

## Fecal Microbiome in Epidemiologic Studies—Letter

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We congratulate Sinha and colleagues on their recent report (1) comparing fecal sample collection methods for epidemiologic studies of the gut microbiome. These data contribute to the increasing body of literature describing robust methodologic frameworks for specimen collection and processing (2, 3). However, their claim that fixation of stool using RNAlater results in "considerable changes to the microbiome diversity" contrasts with previous findings (2, 3), including those from their earlier reports (4, 5). We have previously demonstrated that self-collected stool stabilized with RNAlater or other fixatives yields high fidelity and reproducibility in compositional profiling of DNA and RNA from shotgun sequence data, compared with immediately frozen specimens (3). In addition, fixation offers several distinct advantages crucial for large-scale population-based studies: a straightforward self-collection procedure; sample stabilization without deep-freezing during shipping, receiving, and processing; and versatility for multiple molecular analyses. The authors' finding that specimens preserved in RNAlater had poor correlation with immediately frozen specimens (1) could be explained, for example, by improper fixation resulting from an excess of specimen relative

to preservative volume (1–2 g:2.5 mL, compared with the manufacturer-recommended ratio of 1 g:5–10 mL; Thermo Fisher Scientific).

Several key issues must be addressed before fecal occult blood test (FOBT) cards should be considered the "most practical collection device for field studies," as the authors propose. First, while FOBT cards may have utility for 16S sequencing, can this method assure the stability of specimens for metatranscriptomic, metaproteomic, or metabolomic analyses that are likely to yield significantly richer biologic insights? Second, the dietary (e.g., abstinence from red meat) and medication (e.g., avoidance of NSAIDs) modifications required for FOBT collection within colorectal screening programs may impose undesirable limitations on gut microbiome studies. Finally, as participant self-collection is the most feasible method for cohort studies at scale, how will environmental variation, such as ambient humidity and temperature during desiccation, or time between specimen collection, mailing, and eventual processing affect unfixed sample stability? These factors play important roles in gut microbial characterization for human populations, but none were addressed in the current study. In addition, the rise in popularity of alternative noninvasive tools (e.g., fecal immunochemical testing) may threaten the long-term viability of guaiac FOBT cards for population-based colorectal screening. Therefore, despite their clinical track record and potential cost benefit, it is premature to recommend FOBT cards over more established and validated microbiome specimen collection protocols.

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## Disclosure of Potential Conflicts of Interest

C. Huttenhower is a consultant/advisory board member for Seres Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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