qPCR Report
Enterococcus TaqEnviron Assay
20111110 plate 13 env mm MR-ls.pcrd

June 10, 2014

Organization: SCCWRP
Date Performed: 11/10/2011 12:34:47 PM UTC -08:00
Protocol: Sipp Entero1A.prcl
Sample Volume: 25 µl

1 Standard Curve QC Results

Both enterococcus and sketa standard curves must have an r^2 that is greater than 0.98, and an efficiency that is between 1.87 and 2.1.

Target	Parameter	Value	QC
Entero1A	r-squared	1.00	PASS
Entero1A	Amplification Factor	1.98	PASS
Sketa	r-squared	1.00	PASS
Sketa	Amplification Factor	1.98	PASS

2 NTC and NEC QC Results

Both the NTCs (qPCR blanks) and NECs (extraction blanks) must be non-detects. Detectable signals in any replicates will cause these tests to fail.

Target	Sample	Ct_{Rep1}	Ct_{Rep2}	QC
Entero1A	NEC	N/A	N/A	PASS
Entero1A	NTC	N/A	N/A	PASS
Sketa	NTC	N/A	N/A	PASS

3   Sample Processing and Inhibition Control QC Results

The sketa calibrator internal control Ct on this plate was 21.82, with a standard deviation of NA. In order to pass, the difference between the mean sample sketa Ct (sketaCt$_{mean}$) and the calibrator Ct (i.e., ΔCt$_{mean}$) must be less than 1.7. Note that the threshold level and pass/fail designations assume that the sample has not been diluted.

Additionally, the mean sketa Ct in the NECs was 22.08. A large difference between this value and the calibrator sketa would indicate some sort of problem.

Calibrator Ct	Δ Ct	QC
21.94	-0.14	PASS
21.70	-0.38	PASS

Sample	sketaCt$_{mean}$	ΔCt$_{mean}$	QC
1	22.17	0.09	PASS
1 1:5	24.42	2.34	FAIL
2	21.98	-0.10	PASS
2 1:5	24.44	2.36	FAIL
3	22.07	-0.01	PASS
3 1:5	23.91	1.83	FAIL
4	22.13	0.05	PASS
4 1:5	24.09	2.02	FAIL
5	22.12	0.04	PASS
5 1:5	24.26	2.18	FAIL
6	35.88	13.80	FAIL
6 1:5	38.55	16.48	FAIL
7	22.25	0.17	PASS
7 1:5	24.16	2.08	FAIL
8	22.23	0.16	PASS
8 1:5	24.23	2.15	FAIL
9	21.98	-0.10	PASS
9 1:5	24.19	2.11	FAIL

4 Enterococcus Cell Equivalence Estimation

Cell equivalents (CE) per reaction is calculated using the ΔCt method, in which samples are compared to the calibrator in the following way:

$$\log_{10} CE = \frac{\Delta\text{Ct}_{samp,cal}}{slope} + \log_{10} cal$$

where *slope* is the slope of the enterococcus standard curve (-3.365 for this plate), *cal* is the enterococcus calibrator expected CE, and $\Delta\text{Ct}_{samp,cal}$ is the difference in Ct between the mean of the calibrators and the sample. These values are then transformed to CE per filter (assumed to be 100 ml), and are reported below. Samples indicated to be inhibited by sketa controls are labeled as such. Uninhibited CE estimates that are below the detection limit (set to a Ct of 45) are denoted by "ND". Any detected inhibition among sample replicates causes the mean to be labeled as "inhibited". Remember that these CE estimates (both replicate and mean) are reported on a logarithmic scale.

Sample	Target	Ct_{Rep1}	Ct_{Rep2}	$\log_{10}$ cells/100 ml$_{Rep1}$	$\log_{10}$ cells/100 ml$_{Rep2}$	Mean $\log_{10}$ cells/100 ml
1	Entero1A	39.05	37.08	0.275	0.86	0.659
1 1:5	Entero1A	N/A	39.16	N/A	0.242	Inhibited
2	Entero1A	36.66	36.56	0.985	1.015	1
2 1:5	Entero1A	N/A	N/A	N/A	N/A	Inhibited
3	Entero1A	N/A	38.22	N/A	0.522	N/A
3 1:5	Entero1A	N/A	39.92	N/A	0.016	Inhibited
4	Entero1A	39.16	37.89	0.242	0.62	0.471
4 1:5	Entero1A	N/A	N/A	N/A	N/A	Inhibited
5	Entero1A	36.35	36.23	1.077	1.113	1.095
5 1:5	Entero1A	N/A	N/A	N/A	N/A	Inhibited
6	Entero1A	N/A	N/A	N/A	N/A	Inhibited
6 1:5	Entero1A	N/A	N/A	N/A	N/A	Inhibited
7	Entero1A	N/A	36.29	N/A	1.095	N/A
7 1:5	Entero1A	39.81	37.52	0.049	0.73	Inhibited
8	Entero1A	N/A	38.43	N/A	0.459	N/A
8 1:5	Entero1A	N/A	39.9	N/A	0.022	Inhibited
9	Entero1A	38.17	39.15	0.536	0.245	0.414
9 1:5	Entero1A	N/A	N/A	N/A	N/A	Inhibited