**ANALYTICAL SERVICES, INC. (ASI)**
*Microbiological Testing, Research and Consulting*

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14 July 2009

Paul O'Brien
Miracle Straw
917 SR 92 North
Tunkhannock, PA 18657

**Subject:** **LETTER OF TRANSMITTAL**
**The Miracle Straw™ – Microbiological Challenge Study Report**

Dear Paul,

This document is Analytical Services, Inc.'s (ASI) final report concerning a microbiological challenge study performed The Miracle Straw. Testing was performed in ASI's laboratory in Williston, VT, and was initiated on 25 June 2009.

Following the executive summary below, this report includes the following:

- a brief description of the units tested;
- a description of the test protocol employed;
- an overview of the analytical methodologies used;
- a summary of analytical data; and
- a brief discussion of the results.

**Executive Summary** – Two Miracle Straw units were tested under low throughput, challenge water (low temperature, elevated turbidity, total organic carbon, altered pH, etc. ) conditions to document removal and or inactivation of MS2 coliphage (virus) and *Raoultella terrigena* (bacteria) and removal of *Cryptosporidium parvum* (protozoa) oocysts. The treatment mechanisms were disinfection and filtration. The target reductions for each type of organism were as follow: 4 $Log_{10}$ virus; 6 $Log_{10}$ bacteria and 3 $Log_{10}$ protozoa. Under the test conditions, both units successfully achieved all target log reduction goals.

Thank you for using ASI for your microbiological testing needs. If we may be of further service, please contact us at anytime.

Best regards,

ANALYTICAL SERVICES, INC. (ASI)

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**Study Title:**

**Microbiological challenge testing of two Miracle Straw water treatment devices using challenge water conditions**

## Product Description

Two Miracle Straw units were delivered to ASI by Mr. Paul O'Brien on the day of testing; one unit was a cylindrical Miracle Straw configuration, the other was a backpacker style unit. Both units consisted of prefilters, disinfection by chlorine, a chlorine contact chamber, granular activated carbon filtration and a pleated paper filter. Units were operated by manual intake of water by suction created by withdrawing the plunger element of the unit; water was expressed from the unit by inserting the plunger.

Treatment of water is accomplished by disinfection (chlorine tablet ) and filtration (the NanoCeram pleated filter). Chlorine is neutralized by carbon filtration. Treatment volume varied between the two units; the Straw unit treated approximately 180 mL (6 ounces) per discharge, while the backpack style unit treated approximately 20 mL per discharge.

## Study Design

Two (2) Miracle Straw units were tested using the following three organisms types; MS2 coliphage (ATCC 15597-B1), *Raoultella (*formerly known as *Klebsiella) terrigena* (ATCC 33257), and *Cryptosporidium parvum* (Iowa isolate). The target influent concentrations (dose) and target log reductions as per NSF P248 are shown in Table 1 below.

**Table 1. Challenge Organisms**

| Organism | Target Dose (min.) | Target Log Reduction |
|---|---|---|
| *Raoultella terrigena* | 1.0 x 10e7 CFU/100mL | 6 log |
| MS2 coliphage | 1.0 x 10e7 PFU/L | 4 log |
| *Cryptosporidium parvum* | 5.0 x 10e4 oocysts/L | 3 log |

Units were tested under "challenge water" conditions, except that only water of pH 9 was used (water of pH 5 was not used). The challenge water was prepared using a recipe provided by NSF International to Miracle Straw. The recipe for the challenge water is shown below.

**Table 2. Recipe for P248 Challenge Water #2 as received from NSF International.**

To prepare Phase II (Challenge) water:

110 L RO/DI
Add 150 g Sea Salts
Add 290 mL Sodium Bicarbonate solution (see preparation below)
Add 340 mL of 6g/L Tannic acid solution
Adjust pH
Add 10 g of Fine Test Dust

Sodium Bicarbonate Solution
Adjust pH above 9 with NaOH before adding $NaHCO_3$
3960 g Sodium Bicarbonate ($NaHCO_3$) to 60 L DI $H_2O$

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This recipe was modified by ASI for application to the smaller volumes used in this study. Five liters (%L) of challenge water were prepared for this study (22-fold less than the 110 L specified in the recipe), so the amount of each constitute added was divided by twenty two. Twenty milliliters of sodium bicarbonate solution was prepared and a pro-rated amount added to the challenge water. The challenge water was chilled overnight prior to use.

Challenge Study Protocol

Neutralization of chlorine by the carbon in the Miracle Straw units had been previously tested and was not re-examined prior to this study. All samples were collected in sterile vessels containing sodium thiosulfate to ensure oxidant neutralization upon sample collection (the vessels with sodium thiosulfate have demonstrated the ability to neutralize up to 15 mg/L chlorine).

1. Homogenize challenge water and aliquot 5L challenge water into disinfected carboy with nmagnetic stir bar. Record general water quality parameters before spiking. Dose with microorganisms to desired concentrations as above.
2. Place carboy on stir plate and ensure moderate vortex for continued mixing.
3. Collect 1L aliquots of spiked challenge water in disinfected beakers for use in each test unit. Pour the challenge water into the back pack unit bladder.
4. Backpacker style unit – operate as per manufacturer's instructions, pumping the spiked water from the bladder out into a receiving vessel.
5. Miracle Straw unit – operate as per manufacturer's instructions, withdrawing water from the beaker and expelling into the provided cup.
6. Treat approximately 1.5L through each unit.
7. Back-pack unit Influent - Using a serological pipette, collect one 20 – 25 mL pretreatment (influent) sample from the back-pack unit bladder. Label "Influent 1" and set aside.
8. Miracle Straw Influent - Using a serological pipette, collect one 20 – 25 mL pretreatment (influent) sample from the carboy on the stir plate. Label "Influent 2" and set aside. This sample will be analyzed to establish the starting concentrations of MS2, *Raoultella* and *Cryptosporidium* as described below.
9. Continue treating water with each unit. After approximately 2L, .expel treated product water into a sterile container with sodium thiosulfate. Label "Effluent 1 (back-pack) or Effluent #2 (Miracle Straw)". These samples will be analyzed to determine the concentrations of infectious MS2, culturable *Raoultella* and *Cryptosporidium* present in the treated water, if any.

**Microbiological Methods**

*Raoultella* – Propagation of *Raoultella terrigena* ( ATCC 33257 was performed by subculturing *R. terrigena* to TSA (ASI Lot # 050809SEV02) and incubating at 35 ± 1.0℃ for approximately 16-24 hours. The organism was harvested by rinsing each plate with 2, 5.0mL rinses of sterile phosphate buffered water (Biotrace International lot # FT07192) for a 10 mL total stock suspension.

Enumeration of *Raoultella* was performed by ASI SOP 310-1 using spread plate technique with MacConkey agar (ASI Lot #041509SEV01). Prior to plating, dilutions were performed using sterile laboratory deionized water, and single plates of all dilutions were prepared. All samples were incubated at 35 ± 1.0℃ for approximately 16-24 hrs.

MS2 – Propagation of MS2 coliphage is performed using ASI standard procedure for coliphage propagation. Briefly, a culture of *Escherichia coli* ATCC 15597 is cultured overnight at 35 ± 1.0℃ for approximately 16-24 hours. The *E. coli* is inoculated to TSB (Trypticase Soy Broth) and allowed to incubate on an incubator/shaker for 3-4 hours at 35 ± 1.0℃, and approximately 150 RPM. An aliquot of MS2 stock is inoculated into the *E. coli* host culture and is allowed to incubate overnight at 35 ± 1.0℃. The suspension is then centrifuged to pellet the dead bacterial host, and the remaining supernatant

containing MS2 is poured off. The supernatant containing MS2 is then filtered through a 0.2 µm membrane to remove a remaining bacterial host. The MS2 is then titered to determine concentration by double agar overlay procedure.

MS2 enumeration of the test samples was performed in accordance with ASI Standard Operating Procedure (SOP) 212-2, using double agar overlay procedure using *E. coli* ATCC 15597 as the host, on Trypticase Soy Agar with 2X Trypticase Soy Broth Agar Overlays (ASI Lot # 050809SEV02 and 050409SEV01, respectively). Prior to plating, dilutions were performed using sterile laboratory deionized water, and single plates of all dilutions were prepared. Overlay controls were analyzed along with samples and gave acceptable results. All samples were incubated at 35 ± 1.0℃ for approximately 16-24 hrs.

*Cryptosporidium* – The live, infectious *Cryptosporidium parvum* oocysts used in this study (Iowa isolate, Lot # 16-09) were purchased from Dr. H. Stibbs at Waterborne, Inc. (New Orleans, LA). The oocysts purchased were from experimentally infected calves (shedding date: 15 May 2009), purified from feces by sucrose and Percoll density gradient centrifugation after initial extraction by diethyl ether (purification date: 15 May 2009). The oocysts were shipped to ASI in approximately a 4 mL of PBS with penicillin, streptomycin, genticin, Amphotericin B, and 0.01% Tween 20.

The *Cryptosporidium* influent sample was collected as one 0.5mL aliquot from the previously described influent sample. The aliquot was placed into a Leighton tube for separation and purification by immunomagnetic separation (IMS). The volume was brought up to 12mL with laboratory DI water. Two effluent samples for *Cryptosporidium* analysis (one per unit). Effluent samples were spun separately in 250 mL centrifuges tubes at 1500xG for 20 minutes. They were then aspirated to 5.0 ml, transferred to individual Leighton tubes and processed through IMS, staining and microscopic examination in accordance with EPA Method 1623, except that at the end of IMS when both dissociations were complete, 50µL of the sample concentration was taken from the first slide and put onto a second slide. This was performed to allow enumeration in the event that a high concentration of oocysts was present in the sample.

## Results

The general water quality of the challenge water, prior to spiking with microorganisms, is shown in Table 2. The results of analyzing the influent and effluent samples and log reduction values are summarized in Tables 3 – 5, below.

**Table 2. Water quality parameters of Challenge Water #2 prior to spiking.**

| Parameter | Measurement | Units |
|---|---|---|
| Temperature | 4±1 | ℃ |
| pH | 9.01 | pH units |
| Turbidity | 50 | NTU |
| Total Chlorine | <0.03 | mg/L |
| Total Dissolved Solids | 1419 | mg/L |

(Note: TOC and alkalinity were not measured for this preliminary trial).

**Table 3. *Raoultella* Results**

| Sample | CFU/mL | Log | Log Reduction |
|---|---|---|---|
| Spike Suspension | 5.00E+9 | NA | NA |
| Backpack Influent | 7.30E+06 | 6.86 | NA |
| Backpack Effluent | <2 | <0.3 | >6.56 |
| Straw Influent | 5.50E+06 | 6.74 | NA |
| Straw Effluent | <2 | <0.3 | >6.43 |

**Table 4. MS2 Results**

| Sample | PFU/mL | Log | Log Reduction |
|---|---|---|---|
| Spike Suspension | 1.30E+8 | NA | NA |
| Backpack Influent | 8.50E+04 | 4.93 | NA |
| Backpack Effluent | <1 | 0 | >4.93 |
| Straw Influent | 6.20E+04 | 4.79 | NA |
| Straw Effluent | <1 | 0 | >4.79 |

**Table 5. *Cryptosporidium* Results**

| Dilution | Oocysts/100mL | Log | Log Reduction |
|---|---|---|---|
| Back-pack Influent | 2.14E+04 | 4.33 | NA |
| Back-pack Effluent | <1 | 0.00 | >4.33 |
| M. Straw Influent | 2.22E+04 | 4.35 | NA |
| M. Straw Effluent | 1 | 0.00 | >4.35 |

## Discussion

The units functioned without difficulty and were operated with a minimal amount of training.

Analytical control samples yielded acceptable results. There were no instances of nonconformance recorded with regard to unit operation, sample collection or sample analysis; no data herein is qualified.

Under the test conditions described above, the performance of both Miracle Straw units exceeded the target log reductions established by the client (as summarized below in Table 6).

No target organisms were detected in the effluent of either test unit.

**Table 6. Target log reduction for each organism type and the log reductions achieved by both Miracle Straw units under the test conditions described herein.**

| Organism | Target Log Reduction | Achieved Log Reduction (both units) |
|---|---|---|
| MS2 coliphage | 4 log | Greater than 4 log |
| *Raoultella terrigena* | 6 log | Greater than 6 log |
| *Cryptosporidium parvum* | 3 log | Greater than 4 log |

***Analytical Services, Inc. (ASI) certifies that the testing was performed as described above and that the results obtained were as reported herein. ASI prohibits reproduction of portions of this report without our express written permission. This report may be reproduced only in its entirety as results presented herein must be interpreted in context with the challenge protocol, test water and the type and concentration of microbiological challenge described in this report, and do not guarantee performance under other test conditions.***