

Re: European guideline on indications, performance, and clinical impact of hydrogen and methane breath tests in adult and pediatric patients

Dear Dr Drenth,

We welcome the work of the authors in developing a well-considered clinical guideline aimed to improve consistency in the use of hydrogen (H₂) - methane (CH₄) breath testing.¹


Research we published in 2 January 2021,² which would not have been considered in the *European Guideline*, suggests that recommendation 1.6: "To reduce the risk of H₂ production from oral bacteria, the oral cavity should be rinsed with an antiseptic solution (e.g., chlorhexidine) immediately before the first (baseline) breath measurements are obtained" should be re-evaluated.

In our clinical study we administered a chlorhexidine mouthwash to people presenting for breath testing with basal H₂ or CH₄ concentrations of ≥ 10 ppm.² Data from 69 tests (from 64 participants), indicated that the mouthwash resulted in a substantial reduction in expired H₂ in 46 (66.7%), CH₄ in 64 (92.7%), and both gases in 45 (65.2%) cases. We repeated this protocol at 15–20 min intervals for up to 3 h of breath testing. In 65 tests (94.2%), post-mouthwash gases were consistently reduced, with mean area-under-the curve (AUC) for H₂ reduced by 20.2 ppm (SD 28.1) $p < 0.001$, CH₄ -36.2 ppm (SD 35.1) $p < 0.001$, and H₂+CH₄ -56.0 ppm (SD 40.8) $p < 0.001$.

In individuals with increased basal elevations prior to mouthwash, our analysis suggests that the administration of a single mouthwash immediately before commencing breath testing increases the risk of an apparent early increase in expired gases in the first post-substrate samples, leading to higher numbers of false positive tests. We saw this pattern in 30 out of 43 tests (69.7%), with first post-substrate increases which may have been interpreted as false-positive had breath testing been conducted with a single mouthwash at baseline.

We believe we are the first group to investigate the effects of oral hygiene (with chlorhexidine) within a clinical setting over the duration of breath testing. Our published research demonstrates that pre-test mouthwash oral hygiene may alter test results, with a single mouthwash at baseline resulting in a significant increase in false-positive results in a relatively high proportion of patients with high basal gas concentrations. Our findings have informed our current, larger scale study (ANZCTR #ACTRN12620001318976) that includes evaluating the possible role of the oral microbiome in the

production of exhaled hydrogen and methane gases. In the meantime, we recommend either no pre-test mouthwash or a mouthwash after **every** sample as the most effective way to avoid substantive false positive results.

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2. Erdrich S, Tan ECK, Hawrelak JA, Myers SP, Harnett JE. Hydrogen-methane breath testing results influenced by oral hygiene. *Sci Rep.* 2021;11. <https://doi.org/10.1038/s41598-020-79554-x>