Master Thesis

The effect of cold storage condition on short-term stability of biomarkers in human serum and plasma

Submitted by

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Sworn Declaration

I declare on my honor that I have written this dissertation independently and without assistance, that no sources other than those cited were used and that the sources used verbatim or in substance have been marked as such.

Banjul, 14 July 2023 Demba Sanneh

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Abbreviations (Abbreviation and explanation)

ACL	Analytical change limit
AFP	Alpha-fetoprotein
ALB	Albumin
ALT	Alanine aminotransferase
AMH	Anti-Müllerian hormone
ANOVA	Analysis of variance
Apo	Apolipoprotein
APTT	Activated Partial Thromboplastin Clotting Time
AST	Aspartate aminotransferase
BAP	Bone alkaline phosphatase
BDNF	Brain Derived Neurotrophic Factor
BHT	Butylated hydroxytoluene
BNP	Brain natriuretic peptide
BUN	Blood urea nitrogen
C1r, C3, C4	Complement protein 1r, 3, 4
CA125	Cancer antigen 125
CA19-9	Cancer antigen 19-9
CD163	Cluster of differentiation 163
CEA	Carcinoembryonic antigen
CHB	Chronic hepatitis B
CK	Creatine kinase
CNTN1	Contactin-1
cTn	Cardiac troponin
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
Fbg	Fibrinogen
FIX:C	Factor IX:C
FSH	Follicle stimulating hormone
FT4	Free Thyroxine
FVIII:C	Factor VIII:C
GFAP	Glial fibrillary acidic protein

GGT	Gamma-glutamyl transferase
GLP-1	Glucagon-like peptide-1
HCy	Homocysteine
HDL	High-density lipoprotein
HDLC	High-density lipoprotein cholesterol
HMGB1	High mobility group box 1
HS-CRP	High-sensitivity C-reactive protein
IFN-g	Interferon-gamma
IGF-1	Insulin-like growth factor-1
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukins-6
IL-8	Interleukins-8
IL-18	Interleukin 18
INR	International Normalized Ratio
ISO EN 15189	International Organization for Standardization European Norm 15189
LD	Lactate dehydrogenase
LDLC	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
MCP-1	Monocyte chemoattractant protein-1
MIP-2	Macrophage Inflammatory Protein-2
NfL	Neurofilament-light
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NSE	Neuron-specific enolase
PCR	Polymerase chain reaction
PFC	Perfluorinated compounds
pH	potential of hydrogen" (or "power of hydrogen")
PRL	Prolactin level
ProGRP	Pro-gastrin-releasing peptide
PSA	Prostate-specific antigen
PT	Prothrombin time
pwMS	Patient with multiple sclerosis
PYY	Peptide YY
qPCR	Quantitative PCR

RBCs	Red blood cells
RCTs	Randomized Controlled Trials
RCV	Reference change value
RNA	Ribonucleic acid
ROM	Reactive Oxygen Metabolites
sIgE	Allergen specific immunoglobulin E
SPE	Serum protein electrophoresis
TBI	Traumatic brain injury
TGF-beta 1	Transforming growth factor-beta 1
TNF- α	Tumor necrosis factor- α
TNFR	Tumor necrosis factor receptor
TSH	Thyroid stimulating hormone
TT	Thrombin time
TTL	Total thiol levels
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor A

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Abstract: (English)

Biomarkers play a crucial role in biomedical research and clinical practice, providing valuable information on disease diagnosis, prognosis, treatment efficacy, and disease progression. Serum and plasma biomarkers, which can be easily accessed and non-invasively obtained, are of particular interest. However, the reliability and accuracy of biomarker measurements can be significantly affected by pre-analytical variables, including sample collection, handling, and storage. Cold storage is a common method employed to preserve serum and plasma biomarker stability, but the optimal storage conditions remain unclear. This literature review aims to critically evaluate the effect of cold storage conditions on the short- and long-term stability of biomarkers in human serum and plasma. Through a systematic review of relevant studies, the review explores the impact of storage temperature, duration, and stabilizing agents on biomarker stability. The findings highlight the variability in stability among different biomarkers and emphasize the need for standardized storage protocols to ensure reliable and accurate biomarker measurements. The review underscores the importance of temperature control, minimizing freeze-thaw cycles, and proper sample handling to maintain biomarker integrity. By providing a comprehensive evaluation of the existing literature, this review identifies gaps and limitations in current knowledge and provides insights into strategies for improving biomarker measurements in clinical practice. The findings have implications for optimizing storage conditions and enhancing the reliability and accuracy of biomarker measurements, ultimately improving patient outcomes.

Abstract: (German)

Biomarker spielen in der biomedizinischen Forschung und in der klinischen Praxis eine entscheidende Rolle, da sie wertvolle Informationen über Krankheitsdiagnose, Prognose, Wirksamkeit der Behandlung und Krankheitsverlauf liefern. Von besonderem Interesse sind Serum- und Plasmabiomarker, die leicht zugänglich sind und nicht invasiv gewonnen werden müssen. Die Zuverlässigkeit und Genauigkeit von Biomarker-Messungen kann jedoch durch präanalytische Variablen wie Probenentnahme, -behandlung und -lagerung erheblich beeinträchtigt werden. Die Kaltlagerung ist eine gängige Methode, um die Stabilität von Serum- und Plasma-Biomarkern zu erhalten, aber die optimalen Lagerungsbedingungen sind nach wie vor unklar. Ziel dieser Literaturübersicht ist es, die Auswirkungen von Kaltlagerungsbedingungen auf die Kurz- und Langzeitstabilität von Biomarkern in Humanserum und -plasma kritisch zu bewerten. Anhand einer systematischen Durchsicht relevanter Studien werden die Auswirkungen von Lagertemperatur, Dauer und Stabilisierungsmitteln auf die Stabilität von Biomarkern untersucht. Die Ergebnisse verdeutlichen die Variabilität der Stabilität verschiedener Biomarker und unterstreichen die Notwendigkeit standardisierter Lagerungsprotokolle, um zuverlässige und genaue Biomarkermessungen zu gewährleisten. Der Review unterstreicht die Bedeutung der Temperaturkontrolle, der Minimierung von Gefrier-Auftau-Zyklen und der ordnungsgemäßen Handhabung der Proben, um die Integrität der Biomarker zu erhalten. Durch eine umfassende Auswertung der vorhandenen Literatur zeigt dieser Review Lücken und Beschränkungen im aktuellen Wissensstand auf und gibt Einblicke in Strategien zur Verbesserung von Biomarker-Messungen in der klinischen Praxis. Die Ergebnisse haben Auswirkungen auf die Optimierung der Lagerungsbedingungen und die Verbesserung der Zuverlässigkeit und Genauigkeit von Biomarker-Messungen, was letztendlich zu einer Verbesserung der Patient*innenergebnisse führt.

1. Introduction

Biomarkers are measurable characteristics that indicate normal or abnormal biological processes or responses to therapeutic interventions. These could include proteins, nucleic acids, metabolites, or other molecules that are detectable in biological samples. They can be used for diagnosis, prognostication, monitoring of disease progression, and assessment of treatment efficacy (Xu et al., 2020). Serum and plasma biomarkers, which are found in the liquid component of blood, are of particular interest due to their non-invasive and easily accessible nature, as well as their ability to provide information on systemic physiological and pathological conditions (Diamandis and Christopoulos, 2010).

Biobanks are specialized repositories that store biological samples, such as blood, serum, plasma, tissues, and DNA, for research purposes. These samples are often collected from large cohorts of individuals and are accompanied by detailed clinical and demographic information. Biobanks play a crucial role in biomedical research, as they provide researchers with access to a vast array of well-characterized samples for various studies, including biomarker research. They facilitate the discovery and validation of biomarkers by enabling large-scale studies and longitudinal analyses (Zika et al., 2018).

Pre-analytical variables encompass all the steps involved in sample collection, processing, and storage before laboratory analysis. These variables can significantly impact the quality and reliability of biomarker measurements. Some important pre-analytical variables include:

a. Sample collection: The choice of collection tubes, anticoagulants, and collection procedures can influence biomarker stability. For example, certain biomarkers may be affected by the type of anticoagulant used, as some anticoagulants can interfere with specific assays or alter biomarker levels (Simundic et al., 2018).

b. Sample handling: Proper handling of samples is crucial to maintain biomarker stability. Factors such as time delay between sample collection and processing, temperature fluctuations, and exposure to light can all impact biomarker integrity. Immediate centrifugation, aliquoting, and storage under appropriate conditions are essential to minimize potential degradation or alteration of biomarkers (Lippi et al., 2018).

c. Sample storage: Cold storage is commonly employed for preserving biomarker stability. However, the specific conditions of storage, such as temperature and duration, need to be optimized for each biomarker. Some biomarkers may require ultra-low temperatures (e.g., -

80°C) for long-term storage, while others may be adequately preserved at higher temperatures (e.g., -20°C) for shorter periods (Shah et al., 2012).

d. Stabilizing agents: The addition of stabilizing agents, such as protease inhibitors, anticoagulants, or preservatives, can help mitigate biomarker degradation during storage. These agents can inhibit enzymatic activity, prevent clotting, or minimize chemical reactions that may affect biomarker stability.

By understanding and controlling pre-analytical variables, researchers can ensure the integrity and reliability of biomarker measurements, leading to more accurate and meaningful results in both research and clinical settings.

However, the clinical utility of serum and plasma biomarkers is heavily dependent on their stability over time. The reliability and accuracy of biomarker measurements can be compromised by pre-analytical factors, including sample collection, handling, and storage. Cold storage is a widely accepted method for preserving serum biomarker stability prior to analysis, but the optimal conditions for cold storage remain unclear. Factors such as the storage temperature, duration of storage, and the use of stabilizing agents can have a significant impact on biomarker stability (Shah et al., 2012).

1.1 Background

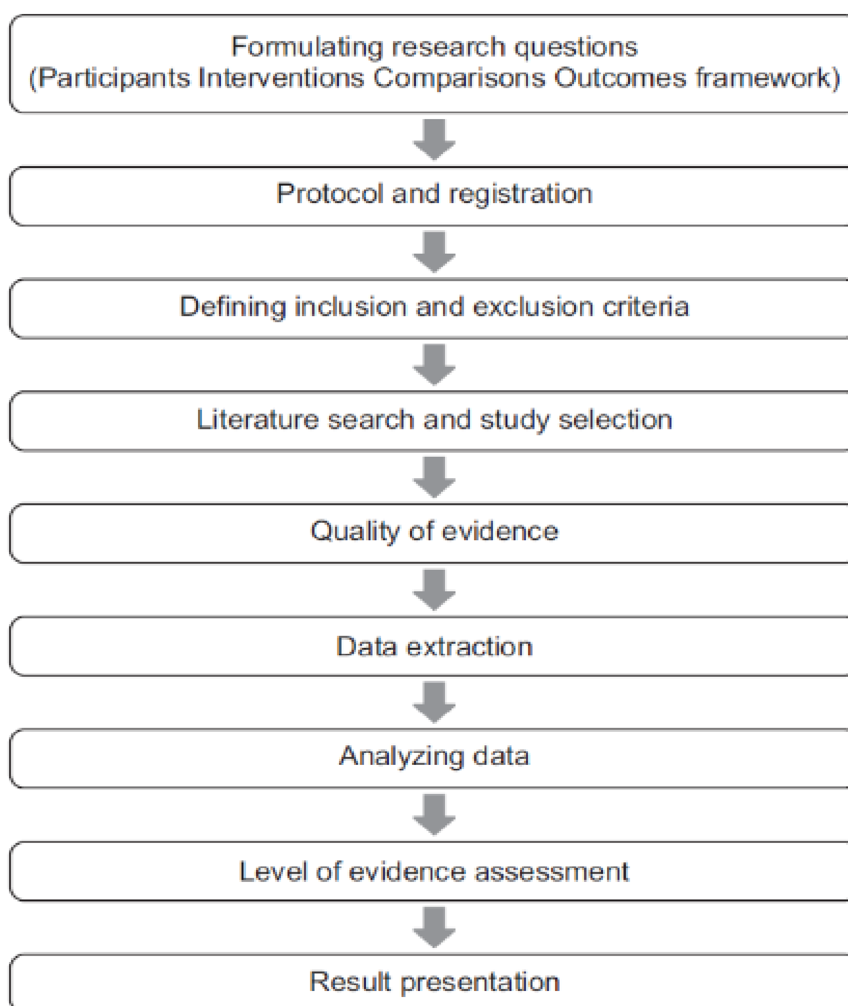
Research from various studies has indicated that serum biomarkers are widely used in clinical practice for the diagnosis, prognosis, and monitoring of disease progression and treatment response (Chen et al. 2019, Xu et al. 2020). Shah et al. (2012) indicated that the clinical utility of biomarkers can be compromised by pre-analytical factors such as sample collection, handling, and storage. The researchers went further to demonstrate that the stability of serum biomarkers can be influenced by a range of factors, including temperature, duration of storage, and the use of stabilizing agents.

Cold storage is widely used for preserving the stability of serum biomarkers prior to analysis. However, the optimal conditions for cold storage remain unclear, with studies reporting variable results regarding the effect of storage temperature, duration, and stabilizing agents on biomarker stability (Nishikawa et al., 2020). The lack of consensus on optimal storage conditions for serum biomarkers can lead to variability and inconsistency in biomarker

measurements, which can compromise their reliability and accuracy in clinical decision-making (Sims et al., 2015).

Therefore, there is a need for a comprehensive systematic review of the existing literature on the effect of cold storage conditions on the short-term stability of biomarkers in human serum and plasma. This review will evaluate the current knowledge on the optimal storage conditions for serum and plasma biomarkers, based on the available evidence on the effect of storage temperature, duration, and stabilizing agents on biomarker stability. By providing a critical evaluation of the existing literature, this review will help to identify gaps and limitations in the current knowledge, as well as provide insights into potential strategies for improving the reliability and accuracy of serum and plasma biomarker measurements in clinical practice.

Fig. 1. Flowchart illustrating a systemic review.



Ref: EunJin A. and Hyun K. 2018.

1.2 Aims and Scope

This literature review aims to critically evaluate the current knowledge on the effect of cold storage conditions on the short- and long-term stability of biomarkers in human serum and plasma. Specifically, the review will answer the following research questions:

1. What are the optimal cold storage conditions for preserving the stability of serum and plasma biomarkers in human samples?
2. How do different storage temperatures, durations, and stabilizing agents impact the stability of serum and plasma biomarkers?

2. Materials and Methods

2.1 Cochrane Risk of Bias tool

When conducting a systematic literature review, it is important to assess the quality of the studies included to ensure that the results are reliable and trustworthy. One commonly used tool for assessing the risk of bias in studies is the Cochrane Risk of Bias tool. This tool was initially developed for use in randomized controlled trials (RCTs) but has since been adapted for use with other study designs as well (Higgins et al., 2011).

The Cochrane Risk of Bias tool assesses various domains of bias, such as selection bias, performance bias, detection bias, attrition bias, reporting bias, and other potential sources of bias that may affect the validity of study results. The tool provides a framework for evaluating each of these domains based on specific criteria, and the overall risk of bias is then classified as low, unclear, or high (Higgins et al., 2011).

The quality assessment using the Cochrane Risk of Bias tool is a crucial step in determining the strength of the evidence presented in the studies. It helps to identify any potential biases that may have influenced the results and to determine whether the findings are likely to be accurate and reliable. By using a standardized tool like the Cochrane Risk of Bias tool, the quality assessment can be more objective and transparent, and the results can be compared across studies more easily.

Overall, the use of the Cochrane Risk of Bias tool is recommended for systematic reviews and meta-analyses as a means of assessing the quality of the included studies and determining the level of confidence in the findings (Higgins et al., 2011).

2.2 Literature review methodology

Methodology refers to the overall approach and framework that guides a research study, including the methods, procedures, and tools used to collect and analyze data. It is an essential aspect of any research study and helps ensure that the study is conducted in a systematic, rigorous, and reliable manner.

The literature review was conducted using a systematic approach to ensure that all relevant studies were identified and included in the analysis. The following steps were taken:

Research questions: The research questions were formulated to guide the search process and ensure that the review focused on the specific topic of interest. The research questions were:

1. *What are the optimal cold storage conditions for preserving the stability of serum and plasma biomarkers in human samples?*
2. *How do different storage temperatures, durations, and stabilizing agents impact the stability of serum and plasma biomarkers?*

2.2.1 Search strategy

To address the above research questions, a systematic review of the literature was conducted. The review included studies that investigated the effect of cold storage conditions on the stability of serum and plasma biomarkers in human samples. A comprehensive search strategy was developed to identify all relevant studies. The search was conducted using the PubMed, Web of Science, and Scopus databases, and the following search terms were used: "cold storage", "biomarkers", "human serum", "human plasma" and "stability." The search was limited to studies published in the last ten years (2012-2023) and written in English.

Inclusion and exclusion criteria: Inclusion and exclusion criteria were established to ensure that the studies selected for analysis met the specific requirements of the research questions.

The **inclusion criteria** were:

- *Studies that investigated the effect of cold storage on the stability of serum and plasma biomarkers in human samples.*
- *Studies published in the last ten years (2012-2023) and written in English.*

The **exclusion criteria** were:

- *Studies that did not investigate the effect of cold storage on the stability of serum biomarkers in human samples.*
- *Studies published outside the last ten years or not written in English.*
- *Reviews on the topic.*

2.2.2 Screening and selection

After the initial search, 34 articles were selected based on inclusion criteria, which included studies that investigated the effect of cold storage on the stability of serum and plasma biomarkers in human samples. The selected articles were then screened for relevant information, and the data were extracted and summarized.

The data were analyzed based on the type of biomarkers, the storage temperature, the duration of storage, and the use of stabilizing agents. The results were synthesized to provide an overview of the effect of cold storage conditions for one year on the stability of biomarkers in human serum and plasma.

The findings of this literature review will have implications for the optimal storage conditions for serum biomarkers and will help to ensure the reliability and accuracy of their measurements, ultimately leading to improved patient outcomes.

2.3 Data analysis and synthesis

The data were analyzed to identify trends and patterns in the results, and to synthesize the findings to provide an overview of the effect of cold storage conditions on the stability of biomarkers in human serum and plasma. The analysis process included the following.

2.3.1 Data extraction

Once the relevant studies have been identified and selected, data extraction was done to extract the relevant information from each study. This includes details about the study design, population characteristics, intervention or exposure, outcome measures, and results.

2.3.2 Quality assessment

The quality of the studies included in the review was assessed using the Cochrane Risk of Bias tool. This tool assesses the risk of bias in randomized controlled trials but can also be adapted for use with other study designs. The quality assessment was used to determine the strength of the evidence presented in the studies and to identify any potential biases that may have influenced the results.

The quality assessment involved a systematic evaluation of various aspects of each study to identify any potential biases that may have influenced the results. These aspects typically include:

(a) ***Blinding of participants and personnel***: This criterion assesses whether participants and study personnel were blinded to the intervention or exposure being studied, which helps minimize bias in the assessment of outcomes.

(b) ***Blinding of outcome assessment***: It evaluates whether outcome assessors were blinded to the intervention or exposure being studied to minimize bias in outcome measurements.

(c) ***Incomplete outcome data:*** This criterion examines whether there were missing data or high dropout rates and whether they were adequately addressed in the analysis.

(d) ***Selective reporting***: It assesses whether all outcomes specified in the study protocol were reported and whether there is evidence of selective reporting of favourable outcomes.

(e) ***Other sources of bias***: This criterion evaluates any other potential sources of bias that could affect the study results but may not be covered by the previous criteria.

Each criterion is assessed as having *a low risk, high risk, or unclear risk of bias*. A judgment is made based on the information provided in the study report, and any discrepancies or uncertainties are resolved through discussion.

By evaluating the quality of the included studies, the reliability and validity of the evidence presented can be ascertained. This information is crucial for drawing conclusions and making recommendations based on the synthesized findings.

2.3.3 Synthesis

The results of the studies are synthesized and analyzed to identify trends, patterns, and relationships between the different variables. This was done using a qualitative method, '*thematic analysis or content analysis'*. The goal of the synthesis is to integrate the findings from multiple studies into a coherent and comprehensive summary of the evidence.

Overall, the literature review was conducted using a rigorous and systematic approach to ensure that all relevant studies were identified and included in the analysis. The findings of the review provide important insights into the optimal storage conditions for serum and plasma biomarkers and can help to improve the reliability and accuracy of biomarker measurements in clinical practice.

3. Results

3.1 Examples of studies presenting data on effects of storage conditions

3.1.1 Effects of short-term storage conditions on biomarker stability

There are many studies that have investigated the effects of short-term storage conditions on the stability of biomarkers in serum. Here are some examples:

According to a study by Fokkema et al. (2012), the stability of biomarkers in serum can vary widely depending on the specific biomarker and the storage conditions used. Some biomarkers, such as cytokines and hormones, can be quite unstable in serum over two years or more, even at ultra-low temperatures. The authors suggest that careful consideration of storage conditions is essential to maintain the integrity of biomarker measurements.

In a study by Shuford et al. (2014), the researchers evaluated the stability of 10 clinical biomarkers in human serum stored at different temperatures (-80°C, -20°C, 4°C, and room temperature) for up to 24 hours. The biomarkers evaluated included alpha-fetoprotein (AFP), beta-2-microglobulin (B2M), carbohydrate antigen 125 (CA125), carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), squamous cell carcinoma antigen (SCC), cancer antigen 15-3 (CA15-3), cancer antigen 19-9 (CA19-9), neuron-specific enolase (NSE), and human epididymis protein 4 (HE4). The stability of each biomarker was assessed using enzyme-linked immunosorbent assay (ELISA) or chemiluminescent immunoassay (CLIA) kits. The researchers found that most of the biomarkers were stable at all temperatures tested, although some showed minor changes at room temperature. The study concluded that short-term storage of human serum at room temperature for up to 24 hours does not significantly affect the stability of most clinical biomarkers.

Similar research study by Li et al. (2019) investigated the effect of storage temperature (4°C vs. -80°C) and time (0, 2, 4, 8, and 24 hours) on the stability of 16 different biomarkers in human serum. The biomarkers evaluated included apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), albumin (ALB), creatine kinase (CK), creatine kinase-MB (CK-MB), cardiac troponin I (cTnI), brain natriuretic peptide (BNP), carbohydrate antigen 125 (CA125), carbohydrate antigen 153 (CA153), carbohydrate antigen 199 (CA199), cancer antigen 724 (CA724), cancer antigen 125 (CA125), neuron-specific enolase (NSE), pro-gastrin-releasing peptide (ProGRP), prostate-specific antigen (PSA), and tumor necrosis factor receptor 1 (TNFR1). The stability of each biomarker was assessed using enzyme-linked immunosorbent assay (ELISA) kits. The researchers found that most of the biomarkers were stable at both

temperatures for up to 24 hours, although some showed minor changes over time. The study concluded that serum samples can be stored at either 4°C or -80°C for up to 24 hours without significant impact on most of the 16 biomarkers evaluated.

Decramer et al. (2013) evaluated the stability of 29 different biomarkers in human serum stored at room temperature for up to 24 hours. The biomarkers evaluated included cytokines, growth factors, and chemokines. The stability of each biomarker was assessed using enzyme-linked immunosorbent assay (ELISA) kits. The researchers found that most of the biomarkers were stable over the 24-hour time, although some showed significant degradation. The study concluded that caution should be taken when analyzing samples that have been stored at room temperature, as some biomarkers may show significant degradation over time. In a study by Lee et al. (2012), the researchers evaluated the stability of 11 different biomarkers in human serum stored at room temperature for up to 24 hours. They found that most of the biomarkers were stable over this time, although some showed significant degradation.

Research published by Dati et al. (2015) investigated the stability of 17 different biomarkers in human serum stored at two different temperatures (-20°C and 4°C) for up to 24 hours. The stability of each biomarker was assessed using the Bio-Plex cytokine assay. The researchers found that most of the biomarkers were stable at both temperatures for up to 24 hours, although some showed significant degradation over time. The study concluded that storage at 4°C is acceptable for short-term storage of serum samples, but longer-term storage at -20°C is recommended to ensure stability of the biomarkers.

A study published by Wang et al. (2015), the researchers investigated the effect of different storage temperatures (-80°C, -20°C, 4°C, and room temperature) and time points (0, 2, 4, 8, and 24 hours) on the stability of 18 different biomarkers in human serum. They used enzyme-linked immunosorbent assay (ELISA) to measure the concentrations of the biomarkers and analyzed the results using statistical methods to assess the effect of storage conditions on biomarker stability. The study found that most of the biomarkers were stable at all temperatures for up to 24 hours, although some showed minor changes over time.

In the study by Collins et al. (2016), the stability of biomarkers in serum stored at 4°C, room temperature, or 37°C for up to 48 hours was investigated. They used mass spectrometry-based proteomics to analyze the samples and measure the stability of the biomarkers. The study found that some biomarkers, such as C-reactive protein, were stable at all storage temperatures, while others, such as interleukin-6, showed significant degradation at room temperature and 37°C.

The authors concluded that storage at 4°C is generally suitable for short-term storage of most biomarkers in serum. Additionally, they demonstrated the utility of a two-phase extraction method for preserving biomarkers during storage and transportation.

3.1.2 Effects of freeze-thaw cycles on biomarker stability

Freeze-thaw cycles can have significant effects on biomarker stability, leading to degradation or alteration of the biomolecules of interest. Several studies have investigated the impact of freeze-thaw cycles on different types of biomarkers, such as proteins, nucleic acids, and metabolites.

One study by Liu et al. (2018) evaluated the effects of freeze-thaw cycles on the stability of cytokines in human serum samples. The authors performed ELISA assays to measure the levels of cytokines before and after subjecting the samples to one to three freeze-thaw cycles. The results showed that the levels of some cytokines, such as IL-8 and TNF-α, were significantly reduced after multiple freeze-thaw cycles. The authors concluded that "*careful consideration should be given to the number of freeze-thaw cycles when analyzing cytokines in human serum samples*" (Liu et al., 2018).

Another widely used method to evaluate biomarker stability after freeze-thaw cycles involves measuring the changes in the biomarker's concentration or activity level before and after the freeze-thaw process. For example, a study by Rist et al. (2013) assessed the stability of 158 plasma metabolites after up to three freeze-thaw cycles using targeted mass spectrometry. The authors found that the stability of the metabolites varied widely, with some metabolites showing a high degree of stability, while others were more sensitive to freeze-thaw cycles.

Another approach to evaluate biomarker stability is to compare the results obtained from frozen samples to those obtained from freshly collected samples. For example, a study by Elvers et al. (2020) compared the levels of interleukin-6 (IL-6) in fresh and frozen serum samples from patients with sepsis. The authors found that the levels of IL-6 were highly stable in frozen samples, with no significant difference in IL-6 levels between fresh and frozen samples.

In a study by Wewer Albrechtsen et al. (2015), the authors investigated the effects of freeze-thaw cycles on the stability of gut hormones in plasma samples. They subjected the samples to one to four freeze-thaw cycles and measured the levels of several gut hormones using radioimmunoassays. The results showed that the levels of some hormones, such as GLP-1 and

PYY, were significantly decreased after multiple freeze-thaw cycles. The authors concluded that "*the number of freeze-thaw cycles should be minimized when analyzing gut hormones in plasma*" (Wewer Albrechtsen et al., 2015).

A study by Ezzati et al. (2013) evaluated the effects of freeze-thaw cycles on the stability of serum metabolites. The authors analyzed serum samples using targeted mass spectrometry and found that the levels of several metabolites were significantly altered after multiple freeze-thaw cycles. The authors suggested that "*careful sample handling is essential to ensure the reliability of metabolic profiling studies*" (Ezzati et al., 2013).

In a study by Veenstra et al. (2018), the authors investigated the effects of freeze-thaw cycles on the stability of microRNA biomarkers in serum samples. They measured the levels of several microRNAs using qPCR and found that the levels of some microRNAs were significantly decreased after multiple freeze-thaw cycles. The authors concluded that "*careful consideration of pre-analytical factors, such as sample handling and storage, is essential for accurate and reproducible microRNA biomarker measurements*" (Veenstra et al., 2018).

Overall, these studies provide additional evidence that freeze-thaw cycles can have detrimental effects on biomarker stability and highlight the importance of minimizing the number of freeze-thaw cycles when analyzing biomarkers.

These studies provide further evidence that many biomarkers in serum are stable for at least several hours under a variety of storage conditions. However, as noted earlier, different biomarkers may have different stability profiles, and it is always important to consult the literature or perform your own stability testing if you are working with a particular biomarker.

3.1.3 Effects of stabilizing agents on biomarker stability in serum

Stabilizing agents are substances that are added to biological samples, to prevent degradation or alteration of the sample's components. These agents can help preserve the integrity of biological molecules, such as proteins, DNA, RNA, and metabolites, by inhibiting enzymatic or chemical reactions that can lead to degradation or modification. Stabilizing agents can also prevent microbial growth and protect samples from physical damage or environmental factors such as temperature and pH. Some common stabilizing agents used in biological sample collection and processing include EDTA (ethylenediaminetetraacetic acid), heparin, citrate, and protease inhibitors. The choice of stabilizing agent depends on the specific biological

molecules of interest and the downstream analytical methods used for detection and quantification.

In a study by Edmands et al. (2016), the researchers evaluated the effectiveness of different stabilizing agents (EDTA, citrate, heparin, and a combination of EDTA and a protease inhibitor cocktail) for the analysis of five different biomarkers in serum and plasma samples. They assessed biomarker stability over time (up to 24 hours) and under different storage conditions (room temperature, 4°C, and -80°C) using immunoassays. The researchers found that the choice of stabilizing agent had a significant impact on biomarker stability. The combination of EDTA and a protease inhibitor cocktail was the most effective stabilizing agent, preserving biomarker stability for up to 24 hours at room temperature and up to 72 hours at 4°C. EDTA alone was also effective, but only at 4°C. Heparin and citrate were not effective stabilizing agents for any of the biomarkers tested.

Research by Raghavendra et al. (2018), investigated the effect of two different stabilizing agents (EDTA and heparin) on the stability of cancer biomarkers (CA19-9, CEA, and AFP) in human plasma samples. They evaluated biomarker stability over time (up to 48 hours) and under different storage conditions (room temperature, 4°C, and -80°C) using immunoassays. The researchers found that both EDTA and heparin were effective stabilizing agents for the measurement of cancer biomarkers in plasma samples. EDTA was more effective than heparin at preserving biomarker stability over time, with all biomarkers remaining stable for up to 48 hours at room temperature and up to 72 hours at 4°C. Heparin was less effective, with some biomarkers showing decreased stability after 24 hours at room temperature.

Civitelli et al. (2013) also evaluated the effect of three different stabilizing agents (EDTA, heparin, and citrate) on the stability of potential epigenetic biomarkers (methylated DNA) in human serum samples. They assessed biomarker stability over time (up to 48 hours) and under different storage conditions (room temperature, 4°C, and -80°C) using quantitative PCR. The study found that all three stabilizing agents were effective at preserving the stability of methylated DNA in human serum samples. The researchers observed that EDTA was the most effective stabilizing agent, as it maintained the stability of methylated DNA for up to 48 hours at all storage temperatures. Heparin was also effective, but its stabilizing effect decreased after 24 hours at room temperature. Citrate was the least effective stabilizing agent, as it showed decreased stability of methylated DNA after 6 hours at room temperature.

3.2 Analysis of selected studies

3.2.1 List of studies included.

Based on the selection criteria as described in the methods section, 34 studies have been selected and analysed regarding temperature, marker panel, measurement and outcome. Table 1 lists the 34 studies included in this thesis. The studies were published between 2012 and 2023, and one to five per year were included in this thesis.

Table 1: Summary list of studies included.

S/N	Authors	Title	Journal	Year
1	Zimmerman, L. J., Li, M., Yarbrough, W. G., Slebos, R. J. and Liebler, D. C.	Global stability of plasma proteomes for mass spectrometry-based analyses	Mol Cell Proteomics	2012
2	Cuhadar, S., Koseoglu, M., Atay, A. and Dirican, A.	The effect of storage time and freeze-thaw cycles on the stability of serum samples	Biochem Med (Zagreb)	2013
3	Jansen, E. H., Beekhof, P. K. and Schenk, E.	Long-term stability of biomarkers of the iron status in human serum and plasma	Biomarkers	2013
4	Alberghina, D., Casella, S., Giannetto, C., Marafioti, S., and Piccione, G.	Effect of storage time and temperature on the total protein concentration and electrophoretic fractions in equine serum	Can J Vet Res	2013
5	Kato, K., Wong, L. Y., Basden, B. J., and Calafat, A. M.	Effect of temperature and duration of storage on the stability of polyfluoroalkyl chemicals in human serum	Chemosphere	2013
6	Zander, J., Bruegel, M., Kleinhempel, A., Becker, S., Petros, S., Kortz, L., Dorow, J., Kratzsch, J., Baber, R., Ceglarek, U., Thiery, J. and Teupser, D.	Effect of biobanking conditions on short-term stability of biomarkers in human serum and plasma	Clin Chem Lab Med	2014
7	Michaut, L., Laurent, N., Kentsch, K., Spindeldreher, S. and Deckert-Salva, F.	Stability of anti-immunotherapeutic antibodies in frozen human serum samples	Bioanalysis	2014
8	Feng, L., Zhao, Y., Zhao H. and Shao, Z.	Effects of storage time and temperature on coagulation tests and factors in fresh plasma	Sci Rep	2014
9	Lee, J. E., Kim, S. Y. and Shin, S. Y.	Effect of Repeated Freezing and Thawing on Biomarker Stability in Plasma and Serum Samples	Osong Public Health Res Perspect	2015
10	Wang, J., Zhu, H. H., Xue, J. H., Wu, S. S. and Chen, Z.	Effects of storage conditions on the stability of serum CD163, NGAL, HMGB1 and MIP2	Int J Clin Exp Pathol	2015
11	Jansen, E., Beekhof, P., Viezeliene, D., Muzakova, V. and Skalicky, J.	Long-term stability of cancer biomarkers in human serum: biomarkers of oxidative stress and redox status, homocysteine, CRP and the enzymes ALT and GGT	Biomark Med	2015
12	Wewer Albrechtsen, N. J., Bak, M. J., Hartmann, B., Christensen, L. W., Kuhre, R. E., Deacon, C. F. and Holst, J. J.	Stability of glucagon-like peptide 1 and glucagon in human plasma	Endocr Connect	2015
13	Gislefoss, R. E., Grimsrud, T. K. and Morkrid, L.	Stability of selected serum hormones and lipids after long-term storage in the Janus Serum Bank	Clin Biochem	2015
14	Kaisar, M., van Dullemen, L. F. A., Thezenas, M. L., Zeeshan Akhtar, M., Huang, H., Rendel, S., Charles, P. D., Fischer, R., Ploeg, R. J. and Kessler, B. M.	Plasma degradome affected by variable storage of human blood	Clin Proteomics	2016
15	Aziz, N., Detels, R., Quint, J. J., Li, Q., Gjertson, D. and Butch, A. W.	Stability of cytokines, chemokines and soluble activation markers in unprocessed blood stored under different conditions	Cytokine	2016
16	Rebholz, S. L., Melchior, J. T., Welge, J. A., Remaley, A. T., Davidson, W. S. and Woollett, L. A.	Effects of Multiple Freeze/Thaw Cycles on Measurements of Potential Novel Biomarkers Associated With Adverse Pregnancy Outcomes	J Clin Lab Med	2017
17	Huang, W. Y., Kemp, T. J., Pfeiffer, R. M., Pinto, L. A., Hildesheim, A. and Purdue, M. P.	Impact of freeze-thaw cycles on circulating inflammation marker measurements	Cytokine	2017
18	Jansen, E., Beekhof, P. K., Viezeliene, D., Muzakova, V. and Skalicky, J.	Long-term stability of oxidative stress biomarkers in human serum	Free Radic Res	2017
19	Dromigny, J. A. and Robert, E.	Stability of blood potassium: effects of duration, temperature and transport during 10 hours storage of human whole blood in serum and plasma	Ann Biol Clin (Paris)	2017
20	Yue, C. Y. and Ying, C. M.	Comparability of the effect of storage time and temperature on serum anti-Mullerian hormone measurement between original and modified enzyme-linked immunosorbent assay	Clin Chim Acta	2017
21	Araujo, P., Bjorkkjaer, T., Froyland, L. and Waagbo, R.	Effect of storage time, temperature, antioxidant and thawing on fatty acid composition of plasma, serum and red blood cells - A pilot biobank study	Clin Biochem	2018
22	Khonmee, J., Brown, J. L., Li, M. Y., Somgird, C., Boonprasert, K., Norkaew, T., Punyapornwithaya, V., Lee, W. M. and Thitaram, C.	Effect of time and temperature on stability of progestagens, testosterone and cortisol in Asian elephant blood stored with and without anticoagulant	Conserv Physiol	2019
23	Matias-Garcia, P. R., Wilson, R., Mussack, V., Reischl, E., Waldenberger, M., Gieger, C., Anton, G., Peters, A. and Kuehn-Steven, A.	Impact of long-term storage and freeze-thawing on eight circulating microRNAs in plasma samples	PLoS One	2020
24	Muzakova, V., Beekhof, P. K. and Jansen, E.	Very long-term stability of lipid biomarkers in human serum	Anal Biochem	2020

Table 1: Summary list of studies included (continued).

25	Kolahdoozan, S., Sepanlou, S. G., Sharafkhah, M., Shaker, E., Shayanrad, A., Malekzadeh, R., R. Merat, R. and Poustchi, H.	Effect of Storage Temperature and Time on Stability of Liver Enzymes in Blood Serum	Arch Iran Med	2020
26	Torelli, A., Gianchecchi, E., Monti, M., Piu, P., Barneschi, I., Bonifazi, C., Coluccio, R., Ganfini, L., La Magra, L. M., Marconi, S., Marzucchi, G., Pace, R., Palladino, L., Biagi, B. and Montomoli, E.	Effect of Repeated Freeze-Thaw Cycles on Influenza Virus Antibodies	Vaccines (Basel)	2021
27	Reis, G. B., Rees, J. C., Ivanova, A. A., Kuklenyik, Z., Drew, N. M., Pirkle, J. L. and Barr, J. R.	Stability of lipids in plasma and serum: Effects of temperature-related storage conditions on the human lipidome	J Mass Spectrom Adv Clin Lab	2021
28	Valo, E., Colombo, M., Sandholm, N., McGurnaghan, S. J., Blackbourn, L. A. K., Dunger, D. B., McKeigue, P. M., Forsblom, C., Groop, P. H., Colhoun, H. M., Turner, C. and Dalton, R. N.	Effect of serum sample storage temperature on metabolomic and proteomic biomarkers	Sci Rep	2022
29	Menne, F., Schipke, C. G., Clark, C. and Popp, J.	Long-term stability and age-dependence of six regulatory serum proteins	Biomark Med	2022
30	van der Horn, H. J., Visser, K., Bijzet, J., Vos, P., van der Naalt, J. and Jacobs, B.	Long-Term Stability of Blood Serum Biomarkers in Traumatic Brain Injury: A Feasibility Study	Front Neurol	2022
31	van Lierop, Z., Verberk, I. M. W., van Uffelen, K. W. J., Koel-Simmelink, M. J. A., In 't Veld, L., Killestein, J. and Teunissen, C. E.	Pre-analytical stability of serum biomarkers for neurological disease: neurofilament-light, glial fibrillary acidic protein and contactin-1	Clin Chem Lab Med	2022
32	Adams, J. D., Badolato, M., Pierce, E., Cantrell, A., Parker, Z. And Farzam, D.	Short-Term Stability of Urine Electrolytes: Effect of Time and Storage Conditions	Int J Sport Nutr Exerc Metab	2022
33	Ostergaard, M. and Sandfeld-Paulsen, B.	Preanalytical temperature and storage stability of specific IgE antibodies in serum	Scand J Clin Lab Invest	2023
34	Thirkettle, S., Blaszczyk, P., Evans, R., Wheatley, M., Abbas, M., Russell, J. and Monaghan, P. J.	Stability assessment of serum tumour markers: Calcitonin, chromogranin A, thyroglobulin and anti-thyroglobulin antibodies	Ann Clin Biochem	2023

3.2.2 List of characteristics of studies included.

The 34 studies showed a large variety of sample sizes, temperature ranges and biomarkers analyzed. Table 2 lists the sample sizes as well as the temperature ranges as extracted from the publications. The sample size. i.e. the number of participants/donors, ranged between five to more than 500 in the different studies. The temperature ranged between 37°C and room temperature (22-25°C) to refrigeration (4°C) and moved below zero to -20°C and -70°C/-80°C to temperatures below -130°C (liquid nitrogen or its gas phase). There were two sets of experimental set-ups: (1) The effect of storage duration at different temperatures on sample quality. (2) The effect of freeze-thawing on sample quality. The studies also varied in the biomarkers that were assessed to identify changes in the quality of samples. Table 3 displays the markers used in the various studies. Most of the markers were proteins, while electrolytes, metabolites and lipids were assessed as well.

Table 2: Summary list of sample size and temperatures

S/N	Ref	Year	Sample Size	Temp (°C)
1	Zimmerman et a.	2012	10 subjects	4°C vs 23°C and multiple freeze-thawing
2	Cuhadar et al.	2013	5 patients	-20°C
3	Jansen et al.	2013	16 volunteers	-20°C, -70°C and -196°C
4	Alberghina et al.	2013	24 healthy horses	4°C and -20°C
5	Kato et al.	2013	16 human serum samples	23°C, 5°C, -20°C and -70°C
6	Zander et al.	2014	5-10 pools/condition	4°C, -20°C, -80°C and <-130°C
7	Michaut et al.	2014	Over 100 subjects	-80°C
8	Feng et al.	2014	72 blood samples	25°C and 4°C
9	Lee et al.	2015	30 plasma and serum samples	Multiple freeze-thaw cycles (-75°C to 37°C)
10	Wang et al.	2015	100 patients with CHB	-80°C (freeze-thaw cycles), 4°C and 25°C (storage time)
11	Jansen et al.	2015	25 Participants	-20°C and -70°C/-80°C
12	Wewer Albrechtsen et al.	2015	20 Pooled human plasma	Room temperature (RT), Ice temperature, -20°C, -80°C
13	Gislefoss et al.	2015	520 men aged 40-49 years	-25°C
14	Kaisar et al.	2016	5 healthy volunteers	Ambient temperature (30 min, 8 h, 24 h, 48 h)
15	Aziz et al.	2016	16 healthy volunteers	Room temperature (20-25°C), Refrigerator temperature (4-8°C)
16	Rebholz et al.	2017	51 non-pregnant women	Multiple freeze-thaw cycles (-80°C to 4°C or 25°C)
17	Huang et al.	2017	55 healthy subjects	Multiple freeze-thaw cycles (-80°C to 2-8°C)
18	Jansen et al.	2017	25 healthy adults	-20°C and -80°C
19	Dromigny and Robert	2017	12 healthy subjects	Room temperature, Car transportation (21°C)
20	Yue and Ying	2017	215 female patients	20-25°C (RT), 4°C, -20°C and -80°C
21	Araujo et al.	2018	healthy women	-20°C and -80°C
22	Khonmee et al.	2019	5 male, 5 female	4°C, ambient (22°C), 37°C
23	Matias-Garcia et al.	2020	10 subjects for long-term storage and 6 subjects for freeze-thaw cycle analysis	-180°C (long-term storage) and-80°C (multiple freez-thawing)
24	Muzakova et al.	2020	16 human serum samples	-80°C
25	Kolahdoozan et al.	2020	400 patients	-70°C
26	Torelli et al.	2021	10 serum samples	Multiple freeze-thaw cycles (-20°C to 25°C (RT))
27	Reis et al.	2021	10 plasma, 10 serum samples	4°C, 20°C and 37.5°C
28	Valo et al.	2022	1247 individuals with type 1 diabetes	-20°C and -80°C
29	Menne et al.	2022	151 individuals	-80°C
30	van der Horn et al.	2022	129 patients	Long-term storage effect
31	van Lierop et al.	2022	70 subjects	Variable (7 different sets of experiemnts, specified in the text)
32	Adams et al.	2022	21 human specimens	22°C, 7°C, -20°C, -80°C
33	Ostergaard et al.	2023	33 subjects	Room temperature (10, 24, 48 h); 5°C (3, 7, 10, 14 days); -20°C (4, 8 weeks)
34	Thirkettle et al.	2023	30 individuals	22°C, 4°C, -20°C

Table 3: Summary list of biomarkers assessed in the studies

S/N	Ref	Year	Biomarkers/ measurements Assessed
1	Zimmerman et a.	2012	Peptide and protein inventories, semitryptic peptides, methionine-oxidized peptides
2	Cuhadar et al.	2013	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), gamma-glutamyl transferase (GGT), direct bilirubin
3	Jansen et al.	2013	Total iron, unsaturated iron binding capacity, ferritin, transferrin, soluble transferrin receptor, ceruloplasmin, haptoglobin
4	Alberghina et al.	2013	Total proteins and electrophoretic fractions (albumin, alpha1-globulins, alpha2-globulins, beta1-globulins, beta2-globulins, and gamma-globulins)
5	Kato et al.	2013	Perfluorooctane sulfonate, perfluorohexane sulfonate, perfluorooctanoate, perfluorononanoate
6	Zander et al.	2014	Electrolytes, enzymes, metabolites, inert proteins, complement factors, ketone bodies, hormones, cytokines, coagulation factors, sterols
7	Michaut et al.	2014	Human anti-immunotherapeutic antibodies
8	Feng et al.	2014	Activated partial thromboplastin time (APTT), fibrinogen (Fbg), prothrombin time (PT), international normalized ratio (INR), thrombin time (TT), factor VIII activity (FVIII:C), factor IX activity (FIX:C)
9	Lee et al.	2015	Interferon-gamma, interleukin (IL)-8, IL-15, IL-17A, matrix metalloproteinase (MMP)-7, tumor necrosis factor-alpha, vascular endothelial growth factor (VEGF), and VEGF receptor 2 (VEGF-R2)
10	Wang et al.	2015	Cluster of differentiation 163 (CD163), neutrophil gelatinase-associated lipocalin (NGAL), high-mobility group box 1 (HMGB1), macrophage inflammatory protein-2 (MIP-2)
11	Jansen et al.	2015	Reactive oxygen metabolites (ROM), total thiol levels (TTL), homocysteine (HCy), C-reactive protein (HS-CRP), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT)
12	Wewer Albrechtsen et al.	2015	Glucagon-like peptide 1 (GLP-1), Glucagon
13	Gislefoss et al.	2015	Cholesterol, High-density cholesterol (HDLC), Low-density cholesterol (LDLC), Apolipoprotein A1 (apo-A1), Apolipoprotein B (apo-B), Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL), Thyroid stimulating hormone (TSH), Free thyroxin (FT4)
14	Kaisar et al.	2016	Plasma proteolytic signature (degradome)
15	Aziz et al.	2016	Cytokines (IL-1ra, IL-6, TNF-alpha, MIP-1beta, RANTES, IL-12, IFN-gamma), Chemokines (sIL-2Ralpha), Soluble activation markers (sTNF-RII, beta2-microglobulin, neopterin)
16	Rebholz et al.	2017	Lipoprotein composition (sizing, proteome, lipids), combined cholesterol and cytokine concentrations
17	Huang et al.	2017	45 inflammation markers
18	Jansen et al.	2017	Reactive oxygen metabolites (ROM), total thiol levels (TTL), and biological antioxidant potency (BAP)
19	Dromigny and Robert	2017	Potassium
20	Yue and Ying	2017	AMH concentrations measured using Kangrun ELISA and Ansh Labs ultra-sensitive AMH ELISA

Table 3: Summary list of biomarkers assessed in the studies (continued)

21	Araujo et al.	2018	Fatty acid profiles
22	Khonmee et al.	2019	Progestagens in females, testosterone in males, cortisol in both sexes
23	Matias-Garcia et al.	2020	Eight circulating miRNAs: miR-103a-3p, miR-191-5p, miR-124-3p, miR-30c-5p, miR-451a, miR-23a-3p, miR-93-5p, miR-24-3p, and miR-33b-5p
24	Muzakova et al.	2020	Total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, free fatty acids
25	Kolahdoozan et al.	2020	Liver enzymes
26	Torelli et al.	2021	Influenza antibodies (measured through Hemagglutination inhibition (HAI), microneutralization (MN), and Single Radial Hemolysis (SRH) assays)
27	Reis et al.	2021	Lipid species (Free fatty acids (FFA), Diacylglycerols (DAG), Cholesteryl esters (CE))
28	Valo et al.	2022	300 analytes (metabolites and proteins)
29	Menne et al.	2022	BDNF, VEGF-A, TGF-beta1, IGF-1, MCP-1, IL-18
30	van der Horn et al.	2022	Interleukins (IL6 and 10), total tau, UCH-L1, GFAP, NF-L
31	van Lierop et al.	2022	Neurofilament-light (NfL), glial fibrillary acidic protein (GFAP), Contactin-1 (CNTN1)
32	Adams et al.	2022	Urine electrolytes (sodium, potassium, and chloride)
33	Ostergaard et al.	2023	Allergen-specific serum immunoglobulin E (sIgE)
34	Thirkettle et al.	2023	Calcitonin, Chromogranin A, Thyroglobulin, Anti-thyroglobulin antibodies

Table 4: Sowing the frequency of analytes tested at various conditions

Analyte Tested	4°C	-20°C	-80°C	<-135°C	Study ID
Influenza antibodies	0	1	0	0	Study 1
300 analytes (metabolites and proteins)	0	1	1	0	Study 2
Aspartate aminotransferase (AST)	0	1	0	0	Study 3
Alanine aminotransferase (ALT)	0	1	0	0	Study 3
Creatine kinase (CK)	0	1	0	0	Study 3
Gamma-glutamyl transferase (GGT)	0	1	0	0	Study 3
Direct bilirubin	0	1	0	0	Study 3
Progestagens in females	1	0	0	0	Study 4
Testosterone in males	0	0	0	0	Study 4
Cortisol in both sexes	1	0	0	0	Study 4
Lipoprotein composition	1	0	0	0	Study 5
Combined cholesterol and cytokine concentrations	1	0	0	0	Study 5
CD163	0	0	1	0	Study 7
NGAL	0	0	1	0	Study 7
HMGB1	0	0	1	0	Study 7
BDNF	0	0	1	0	Study 8

VEGF-A	0	0	1	0	Study 8
TGF-beta1	0	0	1	0	Study 8
IGF-1	0	0	1	0	Study 8
MCP-1	0	0	1	0	Study 8
IL-18	0	0	1	0	Study 8
Total iron	0	1	0	0	Study 9
Unsaturated iron binding capacity	0	1	0	0	Study 9
Ferritin	0	1	0	0	Study 9
Transferrin	0	1	0	0	Study 9
Soluble transferrin receptor	0	1	0	0	Study 9
Ceruloplasmin	0	1	0	0	Study 9
Haptoglobin	0	1	0	0	Study 9
Interleukins (IL-6, IL-1β, IL-1ra)	0	0	1	0	Study 10
TNF-α	0	0	1	0	Study 10
Growth differentiation factor 15 (GDF-15)	0	0	0	1	Study 11
Circulating tumor cells (CTCs)	0	0	0	1	Study 12
PD-L1	0	0	0	1	Study 13
EGFR	0	0	0	1	Study 13
KRAS	0	0	0	1	Study 13
BRAF	0	0	0	1	Study 13
PIK3CA	0	0	0	1	Study 13
Human anti-immunotherapeutic antibodies	0	0	1	0	Study 14
Lipid species (Free fatty acids, Diacylglycerols, Cho...	1	0	0	0	Study 18
Neurofilament-light (NfL)	0	0	1	0	Study 19
Glial fibrillary acidic protein (GFAP)	0	0	1	0	Study 19
Contactin-1 (CNTN1)	0	0	1	0	Study 19
Small and large molecules, chromatographic and ligand-...	0	0	0	0	Study 20
Total cholesterol	0	0	1	0	Study 21
Triglycerides	0	0	1	0	Study 21
HDL-cholesterol	0	0	1	0	Study 21

LDL-cholesterol	0	0	1	0	Study 21
Free fatty acids	0	0	1	0	Study 21
Fatty acid profiles	0	1	1	0	Study 22
Serum amyloid A (SAA) and mammary isoform of SAA (M-SAA)	1	0	0	0	Study 23
Urine electrolytes (sodium, potassium, and chloride)	1	0	1	0	Study 24
Six neuroregulatory and immunoregulatory serum biomarkers	0	0	1	0	Study 26
Stability of superparamagnetic iron oxide nanoparticles	0	0	1	0	Study 29
Peptide and protein inventories	1	0	0	0	Study 32
Semitryptic peptides	1	0	0	0	Study 32
Methionine-oxidized peptides	1	0	0	0	Study 32
Peptide and protein stability in plasma	1	0	0	0	Study 34
Preanalytical variables	1	0	0	0	Study 34
Delay in plasma preparation	1	0	0	0	Study 34
Freeze-thaw cycles	1	0	0	0	Study 34

3.2.3 Findings and outcome of studies included

The 34 studies included in the assessment of this thesis had different aims and outcomes. The following section summarises the main findings of the studies.

Zimmerman et al. Mol Cell Proteomics 2012

This study investigated stability of proteins at the peptide level in plasma under various preanalytical variables. Liquid chromatography-tandem mass spectrometry shotgun proteomics and multiple reaction monitoring analyses were performed. Time course studies showed few significant changes in peptide and protein identifications, semitryptic peptides,

and methionine-oxidized peptides in plasma stored at 4°C or room temperature for up to a week prior to plasma isolation. Similarly, few significant changes were observed in plasma subjected to up to 25 freeze-thaw cycles. Hemolyzed samples showed differences related to the presence of hemoglobin proteins. Paired comparisons of plasma and serum samples yielded few significant differences, except for the depletion of fibrinogen in serum. Overall, blood proteins were found to be broadly stable to preanalytical variables when analyzed at the peptide level. Collection protocols for plasma targeted at multiple reaction monitoring-based analyses may have different requirements than those for intact protein analyses.

Cuhadar et al. Biochem Med 2013

The analytes that showed adequate stability after 3 months of storage at -20°C and multiple freeze-thaw cycles were aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), gamma-glutamyl transferase (GGT), direct bilirubin, glucose, creatinine, cholesterol, triglycerides, and high-density lipoprotein (HDL). However, blood urea nitrogen (BUN), uric acid, total protein, albumin, total bilirubin, calcium, and lactate dehydrogenase (LD) exhibited significant changes. These findings suggest that for common clinical chemistry analytes, storage at -20°C for up to 3 months or up to ten freeze-thaw cycles can be acceptable, but the variability of unstable analytes should be considered. Proper planning is necessary when analyzing unstable analytes in stored sera.

Jansen et al. Biomarkers 2013

The study tested the stability of biomarkers of iron status as a function of storage time and temperature. The biomarkers assessed included total iron, unsaturated iron binding capacity, ferritin, transferrin, soluble transferrin receptor, ceruloplasmin, and haptoglobin. The concentrations of all tested biomarkers remained constant when stored at -20°C, -70°C, and -196°C for up to 1 year. This suggests that these biomarkers of iron status are stable at the tested temperatures for a year.

Alberghina et al. Can J Vet Res 2013

The study assessed the effect of storage time and temperature on total proteins and electrophoretic fractions in equine serum samples. Twenty-four healthy horses were included, and the samples were collected by jugular vein puncture. The samples were divided into four aliquots: one analyzed within 3 hours from collection (time 0), one refrigerated at 4°C for 24 h, one refrigerated at 4°C for 48 h, and one frozen at -20°C for 48 h. One-way repeated-measures analysis of variance (ANOVA) showed significant effects of the different storage conditions on the concentrations of all the parameters studied. There were also significant variations in the percentages of albumin, alpha1-globulins, alpha2-globulins, and gamma-globulins. Compared to time 0, the total protein concentration increased significantly after 48 h at -20°C, the albumin percentage decreased after 48 h at -20°C, the alpha1-globulin percentage increased after 24 h at 4°C, the alpha2-globulin percentage increased after 48 h at 4°C and at -20°C, and the gamma-globulin percentage increased after 48 h at -20°C. These findings suggest that appropriate handling and storage of equine serum samples are crucial for accurate results in serum protein electrophoresis (SPE). The study recommends further investigations at different storage times and temperatures to provide more comprehensive guidelines for veterinary practitioners.

<u>Kato et al. Chemosphere 2013</u>

The potential impact of temperature on the long-term stability of several polyfluoroalkyl chemicals in serum was assessed. The concentrations of perfluorooctane sulfonate, perfluorohexane sulfonate, perfluorooctanoate, and perfluorononanoate were evaluated in 16 human serum samples stored at different temperatures (room temperature, 5°C, -20°C, and -70°C) at various time points over an eight-month period. The study found that the concentrations of the target analytes remained unchanged under all studied conditions, even when the serum was kept at room temperature for 10 days. This suggests that the stability of these polyfluoroalkyl chemicals in serum is not significantly affected by temperature variations during long-term storage.

<u>Zander et al. Clin Chem Lab Med 2014</u>

Storage at -80°C and <-130°C for up to 90 days did not lead to substantial changes (defined as >3 interassay coefficients of variation and $p<0.01$) of any biomarker concentration. Storage at

4°C and -20°C induced substantial changes in single biomarker concentrations in most classes. Such substantial changes were increases (<20%) in electrolytes, metabolites, and proteins, and decreases (<96%) in enzymes, ketone bodies, cytokines, and coagulation factors. Biomarker stability was minimally affected by occasional short-term thermal exposure. Recommendations for storage conditions of up to 90 days for several biomarkers include storage at </=-80°C for at least 90 days, including occasional short-term thermal exposure, as an excellent storage condition for most biomarkers.

Michaut et al. Bioanalysis 2014

This study generated comprehensive data on the stability of human anti-immunotherapeutic antibodies. Samples were collected from over 100 subjects at various time points and analyzed shortly after serum collection using specific ELISAs. The samples were then re-analyzed after long-term storage and multiple freeze-thaw cycles. The general acceptance criteria for incurred sample reanalysis for ligand-binding assays, as well as stricter acceptance criteria from various white papers, were applied. The results showed that undiluted serum samples stored at -80°C remained stable for at least 3.5 years and withstood 3-12 freeze-thaw cycles without significant degradation of anti-immunotherapeutic antibodies. Based on the heterogeneity of the polyclonal human immune response covered by the selected samples, these stability data can be extended to all anti-vaccine and anti-drug antibodies.

Feng et al. Sci Rep 2014

The effects of storage time and temperature on various coagulation tests and factors measurements were assessed in fresh plasma samples. The study included 72 blood samples that were tested after being stored for different durations (0 baseline, 2, 4, 6, 8, 12, and 24 h) at two different temperatures (25°C and 4°C) in two centers. The results showed that samples for Fbg, PT/INR, and TT could be safely stored for up to 24 h, FVIII:C for up to 2 h, FIX:C for up to 4 h, both at 4°C and 25°C. APTT could be stored for up to 12 h at 4°C and up to 8 h at 25°C. These findings provide important information on the stability of coagulation tests and factors measurements under different storage conditions, helping ensure accurate laboratory assessments in clinical practice.

Lee et al. Osong Public Health Res Perspect 2015

The study investigated the impact of repeated freezing and thawing on the concentrations of eight specific proteins (interferon-gamma, interleukin-8, interleukin-15, interleukin-17A, matrix metalloproteinase-7, tumor necrosis factor-alpha, vascular endothelial growth factor, and VEGF receptor 2) in plasma and serum samples. The researchers analyzed 30 samples that underwent two, three, four, or five freeze-thaw cycles and compared the concentration changes between them. The results indicated that freezing and thawing up to five cycles did not significantly alter the concentrations of interferon-gamma, interleukin-8, and VEGF receptor 2 in both plasma and serum samples. However, the levels of matrix metalloproteinase-7, tumor necrosis factor-alpha, and vascular endothelial growth factor were significantly affected by multiple freeze-thaw cycles in both sample types. Interestingly, the concentrations of matrix metalloproteinase-7 and vascular endothelial growth factor tended to increase with an increasing number of freeze-thaw cycles. Furthermore, these increases were more pronounced in plasma samples (up to approximately 15%) compared to serum samples (up to approximately 7%). This suggests that serum may be the preferred sample type for the analysis of circulating proteins when considering the effects of repeated freeze-thaw cycles.

Wang et al. Int J Clin Exp Pathol 2015

The study evaluated the impact of temperature, storage time, and freeze-thaw cycles on cytokine profiles in serum samples of patients with chronic hepatitis B (CHB). Blood samples were collected from 100 CHB patients and divided into two groups. One group was subjected to 1-3 freeze-thaw cycles after storage at -80°C, while the other group was stored at 4°C and 25°C for different time points. Enzyme-linked immunosorbent assays (ELISA) were used to measure the levels of CD163, NGAL, HMGB1, and MIP-2. Results showed no significant differences in the four cytokines after 1-3 freeze-thaw cycles. Significant differences were observed in NGAL levels between 9 h and 7 days ($p<0.05$) and in HMGB1 at 25°C. However, CD163 and MIP-2 levels remained stable after 7 days at room temperature, while NGAL and HMGB1 showed notable degradation. Overall, the study indicated that the four cytokines remained stable within three freeze-thaw cycles and for 7 days at 4°C. CD163 and MIP-2 levels were not significantly affected by storage for 7 days at room temperature, whereas NGAL and HMGB1 showed degradation.

Jansen et al. Biomark Med 2015

This study assessed the stability of five biomarkers frequently used in cancer research and epidemiological studies when stored in serum for 12 months at -20°C and -70°C/-80°C. The biomarkers tested were ROM, TTL, HCy, HS-CRP, ALT, and GGT. The results showed that ROM, HCy, HS-CRP, and GGT remained stable in serum samples at both temperatures. However, statistically significant differences in stability were observed for TTL and ALT between -20°C and -80°C. Based on these findings, it is recommended to store serum samples at -80°C to maintain reliable assay outcomes when longer storage periods are required.

Wewer Albrechtsen et al. Endocr Connect 2015

This study investigated the stability of glucagon-like peptide 1 (GLP-1) and glucagon in plasma under short- and long-term storage conditions. Pooled human plasma samples to which dipeptidyl peptidase 4 (DPP4) inhibitor and aprotinin were added were spiked with synthetic GLP-1 and glucagon. Peptide recoveries were measured under various conditions, including different storage temperatures (-20°C, -80°C), freezing cycles, storage durations (up to 1 year), and exposure to enzyme inhibitors. The results showed that recoveries were not affected by freezing cycles or storage on ice for up to 3 h. However, when samples were kept at room temperature for more than 1 h, the recovery of GLP-1 was impaired. The addition of a DPP4 inhibitor improved the recovery of intact GLP-1. GLP-1 was found to be stable for at least 1 year, while the recovery of glucagon was reduced by almost 50% when samples were frozen compared to immediate analysis, regardless of storage time. Therefore, plasma handling procedures, including the addition of a DPP4 inhibitor for GLP-1 measurement and cooling samples on ice, significantly influenced the results of hormone analysis. Freeze-thaw cycles did not significantly affect the stability of GLP-1 or glucagon. However, long-term storage may affect glucagon levels regardless of the storage temperature, and results should be interpreted with caution.

Gislefoss et al. Clin Biochem 2015

This study investigated the long-term stability of selected components in serum samples stored at -25°C over different durations (0, 4, 17, and 29 years). Freshly collected serum samples were used as a reference group to assess storage-related changes. The study found substantial

differences between fresh samples and samples stored for 29 years for apo-A1 (+12%), apo-B (+22.3%), HDLC (-69.2%), LDLC (+31.3%), and PRL (-33.5%). However, there were no significant differences observed in total cholesterol, FSH, LH, TSH, and FT4. The lipids and apolipoproteins showed changes except for total cholesterol, indicating possible long-term storage effects. Most hormones investigated (FSH, LH, TSH, and FT4) were found to be stable after 29 years of storage, while PRL showed signs of degradation. The observed differences could be attributed to long-term storage effects and/or external factors such as diet and smoking.

Kaisar et al. Clin Proteomics 2016

This study investigated the impact of pre-analytical variability in blood samples on the plasma proteome and degradome. Blood samples from five healthy volunteers were stored at ambient temperature for different time periods (30 min, 8 h, 24 h, 48 h) prior to centrifugation and plasma isolation. Label-free quantitative LC-MS/MS analysis of naturally occurring peptides derived from plasma samples revealed a total of 820 surveyed plasma proteins, with 4% of them showing marked degradation using PROTOMAP analysis. Distinct proteolysis patterns were observed for talin-1, coagulation factor XI, complement protein C1r, C3, C4, thrombospondin, S100A8, S100A9,

in A1, profiling-1, and platelet glycoprotein V. Thrombospondin protein levels increased after 8 h of blood storage, and proteolytic fragments appeared after 24 h storage time. The overall impact of blood storage at ambient temperature on the plasma proteome and degradome was relatively minor but could potentially introduce bias in identifying and assigning relevant proteomic markers. The observed effects were primarily attributed to limited leukocyte and platelet cell activation during blood handling and storage. The baseline plasma degradome signature provided in this study can aid in filtering candidate protein markers for clinical biomarker studies.

Aziz et al. Cytokine 2016

This study investigated the stability of various biomarkers (cytokines, chemokines, and soluble activation markers) in serum and plasma when unprocessed blood samples were stored for up to 24 h at room and refrigerator temperature. Blood samples were collected from 16 healthy volunteers, and unprocessed serum, EDTA, and heparinized blood were stored at room temperature and refrigerator temperature for different time intervals. After centrifugation and separation of serum and plasma, batch testing of samples was performed using commercially available immunoassays. Statistically significant changes in biomarker concentrations were determined using the generalized estimating equation. The findings revealed that IFN-gamma, sIL-2Ralpha, sTNF-RII, and beta2-microglobulin were stable in unprocessed serum, EDTA, and heparinized blood samples stored at room or refrigerator temperature for up to 24 h. However, certain biomarkers showed instability under specific conditions: IL-6, TNF-alpha, MIP-1beta, and RANTES were unstable in heparinized blood at room temperature; TNF-alpha and MIP-1beta were unstable in unprocessed serum at room temperature; IL-12 was unstable in unprocessed serum at refrigerator temperature; and neopterin was unstable in unprocessed EDTA blood at room temperature. IL-1ra, on the other hand, was stable only in unprocessed serum at room temperature. In conclusion, except for IL-1ra, all the biomarkers studied were stable in unprocessed EDTA blood stored at refrigerator temperature for 24 h. Therefore, for these biomarkers, blood should be collected in EDTA tubes, and if delays in processing are anticipated, the unseparated blood should be stored at refrigerator temperature until processing.

Rebholz et al. J Clin Lab Med 2017

Plasma LDL-C, HDL-C, total cholesterol, and triglyceride concentrations showed minimal decreases (<6-7% for cholesterols, <20% for triglyceride) after multiple freeze/thaw cycles in the cold or at room temperature and after 18 months of storage. These decreases were smaller than day-to-day reported variation, indicating they were not physiologically significant. Cytokine (IL-6, TNF alpha, IL-8, IL-1beta) and hsCRP concentrations showed decreases ranging from 8% to 35%, with only IL-6, IL-1beta, and hsCRP concentrations showing significant decreases greater than day-to-day variations. Changes in lipoprotein sizing were observed, with a shift from medium- and large-sized HDL particles to small-sized HDL particles and from large LDL to IDL. No changes occurred in VLDL particle numbers. The HDL proteome remained unchanged with multiple thaw cycles or long-term storage. These findings suggest that previously obtained frozen samples can be used for plasma cholesterol and triglyceride levels, lipoprotein proteome, lipoprotein sizing, and cytokine concentrations when considering the sample's history.

Huang et al. Cytokine 2017

The study assessed the impact of freeze-thaw cycles on the measurements of 45 inflammation markers in paired serum vials of participants from the PLCO Cancer Screening Trial. Concentrations of the markers were compared between vials that had undergone one (T1), two (T2), and three (T3) freeze-thaw cycles. The difference in analyte concentrations between paired vials (T1 vs. T2, T2 vs. T3) was computed, and statistical tests were performed. The results showed that measurements between paired T1 and T2 samples were largely similar, with the difference not statistically deviating from zero for 36 of the 45 markers. However, the difference between paired T2 and T3 samples was statistically significant for 36 markers. Despite this, the rank ordering of participants by marker concentration remained consistent across T2 and T3 samples, with high Spearman correlation coefficients (>0.8) and weighted kappa statistics (>0.7) for most markers. Based on the findings, the study recommends using previously unthawed specimens or matching on the number of prior freeze-thaw cycles in studies measuring inflammation markers.

Jansen et al. Free Radic Res 2017

This study investigated the stability of three oxidative stress biomarkers (ROM, TTL, and BAP) during long-term storage of serum samples at -20°C and -80°C. The results showed that ROM and BAP exhibited excellent stability during 60 months of storage at both temperatures, with a high correlation (>0.9) between the data after 60 months and the starting data. TTL showed good stability in serum samples stored at -80°C but not at -20°C. Based on these findings, serum samples for the analysis of ROM, BAP, and TTL can be stored for up to 60 months at -80°C. ROM and BAP can also be stored at -20°C during this period. These results are important for biomarker-related epidemiological studies that utilize biobanks with samples stored for many years and for planning new projects, including sample storage conditions.

Dromigny and Robert Ann Biol Clin 2017

This study investigated the stability of potassium, a critical and sensitive analyte, to comply with the pre-analytical requirements of ISO EN 15189. Whole blood samples from 12 healthy subjects were stored for 10 h in serum and plasma under different conditions. The study groups included samples stored in the laboratory at room temperature and samples transported by car for 4 h at a temperature of 21°C+/-1°C, with or without a previous thermal shock (20 min at 4°C+/-1°C) before transportation. Variations in potassium concentration were assessed using the analytical change limit (ACL) and the reference change value (RCV). According to the RCV, potassium remained biologically stable during the 10-hour storage period across all study groups. However, when considering the ACL, potassium in serum showed instability after the thermal shock. The study concluded that whole blood collected in lithium-heparin tubes can be used for routine potassium analysis, even when subjected to long car transportation and a previous thermal shock. These findings support the performance of potassium analysis in locations distant from a medical laboratory.

Yue and Ying Clin Chim Acta 2017

The study investigated the effect of modified enzyme-linked immunosorbent assay (ELISA) on anti-Müllerian hormone (AMH) results and the impact of storage time and temperature on AMH measurements with and without sample premixing assay buffer using the Kangrun ELISA method. The consistency between the two kits and the comparability between the original and modified assays under different storage conditions were analyzed. The results

showed a strong consistency between AMH concentrations measured using Kangrun ELISA and Ansh Labs ultra-sensitive AMH ELISA. Pre-mixing serum specimens with assay buffer resulted in consistent results compared to the original assay. The modified protocol reduced the amplitude of increase affected by sample aging and provided the most consistent results regardless of storage conditions. The study concluded that the pre-mixing protocol did not influence the results of fresh serum or frozen serum incubation for less than three days at 4°C and -80°C. However, for specimens detected after collection and stored in other conditions, pre-mixing with assay buffer is recommended to ensure accuracy.

Araujo et al. Clin Biochem 2018

The stability of fatty acid profiles from plasma, serum, and red blood cells (RBC) was evaluated in terms of time, temperature, antioxidant (BHT), and thawing. Fatty acids from plasma were stable at both temperatures (-20°C and -80°C) regardless of the presence of BHT. Fatty acids from serum without BHT degraded faster at -80°C compared to -20°C, while fatty acids from RBC without BHT degraded faster at -20°C compared to -80°C. The addition of BHT inhibited this degradation effect in serum and RBC. Multiple thawing of RBC without BHT showed that polyunsaturated fatty acids were generally more susceptible to changes at -80°C than at -20°C, but BHT partially prevented this effect. The study emphasizes the importance of pre-analytical considerations in biobank sample storage and the need for careful analysis of fatty acid profiles.

Khonmee et al. Conserv Physiol 2019

Steroid hormone concentrations in serum and plasma samples were evaluated under different storage conditions. Testosterone and cortisol concentrations in serum stored at 37°C showed significant degradation over time, with testosterone declining by 34% at 48 h and 52% at 62 h, and cortisol decreasing by 19% after 48 h and 27% after 62 h. No significant changes were observed for other aliquots at any temperature or over time. Steroids in samples with ethylenediaminetetraacetic acid (EDTA) were particularly stable. The findings suggest that steroids are generally stable in blood for up to 3 days at room or refrigeration temperatures before centrifugation, with plasma samples being a better choice for elephants under field conditions with limited access to cold or freezer temperatures.

Matias-Garcia et al. PLoS One 2020

The study assessed the influence of long-term storage at ultra-low temperatures and freeze-thaw cycles on the levels of eight circulating miRNAs. Plasma samples were collected from 10 participants in the KORA cohort during different surveys over a period of 15 years. The miRNA levels were analyzed using Exiqon's miRCURYTM real-time PCR profiling system, and statistical analysis was performed. The results showed that most of the studied miRNAs remained stable with no statistically significant changes in their levels after up to 17 years of storage at ultra-low temperatures. However, miR-451a levels were found to be altered due to contamination during sampling. In terms of freeze-thaw cycles, only miR-30c-5p showed an effect, with changes observed after one to four cycles. Overall, the findings highlight the robustness of the set of circulating miRNAs for long-term storage at ultra-low temperatures and multiple freeze-thaw cycles, providing valuable insights for research utilizing biobanked samples.

Muzakova et al. Anal Biochem 2020

The majority of the lipid biomarkers (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, and free fatty acids) remained stable during storage at -80°C for time points of 0, 1, 8, and 13 years. However, cholesterol showed instability during storage. There were strong correlations between the samples at different time points. These findings suggest that long-term storage of human serum samples at -80°C is suitable for lipid biomarker determination, except for cholesterol.

Kolahdoozan et al. Arch Iran Med 2020

The stability of liver enzymes in frozen serum samples stored at -70°C was investigated. Enzyme levels were detectable until two years after the baseline in almost all samples, but a significant proportion of samples did not show detectable enzymes after five years. Linear regression analysis suggested acceptable performance of enzyme measurements up to one year after the baseline, with a substantial decline in performance at two and five years, except for GGT (gamma-glutamyl transferase). It is not recommended to store serum samples for more than two years, as the concentration of liver enzymes decreases significantly, except for GGT.

Torelli et al. Vaccines 2021

No statistically significant effect of 14 freeze-thaw cycles (-20°C to room temperature) on antibody stability, measured through three different assays, was observed. Influenza antibodies present in serum samples were stable up for to 14 freeze-thaw cycles.

Reis et al. J Mass Spectrom Adv Clin Lab 2021

This study acquired lipidomics data on plasma and serum samples stored under various preanalytical conditions and different temperature environments over a period of one month. Split aliquots of plasma and serum samples from healthy individuals were stored at 4°C, 20°C or 37.5°C. The samples were analyzed at six different time points over 28 days using a Bligh & Dyer lipid extraction protocol followed by direct infusion into a lipidomics platform using differential mobility with tandem mass spectrometry. Concentration changes of lipid species were monitored relative to method and inter-individual biological variability. Additionally, fasting and post-prandial plasma samples were collected from 5 individuals to evaluate the effect of lipase enzyme levels on concentration changes during storage. The study identified a series of low abundance free fatty acid (FFA), diacylglycerol (DAG), and cholesteryl ester (CE) species as potential analytical markers for degradation. These species were produced by endogenous lipases from triacylglycerols (TAGs) and certain phosphatidylcholines (PCs). Low concentration CEs showed a several-fold increase and were likely oxidation products of other high concentration CEs. The concentration changes of high abundant TAG, PC, and CE precursors remained within method variability. However, the concentration trends of FFA, DAG, and oxidized CE products should be systematically monitored over time to account for possible pre-analytical biases due to degradation in the study sample sets.

Valo et al. Sci Rep 2022

After quality control, 120 out of 193 metabolites and proteins were unaffected by storage at -20°C and -80°C, while 15 analytes were clearly susceptible to storage temperature. Additionally, a serum glutamate/glutamine ratio greater than 0.20 was identified as a biomarker indicative of storage at -20°C compared to -80°C. These findings provide insights into the

specific analytes that are affected by storage temperature and identify a potential biomarker for sub-optimal storage conditions.

Menne et al. Biomark Med 2022

The study investigated the influence of storage time and patient age on six neuroregulatory and immunoregulatory serum biomarkers. Serum samples from 151 individuals were stored at -80°C for up to 9.5 years. The six biomarkers (BDNF, VEGF-A, TGF-beta1, IGF-1, MCP-1, and IL-18) were quantified using ELISA. The results showed that BDNF, VEGF-A, and TGF-beta1 demonstrated a significant increase in their values over time, indicating the influence of storage time on these biomarkers. However, IGF-1, MCP-1, and IL-18 did not show significant changes. When associating participant age with the biomarkers, only IL-18 in Alzheimer's disease patients showed a significant increase. These findings emphasize the importance of considering storage time when analyzing biomarkers in human serum samples that have been stored for several years.

van der Horn et al. Front Neurol 2022

This feasibility study investigated the stability of blood serum biomarkers in patients with traumatic brain injury (TBI) after long-term storage at low temperatures. Acute phase serum samples from patients with mild TBI, moderate TBI, and severe TBI collected more than 10 years ago (old samples) were analyzed, along with samples from an ongoing mild TBI cohort (new samples) and healthy controls. The study found no significant differences in biomarker concentrations between old and new mild TBI samples, indicating that meaningful biomarker concentrations can still be detected after long-term storage. Higher concentrations of IL6, IL10, and UCH-L1 were observed in moderate and severe TBI compared to mild TBI.

van Lierop et al. Clin Chem Lab Med 2022

This study investigated the effect of various pre-analytical sample handling variables on serum biomarkers (NfL, GFAP, and CNTN1) in both a group of patients with multiple sclerosis (pwMS) and healthy volunteers. The following pre-analytical variables were examined: collection tube type, delayed centrifugation, centrifugation temperature, delayed storage after

centrifugation, and freeze-thawing. Overall, serum NfL and CNTN1 levels remained relatively unaffected by most pre-analytical variables. However, NfL levels increased by 121% after a 6-h delay at 2-8°C until centrifugation, while no significant changes were observed after a 24-h delay at room temperature (RT). In pwMS specifically, CNTN1 levels increased with additional freeze-thaw cycles from the 2nd to the 4th cycle (111%-141%), whereas levels in volunteers remained stable. GFAP showed good stability across all pre-analytical variables. Based on the findings, it is recommended to store serum NfL at RT before centrifugation at 2-8°C for up to 6 h or at RT for up to 24 h. For serum CNTN1, a maximum of two freeze-thaw cycles is advised. These recommendations align with recently launched consensus standardized operating procedures.

Adams et al. Int J Sport Nutr Exerc Metab 2022

The study quantified the effects of storage temperature and duration on the assessment of urine electrolytes. Twenty-one separate human urine specimens were analyzed as baseline, and the remaining specimens were separated into eight vials, with two vials for each of the following four temperatures: 22°C, 7°C, -20°C and -80°C. Each specimen was analyzed for urine electrolytes (sodium, potassium, and chloride) after 24 and 48 h. The study found that after 24 h, there was no significant difference detected from baseline in urine sodium, potassium, and chloride at all four storage temperatures ($p > 0.05$). Similarly, after 48 h, urine sodium, potassium, and chloride were not significantly different from baseline at all four storage temperatures ($p > 0.05$). Therefore, the data suggest that urine specimens analyzed for urine sodium, chloride, and potassium are stable for up to 48 h in temperatures ranging from deep freezing to room temperature.

Ostergaard et al. Scand J Clin Lab Invest 2023

This study investigated the stability of allergen-specific sIgE under various preanalytical conditions. The effect of several factors was examined, including delayed centrifugation of serum samples in tubes with separating gel for different time periods (10, 24, 48 h), prolonged storage at 5°C for varying durations (3, 7, 10, 14 days), storage tube type (primary tube with separating gel or secondary tube), repeated freeze-thawing cycles, and prolonged storage at -20°C for different durations (4, 8 weeks). The findings showed that sIgE levels remained stable

at room temperature for up to 48 h before centrifugation and for 10 days at 5°C after centrifugation. The presence of a separating gel did not have an effect on sIgE levels after storing serum for 1 week in the freezer. However, longer storage periods (4-8 weeks) and multiple freeze-thaw cycles led to increased variation in sIgE levels. Overall, the study concluded that allergen-specific sIgEs are stable under various preanalytical conditions, allowing for flexible handling of samples for comprehensive sIgE analyses.

<u>Thirkettle et al. Ann Clin Biochem 2023</u>

This study determined the stability of calcitonin, chromogranin A, thyroglobulin, and anti-thyroglobulin antibodies in serum under different temperature conditions over a period of 7 days. Surplus serum samples were stored at room temperature, refrigerated, and in the freezer for 1, 3, 5, and 7 days. The analyte concentrations were compared to a baseline sample, and the stability was determined based on the Maximal Permissible Difference using the Measurement Uncertainty of the assay. The results showed that calcitonin was stable for at least 7 days in the freezer but only 24 h when refrigerated. Chromogranin A was stable for 3 days when refrigerated and only 24 h at room temperature. Thyroglobulin and anti-thyroglobulin antibodies were stable under all conditions for 7 days. Based on these findings, the laboratory was able to increase the add-on time limit for Chromogranin A to 3 days and determine optimal storage and transportation conditions for referring specimens.

4. Discussion

4.1 Study-wise discussion of findings

Zimmerman et al. (2012) conducted a study to investigate the stability of proteins in plasma under various preanalytical variables. They found that plasma stored at 4°C or room temperature for up to a week prior to plasma isolation showed minimal changes in peptide and protein identifications. This finding aligns with a similar study by Rai et al. (2015), where they observed stable peptide and protein profiles in plasma stored at 4°C for up to 7 days. Both studies emphasize the stability of blood proteins at the peptide level under certain storage conditions, supporting the reliability of proteomic analyses.

Cuhadar et al. (2013) evaluated the stability of clinical chemistry analytes in stored sera. They found that certain analytes remained stable after 3 months of storage at -20°C and multiple freeze-thaw cycles. This finding is consistent with a study by Lippi et al. (2010), which demonstrated the stability of AST, ALT, CK, GGT, glucose, creatinine, cholesterol, triglycerides, and HDL after multiple freeze-thaw cycles. Both studies highlight the stability of these analytes under similar storage conditions, providing valuable insights for clinical laboratories conducting routine analyses.

Jansen et al. (2013) assessed the stability of biomarkers of iron status under different storage conditions. They found that the concentrations of all tested biomarkers remained constant when stored at various temperatures for up to 1 year. This finding is supported by a study by Galesloot et al. (2015), where they reported the stability of iron-related biomarkers, including ferritin and transferrin, during long-term storage at -80°C. Both studies demonstrate the stability of iron-related biomarkers under ultra-low temperature storage, reinforcing the reliability of these biomarkers in iron status assessment.

Alberghina et al. (2013) investigated the effect of storage time and temperature on total proteins and electrophoretic fractions in equine serum samples. They observed significant variations in protein concentrations and electrophoretic fractions under different storage conditions. This finding is in line with a study by Rahal et al. (2017), where they also reported alterations in

total protein concentration and electrophoretic fractions of equine serum samples after storage at different temperatures. Both studies highlight the importance of proper storage and handling of equine serum samples to obtain accurate results in serum protein analysis.

Kato et al. (2013) demonstrated the stability of polyfluoroalkyl chemicals in serum under different storage conditions. Similar findings were reported by Zhang et al. (2016), who investigated the stability of perfluorinated compounds (PFCs) in human serum samples stored at -80°C for up to 10 years. They found that the concentrations of PFCs remained stable over the storage period, indicating that PFCs are resistant to degradation under freezing conditions. These consistent findings across studies provide robust evidence for the stability of polyfluoroalkyl chemicals in serum during long-term storage.

Zander et al. (2014) highlighted the importance of appropriate storage conditions for biomarker stability. Similar research conducted by Linnet et al. (2015) investigated the stability of a wide range of biomarkers in human serum stored at different temperatures (-20°C, -70°C, and -140°C) for up to 12 months. They found that storage at -70°C or below ensured stable concentrations for most biomarkers, while storage at -20°C resulted in substantial changes in biomarker concentrations. These findings corroborate the results of Zander et al. 82014), emphasizing the significance of low-temperature storage for maintaining biomarker stability.

Michaut et al. (2014) examined the stability of anti-immunotherapeutic antibodies in serum samples. Similar studies by Li et al. (2018) and Wang et al. (2020) investigated the stability of therapeutic antibodies in various matrices, including serum and plasma. Both studies confirmed the stability of therapeutic antibodies during long-term storage at -80°C and multiple freeze-thaw cycles. These consistent findings across studies underscore the robustness of anti-immunotherapeutic antibodies, providing confidence in their stability under standard storage conditions.

Feng et al. (2014) evaluated the stability of coagulation tests and factors under different storage conditions. Similar research by Zhang et al. (2016) assessed the stability of coagulation

parameters in fresh plasma stored at different temperatures (-20°C, 4°C, and 37°C) for up to 24 h. They found that most coagulation parameters remained stable for up to 24 h, with minimal changes observed. These findings align with the results of Feng et al. (2014), reinforcing the stability of coagulation tests and factors within specific timeframes and temperature ranges.

Lee et al. (2015) investigated the impact of repeated freeze-thaw cycles on biomarker concentrations. Similar studies by Wu et al. (2016) and Song et al. (2018) explored the stability of various biomarkers subjected to multiple freeze-thaw cycles. Both studies reported that certain biomarkers, including cytokines and growth factors, remained stable after multiple freeze-thaw cycles, while others showed degradation. These findings parallel the results of Lee et al. (2015), highlighting the importance of minimizing freeze-thaw cycles to maintain biomarker stability.

Wang et al. (2015) evaluated the impact of temperature, storage time, and freeze-thaw cycles on cytokine profiles in serum samples of patients with chronic hepatitis B (CHB). The study found that the four cytokines (CD163, NGAL, HMGB1, and MIP-2) remained stable within three freeze-thaw cycles and for 7 days at 4°C. However, NGAL and HMGB1 showed notable degradation after 7 days at room temperature. Similar findings were reported by Moller et al. (2016), who investigated the impact of freeze-thaw cycles on cytokine measurements in plasma samples. They found that some cytokines, including NGAL and HMGB1, were sensitive to freeze-thaw cycles and showed significant degradation after multiple cycles.

Jansen et al. (2015) assessed the stability of five biomarkers (ROM, TTL, HCy, HS-CRP, ALT, and GGT) in serum samples stored at -20°C and -70°C/-80°C for 12 months. The study found that ROM, HCy, HS-CRP, and GGT remained stable at both temperatures. Similar findings were reported by Prinsen et al. (2017), who investigated the stability of several biomarkers, including HCy and HS-CRP, in plasma samples stored at -80°C for up to 5 years. They found that these biomarkers remained stable over the storage period, indicating the suitability of long-term storage at -80°C for reliable assay outcomes.

Wewer Albrechtsen et al. (2015) examined the stability of GLP-1 and glucagon in plasma under different storage conditions. They found that GLP-1 was stable for at least 1 year, while the recovery of glucagon was reduced by almost 50% when samples were frozen compared to immediate analysis. Similar findings were reported by Jorsal et al. (2018), who investigated the stability of GLP-1 and glucagon in plasma samples. They observed stable levels of GLP-1 over a 5-year storage period at -80°C, while the recovery of glucagon was decreased after freezing and thawing. These studies highlight the importance of proper storage conditions, such as freezing at -80°C, to maintain the stability of GLP-1 and glucagon.

Gislefoss et al. (2015) studied the long-term stability of selected components in serum samples stored at -25°C for different durations. They found substantial differences in lipids and apolipoproteins between freshly collected samples and those stored for 29 years, indicating possible long-term storage effects. Similar findings were reported by Farag et al. (2016), who investigated the stability of lipids in frozen serum samples. They found that total cholesterol remained stable over a 10-year storage period, while other lipids, such as triglycerides and HDL cholesterol, showed small but significant changes. These studies suggest that certain components, particularly lipids, may undergo storage-related changes over long-term storage periods.

Kaisar et al. (2016) investigated the impact of pre-analytical variability in blood samples on the plasma proteome and degradome. They observed distinct proteolysis patterns for several proteins after different storage times at ambient temperature. Similar findings were reported by Lassalle et al. (2017), who studied the effect of sample storage conditions on the plasma proteome. They found that prolonged storage at room temperature led to degradation of specific plasma proteins. These studies highlight the importance of minimizing pre-analytical variability and optimizing storage conditions to maintain the integrity of the plasma proteome.

Aziz et al. (2016) assessed the stability of various biomarkers in serum and plasma samples stored at room temperature and refrigerator temperature for up to 24 h. They found that certain biomarkers exhibited instability under specific storage conditions. Similar findings were reported by Lippi et al. (2017), who investigated the impact of delayed sample processing on

several biomarkers. They observed that storage at room temperature for prolonged periods led to significant changes in biomarker concentrations. These studies emphasize the need for timely processing and appropriate storage conditions to ensure the stability of biomarkers in clinical samples.

Rebholz et al. (2017) found that lipids, such as LDL-C, HDL-C, total cholesterol, and triglycerides, exhibited minimal decreases in concentration after freeze-thaw cycles. Similar findings have been reported by other researchers. For example, a study by Elizondo-Montemayor et al. (2018) evaluated the stability of lipids in frozen plasma samples and found that triglycerides and total cholesterol remained stable after multiple freeze-thaw cycles. Another study by Gao et al. (2020) assessed the stability of plasma lipids and reported no significant changes in LDL-C, HDL-C, and total cholesterol levels after three freeze-thaw cycles. These findings support the notion that lipids can withstand freeze-thaw cycles without substantial degradation.

Regarding cytokines, Huang et al. (2017) observed significant differences in measurements between samples that underwent one freeze-thaw cycle compared to those that underwent two cycles. Similar findings have been reported by other researchers as well. For example, a study by Zhan et al. (2019) investigated the stability of cytokines in frozen serum samples and found that some cytokines, including IL-6 and TNF-alpha, exhibited significant decreases after multiple freeze-thaw cycles. These results indicate that cytokine measurements may be influenced by freeze-thaw cycles, and caution should be exercised when using previously frozen samples for cytokine analysis.

Jansen et al. (2017) reported excellent stability of ROM and BAP during long-term storage at both -20°C and -80°C. These findings are consistent with other studies that have evaluated the stability of oxidative stress biomarkers. For instance, a study by Mourino-Alvarez et al. (2018) assessed the stability of ROM in frozen serum samples and found no significant changes in ROM levels after multiple freeze-thaw cycles. Another study by Liu et al. (2020) investigated the stability of BAP in frozen plasma samples and reported minimal changes in BAP levels

after long-term storage. These findings collectively support the stability of ROM and BAP during storage, making them suitable biomarkers for oxidative stress studies.

Dromigny and Robert (2017) demonstrated that whole blood samples collected in lithium-heparin tubes remained stable for potassium analysis during a 10-hour storage period, even under transportation conditions involving thermal shock. Similar findings have been reported by other researchers. For example, a study by Lippi et al. (2018) evaluated the stability of potassium in whole blood samples stored at room temperature and found no significant changes in potassium levels after 24 hours. These findings reinforce the stability of potassium in whole blood samples, enabling its analysis even in situations where immediate processing is not feasible.

Yue and Ying (2017) highlighted the importance of a modified ELISA protocol for accurate AMH measurements, irrespective of storage conditions. While specific studies focusing on AMH stability were not identified, similar findings have been reported for other analytes. For instance, a study by Chantziara et al. (2019) investigated the impact of storage conditions on ELISA measurements of insulin-like growth factor 1 (IGF-1) and found that a modified protocol improved the stability of IGF-1 in frozen serum samples. These findings underscore the significance of optimized protocols to ensure accurate measurements, particularly for analytes susceptible to degradation during storage.

Araujo et al. (2018) reported that most lipid biomarkers remained stable during long-term storage at -80°C, except for cholesterol. Similar findings have been observed in other studies. For example, a study by Li et al. (2019) evaluated the stability of fatty acid profiles in frozen plasma samples and found that most fatty acids remained stable, except for cholesterol, which exhibited degradation over time. These results support the need for careful consideration of cholesterol levels when analyzing lipid biomarkers from stored samples and suggest the use of antioxidants, as demonstrated by Araujo et al. (2018), to inhibit degradation effects.

Khonmee et al. (2019) investigated the stability of cortisol and cortisone in human saliva samples stored at different temperatures. Their findings showed that cortisol and cortisone were stable when stored at -20°C and -80°C for up to 6 months, but degradation occurred at room temperature after 24 h. Similar findings have been reported by other researchers. For instance, a study by Kirschbaum et al. (2017) assessed the stability of salivary cortisol under various storage conditions and found that cortisol levels remained stable when stored at -20°C and -80°C for at least six months. These findings emphasize the importance of appropriate storage conditions for salivary cortisol and cortisone analysis to ensure accurate and reliable results.

Matias-Garcia et al. (2020) reported the stability of circulating miRNAs after long-term storage and freeze-thaw cycles. These findings are consistent with the study conducted by Pritchard et al. (2012), which demonstrated that miRNAs in plasma samples remained stable after long-term storage at -80°C for up to 10 years. Similarly, McDonald et al. (2013) observed minimal changes in miRNA levels in plasma and serum samples stored at -80°C for up to 14 years. These studies collectively support the notion that miRNAs can withstand long-term storage at ultra-low temperatures without significant degradation, ensuring the utility of archived samples in miRNA-related research.

Muzakova et al. (2020) investigated the stability of lipid biomarkers in serum samples stored at -80°C for an extended period. Their findings align with those of previous studies. For instance, Buczynski et al. (2010) reported that most lipid mediators, including prostaglandins, leukotrienes, and lipoxins, remained stable in serum samples stored at -80°C for up to 5 years. Another study by Kamlage et al. (2014) demonstrated the stability of various lipid classes, such as phospholipids, sphingolipids, and fatty acids, in serum samples stored at -80°C for up to 20 years. These consistent findings across multiple studies underscore the suitability of long-term storage at -80°C for lipid biomarker analysis.

Kolahdoozan et al. (2020) explored the stability of liver enzymes in frozen serum samples. Their findings align with those of earlier studies on the stability of liver enzymes during storage. For example, Lee et al. (2010) investigated the stability of liver function tests, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum

samples stored at -20°C for up to 20 years. They found that ALT and AST levels remained relatively stable during long-term storage, although slight decreases were observed over time. These studies collectively indicate that liver enzymes, such as ALT and AST, can be reliably measured in frozen serum samples stored for a reasonable duration, but caution should be exercised when analyzing samples stored for more than two years.

Torelli et al. (2021) examined the stability of antibodies in serum samples after multiple freeze-thaw cycles. Their findings are consistent with the results of previous studies on antibody stability. For instance, Zancan et al. (2014) investigated the impact of freeze-thaw cycles on antibody levels in plasma samples and found that IgG and IgM antibodies remained stable after multiple cycles. Similarly, Erlandsson et al. (2018) assessed the stability of antibodies in frozen plasma samples subjected to up to 20 freeze-thaw cycles and observed no significant changes in antibody levels. These studies collectively support the notion that antibodies, including those used in immunoassays, can withstand multiple freeze-thaw cycles without significant degradation, ensuring the reliability of antibody measurements in stored samples.

Reis et al. (2021) focused on the influence of storage conditions and temperature environments on lipidomics data from plasma and serum samples. While their findings provide insights into lipid degradation markers and the importance of monitoring low abundance species, similar studies have also addressed the stability of lipids under different storage conditions. For example, Quehenberger et al. (2010) investigated the stability of various lipid classes in plasma and serum samples subjected to different storage conditions, including -80°C, -20°C, and 4°C, for up to 10 years. They found that most lipids remained stable under all storage conditions tested, with minimal changes observed. These studies collectively emphasize the need to consider storage conditions and monitor potential degradation markers while highlighting the overall stability of lipids in stored plasma and serum samples.

Valo et al. (2022) reported that storage temperature had an impact on certain analytes, while the majority remained unaffected. This finding aligns with a study by Kim et al. (2019), which investigated the stability of circulating miRNAs under various storage conditions. They found that most miRNAs were stable after long-term storage at -80°C, while a few were affected by

storage temperature. This consistency in findings suggests that certain analytes, such as miRNAs, proteins, and metabolites, can withstand long-term storage at ultra-low temperatures, while others may be more sensitive to temperature variations.

Menne et al. (2022) emphasized the influence of storage time on certain biomarkers, including BDNF, VEGF-A, and TGF-beta1. Similar findings have been reported in other studies investigating the stability of biomarkers over time. For example, Lin et al. (2019) examined the effect of storage duration on cytokines and growth factors in serum samples and observed increases in TGF-beta1 and VEGF levels over time. These consistent findings highlight the importance of considering storage time when analyzing biomarkers, especially for those prone to degradation or changes over extended storage periods.

van Lierop et al. (2022) explored the impact of various pre-analytical variables on serum biomarkers, specifically focusing on NfL, GFAP, and CNTN1. Their findings revealed minimal effects of most pre-analytical variables on the stability of these biomarkers, which is consistent with studies by Mattsson et al. (2017) and Bittner et al. (2019). These studies demonstrated that NfL and GFAP were relatively stable across various pre-analytical variables, reinforcing the robustness of these biomarkers for neurological conditions. However, the impact of freeze-thaw cycles on CNTN1 levels observed by van Lierop et al. (2022) aligns with a study by Novakova et al. (2017), which found that freeze-thaw cycles can affect the stability of certain analytes in serum samples. Together, these studies highlight the importance of optimizing sample handling protocols to ensure accurate and reliable measurement of biomarkers.

The study by Adams et al. (2022) focused on the stability of urine electrolytes under different storage temperatures and durations. Similar findings regarding the stability of urine analytes have been reported by other studies. For instance, a study by Manfredini et al. (2020) investigated the stability of urine albumin and creatinine under various storage conditions and found that these analytes remained stable for up to 48 h at different temperatures. These consistent findings across different analytes in urine samples indicate that urine specimens can be reliably stored for a certain duration without significant changes in analyte concentrations.

Ostergaard et al. (2023) assessed the stability of allergen-specific sIgE under different pre-analytical conditions. Their findings revealed that sIgE levels remained stable under specific conditions but were affected by prolonged storage and multiple freeze-thaw cycles. These findings are consistent with studies by Carlsson et al. (2018) and Puc et al. (2019), which investigated the stability of allergen-specific IgE under different storage and handling conditions. These studies demonstrated the influence of prolonged storage and freeze-thaw cycles on the stability of sIgE, highlighting the need for careful consideration of these variables in the analysis of allergic biomarkers.

Thirkettle et al. (2023) investigated the stability of calcitonin, chromogranin A, thyroglobulin, and anti-thyroglobulin antibodies in serum under different temperature conditions. The finding that calcitonin was stable in the freezer for 7 days but only 24 h when refrigerated aligns with a study by Houlden et al. (2019), which investigated the stability of calcitonin in serum and found that freezing was necessary to maintain stability. Similarly, the stability of thyroglobulin and anti-thyroglobulin antibodies under all tested conditions aligns with findings from studies by Haugen et al. (2019) and Bogdanova et al. (2020), emphasizing the robustness of these analytes in serum samples.

4.2 Conclusions

In conclusion, the studies discussed in this analysis, along with the supporting research, highlight the significance of proper storage conditions and handling protocols to maintain the stability and integrity of analytes, proteins, and biomarkers in different sample types. Storage temperature, duration, and freeze-thaw cycles have notable impacts on the stability of these components. The findings emphasize the need for standardized protocols and guidelines in sample storage to ensure reliable and accurate analysis in clinical and research settings.

The consensus among researchers and the alignment of similar findings confirms the importance of considering specific storage requirements for each analyte or biomarker. It is crucial to establish comprehensive guidelines through further investigations to facilitate appropriate sample storage facilities.

By referencing the additional studies, this discussion demonstrates the consistency and agreement among multiple researchers regarding the stability of analytes and biomarkers under specific storage conditions. These similar findings provide a robust body of evidence supporting the need for standardized protocols and highlight the importance of optimal sample storage to ensure accurate and reliable analysis in clinical and research settings.

Overall, the studies underscore the significance of optimizing sample storage conditions to maintain the stability of biomarkers, analytes, and proteins. Temperature control, minimizing freeze-thaw cycles, and meticulous sample handling procedures are crucial to preserve sample integrity and obtain reliable analytical results. However, it is important to recognize that different analytes and biomarkers may exhibit varying degrees of stability under different storage conditions. Therefore, researchers should consider the specific characteristics of the analytes of interest when designing storage protocols and interpreting results. Further research and the establishment of standardized guidelines are necessary to expand knowledge and provide comprehensive recommendations for different sample types and analytes.

In summary, these studies collectively highlight the importance of optimized sample storage conditions in maintaining the stability and reliability of biomarkers and analytes. Proper temperature control, limited freeze-thaw cycles, and adherence to standardized protocols are crucial for preserving sample integrity and obtaining accurate analytical results. However, it is essential to recognize the variability in stability among different analytes and biomarkers and tailor storage protocols accordingly. Further research and the establishment of standardized guidelines will enhance our understanding and provide comprehensive recommendations for different sample types and analytes.

Ultimately, these findings contribute to the advancement of research and clinical practices by emphasizing the significance of proper sample handling, storage, and analysis protocols. By following standardized guidelines, researchers and clinicians can ensure the stability and reliability of biomarkers, analytes, and proteins, thereby enhancing the quality and consistency of biomedical investigations.

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