Institute of clinical chemistry and laboratory diagnostics
Integrated Biobank Jena

Lecture: Biobanking and Quality indicators

Quality indicators (QIs) in preanalytics of biospecimen as a prerequisite for reliable findings in prospective biomedical research

SS 2023

Jena, 05/06/2023

Sven Heiling

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Please contact me if you have any questions regarding this lecture or Biobanking/Preanalytics in general:

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Content

1. Biobanking and sample quality
2. Liquid sample heterogeneity
3. Critical preanalytics
4. Steps to high sample quality
5. Quality indicators (QIs)

Important

Lecture will be online

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What is a biobank?

Cover Credit: Arthur Hochstein

"Think of it as an organic bank account. You put your biomaterial in and earn medical interest in the form of knowledge and therapies that grow out of that deposit — no monetary reward, just the potential that you might benefit from the accumulated data at some later date." – Alice Park 2009

"The key to a powerful biobank is high-quality specimens from as wide a swath of the country's population as possible."

http://content.time.com/time/specials/packages/article/0,28804,1884779_1884782_1884766,00.html

What is a biobank?

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Biorepository and Biobank – attempt of a definition

Biorepository
A facility that collects, catalogs, and stores samples of biological material, such as urine, blood, tissue, cells, DNA, RNA, and protein, from human, animals, or plants for laboratory research.
If the samples are from people, medical information may also be stored along with a written consent to use the samples in laboratory studies.

A biorepository facilitates the dissemination of specimens and the assurance of sample quality and related data.

Biobank
„...is a biorepository that accepts, processes, stores and distributes biospecimens and associated data for use in research and clinical care...“

Biospecimen – a quantity of tissue, blood, urine, or other biologically derived material.

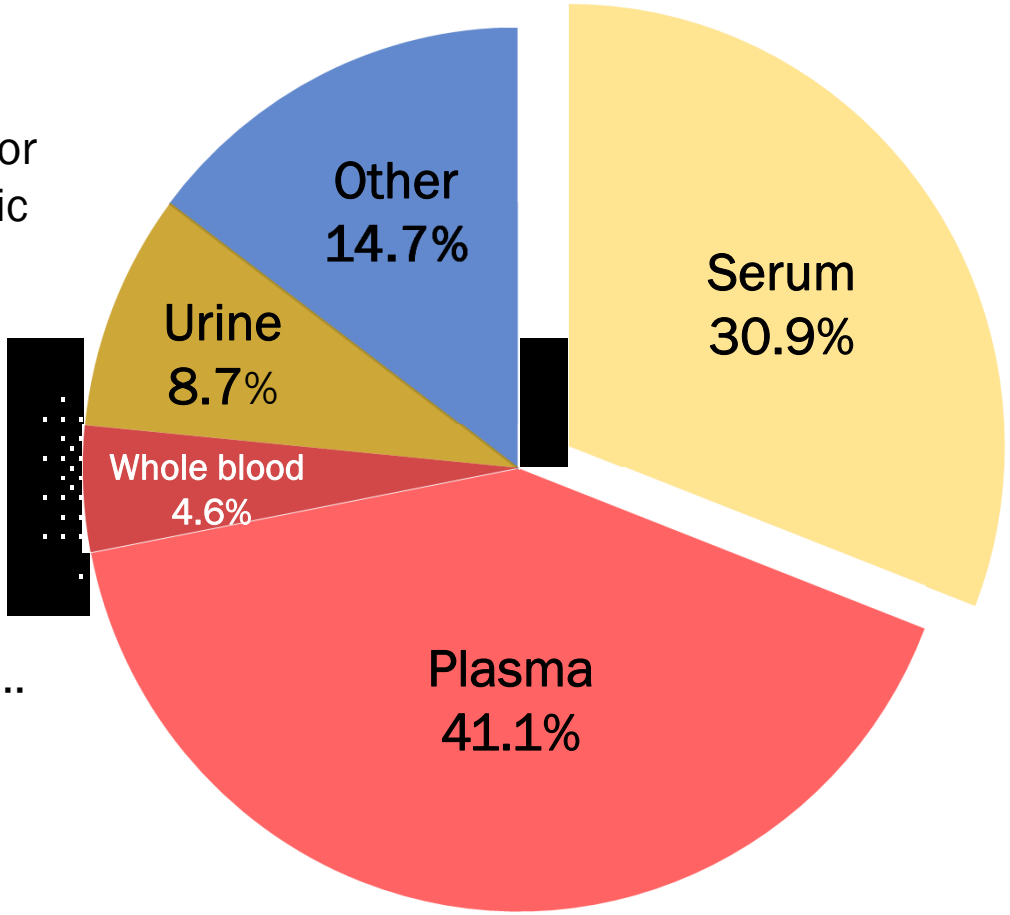
Def. Biorepository – National Cancer Institute (NIH) – https://biolincc.nhlbi.nih.gov/glossary
De Souza and Greenspan 2013 Biobanking past, present and future: responsibilities and benefits*; doi:10.1097/QAD.0b013e32835c1244*

Biobank samples originate from clinical/biomedical research studies or the clinical routine process (diagnostic laboratories)

Biospecimen stored in biobanks are diverse:
Blood (serum, plasma), Urine, Saliva, CSF, Sweat, Milk, Lacrimal, endometrial/peritoneal fluids, microdyalysate, breath and volatiles …

Tissue, cell cultures, DNA, RNA …

Biospecimen in GBA (~ 16.94 Mio. „liquid“ samples)



Source: German Biobank Alliance 2020
~ 35 akademic biobanks (2022)

https://www.bbmri.de/ueber-gbn/german-biobank-alliance/

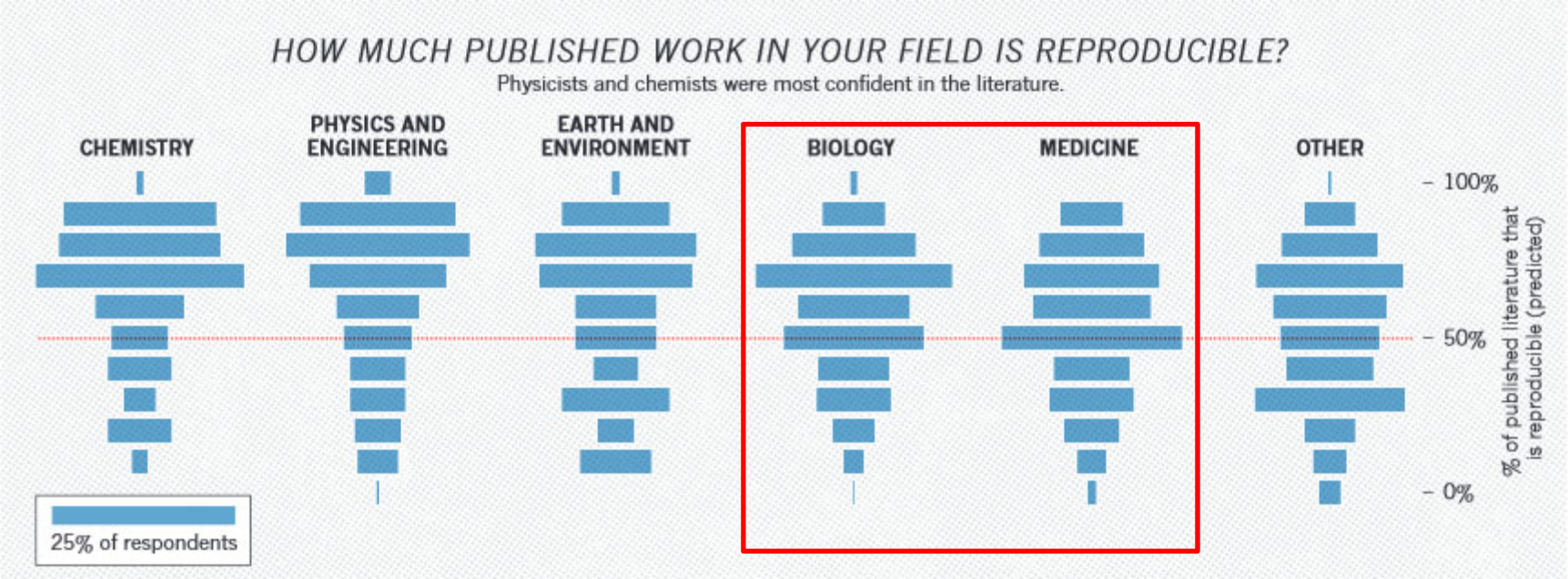
What is most important for biobanks?

Quality of Biospecimen

[1]https://www.freezerchallenge.org/fc-blog/blast-the-ice-jam1642359
[2]https://med.stanford.edu/news/all-news/2009/10/ice-age-over-sustainability-effort-targets-freezers.html
[3]https://www.haus.de/leben/gefrierschrank-abtauen-22156

[4]https://freelims.org/blog/what-is-a-biobank.html
[5]https://www.biobanken-verstehen.de/was-sind-biobanken/

Why do we need high-quality samples?



High-quality, well characterized human biological samples are crucial for **accurate diagnostics** in the clinical routine laboratory and for **reliable, conclusive, reproducible biomedical research.**

Baker, M.(2016), 1500 scientists lift the lid on reproducibility Nature **volume 533**, pages 452–454

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What is a high-quality sample?

It Depends!

- What do you want to do with the samples? (**Fit-for-Purpose**, e.g. suitable sample for diagnostics or research)

- What **analysis/methodology** will you use? (Omics-Technologies, e.g. Next Generation Sequencing, Proteomics, Metabolomics; cell cultures still viable?)

- e.g. DNA extraction -> high yield of good-quality DNA from blood after 24 h storage at room temperature possible (1)

- e.g. for Metabolomics -> the metabolome at the time of extraction and analysis should reflect as closely as possible the original *in vivo* metabolome

(1) Lahiri DK, Schnabel B. DNA isolation by a rapid method from human blood samples: effects of MgCl 2, EDTA, storage time, and temperature on DNA yield and quality.Biochem Genet 1993;31:321– 8.

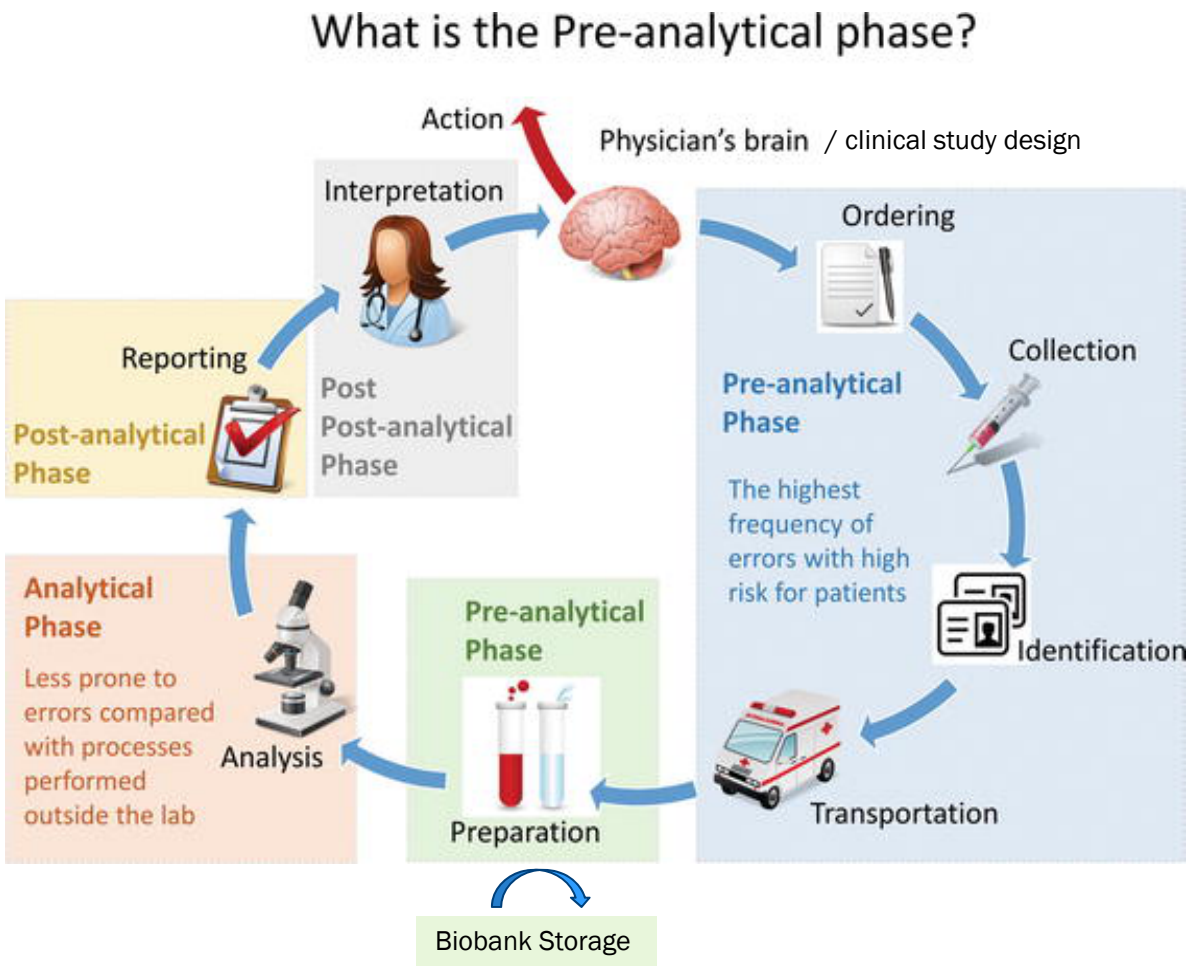
If it depends, how do we assess a high-quality sample?

What affects my samples/biospecimen?

- Sample collection conditions
- Sample additives
- Transportation conditions
- Temperature
- Sample history

Pre-analytical Phase

https://www.syfy.com/sites/syfy/files/styles/blog-post-embedded--mobile/public/scrubs_dr_acula_1_5x06.gif



In the 70ies of the last century, the term "**pre-analytical phase**" was introduced in the literature.

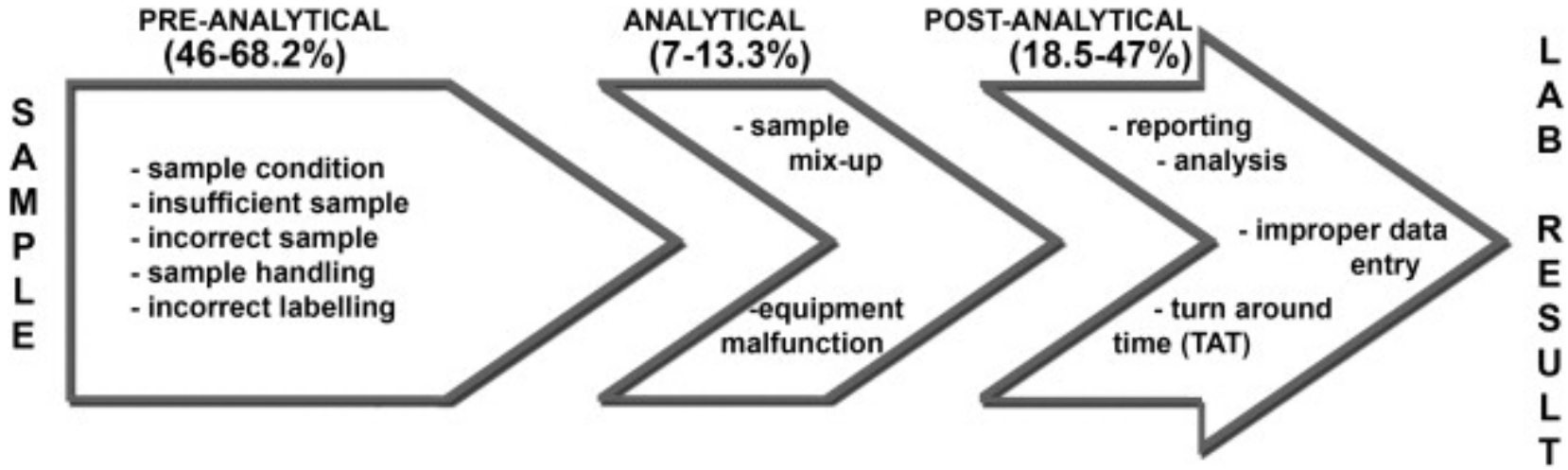
This term describes all **actions and aspects** of the "brain to brain circle" of the medical laboratory diagnostic procedure happening **before the analytical phase.**[1]

Biobank samples originate from clinical/biomedical research studies or the clinical routine process (diagnostic laboratories).

[1]Guder, W. G. (2014) **History of the preanalytical phase**: a personal view Biochem Med (Zagreb), 24(1): 25–30.

[#]Modified - Ramune Sepetiene, Raminta Sidlauskiene and Vaiva Patamsyte **Plasma for Laboratory Diagnostics (2018)** DOI: 10.5772/intechopen.76092

Errors in clinical studies and laboratory processes



- Diverse errors occur at every level of the health care system
- Pre-analytical variation constitutes the majority of laboratory errors

- Def. **Laboratory error**: … is any defect from ordering tests to reporting results and appropriately interpreting and acting on these[1,2]

[1]Bonini PA, Plebani M, Ceriotti F, Francesca Rubboli F. Errors in laboratory medicine. Clin Chem 2002;48:691–8.
[2]ISO/WD TS 22367. Medical laboratories – reduction of error through risk management and continual improvement
Kalra, J. Kalra N., Baniak, N. (2013) Medical error, disclosure and patient safety: A global view of quality care. Clinical Biochemistry 46, 1161-1169

Errors in clinical studies and laboratory processes

Biological and Environmental variability

Collection

Venous blood collection - phlebotomy

Identification and Transport

Sample processing

Long-term storage

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Errors in clinical studies and laboratory processes

Step	Preanalytical variable	Recommendation
Ordering	Ordering forgotten	Laboratory Information System
	Consent: none, improper, lost	Secure consent
	Typing error, improper labeling	Check Spelling
	Incorrect patient ID	Scan IDs, avoid manual typing

- Errors have an impact on the quantity or quality of the biospecimens collected (e.g. sample can be incorrect, might be missed, collected in duplicate or even lost)

- Without / improper or lost consent can limit the analytical value of a sample

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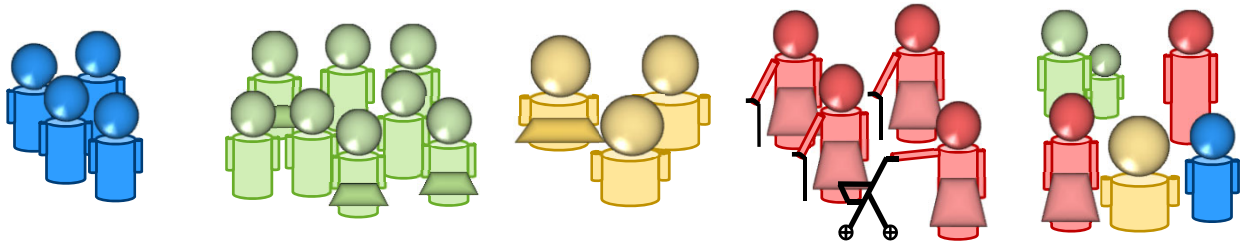
Preanalytics: Sources of sample heterogeneity

Study	A	B	C	D	E

Collection

- *Subjects*
- *Collection conditions*

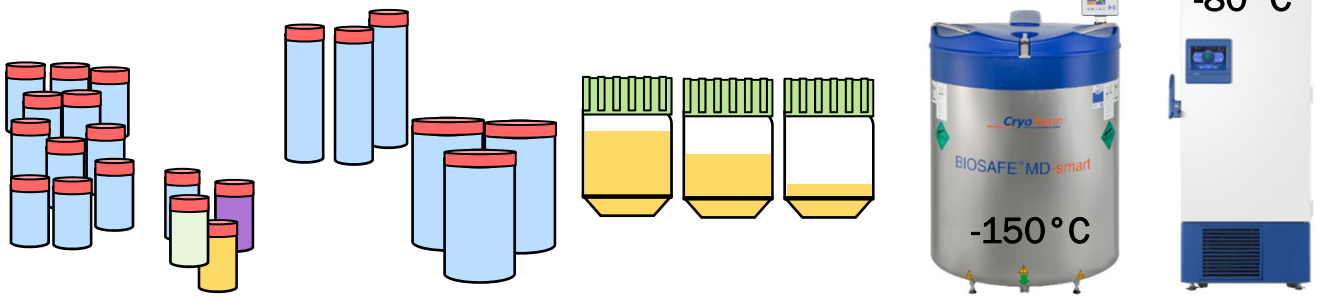


Sample handling

- *Temperature*
- *Time Delays*
- *Protocol variations*
- *Transport*

Storage

- *Sample*
  - *Count*
  - *Volume*
- *Container type*
- *Temperature*



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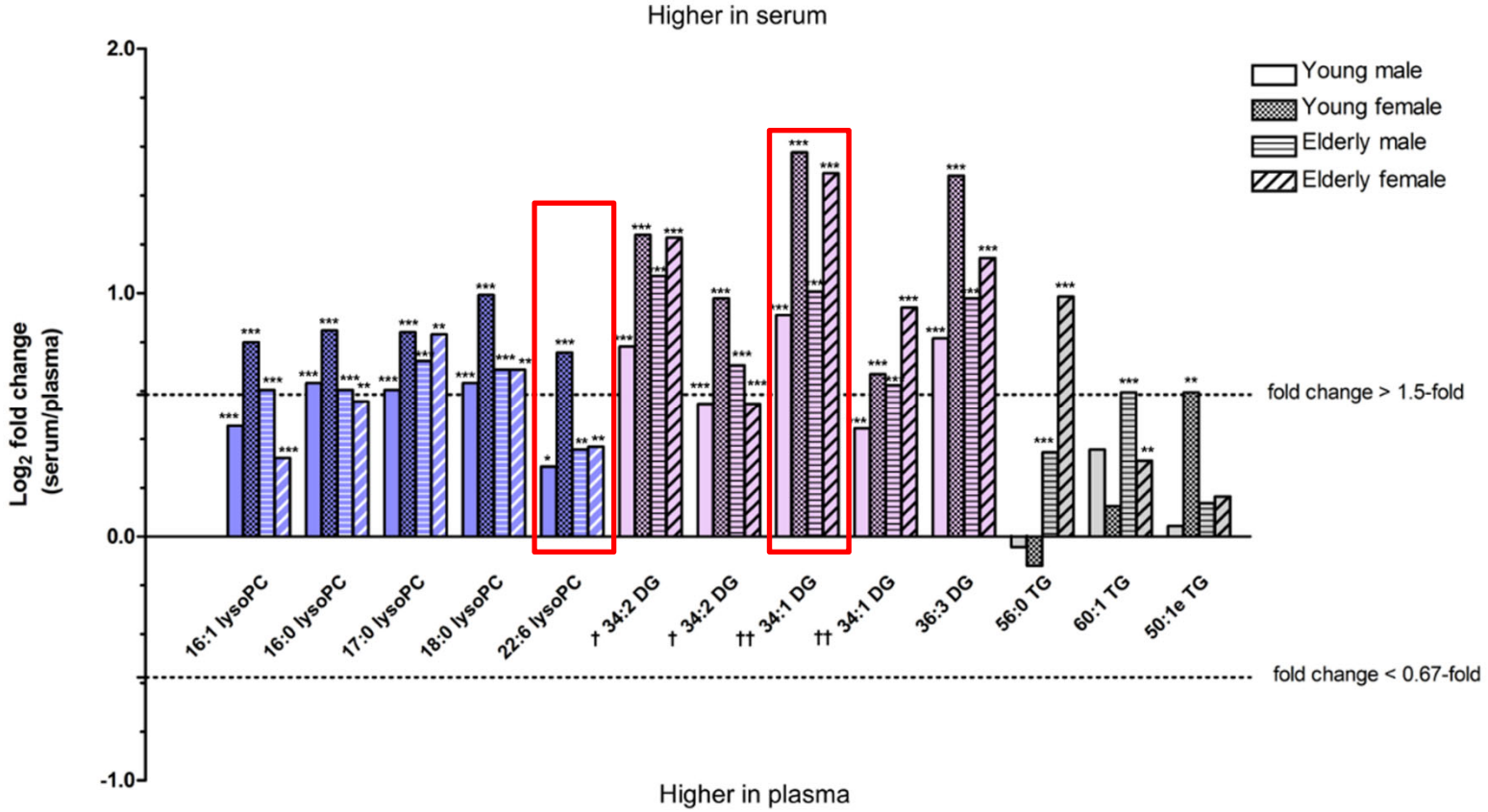
Biological and Environmental variability

Factor	Preanalytical variable
Biological variability	Age, Sex, Ethnicity, Body mass index (BMI), Circardian and Diurnal rhythms
	Hormone status, Menstrual cycle, Pregnancy, Lactation,
	Circardian and Diurnal rhythms
	Fasting/Feeding, Diet, Drugs (Alcohol, Caffeine, etc.) Smoking, Hydration Status
Environmental variability	Seasonal changes, Temperature, Humidity, Moisture, Geographic location, Altitude, Sunlight

[1]Ellervik, C. *et al.* (2015) Preanalytical variables affecting the Integrity of human biospecimens in biobanking, Clinical Chemistry 61:7, 914-934

AGE

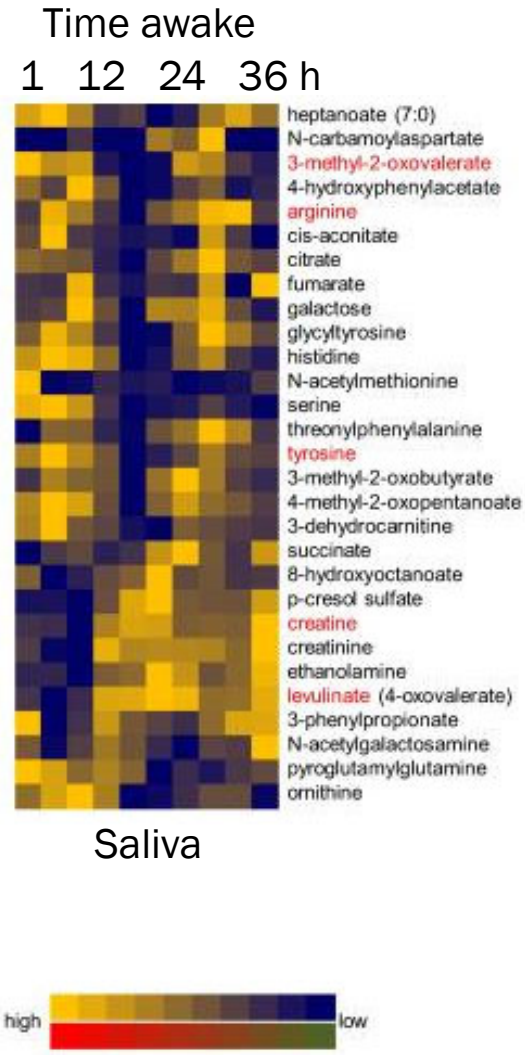


Differences in lipid metabolite levels in human blood samples between plasma and serum.

e.g. 22:6 lysoPC – altered in young females compared to young male, elderly males/females; 34:1 DG male/female

Ishikawa, M. *et al.* (2014)Plasma and Serum Lipidomics of Healthy White Adults Shows Characteristic Profiles by Subjects' Gender and Age https://doi.org/10.1371/journal.pone.0091806

Plasma



Saliva

Diurnal rhythm

The levels of nearly all lipid products were highest at midmorning to noon and were significantly lower at other times of the day.[1]

- In plasma several lipids show a strong diurnal rhythm
- In saliva, largest group with diurnal rhythm were amino acids e.g. Arginine or Tyrosine

Dallmann, R. *et al.* (2012) The human circadian metabolome, Proc Natl Acad Sci U S A. 109(7): 2625–2629.

Collection

Factor	Preanalytical variable	Recommendations
Collection	Biological and Environmental variability	Follow evidence-based literature and guidelines for standardization; Study design
	e.g. Diurnal Rhythm	Fixed time of sample collection
	Incorrect collection (e.g. phlebotomy, puncture)	Educate and train staff and patients (training and E-learning)
	Collection device, Collection device age, Anatomical location of collection, Contamination of specimen,	Use the same tubes Check expiration date Sterile collection
	Tube material, additives	Correct tube for target analysis
	Sample Volume, Filling volume tubes	Proper for analysis According to manufacturer information

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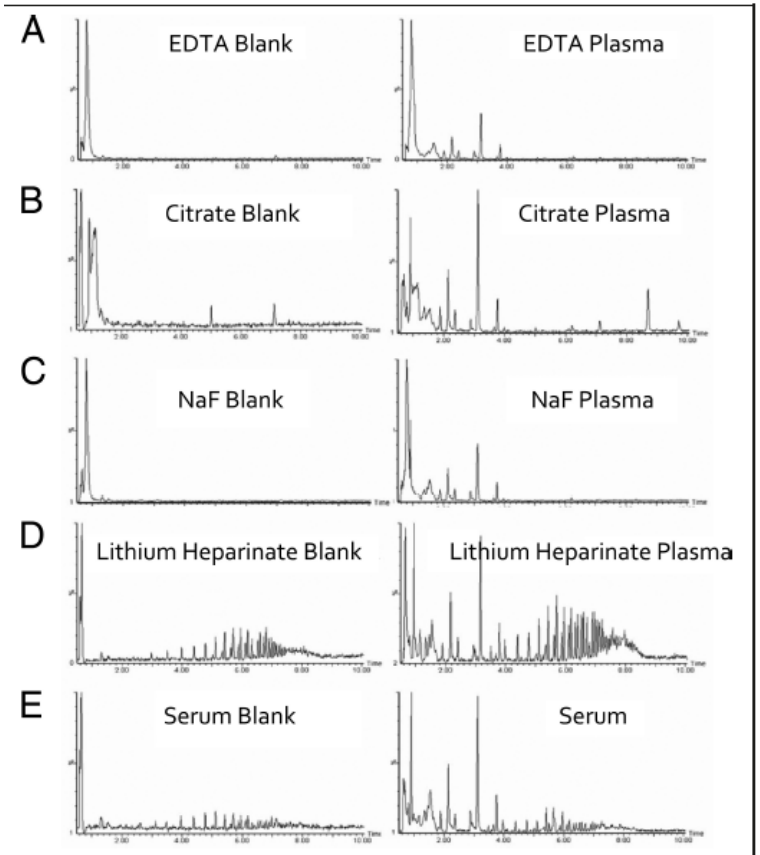

Fig. 1. Investigation of chemical noise arising from different blood collection tubes.
The blanks (50 g/L human albumin in normal saline) and blood samples were collected in commercial blood collection tubes with the following additives: (A), K^+-EDTA; (B), Na^+-citrate; (C), Na^+-fluoride (NaF); (D), lithium-heparinate and (E), kaolin to enhance the generation of serum. (F), Mass spectra of chemical noise [(-CH2CH2O-)n] detected in lithium-heparinate plasma, which exhibits typical polyethylene glycol clusters *(38)*. Typical total ion chromatograms of blanks, plasma, and serum are given.

Continued on page 837

Impact of Collection tube on Mass spectrometry

- Chemical background (in mass spectrometry) of blanks (50g/L human albumin in saline solution) and blood in different sample containers.

- Contaminants present in the blood collection tubes may affect the ionization process during an LC-MS run, thereby suppressing metabolite ionization and/or introducing interfering peaks

Yin et al. 2013 Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. Clinical Chemistry 59, 833-845

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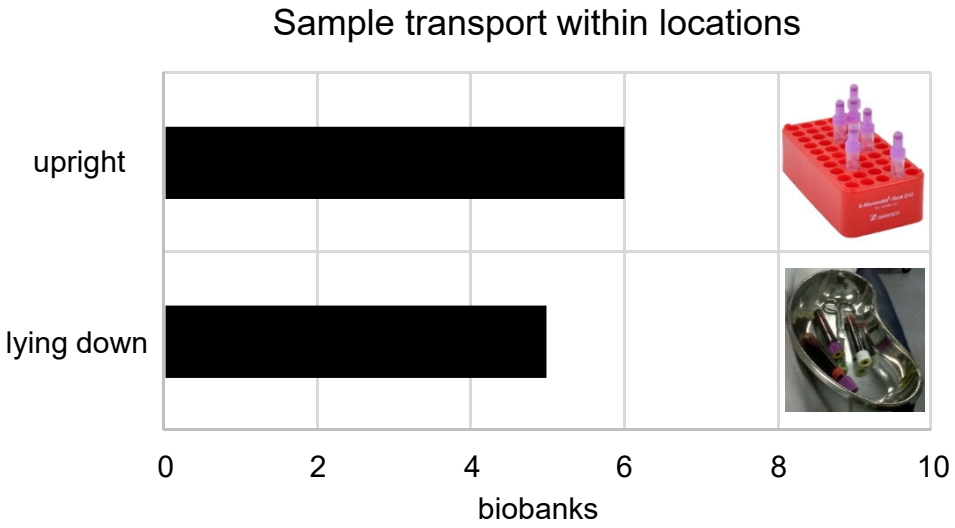
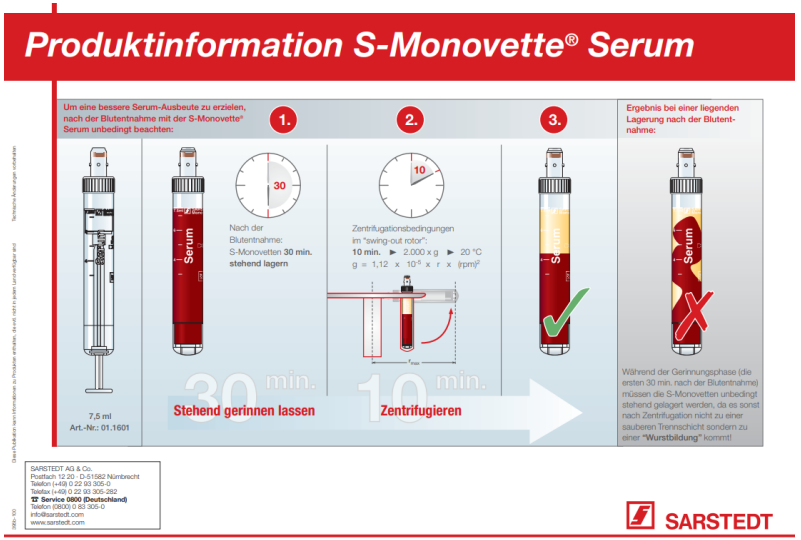
Improper handling of serum





- Serum monovette should be stored upright, otherwise no clean phase separation occurs - "sausage formation" (danger: less yield, unclean collection of serum)

https://dafxbb5uxjcds.cloudfront.net/fileadmin/user_upload/99_Broschueren/NEU/453/20_453_0100_142_tipps_tricks_0819.pdf

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Venous blood collection - phlebotomy

Preanalytical variable
Position of the patient (sitting or laying down)
Technical error such as placement of the tourniquet
Choice of container type and additive
Order of blood withdrawel to reduce carry-over effects
Filling volume of collection container, appropriate mixing

Recommended order of draw

According to Gurr[10]:

Based on BS 4851 (EU Code)	ISO 6710:2017	
		Blood culture
		Serum/serum-gel blood
		Citrate blood
		Heparin/heparin-gel blood
		EDTA blood
		Fluoride/citrate-fluoride blood

According to CLSI[11]:

Based on BS 4851 (EU Code)	ISO 6710:2017	
		Blood culture
		Citrate blood
		Serum/serum-gel blood
		Heparin/heparin-gel blood
		EDTA blood
		Fluoride/citrate-fluoride blood

[10] Gurr et al.; Musterstandardarbeitsanweisung Präanalytik; J Lab Med 2011
[11] CLSI Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, Approved Standard 2007, 6th edition GP 41-A6 (former H3-A6), 27 (26)

Tipps und Tricks in der Präanalytik - Sarstedt

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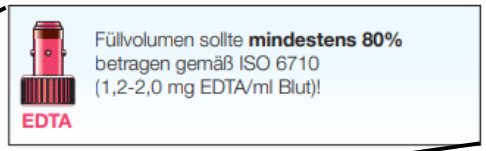
Filling volume



According to ISO 6710 – filling volume should be **at least 80%**

Excerpt SOP – sampling location:

3. Sample processing

Per patient: 2 serum and EDTA monovettes full and 3 serum or EDTA monovettes half-full are collected and processed in the laboratory.

- Allow blood collection tubes to rest for 30 min after blood collection (make sure serum is clotted).

- **Additive K-EDTA is present in twice the concentration**
- **Shear forces are greater with less volume -> risk of hemolysis (17% hemolytic samples)**

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Identification and Transport

Factor	Preanalytical variable	Recommendations
Identification	Correct assignment of the samples to the patients	Laboratory Information and Management System (LIMS)
	Correct assignment of the samples history	LIMS + accompanying documents
Transport	**Environmental exposure** Temperature Shear Force in pneumatic tubes	Fast, secure transport in suitable packaging to the laboratory/biobank Samples cooled? Track temperature Reduce transportation speed

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Unexpected high urine amylase activity[1]

“In the 1960s, before operating a case with acute abdominal pain surgeons ordered to measure amylase in urine in order to exclude pancreatitis as a possible cause of the abdominal pain. After the amylase was increased, the operation was postponed, until continuous symptoms forced to operate. The surgeon said “When looking into the pancreas, there was no sign of pancreatitis, hence the amylase result must be wrong!”. Having no explanation, we asked the nurses to send several patient’s urine having no symptoms. Although most cases exhibited normal amylase activity, some again exhibited increased values. Having asked all persons involved in sampling and transport, it turned out that the nurses collecting and transferring the samples, sometimes held discussions close to the open vessels containing the urine that had to be delivered to laboratory. During this discussions, quite often drops of nurse’s spittle came into contact with the patient’s samples thus contaminating them with amylase from the nurse’s saliva!”

[1]Guder, W. G. (2014) **History of the preanalytical phase:** a personal view Biochem Med (Zagreb), 24(1): 25–30.

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Sample processing

Factor	Preanalytical variable	Recommendations
Processing	Processing Duration	Process rapidly, document date and time of processing
	Time from blood collection to centrifugation (TTC), Time from centrifugation to freezing (TTF)	Important for blood-based samples – document date and time of processing
	Centrifugation speed/force, time and temperature	According to manufacturers recommendations
	Temperature	temperature controlled laboratory
	Aliquotation (volume) in barcoded tubes	Aliquot to secondary tubes, multiple small volume aliquots instead of one large volume aliquot (readable unique 2D coded cryostable tubes)

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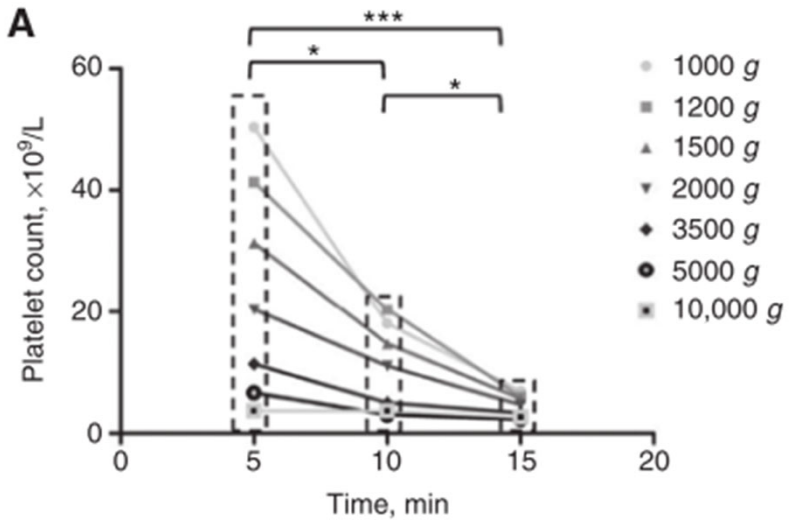
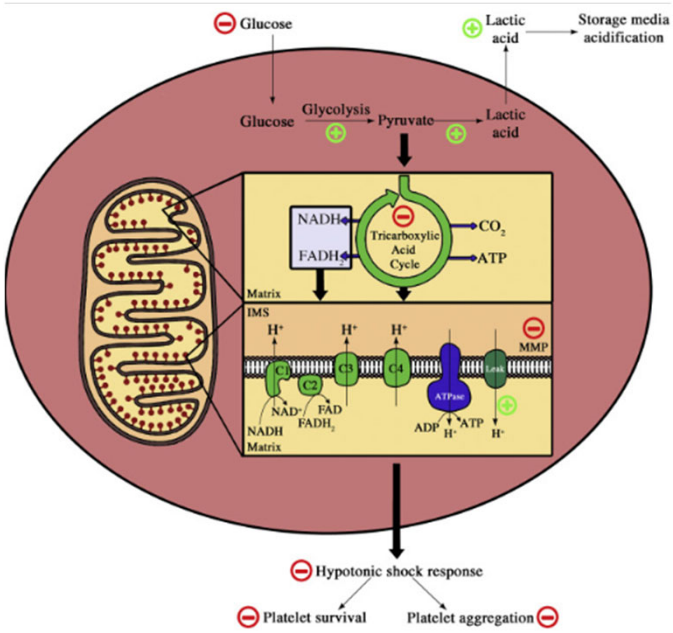
Centrifugation



Platelet count in plasma differs after different centrifugation times/strengths



Storage at room temperature results in consumption of glucose and formation of lactate (acidification), as well as accumulation of immunoactive proteins such as IL-6, IL-8, etc.

Number of platelets influences the change of analytes in blood samples!

Söderström et al. 2016 The effect of centrifugation speed and time on pre-analytical platelet activation, Clin Chem Lab Med, 54: 1913-1920
Ng, M. S. Y., et al. (2018). "Platelet Storage Lesions: What More Do We Know Now?" Transfusion Medicine Reviews **32**(3): 144-154.

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Long-term storage

Factor	Preanalytical variable	Recommendations
Long-term storage	Storage duration, temperature, facility	Store at -80 °C or in the vapour phase of liquid nitrogen
	Freeze-thaw cycles	Avoid multiple freeze-thaw cycles, single-use aliquots only
	Cryotubes, labeling	Barcoded cryotubes suitable for ultra-low temperatures

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Pre-analytical phase - how do we assess a high-quality sample?

What would you do?

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Steps to high sample quality

- Establishment of **standard operating procedures** (SOPs) for sample processing

- **Training of staff** in pre-analytical precautions during sample handling and implementation of SOPs

- Implementation of technical approaches in pre-analytical procedures – **electronic sample tracking**, electronic patient/study participant ID, **electronic time tracking** of pre-analytical processes

- **Synchronizing** of pre-analytical structures and processes between biobanks and clinics

- Mapping and documentation of sample history. Main pre-analytical parameters were included in a labeling system for sample collections in biobanks (**SPREC**),

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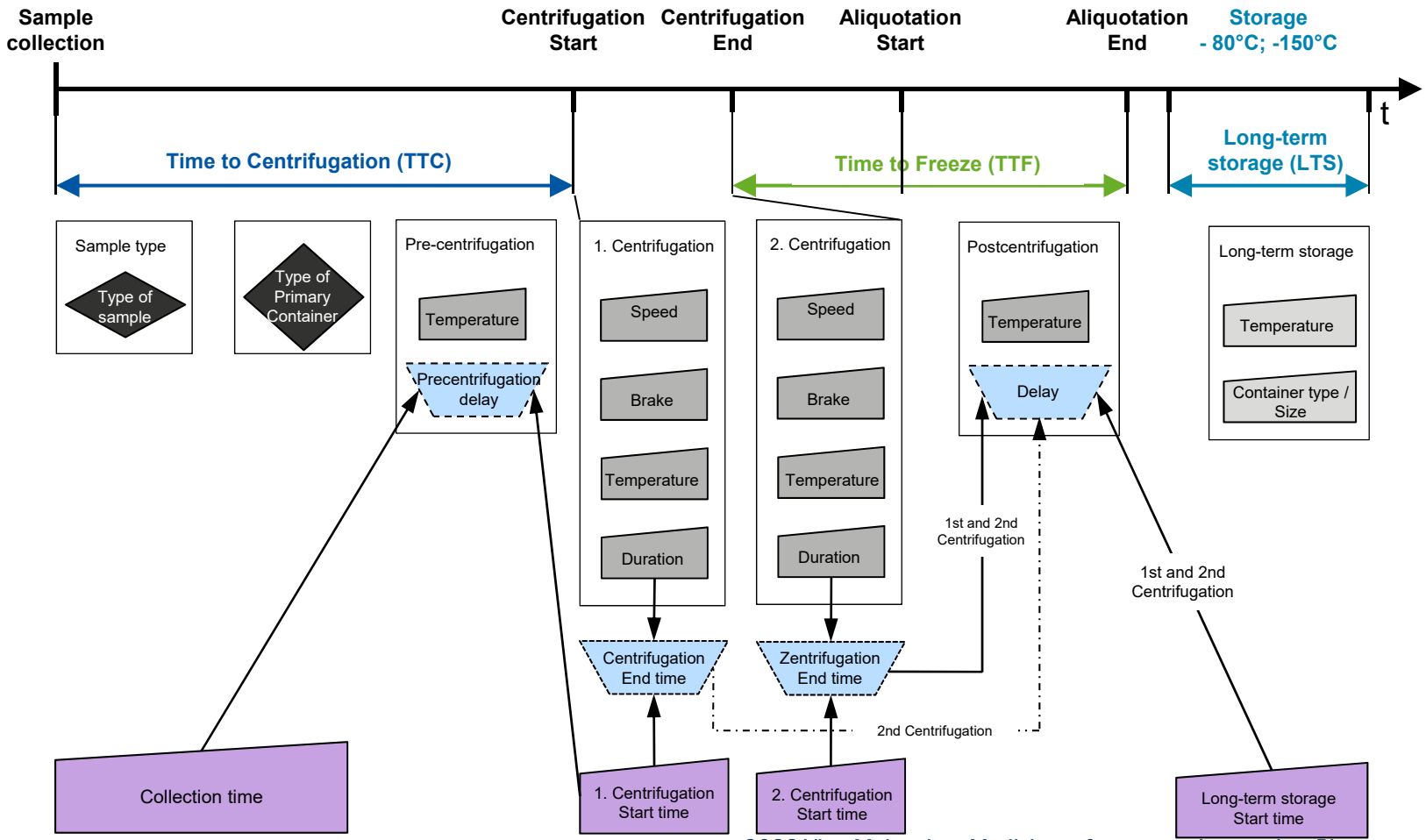
SPREC – Sample PREanalytical Coding for biospecimen – an attempt to improve sample quality registration



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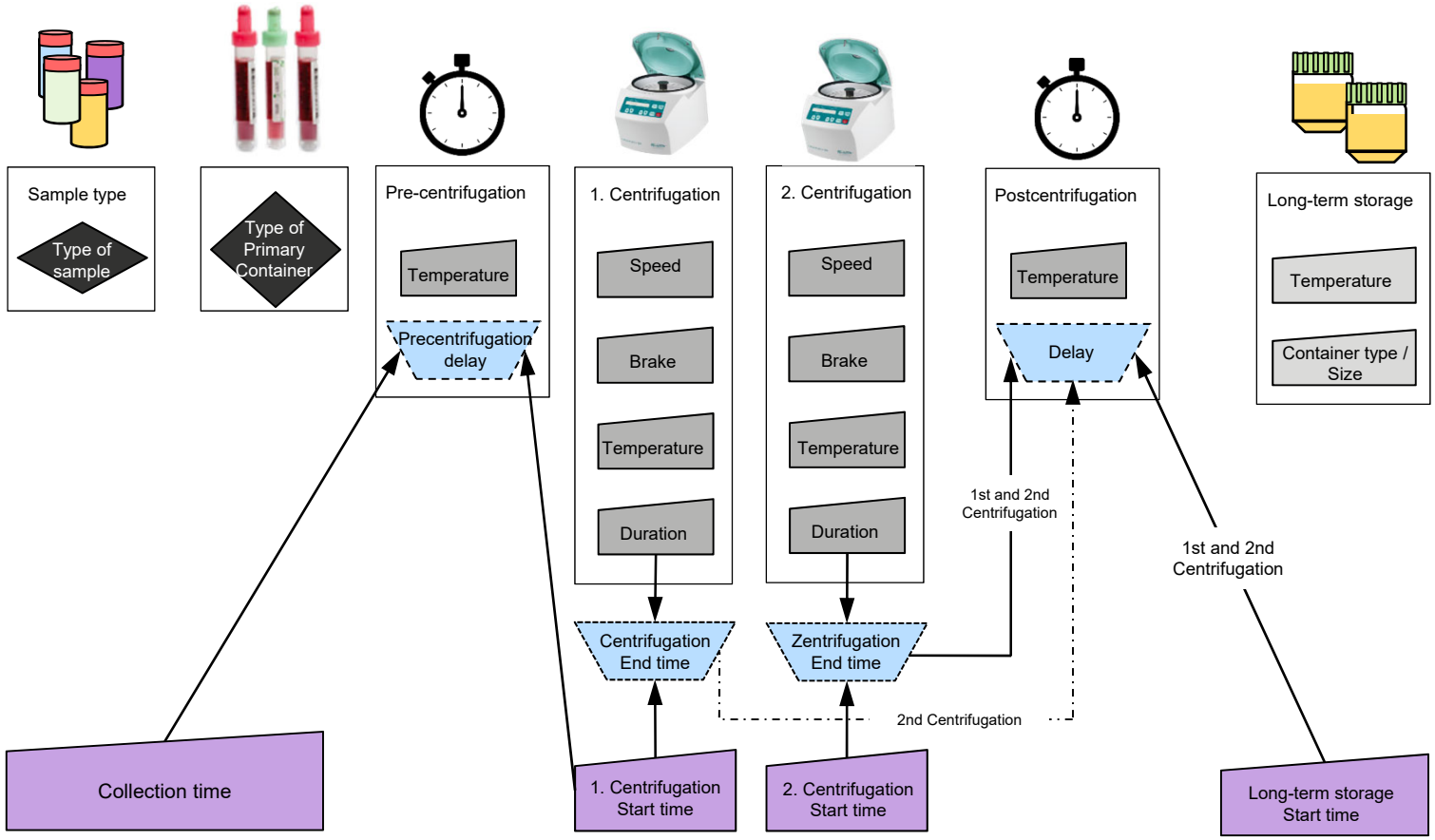
SPREC – Sample PREanalytical Coding for biospecimen – an attempt to improve sample quality registration



SPREC2.0 for liquid samples (7 characters), example SER-SST-A-A-N-B-A

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SPREC – Sample PREanalytical Coding for biospecimen – an attempt to improve sample quality registration

SER-SST-A-A-N-B-A

Ser= Serum; SST = Serum separator tube with clot activator

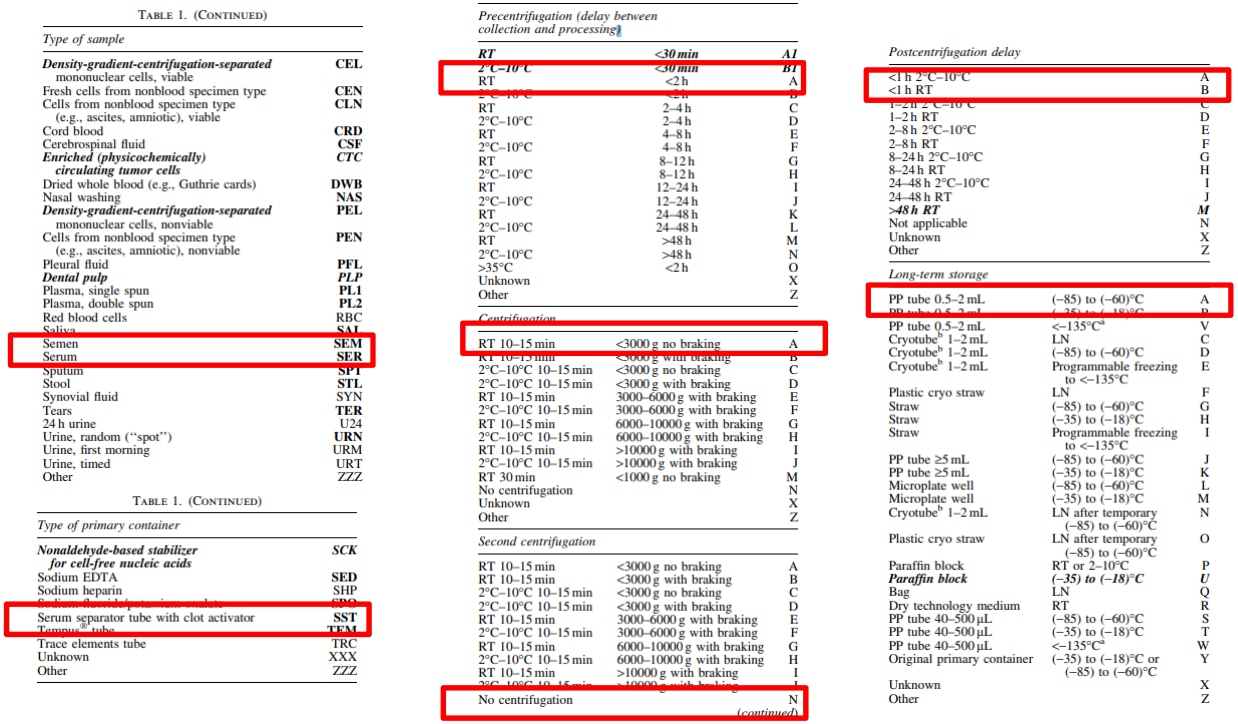

TABLE 1. (CONTINUED)

Type of sample	
Density-gradient-centrifugation-separated mononuclear cells, viable	**CEL**
Fresh cells from nonblood specimen type	**CEN**
Cells from nonblood specimen type (e.g., ascites, amniotic), viable	**CLN**
Cord blood	**CRD**
Cerebrospinal fluid	**CSF**
Enriched (physicochemically) circulating tumor cells	***CTC***
Dried whole blood (e.g., Guthrie cards)	**DWB**
Nasal washing	**NAS**
Density-gradient-centrifugation-separated mononuclear cells, nonviable	**PEL**
Cells from nonblood specimen type (e.g., ascites, amniotic), nonviable	**PEN**
Pleural fluid	**PFL**
Dental pulp	***PLP***
Plasma, single spun	**PL1**
Plasma, double spun	**PL2**
Red blood cells	RBC
Saliva	**SAL**
Semen	**SEM**
Serum	**SER**
Sputum	**SPT**
Stool	**STL**
Synovial fluid	SYN
Tears	**TER**
24 h urine	U24
Urine, random ("spot")	**URN**
Urine, first morning	URM
Urine, timed	URT
Other	ZZZ

TABLE 1. (CONTINUED)

Type of primary container	
Nonaldehyde-based stabilizer for cell-free nucleic acids	***SCK***
Sodium EDTA	**SED**
Sodium heparin	SHP
[illegible]	[illegible]
Serum separator tube with clot activator	**SST**
[illegible]	[illegible]
Trace elements tube	TRC
Unknown	XXX
Other	ZZZ

Precentrifugation (delay between collection and processing)		
RT	***<30 min***	***AI***
2°C–10°C	***<30 min***	***BI***
RT	<2 h	A
[illegible]	[illegible]	B
RT	2–4 h	C
2°C–10°C	2–4 h	D
RT	4–8 h	E
2°C–10°C	4–8 h	F
RT	8–12 h	G
2°C–10°C	8–12 h	H
RT	12–24 h	I
2°C–10°C	12–24 h	J
RT	24–48 h	K
2°C–10°C	24–48 h	L
RT	>48 h	M
2°C–10°C	>48 h	N
>35°C	<2 h	O
Unknown		X
Other		Z

Centrifugation		
RT 10–15 min	<3000 g no braking	A
[illegible]	[illegible]	B
2°C–10°C 10–15 min	<3000 g no braking	C
2°C–10°C 10–15 min	<3000 g with braking	D
RT 10–15 min	3000–6000 g with braking	E
2°C–10°C 10–15 min	3000–6000 g with braking	F
RT 10–15 min	6000–10000 g with braking	G
2°C–10°C 10–15 min	6000–10000 g with braking	H
RT 10–15 min	>10000 g with braking	I
2°C–10°C 10–15 min	>10000 g with braking	J
RT 30 min	<1000 g no braking	M
No centrifugation		N
Unknown		X
Other		Z

Second centrifugation		
RT 10–15 min	<3000 g no braking	A
RT 10–15 min	<3000 g with braking	B
2°C–10°C 10–15 min	<3000 g no braking	C
2°C–10°C 10–15 min	<3000 g with braking	D
RT 10–15 min	3000–6000 g with braking	E
2°C–10°C 10–15 min	3000–6000 g with braking	F
RT 10–15 min	6000–10000 g with braking	G
2°C–10°C 10–15 min	6000–10000 g with braking	H
RT 10–15 min	>10000 g with braking	I
[illegible]	[illegible]	J
No centrifugation		N

(continued)

Postcentrifugation delay		
<1 h 2°C–10°C		A
<1 h RT		B
[illegible]		C
1–2 h RT		D
2–8 h 2°C–10°C		E
2–8 h RT		F
8–24 h 2°C–10°C		G
8–24 h RT		H
24–48 h 2°C–10°C		I
24–48 h RT		J
>48 h RT		***M***
Not applicable		N
Unknown		X
Other		Z

Long-term storage		
PP tube 0.5–2 mL	(−85) to (−60)°C	A
[illegible]	[illegible]	B
PP tube 0.5–2 mL	<−135°C[a]	V
Cryotube[b] 1–2 mL	LN	C
Cryotube[b] 1–2 mL	(−85) to (−60)°C	D
Cryotube[b] 1–2 mL	Programmable freezing to <−135°C	E
Plastic cryo straw	LN	F
Straw	(−85) to (−60)°C	G
Straw	(−35) to (−18)°C	H
Straw	Programmable freezing to <−135°C	I
PP tube ≥5 mL	(−85) to (−60)°C	J
PP tube ≥5 mL	(−35) to (−18)°C	K
Microplate well	(−85) to (−60)°C	L
Microplate well	(−35) to (−18)°C	M
Cryotube[b] 1–2 mL	LN after temporary (−85) to (−60)°C	N
Plastic cryo straw	LN after temporary (−85) to (−60)°C	O
Paraffin block	RT or 2–10°C	P
Paraffin block	***(−35) to (−18)°C***	***U***
Bag	LN	Q
Dry technology medium	RT	R
PP tube 40–500 µL	(−85) to (−60)°C	S
PP tube 40–500 µL	(−35) to (−18)°C	T
PP tube 40–500 µL	<−135°C[a]	W
Original primary container	(−35) to (−18)°C or (−85) to (−60)°C	Y
Unknown		X
Other		Z

A= Precentrifugation delay < 2 h RT
A= 1st Centrifugation RT 10 – 15 min < 3000 g, no braking
N= no 2nd Centrifugation
B= Postcentrifugation time < 1 h RT
A= long-term storage in PP (polypropylen) tube 0.5 – 2 mL (-85 °C –60 °C)

Lehmann et al. 2012 Biopreservation and Biobanking, DOI:10.1089/bio.201200012
Betsou et al. 2018 Standard PREanalytical Code Version 3.0 BIOPRESERVATION AND BIOBANKING 16, DOI: 10.1089/bio.2017.0109

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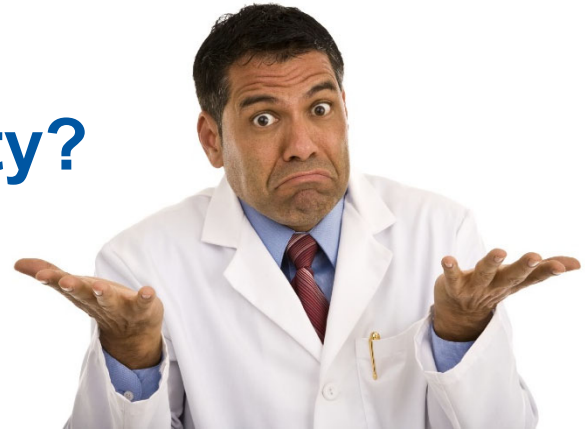
SPREC – Sample **PRE**analytical **C**oding for biospecimen – an attempt to improve sample quality registration

- SPREC system was established to increase transparency of sample history
- Contributes to a consistent sample coding and documentation in biobanks
- It forms the basis for the establishment of standard operating procedures (SOPs) for sample handling processes in laboratories and/or biobanks
- Two different SPREC codes – liquid and solid samples

Are all samples now of high quality?

What about older samples?



Lehmann et al. 2012 Biopreservation and Biobanking, DOI:10.1089/bio.201200012
Betsou et al. 2018 Standard PREanalytical Code Version 3.0 BIOPRESERVATION AND BIOBANKING 16, DOI: 10.1089/bio.2017.0109

What should we do with?

- Biospecimens from pre-existing collection
- Rare biospecimens from patients with rare diseases
- Biospecimen with an insufficient sample history

Retrospective analysis

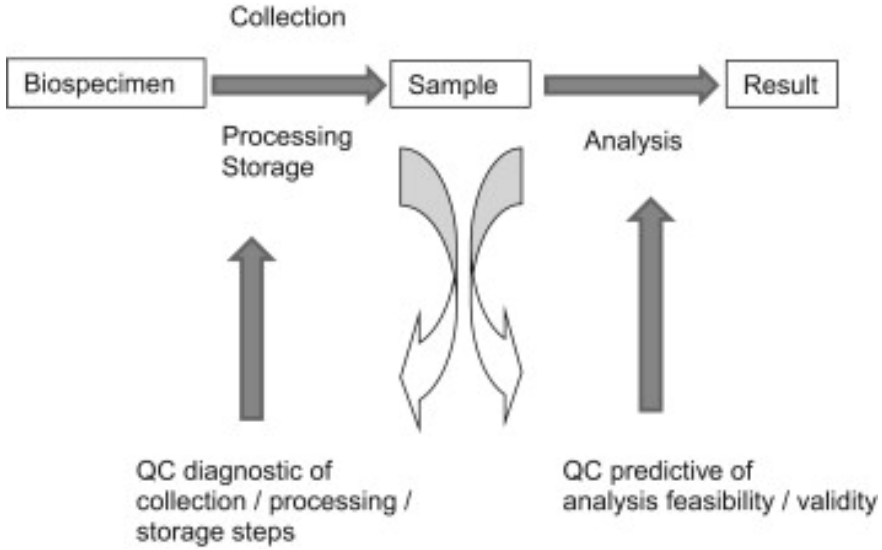
Quality indicators (QI) for biospecimen

Def. QIs are biomolecules used as tools for quality assessment of biospecimen

<u>Categories:</u>

Diagnostic: assess the processing steps of the biospecimen, such as delay of processing or storage conditions

Predictive: assess the feasibility and/or reliability of the downstream analysis (successful method performance)



Betsou *et al.* (2012) Identification of Evidence-Based Biospecimen Quality-Control Tools https://doi.org/10.1016/j.jmoldx.2012.06.008

Selection of Quality Indicators: Challenges/Criteria

- QIs should be characteristic for one specific pre-analytical condition
  - e.g. pre-analytical time delay, handling temperature, freeze-thaw cycles, long-term storage, ambient handling/storage temperature
- monitoring the most important common pre-analytical variations
- QIs should be unaffected by confounders such as
  - sample additives
  - certain disease states and/or
  - clinical conditions and/or
  - therapeutic interventions and/or
  - other confounders
- Precise, robust, reproducible
- QIs should be quantified at moderate costs and require only minimal sample volume

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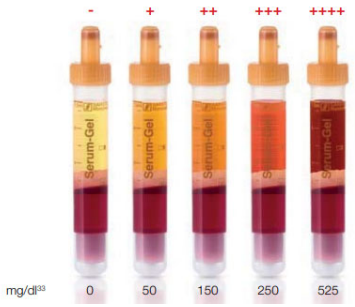
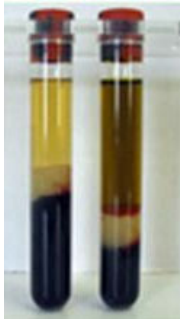
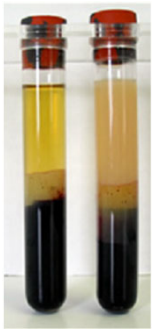
HIL-index: quality assessment in routine laboratory applications

HIL-index (Hemolysis, Icterus (Hyperbilirubinemia), Lipemia-Index) in human liquid biospecimens







Causes of HIL-positive samples:

Hemolytic:	long application tourniquet, mechanical stress on erythrocytes…
Icteric:	increased bilirubin concentration (yellow/brown sample)
Lipemic:	high triglyceride concentration (white haze)

Consequences of HIL-positive samples: Biases in clinical diagnosis, interference with other assays

Analysis: quantification of hemoglobin, bilirubin and triglyceride concentration by automated absorption measurement (absorption spectrum: 500-804 nm)

Hyunh *et al.* Cureus, 2017, DOI:10.7759/cureus.1965

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Metabolomics as resource for quality indicators

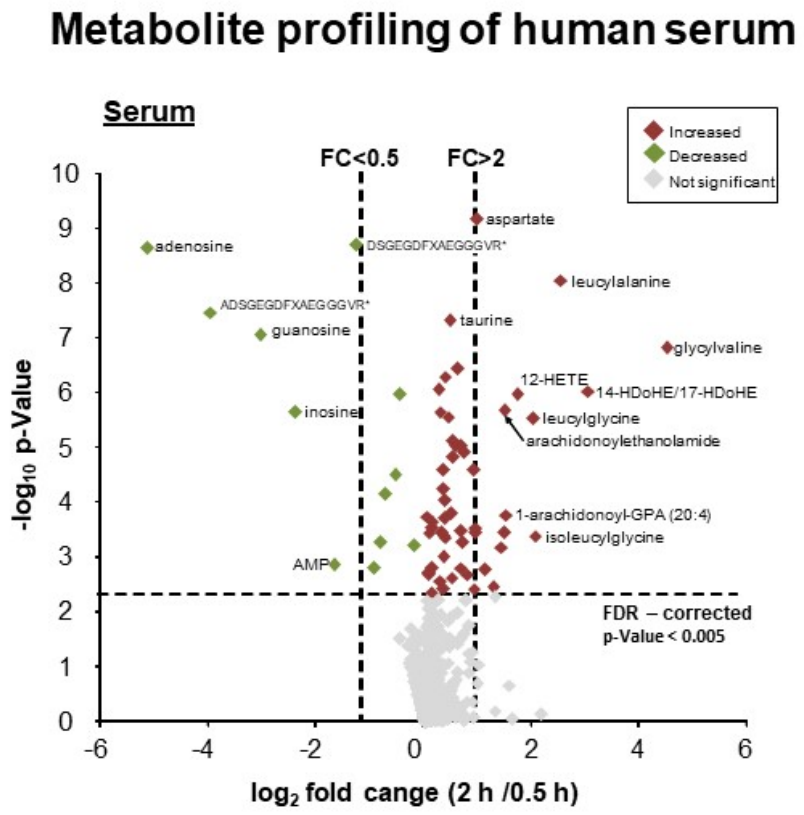
- Metabolomics = basis for establishment of individual phenotypes (biomarkers indicating diseases, nutritional status ...)
- Metabolome mirrors variations within the human body
- Concentration changes of metabolites are mainly due to cellular activities of blood cells and these changes reflect pre-analytical variation in biospecimens
- Several metabolites were identified as QI candidates for the discrimination of individual sample quality
- Need for consistent sample quality and storage conditions in Biobanks for reliable metabolomics analyses/biomarker identification

Obstacles:

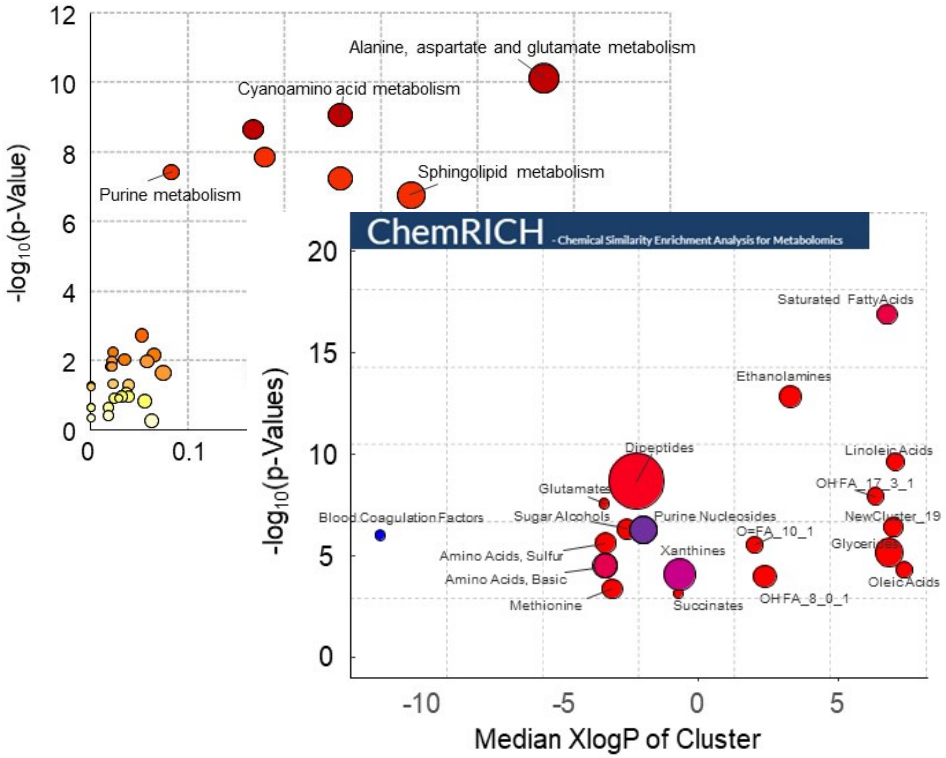
- Heterogeneous sampling and biobanking processes
- Not all biospecimen are well studied (saliva, feaces)

Kirwan et al. 2018 Clinical Chemistry 64:8, 1158-1182; doi:10.1373/clinchem.2018.298620

Metabolomics as resource for quality indicators



Level of significance is p<0.05 (paired T-test). FDR correction was applied using the Benjamini and Hochberg method*. Asterisks indicate putatively identified metabolites. (N=10)



- Metabolites were used for Pathway Enrichment and Chemical Similarity Enrichment Analysis to map individual altered pathways and cluster compounds based on chemical similarity to identify potential quality markers

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Metabolomics as resource for quality indicators

Table 3. Proposed QIs to be considered for further investigation in serum and EDTA plasma.

Potential QIs in Serum	Median Opt. Cutoff	Specificity n+/n > 1 h	Sensitivity 1-(n-/n) < 1 h
HG-ratio	1.41	79% (11/14)	85% (22/26)
XI-ratio	2.06	100% (14/14)	73% (19/26)
		Specificity n+/n > 2 h	Sensitivity 1-(n-/n) < 2 h
Tetranor-12(S)-HETE	0.45 ng/mL	92% (12/13)	100% (7/7)
12(S)-HEPE	8.17 ng/mL	100% (13/13)	86% (6/7)
8(S)-HETE	2.22 ng/mL	100% (13/13)	86% (6/7)
12-HETE	15.14 ng/mL	92% (12/13)	86% (6/7)
12-oxo-ETE	2.62 ng/mL	100% (13/13)	86% (6/7)
Potential Qis in EDTA Plasma	Median Opt. Cutoff	Specificity n+/n > 1 h	Sensitivity 1-(n-/n) < 1 h
HI-ratio	1.54	85% (11/13)	81% (22/27)

The table displays the calculated median optimal cutoffs for all considered QIs in validation sample 1 and 2 in serum and EDTA plasma and applies them in validation sample 3 and 4. n+, measurements with a positive test result; n-, measurements with a negative test result; n > 1 h, 2 h measurements with a TTC > 1 or 2 h; n < 1 h, 2 h, measurements with a TTC < 1 or 2 h.

- HG (hypoxanthine/guanosine)-ratio and XI (xanthine/inosine)-ratio were identified to discriminate with high sensitivity and specificity a TTC > 1 h in serum
- Several eicosanoids were identified to discriminate with high sensitivity and specificity a TTC > 2 h in serum

Heiling *et al.*, Metabolites 2021, 11, 638

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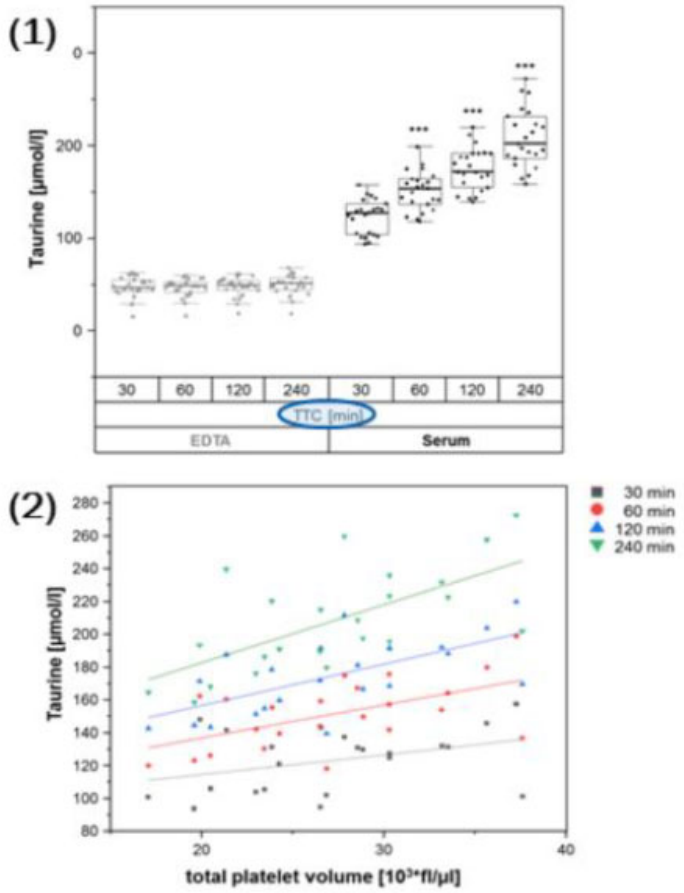
Taurine as serum specific indicator of TTC delay



Copyright: Schwarz *et al.* doi: 10.1089/bio.2019.0004

- concentration increase during serum sample storage (1)
- correlation between individual platelet count and volume (MPV) and Taurine (2)
- Taurine quantification via LC-MS/MS, Ion-exchange chromatography

Metabolome: source for potential Quality indicators – a current selection from the literature

Serum (tested pre-analytical conditions)	EDTA-Plasma (tested pre-analytical conditions)
Amino acids/derivatives/amino acid-scores	
Arginine (TTF[1])	Arginine (TTF[1], Temperature[1])
Ornithine (TTF[1])	Ornithine (TTC[2], TTF[1], Temperature[1])
Aspartate (TTC[2,3,4], TTF[1])	Aspartate (TTF[1,4], Temperature[1])
Glycine (TTF[1])	5-Oxo-proline (TTC[11])
Taurine (TTC[3])	
other metabolites	
Glucose (TTC[4], TTF[7])	Glucose (TTC[4], TTF[5,7])
Pyruvate (TTC[4])	Lactate (TTC[4], TTF[5], Temperature[5,6])
LysoPC C18:0 (TTF[9], Temperature[9])	LysoPC C18:0 (TTF[1,2,8], Temperature[1,2,8])
LysoPC C18:1 (TTF[9], Temperature[9])	Ascorbate/Ascorbic Acid (TTC[12], Temperature[6])
α-Ketoglutarate (TTC[4])	α-Ketoglutarate (TTC[4])

References: next slide

Literatur additions

[1]Breier et al., 2014, Plos One, Vol. 9, Issue 2, doi:10.1371/journal.pone.0089728

[2]Kamlage et al., 2014, Clinical Chemistry 60:2, DOI: 10.1373/clinchem.2013.211979

[3]Kamlage et al., 2018, Metabolites, 8, 6, doi:10.3390/metabo8010006

[4]Liu et al., 2010, Analytical Biochemistry, 406, doi:10.1016/j.ab.2010.07.015

[5]Malm et al., 2016, Biobanking and Biopreservation, Vol. 14, No. 5, DOI: 10.1089/bio.2015.0092

[6]Trezzi et al., 2016, Metabolomics, 12:96, DOI 10.1007/s11306-016-1038-1

[7]Boyanton et al., 2002, Clinical Chemistry, 48:12

[8]Yang et al., 2013, Analytical Chemistry, 85, DOI 10.1007/s11306-016-1038-1

[9]Anton et al., 2015, Plos One, doi:10.1371/journal.pone.0121495

[10]Betsou et al., 2013, JMD Vol.15, No. 1; http://dx.doi.org/10.1016/j.jmoldx.2012.06.008

[11]Jain et al., 2017, Clinica Chimica Acta, 466, http://dx.doi.org/10.1016/j.cca.2017.01.005

[12]Karlsen et al., 2007, European Journal of Clinical Nutrition, 61

Educational objectives

1. What is biobanking and why is it important?
2. Sample quality
3. Preanalytical Source of Errors?
4. SPREC? Function and Components of SPREC
5. Steps to a high sample quality
6. Quality indicators: Definition, Requirements/confounding factors, examples

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