Comparison of Systems for Identification and Differentiation of Species within the Genus *Yersinia*

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Of four tested identification systems (API 20E, API Rapid 32 IDE, Micronaut E, and the PCR-based *Yersinia enterocolitica* Amplification Set), API 20E is still the system of choice for identifying pathogenic *Yersinia* isolates. It provides the highest sensitivity both at the genus and at the species level and has the best cost-effectiveness correlation.

The genus *Yersinia* consists of 11 species. *Y. pseudotuberculosis* and some biovar-serovar combinations of *Y. enterocolitica* are pathogenic for warm-blooded animals and humans. They cause a complex clinical picture known as yersiniosis (1). Slide agglutination tests for the most prevalent pathogenic serovars in combination with identification systems based on biochemical properties are used frequently in routine diagnostic practice. The major disadvantage of this technique is the presence of antigens O:3 and O:9 also in four nonenteropathogenic species, including *Y. intermedia* (1). Members of apathogenic species can be isolated from meat and milk of animals and from the stool or blood of symptomatic and asymptomatic humans (2, 4–7, 20, 21). Hence, correct typing depends on the knowledge of the reliability of the test system used.

In the present study, the identifications (by four systems) of 118 phenetically typed strains (1, 8) from 10 *Yersinia* species (i.e., all except *Y. pestis*) were compared. API 20E (Bio-Merieux, Nürtingen, Germany), API Rapid 32 IDE (Bio-Merieux), and Micronaut E (Merlin, Bornheim-Hersel, Germany) were used as instructed by the manufacturers, except for the incubation of API 20E strips at 28°C (3). For use in the *Yersinia enterocolitica* Amplification Set (Kreatech, Amsterdam, The Netherlands), *Yersinia* strains were incubated in Mueller-Hinton broth at 28°C for 12 h, and a 0.5-μl aliquot was added to a 50-μl PCR mixture containing 5× PCR optimization buffer N (Invitrogen, DeShelp, The Netherlands), PCR Nucleotide Mix (Roche Diagnostics, Boehringer, Mannheim, Germany), and *Taq* polymerase (Applied Biosystems, Weiterstadt, Germany). Optimization of cycling conditions resulted in initial denaturation for 10 min at 94°C and 30 cycles each consisting of denaturation (1 min, 94°C), annealing (1 min, 55°C), and elongation (1 min, 72°C). Labeling and dot blot hybridization (stringent washing at 45°C) were done as described previously (15, 18).

The API 20E system is considered the "gold standard" (9, 13, 14, 17) to which new identification systems have to be compared. For this assay we found an overall sensitivity of 79% (Table 1). Differentiation at the genus level was 91%. All pathogenic *Y. enterocolitica* strains and 95% of apathogenic strains

were correctly identified, resulting in a sensitivity of 96% for the species *Y. enterocolitica*. Sensitivity for *Y. pseudotuberculosis* was 90%. Of the *Y. intermedia* strains, 70% were misidentified. The time needed to get results was 25 h. Our results are in agreement with three preliminary investigations. Archer et al. reported that recording a negative result for the Voges-Proskaner test enhances the sensitivity for *Yersinia* spp. from 66 to 93% (3). Sharma et al. found a sensitivity of 90% for *Yersinia* spp. (19). The sensitivity for *Y. intermedia* was considered to be unacceptable (19). In both studies, only clinical isolates were examined and pathogenicity was not investigated. O'Hara et al. tested 30 *Yersinia* strains, resulting in a sensitivity of 70 or 94% when results were read after 24 or 48 h, respectively (16). The API 20E system in combination with slide agglutination tests is therefore suited for routine detection of pathogenic *Yersinia* isolates. The overall sensitivity of the API Rapid 32 IDE was 86% at the genus level but only 42% at the species level. It identified 92% of the pathogenic *Y. enterocolitica* strains and 85% of the *Y. pseudotuberculosis* strains. An incubation temperature of 28°C increased the number of nonidentified isolates, as could be expected on the basis of reaction kinetics. The low sensitivity might be caused by the weak metabolic activities of the members of the genus. Results were obtained after 5 h. The Micronaut E system is comparable to API 20E in its sensitivity at the genus level (92%) and at the species level (72%). Of the *Y. intermedia* strains, 94% were misclassified. The codes for *Y. intermedia* biotypes have to be revised. Due to the computer program used, reading of the results occurs after 24 h. Giving only "yes" or "no" answers, the *Yersinia enterocolitica* Amplification Set provides significantly less information than the other systems. The sequences of the sets' primers and gene probe are not available. The PCR product is approximately 400 bp long. The overall sensitivity of 85% mimics the actual sensitivity of 80% for the species *Y. enterocolitica* and *Y. intermedia*, causing false-positive reactions. A total of 14 apathogenic *Y. enterocolitica* and 3 *Y. intermedia* strains were misidentified. The reason for this loss in sensitivity may be the presence of various 16S rRNA genomospecies in the investigated strains (10–12). Further drawbacks are the recommended procedure of end labeling the provided oligonucleotide and the lack of DNA of type strains for optimization. The test procedure is time-consuming (two working days) and sophisticated and therefore not suited for routine diagnosis. API 20E turned out to be the most cost-effective test, fol-

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lowed by Micronaut E, API Rapid 32 IDE, and the PCR assay. The reading and computing device for the Micronaut E system is essential and has to be considered as an important cost factor. Detection of pathogenic *Yersinia* isolates is a problem not only for physicians and veterinarians but also for anyone dealing with food and water hygiene. Although reclassification of the genus *Yersinia* was completed in 1988, resulting in seven new species (2, 4, 5, 7, 20, 21), this increase in number is not reflected in the indices of the identification kits. The API 20E index lists seven species, and the API Rapid 32 IDE and Micronaut E indices list only six species each. A correction is overdue. The close relationship of various *Yersinia* species with regard to biochemical characteristics requires an optimal combination of key reactions for differentiation at the species level. None of the four kits tested in the present study solved this problem completely. The most important reactions (Simmons citrate, sorbose, saccharose, melibiose, and rhamnose) for distinguishing *Yersinia* species are not present or are simply interpreted divergently. Additional tube testing should be advised in the indices of the kits. Compared to the API 20E or Merlin E system, there is no advantage in using diagnostic PCR systems based on 16S rRNA gene sequences as long as no clear definition of the connection between genomospecies and phenotypic species exists. Still, seeking the expertise of a reference center is advised in cases of doubt.

TABLE 1. Identification of *Yersinia* species by different identification systems

Species as determined by conventional testing (n)	No. of correct identifications by:				No. (species) of incorrect identifications by:			
	API 20E	API Rapid 32E	Micronaut E	YeAS[a]	API 20E	API Rapid 32E	Micronaut E	YeAS
Y. enterocolitica[T] (1)	1	1	1					1 (NT)
Y. enterocolitica[b] (12)	12	11	12	12		1 (*Hafnia alvei*)		
Y. enterocolitica[c] (58)	55	5	52	44	2 (NT[d]), 1 (*Hafnia alvei*)	6 (NT), 42 (*Yersinia intermedia*), 4 (*Yersinia frederiksenii*), 1 (*Hafnia alvei*)	3 (NT), 2 (*Hafnia alvei*), 1 (*Serratia* sp.)	12 (*Yersinia frederiksenii*), 2 (NT)
Y. pseudotuberculosis (20)	18	17	17	20	2 (NT)	3 (NT)	3 (NT)	
Y. intermedia (17)	3	12	1	14	12 (*Yersinia enterocolitica*), 2 (NT)	4 (NT), 1 (*Yersinia frederiksenii*)	13 (*Yersinia frederiksenii*), 2 (NT), 1 (*Yersinia enterocolitica*)	3 (*Yersinia frederiksenii*)
Other *Yersinia* species (10)	4	4	2	10	1[e] (NT), 3 (*Yersinia enterocolitica*), 2 (NT)	3 (*Yersinia frederiksenii*), 2 (NT), 1 (NT)	4 (*Yersinia enterocolitica*), 1 (NT), 1 (*Yersinia kristensenii*), 1 (*Salmonella pullorum*), 1 (*Citrobacter* sp.)	
Total[f] (118)	93	50	85	100	25	68	33	18

[a] YeAS, *Yersinia enterocolitica* Amplification Set.
[b] *Y. enterocolitica* strains of enteropathogenic serovars.
[c] *Y. enterocolitica* strains of apathogenic serovars.
[d] NT, not typeable.
[e] Indicated as differential diagnosis.
[f] Total percent correct identifications by API 20E, API Rapid 32E, Micronaut E, and the *Yersinia enterocolitica* Amplification set were 79, 42, 72, and 85%, respectively.

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