Use of a solid reagent in the triphenyl tetrazolium chloride test for bacteriuria

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SYNOPSIS The use of a solid reagent in the triphenyl tetrazolium chloride (T.T.C.) test for significant bacteriuria is described. The reagent gave a positive result with 108 (86%) of 126 specimens of urine containing over 100,000 organisms per ml. In comparison with the standard T.T.C. test the modified test gave comparable results. Some possible reasons for the varying results obtained by different users of the T.T.C. test are discussed.

The need for a simple test capable of detecting significant numbers of bacteria in the urine is now well established (*Lancet*, 1964). If a quantitative bacterial count is performed, the presence of more than 100,000 organisms per ml. in carefully collected urine is usually considered to be significant, but as bacteriological procedures of this type require laboratory facilities they cannot be performed by the general practitioner. To overcome this difficulty attempts have been made to devise and improve on simple and reliable chemical tests such as the Griess test (Sleigh, 1965) and the triphenyl tetrazolium chloride test (the T.T.C. test) (Simmons and Williams, 1962).

The T.T.C. test depends on the reduction by bacteria of colourless 2, 3, 5, triphenyl tetrazolium chloride to triphenyl formazan which is red. It has been used successfully in general practice (Constable, 1966) where the preparation of reagent, which requires laboratory facilities, proved to be the main difficulty.

In many laboratory trials the T.T.C. test has detected approximately 85% of urines containing more than 100,000 bacteria per ml., but in others it has been found to be less reliable and in this paper possible reasons for the different results are discussed. A commercial preparation of the T.T.C. test for which laboratory facilities are not required is also evaluated and compared with the test as originally described.

MATERIALS AND METHODS

Triphenyl tetrazolium chloride tests using a commercial

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reagent and quantitative bacterial counts were carried out on 502 urine specimens submitted for bacteriological examination to either Lewisham Group Laboratory or the Bacteriology Department at the Dudley Road Hospital, Birmingham. Standard T.T.C. tests were also performed on 462 of the specimens. The quantitative bacterial counts and standard tests were carried out by the methods described by Simmons and Williams (1962).

The modified test was performed with a commercially prepared T.T.C. reagent from British Drug Houses (B.D.H. bacteriuria reagent). This was made up as dry buffered triphenyl tetrazolium chloride in disposable marked tubes, each of which contained sufficient reagent for one test. To perform the test the urine was mixed by gentle inversion to dissolve the reagent. Tubes were incubated at 37°C. for four hours and examined. A positive result was shown by a red precipitate and a negative result by its absence. A concave mirror was helpful in reading the results.

RESULTS

Similar results were obtained in the two hospitals. When the modified test was compared with quantitative bacterial counting, good correlation was observed between positive results and counts of over 100,000 organisms per ml. (Table I). Two hundred and sixty-two specimens were examined in the Lewisham Group Laboratory. Seventy-one contained more than 100,000 organisms per ml., of which 60 (85%) gave a positive result. One hundred and seventy specimens contained fewer than 10,000 organisms per ml. and only 16 (9%) gave a positive result.

Two hundred and forty specimens were examined at the Dudley Road Hospital and 55 contained more than 100,000 organisms per ml., of which 48 (87%) gave a positive result. One hundred and fifty-eight

TABLE I

RESULTS OF MODIFIED T.T.C. TESTS IN TWO HOSPITALS

Hospital	Total Specimens	Organisms per ml.					
		Less than 10,000		10,000-100,000		More than 100,000	
		Total	T.T.C. +ve	Total	T.T.C. +ve	Total	T.T.C. +ve
Lewisham	262	170	16 (9%)	21	2 (10%)	71	60 (85%)
Dudley Road	240	158	15 (9%)	27	10 (37%)	55	48 (87%)
Combined	502	328	31 (9%)	48	12 (25%)	126	108 (86%)

TABLE II

COMPARISON OF MODIFIED AND STANDARD T.T.C. TESTS ON 462 SPECIMENS OF URINE

Organisms per ml.	No. of Specimens	Positive Tests	
		Modified	Standard
100,000 or more	119	101 (85%)	95 (80%)
10,000 to 100,000	46	10 (22%)	13 (28%)
Less than 10,000	297	27 (9%)	24 (8%)

specimens contained fewer than 10,000 organisms per ml. and 15 (9%) gave a positive result.

When the results from the two hospitals were combined, 126 of the 502 specimens contained more than 100,000 organisms per ml. of which 108 (86%) gave a positive result. Three hundred and twenty-eight specimens contained fewer than 10,000 organisms per ml. of which only 31 (9%) gave a positive result.

A comparison of the modified and standard T.T.C. tests is shown in Table II. Similar results were obtained with both methods. One hundred and nineteen of the 462 specimens examined contained more than 100,000 organisms per ml. One hundred and one (85%) of these gave a positive result with the modified test and 95 (80%) with the standard test. Two hundred and ninety-seven specimens contained less than 10,000 organisms per ml. of which 27 (9%) gave a positive result with the modified test and 24 (8%) with the standard test.

Table III shows the bacterial population of the urines containing more than 100,000 organisms per ml. Ninety-five of the specimens were infected with

TABLE III

PROPORTION OF POSITIVE MODIFIED T.T.C. TESTS ACCORDING TO THE BACTERIAL SPECIES ISOLATED (PRESENT IN NUMBERS EXCEEDING 100,000 PER ML.)

Bacterial Species	No. of Isolations	No. of Positive Tests
<i>Esch. coli</i>	67	58
<i>Proteus spp.</i>	21	15
<i>Kl. aerogenes</i>	5	5
<i>Ps. pyocyanea</i>	2	2
<i>Monilia</i>	1	1
<i>Strep. faecalis</i>	7	7
<i>Staph. albus</i>	4	4
More than 1 organism	19	16

Gram-negative bacilli occurring in pure culture and 80 (84%) gave a positive result.

DISCUSSION

Quantitative bacterial counting is now accepted as a reliable method of distinguishing bacteria causing infections from contaminants in carefully collected specimens of urine. However, it is a relatively time consuming procedure and efforts have been made to find simpler tests which would enable large numbers of specimens to be examined more easily. If a satisfactory method were found it could be used not only in laboratories, but also in general practice thus enabling the family doctor to extend his diagnostic scope (Constable, 1966).

The T.T.C. test has been found to have many of the characteristics required of a test for use in general practice, but in its original form a liquid reagent was used which was highly light sensitive and not easy to prepare. In the modified test a solid reagent is used and dispensed in marked disposable tubes thus eliminating the need for any laboratory facilities. The only apparatus required is a small water bath or block heater for incubation.

In the present investigation the modified T.T.C. test compared favourably with the standard test and detected 86% of specimens containing over 100,000 organisms per ml. The standard test was found to be reliable in several trials (Chard and Cole, 1963; Kincaid-Smith, Bullen, Mills, Fussell, Huston, and Goon, 1964; Pinkerton, Houston, and Gibson, 1965; Deutch and Jespersen, 1964), but other workers, mainly American, have obtained poor results with the test (Steers and Jackson, 1963; Guze and Kalmanson, 1963; Fairley and Barraclough, 1966).

It is unlikely that the different results were due to a difference of technique since a standard procedure was used in most cases, but they could have been due to variation in the colour reagent which would not always have been obtained from the same source. Another possible reason for the different results may be differences in the nature of the specimens examined. For example, Jensen (1965) demonstrated that chemotherapeutic substances in the urine make

the T.T.C. test less sensitive and the number of patients receiving antibiotics in different trials may have varied. Recently pH and osmolarity of the urine have been shown to affect its efficiency as a culture medium and the urine of pregnant women was found to be almost invariably at optimal pH for the growth of *Esch. coli* (Asscher, Sussman, Waters, Davis, and Chick, 1966). Although the pH and osmolarity of the urine are altered in the T.T.C. test, the initial values may affect its sensitivity, and in trials in which it was used to detect asymptomatic bacteriuria of pregnancy the test was found to be reliable and the results were almost identical (Williams and Simmons, 1963; Kincaid-Smith *et al.*, 1964; Pinkerton *et al.*, 1965).

One of the major difficulties in all investigations of urinary tract infection is the collection of specimens suitable for examination, for if sufficient care is not taken the number of contaminating bacteria may exceed 100,000 per ml. Where similar conditions

were artificially created by the addition of bacteria to urine the T.T.C. test was shown to be unreliable (Steers and Jackson, 1963).

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