Bacterial Monitoring

Reproducibility and Comparative Methodology

Background:

Bacterial populations in a sample stream, by nature, are difficult to characterize due to their propensity to form clusters with one another or around other suspended particulate matter. The result of this natural behavior is a heterogeneous distribution of organisms which leads to greater variability in measured outcomes when compared to laboratory chemical measurements of well-mixed parameters. This inherent variability is, in part, why agencies like the Oregon Department of Environmental Quality have regulatory limitations based on monthly geometric means and allow for retesting to occur following an unusually high *E.coli* reading (DEQ OAR 340-041-0009). The use of geometric means as opposed to arithmetic means places less significance on single outlying values and returns a lower value. Measurements are typically reported as the 'most probable number' (MPN) which is itself an estimated value based in Poisson statistics. By definition these values have significantly larger errors than other typical laboratory measurements and any one result actually represents a range of possible outcomes and not a single value, furthermore this range will expand as the measured value increases (Oblinger and Koburger, 1975). For example, the Oregon Department of Environmental Quality has used duplicate *E.coli* measurements to calculate a root mean square error for *E.coli* of 0.3Log, translating to a confidence interval from 7.5 to 29.9 for an MPN measurement of 15 MPN/100ml. In other words, a measured value of 15 MPN/100ml is just as likely to be measured on a sample with an actual bacterial population of 7.5 MPN/100ml as it for a sample with a bacterial population of 29.9 MPN/100ml.

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About LiquID™

The LiquID Station from ZAPS Technologies (pictured below) is an innovative, optical instrument for continuous water quality monitoring. The automated online instrument analyzes a continuous flow-through stream from a pressurized water sample line using multi-spectral light and software algorithms, and uses no reagents nor produces any waste other than the original sample (which is returned or wasted as appropriate). With this method LiquID is capable of monitoring a wide range of water quality parameters in a number of different industry applications, including those relevant to municipal water and wastewater treatment, water reuse systems and industrial process control.

Additional studies have been conducted which demonstrate the reproducibility of duplicate analyses for methodologies such as the IDEXX Quanti-Tray approach. Boubetra et al. (2011) published a compilation of duplicate Quanti-Tray analyses for three different *E.coli* abundance ranges (low, medium and high). This study

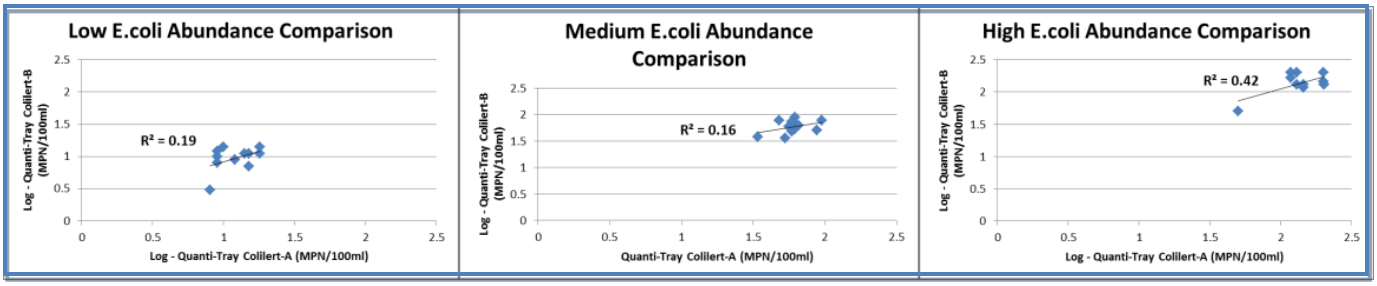


Figure 1A: Duplicate Quanti-Tray measurements plotted for individual population groups [Low (~10 MPN/100ml), Medium (~60 MPN/100ml) and High (~140 MPN/100ml)]. Poor correlations indicate inherent randomness in bacteria sampling and quantification within a restricted population abundance.

was conducted in a joint effort between multiple French National Laboratories and IDEXX Laboratories Inc. the developers of the Defined Substrate Technology (DST) and manufacturer of the Quanti-Tray 2000 test kits. Graphs were reproduced using the data presented in this study and are shown both within individual abundance groups (Figure 1A) and as a complete data set (Figure 1B).

A closer look at the data presented here demonstrates the variability which is inherent in estimating bacteria populations. The poor correlation coefficient for each individual abundance group in this data set is largely the function of the random distribution of bacteria in nature but also includes the mathematically rooted uncertainty associated with MPN estimates.

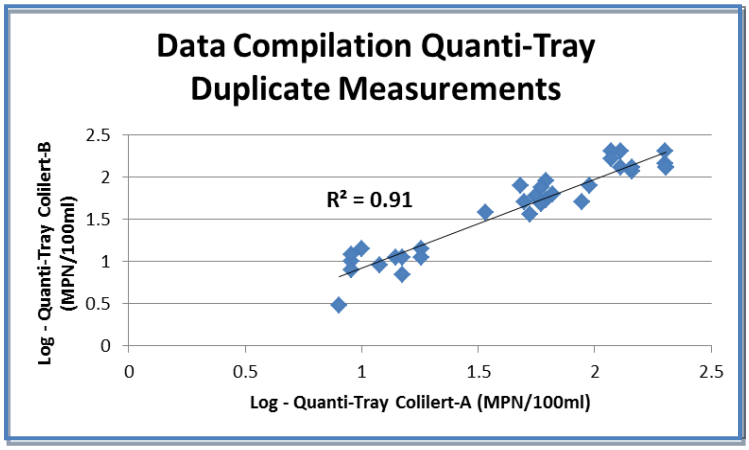


Figure 1B: Data compilation demonstrating a much stronger correlation when evaluated over a larger population range. All data taken from Boubetra et al. (2011).

ZAPS LiquID *E.coli* Output:

Additional uncertainties should also be expected when comparing different methodologies because certain sampling biases can no longer be cancelled, as is the intent of careful sampling protocols defined for a given analytical technique. Taking these factors into account, data generated by the ZAPS LiquID Station can be compared to those data generated by more traditional methods, namely IDEXX Quanti-Tray 2000®. In the diagrams below output from the ZAPS LiquID Station is plotted against grab sample values representative of limited *E.coli* abundances (Figure 2A) and over a wider range of *E.coli* abundances (Figure 2B) collected and

analyzed at multiple certified waste water treatment municipalities. The data set compared in the diagrams below includes 21 time-stamped samples over a similar *E.coli* range investigated by Boubetra et al. (2011).

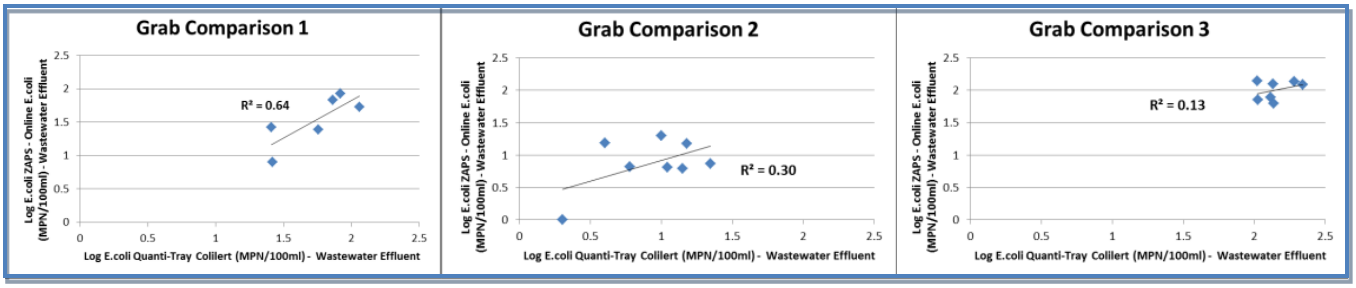


Figure 2A: Data comparing ZAPS LiquID continuous output to certified laboratory Quanti-Tray *E.coli* counts for wastewater effluent samples over limited population abundance. Notice correlations over these limited ranges are equivalent to or better than published duplicate values for the Quanti-Tray method.

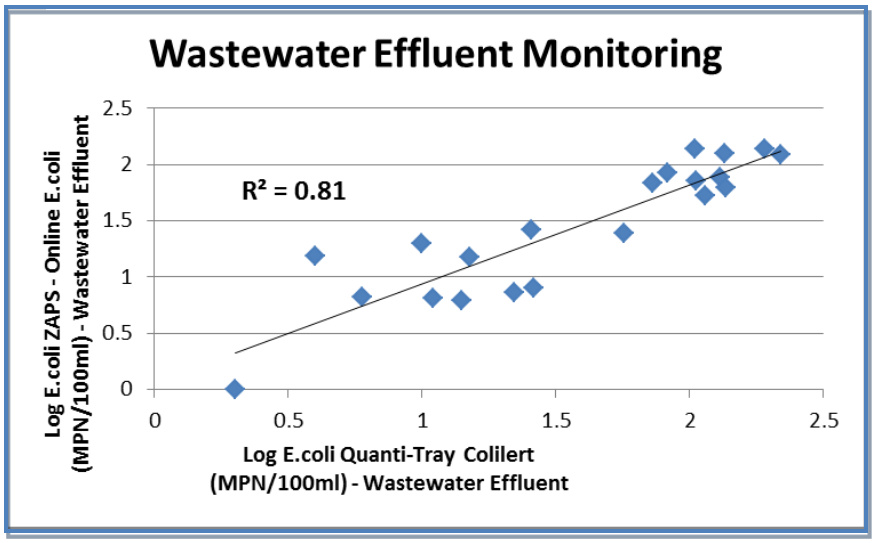


Figure 2B: The same data as in Figure 2A, but plotted as a compilation demonstrating the strong agreement between the ZAPS LiquID output and certified laboratory Quanti-Tray measurements.

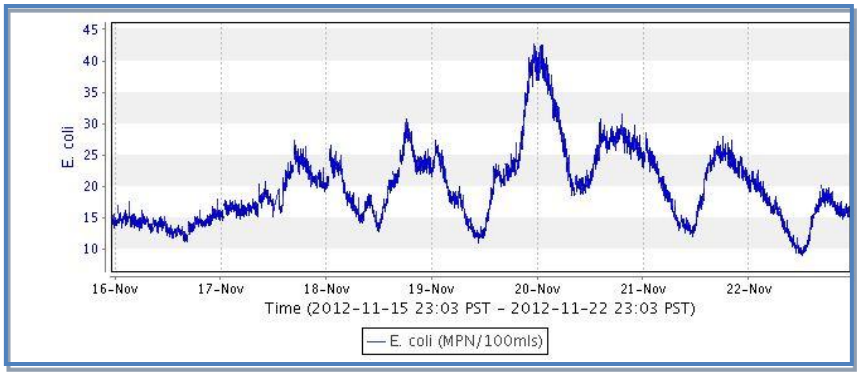
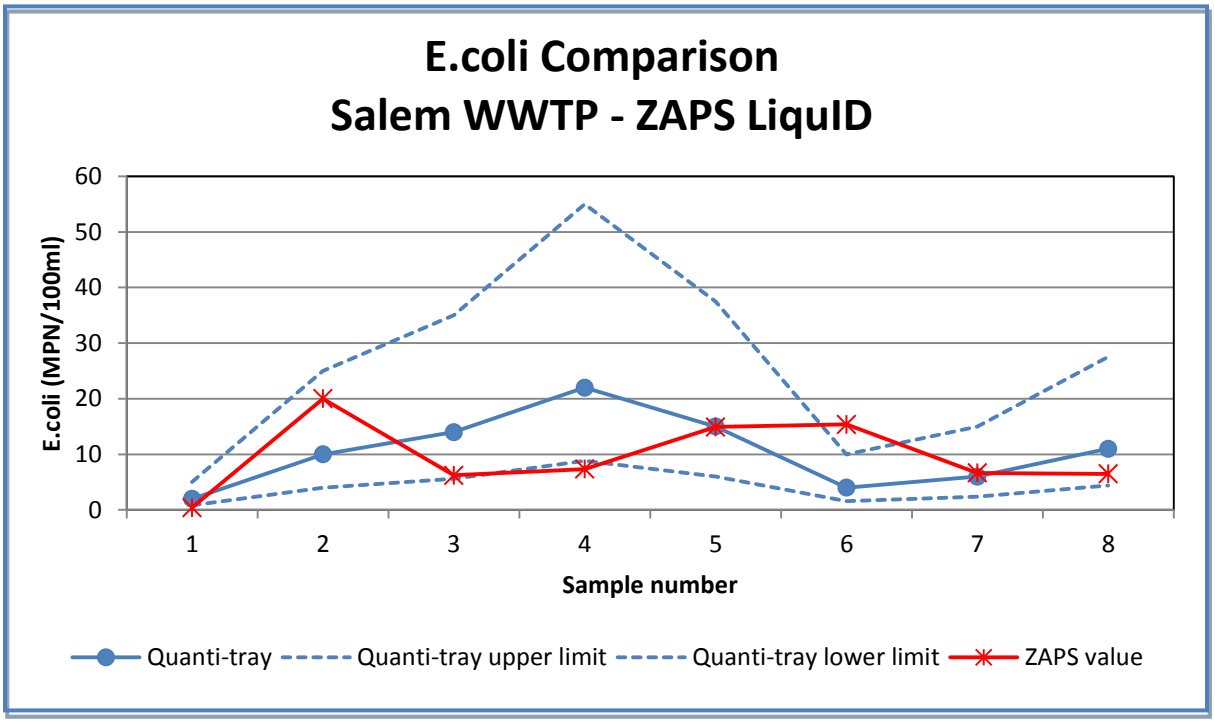


Figure 3: Graphical display of one week of data reported for a signal parameter (*E.coli*) as viewed using the ZAPS Technologies web-user interface (WUI). This one week of data includes 3992 E.coli outputs with a geometric mean for the week of 19.1 MPN/100ml.

ZAPS data (plotted above on the y-axis) represents a single mean value reported by the LiquID at the time of the grab sample. However, each of these mean values is actually calculated from 400 measurements taken each analysis cycle, or about every 2.5 minutes, which allows statistical significance to be placed on each time-stamped output. Additionally, the rapid measurement cycle of the LiquID Station also enables the calculation of average values for varying time intervals giving the user the ability to interpret the difference between natural heterogeneity and an actual significant change in bacterial population. Data from each analysis cycle is reported to the user through a web-user interface (WUI) which updates in near real-time and can be viewed over short (e.g. an half-hour) or long (e.g. a month) durations giving operations managers and scientists a view of how much bacterial communities change over these time intervals. Figure 3 is an example of one week of data collected from the effluent of a wastewater treatment facility and reported via the ZAPS Technology WUI. In total 3992 values were reported over this time interval with a geometric mean of 19.1 MPN/100ml.

Current Case Study:

Focusing more closely on data collected and analyzed at the Salem Wastewater Treatment Facility (Grab Comparison 2, above) and applying an appropriate confidence limit of 0.4Log units the data comparison between the laboratory analyses and the ZAPS LiquID can be more easily visualized.



The diagram presented above demonstrates the agreement between the ZAPS LiquID and the Quanti-Tray laboratory samples in this context. The data presented in this way reveals agreement for 7 of 8 samples in the data set with the lone outlier being sample 6 with a Quanti-Tray value of 4 MPN/100ml (confidence interval of 1.6 to 10 MPN/100ml) and a ZAPS LiquID output of 15 MPN/100ml, which although outside a confidence interval for the Quanti-Tray value does not factor in any variability from sampling etc. Another way to compare the results in this data set is to use them to calculate a geometric mean as is commonly done for regulatory reporting of *E.coli*, doing so for these data results in geometric means of 6.6 MPN/100ml and 8.5 MPN/100ml for the ZAPS output and Quanti-Tray results, respectively.

Regardless of methodology, the quantification of bacterial communities remain, to some degree, an estimate of a naturally heterogeneous distribution which can best be described through increased sampling, a major advantage of the ZAPS LiquID flow through approach.

References:

1. Oregon Bulletin Excerpt, August 1, 2011 – Water Quality Standards – Human Health Toxic Pollutants
2. Oregon Department of Environmental Quality, Willamette Basin Total Maximum Daily Load Program Report, September 21st, 2006
3. Boubetra, A., Le Nestour, F., Allaert, C., and Feinberg, M., Validation of Alternative Methods for the Analysis of Drinking Water and Their Application to Escherichia coli, Applied and Environmental Microbiology, pp. 3360-3367, 2011.
4. Oblinger, J.L. and Koburger, J.A., Understanding and Teaching the Most Probable Number Technqiue, Journal of Milk Food Technology, 38, pp. 540-545, 1975.