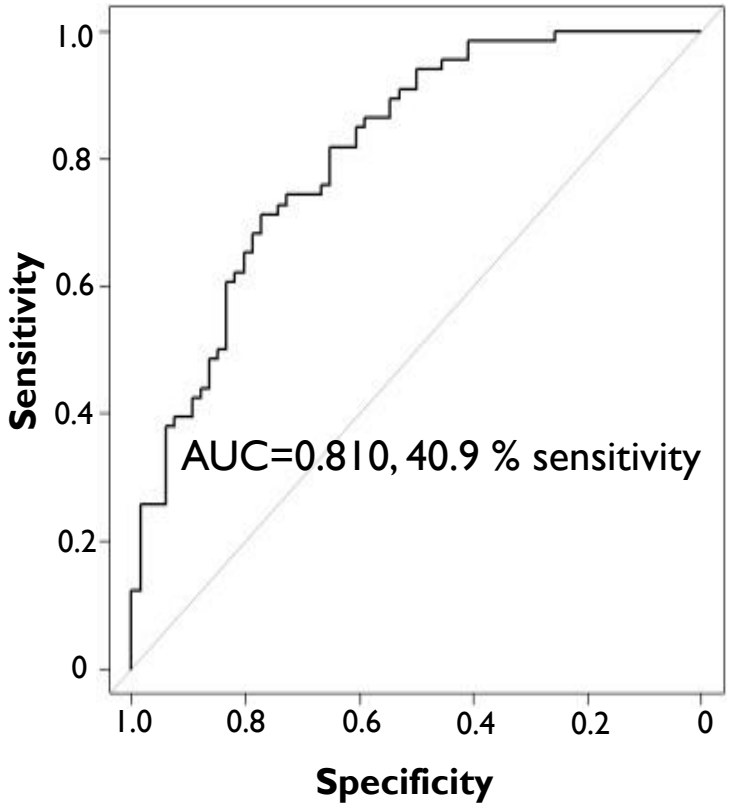
Biomarker Discovery in CHDRN



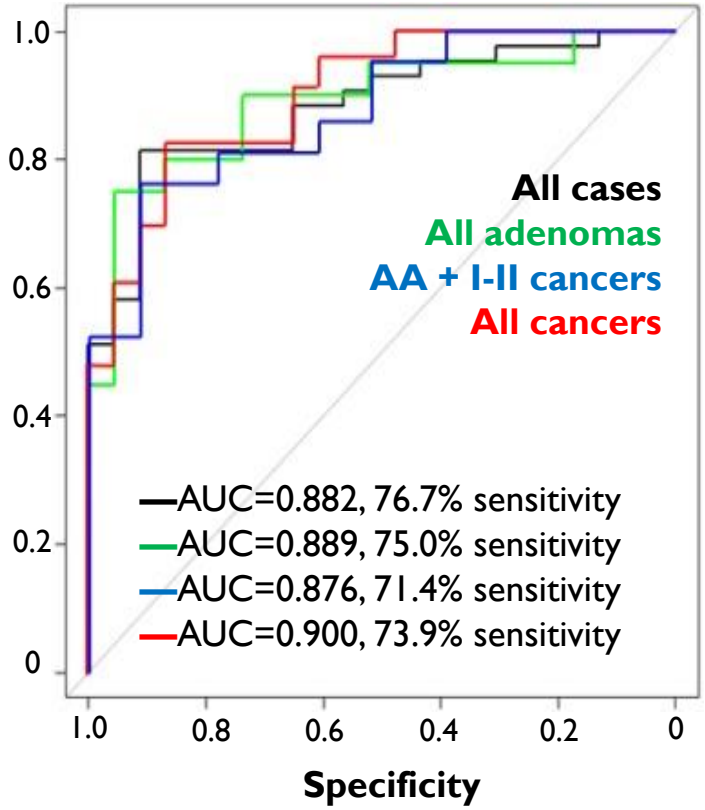
From Rho, et. al, 2016

Biomarker combinations

CHS Prediagnostic plasma



EDRN Diagnostic plasma



BAG4, IL6ST, VWF and EGFR or CD44 were selected

Sensitivities are based on 90% specificity

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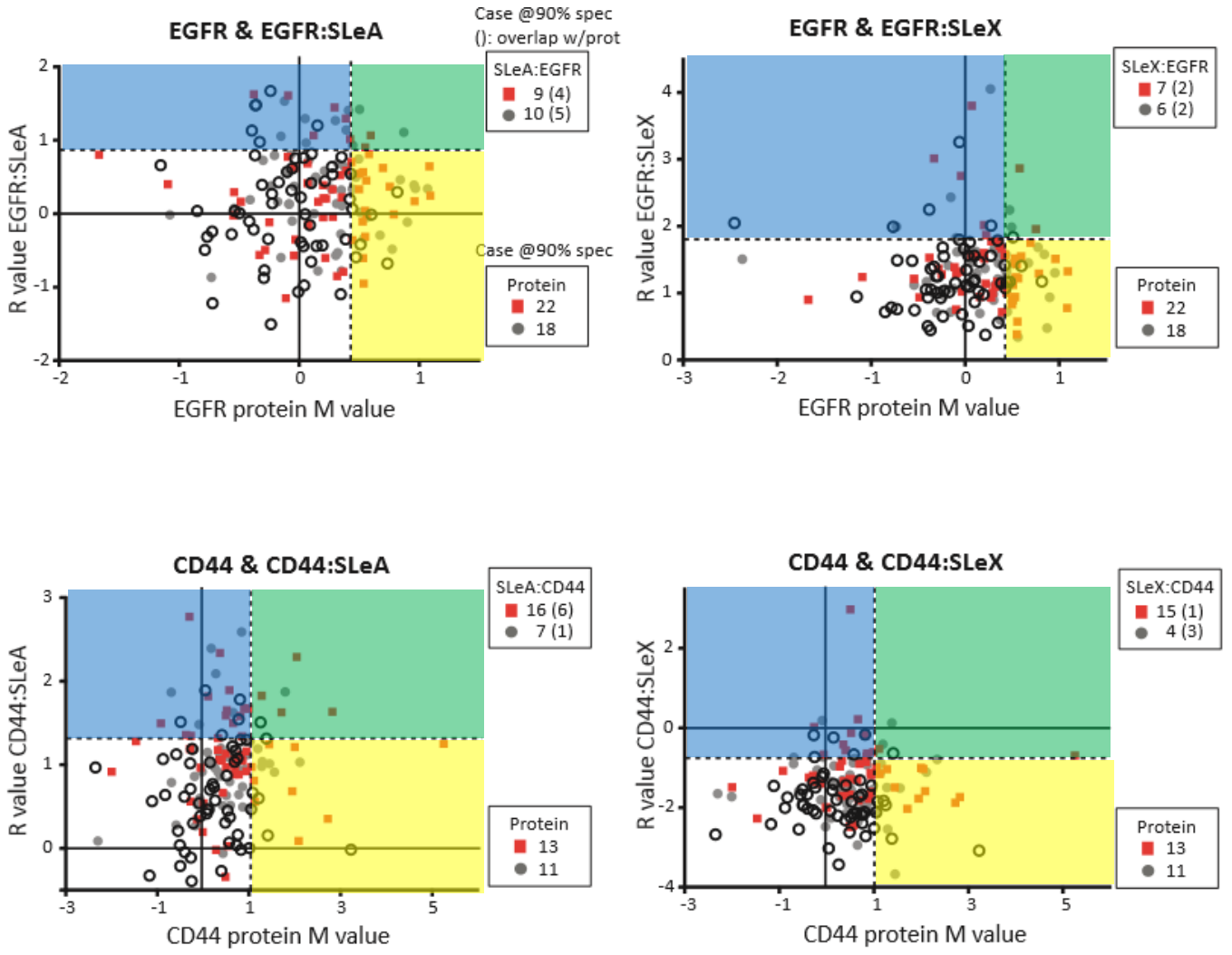
EGFR and CD44 glycosylation



High in SLe
Low in Prot

High in SLe
High in Prot

Low in SLe
High in Prot

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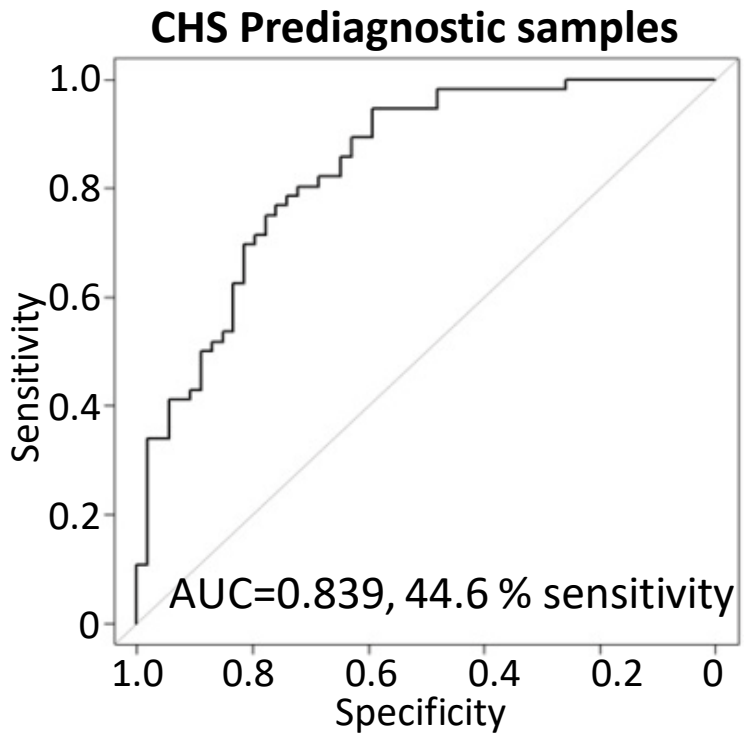
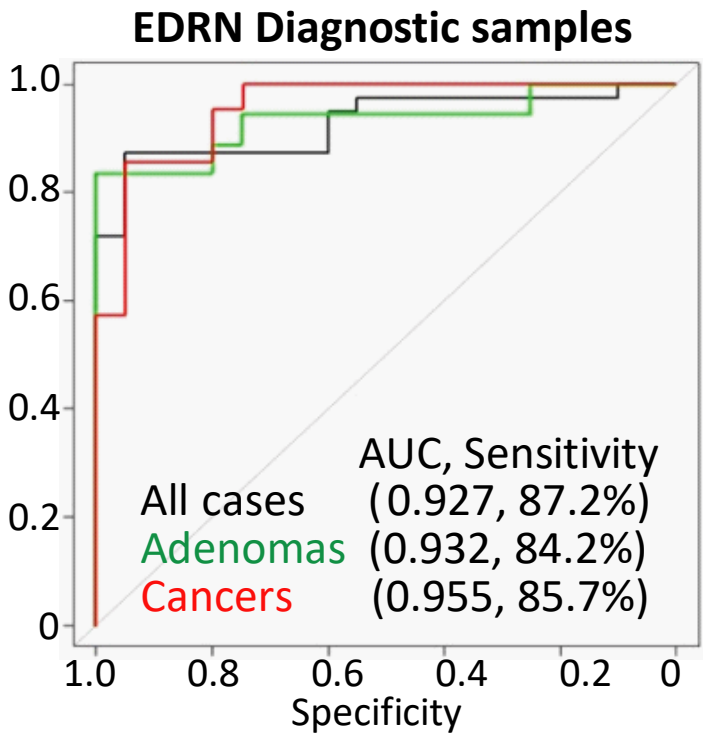
From Rho, et. al, 2018

Improving the protein panel

EGFR glycomics





Additionally, the protein and glycomic hybrid panel had 83.3% sensitivity (AUC=0.933) for the advanced adenoma plus stage I-II cancers

Overall, the glycomic hybrid characteristic added 3.7%-11.9% sensitivity (average 9.4%) at 90% specificity to the four protein marker panel.

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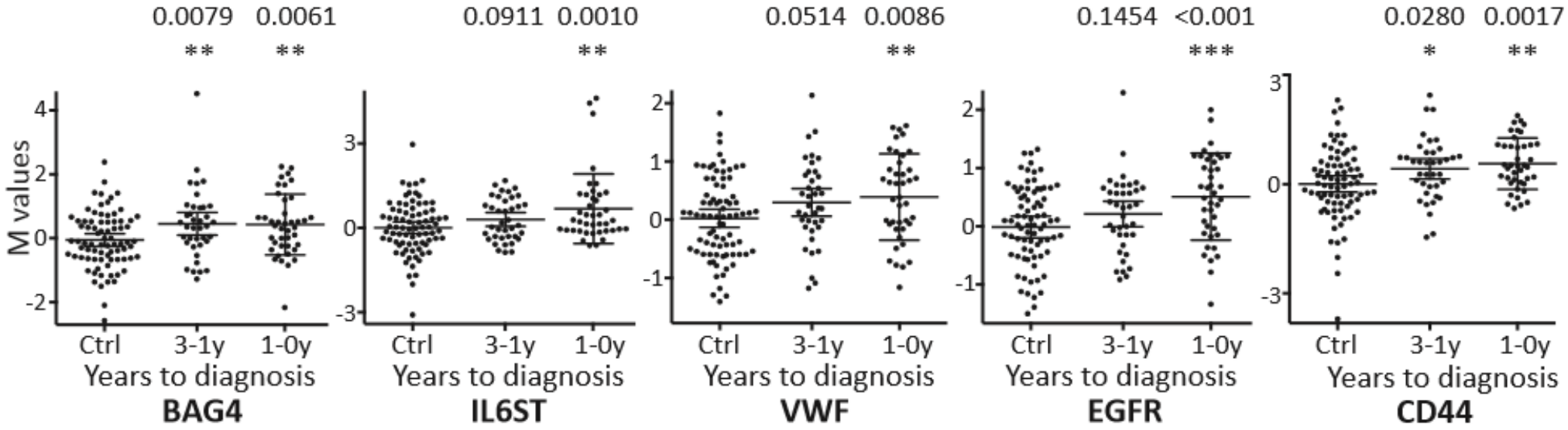
Time prior to diagnosis



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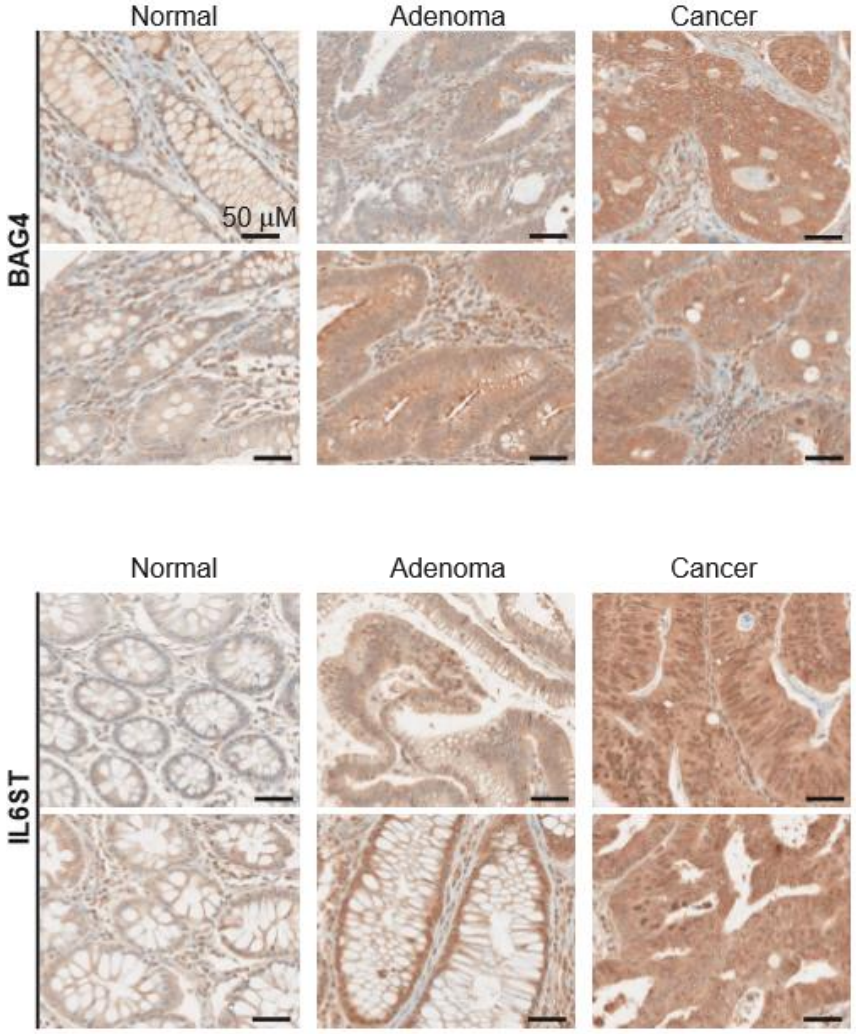
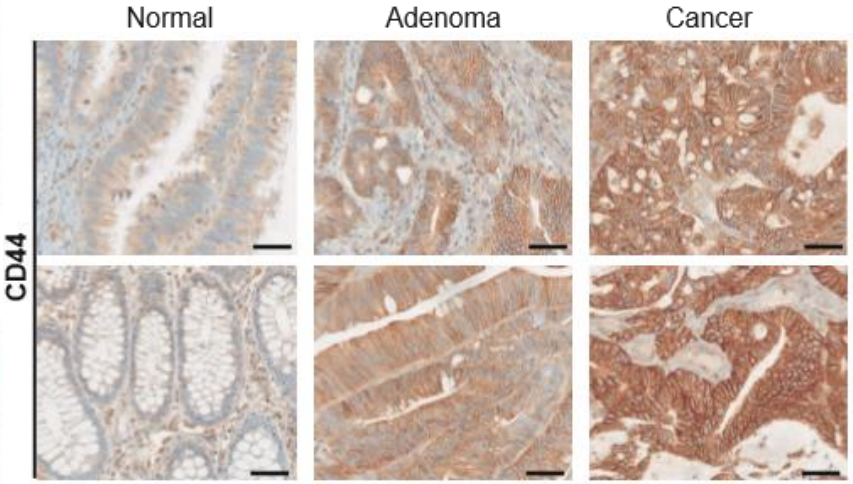
From Rho, et. al, 2018

Are the markers increased in tumor tissue?





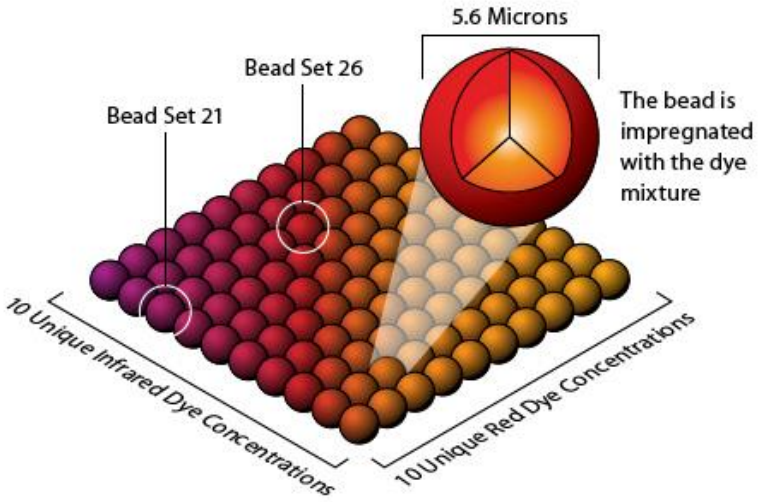
EGFR was also elevated, though not significantly (p~0.1)

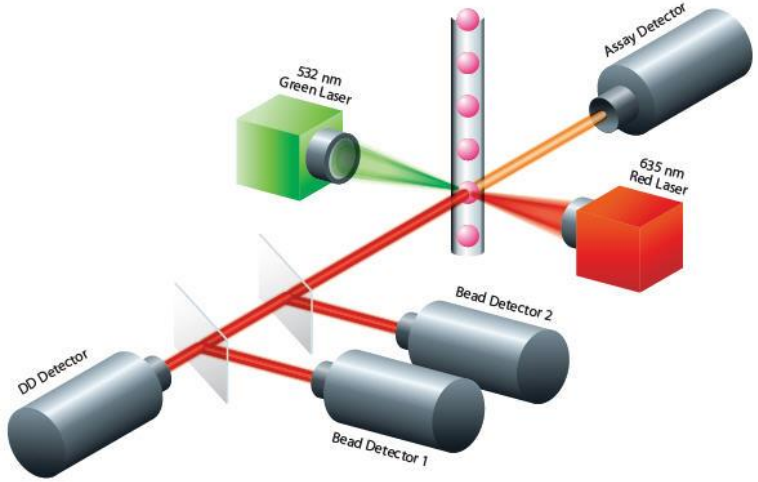
vWF showed no epithelial staining

From Rho, et. al, 2018

Transferring to quantitative, high-throughput techniques: Luminex

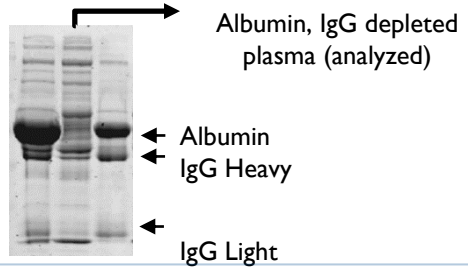
- Bead-based technology
- Suitable for multiplexing
- Antibody-based assay
  - Directly translate microarray platform



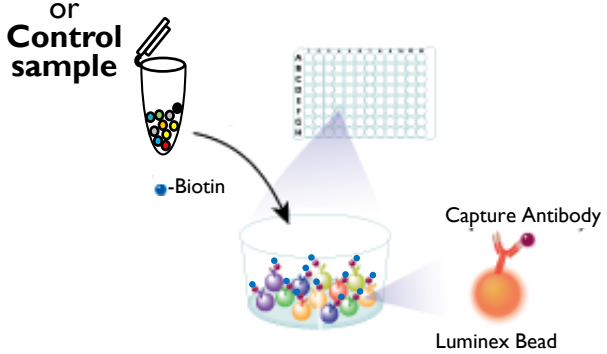


Modified Luminex protocol

1. Depletion of plasma abundant proteins

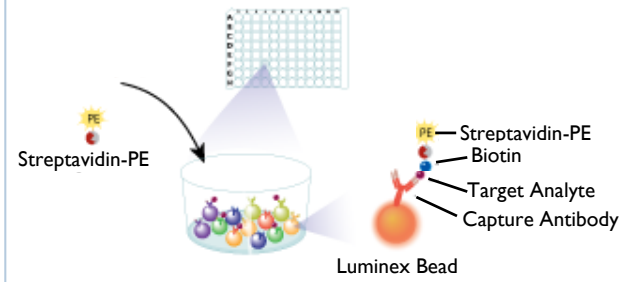


2. Labeling of plasma proteins and array incubation



Antibodies covalently linked to individual beads

3. Incubate with PE-labeled Streptavidin



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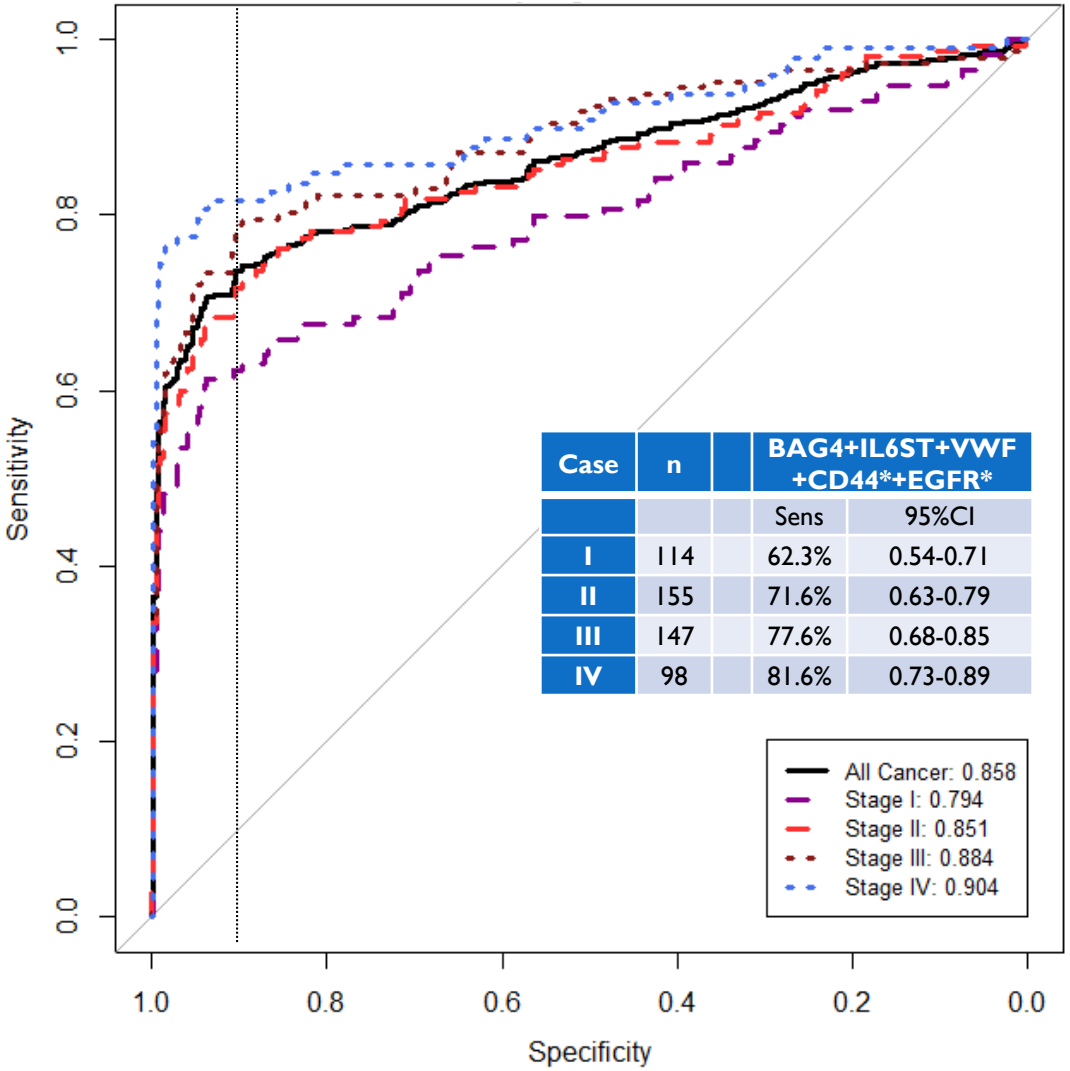
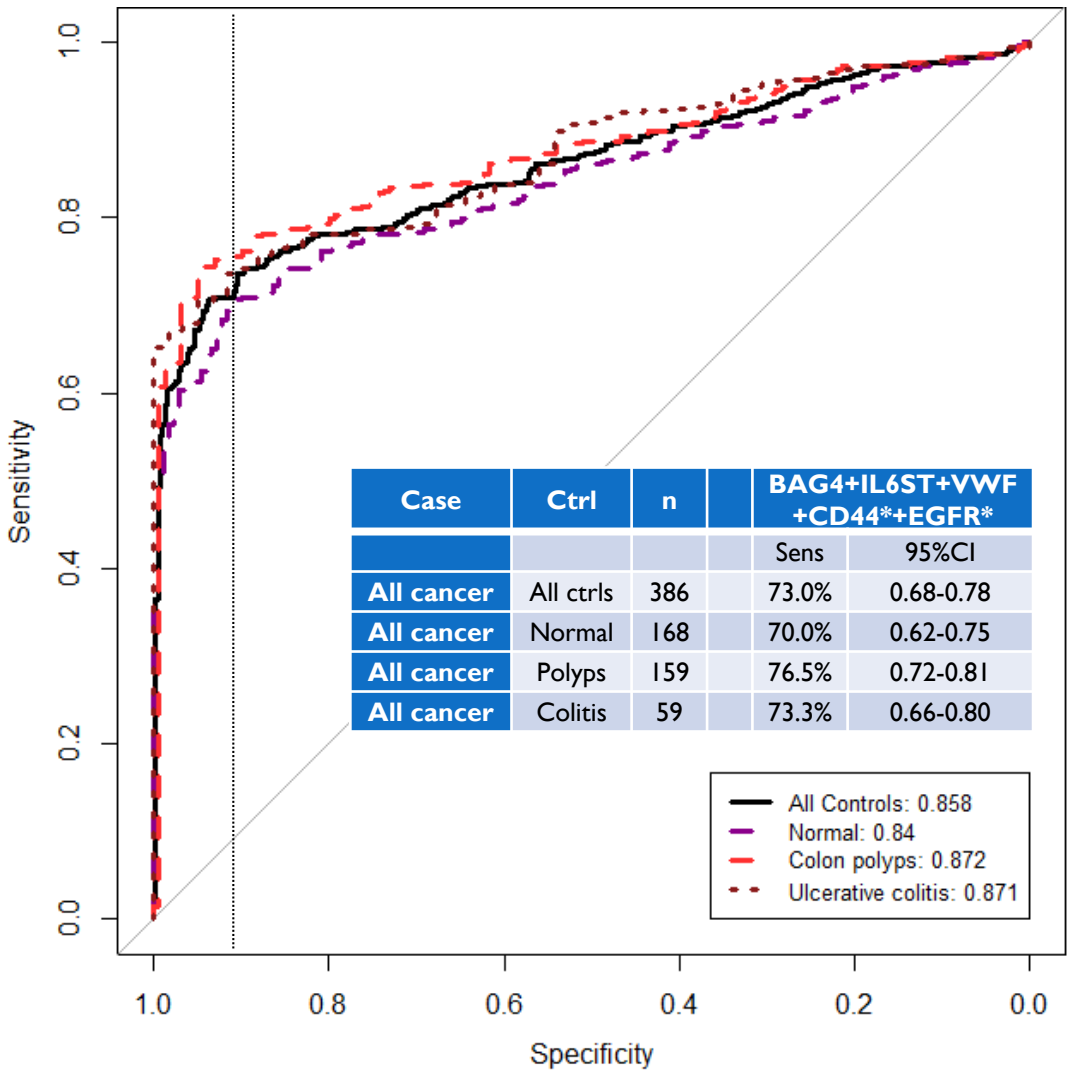
Images adapted from rndsystems.com

Panel performance in Ogaki Samples



Case	n		BAG4+IL6ST+VWF +CD44*+EGFR*	
			Sens	95%CI
I	114		62.3%	0.54-0.71
II	155		71.6%	0.63-0.79
III	147		77.6%	0.68-0.85
IV	98		81.6%	0.73-0.89



Case	Ctrl	n		BAG4+IL6ST+VWF +CD44*+EGFR*	
				Sens	95%CI
All cancer	All ctrls	386		73.0%	0.68-0.78
All cancer	Normal	168		70.0%	0.62-0.75
All cancer	Polyps	159		76.5%	0.72-0.81
All cancer	Colitis	59		73.3%	0.66-0.80

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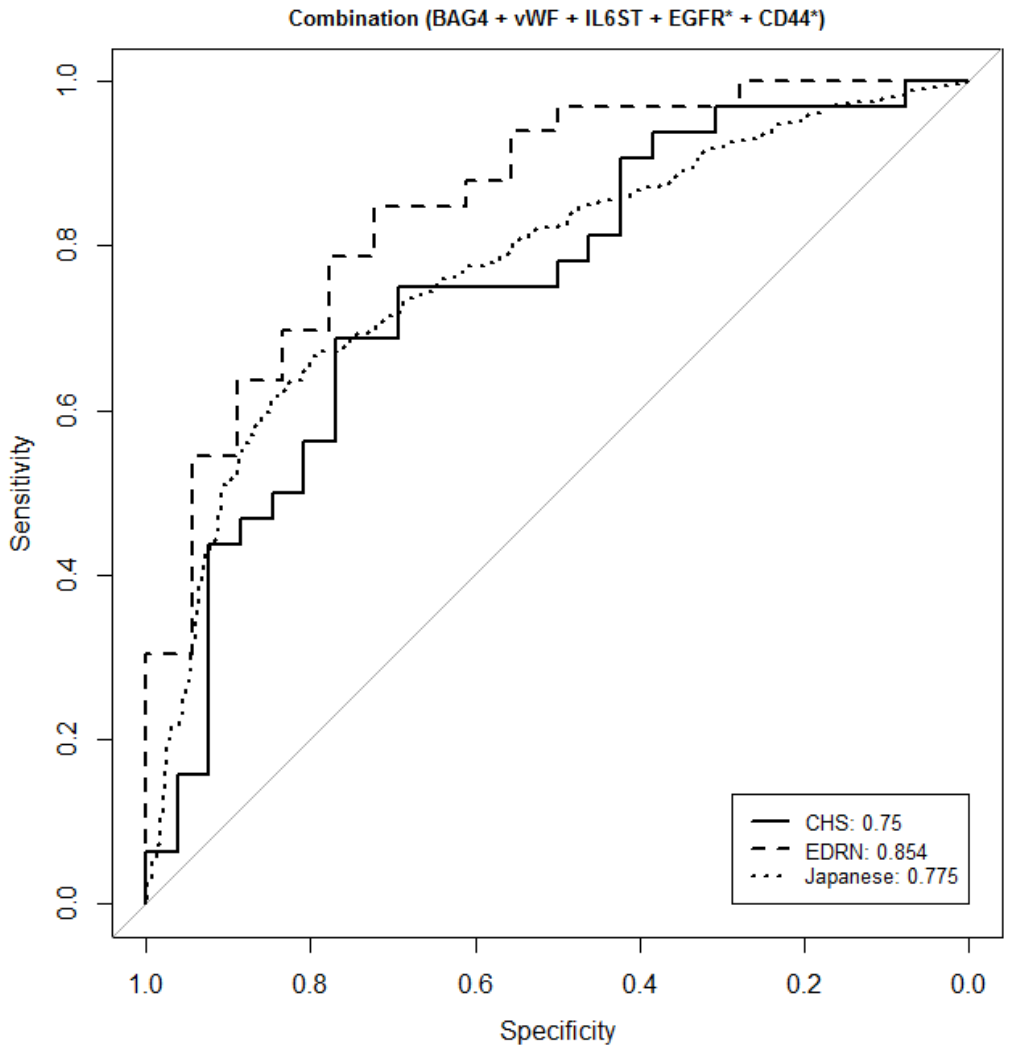
From Rho, et. al, 2018

Panel performance across all 3 sample sets



	CHS	EDRN	Ogaki
AUC	0.75	0.854	0.775
95% CI	0.62-0.88	0.74-0.96	0.74-0.81
Sens	40.6%	54.5%	51.4%
95% CI	0.03-0.72	0.18-0.85	0.41-0.59
Opt Spec	76.9%	72.2%	84.7%
Opt Sens	68.8%	84.8%	61.7%

Equal weight combination

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Protein and glycomic plasma markers for early detection of adenoma and colon cancer

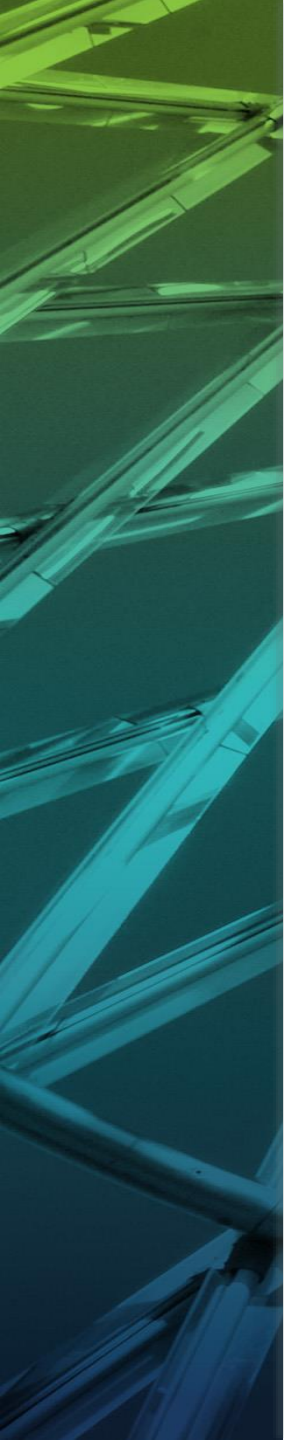
Gut, 2018 Mar, 67(3): 473-484. PMID: 27821646 PMC:5420499

Comparison of our panel to other non-invasive tests

	FHCRC		FIT		Cologuard		CEA		SEPT9	
Sample	Blood		Feces		Feces		Blood		Blood	
Collection site	Drs. office		Home		Home		Drs. Office		Drs. Office	
	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec
Adenoma	72%	90%	15-44%	95%	42%	86%	-	-	18%	80%
Cancer	75-85%	90%	50-79%	95%	92%	86%	40%	30-80%	37-96%	81-99%

Major advantages:

- Blood test vs. fecal test
- Taken at clinic vs. home collection



Creating In-House Antibodies

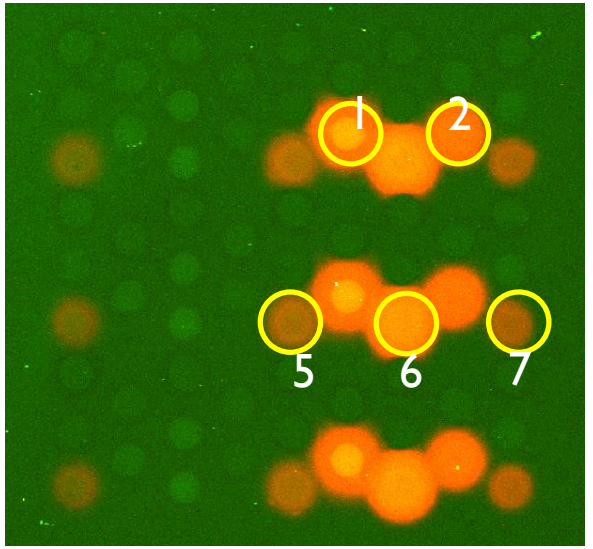
Produce our own monoclonal antibodies to the markers

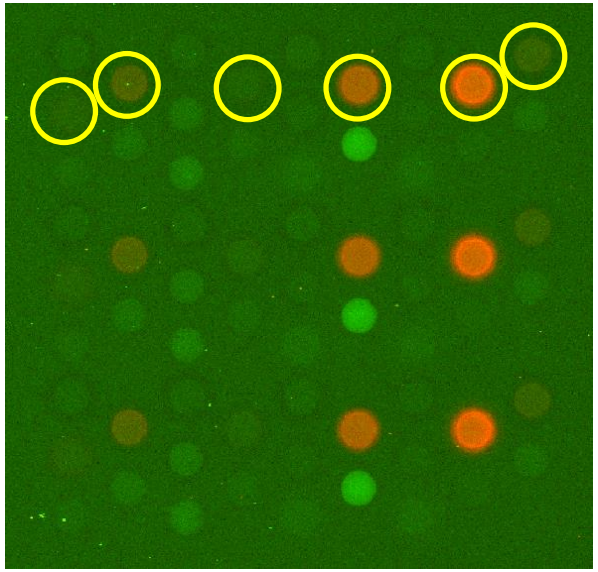
- Helps solve the issue of passing on assays to commercial interests or BRL
- Eliminates loss of access to consistent antibody
- Dramatically reduces cost for testing in large sample sets
- Excellent, cost efficient monoclonal production facility
- Lab has extensive clone screening experience

- Have produced useful monoclonals to BAG4, CD44, IL6ST and EGFR.

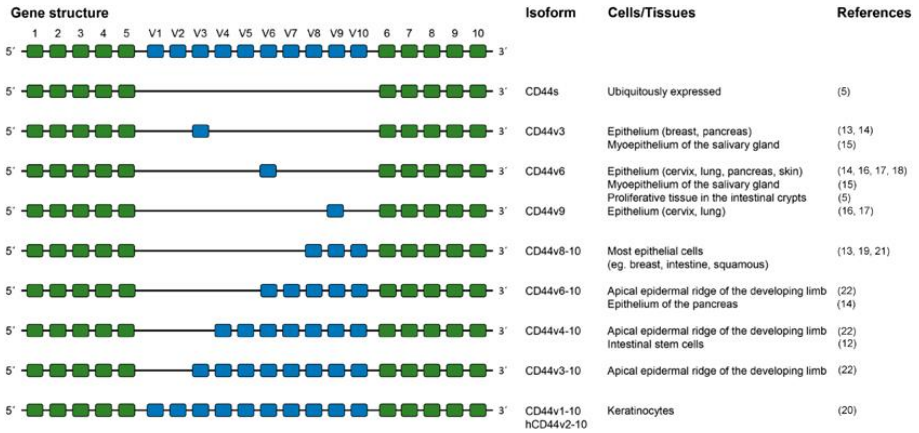
In-House Antibodies

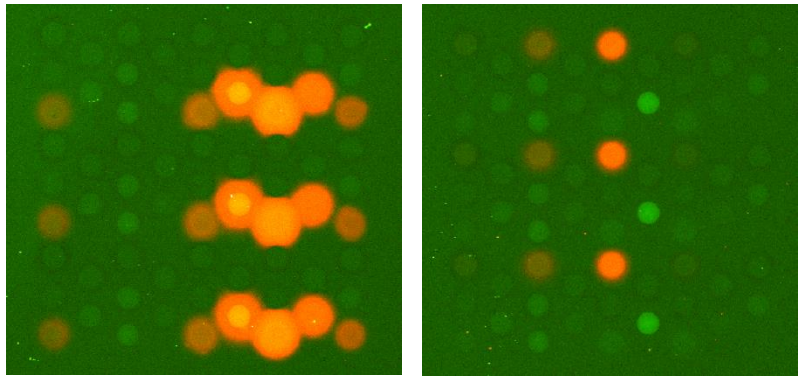
- Commercial Ab Epitope Mapping
  - SDIX 100aa sequence
    - Tiled 20-mers
    - 7aa overlap
- Make KLH-Peptides to Epitope
- Validate mAbs
- Microarray analysis
- WB analysis





CD44 Antibody Development



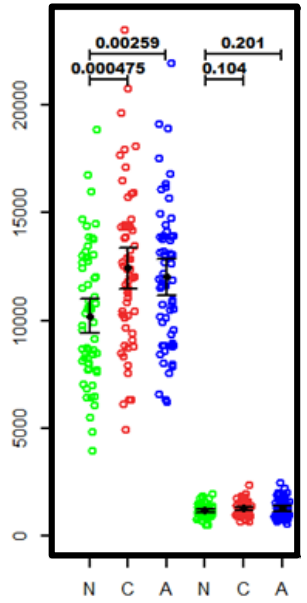


V3 & V4 Binding

V7 Binding

	Peptide	Ab1 (Array)	Ab2 (Array)	Ab3 (Glyco)
Invariant	Pep_Inv_1			
	Pep_Inv_2			
	Pep_Inv_3			
	Pep_Inv_4			
	Pep_Inv_5			
	Pep_Inv_6			
v3	Pep_v3_1			
	Pep_v3_2			
	Pep_v3_3			
v4	Pep_v4_1			
	Pep_v4_2			
	Pep_v4_3			
	Pep_v4_4			
v5	Pep_v5_1			
	Pep_v5_2			
	Pep_v5_3			
v6	Pep_v6_1			
	Pep_v6_2			
	Pep_v6_3			
	Pep_v6_4			
v7	Pep_v7_1			
	Pep_v7_2			
	Pep_v7_3			
	Pep_v7_4			

Strong binding
Moderate binding
Weak binding



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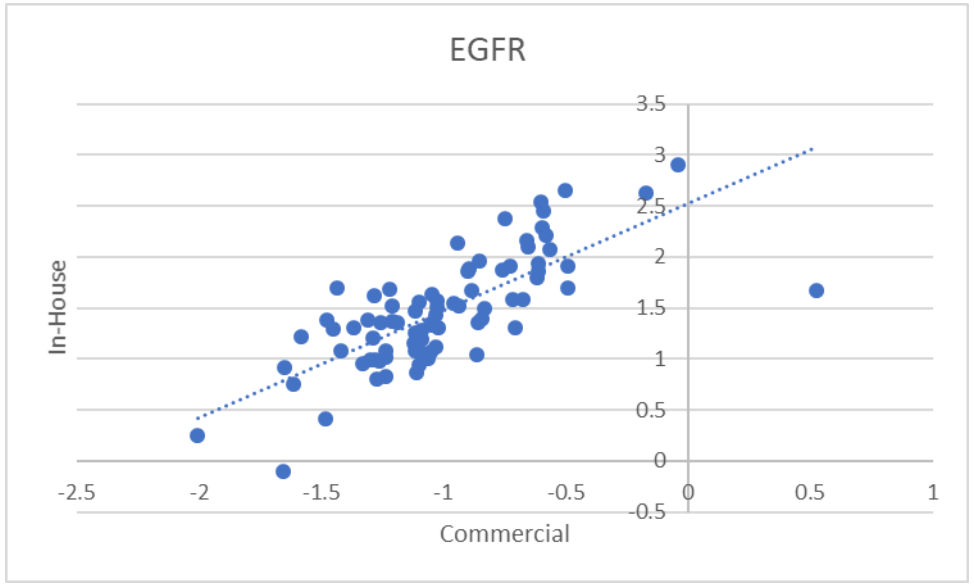
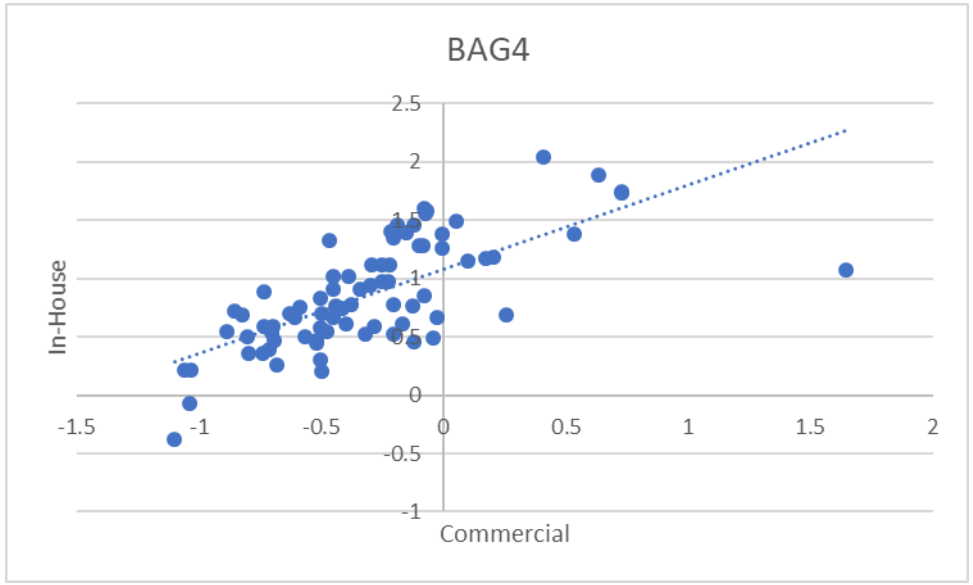
In-House Antibodies





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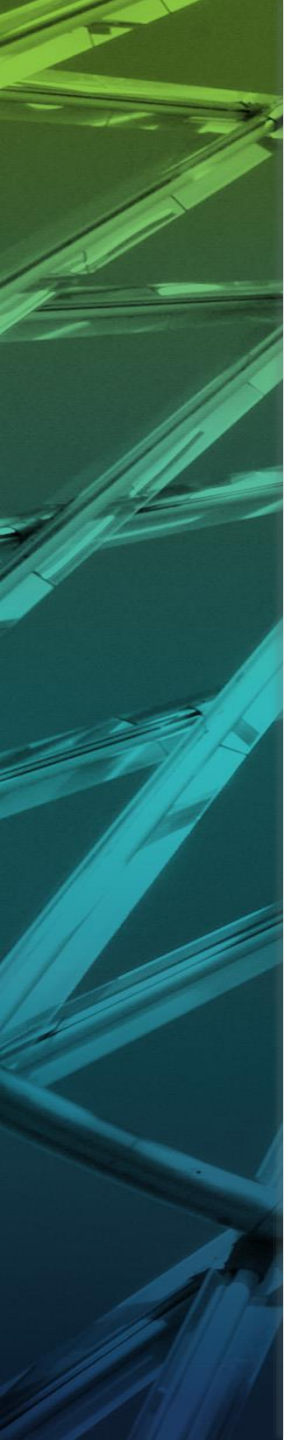


Marker evaluation in large screening cohorts

High-throughput Screens

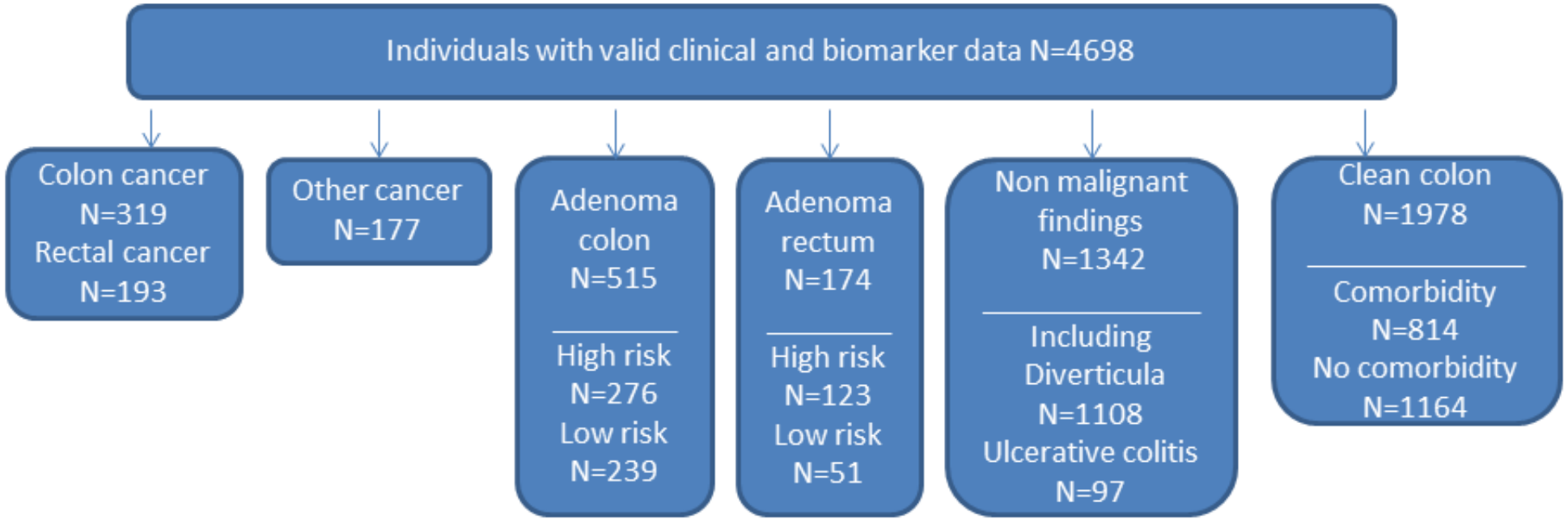
- EGFR
  - Created sandwich assay
    - Capture: In-house Ab
    - Detection: Commercial Ab (commercially biotinylated)
- vWF
  - Created sandwich assay
    - Capture: Commercial Ab
    - Detection: Commercial Ab (commercially biotinylated)
- IL6ST
  - Commercial sandwich assay
- BAG4
  - Created sandwich assay
    - Capture: In-house Ab
    - Detection: Commercial Ab (in-house biotinylation)
- CD44
  - Created sandwich assay
    - Capture: In-house Ab
    - Detection: In-house Ab (in-house biotinylation)
- Sialyl-Lewis A

HRP ELISA

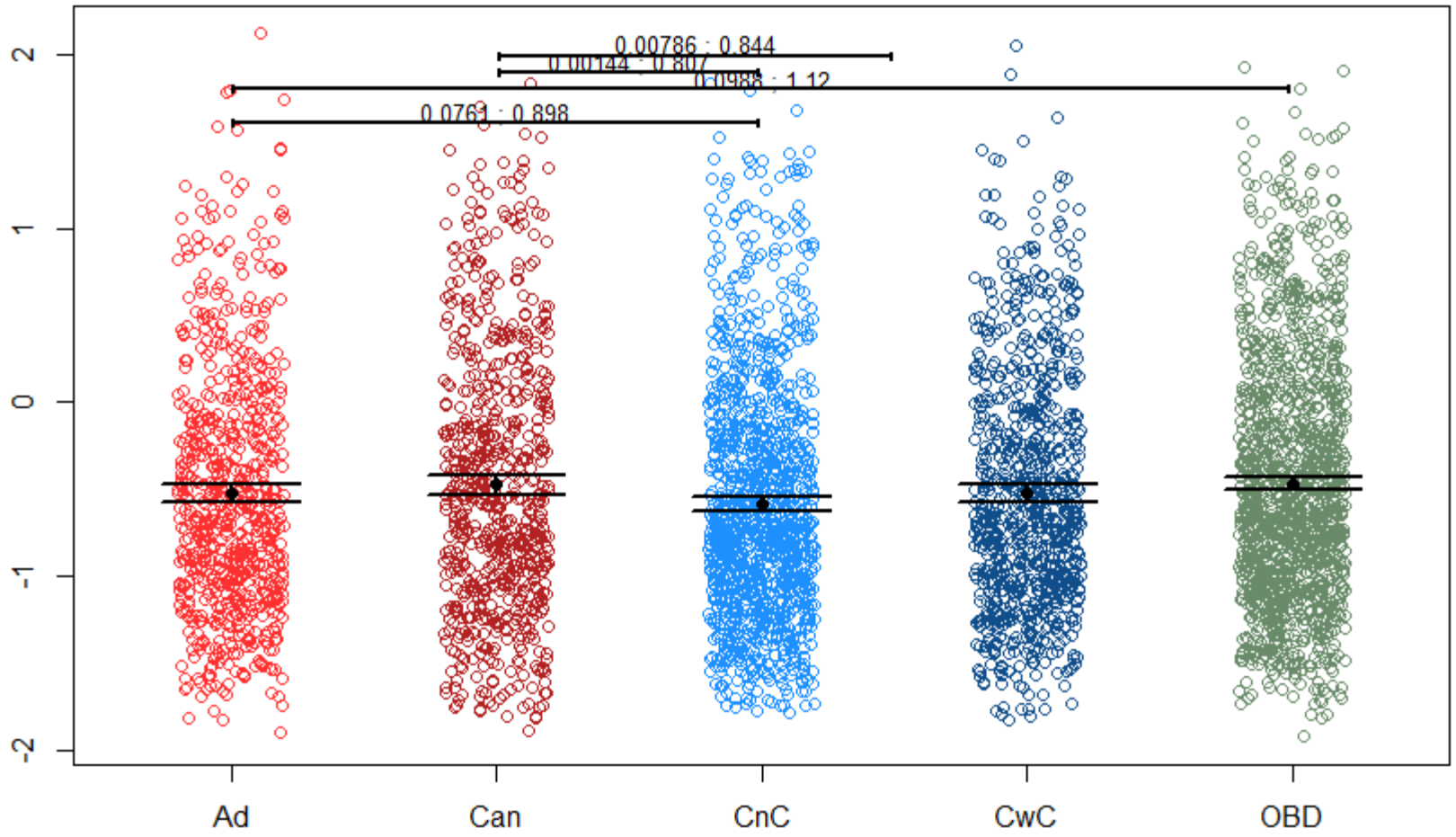
Chemiluminescence

Endoscopy II screening cohort

- Danish Screening Cohort
- Samples collected at time of colonoscopy
- 4,698 samples



EGFR



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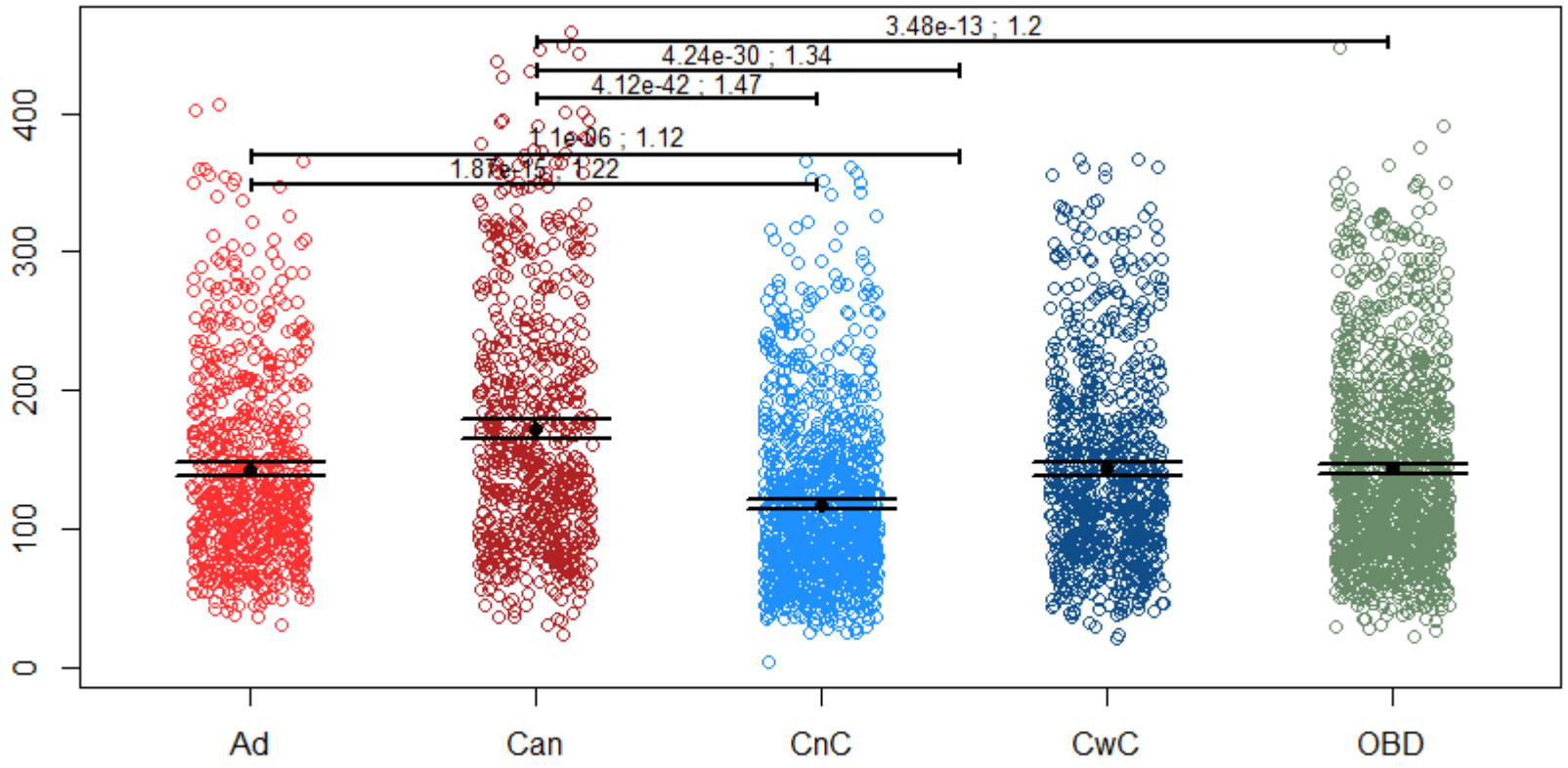
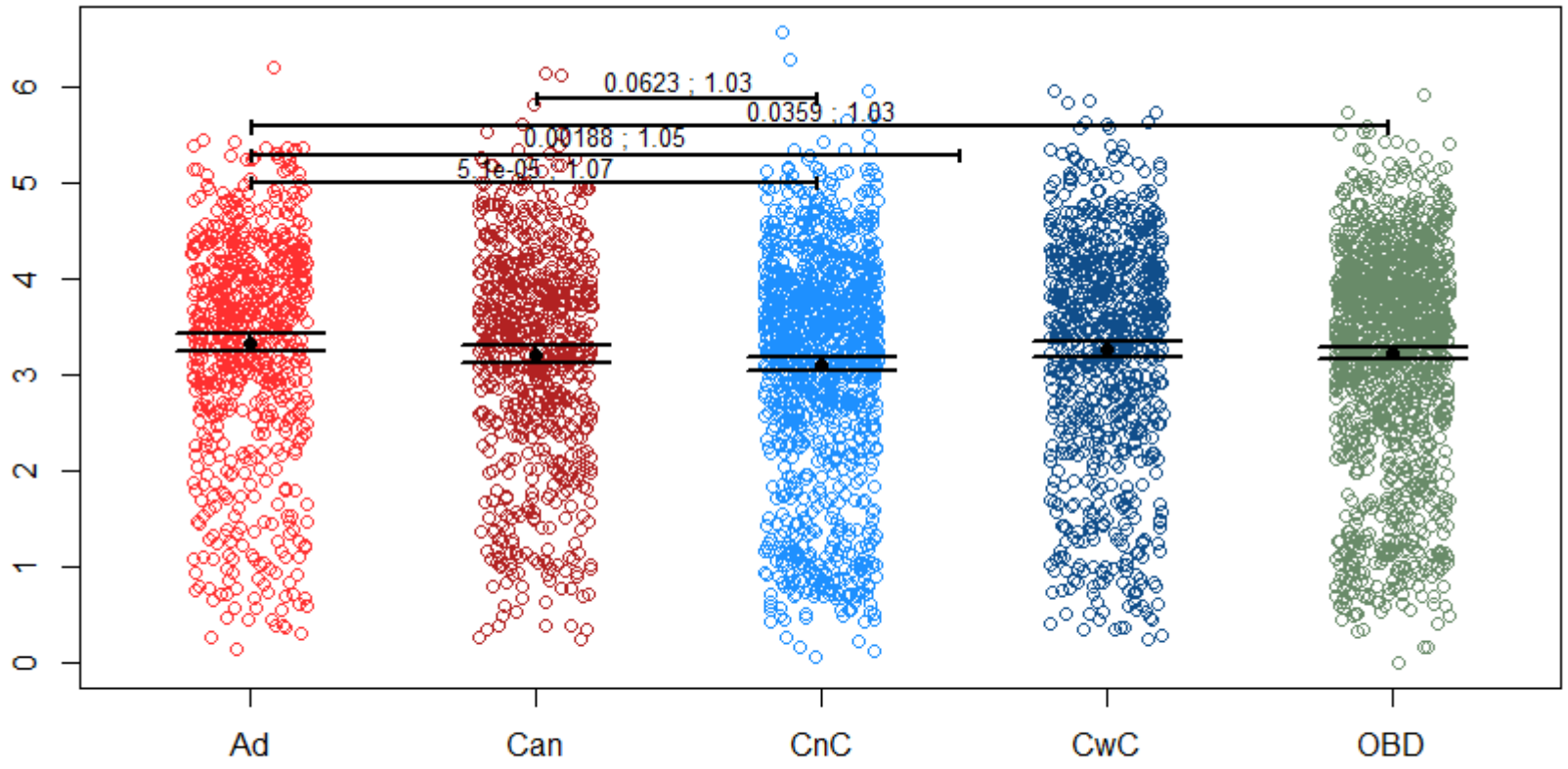
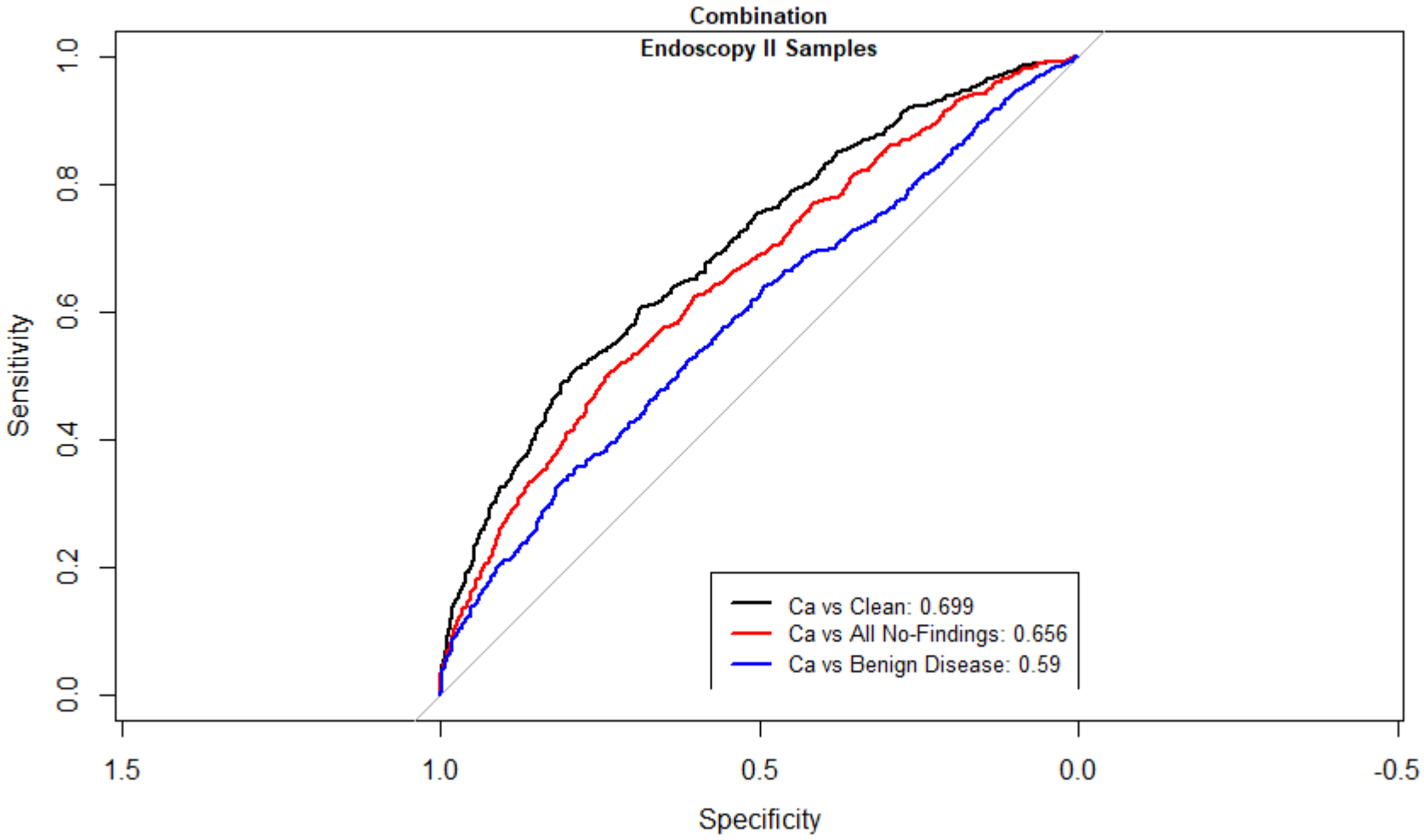
vWF


3.48e-13 ; 1.2
4.24e-30 ; 1.34
4.12e-42 ; 1.47
1e-06 ; 1.12
1.87e-15 ; 1.22
0
100
200
300
400
Ad
Can
CnC
CwC
OBD

IL6ST

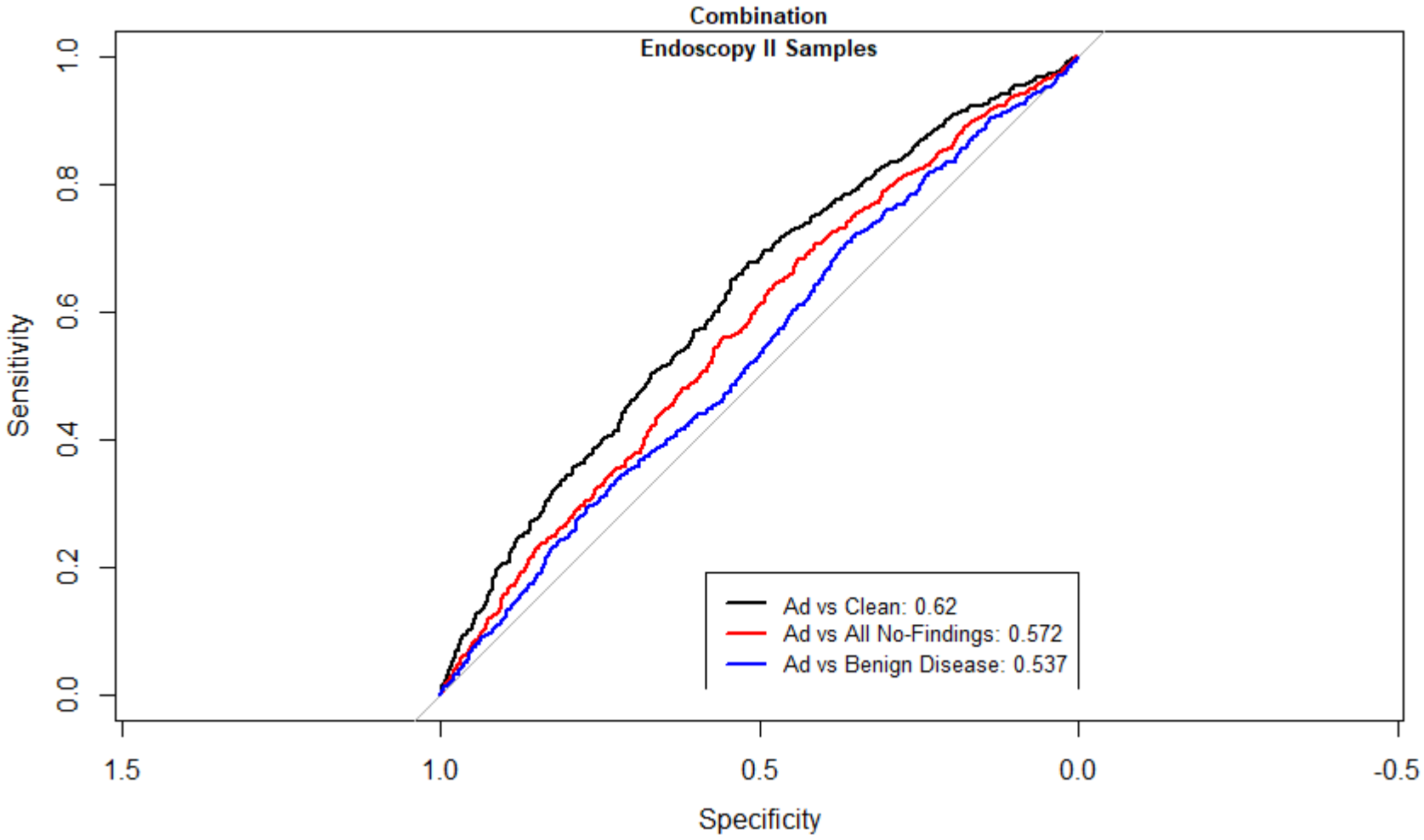

0.0623 ; 1.03
0.0359 ; 1.03
0.00188 ; 1.05
5.1e-05 ; 1.07
0
1
2
3
4
5
6
Ad
Can
CnC
CwC
OBD

Linear combination of 3 markers



FRED HUTCH

Linear combination of 3 markers



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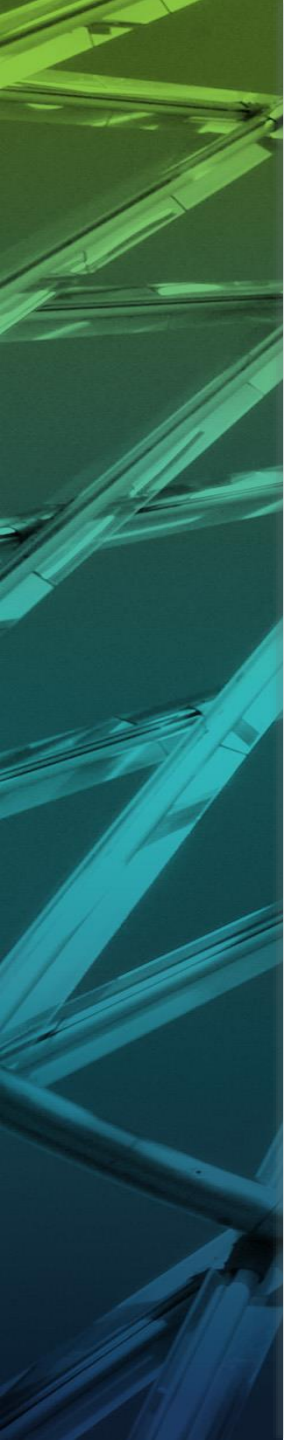
Endoscopy III Screening Cohort

- Danish Screening Cohort with known FIT +/-
  592 Samples
  - 100 FIT-, CRC-
  - 100 FIT+, CRC-
  - 100 Adeno: High & Medium Risk
  - 92 FIT+, CRC+
    - 25 each of Stage I, II, III ; 17 Stage IV
  - 100 FIT-, Cancer+ (to be identified)
- WHI – prediagnostic plasma samples drawn 2 years prior to a CRC diagnosis 284 cases, 568 controls



Questions?

Bowel Evacuation Project

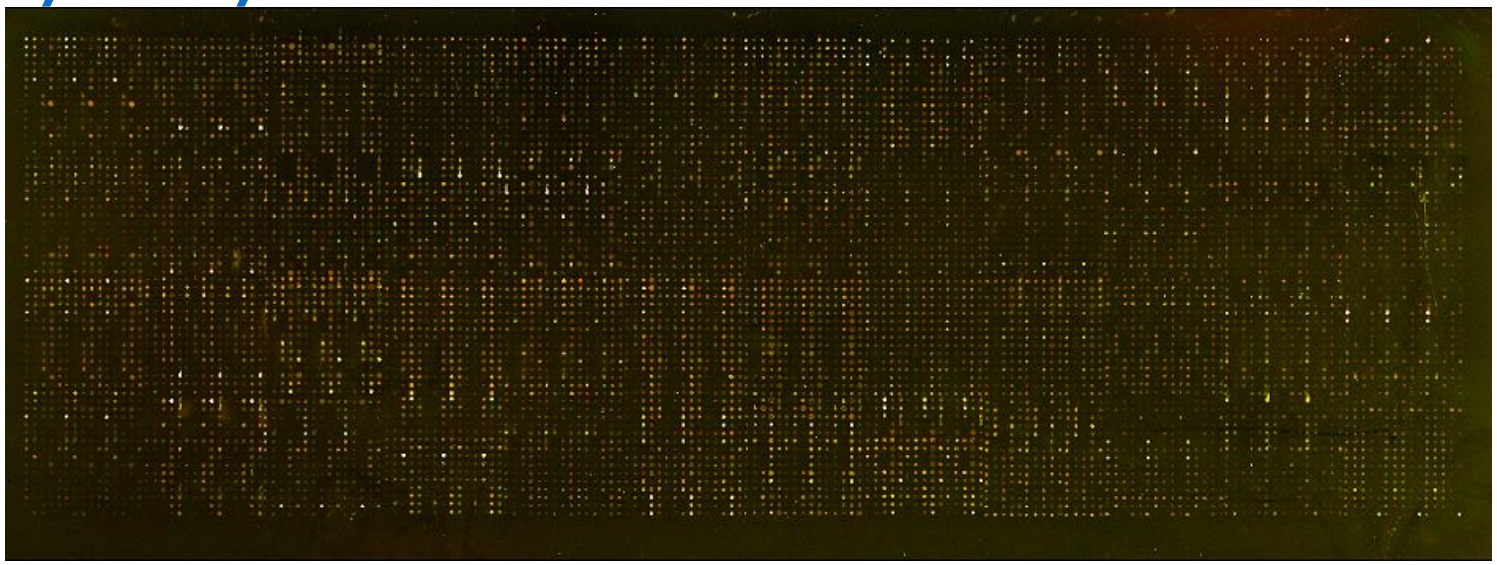
- Effect of bowel prep on protein levels
- Effect of sample prep (centrifugations) on protein levels

- 504 samples from 126 individuals

- More differences from sample prep than bowel prep

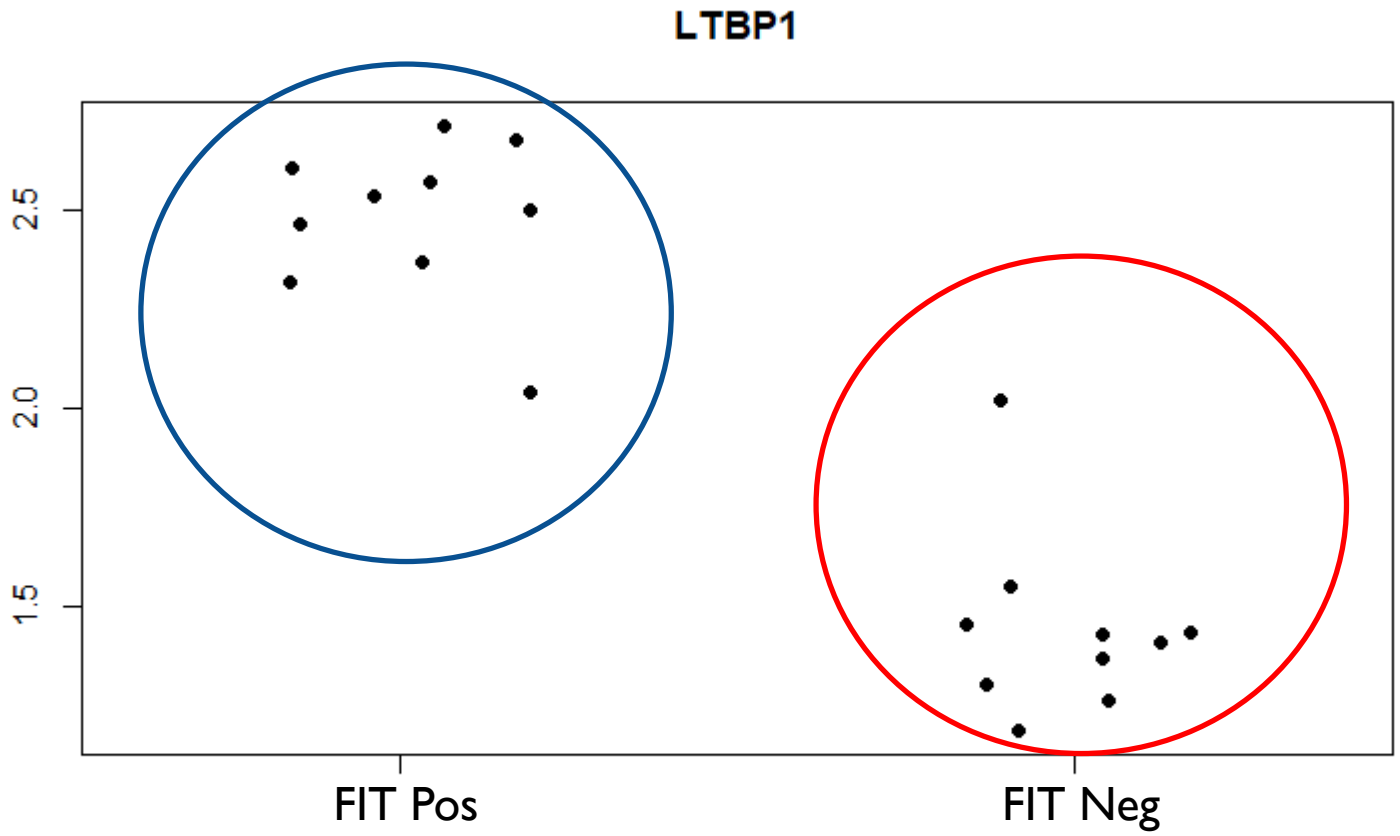
Endoscopy III Samples

- 5 FIT-/CRC+, 5 FIT-/CRC-, 5 FIT+/CRC+, 5 FIT+/CRC-
  - Samples matched on Age, Gender, BMI
- Proteomics on Large-Format Arrays (3600 Antibodies)
- Pathway analyses – enrichR



FIT is a strong confounder

- 2974 fferent (p<0.
- All bu amples



Mixed Effect Model for Statistical Analysis

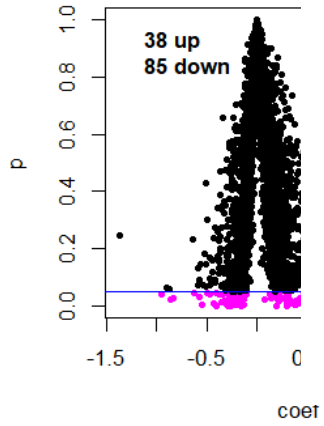
- Combines FIT and Colonoscopy findings into a "case"
  - True or False Positive, True or False Negative

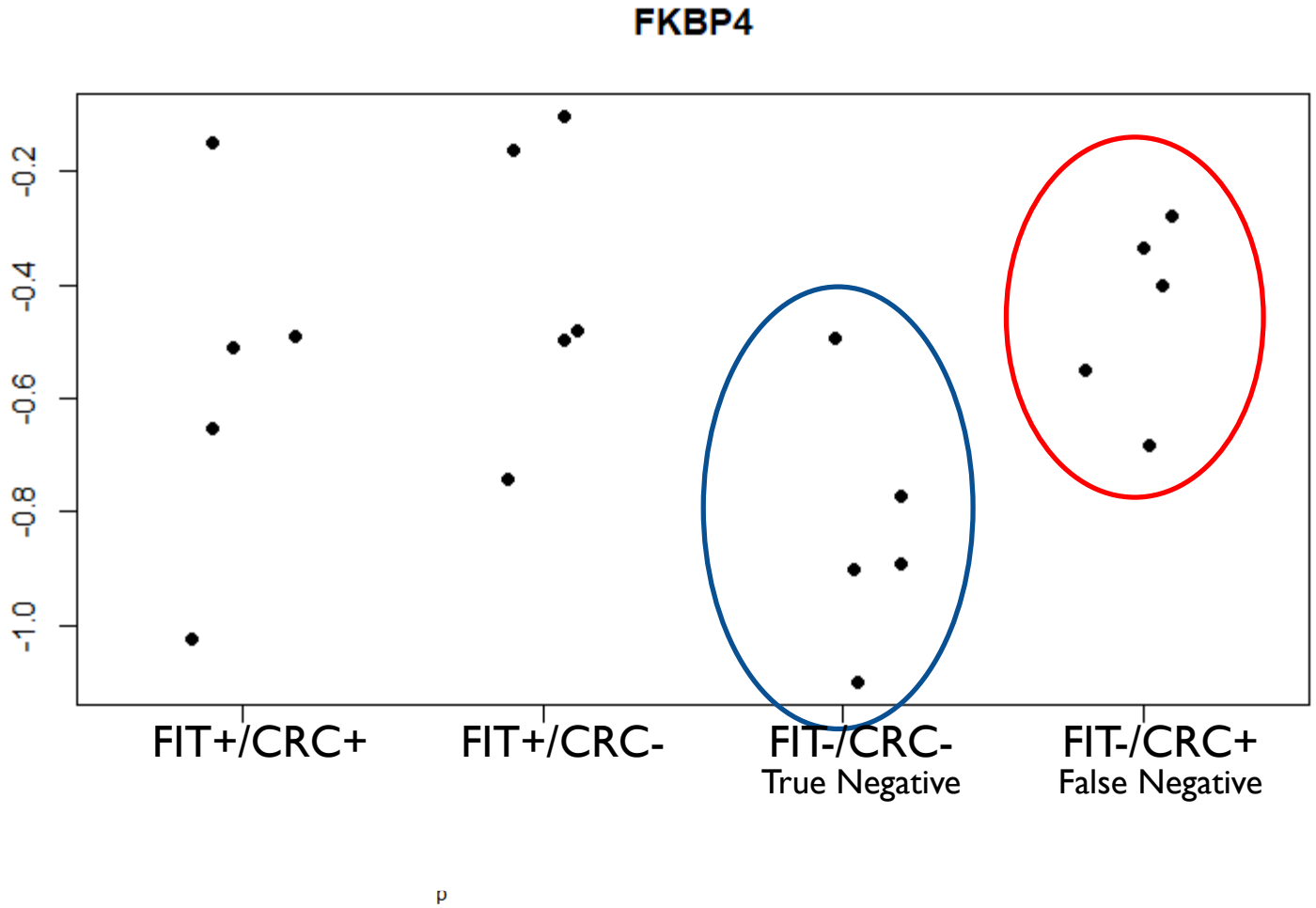
- 5 Pairs of 4 individuals treated as random effect variables
  - Estimating a coefficient for each pair
  - Finding the relationship of the 4 categories within the pair

FIT	Colonoscopy	
	Positive	**Negative**
Positive	True Positive	False Positive
Negative	False Negative	True Negative

False Negative vs True Negative

- 123 c
- 85 ar





FRED HUTCH

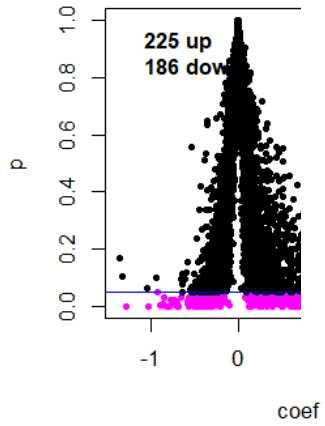
FN vs TN – KEGG & Reactome Analysis

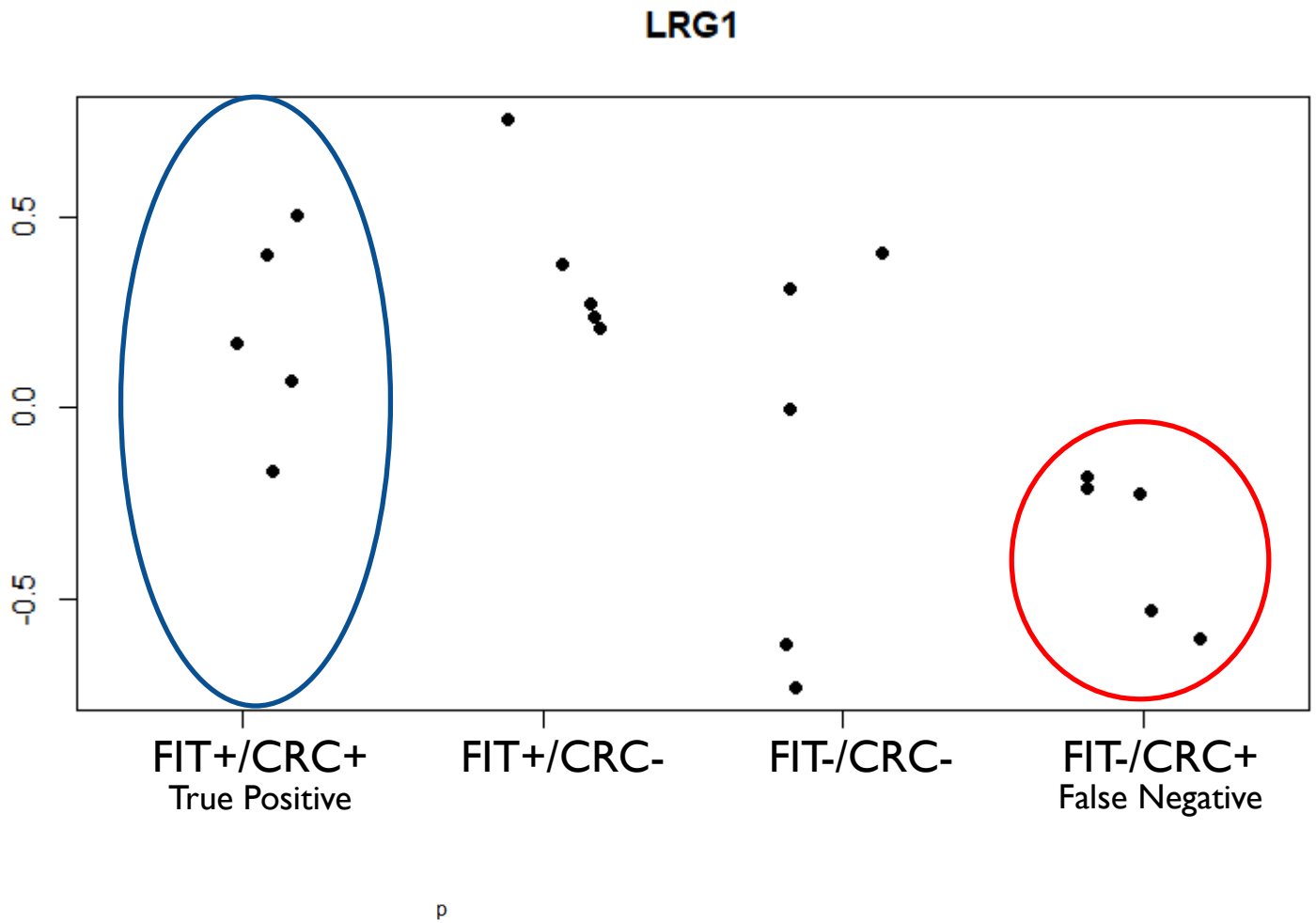
- Intestinal immune network for IgA production (3/15)
- Inflammatory bowel disease (4/31)
- PI3K-Akt signaling pathway (15/159)

- Signaling by Interleukins (12/141)

False Negative vs True Positive

- 441 c
- 225 a





FRED HUTCH

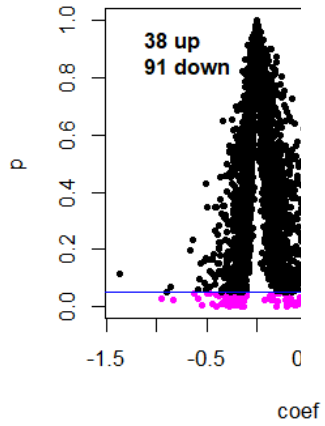
FN vs TP – KEGG & Reactome Analysis

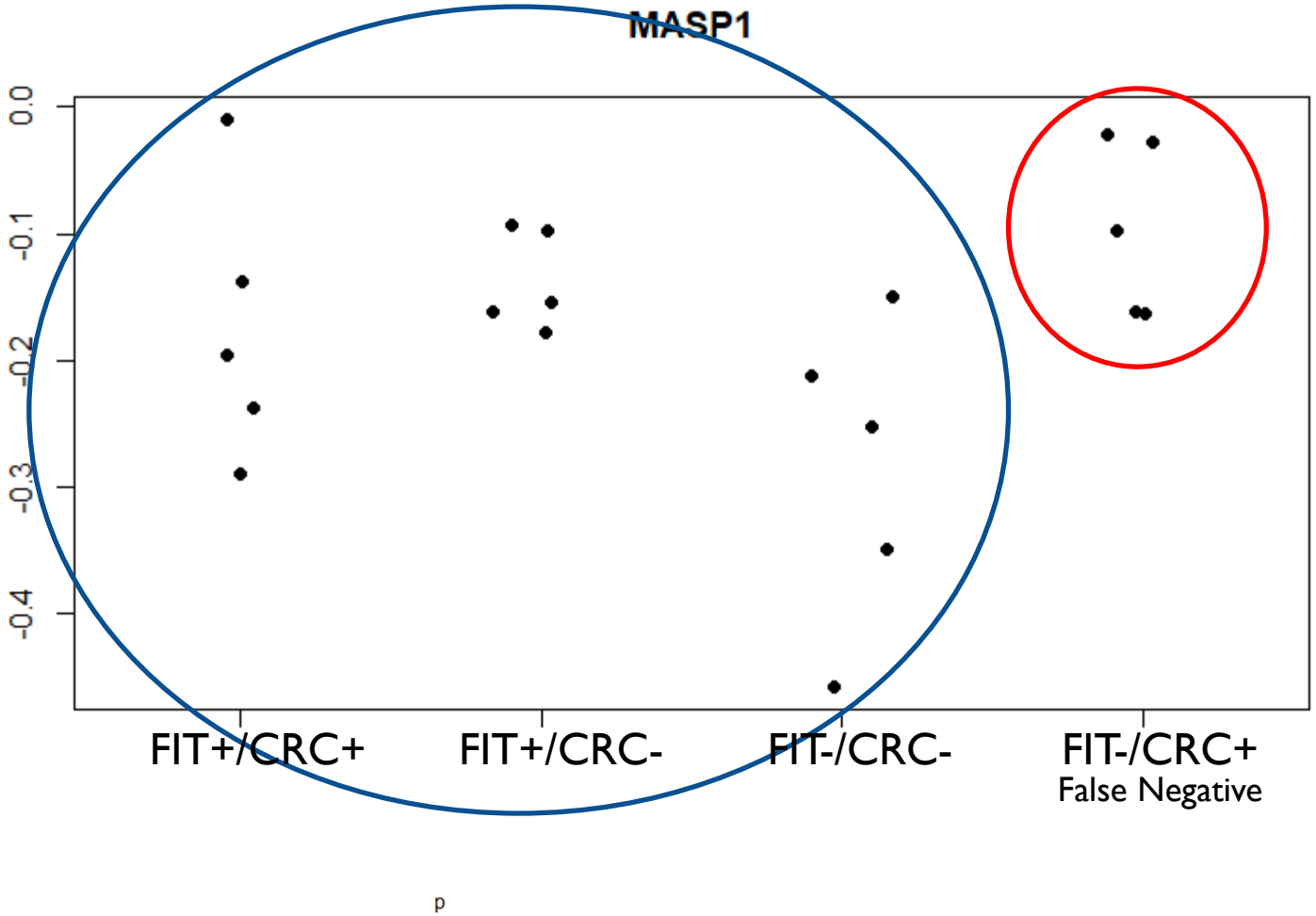
- PI3K-Akt signaling pathway (33/159)
- Focal adhesion (25/99)
- Cytokine-cytokine receptor interaction (28/102)
- Intestinal immune network for IgA production (7/15)

- Immune System (83/356)
- Innate Immune System (56/230)
- Signaling by ERBB2 (11/25)

False Negative vs All Others

- 129 c
- 38 ar



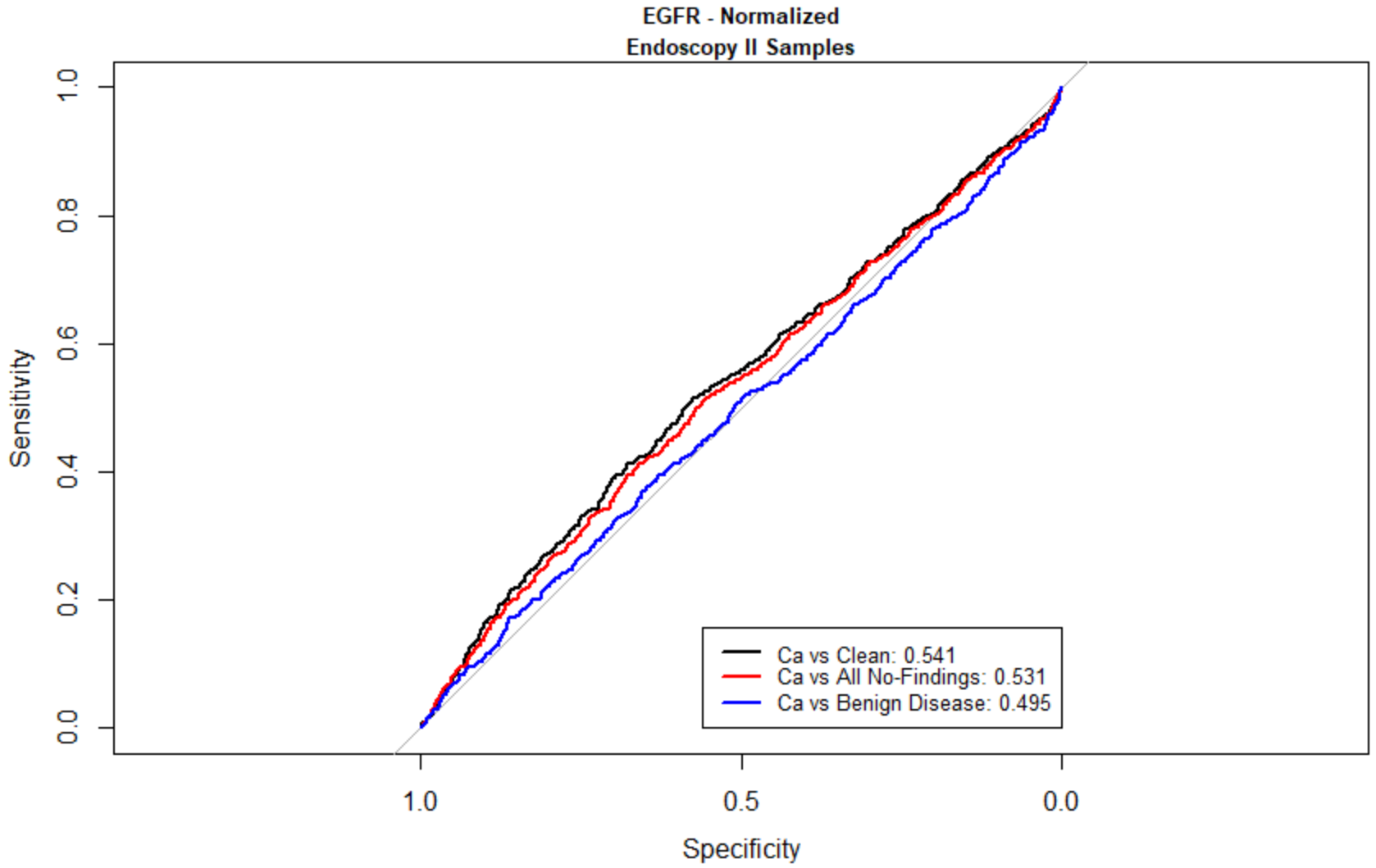


FRED HUTCH

FN vs Others – KEGG & Reactome Analysis

- PI3K-Akt signaling pathway (14/159)
- Inflammatory bowel disease (7/31)
- Transcriptional misregulation in cancer (8/73)

- Signaling by PDGF (11/130)
- MAPK family signaling cascades (8/96)
- Downstream signal transduction (10/123)


EGFR - Normalized
Endoscopy II Samples
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.0
0.5
0.0
Specificity
Ca vs Clean: 0.541
Ca vs All No-Findings: 0.531
Ca vs Benign Disease: 0.495

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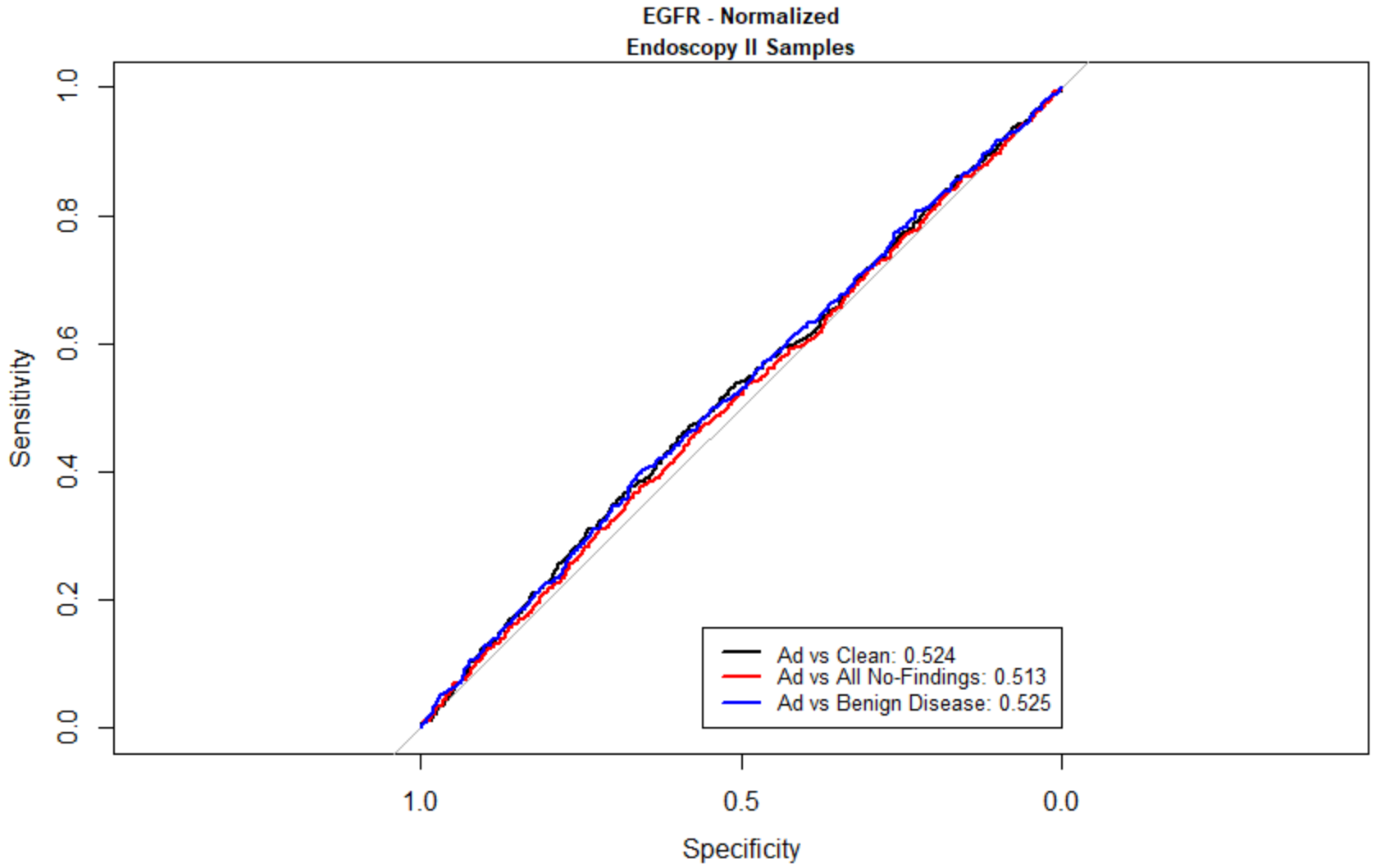
EGFR - Normalized
Endoscopy II Samples
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.0
0.5
0.0
Specificity
Ad vs Clean: 0.524
Ad vs All No-Findings: 0.513
Ad vs Benign Disease: 0.525

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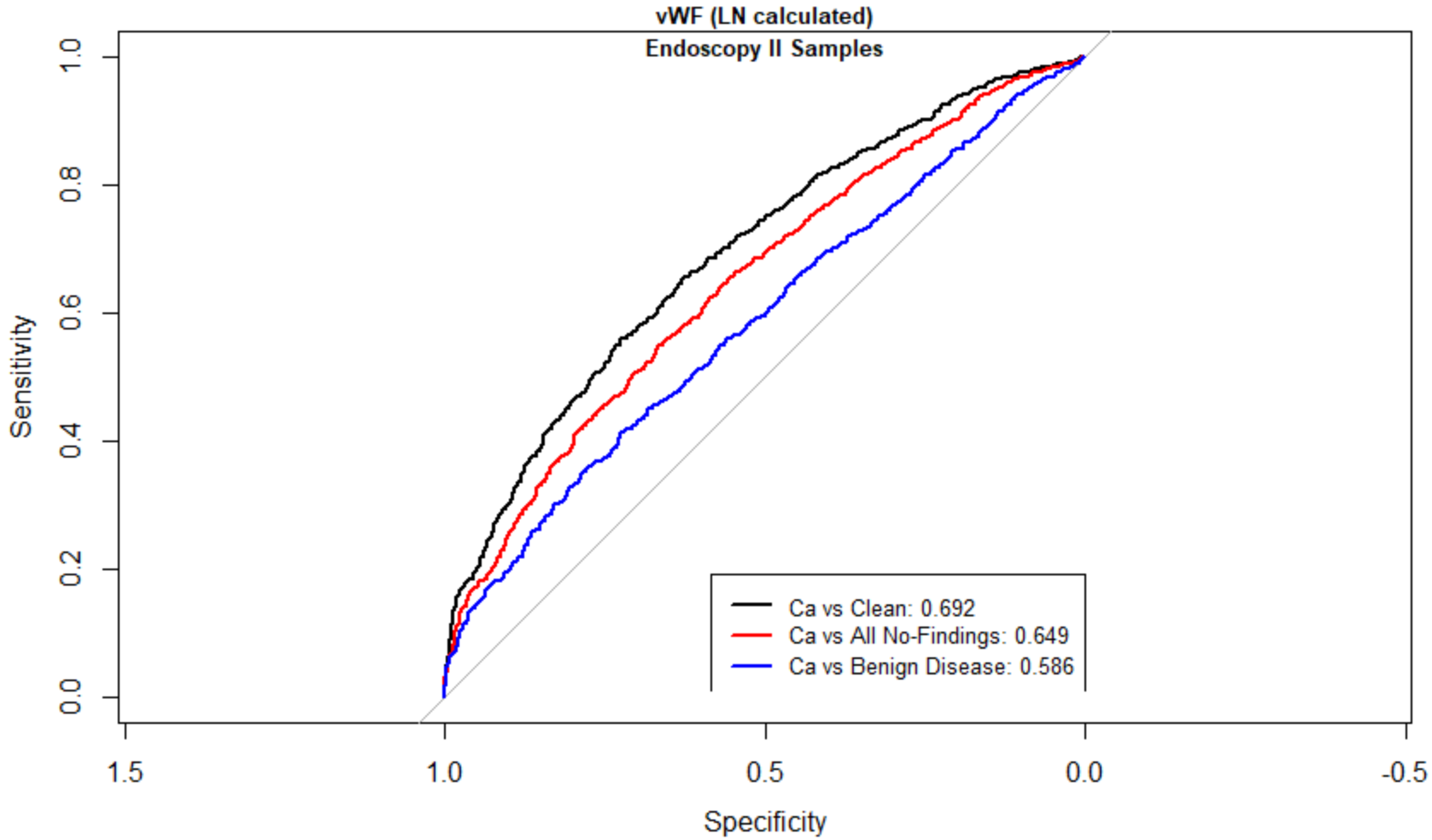
vWF (LN calculated)
Endoscopy II Samples
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.5
1.0
0.5
0.0
-0.5
Specificity
Ca vs Clean: 0.692
Ca vs All No-Findings: 0.649
Ca vs Benign Disease: 0.586

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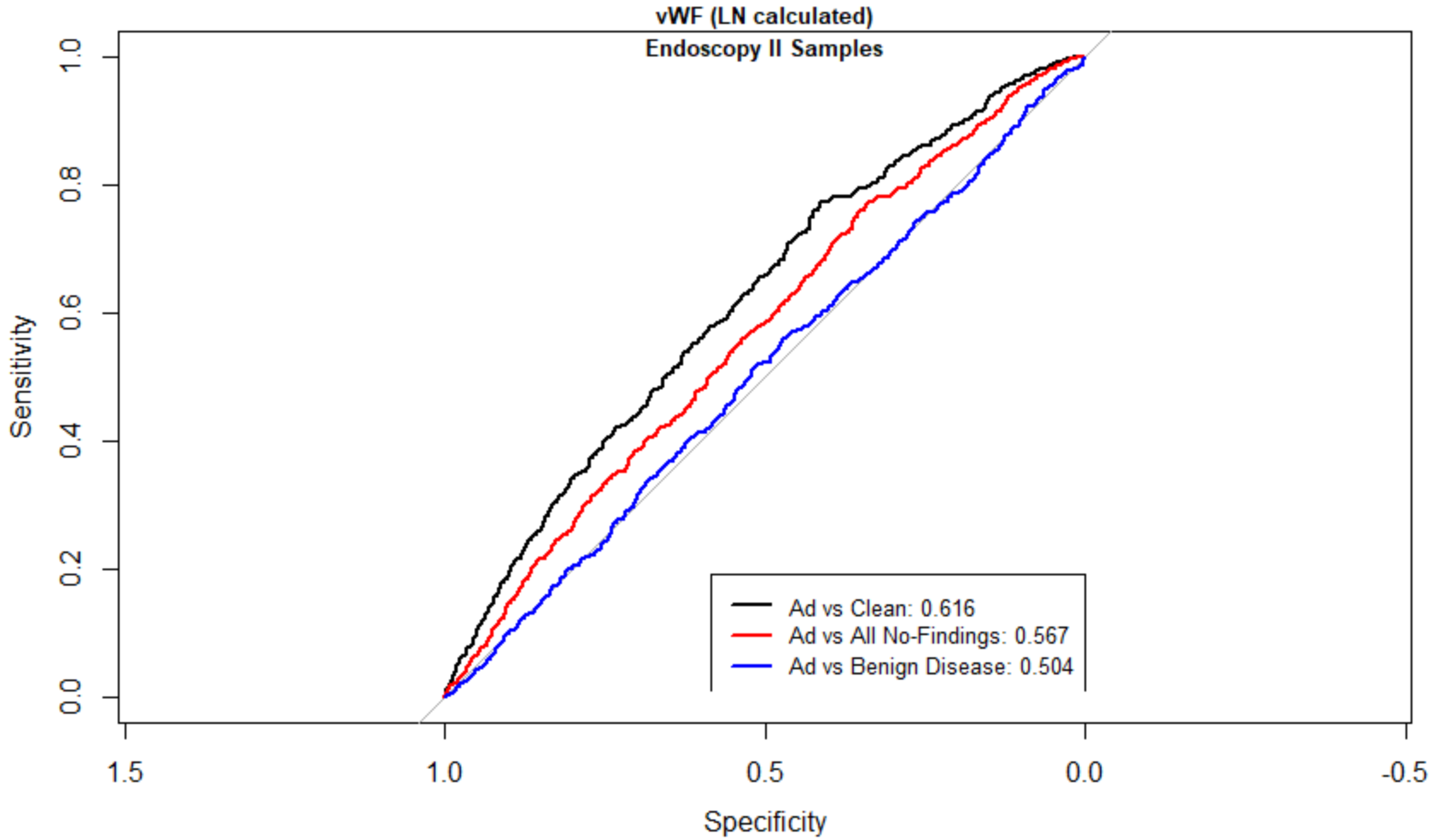
vWF (LN calculated)
Endoscopy II Samples
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.5
1.0
0.5
0.0
-0.5
Specificity
Ad vs Clean: 0.616
Ad vs All No-Findings: 0.567
Ad vs Benign Disease: 0.504

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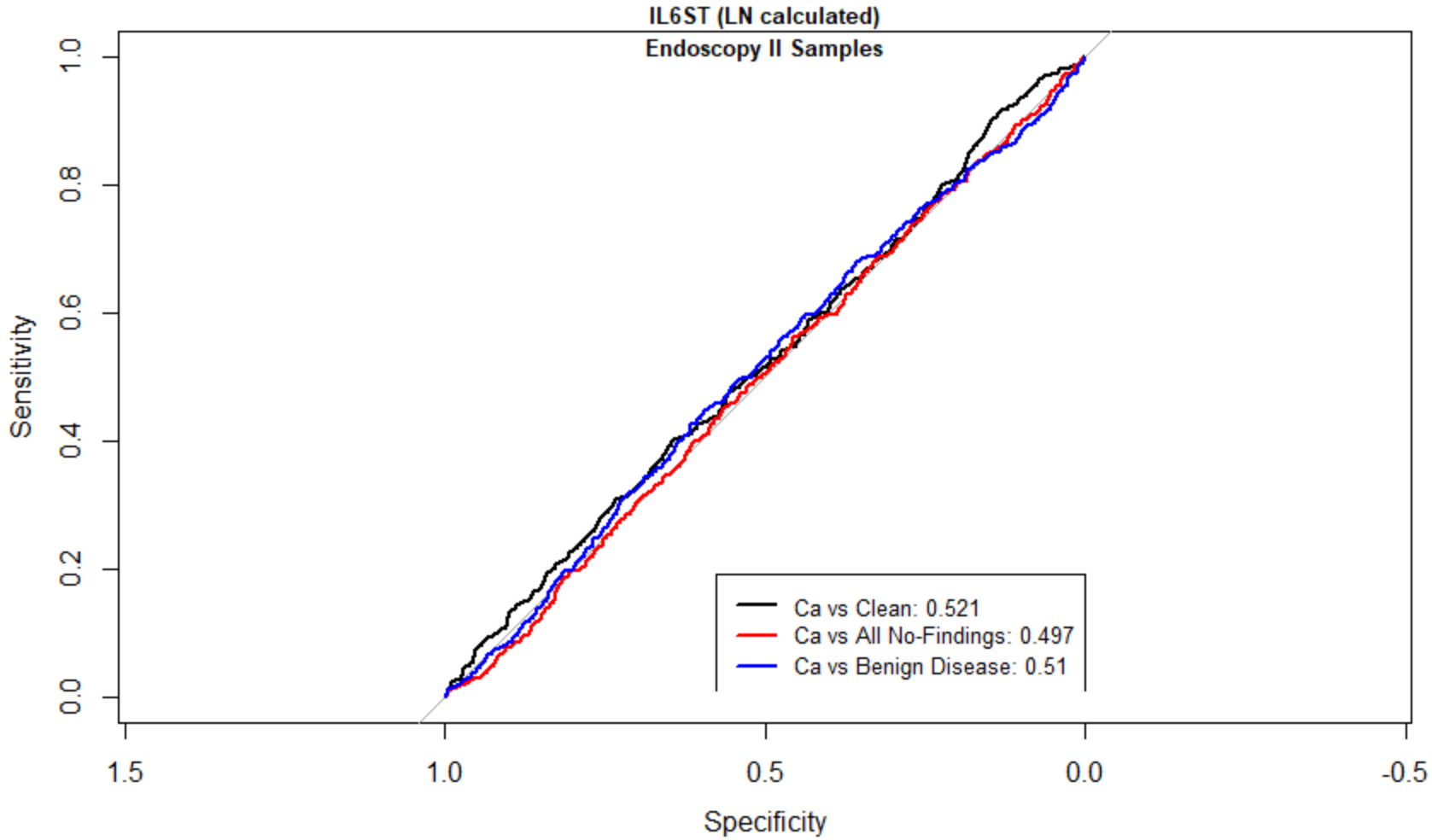
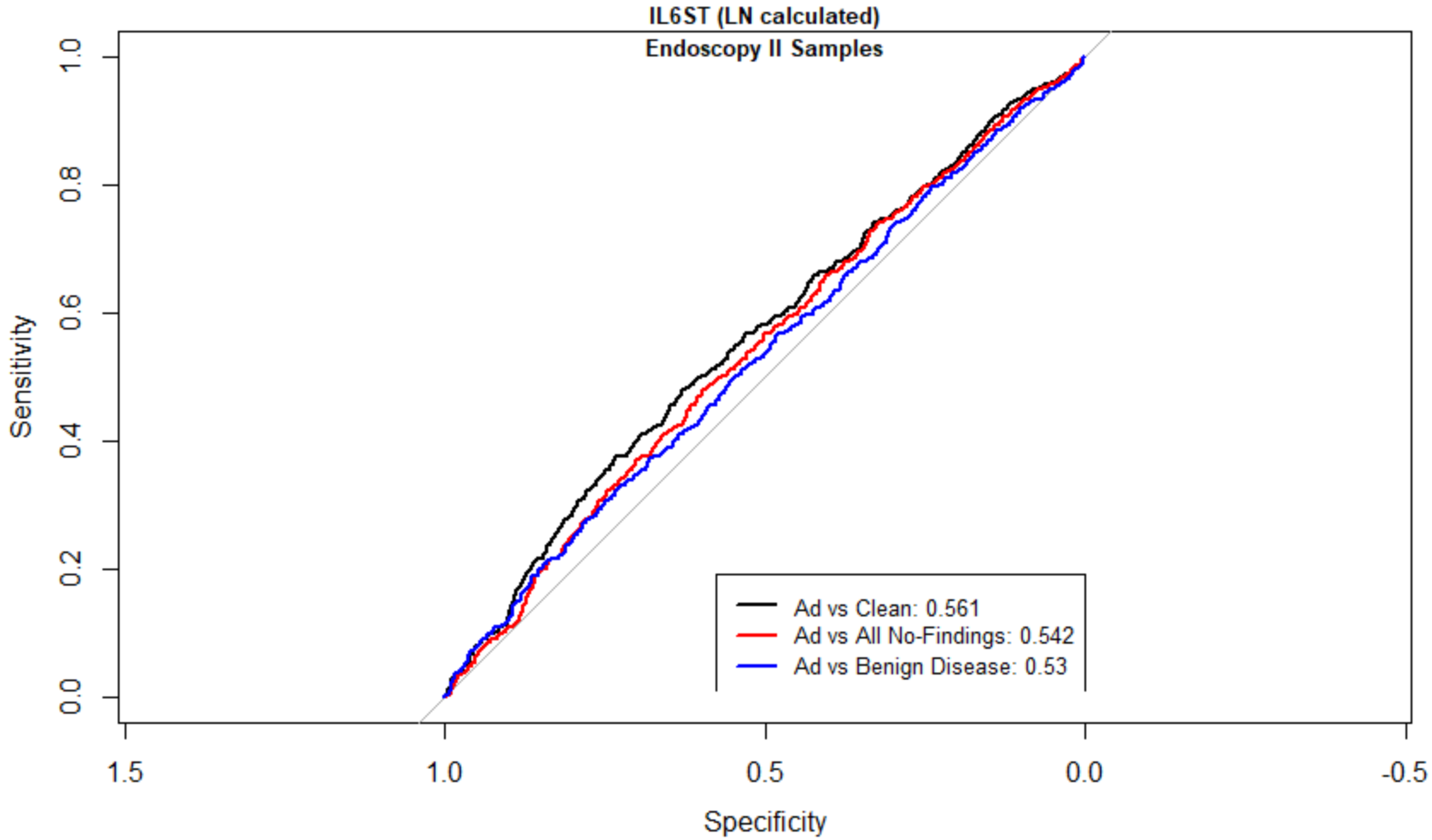

IL6ST (LN calculated)
Endoscopy II Samples
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.5
1.0
0.5
0.0
-0.5
Specificity
Ca vs Clean: 0.521
Ca vs All No-Findings: 0.497
Ca vs Benign Disease: 0.51

FRED HUTCH


IL6ST (LN calculated)
Endoscopy II Samples
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.5
1.0
0.5
0.0
-0.5
Specificity
Ad vs Clean: 0.561
Ad vs All No-Findings: 0.542
Ad vs Benign Disease: 0.53

FRED HUTCH

5 minute Q&A

Chair/Co-Chair/NCI
feed Zoom Chat questions to presenter
and Track Time
NCI and Production Team
answer Chat questions not related to presentations
and use Slack