NOTES

Rapid Test for Acetyl-Methyl-Carbinol Formation by *Enterobacteriaceae*

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A modified Voges-Proskauer test is described which distinguishes within 4 to 8 hours between organisms that can produce acetyl-methyl-carbinol (acetoin) from glucose fermentation and those that cannot.

Gram-negative rods, particularly members of *Enterobacteriaceae*, are the most commonly isolated organisms in clinical microbiology laboratories. Their prevalence and ability to cause disease necessitate rapid and accurate identification, and many biochemical tests have been developed for this purpose. One such test, the Voges-Proskauer (VP) test, is used to distinguish between bacteria that can produce acetyl-methyl-carbinol (acetoin) as an end product of glucose fermentation and those that cannot. The conventional test requires 24 to 48 h. We describe a modification of the VP test which gives results within 1 to 8 h.

The organisms used were 272 strains of gram-negative rods and 2 strains of *Listeria monocytogenes* isolated from clinical specimens identified by conventional means (3, 5) in the clinical laboratory as shown in Tables 1 and 2. The organisms used for the test evaluation consisted of 24-h cultures of the isolates from MacConkey, Mueller-Hinton, or 5% sheep blood (in Trypticase soy agar base) plates. All the media were prepared by using dehydrated media (Difco Laboratories, Detroit, Mich.). The cultures were inoculated to approximately a density of number 3 McFarland standard in 1.2 ml of methyl red/VP broth, prepared in our laboratory by using dehydrated media (Difco). The tubes were incubated at 35°C in a water bath. At 1-, 2-, 4-, and 8-h intervals, 0.2 ml of the suspension was placed in a small tube and VP reagents were added (1). A positive test resulted in the development of a pink to red color within 10 to 20 min.

Results of the positive organisms are shown in Table 1. Of the 65 isolates of *Enterobacter* spp. and *Klebsiella pneumoniae*, 62 (95%) were

positive within 4 h. Isolates of *Aeromonas hydrophila*, *Vibrio cholerae*, *Serratia marcescens*, and *L. monocytogenes* were also positive within 4 h. All other organisms tested gave negative results (Table 2). Since variation in bacteriological media may occur from one lot to another, we tested four different batches of dehydrated MR/VP media, and in all cases correlation with conventional results was 100%.

Several investigators have found that some bacteria can produce neutral end products such as acetylmethylcarbinol (acetoin) and 2,3-butanediol from glucose, and this capability can be used to aid in their identification (6, 7, 9). Acetoin can be oxidized with strong alkali to diacetyl, which gives a color reaction (1, 10). Twenty-four hours to as long as 10 days has been recommended as the incubation time to detect all positive organisms (4). Conventional method requires time and growth of the organism and subsequent degradation of glucose to acetoin, whereas the method described here utilizes the enzymes present in an actively metabolizing culture to convert the glucose to its end products in a very short time (8). The use of heavy inoculum also increases the rapidity of the test. It is not necessary to use sterile materials because the density of organisms prevents overgrowth by contaminants in the time period used. In the 8-h test period, all isolates gave the expected results. When the negative tests were checked at 24 h, 11 of the 18 strains of *Proteus mirabilis* and 4 of the 9 strains of *Acinetobacter calcoaceticus* were positive by the rapid method and negative by the conventional method. All other isolates agreed with conventional results. These delayed false-positive results were not felt to be a significant problem because other tests

TABLE 1. Cumulative numbers of positive organisms tested by the rapid VP method

Organism	No. of isolates	Cumulative no. (%) positive in hours:			
		1	2	4	8
<i>K. pneumoniae</i>	39	14 (37)	27 (69)	37 (94)	39 (100)
<i>Enterobacter cloacae</i>	13	6 (45)	10 (77)	12 (92)	13 (100)
<i>E. aerogenes</i>	10	5 (50)	10 (100)		
<i>E. agglomerans</i>	3	2 (67)	3 (100)		
<i>S. marcescens</i>	12	7 (58)	11 (92)	12 (100)	
<i>A. hydrophila</i>	2	2 (100)			
<i>V. cholerae</i>	1		1 (100)		
<i>L. monocytogenes</i>	2			2 (100)	

TABLE 2. Number of negative organisms tested by the rapid VP method

Organism	No. of strains tested	No. of negative strains in 8 h
<i>E. coli</i>	92	92
<i>P. mirabilis</i>	18	18
<i>P. vulgaris</i>	10	10
<i>P. morganii</i>	12	12
<i>P. rettgeri</i>	12	12
<i>Providencia stuartii</i>	6	6
<i>Edwardsiella tarda</i>	3	3
<i>Salmonella</i> spp., gp. D	2	2
<i>Citrobacter freundii</i>	5	5
<i>A. calcoaceticus</i>	9	9
<i>Pseudomonas aeruginosa</i>	16	16
<i>P. maltophilia</i>	4	4
<i>P. putrefaciens</i>	1	1
<i>Vibrio parahemolyticus</i>	2	2

are more important in identifying these organisms, whereas the VP test is one of the many biochemicals used primarily to separate the *Klebsiella-Enterobacter* group from *Escherichia coli*. The delayed false-positive results with *P. mirabilis* and *A. calcoaceticus* occurred irrespective of whether the colonies were picked from sheep blood agar, MacConkey, or Mueller-Hinton plates.

Benjaminson et al. (2) reported a spot and broth test for rapid acetoin production by *Enterobacteriaceae*. They found that reduction of the volume of medium greatly influenced the VP test; using four different strains of *Klebsiella*, they showed that the test became positive within 2 to 3 h when 0.2 to 0.5 ml of medium was used. However, increasing the volume to 0.75 and 1.0 ml required 7 and 48 h, respectively. They further reported that development of reaction did not depend on the size of inoculum. Our investigations showed that 96% of the organisms

tested gave positive results within 4 h when 1.2 ml of medium was used. Size of the inoculum also influenced the time required for acetoin formation. With light inoculum, 24 to 48 h were required, whereas inoculation to approximate densities of no. 1, 2, and 3 McFarland standards reduced the time to 16, 10, and 4 h, respectively, in over 95% cases. This rapid test represents an improved method of differentiating bacteria by using simple methods and materials already available in the clinical laboratory.

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