

THE INTERPRETATION OF DIRECT DIFFERENTIAL COUNTS OF COLON-AEROGENES ORGANISMS IN WELL WATERS¹

FRED O. TONNEY AND RALPH E. NOBLE

Bureau of Laboratories and Research, Chicago Department of Health

Received for publication, October 28, 1931

Recently developed differential plating methods of enumerating colon-aerogenes organisms are in need of further study from the standpoint of interpretation of findings. Such a method, originally devised by Noble (1928) and later revised and simplified for use in routine water analyses (Tonney and Noble (1931a), has been used by us in studying the sanitary significance of *Bact. coli* and *Bact. aerogenes* in water supplies (Tonney and Noble (1931b)), and also for studying the relative persistence of the two types of organisms in nature (Tonney and Noble (1931c)). The data of these studies seem to indicate that the *Bact. coli* count in this medium has a much closer and more consistent relationship to fecal sources of pollution than has the *Bact. aerogenes* count.

In addition to yielding separate counts of the *Bact. coli* and *Bact. aerogenes* types, as well as the total group count, the differential plating method possesses the inherent advantages of simplicity, accuracy, economy of time, and relative freedom from the complications of symbiosis, which recommend it for more general use.

Being impressed with the promise of the plating method in water analysis, and recognizing its need of evaluation in the light of the accepted standards based on the older fermentative methods, the authors undertook a comparative study of the findings of the two methods as applied to water samples and their cor-

¹ Read before the laboratory section of the American Public Health Association in its sixtieth Annual Convention at Montreal, B.C., September 14, 1931.

relation with a sanitary survey of the sources of fecal pollution. Private well waters were selected as best adapted to the purpose, because of their wide variation in sanitary quality.

Accordingly, 147 well waters, collected from rural districts adjacent to Chicago, were examined during the summers of 1930 and 1931. The colon-aerogenes content was determined in parallel, by direct plating in ferrocyanide-citrate-agar, and by inoculation of fractional portions into lactose broth and subsequent confirmation, by the standard method of water analysis (American Public Health Association, 1925).

The sanitary survey was made by members of the laboratory staff and was directed primarily toward discovery or reasonable exclusion of definite sources of fecal pollution in the environment.

In all instances where inconsistencies occurred in the findings of the two bacteriological methods, "follow-up" samples were collected personally by the bacteriologist and the sanitary survey confirmed. The collection of data was continued until a definite judgment of the safety of the water was formed. Often new factors were discovered in these secondary investigations, which explained apparent discrepancies in the earlier data. In the final sanitary judgment, the wells were classified as (1) satisfactory, (2) doubtful, and (3) unsatisfactory, on the basis of the sanitary survey alone. The bacteriological findings were then correlated with the sanitary judgment in such a way as to show the relation of the final sanitary judgment to:

1. The separate *Bact. coli* count.
2. The separate *Bact. aerogenes* count.
3. The colon-aerogenes index, determined by the standard method of water analysis.
4. The number of extraneous organisms (rough estimate) appearing on the differential plates.

The data are given in figure 1.

To bring out the relationships, the data are arranged as follows, reading from above downward on the chart:

First, in the order of satisfactory, doubtful and unsatisfactory surveys when all bacteriological findings were negative (column 1).

Next, in the order of the colon-aerogenes index by the standard

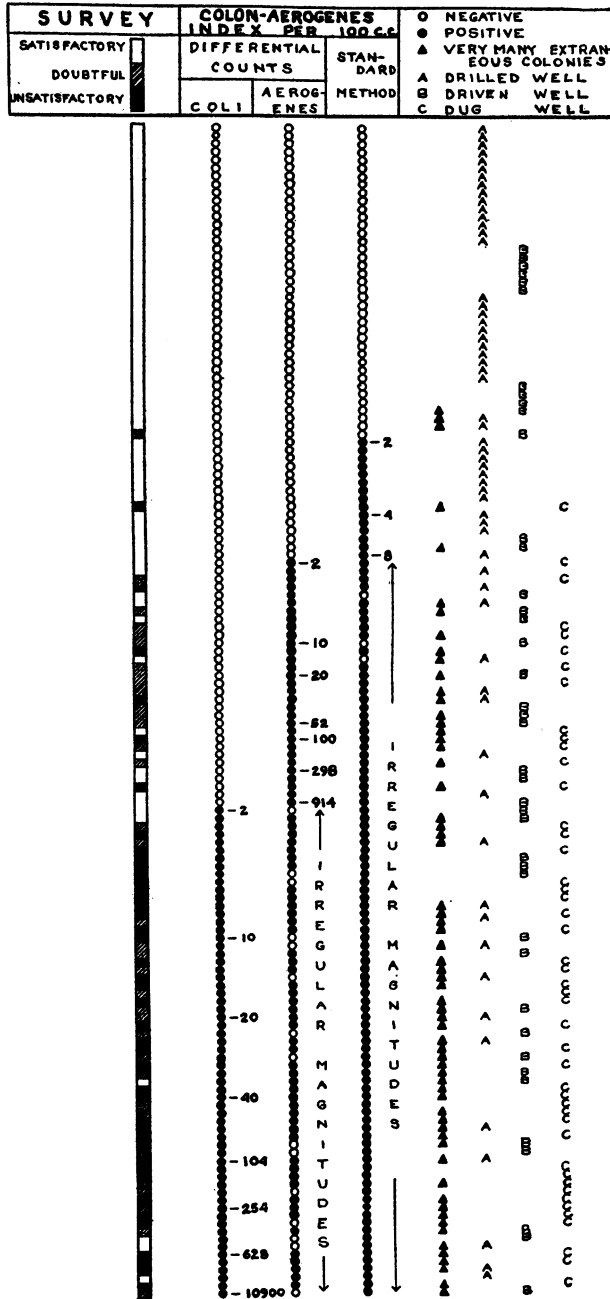


FIG. 1. RELATION OF COLON-AEROGENES FINDINGS TO THE SANITARY SURVEY

method, when the direct differential count of both organisms was negative (column 4).

Next, in the order of the direct *Bact. aerogenes* count, when the *Bact. coli* count was negative (column 3).

And finally, in the order of the direct *Bact. coli* count, irrespective of all other findings (column 2).

On this basis, some very interesting relationships develop.

The clearly satisfactory sanitary surveys, viz.: those in which no source of fecal pollution of the water could be found by repeated inspection, group themselves quite definitely with the negative bacteriological findings by the direct plating method and also in general with the negative findings by the standard method. However, 26.9 per cent of these satisfactory surveys were associated with positive colon-aerogenes findings by the standard method alone. These indices were of low magnitudes (2 to 8 per 100 cc).

A number of doubtful sanitary surveys group themselves with positive *Bact. aerogenes* and negative *Bact. coli* counts by the differential plating method, with the standard method indices usually positive.

The clearly unsatisfactory and the remaining doubtful surveys fall en masse with the direct *Bact. coli* count.

There are interesting sidelights also in the relationships. Thus:

The plates showing numerous extraneous organisms (column 5) which may be considered generally comparable to high bacterial counts in plain agar, are almost without exception identical with the positive *Bact. aerogenes* counts.

The drilled wells (column 6) are grouped primarily with the satisfactory sanitary surveys.

The driven wells (column 7) are fairly well scattered, with a tendency to concentrate in the doubtful and unsatisfactory ranges rather than in the satisfactory.

The dug wells (column 8) fall almost exclusively in the doubtful and unsatisfactory ranges.

From the standpoint of interpretation, the points that stand out in analysis of these data are:

Of the bacterial criteria, the separate *Bact. coli* count seems to

be most consistently associated with the presence of definite sources of fecal pollution. It appears to be the surest and most precise indication of dangerous fecal pollution at present available.

The *Bact. aerogenes* count alone, in the absence of *Bact. coli* in the differential plates, appears to be rather an indication of potential danger of pollution than of actually impending fecal pollution. Its significance seems about parallel to that of a high miscellaneous bacterial content as shown by extraneous forms in the differential plates.

The standard method procedure is found in these studies to be sensitive to both the unsatisfactory and doubtful ranges of sanitary surveys, but is also positive in 26.9 per cent of the wells classed as satisfactory after repeated sanitary surveys. In other words, it is apparently too sensitive to other factors than fecal material and may sometimes be misleading, as a test for actual fecal pollution.

Another factor to be considered in the interpretation of direct differential counts of colon-aerogenes organisms, lies in their value as clues to the solution of special problems of water analysis, such as growths in washers and in wood, when such materials are in direct contact with the water. These growths of *Bact. coli* and *Bact. aerogenes* are very disturbing and are often misleading to those who must pass on the findings of sanitary water analysis. Having in themselves no significance of direct fecal contamination, they nevertheless give the findings of fecal pollution in the water.

Table 1 illustrates the value of the differential plate counts in such special problems of water analysis. It shows:

- a. An infection of a wooden tank with *Bact. aerogenes*.
- b. Infection of a cloth filter with a pure culture of *Bact. aerogenes*.
- c. Infection of a fiber gasket and graphited string packing with *Bact. aerogenes*.
- d. A group of wells found to have washer growths, in some of which the original suggestive findings were confirmed by examination of the washers themselves, and in some of which the water became negative after chlorine disinfection of the washers.

Such growths are suggested when the differential plate count

TABLE 1
Instances of pumping system infections indicated by differential plate counts

	INDEX PER 100 CC.				INDEX PER 100 CC.		
	Differential plate counts		Standard method		Differential plate counts		Standard method
	Coli	Aerogenes			Coli	Aerogenes	
Wooden tank.....	0	12	4	0	2	4	
Cheese cloth bag on pump nozzle.....	0	2,647	1,000	0	0	0	
Fiber gasket and graphited string packing.....	0	2,444	1,000	0	0	10	
	{ 0	66	4	0	0	0	
	{ 0	92	0	0	0	0	
	{ 0	1,200	1,000	0	0	4	
	{ 5,000	200	10,000	0	0	0	
	0	246	10	0	0	0	
Leather washer.....	0	32,600	1,000	2	0	4	
	0	22	6				
	242	20	1,000				
	70	522	1,000				
	0	28	10				
	386	3,700	1,000				

shows one of the two organisms predominating almost to the exclusion of the other. *Bact. aerogenes* is the organism most frequently encountered in this connection.

SUMMARY

A study of 147 samples of water from private wells, collected in rural districts adjacent to Chicago, showed a close correlation between the separate count of *Bact. coli*, and the presence of probable sources of fecal pollution as indicated by the sanitary survey. The separate *Bact. aerogenes* count and the colon-aerogenes index by the standard method (American Public Health Association, 1925) also were uniformly positive in this class of sanitary surveys.

The *Bact. aerogenes* counts in the absence of *Bact. coli* were grouped mainly with doubtful sanitary surveys, in which potential rather than imminent danger of fecal pollution was indicated. The standard method indices were also uniformly positive in this group of sanitary surveys.

The colon-aerogenes index as determined by the standard method (American Public Health Association, 1925) was found to be regularly positive in the groups of unsatisfactory and doubtful sanitary surveys, but was also positive in 26.9 per cent of the wells having satisfactory surveys, in which thorough and repeated search failed to disclose any plausible source of fecal pollution. The differential plates from these samples gave no colon-aerogenes colonies.

REFERENCES

- NOBLE, RALPH E. 1928 A cyanide citrate pour plate medium for direct determination of the colon-aerogenes content of water and sewage. *Jour. Amer. Water Works Assoc.*, **19**, 733.
- TONNEY, F. O., AND NOBLE, R. E. 1931a An improved and simplified cyanide citrate agar for direct enumeration of colon-aerogenes organisms. *Jour. Amer. Water Works Assoc.*, **22**, 1218.
- TONNEY, F. O., AND NOBLE, R. E. 1931b The direct count of colon-aerogenes organisms, its possibilities and promise. *J. I. D.*, **48**, 413.
- TONNEY, F. O., AND NOBLE, R. E. 1931c The relative persistence of *Bact. coli* and *Bact. aerogenes* in nature. *Jour. Bact.*, **22**, 433.