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Total Coliform Method

For the Detection of Coliform including *E. coli* in Pasteurized Dairy Products

Introduction

US dairy products are routinely checked for coliform following the Pasteurized Milk Ordinance. Peel Plate EC (PP-EC) is a simplified total coliform method that uses dual indicators for β-galactosidase and β-glucuronidase to detect total coliform at 32 ºC in 24 hours without the need for confirmatory analysis or gas production interpretation.

Purpose

The purpose of this evaluation was to inter-laboratory evaluate the new method in comparison to the standard method, Violet Red Bile agar (VRB) with BGLB confirmation, and the alternative reference methods, AOAC 989.1 and 991.14, in a variety of dairy products.

Methods

Methods PP-EC (Peel Plate EC Charm Sciences, Inc) and AOAC 991.14 (Petrifilm *E. coli*/Coliform 3M) were evaluated at 32 ºC for inclusivity using 58 coliform strains. In exclusivity study 31 non-coliform were studied. Whole milk 3.3 % fat, chocolate milk 2% fat, heavy cream 35 % fat, and skim milk 1 % were spiked with a variety of heat stressed coliform and *E. coli* strains at 5 different levels split into 5 replicates and mailed. Samples were tested in duplicate by 5 laboratories using VBRA, PP-EC and AOAC 989.1 (Petrifilm Coliform Count 3M). Results were evaluated for repeatability (S_r) and by paired-t-test for statistical difference, 95 % confidence intervals >0.5 log.

Significance

Peel Plate EC demonstrates high selectivity with easy total coliform interpretation in dairy matrices at 32 ºC in 24 hours.

TABLE 2: Exclusivity Organisms

Genus/Species Origin ID Number	32°C PP- EC (CFU/Plate)	Colony Color(s)	35°C PP- EC (CFU/Plate)	Colony Color(s)	32°C AOAC 991.14 (CFU/Plate)	Colony Color(s)	Gas Production (+ or -)	35°C AOAC 991.14 (CFU/Plate)	Colony Color(s)	Gas Production (+ or -)
Acinetobacter baumannii ATCC[1] 19606	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Acinetobacter calcoaceticus ATCC[1] 23055	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Aeromonas caviae ATCC[1] 15468	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Aeromonas hydrophila ATCC[1] 49140	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Alcaligenes faecalis ATCC[1] 8750	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Bacillus amyloliquefaciens ATCC[1] 23842	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Bacillus cereus ATCC[1] 11778	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Bacillus pumilis ATCC 700814	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Bacillus subtilis ATCC[1] 6051	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Corynebacterium jeikeium ATCC[1] 43734	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Edwardsiella tarda ATCC[1] 15947	0	N/A	0	N/A	124	Red	-	128	Red	-
Lactobacillus casei ATCC[1] 11578	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Lactobacillus lactis ATCC[1] 4797	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Lactobacillus lactis ATCC[1] 11494	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Micrococcus luteus ATCC[1] 10240	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Morganella morganii ATCC[1] 25829	0	N/A	0	N/A	149	Purple	-	148	Purple	-
Proteus hauseri ATCC[1] 13315	0	N/A	0	N/A	125	Purple	-	127	Purple	-
Proteus mirabilis ATCC[1] 7002	0	N/A	0	N/A	141	Purple	-	148	Red	-
Proteus vulgaris ATCC[1] 6380	0	N/A	0	N/A	51	Red	-	68	Purple	-
Providencia rettgeri ATCC[1] 14505	0	N/A	0	N/A	37	Blue/Purple	-	31	Purple	-
Providencia stuartii QL11007-5[3]	0	N/A	0	N/A	2	Blue	-	1	Purple/Blue	-
Pseudo. aeruginosa ATCC[1] 27853	0	N/A	0	N/A	16	Pink/red	-	41	Pink/red	-
Pseudo. alcaligenes ATCC[1] 14909	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Pseudo. fluorescens ATCC[1] 13525	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Salmonella enterica subspecies *enterica* serovar Enteritidis ATCC[1] 13076	0	N/A	0	N/A	135	Red	-	146	Red	-
Serratia marcescens ATCC[1] 14756	0	N/A	0	N/A	145	Red	-	149	Red	-
Shigella flexneri ATCC[1] 9199	0	N/A	0	N/A	102	Purple	+	118	Purple	+
Shigella sonnei ATCC[1] 9290	53	Red	68	Red	41	Purple	+	43	Purple	+
Staphylococcus aureus ATCC[1] 6538	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Streptococcus pyogenes ATCC[1] 9696	0	N/A	0	N/A	0	N/A	-	0	N/A	-
Yersinia enterocolitica ATCC[1] 49397	0	N/A	0	N/A	66	Purple	+	71	Purple	+

TABLE 3: Peel Plate-EC Method Total Coliform vs Violet Red Bile Agar with BGLB broth confirmation (VBRA) and 3-M-Petrifilm Coliform Count (AOAC 989.10) Methods with Milk Matrices

Matrix	Fortified micro-organisms ATCC No. (%injury)	Cont. level	Candidate Method			SPC Reference Method							AOAC 989.10 Method						
			Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r	Mean diff.[d]	CI[e] 95% LCL[f]	CI[e] 95% UCL[g]	r^{2h}	Mean	s_r	RSD_r	Mean diff.[d]	CI[e] 95% LCL[f]	CI[e] 95% UCL[g]	r^{2h}
Whole Milk	*Ent. Amnigenus 51816 (66%) E. coli 8739 (71%)*	None	<0.1	nai	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	0.9	0.12	13.8	0.92	0.12	13.2	-0.02	-0.15	0.1		0.35	0.29	81.2	0.55	-0.15	0.1	
		2	1.41	0.07	4.7	1.44	0.11	7.9	-0.03	-0.12	0.07		1	0.13	12.8	0.41	0.32	0.51	
		3	1.55	0.06	4.1	1.64	0.09	5.2	-0.09	-0.18	-0.01	0.99	1.13	0.11	9.4	0.42	0.35	0.47	0.98
		4	1.63	0.07	4.4	1.75	0.08	4.6	-0.12	-0.22	-0.03		1.23	0.11	8.7	0.4	0.3	0.5	
		5	2.38	0.09	3.6	2.53	0.07	2.8	-0.15	-0.22	-0.06		2.57	0.06	2.3	-0.19	-0.25	-0.13	
Whole Milk[i]	*Ent. Amnigenus 51816 (66%) E. coli 8739 (71%)*	None	<0.1	na	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	0.75	0.17	22.9	0.82	0.33	39.7	-0.07	-0.39	0.26		0.72	0.11	15.2	0.03	-0.1	0.17	
		2	1.44	0.1	7	1.56	0.09	5.6	-0.12	-0.21	-0.05		1.34	0.12	9.2	0.1	-0.03	0.23	
		3	1.53	0.07	4.3	1.7	0.06	3.2	-0.17	-0.21	-0.13	0.99	1.47	0.08	5.7	0.06	-0.02	0.14	1
		4	1.71	0.06	3.6	1.85	0.03	1.6	-0.14	-0.18	-0.08		1.57	0.04	2.6	0.14	0.08	0.2	
		5	2.37	0.06	2.7	2.6	0.07	2.6	-0.23	-0.31	-0.15		2.29	0.06	2.7	0.08	0.02	0.14	
Choc. Milk	*Citrobacter freundii 8090 (53%) E. coli 11229 (55%)*	None	<0.1	na	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	0.78	0.14	18	0.9	0.11	12	-0.12	-0.21	-0.14		0.36	0.54	148	0.42	0.26	0.65	
		2	1.14	0.14	12	1.45	0.07	4.6	-0.31	-0.44	-0.19		1.1	0.08	7.4	0.04	-0.1	0.1	
		3	1.46	0.14	9.4	1.76	0.08	4.3	-0.3	-0.39	-0.21	0.97	0.99	0.11	11	0.47	-0.69	-0.31	0.86
		4	1.6	0.1	6.4	1.88	0.06	3	-0.28	-0.36	-0.2		1.93	0.2	10.5	-0.33	-0.01	0.42	
		5	1.95	0.04	2.1	2.12	0.04	1.8	-0.17	-0.21	-0.14		2.17	0.14	6.4	-0.22	0.44	0.04	
Choc. Milk[i]	*Citrobacter freundii 8090 (53%) E. coli 11229 (55%)*	None	<0.1	na	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	0.83	0.12	14.5	1.04	0.11	10.5	-0.21	-0.34	0.09		-0.56	0.58	104	1.27	0.95	1.72	
		2	1	0.38	38.3	1.47	0.08	5.3	-0.47	-0.77	-0.16		0.61	0.16	25.7	0.39	0.27	0.68	
		3	1.59	0.12	7.5	1.85	0.04	2.2	-0.26	-0.33	-0.19	0.93	1.03	0.27	26.2	0.56	0.22	0.64	0.74
		4	1.48	0.16	10.8	1.92	0.03	1.8	-0.44	-0.55	-0.34		1.14	0.2	17.8	0.34	0.16	0.66	
		5	1.72	0.08	4.4	2.13	0.02	0.9	-0.41	-0.47	-0.35		1.03	0.24	23	0.69	0.55	1.08	
Skim Milk	*Ent. cloacae 13047 (61%) E. coli 51813 (50%)*	None	<0.1	na	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	0.46	0.19	41.5	0.35	0.25	70.7	0.11	-0.05	0.27		0.36	0.54	148	0.12	0	0.25	
		2	1.09	0.09	8.1	1.06	0.09	8.3	0.03	-0.06	0.11	0.69	1	0.12	12.5	-0.09	-0.42	0.32	0.84
		3	0.49	0.25	51.1	1.55	0.05	3.1	-1.06	-1.24	-0.88		0.99	0.11	11	0.5	-0.69	-0.31	
		4	1.72	0.18	10.3	2.07	0.2	9.7	-0.35	-0.53	-0.17		1.53	0.08	5.5	-0.21	-0.42	0.01	
		5	2.01	0.14	6.8	2.44	0.08	3.2	-0.43	-0.55	-0.31		2.17	0.14	9.7	0.16	0.02	0.23	
Skim Milk[i]	*Ent. cloacae 13047 (61%) E. coli 51813 (50%)*	None	<0.1	na	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	0.46	0.56	122	0.47	0.54	113	-0.01	-0.51	0.48		0.38	0.57	153	0.08	-0.2	0.36	
		2	1.05	0.14	13.2	1.22	0.1	8.5	-0.17	-0.34	-0.01		1.1	0.12	11	-0.06	-0.16	0.05	0.84
		3	0.45	0.27	59.1	1.41	0.06	3.9	-0.96	-1.15	-0.77	0.77	1.14	0.13	11.3	-0.69	-0.91	-0.46	
		4	1.65	0.23	14.1	2.06	0.92	44.8	-0.41	-0.56	-0.26		2.01	0.13	6.5	-0.36	-0.53	-0.19	
		5	1.87	0.11	6	2.38	0.06	2.6	-0.51	-0.59	-0.44		2.19	0.15	6.8	-0.32	-0.46	-0.19	
Heavy (35%) Cream	*Ent. aerogenes 13048 (54%) E. coli 25922(56%)*	None	<0.1	na	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	1.36	0.56	40.9	1.27	0.51	39.9	0.09	-0.18	0.35	1	1.36	0.56	40.9	-0.36	-0.79	0.07	1
		2	1.92	0.16	8.4	1.81	0.15	8.5	0.11	0.01	0.2		1.92	0.16	8.4	-0.12	-0.24	0	
		3	2.14	0.13	6.2	2.05	0.1	5.1	0.09	-0.04	0.23		2.14	0.13	6.2	-0.24	-0.46	-0.02	
Heavy (35%) Cream[i]	*Ent. aerogenes 13048 (54%) E. coli 25922(56%)*	None	<0.1	na	na	<0.1	na	na	na	na	na		<0.1	na	na	na	na	na	
		1	1.27	0.17	13.6	1.22	0.48	39.2	0.05	-0.35	0.46	0.97	1.27	0.17	13.6	-0.04	-0.4	0.32	1
		2	1.8	0.1	5.8	1.61	0.18	11.3	0.19	0.03	0.35		1.8	0.1	5.8	-0.15	-0.26	-0.04	
		3	2.11	0.11	5.1	2.06	0.09	4.1	0.05	-0.05	0.16		2.11	0.11	5.1	-0.03	-0.2	0.13	

[a]Mean of 5 replicate portions, plated in duplicate, after logarithmic transformation: $Log_{10}[CFU/g + (0.1)f]$.
[b]Repeatability standard deviation.
[c]Relative standard deviation for repeatability.
[d]Mean difference between the candidate and reference methods.
[e]Confidence interval.
[f]95% Lower confidence limit for difference of means. [g]95% Upper confidence limit for difference of means
[h]Square of correlation coefficient.
[i]na- not applicable [j]Independent lab performed

Acknowledgments

Thank you to other laboratory participants: Luke Thevenet and Mike Campbell,Dairygold , Tukwila, WA; Jim Clifford and Lynn Young, Milk Regulatory Consultants, Russellville, MO; Steven Brooks, Melissa Lasch and Mary Bulthaus, Eurofin-DQCI, Mounds View, MO

FIGURE 1: Spiked Chocolate Milk on VRB Coliform Method and Comparative Total Coliform Methods

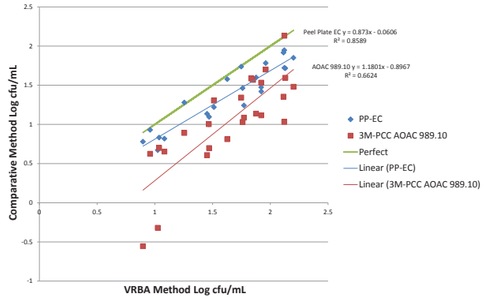


FIGURE 2: Spiked Whole Milk Tested in 5 laboratories in VRB Coliform Method and Compared to Two Other Total Coliform Methods

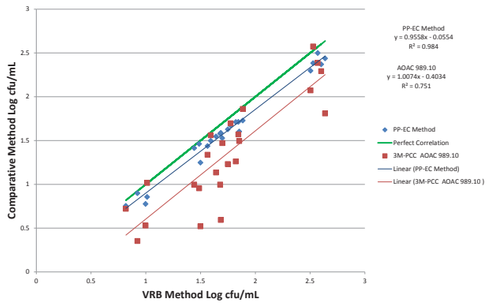


FIGURE 3: Spiked Skim Milk Tested on VRB Coliform Method and Comparative Total Coliform Methods

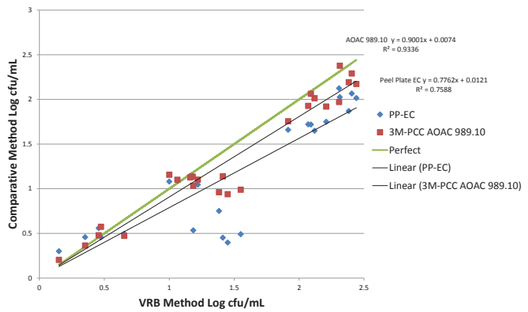
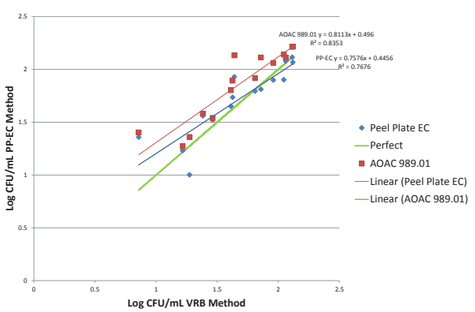


FIGURE 4: Heavy Cream Determination Using VRB and PeelPlate-EC Methods Combined Results of 5 Laboratories



Results

The Peel Plate EC detected 57 of 58 coliform strains, compared to 43 by alternative reference, in inclusivity study shown in ***Table 1***. In exclusivity shown in ***Table 2***, the new method excluded 30 of 31 compared to 28 by alternative reference. ***Table 3*** shows paired statistical analysis of liquid dairy products from two of the evaluation laboratories, Q Labs and Charm Sciences Inc. ***Figures 1-4*** compare log of n=10 means of PP-EC and AOAC 989.1 versus VRB method from all 5 laboratories. The PP-EC was not-significantly different from the VRB reference method except with neat skim at the 3 highest spike levels. Compared to AOAC 989.1 the PP-EC and VRB were biased higher in whole milk and chocolate milk, not significantly different with cream. With skim PP-EC was biased lower in the middle level, highest neat skim milk concentration. Additional skim milk evaluations did not replicate the low bias to the reference methods in neat skim milk suggesting that the bias was a sample anomaly. Replicate pairs from Peel Plate EC had the similar S_r than the reference methods. All *E.coli* tested were detected at 32 ºC but color differentiation was strain and dairy matrix dependent; thus *E. coli* in dairy matrices is only reliably scored as coliform.

TABLE 1: Inclusivity Organisms

Genus/Species Origin ID Number	32°C PP- EC (CFU/Plate)	Colony Color(s)	35°C PP-EC (CFU/Plate)	Colony Color(s)	32°C AOAC 991.14 (CFU/Plate)	Colony Color(s)	Gas Production (+ or -)	35°C AOAC 991.14 (CFU/Plate)	Colony Color(s)	Gas Production (+ or -)
Citro. amalonaticus ATCC[1] 25405	8	Red	38	Red	100	Red	-	93	Red	-
Citrobacter braakii ATCC[1] 43162	138	Red	142	Red	131	Red	-	140	Purple	-
Citrobacter farmer ATCC[1]51633	21	Red	64	Red	126	Purple	-	121	Purple	-
Citrobacter freundiiATCC[1]8090	67	Red	99	Red	129	Purple	+	138	Purple	+
Citrobacter freundii NCTC[2] 9750	63	Red	107	Red	134	Purple	+	141	Purple	+
Citrobacter freundii ATCC[1] 43864	111	Red	133	Red	74	Red	+	129	Red	+
Citro. freundii Q Labs[3] QL11007-10	128	Red	146	Red	105	Red	+	108	Red	+
Citrobacter koseri ATCC[1] 27156	113	Red	119	Red	126	Red	-	134	Red	-
Citrobacter koseri ATCC[1] BAA-895	56	Red	76	Red	88	Red	-	96	Red	-
Citrobacter youngae ATCC[1] 11102	106	Red	137	Red	124	Red	-	139	Red	-
Cronobacter muytjensii ATCC[1] 51329	121	Red	131	Red	112	Red	+	113	Red	+
Crono. sakazakii Q Labs[3] QL11007-9	81	Red	95	Red	136	Purple	+	140	Red	+
Enter. aerogenes ATCC[1] 13048	117	Red	123	Red	130	Purple	+	128	Purple	+
Enter. aerogenes ATCC[1] 35029	126	Red	132	Red	141	Purple	+	147	Purple	+
Enter. aerogenes ATCC[1] 51697	102	Red	98	Red	129	Purple	+	142	Purple	+
Enter. amnigenus ATCC[1] 51816	122	Red	149	Red	121	Purple	+	127	Purple	+
Enter. Cancerogenus QL11010-1[3]	52	Red	56	Red	115	Red	+	117	Red	+
Enterobacter cloacae ATCC[1] 13047	131	Red	139	Red	122	Red	+	132	Red	+
Enterobacter cloacae NBRC[4] 13535	52	Red	51	Red	114	Red	+	119	Red	+
Enterobacter cloacae NBRC[4] 13536	110	Red	118	Red	60	Red	-	42	Red	-
Enterobacter cloacae ATCC[1] 23355	70	Red	84	Red	119	Red	-	146	Red	-
Enterobacter gergoviae ATCC[1] 33028	88	Red	102	Red	108	Purple	-	110	Purple	-
Escherichia blattae ATCC[1] 29907	0	N/A	0	N/A	118	Red	-	109	Red	-
Escherichia coli ATCC[1] 26	15	Purple	15	Purple	3	Blue	+	11	Blue	+
Escherichia coli ATCC[1] 4157	15	Purple	17	Blue	1	Blue	-	11	Blue	-
Escherichia coli ATCC[1] 8677	61	Purple	63	Purple	112	Blue	+	120	Blue	+
Escherichia coli ATCC[1] 8739	80	Red	45	Red	97	Purple	+	111	Purple	+
Escherichia coli ATCC[1] 9637	83	Purple	58	Purple	115	Blue	+	140	Blue	+
Escherichia coli ATCC[1] 10536	46	Red	61	Red	136	Purple	+	150	Purple	+
Escherichia coli ATCC[1] 11229	64	Blue	76	Purple	139	Blue	+	142	Blue	+
Escherichia coli ATCC[1] 13706	50	Blue	53	Blue	104	Blue	+	107	Blue	+
Escherichia coli NBRC[2] 15034	128	Blue	134	Blue	110	Blue	+	110	Blue	+
Escherichia coli ATCC[1] 25922	126	Blue	127	Blue	72	Blue	+	81	Blue	+
Escherichia coli ATCC[1] 35218	131	Purple	148	Purple	69	Blue	+	74	Blue	+
Escherichia coli ATCC[1] 35421	86	Blue	97	Blue	81	Blue	+	62	Blue	+
Escherichia coli O145 NCTC[4] 10279	109	Red	113	Red	139	Blue/Purple	+	143	Blue/Purple	+
Escherichia coli O157:H7 ATCC[1] 43895	117	Red	125	Red	124	Red	+	133	Purple	+
Escherichia coli ATCC[1] 51813	78	Red	83	Red	127	Red	+	125	Purple	+
Escherichia coli Q Labs[3] QL11007-8	93	Purple	90	Purple	72	Purple	+	78	Purple	+
Escherichia coli Q Labs[3] QL11010-2	50	Red	46	Red	74	Red	+	72	Purple	+
Escherichia fergusonii ATCC[1] 35469	13	Red	52	Red	84	Red	+	131	Red	+
Escherichia fergusonii ATCC[1] 35470	16	Red	33	Red	87	Purple	+	116	Red	+
Escherichia hermannii ATCC[1] 33650	32	Red	43	Red	106	Red	+	109	Red	+
Escherichia hermannii ATCC[1] 33651	88	Red	106	Red	128	Purple	+	121	Red	+
Escherichia vulneris ATCC[1] 29943	37	Red	52	Red	82	Red	-	70	Red	-
Hafnia alvei ATCC[1] 51815	139	Red	141	Red	71	Purple	+	73	Purple	+
Klebsiella oxytoca ATCC[1] 43165	107	Red	112	Red	118	Red	+	130	Red	+
Klebsiella pneumonia ATCC[1] 49334	68	Red	62	Red	120	Purple	+	126	Purple	+
Klebsiella pneumonia ATCC[1] 700324	76	Red	78	Red	136	Purple	+	145	Purple	+
Klebsiella pneumonia ATCC[1] 10031	4	Red	11	Red	19	Blue/Purple	-	23	Purple	-
Klebsiella pneumoniae subsp. ***ozaenae*** Type 4 ATCC[1] 11296	17	Red	35	Red	52	Purple	-	56	Purple	-
Klebsiella pneumonia ATCC[1] 13882	54	Red	65	Red	34	Red	+	25	Red	+
Klebsiella pneumonia ATCC[1] 13883	113	Red	120	Red	86	Red	+	99	Red	+
Klebsiella pneumoniae subsp. ***Pneumonia*** ATCC[1] 4352	52	Red	67	Red	22	Purple	+	23	Purple	+
Klebsiella pneumoniae subsp. ***Pneumonia*** Q Labs[3] QL11007-7	120	Red	128	Red	73	Red	+	69	Purple	+
Kluyvera intermedia ATCC[1] 33110	29	Red	2	Red	61	Red	+	49	Red	+
Pantoea agglomerans ATCC[1] 19552	7	Red	36	Red	53	Purple	-	61	Purple	-
Rahnella aquatilis ATCC[1] 55046	82	Red	90	Red	117	Purple	+	123	Purple	+