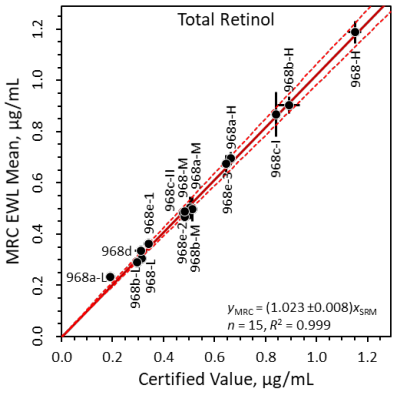
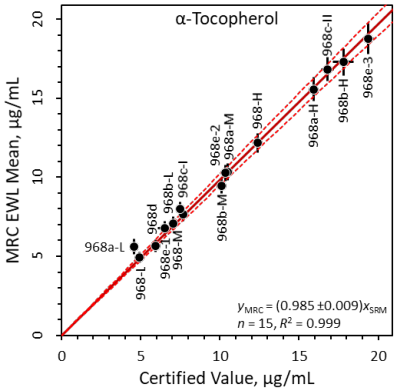
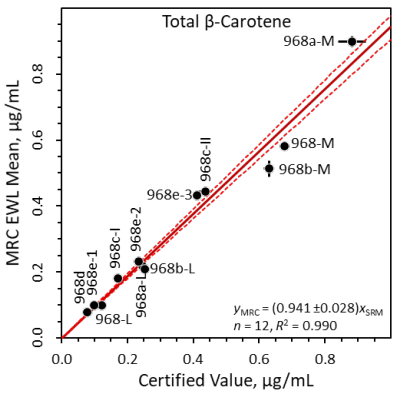
NIST IR 7880-49

# NIST Micronutrients Measurement Quality Assurance Program Sample Integrity Confirmation

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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.

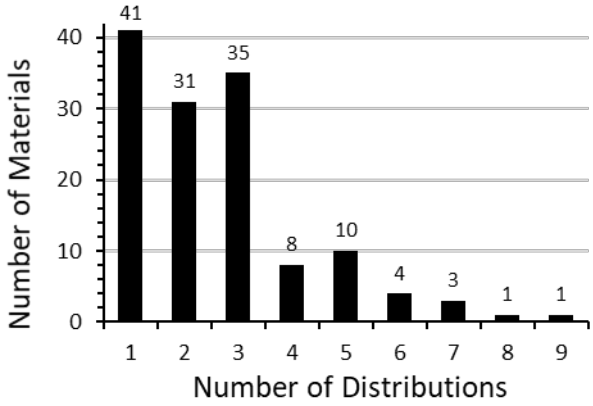


Figure 1. Distribution Frequency for the Unique MMQAP Sample Materials

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)

| | TaC | | TLy | | TbX | | TaX | | TLZ | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | $x_{mean}$ | $s$ | $x_{mean}$ | $s$ | $x_{mean}$ | $s$ | $x_{mean}$ | $s$ | $x_{mean}$ | $s$ |
| 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.

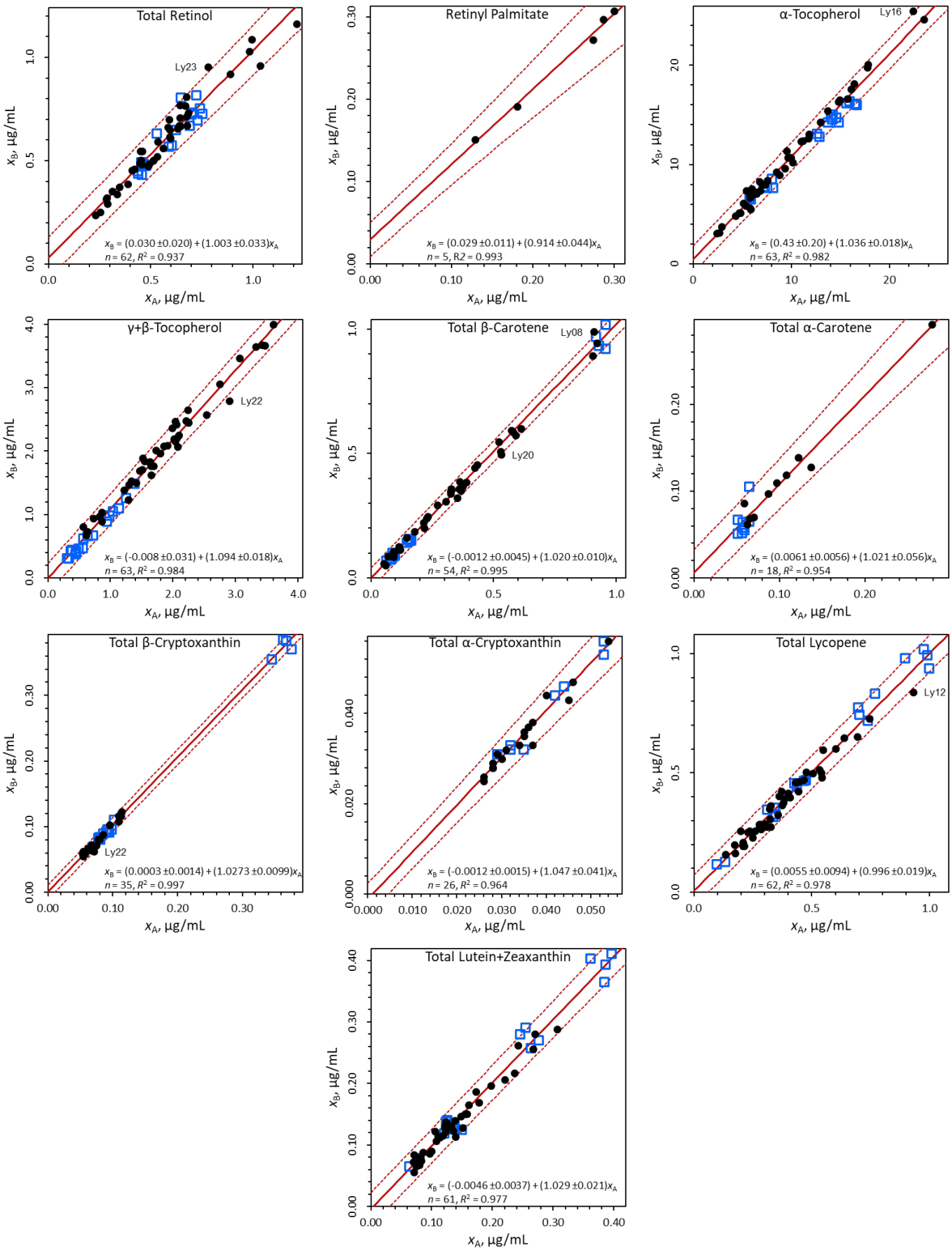


Figure 2. Result of Second Measurement ($x_B$) as Functions of First Measurement ($x_A$)
Solid black circles denote results for MMQAP serum samples; open blue squares denote results for MRC EWL quality assurance plasma materials. Solid red lines represent the linear relationship between the replicates; 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.

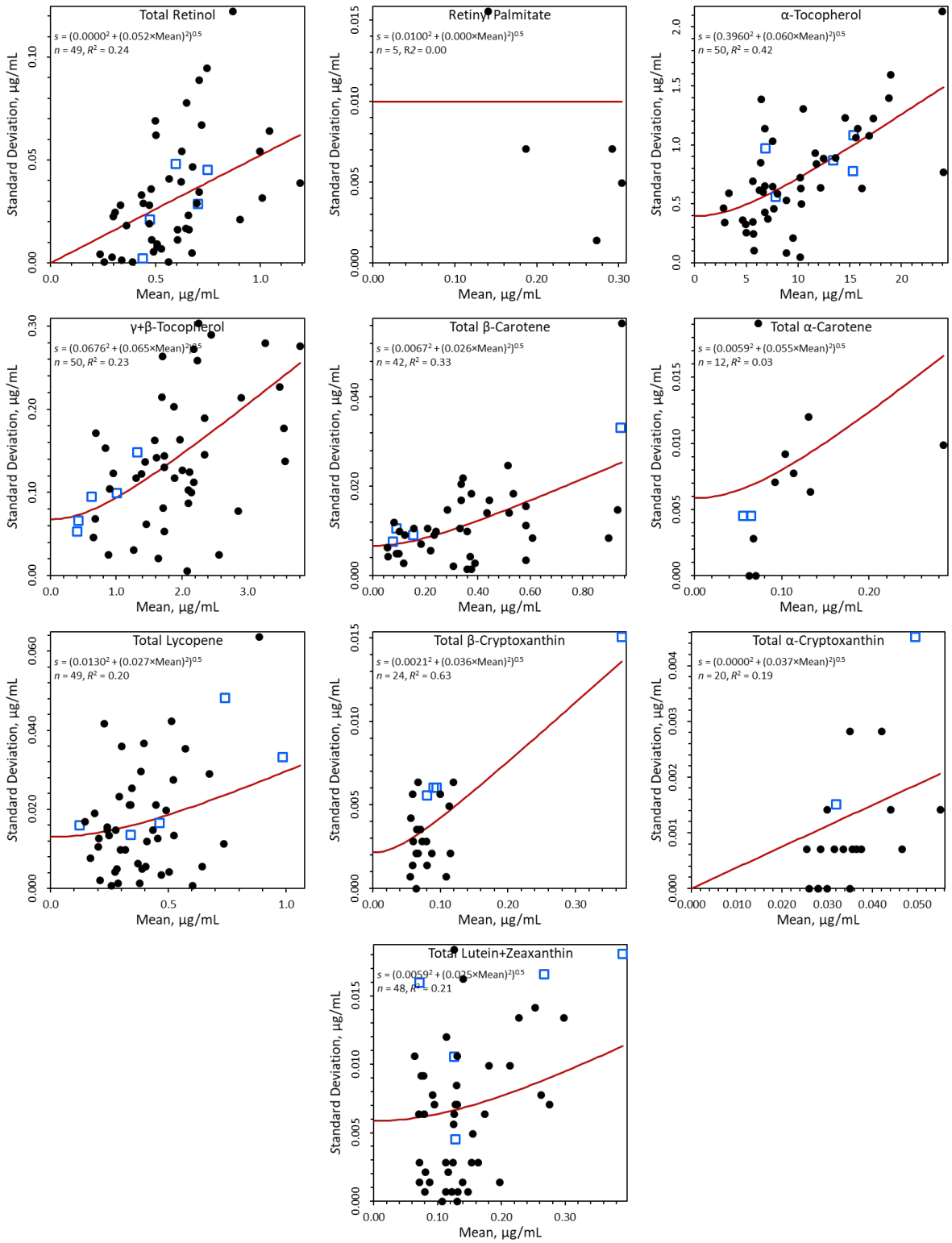


Figure 3. MRC EWL Measurement Repeatability Functions

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 |

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 |

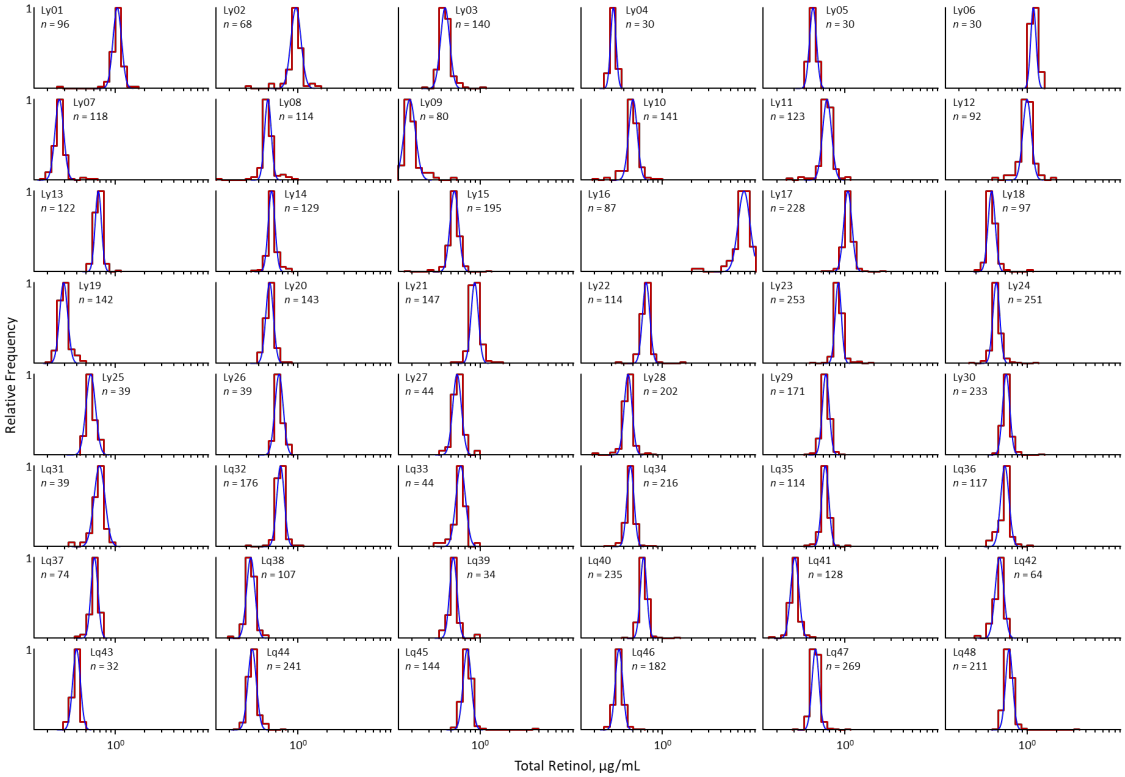


Figure 4. MMQAP Results for Total Retinol

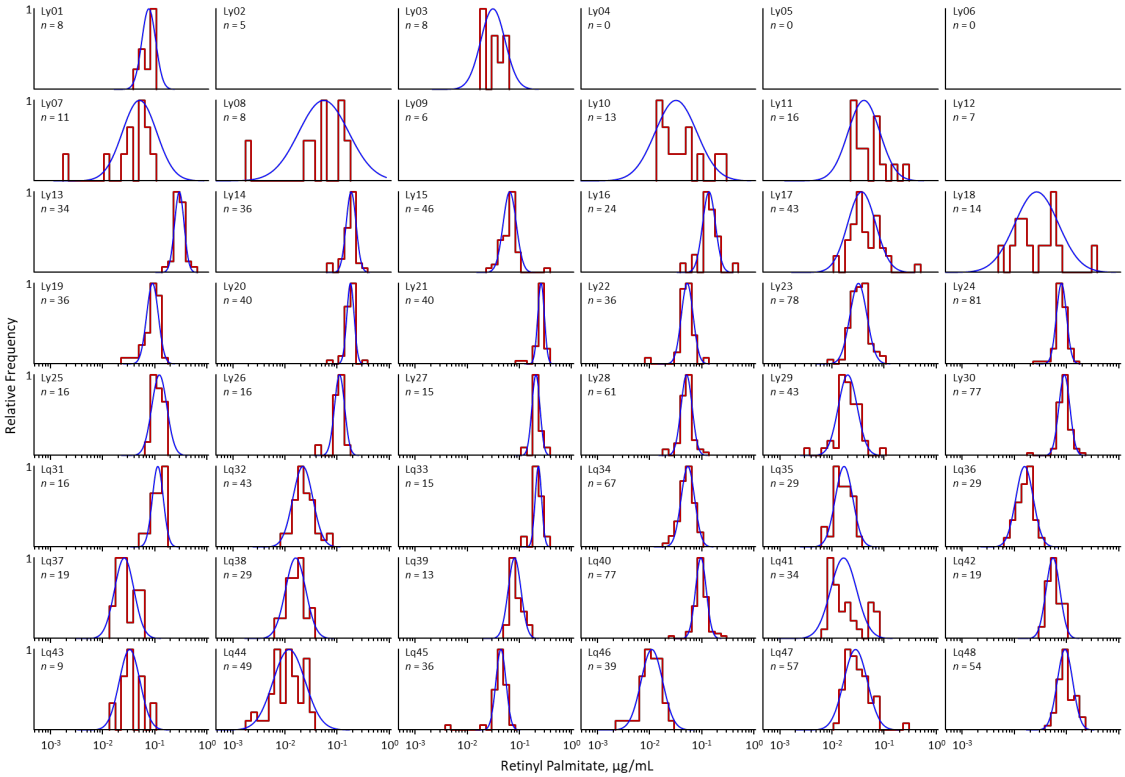


Figure 5. MMQAP Results for Retinyl Palmitate

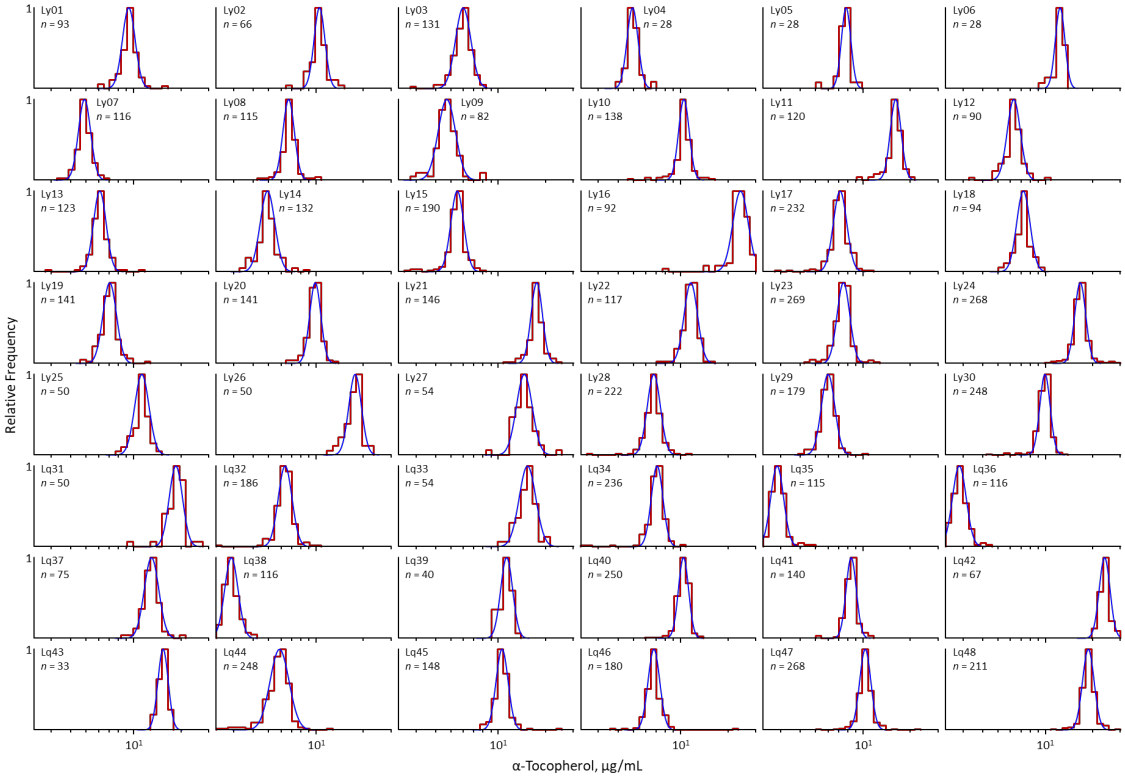


Figure 6. MMQAP Results for α-Tocopherol

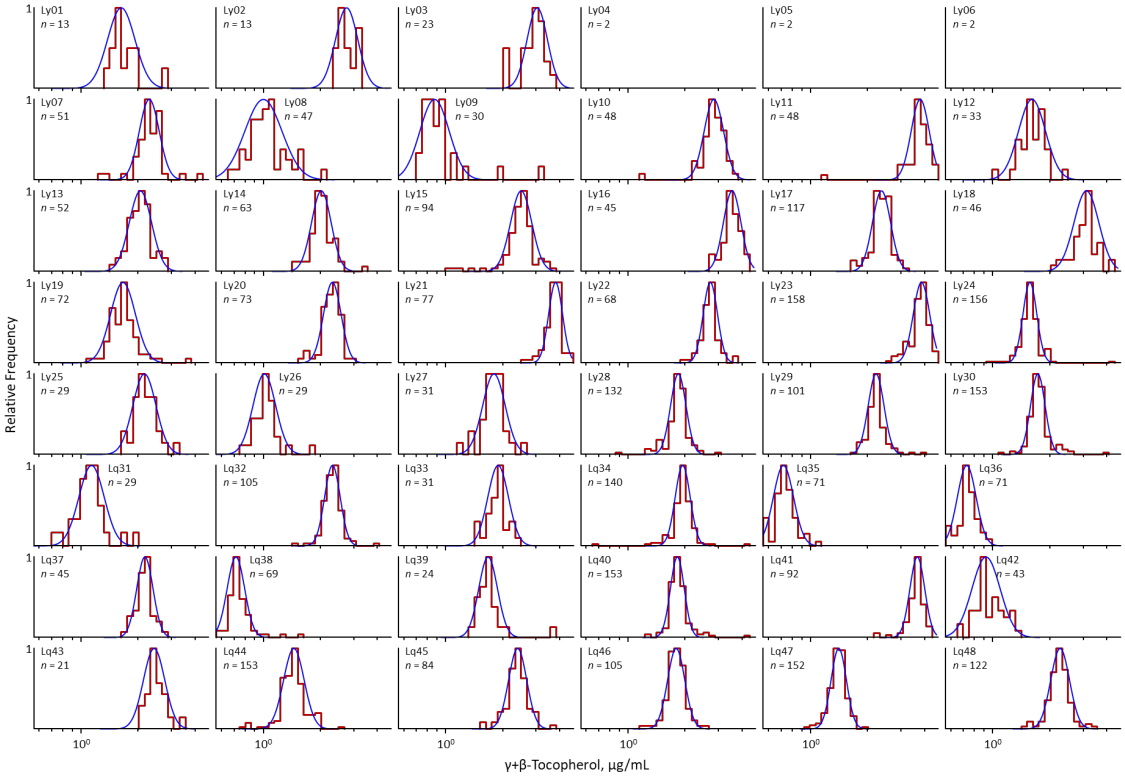


Figure 7. MMQAP Results for γ+β-Tocopherol

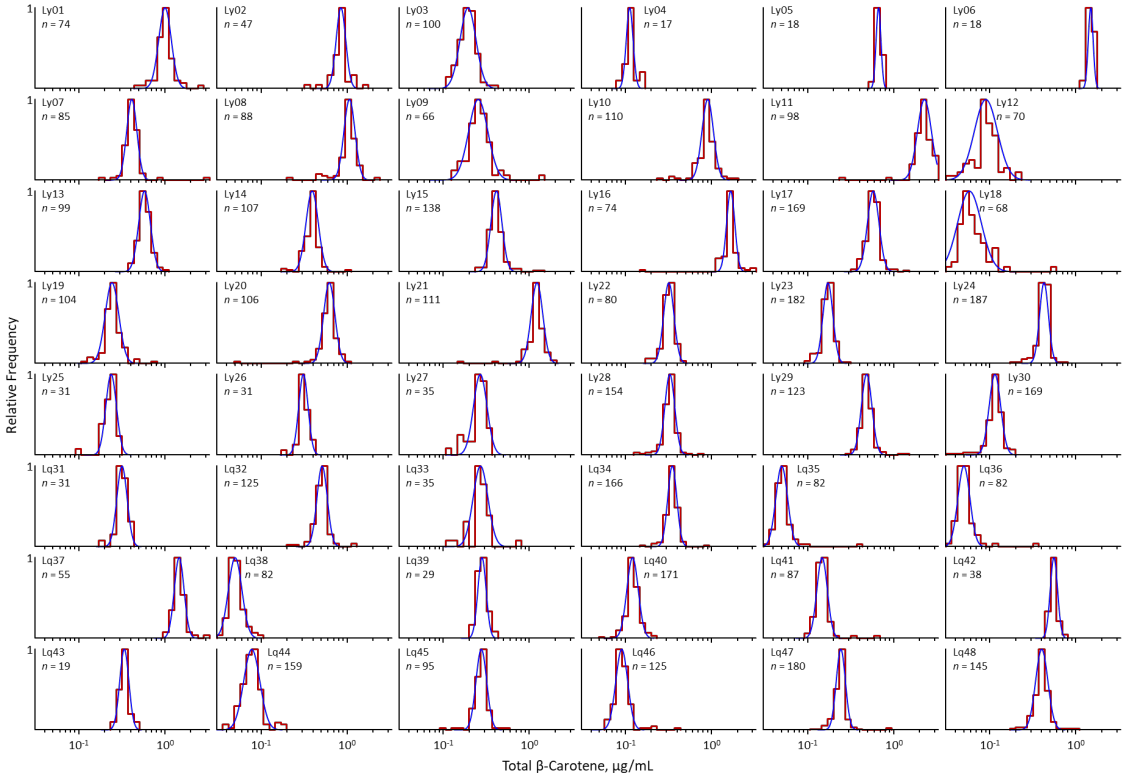


Figure 8. MMQAP Results for Total β-Carotene

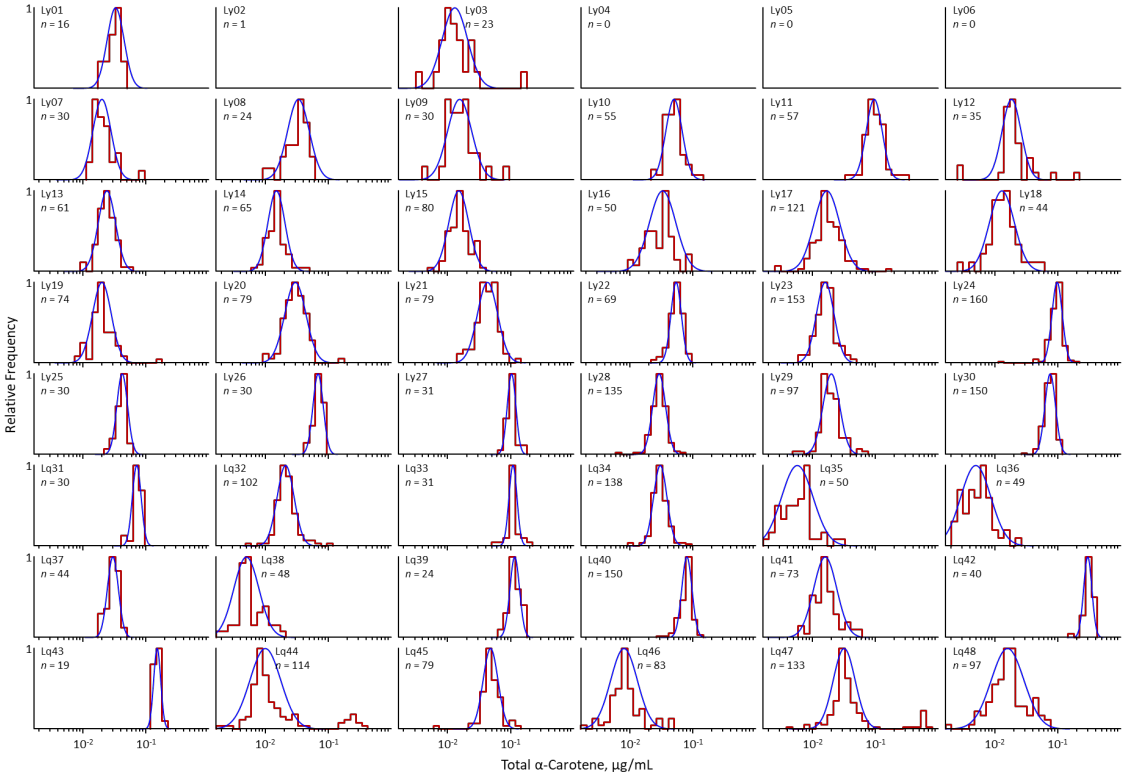


Figure 9. MMQAP Results for Total α-Carotene

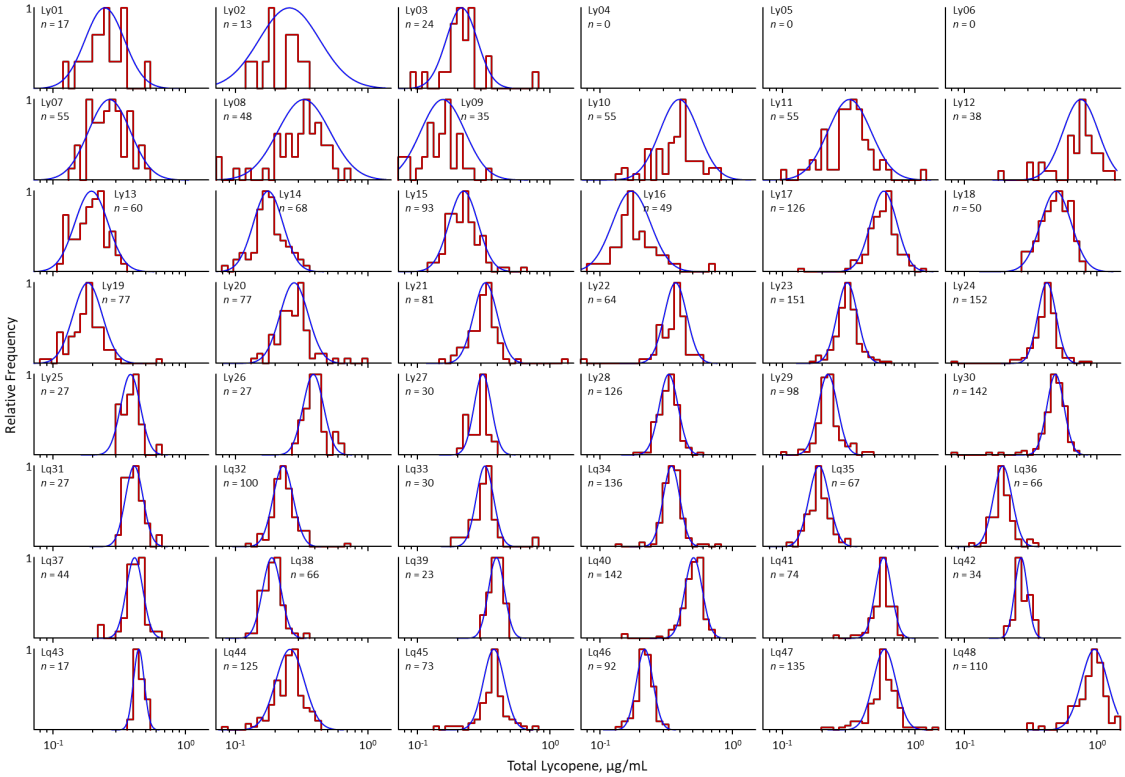


Figure 10. MMQAP Results for Total Lycopene

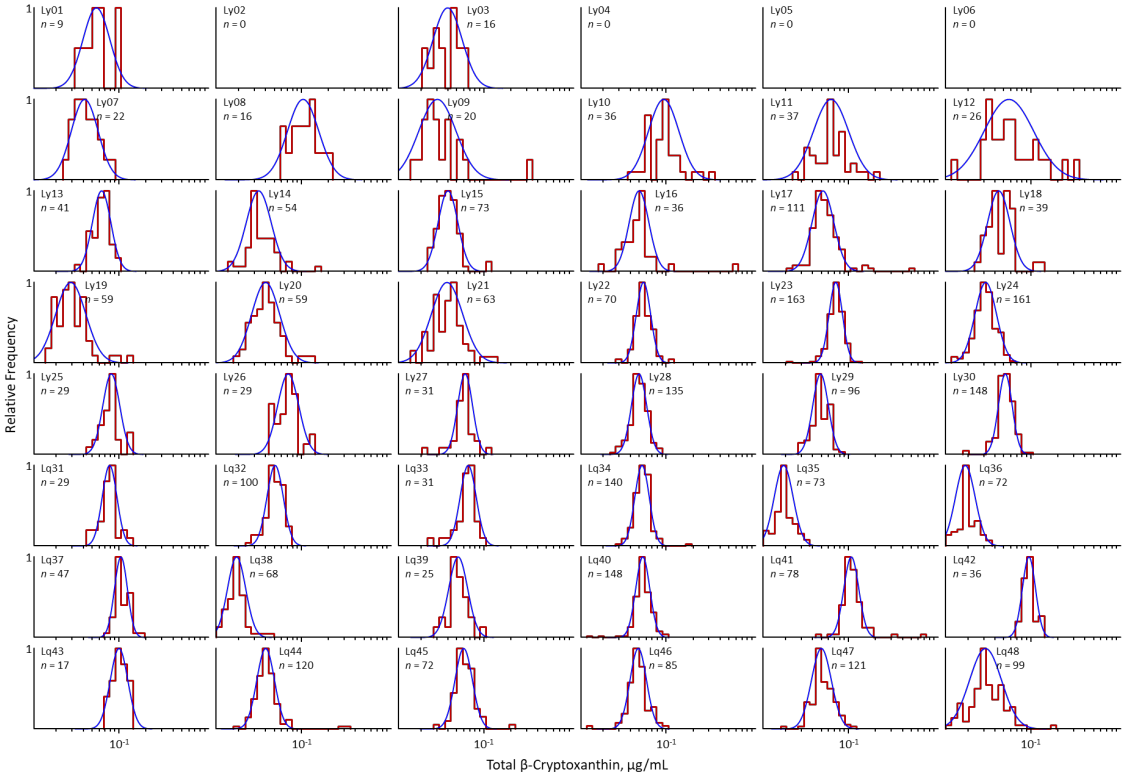


Figure 11. MMQAP Results for Total β-Cryptoxanthin

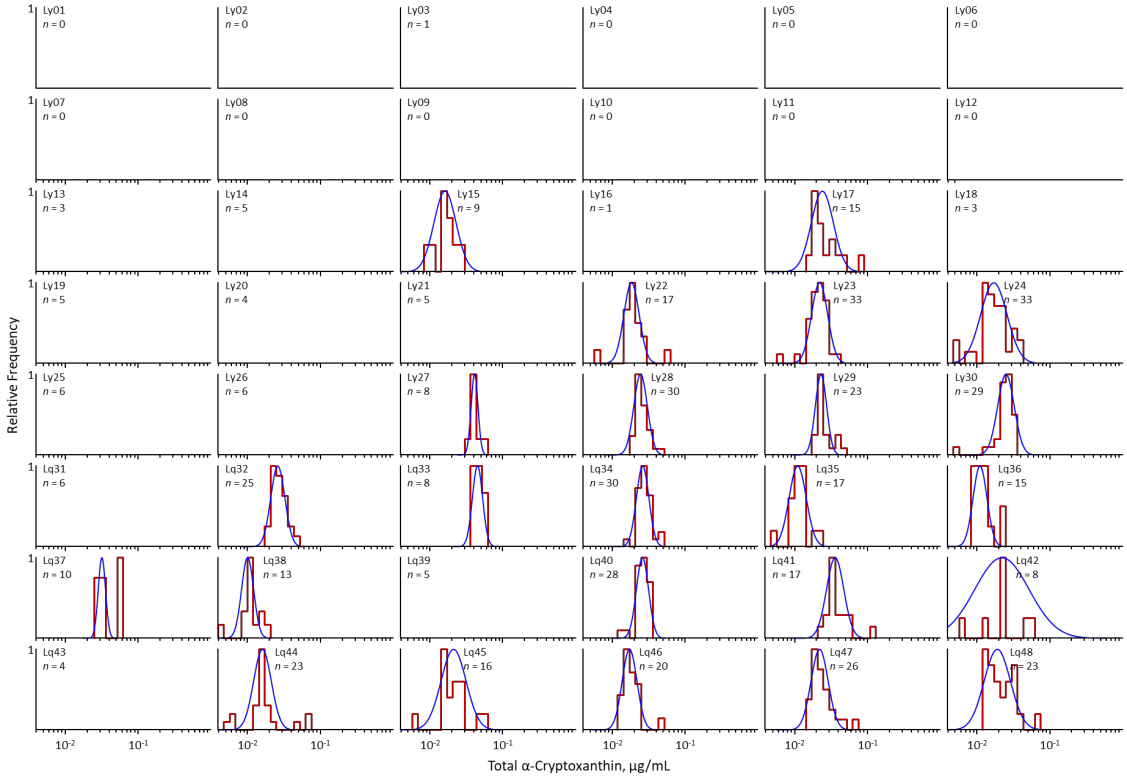


Figure 12. MMQAP Results for Total α-Cryptoxanthin

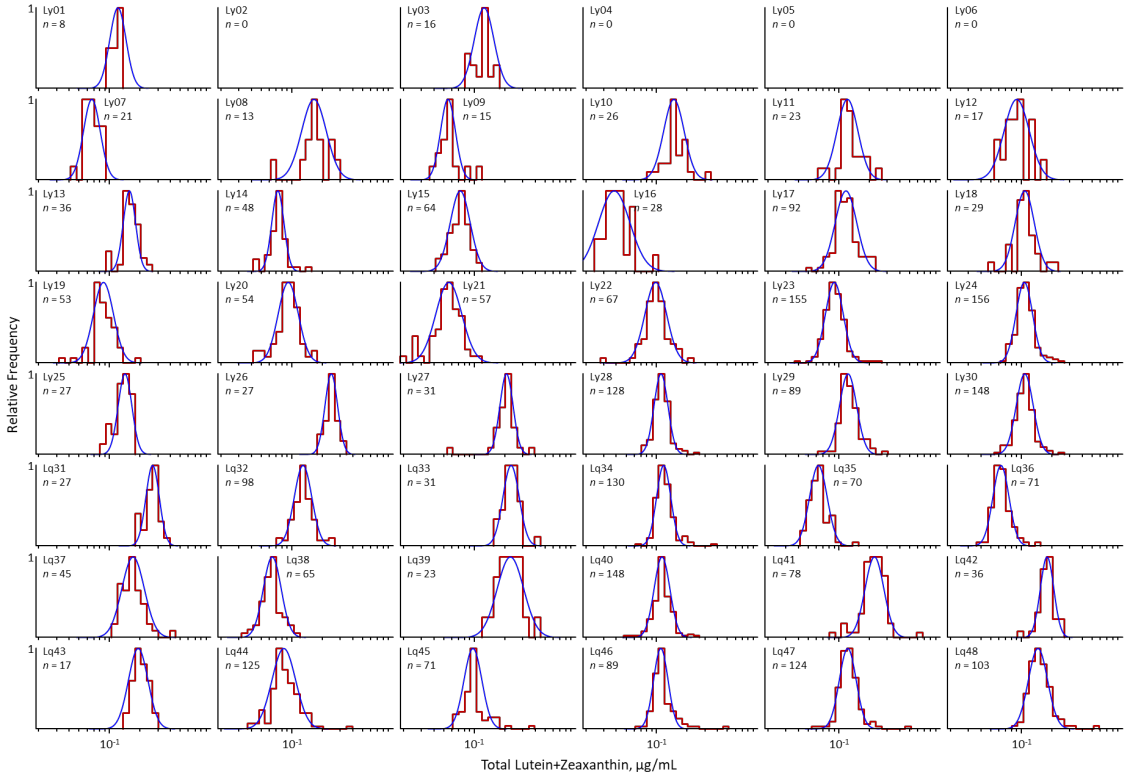


Figure 13. MMQAP Results for Total Lutein+Zeaxanthin

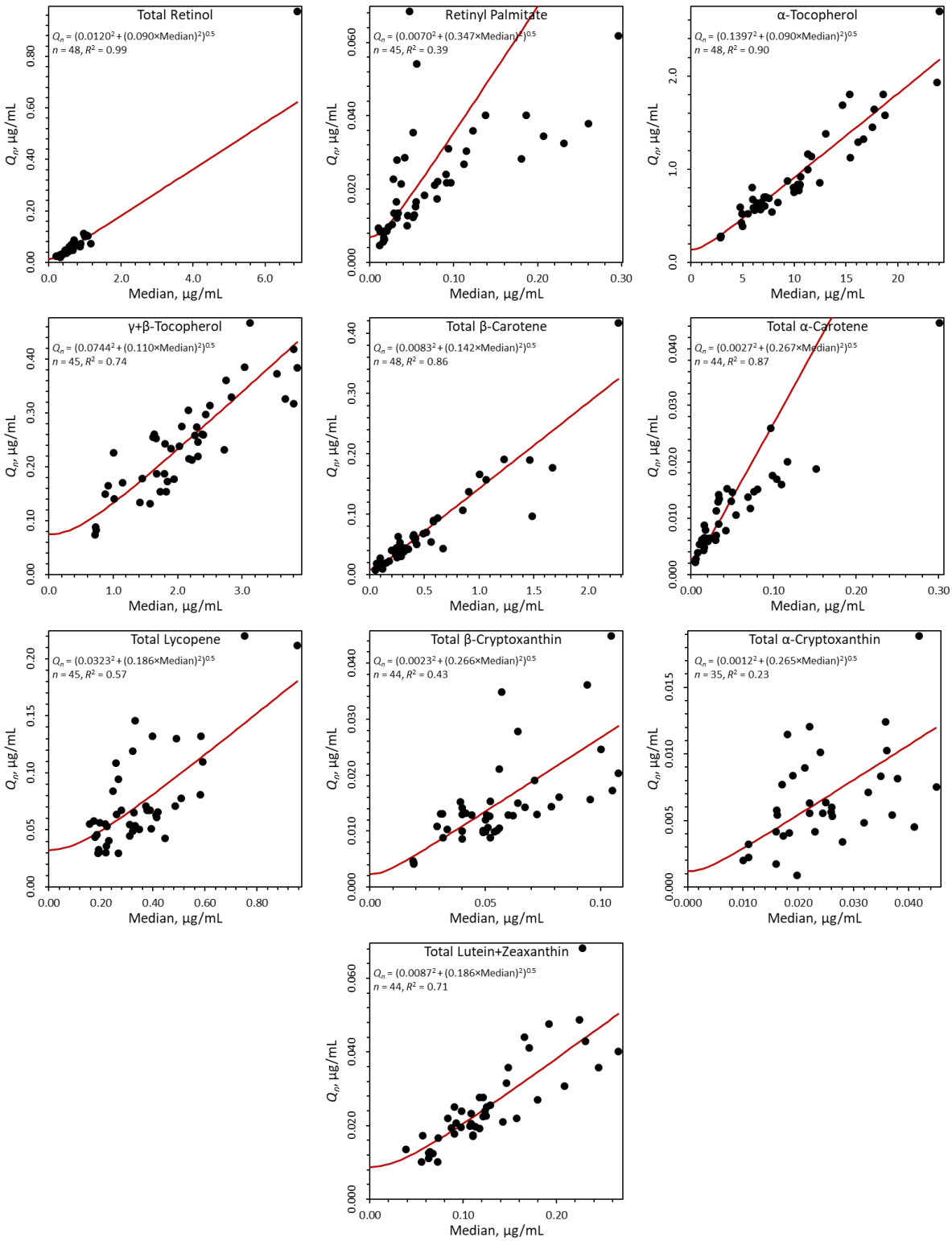


Figure 14. MMQAP Interlaboratory Reproducibility Functions

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 $\sigma_R$ |
|---|---|---|---|---|---|---|---|
| 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, $\sigma_R$), $\varepsilon_{\mathrm{RMSz}} = \sqrt{\sum\frac{((bx-y)/\sigma_R)^2}{n-1}}$.

The $\sigma_R$ 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.

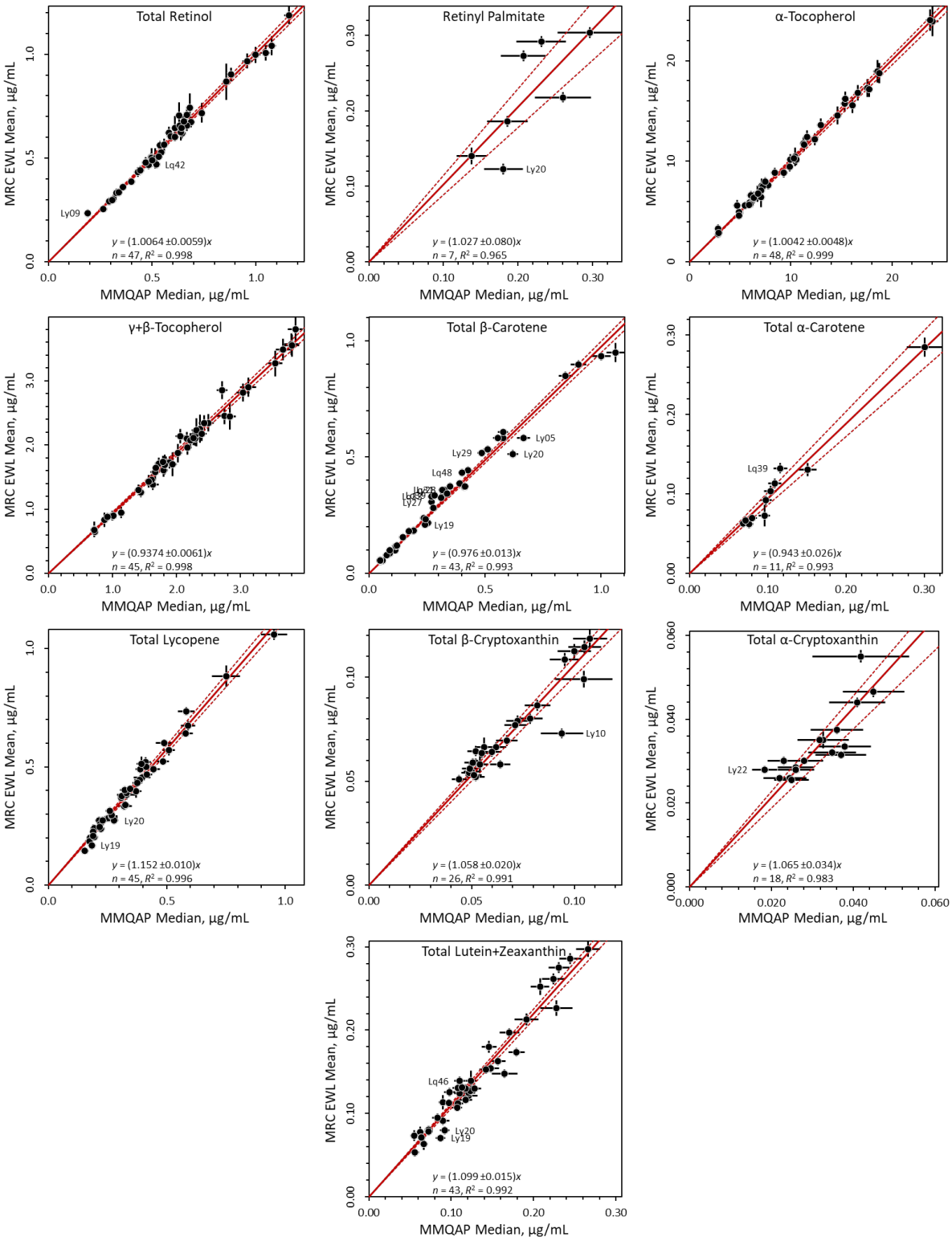


Figure 15. MRC EWL Means as Proportional Functions of MMQAP Medians

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 $\sigma_R$). 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.

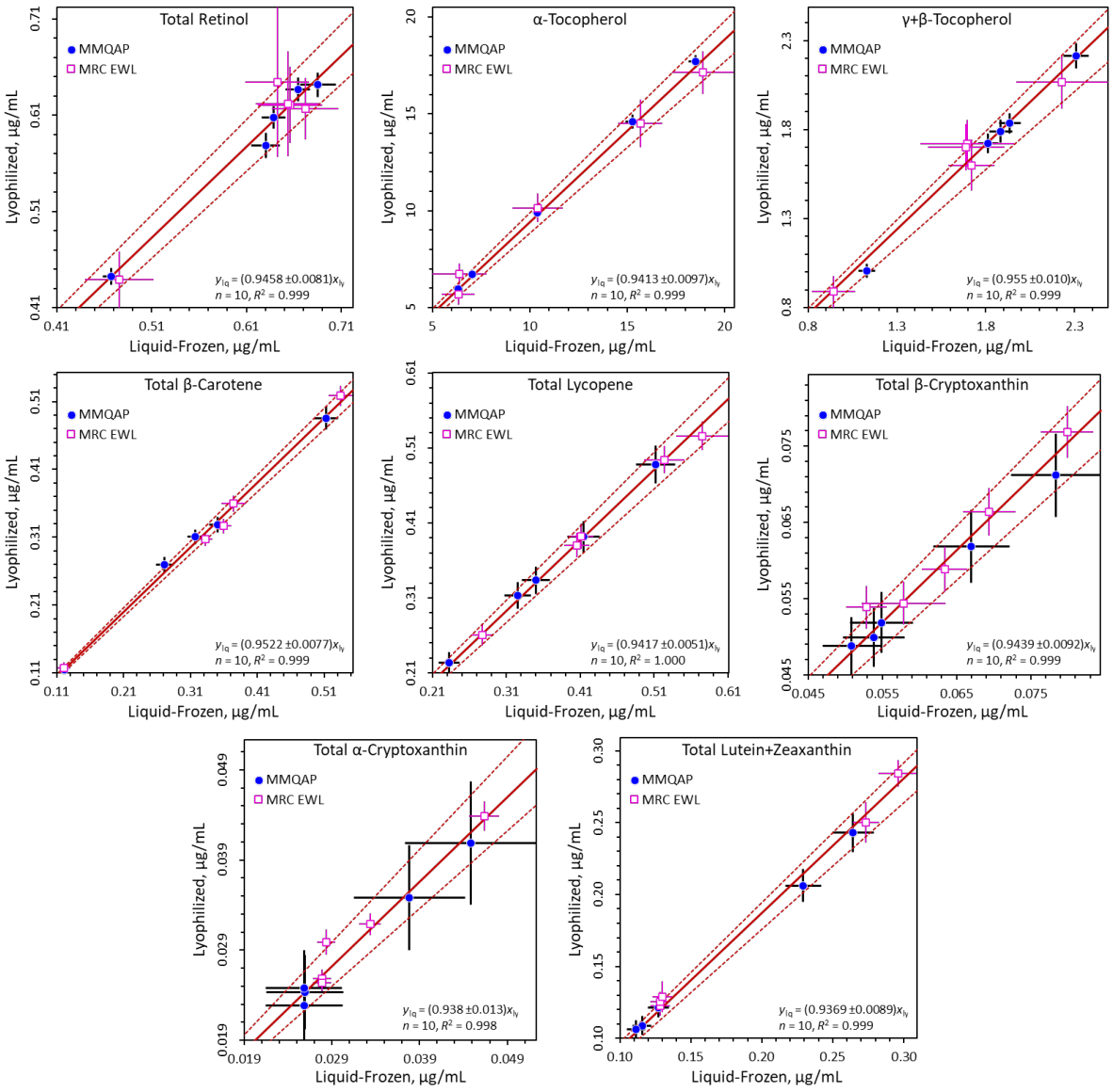


Figure 16. Liquid-frozen Results as Functions of Results for Paired Lyophilized Materials

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].

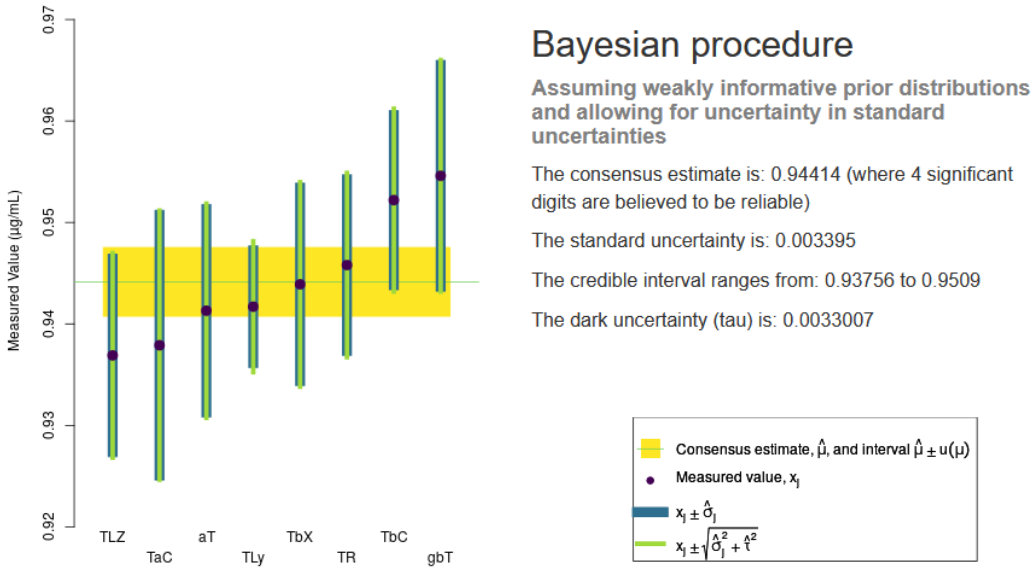


Figure 17. Analysis of Lyophilized/Liquid-Frozen Ratios

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.

## 6. Acknowledgement

The authors recognize the late Margaret C. Kline for her expertise in the production, storage, and documentation of most of the materials used in this study.

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