Are Quantitative Bacterial Wound Cultures Useful?

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Determining if a nonhealing wound is infected can be difficult. The surface of a wound is not sterile and can be colonized with numerous commensal, environmental, and potentially pathogenic microorganisms. Different types of wounds have various clinical presentations, with some signs and symptoms more likely to be present than others depending on the type and location of the wound. Clinicians often order microbiology wound cultures to assist in determining if a nonhealing wound is infected. This minireview briefly summarizes the clinical microbiology of wound cultures, with an emphasis on the history and utility (or lack thereof) of the quantitative wound culture.

Quantitative bacteriology cultures are an important part of the modern clinical microbiology laboratory. Quantitative cultures assist clinicians in determining the threshold above which the bacterial burden of a culture will likely demonstrate clinical significance. Bacterial growth below established thresholds in quantitative cultures typically represents “background noise” of subclinical colonization or inconsequential growth of normal commensal microbiota. The most frequently used quantitative bacterial cultures are urine cultures, where a calibrated inoculation loop is used to inoculate media in order to yield accurate quantitative culture per milliliter of urine. Other less commonly utilized quantitative culturing techniques may be routinely performed depending on the size and scope of the clinical laboratory and can include the use of high-quality liquid specimens such as protected bronchial brushings. Quantitative wound culture techniques were described in large part by research microbiology laboratories in the 1960s and 1970s and were adopted into clinical use thereafter. Quantitative culturing of wounds, particularly biopsy specimens of wounds, involves extensive processing techniques that can be difficult for most clinical microbiology laboratories. Therefore, most nonurine bacterial cultures, including wound cultures, are plated using a semiquantitative technique where cultures are inoculated onto media using a sterile loop that sequentially dilutes the specimen from the first area or quadrant of the medium to the last area or quadrant. Results are often reported as 1+ , 2+, 3+, or 4+ (or as text, using such terms as “trace,” “few,” “moderate,” or “abundant”), depending on which areas or quadrants demonstrate bacterial growth.

Microbiology wound cultures can be the most difficult type of culture to evaluate in the clinical laboratory. Wound culture specimens often arrive in the laboratory with limited information regarding the wound specimen. It is not uncommon for a specimen to arrive in the laboratory with the single word “wound,” with no information provided as to the site and nature of the wound. Wound cultures that are improperly collected and/or grossly contaminated with a wide variety of microorganisms are common. The types of specimens submitted for wound culture typically include tissues, aspirates, and swabs. Tissue and aspirates are the preferred specimens for microbiological wound culture (1, 2, 3, 4). For quantitative wound cultures, tissue is particularly challenging, as the tissue must be accurately weighed, homogenized, and serially diluted prior to inoculation of media for each dilution under aerobic and anaerobic conditions. Variations in biopsy collection, processing, and inoculation can often confuse the interpretation of quantitative wound culture results.

Some clinicians are reluctant to perform tissue biopsy procedures in order to minimize patient discomfort, while others fear complications such as introducing bacteria deeper into noninfected tissue, so swab specimens are submitted for culture. It has been my observation that it is not uncommon for clinicians to aspirate wounds producing a purulent drainage with a syringe (ideal specimens) and then inoculate the aspirate onto a swab (a less than ideal specimen) for culture submission. Traditional swabs are made from cotton, calcium alginate, and Dacron-Rayon. Swabs tend to collect a small fraction of a milliliter of specimen (<0.1 ml), which greatly reduces the amount of bacteria that can be recovered from the swab for bacterial culture. In addition to limited volume collection, traditional swabs tend to retain the collected specimen. A newer generation of swabs made from a flocking process which allows more-efficient specimen release has emerged over the past decade. However, flocked swabs share most of the collection limitations of traditional swabs as they do not collect adequate specimens for comprehensive clinical microbiology wound cultures. Swab culture yields are reduced as multiple types of cultures (aerobic, anaerobic, mycobacterial, and fungal) are requested from a single swab, thus requiring inoculation of many different types of media.

In addition to collection and processing limitations, wound cultures typically require between 36 and 48 h before results are reported. In grossly mixed cultures, separating bacterial isolates can cause even greater delays. This delay in obtaining results is of little use to a surgeon who needs to make a timely decision as to whether or not to close a wound. Delayed culture resulting allows complications such as introducing bacteria deeper into noninfected tissue, so swab specimens are submitted for culture. It has been my observation that it is not uncommon for clinicians to aspirate wounds producing a purulent drainage with a syringe (ideal specimens) and then inoculate the aspirate onto a swab (a less than ideal specimen) for culture submission. Traditional swabs are made from cotton, calcium alginate, and Dacron-Rayon. Swabs tend to collect a small fraction of a milliliter of specimen (<0.1 ml), which greatly reduces the amount of bacteria that can be recovered from the swab for bacterial culture. In addition to limited volume collection, traditional swabs tend to retain the collected specimen. A newer generation of swabs made from a flocking process which allows more-efficient specimen release has emerged over the past decade. However, flocked swabs share most of the collection limitations of traditional swabs as they do not collect adequate specimens for comprehensive clinical microbiology wound cultures. Swab culture yields are reduced as multiple types of cultures (aerobic, anaerobic, mycobacterial, and fungal) are requested from a single swab, thus requiring inoculation of many different types of media.
plex wound culture containing numerous environmental and commensal organisms might serve to confuse the clinical decision-making process should these technologies become integrated into the clinical evaluation of complex wounds.

Whether a tissue, aspirate, or swab is submitted for a wound culture, surface wounds contain various environmental and normal host microbiota that may or may not be involved in the disease process. Problems arise when one attempts to quantitatively assess complex surface or near-surface wound cultures. Even across the surface of a single wound, microbial communities and densities can differ greatly. Diabetic and pressure ulcer culture wounds can resemble fecal specimens containing large amounts of nonnormal intestinal flora, including numerous species of enteric aerobic and anaerobic bacteria. The recovery and characterization of environmental and commensal bacteria obscure clinical evaluation and lead to inappropriate use of broad-spectrum antibiotics.

In accordance with current American Society for Microbiology (ASM)/Infectious Diseases Society of America (IDSA) guidelines (1), most microbiology laboratories do not work up everything in a grossly contaminated culture. However, common primary pathogens such as *Enterococcus* spp., *Staphylococcus aureus*, beta-hemolytic *Streptococcus* spp., and *Pseudomonas aeruginosa* are typically isolated and characterized when present in mixed culture. Clinicians often call the laboratory to request (or demand) everything be worked up in a grossly contaminated culture although there is very limited, if any, positive predictive value of antibiotic susceptibility testing for mixed cultures (7) or positive predictive value of susceptibility testing for infections that will be treated with multiple antibiotics (8).

**SELECTED LITERATURE (QUANTITATIVE CULTURE IS USEFUL)**

The literature investigating the quantitative microbiology of wound cultures spans 50 years and is often discordant. The majority of early investigations supporting the use of quantitative bacterial wound cultures were performed in the 1960s to 1970s. In large part, these studies were undertaken at surgical institutes located with microbiology research laboratories. One of the earliest cited studies indicating the potential usefulness of quantitative culturing was performed in 1964 by Bendy et al., who determined that bacterial counts equal to or greater than 10^6 CFU per milliliter of wound exudate were associated with increased risk of infection (9).

The U.S. Army’s Institute of Surgical Research (ISR) in San Antonio, TX, played a large role in advancing knowledge regarding quantitative wound cultures in the 1960s and 1970s through research by M. C. Robson and J. P. Heggers, who advocated strongly for quantitative biopsy analyses to replace the quantitative fluid cultures initially recommended by Bendy et al. Key research from the ISR was first published in 1967 where Robson and Heggers conducted a retrospective study of 50 granulating wounds demonstrating that 94% of grafted wounds with fewer than 10^5 bacteria per gram of tissue successfully engrafted where only 19% of wounds with greater than 10^5 bacteria per gram successfully engrafted (10). In a similar retrospective study published in 1968, 40 wounds were examined to assess the effects of closing open wounds. Ten wounds were found to have greater than 10^5 bacteria per gram of tissue and did not heal compared to 28 of 30 wounds with fewer than 10^5 bacteria per gram that were successfully closed (11). It was understood at this time that the delay in culture results and increased burden on the microbiology laboratory indicated that this approach was not practical for routine clinical use. Therefore, a more rapid Gram stain technique was developed to quantify bacteria in wounds (12). In 1970, Robson et al. evaluated 94 operative incisional abscesses greater than 5 cm, comparing Gram stain to quantitative cultures. Forty-four of the abscesses were allowed to heal naturally, with a mean patient hospital stay of 22.3 days (maximum, 82 days). Forty-six wounds were subjected to surgical closure once bacterial counts of fewer than 10^6 bacteria per gram of tissue were obtained. Four wounds were not closed due to the presence of beta-hemolytic streptococci. Of the 46 wounds surgically closed, 42 (91%) remained closed and healed, with an average hospital stay of 8 days (13).

Another direct smear and swab culture technique from the Army’s ISR, published in 1976, described correlation of Gram stain and biopsy analyses with a surface swab culture technique (14). The Levine swab technique is frequently cited in the literature as an alternative to quantitative biopsy. The Levine technique is described briefly as follows. A cotton-tipped swab is twirled across a 1-cm-square area for 5 s, pressing the swab into the wound with enough force to cause bleeding. The tip of the swab is then broken off into sterile medium, mixed using a vortex device, serially diluted, and inoculated in pour plates of Trypticase soy agar. Numerous noninvasive swab and surface absorbent techniques were shown to be equivalent to biopsy in the late 1970s and early 1980s, employing various different collection techniques. However, quantitative biopsy may not have been the best comparator for quantitative surface cultures.

**SELECTED LITERATURE (QUANTITATIVE CULTURE IS NOT USEFUL)**

On the other side of the debate regarding the usefulness of quantitative wound cultures is a 1980 article from Freshwater and Su where 285 quantitative biopsy cultures were correlated with clinical signs of sepsis. In this evaluation, 144 (50.5%) cultures grew greater than 10^6 bacteria per gram of tissue. Of the patient specimens growing greater than 10^5 bacteria per gram of tissue, only 29% of cultures with greater than 10^6 bacteria per gram and 36% of cultures with greater than 10^5 bacteria per gram were found to have two or more signs of clinical sepsis. In patients whose cultures grew less than 10^6 bacteria per gram of tissue, there was no relationship between bacterial counts and clinical signs of sepsis. Additionally, four patient cultures with fewer than 10^5 bacteria per gram of tissue demonstrated clinical sepsis and 24 patient cultures with bacterial counts of greater than 10^5 bacteria per gram of tissue showed no signs of sepsis (15). This study, along with others of the period, demonstrated the potential pitfalls in quantitative bacterial culturing of wound infections, with some researchers noting that differences in collecting and processing specimens may have accounted, at least in part, for some of the variability.

Even the Army’s ISR began to question the usefulness of quantitative culturing in 1987 with a study published by McManus et al. where 200 burn biopsy specimens were evaluated in parallel with quantitative microbiology cultures and histopathology to determine the correlation of quantitative culture with tissue invasion. The correlation between negative culture results (counts of fewer than 10^5 bacteria per gram) and negative histopathology was good at 96.1%, consistent with the earlier work by Robson and Heggers. However, positive quantitative microbiology cultures correlated...
with tissue invasion in only 36% of the cases (16). The conclusion of the authors was that quantitative microbiology cultures could be useful in identifying the predominant bacterial species present. However, histological demonstration of tissue invasion was found to be far more predictive of infection and can be accomplished in far less time (16, 17).

In 1996, Steer et al. published back-to-back articles examining the usefulness of quantitative microbiology in burn wound cultures. In the first article, 141 biopsy specimen/swab pairs were evaluated (18). In 18 of the 141 wounds, parallel cultures and biopsy specimens were taken in duplicate (2 biopsy specimens and 2 swabs per wound) to evaluate the differences in adjacent areas of a single wound. In this evaluation, no correlation was observed between the biopsy specimen size and the number of bacteria recovered, suggesting that quantitative biopsy specimen counts per gram of tissue were of limited or no value. In addition to a lack of correlation in biopsy specimen size and colony count, large variations occurred in bacterial counts per gram of tissue when parallel cultures of adjacent biopsy specimens or surface swabs were evaluated from the same patient wound. There was also a large disparity between swab and biopsy specimen culture results, with only 54% of paired biopsy specimen/culture results yielding the same organisms. Several articles demonstrated similar results showing that bacterial counts across the surface of a single wound differed greatly and that the subjective nature of how wounds were sampled, processed, and evaluated complicated comparison of data (15, 19, 20, 21, 22).

In the second of the articles published back to back by Steer et al., 69 biopsy specimen/swab paired specimens were analyzed and compared to clinical signs of sepsis (23). The authors examined both total and threshold (10^4 to 10^7) counts of bacteria per gram and found no relationship between bacterial counts and graft loss. The authors found no correlation between bacterial counts and bacteremia, regardless of total bacterial count or when separately evaluating the presence of S. aureus and that of P. aeruginosa. Many wounds in the evaluation had exceptionally high bacterial counts per gram of tissue and did not show any clinical signs of sepsis progressing to normal healing. Other studies of the time supported these conclusions (15, 24).

The delay in reporting results from quantitative cultures makes clinical management difficult, so direct Gram staining has been used as a surrogate to determine bacterial loads in wounds as described previously (12, 14). Similarly to the literature questioning quantitative biopsy specimen and surface culturing methods, in 2003 Elsayed et al. conducted a prospective analysis of 375 specimens, evaluating Gram stain against quantitative cultures. In this series, there was no significant correlation of bacterial burden in wounds and Gram stain results, with Gram stains demonstrating a sensitivity of only 34.6% compared to culture (25).

ARE QUANTITATIVE CULTURES WORTHWHILE?

So, are quantitative wound biopsy analyses or quantitative wound surface microbiology cultures useful? In my opinion, the answer would be no. Even if one were to discount the body of clinical literature since 1980 questioning the usefulness of quantitative cultures, improvements in wound care have dramatically reduced the morbidity and mortality of wound infections and have diminished the need for extensive quantitation and characterization of wound microbiology culturing. Negative-pressure dressings, improvements in wound bed preparation, improved topical antimicrobials, fibrin sealants, dermal substitutes, more-effective antibiotics, the addition of growth factors, and stem cell therapies have all contributed to improved outcomes for wound healing.

Both advocates and opponents of the quantitative culture argument would agree that bacteria present in wounds can play a role in determining whether or not a wound will become infected. The presence of bacteria in wounds can reflect any combination of surface colonization, biofilm formation, or active invasion and infection. Early advocates of quantitative wound cultures were correct in realizing that infection was related to an out-of-balance condition where bacterial load, variations in host response, and wound type could contribute to determining whether or not a wound would become infected. In the early antibiotic era, quantitative wound cultures were more common than they are today and might have provided some value at the time as indicated by the studies by Robson and Heggers. However, the majority of data published since 1980 have shown little to no benefit of quantitative biopsy analyses or quantitative wound surface cultures, with several studies finding the correlation of culture to infection to be very low at 25% to 39%. The densities of bacteria across the surface of a single wound vary greatly, as do the differences in collection and processing techniques. Processing true quantitative wound cultures is not practical for the average clinical microbiology laboratory, and the time to detection for quantitative cultures, at 24 to 36 h, is not clinically useful.

So, if quantitative culturing from surface or biopsy specimens is not useful, what is? It is widely accepted that the negative predictive value of culture is excellent; therefore, a semiquantitative culture of tissue or exudate will determine if there is growth within less than 24 h for most microorganisms. Characterizing the extent of growth, to include rough descriptions of bacterial diversity, while screening for and characterizing the common pathogenic bacteria such as S. aureus, P. aeruginosa, and the beta-hemolytic streptococci, can provide useful information. Clinicians must be cautious to avoid overinterpreting the significance of microbiology wound cultures in their clinical evaluation of nonhealing wounds.

KEY POINTS

- Wounds vary dramatically in the amount and diversity of bacteria present across the surface of the wound.
- Methods of specimen collection and processing quantitative wound cultures vary greatly.
- Quantitative biopsy analysis is not practical for the clinical microbiology laboratory.
- Numerous prospective studies contradict the usefulness of quantitative cultures.
- Microbiology wound culture results should not supplant clinical judgment.

REFERENCES


2. Paxton A. August 2004. Swapping swabs for syringes and scalpels. CAP
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Minireview


