

## **Evaluation of the HeliFAN Plus Instrument**

### **General**

Metabolic Solutions compared the Fischer HeliFan Plus Instrument against two gas isotope ratio mass spectrometers (Europa Scientific 20-20 and Finnigan BreathMAT Plus). Samples were of two types. First, gas standards made up to specific  $^{13}\text{C}$  delta per mil values were utilized for the evaluation of accuracy. These gas standards had 5%  $\text{CO}_2$  gas with the balance nitrogen gas. Secondly, breath samples collected during several different breath tests were analyzed by both types of instruments. In order to have identical duplicate breath samples, breath was initially collected into a 0.75 liter collection bag (Fischer Analysen Instrumente). A 10 ml sample of this breath collection was transferred under vacuum to an Exetainer tube (Labco, Ltd). The remaining breath sample in the collection bag was evaluated by the HeliFAN instrument while the Exetainer tube was evaluated by the isotope ratio mass spectrometers.

The HeliFAN instrument was evaluated for accuracy, precision, memory carryover and clinical evaluation of breath test results.

### **Accuracy**

Four different gas standards were utilized in this evaluation. The accuracy of the difference between standards was evaluated. Each gas standard was analyzed four times. The mean difference between the HeliFAN and the isotope ratio instruments was  $-0.91$  delta per mil with a standard deviation of  $0.78$  delta per mil. The range of differences was  $-1.89$  to  $0.81$  delta per mil. We conclude that the accuracy of the HeliFAN instrument is excellent and sufficient for breath test measurements.

Eleven breath samples were analyzed in triplicate on both types of instruments. The mean difference between the HeliFAN and the isotope ratio instruments was  $0.53$  delta per mil with a standard deviation of  $1.04$  delta per mil. The accuracy of the HeliFAN instrument improved with breath samples. This was expected since the instrument's spectroscopy is optimized with this type of sample.

### **Precision**

Four baseline (without isotope administration) breath samples were analyzed in quadruplicate on the same day. The  $^{13}\text{C}$  delta per mil results are shown below:

	Collection 1	Collection 2	Collection 3	Collection 4
	-19.94	-19.43	-19.87	-20.33
	-20.59	-19.68	-19.91	-20.04
	-20.55	-19.70	-20.03	-20.19
	-20.88	-19.78	-20.00	-20.25
Mean	-20.49	-19.65	-19.95	-20.20
SD	0.40	0.15	0.08	0.12

There is excellent precision within and between samples. The HeliFAN instrument is precise to measure breath samples.

### **Memory Effect**

The effect of one breath sample on the next sample can result if isotope memory exists. Two gas standards differing by about 20 per mil will be analyzed by the HeliFAN instrument, the low  $^{13}\text{C}$  gas followed by the high  $^{13}\text{C}$  gas. If there is a memory effect, we would observe a change in the delta per mil and standard deviation of the measurements. These results are shown below:

Analysis	Control Gas 1	Control Gas 2
1	-38.09	-18.79
2	-37.76	-18.46
3	-38.14	-18.93
4	-37.76	-18.10
5	-38.17	-18.33
Mean	-37.98	-18.52
SD	0.21	0.34

We observed no measurable memory effect in typical  $^{13}\text{C}$  ranges with breath tests.

### **Clinical Breath Testing**

The HeliFAN instrument was compared to isotope ratio mass spectrometers using several breath tests in development at Metabolic Solutions. These are the results observed:

- a) Gastric Emptying – An  $^{13}\text{C}$ -octanoate labeled meal was used with 16 breath sample collections. The gastric emptying rate,  $T_{1/2}$ , was calculated using all the collection points with isotope ratio analysis and HeliFAN analysis.

$T_{1/2}$  Isotope Ratio = 161.6 minutes

$T_{1/2}$  HeliFAN = 161.6 minutes

Difference = 0.00 minutes

- b) Acetate Metabolism

A small dose of 1- $^{13}\text{C}$ -acetate was administered to a subject. The cumulative percent recovery of  $^{13}\text{C}$  in the breath over 2 hours was determined.

Cum. % Dose Recovered IRMS = 14.69%

Cum. % Dose Recovered FAN = 13.66%

Difference = 1.03%

- c) Glucose Metabolism

A small dose of  $^{13}\text{C}_6$ -glucose was administered to a subject. The cumulative percent recovery of  $^{13}\text{C}$  in the breath over 2 hours was determined.

Cum. % Dose Recovered IRMS = 15.96%

Cum. % Dose Recovered FAN = 14.26%

Difference = 1.70%

- d) Amino Acid Metabolism

A small dose of a 1- $^{13}\text{C}$ -amino acid was administered to a subject. The cumulative percent recovery of  $^{13}\text{C}$  in the breath over 2 hours was determined.

Cum. % Dose Recovered IRMS = 9.66%

Cum. % Dose Recovered FAN = 9.60%

Difference = 0.06%

These results demonstrate a good correlation between isotope ratio mass spectrometry and the HeliFAN instrument.

### **Summary**

We conclude that the HeliFAN instrument provides accurate and precise breath testing results as compared to isotope ratio mass spectrometry. The difference in the  $^{13}\text{C}$  results detected by each instrument will not be meaningful in clinical practice.