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New Method for Early Diagnosis of Celebes Type El Tor Vibrio Carriers

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TABLE 1. *Production of κ-type phage in peptone water culture of Celebes type El Tor vibrio mixed with human feces*

Expt	Initial population of Celebes type El Tor vibrios/ml	No. of plaque-forming units/ml at various incubation times (hr)							
		1	2	3	4	5	6	7	8
A	1.6×10^4	<10 (−)*	6.8×10^2 (+)	1.2×10^3 (++)	1.6×10^3 (++)	1.4×10^4 (+++)	7.6×10^4 (+++)	1.6×10^5 (+++)	6.0×10^5 (+++)
	1.0×10^3	11 (−)	62 (+)	1.4×10^2 (+)	3.0×10^2 (+)	2.2×10^3 (++)	1.3×10^4 (+++)	3.1×10^4 (+++)	4.2×10^5 (+++)
B	1.8×10^2	<10 (−)	<10 (−)	<10 (−)	15 (−)	20 (+)	2.0×10^2 (++)	1.1×10^4 (+++)	— (+++)
	4×10^1	<10 (−)	<10 (−)	<10 (−)	<10 (−)	$<10^2$ (−)	1.0×10^2 (++)	1.0×10^3 (+++)	1.3×10^4 (+++)

*Symbols in parentheses indicate the results of the spot test: −, no lysis; +, several plaques; ++, multiple plaques; +++, complete lysis. The spot test was carried out as follows: a small drop of supernatant of the culture, after centrifugation at 4,000 rev/min for 40 min, was spotted on a plate previously inoculated with indicator organism suspended in semisolid agar.

TABLE 2. *Comparison between the sensitivity of the isolation method of El Tor vibrios and the detection method of κ-type phage after enrichment culture for 8 hr in peptone water containing human feces*

Initial population of Celebes type El Tor vibrios/ml	No. of vibrio colonies detected by isolation culture on agar plates*	No. of plaque-forming units/ml	Spot test
3.6×10^2	—	1.6×10^4	+++
3.6×10^1	5	6.4×10^2	++
3.6	0	2.9×10^2	++
0.4	0	24	+

* Plates of alkaline agar media (*p*H 7.8) were "double streaked" for isolation.

Recently, Takeya and Shimodori (J. Bacteriol. **85**:957, 1963) reported that every known strain of Celebes type El Tor vibrio is lysogenic and produces a characteristic temperate phage which has a very narrow host range. Further studies have revealed that 136 of 138 strains of El Tor vibrio isolated in the epidemic of the West Pacific from 1959 to 1963 were lysogenic and that all the temperate phages liberated from these strains were closely related serologically (Takeya et al., *unpublished data*). Therefore, the temperate phage from Celebes type El Tor vibrio has been tentatively designated as κ-type phage. Our experiences during quarantine work in Japan in the 1963 epidemic showed that the detection of κ-type phage from specimens is a useful method for the early diagnosis of Celebes type El Tor vibrio carriers, because one patient and seven carriers were detected by this method prior to the isolation of the vibrio. Preliminary experimental results to confirm our findings are described here.

Various numbers of cells of Celebes type El Tor vibrio, strain Itazuke, were inoculated in 10 ml of alkaline peptone water (1% peptone, 0.5% NaCl, *p*H 8.2) containing 0.5 g of human feces, and were incubated at 37 C (Table 1). At 1-hr intervals, the culture was centrifuged and the supernatant fluid was subjected to both the "spot test" and the plaque-count method for determining the presence of κ-type phage. *Vibrio comma* strain H218 was used as the indicator strain. After 6 hr of incubation, the presence of κ-type phage could be detected when as few as

400 cells were originally present in 10 ml of culture medium.

The second experiment was carried out to compare the sensitivity of the κ-type phage detection method with that of actual isolation of the vibrio. The results (Table 2) indicated that the κ-type phage detection method is more sensitive than the actual isolation of the vibrio.

The spot test has been adopted for routine work, since this method is much less time-consuming than the plaque-count method and is equally sensitive. Only 8 hr of incubation are necessary to read results with the spot test. In contrast to this, actual isolation of vibrios takes about 12 hr of incubation. Accordingly, early diagnosis of the presence of the vibrio can be made by the spot test. The possible presence of bacteriophages active on the indicator strain H218 will interfere with the results. However, healthy human fecal specimens so far examined did not liberate phages active on the indicator strain. Moreover, the use of a strain of H218 resistant to κ-type phage or the use of immune serum for κ-type phage, or both, can be expected to eliminate such confusion. Obviously, final decision should be postponed until the actual isolation of the vibrio. However, the phage detection method is considered to be a useful accessory method for the early and sensitive diagnosis of Celebes type El Tor vibrio carriers.

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