

This Week's Citation Classic

Watson S W, Novitsky T J, Quinby H L & Valois F W. Determination of bacterial number and biomass in the marine environment. *Appl. Environ. Microbiol.* 33:940-6, 1977.
[Woods Hole Oceanographic Institution, Woods Hole, MA]

A method was developed for indirectly estimating the biomass (as carbon) of bacteria in aquatic environments. A factor for converting acridine orange direct counting to bacterial biomass was also determined. [The *SCI*® indicates that this paper has been cited in more than 280 publications.]

Horseshoe Crabs and Marine Bacteria

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In the late 1960s, Jake Fine, working at the Marine Biological Laboratory (MBL), Woods Hole, Massachusetts, introduced me to the *Limulus* amoebocyte lysate (LAL) test for endotoxin. The test had recently been described¹ by Frederik B. Bang and Jack Levin, two investigators from Johns Hopkins University who had been working on the blood clotting phenomenon of the North American horseshoe crab (*L. polyphemus*) at the MBL. Since the MBL was just across Eel Pond from my laboratory at the Woods Hole Oceanographic Institution (WHOI), it was a simple matter to learn the technique.

Obtaining horseshoe crabs was easy, since John Valois, whose wife Frederica was one of the coauthors and my senior technician, happened to run the MBL supply department. My plan was to make LAL and use it to assess the purity of the membrane fractions I had isolated from some gram-negative marine nitrifying bacteria. Unfortunately, the LAL Levin and Bang had described in their paper, and the first batches of LAL we made, were not sufficiently sensitive for my application. I decided therefore to "spend a few weeks" trying to improve the sensitivity. These few weeks subsequently resulted in a second career—that of producing LAL commercially (Associates of Cape Cod, Inc.). This early research also led to a patent² and to the publication which is the subject of this article.

Apart from my interest in the role of bacteria in the marine nitrogen cycle, I maintained an additional interest in the marine carbon cycle

and thus in the marine bacterial community as a whole. The only knowledge of the abundance of marine bacteria at this time came from traditional plate count or most probable number techniques. Such techniques grossly underestimated the entire bacterial community, since the number and nature of variables (e.g., temperature, pressure, light, salinity, nutrients, etc.) required to grow all the bacteria was largely unknown or uncontrollable. With the advent of acridine orange direct counting (AODC) techniques,³ enumeration of aquatic bacteria was simplified but did not provide information on the contribution of bacteria to the ocean's biomass (as carbon). In addition, counting bacteria through a microscope (oil immersion) while at sea can be a trying experience!

Our initial attempts comparing the LAL test with bacterial number met with failure. The LAL test as originally described by Levin and Bang was only semiquantitative and could not be precisely compared with analytical chemical techniques (total carbon analysis) or with the AODC. We therefore developed a strictly quantitative modification of the LAL test which used the rate of increase in turbidity which occurred when LAL was added to a sample containing endotoxin. We were also fortunate to learn the AODC test firsthand from John E. Hobbie and were able to use the test with slight modification for our work. Combined with carbon analysis, electron microscopy (for bacterial cell volume measurements), and some luck (the majority of marine bacteria are gram-negative), we succeeded in developing a wet chemistry test for bacterial biomass and an alternative to the AODC for bacterial number in the aquatic environment. Likewise, the biomass conversion factor we determined allows AODC to be easily converted to biomass. In fact, the majority of citations to this article use the conversion factor with AODC. It also has been gratifying to see several publications confirm our biomass estimate by other techniques.

Since our original work, one of the coauthors, Novitsky, subsequently adapted the LAL test to determine bacterial biomass in sediments and used the test for a benchmark survey of the Georges Bank.⁴

1. Levin J & Bang F B. The role of endotoxin in the extracellular coagulation of *Limulus* blood. *Bull. Johns Hopkins Hosp.* 115:265-74, 1964. (Cited 300 times.)
2. Sullivan J D & Watson S W. *Limulus* lysate of improved sensitivity and preparing the same. US patent 4,107,077. 15 August 1978.
3. Hobbie J E, Daley R J & Jasper S. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33:1225-8, 1977. (Cited 1,080 times.) [See also: Hobbie J E. Citation Classic. *Current Contents/Agriculture, Biology & Environmental Sciences* 21(4):14, 22 January 1990.]
4. Hobbie J E, Novitsky T J, Rublee P A & Palumbo A V. Microbiology. (Backus D, ed.) *Georges Bank*. Cambridge, MA: MIT Press, 1987. p. 247-51.