

# Causes of Higher RLU Values for AquaSnap Free vs Total

**AquaSnap Free:** Measures all free ATP in a water sample. This product does not contain any surfactant and is essentially measuring all ATP in the sample from every source (except cellular sources).

**AquaSnap Total:** Contains a surfactant to lyse the cellular membrane and break into intracellular ATP sources, which are then mixed with the sources in the collection tube to get a “total” count of ATP from both intracellular and extracellular sources.

If you combine both samples and subtract Free from Total, you should get a theoretical value corresponding to the amount of ATP by cellular contamination in that water source.

**Note:** *The RLU value is not equivalent to a CFU result but a measure of ATP contributed by microbial cells.*

The AquaSnap Total and Free tests are designed to be used for industrial water samples eg. Cooling Tower water and where there is expected to be a high microbial count. Thus, the AquaSnap Total and Free should only be used where there is expected to be greater CFU count than  $10^4$  to  $10^5$  microbial cells depending on organism type.

## Cause of Negative Difference

But what happens if you get a negative number? Or numbers that do not make sense. It could be due to several issues.

- The water source contains a substance that is denaturing the luciferin/luciferase enzymatic mix and causing some enzymatic inhibition in one or more samples. Thus, lowering the amount of ATP measured. This could skew the sample.
- The samples are not “mixed” properly. Total dissolved ATP is different in both samples. This can be fixed by stirring or vortexing the main water sample properly. For example, you have a 1 L sample from which you take your testing samples, the 1L sample should be properly vortexed to have

equal parts dissolved ATP and/or bacteria in both testing samples.

- Ensure the instructions are followed carefully to avoid any user error. ATP based light production is produced on a curve. To test the samples properly, you cannot activate both samples at the same time and test one after another. The first reaction will test normal, the second reaction might test lower or higher. Each sample must be tested using the exact same procedures, on an individual basis.
- Highly contaminated samples can overload the test and instrument. If you are getting very high RLU values for your samples, what is happening is that the enzymatic mix is getting overloaded and may not give accurate values. To fix this, try diluting the main sample with serial dilutions and then quantifying the dilutions.
- Where samples are tested but have lower microbial viable counts than  $10^4$  to  $10^5$  microbial cells, then a combination of test sensitivity and sample variation can show a Free RLU value greater than a Total RLU value.

## Examples and Solutions

**Example 1:** A 20 Gallon sample of water was taken from a freshwater stagnant lake. Testing determined the pH of the water was 7, with no other significant mineral or chemical abnormalities.

Round 1	Total	Free	Difference
John	2558	4784	-2226
Ken	1322	1222	100
Brian	817	1555	-738

It was not mixed. 3 samples were taken by 3 different individuals and arrived at 3 wildly different sample values, some were positive and some were negative. In this case, the most likely cause of the issue is that the sample was not properly stirred and while the values are high, they are not absurdly high. A serial dilution would also help address the high RLU values.

**Example 2:** A sewer line burst, and direct wastewater was mixed in with water from a swimming pool that contains muriatic acid (HCL). A 20-gallon sample was taken and tested.

Samples from the sewer line directly tested 20,000 RLU for Free and 20,000 RLU for total.

Samples from the pool were tested as follows.

<b>Round 2</b>	Total	Free	Difference
John	1223	1453	-230
Ken	1350	1156	194
Brian	1168	1250	-82

What you are seeing here is the direct sewer sample testing so high it is off the charts, the MAX RLU value that you can get from an EnSURE Touch is 20,000 RLU. We know 100% the sample is contaminated.

The pool water is testing much lower. This is because the acidity in the water is causing enzymatic inhibition. The negative values could also be attributed to not stirring the sample properly. The HCL is not only killing the bacteria, but also inhibiting detection to the point that we do not know exactly how clean or dirty this sample really is.

**Example 3:** rainwater from an open-air collection tank is taken in for sample after organic plant material was found decaying in the tank.

<b>Round 3</b>	Total	Free	Difference
John	1223	900	323
Ken	1350	1050	300
Brian	12	1312	-1300

All 3 technicians tested samples. 2 of them came up with very similar numbers, but the third, Brian, activated both tubes and tested free before total. This caused a low value for total as he missed his testing window completely. This is due to user error. The total was tested incorrectly and came up with the difference as a negative value.

**Contact Hygiena Technical Support for further assistance.**

- Phone: 1-888-HYGIENA (1-888-494-4362, option 2)

- Email: [techsupport@hygiena.com](mailto:techsupport@hygiena.com)
- [Submit a Support Ticket](#)
- [Schedule a Microsoft Teams meeting with support](#)