Comparing heterotrophic plate count (HPC) and adenosine triphosphate (ATP) methodologies for bacteriological monitoring in the Greater Vancouver Water District transmission system

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DISCLAIMER

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This project was conducted under the mentorship of Metro Vancouver staff. The opinions and recommendations in this report and any errors are those of the author and do not necessarily reflect the views of Metro Vancouver or the University of British Columbia.

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EXECUTIVE SUMMARY

Heterotrophic plate count (HPC) is a well-established method for monitoring microbial activity in potable water systems such as the Greater Vancouver Water District (GVWD) transmission system. As HPC has the longest turnaround time (five days) of all water quality tests conducted by MetroVancouver, there is interest to explore alternative bacteriological monitoring methods. Rapid adenosine triphosphate (ATP) assay is a feasible HPC replacement for GVWD, based on:

- Literature review, which finds that ATP is 1) more sensitive than HPC, allowing earlier detection of regrowth risk; and 2) not well-correlated with HPC but correlates better than HPC to other indicators of bacterial regrowth, e.g., flow cytometric cell counts.
- Studies by other Canadian utilities (Halifax Water and several Quebec municipalities), which further support that ATP is 1) more sensitive than HPC, which is frequently non-detectable; 2) not well-correlated to HPC but more consistent with free chlorine concentrations.

A preliminary study of ATP testing was conducted using 14 sample sites within the GVWD transmission network from June to July 2022. Results indicate:

- ATP correlates moderately to HPC (R=0.61), and agrees well (98%) based on the rough definitions of high HPC and ATP as >100 CFU/mL and >10 pg/mL, respectively.
- ATP sampling procedures can be modified to facilitate the implementation of ATP testing for MetroVancouver (namely the use of chlorine quenching agents) without sacrificing sample integrity.
- Disadvantages of ATP testing include increased plastic waste and cost of consumables.

If MetroVancouver is interested to further pursue ATP testing, it is recommended that:

- Preliminary ATP testing is further conducted for six months or more to span different seasons and to perform trend analysis.
- ATP and HPC should be jointly assessed until baseline and deviations thereof can be confidently established for ATP, i.e., for six months to a year
- Measures to reduce plastic waste is explored with the test kit manufacturer.

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cATP Celluar adenosine triphosphate

ATP Adenosine triphosphate

BHAA Bactéries hétérotrophes aérobies et anaérobies (HPC equivalent)

CFU Colony forming unit

CWTP Coquitlam Water Treatment Plant

E. coli Escherichia coli

GVWD Greater Vancouver Water District

EPA Environmental Protection Agency (US entity)

HPC Heterotrophic plate count

ME/mL Microbial equivalents per litre

mg/L Milligrams per litre

pg/L Picograms (10^{-12} g) per litre

SCFP Seymour Capilano Filtration Plant

UV Ultraviolet light

1 INTRODUCTION

1.1 Greater Vancouver Water District (GVWD) overview

Greater Vancouver Water District (GVWD) is an entity of MetroVancouver responsible for treating and supplying potable water to 2.7 million residents (MetroVancouver, n.d.-b) in 21 of its member jurisdictions (19 municipalities, one electoral area, and one Treaty First Nation; Wang, 2020). Water is sourced from Seymour, Capilano, and Coquitlam protected Water Supply Areas (MetroVancouver, 2021). Seymour and Capilano source waters are treated at the Seymour-Capilano Filtration Plant (SCFP) through chemically assisted direct filtration, disinfection using UV light and sodium hypochlorite (bleach) injection, and pH adjustment with lime and CO_2 injection (MetroVancouver, 2021). Water from the Coquitlam source is treated at the Coquitlam Water Treatment Plant (CWTP), where unfiltered source water is disinfected through ozonation, UV, and sodium hypochlorite injection, after which the pH is adjusted using soda ash (MetroVancouver, 2021).

Treated water from the SCFP and CWTP are distributed through a water main network with a total length >500 km (Wang, 2020). The transmission system consists of 21 reservoirs, 13 pump stations, and 8 secondary disinfection systems utilizing sodium hypochlorite injection to maintain target free chlorine setpoints (MetroVancouver, n.d.-a, 2021).

1.2 Bacteriological water quality monitoring

Maintaining biological stability in a distribution system and preventing the (re)growth of harmful pathogens is important for ensuring consumer health and safety (Health Canada, 2022). Bacteriological water quality is monitored in the GVWD transmission system through sample collection from approximately 134 sample sites along the GVWD water main, from all 21 reservoirs, before and after each of the two treatment systems (i.e., untreated source water and treated water), as well as from sampling sites in member jurisdiction distribution systems. Samples are analyzed for *Escherichia coli* (*E. coli*) and total coliforms to ensure compliance with regulatory standards for human health risk. Other parameters, including heterotrophic plate count (HPC) turbidity, chlorine residual, and temperature—which are among the suggested parameters in Health Canada guidelines for monitoring biological stability in drinking water distribution systems (Health Canada, 2022)—are analyzed to support the operation of the treatment and

distribution systems. These data are also provided by GVWD to their member jurisdictions as part of the service they provide as a water supplier.

1.2.1 Heterotrophic plate count (HPC)

HPC is a nonspecific culture-based method used to determine the amount of heterotrophic (carbon-eating) bacteria in water (Health Canada, 2012). Generally, water is added to growth media, incubated for several days, and bacterial colonies which form are counted to determine the number of colony-forming units (CFU) per mL of water.

HPC is an indicator of a variety of operational issues in the distribution system, including flow stagnation, low disinfectant residual, increased nutrient concentration, and increased temperatures, all of which could promote bacterial growth in the system and result in increased HPC (Bartram et al., 2003; Health Canada, 2012). It is not, however, an indicator of human health risk (unlike *E. Coli* and total coliforms) and is not regulated in Canada. The recommendation by Health Canada is to monitor HPC in the distribution system for abnormal changes (Health Canada, 2012), the determination for which will vary between different systems. This is consistent with the 1998 European Union Council Directive and the recommendations of the World Health Organization (Robertson & Brooks, 2003). HPC is also not regulated in the US, though the US EPA recommends controlling HPC below 500 CFU/mL to avoid possible interference with *E. Coli* and total coliform tests (Health Canada, 2012). This threshold value is generally less relevant for GVWD, which rarely sees HPC values of this magnitude in the transmission system. Some European countries do regulate HPC in their potable water systems, with limits ranging from 20 to 500 CFU/mL and most commonly at 100 CFU/mL (Payment et al., 2003).

One limitation of the HPC method, and a main driver for this present study, is its turnaround time. MetroVancouver utilizes the spread plate method to inoculate R2A media with 0.5 mL of the sampled water, which needs to be incubated at 28°C for 5 days before counting. This makes HPC the longest turnaround time of all bacteriological water quality tests conducted by MetroVancouver. Eliminating this bottleneck would allow more timely delivery of water quality reports to their member jurisdictions, as well as enable faster response to system conditions.

Other limitations of HPC is it captures only a small fraction of bacteria, as typically only 0.01% of bacteria found in water are heterotrophic, of which only 1% are culturable (Exner et al., 2003).

Indeed, HPC has been found to detect <1% of total cell counts as determined from microscopy or flow cytometry (van der Wielen & van der Kooij, 2010). As well, the small sample volume utilized in HPC makes the method more sensitive to heterogeneity in the water and limits the ability of this method to accurately represent the water tested.

1.2.2 Adenosine triphosphate (ATP) assay

Measuring ATP, the energy molecule used by all living organisms, is increasingly being used for monitoring microbiological activity in water distribution systems (Health Canada, 2022). Commercial kits generally utilize an enzyme (luciferase, also found in fireflies) that reacts with ATP to produce light, and the resulting luminescence is measured and compared against a standard to determine the ATP concentration in the water sample; this in turn is indicative of the number of cells in the water. Like HPC, an ATP test is also nonspecific; as well, it registers non-culturable cells such as nitrifiers, sulphate reducers, etc. (Whalen et al., 2018) as well as eukaryotes (Duda et al., 2015). Of note is that ATP in dead but intact cells will also be picked up by the test, which could lead to underestimating the extent of disinfection, though this concern should be weighed against the fact that ATP tests, in being able to capture a wider range of organisms, can therefore also pick up the impact of disinfectants on a more complete microbial population than HPC (Whalen et al., 2018). Studies have found ATP results correlate strongly to total cell counts as determined by flow cytometry (Health Canada, 2022). One main advantage of ATP is the quick turnaround time, with results being generated in seconds. As well, cellular ATP (cATP) testing kits such as Luminultra Quench-Gone Aqueous (QGA; Fredericton, NB), which capture cells on a filter, test a much larger volume of water than HPC (typically 50–100 mL per test), thus reducing the impact of heterogeneity on sample results.

Table 1: Comparison of HPC method used by MetroVancouver versus Luminultra QGA (Fredericton, NB) cATP test kits.

Characteristics	HPC method (MetroVancouver)	cATP assay (Luminultra QGA)
Time to generate result	5 days	Seconds
Test volume	0.5 mL	50-100 mL
Target of test	Live, culturable bacteria	All intact cells, including eukaryotes

Guidelines for interpreting cATP results have been put forward by Luminultra, with 10 pg/mL as the recommended limit for potable water systems (Table 2; McIlwain, 2020). This is consistent with a Dutch study concluding ATP concentrations below 15 pg/mL is adequate for maintaining HPC below 100 CFU/mL in a non-chlorinated water distribution system (van der Wielen & van der Kooij, 2010).

Table 2: Luminultra guidelines for the interpretation of cATP results in potable water systems (McIlwain, 2020)

cATP	Response
<1 pg/mL	Good control
1–10 pg/mL	Preventative action
>10 pg/mL	Corrective action

2 OBJECTIVES

The purpose of this feasibility study is to assess if ATP testing is a suitable alternative for the HPC method used by MetroVancouver. Two main aspects are considered in assessing suitability:

- **Accuracy and reliability**: can ATP testing provide an equally/more accurate and reliable indication of biological stability, compared with HPC?
- **Operational considerations**: can ATP testing be implemented for MetroVancouver at a reasonable cost, labour, etc.?

This study consists of three components:

- Literature review of ATP and HPC studies involving potable water systems (section 4)
- Review of ATP and HPC studies conducted by Canadian utility operators (Halifax Water and Quebec municipalities; section 5)
- Three-week preliminary ATP testing from GVWD transmission system sample sites from June to July 2022 (section 6)

3 LITERATURE REVIEW

A literature search was conducted for studies comparing HPC and ATP assay for testing microbial activity in drinking water. 10 studies from five countries published between 2001 to 2021 were retained for analysis. Key data from these reports are summarized in Table 3.

3.1 Correlating HPC and ATP

The following observations about correlating HPC and ATP were drawn from the studies' findings:

- **ATP and HPC values generally do not correlate well.**
  Results of HPC and ATP correlations varied widely between the different studies, ranging from R=-0.13 to 0.93 (R=1 signifies perfect positive correlation, R= -1 signifies perfect negative correlation, and R=0 signifies no correlation). In general, however, HPC and ATP do not appear to correlate well, which can be expected considering these are fundamentally different tests which target different microbial populations (Whalen et al., 2018).
- **Cleaner water generally corresponds to weaker correlations between HPC and ATP.**
  Studies finding the highest correlation ($R > 0.8$) tested waters that had high HPC values (maximum HPC up to 10^5–10^6 CFU/mL), whereas studies observing weak correlations ($R < 0.4$) all used waters with comparatively low HPC values (maximum 650 CFU/mL). Poor correlation of HPC and ATP in more pristine water could be attributed to the higher sensitivity of ATP assay: since HPC only captures a small portion of microorganisms, water with low biological activity might be non-detectable for HPC but still register a

range of values for ATP. This would contribute towards a weak correlation between the two parameters.

- **HPC methods with a low upper limit might reduce the strength of observed correlation between HPC and ATP.**
  Studies with a maximum detection limit of 200–300 CFU/mL likely did not have enough resolution to measure samples with higher biological activity, which would contribute towards a weaker correlation. For instance, Hammes et al. (2010) tested samples with a high range of ATP (up to 10^9 pg/mL, or 10^6 microbial equivalents [ME] per mL) but an HPC upper limit of only 300 CFU/mL and reported one of the lowest correlations among the studied papers (R=0.06).

3.2 The value of ATP testing

Despite the poor correlation between ATP and HPC, two points should be highlighted on how ATP may be superior to HPC for monitoring biological activity in distribution systems:

- **Higher sensitivity of ATP allows earlier identification of bacterial regrowth risk than HPC**.
  A two-year longitudinal study by Prest et al. found that ATP values at the treatment plant effluent (i.e., immediately after disinfection with chlorine dioxide) rose in response to increased biological activity associated with increased temperatures during summer months, while the same was not observed for HPC (remained non-detectable). Regrowth was confirmed with elevated HPC and ATP at a downstream sample point (the distribution system [Rotterdam area, The Netherlands] does not maintain residual disinfectant in the distribution network). In other words, ATP testing was sensitive enough to identify elevated bacteriological activity in the treatment effluent, whereas HPC was not able to detect the same until the bacteria had more residence time to proliferate in the distribution network.

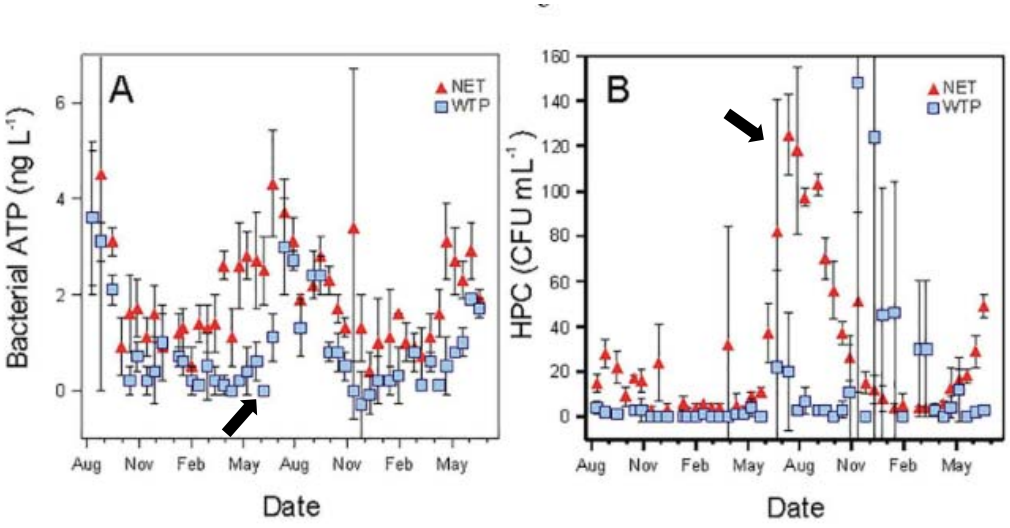

Figure 1: Bacterial (cellular) ATP (Figure 1A) and HPC (Figure 1B) results of treatment plant effluent (WTP) and distribution network sample point (NET), from a two-year study (2012-2014) on a full-scale treatment plant and distribution system in the Rotterdam area of the Netherlands. Elevated ATP at the treatment plant effluent from May to November 2013 (arrow, Figure 1A) corresponded to elevated HPC in the distribution system (arrow, Figure 1B), but no increase in HPC could be observed in the treatment plant effluent. Figure taken from Prest et al. (2016). Arrows added by author.

This long-term study by Prest et al. also illustrates how ATP data is most useful for trend analysis, i.e., a baseline is established for the system from which deviations can then be observed. This philosophy is consistent with Health Canada's guidance on monitoring bacteriological test data (such as HPC) for abnormal changes, rather than prescribing numerical standards.

- **Comparing ATP to other parameters suggest ATP is a more accurate assessment of bacteriological activity than HPC.**

  Several studies compared ATP and HPC results with cell counts obtained via flow cytometry (established method which measures light scattering of fluorescently-labelled cells for rapid cell count). ATP was generally found to correlate better to flow cytometry than HPC, e.g., Nescerecka et al. (2014) determined R=0.87 for ATP and flow cytometry (n=49), versus R=0.42 for HPC and flow cytometry (n=38; coefficients reported as R^2). Several studies also compared ATP and HPC with residual chlorine, again finding a stronger (negative) correlation of ATP than HPC to the same, e.g., Kennedy et al. (2021) determined R= -0.77 for ATP and free chlorine versus R= -0.22 for HPC and free chlorine in a chlorinated distribution system (n=21; R values are Spearman, i.e., nonlinear, correlation coefficient).

Table 3: Summary of literature review on HPC and ATP comparison studies involving drinking water. ATP converted to ME/mL based on 1000 pg/mL = 1 ME/mL (Luminultra). PCA = plate count agar, YEA = yeast extract agar, R2A = Reasoner's 2 agar. R values are Pearson correlation (linear) unless otherwise indicated as Spearman (nonlinear).

Country	Source water	ATP system	HPC media	Correlation	ATP Range (ME/mL)	HPC Range (CFU/mL)	Sodium Thiosulfate	Reference
USA	Chlorinated[a] drinking water (usage point)	Luminultra QGA	R2A	R=0.90 n=106	40–1x10^5	2–3x10^5	Yes	(Duda et al., 2015)
	Chlorinated drinking water (distribution system)	ProMega BacTiter-Glo	-[b]	R=0.13 (Spearman) n=21	10–6000	0.01–2.3	Yes	(Kennedy et al., 2021)
	Chloraminated drinking water (distribution system)	ProMega BacTiter-Glo	-[b]	R=0.37 (Spearman) n=61	10–15000	0.01–24	Yes	(Kennedy et al., 2021)
	Drinking water	New Horizons Diagnostics	R2A	R=0.93 n≈50	-[c]	5–1x10^6	No	(Deininger & Lee, 2001)
Switzerland	Non-chlorinated[a] drinking water (usage point)	Promega BacTiter-Glo (total ATP)	PCA	R=0.56[d] n=200	1000–48000	1–16000	No	(Siebel et al., 2008)
	Non-chlorinated drinking water (usage point, bottled water)	ProMega BacTiter-Glo (total ATP)	R2A	R=0.51[d] n=27	15000–55000	50–9000	No	(Berney et al., 2008)
			PCA	R=0.17[d] n=27	15000–55000	1–630	No	(Berney et al., 2008)
	Surface water, groundwater, non-chlorinated drinking water (usage point), wastewater effluent	ProMega BacTiter-Glo	R2A	R=0.06[d] n=102	250–1x10^6	30–300[e]	No	(Hammes et al., 2010)

Country	Source water	ATP system	HPC media	Correlation	ATP Range (ME/mL)	HPC Range (CFU/mL)	Sodium Thiosulfate	Reference
France	Chlorinated drinking water (distribution system)	New Horizon Diagnostics	R2A	R=0.60 n=64	2–4000	5–10000	No	(Delahaye et al., 2003)
Netherlands	Non-chlorinated[f] drinking water (effluent, usage point)	Celsis	YEA	R=-0.13 n=175	1000–4500	1–140	No	(Prest et al., 2016)
	Non-chlorinated drinking water (treatment system effluent, usage points)	Celsis (total ATP)	R2A	R=0.44[d] n=56	500–13000	1–4500	No	(van der Wielen & van der Kooij, 2010)
Latvia	Chlorinated drinking water (distribution system)	Promega BacTiter-Glo	PCA	R=0.33[d] n=38	0-4.6x10^5	1–220	No	(Nescerecka et al., 2014)
Various	Drinking water	New Horizons Diagnostics	R2A	R=0.86 n≈120	- [c]	1–3x10^5	No	(Deininger & Lee, 2001)

a - not specified by authors
b - IDEXX Quanti-Tray for HPC (does not use culture media)
c - ATP results only reported in RLU
d - authors reported result in R^2
e - detection limit
f - treatment system uses chlorine dioxide for disinfection but no residual disinfectant is maintained in distribution system

4 STUDIES BY CANADIAN UTILITIES

Several Canadian utility operators were invited to share information on ATP and HPC investigations conducted on their distribution systems. Responses were received from Halifax Water and Montreal's Service de l'environnement (regarding a joint study involving multiple Quebec municipalities). Findings from their work are discussed below.

4.1 Halifax Water

4.1.1 Background and methods

Halifax Water provides potable water to 360,000 residents in the city of Halifax (Nova Scotia, Canada) and surrounding areas through nine water treatment systems and a distribution network approximately 1,570 km in water main pipe length (Halifax Water, n.d.-b, 2021). Various treatment technologies are employed in the nine systems, including disinfection by either chlorine gas or sodium hypochlorite (bleach) injection. Residual chlorine is maintained above 0.2 mg/L in the distribution system using sodium hypochlorite (Halifax Water, n.d.-a)

Halifax Water collected ATP data from 11 sampling sites in their distribution system network from January to November 2021, for a total of n=283 samples. This dataset was shared along with HPC and free chlorine results. HPC was conducted by an external laboratory using the spread plate method and R2A media. ATP testing was conducted by Halifax Water using Luminultra QGA. ATP samples were not quenched with sodium thiosulfate.

4.1.2 Results and discussion

Water samples collected by Halifax Water are relatively low in microbial activity: 67% (n=190) are non-detectable for HPC (<1 CFU/mL), and only 2% (n=7) exceed the relatively low upper limit of 250 CFU/mL (compared to studies showing high HPC-ATP correlation, e.g., Duda et al. (2015) with maximum HPC in the range of 10^5 CFU/mL). Median ATP is 0.20 ug/mL and ranges from 0 to a maximum of 7.9 pg/mL (7900 ME/mL), which is still within the recommended limit of 10 pg/mL for corrective action (McIlwain, 2020). ATP demonstrates a higher sensitivity than HPC, as expected, with only 12% of samples (n=34) registering 0 pg/mL (i.e., signal below background [blank] level).

Results show a weak correlation between HPC and ATP (R=0.31, n=283; Figure 2). Excluding samples below and/or above the HPC detection limit (<1 CFU/mL and >250 CFU/mL) does not improve the data fit.

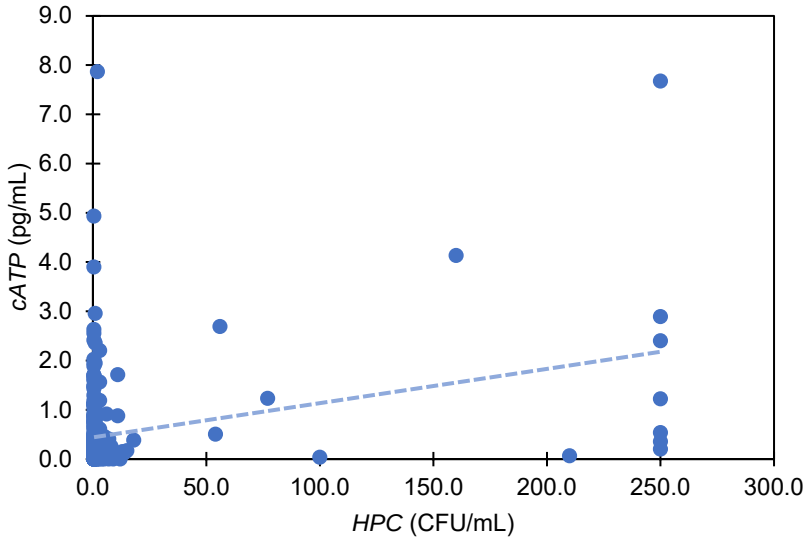

Figure 2: cATP versus HPC for Halifax Water distribution system samples collected January to November 2021. R=0.31 (n=283).

Free chlorine in the samples ranges from 0.02 to 1.47 mg/L (median: 0.57 mg/L). ATP and free chlorine shows a weak negative correlation (R= -0.10), which is at least trending in a more logical direction than HPC and free chlorine which shows a weak *positive* correlation (R=0.12). ATP and HPC results are plotted against free chlorine in Figure 3.

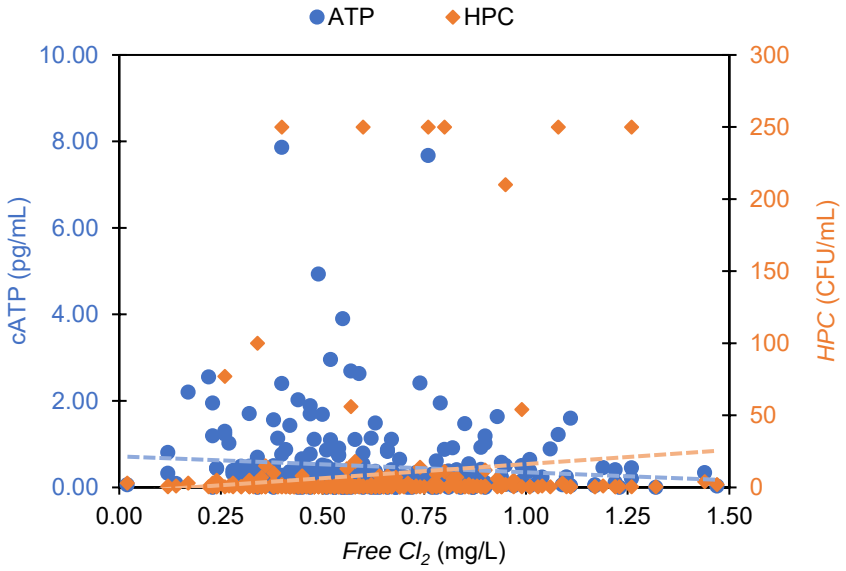

Figure 3: cATP and HPC versus free chlorine for Halifax Water distribution system samples collected January to November 2021. cATP versus free chlorine R= -0.10, HPC versus free chlorine R=0.12 (n=283).

4.1.3 Conclusion and next steps

The study concludes that ATP testing is superior to HPC, as it is more sensitive than HPC (often non-detectable or significantly elevated but rarely in-between) and correlates better to free chlorine. The path forward for Halifax Water is to continue monitoring both HPC and ATP in the distribution system, with the intention of phasing out HPC to fully replace with ATP within a year of this writing.

4.2 Quebec municipalities

4.2.1 Background and methods

As part of PEXEP-D (Programme d'excellence en eau potable – Distribution), five Quebec municipalities (Laval, Longueuil, Montreal, Sherbrooke, Quebec City) collaborated in evaluating ATP assays for water quality assessment of their potable water distribution systems.

The study objectives included:

- Compare ATP and HPC (Bactéries hétérotrophes aérobies et anaérobies; BHAA) to free chlorine in distribution system samples.
- Evaluate replicability of ATP tests.

- Compare two different ATP systems: Luminultra QGA (Fredericton, Canada) and GL Biocontrol Dendridiag (Clapiers, France).

Distribution system samples were collected from each utility from 2019 to 2020, capturing both cold (<5°C) and warm (>20°C) weather periods. A total of n=133 distribution system samples were collected among the five participating utilities. For evaluating ATP replicability, four tests of 10-15 replicates per test were performing using both Luminultra QGA (Fredericton, Canada) and GL Biocontrol Dendridiag (Clapiers, France). ATP samples were collected in bottles precharged with sodium thiosulfate. HPC was performed using the pour plate method by adding 1 mL sample to 15 mL of warm R2A media.

4.2.2 Results and discussion

ATP is found to correlate better to free chlorine than HPC to free chlorine. A significant difference can be observed in ATP levels between samples with free chlorine ≥ 0.1 mg/L versus < 0.1 mg/L (Figure 4A). However, the same is not observed for HPC (Figure 4B). ATP is also found to be more sensitive than HPC, which is non-detectable for 45% of the samples.

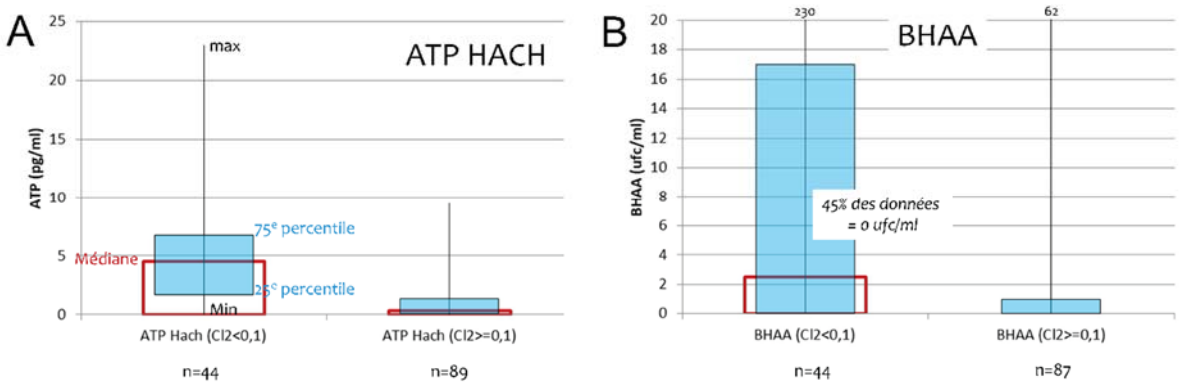

Figure 4: Box and whisker plots of ATP (Fig. A) and HPC (Fig. B) at free chlorine (Cl_2) concentrations below and above 0.1 mg/L, from distribution system samples in five Quebec municipalities collected 2019-2020. ATP results shown for Luminultra QGA (distributed by Hach). Taken from (Besner, 2021)

ATP replicability tests are largely favorable. Standard margin of error for ATP results using Luminultra QGA is determined to be 17-18% (t-test 95% confidence interval divided by sample mean) for three out of the four tests, while tests using GL Biocontrol Dendridiag results in a standard margin of error range of 29-40% (Besner, 2021). One of the tests using Luminultra QGA resulted in a high standard margin of error of 142%, though it should be noted that the

sample had a low average ATP of 0.23 pg/mL which may have contributed towards inflating the error result.

4.2.3 Conclusion and next steps

The study concludes that ATP is more sensitive than HPC and can be a better tool for bacteriological monitoring, particularly at sites with low residual chlorine (< 0.1 mg/L). Further testing for 12 weeks is scheduled to begin summer 2022. Samples will be taken from the treatment plant effluent and distribution network sample sites, and ATP results will be compared between samples with free chlorine concentrations above and below 0.05 mg/L (i.e., expanding to a lower value than the previously investigated 0.1 mg/L).

4.3 Key takeaways

The following observations were drawn from the two utility studies:

- **ATP is more sensitive than HPC.**
  ATP can often produce readings for samples below HPC detection limits, which allows for more sensitive baseline determination and trend analysis.
- **ATP correlates better to free chlorine than HPC to free chlorine**.
  This finding is consistent with other studies of HPC and ATP tests in chlorinated (Kennedy et al., 2021; Stoddart, 2020) and chloraminated (Stoddart, 2020) distribution systems.

5 GVWD PRELIMINARY ATP TESTING

Preliminary testing of ATP assay for MetroVancouver was conducted from June to July 2022, during which select sites along the GVWD water transmission system were analyzed for ATP in addition to routine chemical and bacteriological parameters. Details of this study are discussed below.

5.1 Objectives

The two main goals of the preliminary study are as follows (same objectives presented in section 3 but with additional specifications):

- **Determine if ATP assay is equally or more *accurate and reliable* than HPC for bacteriological monitoring of GVWD transmission system**
  This will include assessing the correlation of ATP to HPC (likely to be poor), as well as comparing how ATP and HPC correlate with other parameters (i.e., free chlorine and turbidity).
- **Assess the validity of an adapted procedure for ATP sampling that is more *operationally feasible* for MetroVancouver**
    - Sodium thiosulfate: use of chlorine quench such as sodium thiosulfate is not recommended by the test kit manufacturer (Luminultra, 2019). As the bacteriological tests conducted by MetroVancouver use bottles precharged with thiosulfate, being able to do the same for ATP will greatly facilitate the incorporation of ATP testing into MetroVancouver's procedures (i.e., avoid the need to wash and prepare separate bottles for ATP). MetroVancouver is therefore interested in evaluating the viability of ATP assessment when samples are quenched with thiosulfate.
    - Hold time sensitivity: ATP tests are best conducted immediately after collection as ATP concentration in the sample can start to change immediately, but having sampling staff perform ATP extractions in the field is not necessarily practical. While test kit manufacturers state ATP extraction within 24 hours is acceptable if samples are kept cool (5°C), an assessment of time dependency on ATP results will be conducted to confirm whether consistent results can be obtained if ATP is analyzed at different times within 24 hours of collection.

5.2 Materials and methods

14 transmission system sampling points were selected for testing, seven from each of the filtered and unfiltered sources. Sites were chosen to cover the range of HPC and free chlorine concentrations typically encountered in the system, and include untreated source water, treated water at the system effluent, and different locations along the transmission network. Samples were collected for three weeks from June to July 2022. Samples analyzed for ATP were collected in plastic Nalgene bottles, either with or without sodium thiosulfate precharge, and tested using Luminultra QGA kits and luminometer (Luminultra PhotonMaster) in accordance with

manufacturer instructions (Appendix). Samples were brought back to the laboratory, extracted (lysed) within 24 hours, and transported to UBC for analysis within 7 days of extraction. Samples and lysate extracts were stored at 5°C and transported in coolers with cold packs. Four sites with historical high/low free chlorine/HPC were selected and analyzed in triplicate for variance studies. Samples analyzed for HPC were collected in plastic Nalgene bottles precharged with sodium thiosulfate. Samples were also analyzed for free chlorine and turbidity from other bottles.

5.3 Results and discussion

5.3.1 Thiosulfate quench and analysis within 24 hours are acceptable practices

Samples were initially collected in both thiosulfate-charged (quenched) and empty (unquenched) bottles and extracted at 6 and 24 hours after collection, as well as 4 hours after collection when possible (samples arrive to lab 4-6 hours after collection depending on samplers' routes). Due to scheduling and sampling issues, only seven of the 14 sites were successfully sampled under both quenched and unquenched conditions. Main ATP results from the quenching and time dependency study are presented in Table 4.

Table 4: ATP results and analysis for samples collected to study thiosulfate quench and extraction time impacts. Values shown are mean ± one standard deviation except cATP. Median cATP, percent difference (calculated as unquenched minus quenched), and agreement with guidelines are calculated from a single time extraction per sample, for n=7 pairs of quenched and unquenched samples. Coefficient of variance is calculated as the standard deviation divided by mean for samples analyzed in triplicates, at all extraction times. More sites were sampled for quenched than unquenched hence differences in n.

	Quenched	**Unquenched**
Median cATP (pg/mL)	0.53 (IQR 0.09–20.2; n=7)	0.32 (IQR 0.25–11.6; n=7)
% difference	29 ± 115% (n=7)	
Coefficient of variance	38 ± 15% (n=19)	27 ± 21% (n=7)
% change from 4hr to 6hr	-50 ± 79% (n=5)	-33 ± 34% (n=3)
% change from 6hr to 24hr	+11 ± 78% (n=13)	-30 ± 46% (n=7)

Median cATP results for quenched and unquenched samples are comparable (0.53 ug/mL and 0.32 pg/mL, respectively), with a larger interquartile range for quenched compared to unquenched samples (n=7). Most notably, the percent difference of quenched/unquenched

(unquenched 29% higher, on average) is comparable with the coefficient of variance calculated from samples analyzed in triplicates from both quenched and unquenched samples (38% and 27%, respectively, which is consistent with other studies e.g., Paul et al. (2014) who determined a variance of 32 ± 16%). As such, any difference in quenched versus unquenched ATP can likely be attributed to inherent variation of ATP tests, though this should be caveated by the large standard deviation of the percent difference value (±115%). Furthermore, 100% of the quenched/unquenched sample pairs (n=7) agree with regards to the ranges for ATP interpretation (Table 2), i.e., quenched samples with cATP <1 pg/mL are also <1 pg/mL when unquenched, etc. Therefore, in all samples analyzed, the same conclusion will be reached for either quenched or unquenched cases.

In terms of time dependency for ATP, results suggest quenched samples decay from 4 to 6 hours after collection (-50%) but the change is less significant from 6 to 24 hours (+11% which is less than the variance of 38% determined for quenched samples). In comparison, unquenched samples decay consistently (around -30%) throughout both time periods to an extent that is slightly above variance (27%). However, all these percent changes over time are still within one standard deviation of the coefficients of variance and are therefore arguably insignificant. Moreover, in all cases, ATP results at all extraction times are consistent with regards to the interpretation ranges, i.e., samples with cATP >10 pg/mL at 4 hours after collection continue to indicate cATP >10 pg/mL at the 6-hour and 24-hour extractions. Therefore, the same conclusion will be reached when analyzed at any time point within 24 hours.

From these results, it is concluded that quenching with thiosulfate and analyzing within 24 hours are acceptable practices which MetroVancouver can adopt for ATP testing.

5.3.2 ATP is consistent with and more sensitive than HPC

Following the conclusion discussed in section 5.3.1, additional samples from the 14 selected sites were collected in the remaining weeks of the study using bottles precharged with thiosulfate (quenched) and analyzed within 24 hours of collection. A total of n=40 samples were collected and analyzed for ATP, as well as HPC, free chlorine, and turbidity.

Median cATP is 0.29 pg/mL and ranges from 0.01 pg/mL to 50.3 pg/mL (untreated source water), which is on the same order of magnitude as the results from Halifax and Quebec municipalities. HPC ranges from <2 CFU/mL to 400 CFU/mL, with 63% (n=25) samples non-

detectable; these values are again comparable to the other Canadian utilities. As expected, ATP proves to be a more sensitive test compared to HPC (none of the samples are non-detectable for ATP).

HPC are plotted against cATP in Figure 5, and correlations (R values) between ATP, HPC, free chlorine, and turbidity are presented in Figure 6:

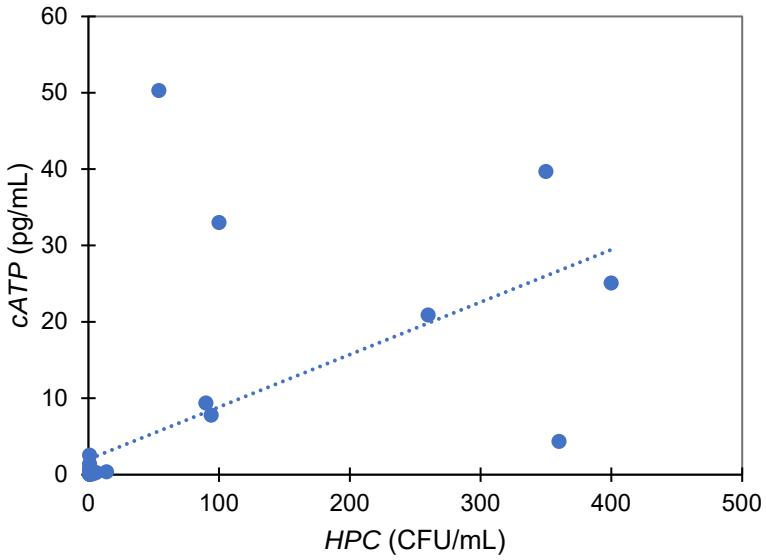

Figure 5: HPC versus cATP for samples collected from MetroVancouver transmission system in 2022 (n=40).

	HPC	Free chlorine	Turbidity
cATP	0.61	0.05	0.08
HPC		0.08	0.08
Free chlorine			-0.14

Figure 6: Correlations between cATP, HPC, free chlorine, turbidity, for samples collected from MetroVancouver transmission system in 2022 (n=40). Idea for correlation matrix from (Kennedy et al., 2021).

Unexpectedly, HPC-ATP shows a moderate correlation (R=0.61), higher than that reported by Halifax (R=0.31) and most of the studies reviewed (Table 3). Also unexpectedly, ATP does not correlate better to free chlorine than HPC, with both parameters showing poor correlations

(R=0.05 and 0.08 for ATP and HPC, respectively). The short duration of the study limits other analyses of the accuracy and reliability of ATP data, which should include assessing ATP over seasonal and temperature changes (sample temperatures are between 8-14°C) as well as analyzing trends (e.g., establishing baselines and identifying whether deviations in ATP are consistent with observations from other parameters).

While guidelines do not exist for HPC values, an approximation definition for high HPC might be considered as 100 CFU/mL based on HPC standards employed in some European countries (US EPA guideline for HPC of 500 CFU/mL is generally too high to be relevant for GVWD; none of the samples collected in this study exceed 500 CFU/mL). This can be compared to the 10 pg/mL cut-off ATP value for prescribing corrective action (Table 2). Per this interpretation, all but one of the samples (n=39, or 98%) agree between HPC and ATP, with the one exception being a high ATP (40 pg/mL) corresponding to a low HPC (54 CFU/mL).

Overall, ATP appears to agree with HPC for MetroVancouver samples, but there is otherwise little evidence to support the use of ATP for bacteriological monitoring of the GVWD transmission system.

5.3.3 Other considerations for ATP testing

Two main operational considerations have been identified through the ATP pilot study:

- Plastic waste: ATP test kits utilize a significant amount of single-use disposable plastic (including pipette tips, filters, syringes), especially compared with HPC testing by MetroVancouver for which the only single-use component are petri dishes.
- Cost: ATP test kits are expensive, partly due to reliance on proprietary consumables and reagents. For utilities that outsource HPC analyses to external laboratories (e.g., Halifax Water), the price difference is likely less significant than utilities such as MetroVancouver which perform in-house HPC analyses.

These factors should be considered in evaluating the feasibility of switching to ATP.

6 CONCLUSION AND RECOMMENDATIONS

ATP testing can feasibly replace HPC testing for bacteriological monitoring of the GVWD transmission system, given the following findings from literature studies and tests by other Canadian utilities:

- ATP is a *more accurate indication* of bacteriological activity than HPC, when corroborated against other parameters such as flow cytometric cell counts and free chlorine concentrations.
- ATP is *more sensitive than* HPC, allowing earlier detection of regrowth and improved trend analysis.

A three-week preliminary test program of ATP sampling for MetroVancouver found:

- ATP is moderately correlated to HPC (R=0.61), which is higher than most literature studies and tests by other Canadian utilities.
- ATP and HPC tests are mostly in agreement (98%) based on the broad categorization of high HPC and ATP as 100 CFU/mL and >10 pg/mL, respectively.
- No other evidence to support the use of ATP as a bacteriological monitoring indicator can be identified from the limited data gathered
- Modifications to ATP sampling protocol to facilitate its implementation by MetroVancouver, particularly the use of thiosulfate quench, should not affect integrity of results.
- Benefits of ATP (faster turnaround time) should be weighed against disadvantages, including increased plastic waste and higher cost of consumables.

If MetroVancouver is interested to further pursue ATP testing, the following is recommended:

- Preliminary ATP testing of the 14 selected sites should be conducted for six months or more, to capture seasonal variations, establish baseline values for ATP, and corroborate trends against other parameters.
- If extended preliminary ATP testing confirms the validity of ATP, a phase-in period of six months to a year is recommended during which both ATP and HPC will be sampled.

HPC should only be discontinued once baseline ATP values and what constitutes abnormal deviations are established.

- Measures to reduce waste should be explored with the test kit manufacturer.

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APPENDIX

Luminultra QGA test kit instructions

LUMINULTRA®
microbial monitoring

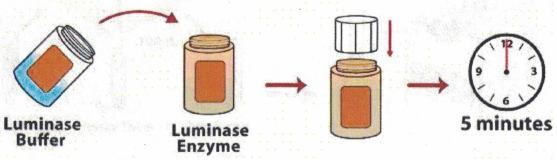
Test Kit Instructions
QuenchGone™ Aqueous (QGA)

PROVIDED

Kit Component	Reagent Volume / Weight	Storage
Luminase Enzyme & Buffer Vials	3mL	20°C
UltraCheck 1 Dropper Bottle	5mL	20°C
UltraLyse 7 Bottle	125mL	20°C
UltraLute (Dilution) Tube	9mL	20°C
Quench-Gone Syringe Filters	-	-
60mL Syringe, PP/Neoprene	-	-
100 to 1250uL Blue Pipet Tips, 96 Rack	-	-
1 to 200uL Yellow Pipet Tips, 96 Rack	-	-
12x55mm Test Tubes	-	-

REHYDRATING LUMINASE

- Gently mix the buffer and **Luminase** enzyme.
- Wait 5 minutes for solution to dissolve.

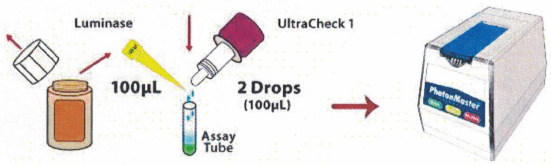


1. ULTRACHECK CALIBRATION (RLU_{ATP1})

- Hold the **UltraCheck1** bottle vertical, **add** 2 drops (100µL) of **UltraCheck1** to a 12x55mm test tube.

Note: Once **UltraCheck 1** is opened, it must be used within 3 months. After 3 months, discard and use a new bottle.

- Pipet 100µL of **Luminase** into the tube.
- Swirl the tube and take reading within 10 seconds.

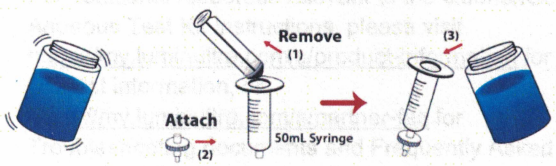


* If $RLU_{ATP1} \leq 5{,}000$ rehydrate a new bottle of Luminase.

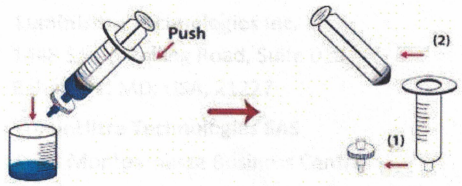
2. CELLULAR ATP ANALYSIS (RLU_{cATP})

2.1 FILTRATION

- Mix sample well.
- Remove the plunger from a 50mL syringe.
- Attach the filter.
- Pour 20-50 mL of sample into the syringe.

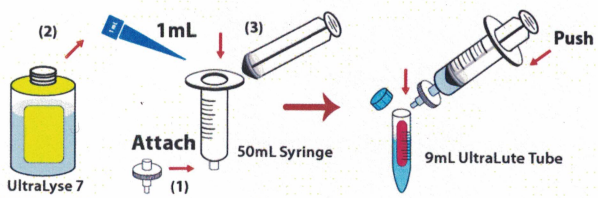


- Slowly push sample through the filter into waste receptacle.
- Detach the filter.
- Remove the plunger.



2.2 EXTRACTION

- Re-attach filter.
- Add 1mL of **UltraLyse 7** to the syringe.
- Filter slowly and collect in a new **9mL UltraLute (Dilution) Tube**.
- Cap and invert three times to mix.



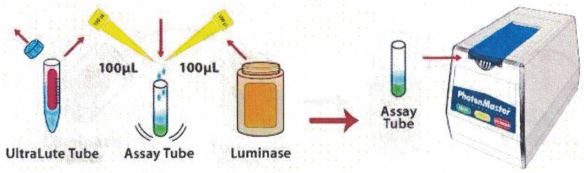
2.3 BACKGROUND MEASUREMENT

- Pipet 100µL of the **Luminase** into a new, clean 12x55mm test tube.
- Put the test tube with **Luminase** into your luminometer and take a reading.
- Record the reading as *Background RLU*. This reading should be below 20 RLU. This tube can then be used for your sample assay (Step 2.4).

TIP: If the results are above 20 RLU, this indicates that some external factors (light, contamination) may be influencing your results. LuminUltra offers cleaning kits for your luminometer. Please contact us to discuss.

2.4 ASSAY

- Add 100µL of the **UltraLute (Dilution)** solution to a 12x55mm test tube.
- Use a new pipet tip to add 100µL of **Luminase** from step 2.3 into the test tube
- Swirl the tube and take reading within 10 seconds.



Calculations

To automatically calculate ATP, use **myLuminUltra Test + Analyze**.

Cellular ATP (**cATP**) represents the amount of ATP contained within living cells and is a direct indication of total living biomass quantity.

$$cATP\ (pg\ ATP/mL) = \frac{RLU_{cATP}}{RLU_{ATP1}} \times \frac{10{,}000\ (pg\ ATP)}{V_{Sample}\ (mL)}$$

Data Interpretation Guidelines

Sample Type	Good Control (pg cATP/mL)	Corrective Action (pg cATP/mL)
High-Purity Water	<0.1	>1.0
Potable Water	<1	>10
Cooling Water *Oxidizing Biocides*	<10	>100
Cooling Water *Non-Oxidizing Biocides*	<100	>1,000

ADDITIONAL RESOURCES

- For additional resources relevant to the QuenchGone Aqueous Test Kit Instructions, please visit https://my.luminultra.com/s/product-information for further product information, or https://my.luminultra.com/s/partner-faq for Troubleshooting documents and Frequently Asked Questions (FAQ).

ORDERING INFORMATION

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