APPENDIX C

Summary of Method 1623 Recovery Analysis

As discussed in Appendix B, problems associated with the Information Collection Rule immunofluorescent assay (ICR IFA) methodology were not uncommon and—as shown by California Department of Water Resources (DWR) experiments—limited the usefulness of the method. Prior to the introduction of the ICR methodology, the US Environmental Protection Agency (EPA) began developing an improved method for detecting protozoan for a special study it planned to conduct (Pontius and Clancy 1999). This study, the Supplemental Survey, was designed to collect protozoan occurrence data from some participating ICR utilities as well as medium- and small-size systems that did not participate in the ICR.

In 1998 and 1999, the EPA introduced Method 1622 (for *Cryptosporidium*) and Method 1623 (for *Cryptosporidium* and *Giardia*) (EPA 1999 and 1999a, respectively). The method represented a significant improvement over the ICR IFA methodology. In the QA/QC of Method 1622 performance, mean recovery of *Cryptosporidium* in spiked reagent water was 35% with a relative standard deviation of 30% (Allen and others 2000).

DWR undertook a Coordinated Pathogen Monitoring Program (CPMP) in 1996 to determine the relative sources of pathogens within the State Water Project (SWP). Unfortunately, at the time of the study, only ICR methodology was available. Based on the problems with the ICR IFA method, the results were unable to answer this question (see Appendix B). DWR was asked to use Method 1623 when it was released in April 1999 to determine the relative sources of pathogens within the SWP.

Prior to again evaluating Delta and SWP waters for *Cryptosporidium* and *Giardia*, it was important to understand Method 1623 capabilities under ambient Delta and SWP conditions. Depending on the site and season, turbidities at key locations in the SWP and the Delta can range from 5 to 200 nephelometric turbidity units (NTUs). Highest turbidities are usually associated with winter runoff when the highest levels of pathogens in surface water would be expected. Although the method is used at utilities across the United States, very little published literature exists on the full method's recoveries in ambient waters with turbidities above 20 NTUs. The EPA validation study tested recovery in turbidities as high as 13.8 NTUs (EPA 1999b), while Clancy and others (1999) reported using turbidities as high as 19.5 NTUs.

In addition to recoveries, the level of variability is also important. Low variability among replicates allows detection of significant differences in pathogen concentrations between sites. Another statistical consideration is the method's performance in different matrices. The EPA's wide acceptance criteria for matrix spikes suggest that the method's recoveries may vary with the matrix (Table C-1). If this is the case, then standard hypothesis testing methods (for example, ANOVA or Kruskal-Wallis) cannot be used to compare site differences. Both statistical approaches assume the method will not perform differently under different environmental circumstances.

Table C-1 Method 1623 Acceptance Criteria for Ongoing Precision and Recovery of Matrix Spike

	Cryptoporidium	*Giardia*
Mean Recovery (as percent)	13 - 111	15 - 118
Precision (as max relative percent difference)	61	30

Adapted from EPA 1999b

The objectives of DWR's Method 1623 study were threefold. The 1st objective was to test the relative capacity of the standard and High Volume Gelman Envirochek™ filters. The standard Gelman filter is commonly used in Method 1623 filtrations. Gelman developed the High Volume filter to filter hundreds of liters of finished water without compromising pathogen recovery. If this filter could be used in ambient waters, then the chances would increase that the full 10 liters could be sampled with turbid storm water samples. A 2nd objective was to determine if it was possible to recover *Cryptosporidium* and *Giardia* from waters collected at key points in the system. Although it was recognized that background water matrices might change with the season, samples were collected once at 4 sites to determine if recoveries were possible under a wide range of water matrices and conditions. Sample sites were the canal into Bethany Reservoir, immediately downstream of the Banks Pumping Plant, the Sacramento River at Hood, the Barker Slough Pumping Plant, and the San Joaquin River near Vernalis. Replicate samples of each water were spiked with 100 organisms/10 L, filtered, and then sent to the laboratory for Method 1623 analysis. The final objective was to compare recovery and variability of spiked samples analyzed by 2 different laboratories.

Comparisons of filtration capacity of the 2 filters found that when the turbidities were high, the High Volume filter could filter about twice the volume of the standard filter; however, neither filter could filter the full 10 liters.

There did not appear to be appreciable differences between recovery and variability of the 2 filter types. In 1 trial *Cryptosporidium* recovery efficiencies were higher with the High Volume filter, while there were no significant differences between *Giardia* recoveries. In a 2nd trial, *Cryptosporidium* recoveries between the 2 filters were not significantly different. Unless noted, all remaining experiments were conducted with High Volume filters.

For both organisms, the lowest recoveries were found in the highest turbidity water (Figure C-1). Recoveries for *Cryptosporidium* ranged from 36% to 75%. Between 11 and 47 NTUs, *Cryptosporidium* recoveries were at or above 50%. Above 47 NTUs, recoveries fell below 50%. Recoveries for *Giardia* ranged from 0.47% to 53%. Unlike *Cryptosporidium* there was a sharp drop in recovery at 47 NTUs in Barker Slough waters. For *Cryptosporidium* the highest variability was associated with spiked samples of Barker Slough water. For *Giardia* the highest variability was associated with spiked samples of water collected immediately above Bethany Reservoir.

Figure C-1 *Cryptosporidium* and *Giardia* Percent Recovery (± std. dev.) vs. Turbidity

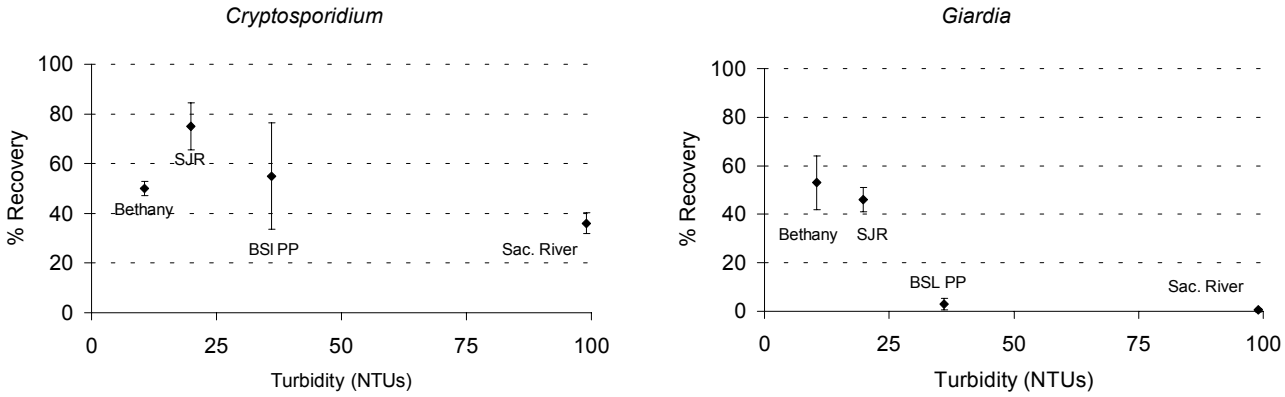

Table C-2 *Cryptosporidium* Average Percent Recovery and Coefficient of Variation by Site

	Turbidity (NTUs)	Average Recovery (%)	Coefficient of Variation (%)
Bethany Reservoir	11	51	6
San Joaquin near Vernalis	20	75	13
Barker Slough Pumping Plant	36	55	38
Sacramento River @ Greenes Landing	99	36	12

Results suggested that changes in *Cryptosporidium* recovery and variability by site were not necessarily influenced by turbidity but from the unique matrix of the water. Table C-2 shows the turbidity, average percent recovery, and coefficient of variation of the sites sampled. Based on coefficient of variations, variation at 99 NTUs was identical to variation at 20 NTUs; however, variation at 36 NTUs was 3 to 6 times higher than at any other turbidity. This suggests a variable other than turbidity is affecting method performance.

Matrix water detection limits were also calculated for each water. Using spiking doses of approximately 100 organisms/10 liters, matrix water detection limits (MWDLs) were calculated at the 99% confidence limit using standard method detection limits (MDLs) equations and an *n* of 3 (40 CFR). Since MDLs are normally calculated in reagent water and do not take into account recoveries, the final MWDL was determined by dividing the MDL value by its percent recovery. Additionally, MDLs are usually calculated with 7 replicates; therefore, a *t* value of 6.965 was used instead of 3.14. Final MWDLs are given in Table C-3. Although an estimate, the process provided a rough idea of the minimum number of organisms under the conditions of this study that a researcher could expect to observe 99% of the time.

One problem with calculating microbial detection limits is that the organisms occur in discrete particles and are not randomly distributed throughout the water column like a chemical contaminant. The EPA has recently begun testing a new method to determine *Cryptosporidium* detection limits in reagent water with Method 1623 (Connell pers. comm.).

Table C-3 Calculated MWDL Based on Initial Spike Dose of Approximately 10 Organisms/L (n = 3)

Cryptosporidium					
Site	sd	t value	MDL (org/L)	Recovery	Recovery Adjusted MWDL (org/L)
Bethany	0.30	6.965	2.1	0.50	4.2
Sac. River	0.40		2.8	0.36	7.9
SJR	0.93		6.5	0.75	8.7
BSI PP	2.08		14.5	0.55	26.3
Giardia					
Site	sd	t value	MDL (org/L)	Recovery	Recovery Adjusted MWDL (org/L)
Bethany	1.10	6.965	7.7	0.53	14.5
Sac. River	0.08		0.58	0.005	122.3
SJR	0.50		3.5	0.46	7.7
BSI PP	0.23		1.6	0.03	61.8

Table C-4 Average Percent Recovery (org/L) ± 1sd by 2 Independent Laboratories

Sample Water	Organism	Lab A	Lab B
San Joaquin River near Vernalis	*Cryptosporidium*	59 ± 9.8	75 ± 9.6
	Giardia	37 ± 3.5	46 ± 5.0[a]

[a] Significantly different, arc sin transform, p = 0.03.

Recovery results between 2 different laboratories were inconclusive (Table C-4). Significant differences in recovery varied by the organism. No significant differences were found between *Cryptosporidium* recoveries, but significant differences were found between *Giardia* recoveries. The experiment was repeated using Barker Slough waters. Unfortunately, the spiking solutions were unknowingly contaminated by the supplier with *G. muris*; therefore, recovery results were not valid. Because of time and financial constraints, no additional comparisons were made.

In conclusion, DWR's Method 1623 study determined that recoveries in ambient water using the Gelman High Volume filter were similar to those using the Gelman standard filter. While neither could filter the full 10 liters in highly turbid water, the High Volume filter could filter twice as much volume as the standard filter. This suggests that the High Volume filter should be used for field sampling.

As verified by EPA studies, Method 1623 recoveries for both organisms were markedly higher than the ICR IFA method. For *Cryptosporidium*, recoveries fell below 50% at 47 NTUs; however, recoveries were still at 36% at 99 NTUs. At lower turbidities, *Giardia* recoveries were also above 50%; however, unlike *Cryptosporidium*, recoveries fell dramatically at 47 NTUs. Average recoveries and coefficient of variation results also suggested that matrix affects could be occurring in samples collected from the San Joaquin River near Vernalis. The variability associated with samples collected from Barker Slough suggested that a large number of samples would be required to accurately assess *Cryptosporidium* concentrations.

While Method 1623 recoveries were higher than the ICR IFA method, the detection limits of the method may still be above ambient concentrations of organisms. Therefore, like the ICR IFA method, there is still a likelihood of false negatives. Also, if detection limits do vary with the matrix, it may be unwise to use a single detection limit across all matrices. Experiments did not determine whether Method 1623 reacts the same in different turbidities or matrices. In order to examine this question, spiked samples of different waters at identical turbidities would have to have been analyzed; however, the EPA's performance criteria for Method 1623 suggest that the method may be influenced by unknown factors in different ambient waters. If this is the case, then both parametric and nonparametric methods of analyses would not be valid. Both families of statistics assume that Method 1623 functions identically in all types of waters. Similarly, if recoveries vary by the matrix, it might be unwise to use 1 recovery rate for all water types.

The study also concluded that given these potential problems, the method might be useful as an indicator of pathogen contamination based on frequency of occurrence. The problems associated with false negatives were still a weakness of the method and could compromise frequency of occurrence data by suggesting that no organisms were present when this was not the case. Based on the patchy distribution of the organisms, any field study would require careful design and a strong QA/QC component. The highest likelihood of success might come from a focused study on a small watershed that had a direct response to local rainfall and that had the potential for high pathogen runoff.

References

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PERSONAL COMMUNICATIONS FOR APPENDIX C

Connell, Kevin, Senior Environmental Scientist, DynCorp IET. 2001. Personal Communication by phone, Jan 18.