C-19

Expert Testimony

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Investigation of FIB and human-specific *Bacteroidales* within Malibu Creek & Lagoon

Summary of UCLA 2009 Study

Investigation of FIB and human-specific *Bacteroidales* within Malibu Creek & Lagoon

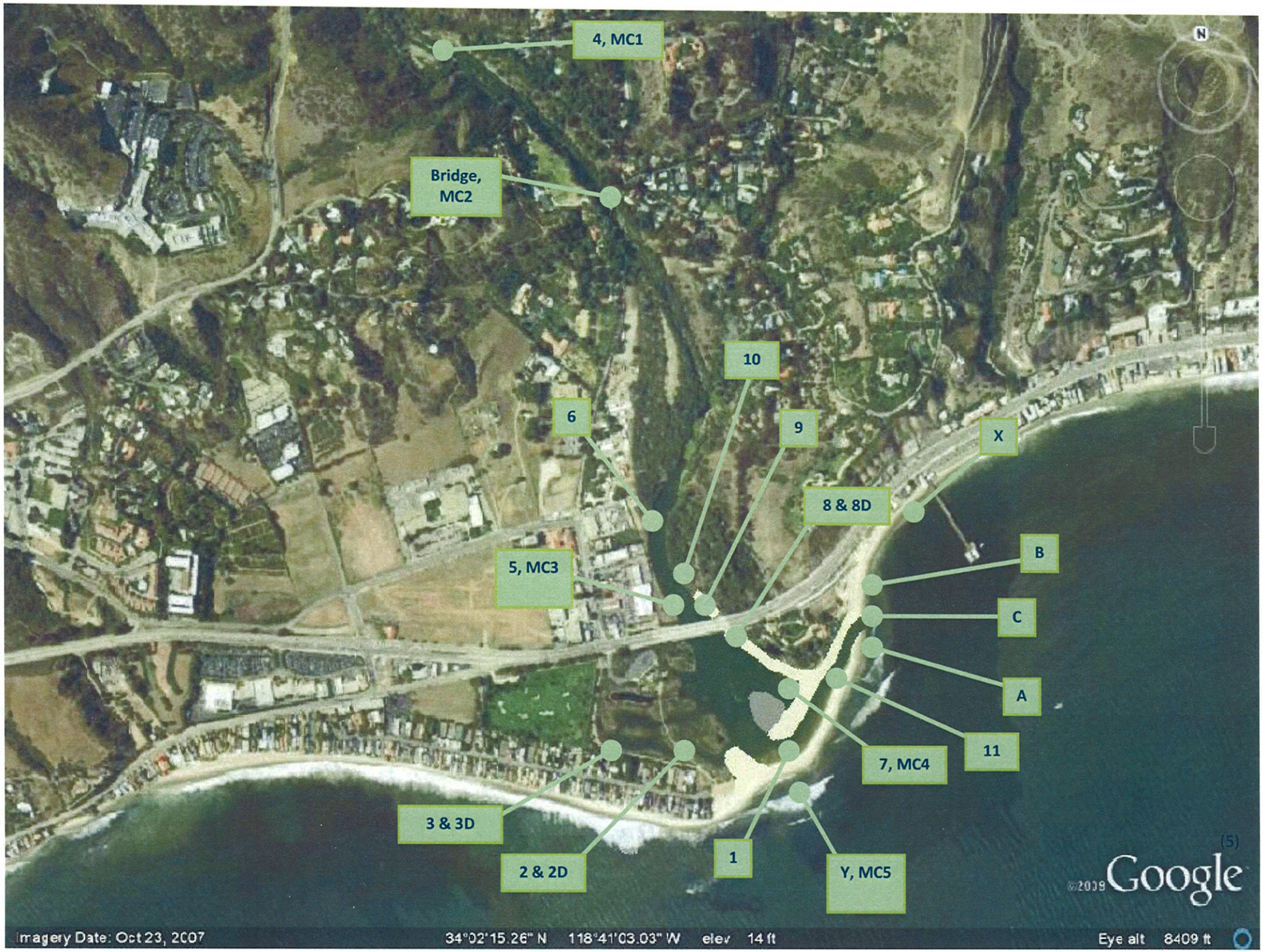
Collaborative study between Dr. Jennifer Jay
and Dr. Richard Ambrose

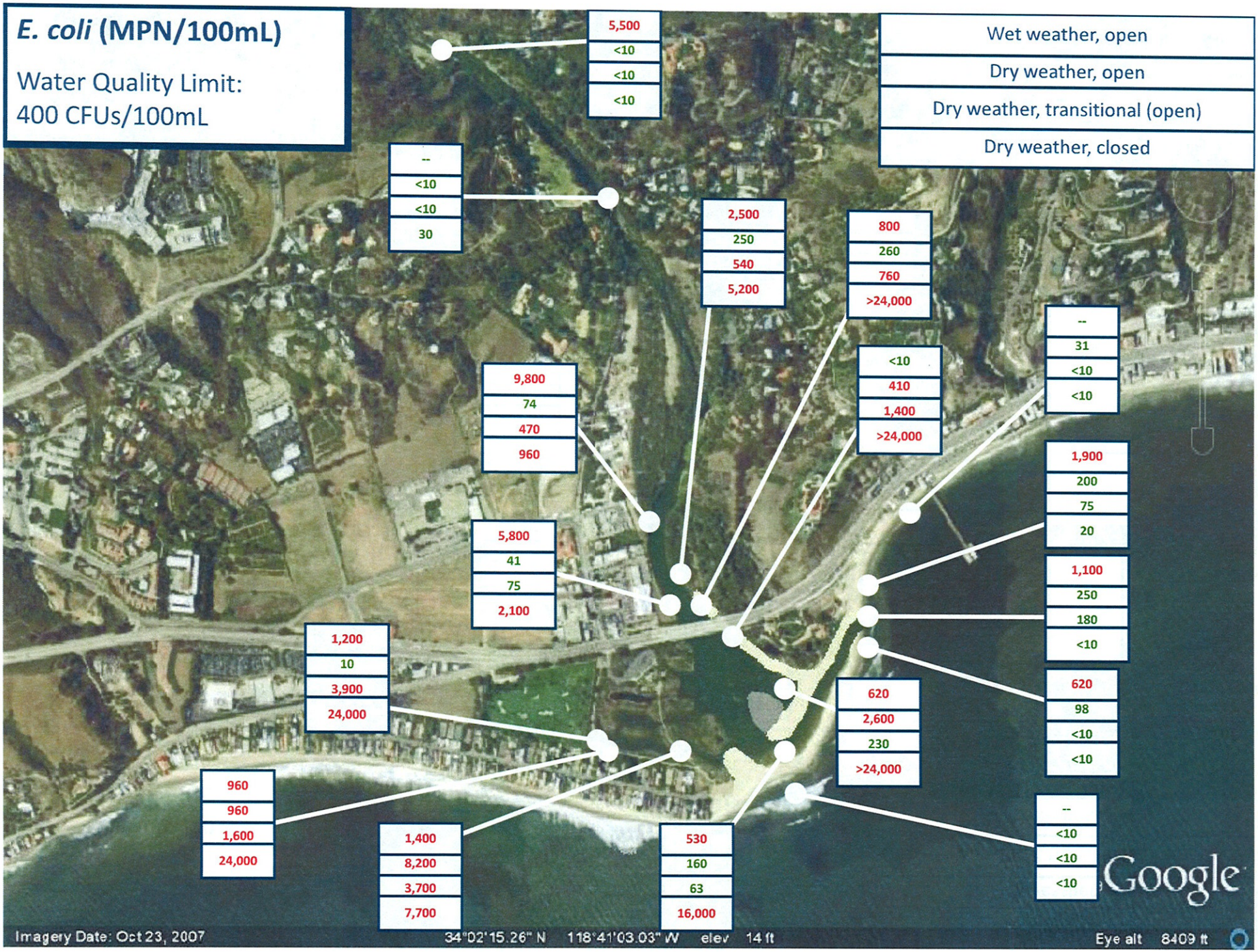
Presentation by Vanessa Thulsiraj, PhD Candidate

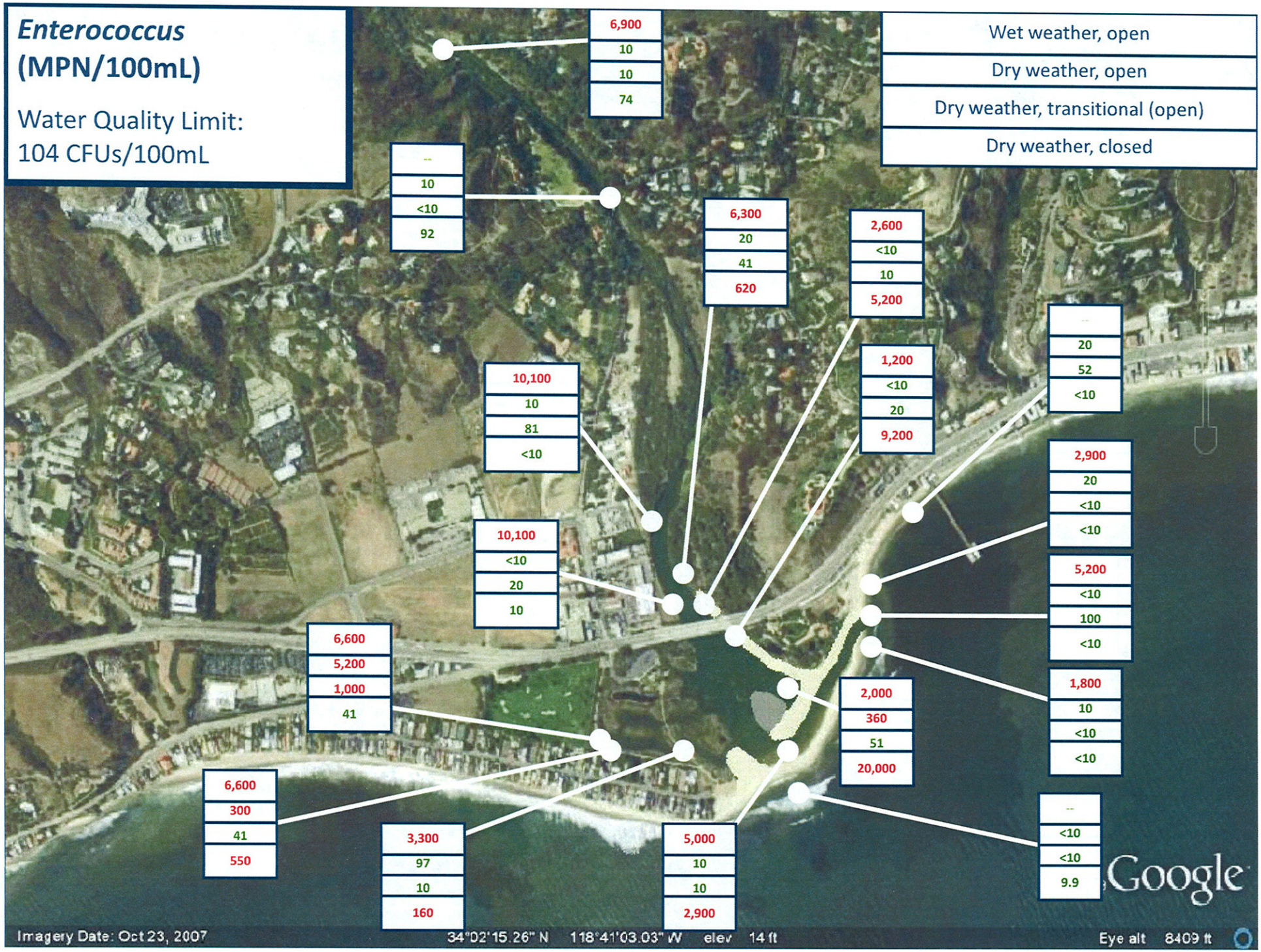
Objectives: Malibu Lagoon

1) Compare FIB concentrations for the following environmental conditions
   a) Wet weather, open lagoon
   b) Dry weather, open lagoon
   c) Dry weather, closed lagoon

2) Compare traditional FIB concentrations to human-specific *Bacteroidales* marker


4, MC1
Bridge, MC2
10
6
9
X
8 & 8D
B
5, MC3
C
A
11
7, MC4
3 & 3D
2 & 2D
1
Y, MC5
N
©2009 Google
Imagery Date: Oct 23, 2007
34°02'15.26" N 118°41'03.03" W elev 14 ft
Eye alt 8409 ft


E. coli (MPN/100mL)
Water Quality Limit:
400 CFUs/100mL
Wet weather, open
Dry weather, open
Dry weather, transitional (open)
Dry weather, closed
5,500
<10
<10
<10
--
<10
<10
30
2,500
250
540
5,200
800
260
760
>24,000
--
31
<10
<10
<10
410
1,400
>24,000
9,800
74
470
960
1,900
200
75
20
5,800
41
75
2,100
1,100
250
180
<10
1,200
10
3,900
24,000
620
98
<10
<10
620
2,600
230
>24,000
960
960
1,600
24,000
1,400
8,200
3,700
7,700
530
160
63
16,000
--
<10
<10
<10
Google
Imagery Date: Oct 23, 2007
34°02'15.26" N 118°41'03.03" W elev 14 ft
Eye alt 8409 ft


Enterococcus (MPN/100mL)
Water Quality Limit: 104 CFUs/100mL
Wet weather, open
Dry weather, open
Dry weather, transitional (open)
Dry weather, closed
6,900 10 10 74
-- 10 <10 92
6,300 20 41 620
2,600 <10 10 5,200
-- 20 52 <10
1,200 <10 20 9,200
10,100 10 81 <10
2,900 20 <10 <10
10,100 <10 20 10
5,200 <10 100 <10
6,600 5,200 1,000 41
1,800 10 <10 <10
2,000 360 51 20,000
6,600 300 41 550
3,300 97 10 160
5,000 10 10 2,900
-- <10 <10 9.9
Google
Imagery Date: Oct 23, 2007
34°02'15.26" N 118°41'03.03" W elev 14 ft
Eye alt 8409 ft

Conclusions: Fecal Indicator Bacteria

- FIB concentrations high throughout stream, lagoon and ocean after rainfall
  - Only time of high FIB upstream and in ocean
- FIB concentrations generally low when lagoon open during wet season
  - Specific hot spot areas
- FIB concentrations high throughout lagoon when lagoon closed

Background

- Griffith et al found detection of *Bacteroidales* to be the most effective method to detect human fecal pollution in various mixtures of fecal sources
  - human-specific *Bacteroidales* in all samples containing human waste, with no false positives
- Members of the order *Bacteroidales* are found exclusively within feces, digestive tract, and other body cavities
- Members of the order *Bacteroidales* from different types of fecal sources exhibit distinct genetic sequences

Methods

- Samples were analyzed for human-specific Bacteroides as in Seurinck et al. (2005)
- A melting point of 76.4°C ± 0.2°C indicated positive, correct amplification of the human-specific Bacteroides
- The presence of environmental interference was assessed by running samples spiked with a 2×10^5 copies / µL dilution of a human-specific Bacteroides plasmid

Methods

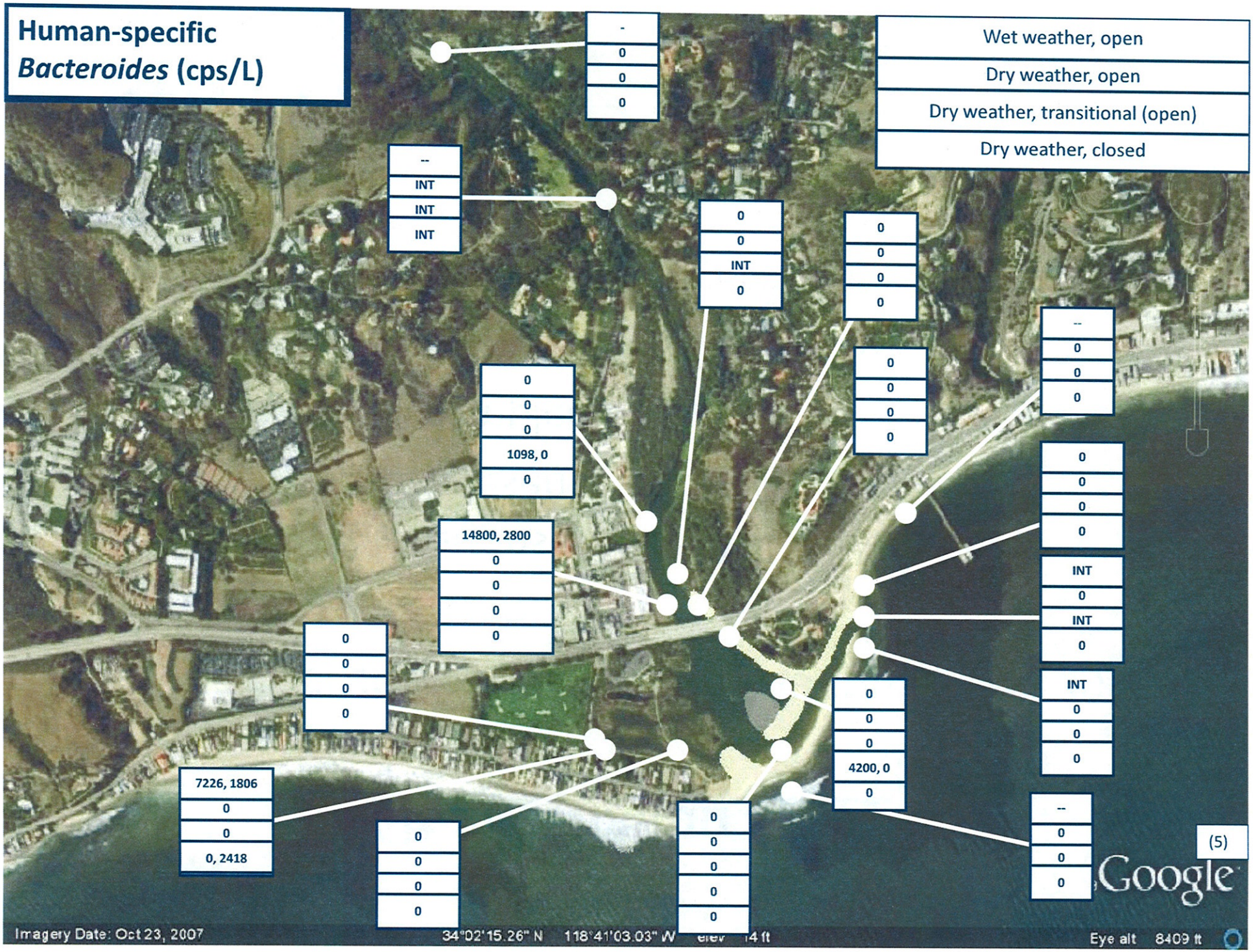
MM = Mastermix
m-H2O = sterile RNAse free H2O
standard/plasmid = we got a Bacteroides plasmid from Dr. Fuhrman's lab. The standard is used both in the SAMPLE+SPIKE and the STANDARD CURVE.

SAMPLE	SAMPLE+SPIKE	STANDARD
1 uL sample 11.18 uL m-h2o 12.82 uL MM	1 uL sample 1 uL standard (plasmid) 10.18 uL m-H2O 12.82 uL MM	1 uL standard (plasmid) 11.18 uL m-H2O 12.82 uL MM

X1	*X1*	Y1	*Y1*	X1+Spike	Y1+Spike	Std-D1	*Std-D1*			
X2	*X2*	Y2	*Y2*	X2+Spike	Y2+Spike	Std-D2	*Std-D2*			
X3	*X3*	Y3	*Y3*	X3+Spike	Y3+Spike	Std-D3	*Std-D3*			
X4	*X4*	Y4	*Y4*	X4+Spike	Y4+Spike	Std-D4	*Std-D4*			
X5	*X5*	Y5	*Y5*	X5+Spike	Y5+Spike	Std-D5	*Std-D5*			
X6	*X6*	Y6	*Y6*	X6+Spike	Y6+Spike	Std-D6	*Std-D6*			
X7	*X7*	Y7	*Y7*	X7+Spike	Y7+Spike	Std-D7	*Std-D7*			
X8	*X8*	Y8	*Y8*	X8+Spike	Y8+Spike	Std-D8	*Std-D8*		Neg control	Neg control
1	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**	**12**
Samples & dups (16 tot)				Spikes, no dups		Std curve + dups			negative controls	

Actual Plate is the dark border with dashed gridlines.

Sample Calculation: $\frac{\text{Human cps/ul}}{\text{ul extract used}} \times \frac{\text{50ul total}}{\text{L filtered}}$


Human-specific
Bacteroides (cps/L)
Wet weather, open
Dry weather, open
Dry weather, transitional (open)
Dry weather, closed
-
0
0
0
--
INT
INT
INT
0
0
INT
0
0
0
0
0
0
0
0
--
0
0
0
0
0
0
0
1098, 0
0
14800, 2800
0
0
0
0
0
0
0
0
INT
0
INT
0
INT
0
0
0
0
0
4200, 0
0
7226, 1806
0
0
0, 2418
0
0
0
0
0
0
0
0
--
0
0
0
(5)
Google
Imagery Date: Oct 23, 2007
34°02'15.26" N 118°41'03.03" W
Eye alt 8409 ft

City of Malibu Study

- 2 week study conducted in Malibu Creek and Lagoon
  - 5 sample locations (MC1 – MC5) were further analyzed for presence of HBM
  - MC1 – MC4 sampled on 4/29
  - MC5 samples on 4/29, 4/30, 5/5, 5/7
  - No detection of HBM in any samples taken during this time (April/May)

Summary

- HBM not detected in dry weather open lagoon (39)
- 5 samples were positive for HBM (7%)
- Human-specific *Bacteroides* marker was detected in dry and wet weather samples in Malibu Lagoon
  - HBM detected in 15% (2/13) wet weather open lagoon samples
    - Sites 3, 5
  - HBM detected in 14% (3/22) dry weather closed lagoon samples
    - Sites 3, 6, 7

Summary

- 74 total samples analyzed for HBM
  - 69 samples did not contain HBM (93%)
- Human-specific *Bacteroides* marker was not detected in any ocean water samples
- Exceedance of FIB water quality health standards occurred at sites where no HBM was detected
- No correlation between FIB and HBM

February 16, 2009

Date	Sample	Cps/uL extract	Duplicate Cps/uL extract	Human cps/L	Duplicate Human cps/L	Average Human cps/L
2/16/09	A	-	-	-	-	-
2/16/09	B	0.0	0.0	0.0	0.0	0.0
2/16/09	C	-	-	-	-	-
2/16/09	1	0.0	0.0	0.0	0.0	0.0
2/16/09	2	0.0	0.0	0.0	0.0	0.0
2/16/09	2D	0.0	0.0	0.0	0.0	0.0
2/16/09	3	5.6	1.4	7226	1806	4516
2/16/09	3D	0.0	0.0	0.0	0.0	0.0
2/16/09	5	7.4	1.4	14800	2800	8800
2/16/09	6	0.0	0.0	0.0	0.0	0.0
2/16/09	7	0.0	0.0	0.0	0.0	0.0
2/16/09	8	0.0	0.0	0.0	0.0	0.0
2/16/09	8D	0.0	0.0	0.0	0.0	0.0
2/16/09	9	0.0	0.0	0.0	0.0	0.0
2/16/09	10	0.0	0.0	0.0	0.0	0.0

March 20, 2009

Date	Sample	Cps/uL extract	Duplicate Cps/uL extract	Human cps/L	Duplicate Human cps/L	Average Human cps/L
3/20/09	A	0.0	0.0	0.0	0.0	0.0
3/20/09	B	0.0	0.0	0.0	0.0	0.0
3/20/09	C	0.0	0.0	0.0	0.0	0.0
3/20/09	X	0.0	0.0	0.0	0.0	0.0
3/20/09	Y	0.0	0.0	0.0	0.0	0.0
3/20/09	Bridge	-	-	-	-	-
3/20/09	1	0.0	0.0	0.0	0.0	0.0
3/20/09	2	0.0	0.0	0.0	0.0	0.0
3/20/09	3	0.0	0.0	0.0	0.0	0.0
3/20/09	3D	0.0	0.0	0.0	0.0	0.0
3/20/09	4	0.0	0.0	0.0	0.0	0.0
3/20/09	5	0.0	0.0	0.0	0.0	0.0
3/20/09	6	0.0	0.0	0.0	0.0	0.0
3/20/09	7	0.0	0.0	0.0	0.0	0.0
3/20/09	8	0.0	0.0	0.0	0.0	0.0
3/20/09	8D	0.0	0.0	0.0	0.0	0.0
3/20/09	9	0.0	0.0	0.0	0.0	0.0
3/20/09	10	0.0	0.0	0.0	0.0	0.0

May 21, 2009

Date	Sample	Cps/uL extract	Duplicate Cps/uL extract	Human cps/L	Duplicate Human cps/L	Average Human cps/L
5/21/09	A	0.0	0.0	0.0	0.0	0.0
5/21/09	B	0.0	0.0	0.0	0.0	0.0
5/21/09	C	-	-	-	-	-
5/21/09	X	0.0	0.0	0.0	0.0	0.0
5/21/09	Y	0.0	0.0	0.0	0.0	0.0
5/21/09	Bridge	-	-	-	-	-
5/21/09	1	0.0	0.0	0.0	0.0	0.0
5/21/09	2	0.0	0.0	0.0	0.0	0.0
5/21/09	3	0.0	0.0	0.0	0.0	0.0
5/21/09	3D	0.0	0.0	0.0	0.0	0.0
5/21/09	4	0.0	0.0	0.0	0.0	0.0
5/21/09	5	0.0	0.0	0.0	0.0	0.0
5/21/09	6	0.0	0.0	0.0	0.0	0.0
5/21/09	7	0.0	0.0	0.0	0.0	0.0
5/21/09	8	0.0	0.0	0.0	0.0	0.0
5/21/09	9	0.0	0.0	0.0	0.0	0.0
5/21/09	10	-	-	-	-	-
5/21/09	11	0.0	0.0	0.0	0.0	0.0

July 18, 2009

Date	Sample	Cps/uL extract	Duplicate Cps/uL extract	Human cps/L	Duplicate Human cps/L	Average Human cps/L
7/18/09	A	0.0	0.0	0.0	0.0	0.0
7/18/09	B	0.0	0.0	0.0	0.0	0.0
7/18/09	C	0.0	0.0	0.0	0.0	0.0
7/18/09	X	0.0	0.0	0.0	0.0	0.0
7/18/09	Y	0.0	0.0	0.0	0.0	0.0
7/18/09	Bridge	-	-	-	-	-
7/18/09	1	0.0	0.0	0.0	0.0	0.0
7/18/09	2	0.0	0.0	0.0	0.0	0.0
7/18/09	2D	0.0	0.0	0.0	0.0	0.0
7/18/09	3	0.0	1.3	0.0	2418	1209
7/18/09	3D	0.0	0.0	0.0	0.0	0.0
7/18/09	4	0.0	0.0	0.0	0.0	0.0
7/18/09	5	0.0	0.0	0.0	0.0	0.0
7/18/09	6	1.4	0.0	1098	0.0	549
7/18/09	7	1.1	0.0	4200	0.0	2100
7/18/09	8	0.0	0.0	0.0	0.0	0.0
7/18/09	9	0.0	0.0	0.0	0.0	0.0
7/18/09	10	0.0	0.0	0.0	0.0	0.0
7/18/09	11	0.0	0.0	0.0	0.0	0.0