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RESEARCH REPORT 42

FECAL AND TOTAL COLIFORM TESTS IN WATER QUALITY EVALUATION

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ABSTRACT

A review of indicator organisms and bacteriological tests of water is presented. The membrane filter fecal coliform count is compared to total coliform counts and MPN. Support is given for use of the fecal coliform indicator in water quality evaluation studies.

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Edited by Ruth L. Hine

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INTRODUCTION

The maintenance of high quality water is one of the most important aspects of environmental engineering. Consideration must be given many criteria to insure that waters are satisfactory for intended uses. Some contaminants are visible or affect the palatability of the water; others, though present in significant quantities, are not detectable by the average human.

Toxic materials or certain minerals in a water supply are undesirable because of their hazardous properties. Water-borne diseases such as typhoid fever, the paratyphoids, dysentery, infectious hepatitis and cholera are often disseminated in water systems. The disease-causing organisms (pathogens) responsible for these illnesses are found in the intestinal discharges of patients or carriers and enter the water supply by some means. Strict regulations, good engineering practices, and immunizations have nearly eliminated the health problems associated with water-borne contamination.

Analyses for chemicals and their toxicity limits to man and animals have been fairly well documented. Accurate and meaningful tests for disease-causing organisms, however, are not infallible in determining whether or not a given water is truly detrimental to health. The Public Health Service Drinking Water Standards have been established with significant data compilation and supporting evidence so that public supplies are rendered reasonably safe from contamination.

Increased water-based recreational activities have created a need for uncontaminated surface waters. Ingestion of pathogenic organisms by a recreationist may cause diseases similar to those experienced by consumers of a contaminated water supply. In the past, bacteriological tests similar to those for drinking water have been used for surface waters, but they have proven unwieldly for this purpose. A simple test which relates more directly to the disease-causing organisms would be of value to maximize the safe potential use of surface waters.

This report gives a brief account of indicator organisms and the necessity for accurate bacteriological determinations. Tests for fecal coliform bacteria have been suggested as better indicators than the old total coliform test. Methods of detection and advantages and disadvantages of each of these bacterial strains is explained. A comparison of the two tests is then made.

INDICATOR ORGANISMS

Bacteria are introduced into waters from many sources; naturally or by man and his activities. Feces from warm-blooded animals, including humans, may, at any time, contain disease-producing microbes consisting of bacterial pathogens, viruses, or intestinal parasites. Most bacteria present in surface waters are not harmful to health, but if pathogenic organisms are ingested, disease or sickness may occur.

There are two reasons for the increased emphasis on the detection of true bacterial contamination in streams. One is that sewered areas have increased in recent years, and increased bacterial discharges have resulted despite advances in waste treatment methods (the disposal of nondisinfected or untreated human wastes is the primary cause of contamination). The second reason for emphasis on detection is that recreation involving increased contact with surface water has resulted in more direct exposure of persons to these bacterially contaminated waters. Few people using contaminated waters for recreation are aware that a hazard exists or that the water is unsafe.

One of the more important laboratory tests to determine water quality is the bacteriological test. This test indicates whether or not a given water is bacterially contaminated, and the extent of such contamination. The test is critical in public supply systems where bacteria may cause an outbreak of disease; however, in surface waters the test is usually not quite as critical, though it may be equally as important to the user. In addition to the laboratory test, other information concerning the probable source and significance of the count must also be obtained in order for the analysis to be meaningful.

When water supplies or surface waters used for recreation are tested, the laboratory is able to determine with reasonable confidence whether or not the water is satisfactory for human use. However, the attempts to isolate pathogenic bacteria have proven to be rather useless and confusing. The 12th edition of Standard Methods for the Examination of Water and Wastewater states that "the isolation of pathogenic bacteria . . . from water and sewage cannot be recommended as a routine practice, inasmuch as the techniques available at the present time are tedious and complicated" (Amer. Public Health Assoc. et al., 1965). It is seldom possible to isolate these intestinal pathogens directly from water because they are present in relatively small numbers, they enter the water sporadically, and a different test is required for each pathogen.

There are two problems that the water pollution investigator must face concerning bacteriological contamination. The first is the choice of a procedure for the quantitative determination of indicator organisms in the water, the second is the determination of the probable native habitat of the micro-organism insofar as it might influence the proposed water uses. Therefore, an indicator organism, one that reasonably reflects the bacterial quality of the water, is needed. Several such indicator systems have been proposed including certain pathogenic bacteria, anaerobic spore-formers, and total bacterial populations, but these have not proven satisfactory. From extensive experience and testing, the coliform has been established as a satisfactory indicator organism. Coliform bacteria and the water-borne enteric pathogens exist under similar conditions, although millions of coliforms occur for every pathogenic bacterium. It has been concluded then, that if coliform counts are relatively low, it is safe to assume that pathogenic bacteria will not be present in quantities hazardous to health.

COLIFORM ORGANISMS

Standard Methods defines the coliform group as "all of the aerobic and facultative anaerobic, Gram-negative, nonspore forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C." (Amer. Public Health Assoc. et al., 1965). These coliform organisms are present on soil, on plants, and on other living things. Coliform bacteria are also present in the feces of all warm-blooded animals in extremely large numbers. The daily per capita excretion rate for humans varies from 125 to 400 billion.

Coliform bacteria are usually prevalent in streams and are especially common during periods following rainfall when there are large amounts of surface runoff. These bacteria enter streams as wash from cities, by-passed and overflow sewage, runoff from soil and certain vegetation, sewage treatment-plant effluents, and from contaminated bottom sediments and sludge deposits. During dry weather it is usually possible to relate coliform counts to waste water discharges because of the small number of bacteria entering a stream from runoff. Coliform numbers increase in a stream from four to eight times the effluent number and reach a maximum about one-half day's travel time downstream from the discharge point (Kittrell and Furfari, 1963). Dilution of waste water with relatively clean stream water contributes to an increase in numbers of coliforms in the receiving stream.

The daily per capita contribution of coliforms to streams does not appear to be affected by small variations in organic nutrient levels. When initial nutrient levels are relatively high, the coliform bacteria tend to decrease more rapidly; however, extremely large numbers of coliforms have been associated with excessively high nutrient levels, especially in streams receiving sugar containing industrial wastes. Caution should also be used in predicting coliform numbers when BOD is high, since high BOD usually indicates some sort of contamination or organic enrichment (Kittrell and Furfari, 1963).

The coliform bacteria have the following advantages for use as an indicator organism: (1) coliforms are constantly found in the human intestine in large numbers, (2) the fate of the coliform organism reasonably reflects that of the pathogenic bacteria, although the coliform bacteria will normally live longer than intestinal pathogens, (3) the coliform organism is easy to isolate and enumerate in the laboratory, and (4) coliforms are normally not pathogenic and are easy to handle. The primary fault of the total coliform test is not in its inability to detect unsafe samples, but that safe samples may show up as unsafe samples. Therefore, because some coliforms are present in nature outside the feces of warm-blooded animals, the samples containing these nonfecal organisms only will also be considered unsafe. This results in declaring water unsuitable for contact recreation when there are no harmful organisms present in the water. It is generally accepted, however, that the absence of the coliform indicates water is safe for human consumption and use. Because of these disadvantages, new organisms are being sought which may be more reliable indicators of sewage pollution.

Investigation into the fecal coliform subgroup of the coliform group indicates that this organism may be a better tool to detect evidence of fecal pollution (Geldreich, 1967). Fecal coliforms exhibit all the characteristics of total coliforms, but are more specific in that they are normally present only in fecal material. If it is accepted that the coliform bacteria of fecal origin are more representative of potential pathogens, then separation of the fecal and nonfecal coliform groups is justified. Most researchers have concluded that fecal coliforms rather than total coliforms are more realistic indicators of sewage pollution.

At the present time there is no way to distinguish fecal coliforms of human origin from those of other warm-blooded animals, and therefore the fecal coliform test is considered indicative of dangerous contamination from all warm-blooded animals. Geldreich (1967) found fecal coliforms in 95.6 percent of the fecal samples from warm-blooded animals, with fecal coliforms in 96.4 percent of the human fecal samples (Table 1).

TABLE 1

Correlation with Coliform Strains from Warm-blooded Animal Feces*

Fecal Source	Fecal Coliform Percentage
Human	96.4
Livestock	98.7
Poultry	93.0
Cats, Dogs, and Rodents	95.3

*From Geldreich (1967), Table 1.

Fecal coliforms may enter suface water by a number of ways, from contaminated soil runoff from storm water, from vegetation and insects, wash from cities, or from direct sewage pollution by man or animals. Fecal coliform bacteria are not naturally present in the intestinal tracts of fish, but may be there due to ingestion of polluted waters. Therefore, because coliforms and pathogens exist in similar environments, it may be possible for fish to carry pathogenic bacteria to unpolluted waters. The fecal subgroup is usually absent, or present in small numbers, in undisturbed soils (less than 2 organisms per gram), but they do become common when the soil is contaminated with sewage-polluted water (Geldreich 1967). Once fecal bacteria are introduced to a soil, they remain there unless they are washed from the soil.

Salmonella organisms have been consistently recovered from certain streams when fecal coliform counts are high (> 1,000/100 ml) (Table 2),

TABLE 2

Detection of Salmonella at Varying Levels of Fecal Pollution in the Red River of the North*

River Station	Fecal Coliforms Per 100 ml	Salmonella Detection**
RR-9	49	None Detected
SH-13	218	None Detected
RR-29	1,030	S. reading, S. infantis
RR-16	1,610	S. heidelberg
RR-12	2,850	S. saintpaul
RR-18	2,950	S. saintpaul, S. thompson
RR-28	2,970	S. reading

* From Geldreich (1967), Table 6.
** Modified Moore swab.

and occasionally detected when fecal counts are extremely low (< 20/100 ml). The recovery of Salmonella when fecal counts are low (< 1,000/100 ml) is due mainly to deficiencies in pathogen detection procedures and unpredictable Salmonella discharges (Geldreich, 1967). Fecal streptococci organisms are also usually present in substantial numbers when fecal coliforms are present.

DETECTION OF COLIFORM ORGANISMS

Total Coliforms

There are several methods presently in use to detect coliform organisms in water. The examination for coliform organisms may be done by either the multiple-tube-fermentation-technique or by the use of the membrane filter. The multiple-tube procedure is one of the best tested and most authenticated available and is used as a basis for water quality standards. This procedure involves a series of preliminary and confirmatory tests in which gas bubbles are formed in small glass vials by the action of coliform bacteria in a lactose broth medium. A statistical analysis, called the most-probable-number (MPN), is then made. The MPN is not an actual count of organisms, but merely an index of the number of coliform bacteria which, more probably than any other number, would give results shown by the laboratory examinations.

There are several things that must be noted when using the MPN analysis: (1) the scale is discontinuous leaving large gaps in the determining and reporting; (2) the number called MPN is actually only representative of a range within which the actual number indicating coliform concentration may be expected to be; (3) it is necessary to have a large amount of data before satisfactory conclusions concerning water quality can be reached; (4) due to the length of the test, it indicates what the water quality was like several days before results are obtained; and (5) the test is not sensitive to large fluctuations in coliform densities.

In recent years, testing for coliform organisms has switched almost entirely to the Membrane Filter Coliform Count (MFCC). Widespread use of this test method has shown its value as a detection device. The membrane filter is a thin, flat, highly porous, flexible plastic disc usually 47 mm in diameter. White grid-marked filters with 0.45 micron pores are usually used for microbiological analyses. The pad's high porosity (80% of filter volume) allows rapid filtration of aqueous suspensions, although if the water is turbid or contains significant algae concentrations, the filter may become clogged and not allow large quantities of water to be filtered. The filter is then placed on a culture medium (M-Endo-MF broth) and after incubation for 24 hours (± 2) at 35°C (± .5°) the appropriate colonies are counted. In addition to being a medium for growth, the M-Endo-MF broth inhibits the growth of some noncoliform bacteria and thus aids in the differentiation of bacteria.

There are several distinct advantages to the membrane filter for detecting coliform organisms: (1) the procedure is relatively simple, (2) the results are obtained in 24 hours instead of the 48 to 96 hours needed for the MPN test, and (3) larger volumes of sample may be analyzed and, therefore, a more representative sample may be obtained.

There are, however, several limitations to the technique: (1) larger numbers of certain noncoliforms are capable of growing on the filter and may complicate the counting procedure, (2) suspended solids may interfere with interpretation of the test, (3) toxic substances may interfere with results, and (4) careful consideration must be given to dilutions of the analyzed water.

The 12th edition of Standard Methods (Amer. Public Health Assoc., 1965) adopts the MFCC as a standard procedure for coliform detection. Modifications in the procedure and especially in the culture medium prior to the publishing of that edition has rendered this test comparable with the MPN analysis. Table 3 shows a comparison of counts from the two methods. Note that the coliform counts from the river samples are usually greater by the membrane filter method, with the overall ratio of MFCC, (referred to as MF in the table), to MPN 1.52 for all samples. Based on these and other analyses, it has been almost unanimously concluded that the membrane filter coliform count is a more accurate determination of total coliform counts than the MPN method.

Fecal Coliforms

The multiple-tube procedure is the only method presently recommended by Standard Methods for the detection of fecal coliforms. The confirmatory total coliform test is run at an elevated temperature (44.5°C) to separate the fecal from the nonfecal strains (Fig. 1). Geldreich (1965) indicated that the multiple-tube test can differentiate between fecal coliforms from warm-blooded animals and coliforms from other sources with a 96 percent accuracy.

TABLE 3

Evaluation of Results with M-FC Medium on Various Waters in Ohio[1]

Sample	Number of Coliform Organisms						Coliform Organism Ratios	
	Total (MPN)		Total (MF)	Fecal (MPN)	MFC			
	Confirmed	Completed			Count	Verified	Total MF / Total MPN	Fecal MFC / Fecal MPN
Wells								
Baas	33	17	4.3	2.0	1.3	1.1	0.25	0.65
Kilb	130	33	37	<2.0	2.8	2.8	1.12	Indr.*
Gilman	278	221	215	<2.0	<0.8	<0.8	0.97	Indr.*
Lakes								
Devou	109	70	36	11	8	8	0.51	0.75
Sharon	221	94	80	<2.0	1.5	1.5	0.85	Indr.*
Burnet	1,700	1,100	380	220	300	270	0.35	1.36
Winton	4,600	3,300	3,300	2,300	2,500	2,500	1.00	1.00
Smith	172,000	49,000	35,000	13,000	5,500	5,500	0.71	0.42
Creeks								
4-Mile	490	141	80	49	22	22	0.57	0.45
8-Mile	350	350	160	23	10	10	0.46	0.44
Taylor	490	490	700	490	550	550	1.43	1.12
Huntley	2,300	1,300	450	14	17	17	0.35	1.21
Upper Cluff	7,000	2,210	900	490	370	370	0.41	0.76
Berkshire	22,000	4,900	10,000	790	1,000	1,000	2.04	1.21
9-Mile	10,900	7,900	15,000	330	550	550	1.90	1.64
Lower Cluff	7,900	7,900	5,300	2,300	1,700	1,500	0.67	0.74
Duck	78,000	49,000	86,000	10,900	6,000	5,400	1.76	0.55
Mill	4,900,000	2,210+	4,800+	221,000	440,000	400,000	2.17	1.99
Lagoon								
Ludlow	49,000	14,100	12,000	1,720	3,600	2,900	0.85	2.09
Rivers								
Little Miami-Kellogg	1,720	1,090	2,100	330	150	150	1.93	0.46
Beechmont	10,900	2,210	3,300	330	290	260	1.49	0.88

Table 3 (contd.)

Sample	Number of Coliform Organisms						Coliform Organism Ratios	
	Total (MPN)		Total (MF)	Fecal (MPN)	MFC			
	Confirmed	Completed			Count	Verified	Total MF / Total MPN	Fecal MFC / Fecal MPN
Newton	10,900	10,900	22,000	3,300	2,800	2,800	2.02	0.85
Great Miami	840	310	470	23	58	46	1.52	2.52
Licking	10,900	10,900	16,000	3,300	3,700	3,700	1.47	1.12
Ohio (mile no.)								
453.9	3,300	3,300	1,300	172	200	180	0.39	1.16
461.6	1,720	700	2,300	172	160	160	3.29	0.93
463.0	460	310	260	130	56	50	0.84	0.43
464.1	700	490	1,300	172	140	140	2.65	0.81
465.0	17,200	17,200	29,000	3,300	1,400	1,100	1.69	0.42
469.8	33,000	17,200	44,000	3,300	6,800	6,800	2.56	2.06
472.4	46,000	14,100	45,000	4,600	4,200	4,200	3.19	0.91
475.1	1,300+	1,300+	3,100+	790,000	1,300+	1,300+	2.30	1.65
482.0	49,000	49,000	52,000	14,100	10,200	10,200	1.06	0.72
485.8	23,000	13,000	64,000	4,900	4,800	4,300	4.92	0.98
513.3	230	230	440	79	58	58	1.91	0.73
517.6	130	27	56	17	12	12	2.07	0.71
522.1	130	11	22	5	2	2	2.00	Indr.*
Domestic Sewage								
Linwood	13,000+	7,900+	19,000+	4,900+	5,400+	5,400+	2.41	1.10
Beechmont	33,000+	17,200+	31,000+	4,600+	6,400+	5,800+	1.80	1.39
Berkshire	54,200+	34,800+	35,000+	4,900+	5,000+	4,500+	1.01	1.02

[1]From Geldreich et al. (1965), Table 1.
* Indeterminate
\+ Thousands

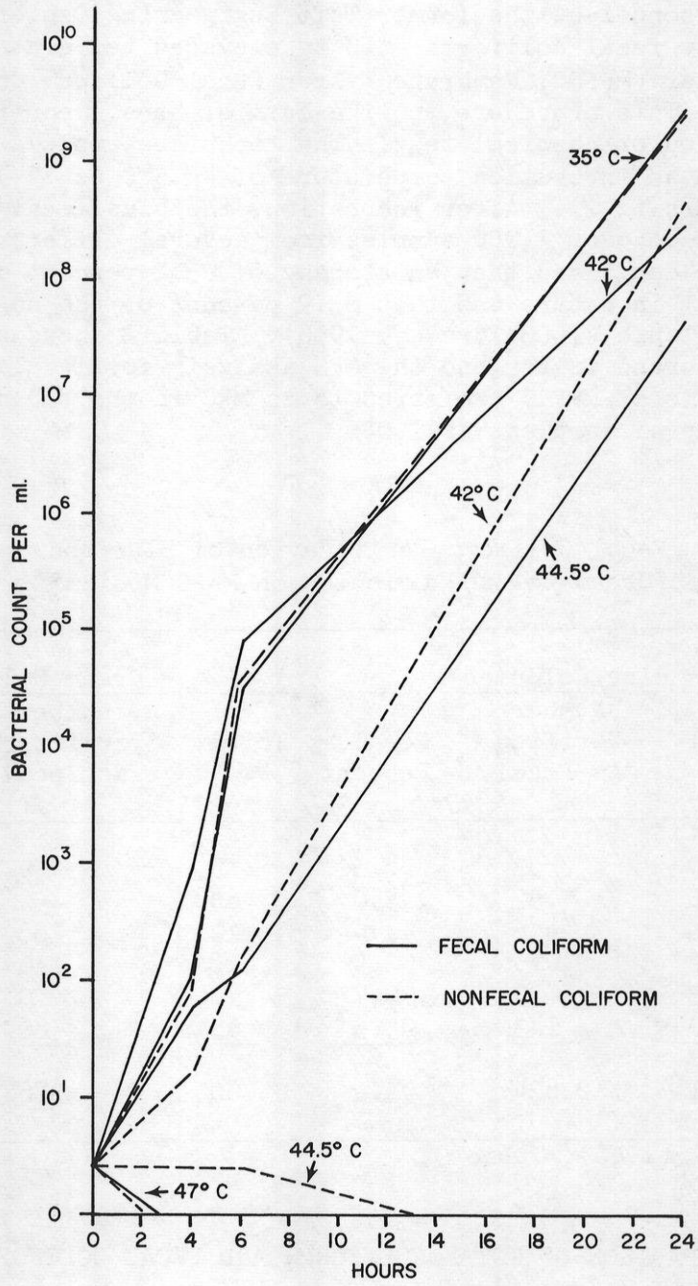

Figure 1. Fecal coliform and nonfecal coliform growth response in EC broth at various temperatures. (From Geldreich, 1967, Figure 1.)

Due to the increasing use of the membrane filter in detecting total coliform organisms, there has developed a need for a similar test for fecal coliforms. Personnel at the former Taft Engineering Center developed a medium to detect fecal coliforms with an elevated temperature test by using a membrane filter (MFFCC, Membrane Filter Fecal Coliform Count) (Geldreich, et al., 1965). This procedure, unlike earlier ones, required no prior enrichment period or chemical test. The technique employs an M-FC Broth base medium and an incubation temperature of 44.5°C (± .5°) in a water bath for 24 hours (± 2). After incubation, the blue fecal colonies are counted. Tests on over 3,000 samples from several different types of sources in Ohio confirmed that an average of 93.2 percent of the blue colonies were truly fecal in nature and that 83.9 percent of the nonblue colonies were nonfecal (Table 4) (Geldreich, 1966). Table 3 shows a comparison between the membrane filter and the MPN analysis for fecal coliforms. The overall ratio of MFFCC (referred to as MFC in the table), to MPN for the entire group of samples was 1.04.

TABLE 4

Fecal Coliform Verification of Blue and Cream-Colored Colonies on M-FC Medium*

	Blue Colonies			Cream Colonies		
Source	Total No.	Number Verified As Fecal	Fecal Percent	Total No.	Number Verified As Nonfecal	Nonfecal Percent
Wells	60	53	88.3	9	9	100.0
Lakes	60	57	95.0	64	53	82.8
Creeks	496	456	91.9	114	88	87.2
Rivers	2,222	2,076	93.4	208	176	84.6
Lagoon	43	39	90.7	0	0	0
Sewage	150	143	95.3	65	60	92.3
Total	3,031	2,824	93.2	460	386	83.9

*From Geldreich (1966), Table 9.

COMPARISON OF TOTAL COLIFORMS AND FECAL COLIFORMS

There are many variables present in trying to relate fecal coliforms to total coliforms. These variables include stream flow, precipitation and runoff, characteristics of the runoff area, season of the year, and existence of effluents. The presence of these variables, together with inconsistencies in sampling, make it nearly impossible to correlate fecal and total coliform counts except in a general way. The following information bears this out.

TABLE 5

Coliform and Fecal Coliform Counts at Two Milwaukee (Lake Michigan) Beaches*

BIG BAY BEACH 1964 Membrane Filter Colonies/100 ml			KLODE PARK 1964 Membrane Filter Colonies/100 ml		
Date	Coliform Group	Fecal Coliform Group	Date	Coliform Group	Fecal Colifrom Group
6/15	10	-	6/15	230	-
6/17	900	26	6/17	230	11
6/19	20	< 4	6/19	30	< 4
6/22	100	18	6/22	150	< 4
6/24	700	190	6/24	310	16
6/26	75	26	6/26	20	29
6/29	20	< 4	6/29	< 10	16
7/1	48	12	7/1	15	8
7/6	3,600	110	7/6	650	20
7/8	13,000	100	7/8	860	18
7/10	340	20	7/10	130	30
7/13	430	50	7/13	230	37
7/15	250	< 5	7/15	45	< 5
7/17	52	9	7/17	19	7
7/20	22,000	1,300	7/20	610	45
7/22	1,800	120	7/22	1,600	92
7/24	1,100	140	7/24	370	75
7/27	1,600	88	7/27	980	82
7/29	6,000	180	7/29	2,700	43
7/31	840	83	7/31	350	43
8/3	830	8	8/3	490	7
8/5	2,000	19	8/5	1,400	47
8/7	50	< 5	8/7	95	5
8/10	840	63	8/10	610	< 5
8/12	290	25	8/12	120	20
8/14	880	28	8/14	63	< 5
8/17	50	< 5	8/17	150	5
8/19	710	40	8/19	740	20
8/21	11,000	1,300	8/21	1,800	180
8/24	90	13	8/24	40	< 5
8/26	< 10	10	8/26	5	< 5
8/28	410	57	8/28	160	23
8/31	130	5	8/31	65	5
9/2	110,000	11,000	9/2	750	15
9/4	370	40	9/4	160	< 5
Geometric Avg.	493.9	65.4	Geometric Avg.	186.2	24.5
Arith. Avg.	5,300	520	Arith. Avg.	474.9	37.4
Median	700	27	Median	230	16
Max.	110,000	11,000	Max.	2,700	180
Min.	< 10	< 4	Min.	5	4

*From Ernest (1965), Tables I and II.

A tabulation of total and fecal counts in shown in Table 3. The average fecal count is about 19 percent of the average total count for all stations, but varies from 2 percent to about 76 percent. Total and fecal coliform counts at two Lake Michigan beaches in the Milwaukee area are shown in Tables 5 and 6. The counts in the latter table are those present after an excessive rainfall and high storm sewer flows. The fecal counts are higher than normal at both beaches, but this is not necessarily the case of total counts especially at Big Bay Beach. The large variability in counts is revealed by the differences in geometric and arithmetic average values in Table 5.

Table 7 is a tabulation of data from the Milwaukee sewage treatment plants and also shows ratios of total to fecal counts. The average fecal values are 13.7 percent, 19.8 percent and 24.7 percent of the average total values for each of the stations shown. Coliform counts from different areas during different seasons of the year are shown in Table 8. This table indicates little correlation between the fecal and total counts either for different seasons or different areas. Coliform densities in

TABLE 6

Comparison of Data Following Excessive Rainfall at Two Milwaukee (Lake Michigan) Beaches*

1964

	Big Bay		Klode	
Date	Coliform Colonies	Fecal Coliform Per 100 ml	Coliform Colonies	Fecal Coliform Per 100 ml
7/20	22,000	1,300	610	45
7/22	1,800	120	1,600	92
7/24	1,100	140	370	75
7/27	1,600	88	980	82
7/29	6,000	180	2,700	43
7/31	840	83	350	43

*From Ernest (1965), Table III.

TABLE 7

Total Membrane Filter Coliform and Membrane Filter Fecal Coliform Concentrations
Milwaukee Jones Island Treatment Plant (All Counts in 1,000's per 100 ml.)*

Month		Total Count on Effluents			Fecal Counts on Effluents			Ratio Total to Fecal		
		Screened Sewage	West Plant	East Plant	Screened Sewage	West Plant	East Plant	Screened Sewage	West Plant	East Plant
1964	Avg.	30,800	1,070	900	5,010	366	221	6.14	2.92	4.08
Aug.	Max.	73,000	3,300	5,000	12,000	1,400	1,100			
	Min.	4,000	140	230	270	33	28			
Sept.	Avg.	51,800	1,800	1,380	6,270	620	560	8.26	2.90	2.46
	Max.	94,000	4,600	2,200	11,000	2,100	1,700			
	Min.	12,000	570	110	1,000	69	100			
Oct.	Avg.	35,600	1,040	1,320	3,450	141	305	10.31	7.37	4.32
	Max.	96,000	3,500	2,900	9,300	510	730			
	Min.	12,000	480	270	660	41	20			
Nov.	Avg.	30,400	1,330	1,090	3,930	108	163	7.73	12.31	6.68
	Max.	88,000	8,800	4,200	8,400	390	660			
	Min.	8,100	120	60	1,600	8	12			
Dec.	Avg.	32,100	780	542	4,070	155	135	7.88	5.03	4.01
	Max.	90,000	2,200	2,600	11,000	430	790			
	Min.	4,900	250	140	700	25	12			
1965	Avg.	15,600	840	354	2,810	324	91	5.55	2.59	3.89
Jan.	Max.	46,000	3,400	1,100	11,000	3,000	250			
	Min.	2,500	140	58	280	39	20			
Feb.	Avg.	12,200	832	343	2,540	188	121	4.80	4.43	2.83
	Max.	27,000	2,300	860	7,200	520	370			
	Min.	1,500	160	100	300	28	27			
Mar.	Avg.	8,190	363	263	1,120	104	71	7.31	3.49	3.70
	Max.	15,000	780	610	2,400	460	200			
	Min.	4,700	140	14	460	30	19			
Apr.	Avg.	8,240	330	273	1,150	69	57	7.17	4.78	4.79
	Max.	26,000	1,000	600	4,100	200	150			
	Min.	2,800	110	60	220	18	16			
May	Avg.	13,200	350	362	1,710	78	99	7.71	4.48	3.66
	Max.	41,000	860	830	4,900	180	180	7.29	5.03	4.04
	Min.	4,700	130	60	400	26	29		(Averages)	

*From Ernest (1965), Table IV.

TABLE 8

Seasonal Variations (Median Values) for Bacterial Discharges in Storm Water and Rain Water from Suburban Areas, Cincinnati, Ohio, and in Agricultural Land Drainage, Coshocton, Ohio*

Source	Date	Total Samples	Season	Total Coliform	Fecal Coliform	Fecal Streptococcus	Ratio FC/FS	Percent Fecal Coliform
Wooded hillside	Feb.62 to Dec.64	278						
			Spring	2,400	190	940	0.20	7.9
			Summer	79,000	1,900	27,000	0.70	2.4
			Autumn	180,000	430	13,000	0.03	0.2
			Winter	260	20	950	0.02	7.7
Street-gutters	Jan.62 to Jan.64	177						
			Spring	1,400	230	3,100	0.07	16.4
			Summer	90,000	6,400	150,000	0.04	7.1
			Autumn	290,000	47,000	140,000	0.34	16.2
			Winter	1,600	50	2,200	0.02	3.1
Business district	Apr.62 to Jul.66	294						
			Spring	22,000	2,500	13,000	0.19	11.4
			Summer	172,000	13,000	51,000	0.26	7.6
			Autumn	190,000	40,000	56,000	0.71	21.1
			Winter	46,000	4,300	28,000	0.15	9.4
Rural	Jan.63 to Aug.64	94						
			Spring	4,400	55	3,600	0.02	1.3
			Summer	29,000	2,700	58,000	0.05	9.3
			Autumn	18,000	210	2,100	0.10	1.2
			Winter	58,000	9,000	790,000	0.01	15.5
Rainwater	Jan.65 to Feb.67	49						
			Spring	< 1.0	< 0.3	< 1.0	-	-
			Summer	< 1.0	< 0.7	< 1.0	-	-
			Autumn	< 0.4	< 0.4	< 0.4	-	-
			Winter	< 0.8	< 0.5	< 0.5	-	-

*From Geldreich et al. (1967), Table I.

urban storm water runoff are tabulated in Table 9. An extreme variance in fecal to total values is noted and no correlation is evidenced. Table 10 shows bacteriological and chemical data from a one-day survey of Cedar Creek in eastern Wisconsin, and again correlation is poor.

TABLE 9

Bacterial Densities in Urban Storm Water Runoff[1]

Rainfall-Runoff Event	Indicator Density - Count/100 ml		
	Total Coliform	Fecal Colifrom	Fecal Streptococcus
March 23, 1966**	152,000	3,200	20,000
July 7, 1964*	920,000	27,000	61,000
August 5, 1965*	2,280,000	31,000	48,000
August 19, 1965*	2,670,000	1,210,000	22,000
Sept. 15, 1965**	45,000,000	430,000	42,000
Sept. 22, 1965**	28,000,000	260,000	290,000
Nov. 24, 1964*	270,000	2,650	5,000
Feb. 6, 1964*	250,000	7,400	8,800
Feb. 10, 1966**	23,900	1,050	6,600

*Flow proportional sample
**Grab Sample
[1]From Evans et al. (1967), Table 2.

TABLE 10

Cedar Creek Stream Survey Results*
July 19, 1967

Sample Location	Mileage	5-day BOD mg/l	DO mg/l	MFCC per 100 ml Total	MFCC per 100 ml Fecal
County Trunk Hwy. "NN" below Big Cedar Lake	30.8	1	7.5	1,000	520
Town road bridge, 1 mile below Little Cedar Lake	27.9	2	3.0	2,000	200
Town road bridge in Cedar Creek	25.8	2	7.6	6,000	180
Wis. Hwy. 60 below Mayfield	23.9	2	8.8	15,000	900
Sherman Road bridge 1 mile south of Jackson	21.3	2	8.9	12,000	1,500
Lagoon Outfall Libby, McNeill & Libby	20.5	6/29/67 72 239 8/10/67 164	-	-	-
Wis. Hwy. 60 bridge above Jackson Sewage Treatment Plant	20.0	3	8.9	13,000	1,800
Jackson Sewage Treatment Plant outfall at bridge	20.0	77	-	-	-
County Trunk Hwy. "G" bridge below Jackson	18.5	3	6.4	22,000	1,100
County Trunk Hwy. "M" bridge	15.4	2	7.2	2,000	110
County Trunk Hwy. "Y" bridge	13.1	4	7.8	200	<100
Wis. Hwy. 143 bridge at Horns Corners	11.8	2	8.3	3,000	50
Covered bridge road 3 miles no. of Cedarburg	9.4	3	12.1	1,300	270
County Trunk Hwy. "I" bridge 3-1/2 miles no. of Cedarburg	8.2	2	11.6	4,000	120

TABLE 10 (contd.)

Sample Location	Mileage	5-day BOD mg/l	DO mg/l	MFCC per 100 ml Total	Fecal
Wis. Hwy. 60 bridge 2 miles no. of Cedarburg	6.3	2	9.9	9,000	1,200
Foot bridge in Cedarburg behind fire dept. bldg.	4.5	2	2.6	4,000	80
Storm Sewer Outfall in Tailrace	4.3	21	-	-	-
County Trunk "T" bridge 1/2 mile above Cedarburg sewage treatment plant	2.0	2	13.2	12,000	130
Cedarburg Sewage Treatment Plant Effluent	1.6	26	-	-	-
Bridge at Hamilton 1/2 mi. below Cedarburg Sewage Treatment Plant at Hamilton	1.2	3	7.8	230,000	3,300
SOUTH BRANCH					
Town Road Bridge 2 miles south of Jackson	0.8	2	6.7	9,000	900

*Kroehn (1967).

Routine sampling at 35 monitoring stations throughout Wisconsin has been conducted for fecal and total coliforms since mid-1965 (Table 11). Through 1968, the data from these samplings have shown very little, if any, trend in total coliform-fecal coliform relationships. Perhaps this inconclusive evidence is due to dissimilar sampling conditions as well as variations in counts. Generally, the fecal counts were much lower than the total coliform counts, the average total count at all monitoring stations for the three and one-half years being 57 times greater than the fecal counts. Average total to fecal ratios over the period in some streams were nearly ten times greater than average ratios in other streams. No correlation of organism populations, either geographically or on the basis of apparent clean (Wolf River) or polluted (Fox River) streams appears to exist. Usually, but not always, the total fecal coliform ratio was higher in the summer than in the winter. The indication here is that perhaps the number of coliforms entering the stream is less in the winter, or that the coliform organism has a faster die-off rate than the fecal coliform in the winter. The large variation in organism counts may be due to substantial changes in organism populations, or, perhaps, to significantly different sampling conditions.

Several sets of samples were collected on the Beaver Dam River below the City of Beaver Dam sewage treatment plant during 1968 (Table 12).

TABLE 11

Total Coliform and Fecal Coliform Counts in Selected Wisconsin Streams*

Date	Rock R. at Afton		Wolf R. at Keshena		Fox R. at Green Bay		Wis. R. at Bridgeport		Chippewa R. at Pepin	
	Total Coliform	Fecal Coliform	Total Coliform	Fecal Coliform	Total Coliform	Fecal Coliform	Total Coliform	Fecal Coliform	Total Coliform	Fecal Coliform
7/65	270		.5	<.1	58		.3	.1	11	.2
8/65	100	39.0	.1	<.1	5		.2	<.1	6	.3
9/65	630	33.0	2.3	.2	16		2.5	<.1	29	.8
10/65	69	7.7	1.1	<.1	14		8.0	.1	42	1.5
11/65	150	8.8	.8	.1	14		6.4	.2	30	2.3
12/65	63	4.5	2.0	.2	46		1.7	<.1	33	1.8
1/66	20	3.8	<.1	<.1	73	19.0	1.1	.3	3.3	1.1
2/66	10	1.9	.1	<.1	35	.5	.2	.1	2.9	1.1
3/66	30	5.1	<.1	<.1	16	3.0	.2	<.1	5	1.1
4/66			.3	<.1	9	<.1	2.3	.1	10	.3
5/66	90	1.9	1.1		4		1.8			
6/66	120	3.8	3.0	<.1	12	<.1	.1	<.1	5.8	<.1
7/66	610	10.0	2.0	<.1	25	1.9	4.0	.2	12	<.1
8/66	310	21.0	1.9	<.1	130	.4	.7	<.1	12	1.0
9/66	280	8.0	.8	<.1	16	1.2	1.7	<.1	15	.2
10/66	37	1.6	1.0	<.1	21	.5	1.9	<.1	130	2.0
11/66	>47	8.0	1.7	<.1	34	.2	6.0	.1	70	1.3
12/66	150	12.0	.6	<.1	71	1.8	120.0	1.3	36	4.1
1/67	75	7.1	1.3	.01	56	1.0	7.0	<.1	35	1.3
2/67	76	12.0	1.2	<.01	32	1.5	.7	.15	36	2.1
3/67	66	2.0	.9	<.01			3.4	.04	31	1.2
4/67	41	2.4	1.3	<.01	16	3.0	1.1	.06	8	
5/67	160	3.1			18	.22	1.8	.07	21	.1
6/67	110	2.0	6.2	.04	28	.4	.9	.02		
7/67	190	4.8	5.0	.07	17	.2	.1	.01	27	.37
8/67	360	4.8					.4	.05	7	.2
9/67	130	2.4	1.9	.01	12	.4	12.0	.15	74	.12
10/67	190	5.6	1.1	>.005	9	.18	3.5	.3	36	.11
11/67	76	4.7	2.7	.015	54	1.8	7.0	.14	71	1.5
12/67	67	7.0	.8	.02	43	3.5	1.8	.03	150	3.5
1/68	320	17.0	.2	<.1	39.0		3.0	.08	34.0	1.8
2/68	68	9.9	.68	.005	50.0	1.9	4.6	.05	30.0	3.2
3/68	38	2.1	4.0	<.1	17.0	.04	2.3	<.1	3.6	.2
4/68	55	2.9	1.6	.005	3.9	.02	2.0	.4	27.0	1.0
5/68	130	2.9	3.3	<.01	11.0	.08	1.8	.07	2.6	.08
6/68	140	2.0	3.4	.01	3.6	.1	3.0	.22	13.0	.09
7/68	120	3.5	.4	.02	1.2	.02	.5	.08	9.0	.2
8/68	17	.4	2.7	.14	2.4	.05	.2	<.01	13.0	.11
9/68	290	21.0	2.2	.01	23.0	1.2	4.0	.16	17.0	.36
10/68	34	.8	2.4	.005	4.9	.08	3.5		18.0	.35
11/68	8.7	.5	.48	.06	11.0	.5	2.8	.10	65.0	3.1
12/68	62	5.9	.38	.005	31.0	1.6	15.0	.10	37.0	2.4

*Partial list of data from 35 monitoring stations, July 1965 to December 1968; (MFCC and MFFCC per 0.1 ml).

TABLE 12

Coliform and Fecal Coliform Counts in Beaver Dam River Below Beaver Dam, Wisconsin Sewage Treatment Plant, Summer 1968*

		Date (1968)								
		Apr. 11	Apr. 16	Apr. 25	May 9	June 6	July 19	Aug. 8	Aug. 22	Aug. 30
Sta. 1 (Avg. of 2)	Total	2.0	190.0	5.5	4.9	20.0	16.5	250.0	85.0	23.0
	Fecal	.25	30.0	.25	.54	.30	.40	4.65	< .1	.35
Sta. 2 ** (STP Eff) Mil. 0.0	Total	21.0	2.7	.69	2.7	1.1	3.4	3.2	11.0	29.0
	Fecal	.20	.06	.06	.30	.15	.05	.26	.21	.76
Sta. 3 (Avg. of 2) Mil. 0.5	Total	320	335	82	90	120	75	405	485	1,400
	Fecal	5.0	22.0	3.0	4.5	4.5	1.8	5.7	5.0	25.5
Sta. 4 Mil. 2.0	Total	180	130	55	140	50	60	270	390	540
	Fecal	4.5	5.0	2.0	8.0	< 1.0	.2	4.7	6.0	15.0
Sta. 5 Mil. 2.8	Total	190	190	56	70	10	90	340	350	480
	Fecal	2.0	2.0	2.0	2.0	1.0	1.8	6.6	5.0	19.0
Sta. 6 Mil. 3.4	Total	150	170	76	110	40	140	710	450	360
	Fecal	1.9	4.9	4.0	2.0	< 1.0	2.1	5.6	5.0	15.0
Sta. 8 Mil. 4.0	Total	90	110	59	70	60	260	360	370	340
	Fecal	1.0	1.9	2.3	2.2	.6	1.6	6.1	3.5	21.0
Sta. 9 Mil. 6.6	Total	25	34	40	43	40	70		210	220
	Fecal	1.0	2.9	2.0	1.1	.6	1.9		2.0	18.0
Sta. 10 Mil. 8.7	Total	16	19	48	26	80	140	300	200	24
	Fecal	6.0	2.1	2.7	.3	.3	2.2	2.2	< 1.0	4.0

* MFCC and MFFCC per 0.1 ml.
** Million per 100 ml.

A good correlation between total and fecal coliforms on any one sampling day did not exist. Generally, the total coliform and fecal coliform counts decreased (or increased) in a like manner downstream, but seldom was the rate uniform. Considering the mean values for the fecal and total counts over the entire sampling period, the fecal count averaged about 2.5 percent of the total count at each station. This was about the same ratio as the counts in the sewage treatment effluent. The average total coliform count for nine samples from the sewage treatment-plant effluent (trickling filter, nondisinfected) was 8,310,000/100 ml while the fecal count averaged 228,000/100 ml. Interference from draw-down activities at Beaver Dam Lake may be the cause of variations in counts on this stream during the sampling period. Although the results of these surveys were inconclusive, they are probably indicative of what further investigations would yield.

As evidenced by the preceding analyses it is apparent that a high correlation between total and fecal coliforms does not exist. Although fecal coliform counts usually represent only a small percentage of total coliform values, occasionally conditions exist when this percentage is much larger. Despite the unrelated results, it is felt that the fecal coliform is a better indicator organism because it is more directly related to sewage contamination than is the total coliform.

WATER QUALITY STANDARDS

The Report of the National Technical Advisory Committee on Water Quality Criteria expressed the opinion "that of the groups or organisms commonly employed in evaluating sanitary conditions in surface waters, fecal coliform is by far the best choice for use in criteria for contact recreation." (Fed. Water Pollution Cont. Admin., 1968). It also stated that localized bacterial standards may be justified if based on sufficient experience, sanitary surveys, or other control and monitoring programs, together with a thorough analysis of the sources of contamination and the degree of threat of pathogens from specific sources.

The best application for fecal coliform detection is in stream pollution studies, wastewater treatment systems, determination of bathing water quality, and other recreational use criteria (Geldreich, 1965). This procedure is not recommended for the examination of untreated water supplies being considered for potable water. Fecal coliforms will be more prominent in recently contaminated waters while insufficiently chlorinated or less recently polluted waters will show a higher percentage of nonfecal coliforms. Use of the fecal coliforms as an indicator does not add greatly to the complexity or expense of sampling and testing water. The report of the Committee on Water Quality Criteria recommended values for recreation activities and also suggested that more research is needed to refine the correlation between fecal coliforms and water-borne disease (Fed. Water Pollution Cont. Admin., 1968).

The Recreation Subcommittee recommends for "secondary contact" recreation (activities not involving significant risk of ingestion) on surface waters generally that an average not exceeding 2,000 fecal coliforms per 100 ml and a maximum of 4,000/100 ml be maintained except in specified mixing zones. The criteria for "secondary contact" activities in waters specifically designed for recreation uses should not exceed a log mean of fecal coliforms of 1,000/100 ml, nor equal or exceed 2,000/100 ml in more than 10 percent of the samples. The recommendation for "primary contact" recreation is that, based on a minimum of not less than five samples collected over a 30-day period, the fecal coliform count shall not exceed a log mean of 200/100 ml nor shall more than 10 percent of the total samples during any 30-day period exceed 400/100 ml (Fed. Water Pollution Cont. Admin., 1968).

The Wisconsin Water Quality Standards do not indicate definite values for fecal coliforms in the recreation criteria. However, it is stated that under special conditions the fecal coliform may be used. Future revisions of the standards may adopt the fecal coliform criteria for recreation waters (Amer. Public Health Assoc. et al., 1965).

COST ANALYSIS

The cost differential in comparing the MPN and membrane filter methods is significant. McCaffrey (1962) in his Illinois study in the early 1960's found that the cost for the membrane filter technique averaged about $0.08 less per test than the corresponding MPN tests. The total cost for materials and labor for the analysis of 33,669 samples with the membrane filter was $9,963.75 or $0.295 per test. The cost of analysis of 36,164 samples by the MPN technique was $13,676.93 or $0.378 per test (Table 13). The laboratories in which these tests were conducted were already equipped to perform the MPN tests; therefore the cost of tubes, incubators, dishwashing equipment, and bench space requirements have not been taken into consideration. If new labs were to be established it is apparent that the overall cost would be higher for the MPN tests than those for the membrane filter tests.

Individual membrane filters cost about 15 cents apiece. Petri dishes to be used for incubation purposes are 3-1/2 to 4 cents apiece and usually are not reused. The MFFCC medium cost is slightly higher than the medium for the MFCC test, but preparation costs are about equal. Although the time required in preparing the fecal coliform sample for incubation is greater than that for the total coliform sample, the time required to count the fecal colonies is less. Therefore the total time required in preparing and counting each individual sample is about equal. It is concluded then that the membrane filter analyses for total and fecal coliforms cost about the same and each is less expensive than MPN method.

OTHER INDICATOR SYSTEMS

There have been additional studies in the field of indicator organism detection. Suggestions are that a combination of the fecal coliform and fecal streptococci may prove to be the best indicator of human fecal pollution in surface waters. If the fecal coliform to fecal strep ratio is less than about 0.7, the contamination is from nonhuman sources (Tables 14 and 15), and if the ratio is greater than 4.3, human contamination is indicated (Table 16) (Geldreich, 1967). Therefore, the use of fecal coliform to fecal strep ratio may further define possible sources of fecal discharge to a stream.

Enterococci may also prove to be better indicators than the coliform organisms because new media have made identification of these organisms easier (Hanes et al., 1964). Results indicate that they are present in sewage in numbers approaching the total coliforms and that they do not multiply in water.

Some studies have been made into the use of organic chemical compounds for the detection of fecal pollution. Steroids offer such a class of compounds because certain steroids are characteristic of wastes from higher forms of life, and their presence has been shown as good evidence of fecal contamination (Murtaugh and Bunch, 1967). Though this test is more time consuming, it may prove to be a better method to detect fecal contamination.

Researchers at the Univeristy of Wisconsin Agricultural Experiment Station (1968) have isolated a new indicator species which is present only in humans (*Pseudomonas aeruginosa*). This organism, which is truly a disease-producer, dies shortly after leaving the human intestine, and was found only in other animals which were recently handled by humans. Further research is being done with this organism.

SUMMARY AND CONCLUSIONS

Surface waters free from chemical and bacterial contamination are necessary to provide a continued recreational asset. Indicator organisms are needed to detect the presence of pathogenic bacteria. The coliform bacteria have been used in the past with great success; however, their use may be too severe in citing contamination of surface waters.

Fecal coliform detection is similar to that for the total coliform and counts appear to show a much better relation to true contamination. Although there is little apparent correlation between the two groups of organisms, the advantages of the fecal strain and the opinion of many researchers indicate that the use of the fecal coliform in detection of surface water contamination is preferred.

TABLE 13

Cost Analysis of Coliform Detection Methods*

MILLIPORE FILTER PROCEDURE
33,669 samples

Materials

Membranes & Pads	35,000 @	$140.00/M	$4,900.00	
MF-Endo broth	8lb.@	8.50/lb.	68.00	
Plastic petri dishes	1,000 @	60.00/M	60.00	
Glass petri dishes	4gr @	94.00/gr.	376.00	
				$5,404.00

Labor

Washing dishes - 4 dishes/min.
Preparing media, pads, close & number dish - 4 dishes/min.
Running sample - 1 min. ea.

Total preparing & running - 1-1/2 min/sample
Reading & recording - 1 min/sample
2-1/2 min. per sample

33,669 samples - 1,403 hr @ $3.25 — 4,559.75

Total cost for 33,669 samples - $9,963.75

COST PER TEST $0.295

MULTIPLE TUBE DILUTION PROCEDURE
36,164 samples

Materials

Tubes lost or broken	4,426 @	$72.50/M	$ 320.88	
Vials lost or broken	2,069 @	70.00/M	144.83	
10 ml. pipettes lost or broken	614 @	98.00/C	601.72	
LT Broth	426 lb.@	7.00	2,982.00	
BG Broth	30 lb.@	8.00	240.00	
				$4,289.43

Labor

Dishwashing, preparing media & glassware
200 samples (100 cans)

Preparing cans, add gas vials, pads	90 min.
Weighing, dissolving, pipetting media, placing in baskets	60 min.

TABLE 13 (contd.)

Loading and unloading autoclaves - three used per batch	105 min.
Collecting & decontaminating media -- two autoclaves per batch	90 min.
Washing & returning glassware	75 min.
Time for 200 samples	420 min.
36,164 samples - 1265 hrs. @ $2.00	$2,530.00
Setting up, reading, transferring, and recording - 3-1/2 min/sample	
36,164 samples - 2110 hr. @ $3.25	6,857.50
	+ 4,289.43 from 1st page
	$13,676.93

COST PER TEST $0.378

*From McCaffrey (1962)

TABLE 14

Bacterial Densities for Separate Stormwater Discharge Systems as Related to Animals Pets and Rodent Fecal Contamination*

Source	Bacterial Densities Per 100 ml Effluent or 1 g Feces		
	Fecal Coliform	Fecal Streptococci	Ratio FC/FS
Stormwater discharge			
Business district	13,000	51,000	0.26
Suburban streets	6,400	150,000	0.04
City park	1,900	27,000	0.70
Agricultural	2,700	58,000	0.05
Animal pets			
Cat	7,900,000	27,000,000	0.30
Dog	23,000,000	980,000,000	0.02
Rodents			
Rat	330,000	7,700,000	0.04
Chipmunk	150,000	6,000,000	0.03
Rabbit	20	47,000	0.0004

*From Geldreich (1967), Table 9.

TABLE 15

Bacterial Densities for Meat Packing House and Dairy Effluents as Related to Farm Animal Fecal Contamination*

Source	Bacterial Densities Per 100 ml Effluent or 1 g Feces: Fecal Coliform	Fecal Streptococci	Ratio FC/FS
Waste effluent			
Meat packing	3,300,000	4,700,000	0.7
Cattle truck wash	3,300,000	40,000,000	0.1
Prison dairy	1,420,000	3,420,000	0.4
Livestock			
Sheep	16,000,000	38,000,000	0.4
Cow	230,000	1,300,000	0.2
Pig	3,300,000	84,000,000	0.4
Poultry			
Duck	33,000,000	54,000,000	0.6
Chicken	1,000,000	3,400,000	0.4
Turkey	290,000	2,800,000	0.1

*From Geldreich (1967), Table 8.

TABLE 16

Bacterial Densities in Various Domestic Sewage and Human Feces*

Sewage	Bacterial Densities Per 100 ml: Fecal Coliform	Fecal Streptococci	Ratio FC/FS
Residential "A"	17,200,000	4,000,000	4.3
Residential "B"	10,900,000	2,470,000	4.4
Residential "C"	340,000	64,000	5.3
Residential "D"	6,300,000	1,720,000	8.6
Human feces	13,000,000**	3,000,000**	4.4

* From Geldreich (1967), Table 7.
**Density per gram.

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