

Supplementary Figure 1. Assessing trace microbial signals obtained from in a subset of spike-in cyst fluid samples. (Left panel) Higher abundant signals. Two samples showed absolute abundances higher than blank controls post-*DECONTAM*, including *Klebsiella sp.* and *Citrobacter sp.* (confirmed bacterial growth in culture). **(Right panel) Lower abundant signals.** Assessing microbial trace amounts otherwise undetectable by wet-lab devices. In a subset of samples, unique bacterial ASVs were detected. All samples were cultured, bacterial growth was not observed after 48 hours.

Supplementary Table 1. Anonymized metadata table with all cyst fluid samples from both experimental runs (with spike-ins and without), along with raw read counts or absolute abundance estimates for all ASVs in cyst fluid samples from the first or second experimental runs, respectively. Taxonomic annotations (column "GTDB_SILVA_NCBI") were verified using all three databases. In cases of mismatches, the annotation with the highest confidence overlap was selected. The column "isCONTAM" reveals potential ASV contamination based on predictions by *DECONTAM* (TRUE-*DECONTAM*), their presence in negative controls (TRUE-BLANK), the absence of bacterial growth in culture (TRUE-noGROWTH), inconsistency across technical replicates (TRUE-Splashome), or positive correlation with previously predicted ASV contaminants (TRUE-CORR).

Supplementary Table 2. Overview of ASVs, annotation, prevalence and predicted contamination class based on predictions by *DECONTAM* (TRUE-*DECONTAM*), their presence in negative controls (TRUE-BLANK), the absence of bacterial growth in culture (TRUE-noGROWTH), inconsistency across technical replicates (TRUE-Splashome), or positive correlation with previously predicted ASV contaminants (TRUE-CORR).