NEUTRAL-RED IN THE ROUTINE BACTERIOLOGICAL EXAMINATION OF WATER[1].

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The research which follows was undertaken with the object of determining how far the neutral-red reaction described by Rothberger could be utilised for the purpose of detecting *Bacillus coli* in water-supplies[2].

In accordance with Scheffler's recommendation I have used glucose media, broth or agar containing 0·5 % of glucose being employed throughout. With regard to the respective merits of broth and agar I am quite in accord with Rothberger, Scheffler, and Hunter that agar media (particularly agar shake-cultures) are best, but I find that in most cases excellent results can be obtained with broth. Glucose neutral-red broth was used in the routine water examination, but for testing individual organisms reliance was placed in preference upon glucose neutral-red agar shake-preparations. All incubations were performed at 37° C., and usually the reaction resulted in 24—48 hrs., but sometimes took several days if broth was used. It is not a matter of indifference what strength of glucose and of neutral-red is used. If neutral-red is added in excess the *B. coli* may not be able to reduce it, as is readily demonstrated by direct experiment. It was found that 0·1 c.c. of a 0·5 % watery solution of neutral-red (Grübler's) added to 10 c.c. of broth or agar gives the best results, and this was the strength employed.

The water was collected in small glass-stoppered bottles of about

[1] MS. received August, 1901.

[2] References to the literature of the neutral-red reaction have on the suggestion of the editors been omitted, as they are given in the preceding paper by Dr Makgill.

TABLE I.

Number	Kind of water	Number of organisms developing at 37° C.	Number of organisms developing at 20° C.	Indol reaction applied to 5 c.c. of the water	Neutral-red test: Amount of water used	Neutral-red test: If reaction obtained	Neutral-red test: Time when obtained	Neutral-red test: If *B. coli* isolated	Remarks
I	Unfiltered public supply	47	705	+	50 c.c.	+	2 days	Yes	
II	,, ,, ,, ...	692	1780	+	50 ,,	+	2 ,,	Yes	*B. coli* isolated from 2 c.c. grown anaerobically. The 50 c.c. not examined
III	A bore hole for a new supply ...	9	24	−	50 ,,	−		No	
IV	Unfiltered public supply	1	42	−	50 ,,	+	3 days	No	
V	A well	20	about 5000	+	50 ,,	+	2 ,,	Yes	
VI	100 c.c. tap water + 1 c.c. sewage	2328	2540	+	100 ,,	+	3 ,,	Yes	Isolated from $\frac{1}{4000}$ c.c. of the sewage
VII	,, ,, ,, + $\frac{1}{10}$ c.c. ,,	312	430	+	100 ,,	+	2 ,,	Yes	
VIII	,, ,, ,, + $\frac{1}{4000}$ c.c. ,,	55	92	+	100 ,,	+	3 ,,	Yes	
IX	,, ,, ,, + $\frac{1}{10,000}$ c.c. ,,	31	76	+	100 ,,	+	3 ,,	Not examined	
X	Filtered public supply	3	8	−	100 ,,	−		No	
XI	A contaminated brook	1370	16,120	+	6 ,,	+	24 hrs.	Not examined	
XII	Same brook as XI after admixture of cemetery drains	1647	15,200	+	6 ,,	+	24 ,,	Not examined	
XIII	A ship's drinking water	18,080	13,000	+	2 ,,	+	3 days	Not examined	
XIV	A well	8	910	−	40 ,,	−		Not	
XV	Unfiltered public supply	16	136	+	40 ,,	+	3 days	Yes	Isolated from the 40 c.c.
					6 ,,	−			
XVI	,, ,, ,, ...	2	61	−	50 ,,	−		Not	
XVII	* ,, ,, ,, ...	18,000	36,800	+	2 ,,	+	24 hrs.	Yes	Isolated from the 2 c.c.
					5 ,,	+	24 ,,		
					40 ,,	+	48 ,,		
XVIII	Drinking water of a ship ...	1560	1450	+	1 ,,	+	4 days	Yes	Isolated from the 1 c.c.
XIX	Filtered public supply	1	7	−	5 ,,	−	—	Yes	Isolated from the 40 c.c.
					40 ,,	Partial	4 days		
XX	,, ,, ,, ...	0	22	+	40 ,,	−		Not	
XXI	Unfiltered public supply	37	282	+	40 ,,	+	48 hrs.	Yes	
XXII	,, ,, ,, ...	33	274	+	40 ,,	+	48 ,,	Yes	

XXIII	Filtered public supply	2	102	+	5 c.c.	−		Not	
					40 ,,	−			
XXIV	* ,, ,, ,, ...	243	500 (about)	+	10 ,,	+	7 days	Not	An organism = *qq* isolated from the 10 c.c. which gives the neutral-red reaction
					40 ,,	+	7 ,,		
XXV	*Unfiltered public supply	about 10,000	Very numerous	−	10 ,,	+	4 ,,	Yes	Isolated from the 10 c.c. and also from 5 c.c. grown anaerobically in glucose formate broth
					40 ,,	+	4 ,,		
XXVI	,, ,, ,, ...	9	150	+	6 ,,	+	4 ,,	Yes	Isolated from the 6 c.c.
					40 ,,	+	3 ,,		
XXVII	A well	6	107	−	10 ,,	−		Not	The 40 c.c. examined
					40 ,,	−			
XXVIII	Unfiltered public supply	7	42		2 ,,	−		Not examined	
					30 ,,	−			
XXIX	Filtered public supply	80	192	+	8 ,,	+	2 days	Yes	Isolated from the 8 c.c.
					40 ,,	+	4 ,,		
XXX	Unfiltered public supply	210	370	+	2 ,,	+	3 ,,	Not examined	A very partial reaction only obtained with the 10 c.c.
					10 ,,	−			
					40 ,,	+	2 ,,		
XXXI	A suspected well	202	1120	+	10 ,,	+	24 hrs.	Yes	Isolated from the 10 c.c.
					40 ,,	+	2 days		
XXXII	Filtered public supply. Same source as XXIV	7	88	+	10 ,,	−	4 ,,	Yes	Isolated from the 40 c.c.
					40 ,,	+			
XXXIII	Filtered public supply	1	188	+	10 ,,	−	4–6 ,,	Yes	Isolated from the 40 c.c. An organism isolated = *pp* which gives a partial reaction
					40 ,,	+			
XXXIV	Unfiltered public supply	16	54	+	5 ,,	+	2 ,,		*B. coli* not isolated, but *bb* isolated from the 5 c.c. which gives marked neutral-red reaction
					40 ,,	+	2 ,,		
XXXV	,, ,, ,, ...	2	206	+	10 ,,	−		Not	The 10 c.c. examined
					40 ,,	−			
XXXVI	A surface-contaminated well ...	1080	about 10,000	+	10 ,,	+	24 hrs.	Yes	Isolated from the 10 c.c.
					40 ,,	+	5 days		
XXXVII	A suspected well	4	254	+	10 ,,	+	5 ,,	Yes	Isolated from the 40 c.c.
					40 ,,	+	3–5 ,,		
XXXVIII	Unfiltered public supply	191	620		5 ,,	+	2 ,,	Yes	Isolated from the 5 c.c.
					40 ,,	+	4 ,,		

* Not packed in ice and only received day after collection, and so valueless for consideration of significance of *B. coli* in water and in relation to the numerical count.

TABLE I. (*cont.*)

Number	Kind of water	Number of organisms developing at 37° C.	Number of organisms developing at 20° C.	Indol reaction applied to 5 c.c. of the water	Neutral-red test: Amount of water used	Neutral-red test: If reaction obtained	Neutral-red test: Time when obtained	Neutral-red test: If *B. coli* isolated	Remarks
XXXIX	Unfiltered public supply	135	402		5 c.c. 40 ,,	+ +	2 days 4 ,,	Yes	Isolated from 5 c.c. grown anaerobically in glucose formate broth. Not found in the 5 c.c. glucose neutral-red broth
XL	A contaminated brook	1350	5250		1 ,, 40 ,,	+ +	4 days 2 ,,	Yes	Isolated from the 1 c.c.
XLI	Unfiltered public supply	0	14	+	10 ,, 40 ,,	– –		Not	The 40 c.c. examined for *B. coli*
XLII	A shallow well	140	980	+	10 ,, 40 ,,	+ +	3 days 5 ,,	Yes	Isolated from the 40 c.c. Could not be found in the 10 c.c.
XLIII	Unfiltered public supply	72	144	+	10 ,, 40 ,,	+ +	3 ,, 3 ,,	Yes	*B. coli* isolated from the 10 c.c.
XLIV	A contaminated brook	4500	4200		5 ,, 40 ,,	+ +	24 hrs. 3 days	Yes	*B. coli* isolated from the 5 c.c.
XLV	Unfiltered public supply	136	320	–	10 ,, 40 ,,	+ +	2 ,, 3 ,,	Yes	*B. coli* isolated from the 10 c.c.
XLVI	Filtered public supply	9	114	–	10 ,, 40 ,,	+ +	3 ,, 3 ,,	Yes	*B. coli* isolated from the 10 c.c.
XLVII	Unfiltered public supply	1	24		10 ,, 40 ,,	– –		Not	The 40 c.c. examined
XLVIII	Filtered public supply	192	370		10 ,, 40 ,,	+ +	3 days 8 ,,	Yes	*B. coli* isolated from the 10 c.c.
XLIX	Drinking water of a ship ...	84	1200		5 ,, 40 ,,	+ +	4 ,, 2 ,,	Yes	Isolated from the 5 c.c.
L	Unfiltered public supply (water from a reservoir)	2100	about 16,000		10 ,, 40 ,,	+ +	24 hrs. 24 ,,	Yes	Isolated from the 10 c.c.

2 oz. (60 c.c.) capacity. After the different amounts required for the various steps of the routine examination were withdrawn, 10 c.c. or a smaller quantity of the water was added by sterile pipette to a tube of glucose neutral-red broth. To the remainder in the bottle, usually about 40 c.c., a second tube of 10 c.c. of glucose neutral-red broth was added. Both were incubated at 37° C. and examined daily. The exact amounts used varied a little, as can be seen from Table I. The liquid in the bottle usually took a longer time to develope the reaction than the more concentrated liquid in the test-tube. At first the neutral-red was added to the broth in batches subsequently to sterilization, but for the last ten to twelve waters the following modification was employed as preferable:—

The 10 c.c. or less of the water is added to the neutral-red broth as before, but instead of adding this *ordinary* glucose neutral-red broth to the remainder in the bottle the contents of a tube of *four times strength* glucose neutral-red broth is now added. Also the neutral-red is added to the broth before sterilization.

If the *B. coli* is present the mixture of broth and water becomes yellow and fluorescent.

Before the value of the reaction applied to detect *B. coli* in water can be affirmed there are obviously two questions which must as far as possible be answered. They are—

(1) If the *B. coli* is present will it always give this characteristic reaction?

And (2) Is the *B. coli* the only organism which may give this reaction under the conditions of the test?

To answer these questions and determine the value of the neutral-red test in routine water examination, fifty waters were systematically investigated bacteriologically. These waters were obtained from very varying sources, some from sources obviously polluted, others from suspected wells, springs, etc., while others were obtained from public water-supplies.

The answer to the first question can be most readily arrived at by considering the following:—

(*a*) In all the cases in which a negative reaction is obtained, is it impossible to find *B. coli*?

(*b*) Do all varieties of *B. coli* give the reaction in neutral-red broth?

(*c*) Are there any retarding or inimical agencies in waters which prevent the development of the reaction?

TABLE II.

Morphological and cultural characters of the B. coli isolated.

Water from which obtained	Broth (24 hrs. growth)	Milk	Indol	Gelatine slope	Motility	Gas production	Standard glucose neutral-red	
							Broth	Agar shake
I	Uniform turbidity no scum	Coagd. in 3 days	+	No liquefaction	Motile. Not active	+	Complete in 24 hrs.	Complete in 24 hrs.
II	,, ,, ,,	,, ,, ,,	+	,, ,,	Actively motile	+	,, ,, ,,	Yellow and fluor. in 2 days
V	Uniform turbidity scum	,, ,, ,,	+	,, ,,	Motile. Not active	+	,, ,, 2 days	,, ,, ,,
VIII	Uniform turbidity slight scum	,, ,, 2 days	+	,, ,,	Sluggishly motile	+	,, ,, 3 days	Complete in 2 days
XV	,, ,, ,,	,, ,, 3 days	+	,, ,,	Motile. Not active	+	,, ,, 24 hrs.	,, ,, ,,
XVII	Uniform turbidity no scum	,, ,, 6-7 ,,	+	,, ,,	Actively motile	+	,, ,, 3 days	Yellow and fluor. in 2 days
XVIII	,, ,, ,,	,, ,, 2 days	+	,, ,,	Sluggishly motile	+	Marked fluorescence 2 days but remains red throughout	Fluorescence and yellow after 3 days
XIX	,, ,, ,,	,, ,, 12 days	+ slight	,, ,,	,, ,,	−	No change 6 days	A commencing yellow in upper layers only after several days
XXI	,, ,, ,,	,, ,, 4 days	+	,, ,,	Motile. Fairly active	+	Fluorescence but red colour remains	Yellow and fluorescence only after several days
XXII	,, ,, ,,	,, ,, 2 days	+	,, ,,	,, ,,	+	Complete in 24 hrs.	Complete in 2 days
XXV	,, ,, ,,	,, ,, 12 days	+	,, ,,	Sluggish motility	+	Marked fluorescence 2 days. Red colour remains	Complete in 3–4 days
XXVI	,, ,, ,,	Not coagulated	+	,, ,,	,, ,,	+	Marked fluorescence 2 days. Red colour remains	Yellow and fluorescence in 2 days

XXIX	Uniform turbidity no scum	Coagd. in 5 days	+	No liquefaction	Very actively motile	+	Complete in 2 days	Complete in 24 hrs.
XXXI	Uniform turbidity slight scum	,, ,, 6 days	+	,, ,,	Motile. Fairly active	+	,, ,, 2 days	,, ,, 2 days
XXXII	Uniform turbidity well-marked scum	,, ,, 4–6 ,,	+	,, ,,	Motile. Very slight	+	,, 24 hrs.	,, ,, 24 hrs.
XXXIII	Uniform turbidity thin scum	,, ,, 2 days	+	,, ,,	Motile. Not marked	+	,, ,, ,,	,, ,, 2 days
XXXVI	Uniform turbidity no scum	,, ,, 11 days	+	,, ,,	Sluggishly motile	+	,, ,, ,,	,, ,, ,,
XXXVII	,, ,, ,,	,, ,, 14 days	+	,, ,,	No true motility	+	,, ,, ,,	,, ,, ,,
XXXVIII	,, ,, ,,	,, ,, 3 days	+	,, ,,	Sluggish ,,	+	,, ,, ,,	,, ,, 3 days
XXXIX	Uniform turbidity slight scum	,, ,, 3 days	+	,, ,,	Actively motile	+	,, ,, ,,	,, ,, 24 hrs.
XL	Uniform turbidity no scum	,, ,, 8 days	+	,, ,,	Motile. Fairly active	+	24 hrs. marked fluorescence. Red colour remains throughout	Lower ⅓ yellow and fluorescent after 3–4 days' growth
XLII	,, ,, ,,	... ...	+	,, ,,	*Sluggishly motile*	+	24 hrs. *orange colour* with marked fluorescence	*Complete in 2 days*
XLIII	,, ,, ,,	,, ,, 7 days	+	,, ,,	Non-motile	−	2 days no change, 4 days orange yellow and fluorescent	A commencing orange in upper layers only after 4 days
XLIV	Uniform turbidity slight scum	,, ,, 2 days	+	,, ,,	Moderate motility	+	Complete in 24 hrs.	Complete in 2 days
XLV	Uniform turbidity no scum	,, ,, 3 days	+	,, ,,	Sluggishly motile	+	,, ,, 2 days	,, ,, ,,
XLVI	,, ,, ,,	,, ,, 4 days	− (10 days)	,, ,,	,, ,,	+	24 hrs. red colour marked fluorescence. Remains red throughout	,, ,, 4 days
XLVIII	Uniform turbidity slight scum	Not coagulated (8 days)	+	,, ,,	Motile. Fairly active	+	Complete in 2 days	,, ,, 2 days
XLIX	Uniform turbidity marked scum	... ...	− (1 week)	,, ,,	Actively motile	+	,, ,, ,,	,, ,, ,,
L	Uniform turbidity thick scum	... ...	+	,, ,,	Motile. Not marked	+	,, ,, ,,	,, ,, ,,

Taking these points in order.

(*a*) As can be seen from Table I., 11 waters gave a negative neutral-red reaction. Of these 10 were examined for *B. coli.* The method of examination is described below.

In none of the 10 waters examined could the *B. coli* be detected. The nearest approach to it was in Water XIX., in which a very partial reaction was obtained, and only after 4 days. From this water an organism was isolated which was probably a *B. coli* but which did not produce gas and gave no true neutral-red reaction when tested in pure culture.

(*b*) Hunter reports that all his *B. coli* gave the test. He however preferred agar cultures, and probably most of the *B. coli* he tested were not examined in neutral-red glucose broth. Scheffler found that all the *B. coli* excluding those organisms incapable of forming gas gave the reaction. Examining the *B. coli* isolated, I found that while the majority of them gave quite typical results with agar shake-cultures, several failed to give complete reactions with broth cultures. For details see Table II. It is noticeable that several gave delayed reactions, and in some the fluorescence disappeared or the red colour returned with time. Nos. XIX. and XLIII. gave very imperfect reactions with neutral-red. As can be seen from the table neither produced gas.

These results agree with Scheffler's in that the absence of gas-producing power was associated with unsatisfactory or absent neutral-red reactions.

(*c*) The reaction is essentially one of reduction, and it is by no means inconceivable that certain conditions, for example the antagonism of co-existing microbes, may prevent any *B. coli* actually present from producing this typical reaction. A thorough investigation of this question could not be made, but throughout the research it was steadily kept in view and a number of accessory experiments were made. The results obtained showed that given an equal start the *B. coli* will generally give the neutral-red reaction in glucose neutral-red broth and water, whether many other organisms are present or not, but that if the water organisms are incubated with neutral-red broth for several days and then the tube or flask is inoculated with *B. coli,* under these circumstances frequently, even usually, no reaction developes. Whether this is due to the *B. coli* not growing, or to the other organisms which have received a start preventing the reduction of the neutral-red, was not determined. Under

the conditions of the test as applied, there is probably very little danger that the other organisms present will prevent the development of the neutral-red reaction by any *B. coli* which are present in the water.

In answering the first question, therefore, the results obtained appear to justify the statement that a negative reaction, while not absolutely establishing the absence of *B. coli* in the water, yet makes its presence very improbable.

The attempt to answer the second question was made along the following lines. (*a*) By endeavouring to find the *B. coli* in all the waters in which a positive reaction was obtained, and (*b*) by endeavouring to find organisms in water other than the *B. coli* which give the reaction.

(*a*) Out of the 50 waters investigated 39 gave a positive reaction. Of these, 34 were specially examined for *B. coli*, and that organism was found in all but three. Of these three waters, in one no neutral-red reducing organisms were obtained, and probably the *B. coli* was present but was missed, while in the other two, organisms not *B. coli*, but which produced the typical reaction, were isolated.

The method adopted for isolating the *B. coli* consisted in brushing[1] the yellow mixture of neutral-red broth and water, usually much diluted, over a series of Petri dishes containing solidified agar. These were incubated at 37° C. for about 24 hrs. and then carefully examined. Usually only a few different kinds of colonies were present and in such cases all the kinds were subcultured and worked out, but where the varieties of colonies were many only those possibly *B. coli* were subcultivated. By incubating at 37° C. throughout, most of the water organisms are kept from growing, while the development of the *B. coli* is favoured. This method is very convenient though not especially delicate. In several cases fresh plates had to be brushed before the *B. coli* could be isolated.

(*b*) The reaction being one of reduction it was hardly to be expected that it would be specific. Indeed Rothberger has shown that

[1] For brushing plates the brusher which gives best results was made as follows. A fairly stout piece of flat indiarubber about $\frac{1}{16}$th inch thick and $\frac{1}{2} \times \frac{3}{4}$ inch in area was fixed into a handle of wire such as is used to make diphtheria swabs. To fix the handle heat the end of the wire red-hot and hammer it flat and fix this into the indiarubber when hot. It readily burns its way into the rubber and when cold the melted indiarubber fixes it firmly. Such brushers can be easily, quickly and cheaply made, and can be sterilized repeatedly in the autoclave without damage. In brushing agar, or gelatine, they do not scratch the surface of the media.

the anaerobes, *B. tetani*, *B. anthracis symptomatici*, and *B. oedematis maligni* will change the colouring matter in the same way. Scheffler reports that he obtained the reaction with 3 out of 13 micro-organisms from spring and river water, and 8 out of 18 intestinal bacteria from man.

A large number of organisms were examined both from the waters which gave a positive reaction and from those which gave a negative reaction. With two exceptions (and one very slightly marked one) no organism other than the *B. coli* gave the reaction. It is important to remember that none of the organisms which would not grow at 37° C. were investigated; as under the conditions of the test they are not important. The neutral-red reacting organisms which are not *B. coli* are of considerable interest. No attempt was made to identify them, and here they are designated *qq*, *bb*, and *pp* respectively.

TABLE III.

	bb	*qq*	*pp*
Morphology	Short small bacilli	Short thick bacilli staining best at the ends	A larger bacillus which produces spores
Motility	Active	Very sluggish or nil	
Broth	Thick scum, broth not uniformly turbid	Uniform turbidity, thick scum	Broth clear with thick scum
Agar slope		Semitrans. growth with crinkled appearance	Opaque smooth white growth
Gelatine slope	White growth, rapid liquefaction	Very translucent bluish growth, slow liquefaction	White growth, fairly rapid liquefaction
Milk	Partial coagulation 2—3 days	No coagulation (1 week)	
Indol production	(7 days) No indol	(10 days) No indol	(7 days) No indol
Gas production	Nil	Nil	Nil
Glucose neutral-red broth	24 hrs. No change 48 hrs. Red colour and fluorescence 3 days. Quite yellow and fluorescent	24 and 48 hrs. No change. 3 days. Quite yellow and markedly fluorescent	24 hrs. to 4 days. No change. 5 days. Slight fluorescence. 7—10 days. Red colour remains but becomes markedly fluorescent 12 days. Fluorescent and orange colour
Glucose neutral-red agar shake	No gas and no fluorescence throughout. The only trace of a reaction is that the upper $\frac{1}{6}$ to $\frac{1}{4}$ gradually becomes orange in colour	No gas and no fluorescence throughout. The only trace of a reaction is that the upper $\frac{1}{6}$ of the agar becomes orange red (3—5 days)	No gas throughout. 2 days upper $\frac{1}{3}$ yellow and fluorescent, the rest red. The yellow part gradually extends until by 6th day it occupies the upper $\frac{1}{2}$

qq was obtained from Water XXIV., *bb* from XXXIV., and *pp* from XXXIII. In Waters XXIV. and XXXIV. no *B. coli* were found, but

in XXXIII. a typical *B. coli* was isolated in addition to *pp*. This latter organism was one which gave only a very partial and incomplete neutral-red reaction. Their characters as worked out are given in Table III.

From the above table it can be seen that these three organisms are perfectly distinct and that two of them, *i.e. bb* and *qq*, gave a complete reaction with neutral-red broth. All three however gave practically no reaction with glucose neutral-red agar shake-cultures. Both *bb* and *qq* were replated to ensure that they were pure cultivations.

From the results obtained, therefore, we cannot say that a positive neutral-red reaction can be taken as certain evidence of the presence of *B. coli*, but the latter was found in 31 out of 34 samples.

Leaving out no. IV., where the failure to find *B. coli* may fairly be ascribed to insufficient examination, we see that out of 44 waters examined by both methods 42 (*i.e.* over 95 %) gave successful results with neutral-red. In other words, if this reaction had been relied upon to detect the *B. coli* without subsequent isolation of the organism, the margin of error would have been less than 5 %, and this too when XXIV. is included which only gave a reaction after 7 days. In ordinary work XXIV. would certainly be excluded, and the margin of error for the samples examined would only be about 3 %.

It will be noticed that the number of positive results obtained is exceedingly high. The 50 waters consisted of the following classes:—

31 public supplies, 10 being filtered and 21 unfiltered.

8 wells, etc., many of which were suspected of being contaminated.

11 obviously contaminated waters.

TABLE IV.

Waters XVII., XXIV. *and* XXV. *are omitted as they cannot be satisfactorily classed.*

General character of water	Number of samples	Neutral-red reaction		*B. coli* looked for		*B. coli* found		Remarks
		+reaction	−reaction	with +reaction	with −reaction	with +reaction	with −reaction	
Bad	25	25	0	20	0	20	0	
Good	19	9	10	9	9	7	0	not found with IV & XXXIV
Suspicious	3	2	1	2	1	2	0	
	47	36	11	31	10	29	0	

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The details of these waters are shown in Table I. In Table IV. the waters are roughly classed into bad, good, and suspicious, this classification being based mainly on the result of their numerical count and in part on a knowledge of their source.

Of great interest are the waters in which the numerical count was satisfactory but which gave the neutral-red reaction. As can be seen from Table V. these were 9 in number and their chief features are reproduced in the table given below.

TABLE V.

Waters with satisfactory numerical count, but which yielded a positive neutral-red reaction.

Water	Number of organisms developing at		If *B. coli* found	Remarks
	37° C.	20° C.		
IV	1	42	Not	
XV	16	136	Yes	+ Reaction with 40 c.c., not with 5 c.c.
XIX	1	7	Yes	Only a very partial reaction and the *B. coli* isolated is not typical
XXVI	9	150	Yes	
XXXII	7	88	Yes	+ Reaction with 40 c.c., not with 10 c.c.
XXXIII	1	188	Yes	,, ,, ,, 40 ,, ,, ,, 10 ,,
XXXIV	16	54	Not	*bb* a reacting organism, not *B. coli*, isolated
XXXVII	4	254	Yes	From a well suspected of being contaminated
XLVI	9	114	Yes	

With regard to these waters it is of interest to notice that XV., XIX. and XXXIII. were from the same public supply, XV. being the water from the reservoir near the gathering grounds and many miles from the town it supplies, XIX. the same water filtered, and XXXIII. the same water but collected from the tap of a neighbouring town supplied from the same source. Into the reservoir from which XV. was taken the only possible source of contamination is a small stream which is contaminated by a small inn on its banks and which runs into the reservoir. The same water was re-examined about a month later (XLI.) but then gave no neutral-red reaction and *B. coli* could not be detected. IV. was examined chemically at the same time and was found quite satisfactory. XLVI. and XXVI. gave satisfactory figures bacteriologically, but samples collected from the same sources, and at the same time, gave chemical evidence of contamination. Thus XLVI. gave free ammonia 0·0034, albuminoid ammonia 0·0174, chlorine 1·0 parts

per 100,000, and considerable sediment consisting of vegetable cells and debris. There was thus evidently marked vegetable contamination. XXVI. gave free ammonia 0·0512, albuminoid ammonia 0·0114, chlorine 1·5 parts per 100,000. Traces of phosphates present. Considerable deposit with vegetable debris and a few animalculae.

The extremely high proportion of positive results is puzzling and naturally arouses suspicions of contamination either in the application of the test or in the collection. With regard to the former especial effort was made to have all apparatus and media sterile, and control experiments were made from time to time. I think possible contamination at this stage may be excluded.

Contamination in collection may possibly have taken place in several instances, as a good many of the samples were collected by sanitary inspectors and others unskilled in collecting water for this purpose, so that the minute printed directions sent out with the sterile bottles may not have been accurately followed. On the other hand a considerable number of the samples were collected by myself and with the greatest care.

It is also necessary to remember that a good many of the specimens were from obviously contaminated sources, while a considerable number of the public supplies were known to be suspicious and several had been repeatedly condemned. Again, a number of them (7 of the 50 waters) were from one source—an unfiltered public supply—and a positive reaction was obtained in 6. This was a water supply in samples of which taken by myself (using glucose formate broth) I was able repeatedly to demonstrate *B. coli* in small quantities of the water. These facts in large part account for the high proportion of positive results obtained. Of the 50 waters only 31 were from quite distinct sources, the same supply having been sometimes examined separately in reservoir, tap, etc., or repeated.

I will only say in this paper that the results are somewhat surprising and tend to make me reconsider the significance of the presence of *B. coli* in water. The detection however of this organism in all the obviously bad waters points strongly to its association with contamination.

Conclusions.

(1) A positive neutral-red reaction obtained as above, while not absolutely diagnostic of *B. coli*, yet in the vast majority of cases points to the presence of that organism.

(2) A negative neutral-red reaction obtained as above does not certainly exclude *B. coli* but renders its presence highly improbable.

(3) The neutral-red test is very readily applied, and with reasonable care fallacies in its employment can be avoided.

(4) It is a test which is of great value in the routine examination of water.