We respectfully submit the following response regarding the retraction of our manuscript, originally published in Gut Pathogens (2021), which investigated the presence and mutational profile of SARS-CoV-2 in patient fecal samples using enrichment next-generation sequencing (NGS):

1. Scientific Rationale and Relevance

This study was conducted in the early months of the COVID-19 pandemic to explore whether SARS-CoV-2 RNA could be detected and sequenced from fecal samples—a biological matrix not typically used for viral genomic surveillance at the time. Our work sought to expand understanding of viral shedding, persistence in the gastrointestinal tract, and the utility of NGS-based methods in detecting whole viral genomes. These questions remain relevant for understanding SARS-CoV-2 biology and its transmission dynamics.

2. Acknowledgment of Inappropriate Content

We recognize that the inclusion of therapeutic details—specifically references to hydroxychloroquine (HCQ), azithromycin, and high-dose vitamin regimens—was inappropriate within the context and scope of this manuscript. The study was not designed, powered, or controlled to assess treatment efficacy. Although we attempted to clearly state the observational nature of these cases, we now acknowledge that even speculative discussion of therapeutic outcomes—particularly involving controversial treatments—may have contributed to confusion or misinterpretation. In hindsight, these details should have been omitted or reserved for a separate, rigorously controlled clinical study.

3. Study Limitations and Intent

This was a small, exploratory study (n=14) intended to assess feasibility and generate hypotheses. We transparently reported sample size, timing of collection, and variability in clinical status. The study was not designed to make claims about infectivity or transmissibility, and we did not assert statistical significance. Rather, we highlighted the potential for NGS to complement existing diagnostics and to study viral genome variation in fecal material.

4. Disclosure and Ethical Compliance

The study was approved by an independent IRB (Advarra), and written informed consent was obtained from all participants. We disclosed that the study was conducted and funded by ProgenaBiome, and all authors' institutional affiliations were listed transparently. Nonetheless, we acknowledge that further clarity on potential conflicts of interest and the exploratory nature of the findings would have strengthened the manuscript.

5. Contributions and Commitment to Scientific Integrity

To our knowledge, this study was among the first to demonstrate full-genome sequencing of SARS-CoV-2 from stool samples, revealing variant-level detail that may inform future virological and epidemiological research. While we regret the inclusion of extraneous clinical treatment discussion, we believe the sequencing methodology and findings are scientifically valid and potentially valuable. We remain committed to scientific rigor and transparency, and we welcome further research to validate and build upon these observations.

6. Support from Subsequent Research

Importantly, subsequent studies have reinforced the clinical and epidemiological value of detecting SARS-CoV-2 in fecal samples. On April 9, 2020, the FDA introduced requirements for screening fecal microbiota transplant (FMT) donor material for SARS-CoV-2 to ensure patient safety. Wastewater surveillance has become a globally accepted strategy for monitoring SARS-CoV-2 prevalence and variants in communities, and multiple studies have demonstrated prolonged viral RNA shedding in stool—even after respiratory clearance. These findings corroborate the original motivation of our study and affirm that the gastrointestinal tract plays a role in SARS-CoV-2 dynamics that merits continued investigation.

7. Response to Journal Editors

The retraction notice (https://gutpathogens.biomedcentral.com/articles/10.1186/s13099-025-00711-6) states that "None of the authors have responded to any correspondence from the editor about this retraction.". This is inaccurate. There was considerable and timely dialog between the authors and the editors from Springer Nature that included our response to each of the editor's questions. A copy of this email correspondence is included below.

From: Andreas Papoutsis [redacted]>
Sent: Friday, June 30, 2023 11:31 AM
To: William Shadbolt <[redacted]>; Dr. Sabine Hazan <[redacted]>
Subject: Re: Responses: Gut Pathogens, Detection of SARS-CoV-2 from patient fecal samples by whole genome sequencing

Hi William,

These are the collective responses to the specific queries that you requested.

Thank you,

Andreas

Get Outlook for iOS

From: William Shadbolt <[redacted]>
Sent: Friday, June 30, 2023 10:40:11 AM
To: Andreas Papoutsis <[redacted]>; Dr. Sabine Hazan <[redacted]>
Subject: RE: Responses: Gut Pathogens, Detection of SARS-CoV-2 from patient fecal samples by whole genome sequencing

Dear Andreas,

Thank you very much for your response. Please could you let me know if the plan is still for your co-authors to answer individually as mentioned by Dr. Hazan in her message from June 6th, or if the below is the collective author response?

If you have any questions or concerns, please do not hesitate to contact me.

Thank you,

William Shadbolt

Publisher

Springer Nature

One New York Plaza, Suite 4600, NY, NY 10004-1562

Phone: +[redacted]

Email: [redacted]

From: Andreas Papoutsis <[redacted]>
Sent: Thursday, June 29, 2023 6:22 PM
To: William Shadbolt <[redacted]>; Dr. Sabine Hazan <[redacted]>

Subject: Responses: Gut Pathogens, Detection of SARS-CoV-2 from patient fecal samples by whole genome sequencing

Hi William, Please find: Responses to Reviewers and Publisher, William Shadbolt Clinical Trial Inclusion criteria 3: "Diagnosis of COVID-19 infection by RT- PCR within 1 week of Screening" Paper: "Of the 14 study participants,

Hi William,

Please find:

Responses to Reviewers and Publisher, William Shadbolt

Clinical Trial

Inclusion criteria 3: "Diagnosis of COVID-19 infection by RT- PCR within 1 week of Screening"

Paper: "Of the 14 study participants, ten were symptomatic and tested positive for SARS-CoV-2 by RT-PCR, two asymptomatic individuals tested negative, and two other asymptomatic individuals did not undergo RT-PCR testing"

It seems that 4/14 (28%) of the participants did not meet the definition for inclusion in the study but were included in the paper's results.

Why were these 4 participants included in the paper's results?

These individuals volunteered to participate in the study and were decided to be included/utilized as negative controls. As the validity of covid PCR testing was not clear early on in the pandemic, (eg initial reporting ~80% accuracy or less) we were interested in assessing if negative RT-PCR patient results would be concordant with NGS testing, which they were. The two volunteers that did not undergo RT-PCR testing were included to simply present information that these asymptomatic individuals did not harbor SARS-CoV-2.

Importantly, these data do not alter nor confound the results/findings of the paper, and although do not fall exactly within the defined inclusion criteria, this information (at the time) was deemed valuable to advancing the understanding of SARS-CoV-2 and covid.

Clinical Trial

"Inclusion criteria 3: "Diagnosis of COVID-19 infection by RT- PCR within 1 week of Screening"

Paper: "While all patients were asked to collect stools at baseline, the samples were collected from 2 to 38 days after RT-PCR testing."

It seems that some number of participants (6/14 (43%) by my count) had samples collected outside the scope of time identified for inclusion in the trial.

Why were these 6 participants included in the paper's results?

Initially we thought a target of 1 week would best capture concordance of RT-PCR positive samples and NGS. However, we soon learned that this target should be expanded based on scholarly publications that highlighted that covid could be detected in stools for at least 4 weeks following infection. Today we now know that in some

people, covid can be detected for this duration, and is also supported in our data set wherein SARS-CoV-2 was detected by NGS in stools from positive nasopharyngeal swab analysis up to 6 weeks later in the most extreme case. The reviewer's notice of discrepancy here warrants an update to the “Inclusion Criteria 3” timeline if so decided/deemed necessary.

Clinical Trial

Expected Study Completion: 2025; Expected Enrollment 250

Paper: Submitted to journal 2 months after trial inception

Why is the trial still recruiting if the results have already been reported?

Continued recruitment of the trial is important to further characterize the various strains of SARS-CoV-2 that have emerged and may continue to emerge to track its evolutionary progression through the population. This may help to better understand the impact of specific mutations that may alter its viability, transmissibility, and contagion.

What explains the large difference between the expected enrollment and the actual?

As mentioned, we were in the midst of an unprecedented pandemic and rushed to share results in efforts to provide valuable information to scientific/medical communities. Early on it was thought that SARS-CoV-2 was a very slow mutating virus (eg like most corona viruses), yet our early data suggested that possibly that was not the case, wherein we identified 33 unique mutations among stool samples in a relatively short time frame (eg less than 2 months).

How were participants recruited?

Patients learnt about the study via various PR outlets and general word of mouth. There was a lot of interest in helping advance information during the early days of the pandemic, and enrollment in the trial was an easy and important contribution that could be made towards these efforts.

We are hopeful and confident that the above responses will satisfy the reviewer and publisher queries.

We look forward to hearing from you.

Thanks,

Andreas