Notes

A Presumptive Test for Vibrios: the "String" Test*

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Cholera diagnostic serum offers the most reliable and rapid means of identification of *Vibrio cholerae* but it is not always available when and where it is needed. A chance observation in the laboratory led to the development of a test for the presumptive identification of vibrios that is well suited for field studies.

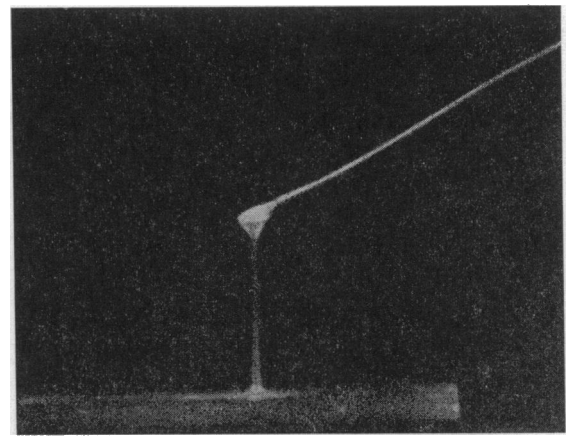
Materials and methods

Stock strains of organisms maintained in the laboratory and freshly isolated strains from patients were used. The vast majority of cultures employed were vibrios—over 2000 different strains of *Vibrio cholerae* and more than 3000 strains of non-cholera vibrios. Also included were strains of other enteric bacteria listed in the accompanying table.

The cellular morphology was determined by microscopic examination of smears stained by the Gram technique. Serological tests were conducted using sera prepared in our laboratory against cholera and non-cholera vibrios (Smith & Goodner, 1965).

Cultures were streaked and isolated colonies were tested after 18–24 hours' incubation at 33°C. Cells from a single colony were smeared on two separate areas on a microscope slide. A loopful of 0.5% sodium deoxycholate in saline was added to one smear and saline to the other. The test was positive if the deoxycholate suspension lost turbidity, gave a mucoid appearance when mixed and formed a "string" when the loop was slowly withdrawn from the drop of suspended organisms (see the figure). The test was recorded as negative when the cells were evenly suspended in the deoxycholate solution plus saline and no "string" was formed. Results were recorded within one minute.

EXAMPLE OF A POSITIVE "STRING" TEST



Results

All strains of *V. cholerae*, as determined by agglutination with group- and type-specific sera, gave positive "string" tests. These strains included organisms designated "classical" and "El Tor" cholera vibrios. Rugose colonies of *V. cholerae* gave weaker positive reactions than smooth colonies.

Gram-negative, curved rods which failed to agglutinate in the cholera diagnostic sera—i.e., non-cholera vibrios—were positive to varying degrees in the string test. These non-cholera vibrios produced a finer and shorter string when the loop was lifted than that observed with *V. cholerae*.

A group of 70 organisms designated as *Vibrio parahaemolyticus* by Japanese workers failed to give positive string tests under any conditions attempted.

None of the other enteric bacteria employed gave a positive test with the deoxycholate solution.

The results are summarized in the table.

Discussion

The "string" test has been found useful in field work. Under conditions which make storage of cholera diagnostic sera impracticable the test has

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SUMMARY OF RESULTS WITH THE "STRING" TEST

Organisms	"String" test [a]
Vibrios:	
V. cholerae (classical or El Tor)	+++
Non-cholera vibrios	+ to +++
V. parahaemolyticus	−
Other enteric bacteria:	
Escherichia	−
Aerobacter	−
Klebsiella	−
Proteus	−
Pseudomonas	−
Salmonella	−
Shigella	−
Alcaligenes	−

[a] +++ = "string" 7 mm or longer; ++ = "string" ca 4 mm-6 mm long; + = "string" 3 mm or less; − = no "string".

been of value in selecting colonies for further testing. The reagent—0.5% sodium deoxycholate—can be prepared as needed and is rather stable. Solutions kept at room temperature and 30°C for one year in closed containers gave results similar to those obtained with freshly prepared material.

The test is not specific for *V. cholerae*; all vibrios tested, except *V. parahaemolyticus* strains, gave a positive result. The "string" test could be considered a general reaction for vibrios. This would appear to limit the usefulness of the test if the objective of the field screening was cholera diagnosis only. However, in view of the increasing number of reported associations of non-cholera vibrios with human diarrhoeal diseases (Aldová et al., 1968; El-Shawi & Thewaini, 1969; McIntyre et al., 1965), preliminary field screening of virtually all vibrios would seem desirable, and for such coverage the "string" test is eminently suitable.

Agents which lower surface tension, such as laundry detergents, can be employed instead of sodium deoxycholate. If these are used, it is necessary to determine the proper conditions with known cultures.

REFERENCES

Aldová, E., Lázničková, K., Štěpánková, E. & Lietava, J. (1968) *J. infect. Dis.*, **118**, 25-31

El-Shawi, N. & Thewaini, A. J. (1969) *Bull. Wld Hlth Org.*, **40**, 163-166

McIntyre, O. R., Feeley, J. C. Greenough, W. B., Benenson, A. S., Hassan, I. & Saad, A. (1965) *Amer. J. trop. Med. Hyg.*, **14**, 412-418

Smith, H. L., Jr & Goodner, K. (1965) *On the classification of vibrios.* In: *Proceedings of the Cholera Research Symposium, Honolulu*, Washington, D.C., US Government Printing Office, pp. 4-8

Cholera O Group I Agglutinating Serum

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The WHO Expert Committee on Biological Standardization (1968) established an International Reference Reagent of Cholera Agglutinating Serum (Ogawa) and discontinued the previously existing International Reference Preparations of Cholera Agglutinating Serum (Ogawa) and Cholera Agglutinating Serum (Inaba). The reference reagent established, specific for cholera strains of the Ogawa serotype, is adequate for the differentiation of the cholera serotypes provided these are proved to be of cholera O group I.

Later, the WHO Expert Committee on Biological Standardization (1969, p. 21) discontinued the International Reference Preparations of Cholera Antigen (Ogawa) and of Cholera Antigen (Inaba). These preparations of antigens were essentially working

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