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Evaluation of a Qualitative Isocitrate Lyase Assay for Rapid Presumptive Identification of *Yersinia pestis* Cultures

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Qualitative rapid tests for isocitrate lyase activity were positive for all of 1,695 *Yersinia pestis* cultures tested but for none of 382 cultures of other gram-negative species. The test is simple, required only commonly available, easily prepared reagents, and provided clear-cut positive or negative results within 20 min.

Hillier and Charnetzky (1, 2) reported high levels of isocitrate lyase activity in cultures of the plague organism, *Yersinia pestis*. A rapid chemical assay for this enzyme, which yielded positive results for all 108 *Y. pestis* cultures tested but for less than 2% of 1,608 cultures of other enteric bacterial species, was described by them and recommended for use in screening procedures to presumptively identify *Y. pestis*.

Rapid, sensitive, specific laboratory tests for reliable presumptive identification of this agent are desirable, especially in those cases in which (i) specific antibiotic therapy may be delayed until laboratory tests would support the diagnosis or (ii) instituting expensive, often labor-intensive, environmental control measures awaits some verification of the diagnosis of a patient's illness as plague. Often only state or provincial health department laboratories or reference laboratories are equipped to perform currently available presumptive or confirmatory identification tests for *Y. pestis* (e.g., fluorescent-antibody, bacteriophage sensitivity, or bacterial agglutination tests).

The purpose of the short study reported here was to evaluate the isocitrate lyase assay for possible recommendation as an adjunct test at the initial clinical laboratory to presumptively identify *Y. pestis*.

The assay for isocitrate lyase, actually a test for production of glyoxylic phenylhydrazone performed by the method described by Hillier and Charnetzky, may be briefly outlined as follows:

(i) Organisms are suspended in glass test tubes (13 by 100 mm) containing 0.1 ml of MOPS-Triton buffer (0.1 M morpholinepropanesulfonic acid [pH 7.7], 5 mM $MgCl_2$, 1 mM Na_2Ca–EDTA, 0.2% Triton X-100). We used a loopful of organisms grown 24 h on blood agar at 28 to 37°C. (ii) Add 0.1 ml of DL-isocitric acid (8 μM) in MOPS-Triton. (iii) Incubate 15 min at ambient temperature (25 to 27°C). (iv) Add 0.05 ml of phenylhydrazine-HCl in 0.4 mM oxalic acid. (v) Heat suspension to boiling and cool immediately by plunging the test tube into an ice water bath. (vi) Add 0.1 ml of 12 N HCl and mix. (vii) Add 0.05 ml of 5% potassium ferricyanide [$K_3Fe(CN)_6$] in water.

Glyoxylic acid, one product of the cleavage of isocitric acid by isocitric lyase, combines with the phenylhydrazine to form glyoxylic phenylhydrazone, which reacts with $K_3Fe(CN)_6$ to form a red precipitate.

Blood agar cultures prepared as viability checks for lyophilized suspensions were available for 1,695 isolates of *Y. pestis* from human, mammalian, and ectoparasite specimens from the United States, South America (Brazil, Bolivia, Ecuador, Peru), Africa (Kenya, South Africa, and Madagascar), the Middle East (Israel, Iran, Yemen), and Asia (USSR, India, Vietnam, Burma, Manchuria). Some were laboratory cultures maintained over long periods of time; some were avirulent, but most were virulent.

Organisms of other species studied included *Yersinia pseudotuberculosis* (72), *Y. enterocolitica* (170), *Salmonella typhimurium* (2), *Salmonella* sp. (1), *Enterobacter cloacae* (1), *Enterobacter agglomerans* (2), *Klebsiella oxytoca* (1), *Klebsiella ozaenae* (1), *Escherichia coli* (3), *Hafnia alvei* (1), *Serratia marcescens* (9), *Francisella tularensis* (43), *Pasteurella multocida* (70), *Aeromonas hydrophila* (2), and *Pseudomonas* sp. (3). (The strains of *K. oxytoca*, *K. ozaenae*, and *E. agglomerans* originally had been misidentified as *Y. pestis*.)

The organisms studied were mostly wild-type cultures isolated at this laboratory from experimental laboratory mice inoculated with test specimens during plague surveillance studies. Many of the *Y. enterocolitica* cultures were obtained from collections of other researchers

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TABLE 1. Results of qualitative isocitrate lyase tests

Culture	No. tested	No. positive (%)
Y. pestis	1,695	1,695 (100)
Y. pseudotuberculosis	72	0 (0)
Y. enterocolitica	170	0 (0)
F. tularensis	43	0 (0)
S. typhimurium	2	0 (0)
Salmonella sp.	1	0 (0)
E. agglomerans[a]	2	0 (0)
E. cloacae	1	0 (0)
K. oxytoca[a]	1	0 (0)
K. ozaenae[a]	1	0 (0)
E. coli	3	0 (0)
A. hydrophila	2	0 (0)
Pseudomonas sp.	3	0 (0)
H. alvei	1	0 (0)
S. marcescens	9	0 (0)
C. freundii	1	0 (0)
P. multocida	70	0 (0)

[a] Strains received originally identified as *Y. pestis*.

and were isolated from human, animal, and environmental (water, soil, or food) specimens. (A complete list and description of cultures studied may be obtained from the senior author.)

Positive results were found for all 1,695 cultures of *Y. pestis*. Negative results were obtained for all 382 cultures of the other bacterial species (Table 1).

The assay proved simple to perform, relatively inexpensive, and required only commonly available, easily prepared reagents. Results were clear-cut and readily interpreted as positive (intense red) or negative (yellow).

The age of the culture at the time of testing for lyase activity appeared to be critical. We did not see positive results for cultures of organisms other than *Y. pestis* except when cultures older than 24 to 30 h were used. Positive results were seen with 20 of 36 strains of *Y. enterocolitica* and 1 of 12 strains of *Y. pseudotuberculosis* inadvertently allowed to incubate for 48 h before performing the assay. Results of tests of 24-h-old subcultures of each of these strains were negative. The results reported in Table 1 are for cultures 18 to 24 h old, only. Hillier and Charnetzky (1) reported that about 2% of cultures of other enteric species gave positive results. Not all of the nonplague organisms possessing the isocitrate lyase would be easily confused with *Y. pestis* in culture, and differentiation would be possible with other tests, probably already used in initial screenings (colonial size and morphology characteristics, stalactite-type growth in broth, lack of motility, phage sensitivity, and biochemical reactions).

Miniature biochemical test kits, available from a variety of commercial sources, have offered rapid, usually reliable, identification of enteric organisms. However, misidentifications, both of nonplague organisms as *Y. pestis* (e.g., *E. agglomerans, K. oxytoca, K. ozaenae, Shigella sonnei*) and of *Y. pestis* as other species, occur often enough to warrant including another test to corroborate the identification.

Our results with the isocitrate lyase assay support those of Hillier and Charnetzky, and therefore we endorse their recommendation that this test be added to those used for presumptively identifying cultures as *Y. pestis* at the initial clinical laboratory. However, because plague remains an internationally notifiable disease, cultures of organisms identified as *Y. pestis* at local clinical laboratories should be sent to state, provincial, federal, or national health department reference laboratories for confirmatory tests and reports.

LITERATURE CITED

1. **Hillier, S. L., and W. T. Charnetzky.** 1981. Glyoxylate bypass enzymes in *Yersinia* species and multiple forms of isocitrate lyase in *Yersinia pestis*. J. Bacteriol. **145**:452–468.
2. **Hillier, S. L., and W. T. Charnetzky.** 1981. A rapid diagnostic test for *Yersinia pestis* based on isocitrate lyase activity. J. Clin. Microbiol. **13**:661–665.