Clinical Chemistry 71:6
677–687 (2025)

Article

Correlations Within and Between Highly Multiplexed Proteomic Assays of Human Plasma

Mary R. Rooney,[a,b] Jingsha Chen,[a,b] Christie M. Ballantyne,[c] Ron C. Hoogeveen,[c] Eric Boerwinkle,[d] Bing Yu,[d] Keenan A. Walker,[e] Pascal Schlosser,[a,f,g] Elizabeth Selvin,[a,b] Nilanjan Chatterjee,[h,i] David Couper,[j] Morgan E. Grams,[k] and Josef Coresh[l,m,*]

INTRODUCTION: The number of assays on proteomic platforms has grown rapidly. The leading platforms, SomaScan and Olink, have strengths and limitations. Comparisons of precision on the latest platforms—SomaScan 11k and Olink Explore HT—have not yet been established.

METHODS: Among 102 participants in the Atherosclerosis Risk in Communities Study (mean age 74 years, 53% women, 47% Black), we used split plasma samples to measure platform precision. CV and Spearman correlations were calculated for each assay. Cross-platform agreement was assessed for overlapping proteins.

RESULTS: SomaScan 11k demonstrated a median correlation of 0.85 for the 10 778 assays and a median CV of 6.8%, similar precision to earlier versions. The 5420 assays on Olink Explore HT exhibited a median correlation of 0.65 and median CV of 35.7%, which was higher than observed in its predecessors (e.g., 19.8% for Olink Explore 3072). Precision of Olink assays was inversely correlated with the percentage of samples above the limit of detection (LOD) ($r = -0.77$). Upon replacing Olink values below the LOD with values half the LOD, the median correlation for Olink assays measured in duplicate increased to 0.79; the median CV decreased to 13.3%. The distribution of between-platform correlations for the 4443 overlapping proteins had peaks at r approximately 0 and at r approximately 0.8. One-tenth of the protein pairs had cross-platform correlations $r \geq 0.8$.

CONCLUSIONS: Precision of these 2 proteomics platforms in human plasma has diverged as the coverage has increased. These results highlight the need for careful consideration in platform selection based on specific research requirements.

Introduction

The number of assays on highly multiplexed proteomic platforms has grown 10-fold over the past 15 years from less than 1000 to ~11 000. As the number of measurable proteins expands, there is a continued need for validation studies of proteomic biomarkers, both in terms of comparisons to earlier versions of the same platform and across different technologies.

The leading aptamer-based and antibody-based platforms have different strengths. For example, Eldjarn et al. (1, 2) demonstrated that the aptamer-based SomaScan 5k (4907 assays, assessed in 35 892 Icelanders) and the antibody-based Olink Explore 3072 (2931 assays, assessed in 46 218 participants of the UK BioBank) had a similar number of *cis*-protein quantitative trait loci among all targets (2120 vs 2101), but SomaScan had fewer *cis*-protein quantitative trait loci among the overlapping targets (1164 vs 1467). Analysis of split plasma measures showed the SomaScan assays to be more precise: median CV of 9.9% vs 16.5% for Olink (1, 2). Head-to-head comparisons of the precision of the newest platforms' versions—SomaScan 11k (>10 700 assays, released in

[a]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States; [b]Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States; [c]Department of Medicine, Baylor College of Medicine, Houston, TX, United States; [d]Department of Epidemiology, University of Texas Health Science Center, Houston, TX, United States; [e]Laboratory of Behavioral Neuroscience, National Institute on Aging, Intramural Research Program, Baltimore, MD, United States; [f]Institute of Genetic Epidemiology, Faculty of Medicine and Medical Center—University of Freiburg, Freiburg, Germany; [g]Centre for Integrative Biological Signalling Studies, University of Freiburg, Freiburg, Germany; [h]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States; [i]Department of Oncology, Johns Hopkins School of Medicine, Baltimore, MD, United States; [j]Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States; [k]Division of Precision Medicine, New York University Grossman School of Medicine, New York, NY, United States; [l]Department of Population Health, New York University Grossman School of Medicine, New York, NY, United States; [m]Department of Medicine, New York University Grossman School of Medicine, New York, NY, United States.

*Address correspondence to this author at: Department of Population Health, New York University Grossman School of Medicine, TRB 227 E. 30th St., Room 707, New York, NY 10003, United States. E-mail josef.coresh@nyulangone.org.

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Received August 16, 2024; accepted February 11, 2025.
https://doi.org/10.1093/clinchem/hvaf030

December 2023) and Olink Explore HT (>5400 assays, released in July 2023)—have not yet been established.

Using data from the Atherosclerosis Risk in Communities (ARIC) Study, we provide estimates of precision based on split plasma samples for the SomaScan 11k and for the Olink Explore HT and report on the cross-platform agreement for proteins overlapping on the SomaScan 11k and Olink Explore HT. We also provide comparisons of SomaScan 11k and Olink Explore HT with clinically used targeted immunoassays.

Materials and Methods

STUDY DESIGN

The ARIC Study is a community-based prospective cohort study that began in 1987–1989 when the participants were 45 to 64 years of age (3). The 15 792 participants were recruited from 4 US communities: suburban Minneapolis, Minnesota; Jackson, Mississippi; Forsyth County, North Carolina; Washington County, Maryland. Visit 2 occurred in 1990–1992 and was attended by 14 348 participants (46–70 years of age). Visit 5 occurred in 2011–2013 and was attended by 6538 participants (66–90 years of age). Plasma samples (never previously thawed) were thawed using a standardized quick thaw and refreeze protocol (see Supplemental Methods). Samples were aliquoted and shipped on dry ice to SomaLogic for SomaScan 11k measurements (visits 2 and 5, samples in duplicate) and to Baylor for Olink Explore HT measurements (visit 5, samples in duplicate). Participants provided written informed consent. The study was approved by a single institutional review board at Johns Hopkins School of Medicine and at each institution.

These analyses are based on data from up to 116 ARIC participants, as indicated (4). Briefly, participants with prevalent coronary heart disease, stroke, or heart failure at visit 5 were not eligible. Participants with incident coronary heart disease (5), stroke (6), or heart failure (7) after visit 5 could be cases, and controls included those who did not have incident coronary heart disease, stroke, or heart failure or who did not die within 5 years of visit 5. Cases were balanced according to categories of age (≥73 or <73 years), sex, race (self-declared; Black or White), and estimated glomerular filtration rate (≥60 or <60 mL/min/1.73 m^2). Controls were frequency matched to the age (within 10 years), sex, race, and estimated glomerular filtration rate groupings of cases.

APTAMER-BASED PROTEOMIC PLATFORM

We quantified 11 083 aptamer assays using the SomaScan 11k (v5.0) platform (8) (SomaLogic) in plasma from 116 ARIC visit 5 participants using highly multiplexed modified DNA-based aptamer technology. We excluded 233 mouse proteins and 72 nonproteins, resulting in a total of 10 778 protein measurements for investigation on the SomaScan 11k platform (including viral proteins SeqId 2769-3 and SeqId 4792-51 for human immunodeficiency viruses). Briefly, protein abundances were quantified with relative fluorescence intensity units, which were calibrated and normalized for plate variation and were standardized using adaptive normalization by maximum likelihood (8). We $\log_2$-transformed relative fluorescence intensity unit values. SomaLogic reports an intraplate CV of 3.3% and an interplate of 3.0% for the SomaScan 11k measurements in plasma (9). One of the 116 participants' samples from visit 5 did not pass SomaScan quality control; 2 additional participants were excluded due to having outliers on one or more of the first 10 protein principal components (defined as >5 SD from the mean). Of the 103 participants with plasma available at visit 2 for SomaScan 11k measurements, there were 100 participants who had SomaScan 11k measurements at both visits 2 and 5.

IMMUNOASSAY PROTEOMIC PLATFORMS

We quantified 5420 antibody assays using the Olink Explore HT platform (10) (Olink Proteomics) on aliquots of plasma from the same ARIC participants using proximity extension assay technology. Briefly, relative protein abundance in plasma was quantified based on the binding of oligonucleotide-labeled antibody pairs to the target protein. These unique hybridization sequences were then amplified with real-time PCR. Olink proteins were reported on a relative and $\log_2$-scale as Normalized Protein eXpression values. Olink reports a median intra-CV of 10.2% and median inter-CV of 8.8% for the Olink Explore HT (10). Eleven participants from visit 5 had samples that did not pass Olink quality control (including a participant whose sample did not pass ARIC quality control for SomaScan); one additional participant was excluded due to having outliers on one or more of the first 10 protein principal components (defined as >5 SD from the mean). Additional details on the Olink Explore HT and information on clinical immunoassays are provided in the Supplemental Methods.

STATISTICAL ANALYSIS

Statistical analysis was performed using *R* version 4.3.0.

Within-Platform Assay Precision. Based on split plasma samples, we calculated means (SDs) of the proteins measured in each batch. Among the 102 participants with usable data on both platforms, we calculated CV (using the Bland-Altman method) (11) and Spearman correlations (r) for each assay available on the SomaScan 11k and Olink Explore HT. These statistics for each protein were summarized using histograms and percentiles. We

examined the precision of each protein according to availability of the aptamer on prior SomaScan platforms (based on SeqId on the SomaScan 5k, 7k, or 11k) and availability of the protein on prior Olink platforms (based on uniprotid, OlinkIDs were not transferable across the Olink 96, Explore 3072, or Explore HT platforms). The SomaScan 11k platform fully includes all 7288 assays on the SomaScan 7k platform and all 5284 assays on the SomaScan 5k platform. Most assays on the Olink 96 platforms and Olink Explore 3072 are found on the Olink Explore HT platform. We also summarized the precision of each assay according to dilution bins.

We conducted several analyses to examine the influence of suggested Olink methods for handling assay values below the limit of detection (LOD) on assay precision (99% of SomaScan 11k assays had >80% of values above the LOD in our study). We defined LOD based on the publicly available validation LOD data (12, 13). In our primary analysis, we report results using plate-normalized results and include all Normalized Protein eXpression protein values as reported to us by Olink (including those below the LOD; intensity normalized values yielded the same overall conclusions). Secondary analyses followed alternative suggested methods (14) for dealing with values below the LOD: (*a*) replacing data below the LOD to a single value (we used the LOD divided by 2), (*b*) excluding proteins assays with a large proportion of samples below the LOD (we used >50% below LOD), or (*c*) treating values below the LOD as missing data.

Between-Platform Assay Comparisons. There were 102 participants with data available on both the SomaScan 11k and the Olink Explore HT for 4443 overlapping assays (targeting 3729 proteins based on SomaScan 11k uniprotID and 3724 based on Olink uniprotID). We calculated the between-platform correlation for each assay across the 102 individuals. We summarized the cross-platform correlations (based on measurements from batch 1) in histograms according to categories of protein detectability, i.e., [0–20%), [20%–50%), [50%–80%), [80–100%), 100% of samples with proteins above the Olink Explore HT LOD values. We summarized the precision of the overlapping assays according to dilution bins.

Comparisons of SomaScan 11k, Olink Explore HT, and Clinical Immunoassays. We included scatterplots for the relative abundance of proteins measured on the SomaScan 11k platform and on the Olink Explore HT data vs absolute measurement of proteins quantified using targeted immunoassays. We generated Spearman correlations of proteins measured on clinical immunoassays [cystatin-C, N-terminal pro-brain natriuretic peptide (NT-proBNP), troponin-T] vs SomaScan 11k. Using visit 2 data, we provide correlations and scatterplots for targeted immunoassays (cystatin-C, NT-proBNP, troponin-T) to the SomaScan 11k measured. We also calculated correlations and included scatterplots for the change in targeted immunoassays vs change in SomaScan 11k from visits 2 to 5.

Results

Plasma samples from 116 participants were assayed, of which 102 had measurements on both the SomaScan 11k and Olink Explore HT platforms at visit 5. The 102 participants were mean age 74 years (SD 5 years), 47% self-identified their race as Black, and 53% were women (Table 1). The mean estimated glomerular filtration rate was 62 mL/min/1.73 m^2 (SD:21), 38% had diabetes, and 78% had hypertension. By design, none of the participants had cardiovascular disease at the time of blood draw, and half of the participants developed incident cardiovascular disease within the 5 years following visit 5. The 100 participants with SomaScan 11k measurements at visit 2 were a mean age of approximately 57 years (SD 5 years) and had fewer cardiometabolic risk factors (Supplemental Table 1).

PRECISION AND RELIABILITY OF PLASMA PROTEINS QUANTIFIED ON THE SOMASCAN 11K

The median Spearman correlation for the 10 778 aptamers (9657 unique proteins based on uniprotID) measured in 102 split samples was 0.85 [interquartile interval (IQI): 0.70–0.94; median SE for *r*: 0.053], and the median CV was 6.8% (IQI: 5.1%–9.3%; CV < 20% for 10 304 assays) (Fig. 1A and Table 2; see Supplemental Table 2 for the individual assays). Assay precision was similar when we stratified aptamers by their prior availability on the SomaScan 5k and 7k platforms (Table 2). Ninety-nine percent of the SomaScan 11k assays had >80% of values above the LOD. Across dilution bins, the median of the correlations and CVs of the SomaScan 11k assays measured in duplicate were similar (Supplemental Table 3).

Similar to visit 5, the precision of the SomaScan 11k using visit 2 samples was high, even based on samples that had been stored for an additional about 20 years. At visit 2, the median Spearman correlation for the 10 778 aptamers measured in 100 split samples was 0.90 (IQI: 0.82–0.95; median SE for *r*: 0.044). The median CV for the 10 778 aptamers measured in split samples was 5.7% (IQI: 4.5%–7.9%) (see Supplemental Table 4 for the individual assays using visit 2 samples).

PRECISION AND RELIABILITY OF PLASMA PROTEINS QUANTIFIED ON THE OLINK EXPLORE HT

For the 5420 Olink antibodies (5416 unique proteins) measured in 102 split samples, the median Spearman correlation was 0.65 (IQI: 0.37–0.90; median SE for

Table 1. Participant characteristics overall and according to case and control status: ARIC Study (2011–2017).[a]

Visit 5 characteristic	Overall	Controls	Incident CVD cases
N	102	51	51
Age, years	74 (5)	74 (4)	75 (6)
Women (%)	54 (53)	30 (59)	24 (47)
Black race (%)	48 (47)	24 (47)	24 (47)
BMI, kg/m^2	30 (6)	29 (5)	31 (6)
eGFR,[b] mL/min/1.73 m^2	62 (21)	64 (21)	60 (21)
Diabetes (%)	38 (38)	14 (28)	24 (48)
Hypertension (%)	80 (78)	36 (71)	44 (86)
Incident CVD (%)	51 (50)	0 (0)	51 (100)
Incident CHD	14 (14)	0 (0)	14 (28)
Incident HF	33 (32)	0 (0)	33 (65)
Incident stroke	15 (15)	0 (0)	15 (29)

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; HF, heart failure.
[a]N (%) for dichotomous variables, mean (SD) for continuous variables. Three participants were missing data on BMI and 2 participants missing data on diabetes status at visit 5 (2011–2013).
[b]eGFR was calculated using the 2021 Chronic Kidney Disease Epidemiology equation incorporating creatinine and cystatin-C.

r: 0.076) and the median CV was 35.7% (IQI: 14.5%–97.1%; CV < 20% for 1804 assays) (Fig. 1A and Table 2). The correlations of assays measured in duplicate varied substantially according to the availability of the assay on prior Olink platforms (i.e., Olink 3072 and Olink 96). Assays available on the Olink 96 and Olink 3072 platforms had median correlations of 0.92 (IQI: 0.83–0.96) and 0.84 (IQI: 0.55–0.94), respectively, whereas the median correlation for the assays added to the newest Olink Explore HT was 0.45 (IQI: 0.28–0.66). The corresponding median CVs rose from 10.9% to 19.8% to 67.9% (Table 2). The median CV for the 4366 Olink Explore HT assays in the 1:1 dilution bin was 47.5%, and the median correlation for assays measured in duplicate was 0.54. Across the remaining dilution bins, the median CV for assays in other dilution bins were lower (8.2%–12.9%) and the median correlations were higher (0.83–0.92) (Supplemental Table 3).

Half of the Olink Explore HT assays had the majority (>50%) of values above the LOD. Forty percent of the assays had >80% values above the LOD. There were 1563 Olink Explore HT assays where all 102 participants had values that were above the published LOD, and their median CV was 12.7%. The precision (CV) of the Olink Explore HT assays was strongly inversely correlated (*r*: –0.77) with protein detectability (i.e., percent of samples above the validation LOD) (Fig. 2). When we replaced Olink values reported below the LOD to half the LOD, the median correlation for the Olink assays measured in duplicate increased to 0.79 (IQI: 0.58–0.93), and the median CV decreased to 13.3% (IQI: 8.7%–21.7%) (Fig. 1A and Table 2; see Supplemental Table 5 for the individual Olink assays). Focusing on moderately to highly detectable proteins (i.e., with a majority of samples above the LOD) left 2446 assays with a median correlation for duplicate measurements of 0.91 (IQI: 0.82–0.96) and median CV of 13.6% (IQI: 9.2%–22.6%). Treating values below the Olink LOD as missing data left 4743 proteins with a median correlation for duplicate measurements of 0.88 (IQI: 0.68–0.96) and a median CV of 17.6% (IQI: 11.2%–28.5%).

In a sensitivity analysis, the overall precision of Olink Explore HT based on results that were intensity normalized were similar to plate-normalized results (median *r*: 0.68, IQI: 0.39–0.92; median CV: 30.6%, IQI: 13.3%–87.0%).

COMPARABILITY OF OVERLAPPING PROTEINS ON SOMASCAN 11K VS OLINK EXPLORE HT

The between-platform median correlation for the 4443 overlapping assays on the SomaScan 11k and Olink Explore HT platforms was 0.14 (IQI: 0–0.58), lower than the 0.33 reported for the earlier versions of these platforms (1, 2). The distribution of the correlations for the overlapping assays had a mode of 0 with another smaller peak at *r* approximately 0.8 (Fig. 1B). About

one-third of the protein pairs had modest to excellent cross-platform correlations (407 proteins with $r \geq 0.8$; 875 proteins with $0.5 \leq r < 0.8$) while the other two-thirds had poor correlations (including 2481 proteins with $r < 0.2$). Approximately one-third of the overlapping proteins had 100% of values above the LOD on Olink Explore HT, and those assays had a median between-platform correlation of 0.52. Conversely, plasma proteins with the majority of samples below the LOD on the Olink platform showed poor correlation to the corresponding SomaScan assay (Fig. 1B). For the overlapping assays, the CVs for the Olink Explore HT assays were strongly inversely correlated ($r = -0.82$) with the percent of samples (in batch 1) above the Olink LOD; the SomaLogic 11k assay CVs were weakly correlated with the percent of samples (in batch 1) above the SomaScan 11k LOD ($r = -0.19$). When we restricted between-platform comparisons to the 2360 assays with greater than 80% of samples above the platform-specific LOD, the median correlation increased to 0.49 (IQI: 0.09–0.75). For the overlapping assays with highly detectable proteins (>80% samples above the LOD), the median CV on the SomaScan 11k was 6.6% (IQI: 5.0%–9.0%), and the median CV on the Olink Explore HT was 11.6% (IQI: 8.4%–17.4%). Summary statistics for the individual overlapping proteins and their precision on each platform are reported in Supplemental Table 6.

After replacing protein values reported below the LOD with the LOD divided by 2, the median correlation for the 4325 proteins on both the SomaScan 11k and Olink Explore HT platforms was 0.13 (IQI: −0.01–0.58), and these results were similar to our primary analyses (median $r = 0.14$).

AGREEMENT OF PROTEINS QUANTIFIED ON THE SOMASCAN 11K AND OLINK EXPLORE HT PLATFORMS VS TARGETED IMMUNOASSAYS

At visit 5, measurements of cystatin-C using clinical immunoassays correlated highly with the cystatin-C measurement from the SomaScan 11k (*r*: 0.92) and from the Olink Explore HT (*r*: 0.93) (Fig. 3). Correlations for visit 5 plasma NT-proBNP measurements with the SomaScan 11k was 0.87 and with the Olink Explore HT was 0.92. At visit 5, the correlation of the targeted

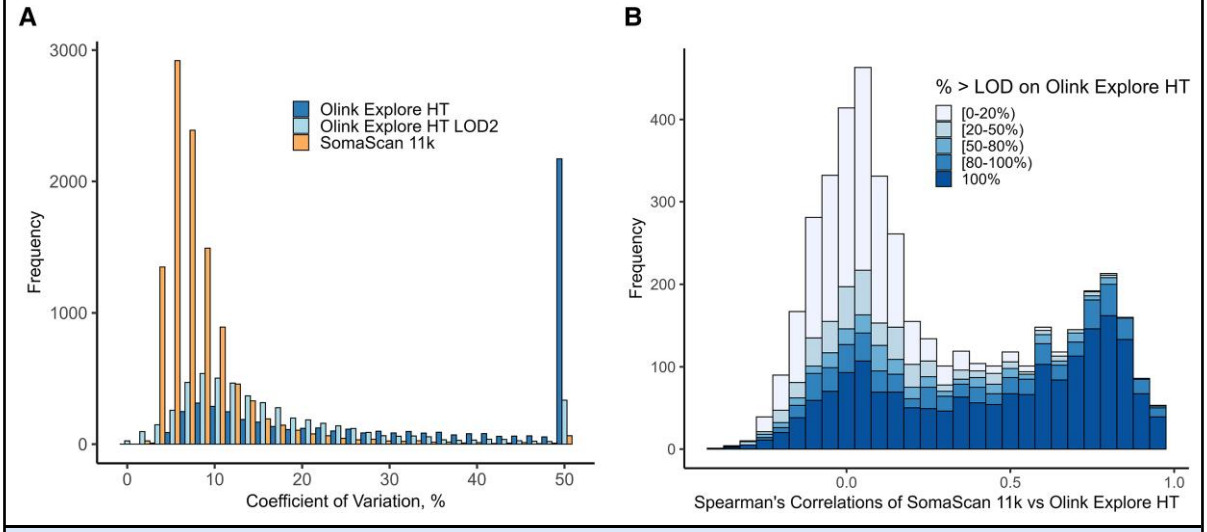

Fig. 1. (A and B), Summary of within-platform precision and between-platform correlations among 102 ARIC Study participants (2011–2013). (A), Precision of proteins measured in duplicate on the SomaScan 11k (10 778 assays) and on the Olink Explore HT (5420 assays) before and after imputing values below LOD to the LOD divided by 2 (LOD2). CVs were capped at 50% for the display of the histogram; (B), Histogram of Spearman correlations for the 4443 overlapping protein assay comparisons on the SomaScan 11k and Olink Explore HT platforms, according to the percentage of samples with protein values above the Olink Explore HT LOD. There were 1841 overlapping Olink proteins with 100% samples with protein values above the LOD, 533 proteins with [80%–100%), 261 proteins with [50%–80%), 407 proteins with [20%–50%), 1399 with less than 20% above the LOD; 2 proteins were missing an Olink LOD. There were 4194 overlapping proteins with 100% samples with protein values above the SomaScan LOD, 216 proteins with [80%–100%), 18 proteins with [50%–80%), 8 proteins with [20%–50%), and 7 with [0–20%) above the LOD. Color figure available at https://academic.oup.com/clinchem.

Table 2. Summary of within-platform precision (overall and according to availability on prior versions) among 102 ARIC visit 5 participants.

	N participants	N proteins	Summary of Spearman *r*				Summary of CV			
			Mean	25th percentile	50th percentile	75th percentile	Mean (%)	25th percentile (%)	50th percentile (%)	75th percentile (%)
SomaScan 11k	102	10 778	0.80	0.70	0.85	0.94	8.6	5.1	6.8	9.3
Assays in SomaScan 11k but not 5k, 7k		3487	0.74	0.62	0.77	0.90	9.0	5.2	7.1	10.2
Assays in SomaScan 7k but not 5k		2303	0.82	0.73	0.87	0.95	8.5	5.1	6.8	9.1
Assays in SomaScan 5k, 7k, and 11k		4988	0.83	0.75	0.88	0.95	8.4	5.0	6.5	8.8
Olink Explore HT (5k)	102	5420	0.61	0.37	0.65	0.90	110	14.5	35.7	97.1
Assays in Olink 5k but not 96, 3k		2591	0.47	0.28	0.45	0.66	173	35.4	67.9	203
Assays in Olink 3k but not Olink 96[a]		2394	0.73	0.55	0.84	0.94	56.5	10.8	19.8	44.2
Assays in Olink 96, 3k, and 5k		435	0.85	0.83	0.92	0.96	26.6	7.6	10.9	18.3
Olink Explore HT (5k, impute < LOD to LOD/2)[b]	102	5160	0.72	0.58	0.79	0.93	24.4	8.7	13.3	21.7
Assays in Olink 5k but not 96, 3k		2410	0.64	0.48	0.70	0.84	31.0	9.0	14.7	24.6
Assays in Olink 3k but not Olink 96[a]		2317	0.78	0.68	0.86	0.95	19.4	8.9	12.7	20.3
Assays in Olink 96, 3k, and 5k		433	0.87	0.84	0.93	0.96	14.1	7.3	10.1	15.4
Olink Explore HT (5k, exclude proteins with >50% of samples < LOD)	102	2446	0.86	0.82	0.91	0.96	35.0	9.2	13.6	22.6
Assays in Olink 5k but not 96, 3k		448	0.78	0.68	0.86	0.94	93.3	13.5	21.8	39.0
Assays in Olink 3k but not Olink 96[a]		1611	0.87	0.82	0.91	0.96	23.2	9.3	13.1	21.4
Assays in Olink 96, 3k, and 5k		387	0.90	0.87	0.93	0.97	17.0	7.3	9.9	14.6
Olink Explore HT (5k, excluding values < LOD)[c]	102	4743	0.75	0.68	0.88	0.96	31.0	11.2	17.6	28.5
Assays in Olink 5k but not 96, 3k		2111	0.68	0.57	0.81	0.95	43.6	15.8	23.6	36.9
Assays in Olink 3k but not Olink 96[a]		2208	0.80	0.75	0.89	0.95	22.0	9.9	14.5	22.6
Assays in Olink 96, 3k, and 5k		424	0.86	0.85	0.93	0.96	14.7	7.5	10.4	15.6

SomaScan 11k results are Adaptive Normalization by Maximum Likelihood and Olink Explore HT (5k) results are plate-normalized. Of the SomaScan platforms listed, SomaScan 5k was released first, followed by SomaScan 7k and then SomaScan 11k. Of the Olink platforms listed, Olink 96 was released first, followed by Olink 3072 (3k) and then Olink Explore HT (5k).

[a]The cardiovascular disease 2, 3, inflammation, organ damage, and cardiometabolic panels data (460 assays) were included as part of the Olink 96 platform.

[b]Impute Normalized Protein eXpression values reported below the LOD to half of the LOD (we excluded 260 assays where all samples had Normalized Protein eXpression values below the LOD in either batch or assays that were missing an LOD).

[c]We excluded 677 assays where <2 samples had a Normalized Protein eXpression value that was above the LOD in both batches or assays that were missing an LOD. The median percentage of observations above the LOD were 55.9% overall and 13.7% in assays in Olink 5k but not 96, 3k; 99.0% proteins in Olink 3k but not Olink 96; and 100% in proteins in Olink 96, 3k, and 5k.

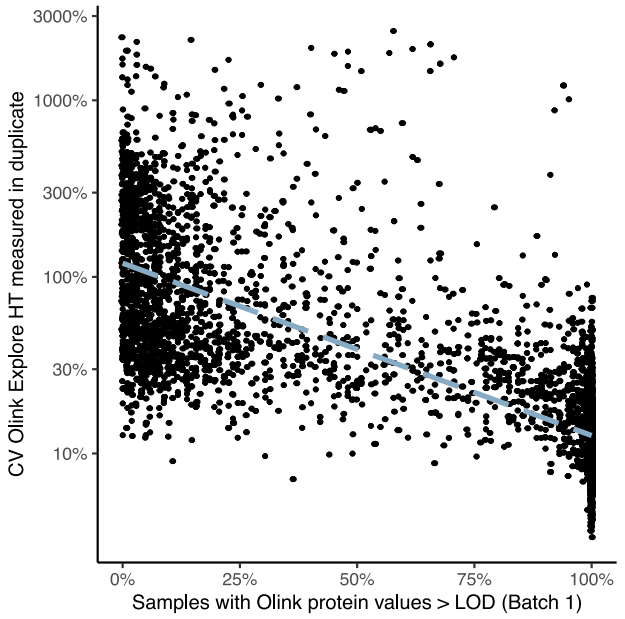

Fig. 2. Scatterplot of the percentage of participants with protein values above the LOD vs CV (displayed on the log_2 scale) on the Olink Explore HT among 102 ARIC participants. Dashed line represents a linear regression of the percentage of participants with protein values above the LOD and the CV for the Olink Explore HT (log_2 transformed). Color figure available at https://academic.oup.com/clinchem.

immunoassay of troponin-T with the SomaScan 11k aptamer was 0.70, and the correlation with the Olink Explore HT protein was −0.03.

At visit 2, the correlations of the cystatin-C (*r*: 0.85), NTproBNP ($r = 0.66$), and troponin-T (*r*: 0.54) with the SomaScan 11k aptamers were lower compared to visit 5 (likely a function of the narrower distribution of these biomarkers in middle age) (Supplemental Fig. 1). The correlations for the change in cystatin-C (*r*: 0.80) and NTproBNP (*r*: 0.76) was high, whereas the correlation of the change in troponin-T for the SomaScan vs targeted was modest at 0.40 (Supplemental Fig. 1).

Discussion

In the ARIC Study, we identified substantial differences in precision within and across the latest large-scale proteomic platforms available. Over 10 300 assays (of 10 778) can be measured with CV < 20% on the latest SomaScan 11k platform based on split samples. Approximately 1800 assays (of 5420) can be measured with CV < 20% on the Olink Explore HT. We demonstrated the substantial impact that different methods for addressing values below the LOD can have on precision within the Olink Explore HT platform. Our findings indicate that the precision of these 2 leading platforms in human plasma has diverged as the number of included proteins has increased.

Our results provide important data on the precision of the latest SomaScan 11k and Olink Explore HT platforms and their cross-platform agreement within an epidemiologic cohort. We found substantial differences in measurements of relative protein abundance across the latest and largest-scale proteomic platforms currently available. Only 30% of the overlapping protein pairs on the SomaScan 11k and Olink Explore HT have modest to excellent agreement. Cross-platform comparison studies of prior proteomic platforms (SomaScan 1.1, 1.3, 5k, 7k; Olink 96, 3072) have reported that approximately half of the overlapping proteins have modest to excellent agreement and that the remaining half have poor agreement (Supplemental Table 8) (1, 2, 4, 15–19). Proteins with excellent cross-platform agreement included plasma proteins with a greater proportion of individuals in our study above the Olink LOD. Proteins with low detectability in plasma tended to have the poorest agreement across platforms. Investigators should be mindful of this potentially large disagreement for most overlapping protein measurements on the latest, large-scale proteomic platforms in community-dwelling populations.

The number of assays available on the leading aptamer-based platform (e.g., SomaScan 5k to 7k to

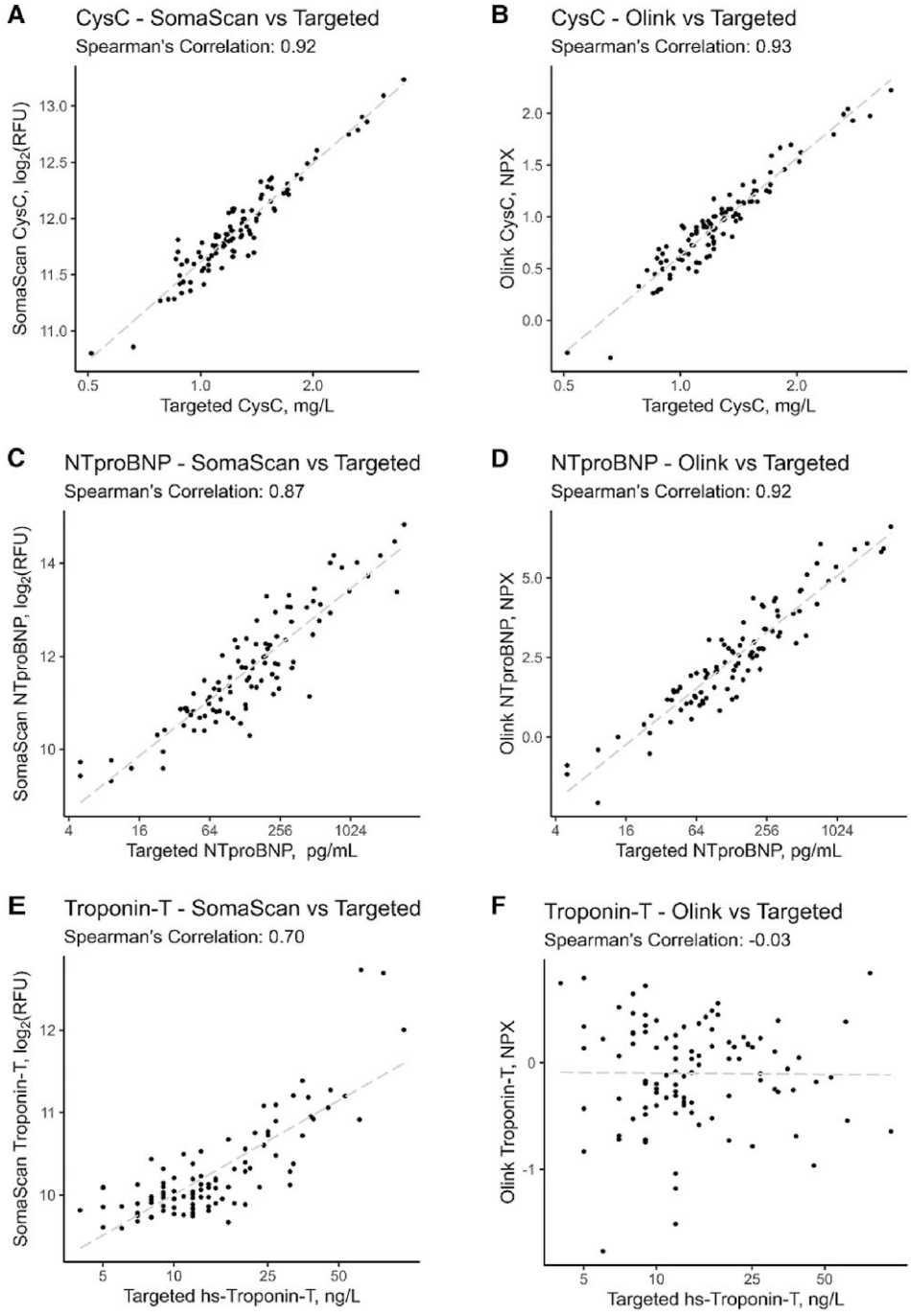

Fig. 3. (A-F), Correlations of SomaScan 11k and Olink Explore HT with targeted immunoassays (cystatin-C, NTproBNP, troponin-T) at visit 5. All values are provided on the log2 scale. The Olink validation LOD (on the Normalized Protein eXpression scale) was −6.17 for cystatin-C (OID45345, 100% samples above LOD on Olink), 0.0074 for NT-proBNP (OID44822, 95% samples above LOD on Olink), and 1.35 for troponin-T (OID40669, 0% samples above LOD on Olink). The SomaScan 11k validation LOD was 6.09 for cystatin-C (SeqId-2609-59, 100% samples above LOD on SomaScan), 8.34 for NT-proBNP (SeqId-7655-11, 100% samples above LOD on SomaScan), and 8.16 for troponin-T (SeqId-5315-22, 100% samples above LOD on SomaScan). Color figure available at https://academic.oup.com/clinchem.

11k) and antibody-based platform (e.g., Olink 96 to Explore 3072 to Explore HT) has grown rapidly. The SomaScan 11k has the largest coverage at >10 700 protein measurements and, in our study, retains high precision based on duplicate measurements (visit 5 median CV: 6.8%; median r: 0.85). These findings are consistent with data from the Baltimore Longitudinal Study of Aging, which reported that the median interplate CVs for the SomaScan 7K and 11K were similar (median CV of 4.5% and 5%, respectively) (20). On Olink Explore HT, the precision of the assays available on the 5 Olink 96 panels measured in ARIC and Olink 3072 was high. However, we found low precision for many assays unique to the Olink Explore HT platform. The plasma proteins unique to Olink Explore HT tended to be low abundance in our study, but precision may increase if the same proteins are found at high abundance in certain disease states or when analyzed using different specimen types. Nevertheless, our results suggest that there are a growing number of low abundance proteins or intracellular proteins on the latest platforms that can be harder to measure precisely in plasma among relatively healthy adults.

We demonstrated how different methods for handling protein values below the LOD can substantially impact results in proteomics studies. In our primary analyses, we analyzed the proteomic data as reported to us by the company (including values below the declared LOD). Within-platform precision and cross-platform correlations were vastly improved when we used different methods (e.g., imputation or exclusion of low detectable proteins) to address the large number of low-value proteins in Olink Explore HT. In our study, we would have excluded 2974 (55%) Olink Explore HT proteins with low detection (i.e., protein below LOD on the majority of samples). Researchers should thoughtfully consider the different methods for handling measurements below the LOD in the context of their own studies.

Our study has limitations. First, the sample size is small, and between-run precision was calculated based on 2 measurements. Future work could consider analyzing proteomic measurements based on additional replicates and comparison to reference methods to assess protein accuracy. Nevertheless, our cross-platform correlations of SomaScan 11k and Olink Explore HT provide important information for investigators when a study has 1 platform but not both. Second, proteomic platforms measure relative abundance (not absolute) of the proteins, and proteins may not be on the same scale across platforms. Third, LOD values were not derived within our study since our study only included 2 Olink plates. We used LOD validation data that is available publicly. Strengths of this study include the largest number of proteins measured using the latest proteomic platforms, the availability of duplicate measurements of both the SomaScan 11k and Olink Explore HT platforms within the same study, and comparisons to clinically used immunoassays. The high level of agreement of the proteomic platforms with cystatin-C provides support for the validity of the laboratory analyses, although the lack of agreement for troponin-T indicates that this may not always be the case. Additionally, the reliability of cystatin-C measured in duplicate was high on both the SomaScan 11k (visit 2 $r = 0.90$; visit 5 $r = 0.96$) and on the Olink Explore HT (visit 5 $r = 0.96$).

In conclusion, the field of proteomics is expanding rapidly, and thousands of proteins, particularly those in higher abundance, can be measured with great precision. We identified substantial differences in precision within and across the latest large-scale proteomic platforms available. Expanding the coverage of human plasma proteomic profiling retains precision with SomaScan's aptamer-based technology. In contrast, newly added antibodies on the latest Olink platform often yielded imprecise values below the LOD of the assay in human plasma. These results highlight the need for careful consideration in platform selection based on specific research requirements.

Data Availability

All data generated in this study are either included in this article (and its Supplementary Material) or available upon reasonable request. Consistent with a prespecified policy for access of ARIC data, requests may be submitted to the ARIC Steering Committee for review. Requests for clinical or proteomic data from individual investigators will be reviewed to ensure that data can be shared without compromising patient confidentiality or breaching intellectual property restrictions. Reasonable requests will be considered and promptly processed.

Author Declaration

A version of this paper was previously posted as a preprint on medRxiv as https://doi.org/10.1101/2024.07.11.24310161.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: ARIC, Atherosclerosis Risk in Communities; LOD, limit of detection; NT-proBNP, N-terminal pro-brain natriuretic peptide; IQI, interquartile interval.

Author Contributions: *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

Mary Rooney (Conceptualization-Equal, Formal analysis-Equal, Methodology-Equal, Visualization-Equal, Writing—original draft-Lead), Jingsha Chen (Formal analysis-Lead), Christie Ballantyne (Methodology-Equal, Writing—review & editing-Equal), Ron Hoogeveen (Methodology-Equal, Writing—review & editing-Equal), Eric Boerwinkle (Writing—review & editing-Equal), Bing Yu (Writing—review & editing-Equal), Keenan Walker (Writing—review & editing-Equal), Pascal Schlosser (Writing—review & editing-Equal), Elizabeth Selvin (Writing—review & editing-Equal), Nilanjan Chatterjee (Writing—review & editing-Equal), David Couper (Writing—review & editing-Equal), Morgan Grams (Writing—review & editing-Equal), and Josef Coresh (Conceptualization-Equal, Funding acquisition-Equal, Methodology-Equal, Supervision-Equal, Writing—review & editing-Lead)

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form.*

Research Funding: The Atherosclerosis Risk in Communities Study has been funded in whole or in part with federal funds from the National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Department of Health and Human Services, under contract nos. (75N92022D00001, 75N92022D00002, 75N92022D00003, 75N92022D00004, 75N92022D00005). This study was funded, in part, by the National Institute on Aging's Intramural Research Program. P. Schlosser was supported by the German Research Foundation (DFG) Project ID 530592017 (SCHL 2292/3-1) and Germany´s Excellence Strategy (CIBSS—EXC-2189—Project ID 390939984). N. Chatterjee was supported by the NIH grants 1R01HG010480-01 and U01CA249866. K.A. Walker was funded by the National Institute on Aging's Intramural Research Program (AG000348-01 and AG000349-01). E. Selvin was supported by NIH/NHLBI grant K24 HL152440. B. Yu was in part supported by R01HL148218. M.E. Grams was supported by grants from the NIH (R01DK108803, K24HL155861) and grants from the National Kidney Foundation. SomaLogic provided the assays at no cost but had no control over the design or analysis of the data.

Disclosures: J. Coresh served on the SomaLogic scientific advisory committee during 2021–2023. K.A. Walker has given unpaid seminars and webinars sponsored or cosponsored by SomaLogic. E. Boerwinkle received an annual consulting fee from Johns Hopkins University. E. Selvin receives royalty payments from Wolters Kluwer for chapters and laboratory monographs in UpToDate on measurements of glycemic control and screening tests for type 2 diabetes and has received support from Abbott Diabetes Care, Roche Diagnostics, Siemens Diagnostics, Ortho Clinical Diagnostics, Abbott Diagnostics, Asahi Kasei Pharma Corp, GlycoMark Corp, as well as donated materials for NIH-supported research. C.M. Ballantyne has received grant/research support paid to institution from Abbott Diagnostics, Akcea, Amgen, Arrowhead, Ionis, Merck, New Amsterdam, Novartis, Novo Nordisk, Roche Diagnostic, NIH, American Heart Association, and American Diabetes Association and has served as a consultant for 89Bio, Abbott Diagnostics, Amarin, Amgen, Arrowhead, Astra Zeneca, Denka Seiken, Esperion, Genentech, Illumina, Ionis, Eli Lilly, Merck, New Amsterdam, Novartis, Novo Nordisk, and Roche Diagnostics. R.C. Hoogeveen is a consultant for Denka Seiken and has received personal fees and has received research grants (to institution) from Denka Seiken for work outside the scope of the submitted work.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: We thank the ARIC Study staff and participants for their important contributions.

References

1. Eldjarn GH, Ferkingstad E, Lund SH, Helgason H, Magnusson OT, Gunnarsdottir K, et al. Large-scale plasma proteomics comparisons through genetics and disease associations. Nature 2023;622:348–58.
2. Eldjarn GH, Ferkingstad E, Lund SH, Helgason H, Magnusson OT, Gunnarsdottir K, et al. Author correction: large-scale plasma proteomics comparisons through genetics and disease associations. Nature 2024;630:E3.
3. Wright JD, Folsom AR, Coresh J, Sharrett AR, Couper D, Wagenknecht LE, et al. The ARIC (Atherosclerosis Risk In Communities) study: JACC Focus Seminar 3/8. J Am Coll Cardiol 2021;77:2939–59.
4. Rooney MR, Chen J, Ballantyne CM, Hoogeveen RC, Tang O, Grams ME, et al. Comparison of proteomic measurements across platforms in the Atherosclerosis Risk in Communities (ARIC) study. Clin Chem 2023;69:68–79.
5. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, et al. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) study: methods and initial two years' experience. J Clin Epidemiol 1996;49:223–33.
6. Rosamond WD, Folsom AR, Chambless LE, Wang C-H, McGovern PG, Howard G, et al. Stroke incidence and survival among middle-aged adults: 9-year follow-up of the Atherosclerosis Risk in Communities (ARIC) cohort. Stroke 1999;30:736–43.
7. Rosamond WD, Chang PP, Baggett C, Johnson A, Bertoni AG, Shahar E, et al. Classification of heart failure in the atherosclerosis risk in communities (ARIC) study: a comparison of diagnostic criteria. Circ Heart Fail 2012;5:152–9.
8. SomaLogic. SomaScan® 11K Assay v5.0 Technical Note SL00000919 Rev 1: 2023-12; 2023. https://somalogic.com/wp-content/uploads/2023/12/SL00000919-Rev-1-2023-12-SomaScan-11K-Assay-v5.0-1.pdf (Accessed March 2024).
9. SomaLogic. Coefficients of variation—SomaLogic. https://somalogic.com/coefficients-of-variation/ (Accessed March 2025).
10. Olink Proteomics. Validation methods and results Olink Explore HT. Contract No.: 1345. 2023. https://7074596.fs1.hubspotusercontent-na1.net/hubfs/7074596/04-Validation%20data/1265-olink-explore-validation-data.pdf (Accessed March 2024).
11. Bland JM, Altman DG. Measurement error proportional to the mean. BMJ 1996;313:106.
12. SomaLogic. SomaScan® Menu. https://menu.somalogic.com (Accessed March 2024).
13. Olink Proteomics. Olink® Explore HT validation data. https://olink.com/resources-support/document-download-center/ (Accessed March 2024).
14. Olink Proteomics. How is the Limit of Detection (LOD) estimated and how is this handled in the data analysis? 2018. https://web.archive.org/web/20231205093211/https://olink.com/faq/how-is-the-limit-of-detection-lod-estimated-and-handled/ (Accessed March 2024).
15. Raffield LM, Dang H, Pratte KA, Jacobson S, Gillenwater LA, Ampleford E, et al. Comparison of proteomic assessment

methods in multiple cohort studies. Proteomics 2020;20:e1900278.

16. Pietzner M, Wheeler E, Carrasco-Zanini J, Kerrison ND, Oerton E, Koprulu M, et al. Synergistic insights into human health from aptamer- and antibody-based proteomic profiling. Nat Commun 2021;12:6822.
17. Haslam DE, Li J, Dillon ST, Gu X, Cao Y, Zeleznik OA, et al. Stability and reproducibility of proteomic profiles in epidemiological studies: comparing the Olink and SOMAscan platforms. Proteomics 2022;22:e2100170.
18. Wang B, Pozarickij A, Mazidi M, Wright N, Yao P, Said S, et al. Comparative studies of 2168 plasma proteins measured by two affinity-based platforms in 4000 Chinese adults. Nat Commun 2025; 16:1869.
19. Katz DH, Robbins JM, Deng S, Tahir UA, Bick AG, Pampana A, et al. Proteomic profiling platforms head to head: leveraging genetics and clinical traits to compare aptamer- and antibody-based methods. Sci Adv 2022;8: eabm5164.
20. Candia J, Fantoni G, Delgado-Peraza F, Shehadeh N, Tanaka T, Moaddel R, et al. Variability of 7K and 11K SomaScan plasma proteomics assays. J Proteome Res 2024; 23:5531–9.