**Supplemental Table 1**. Compound transitions and parameters for thyroid hormones and vitamin D metabolites.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | Abbreviation | Manufacturer | Precursor Ion (*m*/*z*) | Product Ion (*m*/*z*) | DP (V) | EP (V) | CE (V) | CXP (V) |
| Thyroxine | T4 | EC-IRMMa | 777.7 | 731.7 | 100 | 10 | 35 | 50 |
| T4-1 | 777.7 | 604.9 | 100 | 5 | 60 | 30 |
| Thyroxine-13C6\* | T4-13C6 | Cerilliantb | 783.7 | 737.7 | 100 | 10 | 35 | 50 |
| Triiodothyronine | T3 | EC-IRMM | 651.8 | 478.9 | 75 | 5 | 60 | 30 |
| T3-1 | 651.8 | 605.8 | 75 | 10 | 35 | 50 |
| Triiodothyronine-13C6\* | T3-13C6 | Cerilliant | 657.8 | 611.8 | 75 | 10 | 35 | 50 |
| Reverse Triiodothyronine | rT3 | Sigmac | 651.8 | 507.8 | 75 | 5 | 35 | 30 |
| rT3-1 | 651.8 | 605.8 | 75 | 5 | 35 | 30 |
| Reverse Triiodothyronine-13C6\* | rT3-13C6 | Cerilliant | 657.8 | 611.8 | 75 | 5 | 35 | 30 |
| 25-Hydroxy Vitamin D3 | 25(OH)D3 | NIST SRMd 2972a | 401.0 | 386.0 | 55 | 4 | 10 | 18 |
| 3-Epi-25-Hydroxy Vitamin D3 | 3-epi-25(OH)D3 | NIST SRM 2972a | 401.0 | 386.0 | 55 | 4 | 10 | 18 |
| 3-Epi-25-Hydroxy Vitamin D3-*d3*\* | 3-epi-25(OH)D3-*d*3 | Isosciencese | 404.0 | 386.0 | 55 | 4 | 10 | 18 |
| 25-Hydroxy Vitamin D3-13C5\* | 25(OH)D3-13C5 | Isosciences | 406.0 | 388.0 | 55 | 4 | 10 | 22 |
| 25-Hydroxy Vitamin D2 | 25(OH)D2 | NIST SRM 2972a | 413.0 | 395.0 | 55 | 4 | 10 | 22 |
| 25-Hydroxy Vitamin D2-13C3\* | 25(OH)D2-13C3 | Isosciences | 416.0 | 398.0 | 55 | 4 | 10 | 22 |

\* Internal standard

a European Commission, Institute for Reference Materials and Measurements; Gembloux, Belgium

b  Round Rock, TX

c St. Louis, MO

d Gaithersburg, MD

e Ambler, PA

**Supplemental Table 2.** Performance data for previously unpublished mass spectrometry methods used to measure analytes in SRM 1949. Reporting limit (RL) was determined by either three times the standard deviation of the mean plus the mean of the blanks (LOD) or the lowest calibration standard (whichever was higher) for thyroid hormone measurements. The RL is the LOD for trace metals and the limit of quantitation (LOQ) for Vitamin D metabolites. Intra-assay and inter-assay variances are reported as the greatest value among the four levels of SRM 1949. For gravimetric measurement methods, data was converted using a density of 1.0233 g/mL.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | LOD | LOQ | RL | Greatest Intra-assay RSD | Greatest Inter-assay RSD | Analytical Range | R2 of linear regression |
| Thyroglobulin | 0.01 µg/L | 0.1 µg/L | 0.2 µg/L | 9.4 % | 10.8 % | 0.1 µg/L to 100 µg/L | 1.00 |
| Thyroxine | 0.01 nmol/L | - | 22.0 nmol/L | 1.8 % | 1.2 % | 22.0 nmol/L to 218 nmol/L | 1.00 |
| Triiodothyronine | 0.005 nmol/L | - | 0.424 nmol/L | 6.7 % | 5.7 % | 0.424 nmol/L to 3.40 nmol/L | 1.00 |
| reverse Triiodothyronine | 0.012 nmol/L | - | 0.109 nmol/L | 12 % | 15 % | 0.109 nmol/L to 0.876 nmol/L | 1.00 |
| 25(OH)D3 | 0.38 nmol/L | 1.3 nmol/L | 1.3 nmol/L | 3.0 % | 1.6 % | 1.3 nmol/L to 511 nmol/L | 0.999 |
| 25(OH)D2 | 0.50 nmol/L | 1.2 nmol/L | 1.2 nmol/L | 10 % | 14 % | 1.2 nmol/L to 496 nmol/L | 0.999 |
| 3-epi-25(OH)D3 | 0.38 nmol/L | 1.3 nmol/L | 1.3 nmol/L | 4.0 % | 6.7 % | 1.3 nmol/L to 511 nmol/L | 0.996 |

**Supplemental Table 3**. Summary statistics (nmol/L) of the quantifying (quant) and the qualifying (qual) transitions for the thyroid hormones in SRM 1949, Frozen Prenatal Serum (n = 6 vials per level).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| SRM 1949 Level |   | Thyroxine (quant) | Thyroxine (qual) | Triiodothyronine (quant) | Triiodothyronine (qual) | Reverse Triiodothyronine (quant) | Reverse Triiodothyronine (qual) |
| Non-pregnant | Mean | 88.8 | 92.5 | 1.62 | 1.64 | 0.296 | 0.279 |
| Stdev | 1.05 | 0.53 | 0.09 | 0.14 | 0.043 | 0.047 |
| RSD | 1.20% | 0.50% | 5.70% | 9.00% | 14.40% | 17.00% |
| First Trimester | Mean | 129 | 136 | 2.36 | 2.40 | 0.471 | 0.525 |
| Stdev | 2 | 1 | 0.11 | 0.09 | 0.049 | 0.035 |
| RSD | 1.20% | 0.80% | 4.30% | 4.20% | 10.20% | 6.70% |
| Second Trimester | Mean | 147 | 154 | 2.83 | 2.90 | 0.523 | 0.570 |
| Stdev | 1 | 3 | 0.16 | 0.22 | 0.076 | 0.085 |
| RSD | 1.00% | 1.50% | 5.30% | 7.30% | 14.50% | 14.80% |
| Third Trimester | Mean | 146 | 151 | 2.83 | 2.88 | 0.548 | 0.588 |
| Stdev | 1 | 3 | 0.14 | 0.20 | 0.079 | 0.077 |
| RSD | 0.60% | 1.30% | 5.10% | 7.20% | 14.30% | 13.10% |

**Supplemental Table 4**. Analytes on the certificate of analysis for standard reference material 1949, Frozen Prenatal Serum. Values are means ± uncertainty. Information values do not have a calculated uncertainty. Please reference the online certificate for the most up-to-date values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Analyte | Units | Non-pregnant | First Trimester | Second Trimester | Third Trimester |
| Thyroxine | µg/dL | 6.74 ± 0.12 | 9.82 ± 0.17 | 11.14 ± 0.19 | 11.07 ± 0.19 |
| Triiodothyronine | ng/dL | 101.4 ± 6.4 | 147.6 ± 6.6 | 176.0 ± 10.0 | 176.7 ± 10.1 |
| reverse Triiodothyronine | ng/dL | 19.2 | 30.6 | 33.9 | 35.6 |
| 3-epi-25-Hydroxy Vitamin D3 | ng/mL | 1.32 ± 0.06 | 1.43 ± 0.02 | 1.87 ± 0.07 | 1.87 ± 0.04 |
| 25-Hydroxy Vitamin D3 | ng/mL | 24.98 ± 0.28 | 26.01 ± 0.22 | 30.00 ± 0.50 | 29.43 ± 0.41 |
| 25-Hydroxy Vitamin D2 | ng/mL | < 0.5 | 1.20 ± 0.05 | 0.514 ± 0.037 | 0.897 ± 0.057 |
| Vitamin D Binding Protein | µg/mL | 211.5 ± 2.8 | 286.7 ± 3.8 | 349.7 ± 4.3 | 383.4 ± 5.1 |
| Vitamin D Binding Protein | µmol/kg | 4.01 ± 0.05 | 5.43 ± 0.06 | 6.64 ± 0.07 | 7.28 ± 0.08 |
| Copper | μg/dL | 128.5 ± 1.9 | 174.1 ± 2.3 | 214.2 ± 2.5 | 232.4 ± 3.6 |
| Selenium | μg/L | 109.3 ± 1.3 | 105.1 ± 1.9 | 112.7 ± 1.5 | 98.9 ± 2.2 |
| Zinc | μg/dL | 78.8 ± 3.2 | 105.7 ± 3.1 | 101.1 ± 3.7 | 81.7 ± 2.3 |
| Thyroid Stimulating Hormone | mIU/L | 3.6 | 1.4 | 1.1 | 1.1 |
| Thyroglobulin Automated IA | μg/L | 7.80 | 12.2 | 12.7 | 16.7 |
| Thyroglobulin LC-MS/MS | μg/L | 12.0 | 14.2 | 15.6 | 16.5 |
| Thyroglobulin Antibody | IU/mL | 144 | 27.3 | < 1.8 | < 1.8 |
| Serum Density | g/mL | 1.0233 | 1.0234 | 1.0223 | 1.0217 |

**Supplemental Figure Captions**

Figure S1. Comparison of thyroid hormone values in SRM 971, Hormones in Frozen Human Serum (female level is open red circles, male level is solid blue circles), generated by the new liquid chromatography tandem mass spectrometry method compared to the certified values. Five replicates from one bottle per level were analyzed. Both product ions monitored are listed (Tx and Tx-1). For measured values, points are the mean and error bars are ± one standard deviation. For certified values, points are the certified mean and error bars are ± the uncertainty. (A) Triiodothyronine (T3), (B) Thyroxine (T4), (C) Reverse Triiodothyronine (rT3). There are no certified values for reverse triiodothyronine; thus only the measured values are presented.

Figure S2. Comparison of vitamin D metabolite values in SRM 972a, Vitamin D Metabolites in Frozen Human Serum (Level 1 is open circles and Level 2 is solid circles), generated by the high throughput modified method of the reference measurement procedure using liquid chromatography tandem mass spectrometry method compared to the certified values. Three vials were analyzed with duplicate injections. For measured values, points are the mean and error bars are ± one standard deviation. For certified values, points are the certified mean and error bars are ± the uncertainty. (A) 25-hydroxy vitamin D3. (B) 25-hydroxy vitamin D2. (C) 3-epi-25-hydroxy vitamin D2.