VOGES-PROSKAUER TEST: EXPEDITIOUS TECHNIQUES FOR ROUTINE USE

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The standard methods of performing the Voges-Proskauer (VP) test (see Eddy, J. Appl. Bacteriol. 24:27, 1961) require an incubation period of 2 days or more. This is an impediment to the utilization of the test, as time is a precious commodity in many situations. Techniques requiring a shorter incubation period have been proposed by Eddy and also by Pickett and Scott (Bacteriol. Proc., p. 110, 1955), but to our knowledge have not found wide acceptance. In this report, we describe two modifications of the VP test which give reliable results without undue loss of time and expenditure of labor.

Our studies showed (i) that a factor related to the volume of medium greatly influences the development of acetoin in the culture, and (ii) that acetoin is present in detectable amounts in the moist surface growth on media containing sucrose or glucose, or both.

Cultures were obtained from clinical material with the exception of ten strains of the Klebsiella-Aerobacter-Serratia group obtained from W. H. Ewing of the Communicable Disease Center, Atlanta, Ga. Identification was made strictly according to the criteria set forth by Edwards and Ewing (Identification of Enterobacteriaceae, 2nd ed., Burgess Publishing Co., Minneapolis, 1962).

Dehydrated culture media were purchased from BBL with the exception of Triple Sugar Iron (TSI) medium, some of which was obtained from Difco, and Worfel-Ferguson medium, which was prepared according to Edwards and Ewing.

The modified O'Meara and Barritt reagents were prepared and used according to Suassuna et al. (Publ. Health Lab. 19:67, 1961).

Four groups of ten test tubes each, containing 0.2, 0.5, 0.75, and 1.0 ml of MR-VP (BBL) broth, were inoculated with 1 drop (0.04 ml) from a 24-hr nutrient broth culture of a Klebsiella strain. One tube of each set was incubated at 37 C and was tested with Barritt reagent hourly for 7 hr and after 1, 2, and 3 days. This experiment was repeated with four different strains. The tubes with 0.2 ml of medium became positive after 2 to 3 hr of incubation, and were found negative when incubated for 24 hr or more. The tubes with 0.5 ml were positive at 3 hr and were negative after 2 and 3 days of incubation. Those with 0.75 ml remained negative after 7 hr of incubation, were found positive at 24 and 48 hr, and were negative after 3 days. Those with 1.0 ml of medium were found positive only at the 48-hr incubation test.

When working with 0.5-ml volumes of medium, testing cannot be delayed much beyond the period of 24-hr incubation. We inoculated each of 20 strains of Klebsiella into several tubes containing 0.5 ml of medium. All strains gave a positive VP reaction after 24 and 26 hr of incubation; after 28 hr, three of them had faded out. Evidently, this is the point when further metabolization of acetoin starts to prevail over its production under the chosen conditions of the test. Thus, it would appear to be prudent not to rely on tests incubated beyond 24 hr.

We assured ourselves that the development of the reaction does not depend on the size of the inoculum or the degree of aeration. The following technique was adopted.

Method A. Tubes containing 0.5-ml amounts of MR-VP broth were prepared and sterilized as directed by the manufacturer. After inoculation, tubes were incubated at 37 C overnight. Next, three drops of \( \alpha \)-naphthol (5% in 95% alcohol) plus one drop of 40% KOH were added. Tubes were read for definite pink or reddish coloration after standing for 5 min. (Shaking did not markedly improve the development of color. Color was stable for several hours.)

As mentioned before, this procedure loses reliability when incubation of the MR-VP medium is extended beyond 24 hr. If circumstances make it impractical to test within this time limit, the standard VP test should be used, or the following alternative method, which is positive with regularity after 24-hr of incubation and remains positive for 3 days, can be employed.
It can also be used to advantage if no MR-VP medium is available.

Method B. After placing two drops of a 0.5% hydrous solution of creatine in a small test tube or on a spot plate, one heavy loopful of culture from the acid part of TSI medium was added. Next, three drops of α-naphthol and two drops of KOH (of solutions specified for method A) were added. After shaking, waiting 5 min, and shaking again, results were read. A positive test was pink to violet in color. No fading was observed for several hours. Bacteria from TSI slants incubated for up to 72 hr could be used, provided material was taken from the acid part of the slant.

The two techniques were compared with the standard procedures of the VP test with both the Barritt and the O'Meara reagents on 567 strains of Enterobacteriaceae, including 169 Escherichia, 251 of the Klebsiella-Aerobacter-Serratia group, and 96 Proteus. Agreement was excellent with the exception of Proteus. In this group, we experienced large differences in the results with the standard procedures employing the Barritt and the O'Meara reagents, and also between the standard procedures and the new modifications. As noted by Suassuna et al., appearance of acetoin in cultures of Proteus, particularly of P. mirabilis, may vary greatly with conditions of medium and time of incubation. Accordingly, the VP test is of little taxonomic value for the Proteus group (Edwards and Ewing, Identification of Enterobacteriaceae, 1962).

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GERMINATION SYSTEM FOR ENDSPORES OF SARCINA UREA

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The presence of endospores in the motile cococcus Sarcina ureae has been reported by others (Gibson, Arch. Mikrobiol. 6:73, 1935; MacDonald and MacDonald, Can. J. Microbiol. 8:795, 1962; Thompson and Leadbetter, Bacteriol. Proc., p. 49, 1962). However, limited information is available concerning the germination characteristics of spores of this organism. The data of MacDonald (M.S. Thesis, Cornell University, Ithaca, N.Y., 1962) indicate that some of the amino acids (aspartic acid, serine, and proline) and also gelatin produced some germination. Complete germination was accomplished only in complex nutrient media.

To obtain spores for germination studies, S. ureae C1 was grown in the medium recommended by MacDonald and MacDonald. After 4 days of incubation at 22 C, the cells were washed from the plates with cold distilled water. The harvested sporangia, in tetrads, were subjected to 0.5 mg/ml of lysozyme in 0.1 M KCl and agitated overnight on a rotary shaker at 3 C. After repeated washing in cold distilled water, single, clean, refractile spores were obtained. The concentration of spores in the cuvettes was initially adjusted to an optical density of 0.3 as determined in a Bausch and Lomb Spectronic-20 colorimeter. Subsequent changes in optical density were recorded at predetermined time intervals. Incubation of the tubes was carried out at room temperature. Germination systems utilizing 25 mm glucose, 5 mm L-alanine, 5 mm α-aminobutyric acid, or 25 mm urea were tested with and without heat shock at 75 C for 10 min, but produced little or no increase in optical density and, hence, essentially no germination. Significant germination occurred in the presence of 40 mm calcium dipicolinate (CaDPA; Riemann and Ordal, Science 133:1703, 1961). However, as the CaDPA was prepared in a cuvette by the reaction of 40 mm Na2DPA and 40 mm CaCl2, 80 mm NaCl was simultaneously produced and was present in this germination system. To determine whether NaCl stimulated...