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New Version of the Negative Stain

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We have developed a new version of the negative stain which is very quick, reliable, and easy to perform and which uses a waterproof marking pen instead of nigrosin. It is ideal for teaching the negative staining technique to beginning student microbiologists.

We have developed a new version of the negative stain which does not use nigrosin (1). We have made excellent negative stains of bacteria with a black-ink marking pen.

The procedure is extremely simple. First, a bacterial smear (broth or solid medium cultures worked equally well) was air dried and heat fixed. Second, a blunt-tipped marker was brushed lightly in a straight line over the surface of the smear. Only one coat of ink per section was necessary; it dried almost immediately. Of the varieties of water-soluble and water-resistant ink markers tested, water-resistant black-ink markers produced the best negative stains of bacteria, even with thick smears. The Mighty Mark 7000 (Faber-Castell Corp., Lewisburg, Tenn.) was the marker used for the photographs in this paper.

Samples of *Lineola longa*, a long gram-negative rod, *Enterobacter aerogenes*, a short gram-negative rod, and *Pseudomonas aeruginosa*, a short gram-negative rod, were grown on Difco nutrient agar or in Difco nutrient broth overnight at 37°C in an Equatherm incubator (Curtis Matheson Scientific) at a constant temperature. Smears of broth and solid-medium samples were negatively stained and photographed with a Zeiss bright-field photomicroscope at a total magnification of ×970. Kodak Plus-X Pan film was used with a Nikon N2000 through-the-lens-metering camera; exposure times varied from 1/15 to 1/2 s.

The photographs of *L. longa*, *P. aeruginosa*, and *E. aerogenes* (Fig. 1, 2, and 3, respectively) are good examples of how well the stain works with gram-negative bacteria. (The stain works equally well with gram-positive bacteria.) Both the resolution of the specimens and their contrast against the surroundings were good.

The new negative staining technique was easy to use on bacteria and reliable. The technique should be especially useful to instructors in basic microbiology courses, and in

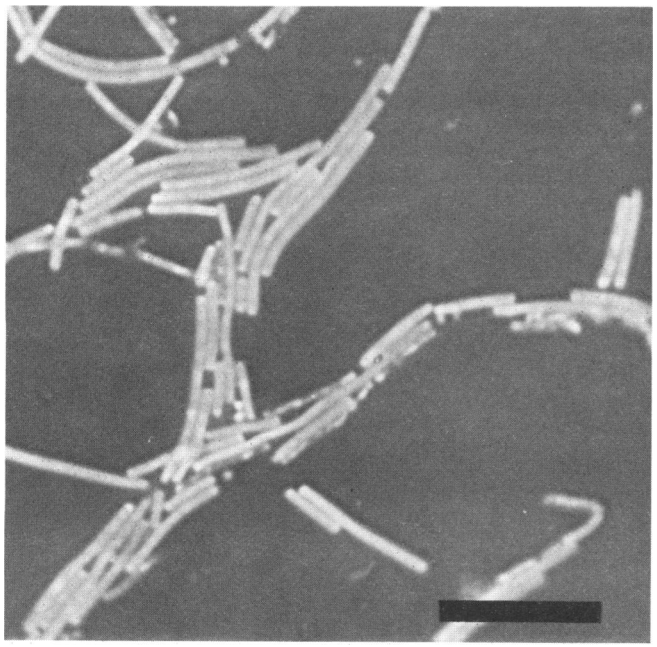

FIG. 1. Negative stain of *L. longa*. Bar = 10 μm.

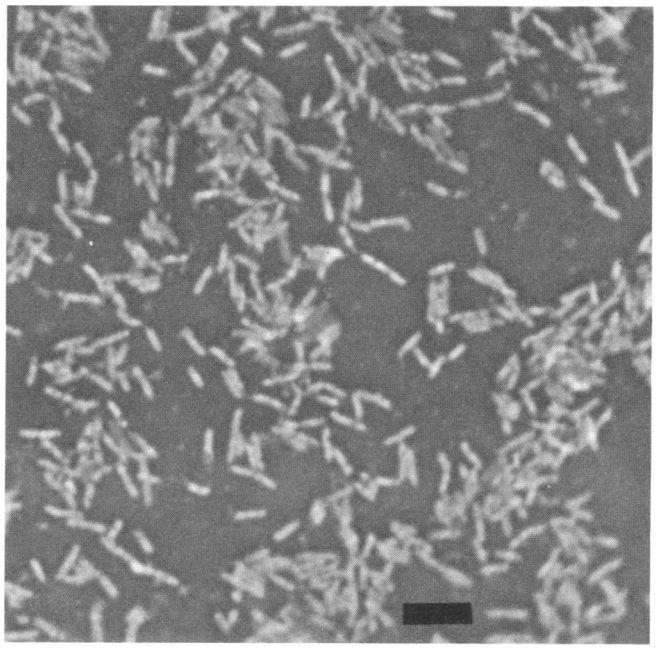

FIG. 2. Negative stain of *P. aeruginosa*. Bar = 4 μm.

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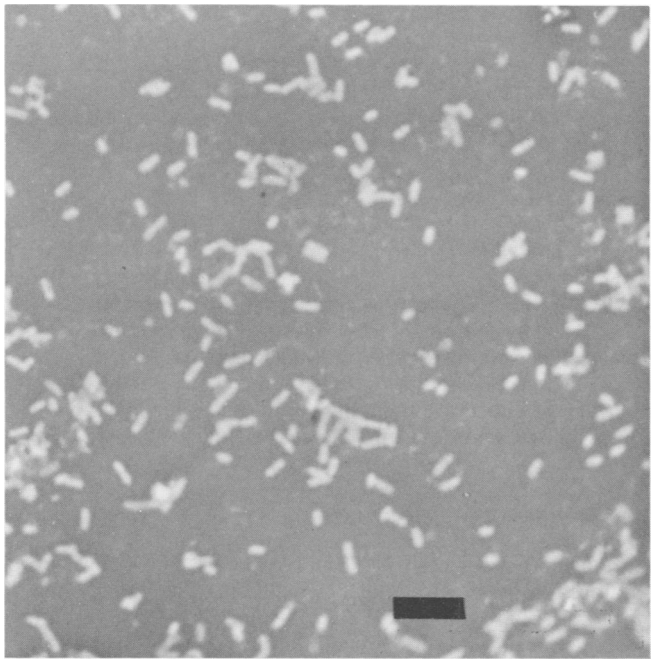

FIG. 3. Negative stain of *E. aerogenes*. Bar = 4 μm.

the future it may assume precedence over the nigrosin technique.

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REFERENCE

1. **Benson, H. J.** 1990. Negative staining, p. 44–45. *In* E. G. Jaffe (ed.), Microbiological applications, 5th ed. William C. Brown publishers, Dubuque, Iowa.