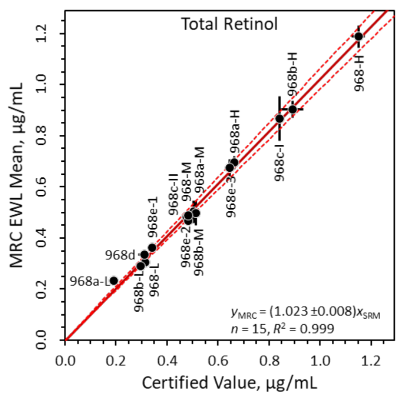
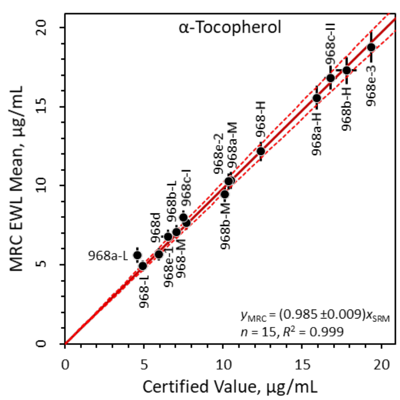
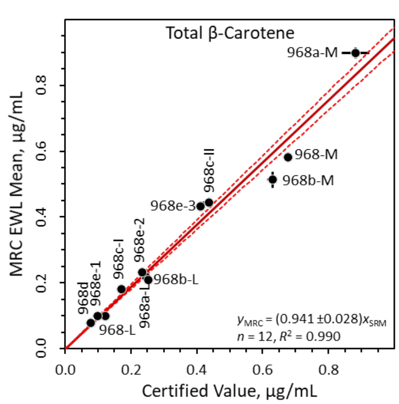
NIST IR 7880-49

NIST Micronutrients Measurement Quality Assurance Program Sample Integrity Confirmation







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NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

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NIST Micronutrients Measurement Quality Assurance Program Sample Integrity Confirmation

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ABSTRACT

From 1984 to 2017, the National Institute of Standards and Technology (NIST) Micronutrients Measurement Quality Assurance Program (MMQAP) coordinated a series of "Round Robin" interlaboratory comparability improvement studies for laboratories that measured fat-soluble vitamins and carotenoids in human serum and plasma. The MMQAP distributed 352 human serum or plasma samples over the course of 79 studies, of which 134 were unique materials. Most of the 95 materials produced prior to 2001 were lyophilized serum; most produced thereafter were liquid-frozen serum. During the period 2000 to 2002 six materials were prepared as {lyophilized, liquid-frozen} pairs to enable direct comparison of sample integrity and stability. While all samples were nominally continuously stored at -80 °C, most materials were subjected to numerous freeze-thaw cycles during sample selection and labeling as well as episodic relocations. This report details the analysis of a set of 48 unique materials produced from 1987 to 2009 that were evaluated synchronously in 2014 by an experienced participant (1995 to 2017) in the MMQAP studies. These materials include 5 of the 6 {lyophilized, liquid-frozen} pairs and the 12 components of the Standard Reference Material® (SRM®) 968 family of certified references materials produced prior to 2014.

KEYWORDS

Carotenoids; Human serum; Lyophilized samples; Liquid-frozen samples; Micronutrients Measurement Quality Assurance Program (MMQAP); Retinol; Tocopherols

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Acronyms

aT	α-tocopherol
cis-R	13-*cis*-retinol
gbT	γ-tocopherol plus β-tocopherol
COA	Certificate of Analysis
LLOQ	lower limit of quantitation
MRC EWL	Medical Research Council of the United Kingdom, Elsie Widdowson Laboratory
NIST	National Institute of Standards and Technology
SRM®	Standard Reference Material®
RP	retinyl palmitate
RR	Round Robin
trans-R	all trans-retinol
TaC	total α-carotene
TbC	total β-carotene
TLy	total lycopene
TaX	total α-cryptoxanthin
TbX	total β-cryptoxanthin
TLZ	total lutein plus zeaxanthin
TR	total retinol
ULOQ	upper limit of quantitation

1. Introduction

From its inception in 1984 until its end in 2017, the NIST Micronutrients Measurement Quality Assurance Program (MMQAP) conducted 79 interlaboratory studies designed to promote improved measurement comparability for fat-soluble vitamins and vitamin-related compounds [1-4]. (Three other studies addressed selenium and zinc.) These studies were named as "Round Robins (RRs)" followed by a sequential index expressed in Roman numerals.

Over its 33-year lifetime, the MMQAP distributed 353 human serum or plasma samples, of which 134 were unique materials. Most (93) of the unique materials were distributed at least twice; 27 were distributed four or more times. The final distribution for several materials was more than 10 years after the initial distribution. Figure 1 displays the distribution of the number of times the unique materials were used in MMQAP RRs.

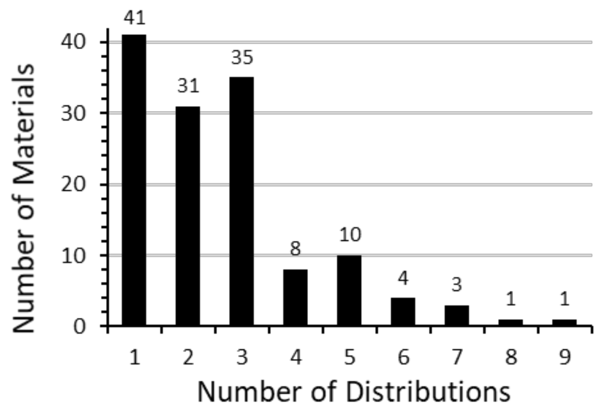

Figure 1. Distribution Frequency for the Unique MMQAP Sample Materials

Of the 95 materials prepared prior to 2001, most (79) were produced as lyophilized samples with the rest prepared as "liquid-frozen" (cooled to -80 °C as quickly as feasible after aliquoting) samples. Based upon results from a stability study of retinol, α-tocopherol and β-carotene in lyophilized and liquid-frozen sera [5], as well as the demonstrated reliability of dry ice shipments, of the 39 materials produced after 2000, nearly all (37) were prepared as liquid-frozen samples. Eighteen (18) of the unique materials were components in one of the NIST Standard Reference Material® (SRM®) 968 family of certified reference materials [6-13], 11 lyophilized and 7 liquid-frozen.

While both the lyophilized and liquid-frozen samples were stored in -80 °C freezers while at NIST and shipped on dry ice to participants, all of the MMQAP materials were episodically relocated during which some thawing may have occurred. All materials distributed in more than one RR were thawed to allow the sample vials to be properly relabeled. To enable direct evaluation of potential differences between the two preparations, six materials were prepared as {lyophilized, liquid-frozen} pairs during the interval from 2000 to 2003. Other than an approximate 5 % difference in measurand concentrations due to the recommended method for reconstituting lyophilized samples, no differences in the nature of any of the fat-soluble measurands nor differences in measurand stability were detected in the MMQAP study data. However, no resources were available for a more sensitive analysis using a more complete longitudinal selection of the archived samples.

In early 2014, Stephen Young of the Medical Research Council of the United Kingdom's Elsie Widdowson Laboratory (MRC EWL) in Cambridge, England inquired about the availability of well-characterized MMQAP samples for use in developing and validating a new liquid chromatographic assay for multiple fat-soluble vitamin-related measurands. This laboratory or its predecessor organizations (Dunn Human Nutrition Unit, MRC Human Nutrition Research) had participated in 34 of the 41 MMQAP RRs since first enrolling in 1995, reporting measurement results for some or all of the 10 measurands listed in Table 1.

Table 1. Measurands Evaluated

Code	Name	Description
TR	total retinol	*trans*-retinol and all *cis*-retinol isomers
RP	retinyl palmitate	
aT	α-tocopherol	
gbT	γ+β-tocopherol	γ-tocopherol and β-tocopherol
TbC	total β-carotene	all isomers of β-carotene
TaC	total α-carotene	all isomers of α-carotene
Tly	total lycopene	all isomers of lycopene
TbX	total β-cryptoxanthin	all isomers of β-cryptoxanthin
TaX	total α-cryptoxanthin	all isomers of α-cryptoxanthin
TLZ	total lutein+zeaxanthin	all isomers of lutein and zeaxanthin

Given the MRC EWL's long experience with and good performance in the MMQAP studies, it was agreed that NIST would provide blind-coded units of 48 selected MMQAP samples in exchange for two independent measurements for each of the 10 measurands[1] detected at quantifiable levels in each material. NIST supplied two units for 46 of the 48 materials. The MRC EWL was not provided with any information about the samples or the expected measurand concentrations until after NIST received the first complete set of measurements in late March 2014. Table 2 lists the sample codes for the 48 selected materials, along with the MMQAP sample numbers, some material descriptions, the MMQAP RR in which the material was first distributed, and the year of that distribution.

None of the materials sent to the MRC EWL had enough remaining samples to be useful in a future RR; many of the samples were the very last of their kind.

The MRC EWL did not provide NIST with information about their new method other than it was based on hexane extraction, solvent exchange, and high-performance liquid chromatographic separation with absorbance and/or fluorescence detection. Variants of this approach [14] have been used by all MMQAP participants since the program's inception.

1 The measurand is "the quantity intended to be measured", not necessarily what is actually measured. Here, the measurands are selected fat-soluble vitamins and carotenoids in human serum or plasma materials. Since many of these measurands are composites of chemically distinctly species (e.g., "total retinol" includes *trans*-retinol and the several *cis*-retinol isomers) and that the components of the composite may have different elution profiles under different chromatographic conditions, measurement differences attributable to differences in measurand identification are relatively common. This is a potential issue for the MMQAP results since chromatographic resolution capabilities have improved from the mid 1980's. At relatively low resolution, a peak may look like a single well-defined, isolated peak. At higher resolution, this peak may show considerable structure, not all of which may 'belong" to the desired measurand.

Note: The MRC closed the EWL in December 2018 [15].

Table 2. Sample Distribution History

Material	MMQAP Sample Numbers									Material Descriptions	1st RR	Year
Ly01	77	91	164							Ctrl D	XII	1988
Ly02	80	144								Ctrl C	XII	1988
Ly03	81	94	112	208						Ctrl B	XII	1988
Ly04	100									SRM 968-L	XV	1989
Ly05	101									SRM 968-M	XV	1989
Ly06	102									SRM 968-H	XV	1989
Ly07	120	147	206								XVIII	1990
Ly08	121	145	187								XVIII	1990
Ly09	148	167								SRM 968a-L	XXII	1991
Ly10	149	159	173							SRM 968a-M	XXII	1991
Ly11	150	161	177							SRM 968a-H	XXII	1991
Ly12	157	160									XXIII	1991
Ly13	170	172	215								XXVI	1992
Ly14	171	176	257								XXVI	1992
Ly15	182	185	216	253							XXVIII	1993
Ly16	191	213									XXX	1994
Ly17	192	199	218	250	278						XXX	1994
Ly18	194	217									XXX	1994
Ly19	200	207	235							SRM 968b-L	XXXII	1994
Ly20	201	209	234							SRM 968b-M	XXXII	1994
Ly21	202	210	227							SRM 968b-H	XXXII	1994
Ly22	247	317	352							1+1 SRM 968c-I, 968c-II	XLIV	1998
Ly23	248	258	263	280	304	318				SRM 968c-I	XLIV	1998
Ly24	249	256	264	284	299	309				SRM 968c-II	XLIV	1998
Ly25	261										XLVII	2000
Ly26	262									Paired with Lq31	XLVII	2000
Ly27	265									Paired with Lq33	XLVIII	2000
Ly28	266	277	282	295	305					Paired with Lq34	XLVIII	2000
Ly29	270	276	367	377	387					Paired with Lq32	XLIX	2001
Ly30	290	300	312	322	333	348	362			Paired with Lq40	LIII	2003
Lq31	260									Paired with Ly26	XLVII	2000
Lq32	267	274	368	380	388					Paired with Ly29	XLVIII	2000
Lq33	268									Paired with Ly27	XLVIII	2000
Lq34	271	275	279	296	308	394a				Paired with Ly28	XLIX	2001
Lq35	285	297	306							Lq38, spiked with *trans*-R	LII	2002
Lq36	286	298	307							Lq38, spiked with 13-*cis*-R	LII	2002
Lq37	287	303								Hemolyzed sera	LII	2002
Lq38	288	293	302								LII	2002
Lq39	291										LIII	2003
Lq40	292	301	313	323	332	349	366			Paired with Ly30	LIII	2003
Lq41	326	331	338	339							LX	2006
Lq42	329	337									LXI	2007
Lq43	330										LXI	2007
Lq44	341	344	351	361	372	419	422	427		SRM 968d-I	LXIII	2008
Lq45	356	360	376	391	435					1.78+1 SRM 968c-I, 968c-II	LXV	2009
Lq46	357	365	375	389	398	403				SRM 968e-I	LXVI	2009
Lq47	358	364	374	386	399	408	416	426	440	SRM 968e-II	LXVI	2009
Lq48	359	363	373	379	400	405	436			SRM 968e-III	LXVI	2009

2. MRC EWL Measurement Results

The MRC EWL evaluated the 48 materials in four batches. Each batch consisted of 24 MMQAP samples and 8 internal quality control plasma materials. Each sample analysis required 20 minutes. The first complete set of results was accomplished in the first and second batches; the second complete set in the third and fourth batches. Table 3 to Table 6 contain the results as provided to NIST. Table 7 provides the summary values.

Table 3. MRC EWL Results for Batch 1: March 19 to 20, 2014

All values reported in µg/mL

Order	Sample [a]	TR	RP	gbT	aT	TLZ	TaX	TbX	Tly	TaC	TbC
1	QA06a	0.700	*b*	0.977	12.626	0.150	*c*	0.085	0.426	*c*	0.089
2	QA07b	0.805	*b*	0.620	15.016	0.280	0.032	0.080	1.019	0.052	0.152
3	Ly02	0.967	*b*	2.449	10.171	0.128	0.029	*c*	0.283	*c*	0.850
4	Ly29	0.585	*b*	2.077	5.789	0.122	0.029	*c*	0.258	*c*	0.528
5	Ly27	0.594	0.274	1.505	13.673	0.242	0.045	0.065	0.378	0.097	0.305
6	Ly06	1.216	*b*	2.540	11.711	0.135	0.026	*c*	0.283	*c*	*d*
7	Ly21	0.888	0.218	3.423	16.415	0.053	*c*	*c*	0.355	*c*	*d*
8	Ly13	0.683	0.300	2.068	6.159	0.161	0.037	0.057	0.229	*c*	0.590
9	Ly25	0.534	*b*	2.037	11.184	0.155	0.031	0.085	0.431	*c*	0.232
10	Ly08	0.512	*b*	0.821	5.972	0.148	0.036	0.095	0.361	*c*	0.909
11	Ly28	0.420	*b*	1.690	6.453	0.122	0.026	0.054	0.385	*c*	0.366
12	QA07a	0.647	*b*	0.568	14.211	0.246	0.035	0.081	0.975	0.056	0.164
13	Ly11	0.674	*b*	3.472	14.809	0.121	*c*	*c*	0.319	0.059	*d*
14	Ly26	0.618	*b*	0.895	17.176	0.286	0.032	0.077	0.491	0.063	0.326
15	Ly16	*d*	0.129	3.070	22.417	*c*	*c*	*c*	0.171	*c*	*d*
16	Ly17	0.996	*b*	2.038	6.801	0.118	*c*	0.052	0.743	*c*	0.613
17	Ly30	0.591	*b*	1.649	9.677	0.123	0.026	0.057	0.532	0.062	0.119
18	QA02b	0.569	*b*	0.420	14.521	0.393	0.046	0.383	0.832	0.067	1.018
19	Ly12	1.036	*b*	1.411	5.803	0.097	*c*	*c*	0.929	*c*	0.091
20	QA11b	0.483	*b*	0.372	6.555	0.140	*c*	0.091	0.346	*c*	0.080
21	Lq38	0.313	*b*	0.630	2.655	0.070	*c*	*c*	0.207	*c*	0.059
22	Lq33	0.646	0.287	1.633	14.918	0.270	0.046	0.067	0.406	0.108	0.324
23	Lq46	0.348	*b*	1.630	6.307	0.138	*c*	0.053	0.237	*c*	0.095
24	Lq48	0.643	*b*	2.021	17.762	0.173	*c*	*c*	1.059	*c*	0.423
25	Lq32	0.639	*b*	2.051	5.770	0.130	0.028	*c*	0.285	*c*	0.521
26	Lq39	0.513	*b*	1.654	10.988	0.236	0.054	0.062	0.542	0.137	0.351
27	Lq34	0.452	*b*	1.516	5.445	0.125	0.028	0.054	0.399	*c*	0.361
28	Lq31	0.677	*b*	0.862	17.815	0.307	0.034	0.079	0.540	0.065	0.360
29	Lq36	0.593	*b*	0.612	2.437	0.073	*c*	*c*	0.213	*c*	0.054
30	QA02a	0.590	*b*	0.366	14.134	0.386	0.044	0.367	0.769	0.051	0.956
31	QA11a	0.467	*b*	0.444	5.749	0.126	*c*	0.093	0.311	*c*	0.087
32	QA06b	0.723	*b*	0.979	13.088	0.125	*c*	0.089	0.458	*c*	0.100
	LLOQ	0.050	0.050	0.200	0.600	0.050	0.025	0.050	0.050	0.050	0.050
	ULOQ	2.5	2.5	10.0	30.0	1.0	0.5	1.0	1.0	1.0	1.0

a The samples listed as "QA" are internal quality assurance plasma materials

b Retinyl palmitate (RP) values only reported if the UV absorbance spectrum of the peak matches standard RP

c Peak response less than the lower limit of quantitation (LLOQ)

d Peak response greater than upper limit of quantitation (ULOQ): highest calibration standard

Table 4. MRC EWL Results for Batch 2: March 24 to 25, 2014

All values reported in µg/mL

Order	Sample [a]	TR	RP	gbT	aT	TLZ	TaX	TbX	Tly	TaC	TbC
1	Ly23	0.781	*b*	3.603	7.558	0.105	*c*	0.077	0.373	*c*	0.177
2	Ly03	0.435	*b*	2.815	5.875	0.129	0.028	*c*	0.273	*c*	0.183
3	Ly22	0.676	*b*	2.906	11.810	0.121	0.028	0.071	0.370	*c*	0.324
4	Ly15	0.531	*b*	2.210	5.363	0.083	*c*	*c*	0.249	*c*	0.375
5	Ly14	0.562	0.181	1.729	4.349	0.082	*c*	*c*	0.208	*c*	0.390
6	Ly05	0.489	*b*	1.913	7.314	0.139	0.035	*c*	0.326	*c*	0.580
7	Ly24	0.448	*b*	1.336	16.064	0.107	*c*	*c*	0.466	0.087	0.432
8	Ly19	0.289	*b*	1.464	6.821	0.075	*c*	*c*	0.174	*c*	0.216
9	QA11A	0.457	*b*	0.336	5.911	0.139	*c*	0.090	0.337	*c*	0.063
10	QA07A	0.754	*b*	0.557	14.567	0.276	0.029	0.078	0.897	0.051	0.148
11	Ly10	0.458	*b*	2.235	9.970	0.158	0.040	0.075	0.445	*c*	0.904
12	Ly18	0.408	*b*	2.750	7.063	0.110	*c*	*c*	0.601	*c*	0.053
13	QA07B	0.726	*b*	0.471	14.721	0.270	0.031	0.083	0.981	0.051	0.142
14	QA06A	0.691	*b*	0.941	12.890	0.138	*c*	0.080	0.437	*c*	0.076
15	QA02A	0.531	*b*	0.305	14.041	0.362	0.042	0.345	0.697	0.065	0.929
16	Ly01	0.984	*b*	1.296	8.799	0.122	*c*	*c*	0.277	*c*	0.922
17	Ly04	0.287	*b*	1.799	4.690	0.086	*c*	*c*	0.326	*c*	0.092
18	QA02B	0.633	*b*	0.314	14.809	0.403	0.044	0.355	0.773	0.105	0.934
19	Ly20	0.449	0.123	1.983	9.316	0.080	*c*	*c*	0.276	*c*	0.531
20	Ly07	0.254	*b*	2.025	4.796	0.071	*c*	*c*	0.307	*c*	0.374
21	Ly09	0.231	*b*	0.726	5.136	0.080	*c*	*c*	0.134	*c*	0.214
22	Lq41	0.282	*b*	3.326	8.505	0.267	0.037	0.114	0.638	*c*	0.147
23	Lq47	0.493	*b*	1.216	9.815	0.151	0.026	0.059	0.694	*c*	0.226
24	Lq45	0.670	*b*	2.095	10.128	0.113	*c*	0.064	0.443	*c*	0.272
25	Lq44	0.335	*b*	1.282	5.832	0.100	*c*	*c*	0.322	*c*	0.071
26	Lq40	0.632	*b*	1.542	9.524	0.132	0.028	0.062	0.547	0.070	0.114
27	Lq37	0.595	*b*	1.848	12.980	0.198	0.035	0.113	0.504	*c*	*d*
28	Lq35	0.643	*b*	0.562	2.888	0.071	*c*	*c*	0.197	*c*	*c*
29	QA06B	0.668	*b*	0.884	12.780	0.135	*c*	0.084	0.441	*c*	0.080
30	QA11B	0.431	*b*	0.309	6.530	0.126	*c*	0.092	0.317	*c*	0.069
31	Lq43	0.387	*b*	2.243	15.737	0.220	0.030	0.109	0.475	0.122	0.327
32	Lq42	0.455	*b*	0.859	23.503	0.178	*c*	0.109	0.302	0.278	0.572
	LLOQ	0.050	0.050	0.200	0.600	0.050	0.025	0.050	0.050	0.050	0.050
	ULOQ	2.5	2.5	10.0	30.0	1.0	0.5	1.0	1.0	1.0	1.0

a The samples listed as "QA" are internal quality assurance plasma materials
b Retinyl palmitate (RP) values only reported if the UV absorbance spectrum of the peak matches standard RP
c Peak response less than the lower limit of quantitation (LLOQ)
d Peak response greater than upper limit of quantitation (ULOQ): highest calibration standard

Table 5. MRC EWL Results for Batch 3: April 1 to 2, 2014

All values reported in µg/mL

Order	Sample [a]	TR	RP	gbT	aT	TLZ	TaX	TbX	Tly	TaC	TbC
1	QA11A	0.479	*b*	0.491	8.125	0.129	*c*	0.098	0.346	*c*	0.079
2	Ly15	0.521	*b*	2.478	5.860	0.074	*c*	*c*	0.228	*c*	0.373
3	Ly07	0.253	*b*	2.171	5.160	0.056	*c*	*c*	0.274	*c*	0.368
4	Ly12	0.959	*b*	1.499	6.681	0.086	*c*	*c*	0.839	*c*	0.084
5	Ly01	1.029	*b*	1.469	8.922	0.121	*c*	*c*	0.284	*c*	0.943
6	Ly26	*e*	*e*	*e*	*e*	*e*	*e*	*e*	*e*	*e*	*e*
7	Ly19	0.293	*b*	1.694	7.350	0.066	*c*	*c*	0.163	*c*	0.201
8	QA02B	0.573	*b*	0.420	15.978	0.365	0.053	0.370	0.718	0.065	0.922
9	QA06B	0.695	*b*	1.100	14.226	0.119	*c*	0.091	0.466	*c*	0.089
10	Ly06	1.161	*b*	2.576	12.611	0.123	*c*	*c*	0.285	*c*	*d*
11	Ly13	0.732	0.307	2.210	7.010	0.165	0.033	0.059	0.251	*c*	0.574
12	Ly05	0.473	*b*	2.092	7.963	0.113	0.036	*c*	0.275	*c*	0.585
13	Ly08	0.499	*b*	0.969	7.588	0.147	0.037	0.103	0.403	*c*	0.989
14	Ly24	0.488	*b*	1.530	17.591	0.107	*c*	*c*	0.471	0.097	0.456
15	Ly14	0.561	0.191	2.017	4.862	0.079	*c*	*c*	0.193	*c*	0.386
16	Ly17	1.087	*b*	2.467	8.264	0.115	*c*	*c*	0.727	*c*	0.601
17	Ly09	0.237	*b*	0.943	6.119	0.067	*c*	*c*	0.158	*c*	0.222
18	QA07B	0.753	*b*	0.669	16.138	0.257	0.032	0.068	0.939	0.055	0.148
19	Lq43	0.388	*b*	2.449	16.633	0.206	0.030	0.116	0.503	0.139	0.358
20	Lq47	0.485	*b*	1.382	10.710	0.128	*c*	*c*	0.653	*c*	0.239
21	Lq42	0.482	*b*	0.895	24.592	0.169	*c*	0.108	0.288	0.292	0.594
22	Lq32	0.672	*b*	2.417	6.978	0.130	0.029	0.053	0.264	*c*	0.547
23	Lq39	0.502	*b*	1.624	12.310	0.217	0.056	0.067	0.482	0.128	0.322
24	QA02A	0.605	*b*	0.450	16.695	0.384	0.053	0.376	0.737	0.065	0.957
25	Lq40	0.656	*b*	1.846	11.377	0.131	0.028	0.065	0.597	0.070	0.127
26	QA07A	0.742	*b*	0.722	16.471	0.263	0.032	0.069	0.997	0.059	0.160
27	QA11B	0.477	*b*	0.460	7.645	0.126	*c*	0.096	0.325	*c*	0.075
28	Lq31	0.670	*b*	1.036	20.075	0.288	0.033	0.081	0.501	0.069	0.358
29	QA06A	0.731	*b*	1.137	14.804	0.121	*c*	0.095	0.467	*c*	0.092
30	Lq37	0.611	*b*	2.079	14.242	0.196	0.035	0.116	0.498	*c*	*d*
31	Lq45	0.765	*b*	2.254	10.199	0.112	*c*	0.064	0.422	*c*	0.293
32	QA00A	0.440	*b*	1.248	7.538	0.063	*c*	*c*	0.098	*c*	*c*
33	QA00B	0.438	*b*	1.255	7.627	0.065	*c*	*c*	0.118	*c*	*c*
	LLOQ	0.050	0.050	0.200	0.600	0.050	0.025	0.050	0.050	0.050	0.050
	ULOQ	2.5	2.5	10.0	30.0	1.0	0.5	1.0	1.0	1.0	1.0

a The samples listed as "QA" are internal quality assurance plasma materials
b Retinyl palmitate (RP) values only reported if the UV absorbance spectrum of the peak matches standard RP
c Peak response less than the lower limit of quantitation (LLOQ)
d Peak response greater than upper limit of quantitation (ULOQ): highest calibration standard
e Failed injection

Table 6. MRC EWL Results for Batch 4: April 7 to 8, 2014

All values reported in µg/mL

Order	Sample [a]	TR	RP	gbT	aT	TLZ	TaX	TbX	Tly	TaC	TbC
1	Ly27	0.650	0.272	1.706	15.413	0.262	0.043	0.068	0.376	0.110	0.308
2	Ly28	0.461	*b*	1.765	7.066	0.126	0.026	0.055	0.392	*c*	0.352
3	Ly25	0.592	*b*	2.160	12.375	0.151	0.032	0.088	0.461	*c*	0.246
4	Ly16	*d*	0.151	3.466	25.433	*c*	*c*	*c*	0.198	*c*	*d*
5	Ly11	0.715	*b*	3.667	16.316	0.129	*c*	*c*	0.349	0.086	*d*
6	Ly30	0.701	*b*	1.764	10.699	0.138	0.025	0.061	0.513	0.062	0.115
7	Ly10	0.546	*b*	2.645	10.681	0.151	0.044	0.071	0.463	*c*	0.892
8	QA02B	0.649	*b*	0.445	16.356	0.411	0.056	0.385	0.745	0.064	0.970
9	Ly18	0.455	*b*	3.053	7.981	0.114	*c*	0.051	0.602	*c*	0.059
10	QA11B	0.493	*b*	0.407	7.774	0.138	*c*	0.110	0.354	*c*	0.078
11	Ly04	0.322	*b*	1.965	5.156	0.088	*c*	*c*	0.362	*c*	0.106
12	Ly20	0.547	*b*	2.368	9.619	0.079	*c*	*c*	0.270	*c*	0.496
13	QA02A	0.623	*b*	0.385	15.921	0.396	0.053	0.363	0.702	0.057	0.918
14	Ly23	0.954	*b*	3.994	8.386	0.122	*c*	0.081	0.364	*c*	0.187
15	Ly29	0.662	*b*	2.069	5.639	0.132	0.031	0.054	0.257	*c*	0.508
16	Ly22	0.810	*b*	2.796	13.066	0.130	*c*	0.062	0.422	*c*	0.348
17	Ly21	0.918	*b*	3.674	18.147	*c*	*c*	*c*	0.325	*c*	*d*
18	Lq34	0.503	*b*	1.889	7.409	0.135	0.028	0.062	0.416	*c*	0.387
19	QA07B	0.817	*b*	0.657	16.156	0.291	0.033	*c*	0.992	0.058	0.157
20	Lq48	0.709	*b*	2.197	19.741	0.187	*c*	*c*	*d*	*c*	0.443
21	Lq38	0.353	*b*	0.727	3.142	0.072	*c*	*c*	0.210	*c*	0.050
22	QA06B	0.732	*b*	1.051	14.225	0.127	*c*	0.096	0.470	*c*	0.090
23	Lq36	0.616	*b*	0.677	3.099	0.069	*c*	*c*	0.195	*c*	*c*
24	QA07A	0.723	*b*	0.635	15.641	0.255	0.032	0.086	0.990	0.058	0.155
25	QA06A	0.704	*b*	1.043	13.698	0.126	*c*	0.092	0.474	*c*	0.097
26	Lq46	0.374	*b*	1.834	7.234	0.140	*c*	0.059	0.256	*c*	0.102
27	Lq41	0.314	*b*	3.647	9.257	0.256	0.038	0.123	0.646	*c*	0.162
28	Lq35	0.769	*b*	0.805	3.729	0.084	*c*	*c*	0.256	*c*	0.058
29	Lq33	0.669	0.297	1.818	16.532	0.280	0.047	0.072	0.398	0.119	0.339
30	QA11A	0.453	*b*	0.451	7.196	0.124	*c*	0.102	0.341	*c*	0.076
31	Lq44	0.337	*b*	1.238	5.484	0.090	*c*	*c*	0.308	*c*	0.088
32	QA00A	0.438	*b*	1.385	8.029	0.079	*c*	*c*	0.132	*c*	*c*
33	QA00B	0.450	*b*	1.486	8.579	0.079	*c*	*c*	0.127	*c*	*c*
	LLOQ	0.050	0.050	0.200	0.600	0.050	0.025	0.050	0.050	0.050	0.050
	ULOQ	2.5	2.5	10.0	30.0	1.0	0.5	1.0	1.0	1.0	1.0

a The samples listed as "QA" are internal quality assurance plasma materials

b Retinyl palmitate (RP) values only reported if the UV absorbance spectrum of the peak matches standard RP

c Peak response less than the lower limit of quantitation (LLOQ)

d Peak response greater than upper limit of quantitation (ULOQ): highest calibration standard

Table 7. MRC EWL Summary Results, µg/mL

	TR		RP		aT		gbT		TbC	
Sample	x_{mean}	s	x_{mean}	s	x_{mean}	s	x_{mean}	s	x_{mean}	s
Ly01	1.007	0.032			8.86	0.09	1.383	0.122	0.933	0.015
Ly02	0.967	*a*			10.17	*a*	2.449	*a*	0.850	*a*
Ly03	0.435	*a*			5.88	*a*	2.815	*a*	0.183	*a*
Ly04	0.305	0.025			4.92	0.33	1.882	0.117	0.099	0.010
Ly05	0.481	0.011			7.64	0.46	2.003	0.127	0.583	0.004
Ly06	1.189	0.039			12.16	0.64	2.558	0.025	*b*	
Ly07	0.254	0.001			4.98	0.26	2.098	0.103	0.371	0.004
Ly08	0.506	0.009			6.78	1.14	0.895	0.105	0.949	0.057
Ly09	0.234	0.004			5.63	0.70	0.835	0.153	0.218	0.006
Ly10	0.502	0.062			10.33	0.50	2.440	0.290	0.898	0.008
Ly11	0.695	0.029			15.56	1.07	3.570	0.138	*b*	
Ly12	0.998	0.054			6.24	0.62	1.455	0.062	0.088	0.005
Ly13	0.708	0.035	0.304	0.005	6.58	0.60	2.139	0.100	0.582	0.011
Ly14	0.562	0.001	0.186	0.007	4.61	0.36	1.873	0.204	0.388	0.003
Ly15	0.526	0.007			5.61	0.35	2.344	0.190	0.374	0.001
Ly16	*b*		0.140	0.016	23.92	2.13	3.268	0.280	*b*	
Ly17	1.042	0.064			7.53	1.03	2.253	0.303	0.607	0.008
Ly18	0.432	0.033			7.52	0.65	2.902	0.214	0.056	0.004
Ly19	0.291	0.003			7.09	0.37	1.579	0.163	0.209	0.011
Ly20	0.498	0.069	0.123	*c*	9.47	0.21	2.176	0.272	0.514	0.025
Ly21	0.903	0.021	0.218	*c*	17.28	1.22	3.549	0.177	*b*	
Ly22	0.743	0.095			12.44	0.89	2.851	0.078	0.336	0.017
Ly23	0.868	0.122			7.97	0.59	3.799	0.276	0.182	0.007
Ly24	0.468	0.028			16.83	1.08	1.433	0.137	0.444	0.017
Ly25	0.563	0.041			11.78	0.84	2.099	0.087	0.239	0.010
Ly26	0.618	*d*			17.18	*d*	0.895	*d*	0.326	*d*
Ly27	0.622	0.040	0.273	0.001	14.54	1.23	1.606	0.142	0.307	0.002
Ly28	0.441	0.029			6.76	0.43	1.728	0.053	0.359	0.010
Ly29	0.624	0.054			5.71	0.11	2.073	0.006	0.518	0.014
Ly30	0.646	0.078			10.19	0.72	1.707	0.081	0.117	0.003
Lq31	0.674	0.005			18.95	1.60	0.949	0.123	0.359	0.001
Lq32	0.656	0.023			6.37	0.85	2.234	0.259	0.534	0.018
Lq33	0.658	0.016	0.292	0.007	15.72	1.14	1.726	0.131	0.332	0.011
Lq34	0.478	0.036			6.43	1.39	1.703	0.264	0.374	0.018
Lq35	0.706	0.089			3.31	0.59	0.684	0.172	0.058	*c*
Lq36	0.605	0.016			2.77	0.47	0.645	0.046	0.054	*c*
Lq37	0.603	0.011			13.61	0.89	1.964	0.163	*b*	
Lq38	0.333	0.028			2.90	0.34	0.679	0.069	0.055	0.006
Lq39	0.508	0.008			11.65	0.93	1.639	0.021	0.337	0.021
Lq40	0.644	0.017			10.45	1.31	1.694	0.215	0.121	0.009
Lq41	0.298	0.023			8.88	0.53	3.487	0.227	0.155	0.011
Lq42	0.469	0.019			24.05	0.77	0.877	0.025	0.583	0.016
Lq43	0.388	0.001			16.19	0.63	2.346	0.146	0.343	0.022
Lq44	0.336	0.001			5.66	0.25	1.260	0.031	0.080	0.012
Lq45	0.718	0.067			10.16	0.05	2.175	0.112	0.283	0.015
Lq46	0.361	0.018			6.77	0.66	1.732	0.144	0.099	0.005
Lq47	0.489	0.006			10.26	0.63	1.299	0.117	0.233	0.009
Lq48	0.676	0.047			18.75	1.40	2.109	0.124	0.433	0.014

a Only one sample of material provided
b Peak response of both samples above ULOQ
c Peak response of one sample below LLOQ
d Injection failure for one sample

Table 7. MRC EWL Summary Results, µg/mL (Continued)

Sample	TaC		TLy		TbX		TaX		TLZ	
	x_{mean}	s	x_{mean}	s	x_{mean}	s	x_{mean}	s	x_{mean}	s
Ly01			0.281	0.005					0.122	0.001
Ly02			0.283	*a*			0.029	*a*	0.128	*a*
Ly03			0.273	*a*			0.028	*a*	0.129	*a*
Ly04			0.344	0.025					0.087	0.001
Ly05			0.301	0.036			0.036	0.001	0.126	0.018
Ly06			0.284	0.001			0.026	*c*	0.129	0.008
Ly07			0.291	0.023					0.064	0.011
Ly08			0.382	0.030	0.099	0.006	0.037	0.001	0.148	0.001
Ly09			0.146	0.017					0.074	0.009
Ly10			0.454	0.013	0.073	0.003	0.042	0.003	0.155	0.005
Ly11	0.073	0.019	0.334	0.021					0.125	0.006
Ly12			0.884	0.064					0.092	0.008
Ly13			0.240	0.016	0.058	0.001	0.035	0.003	0.163	0.003
Ly14			0.201	0.011					0.081	0.002
Ly15			0.239	0.015					0.079	0.006
Ly16			0.185	0.019						
Ly17			0.735	0.011	0.052	*c*			0.117	0.002
Ly18			0.602	0.001	0.051	*c*			0.112	0.003
Ly19			0.169	0.008					0.071	0.006
Ly20			0.273	0.004					0.080	0.001
Ly21			0.340	0.021					0.053	*c*
Ly22			0.396	0.037	0.067	0.006	0.028	*c*	0.126	0.006
Ly23			0.369	0.006	0.079	0.003			0.114	0.012
Ly24	0.092	0.007	0.469	0.004					0.107	0.000
Ly25			0.446	0.021	0.087	0.002	0.032	0.001	0.153	0.003
Ly26	0.063	*d*	0.491	*d*	0.077	*d*	0.032	*d*	0.286	*d*
Ly27	0.104	0.009	0.377	0.001	0.067	0.002	0.044	0.001	0.252	0.014
Ly28			0.389	0.005	0.055	0.001	0.026	0.000	0.124	0.003
Ly29			0.258	0.001	0.054	*c*	0.030	0.001	0.127	0.007
Ly30	0.062	0.000	0.523	0.013	0.059	0.003	0.026	0.001	0.131	0.011
Lq31	0.067	0.003	0.521	0.028	0.080	0.001	0.034	0.001	0.298	0.013
Lq32			0.275	0.015	0.053	*c*	0.029	0.001	0.130	0.000
Lq33	0.114	0.008	0.402	0.006	0.070	0.004	0.047	0.001	0.275	0.007
Lq34			0.408	0.012	0.058	0.006	0.028	0.000	0.130	0.007
Lq35			0.227	0.042					0.078	0.009
Lq36			0.204	0.013					0.071	0.003
Lq37			0.501	0.004	0.115	0.002	0.035	0.000	0.197	0.001
Lq38			0.209	0.002					0.071	0.001
Lq39	0.133	0.006	0.512	0.042	0.065	0.004	0.055	0.001	0.227	0.013
Lq40	0.070	0.000	0.572	0.035	0.064	0.002	0.028	0.000	0.132	0.001
Lq41			0.642	0.006	0.119	0.006	0.038	0.001	0.262	0.008
Lq42	0.285	0.010	0.295	0.010	0.109	0.001			0.174	0.006
Lq43	0.131	0.012	0.489	0.020	0.113	0.005	0.030	0.000	0.213	0.010
Lq44			0.315	0.010					0.095	0.007
Lq45			0.433	0.015	0.064	0.000			0.113	0.001
Lq46			0.247	0.013	0.056	0.004			0.139	0.001
Lq47			0.674	0.029	0.059	*c*	0.026	*c*	0.140	0.016
Lq48			1.059	*c*					0.180	0.010

a Only one sample of material provided
b Peak response of both samples above ULOQ
c Peak response of one sample below LLOQ
d Injection failure for one sample

Figure 2 displays the results from the second set of MRC EWL measurements as functions of the results from the first set. The close agreement demonstrates that the 10-measurand method used was in adequate statistical control. It also demonstrates the near identity of the two samples of the 46 materials for which NIST was able to supply two units.

Figure 3 displays the standard deviations of the two results as functions of the means and an empirical repeatability function fit to these paired values [16,17]. These functions have been used to provide lower-bounds on the empirical standard deviations.

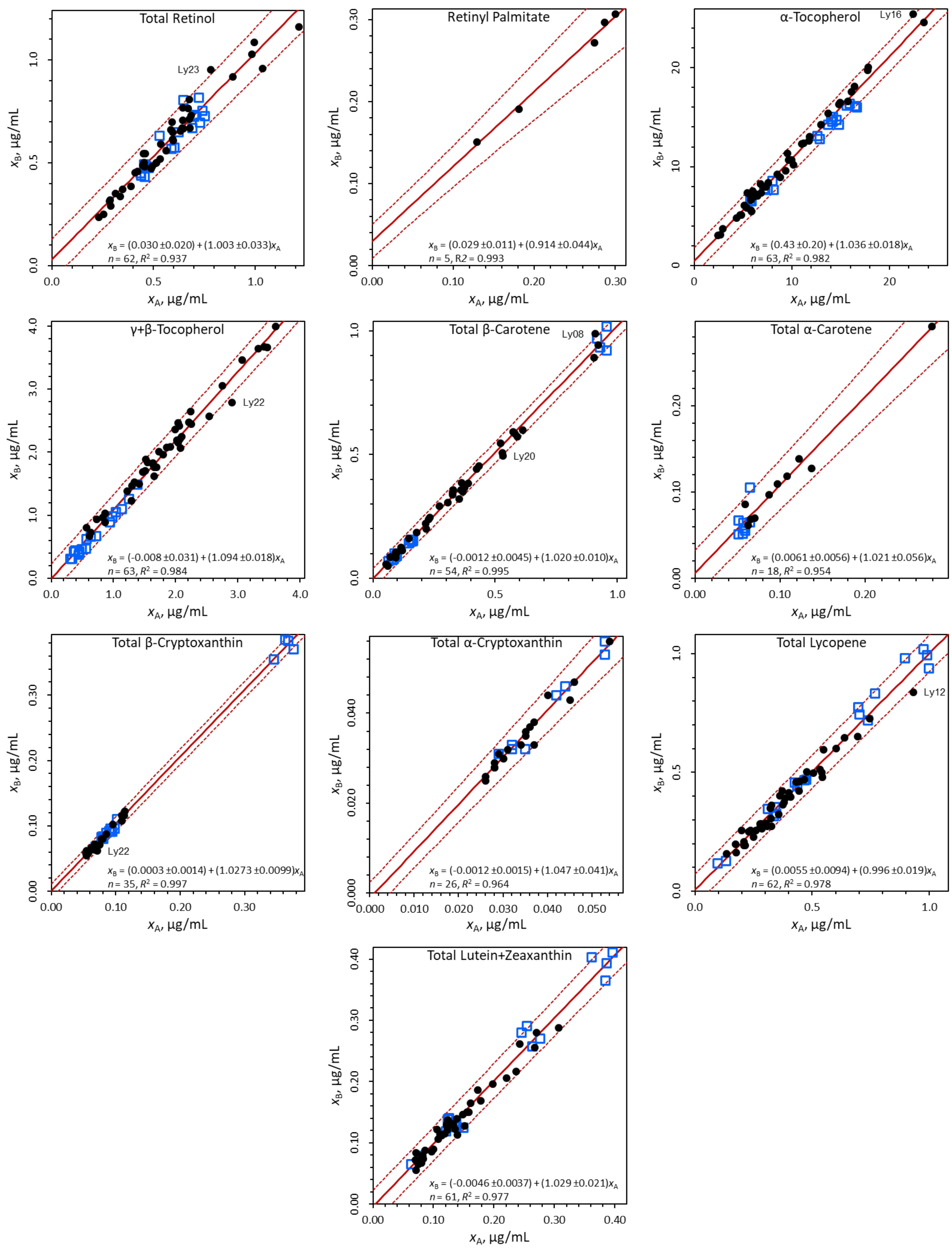

Figure 2. Result of Second Measurement (x_B) as Functions of First Measurement (x_A)

Solid black circles denote results for MMQAP serum samples; open blue squares denote results for MRC EWL quality assurance plasma materials. Solid red lines represent the linear relationship between the replicates; dotted lines encompass an approximate 95 % confidence region. Labels identify samples lying outside the bounds. Results are from independently calibrated measurement runs separated in time by about two weeks.

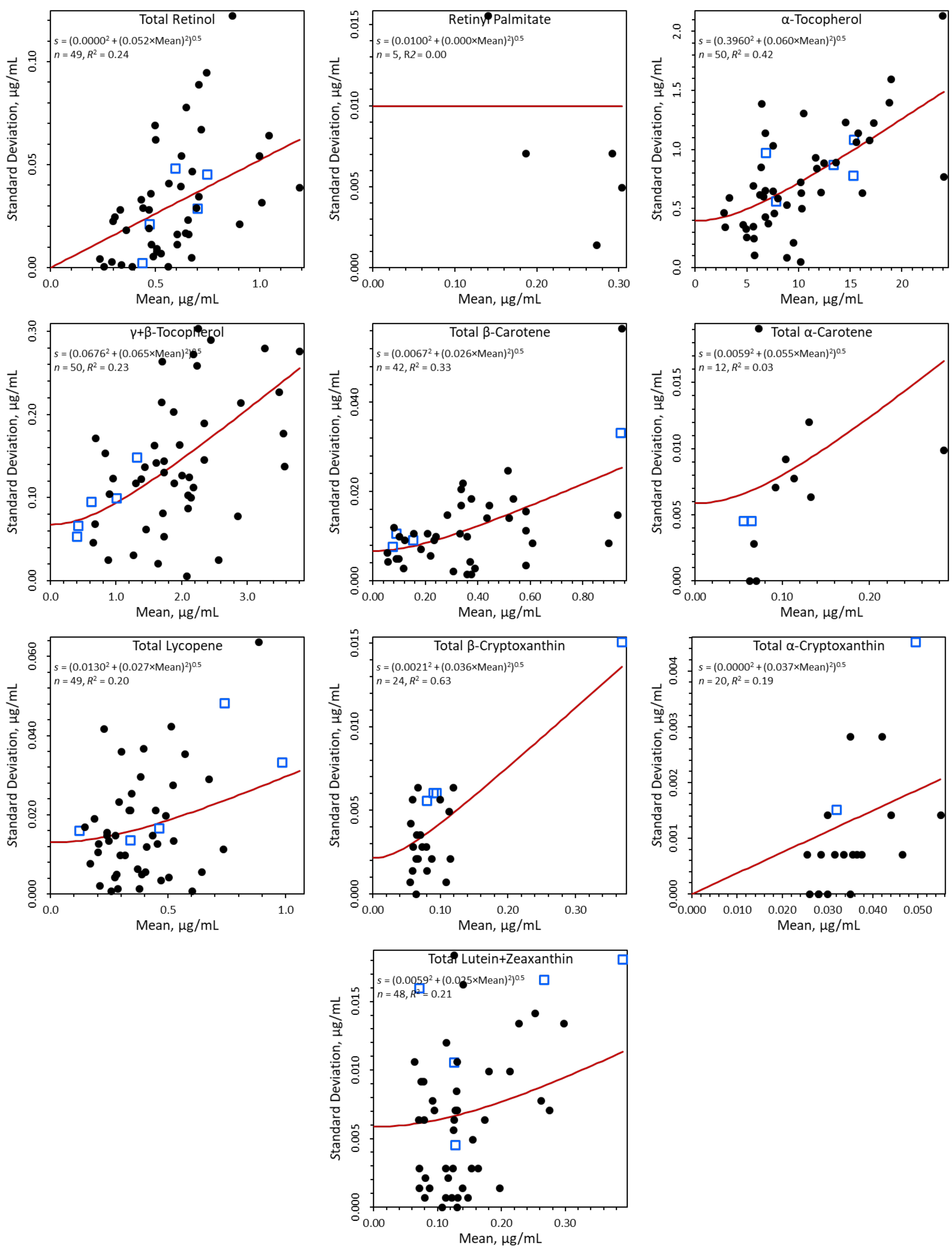

Figure 3. MRC EWL Measurement Repeatability Functions

Solid black circles denote MRC EWL results for MMQAP serum samples; open blue squares denote results for MRC EWL quality assurance plasma materials. Solid red lines represent regression fits to the measurement precision function [16,17]: $s = \sqrt{a^2 + (b \times \text{Mean})^2}$.

3. MMQAP Measurement Results

Table 8 summarizes the MMQAP results for the 48 materials provided to the MRC EWL. The table provides the total number of participant results available for each of the 10 measurands listed in Table 1, the median of those values, and the Q_n robust estimate of standard deviation [18,19]. However, the values are for the complete set of MMQAP results as of the last RR (LXXXII, 2017) rather than those provided to the MRC EWL in April 2014. The complete results for all measurands reported in all of the vitamin-related MMQAP RRs, along with the feedback-analyses provided to the study participants, are available in the NIST.IR.7880 series of reports [20].

Figure 4 to Figure 13 depict the empirical distribution of results for the 10 measurands in all materials for which there were at least eight participant results. The distributions are presented as the exterior trace of 30-bin histograms spanning the range of measurand concentrations among the materials. Because of the relatively wide concentration range for several of the measurands, the distributions are for the base-10 logarithmic transformation of the reported values. Each of the empirical distributions is accompanied by a best-fit lognormal distribution, intended to provide a visual reference for interpreting the sometimes poorly defined empirical distribution.

Figure 14 displays the Q_n estimates as functions of the median values, along with empirical interlaboratory reproducibility functions fit to these paired values [16,17]. These functions have been used to provide lower-bounds on the Q_n values.

Table 8. MMQAP Summary Results, µg/mL

	Total Retinol			Retinyl Palmitate			α-Tocopherol			γ+β-Tocopherol			Total β-Carotene		
Sample	n	x_{median}	Q_n	n	x_{median}	Q_n	n	x_{median}	Q_n	n	x_{median}	Q_n	n	x_{median}	Q_n
Ly01	96	1.046	0.107	8	0.077	0.021	93	9.32	0.88	13	1.630	0.261	74	1.002	0.167
Ly02	68	0.959	0.114	5	0.045	0.013	66	10.53	0.84	13	2.750	0.361	47	0.847	0.107
Ly03	140	0.437	0.048	8	0.031	0.017	131	5.98	0.68	23	3.040	0.386	100	0.194	0.041
Ly04	30	0.313	0.020	0			28	4.95	0.39	2	2.307		17	0.112	0.010
Ly05	30	0.472	0.038	0			28	7.81	0.55	2	2.354		18	0.666	0.044
Ly06	30	1.160	0.075	0			28	12.41	0.86	2	2.960		18	1.487	0.097
Ly07	118	0.266	0.028	11	0.051	0.035	116	4.88	0.43	51	2.291	0.275	85	0.412	0.056
Ly08	114	0.494	0.039	8	0.056	0.054	115	6.71	0.57	47	1.000	0.227	88	1.064	0.158
Ly09	80	0.190	0.025	6	0.010	0.010	82	4.71	0.59	30	0.873	0.150	66	0.255	0.063
Ly10	141	0.498	0.051	13	0.032	0.028	138	10.49	0.82	48	2.836	0.329	110	0.903	0.137
Ly11	123	0.657	0.070	16	0.041	0.029	120	16.13	1.29	48	3.795	0.418	98	2.274	0.417
Ly12	92	0.998	0.102	7	0.047	0.069	90	6.30	0.60	33	1.610	0.256	70	0.090	0.027
Ly13	122	0.668	0.050	34	0.296	0.062	123	6.10	0.56	52	2.057	0.275	99	0.579	0.090
Ly14	129	0.539	0.044	36	0.186	0.040	132	4.92	0.52	63	2.022	0.239	107	0.392	0.064
Ly15	195	0.544	0.055	46	0.065	0.018	190	5.49	0.52	94	2.500	0.314	138	0.415	0.061
Ly16	87	6.890	0.975	24	0.138	0.040	92	24.03	2.69	45	3.542	0.373	74	1.670	0.178
Ly17	228	1.075	0.105	43	0.037	0.022	232	7.15	0.70	117	2.372	0.261	169	0.578	0.088
Ly18	97	0.432	0.037	14	0.028	0.023	94	7.25	0.70	46	3.120	0.467	68	0.058	0.019
Ly19	142	0.294	0.028	36	0.091	0.024	141	7.05	0.70	72	1.665	0.253	104	0.243	0.045
Ly20	143	0.514	0.046	40	0.181	0.028	141	9.90	0.81	73	2.310	0.246	106	0.620	0.094
Ly21	147	0.880	0.075	40	0.260	0.038	146	17.56	1.45	77	3.800	0.317	111	1.227	0.191
Ly22	114	0.681	0.059	36	0.053	0.013	117	11.60	1.14	68	2.716	0.231	80	0.319	0.044
Ly23	253	0.858	0.062	78	0.032	0.012	269	7.51	0.69	158	3.855	0.384	182	0.172	0.023
Ly24	251	0.482	0.039	81	0.080	0.017	268	16.66	1.33	156	1.569	0.132	187	0.426	0.051
Ly25	39	0.558	0.062	16	0.123	0.036	50	11.28	1.17	29	2.160	0.305	31	0.237	0.034
Ly26	39	0.643	0.061	16	0.112	0.027	50	17.74	1.64	29	1.011	0.141	31	0.310	0.039
Ly27	44	0.580	0.061	15	0.207	0.035	54	14.64	1.69	31	1.796	0.243	35	0.269	0.047
Ly28	202	0.444	0.041	61	0.051	0.012	222	6.75	0.65	132	1.840	0.173	154	0.328	0.043
Ly29	171	0.638	0.051	43	0.020	0.009	179	6.00	0.59	101	2.220	0.213	123	0.485	0.068
Ly30	233	0.609	0.051	77	0.091	0.022	248	9.94	0.76	153	1.728	0.154	169	0.115	0.018
Lq31	39	0.687	0.089	16	0.115	0.031	50	18.56	1.80	29	1.136	0.171	31	0.317	0.045
Lq32	176	0.666	0.059	43	0.022	0.010	186	6.37	0.64	105	2.313	0.219	125	0.512	0.071
Lq33	44	0.632	0.072	15	0.231	0.033	54	15.33	1.81	31	1.890	0.234	35	0.271	0.053
Lq34	216	0.469	0.039	67	0.054	0.015	236	7.09	0.61	140	1.941	0.177	166	0.350	0.043
Lq35	114	0.630	0.052	29	0.017	0.006	115	2.85	0.27	71	0.720	0.089	82	0.050	0.008
Lq36	117	0.590	0.058	29	0.016	0.006	116	2.84	0.28	71	0.728	0.083	82	0.050	0.008
Lq37	74	0.610	0.049	19	0.026	0.010	75	13.00	1.38	45	2.172	0.215	55	1.462	0.191
Lq38	107	0.329	0.033	29	0.016	0.007	116	2.90	0.28	69	0.716	0.074	82	0.050	0.008
Lq39	34	0.531	0.046	13	0.081	0.022	40	11.30	1.00	24	1.673	0.188	29	0.282	0.031
Lq40	235	0.640	0.049	77	0.096	0.022	250	10.42	0.77	153	1.819	0.154	171	0.121	0.018
Lq41	128	0.307	0.030	34	0.017	0.009	140	8.41	0.64	92	3.665	0.326	87	0.147	0.020
Lq42	64	0.520	0.052	19	0.055	0.017	67	23.80	1.93	43	0.921	0.166	38	0.555	0.055
Lq43	32	0.399	0.036	9	0.033	0.013	33	15.39	1.13	21	2.430	0.298	19	0.337	0.042
Lq44	241	0.340	0.033	49	0.012	0.009	248	5.87	0.81	153	1.448	0.178	159	0.078	0.017
Lq45	144	0.738	0.069	36	0.044	0.010	148	10.57	0.92	84	2.396	0.260	95	0.277	0.040
Lq46	182	0.358	0.031	39	0.011	0.005	180	6.76	0.58	105	1.794	0.188	125	0.091	0.014
Lq47	243	0.500	0.042	53	0.028	0.014	241	10.30	0.84	139	1.411	0.134	165	0.243	0.029
Lq48	211	0.653	0.055	54	0.094	0.031	211	18.75	1.58	122	2.266	0.259	145	0.401	0.066

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Table 7: MMQAP Summary Results, µg/mL (Continued)

	Total α-Carotene			Total Lycopene			Total β-Cryptoxanthin			Total α-Cryptoxanthin			Total Lutein+ Zeaxanthin		
Sample	n	x_{median}	Q_n	n	x_{median}	Q_n	n	x_{median}	Q_n	n	x_{median}	Q_n	n	x_{median}	Q_n
Ly01	16	0.033	0.009	17	0.246	0.084	9	0.056	0.021	0			8	0.121	0.023
Ly02	1	0.028		13	0.256	0.109	0			0			0		
Ly03	23	0.013	0.006	24	0.215	0.056	16	0.040	0.014	1	0.032		16	0.125	0.025
Ly04	0			0			0			0			0		
Ly05	0			0			0			0			0		
Ly06	0			0			0			0			0		
Ly07	30	0.020	0.006	55	0.267	0.095	22	0.042	0.013	0			21	0.067	0.012
Ly08	24	0.034	0.013	48	0.330	0.146	16	0.105	0.045	0			13	0.165	0.044
Ly09	30	0.016	0.006	35	0.155	0.056	20	0.031	0.013	0			15	0.055	0.010
Ly10	55	0.050	0.015	55	0.396	0.132	36	0.094	0.036	0			26	0.148	0.036
Ly11	57	0.096	0.026	55	0.321	0.119	37	0.064	0.028	0			23	0.121	0.028
Ly12	35	0.019	0.006	38	0.751	0.220	26	0.057	0.035	0			17	0.090	0.025
Ly13	61	0.024	0.007	60	0.195	0.056	41	0.064	0.015	3	0.033	0.007	36	0.157	0.022
Ly14	65	0.015	0.004	68	0.177	0.044	54	0.034	0.010	5	0.016	0.005	48	0.073	0.010
Ly15	80	0.015	0.005	93	0.222	0.053	73	0.040	0.010	9	0.016	0.006	64	0.073	0.017
Ly16	50	0.033	0.014	49	0.173	0.058	36	0.050	0.012	1	0.012		28	0.038	0.014
Ly17	121	0.017	0.008	126	0.583	0.133	111	0.052	0.015	15	0.024	0.010	92	0.117	0.028
Ly18	44	0.013	0.006	50	0.489	0.130	39	0.044	0.013	3	0.036	0.012	29	0.108	0.023
Ly19	74	0.020	0.006	77	0.183	0.046	59	0.029	0.011	5	0.020	0.001	53	0.087	0.019
Ly20	79	0.030	0.011	77	0.278	0.068	59	0.040	0.013	4	0.018	0.012	54	0.092	0.021
Ly21	79	0.043	0.015	81	0.325	0.066	63	0.039	0.015	5	0.016	0.002	57	0.056	0.017
Ly22	69	0.054	0.011	64	0.372	0.071	70	0.056	0.011	17	0.018	0.004	67	0.098	0.024
Ly23	153	0.016	0.005	151	0.309	0.055	163	0.072	0.013	33	0.022	0.006	155	0.090	0.018
Ly24	160	0.098	0.018	152	0.418	0.066	161	0.032	0.009	33	0.017	0.008	156	0.107	0.020
Ly25	30	0.042	0.008	27	0.385	0.068	29	0.082	0.016	6	0.037	0.005	27	0.142	0.021
Ly26	30	0.069	0.014	27	0.388	0.068	29	0.071	0.019	6	0.035	0.008	27	0.245	0.036
Ly27	31	0.104	0.017	30	0.310	0.045	31	0.062	0.013	8	0.041	0.005	31	0.208	0.031
Ly28	135	0.029	0.007	126	0.330	0.054	135	0.050	0.010	30	0.024	0.006	128	0.110	0.017
Ly29	97	0.020	0.006	98	0.221	0.036	96	0.049	0.010	23	0.023	0.004	89	0.123	0.024
Ly30	150	0.076	0.015	142	0.485	0.071	148	0.052	0.009	29	0.025	0.006	148	0.108	0.021
Lq31	30	0.072	0.012	27	0.411	0.063	29	0.079	0.014	6	0.038	0.008	27	0.266	0.040
Lq32	102	0.021	0.006	100	0.230	0.041	100	0.051	0.011	25	0.026	0.006	98	0.129	0.026
Lq33	31	0.109	0.016	30	0.322	0.049	31	0.067	0.014	8	0.045	0.008	31	0.231	0.043
Lq34	138	0.030	0.007	136	0.347	0.051	140	0.054	0.010	30	0.026	0.005	130	0.117	0.019
Lq35	50	0.006	0.003	67	0.189	0.033	73	0.019	0.004	17	0.011	0.003	70	0.063	0.013
Lq36	49	0.005	0.002	66	0.194	0.031	72	0.019	0.005	15	0.011	0.002	71	0.063	0.011
Lq37	44	0.030	0.006	44	0.413	0.061	47	0.105	0.017	10	0.032	0.005	45	0.171	0.041
Lq38	48	0.005	0.002	66	0.189	0.030	68	0.019	0.004	13	0.010	0.002	65	0.064	0.013
Lq39	24	0.116	0.020	23	0.394	0.051	25	0.052	0.013	5	0.042	0.019	23	0.227	0.068
Lq40	150	0.080	0.015	142	0.509	0.078	148	0.055	0.010	28	0.026	0.006	148	0.113	0.020
Lq41	73	0.016	0.007	74	0.580	0.081	78	0.108	0.020	17	0.036	0.010	78	0.224	0.049
Lq42	40	0.301	0.045	34	0.267	0.030	36	0.095	0.016	8	0.022	0.012	36	0.180	0.027
Lq43	19	0.151	0.019	17	0.445	0.043	17	0.100	0.025	4	0.028	0.003	17	0.192	0.048
Lq44	114	0.010	0.005	125	0.259	0.064	120	0.040	0.009	23	0.016	0.004	125	0.083	0.022
Lq45	79	0.048	0.013	73	0.376	0.068	72	0.060	0.013	16	0.021	0.009	71	0.097	0.020
Lq46	83	0.008	0.004	92	0.218	0.030	85	0.049	0.010	20	0.017	0.004	89	0.110	0.018
Lq47	122	0.032	0.013	122	0.590	0.110	112	0.051	0.013	25	0.022	0.006	116	0.124	0.023
Lq48	97	0.016	0.009	110	0.954	0.212	99	0.031	0.013	23	0.019	0.008	103	0.146	0.032

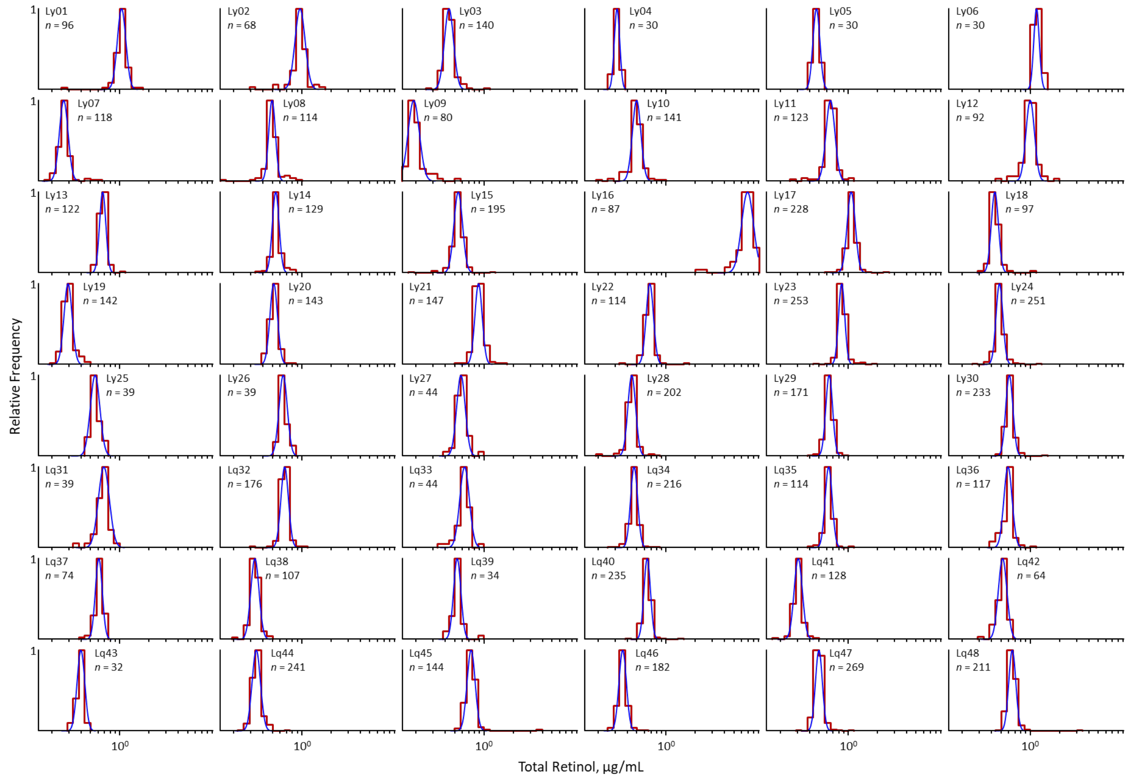

Figure 4. MMQAP Results for Total Retinol

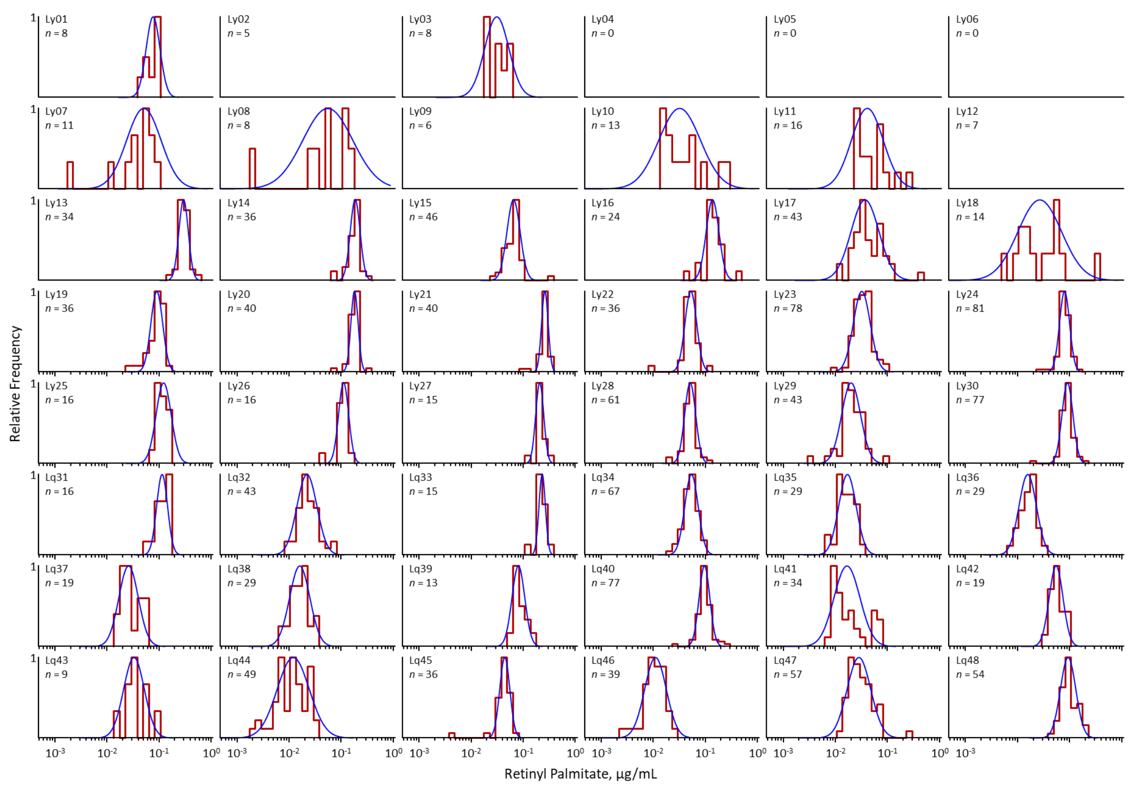

Figure 5. MMQAP Results for Retinyl Palmitate

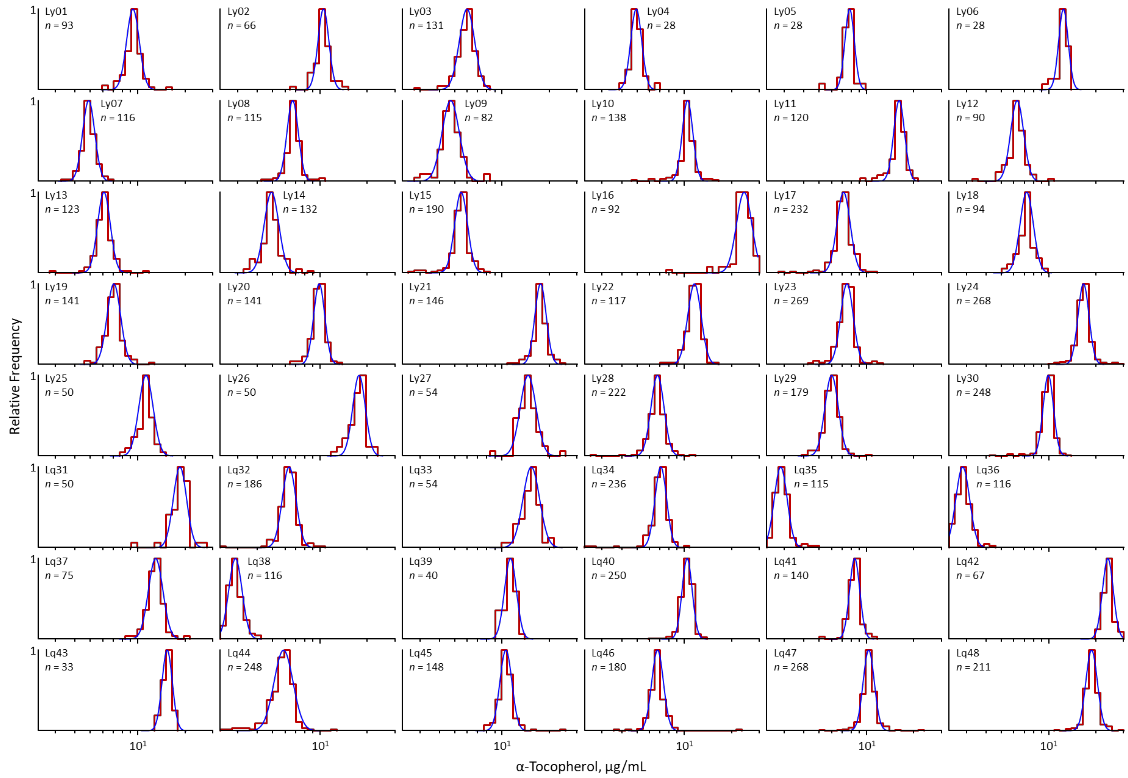

Figure 6. MMQAP Results for α-Tocopherol

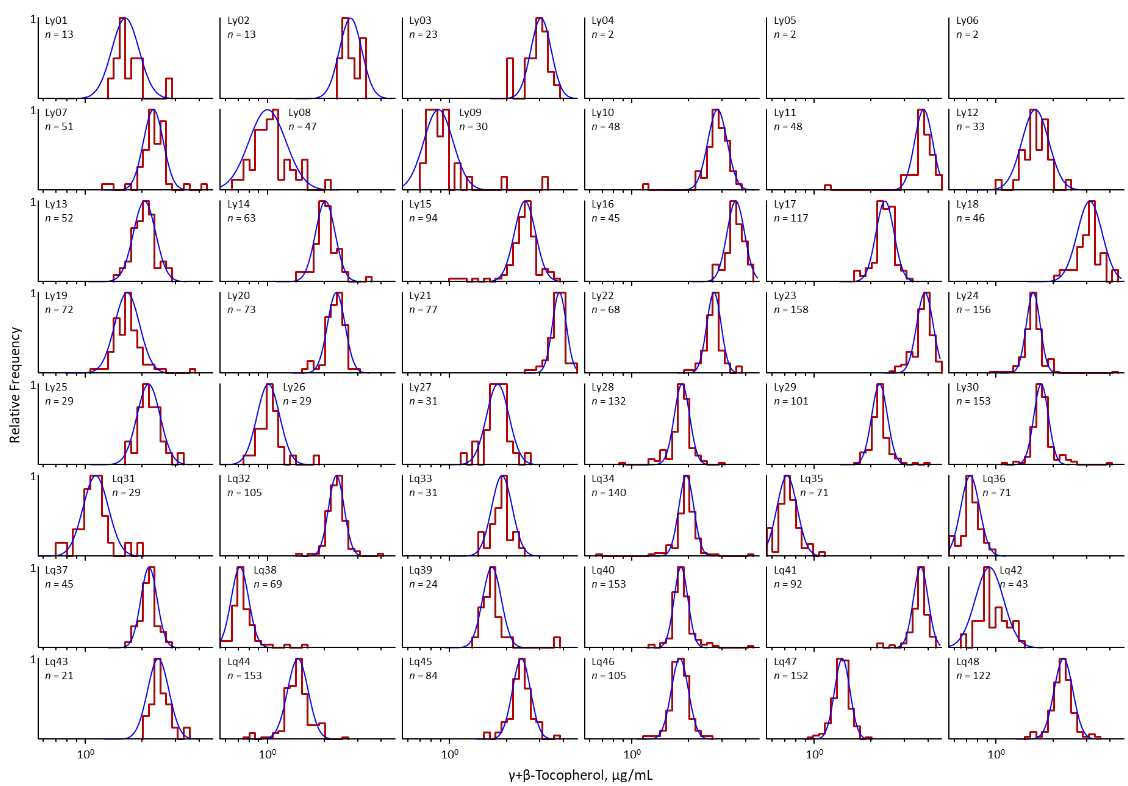

Figure 7. MMQAP Results for γ+β-Tocopherol

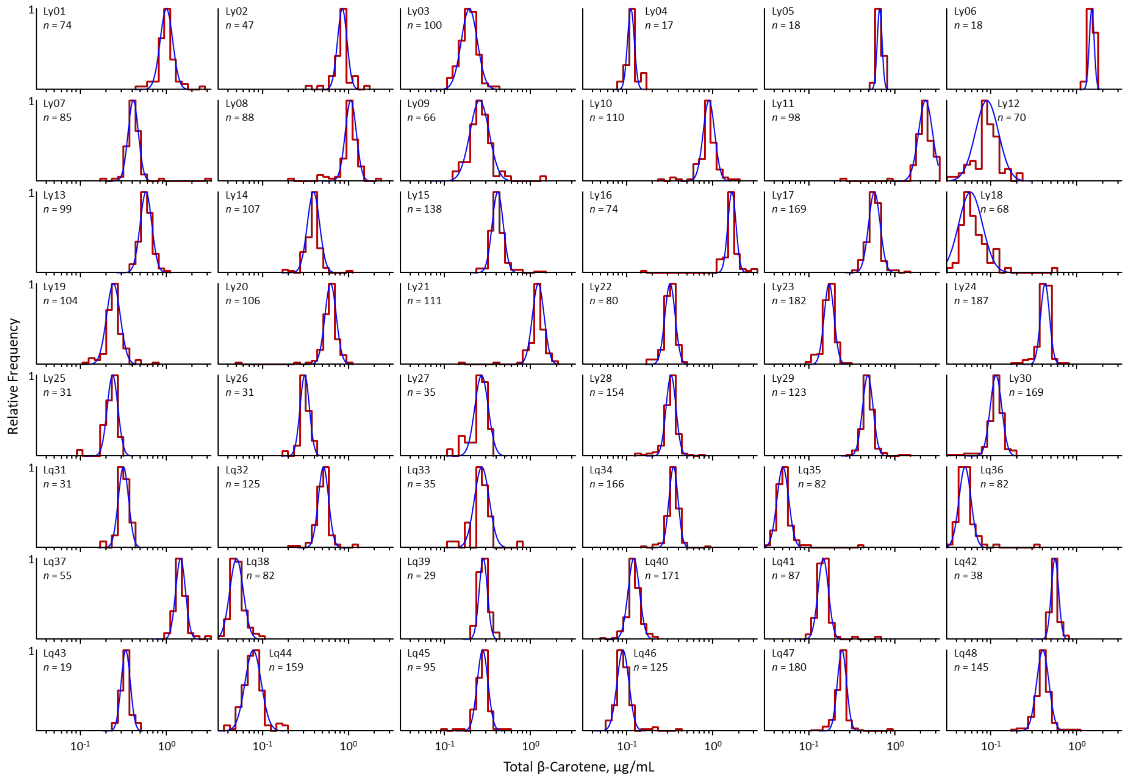

Figure 8. MMQAP Results for Total β-Carotene

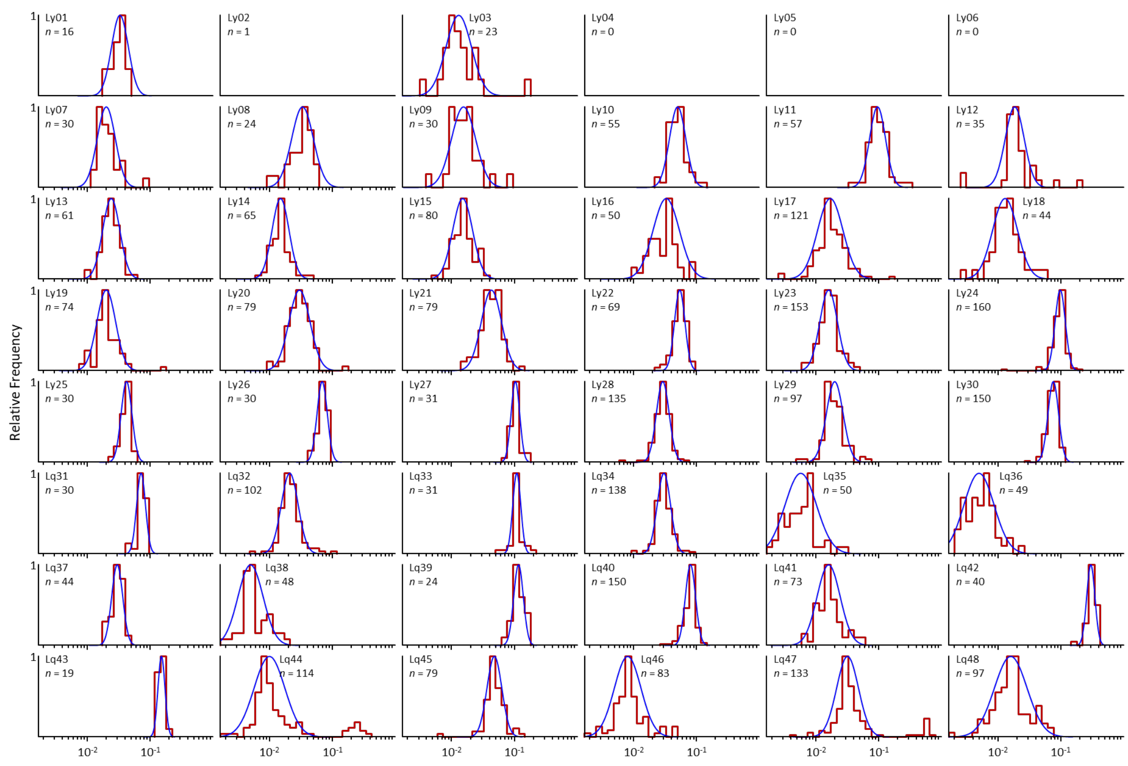

Figure 9. MMQAP Results for Total α-Carotene

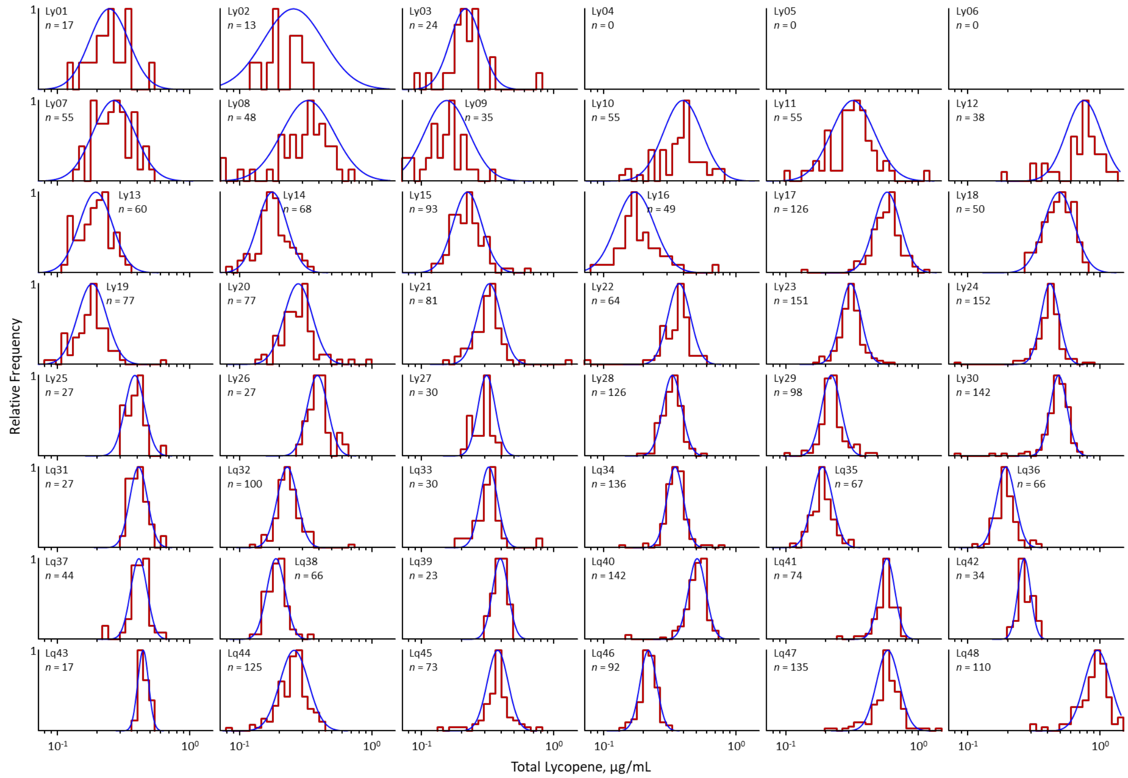

Figure 10. MMQAP Results for Total Lycopene

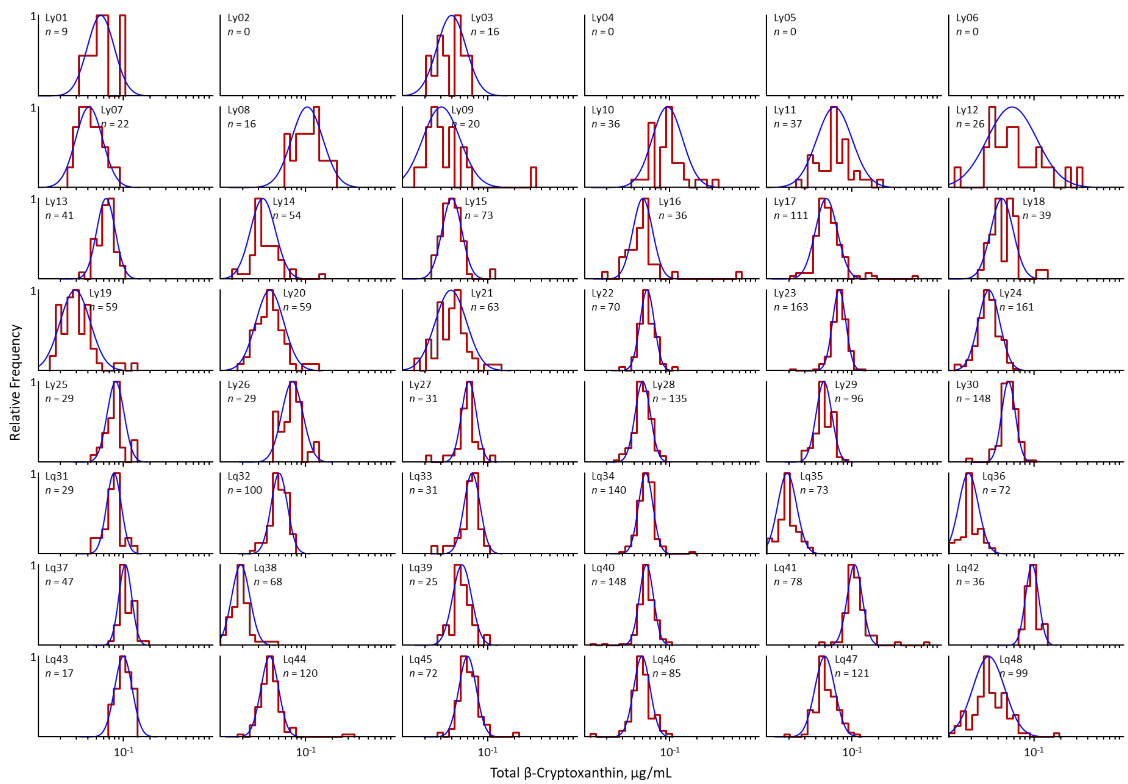

Figure 11. MMQAP Results for Total β-Cryptoxanthin

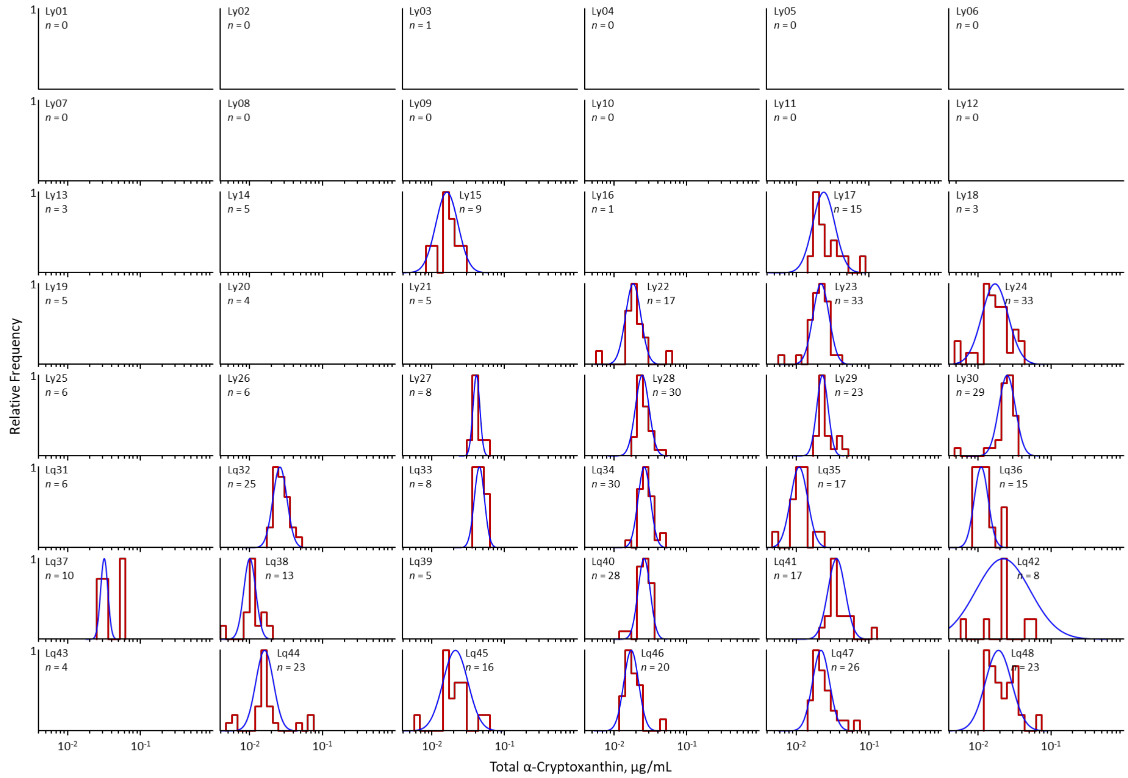

Figure 12. MMQAP Results for Total α-Cryptoxanthin

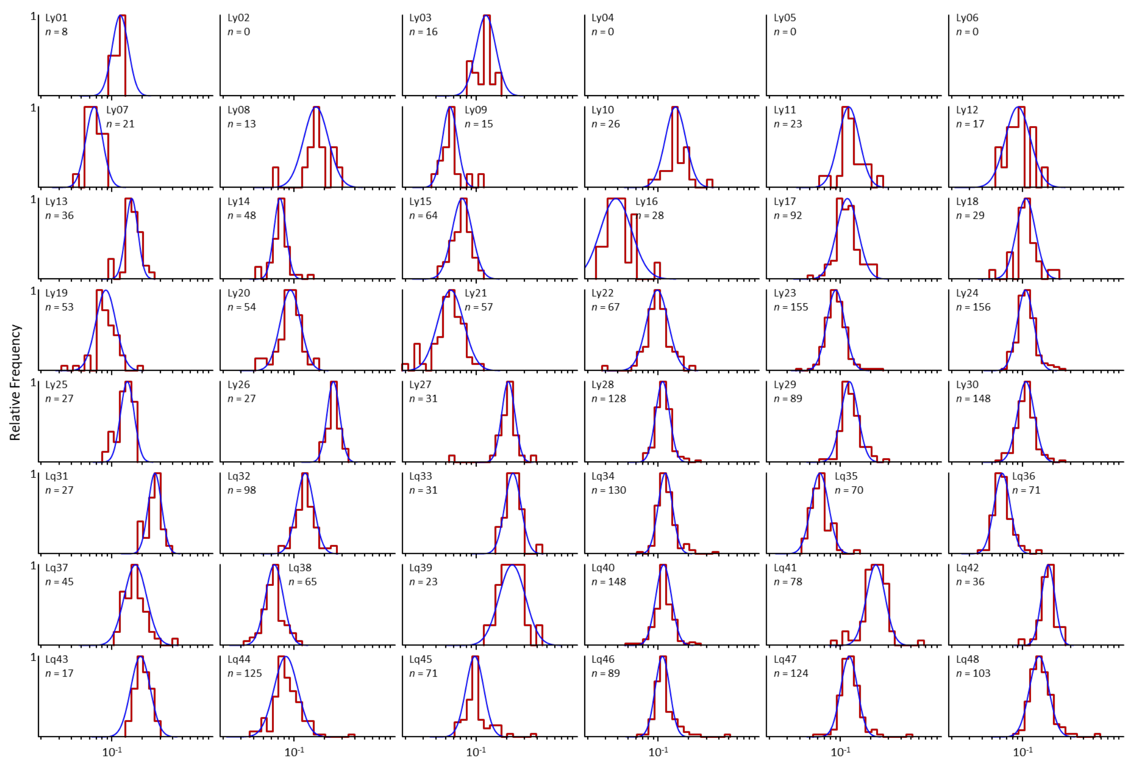

Figure 13. MMQAP Results for Total Lutein+Zeaxanthin

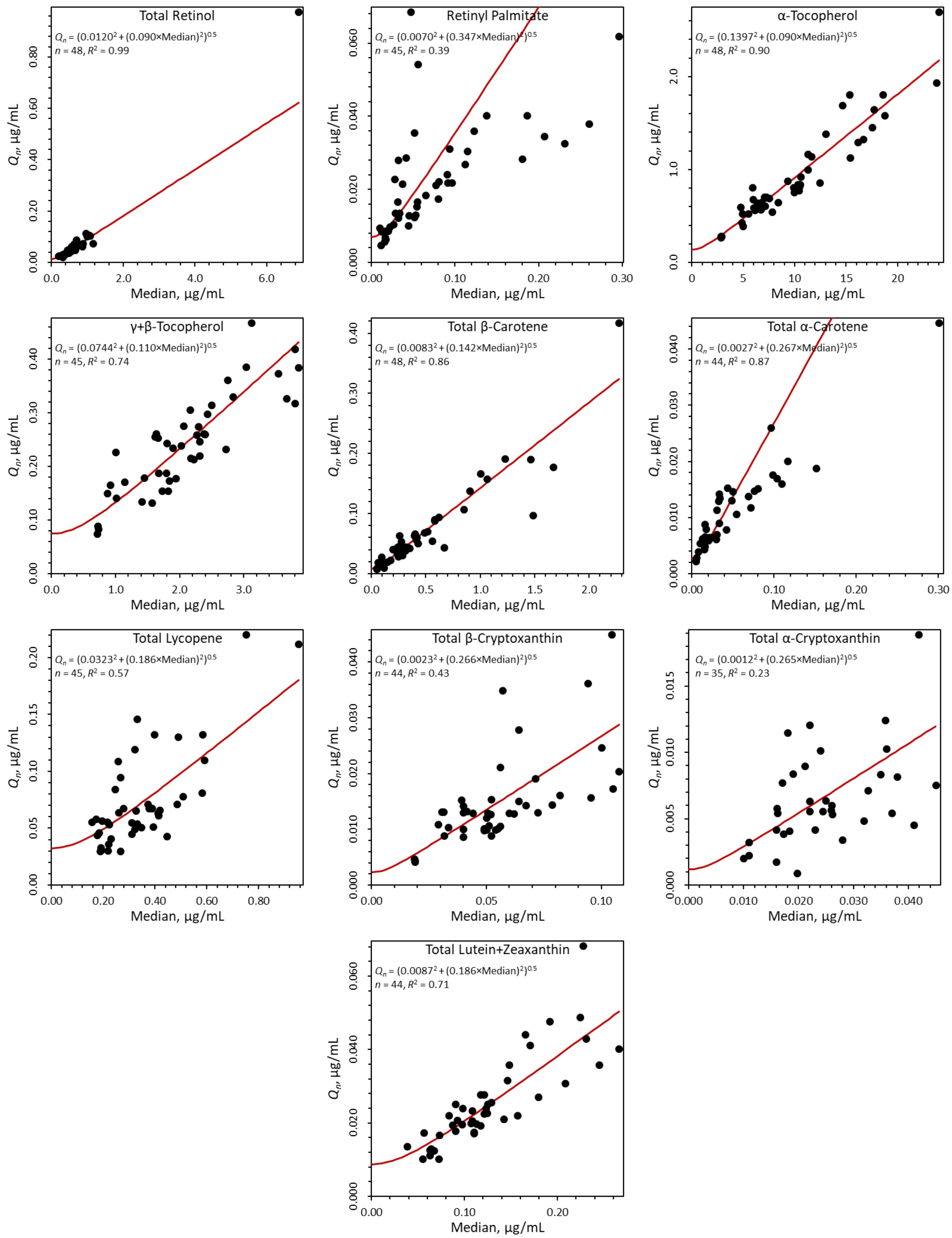

Figure 14. MMQAP Interlaboratory Reproducibility Functions

Solid black circles denote results for MMQAP serum samples. Solid red lines represent regression fits to the measurement precision function [16,17]: $Q_n = \sqrt{a^2 + (b \times \text{Median})^2}$

4. Comparison of MRC EWL and MMQAP Results

Figure 15 displays MRC EWL means as proportional functions of the MMQAP median values, modeling the relationships as $y = bx$, where the y are the MRC EWL means, b are the estimated slopes, and x are MMAP medians. Table 9 lists the regression parameters and four quality metrics.

Table 9. Summary of Proportional Relationships: $y = bx$

Measurand	n	b	$u(b)$	R^2	RMSE µg/mL	RMSE% %	RMSz σ_R
Total Retinol	47	1.006	0.006	0.998	0.025	5.2	0.63
Retinyl Palmitate	7	1.027	0.080	0.965	0.046	22	0.95
α-Tocopherol	48	1.004	0.005	0.999	0.38	5.1	0.55
γ+β-Tocopherol	45	0.937	0.006	0.998	0.091	4.4	0.41
Total β-Carotene	43	0.976	0.013	0.993	0.036	10.0	0.66
Total α-Carotene	11	0.943	0.026	0.993	0.011	10.7	0.38
Total Lycopene	45	1.152	0.010	0.996	0.027	8.5	0.37
Total β-Cryptoxanthin	26	1.058	0.020	0.991	0.007	9.7	0.41
Total α-Cryptoxanthin	18	1.065	0.034	0.983	0.005	16.3	0.61
Total Lutein+Zeaxanthin	43	1.099	0.015	0.992	0.014	11.6	0.55

n number of {MMQAP, MRC EWL} pairs used in the regressions

b slope

$u(b)$ regression-estimated standard uncertainty on the slope

R^2 coefficient of determination, the proportion of the variation in the MRC EWL results that is predictable from the MMQAP results.

RMSE Root mean square error (the expected magnitude of the y residuals), $\varepsilon_{\mathrm{RMSE}} = \sqrt{\frac{\sum(bx-y)^2}{n-1}}$.

RMSE% Relative root mean square error (the expected magnitude of the y residuals relative to the MMQAP medians) expressed as a percentage, $\varepsilon_{\mathrm{RMSE\%}} = 100\sqrt{\sum\frac{((bx-y)/x)^2}{n-1}}$

RMSz Root mean square z-score (the expected magnitude of the y residuals as a fraction of measurement reproducibility, σ_R), $\varepsilon_{\mathrm{RMSz}} = \sqrt{\sum\frac{((bx-y)/\sigma_R)^2}{n-1}}$.
The σ_R values are the long-term measurement reproducibility for a given measurand concentration, estimated as $\sigma_R = \sqrt{\alpha^2 + (\beta_0 x^{\beta_1})^2}$. The parameters used are adapted from those in Table 1 of [1].

These relationships have been estimated without regard to the standard uncertainties. However, the approximate 95 % confidence regions shown in Figure 15 are estimated using Monte Carlo perturbation using Gaussian kernels with variance equal to the squared standard uncertainty.

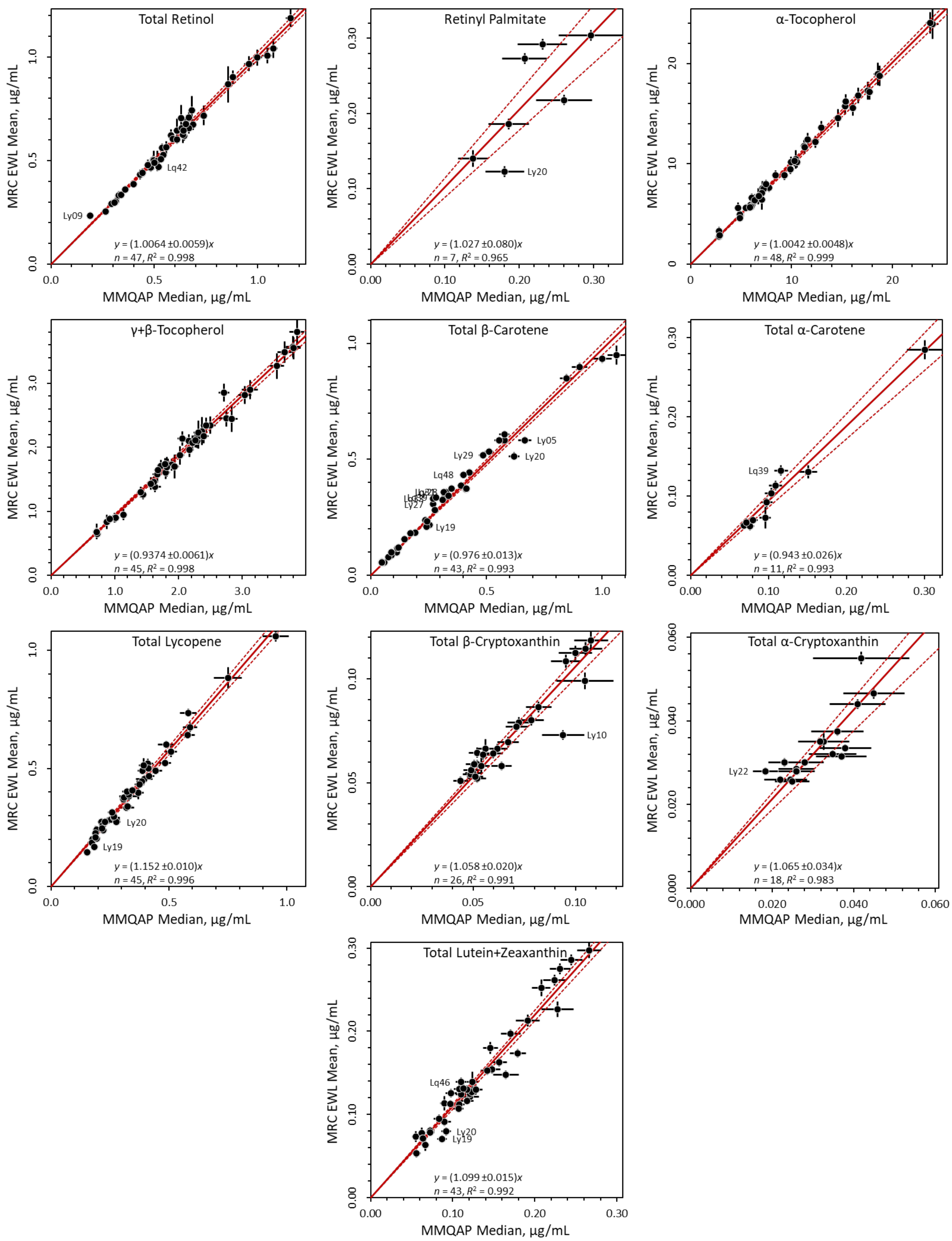

Figure 15. MRC EWL Means as Proportional Functions of MMQAP Medians
Solid black circles denote {MMQAP, MRC EWL} result pairs. Error-crosses represent standard uncertainties. Solid red lines represent regression fits to the function $y = bx$, where y represents the mean MRC EWL result and x the median MMQAP result. Dashed red lines encompass approximate 95 % confidence regions around the linear relationship.

4.1. Standard Uncertainty Estimation

The error bars on the MRC EWL mean values (vertical bars) are estimated as the maximum of the observed standard deviations and the repeatability function divided by √2. In equation form, $u = \text{MAX}(s, \sqrt{a^2 + (bx)^2})/\sqrt{2}$ where *u* estimates the standard uncertainty, *s* is the standard deviation of the two MRC EWL values, *x* is the mean of those values, *a* and *b* are parameters obtained from least-squares optimization using all available $\{x, s\}$ pairs, MAX is the function "take the maximum of these values," and √2 converts the standard deviation of the measurements into the standard uncertainty of the mean of the measurements. The uncertainty functions for the measurands are displayed in Figure 3 and summarized in Table 10.

Table 10. Uncertainty Function Parameters

	MRC EWL			MMQAP			
Measurand	*n*	*a*	*b*	*n*	*a*	*b*	*v*
Total Retinol	49	0.000	0.052	48	0.012	0.090	35
Retinyl Palmitate	5	0.010	0.000	45	0.007	0.347	9
α-Tocopherol	50	0.396	0.060	48	0.140	0.090	37
γ+β-Tocopherol	50	0.068	0.065	45	0.074	0.110	21
Total β-Carotene	42	0.007	0.026	48	0.008	0.142	26
Total α-Carotene	12	0.006	0.055	44	0.003	0.267	20
Total Lycopene	49	0.013	0.027	45	0.032	0.186	22
Total β-Cryptoxanthin	24	0.002	0.036	44	0.002	0.266	19
Total α-Cryptoxanthin	20	0.000	0.037	35	0.001	0.265	4
Total Lutein+Zeaxanthin	48	0.006	0.025	44	0.009	0.186	19

n number of {location, dispersion} pairs used in the parameter optimization
a constant dispersion – for small location values, the error bar cannot be shorter than this value
b proportional dispersion – for large location values, equivalent the coefficient of variation
v the effective degrees of freedom for the MMQAP Q_n values, estimated as the median number of values reported for the measurand over all MMQAP RRs.

The standard uncertainties on the MMQAP median values (horizontal bars) are somewhat similarly estimated as the maximum of the robust Q_n standard deviation of the median and the interlaboratory reproducibility function displayed in Figure 14 and summarized in Table 10. The standard uncertainty of the median of a Gaussian distribution is about $1.25/\sqrt{v}$ the standard deviation (however estimated) of those values where *v* is the effective number of available degrees of freedom – essentially the number of *independently* determined values. Since many participants participated in multiple RRs using the same methods, the number of *independent* values for MMQAP data can be far fewer than the total number of values. For consistency across the measurands and samples (and to keep the uncertainty estimates from becoming unreasonably small), *v* is estimated here as the median number of values per RR. Therefore, an equation appropriate for the horizontal error bars is $u = 1.25 \times \text{MAX}\left(Q_n, \sqrt{a^2 + (bx)^2}\right)/\sqrt{v}$ where *x* is the median of the available results.

4.2. Atypical Sample Identification

All of the relationships between the MRC EWL means and the MMQAP medians are well modeled as proportional; i.e., as straight lines with zero intercept. The expected magnitude of the residuals for all measurands is less than the estimated long-term reproducibility of the MMQAP results (referred to as "eSD" in the MMQAP results, but here symbolized as σ_R). No measurand has systematically degraded in these materials.

However, the MRC EWL means and MMQAP medians are significantly different (at about a 95 % level of confidence) for a few materials with some of the measurands. Materials for which the MRC EWL mean differs significantly from the MMQAP median are identified using a criterion related to the zeta-score (ζ-score) which is widely used in proficiency testing [21] but used here just to identify potentially atypical materials:

$$|\zeta| = \frac{\left|\frac{y}{b} - x\right|}{\sqrt{\frac{u^2(y)}{b^2} + u^2(x)}} > 2$$

where y is the MRC EWL result, b is the slope relating the measured MRC EWL means to the MMQAP medians, x is the MMQAP result, $u(y)$ is the standard uncertainty of the MRC EWL mean, and $u(x)$ is the standard uncertainty of the MMQAP median. Dividing by the slope recalibrates the MRC EWL means onto the MMQAP measurement scale, thus avoiding systematic bias from calibration differences.

Table 11 lists the materials that have been flagged as atypical by this criterion.

Table 11. Potentially Atypical Materials [a]

Material	TR	RP	aT	gbT	TbC	TaC	TLy	TbX	TaX	TLZ	#lo	#hi
Ly20		lo			lo		lo			lo	4	0
Ly19					lo		lo			lo	3	0
Ly05					lo						1	0
Ly10								lo			1	0
Lq42	lo										1	0
Lq39					hi	hi					0	2
Ly09	hi										0	1
Ly22									hi		0	1
Ly27					hi						0	1
Ly28					hi						0	1
Ly29					hi						0	1
Lq31					hi						0	1
Lq33					hi						0	1
Lq46										hi	0	1
Lq48					hi						0	1
Total	2	1	0	0	10	1	2	1	1	3		

a "lo" denotes MRC EWL results that are lower (smaller) than expected based on MMQAP results; "hi" denotes MRC EWL results that are higher (larger) than expected based on MMQAP results.

4.3. Potential Sources of Significant Differences

A number of potential causes could explain the observed disagreements between the MRC EWL and MMQAP results. Unfortunately, these explanations are not mutually exclusive nor is it possible in all cases to retrospectively establish which are most likely. However, the evidence presented in the previous sections of this document can winnow the possibilities.

4.3.1. Incorrectly Identified or Damaged samples

Many of the materials provided to the MRC EWL were prepared, labelled, and stored many years prior to 2014 and had passed through the hands of several record keepers and storage locations. While only samples with adequate history were selected, a mistaken identity or a damaged sample among the archived materials is not inconceivable. However, the generally excellent agreement of the MRC EWL and MMQAP values argues that the materials were properly identified. Additionally, the close agreement among the MRC EWL replicates (Figure 2) argues that if any of the samples for the 46 materials supplied as duplicate units were damaged, then both samples were damaged in the same way across all of the quantifiable measurands.

Material Lq37 was prepared from an unmodified single donor serum that was hemolyzed during primary processing. While this damage is intrinsic to the material, the contamination could plausibly induce measurement differences.

4.3.2. Measurement inaccuracy

The few non-replicated MRC EWL measurements are susceptible to bias relative to the unknown true value of the measurand and/or to underestimation of the uncertainty. However, the excellent agreement between replicates (Figure 2) suggests that the MRC EWL measurement process was in good control throughout and significant bias unlikely. Using the uncertainty functions as lower-bounds on the measured two-sample standard deviations (Figure 3) is intended to minimize underestimation.

MMQAP measurements based on a single distribution are likewise susceptible to bias and uncertainty underestimation. Measurands that were reported by only a few participants are especially susceptible; bias and underestimated uncertainty are less likely to be an issue with materials that were distributed in multiple RRs or were reported by many participants. Using the uncertainty functions as lower-bounds on the observed Q_n estimates (Figure 14) is intended to minimize underestimation, but does not address variability arising from material heterogeneity. Examination of the frequency distributions (Figure 4 to Figure 13) may help identify particularly suspect results.

4.3.3. Sample Matrix

Since the lyophilized materials are reconstituted *with* 1.0 mL water rather than *to* a total volume of 1.0 mL, the measurand concentrations in the lyophilized member of each {lyophilized, liquid-frozen} pair should be somewhat lower than in its liquid-frozen partner. The value of the ratio of concentrations depends on the volume of water actually added, but unless lyophilization changes the nature of some of the measurands the concentration ratios should be about the same across the measurands.

Because the 48 materials provided to the MRC EWL include five {lyophilized, liquid-frozen} pairs, this potential issue was directly explored in Section 5.

4.3.4. Analyte Extraction

The fat-soluble vitamins and vitamin-related compounds do not all extract into hexane with the same efficiency. Extraction protocols adequate for easily extracted compounds may not effectively recover the more difficult. Relatively less complete extraction could lead to lower than expected results for the less readily extractable measurands; relatively more complete extraction could lead to higher than expected results.

4.3.5. Measurand Definition Changes

The identity and description of the MMQAP measurands evolved over the program's 30-year existence. The early RRs did not distinguish between the all-*trans* and *cis* isomers of retinol or the β-carotene isomers; recognition that this was true for all of the carotenoids came considerably later as did recognition that the reported "γ-tocopherol" was the sum of γ- and β-tocopherol.

Enough confusion remained about retinol in 2002 that two materials were prepared from the same serum pool, one spiked with all *trans*-retinol and the other with 13-*cis*-retinol. Results from the analysis of these materials identified several participants who consistently mis-identified their measurand – some who reported "total" who measured only "*trans*" and some who reported "*trans*" who measured "total". The base serum and the two spiked materials were included in the materials sent to the MRC EWL as Lq38, Lq35, and Lq36.

While there were no similarly matched samples available for other measurands, evaluation of the descriptions in use when materials were distributed [20] could help clarify possible measurand identity issues.

4.3.6. Material Changes

Significantly degraded materials should plot well below (to the right-hand side of) the regression-estimated lines. Further, since degradation of just one measurand in a material is unlikely, the degraded material should be identified as atypical in several if not all measurands.

4.4. Evaluation of Significant Differences

4.4.1. Total Retinol

Only two materials are identified as atypical for total retinol.

In Ly09 (SRM 968a-L), the MRC EWL result for total retinol is larger than that for the MMQAP. Total retinol is the only measurand for which this material is flagged as atypical. The material was augmented with α-tocopherol but had native levels of retinol, with a certified "retinol" value equivalent to the MMQAP median [7]. The material's last distribution was as serum 167 in the Spring 1992 RR XXV, prior to resolving the confusion between total retinol and *trans*-retinol. It is plausible the MMQAP (and certified value) result underestimates the total retinol concentration of this material.

In Lq42, the MRC EWL result for total retinol is smaller than that for the MMQAP. The MRC EWL result is likewise smaller than the MMQAP for only three of the seven measurands for which a comparison can be made. The material was produced in 2006 from a single source native serum. The distribution of the two sets of MMQAP results is unexceptional. The two MRC EWL results are adequately similar, ruling out single-sample damage or measurement. The difference appears to be a sample- and measurand-specific measurement anomaly.

4.4.2. Retinyl palmitate

While the available {MMQAP, MRC EWL} pairs are too few for confident assessment, only material Ly20 (SRM 968b-M) is identified as atypical with regard to this measurand. See Section 4.4.11.

4.4.3. α-Tocopherol

No materials are identified as atypical with regard to this measurand.

4.4.4. γ+β-Tocopherol

No materials are identified as atypical with regard to this measurand.

4.4.5. Total β-Carotene

Ten materials are identified as atypical with regard to this measurand.

In both Ly19 (SRM 968b-L) and Ly20 (SRM 968b-M), the MRC EWL means for both materials are smaller than the MMQAP medians. See Section 4.4.11.

In Ly05 (SRM 968-M), the MRC EWL result is smaller than the MMQAP median and the certified value for the measurand (explicitly identified as total β-carotene), however it is quite similar to the non-certified value of 0.55 µg/mL listed for all *trans* β-carotene in the SRM 968 Certificate of Analysis (COA) [6]. The MRC EWL mean is smaller than the MMQAP median for two of the other three measurands for which a comparison is possible. The two MRC EWL results are in excellent agreement. The distribution of the MMQAP results is unexceptional, although based on relatively few measurements. The multi-donor material was augmented with "retinol" and α-tocopherol but the β-carotene level was achieved by nutritional supplementation of some donors. The observed difference for this material may be related to measurand identification issues in nutritionally supplemented sera.

The MRC EWL means for total β-carotene in Ly27, Ly28, Ly29, Lq31, Lq33, Lq39 are larger than the MMQAP medians. All of these materials, along with Ly26, Ly30, Lq32, and Lq34, were prepared from various "normal" native serum pools as {lyophilized, liquid-frozen} pairs in 2000 to 2003. Other than concentration differences related to how the lyophilized materials were reconstituted (see Section 5), the individual pairs have very similar measurand profiles. With the exception of Lq39 for which total α-carotene is also significantly larger, total β-carotene is the only measurand having MRC EWL means significantly different from the MMQAP medians. Except for the somewhat broad distributions of the (Ly27, Lq33) pair, the distributions of the MMQAP results are unimodal and relatively narrow. The MRC EWL results for all material successfully assayed twice are in good agreement. The cause of the significant total β-carotene differences is unlikely to be related to extraction efficiency since the differences are not echoed in the other carotenoids.

Like the {lyophilized, liquid-frozen} pairs, the MRC EWL mean for Lq48 (SRM 968e-III) is larger than the MMQAP median. This multi-donor blended material contains native levels of all measurands, and the two MRC EWL results are in good agreement. The distribution of the MMAQP results is unexceptional, although the median is a bit smaller than the certified value: (0.401 ± 0.016) µg/mL vs. (0.411 ± 0.022) µg/mL.

The materials identified as having significantly larger MRC EWL means all have total β-carotene concentrations between (0.3 and 0.6) µg/mL and lie above but parallel to the line representing the proportional model. This observation is compatible with an ≈0.02 µg positive bias for this relatively large subset of the samples. Peak baseline interpretation and/or measurand identification issues are plausible causes.

4.4.6. Total α-Carotene

While the available {MMQAP, MRC EWL} pairs are too few for confident assessment, only material Lq39 is identified as atypical. As with total β-carotene, the MRC EWL mean is larger than the MMQAP median. The distribution of the MMQAP results is unexceptional, as are the two MRC EWL results. The positive bias for this measurand is likely attributable to the same (unknown) cause of the positive total β-carotene bias.

4.4.7. Total Lycopene

Only materials Ly19 (SRM 968b-L) and Ly20 (SRM 968b-M) are identified as atypical with regard to this measurand; see Section 4.4.11.

4.4.8. Total β-Cryptoxanthin

Only material Ly10 (SRM 968a-M) is identified as atypical, with the MRC EWL mean significantly smaller than the MMQAP median. The distribution of the MMQAP results is broad and poorly defined. The two MRC EWL results agree well and are consistent with the 0.04 µg/mL non-certified value listed in the SRM 968a COA based just on NIST measurements [7]. The measurand "β-cryptoxanthin" was not reported in the MMQAP RRs until the Spring 1991 RR XXII. This material was distributed in RRs "designed to focus primarily on problems associated with selected carotenoid compounds" [22]. The MMQAP median likely overestimates the total β-cryptoxanthin concentration of this material.

4.4.9. Total α-Cryptoxanthin

While the available {MMQAP, MRC EWL} pairs are too few for confident assessment, only material Ly22 [an equal-volume mixture of the serum pools used to produce Ly23 (SRM 968c-I) and Ly24 (SRM 968c-II)] is identified as atypical. The single quantitative MRC EWL result for this material is significantly larger than the MMQAP median; the second result was reported only as being below the measurand's 0.025 µg/mL LLOQ. The distribution of the MMQAP results is unexceptional, with a median close to the average of the medians for Ly23 and Ly24. The MRC EWL result is likely somewhat too high.

4.4.10. Total Lutein + Zeaxanthin

Three materials are identified as atypical with regard to this measurand.

The MRC EWL means for Ly19 (SRM 968b-L) and Ly20 (SRM 968b-M) are smaller than the MMQAP medians. See Section 4.4.11.

The MRC EWL mean for total lutein + zeaxanthin in Lq46 (SRM 968e-I) is significantly larger than the MMQAP median. The two MRC EWL results agree well; the distribution of the MMQAP results is unexceptional. Given the number and consistency of the MMQAP results collected in six RRs, the MRC EWL result is likely somewhat too high.

4.4.11. Materials Ly19 and Ly20

Ly19 (SRM 968b-L) and Ly20 (SRM 968b-M) are the only materials for which the MRC EWL results are significantly smaller than the MMQAP medians for multiple measurands. No significant differences were identified between the MRC EWL and MMQAP results in the related material LY21 (SRM 968b-H). All three of the SRM 968b materials were augmented with *trans*-retinol, α-tocopherol, γ-tocopherol, and retinyl palmitate [8]. The distribution of the MMQAP results for these measurands are unexceptional. Many of the MRC EWL results for the first measurement in these materials are atypically lower than the result for the second. The observed differences plausibly arise from sample-specific reconstitution or extraction difficulties in the MRC EWL Batch 2 set of measurements that were avoided in the Batch 3 and Batch 4 sessions.

5. Comparison of Lyophilized and Liquid-Frozen Materials

The 48 materials provided to the MRC EWL included five {lyophilized, liquid-frozen} pairs, all prepared from various native serum pools from 2000 to 2003 to explore potential significant measurement differences between the lyophilized and liquid-frozen delivery of the same materials. The paired materials are {Ly26, Lq31}, {Ly27, Lq33}, {Ly28, Lq34}, {Ly29, Lq32}, and {Ly30, Lq40}. Figure 16 compares the results for the liquid-frozen member of the pair as functions of the results for its lyophilized partner.

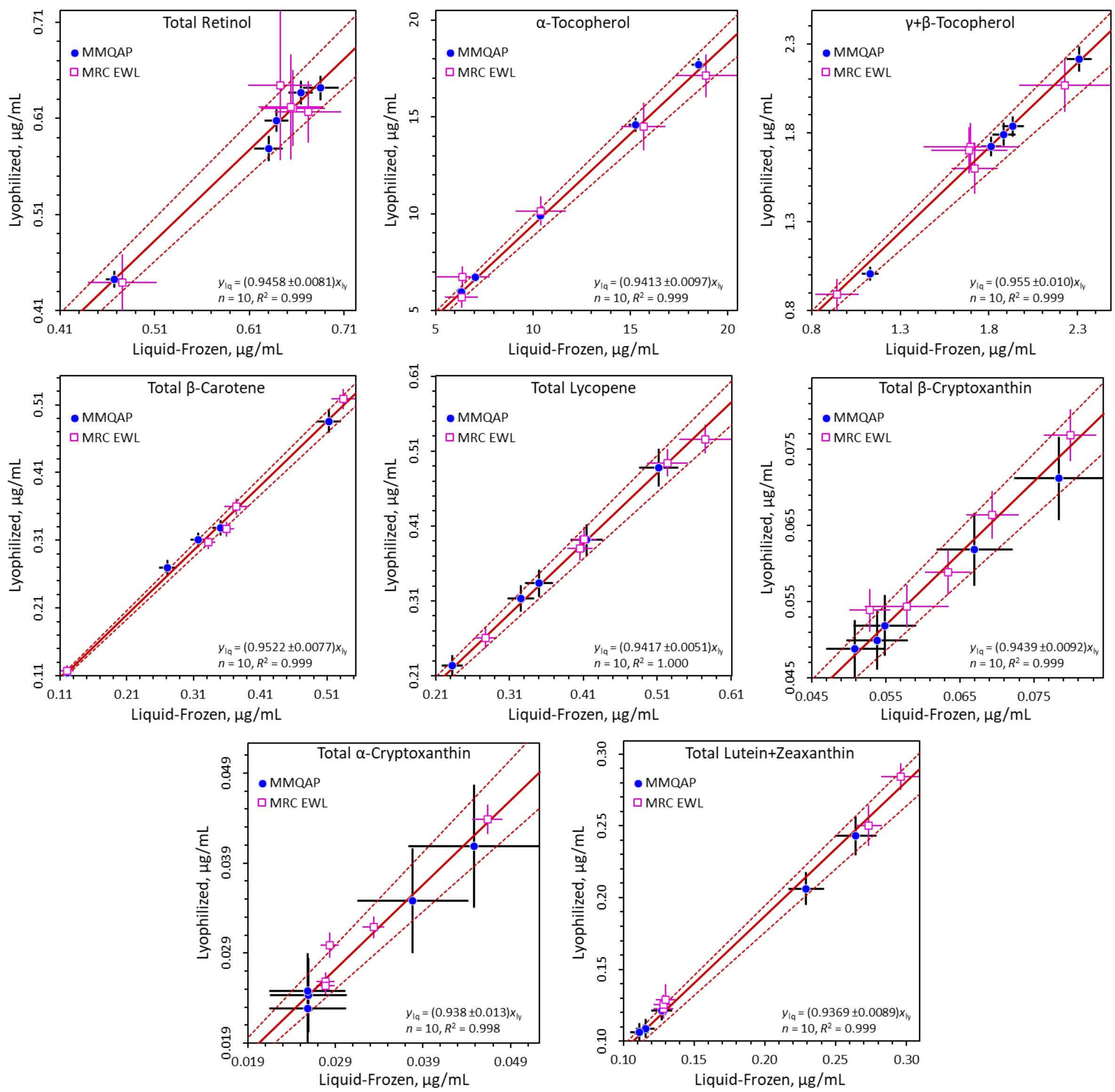

Figure 16. Liquid-frozen Results as Functions of Results for Paired Lyophilized Materials
Solid blue circles represent MMQAP results for the {lyophilized, liquid-frozen} pair sera; open magenta squares represent MRC EWL results. Error-crosses represent standard uncertainties. Solid red lines represent regression fits to the function $y = bx$, where y represents results for the liquid-frozen material and x the results for its lyophilized partner. Dashed red lines encompass approximate 95 % confidence regions around the proportional relationships.

The MMQAP and MRC EWL result ratios agree within their measurement uncertainties for the eight measurands for which complete data are available.

Because participants were instructed to reconstitute lyophilized materials *with* 1.0 mL water rather than attempting to bring the reconstituted volume back *to* the original 1.0 mL, measurand concentrations in the liquid-frozen materials are expected to be uniformly higher than in the slightly over-diluted reconstituted lyophilized materials. Differences in the ratio between the liquid-frozen results and the lyophilized results could indicate changes in extraction efficiency or measurand integrity due to lyophilization. Figure 17 displays an analysis of estimated slopes of the proportional relationships [23].

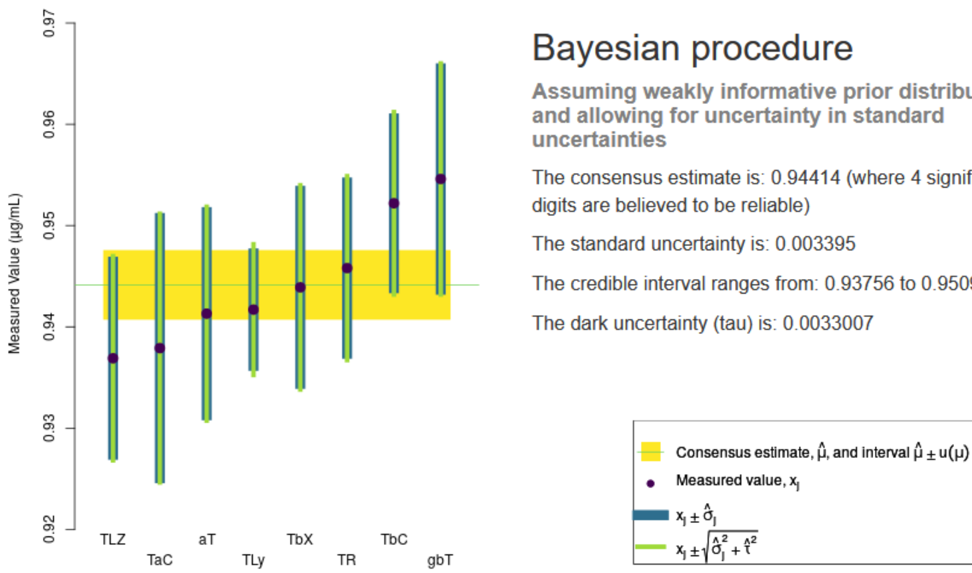

Figure 17. Analysis of Lyophilized/Liquid-Frozen Ratios

The credible range, 0.938 to 0.951, is an approximate 95 % confidence interval on the estimated consensus value of 0.944. The "dark uncertainty" [24] is the between-measurand uncertainty that isn't explained by the standard uncertainties of the slopes. The Bayesian procedure used half-Couchy minimally informative priors for both the within-and between-measurand variances.

While there is significant unexplained variance (dark uncertainty [25]), all of the uncertainty intervals are consistent with the consensus ratio of 0.944 ± 0.003. All of the uncertainty intervals overlap. There are no statistically significant differences among the eight slopes.

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