APPENDIX B

Summary of Pathogen Occurrence in the SWP and QA/QC Work Using the EPA's Information Collection Rule Immunofluorescent Assay (ICR IFA)

The US Environmental Protection Agency (EPA) established the Surface Water Treatment Rule (SWTR) of 1989. The rule set the goals of microbial integrity and focused specifically on reducing risks from *Giardia* cysts and viruses in surface water and groundwater under the direct influence of surface water. One shortcoming of the SWTR was that it did not specifically control for *Cryptosporidium*. A 1993 waterborne disease outbreak from this protozoan in Milwaukee caused 400,000 people to experience intestinal illness, hospitalized more than 4,000 people, and caused at least 50 deaths. The incident demonstrated that drinking water supplies can be vulnerable to this waterborne disease (EPA 1999). The Safe Drinking Water Act Amendments (SDWA) of 1996 mandated that the EPA develop interrelated regulations to control microbial pathogens and disinfectant/disinfection byproducts (D/DBP) in drinking water (EPA 1999a).

One regulation stemming from the amendments to the SDWA was the Information Collection Rule (ICR). One purpose of the rule was to collect occurrence and treatment information to help evaluate the need for possible changes to the SWTR and microbial treatment practices. Among other requirements, the ICR compelled EPA to collect research data on disease-causing microbes (including *Cryptosporidium*) in drinking water sources and indicators of fecal contamination.

The ICR method for pathogens was proposed in February 1994 but was not promulgated until May 1996. One reason for the delay was the scientific issues surrounding the immunofluorescent assay (IFA) method used to quantify *Cryptosporidium* and *Giardia* (oo)cysts (Pontius and Clancy 1999). The first collaborative study of the IFA method was conducted in 1993. Results from 16 laboratories found that recoveries were low and false positives and negatives were common (Clancy and others 1994). Following this study and prior to the rules promulgation, modifications were made in an attempt to improve the method's performance. Two studies examining the modifications found that variability remained high, false positives and negatives were still common, and both inter- and intralaboratory variability was high among the laboratories (Pontius and Clancy 1999). By 1996, promulgation of the ICR had been delayed several times, and the EPA faced issuing the ICR without *Cryptosporidium* testing. The EPA determined that meaningful national data on *Cryptosporidium* occurrence and regulatory decisions could be reached if laboratories achieved on average more than 8% recovery for protozoan cysts. Pathogen monitoring under ICR began July 1996 and ended in December 1998.

The California Department of Water Resources (DWR) used the ICR IFA method to conduct a Coordinated Pathogen Monitoring Program (CPMP) to assess the potential human health threat by microbial contaminants, including *Cryptosporidium*, *Giardia*, and *Clostridium perfringens* in State Water Project (SWP) waters. Total and fecal coliform as well as *E. coli* were also analyzed. For the CPMP, Kern County Water Agency, DWR, and Metropolitan Water District of Southern California (MWDSC) collected samples monthly from October 1996 through April 1998. In addition to monthly samples, storm event samples were collected during the 1st major storm of the wet season and during 2 additional major storm events. Storms that were sampled were based on a number of conditions, but a general guideline of 1 inch of rain in a 24-hour period was used as a trigger to assess a storm event for monitoring. Four flood-related locations were added to the 12-storm event monitoring locations as a result of the January 1997 floods.

Pathogen samples were collected from the SWP's source waters (the Sacramento and San Joaquin rivers) as well as the California Aqueduct. Samples were collected at locations spanning a distance of approximately 600 miles from the northernmost sampling site at Alamar on the Sacramento River to Lake Perris, the terminal reservoir on the East Branch of the California Aqueduct. DWR's Division of Planning and Local Assistance (DPLA) collected source water samples, the Department's Division of Operations and Maintenance (O&M) collected samples within the SWP. Additionally, Kern County Water Agency assisted with sampling at the Check 29 sampling site. The MWDSC collected samples from Castaic and Silverwood lakes. All sampling sites are shown in Table B-1. Based on the first 12 months of data, the number of sampling stations for both storm event and monthly sampling were reduced. Samples collected during the final 6 months of the study focused on locations having the greatest detection frequency. These included the Sacramento River, the San Joaquin River, and Delta sampling locations.

DWR also examined the recovery efficiencies of the ICR method in Delta source waters and SWP waters. In 1 set of experiments, sample water was spiked with certified concentrations of *Cryptosporidium* and *Giardia*. *Cryptosporidium* oocysts were spiked at 4,480 oocysts/200 L (2,240 oocysts/100 L). *Giardia* cysts were spiked at 3,656 cysts/200 L (1,828/100 L). The spiked sample water was then simultaneously filtered into 2 split samples. Waters used for these recovery experiments were collected from 4 sample sites in the CPMP as well as the American River. Sampling sites for the matrix study are also shown in Table B-1. Equipment blanks were also analyzed to examine any loss due to the equipment itself. Laboratory performance was evaluated by directly spiking filters with a known amount of *Cryptosporidium* and *Giardia*, placing the spiked filters in containers of known turbidity matrix water, and sending the filters to 2 independent laboratories for analysis. This design bypassed any loss of (oo)cysts because of filtration and allowed the evaluation of the method itself under different turbidity matrix waters.

Table B-1 Monthly, Storm, and Flood Event Sample Sites for the Coordinated Pathogen Monitoring Program Project

Sample Site	Monthly Sample	Storm Sample	Flood Sample	QA/QC Sample	Sampler[a]
American River @ Fairbairn WTP				X	DPLA
Sacramento River @ Bryte Bend (Alamar Marina)	X	X			DPLA
Sacramento River above Sacramento Regional Wastewater Treatment Plant & Below Confluence with American River @ Miller Park Dock (Miller)	X	X			DPLA
Sacramento River Below Sacramento Regional Wastewater Treatment Plant @ Greenes Landing	X				DPLA
San Joaquin River @ Vernalis at Airport Road Bridge	X	X		X	DPLA
San Joaquin River @ Holt[b]	X				DPLA
Clifton Court @ West Canal Intake Near Radial Gates		X			O&M
Delta-Mendota Canal @ McCabe Road	X	X			O&M
Banks Pumping Plant @ Bethany Reservoir[c]	X	X		X	O&M
Arroyo Valle Creek Inflow to Lake Del Valle (when flowing, about 5 months/year) @ Creek Mouth	X	X			O&M
California Aqueduct, Check 29[d]	X	X		X	KCWA/O&M
Pyramid Lake at the Piru Creek Gaging Station		X			O&M
Pyramid Lake at the Tower in Elderberry Forebay, Release from Elderberry Forebay to Castaic	X				O&M
Castaic Lake @ Elderberry Forebay[e]		X			O&M
Castaic Lake Influent to Jensen Filtration Plant (FP)	X				MWDSC
Silverwood Lake, Influent at Mills FP or Devil Canyon	X			X	MWDSC
Silverwood Lake[f]		X			O&M
Lake Perris @ Outlet Tower	X				O&M
Barker Slough Pumping Plant, North Bay Aqueduct Intake	X	X			O&M
Mokelumne River @ New Hope			X		O&M
Shag Slough @ Liberty Island Bridge			X		O&M
Kern River Intertie Immediately Prior to Confluence with CA Aqueduct			X		O&M
CA Aqueduct @ Mile 241.02 Immediately Upstream of the Kern River Intertie			X		O&M

[a] All QA/QC samples sampled by DPLA.
[b] Samples taken downstream of Stockton Publicly Owned Treatment Works outfall at or shortly after the midpoint of an ebb tide at the sampling site to ensure flow toward the Delta.
[c] Sample collected at the inlet to Bethany Reservoir immediately downstream from Banks Pumping Plant.
[d] Inflow to the San Luis Reach of the CA Aqueduct from Cantua and Salt creeks may be used as a storm event-monitoring trigger for this site.
[e]
1. Fish Creek and Castaic Creek confluence at the lowest debris basin above Elderberry Forebay.
2. Fish Creek - if no water in debris basin.
3. Castaic Creek.
4. Elizabeth arm of lake at the gaging station.

[f]
1. Miller Canyon gaging station.
2. Cleghorn drainage.
3. Sawpit.

Table B-2 ICR IFA Split Matrix Spike Results

	Giardia Recovery 1,828 cysts/spike		*Cryptosporidium* Recovery 2,240 oocysts/spike		Turbidity
Matrix Water Source	cysts/100 L	% recovered	oocysts/100 L	% recovered	NTU
American River @ Fairbarn					2.6
Split Sample 1	5.0	0.27	<5	0	
Split Sample 2	6.7	0.37	<6.7	0	
Background	<6.7		<6.7		
San Joaquin River @ Vernalis					21.5
Split Sample 1	<10	0	<10	0	
Split Sample 2	33.7	1.84	<11.1	0	
Background	10		<10		
Banks at Bethany Reservoir					8.6
Split Sample 1	36	1.96	24	1.07	
Split Sample 2	45.5	2.50	22.6	1.01	
Background	<4.5		<4.5		
Devil Canyon					4.8
Split Sample 1	16.7	0.91	<8.3	0	
Split Sample 2	104.2	5.7	8.3	0.37	
Background	<6.3		<6.3		
California Aqueduct at Check 29					10.8
Split Sample 1	158	8.66	8.3	0.37	
Split Sample 2	57.1	3.12	14.3	0.64	
Background	<8.4		<8.4		
All Samples Average Recovery (SD, n-1)[a]	46.3 (50)	2.53 (2.74)	7.75 (9.59)	0.35 (0.43)	

[a] Standard Deviation with n-1 degrees of freedom

Results of the matrix spike study found that average recoveries were very low for both *Cryptosporidium* and *Giardia*. Recoveries were higher for *Giardia* than for *Cryptosporidium*. The average recovery for spiked *Giardia* cysts was 46.3 cysts/100 L (2.53%). The average recovery for spiked *Cryptosporidium* oocysts was 7.75 oocysts/100 L (0.35%). Recoveries were low regardless of the turbidity or sample location (Table B-2). Low recoveries were also not due to retention of the organisms by the equipment. In all cases, equipment blanks were below the detection limits (Table B-3).

Table B-3 Equipment Blank Results

	Giardia	*Cryptosporidium*	Turbidity
Sample Type	cysts/100L	oocysts/100L	NTU
Equipment Blank			<1
Sample 1	<2.7	<2.7	
Sample 2	<2.7	<2.7	

Table B-4 Recovery Comparisons Between 2 Laboratories, Cysts Seeded (2928 ± 447) and Oocysts Seeded (5532 ± 880)

	Giardia			
Matrix	Laboratory A (8/95 ICR)		Laboratory B (8/95 ICR)	
NTU	cysts/100L	%	cysts/100L	%
60	350	11.9	1,266.7	43.3
10	232	7.92	1,220	41.7
Wastewater	90.4	3.09	1,733.3	59.2
Average Recovery		7.6		48.1
	Cryptosporidium			
Matrix	Laboratory A (8/95 ICR)		Laboratory B (8/95 ICR)	
NTU	oocysts/100L	%	oocysts/100L	%
60	440	7.97	33.3	0.60
10	200	3.6	<10	0
Wastewater	142.5	2.58	50	0.90
Average Recovery		4.7		0.5

Source: DWR 1996

Recovery results from directly spiked filters sent to 2 different laboratories are shown in Table B-4. With the exception of 1 set of *Giardia* results, recoveries were <10%. For laboratory A, average recovery of both *Cryptosporidium* and *Giardia* were similar (4.7% and 7.6%, for *Cryptosporidium* and *Giardia*, respectively). For laboratory B, recovery between the 2 organisms varied by a factor of 10 (0.5% and 48.1% for *Cryptosporidium* and *Giardia*, respectively). Recovery results were not consistent between laboratories. In the case of *Giardia*, laboratory B recoveries were on average 6 times higher than laboratory A recoveries. However, with respect to *Cryptosporidium*, laboratory A recoveries were nearly 10 times higher than laboratory B recoveries.

Monitoring results of the CPMP potentially reflected the poor recoveries observed in the spiking studies. Of the 195 monthly samples collected, 7 tested positive for *Cryptosporidium* (4%). Mirroring the higher recoveries in the spiking studies, *Giardia* detections were more frequent. *Giardia* was detected in 46 of the 195 samples collected (24%). Since the method's recovery was so low and its variability was so high, it was impossible to know whether these results were a true reflection of pathogen occurrence or an artifact of the method.

Because of the high variability and poor recovery for both *Cryptosporidium* and *Giardia*, it was not possible to compare organism concentrations between sampling sites. Therefore, results from all samples were combined to generate summary statistics (Table B-5). Detections were subdivided into monthly samples collected in the first 12 months of the project (Phase I) and the 6 months of sampling focusing on locations with the greatest number of positive detections (Phase II). Pathogen concentrations from samples collected only during rainfall or flood events are shown separately. Summary statistics combining the results of both monthly sampling and rainfall/flood events are also shown

Regardless of the time period (Phase I or Phase II), the geometric means and the ranges for both *Cryptosporidium* and *Giardia* were higher for samples collected during rainfall/flood events than samples collected monthly. For *Giardia*, geometric means for both Phase I and II event sampling were higher than geometric means calculated for Phase I and II monthly sampling. For *Cryptosporidium*, the geometric mean for Phase I storm event data was twice as high as the geometric mean for Phase I monthly samples. However, like all data from this study, interpretation of these patterns are tenuous. With the exception of storm event sampling, the geometric means between *Cryptosporidium* and *Giardia* were similar. However, no statistical comparisons were conducted, and any statistical conclusions would be suspect.

Table B-5 *Giardia* and *Cryptosporidium* Summary Statistics (Total IFA count)

		Giardia (Cysts/100L)			*Cryptosporidium* (Oocysts/100L)			
Study		Number Positive	Range	Geo. Mean	Number Positive	Range	Geo. Mean	N
CPMP Monthly Sampling	Phase I[a]	22% (35 of 158)	2.4 - 92.3	16.6	4% (6 of 158)	9.0 - 26.7	18.0	158
	Phase II[b]	30% (11 of 37)	2.5 - 62.8	15.6	3% (1 of 37)	13.3	N/A	37
	Combined	24% (46 of 195)	2.4 - 92.3	16.4	4% (7 of 195)	9.0 - 26.7	17.2	195
CPMP Rainfall/ Flood Event Sampling	Phase I[a]	33% (9 of 27)	10.05 - 129.8	58.9	30% (8 of 27)	4.4 - 200	35.0	27
	Phase II[b]	35% (9 of 26)	10 - 140	28.5	19% (5 of 26)	10 - 50	22.6	26
	Combined	34% (18 of 53)	10 - 140	40.9	24% (13 of 53)	4.4 - 200	29.6	53
CPMP Combined (Monthly & Rainfall/ Flood Events)	Phase I[a]	24% (44 of 185)	2.4 - 129.8	21.5	8% (14 of 185)	4.4 - 200	26.3	185
	Phase II[b]	32% (20 of 63)	2.5 - 140	20.4	10% (6 of 63)	10 - 50	20.7	63
	Combined	26% (64 of 248)	2.4 - 140	21.2	8% (20 of 248)	4.4 - 200	24.5	248

[a] Phase I: Oct 1996 through Oct 1997
[b] Phase II: Nov 1997 through Apr 1998

Table B-6 Percent Positive *Giardia* Detections for CPMP

Water Type	Sample Location	% Positive Monthly Samples	% Positive Storm/Flood Event Samples
Source and Delta Waters	Alamar	63	67
	Miller	33	100
	Sac River @ Greenes Landing	65	100
	Barker Slough PP	40	29
	San Joaquin River near Vernalis	59	40
	Holt	17	Not sampled
	Delta-Mendota Canal	0	0
	Banks	0	0
SWP Aqueduct and Reservoir Waters	Arroyo	11	0
	Check 29	5	Not sampled
	Elderberry	0	Not sampled
	Jensen	8	Not sampled
	Devil's Canyon	0	Not sampled
	Perris	0	Not sampled

Positive detections of both *Cryptosporidium* and *Giardia* occurred the most frequently in source waters, not the SWP (Tables B-6 and B-7). *Cryptosporidium* was detected in 11% of the source water samples and 5% of the SWP samples. *Giardia* was detected in 50% of the source water samples and in only 2% of the SWP samples.

For *Giardia*, the majority of samples collected from SWP locations were below the detection limit. *Giardia* recovery and variability values were extremely poor; therefore, these distribution patterns must be viewed cautiously. There is the potential that the patterns of occurrence may be incorrect because of false positives or negatives. The high variability of the method also points to the inadequacy of the ICR's single-grab sample describing *Giardia* occurrence in a water body.

Because of poor *Cryptosporidium* recoveries, it is difficult to determine occurrence patterns. Potentially, *Cryptosporidium* may have occurred more frequently in source waters and during storm events. Although logical, the tendency for the ICR method to create false positives cannot rule out the possibility that the increased pathogen numbers during storm events reflects increased false positives from storm water debris.

Table B-7 Percent Positive *Cryptosporidium* Detections for CPMP

Water Type	Sample Location	% Positive Monthly Samples	% Positive Storm/Flood Event Samples
Source and Delta Waters	Alamar	6	0
	Miller	0	50
	Sac River @ Greenes Landing	1	25
	Barker Slough PP	0	14
	San Joaquin River near Vernalis	12	20
	Holt	17	0
	Delta-Mendota Canal	0	25
	Banks	0	25
SWP Aqueduct and Reservoir Waters	Arroyo	0	29
	Check 29	5	Not Sampled
	Elderberry	0	Not Sampled
	Jensen	0	Not Sampled
	Devil's Canyon	0	Not Sampled
	Perris	0	Not Sampled

Like *Giardia* and *Cryptosporidium*, detection frequencies for *Clostridium perfringens* was higher in storm and flood event samples than in monthly samples. Similarly, positive detections occurred more frequently in river and Delta source waters than in the SWP system. The range of positive *Clostridium perfringens* concentrations in monthly samples was 2 to 800 CFUs/100 mL, with a geometric mean of 46.9 CFUs/100 mL. The highest frequency of detection was at the North Bay Aqueduct intake at Barker Slough, which also had the highest geometric mean of all monthly sampling locations for *C. perfringens*.

Like protozoans and *C. perfringens*, total/fecal coliform and *E. coli* detection frequencies and concentrations were highest in the Sacramento River, San Joaquin River, and Delta when compared with the SWP aqueduct and reservoirs. Storm and flood event sample detections, frequency, and geometric means were also higher than those of the monthly samples.

In order to gain information about pathogen levels in floodwaters, additional samples were collected during the January 1997 floods. The highest geometric means for any of the organisms (protozoans or bacteria) were observed in flood event samples. Flood event geometric means were not only higher than monthly values but were also higher than storm event values. Seventy percent of the flood samples tested positive for *Giardia*, while 40% tested positive for *Cryptosporidium*.

The Department's study also conducted correlation analyses to identify possible surrogate indicators for *Cryptosporidium* and *Giardia*. Unfortunately, the quality of data associated with the ICR IFA method precluded any meaningful conclusions from being drawn. Only the relationship between fecal coliform and *E. coli* exhibited a correlation coefficient greater than 80%. Given that *E. coli* is an indicator of fecal contamination, this result is not surprising. Similarly, it is not surprising that no correlation was found between protozoa and bacteria or turbidity. Even if a correlation actually did exist, a lack of correlation would be expected if a method was highly variable and subject to nondetects, false positives, and false negatives.

DWR concluded that the ICR method exhibited poor recovery, accuracy, and precision. Because of these failings, it is impossible to know whether the results obtained from the study accurately reflected pathogen distribution and concentration in source, Delta, SWP Aqueduct, and reservoir waters. Potentially, *Cryptosporidium* and *Giardia* are more prevalent in source and Delta waters than in SWP aqueduct and reservoir waters and occur more frequently at higher concentrations during storm and flood events. The limitations of the method precluded any correlation being drawn between protozoa and bacteria or turbidity concentrations.

The results observed from DWR's study are similar to nationwide ICR results. With 18 months of national ICR data analyzed, the majority of samples have found no detection of either organism. Of the 5,829 samples analyzed, 93% have been nondetect for *Cryptosporidium,* and 81% have been negative for *Giardia* (Allen and others 2000). Like DWR's studies, these results do not mean that the organisms are not present, only that the limitations of the method failed to discern them. The EPA also has not found a correlation between *Cryptosporidium* and bacteria surrogates (Pope pers. comm.).

References

LITERATURE CITED FOR APPENDIX B

Allen MJ, Clancy JL, Rice EW. 2000. "The plain, hard truth about pathogen monitoring." *J Am Water Works Assoc* 92(9):64-76

[DWR] California Department of Water Resources. Division of Planning and Local Assistance. 1996 Aug. *Municipal Water Quality Investigations Program annual report 1995*.

Clancy JL, Gollnitz WD, Tabib Z. 1994. "Commercial labs: how accurate are they?" *J Am Water Works Assoc* 86(5):89

Clancy JL, Bukhari Z, McCuin RM, Matheson Z, Fricker CR. 1999. "EPA method 1622." *J Am Water Works Assoc* 91(9):60-8.

[EPA] United States Environmental Protection Agency.1999. *Microbial and disinfection byproduct rules simultaneous compliance guidance manual.* Office of Water. EPA 815-R-99-015.

[EPA] United States Environmental Protection Agency. 1999a. *Disinfection profiling and benchmarking guidance manual*. Office of Water. EPA 915-R-99-013

Pontius FW, Clancy JL. 1999. "ICR crypto data: Worthwhile or worthless." *J Am Water Works Assoc* 91(9):14-21.

PERSONAL COMMUNICATION FOR APPENDIX B

Pope, Misty, Senior Biologist, DynCorp IET. 2001. Personal communication by phone, Jan 16.