

Supplementary Materials:

Table S1: Concentrations (ng/mL) of the standard curve.

| Compound | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 | Std 7 |
|--|--------|--------|-------|-------|-------|-------|--------|
| retinol | 1600 | 800 | 400 | 200 | 100 | 50 | 25 |
| retinoic acid | 115 | 57.5 | 28.75 | 14.38 | 7.19 | 3.59 | 1.80 |
| 25-OH-D ₃ | 364.35 | 182.18 | 91.09 | 45.54 | 22.77 | 11.39 | 5.69 |
| 1- α -25(OH) ₂ -D ₃ | 165 | 82.5 | 41.25 | 20.63 | 10.31 | 5.16 | 2.58 |
| α -tocopherol | 14000 | 7000 | 3500 | 1750 | 875 | 437.5 | 218.75 |
| γ -tocopherol | 2000 | 1000 | 500 | 250 | 125 | 62.5 | 31.25 |
| α -tocotrienol | 100 | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 |
| K1 | 20 | 10 | 5 | 2.5 | 1.25 | 0.63 | 0.31 |
| MK-4 | 20 | 10 | 5 | 2.5 | 1.25 | 0.63 | 0.31 |
| MK-7 | 30 | 15 | 7.5 | 3.75 | 1.88 | 0.94 | 0.47 |

Abbreviations: Std: Standards and Std1: Original Stock concentrations

Table S2: External standard concentrations spiked into heparin plasma to make QC's.

| Compound | QC 1 | QC 2 | QC 3 |
|--|--------|-------|-------|
| retinol | 1600 | 400 | 100 |
| retinoic acid | 115 | 28.75 | 7.19 |
| 25-OH-D ₃ | 364.35 | 91.09 | 22.77 |
| 1- α -25(OH) ₂ -D ₃ | 165 | 41.25 | 10.31 |
| α -tocopherol | 14000 | 3500 | 875 |
| γ -tocopherol | 2000 | 500 | 125 |
| α -tocotrienol | 100 | 25 | 6.25 |
| K1 | 20 | 5 | 1.25 |
| MK-4 | 20 | 5 | 1.25 |
| MK-7 | 30 | 7.5 | 1.88 |

Abbreviations: QC: Quality Control

Table S3: Mixed model results of sex and generation effects on FSV concentrations. The sex effect was on adults only, not on children.

| Vitamer | Condition | Estimated | p-value |
|------------------|-----------------------|---------------------------------------|-------------|
| | | change | ng/mL |
| <i>Vitamin A</i> | retinol | Effect of sex | -0.004 0.75 |
| | | Effect of generation | 0.26 <0.001 |
| | | Interaction between Sex vs Generation | 0.19 <0.001 |
| | retinoic acid | Effect of sex | -0.01 0.66 |
| | | Effect of generation | 0.17 <0.001 |
| | | Interaction between Sex vs Generation | 0.15 <0.001 |
| | 25-OH-D ₃ | Effect of sex | 0.07 <0.01 |
| | | Effect of generation | -0.1 <0.001 |
| | | Interaction between Sex vs Generation | -0.008 0.82 |
| <i>Vitamin E</i> | α -tocopherol | Effect of sex | -0.02 0.16 |
| | | Effect of generation | 0.2 <0.001 |
| | | Interaction between Sex vs Generation | -0.06 0.02 |
| | γ -tocopherol | Effect of sex | -0.08 0.02 |
| | | Effect of generation | 0.18 <0.001 |
| | | Interaction between Sex vs Generation | -0.02 0.77 |
| | α -tocotrienol | Effect of sex | 0.04 0.4 |
| | | Effect of generation | -0.07 0.05 |
| | | Interaction between Sex vs Generation | 0.004 0.96 |

Table S4: Published reference plasma/serum concentrations of fat-soluble vitamers

| Vitamin | Vitamer | Sample characteristics | Concentrations | Reference | |
|----------------------|--|---|---|-----------|--|
| A | retinol | Middle-aged Chinese women aged 32-75 y (n=404) | 1.22 ± 0.34 µmol/L (252.08 – 446.87 ng/mL) 1.56 ± 0.38 µmol/L (338.02 – 555.73 ng/mL) | 25 | |
| | | Child controls (n=14) | 59 ± 5.88 µg/dL (531.2 – 644.8 ng/mL) Range 28.7-119 µg/dL (287-1190 ng/mL) | 26 | |
| | retinoic acid | Non-diabetic subjects (55.7 ± 9.5 y), men (n=511) and women (n=314) | Men 1.92 (1.32–2.49) ng/mL Women 1.99 (1.38–2.55) ng/mL | 27 | |
| | | Healthy women (n = 36; age 19–47 y) | 9.3 ± 3.7 nmol/L (1.68 – 3.90 ng/mL) | 28 | |
| D | 25-OH-D ₃ | Group of school children (n=479) aged 5-12 y | Males 75.9 ± 21 nmol/L (21.9 – 38.82 ng/mL) Females 70.8 ± 18.3 nmol/L (21.03 - 35.7 ng/mL) | 29 | |
| | | Control subjects (n=208) males and females, age y 25.6±0.5 | Males 93.9±2.7 nmol/L (36.54– 38.70 ng/mL) Females 99.7±2.9 nmol/L (38.78 – 41.10 ng/mL) | 30 | |
| | | Control subjects aged 68.7 ± 7.2 y Caucasian (n = 110) | 114.21 ± 50.6 nmol/L (25.48 - 66.02 ng/mL) | 31 | |
| | α -tocopherol | Healthy children (n=166); 1 month - 18 years | 11.9 - 30 µmol/L (5,125 – 12,921 ng/mL) | 32 | |
| E | | Healthy 20 – 59 y old adults (males (n=33) and females(n=73)) | Males 15.45 ± 10.16 µmol/L (2278.45 - 11,030 ng/mL) Females 15.00 ± 4.54 µmol/L (4,505.22 – 8,416 ng/mL) | 33 | |
| | | Control subjects aged 68.7 ± 7.2 y Caucasian (n = 110) | 19.18 ± 8.85 µg/mL (10330- 29030 ng/mL) | 31 | |
| γ -tocopherol | Control subjects aged 68.7 ± 7.2 y Caucasian (n = 110) | 1.67 ± 1.48 µg/mL (190 – 3150 ng/mL) | 31 | | |
| | Control subjects aged 68.7 ± 7.2 y Mean values (n=675) | 1.98 ± 1.36 µg/mL (620 – 3340 ng/mL) | 34 | | |
| | Male and Female participants aged 56.3 y | 0.04 to 0.61 mg/dL (400- 6100 ng/mL) | 35 | | |
| | α -tocotrienol | Males (n=36) age 21 - 30 y | 34.3 ± 9.6 ng/mL | 36 | |
| | | Males after postprandial diet (n=10) | (1.46 ± 0.52 µmol/L) 399.18 – 840.82 ng/mL | 37 | |
| | | Healthy male adults aged (n=64) age 20–26 y | 9.9 ± 2.5 ng/ml | 37 | |
| | | Placebo male adults aged (n=16) age 20–26 y | 10.31± 3.70 ng/ml | | |

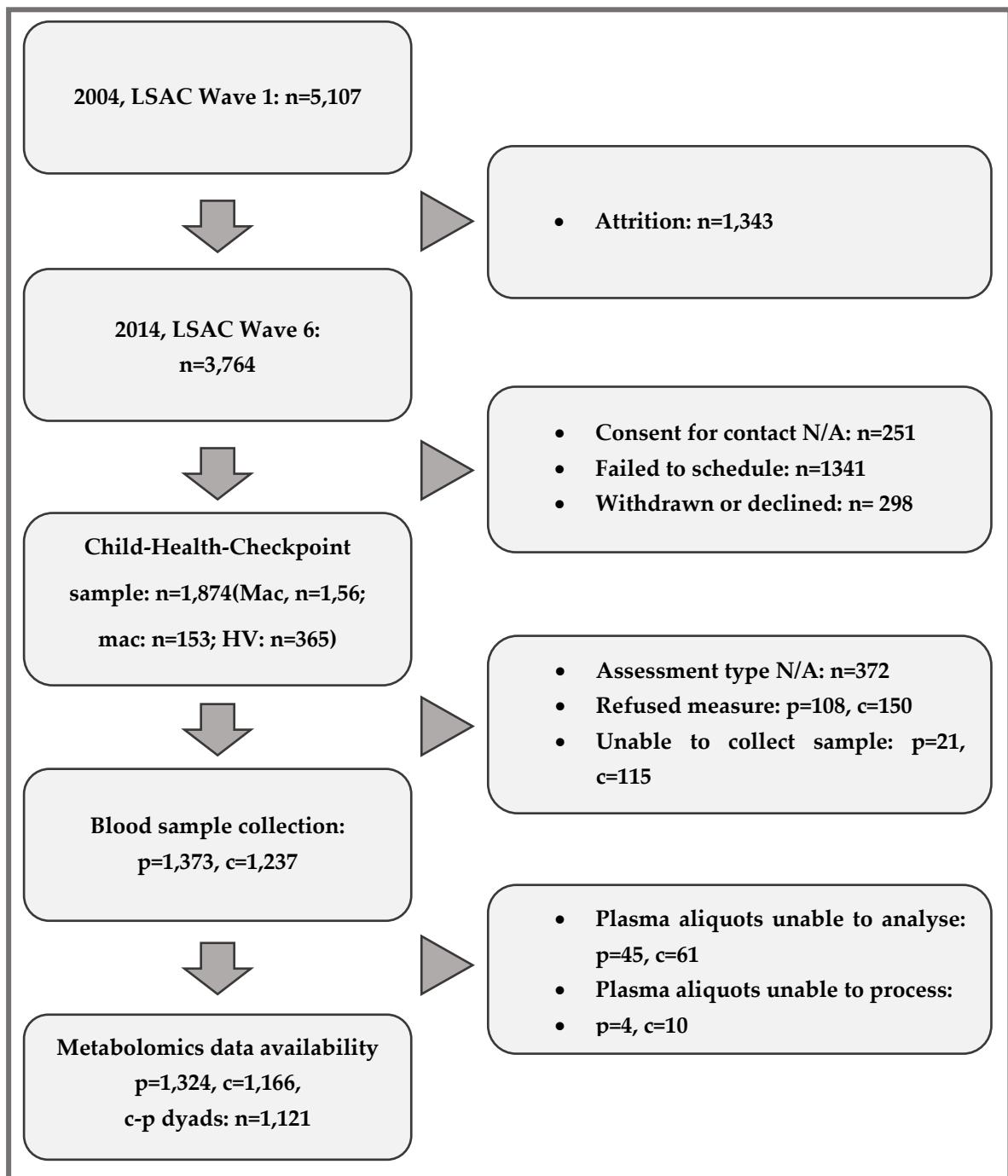


Figure S1: Participant information chart. HV: home visit; LSAC: Longitudinal Study of Australian Children; Mac: main assessment centre; mac: mini assessment centre; p: parent adults; c: children.

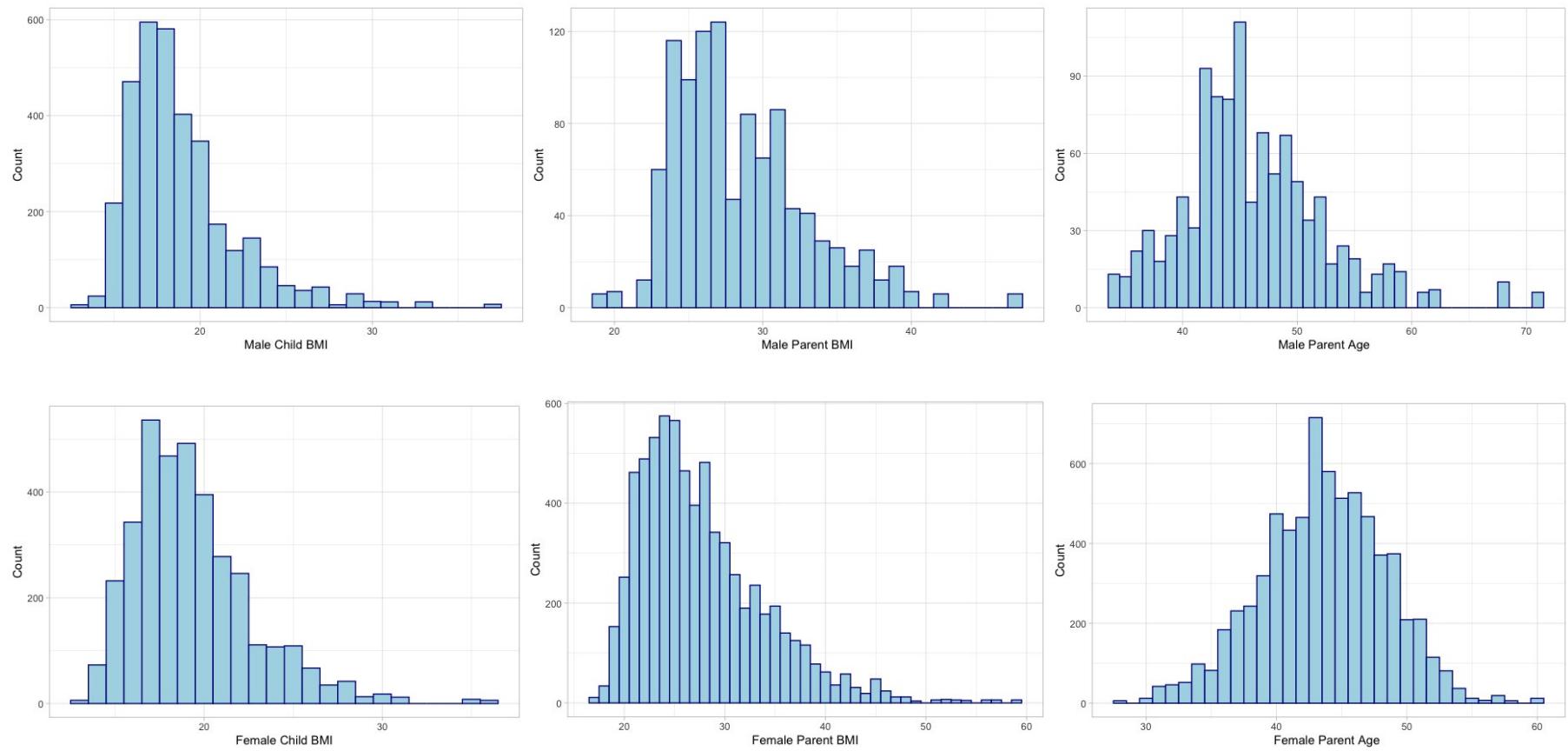


Figure S2: Population characteristics of the LSAC's Checkpoint cohort.

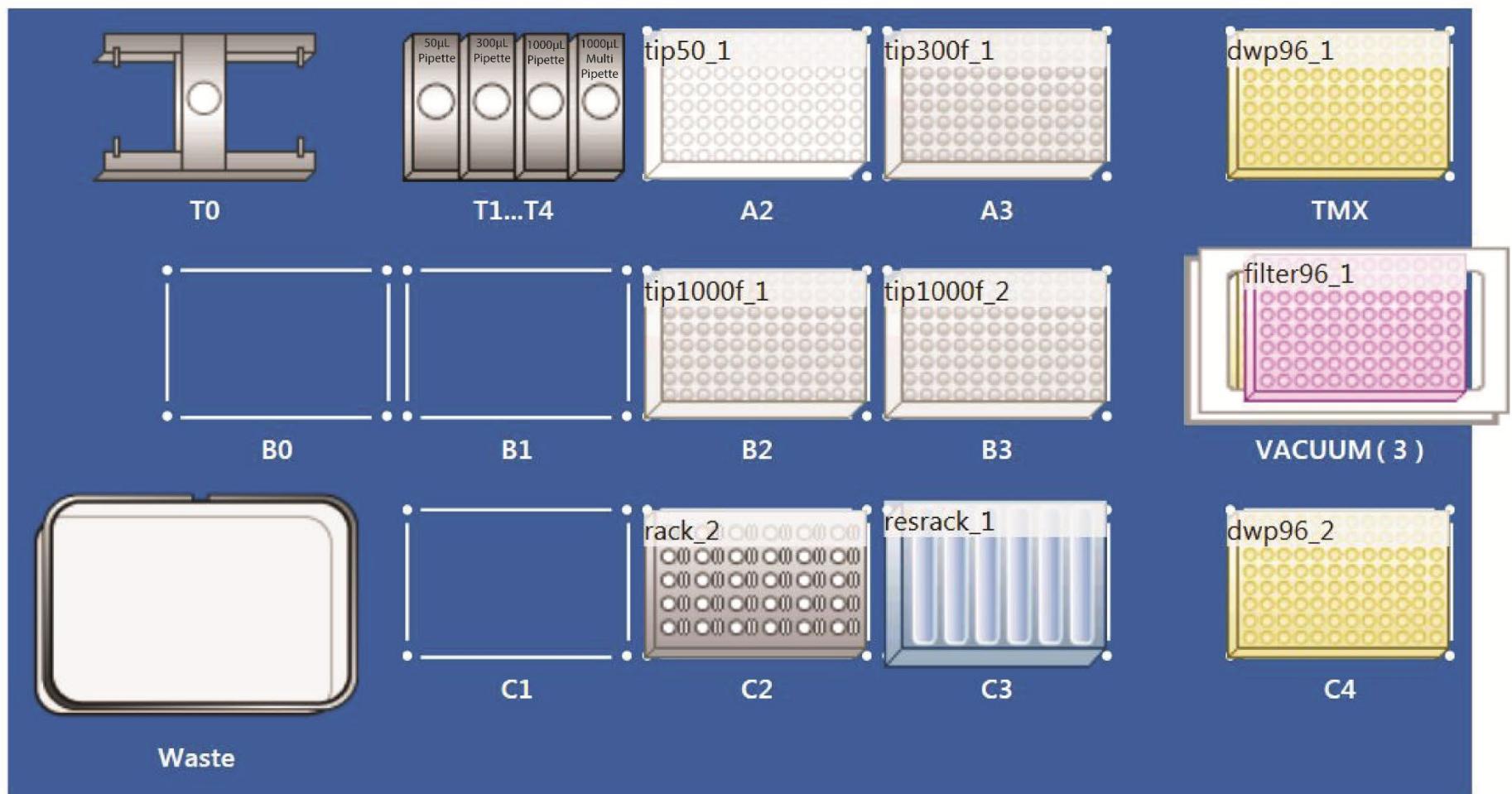


Figure S3: The Eppendorf EpMotion liquid handling robot setup. T0; Plate mover, T1-T4; Automated pipettor, A2, A3, B2 and B3; Filtered pipette tips, dwp96: 96 Deep well plates, Vacuum; vacuum manifold, resrack; reservoirs (3mL and 10mL), rack2; 2mL 2 ml Eppendorf tube holder.

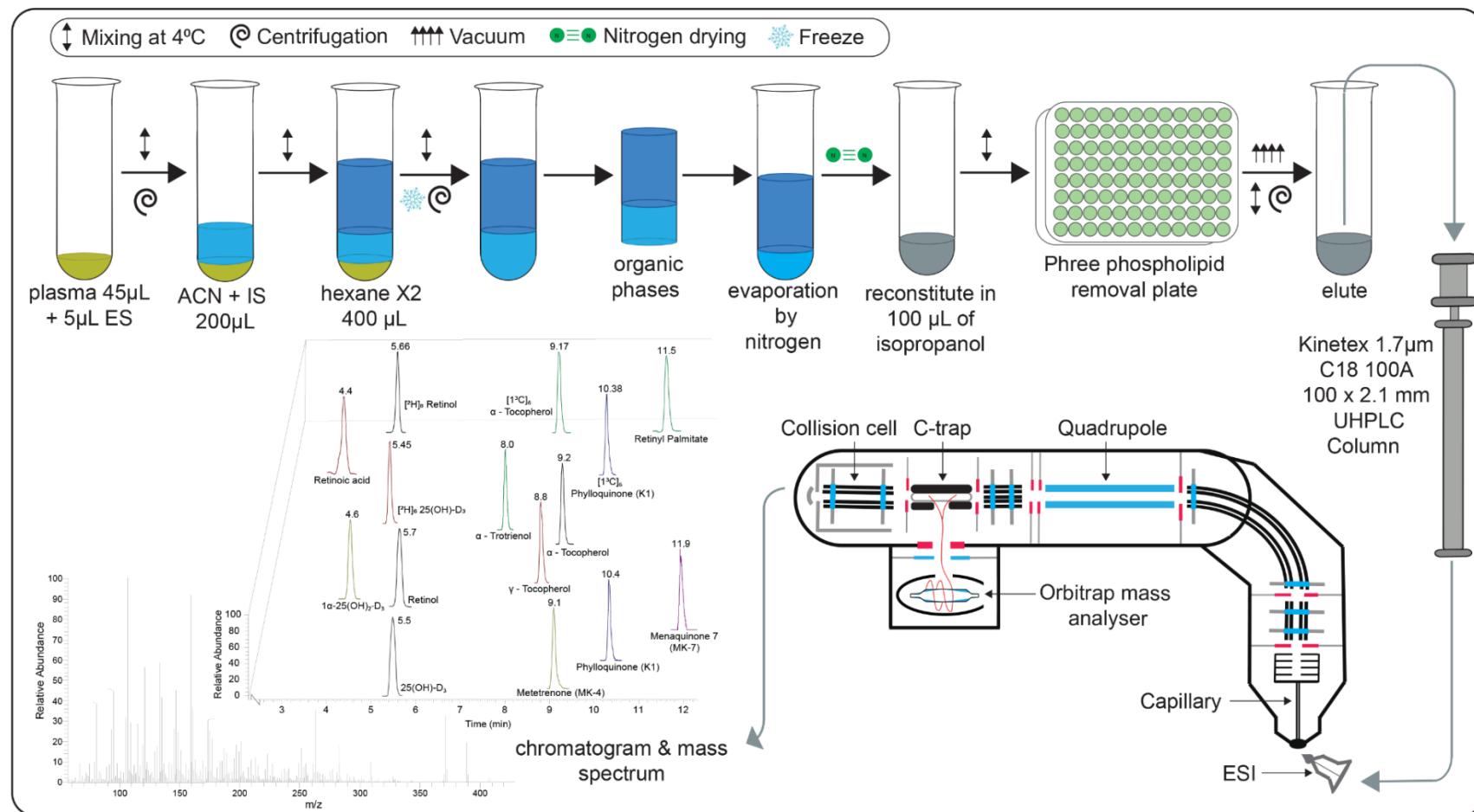


Figure S4: Workflow for sample preparation and LC-MS/MS analysis (Adopted from Arachchige et al., 2021; attribution 4.0 International (CC BY 4.0)).