Automated Detection of *Haemophilus influenzae*

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Addition of heme (X factor) and pyridine nucleotide (V factor) to the medium permits rapid growth of *Haemophilus influenzae*, with evolution of easily detectable amounts of $^{14}$CO$_2$. Radiometric media containing X and V factor should be used when evaluating clinical specimens which might contain *Haemophilus* species.

In 1969, workers in this laboratory introduced a system for the automated detection of bacterial growth as measured by the conversion of carbon-14-labeled substrates to $^{14}$CO$_2$ (3). Since that time, preliminary work supporting this concept has been completed on a comparison of the standard and radiometric microbiological techniques in blood cultures (2, 4), radiometric applications to anaerobic microbiology (H. J. DeBlanc et al., submitted for publication), and radiometric detection of antibiotic effect on bacterial growth (1).

These studies demonstrate that the radiometric detection of bacterial growth with $^{14}$C-labeled substrates is rapid and sensitive for many species of bacteria. As more and more clinical experience is accumulated, we may anticipate species for which the detection system is not optimized. For example, streptococci were not predictably identified by the techniques as initially described; however, this species deficit was corrected by substituting a 10% CO$_2$ wash for the room air used earlier.

The present report describes studies of certain *Haemophilus* species, including the important human pathogen, *H. influenzae*, which are not detectable by the standard radiometric technique. The addition of factors X and V to the medium before inoculation of the organism provides a simple solution to the problem.

Preparation of bacterial inocula. The microorganisms listed in Table 1 were chosen for study. These included six species of *H. influenzae*, two of *H. parahaemolyticus*, and two of *H. parainfluenzae*. The organisms were grown overnight on a chocolate agar slant. A 1-ml amount of Trypticase soy broth (TSB) was poured over the slant, and the organisms were suspended in the TSB by agitation with a loop. The organisms were then added to 9 ml of TSB, giving a concentration of $10^8$ organisms/ml.

Further dilutions were subsequently made with TSB so that final organism concentration of bacteria was $10^1, 10^2$, and $10^4$ per ml. Each concentration was subsequently subcultured with quantitative loops onto chocolate agar.

Radiometric detection. To measure bacterial growth radiometrically, bacteria are inoculated into sealed aerobic culture vials containing $^{14}$C-labeled substrates (Aerobic culture vials 6A, JLI) in 30 ml of the TSB. Radioactive $^{14}$CO$_2$ produced by bacterial action was measured in an instrument (Bactec Bacterial Growth Detector, JLI) that allows automatic sampling at hourly intervals. The details of operation of this device will be published elsewhere (H. J. DeBlanc et al., J. Appl. Microbiol., in press).

Preparation of X, V factor aerobic vials. X- and V-factor-impregnated strips (BBL Taxo strips) were added by sterile technique to aerobic culture vials containing 30 ml of TSB and $^{14}$C-labeled substrates. The vials were then stored at room temperature for up to 40 days before use.

Experimental design. Bacteria in concentrations of $10^1, 10^2, 10^4$ were inoculated into aerobic vials with and without added X, V factor. Radiometric growth indices were automatically measured every 2 h. At 24 h, all cultures that were negative radiometrically were subcultured on chocolate agar. In addition, the lowest concentration that was positive radiometrically was also subcultured onto chocolate agar. All organisms were studied radiometrically in plain medium (TSB) only and in plain medium plus X, V factor.

The influence of X and V factors on the detection of *Haemophilus* species by the radiometric method is summarized in Table 1. When bacteria were inoculated into plain medium only, there was no detectable $^{14}$CO$_2$ release by bacterial metabolism. Also, when these
vials were subcultured at 24 h on chocolate agar, there was no growth at any of the concentrations for seven organisms, whereas for an additional four organisms (K-053, J243, 435, L909) there was growth of only a few colonies at concentrations of 10⁴ and 10⁵. When X and V factors were added to the medium, there was rapid radiometric detection of all concentrations for all strains tested. Subculture on chocolate agar was positive for heavy growth from the vials inoculated with 10 organisms on all species tested.

Iron protoporphyrin (X factor) and pyridine nucleotide (V factor) have been widely used to identify and speciate Haemophilus (5). Since V factor is heat labile, this must be added to the medium after autoclaving. Also, it is important that V factor be in the reduced form, and this presents a potential problem for storage and handling. In three earlier, large-scale comparisons of bacteremia, the inability of the radiometric method to detect Haemophilus species was not recognized. In fact, one Haemophilus of unidentified species was detected (4), and there were no radiometric-negative cultures with positive routine culture Haemophilus in any of the series. This may have been due to patient selection, because the samples were obtained from an adult population in which Haemophilus bacteremia is rare. During the course of CSF simulation studies with Haemophilus, this problem was appreciated. Fortunately, detection is made possible simply by adding X and V factors immediately before use. We have found that these additives are stable in TSB for up to 40 days before use when stored at room temperature. We therefore recommend that X and V factor be added to the radiometric medium, especially for specimens which might contain Haemophilus.

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