DISTRIBUTION OF COLIFORM ORGANISMS IN MILK AND THE ACCURACY OF THE PRESUMPTIVE COLIFORM TEST

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(With 1 Figure in the Text)

It was desired to compare the accuracy of a modified presumptive coliform test with that of the standard technique (Ministry of Agriculture, 1934), but a necessary preliminary was a check on the accuracy of standard methods. Various workers have prepared tables for estimating the bacterial population by the dilution method, based on the number of positive reactions at various levels, notably McCrady (1915, 1918), Halvorson & Ziegler (1933*a*, *b*, *c*) and Ziegler & Halvorson (1935). Their tables are based on the assumption of random distribution of the bacteria. Ziegler & Halvorson (1935) made experiments with bacterial suspensions using sterile tap water at *p*H 7 as their diluent. These writers stated (p. 628): "The fact that the dilution method agrees with other methods (plate count and direct microscopic count) appears to us to justify the use of Poisson's series for the development of the fundamental equations found in earlier papers of this series." A culture of *Bact. coli* was among those used in the experiments. Owing to the physico-chemical structure of milk it may be questioned how far data founded on distribution in water would be applicable to bacterial distribution in milk. Wilson (1935, p. 131) investigating the number of colonies per plate notes that results with a pure culture of *Bact. coli* did not give the gross irregularities which occurred with a mixed flora. These irregularities he ascribes to the uneven distribution of organisms in milk and their aggregation into clumps which are liable to disintegrate. Wilson also found that in milk, cultures of *coli-aerogenes* type showed a strong tendency to clump after passing the 100,000 per ml. level. Fresh milk should not contain such high counts of *coli-aerogenes* forms, and inasmuch as many of the strains found in milk are motile, it might be anticipated that their distribution even in milk would be random. The number of cells in a bacterial clump in milk varies enormously and may attain thousands. Whiting (1923) gives 11·1 and 4·1 as the average (irrespective of type) for two different milk supplies. Breed & Stocking (1920) examined the behaviour of coliform organisms in milk and state that in milk these organisms tend to live as isolated individuals, only

occasionally forming clumps of two, four or rarely more individuals. Using microscopic counts they estimated that the average clump size for coliform bacteria was 1·6 individuals, whereas for the ordinary flora the figures ranged from 2·7 to 18·0 in different samples. Wilson (1935, p. 156) found that 70% of *coli* strains, 47% of *aerogenes*, 84% of *cloacae* and 44% of intermediate strains were motile. Malcolm (1935), investigating 595 strains, finds the following proportions of motile strains: *coli* 88%, *aerogenes* 11%, *cloacae* 90% and 65% intermediate. Chalmers (1928) also finds the majority of *coli* strains motile and the reverse true of *aerogenes* types. All types give a positive reaction in the presumptive coliform test. Wilson (1935) finds that in raw milk the *coli*, intermediate and *aerogenes-cloacae* groups were present in about equal proportions. Malcolm (1933) isolated strains of coliform organisms from market milk in summer and winter periods and found that *coli* types were 40% in summer and 71% in winter, while *aerogenes* types fell from 22% in summer to 7% in winter. This evidence suggests that the majority of coliform organisms in raw milk are likely to be motile and that clumping is unlikely to take place. This would favour a random distribution, but it appears that no extensive trial with milk samples has actually been made.

METHOD OF INVESTIGATION

It was decided to make multiple tests on each sample, each test to be inoculated multifold at more than one level of dilution. This multiple work is statistically highly desirable, but to make these multiple inoculations takes considerable time and at once raises the problem of alteration in the bacterial population during the period of testing. It seemed desirable that the sample should be stored under such conditions that the bacterial population might be expected to be stable when the time of testing arrived, and that during testing the samples (or subsamples) should be so held that the bacterial content would be unlikely to alter during the period of testing.

Each of three workers made seventeen tests, each test consisting of fivefold inoculations into lactose-bile-salt-peptone broth at four different levels. Thus a total of fifty-one tests, each fivefold at four levels, namely, 1 : 10, 1 : 50, 1 : 250 and 1 : 1250, was made on each sample. The testing occupied 3¼ hr. per sample, the three workers working simultaneously.

Treatment of sample

The samples were afternoon milk, sampled from the churn at the farm and received at the laboratory when about 4 hr. old. About 800 c.c. of the 1 qt. sample were placed in a 1 l. shaker bottle and mixed for 5 min. in a soil shaker rotating at 100 r.p.m. About 200 c.c. of the mixed milk were then poured into each of three sterilized 8 oz. kali bottles, provided with rubber bungs, and a fourth bottle similarly filled to be used to check the temperature. All four bottles were stood in iced water and the milk cooled to about 42° F. The subsamples were then stored overnight in a refrigerator, 32–40° F.

The kali bottles used for the subsamples were each fitted with a lead disk, with four holes near the circumference. The disk was held against the bottom of the bottle by copper wire passing through the holes and fastening round the neck.

When testing commenced one and the same worker on all occasions shook each subsample twenty-five times and the bottles were placed in cans of iced water, so filled that the water reached the shoulder of the bottles while the special lead disks ensured the bottle remaining both upright and immersed.

For subsequent tests the bottle was only inverted three times.

No attempt was made to determine the type of the coliform organisms present, and though the samples were all from one farm (this was more with the hope of ensuring a coliform content suitable for demonstration at the dilution levels employed) they extended over a long period, and this would seem likely to prevent the coliform organisms being of one type or in fixed proportions of types. The actual dates were 3 and 16 March, 2 April, 8 and 23 October and 3 and 25 November 1936.

Media

Dilutions were made into 0·9% saline in $6 \times \frac{3}{4}$ in. test-tubes. Inoculations were made into lactose-bile-salt-peptone media (Breed & Stocking, 1920). For each sample a special batch of 6 l. of medium was prepared sufficient for all replications on the one sample.

Dilution technique

The principles of the standard technique (Ministry of Agriculture, 1934) were followed. Straight-sided pipettes, graduated to contain 1 ml., were used. The technique is given below:

(1) Invert the sample bottle three times.

1/10 *dilution.* (2) *Pipette no.* 1. (*a*) Withdraw 1 c.c. whole milk.

(*b*) To remove milk on the outside of the pipette plunge the point through a knife slit in a sterile filter paper fixed over a sterile kilner jar.

(*c*) Blow out into a 9 c.c. saline blank.

(*d*) Mix six times. To do this insert the tip of the pipette and suck up to just over the 1 ml. mark. Withdraw the pipette till the tip is clear of the liquid and blow out. Do not blow through the pipette into the mixture. Repeat five times.

(*e*) Discard the pipette.

1/50 *dilution.* (3) *Pipette no.* 2. (*a*) Mix six times in the 1/10 dilution.

(*b*) Transfer 1 ml. to an 8 ml. saline blank. Blow out only.

(*c*) Transfer a second 1 ml. quantity to the same 8 ml. blank.

(*d*) Mix six times.

(*e*) Discard the pipette.

1/250 *dilution.* (4) *Pipette no.* 3. Commence at 1/50 dilution and proceed as for 3(*a*), (*b*), (*c*) and (*d*).

1/1250 *dilution.* (5) *Pipette no.* 4. Commence at 1/250 dilution and proceed as for stages 3(*a*), (*b*), (*c*) and (*d*).

(6) Using the same pipette transfer 1 ml. of the dilution to a lactose broth tube and in this manner inoculate five tubes, withdrawing 1 ml. on each occasion without mixing.

(7) Still using pipette no. 4, mix the 1/250 dilution 3 times and proceed as in 6.

(8) Repeat no. 7 using the 1/50 dilution.

(9) Repeat no. 7 for the 1/10 dilution.

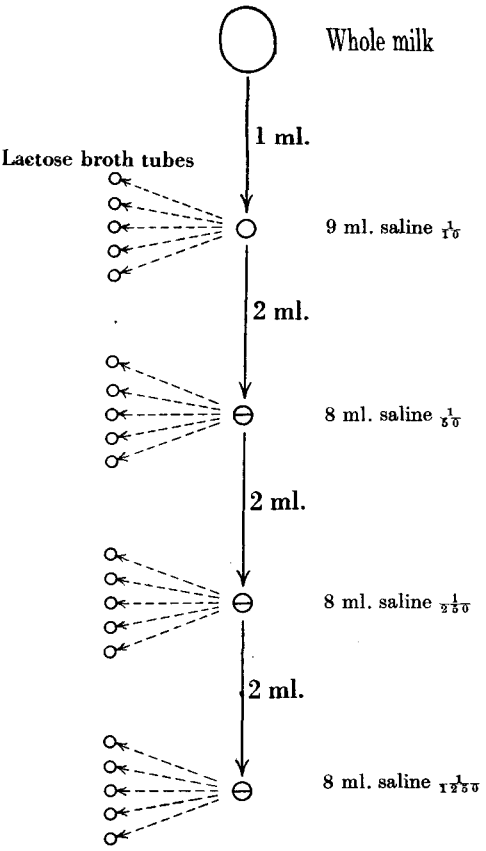
(10) Discard pipette no. 4.

Lactose broth tubes were numbered according to dilution level and in the order of inoculation.

Tubes were incubated for 72 hr. at 37° C.

A diagram of the dilution scheme is given.

Diagram of dilution scheme



Each of three workers repeats the above seventeen times on his own subsample of milk.

THE STATISTICAL ANALYSIS OF THE DATA

The object of the experiment was to test whether the assumption of a Poisson distribution[1] of organisms in parallel tubes is sufficiently accurate to enable an estimate of the number of coliform organisms per ml. in milk to be made from the proportions of sterile tubes observed at the several dilutions.

The conditions under which such a distribution of organisms can be expected to hold are as follows:

(i) The organisms are distributed independently throughout the diluent.

(ii) Each tube offers the same facilities for development.

[1] As far as the writers know, Greenwood & Yule (1917) were the first to apply the Poisson distribution to the estimation of bacterial densities in water. Previous to this McCrady (1915) had hit on the essence of the method, but used the binomial instead of the Poisson series. Fisher (1922) showed how to obtain the maximum likelihood solution from a record of sterile and fertile plates and discussed its accuracy; he also found that taking the value of the count which on the average would give the observed number of sterile plates was quite a good approximation. Halvorson & Ziegler (1933*a*) produced tables to facilitate the calculation of the maximum likelihood solution in certain cases and also discussed the accuracy of the estimate. They give a useful historical discussion in their first paper.

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(iii) The development of each organism is independent of other organisms present.

(iv) Each tube has an equal chance of receiving any organism.

If several organisms are "bound" together in a clump, such a clump can be regarded for this purpose as a single unit; if there is a tendency for clumps to break up as dilution and manipulation proceed, we should still expect a Poisson distribution of units in the parallel tubes at any one dilution, provided the above conditions hold for these units. But in these circumstances the number of bacteria per ml. as estimated from the higher dilutions will be larger than the number as estimated from the lower dilutions.

For each sample of milk, fifty-one sets of five tubes were available at each of four dilutions. If at any one dilution the chance of a tube being sterile is p independently for each tube, the distribution of the number of sterile tubes per set of five should be the binomial $51(q+p)^5$, the successive terms giving the expected number of sets containing respectively 0, 1, 2, 3, 4, 5 sterile tubes. Fitting this binomial to the data, and applying the χ^2 test for goodness of fit, therefore, tests whether the chance of a tube being sterile remains constant at any one dilution.

This does not, however, test whether the distribution of organisms in parallel tubes is Poisson. If, however, this is true at a dilution of $(1/r)$, the number of bacteria per ml. should be given by

$$e^{-m/r} = p_r,$$

where p_r is the proportion of sterile tubes at dilution $(1/r)$; or

$$(m/r) = -\log_e p_r.$$

Since we have four dilutions (1/10, 1/50, 1/250, 1/1250) available for each sample of milk, four estimates of m may be calculated and compared with one another; the extent of their agreement within the limits provided by their standard errors, tests the validity of the Poisson hypothesis.

The results for the seven samples are shown in Tables I–VII. In the first place, the binomials give, on the whole, a very satisfactory fit. Only three values of χ^2 are greater than the 5% point, against about one expected. The irregularities are in sample 4 (dilution 1/50), sample 6 (dilution 1/50) and sample 7 (dilution 1/250). In sample 4, worker *A* found 7 sets of five tubes, all sterile, whereas workers *B* and *C* only found two such sets. In sample 6 (1/50), worker *C* found no sets with four or five sterile tubes, whereas worker *A* found four and worker *B* five such sets. In sample 7 (1/250) the number of sets with three and four sterile tubes were as follows:

No. sterile	*A*	*B*	*C*
3	2	—	6
4	9	9	6

Both *A* and *B* show an excess of sets with four and a defect of sets with three sterile tubes, while *C*'s results are more in accordance with expectation. The

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Table I. *Sample* 1 (3 *March* 1936)

No. sterile	1 : 10 O*	1 : 10 E		1 : 50 O	1 : 50 E		1 : 250 O	1 : 250 E		1 : 1250 O	1 : 1250 E
0	24	21·23		0	0·33		0	0		0	0
1	16	20·34		6	2·87	13·18	0	0	2·19	0	0
2	8	7·79		8	9·98		0	0·15		0	0
3	3	1·49	9·43	16	17·39		1	2·04		0	0·12
4	—	0·14		13	15·15		16	13·43		4	3·75
5	—	0·01		8	5·28		34	35·37		47	47·12
Total	51	51·00		51	51·00		51	50·99		51	50·99

$\chi^2 = 1{\cdot}55$ 5% point = 3·84 | $\chi^2 = 1{\cdot}86$ 5% point = 5·99 | $\chi^2 = 1{\cdot}19$ 5% point = 3·84

* O = observed; E = expected calculated from $51(q+p)^5$.

	1 : 10	1 : 50	1 : 250	1 : 1250
Proportion of sterile tubes (p)	0·1608	0·6353	0·9294	0·9843
Estimated number of bacteria per tube	1·830	0·454	0·073	0·016
Bacteria per ml. (m)	18·30	22·70	18·30	19·75
Standard error	1·43	2·37	4·31	9·88

Weighted mean	19·39	S.E. 1·17
Maximum likelihood estimate	19·57	S.E. 1·19
$m_{(1/10)} - m_{(1/50)}$	−4·40	S.E. 2·77
$m_{(1/50)} - m_{(1/250)}$	+4·40	S.E. 4·92
$m_{(1/250)} - m_{(1/1250)}$	−1·45	S.E. 10·78

Table II. *Sample* 2 (16 *March* 1936)

No. sterile	1 : 10 O*	1 : 10 E		1 : 50 O	1 : 50 E		1 : 250 O	1 : 250 E		1 : 1250 O	1 : 1250 E	
0	30	31·11		2	1·97		—	—		—	—	
1	19	16·16		10	9·03	11·00	—	0·06	6·54	—	—	
2	1	3·36		11	16·56		2	0·84		—	0·03	
3	1	0·35	3·73	21	15·19		5	5·64		1	0·70	9·25
4	—	0·02		5	6·97	8·25	17	18·96		8	8·52	
5	—	—		2	1·28		27	25·51		42	41·75	
Total	51	51·00		51	51·00		51	51·01		51	51·00	

$\chi^2 = 1{\cdot}34$ 5% point = 3·84 | $\chi^2 = 4{\cdot}37$ 5% point = 5·99 | $\chi^2 = 0{\cdot}32$ 5% point = 3·84

* O = observed; E = expected calculated from $51(q+p)^5$.

	1 : 10	1 : 50	1 : 250	1 : 1250
Proportions of sterile tubes (p)	0·0941	0·4784	0·8706	0·9608
Estimated number of bacteria per tube	2·363	0·737	0·139	0·040
Bacteria per ml. (m)	23·63	36·86	34·65	50·00
Standard error	1·94	3·27	6·04	15·81

Weighted mean	27·85	S.E. 1·60
Maximum likelihood estimate	29·65	S.E. 1·75
$m_{(1/10)} - m_{(1/50)}$	*−13·23*	S.E. *3·80*†
$m_{(1/50)} - m_{(1/250)}$	+ 2·21	S.E. 6·86
$m_{(1/250)} - m_{(1/1250)}$	−15·35	S.E. 16·93

† Statistically significant differences are printed in italics.

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Table III. *Sample* 3 (2 *April* 1936)

No. sterile	1 : 10 O*	1 : 10 E		1 : 50 O	1 : 50 E		1 : 250 O	1 : 250 E		1 : 1250 O	1 : 1250 E
0	35	34·63		—	0·30	12·55	—	—	3·45	—	—
1	13	13·94		3	2·66		—	0·02		—	—
2	3	2·24	2·43	13	9·59		1	0·31		—	—
3	—	0·18		14	17·28		3	3·12		—	0·07
4	—	0·01		12	15·57		14	15·76		3	2·86
5	—	—		9	5·61		33	31·79		48	48·07
Total	51	51·00		51	51·01		51	51·00		51	51·00

3 4

$\chi^2 = 0{\cdot}21$ 5% point = 3·84 | $\chi^2 = 4{\cdot}44$ 5% point = 5·99 | $\chi^2 = 0{\cdot}33$ 5% point = 3·84

* O = observed; E = expected calculated from $51(q+p)^5$.

	1 : 10	1 : 50	1 : 250	1 : 1250
Proportion of sterile tubes (p)	0·0745	0·6431	0·9098	0·9882
Estimated number of bacteria per tube	2·597	0·441	0·095	0·012
Bacteria per ml. (m)	25·97	22·07	23·63	14·79
Standard error	2·21	2·33	4·93	8·54

Weighted mean	23·79	S.E. 1·50
Maximum likelihood estimate	24·08	S.E. 1·48
$m_{(1/10)} - m_{(1/50)}$	+3·90	S.E. 3·21
$m_{(1/50)} - m_{(1/250)}$	−1·56	S.E. 5·45
$m_{(1/250)} - m_{(1/1250)}$	−8·84	S.E. 9·86

Table IV. *Sample* 4 (8 *October* 1936)

No. sterile	1 : 10 O*	1 : 10 E		1 : 50 O	1 : 50 E		1 : 250 O	1 : 250 E		1 : 1250 O	1 : 1250 E	
0	46	46·19		11	4·96		0	0·01	12·98	0	—	13·33
1	5	4·62	4·80	10	14·72		2	0·30		0	—	
2	—	0·18		13	17·49		3	2·48		1	0·09	
3	—	—		11	10·38	13·83	8	10·19		1	1·47	
4	—	—		4	3·08		17	20·88		10	11·77	
5	—	—		2	0·37		21	17·13		39	37·66	
Total	51	50·99		51	51·00		51	50·99		51	50·99	

5 17 13 12

$\chi^2 = 10{\cdot}75$† 5% point = 5·99 1% point = 7·82 | $\chi^2 = 3{\cdot}82$ 5% point = 5·99

* O = observed; E = expected calculated from $51(q+p)^5$.

† If also 0 and 1 are grouped $\chi^2 = 1{\cdot}97$, 5% point = 3·84, but the excess of zeros observed is probably real.

	1 : 10	1 : 50	1 : 250	1 : 1250
Proportion of sterile tubes (p)	0·0196	0·3725	0·8039	0·9412
Estimated number of bacteria per tube	3·932	0·987	0·218	0·061
Bacteria per ml. (m)	39·32	49·37	54·56	75·78
Standard error	4·43	4·06	7·73	19·57

Weighted mean	46·64	S.E. 2·76
Maximum likelihood estimate	48·29	S.E. 2·99
$m_{(1/10)} - m_{(1/50)}$	−10·05	S.E. 6·01
$m_{(1/50)} - m_{(1/250)}$	− 5·19	S.E. 8·73
$m_{(1/250)} - m_{(1/1250)}$	−21·22	S.E. 21·04

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Table V. *Sample* 5 (23 *October* 1936)

No. sterile	1 : 10 O*	1 : 10 E	1 : 50 O	1 : 50 E		1 : 250 O	1 : 250 E		1 : 1250 O	1 : 1250 E	
0	50	50·01	12	9·45		0	0·07		0	0·00	
1	1	0·98	13	18·94		2 } 7	0·95	6·22	0	0·01	
2	—	—	20	15·20		5	5·20		0 } 14	0·18	16·37
3	—	—	4	6·10		15	14·30		5	2·24	
4	—	—	2 } 6	1·22	7·42	15	19·67		9	13·94	
5	—	—	0	0·10		14	10·81		37	34·63	
Total	51	50·99	51	51·01		51	51·00		51	51·00	

$\chi^2 = 4{\cdot}34$ (5% point = 5·99) $\chi^2 = 2{\cdot}14$ (5% point = 5·99)

* O = observed; E = expected calculated from $51(q+p)^5$.

	1 : 10	1 : 50	1 : 250	1 : 1250
Proportion of sterile tubes (p)	0·0039	0·2863	0·7333	0·9255
Estimated number of bacteria per tube	5·541	1·251	0·310	0·077
Bacteria per ml. (m)	55·41	62·54	77·54	96·79
Standard error	9·98	4·94	9·44	22·21

Weighted mean	65·13	S.E. 3·95
Maximum likelihood estimate	66·80	S.E. 4·15
$m_{(1/10)} - m_{(1/50)}$	− 7·13	S.E. 11·14
$m_{(1/50)} - m_{(1/250)}$	− 15·00	S.E. 10·66
$m_{(1/250)} - m_{(1/1250)}$	− 19·25	S.E. 24·13

Table VI. *Sample* 6 (3 *November* 1936)

No. terile	1 : 10 O*	1 : 10 E	1 : 50 O	1 : 50 E		1 : 250 O	1 : 250 E		1 : 1250 O	1 : 1250 E	
0	35	33·90	7 } 19	3·36 }	15·50	— }	0·07 }		—	—	
1	13	14·43	12 }	12·14 }		3 } 6	1·00 }	6·45	—	—	
2	2	2·46	15	17·56		3 }	5·38 }		—	0·03	
3	1 } 16	0·21 }	17·11	8	12·69		16	14·49		—	0·70 } 9·25
4	— }	0·01 }		4 } 9	4·59 }	5·25	16	19·53		10	8·52 }
5	— }	— }		5 }	0·66 }		13	10·53		41	41·75
Total	51	51·01	51	51·00		51	51·00		51	51·00	

$\chi^2 = 6{\cdot}09$† (5% point = 5·99) $\chi^2 = 1{\cdot}41$ (5% point = 5·99)

* O = observed; E = expected calculated from $51(q+p)^5$.

† The large χ^2 is probably due to a real excess of sets with 4 and 5 sterile.

	1 : 10	1 : 50	1 : 250	1 : 1250
Proportion of sterile tubes (p)	0·0784	0·4196	0·7294	0·9608
Estimated number of bacteria per tube	2·546	0·868	0·316	0·040
Bacteria per ml. (m)	25·46	43·42	78·88	50·01
Standard error	2·15	3·68	4·87	15·81

Weighted mean	36·37	S.E. 1·72
Maximum likelihood estimate	37·99	S.E. 2·20
$m_{(1/10)} - m_{(1/50)}$	*− 17·96*	S.E. *4·26*†
$m_{(1/50)} - m_{(1/250)}$	*− 35·46*	S.E. *6·11*
$m_{(1/250)} - m_{(1/1250)}$	+ 28·87	S.E. 16·55

† Statistically significant differences are printed in italics.

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Table VII. *Sample* 7 (25 *November* 1936)

No. sterile	1:10 O*	1:10 E		1:50 O	1:50 E		1:250 O	1:250 E		1:1250 O	1:1250 E	
0	44	44·37		8	6·72		—	0·20		—	0·00	
1	7	6·26		14	16·79		3	2·10		—	0·02	
2	—	0·36		18	16·79		11	8·40		—	0·31	
3	—	0·01	6·63	9	8·40		8	16·79		3	3·12	
4	—	0·00		2	2·10	10·70	24	16·79		17	15·76	
5	—	0·00		—	0·20		5	6·72		31	31·79	
Total	51	51·00		51	51·00		51	51·00		51	51·00	

3 + 11 = 14; 14 | 10·7; 20 | 19·21

$\chi^2 = 0{\cdot}80$ — 5% point = 5·99

$\chi^2 = 9{\cdot}16$† — 5% point = 5·99; 1% point = 9·21

* O = observed; E = expected calculated from $51(q+p)^5$.

† Due to an excess of 4 and defect of 3, sterile.

	1:10	1:50	1:250	1:1250
Proportion of sterile tubes (p)	0·0275	0·3333	0·6667	0·9098
Estimated number of bacteria per tube	3·595	1·099	0·405	0·095
Bacteria per ml. (m)	35·95	54·93	101·37	118·16
Standard error	3·73	4·43	11·07	24·65

Weighted mean	48·28	S.E. 2·74
Maximum likelihood estimate	58·64	S.E. 3·48
$m_{(1/10)} - m_{(1/50)}$	*−18·98*	S.E. *5·79*†
$m_{(1/50)} - m_{(1/250)}$	*−46·44*	S.E. *11·92*
$m_{(1/250)} - m_{(1/1250)}$	−16·79	S.E. 27·02

† Statistically significant differences are printed in italics.

three high values of χ^2 are due to these occasional anomalies, such as might easily be produced by some small difference in personal equation or technique.

An examination of the estimates of the number of bacteria per ml., obtained on the assumption of a Poisson distribution of organisms, shows very good agreement between the results at the different dilutions for the three spring samples. The sample (no. 2) taken on 16 March 1936 shows a rise in the estimate of the count at the higher dilutions, but only the rise from 1/10 to 1/50 is significant. All the four autumn samples (nos. 4, 5, 6 and 7) show a tendency for the estimate of the count to rise at the higher dilutions. The differences between consecutive dilutions are not always statistically significant, but the general tendency to rise is manifest. This may very plausibly be attributed to a tendency for clumps to break up at the higher dilutions. Notwithstanding this rise, the assumption of a Poisson distribution of organisms clearly gives a good idea of the magnitude of the count.

Finally two estimates of the number of bacteria per ml. were made for each sample, using the entire series of 1020 ($4 \times 3 \times 17 \times 5$) tubes:

(i) A weighted mean of the results at each dilution using the reciprocals of the error variances as weights.

(ii) The maximum-likelihood estimate, which is the best estimate that can be made from the data.

The two estimates agree very well, the individual differences being, in all cases except one, (sample 7), statistically insignificant. The weighted mean, for these data, is however always slightly below the maximum likelihood estimate but seems near enough to it to be used for practical purposes, being much more easy to calculate. The method of calculating the maximum likelihood estimate is illustrated in the Appendix.[1]

SUMMARY

1. The presumptive coliform test was carried out by each of three workers, at each of four dilutions, on seventeen sets of five tubes for each of seven samples of 18-hour-old afternoon milk, held overnight in the ice chest.

2. With the exception of three slightly anomalous results, the data are consistent with the hypothesis that the chance of a tube remaining sterile is constant for all tubes inoculated from the same sample of milk at any one dilution.

3. The assumption of a Poisson distribution of organisms in parallel tubes is accurate enough, with these data, to give a good idea of the order of magnitude of the number of bacteria per ml.

4. There is, however, a tendency, more marked in our data in autumn than in spring, for the estimated count to rise at the higher dilutions. This may plausibly be attributed to the breaking up of clumps.

APPENDIX

The calculation of the maximum likelihood estimate of the count

The method of calculating the maximum likelihood estimate may be illustrated by the data of sample 1. At dilution 1 : 10 there were in all 41 sterile tubes out of 255; at dilution 1 : 50, 162; at dilution 1 : 250, 237; at dilution 1 : 1250, 251. The likelihood of any value m of the number of bacteria per ml. in the undiluted milk is therefore

$$l = C(e^{-m/10})^{41}\,(1-e^{-m/10})^{214}\,(e^{-m/50})^{162}\,(1-e^{-m/50})^{93} \times (e^{-m/250})^{237}\,(1-e^{-m/250})^{18}\,(e^{-m/1250})^{251}\,(1-e^{-m/1250})^{4},$$

and the value of m, which maximizes this, is the maximum likelihood solution.

[1] The standard error of the maximum likelihood estimate should be less than or equal to that of any other estimate that can be made. The reader may therefore wonder why in this case the standard error of the weighted mean appears to be slightly less. If the weights w used are the reciprocals of the error variances, the error of the weighted mean will be $\sqrt{\{1/S(w)\}}$ provided the correct values of the variances are used. In this case the variances are themselves only estimates, consequently the weights have sampling errors which have been ignored in calculating the error of the weighted mean. The standard error of the weighted mean is therefore somewhat underestimated.

We have:

$$L = \log l = -\frac{41m}{10} - \frac{162m}{50} - \frac{237m}{250} - \frac{251m}{1250} + 214 \log (1-e^{-m/10})$$
$$+ 93 \log (1-e^{-m/50}) + 18 \log (1-e^{-m/250}) + 4 \log (1-e^{-m/1250}),$$

and this is a maximum when $\partial L/\partial m = 0$:

$$\frac{\partial L}{\partial m} = -\frac{41}{10} - \frac{162}{50} - \frac{237}{250} - \frac{251}{1250} + \frac{21{\cdot}4\, e^{-m/10}}{1-e^{-m/10}}$$
$$+ \frac{\frac{93}{50}\, e^{-m/50}}{1-e^{-m/50}} + \frac{\frac{18}{250}\, e^{-m/250}}{1-e^{-m/250}} + \frac{\frac{4}{1250}\, e^{-m/1250}}{1-e^{-m/1250}} = 0.$$

Writing $p = e^{-m/10}$ and reducing, the equation becomes

$$f(p) = -8{\cdot}4888 + \frac{21{\cdot}4\, p}{1-p} + \frac{1{\cdot}86\, p^{1/5}}{1-p^{1/5}} + \frac{0{\cdot}072\, p^{1/25}}{1-p^{1/25}} + \frac{0{\cdot}0032\, p^{1/125}}{1-p^{1/125}} = 0.$$

This may be solved by successive approximation, a rough average of the results at the four dilutions giving a first approximation. If p_0 is this approximation and $p = p_0 + \alpha$, we have

$$f(p) = f(p_0) + \alpha f'(p_0) = 0$$

or

$$\alpha = -f(p_0)/f'(p_0) \quad \text{approximately,}$$

where

$$f'(p) = \frac{21{\cdot}4}{(1-p)^2} + \frac{0{\cdot}372\, p^{-4/5}}{(1-p^{1/5})^2} + \frac{0{\cdot}00288\, p^{-24/25}}{(1-p^{1/25})^2} + \frac{0{\cdot}0000256\, p^{-124/125}}{(1-p^{1/125})^2}.$$

Thus the next approximation is given by $p = p_0 + \alpha$, and the process may be represented as many times as necessary. In the present case, we take,

$$p_0 = e^{-20/10} = e^{-2} = 0{\cdot}1353353,$$

and find

$$f(p_0) = -0{\cdot}294606,$$
$$f'(p_0) = -49{\cdot}6377,$$
$$\alpha = 0{\cdot}0059351,$$

whence

$$p_1 = 0{\cdot}1412704,$$
$$f(p_1) = 0{\cdot}001219,$$
$$f'(p_1) = 50{\cdot}0541.$$

This is clearly accurate enough, the result correct to 4 places being 0·1412 (since the next α is $-0{\cdot}0000243$).

We have also

$$\frac{\partial^2 L}{\partial m^2} = \frac{-p}{10} f'(p),$$

and the standard error of the maximum likelihood solution is given by

$$\sigma_{\hat{m}}^2 = -1 \Big/ \left(\frac{\partial^2 L}{\partial \hat{m}^2}\right) = 1/(0{\cdot}01412)\,(50{\cdot}0541) = 1{\cdot}41,$$

and is therefore equal to 1·19.

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