

Identification of *Yersinia* spp. with the API 20E System

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The ability of the API 20E system to identify 105 clinical isolates of *Yersinia* spp. was compared with those of conventional biochemical tests at 28 and 37°C. Elimination of the Voges-Proskauer test (recorded as a negative result) increased the percentage of correct identifications for *Yersinia* spp. from 66 to 93% when the API 20E strips were incubated at 28°C.

Since the late 1960s, the number of reported cases of yersiniosis in humans has dramatically increased throughout the world (4, 10). This increase was brought about by greater awareness and increased recognition by clinical microbiologists, as well as the advent of newer, more selective plating media (6, 7, 12) with improved enrichment and isolation techniques (9, 11, 13).

Differences in pathogenic potential among the *Yersinia* spp. may make it important to distinguish these organisms. Since *Yersinia* spp. may express biochemical differences at temperatures above and below 30°C, misidentifications can occur with kit systems designed for identification of members of the family *Enterobacteriaceae* at 35 to 37°C (3, 5). Our study was performed to evaluate the ability of the API 20E system (Analytab Products, Plainview, N.Y.) to identify *Yersinia* spp. at 28 and 37°C and to determine some means to increase the percentage of correct identifications.

A total of 105 clinical isolates of *Yersinia* spp., including 65 *Y. enterocolitica*, 15 *Y. intermedia*, 15 *Y. frederiksenii*, 8 *Y. kristensenii*, and 2 *Y. pseudotuberculosis*, were tested with the API 20E kit system at 28 and 37°C. The *Y. enterocolitica* strains were biotyped according to the methods of Wauters (G. Wauters, these d'agregé, University Catholique de Louvain, Vander, Belgium, 1970). There were 30 biotype 1, 5 biotype 2, 15 biotype 3, and 15 biotype 4 *Y. enterocolitica* isolates. The identification of each isolate was confirmed at 28°C according to the conventional biochemical methods of Bercovier et al. (2). The following tests were performed at 28 and 37°C: motility, methyl red, and Voges-Proskauer (VP); and tests for urease, indole, oxidase, Simmons citrate, lysine and ornithine decarboxylase, arginine dihydrolase; β -galactosidase (*o*-nitrophenyl- β -D-galactopyranoside); lipase (Tween 80); DNase; and acid production from D-glucose, lactose, sucrose, maltose, D-mannitol, dulcitol, salicin, adonitol, inositol, D-raffinose, D-trehalose, D-xylose, α -methyl-D-glucoside, D-melibiose, and esculin were also done.

The API 20E tests were performed according to the instructions of the manufacturer, except for a slight modification. Briefly, inocula of *Yersinia* spp. were prepared from blood agar plates incubated overnight at 28°C. A suspension of each organism was made in a 0.85% saline solution and adjusted to a 0.5 McFarland standard. Duplicate API 20E strips were then inoculated and incubated at 28 and 37°C for 18 to 24 h. The biochemical tests were determined, and the

API 20E profile codes were compared with those listed in the Analytical Profile Index.

The temperature of incubation affected the identification of the isolates of *Yersinia* spp. by the API 20E strips (Table 1). Misidentification of genera was not detected. Overall, only 66 and 51% of the *Yersinia* isolates incubated at 28 and 37°C, respectively, were correctly identified by the strips compared with identification by the conventional biochemical tests. In a similar study, Eckwall and Dimander (8) found that 15 of 42 (36%) *Y. enterocolitica* strains at 37°C were not identified successfully by the API 20E system. In our study, *Y. enterocolitica* biotypes 1 and 2 at 28 or 37°C were clearly identified by the strips but biotypes 3 and 4 were not as successfully identified. Less than 50% of biotypes 3 and 4 were correctly identified at either temperature (Table 1). *Y. intermedia* was correctly identified (93%) at 28°C but not (0%) at 37°C. Temperature of incubation had less of an effect on the identification of the remaining species.

The API 20E strips frequently misidentified the *Yersinia* isolates, except *Y. enterocolitica* biotypes 1 and 2, and produced profile codes not listed in the Analytical Profile Index. Fourteen (93%) of the *Y. intermedia* and eight (67%) of the *Y. frederiksenii* were identified as *Y. enterocolitica* at 37°C. These organisms had profile codes listed in the API 20E Analytical Profile Index as "*Yersinia enterocolitica*—Excellent Identification." *Y. kristensenii*, *Y. pseudotuberculosis*, and *Y. enterocolitica* biotypes 3 and 4 also had codes at 28 and 37°C not listed in the API 20E Analytical Profile Index. The inability to ferment melibiose and rhamnose at 37°C resulted in most of the incorrect identifications. Altwegg et al. (1) also noted differences in intensities of reactions of these carbohydrates at 37°C. In contrast, no misidentifications occurred at 28°C except for one strain of *Y. frederiksenii* which was identified as *Y. enterocolitica*.

Although the API 20E Analytical Profile Index did not have codes to include the indole-negative strains of *Y. enterocolitica* (biotypes 3 and 4), the correct identification was increased from 27 and 47, respectively, to 93% by recording the VP test as negative at 28°C (Table 1). Similarly, the correct identification of *Y. frederiksenii* was increased from 13 to 87%. Identification of *Y. intermedia*, *Y. kristensenii*, and *Y. pseudotuberculosis* did not change significantly with or without inclusion of the VP test.

Most clinical laboratories incubate and identify enteric cultures with kit systems at 35 to 37°C. This choice of temperatures may result in misdiagnosed cases or false diagnoses of yersiniosis. Laboratory identification of *Yersinia* spp. is enhanced by an accurate case history, growth

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TABLE 1. Effect of temperature and recording the VP test as negative for identification of 105 *Yersinia* isolates by the API-20E system

Organism (no. of isolates)	% Correct identifications		
	37°C	28°C	28°C recording VP as negative
<i>Y. enterocolitica</i> , biotype 1 (30)	97	100	100
<i>Y. enterocolitica</i> , biotype 2 (5)	100	100	100
<i>Y. enterocolitica</i> , biotype 3 (15)	47	27	93
<i>Y. enterocolitica</i> , biotype 4 (15)	27	47	93
<i>Y. intermedia</i> (15)	0	93	93
<i>Y. frederiksenii</i> (15)	20	13	87
<i>Y. kristensenii</i> (8)	50	75	88
<i>Y. pseudotuberculosis</i> (2)	100	50	50
Total	51	66	93

on selective plating media, and absence of other enteric pathogens. In addition, more profile codes are needed in the API 20E Analytical Profile Index to increase the correct identification of the *Yersinia* spp. at 35 to 37°C. The user is instructed to contact the API Computer Service to obtain the latest information for identification of *Yersinia* spp. In the absence of sufficient profile codes, the identification of suspected *Yersinia* spp. can be significantly enhanced by incubating the API 20E strips at 28°C and recording the VP test result as negative.

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