

Example 6

Capture of *E. coli* from Spiked Water with Particulate Concentration Agents Using a Type I Device

[0214] An isolated colony of *E. coli* (ATCC 51813) from a Tryptic Soy Agar plate (Becton Dickinson, Sparks, Md.) was used to inoculate 5 ml Tryptic Soy Broth (Becton Dickinson, Sparks, Md.) and incubated overnight in a 37° C. incubator. The overnight culture containing approximately 10⁹ colony forming units/ml (CFU/ml) was diluted 1:10,000 (to approximately 10⁵ CFU/mL, hereinafter called “initial diluted suspension”) in filter sterilized 18 megaohm water. Five hundred microliters of the diluted culture were transferred to 50 ml of filter sterilized 18 megaohm water, resulting in a final concentration of about approximately 1000/ml.

[0215] An aliquot (0.5 mL) of 100× Adsorption Buffer (pH 7.2) was added to the 50 mL diluted *E. coli* suspension (hereinafter called “spiked water sample”). The contents were mixed by manual mixing for about a minute.

[0216] An amount of 10 mg of steam sterilized CM-111 was weighed and added to Type I devices prepared as described in example 5. A 10 ml volume of the spiked water sample was added to each device and the devices were capped with surface sterilized Para film. The contents were mixed by shaking manually at room temperature (25° C.) for about 30 seconds.

[0217] After mixing, the devices were incubated for various time periods (1, 5, 10 and 20 minutes, respectively) on a Thermolyne Vari Mix™ rocking platform (Barnstead International, Iowa, 14 cycles/minute). After the incubation the tubes were set on the bench top for 10 minutes to settle the particulate concentration agent, CM-111. After settling, Para film wrapping was removed and the pre-sterilized plunger device was used to pierce the foil seal and deposit the settled CM-111 agent into the lower part of the device. The supernatant was removed by using a pipette, and the lower part of the device (which contained the cell concentration agent) was separated from the upper part of the device using a razor blade. The settled CM-111 concentration agent (in approximately 100 microliters of water) was removed from the device; diluted 1:100 in sterile water, and one-milliliter aliquots of the diluted concentration agent were plated on 3M™ Petrifilm™ Aerobic Count Plate (3M Company, St. Paul, Minn.) according to the manufacturer’s instructions.

[0218] As a control, the initial diluted suspension was further diluted 1:1000 dilution in sterile water and was plated as on 3M™ Petrifilm™ Aerobic Count Plate (3M Company, St. Paul, Minn.) according to the manufacturer’s instructions. The particulate materials were also plated on Petrifilm™ Aerobic Count Plate as sterility controls. The plates were incubated overnight in a 37° C. incubator (VWR Orbital Shaker Incubator, VWR, West Chester, Pa.).

[0219] All plates were analyzed by using 3M™ Petrifilm™ Plate Reader (3M Company, St. Paul, Minn.) according to the manufacturer’s instructions and colony counts were obtained. The results are shown in Table 2. The results were calculated using the following formula:

$$\text{Capture efficiency} = (\text{Number of colonies on concentration agent} / \text{Total number of colonies in the spiked control}) \times 100$$

TABLE 2

Concentration/capture of <i>E. coli</i> from 10 ml sample. All data represent the average of two replicate tests per experiment.		
Sample	% Control	Stdev
1 min	8	4
5 min	34	4
10 min	33	11
20 min	80	10

Example 7

Concentration of *E. coli* Using CM-111 Using a Type III Device

[0220] An isolated *E. coli* (ATCC 51813) colony was inoculated from a streak plate into 5 ml Tryptic Soy Broth (TSB, Becton Dickinson, Sparks, Md.) and incubated at 37° C. for 18-20 hours. This overnight culture at ~10⁹ colony forming units/ml was diluted in sterile-filtered deionized water (MilliQ, Millipore, MA) and spiked in 10 ml of sterile-filtered deionized water to obtain final concentration of 1×10³/ml and 1×10⁴/ml (~1×10⁴/ml cfus and ~1×10⁵/ml cfus total). The spiked water was added to the housing of a Type III device containing 10 mg pre-sterilized (121 deg C., 15 minutes) powder of CM-111 (Cosmetic Microspheres-111, 3M Company, St Paul) and 100 microliters of the 100× Adsorption Buffer (pH 7.2). The housing was sealed with surface sterilized Parafilm and placed on a rocking platform. The capped devices were then incubated at room temperature (25° C.) for 5 minutes contact time on a Thermolyne Vari Mix™ rocking platform (Barnstead International, Iowa, 14 cycles/minute). The devices were then allowed to stand without shaking (to allow the particles to settle by gravity force) for 5 minutes (total elapsed time for rocking and settling=10 minutes), the Parafilm was removed and the Type II device plunger inserted into the housing and was urged toward the bottom of the housing to separate the CM-111 particles from the bulk sample. When the plunger broke the frangible seal, the CM-111 particles, suspended in about 0.1 mL of the liquid sample, was transferred to the lower receptacle of the housing. The CM-111 particles were retrieved from the lower receptacle and transferred to a 1.5 ml sterile microfuge tube. A 100 microliter volume of the BacTiter-Glo™ reagent (Promega, Madison, Wis.) was added to the pellet, mixed by vortexing for 5 seconds on a VWR Fixed Speed Vortex Mixer (3200 rpm for 5 seconds) and read on a tabletop luminometer (FB12 Single Tube Luminometer, Berthold Detection Systems USA, Oak Ridge, Tenn.). A positive control (“100% signal”) was prepared by testing a 100 microliter volume from a 1×10⁵/ml and 1×10⁶/ml suspension of the *E. coli* cells. Results were calculated using the formula below and tabulated in Table 2 below:

$$\text{ATP Signal \% Capture efficiency} = (\text{RLUs on CM-111 pellet} / \text{RLUS from 100\% signal}) \times 100$$

RLU=Relative Luciferase Units.