Comparison of Quantitative and Semiquantitative Culture Techniques for Burn Biopsy

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Wound sepsis is a life-threatening problem for severely burned patients. Evaluation of this condition has been extremely difficult because exposed burned body surfaces are readily available for bacterial colonization. It has been recognized that the degree of bacterial wound contamination has a direct relationship with the risk of wound sepsis. Loebl et al. described a quantitative culture technique (Q technique) of full-thickness eschar biopsy that was useful in prediction of burn wound sepsis, a major cause of death among severely burned patients (6). Observations indicated that patients whose biopsies contained more than 10^5 CFU per g of tissue were likely to develop sepsis (6, 11, 12, 14). Other investigators have successfully applied similar Q techniques to determine graft bed receptiveness and to predict the safety of wound closure (2, 4, 9, 13). Although the degree of bacterial contamination is important in the prediction of wound sepsis, it is necessary to remember that the presence of Streptococcus pyogenes in any numbers constitutes a serious threat to the patient and must be detected by whatever culture method is used.

The Q culture has been recognized as a valuable index for surgeons in the management of severely burned or traumatized patients, but this culture technique is both labor intensive and expensive. Quantitation of bacteria in an eschar biopsy culture involves tissue homogenization, 10-fold serial dilutions, and a minimum of five plated dilutions. In addition, to account for uneven distribution of bacteria in wound tissue, multiple specimens must be obtained to be representative of the bacterial contamination of large wounds. The burn center at Harborview Medical Center routinely obtains one biopsy specimen for each 20% of body surface area burned.

In an effort to reduce the expense of the culture while still providing vital information to the physician, the accuracy and usefulness of a semiquantitative (SQ) biopsy culture was evaluated. The design of the SQ culture was based on a review of Q culture data from the previous year. The SQ culture was examined for reproducibility and compared with the Q technique for accuracy. An evaluation of the ability of the SQ method to provide a predictive index for wound sepsis and a cost comparison between the SQ and Q methods were made.

MATERIALS AND METHODS

Specimen collection. Topical agents were removed from the burn eschar with sterile saline-soaked gauze pads, and the wound was biopsied by making two parallel incisions, approximately 1 to 2 cm in length and 0.5 cm apart. The tissue was lifted with forceps and cut to a sufficient depth to obtain a small portion of the underlying fat. This biopsy method was described by Loebl et al. (6). One biopsy specimen was taken from approximately each 20% of burn area. The tissue was placed in a sterile tube and transported to the laboratory.

Specimen processing. Tissue samples were weighed to the nearest milligram in sterile petri dishes on a Mettler PN163 balance and then cut into small pieces with a sterile scalpel blade. The tissue was placed in 2 ml of sterile physiologic saline containing 0.5% sodium thiosulfate to neutralize remnants of topical iodinated compounds. The tissue was then homogenized in a Ten Broeck tissue grinder (Corning Glass Works, Corning, N.Y.).

Q culture. A 0.1-ml portion of the homogenate was obtained with an Eppendorf pipette and used to make 10-fold serial dilutions in saline with thiosulfate. A 0.1-ml sample from each dilution (10^−1 through 10^−5) was plated on blood agar (BA) plates (heart infusion base; Difco Laboratories, Detroit, Mich.) supplemented with 5% sheep blood (Prepared Media, Tacoma, Wash.). One drop from a Pasteur pipette delivering approximately 0.03 ml of the undiluted homogenate was placed on a BA-MacConkey bi-plate and on a pre-reduced brucella base blood agar plate (brucella base; Difco) supplemented with 5% sheep blood (Prepared Media). Additionally, a brain heart infusion broth (Difco) was inoculated to allow recovery of organisms at concentrations of...
TABLE 1. Comparison of Q and SQ cultures by reporting category

<table>
<thead>
<tr>
<th>Q category</th>
<th>SQ cultures</th>
<th>No. concordant</th>
<th>No. discrepant</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&lt;10^4 CFU</td>
<td>13</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10^4 to 10^6 CFU</td>
<td>10</td>
<td>3*</td>
<td></td>
</tr>
<tr>
<td>&gt;10^6 CFU</td>
<td>41</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Of these SQ cultures, two were greater than 10^6 and one was less than 10^4 by the Q culture method.

less than 10^4 CFU/g of tissue. All aerobic cultures were incubated at 35°C in a 5% CO₂ atmosphere, and the brucella blood plate was incubated at 35°C in a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) under anaerobic conditions to enhance recovery of beta-hemolytic streptococci.

 Colony counts were obtained from plates containing 30 to 300 colonies. The number of CFU per gram of tissue is given by N × D_Q × V × 10/W, where N is the number of colonies counted on a plate of a given dilution, D_Q is the reciprocal of the dilution counted, and V is the volume of the diluent used for tissue homogenization. The factor 10 accounts for the 0.1-ml sample which was plated, and W is the weight of the tissue in grams.

 SQ. From the tissue homogenate used for the Q culture, a 0.1-ml sample was delivered to a BA plate with an Eppendorf pipette. Additionally, a 0.01-ml sample obtained with a calibrated inoculating loop (American Scientific Products, McGaw Park, Ill.) was placed on a BA plate. This method was done in duplicate to assess the reproducibility of colony counts obtained by this procedure. All media were incubated at 35°C as previously described. Exact colony counts were obtained from BA plates with 30 to 300 colonies. If there were more than 300 colonies on both plated dilutions, the factor 300 was used as N for calculations, and the result was considered greater than that value. The number of CFU per gram of tissue is given by N × D_Q × V/W, where N is the number of colonies counted on a plate of a given dilution, D_Q is the reciprocal of the volume of homogenate that was inoculated, V is the volume of diluent used for tissue homogenization, and W is the weight of the tissue in grams. It should be noted that the decision to use 0.1- and 0.01-ml volumes of homogenate was the result of a review of previous Q cultures.

 On the basis of the average sample size of biopsy tissue, the optimal number of colonies to count (i.e., 30 to 300), and the volume of diluent used for tissue homogenization, the volumes of 0.1 and 0.01 ml were chosen to give precise colony counts between 10^3 and 10^6 colonies per g. This range was desired because of the clinical relevance of a 10^6 colony count per g of tissue.

 Reporting of results. Q results were reported as the exact number of CFU per gram of tissue, while SQ results were reported as the following responses: (i) no growth, (ii) less than 10^4 CFU/g of tissue, (iii) more than 10^6 CFU/g, or (iv) the exact colony counts in the range 10^4 to 10^6 CFU.

 Reports with data from positive cultures were called to the attending physician (D.H.) who is Director of the Burn Unit at Harborview Medical Center. The SQ results for each biopsy culture were reported, and the antibiotic therapy response of the physician was recorded. The responses included the following four categories: (i) initiate antimicrobial therapy, (ii) discontinue antimicrobial therapy, (iii) change antimicrobial therapy, and (iv) do not change antimicrobial therapy. Then the Q results for the same culture were reported, and the corresponding antimicrobial therapy response was recorded. Consultation with the physician regarding culture data and therapy response was done either prospectively or retrospectively, depending on the availability of the surgeon.

 Statistical analysis. Comparison of data was done by using a logarithmic scale owing to the wide variations in CFU per gram of tissue from culture to culture. Data were analyzed by the Microstat 4.1 program (Ecosoft Inc., Indianapolis, Ind.).

 RESULTS

 The SQ culture technique was performed in duplicate on each of 78 eschar biopsies to assess the reproducibility of the technique. Of the 78 cultures, 17 biopsies had dilution plates with colony counts in the range of 30 to 300 colonies by the SQ method. A comparison of the CFU per gram of tissue, expressed as the logarithm, for each set of SQ plates for the 17 cultures was made. The absolute difference between duplicate platings ranged from 0.01 to 0.57 with an estimated standard deviation of 0.14.

 Agreement between the SQ and Q culture methods by reporting categories is shown in Table 1. Of 78 cultures, 75 (96%) were in the same reporting categories for both Q and SQ culture procedures. Eleven cultures were sterile by both culture methods. Also, a total of 14 cultures by the Q method had less than 10^4 CFU/g, while 13 of those 14 cultures had less than 10^4 CFU/g by the SQ method. One culture in this category had a colony count of 5.6 × 10^3 by the Q procedure but a colony count of 1.5 × 10^4 by the SQ procedure. A total of 10 cultures were found to be in the range 10^4 to 10^6 CFU/g of tissue by the Q procedure; all 10 of these cultures were also in that range by the SQ technique. In the category of more than 10^6 CFU/g of tissue there were 43 cultures by the Q technique, 41 of which also had more than 10^6 CFU/g of tissue by the SQ technique. The differences in the two discrepant cultures were as follows: 1.5 × 10^6 and 1.6 × 10^6.

 An analysis of the 13 SQ cultures in the category of 10^4 to 10^6 is shown in Fig. 1. Correlations between SQ and Q results were good, with a correlation coefficient of 0.9509. As expected, the greatest deviation between the two methods was seen at the extremes of the 10^4 to 10^6 range. Using the criteria that more than or equal to 10^5 CFU/g of tissue by standard Q culture is indicative of wound sepsis, the SQ method predicted wound sepsis in 46 of 78 cultures and no sepsis in 32 of 78 cultures. Wound sepsis, as predicted by the Q method, actually existed in 48 of 78 specimens and did not exist in 30 of those 78 specimens. There were no false-positive indications of wound sepsis by the SQ method, and only two false-negatives. The positive predictive value of the SQ method was 100% and the negative predictive value was 93.7%.

 Clinical evaluation of the SQ culture data was generated by direct consultation with the attending physician. The SQ data were reported first by telephone to the physician, and his response in terms of antimicrobial therapy was obtained. Then the Q data were reported, and his antimicrobial therapy response was also recorded. The response to the SQ information was the same as the response to the Q results for 76 of 78 cultures. The two cultures that were exceptions had colony counts as follows: (i) Q, 2.5 × 10^5; SQ, >10^6; and (ii) Q, 1.6 × 10^5; SQ, 6.2 × 10^5. In the first culture, with the large
colony count \((10^4)\), an empiric change of antimicrobial therapy was deemed necessary. In the second culture, the Q results indicated to the physician that initiation of antibiotic therapy was necessary, whereas the interpretation of the SQ results was that no change in therapy was necessary.

Table 2 indicates the differences in time and material saving per culture by each method. The SQ culture method represented a reduction from five to two dilution plates. For each plate inoculated the College of American Pathologists (CAP) allows 2.5 work units (1). Therefore, inoculation of three fewer plates with the SQ method represents a saving of \(3 \times 2.5\), or 7.5 work units. Additionally, the Q method requires serial inoculation of five saline tubes, which is not done in the SQ method. Since CAP also allows 2.5 work units per tube inoculation, a saving of 12.5 work units \((2.5 \times 5)\) is gained with the SQ procedure. This results in an overall saving of 20 CAP units \((12.5 \text{ plus } 7.5\) per biopsy culture. The number of CAP units presently accorded to Q wound biopsy cultures at Harborview Medical Center is 66.2. Therefore, a reduction of 20 CAP units for this culture technique represents an approximately 30% saving in technical time. Additionally, there is a reduction of three blood agar and five saline tubes per culture. This represents a 60% reduction in the amount of media used per biopsy culture.

**DISCUSSION**

The quantitative biopsy culture has been described by several authors as a useful indicator for diagnosis and treatment of wound sepsis (6, 8, 11, 14, 15). This type of culture is, however, both labor intensive and expensive for the clinical laboratory. It has been suggested by several investigators (3, 5, 7) that quantitations by Gram stain might be an acceptable compromise, whereas other investigators have felt that quantitation either by culture or Gram stain was not reproducible (10, 16, 17). A semiquantitative culture method involving the use of 1- or 10-µl calibrated loop to plate the homogenate, depending on the weight of the specimen, was proposed by Shulman et al. (13). In his study the results were estimated at a 10^4 CFU/g breakpoint by a graph that related the number of expected CFU on the plates to the weight of the specimen. However, there was no comparison of their method with the standard quantitative biopsy culture. Both the reproducibility of this SQ culture method and the correlation between the SQ and Q culture method were good. Correlation for both comparisons was greater than 90%. A categorical comparison of the SQ results and the Q results showed agreement in 96% of the 78 cultures.

The SQ method had a positive predictive value of 100% and a negative predictive value of 93.7% for wound sepsis. Additionally, the SQ results provided adequate information for antimicrobial management in 76 of the 78 (97%) cultures. If actual effect on the patient is considered, adequate antimicrobial information was actually provided in all except one culture. One of the two cultures in which the responses of the physician differed when given SQ and Q results would not have affected actual patient management. In this case, the culture was one of three submitted the same day. The SQ results of the other two cultures indicated that antimicrobial therapy should be initiated. Therefore, only 1 of the 78 SQ results would have actually resulted in a difference in patient antimicrobial management.

Finally, this SQ method provided substantial cost reduction per culture for the clinical laboratory. There was saving of both technical time and media. Therefore, the SQ method offers a reproducible, efficacious, and less expensive method than the serial dilution culture for burn wound biopsies.

**ACKNOWLEDGMENTS**

We thank the technicians and technologists of Harborview Medical Center for their excellent work and Michel Laurin for his assistance with the statistical analysis.

**LITERATURE CITED**


**TABLE 2. Cost comparison of Q and SQ cultures**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAP units (^a)</th>
<th>Media (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q culture</td>
<td>66.2</td>
<td>13</td>
</tr>
<tr>
<td>SQ culture</td>
<td>46.2</td>
<td>5</td>
</tr>
<tr>
<td>Reduction/culture</td>
<td>20.0</td>
<td>8</td>
</tr>
<tr>
<td>% Reduction</td>
<td>30.2</td>
<td>61.5</td>
</tr>
</tbody>
</table>

\(^a\) Values were computed from CAP workload recording data.

\(^b\) 'Media' represents both plates and tubes used in each culture. A total of five saline tubes and three blood agar plates were eliminated by the SQ method.

FIG. 1. Comparison of Q and SQ culture results for cultures with 10^4 to 10^6 CFU/g of tissue. Solid line represents correlation coefficient of 1.0.


