Application Note

Validation of QGA™ Test Kit for Drinking Water Applications

Background

KWR Watercycle Research Institute (Nieuwegein, The Netherlands) has a long, successful history of applying ATP testing practices to drinking water treatment and distribution infrastructure in the Netherlands. In 2015, KWR and LuminUltra engaged in a study with the aim to validate LuminUltra's 2nd Generation ATP monitoring approach as a possible field-ready methodology that provided laboratory-equivalent results complementary to the tests normally carried out by KWR.

This validation was facilitated by Milispec International (Berkel en Rodenrijs, The Netherlands), who provided training, data analysis and testing materials including a unique field case, pictured in Figure 2.

Objectives

The objectives of this study were to compare LuminUltra's Quench-Gone Aqueous (QGA) test kit combined with its PhotonMaster luminometer to KWR's currently-utilized equipment and methods in the following areas:

1. Compare the limit of detection for each method of measurement.
2. Compare the repeatability of either method.
3. Compare the linearity in response of each method.
4. Compare different mechanisms of RLU to ATP concentration conversion.
5. Finally, evaluate whether or not QGA/PhotonMaster results can be reliably compared to KWR's historical database.

Methods & Materials

Unless otherwise noted, all ATP standards utilized were provided by KWR. All QGA-based measurements were performed on the PhotonMaster in accordance with ASTM D4012, and all KWR measurements were carried out using the current methodology.

Figure 1 – KWR Microbiology Laboratory

Figure 2 – 2nd Generation ATP Field Equipment

Results & Discussion

Low Detection Limit

Through multiple experiments, it was consistently demonstrated that the limit of detection for the LuminUltra QGA reagent system is 0.1 ng/L (0.1 pg/mL) or lower when using a series of controlled ATP standards.

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In one experiment data was collected for eight standards measured in nine-fold and a student's t-test performed to determine if results were significantly different at any concentration.

Table 1 – QGA [ATP] vs. Average & StDev

[ATP] (ng/L)	RLU_{Avg}	RLU_{StDev}
0.0	6.8	0.7
0.10	10.6	1.7
0.25	15.7	1.7
0.50	24.8	1.5
0.75	31.7	1.5
1.0	45.3	3.2
2.5	93.6	6.7
5.0	179.8	8.7

Table 2 – t-Test Data (☺ = p<0.05)

[ATP] (ng/L)	0.0	0.10	0.25	0.50	0.75	1.0	2.5	5.0
0.0	-	☺	☺	☺	☺	☺	☺	☺
0.10	☺	-	☺	☺	☺	☺	☺	☺
0.25	☺	☺	-	☺	☺	☺	☺	☺
0.50	☺	☺	☺	-	☺	☺	☺	☺
0.75	☺	☺	☺	☺	-	☺	☺	☺
1.0	☺	☺	☺	☺	☺	-	☺	☺
2.5	☺	☺	☺	☺	☺	☺	-	☺
5.0	☺	☺	☺	☺	☺	☺	☺	-

It was noted that this low detection limit of 0.1 ng/L or better is at least ten times more sensitive than the method used by KWR.

Repeatability of Results

Controlled ATP standards were utilized to assess the repeatability of the QGA method and the method used by KWR in several experiments.

In one such experiment, a blank and two ATP standards (2 and 100 ng/L respectively) were measured in duplicate with each method.

Table 3 – Repeatability for Three Standards

ATP (ng/L)	KWR RLU_{Avg}	KWR RLU_{StDev}	KWR CV (%)	QGA RLU_{Avg}	QGA RLU_{StDev}	QGA CV (%)
0.0	11	0.7	6.7	5	0.0	0.0
2.0	17	1.4	8.3	85	12.7	15
100	365	4.9	1.4	3420	62.9	1.8

In another experiment, a full range of ATP standards from 0 to 10,000 ng/L were measured with each method.

Table 4 – Repeatability 0 to 10,000 ng ATP/L

ATP (ng/L)	KWR RLU_{Avg}	KWR RLU_{StDev}	KWR CV (%)	QGA RLU_{Avg}	QGA RLU_{StDev}	QGA CV (%)
0.0	13	2	17	6	3	47
1.0	18	1	7.9	46	1	3.1
2.5	24	0	0.0	100	3	2.8
5.0	38	*	*	190	10	5.2
7.5	47	1	1.5	271	5	1.8
10	70	8	12	360	8	2.4
25	118	6	4.8	896	39	4.3
50	243	5	2.0	1795	23	1.3
75	349	24	6.9	2691	69	2.6
100	448	14	3.2	3523	84	2.4
250	1133	17	1.5	8598	76	0.9
500	2207	35	1.6	16236	1661	10
750	3363	25	0.7	26916	272	1.0
1000	4461	116	2.6	34671	105	0.3
10000	44659	1571	3.5	349637	4322	1.2

In both cases, the repeatability of either method was found to be very similar.

Method Linearity

The data from Table 4 was also used to evaluate linearity of each method.

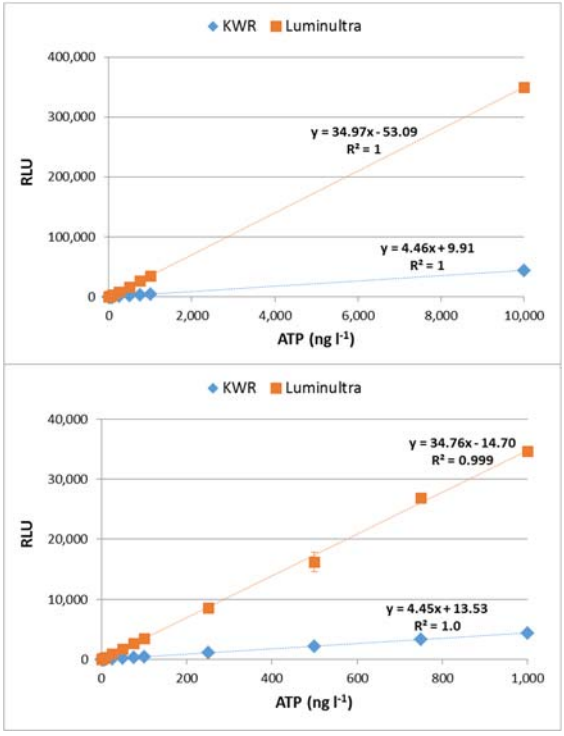

Figure 3 – Linearity of Each Method (Top: 0 to 10,000; Bottom: 0 to 1,000)

It was found that for both methods, linearity was achieved over both the range of 0 to 1,000 ng/L and 0 to 10,000 ng/L, with the former range having slightly superior relationship between RLU and ATP concentration. The slope factor of the QGA/PhotonMaster was approximately eight to nine times higher than for the method used by KWR, confirming the previous conclusions on limit of detection.

ATP Standard Conversions

The calculation of ATP concentration from luminometer RLU values is a critical step in creating reliable ATP data. Both the QGA method and the method KWR uses follow this practice in order to eliminate any noise caused by factors such as luminometer variation, temperature variation, enzyme age, enzyme potency, or matrix inhibition effects.

The methodology used by KWR operates under a highly controlled environment with substantial attention paid to equipment maintenance and a routine calibration program involving the preparation of frozen ATP standards and comparison of current calibration curve to those obtained historically. The average slope factor from the historical database is then used to convert RLU to ATP concentrations. Such an approach is by far the most technically superior, but it is not necessarily convenient for field application.

LuminUltra's QGA test kit is unique in the marketplace in that it is supplied with a liquid-stable dropper bottle of 1 ng/mL (1,000 ng/L or 1,000 pg/mL) ATP standard, which the user is asked to use each time they perform testing so that they can convert raw RLU readings into actual ATP concentrations. This is a considerably easier approach, but one that operates on the assumption of the assay system enzyme and luminometer is always providing a linear response.

Operating under the assumption that a full ATP calibration curve done on the same day as sample measurements is the "gold standard", a number of drinking water samples were tested and subsequently computed according to a slope factor generated from a full calibration curve versus a single-point ATP standard calculation.

Table 5 – Curve vs. Single-Point ATP Standard

Sample	KWR (ng/L) via Curve	KWR (ng/L) via 1 ng ATP/mL
Sample 42-II	5.7	5.7
Sample 43-II	93.8	93.7
Sample 44-II	2.9	2.9
Sample 45-II	73.9	73.8
Sample 46-II	48.3	48.3
Sample 47-II	7.3	7.3
Sample 48-II	100.3	100.2

The results demonstrate that the ATP concentrations obtained are the same from either approach.

Additionally, the results obtained between pipetting 100µL of ATP standard was compared to LuminUltra's recommended practice of adding 2 drops for the equivalent of 100µL for an ATP standard.

Table 6 – Pipet vs. Dropper for ATP Standard

Replicate	Dropper	Pipet
Replicate 1	22461	21737
Replicate 2	20301	22190
Replicate 3	20045	21371
Replicate 4	21505	21078
Replicate 5	20947	20714
AVG	21052	21418
SD	972	573
CV (%)	4.6%	2.7%

The results demonstrate that while there is slightly more variation introduced through the use of a dropper bottle, its savings of an additional pipetting step is of value to operators performing these tests in the field.

Comparison with Real Samples

Finally, an experiment was performed to directly compare calculated ATP results from both the method used by KWR and the QGA/PhotonMaster material combination on a set of drinking water samples.

Table 7 – KWR vs. QGA ATP Measurements on Real Samples

Method	KWR (ng/L)	QGA (ng/L)	% Diff
Sample 42-II	5.7	6.1	**6.8%**
Sample 43-II	93.7	89.2	**-4.8%**
Sample 44-II	2.9	4.9	**68.6%**
Sample 45-II	73.8	44.0	**-40.4%**
Sample 46-II	48.3	22.0	**-54.5%**
Sample 47-II	7.3	7.1	**-1.8%**
Sample 48-II	100.2	58.2	**-42.0%**
Sample 99	931.9	864.5	7.5%
Sample 03	1059.5	1203.7	**12.7%**
Sample 01	1310.4	1434.8	**9.1%**
Sample AA	22.6	19.1	**16.7%**

Seven of the 11 samples tested yielded ±20% the same result, which in general can be considered very good agreement. Three of the 11 samples show a more than 20% lower result with the QGA test. One explanation may be that the method used by KWR measures total ATP (intracellular + extracellular), whereas the QGA method measures only microbial (intracellular) ATP. One of the 11 samples shows a more than 20% higher result for QGA. However, that result is within the same control range as the method used by KWR, as in the Netherlands a result < 5 ng/L (pg/mL) is considered to be a good control level while >10 ng/L (pg/mL) is considered a high value.

Conclusions

The conclusion of this application note is that the 2nd Generation ATP QGA test kit provides a portable and robust, field-ready compliment to the sophisticated ATP test method used by KWR.