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Misinterpretation of Study Data

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In Reply

We thank Power and colleagues for their comments regarding our recent article¹ on metagenomic next-generation sequencing (mNGS). In our article, we expressed concern that their recent reports of a brain microbiome may be confounded by environmental microbial–contaminating sequences in their mNGS data sets.^{2,3}

We make no conclusions about the source of the potential contaminating sequences in Power and colleagues' mNGS data. Environmental contaminants may arise from molecular biological reagents,^{4,5} the laboratory where the sample was acquired, its storage container, and/or from laboratory surfaces and personnel present where the mNGS samples were prepared.

We did not contend the authors performed mNGS on RNA from cerebrospinal fluid. We stated in eFigure 2D in the article's Supplement that we analyzed “publicly available data from...brain specimens” and labeled the figure panel accordingly. In addition, we compared the authors' data with (“no-template”) water controls because cerebrospinal fluid is not an appropriate comparator for a study that asserts that there is a central nervous system microbiome.

The RNA doping experiment showed that for very low input (ie, picogram) nucleic acid samples, adding the RNA of a known sequence substantially reduces (but does not eliminate) the relative abundance of contaminating sequences. This merely establishes the relative mass of reagent contaminants, not that contaminants are absent from high-input nucleic acid samples, including brain tissue.

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Conflict of Interest:

No other disclosures are reported.

We commend the authors for discarding obviously contaminated reagents. However, mNGS reveals the presence of nucleic acid species at levels that are undetectable by conventional spectroscopic methods. Thus, it is essential to perform mNGS on no-template controls regardless of whether nucleic acid can be detected postextraction.

Power et al state that they subtracted “any nonhuman hits that were common across unconnected libraries in a manner similar to [our] background subtraction approach.” We did not subtract common sequences, but rather their rank was lowered in our *z* score–based algorithm to indicate that the organism’s level of abundance was typical for controls.

The variance in the relative abundance of the 4 bacterial classes that were identified in our water controls was generally wider than in the brain mNGS data sets from Power et al. Even within batched mNGS data sets, wide sample-to-sample variation is common because of sample-to-sample variability in contamination levels that arise from nonreagent sources, sample-to-sample variation in input amounts of nucleic acid (see RNA doping experiments), and differences in sample handling during mNGS library preparation.

We commend Power et al for detecting bacterial RNA by in situ hybridization and assessing for bacterial protein immuno-reactivity. However, the former experiments are subject to some of the same potential pitfalls as mNGS, and the latter were not specific for the variety of bacterial taxa on which the claim of a multiple sclerosis brain dysbiosis was made. Given the overlap between the microbiota of laboratory-grade reagents generated by our mNGS protocol with the microbiota identified by Power et al in autopsy brain tissue, more experiments are required to provide definitive evidence that the organisms reported by Power and colleagues truly represent resident brain microbiota.

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References

1. Wilson MR, O’Donovan BD, Gelfand JM, et al. Chronic meningitis investigated via metagenomic next-generation sequencing. *JAMA Neurol.* 2018;75(8):947–955. 10.1001/jamaneurol.2018.0463 [PubMed: 29710329]
2. Branton WG, Ellestad KK, Maingat F, et al. Brain microbial populations in HIV/AIDS: α -proteobacteria predominate independent of host immune status. *PLoS One.* 2013;8(1):e54673 10.1371/journal.pone.0054673 [PubMed: 23355888]
3. Branton WG, Lu JQ, Surette MG, et al. Brain microbiota disruption within inflammatory demyelinating lesions in multiple sclerosis. *Sci Rep.* 2016;6:37344 10.1038/srep37344 [PubMed: 27892518]
4. de Goffau MC, Lager S, Salter SJ, et al. Recognizing the reagent microbiome. *Nat Microbiol.* 2018;3(8):851–853. 10.1038/s41564-018-0202-y [PubMed: 30046175]
5. Naccache SN, Greninger AL, Lee D, et al. The perils of pathogen discovery: origin of a novel parvovirus-like hybrid genome traced to nucleic acid extraction spin columns. *J Virol.* 2013;87(22):11966–11977. 10.1128/JVI.02323-13 [PubMed: 24027301]