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UNIVERSITÄT GREIFSWALD

Wissen
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Seit 1456

SHIP
Leben und Gesundheit in Vorpommern

Universitätsmedizin
GREIFSWALD

Biobanking in der Nationalen Kohorte

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Die Nationale Kohorte

Chronische Krankheiten sind in Deutschland ebenso wie in anderen westlichen Industrieländern die Haupttodesursache. Durch den demografischen Wandel wird die Bedeutung dieser sogenannten Volkskrankheiten in den kommenden Jahrzehnten weiter zunehmen und eine große Belastung für das Gesundheitssystem darstellen. Eine starke Erhöhung der Pazinische Untersuchungen, wiederholte Befragungen und Entnahmen von Blutproben Informationen über die Studienteilnehmer vor der eventuellen Diagnose einer Krankheit gesammelt werden. Somit können für eine Vielfalt von Gesundheitszuständen oder Krankheitskombinationen (Multimorbidität) die Auswirkungen von Lebensstil, Umwelt

tiiert wurden (vor bis zu 25 Jahren), als zahlreiche der heute üblichen Untersuchungstechniken noch nicht verfügbar waren. Des Weiteren werden bei einigen der größeren deutschen Kohortenstudien die Bioproben der für die Forschung interessanten Personen in den nächsten zehn bis 20 Jahren größtenteils aufgebraucht sein.

German National Cohort

200 000 probands

18 study centers

The National Cohort major diseases, major exposures, and risk factors

Major diseases:

- CVD
- Diabetes mellitus
- Cancer
- Neurologic and psychiatric diseases
- Respiratory diseases
- Infectious diseases

Major exposures and risk factors:

- Body composition
- Physical activity
- Physical fitness
- Diet
- Smoking and alcohol consumption
- Psychosocial factors
- Socioeconomic status
- Sleep-related characteristics
- Chronic infections, immune factors, and microflora
- Occupational and environmental exposures

Study design (1)

- population-based prospective cohort
- age range 20 - 69 years
- random sample of inhabitants of defined geographical regions

- Level 1, n = 200.000
- Level 2, n = 40.000 (MRI programme)
- Level 3, n = variable (additional research questions with own financing)

Study design (2)

- Basic examination with interview, questionnaire, medical examination, testing of cognitive functions
- 2.5 h examination programme at level 1 and
  4 h intensive examination programme at level 2
- 5 year basic examination, and a 5 year follow-up examination
- combination of active follow-up (mailed questionnaires every 2-3 years) and passive follow-up (register query)
- collection of biomaterial (blood, urine, saliva, swab, faeces)

Selected Biosamples (pre-final)

Blood ★	Serum* Plasma* Leukozyten* Erythrozyten* RNA DNA ★ PBMC ★	30x 0,19ml* 48x 0,19ml* 4x 0,6ml* 6x 0,2ml* 1x 10ml 1x 9ml 5x 0,2ml	- 180 °C - 180 °C - 180 °C - 180 °C - 80 °C - 80 °C - 180 °C
Urine* ★		12x 0,19ml* 4x 0,2ml*	- 180 °C - 180 °C
Saliva* ★		2x 0,6ml*	- 180 °C
Swab ★		1x 2 Swabs	- 80 °C
Faeces ★		1x nativ 1x 10ml (DNA-Stabilisator)	- 80 °C

* Liquid handling Roboter (ML Starlet, Hamilton Robotics) ★SOP √

Study of Health in Pomerania - SHIP

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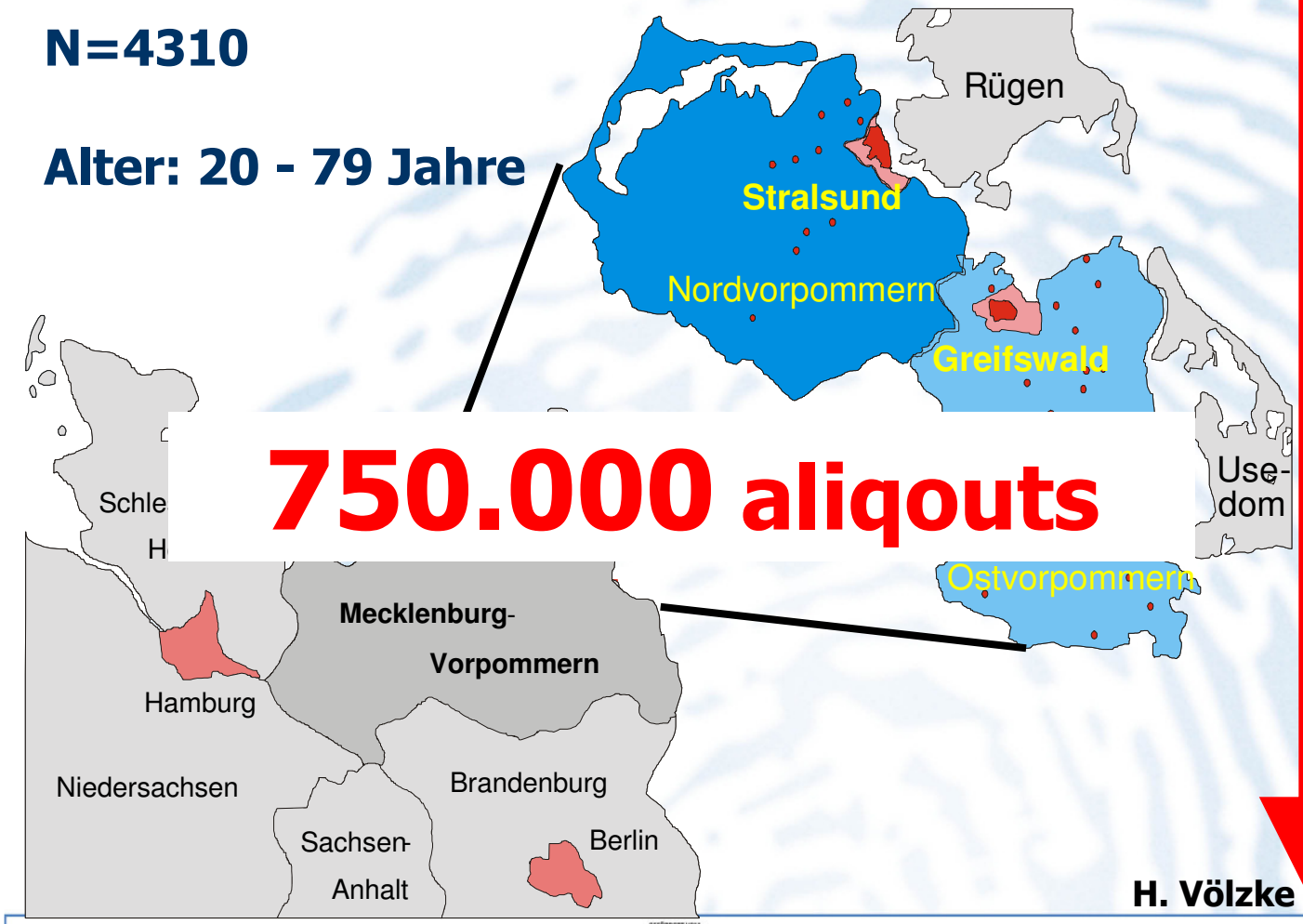
Greifswald Approach to Individualized Medicine

N=4310

Alter: 20 - 79 Jahre



750.000 aliqouts

1997-2001

SHIP-0

2002-2006

SHIP-1

2008-2013

SHIP-2
SHIP-TRENDS

H. Völzke et al. Int J Epidemiol 2010

SPITZENFORSCHUNG & INNOVATION IN DEN NEUEN LÄNDERN

Bundesministerium für Bildung und Forschung

Mecklenburg Vorpommern MV tut gut.

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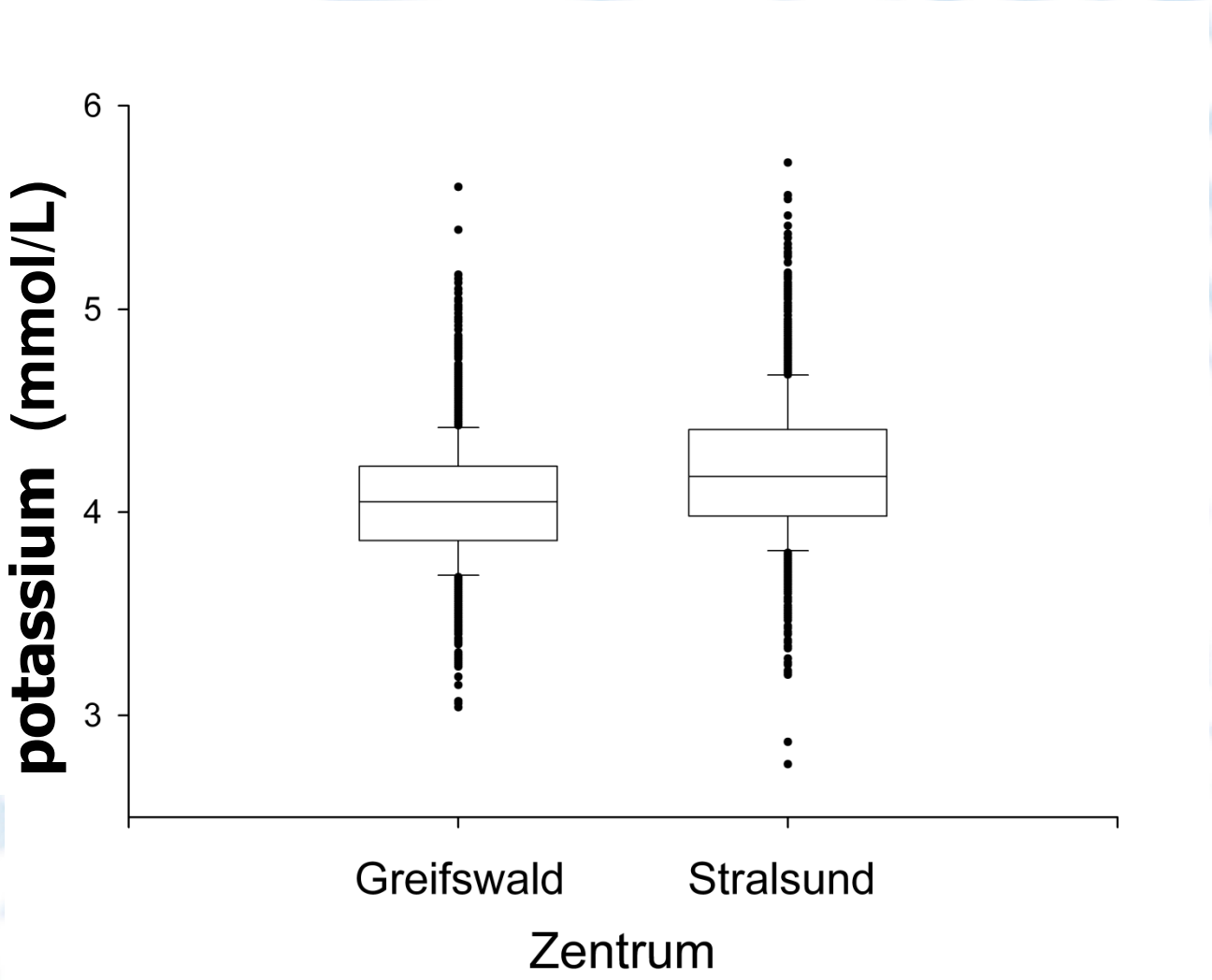
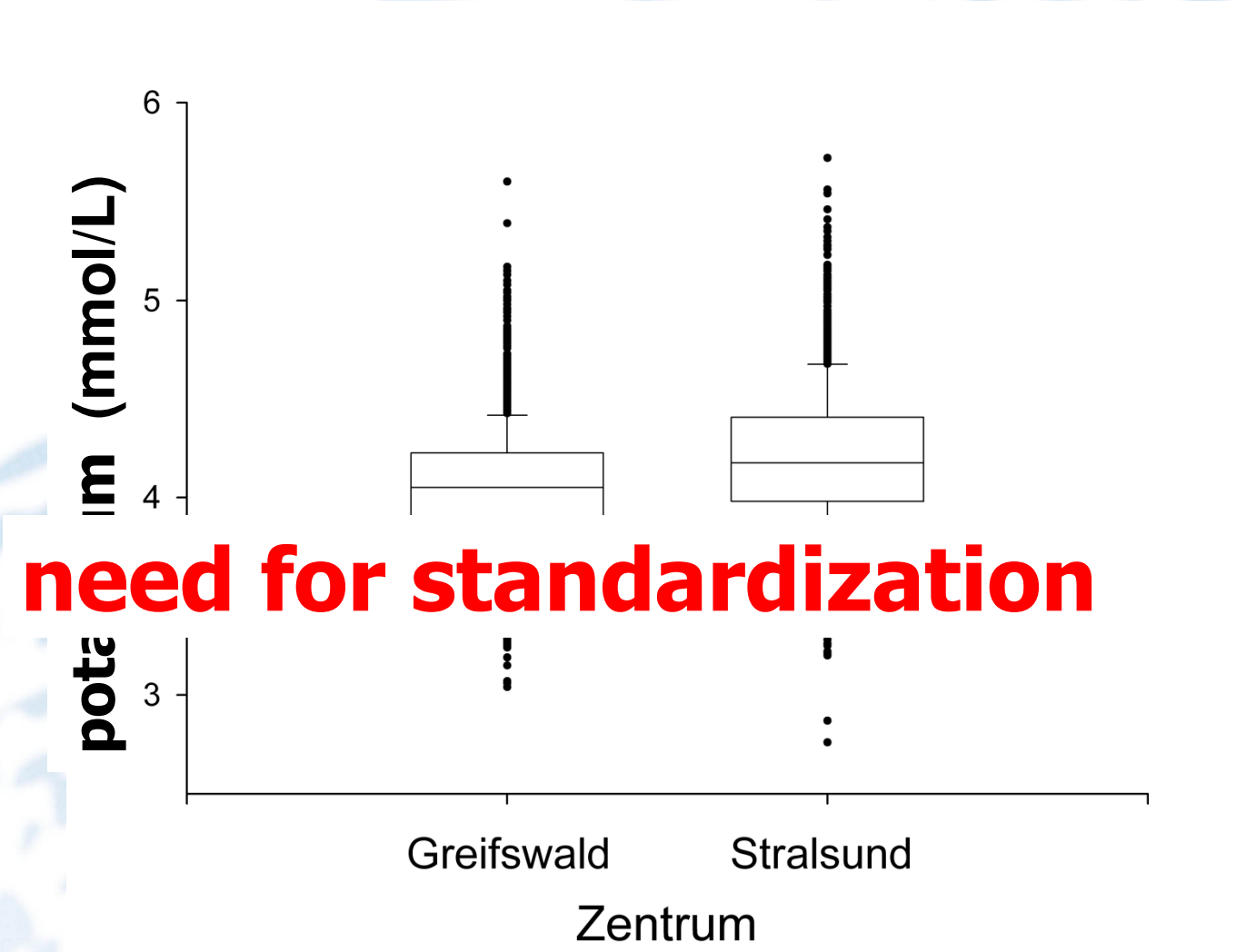
Biobanking in SHIP-0

- Samples were drawn in two examination centers in Stralsund and Greifswald.
- Pre-analytical time period until sample preparation was different.
- In Stralsund the delay was longer than 2 hours until centrifugation.

Pre-analytical problems in SHIP-0



Pre-analytical problems in SHIP-0



need for standardization

Research Biobank

BUILDING BETTER BIOBANKS

NATURE. 7 JUNE 2012, VOL 486,p.141-45

- High-quality biobanking = sample storage + QC-Process
- lack of appropriate QC-tools for sample collection, processing & storage
- research on pre-analytical and analytical storage effects on future biomarker measurements & multi-omics analyses

→ Insufficient provision of biomaterial information for publication (>50%)

Nature. 2011 27;475:454-5

Process

1. indication
2. sample identification
3. sampling
4. transport to the laboratory
5. sample receipt in the laboratory
6. sample distribution
7. **analytics**
8. evaluation of findings
9. delivery of findings
10. Storage of reports

WG: Biobanking

Main Artifacts:

1. artifacts due to cell lysis and cell metabolism

2. artifacts due to the enzymatic degradation of molecular species upon prolonged exposure to 4°C

3. molecular artifacts due to repeated freezing and thawing of stored biomaterials

WG: Biobanking

Avoidance of artifacts requires:

1. prompt and complete separation from serum or plasma of all particulate components of full blood

2. no delay in the aliquotation and freezing

3. volumes small enough to guarantee single use only

WG: Biobanking

High quality can be assured by translating the following major principles into practice:

1. a Laboratory Information Management System (LIMS)
2. local processing of samples
3. automation of almost all steps in preparation, storage, and retrieval of stored materials
4. storage in a central automated biorepository
5. gas phase liquid nitrogen storage

National Cohort – sample processing

Central data management

↑↓

LIMS

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Informed consent

Setting of proband and corresponding case

Printing of barcodes for primary tubes

National Cohort – Primary Tubes

Product	n	Company
EDTA-K-Plasma, 9-10 ml	600.000	Becton Dickinson
EDTA-K-Plasma, 2-3 ml	200.000	Becton Dickinson
Serum without Gel, 9-10 ml	400.000	Becton Dickinson
Serum-Gel, 2-3 ml	200.000	Becton Dickinson
Glucose-determination, 2-3 ml NaF, Citrat buffer; pH: ~ 5.5	80.000	Becton Dickinson, Terumo
Container for urine, 100-120 ml	200.000	Becton Dickinson
Urine, 9-10 ml	200.000	Becton Dickinson
Container for Saliva, 20-30ml	200.000	Sarstedt
Cryo vial 2.0 ml, -80℃ storage	40.000-200.000	Greiner Bio-One
Container, 5 ml	200.000	Greiner Bio-One

National Cohort – sample processing

Central data management

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Informed consent

Setting of proband and corresponding case

Printing of barcodes for primary tubes

Documentation of several time stamps:
of blood collection, centrifugation, aliquoting, etc.

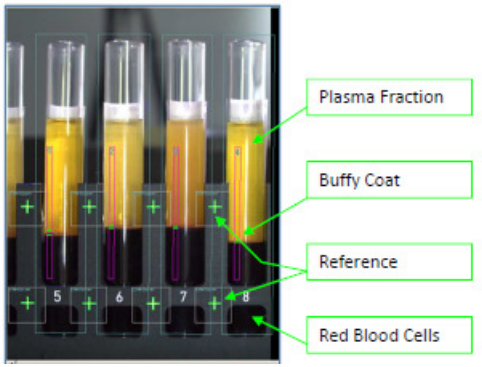
Bidirectional connection with pipetting robot

National Cohort – Pipetting Robot

HAMILTON easyBlood STARlet Workstation

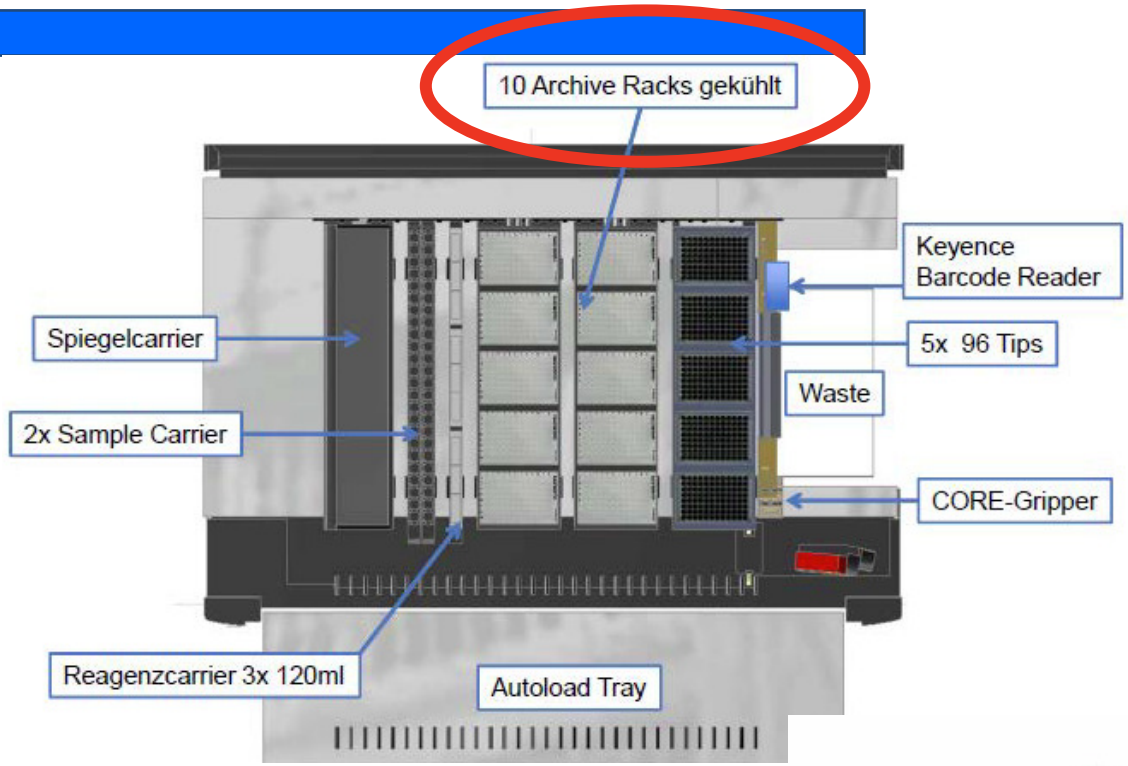
Positive Identification of specimen via barcode

Fraction and volume detection



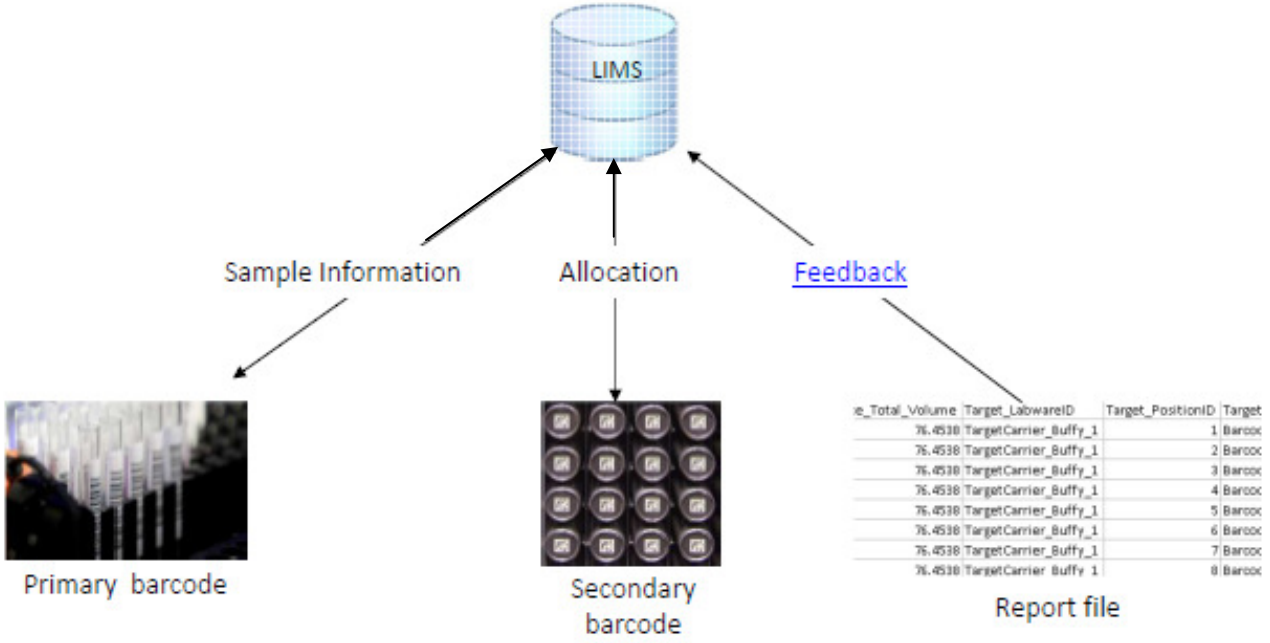
National Cohort – Pipetting Robot

Aliquoting



Scanning of 96 well plates

National Cohort – LIMS and Pipetting Robot



Throughout the process, the location of all samples are monitored and reported.

Aliquoting schema

Material	Anzahl Aliquote	Aliquot Volumen	Ziel Rack
Plasma	48	250 µl	1-3 bzw. 4-6
Serum	30	250 µl	1-3 bzw. 4-6
Erythrozyten	6	250 µl	1-3 bzw. 4-6
Urin	12	250 µl	1-3 bzw. 4-6
Serum	1	600 µl	7 bzw. 8
Urin	4	600 µl	7 bzw. 8
Buffy Coat	3	600 µl	7 bzw. 8

Bei den Level 1 Projekten sind die Aliquotvolumina und Anzahl an Aliquoten pro Materialtyp fest in der Methode hinterlegt. Pro Proband (nicht pro Tube) werden bei Level 1 Projekten folgende Aliquote verteilt.

CentraXX and STARlet EasyBlood

Projektname :	Phasentrennung/Aliquotierung Nationale Kohorte	Hamilton Robotics GmbH		13.11.13	Seite 1 von 53
Dokumentname :	20131113_Systemspecs_144-16_V2_1.doc		Dokumentnr.:	1	Version : 2.1

Systemspezifikationen

HAMILTON
ML STARlet EasyBlood

Pipettierroboter zur
Phasentrennung/Aliquotierung
für die Nationale Kohorte

Content

Inhaltsverzeichnis

National Cohort – sample processing

Central data management

↑↓

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Informed consent

Setting of proband and corresponding case

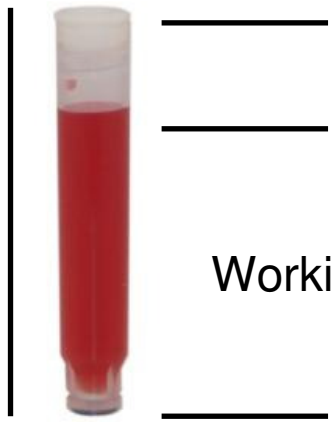
Printing of barcodes for primary tubes

Documentation of several time stamps:
of blood collection, centrifugation, aliquoting, etc

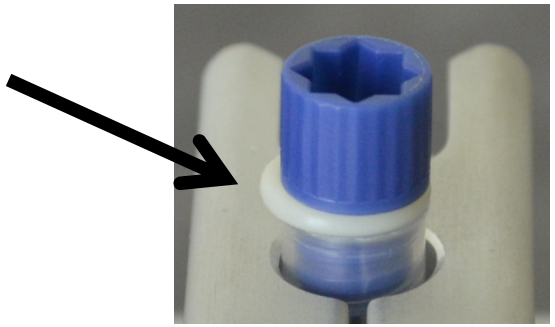
Bidirectional connection with pipetting robot
- Identification of specimen via barcode
- Fraction and volume detection (Hamilton)
- Aliquoting of samples

Tubes

- below -80°C a screw cap has to be used
- usually extern screw caps preferred
- Tubes with screw caps are not allowed to be dipped in LN_2 directly!
- Volume? Water-based samples → expansion of ca. 9%



Working volume = total volume - head

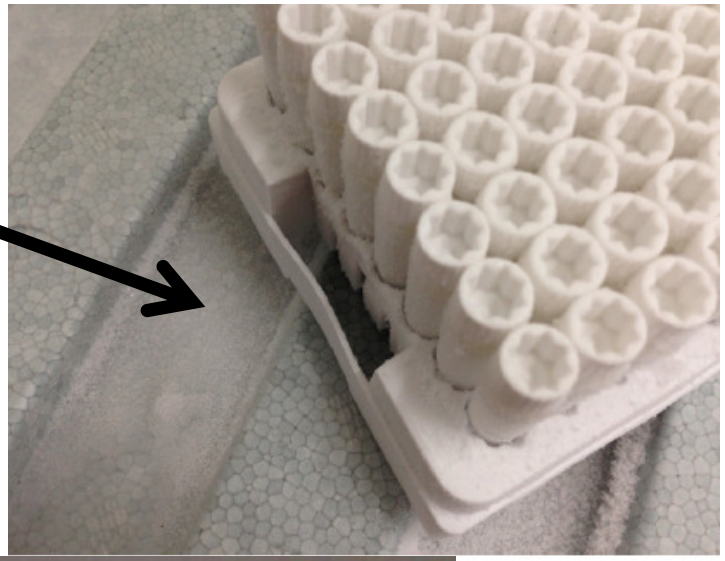


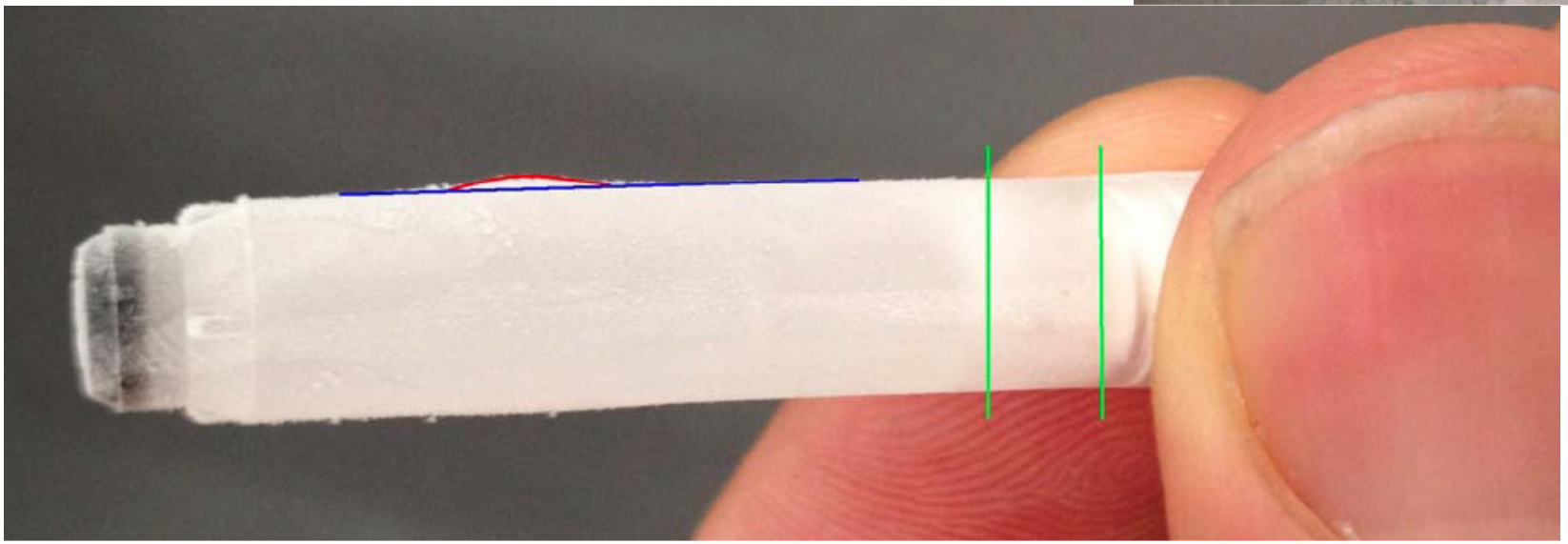
Research Biobank

Repeated freeze-thaw cycles for investigation of:

- Rack Stability
- Tube lock-position (click)
- Tube-Integrity (deformation)
- Tube-lock (leakiness)

Lancet. 1995;346:137-40





Handling tests for tube selection

Cryo tubes for validation purposes: 12 complete racks per tube size

- Biorepository
- Capper/Decapper

Storage:

- 10 racks per Cryo tube
- Cryo tubes are filled with spezified working volume
- Screw caps are closed with specified turning moment
- Tubes are stored at least for 24 h at –80 °C
- refrigerated racks are stored for at least 48 h in the gas phase of liquid nitrogen (-180 °C).

Picking schema

Picking

- 4 out of 10 racks (Rack A, Rack B, Rack C, Rack D) are released from the store. Damaged racks are processed in first line.
- All tubes are removed from 2 racks.
- A full rack (A) and an empty rack (B) are located in the tube picker (-80℃).
- All tubes are transferred from rack A → rack B.
- All tubes are transferred from rack B → rack A.
- This picking process will be repeated three times.
- Racks C and D are processed like racks A and B.

National Cohort – sample processing

Central data management

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Informed consent

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of blood collection, centrifugation, aliquoting, etc

Bidirectional connection with pipetting robot
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- Fraction and volume detection (Hamilton)
- Aliquoting of samples

Scanning of aliquots

Storage in decentral freezers (-80°C)

National Cohort – long term storage

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Transport of frozen samples to Munich Helmholtz Zentrum

Storage in the interim biorepository (up to 2015)

Storage of samples in the final biorepository
reorganization of the samples
collection of follow up samples after 5 years
picking of the samples for scientific purposes

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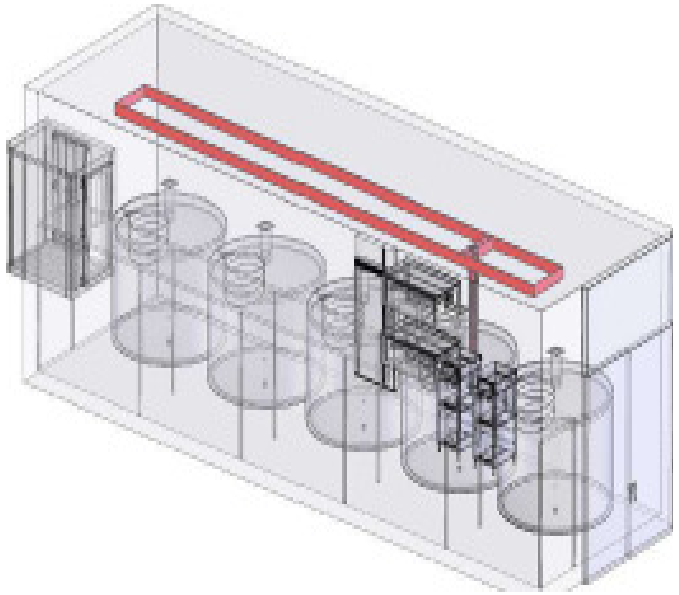
Laboratory analyses

Central data management

Data interpretation

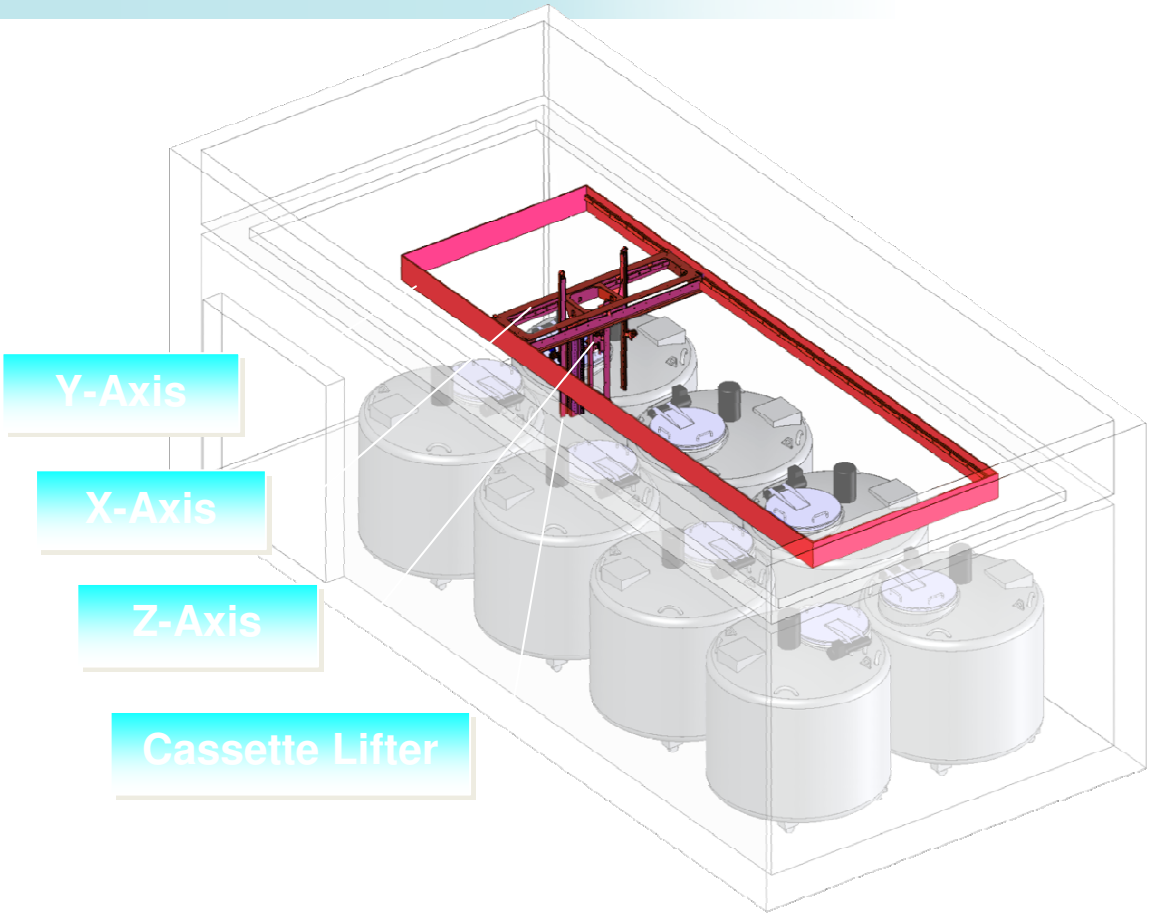
Biorepository – interim solution

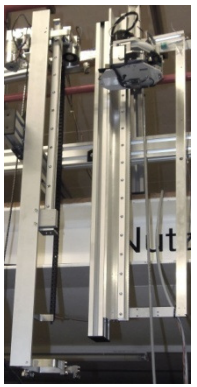


Manual Kryo-repository 10 additional tanks (+4 back up)						R	R
						R	R

LiCONiC INSTRUMENTS

- High Accuracy Cassette Alignment
- Proven Concept
- Felxible Design
- Uninterrupted Climate PN Concept

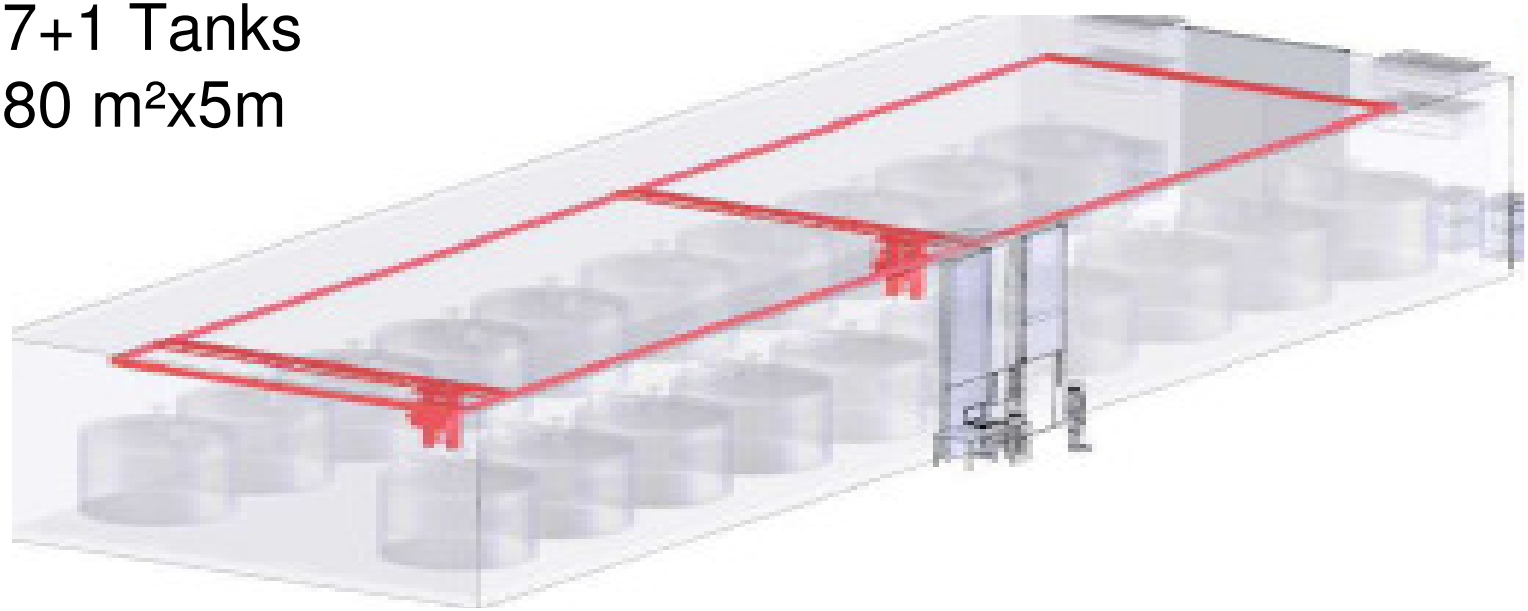




Biorepository of the National Cohort

LiCONiC INSTRUMENTS

Final Biorepository
- Fully Automated
- Tandem Solution
- 17+1 Tanks
- 280 m²x5m



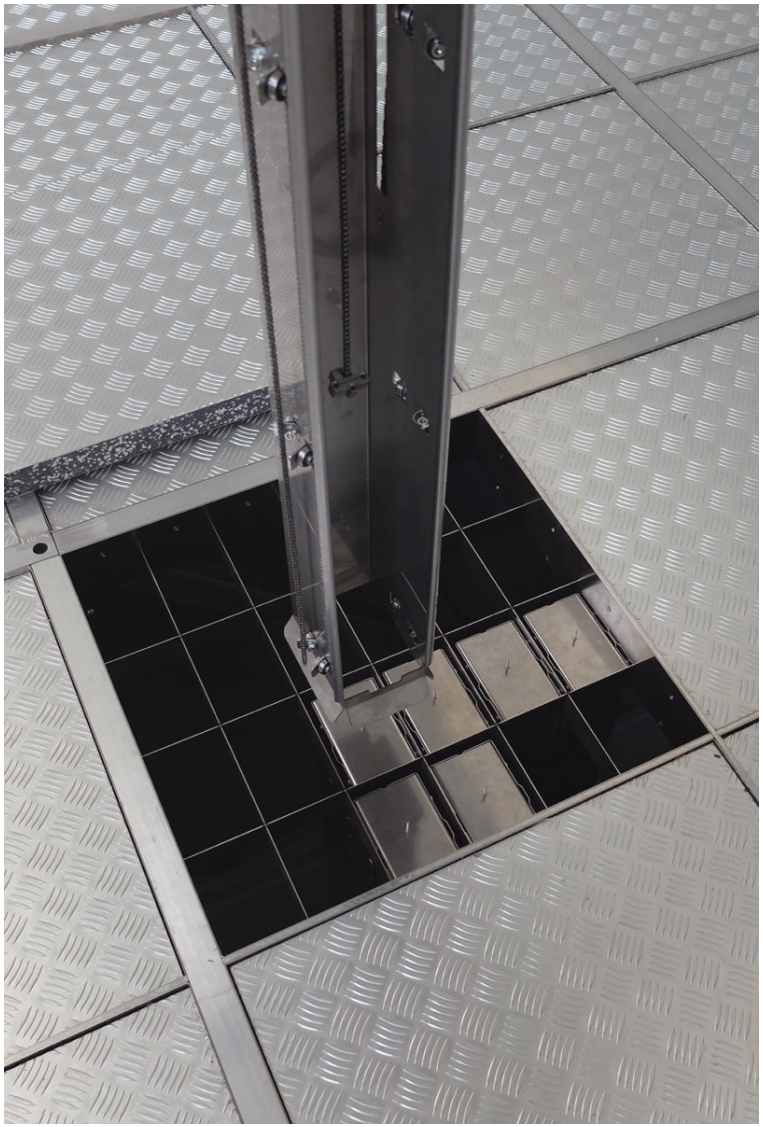
- reorganization of the samples
- collection of follow up samples after 5 years
- picking of the samples for scientific purposes

Biorepository: -80 °C

LiCONiC INSTRUMENTS

Biorepository: -80 °C

LiCONiC INSTRUMENTS



Major principles to reach high sample quality

- local processing of biomaterials
- adherence to stringent SOPs in all study centres (fast separation of cells from plasma/serum, …)
- automation of almost all steps in preparation (pipetting robot at each study centre), storage and retrieval of stored materials
- storage in an automated biorepository
- backup storage at local centres
- many small aliquots (avoidance of freeze thaw cycles)
- storage of most blood and urine samples in gas phase of liquid nitrogen

University and Hansestadt Greifswald - Wieck