qPCR Report
Enterococcus TaqEnviron Assay
new ent template

June 12, 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	0.94	FAIL
Entero1A	Amplification Factor	1.91	PASS
Sketa	r-squared	0.73	FAIL
Sketa	Amplification Factor	2.59	FAIL

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 21.39, 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 21.39. A large difference between this value and the calibrator sketa would indicate some sort of problem.

Calibrator Ct	Δ Ct	QC
21.39	0.00	PASS
21.39	0.00	PASS

Sample	sketaCt$_{mean}$	ΔCt$_{mean}$	QC
Sample1	21.39	0.00	PASS
Sample10	21.39	0.00	PASS
Sample11	21.39	0.00	PASS
Sample12	21.39	0.00	PASS
Sample13	21.39	0.00	PASS
Sample14	21.39	0.00	PASS
Sample15	21.39	0.00	PASS
Sample16	21.39	0.00	PASS
Sample2	21.39	0.00	PASS
Sample3	21.39	0.00	PASS
Sample4	21.39	0.00	PASS
Sample5	21.39	0.00	PASS
Sample6	21.39	0.00	PASS
Sample7	21.39	0.00	PASS
Sample8	21.39	0.00	PASS
Sample9	21.39	0.00	PASS

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:

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

where *slope* is the slope of the enterococcus standard curve (−3.564 for this plate), *cal* is the enterococcus calibrator expected CE, and $\Delta \mathrm{Ct}_{samp,cal}$ 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
Sample1	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample10	Entero1A	29.8	29.78	3.168	3.173	3.171
Sample11	Entero1A	29.8	29.78	3.168	3.173	3.171
Sample12	Entero1A	29.8	29.78	3.168	3.173	3.171
Sample13	Entero1A	29.8	29.78	3.168	3.173	3.171
Sample14	Entero1A	29.8	29.78	3.168	3.173	3.171
Sample15	Entero1A	29.8	29.78	3.168	3.173	3.171
Sample16	Entero1A	29.8	29.78	3.168	3.173	3.171
Sample2	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample3	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample4	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample5	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample6	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample7	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample8	Entero1A	26.65	26.77	4.052	4.018	4.035
Sample9	Entero1A	29.8	29.78	3.168	3.173	3.171