

funds, one may estimate roughly that about \$1 of county funds out of each \$1,000 of total annual income has been spent for the purpose of maintaining a county health unit. The author, therefore, feels justified in the conclusion that a county with a population of from 25 to 50 persons per square mile will be able to support a full-time county health unit. In the poorer counties he feels that some system of state aid is essential to the success of a well rounded rural health program.

The Measurement of Public Health Work—A reprint from the Annual Report of the Milbank Memorial Fund contains an introductory discussion by Edgar Sydenstricker on the measurement of public health work. The subject matter is pertinent in view of the two municipal health demonstrations being supported

in New York State by the Milbank Fund. The author concludes among some of the underlying principles governing the measurement of public health work:

1. Specific activities, rather than the program as a whole, should be measured first. A general health demonstration or a public health administration should not be regarded primarily as a single undertaking or experiment, but rather as a group of experiments, each of which had a definite objective; and the results of each should be measured by the most sensitive index that it is possible to employ.
2. The objectives and methods of a public health effort should be clearly defined.
3. Principles of experimentation should be applied.
4. The use of "experimental" and "control" groups or areas is essential.

LABORATORY

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SANITARY SIGNIFICANCE OF DIFFERENTIAL TESTS FOR THE COLI-AEROGENES GROUP

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OF THE various differential methods which have been applied within recent years to the coli-aerogenes group, only three have been tried to any extent in practical water examination. These are the methyl red, Voges-Proskauer and citrate tests.

Previous work has shown that by the use of these tests the group may be subdivided as follows:

	Chief Source	Differential Tests		
		MR	VP	citrate
Bact. coli and close allies.	feces	+	—	—
Intermediate section.....	soil	—	—	—
Aerogenes-cloacae section..	soil	—	+	+

The coli and the aerogenes-cloacae sections are well known and reviews have been given elsewhere.^{15, 20} The so-called intermediate type is a more recent addition,^{12, 13} the creation of which seems justified because of the finding in apparently unpolluted soils of MR+ cultures which utilize citrate in contrast to the citrate negative fecal coli. These MR+ citrate+

cultures, while not so common as the aerogenes-cloacae types, were nevertheless found in considerable numbers in some soils, and they have been tentatively differentiated from the other two sections.

With the knowledge of certain characteristics which set apart fecal organisms of the colon group from the majority of those found in soil, it might appear at first glance that the application to the sanitary examination of water would offer no difficulties. Experience, however, has been quite the contrary and in spite of a very considerable amount of work the usefulness of the differential tests is still a disputed point. A number have attacked this problem by comparing the proportions of coli and aerogenes types found in water supplies of different sanitary quality, as shown either by sanitary survey or a general knowledge of local conditions. Data of this sort should throw light on the question whether these different types or sec-

TABLE I

SUMMARY OF THE METHYL RED, VOGES-PROSKAUER, AND CITRATE DIFFERENTIATION APPLIED TO CULTURES FROM VARIOUS WATER SUPPLIES
Results obtained in the United States

Investigator	Locality	Character of Water supply	Number of cultures	Percentage distribution			
				MR+ VP-	MR- VP+	citrate	
				-	+	-	+
Greenfield (1916)	Kansas	Ground	158	65.0	35.0		
		Treated	131	70.0	30.0		
		Raw	116	76.0	24.0		
Greenfield & Skourup (1917)	Kansas	Raw: rain	117	37.6	62.4		
		dry	108	62.0	38.0		
		Treated: rain	116	31.8	68.2		
		dry	134	54.5	45.5		
		Tap: rain	193	33.6	67.4		
dry	194	65.8	34.2				
Rogers (1918)	Maine and Washington, D. C.	Various, some polluted, others good quality	134	40.3	59.7		
Stokes (1919)	Maryland	Various, treated and untreated	528	36.4	63.6		
		Sewage	31	58.1	41.9		
Bahlman and Sohn (1924)	Cincinnati, Ohio	Various	1223	61.0	29.5*		
Winslow and Cohen (1918)	New Haven, Conn.	Unpolluted	80	76.0	24.0		
		Stored raw	81	85.0	15.0		
		Polluted	94	76.0	23.0*		
Koser (1924)	Washington, D. C.	High quality	90	73.3*		16.7	
		Polluted (Potomac River)	107	80.4		64.5	
E. R. Houston (1923)	Austin, Texas	Various	154	93.5	6.3		
Lewis (1926)	Barton Spr., Texas	Supposedly good quality	154	79.3	20.7		
Lewis and Pittman (1927)	Texas	High quality	237	78.0	17.7*	34.1	
		Polluted	83	86.7	12.0*	73.5	
Berry and Ey (1926)	Ohio	Wells: good	93	72.0*			
		fair	117	76.9*			
		poor	127	86.6*			

* A small percentage of atypical cultures also found.

tions of the colon group actually make their way into water supplies in such a manner that the determinations of their relative proportions will be significant.

A number of investigations of this nature have now been reported and it should be worth while to summarize the results. Accordingly, all available reports have been reviewed and an effort has been made to bring out the chief points in the following tables. Before examining these in detail, however, it should be understood that certain facts cannot be brought out fully, if at all, in tabular form, and for this reason the results may not be strictly comparable. In the first place, the sanitary quality of the water under investigation is not always clearly stated, or all results from various classes of water may be grouped together. Also, the data have been secured at different times of the year, under widely different conditions and in many different localities. It is also important

to bear in mind that different methods were employed for isolation of the colon group cultures, and this may affect the proportions of the types obtained. In most instances, lactose broth or bile enrichment was followed by picking colonies from Endo or eosinmethylene blue plates. Here, again, there is no agreement as to the number of colonies picked from any one plate. In a few cases direct plating was used for isolation. In view of these many variable factors it is not surprising that different conclusions have been reached.

Table I summarizes the data from the United States. Referring to this let us first consider the results given by the methyl red and Voges-Proskauer tests. Do they show a correlation with the sanitary quality of the water? With the exceptions of Rogers, Stokes, and to some extent Greenfield and Skourup, all have reported a higher percentage of the MR+ VP- section. This holds true for different waters

and for widely different sections of the country. Greenfield and Skourup bring out a certain correlation in that their results depended upon rainfall. In time of drought with little surface wash and with evident pollution the MR+ section predominated. After rain the aerogenes section gained the ascendancy. Rogers also obtained a correlation between these tests and the degree of pollution in several small streams. It is impossible, however, to give his results in detail here. In contrast to the above, Winslow and Cohen found that the MR and VP tests failed to bring out any difference between water supplies of widely different sanitary character. Others have followed the same line of investigation with essentially similar results, and it is quite noteworthy that approximately the same proportion of MR+VP— cultures should be found in waters of widely different quality in the vicinity of New Haven, Conn., in the vicinity of Washington, D. C., and in Texas and Ohio.

The test of citrate utilization has been compared with the other tests and with the sanitary quality of the water in only a few instances.

Since this test serves to set apart certain MR+ organisms found in soil there is a possibility that it might correlate more closely with the sanitary survey. Such was found to be the case in a study of waters of different sanitary quality in the vicinity of Washington, D. C. From waters of high quality 16.7 per cent of the colon group cultures were citrate negative, whereas 64.5 per cent from polluted water were citrate negative. This is a much greater difference than was brought out by the MR and VP tests, which showed 73.3 and 80.4 per cent of MR+VP— cultures. Very recently, Lewis and Pittman have reported somewhat similar results in Texas.

Table II gives the data on these tests which have been reported from other countries. Referring first to the methyl red and Voges-Proskauer tests, we see that the MR+VP— section was found to be the predominating type and the results are quite similar to most of those obtained in this country. Cunningham and Raghavachari⁴ state that pollution is very widespread in their country and that the water supplies in many instances are little better than

TABLE II
SUMMARY OF THE METHYL RED, VOGES PROSKAUER, AND CITRATE DIFFERENTIATION APPLIED TO CULTURES FROM VARIOUS WATER SUPPLIES
Results obtained in other countries

Investigator	Locality	Character of water supply	Number of cultures	Percentage distribution			
				MR+ VP—	MR— VP+	citrate	
				—	+		
A. C. Houston (1911)	England	Raw	243		12.9†		
		Stored	133		5.3		
		Stored & Filtered	156		3.2		
Wood (1919)	England	Various	231	66.7	33.3		
Bardsley (1926)	England	Springs, wells and rivers; majority treated	1441	87.2	11.1*		
Cunningham & Raghavachari (1924)	India	Unfiltered: springs & wells	1500	68.1	31.9		
		impounded surface	755	74.5	25.5		
		rivers	190	69.4	30.6		
Cunningham & Raghavachari (1926)	India	Filtered	2320	70.2	29.8		
		Unfiltered	500	80.0	20.0		
Raghavachari (1926)	India	Unfiltered: springs & wells	594	68.5	31.5	67.2	32.8
		impounded surface	370	72.4	27.6	72.4	27.6
		river	110	69.1	30.9	69.1	30.9
		Filtered	500	61.6	38.4	50.6	49.4
Pawan (1925)	Trinidad	High quality	240	42.5	57.5	18.7	81.3
		Polluted	220	87.3	6.3*	90.9	9.1
Pawan (1926)	Trinidad Chorah R. Lopinot R.	Unpolluted	40			0.0	100.0
		Good	20			5.0	95.0
		Polluted in varying degrees	120			79.2	20.8

† On basis of Voges-Proskauer reaction only

* A small percentage of atypical cultures also found

diluted sewage, so that it may be unfair to expect a great difference between the various classes of water. Pawan,¹⁸ in Trinidad, found some difference between the classes of water upon which he reports, though evidently not so much as expected, for he states that his work substantiates that reported from this country. The citrate test was also used in several of these studies. Raghavachari found it to be of little if any value in India. Pawan on the other hand apparently regards it as useful. He found that it correlated more closely with the quality of the water, as brought out by sanitary survey, than did the other differential tests. Moreover, many MR+ organisms whose frequent appearance in some waters could not be accounted for on the basis of the sanitary survey were found to be citrate+ and thus distinct from intestinal coli.

As already pointed out, these results were obtained under widely different conditions and with different methods of technic, so that they are not strictly comparable, although there would seem to be sufficient data at hand to give one a broad view of the situation. It is evident that the MR and VP tests have been tried fairly extensively in different parts of the world and that many investigators have failed to find any striking correlation between the results of these tests and the quality of the various water supplies. On the whole they appear to have been of little help. The other side of the question is apparent also, for many hesitate to condemn these tests even after obtaining such results. The citrate test has not been used so extensively. Of the five reports on it four bring out some degree of correlation with the sanitary quality of the water. The remaining one found it to be of apparently no value.

QUESTION OF THE USE OF THE DIFFERENTIAL TESTS

Having considered the foregoing results, one may ask whether the differential tests are entitled to a place in our scheme of water examination. Undoubtedly no general answer can be given to this question which will fit all circumstances and different types of water.

In the first place it must be borne in mind that certain details of technic or procedure may account for the lack of correlation with the character of the water supply. The way in which cultures are isolated for testing may be responsible in large measure. We know little

or nothing of the relative rates of growth of the fecal and soil cultures in lactose broth, of their effect upon each other, or of the effect of other miscellaneous water organisms upon them. Obviously, some may be overgrown and thus only one type is isolated and tested. In judging the results of practical application of these tests it is evident that the usual method of selecting cultures may not be the best and it may not give us a true picture of actual proportions in the water. Indeed, this seems to be one of the chief problems confronting the practical use of these tests.

In addition to a consideration of methods, other questions have been raised. It has been pointed out by many that surface washings may be undesirable even though strictly fecal organisms are not found and that the presence of colon group organisms beyond certain limits, irrespective of type, shows the existence of undesirable conditions. Hence the differential tests are regarded as unnecessary. On the other hand, there are a considerable number of reports of the finding of colon cultures, sometimes in fairly considerable numbers, where no source of contamination could be found and where the water was judged to be of high sanitary quality. In many instances these results have been dismissed with the assumption of undiscovered sources of contamination. While this may or may not be the case, the differential tests might aid in interpretation of such results.

In the writer's experience the differential tests have been of most value when applied to raw water supplies of at least fairly good quality. Aside from experimental work, there would seem to be nothing to be gained by applying these tests to a badly polluted water. For example, if colon group organisms are found in 0.01 or 0.001 c.c. amounts it would seem that for practical purposes the differential tests could add little to our information and would scarcely be needed to judge the quality of the water supply.

In contrast to this the differential tests and especially the citrate test appear to be of value in judging water supplies which while not obviously polluted are nevertheless for one reason or another considered to be of questionable quality. The springs and small brooks mentioned in a former publication¹⁴ illustrate the point. Upon sanitary survey they showed no source of pollution at all and yet lactose fermenting colon group organisms were obtained from 10 c.c. amounts almost regularly

and often from 1 c.c. amounts. Upon application of the MR and VP tests the majority of the cultures were MR+ and VP-. Upon application of the citrate test most of them were citrate+ and in this respect could be distinguished from the fecal *Bact. coli*. In this case the citrate test correlated with the sanitary survey and was apparently of value in judging the quality of the water. The recent reports of Pawan^{18, 19} and of Lewis and Pittman¹⁷ seem to confirm this. Houston¹⁰ expressed the opinion that the citrate test might be of considerable value in judging new and doubtful sources of supply. On London waters, however, the test apparently added nothing to the ordinary examination. He states that in general it confirmed the significance of lactose+ indol+ cultures, but sometimes failed to exclude non-typical varieties.

The application of the differential tests to treated or filtered waters presents another problem. Will they give us sufficient information to justify the additional time and labor involved in performing them? This can be answered only by careful study by water works operators. As the matter stands at present it would seem that on the whole there is less need for them here than in the examination of raw waters. In the process of water purification the non-sporing lactose fermenters are used to measure the efficiency of the treatment. By their absence we judge the absence of pathogenes. Although some have found aerogenes to be slightly more resistant to chlorination than coli (and still more resistant than the typhoid bacillus), nevertheless the difference is on the whole not great, and since a margin of safety is always desired, the procedure of aiming at elimination of all

colon organisms would seem to be the proper one. If, however, the differential tests are applied the writer concurs with Hinman⁸ that a more adverse report is justified if the organisms present beyond permissible limits are found to give the differential tests characteristic of the *Bact. coli* of fecal origin.

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