A NOTE UPON THE MAINTENANCE OF CULTURES OF *BACTERIUM TYPHOSUM* FOR TESTING BY THE RIDEAL-WALKER METHOD

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The *British Standard Technique for Determining the Rideal-Walker Coefficient of Disinfectants* (no. 541—1934), in laying down the procedure to be adopted with regard to the test typhoid bacillus culture, recommends that a fresh culture be obtained each month from the National Collection of Type Cultures. In all probability this is the best procedure; it does, however, sometimes happen that a test is required urgently when a fresh culture is not available. The following notes upon our own experience in maintaining cultures may therefore be of some interest. It is our practice to obtain fresh cultures whenever we are called upon to carry out other than routine tests unless a recent one has been obtained, and many of these cultures have now been running for a number of years. On the arrival of the culture from the N.C.T.C. it is subcultured on to an agar slope and "incubated" at room temperature in a dark cupboard. 2% agar is used in a broth prepared by the tryptic digestion of ox heart. Further subcultures are made at intervals of one month. It is our usual practice to start a series of broth cultures from such slopes not less than 14 days after sowing.

The Lister strain of *Bacterium typhosum* is that recognized for the Rideal-Walker test. Another strain, the "S" strain, is now used for the modified technique of the Chick-Martin test.

In 1931 we plated our culture of the Lister strain and carried out some tests upon four separate colonies and upon the mixed growth using an ordinary "black fluid" as the test disinfectant. The Rideal-Walker coefficients obtained in these tests were:

Colony 1	...	7·5, 7·5, 8·0
Colony 2	...	9·1, 9·4
Colony 3	...	10·0, 9·5, 9·5
Colony 4	...	7·5
Mixed growth	...	9·0

There was no visible difference between one colony and another. All gave the typical "sugar" reactions of *Bact. typhosum* except that those giving the lower coefficients gave acid in maltose peptone water, whilst others did not. It is of interest to note that the "S" strain of *Bact. typhosum*, which tends to give lower coefficients than the Lister, also acidifies maltose. Attempts on another occasion to isolate colonies differing in resistance failed.

Since 1931 we have maintained a number of cultures of *Bact. typhosum* on agar at room temperature, determining their resistance to phenol (Rideal-Walker technique) and their effect on maltose in peptone water from time to time. The results of this work are given in Tables I and II. These cultures include those from two of the colonies mentioned above, L 6 and L 7, and some of *Bact. typhosum* "S".

Table I

Culture	Date obtained from National Collection of Type Cultures	Resistance to phenol*			
		1931–2	1933	1934–5	1937
L 1 *Bact. typhosum* "S"	2 December 1931	95–105	95–100	—	—
L 2 ,, ,,	Colony from L 1	—	<95–100	<95	<95, <90
L 3 *Bact. typhosum* Lister	Before December 1931	105–110	95–100	95–105	<95, 95
L 4 ,, ,,	26 November 1931	100–105	95–100	<95– 95	95, 95
L 5 ,, ,,	25 May 1932	—	<95–105	95–100	—
L 6 ,, ,,	Colony from L 3 taken January 1932	100–105	<95–100	<95	<95, 90
L 7 ,, ,,	Colony from L 3 taken January 1932	105–>110	100	100–105	<95, 95
L 8 ,, ,,	Colony from L 4 or L 3	—	100–105	97·5	—
L 10 ,, ,,	28 June 1933	—	95–105	95–97·5	—
L 11 ,, ,,	31 July 1934	—	—	97·5–105	—
L 12 ,, ,,	29 August 1935	—	—	95–97·5	—
63 ,, ,,	30 April 1937	—	—	—	95, 95
61 ,, ,,	16 November 1937	—	—	—	90, 95

* The dilution showing survival of the bacilli after 5 but not after 7½ min.; in each case "1 part of phenol in ..." is understood.

Numerous other cultures have been obtained from the National Collection of Type Cultures, none differed significantly from those maintained by us.

The figures given under 1931–2, 1933 and 1934–5 are limits in a number of tests, often five or more, in the 1937 column they are the results of two tests.

N.B. The B.S.I. technique was introduced during the period under review.

Table II. *Result of cultivation in maltose peptone water*

Culture	1931–2	1933	1937
L 1	A	A	—
L 2	A	A	A
L 3	O	O	O
L 4	O	O	O
L 5	—	O	—
L 6	A	A	A
L 7	O	O	O
L 8	Varies	Varies	—
63	—	—	O
61	—	—	O

A = acid. O = no change.

Table I indicates the dilutions giving "two lives" in the test, that is allowing of survival of the organism up to 5 but not 7½ min. It appears from these results that it is possible to isolate variants from *Bact. typhosum* Lister differing in their resistance to disinfectants and in their capability of fermenting maltose. There is some indication that cultures from colonies forming acid in maltose are more resistant to phenol and tend to give lower Rideal-Walker coefficients than cultures from other colonies. In spite of this, cultures grown monthly on nutrient agar at room temperature under our conditions maintain

a fairly even resistance to phenol for some years. On one occasion during the period under review, we experienced a dying out of some of our cultures. The month-old room-temperature agar slopes, although themselves showing a good growth, failed in some cases to initiate growth on fresh agar slopes or indeed in or on any other medium tried.

The Lister strain of *Bact. typhosum* thus appears capable of giving rise to colonies varying in their resistance to phenol. The more resistant strains may be characterized by production of acid in maltose peptone water. Our tests over a number of years on the mixed culture afford evidence that, so long as no attempt is made to plate out and select colonies for the sowing of future broth cultures, this variation has little effect upon the phenol resistance of the stock cultures.

(*MS. received for publication* 29. XII. 38.—Ed.)