# Accurate Identification of Excessive Methane Gas Producers is Possible by a Single Fasting Measurement of Methane

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## BACKGROUND

The production of methane by intestinal methanogens, has recently received increased attention. Breath methane levels show a good correlation with qPCR of methanogens in stool (1),(2) and are established as relevant in the diagnosis of small intestinal bacterial overgrowth (SIBO) (3). In addition, increased methane production by intestinal methanogens has also been associated with constipation (4), IBS-C (5), obesity and decreased weight loss after bariatric surgery (6),(7), multiple sclerosis (8), and other conditions (3). Breath methane is currently determined together with breath hydrogen using repeated collections of both gases after ingestion of a carbohydrate substrate, often lactulose, in 15 – 20 min intervals until 10 samples have been obtained. Frequent sampling is required to catch a rise of hydrogen emissions, which typically occurs at later time points during the test as the substrate is metabolized by bacteria. In contrast, breath methane levels are typically elevated at baseline and are less prone to change in response to substrate ingestion. If methane emissions are the principal reason for performing the breath test, as is increasingly the case, a spot methane breath test (i.e. a single-time point sample taken after an overnight fast without substrate administration) may be sufficient.

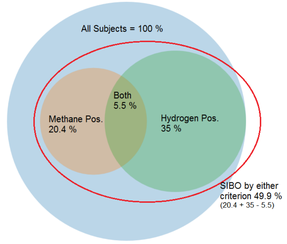
## AIMS

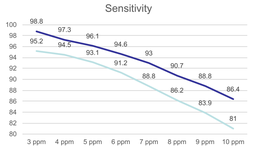
We examined the Commonwealth Laboratories (Salem, MA) nationwide database of consecutive multiple-sample, substrate-administering breath tests to see whether a single methane breath test at baseline is sufficient to classify subjects into low and high-methane emitters. Our results are compared to those from the single institution study of Rezaie et al. (Los Angeles Sample) (9). Our test relies on a different breath collection technique, but is generally comparable to the methodology employed by Rezaie et al. In contrast to the study by Rezaie et al., our samples were sent to a central laboratory for analysis from numerous sites from all 50 states (US Sample). We also examined our database for the influence of a widely-used correction factor that tries to correct for sample contamination with room air.

## METHODS

We identified 11,675 consecutive unique subjects who underwent breath testing for SIBO with lactulose as substrate by Commonwealth Laboratories from October 2014 to September 2015. De-identified patient level data were cleaned by excluding repeat tests from the same subjects. The North American Consensus criteria (10) were used for classification (any methane result ≥10 ppm during breath testing is 'methane-positive; a rise of ≥20 ppm of hydrogen by 90 minutes indicates Small Intestinal Bacterial Overgrowth [SIBO]). We then compared methane and hydrogen high and low producer classifications made using the raw data with 'normalized' data, obtained after multiplication with a correction factor (CF). The analyses were conducted with the following gas chromatographs: Agilent Technologies Gas Chromatograph 7890B (Agilent Technologies, Santa Clara, CA), Quintron Microlyzer SC (Milwaukee, WI), SRI 8610C (SRI Instruments, Torrance, CA), with 13.8%, 21.0% and 65.2% of the samples analyzed by the respective equipment. Depending on patient cooperation, collection method, and analysis work flow, collected air may not represent an alveolar sample. Air from the anatomical dead space or room air may enter the patient's breath sample. In 1979 Niu et al.(11) suggested a correction factor (CF) that normalizes the breath sample for the degree of departure from an alveolar sample. Briefly, normalized [$CH_4$] = observed [$CH_4$] × (5%/observed % $CO_2$). SAS 9.4 was used for the statistical analysis. Commonly used descriptive statistics (mean, SD, C.I., and S.E) were calculated. No hypothesis testing was performed. Data from Rezaie et al. were extracted from the American College of Gastroenterology 2015 meeting abstract (9).

## RESULTS



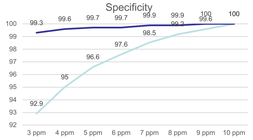




**Figure 1 (left)**: Using corrected results, only 5.5% of subjects are both positive for hydrogen and methane (based on the 10-sample lactulose breath test for SIBO) in the overall sample. Put differently, of those who test hydrogen-positive, 15.7% are also methane positive, and of those who test methane-positive, 27% are also hydrogen positive. Not shown in this figure: The use of a correction factor (5% CO2 as numerator) led to reclassifications Methane-High to Methane-Low in 0.7 % and Methane-Low to Methane-High in 2.1%.

**Figure 2 (left):** These graphs compare how sensitivity and specificity correlate with increasing cut-off values from 3 ppm to 10 ppm. The Los Angeles Sample is in dark blue, the US data set in light blue. Our data are similar to the single institution results of Rezaie et al. In our US Sample, employing the Commonwealth methodology, the optimal cut-off to maximize sensitivity and specificity was ≥4 ppm $CH_4$ (94.5% and 95.0%, respectively) with a minimal difference to the previously proposed ≥5 ppm as cut-off. The sensitivities of both studies follow a parallel course, decreasing with increasing cut-off values. Our US Sample shows the expected reversed course for specificity, i.e., increasing specificity with higher cut-off values, while the curve from the Los Angeles Sample is relatively flat, with higher reported specificities along the spectrum of examined cut-off values.

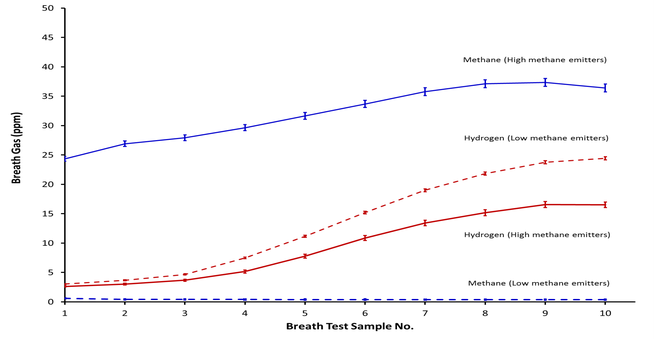



**Figure 3 (above):** X-axis – sample number, y-axis - ppm methane. This graph shows the time course of the average hydrogen production (blue) and methane production (red) in subjects who were either high or low methane producers based on the reference standard (North American Consensus) (mean ± Standard Error) over 10 samples spaced 20 minutes apart (from 1 to 10). Note that the hydrogen measurements of hydrogen-positive subjects that are also methane positive are significantly ($p \leq 0.05$) lower than the hydrogen measurements for the subjects who are hydrogen positive but methane negative.
From top to bottom: Solid blue line: Mean methane values for high-methane emitters. Dashed red line: Mean hydrogen values for low-methane emitters. Solid red line: Mean hydrogen values for high-methane emitters. Blue dashed line: Mean methane values for low-methane emitters. Error bar mean ± Standard Error.

## CONCLUSIONS

- A cut-off value for methane at baseline of either ≥4 ppm, as in our US Sample, or ≥5 ppm, as described in the Los Angeles Sample, are both highly accurate in identifying subjects at baseline that would have been diagnosed as 'methane-positive' in a 10-sample lactulose breath test for SIBO.
- The use of a carbon dioxide correction factor led to few reclassifications, but both raw and corrected data should be reported in research studies.
- A spot methane breath test performed after an overnight fast sensitively and specifically identifies high methane emitters: we propose a consensus of ≥5 ppm to separate patients into groups of high and low methane emitters and believe it to be well supported.

## REFERENCES

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