北京師範大學
BEIJING NORMAL UNIVERSITY

Preserving Urinary Proteins Simply and Economically

Youhe Gao

高友鶴

College of Life Sciences, Beijing Normal University

Biobanking 2015, Barcelona Spain

Why do we need to save urine if it is hardly used?

Urine is a better biomarker source than blood.

My Definition

- **Biomarker is the measurable change associated with a physiological or pathophysiological process.**

Sci China Life Sci, 2013, 56: 1145–1146

Biomarker = Change

In Blood

Homeostasis mechanisms
=minimal changes
=less biomarkers

In Urine

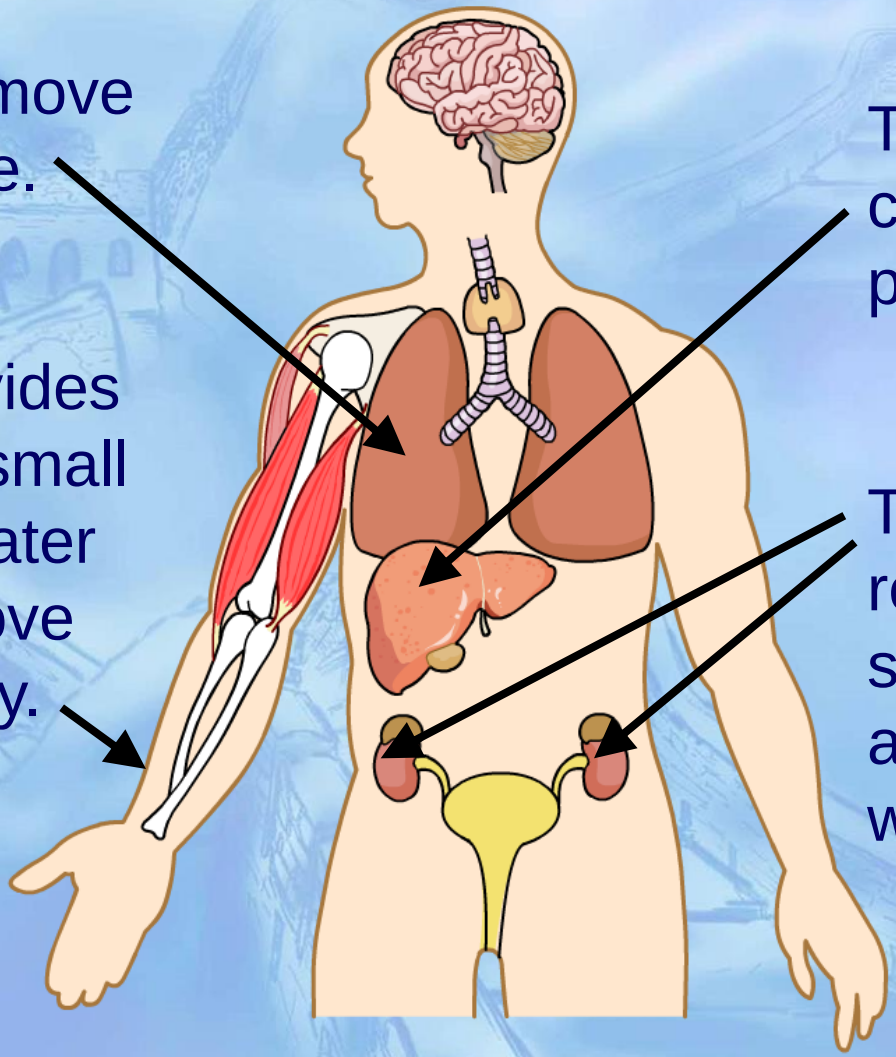
Accumulate all the changes =
Lots of biomarkers

Change removal

Several organs are important in removing waste from the body.

The **lungs** remove carbon dioxide.

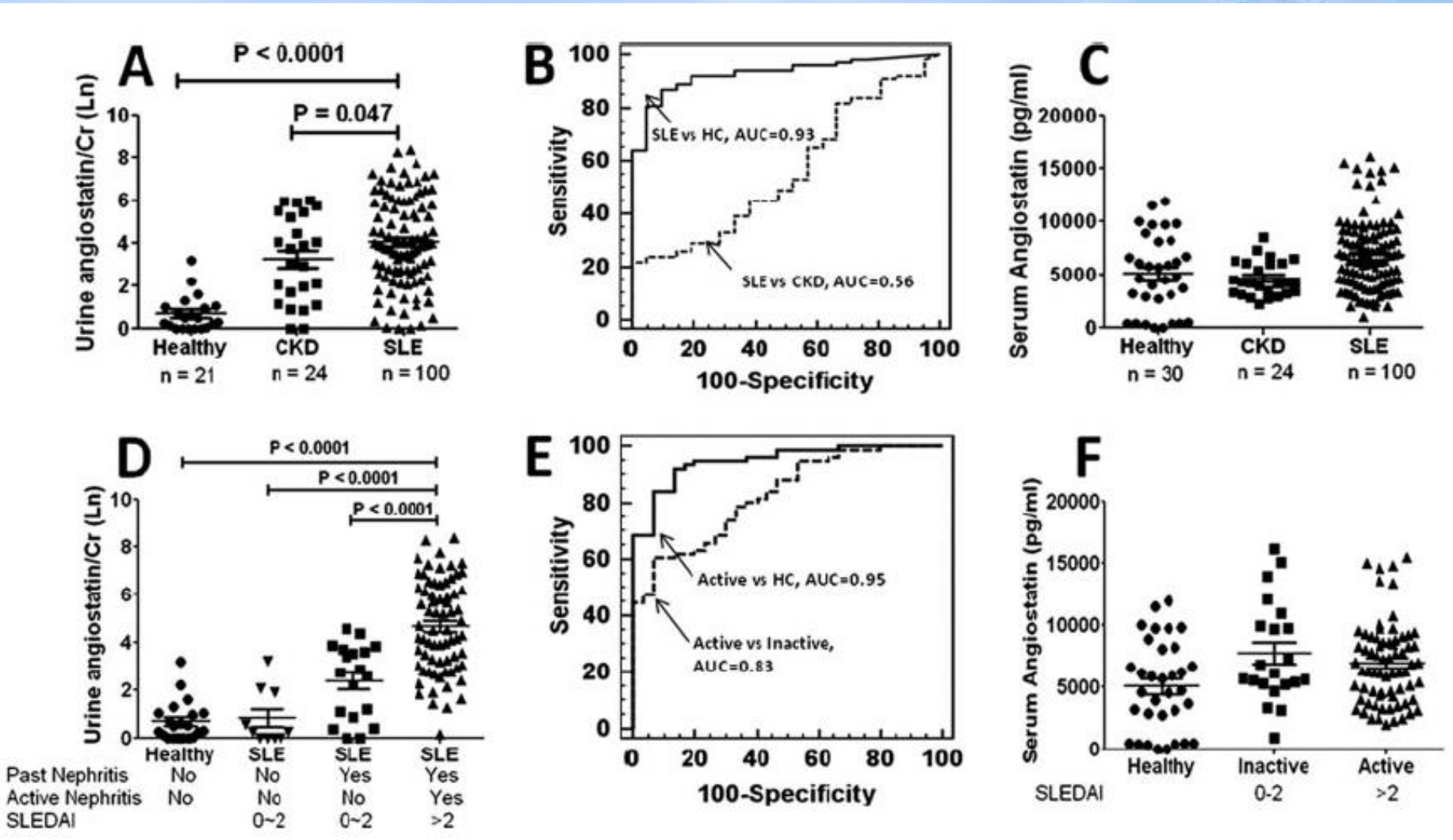
The **skin** provides a surface for small amounts of water and salt to move out of the body.



The **liver** converts excess protein into **urea**.

The **kidneys** remove unwanted substances such as urea, excess water and salt.

Boardworks Ltd 2009



Tianfu Wu et al @ UT Southwestern Medical Center
Molecular & Cellular Proteomics (2013) 12:1170–1179

TABLE III. Frequency of Aberrant Methylation in Urine and Plasma DNA

	GSTP1		RASSF2	
	Urine	Plasma	Urine	Plasma
Positives (%)				
Young asymptomatic males	6%	20%	37%	2%
Biopsy negative	59%	31%	82%	16%
All stages PCa	81%	39%	59%	31%
T1 (n = 47 U, 46 P)	83%	37%	96%	35%
T2 (n = 28 U, 25 P)	71%	32%	82%	16%
T3 (n = 7)	100%	71%	100%	57%
T4 (n = 2)	100%	50%	100%	50%
Median DNA (range), ng/ml				
Young asymptomatic males	0 (0–0.07)	0 (0–0.00[a])	0 (0–0.09)	0 (0–0.00[a])
Biopsy negative	0.001 (0–0.15)	0 (0–0.02)	0.007 (0–0.70)	0 (0–0.04)
All stages PCa	0.008 (0–91.18)	0 (0–0.27)	0.025 (0–112.45)	0 (0–0.19)
T1 (n = 47 U, 46 P)	0.006 (0–91.80)	0 (0–0.18)	0.024 (0–112.45)	0 (0–0.19)
T2 (n = 28 U, 25 P)	0.008 (0–0.88)	0 (0–0.05)	0.022 (0–0.91)	0 (0–0.00[a])
T3 (n = 7)	0.029 (0.001–14.37)	0.001 (0–0.27)	0.042 (0–19.08)	0.0005 (0–0.02)
T4 (n = 2)	n.a. (0.014–0.14)	n.a. (0–0.00[a])	n.a. (0.07–0.08)	n.a. (0–0.00[a])

	HIST1H4K		TFAP2E	
	Urine	Plasma	Urine	Plasma
Positives (%)				
Young asymptomatic males	14%	8%	82%	2%
Biopsy negative	84%	31%	100%	6%
All stages PCa	92%	31%	100%	18%
T1 (n = 47 U, 46 P)	96%	28%	100%	24%
T2 (n = 28 U, 25 P)	82%	28%	100%	4%
T3 (n = 7)	100%	71%	100%	29%
T4 (n = 2)	100%	0%	100%	0%
Median DNA (range), ng/ml				
Young asymptomatic males	0 (0–0.02)	0 (0–0.01)	0 (0–0.24)	0.013 (0–0.00[a])
Biopsy negative	0.004 (0–0.16)	0 (0–0.00[a])	0.052 (0.001–0.94)	0 (0–0.00[a])
All stages PCa	0.008 (0–47.94)	0 (0–0.18)	0.096 (0.004–27.80)	0 (0–0.14)
T1 (n = 47 U, 46 P)	0.008 (0–47.94)	0 (0–0.18)	0.106 (0.01–27.80)	0 (0–0.14)
T2 (n = 28 U, 25 P)	0.008 (0–0.72)	0 (0–0.01)	0.096 (0.01–4.42)	0 (0–0.00[a])
T3 (n = 7)	0.024 (0.00–4.04)	0.0007 (0–0.02)	0.377 (0.01–6.81)	0 (0–0.01)
T4 (n = 2)	0 (0.01–0.06)	0	n.a. (0.08–0.09)	0

Payne, SR et al @ Seattle and Germany
Prostate (2009) 69: 1257–1269

Not just within urinary tract

- This is the first study to demonstrate that analysis of urinary MMPs may be useful in determining disease status in a variety of human cancers, both within and outside of the urinary tract.
- MMP:
  - 72KD
  - 92KD
  - >150KD

Moses MA et al @ Harvard Med School
CANCER RESEARCH (1998) 58, 1395-1399

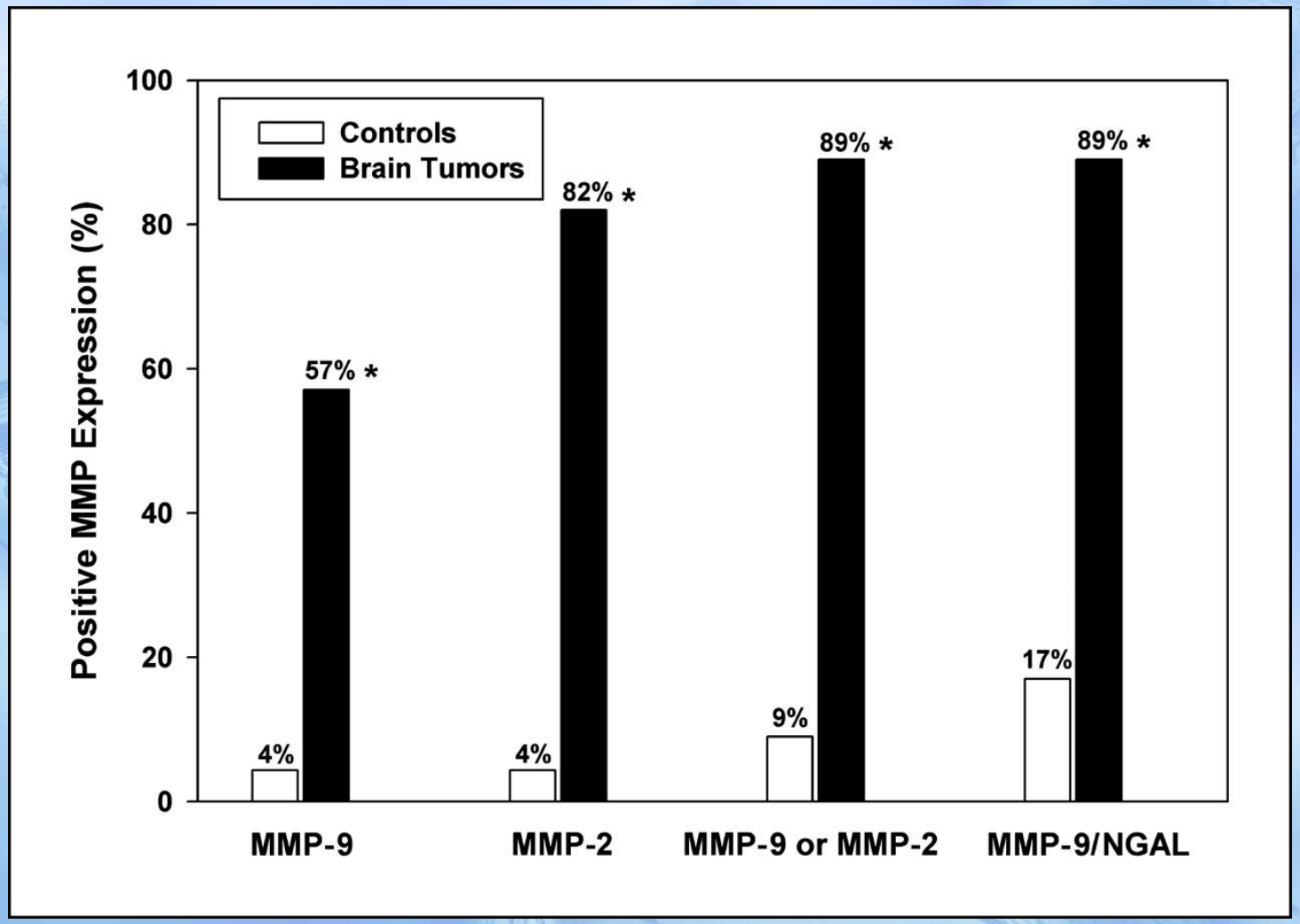
Plasma and Urinary Desmosine as biomarkers for COPD

Table 2 Demographic and desmosine data for group 2 consisting of healthy volunteers and patients with an exacerbation of chronic obstructive pulmonary disease (COPD)

Sample type	Group 2			
	Urine and sputum		Blood	
Group	Healthy volunteers (HV2a)	Patients with 'during an exacerbation' COPD	Healthy volunteers (HV2b)	Patients with 'during an exacerbation' COPD
Number of participants	62	50*	19	102*
Gender (M/F)	24/38	24/26	18/1	43/59
Smoking status (smokers/e-smokers/non-smokers/unknown)	13/41/8/0	31/2/15/2	10/0/9/0	55/33/0/14
Age (years)	22 (21–45)	69 (60–74)†	68 (65–73)	72 (66–79)
Body mass index	25±4	26±7	NA	26±7
FEV_1 (% predicted)	103±13	39±16†	NA	47±18
uDES (ng/mg creatinine)	8 (6–10)	16 (14–22)†	–	–
bDES (ng/ml)	–	–	0.17 (0.12–0.23)	0.30 (0.21–0.37)†

Huang J et al@ UK
Thorax (2012) 67: 502-508

Urine of brain tumor patients contains significantly higher levels of MMP-2, MMP-9, and MMP-9/NGAL compared with control samples.

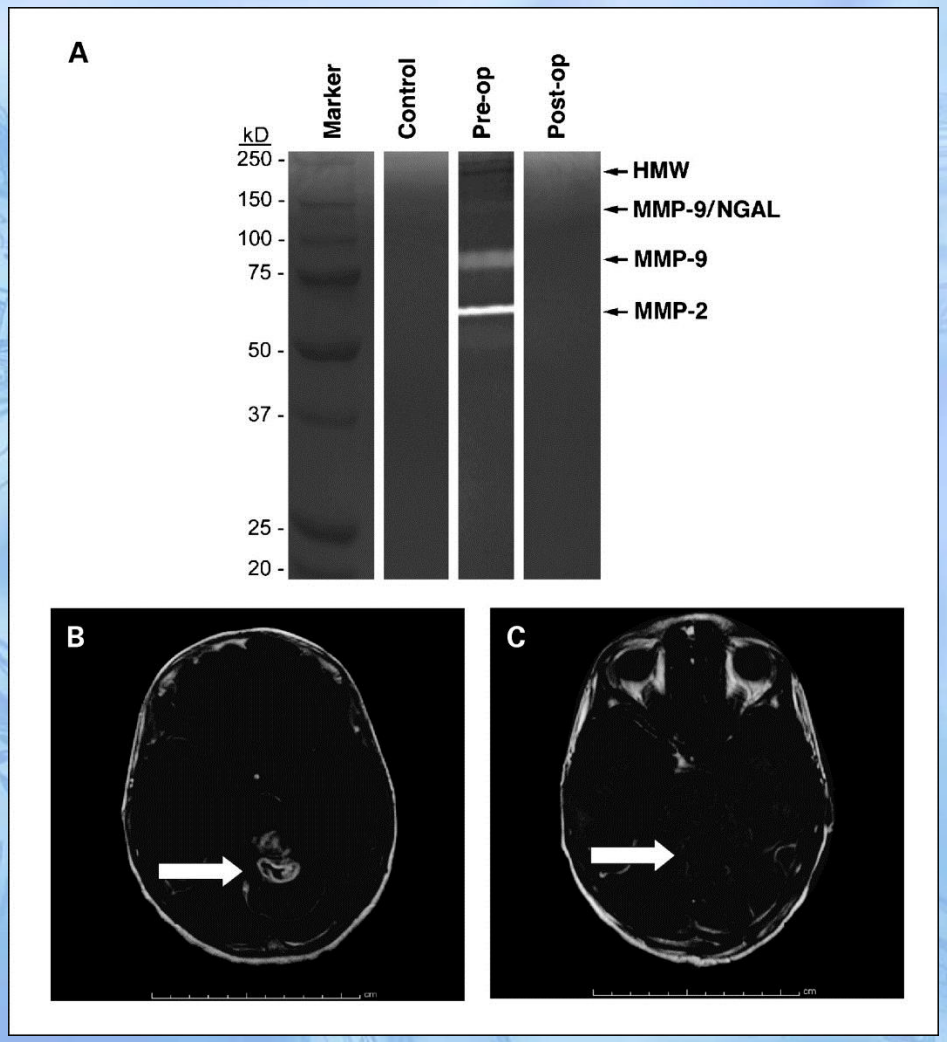


Smith ER et al. @ Harvard Med School
Clin Cancer Res 2008;14:2378-2386

©2008 by American Association for Cancer Research

Clinical Cancer Research

The loss of urinary MMPs after resection of a brain tumor, demonstrating that tumor presence is related to increased urinary MMP activity and removal of that tumor correlates with subsequent clearing of detectable urinary MMP activity.

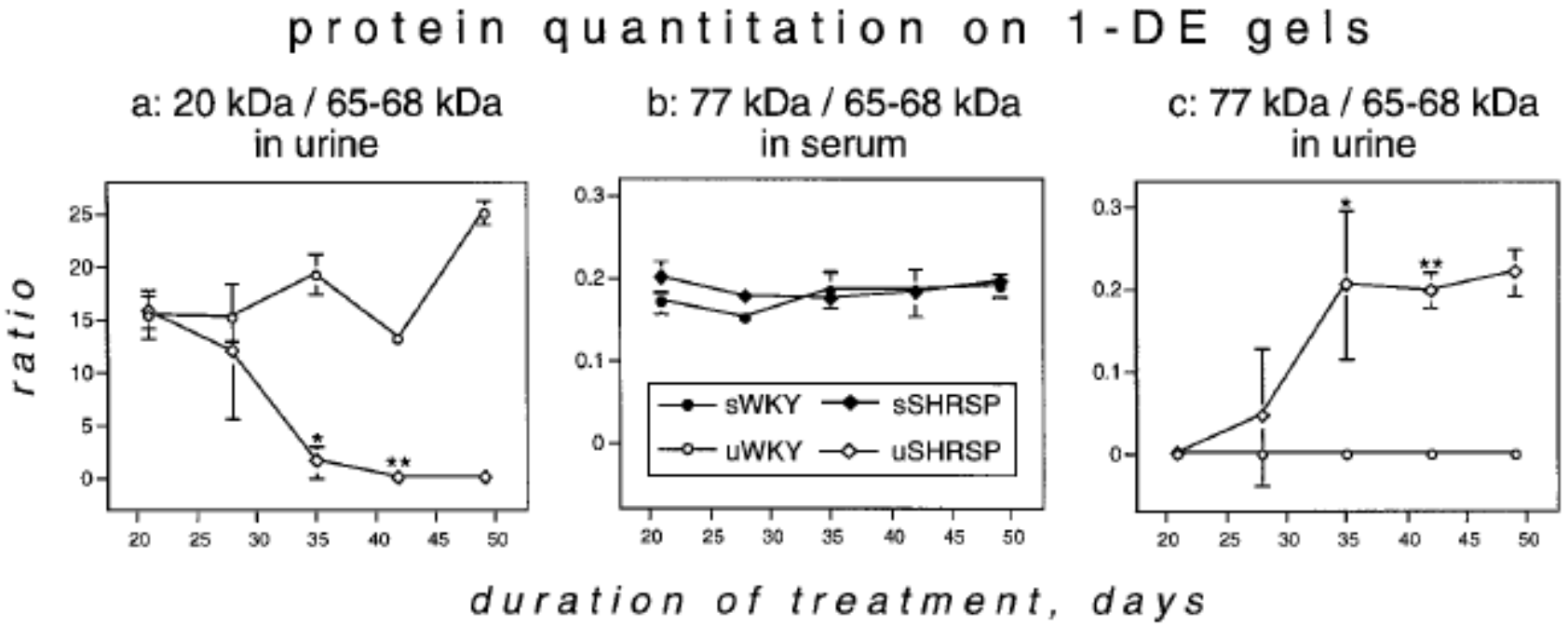


Smith ER et al. @ Harvard Med School
Clin Cancer Res 2008;14:2378-2386

©2008 by American Association for Cancer Research

Clinical Cancer Research

Before Cerebral Ischemia in Rats



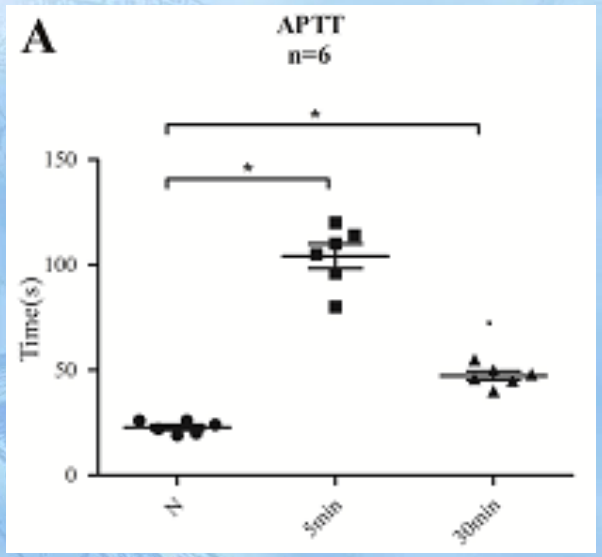
ure 4. Ratio between amount of proteins of different M_r in serum (sWKY, sSHRSP) and in urine (uWKY, uSHRSP). a, 20 kDa/65 to kDa in urine; b, 77 kDa/65 to 68 kDa in serum; c, 77 kDa/65 to 68 kDa in urine. Data are from 1-dimensional electrophoresis (1-DE) under nonreducing conditions on aliquots of serum (0.125 μL) and of urine proteins (3.75 μg) on 4% to 20% T SDS-PAGE gels. :0.05 vs WKY.

Luigi Sironi et al @Italy
Stroke. 2001;32:753-760.

Changes of proteins induced by anticoagulants can be more sensitively detected in urine rather than plasma

Sci China Life Sci, 2014, 57(7): 649-656.

Changes of protein induced by anticoagulants

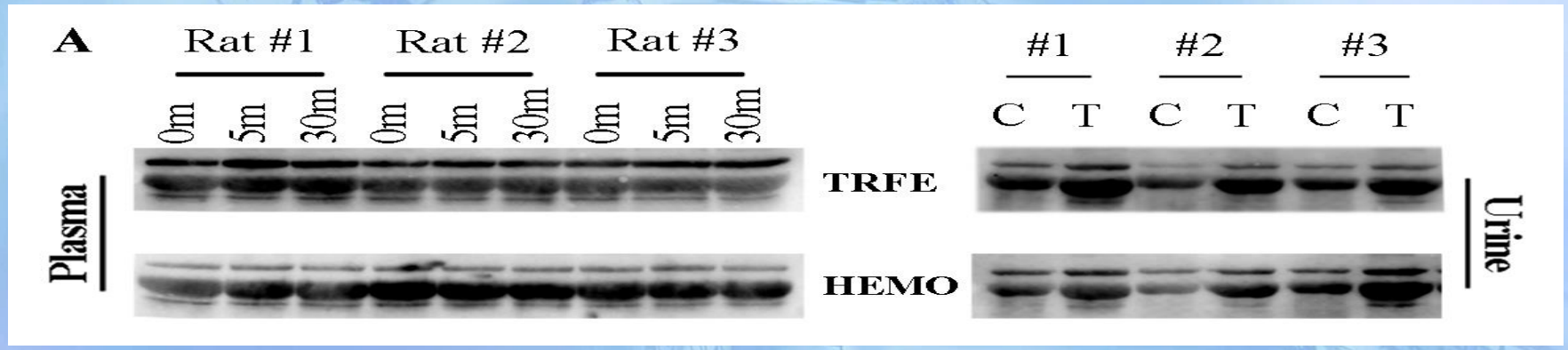


Clotting times increased

Changes

- Heparin
- 27 proteins in urine
- 3 proteins in plasma

- Argatrabon
- 61 proteins in urine
- 1 proteins in plasma



Validation of changes in protein levels

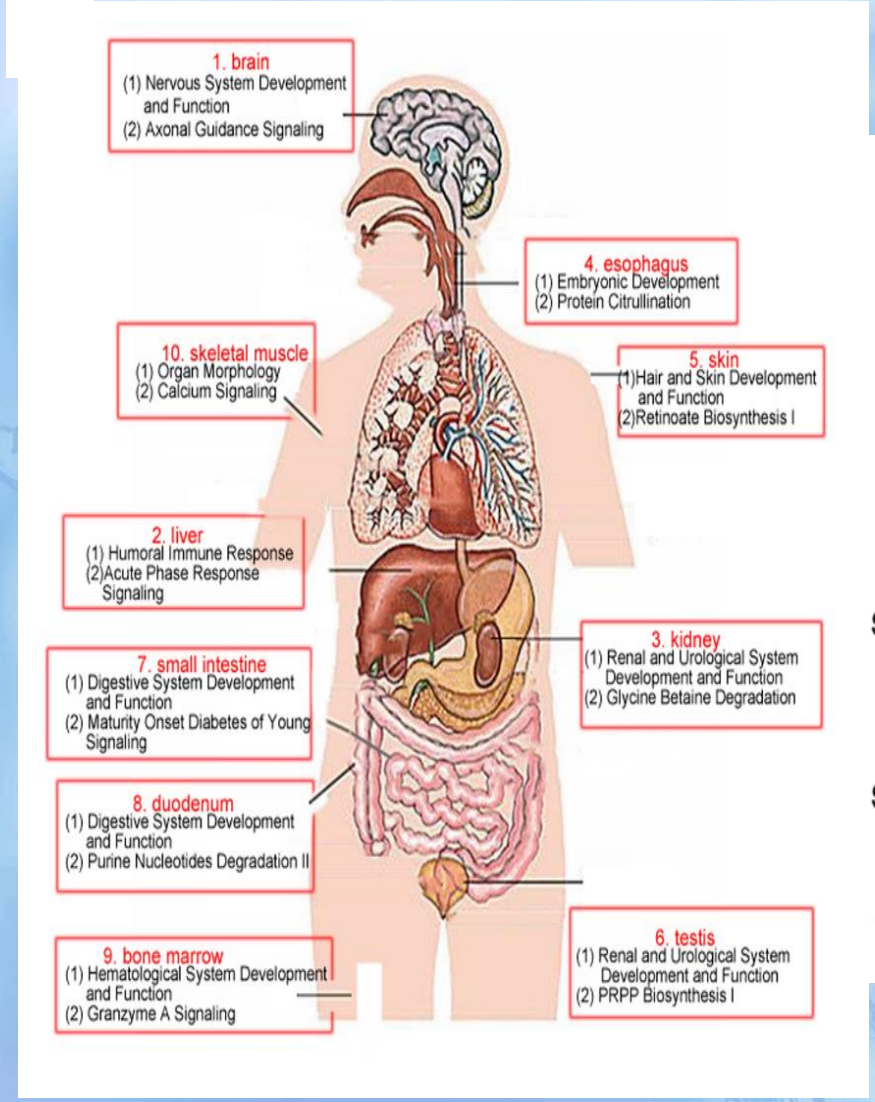
Urine Proteome is Informative

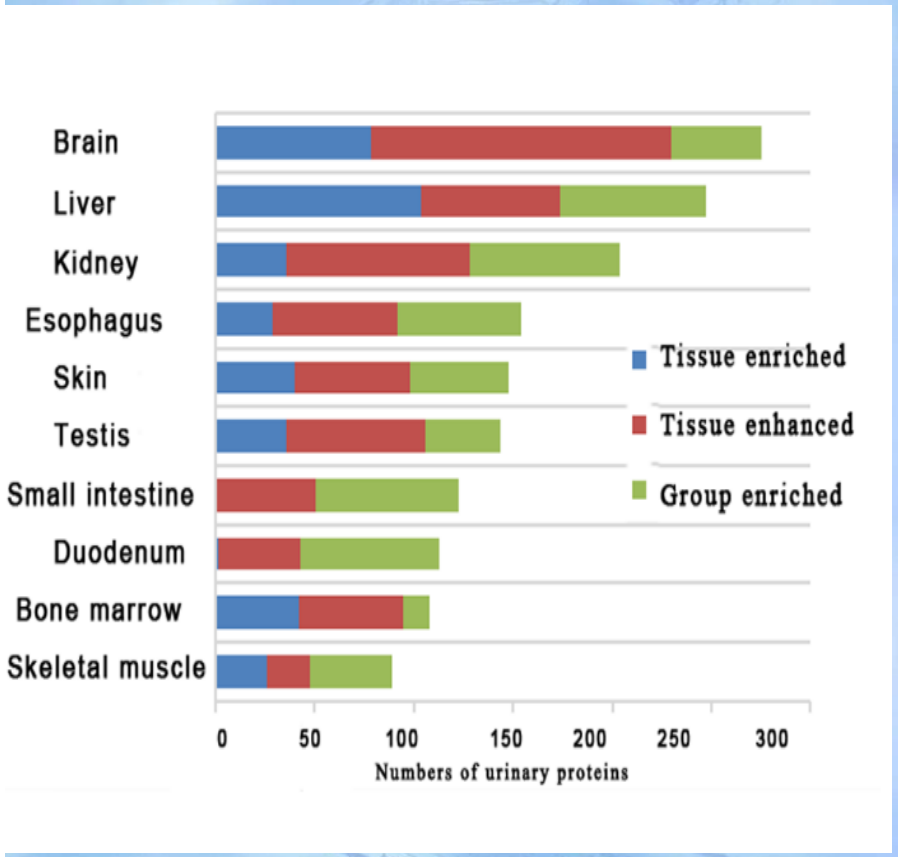
- Up to published result in 2011, 2300 proteins identified totally
- over 6000 with current technology

Low Background

- Actually low protein concentration is better for biomarker discovery
- Low constitutional component is better for revealing the changes

IPA analysis of top 10 tissues



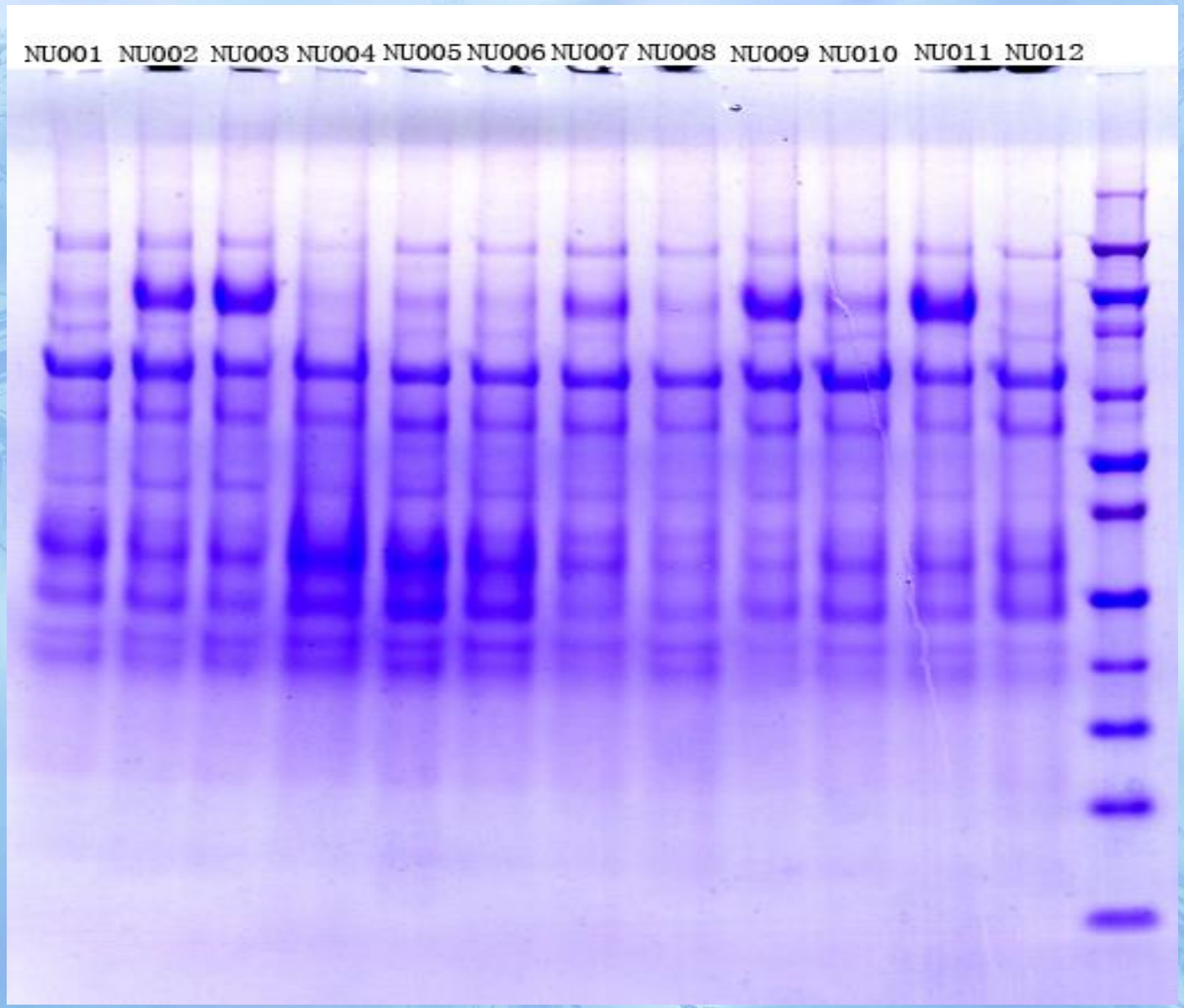


Urine/Blood in Biomarker Studies

Up to 2013-5-29

pubmed - (((blood) OR serum) OR plasma) AND biomarker		pubmed - (urine) AND biomarker		
year	count	year	count	**urine/blood**
2013	3436	2013	302	**8.79%**
2012	20759	2012	1401	**6.75%**
2011	19996	2011	1369	**6.85%**
2010	19097	2010	1288	**6.74%**
2009	17733	2009	1056	**5.95%**
2008	16993	2008	1057	**6.22%**
2007	16509	2007	952	**5.77%**
2006	15186	2006	918	**6.05%**
2005	15389	2005	860	**5.59%**
2004	14208	2004	767	**5.40%**
2003	13417	2003	696	**5.19%**

Individual differences



If urine can reflect physiological changes, it should reflect early pathophysiological changes more sensitively.

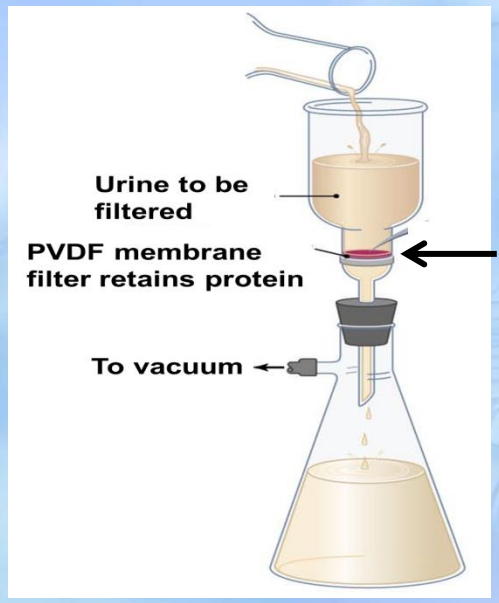
Disadvantages as Biomarker Source

- Big variation
- Need more cases to validate

Problem with samples

- Very diluted
- Protein degradation




Urine to be filtered
PVDF membrane filter retains protein
To vacuum

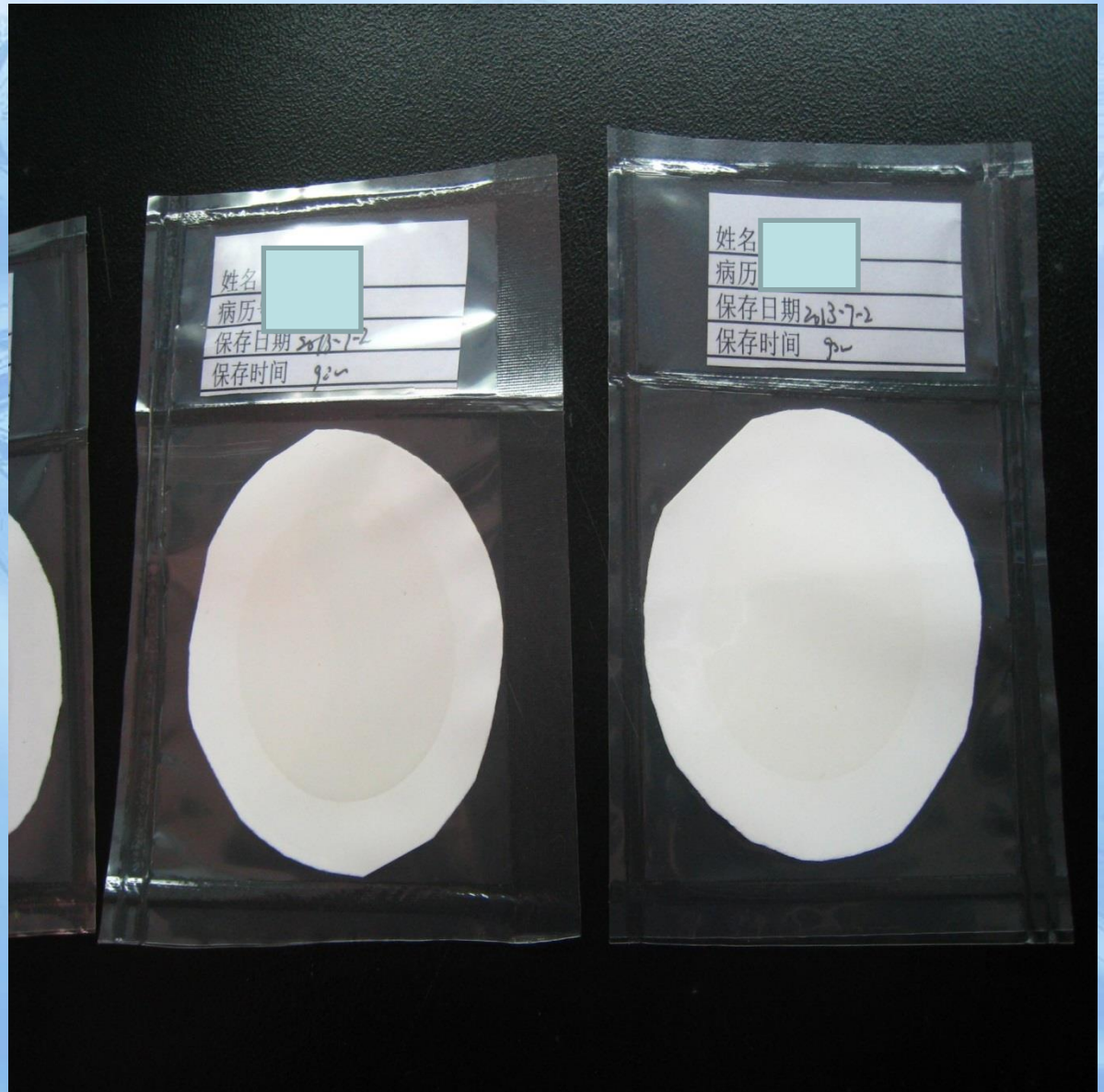

Membrane Solutions

Urimem


healthy-1
healthy-2
healthy-3
healthy-4
10/10/2012 23:43



Urinary Proteins on Membrane



Changed the face of medicine

- “Hard as it is to believe today, a single concept developed by Dr. Henry Plummer (1874 – 1936) at the beginning of the 20th century changed the face of medicine.”



http://www.mayoclinic.org/tradition-heritage/medical-records.html

Changed the face of medicine

- The concept was a centralized medical record, stored in a single repository, and capable of traveling with the patient. (1907)
- Now
  - paperless
  - filmless
  - chartless

http://www.mayoclinic.org/tradition-heritage/medical-

Biobanking's future:

Preserving easily acquired informative samples long-term at room temperature

Urine smells good!


It tastes even better!

Acknowledgement

- Menglin Li
- Lulu Jia
- Liu Liu

- Department of Nephrology, PUMC Hospital:
- Mingxi Li,
- Xuejiao Liu

- Funding from Project 973





北京師範大學
BEIJING NORMAL UNIVERSITY

Thanks!

Youhe Gao (高友鹤)

Blog at Sciencenet (科学网博客),
My Linkedin
gaoyouhe@bnu.edu.cn