

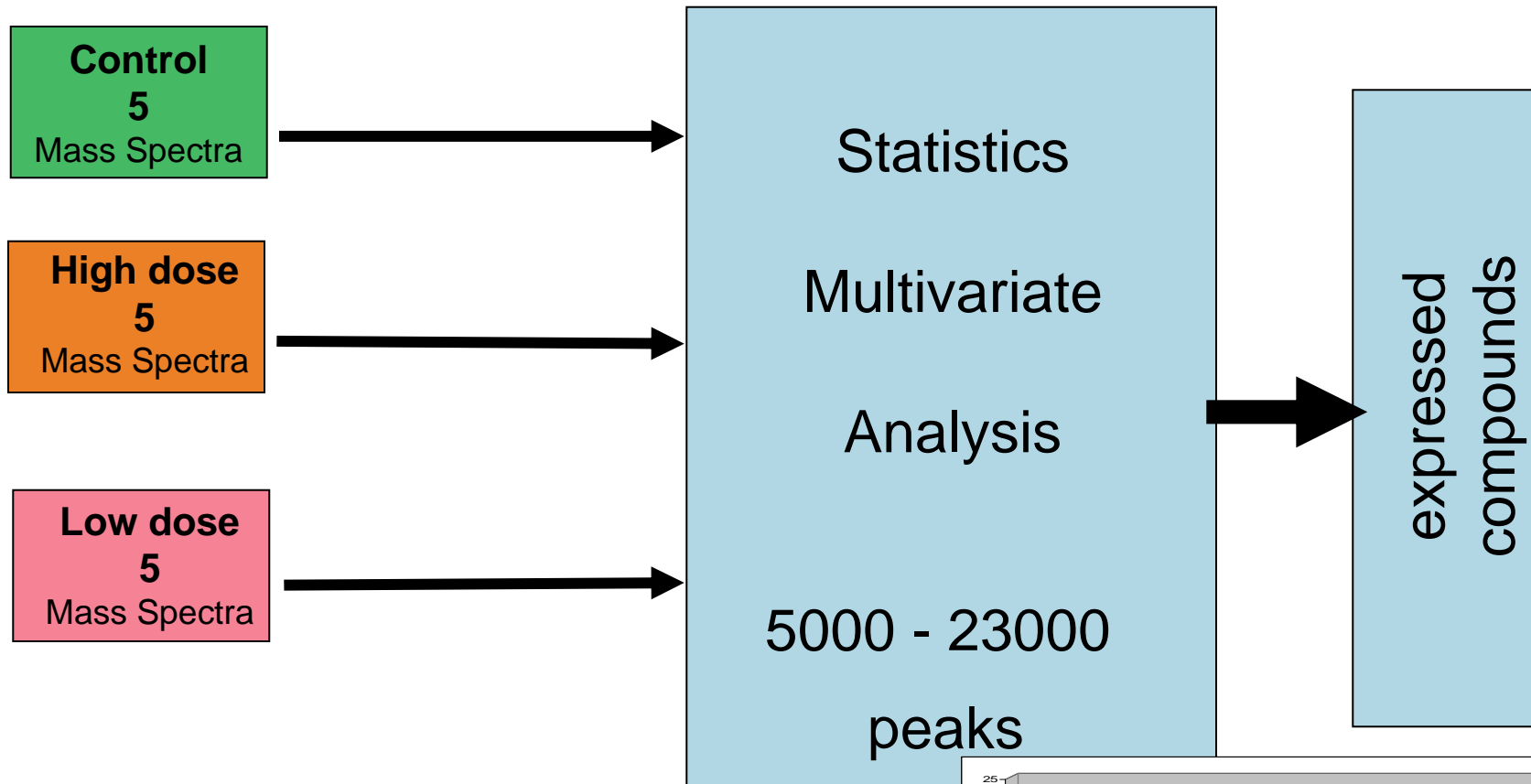
Predictive Metabolomic Profiling in comparison with other Omics Technologies - A toxicology study

K. Olaf Boernsen

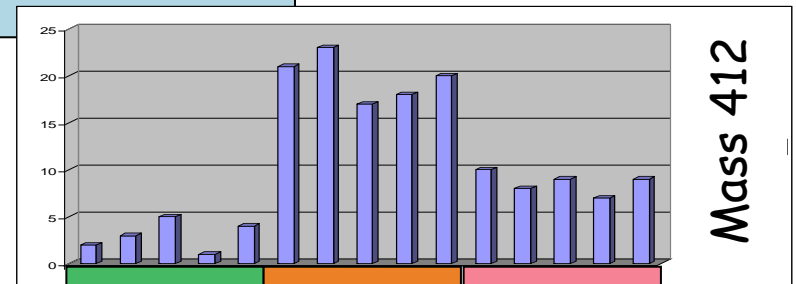
Advion User Meeting 2009



General Biomarker Approach



3 groups each with 5 subjects
Control – High dose - Low dose



Study design

- Rat study with 15 rats
- 3 groups: control, low and high dose administration
 - Pathological effects visible at day 28 in high dose group
 - Drug IMM125 with known Nephrotoxicity
- 5 rats per group
- Plasma sampling at day 1, day 3, **day 14** and day 28
- Analyzed by
 - **Transcriptomics**,
 - Proteomics (DIGE, SELDI),
 - **Metabolomics (LC-MS, NMR, Infusion-MS)**
- Statistical analysis, Cross omics analysis,
 - Expressionist, Genedata

PCA Metabolomics: NMR Urine

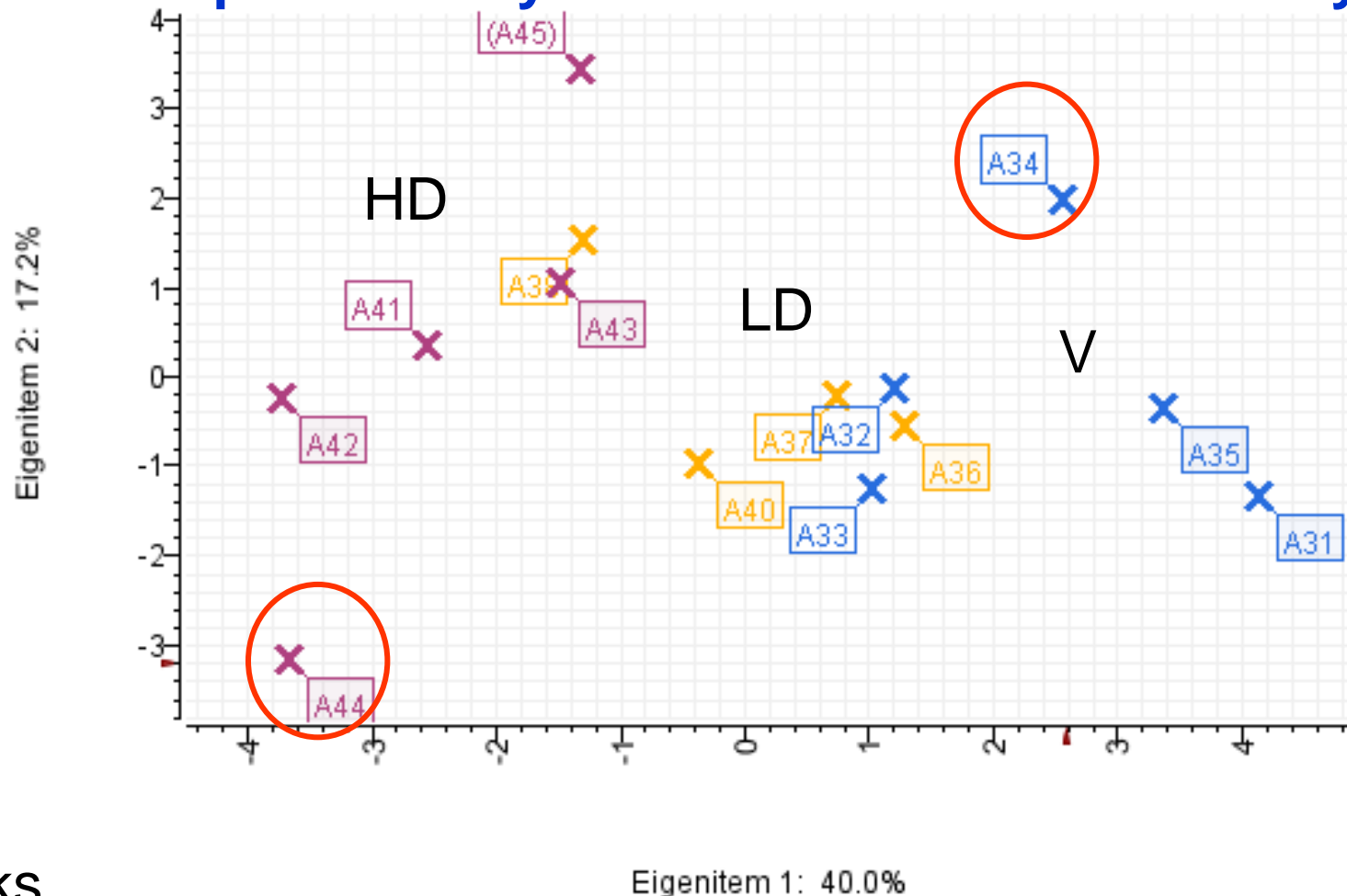
Experiment

- Vehicle d14
- HD d14
- From LD d14

2D Peak

- From 27: T-Test [1]

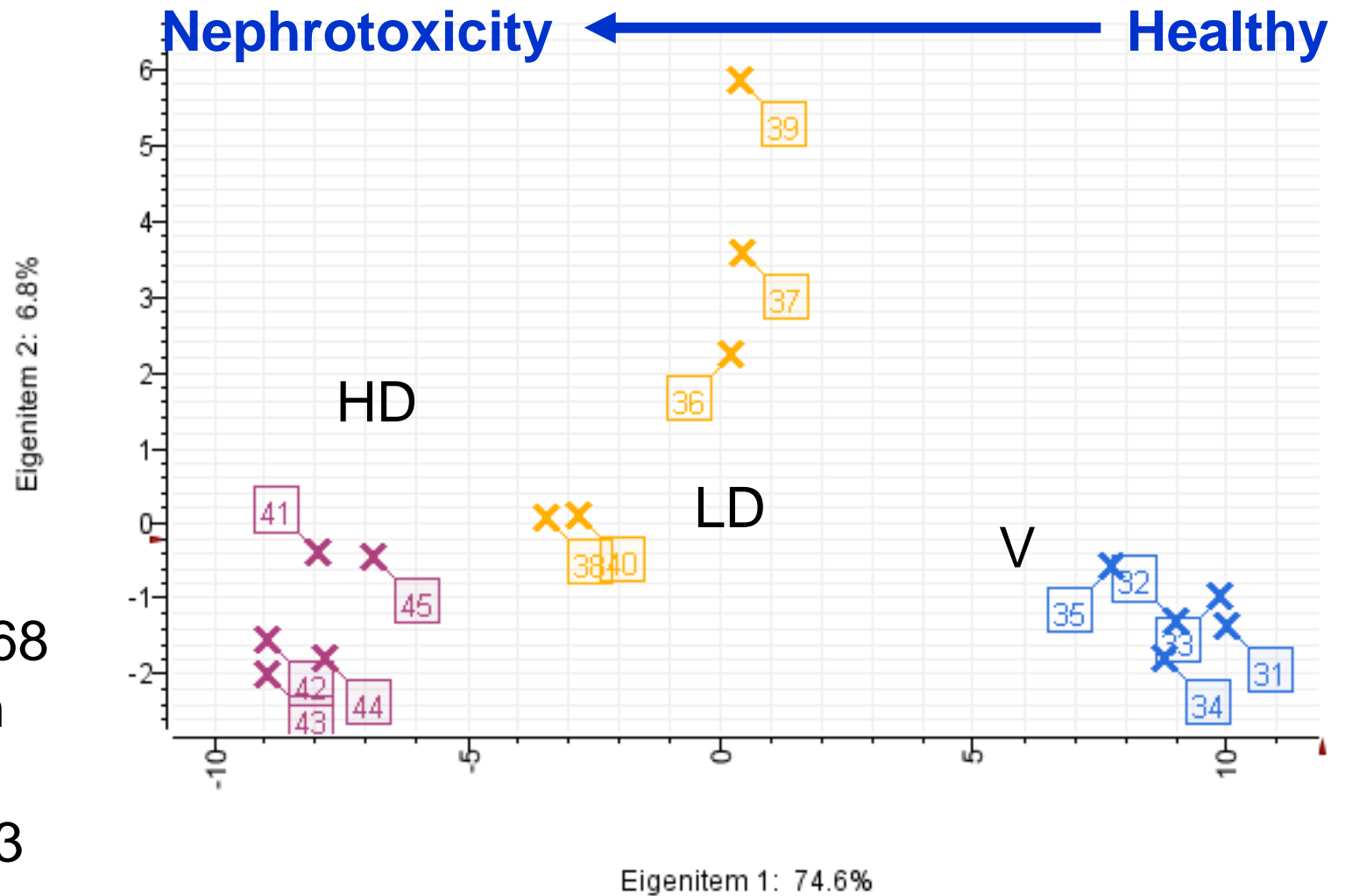
Nephrotoxicity ← Healthy



Separation based on 14 compounds - out of 28 assigned peaks

PCA Metabolomics: UPLC-MS Urine

- Experiment**
- d14 Vehicle
 - d14 Low dose
 - d14 High dose
- 2D Peak**
- From 38: T-Test

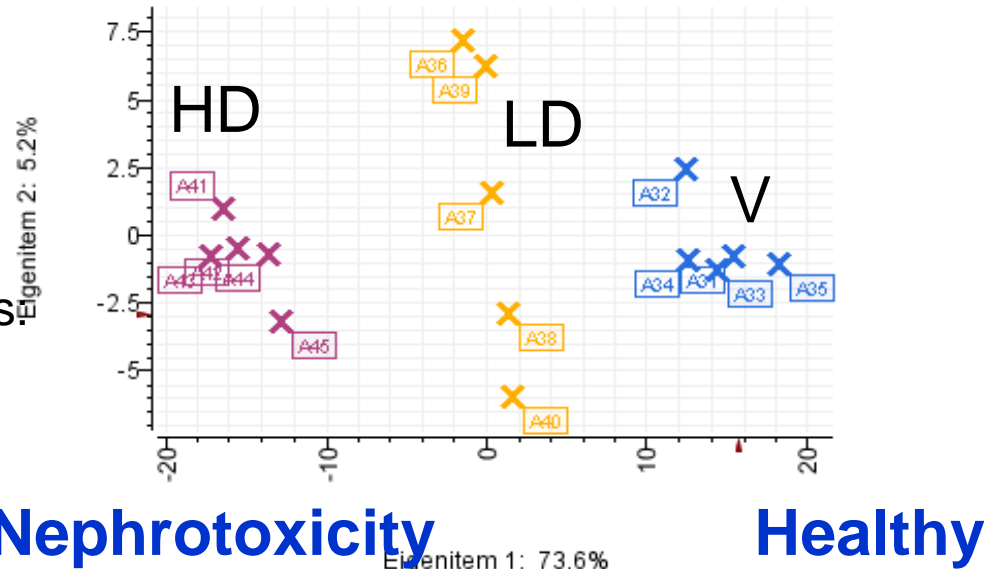


based on 68 peaks with p-values: E-10 to E-3

Comparison Transcriptomics vs Metabolomics

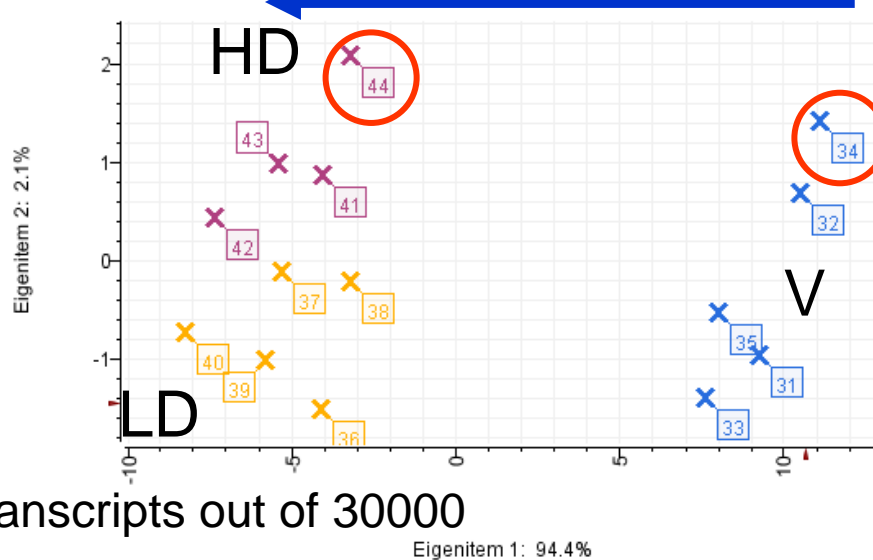
Experiment
 D14 HD
 D14 LD
 V14
2D Peak
 From 34: T-Test

based on 200 peaks p-values E-7 to E-4



PCA
 Metabolomics
 UPLC-MS Serum

Experiment
 Vehicle D14
 Low Dose D14
 High Dose D14
Probe
 From 18: T-Test



based on 53 transcripts out of 30000

PCA
 Transcriptomics
 Affimetrix
 RNA from blood

PCA Metabolomics Infusion-MS: Serum

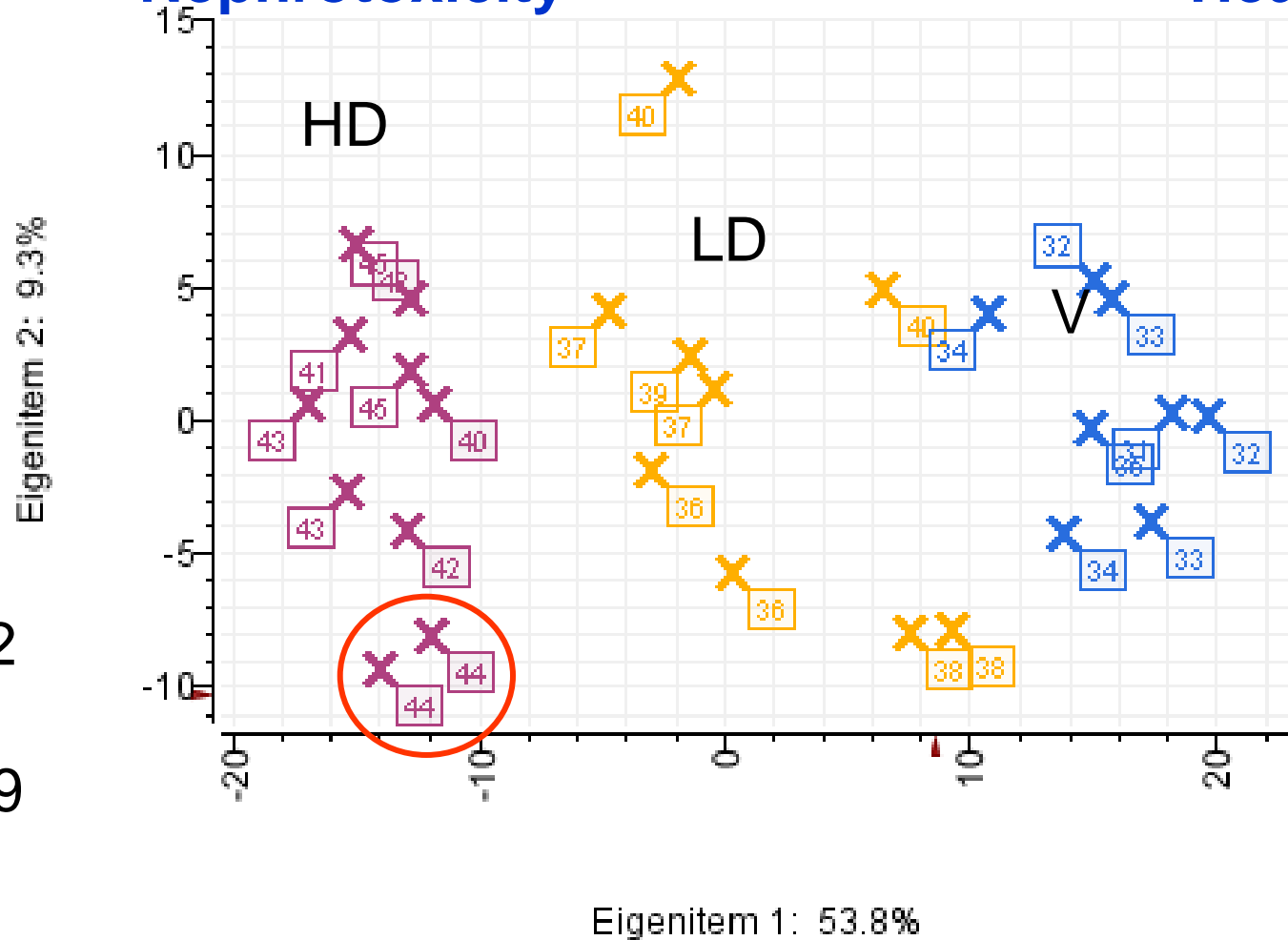
Experiment

- 14days H dose
- Vehicle d14
- d14 L dose

Raw m/z

- From 186: T-Test

Nephrotoxicity ← Healthy



based on 42 peaks with p-values E-9 to E-3

Cross validation results for the histopathology group: Proximal tubular damage

- Transcriptomics
 - Misclassification rate = **0.86 % +- 0.31 %**
 - Misclassification rate normalized by group size = **1.36 %**
- Urine (LC/MS)
 - Misclassification rate = **7.60 % +- 1.30 %**
 - Misclassification rate normalized by group size = **18.07 %**
- Proteomics (DIGE)
 - Misclassification rate = **3.75 % +- 1.39 %**
 - Misclassification rate normalized by group size = **4.35%**
- Serum (Infusion MS)
 - Misclassification rate = **0.35 % +- 0.16 %**
 - Misclassification rate normalized by group size = **0.48%**

Means: An unknown sample will be classified to PTD
with an error of 0.35%

Cross validation results for the histopathology group: Infiltrate mononuclear cells

- Transcriptomics
 - Misclassification rate = **1.40 % +- 0.51 %**
 - Misclassification rate normalized by group size = **1.89 %**
- LC/MS Urine
 - Misclassification rate = **9.67 % +- 1.52 %**
 - Misclassification rate normalized by group size = **20.14 %**
- Proteomics (DIGE)
 - Misclassification rate = **18.50 % +- 2.43 %**
 - Misclassification rate normalized by group size = **44.27 %**
- Infusion MS Serum
 - Misclassification rate = **1.00 % +- 0.30 %**
 - Misclassification rate normalized by group size = **1.03 %**

Means: An unknown sample will be classified to IMC with an error of 1%

Conclusion

- Good study design and sample collection is key
- Metabolomic profiling on day 14 by NMR, UPLC- and Infusion-MS and transcriptomics are able to separate the three groups
- Biological variability can be identified between the different methods
- RNA reached endpoint already with LD
- Infusion-MS of serum showed best misclassification rate
- Prediction for day 3 is possible

Peak capacity and ion suppression

- 17 min gradient has a peak capacity of approximately **300** only
- **Typically longer columns with higher flow rates and 1h gradient have peak capacities of 1000**
- Metabolome may be based on 5000 to 15000 compounds and (200) samples need short analysis times
- Coeluting peaks are a fact
- Coeluting peaks do show ion suppression on standard ESI sources
- Chip based nano ESI is the only method to overcome these hurdles (LC-MS and Infusion-MS)

Acknowledgement

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