Duplicate Sample Testing and Evaluation for the Microbiology Laboratory

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What is True Duplicate?

EPA defines duplicate or collocated samples as:

- **Duplicate samples**: two samples taken from and representative of the same population and carried through all steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variance of the total method including sampling and analysis.
- **Collocated samples**: one of two or more independent samples collected so that each is equally representative for a given variable at a common space and time.

Why Test Duplicate Samples?

- Measure of Precision or an evaluation of the closeness of two or more measurements to each other.
- End game…can the test be repeated under the same conditions and yield the same relative results? How precise is the testing regime at the laboratory?
- Couple with measures to determine accuracy (i.e. Ongoing Recovery and Precision (OPR) samples or the recovery of the known bacterial density of a laboratory enumerated sample or purchased external QC sample).
- Labs can be precise and not accurate and visa versa.

Frequency of Duplicate Sample Testing

- Method specific if stated in the method
- For SM testing, Section 9020B.9.c required for all enumerated/quantitative methods
- Required to be performed at least monthly or more frequently as needed.
- NJ uses one duplicate sample per test run or if the laboratory performs less than 10 test per week with an enumerated method, then the lab can perform a weekly duplicate sample. Other states or data users may require a different frequency.

How to Collect a True Duplicate Sample?

- All samples for microbiology testing are collected as grab samples.
- Three ways to collect a duplicate sample:
  - 1. With two different sample containers at the ready and once the sample site has been prepared, collect one sample and then the next sample in rapid succession.
  - 2. With two different sample containers at the ready and once the sample site has been prepared, hold the two bottles and move back and forth through the flow to fill the two bottles.
  - 3. Collect one large sample volume and aseptically transfer the sample to two smaller containers to submit for testing.

Duplicate Sample Collection

- All sample containers must be verified as sterile before use by the testing lab. The duplicate QC sample must be representative of the routine samples collected. Each matrix is to be tested.
- The sample must be at least 100mls in volume to meet the definition of a grab sample.
- Remember before pouring off a sample and its duplicate from a larger container, the sample must be well shaken (i.e. 25 one foot arcs) before the first aliquot is taken and then again before the second aliquot (the duplicate) is taken. Be sure to use aseptic techniques for the transfer.
- In all cases the samples must be well mixed before an aliquot is removed for testing to ensure an even distribution of the parameter of interest.

Logarithms of a Determined Value

- **Know the difference between logarithmic and exponential equations.** This is a very simple first step. If it contains a logarithm (**for example: $\log_a x = y$)** it is logarithmic problem. A logarithm is denoted by the letters **"log"**.
- If the equation contains an exponent (that is, a variable raised to a power) it is an exponential equation. An exponent is a superscript number placed after a number.
- Logarithmic: $\log_a x = y$
- Exponential: $a^y = x$

SM Approach to the Development of an Acceptance Range (3.27 x mean R)

- Perform duplicate testing on at least 15 positive samples. Record as “D1 and D2” or another recognizable form of sample identification.
- Include all analysts with each performing and equal number of the duplicate sample testing for multiple analysts labs.
- Handle the duplicate sample testing as all real world analyses performed.
- Convert the results to logarithms (number base 10).

Development of the Acceptance Range: Precision of Quantitative Methods

- Standard Methods, 22nd edition, 9020B.9.e
- Reference associated with all approved SM methods and a guideline to use for other method references that do not include information on the acceptance limits for precision.
- If another method source has other requirements they must be followed.

Formula from SM

- If either of the determined duplicate results are <1 add a 1 to both numbers/results before calculating the log. Not sure when this happens for enumerated testing…can you think of any examples?
- Once the log is calculated determine the range (R_{log}) by subtracting the log of the higher of the two results from the other log. No values can be negative for the range. The difference is the range (R_{log}).

Formula from SM

- Calculate the mean of the ranges (R bar).
- Sum all of the log values ($\sum$ of R_{log})
- Divide this value by n
- n = the number of sample run in duplicate for the data set (example in SM uses 15 for this value)
- 3.27 times mean of the range is the acceptance criteria to use

Mean R = ($\sum$ of R_{log}) / n

What's next?

- Once the value for the (mean range x 3.27) is established at the laboratory…
- Run a duplicate sample set. Add a 1 to any values that are <1.
- Transform the results to the log base 10.
- Calculate range of the log values.
- If this value is not ≤ the lab's mean range, then the data set is not acceptable.

Examples

Duplicate Results n = 16		Logarithms of Results		Range of Log
10	15	1.0000	1.1761	0.1761
22	23	1.3424	1.3617	0.0193
35	42	1.5441	1.6232	0.0791
50	60	1.6990	1.7782	0.0792
35	38	1.5441	1.5798	0.0357
120	110	2.0792	2.0414	0.0378
38	34	1.5798	1.5315	0.0483
110	121	2.0414	2.0828	0.0414
6	7	0.7782	0.8451	0.0669
58	67	1.7634	1.8261	0.0627
43	58	1.6335	1.7634	0.1299
32	42	1.5051	1.6232	0.1181
12	11	1.0792	1.0414	0.0378
4	6	0.6020	0.7782	0.1762
71	82	1.8513	1.9138	0.0625
35	47	1.5441	1.6721	0.1280

0.1761 + 0.0193 + 0.0791 + …. +0.0625 + 0.1280 = 1.299 = sum of range of log values

1.299/16 = 0.0812 = mean range

3.27 x 0.0812 = 0.2655 = precision criteria

Examples

- Lab has determined that the precision criteria for the lab is .2655.
- The lab then runs another two sets of duplicate results…

Duplicate Results done on 6/5 and 6/6/15		Logarithms of Results		Range of Log	Acceptable
35	38	1.5441	1.5798	0.0357	Yes
4	20	0.6020	1.3010	0.6990	**No**

And Then What?

- The determined precision criteria when not met also means that there is a 99% probability that the laboratory variability is excessive and discard all analytical results since the last precision check should be discarded or qualified when reported as not meeting QC requirements. Sample collection and analysis should be repeated.
- For our example this would mean that the lab needs to determine the analytical problem, resolve the problem and then repeat the precision study.

Examples of Online Log Calculators (use number base of 10, b=10)

- http://www.1728.org/logrithm.htm
- http://ncalculators.com/number-conversion/log-logarithm-calculator.htm
- https://www.easycalculation.com/log-antilog.php