Quantification of the Human Skin Microbiota
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Abstract
• Understanding how quantities of skin-associated bacteria vary across different body habitats is vital to understanding their ecology and role in affecting human health.
• How does the total number of bacteria counted vary between dry, moist, and sebaceous skin types?
• How does the proportion of live and dead bacteria vary between dry, moist, and sebaceous skin types?

Methods
• Participants: 8 female, 6 male adults
• 12 hours before sampling: no bathing/swimming or applying anything (e.g., lotions) to skin

Results

Table: Summary of Results
<table>
<thead>
<tr>
<th>Method of Quantification</th>
<th>Colony counts (CFU/cm²)</th>
<th>qPCR (# of amplicons per well)</th>
<th>Fluorescence microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony counts</td>
<td>24</td>
<td>2.65 × 10⁸</td>
<td>1.12 × 10⁴</td>
</tr>
<tr>
<td>qPCR</td>
<td>171</td>
<td>2.82 × 10¹</td>
<td>1.01 × 10⁵</td>
</tr>
<tr>
<td>Fluorescence microscopy</td>
<td>626</td>
<td>2.90 × 10¹</td>
<td>3.29 × 10²</td>
</tr>
</tbody>
</table>

Figure 1. Estimates for the mean # CFU/cm² skin sampled determined from colony counts. Error bars: standard error. No statistical significance determined from one-way ANOVA, F₁,₃⁸ = 0.95, p = 0.38.

Figure 2. Mean starting quantity of 16S amplicons per qPCR reaction well. Due to multiple amplification steps, we could not confidently use qPCR data to extrapolate the number of cells/cm² on the skin. Error bars: standard error. No statistical significance determine from one-way ANOVA, F₁,₃⁸ = 0.05, p = 0.89.

Figure 3. Estimates for mean # of cells/cm² skin sampled determined from fluorescence microscopy. Error bars: standard error. Significant differences were found between estimates of live and dead cells for sebaceous sites from multiple comparisons ANOVA, p < 0.05.

Figure 4. Mean cell viability. Cell viability was determined by dividing the # of live cells by the sum of live + dead in each site. Error bars: standard error. Statistical significance between dry and sebaceous (p = 0.004) and moist and sebaceous sites (p = 0.005) was determined from two-tailed t-tests.

Figure 5. Comparison of cell density estimates made from colony counts and fluorescence microscopy. Error bars: mean ± standard error. No statistical significance determined from two-way ANOVA, F₁,₃⁸ = 1.68, p = 0.20.

Discussion
• Colony counts: lower estimates of cell density than what has already been determined from other culture-based studies.
• Why? Colonies only represent the cells that are live and culturable, more colonies could have developed with use of other media and culture conditions.
• qPCR: useful for relative comparisons between sample types but multiple amplification steps prevented us from making confident estimates for cells/cm².
• Next step: do qPCR of genomic DNA, rather than of 16S amplicons, to be able to make more confident estimates of cell density.
• Fluorescence microscopy: provided estimates of abundance for skin-associated bacteria that have been corroborated by both culture-dependent and culture-independent studies.
• Fluorescent dyes CTC and SYTOX Green allowed us to determine that ~5-15% of the skin microbiota is actively respiring.
• Next step: use DAPI stain to determine which cells are alive but not actively respiring (dormant), which may be important for maintaining genetic diversity and nutrient cycling.
• Shown here is the first study comparing multiple methods for quantifying the skin microbiota, including an assay for viability.

References & Acknowledgements
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