Wound infections with multi-drug resistant bacteria increase morbidity and mortality and have considerable socioeconomic impact. They can lead to impaired wound healing, resulting in rising treatment costs. The aim of this study was to investigate an ex vivo human wound infection model. Human full-thickness skin from the operating room (OR) was placed into the Bo-Drum and cultivated for 7 days in an air-liquid interphase. On day 8, the skin was inoculated with either (1) Pseudomonas aeruginosa, (2) Staphylococcus aureus (10^5 CFU, n = 3) or (3) carrier control. 1, 3 and 7 days after inoculation colony forming units in the tissue/media were determined and cytokine expression was quantified. A reliable and reproducible wound infection could be established for 7 days. At this time point, 1.8 x 10^8 CFU/g tissue of P. aeruginosa and 2 x 10^7 CFU/g tissue of S. aureus were detected. Immunohistochemical analysis demonstrated bacterial infection and epidermolysis in infected skin. RT-PCR analysis exhibited a significant induction of proinflammatory cytokines after infection. The BO-drum is a robust, easy-to-use, sterilizable and reusable ex vivo full-skin culture system. For investigation of wound infection, treatment and healing, the BO-drum presents a convenient model and may help to standardize wound research.