Sample Method	Sequence Depth (Gbp)	Total Reads (million)	Bacterial Reads (%)
Biopsy	2.04±0.47	14.5±7.2	26.5±11.5
Cytology Brush	4.22±3.03	41.7±31.9	59.1±23.9*

Mean number ± SD of gigabase pairs sequenced (Gbp), sequenced reads (in millions) after removal of poor quality reads, and bacterial percentage of reads following metatransriptomics sequencing comparing pinch biopsy and cytology brush methods. N=5 samples per group. *, P<0.05 as determined by Student's t-test comparing biopsy to cytology brush.

Class	Mean ± SEM (%)		Dualua	
Class	Biopsy	Cytology Brush	r value	
Methanobacteria	1.08 ± 2.42	1.66 ± 3.71	0.778	
Actinobacteria	56.11 ± 21.16	22.26 ± 14.88	0.019	
Bacteroidia	2.21 ± 4.95	11.58 ± 14.89	0.219	
Bacilli	29.35 ± 5.43	10.82 ± 11.33	0.011	
Clostridia	4.11 ± 7.32	47.71 ± 10.84	0.00007*	
Negativicutes	0.80 ± 1.78	1.28 ± 2.85	0.757	
Betaproteobacteria	0.03 ± 0.05	1.28 ± 2.55	0.306	
Deltaproteobacteria	0.00 ± 0.00	0.65 ± 1.45	0.346	
Epsilonproteobacteria	0.00 ± 0.00	0.07 ± 0.16	0.346	
Gammaproteobacteria	6.31 ± 7.00	2.68 ± 2.32	0.304	

Top 10 bacterial classes identified as different between the two collection methods. Data shown as mean \pm SEM. Three classes noted to be significantly different with p-value <0.05 and one with FDR <0.05 as indicated by the asterisk.

Species	Mean ±	Divolue	
species	Biopsy	Cytology Brush	P-value
Faecalibacterium prausnitzii	2.27 ± 3.36	17.37 ± 11.62	0.023
Finegoldia magna	0.00 ± 0.00	12.49 ± 19.77	0.195
Methanobrevibacter unclassified	1.08 ± 2.42	2.11 ± 4.72	0.676
Parabacteroides unclassified	1.55 ± 3.46	8.52 ± 11.94	0.246
Propionibacterium acnes	53.2 ± 18.44	13.76 ± 12.17	0.004*
Pseudomonas unclassified	5.13 ± 6.44	0.93 ± 2.07	0.202
Ruminococcus lactaris	0.01 ± 0.02	3.27 ± 3.02	0.042
Ruminococcus torques	1.72 ± 3.84	12.22 ± 14.01	0.145
Streptococcus thermophilus	32.14 ± 4.69	13.54 ± 15.18	0.031
Subdoligranulum unclassified	0.13 ± 0.29	4.35 ± 5.54	0.127
Others	2.68 ± 2.09	11.44 ± 7.71	0.039

Top 10 bacterial species identified as different between the two collection methods. Data shown as mean \pm SEM. Four species noted to be significantly different with p-value <0.05 and one with FDR <0.05 as indicated by the asterisk.



Supplemental Figure 1. Heat map demonstrating all 114 metabolites comparing the pinch biopsy versus cytology brush collection methods.



Supplemental Figure 2. Volcano plot demonstrating 114 metabolites identified as significantly up (red), down (blue), or unchanged (gray) comparing the pinch biopsy versus cytology brush collection method. Significance determined by fold change >2.0 and Wilcoxon signed-rank test with FDR adjusted p-value <0.1.

Metabolite	Log ₂ (FC)	P-value (FDR adjusted)
D-Glucono-1-5-lactone 6-phosphate*	-7.86	0.018
Cys-Gly	-7.12	0.018
alpha-D-Glucosamine 1-phosphate*	-6.98	0.018
5-hydroxytryptophan ⁺	-5.45	0.036
2-Hydroxyglutarate/Citramalate	2.74	0.036
Itaconate	2.15	0.036
L-cysteine	-4.59	0.070
D-Erythrose 4-phosphate	-5.57	0.073
N5-Methyl-L-glutamine	-4.38	0.089
N-Acetyl-L-citrulline	-3.47	0.089
СМР	-3.32	0.089

Metabolites identified as significant (fold change, FC >2.0 and paired-t-test with FDR adjusted p-value <0.1) for comparison between pinch biopsy and cytology brush collection method.