A blind spot in an FDA standard leaves significant bacterial survivors after chlorhexidine gluconate preoperative skin preparation

Hannah Duffy[1,2], Nicholas Ashton[2], Abbey Blair[2], Nate Hooper[2], Dustin Williams[1,2,3,4]

[1]Department of Biomedical Engineering, University of Utah, Salt Lake City, UT [2]Department of Orthopaedics, University of Utah, Salt Lake City, UT
[3]Department of Pathology, University of Utah, Salt Lake City, UT [4]Department of Physical Medicine and Rehabilitation, Uniformed Services University, Bethesda, MD

BACKGROUND:

- Skin-flora bacteria are the most significant contributor to surgical site infections (SSIs)[1]
- Presurgical skin preparation (PSP) is the cleansing of skin prior to surgery, usually with alternating scrubs of antiseptic and alcohol
- The FDA requires PSP products to demonstrate a 2-3 $\log_{10}$ reduction of natural flora using a non-destructive skin sampling technique known as the Cup Scrub method[2]
- We developed a porcine model for PSP testing using a microbiological sampling technique known as the Tissue Blend method[3]
- We hypothesized that on-label use of antiseptics approved by the Cup Scrub method would leave viable bacteria underneath the skin's surface. To test our hypothesis, we quantified and characterized the bacterial survivors after applying chlorhexidine gluconate (CHG).

METHODS: We applied the Cup Scrub and Tissue Blend methods to the backs of 7 Yorkshire pigs (Figure 1) following alternating scrubs of 4% CHG and alcohol (n=5 sites/pig). Control skin was used as a baseline (n=5 sites/pig). With 4 treatment groups, 20 samples were taken from each animal for a total of 140 samples.

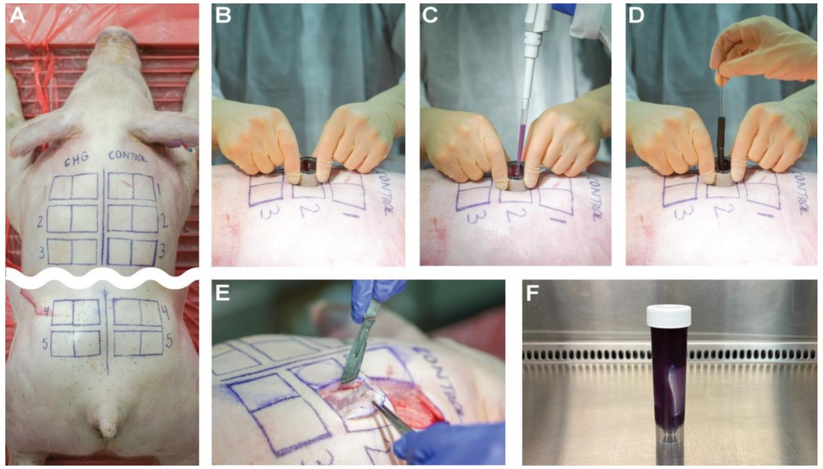
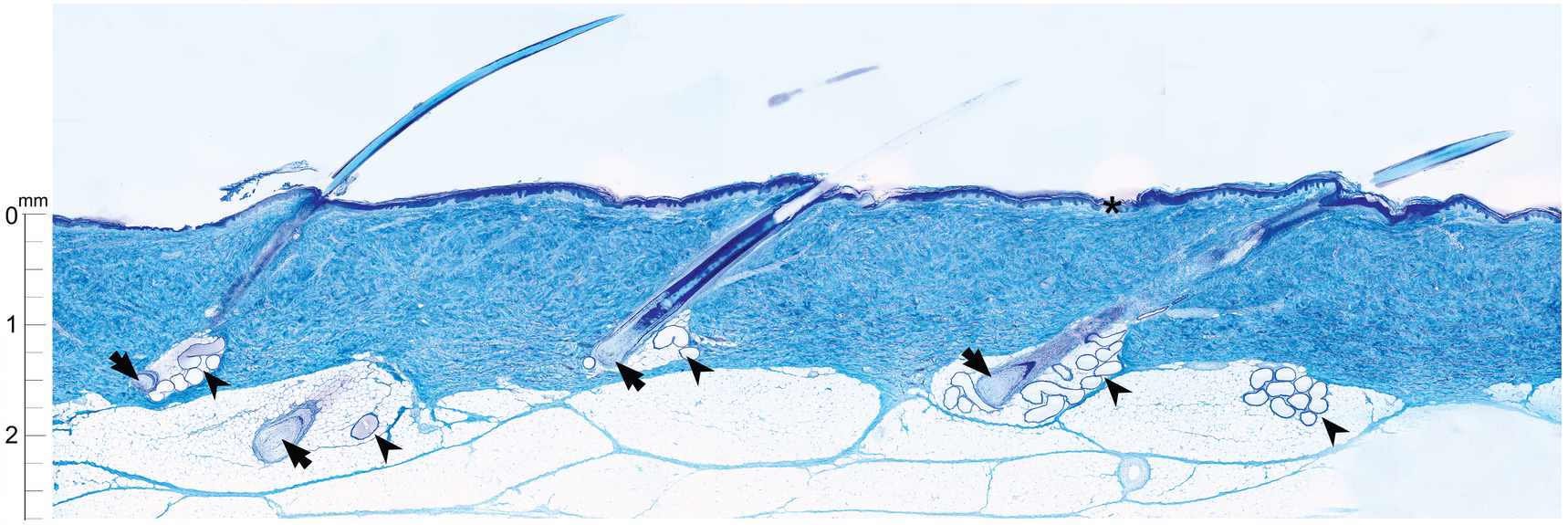


Figure 1: Location and process of sample collection. **(A)** Pig back marked with sample location boundaries. **(B-D) Cup Scrub Method:** A sterile containment cylinder is placed on the skin after applying a PSP. Neutralizing broth is pipetted into the cylinder; the skin is then agitated with a rubber spatula to suspend bacteria into solution. **(E-F) Tissue Blend method:** Full-thickness skin samples were excised, homogenized, serially diluted, and plated on agar.

RESULTS:

- Cup Scrub Method $\log_{10}$ reduction = 1.57 +/- 0.45 CFU/cm^2 (Initial = 2.62 +/- 0.21 $\log_{10}$ CFU/cm^2)
- Tissue Blend Method $\log_{10}$ reduction = 0.23 +/- 0.48 CFU/cm^2 (Initial = 3.46 +/- 0.24 $\log_{10}$ CFU/cm^2)
- Bioburden grouped by location zone was similar across anatomical sites along the pig back.
- Vast majority of bioburden were Gram-positive cocci. Most were from the *Staphylococcus* family



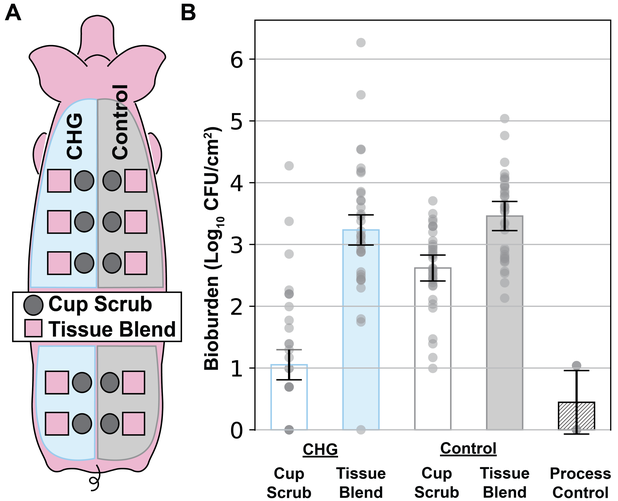


Figure 2: (Above) Longitudinal section (~75 µm thick) of PMMA-embedded pig skin using polarized light microscopy with Sanderson's rapid bone stain. Notable features are shown: the thin stratum corneum (asterisk), the deepest part of the hair follicles (arrow), and the sebaceous glands (arrowhead).

Figure 3: (Left) **(A)** Bioburden is reported as collected for each pig and differentiated by treatment group. Semi-transparent circles represent single data points and error bars show the standard deviation for that treatment group (n=5 per pig). **(B)** Schematic of collection sites.

DISCUSSION:

- The current sampling standard used for FDA approval of PSPs, or the Cup Scrub method, underreports the bioburden in the skin compared to the Tissue Blend method.
- The discrepancy between the methods illustrates a blind spot in how PSP efficacy is measured
- Bacteria in the deep tissue regions survive PSP application; however, they remain undetected using the Cup Scrub method.
- PSP development may be misguided with results that are below the true bioburden.
- The high CFU/cm^2 signal following PSP application shows the importance of readdressing current PSP approaches to mitigate SSI.

References: (1) Wenzel RP. Surgical site infections and the microbiome: An updated perspective. Infect Control Hosp Epidemiol. 2019 May;40(5):590-596. (2) *ASTM Standard Test Method E1173-15* (3) Duffy, H.R et al. A Porcine Model for the Development and Testing of Preoperative Skin Preparations. *Microorganisms* 2022, *10*, 837. **Funding Source:** The authors acknowledge the LS Peery Foundation, the Bone and Biofilm Research Lab (BBRL), and the NSF for the funding to complete this study. **Acknowledgements:** The authors thank the University of Utah Center for Comparative Medicine Center and the entire BBRL staff for their help and technical support. **Contact:** Hannah Duffy via email: hannah.duffy@utah.edu. Please scan the QR code to learn more or connect!

Scan for audio or to connect with me!