COMPARISON OF 6-HOUR AND 24-HOUR INCUBATION PERIODS AT 44° C. AS A CONFIRMATORY TEST FOR *BACTERIUM COLI* TYPE I

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INTRODUCTION

During the discussion of a paper presented by the author to the Society for Water Treatment and Examination at Sheffield in 1952, it was noted that several water bacteriologists were confirming, with a high degree of success, presumptive positive tubes in the coliform test as *Bacterium coli* type I after incubation for 6 hr. only in MacConkey broth at 44° C. (Taylor, 1952).

It was considered that this matter was worth investigating further, and the following study has been carried out. A comparison was made of the number of presumptive tubes confirmed as *Bact. coli* type I after incubation at 44° C. for 6 and 24 hr. in the following media: MacConkey broth, brilliant green bile broth (Mackenzie, Taylor & Gilbert, 1948), peptone water for indole production and lactose glutamic acid medium (Burman & Oliver, 1952) now with brom-cresol purple as an indicator.

METHOD

Routine water samples for bacteriological analysis are inoculated into MacConkey broth and, after a preliminary heating in a water-bath, in order to bring the mixture of ice-cold water and medium rapidly up to the incubation temperature, the tubes are placed in an incubator. The first sorting of tubes takes place after incubation for 18 hr., when the positives are removed for confirmation and the negatives are returned to the incubator. These 18 hr. presumptive positive tubes contain a very high proportion of *Bact. coli* type I and therefore are eminently suitable for comparative work at 44° C.

For the purpose of this investigation each presumptive tube positive in 18 hr. was subcultured into a tube of each of the following media: single strength MacConkey broth, brilliant green bile broth, peptone water (for indole production) and lactose glutamic acid medium.

The results in this series were obtained by using Evans peptone in brilliant green bile medium, in MacConkey broth and in the peptone water. The latter medium was also fortified with 0·03 % of tryptophane. The quality of peptone used in the 44° C. test is of the greatest importance. The tubes were incubated in an accurately controlled water-bath at 44° C. ($\pm$ 0·25° C.) and were examined for acid and gas production, gas production or indole production as the case might be after incubation for 6 and 24 hr.

Tubes not fermented after 6 hr. were returned to the bath and re-examined after a further 18 hr. In the case of the peptone water tube, half of the contents

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was transferred and examined for indole production; if negative, the remainder in the original tube was returned to the bath and examined after a further 18 hr.

A positive result obtained from all four media in 6 hr. or 24 hr. was assumed to be due to the presence of *Bact. coli* type I, and specimens giving it were not examined further. Likewise, a negative result obtained in all four media after incubation for 24 hr. was taken as proof of the absence of *Bact. coli* type I from these particular presumptive positive tubes. In all cases, however, where the results after 24 hr. did not agree, the original cultures or the positive subcultures were plated out on MacConkey agar, single colonies were selected and examined and the coliform type was determined according to its IMViC reactions.

RESULTS

A total of 2478 presumptive cultures was examined. The results are set out in Table 1.

Table 1. *Comparison of positive results from various media after incubation for* 6 *and* 24 hr. *at* 44° C.

	Number of positives at 44° C.		Column (1) expressed as a percentage of column (2)
	6 hr. incubation (1)	24 hr. incubation (2)	
Acid and gas in MacConkey broth	2119	2239	94·6
Gas in brilliant green bile broth	2137	2238	95·5
Indole production	2129	2226	95·6
Acid and gas in lactose glutamic acid	1683	2203	76·4

It will be seen from the table that a high proportion of the presumptive tubes were confirmed as *Bact. coli* type I after the short incubation period of 6 hr. at 44° C. The brilliant green bile medium gave higher results than MacConkey broth medium, indicating that the former medium was at least as good as the latter even for the shorter incubation period. The indole production test also gave very good results at 44° C. but the lactose glutamic acid medium gave lower results particularly in the 6 hr. test. It was concluded that there was little to choose between growth with production of acid and gas in MacConkey broth, growth with production of gas in brilliant green bile broth, and indole production from peptone water, as criteria for a 6 hr. confirmatory test at 44° C. for *Bact. coli* type I.

Table 2 sets out the number of tubes giving false positive results, that is to say, those in which the organism isolated from the positive confirmatory tube was other than *Bact. coli* type I. It will be seen that the numbers were small and relatively even smaller after incubation for 6 hr. than after 24 hr. Furthermore, in the case of the MacConkey broth and brilliant green bile media the majority of the 6 hr. false positives proved to be due to the presence of Irregular type II, an organism which has a strong claim to be closely allied to *Bact. coli* type I (Mackenzie *et al.* 1948); the 24 hr. false positives in these media included Irregular VI as well as Irregular II. The lowest yield of false positives was from the indole test; these were

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due to non-lactose fermenting organisms. When the indole test is done in conjunction with fermentation of MacConkey broth or brilliant green bile broth, a false positive result in both is rare and of no practical importance. For example, in the present series of 2478 tests it occurred on only six occasions (0·24 %) in the 24 hr. results. These false results may be due to mixtures of organisms, each contributing a positive factor, but, on the other hand, it could be that the tubes gave a true result and the fault lay in the failure to select *Bact. coli* type I colonies from the MacConkey agar plates in the check plating and differential technique.

Table 2. *Positive reactions at* 44° C. *due to organisms other than* Bacterium coli *type I*

Incubation period (hr.)	MacConkey broth		Brilliant green bile broth		Indole production from peptone water	
	No. of cultures	Percentage of total positives obtained	No. of cultures	Percentage of total positives obtained	No. of cultures	Percentage of total positives obtained
6	10	0·40	10	0·40	5	0·20
24	27	1·09	27	1·09	14	0·56

The results with lactose glutamic acid as a confirmatory medium were inferior, particularly with regard to the 6 hr. incubation period. Ware (1951) found that some strains of *Bact. coli* would not grow at 44° C. on a chemically defined medium unless glutamic acid and nicotinamide were present. This medium contains the former, but lacks the latter substance, so that more investigation is required into this matter, and for the present the glutamic acid medium cannot be recommended as a suitable substrate for the 44° C. confirmatory test.

DISCUSSION

One of the main objects in the routine bacteriological examination and control of water supplies is rapidity in obtaining results and particularly in the confirmation of presumptive positive tubes in the routine coliform test as due to *Bact. coli* type I, the prime indicator of excretal pollution. This investigation confirms that *Bact. coli* type I may be proved to be present in a water sample in a high proportion of cases within 30 hr. of collection. Such knowledge so quickly gained is of the greatest value to those concerned with water supplies, and marks another great step forward in bacteriological analysis of water.

Thus, if a sample of water is collected on the morning of the first day, inoculated and placed in the incubator by 4 p.m. of that day, 'positives' from the sample which appear by 10 a.m. on the second day may be subcultured into a suitable medium and incubated at 44° C. for 6 hr., and by 4 p.m. on the second day it will be known in the majority of cases whether or not the sample contained *Bact. coli* type I, so that the necessary action can be taken within 30 hr. of collection of the sample. Negative tubes from the 44° C. bath after 6 hr. should be returned to the bath and examined again after the full incubation period.

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