Appendix C

Fecal Coliform Variability Test

June 11, 2003

Memo to: Bacteria sampling files

From: Rick Haley

Subject: Fecal coliform variability test, 5/6/03

On May 6 we conducted a test to obtain more information about fecal coliform variability and to test sampling methods to address that variability. This test was brought about by our own observations that coliform appeared to be extremely variable, both between samples and in duplicates, and by suggestions from outside county government that indicated a need for addressing this variability.

Background

Analysis of our existing fecal coliform data revealed that levels were quite variable from site to site on a given day, and from date to date at a given site. Further inspection revealed that our duplicates (samples taken sequentially at the same time and place, within 15 seconds of each other) were also occasionally quite divergent. We designed an experiment as a first test of short-term temporal variability and lab method variability.

Procedures and results are described beginning on the next page.

Procedure

We conducted our sampling as follows:

1. Prelabel four sample containers with the site number followed by letter a, b, c, d, where:

   a = normal 150-ml grab sample taken at same time as we began to fill 1-L bottle for samples c and d

   b = normal grab sample taken at end of filling of bottle for c and d

   c = one of two 150-ml subsamples taken from a 1-L larger sample

   d = the second of two subsamples samples

2. Obtain samples as follows (two-person team):

   a. Rinse large sample container (1-L) in stream and discard rinse water

   b. Simultaneously and in the same location, begin to fill both the "a" grab sample and the large sample container.

   c. Cap the "a" container and quickly prepare the "b" container while the large sample container continues to fill

   d. Fill the "b" container as the large container is finished filling

   e. Cap the "b" container and homogenize the contents of the large sample container by shaking

   f. Fill the "c" and "d" containers with homogenized sample from the large container

In practice this was accomplished with two people: The first used the sampling wand to obtain samples a and b, while the second obtained the large 1-L sample by wading next to the sampling area. Care was taken to let any disturbance caused by wading drift downstream before sampling occurred, and the wading sampler always stood downstream of the sample area.

Field Sampling

The procedure delineated above was used at the following locations on May 6, 2003:

Baseline Site 12: Nookachamps Cr. at Swan Rd.
19: Hansen Cr. at Hoehn Rd.
21: Coal Cr. at Hoehn Rd.
23: Wiseman Cr. at Minkler Rd.
27: Morgan Cr. at Walberg Rd.

Results

Table 1. Results from fecal coliform variability test conducted at Skagit County Baseline Study sites, May 6, 2003

	Sample results				Calcs - a+b		Calcs - c+d	
Site	**a**	**b**	**c**	**d**	**Mean**	**CV***	**Mean**	**CV***
12	50	80	130	50	65	0.33	90	0.63
19	50	80	13	50	65	0.33	32	0.83
21	80	50	130	50	65	0.33	90	0.63
23	13	8	4	4	11	0.34	4	0.00
27	220	130	240	170	175	0.36	205	0.24
Mean						0.34		0.47

Notes:
Samples a and b were normal grab samples, c and d from homogenized 1-L sample
*CV=Coefficient of variability, which is the standard deviation divided by the mean to give a relative assessment of the degree of variability

The results indicate that for this sample, variability (as indicated by the coefficient of variation) was slightly less between the two "normal" samples taken sequentially (a and b) than for the two samples taken from the homogenized 1-L sample (c and d). This suggests that much of the variability lies in the laboratory and subsampling method. This is surprising in that one would expect the a and b samples to have both temporal variability and lab method variability, while the c and d samples shouldn't have the temporal variability because they were homogenized. However, this experiment did not reveal any of the cases of extreme variability between duplicates that we occasionally see in our regular samples.

Should we decide that further investigation was warranted, here are some options we could pursue:

1. Repeat the experiment without changes in hopes of uncovering one of those instances of wide variability between the two normal duplicates (a and b). The chances of catching one of those might be increased by sampling after rainfall when elevated and more variable fecals might be expected. This method would produce results directly

comparable with the previous experiment and with the duplicates we have taken in the past.

2. Repeat the experiment with a bigger bottle for obtaining the homogenized samples. This would increase the chance of encountering temporal variability because the bigger bottle would take longer to fill, thus increasing the time between taking the a and b samples. It would also increase the amount of sample to be homogenized, which may or may not have an effect on that part of the experiment. You could also increase the temporal variability by simply waiting longer between samples. In this case we would take a 1-L sample, homogenize, and divide it between an "as is" sample and some to be composited with the later sample. After a given time period (5 minutes?), take another 1-L sample, homogenize, take another "as is" sample and add the remainder to the remainder from the first sample. Neither of these methods would produce a and b samples that are comparable to our past duplicates because of the extra time between sampling and the homogenization.

3. Try an experiment that focuses more narrowly on quantifying the lab variability. This could be accomplished by taking a large sample, homogenizing, and taking more than two subsamples – perhaps four or five. This method doesn't quantify temporal variability.

A combination of 1 and 3 or 2 and 3 could yield an indication of temporal, subsampling, and lab variability.