AN EXAMINATION OF THE MODIFIED EIJKMAN METHOD APPLIED TO PURE COLIFORM CULTURES OBTAINED FROM WATERS IN SINGAPORE

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Since the original definition of the coli-aerogenes group of organisms by MacConkey (1905, 1909), research has been continually directed in an endeavour to find a more rapid and definite distinction between its respective members.

In general, for the purposes of water analysis, the coliform organisms can be divided into two main groups: faecal coli, of which *Bact. coli* type I can be considered the typical member, and the non-faecal organisms comprising the intermediate-aerogenes-cloacae (I.A.C.) organisms. All available evidence tends to show that *Bact. coli* type I is by far the most frequent organism in the human and animal intestine. Levine (1921) has summarized the figures of various workers and obtained a percentage ratio of 93·4:6·6 as the ratio of coli:aerogenes in faeces, while Bardsley (1938) has shown that in only a very few cases does the number of I.A.C. organisms exceed the faecal coli in faeces.

It would appear, therefore, that for bacteriological examination of water a quick, sensitive method of detecting *Bact. coli* type I is required, and although this has so far not been obtained, quite good differentiation into groups has been possible by means of the methyl-red, Voges-Proskauer, citrate-utilization and indole-formation tests.

Recent attention has been directed to Eijkman's method for differentiating between faecal and non-faecal coliform organisms. MacConkey broth has been substituted for the original medium of Eijkman, and Wilson (1935) has shown that in England, providing the temperature of incubation is rigidly controlled at 44° C. by means of a thermo-regulated water-bath, practically every strain forming gas at 44° C. in MacConkey broth appears to belong to the faecal *Bact. coli* type I group. Evidence has been brought to show that the discrepancies in results formerly obtained by different workers were largely due to differences in the temperature of incubation and variations in temperature during incubation.

Using a modified Eijkman medium, of 496 pure strains isolated by Wilson 180 out of 193 faecal coli strains grew at 44° C., and of 266 intermediate-aerogenes-cloacae strains only one grew at 44° C. Irregular strains comprising 7·6 % of the total organisms and in general differing from the main groups in either their reactions with the modified Eijkman medium or power to liquefy gelatin, were found. From these results Wilson has extended the classification suggested in the Ministry of Health *Report*, No. 71 (1934), and included eight subgroups of irregular strains.

Mackenzie & Hilton-Sergeant (1938), Bardsley (1938), Dodgson (1938) and

the Metropolitan Water Board *Report* (1938) all agree that in England gas production in MacConkey broth at 44° C. is almost specific for faecal *Bact. coli* type I. Mackenzie & Hilton-Sergeant showed that all samples of faeces examined gave gas at 44° C. and conclude that 'the number of false positives which may arise from the presence of coli organisms other than faecal *Bact. coli* type I is negligible'. Dodgson in his application of the 44° C. MacConkey test to shellfish states that the majority of typical *Bact. coli* grow at 44° C. and all citrate positives are eliminated, but some intermediates and weak citrate positives grew at 44° C. The Metropolitan Water Board *Report* concludes that the 44° C. MacConkey test is practically specific for faecal *Bact. coli* and shows a recovery of over 5 % in excess of the number obtained by the usual differential methods. They prefer a preliminary enrichment by incubation in MacConkey broth at 37° C. before incubation at 44° C., but it is interesting to note that in a number of tubes which were negative at 44° C. *Bact. coli* type I was recovered by other methods.

In temperate climates it may therefore be reasonably concluded that gas production in MacConkey at 44° C. is practically specific for faecal coli, although a few strains of faecal coli are unable to form gas at 44° C.

The most extensive series of observations were made by Ferramola (1940) in the Argentine, where the mean temperature is slightly higher than in England. About 10,000 waters were examined by the method recommended in the Ministry of Health *Report* using the 44° C. MacConkey test. The results obtained were very satisfactory. During the inquiry special observations were made on the specificity of the 44° C. MacConkey test for the detection of faecal coli. Of 963 strains identified as *Bact. coli* type I all but six produced gas at 44° C., while of 105 other coliform strains (excluding four strains of *Bact. coli* type II, the faecal origin of which is in some doubt) not a single one did so. It would appear therefore that in the Argentine the specificity of the 44° C. MacConkey test is as great as it is in England.

In tropical countries, however, the use of the 44° C. MacConkey test has not been so successful. Burke-Gaffney (1932), Webster (1934–5) and Raghavachari & Seetharama Iyer (1938–9), have reported unfavourably on its application. The work of Burke-Gaffney and Webster was conducted at 46° C., and this higher temperature may explain the unsatisfactory results, but Raghavachari has recently shown that of the I.A.C. organisms isolated from natural and treated waters in India about 50 % were capable of producing acid and gas at 44° C. either on direct inoculation and incubation at 44° C. or after previous enrichment by incubation at 37° C. All these organisms were isolated from twelve samples of water.

The higher incidence of I.A.C. organisms in tropical waters may have some significance in this respect. According to Bardsley, in England only 7·6 % of the organisms in the presumptive coliform tubes at 37° C. were due to non-faecal organisms, while in India the percentage ratio of coli:aerogenes in natural waters is said to be 70·2:29·8.

In Singapore the mean temperature is 80° F. and the incidence of intermediate-aerogenes-cloacae types in natural water is high. In the course of this investigation 61 samples of water from wells, reservoirs, etc., have been examined and 125 different coliform organisms have been isolated in pure culture and examined for their ability to give acid and gas at 44° C. in MacConkey broth. All cultures were isolated and typed during the normal examination of water samples submitted to this laboratory, and were obtained by plating presumptive MacConkey positives at 37° C. on to MacConkey agar and isolating separate colonies in peptone water. All results from duplicate strains from the same sample showing identical reactions in methyl-red, Voges-Proskauer, citrate-utilization and indole-formation were omitted from the summary below. All experiments were carried out on fresh 4–8 hr. old cultures of the organisms in peptone water.

The detailed procedure was as follows:

(1) A loopful of each culture was inoculated into 10 ml. of MacConkey's bile salt lactose neutral red broth (in duplicate or quadruplicate) and examined for acid and gas after 48 hr. incubation at 37° C.

(2) A loopful of each culture was inoculated into 10 ml. of MacConkey's bile salt lactose neutral red broth (in duplicate or quadruplicate) and examined for acid and gas after 48 hr. incubation at 44° C. (±0·4° C.).

(3) All MacConkey positives after 48 hr. from (1) were immediately inoculated into MacConkey's bile salt lactose neutral red broth and examined for acid and gas after 48 hr. incubation at 44° C. (±0·4° C.).

(4) All MacConkey positives after 48 hr. from (1) were inoculated into citrate and examined for growth after 48 hr. at 37° C.

(5) All MacConkey positives after 48 hr. from (1) were plated on MacConkey agar, the colonies picked off into peptone water and typed by the usual differential reactions (methyl-red, Voges-Proskauer, citrate-utilization and indole-formation).

(6) All MacConkey positives after 48 hr. from (1) which failed to give acid and gas at 44° C. in (3) after the conclusion of all other tests were again plated on MacConkey agar, the colonies picked off into peptone water and typed by the usual differential reactions.

The following table summarizes the types of coliform organisms and their reactions using the 44° C. MacConkey test:

Type (Ministry of Health plating method)	Total no.	44° C. MacConkey +ve	
		No.	%
Bact. coli type I	42	40	95·2
Bact. coli type II	3	1	33 3
Intermediate type I	22	0	0
Intermediate type II	8	0	0
Bact. aerogenes type I	45	6	13·3
Bact. aerogenes type II	4	1	25 0
Irregular	1	0	0
Total I.A.C.	83	8	9 6

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In the case of two intermediate organisms and two *Bact. aerogenes* type I organisms faint positive growth was sometimes obtained in the 44° C. tubes. As duplicate tubes often gave opposite reactions these have not been classified as 44° C. MacConkey positive. No significant difference was obtained between the series with preliminary enrichment at 37° C. and the series directly inoculated at 44° C.

On the basis of the 44° C. MacConkey test 9·6 % of the organisms examined would be wrongly classified. The main irregularity, however, is in the number of *Bact. aerogenes* type I organisms capable of giving gas at 44° C., 13·3 % giving positive reactions in Singapore, compared with 0·7 % in England (Wilson, 1935) and 63·3 % in India (Raghavachari & Iyer, 1938–9).

It would appear that the MacConkey test at 44° C. cannot be considered entirely specific for faecal *Bact. coli* type I in India and Malaya, and can only be used for differentiating coli into faecal and non-faecal types if this aerogenes-like organism growing at 44° C. is a normal intestinal organism. It seems more feasible, however, that these aerogenes-like organisms are non-faecal but have acquired a resistance to heat due to the higher temperature or their normal habitat, although at present no experimental evidence has been obtained in support of this.

The MacConkey test at 44° C. does, however, offer possibilities of enrichment of the faecal coliform organisms with comparatively small losses in faecal *Bact. coli* type I and, providing differential reactions are carried out on the positive tubes, may well be of assistance in the bacteriological analysis of water in conditions like those in Malaya, where the normal analysis is complicated by the large numbers of I.A.C. organisms.

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