Geometric Means, Statistical Threshold Values, and Microbial Die-Off Rates

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This document outlines how to perform key mathematical steps necessary to develop a microbial water quality profile (MWQP) and then calculate microbial die-off if the MWQP values exceed numerical Geometric Mean (GM) and Statistical Threshold Value (STV) criteria outlined in the Food Safety Modernization Act (FSMA) Produce Safety Rule. There are tools to make the calculations easier, but for those who want to know how to do the math, it is covered here.

The first VERY IMPORTANT POINT!

Here are several web-based and Excel tools that can do the calculations once water samples have been analyzed and test results are in hand. If these tools meet your needs, there may be no need to do the math the long way.

- An Excel spreadsheet, created by the Western Center for Food Safety at UC Davis, can be accessed at wcfs.ucdavis.edu

- An Ag Water App and an Online Calculator, created by University of Arizona, can be accessed at agwater.arizona.edu and agwater.arizona.edu/onlinecalc (respectively)

Some more VERY IMPORTANT POINTS!

This is not a standalone document, it is a supplement to the Produce Safety Alliance (PSA) Grower Training curriculum and information presented in **Module 5: Agricultural Water**. This document simply serves as an example of how to do the math. The full requirements of how the MWQP is developed and used can be found in the FSMA Produce Safety Rule and the PSA Grower Training curriculum.

The MWQP consists of two calculated values: the geometric mean (GM) and the statistical threshold value (STV). This document describes how to calculate the GM and STV using quantified generic *E. coli* water test results. For agricultural water intended to or likely to contact covered produce (other than sprouts) during growing activities, the numerical criteria are:

a. Geometric Mean of 126 or less CFU generic *E. coli* per 100 mL water **AND**

b. Statistical Threshold Value of 410 or less CFU generic *E. coli* per 100 mL water

The initial MWQP described in the FSMA Produce Safety Rule for untreated surface water is based on at least 20 water samples taken over a minimum of 2 years and a maximum of 4 years or, for untreated ground water, at least 4 samples taken over 1 year. The initial MWQP is then updated annually. See **Module 5: Agricultural Water** or § 112.46 in the FSMA Produce Safety Rule for the detailed requirements.

The FSMA Produce Safety Rule also includes several corrective measures that can be taken if the water source does not meet the numerical GM and STV criteria. One of the corrective measures is to apply a time interval from the last water application to harvest to allow microbial die-off. This corrective measure uses a die-off rate of 0.5 log/day (for no more than 4 days) to achieve a calculated reduction of the GM and STV to meet the numerical criteria. This document also provides an example of how to calculate the die-off time interval.

The Fun Begins!

An untreated surface water MWQP of 20 sample test results is included in this factsheet. Growers can follow the steps shown in these example calculations using their own results to get the GM and the STV. These are the calculations behind the MWQP that is calculated for some uses of agricultural water, described above.

Step 1: Collect water samples from the agricultural water source.

Follow laboratory instructions and the requirements of the FSMA Produce Safety Rule to collect water samples from the agricultural water that needs to be tested. This example is based on water samples collected from untreated surface water used as agricultural water. Have the laboratory analyze the sample for the concentration of (e.g., quantified) generic *E. coli* as CFU/100 mL of water.

Step 2: Gather together the sample results.

The example data set includes 20 test results that would be the minimum number of results for the MWQP of an untreated surface water source.

Year	Sample	Result CFU/100mL
1	#1	73
1	#2	260
1	#3	900
1	#4	140
1	#5	5,500
2	#6	120
2	#7	2,300
2	#8	180
2	#9	510
2	#10	210
3	#11	1,200
3	#12	110
3	#13	290
3	#14	2,100
3	#15	220
4	#16	1,600
4	#17	120
4	#18	270
4	#19	67
4	#20	220

Step 3: Log transform the sample results

Log transformation is often done with results that cover a wide range of values, such as these results that range from 67 CFU/100 mL water to 5,500 CFU/100 mL water. To do a log transformation on the results, use the log function on a calculator or a spreadsheet such as Microsoft Excel or OpenOffice Calc. Tables of log values are also available on the internet. The example calculations were done using log base 10. If the results you get do not match the results below, check your calculator's manual or spreadsheet's supporting information. Some log tables, calculators, and spreadsheet functions are based on natural log and will not give the same log (base 10) transformed values as below.

The results from the lab usually should have only 2 significant digits (there are only 2 'real' numbers followed by zeroes that show order of magnitude, like in the value 5,500 above; 2 significant digits are underlined). To avoid rounding errors, calculations are done using 1 extra significant digit. In this fact sheet the calculations are done using 3 significant digits. For log-transformed values, the number of significant digits is the number of decimal places, like in 1.863 below (3 significant digits are underlined). Once calculations are complete, the final result is rounded back down to two significant digits (e.g., 354 becomes 350, 356 becomes 360).

Year	Sample	Result (Log CFU/100mL)
1	#1	1.863
1	#2	2.415
1	#3	2.954
1	#4	2.146
1	#5	3.740
2	#6	2.079
2	#7	3.362
2	#8	2.255
2	#9	2.708
2	#10	2.322
3	#11	3.079
3	#12	2.041
3	#13	2.462
3	#14	3.322
3	#15	2.342
4	#16	3.204
4	#17	2.079
4	#18	2.431
4	#19	1.826
4	#20	2.342

Step 4: Calculate the Geometric Mean (GM)

The GM can be calculated without log transformation. There is a function in some spreadsheets, such as the GEOMEAN function within Excel, that uses regular results. Since the STV has to be calculated, and the STV calculation requires log transformation, the GM calculation here is done using log-transformed results.

The first step in the GM calculation is to take the average of the log results. To get the average, add all the log-transformed results from Step 3 (i.e., 50.972), and then divide by the number of samples (i.e., 20 samples in this example). The average of the 20 log-transformed results in the example data set is 2.549.

Next, convert the average from the log scale to the regular scale. This process is called taking the antilog.

- One way this can be done is to use the antilog function on a calculator to convert the calculated log value (2.549) to a regular scale value.

- Another way to find the regular value is to raise 10 to the power of the log value (the button is labeled 10^y on many calculators. For this example, "y" would be 2.549).

- A third way is to use a spreadsheet function such as the POWER function in Excel. For this example, the formula would be =POWER(10,2.549)

Since the log-transformed value of the GM is 2.549, the regular value of the GM is the antilog of 2.549 ($10^{2.549}$) or 354 CFU/100 mL.

- When using this as a final result it would be rounded to 2 significant digits, or 350 CFU/100 mL.

Step 5: Calculate the Statistical Threshold Value (STV).

Calculate the STV using this formula. Steps 5a through 5d give details of the process.

$$\log(STV) = avg(\log values) + 1.282 * std(\log values)$$

5a) Here is the good news: *avg(log values)* is the log-transformed GM value from Step 4 (i.e., 2.549).

$$\log(STV) = 2.549 + 1.282 * std(\log values)$$

5b) Next, and this may be tricky, calculate the standard deviation of the log-transformed results from Step 3. Use a statistical calculator or a spreadsheet to find the sample standard deviation (the function in Microsoft Excel is STDEV). The process is different for different software and calculators so you may need to look in the instruction manual for the statistical calculator, the help function in the spreadsheet program, or equations from the internet to help. The standard deviation of the log-transformed results from Step 3 is 0.547.

5c) Plug all the values into the log*(STV)* formula. The value 1.282 is a constant that is needed in the calculation (the STV is an estimate of the 90th percentile of the data set). The value 1.282 comes from statistical tables. From here, the math is a little simpler. Do the multiplication and addition, and calculate the value for log*(STV)* of the example data set. It should look like this:

$$\log(STV) = 2.549 + 1.282 * 0.547 = 3.250$$

5d) Using the log value calculated in Step 5c (i.e., 3.250), calculate the regular value using the same process used in Step 4. The STV in the example calculation is the antilog of 3.250, or $10^{3.250}$. The back transformed, or regular, value is calculated to be 1,778 CFU/100 mL.

- When using this as a final result it would be rounded to 2 significant digits, or 1,800 CFU/100 mL

Step 6: Determining if a Corrective Measure is Necessary

Important Note: The next steps use the MWQP that consists of the GM and STV calculated from the full 20 samples. The calculations resulted in a GM of 350 CFU/100 mL and an STV of 1,800 CFU/100 mL.

Comparison of the calculated GM and STV to the numerical criteria shows:

- The GM (350 CFU/ 100 mL water) exceeds the GM criterion (126 or less CFU/100 mL).

- The STV (1,800 CFU/100 mL water) exceeds the STV criterion (410 or less CFU/100 mL).

Step 7: Applying a Time Interval as a Corrective Measure

Several corrective measures are included in the FSMA Produce Safety Rule. One of these is detailed in § 112.45(b)(1)(i)(A), and allows calculation of microbial die-off between the last water application and harvest at a rate of 0.5 log per day, for up to four days. The half-log die-off rate is approximately the same as:

- 68.38% die off over one day (31.62% remains),

- 90.00% over two days (10% remains),

- 96.84% over three days (3.16% remains), and

- 99.00% over four days (1% remains).

Either of the two calculations below results in the same results for the half-log die-off per day adjustment.

1. Multiply the GM by the percent remaining on each day, using the values given above in parentheses

   ○ For the GM of 354 CFU/100 mL calculated in Step 4 (before rounding to 2 significant digits), the amount remaining after one day is 31.62%

     • **Day 1**: 354 x 0.3162 = 112 (round to 110 CFU/100 mL when used as a final value)

2. For each day of die-off, subtract 0.5 from the log(GM) and log(STV) values that were calculated in Step 3 and Step 5, then back transform the adjusted value. Remember the calculation is antilog(y) = 10^y.

   ○ For the GM of 354 CFU/100 mL calculated in Step 4, the adjusted value after one day is 0.5 log lower than the original value. The log-transformed value of 354 is 2.549 (from Step 3).

     • **Day 1**: 2.549 − 0.5000 = 2.049 and $10^{2.049}$ = 112 (round to 110 CFU/100 mL)

The same calculations are done for STV in order to know the minimum time interval that would meet the requirements of this corrective measure, since the adjusted GM and the STV both need to meet the criteria.

- Day 1: 1,780 x 0.3162 = 563 <u>OR</u> 3.250 − 0.500 = 2.750 and $10^{2.750}$ = 562.

  ○ Both round to 560 CFU/100 mL when using as the final value

- Day 2: 1,780 x 0.1000 = 178 <u>OR</u> 3.250 − 1.000 = 2.250 and $10^{2.250}$ = 178

  ○ Both round to 180 CFU/100 mL when using as the final value

Since both GM and STV numerical criteria in the Produce Safety Rule must be met, the 1-day time interval is not enough because the adjusted STV is still over the criterion after 1 day (i.e., 560 CFU/100 mL). After a 2-day interval, the adjusted STV and GM both meet the numerical criteria. The water source would meet the numerical requirements of the FSMA Produce Safety Rule, as long as the produce is not harvested for at least 2 days after the last water application.

Remember that the FSMA Produce Safety Rule only allows a maximum 4-day time interval when using the 0.5 log/day microbial die-off rate, so a 2-log reduction (1% remains) is the biggest adjustment that is allowed.

Summary

The mathematical calculations outlined in this document were designed as instructions for how to calculate the GM and STV values that are part of the MWQP. If the GM or STV of the MWQP sample results are higher than the numerical criteria outlined in the FSMA Produce Safety Rule, growers have the option of applying a corrective measure if they want to continue using it as agricultural water. One corrective measure is to use a time interval for die-off between last application and harvest. Calculations supporting the adjustment for die-off interval were also included in this document. All of these calculations can also be done using tools that are available online.