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# This Week's Citation Classic

**Miles A A & Misra S S.** The estimation of the bactericidal power of the blood.
*J. Hygiene* **38**:732-49, 1938.
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**The survival rate, *p*, of a measured inoculum of *Staph. aureus* in a standard volume, of defibrinated blood, is a reliable quantitative measure of the bactericidal power of blood. The number of viable organisms in the inoculum and in the blood-bacterium mixture may be estimated with the necessary accuracy by counts of colonies developing from measured volumes of the fluid let fall on to the surface of solid media. [The *SCI*® indicates that this paper has been cited over 740 times since 1961.]**

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"I suspect, from the references I have come across, that the paper is most commonly cited for the description of the surface-viable count for bacteria. I devised this while at Cambridge in the early 1930s, in the first place for a class demonstration of the titration of phage preparations, the then recommended method was to seed an agar plate to produce a confluent bacterial lawn, and on each quarter of the plate to spread a volume of phage dilution with a glass spreader. This struck me as messy —a lot of spreaders were needed, the bacterial lawn that subsequently grew was smeared and uneven, and an unknown number of phage particles was removed on the spreader. Instead, I seeded 0.02 ml volumes of graded dilutions of phage suspensions on to the lawn from a dropping pipette, and counted the phages in the drop-areas containing discrete plaques.

" The method was obviously applicable to direct viable counts of bacteria, and had the advantage over both the pour-plate or roll-tube counts, not only because all time-consuming manipulations with molten agar at low temperatures were avoided, but because for organisms requiring them, opaque media like blood agar could be used The disadvantage was statistical, because the coefficient of variation of mean counts tends to diminish with increase in the value of the mean. By the surface-viable method, a maximum of 10-30 discrete colonies —depending on the colony size —can be accommodated in the small drop area. It is therefore less easy to obtain significant differences between the means of, say, 3 replicates of counts of 5-30 organisms than with the same number of replicates of counts in the region of the 300 or so countable in pour-plates or roll-tubes of less dilute suspensions. Accordingly, many more replicates of countable drop-areas are required with surface counts to attain the same level of significance. But even with these restrictions the method proved, for many research purposes, to be a useful substitute for the accepted counting methods.

"This was the state of affairs when at the British Postgraduate Medical School in London, S.S. Misra —who later became an eminent physician in Lucknow —began work with me on the bactericidal power of human blood for *Staph. aureus.* We used the surface count both for direct counts of inocula, and after incubation of blood-bacterium mixtures, for counts of the survivors in 0.02 ml drops of the mixture on blood agar plates.

"The biometrician, J. O. Irwin, then at the London School of Hygiene and Tropical Medicine, devised for us a formula for-estimating the standard error of a ratio, which was needed for comparing differing proportions of the inoculum surviving the bactericidal action of blood. We spent a lot of time investigating the possible fallacies in the interpretation of bacterial survival rates in these conditions, and finally tested 26 normal adults and 6 sufferers from chronic staphylococcal infection; the bloods of the latter proved to be significantly far more bactericidal. But before we could apply the technique further, Misra returned to India, and I went to another job.

"From the scientific literature I have subsequently read, the bactericidal method and the statistical considerations in determining the significance of differences in killing rates appear, like the mule, to have nothing to show in the way of offspring; though as far as my own work is concerned they are coming home to roost in some current work on the bactericidal power of mac - rophage populations. The surface-viable count evidently —and gratifyingly—lives on "