

Supplementary Online Content: Supplement 2

Wilson MR, O'Donovan BD, Gelfand JM, et al. Chronic meningitis investigated via metagenomic next-generation sequencing. *JAMA Neurol*. Published online April 16, 2018. doi:10.1001/jamaneurol.2018.0463

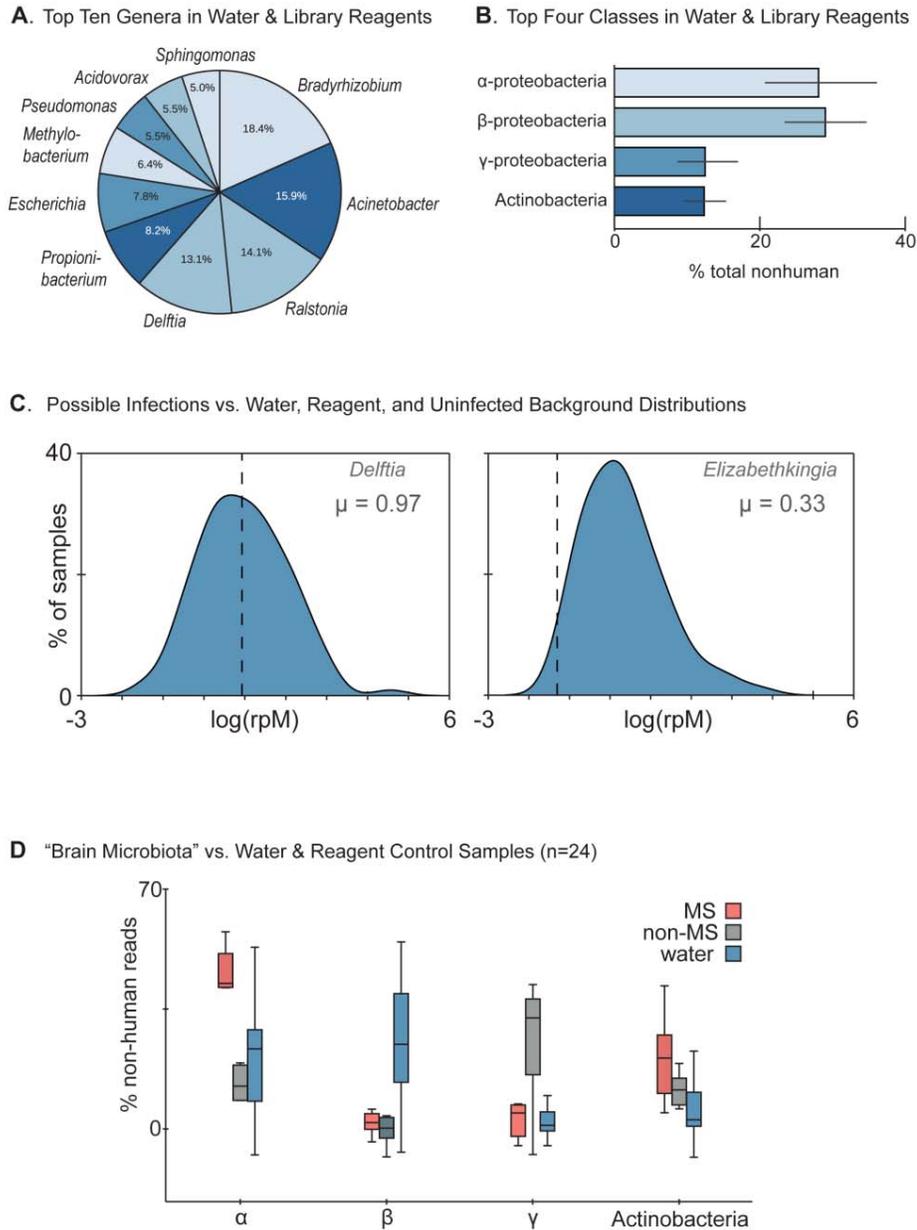
eFigure 1. Background Signature of Reagent and Environmental Contaminants

eFigure 2. RNA Doping Experiment

eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.

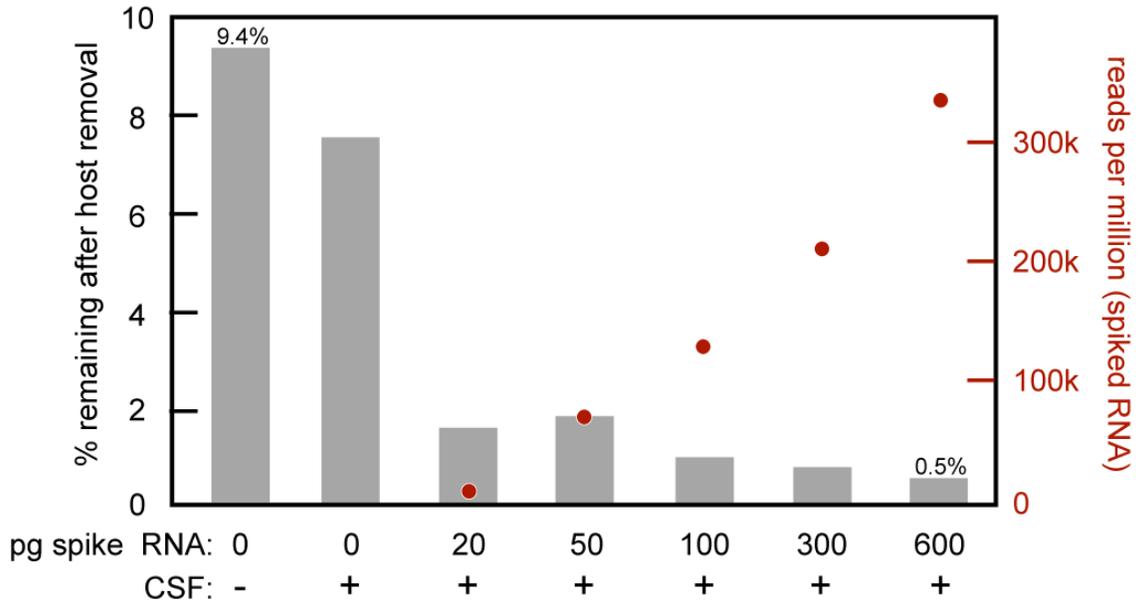
eFigure 1. Background Signature of Reagent and Environmental Contaminants



A, The 10 most abundant genera identified water-only and reagent controls (n=24). 10 bacterial taxa account for ~50% of all non-human (viral, bacterial, fungal, and selected eukaryotes) sequences in the control data. B, Organized at the class-level, four classes of bacteria (α-proteobacteria, β-proteobacteria, γ-

proteobacteria, Actinobacteria) represent >80% of the sequences in the water-only and reagent controls. C, The proportion of *Delftia acidovorans* sequences (dotted line at 0.93) in a reported case of possible CNS infection identified by mNGS¹ is concordant with the expected mean of our control dataset (0.97). Similarly, the proportion of *Elizabethkingia* sequences (dotted line) in another possible infectious case from the same report is significantly lower than the mean abundance in our control dataset. D, Comparison of the observed variance of the four classes of bacteria (B) to publicly available data from a recent report² on microbiota in healthy and multiple sclerosis patient brain specimens suggests that the relative abundances of each taxa in the brain specimens are within the observed variance of our background dataset.

eFigure 2. RNA Doping Experiment



Comparison of the percent non-human sequences (y-axis) found in water (column 1) and a cerebrospinal fluid (CSF) control (column 2) and the decrease in the percent non-human sequences found with increasing amounts of spiked RNA of a known identity (columns 3-7). Added RNA was generated by T7 *in vitro* transcription from a cloned luciferase reporter gene, purified, quantified, and spiked into the CSF at the indicated amounts. These data suggest that common environmental contaminants are present at low picogram quantities, and the addition of only 20 pg is sufficient to suppress the majority of reads not derived from the CSF.

eReferences

1. Salzberg SL, Breitwieser FP, Kumar A, et al. Next-generation sequencing in neuropathologic diagnosis of infections of the nervous system. *Neurol Neuroimmunol Neuroinflamm*. 2016;3(4):e251.

2. Branton WG, Lu JQ, Surette MG, et al. Brain microbiota disruption within inflammatory demyelinating lesions in multiple sclerosis. *Sci Rep*. 2016;6:37344.