



**1000 Patient study of detection of CRC and polyps  
by serum ELISA of altered epigenetic signatures in  
circulating cell free nucleosomes**

*9<sup>th</sup> International Conference of Anticancer Research  
Sithonia, Oct 06-10 2014*

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# Volition's aims

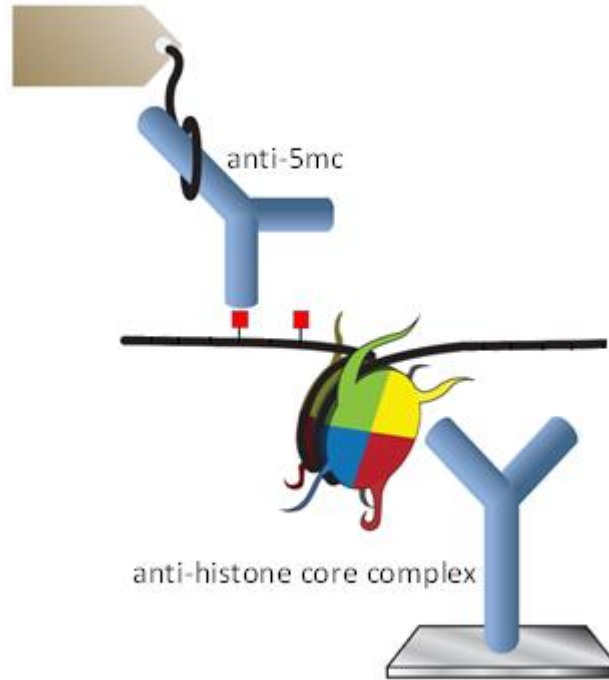
- Detect cancers in simple, cost effective, accurate ELISA blood tests
- Use small volume of serum/plasma (10uL)
- Initial focus on ColoRectal cancer

# Agenda

- Volition NuQ<sup>®</sup> assays
- Background on circulating cell-free nucleosomes
- Example data from cell culture studies
- Pre-analytical studies
- Analytical performance
- Clinical serum studies

# Volition NuQ<sup>®</sup> ELISA tests for epigenetic structures in circulating cell free nucleosomes

Example ELISA for nucleosomes containing methylated DNA in 10uL blood



- 10uL serum/plasma in duplicate
- Cost <\$5 as a research assay
- 20 assays for nucleosomes containing:
  - DNA modifications
  - Histone modifications
  - Histone variants
  - Nucleosome adducts

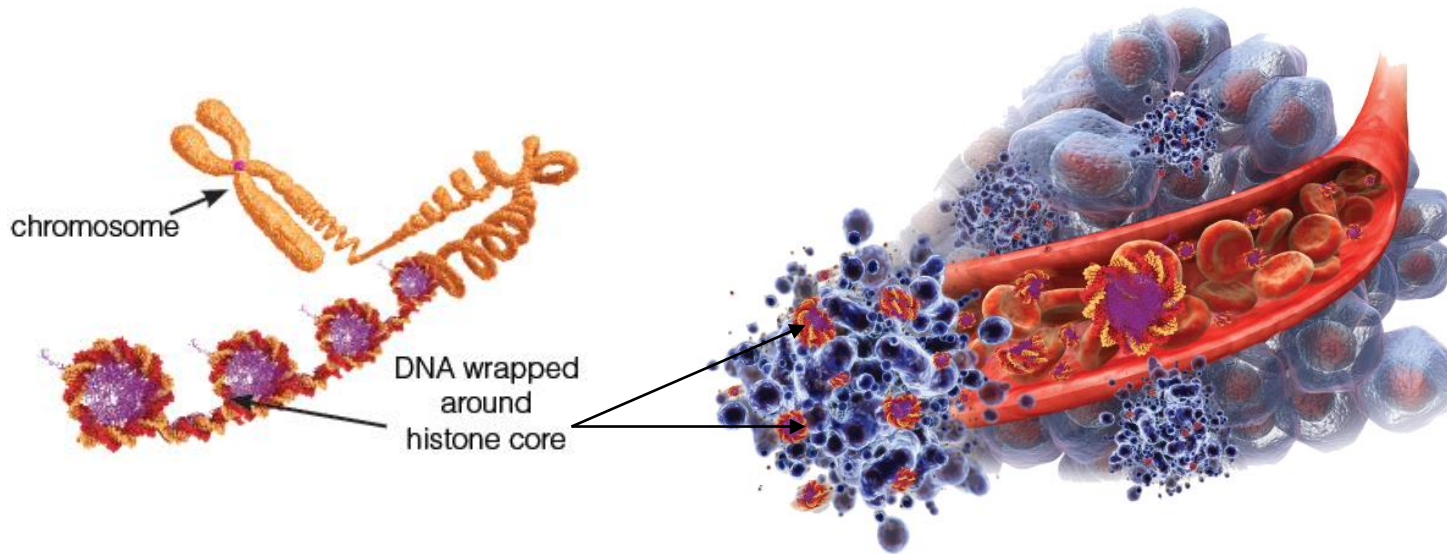
 VOLITION<sup>RX</sup>  
NuQ-X<sup>®</sup> ELISA

# Background: Circulating cell free nucleosomes

- Epigenetic changes in DNA & chromatin of cancer cells<sup>1</sup>
  - Eg; DNA methylation, histone modifications, histone isoforms
- Epigenetic changes occur early in tumorigenesis <sup>1</sup>
- ctDNA circulates as  $\approx$  180bp fragments bound to mononucleosomes<sup>2</sup>
- ctDNA retains genetic mutations of the cancer concerned<sup>2</sup>
- ctDNA retains epigenetic modifications of the cancer concerned<sup>3</sup>

1. Histone Onco-modifications (Review). Fullgrabe J, E Kavanagh E, Joseph B. Oncogene (2011) 30, 3391–3403
2. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Newman AM et al, Nature Medicine (2014) doi:10.1038/nm.3519
3. Sensitive Detection of Colorectal Cancer in Peripheral Blood by Septin 9 DNA Methylation Assay. Grutzmann et al, PLoS ONE 3(11): e3759. doi:10.1371/journal.pone.0003759, 2008

# Background Science

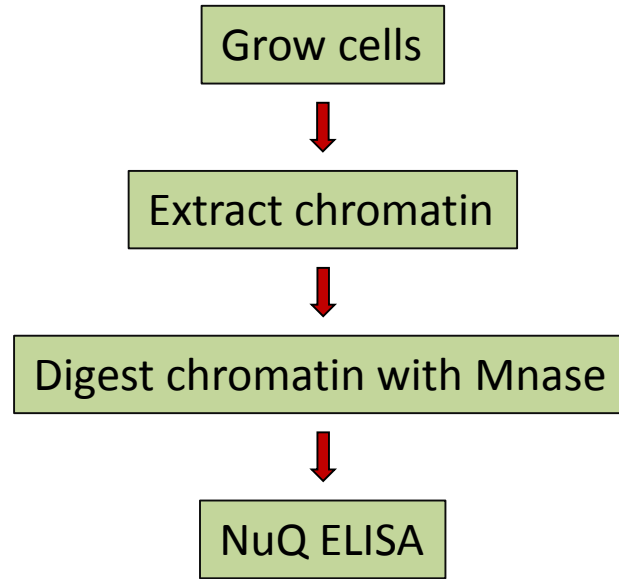


## BASIS OF NUCLEOSOMICS

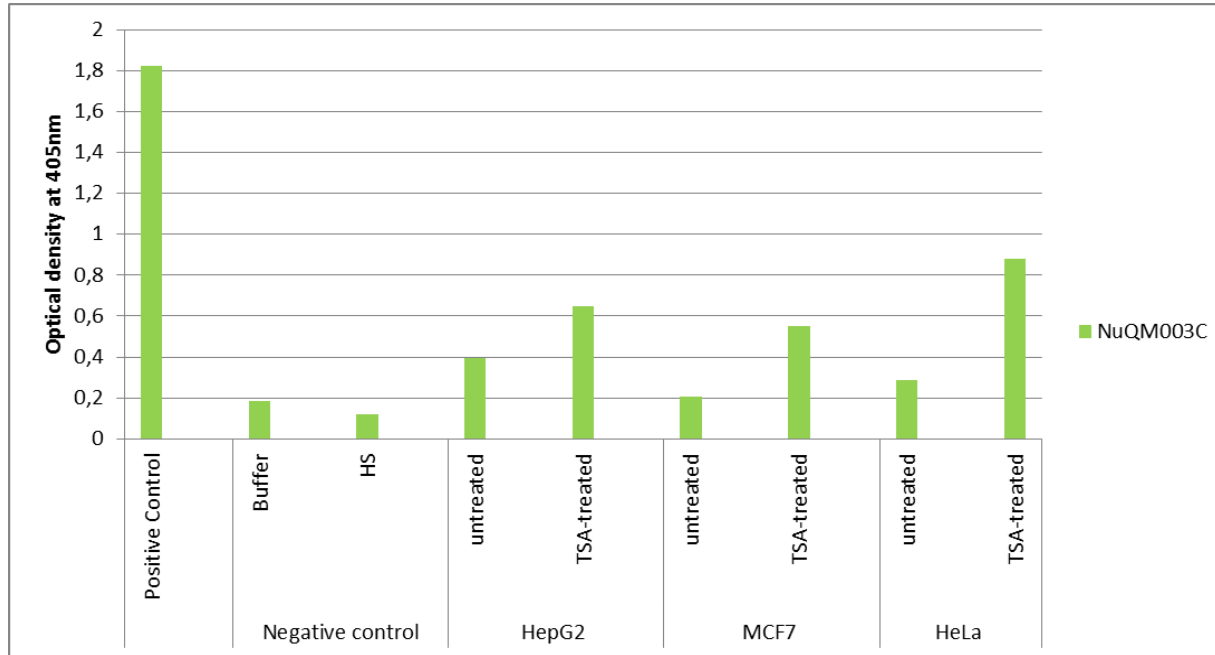
- Genome wide epigenetic analysis of circulating tumour nucleosomes

# Cell Culture

- example NuQ<sup>®</sup> results



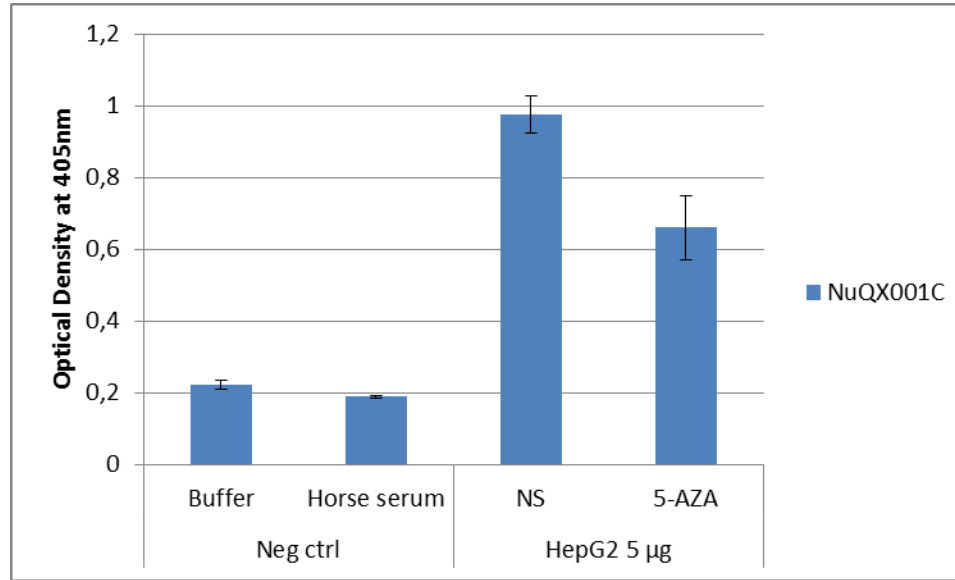
# cfNucleosome bound H4K16Ac response to TSA treatment in cell culture



Detection of H4K16Ac using the NuQM003C ELISA kit

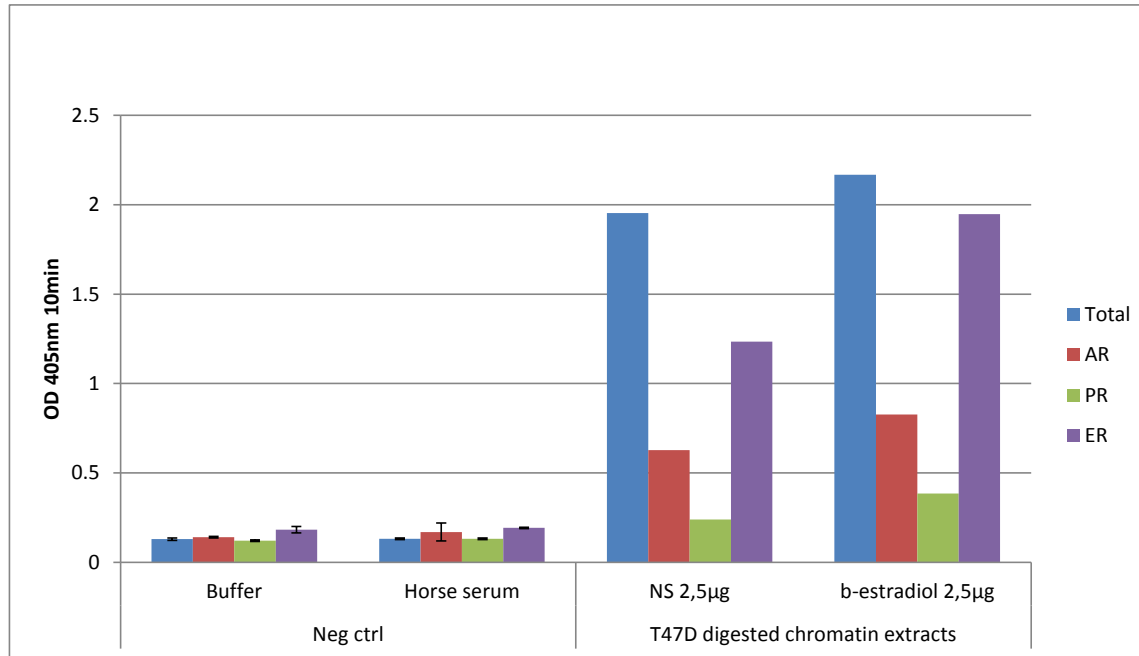


# cfNucleosome bound 5mc response to 5-Azacytidine (DNMT inhibitor) treatment in cell culture



Detection of nucleosomes containing 5mC using the NuQX001C ELISA kit

# cfNucleosome bound ER, AR & PR response to $\beta$ -estradiol treatment in T47D breast cancer cells



# Pre-Analytical Studies

– example results

# Denmark Pre-analytic Studies

## Project 1

± tourniquet and serum taken from top, middle, bottom of tube (240 samples)

## Project 2

44 people before and 1h after colonoscopy (88 samples)

## Project 3

20 people collected day 1, 8am fasted, 8pm non-fasted, 15, 22

## Project 4

12 people collected at 08:00, 12:00, 15:00 (non-fasted) on one day

## Project 5

40 rectal cancer patients collected before surgery, and 6, 24, 48, 72, 96h post surgery

## Project 6

Blood collected 10 healthy and 10 cancer patients

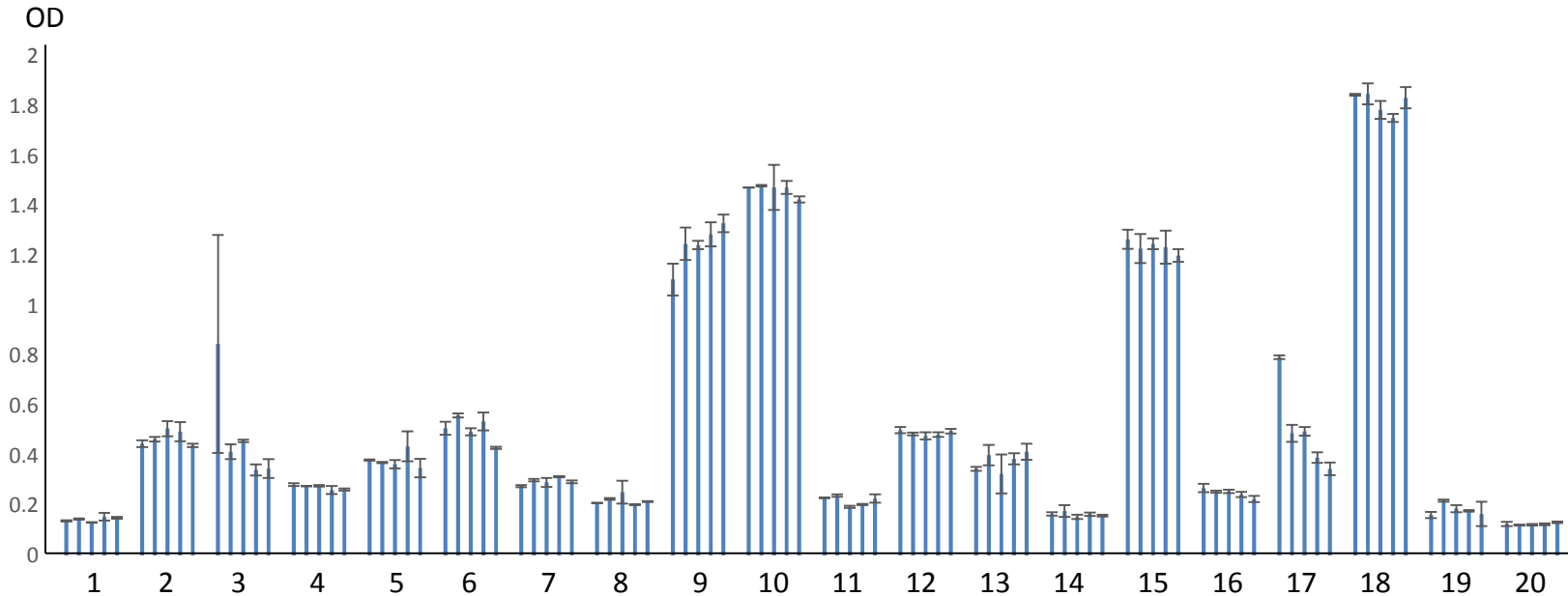
Stored on ice (4°C) and RT (21°C). Then spun at ½, 1, 2, 6, 24, 48, 72h

# Denmark Pre-analytic Studies

## Project 3

20 people collected day 1, 8 (am, fasted), 8 (pm, non-fasted), 15, 22

Assayed for nucleosomes containing methylated DNA



# Analytical Performance

- example results for NuQ assay for cfNucleosomes containing methylated DNA

# Assay Performance – Intra-assay precision (OD)

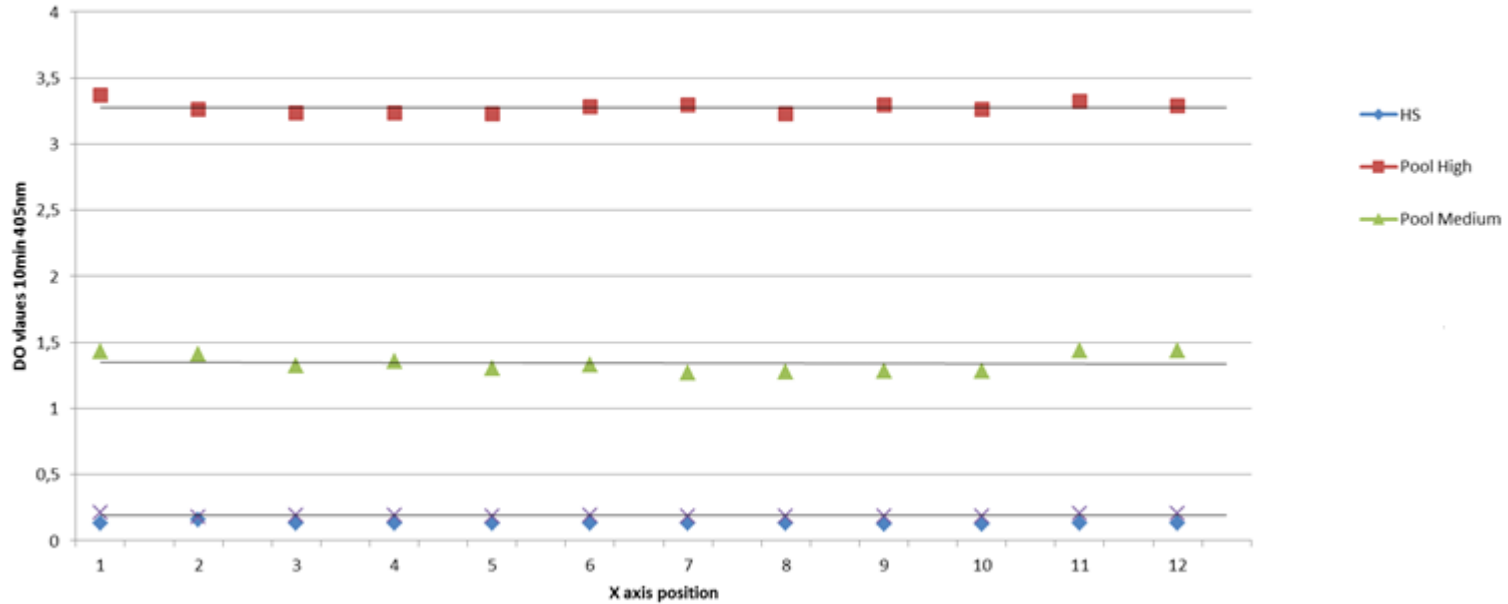
cfNucleosomes containing methylated DNA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pool High	Pool Low	HS	Pool Medium	HS	Pool High	Pool Low	Pool Medium	HS	Pool Low	Pool High	Pool Medium
B	Pool Medium	Pool High	Pool Medium	Pool Low	Pool High	Pool Low	HS	HS	Pool High	Pool Medium	Pool Low	HS
C	Pool Low	HS	Pool Low	Pool High	Pool Medium	HS	Pool Medium	Pool High	Pool Low	HS	Pool Medium	Pool High
D	HS	Pool Medium	Pool High	HS	Pool Low	Pool Medium	Pool High	Pool Low	HS	Pool High	HS	Pool Low
E	Pool High	Pool Low	HS	Pool Medium	HS	Pool High	Pool Low	Pool Medium	Pool Medium	Pool Low	Pool High	Pool Medium
F	Pool Medium	Pool High	Pool Medium	Pool Low	Pool High	Pool Low	HS	HS	Pool High	Pool Medium	Pool Low	HS
G	Pool Low	HS	Pool Low	Pool High	Pool Medium	HS	Pool Medium	Pool High	Pool Low	HS	Pool Medium	Pool High
H	HS	Pool Medium	Pool High	HS	Pool Low	Pool Medium	Pool High	Pool Low	Pool Medium	Pool High	HS	Pool Low

Sample	CV% within-plate
HS	9%
High pool	2%
Medium pool	6%
Low pool	6%

# Assay Performance – horizontal drift (OD)

cfNucleosomes containing methylated DNA

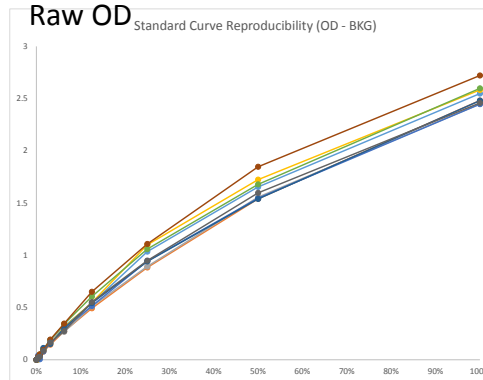
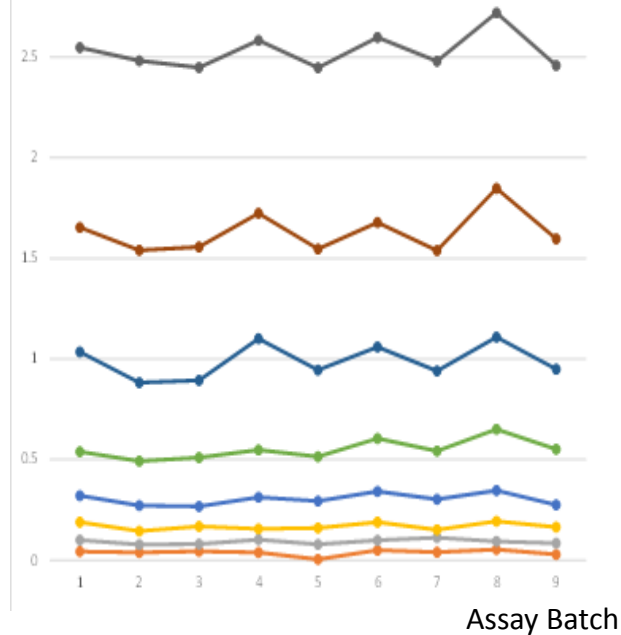




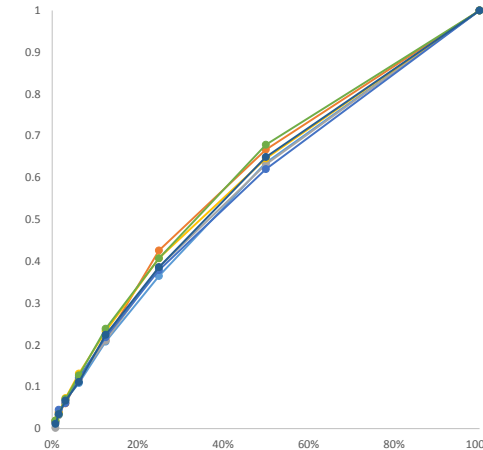
# Assay Performance – std curve stability

cfNucleosomes containing methylated DNA

Raw OD



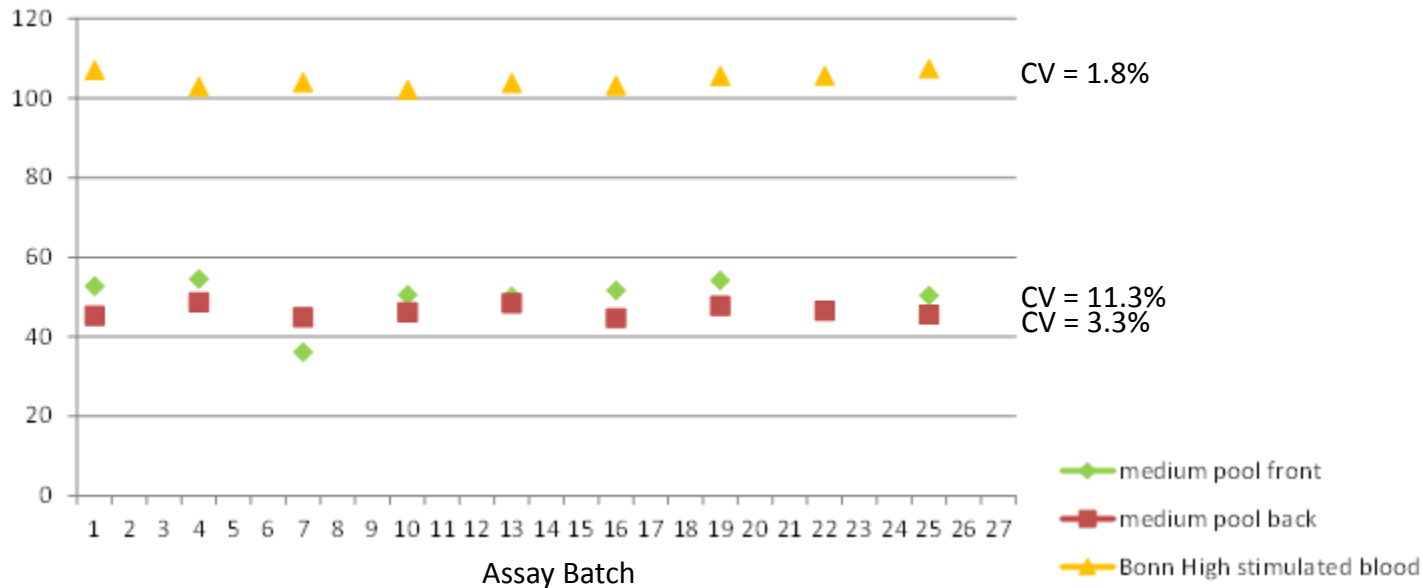
Normalised OD



# Assay Performance – Inter-assay precision (conc)

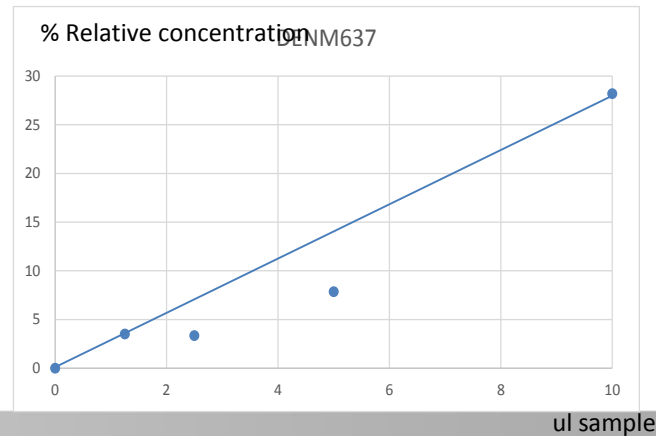
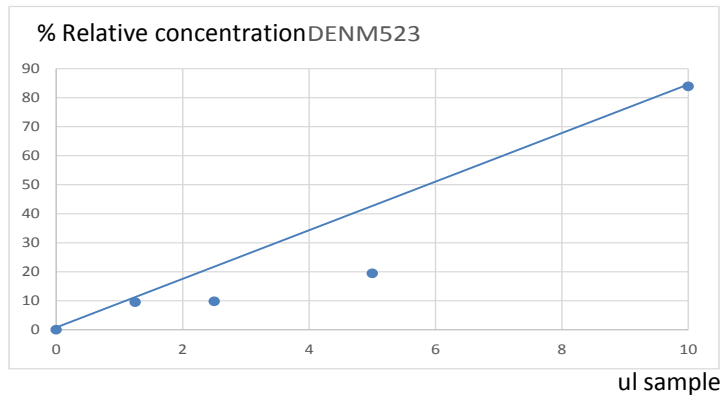
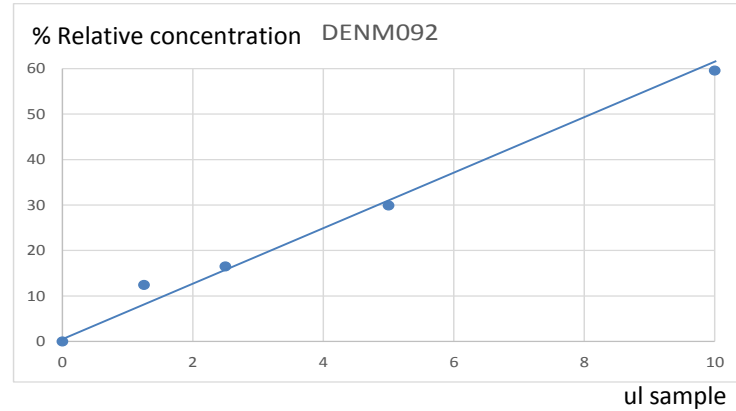
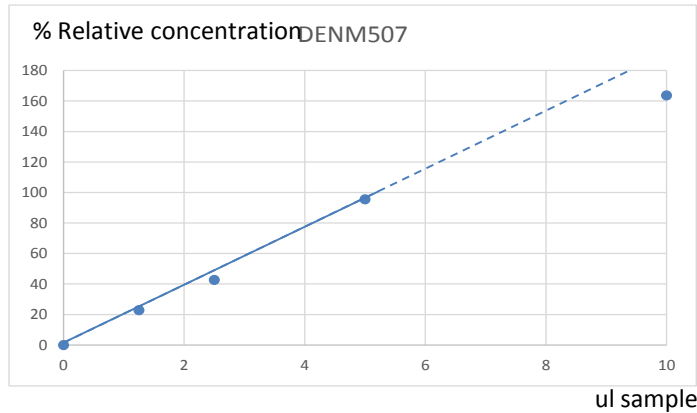
Nucleosomes containing methylated DNA

Relative concentration



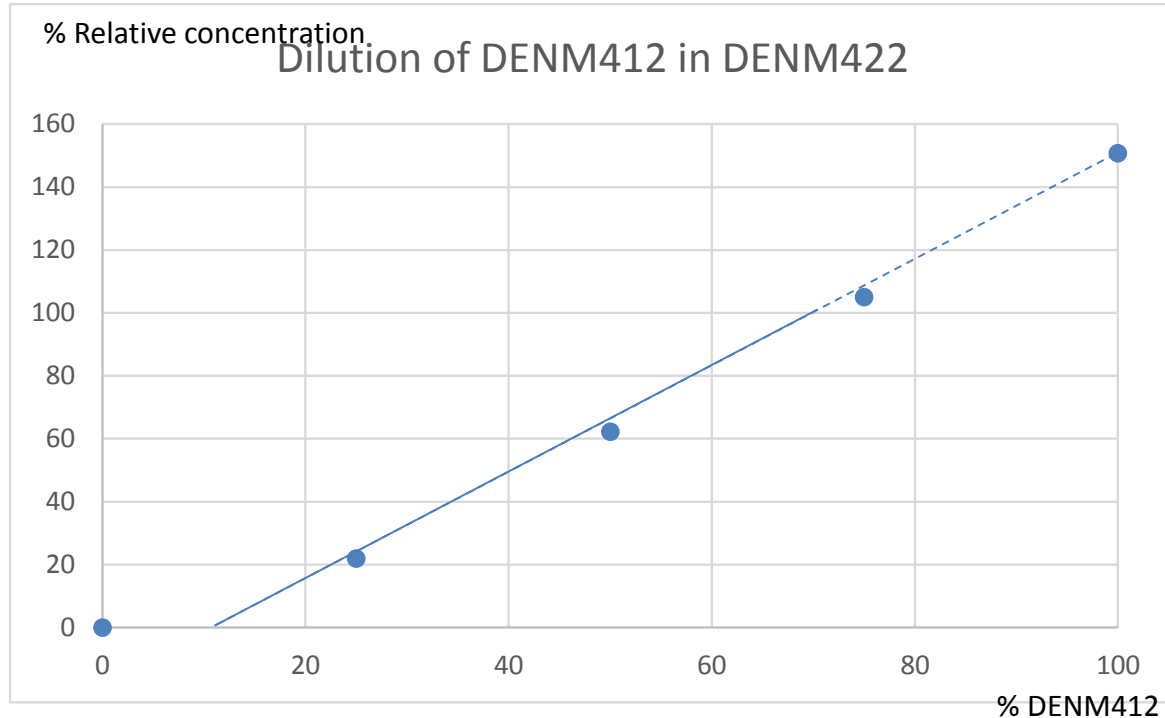
# Assay Performance – Samples diluted in buffer

cfNucleosomes containing methylated DNA



# Assay Performance – Mixed samples

cfNucleosomes containing methylated DNA



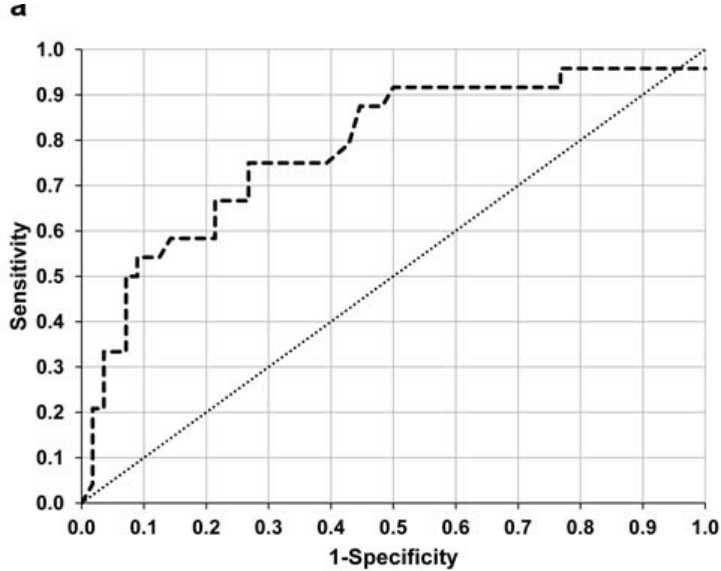
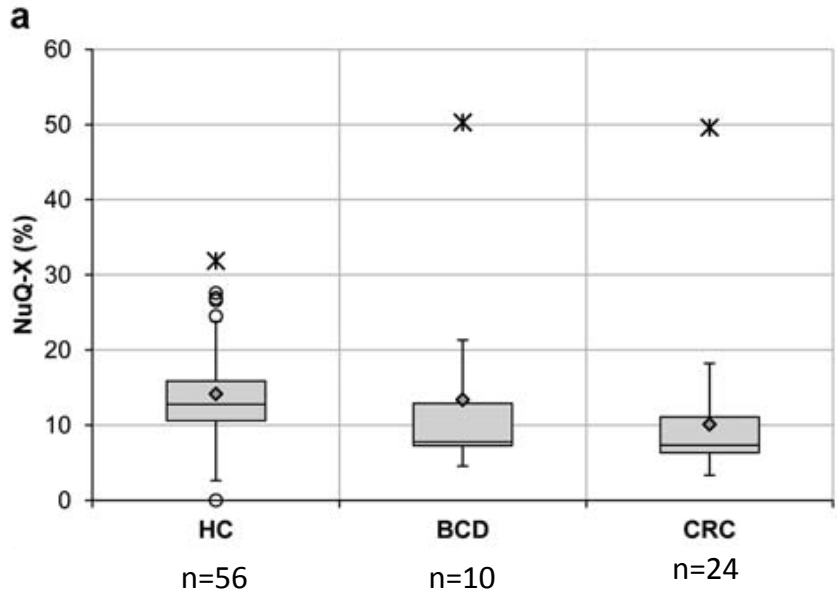
# Bonn Klinikum Clinical Study

Universitätsklinikum Bonn

Single nucleosome biomarker

# Nucleosomics® - Single Nucleosome Biomarker

## Nucleosomes containing 5-methylcytosine



70% Sensitivity / 75% Specificity

# Danish Endoscopy II Clinical Study

Hvidovre Hospital, Copenhagen, Denmark

Panel of nucleosome biomarkers

# Endoscopy II population – 4812 symptomatic subjects referred for colonoscopy in Denmark

• CRC	59 (35 CC & 24 RC)
• Polyp (adenoma)	174
• Other finding	264
• Clean colon + comorbidity	211
• Clean colon + no comorbidity	112
• Other cancer	10
	<hr/>
	830

- All patients referred for colonoscopy for symptomatic reasons
- Data analysis restricted to subjects >50 years of age



# Endoscopy II population – 4812 symptomatic subjects referred for colonoscopy in Denmark

## Work in progress

**Phase I:** analysis of approx 1000 samples

Aim: select panel of NuQ tests to take forward

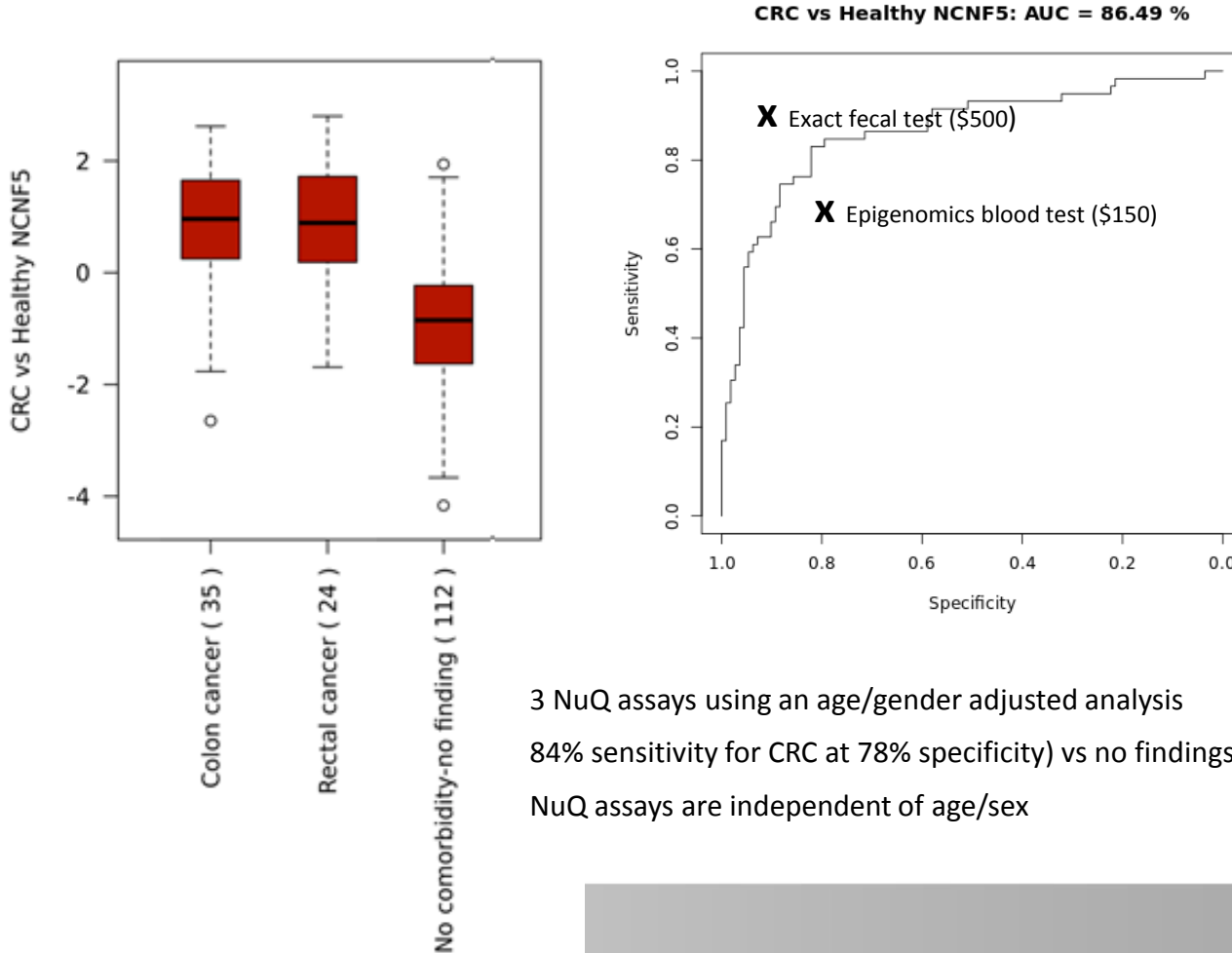
**Phase II:** analysis of remaining 4000 samples

Aim: establish clinical accuracy for CRC

To date:

- 7 NuQ assays performed in serum on 830 samples (aged >50)

# CRC vs no findings, no co-morbidities



# Stage dependence

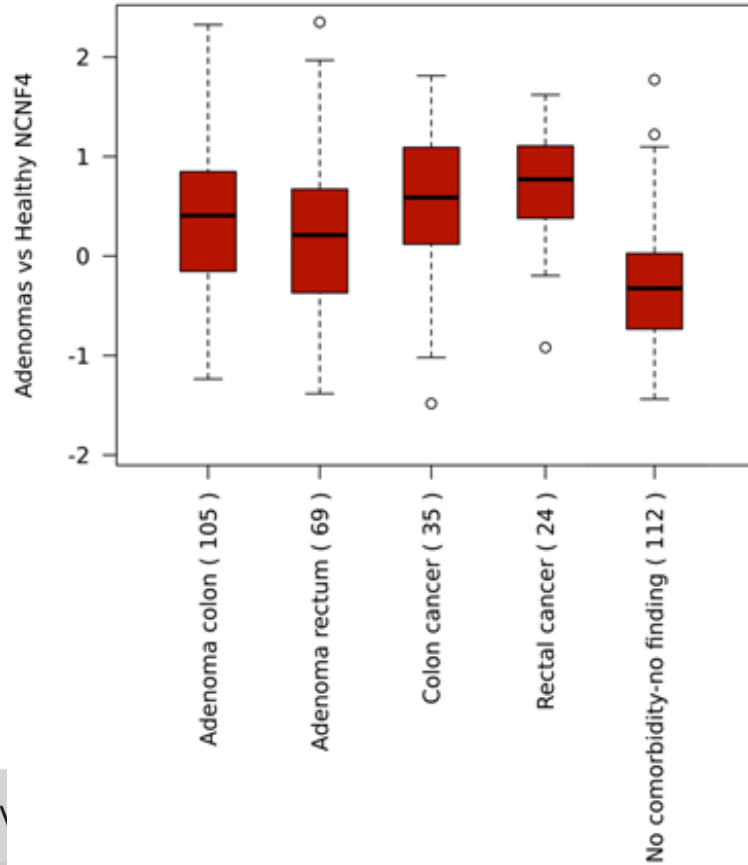
CRC	Stage I	Stage II	Stage III	Stage IV	Total
Detected	6/8	19/20	16/20	9/11	50/59

# Differentiation of Colon & Rectal Cancer

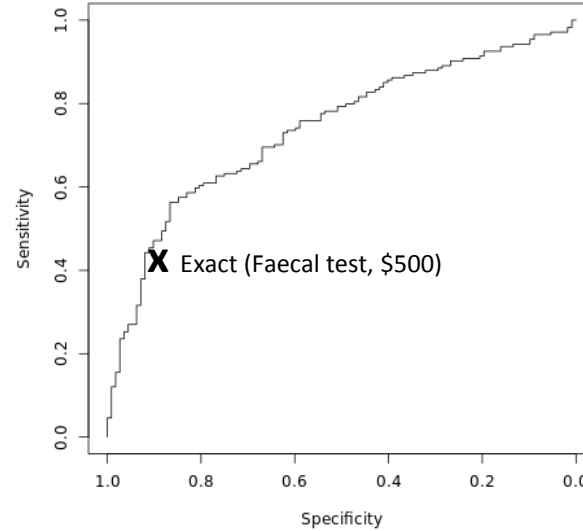
- Panel of nucleosome biomarkers is different for CC and RC
- ROC curve for discrimination gives AUC 68%
- Bodes well for discrimination of other cancer diseases

# Adenomas vs no findings, no co-morbidities

Hvidovre Oct 2014 Adenomas vs Healthy NCNF4



Adenomas vs Healthy NCNF4: AUC = 74.00 %



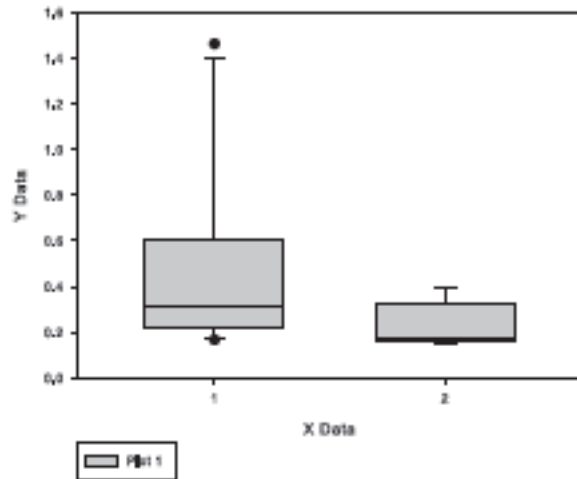
# Pilot Prostate Cancer Study

## Immune Health

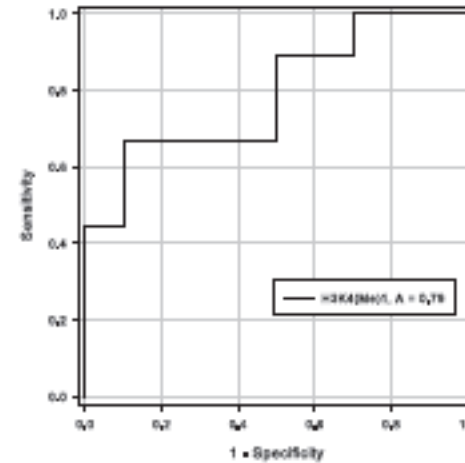
# Immune Health Pilot Prostate Cancer Study

Serum nucleosomes containing 5-methyl-DNA

Box plot

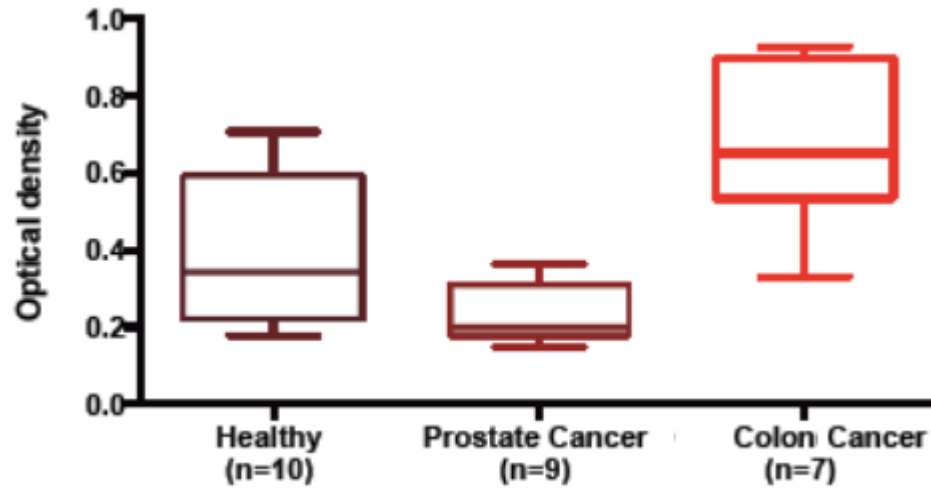


ROC curve



# Immune Health Pilot Prostate Cancer Study

Serum H3K9Me3 in PCA and CC





# Conclusions

- NuQ<sup>®</sup> assays detect drug induced epigenetic changes in cell culture
- NuQ<sup>®</sup> assays detect ColoRectal cancer in a simple panel blood test
- Detects early stage cancers
- Detects adenomas
- Colon and Rectal cancers are discreet diseases wrt NuQ<sup>®</sup> profile
- Also detect and discriminate prostate cancer in pilot studies

# VolitionRx – Current Studies



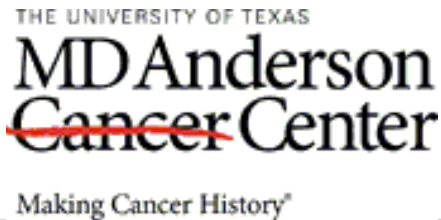
CRC:  
Retrospective study n= 4,800  
Prospective study n= 14,000



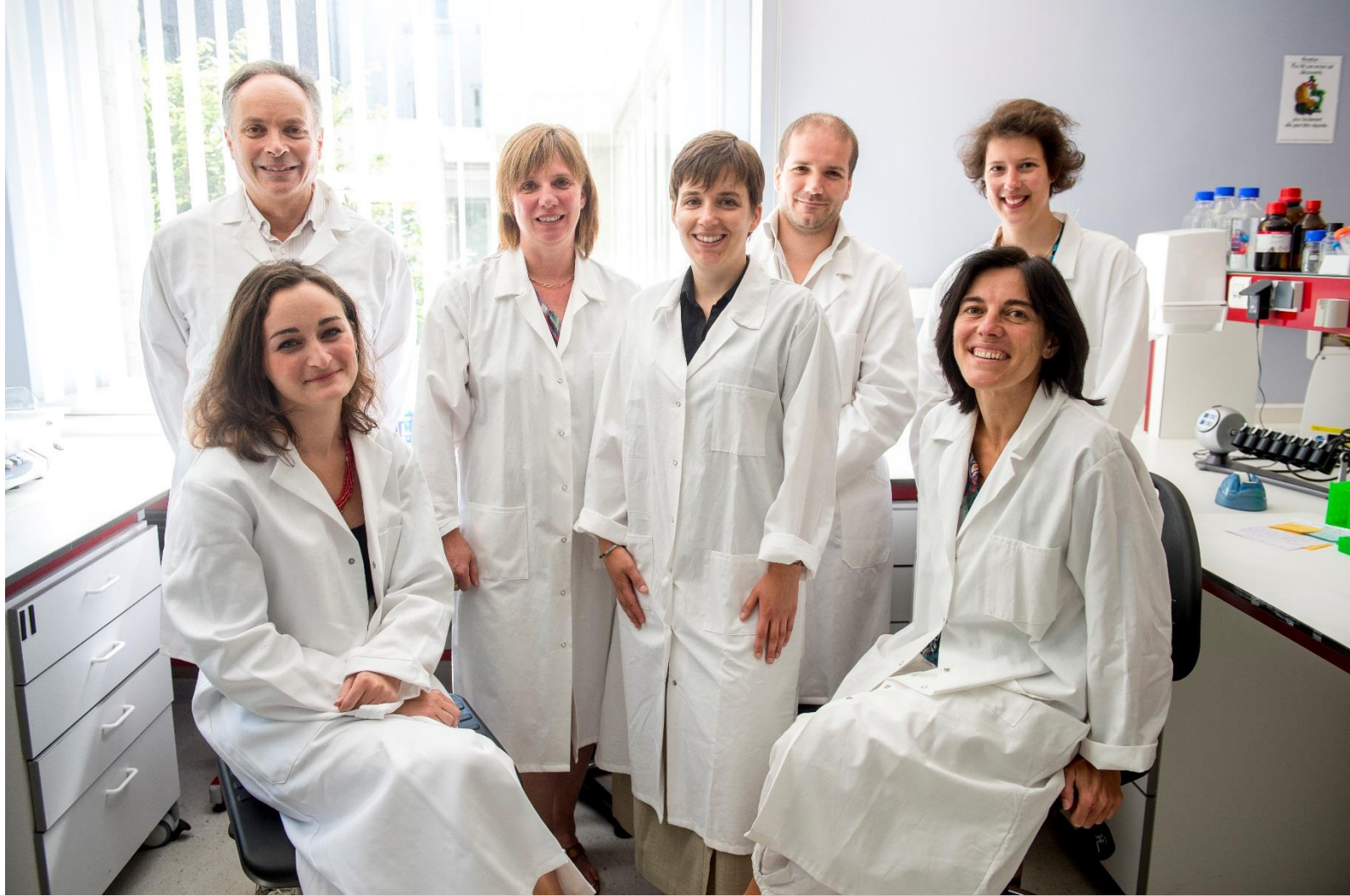
20 most prevalent cancers  
Prospective study n= 4,000



CRC  
Prospective study n= 250



Prostate  
Retrospective study n= TBC



## **Collaborators:**

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