Comparison of the results of food hygiene standard tests obtained by use of 10:90 and 1:9 serial dilutions

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Abstract

According to the microbiological food testing methods released by the Taiwan Ministry of Health and Welfare, 10-fold serial dilutions for quantitative or semi-quantitative microbiological analysis have to be performed with bottles containing 90 ml sterile diluent by adding 10 ml of sample. The tedious and time-consuming preparation of these bottles often limits the test capacity of laboratories.

To simplify the procedure of serial dilution and to reduce the total diluent volume needed for microbiological testing, plate counts obtained from 10:90 serial dilutions of different E. coli cultures as well from beverages and iced products were compared to 1:9 serial dilutions.

The results showed no statistically significant difference between the bacterial counts obtained from 10:90 serial dilutions or from 1:9 serial dilutions of E. coli cultures as well as of E. coli and coliformes plate counts of different beverages and iced products. However, because the E. coli MPN counts were all zero for most samples analyzed, a comprehensive conclusion on the effect of the total dilution volume specifically on E. coli MPN counts wasn't possible.

Purpose

According to the test methods published by the Taiwan Ministry of Health and Welfare on microbiological food testing, 10 ml of sample have to be diluted in 90 ml of diluent for a 10-fold dilution (10:90). Therefore dilution bottles containing 90 ml sterile diluent are needed. The limited preparation capacity of these dilution bottles often limits the test capacity of many laboratories. With this study we wanted to find out if 1 ml of sample diluted in 9 ml (1:9) instead of 10:90 dilutions offer a possibility to improve the capacity of microbiological food testing laboratories without affecting reliability of the testing results.

Comparison of bacterial counts obtained by the 10:90 and 1:9 dilution method applied to E. coli cell cultures

In order to evaluate the results of bacterial counts obtained by the 10:90 and the 1:9 dilution methods we performed plate counts with 18 different E.coli cell cultures. For the statistical analysis of the results a paired t-test was applied. For the 1:9 dilutions we used the Inlabtec Serial Diluter (for more information visit www.inlabtec.com). The experimental setup is shown in Figure 1.

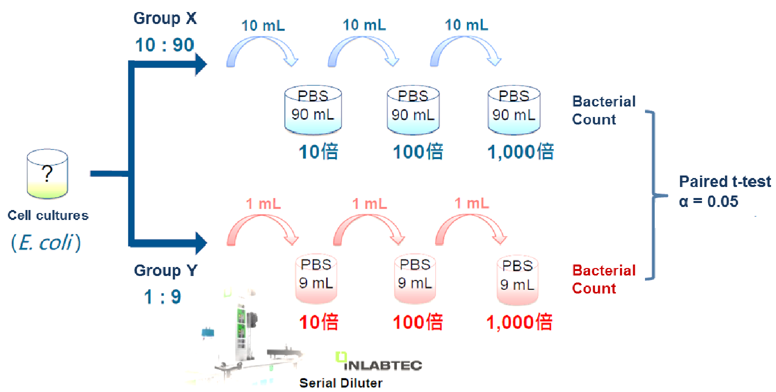

Figure 1: Experimental setup for comparison of bacterial counts obtained by 10:90 and 1:9 dilution methods using E. coli cell cultures

The results of the experiment are shown in Table 1. The probability p associated with a Student's paired t-Test, with a two-tailed distribution is for the primary counts 0.09 and for the resulting total bacterial counts 0.34 (n=18). Both p values are above the significance level $\alpha = 0.05$ and therefore it was no statistically significant difference in the E.coli counts detected between 10:90 and 1:9 dilution volumes used for sample preparation.

Table 1: Results and statistical analysis of bacterial counts obtained from 10:90 and 1:9 dilutions applied to E. coli cell cultures

		Dilution Xi (10:90)			**Dilution Yi (1:9)**				**Dilution Xi (10:90)**	**Dilution Yi (1:9)**	
Sample		Plate count Xi		Av. plate count Xi	Plate count Yi		Av. plate count Yi	Difference $\bar{d}$ = Xi - Yi	Total Bacterial Count Xi	Total Bacterial Count Yi	Difference $\bar{d}$ = Xi - Yi
No.	Dilution factor	plate 1	plate 2		plate1	plate 2					
1	10x	92	83	87.5	97	97	97.0	-9.5	880	970	-90
2	10x	98	97	97.5	120	118	119.0	-21.5	980	1'200	-220
3	10x	112	112	112.0	107	100	103.5	8.5	1'100	1'000	100
4	10x	140	129	134.5	149	137	143.0	-8.5	1'300	1'400	-100
5	10x	161	155	158.0	163	156	159.5	-1.5	1'600	1'600	0
6	10x	179	145	162.0	174	138	156.0	6.0	1'600	1'600	0
7	100x	52	56	54.0	61	69	65.0	-11.0	5'400	6'500	-1'100
8	100x	55	58	56.5	70	81	75.5	-19.0	5'600	7'600	-2'000
9	100x	54	62	58.0	44	60	52.0	6.0	5'800	5'200	600
10	100x	65	69	67.0	77	87	82.0	-15.0	6'700	8'200	-1'500
11	100x	66	72	69.0	70	84	77.0	-8.0	6'900	7'700	-800
12	100x	66	76	71.0	63	70	66.5	4.5	7'100	6'600	500
13	100x	66	75	70.5	60	65	62.5	8.0	7'000	6'200	800
14	100x	65	76	70.5	81	89	85.0	-14.5	7'000	8'500	-1'500
15	100x	73	76	74.5	53	66	59.5	15.0	7'400	6'000	1'400
16	100x	53	58	55.5	66	70	68.0	-12.5	7'600	6'800	800
17	100x	76	77	76.5	69	99	84.0	-7.5	7'600	8'400	-800
18	100x	78	81	79.5	76	82	79.0	0.5	8'000	7'900	100
							Average m$\bar{d}$	-4.44		Average m$\bar{d}$	-211.67
							Standard Deviation S$\bar{d}$	10.62		Standard Difference S$\bar{d}$	911.37
							t Value	-1.77		t Value	-0.99
							Probability* p (n=17)	0.09		p (n=17)	0.34

*: paired t-test, $\alpha = 0.05$, two tailed,

Further, all plate counts were within the internal precision range of the laboratory for the plate count method of ≤ log 0.2108 (Figure 2).

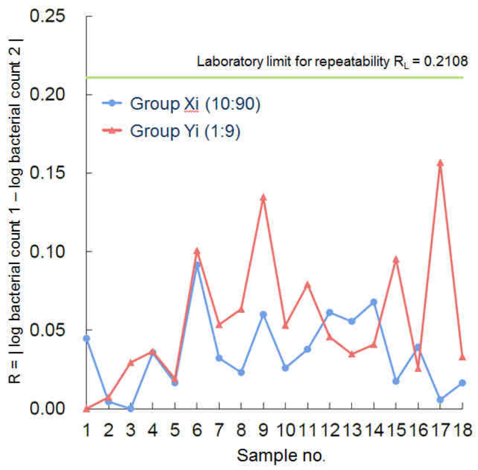

Figure 2: Repeatability R of duplicates of bacterial counts (see Table 1)

Comparison of bacterial counts obtained by the 10:90 and 1:9 dilution method applied to various beverages and iced products

For the comparison of bacterial count of E. coli and coliforms obtained by using 10:90 and 1:9 dilutions, a total of 56 beverage and iced product samples were analyzed. For 11 of these selected samples bacterial counts for the 100 to 1’000-fold sample dilutions were obtained. For the 1:9 dilutions the Inlabtec Serial Diluter was used again. The experimental setup is shown in figure 3.

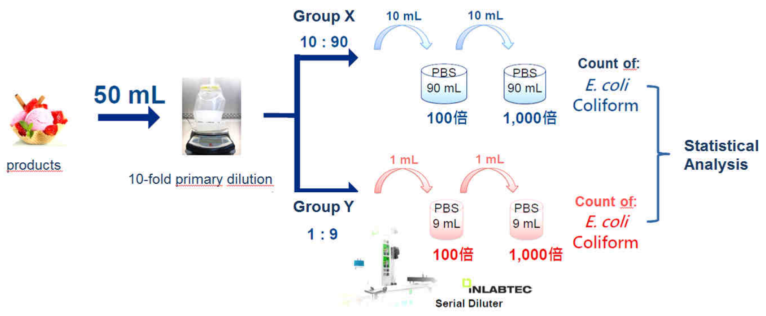

Figure 3: Experimental setup for comparison of bacterial counts obtained by 10:90 and 1:9 dilution methods using beverage and iced product samples.

The results of the experiment are shown in Table 2. The probability p associated with a Student's paired t-Test, with a two-tailed distribution is for the primary counts 0.15 and for the resulting total bacterial counts 0.41 (n=11). Both p values are above the significance level α = 0.05 and therefore it was no statistically significant difference in the E.coli counts detected between 10:90 and 1:9 dilution volumes used for sample preparation.

Table 2: Results and statistical analysis of bacterial counts obtained from 10:90 and 1:9 dilutions applied to beverage and iced product samples

			Dilution Xi (10:90)			Dilution Yi (1:9)				Dilution Xi (10:90)	Dilution Yi (1:9)	
No.	Sample	Dilution factor	Plate count Xi plate 1	plate 2	Av. plate count Xi	Plate count Yi plate1	plate 2	Av. plate count Yi	Difference $\bar{d}$ = Xi - Yi	Total Bacterial Count Xi	Total Bacterial Count Yi	Difference $\bar{d}$ = Xi - Yi
1	Mung bean soup 1	10x	28	26	27.0	28	35	31.5	-4.5	2'700	3'200	-500
2	Ice & lemon	10x	30	28	29.0	39	46	42.5	-13.5	2'900	4'200	-1'300
3	Mung bean soup 2	10x	29	40	34.5	39	45	42.0	-7.5	3'400	4'200	-800
4	Fruit juice & ice	10x	34	41	37.5	30	26	28.0	9.5	3'800	2'800	1'000
5	Black tea & ice	10x	42	33	37.5	38	48	43.0	-5.5	3'800	4'300	-500
6	Mung bean soup & ice	10x	49	70	59.5	52	61	56.5	3.0	6'000	5'600	400
7	Green tea & ice	100x	57	59	58.0	61	61	61.0	-3.0	5'800	6'100	-300
8	Iced soya bean milk	100x	101	95	98.0	120	126	123.0	-25.0	9'800	12'000	-2'200
9	Coffee sorbet	100x	162	159	160.5	140	165	152.5	8.0	16'000	15'000	1'000
10	Mango sorbet	100x	167	169	168.0	185	206	195.5	-27.5	17'000	20'000	-3'000
11	Mung bean soup 3	1000x	107	86	96.5	104	84	94.0	2.5	96'000	94'000	2'000
								Average m$\bar{d}$	-6		Average m$\bar{d}$	-382
								Standard Deviation S$\bar{d}$	12.17		Standard Difference S$\bar{d}$	1'460.01
								t Value	-1.57		t Value	-0.87
								Probability* p (n=10)	0.15		p (n=10)	0.41

*: paired t-test, α = 0.05, two tailed,

Further, all plate counts were within the internal precision range of the laboratory for the plate count method of < log 0.2108 (Figure 4).

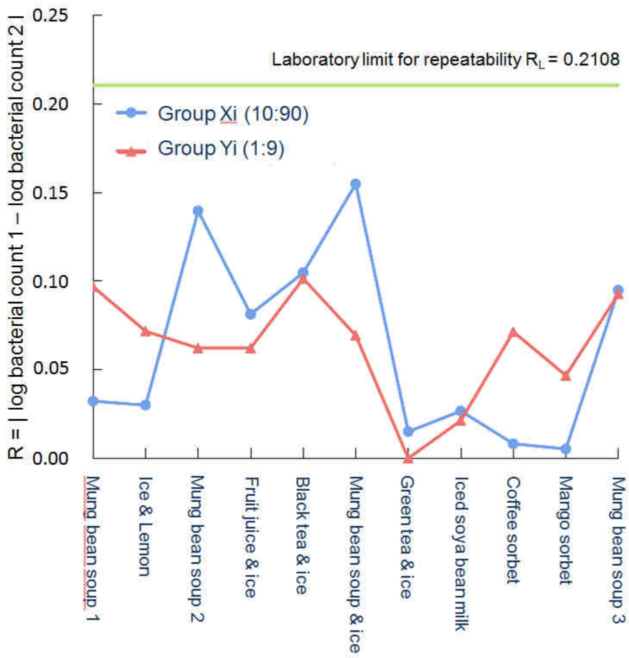

Figure 4: Repeatability R of duplicates of bacterial counts of frozen food/iced drinks (see Table 2)

The Most Probable Number (MPN) test results of beverages and iced products using 10:90 dilutions (total volume 100 ml in test bottles) and 1:9 dilutions (total volume 10 ml in Serial Dilution Bags) are shown in Table 3.

In all of the 56 test samples, the E. coli test results were negative. For samples no. 42 to no. 56 (totally 15), the MPN values of coliformes in both the 10:90 group and the 1:9 group have an overlapping 95 % confidence interval. As has been initially shown there is no statistically significant difference in the test results of coliformes between 10:90 and 1:9 dilutions.

Table 3: MPN-method: Iced products

No.	Sample	E. coli MPN/ml		Coliform MPN/ml	
		Count Xi	Count Yi	Count Xi	Count Yi
		10 : 90	1 : 9	10 : 90	1 : 9
1	Ice cubes A	Negative	Negative	Negative	Negative
2	Ice cubes B	Negative	Negative	Negative	Negative
3	Ice cubes C	Negative	Negative	Negative	Negative
4	Ice cubes D	Negative	Negative	Negative	Negative
5	Ice cubes E	Negative	Negative	Negative	Negative
6	Ice cubes F	Negative	Negative	Negative	Negative
7	Edible ice cube A	Negative	Negative	Negative	Negative
8	Edible ice cube B	Negative	Negative	Negative	Negative
9	Edible ice cube C	Negative	Negative	Negative	Negative
10	Edible ice cube D	Negative	Negative	Negative	Negative
11	Edible ice cube E	Negative	Negative	Negative	Negative
12	Edible ice cube F	Negative	Negative	Negative	Negative
13	Edible ice cube G	Negative	Negative	Negative	Negative
14	Edible ice cube H	Negative	Negative	Negative	Negative
15	Edible ice cube I	Negative	Negative	Negative	Negative
16	Edible ice cube J	Negative	Negative	Negative	Negative
17	Edible ice cube K	Negative	Negative	Negative	Negative
18	Edible ice cube L	Negative	Negative	Negative	Negative
19	Edible ice cube M	Negative	Negative	Negative	Negative
20	Edible ice cube N	Negative	Negative	Negative	Negative
21	Edible ice cube O	Negative	Negative	Negative	Negative
22	Passion fruit & ice A	Negative	Negative	Negative	Negative
23	Black tea & ice	Negative	Negative	Negative	Negative
24	Mung bean soup 3	Negative	Negative	Negative	Negative
25	Milk & ice	Negative	Negative	Negative	Negative
26	Mango & ice A	Negative	Negative	Negative	Negative
27	Mango & ice B	Negative	Negative	Negative	Negative
28	Aulait ice smoothie	Negative	Negative	Negative	Negative
29	Passion fruit & ice B	Negative	Negative	Negative	Negative
30	Lemon & ice	Negative	Negative	Negative	Negative
31	Ice cream cone	Negative	Negative	Negative	Negative
32	Xiaomei ice cream	Negative	Negative	Negative	Negative
33	Bagel ice soda	Negative	Negative	Negative	Negative
34	Jadeite lemon ice	Negative	Negative	Negative	Negative
35	Sorbet	Negative	Negative	Negative	Negative
36	Lemon ice snow	Negative	Negative	Negative	Negative
37	Ice cream original flavor	Negative	Negative	Negative	Negative
38	Denius unsalted cream	Negative	Negative	Negative	Negative
39	Honey black tea	Negative	Negative	Negative	Negative
40	Organic black soybean milk	Negative	Negative	Negative	Negative
41	Red bean milk & ice	Negative	Negative	Negative	Negative
42	Ice cube G	Negative	Negative	3.6	3.6
43	Taro three bean & ice	Negative	Negative	3.6 0.17~18*	7.4 1.3~20
44	Taro ice snow	Negative	Negative	9.2	9.2
45	Milk ice snow	Negative	Negative	23	23
46	Mung bean soup 2	Negative	Negative	23	23
47	Mung bean soup 1	Negative	Negative	23 4.6~94	43 9~180
48	Ormosia ice snow	Negative	Negative	43	43
49	Green tea & ice	Negative	Negative	43	43
50	Black tea ice snow	Negative	Negative	75 17~200	9.4 3.6~38
51	Ice cube H	Negative	Negative	93	93
52	Cheese ice snow	Negative	Negative	240 42~1'000	460 90~2'000
53	Mung bean soup & ice	Negative	Negative	1'100 180~4'100	93 18~420
54	Glacial soya bean milk	Negative	Negative	>1'100 420~--	240 42~1'000
55	Coffee sorbet	Negative	Negative	>1'100	>1'100
56	Xiancao milk ice	Negative	Negative	>1'100	1'100

*: 95 % convidence intervall for MPN

Results and discussions

The study showed that there was no statistically significant difference between bacterial counts obtained using 10:90 dilution or 1:9 dilutions.

For the MPN-method all iced product samples selected for E. coli testing were negative which made a conclusive comparison impossible. Concerning coliform counts samples no. 50, 53 and 54 (see table 3) there was a massive difference detected between 10:90 and 1:9 dilutions. These results are possibly due to operational failures which have to be confirmed by further MPN-testing using both dilution methods.

However, with the aid of the Inlabtec Serial Diluter microbiological food testing according to international food hygiene standards, the inspection capacity of a laboratory can be effectively improved and testing speeded up. Therefore it is worthwhile to continue the evaluation of the 1:9 dilutions for plate counts and the MPN method.