

Using AquaSnap™ Total & Free for Estimating Bacteria Levels

AquaSnap Total & AquaSnap Free test devices provide an easy way to estimate microbial load in a water sample. AquaSnap Total is designed to rapidly assess total ATP levels in liquid samples. Companies use this quick test to assess cleaning practices, monitor processes or bioloads in sealed cooling/heating water systems, and evaluate the efficacy of Clean In Place (CIP) systems by detecting the presence of organic contamination from both microbial and non-microbial residues. AquaSnap Total's honey dipper collection tip is coated with an agent that aids in sample collection and extraction of ATP from microbial and other organic cells. AquaSnap Free does not use the same extraction agent on the dipper, and therefore only detects free (soluble) ATP in the sample. When using both tests on a sample, the difference in results is the estimated microbial bioload of the sample. The larger the difference, the more microbial contamination in the sample. This document compares the Relative Light Unit (RLU) measurements achieved with known dilutions of bacteria (*Escherichia coli* ATCC 9001) using AquaSnap Total and AquaSnap Free ATP detection tests measured with EnSURE and SystemSURE Plus luminometers.

Purpose

To compare RLU measurements with AquaSnap Total and Free using known low levels of *E. coli*.

Procedure

Sample Preparation

E. coli was grown overnight in TSB to an overnight concentration of 1e8 CFU/mL. The bacteria were then diluted in sterile ATP-free Ringers solution; the bacteria were diluted in a reducing 10 fold dilution series to -2, -3, -4 and -5. The counts in each dilution were counted with TSA (Tryptone Soya Agar) 100µL spread plates. 10 replicates per dilution were run.

Assay Method

AquaSnap Total or Free device activity was measured as follows:

1. Remove swab from swab tube
2. Pipette 100µl of bacterial solution directly onto the end of each dipper
3. Replace dipper and activate test
4. Squeeze reagent reservoir twice to dispense the reagent
5. Shake gently to bathe the dipper in the reagent for 10 seconds
6. Measure activity by inserting the device into the luminometer

Results

Results are displayed in Tables 1 and 2.

Conclusion

Hygiene monitoring systems and AquaSnap ATP devices detect low levels of bacteria, down to 1,000 CFU/mL or less. AquaSnap Free only measures free ATP and does not extract ATP from bacterial cells. Thus to estimate microbial ATP, both tests should be used. For more information about AquaSnap, visit www.hygiene.com

Table 1. Average RLU measurements on EnSURE with known dilutions of *E. coli* using AquaSnap Total and Free

Dilution (CFU/mL)	EnSURE		Difference
	AquaSnap Total	AquaSnap Free	
Dilution -2 (1,000,000)	MAX	268	MAX
Dilution -3 (100,000)	1312	41	1271
Dilution -4 (10,000)	187	5	182
Dilution -5 (1,000)	18	0	18
Blank	0	0	0

Table 2. Average RLU measurements on SystemSURE Plus with known dilutions of *E. coli* using AquaSnap Total and Free

Dilution (CFU/mL)	SystemSure Plus		Difference
	AquaSnap Total	AquaSnap Free	
Dilution -2 (1,000,000)	6845	181	6664
Dilution -3 (100,000)	566	29	537
Dilution -4 (10,000)	62	2	60
Dilution -5 (1,000)	7	0	7
Blank	0	0	0