

Independent Laboratory Testing of Hydration Technologies Products

Edward G. Beaudry, D.Sc., P.E.

10-11-06

The products made by HTI use (forward) osmosis to pull water from nearly any source across a semi-permeable membrane to generate a sports drink. The membrane has reverse osmosis rejection properties, which allows the membrane to retain the sugars and salts in the concentrated sports drink syrup, while allowing the water to pass. The membrane has an average pore size of 3-5 Angstroms. This pore size blocks viruses (which are 50-200 times larger), bacteria (over 200 times larger), and parasites (about 10,000 times larger).

Four reports are attached that demonstrate the effectiveness of the products against biological contaminants. They are described below.

Report 9791 from Pacific Analytical Lab, Corvallis, OR

This report consists of nine tests of three X-Pack bags over 16 days of testing (The X-Packs have a maximum recommended lifetime of 10 calendar days.). The first test was started on Monday, April 18, 2005. About 1.7 liters of deionized water were added through the red port of each X-Pack. Then 2.5 ml of a bacterial suspension of Klebsiella and E. coli were added through the red port. Finally, 90 g of HTI Lime Syrup was added through the tube pour-spout. The bags were leaned upright for about 20-23 hours at which time a 100-ml sample and a 1-ml sample were taken of the drink. The challenge water was estimated by placing 1.3 liters deionized water in a container with 2.0 ml of bacterial suspension. A 1- μ l sample of this challenge water was taken an hour or two after starting.

The 100-ml drink sample was analyzed using Colisure with MPN enumeration (Most Probable Number). The analysis for Total Coliforms (Klebsiella plus E. coli) is shown in column 3 of the table on page 2 and at the top of page 3 of the report, and E. coli in column 4. A value of <1 means that no bacteria were detected. The 1-ml drink sample was also analyzed, but first it was diluted to 100 ml using deionized water. The values are reported based on a 100-ml sample, so <100 in columns 5 and 6 means that no bacteria were detected in the 1-ml sample.

The lower table on page 3 of the report shows the bacteria levels that were outside the Hydration Technologies membrane. The values shown are based on a 100-ml sample.

In over two weeks of testing, the X-Pack bags achieved better than 6-log reduction (99.9999+%) of bacteria for all analyses. Bacteria was only detected once in an X-Pack, but it was still at better than 99.9999+% rejection.

Report 11626 from Pacific Analytical Lab, Corvallis, OR

This report consists of ten tests of three X-Pack bags over 16 days of testing. The X-Packs and syrups had been heat-aged by storing at 120°F (49°C) for one month. It was assumed that the heat aging for MREs (meals ready to eat) held, which put one month at 120°F as being equivalent to 55 months at 90°F storage and 100 months at 70°F storage. Procedures

were the same as for Report 9791, except that some samples were taken at 6 hours (marked as pm) and others at 24 hours (marked as am).

In over two weeks of testing, the heat-aged X-Pack bags achieved better than 6-log reduction (99.9999+%) of bacteria for all analyses. Bacteria were not detected in any of the X-Packs.

Anthrax Rejection Testing By Tetracore on a HydroPack

A HydroPack was exposed overnight to a solution containing more than 10^6 colony forming units (CFU) of *Bacillus anthracis Sterne* (anthrax) per ml. Three 250- μ l samples were taken from the drink that was produced overnight. *No anthrax was detected in the drink. In addition, the antigen for anthrax was not detected in the drink.*

Virus Rejection Testing By Cascade Designs

Two HydroPacks, one new X-Pack, one 30-day-old X-Pack (X-Packs have a maximum recommended lifetime of 10 calendar days.), and two HydroWell 24 cartridges were exposed to 3.5×10^4 polioviruses/liter. The HydroPacks and X-Packs were exposed for 24 hours. The HydroWell cartridges were exposed for 2 hours, because they generate 1 liter/h which would require a lot of viruses.

No viruses were detected in the HydroPacks, new X-Pack, and HydroWell 24's. They demonstrated in excess of 4.5-log reduction (99.997+%) of poliovirus. The 30-day-old X-Pack demonstrated 3.1-log reduction (99.92+%) of poliovirus

Pacific Analytical Laboratory, Inc.

529 NW 5th Street
Corvallis, OR 97330

(541) 753-4946

ORELAP ID #OR100009

Water Quality Analysis Report

Ed Beaudry
Hydration Technologies, Inc.
PO Box 1027
Albany, OR 97321

Daytime phone: (541) 917-3335
Fax: (541) 917-3345

Date: May 11, 2005

Report Number: 9791

Sample Information

Sample: Three X Pack bags
Date: April 18, 2005

Method Discussion

Three X Pack bags were analyzed according to the testing plan developed by Hydration Technologies, Inc. (Proposed Testing Plan for Bacteria Rejection of HydroFlow Cartridges). The bags were labeled A, B, and C (050328 Memb., 050415 Prod.) The bags had a bacteria suspension of Escherichia coli (E. coli) and Klebsiella added to the challenge water.

The samples were evaluated for *E. coli* and/or *Klebsiella* using the methods Presence/Absence of coliforms and *E. coli* using Colisure media SM 9223B with MPN enumeration.

Pacific Analytical Laboratory, Inc.

529 NW 5th Street
Corvallis, OR 97330

(541) 753-4946

ORELAP ID #OR100009

Hydration Technologies, Inc.

Date: May 11, 2005

Report Number: 9791

Results

Bacteria concentration in Product Water

Bag ID	Date	MPN* 100 ml		MPN 10 ² dilution	
		Coliform	<i>E. coli</i>	Coliform	<i>E. coli</i>
A	4/19/05 am	<1	<1	<100	<100
B	4/19/05 am	<1	<1	<100	<100
C	4/19/05 am	<1	<1	<100	<100
A	4/20/05 am	<1	<1	<100	<100
B	4/20/05 am	<1	<1	<100	<100
C	4/20/05 am	<1	<1	<100	<100
A	4/21/05 am	<1	<1	<100	<100
B	4/21/05 am	<1	<1	<100	<100
C	4/21/05 am	<1	<1	<100	<100
A	4/22/05 am	<1	<1	<100	<100
B	4/22/05 am	<1	<1	<100	<100
C	4/22/05 am	<1	<1	<100	<100
A	4/26/05 am	<1	<1	<100	<100
B	4/26/05 am	<1	<1	<100	<100
C	4/26/05 am	<1	<1	<100	<100
A	4/27/05 am	<1	<1	<100	<100
B	4/27/05 am	<1	<1	<100	<100
C	4/27/05 am	Present ₍₁₎	NA ₍₁₎	<100	<100
A	4/28/05 am	<1	<1	<100	<100
B	4/28/05 am	<1	<1	<100	<100
C	4/28/05 am	<1	<1	<100	<100
A	4/29/05 am	<1	<1	<100	<100
B	4/29/05 am	<1	<1	<100	<100
C	4/29/05 am	<1	<1	<100	<100

Pacific Analytical Laboratory, Inc.

529 NW 5th Street
Corvallis, OR 97330

(541) 753-4946

ORELAP ID #OR100009

Hydration Technologies, Inc.

Date: May 11, 2005

Report Number: 9791

Bacteria concentration in Product Water

Bag ID	Date	MPN* 100 ml		MPN 10 ² dilution	
		Coliform	<i>E. coli</i>	Coliform	<i>E. coli</i>
A	5/4/05 am	<1	<1	<100	<100
B	5/4/05 am	<1	<1	<100	<100
C	5/4/05 am	<1	<1	<100	<100

* MPN Index – Most Probable Number Index as colony forming units (cfu) per 100 ml

Note – Bold numbers are reportable values.

(1) The seal broke on these MPN samples, therefore they can only be reported as present for Coliform and NA (Not Applicable) or present for *E. coli*.

Bacteria concentration in Challenge Water

Date	MPN	
	Coliform	<i>E. coli</i>
4/18/05 pm	5.2 x 10 ⁸	5.2 x 10 ⁸
4/19/05 pm	1.9 x 10 ⁸	1.9 x 10 ⁸
4/20/05 pm	1.6 x 10 ⁸	1.6 x 10 ⁸
4/21/05 pm	5.9 x 10 ⁷	5.6 x 10 ⁷
4/25/05 pm	1.4 x 10 ⁸	1.2 x 10 ⁸
4/26/05 pm	1.7 x 10 ⁸	1.5 x 10 ⁸
4/27/05 pm	3.4 x 10 ⁸	3.4 x 10 ⁸
4/28/05 pm	4.1 x 10 ⁸	3.9 x 10 ⁸
5/3/05 pm	4.5 x 10 ⁷	4.3 x 10 ⁷

Note – These results are not NELAC certified.

Thank you for your business!

Pacific Analytical Laboratory, Inc.

529 NW 5th Street
Corvallis, OR 97330

(541) 753-4946

ORELAP ID #OR100009

Water Quality Analysis Report

Ed Beaudry
Hydration Technologies, Inc.
PO Box 1027
Albany, OR 97321

Daytime phone: (541) 917-3335
Fax: (541) 917-3345

Date: January 31, 2006

Report Number: 11626

Sample Information

Sample: Three heat aged X Pack bags
Date: 1/10/06

Method Discussion

Three heat aged X Pack bags were analyzed according to the testing plan developed by Hydration Technologies, Inc. (Proposed Testing Plan for Bacteria Rejection of HydroFlow Cartridges). The bags were labeled A, B, and C. The bags had a bacteria suspension of Escherichia coli (E. coli) and Klebsiella added to the challenge water.

The samples were evaluated for *E. coli* and/or *Klebsiella* using the methods Presence/Absence of coliforms and *E. coli* using Colisure media SM 9223B with MPN enumeration.

Pacific Analytical Laboratory, Inc.

529 NW 5th Street
Corvallis, OR 97330

(541) 753-4946

ORELAP ID #OR100009

Hydration Technologies, Inc.

Date: January 31, 2006

Report Number: 11626

Results

Bacteria concentration in Product Water

Bag ID	Date	MPN* 100 ml		MPN 10 ² dilution	
		Coliform	<i>E. coli</i>	Coliform	<i>E. coli</i>
A	1/10/06 pm	<1	<1	<100	<100
B	1/10/06 pm	<1	<1	<100	<100
C	1/10/06 pm	<1	<1	<100	<100
A	1/11/06 pm	<1	<1	<100	<100
B	1/11/06 pm	<1	<1	<100	<100
C	1/11/06 pm	<1	<1	<100	<100
A	1/13/06 am	<1	<1	<100	<100
B	1/13/06 am	<1	<1	<100	<100
C	1/13/06 am	<1	<1	<100	<100
A	1/16/06 pm	<1	<1	<100	<100
B	1/16/06 pm	<1	<1	<100	<100
C	1/16/06 pm	<1	<1	<100	<100
A	1/17/06 pm	<1	<1	<100	<100
B	1/17/06 pm	<1	<1	<100	<100
C	1/17/06 pm	<1	<1	<100	<100
A	1/18/06 pm	<1	<1	<100	<100
B	1/18/06 pm	<1	<1	<100	<100
C	1/18/06 pm	<1	<1	<100	<100
A	1/19/06 pm	<1	<1	<100	<100
B	1/19/06 pm	<1	<1	<100	<100
C	1/19/06 pm	<1	<1	<100	<100
A	1/20/06 am	<1	<1	<100	<100
B	1/20/06 am	<1	<1	<100	<100
C	1/20/06 am	<1	<1	<100	<100

Pacific Analytical Laboratory, Inc.

529 NW 5th Street
Corvallis, OR 97330

(541) 753-4946

ORELAP ID #OR100009

Hydration Technologies, Inc.

Date: January 31, 2006

Report Number: 11626

Bacteria concentration in Product Water

Bag ID	Date	MPN* 100 ml		MPN 10 ² dilution	
		Coliform	<i>E. coli</i>	Coliform	<i>E. coli</i>
A	1/23/06 pm	<1	<1	<100	<100
B	1/23/06 pm	<1	<1	<100	<100
C	1/23/06 pm	<1	<1	<100	<100
A	1/25/06 am	<1	<1	<100	<100
B	1/25/06 am	<1	<1	<100	<100
C	1/25/06 am	<1	<1	<100	<100

* MPN Index – Most Probable Number Index as colony forming units (cfu) per 100 ml

Note – Bold numbers are reportable values.

Bacteria concentration in Challenge Water

Date	MPN	
	Coliform	<i>E. coli</i>
1/10/06 pm	4.4 x 10 ⁸	4.1 x 10 ⁸
1/11/06 pm	3.4 x 10 ⁸	3.4 x 10 ⁸
1/12/06 am	1.9 x 10 ⁸	1.9 x 10 ⁸
1/16/06 pm	1.7 x 10 ⁸	1.7 x 10 ⁸
1/17/06 am	3.1 x 10 ⁷	3.1 x 10 ⁷
1/18/06 am	8.3 x 10 ⁷	8.3 x 10 ⁷
1/19/06 am	6.4 x 10 ⁷	6.4 x 10 ⁷
1/20/06 am	2.2 x 10 ⁸	2.2 x 10 ⁸
1/23/06 pm	5.2 x 10 ⁸	5.2 x 10 ⁸

Pacific Analytical Laboratory, Inc.

529 NW 5th Street
Corvallis, OR 97330

(541) 753-4946

ORELAP ID #OR100009

Hydration Technologies, Inc.

Date: January 31, 2006

Report Number: 11626

1/24/06 pm	2.3×10^8	2.3×10^8
------------	-------------------	-------------------

Note – These results are not NELAC certified.

Thank you for your business!

Filtering Capabilities of the HydroPack for Anthrax Antigen

Procedure

Bacillus anthracis Sterne broth was prepared by streaking a microbank bead of *B. anthracis Sterne* for isolation on a BSA plate. The inoculated plate was incubated in an air incubator over-night at 37°C. Upon cell growth, 250 mL of sterile TSB and one *B. anthracis Sterne* colony was placed into a sterile 1-liter baffled flask. The solution was mixed thoroughly and incubated on a 37°C, orbital shaker at 200 rpm.

After about five hours, an aliquot was removed. A sample was tested in duplicate along with a negative control on the Redline Alert Anthrax kit. Twenty-five microliters of the *B. anthracis Sterne* broth was added to 175 uL of Redline colony isolation buffer. The mixture was vortexed and allowed to sit for two minutes. One hundred and fifty microliters were pipetted into the well of an Anthrax test cartridge. A visual reading and machine reading were recorded at thirteen minutes. If the antigen concentration is not high enough, the SV value remains below 0.17. When this occurs, the broth is allowed to remain in the 37°C orbital shaker. The broth is re-checked every hour in the same manner.

Table 1. 6-17-03/1230 - Anthrax Test strip results.

	Sample 1	Sample 2	Buffer
SV	0.0016	0.0020	0.0017
Reader	NEG	NEG	NEG
Visual	NEG	NEG	NEG

All of the strips returned negative. The broth was placed back in the incubator.

Table 2. 6-17-03/1415 – Anthrax Test strip results.

	Sample 1
SV	0.1294
Reader	POS
Visual	POS

The strip returned an SV less than 0.17; therefore the antigen concentration was too low, and the broth was placed back in the incubator.

Once the broth reached an SV count of 0.17, an aliquot was removed so as to maintain sterile conditions.

Table 3. 6-17-03/1530 – Anthrax Test strip results.

	Sample 1	Sample 2	Buffer
SV	0.2663	0.3907	0.0020
Reader	POS	POS	NEG
Visual	POS	POS	NEG

The strips returned their appropriate results. The SV counts were within range. The broth was ready for dilution and use.

Serial dilutions were then performed according to the following procedure:

Tube Number	Tube Concentration	PBS (mL)	uL of sample from tube
1	1×10^{-1}	4.5	500 uL from stock
2	1×10^{-2}	4.5	500 uL from tube 1
3	1×10^{-3}	4.5	500 uL from tube 2
4	1×10^{-4}	4.5	500 uL from tube 3
5	1×10^{-5}	4.5	500 uL from tube 4
6	1×10^{-6}	4.5	500 uL from tube 5
7	1×10^{-7}	4.5	500 uL from tube 6
8	1×10^{-8}	4.5	500 uL from tube 7
9	1×10^{-9}	4.5	500 uL from tube 8
10	1×10^{-10}	4.5	500 uL from tube 9

Two hundred and fifty microliters of the dilutions ranging from $1:10^6$ through $1:10^{10}$ were spread on Tryptic Soy Agar plates in duplicate and incubated overnight at 37°C .

Table 4. 6-18-03/800 – Colony Counts of B. a. Sterne dilutions.

Concentration	Number of Colonies	Applicable Average
1:10⁶	15	17
	19	
1:10⁷	3	-
	2	
1:10⁸	0	-
	0	
1:10⁹	0	-
	0	
1:10¹⁰	0	-
	0	

The 1x10⁻⁶ concentration plate revealed the best results and was used to calculate the colony forming units per milliliter.

The remaining 245 mL of *B. anthracis Sterne* broth was then added to 10 L of de-ionized water. The “waste water” was mixed and an aliquot taken. The HydroPack was added to the Anthrax infected “waste water” and allowed to soak overnight.

From the aliquot of “waste water”, dilutions were made according to the following procedure:

Tube Number	Tube Concentration	PBS (mL)	ML of sample from tube
1	1x10 ⁻¹	4.5	500 uL from stock
2	1x10 ⁻²	4.5	500 uL from tube 1
3	1x10 ⁻³	4.5	500 uL from tube 2
4	1x10 ⁻⁴	4.5	500 uL from tube 3
5	1x10 ⁻⁵	4.5	500 uL from tube 4

Two hundred and fifty microliters of the original stock sample and the dilutions were spread on TSA plates in duplicate and incubated overnight at 37°C.

The next morning the colonies on all of the TSA plates were counted and then recorded. The number of colony forming units is calculated according to the following equation.

$$\frac{\text{Number of Colonies} * \text{Concentration of Solution}}{\text{Amount of Solution Plated}} = \text{Number of Colony Forming Units per mL}$$

The number of colony forming units in the “waste water” can be calculated in two ways. The first is according to the equation mentioned above. The second is as follows.

$$\frac{\text{Original Broth Concentration} * \text{Amount of Broth Added (mL)}}{\text{Amount of Water Added (mL)}} = \text{Number of Colony Forming Units per mL}$$

Table 5. 6-18-03/800 – Colony Counts of “waste water” and dilutions.

Concentration	Number of Colonies	Applicable Average
1	TNTC	-
	TNTC	
1:10 ¹	TNTC	-
	TNTC	
1:10 ²	TNTC	-
	TNTC	
1:10 ³	334	304
	274	
1:10 ⁴	47	-
	38	
1:10 ⁵	9	-
	1	

“TNTC” means “Too Numerous To Count”. The 1x10⁻³ dilution plate revealed the best results to calculate the number of colony forming units per milliliter.

Table 6. Number of Colony Forming Units.

	CFUs/mL
B. a. Sterne 1:10⁶	6.8 x 10 ⁷
“Waste Water” 1:10³ (From colonies)	1.2 x 10 ⁶
“Waste Water” (Through calculations)	1.6 x 10 ⁶

To discover the filtering performance of the HydroPack, it was opened according to sterile techniques and an aliquot of the sport drink was taken. Two hundred and fifty

microliters of the sport drink were plated in triplicate on TSA plates and allowed to incubate at 37°C overnight. The sports drink was also run in a standard Redline Alert Anthrax kit to detect the presence of Anthrax antigens.

Results

Table 7. Number of Colonies on sports drink TSA plates.

Plate Number	Colonies
1	0
2	0
3	0

This reveals that the filter on the HydroPack succeeded in keeping out the Anthrax.

Table 8. Redline Alert Anthrax Test Results on the sports drink.

	Sample
SV	0.0029
Reader	NEG
Visual	NEG

According to the results, there were not any Anthrax antigens present in the sports drink.

Viral Challenge Results: 13JUN03

Researcher: Lisa Lange

Notebook: MICRO-004

Introduction:

Two FO single use bags, two FO reusable bags and two FO spiral filters were tested with poliovirus to evaluate viral removal.

Test Article:

Article 1: FO single use bag A.

Article 2: FO single use bag B.

Article 3: FO reusable bag New.

Article 4: FO reusable bag Used.

Article 5: FO spiral filter A.

Article 6: FO spiral filter B.

Test Description:

The single use FO bags were tested as follows. Four liters of general (de-chlorinated tap) water were seeded with 40 milliliters of poliovirus 16NOV01. Two single use bags were loaded with one drink powder pouch each. The bags were then placed in a bin containing the stirred challenge water. The tubes of the bag were taped up above the water line to avoid contamination through the green plug which may not be water tight. The bags were left for 24 hours.

The reusable FO bags were loaded with one drink powder pouch each via the green capped tube. Two and a half liters of general (de-chlorinated tap) water were seeded with 25 milliliters of poliovirus 16NOV01. The stirred challenge water was then added via the red bag cap – 1.2 liters to each bag. The bags were then placed in a bin with the tubes of the bag taped up to avoid leakage through the green plug which may not be water tight. The bags were left for 24 hours.

The spiral FO filter systems were set up and tested by Hydration Technologies, Inc. to verify operation. About 500 milliliters of syrup were added to each of the IV bags. Four liters of general (de-chlorinated tap) water were seeded with 40 milliliters of poliovirus 16NOV01. The stirred challenge water was then added to the tube containing the spiral filter – 2 liters to each. The product water tube was placed inside a sample pitcher. The IV drip was started and adjusted to deliver one drip every 6 seconds. The filters were left for about 2 hours.

Viral Test Results:

Test Description	Influent	Effluent	Log Removal
1 (single A)	3.5×10^4	~1000-ml sample. Virions = 0; Wells = 10 (No dilution).	>4.5
2 (single B)	3.5×10^4	~1000-ml sample. Virions = 0; Wells = 10 (No dilution).	>4.5
3 (reuse New)	3.5×10^4	~1000-ml sample. Virions = 0; Wells = 10 (No dilution).	>4.5
4 (reuse Used)	3.5×10^4	~1000-ml sample. Virions = 3.3 Avg; Wells = 1 (No dilution).	3.1
5 (spiral A)	3.5×10^4	~2000-ml sample. Virions = 0; Wells = 10 (No dilution).	>4.5
6 (spiral B)	3.5×10^4	~2000-ml sample. Virions = 0; Wells = 10 (No dilution).	>4.5

Summary:

All except for the used reusable FO bag achieved >4.5 log removal of poliovirus.