

RCM

## Letter to the Editor

To the Editor-in-Chief  
Sir,

**Automated breath hydrogen measurement at the part-per-million level on a continuous-flow isotope-ratio mass spectrometer**

The following report describes a novel use for a continuous-flow isotope-ratio mass spectrometer (CF-IRMS). Hydrogen breath tests are widely used in the clinical setting for the diagnosis of malabsorption, bacterial overgrowth and small intestinal transit time.<sup>1–6</sup> The hydrogen breath test is based on the change in breath H<sub>2</sub> concentration from basal (fasting) levels after a test-dose of an appropriate substrate. Dedicated instruments are available for breath hydrogen analysis, e.g. the exhaled hydrogen monitor (GMI Measurement Instruments Ltd., Renfrew, UK), which utilises an electrochemical detector or the QuinTron MicroLyzer (QuinTron, Milwaukee, WI, USA), which is a miniature gas chromatograph. These instruments are relatively inexpensive, but require manual injection of samples. Hand-held instruments are also available for use in the clinical setting, e.g. the Gastrolyzer breath hydrogen monitor (Bedfont Scientific, Rochester, UK), but these may not be appropriate in the research setting if breath sampling has to begin the day before the study and continue for many hours. Such studies can generate many hundreds of breath samples.

There is increasing awareness of the possible health benefits of non-digestible carbohydrate in the diet. Carbohydrate that has escaped enzymatic digestion in the small intestine provides a substrate for fermentation by colonic bacteria and hence the production of short chain fatty acids and gases, including hydrogen.<sup>7</sup> We

have automated a method of analysing breath hydrogen at the part-per-million (ppm) level on our CF-IRMS, which is routinely used to measure deuterium in body fluids in studies of body composition,<sup>8,9</sup> and to measure total energy expenditure by the doubly labelled water method.<sup>10–12</sup>

Standards (100 ppm H<sub>2</sub>) were prepared by injecting 24 µL reference gas, 5% H<sub>2</sub> in He (Air Products, Special Gases, Crewe, UK), into 12 mL Exetainer gas-sampling vials (Labco, High Wycombe, UK) full of laboratory air using a gas-tight glass syringe. Blank tubes containing laboratory air were also prepared. Multiple replicates of the standard were prepared and analysed in triplicate using the CF-IRMS, weekly over a period of 3 months to determine the shelf-life of samples stored in Exetainers. A calibration curve was prepared with standards (in triplicate) containing 0, 5, 25, 50, 100, 150, and 200 ppm H<sub>2</sub> in air. The CF-IRMS (HYDRA, PDZ Europa, Crewe, UK) was tuned with *m/z* 2 in the deuterium collector (HT 4071 V), which is the high gain Faraday detector with head amplifier feedback resistance 100 GΩ, normally monitoring *m/z* 3 (HT 2715 V). All other settings were the same as for deuterium analysis (electron trap current 600 µA, electron energy –75 eV, ion repeller 32 V, electromagnet current 2.5 A, beam focus 90%) so that switching between modes was trivial and did not disturb instrument stability. The CF-IRMS has an autosampler, which can accommodate up to 200 Exetainer 12 mL gas-sampling vials. Standards and air blanks were analysed at intervals within each batch of samples.

A single healthy subject consumed a test meal containing non-digestible carbohydrate (oats) to compare analysis of breath hydrogen by the IRMS with our current method of breath hydrogen analysis using a breath hydrogen monitor (GMI Measurement Instruments Ltd.) with an electrochemical detector. Breath was sampled every 30 min for 12 h following consumption of the test meal.<sup>13</sup> Alveolar breath samples for analysis by the IRMS can be obtained by blowing

through a straw into an Exetainer, until condensation appears on the wall of the vial. This method is used for <sup>13</sup>C-breath tests,<sup>14</sup> but, in the current study, it was necessary to obtain identical samples for analysis by both the IRMS and breath hydrogen monitor. Therefore, exhaled breath was sampled by blowing into a 600 mL re-usable reservoir (Laerdal Medical, Orpington, UK) via a one-way valve (Ambu, Medicotest UK, St. Ives, UK). Breath samples for analysis on the IRMS were prepared by transferring breath into 12 mL evacuated Exetainers (in duplicate) via a three-way tap (Vygon, Cirencester, UK) using a 50 mL plastic syringe. Breath for analysis using the breath hydrogen monitor was transferred from the reservoir into a 20 mL syringe. The syringe was capped and set aside for analysis the next day. Samples were injected into the breath hydrogen monitor after zeroing on air and calibrating according to the manufacturer's instructions using a certified reference gas (96 ppm H<sub>2</sub>). The bias between methods was determined using the method of Bland and Altman.<sup>15</sup>

The electrochemical detector is zeroed on air; therefore, the readings from this instrument are ppm above the background. There was a substantial blank (50–55 ppm H<sub>2</sub>) when measuring H<sub>2</sub> concentration with the IRMS (Fig. 1). The source of this blank is unclear, but it is not present when pure helium is injected into the IRMS. The area (total beam) of the blank was subtracted from that of the standards before calculation of H<sub>2</sub> concentration using a two-point calibration (0 and 100 ppm H<sub>2</sub> above background) from standards analysed before and after those designated as samples. The standard deviation (SD) of three replicate injections was 3 ppm H<sub>2</sub>. The equation of the standard curve (Fig. 2) was determined by linear regression (gradient 0.96, intercept 0.06, R<sup>2</sup> = 0.996). When preparing samples or standards for breath hydrogen concentration analysis, it is important to use Exetainers with new caps to avoid losses. The shelf-life of samples in Exetainers was greater than 3 months compared with a few hours (as reported by clinical colleagues) for

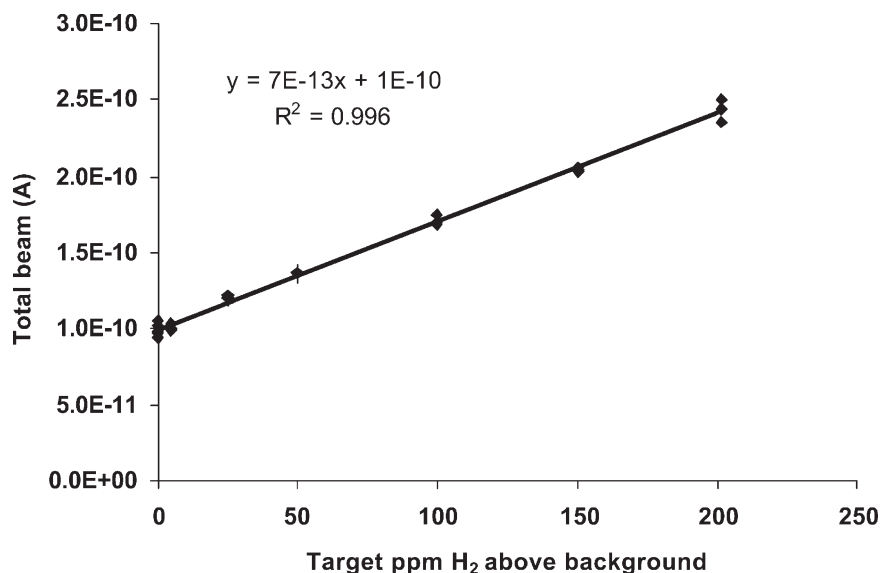


Figure 1. Breath hydrogen calibration curve measured by the IRMS.

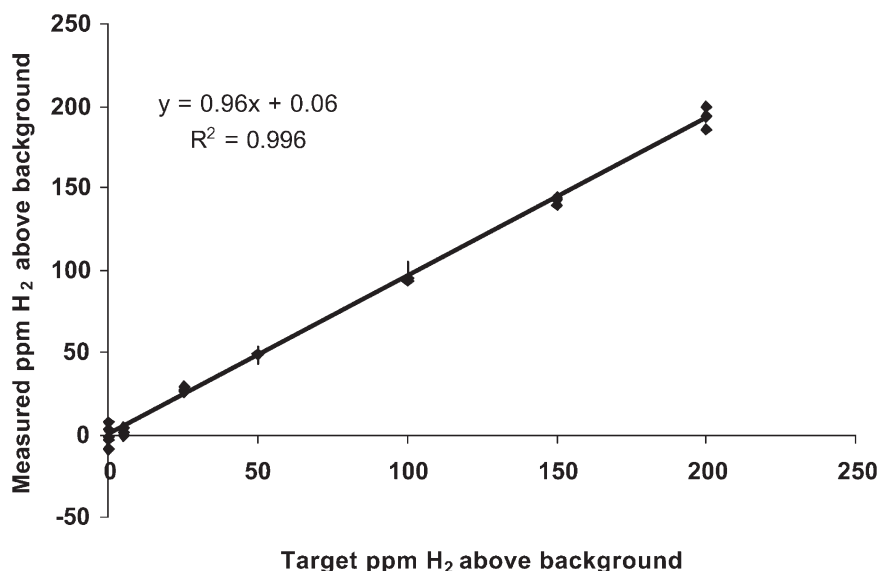


Figure 2. Breath hydrogen calibration curve after subtraction of the blank.

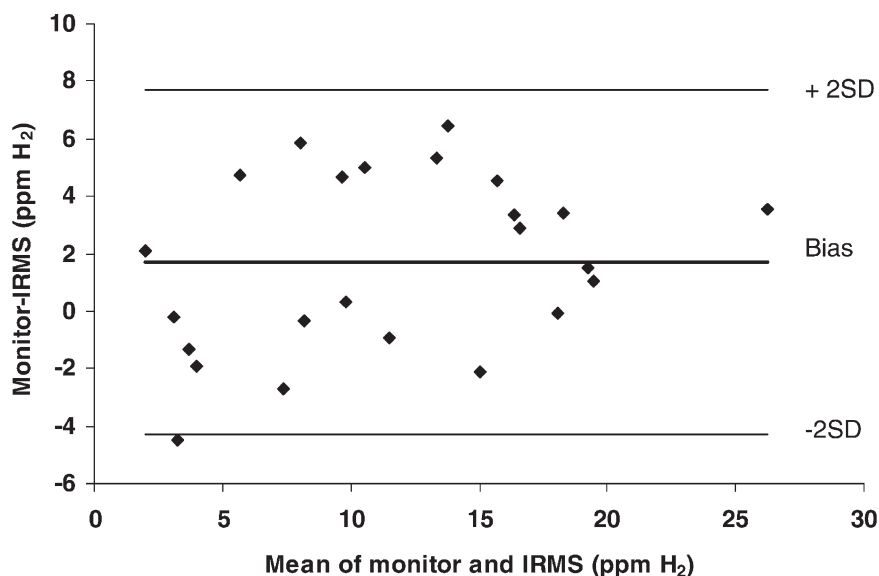


Figure 3. Residual plot of breath hydrogen concentration measured by the breath hydrogen monitor compared with the IRMS.

samples stored in plastic syringes. This characteristic enables samples to be analysed at a site remote from the experimental site. There was a bias of 1.69 ppm H<sub>2</sub> (95% CI 1.68, 1.70) between analysis by the IRMS and using the breath hydrogen monitor (Fig. 3). There was no evidence of proportional errors in the data. Therefore, absolute values of breath hydrogen concentration were used to calculate the bias and limits of agreement.<sup>15</sup> The limits of agreement (mean difference  $\pm$  2 SD) were  $-4.3$  to  $+7.7$ , which is similar to the agreement between one breath sample and the next using a commercially available hand-held breath hydrogen monitor (mean difference 0.5 ppm H<sub>2</sub>, 95% CI  $-7.3$  to  $+8.4$ ) over the range of 0–42 ppm H<sub>2</sub> in the same healthy subject using the same test meal. Therefore, this level of agreement is acceptable.

The CF-IRMS can be used for breath hydrogen analysis, automatically and with high sample throughput, and with no additional expense for laboratories set up to analyse deuterium enrichment in biological fluid samples.

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