

University of Groningen

## Influence of Storage and Inter- and Intra-Assay Variability on the Measurement of Inflammatory Biomarkers in Population-Based Biobanking

van Waateringe, Robert P.; Kobold, Anneke C. Muller; van Vliet-Ostaptchouk, Jana V.; van der Klauw, Melanie M.; Koerts, Jan; Anton, Gabriele; Peters, Annette; Trischler, Gerlinde; Kvaloy, Kirsti; Naess, Marit

*Published in:*

Biopreservation and biobanking

*DOI:*

[10.1089/bio.2017.0001](https://doi.org/10.1089/bio.2017.0001)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2017

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

van Waateringe, R. P., Kobold, A. C. M., van Vliet-Ostaptchouk, J. V., van der Klauw, M. M., Koerts, J., Anton, G., Peters, A., Trischler, G., Kvaloy, K., Naess, M., Videm, V., Hveem, K., Waldenberger, M., Koenig, W., & Wolfenbuttel, B. H. R. (2017). Influence of Storage and Inter- and Intra-Assay Variability on the Measurement of Inflammatory Biomarkers in Population-Based Biobanking. *Biopreservation and biobanking*, 15(6). <https://doi.org/10.1089/bio.2017.0001>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Influence of Storage and Inter- and Intra-Assay Variability on the Measurement of Inflammatory Biomarkers in Population-Based Biobanking

Robert P. van Waateringe,<sup>1</sup> Anneke C. Muller Kobold,<sup>2</sup> Jana V. van Vliet-Ostaptchouk,<sup>1</sup> Melanie M. van der Klauw,<sup>1</sup> Jan Koerts,<sup>2</sup> Gabriele Anton,<sup>3</sup> Annette Peters,<sup>3</sup> Gerlinde Trischler,<sup>4</sup> Kirsti Kvaløy,<sup>5</sup> Marit Naess,<sup>5</sup> Vibeke Videm,<sup>6,7</sup> Kristian Hveem,<sup>5</sup> Melanie Waldenberger,<sup>3</sup> Wolfgang Koenig,<sup>4</sup> and Bruce H.R. Wolffenbuttel<sup>1</sup>

**Background:** In the present study, we examined the effect of sample storage on the reproducibility of several inflammatory biomarkers, including high-sensitivity C-reactive protein (hsCRP), high-sensitivity interleukin-6 (hsIL6), and high-sensitivity tumor necrosis factor alpha (hsTNF $\alpha$ ). In addition, we assessed inter- and intra-assay variability between collaborating biobanks.

**Methods:** In total, 240 fasting plasma samples were obtained from the LifeLines biobank. Samples had been stored for less than 2 or more than 4 years at  $-80^{\circ}\text{C}$ . Measurements were performed at three different laboratories. hsCRP was measured by immunonephelometry and ELISA, hsIL6, and hsTNF $\alpha$  samples were measured with ELISAs from two different manufacturers. For confirmation, similar analyses were performed on samples obtained from a subpopulation of 80 obese individuals. Passing–Bablok regression analysis and Bland–Altman plots were used to compare the results.

**Results:** We observed good stability of samples stored at  $-80^{\circ}\text{C}$ . hsCRP measured on the day of blood draw was similar to levels measured after more than 4 years of storage. There were small interlaboratory differences with the R&D ELISAs for hsIL6 and hsTNF $\alpha$ . We found a linear correlation between the Bender Medsystems ELISA and the R&D ELISA for hsIL6, with significantly higher levels measured with the R&D ELISA. Over 90% of hsTNF $\alpha$  samples measured with the IBL ELISA were below the detection limit of 0.13 ng/L, rendering this assay unsuitable for large-scale analysis. Similar results were found in the confirmation study.

**Conclusion:** In summary, plasma hsCRP showed good stability in samples stored for either less than 2 years or more than 4 years at  $-80^{\circ}\text{C}$ . Both the R&D and Bender Medsystems for hsIL6 measurement yielded similar results. The IBL hsTNF $\alpha$  assay is not suited for use in biobanking samples. Assays for the measurement of inflammatory biomarker assays should be rigorously tested before large sample sets are measured.

**Keywords:** biobanking, assay, storage, variability, ELISA, nephelometry

## Introduction

POPULATION-BASED BIOBANKS are involved in processing and storage of biospecimens for future studies and have, sometimes over decades, also accumulated epidemiological

data. Several new biobanking initiatives have been launched, building on these strengths. The Biobank Standardization and Harmonisation for Research Excellence in the European Union (BioSHaRE-EU) project is based on international collaborative projects between European and

Departments of <sup>1</sup>Endocrinology and <sup>2</sup>Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

<sup>3</sup>Research Unit of Molecular Epidemiology and Institute of Epidemiology II, Helmholtz Zentrum München, Munich, Germany.

<sup>4</sup>Department of Internal Medicine II–Cardiology, University of Ulm Medical Centre, Ulm, Germany.

<sup>5</sup>Department of Public Health and General Practice, Faculty of Medicine, NTNU-Norwegian University of Science and Technology, Trondheim, Norway.

<sup>6</sup>Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, NTNU-Norwegian University of Science and Technology, Trondheim, Norway.

<sup>7</sup>Department of Immunology and Transfusion Medicine, St. Olavs Hospital, Trondheim, Norway.

Canadian institutes and European cohort studies. The project has developed and applied several methods and tools for harmonization and standardization in European biobanks and major biomedical studies.<sup>1</sup> If an efficient organization of these existing resources is implemented, rapid progress can be achieved. This has been impressively demonstrated by the success of genome-wide association studies (GWAS) and the combined analysis of these data in large meta-analyses.<sup>2</sup>

For other new research fields such as metabolomics<sup>3</sup> and epigenomics,<sup>4</sup> the availability of samples of high quality is important. Standardization of sample collection, preanalytics, and harmonization and standardization of high-throughput assays to measure biomarkers such as inflammatory biomarkers are crucial.<sup>5,6</sup> In general, biomarkers are defined as objectively measurable indicators for biological or pathobiological processes or pharmacological responses toward medical treatment.<sup>7</sup> Biomarkers may serve as surrogate endpoints that correlate with clinical endpoints, indicate disease progression and regression under therapy, and may allow outcome prediction. For optimal collaboration between cohort studies or biobanks, harmonization and standardization of analytical procedures of biomarker measurements are essential. Analytical results may be affected by preanalytical conditions and analytical variability.<sup>6,8</sup> For example, different types of samples may be available for analyses, but may yield different results upon measurement (e.g., serum vs. plasma).

Furthermore, biobanks often use samples that may have been stored at different temperatures or storage duration,<sup>9–11</sup> and the amount of sample material may be very limited. Therefore, evaluating the stability of stored samples is important. Finally, results may be based on the use of different assays, techniques, or equipment.

Studies investigating the variability of sample processing, different assays, use of different sample types, and reproducibility of archived samples are scarce, particularly with regard to measurement of inflammatory biomarkers such as cytokines.<sup>12</sup> Aziz et al. examined preanalytical variables on high-sensitivity C-reactive protein (hsCRP) and found that hsCRP levels in serum were not significantly different from plasma samples.<sup>13</sup> In addition, storage of samples at  $-70^{\circ}\text{C}$  for 3 weeks had no effect on hsCRP concentrations. However, some contradictory data regarding long-term storage of hsCRP exist.<sup>14,15</sup> Only a few studies have evaluated and compared different assays to measure other inflammatory markers. Lopez-Campos et al. compared enzyme-linked immunosorbent assay (ELISA) with immunonephelometry for the measurement of hsCRP in patients with stable COPD.<sup>16</sup> Although the serum hsCRP concentrations measured by ELISA and nephelometry correlated well, concentrations measured using ELISA tended to be lower.

The present study aimed to assess the reproducibility of several inflammatory biomarkers after storage for either less than 2 years or more than 4 years at  $-80^{\circ}\text{C}$ . In addition, we examined inter- and intra-assay variability for the measurement of hsCRP, high-sensitivity interleukin-6 (hsIL6), and high-sensitivity tumor necrosis factor alpha (hsTNF $\alpha$ ) between collaborating biobanks. The IBL hsTNF $\alpha$  assay and Bender MedSystems hsIL6 assay were specifically chosen for comparison because they use a smaller sample volume than the R&D ELISA.

## Materials and Methods

### *Participants and sample collections*

Subjects included were participants from the LifeLines Cohort Study.<sup>17</sup> Lifelines is a multidisciplinary, prospective, population-based cohort study examining, in a unique three-generation design, the health and health-related behaviors of persons living in the north of The Netherlands. It started in 2007 and employs a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, physical, and psychological factors that contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics. The methodology has been described previously.<sup>18</sup> All participants were between 18 and 90 years old at the time of enrollment. They provided written informed consent before participating in the study. The study protocol was approved by the medical ethics review committee of the University Medical Center Groningen (UMCG).

### *Laboratory measurements*

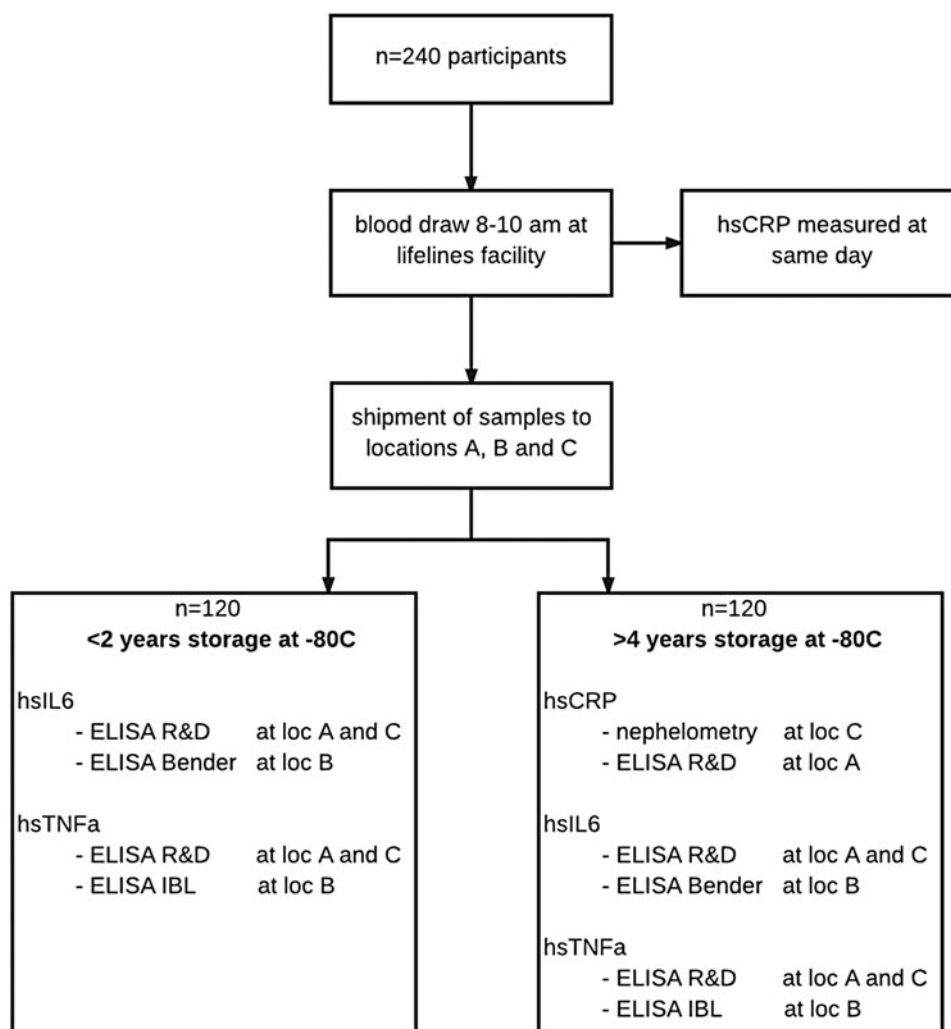
For the current study, a 900- $\mu\text{L}$  plasma sample from each of the 240 participants was selected by the LifeLines Scientific Bureau according to the study proposal. Blood samples were obtained from healthy individuals ( $n=80$ ), individuals with type 2 diabetes ( $n=80$ ), and those with clinical macrovascular disease who reported a previous myocardial infarction ( $n=80$ ). All samples had been drawn by venipuncture in the fasting state, between 8 and 10 a.m. After blood withdrawal, the EDTA tubes were transported at  $4^{\circ}\text{C}$  (1.5 hours) to the LifeLines laboratory. Tubes were centrifuged directly after arrival, and plasma was stored in 0.9-mL aliquots in Thermo Scientific Matrix 2D barcoded 1.0-mL tubes at  $-80^{\circ}\text{C}$ . Only hsCRP was measured on the same day at the department of clinical chemistry at UMCG.

For the current study, stored samples were thawed once and aliquoted in a 96-well polystyrene microplate and stored again at  $-80^{\circ}\text{C}$  before shipment on dry ice 1–2 weeks later to the laboratories in Trondheim, Norway, Ulm in Germany, and the department of clinical chemistry at UMCG, where analysis was performed within 2–4 weeks after receipt. The laboratories have been indicated in this article with a letter (allocated by random number).

More information on the distribution of the samples over the three locations and detection limits of specific assays, as well as storage time, is given in Figure 1 and Table 1.

Serum hsCRP was measured at the day of blood collection at the LifeLines laboratory using latex-enhanced immunonephelometry (Siemens Healthcare Diagnostics). Standardization was based on protein reference ERM DA 470 (CRM 470). The results of the baseline hsCRP measurements were compared with the results from 4-year stored samples using immunonephelometry. Interassay comparison was examined for the R&D ELISA (location A) and immunonephelometry (location C). Due to the limited amount of sample, we skipped hsCRP measurements at location B beforehand.

The R&D ELISA for hsIL6 measurement was used both at locations A and C and used 100  $\mu\text{L}$  of the sample for analysis. The Bender MedSystems ELISA kit (location B) was specifically chosen as it used a smaller volume of sample (50  $\mu\text{L}$ ) for analysis. Measurement of hsIL6 with the Bender MedSystems ELISA was compared with the R&D ELISA at location C.



The IBL ELISA (location B) for the measurement of hsTNF $\alpha$  was specifically chosen for comparison, as it uses a smaller volume of sample (50  $\mu$ L), and was compared with the R&D ELISA used at location C.

For validation of initial results, additional analyses of plasma hsIL6 and hsTNF $\alpha$  were performed by one dedicated analyst in a set of 80 samples obtained from obese individuals who participated in a weight reduction program in The Netherlands (the LOWER study, [www.clinicaltrials.gov](http://www.clinicaltrials.gov), NCT00862953). Those samples had been stored at  $-80^{\circ}\text{C}$  for an average period of 5.5 (range 4–7) years. The results of

hsIL6 measurement using the R&D ELISA were compared with the results obtained by the Bender MedSystems. The results of hsTNF $\alpha$  measurement using the IBL ELISA were compared with the results obtained by the R&D ELISA.

### Statistical analyses

Statistical analyses were performed using SPSS, version 22. Passing–Bablok regression analysis and Bland–Altman plots were created with MedCalc (MedCalc, Ostend, Belgium) to evaluate inter- and intra-assay variation, also between

TABLE 1. ASSAY OVERVIEW FOR DIFFERENT INFLAMMATORY BIOMARKERS

Inflammatory biomarker	Assay	Producer	Standardization	Detection limit	Lowest vs. highest standard	Intra-assay variation	Interassay variation
hsCRP	ELISA	R&D systems	NIBSC 85/506	0.010 mg/L	0.78–50 mg/L	4.4%	6.0%
hsIL6	ELISA	R&D systems	NIBSC 89/548	0.039 ng/L	0.156–10 ng/L	6.9%	9.6%
hsTNF $\alpha$	ELISA	R&D systems	NIBSC 88/786	0.106 ng/L	0.5–32.0 ng/L	8.7%	10.4%
hsIL6	ELISA	Bender MedSystems	NIBSC 89/548	0.030 ng/L	0.08–5.0 ng/L	4.9%	6.0%
hsTNF $\alpha$	ELISA	IBL International	NIBSC 87/650	0.130 ng/L	0.31–20.00 ng/L	8.5%	9.8%
hsCRP	Nephelometry	Siemens Healthcare	CRM 470	0.175 mg/L	0.175–11.00 mg/L	7.6%	unknown

TABLE 2. BASELINE CHARACTERISTICS OF THE 240 PARTICIPANTS IN THE LIFELINES COHORT STUDY

	<i>Healthy</i>	<i>Post-MI</i>	<i>Type 2 diabetic</i>
	N=82	N=83	N=75
Male/female	44/38	61/22	32/43
Age (yrs)	54±11	63±10	62±11
BMI (kg/m <sup>2</sup> )	25.7±3.0	28.0±3.8	30.1±4.5
Fasting blood glucose (mM)	4.9±0.5	5.5±0.9	7.6±1.9
HbA1c (%)	5.6±0.3	5.9±0.5	7.0±1.0
Total cholesterol (mM)	5.6±1.0	4.6±1.1	4.4±0.9
HDL-cholesterol (mM)	1.52±0.39	1.29±0.36	1.28±0.29
LDL-cholesterol (mM)	3.67±0.91	2.86±0.97	2.58±0.80
Triglycerides (mM)	1.10 (0.86–1.54)	1.13 (0.91–1.64)	1.48 (1.14–1.91)

different laboratories, as well as the influence of storage (time) on the measurement of hsCRP, hsIL6, and hsTNF $\alpha$ .

## Results

The characteristics of the study population are shown in Table 2. Individuals who had suffered from an MI were older than healthy subjects and those with type 2 diabetes. Both BMI and hsCRP levels were highest among subjects with type 2 diabetes compared with subjects from the other two groups.

Nephelometry was used to compare the hsCRP results from baseline measurement with the results from samples stored for more than 4 years. Both Figure 2 and Supplementary Figure S1 (Supplementary Data are available online at [www.liebertpub.com/bio](http://www.liebertpub.com/bio)) show identical results for both the baseline samples and samples that had been stored for more than 4 years. For the interassay comparison between the R&D ELISA and immunonephelometry, 200 of the 240 plasma samples were available for analysis; 3 samples were below the detection limit of the ELISA, while 37 samples were above the detectable range.

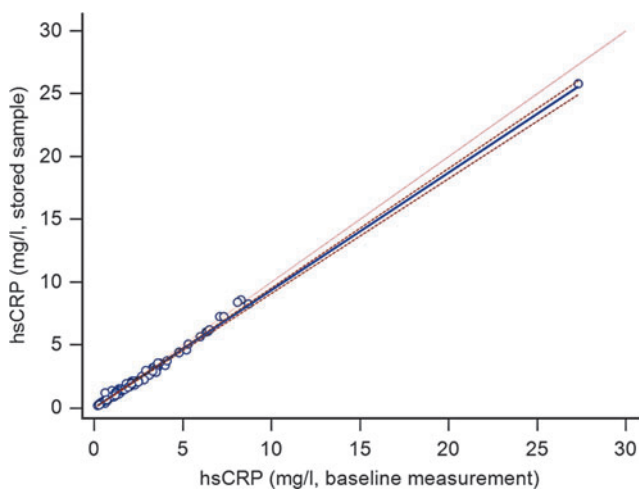
These samples were not measured again in dilution due to insufficient sample material. Plasma hsCRP levels measured by

the R&D ELISA tended to be lower than levels measured with immunonephelometry (Fig. 3 and Supplementary Fig. S2).

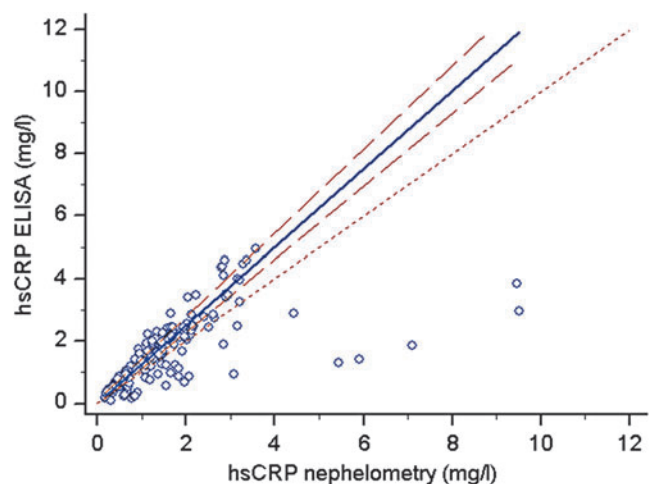
Regarding hsIL6, there were significant differences in the results, as well as differences regarding the detection limit. The R&D ELISA yielded similar results in both laboratories (locations A and C) except for some outliers (Fig. 4).

However, at location A, 38 samples were below the detection limit of the assay, whereas at location C, using the same ELISA, 2 samples had concentrations above 5 ng/l. Next, a linear correlation was found between the results obtained using the Bender MedSystems ELISA (location B) and the R&D ELISA (location C), but with statistically significant higher levels measured by the R&D assay (Fig. 5 and Supplementary Fig. S3). These results were comparable for samples stored up to 2 years and samples stored for more than 4 years showing no effect of storage time on sample stability. With the Bender MedSystems ELISA used at location B, two samples could not be measured due to insufficient sample material, while three samples were below the detection limit of the assay.

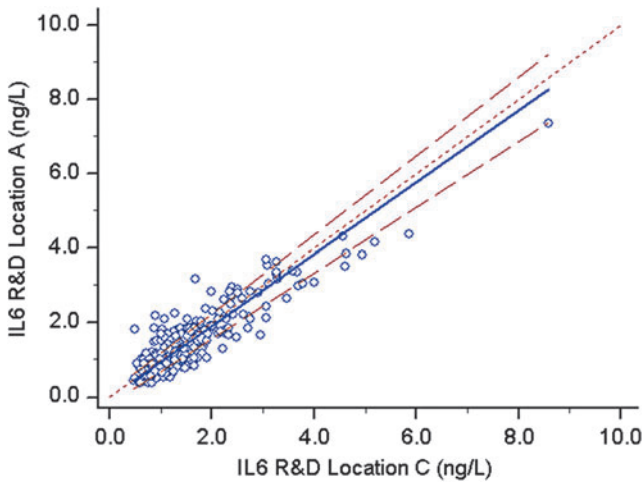
The R&D ELISA for hsTNF $\alpha$  was used at locations A and C. Unfortunately, 177 samples were lost for evaluation due to an error by a technician at location A. Despite



**FIG. 2.** Passing–Bablok correlation between plasma hsCRP measured at the day of blood collection and in plasma samples stored for more than 4 years. All the samples were measured using nephelometry.  $N=116$  samples measured at baseline (the day of blood collection) and after >4 years of storage, in 124 samples, no measurements of hsCRP were performed during screening of the participant.



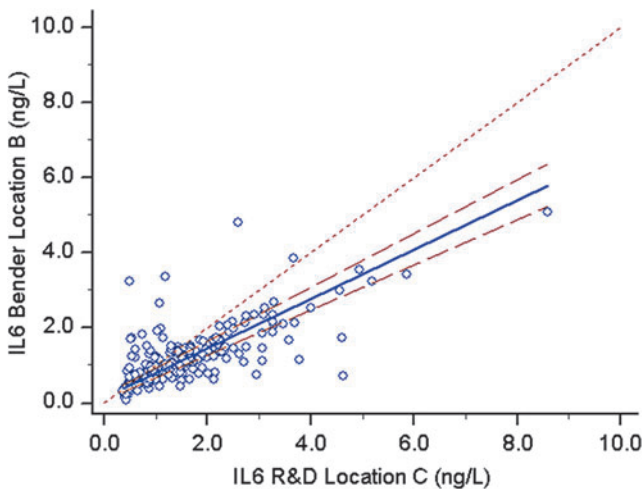
**FIG. 3.** Passing–Bablok correlation for stored plasma hsCRP samples measured with nephelometry (location C) and R&D ELISA (location A). Stored hsCRP samples measured at two locations using different assays. Location A used the R&D ELISA, whereas location C used nephelometry. Two hundred of the 240 samples were available for analysis; 3 samples were below the detection limit of the ELISA, while 37 samples were above the detectable range.



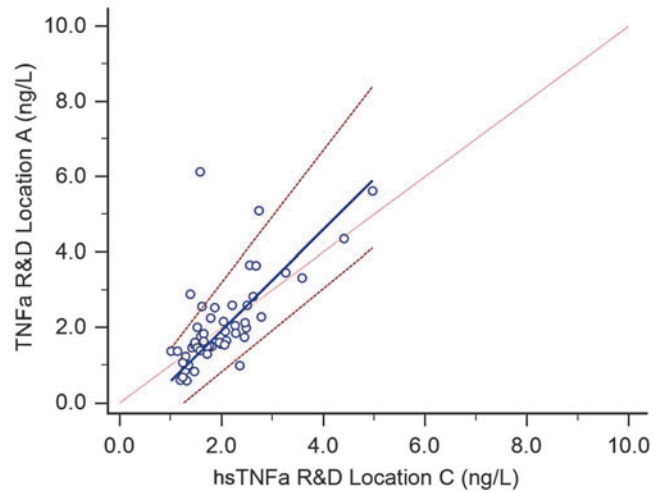
**FIG. 4.** Passing–Bablok correlation for plasma hsIL6 measured with R&D ELISA (location C vs. location A). Stored hsIL6 samples measured at two locations using the same ELISA. Both locations A and C used the R&D ELISA. Two hundred of the 240 samples were available for analysis. At location A, 38 samples were below the detection limit of the assay, whereas at location C, using the same ELISA, 2 samples had concentrations above 5 ng/L. hsIL6, high-sensitivity interleukin 6.

this, a reasonable agreement in the hsTNF $\alpha$  results between locations A and C was observed, except for some outliers (Fig. 6). Since there were only 4-year stored samples available, we were not able to examine the effect of storage time on the samples. At location B, using the IBL ELISA, only 16 samples yielded feasible and measurable results. All other samples proved to be below the detection limit of 0.13 ng/L (Fig. 7).

For confirmation and replication, we performed an additional evaluation of assays for the measurement of plasma hsTNF $\alpha$

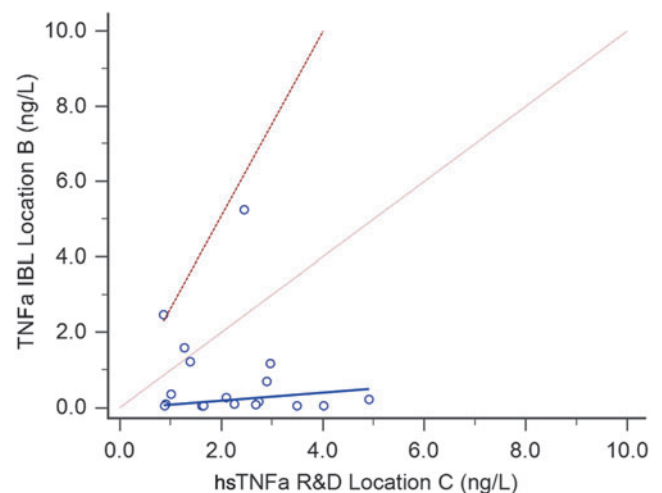


**FIG. 5.** Passing–Bablok correlation for plasma hsIL6 measured with R&D ELISA (location C) and Bender MedSystems (location B). Stored hsIL6 samples measured at two locations using different ELISAs. Location B used the Bender MedSystems ELISA, while Location C used the ELISA from R&D. Two hundred thirty-three of the 240 samples were available for analysis, 4 samples could not be measured due to insufficient sample material, while 3 samples were below the detection limit of the assay.

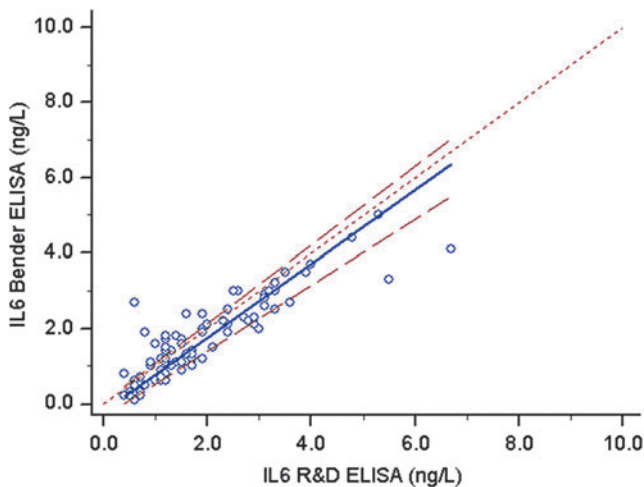


**FIG. 6.** Passing–Bablok correlation for plasma hsTNFa measured with R&D ELISA (location C vs. location A). Stored hsTNFa samples measured at two locations using the same ELISA. Both locations used the R&D ELISA. At location C, one serum sample yielded a result >16 ng/mL. At location A, 7 samples were below the detection limit and one sample yielded results above the detection limit. However, 177 samples (3 ELISA kits) were lost for evaluation due to an error. Only 54 samples could be compared with the results in location C. hsTNFa, high-sensitivity tumor necrosis factor alpha.

and hsIL6 in stored samples (mean 5.5 years, range 4–7 years) obtained from a population of 80 obese individuals (50% males, mean age  $52 \pm 12$  years, BMI  $38.0 \pm 6.2$  kg/m<sup>2</sup>) participating in a weight reduction program. For hsIL6, an excellent correlation was found regarding the results obtained by the R&D ELISA and the Bender MedSystems ELISA (Fig. 8). However, regarding hsTNF $\alpha$  measurements, poor results for the IBL ELISA compared with R&D ELISA, already described above, were confirmed: 66 of 80 samples yielded results below the detection limit set by the manufacturer (Fig. 9).



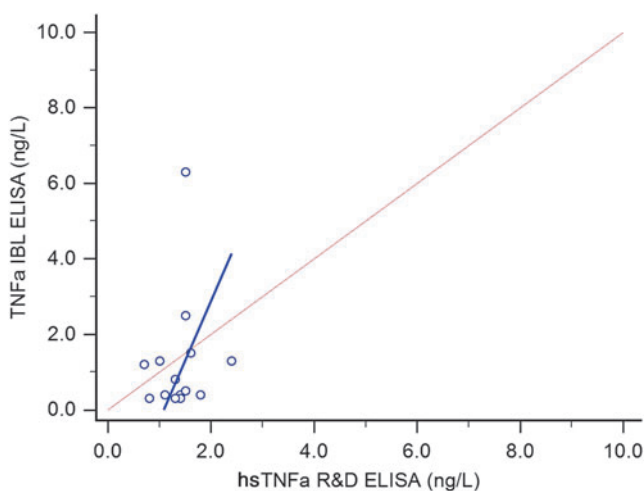
**FIG. 7.** Passing–Bablok correlation for plasma hsTNFa measured with R&D ELISA (location C) and IBL ELISA (location B). Stored hsTNFa samples measured at two locations using different ELISAs. Location B used the IBL ELISA and location C used the R&D ELISA. Sixteen of the 240 samples were available for analysis; the other samples were below the detection limit of the IBL ELISA.



**FIG. 8.** Confirmation study showing Passing–Bablok correlation for plasma hsIL6 measured with R&D ELISA versus Bender MedSystems ELISA in 80 obese subjects participating in a weight reduction program. Stored hsIL6 samples measured using both the R&D ELISA and the Bender MedSystems ELISA.

## Discussion

In the present study, we compared different assays for the measurement of the inflammatory biomarkers, hsCRP, hsIL6, and hsTNF $\alpha$ , and assessed the effect of storage time on the reproducibility of those biomarkers. Our data showed that short- to medium-term storage (less than 2 years, more than 4 years) did not influence the plasma levels of hsCRP and hsIL6 measured by nephelometry and by ELISA, respectively, although small differences between two hsIL6 ELISAs were identified. Concerning the hsTNF $\alpha$  measurements, at one location, the majority of samples was lost due to an analytical error, while the IBL ELISA failed to provide results within the detection limit of the assay.



**FIG. 9.** Confirmation study showing Passing–Bablok correlation for plasma hsTNF $\alpha$  measured with R&D ELISA versus IBL ELISA in 80 obese subjects participating in a weight reduction program. Stored hsTNF $\alpha$  samples measured using both the R&D ELISA and the IBL ELISA. Fourteen of the 80 samples were available for analysis; the other 66 were below the detection limit of the IBL ELISA.

hsCRP is frequently measured in clinical and epidemiological studies. We observed only a moderate agreement between results of nephelometry and ELISA. Similar findings have been reported in a study by Lopez-Campos et al.<sup>16</sup> who reported higher hsCRP concentrations when measured by nephelometry compared with measurements by ELISA.

Our results with the ELISA method were in agreement with Lopez-Campos et al. clearly showing an upper limit of detection. For hsCRP concentrations above 25 mg/L, a sample needs to be diluted and reanalyzed, which is not the case with nephelometry.

Nevertheless, our results of the stored samples showed excellent sample stability after >4 years of storage at  $-80^{\circ}\text{C}$ . These results indicate that the single thaw–freeze step had no influence on hsCRP levels. Other studies regarding long-term storage of hsCRP have reported contradictory results.<sup>14</sup> Doumatey et al. showed that serum hsCRP concentrations remained stable with storage for up to 11 years at  $-80^{\circ}\text{C}$ .<sup>14</sup> This was, however, in disagreement with an article from Japan reporting that hsCRP levels increased over time in samples stored at  $-80^{\circ}\text{C}$  for 13.8 years.<sup>15</sup>

Measurement of hsIL6 with the same R&D ELISA method in different laboratories showed good agreement. However, the fact that 38 samples gave results below the detection limit in one laboratory should be taken into account. It should be noted that we have no data on hsIL6 levels measured on the day of blood collection. There is remarkably little information on studies investigating different assays or the influence of storage time on measurement of IL6. A recent study by Hardikar et al. showed moderate stability of IL6 samples that were stored at  $-80^{\circ}\text{C}$  for less than 13 years.<sup>19</sup>

A previous study examining the influence of short-term storage of several biomarkers showed excellent stability for TNF $\alpha$  in samples stored at  $-80^{\circ}\text{C}$  for 90 days.<sup>20</sup> Although this is encouraging, the relevance of these data for biobanking, where samples have been or will be stored for many years, is limited. As was the case for hsIL6, between-laboratory variation of the R&D hsTNF $\alpha$  ELISA was very small. When biomarker measurements are performed on stored biobank samples, the amount of sample needed for a specific measurement is of great importance. This was the main reason why we chose the Bender MedSystems hsIL6 assay for our comparison studies as the assay required only 50  $\mu\text{L}$  of sample. In contrast, the R&D ELISAs required 100 and 200  $\mu\text{L}$  for hsIL6 and hsTNF $\alpha$ , respectively. A head-to-head comparison between the Bender MedSystems and R&D ELISAs demonstrated a reasonable agreement, although the Bender MedSystems assay gave significantly lower plasma levels of hsIL6.

The majority of hsTNF $\alpha$  samples at location A were lost due to an error by a technician, whereas at location B, many samples were below the detection limit of the assay despite meticulously following specific instructions. Our replication study confirmed that this was not an incidental finding. Despite the low amount of sample needed, we can, currently, not recommend the use of this specific assay. When choosing an assay, it is advisable to thoroughly test all assays needed for the study before measuring samples obtained from long-term storage in biobanks. In addition, the traceability of the standardization of the assay is also very important. These samples are usually expensive samples, with limited amounts of sample material available in storage. For testing purposes, we therefore recommend the use of sample material obtained in

daily practice, samples obtained from (paid) volunteers, or anonymized leftover material from a laboratory or blood bank facility. As far as we know, the LifeLines facility has not split any samples for the purpose of repeated prospective follow-up measurements within the same individual.

In summary, plasma hsCRP and hsIL6 samples showed good stability when stored for either less than 2 years or more than 4 years at  $-80^{\circ}\text{C}$ . Even when the same ELISA method was used, there were small variations in results reported by different laboratories. Although it appears attractive to utilize assays that need only small volumes of samples, such assays should be rigorously tested before large sample sets are measured.

### Acknowledgments

The authors wish to acknowledge all participants of the LifeLines Cohort Study and everybody involved in the setup and implementation of the study. The authors wish to thank Oddrun Storro, Department of Public Health and General Practice of the NTNU-Norwegian University of Science and Technology, and Bettine Haandrikman, Lucy Wagenmakers, and Eduard Heine from the Department of Laboratory Medicine of the UMCG for performing several of the assays.

Part of this article has been published on the BioShare website:

[www.bioshare.eu/sites/default/files/1/D5.9%20Scientific%20paper%20on%20the%20use%20and%20utility%20of%20inflammatory%20markers%20in%20Healthy%20Obese%20individuals.pdf](http://www.bioshare.eu/sites/default/files/1/D5.9%20Scientific%20paper%20on%20the%20use%20and%20utility%20of%20inflammatory%20markers%20in%20Healthy%20Obese%20individuals.pdf)

LifeLines has been funded by a number of public sources, notably the Dutch Government, The Netherlands Organization of Scientific Research NOW [grant 175.010.2007.006], the Northern Netherlands Collaboration of Provinces (SNN), the European fund for regional development, Dutch Ministry of Economic Affairs, Pieken in de Delta, Provinces of Groningen and Drenthe, the Target project, BBMRI-NL, the University of Groningen, and the University Medical Center Groningen, The Netherlands. This work was supported by the National Consortium for Healthy Aging and funds from the European Union's Seventh Framework program (FP7/2007–2013) through the BioSHaRE-EU (Biobank Standardisation and Harmonisation for Research Excellence in the European Union) project, grant agreement 261433. LifeLines (BRIF4568) is engaged in a Bioresource research impact factor (BRIF) policy pilot study, details of which can be found at [www.bioshare.eu/content/bioresource-impact-factor](http://www.bioshare.eu/content/bioresource-impact-factor)

### Author Disclosure Statement

No competing financial interests exist.

### References

- Doiron D, Burton P, Marcon Y, et al. Data harmonization and federated analysis of population-based studies: The BioSHaRE project. *Emerg Themes Epidemiol* 2013;10:12.
- Berndt SI, Gustafsson S, Magi R, et al. Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. *Nat Genet* 2013;45:501–512.
- Inouye M, Kettunen J, Soininen P, et al. Metabonomic, transcriptomic, and genomic variation of a population cohort. *Mol Syst Biol* 2010;6:441.
- Yang J, Loos RJ, Powell JE, et al. FTO genotype is associated with phenotypic variability of body mass index. *Nature* 2012;490:267–272.
- Grizzle WE, Gunter EW, Sexton KC, et al. Quality management of biorepositories. *Biopreserv Biobank* 2015;13:183–194.
- Betsou F, Bulla A, Cho SY, et al. Assays for Qualification and Quality Stratification of Clinical Biospecimens Used in Research: A Technical Report from the ISBER Biospecimen Science Working Group. *Biopreserv Biobank* 2016;14:398–409.
- Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001;69:89–95.
- Ellervik C, Vaught J. Preanalytical variables affecting the integrity of human biospecimens in biobanking. *Clin Chem* 2015;61:914–934.
- Gislefoss RE, Grimsrud TK, Morkrid L. Stability of selected serum hormones and lipids after long-term storage in the Janus Serum Bank. *Clin Biochem* 2015;48:364–369.
- Ikeda K, Ichihara K, Hashiguchi T, et al. Evaluation of the short-term stability of specimens for clinical laboratory testing. *Biopreserv Biobank* 2015;13:135–143.
- Anton G, Wilson R, Yu ZH, et al. Pre-analytical sample quality: Metabolite ratios as an intrinsic marker for prolonged room temperature exposure of serum samples. *PLoS One* 2015;10:e0121495.
- Lee JE, Kim JW, Han BG, et al. Impact of whole-blood processing conditions on plasma and serum concentrations of cytokines. *Biopreserv Biobank* 2016;14:51–55.
- Aziz N, Fahey JL, Detels R, et al. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. *Clin Diagn Lab Immunol* 2003;10:652–657.
- Doumatey AP, Zhou J, Adeyemo A, et al. High sensitivity C-reactive protein (Hs-CRP) remains highly stable in long-term archived human serum. *Clin Biochem* 2014;47:315–318.
- Ishikawa S, Kayaba K, Gotoh T, et al. Comparison of C-reactive protein levels between serum and plasma samples on long-term frozen storage after a 13.8 year interval: The JMS Cohort Study. *J Epidemiol* 2007;17:120–124.
- Lopez-Campos JL, Arellano E, Calero C, et al. Determination of inflammatory biomarkers in patients with COPD: A comparison of different assays. *BMC Med Res Methodol* 2012;12:40.
- Stolk RP, Rosmalen JG, Postma DS, et al. Universal risk factors for multifactorial diseases: LifeLines: A three-generation population-based study. *Eur J Epidemiol* 2008;23:67–74.
- Scholtens S, Smidt N, Swertz MA, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol* 2015;44:1172–1180.
- Hardikar S, Song X, Kratz M, et al. Intraindividual variability over time in plasma biomarkers of inflammation and effects of long-term storage. *Cancer Causes Control* 2014;25:969–976.
- Zander J, Bruegel M, Kleinhempel A, et al. Effect of biobanking conditions on short-term stability of biomarkers in human serum and plasma. *Clin Chem Lab Med* 2014;52:629–639.

Address correspondence to:  
 Bruce H.R. Wolffenbuttel, MD, PhD  
 Department of Endocrinology  
 University of Groningen  
 University Medical Center Groningen  
 HPC AA31, PO Box 30001  
 Groningen 9700 RB  
 The Netherlands  
 E-mail: bwo@umcg.nl