Field Testing the Modified Microscopic Particulate Analysis Method

Peter Wallis and Matthew Henschke
Medicine Hat College, 299 College Dr. SE, Medicine Hat, AB T1A 3Y6

SUMMARY

The Consensus method for the Microscopic Particulate Analysis is improved by combining it with Method 1623 for *Giardia* and *Cryptosporidium*. Laboratory trials reported previously indicate that significant improvements in the recovery of parasite stages and aquatic organisms can be achieved from the filtration of much smaller volumes of water. The protocol devised for sampling and analysis of well water samples was tested by taking samples ten weeks apart at eight well sites servicing Meadow Lake Provincial Park in central Saskatchewan . Some of these water supplies are solar powered and all of them pump raw water only intermittently to cisterns making sampling difficult with the Consensus method. Field sampling time was reduced to one hour per site as opposed to eight hours or more with the old method. Surface water organisms were recovered at all sites along with large numbers of pollen despite a high degree of flocculated iron in the background. MPA analyses were supplemented with measurements of Aerobic Bacteria Spore Formers and presence/absence coliform assays, both of which indicated excellent raw water quality. Risk levels were assessed as medium to low for all sites using the criteria for the Consensus method and as low when evaluated by a risk standard modified to account for the increased sensitivity of the new protocol.

INTRODUCTION

The Consensus method for the Microscopic Particulate Analysis (USEPA, 1992) used to determine if ground water is under the direct influence of surface water (GUDI) suffers from several drawbacks. The three most important issues are:

- Lengthy field sampling time owing to the need to filter 3800 L (8 hours)
- Difficulty in recovering particles including surface water organisms from string wound filters
- Poor recovery of parasites, particularly *Cryptosporidium* oocysts.

Sampling large volumes of water is particularly difficult in small installations which are often not plumbed for direct access to raw water and are set up so that water is only running when filling a storage cistern, necessitating constant drawdown and possible overheating of the pump. Whether eluted by hand washing or mechanical methods, particles are not quantitatively removed from string wound filters. This leads to low recovery of all cells of interest, especially smaller ones such as *Cryptosporidium* oocysts. Method 1623 (USEPA, 2001) for the recovery of *Giardia* cysts and *Cryptosporidium* oocysts represents a large improvement in the recovery of these parasite stages permitting the use of much smaller volumes of water by using more efficient filters and direct binding of target cells with immunomagnetic beads. The pellet remaining after bead extraction is available for further examination so it is logical to replace the original Consensus method with a modified Method 1623 protocol for GUDI determination in a Microscopic Particulate Analysis.

Initial laboratory experiments yielded the recovery results in Table 1 (Wallis and Henschke, 2008; Wallis et al., in press). These results were based on only a few trials but clearly indicated that the Modified 1623 protocol was capable of recovering more cells with greater consistency. This was not surprising for *Giardia* cysts and *Cryptosporidium* oocysts as many previous matrix spike trials (part of

the quality control procedure for Method 1623) demonstrated similar levels of recovery (*Giardia* n = 11, % recovery = 46.8, RSD = 27.0; *Cryptosporidium* n = 11, % recovery = 74.5, RSD = 25.8).

	Giardia Cysts		*Cryptosporidium* oocysts		*Euglena* sp. cells		*Sphaerocystis* sp. cells	
	Consensus	Modified 1623	Consensus	Modified 1623	Consensus	Modified 1623	Consensus	Modified 1623
n	12	5	12	5	12	5	4	5
Mean %	6.5	37.2	0.5	89.2	4.2	36.2	0	2.1
RSD (%)	30.5	25.6	69.1	17.1	92.8	30.4	ND	40.9

Table 1. Percentage recoveries of *Giardia* cysts, *Cryptosporidium* oocysts, *Euglena* sp. and *Sphaerocystis* cells from spiked Filta-Max cartridges pre-loaded with ground water matrix compared to similar trials using the Consensus Method. RSD = Residual Standard Deviation; ND = Not Determined.

The next step in the investigation was to test the field sampling and laboratory protocols in the field to see if the laboratory results could be confirmed. An opportunity arose to do this in Meadow Lake Provincial Park (Saskatchewan) where there are a number of seasonally operated well installations that service campgrounds on the shores of different lakes. These sites are mostly not amenable to sampling with the Consensus method because of the difficulty of filtering 3800 L of water caused by limited pumping capacity and low pressure. Three of these sites are solar powered and all of them are designed to pump only for short times to fill a cistern or pressure tank. The first sampling trip took place in June of 2009, shortly after the campsites had been opened for the season and a second series of samples was obtained in mid-August.

A total of 9 previous samples based on the Consensus method had been taken in Meadow Lake Provincial Park from 2005 to 2008 where that methodology could be used including one of the sampling sites sampled in 2009 (Kimball Lake). No surface water organisms were observed in any of these sites with the exception of one “other” algae (not a diatom) detected on one occasion at Grieg Lake. Low numbers of plant debris and pollen were also sometimes observed but the results of all of the sampling indicated only the lowest level of risk with no ‘risk of surface water contamination’ scoring above 0 at any time (USEPA, 1992). Five of these samples were from the Kimball Lake site which was sampled twice again in 2009 using the modified Method 1623 protocol.

Figure 1. Field sampling at the solar powered pumphouse at First Mustus Lake. Raw well water was pumped into the barrel and then re-pumped through the Filta-Max filter.

It has been proposed that the distribution of aerobic bacterial spores, which are approximately the same size as *Cryptosporidium* oocysts, could provide a useful indication of the efficacy of filtration processes (Brown and Cornwell, 2007). Treated drinking water spore concentrations rarely exceeded 5 spores/L in their study while lake water was found to contain from 300 to 3,000 spores/L. A comparison of aerobic spore occurrence could therefore provide an indication of the filtration capacity of the geologic materials that wells are completed in. The analysis is easy to do, inexpensive and requires only a 100 mL grab sample.

METHODOLOGY

Field Sampling: Well installations at South Waterhen, Jeannette, Floten, First Mustus, Kimball, Matheson, Mistohay, and Pierce Lakes were sampled from June 17-20 and again from August 10-12, 2009. Well water and surface water samples from the adjacent lakes were taken at each site for aerobic spore determination using sterile, 100 mL polystyrene sampling bottles containing $Na_2S_2O_3$ (Idexx). Total and faecal coliform samples were taken during the August sampling trip and temperature measurements from ground water and surface water were recorded. All sampling equipment including hoses and the pump were purchased new and cleaned by flushing with filtered water for 2 minutes before use in field work. The equipment was flushed with the raw water before installing the filter. Filta-Max filters have an optimum working pressure of about 500 kPa (73 psi) which could not be supplied by the pumps in the park. In order to overcome this difficulty, raw outlets (before chlorination) were installed at each of eight sites to be sampled and the *in situ* equipment was used to fill a 200 L polyethylene barrel. 1 g of sodium thiosulphate was added to the barrel at each site to neutralize any chlorine. Approximately 100 L of the well water were then immediately re-pumped through the Filta-Max filter using a 120 V pump (Simer 2825SS-01) powered by a portable generator and the filtered water collected in a polyethylene bucket as shown in Figure 1. The ground water in this area is high in iron and some filters plugged before the full volume could be filtered (minimum volume filtered was 50 L). The filter was removed, bagged and stored in a cooler with ice packs with descriptive paperwork. The filtered water was then used to backflush the Filta-Max housing, pump and barrel in preparation for the next sampling site.

Filter Elution and Sample Preparation: All samples were returned to the laboratory in ice-filled coolers and processing was begun within 96 h of sampling. Filta-Max filters were eluted according to Method 1623 (USEPA, 2001). 1 mL of pellet was processed according to the method and the remainder kept separately for bright-field examination. If the total volume of the pellet was less than 1.0 mL, it was saved along with all the wash solutions and re-concentrated at 1000 X g for 10 min by centrifugation and examined by bright-field microscopy. After bead dissociation, the *Giardia/ Cryptosporidium* suspension was dried on a slide, fixed with methanol and stained with DAPI and monoclonal antibodies conjugated with CY3 (Waterborne Inc.). Pellets for surface water organism detection were transferred to 2.0 mL tubes and concentrated at 2000 X g in a microcentrifuge (Fisher Model 12) for 6 minutes if the pellet volume was less than 200 µL (Jeannette, First Mustus, Kimball and Mistohay samples). Larger pellets were suspended in 2000 µL of filtered (to 1 µm) deionized water and clarified by discontinuous gradient centrifugation using a mixture of Percoll and sucrose adjusted to a specific gravity of 1.23 and centrifugation at 400 X g for 10 minutes with the brake off. The floated material was pipetted off the interface, transferred to a 50 mL tube, and concentrated by centrifugation at 650 X g for 10 minutes. The resulting pellets were transferred to 2.0 mL microcentrifuge tubes and concentrated as above in a microcentrifuge.

Microscopic Examination: *Giardia/Cryptosporidium* slides were read according to the Method 1623 protocol using a Zeiss Axioskop microscope equipped PlanNeofluar 40X and 100 X oil objectives and rhodamine filters. Pellets were resuspended in filtered, deionized water and read in 50 µL aliquots by preparing wet mounts and examining the entire coverslip area using bright-field illumination and 10X or 20 X Achroplan objectives. The entire pellet was examined for the four sites listed above and a minimum of 10% of the pellet suspension was read for the June samples for the Waterhen, Floten, Matheson and Pierce locations. No less than 50% of the resuspended pellets were read for the August samples.

Aerobic Spore Forming Bacteria: The membrane filtration method for aerobic endospores described in Section 9218B of *Standard Methods* (APHA 2005) was used to enumerate spores. Briefly, the 100 mL sample bottles were heated in a water bath to 75 °C for 15 min. and then cooled to room temperature. The entire volume was then filtered through a 0.45 µm membrane filter which was then placed on the surface of a nutrient agar plus trypan blue plate. The plates were incubated at 37 °C for 24 h and colonies were enumerated with the assistance of a low-powered dissecting microscope.

Total and Faecal Coliforms: Defined substrate technology using the Colilert test (Idexx, Westbrook ME) was employed to provide presence/absence data for total coliforms and *Escherichia coli* for the August samples. Samples were taken in the field using 100 mL bottles containing $Na_2S_2O_3$ and kept at 5 °C until they were returned to the laboratory when the contents of one packet of Colilert medium were added to each sampling bottle. Sample holding times ranged from 8 to 48 h and test bottles were incubated for 24 h at 37 °C. Samples were scored as positive for total coliforms if the medium turned yellow and as positive for faecal coliforms if the mixture fluoresced blue under ultra-violet light as per the manufacturer's instructions.

RESULTS

Field Sampling and Filter Elution: Samples were taken at all eight locations within 48 hours with only an hour required at each site. The following volumes were filtered and sediment volumes recovered (Tables 2 and 3). The flow rate through the filters dropped to less than half by the end of the run at Waterhen, Floten, Matheson and Pierce Lakes. All samples contained variable amounts of iron floc and few other recognizable minerals (silica chips and clay were minor constituents). Iron recovered in the pellet increased with the depth of the well. In June, Large amounts of pollen were observed at all sites wherever there was surface water for it to collect in. Much less pollen was in evidence by August along shorelines. The temperature of surface water samples was approximately twice that of ground water samples in the August samples.

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Volume (L)	108	102	87	110	100	76	100	90
Sediment Vol. (L)	3.0	0.2	0.5	0.1	0.1	2.0	0.1	2.0
Filtration Time (min)	30	40	25	25	17	35	22	40

Table 2. Field data for samples taken at Meadow Lake Provincial Park sites, June 2009.

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Volume (L)	100	100	112	100	100	100	100	50
Sediment Vol. (L)	0.5	0.1	0.2	0.1	1.0	2.0	0.1	5.0
Depth of Well (m)	17.7	ND	15.5	11.3	18.3	37.5	7.3	33.5
Temperature – GW	10.6	12.3	9.3	7.7	9.1	10.0	10.4	10.5
Temperature – SW	21.7	20.7	20.5	19.8	22.2	22.6	22.4	20.2

Table 3. Field data for samples taken at Meadow Lake Provincial Park sites, August 2009.

Surface Water Organisms: No diatoms, insect parts, rotifers, nematodes, Crustacea, amoebae or ciliates were found in the June samples. Other algae, plant debris and pollen were observed as recorded in Tables 4 and 5 for the June and August samples respectively. Pollen was particularly abundant in June but less so in August.

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Dist. to Surface Water (m)	65	75	50	50	250	125	45	400
Equivalent Volume (L) examined	21.6	102	87	110	50	7.6	100	9
Other Algae Cells	3	24	2	31	20	1	12	2
Plant Debris	0	31	0	7	0	3	10	0
Pollen	120	1220	500	1680	526	354	191	53
ASFB (surface) /L	TNTC	10	100	150	TNTC	TNTC	250	520
ASFB (well water) /L	0	50	0	0	0	210	10	0

Table 4 . Raw counts (unadjusted for volume) of surface water organisms, pollen and aerobic spores in well water eluates from Meadow Lake Provincial Park sites, June 2009. ASFB = Aerobic Spore Forming Bacteria; TNTC = Too Numerous to Count.

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Dist. to Surface Water (m)	65	75	50	50	250	125	45	400
Equivalent Volume (L) examined	50	100	112	100	50	50	100	25
Other Algae Cells	1	4	3	6	0	1	5	0
Plant Debris	2	14	11	6	7	4	3	0
Pollen	297	24	119	975	8	67	7	41
ASFB (surface) /L	340	0	3500	3300	780	2220	680	2700
ASFB (well water) /L	10	20	0	0	0	0	20	20
Total Coliforms – GW	–	–	–	–	–	–	–	–
Total Coliforms – SW	+	+	+	+	+	+	+	+
Faecal Coliforms – GW	–	–	–	–	–	–	–	–
Faecal Coliforms – SW	+	–	+	+	+	+	–	–

Table 5. Raw counts (unadjusted for volume) of surface water organisms, pollen and aerobic spores in well water eluates from Meadow Lake Provincial Park sites, August 2009. ASFB = Aerobic Spore Forming Bacteria. GW = Ground Water; SW = Surface Water.

Total and Faecal Coliforms: All surface water samples taken in August were positive for total coliforms and five out of eight were positive for faecal coliforms. No ground water samples were positive for either total or faecal coliforms.

***Giardia* and *Cryptosporidium*:** No cysts or oocysts were found in any samples

Aerobic Spore Forming Bacteria: Aerobic spore formers were much more abundant in surface water than in ground water. No ASFB were found in either sample from the Floten, First Mustus and Kimball sites. The Jeannette and particularly the Matheson sample were relatively high in ASFB in June but all other positive samples contained 20 or fewer spores.

DISCUSSION

The presence of surface water organisms in well water is thought to demonstrate hydraulic connection and communication between the two and therefore provide a measure of the risk of contamination with pathogens which could be present in surface water, particularly *Giardia* and *Cryptosporidium*. The original Consensus method uses the raw numbers of diatoms, other algae, insects/larvae, rotifers and plant debris, described as 'Primary Particulates' to calculate concentrations per 100 US gal. (380 L).

Pollen, nematodes, Crustacea, amoebae and ciliates/flagellates are considered to be 'Secondary Particulates' which do not contribute to risk as they are commonly found in shallow ground water even when there was no nearby surface water. The concentrations of Primary Particulates per 100 gal. are then compared to ranges which correspond to estimates of risk described as Not Significant, Low, Moderate, Heavy and Extremely Heavy. Each of these is assigned a relative risk value and the values are summed. 'Relative' means that more obvious indicators of surface water such as diatoms and other algae receive heavier weighting. The presence of any parasites automatically moves a sample into the highest risk level. The sums are then assigned to relative risk ranges with values of 0 to 9 considered to be low risk, 10-19 moderate and >20 as high. All of the categories span a wide range and the whole analysis is only semi-quantitative and rather arbitrary but they have force of law in the United States under the Safe Drinking Water Act of 1986. If a well is used as a potable water source and it falls into a high risk category, then it must be treated as surface water at considerably greater expense than that required for ground water (chlorination at most). Furthermore, an MPA test is not considered to be sufficient make a GUDI determination by itself (even when repeated) in the absence of supporting hydrogeological evidence.

The determination of relative risk takes into account the difficulty in recovering cells and organisms from string wound cartridge filters, the best that were available when the method was developed. This is partially reflected in the large volume to be filtered specified by the method in order to maximize the chance of finding anything. If more efficient techniques for particle recovery are used, the risk scales need to be re-examined in order to preserve the validity and comparability of the results. The results of this study can be used as a starting point.

Based on the original risk ranges and calculations in the Consensus method, the June samples from Meadow Lake Provincial Park produce the following relative risk factors (Table 6). The results show that most of the sites fall into the moderate risk level with the exception Waterhen, Floten and Pierce Lakes. Previous determinations of risk, including Kimball Lake, were all 0 based on the Consensus method protocol. There is no obvious correlation with distance of the wells from the lakes.

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Other Algae Cells	53	89	9	107	152	50	46	84
Plant Debris	0	115	0	24	0	150	38	0
Pollen	2111	4545	2184	5804	3998	17700	726	2238
Calculated Risk Factor	9	11	4	12	12	11	10	9

Table 6 . Risk of surface water contamination in June based on the data from Table 4 using the original criteria specified in the Consensus method for Microscopic Particulate Analysis. Concentrations are adjusted to be per 100 US gal. (380 L).

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Other Algae Cells	8	15	10	23	0	8	19	0
Plant Debris	15	53	37	23	53	30	11	0
Pollen	2257	91	404	3705	61	509	27	623
Calculated Risk Factor	4	4	5	9	1	5	4	0

Table 7 . Risk of surface water contamination in June based on the data from Table 5 using the original criteria specified in the Consensus method for Microscopic Particulate Analysis. Concentrations are adjusted to be per 100 US gal. (380 L).

The data in Table 1 show that the modified 1623 method used in this study is approximately half an order of magnitude more efficient at recovering particles. Adopting a conservative approach, it is proposed that the relative risk scales be adjusted downward by allowing four times higher concentrations

of Primary Particulates. If concentrations per 100 L with the same ranges and risk allocations were used instead it would reflect the improved recovery efficiency of the new method and put the concentrations into the same units commonly used for *Giardia* and *Cryptosporidium* for Method 1623. By these criteria, the risk from each site would be adjusted to those values reported in Tables 8 and 9.

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Other algae cells	14	24	2	12	12	11	10	9
Plant Debris	0	30	0	6	0	39	10	0
Pollen	556	1196	575	1527	1052	4658	191	589
Calculated Risk Factor	4	10	4	9	9	5	4	9

Table 8. Risk of surface water contamination in June based on the data from Table 4 using the concentration of Primary Particulates per 100 L and maintaining the ranges specified in the original Consensus method.

	Waterhen	Jeannette	Floten	First Mustus	Kimball	Matheson	Mistohay	Pierce
Other algae cells	2	4	3	6	0	2	5	0
Plant Debris	4	14	10	6	14	8	3	0
Pollen	594	24	106	975	16	134	7	164
Calculated Risk Factor	4	4	4	4	0	4	4	0

Table 9. Risk of surface water contamination in August based on the data from Table 5 using the concentration of Primary Particulates per 100 L and maintaining the ranges specified in the original Consensus method.

This re-calculation moves all of the sites into the lowest risk level with the exception of Jeannette Lake in June. The lowest risk levels are consistent with the large differences in temperature between surface and ground waters and the absence of any coliform bacteria in the August samples. The negative results for *Giardia* cysts and *Cryptosporidium* oocysts in all ground water samples, despite the high recovery levels offered by Method 1623, further support this hypothesis.

The Aerobic Spore Forming Bacteria and pollen data are interesting but both are found in soil as well as water. Algal cells could theoretically be found in shallow standing pools of water but there are no such water bodies in the vicinity of these sample sites at Meadow Lake Provincial Park. The shoreline areas where the wells are situated consist of glacial deposits with sandy beaches and porosity is high. Pollen grains are larger than spores, *Giardia* cysts and *Cryptosporidium* oocysts (Figure 2) but were found in high numbers in all of the June water samples examined, particularly conifer pollen. There was no way of estimating the age of the pollen grains observed but they appeared to be fresh and not broken apart. Pollen was visible at that time in large quantities at every shoreline and blanketed the entire area. Pollen grains do not contribute to the calculation of risk but their presence in high numbers does suggest that there is communication between the surface and well water at all sites. By August, pollen was visibly absent from the shorelines of adjacent lakes and was present in reduced numbers in ground water samples. Furthermore, many of the pollen grains were fragmented or otherwise in poor condition.

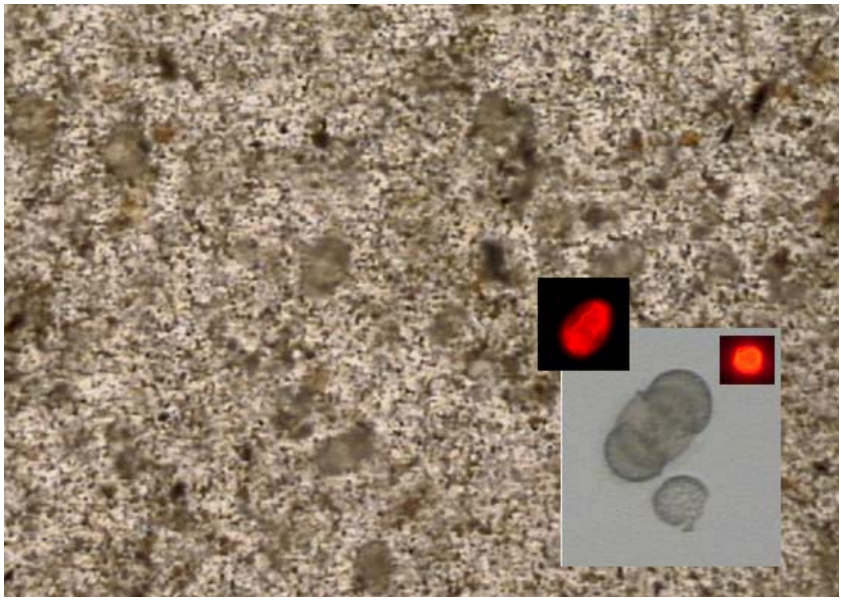

Figure 2. Pollen in ground water from First Mustus sample. Insets show details of pollen grain, *Cryptosporidium* oocysts (round) and *Giardia* cyst (oval) to approximate scale.

The absence of spores in a ground water sample may indicate good filtration capacity of the porous medium that the well was finished in but their presence does not mean that the microbiological quality of the water is bad. No spores were found in well water samples from Waterhen, Floten, First Mustus, Kimball and Pierce Lakes but they were present in all surface water samples taken (Tables 4 and 5). Brown et al. (2007) report that aerobic spores were commonly found at concentrations of 5-10/L in treated water samples from the utilities they sampled in the United States and, presumably, these concentrations indicate a high level of water filtration. In the Meadow Lake samples taken in June 2009, only the Matheson sample exceeded this level (47 spores/L). Pollen never exceeded 10/L in the August ground water samples.

Temperature, coliform, ASFB and *Giardia*/*Cryptosporidium* data all indicate a very low level of risk for these wells. It is surprising, therefore, that any algae cells were found in the ground water samples, especially from the Pierce site which is 250 m from the nearest surface water and also that there was no obvious relationship between the number of cells counted and the distance to the lake. The sandy nature of the soil prevented any accumulation of water in puddles and the weather conditions were generally dry. Elevation data for the standing water level is only available at the Kimball site but it indicates that the lake is lower and ground water is presumably moving to recharge surface water, not surprising in a system that could best be described as a large sandbox. Great care was taken to prevent any contamination of samples with algae during sampling, handling and analysis. The presence of large numbers of pollen, particularly in the June samples, suggest that there is communication with the surface but it is not necessarily from the lake. The pollen spike in the June samples was coincident with the peak production of pollen from the ubiquitous Jack Pines in the area suggesting that their passage into ground water was rapid. Similarly, the algae cells that were observed were in good condition. Algae can occur in soil wherever there is moisture and light so it is possible that there was movement of water down the sides of well casings during precipitation events. This was impossible to evaluate in the field as all the wellheads were covered by pump shacks or concrete covers. Conditions were dry before and during each sampling period but the heaviest precipitation of the summer fell at the east end of the park at the end of the June trip. The rainfall record from the nearest meteorological station, Dorintosh at the southeastern entrance to the park, are

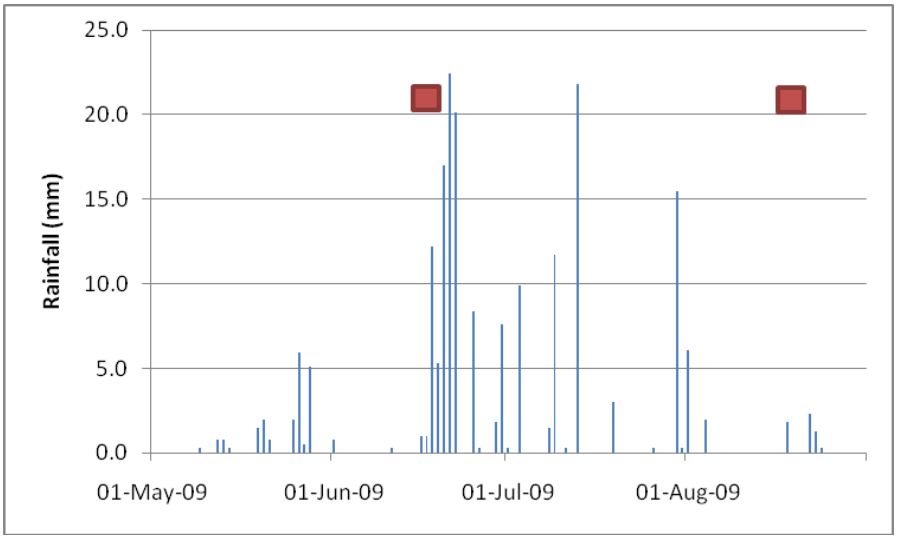

Figure 3. Rainfall record from Dorintosh during the summer of 2009. Sampling intervals are indicated by red boxes.

given in Figure 3 but it should be noted that rainfall events in the summer are very local and the park is large so it is possible that local rain showers occurred that are not accurately captured by these data.

The increased sensitivity of this method explains why algae were found in these samples compared to previous analyses made with the original method but not why there was anything there in the first place.

CONCLUSION

Data from the two sets of samples taken in the summer of 2009 using the modified Method 1623-MPA protocol indicate that the level of risk posed by the wells at the eight sites at Meadow Lake Provincial Park is low or at worst, moderate. Chlorophyll containing algae were present in many samples, however, showing that there is some communication with surface water. No correlation of surface water organisms with the distance to the nearest surface water body is evident and precipitation did not appear to be a factor. The absence of coliform bacteria and low counts of Aerobic Spore Forming Bacteria in well water samples are additional indicators of good water quality.

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