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Deciphering the Impact of Contaminating Microflora in DNA Extraction Reagents on mNGS Workflows

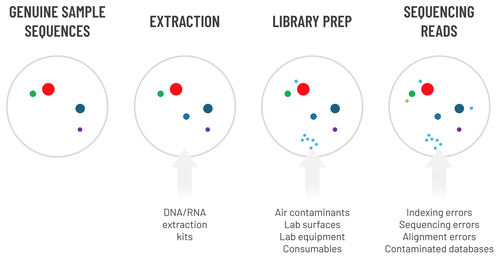
CONCLUSION

1. Background microflora is predominantly contributed by contaminating microorganisms in extraction reagents.
2. They are largely reagent-lot specific, suggesting that manufacturers should provide background microflora data on a per lot basis.
3. Similar to other studies[2], blood from healthy individuals were not associated with a common "microbiome".
4. Novel biocomputational tools[3], together with disciplined use of appropriate (extraction) controls, will be able to account for background microflora from extraction reagents, as well as signals from spurious environmental contamination events.

References
[1]Juraszet al Frontiers in Microbiology 2021 v12: 745076 [2] Tan et al Nature Microbiol 2023 [3] Davis et al. Microbiome (2018)6:226

INTRODUCTION

- The advent of Next-generation sequencing (NGS) have led to an explosion of shotgun metagenomic NGS (mNGS) studies.
- However, shotgun mNGS also detects nucleic acids from contaminants, which can confound the interpretation of microbiome data.
- Such contamination effects are common, as several studies have found contaminant microbial DNA in laboratory reagents, surfaces and environment[1].
- There is no established criterion for identifying or excluding contaminants. mNGS reagent manufacturers do not guarantee the absence of contaminating DNA in their products[1].
- The objective of this study is to:
  a. profile the background microflora in common DNA extraction reagents used for mNGS, and
  b. to understand the reproducibility of this background between manufacturing lots and replicate runs.



METHODS

- Extraction blanks were generated from 4 brands of DNA extraction reagents: Micronbrane (M), and other brands Q, R and Z.
- Extraction protocols were executed using either:
  a. molecular-grade (DNA-free) water (MBG), or
  b. ZymoBIOMICS Spike-in Control Particles D6320 (SICP) as input.
- To assess batch-to-batch variability, 3 lots were tested for M and Q. All tests were conducted in triplicates.
- All resultant eluates were subjected to library preparation using Unison Library Preparation Kit (Micronbrane) and sequenced on MiSeq (Illumina). NGS data was interpreted using in-house PaRTI-Cular software.

Table 1. Experimental plan and study design.

Brand of Extraction Kit	Lot	Molecular Biology Grade Water (MBG) input		ZymoBIOMICS Spike-In Control D6320 (SICP) Input	
		Denotation	Triplicates	Denotation	Triplicates
M (Micronbrane)	1	MA-MBG	MEK-01-MBG1-MO1019 MEK-01-MBG2-MO1019 MEK-01-MBG3-MO1019	–	–
	2	MB-MBG	MEK-01-MBG1-1109211 MEK-01-MBG2-1109211 MEK-01-MBG3-1109211	–	–
	3	MD-MBG	MEK-01-MBG1 MEK-01-MBG2 MEK-01-MBG3	MEK-01-SICP	MEK-01-SICP1 MEK-01-SICP2 MEK-01-SICP3
Q	1	Q-MBG	Q-MBG1 Q-MBG2 Q-MBG3	Q-SICP	Q-SICP1 Q-SICP2 Q-SICP3
	2	Q-15-MBG	Q-15-MBG1 Q-15-MBG2 Q-15-MBG3	–	–
	3	Q-19-MBG	Q-19-MBG1 Q-19-MBG2 Q-19-MBG3	–	–
R	1	R-MBG	R-MBG1 R-MBG2 R-MBG3	R-SICP	R-SICP1 R-SICP2 R-SICP3
Z	1	Z-MBG	Z-MBG1 Z-MBG2 Z-MBG3	Z-SICP	Z-SICP1 Z-SICP2 Z-SICP3

RESULTS

1. Top 20 ranked species were compared among various group of test results. Heatmap and PCA were generated from microbial_reads% of detected microbial targets from each sample.
2. Comparison of heatmap and PCA ***(Fig. 2)*** between various brands suggested that:
   a. Background microflora are different between brands.
   b. MBG and SICP have similar profiles.
   c. Spurious microbial calls may be attributed to random environmental contamination events.
3. All 3 different lots of M and Q exhibited different background microflora, suggesting that background may be largely lot-specific ***(Fig 3 and 4)***.
4. Heatmap was plotted using sequence data from 30 SICP controls done in this lab, was compared with SICP data generated at the manufacturer's (Micronbrane) site. All samples were from the same extraction lot ***(Fig 5)***.
5. Many contaminants were consistently detected. Interestingly, some environmental contaminants may be peculiar to study site.
6. Heatmap was plotted using sequence data from blood samples from 10 healthy controls. These were compared with data generated from SICP controls ***(Fig 6)***.
7. Profiles from blood of healthy individuals were not distinguishable from those from SICP samples, suggesting that a consistent "microflora" cannot be detected in healthy blood.

FIGURE 2-4

Fig 2. Comparison between brands M (MEK01), Q, R and Z using (a) heatmap and (b) PCA. Input were either MBG or SICP.

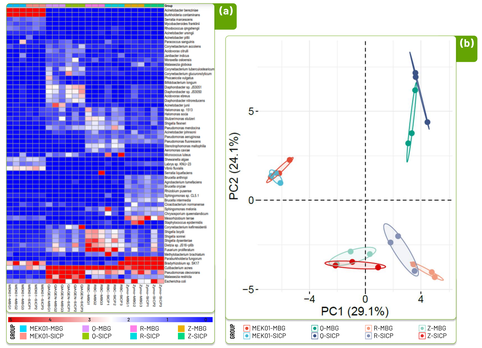


Fig 3. Comparison between 3 lots of M (MA, MB, MC) using. (a) heatmap and (b) PCA. Input was MBG.

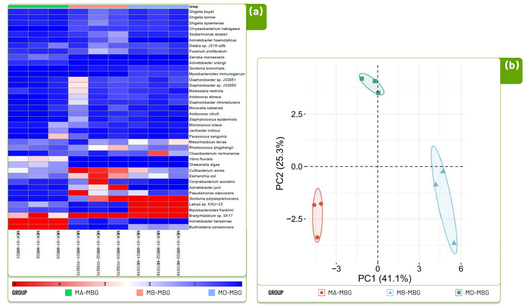


Fig 4. Comparison between 3 lots of Q (Q-MBG, Q-15-MBG, Q-19-MBG) using (a) heatmap and (b) PCA. Input was MBG.

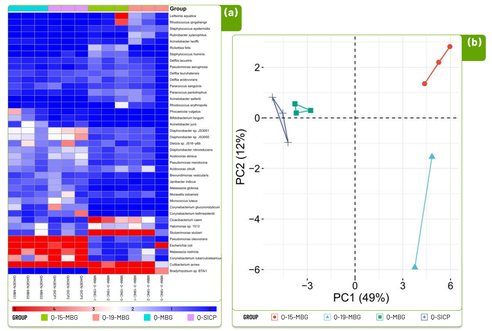
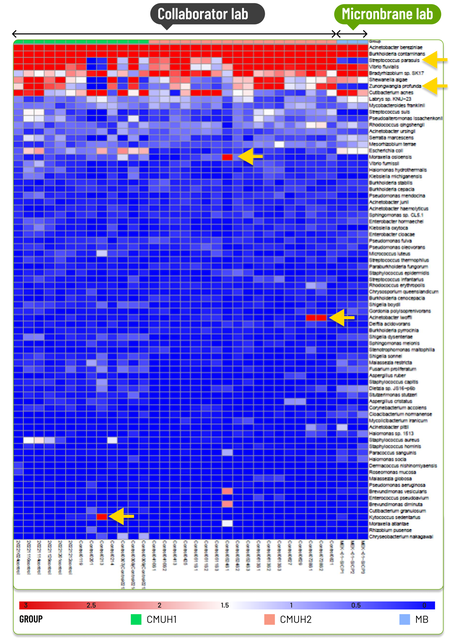


Fig 5. Comparison between SICP done at two locations (this lab and manufacturer's lab). Contaminants peculiar to this study site are marked with yellow pointers (←) in the heatmap.



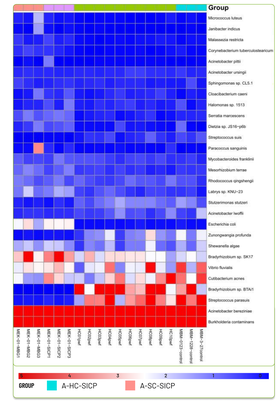


Fig 6. Comparison between profiles generated from healthy blood and SICP controls.

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