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Quality of Biomarkers and Testing Cycle – Biomarkers Meet Biobanks

Webinar, July 6th, 2021

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Biobank Graz (Medical University)

Biobanks – The Basics

Definition

“biorepository that accepts, processes, stores and distributes biospecimens and associated data for use in research and clinical care”

Goal/Importance

Collect, store and disseminate biospecimens and related data

Maintenance standards in different processes: collection, processing, storage, tracking and shipment of biospecimens are key to the outcome of a multitude of studies

Yvonne G. De Souza and John S. Greenspan. 2013. Biobanking Past, Present and Future: Responsibilities and Benefits. AIDS. 28; 27(3): 303–312.

Types of Biological Samples – Tissue Sampling

Biospecimens – Collection Method

- Invasive: blood, tissue, etc.
- Less-invasive: dried blood spots
- Non-invasive: urine, saliva, etc.

Biobank Graz – Sample Material

Paraffin material

Paraffin block
Original paraffin section
Paraffin section
Paraffin shavings
Tissue microarray

Cryopreserved tissue

Cryo tissue
Cryo section

Body fluids and other samples

EDTA whole blood	Cumulus cells	Liquor
Serum	Follicular fluid	Liquor cells
Buffy coat (= EDTA buffy coat)	Fertillized egg supernatant	PAXgene® Blood RNA
Plasma: - EDTA plasma - Na citrate plasma - LI-HEP plasma	Supernatant control	Saliva Synovial fluid Stool sample
Urine: - Spontaneous - 24-hour urine	Seminal plasma	Other:

Blood samples IVF samples other

Urine

Ellervik C, Vaught J. Preanalytical variables affecting the integrity of human biospecimens in biobanking. Clin Chem. 2015 Jul;61(7):914-34. doi: 10.1373/clinchem.2014.228783. Epub 2015 May 15. PMID: 25979952.

Quality

ISO 9000:2000 Definition

- "The totality of features and characteristics of a product or service that bears on its ability to satisfy stated or implied needs".
- Briefly, one can say that a product has good quality when it "complies with the requirements specified by the client".
- QMS (Quality management system) – e.g. ISO
  …to ensure quality and to measure quality

International Organizational for Standardization. 1992. ISO 9000:2000 international standards for quality management. Genève, Switzerland: International Organization for Standardization.

Quality Management (QM)

QM Phases

- Four main components of QM:

  I. Quality planning

  II. Quality assurance (QA)

  III. Quality control (QC)

  IV. Quality improvement

Figure was taken and adapted from: http://www.adageinnovations.com/quality-assurance/

Quality Management (QM)

Difference between QA and QC

Quality Assurance (QA):

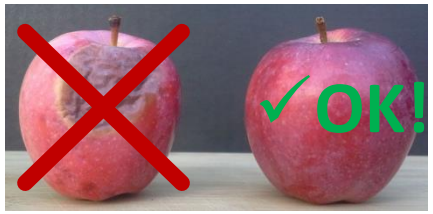
PROCESS- focused

- Ensuring that the correct steps are done in the correct way.
- Monitoring and verifying that all processes are followed and are effective.
- Then (hopefully) the results will turn out as expected.

Quality Control (QC):

PRODUCT- focused

- Checking of the actual results to ensure that things are as expected.



Quality Management (QM)

QA and QC in Biobanking

Quality Assurance (QA):

PROCESS- focused concept

- Assure that sample collection, processing of samples and storage processes are optimized to minimize defects/problems.

  ⇒ Sample handling according to SOPs!

  ⇒ Standardization! E.g. ISO, CEN/TS

  ⇒ Documentation!

Quality Control (QC):

PRODUCT- focused concept

- Testing biospecimens for their quality using standardized and validated sample analysis and quality control methods.

  ⇒ Different sample testing methods for different biomolecules / biomarkers

Quality of Biomarkers

Biomarker definition

- "A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention".

- Types of Biomarkers:
  - Diagnostics
  - Monitoring
  - Pharmacodynamic / response
  - Predictive
  - Prognostic

Califf R. M. (2018). Biomarker definitions and their applications. Experimental biology and medicine (Maywood, N.J.), 243(3), 213–221. https://doi.org/10.1177/1535370217750088

Quality of Biomarkers

Testing Cycle

- Series of activities, starting with the clinical question in the clinician´s mind, leading to test selection, sample collection, transport to the laboratory, analysis, reporting back to the clinician, and final interpretation and decision making by the clinician

- These activities have been traditionally separated into 3 phases: pre-analytical, analytical and post-analytical

- Some authors have introduced the “pre-pre-” and “post-post-” analytical phases (these phases are more error-prone)

Hawkins R. Managing the pre- and post-analytical phases of the total testing process. Ann Lab Med. 2012;32(1):5-16. doi:10.3343/alm.2012.32.1.5

Quality of Biomarkers

Preanalytical Phase

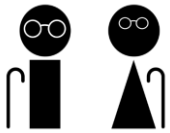
- Mostly meets Biobanks
- All processes that occur until the analysis of the biospecimen
- All factors in handling that affects integrity of the samples, and later the results of the analysis
- Most errors (62%) in clinical laboratories are due to preanalytical factors
- The quality of a study will mostly depend on the pre-analytical factors (integrity of samples)

Szecsi PB, Odum L. Error tracking in a clinical biochemistry laboratory. Clin Chem Lab Med 2009;47:1253–7
Ellervik C, Vaught J. Preanalytical variables affecting the integrity of human biospecimens in biobanking. Clin Chem. 2015 Jul;61(7):914-34. doi: 10.1373/clinchem.2014.228783. Epub 2015 May 15. PMID: 25979952.
Carraro P, Zago T, Plebani M. Exploring the initial steps of the testing process: frequency and nature of prepreanalytic errors. Clin Chem 2012;58:638 – 42

Quality of Biomarkers

1. Pre-analytical Phase

1.1. Biological/environmental Factors



Gender/Age

Time of Day/ season

Weight

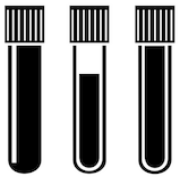
Alcohol/ coffee

Smoking

Medicine

Sunlight

1.2. Technical Factors



Collection → Processing → Transportation → Storage

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Quality of Biomarkers – Pre-analytical Phase

1.1. Biological/environmental Factors

- **Gender** – 80% differences
  - ✓ Specific hormones
  - ✓ Geographic and ethnical differences
  - Black vs Caucasian population → lower leukocyte counts, higher vit. B12, lower granulocyte and monocyte concentrations

- **Age** – different effect according to the biomarker
  - Decreased: Albumin, Ca, creatinine clearance, pO2
  - Increased: cholesterol, sedimentation rate, ferritin, Glu

- **Body Weight** – several biomarkers increase with body weight
  - Increased: Cholesterol, Tryglicerides, uric acid, cortisol, insulin

- **Alcohol** – different effect according to the biomarker
  - ✓ Distinction between acute and chronic consumption
  - Increased: liver enzymes (Gamma-GT, AST/ALT)
  - Decreased: Folic acid, Vit. B6

(2012) *Handhabungs-empfehlungen für die Präanalytik*. Meißner, D. https://shop.gbo.com/de/austria/products/preanalytics/
(2018) Tipps and tricks in der Präanalytik. Lichtinghagen, R. https://www.sarstedt.com/en/home

Quality of Biomarkers – Pre-analytical Phase

1.1. Biological/environmental Factors

- **Coffee** – different effect according to the biomarker
  - Increased: cortisol (>40% with 2 cups), adrenaline, noradrenaline

- **Smoking** – different effect according to the biomarker
  - ✓ Distinction between "spontaneous" and chronic consumption
  - ✓ Even consumption of 1 cigarette → significant changes in serum (< 1h)
  - Differences >10% smokers vs non-smokers: angiotensin, prolactin, ß-carotenoids, fibrinogen, copper, cadmium, monocytes, lymphocytes…

- **Medicine** – each drugs causing its own effect
  - Penicillin & Ibuprofen: increase K and thromboplastin in plasma

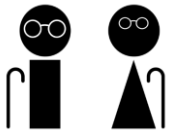
- **Sunlight** – different effect according to the biomarker, daily rhythm and season
  - Daily rhythm: some biomarkers max. morning (ACTH), others noon (Fe, K, eosinophils) or evening (creatinine, urea, TSH, acid phosphates)
  - Seasonal: vit. D higher in summer than winter

(2012) *Handhabungs-empfehlungen für die Präanalytik*. Meißner, D. https://shop.gbo.com/de/austria/products/preanalytics/
(2018) Tipps and tricks in der Präanalytik. Lichtinghagen, R. https://www.sarstedt.com/en/home

Quality of Biomarkers

1. Preanalytical Phase

1.1. Biological/environmental Factors



Gender/Age

Time of Day/ season

Weight

Alcohol/ coffee

Smoking

Medicine

Sunlight

1.2. Technical Factors

Collection → Processing → Transportation → Storage

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Quality of Biomarkers – Pre-analytical Phase

1.2. Technical Factors

Collection

- Obtaining tissue or fluids which represents the kind and quality of the whole sample for clinical (diagnosis, treatment and recovery) and research purposes
- Biopsies, blood draws or donations, saliva, urine, needle punctures, etc. Blades, scalpels, scissors, lancet, tubes with additives, needles, plastic containers
- Forgotten, incorrect, duplicate, cold and warm ischemia, contamination of specimen, insufficient sample volume, diluted samples, short-term storage
- Example: first blood draw order is the most representative (blood composition depends on blood draw); using a too-thin needle might produce hemolysis

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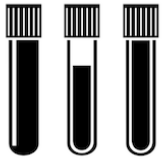
Quality of Biomarkers – Pre-analytical Phase

1.2. Technical Factors

Processing



- Process of keeping the biospecimens free from damage or decay over the time
- Automated systems (bar-code reading, decapping, fractioning, aliquoting), T°-controlled centrifuges, labels (unique, adhesive, cryostable, etc.), material (tubes, swabs, etc.)
- Processing duration, aliquot volume, rough handling and pipetting, mislabelling (unlabelling), wrong ID pairing of primary and secondary tubes.
- Example: processing time reduction increases DNA yield (blood); aliquot volume help reducing freeze-thaw cycles; smooth handling avoids analytes degradation

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Quality of Biomarkers – Pre-analytical Phase

1.2. Technical Factors

Transportation

- Act, process, or activity of taking biospecimens from one place to another either by foot or in a vehicle trying to limit the degradation of the sample.
- Transportable coolers, refrigerators or freezers, special packing, dry ice
- Environmental exposures (season, distance, duration, method of transportation), receiver not on duty, wrong packing-labelling, sent to wrong laboratory
- Example: wrong packing or labelling might cause delays (sample integrity); tubes transported vertically/ smooth handling-transport (hemolysis)

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Quality of Biomarkers – Pre-analytical Phase

1.2. Technical Factors

Storage

- Process of long-term preservation for bioespecimens; ideally they remain equivalent to freshly-collected specimens
- Fridges, freezers, cabinets and drawers, tanks and LN_2
- Time from processing to storage, storage duration, T°, and facility, environmental factors (humidity, moisture, dehydration, evaporation, etc.), no labelling/destroyed labelling, microbiological contamination, missing aliquots, freeze-thaw cycles
- Example: thaw and freezing cycles might affect stability of analytes; high humidity might lead to microbiological contamination

Ellervik C, Vaught J. Preanalytical variables affecting the integrity of human biospecimens in biobanking. Clin Chem. 2015 Jul;61(7):914-34. doi: 10.1373/clinchem.2014.228783. Epub 2015 May 15. PMID: 25979952.

Quality of Biomarkers – Pre-analytical Phase

Analytical Phase

- (Normally) out of control from Biobanks
- Occurs during the actual testing of the specimen
- All methods for performing tests that might impact the results obtained
- Best performing phase (15% of errors) in clinical laboratories
- Differences in the methods

Szecsi PB, Odum L. Error tracking in a clinical biochemistry laboratory. Clin Chem Lab Med 2009;47:1253–7
Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem. 2007 Jul;53(7):1338-42. doi: 10.1373/clinchem.2007.088344.
Sikaris K. Performance criteria of the post-analytical phase. Clin Chem Lab Med. 2015 May;53(6):949-58. doi: 10.1515/cclm-2015-0016. PMID: 25894643.

Quality of Biomarkers – Pre-analytical Phase

Analytical Phase – Examples interfering in Quality

- Equipment malfunction
- Reference interval incorrect → statistical confidence limits for the typical spread of results that usually comes from a healthy reference population
- Errors in calibration → (pipetting) errors dissolving the calibrator (wrong amount), improper units, calculation errors
- Sample mix-ups
- Interference (endogenous or exogenous) → any effect of a substance that is not the analyte to be determined
- Undetected failure in quality control (matrix effect) → use sample that mimics the patient sample

Sonntag O. Analytical interferences and analytical quality. Clin Chim Acta. 2009 Jun;404(1):37-40. doi: 10.1016/j.cca.2009.03.031. Epub 2009 Mar 21. PMID: 19306857.
Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem. 2007 Jul;53(7):1338-42. doi: 10.1373/clinchem.2007.088344.

Quality of Biomarkers

Post-analytical Phase

- Out of control from Biobanks
- Occurs after the actual testing of the biospecimen
- Processes following the examination of the samples (review of the results)
- This phase is also prone to errors (23%) in clinical laboratories

Szecsi PB, Odum L. Error tracking in a clinical biochemistry laboratory. Clin Chem Lab Med 2009;47:1253–7
Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem. 2007 Jul;53(7):1338-42. doi: 10.1373/clinchem.2007.088344.
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Quality of Biomarkers

Postanalytical Phase – Examples interfering in Quality

- **Incorrect interpretation (37%)**
- **Failure in reporting/addressing the report**
- **Excessive turn-around-time**
- **Improper data entry and manual transcription error**
- **Delayed/missed reaction to laboratory reporting**
- Erroneous validation of analytical data
- Failure/delay in reporting critical values
- Inappropriate/inadequate follow-up plan

Quality of Biomarkers

Error Types Affecting Quality

- Cognitive errors/errors of intention (mistakes) → due to poor knowledge or judgement
- Non-cognitive errors/errors of action (slips/lapses) → due to interruptions in a process during routine analysis involving automated analyzers
- Most of the errors are non-cognitive → easily avoided

Amitava Dasgupta, Amer Wahed, Chapter 3 - Pre-Analytical Variables, Clinical Chemistry, Immunology and Laboratory Quality Control, Elsevier, 2014, Pages 35-45, ISBN 9780124078215,

Quality of Biomarkers

What can we do to avoid errors in the pre-, post- & analytical phases?

- Standardization/Check-lists/Use of SOPs → pre-analytical and analytical phases
- Certification/Accreditation (audits) → pre-analytical and analytical phases
- Use of proper controls → analytical phase
- Personnel training/Competency evaluation → all phases
- Improving the work environment → all phases
- Quality improvement programs → all phases

Amitava Dasgupta, Amer Wahed, Chapter 3 - Pre-Analytical Variables, Clinical Chemistry, Immunology and Laboratory Quality Control, Elsevier, 2014, Pages 35-45, ISBN 9780124078215,

Quality of Biomarkers

Importance of Tackling these Problems & Errors

- High quality samples & reliable biomarkers
- Discovery of new biomarkers
- Increased reproducibility
- Proper diagnosis & treatment of patients
- Potential adverse outcomes for patients → qualitative results with clear links to therapy or management decisions

Biomarkers Meet Biobanks

Standardization by Biobanks

- CEN / TS (European standards for the pre-analytical processing of human biological samples)
- ISO

ISO 20387:2018
Biotechnology -- Biobanking -- General requirements for biobanking

- New EU regulations (MDR, IVDR)
  - Regulation (EU) 2017/745 on medical devices (MDR)
  - Regulation (EU) 2017/746 on in-vitro diagnostic medical devices (IVDR)

Biomarkers Meet Biobanks

What can a Biobank offer?

- Quality control and assurance of paraffin and fluid biospecimens, nucleic acids, cells, tissues, etc. → **high sample quality**
- **Standardized** processing and procedures
  - ✓ If not possible → **Documentation**
- **Sample analysis services** for QC and characterization (DNA quantification, sequencing, miRNA profiling, etc.
- **Optimization** and **validation** of sample testing methods
- **Sample characterization**
- **Customized assays**

Summary – Quality of Biomarkers

Quality

- Definition, importance, main components of QM, difference between QA and QC, QA and QC in a BioBank and relevant regulations (CEN/TS, ISO, MDR)

Testing Cycle of Biomarkers

- Pre-analytical phase (biological/environmental and technical factors; examples), analytical phase (examples) and post-analytical phase (examples)

Errors

- Error types, avoiding errors, importance of avoiding errors

Biobanks

- Definition, sample types, standardization, sample analysis and QC, what a BB can offer

Take-home Message – Quality of Biomarkers

Biomarkers

- Their **quality** might be **affected by many** different **factors** through all the testing cycle **and prone to human/technical errors**

Biobanks

- Are meant to guarantee high standards for sample processing to **provide** you with **high quality biomarkers**. When not possible, documentation
- Provide you with a variety of **valuable services**

Thank You !

Biobank Graz Team

Codex Project Team

Medical University of Graz | Biobank

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