
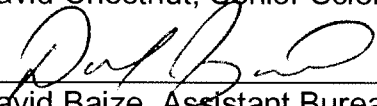
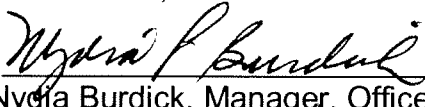
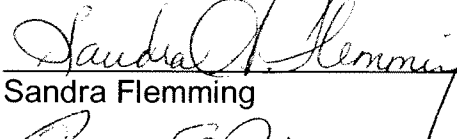


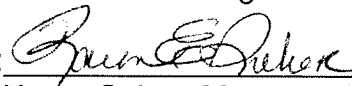
QAPP Addendum – June 1, 2009

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This Addendum modifies Section B-4. Analytical Methods of the approved QAPP for this project.

Background

The *Escherichia coli* (*E. coli*) and *Enterococcus* tests being employed in the Evaluation of Alternative Freshwater Pathogen Indicators study uses the IDEXX Quanti-Tray/2000, a tray with 49 large and 48 small wells, for enumeration, and reagents that are selective for the targeted organism(s). The quantification of the targeted bacteria is based on the Standard Methods Most Probable Number (MPN) approach using the number of large and small wells exhibiting a positive result.

There are 2450 different combinations of positive well counts, but only 1496 different possible MPN values because multiple combinations result in the same MPN value. For example, 17 different combinations result in 51.2.

The distribution of possible MPN values shows much better resolution between values within a range near the lower values and a wider spread of numbers towards the higher end. For instance there is relatively good discriminatory power around 100, i.e. it is possible to get more values between numbers. There are 12 different positive well combinations that yield values between 99.0 and 99.9. There is only one possible way to get 260 (260.3) or 264 (264.6), and the difference between consecutive values gets larger as you get to the higher values, for example the possible values progress from

593.8, 601.5, 613.1, etc. It isn't possible to get 604. The maximum value on the MPN chart is >2419.6.

To date the *E. coli* results are producing many values in the range of 300 to 800, where the MPN table has less discriminatory power, and several values reported as >2419.6.

IDEXX recommends dilution to get within the MPN range needed, but the least dilution necessary is preferable. As part of an e-mail exchange, Krista Doucette, Water Technical Support, IDEXX Laboratories, Inc., said, "Typically, the least diluted sample which gives a readable tray with a mix of positive and negative wells (such as 80% positive/20% negative) would be the reportable MPN result. Sensitivity may be lost with each increasing dilution, but not any more so in the confidence limits. When multiplying the confidence limits by the dilution factor the range stays comparable to the range of an undiluted sample with a higher MPN value. For example if an undiluted sample gave an MPN of 920.8 95% Conf. 620.5-1282.0 and with a 1:2 dilution sample with an MPN of 452.0 after calculations would be MPN 904.0 95% Conf 715.2-1113.4. These numbers are not significantly different and as mentioned above we suggest using dilutions as per the attached reference from Methods for General and Molecular Biology, American Society for Microbiology, which references the most reliable data comes from the level in which 20 % of the cultures are negative."

If a 1 to 4 dilution using 25 ml sample, 75 ml sterile deionized (DI) water were used, then to account for the dilution, the MPN value from the table would be multiplied by 4, and the associated 95% confidence limits would also be multiplied by 4. With 49 large and 48 small wells, 80% positive wells are 39 and 38 respectively, for an MPN of 180.7. So the 1 to 4 dilution using 25 ml sample, 75 ml DI water would equate to 722.8, which allows for greater resolution of values within the ranges that have been observed.

Values of 236, 299, 409, and 576 are numbers from the 1986 EPA criteria for *E. coli*. Using the MPN table values and multiplying by 4, as in a 1 in 4 dilution (25 ml sample, 75 ml DI water), and comparing the resolution indicated by the number of combinations that give an actual number greater than 235 and less than 237, there is better resolution with the dilution results, 9 possible values within the range compared to only 4 without dilution. Similarly for values greater than 298 and less than 301, there are 12 possible values with dilution vs. 2 without dilution. Looking at values greater than 408 and less than 411, there are 8 possible values with dilution and only 2 without. For values greater than 574 and less than 579, there are 7 possible values at 1 to 4 dilution and only 1 possible value without.

With a 1 to 4 dilution (25 ml sample, 75 ml DI water) we get much better resolution in the range of values important for picking a standard within EPA acceptable ranges. While this does somewhat reduce resolution for very low values (i.e. <40) this range will be below any of the possible criteria for *E. coli*.

Considering the impact and importance of setting a new statewide pathogen standard to be protective of human health that will impact a wide range of BOW activities and the regulated community it is imperative that there is adequate resolution within the results to evaluate the different available criteria options.

Changes

Beginning with the samples collected the week of June 15, 2009, a 1 to 4 dilution (25 ml of sample, 75 ml of sterile DI water) will be used for all *E. coli* samples from all sample locations for the duration of the study.

The dilution will be accomplished by pipetting 25 ml of the sample into the Colilert sample bottle and then filling the bottle to the 100 ml line with Sterile DI water.

There are no changes proposed for the *Enterococcus* analysis.