## Experimental conditions for PvW and acceptance protocol

### Additional information regarding 1.1 and 1.2 of PvW:

Samples will be made available in order to determine the parameters to be reported for 1.1 and 1.2 of PvW. During enrolment, the supplier will state whether the offered GC-IRMS instrument meets the requirements and reports the results as described below. Then, during the acceptance phase, the assay will be transferred to the new MS instrument to verify that the claimed parameters are equal to or better than those reported during enrolment.

The mentioned configuration is used to indicate the desired levels and does not imply a preference for any specific bidder, system or type.

### Procedure

Samples are available upon request from the Stable Isotope Laboratory. Detailed information will be provided on the analytes, the theoretical enrichment value and the preparation procedure.

### Additional information samples deuterated amino acid enrichment analysis (PvW 1.1)

A standard curve for selected amino acids in pyrolysis mode (duplicate injections): deuterated isotopologues of valine, leucine, isoleucine, methionine, phenylalanine, lysine and histidine.

Samples contain derivatized chemical standards (N-ethyoxycarbonyl ethyl esters of amino acids dissolved in ethyl acetate).

### GC-conditions deuterated amino acid enrichment analysis (PvW 1.1)

GC-conditions: VF17ms 30x0.25mmx0.50µm, carrier: 0.8 ml/min constant flow, GC temperature gradient: initial temperature 60°C, hold time 0.5 min; increase temperature at 15°C/min to 150°C, then at 4°C/min to 220°C, and then at 8°C/min to 300°C, hold time 7 min.

Gradient and column type may be changed (but need to be specified) to improve results, with a maximum run time of 55 min.

### Additional information samples $CO_2$ analysis (PVW 1.2)

A standard curve for both enrichment of $^{13}CO_2$ in exetainer breath tubes at low abundances (enrichment curve 4.4 µmol/12ml $CO_2$ ranging from 0 to 0.11 APE and a concentration curve ranging from 0 to 7.4 µmol/12ml $CO_2$ are provided. The standards are prepared by the liberation of carbonate using phosphoric acid.

### GC conditions $CO_2$ analysis (PvW 1.2)

PoraBOND Q column (25m x 0.32 mm; Agilent Technologies), 30°C constant temperature, carrier flow 1.5 ml/min, 75 µl headspace injection

Gradient and column type may be changed (but need to be specified) to improve results, with a maximum run time of 10 min.

**Reporting of results**

The following results need to be reported:

- area 2, area 3 and delta values for the amino acid experiment (PvW 1.1)
- area 44, area 45 and delta values for the CO2 experiment (PvW 1.2)
- chromatograms of at least 5 measurements per experiment (PvW 1.1 and 1.2)
- calculated results are reported in excel (see below) (PvW 1.1 and 1.2)
- any deviations from the experimental conditions described below (PvW 1.1 and 1.2)

**Calculation of results**

- Measured delta values are plotted on the y-axis against the theoretical MPE values (as provided by the laboratory) on the x-axis. A linear calibration curve is fitted in Excel using a trendline without a fixed intercept. The resulting linear equation ($y = ax + b$) and the coefficient of determination ($R^2$) are reported.
- The difference between duplicate injections is calculated as (result a + result b)/average of results. The average of all differences between duplicate injections is reported for PvW 1.1.2, including the lowest and highest difference observed.
- The CV% of triplicate injections is calculated as the standard deviation of area 44 of triplicate injections, divided by the average area 44 of triplicate injections. The average of all CV% of triplicate injections is reported for 1.2.2, including the lowest and highest CV% observed.