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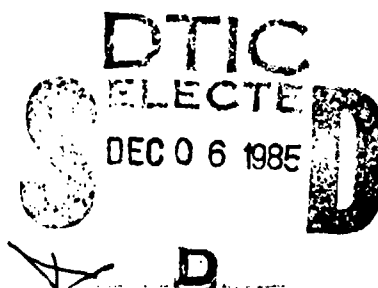
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## A Rapid Section Technique for Burn Wound Biopsy

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At the US Army Institute of Surgical Research, histologic diagnosis of infection using burn wound biopsies has proved superior to quantitative culture methods and is considered the most accurate method for distinguishing between microbial colonization and invasive infection of burn wounds. Although the frozen section technique is faster and is often useful, rapid section technique is the method of choice for histologic evaluation of burn wound biopsies. This technique takes about four hours to complete, yields permanent sections, and can be accomplished in any conventional histology laboratory. (Keyrings)

Burn therapy has made significant advances during the past four decades.<sup>1</sup> The development of topical chemotherapeutic agents, the use of physiologically-based fluid resuscitation, and the availability of broad-spectrum antibiotics, as well as increased understanding of burn pathophysiology and infection, have extended patient survival.<sup>2-4</sup> Effective topical antimicrobial therapy has reduced the overall incidence of burn wound sepsis, but burn wound infection still occurs in individual patients, particularly those with extensive burns requiring prolonged care.<sup>5-11</sup>

Although qualitative and quantitative culture techniques are useful for identifying specific microorganisms, they do not permit accurate differentiation between colonization and invasion, and the time required for culture growth delays diagnostic confirmation, particularly in the case of fungal infection.

An adaptation of the one-hour rapid histologic examination of unburned tissue permits application of that technique to burned tissue.<sup>11</sup> A rapid section technique for processing wound biopsy specimens has been used since the late 1960s at this Institute to prepare sections for histologic evaluation of the microbial status of the burn wound. This technique of histologic examination of biopsy specimens which appears to be the most accurate and reliable means for differentiating burn wound colonization from burn wound infection, is described.

## Materials and Method

Burn wound biopsies are obtained using conventional incision biopsy techniques.<sup>12-14</sup> The incisional biop-

sies measure  $0.5 \times 0.5 \times 0.5$  cm to  $1 \times 1 \times 1$  cm and weigh 100-500 mg each. Tissues for histologic evaluation are received from surgery in 10% neutral buffered formalin solution and examined by a pathologist, who chooses the best areas for evaluation (generally off-color, ie, brown, black, or green). Sections 1-2 mm thick are then processed immediately by an automated tissue processor\* with heat and vacuum. The materials and method for this rapid section technique are summarized in Table 1. Laboratories that do not have this tissue processor can process the tissue manually using Coplin jars, as detailed in Table 2. Within about four hours the biopsy sections can be evaluated by the pathologist; fixation takes 20 minutes; Autotechnicon processing, two hours; embedding into paraffin, 30 minutes; cutting, 30 minutes; and staining, 40 minutes. Brown Hopps Gram's stain, the McManus PAS stain method for glycogen, and Harris hematoxylin and eosin stains are used to stain the tissue sections of each paraffin block.<sup>15-17</sup> Other useful stains and the choice of stains for specific microorganisms are shown in Table 3.

## Discussion and Conclusions

In the past, surface swab cultures have been used for identification of organisms present on burn wounds; more recently, both quantitative surface cultures and quantitative cultures of burn wound biopsies have been proposed as means of detecting burn wound infection.<sup>18,19</sup> However, clinical experience and recent re-

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\*Autotechnicon Ultra, Technicon Instruments Corp. Tarrytown, New York.

**Table 1. Rapid Section Technique**

Transport biopsy in 10% buffered neutral formalin solution  
 Select best areas for sectioning  
 Cut biopsy into sections 1–2 mm thick  
 Fix in preheated 10% buffered neutral formalin with magnetic rotation stirrer (60–65C) for 20 minutes  
 Process in automated processor—2-hour cycle—or manually  
 Infiltrate with paraffin in vacuum infiltrator for 15 minutes  
 Embed in paraffin  
 Cut at 5 $\mu$  or less—3 slides of each specimen.  
 Stain one slide each with Harris H&E stain, Brown Hopps' gram's stain, and McManus PAS stain; alternative or additional stains may include PAS-Giemsa stain and Gomori's silver methenamine stain  
 Coverslip and label

**Table 2. Manual and Automated Techniques for Biopsy Tissue Processing**

Manual		Automated	
Solvent	Duration %	Solvent	Duration (min)
10% Buffered formalin	20	10% Buffered formalin	20
95% Ethanol	5	70% Ethanol	5
100% Ethanol	5	80% Ethanol	5
		95% Ethanol	5
100% Ethanol	5	100% Ethanol	5
( $\frac{1}{2}$ Ethanol)			
50% and	5	100% Ethanol	10
( $\frac{1}{2}$ xylene)			
100% Acetone	5	100% Ethanol	10
( $\frac{1}{2}$ Acetone)			
50% and	7 $\frac{1}{2}$	100% Ethanol	15
( $\frac{1}{2}$ xylene)			
		Histoclear	5
		Histoclear	10
		Histoclear	10
		Paraffin	10
		Paraffin	5
		Paraffin infiltrator	15

**Table 3. Stains for Histologic Slides and Ratings**

Stains	Burn Depth	Purpose*		
		Bacteria	Fungi	Viral
H&E	●	○	○	○
Gram	□	●	○	□
PAS	○	□	●	□
Wright-Giemsa	○	□	○	●
PAS-G	○	□	□	□
Silver methenamine	—	—	●	—
Gridley	—	—	●	—

\*Ratings: ● = excellent; ○ = good; □ = fair; — = poor.

ports indicate that neither method is reliable in differentiating microbial colonization from invasion infection.<sup>20,21</sup> Cultures are useful primarily for identifying the specific infecting microorganisms. Histologic examination of the burn wound biopsy in our experience is the most accurate and reliable method of differentiating wound colorization from burn wound infection and evaluating the burn wound as a source of systemic sepsis.<sup>22,23</sup> Although punch biopsy has been recommended by some authors, incisional biopsy has proved superior, providing adequate tissue for processing, maintaining the integrity of the viable-nonviable tissue interface, and ensuring inclusion of viable tissue in the biopsy sample.<sup>24,25</sup>

There are several important cautions in the preparation of satisfactory slides by the rapid section technique. The tissue sample should be at least 0.5 × 0.5 × 0.5 cm and weigh 100 mg to permit adequate evaluation. The harvested tissue sample should be fixed immediately in the 10% buffered neutral formalin and delivered promptly to the histopathology laboratory. Cutting the tissue into thin sections (1–2 mm) permits adequate fixation in the preheated formalin and proper tissue dehydration during processing. The temperature of the preheated formalin must not exceed 65C to prevent processing-related tissue damage. Manual processing can be completed faster than by the automated method but produces slides of poorer quality. If the automated method is used, we recommend a two-hour rather than a one-hour processing cycle because burn tissue requires longer dehydration for production of good slides. On cutting the paraffin tissue block with the microtome, the section must be 5 $\mu$  or less in thickness, since identification of organisms is difficult if not impossible in thicker sections. Another critical point is proper decolorization of Gram stains, since heavy stain precipitation makes visualization of individual

organisms, particularly gram-negative bacilli, difficult.

Hematoxylin/eosin stains are best for differentiating eschar from viable tissue and estimating burn depth. Although viral inclusion bodies, fungi, and some bacteria can be detected in H&E-stained sections, special stains are always required for confirmation of such organisms. Several fungal stains are available (see Table 3), but we routinely use McManus PAS stain because it is fastest and simplest. Viral study of smears of subvesicular scrapings on glass slides is facilitated by Wright-Giemsa stain.

The pathologist must be wary of artifacts such as stain precipitates, silver particles from topical creams, tattoo stain, melanin granules, and hemosiderin pigments—all of which may be confused with bacteria. Occasionally, the elastic tissue of the dermis may be confused with fungal hyphae; this source of error can be eliminated by examination of both H&E- and PAS-stained sections, since the elastic tissue does not stain in hematoxylin/eosin.

Histologic examination is the preferred method for diagnosis of burn wound infection. It permits discrimination between invasive infection and colonization, a differentiation not possible with other methods. Of the methods available for histologic examination, we prefer the rapid technique. The frozen section technique, when available, requires less time and should be used when speed is of the essence, but any frozen section diagnosis must be verified with permanent sections. Extensive experience with the rapid section method supports our recommendation of its use as the standard for examination of burn wound biopsy material.

## References

1. Pruitt BA Jr: Forces and factors influencing trauma care. 1983

- AAST presidential address. *J Trauma* 24: 463-470, 1984.
2. Moncrief JA, Lindberg RB, Switzer WE, et al: Use of topical antibacterial therapy in treatment of the burn wound. *Arch Surg* 92: 558-565, 1966.
3. Pruitt BA Jr, Curreri PW: Burn wound and its care. *Arch Surg* 103:461-468, 1971.
4. Pruitt BA Jr: Fluid resuscitation for extensively burned patients. *J Trauma* 21 (Suppl 8):690-692, 1981.
5. Mason AD Jr: The mathematics of resuscitation. 1980 AAST presidential address. *J Trauma* 20(12): 1015-1020, 1980.
6. Pruitt BA Jr, McManus AF: Opportunistic infection in severely burned patients. *Am J Med* 76 (Suppl 3A):146-154, 1984.
7. Yurt RW: Increased susceptibility to infection related to extent of burn injury. *Arch Surg* 119:183-188, 1984.
8. Robson MC, Heggors JP: Bacterial quantification of open wounds. *Milit Med* 134:19-24, 1969.
9. Georgiade NG, Lucas MC, O'Fallon WM, et al: A comparison of methods for the quantitation of bacteria in wounds. I. Experimental evaluation, and II. Clinical evaluation. *Am J Clin Pathol* 53:35-42, 1970.
10. Levine NS, Lindberg RB, Mason AD Jr, et al: The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. *J Trauma* 16: 89-94, 1976.
11. Robinson HD, Fayes AW: One-hour processing of tissue. *Technical Bulletin of Registry of Medical Technologies*. 34(12):211-212, 1964.
12. Pruitt BA Jr, Foley FD: The use of biopsies in burn patient care. *Surgery* 73:887-897, 1973.
13. Loebl EC, Marvin JA, Heck EL, et al: The method of quantitative burn wound biopsy cultures and its routine use in the care of the burned patient. *Am J Clin Pathol* 61:20-24, 1974.
14. Baxter, CR, Curreri PW, Marvin JA: The control of burn wound sepsis by the use of quantitative bacteriological studies and subeschar clysis with antibiotics. *Surg Clin North Am* 53:1509-1518, 1973.

15. Brown RC, Hopps HC: Staining method for gram-positive and gram-negative bacteria, in Luna LG (ed): *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, ed 3. New York, McGraw-Hill Book Co., 1968.
16. Magee CM, Rodeheaver G, Edgerton MT, et al: A more reliable gram staining technic for diagnosis of surgical infections. *Am J Surg* 130: 341-346, 1975.
17. McManus JFA: Staining method for gram-positive and gram-negative bacteria, in Luna LG (ed): *Manual of Histologic Staining Methods of The Armed Forces Institute of Pathology*, ed 3. New York, McGraw-Hill, Book Co., 1968, pp 224-225.
18. Lawrence JC, Lilly HA: A quantitative method for investigating the bacteriology of skin: Its application to burns. *Br J Exp Pathol* 53:550-559, 1972.
19. Bharadwaj R, Phadke SA, Joshi BN: Bacteriology of burn wound using the quantitative full thickness biopsy technique. *Indian J Med Res* 78:337-342, 1983.
20. Woolfrey BF, Fox JM, et al: An evaluation of burn wound quantitative microbiology: I: Quantitative eschar cultures. *Am J Clin Pathol* 75:532-537, 1981.
21. Parks DH, Linares HA, Thomson PD: Surgical treatment of burn wound sepsis. *Surg Gynecol Obstet* 153:374-376, 1981.
22. Pruitt BA Jr: Biopsy diagnosis of surgical infections. *N Engl J Med* 310:1737-1738, 1984.
23. Kim SH, Hubbard GB, McManus WF, et al: Frozen section technique early burn wound biopsy: a comparison with the rapid section technique. *J Trauma* (in press).
24. Kahn AM, McGrady VL, Rosen VJ: Burn wound biopsy: Multiple uses in patient management. *Scand J Plast Reconstr Surg* 13:53-56, 1979.
25. Bruck HM, Nash G, Stein JM, et al: Studies on the occurrence and significance of yeasts and fungi in the burn wound. *Ann Surg* 176: 108-110, 1972.

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