Chapter 6

Accelerated Detection of Microbes Utilizing an Organic Particle Catalyst in the Total Coliforms and *Escherichia coli* MMO-MUG (Colilert®) test

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Additional information is available at the end of the chapter

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1. Introduction

In 1992, the United States Environmental Protection Agency (EPA) approved the first dual total coliform and *Escherichia coli* (*E. coli*) direct detection test for public drinking water [1]. The MMO-MUG test, commercially known as Colilert® (Idexx Laboratories Inc., Westbrook, ME), could detect 1 total coliform or 1*E. coli* in a 100 mL sample in 24 hours of incubation [2]. Over the next decades, the original MMO-MUG and variants have achieved worldwide use with consistently robust accuracy. Recently one form of the MMO-MUG, Colilert-18®, achieved European Union recognition as an ISO standard for total coliforms and *E. coli* [3].

One barrier to the utilization of new methods has been the need for an 18 to 24 hour incubation time. One form of the MMO-MUG, Colilert-18®, has a shorter incubation time than the original but requires an inconvenient and time consuming pre-heating step. Employing a new strategy that fosters the development of biofilm by incorporation of inert natural particles in the sample was reported to significantly reduce the incubation time of the MMO-MUG drinking water tests and other microbiological analyses.

The natural particles (see Figure 1) are made as a dried hydrophilic colloid extract obtained from *Gelidium cartilagineum*, *Gracilaria confervoides*, *Pterocladia lucida* and related algae of the class *Rhodophyceae*. These particles (Colloidands®, Pilots Point LLC, Sarasota, FL) have no innate significant nutritive value with the following composition: total fat 0%, saturated fat 0%, polyunsaturated fat 0%, monounsaturated fat 0%, cholesterol 0% sodium <1 mg/L, potassium 23 mg/L; total carbohydrate 0.7 g/L, fiber 0.1 g/L, sugar 0 g/L, protein 0 g/L, iron 10 g/L.

The particles are a heterogeneous natural mixture:

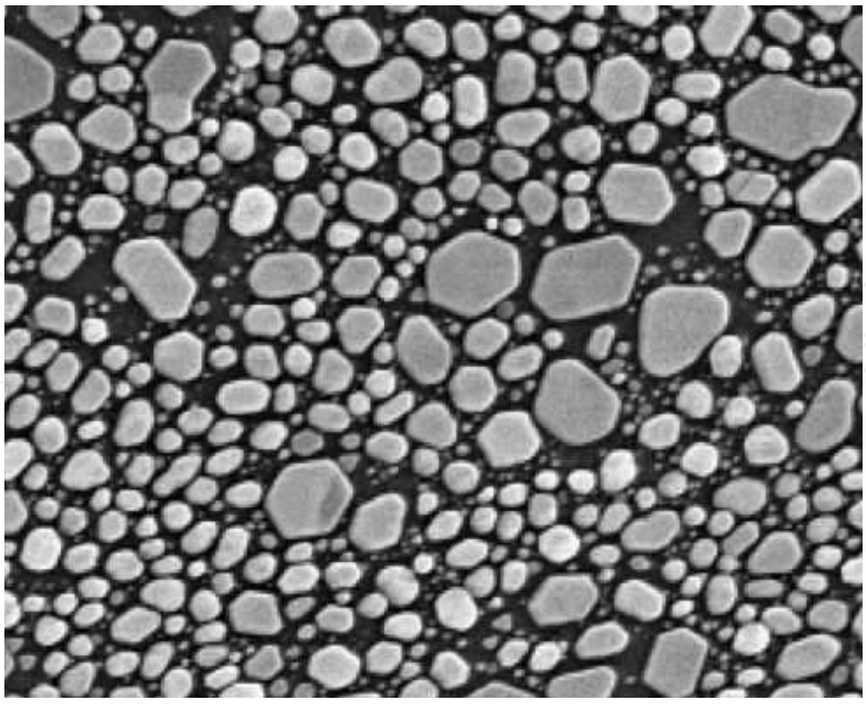

Figure 1. High resolution photomicrograph of the phycocolloid particles (Colloidands®, Pilots Point LLC, Sarasota, FL)

A study was conducted with the original MMO-MUG (Colilert®) to determine if from cold water < 8°C the incorporation of the particles could significantly reduce detection time of total coliforms and *E. coli*

2. Materials and methods

2.1. Activity of the particles

In order to establish that the particles were acting as a physical catalyst, the Colisure® variation of the MMO-MUG test was used. This test uses a yellow/gold beta-galactosidase substrate that becomes red/magenta when positive. Accordingly, the exact physical location of the beginning of the development of color could be directly determined photographically.

Colloidands® particles (Pilots Point LLC, Sarasota, FL) were added to the Colisure® test at 10 grams per liter. Both the standard Idexx *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) quality control bacteria were utilized. Photographic records were taken each hour of incubation at 35°C for 20 hours at a concentration of 10 bacteria per mL.

2.2. Level of sensitivity

2.2.1. Quality control Klebsiella and Escherichia coli (Idexx Laboratories Inc., Westbrook, Maine)

Figure 2 and Figure 3 describe the complete protocol by which the MMO-MUG (Colilert®) was examined for its ability to detect 1 total coliform and 1 *E. coli* in 16 hours. For the purpose of

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studying universally available bacteria, the standard quality control strains available from the manufacturer of Colilert® (Idexx Laboratories Inc., Westbrook, ME) were utilized. Virtually all water testing laboratories use these for quality control or have access to them.

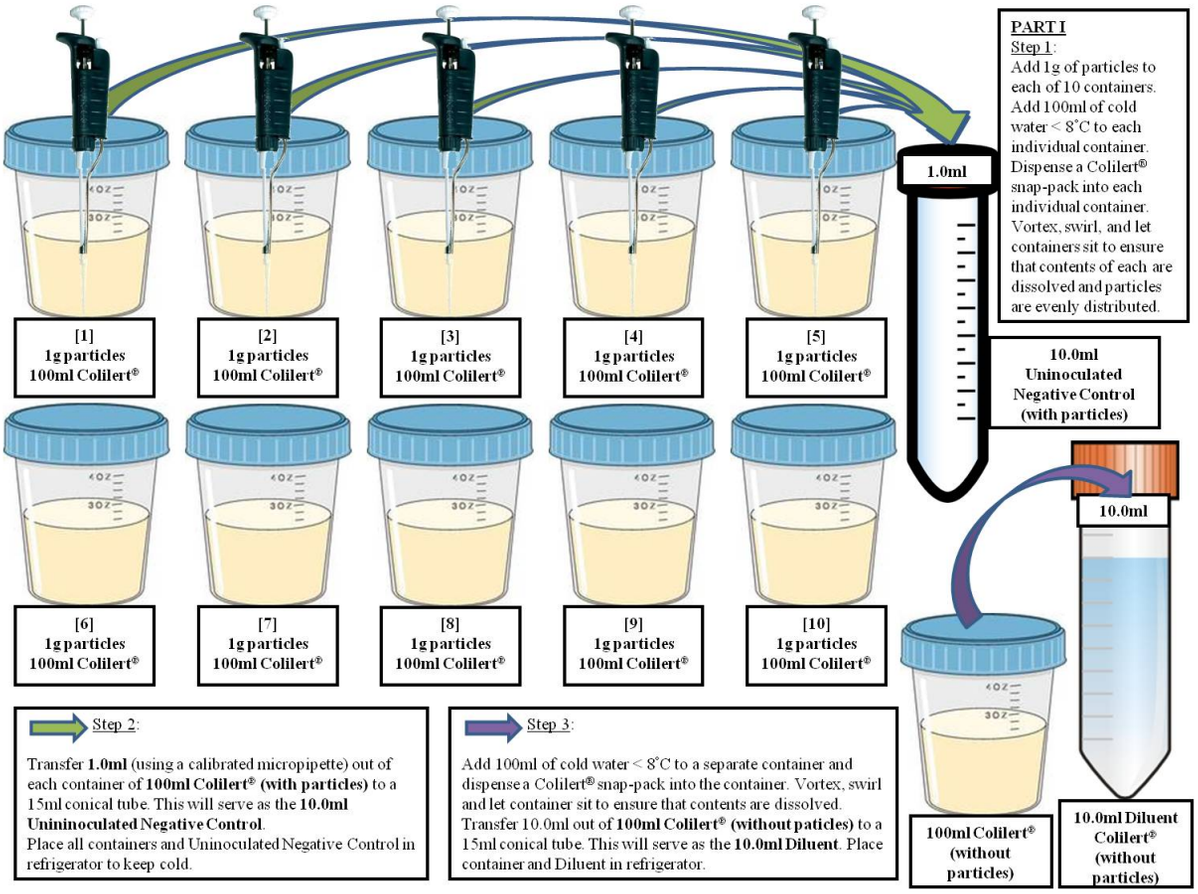

Figure 2. Test Protocol Part I for the detection of 1 total coliform *Klebsiella* and 1 *E. coli* in 16 hours

Analysis was made both by visual observation and also by an instrument. Visual observation: Yellow color development (for total coliforms and *E. coli*) and fluorescence (*E. coli*) was determined each hour while there was a person in the laboratory. The observations were structured to best represent the optimum work pattern in the standard drinking water laboratory: inoculation no later than 4 pm (1600 hours) and the reading of results at 8 am (0800 hours) the next day.

Instrumented Analysis: Figure 4 presents the Pilots Point Monitor (Pilots Point LLC, Sarasota, FL) that was used. This instrument utilizes white light sent through a sample and determines the change in three parameters: Luminosity, or white to black (a measure of turbidity), called the "L" value; a change from red to green (called the "a" value), and a change from blue to yellow (called the "b" value). See Figure 5. Measurements are made every 15 minutes. The instrument can measure light changes from 365 nm through the visible spectrum therefore it can detect both the color change produced by total coliforms and the fluorescence produced by *E. coli*.

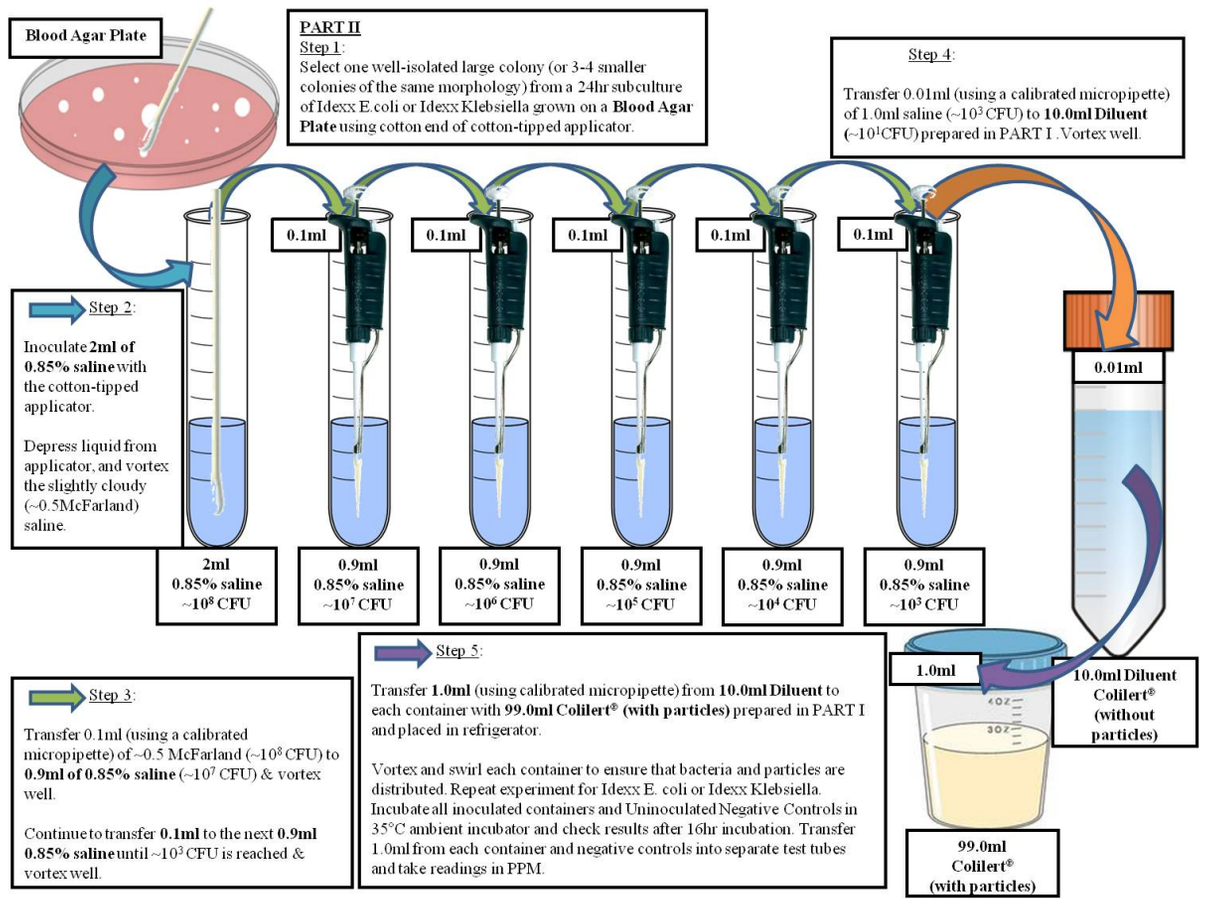

Figure 3. Test Protocol Part II for the detection of 1 total coliform *Klebsiella* and 1 *E. coli* in 16 hours

Figure 4. Pilots Point Monitor® (PPM60®)

2.3. Isolates from source water

Lake source water from the supply to the Regional Water Authority (New Haven, CT) was obtained. While protected from human intrusion, there are abundant animal life, particular deer, rabbit, and small animals. This is the same water source that was used in the original certification of the MMO-MUG test [4].

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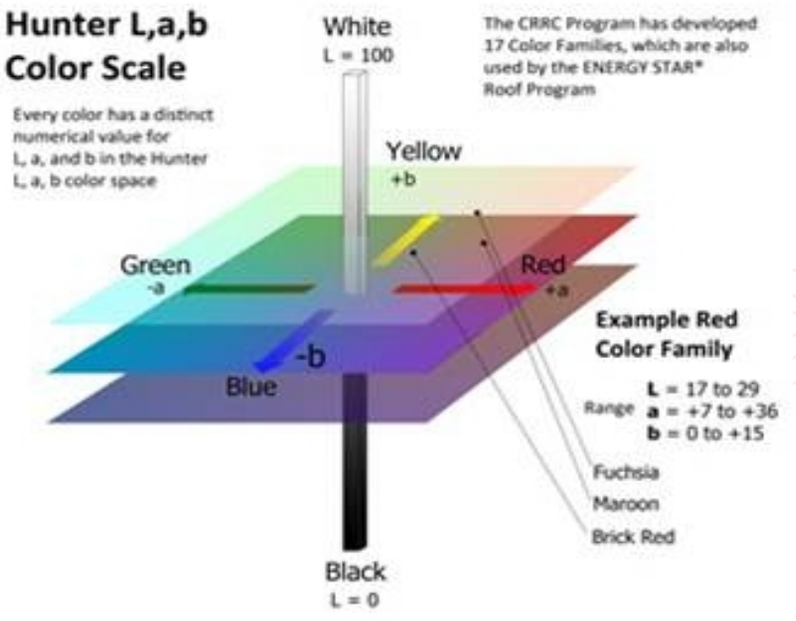


Figure 5. Principle of Pilots Point Instrument

The same protocol as for the quality control bacteria described in the protocol (see Figure 2 and Figure 3) was used. To avoid possible enhancement of enzyme stimulation by the substrates present in the MMO-MUG formula, the source water samples were processed by the classical membrane filtration method as described in Standard Methods [5]. Bacteria consistent with total coliforms and *E. coli* were identified to species (API® ID strip range, bioMérieux, Durham, NC).

3. Results

3.1. Activity of the particles

Figure 6 presents the results of the Colloidands® particle analysis. The top tube is inoculated with Idexx *Klebsiella*, the bottom tube with Idexx *E. coli*. The original color of the Colisure® is yellow/gold; a positive occurs when the hydrolysable substrate is cleaved during growth and multiplication. The particles have largely sunk to the bottom of the tube. It is clearly shown that the red/magenta color is overwhelmingly seen in the particle layer at the bottom of the test tube. Over time, the positive color spreads fully into the liquid layer. The observation that the particle layer consistently is where the color develops first substantiates the ability of the particles to accelerate the growth and multiplication of the target bacteria.

3.2. Level of sensitivity

Table 1 and Graph 1 present the visual observation of the Colilert® test with and without phycocolloid particles at low numbers of *E. coli*, Table 2 and Graph 2 present the visual

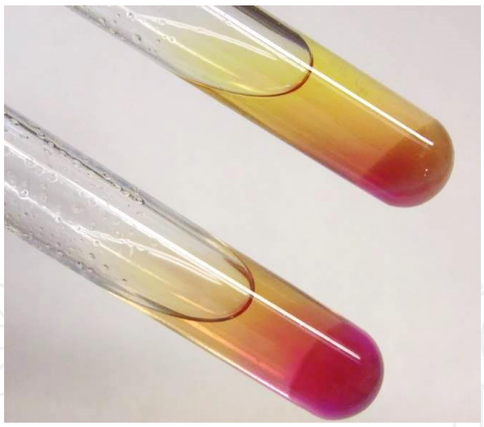


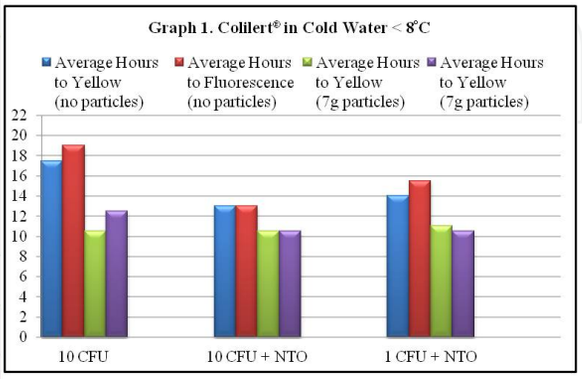
Figure 6. Growth of total coliform *Klebsiella* and *E. coli* utilizing phycocolloids as catalysts

observation of Colilert® with *Enterobacter cloacae* (*E. cloacae*) added, Table 3 and Graph 3 present the observation with *K. pneumoniae* added, and Table 4 and Graph 4 present the observation with *E. coli* added. The water was < 8°C and there were 7 grams of Colloidands® particles per liter of Colilert®. Overall the presence of the particles decreases the time to a positive, both for *K. pneumoniae* and *E. coli* demonstrating the efficacy of the particles. Specifically both for the Idexx *Klebsiella* and Idexx *E. coli*, Colilert® is able to detect 1 bacterium in 100 mL of water in 16 hours or less as seen in Figure 7.

E. coli added count	Hours to Yellow (no particles)	Hours to Fluorescence (no particles)	Hours to Yellow (7g particles)	Hours to Fluorescence (7g particles)
10 CFU (test a)	16	16	10	10
10 CFU (test b)	19	22	11	15
10 CFU + NTO (test a)	13	13	11	11
10 CFU + NTO (test b)	13	13	10	10
1 CFU + NTO (test a)	14	16	11	11
1 CFU + NTO (test b)	14	15	11	10

Table 1. Colilert® in Cold Water < 8°C

E. coli added count	Average Hours to Yellow (no particles)	Average Hours to Fluorescence (no particles)	Average Hours to Yellow (7g particles)	Average Hours to Fluorescence (7g particles)
10 CFU	17.5	19	10.5	12.5
10 CFU + NTO	13	13	10.5	10.5
1 CFU + NTO	14	15.5	11	10.5



Graph 1. Comparison of Time to Detection of MMO-MUG (Colilert®) with and without phycoccolloids. CFU (colony forming units), NTO (non-target organisms), Neg (negative)

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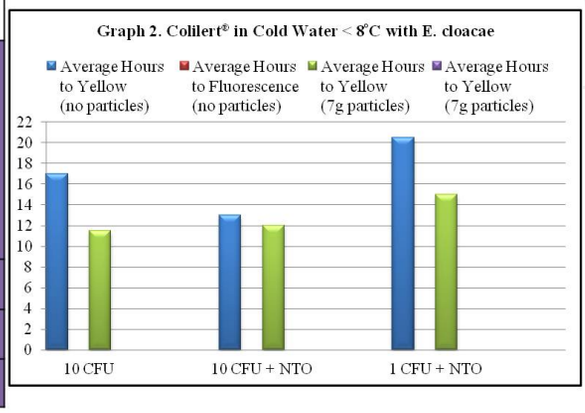
E. cloacae (24 isolates)	Hours to Yellow (no particles)	Hours to Fluorescence (no particles)	Hours to Yellow (7g particles)	Hours to Fluorescence (7g particles)
10 CFU (test a)	16	Neg	12	Neg
10 CFU (test b)	18	Neg	11	Neg
10 CFU + NTO (test a)	13	Neg	12	Neg
10 CFU + NTO (test b)	13	Neg	12	Neg
1 CFU + NTO (test a)	21	Neg	15	Neg
1 CFU + NTO (test b)	20	Neg	15	Neg

Table 2. Colilert® in Cold Water < 8°C with E. cloacae

E. cloacae (24 isolates)	Average Hours to Yellow (no particles)	Average Hours to Fluorescence (no particles)	Average Hours to Yellow (7g particles)	Average Hours to Fluorescence (7g particles)
10 CFU	17	Neg	11.5	Neg
10 CFU + NTO	13	Neg	12	Neg
1 CFU + NTO	20.5	Neg	15	Neg

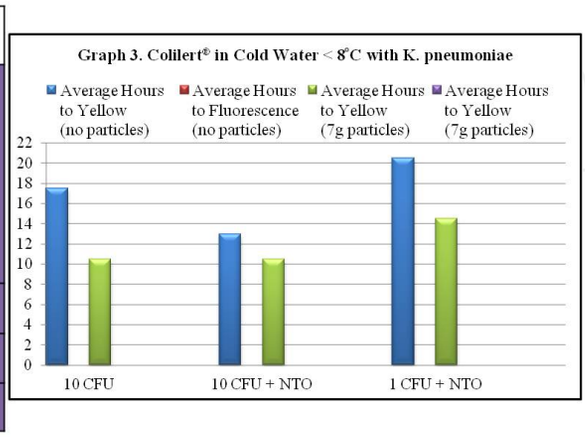


Graph 2. Comparison of Time to Detection of MMO-MUG (Colilert®) with and without phycocolloids with *E. cloacae* added. CFU (colony forming units), NTO (non-target organisms), Neg (negative)

K. pneumoniae (31 isolates)	Hours to Yellow (no particles)	Hours to Fluorescence (no particles)	Hours to Yellow (7g particles)	Hours to Fluorescence (7g particles)
10 CFU (test a)	16	Neg	10	Neg
10 CFU (test b)	19	Neg	11	Neg
10 CFU + NTO (test a)	13	Neg	11	Neg
10 CFU + NTO (test b)	13	Neg	10	Neg
1 CFU + NTO (test a)	21	Neg	15	Neg
1 CFU + NTO (test b)	20	Neg	14	Neg

Table 3. Colilert® in Cold Water < 8°C with K. pneumoniae

K. pneumoniae (31 isolates)	Average Hours to Yellow (no particles)	Average Hours to Fluorescence (no particles)	Average Hours to Yellow (7g particles)	Average Hours to Fluorescence (7g particles)
10 CFU	17.5	Neg	10.5	Neg
10 CFU + NTO	13	Neg	10.5	Neg
1 CFU + NTO	20.5	Neg	14.5	Neg

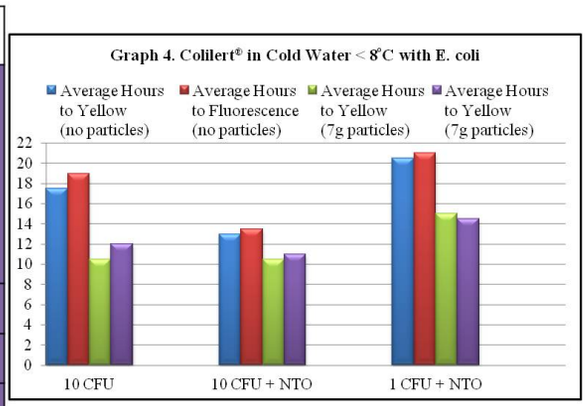


Graph 3. Comparison of Time to Detection of MMO-MUG (Colilert®) with and without phycocolloids with *K. pneumoniae* added. CFU (colony forming units), NTO (non-target organisms), Neg (negative)

E. coli (8 isolates)	Hours to Yellow (no particles)	Hours to Fluorescence (no particles)	Hours to Yellow (7g particles)	Hours to Fluorescence (7g particles)
10 CFU (test a)	16	16	10	10
10 CFU (test b)	19	22	11	14
10 CFU + NTO (test a)	13	14	11	11
10 CFU + NTO (test b)	13	13	10	11
1 CFU + NTO (test a)	21	21	15	14
1 CFU + NTO (test b)	20	21	15	15

Table 4. Colilert® in Cold Water < 8°C with E. coli

E. coli (8 isolates)	Average Hours to Yellow (no particles)	Average Hours to Fluorescence (no particles)	Average Hours to Yellow (7g particles)	Average Hours to Fluorescence (7g particles)
10 CFU	17.5	19	10.5	12
10 CFU + NTO	13	13.5	10.5	11
1 CFU + NTO	20.5	21	15	14.5



Graph 4. Comparison of Time to Detection of MMO-MUG (Colilert®) with and without phycoccolloids with *E. coli* added. CFU (colony forming units), NTO (non-target organisms), Neg (negative)

Figure 7 presents the actual pictures of the replicates of the analysis of the Colilert® test to detect 1 bacterium per 100 mL. As can be seen, each of the 100 mL samples was clearly positive. As shown in Figure 6, the particle layer at the bottom of the water collection vessel is densely colored. The liquid is also clearly positive.

Figure 8 and Figure 9 present the results of the testing shown in Figure 7 as follows: from the 100 mL samples in Figure 7, 1 mL of supernatant was added to a 13 x 100 mm polystyrene test tube and placed in the PPM instrument. Readings in the PPM instrument were taken and a definite change in the "L", "a", and "b" values can be seen which indicate positive results for both quality control Idexx *E. coli* and Idexx total coliform *Klebsiella*. Because the light path in the test tube is much smaller than in the 100 mL vessel, the time required for a positive in a 100 mL sample is longer.

In all configurations – 1 mL in a test tube and 100 mL in a water collection vessel – the phycocolloids significantly decreases the time to detect the quality control Idexx total coliform *Klebsiella* and the Idexx quality control *E. coli*. In addition, the phycocolloids decreases the time to detect 1 bacterium in 100 mL of cold water < 8°C to within 16 hours.

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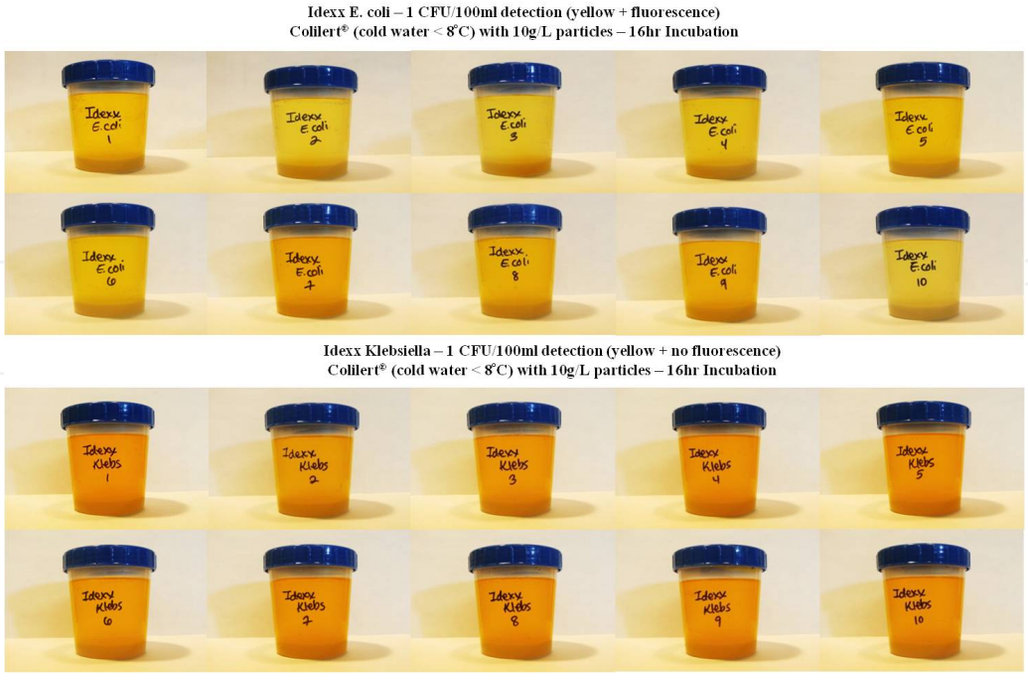

Figure 7. Replicate Testing with Idexx Quality Control *E. coli* and *Klebsiella* showing the ability of Colilert® to detect 1 bacterium per 100 mL within 16 hours in a cold water <8°C sample with phycocolloids added

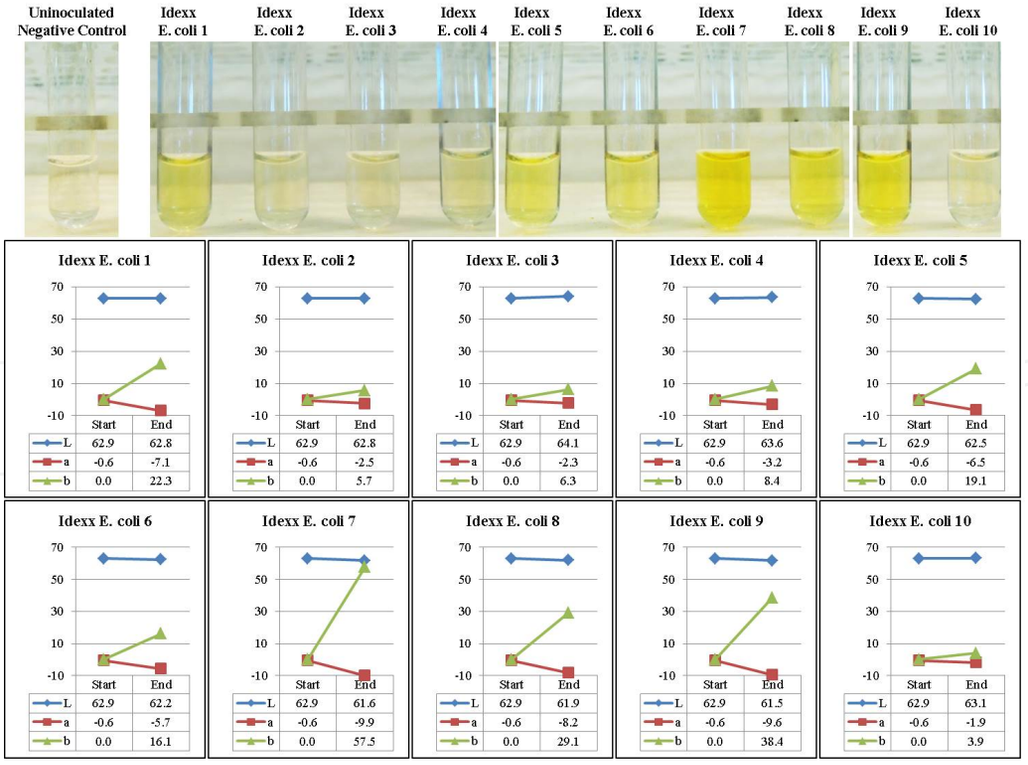

Figure 8. PPM Readings of 10 Quality Control *E. coli* at the 1 CFU/100 mL level

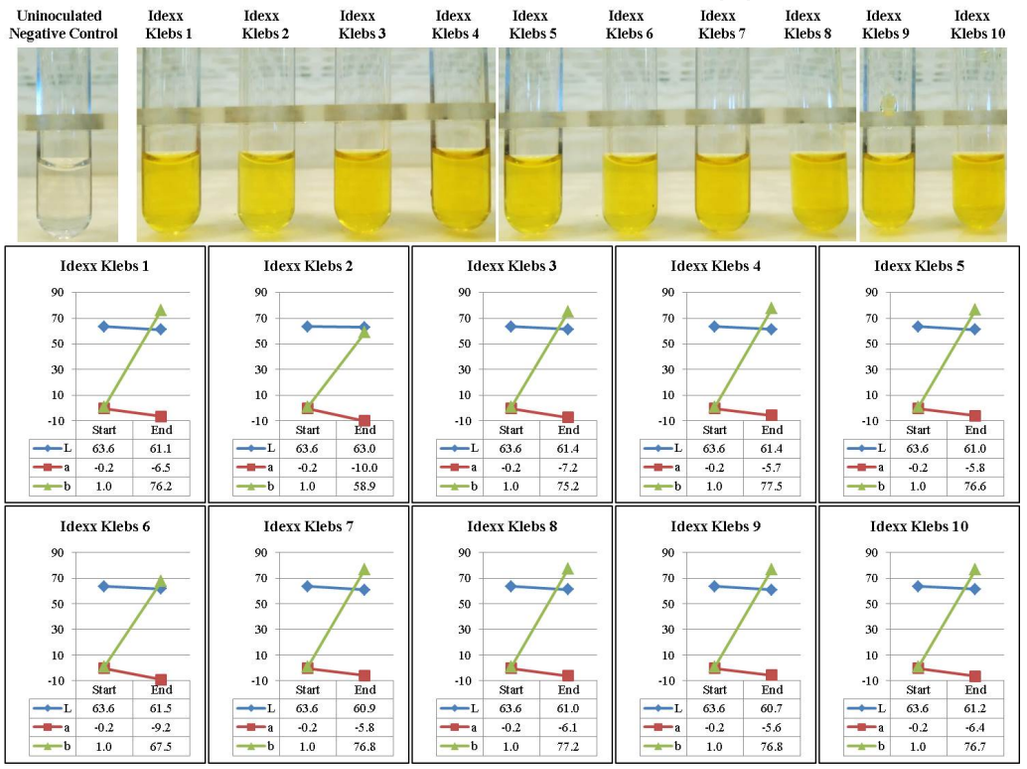

Figure 9. PPM Readings of 10 Quality Control *Klebsiella* at the 1 CFU/100 mL level

Figure 10 and Figure 11 present 1 mL duplicate data from the PPM instrument on detection of 10 bacteria per mL of Idexx *E. coli* in Colilert® with the addition of phycocolloids in cold water < 8°C. The "b" value on the graph indicates the color change from colorless (Colilert® negative) to yellow (Colilert® positive). Once the yellow color starts to develop, a strong yellow color is observed in a two hour time period. In the experiment shown in Figure 10, the time to detection was 8.5 hours. In the experiment shown in Figure 11, the time to detection was 9.5 hours. The phycocolloid particles not only reduces the time to positive detection but also reduces the time bacteria spend in lag phase and increases the multiplication of bacteria in log phase as seen in the graphs.

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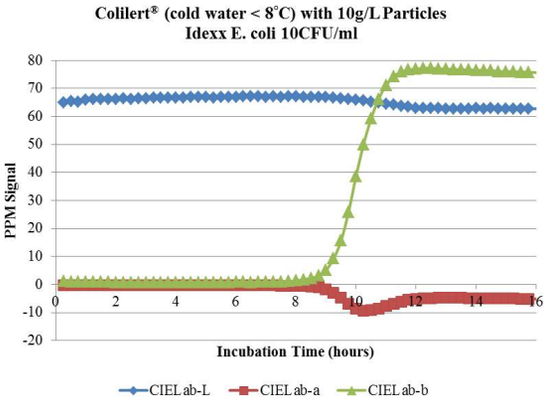
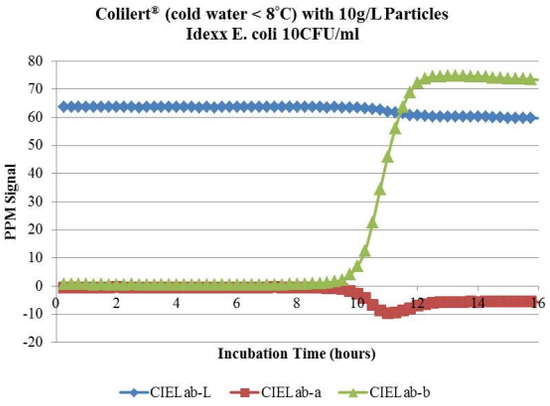


Time [ms]	Hours	Temp. [°C]	CIELab-L	CIELab-a	CIELab-b
900031	0.250009	33	64.83603	-0.27424	1.212597
1800062	0.500017	33.5	65.44651	-0.34088	1.051462
2700093	0.750026	33.5	65.26589	-0.33963	1.018691
3600125	1.000035	33.5	65.7773	-0.27013	0.935626
4500156	1.250043	33.5	66.1361	-0.34136	0.899315
5400187	1.500052	33.5	66.02613	-0.32318	0.873601
6300203	1.750056	33.5	66.12133	-0.31909	0.861633
7200218	2.000061	33.5	66.17171	-0.36201	0.832033
8100234	2.250065	33.5	66.28529	-0.2887	0.836647
9000265	2.500074	33.5	66.11073	-0.36544	0.802457
9900281	2.750078	33.5	66.43523	-0.24322	0.818241
10800312	3.000087	33.5	66.48994	-0.32371	0.80719
11700359	3.2501	33.5	66.58561	-0.29621	0.817478
12600375	3.500104	33.5	66.68216	-0.3382	0.813389
13500406	3.750113	33.5	66.64419	-0.31379	0.807405
14400437	4.000121	33.5	66.70656	-0.31188	0.825918
15300453	4.250126	33.5	66.68128	-0.2988	0.82618
16200484	4.500134	33.5	66.8167	-0.30628	0.809467
17100500	4.750139	33.5	66.79412	-0.29966	0.800812
18000562	5.000156	33.5	66.89322	-0.26998	0.81948
18900593	5.250165	33.5	66.7479	-0.34866	0.799978
19800609	5.500169	33.5	66.92468	-0.28732	0.819755
20700625	5.750174	33.5	66.99007	-0.29558	0.819588
21600656	6.000182	33.5	66.98164	-0.29019	0.846958
22500687	6.250191	33.5	67.05287	-0.26909	0.850356
23400703	6.500195	33.5	67.04262	-0.32964	0.836694
24300734	6.750204	33.5	67.06235	-0.29391	0.852704
25200750	7.000208	33.5	66.95059	-0.38117	0.875723
26100781	7.250217	33.5	67.06941	-0.36445	0.936592
27000812	7.500226	33.5	66.97546	-0.40987	0.987101
27900843	7.750234	33.5	67.05117	-0.47147	1.140034
28800859	8.000239	33.5	67.07195	-0.49791	1.384532
29700875	8.250243	33.5	66.95478	-0.62513	1.76003
30600906	8.500252	33.5	66.97697	-0.80168	2.273226
31500937	8.75026	33.5	66.94083	-1.11115	3.066063
32400953	9.000265	33.5	66.91933	-1.75732	5.134702
33300984	9.250273	33.5	66.57552	-3.03569	9.248198
34201000	9.500278	33.5	66.46468	-4.64866	15.60215
35101015	9.750282	33.5	66.05341	-6.90302	25.88704
36001062	10.0003	33.5	65.86639	-8.66067	38.68569
36901093	10.2503	33.5	65.56187	-9.30381	49.99931
37801109	10.50031	33.5	65.14212	-9.17062	59.20099
38701125	10.75031	33.5	64.86913	-8.52239	66.13572
39601156	11.00032	33.5	64.48917	-7.73296	71.07744
40501187	11.25033	33.5	64.16003	-6.84321	74.22168
41401203	11.50033	33.5	63.60147	-6.05825	75.86057
42301234	11.75034	33.5	63.33666	-5.50708	76.64969
43201250	12.00035	33.5	62.97827	-5.21466	76.86161

Figure 10. Actual PPM Data for detection of Idexx *E. coli* at 10 CFU/mL. Positive detection at 8.5hr incubation.



Time [ms]	Hours	Temp. [°C]	CIELab-L	CIELab-a	CIELab-b
900031	0.250009	32.5	63.69099	-0.49168	0.689805
1800047	0.500013	33.5	63.66232	-0.49499	0.678027
2700063	0.750018	34	63.61729	-0.52235	0.62027
3600094	1.000026	34.5	63.64006	-0.4952	0.630665
4500110	1.250031	35	63.6042	-0.4476	0.581539
5400141	1.500039	35	63.6333	-0.43148	0.597847
6300156	1.750043	35	63.60711	-0.45547	0.561321
7200172	2.000048	35	63.68177	-0.39294	0.553584
8100203	2.250056	34.5	63.60659	-0.51785	0.549924
9000235	2.500065	34.5	63.63307	-0.46173	0.594509
9900266	2.750074	34.5	63.54198	-0.50387	0.570631
10800281	3.000078	34.5	63.62386	-0.49049	0.586247
11700297	3.250083	34.5	63.56142	-0.45276	0.549948
12600313	3.500087	34.5	63.63914	-0.45624	0.573933
13500344	3.750096	34	63.57871	-0.4375	0.570476
14400360	4.0001	34	63.62157	-0.42224	0.568533
15300375	4.250104	33.5	63.58437	-0.47004	0.54239
16200391	4.500109	33.5	63.62916	-0.42123	0.539017
17100406	4.750113	33.5	63.44792	-0.43824	0.511515
18000422	5.000117	33.5	63.624	-0.42686	0.517488
18900453	5.250126	33	63.53188	-0.52026	0.558603
19800485	5.500135	33.5	63.63403	-0.4344	0.534225
20700516	5.750143	33	63.64838	-0.44346	0.56057
21600547	6.000152	33	63.60928	-0.45085	0.553536
22500594	6.250165	33	63.66185	-0.42686	0.540173
23400641	6.500178	33	63.6083	-0.40844	0.541472
24300672	6.750187	32.5	63.65443	-0.48518	0.583649
25200703	7.000195	32.5	63.59076	-0.44426	0.556648
26100735	7.250204	32.5	63.65409	-0.36755	0.569105
27000766	7.500213	32.5	63.68359	-0.40975	0.582528
27900797	7.750221	32	63.61787	-0.46471	0.638187
28800813	8.000226	32	63.66132	-0.50753	0.677574
29700844	8.250234	32	63.61823	-0.57426	0.753474
30600875	8.500243	32	63.63292	-0.55173	0.880742
31500891	8.750248	32	63.68202	-0.61023	1.035964
32400906	9.000252	31.5	63.35884	-0.76452	1.257169
33300922	9.250256	31.5	63.56632	-0.84788	1.640439
34200953	9.500265	31.5	63.53264	-1.04955	2.222466
35100985	9.750274	31.5	63.37869	-1.71554	4.102373
36001016	10.00028	31.5	63.34806	-2.71517	7.161081
36901031	10.25029	31.5	63.17438	-4.27178	12.50897
37801047	10.50029	31.5	62.89819	-6.66636	22.5172
38701063	10.7503	31.5	62.65871	-8.64917	34.42073
39601094	11.0003	31.5	61.97878	-9.5205	45.94672
40501110	11.25031	31.5	61.67507	-9.43205	56.04141
41401141	11.50032	31.5	61.36737	-8.74519	63.68078
42301172	11.75033	31.5	60.84645	-7.86057	68.94051
43201203	12.00033	31.5	60.75281	-6.99872	72.19634

Figure 11. Actual PPM Data for detection of Idexx *E. coli* at 10 CFU/mL. Positive detection at 9.5hr incubation.

4. Discussion

Since the introduction of the MMO-MUG (Colilert®) test in 1989 for the simultaneous detection of total coliforms and *E. coli* from drinking water, wastewater, bathing water, and other uses, attempts to decrease the time to a positive have been met with limited success. A variation of the Colilert® test, Colilert-18®, reduced the time from 24 hours to 18 hours incubation but requires the heating of the water sample adding an additional hour or two. Water samples are transported to the laboratory chilled by regulation. However, because many water samples arrive and are processed at the end of the work day after collection, the time to a result is often in the middle of the work day, thus delaying remediation and response and adversely affecting the public's health protection.

The two types of bacteria that generally exist are describes as either sessile (a unit that attaches to a surface or exist in a biofilm) or planktonic (existing freely in bulk solution). Antonie van Leuwenhoek described biofilms in 1674 as "animalcules" through observation of material scraped from human tooth surfaces with his microscope, but with advances in technology, biofilms can more accurately be described [6]. Biofilms can be described as microbial communities that are sessile and grow on surfaces surrounded by a matrix of extracellular polymeric substances; microcolonies are distinct communities of bacterial cells of one or many different species that are surrounded by a matrix. The advantages of biofilm formation by bacteria is that it provides protection against antibiotics, disinfectants, and environments that are constantly changing [7]. The key to the catalyst activity of the phycocolloids is their ability to interact with the bacteria and the attendant production of microcolonies. The bacteria multiply much faster when attached to the particles as microcolony biofilm.

The micro particles increase the surface area in the liquid broth and allow microbes that multiply in vitro to establish a biofilm. In effect, the micro particles act in an analogous way as a catalyst does in a chemical reaction. In the microbiology area, the micro particles provide multiple attachment surfaces for the microbes to "establish residence". Microbes prefer surfaces on which to grow and multiply rather than being free in a liquid environment. For example, the microbes may experience quorum sensing, which accelerates the generation of a biofilm. The biofilm is produced when the microbes multiply, and it yields colonies of microbes that are held together by external capsules, pili, and glycocalyxes of the microbes which, in the broad context, are surface components, such as polysaccharides, proteins and/or mixtures thereof. The micro particles are static, in that they are not consumed but serve as a physical structure that provides shelter and attachment and promotes the multiplication and expression of the target microbe. There may be attached nutritive elements on the micro particles that serve to stimulate the development of the bacterial nidus. The micro particles may be colloidal, in suspension, or a combination. Any materials or structures that encourage the growth of microbes on a biofilm are highly preferred for use in this invention. Supporting the catalytic activity of the phycocolloids is the observation that growth starts first at the bottom of the test tubes, where particles have gravitationally settled and microbial biofilms have developed, attached to the particles. Additional particles are distributed with the remainder of the admixture, but have not yet been associated with metabolizable substrate creating a color

change. The image of the test tubes illustrates well how the particles expedite the detection of the bacteria (i.e., *E. coli*) targeted by the metabolizable substrate of the test mixture, and the increased sensitivity associated therewith; i.e., the micro particles provide surface area or attachment surfaces for the *E. coli* microbes to establish residence, grow, and multiply (e.g., creating biofilms), which growth is indicated by the color change created when the nutrient portions of the metabolizable substrate are metabolized by the microbes.

This report demonstrates that phycocolloids introduced into the classical Colilert® formula significantly decreases the time to a positive. By visual observation with both standard quality control *Klebsiella* and *E. coli*, plus with total coliforms and *E. coli* isolated from source water, it was demonstrated that Colilert® could detect 1 target bacterium in 100 mL of cold water < 8°C in repetitive experiments. The detection in cold water < 8°C is particularly important because the Colilert-18® variation, while rated positive in 18 hours, requires the pre-heating of the water sample before the test is started, thus effectively increase the time to approximately 20 hours.

The 16 hour benchmark is particularly important for laboratory work flow. It provides the ability to perform a 4 to 8 test – in by 4 pm, finished by 8 am, the optimum for the work flow. Further enhancing the time to detection is the use of the PPM instrument. With it, detection of 1 total coliform and 1 *E. coli* in cold water < 8°C was decreased by several hours; furthermore the instrument signals a positive at the time it is positive.

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References

[1] International Organization for Standardization (ISO). (2012). *ISO 9308-2:* Water quality--Enumeration of *Escherichia coli* and coliform bacteria--Part 2: Most probable number method. ISO, Geneva Switzerland.

[2] Environmental Protection Agency (EPA). (1992) National Primary Drinking Water Regulations, Analytical Techniques: Coliform Bacteria: Final Rule. *Federal Register* 57(112):24744-24747.

[3] Edberg, S.C., M.J. Allen, D.B. Smith, and the National Collaborative Study. (1989) National field evaluation of a defined substrate method for the simultaneous detec-

tion of total coliforms and *Escherichia coli* from drinking water: Comparison with presence-absence techniques. *Applied and Environmental Microbiology* 55(4):1003-1008.

[4] Edberg, S.C., M.J. Allen, D.B. Smith, and N.J. Kriz. (1990) Enumeration of total coliforms and *Escherichia coli* from source water by the defined substrate technology. *Applied and Environmental Microbiology* 56(2):366-369.

[5] American Public Health Association (APHA). (1992) Standard Methods of Water and Wastewater. 22nd ed. American Public Health Association, American Water Works Association, Water Environment Federation publication. APHA, Washington D.C.

[6] Costerton, J.W. Introduction to biofilm. (1999) *International Journal of Antimicrobial Agents*, 11(3-4):217–221.

[7] Garret T.R., A. Bhakoo, and Z. Zhang. Bacterial adhesion and biofilms on surfaces. (2008) *Progress in Natural Science*, 18:1049–1056.