

1 **Title:** Human faecal collection methods demonstrate a bias in microbiome composition by
2 cell wall structure.

3

4 **Authors:**

5 Emma-Jane Watson¹, Jennifer Giles¹, Benjamin L. Scherer¹ and Dr Paul Blatchford^{2,*}.

6

7 ¹ CSIRO Health and Biosecurity, Gate 13 Kintore Avenue, Adelaide, South Australia 5000,
8 Australia

9 ² Zespri International Limited, 400 Maunganui Road, Mt Maunganui 3149, New Zealand.

10 * paul.blatchford@zespri.com

11

	Mean Bacterial Abundance (%) ±SEM		Gram Status	Percent Difference	P- Value
	Fresh frozen	Collection tube			
<i>Bacteroides</i>	4.66 ± 0.65	17.48 ± 1.25	Negative	-12.82	<0.001*
<i>Prevotella</i>	1.37 ± 0.61	7.66 ± 1.81	Negative	-6.29	0.192
<i>Alistipes</i>	0.69 ± 0.08	2.61 ± 0.26	Negative	-1.92	<0.001*
<i>Phascolarctobacterium</i>	0.28 ± 0.06	1.53 ± 0.3	Negative	-1.25	0.520
<i>Barnesiella</i>	0.31 ± 0.07	1.27 ± 0.21	Negative	-0.96	<0.010*
<i>Oscillibacter</i>	0.43 ± 0.06	1.22 ± 0.11	Negative	-0.79	<0.001*
<i>Parabacteroides</i>	0.19 ± 0.03	0.96 ± 0.11	Negative	-0.77	<0.001*
<i>Sutterella</i>	0.04 ± 0.02	0.5 ± 0.11	Negative	-0.46	0.048*
<i>Odoribacter</i>	0.06 ± 0.01	0.45 ± 0.03	Negative	-0.39	<0.001*
<i>Faecalibacterium</i> †	6.02 ± 0.46	6.34 ± 0.32	Positive	-0.32	1.000
<i>Parasutterella</i>	0.04 ± 0.01	0.34 ± 0.06	Negative	-0.3	<0.001*
<i>Paraprevotella</i>	0.04 ± 0.02	0.29 ± 0.11	Negative	-0.25	1.000
<i>Coprobacter</i>	0.02 ± 0	0.25 ± 0.05	Negative	-0.23	<0.001*
<i>Cerasicoccus</i>	0.01 ± 1	0.19 ± 0.06	Negative	-0.18	0.280
<i>Butyrivibrio</i>	0.28 ± 0.16	0.43 ± 0.24	Negative	-0.15	0.420
<i>Haemophilus</i>	0.05 ± 0.02	0.17 ± 0.05	Negative	-0.12	0.006*

<i>Acidaminococcus</i>	0.05 ± 0.03	0.15 ± 0.06	Negative	-0.1	0.280
<i>Desulfovibrio</i>	0.03 ± 0.01	0.12 ± 0.04	Negative	-0.09	0.680
<i>Megasphaera</i>	0 ± 0	0.09 ± 0.06	Negative	-0.09	0.400
<i>Victivallis</i>	0 ± 0	0.09 ± 0.03	Negative	-0.09	<0.001*
<i>Clostridium IV</i>	0.67 ± 0.17	0.76 ± 0.13	Variable	-0.09	0.680
<i>Clostridium XIVb†</i>	0.21 ± 0.03	0.29 ± 0.04	Positive	-0.08	0.052
<i>Methanomassiliicoccus†</i>	0 ± 0	0.06 ± 0.04	Positive	-0.06	0.520
<i>Cloacibacillus</i>	0 ± 0	0.04 ± 0.03	Negative	-0.04	0.660
<i>Megamonas</i>	0.06 ± 0.05	0.09 ± 0.06	Negative	-0.03	0.110
<i>Succiniclasicum</i>	0.07 ± 0.05	0.1 ± 0.07	Negative	-0.03	1.000
<i>Akkermansia†</i>	0.9 ± 0.19	0.87 ± 0.17	Negative	0.03	1.000
<i>Enterococcus</i>	0.05 ± 0.03	0.02 ± 0.01	Positive	0.03	1.000
<i>Lactobacillus</i>	0.23 ± 0.11	0.16 ± 0.09	Positive	0.07	0.003*
<i>Faecalitalea</i>	0.19 ± 0.09	0.11 ± 0.06	Positive	0.08	0.660
<i>Methanosphaera</i>	0.13 ± 0.09	0.05 ± 0.03	Positive	0.08	0.600
<i>Slackia</i>	0.24 ± 0.06	0.14 ± 0.04	Positive	0.1	0.400
<i>Dialister†</i>	0.8 ± 0.16	0.66 ± 0.13	Negative	0.14	0.880
<i>Intestinibacter</i>	0.5 ± 0.1	0.33 ± 0.08	Positive	0.17	0.380
<i>Senegalimassilia</i>	0.42 ± 0.09	0.18 ± 0.05	Positive	0.24	0.600
<i>Holdemanella</i>	0.71 ± 0.19	0.42 ± 0.16	Positive	0.29	1.000
<i>Butyricicoccus</i>	0.89 ± 0.14	0.54 ± 0.06	Positive	0.35	0.360
<i>Terrisporobacter</i>	0.68 ± 0.18	0.3 ± 0.1	Positive	0.38	0.360
<i>Streptococcus</i>	0.78 ± 0.13	0.34 ± 0.07	Positive	0.44	0.002*
<i>Teuricibacter</i>	0.64 ± 0.22	0.2 ± 0.08	Positive	0.44	0.150
<i>Coprococcus</i>	3.07 ± 0.24	2.61 ± 0.22	Positive	0.46	0.240
<i>Gemmiger†</i>	2.16 ± 0.22	1.54 ± 0.18	Negative	0.62	0.086
<i>Ruminococcus_L</i>	2.18 ± 0.24	2.05 ± 0.22	Positive	0.76	0.30*
<i>Lachnospiracea_is</i>	4.45 ± 0.26	3.43 ± 0.2	Positive	1.02	0.019*
<i>Roseburia</i>	6.32 ± 0.72	5.25 ± 0.51	Positive	1.07	1.000
<i>Fusicatenibacter</i>	3.59 ± 0.4	2.47 ± 0.28	Positive	1.12	0.118
<i>Anaerostipes</i>	3.4 ± 0.35	2.16 ± 0.23	Positive	1.24	0.009*
<i>Methanobrevibacter</i>	1.91 ± 0.52	0.58 ± 0.14	Positive	1.33	1.000
<i>Clostridium_ss</i>	2.36 ± 0.51	0.98 ± 0.2	Positive	1.38	0.066
<i>Dorea</i>	3.18 ± 0.27	1.8 ± 0.15	Positive	1.38	<0.001*
<i>Romboutsia</i>	2.49 ± 0.48	1.07 ± 0.21	Positive	1.42	0.005*
<i>Bifidobacterium</i>	3.72 ± 0.69	2.06 ± 0.42	Positive	1.66	0.076
<i>Ruminococcus_R</i>	6.3 ± 0.5	3.36 ± 0.38	Positive	2.94	<0.001*
<i>Collinsella</i>	5.98 ± 0.82	2.95 ± 0.48	Positive	3.03	0.010*
<i>Blautia</i>	16.05 ± 0.98	6.85 ± 0.45	Positive	9.2	<0.001*

12

13 **Supplementary Table 1: Microbial relative abundance (%) at genus-level.**

14 *Fifty-five bacterial genera in fresh frozen samples compared to DNA collection tube samples*

15 *with associated **Gram** stain classification. There are six exceptions to the cellular wall*

- 16 *structure trend, denoted by τ . Significant differences between methods were determined with the*
- 17 *Wilcoxon Signed Rank test after False Discovery Rate (FDR) correction ($P^* < 0.050$).*
- 18 *Ruminococcus R – Ruminococcus within the Ruminococcaceae family; Ruminococcus L –*
- 19 *Ruminococcus within the Lachnospiraceae family; Lachnospiraceae IS – Lachnospiraceae incertae*
- 20 *sedis.*