

## METHOD OF QUANTITATIVE BACTERIAL COUNT IN BURN WOUND

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### ABSTRACT

**Objective:** To describe a technique for conducting Quantitative bacteriology in burn wounds, which is a very important tool in the management of burn wound sepsis.

**Methodology:** Technique was used in 21 patients with burn wound injury. Biopsy for the bacteriology was taken with the help of punch forceps. Then it was directly put into sterilized pre weighed homogenizer bag containing 1ml normal saline. This bag was re-weighed. Weight of the tissue was obtained and bag was homogenized in homogenizer (Stomacher Lab Blender-80). Four fold dilutions were made of homogenate specimen. From each dilution tube, 0.01ml was then inoculated on blood agar plates, incubated for 24 hours and number of colonies counted by formula.

**Results:** Four specimens were taken each day and repeated on alternate days for up to 6 days. Out of twenty one patients there were 10 male and 11 female with mean age of 25.95 years. Total body surface area burnt was from 9-41%. Bacterial counts obtained varied from 1.45-8.89.

**Conclusion:** Quantitative bacteriology is simple by the above method and should be employed in every public and private sector burn center.

**KEY WORDS:** Burn wound, Burn sepsis, Quantitative bacteriology, Wound biopsy.

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### INTRODUCTION

Infection in the burn wound continues to be the main cause of morbidity and mortality in patients who are admitted to hospital with major thermal injuries. Infection causes 50%

to 60% of deaths in burn patients in spite of intensive therapy with antibiotics both topically as well as intravenous.<sup>1</sup>

Skin is never sterile. It is colonized by two major groups of organisms, its resident flora and transient flora. Although bacteria are not normally recoverable from skin surface or from sweat glands, they can be identified in hair follicles, particularly near the orifices of the sebaceous glands.

Bacteria of resident flora are resistant to heat injury in approximately the same proportion as are skin cells. Those on the surface are heat killed as are the surface cells so that initial swab cultures are usually sterile, however bacteria in the hair follicles and sweat glands, survive and quantitative counts of biopsied specimen show the same  $10^3$  bacteria per gram of tissue as found in the tissue prior to burning.<sup>2</sup>

As the bacteria proliferates following burn injury and reach levels of greater than  $10^5$  bacteria per gram of tissue, they will break out of the hair follicles and glands and begin to

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migrate through the tissue, colonizing along the dermal-subcutaneous interface.<sup>3</sup> Peri-vascular growth is accompanied by thrombosis of vessels, necrosis of any remaining dermal elements, converting partial thickness burn to full thickness loss.<sup>4</sup>

Bacterial proliferation occurs in the subeschar tissue and when level of bacterial growth exceeds  $10^6$  or  $10^7$  invasion into blood stream is more common and causes septicemia.<sup>5</sup>

Multiple studies from United States Army Institute of Surgical Research made invasive burn sepsis synonymous with bacterial level of  $10^5$  or greater than  $10^5$  bacteria per gram of tissue and it's monitoring an important tool in predicting and preventing invasive burn wound sepsis.<sup>6-8</sup>

There are various modalities of monitoring sepsis in burn patients in Pakistan. Among them wound swab and blood cultures are most commonly employed.

Surface wound swabs currently used in many centers in Pakistan do not give the exact count of pathogens involved in burn wound sepsis; they just throw light on the qualitative data.<sup>9,10</sup> Blood cultures on the other hand fail to yield positive results at times.<sup>11,12</sup>

Therefore in order to monitor burn wound, quantitative bacteriology is essential and there should be a technique for monitoring bacterial counts. In this paper, a simple technique, originally published by Lobel<sup>13</sup> in 1973 is described with few modifications.

## METHODOLOGY

Surface of the wound is first washed with normal saline in order to wash topical agents. It is then washed with solution of cetrimide and chlorhexidine (Savlon), in order to remove contaminants. A biopsy for the bacteriology was performed with the help of No.3 or 4 dermal punch. Specimen was then elevated with the help of tooth forceps. Biopsy was directly put into sterilized pre weighed homogenizer bag containing 1ml normal saline (Fig-1). This bag was re-weighed. Weight of the tissue was obtained by subtracting the first weight from the second reading by formula



Figure-1: Homogenizer Bag

$$C=B-A$$

Where C= weight of the tissue  
B=weight of the bag with saline and specimen.  
A=weight of the bag with saline.

The bag was then homogenized in homogenizer (Stomacher Lab Blender-80) (Fig-2) for 30 seconds.

In safety bacteriological chamber (Fig-3), 0.5ml of homogenate is taken from homogenizer bag and is placed in already prepared dilution test tube containing 4.5ml of normal saline. It is properly mixed and then 0.5ml is transferred to second tube of 4.5ml dilution and juster tip is discarded. With new sterilized tip it is again mixed and 0.5ml is transferred to 3<sup>rd</sup> tube and tip is discarded. In this way four dilutions from each parent bag is made i.e. 1/10,1/100,1/1000 and 1/10,000.(Fig-4)

Already prepared blood agar plates are taken and divided into four quadrants as 1/10,1/100,1/1000 and 1/10,000.Each quadrant receive 0.01ml of homogenate, dropped over



Figure-2: Lab Blender Stomacher



Figure-3: Safety Chamber for inoculation

the blood agar plate from a height of 2.5cm from respective test tubes. Plates were then placed in incubator at 37°C for 15 minutes with lids slightly open at one corner. Lids are then replaced back on plates and plates are incubated for 24 hours. The numbers of colonies are counted at the end of 24 hours (Fig.5). Colony count per gram of tissue was obtained by the following formula of Miles & Misra.<sup>14</sup>

$$\text{CFU/gm. of tissue} = C \times D \times 1 / W \times 0.01$$

Where "C" is the total number of CFU, "D" is the dilution factor, "W" is the weight of the tissue, "1" is the volume of normal saline and "0.01" is the volume of inoculum.

### RESULTS

This methodology was applied in 54 patients. One specimens was taken each day and repeated on alternate days for up to six days. In this way in one patient, 4 specimens were taken and in 54 patients, 216 specimens were studied. The bacterial counts obtained were a part of another series.<sup>15</sup>

In addition to these 54 patients, this technique was employed in 21 burn patients, whose wounds were clinically ready for receiving skin



Figure-4: Ten four dilutions of inoculum are made

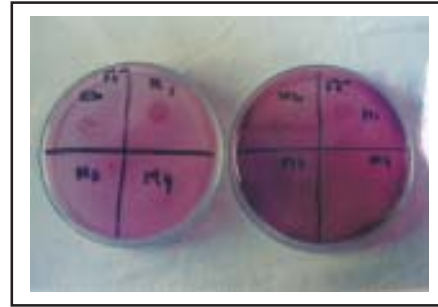


Figure-5: Culture plates with growth

grafts. Their mean age vary from 15 years to 50 years with mean age of 25.95 with SEM ±2.16. The total body surface areas burnt vary from 09% to 41% with a mean burn of 21.33 with SEM ±1.99. The bacterial counts obtained are shown as in Table-I.

### DISCUSSION

At every burn center the main threat of mortality is infection and sepsis. It has been shown by the Pruitt and Currari<sup>16</sup> that organisms which are responsible for sepsis are those which are present in the sub eschar tissue. Due to compromised state of immunity in burn

Table-I: Bacterial counts in log scale along with burn extent, gender and age of the patients.

S.No	Burn Extent (percentage)	Sex		Age (Years)	Bacterial Count (log Scale)
		M	F		
1	17		F	18	7.37
2	33		F	20	4.59
3	35		F	40	4.43
4	41		F	20	4.94
5	09	M		15	6.48
6	22	M		45	7.23
7	15	M		22	8.89
8	20	M		50	4.45
9	29	M		22	5.05
10	21	M		22	6.07
11	25			22	4.48
12	21	M		18	5.00
13	37		F	24	3.76
14	17		F	25	1.45
15	16		F	25	4.57
16	15	M		30	5.98
17	14	M		14	4.90
18	11	M		42	4.98
19	09		F	22	3.09
20	24		F	26	4.85
21	17		F	23	3.99
Mean 21.33		10	11	Mean 25.95	
SEM ± 1.99				SEM ± 2.16	

patients these organisms multiply and when they reach the critical level of  $10^5$  organisms per gram of tissue they pass along blood vessels, cause coagulation and then invade and spread into blood stream causing septicemia.

Therefore main aim is to keep the bacterial count below critical level of  $10^5$  organisms per gram of tissue. This is why those topical anti bacterial creams are preferred in the management of deep burn wound which have power of penetration in the burn eschar like silversulpha diazine and mefinide acetate. Burn wound excision significantly reduces burn wound colonization.<sup>17</sup>

In the light of above pathogenesis of sepsis it is essential to monitor burn wound with regard to quantitative bacteriology.<sup>18</sup> There are various methods of doing this including Dry Surface Swab, Contact plates, Gauze Capillary method, Wound Biopsy, Slide smear technique and Absorbent disc technique.

Dry Surface Swab<sup>19</sup> is most commonly used in burn centers in Pakistan. Dry swabs produce qualitative data. This is an inexpensive technique, but it does not differentiate surface contamination from deeper wound infection. Moreover a swab culture of the surface does not sample the subeschar space where microbial proliferation occurs prior to invasion of underlying viable tissues. Even quantitative swab cultures of the wound surface may be misleading by virtue of either falsely low or falsely high counts.<sup>20</sup> Falsely low counts are mostly commonly due to effect of residual topical agent but may also come about by culturing a non-representative desiccated area of the wound or by desiccation of the swab prior to plating. Falsely high counts can result from culture of sloughing eschar or of pooled wound exudates and from incubation of the swab in transport media prior to plating. Extrinsic contamination is another possibility.

A contact plate involves the contact of the culture media with area of the wound to be studied. Contact plates provide quantitative information and when selective media are used, can produce qualitative information as well.<sup>21</sup> The use of contact plates as quantitative test for bacterial assay of burn wound is limited

because of the confluent growth of the bacteria on it after its application to moist burn areas.<sup>22</sup> Furthermore contact plates take more time to give results and do not sample the subeschar space. Gauze Capillary method was described by Brentano et al in 1966<sup>23</sup> and Brentano & Gravens in 1967.<sup>24</sup> It is superior to dry swab method in recovering the bacteria from the burn wound but it is painstaking and time consuming.

Wound Biopsy is the most reliable and accurate means for monitoring the microbial proliferation of burn wound and diagnosing the incidence of infection in biopsy sampling. At the time of biopsy, tissue should be dissected and one half should be sent to the pathology laboratory where it is processed and examined while the other half is sent for quantitative wound culture.<sup>2,4,8,13,25</sup>

Histological signs characteristic of burn infection are presence of microorganisms in unburned subeschar tissue at viable/nonviable tissue interface, hemorrhage present in unburned subcutaneous tissue, exaggeration of the normally mild inflammatory response present in viable tissue immediately adjacent to the burn, microbial invasion of the small vessels of the specimen and peripheral and perilymphatic proliferation of organisms.

Disadvantage of biopsy method is that it is invasive, needs surgical preparation and is frightening to patients.<sup>26</sup>

Slide smear technique<sup>27</sup> is very much similar to our technique except that after homogenization the homogenate is not inoculated on blood agar plates, instead 0.2ml of homogenate is spread over a glass slide and dried at 45°C for 15 minutes. After the slide is fixed, it is Gram stained and examined under microscope. The presence of single organism in the entire field is regarded as equivalent of the apparently critical level of  $10^5$  bacteria per gram of tissue. Advantage of this technique is that it is simple, rapid and easy to perform. Disadvantage is its dubious accuracy. Absorbent disc technique described by Bruce Williams<sup>28</sup> is the refinement of gauze capillary method.

The method adapted by us is similar to described by Lobel.<sup>13</sup> The difference is that of

homogenization which is essential step for freeing the bacteria embedded in the deep eschar tissue. We have used Lab blender Stomacher 80 which ensures a sterile and safe method. Lawmence<sup>19</sup> in 1972 described an open method. Baxter<sup>4</sup> homogenizes the tissue with the help of knife after suspending it in 1 to 2ml of normal saline. Homogenization can be done with mortar and pestle. Some have used Polytron homogenizer.<sup>27</sup> The other difference is that we have taken biopsy sample with the help of Punch biopsy whereas Lobel has used scalpel. The Technique adapted for the bacterial quantification is that of Miles and Misra.<sup>14</sup>

### CONCLUSION

Quantitative bacteriology of the burn wound should be the main investigative tool for monitoring the bacterial count in burn wound in order to prevent bacterial count reaching the critical level of  $10^5$  per gram of tissue. The method described is simple and can be easily adaptable. We would recommend that it should be employed in every public and private sector burn center in Pakistan.

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