

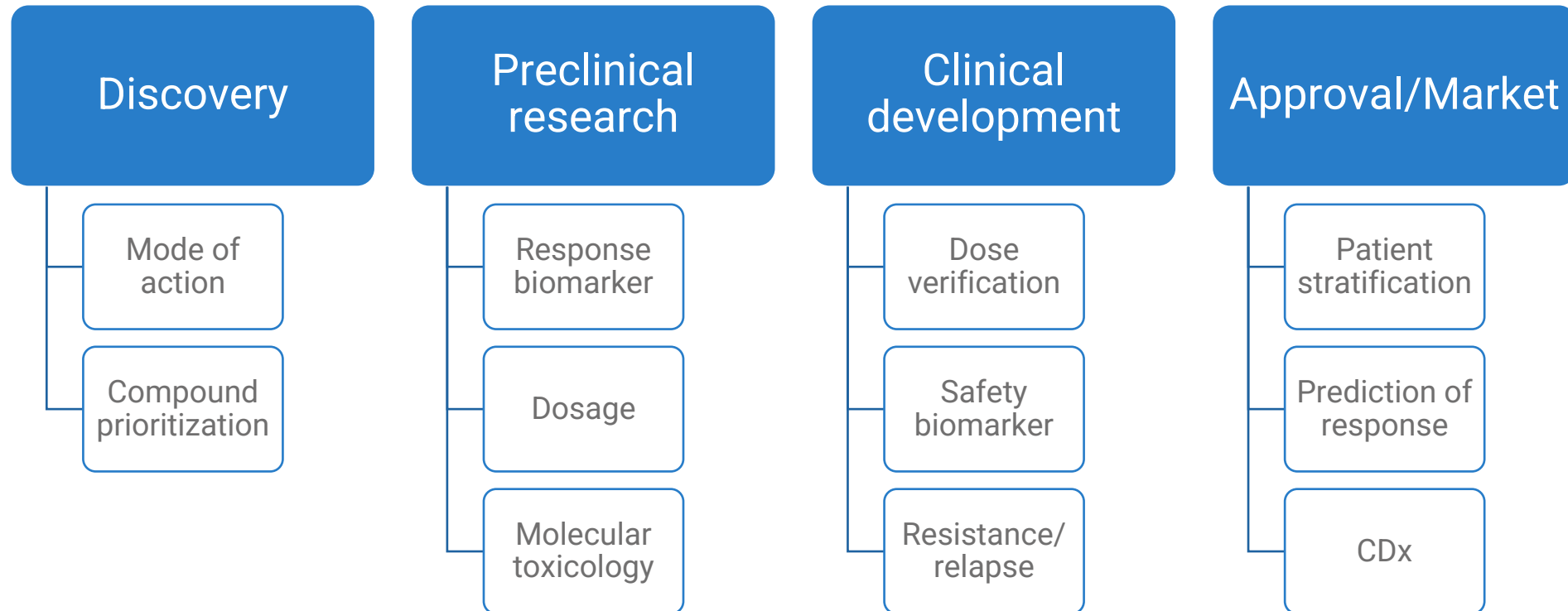
Metabolomics screening kits for use in clinical trials – fit for purpose?

- Heike Wiese -

NUVISAN

➤ Why metabolomics?

Through the measurement of metabolites, a person's metabolic make-up can be profiled at any given moment, to understand and predict the impact of external influences.



➤ Biocrates Kits –Targeted Metabolomics using LC-MS/MS



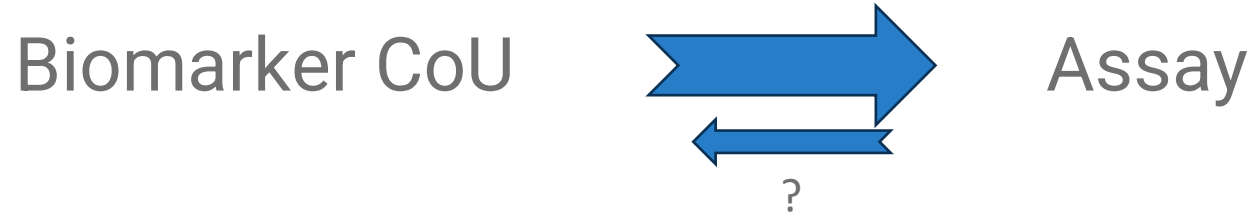
Ready to use kits including

- System suitability test samples
- Calibration standards
- Quality controls
- Internal standards
- Sample preparation plate
- Sample preparation protocol
- Instrument specific methods for Agilent, Sciex and Waters Triple Quads
- Software enabling sample registration and data analysis
- Chromatography columns

7 Kits available ranging from 17 steroids up to 1,019 metabolites from 39 biochemical classes

AbsoluteIDQ[®] Bile Acids and MxP[®] Quant 500 Kits

Bile Acids



Bile Acids Kit:

- 20 Bile Acids (16 human)
- Quantification via LC-MS/MS
- 7-point calibration curve for each analyte
- ISTD normalized

Quant 500 Kit:

- Coverage of 630 metabolites from 26 biochemical classes
- Quantification via LC- and FIA MS/MS, positive and negative polarity
- Different analytical qualities
- ISTD normalized

➤ Data Quality

High percentage of analytes yield relative quantitative results

- ➔ Quantitative or relative quantitative
- ➔ Reproducible values
- ➔ Able to identify concentration differences and trends between groups
- ➔ Pre-validated calibration and quantification ranges

Analytical classification	Abbreviation	Validation criteria	
Quantitative 54	Q	LC	<ul style="list-style-type: none"> - 7-point calibration used - CV < 15% (CV < 20% at LOD) - accuracy 85 - 115% (accuracy 80 - 120% at LOD) - corresponding ISTD used
		FIA	<ul style="list-style-type: none"> - 1-point calibration used - CV < 20% - accuracy 80 - 120% - corresponding ISTD used
Quantitative with restrictions 51	QR	LC	<ul style="list-style-type: none"> - 1-point calibration or - CV < 20% at entire concentration range or - accuracy 80 - 120% at entire concentration range
Relative quantitative 455	RQ	LC	<ul style="list-style-type: none"> - CV < 20% - accuracy not verified
		FIA	<ul style="list-style-type: none"> - CV < 30% - accuracy not verified
Not validated 66	NV		<ul style="list-style-type: none"> - analyte concentration < LOD*
Invalid 4	IV		<ul style="list-style-type: none"> - validation failed

➤ Bile Acid Kit validation

Intended context of use

Disease modulation biomarker (understanding the drug's mode of action), pharmacodynamic response biomarker regarding the liver, exploratory biomarker

FFP validation design

- A&P runs with 3 Kit QC level (lyophilized) + self-made QC (spiked plasma)
- Carry over
- Stabilities: FT, BT with self-made QC, processed sample stability of all QCs
- Stability of analytes from isochronic samples

Acceptance criteria

according to biocrates kit specifications:

Accuracy calibration samples $\pm 15\%$

Accuracy for QCs $\pm 30\%$ ($\pm 45\%$ for QC 3)

Accuracy for self-made QCs: $\pm 25\%$

Carry-over: $<20\%$ of LLOQ



Validation results

Self-made QC (A&P 3):

	CA	CDCA	DCA	GCA	GCDCA	GDCA	GLCA	GUDCA	LCA	TCA	TCDCa	TDCA	TLCA	TMCA(a+b)	TUDCA	UDCA
mean	151.5	139.8	182.5	237.5	1164.8	271.3		121.3	277.8	2197.7	131.0	33.7	203.2	937.0	732.8	1386.8
cv [%]	5.6	4.8	3.1	4.2	1.2	2.3		3.6	1.8	3.5	2.8	3.0	2.3	2.6	3.3	3.7
bias [%] to nominal	9.7	7.2	6.9	-4.5	5.1	12.5		6.9	-7.8	-0.9	6.1	6.6	10.4	-2.0	4.1	0.7

Kit QCs:

		CA	CDCA	DCA	GCA	GCDCA	GDCA	GLCA	GUDCA	LCA	TCA	TCDCa	TDCA	TLCA	TMCA(a+b)	TUDCA	UDCA
A&P 1	CV	5.2	2.6	3.0	5.2	2.8	5.4	7.7	4.4	6.7	2.6	2.8	3.9	2.9	1.8	4.3	4.0
	bias	-14.9	-27.0	-9.3	-12.9	-4.0	-11.3	-13.5	-6.8	-18.7	-10.1	-25.0	-2.8	-17.8	-10.4	-6.9	-14.6
A&P 2	cv [%]	8.0	2.2	3.5	2.9	4.0	2.3	3.7	3.2	5.3	4.7	5.5	3.7	5.3	1.3	2.3	5.8
	bias [%]	-15.5	-25.8	-1.6	-7.1	-12.4	-11.5	-11.0	-6.6	-16.8	-11.4	-25.9	-7.3	-18.7	-14.4	-12.2	-6.7
A&P 3	cv	4.3	4.9	2.2	3.5	3.2	4.9	2.3	4.2	3.6	2.4	5.0	4.9	4.6	3.5	5.0	11.6
	bias	2.5	-21.5	4.7	-2.9	8.7	-0.3	1.4	4.2	-3.0	-6.2	-19.7	5.2	-3.0	-6.6	-1.2	-6.4

Problem identified: QC reconstitution!

Stability

Benchtop stability: 48 h at RT

Freeze-thaw: 3 cycles

Processed sample stability: 36 h

Long-term stability (isochronic samples): up to 3 years at -20 & -75 °C

- Validation successful
- Identified problems: slight carry over not identified in system suitability test and insufficient QC reconstitution (improve reconstitution procedure)

➤ Compliance to GxP requirements?

Proper sample management



Documentation



SOPs – biocrates Processes



Validated instruments/lab equipment



Data analysis – software ?

Data handling and post processing ? **X**

QC checking ?

Reporting ?

Data analysis software: WebIDQ

Create project

Active



Project code

001

Project name

demo project

Description

here you can enter a description

Contacts

Heike Wiese



Project team

Lena Maier

Anna-Lena Birkert

Cancel

Save

LIMS

Quantification

Results



Delete measurements

Export worklist to MS

Plate run	Run time ↑	OP type	OP	Plate prod...	Plate valid...
1052344946-1	2023-07-20 09:17:46	LCMS	BA02-0-5813		OK

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10		
B1		B3	B4	B5	B6	B7	B8	B9	B10		
C1		C3	C4	C5	C6	C7	C8	C9	C10		
D1		D3	D4	D5	D6		D8	D9	D10		
E1	E2	E3	E4	E5	E6		E8	E9	E10		
F1	F2	F3	F4	F5	F6		F8	F9	F10		
G1	G2										
H1											

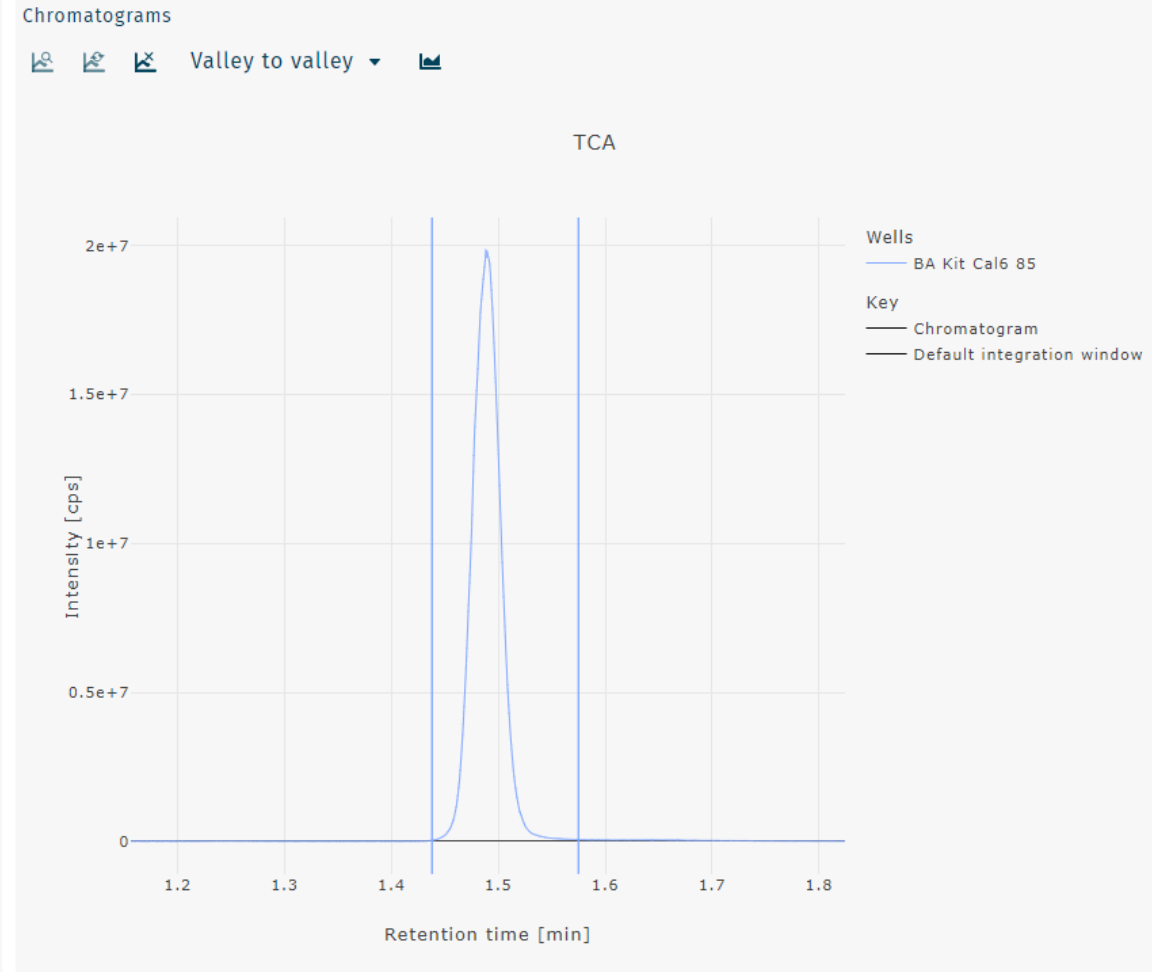
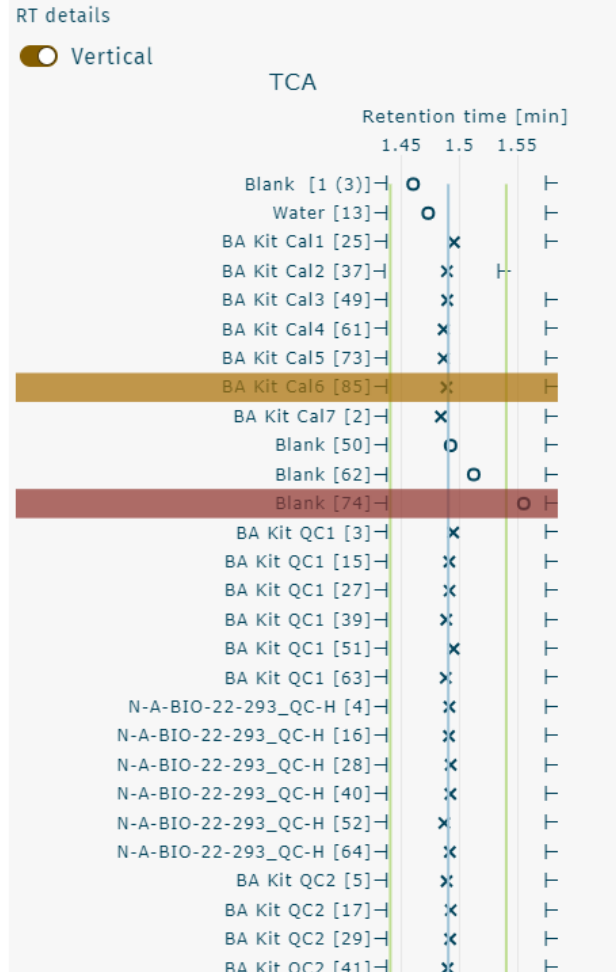
Data analysis software: WebIDQ

Wells

Sample identifier	Well
Blank	1 (3)
Water	13
BA Kit Cal1	25
BA Kit Cal2	37
BA Kit Cal3	49
BA Kit Cal4	61
BA Kit Cal5	73
BA Kit Cal6	85
BA Kit Cal7	2
Blank	50
Blank	62
Blank	74
BA Kit QC1	3
BA Kit QC1	15
BA Kit QC1	27
BA Kit QC1	39
BA Kit QC1	51
BA Kit QC1	63
N-A-BIO-22-293_QC-H	4
N-A-BIO-22-293_QC-H	16
N-A-BIO-22-293_QC-H	28
N-A-BIO-22-293_QC-H	40
N-A-BIO-22-293_QC-H	52
N-A-BIO-22-293_QC-H	64
BA Kit QC2	5
BA Kit QC2	17
BA Kit QC2	29
BA Kit QC2	41
BA Kit QC2	53
BA Kit QC2	65
BA Kit QC3	6
BA Kit QC3	18

Metabolites

Name ↑	RT
CA	2.646
CA-ISTD	2.639
CDCA	3.300
CDCA-ISTD	3.289
DCA	3.392
GCA	1.481
GCA-ISTD2	1.472
GCDCA	2.286
GDCA	2.414
GLCA	2.857
GLCA-ISTD2	2.855
GUDCA	1.260
GUDCA-ISTD	1.251
HDCA	2.656
HDCA-ISTD	2.556
LCA	3.735
LCA-ISTD	3.733
MCA(a)	1.948
MCA(b)	2.090
MCA(o)	1.866
TCA	1.489
TCA-ISTD2	1.475
TCDCa	2.284
TCDCa-ISTD2	2.275
TDCA	2.414
TLCA	2.836
TMCA(a+b)	0.878
TUDCA	1.268
TUDCA-ISTD	1.260
UDCA	2.574



Data analysis software: WebIDQ

☆ **Validate** Exclude ?

Summary

Plate run	1052344946-1
Run time	2023-07-20
OP	BA02-0-5813
Plate validation	OK
Note	<input type="checkbox"/>

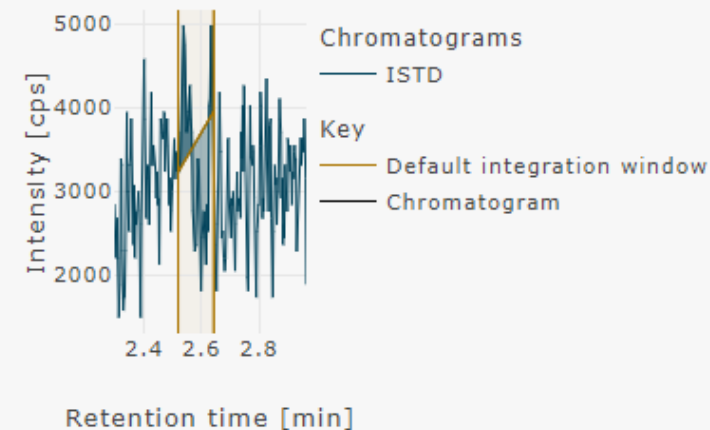
LOD configuration

The LOD was calculated using 1 zero sample.

Plate view

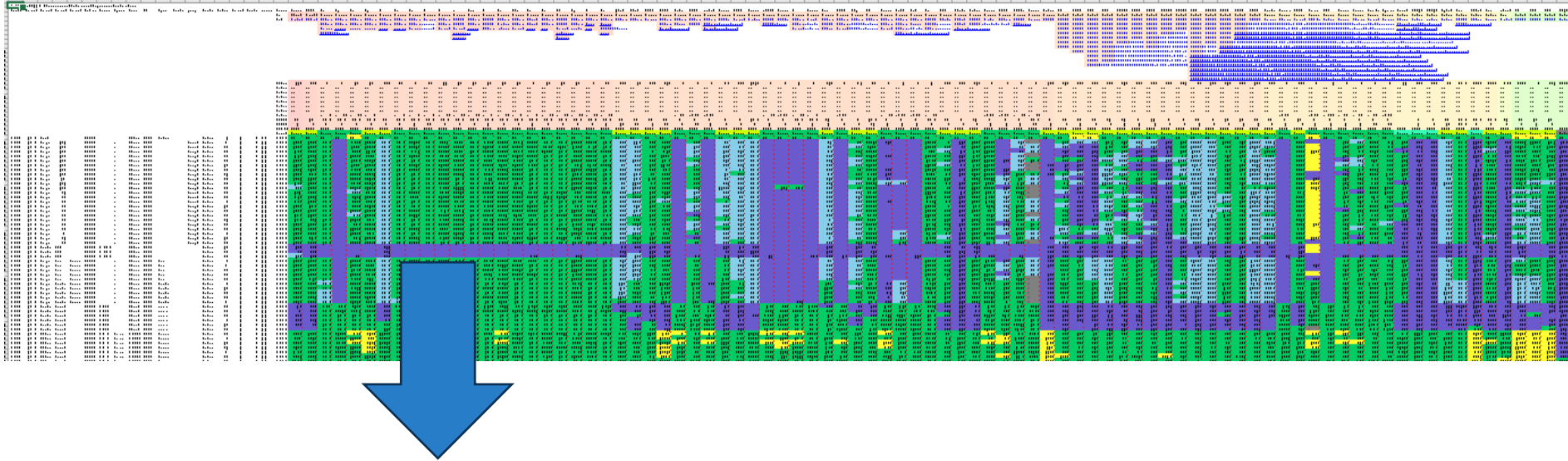
A1	A2	A3	A4	A5	A6	A7	A8	A9	A10		
B1		B3	B4	B5	B6	B7	B8	B9	B10		
C1		C3	C4	C5	C6	C7	C8	C9	C10		
D1		D3	D4	D5	D6		D8	D9	D10		
E1	E2	E3	E4	E5	E6		E8	E9	E10		
F1	F2	F3	F4	F5	F6		F8	F9	F10		
G1	G2										
H1											

Chromatograms



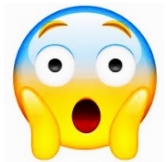
➤ Data export

Results can be exported in excel or text format (csv, txt)



Client specific dta format?

Manual transformation prone to error
- therefore requires 100 % QC check



Best way: validated script
- new for every client and project
- you need a bioinformatician



Summary & Acknowledgement

- FFP Validation successful for Bile Acid Kit with good accuracy and precision
- FFP Validation for Quant 500 Kit ongoing
- Good quality data
- Challenges in data integrity and transfer
 - Does anyone use “omics” kits?
 - What level of quality check is applied?
 - Did anyone ever get feedback from authorities?

Let's discuss – any feedback welcome

Thank you

BioA Neu-Ulm....

... and the people who turn
science into Life Science:

Lena Maier

Anna-Lena Birkert

Anastasiia Shevchuk

QC & Reporting team

Dr. Manuel Kratzke and Stefan
Ledinger from biocrates