



## **WG3 Activities**

- Posting protocols for urine collection
- Development and characterization of standard urine sample
- Position paper on minimal requirements for scientific reporting of biomarker discovery data

# Post urine collection protocols (SOP) to be used by clinicians and researchers ([www.eurokup.org](http://www.eurokup.org))

[Home](#)

## Protocols

This site summarizes Standard Operating Procedures (SOPs) developed and employed by EuroKUP members for biological specimen (urine-tissue) collection. These protocols have been widely employed for proteomics analysis and are therefore recommended to be used in the clinical setting

- **Collection of urine samples for peptidome (e.g. LC-MS or CE-MS) analysis**

To achieve standardization in the clinical setting and facilitate data comparability, a detailed protocol for urine collection has been prepared and can be downloaded [here](#)

Collection of urine samples for proteome analysis

A detailed protocol for urine collection employed in Prof Roz Bank's laboratory is described in the attached file

**Attachment**

**Size**

[PROCEDURES FOR URINE SAMPLE PROCESSING v1 1 - Basic.doc](#)

**28 KB**

Can we agree on minimal SOP for urine collection?

Mischak

Midstream, second morning urine

Collect in urine monovette

Do not use additives

Do not spin (only if turbid) 

Freeze in -20oC within 3hrs (RT? 4°C? ice?)

Do not centrifuge after thawing

Banks

Midstream urine

Collect in sterile urine collector

Place on ice ASAP 

Add PI, adjust pH to 7.0 **X**

Remove particulate matter by filtering  
Centrifuge at 2000g for 10min

Aliquot, freeze in -20oC



Collect midstream, second morning

In Sterile urine collector or monovette (*volume 2-50mls depending on technology*)

Do not use additives

*Centrifuge or not before aliquoting*

Freeze in -20oC within 3hrs (*in between leave at RT or ice?*)

Do not centrifuge after thawing (at least without dissolving precipitates)

## **Development of “standard” urine sample for comparability and standardization in clinical proteomics**

Harald Mischak, Walter Kolch, Michalis Aivaliotis, David Bouyssié, Magali Court, Hassan Dihazi, Gry H. Dihazi, Julia Franke, Jérôme Garin, Anne Gonzalez de Peredo, Alex Iphöfer, Lothar Jänsch, Chrystelle Lacroix, Manousos Makridakis, Christophe Masselon, Jochen Metzger, Bernard Monsarrat, Michal Mrug, Martin Norling, Jan Novak, Andreas Pich, Andrew Pitt, Erik Bongcam-Rudloff, Justyna Siwy, Hitoshi Suzuki, Visith Thongboonkerd, Li-Shun Wang, Jérôme Zoidakis, Petra Zürlbig, Joost P. Schanstra, and Antonia Vlahou



# Development of “standard” urine sample

## Background/Problem statement

Lack of inter-lab and inter-platform comparability in urine proteomics

Lack of appropriate quality controls (platform validation relies on purified proteins)

In depth characterization of urine proteome requires concerted “multi-platform” analysis

## Objectives of the initiative

Generate a “real life” reference sample to be freely available to urine proteomics investigators

Characterize in depth this sample by different platforms and methods

Make data freely available: Set the foundation for comprehensive compilation of urine proteome

Generation of 2 normal pools: Male-Female  
Collection protocol: Midstream morning urine  
 7 male 8 female volunteers

**Clinical characterization**

Analyte or Parameter	male	female
<b>Age</b>	36 ± 6	28 ± 6
<b>BMI</b>	22.5 ± 1.1	22.4 ± 2.8
<b>GFR (CG)</b>	108 ± 6	104 ± 9
<b>Diastolic BP</b>	77 ± 4	71 ± 6
<b>Systolic BP</b>	126 ± 4	112 ± 7
Creatinine	879000,00	787000,00
total protein	14600,00	18400,00
Albumin	3500,00	4600,00
IgA	557,70	258,20
HAA-IgA	0,22	0,19
CD14	10,15	16,37
Ngal	2,20	8,89

Freeze collected samples  
 Thaw on ice, sonicate, combine per gender (>2,500mls )  
 Aliquot in 1, 10 and 50mls

Distribution of samples to multiple laboratories



Analysis by multiple proteomics technologies

CE-MS  
LC-MS/MS (Orbitrap MS)                      →                      Analysis of peptidome

2DE-MALDI TOF MS  
1DE-LC MS/MS (LTQ-FT Ultra; GeLCMS)                      →                      Analysis of proteome

**In depth characterization of proteome and peptidome**

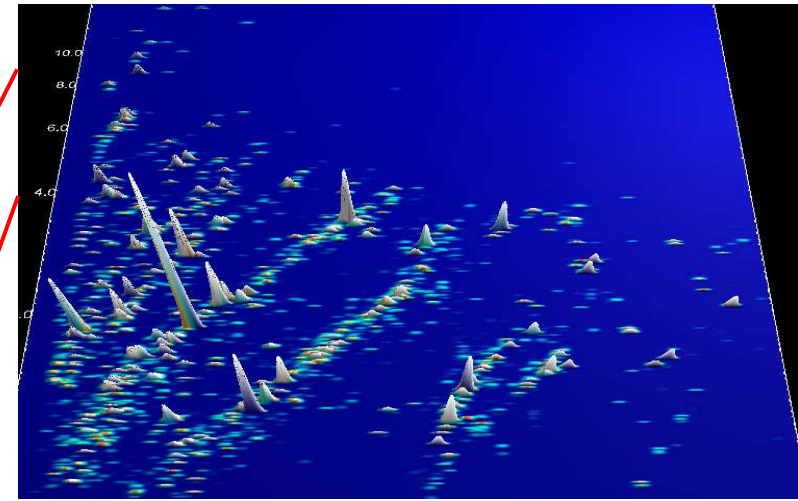
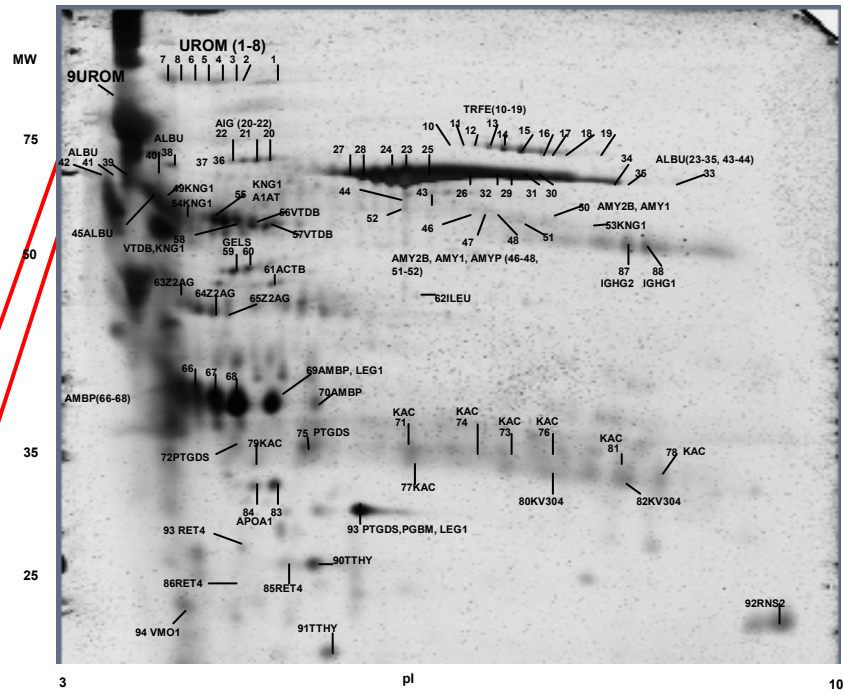
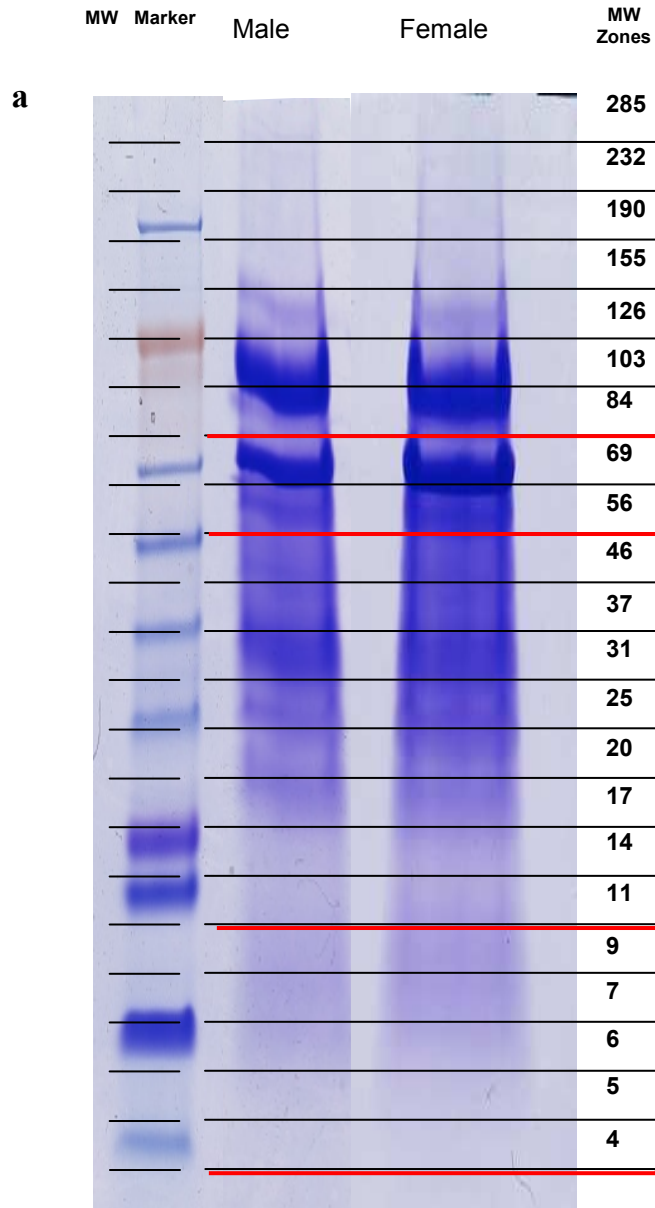
Compile lists of identified proteins and peptides

Collect data on protein PTMs and relative quantities

Absolute quantification of peptides

GeLC-MS

2-DE-MS

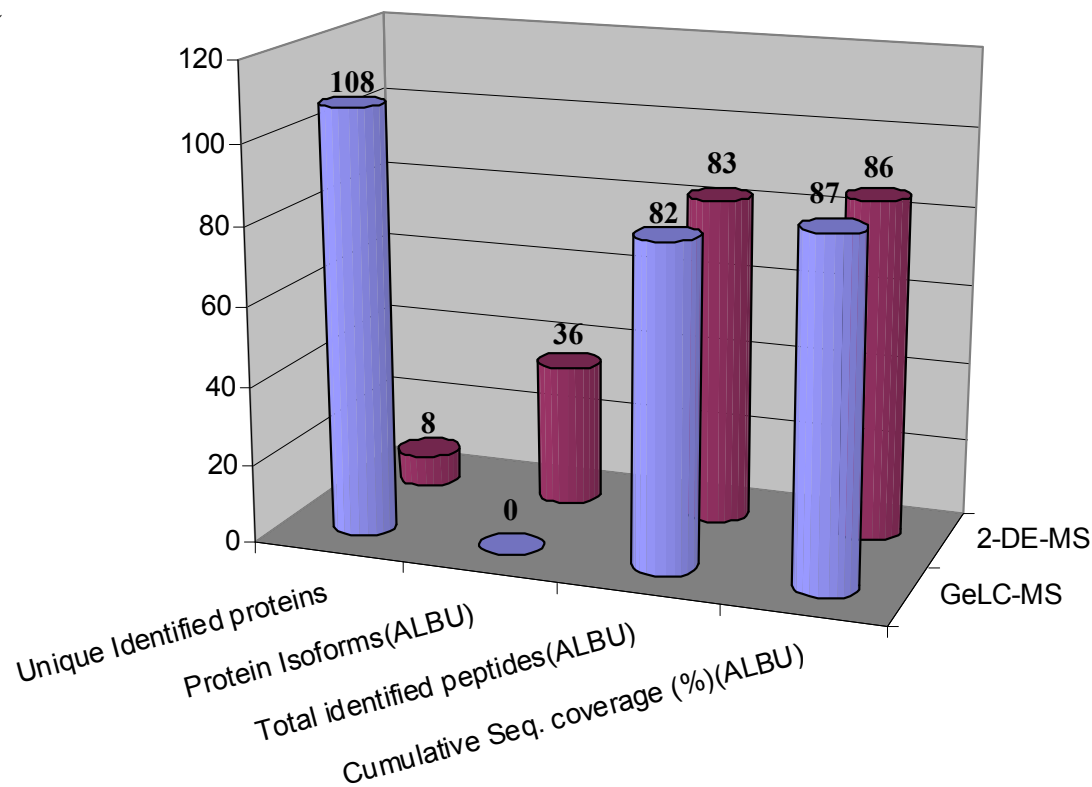


CE-MS

a

## Analysis of Proteome: Platform Complementarity

Mass Zone 56-69 kDa



I

### ALBU: GeLC-MS

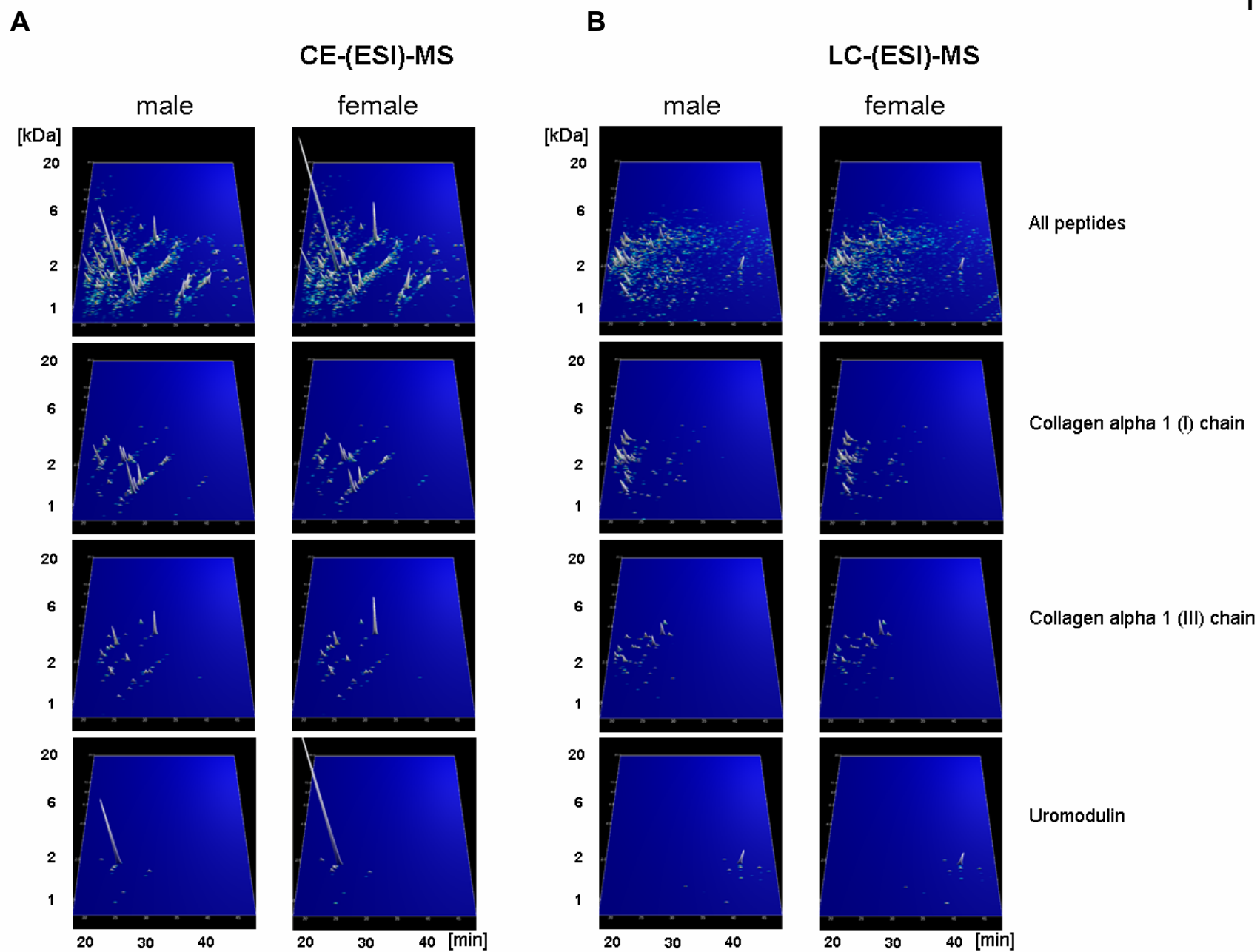
### ALBU: 2-DE-MS

II

1	MKWVTFISLL	FLFSSAYSRG	VFRRDAHKSE	VAHRFKDLGE	ENFKALVLIA	1	MKWVTFISLL	FLFSSAYSRG	VFRRDAHKSE	VAHRFKDLGE	ENFKALVLIA
51	FAQYLQQCPF	EDHVKLVNEV	TEFAKTCVAD	ESAENC DKSL	HTLFGDKLCT	51	FAQYLQQCPF	EDHVKLVNEV	TEFAKTCVAD	ESAENC DKSL	HTLFGDKLCT
101	VATLRETYGE	MADCCAKQEP	ERNECFLOHK	DDNP NLPRLV	RPEVDVMCTA	101	VATLRETYGE	MADCCAKQEP	ERNECFLOHK	DDNP NLPRLV	RPEVDVMCTA
151	FHDNEETFLK	KYLYEIARRH	PYFYAPELFF	FAKRYKAAFT	ECCQAADKAA	151	FHDNEETFLK	KYLYEIARRH	PYFYAPELFF	FAKRYKAAFT	ECCQAADKAA
201	CLLPKLDELRL	DEGKASSAKQ	RLKCASLQKF	GERAFKAWAV	ARLSQRFPKA	201	CLLPKLDELRL	DEGKASSAKQ	RLKCASLQKF	GERAFKAWAV	ARLSQRFPKA
251	EFAEVS KLVT	DLTKVHTECC	HGDLL ECADD	RADLAKYICE	NQDSISSK LK	251	EFAEVS KLVT	DLTKVHTECC	HGDLL ECADD	RADLAKYICE	NQDSISSK LK
301	ECCEKPLLEK	SHCIAEVEND	EMPADLPSLA	ADFVESKDVC	KNYAEAKDVF	301	ECCEKPLLEK	SHCIAEVEND	EMPADLPSLA	ADFVESKDVC	KNYAEAKDVF
351	LGMFLY EYAR	RHPDYSVLL	LRLAKTYETT	LEKCCAAADP	HECYAKVFDE	351	LGMFLY EYAR	RHPDYSVLL	LRLAKTYETT	LEKCCAAADP	HECYAKVFDE
401	FKPLVEEPQN	LIKQNC ELFE	QLGEYKFQNA	LLVRYTKKVP	QVSTPTLVEV	401	FKPLVEEPQN	LIKQNC ELFE	QLGEYKFQNA	LLVRYTKKVP	QVSTPTLVEV
451	SRNLGKVGSK	CCKHPEAKRM	PCAEDYLSV	LNQLCVLHEK	TPVSDRVTKC	451	SRNLGKVGSK	CCKHPEAKRM	PCAEDYLSV	LNQLCVLHEK	TPVSDRVTKC
501	CTESLVNRRP	CFSALEVDET	YVPKEFNAET	FTFHADICTL	SEKERQIKKQ	501	CTESLVNRRP	CFSALEVDET	YVPKEFNAET	FTFHADICTL	SEKERQIKKQ
551	TALVELVKHK	PKATKEQLKA	VMDDFAAFVE	KCKKADDKET	CFAEEGKLV	551	TALVELVKHK	PKATKEQLKA	VMDDFAAFVE	KCKKADDKET	CFAEEGKLV
601	AASQAALGL					601	AASQAALGL				

X: unique identifications

Figure 3





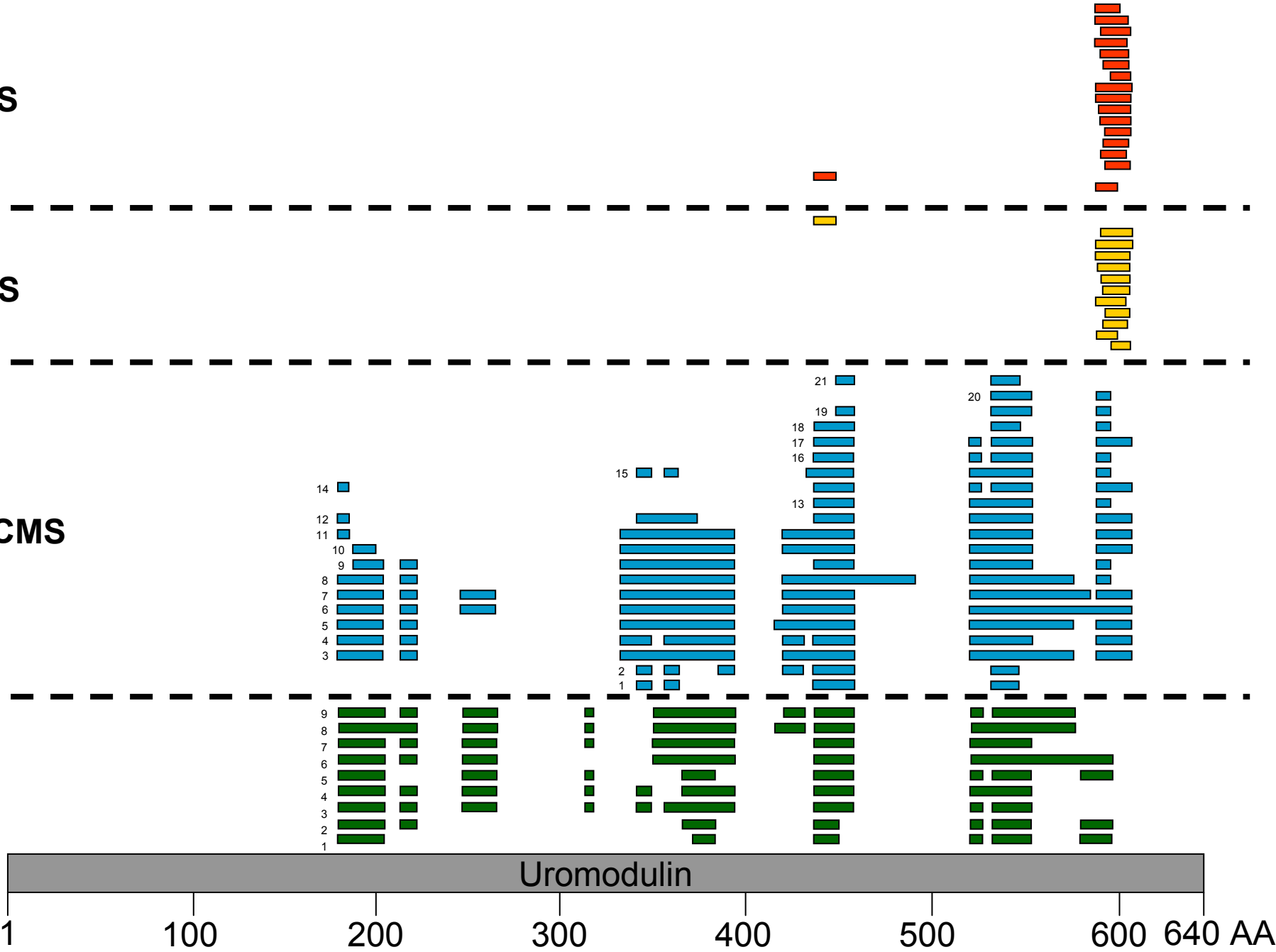
# Compilation of sequence information

LCMS

CEMS

GeLCMS

2DE



## Albumin coverage

### Legend

>40

45-70

80<

45-70+80<

>40+80<

>40+45-70

All ranges

<b>MKWVTFIS</b>	<b>LLFLFSSA</b>	<b>YSRGVFRR</b>	DAHKSEVA	HRFKDLGE	ENFKALVL
IAFAQYLQ	QCPFEDHV	KLVNEVTE	AKTCVAD	ESAENC DK	SLHTLFGD
KLCTVATL	RETYGEMA	DCCAKQEP	ERNECF LQ	HKDDNP NL	PRLVRPEV
DVMCTAFH	DNEETFLK	KYLYEIAR	RHPYFYAP	ELLFFAKR	YKAAFTEC
CQAADKAA	CLLPKLDE	LRDEGKAS	SAKQRLKC	ASLQKFGE	RAFKAWAV
ARLSQRFP	KAEFAEVS	KLVTDLTK	VHTECCHG	DLLECADD	RADLAK
CENQDSIS	SKLKECCE	KPLLEKSH	CIAEVEND	EMPADLPS	LAADFVES
KDVCKNYA	EAKDVFLG	MFLYEYAR	RHPDYSV V	LLLRLAKT	YETTLEKC
CAAADPHE	CYAKVFDE	FKPLVEEP	QNLIKQNC	ELFEQLGE	YKFQNAL L
VRYTKKVP	QVSTPTLV	EVSRLNGK	VGSKCCKH	PEAKRMPC	AEDYLSV V
LNQLCVLH	EKTPVSDR	VTKCCTES	LVNRRPCF	SALEVDET	YVPKEFNA
ETFTFHAD	ICTLSEKE	RQIKKQTA	LVELVKHK	PKATKEQL	KAVMDDFA
AFVEKCCK	ADDKETCF	AEEGK KLV	AASQAALG		

## **Summary**

A urine sample has been generated to be used as reference in clinical proteomics

Characterized in depth by a variety of proteomics platforms

Samples are freely available

Data will be become available in the EuroKUP website

## **Future Work**

Continuous collection of proteomics data on this sample

Need to integrate available results (in collaboration with WG4, e-Lico)

Need to establish in the EuroKUP website systems for protocol, data reporting, processing, comparison, mining (in collaboration with WG4)

## **Recommendations for biomarker identification and classifier validation in clinical proteomics**

Harald Mischak, Günter Allmaier, Rolf Apweiler, Teresa Attwood, Marc Baumann, Ariela Benigni, Samuel E. Bennett, Rainer Bischoff, Erik Bongcam-Rudloff, Giovambattista Capasso, Joshua J. Coon, Patrick D'Haese, Anna F. Dominiczak, Mohammed Dakna, Hassan Dihazi, Jochen H. Ehrich, Patricia Fernandez-Llama, Danilo Fliser, Jorgen Frokiaer, Jerome Garin, Mark Girolami, William S. Hancock, Marion Haubitz, Denis Hochstrasser, Rury Holman, Joachim Jankowski, Bruce A. Julian, Jon B. Klein, Walter Kolch, Theo Luider, Ziad Massy, William B. Mattes, Franck Molina, Bernard Monsarrat, Jan Novak, Karlheinz Peter, Peter Rossing, Marta Sánchez-Carbayo, Joost P. Schanstra, O.John Semmes, Goce Spasovski, Dan Theodorescu, Visith Thongboonkerd, Raymond Vanholder, Timothy Veenstra, Eva Weissinger, Tadashi Yamamoto, and Antonia Vlahou

(submitted)

Scientific context: biomarker discovery studies result in high hopes most of which cannot be validated

This initiative:

- 1) Demonstrates different ways of data interpretation
- 2) Pinpoints the major errors made in earlier research
- 3) Highlights good practice, correct use of statistics to reveal valid biomarkers
- 4) Basis for mandatory requirements for reporting proteomic biomarkers (scientists, journals, agencies)

## **Scientific reporting of proteomic biomarker data should include:**

\*A description of subjects and sampling (demographical, clinical data)

\* Pre-analytical handling and variability

\* Platform performance, including data processing

\*Proper statistical evaluation

- Sample size affects number of biomarkers

- WT-Adjustment for multiple testing

- Evaluation of the biomarkers/profiles in an independent test set is needed

(Dakna et al, submitted)

## Points to be discussed

- Can we reach a consensus on minimal requirements for urine collection?

Generate this consensus protocol  
Send it to members (EuroKUP, HUKPP)

- Expand on standard urine data
- Compilation of sequence information (with WG4 and e-Lico)
- Deposit data in EuroKUP website  
With WG4: systems for protocol, data reporting...

Data providers → deposit to website → STSMs

- Generation of compiled 2DE map (F Molina)