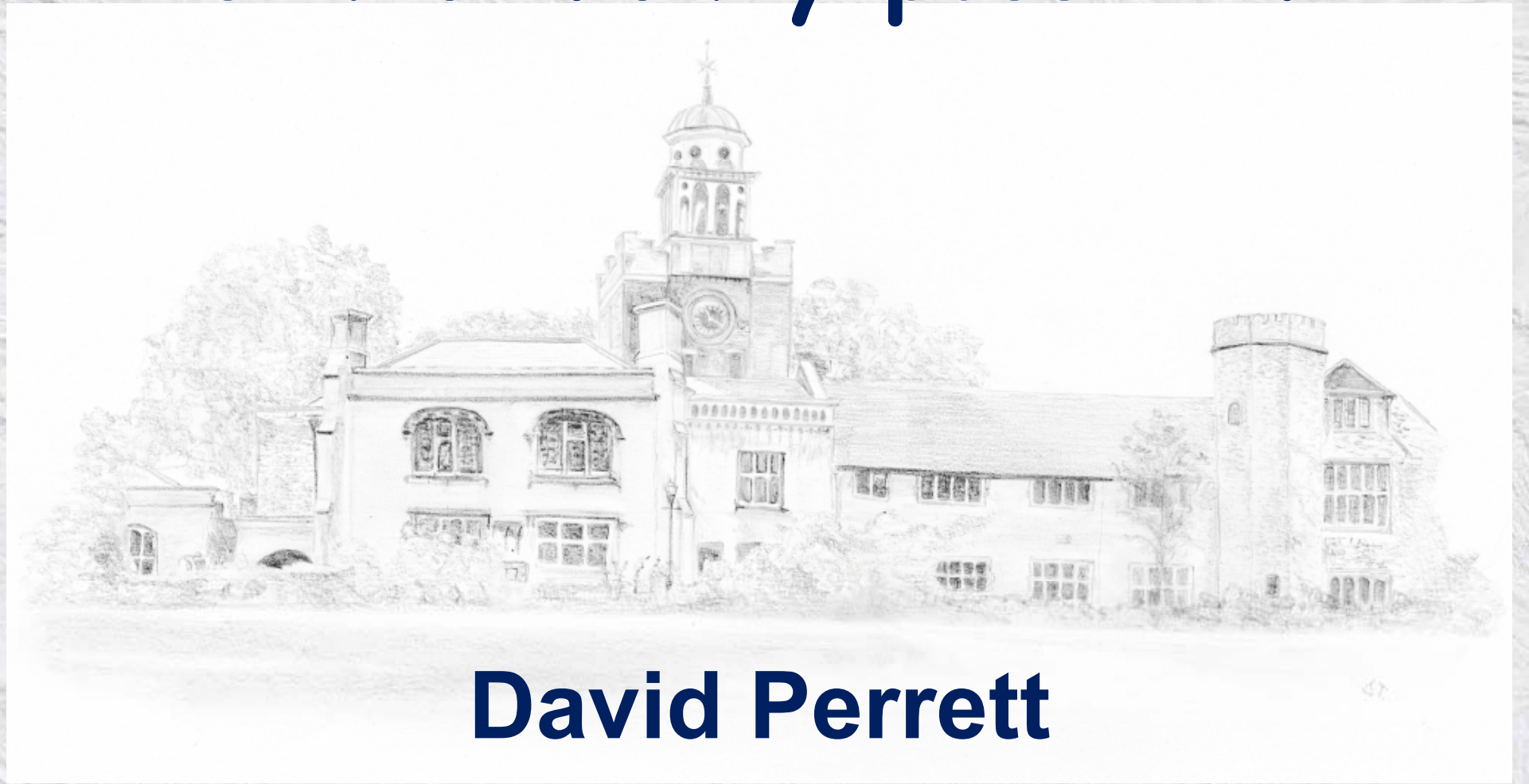
Validating BioMarkers
Is it clinically possible?



David Perrett

Professor of Bioanalytical Science
Barts & the London School of Medicine & Dentistry
Queen Mary University of London

Clinical Biomarkers

Endogenous compounds those levels change in clinical conditions

Measurement of their concentrations in urine or blood samples may aid the diagnosis of a disease *and/or* monitor therapy

Almost daily announcements

1GM Friday September 4 2015 | THE TIME

W

ooks

s and lies
er Boyes reviews
Hastings's
history of the
nd World War

Sport

The power and the glory
Meet the men putting the muscle into England's World Cup campaign

Blood test detects eight out of ten with lung cancer

Kat Lay Health Correspondent

A blood test for lung cancer on the NHS is a step closer after "exciting" early results from a trial of 10,000 Scottish smokers.

The study invites people who are over 50 and have smoked the equivalent of a pack of cigarettes a day for 20 years to have regular testing.

Initial results, to be presented on Monday at the International Association for the Study of Lung Cancer's 16th world conference, suggest that the EarlyCDT-Lung cancer blood test picks up eight out of ten lung cancers.

While regular screening with CT scans has been proven to save lives by picking up early lung cancer, it is expensive and not available on the NHS. However, adding in regular blood tests and only sending people with a positive result for CT scans would be much more affordable.

The £100 test involves scanning the blood to read alerts from the body's own immune system that indicate cancer.

Lung cancer is one of the deadliest cancers, largely because it is generally picked up late, once it has already started to spread.

Dr Neal Navani, a lung cancer specialist from UCLH, said: "These results are very exciting. Previous data on the EarlyCDT-Lung cancer blood test suggested that it was able to detect four cases out of 10 with lung cancer.

"The preliminary results from the ECLS trial indicate that the test may be far more sensitive than previously thought, detecting eight out of 10 cases.

The trial also confirms that when a test is positive for lung cancer, it is correct 90 per cent of the time.

"If this is proven in the final results of the trial, then this makes the EarlyCDT blood test a very important tool for the detection of lung cancer."

Oncimmune Limited, the company behind the trial, believes it could save the NHS between £40,000 and £50,000 per lung cancer patient through earlier diagnosis.

While it costs about £50,000 to treat someone who is terminally ill with lung cancer until their death, largely because of medication costs, if a tumour is detected early enough, it can be removed with only surgery, a much cheaper option.

The full results of the trial, due in about three years, will show how people given the blood test fared compared with those just given standard care, to determine whether the early detection can save lives — and whether the numbers of cancers detected justify a full screening programme.

Professor Frank Sullivan, the lead investigator, said they believed the current test predicted two years ahead and said that they might re-test some early recruits a second time "to see whether their negative test may have become positive and the positive tests who have not been shown to have a lung cancer may have changed".

Dr James Jett, a lung cancer specialist at National Jewish Health in the United States, said: "These preliminary results are very exciting and could change the landscape of screening for lung cancer."

Blood test for ageing will ×

nes.co.uk/tto/health/news/article4549368.ece

News | Opinion | Business | Money | Sport | Life | Arts | Puzzles | Papers | Irish news | Monday, September 7

Welcome to your preview of The Times

Blood test for ageing will predict dementia

Oliver Moody Science Correspondent
Last updated at 1:00AM, September 7 2015

A groundbreaking blood test that determines a person's "biological age" will help to predict and diagnose dementia, scientists say.

The gene analysis technique, developed by researchers in Britain, Sweden and the United States, is the first to give an accurate impression of how healthily people are ageing. It would allow doctors to estimate their risk of a wide range of chronic illnesses and speed up the development of new drugs for Alzheimer's disease.

The gene analysis technique is the first to give an accurate impression of how healthily people are ageing
Science Photo Library/Corbis

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Behind the story:

Hospitals make dementia confused

Dementia sufferers may become distressed and confused when seeking treatment as some hospitals fail to create secure...
Last updated at August 12 2015

Post a comment

GPs warn of lack of supp patients

Shortage of care lets down hundr vulnerable people and often ends taxpayer more,... Last updated at J

Post a comment

but where do most end up?

Criteria for a good clinical biomarker

High specificity

High sensitivity

Metabolism / physiological meaning understood

No circadian or day-to-day variations

Easy collection of the biosample

If in urine – no volume correction required

Chemically stable

Cheap assays – cost effective

Know its value to the clinician

… Few, if any, biomarkers approach this ideal

Biomarker assay types

Analyte group assays / information rich assays / screening assays

- HPLC, CE, NMR, LC-MS, etc.

This is general screening in Clinical Biochemistry

Analyte specific assays

- immuno-assay, LC-MS for proteins

This is routine Clinical Biochemistry

DNA and RNA (genetic) analysis

Becoming routine especially in Cancer Diagnosis

Types of biomarkers

Small endogenous compounds

e.g. creatinine
Homocysteine
Adrenaline

Medium endogenous compounds

e.g. Peptide hormones, insulin

Large molecules

e.g. Proteins
i.e. PLAP, bone ALP, CRP
Immunoglobulins
Enzymes
i.e TPMTase

Biophysical effects

e.g. cell counts
ESR
Osmolality
B.P.

Endogenous Biomarkers

dynamic concentrations

complex sample classes

intra + extra-cellular

always present

many & multi-phase matrices

mM – femtomoles

many unstable post collection

Protein/peptide
(Proteomic)
biomarkers

Some protein/peptide biomarkers

Bone markers

- Bone alkaline phosphatase
- Acid resistant alkaline phosphatase
- Osteocalcin

Cardiac markers

- Various enzymes e.g. LDH

Haematological markers

- Abnormal Hb e.g. $HbA1_C$ HbA2

Tumour markers

- Carcinoembryonic antigen (CEA)
- Carbohydrate antigen (CA19-9)
- Cancer antigen (CA125)
- Cancer antigen (CA15-3)
- Prostate specific (PSA)
- α-fetoprotein (AFP)
- Human chorionic gonadotrophin (HCG)

Protein biomarkers – Some Quality Problems

Collection	Instability on collection Instability on storage
Enzyme assay	Immunodetectability Enzyme Activity Isoenzymes
Immunoassay	Antibody specificity between assays Cross-reactivity Isoenzyme
Separation Methods	Throughput Resolution

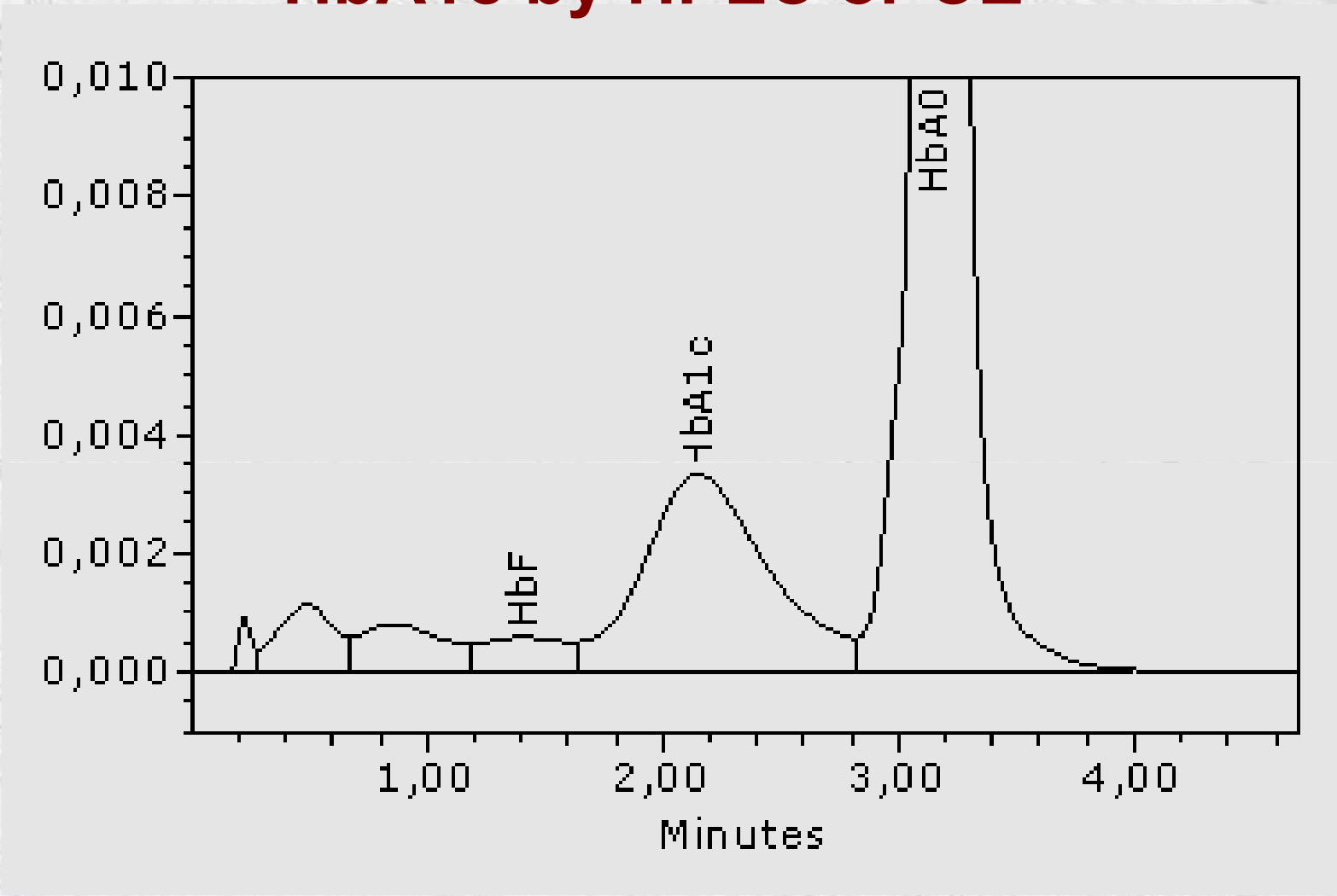
Uses for cancer biomarkers

Use	Example
Estimate risk of cancer	BRCA1 germline mutation (breast & ovarian CA)
Screening	Prostate specific antigen (prostate cancer)
Differential diagnosis	Immunohistochemistry (FISH) to determine tissue of origin
Determine disease prognosis	21 gene recurrence score (breast cancer)
Predict response to therapy	KRAS mutation and anti-EGFR antibody (colorectal cancer) Estrogen receptor expression (breast cancer)
Monitor for disease recurrence	CEA (colorectal cancer) AFP, LDH, βHCG (germ cell tumour)
Monitoring response or progression in metastatic disease	CA15-3 and CEA (breast cancer)

Henry & Hayes, *Cancer Biomarkers* **Molecular Oncology** 6, 2012, 140-146

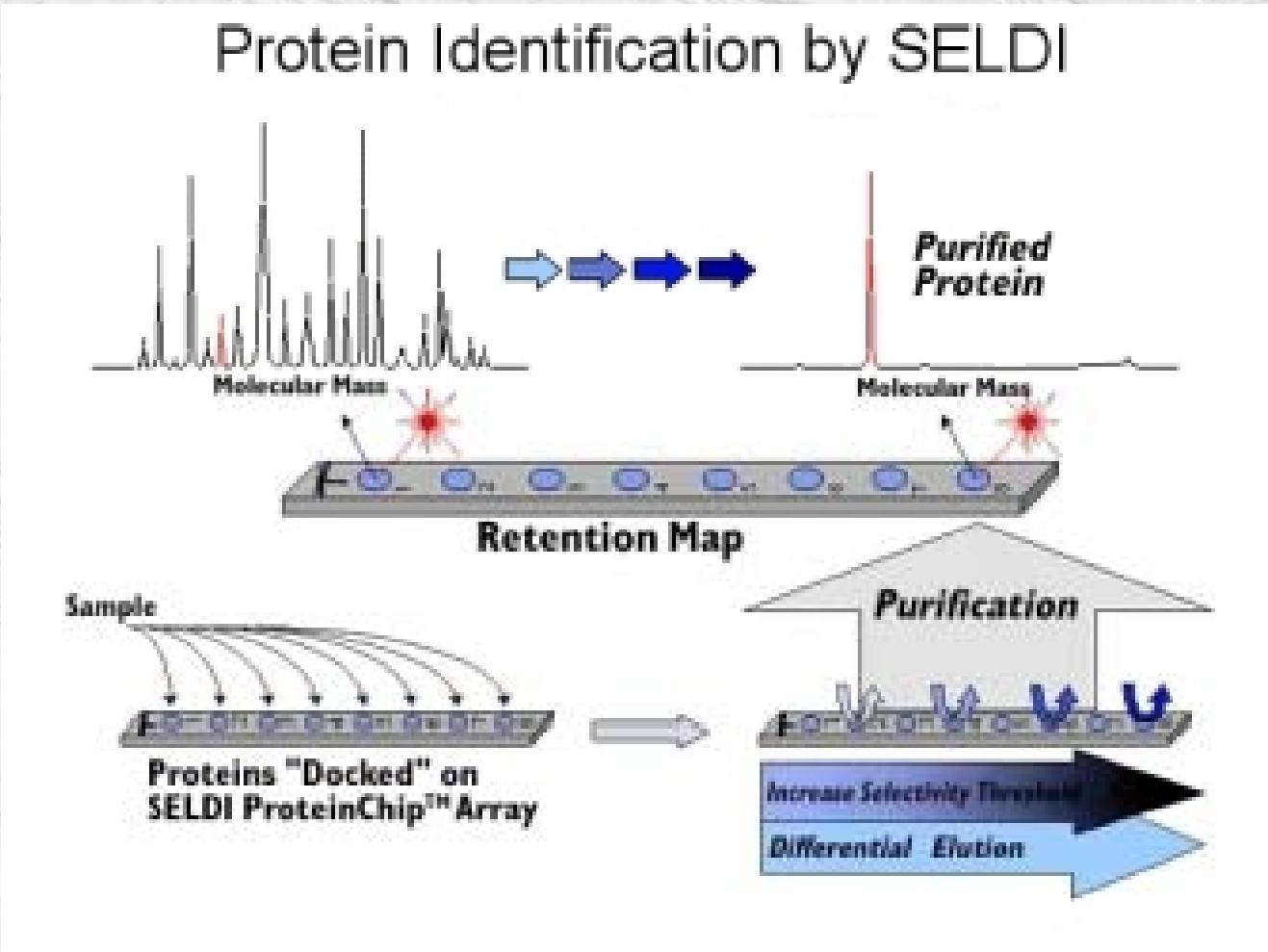
A confirmed protein biomarker

HbA1c by HPLC or CE



Weak cation exchange column

Early Proteomics based biomarker work was based on SELDI



SELDI can detect 200-300 features in a sample. It has been used to find biomarkers from everything from blood to tears.

Biomarker searching

Will typically take 50 samples per condition

Needs to find 10 differences in proteins per condition to have a 90% chance of finding a 2-fold difference

Validation will take 1000s of samples

Finally the assay will have to be converted to something that can be done in a routine clinical lab. and not use proteomics

Risk of Bias in Reports of *In Vivo* Research:
A Focus for Improvement

Malcolm R. Macleod et al.

Plos Biology Published: October 13, 2015

Data for 2,671 publications reporting drug efficacy in eight animal disease models

Randomisation was reported in only 662 publications **(24.8%),**

Blinded assessment of outcome in 788 **(29.5%),**

a sample size calculation in just 20 (0.7%),

a statement of potential conflict of interest in 308 (11.5%).

Early Protein biomarker work has largely been discredited

Biomarkers with similar masses kept being rediscovered.

When the proteins were identified, they were often abundant serum proteins. There are >500,000 protein variants in the human body

The initial studies used VERY selective patient populations

Multi-center studies fail to validate the biomarkers in the clinic

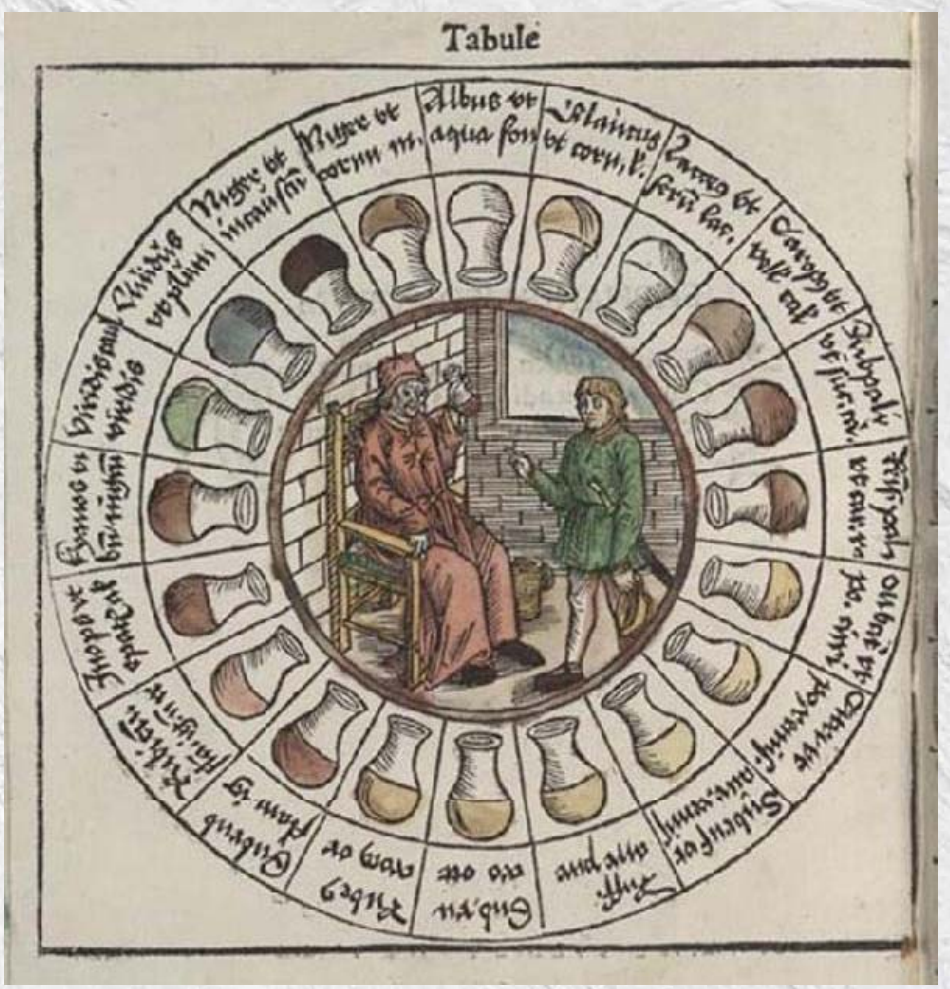
Realization by analytical chemists that serum and other biofluids are not all the same and incredibly complex

Realization that biofluids are incredibly variable and "fragile"

Realization that clinical diagnoses are not perfect

Small Molecule
(Metabolonomic)
Biomarkers

Urinalysis was the first lab test performed in medicine and has been used for several thousand years.



Echeverry, Hortin & Rai Methods Mol Biol. 2010;641:1.
Introduction to urinalysis: historical perspectives and clinical application.

Biomarkers are not new!

1670 Willis discovered sweetness of diabetic urine
1675 Dobson showed that it was sugar
1715 Chevral showed the sugar was glucose

1847 Bence-Jones protein in urine - marker of multiple myeloma

1848 Sir Alfred Garrod (1819-1907) **measured differences in blood urate** *(the thread test)* **in gout**

1897 Biernacki - Erythrocyte sedimentation rate (ESR)
Inflammation – arthritis

1935 Shannon - Serum creatinine in renal clearance failure

1940 Waaler - Rheumatoid Factor

Serendipity?

Some biomarker applications

System	*Examples*
Skeleton	Collagen crosslinks, Alkaline phosphatase, PICP
Neuroendocrine	Catecholamines, 5-HT , 5-HIAA, HVA, VMA
Oncology	X-links, CA125, PSA
Reproduction	Ostrogens, HCG
Inflammation	Neopterins, Histamine, CRP
Cardiovascular	Taurine, Homocysteine, Adenosine
Oxidative stress	8-OxodG, Nitrate/nitrite, GSH/GSSG
Ischaemia	ATP/ADP, hypoxanthine, GSH/GSSG
Vitamin status	Vitamins C, D, B_6, B_{12},

Some aspects of validation

Patient cohort – number(s)
diagnosis
controls

Sample – plasma and serum are different
Sampling
Sensitivity & selectivity
Analytical validation
Clinical interpretation

Validation of Biomedical Assays

Linearity

Intra-assay variation

Inter-assay variation

Analyte carryover determination

L.O.D.

L.O.Q.

Sample / standard stability studies

Recovery - Is it possible?

Comparison to other assays

Comparison of results to published data.

Compare to a definitive assay

References

VAM Web site www. Vam.org

FDA rules are given in Bioanalytical Method validation May 2001

Shah VP et al Pharm. Res 1993

Shah VP et al. Bioanalytical method validation--a revisit with a decade of progress. Pharm Res 2000;17:1551

HPLC with
fluorescence and electrochemical detection
has proved to be
both
sensitive and specific
in the clinical laboratory

Possible Biomarkers of Cardiovascular Performance

Plasma homocysteine

Nitrate & nitrite in plasma & urine

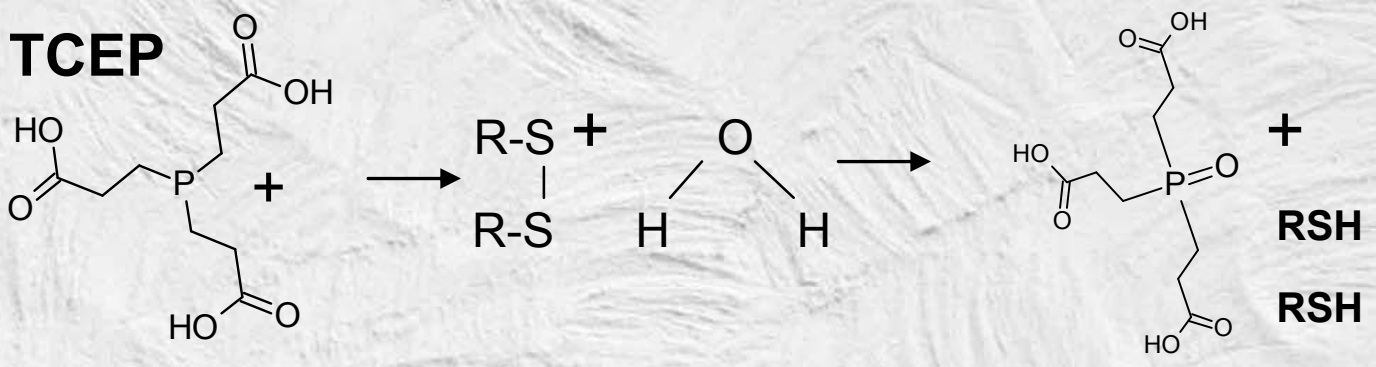
Nitroso – thiols in cells

Plasma taurine

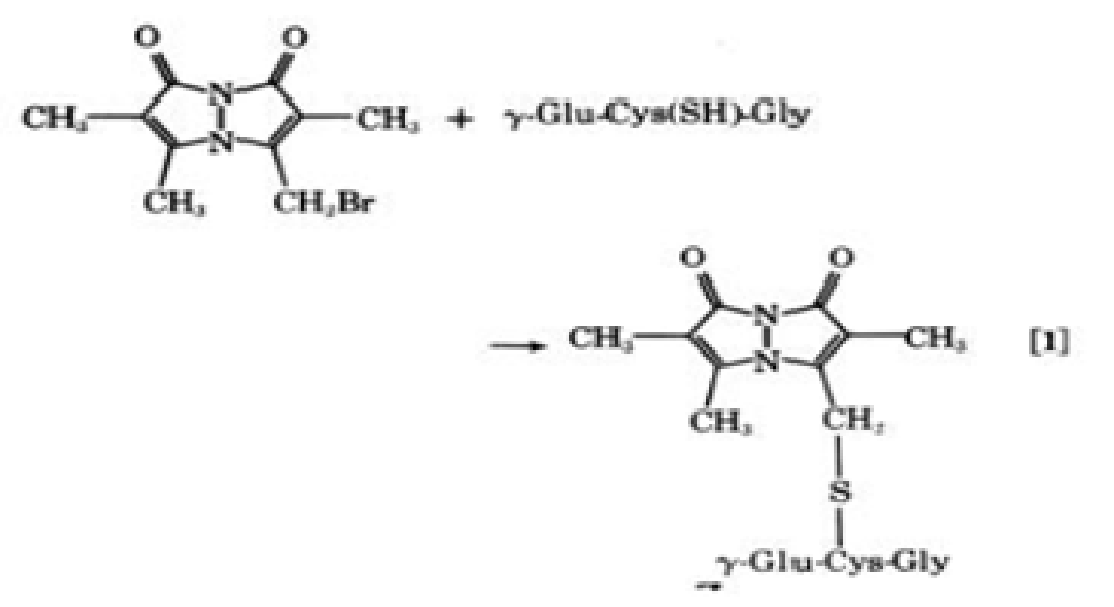
Plasma adenosine

Total Plasma Homocysteine pre - assay

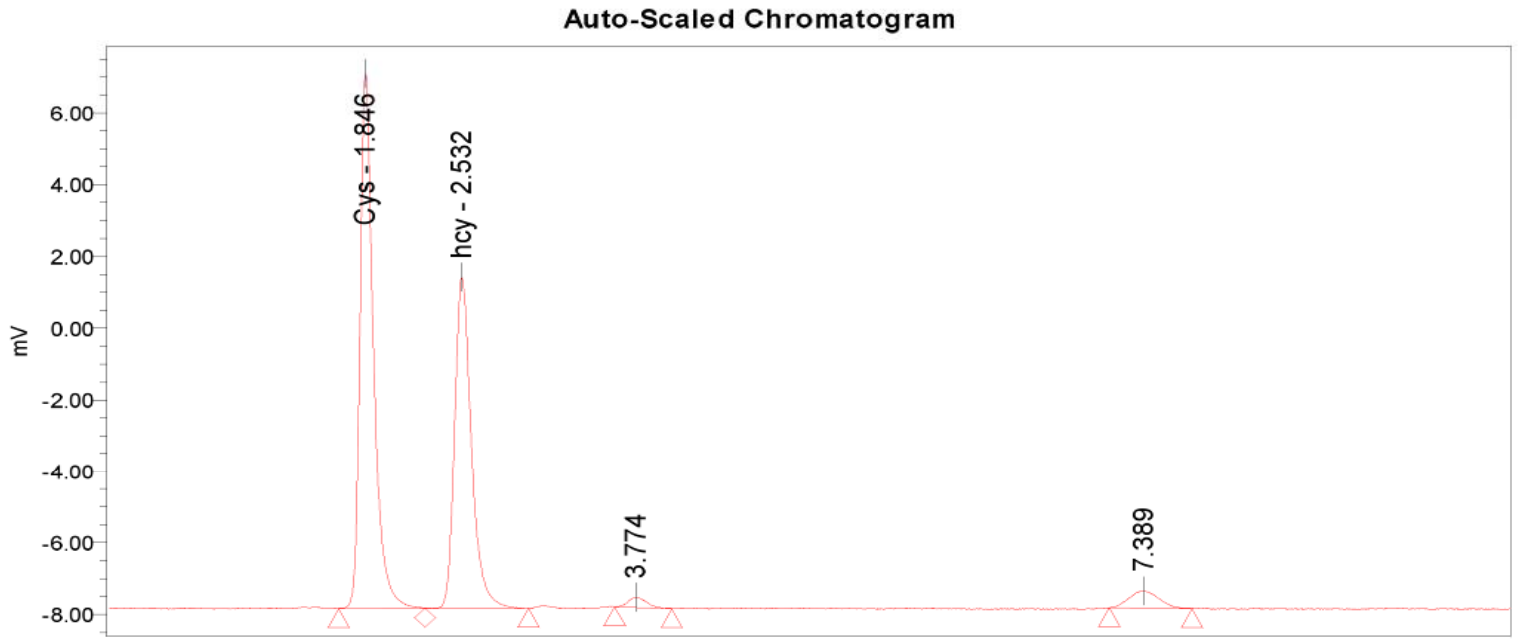
Reduction with TCEP



Derivatisation with monobromobimane



Total Plasma Homocysteine HPLC - Assay

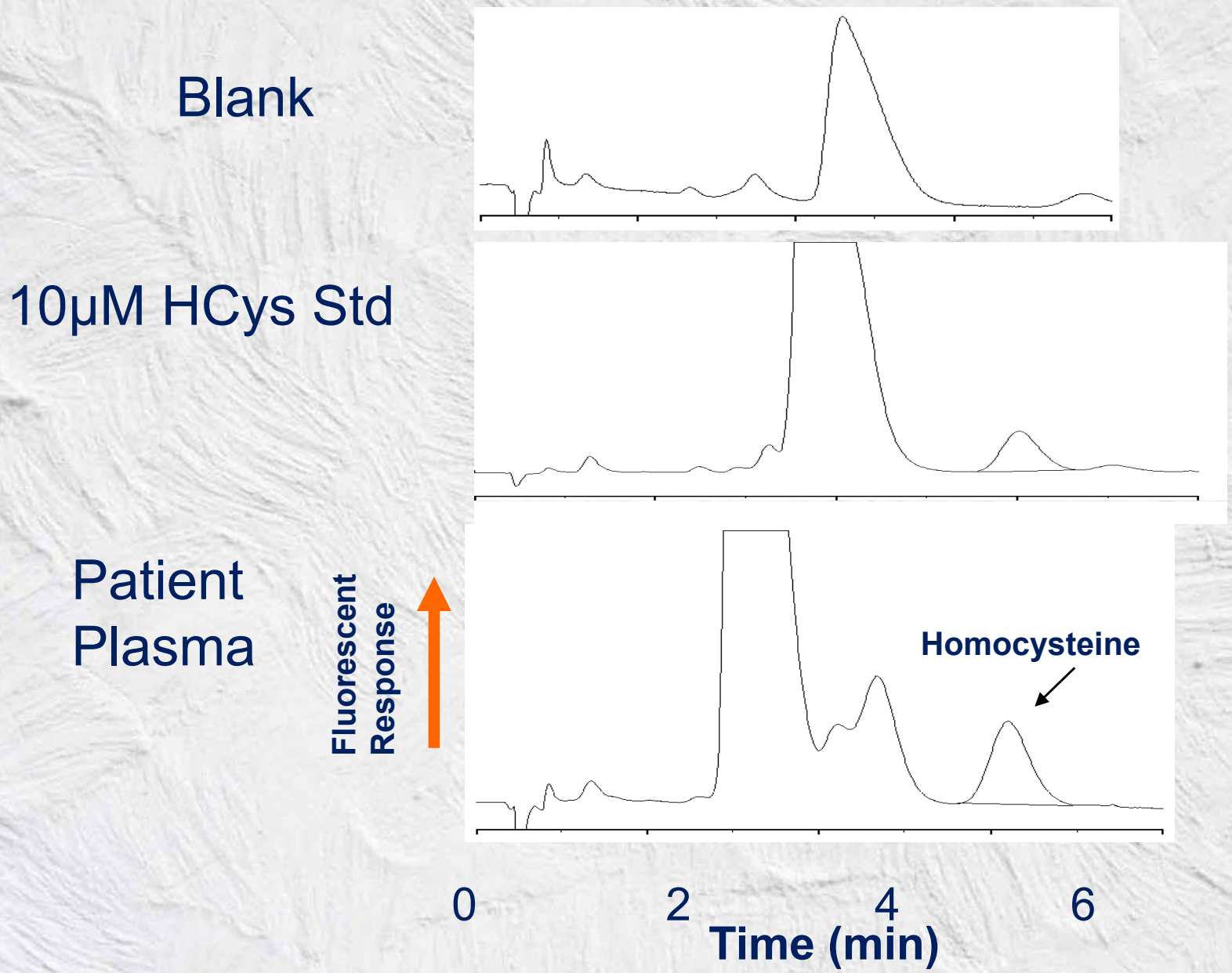


Peak Results

	Name	RT	Area	Height	Amount	Units
1	Cys	1.846	100695	14951		
2	hcy	2.532	77247	9262		
3		3.774	2543	275		
4		7.389	7161	479		

Symmetry Shield™ RP18, 100 x 4.6mm, 5µ
10µL injection
Eluent 50 mM ammonium acetate pH 4.0 + acetonitrile (90:10)
1mL/min
Detection EX 385nm em 470nm

High sensitivity assay of total homocysteine in plasma following bimane derivatisation



Plasma t-Homocysteine and other markers in diabetics

Variant Parameters	Normal males	Male subjects	Normal females	Female subjects
Number	59	120	39	76
Age in years	44.0 ±12.0 (25-76)	60.3 ±11 (24-85)	43 ±14 (24-74)	60 ±13 (33-90)
t-Hcy µmol/L	8.8 ±5.3 (1.0-21)	13.4 ±5.2 (1.0-35).	5.0 ±4.7 (1.0-18)	13.8 ±5.3 (2.3-32)
FBS mmol/L	6.5 ±2.4 (3.7-14)	9.2 ±4.9 (3.3-25)	6.2 ±2.0 (3.9-13)	8.8 ±4.6 (4.4-25)
HbA1c %	5.5 ±0.8 (4.5-7.7)	6.7 ±1.9 (4.1-12).	5.6 ±1.1 (4.1-8.8)	6.9 ±1.9 (4.5-13)
Glycated Protein µmol/L	124 ±85. (3.0-310)	339 ±119 (39-927)	133 ±77 (42-322)	327 ±131 (45-930)
BUN mmol/L	4.8 ±1.3 (2.8-8.3)	6.3 ±2.8 (0.3-21)	4.1±1.5 (2.2-10)	6.2 ±4.6 (1.5-340).
S-creat µmol/L	79.7 ±12.3 (50-112)	90±24 (53-182)	54 ±12.3 (35-89)	78.6 ±66 (31-593)



In-bred, transgenic, diet-controlled, environmentally-controlled animals have revolutionised our understanding of many diseases but clinical biomarkers are not about mice






The trouble is us!

'if it were not for the great variability among individuals medicine might well be a science and not an art'

Sir William Osler 1892

Wouldn't it be nice if we all had the same composition!

Biological Diversity in Man

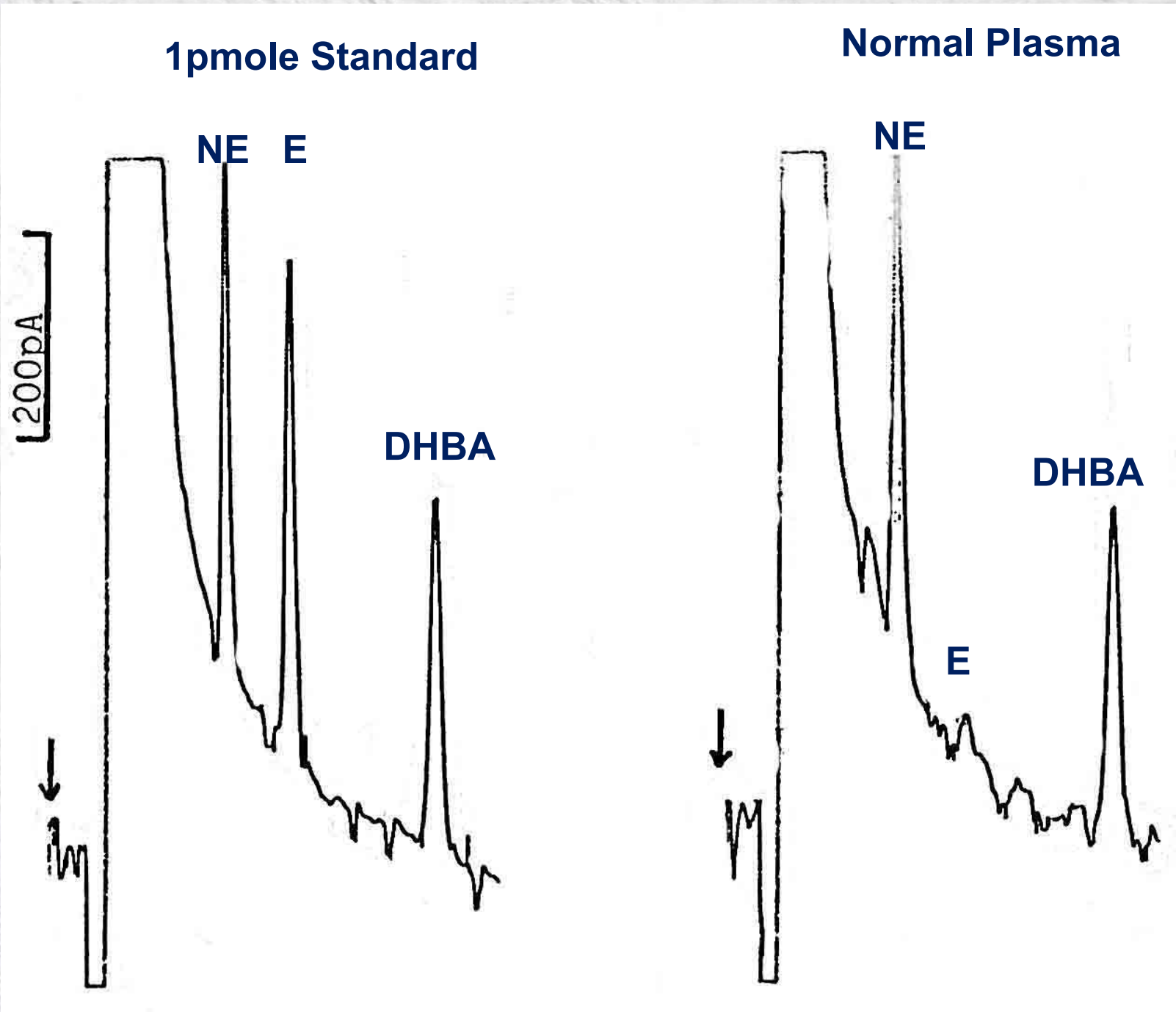
But our DNA is 99.7% common

Some biological variation

	CV (within subject)	CV (between subjects)	CV analytical
Plasma sodium	1	1	1
Plasma creatinine	4	13	2
Plasma urate	9	17	5
Plasma urea	12	18	6
Plasma alk phos	6	25	3
Plasma LDH	7	15	4
Urinary albumin	36	55	18
Urinary creatinine	24	25	12

(source - westgard.com/biodatabase)

Catecholamines in human plasma –HPLC-EC

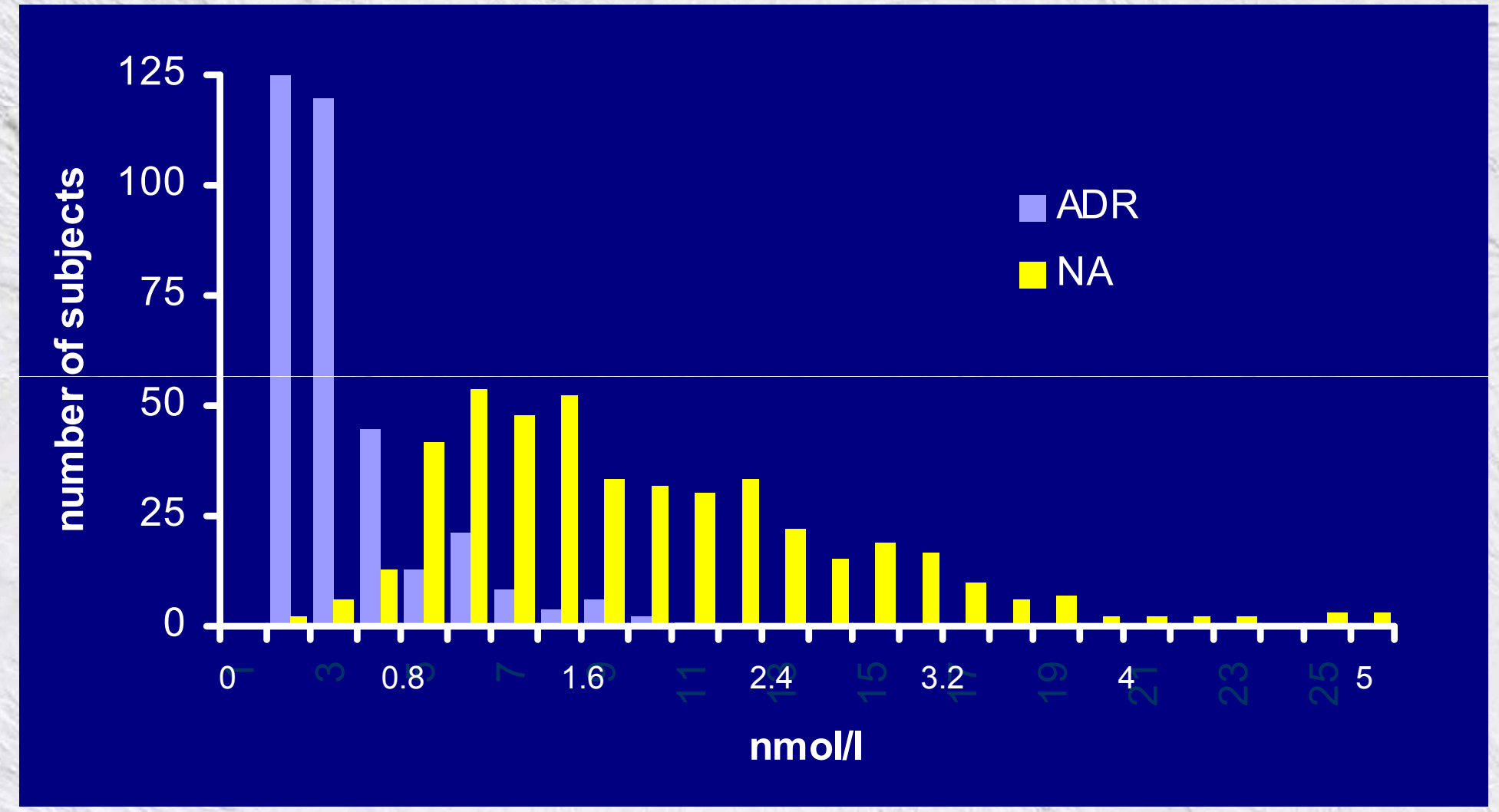


Plasma catecholamine references levels in a 'normal' populations

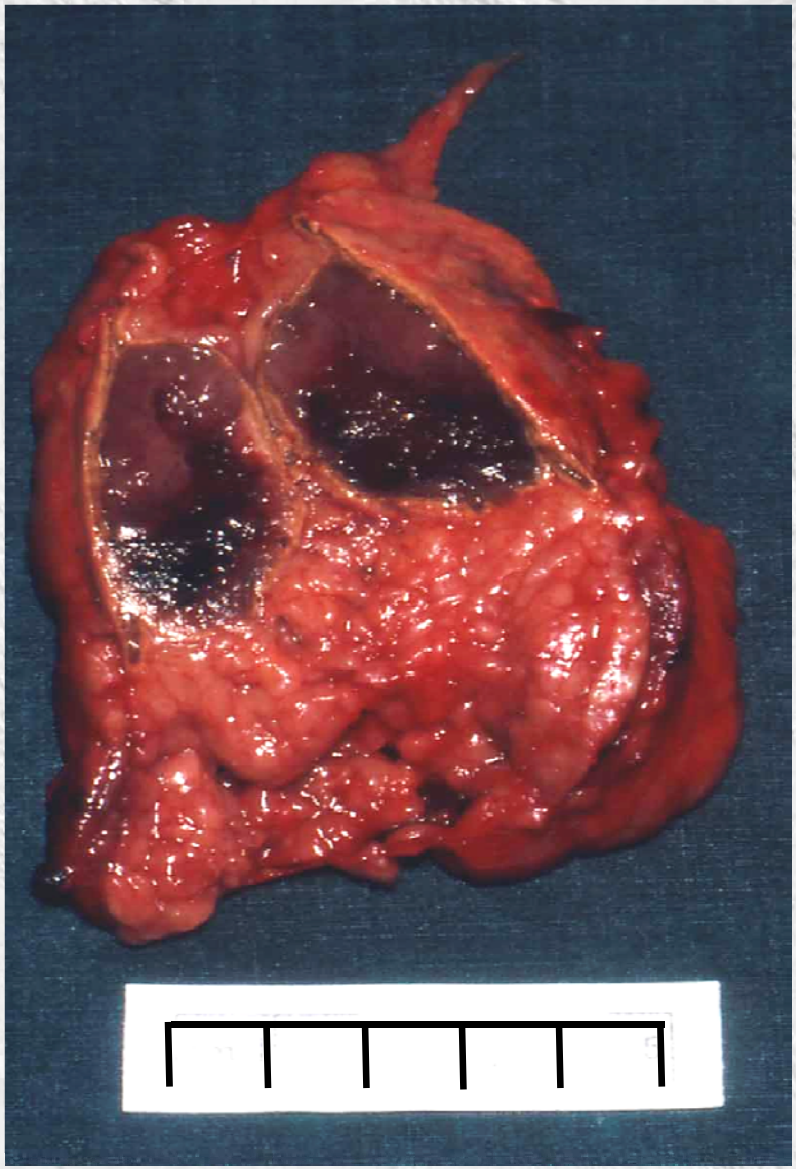
Noradrenaline	Adrenaline	n
pmol/L	pmol/L	
450 – 2490	20 – 460	139
470 – 4120	30 – 1310	545
460 – 3080	60 – 1070	181
840 – 3300	<99 – 480	47
710 – 4020	10 – 3128	
470 – 2940	210 – 453	
410 – 3580	20 – 580	51

From review by Peaston & Weinkove Ann Clin Biochem 41, 2004

Distribution of plasma catecholamine levels in a 'normal' population (n=583)

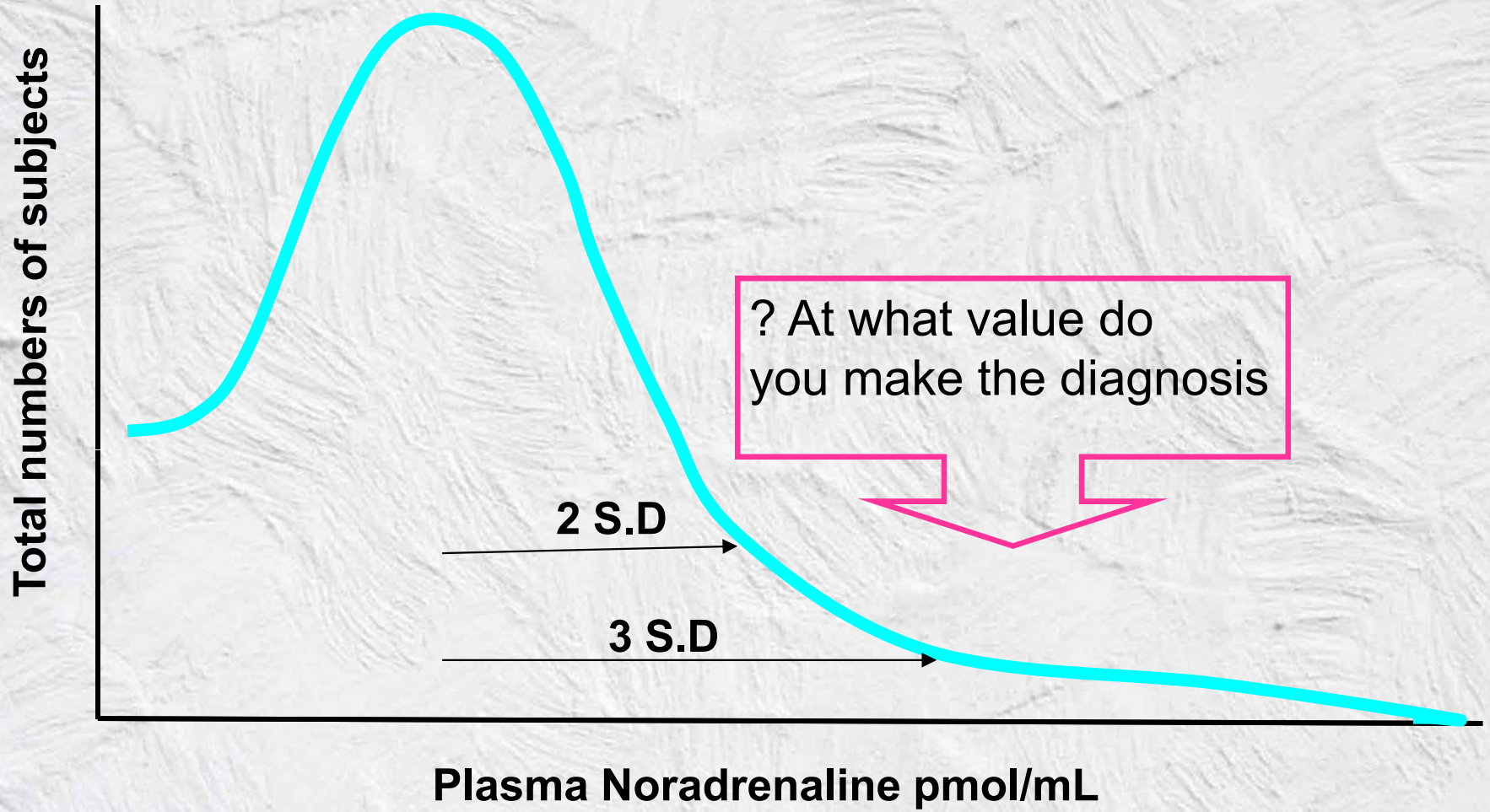


Phaechromocytoma



Catecholamine secreting adrenal tumour that causes massively high BP

Population Variation



Why does clinical population data vary so much?

Subject dependent variables

Physiological variables

Sample/analyte / assay variations

Some additional criteria for validation of Biomarker Assays

Intra-individual variation (timings, diet)

Inter- individual variation (age, sex)

Inter-ethic group variations

Suitability of creatinine correction for urine samples

‘Total’ sample stability studies

Recovery - is it really possible?

Comparison to other assays

Comparison to published normal ranges

Some more criteria for clinical validation of Biomarker Assays

The following are essential in understanding the utility of Bioassays

1. True positive: the patient has the disease and the test is positive

2. False positive: the patient does not have the disease but the test is positive.

3. True negative: the patient does not have the disease and the test is negative

4. False negative: the patient has the disease but the test is negative.

Sensitivity & Specificity of Biomarker Assays

$$\text{Sensitivity} = \frac{\text{Number of true positives}}{\text{True positives} + \text{False negatives}}$$

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{True negatives} + \text{Positives negatives}}$$

How likely is it that this patient has the disease given positive test result?

$$\text{Positive predictive value} = \frac{\text{Number of true positives}}{\text{True positives + False positives}}$$

How likely is it that this patient does not have the disease given that the test result is negative?

$$\text{Negative predictive value} = \frac{\text{Number of true negatives}}{\text{True negatives + False negatives}}$$

Practical Quality in Clinical Biomarkers

Internal quality control

Use of appropriate local reference ranges

NEQAS

SAS Laboratories

Quality models for clinical biomarkers

Sample size must be large enough

At least 120 controlled subjects in the learning set

Even more for a multifactorial disease

See Petersen et al, Ann Clin Biochem 39, 2002, 543

If one biomarker does not work try combinations

Urine test for early stage pancreatic cancer *possible* after biomarker discovery

A combination of *three proteins* in *urine* can accurately detect early-stage pancreatic cancer, QMUL researchers have found.

The discovery could lead to a *non-invasive, inexpensive* test to screen people at *high risk* of developing the disease.

QMUL press release July 2015

Screening in O&G

The Mother

e.g. Pregnancy testing (ß-HCG)

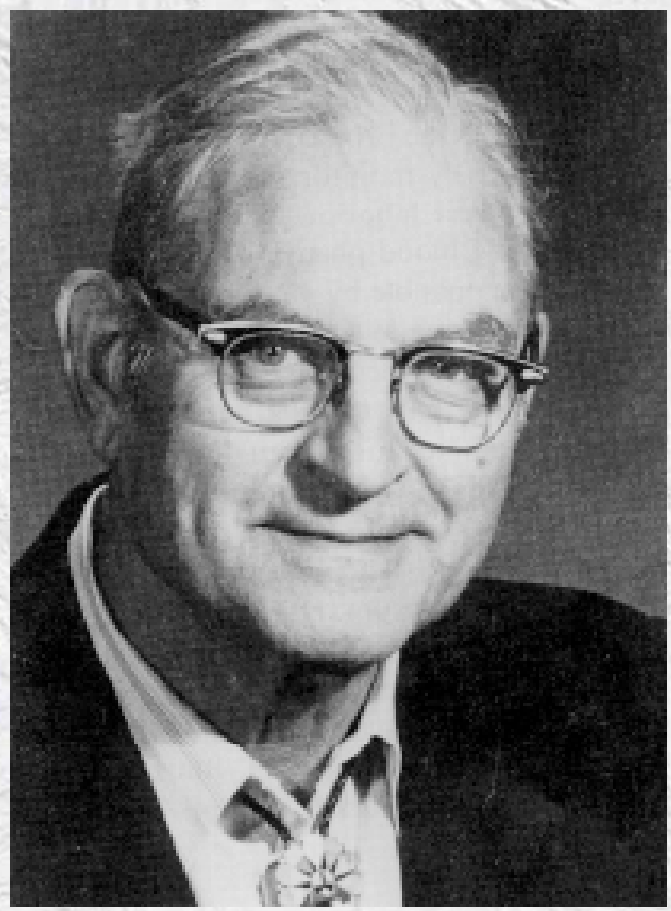
The Parents about the foetus

e.g. Down Syndrome (Triple test)
Pre-eclampsia
Foetal alcohol syndrome

Direct screening of the newborn

e.g. In-born errors of metabolism
Neuroblastoma

Robert Guthrie 1916–1995



1959 **Guthrie test invented and adapted to work with blood spots**

1961 Trial with 3000 families

1962 USA funds a major trial

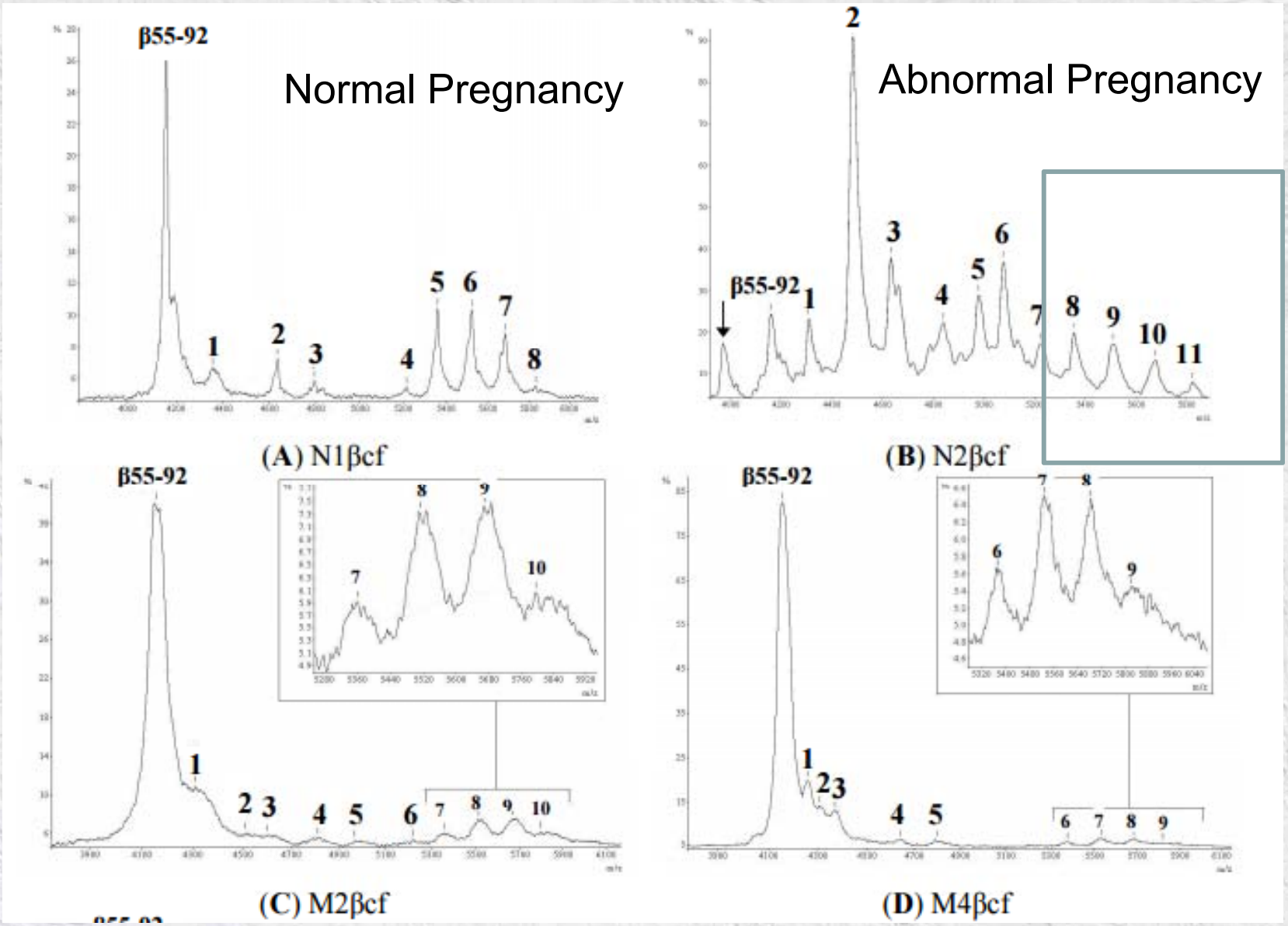
1963 Method paper published in Pediatrics

1965 Guthrie testing mandatory in NY state

Amino acid disorders diagnosed by tandem MS

Disease	Pittsburgh	Kuwait
Maple syrup urine disease (MSUD), clinical variant	2	30
MSUD, intermediate/intermittent variants and E_3 deficiency	None	8
Hyperphenylalaninemia (classical PKU)	16	16
Other types of hyperphenylalaninemias	9	2†
Tyrosinemia type 1	8††	3
Tyrosinemia type 2[1]	8††	3
Hypermethioninemia due to cystathionine ß -synthase deficiency (homocystinuria)	3‡	31
Cystathioninuria	None	1
Cystinuria	None	1
Hyperprolinemia	None	7
Histidinemia	None	1
Nonketotic hyperglycinemia	None	12
Argininosuccinic aciduria	None	13
Citrullinemia	None	7
Ornithine transcarbamylase deficiency	None	4
Lysinuric protein intolerance	None	1

ß-HCG screening of urine by MALDI-MS



Dr Ray Iles – MAP labs

Lab

SC
TECH
INVE

in assc
News

Urine test could warn of miscarriage risk

HOME tests can tell you if you're pregnant – but what if they could reveal if you're expecting twins, or likely to miscarry?

Using algorithms to predict the outcome of a pregnancy based on the proteins in urine could make this a reality.

The test was developed by MAP Diagnostics in Hertfordshire. Its founder Stephen Butler said: 'We want to inform parents of their potential success of having a healthy child.'

Embryos secrete proteins that appear in the urine of their mothers. Rather than a pee stick, Butler's test uses a small mass spectrometer, commonly found in hospitals, to identify these.

To make their predictions, the team use an algorithm they developed by

121 women who were between six and ten weeks pregnant. By identifying differences in the protein it could detect patterns that seemed to be linked to miscarriage.

Other algorithms can predict twins, or if the fetus is likely to be carry chromosomal abnormalities.

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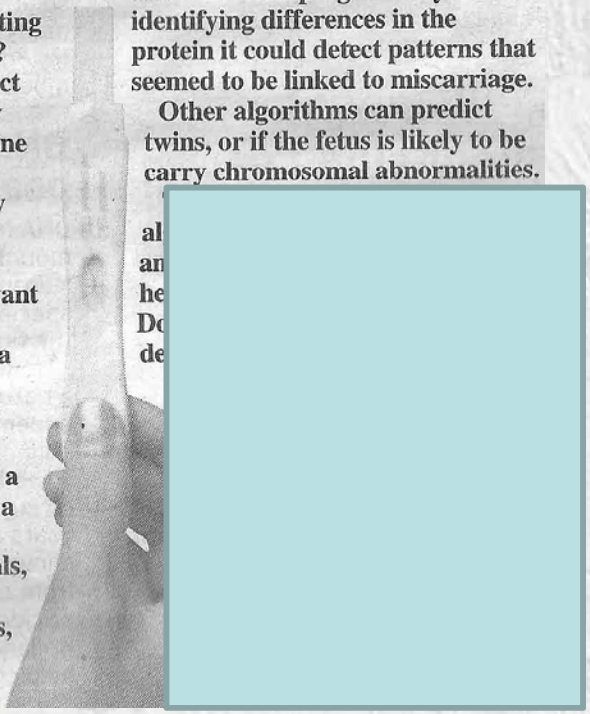

So to validate a biomarker you need….

As well as my earlier specifications

Large numbers of confirmed subjects

Excellent sensitivity & selectivity

Time – many years

Multiple trials – meta-analysis

Acceptance by clinicians

A good reason for doing it - NICE guidelines, Ethics

It must be better, in all respects, than that already available

Thank you

Any Questions