**SUPPLEMENTARY MATERIAL**

**DBS assay validation**

*Precision - Repeatability and Intermediate precision*

Repeatability and intermediate precision of the method were evaluated using venous dried human whole blood samples (DBS) with endogenous plasma 25(OH)D values at 3 different levels at four occasions (n=6 for each occasion). 25(OH)D results are shown in Supplemental Table 1.

Supplemental Table 1. 25(OH)D3 in DBS by LC-MS/MS, repeatability and intermediate precision. Measurements were performed using LC-MS/MS in six parallels at 3 levels at 3 different occasions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Level | Occ. 1  mmol/L | Occ. 2  mmol/ | Occ.3  mmol/ |  | Intermediate precision  RSD% |
| Low | |  | | --- | | 33.1 | | 24.4 | | 33.5 | | 23.7 | | 30.2 | | 31.2 | | |  | | --- | | 36.6 | | 33.0 | | 28.0 | | 28.6 | | 24.8 | | 31.6 | | |  | | --- | | 29.2 | | 30.9 | | 29.4 | | 25.6 | | 23.2 | | 23.4 | |  | 13.9 |
| Repeatability  RSD% | 13.4 | 12.5 | 11.3 |  |  |
| Med | |  | | --- | | 69.2 | | 68.5 | | 89.0 | | 78.3 | | 65.1 | | 67.7 | | |  | | --- | | 75.5 | | 74.4 | | 66.5 | | 59.0 | | 70.3 | | 70.8 | | |  | | --- | | 72.4 | | 63.2 | | 69.6 | | 66.3 | | 60.3 | | 71.2 | |  | 9.9 |
| Repeatability  RSD% | 11.3 | 7.9 | 6.4 |  |  |
| High | |  | | --- | | 142.7 | | 135.7 | | 143.7 | | 154.1 | | 143.5 | | 139.0 | | |  | | --- | | 127.2 | | 154.6 | | 154.6 | | 133.0 | | 135.2 | | 154.0 | | |  | | --- | | 129.4 | | 122.1 | | 138.6 | | 158.7 | | 159.6 | | 153.6 | |  | 8.1 |
| Repeatability  RSD% | 4.0 | 8.1 | 10.1 |  |  |

*Accuracy – plasma*

As 25(OH)D usually is analyzed in serum or plasma, certified SRMs are only available as serum or plasma samples. Accuracy in plasma of the method is thus first evaluated by analyzing serum or plasma reference materials from several sources, Supplemental Table 2.

Supplemental Table 2. Nominal values for 25(OH)D3 in reference material NIST SRM 1950 and 5 DEQAS samples

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference material | Nominal nmol/L[[1]](#footnote-1) | Found  (nmol/L) | Accuracy % | N | CV% |
| NIST SRM 1950\* | 61.8 | 65.2 | 105.3 | 4 | 2.5 |
| DEQAS-491\*\* | 33.4 | 31.2 | 93.4 | 9 | 4.8 |
| DEQAS-492\*\* | 51.6 | 51.2 | 99.2 | 9 | 4.0 |
| DEQAS-493\*\* | 91.8 | 89.5 | 97.5 | 9 | 3.0 |
| DEQAS-494\*’ | 71.9 | 69.2 | 96.2 | 9 | 5.9 |
| DEQAS-495\*\* | 97.6 | 99.9 | 102.4 | 9 | 5.5 |

\*NIST analysed 9th Nov 2016,

\*\*DEQAS analyzed 19th and 25th May 2016.

*Acceptance Criteria – Accuracy in plasma*

The accuracy of plasma is regarded as sufficient as long the result of each reference material is within relative value of ±15% of the given value from the reference source.

As seen in Supplemental Table 2, all the results of reference material are within the acceptance criteria, and thereby regarded as sufficient.

Vitas is participating in the Vitamin D External Quality Assessment Scheme (DEQAS), along with about 1200 other laboratories in 54 countries. Every 3rd month, 5 plasma sample, with unknown concentrations, are distributed to each laboratory.

In total, about 30 different methods are used to analyze the 5 samples distributed. The results submitted are compiled and a report is received from DEQAS. The results submitted are compared with a target value (NIST) and method mean.

Please see Supplemental Fig. 1 for graphic presentation of DEQAS results submitted in 2016.

Supplemental Fig. 1. DEQAS results submitted by Vitas AS in 2006

Vitas mean deviation from target value from January 2012-YTD is -0.4%.

**Accuracy in DBS**

25(OH)D is bound to Vitamin D binding protein (VDBP) which id distributed 100% in plasma fraction of the blood. When analyzing DBS with a known plasma value of 25(OH)D its HcT value is used to convert the plasma value to whole blood value.

Given equally number of punches of calibrator and samples are used, a set factor, for men and women, is used to convert the whole blood results for unknown samples back to plasma results before reported to costumers.

Sample material to be received is DBS. The accuracy of the method it-self is evaluated by using reference material. The accuracy in DBS compared to plasma, must be done by analyzing plasma and DBS from the same person. Plasma and DBS from 78 individuals is analyzed to evaluate the accuracy in DBS.

Supplemental Fig. 3. Correlation of DBS and plasma for 78 samples analysed at Vitas AS

y= 0.8491x + 7.7529

r= 0.973

**Stability**

Whole blood was collected from 3 individuals and DBS cards made. The DBS cards were packed according to the user manual for the DBS vitamin D kit.

DBS cards were stored at 25°C and 50°C, and placed in -20°C after 1,3,6,8,10,14,17 and 21 days. All samples were analysed together. The results are compared to the baseline result.

Supplemental Fig. 4. Stability of 25(OH)D3 on DBS at 25°C for 3 individuals for 21 days.

Supplemental Fig. 5. Stability of 25(OH)D3 on DBS at 50°C for 3 individuals for 21 days

For both temperatures inspected, results for the three individuals show no trending, confirming stability of 25(OH)D3 on DBS over 21 days.

1. <https://www-s.nist.gov/srmors/certificates/1950.pdf> [↑](#footnote-ref-1)